



## **The EU FP7 NanoDefine Project**

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

**Critical review manuscript with real-world performance data for counting, ensemble and separating methods including in-build mathematical conversion to number distributions submitted for publication**

### **NanoDefine Technical Report D3.3**

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### **The NanoDefine Consortium 2016**

## **NanoDefine in a nutshell**

The EU FP7 NanoDefine project was launched in November 2013 and will run until October 2017. The project is dedicated to support the implementation of the EU Recommendation on the Definition of Nanomaterial by the provision of the required analytical tools and respective guidance. Main goal is to develop a novel tiered approach consisting of (i) rapid and cost-efficient screening methods and (ii) confirmatory measurement methods. The "NanoDefiner" eTool will guide potential end-users, such as concerned industries and regulatory bodies as well as enforcement and contract laboratories, to reliably classify if a material is nano or not. To achieve this objective, a comprehensive inter-laboratory evaluation of the performance of current characterisation techniques, instruments and software is performed. Instruments, software and methods are further developed. Their capacity to reliably measure the size of particulates in the size range 1-100 nm and above (according to the EU definition) is validated. Technical reports on project results are published to reach out to relevant stakeholders, such as policy makers, regulators, industries and the wider scientific community, to present and discuss our goals and results, to ensure a continuous exchange of views, needs and experiences obtained from different fields of expertise and application, and to finally integrate the resulting feedback into our ongoing work on the size-related classification of nanomaterials.

### **Bibliographic data**

NanoDefine Technical Report D3.3

Report title: Critical review manuscript with real-world performance data for counting, ensemble and separating methods including in-build mathematical conversion to number distributions submitted for publication

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Publication date: 01/12/2016

Publisher: The NanoDefine Consortium

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Place of publication: Wageningen, The Netherlands

Citation:

Frank Babick, Johannes Mielke, Dan Hodoroaba, Stefan Weigel, Wendel Wohlleben: *Critical review manuscript with real-world performance data for counting, ensemble and separating methods including in-build mathematical conversion to number distributions submitted for publication*; NanoDefine Technical Report D3.3; NanoDefine Consortium, Wageningen, **2016**.

URL: <http://www.nanodefine.eu>

The deliverable was designed to be published as a scientific paper, see *J. Nanopart. Res.*, 18:158, **2016**; doi: 10.1007/s11051-016-3461-7 as Open Access article available on:

<http://link.springer.com/article/10.1007/s11051-016-3461-7>

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## IV. Summary

The deliverable reprints the draft for a scientific paper to be submitted to *ACS nano* and provides some introductory remarks.

The content of the paper is the assessment of the performance of (conventional) measurement techniques (MTs) with respect to the classification of disperse materials according to the EC recommendation for a definition of nanomaterial. This performance essentially refers to the accurate assessment of the number weighted median of (the constituent) particles. All data and conclusions are based on the analytical study conducted within task T3.2 (real-world performance testing). It comprised different types of MTs (imaging, counting, fractionating, spectroscopic and integral) as well as different types of materials. Beside reference materials with well-defined size distribution (= quality control materials) the study also included several commercial powders (representative test materials; variation of particle composition, morphology, coating, size range and polydispersity). The analytical measurements were realised by previously selected laboratories within the NanoDefine consortium. In order to ensure comparability of measurement results, the participants were guided to use uniform protocols in sample preparation, conducting measurements, data analysis and in reporting results. Corresponding documents have been made public, in order to support the reviewing process of the paper, respectively to ensure the reproducibility of data by other users under the same conditions.

Preliminary results of the analytical programme have already been briefly presented in deliverable D3.2. The scientific paper now relies on a comprehensive set of revised measurement data reported in uniform templates, completely describes the experimental procedures and discusses the MTs' performance for selected materials in detail. Even more, the study is summarised and evaluated, which leads to recommendations for the use of MTs within a tiered approach of NM characterisation. In addition, the paper critically examines the factors that may affect the outcome of such a comparison among different MTs.

In order to keep the paper concise and readable, we can neither present all results nor discuss all facets of the MT comparisons. This necessary limitation is addressed by providing additional data as supplementary material to online readers of the journal.

## V. Organisation of the scientific paper

### Content and intended outcome

The scientific paper reflects the measurement results of task T3.2 (real-world performance testing of existing MTs), i. e. the size distributions obtained for quality control materials and test materials with selected MTs. Since the criterion for NM identification is the number weighted median size of (the smallest external dimension of the constituent) particles ( $x_{50,0}$ ), the paper mainly presents number weighted size distributions ( $Q_0$ ). However, the impact of converting intrinsically measured size distributions into  $Q_0$  also is addressed in a special section. As the performance evaluation requires to know whether a material is a NM or not, it was agreed to regard electron microscopy (EM) as a reference MT. This is justified by its principal characteristics (enables distinction between constituent particles and aggregates, facilitates direct measurement of external dimensions of particles, intrinsically yields  $Q_0$ ), but also by the outcome of this study (consistency of  $x_{50,0}$  values within a certain group of MTs is best for EM).

In order to keep the paper concise and readable, it restricts the detailed discussion (including graphical representation of measured  $Q_0$ ) to selected quality control materials and materials, which are considered good representatives of a certain group of materials (quality control materials with narrow and wide size distribution, test materials composed of sphere-like and clearly non-spherical particles). The discussion includes an organic and an inorganic substance, for each of which two different grades are commercially available (one supposed to be NM, the other not). Beside comparing number weighted size distributions of different MTs, the paper addresses key issues that determine the degree to which such a comparison is meaningful (incl. sample preparation and data analysis).

The intended outcome of the paper is an evaluation of all examined MTs whether and under which conditions they can be used for the purpose of NM identification and how they can be implemented within a tiered approach of characterisation.

Finally, the paper does not deal with specific on-going developments within NanoDefine tackled by WPs 4 and 5 (e. g. developments in sp-ICP-MS, PTA, AF4) nor does it present any result for the test materials of complex composition (e. g. BAM-12a, tomato soup with pyrogenic silica).

### Formal aspects

As the paper refers to the results of task T3.2, which have not been published before, it is an original research paper (in contrast to the deliverable title). However, it is considered as a major reference for an intended review paper, which has a wider view on NanoDefine activities (e. g. the sample preparation organised within WP2) and discusses them in context with other scientific publications in this field. This review paper will allow for a more elaborate discussion of ("political evaluation") of the NanoDefine outcome, including a presentation of the performance criteria developed in WP7 (and WP3).

The consortium partners achieved consensus in submitting the paper to *ACS nano*, which is a highly ranked journal in the field of nanoscience and nanotechnology (impact factor > 12). The journal does not impose any quantitative restriction on the length of original research papers, however it asks for "concise, yet comprehensive reports ... presenting an advance of immediate, broad, and lasting impact". Nevertheless, the abstract should comprise approximately 250 words and an unheaded introduction of 1000 words is required, which "expands the background of the work, with relevant references but not a complete survey of the literature".

Furthermore, NanoDefine partners feel obliged to the rules of good scientific practice, which includes considering the current state-of-the-art (ensured by the selection of partners and MTs), drawing conclusions based on (measurement) data (ensured by critically reviewing the draft within NanoDefine), and making all (measurement) data accessible for later validation (which is realised by providing all measurement reports as supplementary material to the journal and its readers). The rules also require that authorship results from a significant intellectual contribution. To fit this latter point it was decided to distinguish between authors, who were actively involved in writing the scientific paper, in interpreting the results and in deriving its conclusions, and in further contributors, who have been solely involved by conducting the measurements within task T3.2 according to the distributed protocols.

The need to reveal the logical chain of our conclusions, which includes reporting all measurements data and comprehensively providing values and figures, as well as the request to concisely present or results led to a rather lengthy appendix and a quite comprehensive document for the supplementary data. These data will be publicly accessible and should be therefore also checked for IPR issues.

### Changes in nomenclature

During the first year of the NanoDefine project a lot of effort was spent in the development of a uniform nomenclature for both, measurement techniques used and material systems examined. However, for the scientific paper it appears favourable to introduce a new nomenclature of materials (see table below) and to modify the terms of a few measurement techniques:

- *calibrant* has changed to *quality control material*, the colloquial use of *calibrant* within NanoDefine does not reflect the real use of the corresponding materials and the quality of their characterisation protocols, the new term is a compromise
- *test substance* has changed to *representative test material*, in accordance to Roebben et al., *J. Nanopart. Res.*, 15:1455, 2013
- AF4-MALS has changed to AF4-LS because the instrumentation employed within task T3.2 did not make use of a multi-angle detection system, but only of the light intensity detector at 90°.
- AUC-RI has changed to *cuvAC-RI* because the centrifugal accelerations used in analysing the NanoDefine materials was in the same order of magnitude as those of the two other AC instruments (cf. Table S-1). Hence, the only criteria for the distinction of AC techniques are the measurement zone (disc or cuvette, which further means line-start or homogeneous method of sample feeding) and the detection system (light turbidity sensor or interferometric optics for refractive index measurement). Consequently, the two other AC techniques needed also a new terminology (discAC-turb instead of discAC, *cuvAC-turb* instead of *cuvAC*).

**Table 0:** material codes within NanoDefine and for the scientific paper

NanoDefine	paper	material	description
ID-19	QCM1	polystyrene	spherical, monomod., nano
ID-17	QCM2	colloidal SiO <sub>2</sub>	spherical, monomod., nano
ID-16	QCM3	colloidal Au	spherical, monomod., nano
ID-21	QCM4	colloidal Ag	spherical, monomod., nano
ID-20	QCM5	polystyrene	spher., trimod., nano + sub-μ
ID-18	QCM6	colloidal SiO <sub>2</sub>	spher., trimod., nano + sub-μ
IRMM-387	RTM1	BaSO <sub>4</sub> , UF	compact constituents, aggr., nano
IRMM-381	RTM2	BaSO <sub>4</sub> , fine	compact constituents, aggr., non-nano
IRMM-388	RTM3	coated TiO <sub>2</sub>	compact constituents, non-nano
IRMM-384	RTM4	CaCO <sub>3</sub>	cigar-like, non-nano
IRMM-385	RTM5	kaolin	platelets, nano + sub-μ
BAM-12a-1	RTM6	fumed SiO <sub>2</sub>	fractal aggregates of nanopart.
IRMM-380	RTM7	organic pigment Y83, transp.	needles, aggr., nano
IRMM-386	RTM8	organic pigment Y83, opaque	needles, aggr., non-nano
IRMM-389	RTM9	basic methacrylate copolymer	compact constituents, micro

## VI. Critical remarks on T3.2

Independently from what is being presented in the scientific paper the partners of the NanoDefine consortium should be aware of several short-comings of the analytical study.

At first, the quality of the study is impaired by considerable variations in sample preparation procedures for the test materials – despite all good intentions. Apart from a variation of dispersing agents there are substantial differences in the energy density used to disperse the materials by the different participant laboratories. Additionally, data on the nominal power of ultrasonic instruments are not complete, while measured values of electric power consumption and calorimetric energy input are rare. This is in striking contrast to the expertise of the consortium, to the initially achieved agreement among the NanoDefine partners, and – most of all – to the requirements on a conclusive study. The failure in ensuring uniform dispersion procedures is obviously due to an underestimation of time for their development and to the lacking quality of dispersion protocols provided to the partners (“NanoDefine Preliminary Dispersion protocols 16\_01\_2015.docx”; a “translation” of sonication with vial tweeter to that with ultrasonic horns is just as missing as any data on energy density values, which are needed to prepare samples of different size; data on the dispersibility are made available for just a few of the NanoDefine materials). However, for well-dispersible materials the comparability is not really affected by the variation of dispersing procedure because dispersion quickly yields “smallest dispersible units” or primary particles – this concerns IRMM-387 (in paper: RTM1) and IRMM-388 (RTM3). Yet for other materials, particle size strongly depends on the degree of dispersion (energy input), which means that comparison of measurement results is not allowed (in a strict sense) – IRMM-381 (RTM2) and IRMM-386 (RTM8). For a third group of materials, there are no data on dispersibility, the dependence on energy input and stabilizing surfactants; an assessment of measurement an assessment of comparability is thus not possible – IRMM-380 (RTM7), IRMM-384 (RTM4), IRMM-385 (RTM5). The reason why for most test materials a relatively good comparability of size distributions is observed is the “harmonising” effect of conversion into  $Q_0$ , which virtually eliminates all measurement signals of coarse, non-dispersed agglomerates.

A further aspect to be considered for the evaluation of MTs refers to the diversity of instruments used within a certain category of MTs. In particular, the two ALS instruments are not really identical with respect to the measurement principle (one just analyses the forward scattering, the second additionally evaluates scattering at large angles and at different wavelengths). However, since in both cases the reliability was not sufficient, the difference was not important for the performance evaluation (even more in practice the principal differences among all commercial ALS instruments are even larger). A similar situation applies to the two PTA instruments, one of which being probably more mature in design and data analysis than the other, but both are restricted in evaluating particles far below 100 nm.

Apart from sample preparation and the employed specific instrumentation used, the quality of measurement signals (data) should be considered when being used to evaluate a MT’s performance. Within task T3.2 this mainly concerns measurements with USSP, which requires relatively high sample concentrations ( $\geq 1$  vol.-%) and sample sizes (40 ml – 100 ml). Since batches of the test materials were rather small, most USSP tests were performed at the lowest possible particle concentration, thus, producing large uncertainties (because bias by false model parameters like liquid viscosity is relatively large). Questions on the data quality, or better sample quality, arose also for some quality control materials. In particular for ID-16 (QCM3, nano-Au), ID-17 (QCM2, nano SiO<sub>2</sub>) and ID-21 (QCM4, nano Ag) we find indications for agglomerates (e. g. due to a slow destabilisation of the suspension) in the measurement data (mainly for AC techniques and DLS; for Au even in sp-ICP-MS). However, since conversion into  $Q_0$  reduces impacts of agglomerates (at least for AC and DLS) this did obviously not affect the classification of the respective MTs.

Last but not least, the analytical matrix (Table 4) contains several gaps that are not explainable with technical reasons (note that the two test materials IRMM-382 and IRMM-383 – MWCNT and nano-steel, respectively – are already excluded). This specifically applies to AF4-LS, for which the present set of data does not support any conclusion on usefulness. The matrix contains further inconsistencies, which may be difficult to explain afterwards. For instance, both organic pigments, IRMM-380 (RTM7) and IRMM-386 (RTM8), could not be analysed with AUC-RI (deposition on cuvette walls), but showed no apparent problems with discAC, cuvAC and DLS

## How reliably can a material be classified as a nanomaterial? Available particle sizing techniques at work

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*Currently established or discussed regulatory frameworks require the classification of materials (nano or non-nano) as specified by respective definitions. This brings up the question if currently available techniques for particle size determination are capable of correctly classifying materials that potentially fall under these definitions. In this study, a wide variety of characterisation techniques, including counting, fractionating and spectroscopic techniques, has been applied to the same set of materials under harmonised conditions. The selected materials comprised well defined quality control materials (spherical, monodisperse) as well as industrial materials of complex shapes and high polydispersity. As a result, each technique could be evaluated with respect to the determination of the number weighted median size. Recommendations on the most appropriate and efficient use of techniques for different types of material are given.*

Recent years have seen a large increase in the interest for the development and application of nanomaterials. Along with this, safety concerns were raised. They were firstly based on known adverse health effects of particulate airborne matter (fine dust), and secondly on the experience from other – at that time – new materials with excellent technical properties that after years or even decades of use turned out to have serious adverse effects, e. g. polychlorinated biphenyl (PCB) or asbestos.

Resultantly, comprehensive efforts into the risk assessment of nanomaterials were initiated and continuously are carried out. Along with this came the need for a definition of nanomaterial for regulatory purposes. The European Commission (EC) recommended a definition in 2011 [1] which focuses on a number based sized distribution (50 % of particles smaller than 100 nm, including constituent particles in agglomerates and aggregates (cf. box). The ISO definition (ISO/TS 80004-1) relies on the same size criterion, but does not involve a number based percentage cut-off.

*'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. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 % and 50 %. By derogation [...], fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials.*

As the EC definition is not restricted to materials intentionally designed to be smaller than 100 nm, it includes virtually all particulate materials with a size distribution into the range below 100 nm. This includes many conventional materials such as pigments, fillers, additives etc. and thus considerably affects the chemical industry, large enterprises, but also small and medium sized ones.

The meaningful implementation of any nanomaterial definition, the EC definition in particular, requires the availability of suited analytical instrumentation and methodologies, yielding consistent and reliable data. Given the broad impact and thus characterisation need for various materials in industry and small and medium-sized enterprises, the respective technologies also need to be

widely available, cost efficient and robust.

The EC recommendation for a definition by a size cut-off in number metrics, supported by a size cut-off in surface metrics as proxy, was a paradigm change without metrological guidance. Interlaboratory comparisons dedicated to measurement of size of nanoparticles as reported in the literature considered number metrics only for techniques with an inherently counting detection principle, such as aerosol quantification by condensation particle counters [2,3,4], or imaging analysis by electron microscopy [5,6,7]. On the other hand, sizes in volume or surface metrics are critical properties for the performance of commercial particulate materials, and are often part of the product specifications, e. g. to differentiate opaque pigments (non-nano) from transparent pigments (nano) [8]. Accordingly, techniques for the determination of sizes in volume or surface metrics are well-established, validated by several interlaboratory comparisons (e. g. [9,10,11]), and subject to international standardisation (e. g. within ISO/TC 24/SC 4, cf. Table 3 in Appendix B). However, care must be taken when referring to these documents that the specific applicability of the measurement techniques to size ranges including also the sub-100 nm part is not addressed systematically. In the wake of the EC the definition recommendation, several authors provided experimental data on the classification by available techniques in volume metrics [9,12,13,14], surface metrics [15], microscopic counting metrics [16,17], or proposed novel methods [18].

However, conceptual reviews lamented quite correctly that all these contributions remained very limited in the diversity of test materials in cross-correlation of techniques with different inherent metrics, or in both – and asked for experimental data to support a self-consistent and widely applicable guidance [12,19,20,21,22,23].

Up to date, a systematic evaluation of potentially suited measurement techniques was not carried out on real world materials, i. e. industrial materials with complex shapes and broad size distributions. Extensive interlaboratory comparison exercises dedicated to the determination of the nanoparticle size distribution have been performed in the recent years almost exclusively on rather idealized, “user-friendly” materials such as spherical, monodisperse and well dispersible silica, polystyrene or gold nanoparticles, many of them being certified reference materials [1,9,24,25,26]. In some studies various measurement techniques, mostly TEM, SEM, DLS, AFM, PTA, SAXS and AC, are taken into account, the corresponding measurement uncertainty budgets are more or less rigorously calculated and the results of the various techniques are compared. Other interlaboratory comparisons are carried out also on well-defined nanoparticle samples, but using only one particular sizing technique in different laboratories, e. g. TEM [5], PTA [27] or BET [15]. Such systematic studies with a more solid metrological background are a prerequisite for the generation of standard procedures to be applied to the classification of a (nano)material according to the EC definition by using a specific measurement technique.

The NanoDefine project was set up to support the implementation of the EC definition in all regulatory contexts by an integrated analytical approach. This approach involves the performance evaluation of existing techniques, improvements in instruments and software, development of sample preparation and measurement methods for selected target materials, and provision of guidance by a method manual and an e-tool for selection of the most appropriate (combination of) methods and classification of materials according to the EC definition. The analytical concept consists of a tiered approach, applying techniques of increasing complexity and complementary measurement principles, but also suggesting adequate procedures for sampling, sample preparation, measurement and data evaluation as well as plausibility checks and minimum performance requirements.

In this paper data from a large-scale study on the capability of different measurement techniques to correctly classify particulate materials according to the recommended NM definition are presented. This the first European coordinated initiative destined to evaluate experimentally the performance of most of currently applied particle sizing techniques for the characterisation of a broad variety of quality control materials and real world test materials under harmonised conditions for sample preparation, data analysis and reporting. This delivers a unique data set that allows to draw conclusions and to give recommendations on possible use and limitations of these techniques.

## 1 Design of the analytical study

Measurement techniques (MTs) that are relevant in the context of NM classification are rather di-

verse with respect to their measurement principles, their historical fields of applications and the scientific domains concerned. For this reason, it was neither possible nor meaningful to conduct the analytical study in just one laboratory. Instead, the experimental work was shared among nine participants with long experience and acknowledged expertise for specific MTs. Some MTs were available at more than one participant, this enhancing the quality of the study. In addition, evaluating the „real-world“ performance of MTs requires that the particulate samples reflect the real diversity of particulate materials with respect to chemical composition, particle morphology and size range. For this reason a rather large set of materials had to be selected as representative. In total, the analytical study comprised 174 successful material analyses (not all analyses yielded meaningful results of particle size). For the evaluation of the significance and inter-comparability of such a large and complex data set it was important to ensure that the single steps of the analytical chain are identical or at least similar among the different laboratories and do not differ principally among the materials. This chain includes sampling, primary sample preparation (yielding stock suspensions), secondary sample preparation (for feeding the instrument), instrument preparation (regular qualification, calibration, equilibration, settings), the actual measurement, data analysis and reporting all steps.

The following sections reflect the main ideas in selecting materials and measurement techniques, and they describe the concepts of sample preparation, measurement and data analysis. More details are provided in the annexes and as supplementary material.

## 1.1 Particle systems

In order to assess the performance of the selected MTs for the purpose of identifying NMs, a set of 15 different particulate materials was selected and supplied to the participants of the analytical study. These materials can be grouped into quality control materials (intended to qualify the sizing techniques), and representative test materials (intended to reflect the variability of “real-world” materials). The quality control materials are composed of individual, i. e. (virtually) non-aggregated, particles of spherical or sphere-like shape. In addition, the impact of sample preparation was diminished by providing the quality control materials as stabilised suspensions. The study used quality control materials with a rather narrow or with deliberately wide, even trimodal size distributions.

