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Harald Bosse, Egbert Buhr, Thorsten Dziomba,
Vasile-Dan Hodoroaba, Tobias Klein, Michael Krumrey (Hrsg.)

NanoWorkshop 2018:
Workshop on Reference Nanomaterials

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Current situation and needs:
development, measurement, standardization

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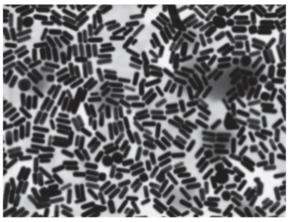

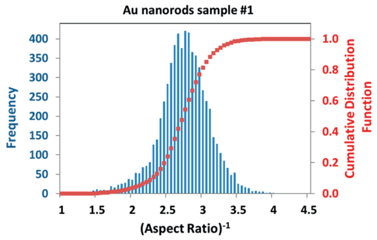

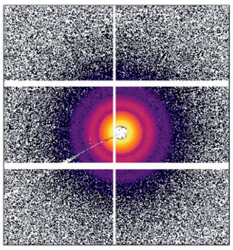

NanoWorkshop 2018

Workshop on Reference Nanomaterials Current situation and needs: development, measurement, standardization

PTB Berlin-Adlershof
BESSY II
Germany

May 14 & 15, 2018

Organized by

PTB
Physikalisch-Technische Bundesanstalt
Braunschweig und Berlin

BAM
Bundesanstalt für
Materialforschung
und -prüfung

Sponsored by

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Physikalisch-Technische Bundesanstalt
Braunschweig und Berlin

BAM
Bundesanstalt für
Materialforschung
und -prüfung

Report on the BAM-PTB Nano Workshop on May14/15 2018 in Berlin-Adlershof at BESSY II

The **open workshop** was jointly organized by a team from BAM and PTB. The Call for Papers (see attachment, open until March14) was announced on Jan. 31, 2018 with the title:

Workshop on Reference Nanomaterials -
Current situation and needs: development, measurement, standardization

The **Scope** of the workshop was described in the announcement as: The workshop provides a forum for discussion on progress in development, characterization and application of reference nanomaterials. Results of recently finished national and international research projects will be presented. Moreover, the need for future developments will be addressed.

Attendees of the workshop were expected to be experts from academia, research institutes, regulatory bodies and industry working or interested in the area of reference nanomaterials.

The following **structure** of the workshop was planned: The main focus of the first day is on reference material needs and development, while the second day will focus on reference material characterization and standardization. The workshop will consist of presentations and discussions, poster session, lab tour to the PTB laboratories at BESSY II and a get-together at the end of the first day.

Overall **111 participants** from **13 countries** and the European Joint Research Centre registered for the workshop which was organized on two half days with **16 oral presentations** and **42 poster presentations** grouped in 2 sessions and 10 topical areas.

Participants statistics: The 111 registered attendees came from 13 different countries. In detail: AL: 1, AU: 1, BE: 4, CH: 2, CN: 1, DE: 84, ES: 2, FI: 1, FR: 5, IT: 6, PT: 1, RO: 1, UK: 1 and EU-JRC: 1. The affiliation of the participants was as follows: Industrial Company: 12; Industry Association: 1; National Metrology Institute or Designated Institute: 80 (including several PhD students working at BAM and PTB); Other Governmental Research Institute: 6; Research Institute: 6; University: 4; Standardization Organization: 1; EU-Joint Research Center: 1.

The **program** and the **abstracts of all contributions** were compiled in the abstract book. Moreover, **pdf files of the presented posters** and **oral presentations** are attached to this report.

The **main objective of the workshop** was to present, discuss and reflect on the latest developments in the area of **Reference Nanomaterials** starting from an analysis of the current situation and then looking ahead guided by the following questions:

Is there a need for:
- new or improved types of (certified) **reference materials**?
- new or refined **measurement methods** or new approaches?
- new or further developed **international standards**?

A summary of the **observations, analysis and conclusions of the workshop** is given below including a table of links to information on existing reference nanomaterials.

Observations, analysis and conclusions of the workshop:

Addressees, broad spectrum of attendees:

- It was generally welcome that the Workshop successfully tried to bring together key stakeholders from industry and researchers from different disciplines and institutes. The general perception is that there is too often a gap between the needs of industry, the challenges resulting e. g. from the EC recommendation for a definition of nanomaterials, referred to by several industry representatives, and the practical support given by guidelines, standards and the available reference materials. It is targeted that more representatives from companies and associations commercially dealing with the broad range of nanomaterials in very different branches get involved and join efforts with metrologists, standardizers and regulators to ensure a safe, practical and efficient application of these materials, aiming at bridging this gap, based on more exchange and more joint initiatives to achieve a better mutual understanding.
- While industry urgently needs quick, uncomplicated and inexpensive methods e. g. to decide whether a material is a nanomaterial, national metrology institutes and other reference institutes need to further develop instrumentation, methods and reference materials on the highest level to ensure accuracy and traceability. This results in different priorities e. g. for measurement methods, which makes it difficult to establish guidelines and procedures considered generally valid for both sides. NMIs will, among others, further focus on the development of uniform, pure, clean and homogeneous reference materials to be calibrated with smallest possible uncertainties. industry, on the other hand, typically needs to cope with industrially relevant materials, sometimes in large quantities, thus needing to ensure that representative samples are drawn from these masses, prepare them efficiently and have a reliable measurement technique at hand, specifically adjusted to their particular needs. This needs to be reflected in guidelines and standards, and in a better guidance through these, to make it easier for such a heterogenous usership to find the information that fits the purpose.

Scope of the NanoWorkshop 2018:

- The short title "NanoWorkshop 2018" was mainly chosen for reasons of brevity and does not claim to provide a general forum on nanotechnology, as there are both many academic and commercial conferences, seminars, exhibitions and fairs dealing with the broad range of nanotechnologies and their applications.
- As the full title "Workshop on reference nanomaterials - current situation and needs: development, measurement, standardization" reveals, the focus was deliberately put on nanomaterials, as it is generally felt that the safe, efficient and traceable characterization of these materials remains an urgent challenge in many branches and continuously requires more exchange within the broad spectrum of stakeholders.
- Contrary to the many other seminar series organized by NMIs, this one does not focus on a specific measurement technique, but tries to stimulate discussions across different methods and user groups for the benefit of better nanomaterial characterization. Consequently, one criterion for the selection of contributions was whether they help with nanomaterial measurements, rather than the excellence of a particular measurement instrument or method.
- It is generally understood that nanometrology is by far broader than what such a two half-day workshop can cover, e. g. dimensional nanometrology, and participants and readers are therefore advised to refer to seminar series such as NanoScale, with the next one scheduled for October 15-16, 2019 at the PTB in Braunschweig, and their proceedings that have a clear focus on dimensional nanometrology and related measurement techniques. Similarly, element-analytical methods definitely play a decisive role for the characterization of nanomaterials that they deserve their own conferences and can only be briefly handled in this Workshop.

- The Workshop mainly focuses on particulate nanomaterials, while 2D nanomaterials such as graphene provide a multitude of exciting applications as well; as many of their characteristics and applications are unique to 2D materials only, they can certainly be dealt with in a specific event
- Similarly, semiconductor technology is closely linked with nanometrology, and many preparation techniques rely on methods developed for or mainly used in semiconductor technology and as such found their way into the Workshop. On the other hand, semiconductor technology itself is handled within so many other events and, consequently, cannot be in the focus of this Workshop.

General observations:

- As so often with nanotechnology, many materials and methods may be used in a different context as well, with the potential of synergy effects:
  - image analysis (e.g. identification of nanoparticles) and data fusion from different measurement methods to provide a holistic view: exchange and adjustment of strategies and algorithms
  - documentation (e.g. lifecycle of nanomaterials from raw fabrication via conditioning and sampling to sample preparation and its measurement) and data management (raw measurement data, documentation of data treatment and analysis steps to the processed data and their assigned measurement uncertainty)
  - nano-pillars used for nano-mechanical investigations and optical nano-gratings
  - mutual support by (vitual) nanocentres, i. e. better networking of preparation and measurement facilities, to make it easier to fabricate a certain nanomaterial or have it investigated without establishing the whole fabrication and measurement infrastructure on one's own, by referring to an established facility excellent in the field with long-term experienced experts for best value-for-money
- Industry needs easy-to-use uncomplicated reliable measurement techniques. A huge preparation effort is depreciated. For many industrial inspections, even SEM is too labour-intensive in terms of preparation, measurement and analysis so that applied, automated routines need to be implemented and validated to make it efficient. Methods like AFM, often involving an even greater preparation effort, do play an important role in the profound investigation in the background, but are in most cases regarded unpractical for everyday checks.
- Especially in backstage discussions, it became evident that sample preparation is often much more challenging in real life than suggested in some official presentations. Many participants encourage developers of standards and guidelines to adequately tackle this critical issue. Too often, the success of a preparation for the envisaged measurement obviously depends strongly on certain parameters that are in many cases unknown, sometimes leading to the unsatisfactory situation that a successful preparation depends on a certain person - for whatever reasons. On the other hand, uniform procedures for successful preparation are hard to set, as the available form of a nanomaterial may often differ from manufacturer to manufacturer or even from batch to batch, and in most cases, a nanomaterial is neither sufficiently pure nor its state and history adequately documented. An inherent challenge is that most of them may easily vary with the ambient conditions they are exposed to. Agglomeration and aggregation are a notorious challenge, and the application of sonication as well as the behavior of auxiliary agents in the preparation process therefore deserve careful consideration among many other parameters.
- In the past few years, significant progress has been achieved in the development of measurement methods for nanomaterials, incl. various initiatives to guide users through different characterization methods to help find an efficient, affordable path to the particular characterization needed, e. g, with decision trees. A prominent example is the NanoDefine e-tool with an implemented tiered approach to decide whether a material is a nanomaterial. However, many participants marked that most of these achievements, incl. many standardization projects, were based on third-party funding, and voiced the concern that continuity and sustainability are at risk once the funding ends.

Conclusions / main topics addressed in the oral presentations:

- The potential of nanotechnology was highlighted in the welcome address of Prof. U. Panne, president of BAM, by reminding the workshop on the famous words of **Richard Feynman: There is plenty of room at the bottom** in his talk nearly 60 years ago.
- H. Rauscher presented the analysis of the JRC survey from 2017 on the needs for certified and non-certified reference materials and representative test materials in the area of nanotechnology. The importance and need for reference nanomaterials was pointed out in particular for **areas under regulation** (cosmetics, food, …).
- In the talk by M. Reuter from VCI, the **tiered approach** was regarded as a reasonable approach to nanomaterial characterization in industry. However, there is still a need for measurement methods which would allow **the number of borderline materials in tier 1 testing** to be reduced or screening of manufactured nanomaterials.
- One important output of the European NanoDefine joint research project funded within the FP 7 was the development of an expert system, called "**NanoDefine e-tool**" (http://www.nanodefine.eu/index.php/nanodefiner-e-tool), which uses **expert knowledge** to guide interested users which types of measurement method could be reasonably chosen to investigate a nanomaterial of interest with respect to the EC recommendation for the definition of nanomaterial (talk by H. Rauscher on NanoDefine)
- In the presentation by H. Bresch from BAM on the OECD Test Guideline on particle size & size distribution of Manufactured Nanomaterials a need was indicated for **test materials with a longer availability** of about 20 years.
- In the talk given by A. Meyer-Plath from BauA a clear need for standards for the **mechanical properties of high aspect ratio materials** to support investigations concerning their possible toxicity and health risk was identified. Moreover, cooperations to extract **accurate dimensional information from SEM images** of high aspect ratio materials (width, length, …) were regarded as very helpful.
- Promising possibilities to generate **well defined nanoobjects of different shape** (bi-pyramids, platelets, prisms, …) by hydrothermal techniques were presented in the talk by V. Maurino from University of Torino.
- M. Krumrey from PTB presented results on absolute scattering intensity measurements in SAXS experiments, which allowed **nanoparticle concentrations** in solution to be traceably determined.
- In the talk by M. Stintz on **ISO Standardization Activities on Reference Nanomaterials** also more complex nanoparticle distributions like e.g. **picket-fence distributions** were mentioned to be now treated in international standardization projects.
- There is a clear **need to specify the magnetic properties of magnetic reference nanomaterials**, which is not the case up to now for the available magnetic reference nanomaterials as was pointed out by U. Steinhoff from PTB in his talk.
- In the presentation by E. Buhr on "TSEM: a traceable method for nanoparticle metrology" it was pointed out that there are measurement methods for nanoparticle size and size distributions (namely SAXS and TSEM by PTB), specified in the **KCDB of the BIPM**, i.e. these specifications have undergone a peer review of metrology experts worldwide.
- The question to what degree the **measurement results of different measurement methods for nanoparticle characterization can be made comparable** by proper signal modelling was discussed in the talk by V. Coleman from NMIA.

- A. Thünemann from BAM pointed out in his presentation entitled “Challenges of synthesis and analysis of core-shell nanoparticles for reference materials” that long-term stable nanomaterials in solution are always **core-shell type nanomaterials**.
- In the talk by R. Theissmann from Kronos an approach to **sectioning of manufactured nanomaterials (TiO_2)** was shown, which allowed the content of nanomaterial in a product to be accurately determined - according to the EC recommendation for definition of nanomaterial - by **highly automated SEM image acquisition and analysis**.
- D. Hodoroaba from BAM gave a presentation on “Shape controlled TiO_2 nanoparticles as candidates for nano-CRM’s – an ISO case study” in which bi-pyramidal TiO_2 nanoobjects were identified as good candidates for a **shape and size reference nanomaterial**.
- Hyphenated **ICP-MS** was presented as a good **method for size and number-based concentration measurements** in the talk by D. Bartczak from LGC. A CCQM intercomparison on colloidal Au NP and a parallel VAMAS study open for everybody to participate was started. The coupling of ICP-MS results and A4F results is also currently investigated.
- In the talk by S. Weigel from BfR the question “Can labelled nanoparticles as internal standards improve the reliability of the quantitative analysis of nanomaterials in complex matrices?” was discussed. The ICP-MS method has been investigated as a method for characterization of (labelled) **nanoparticles in complex matrices**.

Overview of topics discussed in the oral and poster contributions

a) Development of **reference materials**
b) Development of **measurement/classification methods**
c) Development of **international standards**

Code	**Title of talk**	**a)**	**b)**	**c)**	Need for **reference materials**	Need for **measurement / classification methods**	Need for **international standards**
Oral							
1	Needs for certified and non-certified reference materials and representative test materials in the area of nanotechnology – Analysis of a survey held by JRC in 2017	x	x		In particular for areas under regulation (cosmetics, food …)	In particular for areas under regulation (cosmetics, food …)	In particular for areas under regulation (cosmetics, food …)
2	Expectations, results and follow-up: industry perspective in developing a measurement strategy for nanomaterial		x			Measurement methods to reduce the number of borderline materials in tier 1 testing	
3	The NanoDefine decision framework and “NanoDefiner” e-Tool		x	x		To decide if a material falls under the EU definition of a nanomaterial	
4	OECD Test Guideline on particle size & size distribution of Manufactured Nanomaterials	x	x	x	Test materials with a longer availability of about 20 years		New technical guideline or guidance document on particle size and size distribution of manufactured nanomaterials
5	Health Issues of Micro- and Nanoscale High Aspect Ratio Materials (HARM): Need for Reference Materials and Testing Standards		x		Standards for the mechanical properties of high aspect ratio materials	Method to extract accurate dimensional information from SEM images of high aspect ratio materials	

Code	**Title of talk**	**a)**	**b)**	**c)**	Need for **reference materials**	Need for **measurement / classification methods**	Need for **international standards**
6	Synthesis of size and shape controlled TiO2 nanoparticles: possible CRM's candidates for size, shape and functional properties	x					
7	Traceable nanoparticle characterization using Small-Angle X-ray Scattering (SAXS)		x			SAXS measurements to traceably determine nanoparticle concentrations	
8	Challenges of synthesis and analysis of core-shell nanoparticles for reference materials	x	x		Core-shell type nanomaterials	For core-shell type nanomaterials	
9	ISO Standardization Activities on Reference Nanomaterials		x	x	More complex nanoparticle distributions	Measurement methods for more complex nanoparticle distributions	For measurement of more complex nanoparticle distributions
10	Standardisation of magnetic nanomaterials: Steps towards magnetic reference particles		x	x	Magnetic reference nanomaterials	To specify the magnetic properties of nanomaterials	On the specification of magnetic properties of nanomaterials
11	TSEM: a traceable method for nanoparticle metrology		x			Which have undergone a peer review and are listed in the KCBD of the BIPM	
12	Measurands and method dependent reference values: can one global particle size reference value fit all?		x			Proper signal modeling in order to make results from different measurement methods comparable	
13	TiO2 Pigment Sizing: Constituent, Aggregates and Primary Particles		x	x			
14	Shape controlled TiO2 nanoparticles as candidates for nano-CRM's – an ISO case study	x	x	x	Non-spherical shape		3-D reconstruction of nano objects using TEM (Preliminary work item of ISO)
15	Reference methods for the determination of the nanoparticle mass- and number-based concentration using hyphenated ICP-MS		x				

Code	**Title**	**a)**	**b)**	**c)**	Need for **reference materials**	Need for **measurement / classification methods**	Need for **international standards**
16	Can labelled nanoparticles as internal standards improve the reliability of the quantitative analysis of nanomaterials in complex matrices?	x	x				
Poster							
CLN-01	The Malta Project – A European Initiative to Develop and Amend OECD Technical Guidelines for Testing of Nanomaterials			x	Suitable reference nanomaterials for physico-chemical characterization for ecotoxicology and human health	Physico-chemical characterization for ecotoxicology and human health	Test guidelines and guidance documents, e.g. as OECD documents
HSN-01	Quantification of Silver Nanoparticles at Single Cell Level by Mass Cytometry		x				
HSN-02	OECD Test Guidelines Development for Chemicals Safety Assessment of Nanomaterials	x	x	x	Quite different demands due to different measurement variables	Validated, standardized and harmonized test methods for a variety of measurands	Test guideline for safety assessment
HSN-03	Assessing potential risks of nanomaterials - Genotoxicity screening with the fluorometric γ-H2AX assay and automated microscopic detection		x		Nanomaterials as positive (and negative) genotoxicity standards	Standardized, efficient and automated test procedure for the genotoxicity of functional nanomaterials	
MAN-01	Reference samples for Magnetic Force Microscopy	x	x				
NCM-01	Nanocrystals as labeling reagents for Imaging Mass Cytometry	x	x				
NCM-02	Studying nanoparticle-cell interaction by ICP-MS based techniques		x				
RMN-01	Toward a reference sample for atom probe tomography	x	x		Reference materials for atom probe tomography		

Code	**Poster title**	a)	b)	c)	Need for **reference materials**	Need for **measurement / classification methods**	Need for **international standards**
RMN-02	Creating the Silver Standard: Development and Applications of a Silver Nanoparticle Reference Material	x					
RMN-03	Microwave-assisted high-speed silver nanoparticle synthesis	x					
SDN-01	Development of a hybrid metrology combining AFM and SEM techniques for measuring the characteristic dimensions of a nanoparticle population		x		Perfectly spherical nanoparticle reference materials	Hybrid metrology, e.g. combining AFM and SEM	
SDN-02	Contribution to accurate Spherical Gold Nanoparticles analysis (size, size distribution) by SpICPMS and SAXS		x				
SDN-03	Improved traceability chain of nanoparticle size measurements – the new EMPIR project nPSize				Non-spherical shapes, polydisperse samples, accurate particle number concentration	Reliable analysis of nanoparticle size and comparable results of physically different methods	Standards for measurement of non-spherical nanoparticles
SDN-04	Advanced screening method using volume-specific surface area (VSSA) for nanomaterial identification of powders		x		Reference powders for measurement of volume-specific surface area (VSSA)	Measurement of volume-specific surface area (VSSA) for real-world samples	
SDN-05	Controlled electrospray deposition of nanoparticles for improved analysis by electron microscopy		x				
SDN-06	Software development and instrument development for characterization of nano (C) RMs		x		A wide range of nano-CRMs that mimic real-life practical samples		
SDN-07	Dimensional characterization of mono-disperse sub 6 nm colloidal semiconductor nanocrystals	x	x				

Code	**Poster title**	**a)**	**b)**	**c)**	Need for **reference materials**	Need for **measurement / classification methods**	Need for **international standards**
SDN-08	In-Situ SAXS Techniques		x				
SDN-09	Measurements of biological particles and nanocharacterization methods		x				
SSN-01	Chemical Anaylsis of Core-Shell Nanoparticles using ToF-SIMS and XPS	x	x				
SSN-02	Synthesis and systematic characterization of core-multi-shell NaYF4:Er3+, Yb3+ @SiO2@Au nanoparticles for the enhancement of fluorescence emissions	x	x				
SSN-03	Characterization of core-shell quantum dots of different particle architecture on the ensemble and single particle level	x	x				
DNS-01	SI traceable characterization of nanoscaled materials by X-ray spectrometry	x	x				
DNS-02	Analytical and topographical Reference Material for the Nanoscale	x	x				
DNS-03	Directed self-assembly of Block Copolymers for Lateral Length Standards at the Nanoscale	x					
DNS-04	Reference-free GIXRF-XRR based qualification of nanolayers and nanostructures as potential calibration samples	x	x				
DNS-05	OECD Test Guideline on Particle Size and Particle Size Distribution of Manufactured Nanomaterials: Simultaneous Measurement of Length and Diameter of Fibers		x	x		Simultaneous measurement of length and diameter of nanofibers	OECD test guideline on simultaneous measurement of length and diameter of nanofibers
DNS-06	PillarHall LHAR structure for thin film conformality measurements	x					
DNS-07	Traceable 3D nanometrology	x	x		Tip characterizers, standards for hybrid metrology, nanoparticles	3D nanometrology techniques	

Code	**Poster title**	**a)**	**b)**	**c)**	Need for **reference materials**	Need for **measurement / classification methods**	Need for **international standards**
DNS-08	The need of a reference for APT-AFM tip reconstruction		x		Tip characterizer	Combination of atom probe tomography (APT) and AFM	
DNS-09	The Research of Nanometer Geometry Standard Reference Materials in NIM	x	x				
MPN-01	Nanoscale reference artefacts for contact resonance spectroscopy and force-distance curve techniques	x	x				
MPN-02	Development of a Method for Measuring the Rigidity of Nanofibers		x				
MPN-03	Crystalline Si and GaN micro- and nanopillars as reference artefacts for nano-mechanical characterization	x	x			Nano-mechanical characterization	
OPN-01	Long-range ordered gold-coated silicon nanowires for surface-enhanced Raman spectroscopy	x	x				
OPN-02	Synthesis and optical characterization of ternary AIS/ZnS quantum dots	x	x				
OPN-03	Sandwich assay of Magnetic and Gold Nanoparticles for Purification and Quantification of Tau-Protein using ID-SERS		x			Detection of tau-protein, a biomarker for Alzheimer’s disease	
OPN-04	Raman spectroscopy for identification and quantification of different polymorphic forms of titanium dioxide in nanoparticle mixtures		x				
OPN-05	Optical characterization of Cd-free AgInS/ZnS quantum dots		x				
OPN-06	Simple and Validated Methods for Quantifying Functional Groups, Ligands, and Biomolecules on Nanomaterials		x				

Code	**Poster title**	**a)**	**b)**	**c)**	Need for **reference materials**	Need for **measurement / classification methods**	Need for **international standards**
OPN-07	Functional group analysis on nanoparticles with simple optical assays						
OPN-08	The Future of Scattering for Nanomaterial Characterisation		x			Modular SAXS approach	
OPN-09	Investigation of upconverting nanoparticle growth utilizing in-situ luminescence monitoring in combination with offline small-angle X-ray scattering (SAXS) and transmission electron microscopy (TEM)	x	x				

Information on existing reference nanomaterials

The following list provides information about existing reference nanomaterials:

Institute	Country	Material Type	Material properties	Link
BAM	DE	Certified Reference Material (CRM) Quality Control Materials (QCM) Reference Material (RM)	Flatness, Film thickness, Single step, periodic step, step grating, Lateral X-Y-axis, 1-dim, Lateral X-Y-axis, +2-dim, critical dimensions, 3-dimensional, Nanoobjects/nanoparticles/nanomaterial Nanocrystallite materials, Porosity Depth profiling resolution	http://www.nano-refmat.bam.de/en/
JRC	EC	JRC NANOMATERIALS REPOSITORY: Titanium Dioxide, Zinc Oxide, Silicon Dioxide, Cerium Dioxide, Gold, MWCNT, Graphene, Nanoclay	List of Representative Nanomaterials: Particle Size, Particle size distribution; Length, Diameter, surface area	https://ec.europa.eu/jrc/en/scientific-tool/jrc-nanomaterials-repository https://ec.europa.eu/jrc/sites/jrcsh/files/JRC%20Nanomaterials%20Repository-List%20of%20Representative%20Nanomaterials-201606.pdf
NIST	US	Standard Reference Materials	Nanoobjects/nanoparticles/nanomaterial	https://www.nist.gov/srm => search for ‘nano’
PTB	DE	Nanoscale dimensional standards	1D pitch, 2D pitch, step height, linewidth (CD), flatness, layer thickness, roughness,	https://www.nanoscale.ptb.de/nanoscale-standards.html
PTB	DE	Nanoforce standards	stiffness	https://www.nanoscale.ptb.de/nanoscale-standards.html

Workshop on Reference Nanomaterials
Current situation and needs: development, measurement, standarization

May 14

Focus: reference material needs and development

	Day 1	**Page**
10:30-11:00	Registration	
11:00-11:15	Welcome and Introduction by BAM and PTB	
11:15-11:45	**Needs for certified and non-certified reference materials and representative test materials in the area of nanotechnology – Analysis of a survey held by JRC in 2017** H. Rauscher[a], G. Roebben[b], A. Held[b] [a]Joint Research Centre of the European Commission, Ispra, Italy [b]Joint Research Centre of the European Commission, Geel, Belgium	25, 97-107
11:45-12:15	**Expectations, results and follow-up: industry perspective in developing a measurement strategy for nanomaterial** M. Reuter VCI (German Chemical Industry Association), Science and Research, Frankfurt, Germany	26-27, 108-113
12:15-12:45	**The NanoDefine decision framework and “NanoDefiner” e-Tool** H. Rauscher[a], A. Mech[a], C. Gaillard[a], H. Marvin[b], W. Wohlleben[c], D. Hodoroaba[d], F. Babick[e], C. M. Friedrich[f], R. Bruengel[f], J. Rückert[f], A. Ghanem[g], S. Weigel[h] [a]European Commission Joint Research Centre – Directorate Health, Consumers and Reference Materials, Italy [b]RIKILT, Wageningen UR, Netherlands [c]BASF SE, Germany [d]Bundesanstalt für Materialforschung und –pruefung (BAM), Germany [e]Technische Universität Dresden, Germany [f]University of Applied Sciences and Arts Dortmund, Germany [g]Solvay – Research & Innovation Centre Brussels, Belgium [h]Bundesinstitut für Risikobewertung (BfR), Germany	28, 114-124
12:45-12:55	*Group Photo*	
12:55-13:45	*Lunch*	
13:45-15:00	**Poster Session I**	
15:00-15:20	**OECD Test Guideline on particle size & size distribution of Manufactured Nanomaterials** H. Bresch[a], A. Schmidt[a], V. Bachmann[b], K. Kämpf[b], T. Kuhlbusch[b], K. Schwirn[c], D. Völker[c] [a]Federal Institute for Materials Research and Testing (BAM), Berlin, Germany [b]Federal Institute for Occupational Safety and Health (BAuA), Germany [b]German Environment Agency (UBA), Dessau-Roßlau, Germany	29, 125-132
15:20-15:40	**Health Issues of Micro- and Nanoscale High Aspect Ratio Materials (HARM): Need for Reference Materials and Testing Standards** A. Meyer-Plath, K. Kämpf, E. Heunisch, D. Broßell, R. Packroff BAuA-Federal Institute for Occupational Safety and Health, Berlin and Dortmund, Germany	30, 133-145
15:40-16:00	*Coffee break*	

Workshop on Reference Nanomaterials
Current situation and needs: development, measurement, standarization

May 14

Focus: reference material needs and development

	Day 1 continued	**Page**
16:00-16:20	**Synthesis of size and shape controlled TiO_2 nanoparticles: possible CRM's candidates for size, shape and functional properties** V. Maurino,[a] L. Pellutiè,[a] F. Pellegrino,[a] E. Ortel,[b] V.-D. Hodoroaba,[b] R. Isopescu[c] [a]Dipartimento di Chimica and Centro Interdipartimentale NIS, Università di Torino, Turin, Italy [b]BAM - Federal Institute for Materials Research and Testing Division 6.8 Surface Analysis and Interfacial Chemistry, Berlin, Germany [c]R&D Consultanta si Servicii, Bucharest, Romania	31, 146-162
16:20-16:40	**Traceable nanoparticle characterization using Small-Angle X-ray Scattering (SAXS)** M. Krumrey, A. Schavkan Physikalisch-Technische Bundesanstalt (PTB), Berlin, Germany	32, 163-176
16:40-17:00	**Challenges of synthesis and analysis of core-shell nanoparticles for reference materials** A. F. Thünemann Bundesanstalt für Materialforschung und -prüfung (BAM), Berlin, Germany	33, 177-188
17:10-18:30	Tour of PTB labs at BESSY II	
18:45-20:45	*Get Together and Dinner –previous registration required* *esswirtschaft Adlershof, Rudower Chaussee 24*	

Workshop on Reference Nanomaterials
Current situation and needs: development, measurement, standarization

May 15

Focus: reference material characterization and standardization

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08:00-08:30	Registration	
08:30-09:00	**ISO Standardization Activities on Reference Nanomaterials** M. Stintz TU Dresden, Dresden, Germany	34, 189-199
09:00-09:20	**Standardisation of magnetic nanomaterials: Steps towards magnetic reference particles** U. Steinhoff[a], R. Bleul[b], R. Rüttinger[c], A. Price[d], F. Wiekhorst[a] [a]Physikalisch-Technische Bundesanstalt, Berlin, Germany [b]Fraunhofer ICT-IMM, Mainz, Germany [c]DIN e. V., Berlin, Germany [d]BSI Group, London, United Kingdom	35, 200-208
09:20-09:40	**TSEM: a traceable method for nanoparticle metrology** E. Buhr, D. Bergmann Physikalisch-Technische Bundesanstalt (PTB), Braunschweig, Germany	36, 209-218
09:40-10:00	**Measurands and method dependent reference values: can one global particle size reference value fit all?** V. A. Coleman, Å. K. Jämting, M. A. Lawn, B. Babic and J. Herrmann National Measurement Institute Australia, Lindfield, Australia	37, 219-227
10:00-11:20	**Poster Session II *and Coffee break***	
11:20-11:40	**TiO_2 Pigment Sizing: Constituent, Aggregates and Primary Particles** R. Theissmann, T. Koch KRONOS International, Inc., Leverkusen, Germany	38, 228-244
11:40-12:00	**Shape controlled TiO_2 nanoparticles as candidates for nano-CRM's – an ISO case study** V.-D. Hodoroaba[a], K. Yamamoto[b], E. A. Grulke[c] [a]Federal Institute for Materials Research and Testing (BAM), Berlin, Germany [b]National Institute of Advanced Industrial Science and Technology, Japan [c]University of Kentucky, Chemical & Materials Engineering, Lexington, KY, USA	39, 245-255
12:00-12:20	**Reference methods for the determination of the nanoparticle mass- and number-based concentration using hyphenated ICP-MS** D. Bartczak, S. Nunez and H. Goenaga-Infante LGC Limited, Teddington, United Kingdom	40, 256-265
12:20-12:40	**Can labelled nanoparticles as internal standards improve the reliability of the quantitative analysis of nanomaterials in complex matrices?** S. Weigel[a,b], L.-J. Ellis[c], A. Auger[d], C. Cascio[a,e], E. Valsami-Jones[c] [a]RIKILT Wageningen UR, Wageningen, The Netherlands [b]present: German Federal Institute for Risk Assessment, Berlin, Germany [c]School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham, United Kingdom [d]CEA, Grenoble, France [e]present: European Food Safety Authority (EFSA), Parma, Italy	41, 266-274
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Workshop on Reference Nanomaterials
Current situation and needs: development, measurement, standarization

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Poster Session I

Topic	Poster Title
CLN-01	**The Malta Project – A European Initiative to Develop and Amend OECD Technical Guidelines for Testing of Nanomaterials** E. Heunisch, V. Bachmann, T. Kuhlbusch / BAuA, DE
HSN-01	**Quantification of Silver Nanoparticles at Single Cell Level by Mass Cytometry** N. Jakubowski, A. Lopez-Serrano Oliver, A. Peddinghaus, D. Wittke, A. Haase, A. Luch, A. Grützkau, S. Baumgart BAM, DE; DRFZ, German Rheumatism Research Centre Berlin, DE; BfR, DE
HSN-02	**OECD Test Guidelines Development for Chemicals Safety Assessment of Nanomaterials** K. Schwirn, D. Völker, J. Ahtiainen, A. Schmidt, H. Bresch, K. Kämpf, V. Bachmann, T. Kuhlbusch UBA, DE; Drumsö Ecotox Consultancy, FI; BAM, DE; BAuA, DE
HSN-03	**Assessing potential risks of nanomaterials - Genotoxicity screening with the fluorometric γ-H2AX assay and automated microscopic detection** M. Wegmann, T. Jochum, M. Hannemann, V. Somma, K. Hoffmann, J. Niehaus, D. Roggenbuck, and U. Resch-Genger BAM, DE; Fraunhofer IAP, DE; MEDIPAN GMBH, DE
MAN-01	**Reference samples for Magnetic Force Microscopy** S. Sievers, H.W. Schumacher / PTB, DE
NCM-01	**Nanocrystals as labeling reagents for Imaging Mass Cytometry** J. Saatz, B. Grunert, N. Jakubowski / BAM, DE; nanoPET, DE
NCM-02	**Studying nanoparticle-cell interaction by ICP-MS based techniques** H. Traub, T. Büchner, D. Drescher, V. Merk, J. Kneipp, N. Jakubowski BAM, DE; Humboldt University Berlin, DE
RMN-01	**Toward a reference sample for atom probe tomography** M. Dialameh, C. Fleischmann, J. Bogdanwicz, N. De Leo, L. Boarino, W. Vandervorst / Imec, BE; Politecnico di Torino, IT; INRIM, IT; KU Leuven, BE
RMN-02	**Creating the Silver Standard: Development and Applications of a Silver Nanoparticle Reference Material** C. Kästner, H. Sieg, L. Böhmert, A. Lampen, A F. Thünemann / BAM, DE; BfR, DE
RMN-03	**Microwave-assisted high-speed silver nanoparticle synthesis** P. E. J. Saloga, C. Kästner, A. F. Thünemann / BAM, DE
SDN-01	**Development of a hybrid metrology combining AFM and SEM techniques for measuring the characteristic dimensions of a nanoparticle population** L. Crouzier, A. Delvallée, N. Feltin, S. Ducourtieux, L. Devoille, G. Noircler, C. Ulysse, O. Taché, E. Barruet LNE, FR; Institut P' D1, FR; Centre de Nanosciences et de Nanotechnologies C2N, FR; CEA Saclay, FR

Topics Poster Session I, May 14

CLN: Classification of NM
HSN: Health & Safety of NM
MAN: Magnetic properties of NM
RMN: RM and Need
SDN: Size and size distribution of NM
SSN: Shape & (core-shell) structure of NM
NCM: NM in complex matrices

Workshop on Reference Nanomaterials
Current situation and needs: development, measurement, standarization

May 14

Poster Session I

Topic	Poster Title
SDN-02	**Contribution to accurate Spherical Gold Nanoparticles analysis (size, size distribution) by SpICPMS and SAXS** V. Geertsen, O. Taché, E. Barruet, J.L. Lacour, F. Gobeaux, A. Thil NIMBE / CEA Paris-Saclay, FR
SDN-03	**Improved traceability chain of nanoparticle size measurements – the new EMPIR project nPSize** V.-D. Hodoroaba, H. Bosse / BAM, DE; PTB, DE
SDN-04	**Advanced screening method using volume-specific surface area (VSSA) for nanomaterial identification of powders** P. Kuchenbecker, D.-V. Hodoroaba, W. Wohlleben, F. Lindemann BAM, DE; BASF; DE
SDN-05	**Controlled electrospray deposition of nanoparticles for improved analysis by electron microscopy** J. Mielke, P. Dohányosová, P. Müller, S. López-Vidal, V.-D. Hodoroaba BAM, DE; RAMEM S.A., ES; BASF SE, DE
SDN-06	**Software development and instrument development for characterization of nano (C) RMs** B.R. Pauw, I. Bressler, T. Snow, A. Thünemann / BAM, DE
SDN-07	**Dimensional characterization of monodisperse sub 6 nm colloidal semiconductor nanocrystals** J. C. Porsiel, A. Schirmacher, E. Buhr, G. Garnweitner TU Braunschweig, DE; PTB, DE
SDN-08	**In-Situ SAXS Techniques** Q. Saadeh, B.R. Pauw, A.F. Thünemann, J. Günster / BAM, DE
SDN-09	**Measurements of biological particles and nanocharacterization methods** C. Sousa, I. M. Pinto, D. Y. Petrovykh / INL, PT
SSN-01	**Chemical Anaylsis of Core-Shell Nanoparticles using ToF-SIMS and XPS** T. Heinrich, A. Müller, M. Schneider, K. Sparnacci, W.E.S. Unger BAM, DE; Università del Piemonte, IT
SSN-02	**Synthesis and systematic characterization of core-multi-shell NaYF4:$Er^{3+,}$ Yb^{3+} @SiO_2@Au nanoparticles for the enhancement of fluorescence emissions** M. Saleh, C. Kembuan, F. Frenzel, B. Rühle, C. Würth, C. Graf, U. Resch-Genger BAM, DE; Freie Universität Berlin, DE; Hochschule Darmstadt, DE
SSN-03	**Characterization of core-shell quantum dots of different particle architecture on the ensemble and single particle level** F. Weigert, U. Resch-Genger / BAM, DE

Topics Poster Session I, May 14

CLN: Classification of NM
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Poster Session II

Topic	Poster Title
DNS-01	**SI traceable characterization of nanoscaled materials by X-ray spectrometry** B. Beckhoff, P. Hönicke, Y. Kayser, B. Pollakowski-Herrmann, C. Seim, C. Streeck, R.Unterumsberger / PTB, DE
DNS-02	**Analytical and topographical Reference Material for the Nanoscale** I. Busch, S. Bütefisch, O. Lenck, L. Koenders, W. Unger, T. Weimann, M. Weinert / PTB, DE; BAM, DE
DNS-03	**Directed self-assembly of Block Copolymers for Lateral Length Standards at the Nanoscale** F. Ferrarese Lupi, G. Aprile, E. Cara, M. Dialameh, N. De Leo, L. Boarino Nano Facility Piemonte, IT; INRIM, IT
DNS-04	**Reference-free GIXRF-XRR based qualification of nanolayers and nanostructures as potential calibration samples** P. Hönicke, V. Soltwisch, Y. Kayser, B. Beckhoff, F. Scholze, T. Weimann, M. Krämer / PTB, DE; AXO DRESDEN, DE
DNS-05	**OECD Test Guideline on Particle Size and Particle Size Distribution of Manufactured Nanomaterials: Simultaneous Measurement of Length and Diameter of Fibers** K. Kämpf, V. Bachmann, H. Bresch, A. Schmidt, T. Kuhlbusch, K. Schwirn, D. Völker / BAuA, DE; BAM, DE; UBA, DE
DNS-06	**PillarHall LHAR structure for thin film conformality measurements** V. Korpelainen, O. M. E. Ylivaara, M. Ylilammi, M. Utriainen, Feng Gao and R. L. Puurunen / VTT, FI; Aalto University, FI
DNS-07	**Traceable 3D nanometrology** V. Korpelainen, M. Valtr, G. Zeng, A. Yacoot, N. Sebaihi, G. Dai, R. Koops, T. Hausotte, Yiting Wu, G. Papageorgioui and P. Dimitrakisi, H. Spruit, E. van Zeijl, G.B. Picotto and F. Meli VTT, FI; CMI, CZ; DFM, DK; NPL, UK; PTB, DE; SMD, BE; VSL, NL; FAU, DE, NCSR Demokritos, GR; TNO, NL; INRIM, IT; METAS, CH
DNS-08	**The need of a reference for APT-AFM tip reconstruction** J. Op de Beeck, C. Fleischmann, K. Paredis, W. Vandervorst IMEC, BE; KU Leuven, BE
DNS-09	**The Research of Nanometer Geometry Standard Reference Materials in NIM** Y. Shi, S. Zhang, S. Gao, X. Hu, S. Li, W. Li, Q Li, L. Huang / NIM, CN
MPN-01	**Nanoscale reference artefacts for contact resonance spectroscopy and force-distance curve techniques** M. Fahrbach, M. Bertke, G. Hamdana, L. Krieg, T. Voss, B. Cappella, E. Peiner TU Braunschweig, DE; BAM, DE

Topics Poster Session II, May 15

DNS: Dimensional nanostandards
MPN: Mechanical properties of NM
OPN: Optical properties of NM

Workshop on Reference Nanomaterials
Current situation and needs: development, measurement, standarization

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Topic	Poster Title
MPN-02	**Development of a Method for Measuring the Rigidity of Nanofibers** R. Fortini, D. Kehren, A. Meyer-Plath, H. Sturm / BAM, DE; BAuA, DE
MPN-03	**Crystalline Si and GaN micro- and nanopillars as reference artefacts for nano-mechanical characterization** P. Puranto, J. Langfahl-Klabes, G. Hamdana, M. Bertke, M. F. Fatahilah, Feng Yu, A. Felgner, Min Xu, F. Pohlenz, T. Granz, E. Peiner, A. Waag, H. S. Wasisto, Z. Li, U. Brand PTB, DE; TU Braunschweig, DE; Indonesian Institute of Sciences (LIPI), ID
OPN-01	**Long-range ordered gold-coated silicon nanowires for surface-enhanced Raman spectroscopy** E. Cara, F. Ferrarese Lupi, L. Mandrile, A. Giovannozzi, M. Dialameh, N. De Leo, L. Boarino / INRIM, IT
OPN-02	**Synthesis and optical characterization of ternary AIS/ZnS quantum dots** L. Dhamo, J. X. Soares, U. Resch-Genger / BAM, DE; University of Porto, PT
OPN-03	**Sandwich assay of Magnetic and Gold Nanoparticles for Purification and Quantification of Tau-Protein using ID-SERS** C. Frank, V. Maurer, J. C. Porsiel, S. Zellmer, S. Wundrack PTB, DE; TU Braunschweig, DE
OPN-04	**Raman spectroscopy for identification and quantification of different polymorphic forms of titanium dioxide in nanoparticle mixtures** L. Mandrile, C. Portesi, A.M. Rossi / INRIM, IT
OPN-05	**Optical characterization of Cd-free AgInS/ZnS quantum dots** I. Martynenko, J. Soares, J. Pauli, U. Resch-Genger / BAM, DE
OPN-06	**Simple and Validated Methods for Quantifying Functional Groups, Ligands, and Biomolecules on Nanomaterials** N. Nirmalananthan, M. Moser, D. Geissler, T. Behnke, R. Schneider, U. Resch-Genger / BAM, DE
OPN-07	**Functional group analysis on nanoparticles with simple optical assays** N. Nirmalananthan, M. Moser, T. Behnke, D. Geißler, U. Resch-Genger/BAM,DE
OPN-08	**The Future of Scattering for Nanomaterial Characterisation** B.R. Pauw, A.Thünemann / BAM, DE
OPN-09	**Investigation of upconverting nanoparticle growth utilizing in-situ luminescence monitoring in combination with offline small-angle X-ray scattering (SAXS) and transmission electron microscopy (TEM)** S. Radunz, A. Schavkan, S. Wahl, C. Würth, H. R. Tschiche, U. Resch-Genger BAM, DE; PTB, DE; Humboldt University Berlin, DE

Topics Poster Session II, May 15

DNS: Dimensional nanostandards
MPN: Mechanical properties of NM
OPN: Optical properties of NM

Abstracts
Oral Presentations

Needs for certified and non-certified reference materials and representative test materials in the area of nanotechnology

- Analysis of a survey held by JRC in 2017

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Keywords: Certified reference material, Representative test material, Nanomaterial

Between February 2017 and May 2017, the Joint Research Centre of the European Commission held a survey on the needs for certified and non-certified reference materials and representative test materials in the area of nanotechnology. The focus of the survey was on the data needed for the implementation of (European) legislation and regulations. This presentation analyses the survey outcome and summarises the needs of the nanomaterial community for (certified) reference materials (CRMs and RMs) and representative test materials (RTMs).

A brief introduction will first be given to indicate the differences between the mentioned benchmark materials (CRMs, RMs and RTMs), which play different, but equally important roles in the measurement systems that underpin the reliability of data obtained on nanomaterials [1]. Then a short overview of the survey design and set-up will be given, indicating the background and geographical origin of the 646 individually contacted stakeholders, as well as the differences in response rate between types of stakeholders (government officials, public research organisations, industry, academia, consultants, NGOs and standardization bodies). Then the reason for each of the eight survey questions will be explained and the corresponding results shown, as for example illustrated in Figure 1 and Figure 2. A comparison will be made between the needs identified in the survey and the already available benchmark materials.

The main outcome of the survey is a list of currently lacking benchmark materials, the production of which should be prioritised, based on the survey outcome. To conclude, the suggested routes for collaboration on the production of these benchmark materials will be presented and evaluated.

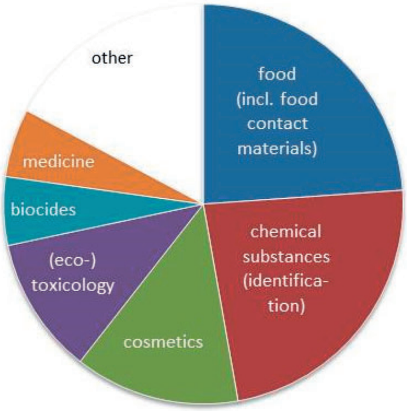

Figure 1: Main regulatory areas with RM/RTM needs identified by the survey participants.

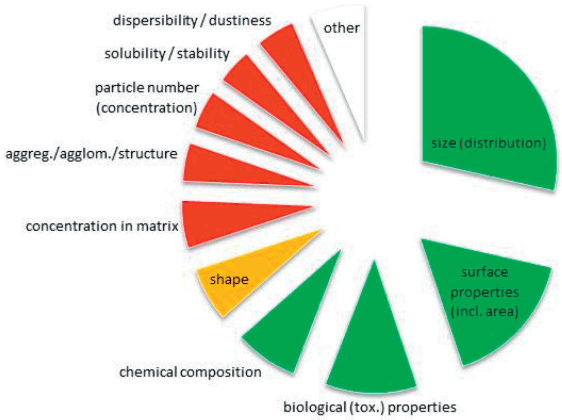

Figure 2: Nanomaterial properties judged most relevant by the survey participants

List of references:

1. Reference materials and representative test materials: the nanotechnology case, G. Roebben, K. Rasmussen, V. Kestens, T. P. J. Linsinger, H. Rauscher, H. Emons, H. Stamm, J. Nanoparticle Research, Vol. 15, citation ID 1455, DOI 10.1007/s11051-013-1455-2 (2013)

Abstract: 01-2018

Expectations, results and follow-up: industry perspective in developing a measurement strategy for nanomaterials

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Keywords (up to 3): Implementation of the nanomaterial definition of the EC for regulatory purposes, tiered measurement strategy for nanomaterials, challenges for industry

Industry has jointly together with federal institutes and research organisations undertaken several initiatives and research projects to leverage the development of a measurement strategy for nanomaterials. There are severe challenges to implement the recommendation of the European Commission on the definition of nanomaterials from 2011 for regulatory purposes in respect to industrially manufactured materials in the nanoscale. This is mainly due to the morphology and size distribution from particles out of industrial processes. So the chemical industry associations have developed a tiered measurement strategy that has productively been taken up e.g. by the FP7 project NanoDefine.

At the end of scientifically successful research projects the possibility to implement the recommendation of the EC effectively and without conflicting assignments still is in question. The research that has been undertaken has empowered industry finally formulating the challenges even more precisely. So this presentation will try to sum up these challenges and wants to present stimuli for the further discussion and follow-up activities.

Abstract: 02-2018

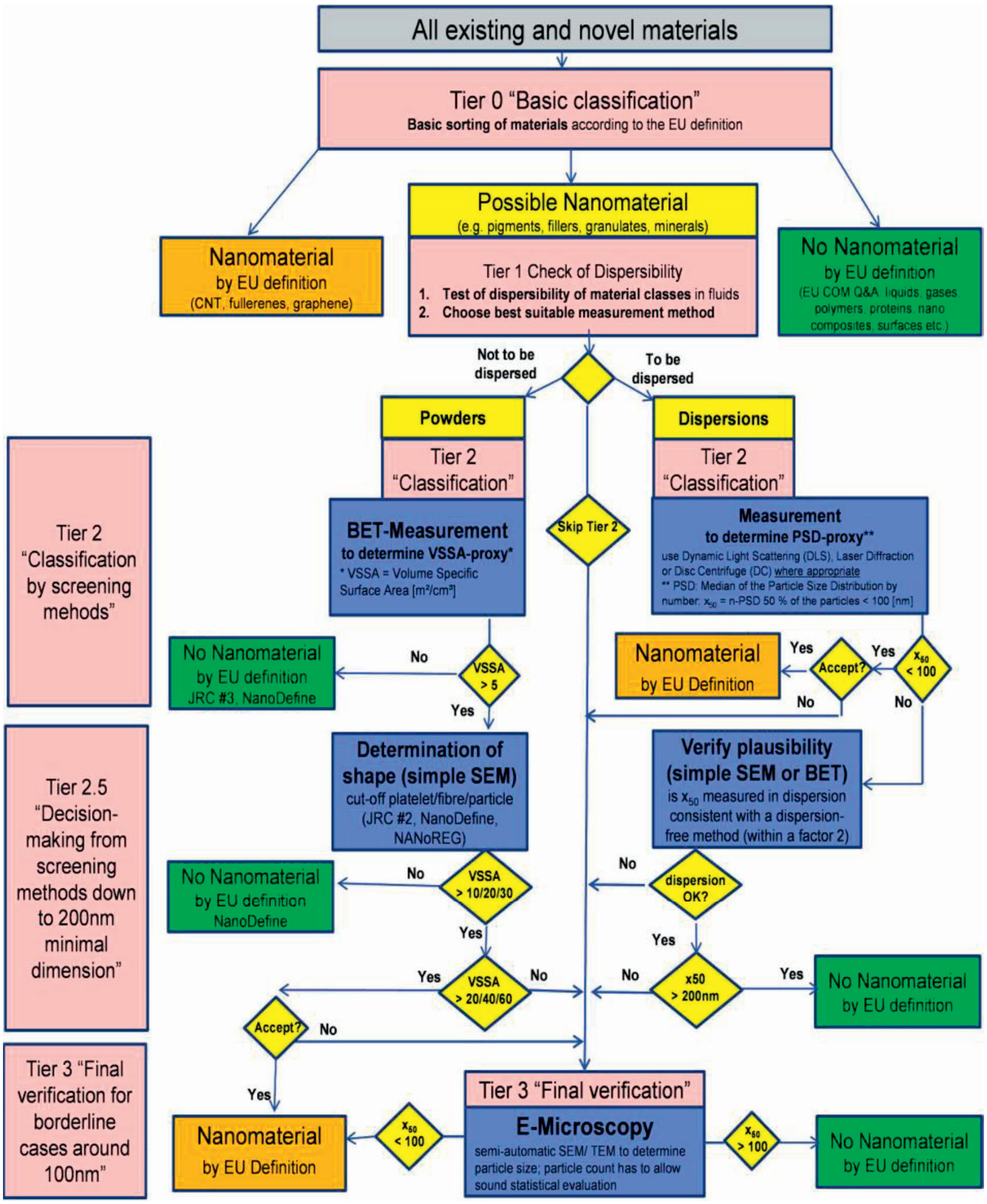

Figure 1: The tiered measurement strategy of VCI/VdMi and Eurocolour

List of references:

1. A tiered measurement strategy to implement the EC recommendation for a nanomaterial definition by VCI in cooperation with Verband der Mineralfarbenindustrie e.V. (VdMi) and Dachverband der Pigment, Füllstoff- und Farbstoffindustrie Eurocolour/CEFIC, December 2015 (1)

The NanoDefine decision framework and “NanoDefiner” e-Tool

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Keywords: Definition of nanomaterial, Regulation

The European Commission's recommendation on the definition of nanomaterial [2011/696/EU] is broadly applicable across different regulatory sectors and requires the quantitative size determination of constituent particles in samples down to 1 nm. A material is a nanomaterial if 50 % or more of the particles are in the size range 1-100 nm. The implementation of the definition in a regulatory context challenges measurement methods to reliably identify nanomaterials and ideally also non-nanomaterials as substance or product ingredient as well as in various matrices.
The EU FP7 NanoDefine project [www.nanodefine.eu] addressed these challenges by developing a robust, readily implementable and cost-effective measurement strategy to decide for the widest possible range of materials whether it is a nanomaterial or not. It is based on existing and emerging particle measurement techniques evaluated against harmonized, material-dependent performance criteria and by intra- and inter-lab comparisons. Procedures were established to reliably measure the size of particles within 1-100 nm, and beyond, taking into account different shapes, coatings and chemical compositions in industrial materials and consumer products. Case studies prove their applicability for various sectors, including food, pigments and cosmetics.
A main outcome is the establishment of an integrated tiered approach including rapid screening (tier 1) and confirmatory methods (tier 2), a decision support flow scheme and a user manual to guide end-users, such as manufacturers, in selecting appropriate methods. Another main product is the “NanoDefiner” e-Tool which implements the flow scheme in a user-friendly software and guides the user in a semi-automated way through the entire decision procedure. It allows a cost-effective selection of appropriate methods for material classification according to the EC's nanomaterial definition and provides a comprehensive report with extensive explanation of all decision steps to arrive at a transparent identification of nanomaterials as well as non-nanomaterials for regulatory purposes.
The project has received funding from the European Union’s Seventh Programme for research, technological development and demonstration under grant agreement No 604347.

Abstract: 03-2018

OECD Test Guideline on particle size & size distribution of Manufactured Nanomaterials

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Keywords (up to 3): standardization, particle size, size distribution

The properties of nanomaterials are influenced not only by their chemical composition but also by physical properties (such as size, geometry and crystal structure). Particle size and size distribution is considered as one major information for nanomaterial identification and characterization. This information is also important for a reliable risk assessment, for instance to interpret and compare test results and - in future – to forecast interaction and effects of nanomaterials. The particle size distribution is also proposed to decide whether a substance or a form of a substance can be considered as a nanomaterial in the EU. Especially in borderline cases, a standardized and comparable measurement methodology is therefore essential.

In the past years, the OECD Working Party on Manufactured Nanomaterials reviewed existing OECD Test Guidelines (TGs) for their applicability to nanomaterials. An important outcome was that TGs are in principle applicable. However, in several cases adaptations and further guidance is needed to address the specific needs for nanomaterials. The existing standardized test methods of the OECD for physical-chemical particle size characterization have not been developed for nanomaterials in particular. Thus, a high demand for an adaptation of the TG was identified. To meet the need for an appropriate TG on physical-chemical characterisation, Germany submitted a proposal for developing a TG on particle size and size distribution for nanomaterials to the OECD Test Guideline Programme. The activity is funded by the German Federal Ministry of the Environment, Nature Conservation, Building and Nuclear Safety and coordinated by UBA which commissioned BAM and BAuA with the preparation of the Test Guideline and the performance of the underlying experimental work. The aim of the project is the development of a harmonized and international agreed test protocol for a valid and reproducible determination of particle size and size distribution for sphere-like particles and fibres.

For e.g. sphere-like particles several challenges have to be considered to find a pragmatic way forward like different measuring methods providing different results for the size distribution of the particles. This is e.g. caused by the different measuring principles of the methods. Each method measures a specific parameter that ultimately determines particle size. There are three major challenges for the measurement of nanoparticles. First, the measured quantity differs for each method (Scattered light intensity, electric mobility, etc.). Second, the diameter of the measured nanomaterial can be calculated and thus presented differently (Feret Diameter, Hydrodynamic Diameter, etc.). Third, a measuring method provides a size distribution which is measured either mass-based, surface-based or number-based. A conversion between the results requires additional parameters and thus possibly increases the measurement error.

In addition to the technical differences, the individual parameters are strongly influenced by the structure and material of the nanoparticles. The suitability of measurement methods therefore differs with the material of the nanomaterial. As a result, it is possible that two very different results are obtained for the particle size distribution using two different methods, which are nevertheless both correct.

The above listed issues and a way forward toward the TG will be discussed in the presentation. It will focus on methodology for the characterization of sphere-like nanoparticles while the part concerning nanofibers will be presented on a poster by BAuA.

Abstract: 04-2018

Health Issues of Micro- and Nanoscale High Aspect Ratio Materials (HARM): Need for Reference Materials and Testing Standards

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Keywords: Toxicological grouping of fibres, flexural rigidity, agglomeration state

The last decades have seen a strong increase in the availability and use of novel micro- and nanoscale engineered high aspect ratio materials (HARM, HAR materials). HARM is a generic term for fibrous materials that is not limited to compact fibres. Instead, it also comprises fibrous objects of elongated shape in percolated, agglomerated, entangled or aggregated state.

According to the fibre definition of the World Health Organization (WHO), HAR materials with a length greater than 5 µm, a diameter less than 3 µm and aspect ratio exceeding 3:1 are to be considered respirable. Upon inhalation they may be delivered to deep lung tissues and reach the pulmonary alveoli. Exposure of humans to insoluble HAR materials of WHO dimension that persist in the lung tissue for a long duration lead to severe health risks. The effect is called fibre pathogenicity paradigm and was learned from the high death toll still being paid for industrial use of asbestos.

The carcinogenicity of some HAR materials is attributed to failing clearance of alveoli from biodurable respirable dusts due to frustrated phagocytosis of alveolar macrophage cells, or to their translocation into lung-lining mesothelial tissue. Better understanding of these mechanisms may justify toxicological grouping of HAR materials. Such grouping is indispensable since only a minor fraction of the multitude of already engineered HAR materials can be tested in animal experiments. It requires reliable testing standards and reference materials for determining the following key properties of a HARM sample

1. To determine the WHO fibre fraction: Pairwise distribution of length and diameter
2. To determine a material's hierarchical structure: State and type of agglomeration
3. To predict biodurability: Solubility properties
4. To estimate phagocytosis and translocation properties: Correlation between diameter and flexural rigidity.

Providing robust and reliable testing standards for HAR materials ranging from micro- to nanoscale diameters with aspect ratios sometimes exceeding 1000:1 is a challenge. While significant progress has been achieved for individual length and diameter measurement of fibres, (1) pair-wise assessing of both measurands requires intelligent microscopy strategies to estimate respirable fractions. Agglomeration state characterisation (2) is essential both for morphological description and exposure assessment of fibrous. It requires standard dispersion operations as well as categorization and counting rules. HARM *in vitro* solubility testing standards (3) are needed that correlate well to *in vivo* results and are suited to predict biodurability. Finally, there are no standards for measuring flexural rigidity (4) of nanoscale HAR materials that may enable predicting phagocytosis and translocation properties.

Such developments require reference HAR material sets that are simultaneously standardized with respect to measurand pairs (1) length and primary particle diameter, (2) state of agglomeration, and (3,4) primary particle diameter and chemical composition.

Future progress in toxicological grouping of HARM will therefore rely on progress made in the field of reference materials and standardized testing.

Abstract: 05-2018

Synthesis of size and shape controlled TiO_2 nanoparticles: possible CRM's candidates for size, shape and functional properties

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Keywords: Anatase, shape control, hydrothermal synthesis

Titanium dioxide is one of the most studied metal oxides due to its chemical, surface, electronic and (photo)catalytic properties, providing this material of multisectorial applications ranging from healthcare, photocatalysis, smart materials with self cleaning and self sterilizing properties and solar energy harvesting. However it is difficult to correlate the functional properties of TiO_2 nanomaterials to the properties at single nanoparticle level due to the high polydispersity in shape, size and surface features of the currently available TiO_2 nanoparticles (NPs). Although intensive experimental and theoretical studies have been conducted on the reactivity of different surfaces of metal oxides such as TiO_2 [1,2] much less attention is paid on the dependence of functional properties, like photocatalytic activity, dye adsorption, open circuit potential and fill factor in dye sensitized solar cells, on crystal facets in different orientations [3]. One of the goal of SETNanoMetro project was the development of design rules to tune crystal facets of TiO_2 NPs in order to optimize and control functional properties. In the present work we have developed a series of design rules in order to obtain sets of anatase TiO_2 NPs with low polydispersity and to tune their shape and size by hydrothermal processing of Ti(IV)-Triethanolamine complex in presence of different shape controllers [4]. Through a careful experimental design, a predictive soft model was developed. The model is able to predict the synthesis outcome allowing to tune the shape factor from 5 (prisms) to 1.5 (bipyramids) to 0.2 (platelets). This allows to control the main crystal facets exposed ranging from (100) to (001). Due to the dependence of functional properties of nanomaterials on shape distribution and not only size, the availability of NPs sets with uniform and well defined and tunable shapes can be of paramount relevance in order to produce reference nanomaterials for shape measurement [5].

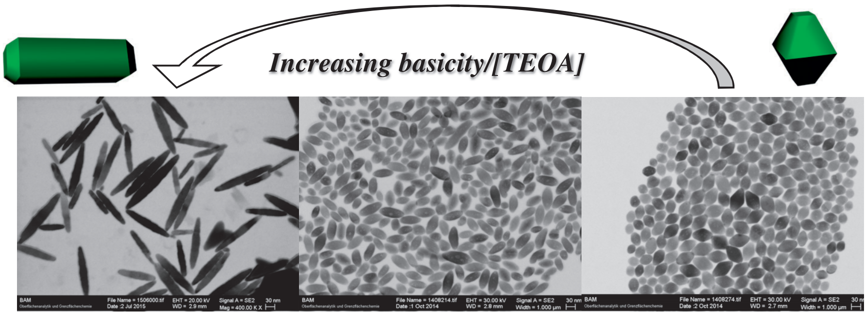

Figure 1: Shape tunability of anatase TiO_2 NPs synthesized by the hydrothermal procedure developed in this work

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Abstract: 06-2018

Traceable nanoparticle characterization using Small-Angle X-ray Scattering (SAXS)

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Keywords: SAXS, nanoparticle size, continuous contrast variation

Small Angle X-ray Scattering (SAXS) is an ensemble method where nanoparticles can be studied in liquid suspension. Due to the X-ray wavelength well below 1 nm, SAXS is ideally suited for the investigation of nanoparticles. The difference of the electron density between the nanoparticles and the suspending medium gives rise to X-ray scattering in a small angular range (typically < 5°) around the transmitted beam (Fig. 1). For sufficiently monodisperse particles, the observed oscillations of the scattered intensity as a function of the momentum transfer *q*, which is directly related to the scattering angle and the wavelength (or photon energy) of the incident X-rays, enable the traceable size determination of nanoparticles [1]. The particle size determination of nanoparticles using SAXS is already covered by the ISO standard 17867:2015, while activities are ongoing in ISO TC 24 / SC 4 and in EMPIR projects towards standardization of nanoparticle concentration determination. Here, the absolute scattering cross section has to be measured and the electron density difference between the particles and the suspending medium needs to be known.

In the synchrotron radiation laboratory of PTB at the electron storage ring BESSY II, all relevant parameters for a traceable size determination of nanoparticles with SAXS are determined directly with low uncertainty: the wavelength, the sample-detector-distance and the pixel size of the area detector [2]. For the concentration determination, the detectors for the incident photon flux and the area detector are absolutely calibrated. For nanoparticles with low density, the electron density and the inner structure of the particles can be determined by continuous contrast variation (Fig. 2) [3].

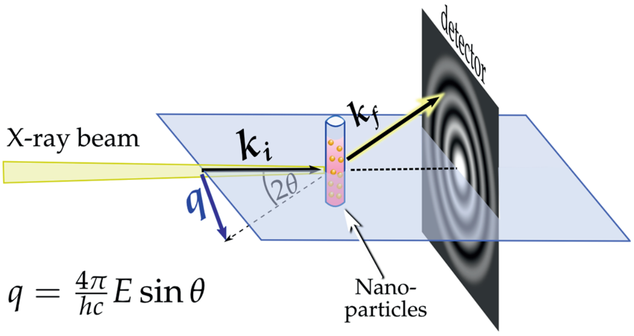

Fig. 1: Principle of SAXS

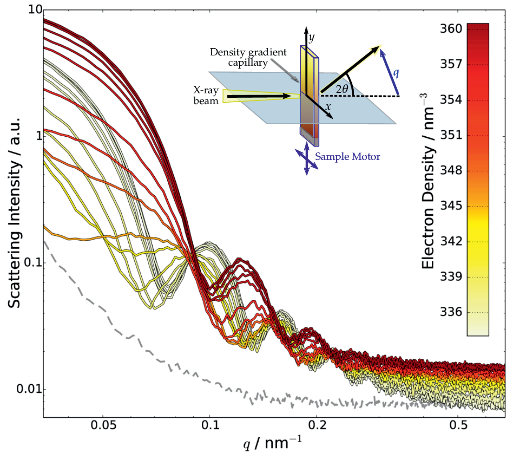

Figure 2: Continuous contrast variation

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Challenges of synthesis and analysis of core-shell nanoparticles for reference materials

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Keywords: Small-angle X-ray scattering, SAXS, core-shell nanoparticles

Core-shell nanoparticles are widespread in nature, industrial applications and nanotechnology research. Facile ways of modern synthesis will be discussed and possibilities to reveal their structures with small-angle X-ray scattering (SAXS). A recent review on using block copolymer templates as one of the most reliable routes for tuning size and shape of nanoparticles is provided by Li et al.[1] Ferritin and apoferritin are archetypical examples for protein-based core-shell nanoparticles. Their structures are easily accessed by synchrotron SAXS[2] but also with commercial instruments and allow fast performance tests.[3] SASfit[4] is a suitable program tool based on classical curve fitting and McSAS[5] is a complementary program based on a Monte Carlo technique. Detailed refinements of SAXS data evaluation are on the way for better data analysis.[6] A sub nanometer resolution is state-of-the-art for quantification of the size distribution of polyacrylic acid stabilized silver nanoparticles.[3] Such particles are useful in catalysis.[7] It was observed that the catalytic activity can be tuned easily by varying the shell material of the particles (see Figure 1).

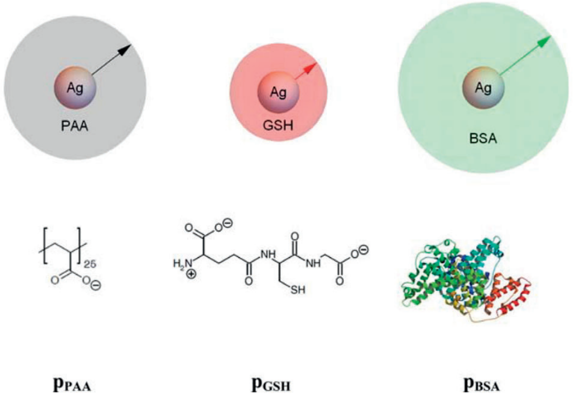

Figure 1: Chemical structures of the (macro)molecules that form a shell around the silver cores and provide long-term stability in dispersion. Shell thicknesses are indicated by arrows. Parent particles p_{PAA} are stabilized with poly(acrylic acid) (PAA), MW = 1800 g mol^{-1} (left). The p_{GSH} are stabilized with glutathione, which is a tripeptide formed by glutamic acid, cysteine, and glycine, MW = 307 g mol^{-1} (middle). The p_{BSA} are stabilized with bovine serum albumin (BSA); MW = 66463 g mol^{-1} (right). (Figure is from[7])

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ISO Standardization Activities on Reference Nanomaterials

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Keywords: ISO, Particle Characterization, Nanotechnology

ISO is not producing reference materials but relevant Technical Committees (TC) generate written standards with requirements, e.g. ISO 17034:2016 "General requirements for the competence of reference material producers". It was prepared by the ISO Committee on Conformity Assessment (CASCO), in collaboration with the ISO Committee on Reference Materials (REMCO). It understands a reference material as sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process.

In the field of nanotechnology ISO/TC 229 published a first technical specification ISO/TS 16195:2013 "Nanotechnologies -- Guidance for developing representative test materials consisting of nano-objects in dry powder form".

But also other TCs deal with relevant requirements, especially for particle characterization ISO/TC 24/SC 4, working group 11 "Sample preparation and reference materials". Managed by its convener Dr. Linsinger from EC JRC in Geel a first TS for Particle size distribution measurement by ensemble techniques has been published: ISO/TS 14411-1:2017. Preparation of particulate reference materials - Part 1: Polydisperse material based on picket fence of monodisperse spherical particles. A second project is currently ongoing: ISO/WD 14411-2:2017 "Preparation of particulate reference materials-Part 2: Polydisperse spherical particles". For instance "sufficiently homogenous" turned out to be one limiting factor for broad particle size distributions. But even more narrow distributions need special sample preparation methods [1] to get certifiable characteristic parameters.

Additionally a not so common "intended use" will be addressed – a nanostructured reference material for agglomerate stability testing in ultrasonic dispersion procedures with defined suspension volume specific energy input [2].

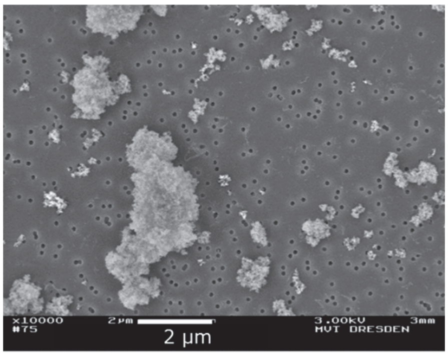

Figure 1: Nanostructured precipitated silica

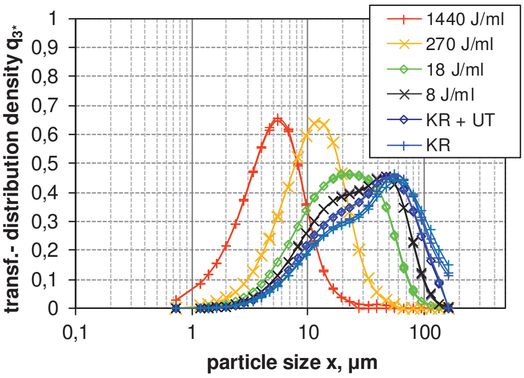

Figure 2: PSD of precipitated silica after ultrasonication

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Standardisation of magnetic nanomaterials: Steps towards magnetic reference particles

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Keywords: magnetic nanoparticles, reference materials, standardisation

Magnetic nanoparticles (MNP) comprise one of the largest nanomaterial families with an annual turnover of MNP dependent products of over 2 billion Euros generated by European companies alone. MNP are mainly employed in biomedicine for in-vitro diagnostics, for the separation and extraction of cells, viruses, proteins, and DNA from blood. In addition, new cancer therapies and further technical applications like magnetic bearings or loudspeakers make intensive use of MNP. Besides these "accepted" uses, we also find MNP to appear in the EU catalogue of nanomaterials in cosmetics.

Despite these advances, a unified terminology, standardized measurement procedures and certified reference materials (CRM) for MNP specific magnetic properties still have to be developed. ISO/TC 229 is working on the first standard for MNP: ISO 19807-1 "Liquid suspension of magnetic nanoparticles", which will define main characteristics and appropriate measurement methods. A second ISO standard (ISO 19807-2) is under preparation defining superparamagnetic beads used for nucleic acid extraction.

Developing any CRM should follow the guidance set out in ISO Guide 35:2016 on characterization of reference materials (RMs). The production of RMs is decisive for the improvement and maintenance of a measurement system for MNP and the increasing confidence in this novel nanomaterial. Currently, only MNP quality control materials (QCM) with adequate homogeneity and stability are used by MNP manufacturers, but these are not sufficiently characterized to be used for method calibration or to provide metrological traceability.

Paving the way towards CRM MNP, one should consider, that also the procedural standards for MNP magnetic characterisation need further improvement. Currently, only laboratory measurement standards are available, that ensure consistency of MNP measurement results within a laboratory. Early informal interlaboratory comparisons of MNP performance in biomedical applications based on these laboratory standards demonstrated the need for development of more general standard operating procedures (SOP). Robust SOP for MNP characterisation are a prerequisite for any MNP CRM development. On the other hand, it seems necessary to have at least calibration reference MNP materials (CAL) available, to develop measurement standards for MNP specific properties at ISO or CEN level that can be certified. CAL are quality control materials characterised by an assigned property value and uncertainty that is reproduced by any calibration sample used according to the specification. Only when MNP CAL and proper magnetic measurement standards will be available, also MNP CRM can be developed. Thus, any quality level of MNP RM is interlinked with the existence of appropriate magnetic measurement standards. To solve this hen and egg problem, both MNP RM and MNP SOP should be developed in parallel, eventually leading to convergence on a higher level.

The previous FP7 project "NanoMag" and the current EMPIR project "MagNaStand" contributed to the development of MNP terminology. Generally acceptable SOP for characterisation of MNP static and dynamic magnetisation, MNP performance in imaging, hyperthermia and magnetic separation are currently under preparation. The next step will then be the development of stable MNP RM with defined magnetic properties in these measurement procedures. This will consecutively be tested in interlaboratory comparisons, but not yet on VAMAS or OECD level.

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Abstract: 10-2018

TSEM: a traceable method for nanoparticle metrology

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Keywords: nanoparticle size, scanning electron microscopy, metrology

Traceable measurements of the lateral dimensions of nanoparticles are accomplished by means of a calibrated scanning electron microscope operated in transmission mode (TSEM) [1]. Nanoparticles under test are deposited on thin support films equal to those commonly used in transmission electron microscopy. Application of the bright-field imaging mode yields black-and-white images of the nanoparticles similar to shadow casts. An automatic image analysis tool determines the boundary and size of every particle and furthermore enables the analysis of a series of TSEM images containing thousands of particles to attain a particle size distribution.

An accurate determination of the particle boundary within the black-white transition requires a-priori information on the threshold level to be applied. For this purpose, expected TSEM signals are modelled by Monte Carlo simulations based on first-principle electron scattering theory considering relevant instrument and particle properties [2].

Traceability to the SI unit ‘metre’ is achieved by means of two-dimensional gratings which in turn are calibrated using a laser diffractometer with known wavelengths. With the current setup, the diameter of nanoparticles of different material classes (gold, silver, silica, latex) and sizes ranging from about 7 nm to 300 nm can be measured with uncertainties in the range of 1 nm to 4 nm, also confirmed by international comparison studies [3,4].

Applications of the technique include the calibration of spherical reference nanoparticles, but also the characterization of non-spherical nanoparticles (for example, the aspect ratio of gold nanorods [5], see Fig. 1 and 2). An extension of the TSEM technique exploits the minimum TSEM signal strength inside the particles which continually decreases with increasing particle thickness [6]: this additionally enables the measurement of the vertical particle size.

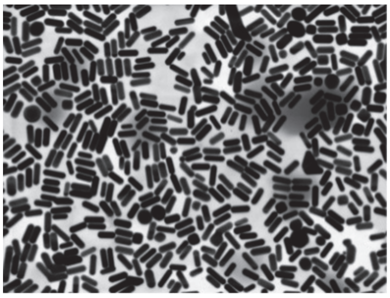

Figure 1: TSEM image of Au nanorods

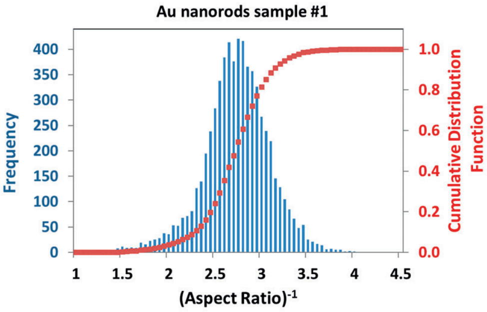

Figure 2: Histogram of Au nanorod aspect ratios

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Measurands and method dependent reference values: can one global particle size reference value fit all?

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Keywords: size, nanoparticle, reference value

It is well known that different measurement techniques for "nanoparticle size" have different measurands (see for example references [1,2]). Most markedly, atomic force microscopy (AFM) consistently measures smaller diameters and dynamic light scattering (DLS) larger diameters when compared with other methods [2] Because of this, nanoscale reference materials are typically accompanied by certificates assigning specific particle diameter values to different methods [3]. This method-dependent approach to particle size determination is consistent with the definition of a measurand given in the 2nd edition of the International Vocabulary of Metrology (VIM), the "particular quantity subject to measurement".

The concept of a global reference value for nanoparticle size is more closely aligned with the approach in the 3rd edition of the VIM, the "quantity intended to be measured". This has been explored in a recent supplementary comparison within the Asia Pacific Metrology Programme (APMP.L-S5 - Supplementary comparison on nanoparticle size, currently in revision to Draft B status). In adopting such an approach to particle size measurement, adequate measurement models that allow for the correction of method dependent data must be developed, and appropriately factored into uncertainty analysis to ensure consistency when comparing results across different measurement techniques. Indeed, the use of global reference values for particle size measurement may be favoured by metrology institutes considering applying the broad-scope approach to calibration and measurement capability (CMC) claims for publication in the Key Comparison Database (recommendations arising from the review of the Mutual Recognition Arrangement of the International Committee for Weights and Measures, CIPM MRA).

Here, we present experimental results obtained at the National Measurement Institute Australia that explore some of these concepts, using measurements made on gold, silica and polystyrene reference materials. In particular, we present our investigations into tip-induced particle deformation during AFM imaging, and the impact of small deviations from sphericity on the measurement of particle size made by other particle characterization instrumentation such as DLS and differential centrifugal sedimentation.

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TiO_2 Pigment Sizing:
Constituent, Aggregates and Primary Particles

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Keywords: Cross-Section, Primary Particles, Aggregates

A strong demand for reliable characterization methods of particulate materials is triggered by the prospect of forthcoming national and international regulations concerning the classification of nanomaterials. Scientific efforts towards standardized number-based sizing methods have so far been concentrated on model systems, such as spherical gold or silica nanoparticles. However, for industrial particulate materials, which are typically targets of regulatory efforts, characterization is in most cases complicated by irregular particle shapes, broad size distributions and a strong tendency to aggregation and agglomeration. Established and standardized sizing methods as well as certified reference materials that overcome these obstacles are still lacking. By using the example of titanium dioxide, this contribution presents a combination of advanced sample preparation and a sophisticated counting algorithm, which is based on the analysis of cross-sections prepared from embedded particles. The data presented demonstrate that many typical difficulties of particle sizing based on projection-images are overcome by the use of section-images. The developed image analysis procedure does not only allow the identification of primary particles, but moreover allows to identify and quantify size and size distribution of primary particles, aggregates and constituents on a statistically sound basis of several thousand particles. In other words, a robust, reproducible and statistically reliable method is presented, which leads to number-based size distributions of pigment-grade titanium dioxide, and therefore allows reliable classification of this material according to forthcoming regulations.

Based on the presented results, practical needs for reference materials are developed, which mainly include the requirement that the size of a reference material needs to be referenced in terms of section-size and projection-size to allow a wider variety of sample preparation methods. Especially with respect to the preparation as cross-section, aggregate-size and primary-particle size need to be given as well, together with the shape descriptors that are used to distinguish them.

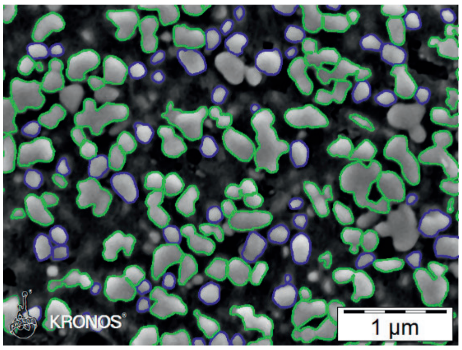

Figure 1: SEM of TiO2 white pigment prepared as cross-section; marked blue are primary particles; marked green are aggregates.

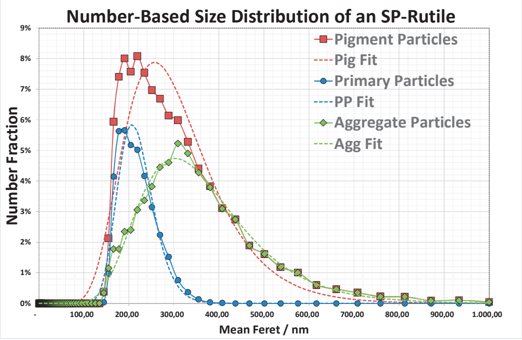

Figure 2: Deconvolution of the constituent size of a white pigment in terms aggregates and primary particles based on particle shape.

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Shape controlled TiO_2 nanoparticles as candidates for nano-CRM's – an ISO case study

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Keywords: titanium oxide, nanoparticles, shape-controlled

Extraction of true, 3D shape (and size) of non-spherical nanoparticles (NPs) is associated with errors by conventional 2D electron microscopy using projection images. Significant efforts within the ISO technical committee TC 229 'Nanotechnologies' are aimed at establishing accurate TEM and SEM measurement of NP size and shape as robust, standard procedures [1]. Study groups have been organizing inter-laboratory comparisons on well-selected NP systems according to the market needs, such as aggregated titania nano-powder for which size and shape distribution of primary crystallites of irregular shape must be measured accurately [2]. To be noticed is e. g. the fact that the measurement procedure allows only manual selection of the particles clearly distinguishable for analysis as well as manual definition of the contour of the imaged NPs.

An inter-laboratory exercise on titania NPs (pure anatase, grown by hydrothermal synthesis [3]) of well-defined non-spherical shape, i.e. bipyramidal, see Figure 1, has been recently started within ISO/TC 229 under similar conditions as for the irregular shaped titania. Figure 2 shows a representative TEM micrograph with particles tracked manually according to the measurement protocol. Overlapped particles were allowed to be considered, as long as they are clearly distinguishable. One decisive NP selection criterion was to analyze only those NPs with a roundness value below 0.7, i.e. the NPs laying on the support foil and, hence, with projection areas clearly deviating from perfect circles (R=1). The overall evaluation (for 15 labs) of the size descriptors (area, Feret, minFeret, perimeter) and shape descriptors (aspect ratio, roundness, compactness, extent) by analysis of variance is just to be finished and included in ISO/WD 21363 Nanotechnologies -- Protocol for particle size distribution by transmission electron microscopy.

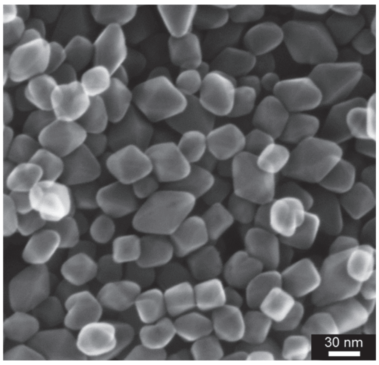

Figure 1: Representative SEM micrograph with the bipyramidal titania NPs

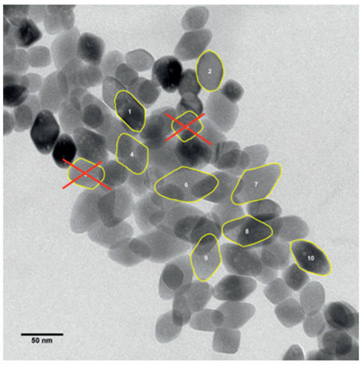

Figure 2: Exemplary TEM image of the bipyramidal TiO_2 NPs as prepared by BAM on carbon TEM grids, included in the measurement protocol distributed to the ILC participants

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Abstract: 14-2018

Reference methods for the determination of the nanoparticle mass- and number-based concentration using hyphenated ICP-MS

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Keywords: particle concentration, size-resolved IDMS, spICP-MS

Engineered nanomaterials (NM) have found variety of industrial applications, ranging from paints and coatings, microelectronic devices, through to food, cosmetics and clothing, with over 1300 NM-containing consumer products currently available on the market[1]. Widespread and rapidly growing use of NM has raised safety concerns with regards to an uncontrolled human exposure, as well as possibly adverse effects on the environment. To underpin the metrology supporting scientific efforts aiming to increase the understanding of the potential impact of NMs on human and the environment, well-characterised set of nanoparticle (NP) reference materials (RM) is required[2].

RM, ranging from simple water suspensions, through to complex matrices will allow instrument calibration as well as appropriate method development and validation. They will also be invaluable for the quality control purposes aiding in manufacture of more efficacious and safer products. However, RM certified for number- and/or mass-concentration of NP are either not currently available or very scarce, respectively.

This lecture will discuss the potential and remaining challenges in the determination of particle mass- and number-based concentration using inductively coupled plasma mass spectrometry (ICP-MS), with focus on (i) ICP-MS hyphenation to asymmetric flow field-flow-fractionation (AF4) with double isotope dilution (ID) calibration for the mass-based determination of silica NP and (ii) the application of SI traceable spICPMS methodology for the determination of number-based concentration of colloidal gold NP, being the mass concentration of NP relevant to future risk assessment and the number-concentration of NP relevant to NP identification and characterisation. Finally, the analytical figures of both methods, including their measurement uncertainty and their feasibility for RM characterisation will also be discussed.

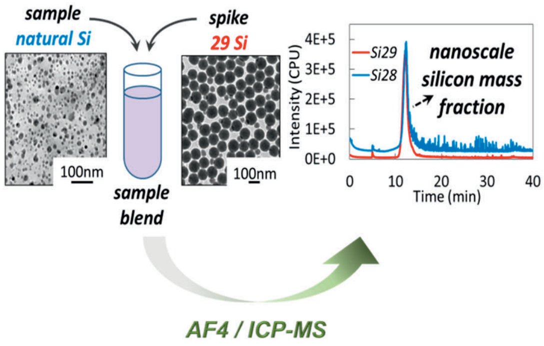

Figure 1. Schematic representation of size-resolved ID approach for mass-concentration determination of NP RM.

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Can labelled nanoparticles as internal standards improve the reliability of the quantitative analysis of nanomaterials in complex matrices?

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Keywords: labelling, silica, titania

The reliable quantification of nanomaterials (NMs) in complex matrices such as food, cosmetics and biological and environmental compartments can be challenging due to interaction of particles with matrix components and analytical gear (vials, tubing etc.). The resulting losses along the analytical process (sampling, sample extraction, clean-up, separation, detection) hamper the reliable quantification of the target NM in these matrices as well as the comparability of results and thus a meaningful interpretation of nanosafety studies. These issues can be overcome by the addition of known amounts of internal/recovery standards to the sample at the beginning of the analytical process. These standards need to mimic as closely as possible the behaviour of the target analytes in the analytical process which is mainly defined by the size, surface properties (e.g. the coating in case of coated particles), shape and density. At the same time they need to carry a tag that can be quantified independently of the target analyte. Since inductively coupled plasma mass spectrometry (ICP-MS) is often used for the identification and quantification of NMs the tagging with either low-abundant isotopes of the target analyte or with chemically related rare elements is a promising approach.

Within the EU project NanoDefine labelled analogues were synthesized for two frequently used nanomaterials: silica and titania. For silica, core-shell nanoparticles were prepared that consisted of a cobalt core and a silica shell. For nano-scale titania as it is used in sun screen as UV protection, first the structure of commercial materials was analysed. Then particles that mimic these materials in terms of shape, size and surface coating were prepared, but doped with hafnium or zirconium in the titania core. All were fully characterised and examples will be shown. These efforts have shown the feasibility of synthesizing respective materials. The next step is to test their behavior within the analytical process and to demonstrate that they behave reproducibly and in the same way as the target analytes.

The presentation will focus on the discussion of the applicability of the use of labelled internal standards for the analysis of nanomaterials. While this approach is well established for traditional analytes (e.g. molecules) it is more difficult to implement for nanomaterials because the target analyte is not one unique entity. Nanomaterials of the same nominal composition, even if from the same batch, vary in size, shape and surface properties (within the given specifications). Labelled analogues would either have to represent these properties or span the same characteristics as the target material.

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Abstracts
Poster Presentations
May 14

The Malta Project – A European Initiative to Develop and Amend OECD Technical Guidelines for Testing of Nanomaterials

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Keywords: OECD Technical Guideline

The European Commission proposed a number of changes in the REACH annexes concerning testing of nanomaterials. However validated, standardized and harmonized test methods for nanomaterials are currently not available for all endpoints. The Malta Project is a common initiative of the European Commission together with European countries to work on filling this gap. The purpose is to adapt or develop new OECD test guidelines (TGs) and guidance documents (GDs) to be applicable for nanomaterials as they are mutually accepted by regulatory bodies worldwide.

Relevant knowledge and methods, gathered in research projects, should be used to develop and validate OECD standards. All work will be performed in close collaboration with the OECD Working Party on Manufactured Nanomaterials (WPMN), the OECD Working Group of National Coordinators of the Test Guidelines Program (WNT) and the ECHA Nano Materials Expert Group (NMEG). The TGs will be developed in international groups to ensure acceptance by the OECD member states.

The development/amendment of TGs/GDs includes experimental work, the translation of scientific results for regulatory methodical adaptation, development of standard operation procedures, their validation and the implementation at OECD. In order to validate the SOPs and to ensure their applicability and reproducibility several ring tests are planned. For quality assurance well characterized reference nanomaterials will be required.

The Malta Project covers endpoints, foreseen to be addressed in the revised REACH annexes in the fields of phys-chem characterization, human health and ecotoxicity. Currently addressed endpoints are e.g. the specific surface area, dustiness, solubility in water and biological media, surface chemistry, in vitro genotoxicity, skin sensitization, toxicokinetics, environmental transformation and bioaccumulation.

Further endpoints are discussed and some are still open. Member states should commit themselves for the development/amendment of a TG. An overview of the foreseen tasks of the submitted OECD Project Proposals will be given.

Abstract: CLN-01-2018

Quantification of Silver Nanoparticles at Single Cell Level by Mass Cytometry

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Keywords: Nanoparticles, cells, mass cytometry

Silver nanoparticles (AgNPs), have a high scientific and commercial impact due to their important antibacterial properties. However, there are serious concerns about their toxicological adverse effects because of their broad range of applications. Particularly, the impact of AgNPs on cells is not very well understood yet and there is a current demand to develop analytical methodologies providing information about the interaction and distribution of AgNPs at a single cell level. In this research, mass cytometry was used to introduce a new quantitative approach to study the uptake of AgNPs by individual THP-1 macrophages as a cell model system.[1] Here, we show that this methodology provides not only multi-variate phenotypic information of individual cells but enables the quantitative analysis of AgNPs associated to cells in a single measurement by performing an external calibration using AgNPs suspension. Using differentiated THP-1 cells, we monitored and quantified the uptake of 50 nm AgNPs in a time and dose-dependent manner by mass cytometry. 7 to 120 AgNPs per cell (2 to 89 fg Ag/cell) were determined after exposure of differentiated THP-1 cells to low AgNPs concentrations of 0.1 and 1.0 mg L^{-1}, at time points of 4 and 24 h. The results were validated by mass cytometric analysis of digested cells working as a conventional inductively coupled plasma mass spectrometry, ICP-MS. This study demonstrates the power of single cell analysis by mass cytometry even for low doses experiments as a new analytical tool for hitherto unaddressed questions in nanotoxicology.

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OECD Test Guidelines Development for Chemicals Safety Assessment of Nanomaterials

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Keywords: Standardization, Particle size distribution, OECD Test Guideline

The OECD test guidelines (TGs) for testing chemicals have been widely used for regulatory purposes all over the world since the establishment of the Mutual Acceptance of Data (MAD) principle in 1984. This MAD principle ensures that, if a chemical is tested under the Good Laboratory Practice (GLP) conditions accordingly to an OECD TG, the data should be accepted in all OECD countries. The TGs have been developed, harmonized, internationally validated (round robin tests) and adopted by OECD countries to be used for the physical-chemical characterisation, fate estimation, and hazard identification for risk assessment of various chemicals. In addition to the TGs, OECD Guidance Documents (GDs) usually provide guidance on how to use TGs and how to interpret the results. These GDs do not have to be fully experimentally validated, and hence they are not under MAD, but they are based on relevant published scientific research.
But are the existing TGs and the related GDs applicable and adequate for the regulatory testing of nanomaterials? In general, it is accepted that most of the "endpoints" or more precisely measurement variables are applicable also for nanomaterials. However, for some endpoints new or amended TGs are needed. In addition, several GDs are needed to give more precise advice on the test performance in order to gain regulatory relevant data on nanomaterials.

The poster will present the status quo on recent TGs and GDs development for nanomaterials at OECD level with relevance for physical-chemical characterisation. Emphasis will be given to the proposed OECD TG on particle size and size distribution for manufactured nanomaterials. The development of such a TG is of special importance as particle size and size distribution is considered as major information for nanomaterial identification and characterization. A reliable and reproducible characterisation of particle size and size distribution is also needed for chemicals risk assessment of nanomaterials, for instance to interpret and compare test results and - in future – to forecast interaction and effects of nanomaterials. The presented poster will illustrate the way from the idea for a new TG and new GD to an accepted OECD TG/GD.

Abstract: HSN-02-2018

Assessing potential risks of nanomaterials - Genotoxicity screening with the fluorometric γ-H2AX assay and automated microscopic detection

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Nanoparticles, Quantum dots, Surface chemistry, Cell test, Genotoxicity, Toxicity screening

The increasing use of nanomaterials in the material and life sciences and the synthesis of an ever increasing number of new functional nanoparticles raises also concerns of nanotoxicity, calling for standardized test procedures[1,2] and efficient approaches to screen the potential genotoxicity of these materials. Aiming at the development of fast and easy to use, automated microscopic methods for the determination of the genotoxicity of different types of application-relevant nanoparticles, we assess the potential of the fluorometric γH2AX assay for this purpose. This assay, which can be run on an automated microscopic detection system, relies on the detection of DNA double strand breaks (DSB) as a sign for genotoxicity[3]. Here, we present first results obtained with broadly used nanomaterials like CdSe/CdS and InP/ZnS quantum dots as well as iron oxide, gold, and polymer particles of different (known) surface chemistry with previously tested colloidal stability and different cell lines like Hep-2 and 8E11 cells, which reveal a dependence of the genotoxicity on the chemical composition as well as the surface chemistry of these nanomaterials. These studies will be also used to establish nanomaterials as positive and negative genotoxicity controls or standards for assay performance validation for users of this fluorometric genotoxicity assay. In the future, after proper validation, this microscopic platform technology will be expanded to other toxicity assays.

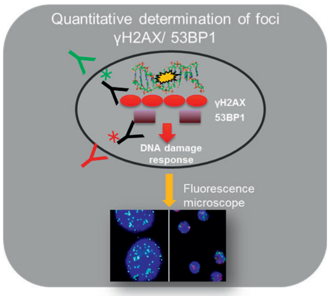
Quantitative determination of foci
γH2AX/ 53BP1

Figure 1: Detection of DSB with γH2AX assay

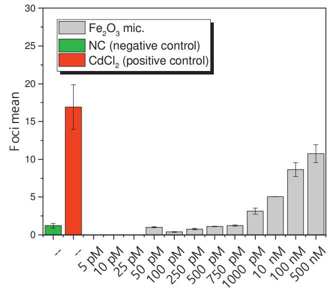

Figure 2: Genotoxicity of iron oxide nanoparticles

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Reference samples for Magnetic Force Microscopy

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Keywords: high resolution magnetic field sensing, reference samples, deconvolution

Magnetic field imaging techniques with sub micrometer resolution are of increasing scientific and industrial interest as they are used for the characterization of e.g. magnetic nanostructures like domain walls as well as for magnetic sensors, scales and storage devices.

Some imaging techniques, like scanning Hall, scanning magnetoresistive imaging, magnetic force microscopy (MFM) and magneto-optical imaging using Faraday indicator films (MOIF) can in principle be calibrated to yield quantitative spatially resolved magnetic field data $\boldsymbol{H}(x,y,z)$. Deconvolution approaches even allow an extraction of field data on length scales smaller than typical sensor dimensions, if the physical principles of the sensor mapping function are understood, as it is the case for all techniques mentioned above. However, for nanoscale sensors unavoidable manufacturing-related deviations in geometry and material parameters may significantly impact the sensor sensitivity, in particular when measuring nanoscale structures. Therefore, each individual sensor has to be calibrated, and if subject to wear processes this calibration may even be required before and after any actual measurement. Since the sensitivity typically shows a strong dependency on the dimensional features of the stray field distribution, a calibration has to be performed in an application specific stray field distribution. Nano- and micro scale magnetic field features can only be observed next to the surface of a magnetically or mechanically micro- or nano-patterned magnetic reference material. Such magnetic reference samples for different applications are developed at PTB within the EMPIR project NanoMag. We here will discuss demands and possible implementations of such reference materials for different calibration purposes.

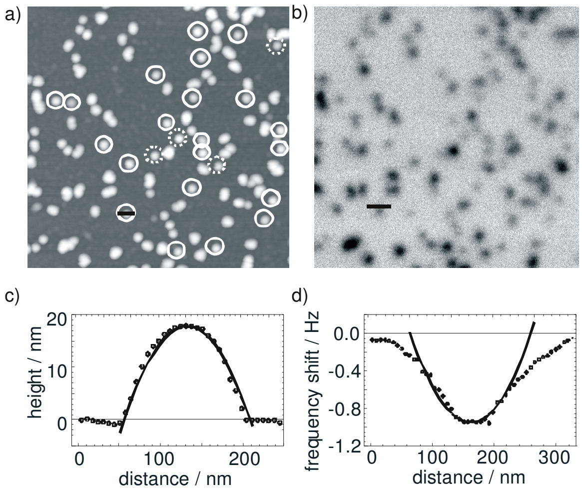

Figure 1: Magnetic nanoparticles for MFM tip calibration.

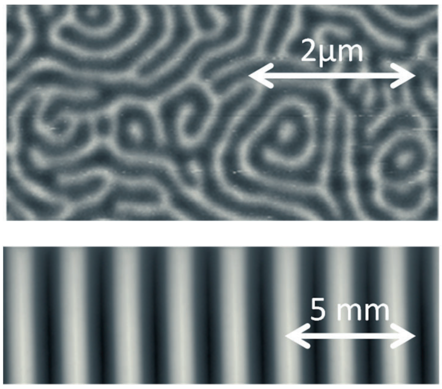

Figure 2: Maze domain pattern in a CoPt sample (top) and a magnetic scale (bottom) for the calibration of magnetic sensors with active areas in or below the micrometer range and up to the millimeter range, respectively.

Nanocrystals as labeling reagents for Imaging Mass Cytometry

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Nanocrystals, lanthanide, immuno-assays

Imaging of elemental distributions in single cell assays as well as tissue sections can be performed by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). This powerful technique offers precise spatially resolved measurements at the trace and ultratrace level and has been established as an excellent tool to answer analytical, biological and biomedical questions. To date, imaging mass cytometry is already able to simultaneously detect up to 40 cellular targets due to conjugation of isotopically pure lanthanides to affinity binders, e.g. antibodies.

To further enhance the ability of multiparametric analysis to more than 100 analytes at once, we investigated lanthanide nanocrystals as new, highly sensitive metal tags for identification of targets in clinical cell assays and tissue samples. Multiparametric analysis will be possible by encoding the lanthanide composition of nanocrystals associated to the affinity binders. Nanocrystals showed remarkable potential for sensitive detection in MS due to high stability and signal amplification compared to e.g. polymer tags, carrying fewer metal atoms.

Synthesis of functionalized lanthanide nanocrystals for further bioconjugation was performed with high reproducibility and monodisperse size distribution. For proof of principle, the uptake and distribution of these nanocrystals within the monolayered cell line A549 were investigated by mapping the intensities at subcellular resolution using LA-ICP-MS. It could be shown, that the cells were efficiently labeled with the nanocrystals. Additionally, the bioconjugation of the nanocrystals to antibodies and particularly the preservation of the antibody specificity was investigated using Dot Blot experiments. All in all, the results imply high sensitivity and the possibility of multiparametric analysis by doting various lanthanides into the nanocrystals.

Abstract: NCM-01-2018

Studying nanoparticle-cell interaction by ICP-MS based techniques

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Keywords: Nanoparticles, cells, laser ablation ICP-MS

Nanoparticles (NPs) have found a wide range of applications in research and industry. Thereby the interaction of NPs with biological systems like cells has become a major field of interest, ranging from medical applications to nanotoxicology. Size, shape and surface modification of the nanomaterials determine the uptake rate and pathway into the cells, and therefore impact specific cell components and processes.

Inductively coupled plasma mass spectrometry (ICP-MS) is a well-established analytical method offering high sensitivity and multi-element capability. By coupling a laser ablation (LA) system to an ICP-MS the analysis of different kinds of solid samples is possible. In recent years, it was shown that LA-ICP-MS can provide quantitative as well as distribution information of metal containing nanoparticles (NPs) in cell samples.

Here LA-ICP-MS was applied for the imaging of individual fibroblast cells to study the uptake and intracellular processing of NPs. Our results show that LA-ICP-MS can be used to localize nanoparticle aggregates within cellular compartments. The studied NPs accumulate in the perinuclear region in the course of intracellular processing, but do not enter the cell nucleus.[1, 2] The uptake efficiency depends strongly on the physico-chemical properties of the nanostructures[3, 4] as well as on the incubation conditions like concentration and incubation time.[1, 4]
ICP-MS was used to determine the composition of the nanomaterials as well as the number of NPs in cells after acid digestion of the samples.

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Abstract: NCM-02-2018

Toward a reference sample for atom probe tomography

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Keywords: Reference sample, Atom probe tomography, interlaboratory comparison

Atom probe tomography (APT) is an analytical technique capable of providing in-depth elemental information at sub-nanometer resolution (lateral and depth) with a relatively high sensitivity. Therefore, it is seen as an important enabler in diverse fields of applications such as semiconductor industry, metallurgy etc. Over the last decade, APT has seen considerable progress in instrumental development and the theoretical understanding of its underlying physics. In contrast to this, universal analysis procedures, uncertainty assessment and widespread comparison of APT data are still lacking, mainly due to the absence of appropriate standards and reference materials. This is not surprising, given the high complexity paired with the low reproducibility of current APT sample preparation procedures [1]. The underlying physical principle of APT, i.e. laser-assisted atom-by-atom field evaporation, requires a tip-shaped sample with an endpoint radius below 100 nm. Current practise in the field is to use dual-beam instruments that combine a scanning electron microscope and a focused ion beam (FIB), to nanofabricate a sharp tip from the sample. The latter is however very time consuming and, due to its manual nature, distant from manufacturing many sample in a reproducible manner for comparative studies.

To address these limitations, we have developed a novel sample preparation scheme that yields APT samples with minimized tip-to-tip variations i.e. with reproducible tip radii, taper angles and heights. With this approach we strongly improve the assessment of the uncertainty budget in APT by eliminating the impact of tip geometry and its properties, as the latter might be strongly modified during the FIB preparation. Our protocol is based on standard lithography and etching techniques, hence omitting the use of a manual SEM-FIB step, and can be easily upscaled towards full wafer processing with a high degree of automation. As initial test vehicle, we have optimized the procedure for the preparation of APT tips starting from a boron-doped (~1E20 atoms/cm^3), epitaxially grown SiGe (25% Ge) layer (100 nm) on a 300 mm Si (100) wafer. The complementary characterization of the same (or similar) wafer using different (reference-free) analysis techniques is done to extract the layer thickness, composition and dopant concentration. This complete characterization together with the repeatability in forming the different APT samples, will form the cornerstone to establish a potential reference sample for APT. Tthese well-characterised APT tips will be used to initiate an interlaboratory study (e.g. under the VAMAS umbrella [2]), addressing the uncertainty budget in APT analysis related to the analysis of Boron (as a dopant) and Ge (as an alloy element).

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Creating the Silver Standard: Development and Applications of a Silver Nanoparticle Reference Material

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Keywords: silver nanoparticles, SAXS, artificial digestion

The utilization of silver nanoparticles in consumer related products has significantly increased over the last decade, especially due to their antimicrobial properties. Today they are used in a high variety of products ranging from food containers over children toys and textiles. Therefore, research on the toxicological potential of silver nanoparticles becomes increasingly important for a high amount of studies. Unfortunately, the results of these studies are extremely diverse and do not lead to a consistent evaluation. The central problem lies in the use of a wide range of silver nanoparticles, which show a broad size distribution. To overcome this problem we report on the synthesis and application of small silver nanoparticles with a narrow size distribution ($R = 3.1$ nm, $\sigma = 0.6$ nm). The poly(acrylic acid) stabilized particles are thoroughly characterized by small-angle X-ray scattering, dynamic light scattering and UV/Vis spectroscopy. The particles are highly stable and show no aggregation for more than six months. It is foreseen to use these thoroughly characterized nanoparticles as reference material to compare the catalytic and biological properties of functionalized silver nanoparticles. As a first step the particles are used in the first world-wide inter-laboratory comparison of SAXS.[1] Furthermore, the stabilizing ligand PAA can be easily exchanged by biomolecules to modify the surface functionality. Replacements of PAA with glutathione (GSH) and bovine serum albumin (BSA) have been performed as examples. With this flexible system first applications regarding biological application in an artificial digestion procedure have been performed. Thereby the changes in size distribution and aggregation state were monitored by SAXS (Figure 1).[2] Additionally these particles show a high catalytic activity of (436 ± 24) L g^{-1} s^{-1} in the reduction of 4- nitrophenol to 4-aminophenol. This activity is two orders of magnitude higher than for other silver particles in the literature.[3]

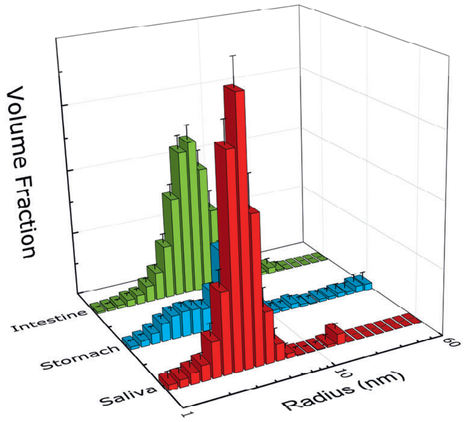

Figure 1: Volume-weighted size distributions derived by SAXS measurements of silver nanoparticles in the presence of food components at the three digestion steps: saliva, stomach and intestine (red, blue and green bars, respectively).

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Microwave-assisted high-speed silver nanoparticle synthesis

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Keywords (up to 3): SAXS, Microwave Synthesis, Silver Nanoparticles

Ever since increasing a reaction's yield while shortening the reaction time is the main objective in synthesis optimization. Microwave reactors meet these demands. In literature however their usage is under discussion due to claims of the existence of non-thermal effects resulting from the microwave radiation. Especially for nano-reference-material syntheses it is of crucial importance to be aware of influences on the reaction pathway. Therefore, we compare ultra-small silver nanoparticles with mean radii of 3 nm, synthesized via conventional and microwave heating.

We employed a versatile one-pot polyol synthesis of poly(acrylic acid) (PAA) stabilized silver nanoparticles, which display superior catalytic properties. No microwave specific effects in terms of particle size distribution characteristics, as derived by small-angle X-ray scattering (SAXS) and dynamic light scattering (DLS), are revealed. Due to the microwave reactor's characteristics of a closed system, syntheses can be carried out at temperatures beyond the solvent's boiling point. Particle formation was accelerated by a factor of 30 by increasing the reaction temperature from 200 °C to 250 °C. The particle growth process follows a cluster coalescence mechanism.

A post-synthetic incubation step at 250 °C induces a further growth of the particles while the size distribution broadens. Thus, utilization of microwave reactors enables an enormous decrease of the reaction time as well as the opportunity of tuning the particles' size. Possibly, decomposition of the stabilizing ligand at elevated temperatures results in reduced yields. A temperature of 250 °C and a corresponding reaction time of 30 s represent a compromise between short reaction times and high yields.

Development of a hybrid metrology combining AFM and SEM techniques for measuring the characteristic dimensions of a nanoparticle population

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Keywords (up to 3): nanoparticle size, hybrid metrology, microscopy-based techniques

This study proposes a novel approach of hybrid metrology combining AFM (Atomic Force Microscopy) and SEM (Scanning Electron Microscopy) measurements for measuring the characteristic dimensions of nanoparticles (NP) in 3D with controlled uncertainties. This method takes advantage of the complementary nature of both techniques: SEM gives no quantitative information about the NP heights whereas the uncertainty associated with AFM measurements of NP maximum point is close to 1.5 nm [1]. Conversely, the lateral dimensions measured by AFM are impacted by tip / NP convolution whereas SEM of latest-generation SEM equipped with FEG (Field Emission Gun) can reach a resolution of 1 nm in the XY plane.

In order to check the consistency of measurements performed by AFM and SEM, a comparison of the mean diameter of spherical silica NP measured by both techniques had been carried out in a previous study [2]. However, this comparison had not been performed exactly on the same population.

In the meantime, a specific repositioning device consisting of silicon wafer with lithographed landmarks has been developed to easily find an area of interest. Henceforth, it is possible to determine the AFM height and SEM lateral diameter on the same set of NP. The proof-of-concept has been tested on various samples of silica NP synthesized by Stöber method [3] and reference silica NP with sizes ranging from 5 nm to 110 nm (Figure 1). Although the spherical nature requires equality between AFM height and SEM lateral diameter, a systematic discrepancy was observed, especially for smaller NP.

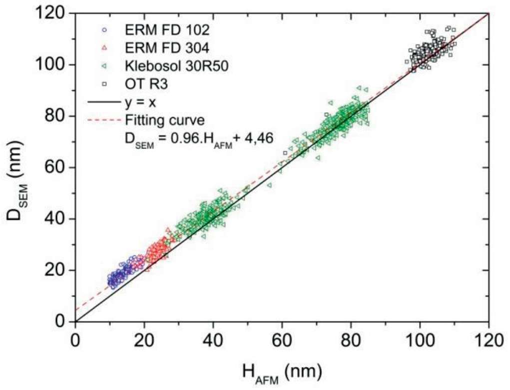

Figure 1: Comparison between height measurements performed by AFM and lateral diameters measured by SEM on various populations of silica nanoparticles (FD-304, FD-102, Klebosol 30R50, OTR3).

An analogy with PSL (polystyrene latex) NP presenting known deformation [4-5] suggests that mechanical properties of NP could play a role in measured discrepancies. But, the main contributions come from the sphericity deviation of smaller silica NP as well as their orientation on the substrate.

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Contribution to accurate Spherical Gold Nanoparticles analysis (size, size distribution) by SpICPMS and SAXS

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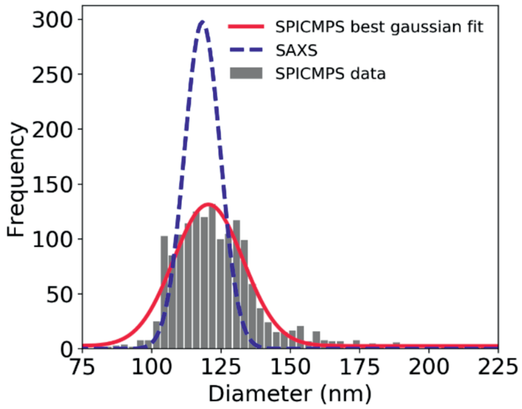
Keywords:

Small-Angles X-Ray Scattering (SAXS) has been established as a metrological method for the determination of nanoparticles size and size distribution. Modern SAXS Laboratory experiments1, by involving synchrotron-based instrumentation at lower price and very stable X-ray source, are more and more used in nanomaterials domain. In the frame of the EMPIR Innanopart project, we have developed a methodology for the size, size distribution and concentration determination of spherical nanoparticles. This protocol involves a precise sample preparation, and a set of homemade software tools for the data processing - from the acquisition, the absolute scaling, to the analysis.
spICPMs is not a metrological traceable technique but has many strengths to become a useful complement of nanoparticle characterization methods such as SAXS and microscopy. It can also measure highly diluted nanoparticles suspensions which is not the case of Dynamic Light scattering (DLS) or SAXS. Finally, ICPMS analyzes inorganic ions in liquid solution in a very large range of concentration, which should allow linear diameter measurement range over at least 3 orders of magnitude.
In this work, we confront spICPMS with SAXS in order to investigate the method and the developed protocols on a set of commercial spherical Gold Nanoparticles.



Comparison between SAXS and spIPCMS method for the determination of size of spherical Gold Nanoparticles

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Improved traceability chain of nanoparticle size measurements – the new EMPIR project nPSize

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Keywords: traceable size, nanoparticles, shape

Coming as response to the needs expressed by The European Commission mandating CEN, CENELEC and ETSI to develop European standards for methods that can characterize reliably manufactured nanomaterials, a new European metrology research project '***nPSize*** *- Improved traceability chain of nanoparticle size measurements*' has received funding for the next three years. The project will develop methods, reference materials and modelling to improve the traceability chain, comparability and compatibility for nanoparticle size measurements to support standardization.

nPSize has selected only those nanoparticle sizing techniques which are able to provide traceable results: electron microscopy (SEM, TSEM and TEM), AFM and SAXS. Metrologists from national metrological or designated institutes (PTB, LNE, LGC, VSL, SMD and BAM) will work together with scientists with know-how in development of new nano reference nanoparticles (CEA, University of Turin, LGC, BAM) and with experts in advanced data processing, e.g. by machine learning (POLLEN). With the support of DIN, the project outcomes will be channelized to standardization bodies such as ISO/TC 229 'Nanotechnologies'/JWG 2 'Nanoparticle Measurement and Characterization' (SEM, TSEM and TEM), CEN/TC 352 'Nanotechnologies' (SEM, TSEM and TEM), ISO/TC 201/SC 9 (AFM), ISO/TC 24/SC 4 (SAXS).

Three technical work packages will ensure input for impact to standardization community, nanoparticle manufacturers, instrument manufacturers, and (accredited) service laboratories:

- WP1 Performance and traceability of characterization methods
- WP2 Reference materials - Preparation and Characterization
- WP3 Modelling and development of measurement procedures

Well-defined non-spherical nanoparticles shapes such as cubes, platelets, bipyramids, rods/acicular will be developed, with mono- and polydisperse size distribution, as well as with accurate particle number concentration (by SAXS and isotopically enrichment for ICP-MS).

Physical modelling of the signal for TSEM, SEM, 3D-AFM and SAXS will be used to feed machine learning modeling from a-priori measurement data. Further, data fusion will be developed for hybrid sizing techniques: SEM with TSEM/TEM, SEM/TSEM with AFM, SEM/TSEM with SAXS with the final aim of improving the true shape and size of non-spherical nanoparticles by a better estimation of the measurement uncertainties.

In the second half-time of the project dedicated workshops (focused on method improvement and reference materials development) will be organized to disseminate the gained knowledge to end-users. Further, a data library with relevant tagged measurement data is planned to be organized and made publicly available. Inter-laboratory comparisons based on the newly developed multi-modal nano reference materials will be organized preferably within VAMAS/TWA 34 'Nanoparticle populations'.

EMPIR EURAMET
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Abstract: SDN-03-2018

Advanced screening method using volume-specific surface area (VSSA) for nanomaterial identification of powders

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Keywords: VSSA, nanomaterial screening, nano-powder characterization

Abstract: The EC's recommendation for a definition of nanomaterial (2011/696/EU) should allow the identification of a particulate nanomaterial based on the number-based metric criterion according to which at least 50% of the constituent particles have the smallest dimension between 1 and 100 nm. However, it has been recently demonstrated that the implementation of this definition for regulatory purposes is conditioned by the large deviations between the results obtained by different sizing methods or due to practical reasons such as high costs and time-consuming (SEM, TEM) [1].

Within the European project NanoDefine (www.nanodefine.eu) a two-tier approach has been developed, whereby firstly a screening method is applied for the rough classification as a nano-/non-nanomaterial, and for borderline cases a confirmatory method (imaging methods or field flow fractionation) must be considered.

One of the measurement methods well suited to particulate powder is the determination of volume-specific surface area (VSSA) by means of gas adsorption as well as skeletal density. The value of 60 m^2/cm^3 corresponding to spherical, monodisperse particles with a diameter of 100 nm constitutes the threshold for decisioning if the material is a nano- or non-nanomaterial. The correct identification of a nanomaterial by VSSA method is accepted by the EU recommendation.

However, the application of the VSSA method is associated also by some limitations [2]. The threshold of 60 m^2/cm^3 is dependent on the particle shape, so that it changes considerably with the number of nano-dimensions of the particles. For particles containing micro-pores or having a microporous coating false positive results will be produced. Furthermore, broad particle size distributions make necessary to adjust the threshold. Based on examples of commercially available ceramic powders, the applicability of the VSSA approach will be tested (in relation with SEM and TEM measurements) in order to expand the actual knowledge and to improve this good available and agglomeration tolerant method.

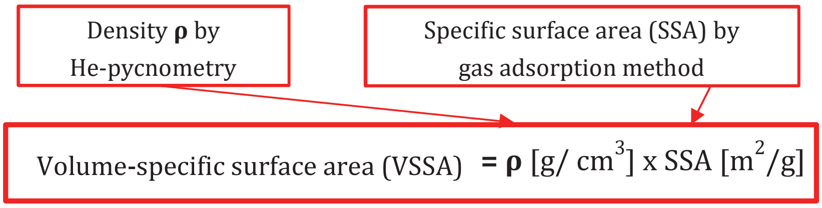

Figure 1: Determination of the volume-specific surface area (VSSA)

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Controlled electrospray deposition of nanoparticles for improved analysis by electron microscopy

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Keywords: electrospray, deposition efficiency, imaging

Of many experimental techniques for measuring particle sizes and size distributions, electron microscopy (EM) is considered as the gold standard, especially in the nano range (1–100 nm). Furthermore, high-resolution X-ray spectroscopy (EDX) in conjunction with EM can be applied to individual NPs. Preparation of an EM sample for generic particulate materials is a difficult task. Usually, the particles in a suspension are deposited on a support. However, this procedure includes the drying of larger solvent amounts on the substrate itself, and this can affect the spatial distribution of the deposited particles. One possibility to overcome this is the use of an electrospray system, where the suspension of particles is sprayed onto the substrate in charged droplets that are so small that they either dry off on the substrate immediately without affecting the position of particles, or even already during their flight time to the substrate. Additionally, the charging of particles minimizes agglomeration and aggregation, maximizing the collection of the EM grids. The prototype of an electrospray deposition system from RAMEM under its trademark IONER (www.ioner.eu) was tested.

Electrospray is theoretically described since a long time,[1] but no dedicated commercial instruments are available for the preparation of TEM grids yet, apart from electrostatic deposition of aerosols.[2] Several materials have been sprayed onto TEM grids and the resulting particle distributions were evaluated. Operation parameters such as the sample flow-rate, capillary – substrate distance, electric field strength and sampling period length have been optimized. It was found that the particles deposited by electrospray generally show a much more homogeneous spatial distribution on the substrate and a substantial increase of the number of single particles (suited to automatic analysis), see e.g. Figure 1.[3]

The project has received funding from the European Union's Seventh Programme for research, technological development and demonstration under grant agreement No 604347.