Representative test materials are commercial powders, for which appropriate dispersion procedures had to be developed before starting the characterisation study. The list of representative test materials comprised mined and manufactured materials, inorganic and organic ones, materials with amorphous or crystalline phase structure, colour pigments as well as non-light-absorbing materials. In addition, various types of particle morphology are represented (general irregularly-shaped particulates, needles, platelets; weakly and strongly bound agglomerates, compact and fractal-like aggregates). Moreover, the study included two material pairs, which referred to different granulometric grades of one substance.

These representative test materials represent conventional nano- and non-nano particulate materials with kiloton-to-Megaton production quantities [28,29,30,31] for the industry segments of paper and packaging, automotive coatings, plastics in consumer equipment, paints, anti-caking additives in food and feed. The representative test materials do not cover the important classes of reactive or otherwise instable particulates, such as cements and volatile organics respectively; they further do not cover macroscopic particulate materials such as polymer granulates and pellets with constituent particles above 100 µm in volume or surface metrics. Reactive and macroscopic materials pose additional challenges, as they are intended to change their physical and chemical properties just after suspending, dissolution or melting, for which reason the particle size can be severely affected by the milieu (dispersion medium, temperature, pH etc.). However, one substance (RTM9, basic methacrylate copolymer) represents such an intermediate of relatively larger size.

A brief survey of all quality control materials and test materials is given in Table 1 (Appendix A). It also provides pieces of information on the respective polydispersity and NP content, which were all derived from number weighted distributions of the minimum Feret diameter as measured with a high-resolution SEM (cold field emitter SEM).

## 1.2 Sample preparation

Sample preparation constitutes a crucial step within the analytical chain, because it determines the state of dispersion which prevails during the measurement. Two phases of sample preparation can be distinguished: a primary phase that provides well-dispersed and stabilised stock suspensions for analysis with different instruments, and a secondary phase that comprises all measures to transfer samples of the stock suspension into the measurement zone. The former is intended to adjust the state of dispersion. In the context of NM characterisation, it aims at the individualisation of the constituent particles or at least at an utmost feasible degree of desagglomeration. In contrast, secondary sample preparation is to conserve the (once achieved) state of dispersion (i. e. to avoid re-agglomeration) when the sample is adapted to the measurement instruments by dilution or addition of various agents (e. g. rheological or colourising agents, electrolytes).

The specific feature of this analytical study is the fact that apart from BET all characterisation methods are based on suspension samples – even EM analysis. Yet, only the quality control materials and representative test material RTM6 were provided as well-dispersed and stabilised suspensions to all participants. These materials did not require sophisticated steps for primary sample preparation; slight agitation (shaking, stirring, short bath sanitation) ensured re-suspension of settled particles and homogenisation of local particle concentration. If the measurement required a dilution this was realised with filtered, de-ionised water. The spray-DEMA analysis forms an exception, because the employed electro-spraying required sample dilution in a particle-free ammonium acetate buffer.

All other representative test materials were provided as powder, which meant that the preparation of well-dispersed and stabilised stock suspensions had to be conducted by the participating laboratories. For this purpose dispersion protocols were developed for each representative test material and provided to all partners. These protocols define the wetting agents, stabilising additives and parameters of dispersion, which yielded finest size distributions with *cuvAC-turb* or *DLS*. In each case, ultrasonication served as chief technique for desagglomeration because the stress intensities within cavitation fields are comparatively high [32]. In addition, participants were advised to control the final state of dispersion via the energy density [33,34]. However, implementing the energy density concept in practice was more difficult than expected. This is because the participants worked with different types of ultrasonication disperser (probe sonicators and vial tweeters) and handled different sample sizes (a few millilitre up to 200 ml); an accurate determination of the energy input is particularly challenging for vial tweeters and minute sample sizes. Additionally to the differences induced by the local set-ups used for de-agglomeration, also the re-agglomeration up to the completion of data acquisition is a potential source of differences between labs. Yet, we benefit from the fact that some representative test materials proved to be well dispersible in the sense that low energy density suffices to either individualise all constituents or to decompose agglomerates in rigidly bound, hardly dispersible aggregates (e. g. RTM1, RTM3 or RTM9). Regarding the quality of our analytical study, we therefore expected for representative test materials RTM7 and RTM8 a significant impact of sample dispersion on the comparability of measurement results.

Details on sample preparation are provided in A.2.

## 1.3 Measurement techniques

One of the most crucial tasks in planning this study was the selection of the measurement techniques (MTs) since an all-embracing set of MTs would be neither meaningful nor feasible. Hence, different criteria were defined for the selection process, including applicability to the nano-range (< 100 nm), ability to directly measure number weighted size distributions, availability (for industry, academic world and legal authorities), and accessibility to the project consortium. These criteria emphasise different aspects and were treated as non-exclusive. The final decision on the MT selection was taken after an expert survey.

The following MTs were eventually selected: transmission electron microscopy (TEM), scanning electron microscopy (SEM), single-particle inductively coupled plasma mass-spectrometry (sp-ICP-MS), particle tracking analysis (PTA), differential electrical mobility analysis on sprayed suspensions (spray-DEMA), analytical centrifugation in disc centrifuges with turbidity detector (*discAC-turb*), analytical centrifugation in cuvette centrifuges with turbidity detector (*cuvAC-turb*), analytical centrifugation in cuvette centrifuges with refractive index measurement (*cuvAC-RI*),

asymmetric flow field-flow-fractionation with light scattering detection (AF4-LS), dynamic light scattering (DLS), angular light scattering (ALS), small angle X-ray scattering (SAXS), ultrasonic attenuation spectrometry (USSP) and gas adsorption analysis based on the BET-method (BET). The main features of these techniques are explained in Table 3 in Appendix B.

It is clear that the selected MTs could be easily supplemented by other MTs, especially by new developments, which explicitly aim at the characterisation of NMs (e. g. differential surface plasmon microscopy [35]). In addition, some measurement principles, which can be technically realised in various ways, are only represented by one (frequently used) MT (e. g. AF4-LS as one type of field-flow-fractionation techniques). Last but not least, some MTs were excluded from this study because of their very limited availability (e. g. SANS). Nevertheless, we believe that our list is a representative collection of available and employed MTs in the field of NM characterisation. It does not only contain established MTs (e. g. AC and ALS), but also relatively new developments (e. g. spICP-MS, PTA).

The selected MTs can be distinguished with respect to the way of particle quantification (by counting, via fractionation, from spectroscopic signals; or integral signal instead of resolving the size distribution) and with respect to the probed particle property. Such properties may be particle volume or mass, based on particle mobility (incl. diffusion coefficient, settling velocity, electric mobility) or related to a scattering pattern. In the case of image analysis, the selection is up to the operator; in this study only the minimum Feret diameter was considered (as an estimate of smallest external dimension). A further distinction refers to the question, if the MT intrinsically determines the sum or the (transformed) density function of the size distribution ( $Q(x)$  or  $q(x)$ , respectively  $q^*(x)$ ).

Based on their technical characteristics it is possible to express some expectations on the performance of the selected MTs. The first point is that only image analysis offers the chance to directly measure the external dimensions of particles. For isometric and elongated (i. e. needle-like or fibrous) particles it provides good estimates of the smallest external dimension, but it may be a challenge to do so for flat, platelet-like particles. Scattering patterns, which can be considered as 2D-transforms of the 3D-morphology, principally reflect all external dimensions including the smallest one. Yet this requires that the pattern is measured in high resolution at the relevant scattering angles; for nanoparticles this is only possible with SAXS (and SANS). In contrast, mass and mobility-based properties cannot resolve the particle morphology; although mobility is affected by it. A typical order of length scale is: largest dimension > hydrodynamic diameter > volume (or mass) equivalent diameter > Stokes diameter > VSSA equivalent diameter > smallest dimension. For particle aggregates, mass and mobility are always affected by the aggregates outer dimension, but also by the internal aggregate structure. In the worst case (for the purpose of NM classification), the corresponding equivalent diameter is close to the diameter of the aggregate's convex hull; in the best case, they are upper limits of the constituent particles. For fractal aggregates it was demonstrated both theoretically and experimentally, that the volume equivalent and Stokes diameters are considerably smaller than hydrodynamic or any geometric aggregate diameter [36,37].

A further consideration refers to the quantification of size fractions. If particles are not counted, but quantified by physical properties (e. g. by weight, turbidity or scattering intensity), then signals of coarse particles may outweigh those of the fine ones. Consequently, the minimum size would be overestimated and the quantity of fine size fractions underestimated. Obviously, this problem is particularly relevant for high polydispersity.

An additional aspect with respect to the analytical task defined by the recommended definition for NMs is the distinction between MTs that – by measurement principle – determine sum functions of the size distribution and MTs that inherently measure density functions. The former group comprises all counting and some fractionating techniques (e. g. EM and cuvAC), while the latter is mainly formed by the spectroscopic techniques (e. g. DLS; but also discAC-turb). This is relevant because the requested median value  $x_{50,0}$  is a characteristic of the sum function.

All these considerations mean that an imaging technique would be first choice for an identification of NM according to the recommended definition. Therefore, electron microscopy (EM) techniques are regarded as reference MTs within this study.

#### 1.4 Particle size measurement and data analysis

From a metrological point of view, the experimental programme of this study must be regarded

highly ambitious. Not only that the different MTs determine different intrinsic type of quantities, but these different MTs were placed at different institutions (with specific backgrounds in particle characterisation) and thus operated by different people (with varying expertise and individual preferences). To ensure comparability of measurement results under such conditions requires a common strategy on handling samples and conducting as well as analysing measurements. In a strict sense the final results may be compared only if they are traceable, i. e. provided with realistic measurement uncertainty budgets. Even for experienced operators the quantitative evaluation of whole traceability chain is a challenging task. In this study the following measures were implemented (cf. supplementary data):

- protocols for ensuring a uniform and reproducible state of dispersion at measurement (which goes beyond the sample preparation, described in section 1.1)
- guidelines for ensuring similar and optimum measurement conditions when working with different instruments of a MT (e. g. in case of spray-DEMA or DLS) or measurement principle (e. g. for all AC instruments)
- rules for replicating measurements in order to estimate method repeatability (i.e. precision)
- set of consistent values for model parameters (e. g. refractive index, cf. supplementary data S.5)
- a template for reporting the measurement data in a harmonised way (i. e. reporting identical parameters of particle size distribution) and the experimental conditions
- request to check the instruments performance with reference materials before starting the experimental programme (qualification of the instruments)

Despite these measures, it was not possible to completely exclude variations in the state of dispersion or to conduct the measurements always at the instrument's optimum settings. In addition, it was not feasible to evaluate the method repeatability and intermediate precision at the same level of sophistication because the effort of measurement did considerably vary. For instance, the total time expenditure for a DLS measurement is less than 1 hour, but may expand to more a few hours for electron microscopy. As far as we were aware of such imperfections they are considered in the evaluation of the experimental data (section 3).

After having conducted the measurements, size distributions were calculated with the conventional instrument software (usually commercial software provided by the instrument's manufacturer). We deliberately refrained from using specialised high-end research algorithms, which would distort the "real-world" performance of existing measurement techniques. This means, for instance, that for all but the imaging MTs the particles were considered homogeneous spheres. A few MTs require a manual pre-treatment of the measured data by experienced operators (e. g. for handling of noise or outliers). When such a pre-treatment is part of the usual analysis procedure it was allowed as long as it followed clear rules (cf. supplementary data S.2). For the purpose of our study, we primarily compared number weighted size distributions  $Q_0(x)$ . This required the conversion of the intrinsically measured size distributions for some of the MTs (cf. Table 3), yet instrument software frequently provides size distributions in any type of quantity (TOQ). The conversion into a number weighted size distribution may involve a prior smoothing of measured data (e. g. cuvAC) and/or may employ a model for the intrinsic type of quantity, which needs additional material properties (e. g. when intrinsically measuring an extinction weighted size distribution). The impact of conversion procedures on the MT's performance is discussed in section 3.2).

## 2 Results

Originally, it was intended to analyse each material with each of the MTs. However, in some cases it was not possible to conduct measurements because of restrictions set by the sample or by the MT: For instance, BET measurements were not possible for the quality control materials, which were supplied as dilute suspensions. USSP measurements could be conducted only on a small set of materials, because the relatively large sample quantities required by the employed instruments (approx. 1 g to 5 g particles) were not available. Organic samples cannot be analysed by spICP-MS. Nanoparticulate BaSO<sub>4</sub> could not be analysed with sp-ICP-MS because this substance although classified as "hardly soluble" starts dissolving under the extreme dilution re-

quired for spICP-MS analysis (in line with the dissociation constant). For some techniques (e. g. ALS and PTA), it was not possible to characterise the finest quality control materials (i. e. QCM2, QCM3, QCM4) since the detection limits for these materials are far above 100 nm. In addition, we encountered difficulties during the analysis of a few materials, which were related to sample preparation rather than to technical limitations. For example, when samples were stabilised with surfactants, their spray aerosolisation for DEMA would be impeded due to foaming. All combinations, which did not allow reliable measurements are indicated as "n.m." (not measurable) in Table 4 (Appendix D).

The realised measurement programme remains significant to conclude on the principal performance of the selected MTs for the identification of NMs. This section will first present and summarise the results of the quality control materials. In a second step, results of selected representative test materials are shown in detail. In order to focus on the accurate determination of the number weighted median size ( $x_{50,0}$ ), only the cumulative functions of the number weighted size distributions ( $Q_0(x)$ ) are shown. In this paper, the term "size" either refers to the equivalent diameter specific to the respective MT or to the minimum Feret diameter determined by imaging techniques (i. e. TEM and SEM). Values and graphs for the density functions and for the intrinsically measured size distributions (i. e. weighted in the intrinsic type of quantity) are reported in the measurement reports, which are provided as supplementary data.

Eventually a survey of all number weighted medians is given in Table 4.

## 2.1 Quality control materials

The employed quality control materials consisted of spherical particles (or at least particles with similar shape) of varying chemical nature (metals, oxide, polystyrene). Four quality control materials show a monomodal and relatively narrow size distribution (QCM1, QCM2, QCM3 and QCM4), while two others are polydisperse and multimodal (QCM5 and QCM6). It turned out that the performance of the MTs depended on to which group the quality control material belongs. For this reason the two groups will be separately discussed. Yet, one can also observe some common features:

In general, there is a rough agreement among the intrinsically measured size distributions within a class of characterisation techniques at least for well stabilised suspensions. That means the number weighted size distributions ( $Q_0$ ) of EM techniques are mostly in good agreement. Also, the extinction and volume weighted size distributions ( $Q_{ext}$ ) of AC techniques agree fairly well, and the same applies to the intensity weighted size distributions ( $Q_{int}$ ) of DLS and AF4-LS.

The intrinsically measured size distributions (e. g.  $Q_{int}$  or  $Q_{ext}$ ) of non-counting MTs like DLS or AC sometimes show coarse particle fractions (even > 100 nm), which virtually "disappear" after conversion into  $Q_0$  (QCM2 and QCM3). Such coarse fractions imply that the particle system had experienced some degree of agglomeration (either in the original sample or after having been fed to the measurement system), yet their detection is typically related to the relatively high sensitivity towards coarse particles and agglomerates. In this regard, conversion can improve the apparent performance of a MT with respect to NM identification, since this specific analytical task requires ignoring aggregates and agglomerates.

On the other hand, one can also observe that conversion into  $Q_0$  may considerably amplify slight differences prevailing in the  $Q_{ext}$  or  $Q_{int}$ , in particular when these differences refer to the quantification of fine particles (e. g. discAC-turb and cuvAC-turb for QCM3, cf. discussion in section 3.2).

### 2.1.1 Quality control materials with narrow size distribution

Three of the four quality control material materials (QCM1, QCM2, QCM3) had narrow size distributions in the range of 10 nm to 100 nm, while the fourth (QCM4) consisted of particles smaller than 10 nm. Graphs of the number weighted size distributions for all quality control materials are provided in the supplementary data.

A general observation for these quality control material materials is that the differences among the results of different MTs or instruments of the same MT increase as the particle size goes down. This even applies to the electron microscopy data, which typically agree very well for particle systems with low polydispersity, but significantly deviate from each other for the finest quality control material QCM4. However, this behaviour in the very low size range has no significant

consequences on the NM classification according to the EC definition.

A further observation is that some of the MTs did not allow for a characterisation of these quality control materials because their particle size was beyond the accessible measurement range. This applies to ALS and partly to PTA, spray-DEMA and sp-ICP-MS. PTA could be only used for the coarsest quality control material (QCM1,  $x_{50,0} \sim 45$  nm), for which it generated size distributions close to those of EM. Spray-DEMA was only applicable to particle systems  $\geq 10$  nm, which is the lower detection size limit of the instrumentation employed. In contrast to PTA or spray-DEMA, the limited applicability of sp-ICP-MS depends to a large extent on the material properties. In particular, polymer (organic) particles are not amenable to ICP-MS. Measurement of SiO<sub>2</sub> particles is hampered by lower sensitivity, high background and isobaric interferences so that characterisation in the nano-range is not facilitated; hence, sp-ICP-MS was only applied to the Au-quality control material (QCM3). Last but not least, BET and USSP could not be applied to the quality control materials, since the sample size (mass of particles, total suspension volume) was insufficient, and – in case of USSP – because particle concentrations were too low.

The following paragraphs briefly address the performance of the selected MTs.

In this study, PTA and sp-ICP-MS are the only counting techniques in this study that do not rely on image analysis. Since both were just applicable to only one of the narrowly distributed quality control materials, conclusions on their general performance could not be drawn. PTA worked very well for QCM1, whereas sp-ICP-MS clearly overestimated the size of the Au-quality control material (QCM3) – cf. supplementary data and Table 4. While the focus of this study lies on the determination of the number weighted median  $x_{50,0}$ , the measured size distribution may reveal considerably more details on the state of dispersion. This holds particularly true for counting techniques as they inherently yield highly resolved size distributions.

Among the regarded MTs spray-DEMA has some unique features. Even though relying on a fractionation of the particle system it intrinsically measures number weighted size distributions. In addition, it is the only aerosol-based MTs within the analytical study. However, the aerosolisation of suspended particles coincides with "residual particles", which are non-intentionally generated particles from dissolved electrolytes or surfactants etc. These particles typically show an exponential size distribution, which superposes the size distribution of the test specimen. Spray-DEMA therefore requires the elimination of "residual particles", a task which can be conducted physically by electro-spraying or during data analysis (assuming that the modes of residual and relevant particles are clearly separated). For the narrowly distributed quality control materials spray-DEMA was in good accordance with EM-results (almost perfect agreement for QCM1, slightly coarser size values for QCM2 and slightly finer ones for QCM3). Moreover, the differences between the two instruments employed are marginal.

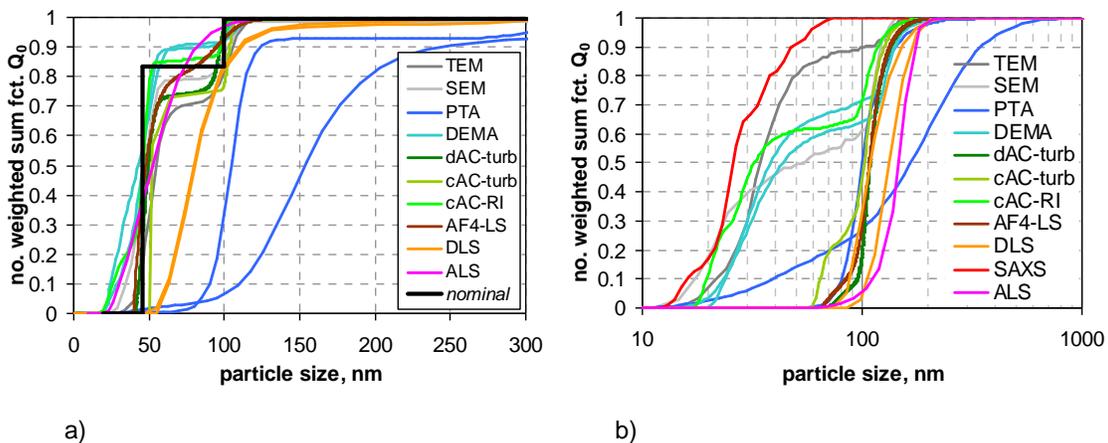
A further group of MTs are formed by the AC techniques, for which generally only little result variation among the three techniques (discAC-turb, cuvAC-turb, cuvAC-RI) is observed, although the principles of fractionation and quantification are qualitatively different. Deviations from each other are most pronounced for the finest particle system (QCM4, i. e. 5 nm Ag). A consistent explanation of this behaviour cannot be given; probably a combination of different effects is responsible. Brownian motion is incorporated in the cuvAC-RI data analysis, but is ignored in classical cuvAC-turb and discAC-turb data analysis. Further, the plasmon resonances of Ag and Au constitute a size-dependent modulation of the optical signal that is not corrected by any of the AC evaluations, which assume that real and imaginary parts of the refractive index are uniform for all particles.

A somewhat unexpected outcome of the quality control material characterisation is the similar performances of DLS and AF4-LS. Both agree fairly well with each other (provided that the void peak signal in AF4-LS can be clearly separated, cf. section 3.2 and S.2). In addition, the results of the two different DLS instruments almost perfectly match. However, when compared to EM techniques the  $Q_0$  of both techniques are not highly reliable. While the covered size range is in accordance with EM, the number weighted median is once underestimated (QCM1, QCM3), then overestimated (QCM4), and also fits to the EM result (QCM2). This is not really unexpected, since DLS intrinsically weighs size fractions according to their scattering strength, which is roughly proportional to the squared volume within the nano-range and thus matches the trend of insensitivity towards the finest particles. In addition, DLS requires a numerical inversion procedure of the spectral signal (time correlation function), which inevitably introduces some bias on the shape of the distribution function [38]. If, for instance, this bias artificially creates a small fraction of fine particles within  $Q_{int}$  this may result into a significant overestimation of fine particles within  $Q_0$ .

A last MT that was employed to the narrowly distributed quality control materials is SAXS, which appears to be very close to the EM results (QCM2 & QCM4). This MT benefits from its high sensitivity to structures in the nano-range and from the fact that the analysed scattering signals are essentially surface weighted ( $Q_2$ ), which keeps possible negative impacts by conversion small.

### 2.1.2 Quality control materials with broad size distribution

Two quality control materials, QCM5 and QCM6, possessed a relatively high polydispersity (cf. Table 3), but they differed in the details of the size distribution. Quality control material QCM5 was composed of three narrowly distributed PSL samples, which led to a rather artificial shaped size distribution with distinct peaks in and beyond the nano-range (yet only in  $Q_3$  the peak at is clearly visible). In contrast, quality control material QCM6 was a polydisperse, commercial slurry with three not very distinct size modes. The most interesting feature of both quality control materials is the simultaneous presence of nano and non-nano particles (i. e. the existence of size fractions below and above the critical value of 100 nm). The particles in both quality control materials were coarser than for the systems in section 2.1.1, for which reason ALS and PTA could now be employed.



**Figure 1:** number weighted sum functions of a) QCM5 (trimodal PSL) and b) QCM6 (trimodal  $\text{SiO}_2$ ); from measurements with TEM, SEM, PTA, spray-DEMA, discAC-turb, cuvAC-turb, cuvAC-RI, DLS, AF4-LS, ALS and SAXS

A first glance at the results of size analysis (Figure 1) reveals that the differences among the various MTs are much more pronounced than for the narrowly distributed quality control materials. In the case of QCM5 (with clearly separated modes), most MTs were able to recover the whole size range and even reflect the multimodal shape of the size distribution. However, only few MTs did accurately determine the nominal composition, which was originally defined in terms of mass ratio (cf. Figure S-19, supplementary data). Best performance is achieved by AC techniques, followed by EM and AF4-LS. A different situation prevails for QCM6, where only few MTs indicate a multimodal size distribution. A clear distinction between a significant nanoparticle fraction at around 25 nm and a fraction at around 120 nm is only achieved by EM, spray-DEMA and cuvAC-RI. The two latter MTs also indicate the presence of third size fraction in the range from 60 nm to 100 nm with a relative weight of approx. 3 % by number. The existence of this fraction is further confirmed by the two other AC techniques and by AF4-LS, which however did not detect the fine mode at 25 nm. The remaining MTs evaluate the particle system as monomodal. While most MTs could not detect the fine NPs in this broadly distributed particle system, SAXS appears virtually insensitive to the coarse particles.

The examples of the two quality control materials show that a simple evaluation of a MT's performance is not possible. Even for the EM techniques there is no unambiguous picture: While they agree with respect to the size of the different modes, they quantify these modes differently. This is particularly visible for QCM6. This effect can be generally explained by the high sample surface sensitivity, i. e. better visibility of smaller particles onto bigger particles, in SEM than in TEM. Most small particles situated behind larger particles are invisible for both SEM and TEM. Hence, the observed difference can be attributed to a combination of insufficiently good sample preparation with technique detection capabilities.

Of all the other MTs only spray-DEMA showed a (more or less) good agreement with the EM techniques. This is certainly favoured by the fact that both quality control materials are well above the lower size limit of this MT (=10 nm) and that it intrinsically yields number weighted size distributions.

All AC techniques recovered the multimodal shape of quality control material QCM5 fairly well (with regard to both, size and quantity), but they did not perform uniformly for QCM6. Only cu-vAC-RI resolved the trimodal shape and detected the finest particle fraction at around 25 nm. The two other AC techniques, which rely on turbidity measurement, were obviously blind for the fine particles. Nevertheless, both did identify the third mode in the range from 60 nm to 100 nm, which is not seen by most other techniques. Regarding the main features of the distribution functions, there is only minor discrepancy between the turbidity based AC techniques and AF4-LS.

Unlike with the narrowly distributed quality control materials, the performance of DLS and AF4-LS differs for the multi-disperse quality control materials, especially for QCM5. Obviously, the fractionating step by AF4 facilitates the detection of the finest particle mode (at 50 nm), which is not seen by DLS. Yet, for quality control material QCM6 both techniques ignore the finest size fraction around 30 nm, which is certainly related to extremely strong dependency of scattering intensity on size in the nano-range (the 30 nm mode of QCM6 scatters approx. 1300 times less than the 100 nm mode; whereas for QCM5 the finest mode of 45 nm scatters approx. 120 times less than the 100 nm mode).

It is interesting to note that ALS performs similarly as AF4-LS. That is, it agrees rather well with EM results for the multi-disperse trimodal quality control material QCM5, but clearly ignores the 30 nm mode of QCM6. For this quality control material, the number weighted median is among the coarsest ones.

The largest deviation from EM results of QCM6 is observed for one of the PTA instruments. In the case of QCM5, both PTA instruments underestimated the NP content and clearly failed to classify this material as nanomaterial.

While the "optical" MTs (i. e. DLS, ALS, PTA) are prone to underestimate the amount of NPs, thus to overestimate the number weighted median  $x_{50,0}$ , the opposite behaviour is demonstrated for SAXS. While this MT performed rather well for the narrowly distributed quality control materials it clearly underestimated the maximum particle size for QCM6. This is related to the MT's lacking sensitivity for particles well above 100 nm. Regarding the  $x_{50,0}$  of this quality control material the impact is rather marginal. However, the deviations may become large for size distributions with increased polydispersity (maximum particle size at 1  $\mu\text{m}$  or above) and generally for all non-nanomaterials.

### 2.1.3 Conclusions on quality control materials

The results of the quality control material materials have shown that a MT's performance depends on material, mean particle size, width of the size distribution and shape of the distribution function. In general we can state that the size determination of the various MTs is rather reliable for narrowly distributed particle systems, i. e. number weighted size distributions are consistent with the results of electron microscopy (the difference with respect to  $x_{50,0}$  is less than 20 % for almost all MTs). This holds true, as long as the particle size falls into the respective measurement range. Several MTs have a lower size limit well above 1 nm (e. g. sp-ICP-MS, PTA, ALS, AF4-LS), which restricts their applicability and reduces their general reliability regarding the quantification of nanoparticle fractions. In principle, one should also regard the upper size limits. Yet for the quality control materials (maximum size: approx. 350 nm for QCM5) this was just relevant for SAXS, where the upper size limit is approx. 100 nm for conventional SAXS instrumentation. The quality control material analysis has also illustrated the existence of further applications limits, which refer to minimum values for concentration of suspended particles, sample volume or total particle mass. Such limits are particularly relevant for BET and USSP, which both could not be applied to the suspension quality control materials selected here; however, powders of certified reference materials for BET are available from NIST, BAM, and IRMM.

Most non-counting MTs are seriously challenged by highly polydisperse samples because the sensitivity towards a given particle fraction is typically increases with size (e. g. a nanoparticle's contribution to RI-increment is proportional to its volume, while its scattering intensity is proportional to the squared volume). As a result, the quantity of fine particles is typically underestimated, and the resulting median particle size is too coarse. In principle, this effect should be more

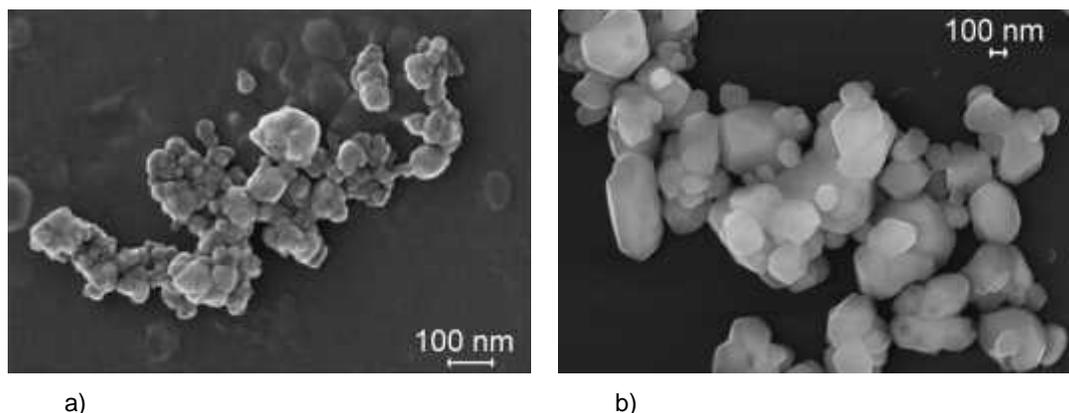
emphasised for spectroscopic MTs (DLS, ALS) than for fractionating MTs (e. g. AC, AF4-LS); this hypothesis could be partially confirmed (QCM5). However, results on QCM6 (polydisperse SiO<sub>2</sub> suspension, i.e. particles with low optical contrast) indicate that the a priori superior performance of fractionating MTs still requires that the measurement signals of the fine particles are sufficiently high for detection. In other terms: The performance of a MTs with respect to the measurement of number weighted size distribution is less affected by the way of quantification (via fractionation or from spectroscopic signals), than by its intrinsic TOQ (proportionality to number, surface, volume, squared volume etc.).

Last but not least, it should be also noted that even the reference MTs, i. e. TEM and SEM, did not produce unambiguous results for the quality control materials with high polydispersity. While their results agree in size range and modal values they differed considerably with respect to the quantity of the size modes due to the reasons described above. In general, this may result in a tremendous error of the number weighted median, which is critical in the context of the recommended NM definition. It is unlikely that the differing results are related to the image analysis because the quality control materials consist of well-stabilised, isolated and spherical particles. Instead the example points to the Achilles heel of EM analysis: the preparation of a representative sample of the particle system.

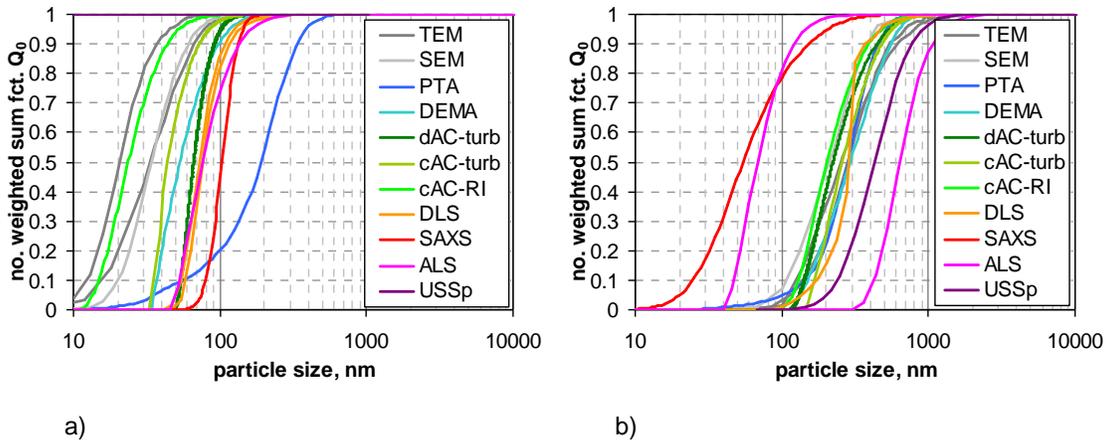
## 2.2 Representative test materials

Beside the quality control materials, the analytical programme comprised nine representative test materials, which are commercial powders consisting of non-spherical, frequently aggregated particles at several size scales (cf. section 1.1). This section presents the results of four of these materials in more detail; those of the remaining materials are presented in the supplementary data. A survey of all number weighted median values is found in Table 4.

At first, the results of samples RTM1 and RTM2 are discussed. Both are BaSO<sub>4</sub> powders, yet they differ in size. In each case the powder consist of particle aggregates, with constituent particles of compact shape (Figure 2). Preliminary investigations on the right dispersion procedure indicated that a virtually complete disintegration of particle aggregates was possible for the "coarse" BaSO<sub>4</sub> sample RTM2, whereas for the "fine" sample RTM1 only a reproducible state of aggregation was achieved.



**Figure 2:** SEM images of a) RTM1 (BaSO<sub>4</sub>, ultrafine grade) and b) RTM2 (BaSO<sub>4</sub>, fine grade)



**Figure 3:** number weighted sum functions of a) RTM1 (ultrafine BaSO<sub>4</sub>) and b) RTM2 (fine BaSO<sub>4</sub>, right); from measurements with TEM, SEM, PTA, spray-DEMA, discAC-turb, cuvAC-turb, cuvAC-RI, DLS, SAXS, ALS and USSP

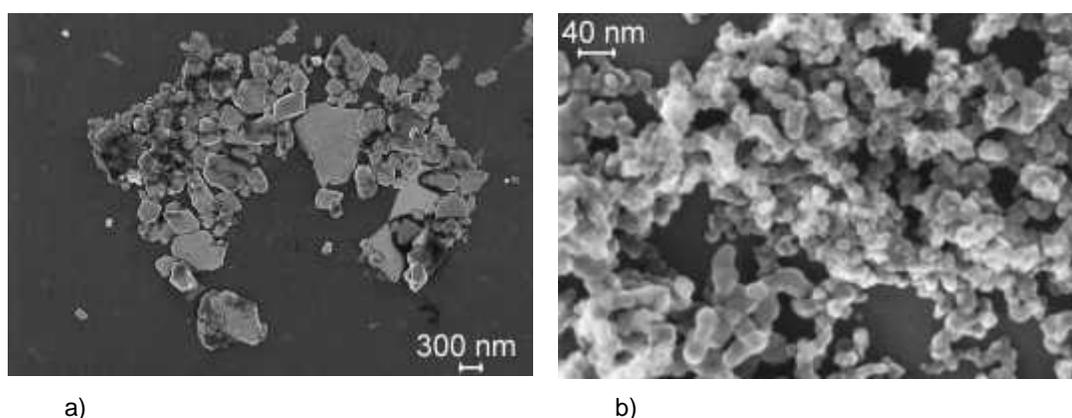
The number weighted size distributions of both materials are depicted in Figure 3. For sample RTM1 (the nano-sized sample) there are obviously considerable variations among all MTs, with EM techniques being among those with the finest size distribution ( $x_{50,0} \approx 30$  nm). EM should deliver the smallest median size for disperse systems that consists of particle aggregates. In detail one observes a high similarity between the DLS and ALS results ( $x_{50,0} \approx 75$  nm) and that the two DLS curves are almost identical. Spray-DEMA yields a slightly finer result ( $x_{50,0} \approx 53$  nm), while the AC techniques are rather inconsistent (cuvAC-RI:  $x_{50,0} \approx 24$  nm, cuvAC-turb:  $x_{50,0} \approx 48$  nm, discAC-turb:  $x_{50,0} \approx 66$  nm). Surprisingly, SAXS virtually ignores particles below 70 nm and yields a relatively coarse value of the number weighted median ( $x_{50,0} \approx 103$  nm). The coarsest distribution function is obtained with PTA ( $x_{50,0} \approx 200$  nm), even though the presence of nanoparticles is indicated. Based on the measurement principle, PTA should be similar as DLS (since both probe the particle diffusion). The observed discrepancy is probably related to different sensitivities for very fine particles. There is also a result of USSP (cf. Table 4), which is, however, hardly reliable ( $x_{50,0} < 1$  nm) – probably due to the relatively low particle concentration ( $\approx 1$  vol.-%). Summarising, the “real-world” sample RTM1 excellently illustrates the discrepancies among MTs in the case of aggregated systems. Electron microscopy, which can probe at least a part of the constituent particles, systematically provides finer values than e. g. DLS, which is sensitive to hydrodynamic diameter of aggregates; for RTM1 the deviation amounts to a factor of approx. 2.5.

In contrast to RTM1, the other BaSO<sub>4</sub> sample (RTM2) is clearly a non-nano material as almost all MT indicate. Apart from ALS, USSP and SAXS there is a relatively good agreement among all MTs (including PTA), with number weighted medians in the range of 203 nm (cuvAC-RI) to 293 nm (spray-DEMA), which coincides with the range obtained by EM techniques (212 nm for SEM, and 280 nm for TEM). For USSP ( $x_{50,0} \approx 410$  nm), the reliability is again poor as the particle concentration was at the very lower limit of application (1 vol.-%). The most remarkable feature of the RTM2 analysis is the striking difference between the two ALS-results. It should be mentioned that the difference is much smaller in the intrinsically measured volume weighted size distribution ( $Q_3$ ) and is instead induced by different concepts of data analysis. In section 3.2, a more detailed explanation of such effect will be provided. A further intriguing outcome is the performance of SAXS, which gives the finest size distribution and indicates a high NP content. This is in line with SAXS performance for QCM6 (Figure 1b) and reveals a severe shortcoming of this MT with respect to the identification of non-nano materials.

Since the two samples RTM1 and RTM2 are different grades of the same substance (BaSO<sub>4</sub>) it is interesting to see to which extent the MTs did reflect the difference in number weighted size distribution ( $Q_0$ ). In this regard, most MTs (i. e. spray-DEMA, AC techniques, DLS) performed fairly well. However, PTA and ALS clearly failed under this aspect because the results of the two grades are (partly) quite similar. For SAXS the evaluation would be even reverse (RTM1:  $x_{50,0} \approx 103$  nm, RTM2:  $x_{50,0} = 54$  nm), due to the MT's insensitivity to coarse particles. An evaluation of USSP was not possible since the measurement conditions (low particle concentration) could not ensure sufficient reliability. On the other hand, the example implies that this MT may encounter similar limits of application in practice. Finally, attention is drawn to the BET equivalent minimum

size ( $x_{\text{BET,min}}$ , Table 4), which were calculated from volume specific surface area (VSSA) as determined by BET method under the assumption of spherical particles (cf. appendix E.1). The two grades are clearly differentiated, but for RTM1, the  $x_{\text{BET,min}}$  is notably, yet not greatly larger than the number weighted medians of EM (25 %), whereas for RTM2 the  $x_{\text{BET,min}}$  is more than twice as large as the  $x_{50,0}$  of EM techniques.

A second pair of representative test materials, which will be examined in detail are samples RTM5 (kaolin) and RTM6 (fumed silica), which are well known for the non-spherical morphology of the dispersed phase. The kaolin sample consists of platelet-like particles, which are partially aggregated (Figure 4a), while fumed silica is composed of highly porous, fractal-like aggregates of nano-sized particles (Figure 4b). The aggregates of both materials are rather firm and impede a complete deagglomeration. In particular for fumed silica it is known that even intense dispersion by ultrasonication leaves aggregates with dozens or even hundreds of constituent particles [37,39,40]. In contrast, kaolin aggregates are typically formed by just a small number of constituents. The major problem of this material is that imaging techniques are biased by the preferential orientation of platelets parallel to the substrate, i. e. the smallest external dimension is typically not accessible to 2D imaging techniques of this type of material.



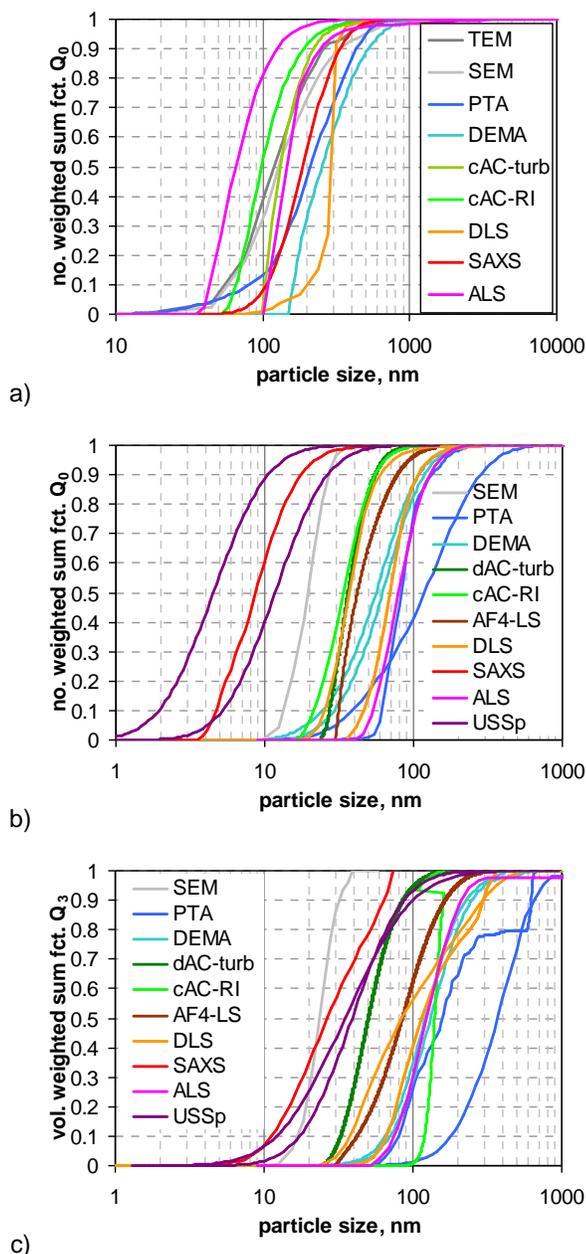
**Figure 4:** SEM images of a) RTM5 (kaolin) and b) RTM6 (fumed SiO<sub>2</sub>)

The graphs of the measured size distributions are presented in Figure 5. For both materials a considerable variation among all curves is observed.

The variation seems less pronounced for kaolin, where the results of TEM and SEM ( $x_{50,0} \approx 125$  nm) are similar to those of the AC techniques ( $x_{50,0} \approx 100$  nm ... 130 nm). However, one should keep in mind that the EM results are biased toward larger values, since the images emphasise the largest external dimension and conceal the smallest one. The equivalent diameters measured by mobility-based MTs should be lower, as these are affected by all external dimensions, and should deliver a value between thickness and lateral diameter. But the measured diameters are even larger than those from EM, and specifically those measured as hydrodynamic diameter are coarser (PTA:  $x_{50,0} = 212$  nm, spray-DEMA:  $x_{50,0} = 252$  nm, DLS:  $x_{50,0} = 290$  nm) than those of centrifugation ( $x_{50,0} < 110$  nm). The general picture is quite consistent with an aggregated suspension, but the results of AC techniques with turbidity detectors and of DLS should be treated with care, since the conversion into  $Q_0$  assumed spherical shape for the optical models, which is far from reality. The analytical programme included ALS measurements with two different instruments. Their results considerably deviate from each other, which is primarily due to the limited size range of one instrument and further enhanced by conversion. There is also the result obtained by SAXS ( $x_{50,0} = 187$  nm), which fits well to the size range covered by the other MTs. In addition, the kaolin sample RTM5 was characterised by USSP. The number weighted median is not meaningful ( $< 1$  nm, cf. Table 4), yet the volume weighted median seems to be rather plausible (65 nm), which is in accordance with theory that the equivalent diameter of USSP is close to the VSSA equivalent diameter [41]. Since the particle concentration was sufficiently high (3 vol.-%) the results for  $Q_0$  are probably due to the conversion and the simplifying assumption of spherical particles in data analysis.