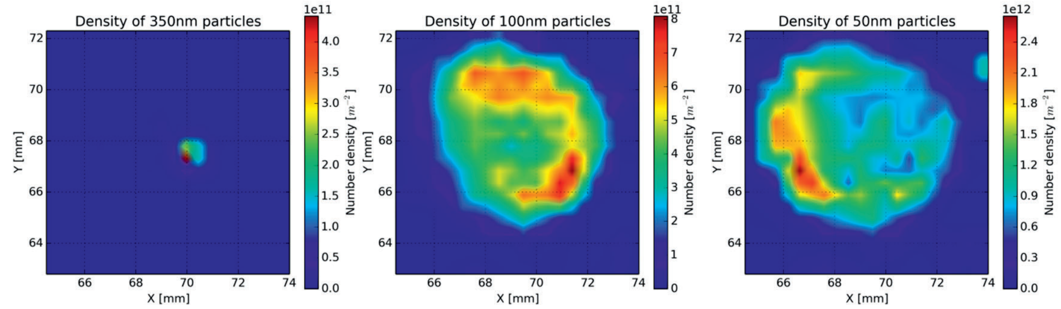

Figure 1: Spatial distribution of the number densities of each particle mode of a tri-modal PSL sample (50 nm + 100 nm + 350 nm), deposited on silicon, as extracted from SEM micrographs at 3 kV.

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Software development and instrument development for characterization of nano (C)RMs

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Keywords: Scattering, Correction, Analysis

It is never nice to admit to one's inabilities, however, with access to an extensive range of analytical instruments rapidly delivering a vast quantum of data, the manual analysis of all data becomes infeasible. Therefore, we look towards increasingly automated analysis, performed by software, to facilitate the characterization of our systems. However, this infers that we must know and communicate the limits and caveats of our methods, so that researchers may add a well-founded degree of confidence to the assessments.

We have discovered a number of limits of our techniques through software development efforts, both for instrumentation, analysis in addition to data correction. Building on this experience, we are now starting to explore further boundaries using candidate nano-CRMs. Through definition of such boundaries, we concurrently elucidate where our technique can be applied successfully. Using small-angle scattering (SAS) as an example, this talk highlight what limits we have managed to discover, and how they were found. Examples are proposed for the further exploration of the application boundaries, alongside some strategies for overcoming limitations.

A broader future perspective can be gained by taking the outsider position: a methodologically sound researcher will apply a range of techniques to a given sample and weigh the results by their respective confidence metrics. This can only be done if the limits and strengths of all the techniques are well-characterized, for which a wide range of nano-CRMs are required that mimic real-life practical samples. A pilot nano-CRM SAS investigation will be highlighted with lessons learnt and future plans discussed.

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Abstract: SDN-06-2018

Dimensional characterization of monodisperse sub 6 nm colloidal semiconductor nanocrystals

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Keywords: standardization, nanocrystals, small angle X-ray scattering

Due to their unique electronic and optical properties, as well as their promising application in many modern technologies, the demand for semiconductor nanocrystals has rapidly increased over the past few years. While the synthesis of monodisperse nanoparticles is possible via various methods, each sample still has to be characterized and validated for its use. To assess all features like size, concentration, polydispersity and morphology, most commonly UV/VIS-spectroscopy and transmission electron microscopy (TEM) are combined. TEM is known to be a less accessible and strongly time consuming method. As a possible faster but, in terms of accuracy, equal alternative we explored small angle X-ray scattering (SAXS) as a metrological precision tool. SAXS measures the sample as an ensemble and furthermore provides information towards particle interaction while enabling a direct measurement of dispersions, not requiring an elaborate sample preparation prior to the measurement, and not being restricted to a local focus. This makes SAXS a valuable tool in modern research as well as in quality control.

The metrology of nanocrystals essentially depends on correlating models which comprehend their optoelectronic and physical properties. Extensive measurements were done on the well-known cadmium selenide system. The synthesis of monodisperse semiconductor nanoparticles, including an investigation of different synthesis approaches, utilizing various ligands/shells or solvents, constituted the base of the routine within this work. The correlation of the collected data from each method to the most accessible features is in great accordance with the literature. The processing of further experimental data will yield a comprehensive model function fully characterizing any monomorphic sample.

While metrological references in the nanometer range are rare, the development of new standards is crucial to the promotion of novel applications in the fields of nanotechnology. SAXS is able to characterize and evaluate with high precision and showed great potential to support these goals.

In-Situ SAXS Techniques

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Keywords: SAXS, Nano-particles alignment.

This project studies the orientation of nanoparticles under the influence of external stimuli such as electric fields, magnetic fields and ultra-sonic vibrations. A set of functional sample holders that fit inside the sample chamber (image below) of a state-of-the-art Small Angle X-ray Scattering (SAXS) machine, called the “Multi-scale Analyzer for Ultrafine Structures” (MAUS).

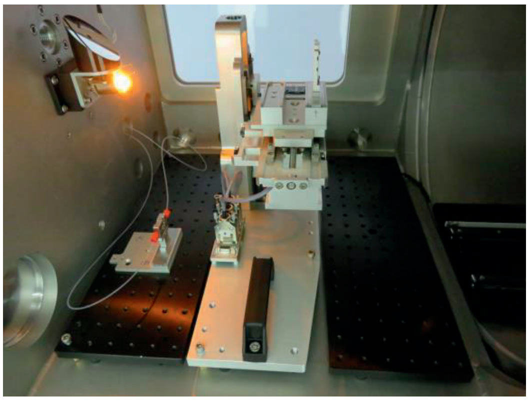

Figure 1: The sample chamber of the MAUS.

The MAUS has been custom engineered to serve as a miniaturized synchrotron, thus enabling standard material to be characterized to a high standard. Our work is needed to detail the fine characterization of reference nano-particles, not only on the nano-scale, but also coupled with external agents.

A second aim of this project is to verify a few proof-of-concept designs for the alignment of nano-particles. Where the alignment of nano-particles In-Situ is intended to further develop 3D printing technologies, and SAXS is an ideal choice to study the alignment of an oriented ensemble.

Measurements of biological particles and nanocharacterization methods

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Keywords: nanoparticles, bacteria, size

The unique physicochemical properties of nanoparticles (NPs) are the basis for their potential biomedical applications, whereby NPs interacting with biological particles provide a means for detecting, monitoring, or controlling biological functions via engineered non-biological (magnetic, electronic, optical, mechanical) properties of NPs. Characterization of samples that include nanoscale interactions between non-biological NPs and biological particles (Figure 1) requires considering both biological and physicochemical properties of the system [1].

Bacterial cells are an example of biological particles that are practically important in biomedical and food sectors. Having typical features with sizes in the sub-micron and nanoscale range also makes bacterial cells a convenient model for extension and validation of nanocharacterization methods from solid inorganic NPs to soft biological particles [1]. While some biological methods are available for characterizing bacterial cells in suspension, reliable measurements of their physicochemical properties remain challenging, even for basic parameters, such as size distribution and concentration (Figure 2).

As a model system for quantitative characterization of biological particles (Figures 1–2), we are using *Staphylococcus aureus* (*S. aureus*) bacteria, which offer the advantages of nearly spherical shape and of robust viability under a wide range of experimental conditions [1]. The *ca*. 1 µm diameter of live *S. aureus* cells also makes them representative of the challenges encountered in the characterization of bacterial cells. In microscopy, for example, the apparent size of individual *S. aureus* bacteria changes dramatically as they are prepared for measurements with increased spatial resolution: from confocal optical microscopy, to environmental scanning electron microscopy (SEM), to SEM in vacuum.

As an example of using *S. aureus* bacteria as a model system, we describe an analytical protocol [1] that starts from NP–cell interactions in solution and preserves their evidence in dried samples to be subsequently revealed in high-resolution SEM images (Figure 1). The validation of this protocol also provides insight into a quantitative comparison of a physicochemical parameter (colloidal stability) of NPs and bacteria based on combining systematic physicochemical (sedimentation), biological (colony forming units, CFU), and optical (optical density, OD) measurements.

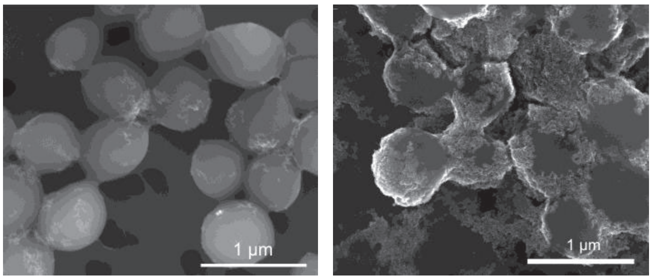

Figure 1: SEM images of the pellet (left) and supernatant (right) after differential sedimentation of an NP–cell mixture.

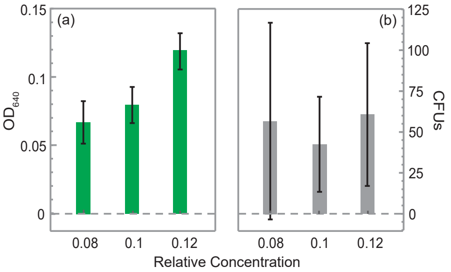

Figure 2: Cell concentration measurements for *S. aureus* dilution series with 20% increments.

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Chemical Analysis of Core-Shell Nanoparticles using ToF-SIMS and XPS

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Keywords: core-shell, XPS, ToF-SIMS

The analysis of nanomaterials is currently an important task - especially in case of risk assessment – as the properties of these material class are not well understood. The rather high surface area of these objects renders their interactions significantly different to their corresponding bulk. Thus, the surface's chemical composition must be investigated to get a better understanding and prediction of the nanomaterials' behavior. ToF-SIMS and XPS have proven to be powerful tools to determine the general chemical composition. The superior surface sensitivity of ToF-SIMS furthermore allows us to study mainly the utmost atomic layers and thus gives us an idea of the interactions involved.

Here, we present initial data on the analysis of Hyflon®-polystyrene core-shell nanoparticles which can be used as a model system due to the known preparation and a rather good chemical as well as physical separation of core and shell. Furthermore, principle component analysis (PCA) will be used to detect the influence of sample preparation and for a better separation of different samples. ToF-SIMS imaging is desired to be implemented for single particle detection as well.

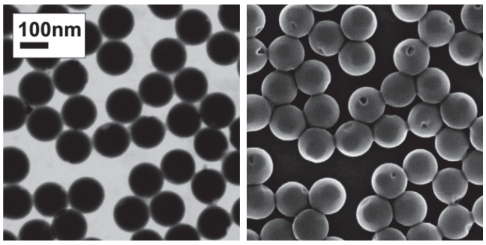

Figure 1: T-SEM and SEM images of Hyflon®-polystyrene core-shell nanoparticles.

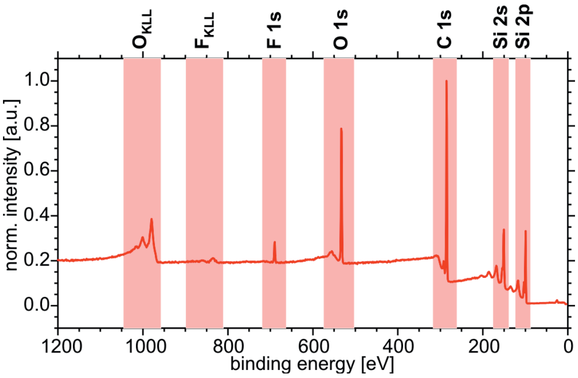

Figure 2: Survey XP spectrum of Hyflon®-polystyrene core-shell nanoparticles.

Synthesis and systematic characterization of core-muti-shell $NaYF_4$:Er^{3+}, Yb^{3+}@SiO_2@Au nanoparticles for the enhancement of fluorescence emission

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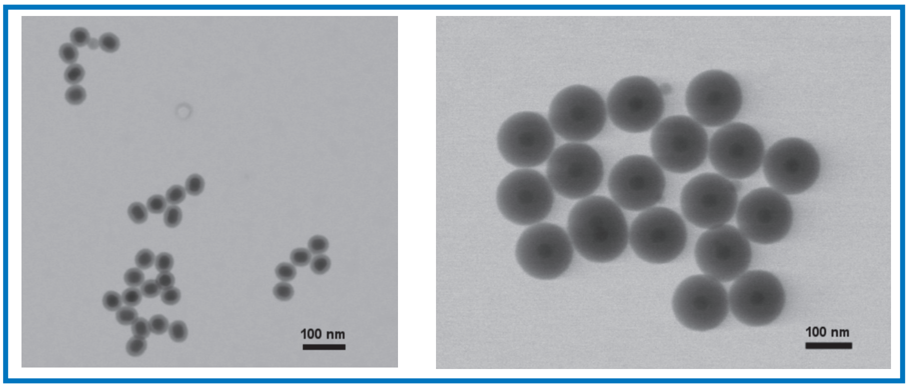
Keywords: upconversion nanoparticles, core-shell nanoparticles, plasmonic enhancement

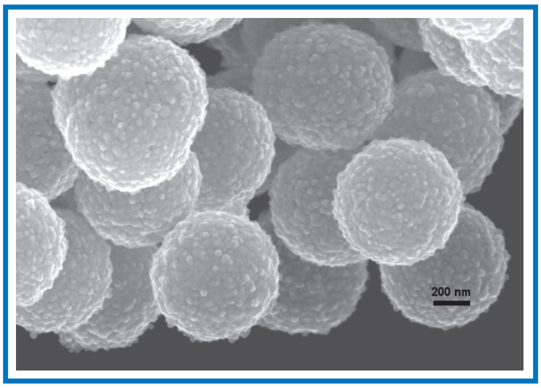
Lanthanide doped $NaYF_4$ nanoparticles (upconversion nanoparticles, UCNP) have been reported as fluorescence reporters in biological applications and *in vivo* imaging[1,2] due to their high photostability, specific and narrow emission bands, long luminescence lifetimes, and low toxicity. However, the use of UCNP in these applications usually requires specific functionalities and hence surface modification of the UCNP. An elegant and versatile approach to address this issue and implement different functionalities is the synthesis of core-shell particles with an UCNP core and shell that can be readily functionalized depending on the intended application (such as silica). Systematic synthetic and analytical approaches on coating the UCNPs with a silica shell/multi shells is highly important for the validation of the different applications of UCNP. Adding a silica shell on the UCNP often decreases the fluorescence intensity of the nanoparticles. The luminescence of these nanocrystals can be enhanced by plasmonic interactions.[3,4]

In this work, UCNP consisting of $NaYF_4$ as the host crystal, containing 18 % Yb^{3+} and 2 % Er^{3+} as sensitizer and activator, respectively, were used. The monodisperse luminescent UCNP with diameters of 18-30 nm were synthesized *via* a thermal decomposition process. Subsequently, silica nanoshells of adjustable thicknesses were grown onto these cores. However, growing such a shell using well-stablished standard approaches usually reduces the upconversion fluorescence intensity of the NP. [5], [6]

To enhance the typically low upconversion fluorescence, the UCNP@SiO_2 systems are further coated with a gold shell. Depending on the thickness of the gold and silica shells and the spectral position of the surface plasmon resonance peak, we expect a plasmonic enhancement of the UCNP luminescence upon illumination with NIR light. [3]



$NaYF_4$: 18 % Yb^{3+} and 2 % Er^{3+} coated with silica shells of different thicknesses (UCNP@SiO_2)



UCNP@SiO_2@Au

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Abstract: SSN-02-2018

Characterization of core-shell quantum dots of different particle architecture on the ensemble and single particle level

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Keywords: quantum dot, photoluminescence, single particle studies, quantum yield, size distribution

The optical properties of semiconductor quantum dots (QDs) and quantum rods (QRs) are controlled by constituent material, particle size, and surface chemistry, specifically the number of dangling bonds favoring nonradiative deactivation. This can lead to a distribution of photoluminescence (PL) quantum yields amongst the QD particles, i.e., mixtures of “bright” and “grey” or “dark” QDs. Particularly the number of absorbing, yet not emitting particles can have a significant effect on the PL quantum yield obtained in ensemble measurements, leading to its underestimation. The “dark fraction” is not assessable in common ensemble measurements; it can be probed only on a single particle level using a confocal laser scanning microscope coupled with an AFM.

We present here a study of core-shell CdSe QDs with different shells and surface chemistries on the ensemble and single particle level, with special focus on thick shell QDs that have been also assessed by SAXS and XPS regarding size, size distribution, and thickness of the passivation shell. Special emphasis was dedicated to correlate particle brightness, blinking, dark fraction, and decay kinetics of the single QDs with the ensemble PL quantum yields and particularly the PL decay kinetics. Moreover, the luminescence properties of single particle core-shell CdSe QDs were compared to those of hydrophilic ternary AgInS/ZnS QDs. The results of this study reveal e.g. a different blinking behavior of these materials. In the future, such single particle studies can help to identify new synthetic routes and surface modifications to homogeneous ensembles of colloidally and photochemically stable QDs with a PL quantum yield close to unity, which could present interesting candidates for new nanometer-sized reference materials.

Abstract: SSN-03-2018

Abstracts
Poster Presentations
May 15

SI traceable characterization of nanoscaled materials by X-ray spectrometry

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Keywords: physically traceable reference measurements, X-ray spectrometry, non-destructive quantitative analysis, chemical speciation

Reference-free X-ray spectrometry (XRS) [1] is a physically traceable element-sensitive method enabling the quantitative compositional and chemical analysis of nanoscaled samples such as nanolayers, nanoparticles or near-surface implantations. The traceability of the method is based on well-known synchrotron radiation and radiometrically calibrated instrumentation [1] as well as on the knowledge of the atomic fundamental parameters [2], and is independent of electric or magnetic properties of the sample.

This allows for a traceable analysis without any reference materials and offers a direct access towards atomic mass depositions with sub-monolayer sensitivity, allowing for the qualification of nanoscaled calibration samples. In addition to layer-type depositions, elemental depth distributions can be determined by grazing incidence XRS within depth scales ranging from a few to several hundred nanometers [3], whereas the combination with X-ray absorption spectroscopy provides depth-resolved chemical speciation capabilities [4,5].

These experimental capabilities and the instrumental developments [6] opened up alleys for various applications. For example, the molecular density of organic monolayers used for surface functionalization was determined, which allows for establishing a traceability chain for X-ray photoemission spectroscopy [7,8]. Artificial nanostructures as potential calibration samples for 3D analytical techniques as e.g. atom probe tomography [9] can be qualified by applying combined or hybrid methodologies using quantitative GIXRF and X-ray reflectometry. A procedure has been developed and validated for a depth-sensitive and non-destructive analysis of the chemical binding state of complex structures consisting of multiple thin layers [4,5]. Traceable vibration-reduced scanning X-ray fluorescence analysis with a scanning X-ray transmission microscopy option at the sub 100 nm ranges has been realized and may contribute to the analytical qualification of 3D nanostructures.

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Analytical and topographical Reference Material for the Nanoscale

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Keywords: Step height Standard, Element Analysis

Sustainable technological progress in the field of nanotechnology makes it necessary to make the knowledge and skills gained in basic research accessible to general application in industry. A key component of this task is the availability and the reliability of suitable reference materials, e. g. for resolution verification. It is only through them that complex methods can be applied to the general application of nanotechnology.

The availability of suitable nano-reference materials makes it necessary to develop new, innovative manufacturing methods, as a simple shrinking of dimensions leads to a technological impasse: The achievable uncertainties remain unacceptably high, the production and traceability of such standards is labour and cost-intensive and finally the application requires extensive know-how.

For this reason, two European collaborative research projects have been carried out in recent years with the aim of providing reference materials for topographic analysis in the nano- and sub-nanometer range (JRP CRYSTAL). The second project aimed to develop lateral resolution standards for near-surface elemental analysis (JRP SurfChem).

In the CRYSTAL project, the self-organization of silicon crystal surfaces was successfully used for the production of step height standards in the sub-nanometer range ($h = 0.314$ nm). For this step height, a standard uncertainty of about 25 pm was achieved; an improvement of more than one order of magnitude. The use of self-organization also shortens the traceability chain and thus cost of such standards. Furthermore, the application of such standards can be simplified.

For imaging surface chemical analysis as XPS, AES and SIMS at the sub µm scale, PTB has developed a novel method for the fabrication of standards for measuring and benchmarking the lateral resolution achievedwithin the framework of the EMRP JRP SurfChem.

The greatest challenge here is to minimize the topography of the Material A in Material B pattern on the standard's surface which may occur as steps at the corresponding material boundaries Having so-called "NoStep" standards, produced with this new, innovative technique, the vertical step at the boundaries of element patterns was reduced by a factor of five compared to competing standards manufactured by conventional lithographic techniques. But in the end, the decisive factor of this new standard is not only the reduction of the step between patterns of different material, but also the fact that the new "NoStep" standard displays an innovative pattern for a measurement of lateral resolution for element analysis. Highly requested by instrument manufacturers and users and it is well suited to underpin an implementation of the revised ISO Standard 18516 developed by TC 201 "Surface Chemical Analysis".

Directed self-assembly of Block Copolymers for Lateral Length Standards at the Nanoscale

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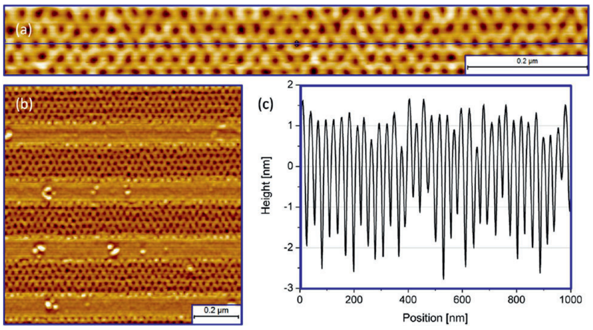
Keywords: Lateral length standard, Block Copolymer, Nanometrology

The miniaturization process that is currently involving the development of nanotechnology requires the parallel implementation of reliable characterization techniques and suitable standards for the dimensional characterization of structures having a minimum features size at sub-10 nm level. Industry and calibration laboratories need lateral length standards at the nanometric scale for the resolution certification of a variety of measuring instruments, such as Scanning Probe Microscopes (SPM), Optical Phase Shift Microscopes, and Scanning Electron Microscopes (SEM). The implementation of traceable measurement capabilities in surface analysis, material science and biophysics requires the introduction of new types of reference samples for length metrology at the nanoscale. In particular a new paradigm is necessary for the realisation of nanometric lateral length standards, possibly employing invariants of nature, like self-organized structures at the nanoscale. In this context one interesting solution in order to satisfy this lack is represented by the directed self-assembly (DSA) of Block Copolymers (BCP) inside gratings of multiple trenches. Recently we have demonstrated that the possibility to carefully tune the characteristic dimensions of the nano-domains confined inside periodic gratings, allows envisioning a strategy to use the DSA of BCP as a tool for the fabrication of lateral length standards. [1]



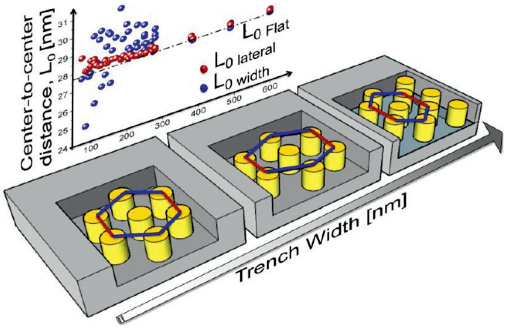


Figure 1: (a, b) AFM micrographs corresponding to the DBC pattern confined inside the periodic trenches. (c) Height profile through the blue line marked in (a).

As preliminary result a prototype transfer standard with cylindrical holes (Figure 1) was used to calibrate the linear correction factor $c(\Delta x')_{xx'}$ for a scan length of $\Delta x' = 1$ µm with a relative standard uncertainty of only 1.3%. A second-order nonlinearity correction factor was found to be significant and estimated to influence the AFM measurement up to a value of 0.4 % of the average period.

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Reference-free GIXRF-XRR based qualification of nanolayers and nanostructures as potential calibration samples

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Keywords: reference-free XRF, traceable quantification, calibration samples

In most cases, bulk-type or micro-scaled reference-materials do not provide optimal calibration schemes for analyzing nanomaterials as e.g. surface and interface contributions may differ from bulk, spatial inhomogeneities may exist at the nanoscale or the response of the analytical method may not be linear over the large dynamic range when going from bulk to the nanoscale. Thus, we have a situation where the availability of suited nanoscale reference materials is drastically lower than the current demand. The reference-free X-ray fluorescence (XRF) technique of PTB [1] can address this disparity, as it enables an SI trList of aceable quantitative characterization of nanomaterials without the need for reference or calibration standards. This opens a route for the XRF based qualification of calibration samples. In this work, we will summarize two different activities in this direction.
As a first example, we use physical vapor deposition techniques for the development of layer-like reference samples with mass depositions in the ng/mm^2 (??)-range and well below. Several types of reference samples were fabricated: multi-elemental layer (see Fig. 1) and extremely low areal mass density (sub-monolayer) samples for various applications in XRF and total-reflection XRF analysis. Those samples were quantitatively characterized using synchrotron radiation and employing PTB´s reference-free XRF approach.
In a second example, we work on the development of nanostructures as calibration samples. Several lithographic 2D and 3D nanostructures have been fabricated and characterized using the reference-free GIXRF methodology of PTB [2]. Here, an advanced and novel calculation scheme for the intensity distributions within the X-ray standing wave field (XSW) is required (see Fig. 2). In addition to the traceable quantification of elemental mass depositions, this allows for a determination of in-depth elemental distributions and the dimensional properties of the nanostructures [3].

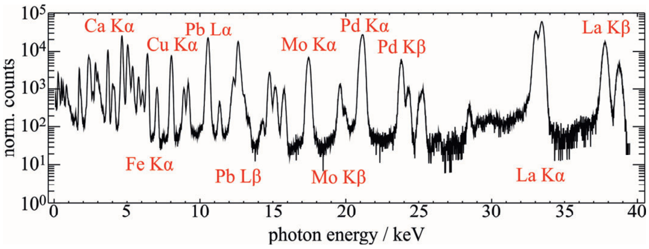

Figure 1: Sum spectrum for one of the potential calibration samples demonstrating the wide selection of useable fluorescence lines

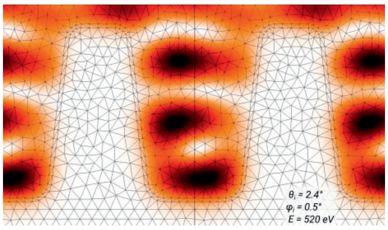

Figure 2: Calculated XSW intensity distribution for the S_3N_4 grating

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OECD Test Guideline on Particle Size and Particle Size Distribution of Manufactured Nanomaterials: Simultaneous Measurement of Length and Diameter of Fibers

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Keywords: fibers, particle size distribution, electron microscopy

The particle size distribution is considered the most relevant information for nanoscale property identification and material characterization. In order to measure particle size distributions, a plenitude of techniques are available. Most of them rely on the assumption of spherical particles or are not capable to measure the diameter and length of elongated particles simultaneously. Such techniques are therefore not applicable to nanoscale fibers, i.e., materials with a diameter below 1000 nm and a length-to-diameter ratio greater than 3.

Methods suited to report parameters relevant for fiber morphology characterization include imaging techniques of sufficient resolution, e.g., electron microscopic techniques (TEM, SEM). While the imaging approach allows determining the length and diameter directly and simultaneously, it has the drawback of being a relatively slow technique generally providing only small measurement statistics. Thus the following aspects need to be considered for reproducible and accurate characterization of fibers:

- Required number of imaged fibers for sufficient statistical significance
- Trade-off between diameter resolution and combined diameter and length measurement
- Dealing with aggregated, bundled and tangled fibers
- Need for automated image acquisition
- Need for software assisted fiber detection

Currently, an OECD test guideline that covers particle size distributions of nanofibers is missing. The development of such a harmonized and internationally-agreed test protocol for the determination of particle size and size distribution for nanoscale fibers is part of the German proposal for developing an OECD test guideline on particle size and size distribution for nanomaterials.

The present poster presents the project's objectives together with first results.

Abstract: DNS-05-2018

PillarHall LHAR structure for thin film conformality measurements

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Keywords: Thin film conformality, Atomic layer deposition (ALD), lateral high-aspect-ratio (LHAR) structure

The downscaling of future semiconductor devices with increasing 3D character leads to increasing demand of highly conformal thin films. Atomic layer deposition (ALD), based on the use of repeated, self-terminating reactions of typically at least two compatible reactants on a solid substrate, is often the only technique that can meet the conformality specifications. Conformal films made by ALD are also needed in other fields with intrinsic three-dimensionality requirements, such as microelectromechanical systems (MEMS), energy applications, and high-surface-area catalysts.

This work continues on earlier work on conformality analysis [1–5]. All-silicon microscopic lateral high-aspect-ratio (LHAR) structure prototypes have been designed and fabricated with an improved design (PillarHall Prototype 3B). The LHAR structures consist of a lateral gap of typically 500 nm (in some cases, 100 to 2000 nm) in height under a polysilicon silicon membrane, supported by silicon pillars. The gap length varies from 1 to 5000 µm, giving aspect ratios (length vs height) for the typical ~500 nm gap of 2:1 to 10 000:1. In some PillarHall chips, unique distance indicator lines have been etched directly onto the silicon membrane to provide an accurate internal length scale. Each distance indicator (every 100 µm) is unique, providing a means to detect length even when the LHAR structure opening is not visible. Applications are predicted in both non-destructive top-view microscopy analysis and cross-sectional scanning electron microscopy (SEM).

Chips are available at VTT. We are interested in research cooperation.

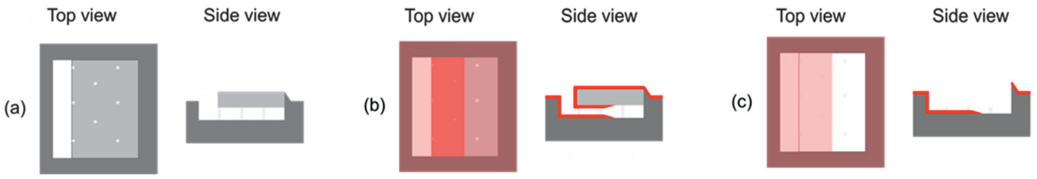

Figure 1: Schematic representation (not in scale): (a) uncoated structure, (b) structure coated with a film, and (c) coated structure, with top membrane removed.

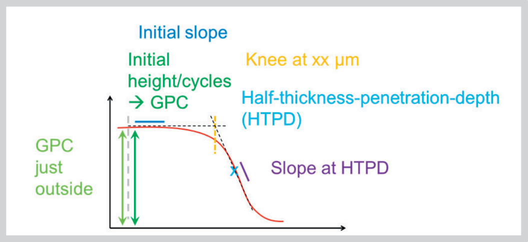

Figure 2: Experimental parameters to be recorded from thickness line scans

Acknowledgements: This work has been partly funded by the Tekes PillarHall project and the Finnish Centre of Excellence on Atomic Layer Deposition.

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Abstract: DNS-06-2018

Traceable 3D nanometrology

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Keywords: 3D measurements, tip characterization, hybrid metrology

Traceability to the SI units is needed in all quantitative measurements. Currently there is insufficient traceability to the SI metre for true 3D nanomeasurements, because the existing level of uncertainty in measurements (5 nm) does not meet the requirements of industry and scientific research. 3D metrology differs from one dimensional (1D) height and pitch metrology, where the impact of tip geometry and the tip sample interaction at the top/bottom planes are self-compensating. Instead, measurements of 3D structures are bi-directional, where the effect of tip geometry and tip sample interactions at the left and right sidewalls are different.

In EMPIR project 3DNano [1], we develop new traceability routes for 3D nanomeasurements. Both bottom-up and top-down methods are being developed. The bottom-up approach is based on the cross-section of TEM structures with atomic resolution. In such a way, the critical dimension (CD) can be directly linked to the atomic spacing in the crystal lattice, which has been measured using a combined optical interferometer and x-ray interferometry. In the top-down approach, optimisation of tools and measurement procedures for true 3D measurands is ongoing. The noise level, scanning speed, and range of the metrological AFMs developed at European NMIs are being improved.

In the project new reference materials were designed which will be manufactured and characterized soon. New tip characteriser standards suitable for hybrid metrology and nanoparticle samples are developed and/or tested.

Structure/tip deformation in AFM due to the measurement force and humidity will be theoretically and experimentally investigated.

Industrial solutions for hybrid metrology are being studied and methods and software for data fusion from different tools (TEM, AFM, SEM and optical scatterometer) will be established. So far a software module for Gwyddion [2] for data fusion has been developed.

The goal of the project is to establish traceability for 3D nanomeasurements in Europe.

This project has received funding from the EMPIR programme co-financed by the Participating States and from the European Union’s Horizon 2020 research and innovation programme

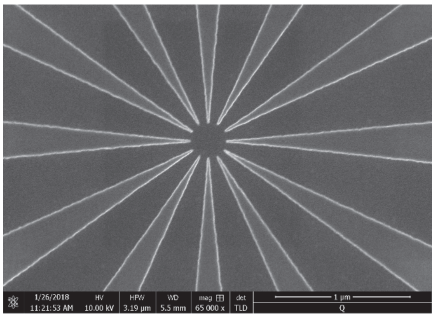

Figure 1: Siemens star structure for tip characterization

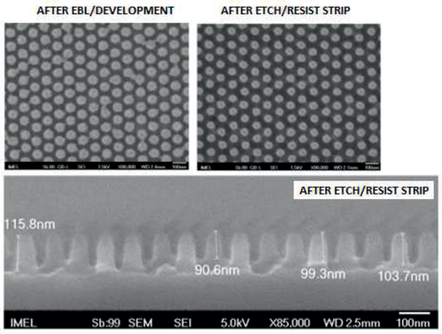

Figure 2: hexagonal structure optimized for both scatterometry and AFM/SEM methods.

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The need of a reference for APT-AFM tip reconstruction

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Keywords: APT-AFM, tip reconstruction, tip-sample convolution

Current industry needs for nanoscale topographic characterisation are pushing atomic force microscopy (AFM) technology to its limits, demanding high resolution and artifact free imaging. [1] The need for highly reliable 3D data is dual. First and foremost, the semiconductor industry is putting high demands on the imaging capabilities of AFM by manufacturing increasingly complex 3D nanodevices to characterise. [1] Secondly, novel concepts such as atom probe tomography-AFM (APT-AFM), in which a needle shaped APT specimen is measured by the AFM probe, create a new series of challenges. [2] These arise from the fact that the apex and aspect ratio of an APT specimen are comparable to those of the AFM probe itself. As the APT-AFM approach is a very recent development, dedicated reference structures are not yet existing.

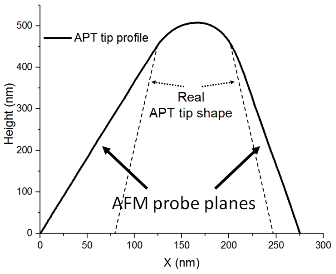

Figure 1: Convolution artifacts in APT-AFM result in an apparent lower aspect ratio APT tip.

It is of great importance to characterise the AFM probe before/after APT-AFM measurements, as its properties are inherently linked to the tip-sample interaction. In APT-AFM, the AFM probe interacts with the APT specimen apex as well as with a large part of the tip side walls due to the very high aspect ratio of the atom probe specimen. Knowledge on the shape of the AFM probe is therefore crucial to understand possible convolution artefacts (Fig. 1) and probe damage resulting from this challenging tip-on-tip configuration and to identify the convoluted regions.

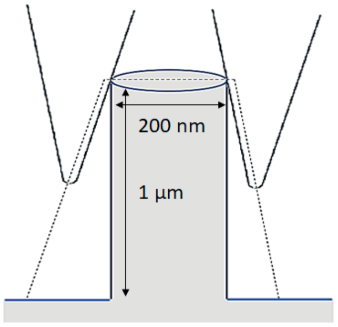

Figure 2: The proposed reference structure. The resulting image (dotted line) allows to reconstruct back the shape of the AFM probe.

A promising approach to assess the 3D geometry of the AFM probe is to measure a dedicated tip charactiser. In this measurement the detrimental effect of tip-sample convolution is turned to the users advantage (Fig. 2) as it allows to extract the shape of the AFM probe using algorithms such as the blind tip reconstruction method. [3] Ideal geometrical properties of such a characteriser have been suggested in literature but access to those samples remains scarce.[4] In addition, the APT-AFM concept poses more stringent requirements regarding the height of the characteriser sample.

The ideal characteriser dimensions are proposed to be the following. A cylindrical structure (radius≈ 100 nm) out of a hard material, e.g. silicon, with straight sidewalls (cone angle ≈ 0°) allowing reconstruction of high-aspect-ratio probes. Furthermore, the radius of curvature at the top edges should be as small as possible as the error on the tip reconstruction increases with increasing curvature radius. [4] Also, the height should correspond to the region of interest of the APT tip as scanned by the AFM which should be ≈ 1 µm. Finally, a pitch of around 3 µm should ensure no interaction with neighboring pillars.

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Abstract: DNS-08-2018

The Research of Nanometer Geometry Standard Reference Materials in NIM

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Keywords: Standard Reference Materials, nanometer geometry

Abstract: The research of micro/nanometer geometry Standard Reference Materials (SRM) in National Institute of Metrology will be introduced, which is the guarantee of measurement accuracy and uniformity, trace the measurement results to the International System of Units (SI) at the same time. Currently several micro/nanometer geometric dimension standard devices and series of Standard Reference Materials have been developed, which preliminarily established the national nanometer standard traceability systems.

Millimeter range metrological nano-geometric standard device developed by NIM is introduced, as shown in Figure 1, which is used to calibrate the nanometer geometrical SRM, and which is equipped with homodyne interferometers for measuring the relative position between the sample and the probe, with the measurement results directly traced to the definition of the “Meter”. The standard device has two kinds of measurement range 50 mm × 50 mm× 2 mm and 100μm × 100μm × 3μm, with measurement uncertainties ≤20nm and ≤2nm respectively, which can calibrate horizontal and vertical direction structures, such as step height, one dimension and two dimensions grids, and nanometer roughness.

One dimension grating pitch of 400 nm fabricated by electron beam lithography is calibrated with this instrument and the gravity center method is applied to evaluate the pitch, as shown in Figure 2. To establish the quality assurance system in micro and nano scale, one dimension and two dimensions grids SRMs, ranging from 70nm to 10000nm，are widely used in the calibration of magnification factors of scanning electron microscopy (SEM). A series of step height (50nm-1000nm) standard samples are also developed to calibrate AFM in vertical direction, as shown in Figure 3. Step height samples under 25nm are fabricated with atomic layer deposition (ALD) technique and lithography to meet the demand of small size step, as shown in Figure 4.

Figure 1: Nano-geometric standard device

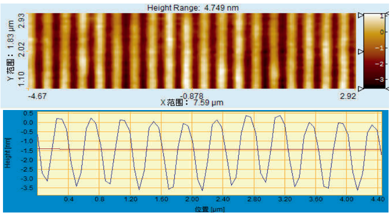

Figure 2: 400nm pitch grating CRM

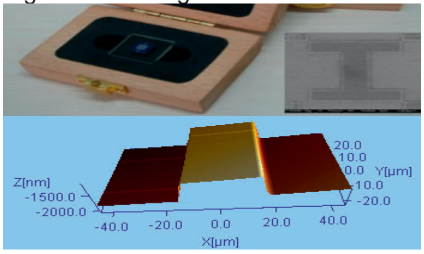

Figure 3: Nano step height standards

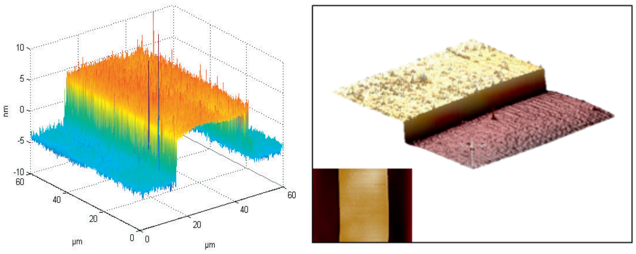

Figure 4: 8nm step height standard.

Abstract: DNS-09-2018

Nanoscale reference artefacts for contact resonance spectroscopy and force-distance curve techniques

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Keywords: thin-layer PEDOT, silicon pillar array, Young's modulus

Tactile probing using micromechanical piezoresistive silicon cantilevers has been demonstrated to be suitable for high-speed measurements on industrially manufactured parts [1]. In addition to surface quality, mechanical properties of thin layers can be measured by operating such probes in a contact resonance (CR) mode [2-5]. However, much effort is required to determine quantitative values, e. g. Young's modulus, from the measured contact resonance frequencies. Alternatively, reference samples can be used. These have to be calibrated, e. g. using force-distance (FD) curves measured with an atomic force microscope (AFM) [6]. Thin films of poly(3,4-ethylenedioxythiophene), i.e. PEDOT are selected, which is an organic semiconductor with exceptional environmental stability and electrical conductivity for organic electronics, biosensors, etc. [7]. Furthermore, three-dimensional, silicon micro-pillar arrays are investigated.

PEDOT films are deposited on silicon substrates using oxidative chemical vapor deposition (oCVD). Their thickness is subsequently measured using a profilometer. Different oCVD parameters yield thicknesses in the range of 20 nm up to 300 nm. Silicon micro-pillar arrays are fabricated from a bulk (100)-silicon wafer using nanoimprint lithography (NIL) and cryogenic deep reactive ion etching (cryo DRIE) [8]. The resulting pillars are designed to be 1.6 µm in diameter, 4 µm in height, and to have a 4 µm pitch. For CR measurements commercial 5-mm-long, 200-µm-wide and 50-µm-thick piezoresistive microprobes (CAN-50-2-5) are employed [9] mounted on multilayer piezo actuators for dynamic excitation [10]. Measurements using the second out-of-plane vibration mode show the best sensitivity and reproducibility. FD measurements are performed using the common optical lever system of a Cypher AFM.

Resonance frequencies f_2 obtained on PEDOT films of different thickness are shown in Fig. 1. They reveal a linear decrease of f_2 with increasing film thickness with a correlation coefficient of -0.986. The different frequency errors of the data points can be ascribed to different non-uniformities of the layer thickness of the investigated samples. A second CAN-50-2-5 microprobe is used for CR measurements on the silicon pillar array, yielding slightly lower values of f_2. Figure 2 shows (in blue) the measured resonance frequency in dependence of the position on the pillar array. The simultaneously determined height plot (in red) shows an explicit correlation with f_2. Distinctly lower values of f_2 are found when the probe is in contact with the pillars top surface, which is due to higher compressibility of the pillars compared to the bulk. We conclude the investigated PEDOT and 3D pillar samples may be suitable artefacts for calibrating the thin-layer scanning CR method.

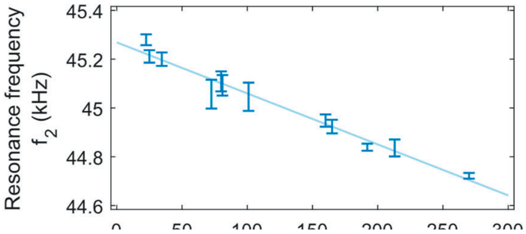

Figure 2: CR measured on PEDOT films

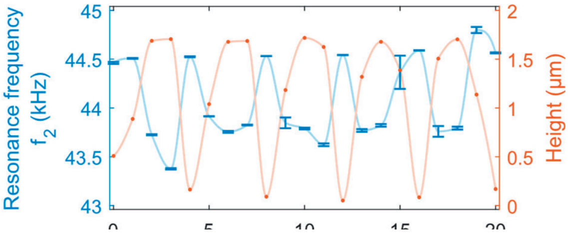

Figure 1: CR measured on silicon pillar array

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Development of a Method for Measuring the Rigidity of Nanofibers

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Keywords: Nanofibers, Rigidity, Resonance

Over the past decades, the growing industry working for lightweight constructions has demanded materials exhibiting improved properties such as higher mechanical modulus and strength, better chemical resistance and lower costs of production.

It is known that especially nanofibers tend to enhance the mechanical, thermal and electrical properties of polymers. Therefore, some types of nanofibers are already being used embedded in polymeric matrix of sports equipment, aircraft parts and automobile components. However, nanofibers are a relatively new material category, the consequences of which for mankind are not yet fully known. Nanofibers detached from polymeric materials can be released into the environment during, e.g., processing and aging [1]. The risks in dealing with such nanofibers are generally unknown, even if further studies of health aspects are being carried out [2,3]. Problems always arise when airborne fibers reach the alveolar region of the lung where their deposition is countered by macrophage cells. If the fibers can penetrate the cells upon digestion, it will lead for example to a frustrated phagocytosis. The mechanical rigidity of nanofibers is a key factor in assessing the risks for cell penetration. Common methods describe the geometry of nanofibers. These methods are necessary, but not sufficient to describe the mechanical properties, as for during manufacture and aging these properties may change; the modulus and strength values may differ from those of the original fibers.

Different experimental techniques for nanomechanical testing have been studied using atomic force microscope, scanning electron microscope and transmission electron microscope, showing promising results [4].

This project aims to evaluate the bending stiffness of CNTs with an experimental setup inside a Dynamic Scanning Electron Microscope [5]. Based on the Euler-Bernoulli beam theory, the Young's modulus of CNTs can be obtained using the resonance frequency of mechanically excited nanofibers, its dimensions and density. The resonance frequency is acquired by extracting the signals of the secondary electrons when the nanofibers show significant vibration movement, also possible to be seen on the microscope images. The electronic signals of the secondary electrons are filtered and treated by a lock-in amplifier, so the corresponding amplitude and phase values can be evaluated. This method could be applied to any nanofiber that is shaped similar to a symmetric beam.

Example of a TiO_2 fiber vibration at resonance frequency in the Dynamic Scanning Electron Microscope at BAM.

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Crystalline Si and GaN micro- and nanopillars as reference artefacts for nanomechanical characterization

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Keywords: micropillar, compressibility, nanoindentation

Micro- and nanopillars are increasingly used for energy harvesting, LED and tactile force applications. Their mechanical properties are of great interested, since such high aspect ratio structures might enable additional elastic relaxation of strain and hence possibly increasing the acceptable lattice mismatch in heterostructures. Whereas the mechanical properties of bulk micro materials can be easily measured using nanoindentation, transferring this technique to high aspect ratio micropillars is complex. E.g., a depth dependence of the modulus of nanopillars was observed and explained by Li et al. considering the compressibility of the high aspect ratio pillars [1]. This mainly depends on the pillar diameter and height and can be theoretically taken into account. Both, height and diameter can be measured by scanning electron microscopy or optical microscopy. Thus, micro- and nanopillars made of different materials can serve as indentation modulus reference, provided nanoindentation instruments carefully calibrated for force, indentation depth and contact area are used for the measurements. Nanoindentation measurements at different crystalline silicon [2] and GaN [3] micropillars with aspect ratios of >1.5 have been carried out using a Hysitron Triboindenter TI-950 with a Berkovich indenter tip. To locate the pillar selected for indentation each pillar was carefully scanned by the tip using a scan area of 2 µm × 2 µm. To validate the correction method, two further measurements were made for each pillar sample. One comparison measurement was taken near the pillar at the base and a further measurement was taken on a second bulk sample, made of the same material using the same processing. A matrix of indentations with different depths have been made over an area of 20 µm × 20 µm. The main result is that the indentation moduli of bulk material and micropillars are in a good agreement. However, the roughness of the samples influenced the nanoindentation measurements and needs to be sufficiently low. The roughness contribution will be investigated in more detail in the future.

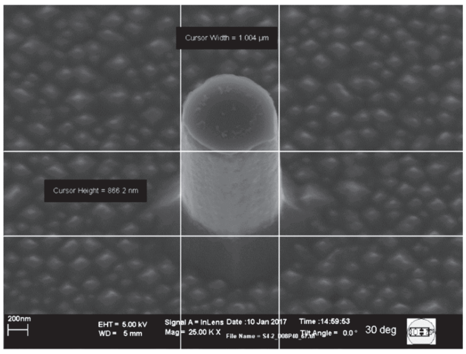

Figure 1: SEM image of a Si <100> micropillar (Ø = 1.004 µm, height = 1.732 µm)

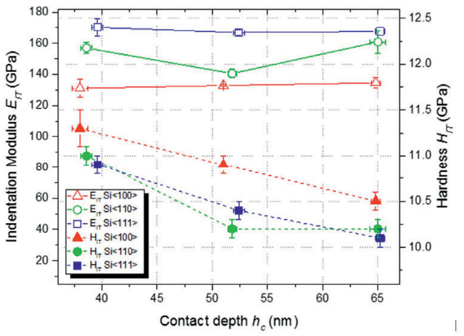

Figure 2: Corrected indentation modulus and hardness of different crystalline Si micropillar samples

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Abstract: MPN-03-2018

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Keywords: SERS-active substrate, homogeneity, silicon nanowires

Surface-enhanced Raman spectroscopy is a powerful vibrational spectroscopy technique presenting strong surface sensitivity. The fabrication of nanostructured substrates is tailored to control the location and distribution of hot spots, localized regions of extremely large enhancement, to fulfill important requirements concerning the chemical detection level as well as the uniformity of the substrate. Recently, the flexibility of metal-coated nanowires used as SERS substrates has been highlighted as an important feature, because it allows the nanowires to bend and trap the probe molecule in the hot spots generated at the tip-to-tip site producing a strong enhancement[1-3].

An effective and unconventional approach to the fabrication of flexible silicon nanowires over large area is discussed; it is constituted of a combination of nanospheres lithography (NSL) and metal-assisted chemical etching (MACE). NSL is a lithographic tool based on the self-assembly of spherical colloids in a hexagonally close-packed monolayer. Matching NSL to MACE, an anisotropic wet etching technique, leads to the realization of nanowires with tunable aspect ratios and flexibility as well as high degree of order and controlled geometrical parameters.

The matrix of flexible gold-coated silicon nanowires was successfully tested as SERS substrate and the influence of the long-range order of the nanostructures on the enhancement was investigated. The platform showed remarkable results in terms of enhancement and homogeneity and the optimization of the fabrication technique is promising to broaden the field of applicability of this substrate.

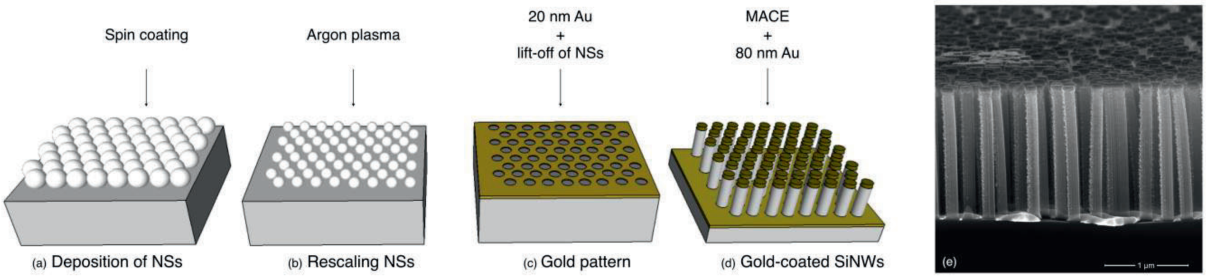

Figure 1: (a)-(d) Fabrication scheme of the SERS-active substrate. (e) Gold-coated silicon nanowires cross-section SEM image.

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Synthesis and optical characterization of ternary AIS/ZnS quantum dots

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Keywords: quantum dots, microwave synthesis, photoluminescence

Ternary semiconductors quantum dots (t-QD) are Cd-free nanocrystals made from I-III-VI group elements like silver or copper, indium and sulfide yelding CIS ($CuInS_2$) or AIS ($AgInS_2$) QDs. They are interesting alternatives to Cd-based QDs for applications as solar concentrators or optically active material for solar cells, light emitting diodes (LED) or reporters for diagnostic assays.

To avoid ligand exchange procedures for high quality QDs, which are commonly synthesized in high boiling apolar solvents with apolar surface ligands, which are required for bioanalytical application, AIS QDs are synthesized in aqueous solution by a microwave-assisted procedure. The surface of these QDs is passivated by a ZnS shell to enhance photoluminescence quantum yield (PL QY) and prevent material decomposition and oxidation. The resulting AIS/ZnS QDs exhibit broad PL spectra in the visible and near infrared, tunable by size and chemical composition.

Here we show the simple synthetic procedure and a spectroscopic study of different AIS QDs evaluating their PL properties and stability, PL QY, and PL decay kinetics. The analyzed sample showed long lifetimes, relatively high QY (50%) and good stability.

The simple aqueous synthesis together with the tunable emission color, the high PL QY, the high absorption coefficients and the long luminescence lifetimes make these t-QDs promising Cd-free materials for many different applications in the material and life sciences.

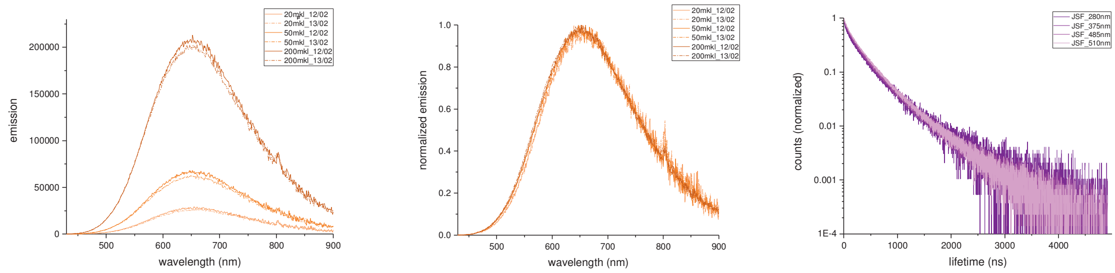

Figure: a) and b) PL stability of AIS/ZnS with different concentration, c) PL decay kinetics of AIS/ZnS QDs; All samples are studied in water

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Abstract: OPN-02-2018

Sandwich assay of Magnetic and Gold Nanoparticles for Purification and Quantification of Tau-Protein using ID-SERS

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Keywords: ID-SERS, gold nanoparticles, magnetic nanoparticles

Metallic and magnetic nanoparticles provide versatile sensing platforms for biological and biomedical applications such as the detection and quantification of large biomolecules serving as diagnostic markers (biomarkers) in human medicine. Here, a sandwich immuno-assay for surface-enhanced Raman scattering (SERS) based determination of tau-protein, one of the established biomarkers for Alzheimer's disease, will be established. Up to now the Alzheimer's disease is only reliable diagnosed post mortem. The developed assay will be used to separate the target analyte from the matrix with the help of magnetic nanoparticles as well as for the quantification of the protein by utilisation of a sensitive SERS active marker coupled to gold nanoparticles. This approach makes this method suitable for very low sample volumes and analyte concentrations typically found in cerebrospinal fluid (CSF) samples while highest accuracy is achieved through the isotope dilution approach.

Gold nanoparticles were produced including a nucleation step and a growth step[1]. The size and the Ostwald ripening of the particles were monitored over time to ensure repeatable Raman intensities. The magnetic nanoparticles were produced using a top-down process. Both, the build-up of the gold nanoparticle site as well as the magnetic nanoparticle side were carefully characterized using several techniques like UV/Vis, XRD, TEM, DLS, TGA, SAXS and zeta potential measurements.

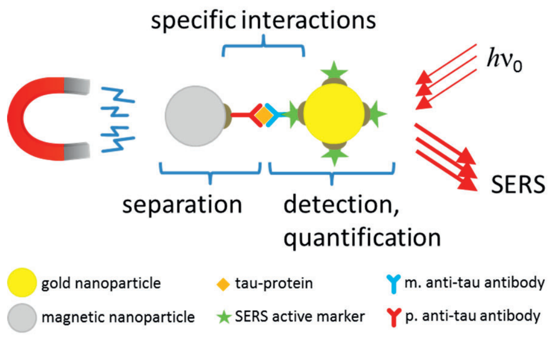

Figure 1: Schematic depiction of the sandwich assay in its natural isotopic composition

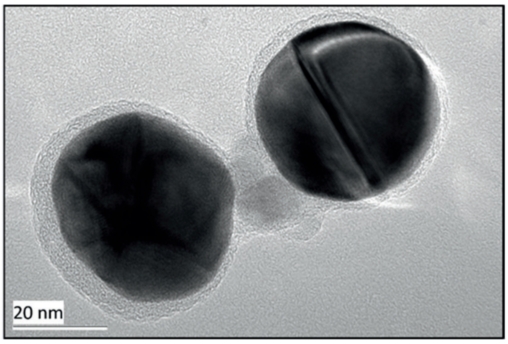

Figure 2: TEM image of the sandwich assay (the black spheres are the gold nanoparticles)

Acknowledgement: This project has received funding from the EMPIR programme co-financed by the Participating States and from the European Union's Horizon 2020 research and innovation programme.

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Raman spectroscopy for identification and quantification of different polymorphic forms of titanium dioxide in nanoparticle mixtures

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Keywords: TiO_2 nanoparticles mixture, Raman spectroscopy, method validation

Titanium dioxide (TiO_2) is one of the most commonly used semiconductor materials. It is employed in a wide range of applications including chemical, medical, biological, environmental, and solar energy fields. TiO_2 occurs in nature as three different polymorphs, which provide different chemical-physical properties. Polymorphs are known as rutile (tetragonal), anatase (tetragonal) and brookite (orthorhombic). These phases exhibit different properties and consequently different photocatalytic performances. In addition, mixed-phase TiO_2 have been demonstrated to have enhanced photocatalytic activity relative to pure-phase TiO_2.Titanium dioxide is the most important white pigment used in the polymer industry. The rutile pigments are preferred over anatase pigments, because they scatter light more efficiently, are more stable, and are less likely to catalyze photodegradation. The present work is focused on the development of an analytical method based on Raman spectroscopy that in combination of chemometric analysis is able to classify and quantify different phases in TiO_2 binary mixtures by Raman spectroscopy. Raman spectroscopy analysis represents a rapid and simple method to distinguish different crystallographic structures of the same compound. Due to the different spatial organization of atoms into the elementary cell of the crystal, anatase, rutile and brookite, are characterized by different crystal vibrational modes which provide significantly different Raman spectra (Figure 1). Raman spectroscopy is able to distinguish different TiO_2 polymorphs, and also it allows the components' fraction to be quantified on the basis of mutual intensities of characteristic signals for each polymorph. A Discriminant Analysis (DA) was performed for the identification of the three pure phases (anatase, rutile and brookite) and the related binary combinations (anatase-brookite, anatase-rutile and rutile-brookite) (Figure 2). Besides, Partial Least Squares (PLS) was used in order to quantify the amount of the polymorphs in the mixtures.

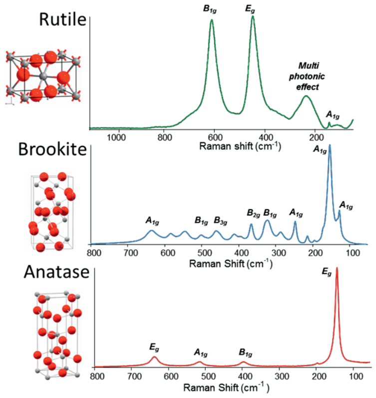

Figure 1 TiO2 allotropes fingerprint

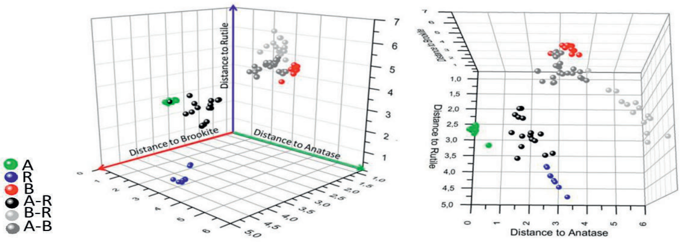

Figure 2 Cooman's Plot for the DA classification model for the distinction of pure allotropes and binary mixtures.

Optical characterization of Cd-free AgInS/ZnS quantum dots

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Keywords: defect photoluminescence, photoluminescence quantum yield, nanocrystals

A quantum dot (QD) is a semiconductor nanoparticle with a size in the quantum confinement region that shows unique size-tunable optical properties including a large absorption cross section and photoluminescence (PL) with a high quantum yield (PL QY). Novel Cd-free ternary quantum dots (t-QDs) are nanocrystals made of I-II-VI groups semiconductors like Cu-In-S (CIS) or Ag-In-S (AIS) with e.g. ZnS passivation shells.[1] Such t-QDs have a complicated composition and crystal lattice and thus, relatively high defect densities. Therefore, AIS QDs usually have broad defect photoluminescence with fwhm > 300 eV with large Stocks shift of > 500 eV and long > 100 ns PL lifetimes.[2] Also, due to the high density of defect states, the absorption bands of ternary QDs have show tailing at energies lower than the actual band gap (so-called Urbach tail). This renders the accurate determination of the excitation energy/wavelength dependence of the PL properties in the whole spectral range of t-QD absorption relevant since excitation to the band gap states or to the defect-related “tail” states can differently affect the t-QD PL properties.

We present here a study of the optical properties of hydrophilic AIS/ZnS QD samples of different size, chemical composition, and capping ligands like mercaptopropionic acid (MPA) and D-penicillamine (D-Pen). Prior the measurements we traceably calibrated our fluorescence spectrometer with physical transfer standards. Then, the PL QYs of AIS/ZnS QD were measured as a function of the excitation energy varying from 2.1 to 3.5 eV relatively to Rhodamine 6G in ethanol as quantum yield standard (PL QY of 95%).

Our measurements reveal that these AIS/ZnS QDs do not exhibit an excitation energy dependence of PL QY in ensembles and show a linear red shift and a narrowing of the defect PL band with excitation below the band gap regardless of size, elemental composition, and chemical nature of the surface capping ligands. The observed linear PL shift and PL narrowing is attributed to an intermix of size and electronic states´ selection and allows to determine the effective band gap of AgInS/ZnS quantum dots. Our results underline the potential of PL spectroscopy for the study of the electronic energy structure and determination of effective bandgap of I-II-VI group QDs.

List of references:

1. Girma, W. M.; Fahmi, M. Z.; Permadi, A.; Abate, M. A.; Chang, J.-Y., Synthetic strategies and biomedical applications of I-III-VI ternary quantum dots. *J Mater Chem B* **2017,** *5* (31), 6193-6216.
2. Raevskaya, A.; Lesnyak, V.; Haubold, D.; Dzhagan, V.; Stroyuk, O.; Gaponik, N.; Zahn, D. R. T.; Eychmüller, A., A Fine Size Selection of Brightly Luminescent Water-Soluble Ag–In–S and Ag–In–S/ZnS Quantum Dots. *J. Phys. Chem. C* **2017,** *121* (16), 9032-9042.

Simple and Validated Methods for Quantifying Functional Groups, Ligands, and Biomolecules on Nanomaterials

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Nanoparticles, Surface chemistry, Multimodal reporters, Surface functionalization

Many applications of nanomaterials in the life sciences require the controlled functionalization of these materials with ligands like polyethylene glycol (PEG) and/or biomolecules such as peptides, proteins, and DNA. [1,2] This enables to tune their hydrophilicity and biocompatibility, minimize unspecific interactions, improve biofunctionalization efficiencies, and enhance blood circulation times. Moreover, it is the ultimate prerequisite for their use as reporters in assays or the design of targeted optial probes for bioimaging. At the core of these functionalization strategies are reliable and validated methods for surface group and ligand quantification that can be preferably performed with routine laboratory instrumentation, require only small amounts of substances, and are suitable for many different types of nanomaterials. [1,3]

We present here versatile and simple concepts for the quantification of common functional groups, ligands, and biomolecules on different types of organic and inorganic nano-materials, using different types of optical reporters and method validation with the aid of multimodal reporters, method comparisons, and mass balances. [3,4]

List of references:

1. K. E. Sapsford et al., Anal. Chem. 2011, 83, 4453-4488.
2. J. Hühn et al., Chem. Mater. 2017, 29, 399-461.
3. A. Hennig et al., J. Am. Chem. Soc. 2012, 134, 8268-8276.
4. M. Moser et al., Anal. Chem. 2016, 88, 8624-8631.

Abstract: OPN-06-2018

Functional group analysis on nanoparticles with simple optical assays

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Keywords: cleavable probes, conventional dyes, nanoparticles, optical assays

Polymer nanoparticles (NPs) are of increasing importance for a wide range of applications in the material and life sciences. This includes their application as carriers for e.g., analyte-responsive ligands for DNA sequencing platforms, drugs as well as dye molecules for use as multichromophoric reporters for signal enhancement in optical assays or the fabrication of nanosensors and targeted probes in bioimaging studies. All these applications require surface functionalization of the particles with e.g., ligands, sensor dyes, or analyte recognition moieties like biomolecules, and subsequently, the knowledge of the chemical nature and total number of surface groups as well as the number of groups accessible for coupling reactions. Particularly attractive for the latter are optically active reporters together with sensitive and fast optical assays, which can be read out with simple, inexpensive instrumentation. [1]

We assessed a variety of conventional and newly developed colorimetric and fluorometric labels for optical surface group analysis, utilizing e.g., changes in intensity and/or color for signal generation. Moreover, novel cleavable and multimodal reporters were developed which consist of a reactive group, a cleavable linker, and an optically active moiety, chosen to contain also heteroatoms for straightforward method validation by elemental analysis, ICP-OES, ICP-MS or NMR. In contrast to conventional labels measured bound at the particle surface, which can favor signal distortions by scattering and encoding dyes, cleavable reporters can be detected colorimetrically or fluorometrically both attached at the particle surface and after quantitative cleavage of the linker in the transparent supernatant after particle removal e.g., by centrifugation. Here, we present first results obtained for the optical quantification of carboxylic and amino groups on a series of self-made polystyrene NPs with different types of labels and compare their potential and drawbacks for surface group analysis. [2]

List of references:

1. K. E. Sapsford et al., Anal. Chem. 2011, 83, 4453.
2. M. Moser, N. Nirmalananthan et al., *Anal. Chem.* 2018, accepted (DOI: 10.1021/acs.analchem.8b00666)

The Future of Scattering for Nanomaterial Characterisation

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Corresponding author: brian.pauw@bam.de

Keywords: Future, Scattering, Characterization

The current range of nano-metrological applications of SAS indicate that it ought to be flexible enough to adapt to broadening future applications, and further solidify its status as a reliable and useful characterisation technique. A question is thus raised that lies central to the future heading: "How good do we need to be, and where can we improve?".

The metrological approach to answering this is defined by numerical quality: estimating and improving the data accuracy and consistency, and determining its effects on the resultant morphological parameters. The numerical quality is dictated by both instrumentation and data correction methods. While we are now enjoying the luxury of bright sources, scatterless collimation, and largely problem-free detection solutions, some questions on (the necessity of) data corrections and reproducibility aspects remain to be answered [1].

The personal approach to the central question is defined by our communication: where and how (much) do we need to improve our outreach efforts? Fortunately, the "lookingatnothing.com" weblog has a valuable side-effect: readers of the weblog have been in contact to discuss unclear aspects of small-angle scattering, which serve as powerful indicators (or reminders) of underdeveloped SAS aspects in need of reinforcement or reiteration. Their questions highlight our need to develop and concatenate introductory methods and materials, data correction software and methods, and data analysis guidelines.

Lastly, some words of warning may be appropriate, which may prevent alienation of the casual user. Such risks lie, for example, in the development of "black box" SAXS instruments that distance the user from the straightforward technique and its not-so-straightforward caveats, improper application or over-interpretation of the result effecting dissonance in results, or the lack of interoperable data formats and standard analysis methodologies.

This poster will thus provide a broad overview of the methods for the current state and future aspects of the technique. It will highlight areas of interest and encourage good practices in small-angle scattering. If all the pieces fall into place, our future can be amazing indeed.

1. [1] B. R. Pauw, A. J. Smith, T. Snow, N. J. Terrill, A. F. Thünemann, The modular SAXS data correction sequence for solids and dispersions, *Journal of Applied Crystallography*, 50: 1800–1811, DOI: 10.1107/S1600576717015096

Investigation of upconverting nanoparticle growth utilizing in-situ luminescence monitoring in combination with offline small-angle X-ray scattering (SAXS) and transmission electron microscopy (TEM)

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Keywords: Nanoparticles, Synthesis, Upconverting Materials, Small-Angle X-ray Scattering (SAXS)

Many applications of nanoparticles require defined physico-chemical properties of the used materials like chemical composition, size distribution, surface chemistry/functionalization, and optical features. Many of these properties can be tuned by synthesis, which requires a very good understanding of the fundamental mechanisms of particle growth. This is particularly relevant for luminescent nanomaterials like lanthanide-based nanocrystals revealing conventional downconverted and upconverted luminescence. Upconversion (UC) is a non-linear process occurring in defined lanthanide-doped systems like Yb^{3+} and Er^{3} co-doped $NaYF_4$ nanocrystals, which show a strong luminescence in the visible region (540 nm, 650 nm) after excitation in the NIR (980 nm), that is strongly size and crystal phase dependent. Therefore, this luminescence can be used for monitoring the particle growth during synthesis.[1] In addition, further techniques to determine the size of the nanoparticles such as small-angle x-ray scattering (SAXS) transmission electron microscopy (TEM) are used.[2]

We herein present the synthesis of $NaYF_4$:Yb^{3+}(20%),Er^{3+}(2%) upconversion nanoparticles which is assessed by in-situ luminescence monitoring and offline small-angle X-ray scattering (SAXS) as well as transmission electron microscopy (TEM) measurements. Various stages of particles growth were investigated, with the combined results from these optical and sizing methods giving insights into the reaction mechanism during particle formation.

List of references:

1. John D. Suter et al., J. Phys. Chem. C 2014, 118, 13238-13247
2. Thorben Rinkel et al., Nanoscale 2014, 6, 14523

Abstract: OPN-09-2018

Oral Presentations

Needs for certified and non-certified reference materials and representative test materials in the area of nanotechnology – Analysis of a survey held by JRC in 2017

H. Rauscher, G. Roebben, A. Held
BAM-PTB Workshop, 14 May, 2018

EC-JRC certified reference materials

Colloidal silica ERM®-FD102

- **General goal of JRC CRMs:** provide reliable reference points in complex measurement systems
- **Available JRC CRMs in nano-area:** certified particle size parameters and surface area values
- **Upcoming JRC CRMs in nano-area:** nanoparticle shape and surface charge (density) analysis
- https://crm.jrc.ec.europa.eu/

EC-JRC representative nanomaterials

- Produced, e.g., in support of OECD WP on Manufactured Nanomaterials, NanoChOp, NanoDefine, …
- Many available through the JRC Nanomaterials Repository

JRC ID	Substance	JRC ID	Substance
JRCNM01001a	Titanium Dioxide	JRCNM02000a	Silicon Dioxide
JRCNM01002a		JRCNM02001a	
JRCNM01003a		JRCNM02002a	
JRCNM62001a		JRCNM10404a	
JRCNM62002a		JRCNM02004a	
…		…	

https://ec.europa.eu/jrc/en/scientific-tool/jrc-nanomaterials-repository

European Commission

Outline

- Goal of the survey
- Metadata
- Main findings per topic
- Summary

European Commission

Goal

- **Help establish priorities for (certified) reference materials (CRM, RM) and representative test materials (RTM) for nanomaterials**

- **Collect ideas for ways to increase collaboration on production of nanomaterial CRMs, RMs, RTMs with other stakeholders (Member State laboratories, industry, academia, …)**

Tool: a stakeholder survey

- **8 open questions (not multiple-choice)**

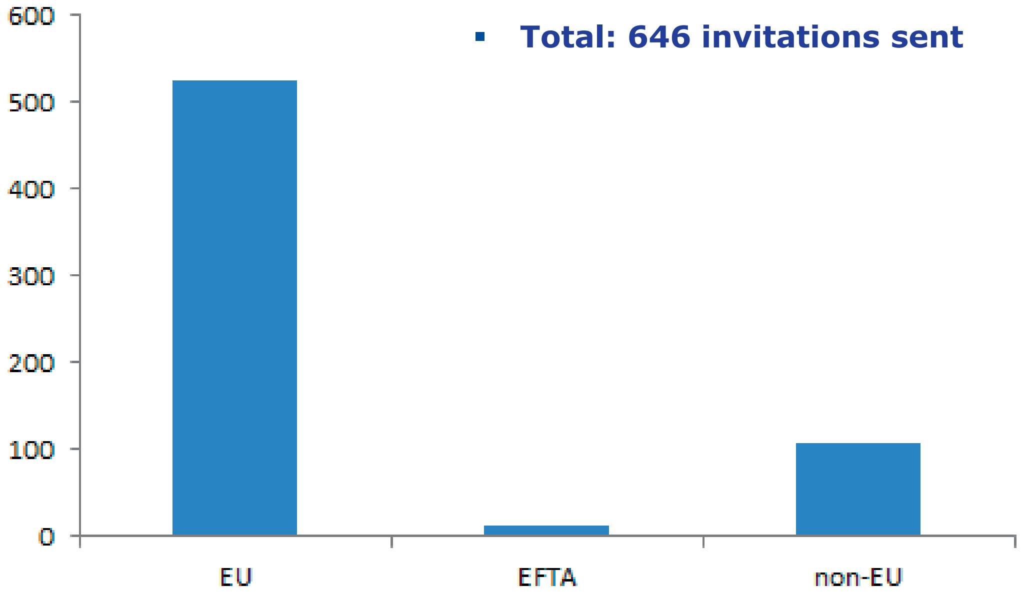
- **Distributed via e-mail**

- **1st distribution of the survey: 24/2/2017**
  - about 400 addresses obtained from JRC colleagues, from DGs, from ECHA, EFSA, …

- **Later distributions:**
  - to individuals / organisations suggested by earlier participants

- **Last distribution of the survey: 19/5/2017**

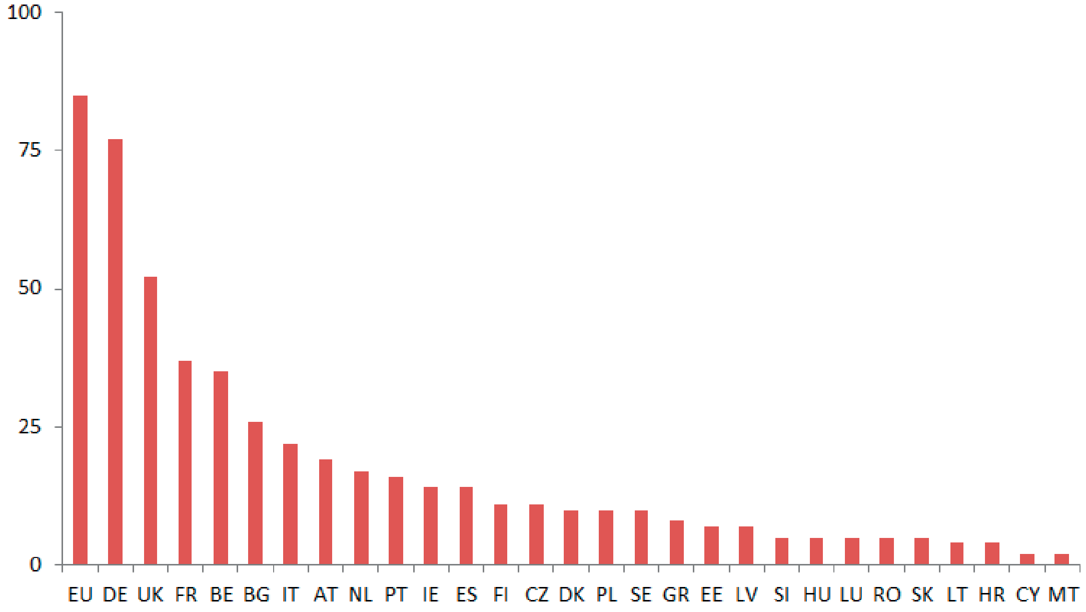
Survey invitations: EU vs. international



- **Total: 646 invitations sent**

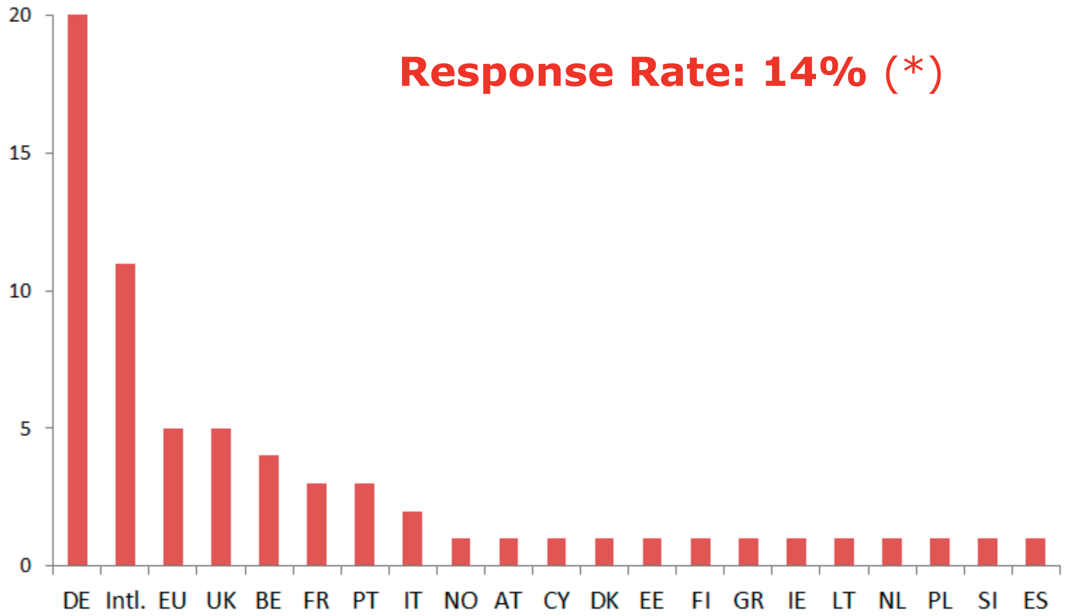
- EU = from 28 EU member states + EU-wide organisations

Within-EU distribution of surveys



- **At least 1 contact per Member State**

Geographical distribution of responses

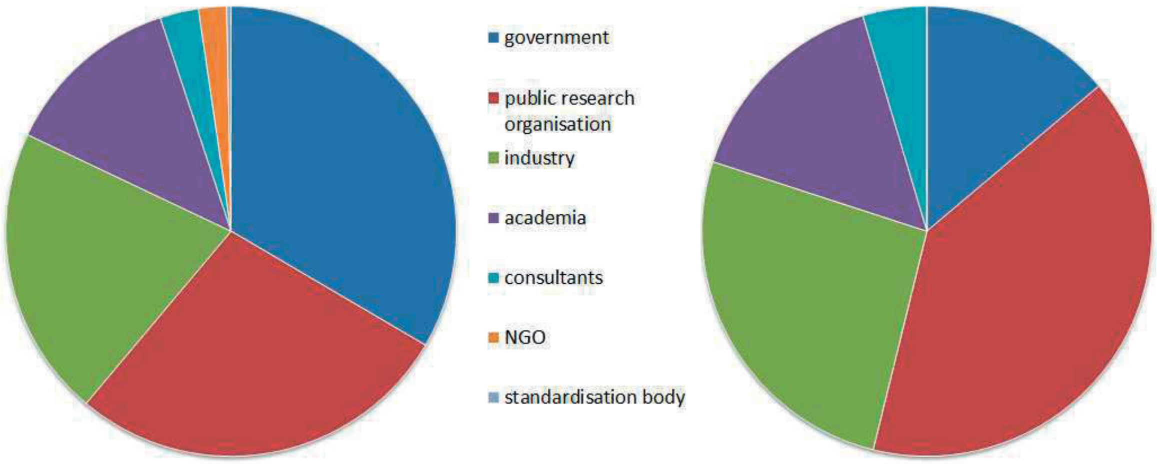


Response Rate: 14% (*)

(*) after correction for mail errors, retirements, declined, …

European Commission

Distribution over types of stakeholders

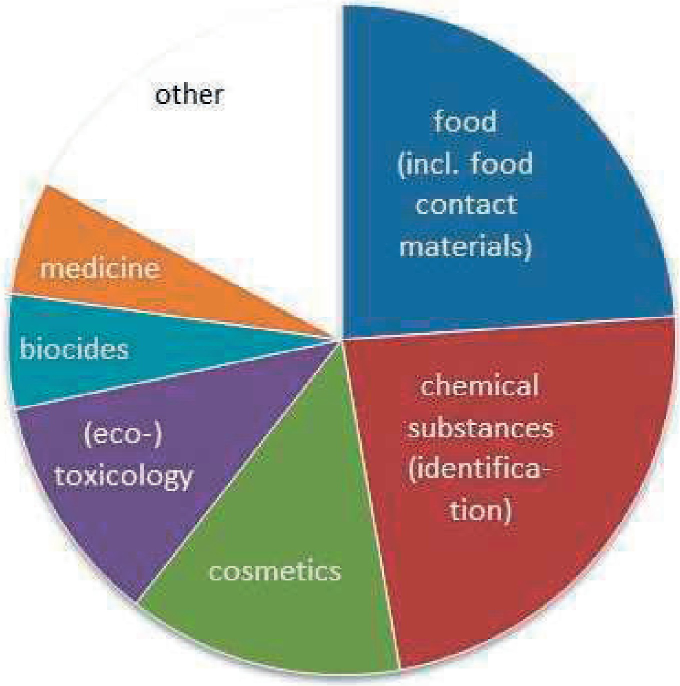


Invitations

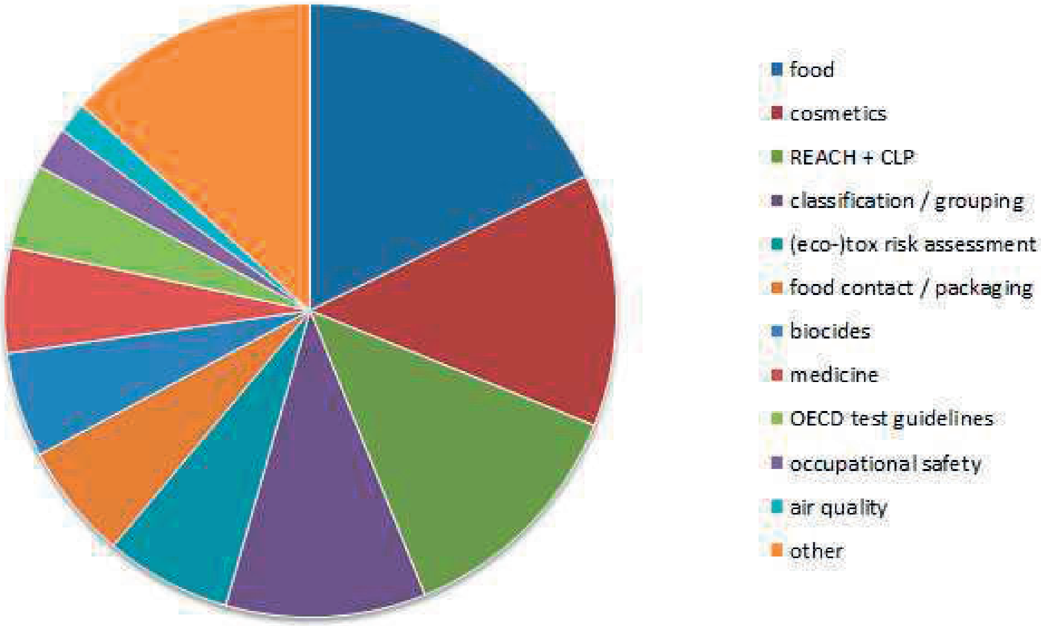
Answers

European Commission

Q1(1): Policy areas with RM/RTM needs

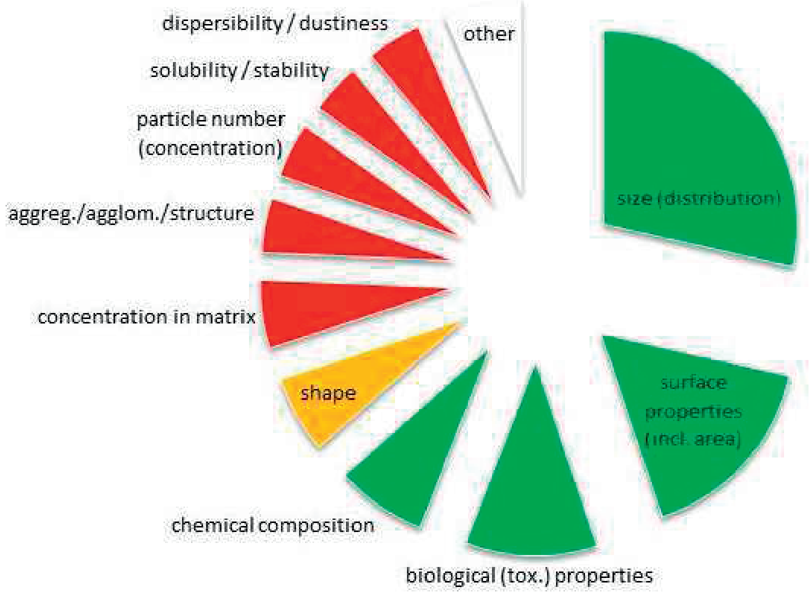


Q1(2): Policy areas with RM/RTM needs



(More detailed than graph on previous slide)

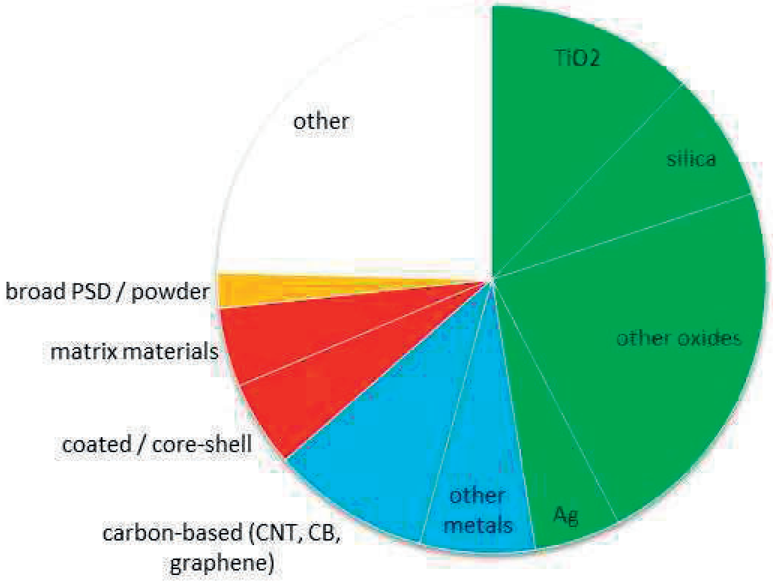
Q2: Relevant nanomaterial properties



Green: partly available from JRC & others
Yellow: work in progress by JRC & others
Red: not yet in JRC work programme

European Commission

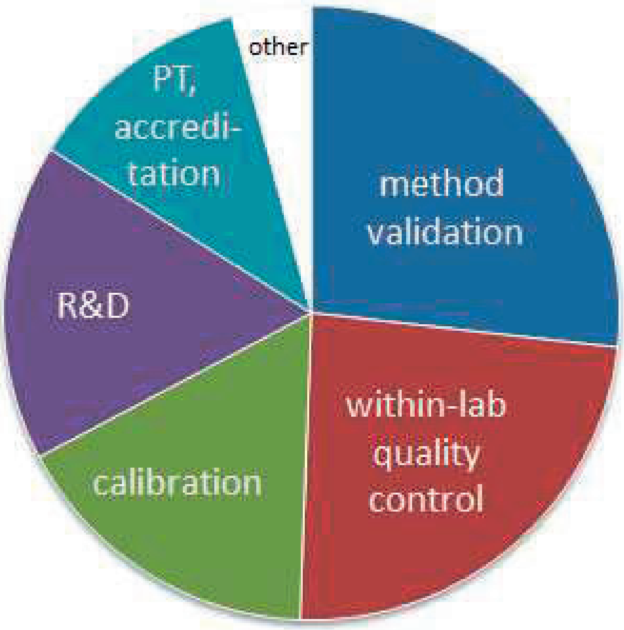
Q3: Relevant nanomaterials



Green: partly available from JRC & others
Yellow: work in progress by JRC & others
Red: not yet in JRC work programme
Blue: available from other (RM producers)

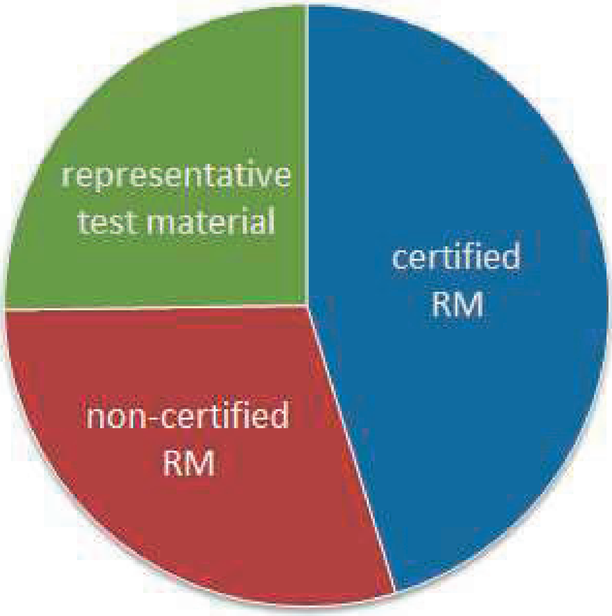
European Commission

Q4: Intended use of CRM/RM/RTM



European Commission

Q5: Preferred status (CRM? RM? RTM?)

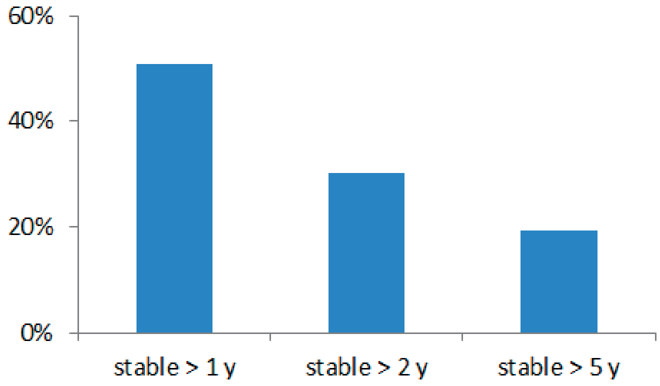


Comment 1:
Several participants require that the reference materials should also be recognised and accepted as such outside the EU.

Comment 2:
Many of those who require CRMs, will accept RMs and RTMs as temporary solutions.

European Commission

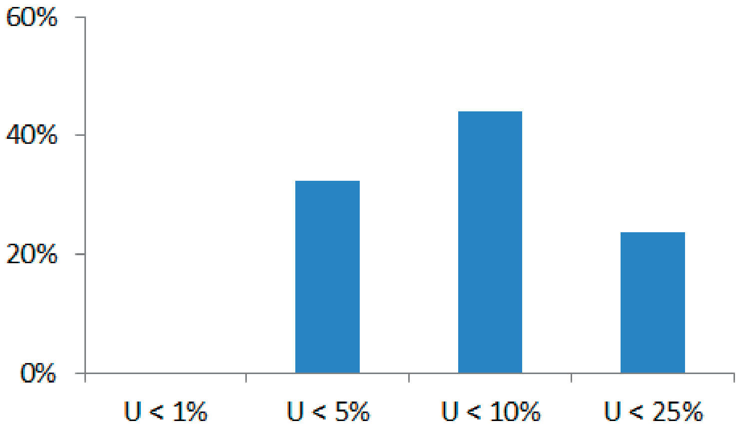
Q6 (1): Stability requirements



- **Minimum stability: 1 year**
- **For certain applications: > 5 years**
- **+ permanent availability**
  - **comparable across batches**

European Commission

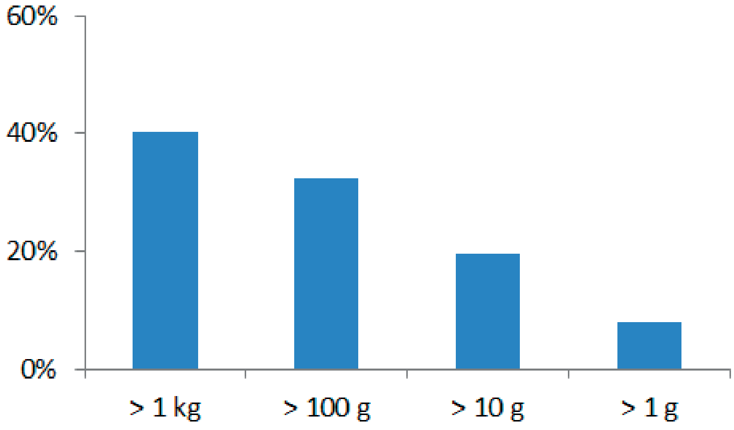
Q6 (2): Uncertainty (U) requirements



- **Maximum U (95% confidence level): 5 % to 10 %**
- **For certain applications: 25 % acceptable**
- **General request:** $u_{homogeneity} < u_{method}$

European Commission

Q7 Amounts of CRM/RM/RTM



- **Large variety: gram to kilogram**
- **Strongly depending on intended use**
- **Conclusion: often industrial sourcing needed**

European Commission

Q8: Collaboration?

- **Broad support and understanding for the need of a collaborative approach**
  - E.g. certification via interlaboratory studies
- **Stakeholders to be involved:**
  - Industry, NMI's, academia, research institutions, qualified / accredited (member state) labs, standards bodies (ISO, OECD)
- **Possible frameworks:**
  - Ad-hoc projects (H2020, EMPIR, VAMAS)
  - JRC-lead (CRM certification) projects with qualified sub-contractors
  - ESFRI European Strategy Forum on Research Infrastructures

European Commission

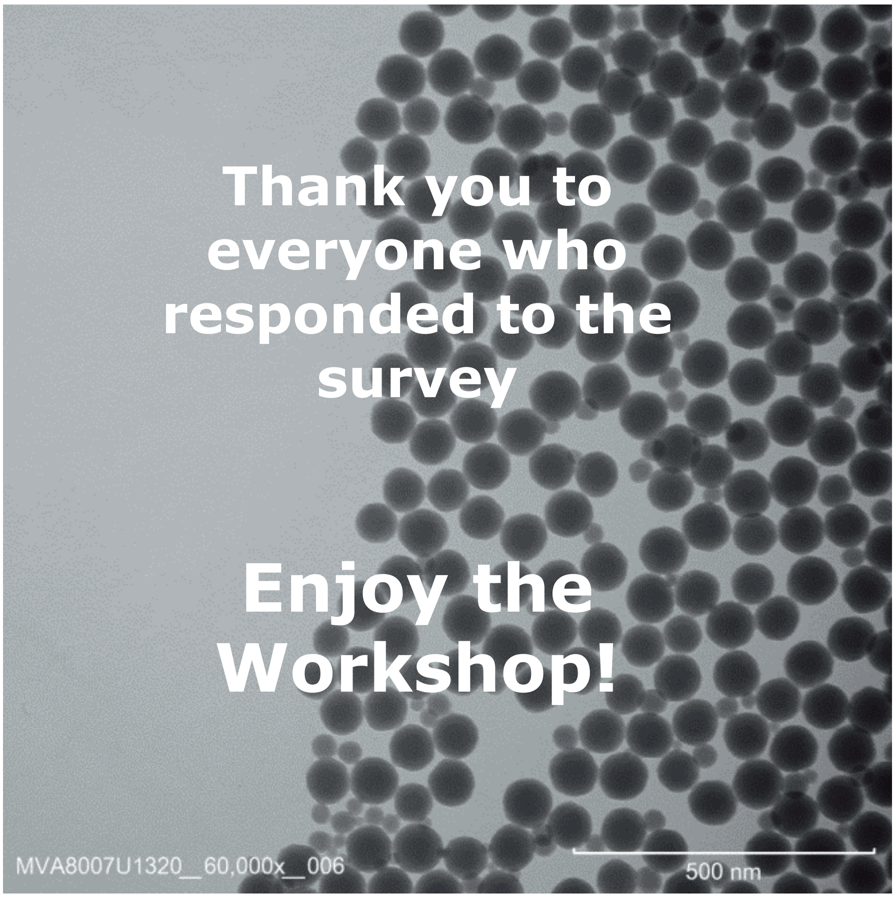
Concluding comments

- **Current JRC activities are broadly in line with the needs of the EU stakeholders**

- **Collaboration possibilities exist, e.g.:**
    - Production of CRM/RM/RTM under JRC-lead supported by qualified subcontractors
    - (Pre-)normative work under OECD, CEN, ISO, VAMAS, …

- **Other proposed collaboration routes are questionable**
    - Ad-hoc projects: Sustainability? End-responsibility? Acceptance by customers?
    - ESFRI: realistic?

European Commission



Thank you to everyone who responded to the survey

Enjoy the Workshop!

Expectations, results and follow-up: Industry perspective in developing a measurement strategy for nanomaterials

Reuter (VCI), BAM-PTB “Workshop on Reference NM - measurement, standardization”

VERBAND DER
CHEMISCHEN INDUSTRIE e.V.
WIR GESTALTEN ZUKUNFT.
VCI

Many industrial projects
– e.g. Joint JRC-Eurocolour Project & Report 11/2014 for pigments

Examples for pigments and extenders
used in the JRC Eurocolour project:

1 Al-Co-Blue Spinell Pigment $CoAl_2O_4$

2 Pigment Yellow 83/Organic Pigment

3 Pigment Red 101/Fe_2O_3

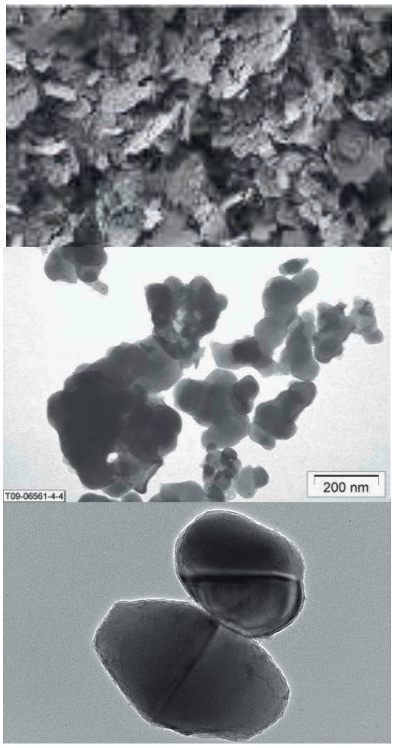
4 Pigment Yellow 42 (Trans. FeOOH)

5 TiO_2 Rutile

6 TiO_2 Anatase

7 “Metal Pigment 2” – Cu/Zn alloy

8 Pyrogenic Silica

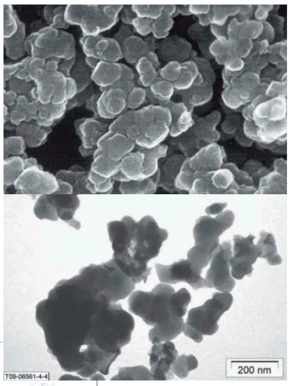


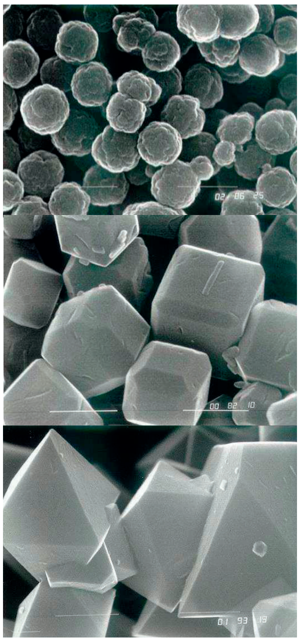
Verband der Mineralfarben-industrie e. V.
Eurocolour pigments, dyes and fillers
VCI

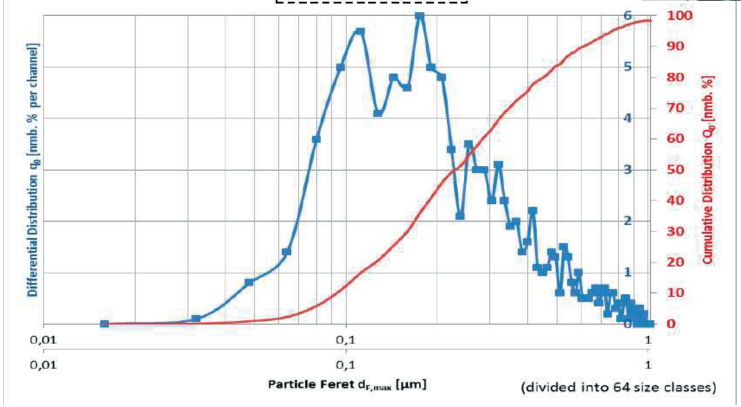
Particle Forms and Particle Size Distribution

The **particles** in question **are** typically

1. **irregularly formed**
2. **aggregated**
3. **part of a distribution** at least an order of magnitude wide







Verband der Mineralfarben-industrie e. V. Eurocolour pigments, dyes and fillers VCI

Implementation of EU Recommendation on the definition of NM (2011/696/EU) – to be revised

Requirements for implementation:

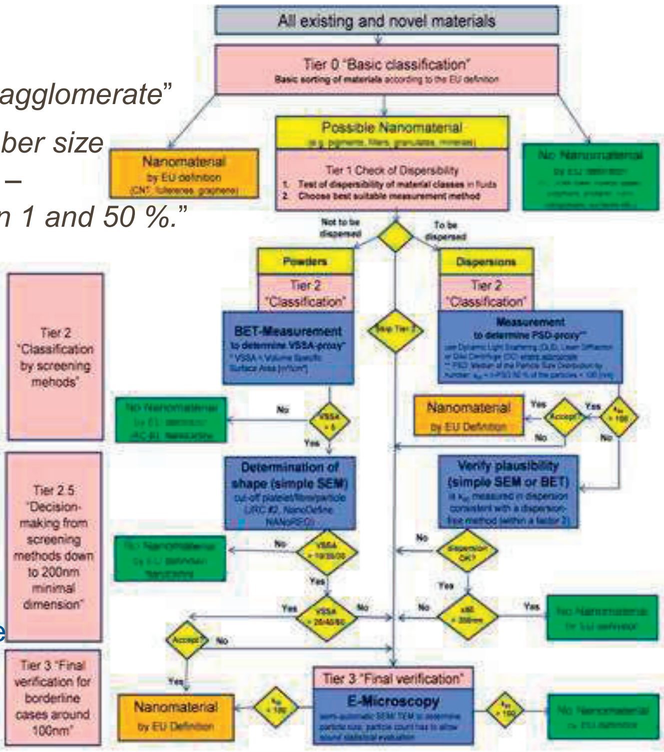
- *"manufactured material"*
- *"unbound state or as an aggregate or as an agglomerate"*
- *"for 50 % or more of the particles in the number size distribution … in the size range 1 - 100 nm" – "… may be replaced by a threshold between 1 and 50 %."*

Challenges for applicability:

- Methods & strategies for **"real and existing materials"**
- Measurements must be able to determine a given material being "nano or non-nano"
- industry needs strategies to determine product families
- Measurement equipment and know-how & training needs to be accessible

Expectations:

- **Implementation of a "Tiered measurement strategy" (2012 / 2015)**



Verband der Mineralfarben-industrie e. V. Eurocolour pigments, dyes and fillers VCI

Expectations of industry for a measurement strategy in more detail

- **Strategies for "Conflicting Assignments"**

Requirements for implementation *and challenges for applicability*:

Measurements must be able to regulatory compliantly determining a given material being "non-nano" as well as "nano"

Expectations:

1. *Regulatory compliance of measurement methods & guidance on analytical application of specific methods & guidance on compliant documentation*
2. *Tools for selecting the most appropriate measurement method(s) and measurement techniques (algorithms, evaluation of results)*

That implies ...

- **Industry needs Strategies for "Screening & methods"**

Requirements for implementation *and challenges for applicability*:

Methods have to be available and should be applicable for SMEs and companies within the industrial value chains

Expectations:

1. *Implementation of **standard (screening)methods**:*
   - ***BET-** and **non EM-methods**,*
   - *recommendations for **application** of EM (EM/TEM),*
   - *calibrating **VSSA** as a screening method via TEM (for specific product families, for non-dispersed powder materials with VSSA > $10/20/30_{shape}$ m²/cm³),*
   - *screening-methods for dispersed / suspended materials with a x_{50} > 100 nm*
2. *Practical testing, SOPs*

Lessons learned from the project NanoDefine: Messages from industry @ Science

- **Results of an evaluation of the NanoDefine Project for implementation of a regulatory compliant measurement strategy for NM:**

What does industry technically need to comply with regulation?

1. Project confirms: **Sample preparation is critical step & remains challenging**
2. **Regulatory compliance**: Progress made BUT **still strong limitations for "borderline substances"** (at date **"factor 2,5"**)
3. **Tiered approach not fully accomplished – "tiered approach" would mean**:
   - **avoid regular use of tier 1 methods (EM)**
   - **adequate and reliable use of screening methods (**BET + non EM-methods)
   - development of **strategies to measure product families** within value chain
4. Still difficulties to **measure agglomerates & aggregates: Measuring coated NP**, differentiation of **"constituent particles"**
5. Very interesting **new methods** (e.g. AF4 with "uncomplicated sample prep"): Time frames to develop those as standard applications acceptable?
6. Promising **"E-Tool"** ("NanoDefiner") **but "practical validation"** by industry e.g. pigments & extenders producers still **crucial**

In more detail: Tier 1 method Electron Microscopy

- The **presence of any significant aggregation/agglomeration** may make it impossible to realize the advantages of EM due to the difficulties of analysing complex three dimensional structures using a method which produces a two dimensional projection.
- A wide **variety of sample preparation and counting** are used and must be adopted to needs of the material in question.
- Particles need to be detected manually. If two dimensional enumerations are necessary this may **last two to three days for each sample**.
- The minimum number of particles which has to be counted is practically given by the requirement, that the statistical relevance of the tailings of a particle size distribution needs to be ensured.

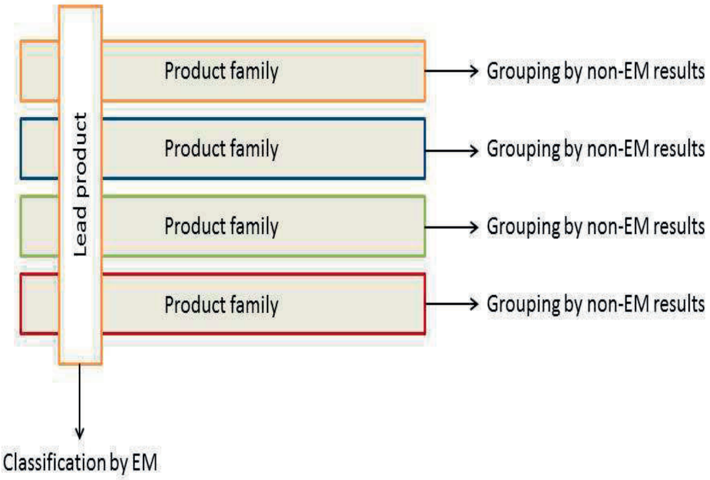
In more detail: The “Product Family Approach”

- **Requirement:**

In some cases product families car defined in which it can be unequivocally determined which specimen has a higher mean partic size than the other.

- **Approach:**

In these cases **it may be sufficient to characterize one member**, the lead, of the family by electron microscopy and do following grouping by other means.



Lessons learned form the project NanoDefine: Messages from industry @ politics

- **Results of an evaluation of the NanoDefine Project for implementation of a regulatory compliant measurement strategy for NM:**

What does industry regulatory need to comply with regulation?

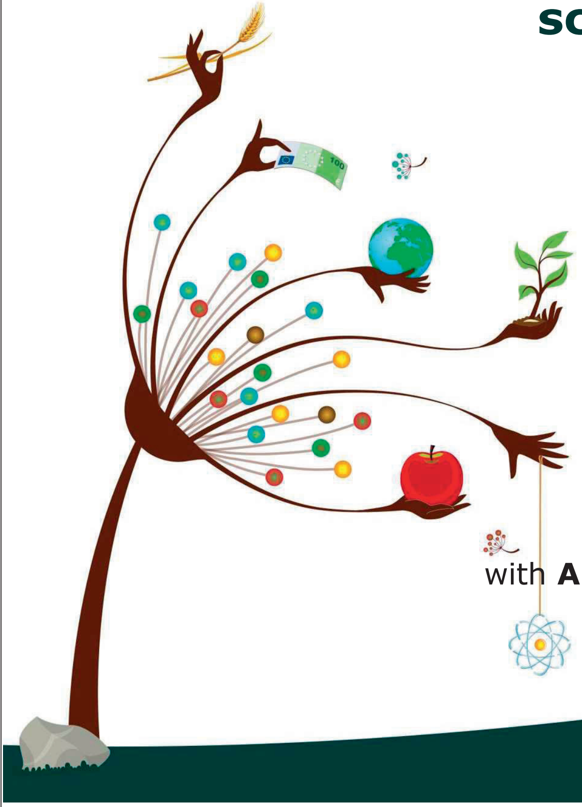
1. Problems to practically **implement a measurement strategy when measuring “borderline substances”**
2. Regulatory compliant **measurement methods & strategies to determine product families**
3. Focus on **methods with wide spread use**
4. Fostering **know-how & training** on analytical methods for NM within the value chains

VCI is happy to discuss follow-ups …

- **First thoughts**
  to "establish a real follow-up of the NanoDefine project":
    - ➔ no "re-invention" of methods & strategies
    - ➔ practicability of results is still lacking
    - ➔ industry & authorities still in need of progress in measurement methods
  - **Possible scope: "NanoDefine Implementation & Testing"**
    - **- Identification of hot-spots for practical implementation**
      (e.g. SOPs, screening methods, automated EM, …)
    - **- Establishment "of industrial value chain peer group" for testing methods** to further refine the "NanoDefiner"
- **VCI is happy to discuss recommendations**
  - **To meet industry requirements on methods & strategies**
  - **on follow-up activities, a follow-up project …**

The European Commission's science and knowledge service

Joint Research Centre



The NanoDefine Decision Framework and NanoDefiner e-Tool:

a practical guide to the identification of nanomaterials

Hubert Rauscher
European Commission Joint Research Centre
with **A. Mech, C. Gaillard, H. Marvin, W. Wohlleben, D. Hodoroaba, F. Babick, C. M. Friedrich, R. Bruengel, J. Rückert, A. Ghanem, S. Weigel**

Joint BAM and PTB Workshop on Reference Nanomaterials
Current situation, measurement methods, standardization and future needs
Berlin, 14-15 May 2018

The European Commission definition of Nanomaterial

COMMISSION RECOMMENDATION of 18 October 2011 (2011/696/EU)

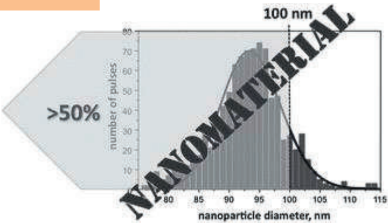
Need to know how to consider aggregate/agglomerates

Refers to constituent (primary) particles

"Nanomaterial" means a natural, incidental or manufactured material containing particles, in **an unbound state or as an aggregate or as an agglomerate** and where, for **50 % or more** of the particles in the **number size distribution**, one or more external dimensions is in the size range **1 nm - 100 nm**"

Methods of directly "counting" particles?
Number based vs. mass based measurements

Methods needed which cover this range



Commission Recommendation of 18 October 2011 on the Definition of nanomaterial (2011/696/EU)

- Recommendation (legally not binding), developed for regulatory purposes
- No relation to hazard or risk intended
- Size as the only defining property

- Important for:
  - Biocidal Products Regulation 258/2012
  - Medical Devices Regulation EU/2017/745

  - REACH - working definition, amended Annexes

  - Regulations adopted before the publication of the EC Recommendation (Cosmetic Products, Food Products) have different definition

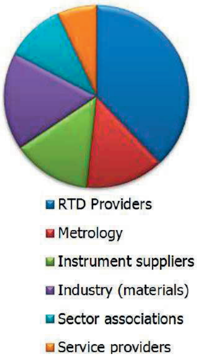
NanoDefine project and NanoDefiner e-Tool

Objective:

To provide the affected industries and regulatory agencies with the tools that **support the implementation** of the European Commission's recommendation **for a definition of nanomaterial** in all relevant regulatory contexts.

- Collaborative FP7 project 11/2013 – 10/2017

- 28 partners: industry, regulators, research



"Tools" for the practical implementation of the nanomaterial definition

- **Decision flow scheme**

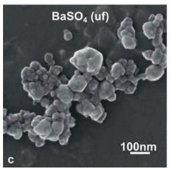
  to identify nanomaterials **and** non-nanomaterials

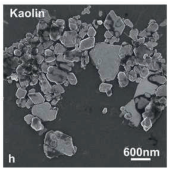
- **NanoDefiner E-tool**
  - user-friendly decision support software implementing the flow scheme
  - web-based and standalone versions
    (Free access after registration)
  - Guided workflow
  - Generates comprehensive, transparent report with reasoning

- **NanoDefine Methods Manual**

- **Tested on commercial industrial materials**

  representing the majority of different types of particulate materials on the market





www.nanodefine.eu

Methods evaluation and development

Sizing techniques were **evaluated according to uniform performance criteria** with a view to measurement requirements resulting from the EC's nanomaterial definition

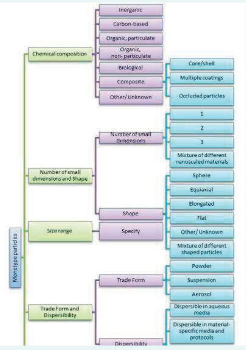
Methods and their performance (selection)

		Methods recommended by NanoDefine method evaluation (Babick et al. 2016)								Methods not evaluated by NanoDefine
		TEM	SEM	BET	DEMA spray	AC - turb	AC-RI	DLS	sp ICP-MS	AFM
MAIN (selected) CRITERIA FOR MONOTYPE PARTICLES										
CHEMICAL COMPOSITION	Inorganic	X	X	X	X	X	X	X	X	X
	Carbon based	X	X	X	X	X	X	X		X
	Organic particulate	X	X	X	X	X	X	X		X
	Biological	X	X		X	X	X	X		X
COMPOSITE	Core shell	X	X	X	X	X	X[1]	X	X	X
	Multiple coating	X	X	X	X	X[1]	X[1]	X	X	X
	Occluded	X	X	X	X	X[1]	X[1]	X	X	X
SMALL DIMENSIONS	1	X	X[2]	X						X
	2	X	X	X						X
	3	X	X	X	X	X	X	X	X	X
	Mixture	X	X							X
SHAPE	Spherical or equiaxial	X	X	X	X	X	X	X	X	X
	Elongated	X	X	X						X
	Flat	X[2]	X[2]	X						X
	mixture	X	X[3,4]							X
Size range (nm)		1 nm-1000 µm	10 nm - 1000 µm	1 nm -10 µm	2 nm - 1 µm	5 nm- 100 µm	1 nm – 1 µm	3 nm – 5 µm	20 nm -1 µm	1 nm -10 µm
TRADE FORM	Powder	X[3]	X*	X						X[2]
	Suspension	X[3]	X[3]		X	X	X	X	X	X[2]
	Aerosol				X					
Electron beam sensitive				X	X	X	X	X	X	X
Sensitivity to vacuum					X	X	X	X	X	X
Analysis temperature (C)		15-40	15-40	15-40	10-40	5-60	5-60	5-60	15-40	15-40
Conductive materials		X	X	X	X	X	X	X	X	X

Can be material dependent

Material classification system

The NanoDefine MCS is method-driven to match materials with appropriate size measurement techniques.



Methods evaluation and development

Sizing techniques were **evaluated according to uniform performance criteria** with a view to measurement requirements resulting from the EC's nanomaterial definition

Machine-readable

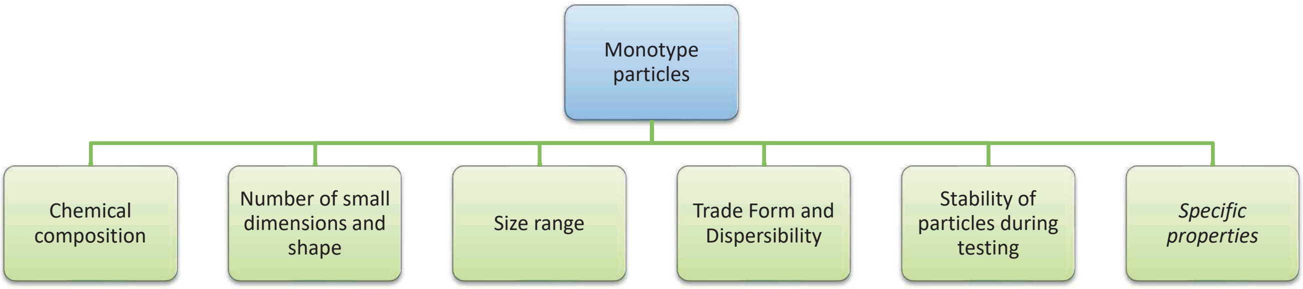
Knowledge Base

Includes:

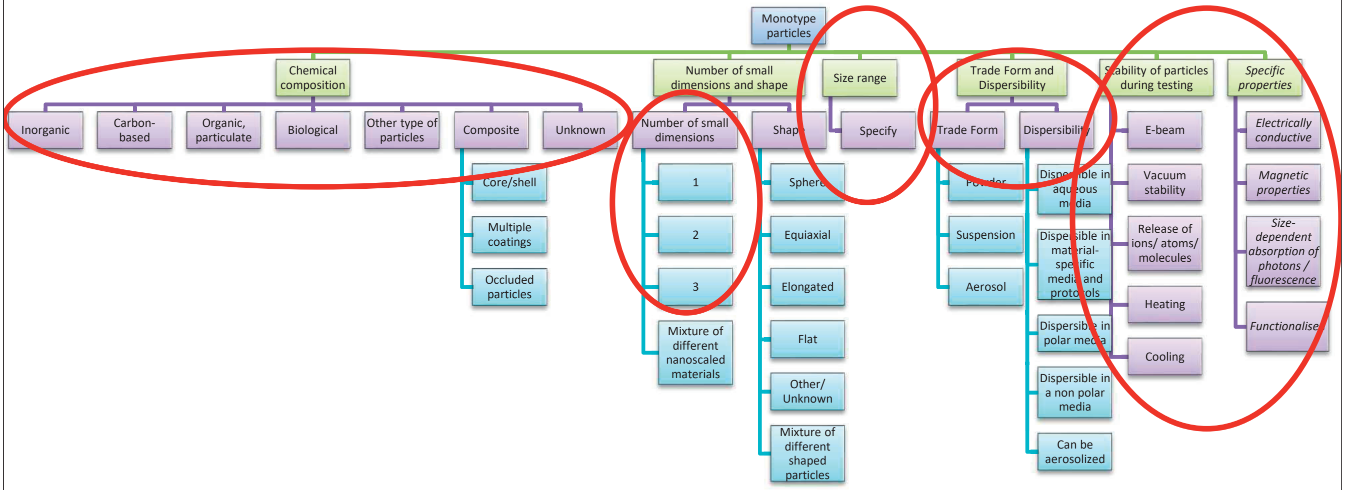
- All techniques and their performance
- Material characteristics and templates
- Priorities
- Built-in decision rules

Material classification system

- The material is classified according to several parameters that are most important for size measurement methods
- Main properties:

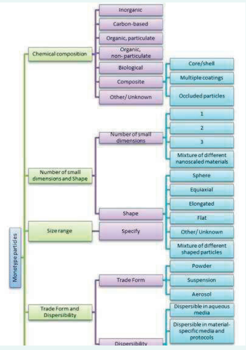

Monotype particles
Chemical composition
Number of small dimensions and shape
Size range
Trade Form and Dispersibility
Stability of particles during testing
Specific properties

Complete material classification system for monotype particles


Monotype particles
Chemical composition
Inorganic
Carbon-based
Organic, particulate
Biological
Other type of particles
Composite
Core/shell
Multiple coatings
Occluded particles
Unknown
Number of small dimensions and shape
Number of small dimensions
1
2
3
Mixture of different nanoscaled materials
Shape
Sphere
Equiaxial
Elongated
Flat
Other/ Unknown
Mixture of different shaped particles
Size range
Specify
Trade Form and Dispersibility
Trade Form
Powder
Suspension
Aerosol
Dispersibility
Dispersible in aqueous media
Dispersible in material-specific media and protocols
Dispersible in polar media
Dispersible in a non polar media
Can be aerosolized
Stability of particles during testing
E-beam
Vacuum stability
Release of ions/ atoms/ molecules
Heating
Cooling
Specific properties
Electrically conductive
Magnetic properties
Size-dependent absorption of photons / fluorescence
Functionalised

Material classification system

The NanoDefine MCS is method-driven to match materials with appropriate size measurement techniques.