In summary, most MTs – including TEM and SEM – would classify the material as “non-nano”. Indications that the number weighted median of the smallest dimension – thickness – is smaller than 100 nm stem from BET (specific surface area,  $x_{\text{BET,min}} = 48$  nm), ALS (scattering pattern) and

USSP (acoustophoretic mobility).



**Figure 5:** number weighted sum functions of a) RTM5 (kaolin = platelets), and b) RTM6 (fumed SiO<sub>2</sub> = aggregates); c) volume weighted sum functions of RTM6; from measurements with TEM, SEM, PTA, spray-DEMA, discAC-turb, cuvAC-turb, cu-vAC-RI, DLS, SAXS, ALS and USSP

Sample RTM6, a fumed silica consisting of fractal-like aggregates, was considered to be the most critical in our study, since i) the sizes of the constituent particles and the aggregates typically differ by one order of magnitude and ii) the aggregate porosities are large enough to allow some degree of interstitial flow. Previous studies have already shown that this leads to severe deviations among the various equivalent diameters. This expectation is met by the measurement results (Figure 5b), which yield number weighted medians in the range of a few nanometres up to > 100 nm. The reference value by SEM ( $x_{50,0} \approx 20$  nm) refers to the constituent particles and is rather consistent with the BET equivalent minimum size ( $x_{\text{BET,min}} \approx 14$  nm). SAXS, which also probes the size of the constituent particle, yields a significantly smaller value ( $x_{50,0} \approx 8$  nm). The discrepancy is probably caused by the conversion of SAXS data into  $Q_0$ ; by volume weighted medians the two MTs are fairly close (SEM:  $x_{50,0} \approx 24$  nm, SAXS:  $x_{50,0} \approx 26$  nm) – in other terms:

SAXS probably overestimates the polydispersity. In contrast to SEM, BET and SAXS, the remaining MTs do not measure the constituent particles, but reflect properties of the aggregates. Apart from USSP and one PTA, they yield number weighted medians between 37 nm and 82 nm. The curves imply some systematic differences (e. g. that AC techniques determine finer size distributions than those measuring the hydrodynamic mobility. But also the conversion to number metrics contributes and is the main cause of the unreliable  $Q_0$  of USSP. The present material is a rare case where the ALS result matches the mobility-based data. The two PTA instruments yield significantly different results (similar as for QCM5, cf. Figure 1a).

### 2.3 Summary of all experimental data

The previous sections showed exemplary results of particle size analysis for different types of particulate materials. The complete set of analysis results is provided in the supplementary data. Below, these size analyses are summarised in order to discuss the performance of the different MTs. The summary focuses on the number weighted median ( $x_{50,0}$ ) as the decisive parameter for the classification of particulate materials according to the EC definition. According values are presented in Table 4 (Appendix D).

At first, we look at the electron microscopy results because EM techniques had already been identified as most appropriate for the NM classification (cf. section 1.3). This study comprised analyses by two TEM and one SEM instruments. Only the latter was applied to all materials of this study, for which reason its number weighted median sizes are taken as reference for all other MTs. A parity plot of TEM versus SEM results (Figure 6a) reveals a good agreement (within a factor of 1.2) among the three EM results for the majority (8 of 15) of the particulate materials. However, for RTM9 a considerable deviation between SEM and TEM is observed, which is due to improper sample preparation. For three further materials (QCM4, QCM6 and RTM1) the ratio between the largest and smallest  $x_{50,0}$  values lies between 1.5 and 2, and for two other materials the relative deviation amounts to values between 20 % and 50 %. There are manifold sources for such deviations: Improper sampling, sample preparation or particle deposition on the substrate may affect the representativeness of the imaged particles. In addition, the identification of constituent particles and their characterisation is affected by software settings (threshold values, separation algorithms, etc.). In summary, EM techniques provided consistent  $x_{50,0}$  results for most, yet not all materials. The example of RTM5 (kaolin) has even shown (see previous section) that they might systematically overestimate the smallest external dimension. For this reason EM results cannot be used alone for the NM classification.

Keeping in mind the possible bias of the SEM values we subsequently use them for the evaluation of the other MTs. The outcome for non-imaging counting techniques (PTA, spICP-MS) is shown in Figure 6b. The graph shows that PTA has a general problem to measure particles in the range below 100 nm. This holds particularly true for one of the two instruments (by different manufacturers) employed in this study, which systematically overestimated the number weighted median in the nano-range and even failed to identify NMs as such. The second instrument performed better, especially for the non-aggregated quality control materials. However, both instruments *struggled* with detection of very fine nanoparticles, for which reason they did not deliver meaningful results for materials QCM2, QCM3 and QCM4. It should be noticed that PTA generally performed better for particle sizes from 100 nm to 1000 nm. For them, the deviation from SEM results with respect to the number weighted median ( $x_{50,0}$ ) is here less than factor 2 (btw. 1.3 and 1.8).

For sp-ICP-MS, only a reduced amount of measurement data is available, as only two materials (QCM3, RTM3) were accessible to size analysis. In both cases, the MT performed quite well, that is the number weighted medians are close to the SEM value (deviation for QCM3: 37 %, for RTM3: 2 %). However, the lacking applicability to non-metal containing particles (i. e. the majority of quality control materials and representative test materials) illustrates that this MT cannot serve as a universal tool for the classification of NM.

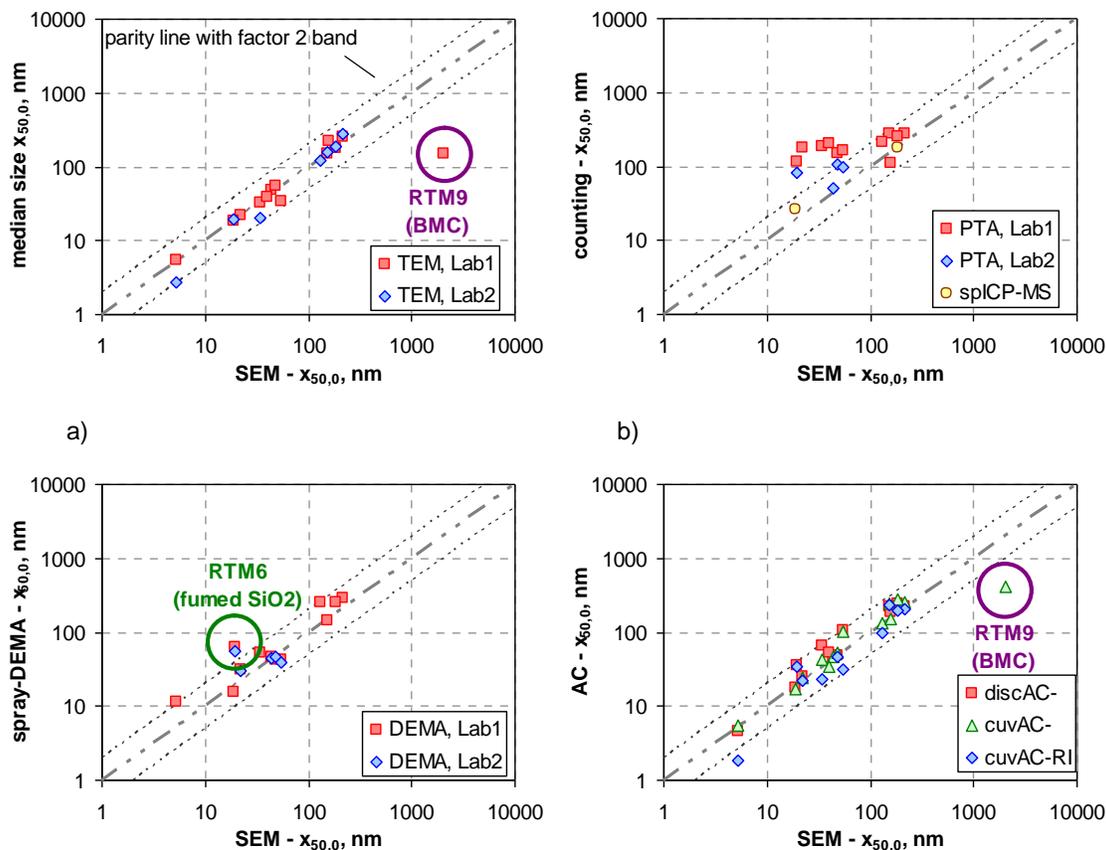
Figure 6c summarises the results of spray-DEMA, which is a representative for fractionating techniques with a counting particle detector. Within this study one commercial measurement system was independently used by two different labs. The figure reveals that their results are highly consistent (less than < 20 %, yet for just 5 materials) and the number weighted medians deviate from SEM values typically by factors smaller 1.5 (for 8 of 11 materials). Only for the fractal aggregates of RTM6 (fumed silica), spray-DEMA overestimated the size by a factor of approx. 3.

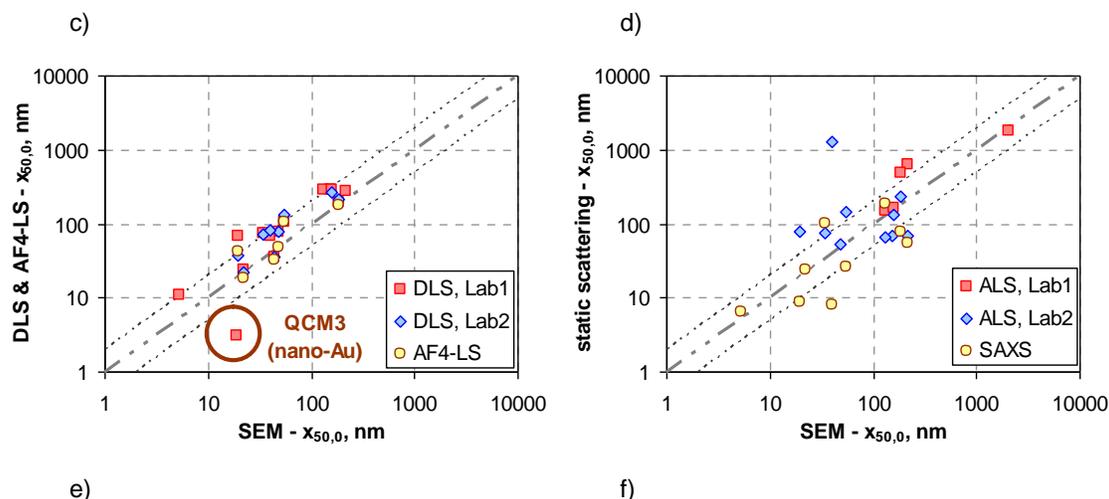
A further group of MTs is analytical centrifugation (AC), of which this study comprised disc-AC with a turbidity sensor (discAC-turb), cuvette-AC with a turbidity sensor (cuvAC-turb) and cuvette-AC with refractive index measurement (cuvAC-RI). The results of the three AC techniques are quite coherent for most of the materials as Figure 6d shows. The maximum variation is less than factor 1.5 for 10 of 14 materials. Large inconsistency (> factor 2) was observed for just three materials (QCM4, QCM6 and RTM1), which was only partly understood (cf. previous section). Regarding the conformity with SEM analyses, one observes that for 10 of 15 materials the number weighted medians ( $x_{50,0}$ ) differ by less than 50 %. There are only two materials, for which they deviate by a factor above 2: QCM3 (which is clearly nano, < 10 nm) and RTM9 (which is clearly non-nano, >1  $\mu$ m).

Figure 6e comprises data of DLS and AF4-LS. DLS results were obtained by similar instruments in two different laboratories. They are quite consistent with a deviation <25 % for most materials (exception: RTM6, for which results differ by a factor of 1.9). However, DLS typically yields larger number weighted medians than SEM – independent of size range. The exceptions are the monodisperse QCMs 1 and 2 (deviation less than 20 %) and QCM3 (underestimation of size). The factor of overvaluation is less than 2.5 for 13 of 14 materials, but amounts to 3.5 for the fractal-like aggregates of RTM6.

In this study, AF4-LS performed essentially similar as DLS, yet the statement relies on the results of just 6 materials. Good agreement with SEM (below 25 %) was observed for the monodisperse QCMs and for RTM3 (TiO<sub>2</sub>), which consists of non-aggregated, compact particles with moderate polydispersity, whereas the polydisperse QCM6 and RTM6 were overestimated in size (up to factor 2.1).

A last graph of this series (Figure 6f) corresponds to the static scattering techniques ALS and SAXS. Both techniques defy an unambiguous evaluation and show contradictory results. For ALS two different instruments were employed, which allows an evaluation of consistency. Obviously, it is rather poor: Only in 1 of 5 materials do the results differ by less than 100 % (for RTM1 they differ even by a factor of 9). This is in line with reports by other authors [11] on the low reliability of ALS data in the submicrometre range. In addition, number weighted medians by ALS deviate considerably and non-systematically from those by SEM (factor is above 2 for 7 of 11 materials).





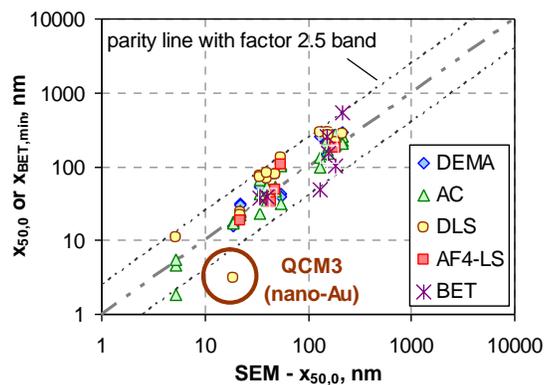
**Figure 6:** parity plots of the number weighted medians  $x_{50,0}$  as determined by the various MTs vs. the SEM value (cf. Table 4), lines indicate parity and deviation from parity by a factor of 2; a) imaging techniques (TEM), b) non-imaging counting techniques (PTA, spICP-MS), c) fractionating techniques with a counting detector (spray-DEMA), d) AC techniques (discAC-turb, cuvAC-turb, cuvAC-RI), e) mobility-based techniques with a light scattering detector (DLS, AF4-LS), f) static scattering techniques (ALS, SAXS)

Similarly, SAXS also deviates notably from SEM for the majority of materials (well above factor 2). However, good agreement with SEM (<20 %) is found for the monomodal QCMs 2 and 4. For the fractal aggregates of RTM6, the number weighted median ( $x_{50,0}$ ) was underestimated by approx. 50 %, which is probably related to conversion issues (see discussion in section 2.2), yet also proves that SAXS probed the constituent particles rather than a property of the particle aggregates. Not all SAXS results are really understood. For instance, why for RTM5 (kaolin) the  $x_{50,0}$  by SAXS was approximately treble the BET equivalent minimum size, or why the number weighted median of RTM7 was 5 times smaller than that of EM techniques. In total, both MTs are not appropriate for the classification of NM. However, SAXS – unlike ALS – is not really well-explored and the MT is still experiencing significant developments.

The graphical summary of Figure 6 does not include the only acoustical MT in this study, USSP, which has the principal advantage of covering a broad size range (10 nm ... 100  $\mu$ m), but also requires a relatively high minimum particle concentration for reliable measurements. The latter proved to be a critical aspect in this study, where sample volumes and particle concentrations had to be kept low in order to ensure homogeneity among all samples sent to the participants. In this regard, the study could not accurately reflect the MT's performance, whereas in industrial practice sample volume may be of minor relevance. The USSP showed rather interesting results for the volume weighted size distributions of RTM5 (kaolin with platelet-like particles) and RTM6 (fumed silica), because it was similar to the BET and SEM values, respectively. However, after conversion into  $Q_0$  the physical plausibility was lost, which shows a need to improve data analysis.

The parity plots of Figure 6 are a simple and comprehensible way of evaluating the performance of selected MTs with respect to internal consistency and inter-comparability. Our study clearly showed the practical limitations of measuring the number weighted median size ( $x_{50,0}$ ) of the constituent particles of particulate materials. Even EM techniques cannot per se claim high accuracy and reliability, even though for most materials the results of those techniques are quite consistent. The parity plots also indicate deficiencies of PTA, ALS and SAXS in measuring the number weighted median size ( $x_{50,0}$ ) over the complete size range of 10 nm to 1000 nm, which constitutes a severe obstacle for the applicability to NM classification. The graphs also show that all non-counting MTs that are based on the hydrodynamic mobility (i. e. spray-DEMA, AC techniques, AF4-LS and DLS) performed similarly with respect to the  $x_{50,0}$  determination. This was already noticed in the previous sections when discussing the shape of the number weighted size distributions. Such an outcome was not really expected because of the considerable differences in equivalent diameter and intrinsic TOQ. Yet, only in a few cases these differences are relevant. This refers to particle aggregates with high porosity, for which AC techniques determine finer size

distributions, and to materials with high polydispersity, for which spray-DEMA may be more reliable for the fine particle fractions than the others (cf. quality control material QCM6, Figure 1). AC techniques, AF4-LS and DLS require the conversion of intensity, extinction or volume weighted size distributions into  $Q_0$ . This conversion is the most critical step of these techniques since it amplifies noise, artefact modes etc. In some cases this may eventually lead to a misclassification of the substance (see discussion in section 3.2). For DLS this effect is expected to be even more pronounced since the conversion bias adds to that of inverting the spectral signal. Although this holds generally true, it was not particularly relevant in this study. Hence, all four mobility-based MTs (spray-DEMA, AC techniques, AF4-LS and DLS) may serve as screening techniques for classification of real-world particulate materials, even though the performances for RTM6 (fumed SiO<sub>2</sub>) and RTM9 (BMC) were partly rather poor. When these materials are excluded from the study we can show that almost all values of the number weighted median ( $x_{50,0}$ ) agree with the corresponding value of SEM by a factor of 2.5 (Figure 7). That means, when one of these techniques finds number weighted medians ( $x_{50,0}$ ) above 250 nm this implies for most materials that they are not a nanomaterial according to the EC definition. Similarly, a  $x_{50,0}$ -value below 40 nm strongly indicates that the material is a nanomaterial. Likewise, the volume, extinction or intensity weighted medians, which are intrinsically measured by AC, DLS or AF4-LS), classify a material as nanomaterial if they are smaller than 100 nm (i. e. a first evaluation of materials is possible without the need of conversion). If the measurements results are close to the 100 nm borderline, a NM classification requires the characterisation by at least one further MT.



**Figure 7:** parity plot of the number weighted medians  $x_{50,0}$  as determined by non-counting, mobility based MTs: spray-DEMA, AC (discAC-turb, cuvAC-turb, cuvAC-RI), DLS and AF4-LS; additionally with BET equivalent minimum size (cf. appendix E.1); the SEM value (cf. Table 4), lines indicate parity and deviation from parity by a factor of 2.5; in contrast to Figure 6, the RTM6 and RTM9 are excluded

Last but not least, the study also employed gas adsorption measurements according to the BET method for estimating the VSSA and its corresponding mean value of the smallest particle dimension. This mean size is generally in agreement with EM techniques within a factor 2.5, which is the same tolerance range as found for the non-counting, mobility-based techniques (see also Figure 7). However, BET results may be misleading for materials with internal or coating microporosity (see RTM3 in Table 4). In the borderline region close to the 100 nm cut-off, BET requires confirmation by a second MT. However, the BET analysis facilitates an estimation of the size of constituent particles and may even help to correct biased data from EM techniques, as evidenced by the case of platelet particles (see TS 5 in Table 4). Beside the access to the smallest dimension, the advantage of BET is that no dispersion protocol is required, so that artefacts are avoided for hydrophobic or soluble NMs (see TS 7 and RTM9 respectively in Table 4).

### 3 Discussion

The question for the reliability of NM classification with currently available MTs may provoke quick and general answers. Yet in practice, the performances of MTs depend also on the specific material, the quality of measurement procedures (incl. sample preparation) and the appropriateness of data analysis. For this reason it is not sufficient to select appropriate MTs for the identification of NMs, but incorporate these techniques in characterisation methods, which define all steps from sampling to data analysis. A further aspect is the precision with which the measure-

ment results are obtained. Consequently, our discussion will therefore first look on the general data quality, subsequently discuss the potential impact of the steps of analysis on measurement result, before it evaluates the outcome of this study.

### 3.1 Estimation of measurement uncertainty within this study

The evaluation of the accuracy of the results relies on the assessment of its two components, precision - including repeatability, intermediate precision and reproducibility – and trueness. While precision is in principle easily to evaluate for all the employed measurement techniques, but practically time-consuming, the evaluation of trueness, i.e. of the deviation of the result obtained from its real value, is generally a challenging task necessitating considerable expert knowledge of the individual technique, instrument and software. An overview of the relative repeatability and intermediate precision attained in this analytical study can be seen in Table 6 (appendix E.2). Note the small values (below 5%-rel.) for the majority of techniques and materials. The estimation of trueness has been performed in this study partly by means of using the quality control materials, i.e. samples with well-known particle size distribution. Further sources of measurement uncertainties generating systematic (bias) deviations have been already addressed in the previous sections on a more material related basis and are discussed qualitatively in the next sections on a rather more methodical basis (robustness, sample preparation, data reduction, etc.).