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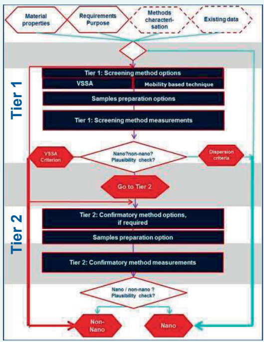
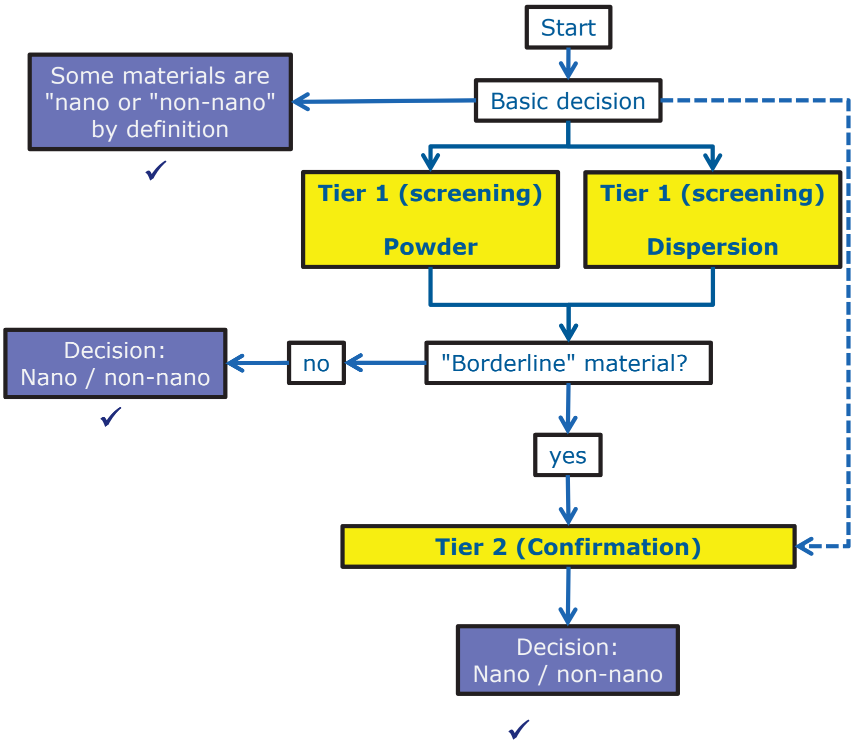
Decision flow scheme

- guides users through the whole process
- 2-tiered approach
- screening and confirmatory methods
- 'nano' or 'non-nano' classification.



NanoDefine – decision flow scheme (simplified)

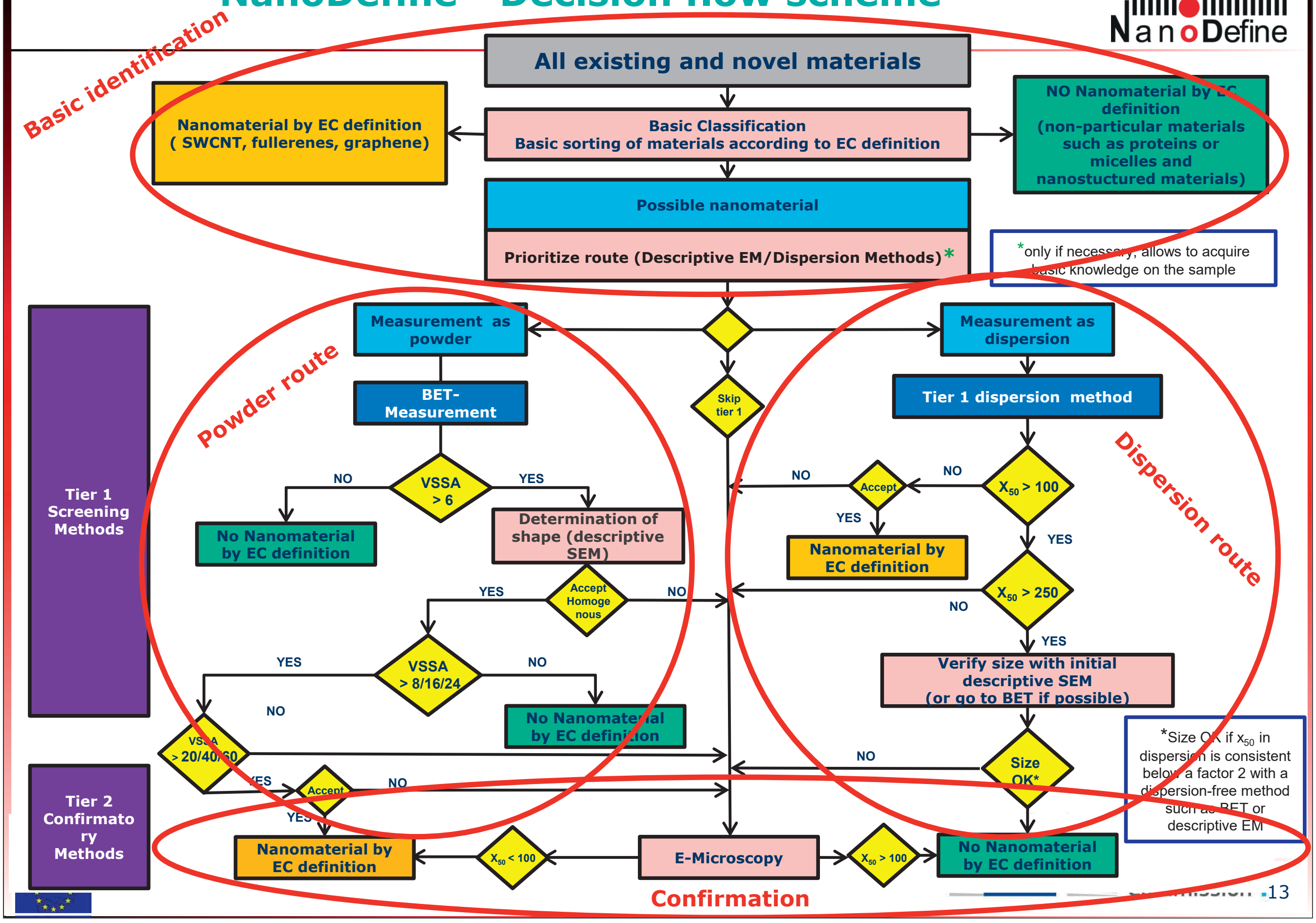


Decision scheme

- 2-tiered approach
- Screening/confirmation
- **Identification 'nano' or 'non-nano'**

- **Cost efficient**
  - Early decisions possible
- **Flexible**:
  - Choice of methods
  - Tier1 / tier 2

NanoDefine - Decision flow scheme



Material classification system

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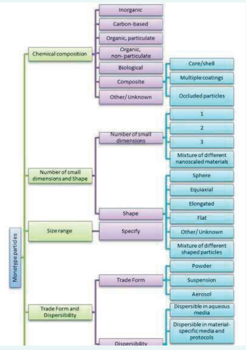

Methods evaluation and development

Sizing techniques were evaluated according to uniform performance criteria with a view to measurement requirements resulting from the EC's nanomaterial definition

Decision flow scheme

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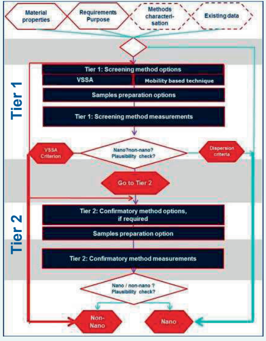


Machine-readable

Knowledge Base

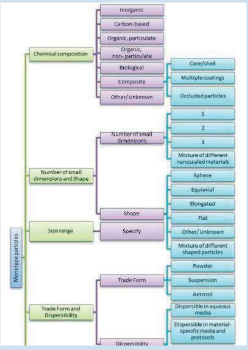
Includes:

- All techniques and their performance
- Material characteristics and templates
- Priorities
- Built-in decision rules

Nanomaterial ⟷ No nanomaterial

Material classification system

The NanoDefine MCS is method-driven to match materials with appropriate size measurement techniques.



Methods evaluation and development

Sizing techniques were evaluated according to uniform performance criteria with a view to measurement requirements resulting from the EC's nanomaterial definition

Decision flow scheme

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- screening and confirmatory methods
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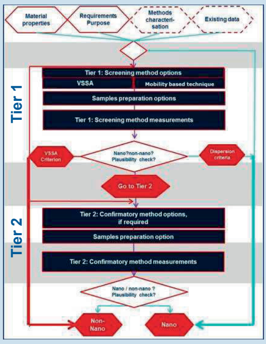


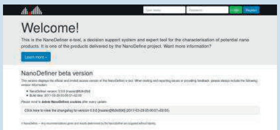
Machine-readable Knowledge Base

Includes:

- All techniques and their performance
- Material characteristics and templates
- Priorities
- Built-in decision rules

NanoDefiner eTool and Manual

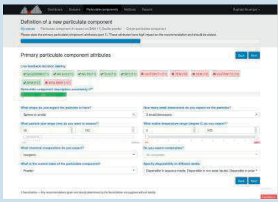
https://labs.inf.fh-dortmund.de/NanoDefiner



Welcome!

This is the NanoDefiner e-tool, a decision support system and expert tool for the characterisation of potential nano products. It is one of the products delivered by the NanoDefine project. Want more information?

NanoDefiner beta version



Definition of a new particulate component

Primary particulate component attributes

Screenshots

eTool workflow

- Dossier creation
- Particulate material description
- Technique selection
- Result evaluation
- Report generation

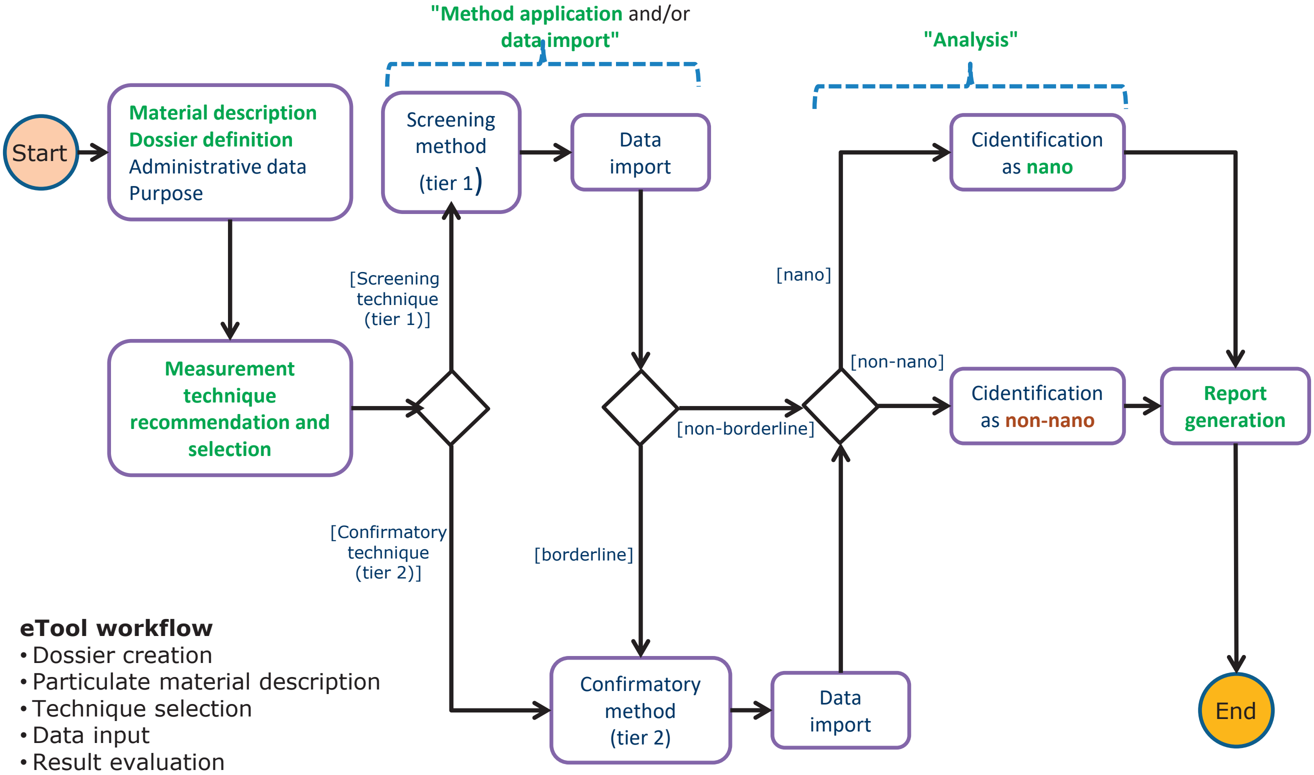
eTool features

- Free use with registration
- Platform independent
- Guided workflow
- Comprehensive dossiers
- Material classification system
- Transparent method selection
- Reports with reasoning
- Extensive knowledge base
- Institutionalisation and internationalisation

Nanomaterial

No nanomaterial

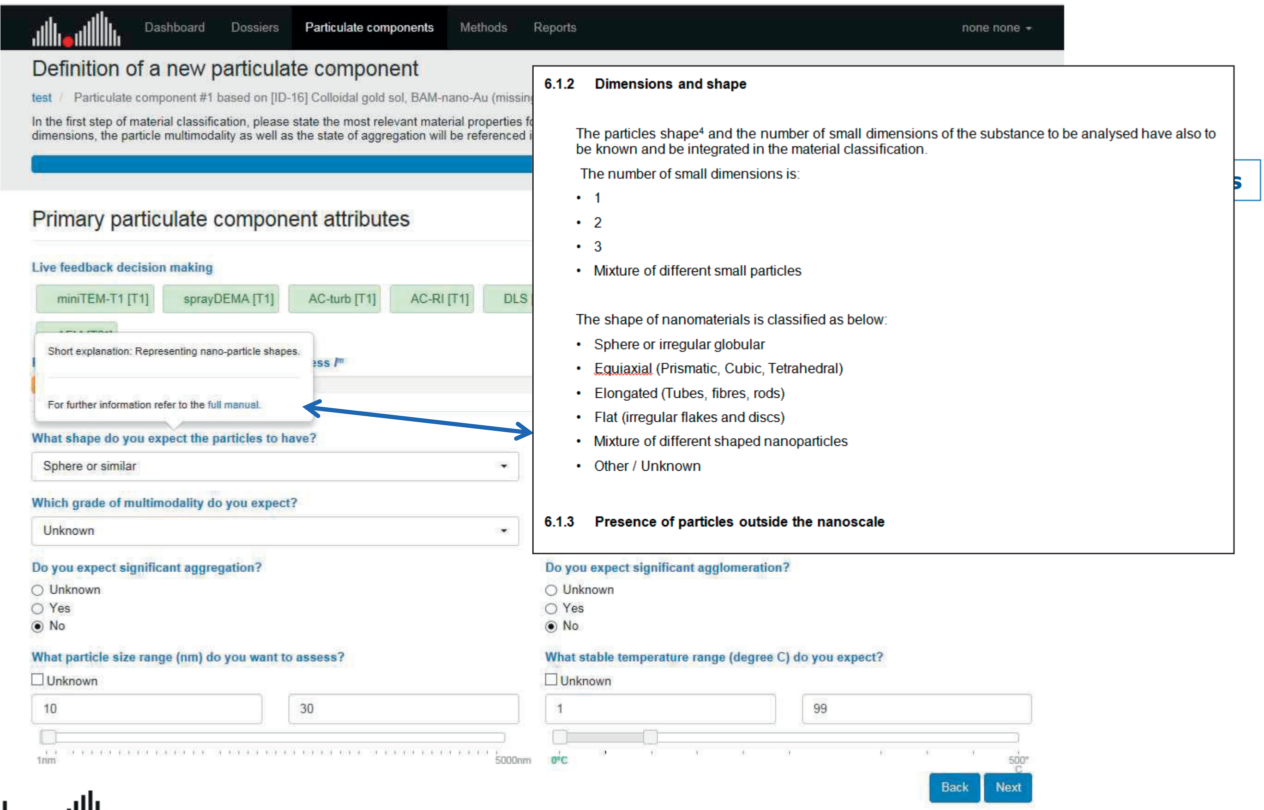
E-tool: flow scheme



eTool workflow

- Dossier creation
- Particulate material description
- Technique selection
- Data input
- Result evaluation
- Report generation

NanoDefiner e-tool



Definition of a new particulate component

test / Particulate component #1 based on [ID-16] Colloidal gold sol, BAM-nano-Au (missin

In the first step of material classification, please state the most relevant material properties f
dimensions, the particle multimodality as well as the state of aggregation will be referenced i

Primary particulate component attributes

Live feedback decision making

Short explanation: Representing nano-particle shapes.

For further information refer to the full manual.

What shape do you expect the particles to have?
Sphere or similar ▾

Which grade of multimodality do you expect?
Unknown ▾

Do you expect significant aggregation?
○ Unknown
○ Yes
● No

Do you expect significant agglomeration?
○ Unknown
○ Yes
● No

What particle size range (nm) do you want to assess?
☐ Unknown
10 | 30

What stable temperature range (degree C) do you expect?
☐ Unknown
1 | 99

6.1.2 Dimensions and shape

The particles shape[4] and the number of small dimensions of the substance to be analysed have also to be known and be integrated in the material classification.

The number of small dimensions is:

- 1
- 2
- 3
- Mixture of different small particles

The shape of nanomaterials is classified as below:

- Sphere or irregular globular
- Equiaxial (Prismatic, Cubic, Tetrahedral)
- Elongated (Tubes, fibres, rods)
- Flat (irregular flakes and discs)
- Mixture of different shaped nanoparticles
- Other / Unknown

6.1.3 Presence of particles outside the nanoscale

NanoDefiner e-tool

Predefined materials

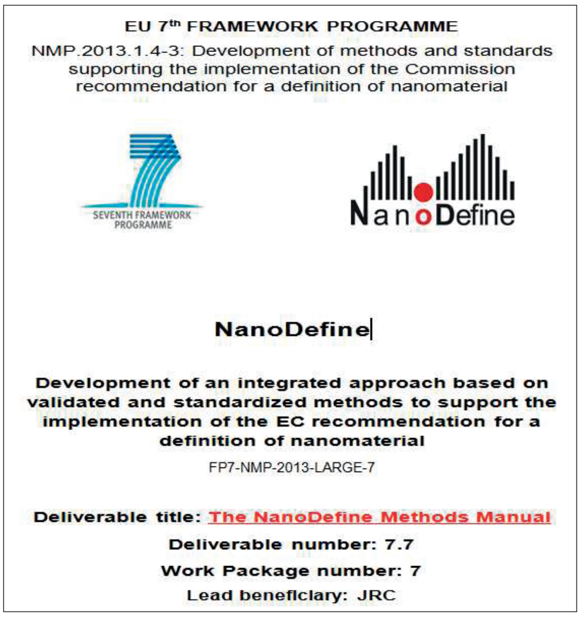
Reference/representative test material	NanoDefine code	Paper code [2]
Colloidal Au (monomodal)	ID-16	QCM3
Colloidal SiO_2 (monomodal)	ID-17	QCM2
Colloidal SiO_2 (trimodal)	ID-18	QCM6
Polystyrene (monomodal)	ID-19	QCM1
Polystyrene (trimodal)	ID-20	QCM5
Colloidal Ag (monomodal)	ID-21	QCM4
Zeolite	BAM-11	–
Organic pigment Y83 (nano grade)	IRMM-380	RTM7
$BaSO_4$ (fine grade)	IRMM-381	RTM2
Multi-walled carbon nanotubes	IRMM-382	–
Nano steel	IRMM-383	–
$CaCO_3$ (fine grade)	IRMM-384	RTM4
Kaolin	IRMM-385	RTM5
Organic pigment Y83 (coarse grade)	IR	
$BaSO_4$ (ultrafine grade)	IR	
TiO_2 (coated)	IR	
Basic methacrylate copolymer	IR	

Predefined methods

Measurement technique, non-assessed *emphasized*	Tier
Atomic Force Microscopy (AFM)	*2*
Scanning Electron Microscopy (SEM)	2
Transmission Electron Microscopy (TEM)	2
Analytical Centrifugation, turbidity (AC-turb)	1
Analytical Centrifugation, refractive index (AC-RI)	1
Asymmetric Flow FFF, Multi-angular Light Scattering (AF4-MALS)	*1*
Brunauer–Emmett–Teller (BET)	1
Dynamic Light Scattering (DLS)	1
Mini Transmission Electron Microscopy (miniTEM)	*1*
Particle Tracking Analysis (PTA)	*1*
Single Particle Inductively Coupled Plasma Mass Spectrometry (spICP-MS)	*1*
Spray Differential Electrical Mobility Analysis (sprayDEMA)	1
Tunable Resistive Pulse Sensing (TRPS)	*1*
Ultrasonic Spectroscopy (USSP)	*1*
Angular Light Scattering (ALS)	–
Small-Angle X-ray Scattering (SAXS)	–
X-ray Diffraction (XRD)	–

NanoDefine Methods Manual

- **The NanoDefine Methods Manual**: Comprehensive Vademecum covering **all aspects** to implement the EC nanomaterial definition, including
  - Assessment of measurement methods
    - Technical method performance
    - Applicability to material groups
    - Developments in the project
  - Standard operating procedures
  - Steps necessary to identify nano- and non-nanomaterials
  - will be the basis for JRC **Guidance** on the nanomaterial definition (in preparation)
  - E-tool manual



EU 7th FRAMEWORK PROGRAMME

NMP.2013.1.4-3: Development of methods and standards supporting the implementation of the Commission recommendation for a definition of nanomaterial

SEVENTH FRAMEWORK PROGRAMME

NanoDefine

NanoDefine

Development of an integrated approach based on validated and standardized methods to support the implementation of the EC recommendation for a definition of nanomaterial

FP7-NMP-2013-LARGE-7

Deliverable title: The NanoDefine Methods Manual

Deliverable number: 7.7

Work Package number: 7

Lead beneficiary: JRC

will become a public JRC report

www.nanodefine.eu

European Commission

Interlaboratory validation efforts

(collaboration with VAMAS)

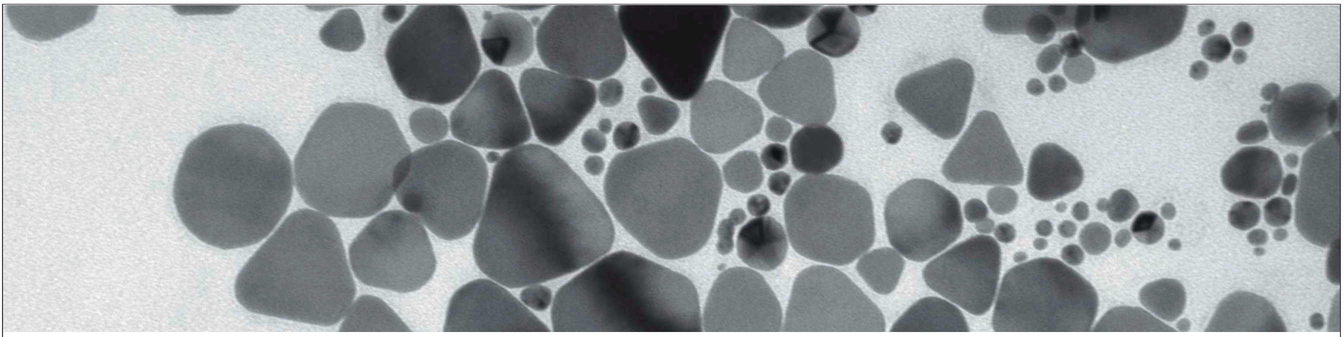
- 8 methods selected which were previously single-laboratory validated
- 3x analytical centrifugation (cuvette-type AC, disc-type AC and analytical ultracentrifugation)
- 3x spICP-MS
- 1x Asymmetric-Flow Field Flow Fractionation (AF4)
- 1x software for the automated analysis of transmission electron microscopy (TEM) images (NanoDefine ParicleSizer).

Results

- Two methods, the NanoDefine ParticleSizer software and the Cuvette CLS method can be considered as successfully validated in the interlaboratory studies.
- For three methods, Disc-CLS, AUC and AF4, the interlaboratory study cannot be considered successful due to the lack of sufficient data, even though the results are promising.
- No validation of spICP-MS methods (reproducibility problems)

Note: no time for a training of the laboratories and very little time for the implementation of the methods.

European Commission



Summary and Outlook

- NanoDefine developed a comprehensive framework supporting the regulatory identification of nanomaterials
- Tools include: Decision Flow Scheme, NanoDefiner e-Tool and Manual
- The tools can be adapted to changes in the regulatory requirements
- Flow scheme tested manually and with e-tool
- JRC is preparing Guidance on the implementation of the EC definition of nanomaterials which will benefit from NanoDefine results
- Continue with method validation
- Work towards standardisation planned
- Reference materials are needed

NanoDefine

European Commission

Many thanks – stay in touch!

JRC Science Hub:
ec.europa.eu/jrc

Twitter and Facebook:
@EU_ScienceHub

LinkedIn:
european-commission-joint-research-centre

YouTube:
JRC Audiovisuals

Vimeo:
Science@EC

NanoDefine

www.nanodefine.eu

European Commission

BAM
Bundesanstalt für
Materialforschung
und -prüfung

Sicherheit in Technik und Chemie

OECD TEST GUIDELINE ON PARTICLE SIZE AND SIZE DISTRIBUTION OF MANUFACTURED NANOMATERIALS

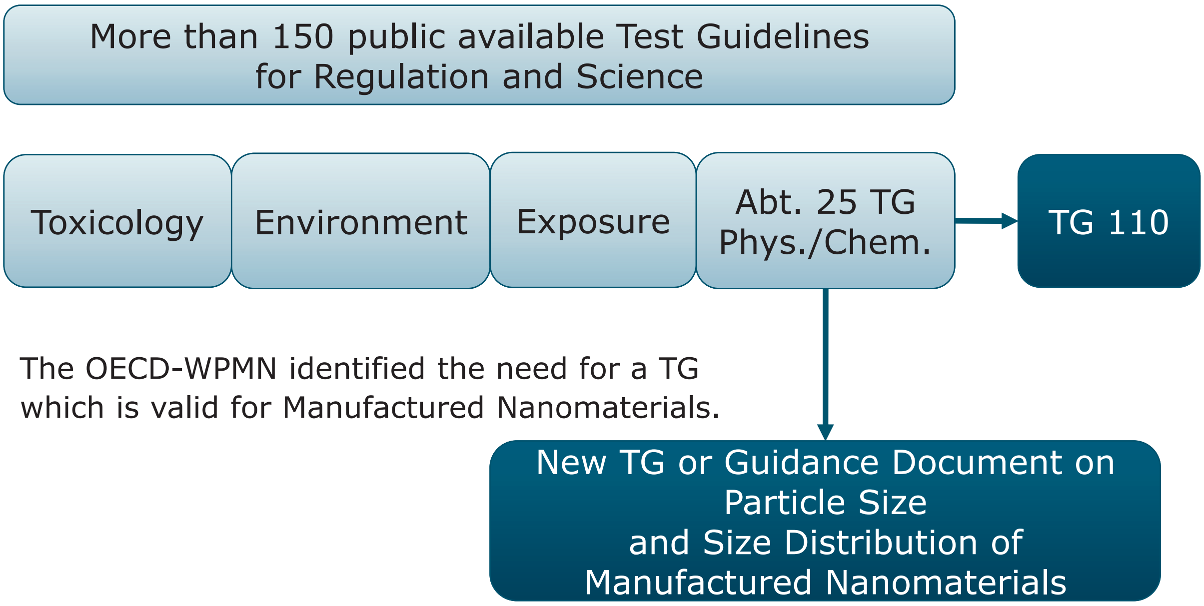
Harald Bresch[1], Alexandra Schmidt[1],
Volker Bachmann[2], Kerstin Kämpf[2], Thomas Kuhlbusch[2],
Kathrin Schwirn[3], Doris Völker[3]

[1]Bundesanstalt für Materialfoschung und –prüfung (BAM)
[2]Bundesanstalt für Arbeitsschutz und Arbeitsmedizin (BAuA)
[3]Umweltbundesamt (UBA)

www.bam.de

OECD Test Guidelines

BAM



More than 150 public available Test Guidelines for Regulation and Science

- Toxicology
- Environment
- Exposure
- Abt. 25 TG Phys./Chem. → TG 110

The OECD-WPMN identified the need for a TG which is valid for Manufactured Nanomaterials.

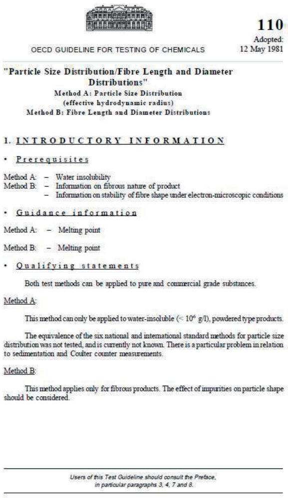
New TG or Guidance Document on Particle Size and Size Distribution of Manufactured Nanomaterials

→ Poster contribution Kathrin Schwirn (UBA)

OECD TG 110: Particle Size Distribution Fibre Length and Diameter Distribution

BAM

- Published 1981
- Valid for particles with d > 0.35 µm
- Valid for fibres with d > 0.2 µm
- Some standards are meanwhile withdrawn
- Adressed methods:
  Centrifugation, TEM, SEM and Coulter Counter



OECD GUIDELINE FOR TESTING OF CHEMICALS

110
Adopted:
12 May 1981

"Particle Size Distribution/Fibre Length and Diameter Distributions"
Method A: Particle Size Distribution
(effective hydrodynamic radius)
Method B: Fibre Length and Diameter Distributions

1. INTRODUCTORY INFORMATION

- **Prerequisites**

Method A: – Water insolubility
Method B: – Information on fibrous nature of product
– Information on stability of fibre shape under electron-microscopic conditions

- **Guidance information**

Method A: – Melting point

Method B: – Melting point

- **Qualifying statements**

Both test methods can be applied to pure and commercial grade substances.

Method A:

This method can only be applied to water-insoluble (< 10^{6} g/l), powdered type products.

The equivalence of the six national and international standard methods for particle size distribution was not tested, and is currently not known. There is a particular problem in relation to sedimentation and Coulter counter measurements.

Method B:

This method applies only for fibrous products. The effect of impurities on particle shape should be considered.

Users of this Test Guideline should consult the Preface, in particular paragraphs 3, 4, 7 and 8.

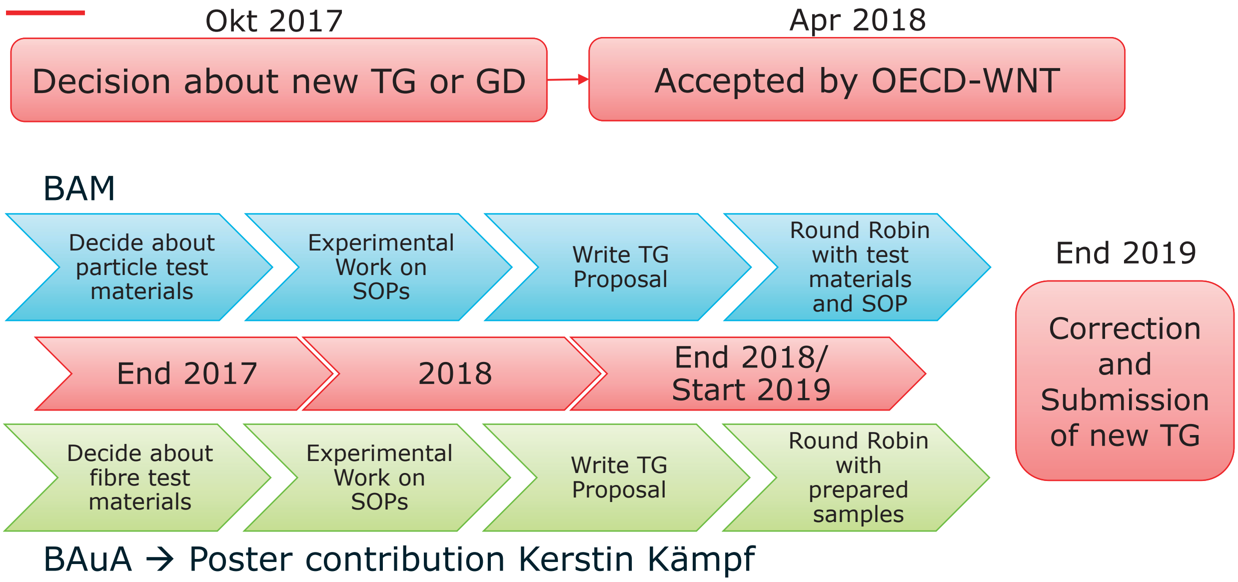
Germany has agreed to develop a new Test Guideline on Particle Size and Size Distribution of Manufactured Nanomaterials

BMU/UBA assigned the development to BAM (Nanoparticles) and BAuA (Nanofibres) .

Nanofibres: → Poster contribution Kerstin Kämpf (BAuA)

Presumable project timeline

BAM



Okt 2017
Decision about new TG or GD

Apr 2018
Accepted by OECD-WNT

BAM

Decide about particle test materials

Experimental Work on SOPs

Write TG Proposal

Round Robin with test materials and SOP

End 2017

2018

End 2018/
Start 2019

Decide about fibre test materials

Experimental Work on SOPs

Write TG Proposal

Round Robin with prepared samples

BAuA → Poster contribution Kerstin Kämpf

End 2019
Correction and Submission of new TG

OECD TG on Particle Size and Size Distribution of Manufactured Nanomaterials

General

- The TG has to address particles/fibres with d < 100 nm
- The validity should cover d < 1000 nm
- Nano-relevant properties need to be addressed

Method

- The measurement principles for nanoparticles (NP) deliver different diameters (Aerod., Hydrod, Mobility, Optical, ...)
- The measurement method might change the NP (Vacuum, radiation damage)

Notation

- There is still no agreement about the main measure for a particle size distribution (volume, surface, number)
- Units are generally not used as proposed by ISO 9276.
- In most reports the errors and uncertainties are not addressed properly.

Methods for measuring the size of Manufactured Nanomaterials *

Commonly used methods	
TEM	
SEM	incl. TSEM/ESEM
DMA	(SMPS/DMAS/FMPS)
DLS	Prop. combined with FFF
CLS	Different versions
NTA	(PTA)
SAXS	Prop. combined with FFF
AFM	
spICP-MS	

Specific and uncommon methods	
DOSI-NMR	NMR-active
Ultrasonication	Calculated
XRD	Crystalline
GiXRF	< ca. 50 nm
ToF-AMS	> ca. 70 nm
To be continued	…

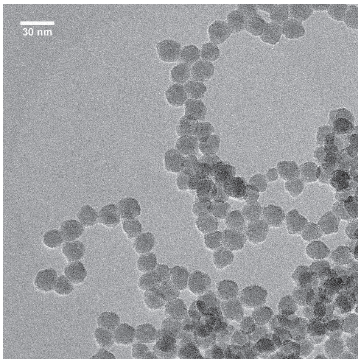
* Based on the OECD-WPMN Series on the Safety of Manufactured Nanomaterials, No. 65 (2016)

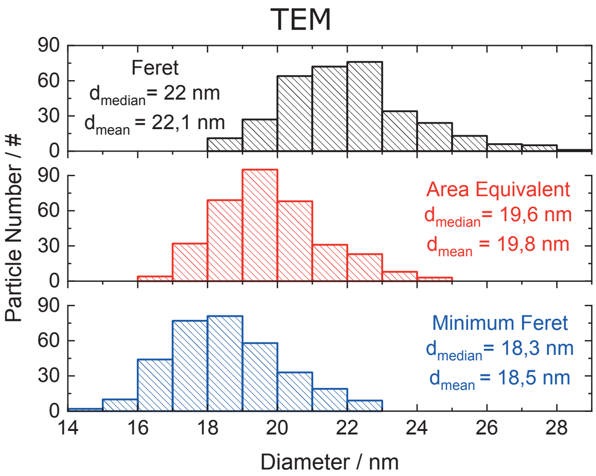
Selection of test materials

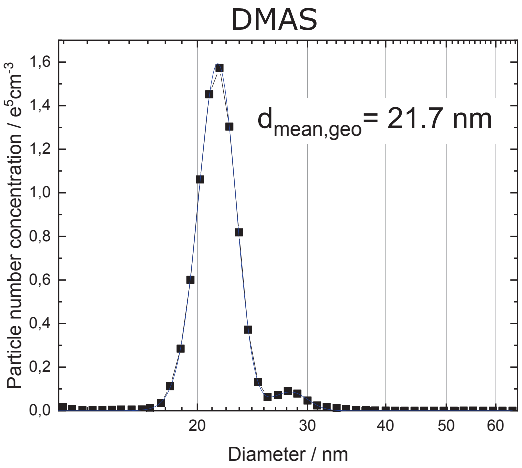
	Ag N001 (BAM)	SiO_2 (KRISS)	SiO_2 (KRISS)	ZnO NM110 (JRC)	PSL Mix (NIST)	TiO_2 /Al (IRMM)	PSL Mix (NIST)
	17 nm	20 nm	50 nm	<100 nm	80/120 nm	200-300 nm	80/900 nm
Core-Shell Diameter	X					x	
Functionalisation	X					x	
Not ideal spherical				x		x	
Metal	X						
Anorganic		x	x	x		x	
Organic					x		x
Solubility				x			
Amorph		x	x		x		x
Crystalline	X			x		x	
Narrow Mixture					x		
Mixture two decades							X
Radiation damage		x	x				
Hydrophob					x		X

Example SiO_2 20 nm, monodisperse distribution

Images with kind regards to BAM FB 5.1 (I. Dörfel)







Example SiO_2 20 nm / 50 nm monodisperse distribution

Method	20 nm	50 nm
TEM (max Feret)	22.1 nm	54.5 nm
TEM (Area)	19.8 nm	49.9 nm
TEM (min Feret)	18.5 nm	48.0 nm
DMAS (Mobility)	21.7 nm	54.0 nm
SEM (max Feret)	21.3 nm	57.3 nm
SEM (Area)	18.5 nm	51.0 nm
SEM (min Feret)	17.6 nm	48.7 nm
DLS (Hydrodyn.)	19.3 nm	54.7 nm

Example ZnO NM110 – TEM polydisperse distribution

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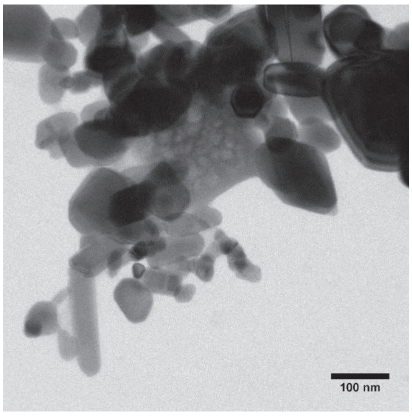
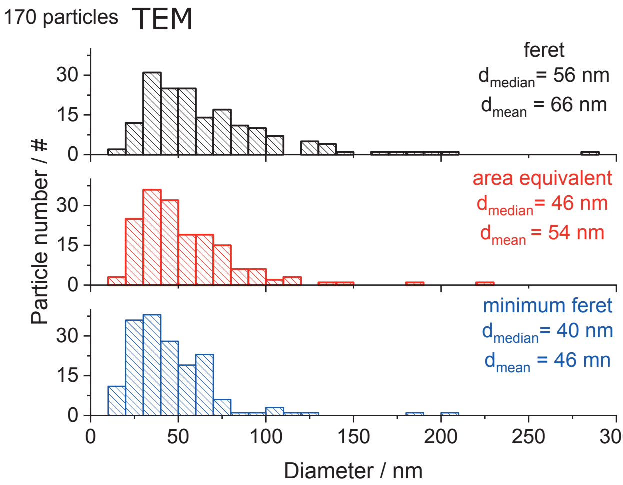
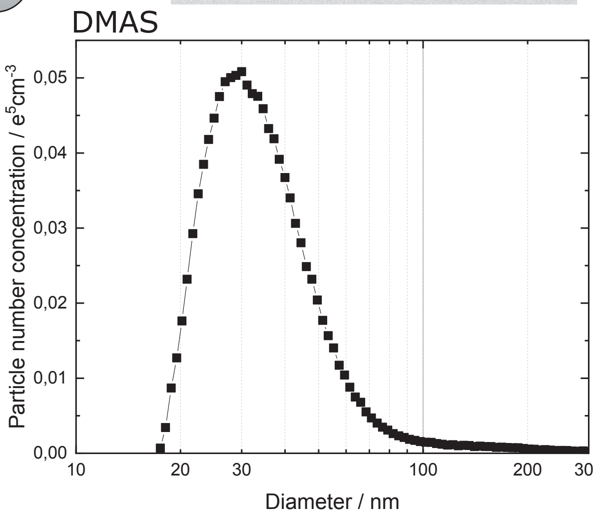


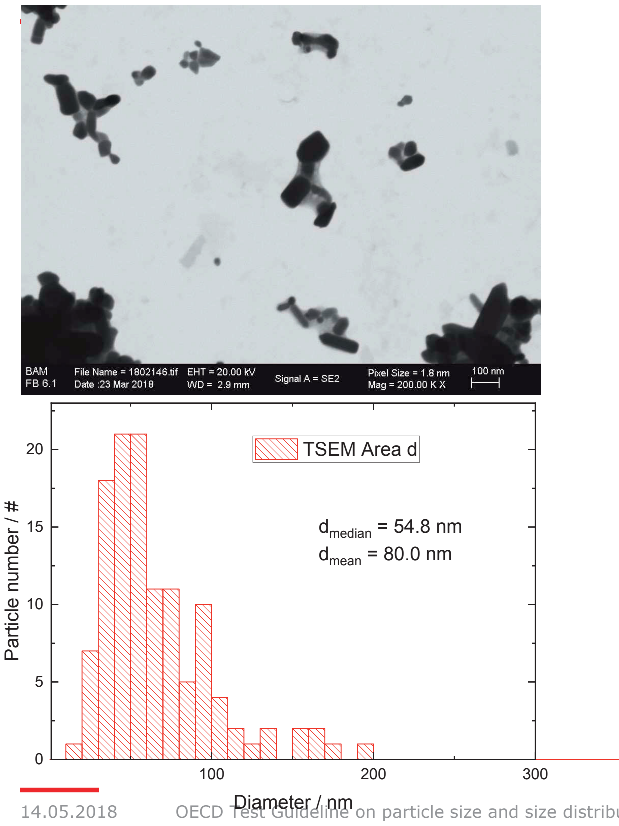
Possible reasons for differences:
a) Dissolution
b) Hidden particles
c) Shape dependence
d) Image statistics

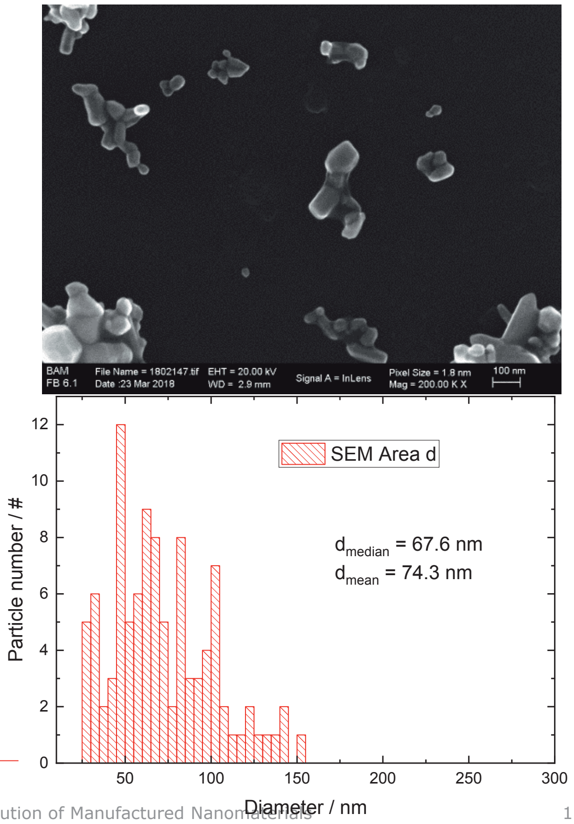




ZnO NM110 – SEM

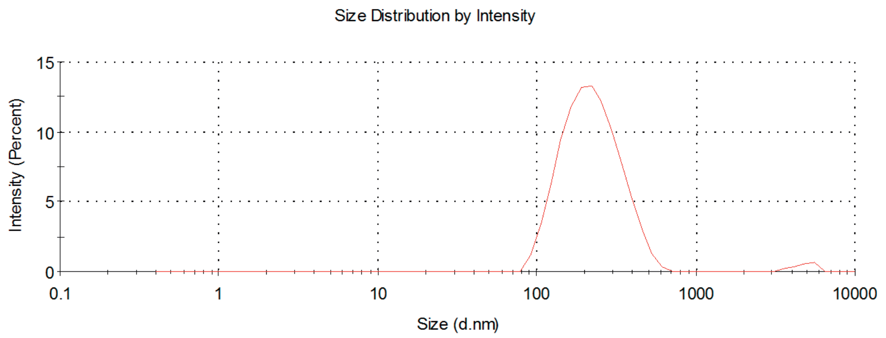
Images with kind regards to BAM FB 6.1 (V.-D. Hodoroaba)



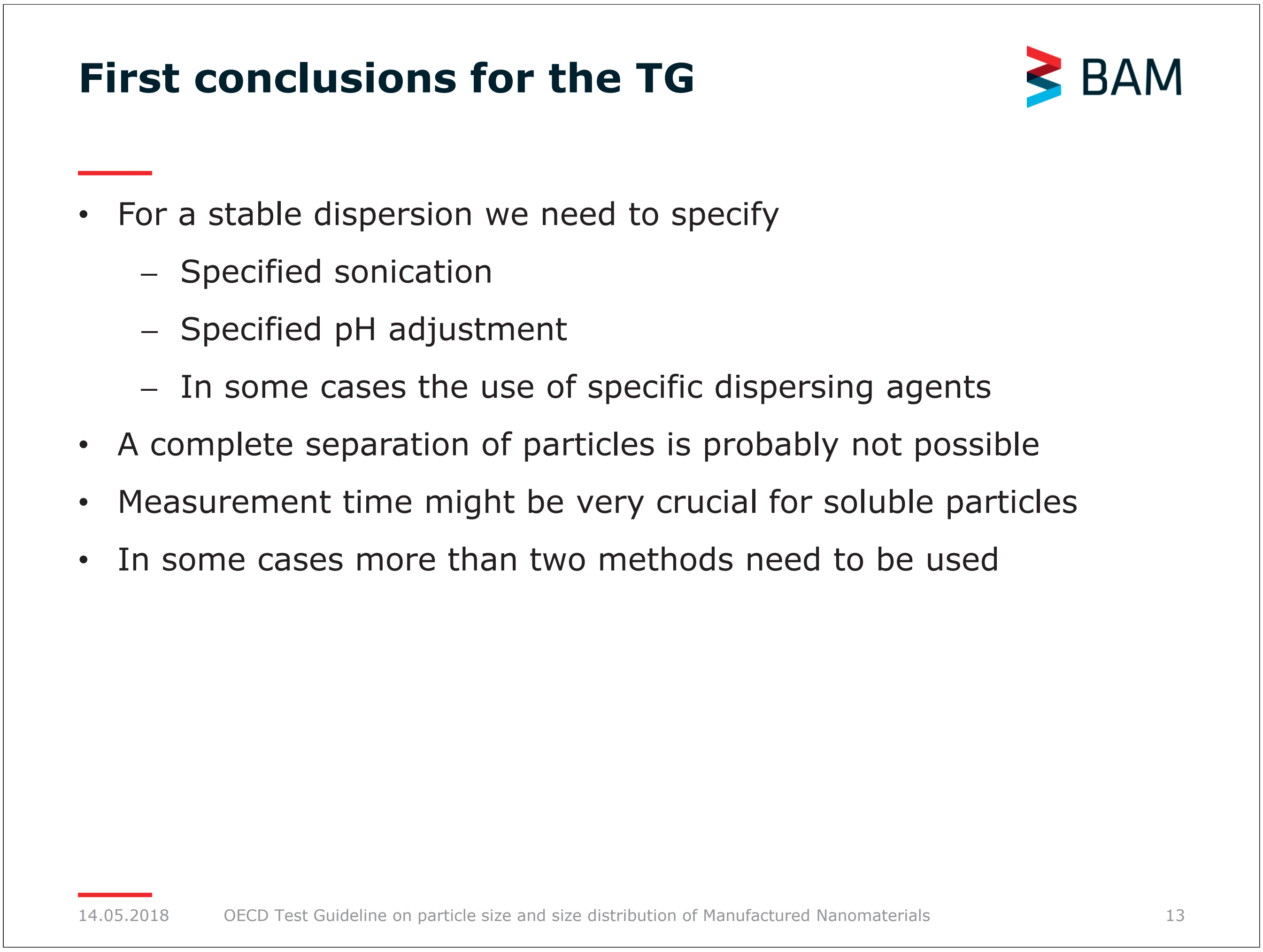


ZnO NM110 - DLS

Results with kind regards to BAM FB 1.3

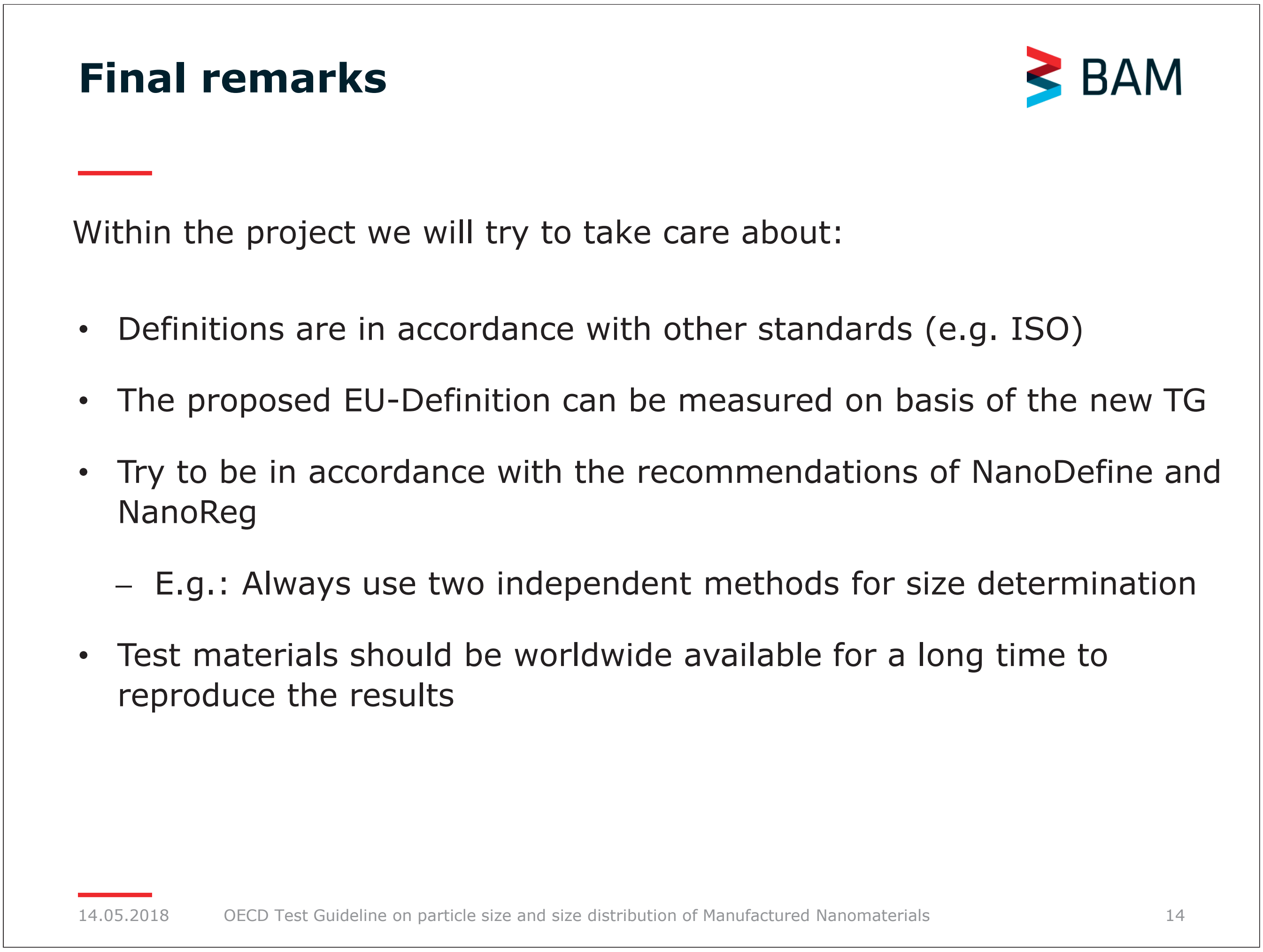


Messung	1	2	3	4	5
D_{AV}/nm	207	204	206	207	203



First conclusions for the TG

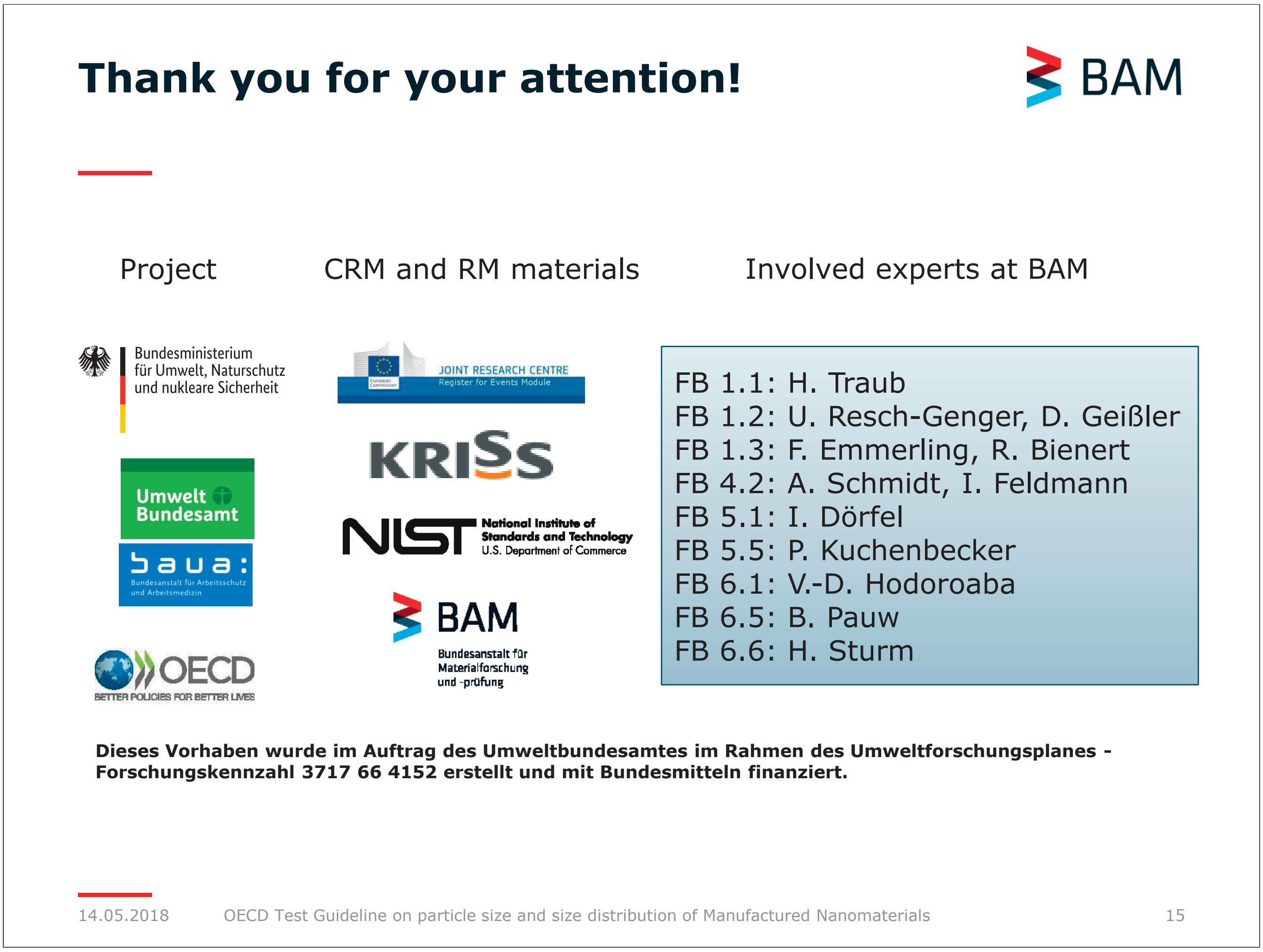
- For a stable dispersion we need to specify
  - Specified sonication
  - Specified pH adjustment
  - In some cases the use of specific dispersing agents
- A complete separation of particles is probably not possible
- Measurement time might be very crucial for soluble particles
- In some cases more than two methods need to be used



Final remarks

Within the project we will try to take care about:

- Definitions are in accordance with other standards (e.g. ISO)
- The proposed EU-Definition can be measured on basis of the new TG
- Try to be in accordance with the recommendations of NanoDefine and NanoReg
  - E.g.: Always use two independent methods for size determination
- Test materials should be worldwide available for a long time to reproduce the results



Thank you for your attention!

BAM

Project

Bundesministerium für Umwelt, Naturschutz und nukleare Sicherheit

Umwelt Bundesamt

baua: Bundesanstalt für Arbeitsschutz und Arbeitsmedizin

OECD BETTER POLICIES FOR BETTER LIVES

CRM and RM materials

European Commission JOINT RESEARCH CENTRE Register for Events Module

KRISS

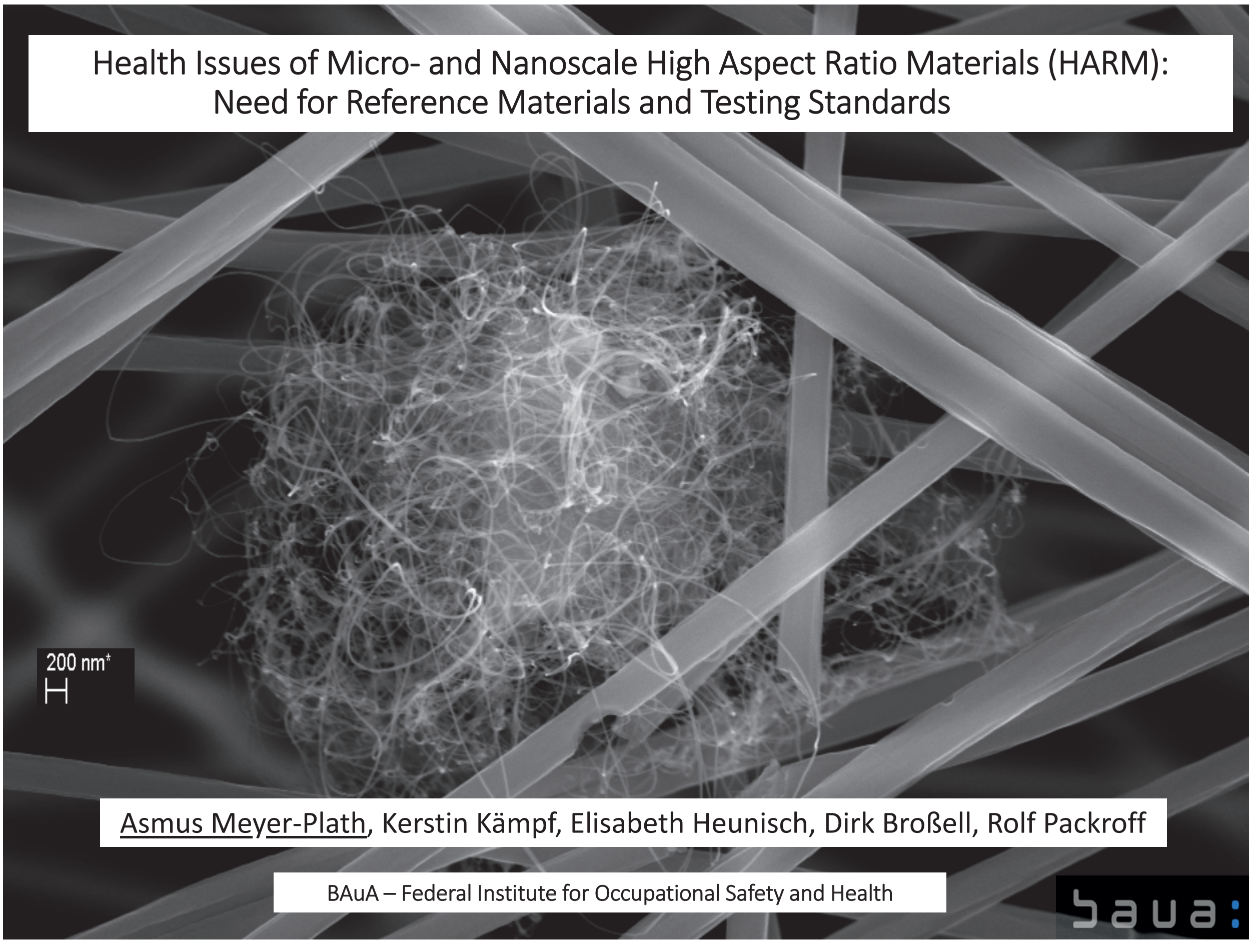
NIST National Institute of Standards and Technology U.S. Department of Commerce

BAM Bundesanstalt für Materialforschung und -prüfung

Involved experts at BAM

FB 1.1: H. Traub
FB 1.2: U. Resch-Genger, D. Geißler
FB 1.3: F. Emmerling, R. Bienert
FB 4.2: A. Schmidt, I. Feldmann
FB 5.1: I. Dörfel
FB 5.5: P. Kuchenbecker
FB 6.1: V.-D. Hodoroaba
FB 6.5: B. Pauw
FB 6.6: H. Sturm

Dieses Vorhaben wurde im Auftrag des Umweltbundesamtes im Rahmen des Umweltforschungsplanes - Forschungskennzahl 3717 66 4152 erstellt und mit Bundesmitteln finanziert.

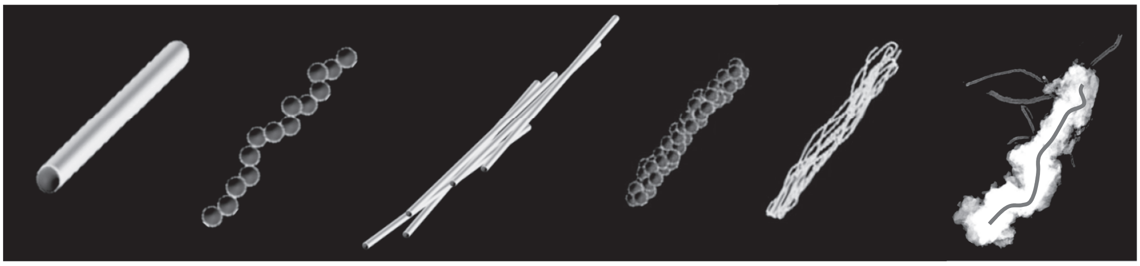


Health Issues of Micro- and Nanoscale High Aspect Ratio Materials (HARM): Need for Reference Materials and Testing Standards

Asmus Meyer-Plath, Kerstin Kämpf, Elisabeth Heunisch, Dirk Broßell, Rolf Packroff

BAuA – Federal Institute for Occupational Safety and Health

The Basics – What is a Fibre? – What is HARM?



Individual fibre **Particle percolation** **Fibre bundle** **Aggregate** **Agglomerate** **Composite particle with protruding or contained fibres**

Everything that [in a blurry image] **looks like a Fibre …**
… not only compact fibrous objects!

We therefore suggest using the more general term:

High Aspect Ratio Material (HARM), not Fibre.

What HARM types may cause harm? …

The most prominent High Aspect Ratio Material ...

ASBESTOS DUST IS A KILLER

Worldwide, more than 100,000 people a year die from lung diseases caused by exposure to asbestos 20–40 years ago

If you do accidentally disturb asbestos-containing materials:
- stop work immediately
- move everyone away from the area
- do not remove equipment or materials
- close, seal or lock off the area
- put up warning signs
- report it
- call a specialist contractor to clean up

Don't become one of tomorrow's statistics – protect yourself today

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WORKING TOGETHER TO BEAT OCCUPATIONAL CANCER
The Institution of Occupational Safety and Health is campaigning to stop thousands of untimely deaths due to work-caused cancer.
www.notimetolose.org.uk

New IOSH Campaign
„NO TIME TO LOSE“

Promoted during the

Congress of the
International
Commission on
Occupational **H**ealth
(ICOH) 2018

Dublin 2018

Focus at BAuA: Health Issues Related to Inhalative Exposure

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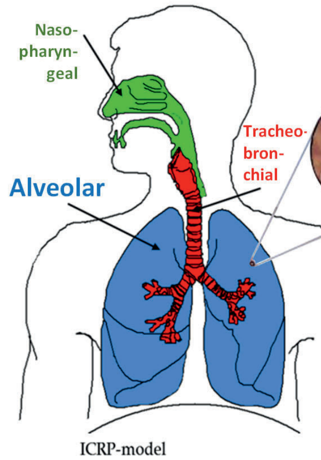
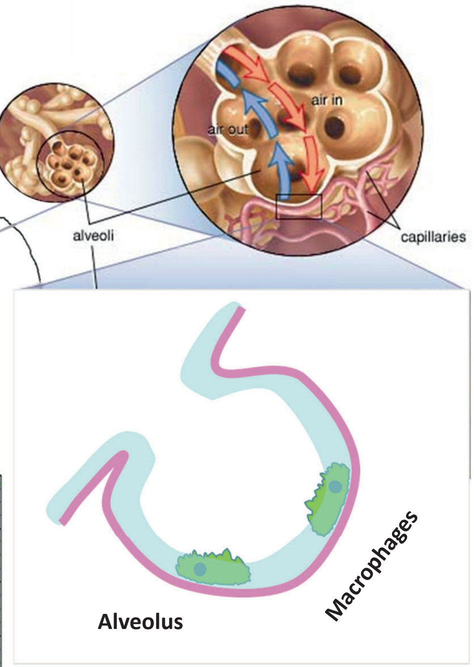
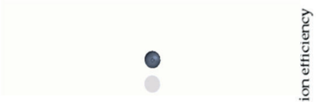
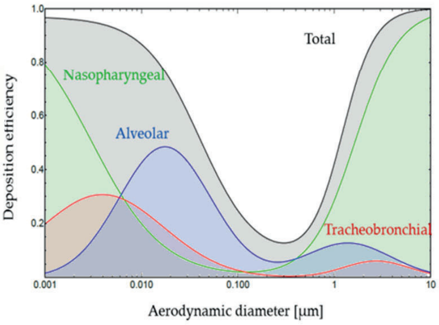






In direction of flight, fibres look and fly like particles



This is a nanomaterial workshop!

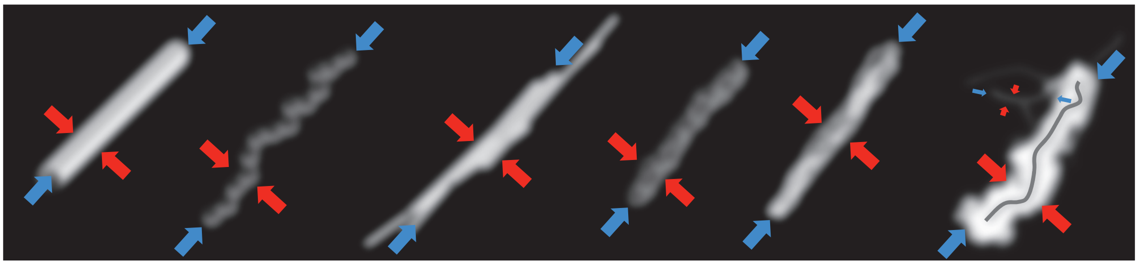
Why mention Asbestos and address HARM and not restrict to High Aspect Ratio Nanomaterials (HARN)?

For our lung, alveolar respirability matters !

The size limit of 100 nm does help to identify toxic objects.

WHO Fibre Definition* developed for Asbestos and MMMFs

*[World Health Organization, Determination of airborne fibre number concentrations: a recommended method, by phase-contrast optical microscopy (membrane filter method), 1997]



Fibre Definition of WHO*

- **Thinner than 3 µm*** => **Alveolar respirability**
- **Longer than 5 µm** => **Failing alveolar clearance**
- **3x longer than thick** => **Elongated shape, i.e., fibre character**

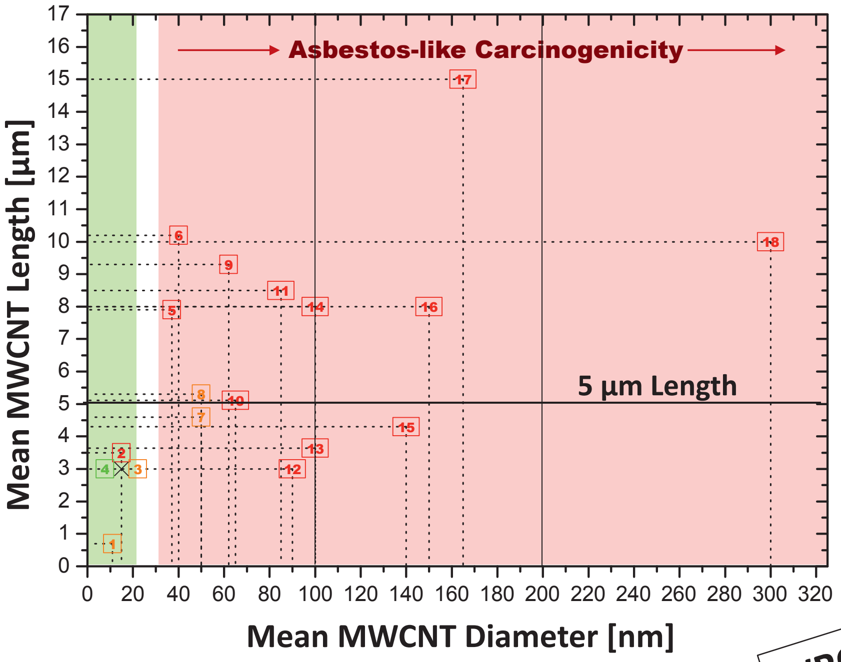
* **WHO Counting limit 200 nm**

WHO addresses long respirable fibres, which implies an upper limit on diameter.

However, according to the WHO counting rules*,
fibres thinner than 200 nm remain uncounted.

So, what about sub-200-nm fibres? …

Fibre Hazard Assessment by means of Intraperitoneal Testing
of Multi-Walled Carbon Nanotubes (MWCNT)



25 Years
1991-2017

[1] Muller, J., et al. (2009)
Toxicol. Sci. 110(2): 442-448
[2,17] Murphy, F. A., et al. (2011)
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[18] Sargent, L. M., et al. (2014)
Part. Fibre Toxicol. 11: 3

Data compiled by
[F. Herzberg, BfR]

F. Herzberg, D. Kehren, A. Meyer-Plath

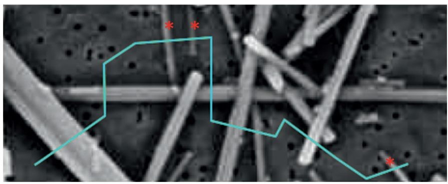
The CNT community used to say
"Asbestos – That's a complete different story!"

WRONG!

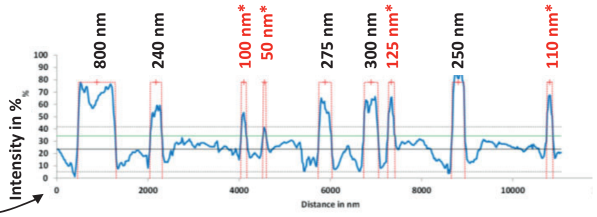
… and there are nanoscale asbestos fractions
thinner than 200 nm that remain currently uncounted*

***DGUV 213-546**

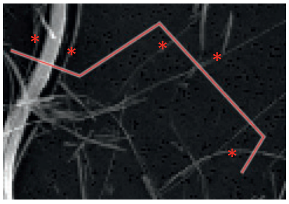
Krokydolith



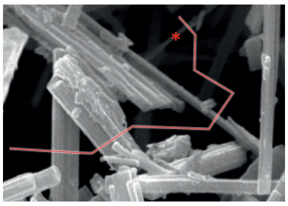
[Creutzenberg, O., et al. (2005). Schriftenreihe der BAuA.]



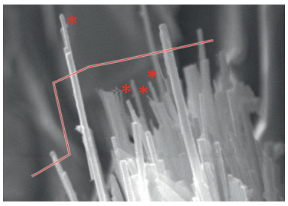
Chrysotile



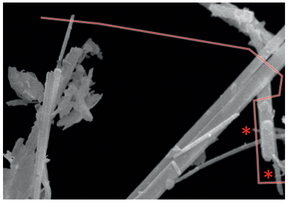
Amosite



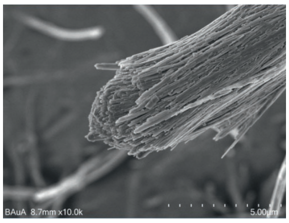
Winchite-Richterite

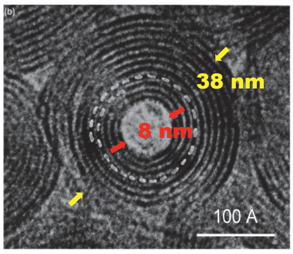


Anthophyllite



[http://usgsprobe.cr.usgs.gov/picts2.html]





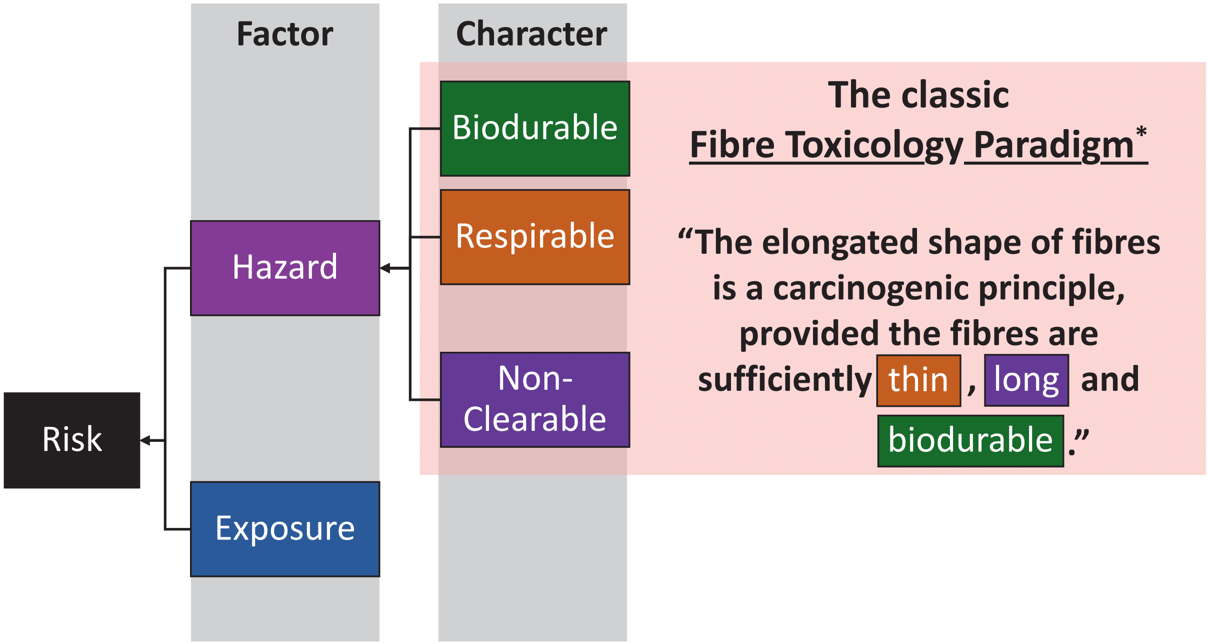
[Lotz, B. and S. Z. D. Cheng (2005). Polymer 46(3): 577-610.]

Chrysotile is a bundled nanoscroll !

… and here meet the Nano and Asbestos world !

A. Meyer-Plath

HARM-related Risk – According to the Fibre Toxicology Paradigm



Factor

Character

Biodurable

Respirable

Non-Clearable

Hazard

Risk

Exposure

The classic

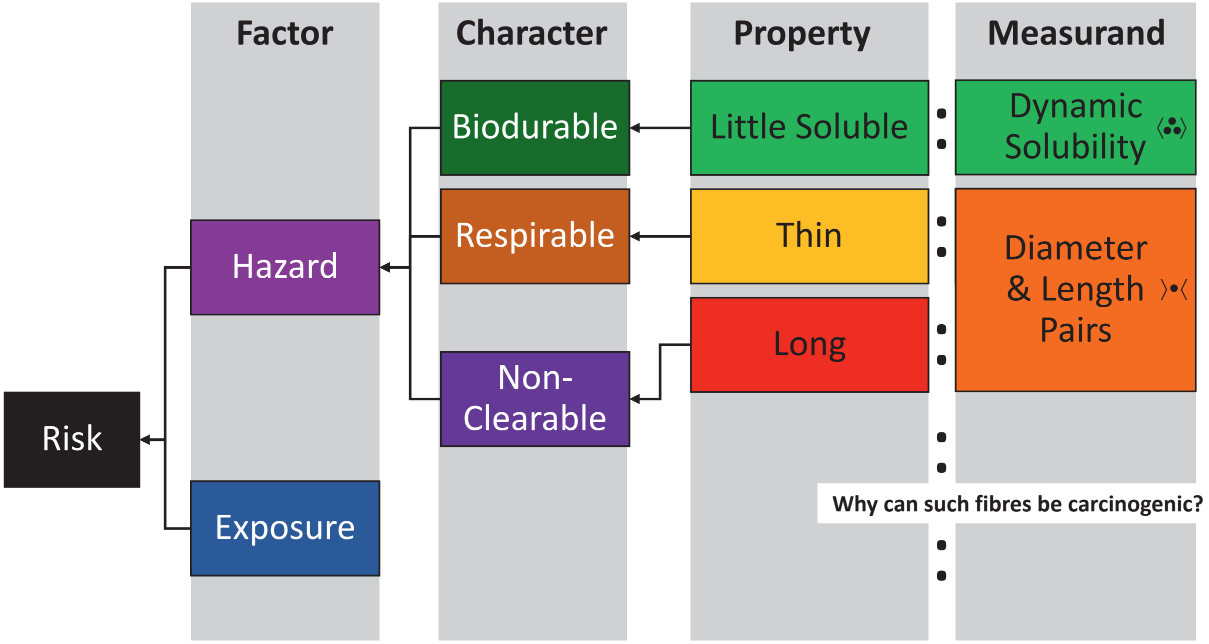
<u>Fibre Toxicology Paradigm</u>*[*]*

“The elongated shape of fibres is a carcinogenic principle, provided the fibres are sufficiently thin , long and biodurable .”

* [Pott, F. and K. H. Friedrichs (1972). <u>Naturwiss.</u> **59**(7): 318.]
[Stanton, M. F. and C. Wrench (1972). <u>J. Nat. Cancer Inst.</u> **48**: 797-821.]

What properties do we have to assess in detail?

HARM-related Risk – According to the Fibre Toxicology Paradigm



Factor

Character

Property

Measurand

Biodurable

Little Soluble

Dynamic Solubility ⟨∴⟩

Respirable

Thin

Diameter & Length Pairs ⟩•⟨

Long

Non-Clearable

Hazard

Risk

Exposure

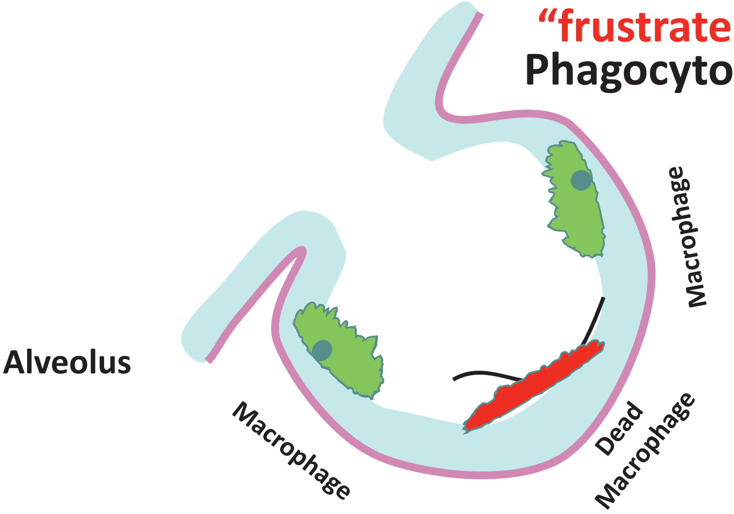
Why can such fibres be carcinogenic?

⟨∴⟩ : Ensemble based. ⟨•⟩ : Individual particle based, but averaged. ⟩•⟨ : Individual particle specific.

According to the Fibre Toxicology Paradigm,
the Clearance Mechanism of the Alveoli may fail for HARM

× No Clearance !!!

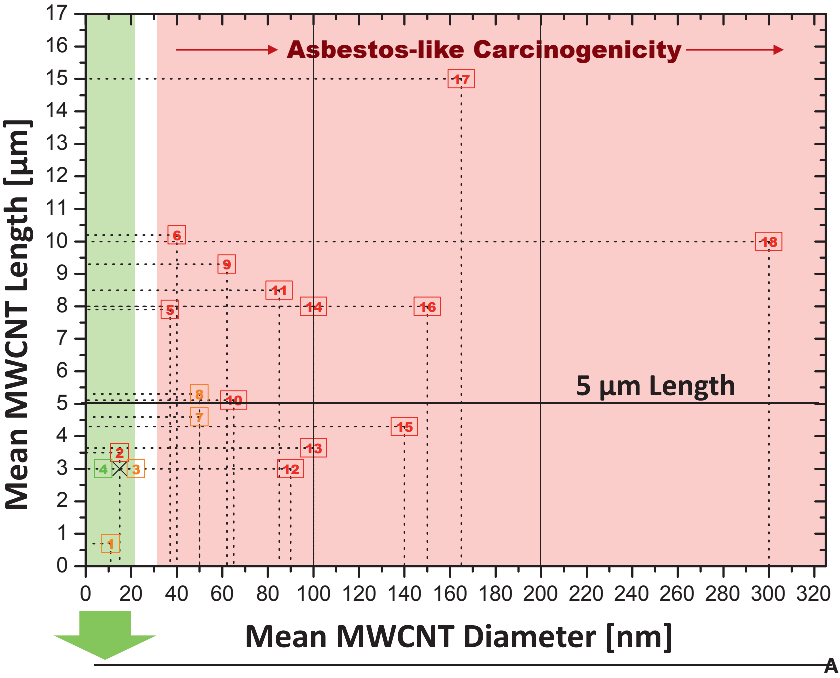
“frustrated” Phagocytosis



Long-term alveolar persistence of HARM objects may induce chronic inflammations that may induce cancer.

What properties of HARM may impede phagocytosis ?

Fibre Hazard Assessment by means of Intraperitoneal Testing
of Multi-Walled Carbon Nanotubes (MWCNT)



25 Years

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[18] Sargent, L. M., et al. (2014)
Part. Fibre Toxicol. 11: 3

Data compiled by
[F. Herzberg, BfR]

And: By what mechanism could the diameter of a fibre affect its hazard? …

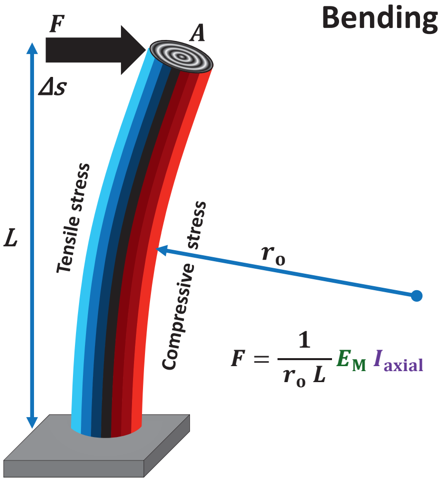
This data also raised a NEW question:

Is the Fibre Toxicology Paradigm not applicable to thin fibres?

F. Herzberg, D. Kehren, A. Meyer-Plath

Let’s don’t talk about diameter but introduce a more relevant fibre property:

Flexural Rigidity



$$F = \frac{1}{r_o\,L} E_M\, I_{\text{axial}}$$

Required Bending Force

Material Property

Elastic Modulus

$$F \sim \boxed{E_m \cdot I_{axial}} \leftarrow \textbf{Rigidity}$$

Geometric Property

Axial Second Moment of Area

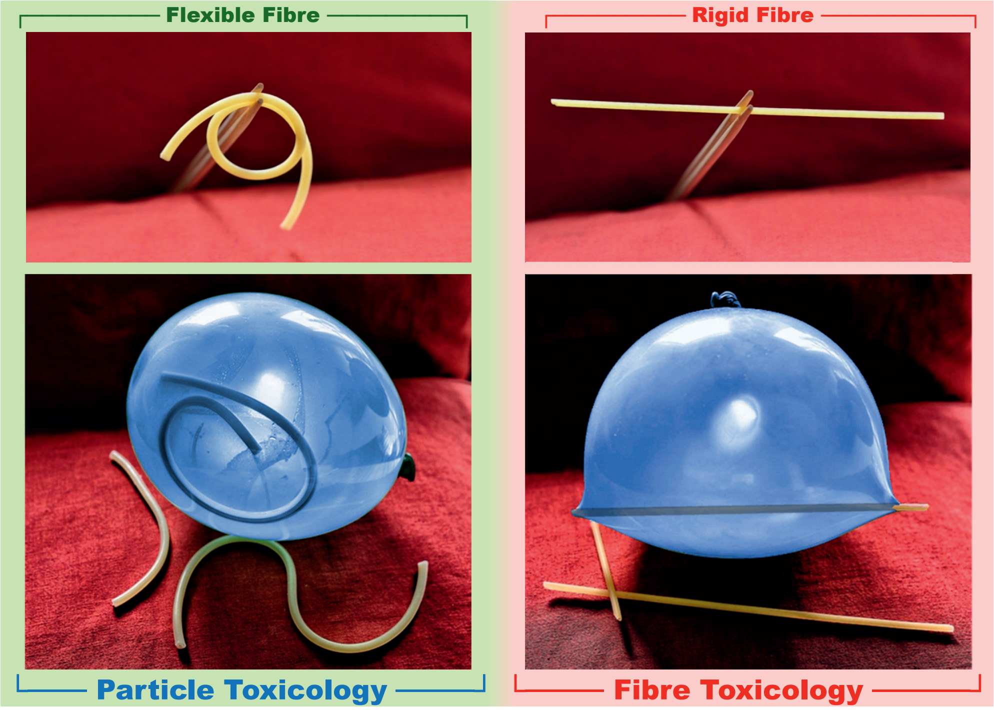
$$I_{\text{axial}} = \int_{Disk(r)} \vec{x} \cdot \begin{pmatrix} 1 \\ 0 \end{pmatrix} d\vec{x} = \frac{\pi}{4} r^4$$

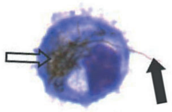
Fibre Cross-section Area

By what mechanism could Rigidity affect fibre hazard? …

We propose Fibre Rigidity as additional Toxicological Parameter

… since Alveolar Macrophages perform the *Al-dente* Test !



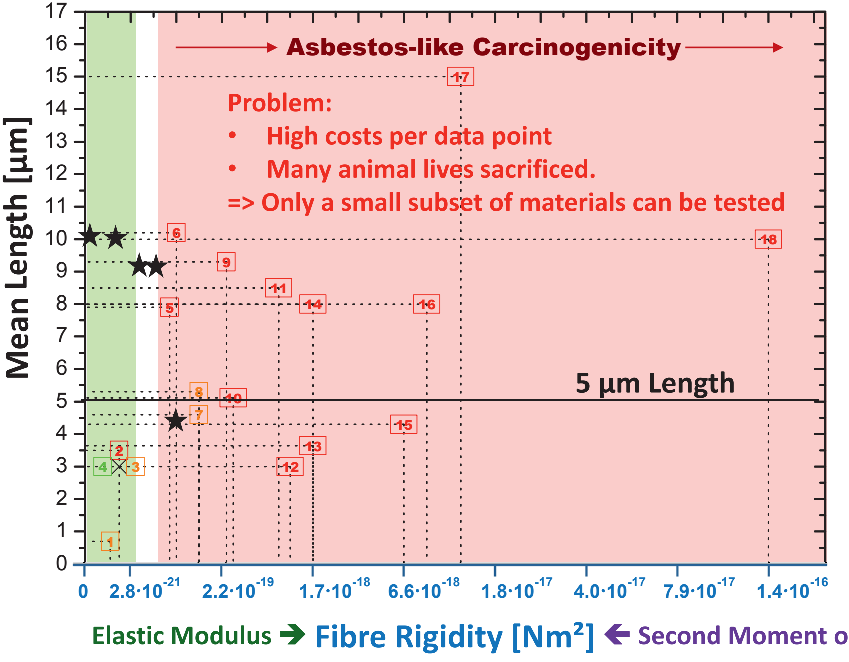


[Donaldson et al. Particle Fibre Toxicol. 2010, 7:5]

What could be the benefit of considering a fibres Rigidity?

Transfer of toxicological findings from MWCNTs to other HAR Materials using Rigidity

BAuA-initiated ★ i.p. testing to close data gaps



Problem:
- High costs per data point
- Many animal lives sacrificed.

=> Only a small subset of materials can be tested

for universal rigidity-related toxicological hazard grouping

1991-2017

[1] Muller, J., et al. (2009)
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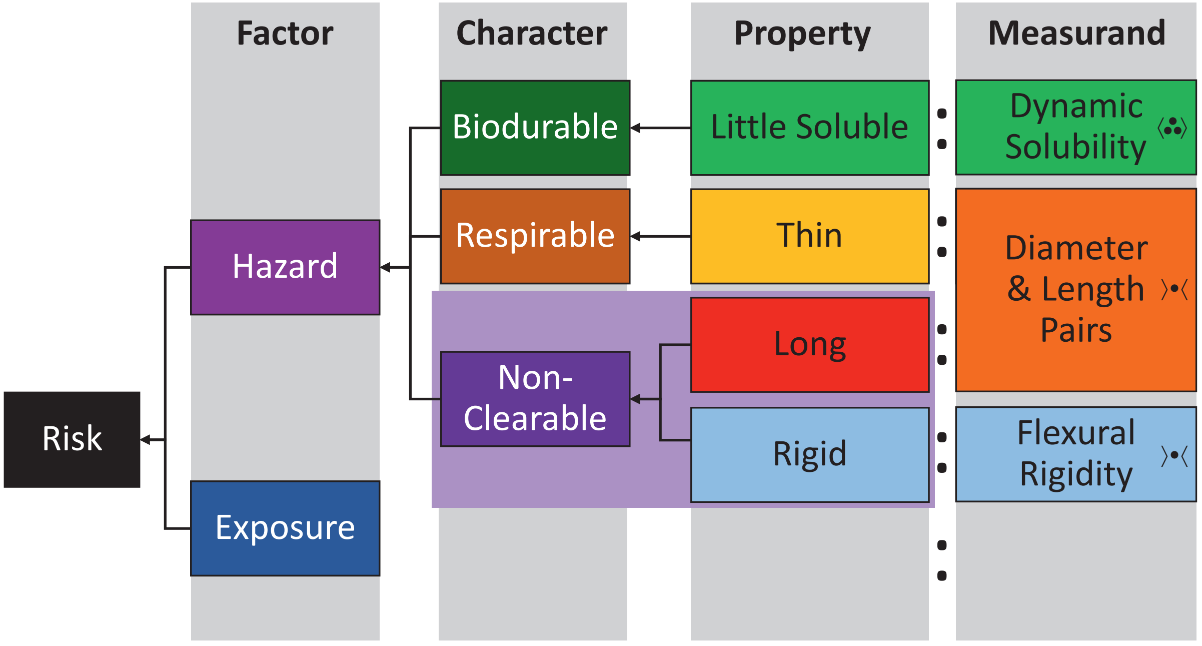
[F. Herzberg, BfR]

Our Hypothesis:
Rigidity will allow
using & extending
this valuable data to other materials.

HARM-related Risk – So we also need to measure Rigidity!



Factor | **Character** | **Property** | **Measurand**

Risk

Hazard

Exposure

Biodurable

Respirable

Non-Clearable

Little Soluble

Thin

Long

Rigid

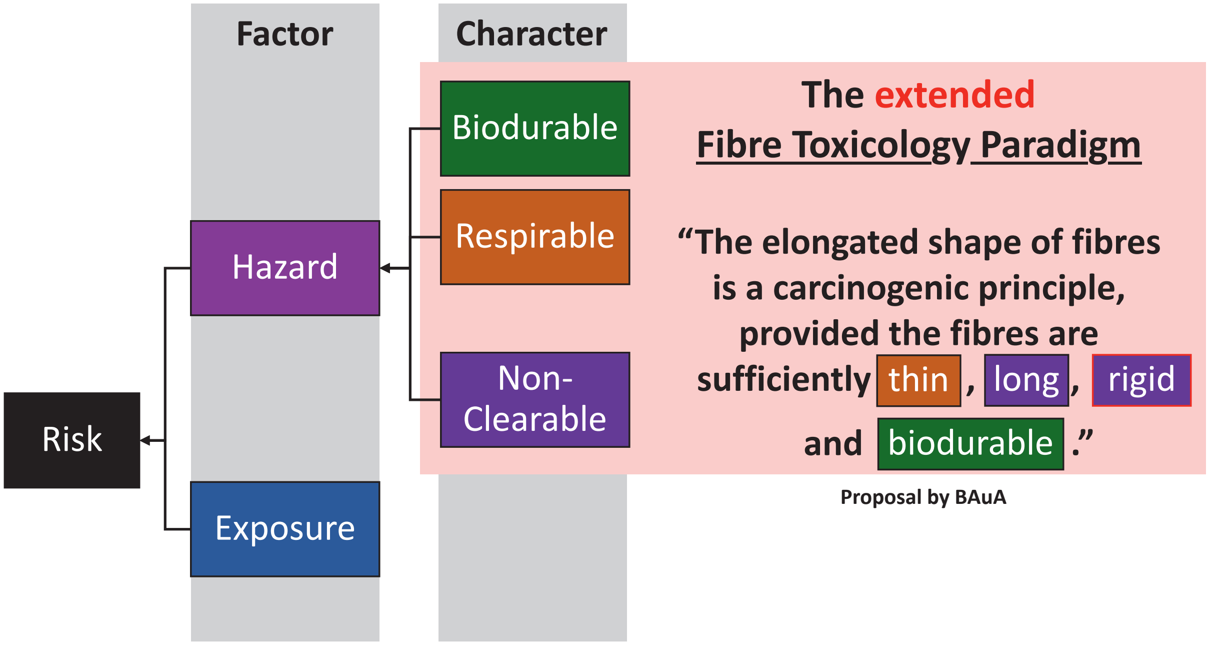
Dynamic Solubility ⟨∴⟩

Diameter & Length Pairs ⟩•⟨

Flexural Rigidity ⟩•⟨

⟨∴⟩ : Ensemble based. ⟨•⟩ : Individual particle based, but averaged. ⟩•⟨ : Individual particle specific.

HARM-related Risk – The Extended Fibre Toxicology Paradigm



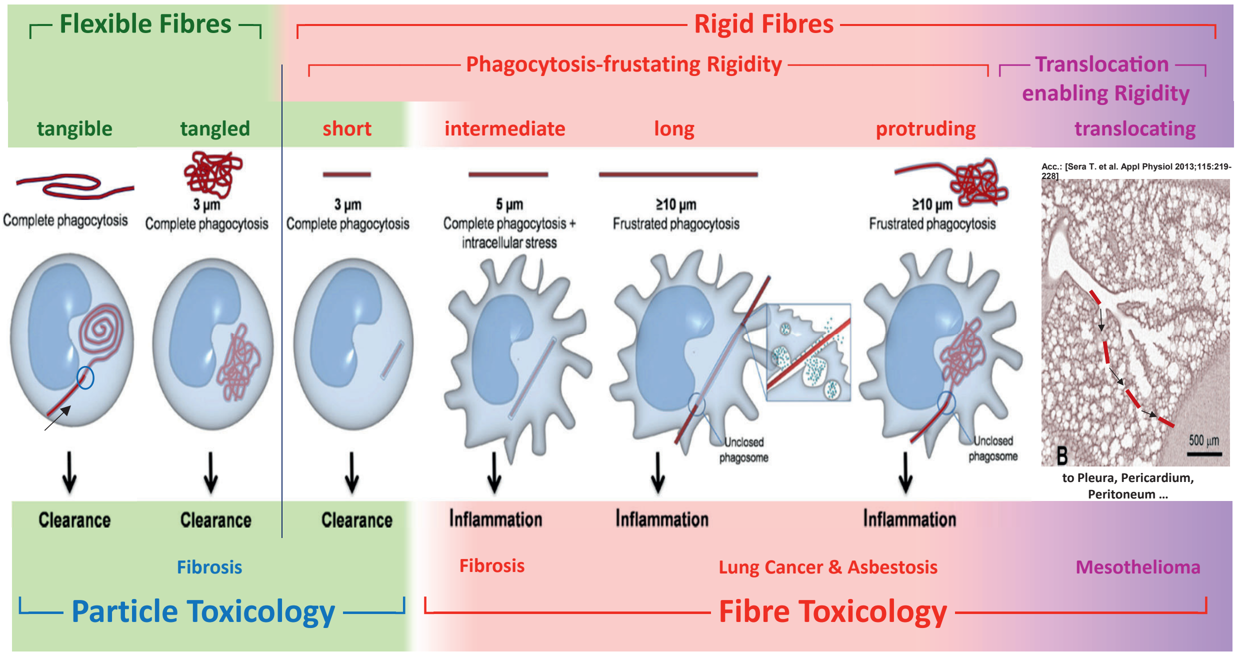
The extended Fibre Toxicology Paradigm

“The elongated shape of fibres is a carcinogenic principle, provided the fibres are sufficiently thin, long, rigid and biodurable.”

Proposal by BAuA

The property Rigidity may require even more refinement …

Rigidity-extended Fibre Toxicology

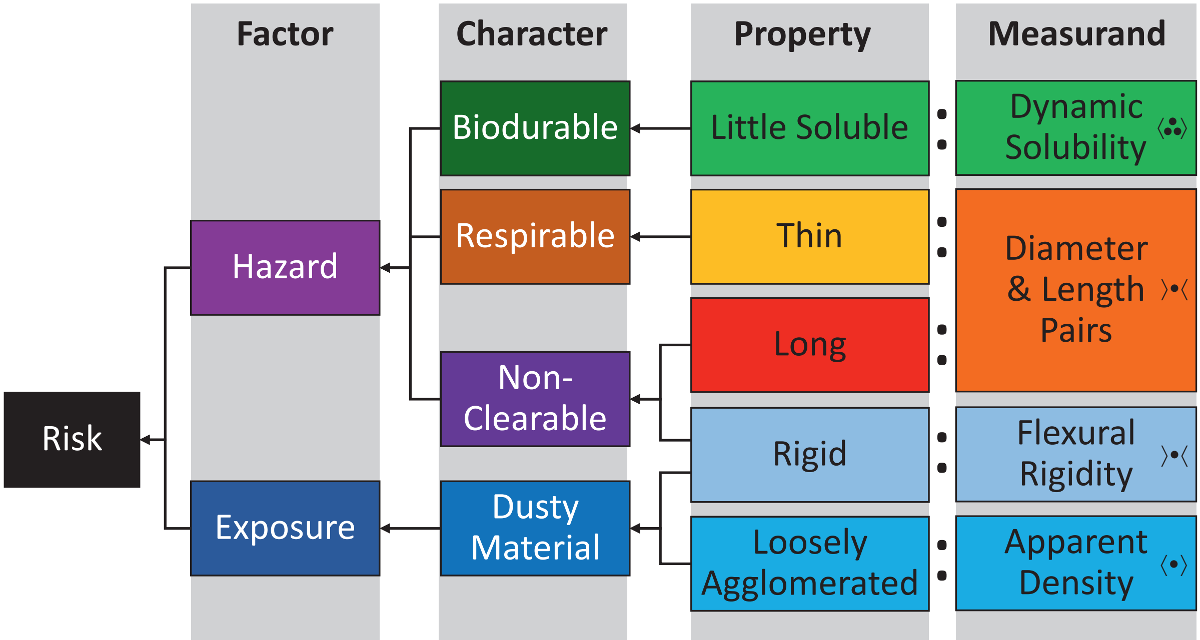


Our Hypothesis

Rigidity may be of central importance for the understanding of the toxicology of biodurable HARM.

Cell drawings inspired by [Schinwald A. et al., Particle and Fibre Toxicology 2012, 9:34]

Let’s not forget : The Exposure Factor of HARM-related Risk

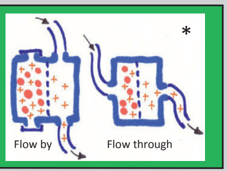
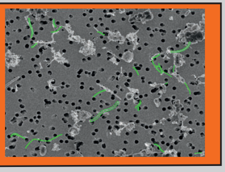


Factor	Character	Property	Measurand
	Biodurable	Little Soluble	Dynamic Solubility ⟨∴⟩
Hazard	Respirable	Thin	Diameter & Length Pairs ⟩•⟨
	Non-Clearable	Long	
		Rigid	Flexural Rigidity ⟩•⟨
Exposure	Dusty Material	Loosely Agglomerated	Apparent Density ⟨•⟩

Risk

⟨∴⟩ : Ensemble based. ⟨•⟩ : Individual particle based, but averaged. ⟩•⟨ : Individual particle specific.

Our Test Method Developments

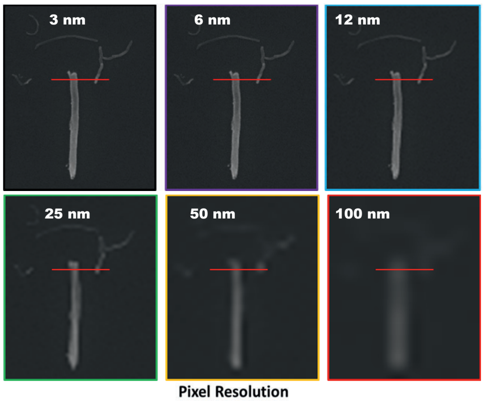
Measurand	Test Method	
Dynamic Solubility ⟨∴⟩	Flow Cell Dissolution*: Ion Leaching & Residual Mass 	• **nanoGRAVUR (BMBF)**
Diameter & Length Pairs ⟩•⟨	Electron Microscopy with Large Image Acquisition or Active Fibre Tracking, Fibre Analysis Software 	• **OECG TG (BMUB)** ➔ **Poster DNS-05**

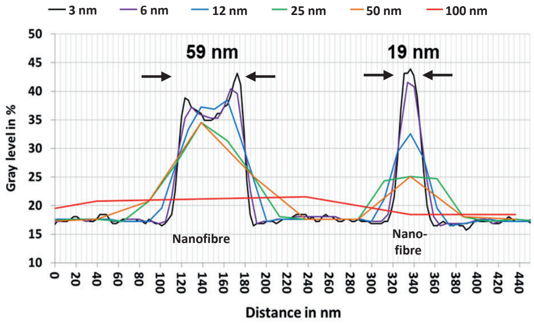
*Results of nanoGRAVUR

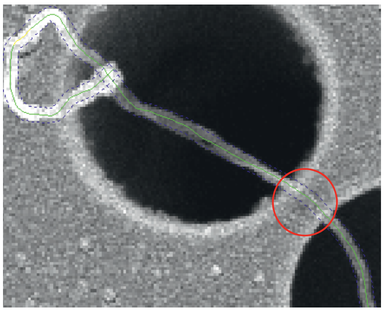
⟨∴⟩ : Ensemble based. ⟨•⟩ : Individual particle based, but averaged. ⟩•⟨ : Individual particle specific.

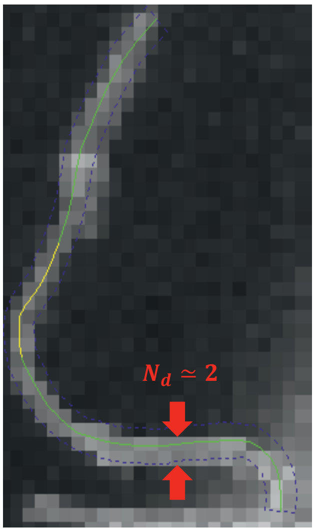
Example: How is the Diameter to be measured?

The fibre must be resolvable!
Chose the pixel resolution accordingly!



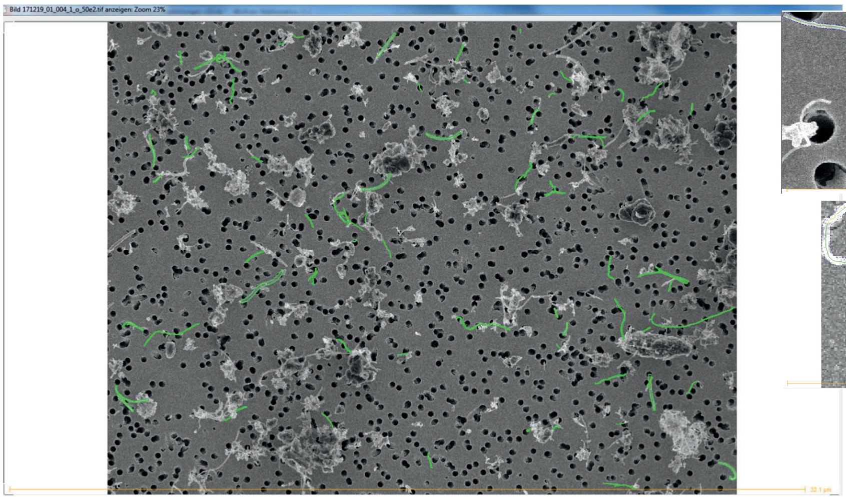


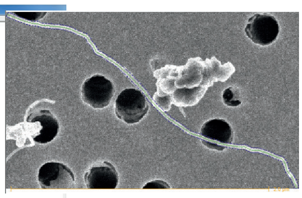


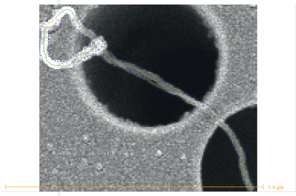


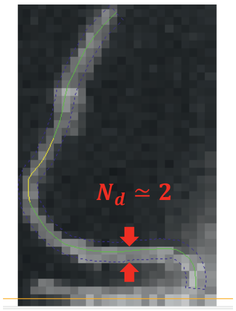
- **How to define the diameter in low SNR situations?**
- **What is the acceptable minimum Number of pixels per diameter N_d?**
- **$d_{\min}$ and N_d determine the necessary pixel resolution.**
- **SEM Magnification is not a *very* useful parameter in times of varying image pixel dimensions.**
- **Pixel resolution must be specified according to the diameter $d_{\min}$ to be measured.**

Statistics of Diameter and Length Pairs using BAuA's FibreDetect Software









Problem

- **The Image Pixel Dimension N_x x N_y limits the accessible Aspect Ratio**

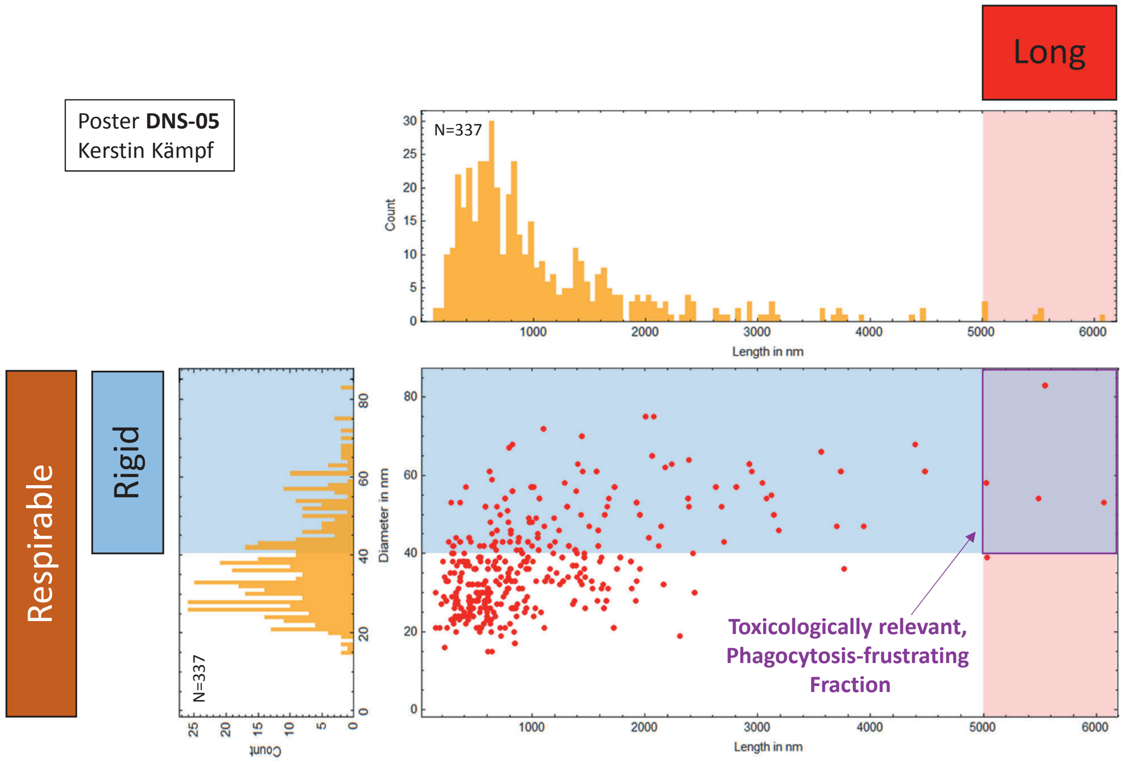
N_d: Pixels per Diameter, $\boxed{\text{Aspect Ratio } AR \simeq \frac{N_y}{N_d}}$

- **Large images or Active Fibre Tracking during SEM imaging are required for HAR !**

Length ℓ, Diameter $d = \frac{\ell}{AR}$, Diameter Resolution $\Delta d = \frac{d}{N_d}$

Example
Length and Diameter Measurement with Paired Values

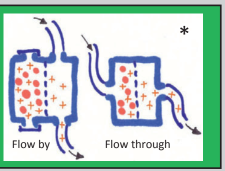
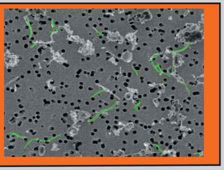
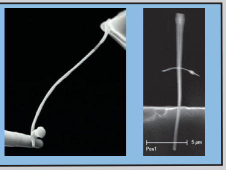
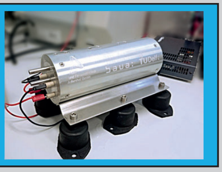
Poster **DNS-05**
Kerstin Kämpf



Toxicologically relevant, Phagocytosis-frustrating Fraction

Fibres > 5 µm

Our Testing Method Developments

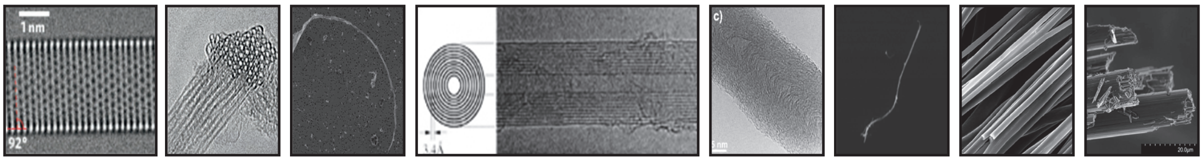
Measurand	**Test Method**		
Dynamic Solubility ⟨⁂⟩	Flow Cell Dissolution*: Ion Leaching & Residual Mass		• **nanoGRAVUR (BMBF)**
Diameter & Length Pairs ⟩•⟨	Electron Microscopy with Large Image Acquisition or Active Fibre Tracking, Fibre Analysis Software		• **OECG TG (BMUB)** ➔ **Poster DNS-05**
Flexural Rigidity ⟩•⟨	Tensile Testing and Modal Analysis		• **F2365: Rigidität** ➔ **Poster MPN-02**
Apparent Density ⟨•⟩	Combination of DMA & BAuA's Nano-PMC		Ratio of Agglomerate Mass and Aerodynamic Diameter [Brossell at al. DOI:10.1080/02786826.2015.1045964]

*Results of nanoGRAVUR

⟨⁂⟩ : Ensemble based. ⟨•⟩ : Individual particle based, but averaged. ⟩•⟨ : Individual particle specific.

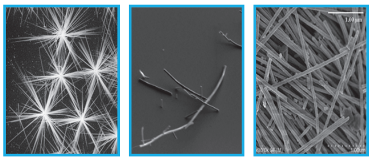
The World of HARM is too big to be Hazard-tested in Animal Studies !

Carbon Fibres



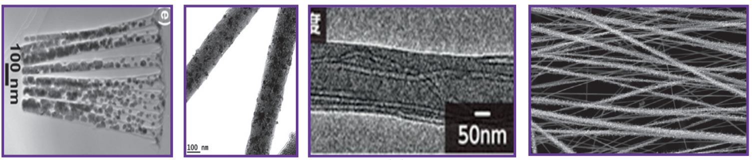
[DOI: 10.1088/0953-8984/24/31/313202] [ww.ifw-dresden.de] [BAuA] [DOI:10.1038/354056a0] [DOI: 10.1088/1367-2630/5/1/126] [BAuA] [BAuA] [BAuA]

Crystallites



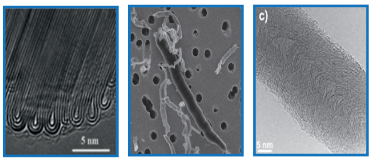
[DOI: 10.1088/0957-4484/18/35/355306] [BAuA] [DOI: 10.1007/s11671-009-9357-z]

Composites



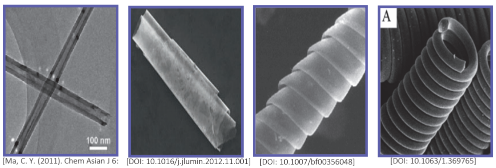
[DOI: 10.1039/B614300H] [A. Meyer-Plath, BAM] [www.nitta.co.jp/en/?post_type=newtech&p=7519] [A. Meyer-Plath, BAM]

Stacks



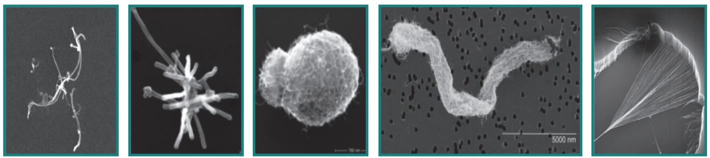
[DOI: 10.1002/cphc.201000298] [BAuA] [DOI: 10.1088/1367-2630/5/1/126]

Scrolls



[Ma, C. Y. (2011). Chem Asian J 6: 1331] [DOI: 10.1016/j.jlumin.2012.11.001] [DOI: 10.1007/bf00356048] [DOI: 10.1063/1.369765]

Agglomerates



[BAuA] [BAuA] [BAuA] [BAuA] [CSIRO ScienceImage 1074]

We will only manage to assess
the hazard potential of HARMs
by reliable Toxicological Grouping Strategies.

These rely on the availability of
Reference Test Methods and Materials !

Please Support the Development of Tests and Materials for Toxicological Grouping of HARM!

Measurand	Test Method	Reference Material
Dynamic Solubility	Dissolution Progress Testing	Standard Solvents and Materials
Diameter & Length Pairs	Electron Microscopy	Fibre Ensembles of known, preferably narrow Diameter or Length Distribution, very High Aspect Ratio Samples
Flexural Rigidity	Tensile or Flexural Rigidity Testing	Fibres of known Elastic Modulus and Density or of known Rigidity
Apparent Density	Volume and Mass Measurement	Standard Agglomerates

Thank You!

Synthesis of Shape controlled TiO_2 nanoparticles: possible CRM's candidates for size, shape and functional properties

Valter Maurino, ***[a]*** ***Letizia Pellutiè,[a] Francesco Pellegrino,[a] Erik Ortel,[b] Vasile-Dan Hodoroaba,[b] Raluca Isopescu,[c]***

a. Dipartimento di Chimica and Centro Interdipartimentale NIS, Università di Torino, Via Giuria 7, 10125, Turin, (Italy).
b. BAM - Federal Institute for Materials Research and Testing Division 6.8 Surface Analysis and Interfacial Chemistry, DE-12200, Berlin.
c. R&D Consultanta si Servicii, 21 Tudor Arghezi Street, Bucharest, 020943, Romania

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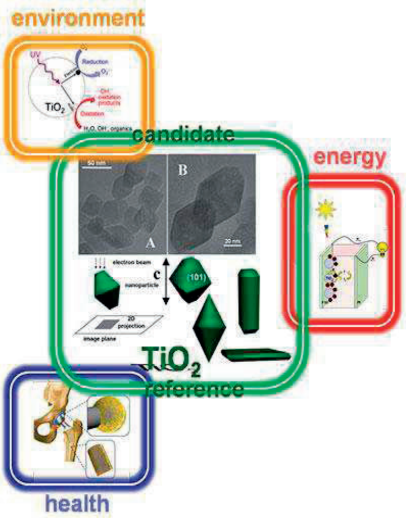
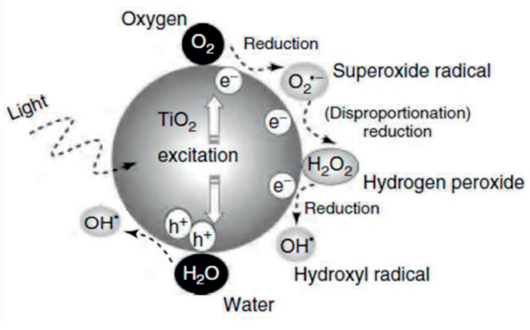
SETNANOMETRO



Why TiO_2



- ✓ Heterogeneous Photocatalysis
- ✓ Smart materials production
- ✓ Energy Conversion and Storage (DSSC, Lithium ion batteries)
- ✓ Health

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C. Minero, V. Maurino. D. Vione, In Photocatalysis and Water Purification: From Fundamentals to Recent Applications, P. Pichat ed, Wiley ,2013
U. Diebold, Surf Sci Rep 2003, 48, (5-8), 53-229, and references therein.

Motivation

Oxidic nanomaterials offered and still promise real, effective and efficient innovation in strategic sectors for mankind (energy production and use, sustainable development, environment protection and restoration, healthcare practices and systems). However, in particular for TiO_2 nanomaterials, a complete comprehension of the phenomena involved at molecular and atomic level is hindered by the wide heterogeneity of:

- types of TiO_2-based nanoparticles (NPs) developed, each of them often exhibiting a rich dimensional and morphological variety,
- surface and/or interfacial molecular states,
- assembly/aggregation states of the NPs to form nanostructured and nano-enabled systems,
- measurement conditions of the functional performances of these systems.