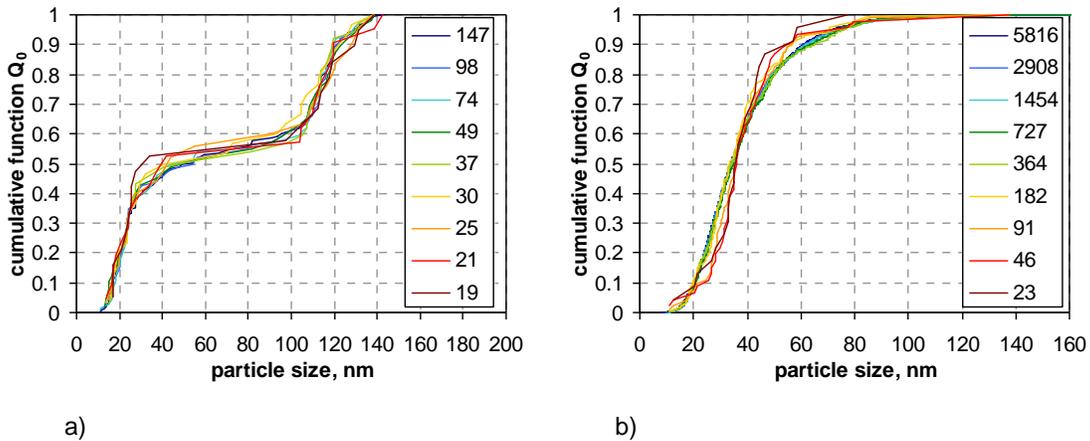
Without a rigorous evaluation of the measurement uncertainty budgets associated to the results obtained in this study, a direct comparison of all the results generated by different techniques is in fact hardly possible. Nevertheless, the comparison was done taking the results obtained by electron microscopy as a reference and discussing the potential sources of uncertainties for each technique in part in a semiquantitative way. For the electron microscopy systematic, metrological studies [4,5,6,7] have been recently carried out by various research groups with the purpose of estimation of the measurement uncertainties related to model samples (such as the quality control materials in this study). However, for most of the other measurement techniques the metrological basis for application in the size range relevant for this analytical study is simply missing.

The present analytical study does not claim to be a metrological interlaboratory comparison of a large variety of complex, real-world materials with almost all available sizing techniques. After this unique systematic analytical study the NanoDefine project has planned to identify and group techniques and materials in order to proceed further with intra- and interlaboratory validation which may be even finally be standardized.

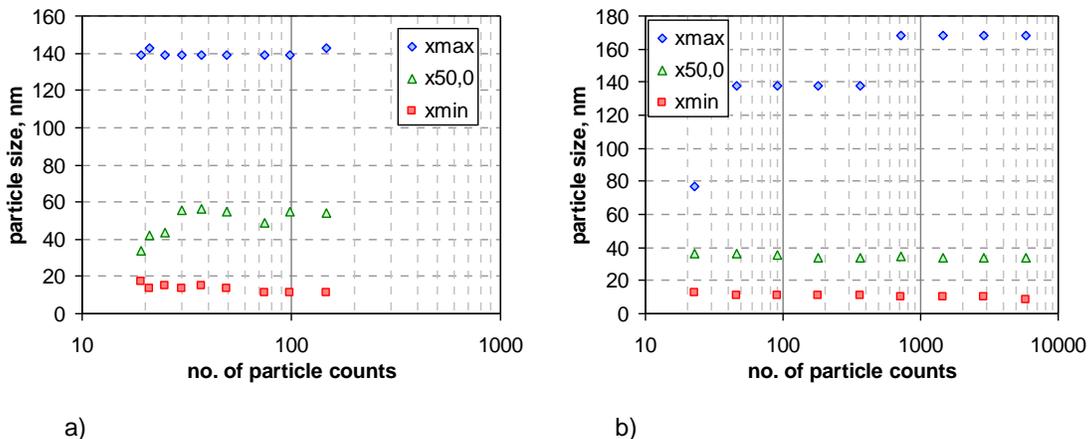
This study aimed at a first evaluation of several MTs whether they can support the classification of particulate material according to the EC definition of nanomaterials. For this purpose it was necessary to cover a wide range of different materials and potential MTs, rather than to conduct an interlaboratory comparison which obeys certain metrological standards. Hence, this study gathered little data on the quality of the measured size distribution with respect to accuracy, intermediate precision and repeatability. In particular, it was not possible to quantify the “trueness” of  $x_{50,0}$  for most materials, as such analyses need verified reference values for a material of similar morphology. However, most participants conducted at least 2 repeated runs of up to 3 aliquots what allows assessing the uncertainty related to precision. The respective effort depended on the analysis costs (e. g. most DLS results are averaged from 30 individual measurements at 3 aliquots, while most SAXS results are averaged from just 2 individual measurements). Corresponding values of precision-uncertainty are listed in Table 6. It is remarkable that most values are smaller than 20 %, which is clearly less than the deviation among the different MTs. For instance, the measured  $x_{50,0}$  values by DLS for RTM6 varied with 16 % relative standard deviation while the they differ to  $x_{50,0}$  of discAC-turb and SEM by a factor of 1.92 and 3.5, respectively.

A particular situation exists for counting techniques, for which the number of analysed particles is typically much lower than for fractionating or spectroscopic techniques (e. g. assuming a typical scattering volume of  $10^6 \mu\text{m}^3$  in DLS [42] means for a suspension with 0.01 vol.-% 100 nm particles that at each instant approx.  $2 \times 10^5$  particles contribute to the signal). It is therefore necessary to understand, to which degree the measured number weighted size distribution is affected by sample size. We examined this effect for the EM techniques, since they are considered most appropriate for the implementation of NM classification. For this purpose, the set of particle images was divided in random subsets, from which the size distributions were subsequently derived. Exemplary results for QCM1 and RTM1 are plotted in Figure 8 and Figure 9; they reveal that a large sample size is mainly required to smooth the shape of the distribution function and to accurately

resolve the edges of the size distribution. However, median values are relatively robust parameters which can be reliably determined with as few as 200 particles – provided that these 200 particles as well as all particles available for the image analysis are a representative sample of the disperse system.



**Figure 8:** impact of sample size (no. of particles) on number weighted size ( $x_{\text{Ferret,min}}$ ) distribution by SEM; for a) QCM6 and b) RTM1



**Figure 9:** impact of sample size (no. of particles) on the parameters of number weighted size distributions by SEM (maximum, median and minimum size); for a) QCM6 and b) RTM1

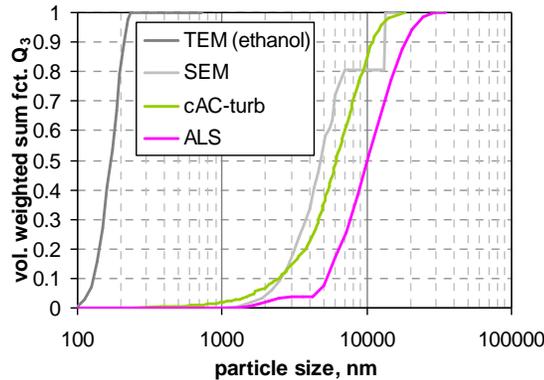
### 3.2 Influence of the characterisation methodology on the quality of measurement data

As explained in section 1.4, this study ensured defined conditions for the whole analytical chain by providing protocols, guidelines and data sets to all participants. However, what is a prerequisite for comparing different results may also introduce bias into the analytical results, for instance when the procedures described in protocols are inappropriate for a given material or when instrument software is fed with erroneous model parameters. This section illustrates the importance to properly define the conditions of measurement.

#### 3.2.1 Impact of sample preparation

EM is generally regarded as reference technique, but RTM9 is an intriguing example on impact of sample preparation on the trueness for EM techniques. For the hydrophilic copolymer RTM9 it was highly difficult to develop a dispersion protocol that allowed reproducible size analysis. Since such a protocol was not available at the beginning of the study, one participant tried a frequently used preparation procedure for TEM. It consists of wetting the hydrophilic powder in ethanol before placing it on the TEM substrate. However, since the copolymer dissolves in ethanol, the

state of dispersion is not defined and in any case not representative for the original material. The finally obtained size distribution was thus considerably different to those derived by other MTs (including SEM) for a dispersion protocol based on aqueous solutions.

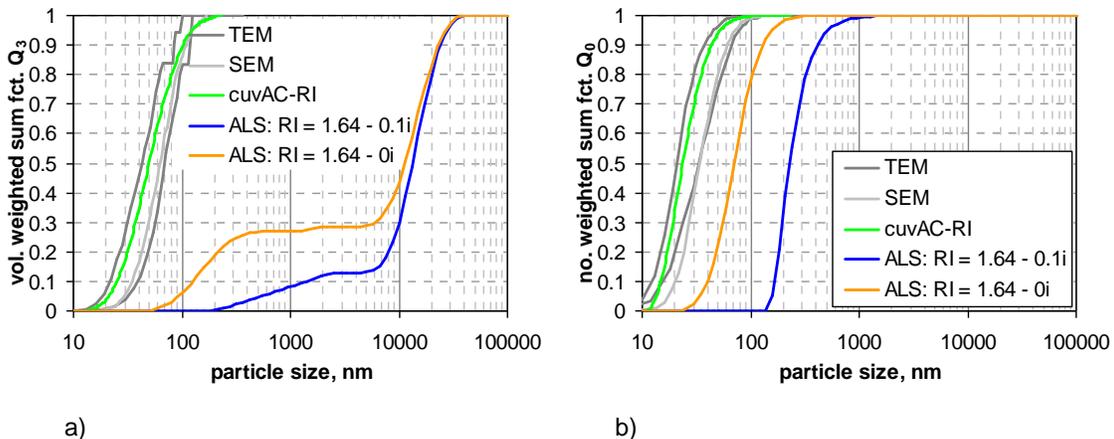


**Figure 10:** volume weighted sum functions of RTM9 (BMC), from measurements with TEM (powder dispersed in ethanol), SEM, cuvAC-turb, and ALS

### 3.2.2 Impact of data analysis

Data analysis forms a further particularly critical point in the whole chain of characterisation. However, while inappropriate sample preparation frequently leads to visual effects (flocculation, sedimentation, opalescence or clearing etc.), artefacts due to wrong data analysis remain commonly concealed. Two aspects of data analysis are being considered: the dependency on model parameters and the impact of inversion algorithms; both are also related to the conversion of intrinsically measured size distributions into number weighted size distributions.

Apart from imaging techniques all MTs rely on models that relate the measured signals to particle size and also to particle number. These models often require values of certain material properties as input parameters (cf. Table S-2, supplementary data S.5). Optical MTs normally need the particles' refractive index (RI) either for sizing (e. g. ALS) or for conversion to  $Q_0$  (e. g. AC-turb). Figure 11 shows an example for the influence of RI on the result of ALS analysis. The graphs refer to representative test material RTM1 and display measurement results of TEM, SEM and cuvAC-RI (i. e. MTs whose resulting size distribution is independent of a specific RI value) as well as those of ALS, when analysed with a real and with a complex value of the particle refractive index. Adding an imaginary part to the RI (which is actually real) yields a significant change in the volume weighted size distribution ( $Q_3$ ) and a large qualitative difference in the number weighted one ( $Q_0$ ). The comparison with AC confirms that the correct RI value yield plausible results for  $Q_0$ .

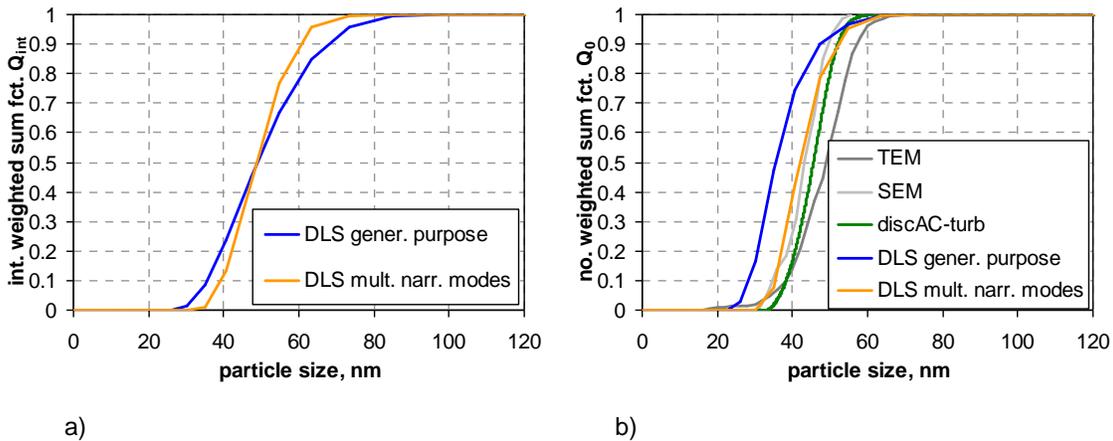


**Figure 11:** ALS result for RTM1, impact of RI values on a) volume and b) number weighted size distribution (additionally results of TEM (2×), SEM and cuvAC-RI)

A further example of the potential impact of RI on ALS results is shown in Figure S-20 for repre-

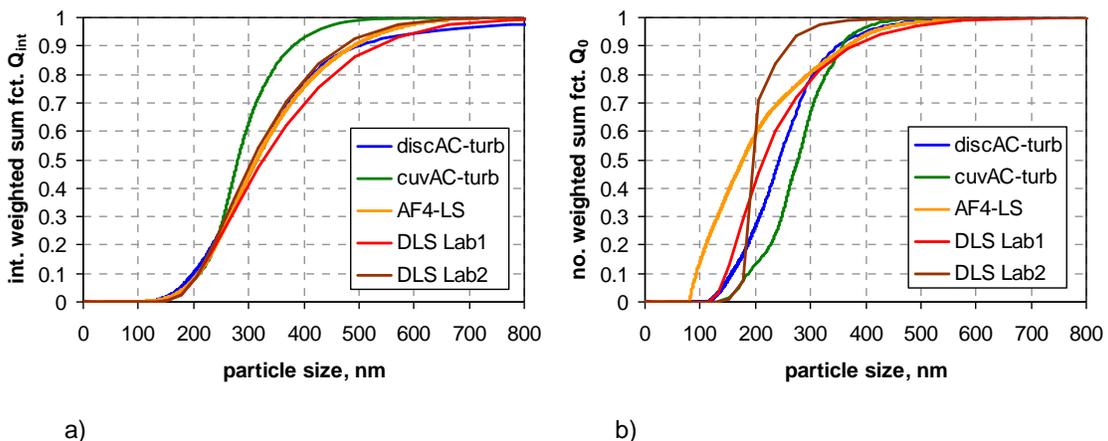
sentative test material RTM4, which is  $\text{CaCO}_3$  powder in its calcite phase. Calcite is a uniaxial crystal, for which the RI depends on the crystal orientation. There are two principal RI values: for the “ordinary ray”, i. e. axis is parallel to incident light, and for the “extraordinary ray”. Values are 1.66 and 1.48, respectively. In practice, crystals will be randomly aligned during an ALS measurement zone, thus the orientation averaged RI (1.53) applies. The figure demonstrates that data analysis with the “wrong” RI value can result in huge mis-evaluation of the number weighted median. Again the impact of erroneous RI value is considerably amplified at conversion.

Spectroscopic measurement techniques like DLS, ALS, SAXS and USSP derive the size distribution from a distributed signal (spectrum), for which purpose numerical algorithms are employed that impose bias on the distribution shape (e. g. on smoothness and non-negativity). The outcome of this spectrum inversion depends on the selected algorithm and its parameterisation. For the analysis of DLS data of quality control material QCM1 this is shown Figure 12. While the algorithm settings only affect the calculated distribution width in the intrinsically measured  $Q_{\text{int}}$ , it is decisive for the median of the number weighted size distribution.



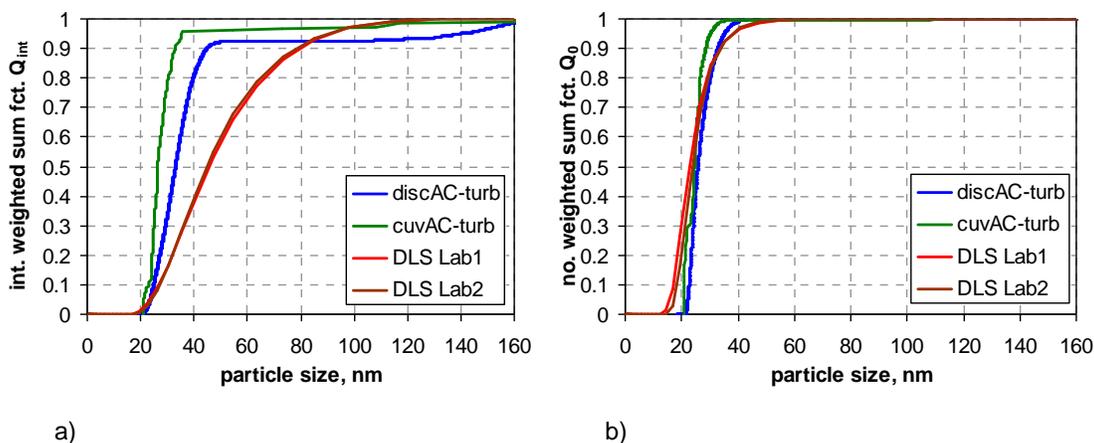
**Figure 12:** DLS result for QCM1, impact of analysis settings (“general purpose, “multiple narrow modes”) on a) the intrinsically measured intensity weighted and on b) the derived number weighted size distribution (in case of the latter: additionally results by TEM, SEM & d discAC-turb)

All three examples on data analysis demonstrated that for non-counting MTs the detrimental effect of inappropriate data analysis may become notably magnified by conversion. This effect applies to any “perturbation” of measured size distribution (e. g. caused by improper dispersion procedures, agglomeration or contaminant particles). A kind of worst-case scenario is depicted in Figure 13, which plots the intrinsically measured (intensity or extinction weighted) and the number weighted size distribution of representative test material RTM3 for AC techniques, AF4-LS and DLS (all are mobility-based). The results are rather similar when presented as extinction or intensity weighted size distributions (cf. Figure 13a), whereas conversion into  $Q_0$  leads to considerable differences (Figure 13b).



**Figure 13:** RTM3 (coated TiO<sub>2</sub>) results of mobility-based MTs with particle quantification by light extinction or light scattering; a) intrinsically measured intensity and extinction weighted and b) derived number weighted sum functions

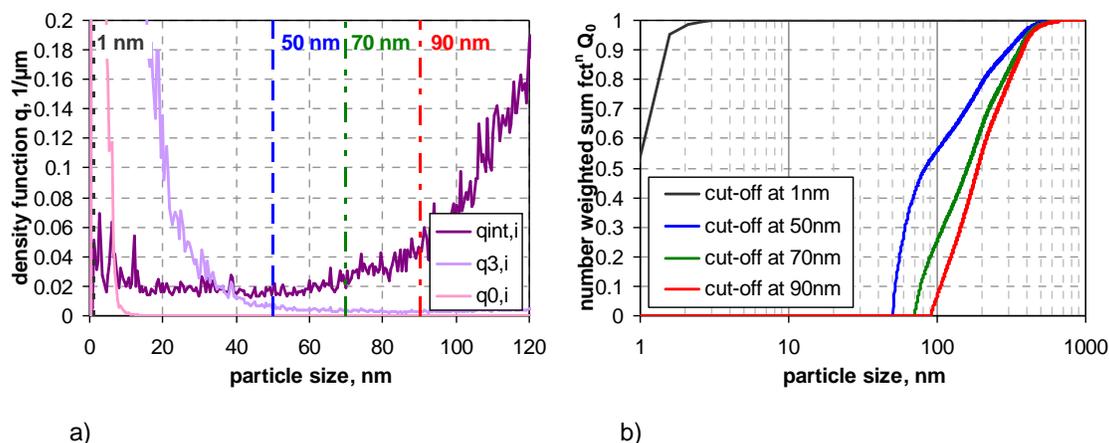
However, conversion may also give rise to a suppression of differences that occur in the intensity and extinction weighted size distributions. Such a “harmonisation-scenario” was encountered for quality control material QCM2 (Figure 14), where clear differences among the intrinsic results are seen – predominantly with respect to the coarse particle content. The reasons of this are not definitively clear, but they are certainly related to a different sensitivity towards coarse particles / agglomerates or due to loss of sample stability in some measurements. After conversion to  $Q_0$  the coarse particle fractions are virtually disappeared. Similar observations were made for representative test material RTM1 (cf. Figure S-22).



**Figure 14:** QCM2 (nano SiO<sub>2</sub>) results of mobility-based MTs with particle quantification by light extinction or light scattering; a) intrinsically measured intensity or extinction weighted and b) derived number weighted sum functions

### 3.2.3 Impact of data pre-treatment

A further aspect of the reliability of measured size distribution is the restriction of the  $Q_3$  size range and its implications on the conversion to  $Q_0$ . The size range, which is spanned by the measured size distribution, is primarily determined by the sensitivity of the MT (e. g. towards scattered light) but can be also defined by the settings of the analysis algorithm. Specifically, for spray-DEMA, discAC-turb and AF4 the minimum size has to be selected or confirmed by the operator in order to remove the “residual” particles from baseline shift and void peak, respectively (cf. supplementary data S.2). This minimum size setting may severely affect the NM classification as demonstrated in Figure S-23 and Figure 15 on results for discAC-turb and AF4-LS: the wider the size interval is chosen, the stronger the  $1/\text{size}^3$  factor during conversion suppresses those modes that are statistically significant in  $Q_3$ . Effectively, the conversion to  $Q_0$  amplifies noise and delivers completely misleading results. Consequently, within this study any data treatment is considered to have followed defined and harmonised rules to cut off “residual particles” if the local minimum in the fractional number concentration was clearly separated from the main size distribution.



**Figure 15:** impact of setting a lower size limit on  $Q_0$  for RTM3 (coated  $\text{TiO}_2$ ) when measured with AF4-LSa) detail of the originally measured density functions with possible cut-off values for the lower size limit, b) sum functions of number weighted size distribution for different values of minimum particle size

### 3.3 Consequences for the implementation of the recommended NM definition in industrial practice

Our analytical study on the characterisation of quality control material particle systems and commercial powders with the most common MTs for particle sizing has clearly demonstrated that there is no single MT that ensures reliable identification of NM for all kinds of materials. This also applies to imaging techniques, like TEM or SEM, which are frequently considered as the only possibility to finally suggest whether a material is a NM or not. Since the expenditure of time and staff for imaging techniques is rather high, there is a strong demand for MTs that allow a fast, cheap and reliable classification of materials that are definitely nano or clearly non-nano. This leads to a tiered characterisation strategy:

The approach distinguishes among three levels of characterisation methods or MTs. *Tier 1 (powder)* facilitates a rough evaluation based on integral properties of powders. In contrast, *tier 1 (suspension)* aims at the determination of size distributions of suspended, i. e. individualised, particles. However, the measured distribution functions are not intrinsically number weighted or not related to the constituent particles (but to the morphology of aggregates). In spite of this, these MTs can be used for a screening decision. While the analysis within any of the *tier 1* MTs may be fast, it does not warrant absolute certainty with respect to NM classification. Unambiguous decisions are only possible within *Tier 2* (confirmatory techniques), in which the number weighted size distributions of (constituent) particles are directly measured and their median values ( $x_{50,0}$ ) can be thus determined with high accuracy. More details on an integrated decision tree are prepared for publication in the NanoDefine public deliverable D7.10.