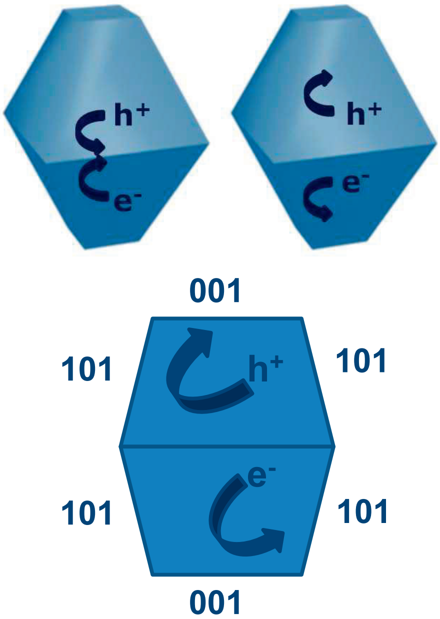
A set of NPs with controlled size and shape, surface properties at molecular level and high stability, obtained though reproducible synthetic methods, can be the basis of:

i - calibration standards and certified reference materials in nanometrology;

ii – reference material to ensure traceability in the measurements of functional properties;

iii – improve the understanding of structure/functional properties at nano and molecular level

Size, Shape and functional properties

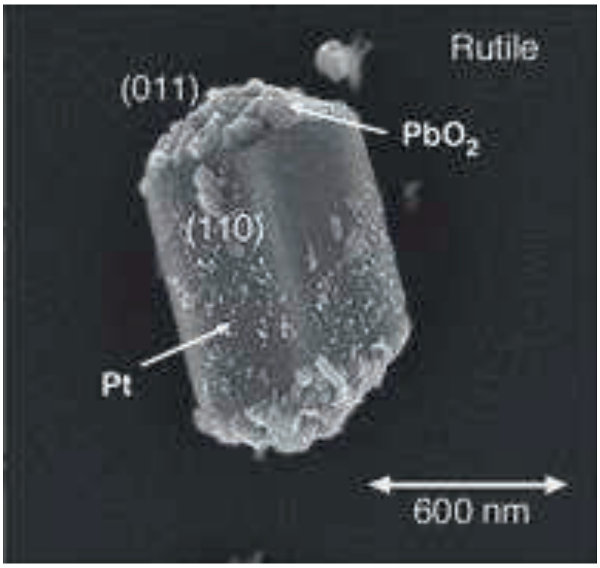


- **Size:** Optimization of Interfacial Transfer/Recombination Ratio

Gerischer, H., Electrochim. Acta, 1995. 40(10).
C. Minero, D. Vione, Applied Catalysis B: Environmental, 2006. 67: p. 257–269.

- **Shape:** Different Surfaces have Different Reactivities

Longoni et al., Nano Lett., 2017, 17 (2), pp 992–1000
D'Arienzo et al., J. Am. Chem. Soc., 2011, 133 (44), pp 17652–17661



Photocatalytic deposition of

PbO_2 (from Pb^{2+}, oxidative)
$Pb^{2+} + 2h^+ (VB) + 2H_2O \leftrightarrows PbO_2$

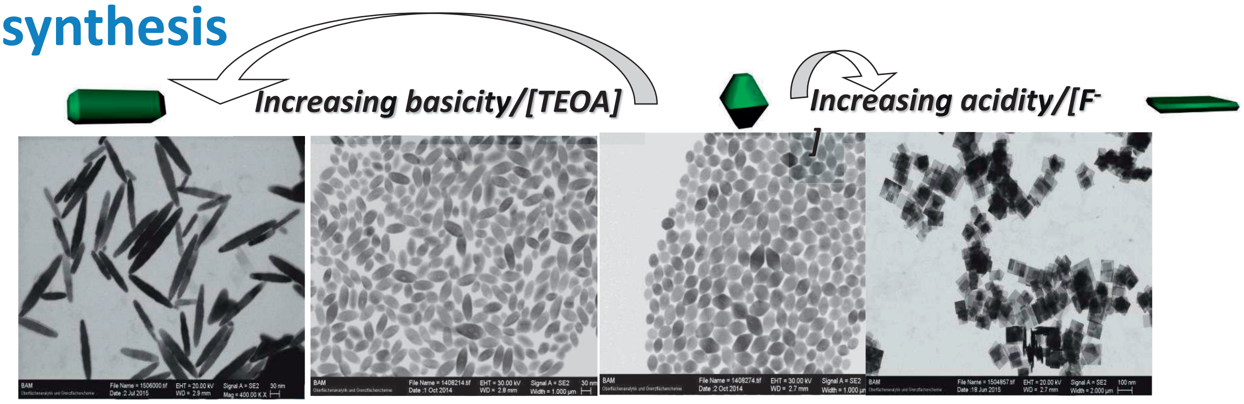
Pt (from H_2PtCl_6, reductive)
$PtCl_6^{2-} + 4e (CB) \leftrightarrows Pt + 6 Cl^-$

On a Rutile shape controlled particle

Ohno et al, ***New J. Chem.***, 2002,**26**, 1167-1170

Motivation and Goal

Setting Design Rules to obtain set of TiO_2 NPs with tunable size and shape by **Hydrothermal synthesis**

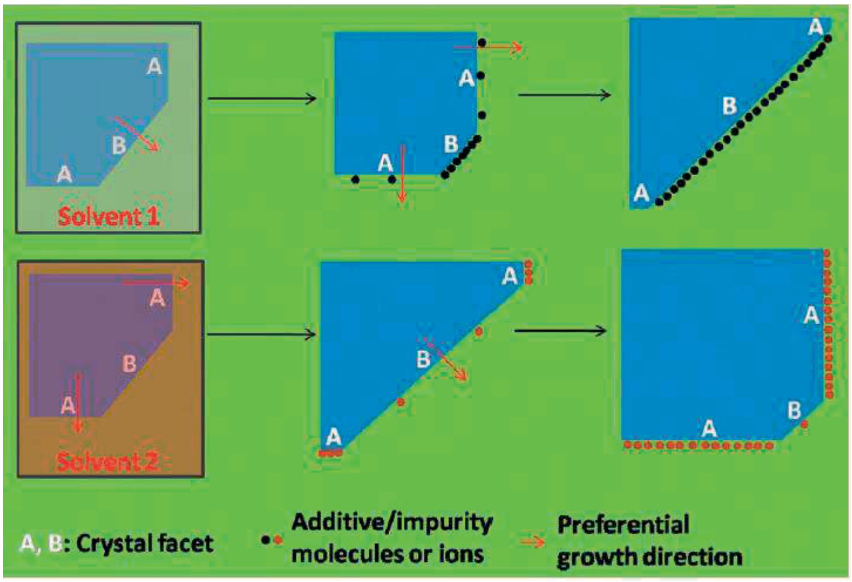


Key points in HT synthesis

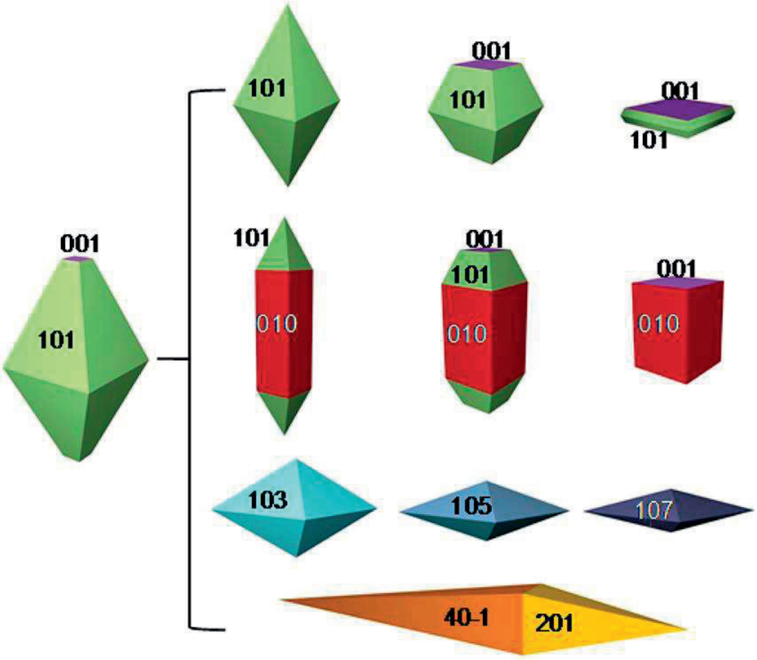
- FEEDER (PRECURSOR) and MINERALIZER
- SHAPE CONTROLLERS
  - – Oxygen ligand (water, aminoalcohols, glycols): bipyramids
  - – Fluoride: platelets (Yang et al 2008)
  - – Sulfate: prisms
  - – Carboxylates: prisms
  - – pH > 12, brookite,
  - – pH < 2, high [Cl^-]: rutile

Liu et al Chem. Rev., 2014, 114 (19), pp 9559–9612

Ong et al , Nanoscale, 2014, 6, 1946-2008

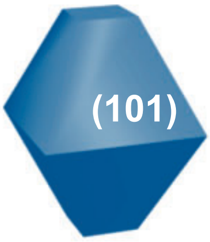


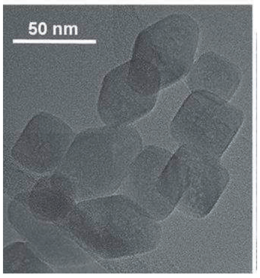
Liu et al Chem. Rev., 2014, 114 (19), pp 9559–9612



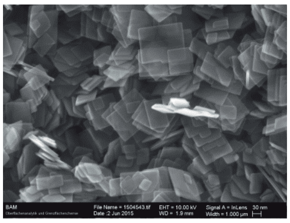
SHAPE CONTROLLERS

- **Bipyramids:** *oxygen ligand (water, aminoalcohols, glycols);*



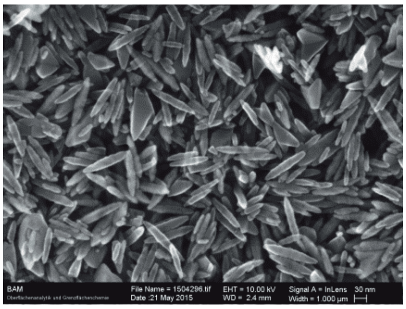


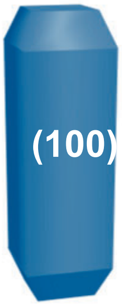
- **Platelets:** *fluoride* (Yang et al 2008);



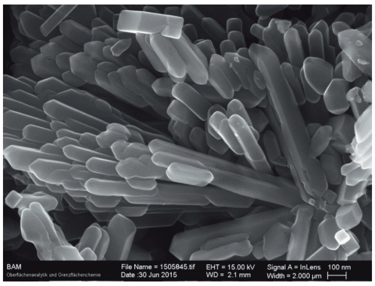
1. Liu et al Chem. Rev., 2014, 114 (19), pp 9559–9612;
2. Ong et al. , Nanoscale, 2014, 6, 1946-2008

- **Prisms:** *sulfate and carboxylates*



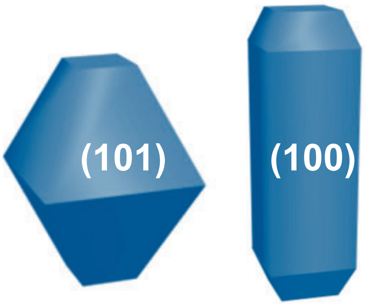


- **Brookite:** *pH > 12;*
- **Rutile:** *pH < 2, high $[Cl^-]$;*



1. Liu et al., Chem. Rev., 2014, 114 (19), pp 9559–9612;
2. Ong et al., Nanoscale, 2014, 6, 1946-2008

Synthesis of Anatase Nanoparticles

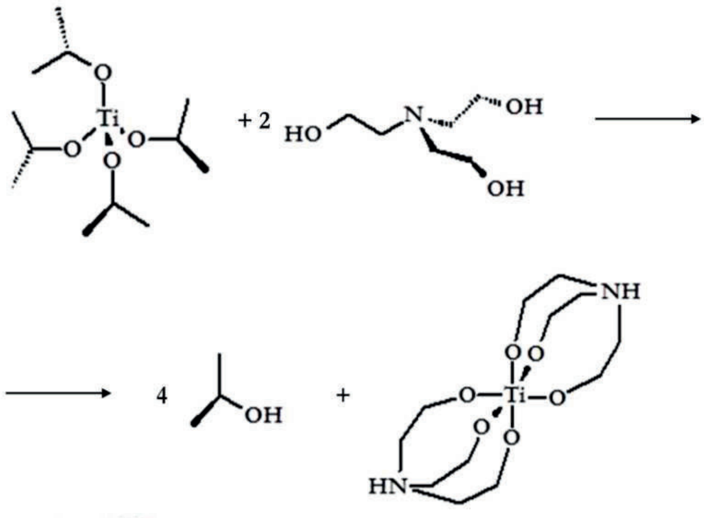


- **Bipyramids and Prisms:** *hydrothermal synthesis starting from a Ti precursor Ti(TeoaH)$_2$ and Triethanolamine (TeoaH$_3$) as shape controller;*

- **Platelets:** solvo*thermal synthesis starting from Titanium(IV) butoxide Ti(BuO)$_4$ and Hydrofluoric Acid (HF) as shape controller;*

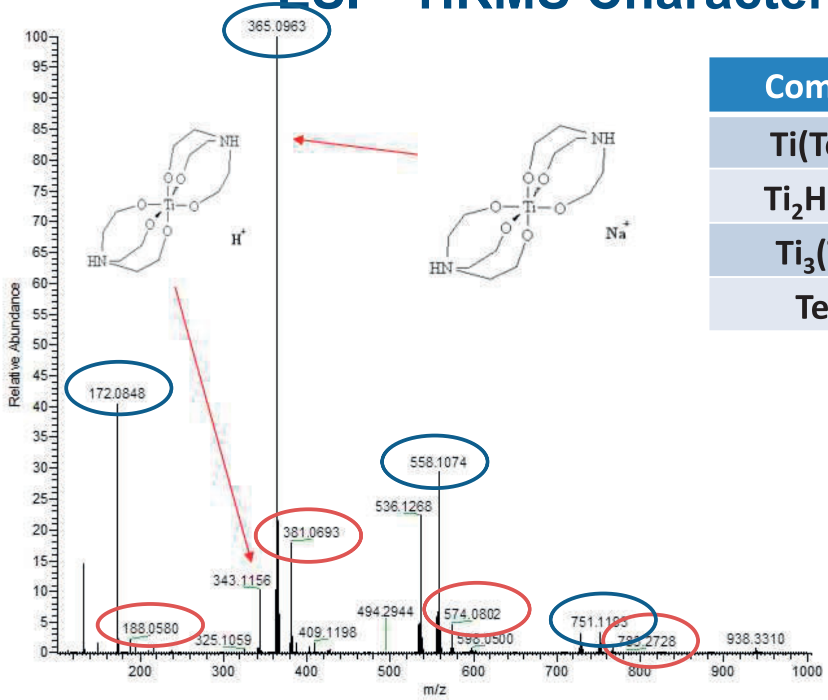
Bypiramidal and Prismatic Anatase NPs

Homogeneous nucleation starting from a water soluble precursor (no organic solvents)[1]: Ti(TeoaH)$_2$



Deiana C., et al. Phys. Chem. Chem. Phys., 15 (2013), 307-315.

ESI – HRMS Characterization



Compound	m/z
$Ti(TeoaH)_2$	343.1343
$Ti_2H(Teoa)_3$	536.1559
$Ti_3(Teoa)_4$	729.2786
$TeoaH_3$	150.1122

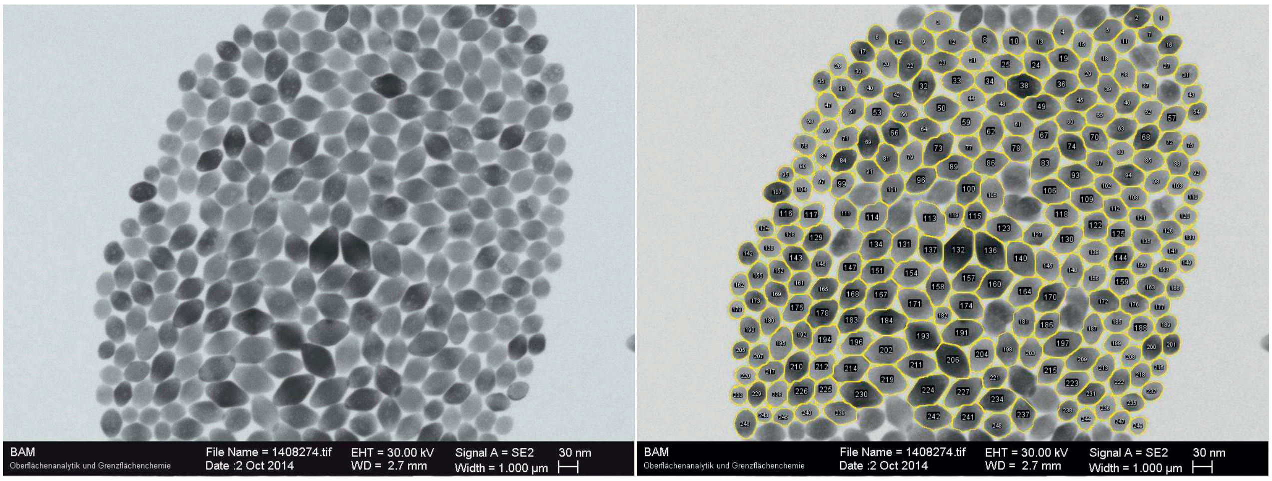
Na^+

K^+

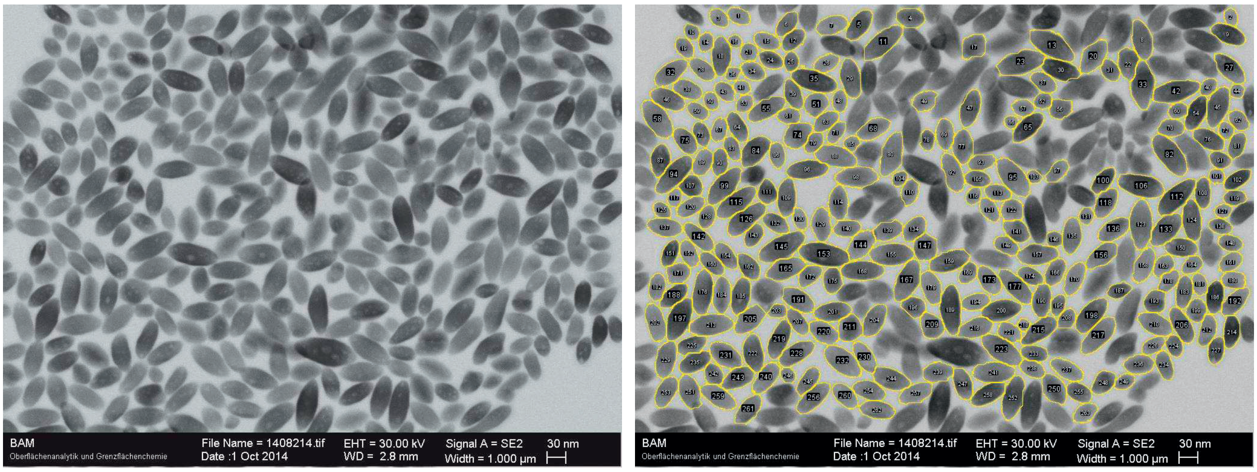
V. Lavric et al, *Cryst. Growth Des.*, 2017, 17 (11), pp 5640–5651

- **Orthogonal Fractional Second Order CCD expl design**
- The **process parameters** and their limits are:
  - Z1 - $Ti(HTeoa)_2$ initial concentration: 10-120 mM
  - Z2 - Added H_3Teoa (Triethanolamine): 0-80 mM
  - Z3 - Initial pH: 8-12
  - Z4 - Temperature: 120-220 °C
- The **product characteristics** which will be measured for each experiment are:
  - Y1 - Hydrodynamic radius (DLS)
  - Y2 - Polydispersity index (standard deviation of the relevant mode of the size distribution)
  - Y3 - ***Shape parameter*** (and its standard deviation) (c/a obtained from the ellipsoid fitted to the particles T-SEM contour)

Material	Y1 BAM R_H (major-minor)	Y1 R_H number mode 1	Y2 SD R_H number mode 1	R_H number mode 2	R_H mass mode 1	SD R_H mass mode 1	R_H mass mode 2	Y3 Shape parameter Major/Minor	Y3 SD Major/Minor
UT_2-01	9	7	0.26	24	7	0.26	32	1.52	0.55
UT_2-02	29	20	1.06	58	20	1.08	63	1.98	0.86
UT_2-03	16	15	1.38	35	15	1.51	50	1.38	0.41
UT_2-04	22	17	1.12	68	17	1.16	73	5.5	2.63
UT_2-05	12	14	0.75	35	14	0.77	39	1.37	0.46
UT_2-06	20	18	1.65	44	18	1.78	53	5.46	2.08
UT_2-07	10	18	4.41		22	7.14		1.69	0.76
UT_2-08	25	17	0.99	126	17	1.02	154	2.33	1.14
UT_2-09	18	20	3.63		22	4.79		1.38	0.39
UT_2-10	19	19	3.72		21	5.00		1.76	0.59
UT_2-11	16	17	2.89		19	3.60		1.33	0.36
UT_2-12	19	21	3.39		23	4.02		1.5	0.41
UT_2-13_01 HCl	12	13	0.68	35	13	0.70	43	1.41	0.51
UT_2-13_02 $HClO_4$	10	17	1.49	40	18	1.61	43	1.38	0.52
UT_2-14	-	253	23.7		260	23.4		NA	NA
UT_2-15	-	2	0.28	12	2	0.32	16	NA	NA
UT_2-16	20	20	3.58		22	4.61		1.4	0.30
UT_2-17	18	19	3.42		21	4.44		1.49	0.43
UT_2-18	18	18	3.89		21	5.69		1.39	0.35
UT_2-19	18	19	3.42		21	4.41		1.37	0.36
UT_2-20	18	19	3.04		21	3.68		1.38	0.39



T-SEM images of sample UT_2-16, shape factor 1.4, R_H 20 nm.



T-SEM images of sample UT_2-10, shape parameter 1.76, R_H = 19 nm

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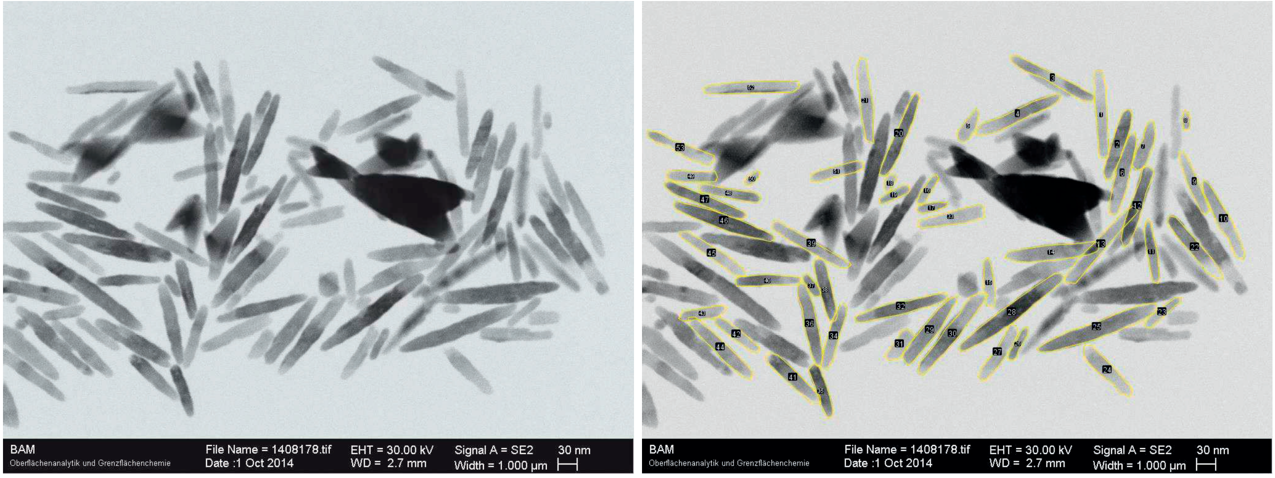


T-SEM images of sample UT_2-04. Shape parameter 5.5, R_H = 17 nm

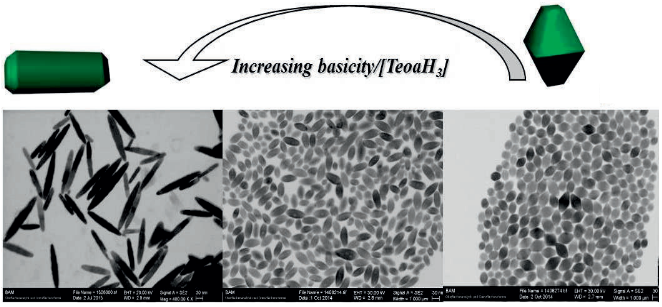
Soft Model Validation

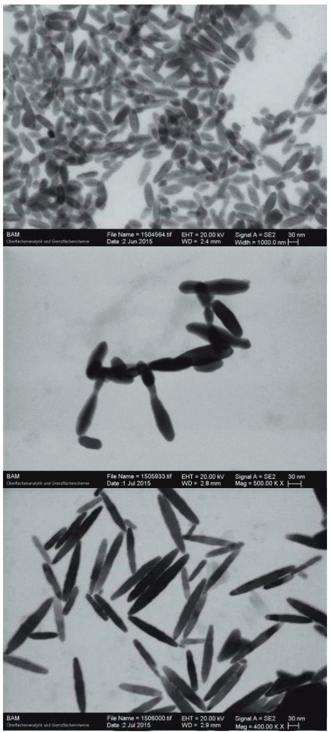
Two soft model derived from the experimental design:

- ➢Polynomial model
- ➢Artificial neural network model
  - ▪Validation experiments

Shape Parameter from 1.4 to 6.2

R_h from 10 to 30 nm





Shape parameter predicted: 2

Shape parameter measured: 2.3

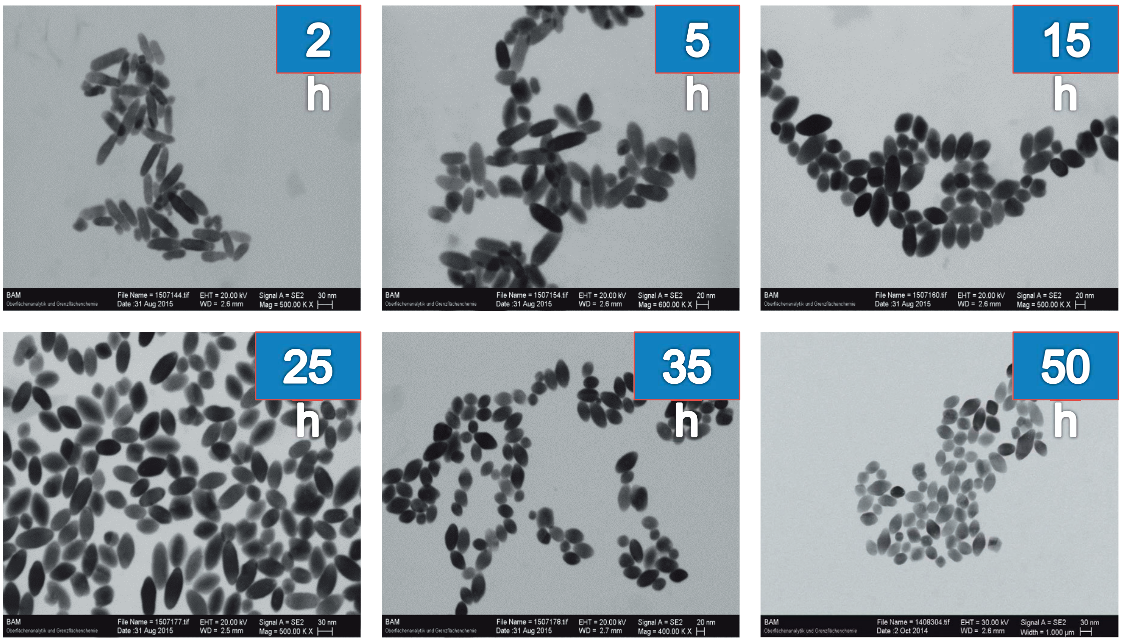
Shape parameter predicted: 3

Shape parameter measured: 3.1

Shape parameter predicted: 4

Shape parameter measured: 6.2

Bipyramid Growth Mechanism



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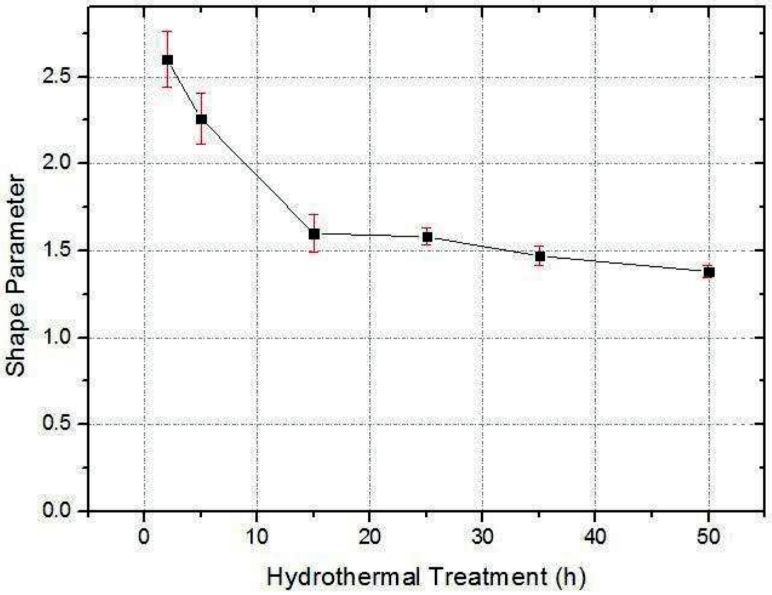
V. Lavric et al, *Cryst. Growth Des.*, 2017, 17 (11), pp 5640–5651

Bipyramid Growth Mechanism



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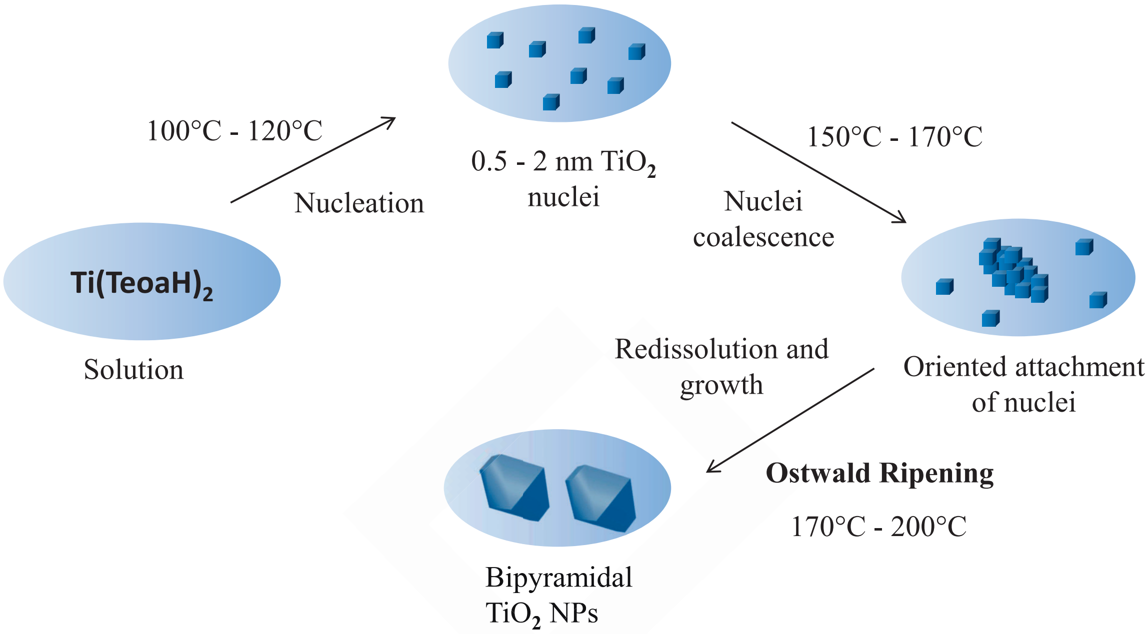
Bipyramid Growth Mechanism



Ti(TeoaH)$_2$

Solution

100°C - 120°C

Nucleation

0.5 - 2 nm TiO_2 nuclei

150°C - 170°C

Nuclei coalescence

Oriented attachment of nuclei

Redissolution and growth

Ostwald Ripening

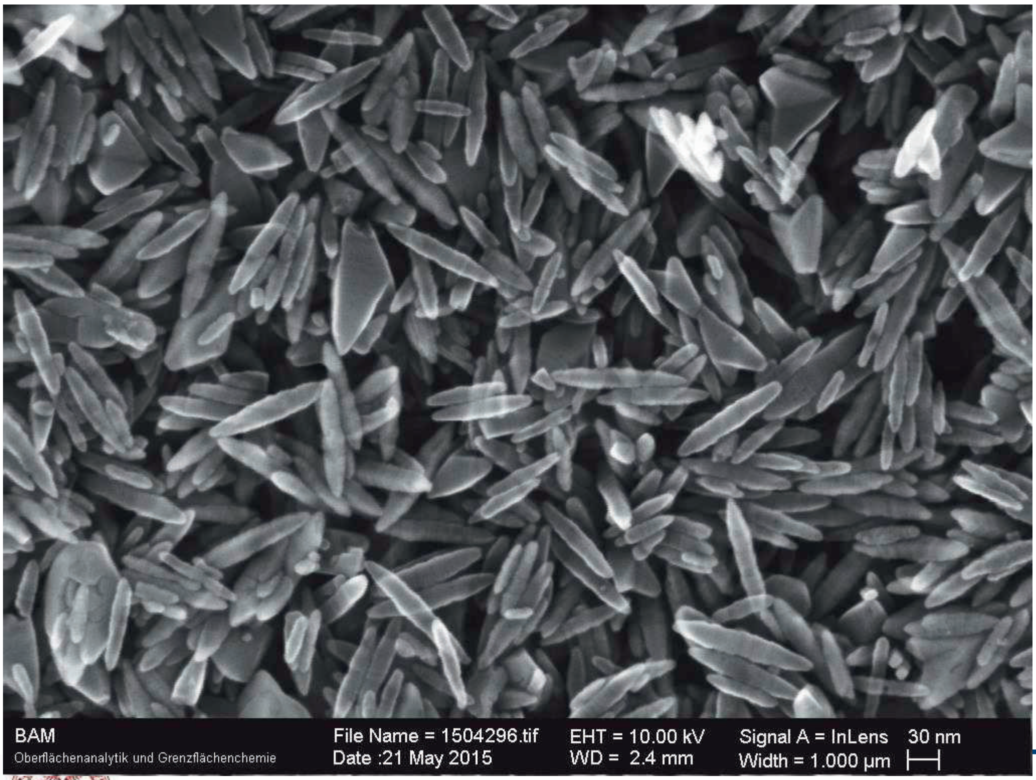
170°C - 200°C

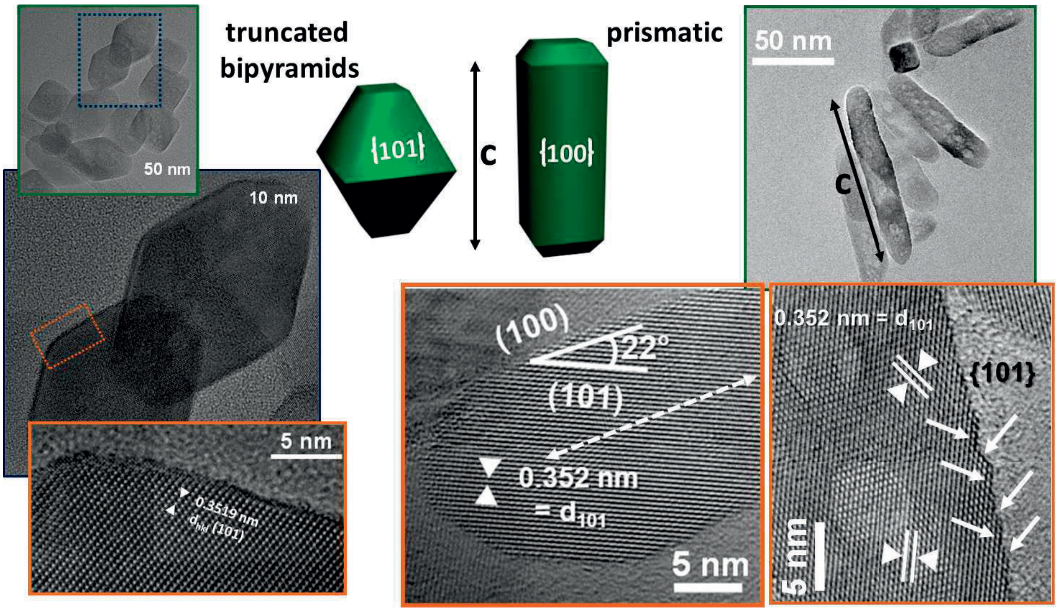
Bipyramidal TiO_2 NPs

Results and Conclusion on elongated and bipyramidal Anatase NPs

- A variation of the size parameter (hydrodynamic radius) between 10 and 25 nm is obtained.
- A variation of the shape parameter c/a between 1.35 and 5.5 is obtained. The shape parameter of perfect anatase bipyramids is 2.5.
- Above initial pH 12, and, concurrently, temperatures below 170 °C, the HT synthesis results in the production of substantial amount of brookite phase instead of anatase.
- The increase in Temperature leads to bigger particles with good bipyramidal shapes.
- The increase in the shape parameter, leading to high c/a values, is favoured at initial pH between 11 and 12. In this pH range, the shape parameter increases with the decrease of the temperature. The presence of high [TEOA] disfavour the formation of brookite
- ***A soft model for the prediction of synthesis parameters from the desired size and shape of the Anatase NPs was set up***

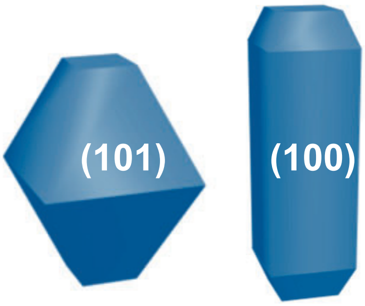
F. Pellegrino et al., work in preparation


BAM
Oberflächenanalytik und Grenzflächenchemie
File Name = 1504296.tif
Date :21 May 2015
EHT = 10.00 kV
WD = 2.4 mm
Signal A = InLens
Width = 1.000 µm
30 nm


50 nm
truncated bipyramids
{101}
c
{100}
prismatic
50 nm
c
10 nm
5 nm
0.3519 nm
d_hkl (101)
(100)
22°
(101)
0.352 nm = d101
5 nm
0.352 nm = d101
{101}
5 nm

Synthesis of Anatase Nanoparticles

➢**Bipyramids and Prisms:** *hydrothermal synthesis starting from a Ti precursor Ti(TeoaH)$_2$ and Triethanolamine (TeoaH$_3$) as shape controller;*



➢**Platelets:** *solvothermal synthesis starting from Titanium(IV) butoxide Ti(BuO)$_4$ and Hydrofluoric Acid (HF) as shape controller;*

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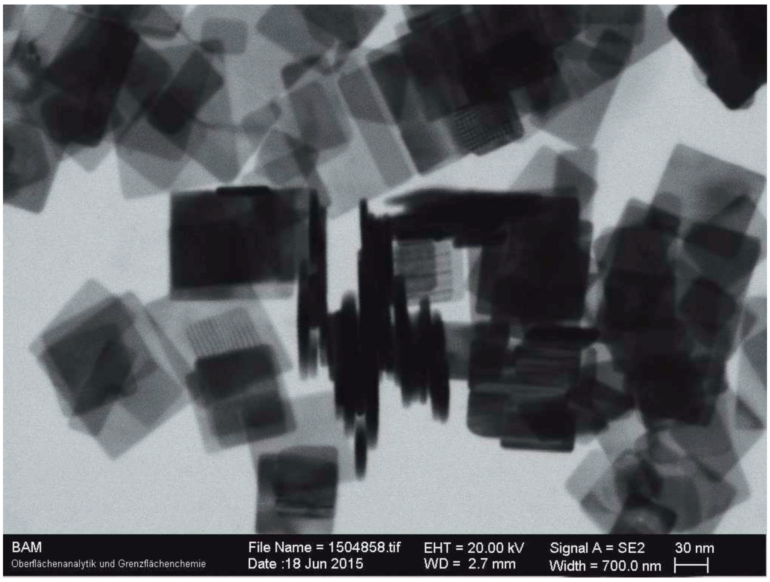
- Platelets



Ti(OBu)$_4$ + HF conc

Very low shape factor

Nearly 0.1

Problem: as synthesized contains 0.5-1% F, probably 50% at the surface

Han et al J. AM. CHEM. SOC. 2009, 131, 3152–3153

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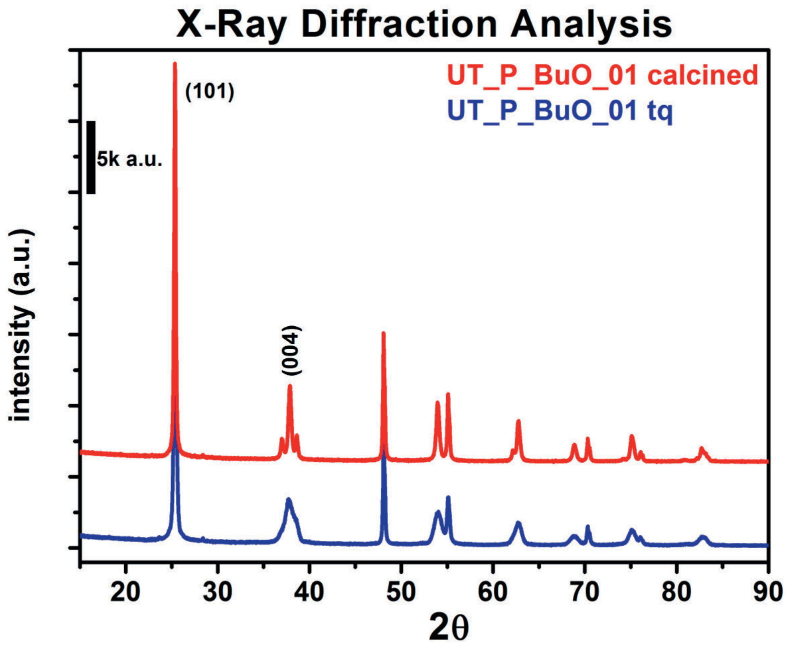

Calcination presumably removes surface F, but nearly half the surface area: platelets stick on (001) planes

L. Mino, F, Pellegrino, S.Rades, VD Hororoaba, V Maurino, G. Spoto, G. Martra ACS Applied Nano Materials submitted

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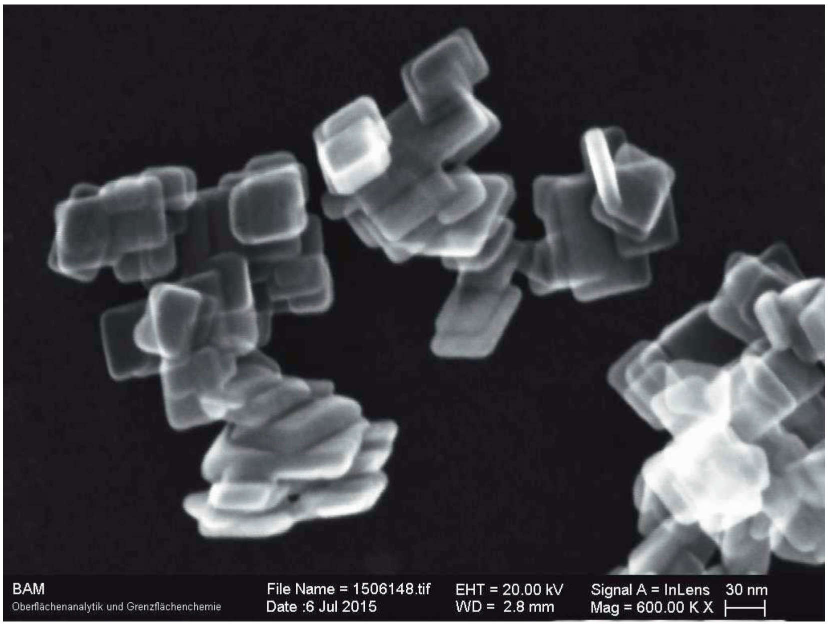

The presence of chloride increase the shape factor, probably adsorbing on (100) (101) facets

- Ack: **EU FP7 SETNanoMetro**
  - V.D. Hodoroaba (BAM)
  - E. Ortel (BAM)
  - S. Rades (BAM)
  - V. Lavric (University “Politehnica” of Bucharest)
  - R. Isopescu (University “Politehnica” of Bucharest)
  - Chiara Deiana (UNITO)
  - Gianmario Martra (UNITO)
  - Giuseppe Spoto (UNITO)
  - A.M. Rossi (INRIM)
  - C. Portesi (INRIM)

SETNANOMETRO

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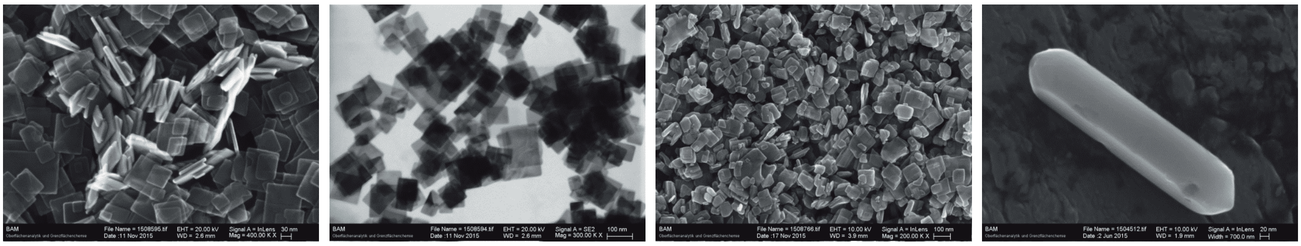
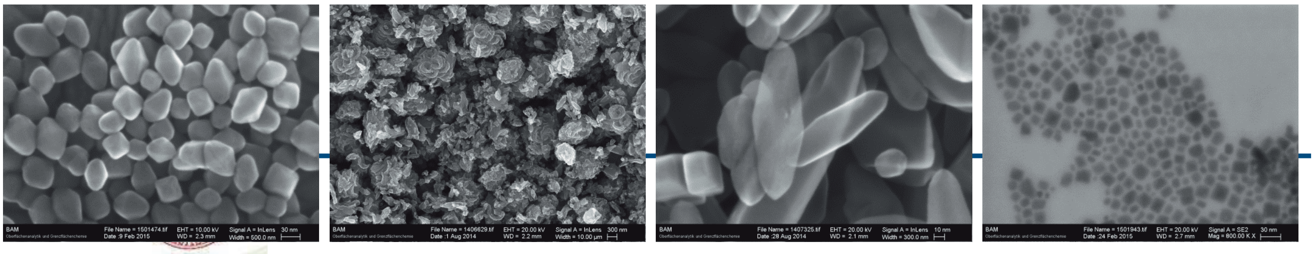


Thanks for your attention



X-ray Radiometry

Traceable nanoparticle characterization using Small Angle X-ray Scattering (SAXS)

Michael Krumrey

Physikalisch-Technische Bundesanstalt

PTB – BAM Workshop on
Reference Nanomaterials

PTB Berlin-Adlershof (BESSY II)
14th May 2018

Outline

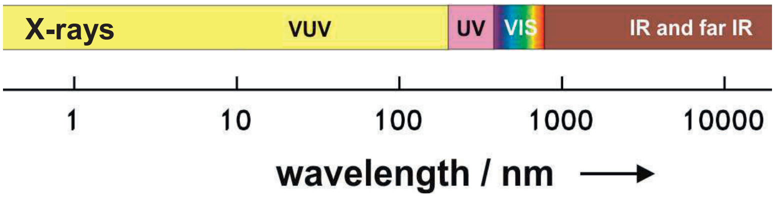
X-ray Radiometry

1. Small-Angle X-ray Scattering (SAXS)
   Why using X-rays for the characterization of nanoparticles (NPs) ?
2. PTB primary approach:
   SAXS with synchrotron radiation in the PTB laboratory at BESSY II
3. Size determination for NPs in liquid suspension with SAXS
4. Concentration determination of NPs with SAXS
5. Continuous contrast variation for density and size determination of core/shell nanoparticles
6. Conclusion

Small Angle X-ray Scattering (SAXS)

X-ray Radiometry

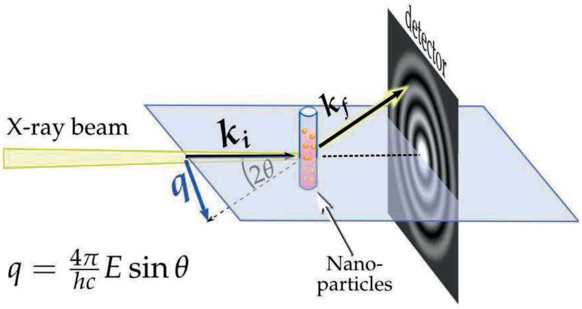
Why X-rays?

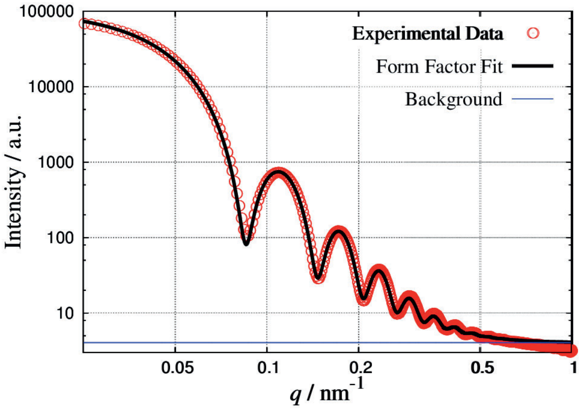


- Wavelength of the radiation < 0.5 nm, well suited to study particles in the diameter range from a few nm up to several hundred nm
- SAXS is an ensemble technique (like DLS)
- Particles can be investigated in suspension
- Scattering sensitive to electron density contrast
- Straightforward scattering theory (form factor), traceability!

Traceable SAXS

X-ray Radiometry





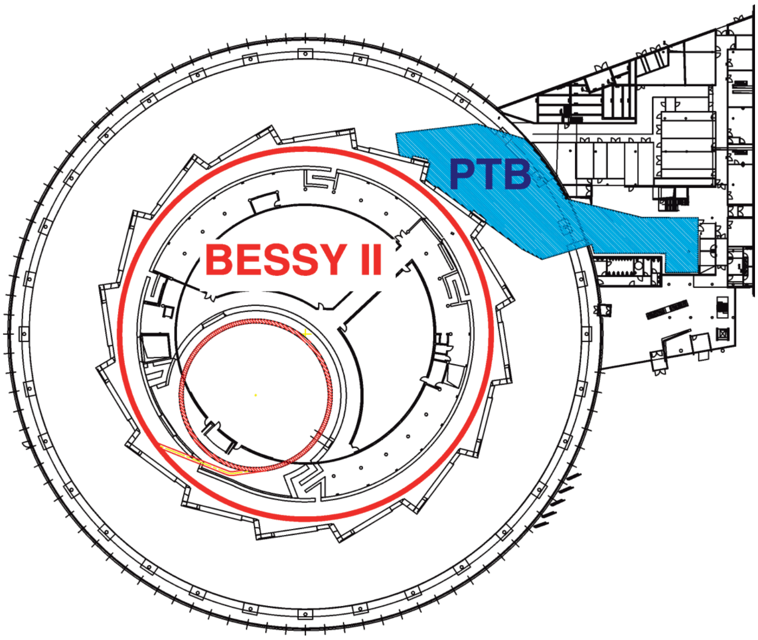
Model fitting for sufficiently monodisperse particles:
- period of oscillations can be connected to the X-ray wavelength
- size can be made traceable to the SI unit meter

For size measurements, only the *q*-axis needs to be traceable

For concentration measurements, also the 'differential scattering cross section' needs to be determined on an absolute scale

Synchrotron radiation: electron storage ring

X-ray Radiometry



Main parameters:

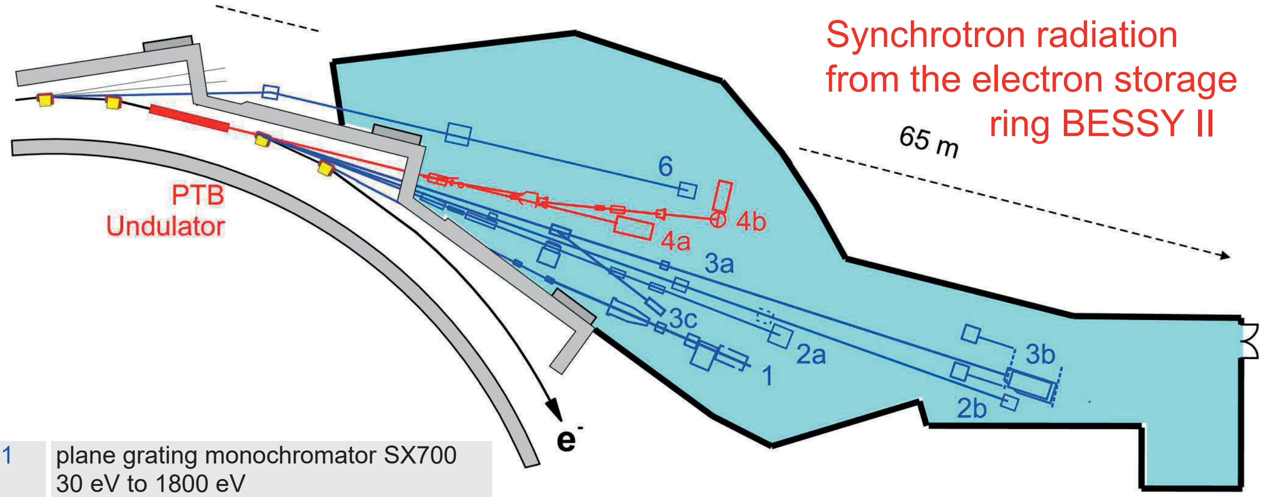
ring circumference	240 m
electron energy	1.7 GeV
max. ring current	300 mA
beamlines	50

Operated by: Helmholtz-Zentrum Berlin (HZB)

PTB laboratory at BESSY II in Berlin

X-ray Radiometry

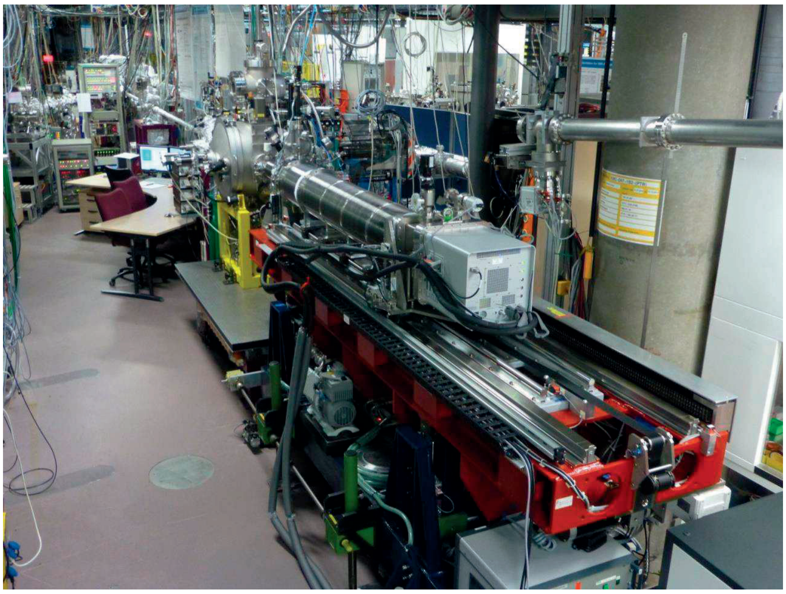
Synchrotron radiation from the electron storage ring BESSY II



1	plane grating monochromator SX700 30 eV to 1800 eV		
2a	**four-crystal monochromator 1.75 keV to 10 keV (0.7 nm to 0.1 nm)**	3c	deflected undispersed bending magnet radiation, EUV irradiation test station
2b	X-ray pencil beam facility (XPBF), astrophysics optics characterization	4a	undispersed undulator radiation Compton backscattering
3a	undispersed bending magnet radiation	4b	plane grating monochromator (PGM) at undulator, 40 eV to 1900 eV (30 nm to 0.65 nm)
3b	normal incidence monochromator 3 eV to 35 eV	4c	deflected undispersed undulator radiation EUVL metrology test station
5	*BAM*line, 8 keV to 60 keV (outside PTB laboratory)	6	X-ray parallel beam facility (XPBF 2.0), astrophysics optics characterization

Detector and SAXS set-up

X-ray Radiometry



PTB PILATUS 1M hybrid pixel detector:

- **vacuum-compatible**
- detector area:
  17 cm x 18 cm
- pixel size: (172 µm)²
- operational down to
  1.75 keV

HZB SAXS set-up:

- sample – detector distance: 1.4 m to 4.3 m

J. Wernecke, C. Gollwitzer, P. Müller and M. Krumrey, J. Synchrotron Rad. **21**, 529 (2014)

ISO/TC 24/SC 4: Particle characterization

X-ray Radiometry

WG 10: SAXS

INTERNATIONAL
STANDARD

**ISO
17867**

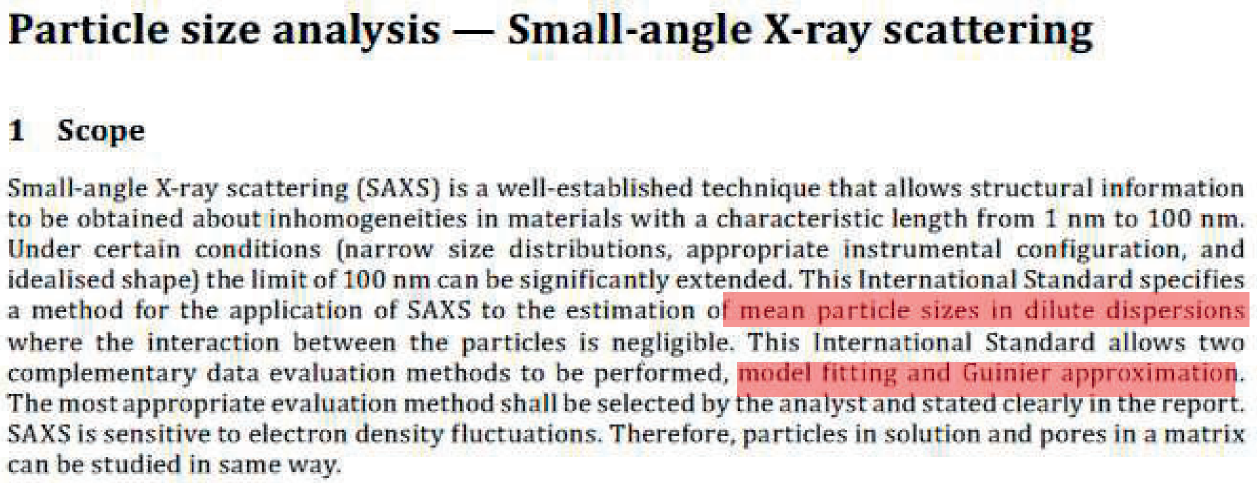
First edition
2015-05-01

Particle size analysis — Small-angle X-ray scattering

Analyse granulométrique — Diffusion des rayons X aux petits angles

ISO/TC 24/SC 4: Particle characterization

WG 10: SAXS



Particle size analysis — Small-angle X-ray scattering

1 Scope

Small-angle X-ray scattering (SAXS) is a well-established technique that allows structural information to be obtained about inhomogeneities in materials with a characteristic length from 1 nm to 100 nm. Under certain conditions (narrow size distributions, appropriate instrumental configuration, and idealised shape) the limit of 100 nm can be significantly extended. This International Standard specifies a method for the application of SAXS to the estimation of mean particle sizes in dilute dispersions where the interaction between the particles is negligible. This International Standard allows two complementary data evaluation methods to be performed, model fitting and Guinier approximation. The most appropriate evaluation method shall be selected by the analyst and stated clearly in the report. SAXS is sensitive to electron density fluctuations. Therefore, particles in solution and pores in a matrix can be studied in same way.

Current work in WG 10 on extension to include:

- additional data evaluation methods
- refined data correction, size **distribution** determination

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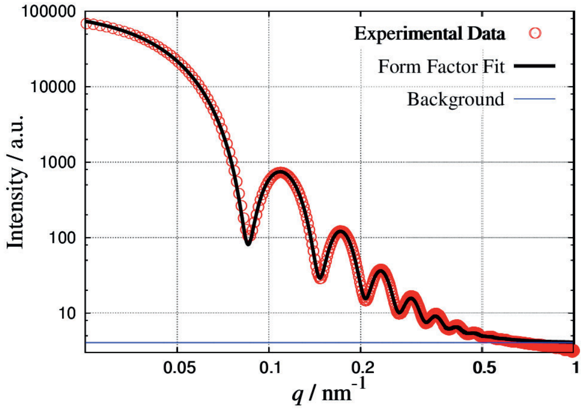
Traceable size determination with SAXS

Guinier approximation:

- scattered intensity dominated by larger particles
- strongly affected by agglomeration
- **not** suited for traceable size determination



Model fitting for sufficiently monodisperse particles:

- period of oscillations can be connected to the X-ray wavelength
- can be made traceable to the SI unit meter

$$I \approx \langle f^2(q,R) \rangle$$

$$f(q,R) = \frac{4}{3}\pi R^3 \cdot 3\frac{\sin qR - qR\cos qR}{(qR)^3}$$

Uncertainties and traceability

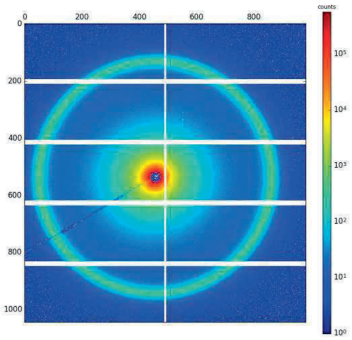
X-ray Radiometry

Contributions to the uncertainty budget of *q*

$$q = \frac{4\pi}{hc} E \sin\theta$$

- **Photon energy** (or wavelength) of the radiation:
  traceable to the lattice constant of silicon by back-reflection from a silicon crystal, $u_{\mathrm{rel}} \lesssim 10^{-4}$
- **Distance** between sample and detector:
  triangulation using calibrated length measurement system, $u_{\mathrm{rel}} \lesssim 10^{-3}$
- **Pixel size**:
  displacement measured using calibrated length measurement system, $u_{\mathrm{rel}} \lesssim 10^{-4}$

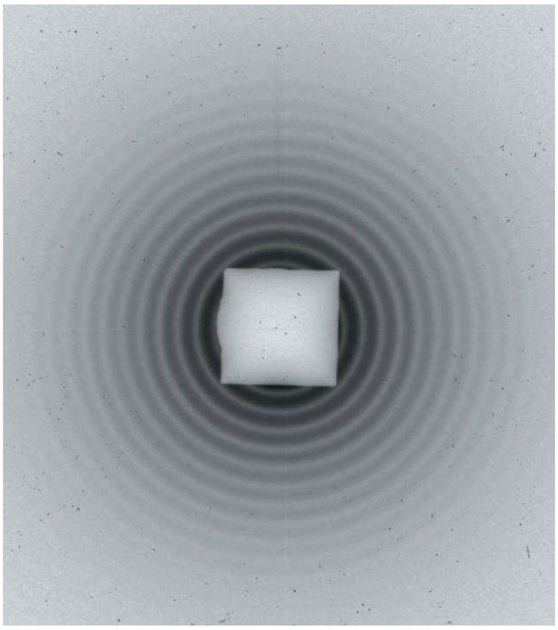
Alternative for lab instruments:
use of 'standard' with known peaks on the *q*-axis, e.g. Ag-behenate

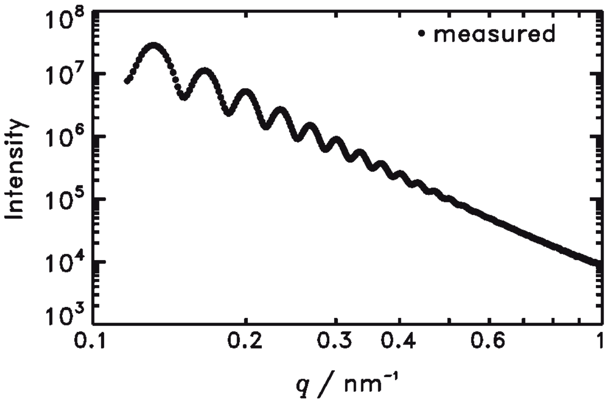


SAXS at the PTB FCM beamline

X-ray Radiometry

PMMA „192 nm“
Microparticles GmbH

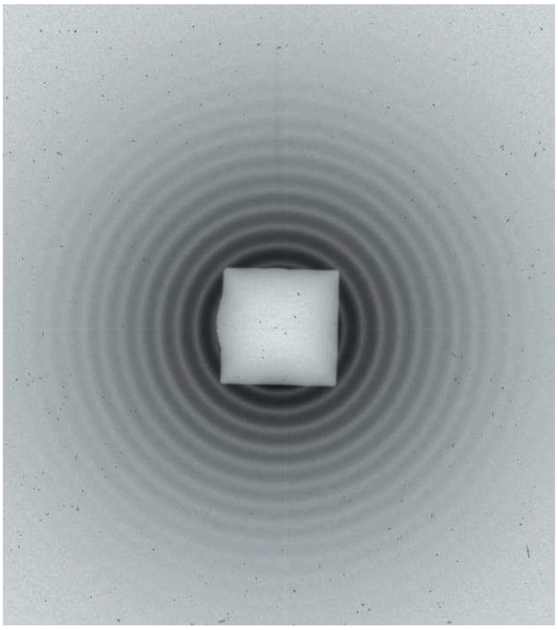


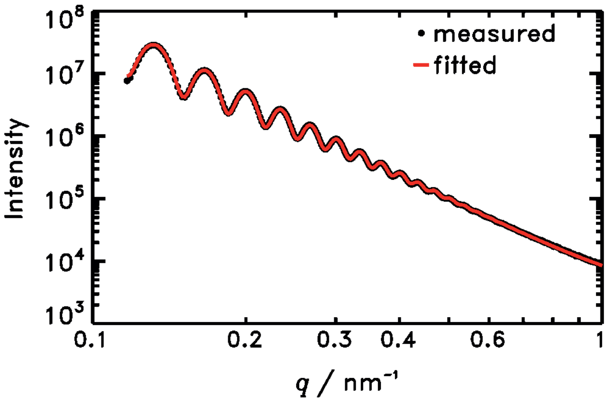


SAXS at the PTB FCM beamline

X-ray Radiometry

PMMA „192 nm“
Microparticles GmbH





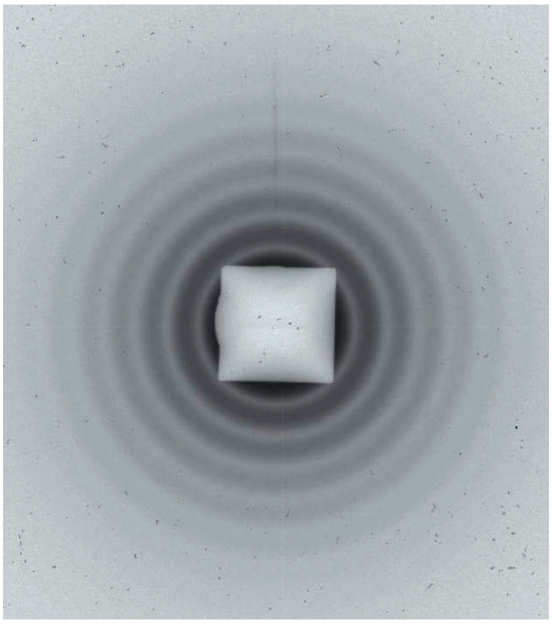
Mean diameter: (188.1 ± 1.3) nm
(number weighted)

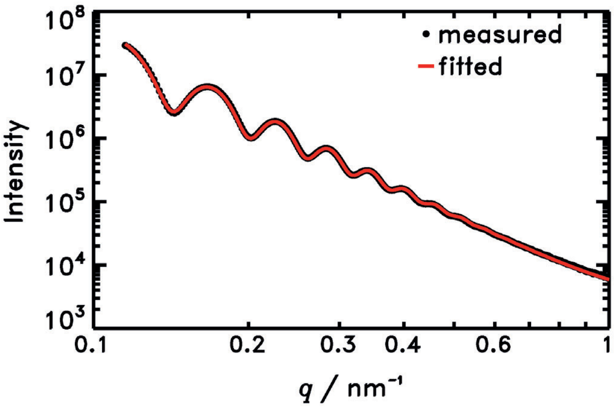
FWHM: 11.1 nm
(Gaussian, polydispersity 6 %)

SAXS at the PTB FCM beamline

X-ray Radiometry

PMMA „108 nm“
Microparticles GmbH





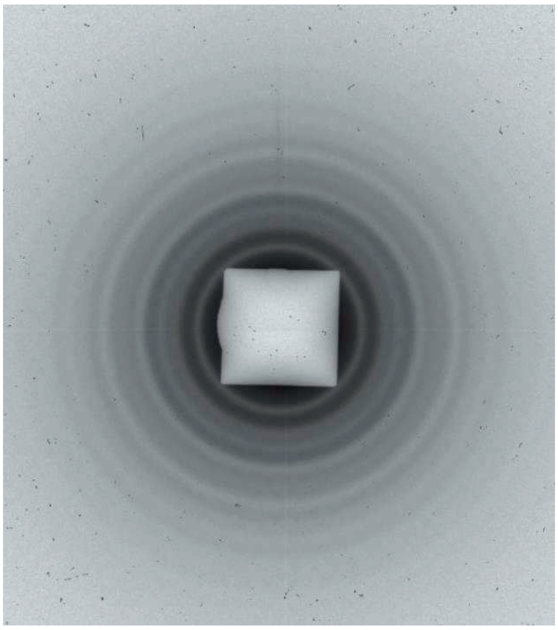
Mean diameter: (109.0 ± 0.8) nm
(number weighted)

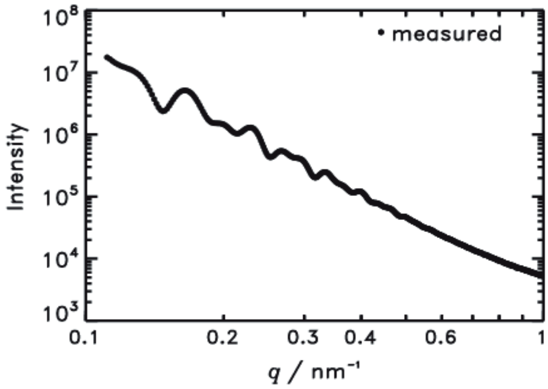
FWHM: 12.0 nm
(Gaussian, polydispersity 11 %)

SAXS at the PTB FCM beamline

X-ray Radiometry

Mixture of both PMMA particles

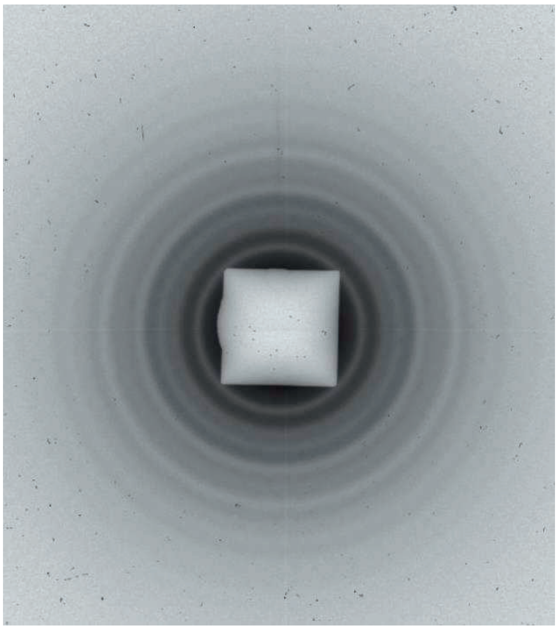


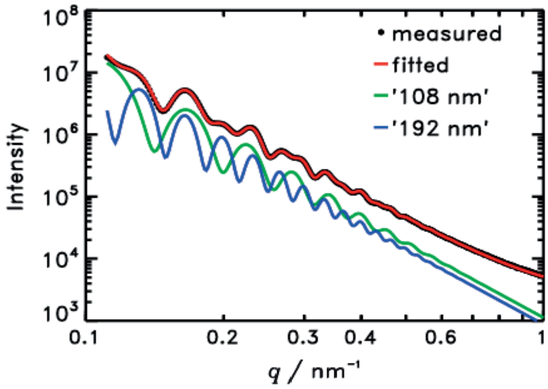


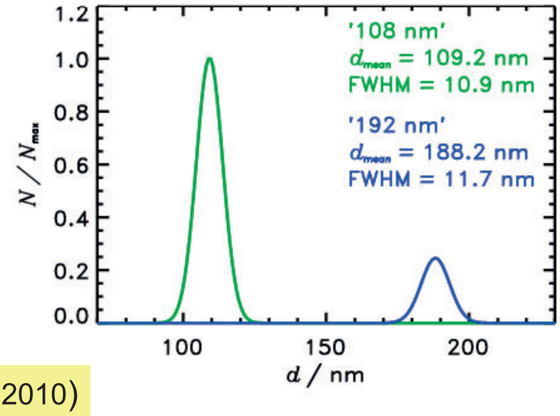
SAXS at the PTB FCM beamline

X-ray Radiometry

Mixture of both PMMA particles



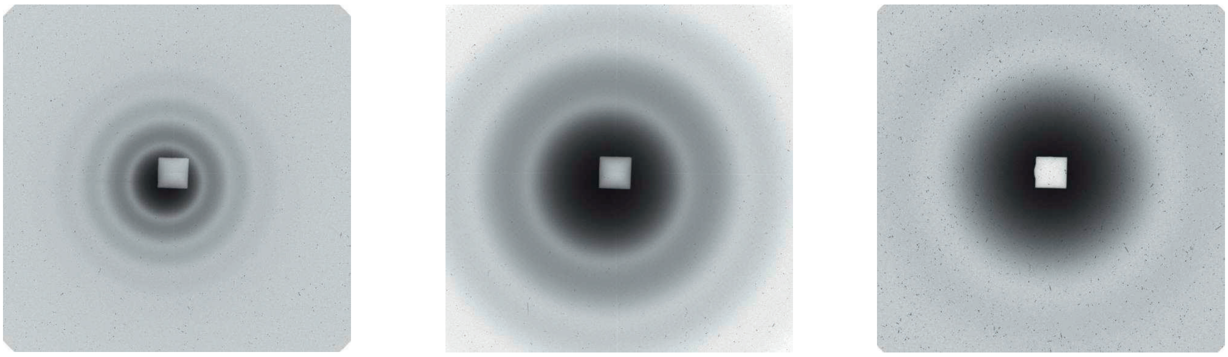


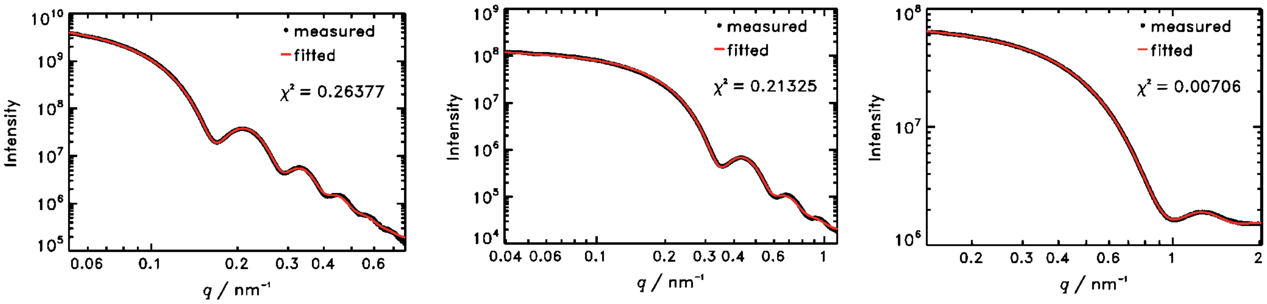


G. Gleber *et al.*, J. Phys. Conf. Ser. **247**, 012027 (2010)

NIST gold reference nanoparticles

X-ray Radiometry





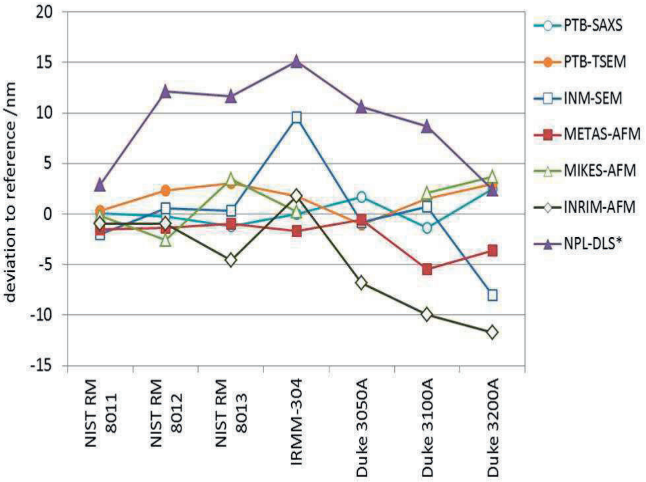
Mean diameters (number weighted)

RM 8013 "60 nm"	RM 8012 "30 nm"	RM 8011 "10 nm"
(52.8 ± 0.5) nm	(25.3 ± 0.5) nm	(8.84 ± 0.25) nm

First European NMI nanoparticle comparison

X-ray Radiometry

Plus: Traceable Characterization of Nanoparticles (2008 – 2011)



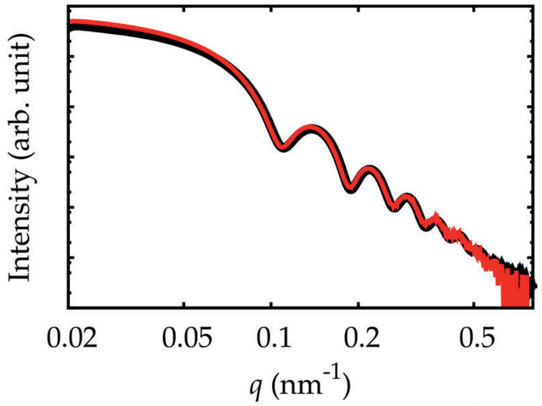
In total, SAXS had the smallest deviations from the reference values.

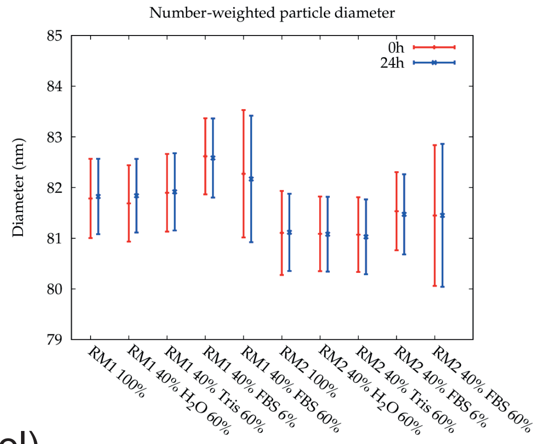
F. Meli *et al.*, Meas. Sci. Technol. **23**, 125005 (2012)

Reproducibility of size determination

X-ray Radiometry

Chemical and optical characterisation of nanomaterials in biological systems





Mean diameter of silica nanoparticles (Klebosol)
- low uncertainties
- high reproducibility
- very weak influence of (biological) environment, in contrast to other methods!

C. Gollwitzer *et al.*, Anal. Methods **8**, 5272 (2016)

Particle concentration measurement with SAXS

X-ray Radiometry

For size measurements, only the *q*-axis needs to be traceable, for concentration measurements, also the 'intensity' needs to be traceable (differential scattering cross section):

$$\frac{d\Sigma}{d\Omega}(q) = r_e^2 \cdot C \cdot \Delta\rho_e^2 \int_0^\infty g(R) \cdot S(q,R) \cdot |f(q,R)|^2 dR + BG$$

Electron radius

Concentration

Electron density difference to matrix

Size distribution function

$$\frac{d\Sigma}{d\Omega}(q) = \frac{I_{meas}(q)}{\Phi_0 \cdot T \cdot \Delta\Omega \cdot t_{exp} \cdot \eta_{QE} \cdot w}$$

Measured scattered intensity

Sample thickness

Detection efficiency

Exposure time

Solid angle

Transmittance

Incident photon flux

Particle concentration measurement with SAXS

Incident photon flux

Responsivity of a semiconductor photodiode

Detection efficiency

of the large-area PILATUS detector (no direct SR beam!)

both based on PTB X-ray detector calibration

Alternatives for lab instruments:

- use of hybrid-pixel detector with high dynamic range
- calibration standard like e. g. glassy carbon (NIST)

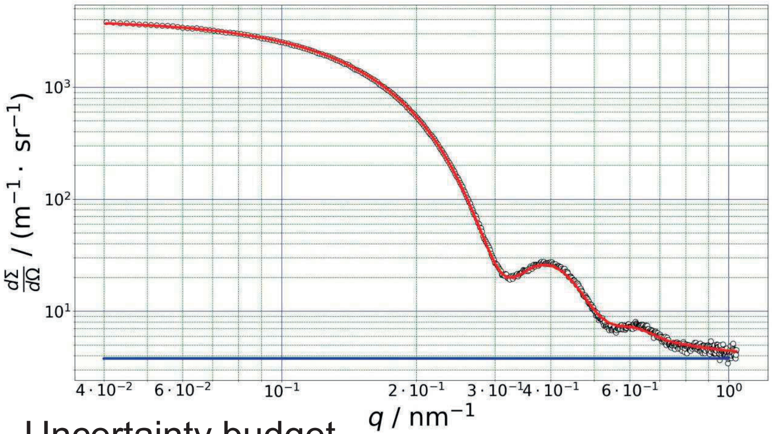
Electron density ρ_e:

for low densities: contrast variation
(or known from other sources)

$$\rho_e = (\rho \cdot Z \cdot N_A)/A$$

Close to absorption edges better: **effective** electron density (ASAXS)

Example for NP concentration from SAXS



Au 30 nm

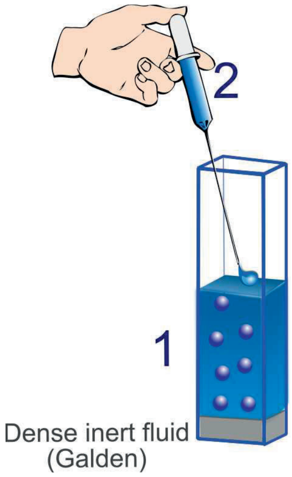
$\rho = (19.3 \pm 0.5)$ g/cm³

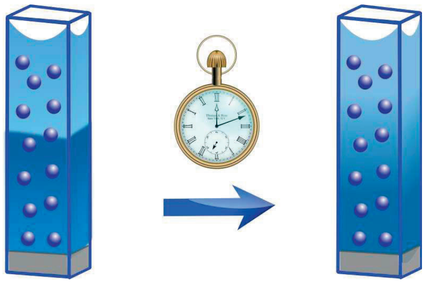
Uncertainty budget

Input quantity x_i	x_i/unit	$U(x_i)$/unit	U_C/ml^{-1}
Energy E	8000.0 eV	0.8 eV	$5.6 \cdot 10^8$
Pixel size s	172.1 μm	0.2 μm	$6.1 \cdot 10^9$
Sample-detector distance L	4511 mm	5 mm	$5.7 \cdot 10^9$
Incoming flux Φ_0	$6.8 \cdot 10^9$ ph/s	$6.8 \cdot 10^7$ ph/s	$1.85 \cdot 10^9$
Sample transmission T_{total}	2.00 %	0.02 %	$1.85 \cdot 10^9$
Exposure time t	60.00 s	< 0.06 s	$1.85 \cdot 10^8$
Detector quantum efficiency $\eta_{QE,8\,\mathrm{keV}}$	97 %	3 %	$6.0 \cdot 10^7$
Sample thickness w	1.01 mm	0.03 mm	$6.03 \cdot 10^8$
Fitting Parameter $Nfit$	$2.75 \cdot 10^{-5}$	$9.0 \cdot 10^{-7}$	$4.44 \cdot 10^9$
Contrast $(\Delta\rho)^2$	$1.88 \cdot 10^7$ nm^{-6}	$1.05 \cdot 10^6$ nm^{-6}	$1.03 \cdot 10^{10}$
Concentration C	$1.85 \cdot 10^{11}$ ml^{-1}		$1.3 \cdot 10^{10}$

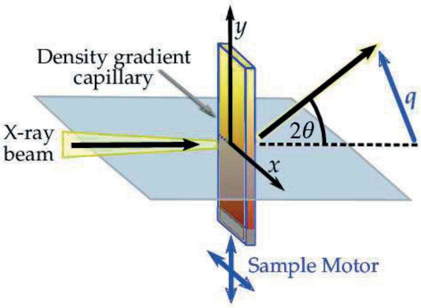
Continuous contrast variation

X-ray Radiometry





Electron density gradient develops in a capillary

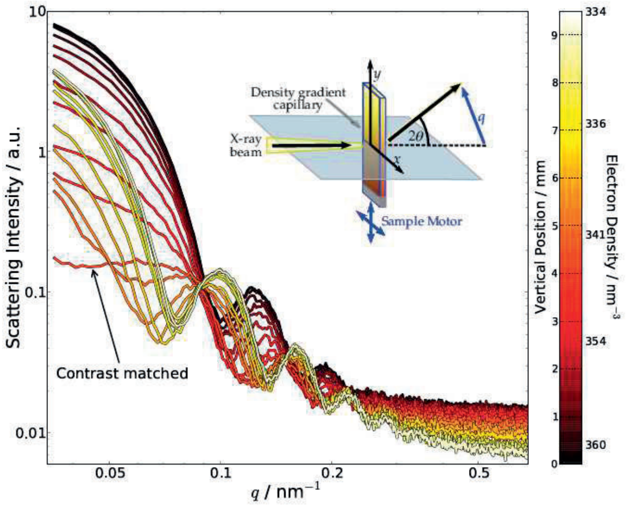


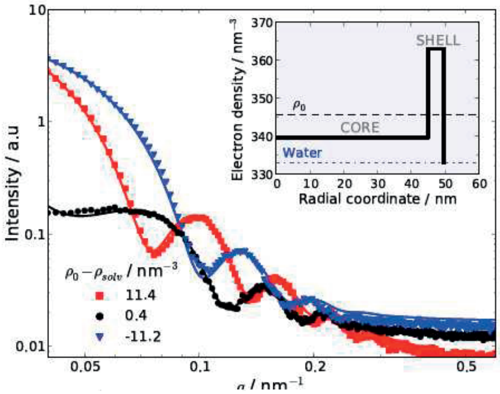
Electron density difference depends on vertical position *y*

Core/shell nanoparticles

X-ray Radiometry

Polymeric core/shell NPs, 8.8 keV,
measurement every 0.5 mm on vertical axis





	R (nm)	ρ_0 (nm^{-3})
Core-shell fitting	49.7±2.8	345.9±1.5
Isoscattering point*	50.5	-
Radius of gyration	47.6**	343.7
Zero-angle intensity	-	346.0

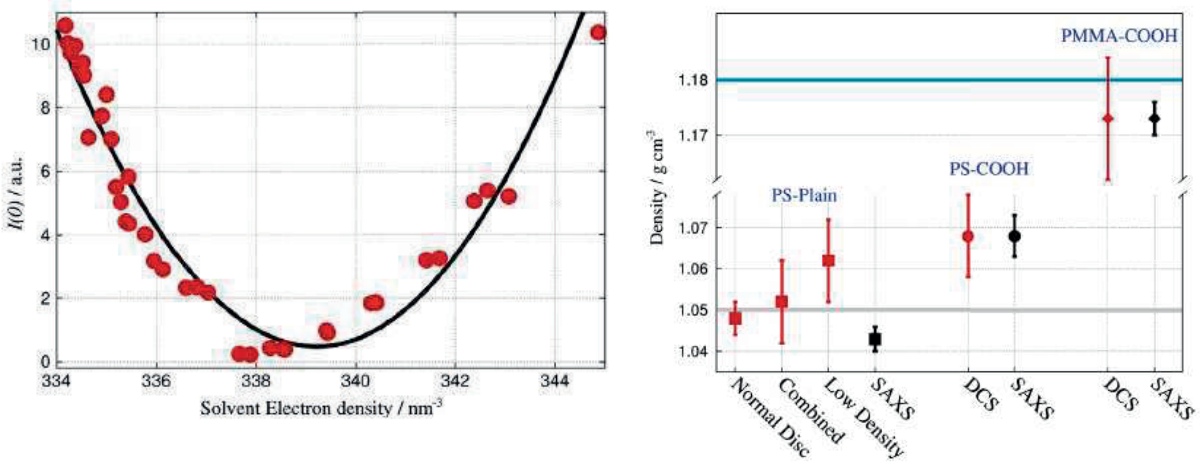
Has also been applied e.g. for nanodrugs (Doxil / Caelyx)

R. Garcia-Diez, C. Gollwitzer and M. Krumrey, J. Appl. Cryst. **48**, 20 – 28 (2015)

Electron density determination

X-ray Radiometry

Continuous contrast variation



Electron density of polymeric colloids compared to results from DCS

R. Garcia-Diez, A. Sikora, C. Gollwitzer, C. Minelli, M. Krumrey, Europ. Polymer J. **81**, 641 (2016)

Conclusions

X-ray Radiometry

- Traceable size determination of spherical nanoparticles using Small-Angle X-ray Scattering (SAXS) has already been established, ISO standard 17867:2015 is available.
- Nanoparticle concentration determination with low uncertainties requires the determination of all relevant parameters (including the density of the nanoparticles), work on ISO standard 23484 has just been started.
- Continuous contrast variation can be used to determine the particle density and the size of core/shell nanoparticles

Acknowledgements

X-ray Radiometry

PTB

- Alexander Schavkan
- Christian Gollwitzer (now at BAM)
- Raul Garcia-Diez (now at HZB)
- Peter Müller
- Levent Cibik

HZB Helmholtz Zentrum Berlin

- Armin Hoell

IN NANO PART
Innovative Nanoparticle Metrology

NPL National Physical Laboratory

- Alex Shard
- Caterina Minelli
UK

EURAMET
European Association of National Metrology Institutes

BAM

Bundesanstalt für
Materialforschung
und -prüfung

Sicherheit in Technik und Chemie

14.05.2018

CHALLENGES OF SYNTHESIS AND ANALYSIS OF CORE-SHELL NANOMATERIALS FOR REFERENCE MATERIALS

Andreas F. Thünemann

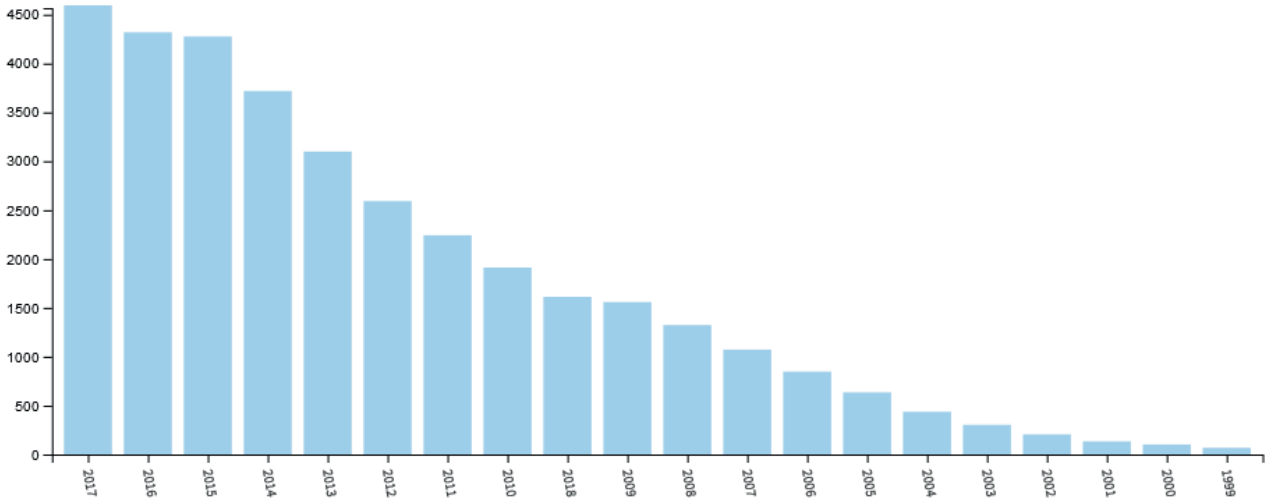
BAM

Outlook:

- Importance of core-shell nanoparticles
- Selected examples of core-shell particles
- Trojan horses jumping over the blood-brain barrier
- SAXS: A method to look into nanoparticles

Importance of core-shell nanoparticles

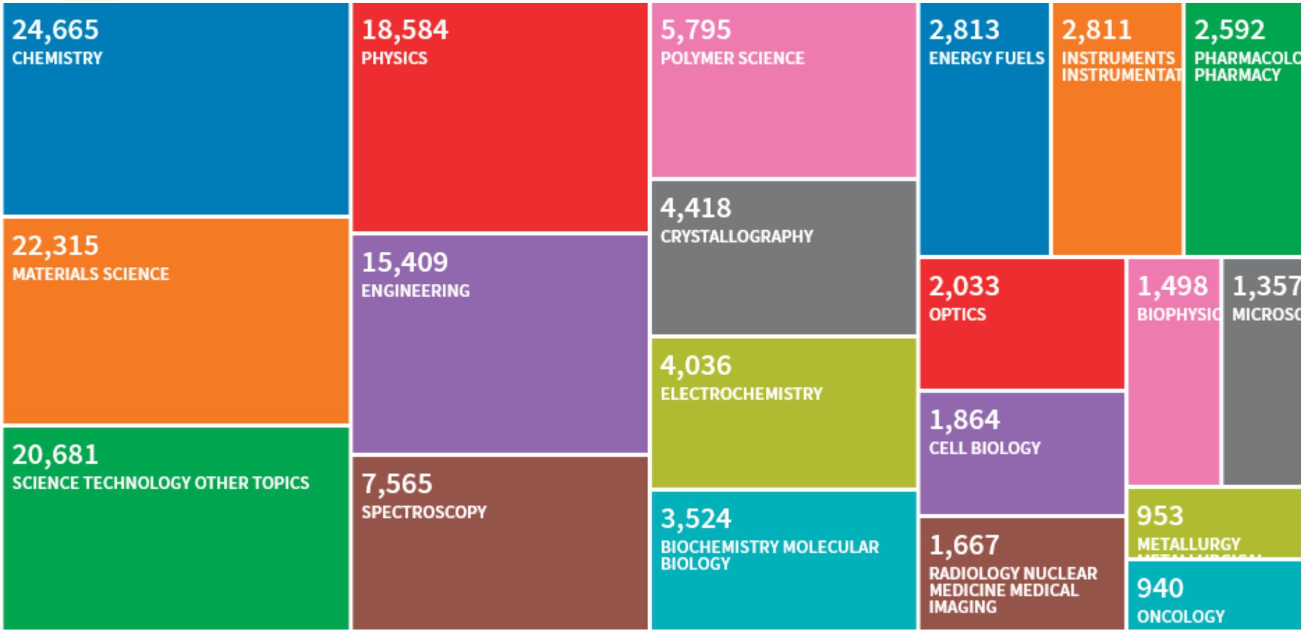
Publications found in "Web of Science"



Showing 34,175 records for **TOPIC:** (Core-shell nanoparticle*)

Importance of core-shell nanoparticles

Research areas according to "Web of Science"



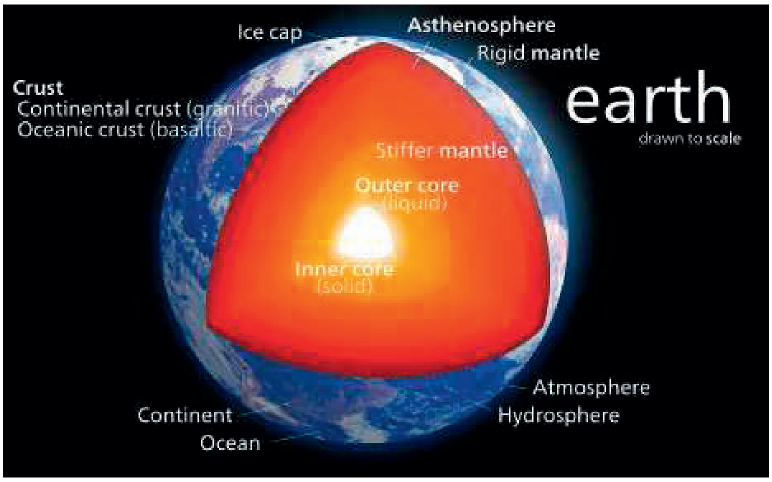
Showing 34,175 records for **TOPIC:** (Core-shell nanoparticle*)

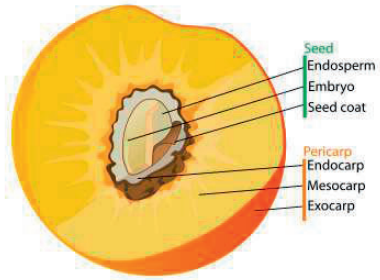
Selected examples of core-shell particles

Different length scales and morphologies

radius = 6371 km

radius ca. 6 cm



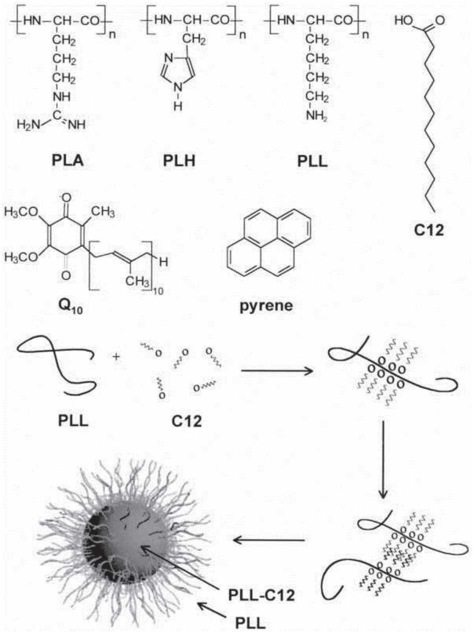


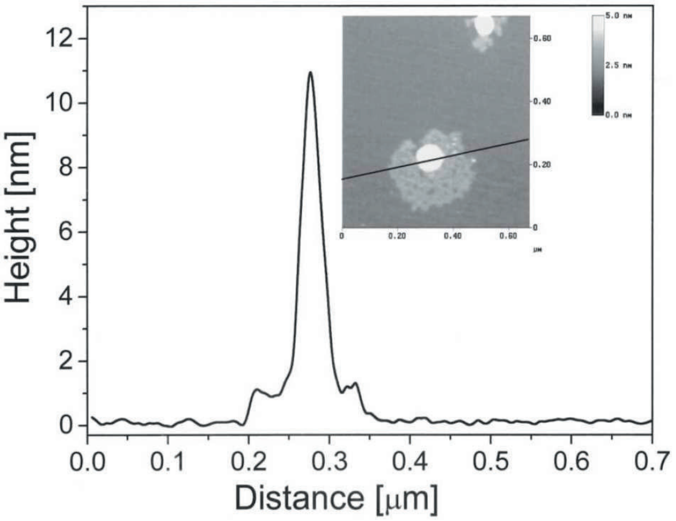
https://en.wikipedia.org/wiki/Structure_of_the_Earth

https://en.wikipedia.org/wiki/Drupe

Selected examples of core-shell particles

Different length scales and morphologies



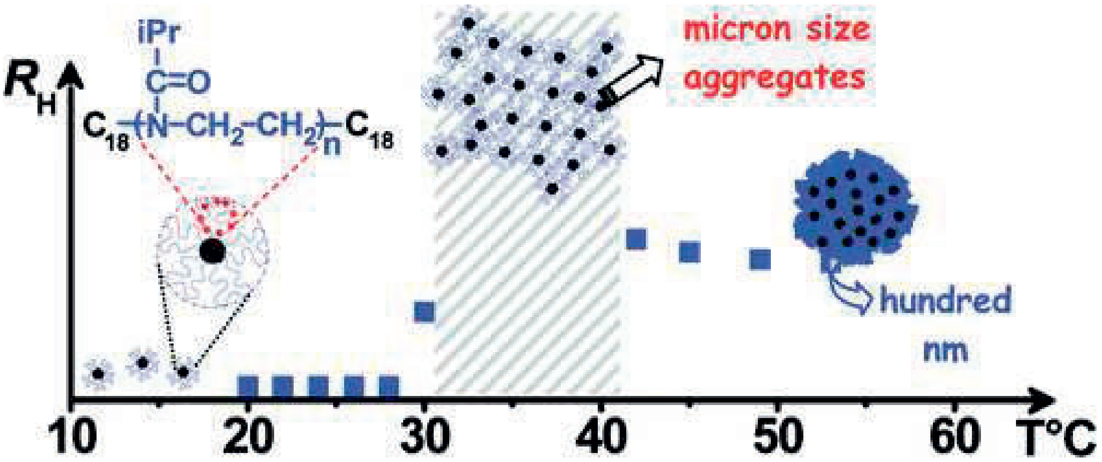


Radius: 100-200 nm
Core radius: 50-100 nm

S. General, A.F. Thünemann International Journal of Pharmaceutics 2001, 230, 11-24

Selected examples of core-shell particles

Different length scales and morphologies



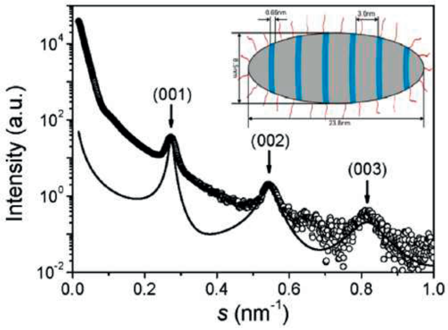
Radius: 7-12 nm
Core radius: 1.3 nm

F. R. Obeid, A. F. Thünemann, F. Winnink et al. Macromolecules 2009, 42, 2204-2215

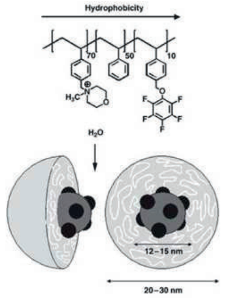
Selected examples of core-shell particles

Different length scales and morphologies

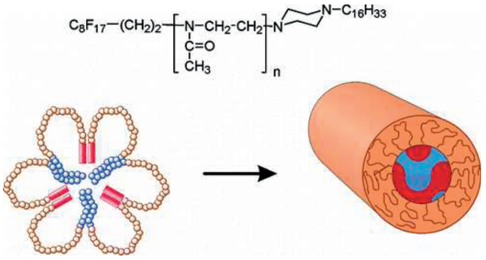
Like an American football with filling



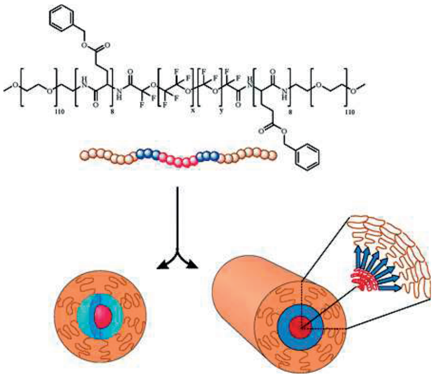
Macromolecules 2006, 39, 9337-9345



Like a doughnut with a raspberry filling
Angew. Chem. Int. Ed. 2005, 44, 5262-5265



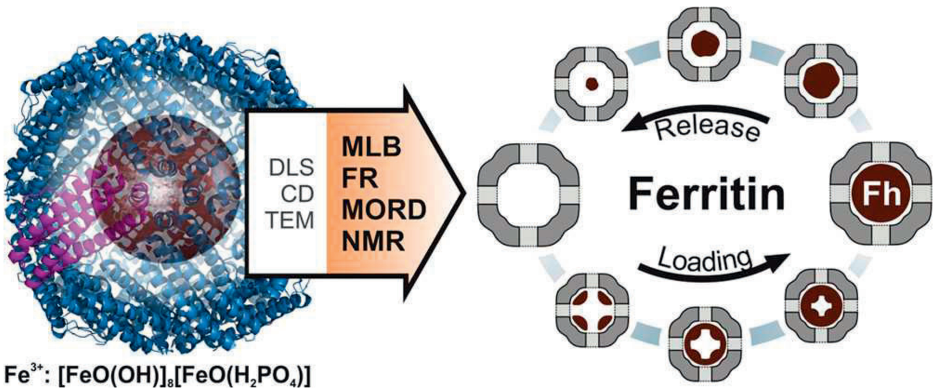
Langmuir 2005, 21, 7214-7219



Langmuir 2006, 22, 2506-2510

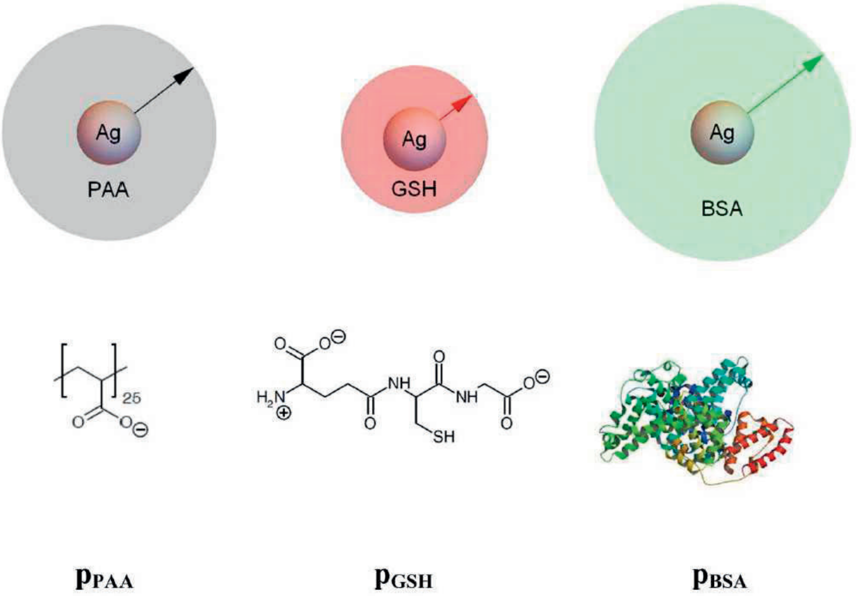
Selected examples of core-shell particles

Different length scales and morphologies



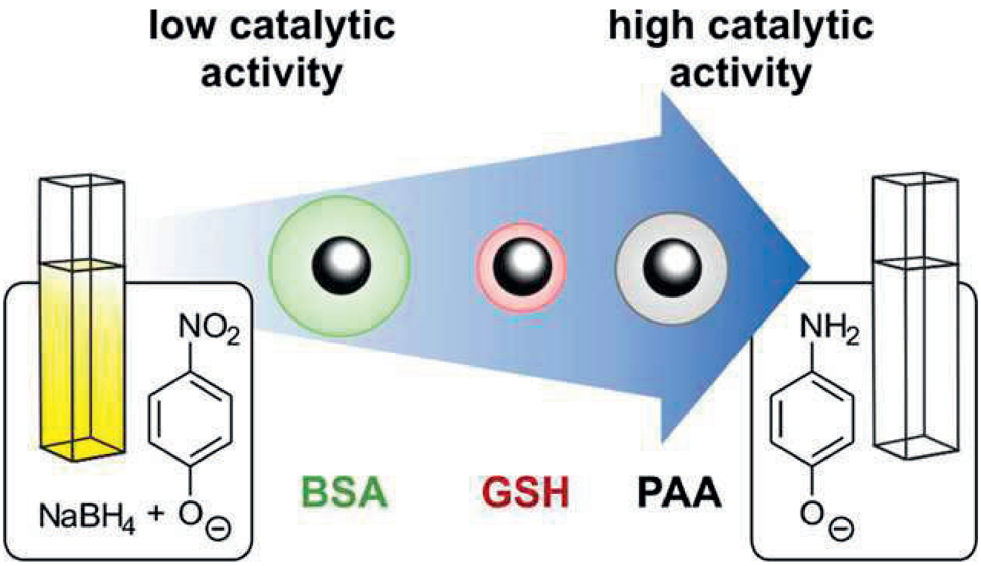
Koralewski et al. *ACS Appl. Mater. Interfaces* 2018, 10, 7777-7787

Selected examples of core-shell particles Catalytic Active silver Nanoparticles



C. Kästner, A. F. Thünemann *Langmuir* 2016, 32, 7383-7391

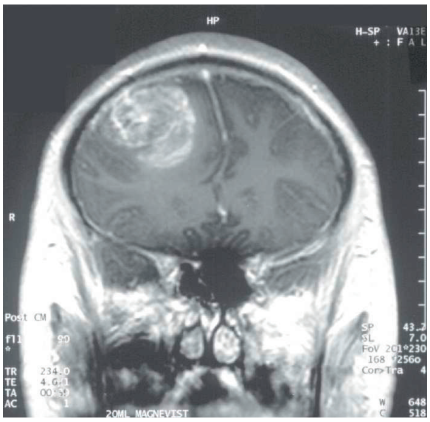
Selected examples of core-shell particles Catalytic Active silver Nanoparticles



C. Kästner, A. F. Thünemann *Langmuir* 2016, 32, 7383-7391

Trojan horses jumping over the blood-brain barrier

- An intact blood-brain barrier ensures that only selected substances enter the brain.
- Particles have no access to the brain, not even nanoparticles.
- This is useful in a healthy brain, but a huge problem in the treatment of diseased brains (e.g. glioblastomas).



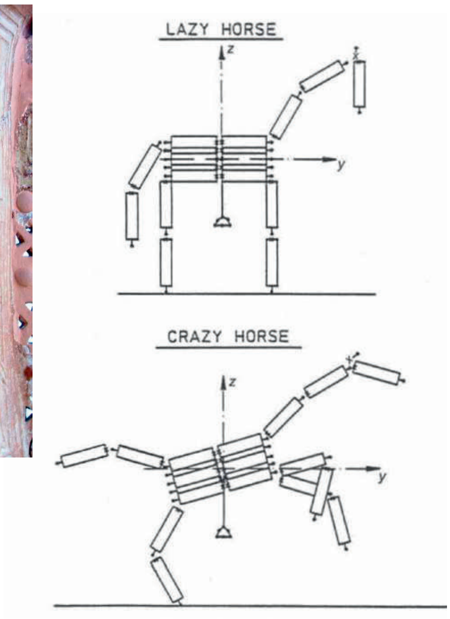
MRI with contrast of a glioblastoma in a 15-year-old male (https://en.wikipedia.org/wiki/Glioblastoma)

Here, something like a Trojan horse is needed to access the brain.

Trojan horses jumping over the blood-brain barrier

The Mykonos vase (750 to 650 BC)

https://en.wikipedia.org/wiki/Trojan_Horse

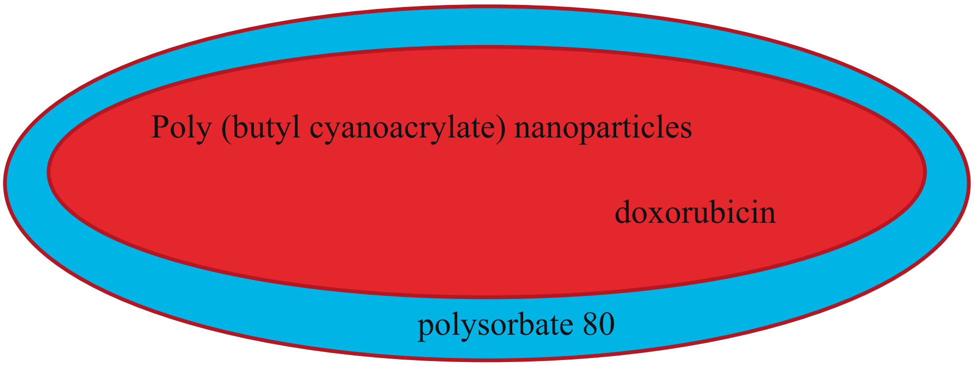


Scattering Methods
and their Application
in Colloid
and Interface Science

OTTO GLATTER

Otto Glatter
2018, Book Elsevier

Trojan horses jumping over the blood-brain barrier



Poly (butyl cyanoacrylate) nanoparticles

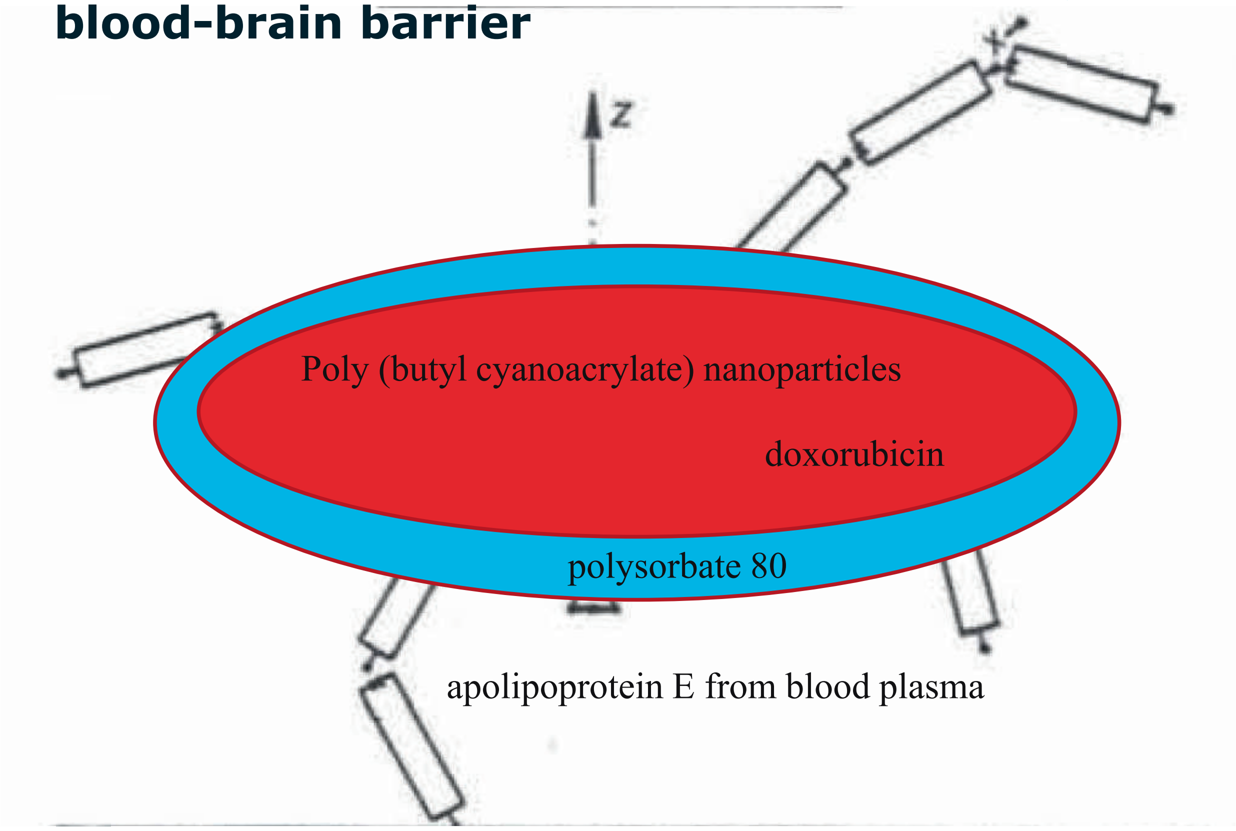
doxorubicin

polysorbate 80

apolipoprotein E from blood plasma

J. Kreuter Advanced Drug Delivery Reviews 47 (2001) 65–81

Trojan horses jumping over the blood-brain barrier



Poly (butyl cyanoacrylate) nanoparticles

doxorubicin

polysorbate 80

apolipoprotein E from blood plasma

J. Kreuter Advanced Drug Delivery Reviews 47 (2001) 65–81

Small-Angle X-ray scattering (SAXS)

A method to look into nanoparticles

My favorite method for core-shell particles
with diameters
In the range of 1 to 100 nm

Small-Angle X-Ray Scattering

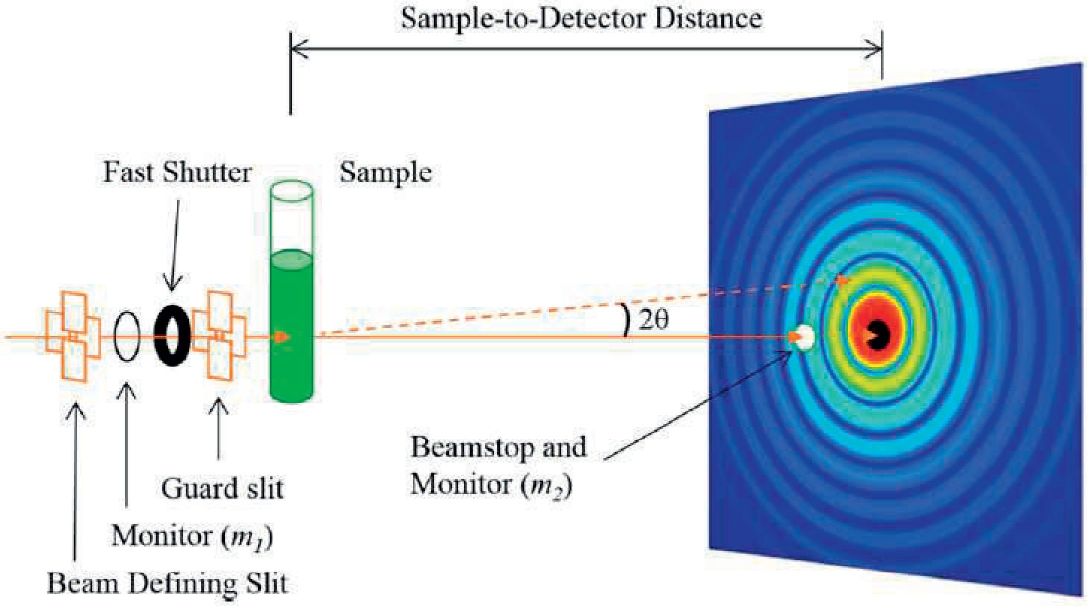
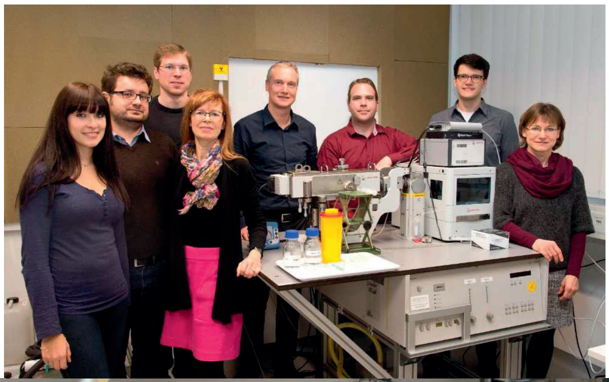
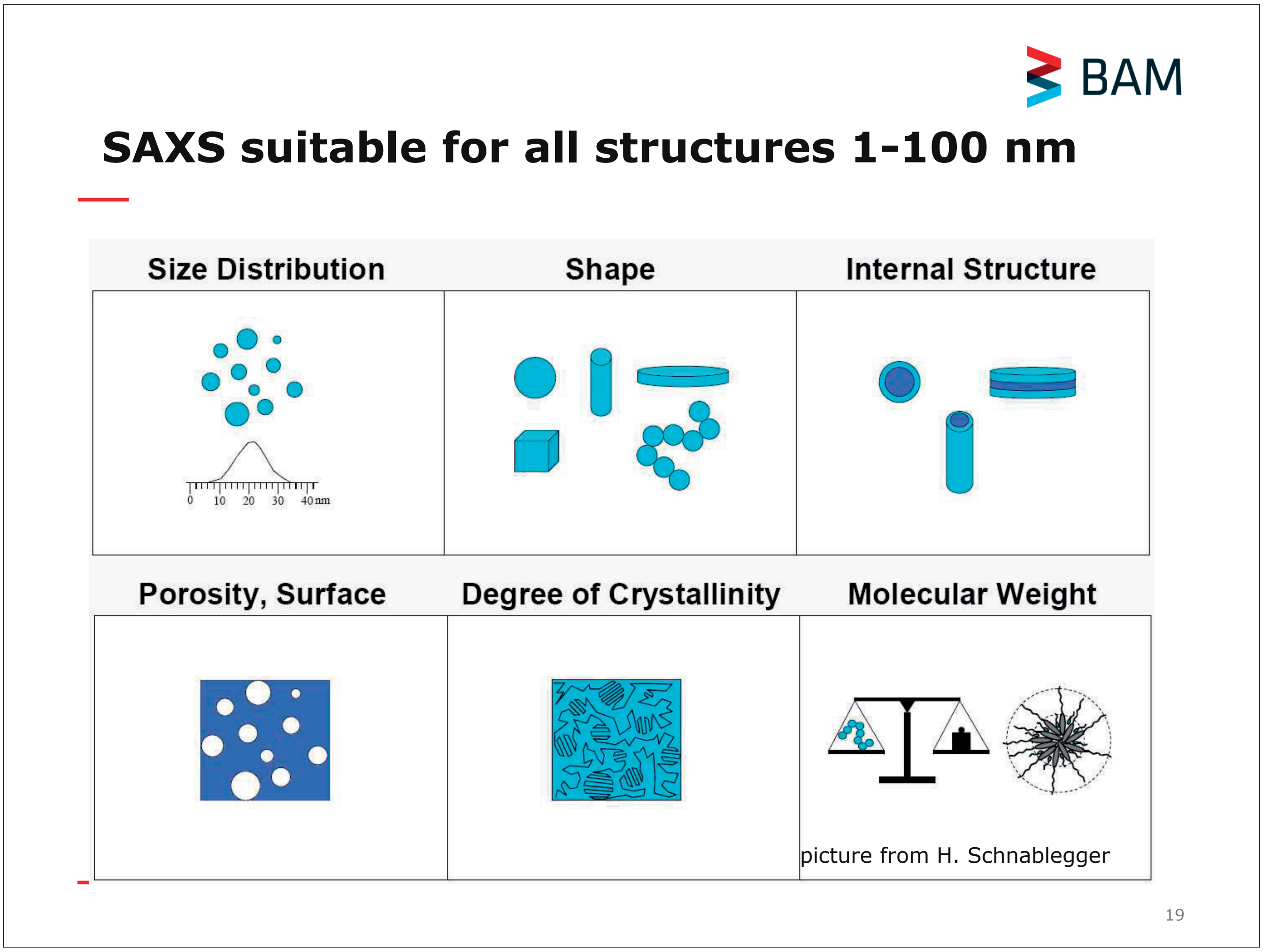


Figure 1. Schematic for the optical components of the 12ID-B SAXS beamline at the Advanced Photon Source (APS).

DOI: 10.1021/acs.chemrev.5b00690
Chem. Rev. 2016, 116, 11128–11180

Small-Angle X-Ray Scattering in BAM 6.5

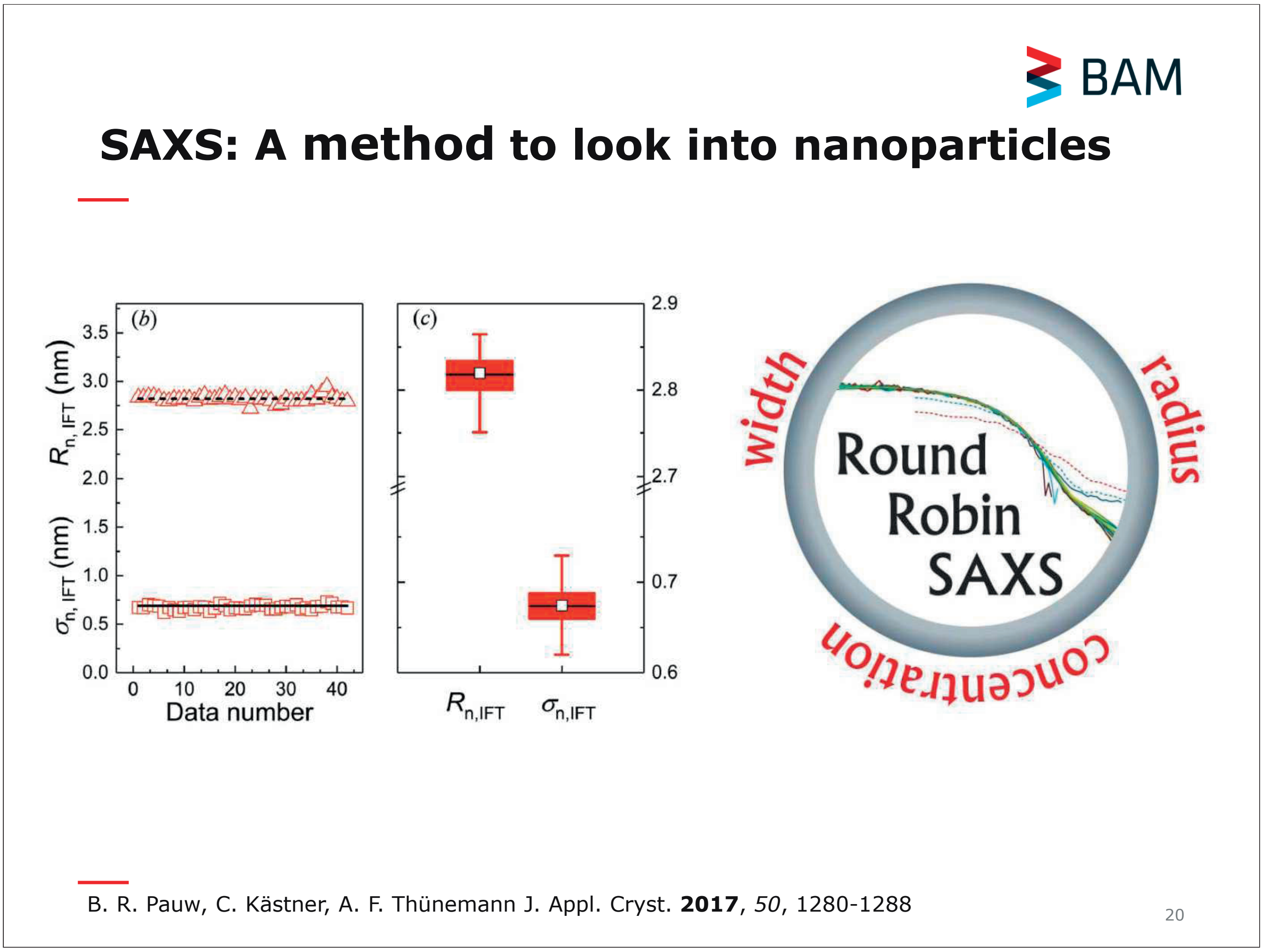




SAXS suitable for all structures 1-100 nm

Size Distribution	Shape	Internal Structure
Porosity, Surface	**Degree of Crystallinity**	**Molecular Weight**

picture from H. Schnablegger



SAXS: A method to look into nanoparticles

B. R. Pauw, C. Kästner, A. F. Thünemann J. Appl. Cryst. **2017**, *50*, 1280-1288

SAXS: A method to look into nanoparticles

Inhouse SAXS software development

Classical curve fit

Monte Carlo approximation

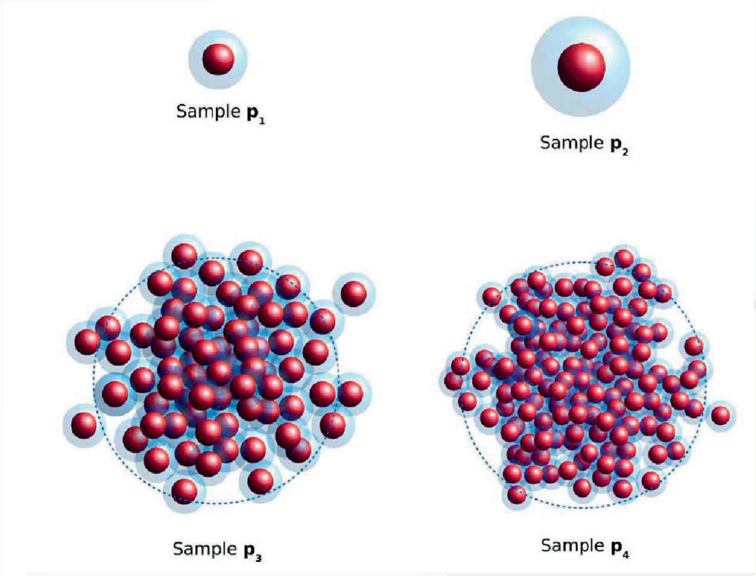
SASfit: I. Breßler, J. Kohlbrecher, A. F. Thünemann J. Appl. Cryst. **2015**, *48*, 962-969

McSAS: I. Breßler, B. R. Pauw, A. F. Thünemann J. Appl. Cryst. **2015**, *48*, 1587-1598

SAXS: A method to look into nanoparticles

SAXS analysis of multi-core iron oxide magnetic nanoparticles

See EU-Project NanoMag



W. Szczerba, A. F. Thünemann et al. *J. Appl. Cryst.* **2017**, *50*, 481-488

SAXS: A method to look into nanoparticles

Reference Materials

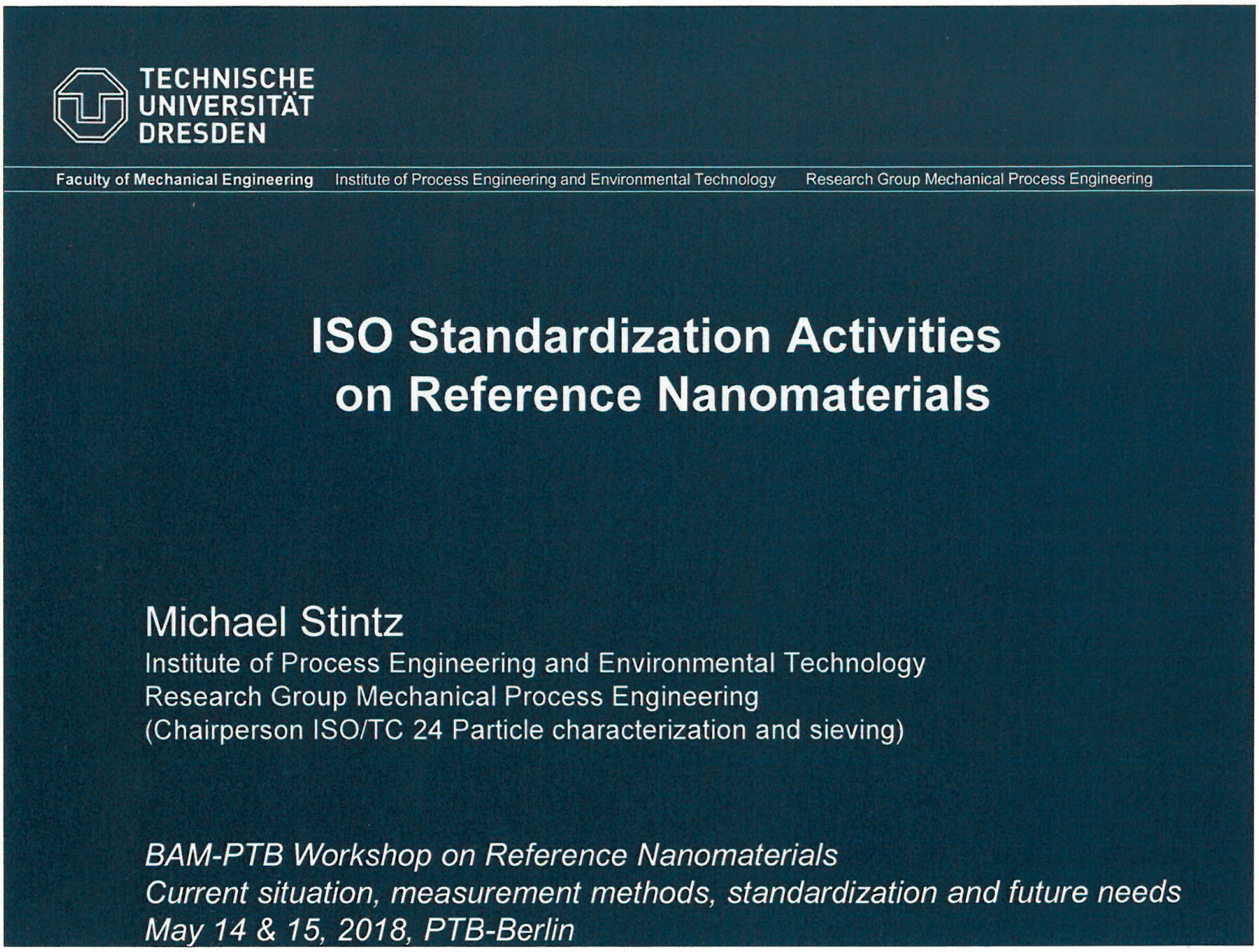
Synthesis and characterization of Nanoparticles

Monitoring reactions of Nanoparticles (Nanoscale 2018 in press)

Summary:

- Long-term stable nanoparticles have a core-shell structure
- We offer the production of custom-made particles
- Characterization is performed (among other methods) with in-house SAXS

Many thanks to all members of my group!
Thank you for your attention!



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Faculty of Mechanical Engineering Institute of Process Engineering and Environmental Technology Research Group Mechanical Process Engineering

ISO Standardization Activities on Reference Nanomaterials

Michael Stintz
Institute of Process Engineering and Environmental Technology
Research Group Mechanical Process Engineering
(Chairperson ISO/TC 24 Particle characterization and sieving)

BAM-PTB Workshop on Reference Nanomaterials
Current situation, measurement methods, standardization and future needs
May 14 & 15, 2018, PTB-Berlin

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Agenda

MVT

Faculty of Mechanical Engineering Institute of Process Engineering and Environmental Technology Research Group Mechanical Process Engineering

Standardization for nanotechnologies, -materials

Nanoobjects in Nanomaterial Terminology

ISO Standardization Activities on Reference Nanomaterials

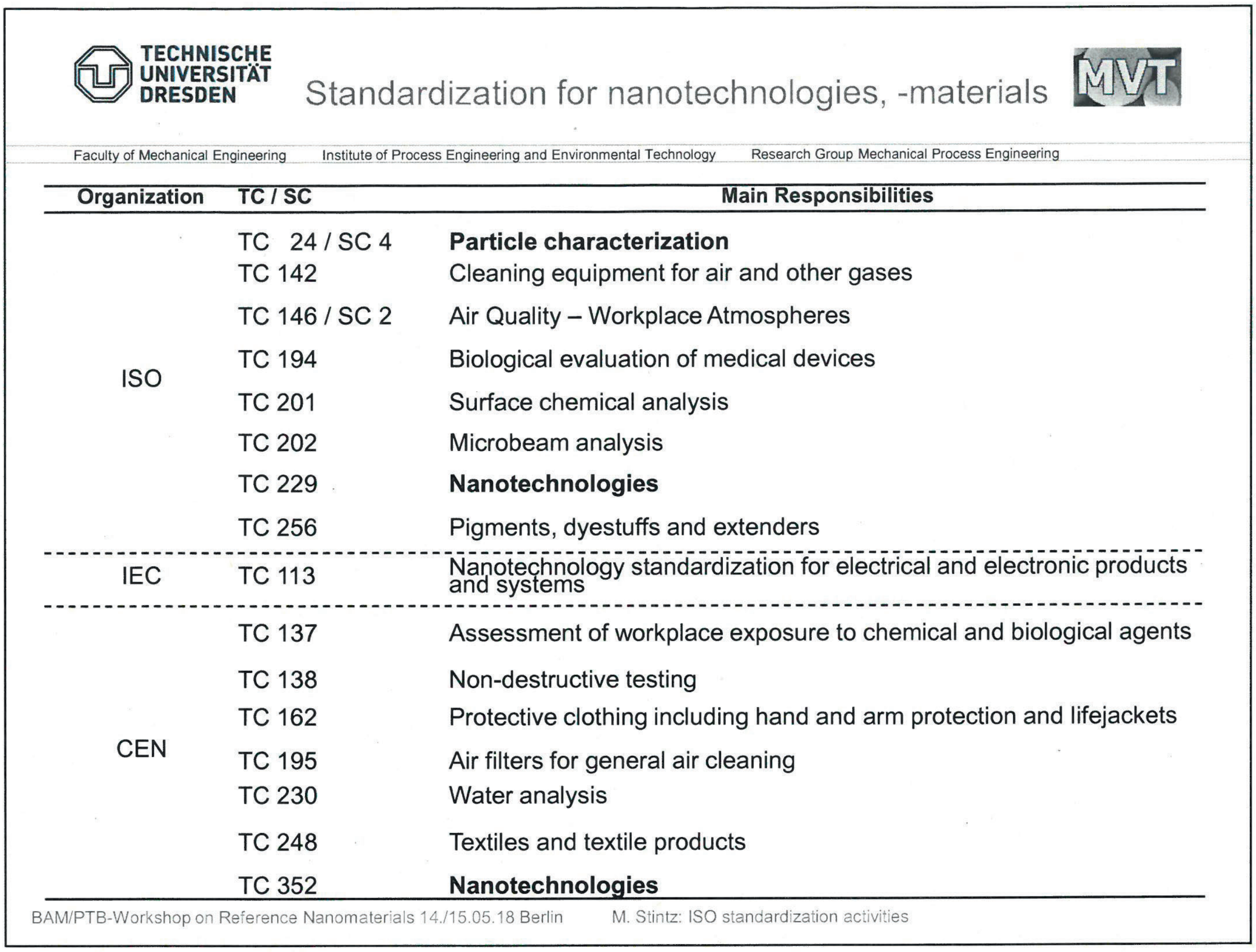
  ISO/TC 229 Nanotechnologies

  ISO/TC 24/SC 4 Particle Characterization

DIN NMP Nanotechnologie Spec

New candidate for reference material

BAM/PTB-Workshop on Reference Nanomaterials 14./15.05.18 Berlin M. Stintz: ISO standardization activities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Standardization for nanotechnologies, -materials

Faculty of Mechanical Engineering Institute of Process Engineering and Environmental Technology Research Group Mechanical Process Engineering

Organization	TC / SC	Main Responsibilities
ISO	TC 24 / SC 4	**Particle characterization**
	TC 142	Cleaning equipment for air and other gases
	TC 146 / SC 2	Air Quality – Workplace Atmospheres
	TC 194	Biological evaluation of medical devices
	TC 201	Surface chemical analysis
	TC 202	Microbeam analysis
	TC 229	**Nanotechnologies**
	TC 256	Pigments, dyestuffs and extenders
IEC	TC 113	Nanotechnology standardization for electrical and electronic products and systems
CEN	TC 137	Assessment of workplace exposure to chemical and biological agents
	TC 138	Non-destructive testing
	TC 162	Protective clothing including hand and arm protection and lifejackets
	TC 195	Air filters for general air cleaning
	TC 230	Water analysis
	TC 248	Textiles and textile products
	TC 352	**Nanotechnologies**

BAM/PTB-Workshop on Reference Nanomaterials 14./15.05.18 Berlin M. Stintz: ISO standardization activities


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International Standardization / TC Structure

Faculty of Mechanical Engineering Institute of Process Engineering and Environmental Technology Research Group Mechanical Process Engineering

ISO/TC 24/SC 4 „Particle Characterization"

WG1 Results representation, WG2-17 Measurement methods

"vertically", measurement methodology oriented

ISO/TC 229 „Nanotechnologies"

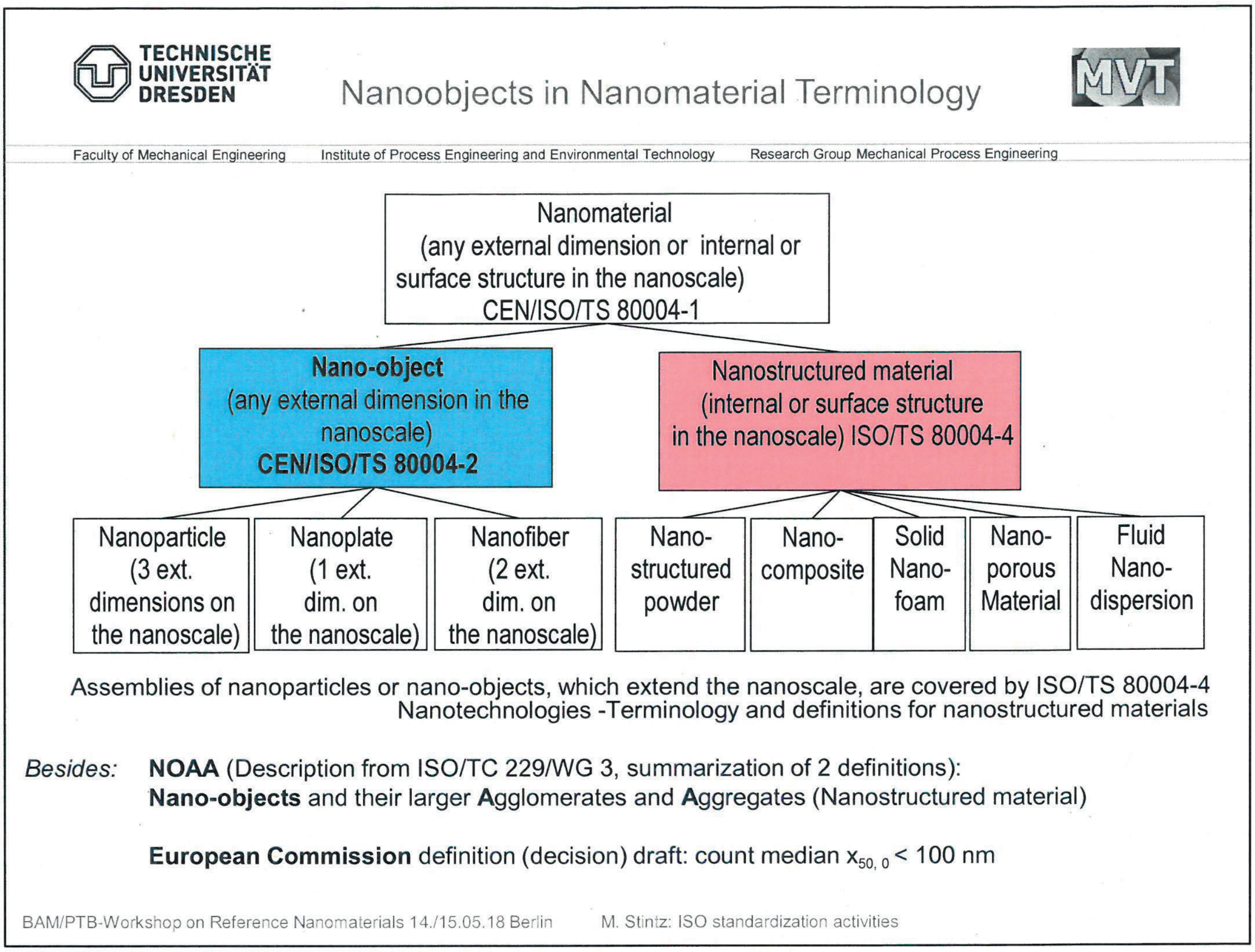
WG1 Terminology, WG2 Measurement, WG3 HSE-aspects, WG4 Material spec., WG5 Product spec.

"horizontally", interdisciplinary, application oriented

CEN/TC 352 „Nanotechnologies"

WG1 Measurement, WG2 Commercial aspects, WG3 HSE-aspects

BAM/PTB-Workshop on Reference Nanomaterials 14./15.05.18 Berlin M. Stintz: ISO standardization activities



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Nanoobjects in Nanomaterial Terminology

MVT

Faculty of Mechanical Engineering Institute of Process Engineering and Environmental Technology Research Group Mechanical Process Engineering

Nanomaterial
(any external dimension or internal or surface structure in the nanoscale)
CEN/ISO/TS 80004-1

Nano-object
(any external dimension in the nanoscale)
CEN/ISO/TS 80004-2

Nanostructured material
(internal or surface structure in the nanoscale) ISO/TS 80004-4

Nanoparticle (3 ext. dimensions on the nanoscale)

Nanoplate (1 ext. dim. on the nanoscale)

Nanofiber (2 ext. dim. on the nanoscale)

Nano-structured powder

Nano-composite

Solid Nano-foam

Nano-porous Material

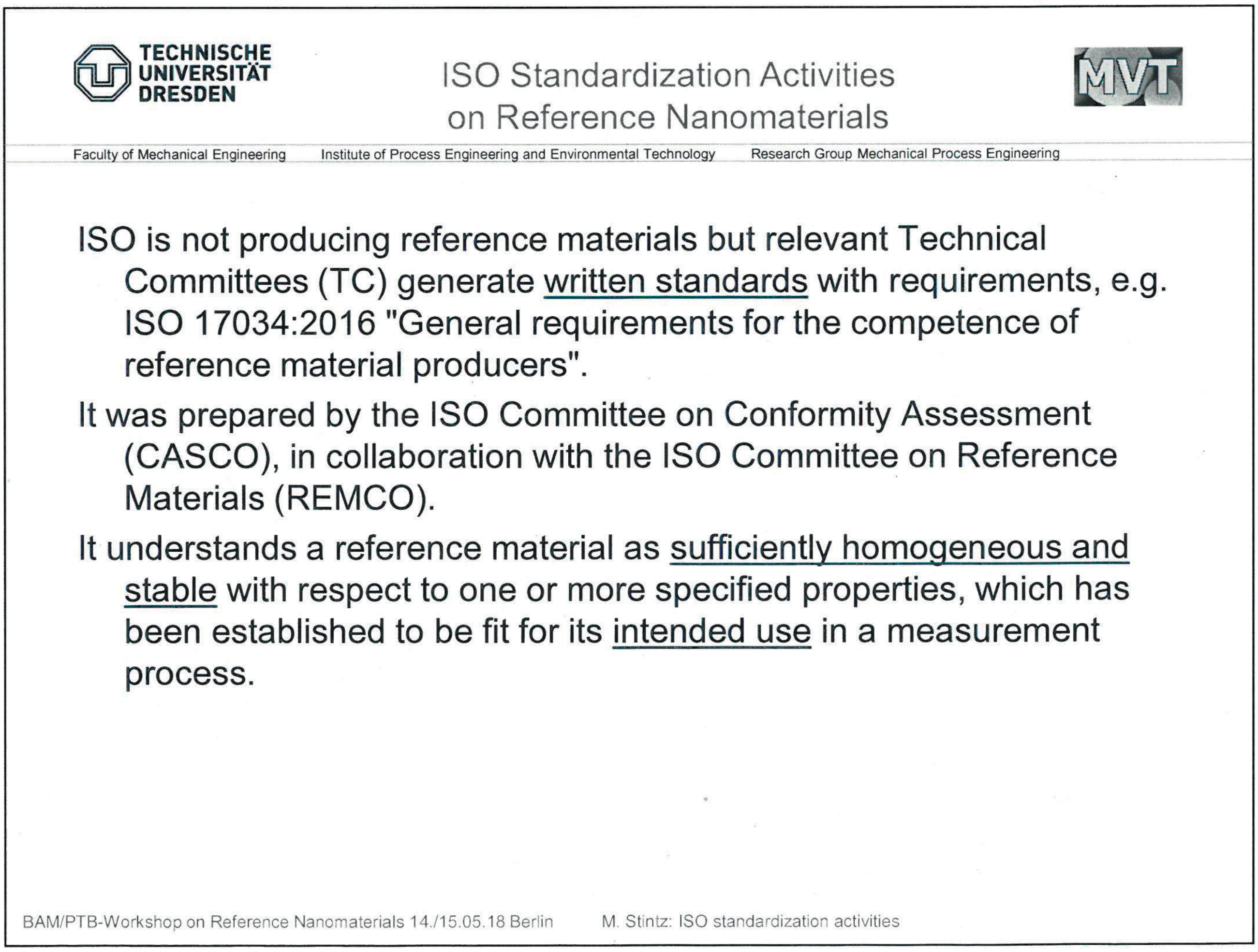
Fluid Nano-dispersion

Assemblies of nanoparticles or nano-objects, which extend the nanoscale, are covered by ISO/TS 80004-4
Nanotechnologies -Terminology and definitions for nanostructured materials

Besides: **NOAA** (Description from ISO/TC 229/WG 3, summarization of 2 definitions):
Nano-objects and their larger **A**gglomerates and **A**ggregates (Nanostructured material)

European Commission definition (decision) draft: count median $x_{50,0} < 100$ nm

BAM/PTB-Workshop on Reference Nanomaterials 14./15.05.18 Berlin M. Stintz: ISO standardization activities



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ISO Standardization Activities on Reference Nanomaterials

MVT

Faculty of Mechanical Engineering Institute of Process Engineering and Environmental Technology Research Group Mechanical Process Engineering

ISO is not producing reference materials but relevant Technical Committees (TC) generate written standards with requirements, e.g. ISO 17034:2016 "General requirements for the competence of reference material producers".

It was prepared by the ISO Committee on Conformity Assessment (CASCO), in collaboration with the ISO Committee on Reference Materials (REMCO).

It understands a reference material as sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process.

BAM/PTB-Workshop on Reference Nanomaterials 14./15.05.18 Berlin M. Stintz: ISO standardization activities

ISO/TC 229 "Nanotechnologies" JWG 2

In the field of nanotechnology ISO/TC 229 published a first technical specification ISO/TS 16195:2013 "Nanotechnologies -- Guidance for developing representative test materials consisting of nano-objects in dry powder form", which is now under revision.

Scope:

This technical specification provides guidance for developing representative test materials consisting of nano-objects in dry powder form, to enable test method development and improve comparability of data for nanotechnology applications.

This guidance includes the physico-chemical properties (specifically, size and shape, specific surface area, crystal structure, and bulk chemical composition) that are required to be measured and reported with the representative test material.

ISO/TS 16195:2013 - normative example:

6 Contents of verification report

6.3 Measurement result

The verification report shall contain the following items with respect to measurement conditions and results of the physico-chemical properties specified in 4.2 with statements about their homogeneity and stability. The measurement conditions shall also accompany the evaluation of homogeneity and stability.

6.3.1 Size and shape of nano-objects

a) Measurement method including detailed sample preparation (e.g., sampling, dispersion, drying and degassing), calibration, and data analysis.

b) Graphical representation or tables showing size distrib. of constituent particles and their aggregates.

c) Measures and types of the particle size according to ISO 9276-1.

d) Representative size of constituent particles and their aggregates.

e) Shape of constituent particles and their aggregates.

f) Definition of the shape, if the shape is described on the basis of other documents

g) Image showing representative constituent particles and their aggregates with its scale bar.

6.3.2 Specific surface area

a) Pre-treatment and degassing conditions.

b) Experimental procedure for adsorption isotherm determination, e.g. volumetric or gravimetric, static or continuous gas admission, calibration of dead volume or buoyancy.

c) Adsorptive (chemical nature, purity).

ISO/TC 229 "Nanotechnologies" JWG 4

In the same TC JWG4 circulates the draft for a CD - ISO/TS 17200 Nanotechnologies – Nanoparticles in powder form – Characteristics and measurement methods, this management challenge must be solved.

Scope:

This International Standard (IS) specifies fundamental characteristics to be measured of an engineered powder sample to determine the size, the chemical composition and the specific surface area of nanoparticles in powder. This IS is applied to both particles that have a covering material on the surface of a core material and that do not have it. The IS also specifies measurement methods for determining each of these characteristics.

Excluded in this IS are characteristics that pertain to industrial applications of nanoparticles in powder form and detailed measurement protocols as well as characteristics related to health, safety, and environmental issues.

ISO/TC 229 "Nanotechnologies" JWG 4

ISO/TS 17200 Nanotechnologies – Nanoparticles in powder form – Characteristics and measurement methods

Table1—Characteristics and measurement methods

Characteristic		Measurement method
Chemical content	Chemical compounds content	Chemical analysis
	Crystallographic component content	XRD method
Surface area	Specific surface area	Gas adsorption method
Particle size	Primary particle size	TEM or SEM
	Crystallite size	XRD method

ISO/TC 24/SC 4 Particle Characterization

Also other TCs deal with relevant requirements, especially for particle characterization ISO/TC 24/SC 4, working group 11 "Sample preparation and reference materials“.

Managed by its convener Dr. Linsinger from EC JRC in Geel a first TS for Particle size distribution measurement by ensemble techniques has been published: ISO/TS 14411-1:2017. Preparation of particulate reference materials - Part 1: Polydisperse material based on picket fence of monodisperse spherical particles.

ISO/TS 14411-1:2017 picket fence

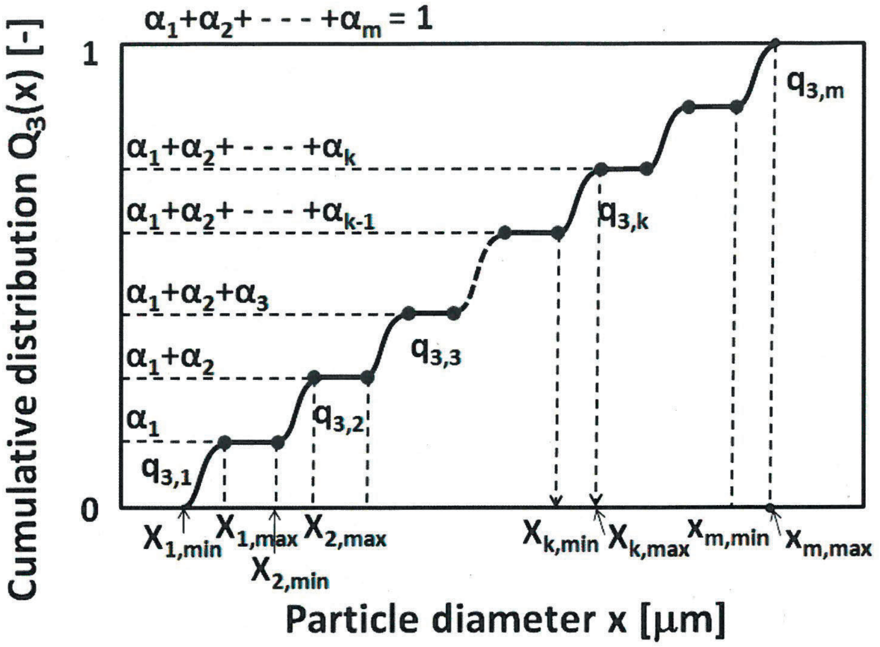
Scope:

This Technical Specification describes the preparation of polydisperse spherical particles based on a picket fence of quasi-monodisperse reference materials, the characterisation of its monodisperse components with acceptable uncertainty and the estimation of the uncertainty of the mixture of these particles.

This type of material should be suitable for all particle characterization methods within the appropriate limits of the techniques. One example usage of these reference materials is the reliability test of the image analysis and other related methods.

This report limits itself to the technical specificities of preparation beyond the general requirements for certified and non-certified reference materials as described in ISO Guides 30, 31, 34 and 35.

ISO/TS 14411-1:2017 picket fence



Particle size distribution composed of several kinds of quasi-monodisperse particles

---

ISO/TC 24/SC 4 Particle Characterization

A second project is currently ongoing: ISO/WD 14411-2:2017 "Preparation of particulate reference materials- Part 2: Polydisperse spherical particles".

Scope:

This international standard describes the specifications for spherical polydisperse particulate reference materials with acceptable uncertainty in particle size distribution and describes protocols for their characterisation. These reference materials will have appropriaye quality for applied industry use. One example usage of these reference materials is the reliability test of the laser-diffraction and other related methods.

This international standard expresses polydispersity and the related uncertainties in size. Users should take notice that small variations in size may imply large variations in cumulative distribution.

This document describes the necessary requirements of particulate reference materials, which should be used to the reliability test of various types of particle size measurement apparatus.

DIN Spec 52407 en.

DIN Spec 52407 Nanotechnologies — Methods for preparation and assessment for particle measurements with atomic force microscopy (AFM) and transmission scanning electron microscopy (TSEM)

For instance "sufficiently homogenous" turned out to be one limiting factor for broad particle size distributions. But even more narrow distributions need special sample preparation methods [1] to get certifiable characteristic parameters:

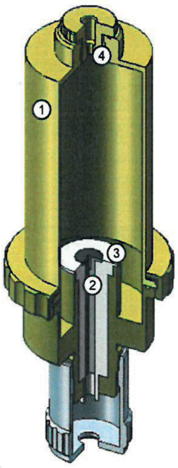
Scope

This DIN SPEC contains a collection of methods for the reliable and reproducible preparation of closely distributed reference nanoparticles on even surfaces as well as their size measurement and analysis.

The methods described are applicable to the preparation and examination of individual particles or particles forming chains as well as monolayers in the size range below 1 µm.

DIN Spec 52407 Preparation of aerosols

Electrostatic precipitation

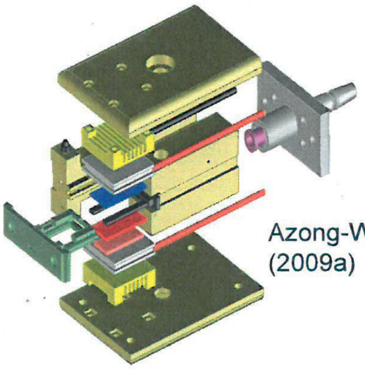


1 cover
2 electrode
3 dielectric
4 aerosol pipe tunnel

Parameters:
Flow rate: 0.3 L·min^{-1}
Flow velocity: 0.9 mm·s^{-1}
Unipolar charching: -3 kV
Electrical field:13 kV

(Dixkens & Fissan (1999) *Aerosol Sci. Technol.;* 30 (5) : 438 – 453) [13]

Thermal precipitation



Azong-Wara et al. (2009a)

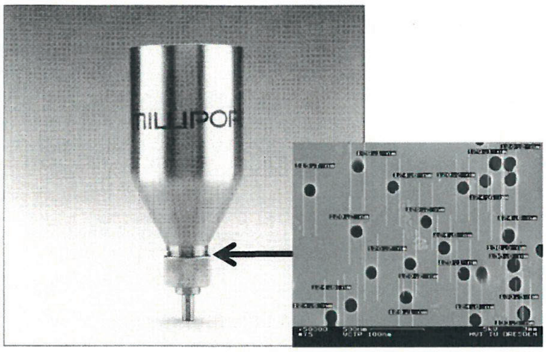
Thermophoretic effect: particle motion warm→cold (= substrat)
Parameters:
Flow rate: 2,0 L·min^{-1}
ΔT = 15 K

TECHNISCHE UNIVERSITÄT DRESDEN

DIN Spec 52407 Preparation of suspensions

Faculty of Mechanical Engineering Institute of Process Engineering and Environmental Technology Research Group Mechanical Process Engineering

Membranefiltration

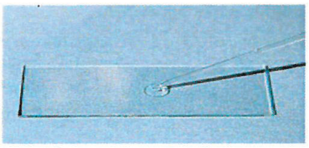


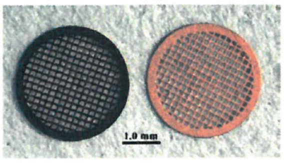
Vakuum-Filterhalter aus Edelstahl 316

- stabilised suspension - homogenising
- dispergating and diluting
- 0,1 – 2 ml filtration with Track etch – membrane at pressure -40 … -70 kPa
- suction until dry
- fix membrane on tab sample stage

Drying method

- stabilised suspension - homogenising
- dispergating and diluting
- droplet sSuspension on substrate
  - drying in exsikator



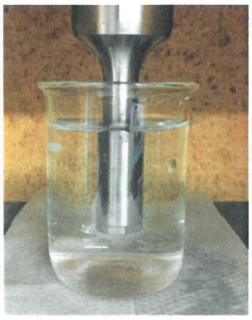


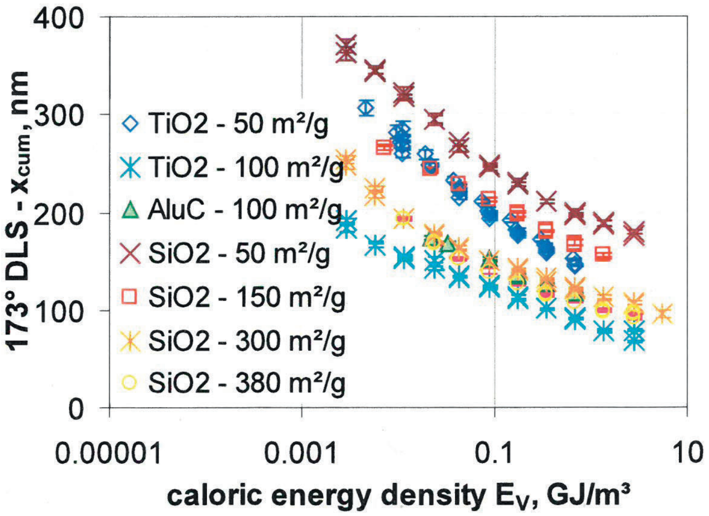
Other:
Critical-Point-Drying, Freezing and drying...

TECHNISCHE UNIVERSITÄT DRESDEN

New need for reference material

Faculty of Mechanical Engineering Institute of Process Engineering and Environmental Technology Research Group Mechanical Process Engineering

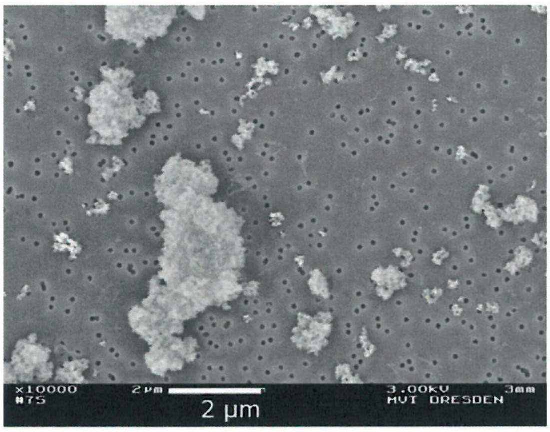




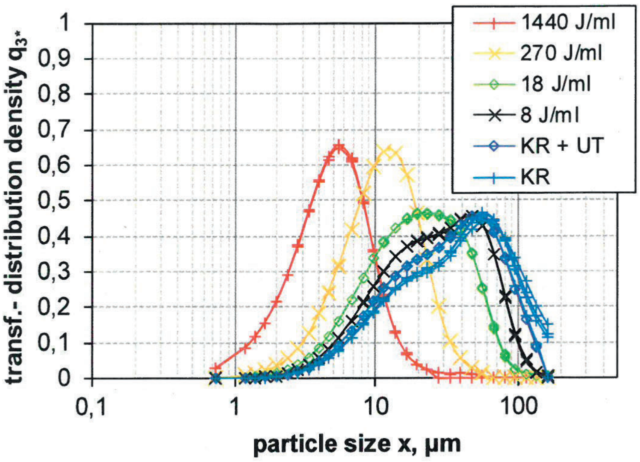
Dependency of average hydrodynamic diameter on caloric energy density [2]
PSD for ultrasonication of several grades (specific surface area) of different pyrogenic substances; measurement by means of DLS (scattering angle of 173°)

New candidate for reference material

Additionally a not so common "intended use“: a nanostructured reference material for agglomerate stability testing in ultrasonic dispersion procedures with defined suspension volume specific energy input.



Nanostructured precipitated silica

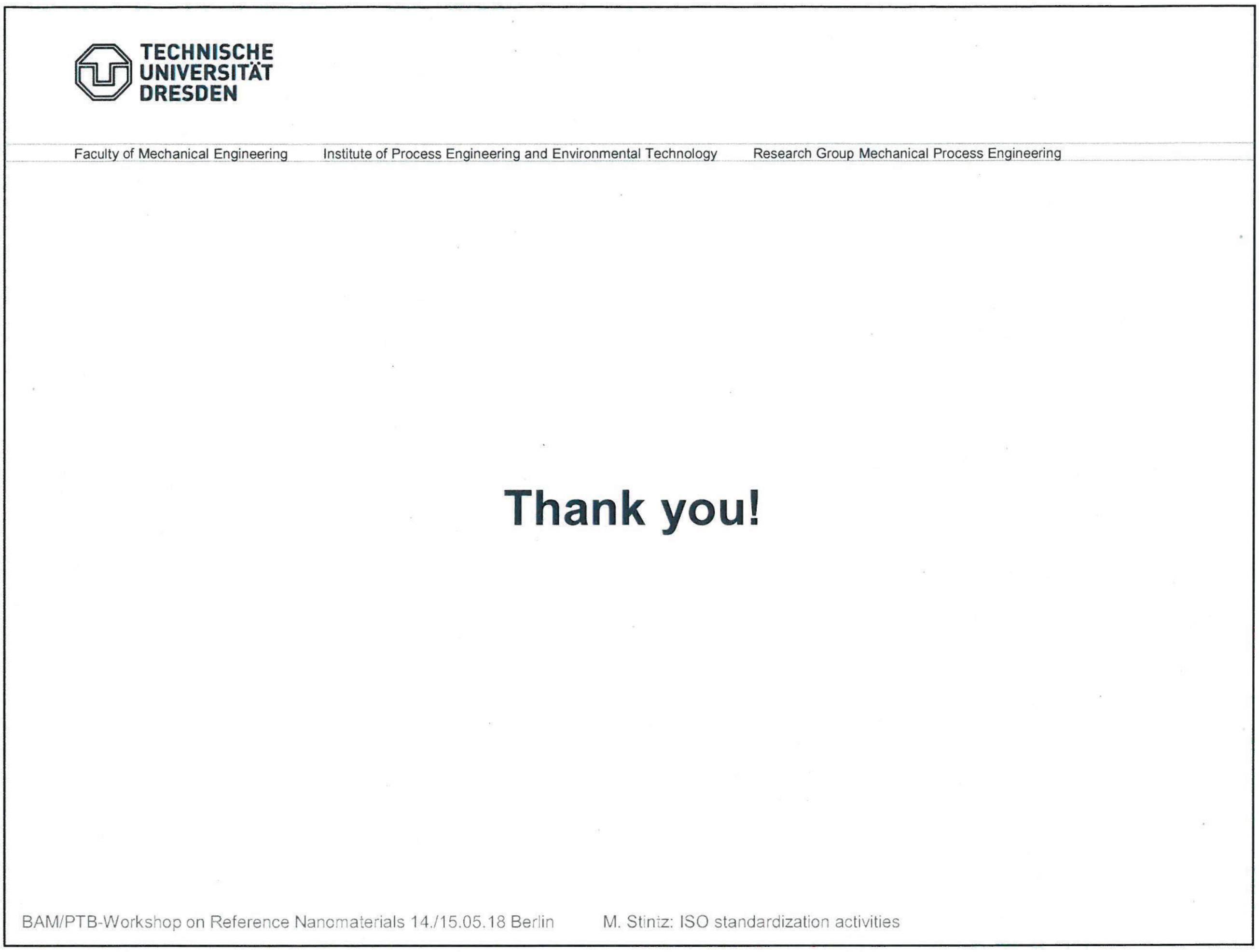


PSD of precip. silica after ultrasonication

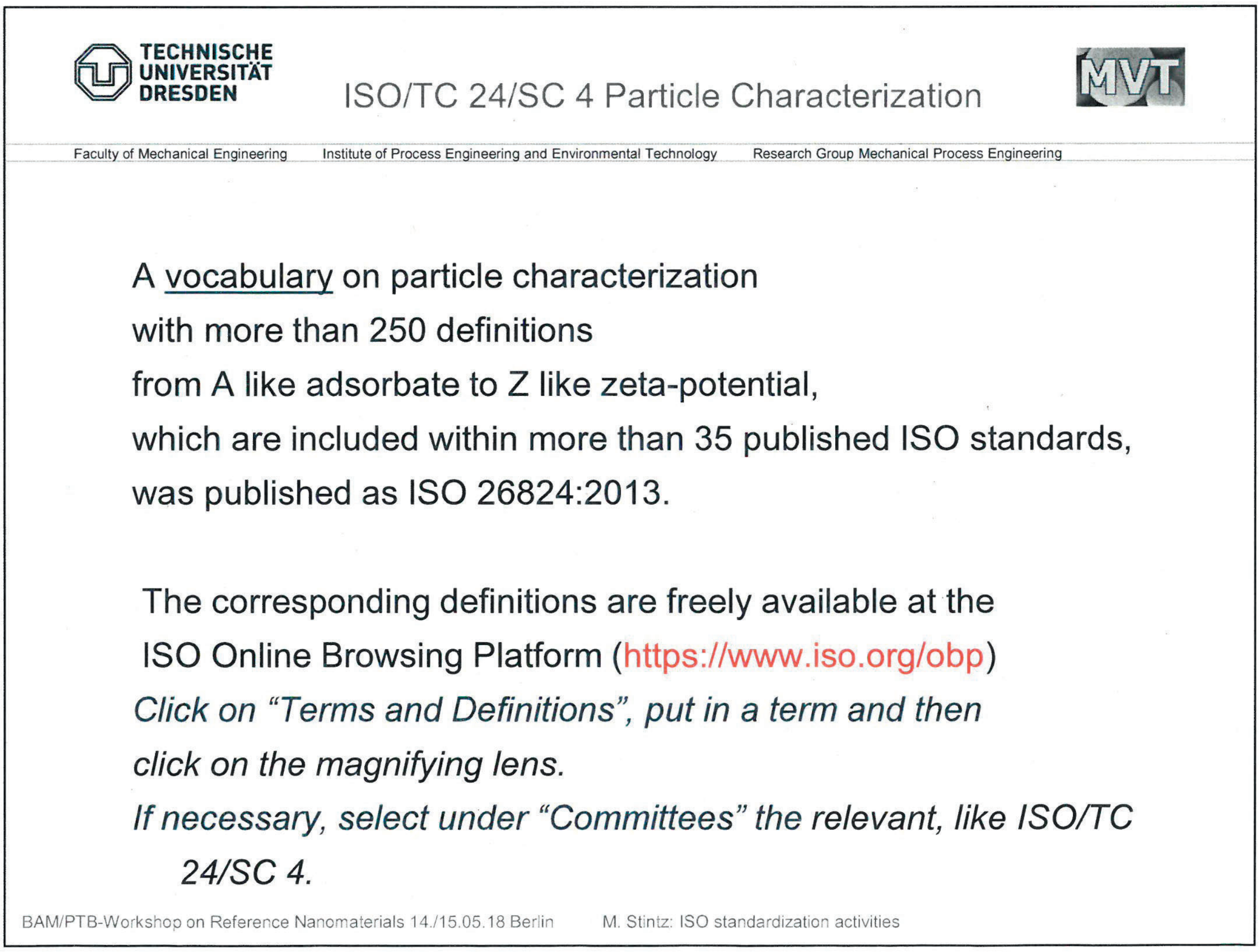
Bibliography

[1] DIN SPEC 52407:2015-03 Nanotechnologies – Methods for preparation and assessment for particle measurements with atomic force microscopy (AFM) and transmission scanning electron microscopy (TSEM) (in German)

[2] R.R. Retamal Marín, F. Babick, M. Stintz, Ultrasonic dispersion of nanostructured materials with probe sonication − practical aspects of sample preparation, Powder Technology, Volume 318, 2017, Pages 451-458



Thank you!



ISO/TC 24/SC 4 Particle Characterization

A vocabulary on particle characterization
with more than 250 definitions
from A like adsorbate to Z like zeta-potential,
which are included within more than 35 published ISO standards,
was published as ISO 26824:2013.

The corresponding definitions are freely available at the ISO Online Browsing Platform (https://www.iso.org/obp)

Click on "Terms and Definitions", put in a term and then click on the magnifying lens.

If necessary, select under "Committees" the relevant, like ISO/TC 24/SC 4.

Standardisation of magnetic nanomaterials:

Steps towards magnetic reference nanoparticles

Uwe Steinhoff[1], Regina Bleul[2], Roman Rüttinger[3], Alex Price[4], Frank Wiekhorst[1]

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[2]Fraunhofer IMM, Mainz, Germany
[3]DIN e. V., Am DIN-Platz Burggrafenstraße 6, 10787 Berlin, Germany
[4]BSI Group, 389 Chiswick High Road, London, W4 4AL, UK

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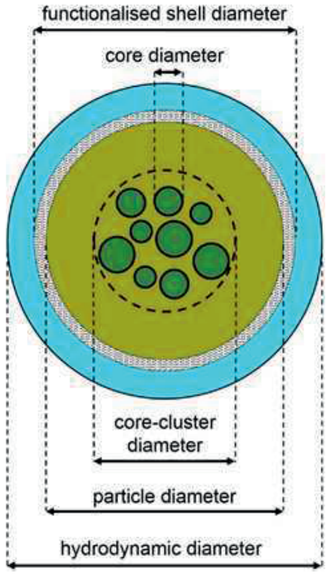
Magnetic nanoparticles (MNP)

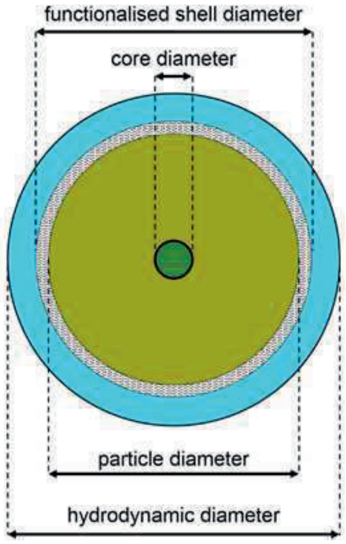
MNPs are **complicated nano-composites**.

They consist of one or several **magnetic crystallites** (iron oxide) **in a non-magnetic matrix**.

Multi-core nanoparticle



Single-core nanoparticle



Sizes
core: 5 – 50 nm
nanoparticle: 7 – 100 nm

Wells, J., et al.: (2017). Standardisation of magnetic nanoparticles in liquid suspension. *Journal of Physics D: Applied Physics*, *50*(38), 383003. DOI:10.1088/1361-6463/aa7fa5

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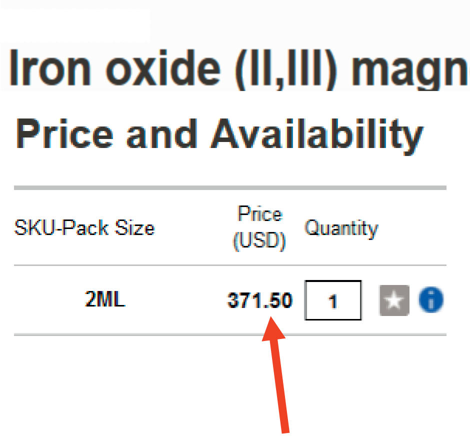
Magnetic nanoparticles – a valuable material class

MNP in suspension liquid

MNP powder

MNP in beads in suspension



Iron oxide (II,III) magnetic nanoparticles solution

Price and Availability

SKU-Pack Size	Price (USD)	Quantity
2ML	371.50	1

Price

7 x higher than pure silver or

3 x higher than Coco Chanel No. 5

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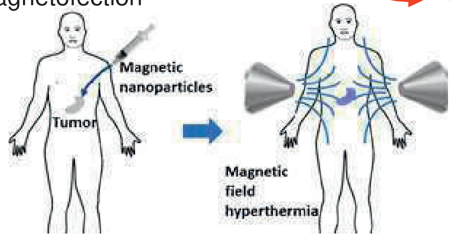
Why are MNPs important ?

Biomedical applications of magnetic nanoparticles (MNP)

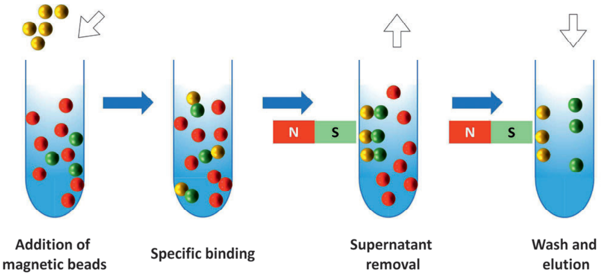
- **new local cancer therapies**
  - **magnetic hyperthermia**
  - magnetic drug targeting
  - DNA magnetofection

MagForce AG **market capitalisation** Feb 2018:
~**170 million €**
+ many more companies



In-vitro-diagnostics:

- **separation** of cells, proteins or DNA from blood



Total global **market turnover** 2014:
>**2.4 billion €**

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What is already standardized for MNPs?

- ISO has already over 40 standards for nanotechnology.
  A number of them applies also to MNPs.

- standardized: **nanoparticle vocabulary**, **analysis of geometry and shape**

General
- ISO/TS 80004 **Vocabulary**
  - Core terms, Nano-objects, Nanostructured materials, Nano-object characterization
- ISO/TS 17200 Nanoparticles in **powder** form -- Characteristics and measurements
- ISO 9276 Representation of results of **particle size** analysis
- ISO 26824:2013 Particle characterization of particulate systems – **Vocabulary**
- Representation of **particle shape** analysis, **DLS**, **SAXS**, **dispersions** in liquids

Specific
- **SAXS**: ISO 17867 Particle size analysis -- Small-angle X-ray scattering
- **DLS**: ISO 22412 Particle size analysis -- Dynamic light scattering

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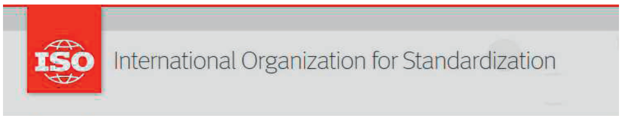
Ongoing MNP standardisation at ISO

In spring 2015, ISO/TC229 "Nanotechnologies" started a new development on MNP.



International Organization for Standardization

ISO/DTS 19807-1
Nanotechnologies - Magnetic nanomaterials - Part 1: Magnetic nanosuspensions - Characteristics and measurements

ISO 19807-1 will be a **material specification**

Purpose: Guideline for **labelling** of MNP suspensions

- Definition of the relevant physical properties of an MNP suspension
- Mentioning of relevant measurement methods
- Link to other applying ISO documents

The document follows ISO/TS 12805 "Guidance on specifying nano-objects".

Since 2016, another new ISO standard is under preparation:
TC229 WG4 PG14
"**Nanostructured superparamagnetic beads suspension for nucleic acid extraction**"

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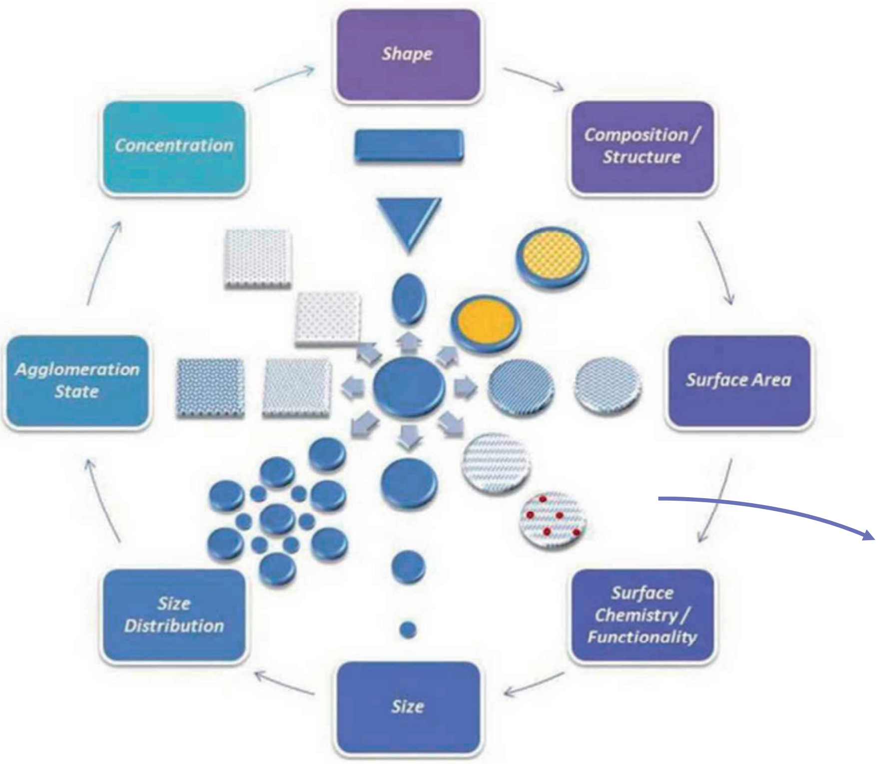
ISO 19807 Characteristics of magnetic nanoparticles



D. Imbraguglio et al., *Nanometrology.*
In:E. Bava et al., eds.
Metrology and Physical Constants
DOI 10.3254/978-1-61499-326-1-193, 2013

The document follows ISO/TS 12805 “Guidance on specifying nano-objects”.

Magnetic properties (depend on all other properties)

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ISO 19807 Definition of **magnetic** MNP properties

- Curie temperature
- magnetic susceptibility (volume, mass, molar)
- magnetic moment
- magnetization (volume, mass, molar)
- magnetoviscosity
- pyromagnetic coefficient
- magnetic relaxivity
- saturation magnetisation
- superparamagnetism
- …

→ Definition of terms, SI units, mentioning of measurement methods

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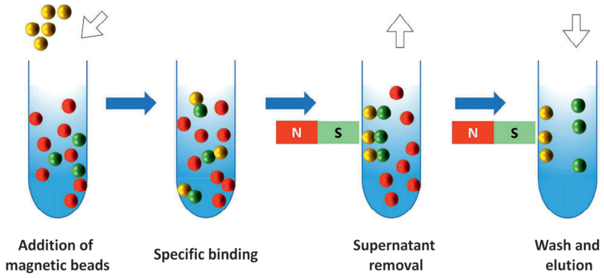
ISO 19807 Magnetic measurement methods for MNP properties

- static (DC) magnetisation measurement
- magnetic susceptibility measurement
- magnetic field hyperthermia
- magnetic resonance spectroscopy

→ No accepted measurement standards available.



For the economically most important application, magnetic separation, there is even **not a definition in the new ISO standard.**

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Current situation of MNP reference materials

- All companies:
    - **Almost never state magnetic properties** in their Technical Data Sheets
- Big companies:
    - use **in-house standards, no** public documentation
- SMEs and academia:
    - depending on the application, so-called „**gold-standards**“ have been established (e.g. Resovist® for imaging applications)
    - These are **not certified** and prone to **batch-to-batch variations**
    - „**gold-standards**“ typically come without a special parameter, but rather **with a specific function** (imaging contrast, heat generation, efficient magnetic separation, …)

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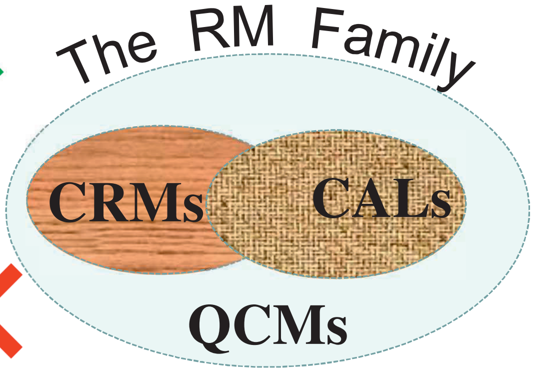
Reference materials for magnetic nanoparticles

QCM – quality control material
homogeneity, stability fit for intended use ✓

CRM – certified reference material
*specified property measured by metrologically valid procedure, **certificate with property and uncertainty and statement of metrological traceability*** ✗

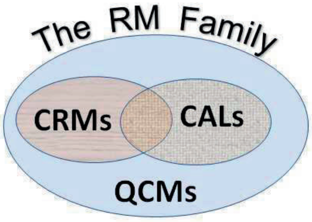


CAL – calibrant
reference materials used for calibration,
***specified property and uncertainty** in a useful range for calibration* ✗

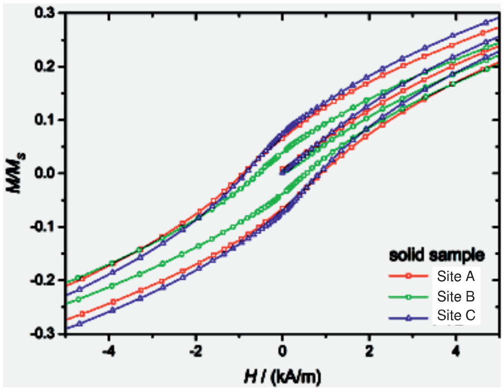
modified after Emons, H. (2006).
The 'RM family'—Identification of all of its members.
Accreditation and Quality Assurance, *10*(12), 690-691.

Ad-hoc ring comparisons of quality control material

Remember: **QCM** – quality control material
homogeneity, stability fit for intended use



DC-magnetisation M(H) measurement
3 participants

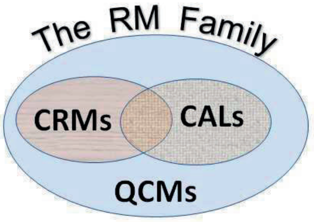


source: EU FP7 NanoMag project 2016

Differences attributed to
- material aging
- different sample preparation
- different measurement protocol

Ad-hoc **ring comparisons** of **quality control material**

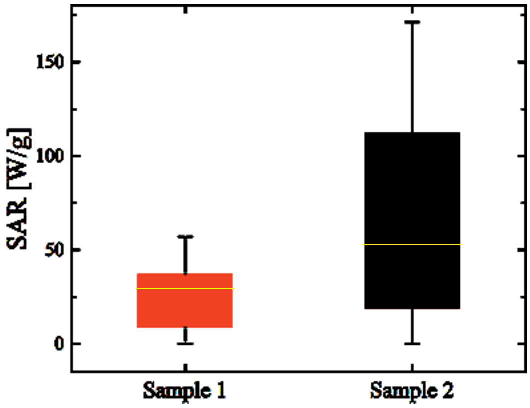
Remember: QCM – quality control material
homogeneity, stability fit for intended use



Magnetic field hyperthermia*
measurement of
energy dissipation (SAR)
in a magnetic AC field

12 participants

Differences attributed to ??



source: EU COST 1402 RADIOMAG network 2017

*Clinical trials in humans ongoing!

Lessons learned (?)

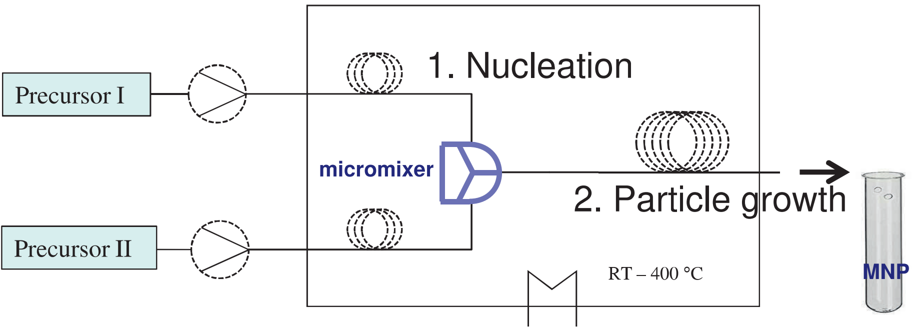
	MNP reference material	**Measurement procedure for magnetic MNP property**
pre-requisite	reproducible synthesis procedure	reproducible sample preparation and measurement protocol
development and testing	needs **standardized measurement procedure**	needs **MNP reference material**

How to go forward?

- Incremental improvement of measurement **procedures** and **reference materials**, **simultaneously**
- development of detailed **measurement procedures** including full **uncertainty budgets** fo magnetic properties of MNP
  → EMPIR project MagNaStand 2017-2020
- development of synthesis strategies for magnetic nanomaterials with tunable and controlled magnetic properties (**micromixer**)

micromixer device

exact control of:
- reagents
- reaction time
- temperature



Outlook – Roadmap

2018: ISO standard on magnetic nanoparticles ISO 19807-1

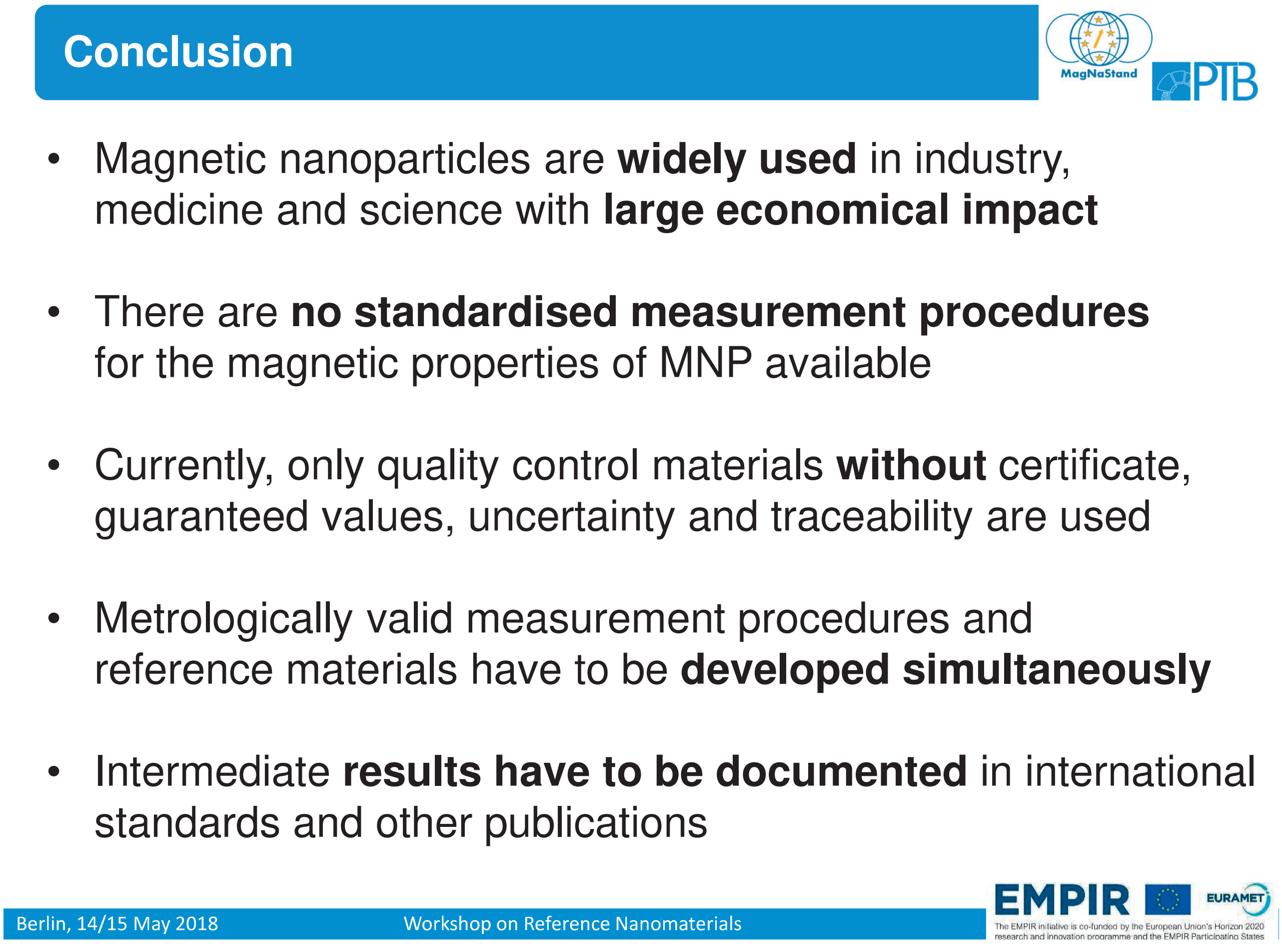
2018 ff: Standard operating procedures for measurement of magnetic MNP properties

2019: New synthesis of MNP with tunable properties using the micromixer approach

2019 ff: Ring comparisons of candidate RM MNP using harmonized measurement protocols

2020 ff: ISO measurement standards

2022 ff: VAMAS interlaboratory comparisons of RM MNP



Conclusion

- Magnetic nanoparticles are **widely used** in industry, medicine and science with **large economical impact**
- There are **no standardised measurement procedures** for the magnetic properties of MNP available
- Currently, only quality control materials **without** certificate, guaranteed values, uncertainty and traceability are used
- Metrologically valid measurement procedures and reference materials have to be **developed simultaneously**
- Intermediate **results have to be documented** in international standards and other publications

Berlin, 14/15 May 2018 Workshop on Reference Nanomaterials

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Physikalisch-Technische Bundesanstalt
Braunschweig and Berlin
National Metrology Institute

TSEM: a traceable method for nanoparticle metrology

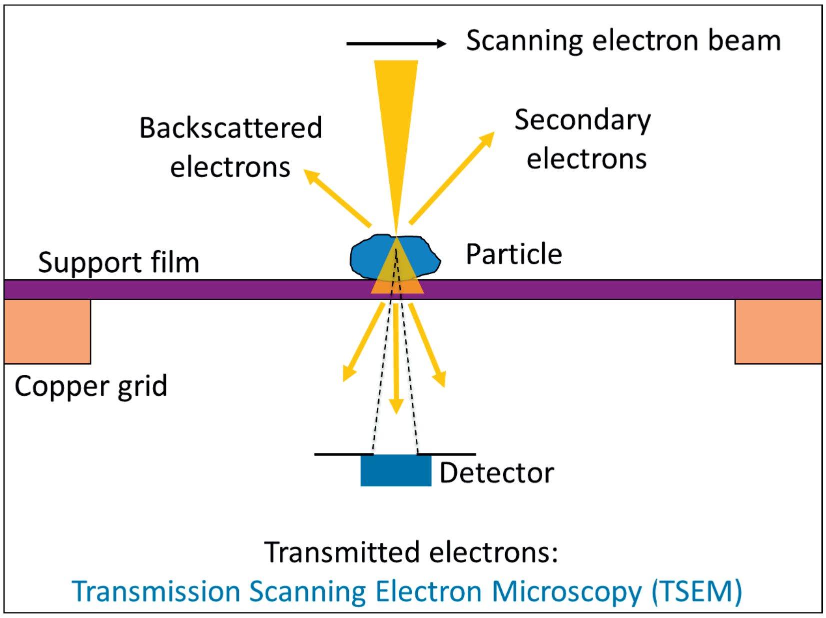
E. Buhr, D. Bergmann, T. Klein
Working Group “Electron Microscopy”

Workshop on Reference Nanomaterials
May 14 & 15, 2018, Berlin

Our mission:

To advance the dimensional measurement of nanoparticles through the development of **traceable measurement methods**

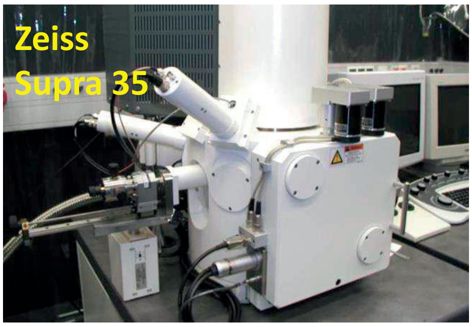
Our approach: Transmission Scanning Electron Microscopy (TSEM)

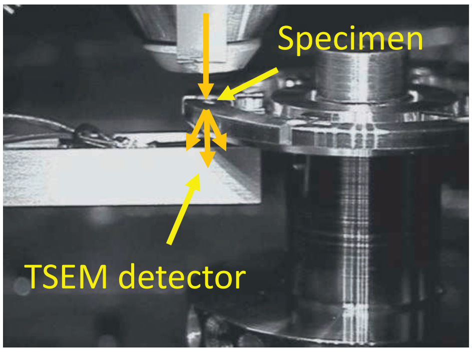


Transmitted electrons:
Transmission Scanning Electron Microscopy (TSEM)

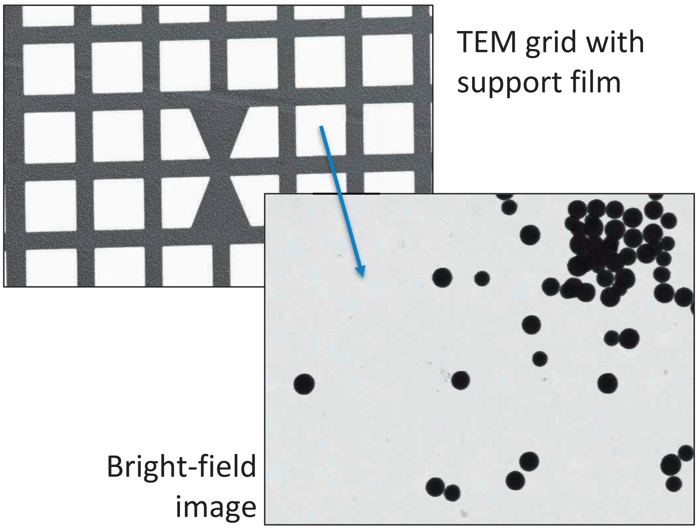
Meas. Sci. Technol. **20** (2009) 084025

Current TSEM implementation at PTB





- Schottky field emission cathode, 30 keV
- TEM grids
- Bright-field imaging mode



TEM grid with support film

Bright-field image

Result: Number-based size data as required by the EC recommendation on the definition of a nanomaterial

---

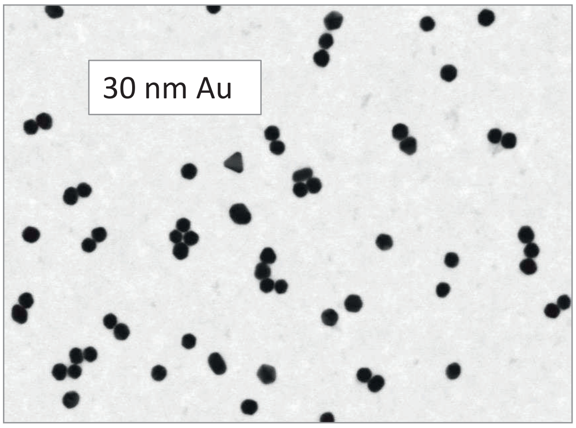
Nanoparticle Metrology using TSEM

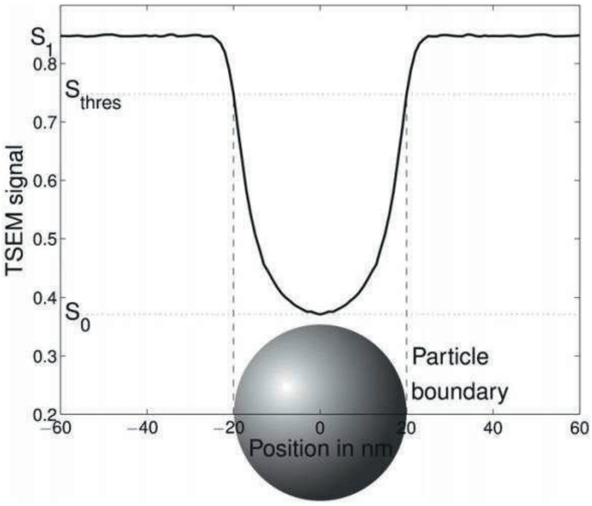
Powerful features

- High-contrast imaging due to low electron energy
- Size, size distribution and particle form
- Agglomerates, artefacts clearly visible
- SI traceability for size data

Challenges for highly accurate data

- Representative preparation
- Many particles required for good statistics:
  Automatic image analysis necessary
- Physical modelling of imaging process

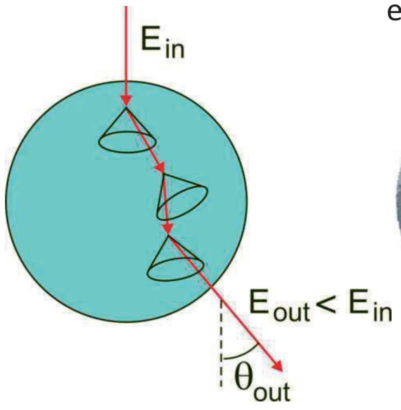




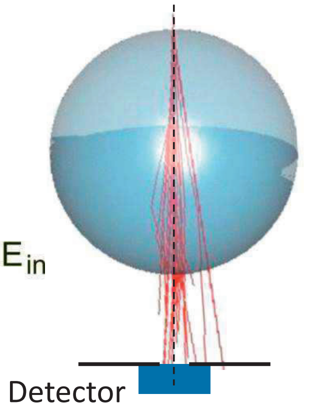
TSEM: Signal modelling

Parameters	Relevant data
Incident electron beam	Electron energy, beam profile
Electron scattering in solid states	Cross sections for elastic and inelastic scattering
Detection process	Angular and energy sensitivity of detector

Single electron



Ensemble of electron trajectories



Monte Carlo simulation of electron scattering

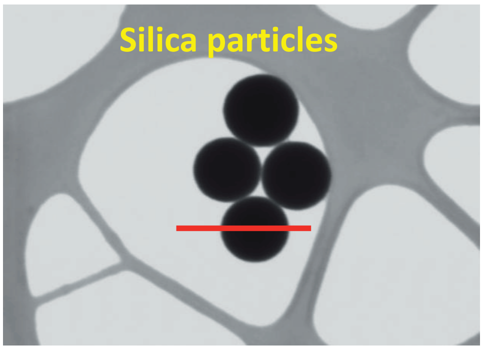
Topical review:

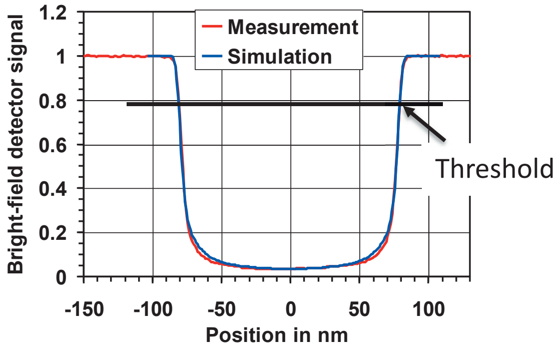
J. Phys. D: Appl. Phys. **42** (2009) 183001

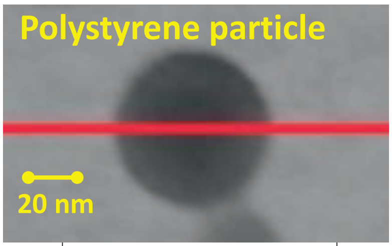
Application to nanoparticles:

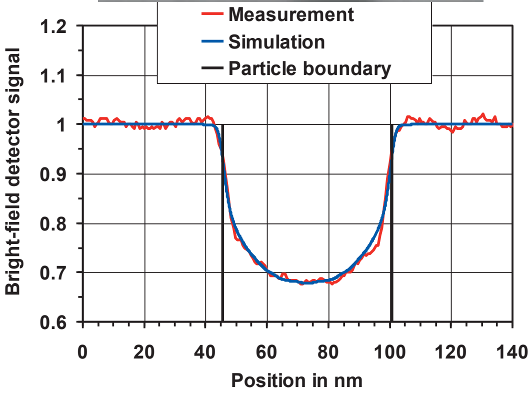
Meas. Sci. Technol. **28** (2017) 034002

TSEM: Signal profiles







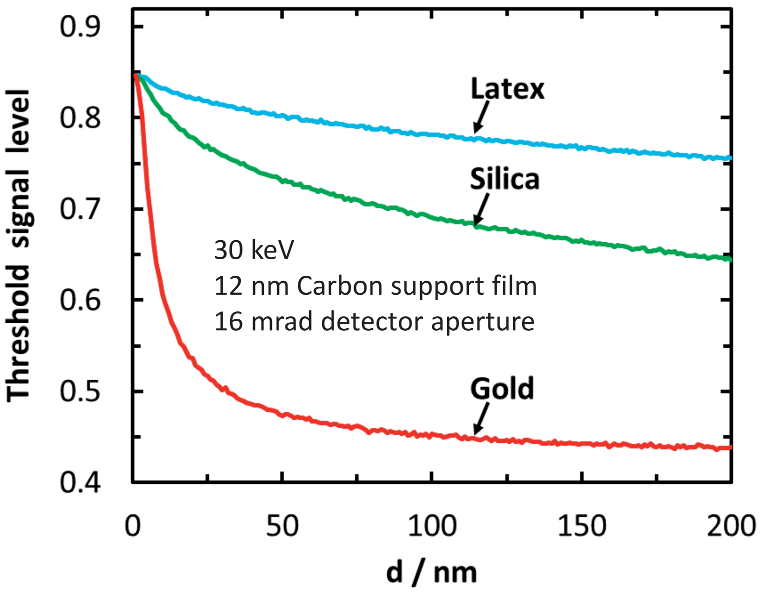


- Good agreement between experiment and simulation
- Signal at particle boundary ('threshold') not at 50% !

TSEM threshold values

Threshold depends on

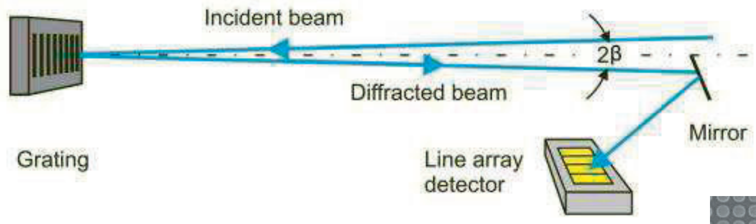
- Particle material
- Particle size
- Instrument parameters:
  - Electron energy
  - Beam size and aperture
  - Detector geometry

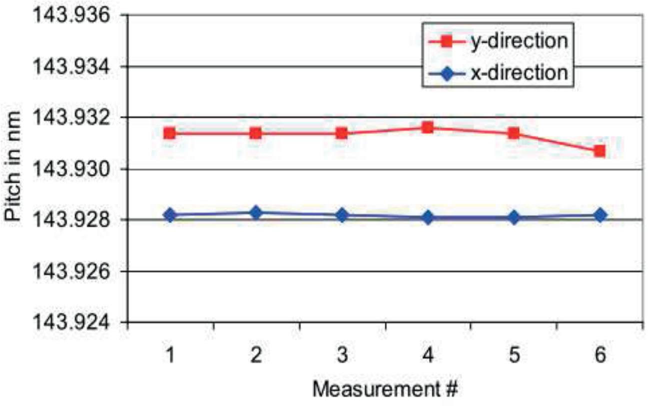


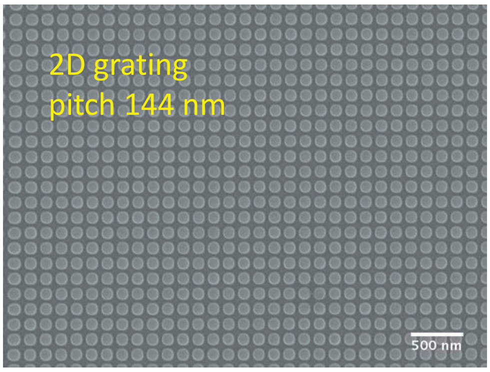
- ➢ Each particle with different size has individual threshold
- ➢ Iterative and automatic analysis

Traceability

- Calibration of pixel size with a 2D grating [1,2]
- Calibration of the 2D grating [3] with a laser diffractometer [4]:







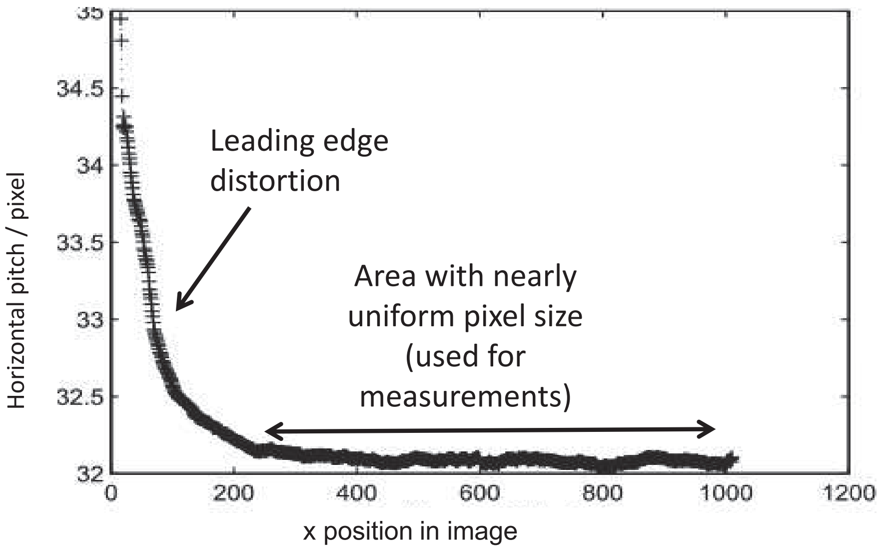
[1] *Meas. Sci. Technol.* 22 (2011) 094002
[2] *Adv. Imaging and Electron Phys*. 171 (2012) 297

[3] *Proc. SPIE* **6922** (2008) 69223J
[4] *Meas. Sci. Technol.* **18** (2007) 667

Traceability: Pixel size calibration

Typical calibration results show:

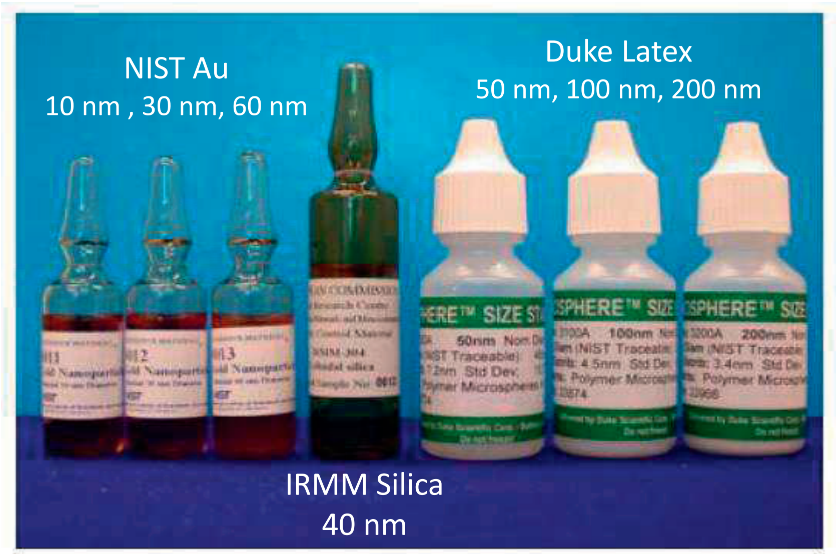
- Pixel size in x-direction non-uniform (leading edge distortion)
- Pixel size in x- and y-direction slightly different (< 0.6 %)
- Pixel sizes indicated by instrument (scale bar) deviate by up to about 3 %



Typical uncertainty of pixel size < 0.25 %

European comparison measurements

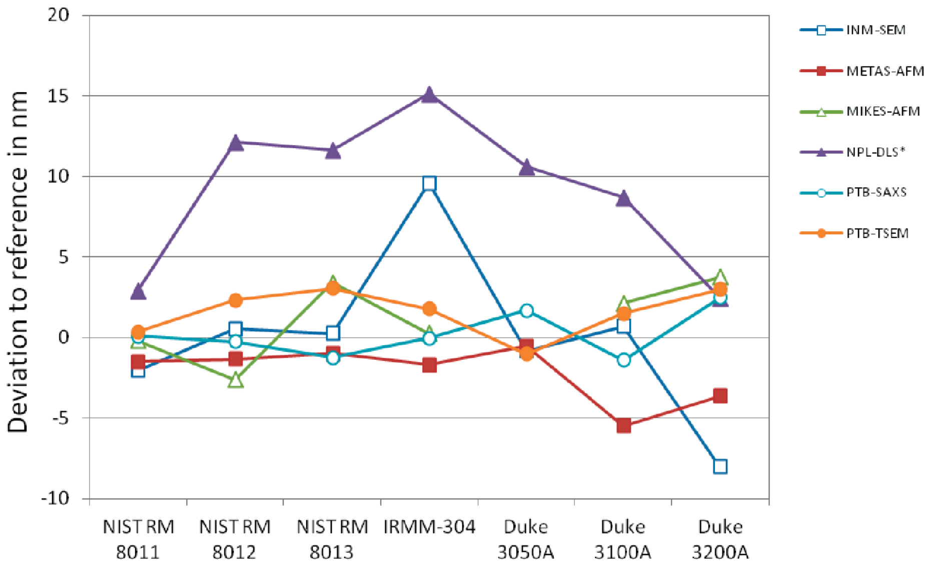
Comparison measurements of reference nanoparticles among European metrology institutes



Methods: AFM, SEM, TSEM, DLS, SAXS

Meas. Sci. Technol. **23** (2012) 125005

European comparison: Results



References values in nm: 8.7 25.4 54.6 25.7 45.5 97.0 197.7

Uncertainty of reference values: < 1.4 nm for particles below 100nm
1.5% for larger particles

Meas. Sci. Technol. **23** (2012), 125005

Uncertainty of TSEM particle size measurements

Parameters affecting the measurement uncertainty of the mean diameter *d* of monodisperse, nearly spherical particles:

Parameter	Contribution
Image analysis, selection of particles	significant
Simulation/ modelling	significant
Reproducibility (incl. digitalization)	significant
Sample preparation	significant
Particle contamination	medium
Statistics (thousand particles)	minor
Determination of grey levels	minor
Pixel size	minor

BIPM Data Base on Calibration and Measurement Capabilities (CMCs)
PTB‘s TSEM implementation:
Expanded Uncertainty (95% confidence) U = 2 nm + 0.02*d* https://kcdb.bipm.org/

Participation in European R&D projects related to nanoparticle metrology using TSEM

iMERAplus JRP “Traceable Characterization of Nanoparticles” (2008 – 2011)

EMRP JRP “Metrological characterisation of micro-vesicles from body fluids as non-invasive diagnostic biomarkers” (2012 – 2015)

EMRP JRP “Emerging Requirements for Measuring Pollutants from Automotive Exhaust Emissions“ (2011 – 2014)

EMPIR JRP “Metrology for light absorption by atmospheric aerosols” (2017 – 2020)

EMPIR JRP “Improved traceability chain of nanoparticle size measurements” (2018 – 2021)

ISO TC 229 Nanotechnologies

Study group “Particle size distribution by TEM”

Aim: Development of an **ISO standard** (ISO 21363) serving as a guide line for industrial practice

Experimental case study, objectives:

- Use of ‘real life’ materials
- Procedures should be automated as much as practical
- Visualizing and fitting the distribution data to reference models

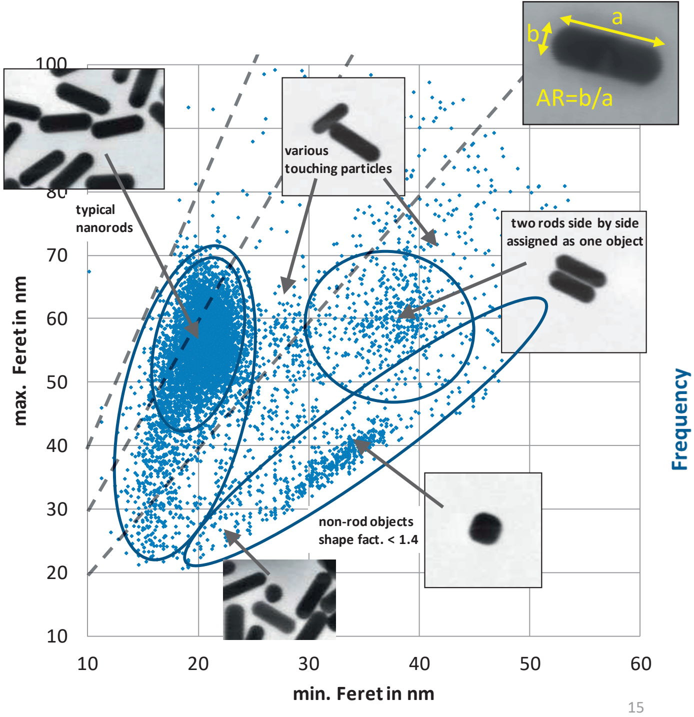
	Titania	Gold nanorods	Carbon black	Bimodal silica	Gold NPs	Bipyramidal TiO2
Material	Commercial rutil, Tayca	NCNT China	Cabot Corp.	IRMM ERM-FD102	NIST RM8012	EU/FP7 SETNano Metro
Published	yes	yes	yes		yes	

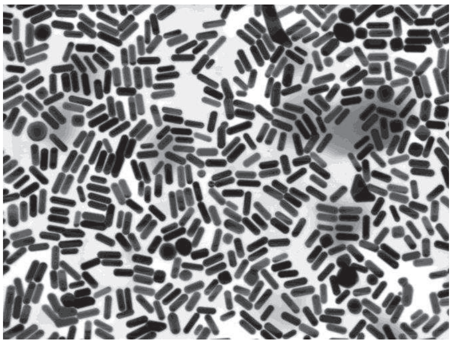
Presentation of Dan Hodoroaba (today 11:40):
Shape controlled TiO_2 nanoparticles as candidates for nano CRM‘s – an ISO case study

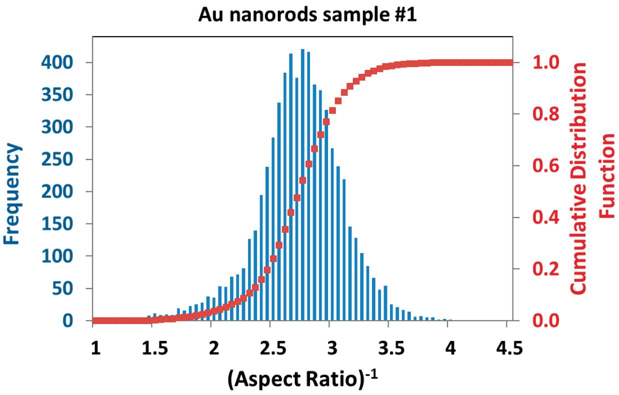
ISO TC 229 TEM Study Group

Gold nanorods, mesoshape descriptors

Tasks:
automatically remove touching particles,
distinguish rods from cubes



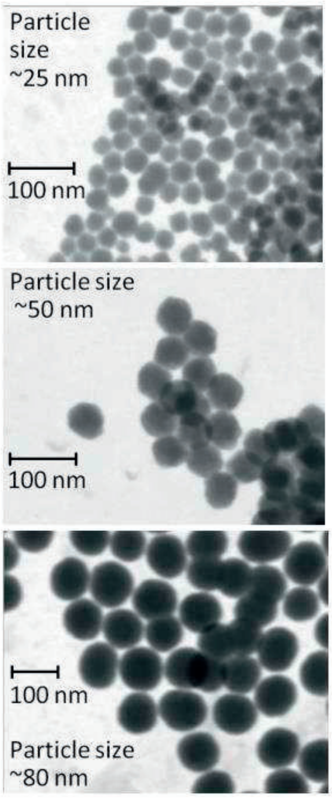


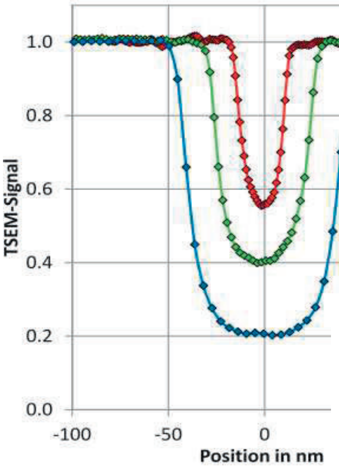


Metrologia **55** (2018) 254

TSEM: Measurement of particle thickness

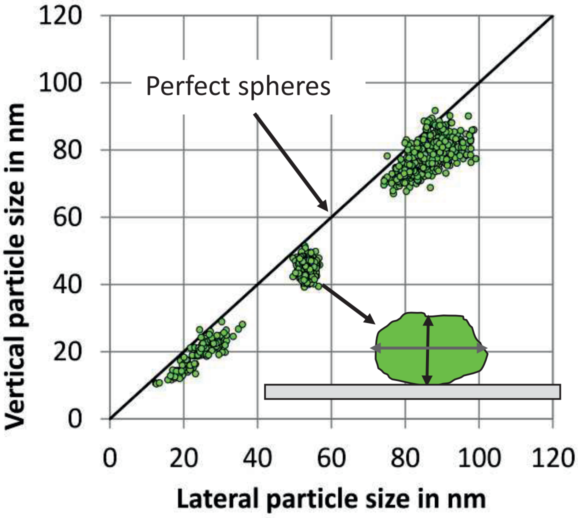
SiO_2 particles





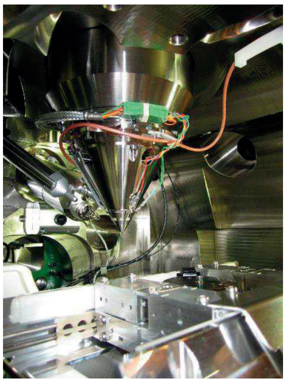
Contrast of TSEM signal is a function of particle thickness

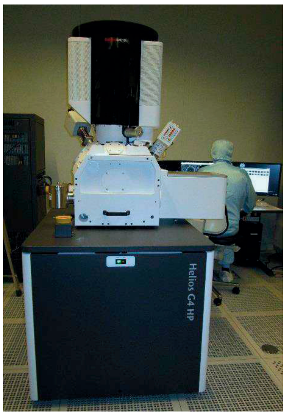
Measured vertical vs lateral size (SiO_2 particles)

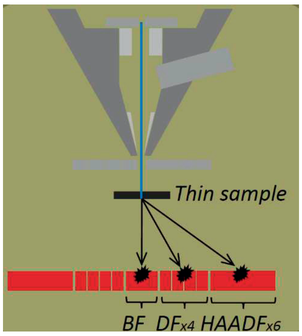


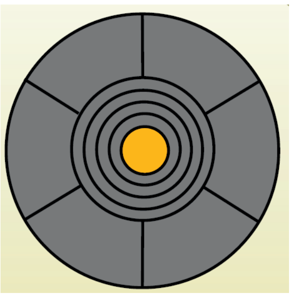
Meas. Sci. Technol. **28** (2017) 034002

New PTB SEM: Enhanced TSEM capabilities





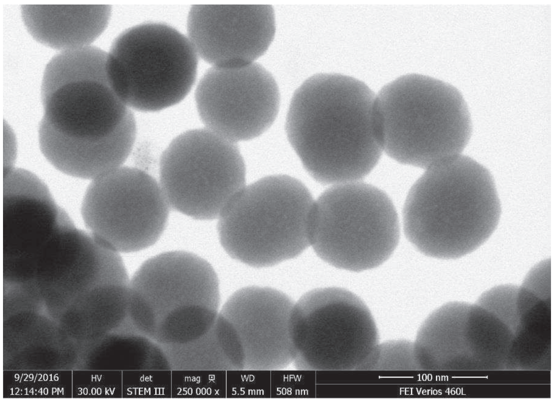




FEI Helios G4

- Improved spatial resolution
- Four annular dark-field detectors
- Segmented HAADF detector
- Simultaneous capture of four TSEM signals
- TSEM detector energy range 5 kV to 30 kV: Enhanced contrast for low-Z particles

80 nm Silica particles



Summary and outlook

TSEM technique offers

- traceable size determination of spherical nanoparticles
- expanded uncertainty (95% confidence): 2 nm to 8 nm depending on particle size (7 nm to 300 nm)
- simultaneous measurement of particle thickness

improvements expected with the new PTB SEM

TSEM technique has been applied successfully to measure

- typical reference nanoparticles (gold, silver, silica, polystyrene)
- aerosol particles, biological micro vesicles

What next?

- Extension to non-spherical particles
- Traceable characterization of soot particles
- Application to other ‘real world‘ particles, e.g. titania
- Exploration of additional possibilities of dark-field TSEM imaging

Thank you for your attention!

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Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

Measurands and method dependent reference values: can one global particle size reference value fit all?

Victoria Coleman
a/g Section Manager, Nanometrology
Chair, Technical Committee Materials Metrology, Asia Pacific Metrology Programme

Malcolm Lawn, Bakir Babic, Åsa Jämting and Jan Herrmann
Nanometrology Section, National Measurement Institute Australia
36 Bradfield Road, West Lindfield, NSW 2070

200 nm

Overview

- About NMIA
- Nanometrology at NMIA
  - mSPM
  - Nanoparticle characterisation
- Measurands
  - Particle deformation – AFM
- Nanoparticle Geometry and measurands
- Questions/ Discussion / Feedback

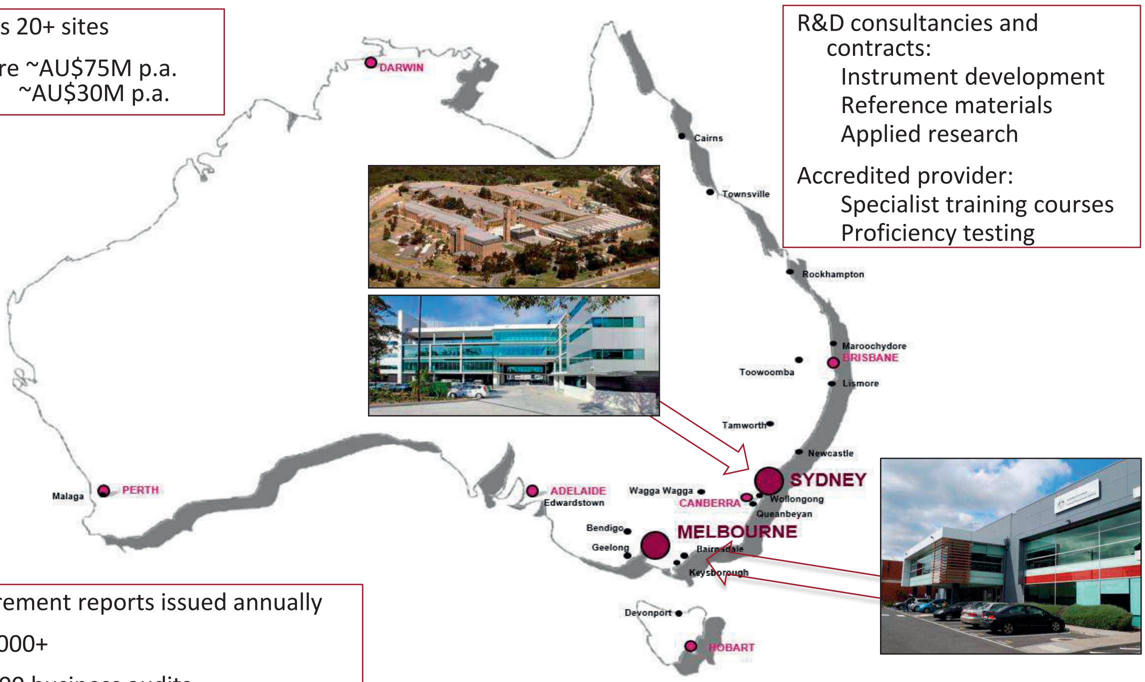
NMIA:
Geographical footprint + some statistics

~330 staff across 20+ sites

Total expenditure ~AU$75M p.a.
revenue ~AU$30M p.a.

R&D consultancies and contracts:
- Instrument development
- Reference materials
- Applied research

Accredited provider:
- Specialist training courses
- Proficiency testing



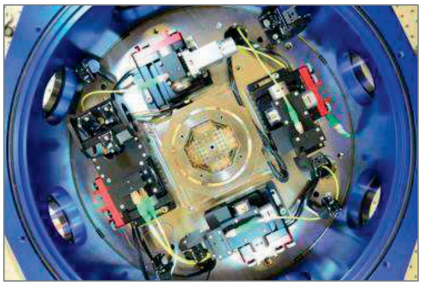
15,000+ measurement reports issued annually

Client list of 10,000+

Conducted 11,000 business audits
tested 10,000 measuring instruments
inspected 29,000 lines of packaged goods

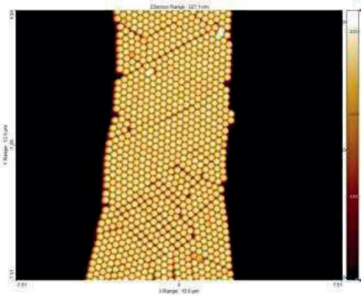
Linked into National Quality Infrastructure
(with NATA, Standards Australia, JASANZ)

NMIA Nanometrology:
Who we are and what we do



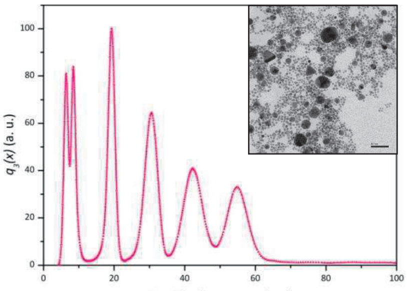
mSPM (primary standard) development and measurement (Traceability)

- Forces
- QTF
- FM non-contact



Accurate nanoscale measurements (AFM)

- Imaging forces
- Particle deformation

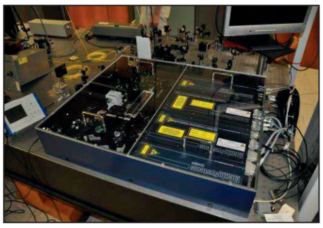


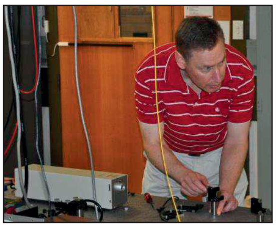
Accurate nanoscale measurements (other techniques)

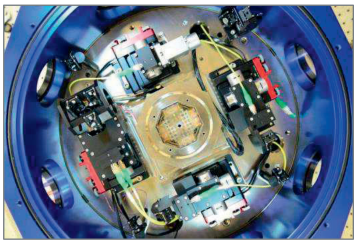
- Surface ligands (+SANS)
- TiO_2 pharmacokinetics
- Complex matricies (nano in food etc.)
- Microplastics

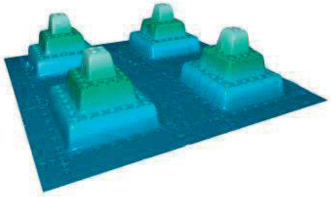
Active in: ISO TC229 (+TC24, TC281), APMP, VAMAS, OECD WPMN

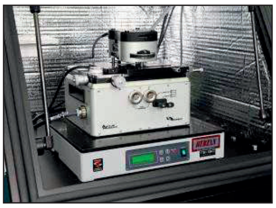
What we do:
SI traceable nanoscale dimensional measurement

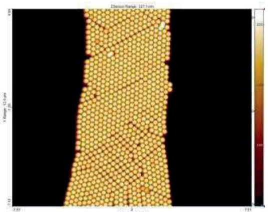

Realisation of the SI metre
at NMIA


Calibration of mSPM
interferometry laser


mSPM
measurement


measurement standards
"nano rulers"


Calibration of
user instruments


Accurate nanoscale
measurements

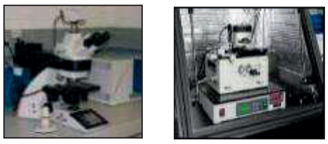
Comprehensive nanoparticle characterization laboratory
More info at www.measurement.gov.au/nano

Dimensional properties ('size')

Light scattering

Separation techniques

Microscopy



Other

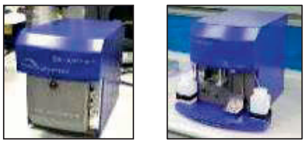
Other physical and chemical properties

Surface area & porosity

Chemical identity

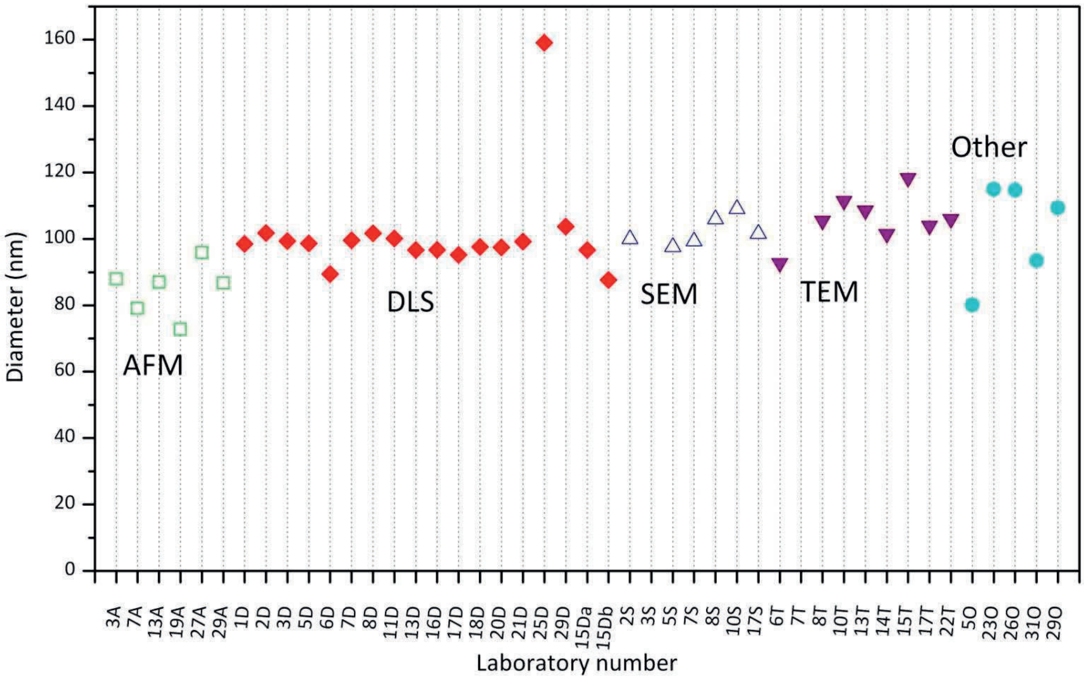
Mass/density

Surface charge



Method Divergence

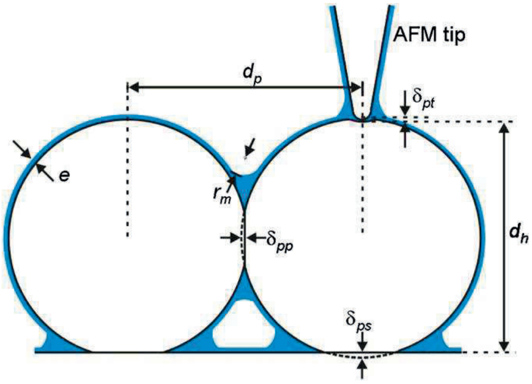
Nanoparticle diameter measurements vary between measurement techniques



Australian Nanoparticle Size Interlaboratory comparison data, 2012

Method Divergence

AFM (particle height)

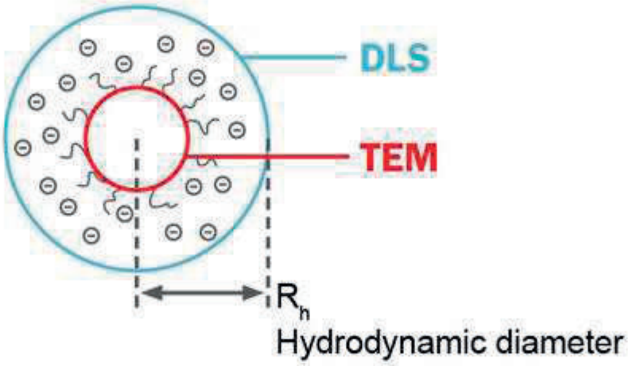


J Garnaes, Meas. Sci. Technol. **22** (2011) 094001

What exerts force on the particle?

- AFM tip
- substrate
- water meniscus layer – imaging in air
- adjacent particles
- Material properties

DLS (hydrodynamic diameter)



We measure the autocorrelation function of the scattered light intensity. This gives us an average diffusion coefficient that can be used to determine an intensity weighted average hydrodynamic diameter, via Stokes Einstein equation.

Method divergence:

What is the measurand?

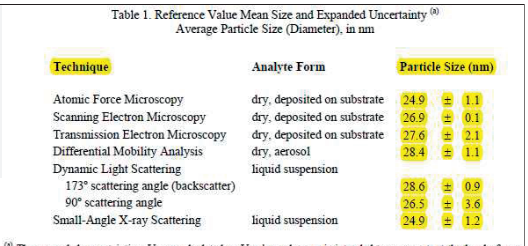


Table 1. Reference Value Mean Size and Expanded Uncertainty [(a)]
Average Particle Size (Diameter), in nm

Technique	Analyte Form	Particle Size (nm)
Atomic Force Microscopy	dry, deposited on substrate	24.9 ± 1.1
Scanning Electron Microscopy	dry, deposited on substrate	26.9 ± 0.1
Transmission Electron Microscopy	dry, deposited on substrate	27.6 ± 2.1
Differential Mobility Analysis	dry, aerosol	28.4 ± 1.1
Dynamic Light Scattering	liquid suspension	
173° scattering angle (backscatter)		28.6 ± 0.9
90° scattering angle		26.5 ± 3.6
Small-Angle X-ray Scattering	liquid suspension	24.9 ± 1.2

[(a)] The expanded uncertainties, U, are calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined standard uncertainty calculated according to the ISO and NIST Guides [2]. The coverage factor, k, for 95 % expanded uncertainty intervals is based on a t multiplier with the appropriate associated degrees of freedom.

NIST Report of Investigation, RM 8012 (2007)

Measurand:

VIM 2nd edition: “particular quantity subject to measurement”
VIM 3rd edition: “quantity intended to be measured”

Taking the VIM 3rd edition approach, if we intend to measure the ‘hard-sphere’ equivalent number distribution, we can use measurement models to correct our data.

AFM

- Include deformation between the substrate and the particle.

We have followed VIM3 approach in APMP.L-S5 (Intercomparison on nanoparticle size) which will shortly be released in Draft B form

DLS

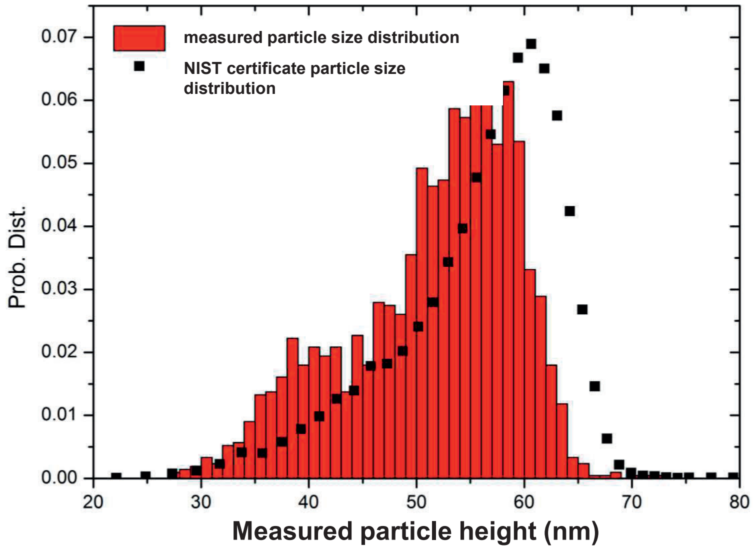
- Include contribution of three additional biases:
  - δD_1 width of particle size distribution (Q-correction)
  - δD_2 scattering angle and concentration dependence
  - δD_3 adsorbed water layer.

measurement.gov.au

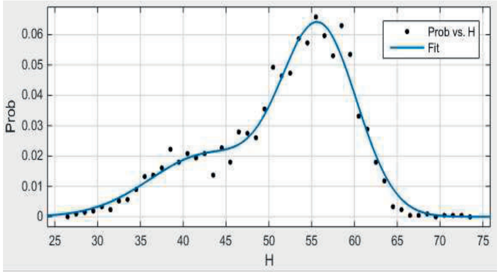
AFM imaging

Non-Contact (minimal imaging force)

NIST SRM® 1964 (nominally 60 nm diameter polystyrene) attached to a mica substrate.