The analytical strategy of a tiered approach is to quickly conclude on the type of a material within *tier 1*. On this level the critical quantity, i. e.  $x_{50,0}$ , is only indirectly measured or derived from an empirical correlation rule, which means that any prior knowledge on the material (e. g. by qualitative imaging) should be used to support the decision. In best case, *tier 1* leads to the clear statement that a material is nano or that is not, otherwise confirmative techniques from *tier 2* have to be employed.

Good candidates for *tier 2* are evidently EM techniques. The measurement results of TEM and SEM (even among different instruments) were typically quite close and the NM classification always consistent. In addition, they completely cover the relevant size range. On the other hand, our results indicated that sample preparation is a crucial issue for the reliability of EM results – in particular for highly polydisperse materials (cf. Figure 1b) – and that for platelet-like particles the smallest external dimension is difficult to assess for two-dimensional images. Other authors [43] reported on the shrinkage of particles during TEM analyses (due to vacuum and electron beam), which can further affect the classification of materials. Hence, EM techniques will serve as a powerful tool for the NM classification, yet its applicability does not cover all materials. Generally, the plausibility of EM results should always be checked (e. g. with BET) before used for classifi-

cation.

The most prominent example for a *tier 1 (powder)* technique is the determination of the VSSA by means of gas adsorption measurements according to the BET method. This selection supposes that the VSSA or the BET equivalent minimum size  $x_{\text{BET,min}}$  strongly correlates with the number weighted median  $x_{50,0}$  of the smallest external dimension of the (constituent) particles. This is obviously difficult to ensure for all types of materials, but for the materials of the present paper  $x_{\text{BET,min}}$  deviated not more than a factor 2.5 from  $x_{50,0}$  by SEM or TEM, and is hence good enough for screening down to a borderline region. This finding is rationalised by the BET metrics depending on the minimum size of constituent particles without the need to disperse and disaggregate. However, BET fundamentally cannot provide size distributions. Employing BET within a tiered approach for NM classification would be highly attractive, since BET results are frequently employed by industry to distinguish different grades of particulate materials.

For *tier 1 (suspension)*, there are quite a lot of potential MTs, but not all of them provide the required applicability to size range and not all reliably determine number weighted size distributions. In view of the results of our analytical study we propose spray-DEMA, all AC techniques and DLS as candidates for the *tier 1 (suspension)* techniques. Similar to BET, we found for these techniques that the number weighted median ( $x_{50,0}$ ) differed from the corresponding SEM value by a factor of less than 2.5 for almost all materials (Figure 7). Screening by *tier 1 (suspensions)* fails for materials that are composed of highly-porous, fractal-like aggregates – e. g. pyrogenic metal oxides. Such materials should be screened within *tier 1 (powder)*, i. e. by BET. Likewise, BET analysis is misleading for NM classification, when the material is microporous (not in this study). In this case, screening should be accomplished by *tier 1 (suspension)* techniques. These examples demonstrate the importance of the above mentioned “prior knowledge” for the selection of a characterisation strategy and the interpretation of its results.

Our recommendations reflect the current state-of-the-art and the results obtained on real-world materials in the present study. Yet, there are more promising candidates in the present study, which experience on-going developments driven by the need for accurate characterisation of NMs. For instance, the element-selective detection principle of sp-ICP-MS represents a restriction of applicability that is inherent to the technique, but it also represents a unique asset for the selective analysis of particles contained in formulations and consumer products; improvements in the sensitivity for lighter elements have been achieved, and also the lower size limit is constantly improving. The size range limitation is removed by the optional coupling of AF4-ICP-MS, which bears equally great potential especially for complex formulations, but was not ready for evaluation in the current study. The well-established AF4-LS was limited by the LS detection principle in some cases, but final conclusions cannot be drawn yet due to the lack of data.

Similar to sp-ICP-MS, the applicability of PTA to the nano-range is currently restricted to a small range just below 100 nm or to strongly scattering materials. In order to consider PTA as a screening technique, it is necessary to enlarge the accessible size range to finer values. The thus required enhancement of sensitivity may be realistically achieved within the next years.

A further potentially interesting technique is SAXS, which has proven as an excellent analytical tool for the characterisation of nanoparticles in this study. However, it obviously fails to correctly classify non-nano materials. It would be useful to expand the upper limit from approx. 100 nm to the  $\mu\text{m}$ -range, which needs SAXS instrumentation for highly intense X-rays scattered at ultra-small angles (USAXS) – currently only available at synchrotrons. Whether or not this can be realised in laboratory instrumentation is questionable. An alternative approach might be to employ SAXS for the measurement of the specific surface area of the (constituent) particles. Currently, ALS is also excluded from the tiered approach because results in the submicrometre range are not very consistent (in contrast to those for  $x > 1 \mu\text{m}$ ). The reasons are rather fundamental (light scattering pattern of nanoparticles are not finely structured and are not very intense). Additional problems may result from the aspiration of commercial instruments to measure nanoparticles and micrometre particle with one optical setup; a focus on particles below  $1 \mu\text{m}$  could improve the situation, but is not very likely to be seen in commercial instruments. Finally, the analytical study cannot currently encourage to use USSP within tier 1 because of the practical limitations observed. Even though, the MT exists in different types of instrumental configurations (sample size from a few  $\mu\text{l}$  to hundreds of ml), its major restriction is the need for particle concentrations in the order of vol.-%. Moreover, the conversion into  $Q_0$  sometimes yielded results beyond any physical meaning, which indicates that data analysis should be still improved.

## 4 Conclusions

The implementation of the EC recommendation for a definition of nanomaterial (NM) in industry and legal institutions is a tremendous analytical challenge. We evaluated the performance of measurement techniques (MTs) on both quality control materials and on real-world particulate materials, and found that no single MT can be recommended for a guidance but that a tiered approach is required that combines different MTs and employs prior knowledge on the material (physico-chemical properties as well as general morphological properties of the particles). The tiered approach comprises screening techniques (*tier 1 (powder)* and *tier 1 (suspension)*) as well as confirmatory techniques (*tier 2 (imaging)*). *Tier 1* techniques are intended to provide “clear statements” whether a material is a nanomaterial or not, or whether a more profound analyses by *tier 2* techniques is required because the number weighted median  $x_{50,0}$  is close to the borderline of 100 nm. They either probe integral properties of the particle system (e. g. VSSA) or determine the distribution of equivalent diameters rather than the geometric lengths of the external dimension. In addition, the intrinsically measured size distributions are typically non-number-weighted. For this reason, they are expected to perform well for low and moderate polydispersity. Moreover, most techniques that are relevant for *tier 1 (suspension)* cannot resolve the internal structure of particles aggregates and probe aggregate properties. Their ability to reliably assess particulate materials according to the EC definition is therefore restricted to materials that consist of particles as individual entities or of well-dispersible aggregates. Based on our data, matching nano / non-nano classification by *Tier 1 (powder)* indicates that this validity criterion is fulfilled. Otherwise, *Tier 2 (imaging)* can help.

This study yields recommendations for the MTs that can be attributed to the different tiers based on their proven performance for real-world materials: *Tier 1 (powders)* can rely on BET, but only outside the borderline region whose limits we explore in details elsewhere [44]. *Tier 1 (suspension)* can be realised with spray-DEMA, all AC techniques or DLS, yet similar as for BET they are inconclusive for a borderline region and certain particle morphologies. Further candidates for *tier 1*, which we tested, provided no reliable classification (ALS, PTA, SAXS), or were not ready for a final assessment (AF4, sp-ICP-MS). *Tier 2 (imaging)* measurements can be conducted with TEM or SEM, which give access to the constituent particles of aggregates and to the smallest external dimension of particles for most materials. However, the preparation of representative samples constitutes a major source of uncertainty and ambiguity for tier 2, and the determination of the smallest external dimension remains challenging (if possible) for several classes of morphology, e. g. for three-dimensional aggregates and two-dimensional platelets.

Inconsistent results occur with both *tier 1* and *tier 2* techniques for highly polydisperse samples: for most screening techniques because of their relative insensitivity towards the fine size fractions; for imaging techniques because any particle deposition process on substrates is affected by particle size. We also observed ambiguity in *tier 1* and *tier 2* results when the materials were composed of indispersible aggregates comprising a large number of constituent particles. In these cases, the *tier 1 (powder)* might be preferable for a pragmatic implementation. Despite these challenges, our results suggest that reliable NM identification is possible for a broad range of real-world substances.

Finally, we can extrapolate from our study that the classification of mixtures of different substances is probably rather difficult and prone to artefacts. The interpretation of particle sizing results for such materials can be critically misleading if, for instance, the turbidity of a mixture is solely determined by one light-absorbing component (e. g. in AC-turb), or when the scattering signal of a mixture is dominated by the component with highest optical or electron density contrast (e. g. DLS, SAXS and EM).

## 5 Symbols, abbreviations and acronyms

symbol	description	SI-unit
$Q_A(x)$	sum function weighted in quantity <i>A</i> (number like “3” refer to geometric properties, abbreviations like “ext” to physical properties)	1/m
$q_A(x)$	density function weighted in quantity <i>A</i>	1/m
$q^*_A(x)$	transformed density function in quantity <i>A</i>	-

$x$	particle size, equivalent diameter	m
$x_{\text{BET}}$	BET equivalent diameter (i. e. computed from VSSA)	m
$x_{\text{BET,min}}$	BET equivalent minimum size (computed from VSSA assuming a certain type of morphology)	m
$x_{50,0}$	median size of the number weighted size distribution	m
$x_{\text{Feret,min}}$	minimum Feret diameter (= distance between parallel tangents)	m
$x_{\text{hd}}$	hydrodynamic diameter (for equivalence to hydrodynamic drag)	m
$x_{\text{mob}}$	mobility diameter (for equivalence to electric mobility of aerosol particles)	m
$x_{\text{Stokes}}$	Stokes diameter (settling velocity equivalent diameter)	m
$x_{\text{V/S}}$	volume / surface equivalent diameter	m

AC	analytical centrifugation
AF4	asymmetric flow field-flow-fractionation
AFM	atomic force microscopy
ALS	angular light scattering
BET	Brunauer–Emmett–Teller Method
DLS	dynamic light scattering
DEMA	differential electrical mobility analysis
EC	European Commission
EM	electron microscopy
MT	measurement technique
NM	nanomaterial
NP	nanoparticle(s)
PSL	polystyrene latex
PTA	particle tracking analysis
RI	refractive index
SANS	small-angle neutron scattering
SAXS	small-angle X-ray scattering
SEM	scanning electron microscopy
SOP	standard operating procedure
spICP-MS	single particle inductively coupled plasma mass spectrometry
TEM	transmission electron microscopy
TOQ	type of quantity
USSP	ultrasonic spectroscopy
VSSA	volume-specific surface area

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## 7 Acknowledgement

The article presents results of the European Research project 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*), which received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under Grant Agreement n° 604347. In addition, the authors would like to thank Antoine Ghanem (Solvay SA), Dora Mehn (JRC-IHCP) and Gert Roebben (JRC-IRMM) for their helpful discussions and their comments to the manuscript.

## 8 Supplementary data

The large extent of the analytical study required to restrict on the main features of the methods employed and to show only a selected set of measurement results. More details on the experimental programme and a complete set of size distributions are provided as supplementary data under <http://xx>. In addition all measurement reports and guidance documents are publicly available at the NanoDefine homepage <http://www.NanoDefine.eu>.

## Appendix A Materials

### A.1 Brief description of materials

**Table 1:** brief characterisation of quality control materials (QCM) and representative test materials (RTM) employed in this study: substance, morphology, polydispersity with respect to number weighted distribution of minimum Feret diameter from SEM measurement and cumulative sum at 100 nm

code	material	description	$x_{99,0}/x_{1,0}$ ( $x_{max}/x_{min}$ )	$Q_0(100nm)$	misc.
QCM1	polystyrene	spherical, monomod., nano	1.7 (1.8)	100 %	
QCM2	colloidal SiO <sub>2</sub>	spherical, monomod., nano	3.0 (5.2)	100 %	
QCM3	colloidal Au	spherical, monomod., nano	1.7 (2.4)	100 %	
QCM4	colloidal Ag	spherical, monomod., nano	3.8 (54)	100 %	
QCM5	polystyrene	spher., trimod., nano + sub- $\mu$	12 (14)	88 %	
QCM6	colloidal SiO <sub>2</sub>	spher., trimod., nano + sub- $\mu$	10 (13)	61 %	
RTM1	BaSO <sub>4</sub> , UF	compact constituents, aggr., nano	7.1 (21)	99.1 %	
RTM2	BaSO <sub>4</sub> , fine	compact constituents, aggr., non-nano	8.5 (16)	7.3 %	
RTM3	coated TiO <sub>2</sub>	compact constituents, non-nano	4.5 (16)	5 %	rutile, coating < 4 nm
RTM4	CaCO <sub>3</sub>	cigar-like, non-nano	8.7 (15)	22 %	calcite*
RTM5	kaolin	platelets, nano + sub- $\mu$	24 (90)	32 %	
RTM6	fumed SiO <sub>2</sub>	fractal aggregates of nanopart.	2.9 (3.6)	100 %	
RTM7	organic pigment Y83, transparent	needles, aggr., nano	3.5 (4.6)	100 %	
RTM8	organic pigment Y83, opaque	needles, aggr., non-nano	6.1 (7.2)	10 %	
RTM9	basic methacrylate copolymer	compact constituents, micro	14 (40)	14 %	

\* verified by Raman-spectroscopy

## A.2 Sample preparation

**Table 2: main features of the sample preparation procedures employed**

code (material)	wetting agent	dispersing agent	conc. at dispersion	volume	pre-treatment	energy density
RTM1 (BaSO <sub>4</sub> , UF)	not required	2 mg/ml SHMP	0.1-0.26 wt.-%	2..100 mL	vortexing	0.2...5 kJ/ml
RTM2 (BaSO <sub>4</sub> , fine)	not required	2 mg/ml SHMP	0.1-0.26 wt.-%	2..200 mL	vortexing	0.3...5 kJ/ml
RTM3 (coated TiO <sub>2</sub> )	ethanol	2 mg/ml SHMP	0.01 wt.-%	2..100 mL	US bath	0.3...5 kJ/ml
RTM4 (CaCO <sub>3</sub> )	not required	2 mg/ml SHMP	5 wt.-%	2..70 mL	stirring	0.3...2 kJ/ml
RTM5 (kaolin)	not required	0.1 mg/ml TSPP	6 wt.-%	2..70 mL	US bath	0.3...3 kJ/ml
RTM6 (fumed SiO <sub>2</sub> )	this material was provided as suspension					
RTM7 (pigment Y83)	methanol / 2-propanol	0.5 wt.-% SBNS	0.01-0.1 wt.-%	2..100 mL	complex	0.3...5 kJ/ml
RTM8 (pigment Y83)	methanol / 2-propanol	0.5 wt.-% SBNS	0.01 wt.-%	2..100 mL	US bath	0.3...1 kJ/ml
RTM9 (BMC)	stearic acid	SDS	1 wt.-%	2..100 mL	stirring	0.3 kJ/ml

dispersing agents:

SDS sodium dodecyl sulphate (CAS No. 151-21-3)

SHMP sodium hexametaphosphate (CAS No. 10124-56-8)

SBNS Sodium butyl naphthalene sulphonate (CAS No. 52628-07-6)

TSPP Tetra-sodium pyrophosphate (CAS No. 7722-88-5)

## Appendix B Brief description of the employed measurement techniques

*Transmission electron microscopy (TEM) and scanning electron microscopy (SEM).* These analytical techniques rely on the various interactions of accelerated electrons with a specimen. Eventually scattered or transmitted electrons are used to create highly resolved images of the specimen (i.e. down to a few nm or even below) [45]. The distinction between TEM and SEM refers to the different detection modes and coincides with different electron energies, thus maximum resolution. This classical scheme is currently becoming obsolete, since hybrid techniques (e. g. SEM in transmission mode – TSEM) steadily gain attraction [46]. In the context of particle characterisation TEM and SEM are highly appreciated for visualising the particles' morphology (shape, state of aggregation etc.). Yet they can also reliably be employed for the determination of particle size distributions [25]. The key issue is the preparation of a representative sample – in particular for high polydispersity, considerable variation of particle shape or mixture of different particulate phases.

*Single particle inductively coupled plasma mass spectrometry (sp-ICP-MS).* Mass spectrometers operated in a mode of high time resolution are able to distinguish between the background signal of dissolved matter and peak signals originating from isolated particles. In this way it is possible to measure the mass of individual particles [47]. The minimum size that can be resolved depends on the chemical composition of the particles, since the intensity of ICP-MS signals correlates with the atomic weight of the elements. The nano-range accessible for most metals (atomic weight > 19) and the corresponding metal oxides. However, this also means that spICP-MS does not support the NM classification for a significant number of particulate materials, including organic pigments and silica products.

*Particle tracking analysis (PTA).* The technique is based on the visualisation of fine colloidal particles by their scattered light when illuminated against a dark background (dark field microscopy, ultramicroscopy). The inherent Brownian motion of such fine particles leads to a steady displacement of the observed scattering pattern, which can be quantified as average displacement length per time step and thus reveals the translational diffusion coefficient of each particle [48]. Though commercial instruments are rather new, the technique was already demonstrated by Perrin [49] for particles in year 1909.

*Differential electrical mobility analysis on sprayed suspensions (spray-DEMA).* DEMA is a classical technique for aerosol characterisation, which consists of three components: defined charging of the particles, their classification according to the electric mobility, and the quantification of classified particles. The most common system, also employed in this study, is based on a bipolar charger, a sequential aerosol classification process and an optical condensation particle counter. It allows the determination of fractional number concentrations for particles in the size range of 10 nm to approx. 1  $\mu\text{m}$  [4,50]. For non-spherical particles, the MT probes the *mobility equivalent diameter*, which is very similar, but not identical to the hydrodynamic equivalent diameters measured with PTA, AF4 or DLS (i. a. because of the velocity slip at particle surface). In this study, DEMA was solely applied to aerosolised particles from (aqueous) suspensions. The aerosolisation was achieved by conventional atomisation (purely mechanical spray generation) and electro-spray generation (spray generation in an electric field). For practical reasons, the latter was only applied to materials with maximum particle size below 200 nm.

*Analytical centrifugation (AC).* Centrifugation results in a classification of suspended particles in accordance to their mass and hydrodynamic mobility. Both properties define the settling velocity or more generally the sedimentation coefficient; the corresponding equivalent diameter is called Stokes diameter. There are different technical realisations of analytical centrifugation. Disc centrifuges inherently measure a scaled density function of the size distribution, whereas cuvette centrifuge primarily determine the cumulative function. Further differences are related to the quantification of particles. This study employs turbidity detectors and interference optics. The former weigh size fraction with respect to the particle extinction cross section, the latter by means of the relative refractive index increment. That means that for instance for non-opaque nano-particles the measured size distribution is intrinsically  $x^6$ -weighted when using turbidity sensors, while refractive index measurements intrinsically yield an  $x^3$ -weighted size distribution.

*Asymmetric flow field-flow-fractionation with light scattering detection system (AF4-LS).* This MT is a field-flow-fractionation technique, for which the cross field is a volume flux through one wall of the fractionation channel [51]. This has the advantage that both, migration to wall and counteracting diffusion, are both determined by the particles hydrodynamic mobility. In addition, this princi-

ple facilitates classification down to 1 nm. The eluent of the fractionation channel contains (more or less) narrow size fractions, the mean size of which increases in a defined manner with retention time (normal mode of operation). The concentration of each size fraction is measured by an appropriate detector. In this study a light scattering detector was used for this purpose.

*Dynamic light scattering (DLS).* The Brownian motion of colloidal particles results in an erratic fluctuation of scattered light, which in turn can be used to determine the diffusion coefficient of the particles or the distribution of the hydrodynamic (equivalent) diameter. There are different technical realisations of DLS, which differ with respect to the impact of scattered light and to the quantification of the intensity fluctuation. Yet, all of them intrinsically measure a (scattered light) intensity weighted size distribution [52,24].

*Angular light scattering (ALS).* This term comprises a set of MTs that were originally developed for different size ranges and thus deviate considerably with respect to technical design and performance in particle sizing [52]. All instruments employed in this study are designed for measuring particles up to several tens of micrometres, for which reason they highly resolve the scattering pattern at small scattering angles (traditionally called *laser diffraction spectroscopy*). Additionally they measure the scattering intensity at moderate scattering angles and even in the backward direction (traditionally called *static light scattering*). One instrument also uses a wavelength shift in order to increase the sensitivity for particles below 100 nm. It should be noted that previous interlaboratory studies evaluated the performance of such hybrid ALS instruments as rather weak for the submicrometre range [11,53]. The dependence of the light scattering pattern on the particle morphology is not simple and depends on size range, principal optical properties and particle alignment. For large micrometre particles it essentially reflects the orientation averaged projection area, while for nanoparticles it depends on the orientation averaged pair distribution function of surface elements.

*Small angle X-ray scattering (SAXS).* The small wavelengths of X-rays allow a characterisation of structures and objects in the nano-range and even sub-nano range [25,54]. With regard to particle size analysis only small scattering angles are relevant. In contrast to numerous other MTs, SAXS facilitates the distinction between aggregates and constituent particles. Moreover, it can also roughly resolve the shape of the constituent particles, which is important in context of the recommended NM definition. When SAXS is employed for the determination of size distributions, it relies on scattering signals, which are weighted by the particle surface. However, in practice the calculated size distribution are either presented volume or number weighed – a procedure that is also applied for ALS.

*Ultrasonic attenuation spectroscopy (USSP).* The manifold interactions between particles and acoustic fields result in sound attenuation and a shift of the sound speed. For colloidal particles the interactions are mainly dissipative (i. e. sound scattering is negligible) and are related to the visco-inertial and thermal coupling between particles and continuous phase. The relevance of these phenomena depends on material properties and the ratio of particle size to wavelength. Scanning through a certain frequency range thus allows for the determination of particle size [55,56]. Size distributions measured by USSP are volume weighted (to first approximation), which ensures the applicability to a broad measurement range. The impact of shape cannot be described in simple and at once accurate and universal terms. If the deviation from spherical shape is not too large, the equivalent diameter is approximately VSSA equivalent [41]. In contrast to most other MTs, USSP requires relatively large particle concentrations (> 1 vol.-%), since the contribution of particle to sound attenuation has to be significant against the sound absorption in the dispersion medium.

*Gas adsorption analysis based on the method of Brunauer, Emmett and Teller (BET).* Gas adsorption on powders is a long-established way for the determination of the specific surface area. The specific BET method of measurement and data analysis is frequently used to distinguish different grades of particulate products, even though the weaknesses of this method are well-known (e. g. its insensitivity to microspores, its dependency on local distribution of surface energy). Volume specific surface areas (VSSA) are integral properties of particle systems and correspond to a characteristic mean size. If the principal shape of the particles is known, it is even possible to calculate an average value for the smallest external dimension of the particles.

**Table 3:** brief description of measurement techniques (MT) employed in this study

MT acronym	type of MT	particle property	intrinsic result	sample form	standards
TEM	counting (via image analysis)	minimum Feret diameter	$Q_0(x_{\text{Feret,min}})$	powder or suspension	ISO 29301, ISO 13322-1
SEM	counting (via image analysis)	minimum Feret diameter	$Q_0(x_{\text{Feret,min}})$	powder or suspension	ISO/TS 24597 ISO 13322-1
sp-ICP-MS	counting	mass	$Q_0(x_v)$	suspension	ISO TS 19590
PTA	counting	diffusion coeff.* (mobility-based)	$Q_0(x_{\text{hd}})$	suspension	ISO/CD 19430
spray-DEMA	fractionation	electric mobility	$q^*_0(x_{\text{mob}})$	suspension	ISO 15900
discAC-turb	fractionation	settling velocity (mobility-based)	$q_{\text{ext}}(x_{\text{Stokes}})$	suspension	ISO 13318, ISO 15825
cuvAC-turb	fractionation	settling velocity (mobility-based)	$Q_{\text{ext}}(x_{\text{Stokes}})$	suspension	ISO 13318, ISO 15825
cuvAC-RI	fractionation	settling velocity (mobility-based)	$Q_{\text{RI}}(x_{\text{Stokes}})$	suspension	ISO 13318, ISO 15825
AF4-LS	fractionation	diffusion coeff.* (mobility-based)	$q^*_{\text{int}}(x_{\text{hd}})$	suspension	
DLS	spectroscopic	diffusion coeff.* (mobility-based)	$q^*_{\text{int}}(x_{\text{hd}})$	suspension	ISO 22412, ISO 13321
ALS	spectroscopic	pair distribut. of (projected) surface elements	$q^*_2(x_{\text{ALS}})$	suspension	ISO 13330
SAXS	spectroscopic	pair distr. of surface elements	$q^*_2(x)$	suspension	ISO 17867
USSP	spectroscopic	acoustophoretic mobility <sup>▲</sup>	$q^*_3(x_{\text{US}})$	suspension	ISO 20998
BET	integral	sp. surface area	$x_{\text{BET,min}}^{\blacklozenge}$	powder	ISO 9277

\* translational diffusion coefficient

▲ apparent diffusion coefficient

▼ for aqueous suspensions of most solid particles in the submicrometre range

◆ explanation of this parameter in appendix E.1

## Appendix C Measures to ensure the comparability of size analysis

The most critical issue when comparing results of particle size characterisation is to ensure that the state of dispersion was identical at each measurement. The uniform SOPs for sample preparation, which are described in appendix A.2 aim at an identical sample state before they are being fed to instrument (cf. section 1.2). In our study, this sample state may have to be changed for size measurement, because some MTs require rather high particle concentrations (i. e. several thousands ppmv, as for cuvAC), while others need highly diluted suspension samples (i. e. in the order of ppmv, as for PTA). In addition, the measurement principle may require a certain ionic strength of the dispersion medium (e. g. for spray-DEMA), which means that an electrolyte has to be added to the sample or that the salt content has to be reduced. In order to avoid any undesirable effect related to this final step of sample preparation uniform rules were set at the beginning of the experimental programme (e. g. further sample dilution only with dispersion medium cleaned with syringe filter cut size 0.02 µm; cf. guidance document on conducting measurements → supplementary data). In spite of such efforts, the experimental practices/reality revealed difficulties in keeping the state of dispersion constant during the measurement.

A further aspect for the comparability of measurements is the potential influence of measurement conditions. In principle, each expert knows best how to set the measurement parameters for obtaining highly reliable results, yet frequently there is a slight impact of the instrumental settings on the measured size distributions (e. g. laser intensity in DLS, since detection optics reacts non-linearly for high light intensities; e. g. duration of DLS measurement, because it affects the signal/noise ratio, e. g. the width of frequency range in USSP, e. g. centrifugal speed in AC can affect the agglomeration behaviour). In order to reduce such effects, we also gave guidance on instruments settings. That was particularly useful for those MTs that were used by two partners (sometimes even with the same type of commercial instrument, cf. Table S-1). The guidelines did also contain advice on determination of statistical uncertainty (e. g. on the number of replicate measurements) for each MT (some MTs allow a rather inexpensive replication of measurement, while others require quite a lot of time for conducting one run).

Measurement data of non-imaging MTs were always analysed by assuming spherical particles. Material properties that are required for data analysis and that may exert a large impact on the measured size or on the conversion into number weighted size distributions were provided as a consistent data set to all partners (cf. S.3).

Eventually all partners were obliged to document sample preparation, measurement conditions, parameters for data analysis and obtained size distributions (number weighted size distribution and – if applicable – the intrinsically measured size distribution) in a measurement report template. Also, all observations or considerations with relevance for quality of measurement data (e. g. large signal noise) were to be reported. All measurement reports are provided in the supplementary data.

## Appendix D Number weighted median sizes

Table 4 shows all measured values of the number weighted medians  $x_{50,0}$  and evaluates these results based on the conformity with SEM regarding the NM classification. However, classification by SEM is not necessarily correct, as the example of the kaolin sample has indicated (cf. section 2.2).

**Table 4:** compliance with classification by SEM; **green** = conformity with SEM evaluation, **red** = clear deviation, **amber** = close to critical cut-off (100 nm), **n.m.** = not measured for instrumental or practical reasons, **brackets** = considered little reliable

code (material)	SEM	TEM	spICP -MS	PTA	DEMA spray	disc AC-tu.	cuv AC-tu.	cuv AC-RI	AF4- LS	DLS	SAXS	ALS	USSP	BET <sup>*</sup>
QCM1 (polystyrene)	43	49	n.m.	50	47 45	46	46	n.m.	33	36 35	n.m.	n.m.	n.m.	n.m.
QCM2 (colloidal SiO <sub>2</sub> )	22	22	n.m.	n.m.	32 30	26	25	23	18	24 23	24	n.m.	n.m.	n.m.
QCM3 (colloidal Au)	19	18 20	26	n.m.	16	18	17	n.m.	n.m.	3	n.m.	n.m.	n.m.	n.m.
QCM4 (colloidal Ag)	5	5 3	n.m.	n.m.	n.m.	5	6	2	n.m.	11	6	n.m.	n.m.	n.m.
QCM5 (3-mod PSL)	48	55	n.m.	153 104	43 46	50	52	52	48	80 80	n.m.	52	n.m.	n.m.
QCM6 (3-mod SiO <sub>2</sub> )	55	35	n.m.	166 100	43 38	109	102	32	108	109 132	26	147	n.m.	n.m.
RTM1 (BaSO <sub>4</sub> , UF)	34	33 21	n.m.	(191)	53	66	43	24	n.m.	76 72	103	76	(1)	37
RTM2 (BaSO <sub>4</sub> , fine)	212	253 281	n.m.	281	293	223	258	203	n.m.	285	54	635 69	410	545
RTM3 (coated TiO <sub>2</sub> )	185	180 185	182	254	253	243	277	201	177	195 215	80	483 237	315	102
RTM4 (CaCO <sub>3</sub> )	154	153 161	n.m.	274	148	225	248	232	n.m.	294	n.m.	160 68	415	260
RTM5 (kaolin)	129	(121)	n.m.	210	252	n.m.	132	98	n.m.	290	187	152 66	(1)	48
RTM6 (fumed SiO <sub>2</sub> )	20		n.m.	118 82	62 56	36	n.m.	34	42	69 37	9	79	5 12	14
RTM7 (pigment Y83)	40	39	n.m.	205	n.m.	52	34	n.m.	n.m.	68 81	8	(1307)	n.m.	40
RTM8 (pigment Y83)	157	221	n.m.	114	n.m.	186	153	n.m.	n.m.	292 269	n.m.	168 133	n.m.	152
RTM9 (BMC)	2026	(149)	n.m.	n.m.	n.m.	n.m.	413	n.m.	n.m.	n.m.	n.m.	1837	n.m.	4084

\* column lists the BET equivalent minimum size, cf. appendix E.1

The table specifies the conformity with classification by SEM by means of text font colour. If the number weighted medians of a MT relates to the critical value of 100 nm in the same way as SEM does, the numbers are printed in **green**. A clear discrepancy is highlighted by **red**, while **amber** is used, when the values are close to 100 nm. This colour code does not represent quantitative agreement with SEM, only agreement of the resulting classification. In addition, there are a few values set in brackets (e. g. USSP for RTM1), which indicates low reliability based on prior considerations (e. g. because the particle concentration was actually too low for USSP analysis). Additionally, the table contains several fields filled with "n.m.", which means that material could not be analysed due to instrumental or practical reasons (e. g. because particle size was beyond measurement range, concentration was too low, sample size too small, suspension could not be stabilised for the specific MT, or foaming impeded a reliable spraying). In several instances the

table states two values, which are results by different labs with and without identical instrumentation (cf. Table S-1, supplementary data S.4).

## Appendix E Further results

### E.1 BET measurements

The BET equivalent diameter  $x_{\text{BET}}$  is the diameter of a sphere with the same VSSA as the particle system when the VSSA is determined via gas adsorption according to the method of Brunauer-Emmett and Teller:

$$x_{\text{BET}} = \frac{6}{S_V}$$

For long fibres or thin sheets it is not really meaningful to work with this equivalent diameter, instead the VSSA is used to compute the diameter of the fibre  $d_{\text{fibre}}$  or the thickness of the sheet  $u_{\text{sheet}}$ , respectively:

$$d_{\text{fibre}} = \frac{4}{S_V}$$

$$u_{\text{sheet}} = \frac{2}{S_V}$$

Both parameters represent the smallest external dimensions of the considered object, which is also relevant for the NM classification. Therefore a BET equivalent minimum size  $x_{\text{BET,min}}$  is employed as characteristic parameter for the NM classification [57]:

$$x_{\text{BET,min}} = \frac{2 \cdot D}{S_V},$$

in which  $D$  indicates the number of small dimensions of the particles. The value should be set to 2 if the aspect ratio exceeds a value of 3, and to 1 if the aspect ratio is smaller than 0.25. Values of BET measurement and the corresponding  $x_{\text{BET,min}}$  data are listed for all representative test materials in Table 5.

**Table 5:** VSSA derived from BET analyses (in mass specific surface area) and skeletal density, corresponding BET equivalent minimum size and dimensionality (cf. Table 1)

code (material)	$S_{\text{m,BET}}$ m <sup>2</sup> /g	$\rho$ g/cm <sup>3</sup>	$S_V$ m <sup>2</sup> /cm <sup>2</sup>	D	$x_{\text{BET,min}}$ nm
RTM1 (BaSO <sub>4</sub> , UF)	36.9	4.4	162.4	3	37.0
RTM2 (BaSO <sub>4</sub> , fine)	2.5	4.4	11.0	3	545.5
RTM3 (coated TiO <sub>2</sub> )	14.8	3.99	59.1	3	101.6
RTM4 (CaCO <sub>3</sub> )	5.8	2.657	15.4	2	259.6
RTM5 (kaolin)	16	2.61	41.8	1	47.9
RTM6 (fumed SiO <sub>2</sub> )	200	2.2	440	3	13.6
RTM7 (pigment Y83)	67.7	1.484	100.5	2	39.8
RTM8 (pigment Y83)	17.5	1.5	26.3	2	152.4
RTM9 (BMC)	1.3	1.13	1.5	3	4084

## E.2 Uncertainty with respect to repeatability (and intermediate precision)

This study aimed at a first evaluation of several MTs whether they can support the classification of particulate material according to the EC definition of nanomaterials. For this purpose it was necessary to cover a wide range of different materials and potential MTs, rather than to conduct an interlaboratory comparison which obeys certain metrological standards. Hence, this study gathered little data on the quality of the measured size distribution with respect to accuracy, intermediate precision and repeatability. In particular, it was not possible to quantify the “trueness” of  $x_{50,0}$  for most materials, as such analyses need verified reference values for a material of similar morphology. However, most participants conducted at least 2 repeated runs of up to 3 aliquots which allows assessing the uncertainty related to precision. The respective effort depended on the analysis costs (e. g. most DLS results are averaged from 30 individual measurements at 3 aliquots, while most SAXS results are averaged from just 2 individual measurements). Corresponding values of precision-uncertainty are listed in Table 6. It is remarkable that most values are smaller than 20 %, which is clearly less than the deviation among the different MTs. For instance, the measured  $x_{50,0}$  values by DLS for RTM6 varied with 16 % relative standard deviation while they differ to  $x_{50,0}$  of discAC-turb and SEM by a factor of 1.92 and 3.5, respectively.

**Table 6:** relative uncertainty of the number weighted medians shown in Table 4 with respect to repeatability and intermediate precision – provided that data allowed such analysis; colours indicate degree of uncertainty: **green** for values  $\leq 5$  %, **red** for values  $\geq 20$  %, **amber** for values between 5 % and 20 %

code (material)	SEM	TEM	spICP -MS	PTA	DEMA spray	disc AC-tu.	cuv AC-tu.	cuv AC-RI	AF4- LS	DLS	SAXS	ALS	USSP	BET
QCM1 (polystyrene)				0.2 % 1.3 %	4.0 % 0.7 %					5.8 %				
QCM2 (colloidal SiO <sub>2</sub> )				3.5 % 0.3 %	3.0 %	16.6 %				6.3 %				
QCM3 (colloidal Au)			4.8 %	1.9 %	0.1 %	1.8 %				32 %				
QCM4 (colloidal Ag)					2.5 %	1.8 %				7.1 %	6.2 %			
QCM5 (3-mod PSL)				1.2 % 1.7 %	1.2 % 0.7 %	3.8 %	3.8 %			9.9 %				
QCM6 (3-mod SiO <sub>2</sub> )				3.4 % 1.8 %	0.6 % 4.0 %	0.6 %				3.0 %	0.3 %			
RTM1 (BaSO <sub>4</sub> , UF)				6.7 %	0.6 %	18.0 %	3.9 %	7.0 %		3.5 %				1.1 %
RTM2 (BaSO <sub>4</sub> , fine)				5.3 %		8.0 %				0.7 %				20 %
RTM3 (coated TiO <sub>2</sub> )	1.6 %		1.6 %	0.6 %	0.5 %		1.5 %			0.6 %	0.7 %			2.7 %
RTM4 (CaCO <sub>3</sub> )				1.8 %	1.2 %					0.4 %				1.7 %
RTM5 (kaolin)				1.9 %	0.7 %		1.9 %			3.1 %	0.5 %			2.5 %
RTM6 (fumed SiO <sub>2</sub> )				5.4 % 7.3 %	1.0 %	2.5 %				16 %	4.7 %		19.0 %	
RTM7 (pigment Y83)				1.1 %		14 %	1.5 %			9.6 %	4.9 %			6.9 %
RTM8 (pigment Y83)				1.3 %		2.0 %	56 %			0.2 %				5.1 %
RTM9 (BMC)														7.7 %

## Supplementary data

### S.1 Guidelines for ensuring high-level SOPs for conducting measurements

currently not yet accessible

### S.2 Manual pre-treatment of measurement data

The data analysis could include a manual data treatment, which had to be declared in the measurement report and which should be in accordance to lab-internal standard procedures. There are three situations, when pre-treatment was considered justified: i) the removal of outliers from a series of measurements (e. g. when gas bubbles spoil the quality of the correlation function in DLS measurement), ii) the manual setting of the minimum particle size, and iii) the manual setting of the maximum particle size. The lower limit of the size range needs to be manually adjusted for discAC-turb (where the steady baseline shift in addition to noise produces uncertainty with respect to the finest particle fractions), for spray-DEMA (where non-intentionally generated salt particles in the nano-range cannot be completely avoided), for AF4-techniques (where typically an initial "void peak" is observed before fractionation takes place), and for imaging techniques (when automated software algorithms erroneously identify debris from dispersion agents as particles). Note that some instruments may employ rules for defining minimum size within the data analysis software (e. g. threshold values for particles signals in sp-ICP-MS). For some MTs it is sometimes necessary to define an upper limit of the size range. This holds true for DLS, where inversion algorithms may attribute all deviations between measured and ideally shaped correlation function to the coarsest size class, which generates a clearly separated, non-physical artefact mode. It also applies to AF4-techniques, where a release peak occurs, after the cross-field has been turned off.

### S.3 Measurement reports

currently accessible under:

<https://cloudstore.zih.tu-dresden.de/public.php?service=files&t=df134ce4a94b90QCM4a3da0a16a050ba2b>

(password: NanoDefine)

S.4 Measurement instruments and partners involved**Table S-1:** measurement systems employed, their characteristic parameters during the analytical study and institutions where analyses were conducted

MT acronym	instrumentation	Characteristic parameters (during measurement)	institution
TEM	STEM, HD 2700	200 kV	EAWAG
	FEI Tecnai G2	200 kV	BASF
SEM	Zeiss Supra 40 (Zeiss, DE)	20 kV	BAM
sp-ICP-MS	Nexion 350 (Perkin Helmer)		RIKILT
PTA	NS500, HS (Malvern)	405 nm	Malvern
	Zetaview (Particle Metrix, DE)	403 nm	TUD
spray-DEMA	SMPS (TSI, USA)		LNE
	SMPS (TSI, USA)		TUD
discAC-turb	DQCM24000 UHR (CPS Instruments)	405 nm, 25000 g	JRC-IHCP
cuvAC-turb	LUMiSizer (LUM, DE)	470 nm, 300 g ... 2250 g	TUD
cuvAC-RI	XLI Proteome Lab Version (Beckman, UK)	670 nm, 70 g ... 27000 g	BASF
AF4-LS	Eclipse DUALTEC (S/N 4032), Wyatt Technology		UNIVIE
DLS	Zetasizer Nano ZS (Malvern, UK)	632 nm, 173°	BAM
	HPPS (Malvern, UK)	632 nm, 173°	TUD
ALS	Mastersizer 2000 (Malvern, UK)	632 nm & 470 nm, 0.2°...135°	BAM
	HELOS BR (Sympatec, DE)	632 nm, 0.5° ... 37°	TUD
SAXS	SAXSess (Anton Paar, AUT)	Cu X-ray tube	BAM
USSP	each: DT 1200	3 MHz ... 100 MHz	BAM
	(Dispersion Technol., USA)	3 MHz ... 100 MHz	TUD
BET	multi-point-BET with N <sub>2</sub> (ISO 9277)	77.3 K, p/p <sub>0</sub> = 0.001 ... 0.3	BAM, BASF

## List of partners:

BAM:	Bundesanstalt für Materialforschung, Division 6.8 Surface analysis and interfacial chemistry (coordination of BAM activities), 12205 Berlin, GERMANY
BASF:	BASF SE, Department of material physics, 67056 Ludwigshafen, GERMANY
EAWAG:	Eidgenössische Anstalt für Wasserversorgung, Abwasserreinigung und Gewässerschutz, Particle laboratory, 8600 Dübendorf, SWITZERLAND
JRC-IHCP:	Joint research centre – Institute of health and consumer protection, Nanobiosciences unit, 21027 Ispra (VA), ITALY
LNE:	Laboratoire National de Metrologie et d'Essais, Paris, FRANCE
Malvern:	Malvern Ltd, Amesbury, SP4 7RT, UNITED KINGDOM
RIKILT:	RIKILT Institute of food safety, 6700 AE Wageningen, NETHERLANDS
TUD:	Technische Universität Dresden, Research group mechanical process engineering, 01062 Dresden, GERMANY
UNIVIE:	Universität Wien, Department of environmental geosciences, 1090 Vienna, AUSTRIA

S.5 Values for skeleton density and refractive index**Table S-2:** skeleton density, and refractive index for several wavelengths

code (material)	..., g/cm <sup>3</sup>	m <sub>405nm</sub>	m <sub>470nm</sub>	m <sub>530nm</sub>	m <sub>633nm</sub>	m <sub>670nm</sub>	m <sub>865nm</sub>
QCM1 (polystyrene)	1.050	1.624	1.608	1.598	1.587	1.584	1.576
QCM2 (colloidal SiO <sub>2</sub> )	2.305	1.469	1.463	1.459	1.455	1.454	1.45
QCM3 (colloidal Au)	19.3	1.46-1.96i	1.28-1.88i	0.569-2.26i	0.155-3.36i	0.140-3.74i	0.175-5.48i
QCM4 (colloidal Ag)	10.5	0.170-2.03i	0.142-2.64i	0.140-3.15i	0.140-3.98i	0.140-4.27i	0.140-5.75i
QCM5 (3-mod PSL)	1.050	1.624	1.608	1.598	1.587	1.584	1.576
QCM6 (3-mod SiO <sub>2</sub> )	2.305	1.469	1.463	1.459	1.455	1.454	1.45
RTM1 (BaSO <sub>4</sub> , UF)	4.4	1.697	1.668	1.652	1.634	1.630	1.617
RTM2 (BaSO <sub>4</sub> , fine)	4.4	1.697	1.668	1.652	1.634	1.630	1.617
RTM3 (coated TiO <sub>2</sub> )	3.99	3.23	2.99	2.87	2.77	2.74	2.66
RTM4 (CaCO <sub>3</sub> )	2.657	1.551	1.541	1.534	1.525	1.522	1.507
RTM5 (kaolin)	2.61				1.56		
RTM6 (fumed SiO <sub>2</sub> )	2.2	1.469	1.463	1.459	1.455	1.454	1.45
RTM7 (pigment Y83)	1.484	1.47-0.457i	1.92-0.42i	1.93-0.07i	1.75-0.029i	1.73-0.023i	1.72-0.03i
RTM8 (pigment Y83)	1.5	1.47-0.457i	1.92-0.42i	1.93-0.07i	1.75-0.029i	1.73-0.023i	1.72-0.03i
RTM9 (BMC)	1.13	1.391	1.387	1.384	1.381	1.381	1.378
water (H <sub>2</sub> O)	0.997	1.343	1.338	1.335	1.332	1.331	1.328

The extent to which the values of material properties affect the measured particle size (distribution) may be weak for one particle system and rather huge for others; they can affect the measured particle size or “just” the conversion to number weighted size distributions. In some case it is possible to quantify the relative uncertainty  $U_x = |\Delta x/x|$  of measured size ( $x$ ) as function of the relative uncertainty in material properties like density (...):

- sp-ICP-MS: impact of density on size  $U_x = \frac{1}{3}U_{\rho}$
- mobility based MTs (PTA, AF4-LS, AC techniques, DLS): reciprocal impact of liquid viscosity ( $\eta$ ) on size  $U_x = U_{\eta}$
- AC techniques: impact of density contrast ( $\Delta\rho$ ) on size  $U_x = \frac{1}{2}U_{\Delta\rho}$
- conversion from  $Q_{ext}$  to  $Q_0$  or from  $Q_{int}$  to  $Q_0$  (AF4-LS, AC techniques, DLS): nonlinear impact of RI
- ALS: nonlinear impact of RI on size distribution
- USSP: nonlinear impact of density contrast ( $\Delta\rho$ ) and liquid viscosity ( $\eta$ ) on size distribution
- BET: reciprocal impact of density on size  $U_x = U_{\rho}$

Only few MTs determine the particle size without the need to account for material properties (TEM, SEM, SAXS).