Measured particle size distribution
of 2113 particles derived from 5 images.



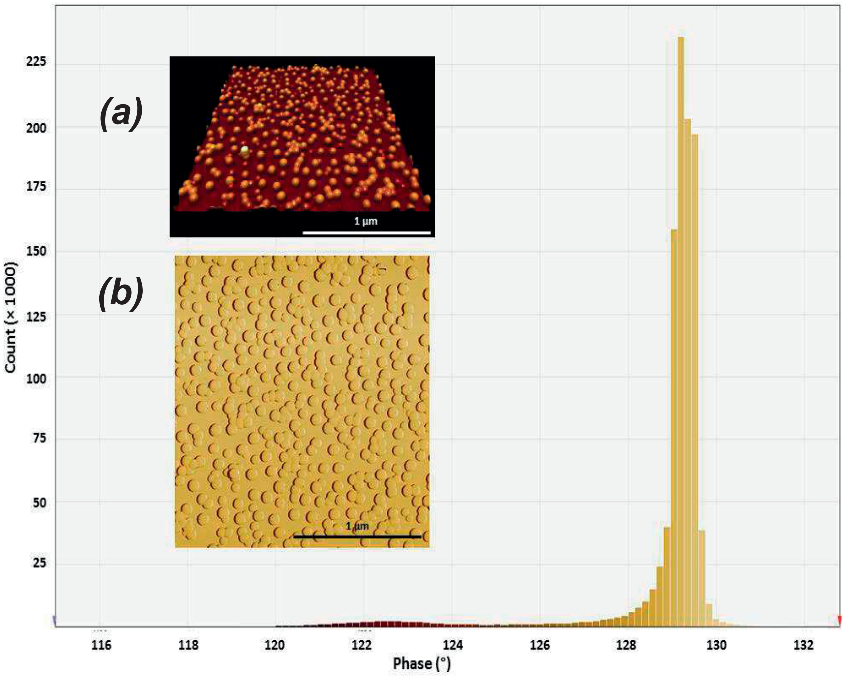
Fit of polynomial curve to measured particle size distribution

- Modal value of fitted curve: **57 ± 2 nm**.
- Dominant contributions to uncertainty: curve fitting and instrument calibration.
- Difference between measured and certificate value (-3 nm) is consistent with modelled deformation caused by particle attachment to substrate*.

*Garnaes, J., (2011) Meas Sci Tech, 22(9), p.094001

M. A. Lawn et al, in preparation

measurement.gov.au



***(a)*:** non-contact mode topography image.

(b)*:** phase image of ***(a).

Main plot: pixel histogram of phase image ***(b)***.

Demonstrates cantilever phase remaining above the resonance phase (90°), and therefore in non-contact mode, across the whole image.

Imaging parameters:

Cantilever: Budget Sensors TAP150AL-G
Spring constant: k = 2.2 nN/nm
Cantilever resonant frequency: 128.42 kHz
Drive frequency: 128.78 kHz
Cantilever free-air amplitude: A_0 =40 nm
Feedback set-point: A = 34.4 nm (A/A_0 = 80%)

Scan size: 2µm × 2µm
Pixel resolution: 1024 × 1024
Scan speed: 100 nm s^{-1}
Scan time: **14 hours**
Number of particles per image: ~400

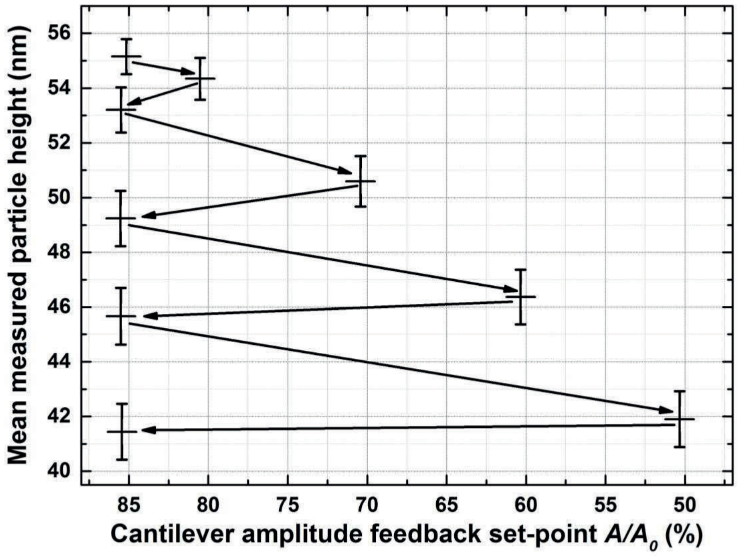
M. A. Lawn et al, in preparation

measurement.gov.au

What about tapping mode?

Influence of set point and repeat imaging

Asylum Research *MFP-3D* in AFM ***intermittent contact mode***



Plot of mean measured particle height results derived from a series of AFM images repeated scans of the same area of particle sample. Arrows between points indicate the order of image acquisition

Intermittent contact (tapping) mode:

- Cantilever free-air amplitude: A_o *(nm)*
- Cantilever amplitude feedback set-point: A/A_o *(%)*

Particle material: polystyrene

Particle size: nominally 60 nm dia.

Substrate: poly-l-lysine functionalised mica

Cantilever spring constant: k = 42 N/m

Cantilever free-air amplitude: A_o = 60 nm

Image size: 2 µm × 2 µm

Particles per image: ~160

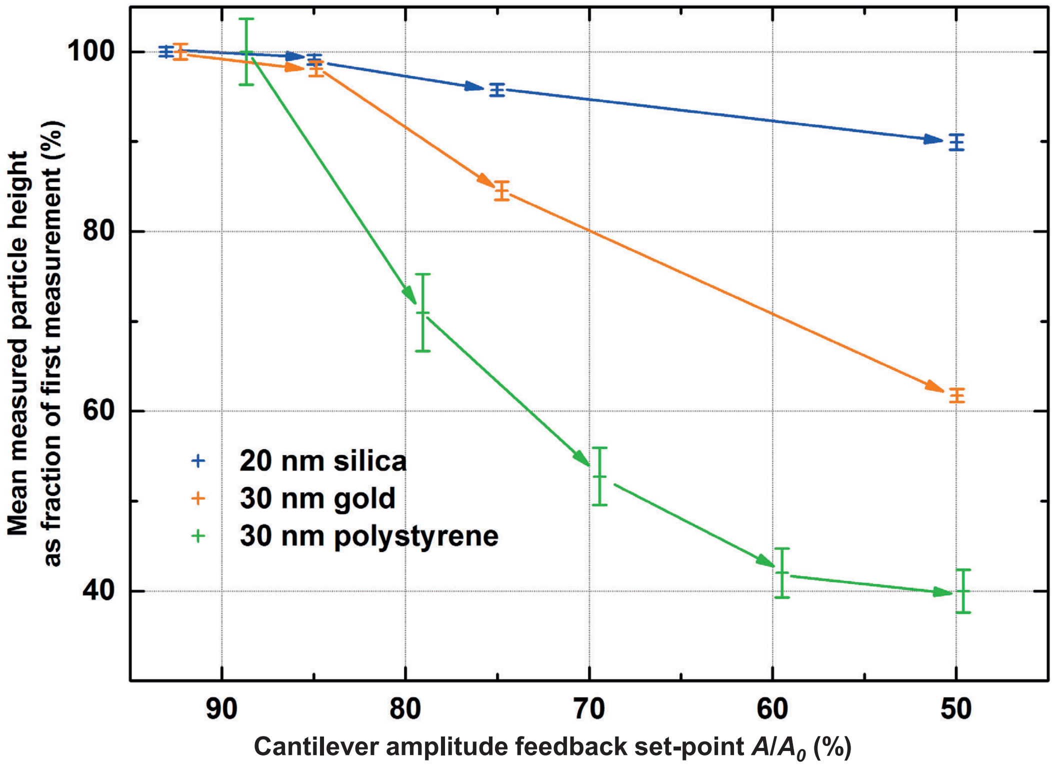
Pixel resolution: 512 × 512

Scan speed: 5 µms^{-1}

M. A. Lawn et al, in preparation

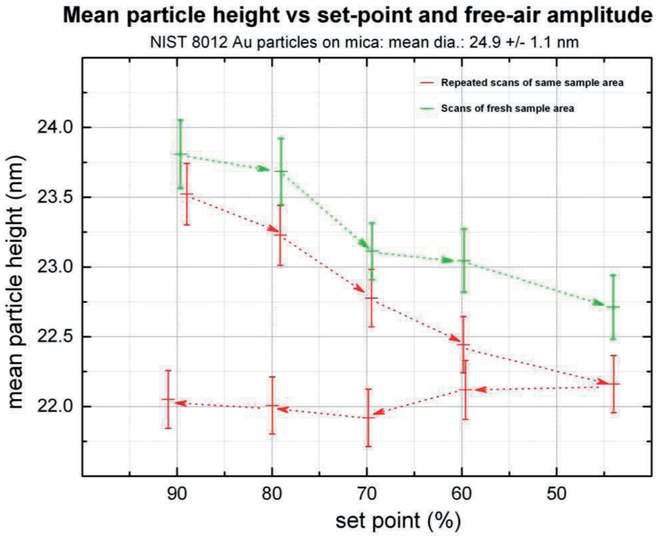
measurement.gov.au

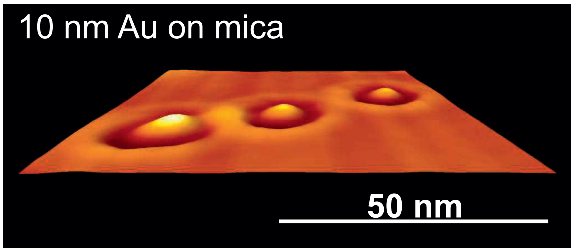
What about tapping mode?
Variation with particle material



M. A. Lawn et al, in preparation

What about tapping mode?
Substrate deformation





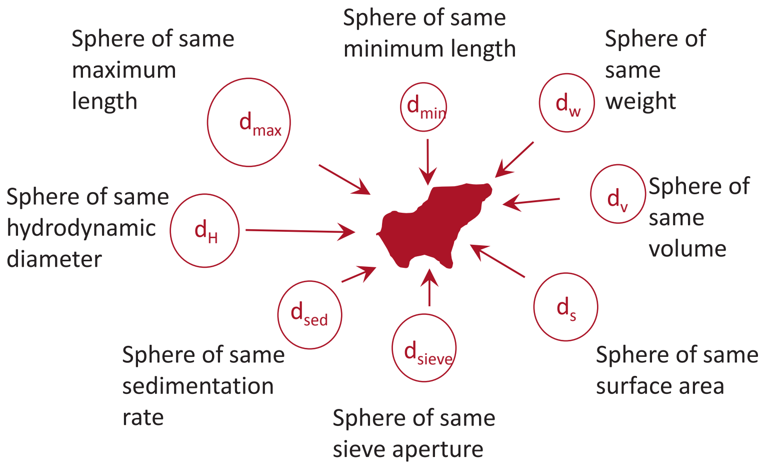
Softer substrates can deform beneath harder attached particles, especially if particle radius is similar or less than radius of tip.

material	E_{bulk} (GPa)	$E_{particle}$(GPa)
polystyrene	3 to 3.5	2 to 8
gold	78	100
silica	72	69
silicon	169	-
muscovite mica	20 – 80	-

M. A. Lawn et al, in preparation

What is the measurand - Non-spherical particles

VIM 2nd edition: “particular quantity subject to measurement”
VIM 3rd edition: “quantity intended to be measured”



Sphere of same maximum length

Sphere of same minimum length

Sphere of same weight

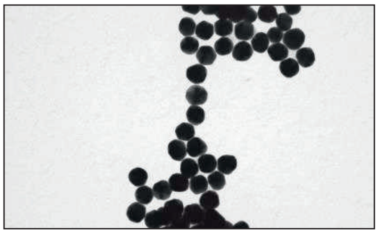
Sphere of same hydrodynamic diameter

Sphere of same volume

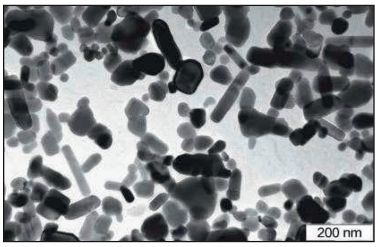
Sphere of same sedimentation rate

Sphere of same sieve aperture

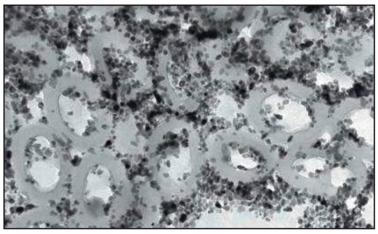
Sphere of same surface area



Au Reference Material

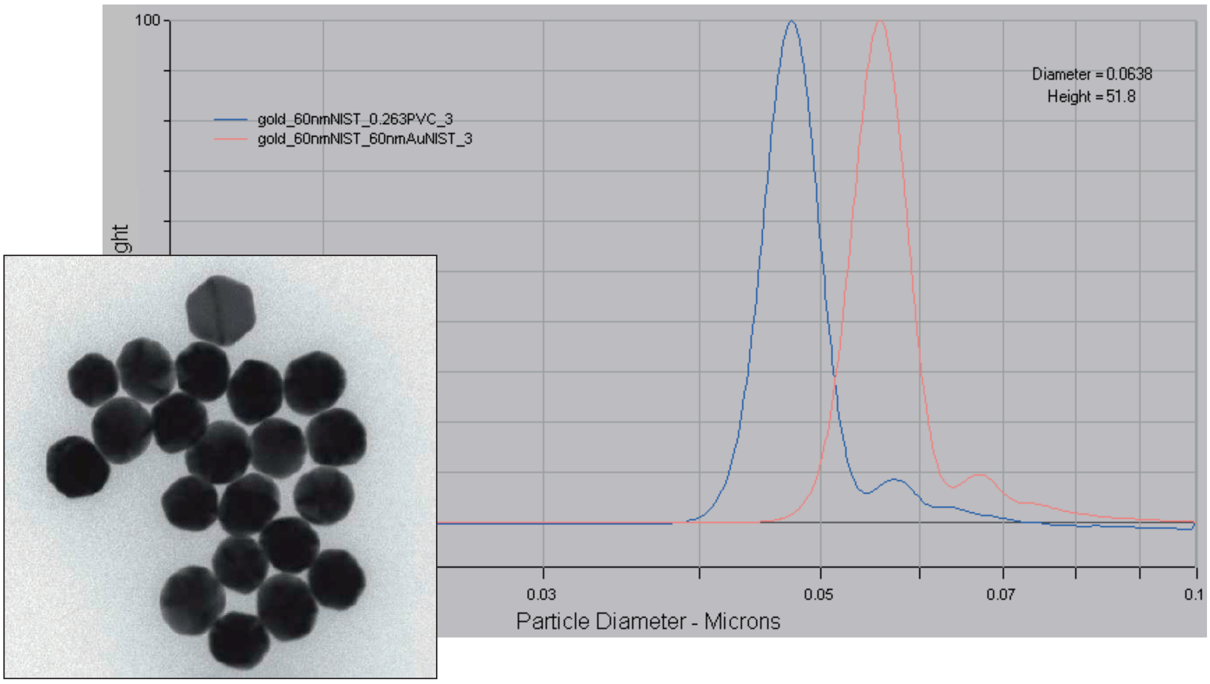


ZnO powder



ZnO in formulation

Deviations from spherical Gold NPs and DCS (CLS)

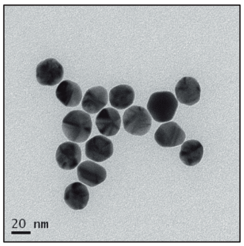


60 nm Au

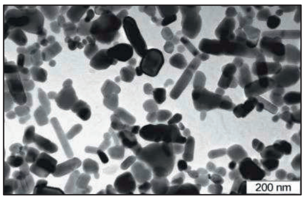
What helps industry?
How should NMIs respond?

“quantity intended to be measured”

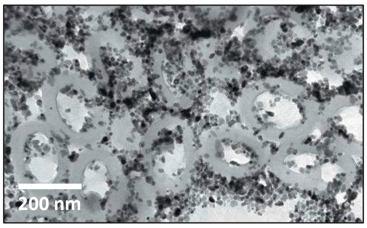
“particular quantity subject to measurement”



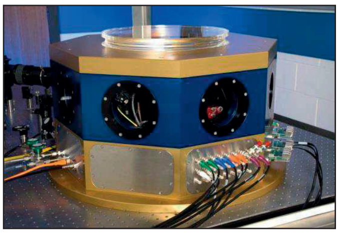
30 nm Au Reference Material



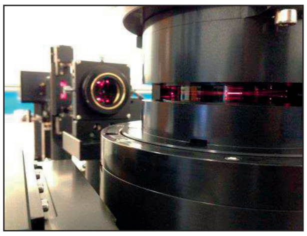
Commercial ZnO powder



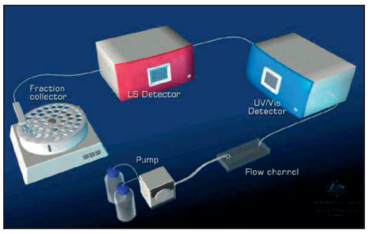
ZnO in sunscreen



Primary nanoscale standard



Commercial instrumentation



Multi (component and disciplinary) solution

Global reference values

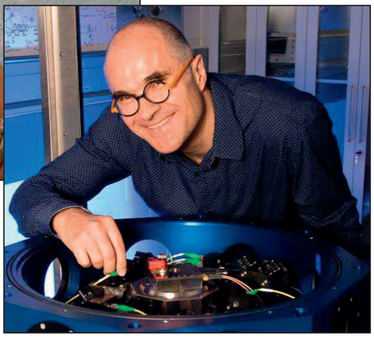
>> U_{meas} →

Method dependant reference values

Bakir Babic
(Chris Freund)
mSPM

Malcolm Lawn
Accurate nanoscale measurements (AFM)

Åsa Jämting
Victoria Coleman
Accurate nanoscale measurements (other techniques)



Jan Herrmann
General Manager: Physical Metrology

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TiO_2 – Pigment Sizing: Constituents, Aggregates and Primary Particles

Dr. R. Theissmann
KRONOS INTERNATIONAL, Inc., Leverkusen

Berlin, May 2018

Agenda

- Motivation
- Industrial Relevance
- TiO_2 – Pigment Particles
- Measurands and Sample Preparation
- Standardizing the Measurement Conditions
- Data Post – Processing
- Repeatability and Replicability Testing
- Implications for Reference Materials

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Motivation

Motivation – sizing an „industrial“ material

Large amounts (>5Mio tons / year)

Stan-dardized, reliable, fast

easy to use, user inde-pendent, cheap

- Suitable for lot / palette testing
- Easily reproducible for customers / authorities / competitors
- Fast and cheap is preferred
- Needs to allow to distinguish *nano – micro*:
  - **d50 < 100nm**

Motivation – sizing a „real“ material

irregular shape

significant size distribution

strong aggregation / agglomeration

Increased demands on

- **Sample preparation and**
- **Sample statistics (e.g. projection / section average)**

compared to model-systems, such as spheres or cubes.

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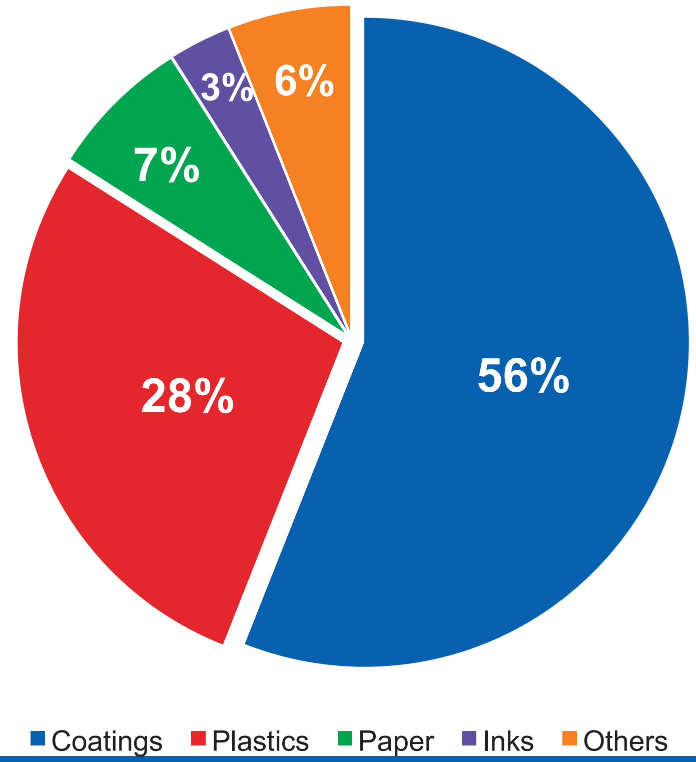
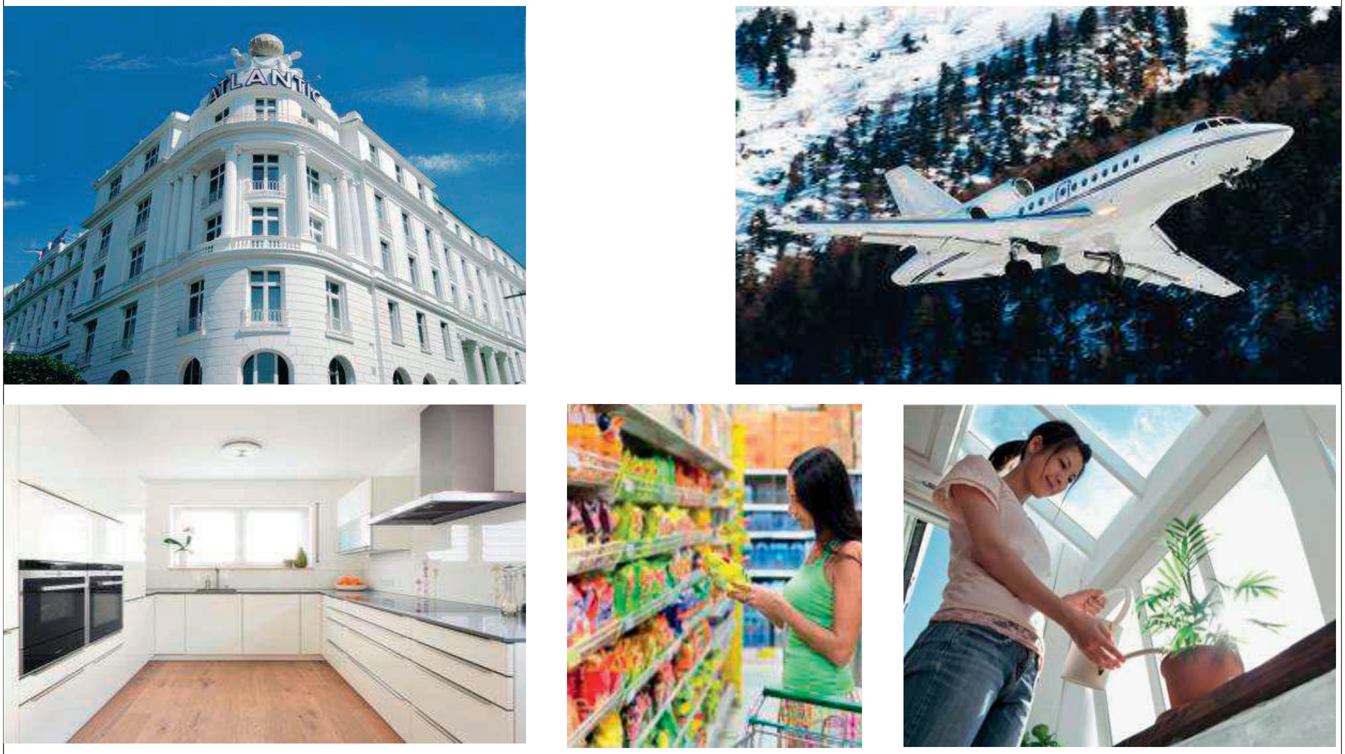
Industrial Relevance

Industrial Use of TiO_2: more than 5 Million tons / year



TiO_2 in Architectural and Industrial Coatings, Laminates, Inks

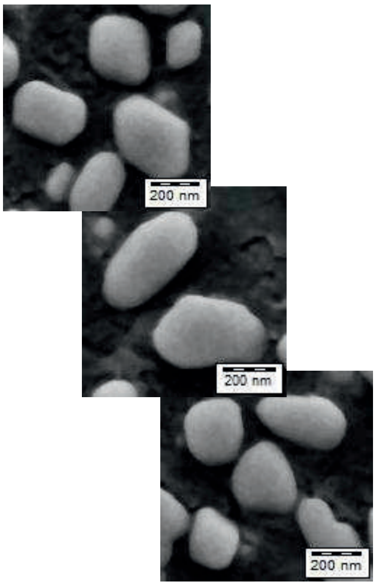


TiO$_2$ – Particle Sizing

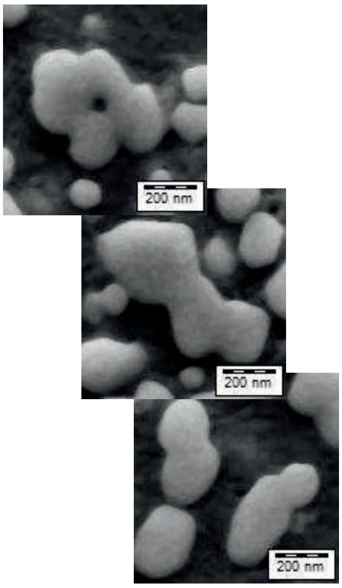
Introduction: TiO$_2$-Particles

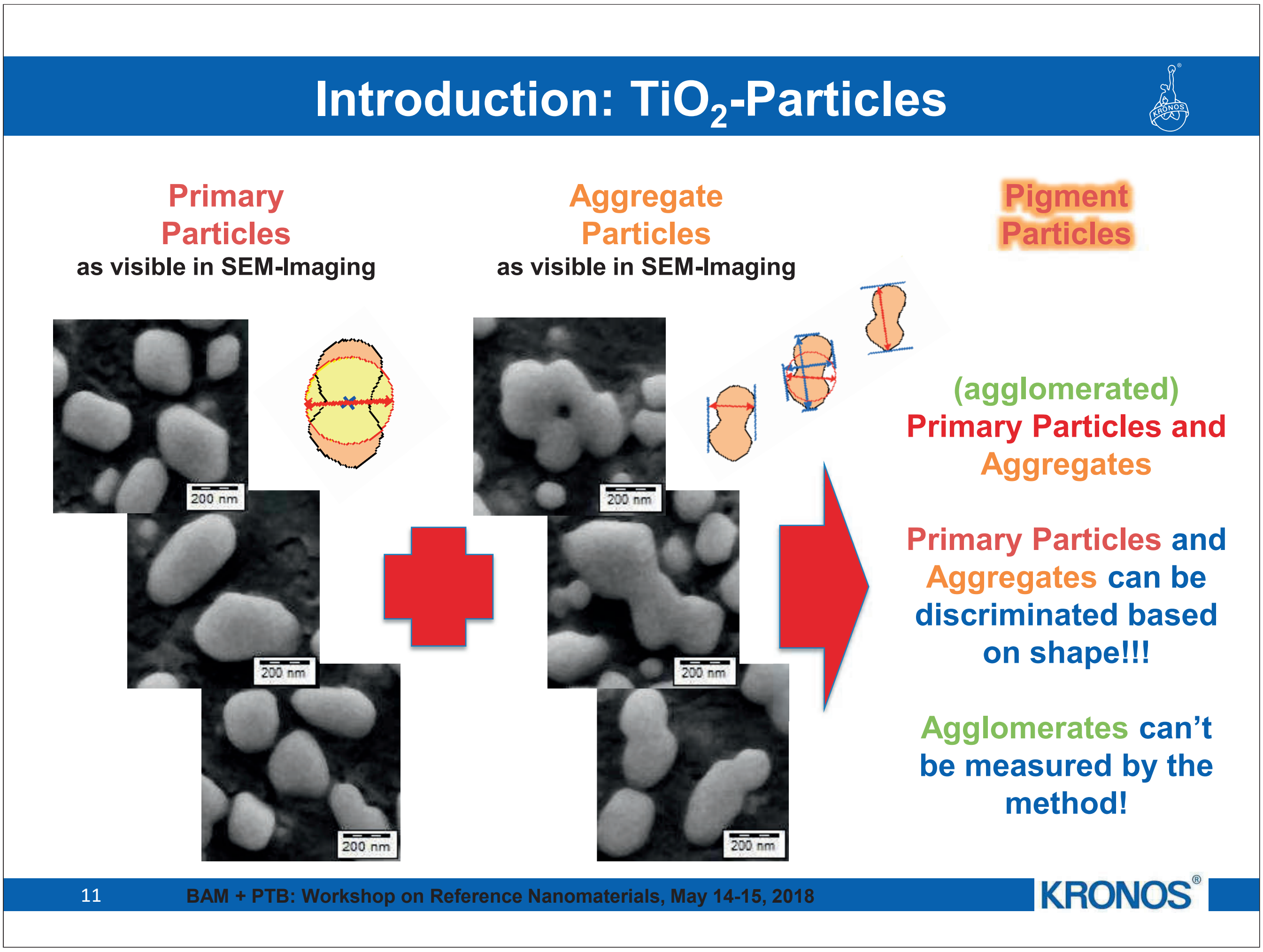
Twinned Primary Particles
as visible in TEM-Imaging

Primary Particles
as visible in SEM-Imaging



Aggregate Particles
as visible in SEM-Imaging





Introduction: TiO_2-Particles

Primary Particles
as visible in SEM-Imaging

Aggregate Particles
as visible in SEM-Imaging

Pigment Particles

(agglomerated) Primary Particles and Aggregates

Primary Particles and Aggregates can be discriminated based on shape!!!

Agglomerates can't be measured by the method!

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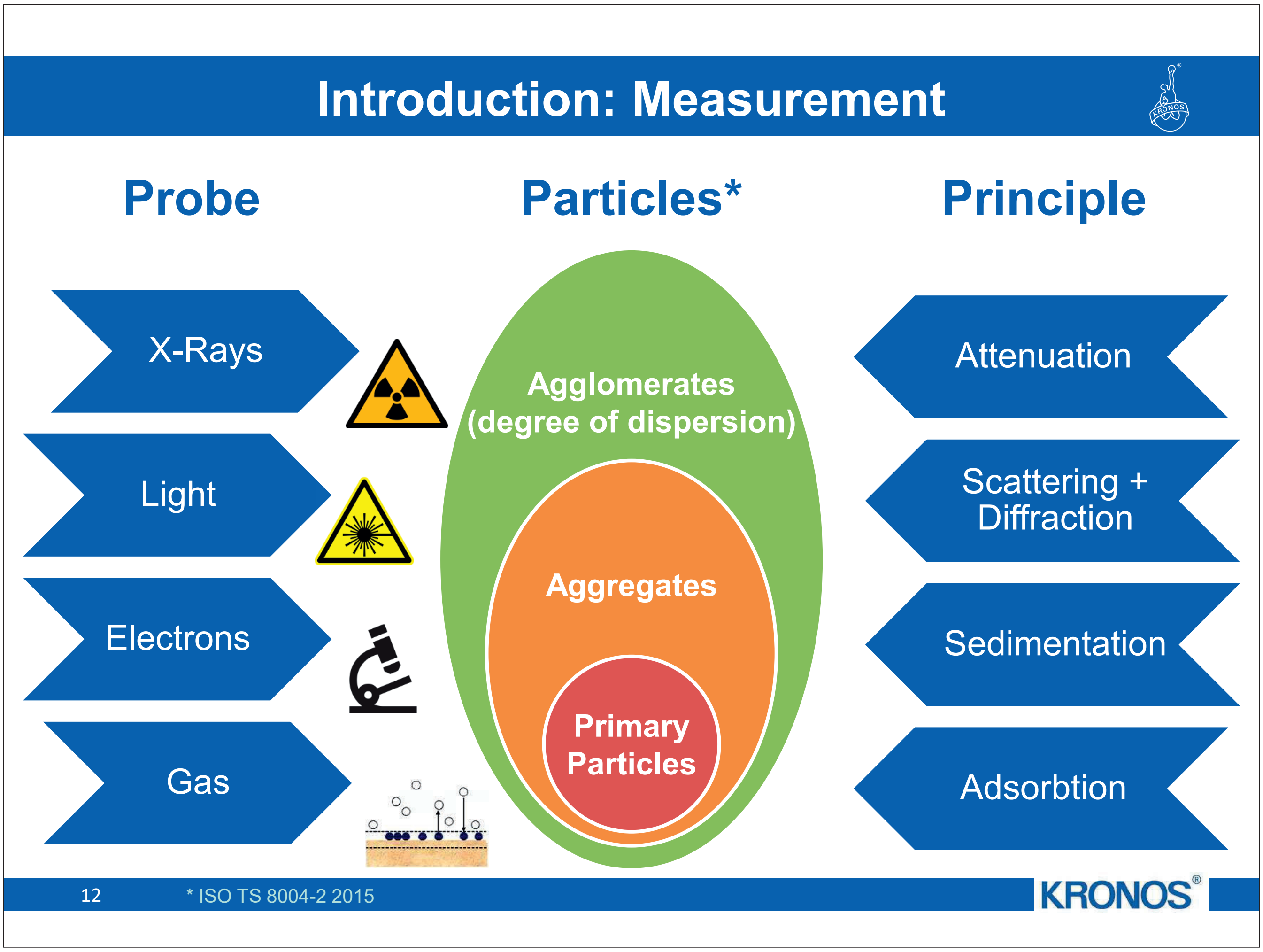

Introduction: Measurement

Probe	Particles*	Principle
X-Rays	Agglomerates (degree of dispersion)	Attenuation
Light	Aggregates	Scattering + Diffraction
Electrons	Primary Particles	Sedimentation
Gas		Adsorbtion

* ISO TS 8004-2 2015

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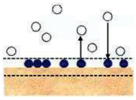
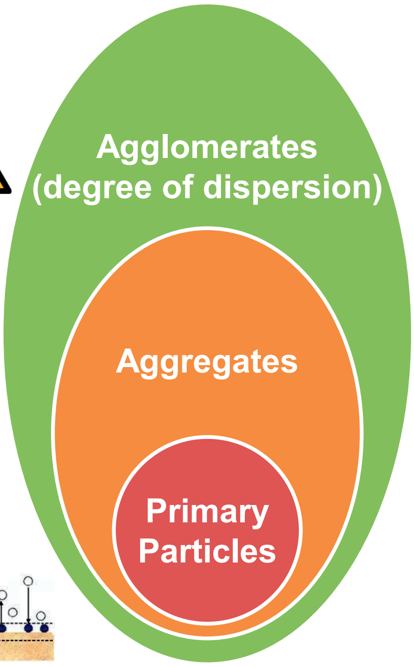
Introduction: Measurement

Measurand

- Hydro-dynamic Radius
- Stokes Diameter
- ECD, FERET
- Surface Area



Particles*



Coincidences

Perfect Dispersion:
Particle Size and Aggregate Size coincide

Perfect Milling:
Aggregates are milled down to primary particles, Aggregate Size and Primary-Particle Size coincide

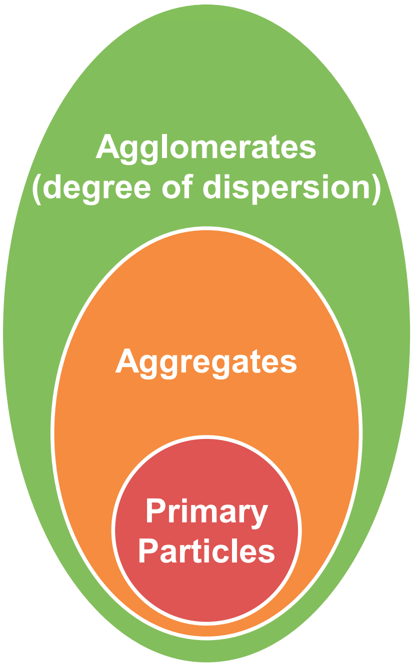
Perfect Dispersion + Perfect Milling:
Particle Size and Primary-Particle Size coincide

* ISO TS 8004-2 2015

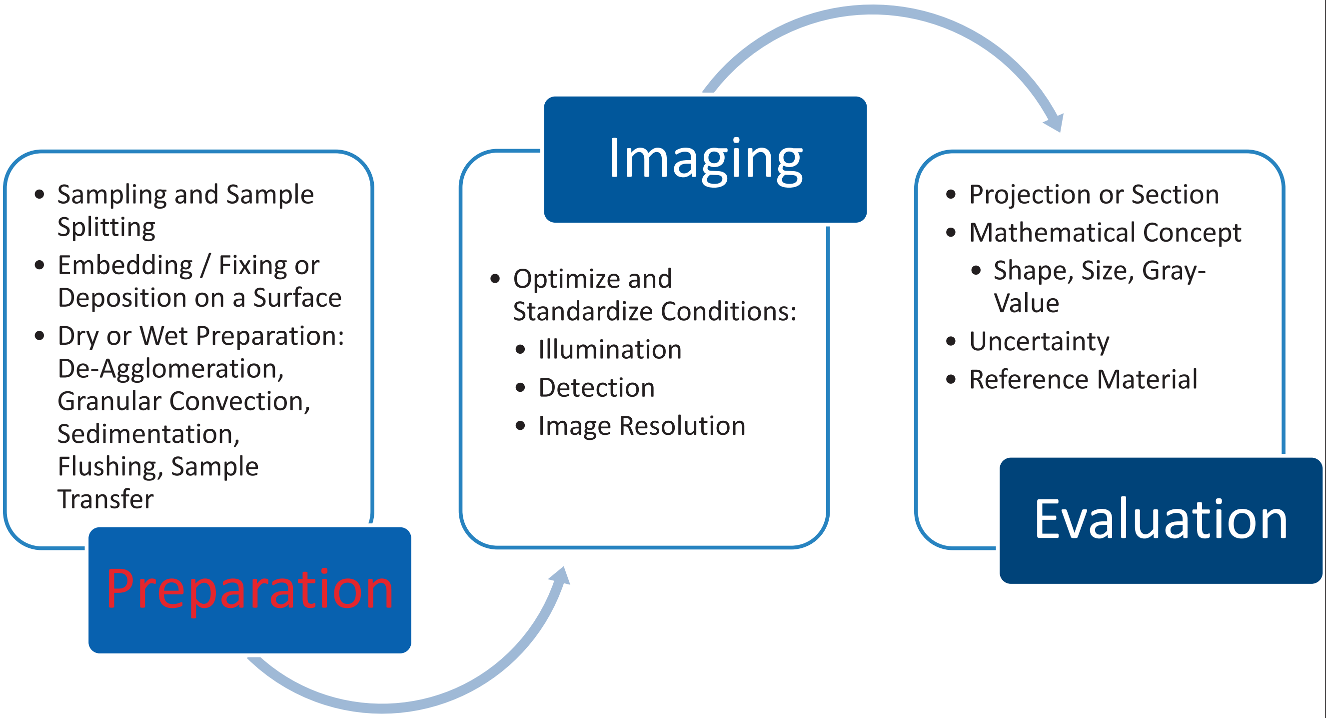
Introduction: Sample-Measurement

Method	Size (d_{50m})
Masersizer 2000	460nm
Disc-centrifuge	340nm
Sedigraph 5100	360nm
SEM	358nm 247nm

K2360



Route to a Representative Size Distribution



Preparation

- Sampling and Sample Splitting
- Embedding / Fixing or Deposition on a Surface
- Dry or Wet Preparation: De-Agglomeration, Granular Convection, Sedimentation, Flushing, Sample Transfer

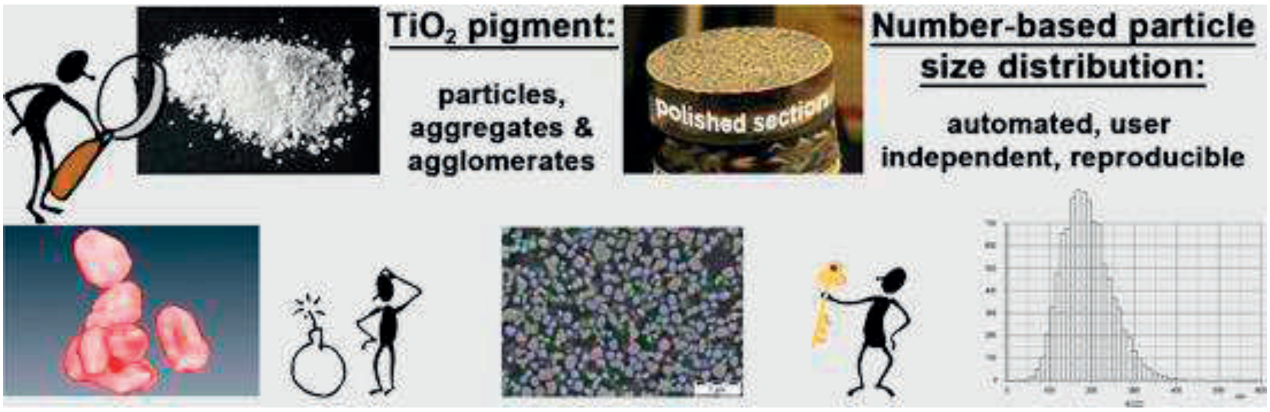
Imaging

- Optimize and Standardize Conditions:
  - Illumination
  - Detection
  - Image Resolution

Evaluation

- Projection or Section
- Mathematical Concept
  - Shape, Size, Gray-Value
- Uncertainty
- Reference Material

One possible solution…



Sample is selected by repeated splitting (Sample Riffler)

✓ Representative, macroscopic amount of Sample

Dry Mixing of sample and <u>solid</u> resin, hot-mounting, grinding and polishing

✓ No Sedimentation or Flushing

Use of standard imaging conditions and evaluation routine

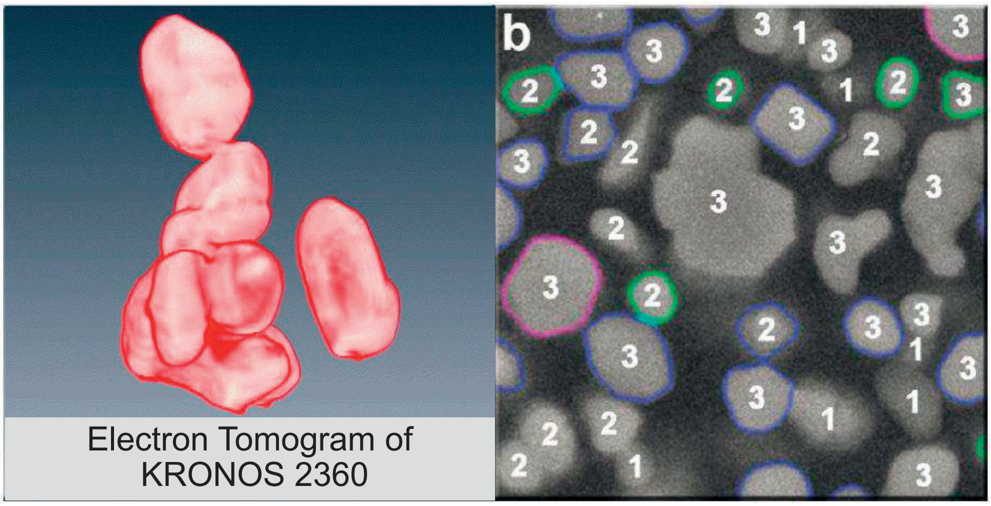
✓ Reproducible data analysis without user based decisions

Theissmann, R.; Kluwig, M.; Koch, T. *Beilstein J. Nanotechnol.* **2014,** *5,* 1815–1822.

Results

Measurand: ***Section Size***

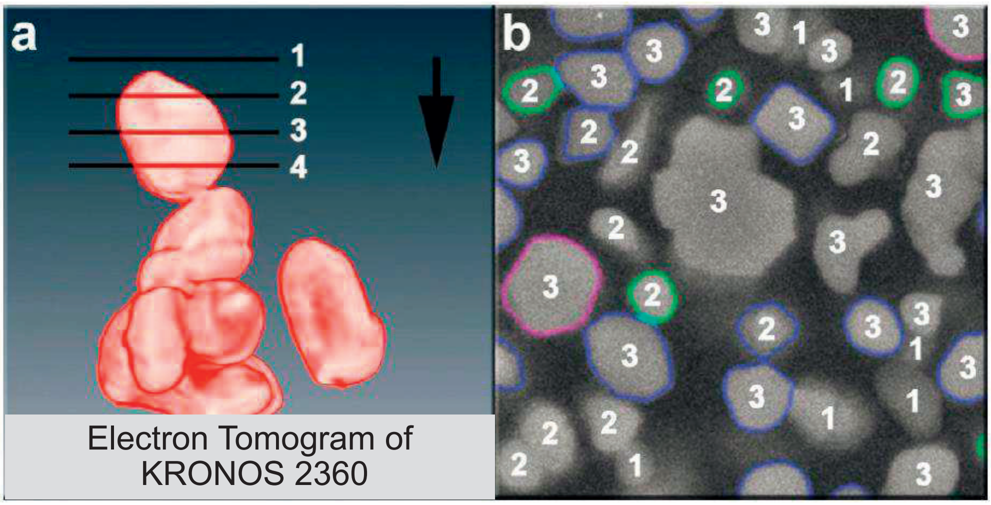
(Opposing the *Projection Size* in most other preparation methods)


Electron Tomogram of KRONOS 2360

Results

Measurand: ***Section Size***

(Opposing the *Projection Size* in most other preparation methods)


Electron Tomogram of KRONOS 2360

Sample Preparation and Imaging

Representative Sample

- repeated splitting of the "*as delivered*" sample

Immobilizing / Fixing the Sample

- dry mixing and hot mounting without sedimentation or flushing

Representative Cross-Section

- grinding and polishing (standard metallurgical preparation)

Reproducible Microscopy-Data

- standard imaging conditions (e.g. 5kV, 7mm, ETD, 20pix @ d_{10})

Theissmann, R.; Kluwig, M.; Koch, T. *Beilstein J. Nanotechnol.* **2014,** *5,* 1815–1822.

Sample Image

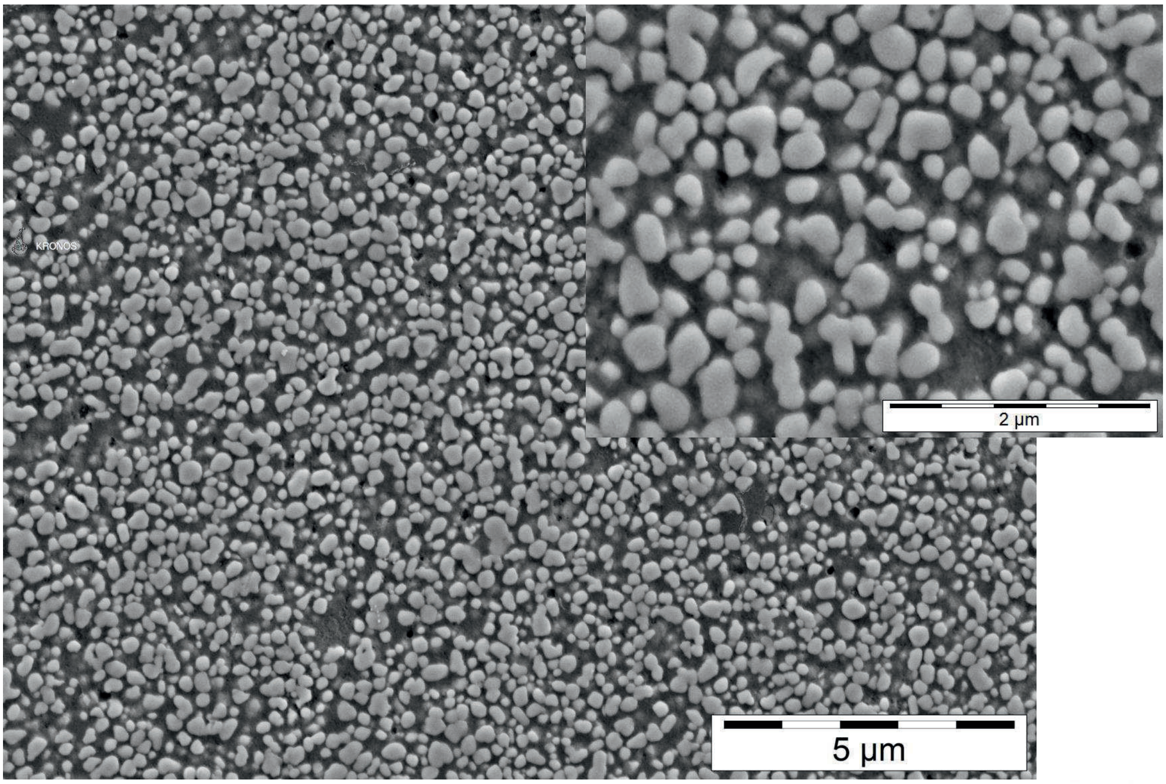


Image Post Processing

Noise Filtering and Gray-Value Adjustment

- Median-Filter or Rank-Filter, auto brightness / contrast

Binarization

- With automated detection of threshold

Watershed transform

- Separate aggregates and touching particles

Connectivity Filter

- Removal of isolated pixels

Mask original- with binary image

- Separated (primary) particles with accessible gray values

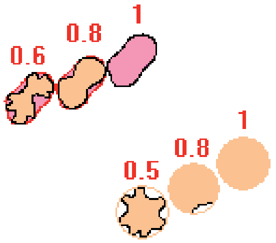
Theissmann, R.; Kluwig, M.; Koch, T. *Beilstein J. Nanotechnol.* **2014,** *5,* 1815–1822.

Data Analysis / Filtering

PRIMARY PARTICLES:
convex particles with similar grey values

Shape-Based Filtering

- ✓ Convexity > 0.90
- ✓ Formfactor > 0.86



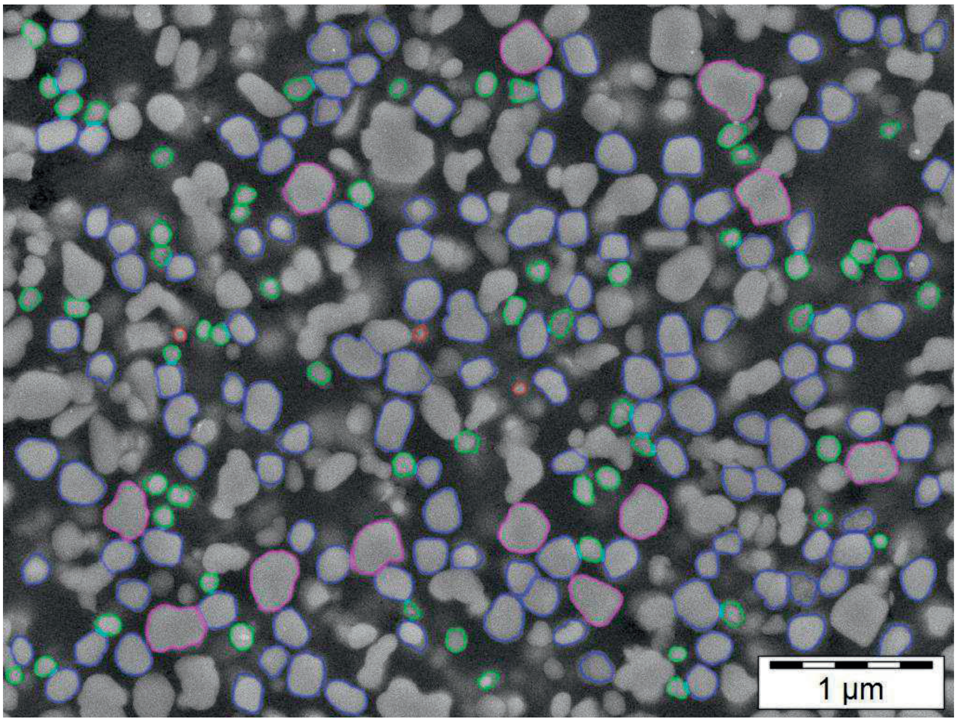
Assumptions for Grey-value (GV) based filtering

- ✓ the mean grey-values and its standard deviations are normal-distributed
- ✓ asymmetries in both distributions are caused by detection errors
- ✓ Filters (thresholds) are applied to make the grey-value distributions symmetric and remove detection errors.
- ➢ Software: Olympus-SIS, Analysis (iTEM, Scandium)

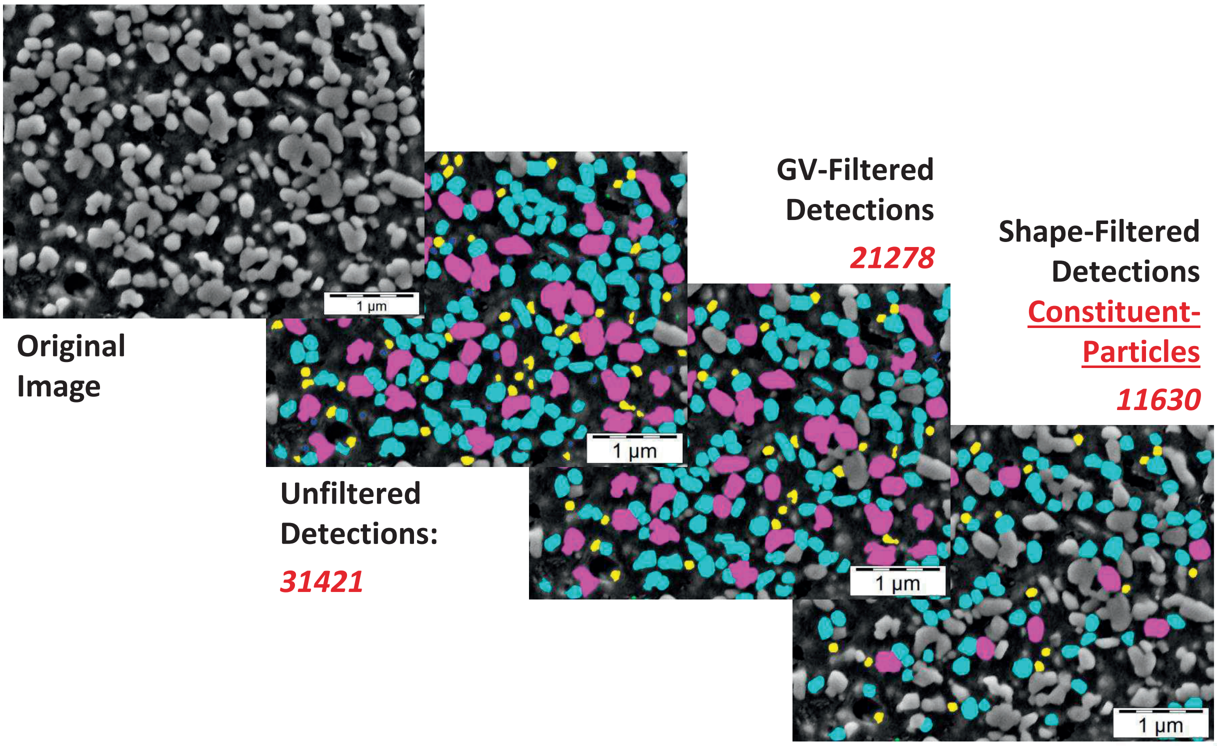
SEM Imaging and Post-Processing

Post-Processing Steps:

1. Original Image
2. Noise Filtering
3. Binarization
4. Watershed Transformation
5. Masking **the filtered original**
6. **Detection of** Primary Particles
7. Morphologic Filtering + Grey-Value Filtering: Colorcoded Primary Particles



Details on Primary-Particle filtering



Original Image

Unfiltered Detections:

31421

GV-Filtered Detections

21278

Shape-Filtered Detections

Constituent-Particles

11630

Internal repeatability / replicability Testing

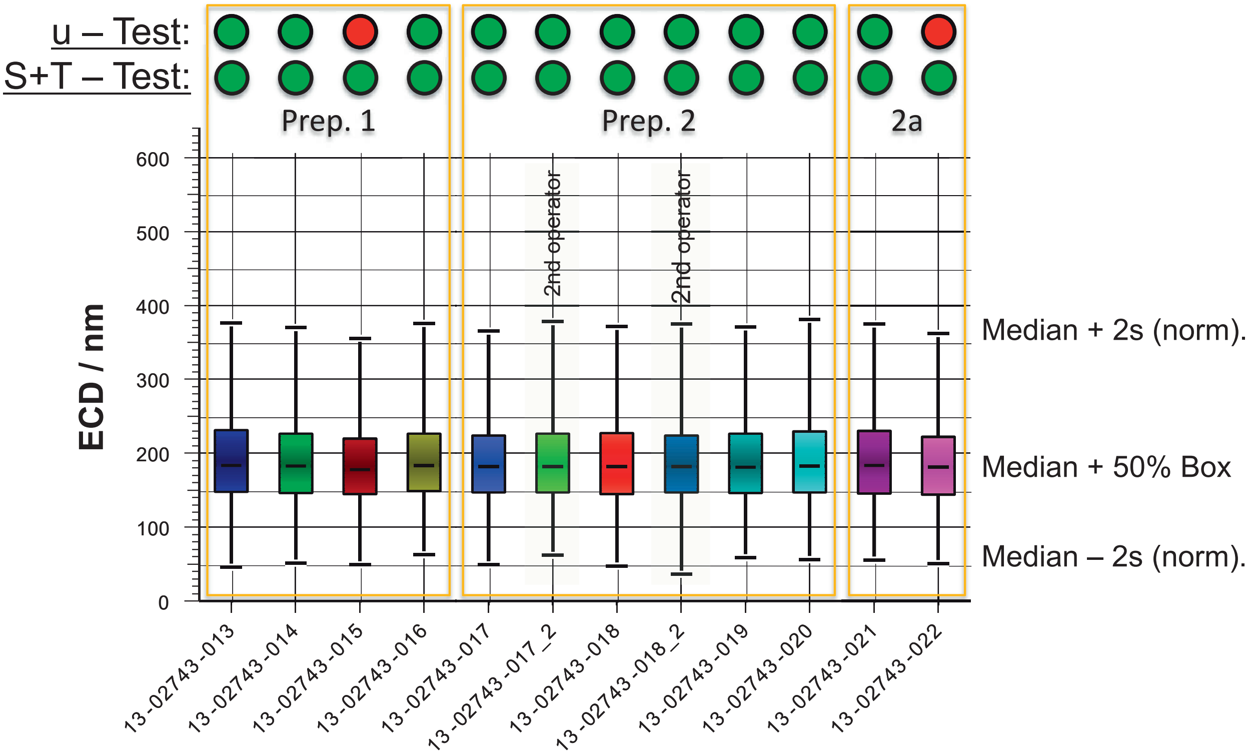
- Eight samples were prepared in two independent series à four samples
- Eight Measurements were done under standard conditions by one operator
- Repeated Measurements were done by a second operator
- Results were tested using the u-Test after Wilcoxon, Mann and Whitney
- Additional Testing was done using the Rank-Dispersion Test of Siegel and Tukey (S+T – Test)

	primary particles - preparation series I					
	13-02743-013	13-02743-014	13-02743-015	13-02743-016	Mean	Std. - Dev.
d16 / nm	130	130	127	133	130	2
d50 / nm	183	182	178	184	182	3
d84 / nm	247	245	240	246	245	3

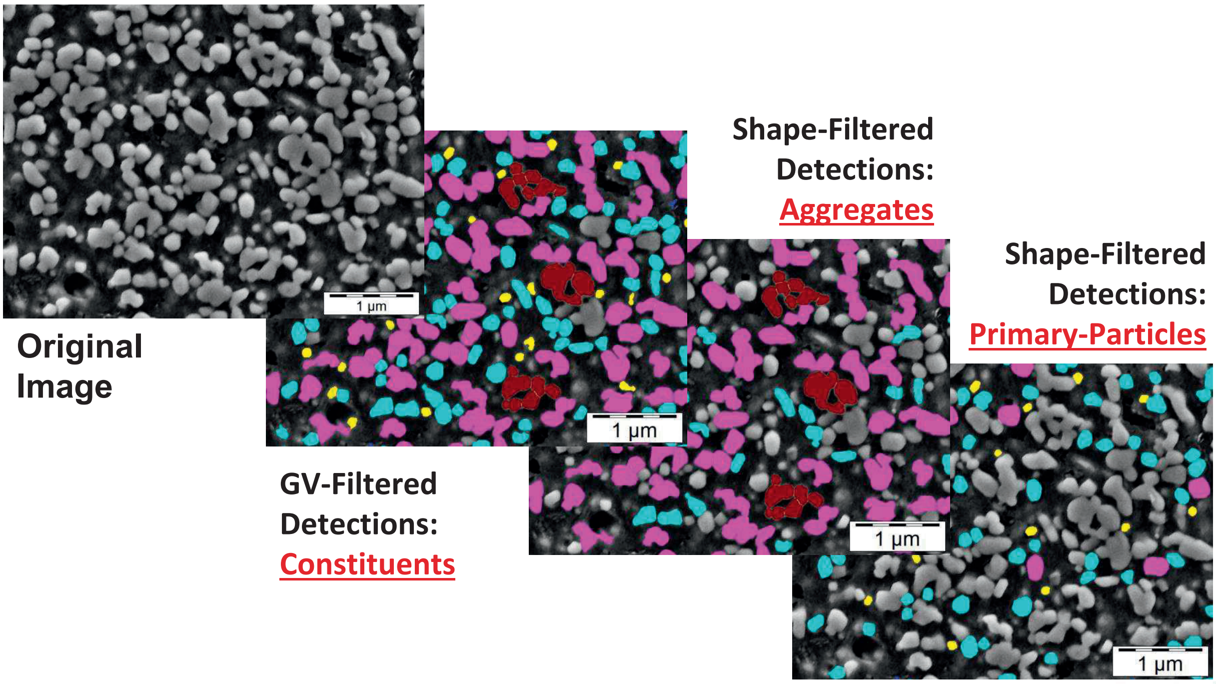
	primary particles - preparation series II							
	13-02743-017	13-02743-017b	13-02743-018	13-02743-018b	13-02743-019	13-02743-020	Mean	Std. - Dev.
d16 / nm	129	131	128	129	129	130	129	1
d50 / nm	182	182	182	182	181	183	182	1
d84 / nm	244	247	245	245	244	244	245	1

Theissmann, R.; Kluwig, M.; Koch, T. *Beilstein J. Nanotechnol.* **2014,** *5,* 1815–1822.

Internal repeatability / replicability Testing



Details on Pigment-Particle deconvolution



Original Image

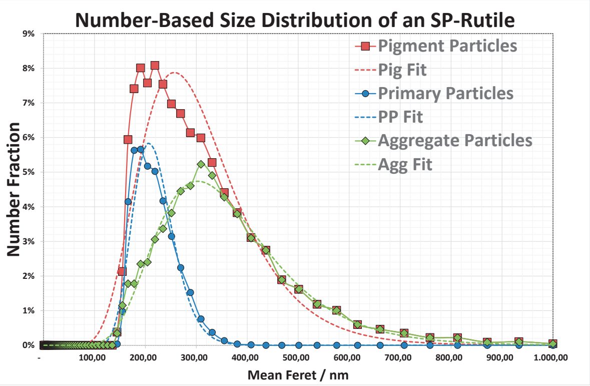
GV-Filtered Detections: Constituents

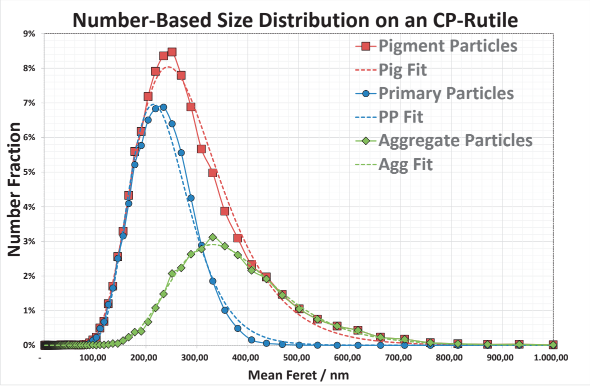
Shape-Filtered Detections: Aggregates

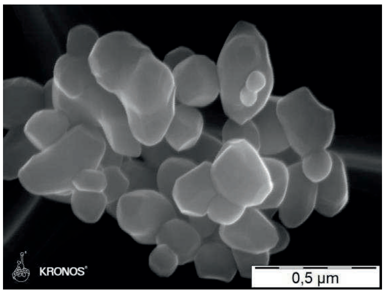
Shape-Filtered Detections: Primary-Particles

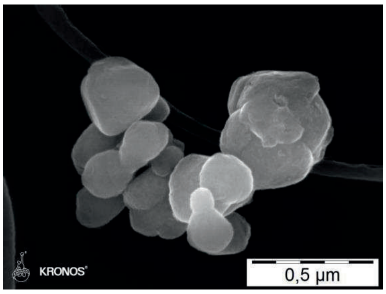
Deconvolution of Pigment-Particles

Comparison of two commercial Rutile Grades



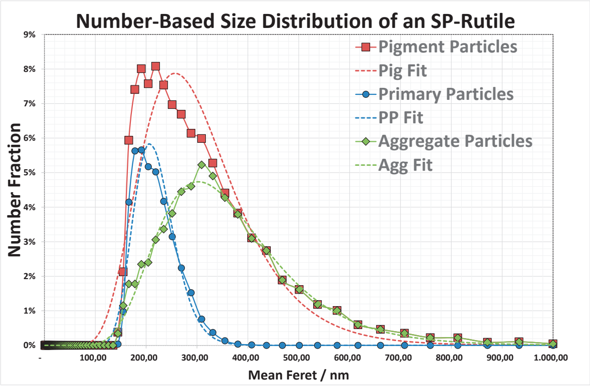


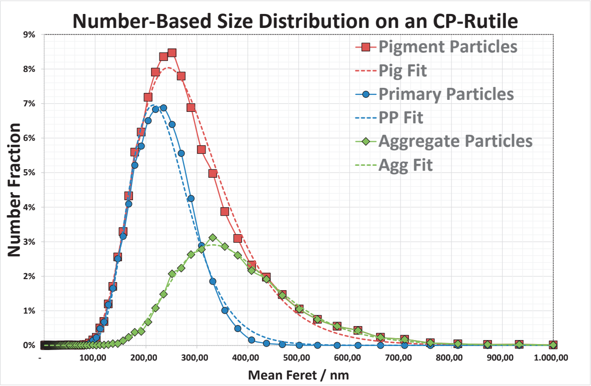




Deconvolution of Pigment-Particles

Comparison of two commercial Rutile Grades





	primary particles		pigment particles	
	number-based	volume-based	number-based	volume-based
measurand	measurement	*fit*	measurement	*fit*
d16	161	*174*	178	*207*
d50	189	*213*	241	*318*
d84	230	*264*	366	*505*
#	6.860	*6.860*	13.390	*1.690*
$\sigma_{ln}/ln(10)$	0,08	*0,09*	0,16	*0,19*
% PP	100%	*100%*	39%	*12%*
% Agg	0%	*0%*	61%	*88%*

	primary particles		pigment particles	
	number-based	volume-based	number-based	volume-based
measurand	measurement	*fit*	measurement	*fit*
d16	151	*197*	169	*240*
d50	199	*248*	234	*358*
d84	255	*318*	331	*553*
#	20.622	*20.662*	26.795	*26.795*
$\sigma_{ln}/ln(10)$	0,11	*0,10*	0,15	*0,18*
% PP	100%	*100%*	68%	*33%*
% Agg	0%	*0%*	32%	*67%*

Summary and Conclusions: Primary Particles

The presented method was proven ***precise***, ***repeatable*** and ***replicable*** for sizing of TiO_2 – Pigment Particles:

- Representative Sample
- Measurement under standardized conditions
- Fully automated image post-processing and evaluation
- Statistically relevant number of detections

Summary and Conclusions: Pigments

The presented method allows to distinguish between primary particles, aggregates based on particle-shape:

- defined shape thresholds (convexity, shape-factor, aspect-ratio, fractal dimension, etc.)
- In case of a „perfect" dispersion, pigment size and stokes diameter measured by disk-centrifuge are shown to coincide

---

Implications on Reference-Materials

We need a "one for all" polydisperse Reference-Material for EM-Measurements with given *Median*, *Sigma – Intervals / selected Quantiles* and *Skewness* certified for…

- primary-particle size (projections and sections)
- constituent size (projections and sections)
- aggregate size (projections and sections)
- defined shape thresholds for primary particles and aggregates (convexity, shape-factor, aspect-ratio, fractal dimension, etc.)
- Correlation to other, practically relevant measurands (e.g. stokes diameter)

Thank You for Your Attention

BAM
Bundesanstalt für Materialforschung und -prüfung

Sicherheit in Technik und Chemie

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SHAPE CONTROLLED TiO_2 NANOPARTICLES AS CANDIDATES FOR nano-CRM‘S – AN ISO CASE STUDY

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Outline

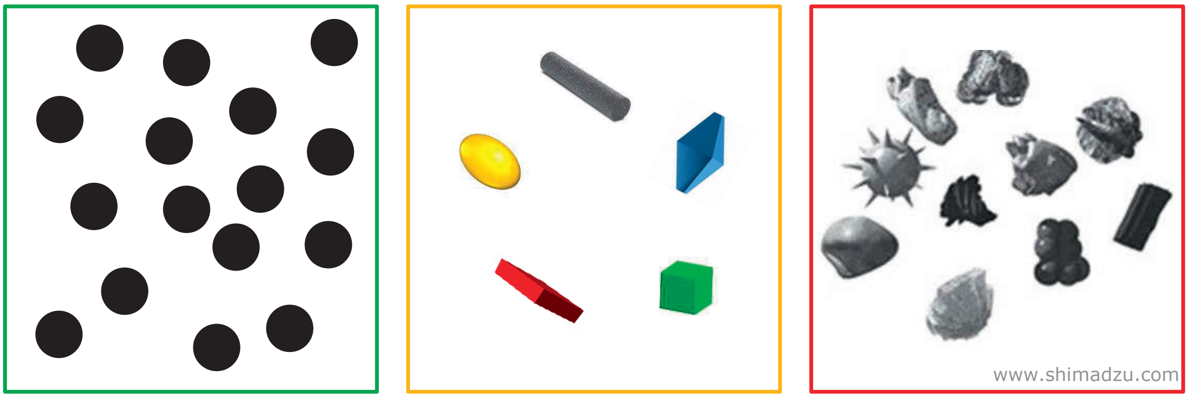
- Need of shape-controlled NPs
- Our proposal: TiO_2 (bipyramidal,…) NPs
- NP characterization (by electron microscopy) framework
- An ILC case study of ISO/WD 21363 *NPS&SD by TEM*
- Measurement and Image Analysis Protocol
- Size & shape descriptors and Data analysis (models)
- Conclusions & Outlook

Accurate size & shape as p-c parameters for (eco)toxicological testing

Lists of minimum p-c characteristics of NM to be assessed for eco(toxicological) testing:

- Testing Programme of **OECD WPMN** (OECD 2009): *Agglomeration/ aggregation, water solubility, crystalline phase, dustiness, crystallite size, representative TEM picture(s), particle size distribution, specific surface area, zeta potential, surface chemistry, photocatalytic activity, pour density, porosity,…*
- **ISO**/TR 13014: *agglomeration/aggregation, solubility, shape and size, PSD, specific surface area, surface charge, surface chemistry, composition*.
- **ISO**/TR 12885: '…*Nanotechnology presents us with new challenges as the properties of NMs now depend on size and shape as much as the more conventional factors of chemical structure and composition. Measuring these additional attributes will be necessary to accurately assess NMs in the workplace…*'
- **Research projects** (Stone *et al*, 2010, …): *aggregation state, crystal structure, shape and size, PSD, surface area, surface charge*.
- …

Nanoparticle shape; Problem: Filling the gap between simple - complex


www.shimadzu.com

Need for shape-controlled nanoparticles as a metrological bridge between model/lab samples and real-world/complex nanoparticles

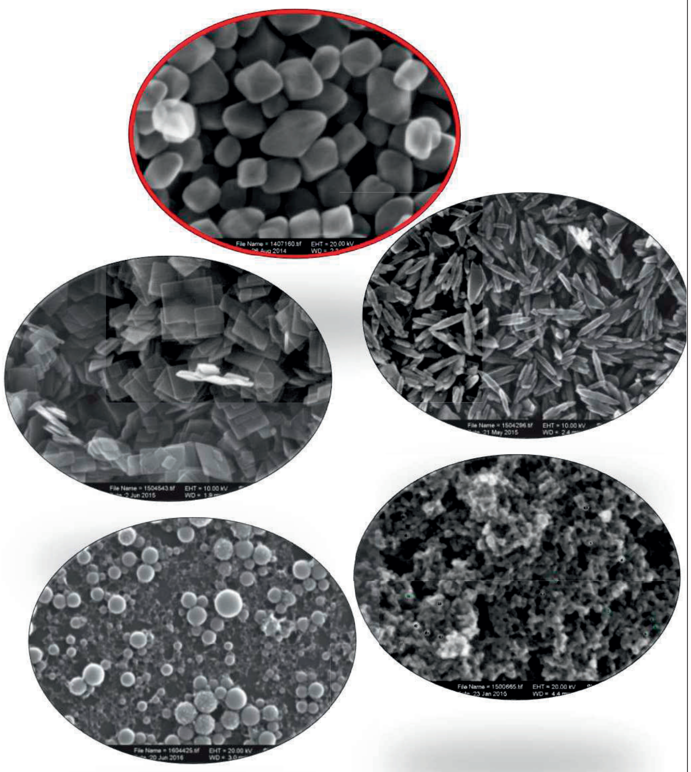
Availability of shape-controlled NPs

BAM

- Shape controlled TiO_2 NPs, (HT) manufactured within EU/FP7 project *SETNanoMetro* as monodisperse platelets, bipyramids and elongated; also round TiO_2 NPs and irregular available.

- NP size and in particular shape (crystal facet engineering) are of interest for large-scale applications

- Candidate as a reference material with certified aspect ratio (and PSD)



NP Characterisation Framework: ISO, CEN, VAMAS, BAM & Co

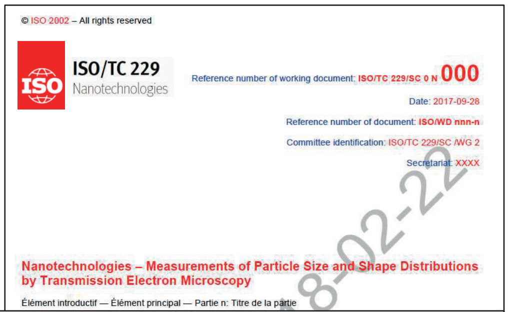
BAM

- ISO/WD 21363 Nanotechnologies -- *Measurements of Particle Size and Shape Distributions by TEM*

- ISO/WD 19749 Nanotechnologies -- *Measurements of Particle Size and Shape Distributions by SEM*

- ISO/PWI 22292 Nanotechnologies -- *3-D Reconstruction of Nano-objects Using TEM*

- VAMAS/TWA 34 Nanoparticle Populations – e.g. *Assessment of a Quantitative NM Definition*



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ISO/TC 229
Nanotechnologies

Reference number of working document: ISO/TC 229/SC 0 N 000

Date: 2017-09-28

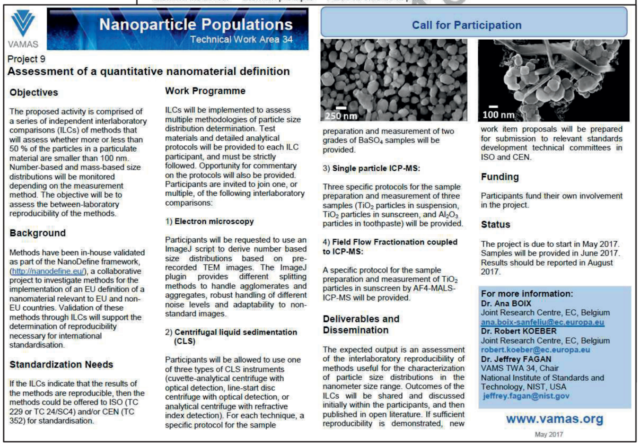
Reference number of document: ISO/WD nnn-n

Committee identification: ISO/TC 229/SC /WG 2

Secretariat: XXXX

Nanotechnologies – Measurements of Particle Size and Shape Distributions by Transmission Electron Microscopy

Élément introductif — Élément principal — Partie n: Titre de la partie



VAMAS

Nanoparticle Populations
Technical Work Area 34

Call for Participation

Project 9
Assessment of a quantitative nanomaterial definition

Objectives

The proposed activity is comprised of a series of independent interlaboratory comparisons (ILCs) of methods that will assess whether more or less than 50 % of the particles in a particulate material are smaller than 100 nm. Number-based and mass-based size distributions will be monitored depending on the measurement method. The objective will be to assess the between-laboratory reproducibility of the methods.

Background

Methods have been in-house validated as part of the NanoDefine framework, (http://nanodefine.eu/), a collaborative project to investigate methods for the implementation of an EU definition of a nanomaterial relevant to EU and non-EU countries. Validation of these methods through ILCs will support the determination of reproducibility necessary for international standardisation.

Standardization Needs

If the ILCs indicate that the results of the methods are reproducible, then the methods could be offered to ISO (TC 229 or TC 24/SC4) and/or CEN (TC 352) for standardisation.

Work Programme

ILCs will be implemented to assess multiple methodologies of particle size distribution determination. Test materials and detailed analytical protocols will be provided to each ILC participant, and must be strictly followed. Opportunity for commentary on the protocols will also be provided. Participants are invited to join one, or multiple, of the following interlaboratory comparisons:

1) **Electron microscopy**

Participants will be requested to use an ImageJ script to derive number based size distributions based on pre-recorded TEM images. The ImageJ plugin provides different splitting methods to handle agglomerates and aggregates, robust handling of different noise levels and adaptability to non-standard images.

2) **Centrifugal liquid sedimentation (CLS)**

Participants will be allowed to use one of three types of CLS instruments (cuvette-analytical centrifuge with optical detection, line-start disc centrifuge with optical detection, or analytical centrifuge with refractive index detection). For each technique, a specific protocol for the sample preparation and measurement of two grades of $BaSO_4$ samples will be provided.

3) **Single particle ICP-MS:**

Three specific protocols for the sample preparation and measurement of three samples (TiO_2 particles in suspension, TiO_2 particles in sunscreen, and Al_2O_3 particles in toothpaste) will be provided.

4) **Field Flow Fractionation coupled to ICP-MS:**

A specific protocol for the sample preparation and measurement of TiO_2 particles in sunscreen by AF4-MALS-ICP-MS will be provided.

Deliverables and Dissemination

The expected output is an assessment of the interlaboratory reproducibility of methods useful for the characterization of particle size distributions in the nanometer size range. Outcomes of the ILCs will be shared and discussed initially within the participants, and then published in open literature. If sufficient reproducibility is demonstrated, new work item proposals will be prepared for submission to relevant standards development technical committees in ISO and CEN.

Funding

Participants fund their own involvement in the project.

Status

The project is due to start in May 2017. Samples will be provided in June 2017. Results should be reported in August 2017.

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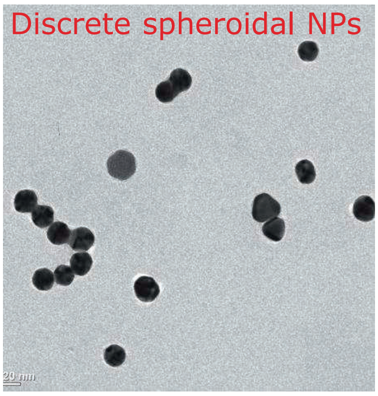
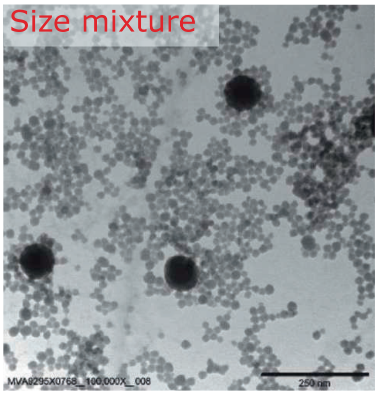
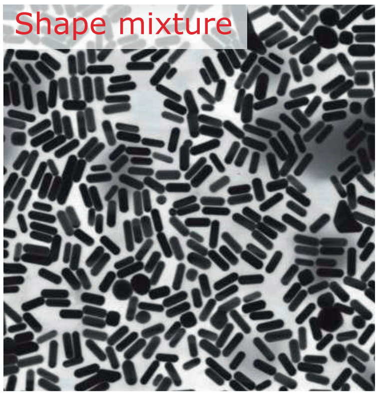
May 2017

NP Characterisation Framework: ISO/WD 21363 (1)


Discrete spheroidal NPs


Size mixture

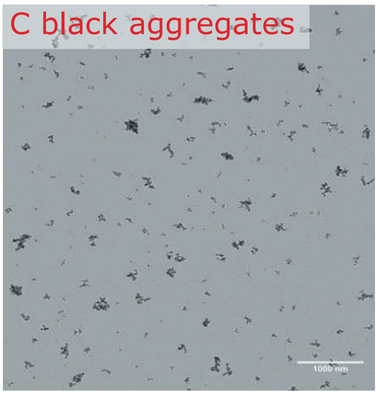

Shape mixture

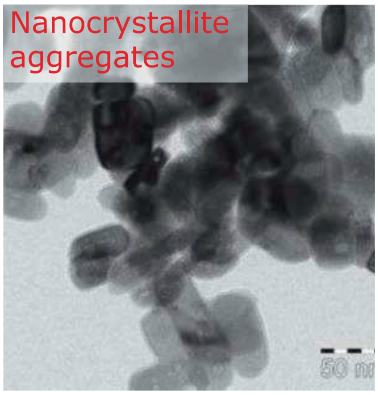
- Automated image capture
- data quality assessment
- meas. uncertainties of distribution means and spreads

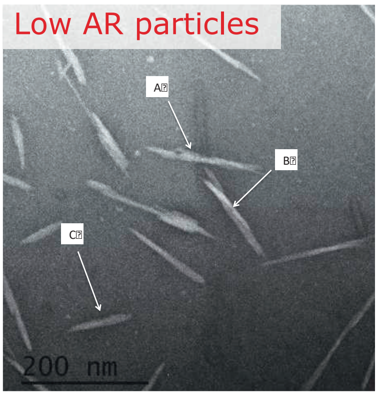
- Differentiation between datasets
- criteria for separating data clusters
- intermediate precision of separated clusters

- Criteria for identifying touching nanorods
- differences in size and shape distributions quantified

NP Characterisation Framework: ISO/WD 21363 (2)


C black aggregates


Nanocrystallite aggregates


Low AR particles

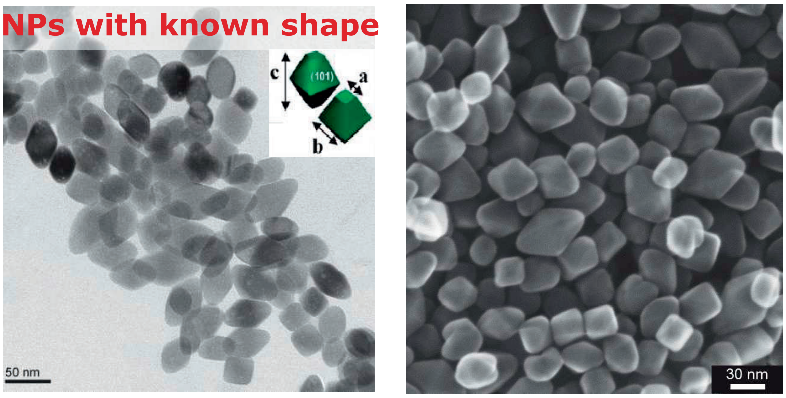
- Aggregate distributions
- meas. uncertainties of prior standard descriptors is high
- 4 shape categories of aggregates are non-uniformly distr. across data ranges, leading to multimodal distribs.

- Manual outlining
- lognormal PSD is preferred (to normal)
- AR descriptor parameters had the lowest meas. uncert.
- meas. uncert. should be suppl. by visualiz. techniques for better understanding.

- Three analysis methods are compared with data for low aspect ratio particles.

NP Characterisation Framework: ISO/WD 21363 (3)

NPs with known shape



- Reproducibilities are compared by ANOVA for three instrument types: TEM, TSEM and miniTEM
- Manual *vs* automated tracing
- One grid tested multiple times
- Analysis of the same particles by six labs.

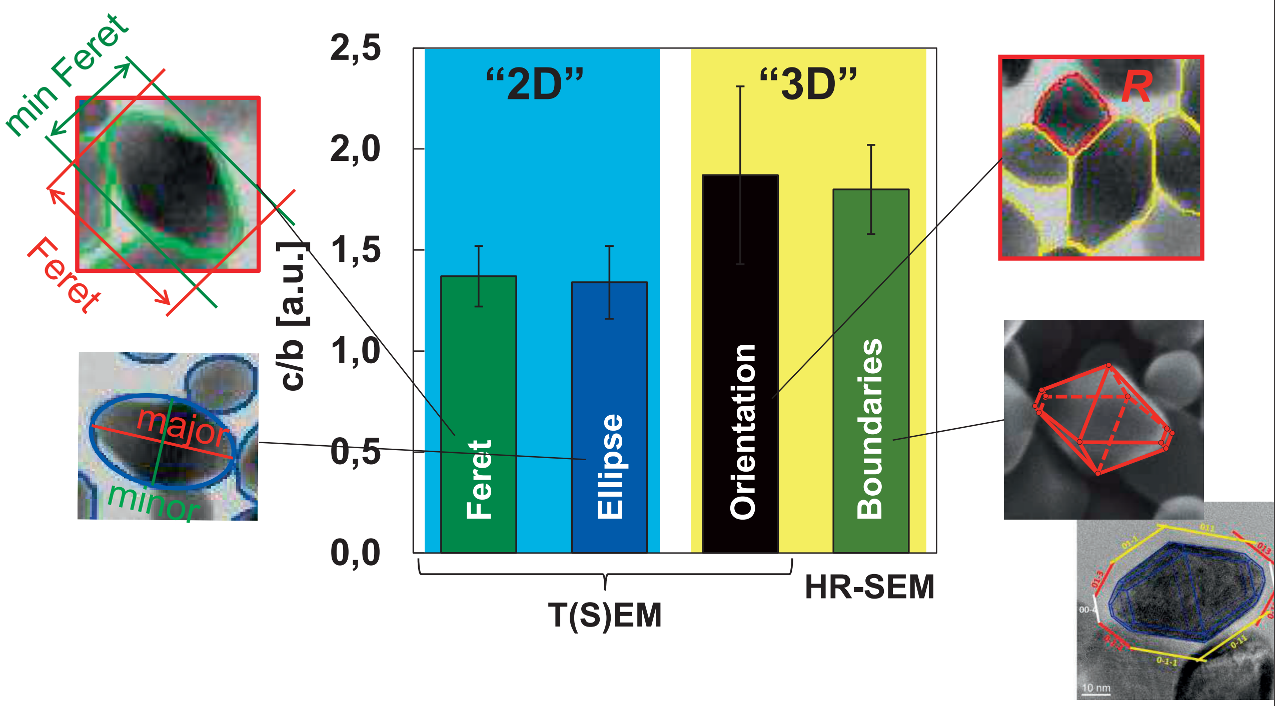
ISO/WD 21363 *NPS&SD by TEM* Case study #H: NPs with known shape

Lab #	TEM/ SEM	Notes
1	TSEM	√
2a	TEM	√
3	TEM	√
4	TSEM	√
5	TSEM	automated
6	TEM	√
7	TEM	√
8a	miniTEM	manual
8b	miniTEM	automated
9	TEM	√
10a	TEM	automated
10b	TEM	Operator 1
10c	TEM	Operator 2
11	TEM	√
12	TEM	√
13	TEM	√
14	TEM	√
15	TEM	√
16	TEM	same grid as 2a
2b	TEM	same grid as 2a
20	***Data sets***	

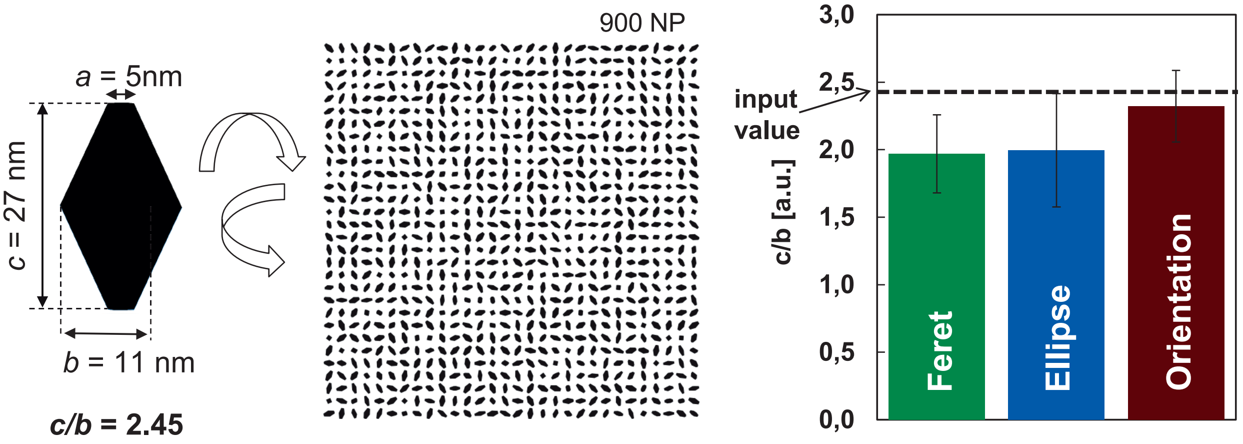
Labs and countries:

BAM a, b1, b2 (D)
PTB (D)
AIST a, b (JP)
CodaCerva (B)
BASF (D)
Vironova a, b (S)
Evonik (D)
Kronos a, b (D)
KRISS (KR)
Ishihara (JP)
NIOSH (USA)
DuPont (USA)
FDA (USA)
NMIA (AU)

Setting the Analysis Protocol
What (descriptors) and how to analyse?



Setting the Analysis Protocol
What (descriptors) and how to analyse?



- Orientation approach shows good agreement with input value

Measurement and Analysis Protocol - as distributed on July, 2016 (1)

1) Specimen: 1 (+1) TEM grid with bi-pyramidal TiO_2 NPs on Carbon membrane.

- Measure the sample as it is.

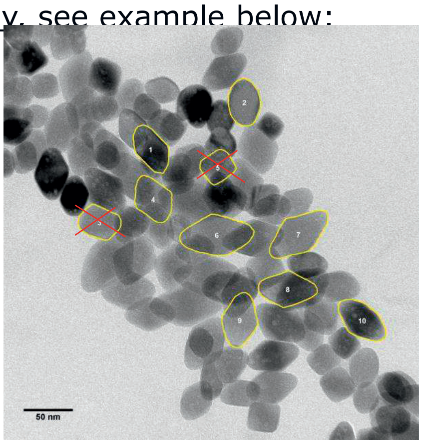
2) Descriptors for scale and shape to be measured:

a. *Area*
b. *(Maximum) Feret*
c. *Minimum Feret*
d. *Elliptical diameters*
e. *Roundness* <0.7, with roundness $R = 4 \cdot \frac{Area}{\pi \cdot MaximumFeret^2}$
f. *Perimeter of convex hull envelope*
g. *Area of convex hull envelope*
h. *Ruggedness* (or *Fractal dimension*)
i. Note that further descriptors such as: *Equivalent circular diameter*, *Equivalent perimeter diameter*, *Aspect ratio*, *Ellipse ratio*, *Extent*, *Circularity*, *Compactness, Convexity, Solidity* will be extracted by BAM from the data reported.

Measurement and Analysis Protocol - as distributed on July, 2016 (2)

3) TEM operation mode: BF TEM imaging is recommended; dark field, STEM and also SEM in transmission mode (TSEM) are accepted, too.

4) Tracing of particle shape: distinguishable, lying particles, i.e. with a roundness R <0.7 should be measured manually, see example below:



5) Number of particles to be measured: at least 500 lying NPs with R <0.7!

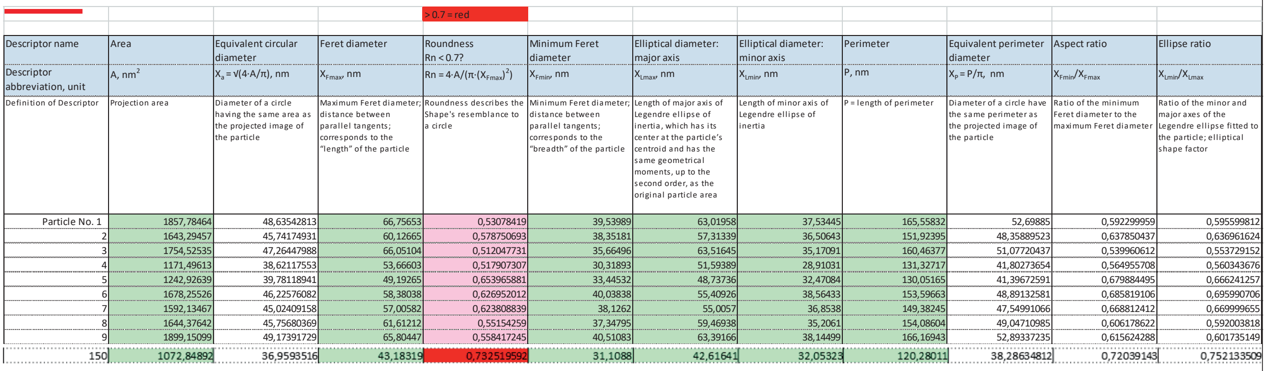
6) Resolution of the captured image: max. 0.5 nm/pixel

7) Analysis software: *ImageJ* is strongly recommended

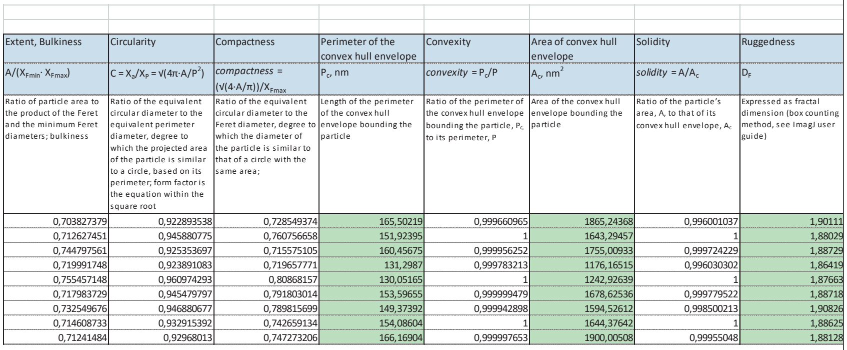
6) Reporting data format: use the xls-template

7) Time scale: three weeks after receipt of the samples

Measurement and Analysis Protocol - xls template provided by BAM (3)

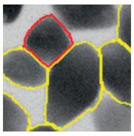


				> 0.7 = red							
Descriptor name	Area	Equivalent circular diameter	Feret diameter	Roundness Rn < 0.7?	Minimum Feret diameter	Elliptical diameter: major axis	Elliptical diameter: minor axis	Perimeter	Equivalent perimeter diameter	Aspect ratio	Ellipse ratio
Descriptor abbreviation, unit	A, nm^2	$X_a = \sqrt{(4 \cdot A/\pi)}$, nm	X_{Fmax}, nm	$Rn = 4 \cdot A/(\pi \cdot (X_{Fmax})^2)$	X_{Fmin}, nm	X_{Lmax}, nm	X_{Lmin}, nm	P, nm	$X_P = P/\pi$, nm	X_{Fmin}/X_{Fmax}	X_{Lmin}/X_{Lmax}
Definition of Descriptor	Projection area	Diameter of a circle having the same area as the projected image of the particle	Maximum Feret diameter; distance between parallel tangents; corresponds to the "length" of the particle	Roundness describes the Shape's resemblance to a circle	Minimum Feret diameter; distance between parallel tangents; corresponds to the "breadth" of the particle	Length of major axis of Legendre ellipse of inertia, which has its center at the particle's centroid and has the same geometrical moments, up to the second order, as the original particle area	Length of minor axis of Legendre ellipse of inertia	P = length of perimeter	Diameter of a circle have the same perimeter as the projected image of the particle	Ratio of the minimum Feret diameter to the maximum Feret diameter	Ratio of the minor and major axes of the Legendre ellipse fitted to the particle; elliptical shape factor
Particle No. 1	1857,78464	48,63542813	66,75653	0,53078419	39,53989	63,01958	37,53445	165,55832	52,69885	0,592299959	0,595599812
2	1643,29457	45,74174931	60,12665	0,578750693	38,35181	57,31339	36,50643	151,92395	48,35889523	0,637850437	0,636961624
3	1754,52535	47,26447988	66,05104	0,512047731	35,66496	63,51645	35,17091	160,46377	51,07720437	0,539960612	0,553729152
4	1171,49613	38,62117553	53,66603	0,517907307	30,31893	51,59389	28,91031	131,32717	41,80273654	0,564955708	0,560343676
5	1242,92639	39,78118941	49,19265	0,653965881	33,44532	48,73736	32,47084	130,05165	41,39672591	0,679884495	0,666241257
6	1678,25526	46,22576082	58,38038	0,626952012	40,03838	55,40926	38,56433	153,59663	48,89132581	0,685819106	0,695990706
7	1592,13467	45,02409158	57,00582	0,623808839	38,1262	55,0057	36,8538	149,38245	47,54991066	0,668812412	0,669999655
8	1644,37642	45,75680369	61,61212	0,55154259	37,34795	59,46938	35,2061	154,08604	49,04710985	0,606178622	0,592003818
9	1899,15099	49,17391729	65,80447	0,558417245	40,51083	63,39166	38,14499	166,16943	52,89337235	0,615624288	0,601735149
150	1072,84892	36,9593516	43,18319	0,732519592	31,1088	42,61641	32,05323	120,28011	38,28634812	0,72039143	0,752133509

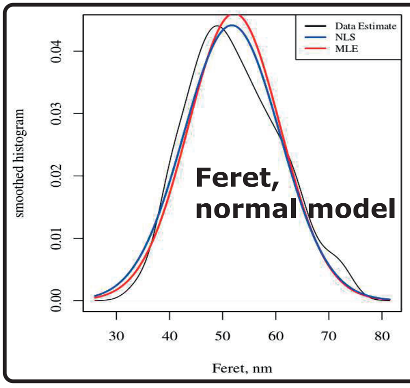


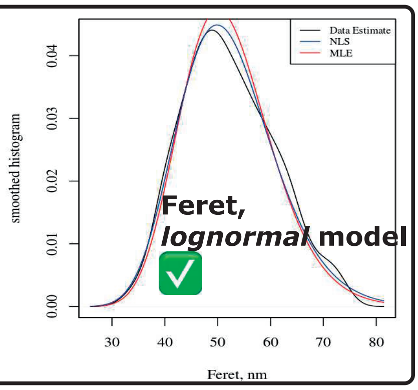
Extent, Bulkiness	Circularity	Compactness	Perimeter of the convex hull envelope	Convexity	Area of convex hull envelope	Solidity	Ruggedness
$A/(X_{Fmin} \cdot X_{Fmax})$	$C = X_a/X_P = \sqrt{(4\pi \cdot A/P^2)}$	*compactness* = $(\sqrt{(4 \cdot A/\pi)})/X_{Fmax}$	P_c, nm	*convexity* = P_c/P	A_c, nm^2	*solidity* = A/A_c	D_F
Ratio of particle area to the product of the Feret and the minimum Feret diameters; bulkiness	Ratio of the equivalent circular diameter to the equivalent perimeter diameter, degree to which the projected area of the particle is similar to a circle, based on its perimeter; form factor is the equation within the square root	Ratio of the equivalent circular diameter to the Feret diameter, degree to which the diameter of the particle is similar to that of a circle with the same area;	Length of the perimeter of the convex hull envelope bounding the particle	Ratio of the perimeter of the convex hull envelope bounding the particle, P_c, to its perimeter, P	Area of the convex hull envelope bounding the particle	Ratio of the particle's area, A, to that of its convex hull envelope, A_c	Expressed as fractal dimension (box counting method, see ImagJ user guide)
0,703827379	0,922893538	0,728549374	165,50219	0,999660965	1865,24368	0,996001037	1,90111
0,712627451	0,945880775	0,760756658	151,92395	1	1643,29457	1	1,88029
0,744797561	0,925353697	0,715575105	160,45675	0,999956252	1755,00933	0,999724229	1,88729
0,719991748	0,923891083	0,719657771	131,2987	0,999783213	1176,16515	0,996030302	1,86419
0,755457148	0,960974293	0,80868157	130,05165	1	1242,92639	1	1,87663
0,717983729	0,945479797	0,791803014	153,59655	0,999999479	1678,62536	0,999779522	1,88718
0,732549676	0,946880677	0,789815699	149,37392	0,999942898	1594,52612	0,998500213	1,90826
0,714608733	0,932915392	0,742659134	154,08604	1	1644,37642	1	1,88625
0,71241484	0,92968013	0,747273206	166,16904	0,999997653	1900,00508	0,99955048	1,88128

- green cells: to be filled mandatory
- white cells: calculated automatically
- pink cells: *R*
- red cells: *R*>0.7

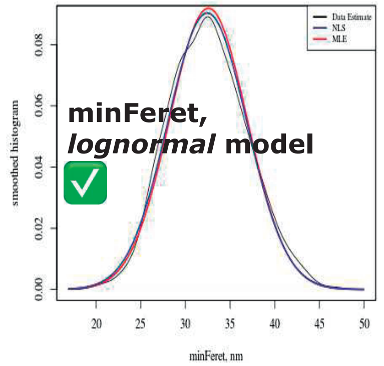


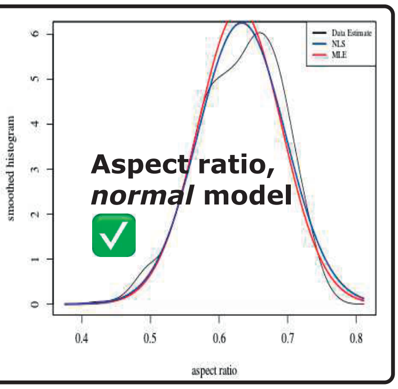
Data Analysis - Reference models for size and elongational shape descriptors





Lab #15





Data Analysis - Grand means of size & shape descriptors, normal or lognormal?

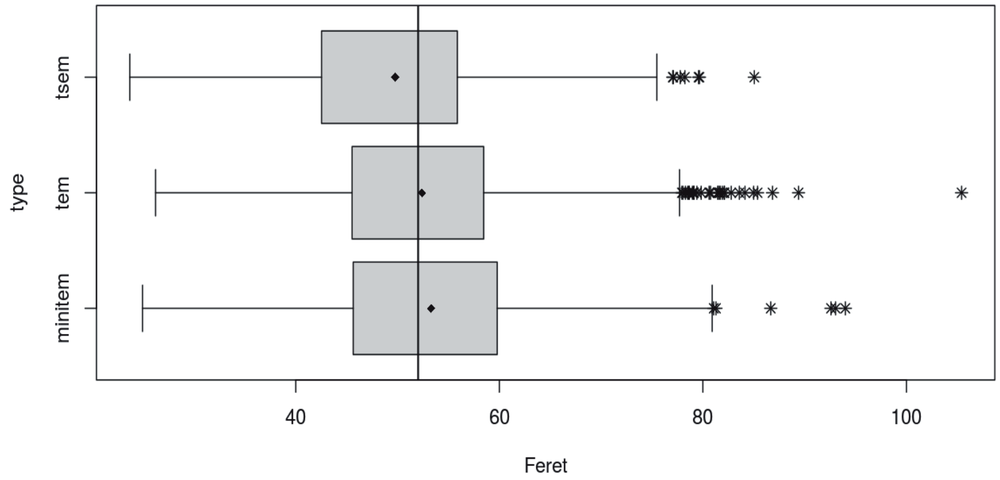
		Feret		minFeret		aspect ratio	
lab	**instr**	**scale**	**spread**	**scale**	**spread**	**scale**	**spread**
1a	TSEM	3.924	0.1706	3.492	0.1127	0.6520	0.0619
3	TSEM	3.895	0.2097	3.468	0.1802	0.6563	0.0668
6	TSEM	3.772	0.1760	3.393	0.1197	0.6853	0.0519
9a	miniTEM	3.927	0.1804	3.531	0.1361	0.6638	0.0584
9b	miniTEM	3.973	0.2001	3.531	0.1391	0.6429	0.0678
18a	TEM	3.990	0.1524	[illegible]	0.1267	[illegible]	0.0576
18b	TEM	3.879	0.1694	3.442	0.1330	[illegible]	[illegible]
15	TEM	3.941	0.1734	3.477	0.1366	[illegible]	0.0638
10	TEM	3.961	[illegible]	3.504	0.1311	0.6371	0.0769
17	TEM	3.927	0.1899	3.499	0.1440	0.5477	0.0635
11a	TEM	3.852	0.1945	3.424	0.1450	0.6569	0.0654
11b	TEM	3.929	0.1890	3.490	0.1493	0.6507	0.0680
14	TEM	3.997	0.1562	3.535	0.1269	0.6344	0.0578
18c	TEM	4.017	0.1880	3.528	0.1293	0.6196	0.0672
	average	**3.93**	**0.180**	**3.49**	**0.136**	**0.640**	**0.064**
	st dev	**0.0641**	**0.0161**	**0.0431**	**0.0160**	**0.0314**	**0.0061**
	C_v, %	**1.63**	**8.95**	**1.24**	**11.7**	**4.91**	**9.54**
	U_{ILC}, %	**3.38**	**18.5**	**2.56**	**24.3**	**10.2**	**19.7**

Size descriptors (lognormal)

Shape descriptor (normal)

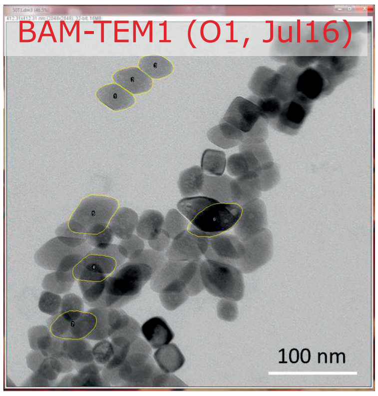
statistic	**normal**			
	Feret		**minFeret**	
	mean	**spread**	**mean**	**spread**
average	**51.2**	**9.13**	**32.8**	**4.45**
stdev	**3.19**	**0.900**	**1.35**	**0.538**
Cv, %	**6.24**	**9.86**	**4.13**	**12.1**
U_{ILC}	**12.9**	**20.4**	**8.54**	**25.0**

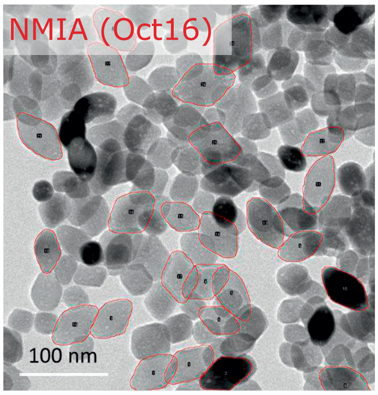
Data analysis
Comparison: TEM, TSEM, miniTEM

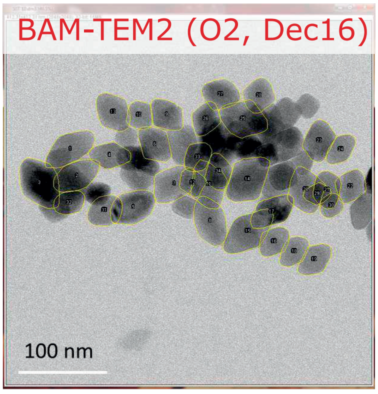


- Similar results occur for ANOVA analysis of the *minFeret* and *aspect ratio* descriptors by instrument type
- The *p*-value for this set of instruments is <0.05: means of the individual datasets are different from the grand mean

Data analysis
Reproducibility: BAM1, NMIA, BAM2



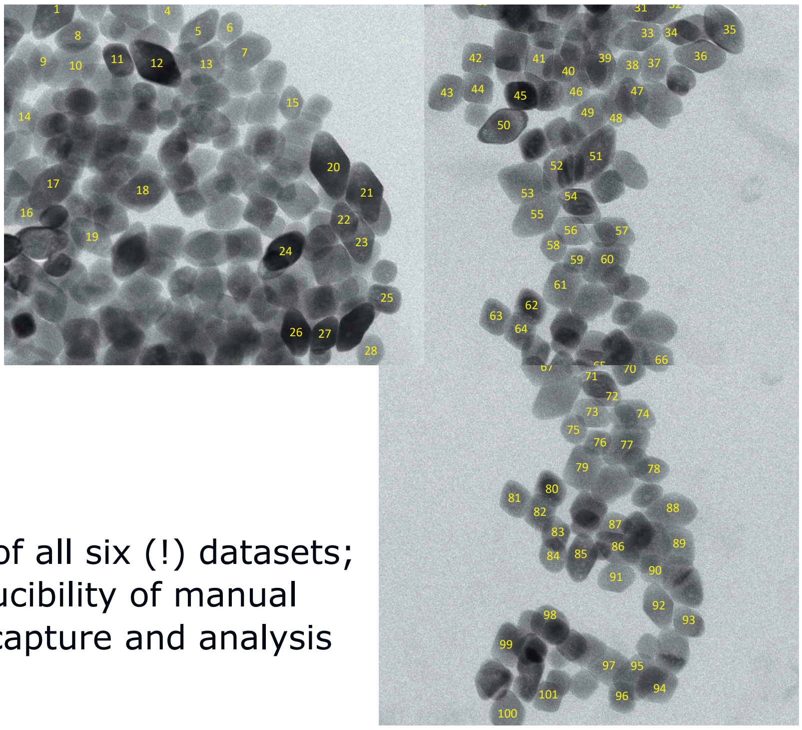




	ANOVA *p*-values for sequential tests on same TEM grid		
Descriptor	**Lab 1a vs Lab 2**	**Lab 2 vs Lab 1b**	**Lab 1a vs Lab 1b**
Size			
Area	0.220	2e-06	2e-06
Perimeter	1e-06	1e-06	1e-06
Feret	0.002	2e-06	2e-06
minFeret	0.009	2e-06	2e-06
Elongational shape			
Aspect ratio	0.780	2e-06	2e-06

Data analysis - Reproducibility of NP datasets taken on same images

Descriptor	ANOVA *p*-values	Rank order
Size		
Area	0.802	4
Feret	0.316	7
minFeret	0.165	8
Perimeter	0.621	5
ECD	0.560	6
EPD	0.165	8
Elongational shape		
Aspect ratio	0.969	**1**
Roundness	0.826	**3**
Compactness	0.830	**2**



- Excellent similarity of all six (!) datasets;
- implies high reproducibility of manual tracing + software capture and analysis

Conclusions and Outlook

1. Consistent results of 20 data sets have been reported
- TEM *vs* TSEM *vs* miniTEM
- Manual *vs* automated NP tracing
- Reproducibility: BAM1, NMIA, BAM2
- Reproducibility of datasets on same NPs/image

2. Report included in the case study of ISO/WD 21363 Nanotechnologies -- *Measurements of Particle Size and Shape Distribution by TEM*
- We can measure and analyse in compliance with ISO

3. Bipy TiO_2 NPs: suited as nano-CRM for size and shape
- *'…Clearly, the data reproducibility is high, supporting the concept that this sample could be certified for both size and shape descriptors….'*
- Enough material and ist prep?: new EMPIR *nPSize* project

4. Step II: distribution of TiO_2 NP suspension for prep on TEM grid in each lab acc. to same protocol + measurement & data analysis as in step I
- Comparison with step I and later inclusion into ISO standard

17NRM04 nPSize - Improved Traceability Chain of Nanoparticle Size Measurements

EMPIR

Vasile-Dan Hodoroaba (BAM) and Harald Bosse (PTB)

CONSORTIUM

NEEDS
- **Reliable analysis** of nanoparticle size and **comparable results** of physically different methods for:
  - Nanoparticle manufacturers
  - Service/accredited laboratories
  - Analytical instrument makers
- EC mandate M/409: *'…utmost importance to translate European excellence in nanoscience into safe and commercially marketable products and processes'*
- EC mandate M/461: *'…requires to develop standards activities regarding nanotechnologies and nanomaterials …for safe, integrated and responsible use of nanomaterials…'*
- **Regulatory** requirements/classification of nanomaterials acc. to EC definition (2011/696/EU)

OBJECTIVES
- Establish EU capability of traceable measurement of NP size and shape
- Lower uncertainties of NP size measurement by:
  - new nano-CRMs,
  - new models (physical and machine learning),
  - 3D method combination
- New ISO and CEN standards on accurate NP size measurement
- Guidance and knowledge transfer

WP1 Performance and traceability of Characterization Methods
- Assesment of method performance of traceable sizing methods EM, SAXS and AFM on NP size, shape, polydispersity and number concentration
  - current state
  - with new, dedicated RMs (developed in WP2) and models (WP3)
- Public data library for WP3
- Steps to extend traceability from spherical to non-spherical NP shapes

WP2 Reference materials - Preparation and Characterization
- Analysis of requirements (WP1 & 4)
- Development of well-defined non-spherical shapes: cubes, platelets, bipyramids, rods/acicular
- mono- and polydisperse
- accurate particle number concentration (SAXS and isotopically enrichment for ICP-MS)
- Re-validation loop via improved WP3 measurement procedures

WP3 Modelling and development of measuring procedures
- Physical modelling (TSEM, SEM, 3D-AFM, SAXS)
- Machine learning modeling from a-priori measurement data
- Physical model input into machine learning
- Data fusion for hybrid sizing techniques: SEM & TSEM/TEM, SEM/TSEM & AFM, SEM/TSEM & SAXS (with WP1)

WP4 CREATING IMPACT
- ISO/TC 229 'Nanotechnologies'/JWG 2 'Measurement & Characterization' (SEM, TSEM, TEM)
- ISO/TC 24/ SC 4 'Particle characterization' (SAXS)
- ISO/TC 201/SC 9 'Scanning probe microscopy' (AFM)
- CEN/TC 352 'Nanotechnologies'/ WP1 'Measurement, characterization and performance evaluation'
- **Dedicated workshops** (EM, SAXS, AFM) for end-users
- **New nano-CRMs** candidates
- **Public data library** with tagged measurement data
- **Signal modeling** and **machine learning** procedures published

BEYOND STATE-OF-THE-ART
- Improved nanoparticle size traceability chain by:
  - Reduction of method- and material dependent discrepancies
  - Estimation of the influence of particle shape and size distribution
  - More accurate dimensional characterization of nanoparticles by combining results of different methods
- Standardization: Launching European standardization projects on nanoparticle size and shape in line with ISO

Acknowledgement

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ISO/TC 229/ JWG 2 *Measurement and Characterization*

ISO/TC 229
Nanotechnologies

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CD Chan (DuPont)
J Zheng (FDA)
V Coleman, J Hermann (NMIA)

Reference methods for the determination of the nanoparticle mass- and number-based concentration using ICP-MS

D. Bartczak, S. Nunez and H. Goenaga-Infante, *LGC Limited*

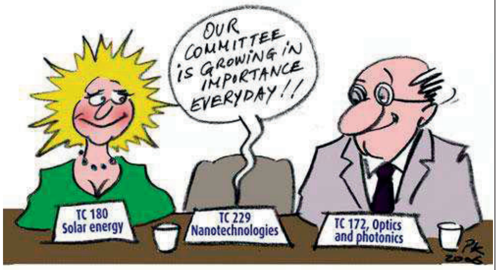
15 May 2018

Outline

- Introduction: Increasing use of nanotechnology and need for reliable particle mass and number-based characterization
- Application 1: SI traceable *spICPMS* methodology for the determination of *number based concentration* of colloidal Au NPs
- Application 2: size-specific *AF4/IDMS* for the quantification of the nanoscale element *mass fraction* in complex food matrix
- Summary

Need for reliable characterisation of NM

- **Nanotechnology** is increasingly being used to overcome scientific, commercial and industrial challenges
- **Nanoparticles** are incorporated in vast number of products, including cosmetics, medicines and food additives
- However to date…

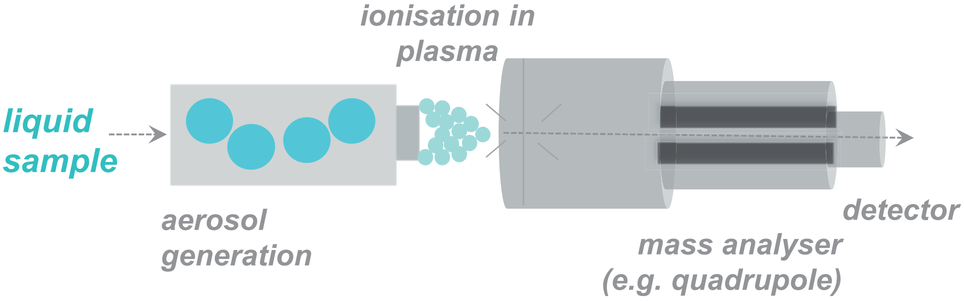


http://www.iso.org/iso/nanotech-2.jpg

there are no metrologically validated methods to measure mass and number concentration of particle fraction in colloidal suspension

- Validated methods will help the industry
  - ✓ to ensure the quality and efficacy of commercial products
  - ✓ to comply with the emerging regulation (e.g. food *1169/2011* and cosmetic *223/2009*)

Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) principle



ionisation in plasma

liquid sample

aerosol generation

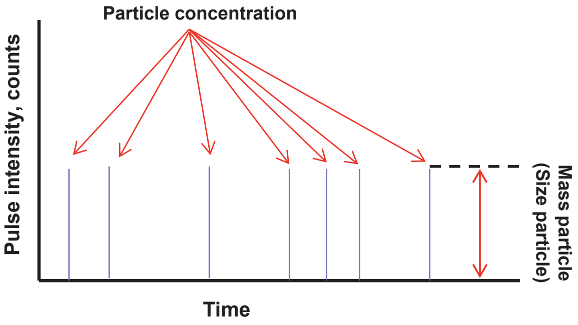
detector

mass analyser (e.g. quadrupole)

- Fine aerosol of a sample is introduced into hot (6000 - 10000 K) Ar plasma
- Plasma is of sufficient energy to dry the aerosol and form analyte atoms, which are simultaneously ionised
- Ions are then sorted and quantified based on their mass-to-charge (*m/z*) ratio

Application 1: spICP-MS as an attractive technique for size and number-based concentration determination

- Measurement of the signal intensity produced by a single particle
- Each transient peak or pulse represents a single particle event
- Samples are very diluted so that only a single particle reaches the detector (*less matrix effects*)



signal Intensity → particle mass → ***particle size***

$$d_p = \sqrt[3]{\frac{6m_p}{\pi\rho_p}\cdot 10^4}$$

signal frequency (number of pulses) → ***particle concentration***

, where:

d_p – particle diameter

m_p – particle mass

ρ_p – particle density

- Also from baseline signal information about the dissolved ions

---

Application 1: Particle number-based concentration with spICP-MS

Key parameters to consider:

Number-based concentration →

$$C(\mathrm{NP/ml}) = \frac{Df\cdot N}{\eta\cdot V\cdot T}$$

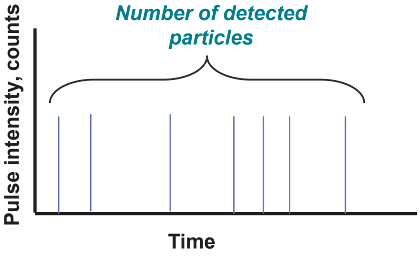
Sample dilution

Number of detected particles

Acquisition time (min)

Sample uptake (ml/min)

Transport efficiency



Use of Reference Material

Waste collection approach

Use of a balance to measure the amount of sample going into the ICPMS

Application 1: *Revisited* waste collection approach vs. frequency method

- Approach based on the **revisited waste collection** method:
  - ✓ Balance used to measure nebuliser and uptake flows

$$\eta = \frac{Slope\ of\ the\ nebuliser\ flow}{Slope\ of\ the\ uptake\ flow}$$

  - ✓ Traceable to the SI Kg, unit 1(Si brochure, 8th ed.)
  - ✓ Does not require material pre-characterisation

- Approach based on the **particle frequency method**:
  - ✓ In the absence of certified particle number value, the following particle characteristics are considered:

diameter *(TEM, certified)*

density *(for gold might be taken as bulk)*

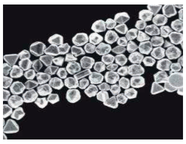
$$\eta = \frac{N \cdot 4 \cdot d^3 \cdot \rho \cdot \pi}{C_m \cdot 3 \cdot 8 \cdot m}$$

mass concentration *(measured in-house)*

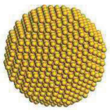
sample uptake *(measured)*

  - ✓ Requires characterisation of the above parameters to ensure traceability!

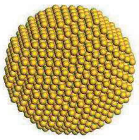
Application 1: Performance comparison of the two approaches



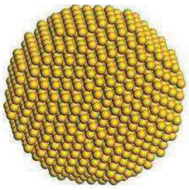
Material: Au NPs



30 nm



60 nm



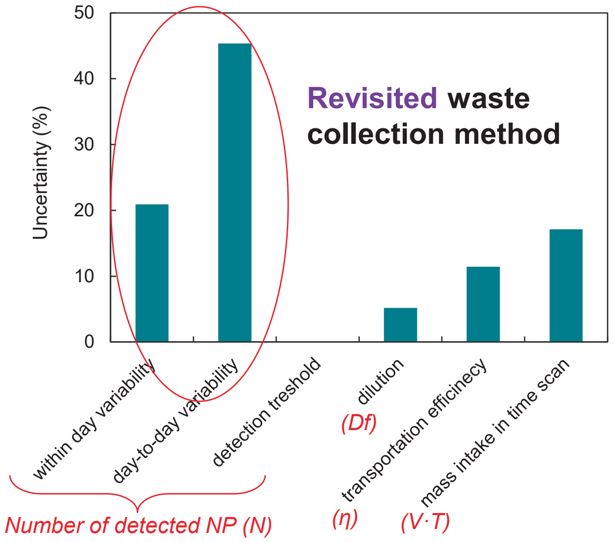
100 nm

	Expected* values ↓	**Revisited waste collection method** ↓	**Frequency method** ↓
Sample	**Particles/kg (~15 % U)**	**Particles/kg (Mean ± U, k=2) (RSU, %)**	**Particles/kg (Mean ± U, k=2) (RSU, %)**
30nm Au NPs	$1.8 \cdot 10^{14}$	$(1.8 \pm 0.2) \cdot 10^{14}$ (9%)	$(1.9 \pm 0.2) \cdot 10^{14}$ (12%)
60nm Au NPs	$2.9 \cdot 10^{13}$	$(2.8 \pm 0.3) \cdot 10^{13}$ (10%)	$(2.9 \pm 0.4) \cdot 10^{13}$ (13%)
100nm Au NPs	$4.2 \cdot 10^{12}$	$(4.1 \pm 0.3) \cdot 10^{12}$ (7%)	$(4.4 \pm 0.4) \cdot 10^{12}$ (10%)

*Calculated using TEM size and mass concentration measured with bracketing exact matching approach with internal standard

- The two methods agreed very well within the uncertainty and with the expected values
- Relative expanded uncertainties for the revisited waste method were between 7-10%
- Relative expanded uncertainties for the frequency method were between 10-13%

Application 1: Uncertainty budget



- Uncertainty budget for the two methods is different
- Main contributing factors in the **revisited waste collection method** is the variability in the number of detected NPs (*N*)

Nunez, Bartczak, Goenaga-Infante *manuscript in preparation*

Application 1: Summary

- Methodology for SI traceable determination of NP number concentration with spICPMS was developed by revisiting the waste collection approach
- The impact of key parameters on the accuracy and uncertainty of the number concentration data was evaluated, obtaining RSU (k=2) <10%
- Key parameter contributing to the overall uncertainty budget was variability in the number of detected NPs (*N*)
- Obtained results for 30nm, 60nm and 100nm Au NPs using the methodology proposed here were in agreement with the particle frequency method and expected particle number concentration

Currently…

- Applicability of the revisited waste method to more complex samples, including non-spherical or less amenable to spICPMS (e.g. Si, Ti-based)
- **CCQM** Intercomparison at the NMI level for the determination of number-based concentration of colloidal AuNPs (coordinator: Heidi Goenaga-Infante LGC, UK)
- Parallel **VAMAS** study open to all interested participants(Coordinator: Caterina Minelli NPL, UK)

Reference materials from these parallel VAMAS and CCQM studies will become available soon

Application 2: Analytical techniques for characterisation of NM in complex liquid matrices

- There are several analytical techniques available…

*…However, they are not easily or directly compatible with highly complex matrices, like **biological serum, food or cosmetics***

- Majority require sample pre-cleaning or chemical processing affecting NP *(i.e. induced aggregation)…*

Chemical pre-treatment

complex sample

aggregation

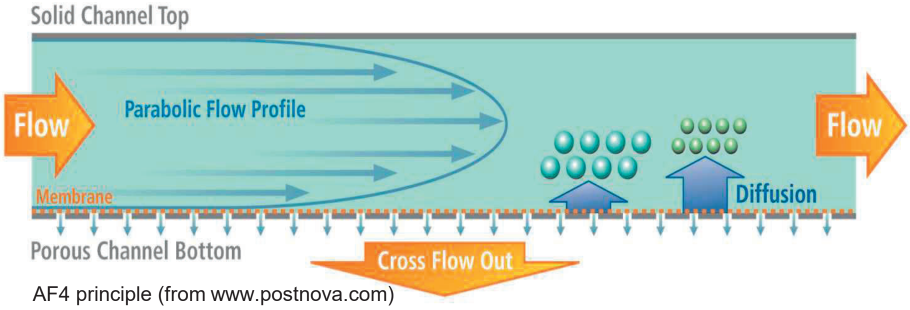
*…Therefore, **asymmetric flow filed-flow fractionation** (AF4) based methods have gained popularity*

- AF4 offers gentle separation – reduction of sample carry over and suitability to complex targets such as proteins, labile colloids and nanomaterials

---

Application 2: Advantages of coupling AF4 and ICP-MS

AF4 coupled to ICP-MS has emerged as an efficient method for the characterisation of **NMs in suspensions** as it:

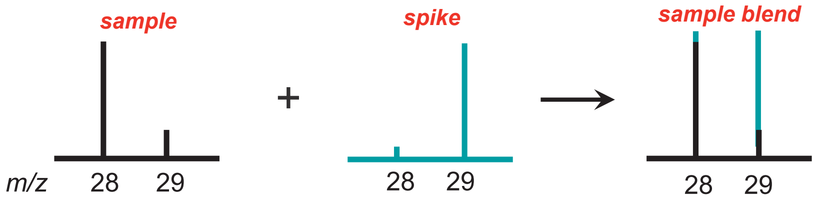


AF4 principle (from www.postnova.com)

- Offers quantitative information on chemical composition across the size distribution of particulate fraction (size-based elemental speciation)
- ICP-MS detection offers excellent LODs and multi-element capability
- Provides atomic ratio distributions
- Typical flow rates are very similar for both instruments

Application 2: Single IDMS strategy for characterisation of silica in food

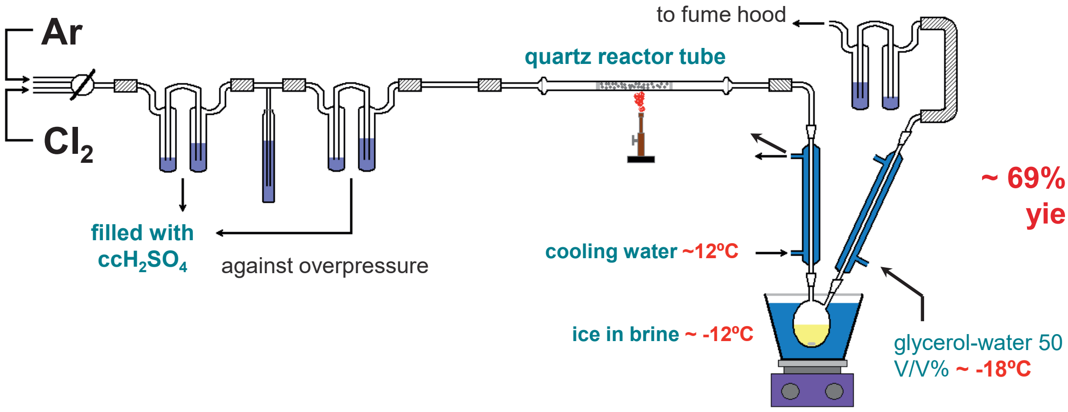
- Isotopically enriched analogue (**spike**) of the analyte element is added to a known mass of sample to form **sample blend**
- Sample blend is allowed to **equilibrate**
- **Ratio of two isotopes** in the sample blend is measured using ICPMS



- **Single IDMS** relies on accurate quantification of the **spike** (separate step)
- Measured ratio of isotopes requires correction for:
  - Mass bias
  - Detector linearity (if ratio is not 1)

Application 2: Synthesis of isotopically (^{29}Si) enriched silica nanoparticles (spike)

- ***Step 1***: synthesis of ^{29}Si enriched precursor (TEOS)



~ 69% total yield

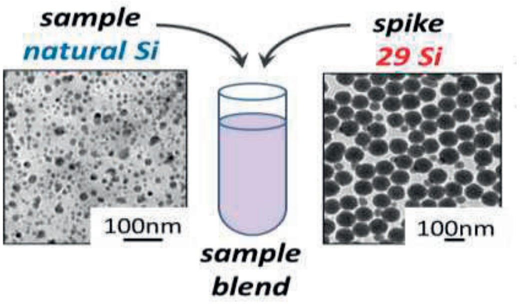
- ***Step 2***: synthesis of ^{29}Si enriched silica *via* Stöber method

$$\mathrm{Si(OEt)_4} \xrightarrow[25^{\circ}\mathrm{C}\text{, } 24\ \mathrm{h}]{25\%\ \mathrm{NH_3}} \left[\mathrm{SiO_2}\right]_n$$

Pálmai, Szalay, Bartczak, Varga, Nagy, Gollwitzer, Krumrey, Goenaga-Infante *J Colloid Interface Sci* **2015**, 445, 161-165

Application 2: characterisation of silica in food by IDMS in practice

*Silica in **coffee creamer** and **spike** are different in **size** and **density** but show similar behaviour in AF4 system in the presence of the **matrix***



Sample blend

coffee creamer (powder)
spike (suspension)
water

→

Equilibration

(2h mixing at room temp.)

→

Analysis with AF4/ICPMS

sample blend:
3 replicate injection

mass bias: in-between

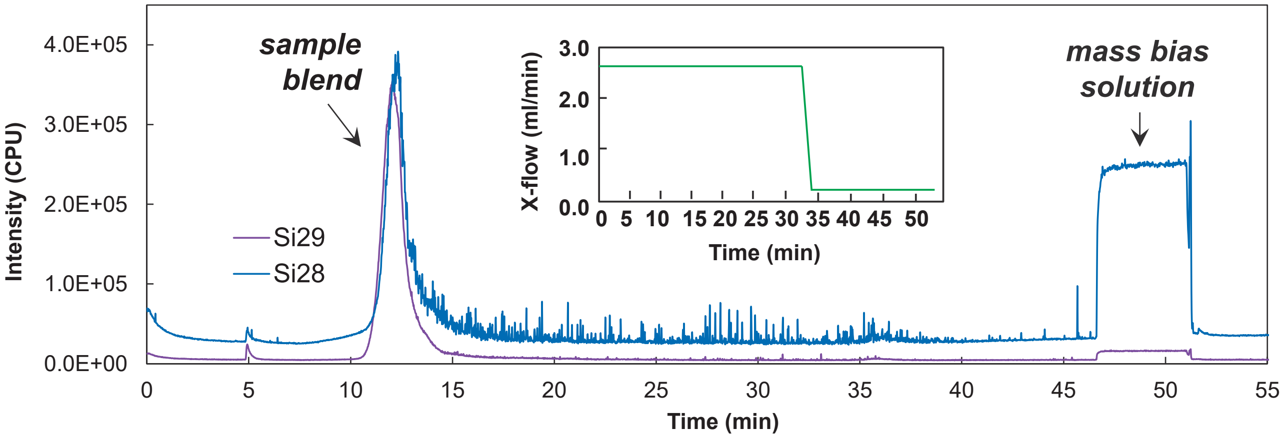
In a separate step analysis of spike:

- ✓ Si content quantification *via* total reverse IDMS
- ✓ Recovery from membrane in the AF4 estimated

Bartczak, Nunez, Pálmai, Hill, Petrov, Varga, Goenaga-Infante *manuscript in preparation*

Application 2: AF4/ICP-IDMS fractorgams of sample blend

- Typical fractogram of the **sample blend**:



- **AF4 conditions**:
  - ✓ membrane: 10 kDa regenerated cellulose
  - ✓ spacer: 500 µm
  - ✓ x-flow: 2.5 ml/min
  - ✓ carrier: ultra-pure water

Bartczak, Nunez, Pálmai, Hill, Petrov, Varga, Goenaga-Infante *manuscript in preparation*

Application 2: AF4/IDMS results

The amount of ***nanoscale Si*** *calculated from* ***IDMS equation***

$$C_X = C_Y \cdot \frac{m_Y}{m_X} \cdot \frac{W_X}{W_Y} \cdot \frac{A_Y^b}{A_X^a} \cdot \left(\frac{R'_B - R_Y}{1 - R'_B \cdot R_X} \right)$$

where: ***X*** – sample; ***Y*** – spike ; ***B*** – blend

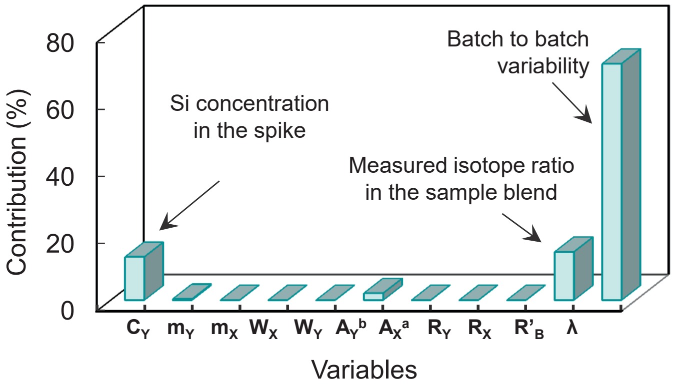
107.0 (4.3) mg/kg

mean (u), k=1

Uncertainty budget

(main contributing factors):

- ✓ Batch-to-batch variability
- ✓ Si concentration in the **spike** (*via reverse total IDMS*)
- ✓ Measured isotope ratio in **sample blend**



<u>Bartczak</u>, Nunez, Pálmai, Hill, Petrov, Varga, Goenaga-Infante *manuscript in preparation*

Application 2: Summary

With size-specific single AF4/IDMS it was possible to:

- Reliably characterise the mass content of **nanoscale silicon** in a complex ‘real life’ sample
- Relative measurement uncertainty of around **4%** (RSU, k=1) was achieved
- Major contributing factors to measurement uncertainty were: **spike** concentration, **isotope ratio** in the sample blend, blend-to-blend **variability**

ACKNOWLEDGEMENTS

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Department for Business Innovation & Skills

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IN NANO PART
Innovative Nanoparticle Metrology

Thank you
for your attention!

GERMAN FEDERAL INSTITUTE
FOR RISK ASSESSMENT

Bundesinstitut für Risikobewertung

Can labelled nanoparticles as internal standards improve the reliability of the quantitative analysis of nanomaterials in complex matrices?

S. Weigel, C. Cascio (ex-RIKILT, now BfR, EFSA)
Laura-Jayne Ellis, E. Valsami-Jones (Univ. of Birmingham)
A. Auger (CEA)

Quantification of NM in complex matrices

- Biological compartments, test media
  - dose determination
  - exposure
  - toxicokineteics
- Consumer products: Cosmetics, food, food contact materials
  - content, levels
  - regulatory purposes: labelling, maximum levels
  - exposure
- Environmental matrices
  - content, levels
  - ecotoxicology

Quantification of NM in complex matrices

- Challenges along the analytical process

sampling → conservation, processing → sample preparation → measurement → evaluation

  - losses (adsorption, agglomeration, aggregation, transformation)
  - incomplete, varying extraction efficiencies
  - matrix effects
- Result: unreliable detection and quantification

Improvements in accuracy (trueness, precision)

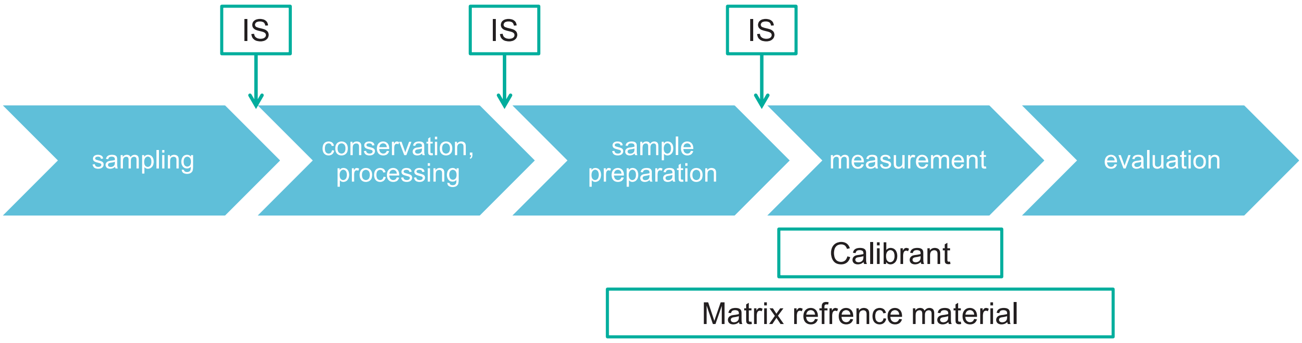
- Optimisation of process
  - sample preparation (extraction, clean-up)

- Use of (certified) matrix reference materials (CRM)
  - limited availability
  - validation of process/method, not individual sample
  - very specific for one matrix, related cases may not be covered (e.g. meat: chicken ≠ turkey)

- Use of internal standards
  - added to sample
  - compensates for losses, matrix effects

Internal standards

- Compensation for
  - sample volume (evaporation, dilution, splitting)
  - analyte losses
  - matrix effects



IS

IS

IS

sampling

conservation, processing

sample preparation

measurement

evaluation

Calibrant

Matrix refrence material

Internal standards

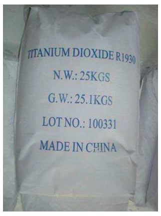
- Requirements
  - same behaviour as target analyte
    - log Kow
    - reactivity
  - possibility to be distinguished from analyte, matrix
    - mass
    - spectra
    - retention times
- Labels
  - different chemical structure, same behaviour (e.g log K_{ow})
  - chemical label (e.g. additional $–CH_3$ group)
  - isotopic label
    - stable isotopes (e.g. ^{13}C, ^{15}N, $^{2}H/D$)
    - radioisotopes

Internal standards for nanomaterials

- Requirements
  - „same behaviour as target ~~analyte~~ particles“
    - size, shape
    - surface properties
      - chemistry
      - zeta potential
    - density
  - possibility to be distinguished from analyte, matrix
- Labels
  - tags on surface (e.g. organic molecules, fluorescence)
  - tags inside
    - core-shell (shell = target NP, core = tag)
    - doping (same structure as target NP, but doped with n % of tag)

NanoDefine

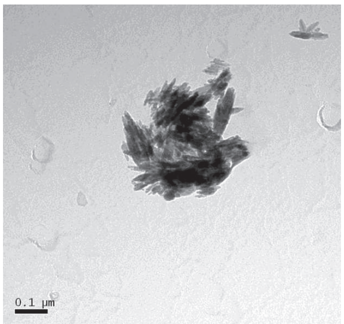
- Titania
  - used in food (food additive E 171, pigment grade ~ 200 nm)
  - very frequently used in sun screen (nano grade, ~ 40 nm)



- Silica
  - frequently used in food (food additive E 551)
  - many other applications (but different sizes, shapes, functionalisations etc.)

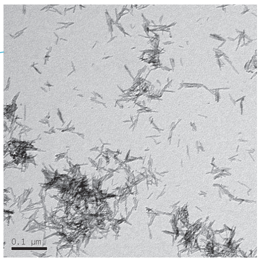
NanoDefine: Titania (U Birmingham)

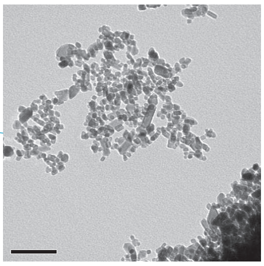
- Analysis of a commercial sun screen product
  - Particulate content:
    - TiO_2 (nano)
    - TiO_2 (pigment)
    - Fe-oxides (pigment)
  - Properties of TiO_2 (nano)
    - composition: TiO_2, alumina, stearic acid
    - rutile phase
    - rod shape
    - size (TEM) ~ 65 ± 25 nm

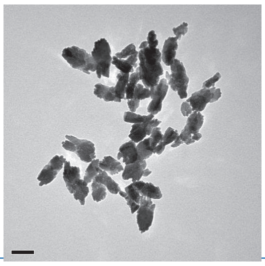


NanoDefine: Titania (U Birmingham)

- Synthesised particles
  - TiO_2 with alumina coating
    - rod shape, 40 nm (similar to target NP)
  - Hf doped TiO_2 with surface coating
    (a: acetate; b: stearate)
    semi-sperical, a:30 x 12 nm; b: 20x8 nm
  - Zr doped TiO_2
    semi-sperical, 42 x 12 nm
  - Hf doped ZrO_2
    ellipsoid, 166 x 54 nm







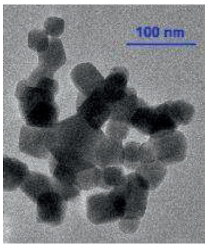
Ellis et al., 2018, RSOS, accepted

NanoDefine: Titania (U Birmingham)

- Challenges
  - synthesis route established to mimic size and shape of target NP did not work with dopants, results irreproducible + shape changed by Al and organic coating
  - other routes result in different shapes and sizes
  - low concentrations of dopants

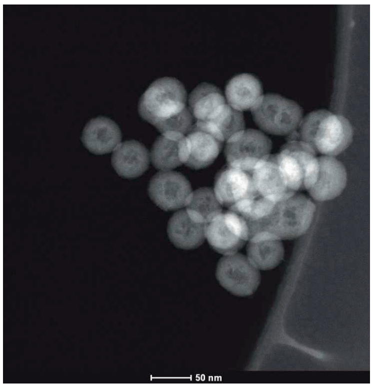
NanoDefine: Silica (CEA)

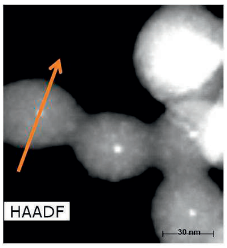
- Synthesised particles
  - In_2O_3
    - generic mimic of oxidic NP (SiO_2, Al_2O_3, TiO_2)?
    - cubic/sperical, 40 nm

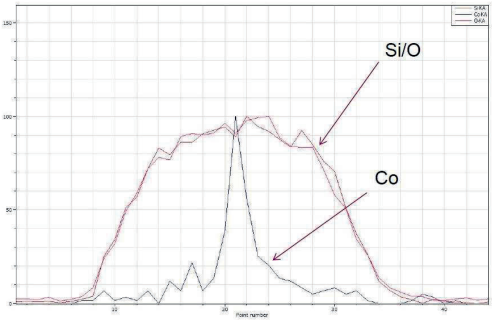


NanoDefine: Silica (CEA)

- Synthesised particles
  - SiO_2 shell, Co core
    - sperical, 50 ± 9 nm
    - core 17 ± 3 nm
    - shell width 16 ± 8 nm







Next steps

- Evaluation of the synthesised particles
  - determination of
    - recovery rates (in relation to target NP)
      - pure dispersions
      - relevant matrices
    - stability
    - repeatability, reproducibility
  - Joint exercise: RIKILT, DTU, BfR

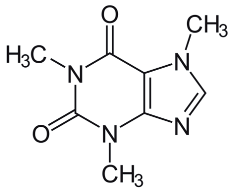
Specific challenges to NM internal standards

- Diversity of NM
  - Commercial UV absorbing TiO2

TTO NJE8	**JTTO MS7**	**UV Balance Powder 100 NJE8**	**TiO_2 TA-100**	**NanoDefine BAM 13B**
TiO_2, alumina, Jojoba esters	TiO_2, alumina, methicone	TiO_2, alumina, Jojoba esters	TiO_2, alumina, silica	TiO_2, alumina, stearic acid
rutile	rutile	rutile	rutile	rutile
rods	rods	sperical	sperical	rods
92 nm	79 nm	71 nm	40 nm	65 nm

Specific challenges to NM internal standards

- Diversity of NM
  - Conventional chemicals
    - one defined entity
      - e.g. caffeine, $C_8H_{10}N_4O_2$, M=194
        IS e.g. $^{12}C_5{}^{13}C_3H_{10}{}^{14}N_2{}^{15}N_2O_2$, M=199
  - Nanomaterials
    - many entities under one nominal name
      - e.g. TiO2
        1 chemical x 8 modifications x *n* shapes x *m* sizes x
        o size distributions x ...



Discussion

- Can labelled nanoparticles as internal standards improve the reliability of the quantitative analysis of nanomaterials in complex matrices?
- Under which conditions?
- Are there other means to achieve reliability in NM quantification?

GERMAN FEDERAL INSTITUTE FOR RISK ASSESSMENT

BfR
Bundesinstitut für Risikobewertung

Thank you for your attention

Stefan Weigel

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Poster Presentations
May 14

The Malta Initiative – A European Activity to Develop and Amend OECD Test Guidelines for the Testing of Nanomaterials

Elisabeth Heunisch[a], Volker Bachmann[a], Thomas A. J. Kuhlbusch[a], Kathrin Schwirn[b], Anke Jesse[c]

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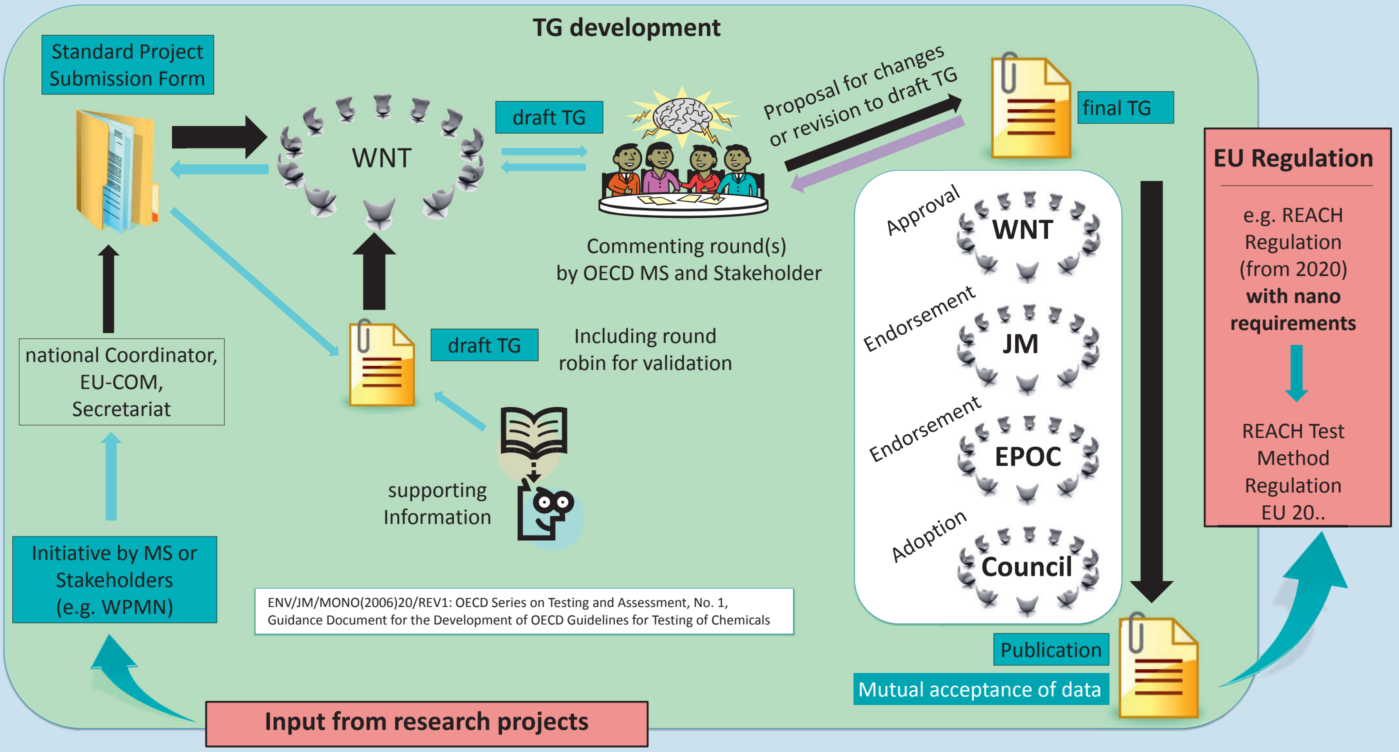
The REACH Committee has accepted nanomaterial specific changes to the REACH-Annexes proposed by the European Commission with effect of Jan. 1st, 2020. With these amendments to the annexes amongst others transparency in registration dossier and chemical safety report will be achieved and characterization and the information requirements for nanomaterial will be complemented. However validated, standardized and harmonized test methods for nanomaterials are lacking for several endpoints. Therefore the Malta Initiative was started as a common activity of the European Commission and Member States (MS) to work on filling these gaps. The purpose is to adapt existing or develop new OECD test guidelines and guidance documents to be applicable for nanomaterials as the guidelines are mutually accepted by all OECD members worldwide.

The way from the idea to the TG and its application in (EU) Regulation



ENV/JM/MONO(2006)20/REV1: OECD Series on Testing and Assessment, No. 1,
Guidance Document for the Development of OECD Guidelines for Testing of Chemicals

Endpoints currently covered by the Malta Initiative

Area	Endpoint in REACH	Existing Test Guideline or Standard	Content related EU Research projects	Activities within Malta Initiative
Pchem	Annex VII 7.7 Water solubility	OECD TG105 - considered not to be applicable for nanomaterials	NanoFASE, NanoREG, NanoREG2, Prosafe, ACENANO, CERASAFE, GRACIOUS, HISENTS, NanoFARM, PATROLS, SKHINCAP, NanoMile, SUN	DK - Determination of solubility and dissolution rate of nanomaterials in water and relevant synthetic biologically mediums
	Annex VI 2.4.3. Description of surface functionalization		NanoREG, QualityNANO, ACENano, Solnanotox, GRACIOUS, CERASAFE, HISENTS, npSCOPE, PATROLS	DK - Identification and quantification of the surface chemistry and coatings on nano- and microscale materials
	Annex VII 7.14 Dustiness	*CEN TC137* *VDI 2263 Part 9* *EN 60079 Part 10-2*	Calibrate, CERASAFE, EC4SafeNano, GRACIOUS, NanoReg2,	FR - New TG on Determination of the Dustiness of Manufactured Nanomaterials
	Annex VI 2.4.5. Surface area	*ISO 9277:2010* *CEN/TS 17010:2016*	ACENANO, CERASAFE, npSCOPE	JRC - New TG on Determination of the Specific Surface Area of Manufactured Nanomaterials
ENV	Annex IX 9.3 Fate and behaviour in the environment	OECD TG305 - additional GD for nanomaterials needed	NanoFase	ES - Studies on bioaccumulation of nanomaterials in fish
	Annex VII 9.2 Degradation	OECD TG301 / 306 / 307 / 308 / 309 / 310 / 311 - in many cases not relevant for nanomaterials	NanoFASE , ACENANO, GRACIOUS, NanoFARM, NanoMile	AT - Aquatic (Environmental) Transformation of Nanomaterials
HH	Annex VIII 8.8.1 Assessment of the toxicokinetic behaviour	OECD TG417 / 428 - amendments for nanomaterials required ISO 10993-16	NanoREG, HISENTS, MARINA	NL - Development of new Test Guideline on toxicokinetics or Amendments to OECD TG 417 to accommodate nanomaterials
	Annex VII 8.1 Skin irritation or skin corrosion - *in vitro* 8.3 Skin sensitization - i*n vivo*	OECD TG430 / 431 / 435 / 439 – not considered applicable for nanomaterials OECD in vivo local lymph node assays	SKHINCAP	CH - Applicability of the TG 442D in vitro skin sensitisation for nanomaterials

Umwelt Bundesamt

Federal Ministry
for the Environment, Nature Conservation
and Nuclear Safety

baua:

Quantification of Silver Nanoparticles at Single Cell Level by Mass Cytometry

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[1]Bundesanstalt für Materialforschung und –prüfung (BAM), [2]Bundesanstalt für Risikobewertung (BfR); [3]Deutsches Rheumaforschungszentrum, Ein Leibniz Institut, [1,2,3]Berlin, Germany

Introduction

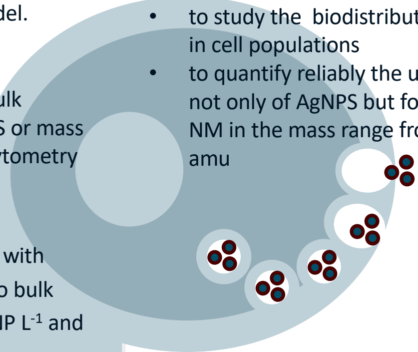
Nano material (NM) is increasingly used in many different products and applications. Thus, potential human exposure is increasing and therefore there is an urgent need to develop robust analytical methods that allow for precise and fast quantification of NM uptake and even can provide information on the level of individual cells.

In this research, mass cytometry was used to introduce a new quantitative approach for nanosilver by performing an external calibration using a silver nanoparticle (AgNPs) suspension to study the uptake of 50 nm AgNPs by individual THP-1 macrophages as a cell model.

Conclusion

In comparison to conventional bulk analysis of digested cells (ICP-MS or mass cytometry liquid mode), mass cytometry in single cell mode enables

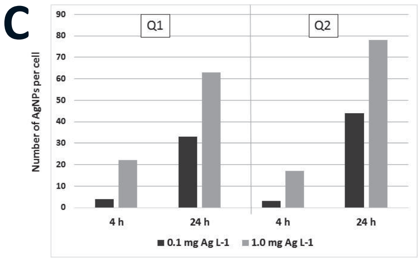
- phenotyping of single cells by using specific metal-labelled antibodies
- to distinguish between non-cell and cell associated AgNPs
- to study the biodistribution of AgNPs in cell populations
- to quantify reliably the uptake per cell not only of AgNPS but for potential NM in the mass range from 75 to 209 amu



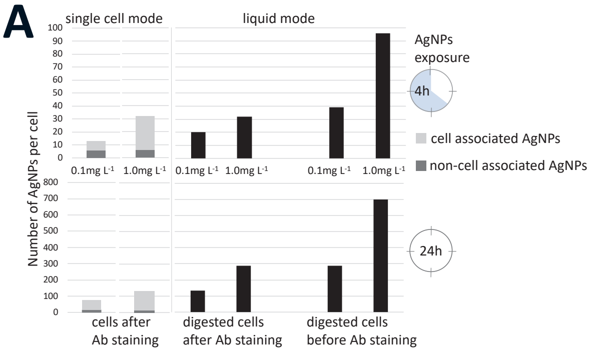
Results

- Our new quantitative approach by measuring cells and calibrating the CyTOF with AgNPs revealed similar results at 4h AgNPs exposure time when comparing to bulk analysis of digested cells by including free AgNPs: 13 vs 20 NPs at 0.1 mg AgNP L^{-1} and 32 vs 32 NPs at 1.0 mg AgNP L^{-1} (Figure A).
- At higher exposure time of 24h the results differ by a factor 2 suggesting an increasing saturation of the detector in single cell mode (Figure A).
- Decreasing numbers of AgNPs after Ab staining could be referred to loosely attached AgNPs to the cell surface that were washed off during staining procedure (Figure A).
- Two distinct phenotypes of THP-1 macrophages show different AgNPs distribution patterns depending on AgNPs exposure time and doses (Figure B) but the mean number of AgNPs per cell was very similar (Figure C).

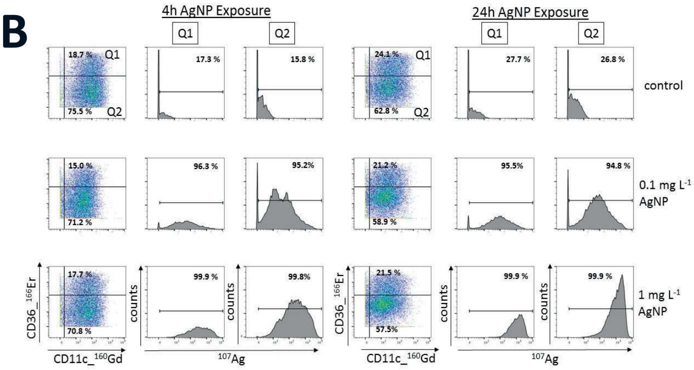
C



A



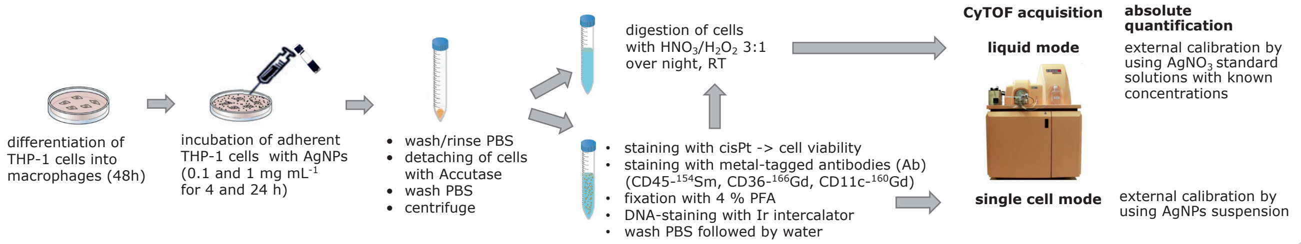
B



Differentiated THP-1 cells exposed to 0.1 and 1.0 mg AgNPs L^{-1} for 4 and 24 h: **Figure A**: Number of AgNPs per cell obtained from two quantitative approaches: single cell mode versus conventional ICP-MS method operating CyTOF in liquid mode. ^{107}Ag intensities were measured on single cells applying minimal pre-gating in order to compare results to the bulk analysis of digested cells before and after Ab staining. **Figure B**: Dot plots of THP-1 macrophages show two distinct cell populations (Q1: $CD36^{+}CD11c^{+}$ and Q2: $CD36^{-}CD11c^{+}$). ^{107}Ag intensities are illustrated in histograms. **Figure C**: Bar diagram shows the number of AgNPs per cell of Q1 and Q2.

Methods

Cell assay and CyTOF measurement of single and digested cells for quantification of AgNPs per single cell



differentiation of THP-1 cells into macrophages (48h)

incubation of adherent THP-1 cells with AgNPs (0.1 and 1 mg mL^{-1} for 4 and 24 h)

- wash/rinse PBS
- detaching of cells with Accutase
- wash PBS
- centrifuge

digestion of cells with HNO_3/H_2O_2 3:1 over night, RT

- staining with cisPt -> cell viability
- staining with metal-tagged antibodies (Ab) (CD45-^{154}Sm, CD36-^{166}Gd, CD11c-^{160}Gd)
- fixation with 4 % PFA
- DNA-staining with Ir intercalator
- wash PBS followed by water

CyTOF acquisition

liquid mode

single cell mode

absolute quantification

external calibration by using $AgNO_3$ standard solutions with known concentrations

external calibration by using AgNPs suspension

Fundings: EU H2020-MSCA-IF-2014-654496, BfR AZ1329-539

OECD Test Guidelines Development for Chemicals Safety Assessment of Nanomaterials

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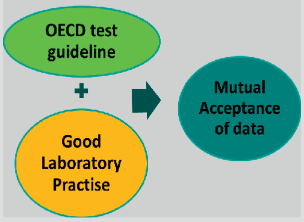
The OECD test guidelines (TGs) for testing chemicals have been widely used for regulatory purposes all over the world since the establishment of the Mutual Acceptance of Data (MAD) principle in 1984. This MAD principle ensures that, if a chemical is tested under the Good Laboratory Practice (GLP) conditions accordingly to an OECD TG, the data should be accepted in all OECD countries. The TGs have been developed, harmonized, internationally validated (round robin tests) and adopted by OECD countries to be used for the physical-chemical characterisation, fate estimation, and hazard identification for risk assessment of various chemicals. In addition to the TGs, OECD Guidance Documents (GDs) usually provide guidance on how to use TGs and how to interpret the results. These GDs do not have to be fully experimentally validated, and hence they are not under MAD, but they are based on relevant published scientific research.

But are the existing TGs and the related GDs applicable and adequate for the regulatory testing of nanomaterials? In general, for nanomaterials it is accepted that most of the "endpoints" or more precisely measurement variables are applicable. However, for some endpoints new or amended TGs are needed. In addition, several GDs are needed to give more precise advice on the test performance in order to gain regulatory relevant data on nanomaterials.

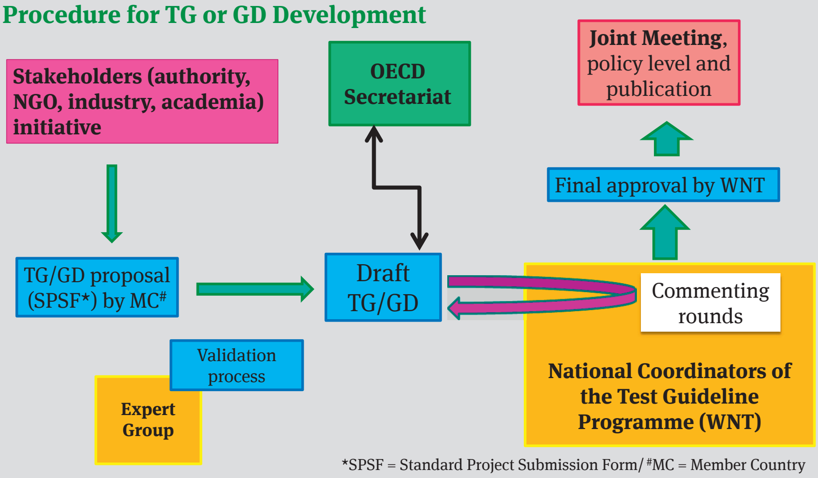
Why do we need OECD TGs ?



- *For testing intrinsic properties of chemicals (substances)*
- *Binding OECD countries and selected non-members*

- *Avoid duplication of testing*
- *Reduction of animals testing*
- *160 million euros saved each year (2010)*
- *"easily" adopted to EU regulation (440/2008) for EU regulatory needs*

Procedure for TG or GD Development



*SPSF = Standard Project Submission Form/ #MC = Member Country

Difference between OECD TGs and GDs

Test Guideline	Guidance Document
Regulatory need explained by the SPSF*	Regulatory need
Covered by MAD	Not covered by MAD
Fixed test protocol with **validity criteria**	Can be a test method, or it provides technical guidance for the use of test guidelines
Thorough **experimental validation** needed with fixed protocol	Lower level of scientific validation needed, e.g. can be based on published resent literature
Takes time and resources and is more cumbersome to update	Faster to develop and revise

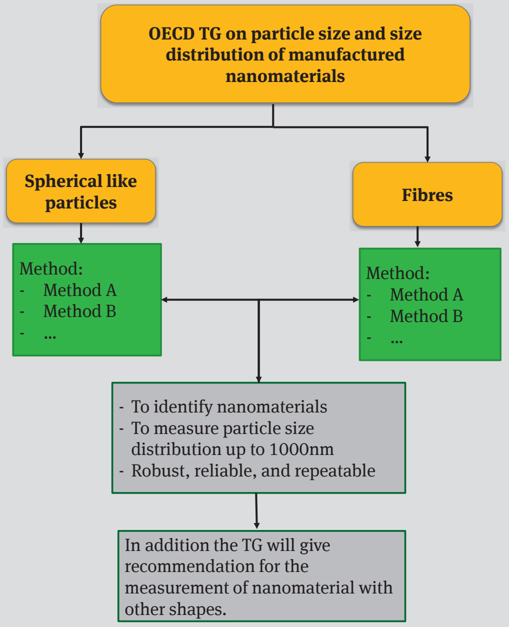
*SPSF = Standard Project Submission Form

List of current projects on TG development for the physical-chemical characterisation of nanomaterials approved by WNT

- TG on particle size and size distribution of manufactured nanomaterials (lead by Germany)
- TG on specific surface area of manufactured nanomaterials (lead by EU JRC)

The development of the TG on particles size and size distribution of manufactured nanomaterials is accompanied by an international advisory group taking into account recent progress in relevant European and international research projects as well as standardisation activities of ISO and CEN.

Development of a OECD TG on particle size and size distribution of manufactured nanomaterials



References:

OECD, 2009: GD 1 Guidance Document for the Development of OECD Guidelines for Testing of Chemicals (as revised in 2009).

OECD, 1998: OECD Series on principles of Good Laboratory Practice and compliance monitoring number 1.

OECD, 2005: GD 34 Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment.

Research and activities of UBA at: https://www.umweltbundesamt.de/en/topics/chemicals/nanotechnology

Umwelt Bundesamt

NanoGenotox - Automatable Determination of Genotoxicity of Nanoparticles with DNA-based Optical Assays

BAM
Bundesanstalt für
Materialforschung
und -prüfung

M. Wegmann[1], K. Hoffmann[1], M. Hannemann[2], V. Somma[2], T. Jochum[3], D. Roggenbuck[2], J. Niehaus[3], U. Resch-Genger[1]
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Introduction

The increasing use of nanomaterials and the continuous emergence of new nanoparticles call for efficient, preferably standardized test procedures to screen these materials for their potential cyctoxicity and genotoxicity.[1,2] Aiming at the development of fast and easy to use, automatable microscopic methods for genotoxicity testing of nanomaterials, we assess the feasibility of the fluorometric *γ-H2AX*[3] *assay* for this purpose, that detects DNA double strand breaks (DSB). Here, we provide first results of cell tests obtained with different nanoparticles, especially cadmium (Cd) containing semiconductor nanomaterials of different surface chemistry.

Synthesis and Modification

Cd-containing (CdSe/CdS) and Cd-free (Fe_2O_3, InP, Au) nanoparticles were produced in a continuous flow reactor by CAN GmbH, using apolar surface ligands. The surface of these nanoparticles is then modified to realize water dispersibility[4] (Fig. 1).

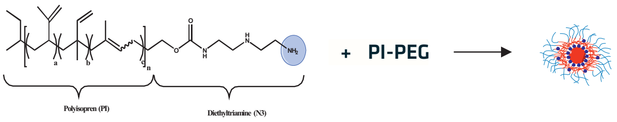

Figure 1
Micellar encapsulation of the particles to yield stable dispersions in water.

Characterization of the nanoparticles

Spectroscopic measurements (absorption spectra; for luminescent materials also photoluminescence (PL) spectra, PL quantum yields, PL decay kinetics) of the nanoparticles were performed at BAM to assess potential interferences with the fluorescent reporters used in the *γ-H2AX assay* and to monitor the longterm colloidal and chemical stability of the dispersed nanoparticles.

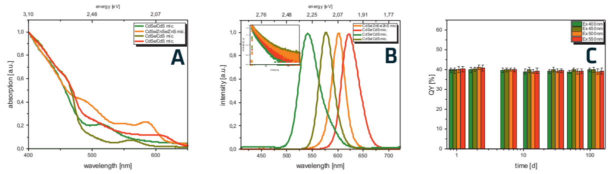

Figure 2
(A) Absorption spectra, (B) emission spectra and PL decays at different excitation wavelengths, and (C), longterm stability of the PL quantum yield of CdSe particles with an elongated CdS shell in water, respectively.

Quantification of functional groups on particle surfaces

The chemical nature and amount of functional groups on particle surfaces together with their shape and chemical composition can affect their genotoxicitiy. For surface group analysis, different methods like conductometry and optical assays using different reporters were employed. This enables to distinguish between amine groups that point to the encapsulated nanoparticles and amino groups that are located at the micelle surface. The latter should be accessible for the conjugation of the particles to biomolecules.

SCNC	Inorg. shell	Shape	Ligand (L)	Size by DLS [nm]	NP conc. (µM)	Ligand conc. (µM)
No (blank)		spherical	PI-N3/PI-PEG	20	5	5500
CdSe	-	spherical	PI-N3/PI-PEG	29	5	1720
CdSe	CdS	spherical	PI-N3/PI-PEG	29	5	1650
CdSe	CdS/ZnS	spherical	PI-N3/PI-PEG	55	5	1900
CdSe	CdS	elongated	PI-N3/PI-PEG	54	9	1100

Figure 3
Conductometric determination of the total number of amine groups..

Detection of DSB

DNA DSBs can be determined efficiently with the *γ-H2AX* assay. Immunfluorescence staining with a *γ-H2AX* antibody enables the visualization of DSB-related nuclear focis which can be read out with the automated AKLIDES® system.[5]

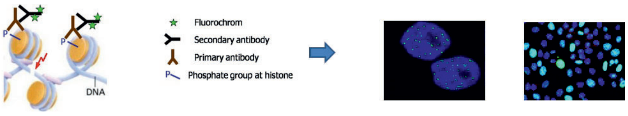

Figure 4
DSB recognition via the fluorometric γ-H2AX assay.

Different concentrations of the various nanoparticles were studied in Hep2 cells to identify suitable parameters for the automated DBS detection and quantification with the *γ-H2AX assay* and to establish assay-specific positive and negative standards.

Conclusion and Outlook

We developed a new detection method for genotoxicity screening of nanomaterials, based on the *γ-H2AX assay* and the automated microscopic platform AKLIDES®. Supported by the ongoing development of reference materials for instrument performance validation, this will provide the basis for better comparable and reliable results of genotoxicity studies.

References

(1) Landsiedel, R. et al., Reviews in Mutation Research 2009, 681, 241. (2) Henriksen-Lacey, M. et al., Bioconjugate Chem. 2016, 28, 212. (3) Willitzki, A. et al., Cytometry Part A 2013, 83, 1017. (4) Ostermann, J. et al., Beilstein journal of nanotechnology 2015, 6, 232. (5) Runge, R. et al., International journal of radiation biology, 2012, 88, 439.

Acknowledgements

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Bundesministerium für Bildung und Forschung

Physikalisch-Technische Bundesanstalt
National Metrology Institute

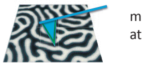
S. Sievers and H. W. Schumacher

Reference samples for Magnetic Force Microscopy

Spatially resolved magnetic field detection

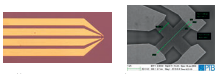
… on the micro- and nanoscale

- **with magnetic probes**
- **Magnetic Force Microscopy (MFM)**



magnetically coated tip
at lift height d

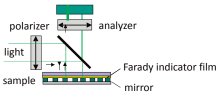
- **Scanning Hall Magnetometry**



[3]

Hall sensors on cantilevers

- **with magnetooptical indicator films (MOIF)**



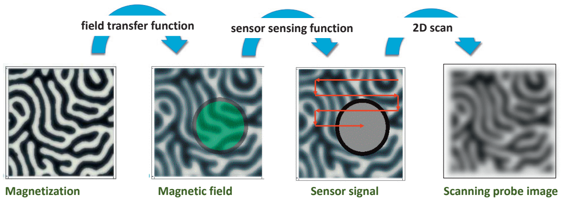
Motivation:

- To enable quantitative magnetic field measurements on scales from millimeters down nanometers
- To Support research and development in areas like magnetic data storage, magnetic sensing, magnetic scales for positioning systems
- To support fundamental physics, e.g. detection and analysis of Skyrmions

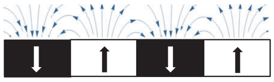
> Quantitative field measurements require sensor calibration based on nanoscale reference samples

Imaging principles of scanning magnetic probes

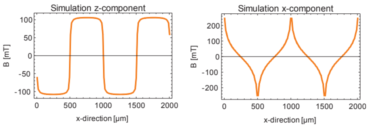
- Magnetic probes detect the magnetic stray field of magnetic nanostructures or domain patterns
- The signal is mapped with a sensor with a characteristic sensing function relating the field distribution over the active sensor area and the scalar sensor signal (Hall voltage, phase shift)
- The image results from a 2D scan that maps the sensor system as a function of sensor position



Nanomagnetic field characteristics



1) Stray field orientation varies on the scale of characteristic pattern dimensions
2) 3D field vectors with high field contributions in all spatial directions

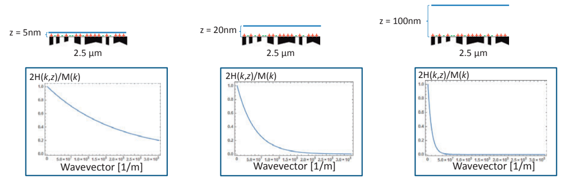


Example:
stray field distribution above a typical commercial magnetic scale
pole width: 1mm
z-distance: 6.725 µm

3) Rapid decay of stray field with distance from sample

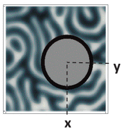
$$\boxed{H_z(\boldsymbol{k},z) = \frac{1}{2}M(\boldsymbol{k})\cdot e^{-kz}}$$

field transfer function for perpendicularly magnetized samples



Signal Generation in scanning magnetic microscopy

Deconvolution possible, if I) sensor response is linear with respect to the field and
II) is a weighted sum of contributions from different parts of sensor volume



$$Signal\,(x,y) \sim \sum^{SensorArea} H(x+x',y+y')\,S(x',y')\Delta x'\Delta y'$$

Correlation integral → Fourier transform [1]

$$\tilde{Signal}(k_x,k_x) \sim \breve{H}(k_x,k_x)\cdot\check{S}^*(k_x,k_x)$$

Sensor transfer function ***S***

$$\check{S}^*(k_x,k_x) = C\cdot\frac{\tilde{Signal}(k_x,k_x)}{\breve{H}(k_x,k_x)}$$

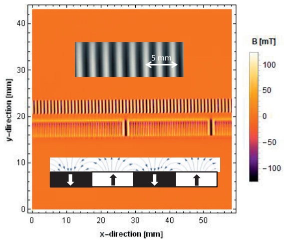
1) Sensor transfer function ***S*** can be determined from well known magnetic field distribution.

$$\breve{H}(k_x,k_x) = C\cdot\frac{\tilde{Signal}(k_x,k_x)}{\check{S}^*(k_x,k_x)}$$

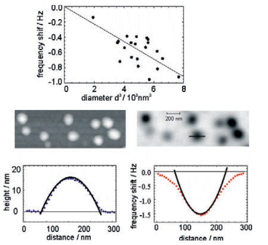
2) Stray field of arbitrary samples can be determined from the measured signal by a deconvolution with the known transfer function.

Reference samples for magnetic imaging

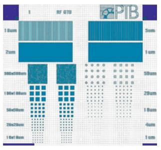
- Calculable stray field distribution, e.g.:
  - homogeneously magnetized spheres
  - perpendicularly magnetic materials with
    - lithographically defined patterns
    - intrinsic domain pattern or
    - written magnetic pattern
  - current carrying coils
- Magnetic field contributions over the spatial wavelength area relevant for the sample under test
- Well known features (domain wall width, edge properties, saturation magnetization, …)



MOIF image of a periodically up and down magnetized scale

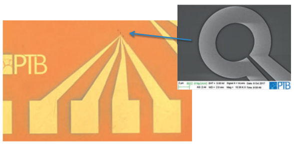


MFM images of magnetite magnetic nano-particles (MNP) poured onto a Si substrate [2]

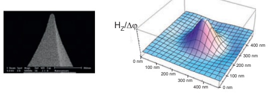


Litographically patterned hard magnetic thin films

2.5 µm



Current carrying coils



PMA films with maze domain pattern, MFM tip and its sensor transfer function

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[1] Hug, H. et al. (1998). Quantitative magnetic force microscopy on perpendicularly magnetized samples. Journal of Applied Physics. 83. 5609-5620. 10.1063/1.367412.
[2] Sievers, S. et al. (2012). Quantitative Measurement of the Magnetic Moment of Individual Magnetic Nanoparticles by Magnetic Force Microscopy. Small, 8: 2675-2679. doi:10.1002/smll.201200420
[3] M. Gerken, PTB AG 2.52 and A. Solignac, CEA, France

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Nanocrystals as Labeling Reagents for Bioimaging of Clinical Cell Assays

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Bundesanstalt für Materialforschung und -prüfung

Introduction

Early and sensitive detection of diseases like breast cancer is crucial for their optimal treatment. Clinically established Single Cell **Mass Cytometry** allows the sensitive **detection of several biomarkers at once** by measurements of cell suspensions using mass spectrometry (MS). [1] In comparison, laser ablation inductively coupled plasma **(LA-ICP) MS** works as a **bioimaging** tool for **tissue samples**, able to use the same metal affinity tagging reagents. Opposed to fluorescence immunoassays, this method might permit quantification of the analytes in the future.

Aim

This project aims at the **development of new labeling reagents** optimized for **multiparametric** analysis and bioconjugation to biomarker associated antibodies. **Nanocrystals (Eu:GdVO$_4$-PAA)** offer high stability, as well as sensitivity in MS allowing signal amplification compared to other tagging reagents available and give the option for **multimodal imaging** of single cells or tissues (e.g. fluorescence microscopy, magnetic resonance imaging (MRI) and LA-ICP-MS (Imaging Mass Cytometry).

Conclusion

- Eu:GdVO$_4$-PAA synthesis shows great reproducibility in composition and size
- Surface functionalization with poly(acrylic)acid (PAA) groups allows bioconjugation
- A549 cells could be efficiently labeled with the nanocrystals
- LA-ICP-MS proves the nanocrystals can be detected with high sensitivity and allow high spatial resolution analysis.

Acknowledgement

This work was funded by the by the MNPQ-program of the federal Ministry of Economic Affairs and Energy (BMWi) (MNPQ project 1176 07/13)

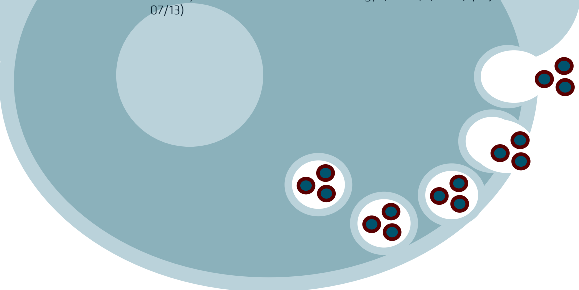


Figure 2: Concept of endocytosis

Nanocrystals might be enclosed within early endosomes, phagosomes or macropinosomes to be internalized by cells [2]

Results

Multimodality

- Nanocrystals demonstrate contrast enhancing properties in MRI with increasing Gd^{3+} concentrations + fluorescence by excitation with UV light due to doping with Eu^{3+} (Fig. 1)

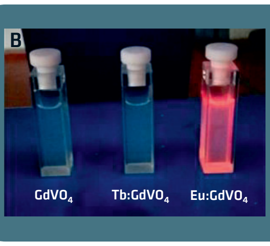
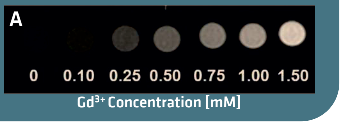


Figure 1:
(A) T1-weighted images of nanocrystals measured at 1 T.
(B) Fluorescence of nanocrystal solution with UV excitation (254 nm)



LA-ICP-MS

- Incubation of A549 cells with nanocrystals results in their uptake, possibly by endocytosis (Fig.2 and Fig.3)
- Spatial distribution of ^{158}Gd, ^{153}Eu and ^{51}V and ^{193}Ir signals measured by LA-ICP-MS correlate well, confirming that the cells were efficiently labeled with the nanocrystals (Fig.3). High intensities of Gd, Eu and V allows high spatial resolution (down to 4 µm * 4 µm/s was tested).

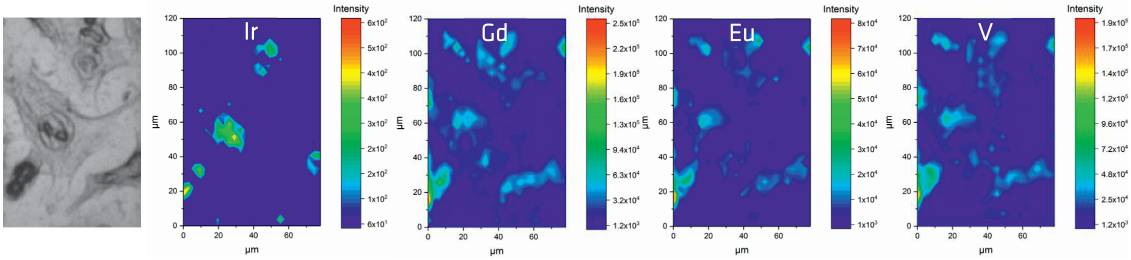


Figure 3: Microscopic image and intensity distribution maps of ^{158}Gd, ^{153}Eu, ^{51}V, and ^{193}Ir in A549 cells - LA-ICP-MS, resolution 6 µm * 6 µm/s

Methods

Nanocrystal Synthesis

- Synthesis of the poly(acrylic)acid (PAA) functionalized Eu:GdVO$_4$ nanocrystals was performed via solvothermal synthesis.

Magnetic Resonance Imaging

- MR images were obtained using a 1 T nanoScan PET/MRI (Mediso Medical Imaging Systems)
- Imaging experiments were performed with a T1-weighted sequence, as the contrast should be primarily enhanced by the T1-relaxation time.

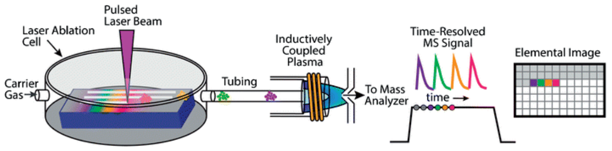


Figure 4: Imaging by laser ablation ICP-MS [3]

Cell assay and LA-ICP-MS analysis

- Human lung cancer cells (A549) were cultivated in Dulbecco´s Modified Eagle´s medium (10% FCS, 1% Penicillin) at 37°C with 5% CO$_2$
- Cells seeded on coverslips were incubated with nanocrystals for 6 h by addition of nanocrystals directly to fresh culture medium
- Cells were washed, fixed and dehydrated by an alcohol series and Ir-intercalator staining (of nucleic acids)
- LA-ICP-MS (New wave 213, ESI and Element XR, Thermo Fisher Scientific) resolution 6 µm * 6 µm/s with 3 µm overlap, repetition rate 20 Hz, fluence 0.5 J/cm^2

References

[1] Ornatsky, O., et al., Highly multiparametric analysis by mass cytometry. Journal of Immunological Methods, 2010. 361(1): p. 1-20.

[2] Iversen, T.-G., T. Skotland, and K. Sandvig, Endocytosis and intracellular transport of nanoparticles: Present knowledge and need for future studies. Nano Today, 2011. 6(2): p. 176-185.

[3] Gundlach-Graham, A. and D. Günther, Toward faster and higher resolution LA–ICPMS imaging: on the co-evolution of LA cell design and ICPMS instrumentation. Analytical and Bioanalytical Chemistry, 2016. 408(11): p. 2687-2695.

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Studying nanoparticle-cell interaction by ICP-MS based techniques

Heike Traub[1], Tina Büchner[2], Daniela Drescher[2], Virginia Merk[2], Janina Kneipp[1,2] & Norbert Jakubowski[1]
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[2] Humboldt-Universität zu Berlin, Department of Chemistry, Berlin, Germany

Introduction

- Interaction of nanoparticles (NPs) with cells is crucial to medical applications in diagnostics and therapy as well as risk assessment.
- Uptake and intracellular behaviour depend on the physiochemical properties of the NPs like size, shape and surface modification.
- Inductively coupled plasma mass spectrometry (ICP-MS): Powerful analytical technique offering high sensitivity and multi-element capability
- Coupling with laser ablation (LA) system enables measurement of spatial elemental distribution.

Objective

Evaluation of nano-bio interaction by ICP-MS based techniques

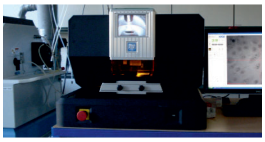
Experimental

Cell culture & incubation experiments

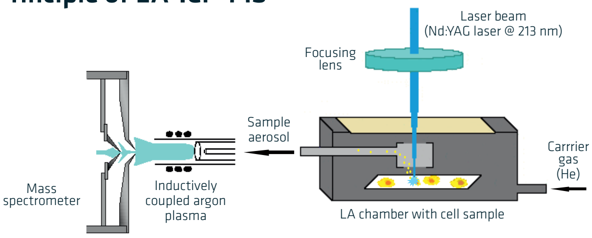
- Cultivation of fibroblast cells (cell line 3T3, DSMZ, Germany) under standard conditions.
- Incubation with different metal-containing NPs at varying conditions.
- For LA: Fixation, dehydration

ICP-MS & LA-ICP-MS

- ICP-MS with pneumatic nebulization (iCAP Qc; Thermo, Germany) after acid digestion of NP suspensions or cell pellets.
- LA systems NWR213 or NWRimage with Two-Volume LA chambers (ESI, USA) coupled to ICP-MS Element XR (Thermo, Germany).
- LA parameters: Continuous line scanning at different laser spot sizes (1 µm, 4 µm, 15 µm)



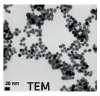
Principle of LA-ICP-MS

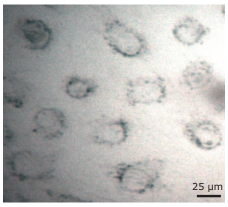


Results & Discussion

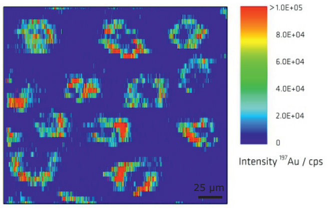
Au NP uptake by fibroblast cells [1, 2]

Incubation of cells with Au NPs (∅ 14 ± 3 nm) for 1, 3 and 24 h



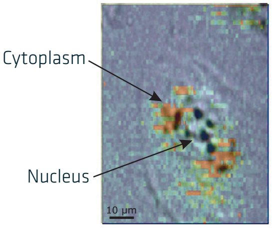


Bright field image of cells incubated with NPs for 24 h before LA



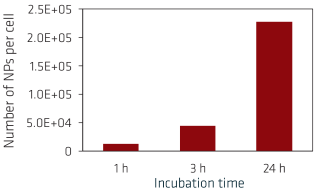
Corresponding LA-ICP-MS image of Au intensity distribution
(pixel size 0.4 µm x 5 µm)

LA imaging at 1 µm spatial resolution
(pixel size 1 µm x 1 µm)



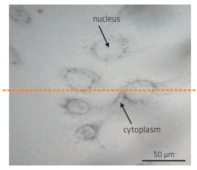
Overlay of bright field image and LA-ICP-MS image

ICP-MS after digestion of cell pellets → Number of Au NPs per cell at different incubation times

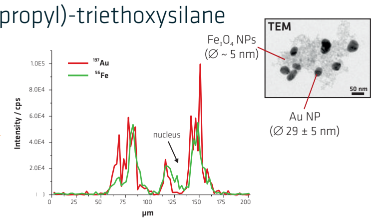


Localisation of magnetic Au-Fe_3O_4 composite nanostructures in fibroblast cells [3]

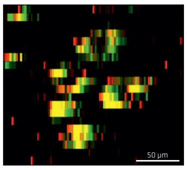
Incubation for 24 h with Fe_3O_4 NPs linked to Au NPs using (3-aminopropyl)-triethoxysilane



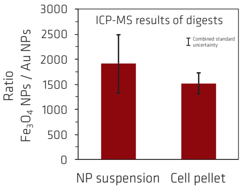
Bright field image before LA



Single line scan, LA spot ∅ 15 µm



LA-ICP-MS:
Composite image of Au and Fe intensity distribution
red → Au
green → Fe
yellow → high Au and Fe intensity



- Spatial overlap of Au and Fe signals and similar ratio Fe_3O_4/Au in NP suspension and cell pellets. → Stability of composite structures during cellular uptake and processing.
- NPs found in cytoplasm, but do not enter the nucleus.
- ICP-MS after digestion of cells: $2.8 \cdot 10^4$ Au NPs and $4.2 \cdot 10^7$ Fe_3O_4 NPs per cell

References
[1] T. Büchner, D. Drescher, H. Traub, P. Schrade, S. Bachmann, N. Jakubowski, J. Kneipp, Anal. Bioanal. Chem. 406 (2014) 7003
[2] D. Drescher, H. Traub, T. Büchner, N. Jakubowski, J. Kneipp, Nanoscale 9 (2017) 11647
[3] T. Büchner, D. Drescher, V. Merk, H. Traub, P. Guttmann, S. Werner, N. Jakubowski, G. Schneider, J. Kneipp, Analyst 141 (2016) 5096

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Toward a reference sample for atom probe tomography

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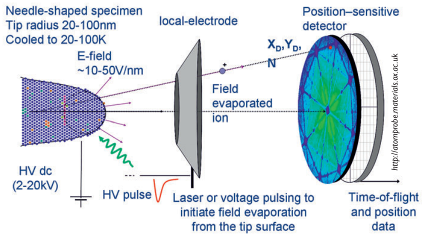
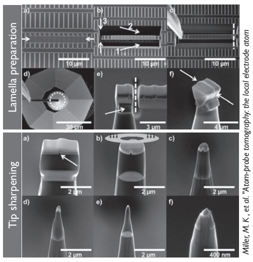
Introduction

Atom probe tomography (APT) is an analytical technique capable of providing in-depth elemental information at sub-nanometer resolution (lateral and depth). Despite the recent progresses in instrumental development, the uncertainty assessment and widespread comparison of APT data are still lacking, mainly due to the absence of appropriate standards and reference materials. To address these limitations, we have developed a novel sample preparation, based on standard lithography and etching techniques, that yields repeatable APT samples with minimized variation in the tip geometry (radius, taper angles and heights). As an initial test vehicle, we have optimized the procedure for the preparation of APT tips starting from a boron-doped (~1E20 atoms/cm^3), epitaxially grown SiGe (25% Ge) layer (100 nm) on a 300 mm Si (100) wafer. The complementary characterization of the same (or similar) wafer using different (reference-free) analysis techniques is done to extract the layer thickness, composition and dopant concentration. This complete characterization together with the repeatable sample preparation, will form the cornerstone to establish a potential reference sample for APT as well as initiate an interlaboratory study (e.g. under the VAMAS umbrella).

Atom probe tomography (APT)



Sample preparation using FIB



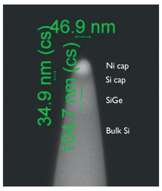
Miller, M. K., et al. "Atom-probe tomography: the local electrode atom probe." Springer Science & Business Media, 2014.

Not suitable for the preparation of reference samples:

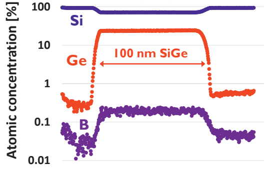
- ❖ Not repeatable: tip to tip variation
- ❖ Ga induced damage (amorphization, implantation, ...)

Motivation of this work

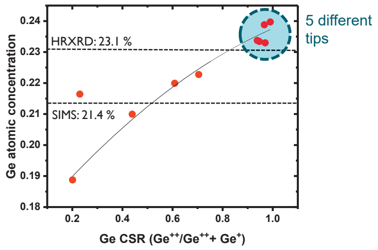
- ❖ Prepare reference samples for APT using an alternative preparation technique (no FIB).
- ❖ Define the uncertainty budget in APT compositional analysis by measuring well characterized/similar reference tips
- ❖ Launch an interlaboratory comparison study (VAMAS) for APT using the prepared reference samples.
- ❖ Underpin fundamental studies (e.g. laser - tip interaction and the role of tip shape on the APT quantification).
- ❖ Study the link between experimental parameter and APT quantification (using comparable tip shapes).



APT depth profile

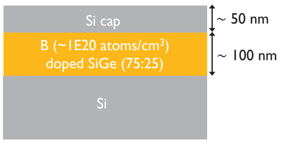


Variation in measured Ge concentration with experimental conditions



Characterization of potential reference sample

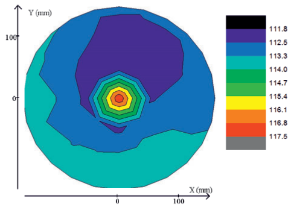
Reference wafer



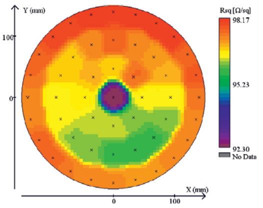
Measurands:

- ❖ **Ge concentration**
- ❖ **B concentration**

Uniformity of SiGe thickness (XRD)

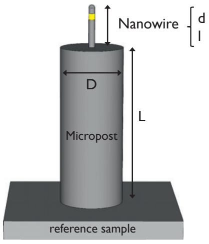


Sheet resistance (4PP)



Characterization techniques: Reference free GIXRF, SIMS, XRD and 4PP.

Geometrical design of the APT reference sample



Based on finite element simulations:

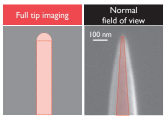
- "Nanowire on micropost" geometry enables full tip imaging.
- Field of view is mainly driven by the length L and diameter D of the micropost.
- The dimension of the nanowire was fixed (l=600, d=80 nm) to maximize the electric field.

1) Maximizing electric field enhancement at tip apex:

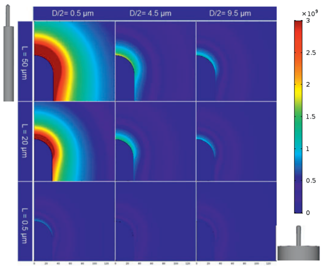
$$F = \frac{V}{r\,k_f}$$

F: electric field at the apex
V: electric potential
R: tip radius
K_f: field factor

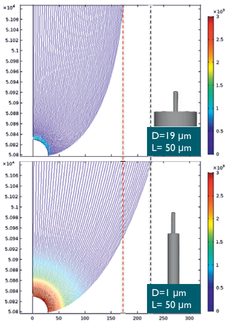
2) Maximizing field of view:



Finite element simulation of the electric field around the tip apex for various Micropost geometries (D, L):



Finite element simulation of electric field lines and ion trajectories:

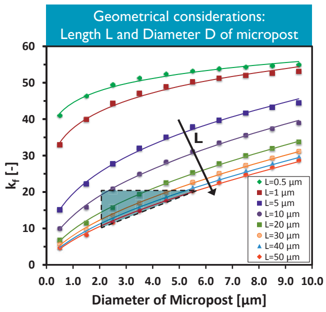


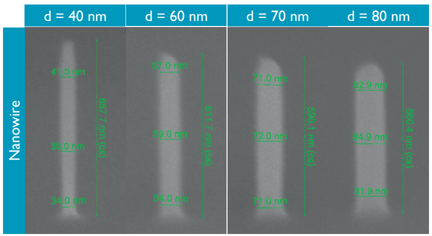
- ❖ No Ga induced damage
- ❖ Constant diameter over entire tip length
- ❖ High field of view
- ❖ Reproducible and repeatable sample geometry
- ❖ Up-scalable towards full wafer processing

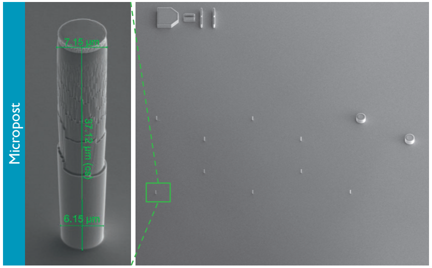
Preparation of potential reference sample

- ❖ Sample preparation is based on standard lithography and etching techniques used in micro electronics.
- ❖ Dimensions (D, L) of the Micropost were identified based on the finite element simulations to maximize:
  - Electric field enhancement ($F \geq 1\times10^{10}$ V/m): $k_f < 20$
  - Field of view (for full tip imaging): $k_f > 8$

Geometrical considerations:
Length L and Diameter D of micropost







Acknowledgments

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Creating the Silver Standard: Development and Applications of a Silver Nanoparticle Reference Material

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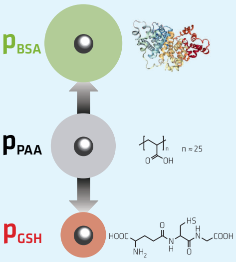
Introduction

Silver nanoparticles are used in a high variety of applications due to their special optical and antibacterial properties.[1] Nevertheless, their toxicological potential is still under discussion due to contrary studies in this field.[2,3] The essential problem is the use of polydisperse, non uniform particles which makes it impossible to define their size dependent catalytical and biological properties.

Here, we present a possibility to synthesize stable, uniform silver nanoparticles in a highly reproducible way with an upscalable variant. The particles are thourougly characterized and show a high catalytic activity in the standard reaction of the reduction of 4-nitrophenol. Additionally, the particles are used in an artificial digestion to monitor changes in size and size distribution width.

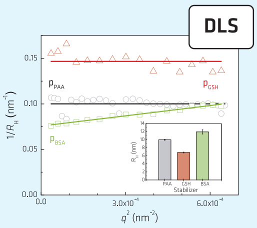
Particle Systems

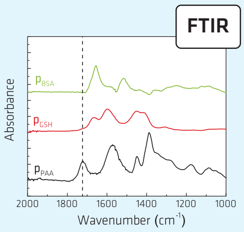


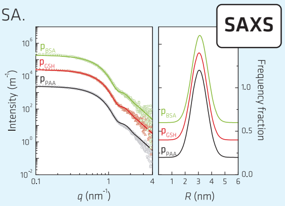
We synthesized three different particle systems with the same silver core. For the starting particles we used a modified polyol process with poly-(acrylic acid) as stabilizer (PAA). These particles were transfunctionalized with bovine serum albumin (BSA) and glutathione (GSH). This results in a higher biocompatibility and therefore a better applicability for biological studies.

Characterization

With the help of small-angle X-ray scattering (SAXS) the core sizes of the particles were determined (R_c = 3 nm). The particles show no sign of aggregation due to the abscence of differences in the particle sizes. In comparison with dynamic light scattering (DLS) the thicknesses of the ligand shells were determined. Depending on the stabilizer they amount to 7.0 ± 0.2 nm (p_{PAA}), 3.8 ± 0.2 nm (p_{GSH}) and 8.9 ± 0.5 nm (p_{BSA}). The direct evidence of the successful ligand exchange was observed with FTIR. Here, the carbonyl vibration band of PAA (1723 cm^{-1}) vanishes with the exchange of the ligand shell with GSH or BSA.

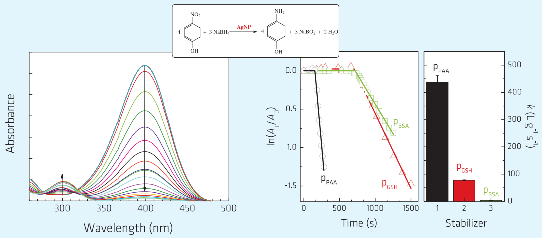






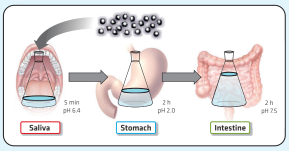
Catalysis

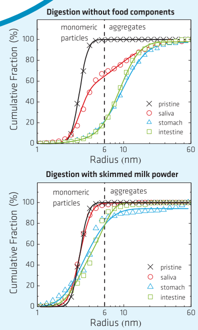
Investigation of the catalytic activity in dependence of the stabilizing ligand with the help of a standard reaction: reduction of 4-nitrophenol to 4-aminophenol with sodium borohydride (see reference [4])



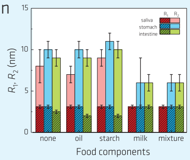
To the best of our knowledge the p_{PAA} particles display the highest catalytic activity reported for silver nanoparticles until now. In contrast, p_{GSH} and p_{BSA} show a significant lower activity in the catalysis.

Artificial Digestion





During artificial digestion of ultra-small silver nanoparticles, the size and size distribution remain in a nanoscale but undergo a transformation in size. It was observed that skimmed milk powder shows a stabilizing and protecting effect. In this case the size distribution did not change significantly, whereas the addition of oil or starch shows a transformation in the size distribution. (see reference [5])



Conclusion

We have shown that ultra-small silver nanoparticles with a core radius of 3 nm can be produced in large quantities. The original PAA stabilizer can be easily replaced by other stabilizers to tune the surface on demand, as has been demonstrated by using BSA and GSH. Of high interest is that the PAA stabilized particles display the highest catalytic activity of silver nanoparticles reported to date for the reduction of 4-nitrophenolate to 4-aminophenolate. The artificial digestion of these particles revealed that they remain nanoscaled and are strongly influenced by food components. The particles are available from the authors on request.

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[5] Kästner, C., Lichtenstein, D., Lampen, A., Thünemann, A. F., *Colloids Surf. A* **2017**, 526, 76–81.

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Microwave-assisted high-speed silver nanoparticle synthesis

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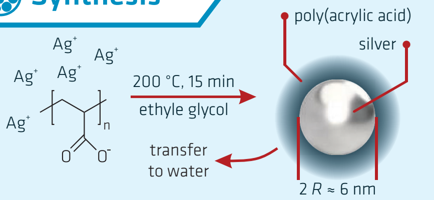
Introduction

▶ Microwave-facilitated syntheses have become popular in the late 20th century since they offer significantly increased heating as well as reaction rates.[1]

▶ The use of microwave synthesis reactors is under controversial discussion in literature as so called non-thermal effects might exist.[2] These could alter the reaction pathway and its outcome.

▶ We compare the syntheses of ultra-small, spherical silver nanoparticles with ca. 3 nm radius both via conventional and microwave heating. Reactions were performed at the solvent's boiling point and also at higher temperatures and corresponding pressures in a microwave reactor.

Synthesis

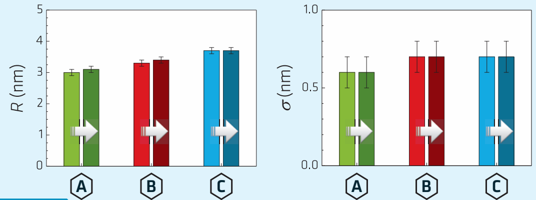


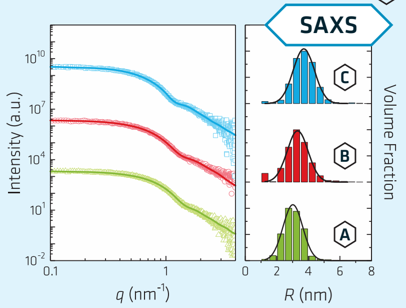
Synthesis	**A**	**B**	**C**
Heating	Conventional	Microwave	Microwave
Vessel	Glass	Glass	Silicon Carbide

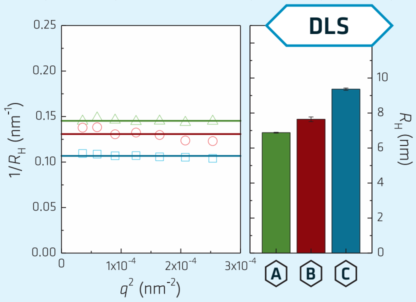
Comparison

▶ Using small-angle x-ray scattering (SAXS), the particles' core radii R and radii distribution widths σ were determined. Upon particle transfer to water, these values increased by not more than 0.1 nm (right bars).

▶ Multi-angle dynamic light scattering (DLS) was used to investigate the particles' size inclusive of the PAA shell. Contrary to the heat-type independent core radii, the hydrodynamic radii R_h are slightly larger for microwave-assisted syntheses. No signs of particle aggregation were found.



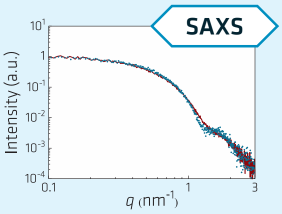


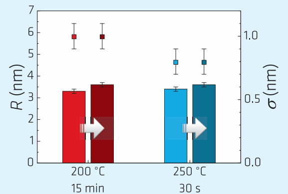


Acceleration

▶ Assuming a doubling of the reaction rate with a temperature increase of 10 K, the particle synthesis should be completed within 30 s at a temperature of 250 °C.

▶ In fact, both synthesis routes yield particles with mean radii (bars) and radii distribution widths (points) that agree within their uncertainties. An overlay of the normalized scattering curves demonstrates their approximate congruence.





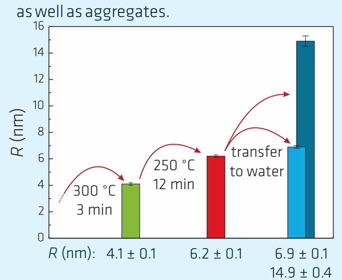
Growth

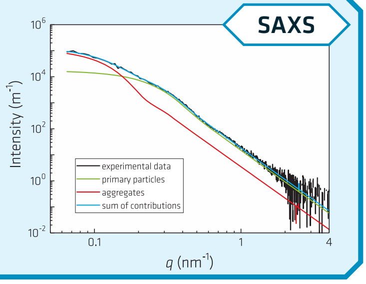
▶ At 300 °C, the particle synthesis is certainly completed after 3 minutes.

▶ The resulting primary particles can be further grown at elevated temperatures. This is accompanied by a broadening of the size distribution.

▶ Unfortunately, aggregates formed upon particle transfer into water. This is reflected in a second particle population.

▶ The SAXS data can correspondingly be modeled as a sum of scattering contributions by primary particles as well as aggregates.





Conclusion

▶ Ultra-small silver nanoparticles with 3 nm radius and a narrow size distribution can be produced in a one-pot synthesis via conventional and microwave heating. While the heating source and vessel material have no substantial impact on the particles' core size, their shell thickness depends on the heating type.

▶ An increase of the reaction temperature by 50 K leads to a reduction of the reaction time by 97 %. Whereas the particle parameters remain nearly unchanged, the particle losses increase. Time- and material-saving reaction routes must be weighed up against each other.

▶ Incubation at elevated temperatures leads to particle growth and offers the opportunity of tuning the particle size. However, a better control over the dispersity must be achieved.

▶ During this study, no potential specific or non-thermal microwave effects were observed.

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[2] Baghbanzadeh, M.; Carbone, L.; Cozzoli, P. D.; Kappe, C. O., *Angew. Chem. Int. Ed.* **2011,** *50*, 11312-11359.

Publication

This work has been published in Langmuir:
Saloga, P. E. J.; Kästner, C.; Thünemann, A. F., *Langmuir* **2018**, *34* (1), 147-153.

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Development of a hybrid metrology combining AFM and SEM techniques for measuring the characteristic dimensions of a nanoparticle population

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Context

We propose a novel approach of hybrid metrology combining AFM (Atomic Force Microscopy) and SEM (Scanning Electron Microscopy) measurements for measuring the **characteristic dimensions of nanoparticles (NP) in 3D with controlled uncertainties**.

This method takes advantage of the complementary nature of both techniques: SEM gives no quantitative information about the **NP heights** whereas the uncertainty associated with AFM measurements of NP maximum point is close to 1.5 nm [1].

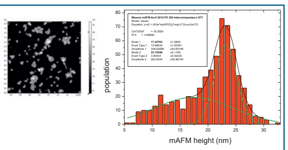
Conversely, the **lateral dimensions** measured by AFM are impacted by tip / NP convolution whereas SEM of latest-generation SEM equipped with FEG (Field Emission Gun) can reach a resolution of 1 nm in the XY plane.

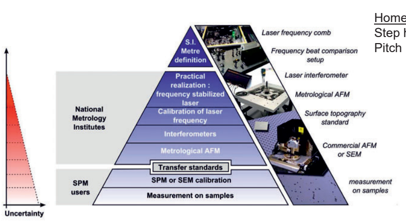
In order to check the consistency of measurements performed by AFM and SEM, a comparison of the mean diameter of spherical **silica NP** measured by both techniques had been carried out in a previous study [2]. However, this comparison had not been performed exactly on the same population. In the meantime, **a specific repositioning device** consisting of silicon wafer with lithographed landmarks has been developed to easily find an area of interest. Henceforth, it is possible to determine the AFM height and SEM lateral diameter on **the same set of NP**. The proof-of-concept has been tested on various samples of silica NP synthesized by Stöber method and reference silica NP with sizes ranging from 5 nm to 110 nm .

1. Delvallée, A.; Feltin, N.; Ducourtieux, S. Metrologia 2016, 53 Number 1
2. Delvallée, A.; Feltin, N.; Ducourtieux, S. Measurement Science and Technology 2015, 26 Number 8

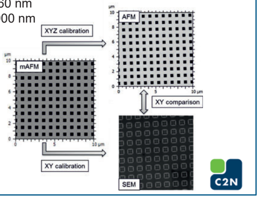
Traceability

- Traceability ensured by LNE's metrologial AFM (mAFM).
- Laser interferometry creates a link between the measurement at the nanoscale and SI meter definition.
- Pitch and step height measurements performed by mAFM .
- Reference values disseminated through transfert standard

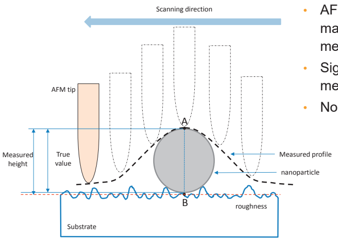




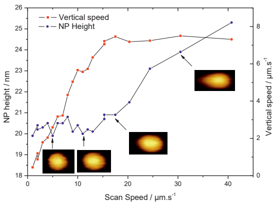
Homemade transfert standard :
Step height: 60 nm
Pitch (XY) : 900 nm



Uncertainty budget established for AFM measurements



- AFM height defined as the difference between the NP maximum point (marked A) and the reference plane (= mean plane of roughness)
- Significant error associated with lateral diameter measurements due to tip/ sample convolution.
- No convolution impacts the NP highest point measurement

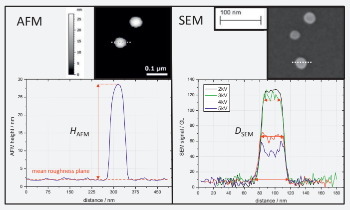


Silica NP : D_m=79 nm

Main components

Repeatability	0.4 nm
Calibration (VLSI)	0.7 nm
Scanning speed (4 µm/s)	0.24 nm
Z noise level	0.16 nm
Repeatability	0.08 nm
Roughness (S_q)	0.08 nm

$$U_{AFM}(k=2) = 1{,}5 \text{ nm}$$



Uncertainties

Type A : random errors (repeatability)

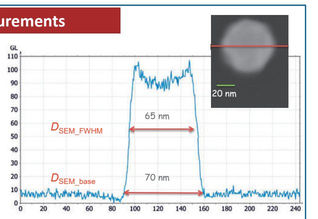
Type B : systematic errors (calibration, drifts…)

Uncertainty budget established for SEM measurements

- No metrological information along Z axis.
- FEG-SEM with a resolution reaching 1 nm in XY plane.

Nevertheless :

- Issue linked to convolution gaussian e-beam / NP: unknown size / shape → FWHM measurements on the profile .



True value ?

FD-304 NP : D_m=26.3 nm
(calibation certificate : 27.8 nm ± 0.75 nm)

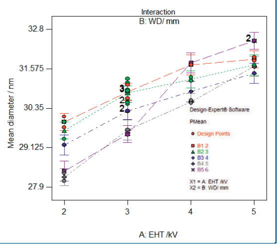
Main components

Repeatability	0.5 nm
Calibration (VLSI)	0.8 nm
Resolution (e-beam R50)	1.7 nm
EHT effect	1.4 nm
WD effect	0.5 nm
Interaction EHT/WD	0.6 nm

$$U_{SEM-FWHM}(k=2) = 2{,}6 \text{ nm}$$

Interaction electron / NP modelling?

Influence of WD and EHT on the measurement of the mean of nanoparticle diameter (DOE, ANOVA)



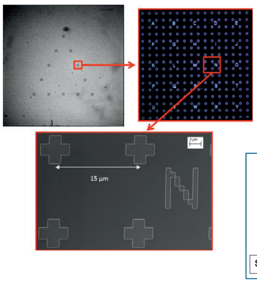
Direct comparison between AFM and SEM measurements

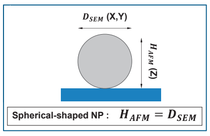
Using the complementary nature of both techniques (AFM/SEM) requires the development of specific tools enabling to rapidly find a area of interest (≈ µm²).

Objective :

- Assessing **repeatability / reproducibility** of the measuring method on a NP population.
- Comparing the AFM and SEM measurements performed on the same set of NP.

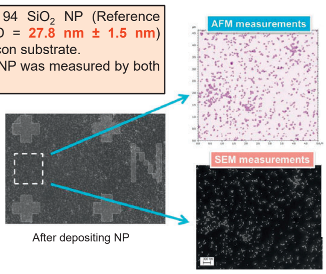
→ Development of a specific lithographed silicon substrate: array of marking patterns (crosses and letters) **visible with an optical microscope.**



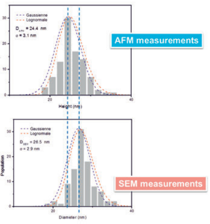


Spherical-shaped NP : $H_{AFM} = D_{SEM}$

Area of interest with 94 SiO_2 NP (Reference material, FD304 JRC, D = **27.8 nm ± 1.5 nm**) deposited on marked silicon substrate. **Exactly** the same set of NP was measured by both techniques (AFM / SEM)



After depositing NP

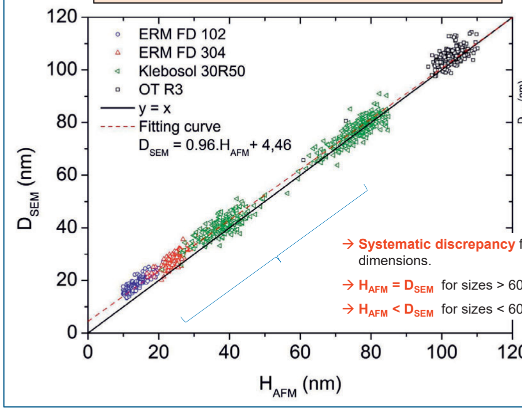


Discrepancy
D_{SEM}-H_{AFM} = 2.1 nm

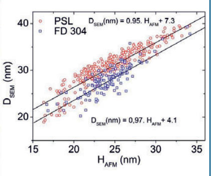
Measurements performed on a series of nanosilica samples ranging from 1 to 100 nm

Comparison AFM / SEM measurements carried out on four silica NP populations coming from different providers

ERM FD 102 : reference material (certified by SEM)
ERM FD 304 : reference material (certified by DLS)
30 R 50 : colloidal nanosilica manufactured product
OT R3 : colloidal nanosilica synthetized in laboratory(CEA)



Comparison of SEM and AFM measurement on a same set of PSL and SiO_2 NP

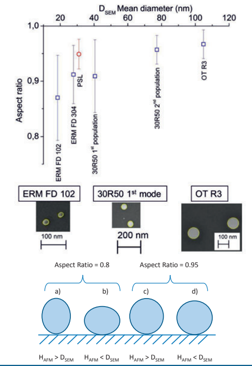


→ **Systematic discrepancy** for NP with the smallest dimensions.

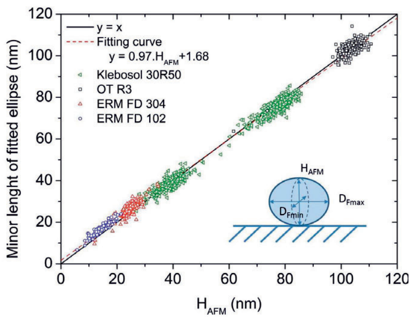
→ **H_{AFM} = D_{SEM}** for sizes > 60 nm.

→ **H_{AFM} < D_{SEM}** for sizes < 60 nm

Aspect ratio measured for each batch of NP (100 NP measured for each population).
Error bars represent the sphericity distribution.



Comparison of minimal Feret diameter obtained with a fitted ellipse (SEM) and height measurement (AFM) taking into account the theoretical deformation on a same set of FD 102, FD 304 NP, 30 R 50 and OT R3 NP.



Conclusions

- First step towards an approach of hybrid metrology combining AFM/SEM
- Method for measuring **characteristic dimensions** of a NP population in 3D.
- Method has been validated with **spherical-shaped nanoparticles**.
- The study shows that aspect ratio of silica NP decreases with size
- Discrepancies between AFM and SEM measurements are mainly explained by the sphericity deviation of smallest silica NP.

Accurate protocol for nanoparticles analysis (size, size distribution, concentration) by SAXS and spICPMS

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Small Angles X-Ray Scattering (SAXS) has been established as a metrological method for the determination of nanoparticles size and size distribution. Modern SAXS Laboratory experiments , by involving synchrotron-based instrumentation at lower price and very stable X-ray source, are more and more used in nanomaterials domain. In the frame of the Innanopart project we have developed a methodology for the size and concentration determination of spheric Silica nanoparticles. This protocol involves a precise sample preparation, and a set of homemade software tools for the data processing - from the acquisition, the absolute scaling, to the analysis.

spICPMs is not a metrological traceable technique but has many strengths to become a useful complement of nanoparticle characterization methods such as SAXS and microscopy. It can also measure highly diluted nanoparticles suspensions which is not the case of Dynamic Light scattering (DLS) or SAXS. Finally, ICPMS analyzes inorganic ions in liquid solution in a very large range of concentration, which should allow linear diameter measurement range over at least 3 orders of magnitude by spICPMS.

In this work, we confront spICPMS, as a counting technique, with SAXS in order to investigate the method and the developed protocols on a set of commercial Gold Nanoparticles.

Accuracy of measurement instruments➔ Quality of nanoparticle synthesis

PROTOCOL FOR ACCURATE SIZE AND CONCENTRATION ESTIMATION BY SAXS

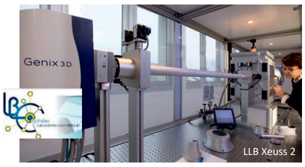
SWAXSLab platform

- 4 experiments (SAXS, USAXS, WAXS, XEUSS)
- 150m2 dedicated
- Chemistry laboratory and workshop


High Performance SAXS

High Performance SAXS (CEA design)

Rigaku Rotating Anode Generator , Fixed configuration
Designed for in-situ experiment (acquisition time ~ 1min)



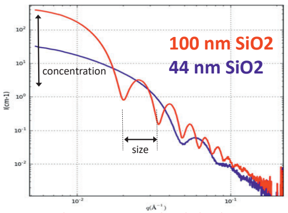
LLB Xeuss 2

SAXS/GISAXS experiment (XENOCS XEUSS 2.0)

Collaboration with LLB Neutron Small-Angle Team

- A polyvalent experiment (sample in the air or under vacuum, GISAXS stage, variable Distance)
- Pilatus 1M detector , Stable Cu X-Rays micro Source

ABSOLUTE INTENSITIES CALIBRATIONS



Scattered Xrays in normalized absolute Intensities (cm-1) are proportionals to NP concentration

$$I(q) = \frac{\eta 1}{\eta 2} \cdot \frac{Cij}{\phi_0} \cdot \frac{1}{dt} \cdot \frac{D^2}{p^2} \cdot \frac{1}{thickness}$$

Number of counts detected

Sample/Detector distance (±0.1%)

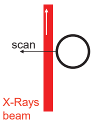
Efficiency detectors constants (=1)

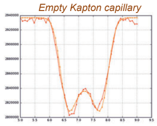
Integrated Transmitted Flux (very constant)

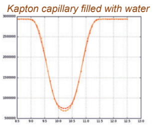
Detector Pixel size

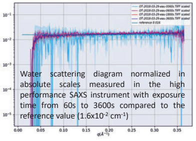
- **How to determine the sample thickness ?**

Using the same sample cell (capillaries) for all
Measuring the sample thickness with a well known sample (water)
We have developed a protocol for determining the sample thickness from a « scan process »




Empty Kapton capillary

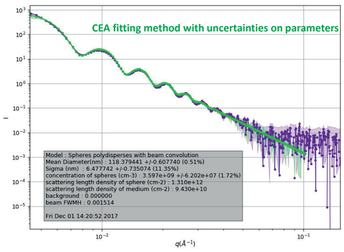

Kapton capillary filled with water


Water scattering diagram normalized in absolute scales measured in the high performance SAXS instrument with exposure time from 60s to 3600s compared to the reference value (1.6x10-2 cm-1)

Internal thickness : 0.138 mm Datasheet : 0.142 mm Determined from Water transmission : 0.141 mm

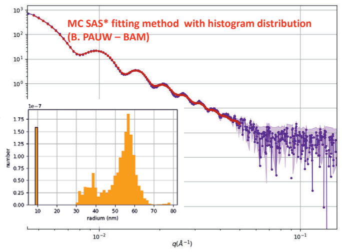
And calibration samples :
NIST Glossy Carbon ,Lupolen (measured at PTB), AgBH, Tetradecanol (shark peaks for D determination)

Analysis SAXS Software integration : pySAXS



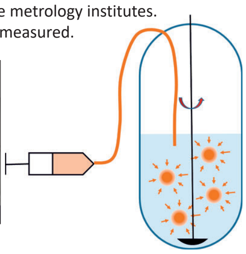
Model : Spheres polydisperses with beam convolution
Mean Diameter(nm) : 118.379441 +/-0.607740 (0.51%)
Sigma (nm) : 6.477742 +/-0.735074 (11.35%)
concentration of spheres (cm-3) : 3.597e+09 +/-6.202e+07 (1.72%)
scattering length density of sphere (cm-2) : 1.310e+12
scattering length density of medium (cm-2) : 9.430e+10
background : 0.000000
beam FWMH : 0.001514

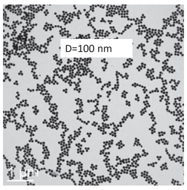
Fri Dec 01 14:20:52 2017



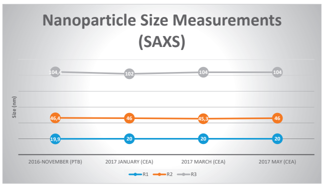
Innanopart project : SiO2 Monodisperses nanoparticules synthesis with controled size

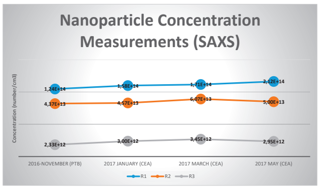
The CEA work in Innanopart project was to synthesis spheric SiO2 nanoaparticles as monodisperses as pssible in the range of 20-200 nm.
3 batchs of solution (18 nm, 44 nm, 100 nm) were selected and provided to the consortium and were measured by the metrology institutes.
The concentration and size were regulary measured.





S. Fouilloux, J. Daillant, A. Thill
Colloids and Surfaces A: Physicochem. Eng. Aspects 393 (2012) 122– 127
Single step synthesis of 5–30 nm monodisperse silica nanoparticles: Important experimental parameters and modeling

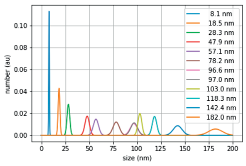

Nanoparticle Size Measurements (SAXS)


Nanoparticle Concentration Measurements (SAXS)

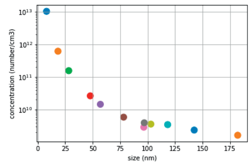
- Need to estimate the quantities of reagent product -> Need to measure the size and concentration by SAXS
- Need to control the injection during the synthesis
- **CEA Project 2017-2018 for a controled synthesis of nanoparticles monitored by SAXS**
- **Projet EMPIR npSize 2018**

CONTRIBUTION TO ACCURATE SPHERICAL GOLD NANOPARTICLES SP-ICPMS ANALYSIS A COMPARISON WITH SAXS

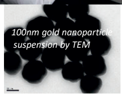
Commercials Gold nanoparticles measured by SAXS



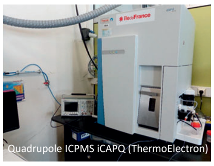
size



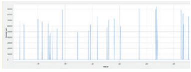
Concentration


100nm gold nanoparticle suspension by TEM

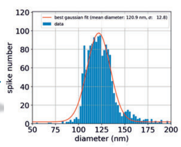
SP-ICPMS data treatment processing code


Quadrupole ICPMS iCAPQ (ThermoElectron)

Raw datas from the spectrometer



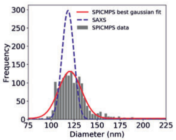
Histogram (nb/nm)



SP-ICPMS protocol improvement

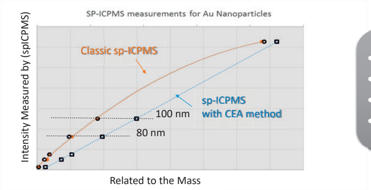
	Supplier	SAXS		SAXS+ICPMS	DLS	
Standard	[AuNPS] p/mL	d (nm)	σ (nm)	[AuNPS]ᵃ p/mL	[AuNPS]ᵇ p/mL	d (nm)	σ (nm)
30nm	1.8E+11	28.3	4	1.6E+11	1.7E+11	50.7	21
50nm	3.5E+10	53.3	6.5	2.8E+10	3.1E+10	64.4	24
60nm	1.9E+10	57.1	7.7	1.5E+10	1.8E+10	81.5	30
80nm	7.8E+09	78.2	9.4	5.9E+09	7.3E+09	102.6	33
100nm	3.8E+09	96.6	10.6	4.0E+09	4.4E+09	121	35
150nm	3.6E+09	142.5	13	2.4E+09	3.1E+09	178	48

	Au Samples	
	125nm	135
SAXS Gaussian distribution mean diameter (nm)	103	118.3
SAXS Gaussian distribution sigma (nm)	5.7	6.6
CLASSIC sp-ICPMS (nm)	87	96
Difference ICPMS / SAXS	-15%	-19%
IMPROVED SPICPMS Gaussian distribution mean diameter (nm)	106	121
SPICPMS Gaussian distribution s (nm)	8	13
Difference ICPMS / SAXS	3.50%	2.20%



Histogram (nb/nm)
spICPMS-SAXS comparison

SP-ICPMS measurements improvements



- Density measurement ?
- Improvement of spICPMS data treatment
- Improvement of spICPMS hardware
- No need of calibration datas

17NRM04 nPSize - Improved Traceability Chain of Nanoparticle Size Measurements

The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States

Vasile-Dan Hodoroaba (BAM)
and Harald Bosse (PTB)

CONSORTIUM

NEEDS

- **Reliable analysis** of nanoparticle size and **comparable results** of physically different methods for:
  - Nanoparticle manufacturers
  - Service/accredited laboratories
  - Analytical instrument makers
- EC mandate M/409: ‚*…utmost importance to translate European excellence in nanoscience into safe and commecially marketable products and processes.*‘
- EC mandate M/461: ‚*…requires to develop standards activities regarding nanotechnologies and nanomaterials …for safe, integrated and responsible use of nanomaterials…*‘.
- **Regulatory** requirements/classification of nanomaterials acc. to EC definition (2011/696/EU)

OBJECTIVES

- Establish **EU capability** of **traceable measurement** of NP size and shape
- **Lower uncertainties** of NP size measurement by:
  - **new nano-CRMs**,
  - **new models** (physical and machine learning),
  - 3D method **combination**
- New **ISO and CEN standards** on accurate NP size measurement
- Guidance and knowledge transfer

WP1 Performance and traceability of Characterization Methods

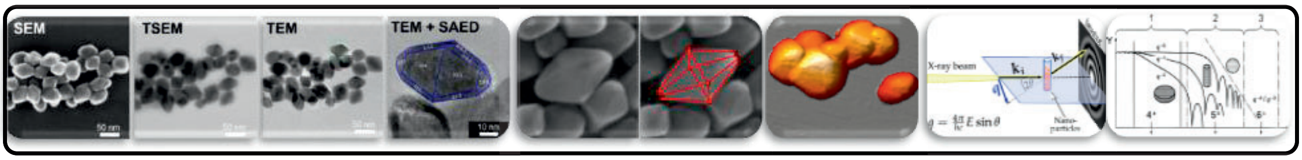
- Assesment of method performance of traceable sizing methods EM, SAXS and AFM on NP size, shape, polydispersity and number concentration
  - current state
  - with new, dedicated RMs (developed in WP2) and models (WP3)
- Public data library for WP3
- Steps to extend traceability from spherical to non-spherical NP shapes

WP2 Reference materials - Preparation and Characterization

- Analysis of requirements (WP1 & 4)
- Development of well-defined non-spherical shapes: cubes, platelets, bipyramids, rods/acicular
- mono- and polydisperse
- accurate particle number concentration (SAXS and isotopically enrichment for ICP-MS)
- Re-validation loop via improved WP3 measurement procedures

WP3 Modelling and development of measuring procedures

- Physical modelling (TSEM, SEM, 3D-AFM, SAXS)
- Machine learning modeling from *a-priori* measurement data
- Physical model input into machine learning
- Data fusion for hybrid sizing techniques: SEM & TSEM/TEM, SEM/TSEM & AFM, SEM/TSEM & SAXS (with WP1)



WP4 CREATING IMPACT

- **ISO/TC 229** ‘Nanotechnologies’/JWG 2 ‘Measurement & Characterization’ (SEM, TSEM, TEM)
- **ISO/TC 24/ SC 4** ‘Particle characterization’ (SAXS)
- **ISO/TC 201/SC 9** ‘Scanning probe microscopy’ (AFM)
- **CEN/TC 352** ‘Nanotechnologies’/ WP1 ‘Measurement, characterization and performance evaluation’

ISO/TC 229 Nanotechnologies | ISO/TC 24/SC 4 Particle characterization | ISO/TC 201/SC 9 Scanning probe microscopy | CEN/TC 352 - Nanotechnologies European Committee for Standardization

- **Dedicated workshops** (EM, SAXS, AFM) for end-users
- **New nano-CRMs** candidates
- **Public data library** with tagged measurement data
- **Signal modeling** and **machine learning** procedures published

BEYOND STATE-OF-THE-ART

- Improved nanoparticle size traceability chain by:
  - Reduction of method- and material dependent discrepancies
  - Estimation of the influence of particle shape and size distribution
  - More accurate dimensional characterization of nanoparticles by combining results of different methods
- Standardization: Launching European standardization projects on nanoparticle size and shape in line with ISO.

STAKEHOLDERS

CEN/TC 352 - Nanotechnologies
European Committee for Standardization

- Nanoparticle manufacturers
- Service/accredited laboratories
- Analytical instrument makers

Advanced screening method using volume-specific surface area (VSSA) for nanomaterial identification of powders

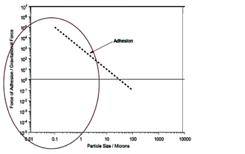
Kuchenbecker, P.; Hodoroaba, V.-D.; Lindemann, F. (all BAM)
Wohlleben, W. (BASF)

BAM
Bundesanstalt für
Materialforschung
und -prüfung

Introduction

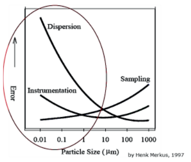
Determining the particle size of submicron and nanoparticles is challenging, but necessary. For the identification as nano- or non-nanomaterial according to EC recommendation (2011/696/EU), the size of the individual particles is decisive. For most common measurement methods for particle size determination it is necessary to initially disperse the particles in a suitable liquid. Many common methods such as dynamic light scattering (DLS), centrifugal liquid sedimentation (CLS) or ultrasound attenuation spectroscopy (US) can reliably determine the size of nanoparticles, if they are properly separated and stabilized (e.g. in reference suspensions). However, with decreasing particle size, the adhesion forces increase strongly (*see top right*), making it more difficult to successfully deagglomerate the particles and to assess the result of this process. Therefore, the preparation of the dispersion determines substantially the measurement uncertainty (*see below right*).
Preferably, fast and cost-efficient methods are desired to gain information about particle sizes, especially for the identification of nanomaterials. In order to not have to use hardly available and expensive methods such as SEM / TEM for all powders, a screening method is useful.
For this purpose, this poster presents the method of the volume-specific surface area (VSSA) by gas adsorption and density measurement.

Adhesion forces



From: Aerosol Science, Ed. C N Davies, Academic Press, London and New York, 1966

Measurement errors



by Henk Merkus, 1997

Volume-specific surface area (VSSA) method

Density ρ by He-Pycnometry

Specific surface area (SSA) by gas adsorption method

Volume-specific surface area (VSSA) = ρ [g/ cm³] x SSA [m²/g]

VSSA of monomodal spherical particles with d = 100 nm ➡ 60 m²/ cm³

➡ 60 m²/ cm³ is the cutoff between nano- and non-nanomaterials

Advantages:

- Agglomeration-tolerant
- integral property
- without major sample preparation, except for degassing
- He-pycnometry and gas adsorption method are basic and routinely applied in ceramic laboratories
- positive classification as nanomaterial by means of VSSA is allowed by the EU definition

Disadvantages:

- the cutoff of 60 m²/ cm³ is valid only for
  - ➢ spherical
  - ➢ non-porous and
  - ➢ monodisperse

  particles

Corrections needed for:

- shape factor
- separate internal from external surface
- taking into account polydispersity

Corrections

1. Shape dependent cutoff value, as introduced by Roebben et al. 2014

$$\mathrm{VSSA}_{cutoff} = 60\ \mathrm{m^2/cm^3} \times \frac{D}{3}$$ with D the number of small particle dimension

shape	spheres, cubes	needles , fibers	platelets
D	3	2	1

2. Deviation from sphericity for particles having 3 similar dimensions

VSSA = ρ [g/ cm³] x SSA [m²/g] x F with F the shape descriptor ⟶ work is in progress

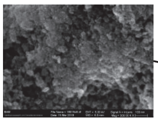
3. For the case of inner porosity: separation of inner area fraction from outer surface

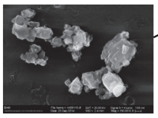
Taking into account the curses of measured isotherms and the value of the BET-parameter c (equation (1) ISO 9277). If there are indications of micropores, the t-plot or the α_S-method should be used to extract the area fraction of the micropores (ibid, Annex C).

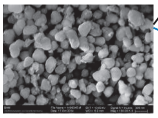
4. Influence of the polydispersity (e.g. according to ISO 9276-5: logarithmic normal probability distribution)

$$\mathrm{VSSA} = \frac{6000}{d_{50,p}} \times e^{(p-2{,}5)\,\sigma^2}$$ with d_{50} the median [nm], p the dimensionality of the distribution and σ the standard deviation of the density distribution

Examples







Material	Density by He-pycnom. [g/ cm³]	BET [m²/ g]	Area fraction of micropores acc. to 3. [m²/ g]	VSSA [m²/ cm³]	D	VSSA Cutoff value acc. to 1. [m²/ cm³]	Screening Decision by VSSA	$d_{50,0}$ by SEM (minFeret) [nm]	Confirmatory Decision by SEM
TiO_2 #1	3.45	248.3	not found	856.6	3	60	nano	≤10	nano
Zeolite	2.07	346.6	317.8	59.6	3	60	borderline	118.2	non-nano
TiO_2 #2	3.99	12.6	not found	50.3	3	60	borderline	183.7	non-nano

Consideration of deviation of sphericity (2.) and polydispersity (4.)

Material	$d_{50,0}$ by DLS [nm]	σ by DLS	Density distribution model (approx.)	VSSA calculated for ideal spheres acc. to 4. [m²/ cm³]	D	VSSA measured [m²/ cm³]	Estimated value F (shape descriptor)	Estimated VSSA cutoff value acc. to 2. [m²/ cm³]	Screening Decision by VSSA
TiO_2 #2	156	0,31	log normal	30,2	3	50,3	1,7	102	non-nano

Outlook : Suitable reference materials for VSSA method including corrections are under discussion at BAM.

References:
1. G. Roebben, H. Rauscher et al. , Towards a review of the EC Recommendation for a definition of the term "nanomaterial" - Part 2: Assessment of collected information concerning the experience with the defintion. European Commission, Joint Research Centre, 2014
2. W. Wohlleben, J. Mielke et al. , Reliable nanomaterial classification of powders using the volume-specific surface area method, J. Nanopart. Res., 2017, 19, 61
3. ISO 9277:2010 Determination of the specific surface area of solids by gas adsorption – BET method.
4. ISO 9276-part 1, 2, 5 and 6: Representation of results of particle size analysis

Contact: petra.kuchenbecker@bam.de

Sicherheit in Technik und Chemie

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Development of an integrated approach based on validated and standardized methods to support the implementation of the EC recommendation for a definition of nanomaterial

Controlled Electrospray Deposition of Nanoparticles for Improved Analysis by Electron Microscopy

J. Mielke[a], P. Dohányosová[b], P. Müller[c], S. López-Vidal[b] and V.-D. Hodoroaba[a]

[1] Federal Institute for Materials Research and Testing (BAM), Berlin, Germany; e-mail: Dan.Hodoroaba@bam.de
[2] RAMEM Ltd., Madrid, Spain
[3] BASF SE, Ludwigshafen, Germany

Introduction

Electrospray (ionization, ESI) deposition principle:

- deposition of (nano)particles from a suspension on a support suited to high-resolution imaging (SEM, TEM, EDS, SPM, etc.) [1]
- by spraying of charged particles on a TEM grid

Advantages vs. drop-on-grid:

- small droplets
- drying during flight /or on the grid
- avoiding on-grid agglomeration

Advantages against other ESI systems:

- works at atmospheric pressure
- versatile: capillary diameter adjustable

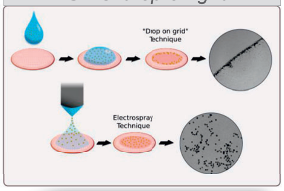
Further details:

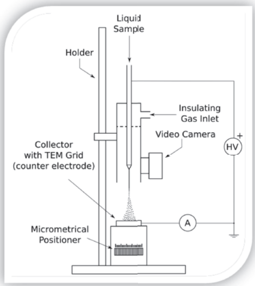
- prototype developed by RAMEM
- tested at BAM on representative *NanoDefine* materials [2,3]
- size of primary droplets [4]:

$$d = G(\varepsilon)\sqrt[3]{\frac{Q\varepsilon\varepsilon_0}{k_e}}$$

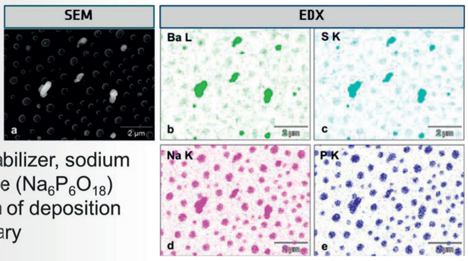
k_e: electric conductivity
Q: flow rate
ε: dielectric constant ('*0*' for vacuum)
$G(\varepsilon_0)$: size of primary droplets [4]

ESI vs. drop-on-grid

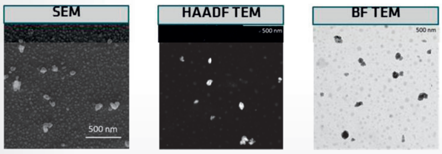


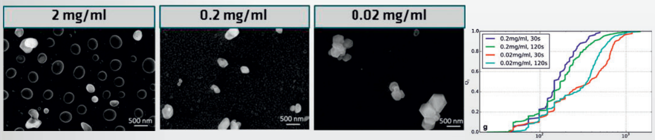


ESI deposition optimization (fine grade $BaSO_4$)



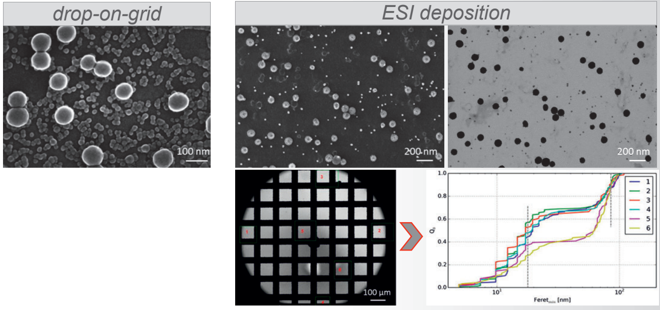
- Co-deposition of stabilizer, sodium hexametaphosphate ($Na_6P_6O_{18}$)
- Further optimization of deposition parameters necessary





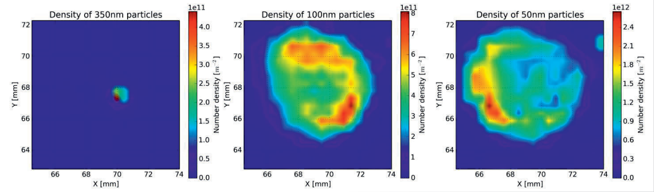
- Agglomerates size increases with decreasing stabilizer concentration
- Agglomerates size does not depend on deposition time → agglomerates present in suspension
- Same behavior for ultrafine $BaSO_4$ [5]

ESI vs. drop-on-grid (bi-modal SiO_2 - ERM FD102)



- deposition of particles without agglomeration
- no artifacts due to stabilizer
- more larger particles in center of TEM grid (higher inertia)
- deposition efficiency: unknown due to unknown concentration of certified suspension

ESI deposition efficiency (Tri-modal (50 + 100 + 350 nm) PSL)



Particle modes /nm	*Nr. of sprayed particles*	*Nr. of deposited particles*	***Deposition efficiency (%)***
350	*296.800*	*320.533*	***108***
100	*12.740.000*	*18.351.122*	***144***
50	*65.380.000*	*54.805.011*	***84***

- known suspension number concentration → known deposition efficiency

Conclusions

- ESI: promising deposition technique for high-res imaging [6]
- Particle distribution on TEM grids: homogeneous & no losses
- Suspension must be appropriate (stabilizer may induce artefacts)
- ESI parameters need to be optimized for each material

Acknowledgement

The authors thank K Löschner & M Correia (Technical University of Denmark), E Verleysen (CODA-CERVA) for the development of the dispersion protocols for the *NanoDefine* substances as well as R Koeber (JRC-IRMM) for providing the *NanoDefine* materials.

Literature

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[4] A Gomez *et al*, 2011 Fundamentals of cone-jet electrospray. In Aerosol Measurement: Principles, Techniques & Applications, P Kulkarni et al (Eds), pp. 435–448, 3rd ed. Hoboken, NJ: John Wiley & Sons, Inc.
[5] J Mielke *et al*, 2017 *Microsc Microanal* **23**, 163–172.
[6] I Mugica *et al*, 2017 *Sci Total Environ* **609**, 349-359

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Dimensional characterization of monodisperse sub-6 nm colloidal semiconductor nanocrystals

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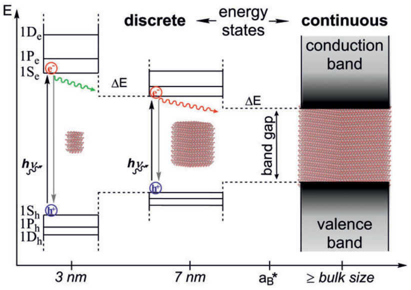
Introduction

Semiconductor nanocrystals are increasingly applied in nanotechnology thanks to their special optoelectronic behavior which varies with size, shape and surface functionalization. These applications often require monodisperse and monomorphic particles. Hence, a thorough characterization of the particles is inevitable.

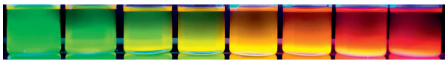


Objectives

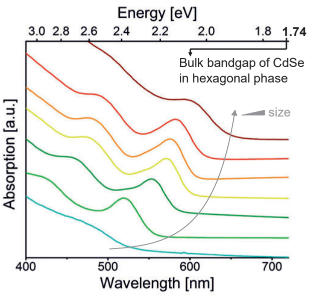
- **Synthesis** of highly monodisperse crystalline cadmium selenide (CdSe) nanoparticles as a case study.
- **Implementation** of small angle X-ray scattering (SAXS) as a high precision and more time efficient alternative to transmission electron microscopy (TEM).
- **Correlation** of the determined dimensions for each specimen with its readily accessible optoelectronic properties to develop a comprehensive model function for a metrological application and estimation of correlation uncertainties.

Methods

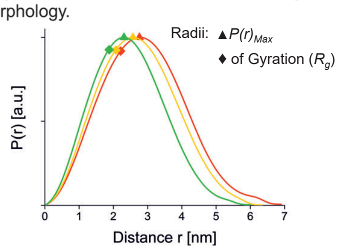
UV/VIS spectroscopy reveals the energetic states within a nanocrystal. The first absorption peak on the lower energy side describes the characteristic band gap energy. Undergoing a *Stokes* shift, the fluorescence can be detected at slightly higher wavelengths.

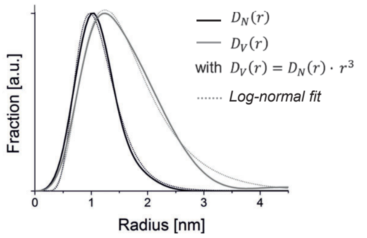


Excitation of CdSe ($D_{Mean,N}$ from 3.0 to 4.9 nm) at λ = 365 nm.

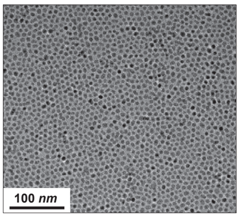


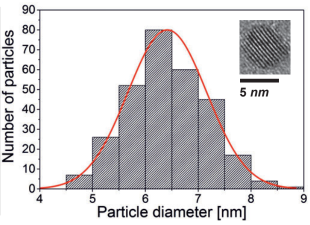
SAXS detects the radiation which has been scattered by the particles and translates the signal model free via an indirect Fourier transformation into real space. The readily accessible pair distance distribution function (*P(r)*), as well as the volume (D_V) and number (D_N) weighted size distributions for spheres, give clear indications about their size, polydispersity and morphology.



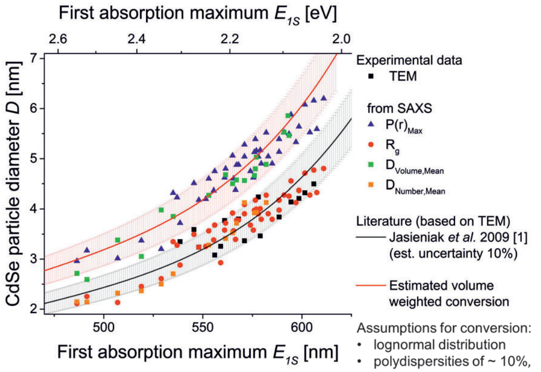


TEM visualizes the particle morphology in a 2D projection, however requires defined sample preparation for reliable size determination. In comparison with SAXS it is important to consider that the gathered size distribution is number weighted, although for point-like size distributions $D_V \approx D_N$.





Correlation and Uncertainties



Assumptions for conversion:

- lognormal distribution
- polydispersities of ~ 10%,
- $\sigma_{D_N} = \sigma_{D_V} = \sigma = 0.3$ and
- $D_{N,Median} \approx D_{N,Mean}$, then:

$$D_{V,Mean} = D_{N,Mean} \cdot e^{(3\sigma^2)}$$ [2]

Dimensional limitations:

- Absorption spectroscopy for ***ΔE_{1S}* ≥ 620 nm** is mainly limited by the progressively **bulk-like behavior** which causes an increasing overlap and broadening of transition energies.
- The decreasing electron density and scattering contrast requires **adequate sample preparation** and clearly **defined background** noise.

Uncertainties:

- The **polydispersity** strongly affects the ratio of $D_{V,Mean}$ and $D_{N,Mean}$ and the visibility of each fraction within a sample.
- It is acceptable to **transform** a number into a volume basis, while vice versa can lead to undefined **errors** and noise amplification by the factor of 10^3.

[1] Jasieniak et al., *J. Phys. Chem. C*, **2009**, 113 (45), pp 19468-19474
[2] El-Hilo and Chantrell, *J. Magn. Magn. Mater.* **2012,** *324,* 2593

Conclusions and Outlook

- SAXS has been successfully implemented in the analytical process and provides particle dimensions nearly identical to the TEM analysis and the literature.
- While the correlating model is reproducible and in good accordance with the literature, further data is required to give a more defined estimation about the measurement uncertainties.
- The assurance of a high sample quality (narrow size distribution, uniform morphology) is essential for a proper metrological characterization.
- Future work will focus on the extension of the correlating model for particles at the lower resolution border and on a comprehensive number and volume weighted model function which is able to fully describe a sample solely by absorption spectroscopy.

Acknowledgments

The authors thank the Laboratory for Nano and Quantum Engineering (LNQE), Leibniz University Hannover, for the TEM instrument and B. Temel for the measurements. We also gratefully acknowledge the support of the Braunschweig International Graduate School of Metrology B-IGSM and the DFG Research Training Group GrK1952 "Metrology for Complex Nanosystems".

IN-SITU SAXS TECHNIQUES

BAM
Bundesanstalt für
Materialforschung
und -prüfung

Q. Saadeh, B.R. Pauw , A.F. Thünemann and J. Günster

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Project's Prelude

We aim to enhance the capabilities of our upcoming Two-Photon-Polymerization (2PP) 3D printer, to let it produce arrays of precisely aligned nanoparticles. As heterogeneous functional nanostructures can be built with arrays of oriented nanoparticles; enhanced functionality can be achieved in the fields of electrochemistry, energy storage, nanoelectronics, amongst others.[1]

The feasibility of orienting nanoparticles using magnetic and electric fields is under investigation using Small Angle X-ray Scattering (SAXS). A set of ad hoc functional sample holders, sample stages and other In-Situ SAXS solutions are being developed, and incorporated in the state-of-the-art SAXS machine, the "Multi-scale Analyzer for Ultrafine Structures" (MAUS).

As a proof of concept, we started a few experiments to orient superparamagnetic nanoparticle solutions (Resovist®), where the outcome will help to understand the mechanics of field-particle interactions, and help to further evolve the adequate needed set of corrections and analyses to the SAXS data, in particular those for oriented samples.

Two-Photon Polymerization

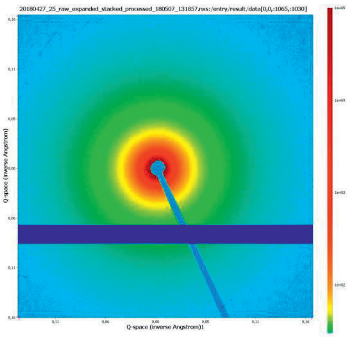
Fabrication of micro-structures with a resolution down to at least 100 nm is now feasible, where pulses of femto-second lasers can locally cure very small volumes (voxels) of photo-initiator-enhanced resins. The high-resolution in fabrication of this technology is due to the fact that two-photon absorption is a non-linear, "illegal" optical process. [1]

Resovist®

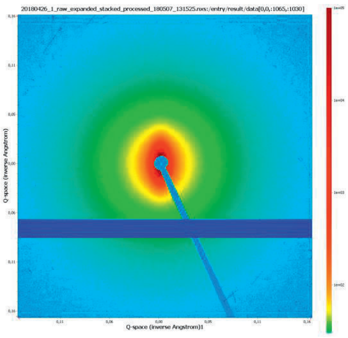
Resovist® is an organ-specific Magnetic Resonance Imaging (MRI) contrast agent. It consists of an aqueous suspension of superparamagnetic iron oxide nanoparticles coated with carboxydextran, containing 540 mg/mL Ferucarbotran, corresponding to 28 mg/mL of iron. The radii of the majority of the nanoparticles (>99%) range from 4 to 13 nm (less than 1% of the particles have radii up to 21 nm). [2]

Proof of Concept Experiments

Resovist® was selected as it contains superparamagnetic iron oxide nanoparticles (and we had it lying around), which can be magnetized easily. The sample stages developed until now consist of two cylindrical magnets, with diameter of 30 mm and height of 15 mm. The samples' response is investigated In-Situ using a flow-through capillary. [3] Future developments will include more complicated magnetic field structures, as well as more variable-field options



A



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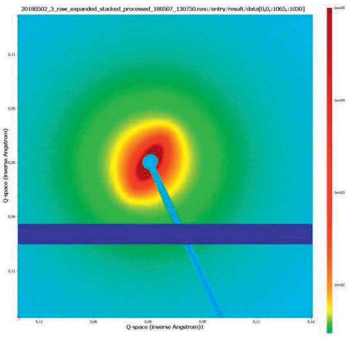

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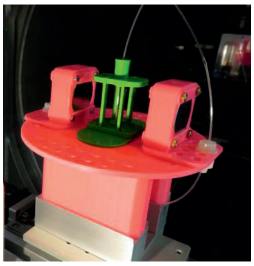

D

E

Figures A to E:

Figure A represents the SAXS pattern of Resovist® without the influence of an external magnetic field. Figure B, shows the SAXS pattern after putting Resovist® between two magnets in an attractive configuration as shown in figure D (horizontal magnetic field). Figure C shows the SAXS pattern with Resovist® placed between two tilted magnets (60°) in an attractive configuration, shown in figure E, showing no evidence of capillary forces affecting the sample. Our working hypothesis is that the Resovist® aligns in a chain conformation along the field lines.

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2 A.F. Thünemann et al, Anal. Chem. 2009, 81, 296–301.
3 Magnetism and Magnetic Materials. By J. M. D. Coey. Cambridge University Press (25 Mar 2010)

Chemical Analysis of Core-Shell Nanoparticles using ToF-SIMS and XPS

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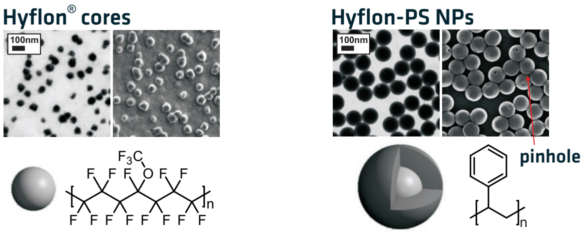
[2] Università del Piemonte Orientale, 15121 Alessandria, Italy

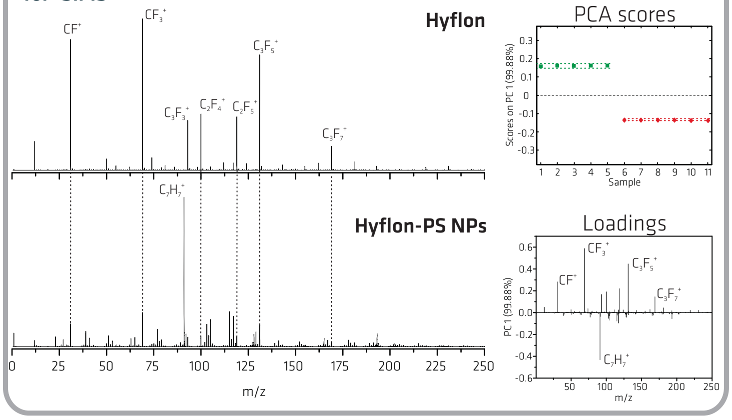
Motivation

The surface chemical composition of nanomaterials should be investigated to get a better idea and prediction of its interaction with its environment. ToF-SIMS and XPS have proven to be powerful tools to determine the general chemical composition. The superior surface sensitivity of ToF-SIMS furthermore allows us to study mainly the utmost atomic layer and thus gives us an idea of the interactions involved. Here, we present initial data on the analysis of Hyflon®-polystyrene core-shell nanoparticles (NPs) which can be used as a model system due to the known preparation and a rather good chemical separation of core and shell.

The System investigated by SEM



ToF-SIMS



Conclusion

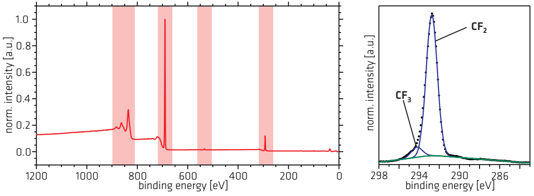
The inhomogeneity of the shell observed by EM is confirmed by XPS and ToF-SIMS due to the observed fluorine signals. Sputtering mainly shows a negative correlation between shell and core signals in ToF-SIMS. It is also interesting to see that the core signals are visible even in the spectra of the core-shell particles. This could be due to the very high ionisation yield of flourine containing signals which leads to the detection of the core-signals even when a small amount of core-shell particles are defective. Finally, the highly concentrated particles show a near to ideal point-to-point reproducibility shown by PCA. For single particle detection however, diluted particles seem to be ideal.

Outlook

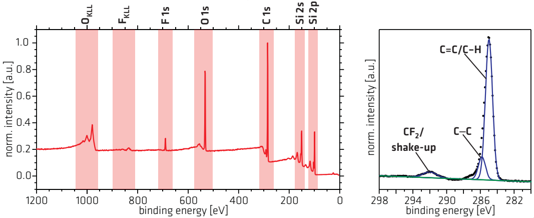
In the future, we want to verify the shown initial ToF-SIMS data by changing the sputter-ions to oxygen. The experiments will also be done with NM that has an inorganic core and an organic shell for better signal separation. The preparation is crucial for a working analysis and we have yet to find a procedure that works for our systems as seen by PCA.

XP spectroscopic investigation (hν = 1486.6 eV)

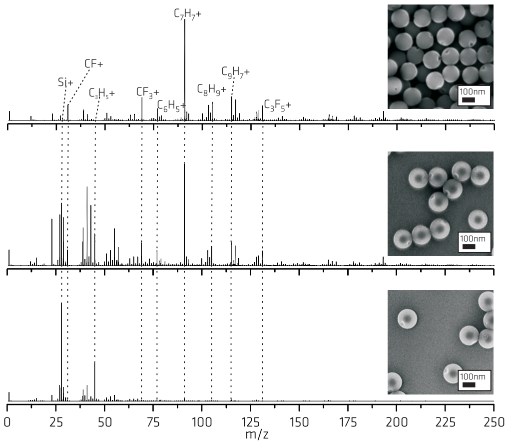
Hyflon® cores



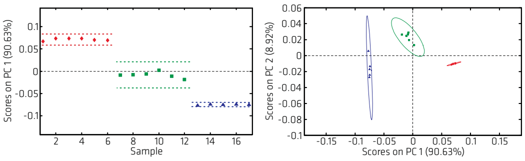
Hyflon®-PS core-shell nanoparticles



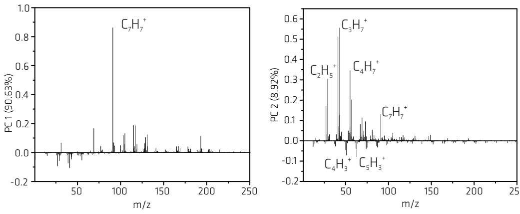
Concentration dependendent ToF-SIMS and PCA



PCA scores



Loadings



Acknowledgements
The authors want to thank S. Benemann for operating the electron microscope and are grateful to J.-M. Stockmann and Dr. J. Radnik for operating the XPS instrument at BAM 6.1. The authors acknowledge financial support from the European Metrology Programme for Innovation and Research (EMPIR) as part of the InNanoPart 14IND12 project and from the ACEnano project both funded by the European Union's Horizon 2020 research and innovation programme.

Analytical and Characterisation Excellence

Synthesis and systematic characterization of core-muti-shell $NaYF_4$:Er^{3+}, Yb^{3+}@SiO_2@Au nanoparticles for the enhancement of fluorescence emission

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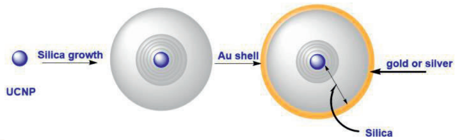
Aim of this work

In the present work, we aim to explore how far the upconversion nanoparticles (UCNP) emission intensity can be enhanced by the aid of plasmonic interactions using a gold shell. The distance between the UCNP core and the gold shell is varied by adding a silica spacer of different thicknesses. Different synthetic routes were used until the final desired thickness was achieved.

Introduction

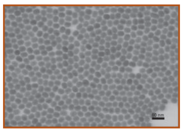
UCNP are widely used fluorescent reporters in biological applications and in vivo imaging.[1,2] due to high photostability, specific and narrow emission bands, long luminescence lifetimes, and low toxicity. However, the use of these nanoparticles requires a surface modification to render UCNPs water dispersible. This is often done with silica coating which leads to fluorescence quenching. To enhance the fluorescence, a gold shell cab be used.

In this work, multi step silica coatings were performed followed by adding a gold shell on the nanoparticles as shown in the following figure:.

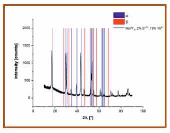


Results

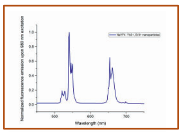
In this work, **β**-phase UCNP consisting of $NaYF_4$ as the host crystal, containing 18% Yb^{3+} and 2% Er^{3+} as sensitizer and activator, respectively, were used. The monodisperse luminescent UCNP with a diameter of 30 nm were synthesized via a thermal decomposition procedure.



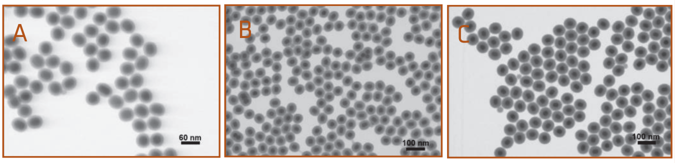
STEM image of $NaYF_4$ doped with 18% Yb^{3+} and 2% Er^{3+}. The average diameter: 30 ± 1 nm.



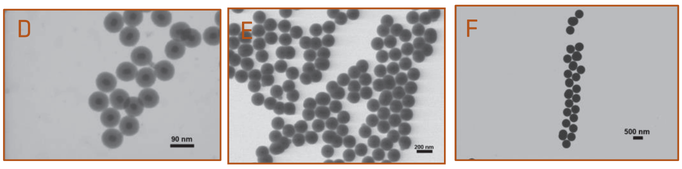
XRD measurements of the UCNP confirm that the crystal structure of the particles is β-phase.



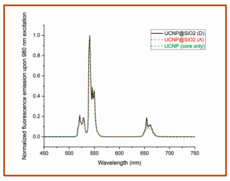
Emission of the UCNP in cyclohexane upon excitation at 980 nm.



Stepwise silica coating of the UCNP. A, The total average diameter is 47 nm and the average shell thickness is 8.5 nm. B, The total average diameter is 57 nm and the shell thickness is 13,5. C, The total average diameter is 74 nm and the shell thickness is 22 nm.



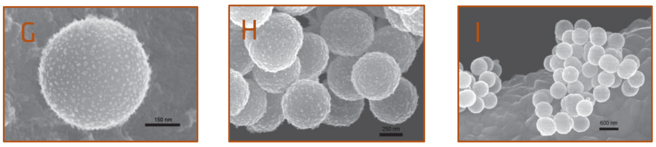
D, The total average diameter is 80 nm and the shell thickness is 25 nm. E, The total average diameter is 178 nm and the shell thickness is 74 nm. F, The total average diameter is 472 nm and the shell thickness is 221 nm.



Emission specta of UCNP sample (core only), UCNP@SiO_2 (A) with an average silica shell thickness of 8,5 nm, and UCNP@SiO_2 (D) with an average silica shell thickness of 25 nm.

Gold coating for plasmonic enhancement

Gold clusters are formed on the APS functionalized UCNP@SiO_2 nanoshells through reduction of $HAuCl_4$.[3] Semi-closed or closed gold nanoshells are formed upon further reduction using a procedure published by Graf et. al..



G. UCNP@SiO_2@small Au clusters, the total SEM diameter is 472 nm. H & I are UCNP@SiO2@Au closed shell the total SEM diameter is 522 nm, the UCNP@SiO_2 total diameter is 472 nm and the diameter of UCNP core is 32 nm. The average gold shell thickness is 25 nm.

Conclusion and outlook:

The synthetic conditions for obtaining UCNP@SiO_2@Au core-shell nanoparticles with precisely tuneable silica shell thicknesses were investigated. A gold shell on the UCNP@SiO_2 nanoparticles is expected to give rise to a noticeable enhancement of particle brightness and fluorescence, given that the thicknesses of the silica shell and the gold coating can be controlled and fine-tuned. First single particle studies revealing shortening of the Er^{3+} lifetimes suggest that plasmonic enhancement occurs.

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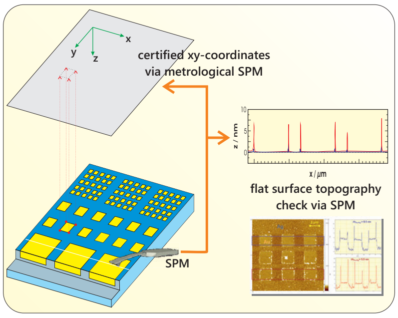
Poster Presentations
May 15

Analytical and topographical Reference Material for the Nanoscale

I. Busch [1], S. Bütefisch [1], O. Lenck [1], L. Koenders [1], W. Unger [2], T. Weimann [1], M. Weinert [1]

Introduction

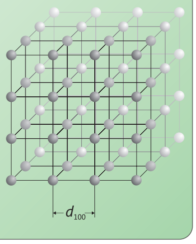
- Reliable standards are required to support research and development as well as end-user in application
- Appropriate standards have to fulfill three requirements: small uncertainty, easy to use and low overall costs of application
- For calibration of microscopes at nanoscale and/or element analysis special requirements for standards are given, which are challenging in manufacture



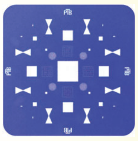
Crystalline sub-Nanometer Standards

Invariants of nature and self-organisation

- Crystalline lattice is forming a regular pattern with highly reproducible and accurate spacing (invariant of nature)
- Self-organisation is used to propagate the lattice planes to the surface of standards
- the formation of mono-atomic steps is controlled by appropriate processing parameter



Production of mono-atomic step height standards with large terraces



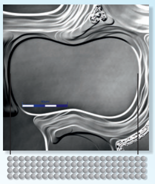
Lithographic preparation

Self-organisation by annealing

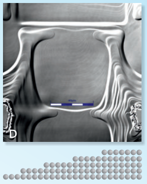
Atomic steps

Results atomic step height standards

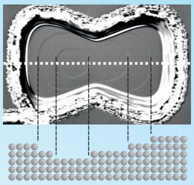
- smallest height is reduced from 6 nm to 0.3 nm
- uncertainty is reduced by a factor of 20
- reduction of cost (production/application)
- a priori information of standards can be used to simplify application of standards
- automatisation of data evaluation is in preparation



Atomically Flat Surface



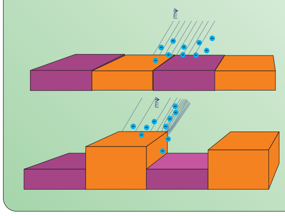
Staircase Structure



Amphi Theater Structure

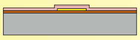
Element Specific NoStep Lateral Standards

Surface analysis with electrons

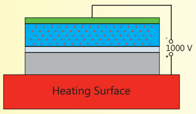


- Lateral resolution is sensitive to vertical steps at inlay boundaries
- Additional electron emmision through side walls is leading to lateral crosstalk

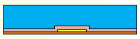
Manufacture of NoStep Standards



- Lithographic preparation of inlay pattern
- Wafer surface serves as reference plane

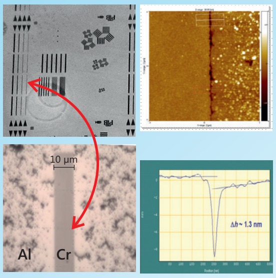


Anodic bondig of pyrex wafer



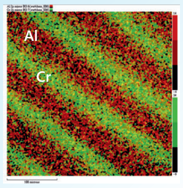
Initial wafer is removed by etching

Results for NoStep



- Lateral standard for element sensitive imaging (e.g. iXPS)
- Upside down manufacture for step reduction
- Reduction of topographic steps between metallic inlays by a factor of five

The lateral crosstalk due to large steps at inlay boundaries is successfully removed at *NoStep* standards, which is confirmed by iXPS measurements.



Acknowledgement

This work was performed in the project *Crystalline surfaces, self assembled structures and nano-origami as length standards in (nano)metrology (CRYSTAL)*, which is jointly funded by the EMRP participating countries within EURAMET and the European Union.

This work was performed in the project *Traceable quantitative surface chemical analysis for industrial applications (SurfChem)*, which is jointly funded by the EMRP participating countries within EURAMET and the European Union.

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Directed self-assembly of Block Copolymers for Lateral Length Standards at the Nanoscale

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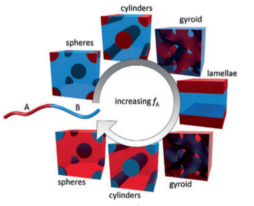
NanoFacility Piemonte, Istituto Nazionale Ricerca Metrologica, Strada delle Cacce 91, 10135 Torino, Italy

Introduction

The direct self-assembly (DSA) of block copolymers (BCPs) is widely used as patterning and nanofabrication technique, combining the top-down and bottom-up approaches to the BCP capability of phase separate into the small size and high-density features of different shape. These characteristics suggest the possibility of using the BCPs in order to address the gap in nano-structured lateral standards for nano-metrology, consequently supporting the miniaturization processes involved in the semiconductor industry and in nanostructured device fabrication.

BCP Synthesis

Self-Assembly

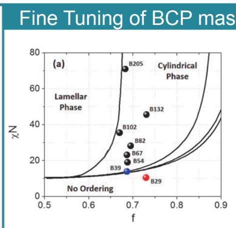


Rapid Thermal Processing

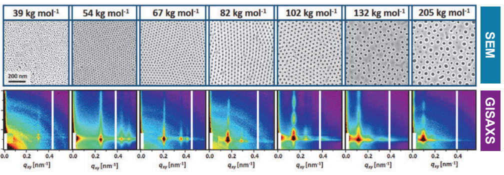
International Application No.: *PCT/IB2014/061324*

Ferrarese Lupi et al. **Nanotechnology**, 24, 315601, 2013

Fine Tuning of BCP masks

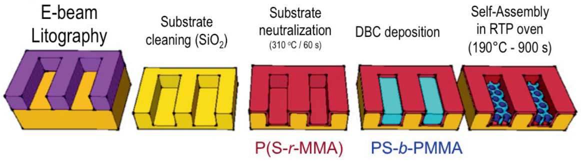


Sample	*h* (nm)	T_A (°C)	*d* (nm)	$L_{0\,SEM}$ (nm)	$L_{0\,GISAXS}$ (nm)
B39	33	180	12.0 ± 2.0	24.0 ± 1.0	23.5 ± 0.2
B54	29	200	13.0 ± 1.0	28.8 ± 0.5	28.3 ± 0.3
B67	27	250	17.0 ± 1.0	35.0 ± 1.0	34.4 ± 0.4
B82	34	250	19.0 ± 2.0	42.9 ± 0.7	43.9 ± 0.7
B102	33	230	22.7 ± 1.5	47.0 ± 1.0	48.7 ± 0.9
B132	28	240	28.6 ± 1.6	59.0 ± 4.0	62.1 ± 1.4
B205	35	240	30.0 ± 4.0	73.0 ± 8.0	74.0 ± 2.0

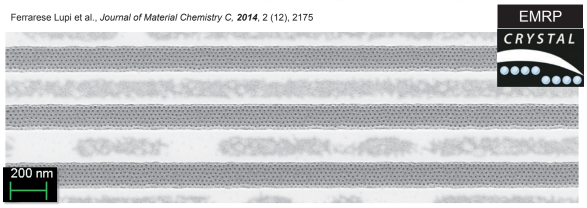


Ferrarese Lupi et al., *ACS Appl. Mater. Interf.* **2014**, 6, 7180-7188

Directed self-assembly

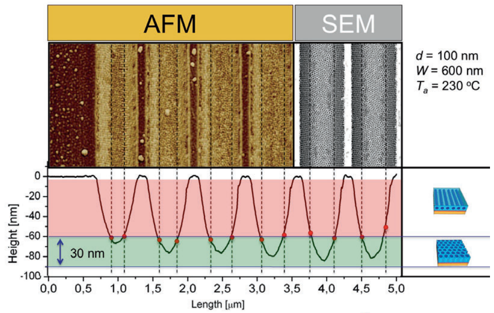


Ferrarese Lupi et al., *Journal of Material Chemistry C*, **2014**, 2 (12), 2175

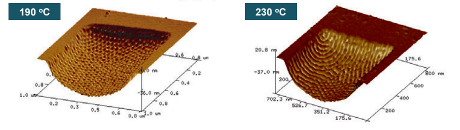


Ferrarese Lupi et al., *ACS Appl. Mater. Interf.* **2014**, 6, 21389

Morphological Analysis

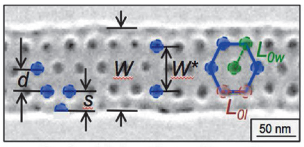


d = 100 nm
W = 600 nm
T_a = 230 °C



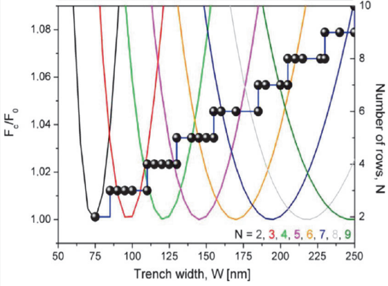
Ferrarese Lupi et al. **ACS Appl. Mater. & Interfaces** 7, 23615, **2015**

L_0 Measurement



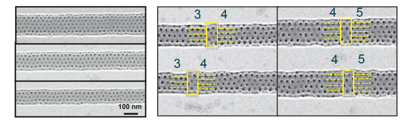
$$\lambda = \frac{W}{NL_0}$$

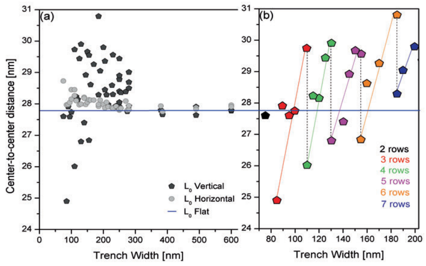
$$\frac{F_c}{F_0} = \frac{1}{3}\left[\lambda^2 + \frac{2}{\lambda}\right]$$



COMMENSURATED TRENCHES

NOT COMMENSURATED TRENCHES





Aprile et al. **ACS Appl. Mater. & Interfaces** 9, 15685, **2017**

AFM Calibration

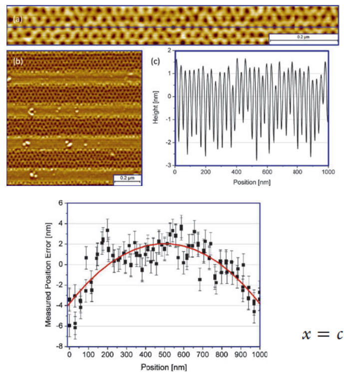
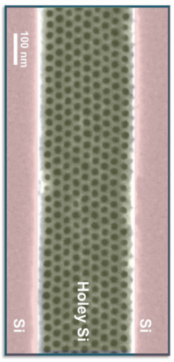


Table 1. Input Parameters and Associated Uncertainty for the Estimation f the Linear Correction Factor $c(\Delta x')_{xx}$ for a Scan Length of $\Delta x' = 1\ \mu m$

quantity (unit)	x_i	$u(x_i)$
observed average center-to-center distance L_0^{Obs} (nm)	27.70	0.01
average center-to-center distance of the DBCs L_0^{GISAXS} (nm)	28.30	0.30
deviation due to the limited number of sampled distances δ_{Samp} (nm)	0	0.20
deviation due to tip shape and friction δ_{Tip} (nm)	0	0.07
deviation due to drift and experimental control parameters δ_{Exp} (nm)	0	0.11
deviation due to leveling and image processing $\delta_{ImagPro}$	0	0.05
linear correction parameter $c(\Delta x')_{xx'}$	1.02	0.014

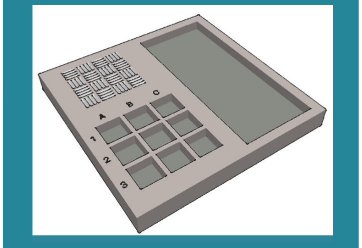
$$x = c(\Delta x')_{xx'}x' + c(\Delta x')_{xx'^2}x'^2 + \cdots \qquad c(\Delta x')_{xx'} = \frac{L_0^{GISAXS}}{L_0^{Obs}}$$

Pattern Transfer



Length Standards for nano Metrology

Patent Title: DISPOSITIVO CAMPIONE DI RIFERIMENTO PER LA CALIBRAZIONE DI MISURE DI LUNGHEZZA E RELATIVO PROCEDIMENTO DI CALIBRAZIONE



Italian Application No. 102016000052889
23/05/2016

Acknowledgments

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Physikalisch-Technische Bundesanstalt
Braunschweig und Berlin
National Metrology Institute

Reference-free GIXRF-XRR based qualification of nanolayers and nanostructures as potential calibration samples

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Introduction

In most cases, bulk-type or micro-scaled reference-materials do not provide optimal calibration schemes for analyzing nanomaterials as e.g. surface and interface contributions may differ from bulk, spatial inhomogeneities may exist at the nanoscale or the response of the analytical method may not be linear over the large dynamic range when going from bulk to the nanoscale. Thus, we have a situation where the availability of suited nanoscale reference materials is drastically lower than the current demand.

Reference-free XRF

Reference-free X-ray fluorescence (XRF), being based on radiometrically calibrated instrumentation, enables an SI traceable quantitative characterization of nanomaterials without the need for any reference material or calibration specimen. This opens a route for the XRF based qualification of calibration samples.

Sherman equation for K fluorescence

$$I_{i,j} = I_0(E_k)\varepsilon_{eff}(E_{i,j})\frac{d\Omega}{4\pi}\, e^{-\mu_s(E_k)\rho x/\cos(\Psi_1)}$$

absorption

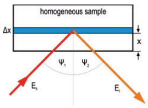
$$W_i \tau_i(E_k)\omega_i T_{i,j}\frac{1}{\cos(\Psi_1)}\Delta x$$

fluorescence production

$$e^{-\mu_s(E_{i,j})\rho x/\cos(\Psi_2)}$$

absorption

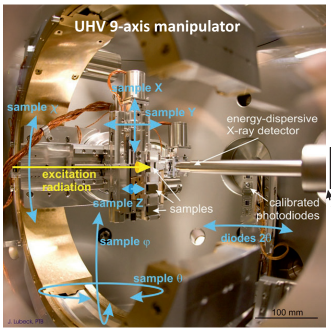
instrumental parameters:
$\varepsilon_{eff}(E_{i,j})$, $d\Omega/4\pi$
atomic fundamental parameters:
μ_S, τ, ω_i, T_i, ρ
specimen composition
W_i weight fraction of element i



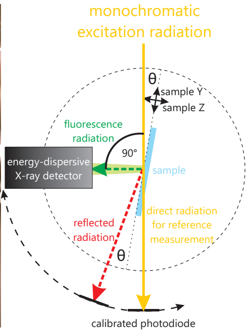
J. Anal. At. Spectrom. (2008) **23**, 845 - 853

Experimental capabilities

• TXRF, GIXRF and XRF	Accessible photon energy range:
• NEXAFS / XANES	80 eV – 1875 eV @ PGM beamline
• EXAFS	1.740 keV – 10.5 keV @ FCM beamline
• XRR	6.5 keV – 80 keV @ BAMline



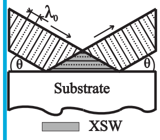
View into the experimental chamber



Sketch of the experimental setup

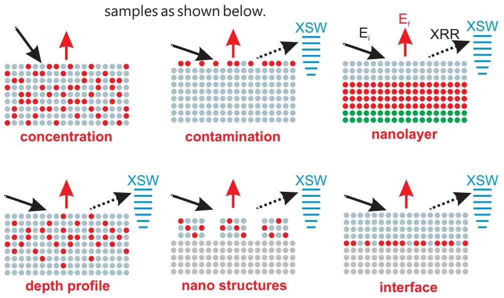
Rev. Sci. Instrum. (2013) **84**, 045106

Reference-free XRF in grazing incidence geometry



Principle of GIXRF

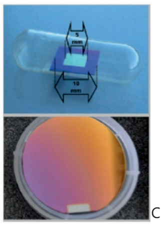
Grazing incidence XRF is based on a variation of the incident angle of the exciting radiation. Due to the interference between incident and reflected beam an X-ray standing wave field (XSW) arises and strongly modifies the local intensity. By scanning the angle, the depth dependent changes of the XSW can be used as a nanoscaled depth sensor in order to gain dimensional information about the sample. In conjunction with the reference-free setup, this can be used to reveal quantitative information about different types of samples as shown below.



Sketch of the different accessible sample types for characterization with reference-free GIXRF

Materials (2014) **7(4)**, 3147-3159

Qualification of nanolayer calibration samples

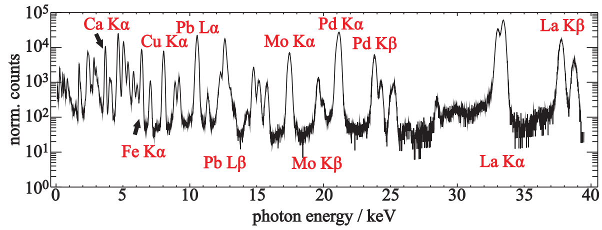


Several types of nanolayer calibration samples were fabricated and qualified using reference-free XRF techniques. Multi-elemental layer and single element sub-monolayer samples for various applications in XRF and TXRF analysis on silicon nitride membranes and silicon wafers were studied. Both types are very close to an ideal calibration sample (negligible self-attenuation, no secondary excitation).

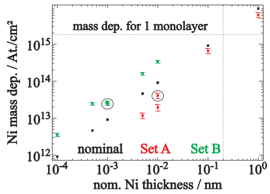
Calibration samples for XRF and TXRF applications

	Lineset	E_0	Sample C_0	Sample $10C_0$	Ratio $\frac{10C_0}{C_0}$
La	**L3**	**5.75**	12.9 (1.1)	122.7 (10.2)	9.5 (1.2)
Pd	**L3**	**5.75**	4.3 (0.4)	36.6 (2.7)	8.5 (1.0)
Mo	**L3**	**5.75**	0.56 (0.07)	5.0 (0.4)	8.9 (1.3)
Fe	**K**	**13.5**	4.53 (0.31)	40.3 (2.8)	8.9 (0.9)
Cu	**K**	**13.5**	2.4 (0.2)	22.6 (1.7)	9.4 (1.0)
Ca	**K**	**5.75**	18.7 (1.6)	171.9 (14.2)	9.2 (1.1)
Pb	**L3**	**13.5**	7.1(0.5)	74.3 (5.2)	10.5 (1.1)
		keV	**ng mm $^{-2}$**	**ng mm $^{-2}$**	

SI traceable quantification of the mass depositions

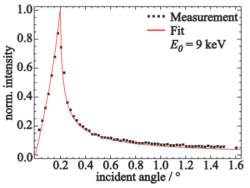


Wide selection of coating elements to enable calibration in a broad energy range



Broad range of available surface coverages far below a single monolayer

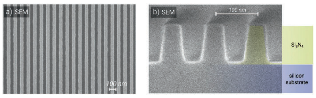
In cooperation with AXO Dresden GmbH



Ultra-thin layer-like elemental distribution without agglomeration and crystallization artefacts for TXRF calibration

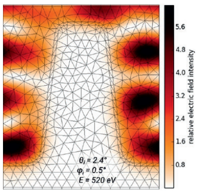
Spectrochimica Acta B (2018) **145**, 36-42

Qualification of nanostructure calibration samples - 2D objects

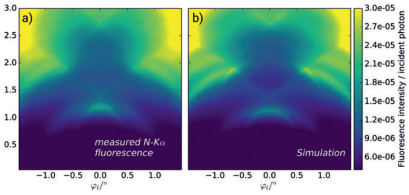


SEM images of the characterized Si_3N_4 grating structure

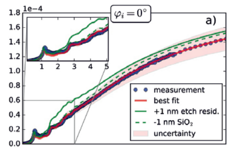
In a second example, we work on the development of nanostructures as calibration samples. Several lithographic 2D grating structures have been fabricated and characterized using the reference-free GIXRF methodology of PTB. Here, an advanced calculation scheme based on the finite element method for the intensity distributions within the X-ray standing wave field (XSW) is required. In addition to the traceable quantification of elemental mass depositions, this allows for a determination of in-depth elemental distributions and the dimensional properties of the nanostructures.



FEM calculation of the XSW intensity distribution



Comparison of an FEM calculated fluorescence intensity map to the experimental data as a funcion of the incident and the azimuthal angle



Comparison of the experimental data to the FEM calculated data

Nanoscale (2018) **10**, 6177-6185

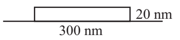
Qualification of nanostructure calibration samples - 3D objects

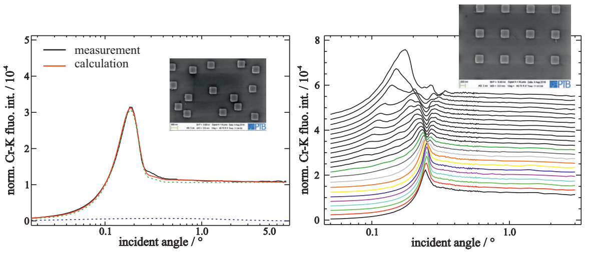
In principle, the methodology is also well suited to characterize 3D nanostructures for calibration sample applications. Here, electron beam lithography was used to fabricate regular and irregular ordered chromium pads with nominal sizes of 300 x 300 x 20 nm³. They were also characterized using the reference-free GIXRF methodology of PTB.

Here, the experimental data from the irregular sample is modeled using the effective density approach. The nanostructures are approximated as a layer of Cr with reduced density. The density can be calculated as a function of the dimensional parameters of the structures.

The regular structures also show a strong dependence on the azimuthal angle. However, they cannot be modeled using the effective density approach requiring also the FEM based technique. As this results in a much larger computational effort as compared to the 2D gratings, it could not be performed so far. Due to the high amount of features in the data, we expect a high sensitivity for both the dimensional parameters and the elemental distributions.

Schematic view of nano-squares





Comparison of an experimental GIXRF curve for irregularly and regularly ordered chromium nanoblocks (see insets). For the irregular blocks also a calculated GIXRF signal assuming ideally shaped rectangles is shown.

Phys. Status Solidi A (2018) **215**, 1700866

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This work was funded through the European Metrology Research Programme (EMPIR) from the Projects „3dMetChemIT“ and „3DStack“. The European Metrology Programme for Innovation and Research (EMPIR) is jointly funded by the participating countries within EURAMET and the European Union.

OECD Test Guideline on Particle Size and Particle Size Distribution of Manufactured Nanomaterials: Simultaneous Measurement of Length and Diameter of Fibers

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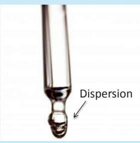
[c] German Environment Agency (UBA), Wörlitzer Platz 1, 06844 Dessau-Roßlau

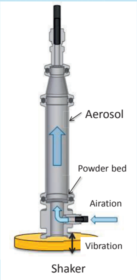
Introduction

The particle size distribution is considered the most relevant information for nanoscale property identification and material characterization. In order to measure particle size distributions, a plenitude of techniques are available. Most of them rely on the assumption of spherical particles or are not capable to measure the diameter and length of elongated particles simultaneously. Such techniques are therefore not applicable to nanoscale fibers, i.e., materials with a diameter below 1000 nm and a length-to-diameter ratio greater than 3. For fibrous materials an imaging technique (SEM, TEM) has to be applied in order to perform a morphological analysis and to obtain the material characterization required for regulatory purposes.

Sample preparation

- ➢ Different methods: s. picture
- ➢ Protocol depends on material
- ➢ Can lead to large errors



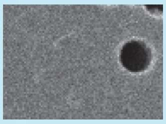


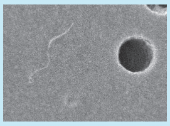
- ➢ TG will contain quality criteria for sample preparation
  - ▪ high single fiber content
  - ▪ Preparation must not change the sample!

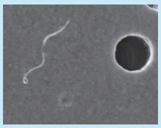
Image acquisition

Resolution of the methods

- ▪ SEM: Resolution > 5 nm
- ▪ TEM : Resolution < 1nm


pixel size = 12.4 nm


pixel size = 8.3 nm

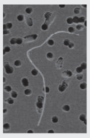
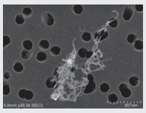


pixel size = 0.5 nm

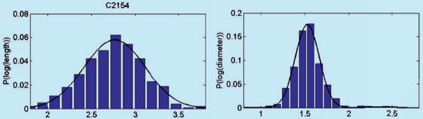
- ➢ Pixel size should be half of the smallest diameter to be analyzed or smaller
- ➢ Brightness and contrast have to be optimized for fiber detection

Data evaluation

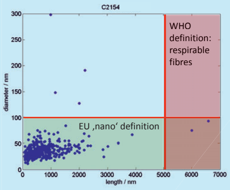
Differentiation between morphological classes:

Class I: single fibres	**Class II**: Cluster, Aggregates/ Agglomerates
	

- ➢ Length and diameter distributions are determined for single fibers



- ➢ Concurrent determination of length and diameter for regulatory context necessary



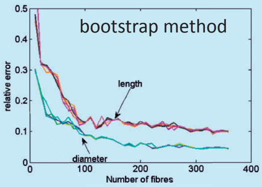
Protocol for measurement of length and diameter distribution of fibers with SEM/TEM

Uncertainty analysis

Sources of errors in data evaluation:

- ▪ Statistical sampling
- ▪ 2D projection of 3D objects
- ▪ Classification error
- ▪ Differences between evaluators

- ➢ Error estimation for median:

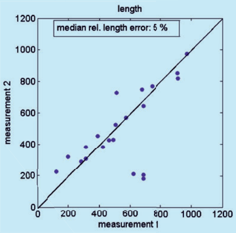


About 200 fibers have to be counted.

- ➢ 2D projection of 3D object

Fiber length is underestimated.

- ➢ Different evaluators



- ▪ Rel. Error (length) < 10 %
- ▪ Rel. Error (width) > 20 %

SOP Validation: Round Robin Test

Goal:

- ▪ Validate SOP for the method
- ▪ Determine interlaboratory reproducibility
- ▪ Planned for 2018/2019

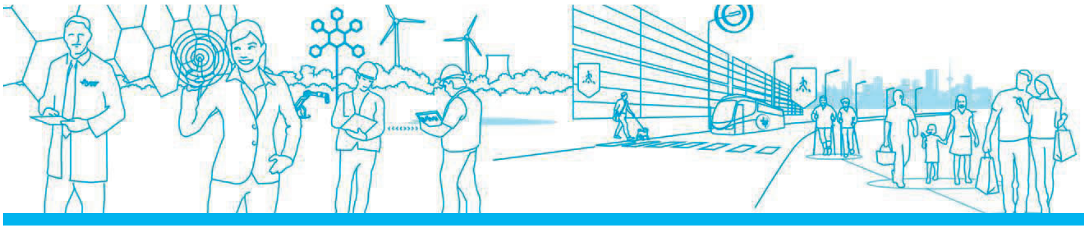
Materials:

	material	preparation	length / µm	diameter / nm	method
1	MWCNT	Aerosol	0.60	33	SEM
2	SiC	Dry	10 - 100	100 – 600	SEM
3	NanoAg	Dispersion	20-60	120	SEM
4	TiO_2	Aerosol	5-10	10	SEM/TEM
5	SWCNT	Dry	5-50	<2	TEM

Conclusion and Outlook

For harmonizing the determination of the length and width distributions of fibers the new OECD test guideline will address:

- ➢ sample preparation (optimized for each material, general quality criteria given)
- ➢ image acquisition (appropriate resolution to picture fibres as a whole)
- ➢ data evaluation (determine diameter and length concurrently → applicability of different regulatory definitions) and
- ➢ uncertainty analysis (quality of the results critically depend on the sample preparation and data evaluation)
- ➢ classification rules have to be formulated and followed accurately in order to optimize reproducibility of the method
- ➢ SOP will be validated in an international round robin test, planned for 2018/2019
- ➢ Reference materials needed for validation and quality control



TECHNOLOGY FOR BUSINESS

PillarHall LHAR structure for thin film conformality measurements

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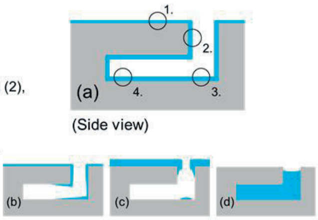
[2]Aalto University, Finland

1. Conformal film covers complex 3-D structures with same thickness and properties

Conformal film, schematic example:

a. Optimized ALD
→ Same film top (1), sidewall (2), bottom (3), deep within (4)

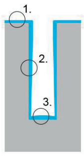


Partly conformal & non-conformal films, schematic examples

b. Partly conformal, typical for CVD
c. Noncormal with overhang, from line-of-sight deposition
d. "Superconformal", i.e., preferential filling inside the 3-D feature

2. Traditional way of measuring conformality: cross-sections of trenches etched into silicon

Aspect ratio (height/width, not in scale) max ~50:1



Vertical HAR procedure:

Measure thickness
1. Top
2. Sidewall
3. Bottom

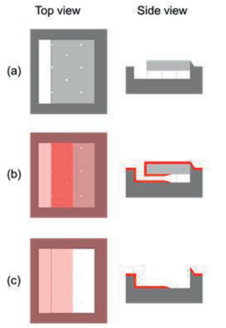
If thinner in trench
→ conformality <100%

Calculate conformality:
- Bottom/top xx%
- Sidewall/top yy%

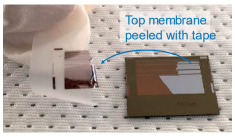
- Measurement takes time & effort, accuracy operator-dependent
- Point-per-point analysis, wafer mapping quasi-impossible

3. The PillarHall® approach

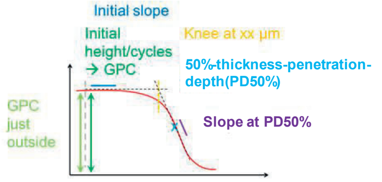
"Turn your thinking 90 degrees"



Analysis after removal of top membrane



LEFT: Schematic representation (not in scale): (a) uncoated structure, (b) structure coated with a film, and (c) coated structure, with top membrane removed.



Experimental parameters to be recorderd from thickness line scans

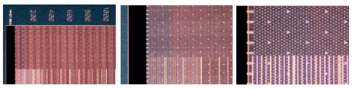
4. Internal length scale

- There are two kind of distance indicators on the chips.
  - Distances of the pillars ("dots" in figures).
  - Distance indicator lines processed on the top membrane
- The pillars are visible also when the membrane is removed.
- Distances between the pillars are known.

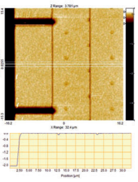
Distance indicator lines
- Easy and accurate determination of the film penetration depth.
- Unique pattern structure
  - Measurement possible also when the opening of the structure and the end of the film can not be observed at the same time.

5. Results

RIGHT.
- *Dark field microscope images of chip coated with 50 nm thick Al_2O_3 grown by ALD*
- *Gap height 500 nm*
- *Penetration depth ~130 µm*



- Line position calibration by QuickVision (QV) optical coordinate measuring machine.
- The lines near the opening are measured using atomic force microscope (AFM).
- The combination of two methods reduces uncertainty caused by the detection the zero point. i.e. the edge of the membrane.



LEFT.
- *An AFM image of the first two lines (top).*
- *An averaged line profile showing the scale lines (down).*
- *The averaging area is shown in the AFM image.*

Uncertainty estimates
- Standard uncertainty u_c = 0.3 µm (QV).
- u_c = 0.1 µm (AFM)
- The main uncertainty components:
  - Detection of the lines (QV & AFM)
  - Tip size (AFM)

6. Traceability

VTT MIKES is a national metrology institute (NMI) in Finland, thus the measurements have in-house traceability to the definition of the metre.

7. Conclusion

- PillarHall® test structures transform the way conformality analysis is made, enabling quick quantitative analysis of thin film conformality
- Calibrated internal length scale processed on top of the membrane allows traceable distance measurement.
- Chips are available at VTT. We are interested in research cooperation.

References

[1] F. Gao, S. Arpiainen, R. L. Puurunen, J. Vac. Sci. Technol. A (letter) 33 (2015) 010601. Microscopic silicon-based lateral high-aspect-ratio structures for thin film conformality analysis. http://dx.doi.org/10.1116/1.4903941

[2] M. Mattinen, J. Hämäläinen, F. Gao, P. Jalkanen, K. Mizohata, J. Räisänen, R. L. Puurunen, M. Ritala, M. Leskelä, Langmuir 32 (2016) 10559-10569. Nucleation and Conformality of Iridium and Iridium Oxide Thin Films Grown by Atomic Layer Deposition. http://dx.doi.org/10.1021/acs.langmuir.6b03007

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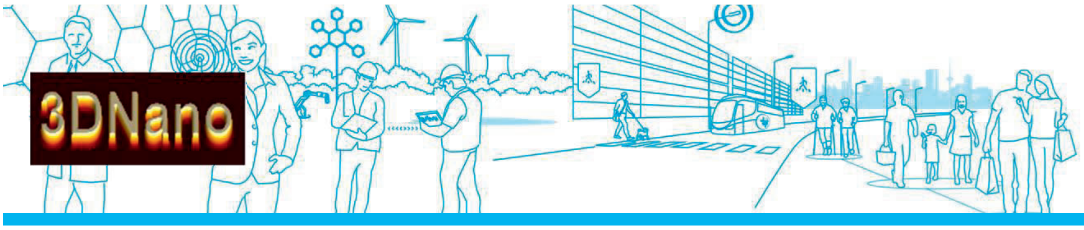

3DNano

MIKES METROLOGY

Traceable 3D nanometrology

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[1] VTT, Finland, [2] CMI, Czech Republic, [3] DFM, Denmark, [4] NPL, United Kingdom, [5] SMD, Belgium, [6] PTB, Germany, [7] VSL, Netherlands [8] FAU, Germany, [9] NCSR Demokritos, Greece, [10] TNO, Netherlands, [11] INRIM, Italy, [12] METAS, Switzerland,

Introduction / background

Traceability to the SI units is needed in all quantitative measurements. Currently there is insufficient traceability to the SI metre for true 3D nanomeasurements, because the existing level of uncertainty in measurements (5 nm) does not meet the requirements of industry and scientific research. 3D metrology differs from one dimensional (1D) height and pitch metrology, where the impact of tip geometry and the tip sample interaction at the top/bottom planes are self-compensating. Instead, measurements of 3D structures are bi-directional, where the effect of tip geometry and tip sample interactions at the left and right sidewalls are different.

Objectives

1. Reduce uncertainty < 1 nm.
2. Widen understanding of tip sample interaction.
3. Reference material for 3D nano.
4. Improvement of MAFMs: the noise level, scanning speed, and range.
5. Hybrid metrology: data fusion & instrument fusion.

Traceability routes for 3D nanomeasurements

Bottom-up approach

- Based on the cross-section of TEM structures with atomic resolution, and combined optical interferometer and x-ray interferometry.
- The critical dimension (CD) can be directly linked to the atomic spacing in the crystal lattice.

Top-down approach

- Tools and measurement procedures.
- The noise level, scanning speed, and range of the MAFMs.

Reference materials

- New tip characterizer and standards suitable for hybrid metrology were designed and will be characterized.
- Nanoparticle samples are selected, purchased and will be tested.

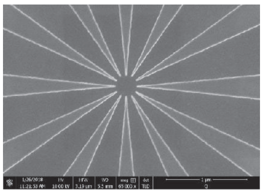


Figure 1: Siemens star structure for tip characterization. (TNO)

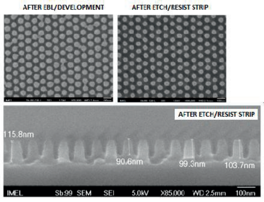


Figure 2: Hexagonal structure optimized for both scatterometry and AFM/SEM methods. (NCSR Demokritos)

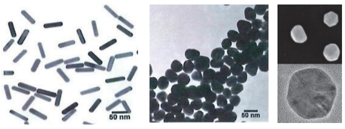
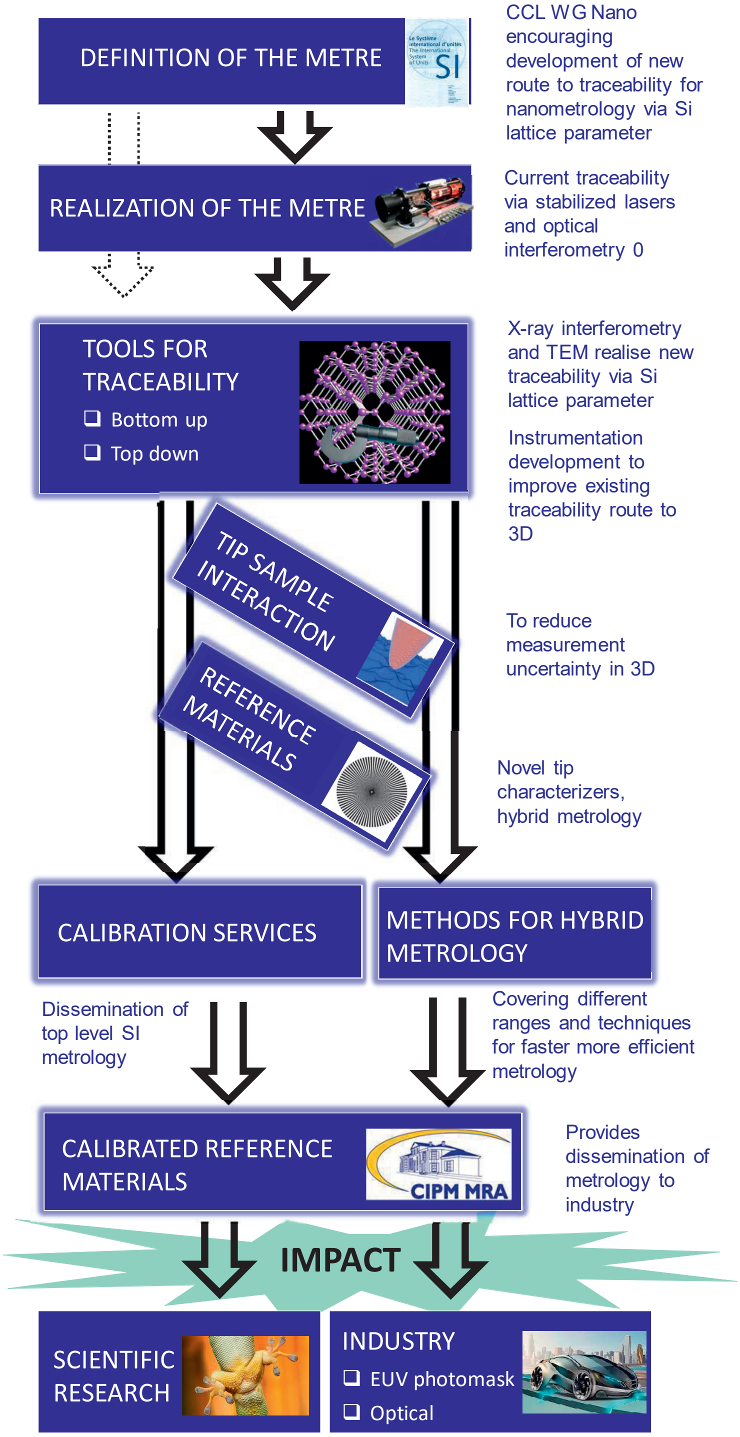


Figure 3: Nanoparticle samples. Gold Nanorods 58 nm x 16 nm (nanoComposix), Silver Nanospheres (nanoComposix), Gold Nanoparticles 25 nm (NIST)



DEFINITION OF THE METRE

CCL WG Nano encouraging development of new route to traceability for nanometrology via Si lattice parameter

REALIZATION OF THE METRE

Current traceability via stabilized lasers and optical interferometry 0

TOOLS FOR TRACEABILITY
❑ Bottom up
❑ Top down

X-ray interferometry and TEM realise new traceability via Si lattice parameter

Instrumentation development to improve existing traceability route to 3D

TIP SAMPLE INTERACTION

To reduce measurement uncertainty in 3D

REFERENCE MATERIALS

Novel tip characterizers, hybrid metrology

CALIBRATION SERVICES

METHODS FOR HYBRID METROLOGY

Dissemination of top level SI metrology

Covering different ranges and techniques for faster more efficient metrology

CALIBRATED REFERENCE MATERIALS

Provides dissemination of metrology to industry

IMPACT

SCIENTIFIC RESEARCH

INDUSTRY
❑ EUV photomask
❑ Optical

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EMPIR EURAMET
The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States

The need of a reference for APT-AFM tip reconstruction

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Corresponding author: Jonathan.OpdeBeeck@imec.be

The APT-AFM concept

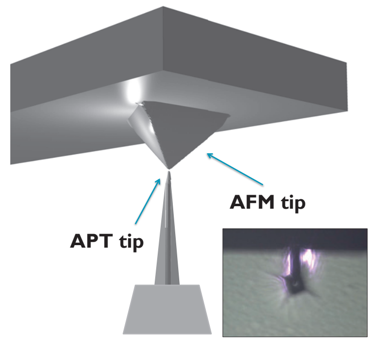


Figure 1: Schematic depicting (to scale) the APT-AFM concept. Inset shows the optical image when the tips are aligned.

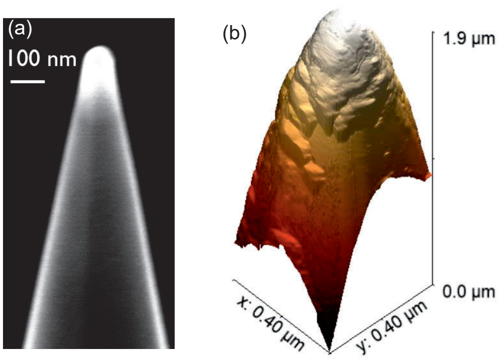


Figure 2: Determination of the atom probe tip shape through SEM (2D) as opposed to AFM (3D).

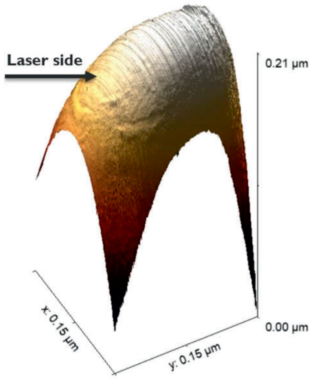


Figure 3: Asymmetric atom probe tip after laser-assisted APT analysis as measured by AFM.

The problem statement

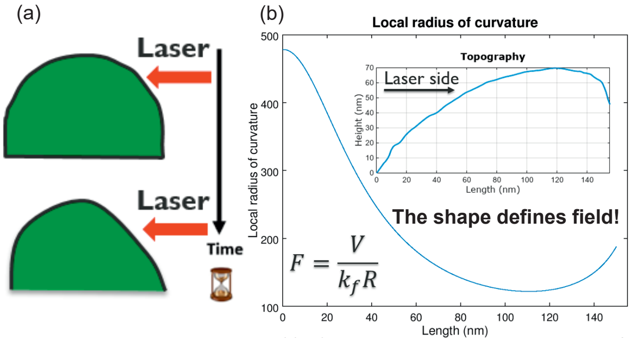


Figure 4: Non-hemispherical shape (a) of the atom probe tip as measured by AFM (b). The tip is blunted on the laser side, resulting in a larger local radius affecting the local electric field and therefore the ion trajectories.

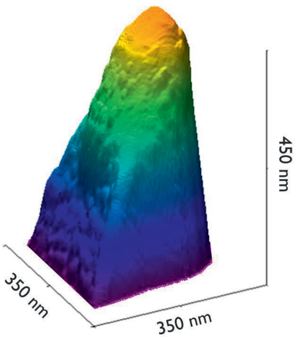
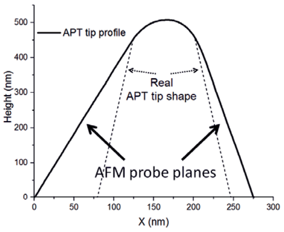


Figure 5: Convolution artifacts in APT-AFM result in an apparent larger shank angle of the APT tip. [1]

The challenge is:

- To align and measure a sharp atom probe tip with an even sharper scanning probe microscopy tip.
- Understand tip-sample interactions and convolution artefacts.
- Perform highly reliable measurements with high resolution.



Reference standard to determine AFM tip shape

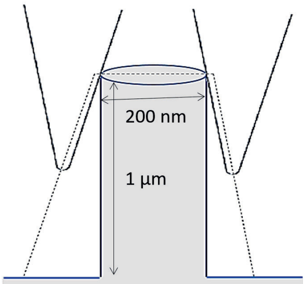


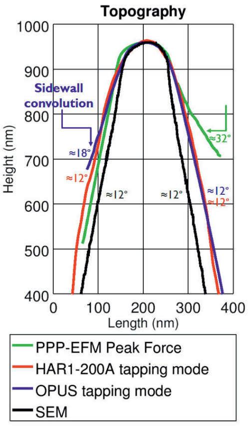
Figure 7: By measuring a well-defined reference structure the shape of the AFM probe can be retrieved.

Dedicated reference structures:

- High aspect ratio pillar.
- Tip-sample convolution is turned to the users advantage.
- Extract the shape of the AFM probe using the blind tip reconstruction method.[2]

Requirements:

- A cylindrical structure (radius≈ 100 nm) and hard material.
- Sidewalls (cone angle ≈ 0°) allow reconstruction of high-aspect-ratio (HAR) probes.
- Height should correspond to the region of interest of the APT tip (1 µm).



Imaging using different AFM probe shapes shows:

- Convolution artifacts using conventional probes.
- The correct shank angle using a fib-milled HAR-probe.

Convolution can be mitigated!

Figure 6: Line profile comparison of the APT tip shape as measured by SEM and 3 different AFM probes.

Summary

- **The challenge is to understand and resolve tip convolution artefacts.**
- **Knowledge on the tip shape is critical to asses the reliability of the AFM measurement.**

References

1. I.C Fleischmann, K Paredis et al. 2018, in review with Ultramicroscopy.
2, J.S.Villarubia 1994, Surface Science 321, 287-300.

Acknowledgments

This work has been carried out in the frame work of Project 11D5118N, funded by a SB PhD FWO grant.

Atom probe tomography (APT) holds great potential:

- High spatial resolution (~0.2-0.3 nm).
- High elemental sensitivity in 3 dimensions.
- 3D information.

Atom probe tomography principle:

- Voltage/laser pulses trigger atom-by-atom field evaporation.
- Position sensitive detector and time-of-flight mass spectrometer reveal spatial and chemical information.

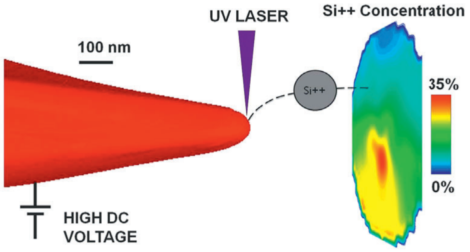


Figure 8: Schematic depiction of the working principle of atom probe tomography.

The Research of Nanometer Geometry Standard Reference Material in NIM

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National Institute of Metrology, China
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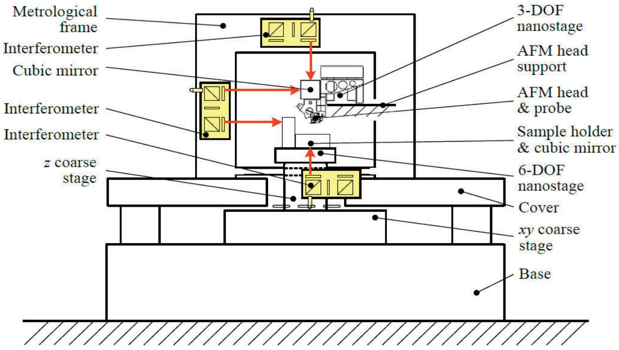
I. Introduction

Several micro/nanometer geometric dimension standard devices and corresponding certified reference materials (CRMs) are developed to guarantee the measurand accuracy，uniformity and traceability of measurement results to the International System of Units, which preliminarily established the national nanometer standard traceability systems.

II.Standard Device

A large range metrological AFM is developed.

- Measurement volume
  50 mm × 50 mm× 2 mm, $U \leqq 20$nm (k=1) ,
  100 μm × 100 μm × 3 μm, $U \leqq 2$nm (k=1).
- Homodyne 8-pass interferometers for measuring the relative position between the sample and the probe, with the measurement results directly traced to the the International System of Units.
- Capability of calibration horizontal and vertical direction structures, such as step height, 1D and 2D grid, and nanometer roughness.



Millimeter range metrological nano-geometric standard device

II. Calibration

Wavelength Primary Standard

↓

Standard Device
(Millimeter range metrological nano-geometric standard device)

↓

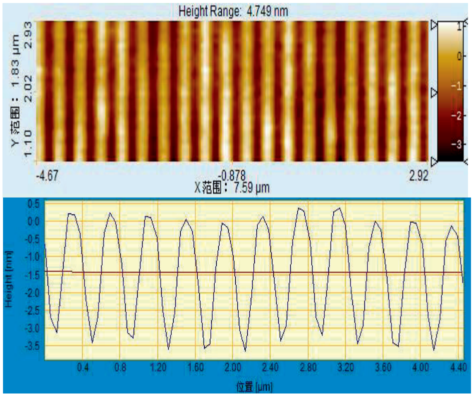
CRMs
(Grating Pitch/Step Height CRMs etc.)

↓

Devices
(AFM/SEM/Profiler)

III. Grating Pitch CRMs

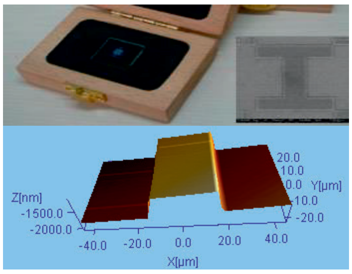
- 1D grating pitch of 400 nm fabricated by electron beam lithography is calibrated with this instrument and the gravity center method is applied to evaluate the pitch
- To establish the quality assurance system in micro and nano, 1D and 2D grid CRMs, ranging from 70nm to 10μm，are widely used in calibration of magnification factors of scanning electron microscopy(SEM).



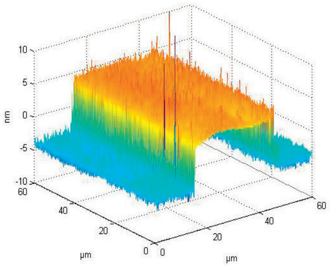
Profile of 400nm pitch grating CRM

IV.Step Height CRMs

- A series of step height (50nm-1000nm) standard samples are also developed to calibrate AFM in vertical direction.
- To meet the demand of step height standard smaller than 25 nm, the novel step height samples are fabricated with atomic layer deposition (ALD) technique and lithography. The step height samples are calibrated and the sample stability is also tested.



Nano step height standards



18 nm step height standard fabricated with ALD

V. Conclusion

- The nano-geometric standard device and nano step height and grating reference materilas are developed to establish the traceability in horizontal and vertical direction for nano measurement instrument.

Presented at the Workshop on Reference Nanomaterials, Berlin, Germany, May 14th & 15th 2018

Technische Universität Braunschweig

LENA Laboratory for Emerging Nanometrology

Institut für Halbleitertechnik

Nanoscale reference artefacts for contact resonance spectroscopy and force-distance curve techniques

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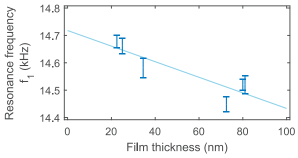
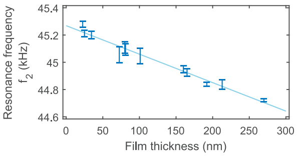
Motivation

- Demand for measurement systems, designed to monitor equipment and workpieces [1]
- Tactile probing, using Micromechanical piezoresistive silicon cantilevers, is suitable for high-speed measurements [2]
  - → Measurement of surface profiles [2]
  - → Information about mechanical properties via contact resonance (CR) spectroscopy
    - → Elasticity [3]
    - → Viscosity [3]
- Determining quantitative values, e. g. indentation modulus, from the measured contact resonance frequencies requires much effort
  - → Use reference samples as an alternative
- Two types of reference artefacts
  - → Thin films of poly(3,4-ethylenedioxythiophene), i.e. PEDOT
  - → Three-dimensional silicon micro-pillar array

PEDOT films CR

- Deposited on silicon substrates using oxidative chemical vapor deposition (oCVD)
- The films' thickness is measured using a profilometer
- Different oCVD parameters yield thicknesses in the range of 20 nm up to 300 nm
- CR measurements using the first (f_1) and second (f_2) out-of-plane vibration mode
- Different static forces used in the first and second mode
  - → 129 µN using the first mode
  - → 258 µN using the second mode

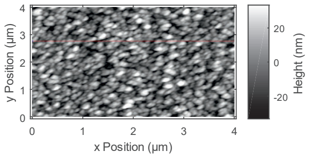


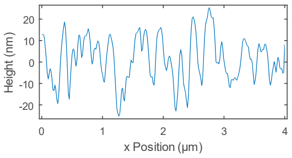


- Both modes show decreasing frequencies with increasing film thicknesses
- Measurements in the second mode show a better correlation coefficient
  - → -0.89 vs. -0.99
- Mean frequency errors of 32.5 Hz enable a thickness-resolution of 16 nm

PEDOT films FD

- PEDOT films have to be calibrated
  - → Force distance (FD) measurements determine elastic modulus
- PEDOT surface consists of individual grains (Ø ca. 150 nm)
  - → Increased surface roughness
  - → Optimization of deposition parameters needed to allow FD measurements



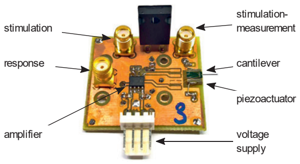


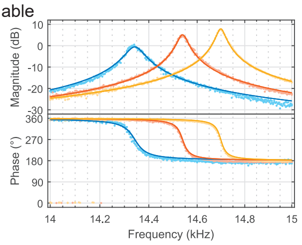
Acknowledgement

The authors thank Angelika Schmidt, Juliane Breitfelder and Kerstin Franke for their valuable technical support. M. Fahrbach is grateful for the support from European Union by funding of the European Metrology Program for Innovation and Research (EMPIR) project "Multifunctional ultrafast micro-probes for on-the-machine measurements (17IND05 MicroProbes). M. Bertke and G. Hamdana are grateful for funding by the Ministry of Science and Culture of Lower Saxony (MWK), Germany in the QUANOMET project and by the German Research Foundation (DFG) under no. PE 885/3-1, respectively. Linus Krieg gratefully acknowledge support by the DFG Research Training Group GrK 1952/1 'Metrology for Complex Nanosystems', the Braunschweig International Graduate School of Metrology B-IGSM, 'Niedersächsisches Vorab' through 'Quantum- and Nano-Metrology (QUANOMET)' initiative within the project NL-3 'Sensors' and the DFG Research Unit FOR 1616: Dynamics and Interactions of Semiconductor Nanowires for Optoelectronics.

CR measurement setup

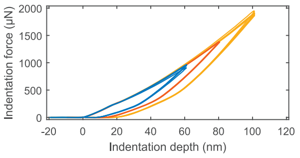
- Commercial 5-mm-long, 200-µm-wide, 50-µm-thick piezoresistive cantilevers
  - → CAN-50-2-5 by *CiS Forschungsinstitut für Mikrosensorik GmbH* [4]
- Cantilevers mounted on multilayer piezo actuators for dynamic excitation
  - → PL055.30 PICMA-Chip piezo actuator by *PI Ceramic GmbH* [5]
- Cantilever and piezo actuator are mounted on a carrier PCB
- Carrier PCB mounted on YZ piezo stage
- Cantilever gets pressed against sample with controllable force
- Resonance measurement using PLL circuit
- Evaluation using analytical model
  - → Cantilever geometry has to be known
  - → Tip wear not considered
- Evaluation using calibrated samples
  - → Indentation modulus directly available

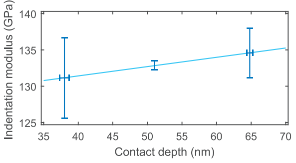




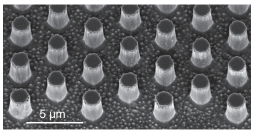
Silicon pillar array

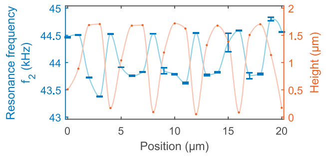
- Fabricated from bulk (100)-silicon using nanoimprint lithography (NIL) and cryogenic deep reactive ion etching (cryo DRIE)
- 1.6 µm diameter, 4 µm height, 4 µm pitch [6]
- Indentation modulus measured by nanoindentation





- Measured CR frequency values f_2 lower on top of pillars
  - → Higher compressibility compared to the bulk
- Measured pillar height contains information about the cantilever's tip geometry
  - → Tip height can be calculated
- → Tip wear can be monitored





Conclusion & Outlook

- Silicon pillar arrays can be used to calibrate samples and monitor probe-wear
- PEDOT films show great promise, however optimization for use as reference needed
- Different fabrication processes will be explored to reduce surface roughnesses
- Further CR-AFM and nanoindentation measurements will be conducted

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Development of a Method for Measuring the Rigidity of Nanofibers

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Introduction

- Airborne fibers that reach the respiratory system may cause serious, asbestos-like health problems in lungs. Incomplete phagocytosis of fibers by alveolar macrophages and cell lesions may induce chronic inflammation. Flexural rigidity is a material property that is believed to govern the hazard of fibers. Here a method is presented that aims at measuring the flexural rigidity of fibers with diameters down to the nanoscale.
- The mechanical rigidity of fibers may change from the original value due to manufacture and aging.

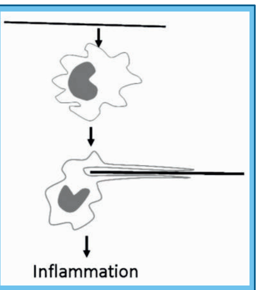


Fig. 1: Example of a frustrated phagocytosis during digestion of rigid fibers [1]

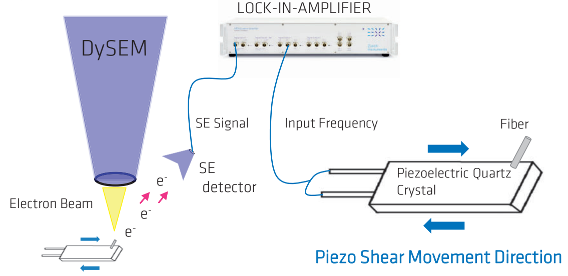
Goal

This project aims to evaluate the flexural rigidity (E) of carbon nanotubes (CNTs) by detecting the resonance frequency of fibers with an experimental setup inside a Dynamic Scanning Electron Microscope (DySEM) [2].

The Method

- Based on Euler-Bernoulli beam theory, the Young's Modulus of CNTs can be obtained using the resonance frequency of mechanically excited fibers.
- To acquire the resonance frequency during excitation, the signals of secondary electrons (SE) are filtered and treated by a lock-in amplifier, that sorts out the corresponding amplitude and phase values.
- This method could be applied to any nanofiber that has a shape similar to a symmetric beam.

Experimental Set Up



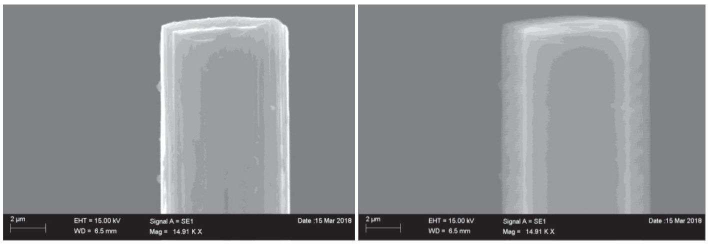


Fig 2: SEM image of a carbon fiber of 7 µm diameter without input frequency (left) and at resonance frequency of 15.2 KHz at the fundamental mode (right).

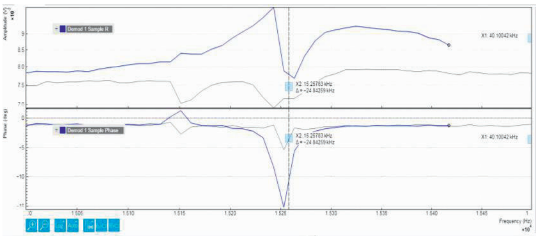
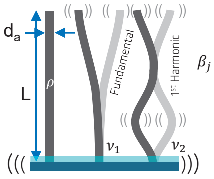


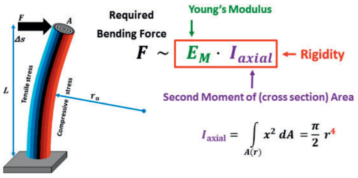
Fig 3: SE signals of the fiber at resonance frequency (ϑ_i)

Euler-Bernoulli beam theory



$$E = \rho \left(\frac{\nu_j 8 \pi L^2}{\beta_j^2 d_a} \right)^2$$

$$\beta_j = 1.875, 4.694, 7.855, \dots \quad \text{for} \quad j = 1, 2, 3, \dots$$



$$F \sim \boxed{E_M \cdot I_{axial}}$$

$$I_{\text{axial}} = \int_{A(r)} x^2 \, dA = \frac{\pi}{2} r^4$$

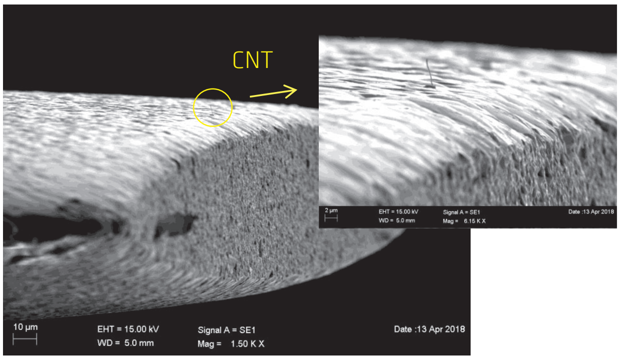
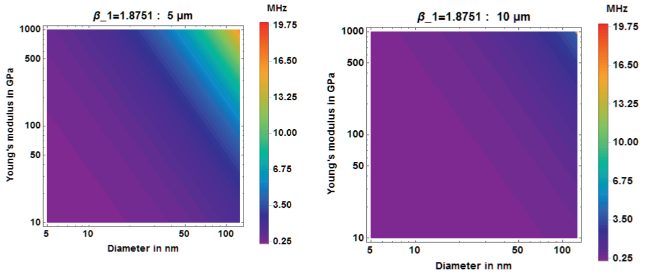


Fig 4 (left) and (right): Carbon Nanotube fixed on the tip of a 12 MHz piezo quartz

Estimation of resonance frequency and Young's modulus for CNTs with different dimensions



Acknowledgement
We thank BAuA for funding this project.
We thank ZELMI at TU Berlin for helping with the manipulation of CNTs on piezo quartz.

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[2] Schröter, Ritter, Holscheider, Sturm "Enhanced DySEM imaging of cantilever motion using artificial structures patterned by focused ion beam techniques" *J. Micromech. Microeng.* **26** (2016) 035010.

Long-range ordered gold-coated silicon nanowires for surface-enhanced Raman spectroscopy

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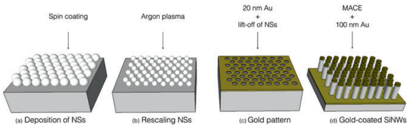
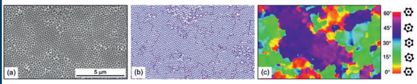
FABRICATION

Large-scale unconventional methods are used for the realisation of novel 3D plasmonic nanostructures. Au-coated flexible silicon nanowires has been successfully tested [1].

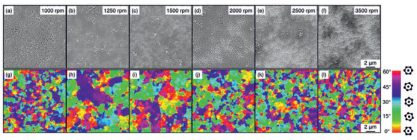
Combination of nano spheres lithography (NSL) and metal-assisted chemical etching (MACE).



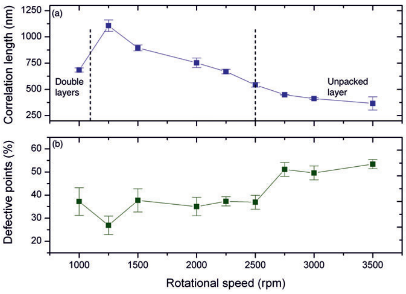
Quantitative evaluation of the degree of order of the nanosphres is achieved through image processing based on Delaunay triangulation.



The self-assembly of the nano spheres is affected by the dynamic conditions during spin coating.



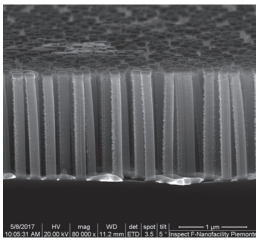
By elaborating the colour maps it is possible to calculate the correlation length ξ, the distance in which the coherency of the order is preserved.
Low velocities trigger the surface tension that pulls nanoparticles together, resulting in the maximum ξ and in the lowest number of defects.



[1] Kara S., et al. 2016, RSC Adv., 6, 93649–93659

FUNDAMENTALS

Flexible gold-coated Si nanowires for SERS analytical measurements

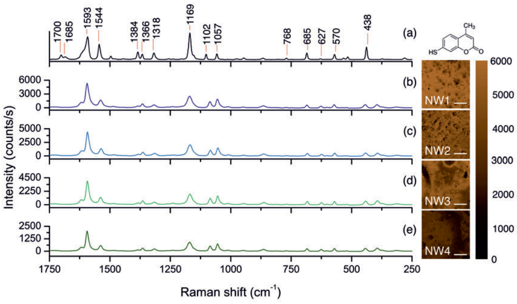


Requirements:

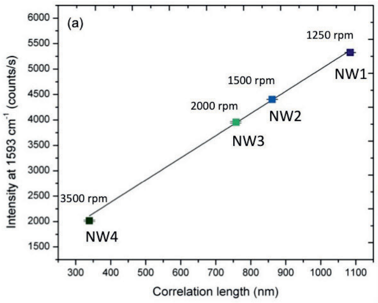
- large area;
- favorable chemical detection level;
- stability and reproducibility;
- homogeneity.

SURFACE-ENHANCED RAMAN SPECTROSCOPY

SERS measurements were carried out using 7-mercapto-4-methylcoumarin (MMC) as probe molecule on four substrates corresponding to different values of ξ for which we completed the fabrication to obtain flexible nanowires.



The linear increasing trend of the intensity of the SERS signal at 1593 cm^{-1}, due to the conjugated -C=C- stretching mode of MMC, can be motivated by the increase of the order of the nanostructures that influence both the density of the nanowires and the bending configuration for the formation of the hot spots.



Enhancement factor equal to $1.6 \cdot 10^6$ calculated on substrate NW1 with the following formula [2]

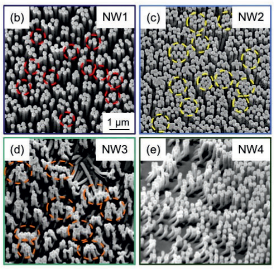
$$EF = \frac{I_{SERS} N_{Ref}}{I_{Ref} N_{SERS}}$$

[2] Le Ru E. C., et al. 2007, *J. Phys. Chem. C* 111, 13794–13803

MORPHOLOGY

Scanning electron microscopy (SEM) provides a morphological characterisation of the high aspect-ratio nanowires. Morphology is crucial in this surface-sensitive technique.
Determination of the active areas:

- hot spots at the tip-to-tip sites
- ensembles of two to five nanowires produce larger enhancement

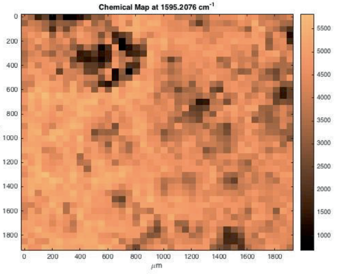


HOMOGENEITY

The homogeneity was evaluated through the calculation of the relative standard deviation (RSD) on the Raman color map of the most ordered substrate up to 4 mm^2.

Intra-map RSD: pixel-to-pixel variability within single maps
Inter-maps RSD: repeated measurements of equal areas

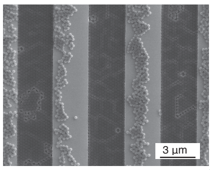
- RSD < 20% states acceptable homogeneity level over large area (mm^2);
- minimum area that ensures good repeatability (500 x 500) μm^2



Scanned area (μm^2)	Number of spectra	Intra-map RSD (%)	Inter-maps RSD (%)
150 × 150	9	12.1	12.8
300 × 300	36	16.4	13.6
450 × 450	81	16.2	10.7
600 × 600	144	17.4	8.0
750 × 750	225	18.1	7.3
900 × 900	324	17.9	6.8
1000 × 1000	400	18.1	6.6
2000 × 2000	1600	20	–

FUTURE PERSPECTIVE

By pre-patterning of topographical micro-sized templates on the substrate with direct laser writing (DLW), it is possible direct to the self-assembly of the nano spheres and boost the degree of order over large area together with the enhancement and the homogeneity.
Moreover topographical templates can be used for microfluidics application and multiplexed analysis.



The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States

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Synthesis and optical characterization of ternary Quantum Dots: $AgInS_2$/ZnS

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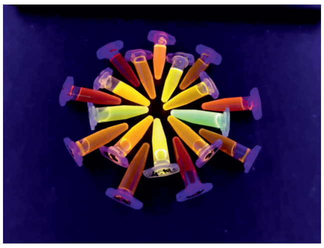
Introduction

Ternary semiconductor quantum dots (t-QDs) like $AgInS_2$ (AIS) QDs are interesting alternatives to Cd-based QDs for applications as optically active materials in light-emitting diodes (LEDs), solar concentrators, and solar cells as well as as biodiagnostic tools. [1] AIS QDs exhibit broad photoluminescence (PL) spectra in the visible and near infrared. The spectral position of these PL bands is tunable by QD size and chemical composition (ratio of components or doping). In order to enhance the PL quantum yield (PL QY or Φ_{pl}) and prevent material decomposition and oxidation, the surface of the AIS QDs is passivated with a ZnS shell. Here we show a spectroscopic study of differently colored AIS QDs synthesized in water by a Microwave (MW)-assisted procedure.

Experimental

Synthesis

Core $AgInS_2$(AIS) QDs and core-shell AIS/ZnS Qds we synthesized in water. Different amounts of Ag and liga enable spectral tuning, thereby largely covering the solar spectra, and size selected precipitation yields t-QD with sizes from 2 nm to 4 nm.



Emission

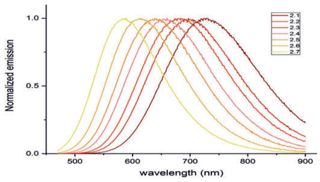
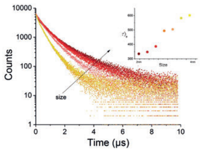


Fig 2: PL spectra of size-selected AIS Qds in water.

The size selected QDs show broad PL spectra with band maxima shifted to higher wavelength with size.

Acknowledgements:
We gratefully acknowledge financial support from the German Research Council (DFG; RE1203|12-3)



$$\langle\tau\rangle_a = \frac{a_1 \cdot \tau_1 + a_2 \cdot \tau_2}{a_1 + a_2}$$

Fig 3: PL decay and average lifetimes $<\tau>_a$

The mean PL lifetime increases with increasing QD size.

Setup for measuring quantum qields

$$\Phi_{pl} \quad \frac{N_{em}(\lambda)}{N_{abs}(\lambda)} = \frac{\#\ emitted\ photons}{\#\ absorbed\ photons}$$

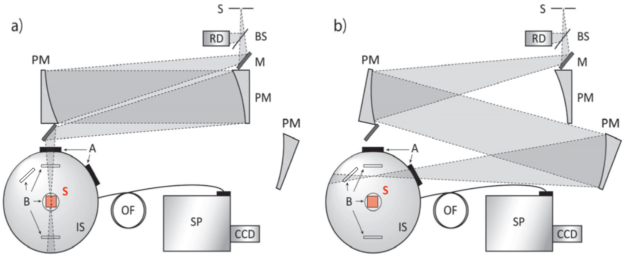


Fig 1: Schematic drawing of the integrating sphere setup used. S: exit slit of the excitation monochromator; RD: reference detector; BS: beam splitter; M: plane mirror; PM: parabolic mirror; A: aperture; B: baffle; IS: integrating sphere; OF: optical fiber; SP: spectrograph; CCD: charge-coupled device; S: sample.
a) Absolute QY measurements performed by direct illumination of the sample; b) absolute QY measurements done by indirect illumination of the sample.

PL QY measurements

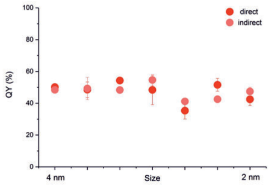


Fig 4: PL QY measurements, done directly (red) and indirectly (pink).

PL QY of AIS QDs is ca. 50% , independent of size.

Conclusion and outlook

The simple aqueous synthesis that avoids further ligand exchange for bioanalytical applications, the tunable emission color, high absorption coefficients, PL QY ~50%, and the long lifetime make these t-QDs promising Cd-free materials as biodiagnostic tools or optically active materials for devices.
The lifetime can be extended by increasing QD size while keeping PL QY constantly high.

References

[1] W. Girma *et al.*, *J. Mat. Chem. B. (2017), 5, 6193*
[2] A.E. Raevskaya *et al., J Phys. Chem. C (2017) 121, 9032*
[3] C. Wuerth, Ute Resch-Genger, *Appl. Spectrosc. (2015) 69, 6, 749*

Physikalisch-Technische Bundesanstalt
National Metrology Institute

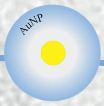
C. Frank[1], V. Maurer[2], J. C. Porsiel[2], S. Zellmer[2],
S. Wundrack[1], G. Garnweitner[2], R. Stosch[1]

Sandwich Assay of Magnetic and Gold Nanoparticles for Purification and Quantification of Tau Protein Using ID-SERS

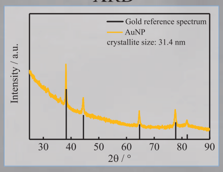
Motivation

More than 4 million people in the European Union suffer from Alzheimer‘s disease and this number will likely double within the next 20 years. Additionally, even if detected, then often only in advanced stages of the disease. Up to now, the Alzheimer’s disease is only reliable diagnosed post mortem. Tau-protein is an established biomarker for this dementia form and magnetic and metallic nanoparticles provide versatile sensing platforms for isolating and quantifing this large biomolecule.

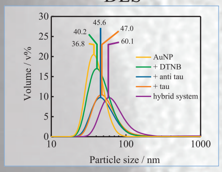
Synthesis route of the AuNP site



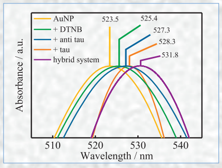
XRD

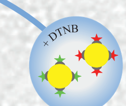


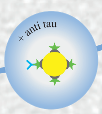
DLS

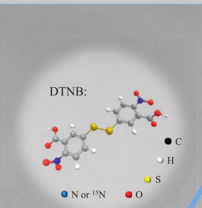


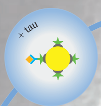
UV/Vis



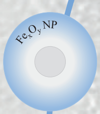




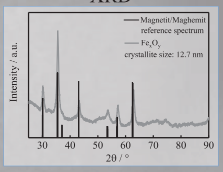




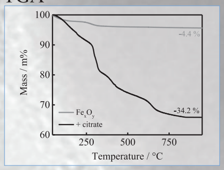
Synthesis route of the Fe_xO_y NP site



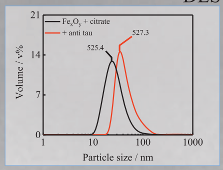
XRD

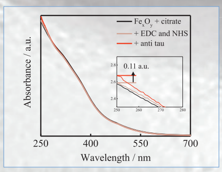


TGA

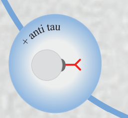


DLS

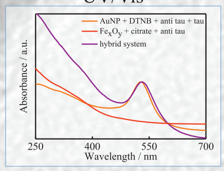




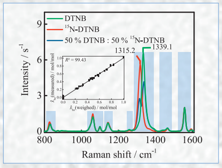
UV/Vis

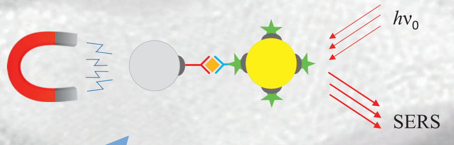


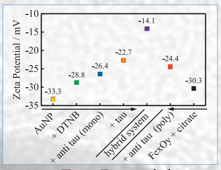
UV/Vis



SERS

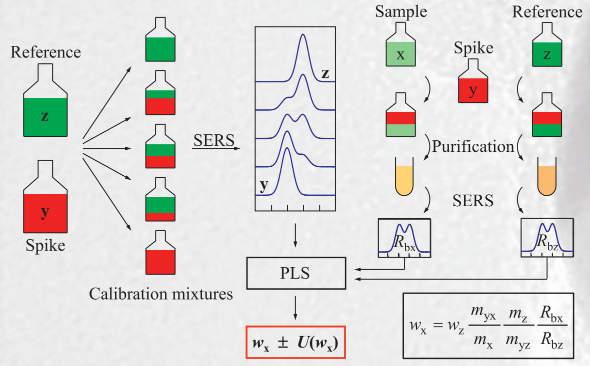






Zeta Potential

Quantification Principle of ID-SERS*

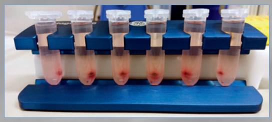


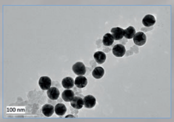
$$w_x = w_z \frac{m_{yx}}{m_x} \frac{m_z}{m_{yz}} \frac{R_{bx}}{R_{bz}}$$

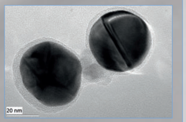
$$w_x \pm U(w_x)$$

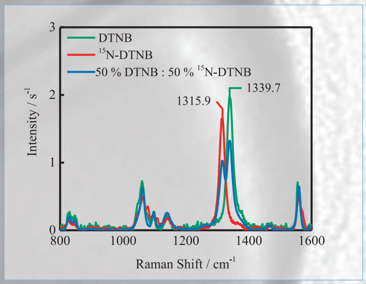
- SI traceability can be achieved via the reference
- Measurement is independent of reference and spike purity
- After spiking, sample loss does not affect the result

First Measurements of the Hybrid Complex









- No significant bond shift when measureing DTNB on AuNP compared to the hybrid complex
- The simplified model generated from DTNB/^{15}N-DTNB on AuNP can be consulted to quantify tau protein in the hybrid complex

Project homepage (15HLT02, ReMiND): http://projects.ptb.de/emrp/remind-project.html

* S. Zakel, S. Wundrack, G. O‘Connor, B. Güttler, R. Stosch, *J Raman Spectrosc* (2013) 44, 1246.

20 nm

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Simple and Validated Methods for Quantifying Functional Groups, Ligands, and Biomolecules on Nanomaterials

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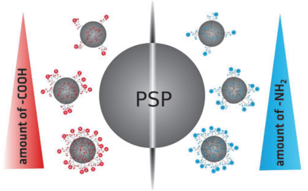
Bundesanstalt für Materialforschung und -prüfung (BAM), Richard-Willstätter-Str. 11, D-12489 Berlin, Germany

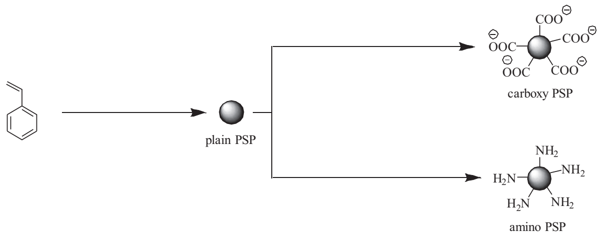
Introduction and Motivation



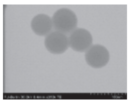
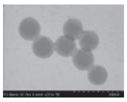
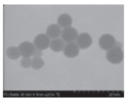
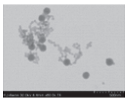
- Nanoparticles (NP) are widely used as drug carriers, fluorescent sensors, and multimodal reporters in optical assays and bioimaging [1]
- Application-relevant properties of NPs are size, shape, concentration, and especially the chemistry and number of surface functional groups (FG), which are responsible for colloidal stability, biocompatibility, toxicity, and subsequent (bio)functionalization [2,3]
- Need for reliable and validated methods for surface group and ligand quantification that can be preferably performed with routine laboratory instrumentation, require only small amounts of substances, and are suitable for many different types of nanomaterials.
- **Objectives: Develop versatile and simple concepts for the quantification of common functional groups, ligands, and biomolecules on different types of organic and inorganic nanomaterials, using different types of optical reporters and method validation with the aid of multimodal reporters, method comparisons, and mass balances.**

Synthesis and Characterization of PSP

Reaction mechanism for the synthesis of amino and carboxy PSP



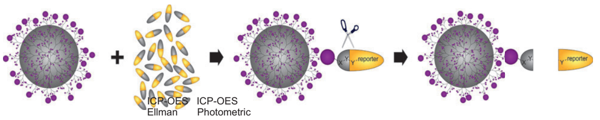
Characterization results of carboxy PSP

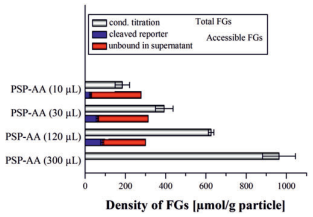
	PSP -COOH			
	10 µL AA	30 µL AA	120 µL AA	300 µL AA
Hydrodynamic diameter [nm]	132 ± 24	120 ± 5	113 ± 4	polydispere
PDI	0.280	0.132	0.142	
SEM				

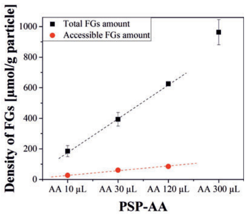
- Microemulsion-based synthesis provides monodisperse, spherical PSP with sizes between 110-130 nm
- High amount of co-monomer acrylic acid (AA) leads to polydisperse suspension with small AA particles

Quantification of Functional Groups

Quantification with cleavable reporters







- Cleavable reporters enable quantification via optical spectroscopy without interferences from NP light scattering, and validation via ICP-OES and photometric Ellman's test
- FG densities of 200-600 µmol/g (total) and 25-90 µmol/g (ca. 15% accessible) were determined

Conclusion and Outlook

- Stable, monodisperse, 100 nm-sized PSP with well-defined FG densities were synthesized via emulsion polymerization
- FG quantification was successfully performed with conductometric titration (total amount of FG) and cleavable reporters (amount of FG accessible for functionalization or modification)

Acknowledgements

This project has received funding from the EMPIR programme (project 14IND12 Innanopart) co-financed by the Participating States, from the European Union's Horizon 2020 research and innovation programme. We gratefully acknowledge financial support from the Federal Ministry for Economic Affairs and Energy (BMWi-10/12).

References

[1] K. E. Sapsford, K. M. Tyner, B. J. Dair, J. R. Deschamps, I. L. Medintz, *Anal. Chem.* **2011**, *83*, 4453-4488.
[2] A. Hennig, P. M. Dietrich, F. Hemmann, *et. al.*, *Analyst* **2015**, *140*, 1804-1808.
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Multimodal cleavable reporters for the optical quantification of functional groups on nano- and microparticles

BAM
Bundesanstalt für
Materialforschung
und -prüfung

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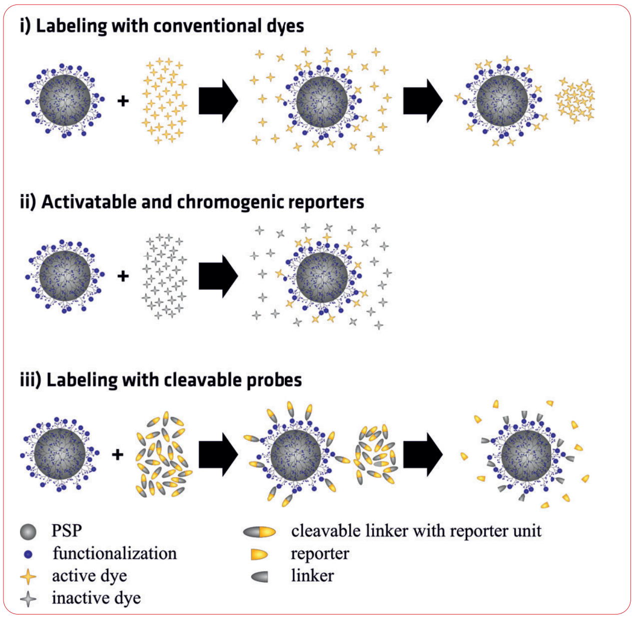
Introduction and Motivation

- Nanoparticles (NP) are used in the life sciences and medicine as carriers for dye molecules and drugs, multichromophoric reporters in optical assays, targeted probes in bioimaging studies, and as nanosensors.[1]
- Application-relevant properties of NP are e.g. size, morphology, concentration, and surface functionalization.
- The surface chemistry is responsible for colloidal stability, subsequent (bio)functionalization, interaction with biological systems, biocompatibility, and toxicity.[2,3]

  ⟶ **Quantication of surface functionalities is essential**
- No standardized methods and reference materials available for functional group analysis and quantification.[2]

Objectives: To develop simple, fast, robust, and inexpensive optical methods and multimodal reporters for quantification of total and accessible -COOH and -NH_2 groups on NP

Labeling Strategies



Application potential of the methods assessed.

	Conductometry	[i] Convent. dye	[ii] Precursor	[iii] Cleavable
Total amount	+	−	+[a]	−
Accessible amount	−	+	+	+
Unbound	−	+	−/+[b]	+
On surface	+	−	+	−/+
Cleaved from surface	−	−	−	+
Mass balance	−	−	−	+
Spectrophotometry	−	+	+	+
Fluorometry	−	+	+	−

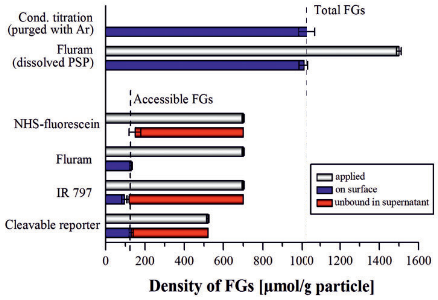
[a] after previous particle dissolution, [b] detectable with complementary methods, e. g., MS

Results

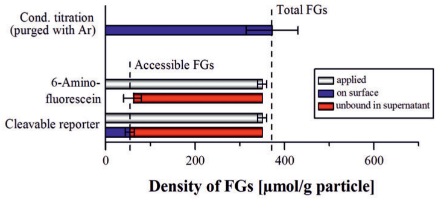
- Quantification of functional groups (FGs) was done for micro- and nanometer particles (1 µm and 100 nm) with different degrees of surface functionalization

Results of optical assays for 100 nm-sized particles

-NH_2



-COOH



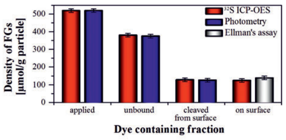
- Total amount of FGs was determined via conductometric titration; accessible amount of FGs was quantified with three labeling strategies and different reporters

  ⟶ 13% of the total amino and 16% of carboxy groups are accessible for (bio) functionalization

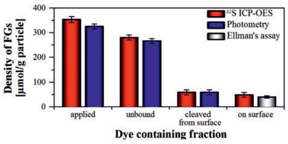
Validation of accessible surface functions

- Validation via ICP-OES and mass balance only possible with cleavable reporters (method iii)

-NH_2



-COOH



- ICP-OES and Ellman‘s assay confirm the photometry results of the cleavable reporters [4]

Conclusion and Outlook

- Cleavable reporter for quantification of -COOH and -NH_2 were developed
- Cleavable multimodal probes elegantly circumvent particle scattering, only need inexpensive instrumentation, and allow validation with different methods
- Outlook: Design of cleavable fluorescent multimodal probes is ongoing

Acknowledgements

This project has received funding from the EMPIR programme (project 14IND12 Innanopart) co-financed by the Participating States, from the European Union's Horizon 2020 research and innovation programme. We gratefully acknowledge financial support from the Federal Ministry for Economic Affairs and Energy (BMWi-10/12) and support from the MI programm of BAM ((Ideen_2015_56).

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[1] K. E. Sapsford, K. M. Tyner, B. J. Dair, J. R. Deschamps, I. L. Medintz, Anal. Chem. 2011, 83, 4453-4488.
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Sicherheit in Technik und Chemie

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Investigation of upconverting nanoparticle growth utilizing in-situ luminescence monitoring in combination with offline small-angle X-ray scattering (SAXS) and transmission electron microscopy (TEM)

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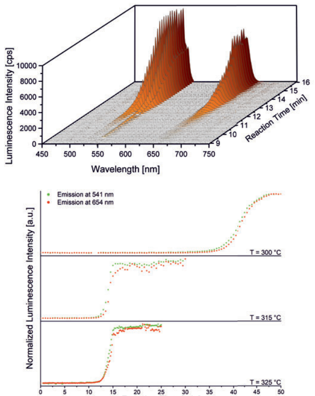
Introduction

- Upconversion is a non-linear, multiphotonic process which is used for applications in various optical devices or sensitive bio-probes [1]
- Colloidal inorganic nanocrystals showing NIR-to-visibile upconversion have a great potential in particular as nanolabels for bioimaging
- Nanoparticles have unique size-/shape-dependent optical properties controlled by synthesis [2]
- Proper tuning of these properties requires deeper understanding of nanoparticle formation [3]

Objective

- Investigation of upconverting nanoparticle (UCNP) growth utilizing state-of-the-art analytical methods such as in-situ and time-resolved luminescence spectroscopy, small-angle X-ray scattering (SAXS) and transmission electron microscopy (TEM)

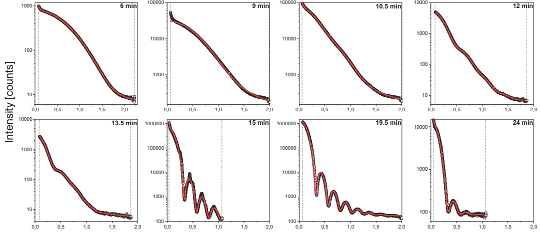
In-situ luminescence monitoring



- Strong increase of the luminescence intensity during synthesis (α ⇨ β transition)
- Onset of luminescence increase is temperature dependent
- Sigmoidal time-dependency of luminescence increase indicates a size limited growth mechanism
- Duration of α ⇨ β phase transition varies from 5 to 10 minutes for 325/315 °C and 300 °C, respectively

Small-angle X-ray scattering (SAXS)

- Fitting of scattering curves depicts evolution of nanoparticles (assumption: spherical particles)

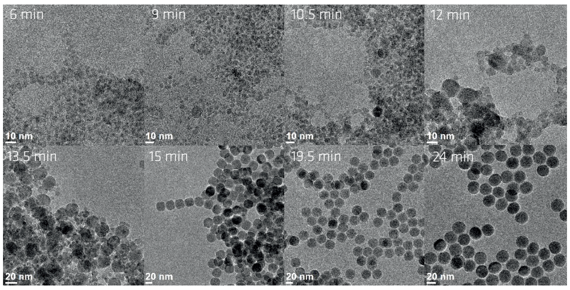


SAXS scattering curves and corresponding fits.

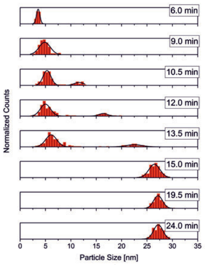
Table 1: Nanoparticle sizes determined by SAXS and TEM and amplitude-weighted luminescence lifetimes for samples collected during synthesis.

Reaction time [min]	Size **α**-phase NCs (SAXS) [nm]	Size **β**-phase NCs (SAXS) [nm]	Size **α**-phase NCs (TEM) [nm]	Size **β**-phase NCs (TEM) [nm]	τ_{Lum} (541 nm) [µs]	τ_{Lum} (655 nm) [µs]	τ_{Lum} (940 nm) [µs]
6.0	4.06 ± 1.81	-	3.50 ± 0.31	-	c	c	c
9.0	4.18 ± 2.24	-	4.84 ± 1.10	-	55 ± 6	87 ± 9	14 ± 2
10.5	4.88 ± 1.44	9.02 ± 2.98	5.29 ± 0.97	11.28 ± 1.05	59 ± 6	114 ± 12	18 ± 2
12.0	5.30 ± 1.78	16.96 ± 0.82	4.98 ± 1.34	16.38 ± 1.54	59 ± 6	131 ± 14	25 ± 3
13.5	6.46 ± 1.36	22.34 ± 1.34	6.11 ± 1.20	22.50 ± 2.24	79 ± 8	162 ± 17	49 ± 5
15.0	-	26.82 ± 0.48	-	26.46 ± 1.09	102 ± 11	226 ± 23	107 ± 11
19.5	-	27.00 ± 1.06	-	27.17 ± 0.89	112 ± 12	219 ± 22	119 ± 12
24.0	-	26.54 ± 1.14	-	27.21 ± 0.91	116 ± 12	234 ± 24	129 ± 13

Transmission electron microscopy (TEM)



TEM images of samples collected at different times during synthesis.

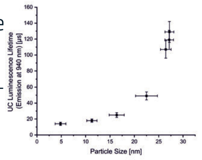


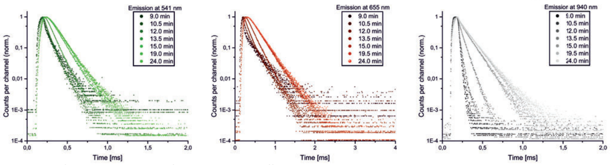
Size histograms derived from TEM data.

- Size distribution (determined from TEM images) confirms particle growth
- α ⇨ β phase transition occurs within 5 minutes
- Final size and spherical shape adopted after 24 min (for synthesis at 325 °C)

Time-resolved luminescence spectroscopy

- Luminescence decay curves collected for the emissions at 541 nm, 655 nm and 940 nm
- Luminescence lifetimes show a typical non-linear increase dependent on UCNP size

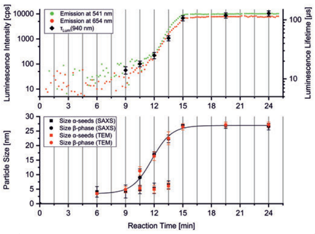




Decay curves of upconversion emissions of samples collected at different times during synthesis

Combination of in-situ & time-resolved luminescence spectroscopy, SAXS & TEM

- Luminescence intensity & lifetimes correlate with particle size evolution
- Sizes of nanoparticles derived from SAXS and TEM measurements are in good agreement



Top) Real time luminescence measurements during the UCNP synthesis. Bottom) Nanoparticle diameter evolution during the synthesis derived from SAXS (black) and TEM (red) measurments.

Conclusion and Outlook

- UCNPs were succesfully synthesized and characterized
- Various stages of UCNP growth were tracked using different analytical methods including real time in-situ & time-resolved luminescence spectroscopy, SAXS and TEM measurements
- Additional size determination will be performed using inductively coupled plasma - mass spectrometry (ICP-MS)

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