## S.6 SEM images of quality control materials

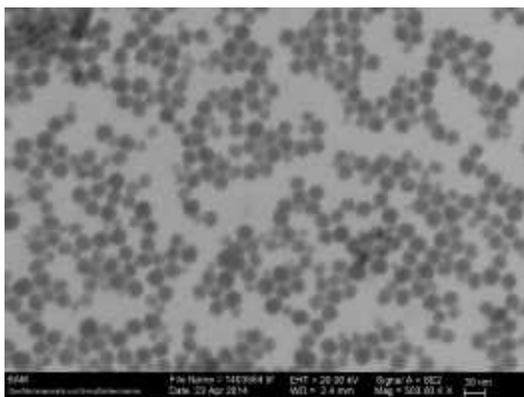


Figure S-1: SEM image of **QCM2: nano-sized SiO<sub>2</sub>**

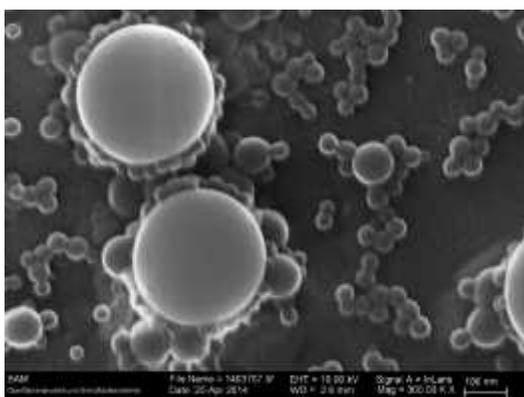


Figure S-2: SEM image of **QCM5: trimodal PSL**

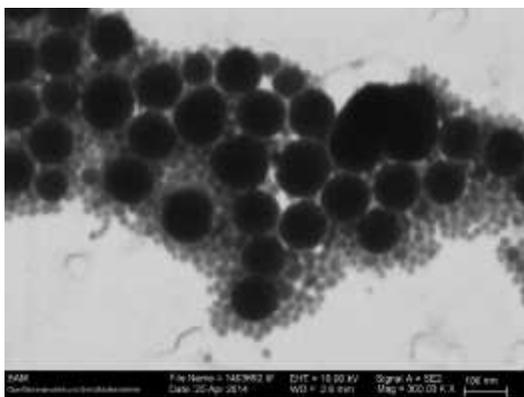
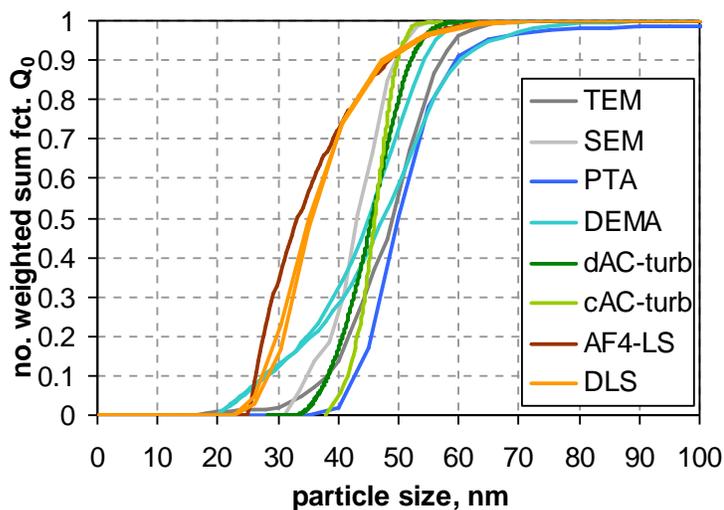
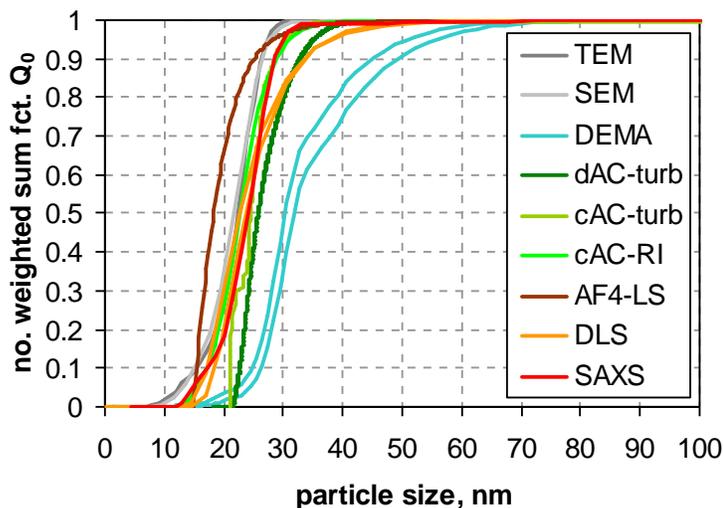


Figure S-3: SEM image of **QCM6 (3mod SiO<sub>2</sub>)**

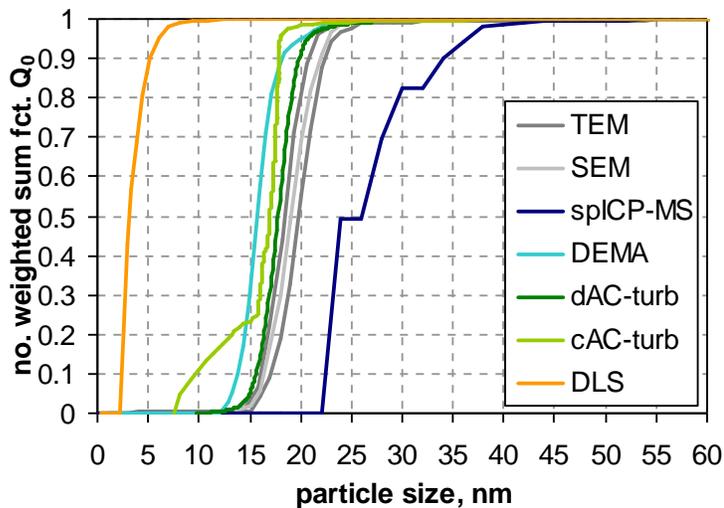
S.7 Graphical representation of number weighted size distributions



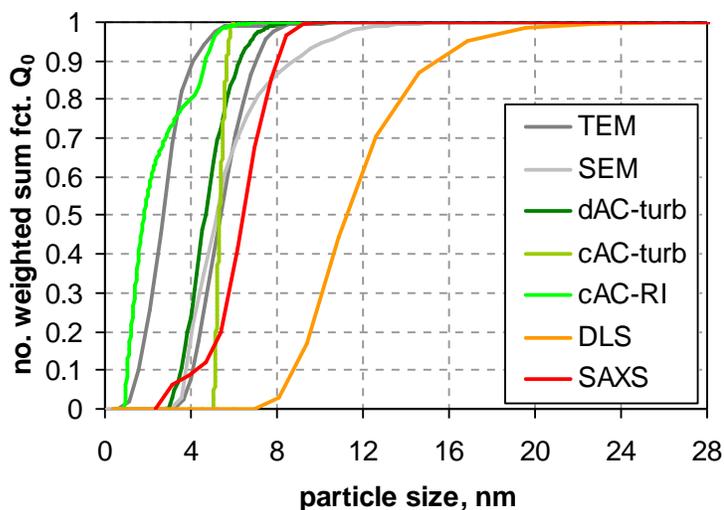
**Figure S-4:** QCM1 (monomodal polystyrene), number weighted sum functions from measurements with TEM, SEM, PTA, spray-DEMA (2×), discAC-turb, cuvAC-turb, cuvAC-RI, AF4-LS, and DLS (2×)



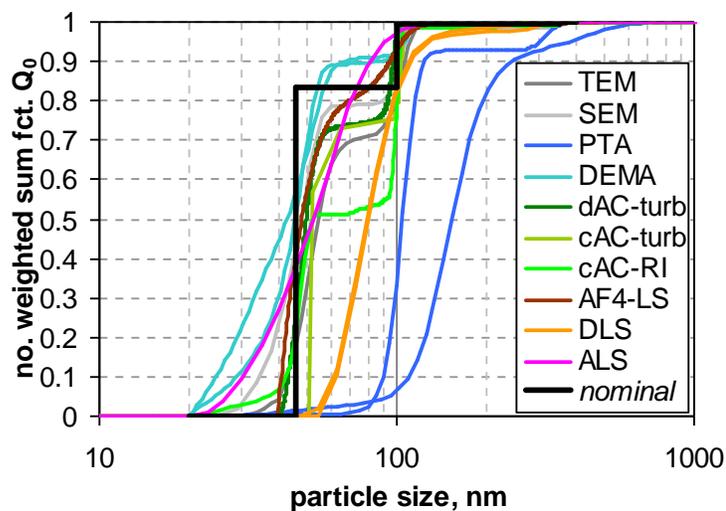
**Figure S-5:** QCM2 (colloidal  $\text{SiO}_2$ ), number weighted sum functions from measurements with TEM, SEM, spray-DEMA (2×), discAC-turb, cuvAC-turb, cuvAC-RI, AF4-LS, DLS (2×), and SAXS



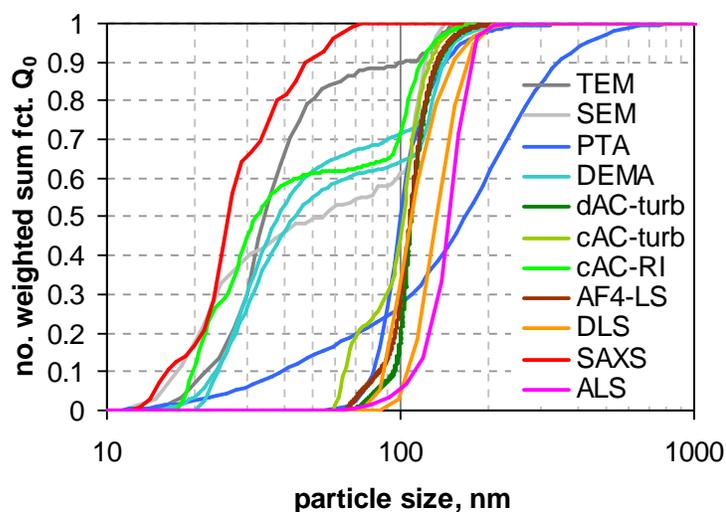
**Figure S-6:** QCM3 (colloidal Au), number weighted sum functions from measurements with TEM (2 $\times$ ), SEM, sp-ICP-MS, spray-DEMA, discAC-turb, cuvAC-turb, and DLS



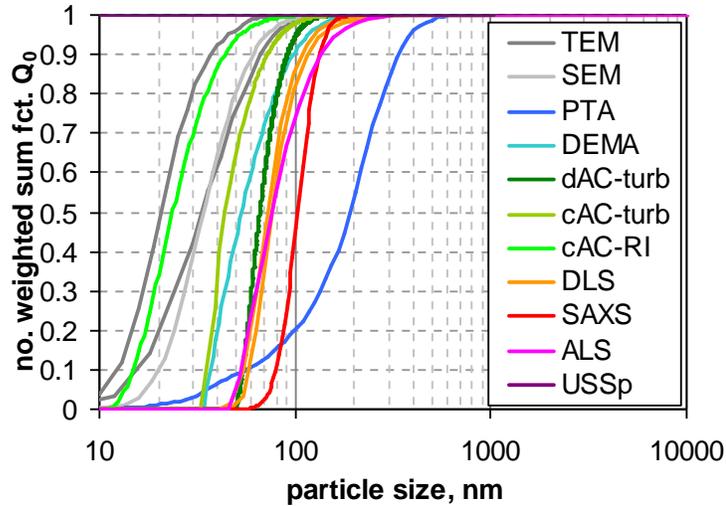
**Figure S-7:** QCM4 (colloidal Ag), number weighted sum functions from measurements with TEM (2 $\times$ ), SEM, discAC-turb, cuvAC-turb, cuvAC-RI, DLS and SAXS



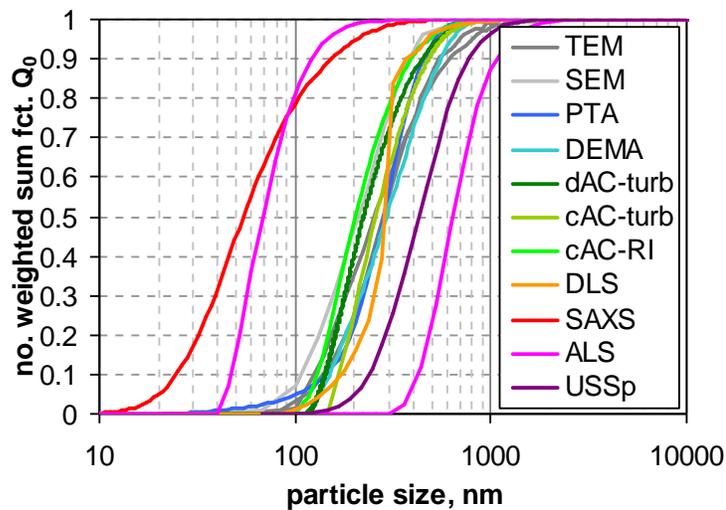
**Figure S-8:** QCM5 (trimodal polystyrene), number weighted sum functions from measurements with TEM, SEM, PTA (2×), spray-DEMA (2×), discAC-turb, cuvAC-turb, cuvAC-RI, AF4-LS, DLS (2×), and ALS



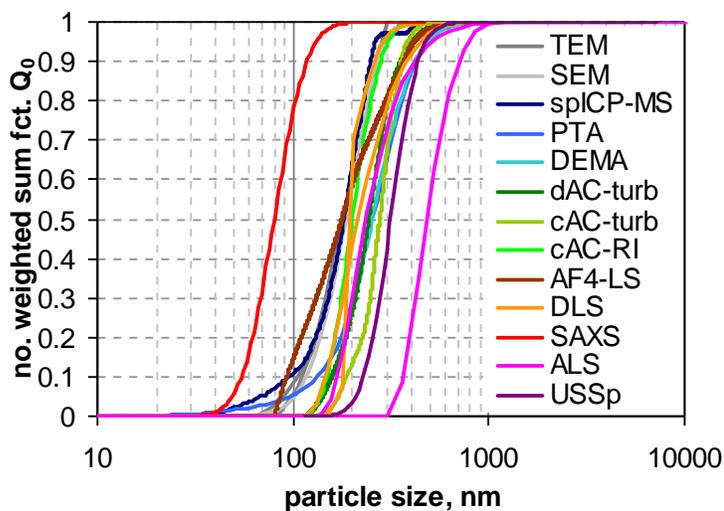
**Figure S-9:** QCM6 (trimodal  $\text{SiO}_2$ ), number weighted sum functions from measurements with TEM, SEM, PTA (2×), spray-DEMA (2×), discAC-turb, cuvAC-turb, cuvAC-RI, AF4-LS, DLS (2×), SAXS, and ALS



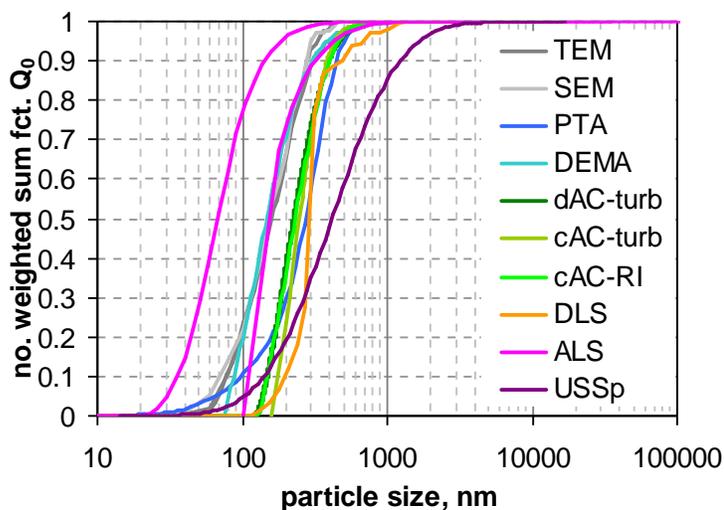
**Figure S-10:** RTM1 (BaSO<sub>4</sub>, ultrafine), number weighted sum functions from measurements with TEM (2×), SEM, PTA, spray-DEMA, discAC-turb, cuvAC-turb, cuvAC-RI, DLS (2×), SAXS, ALS, and USSP



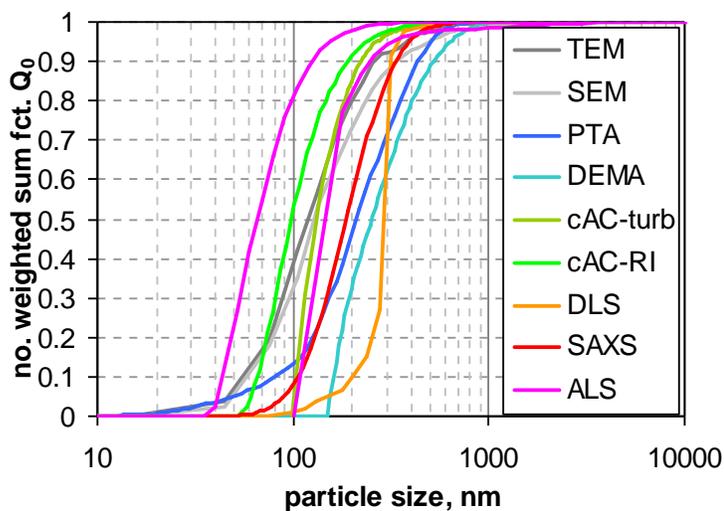
**Figure S-11:** RTM2 (BaSO<sub>4</sub>, fine), number weighted sum functions from measurements with TEM (2×), SEM, PTA, spray-DEMA, discAC-turb, cuvAC-turb, cuvAC-RI, DLS, SAXS, ALS (2×), and USSP



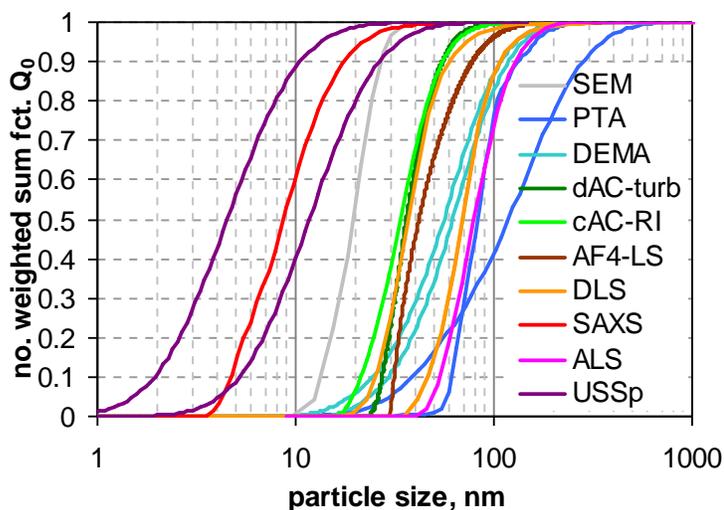
**Figure S-12:** RTM3 (coated  $\text{TiO}_2$ ), number weighted sum functions from measurements with TEM (2 $\times$ ), SEM, PTA, spray-DEMA, discAC-turb, cuvAC-turb, cuvAC-RI, DLS (2 $\times$ ), SAXS, ALS (2 $\times$ ), and USSP



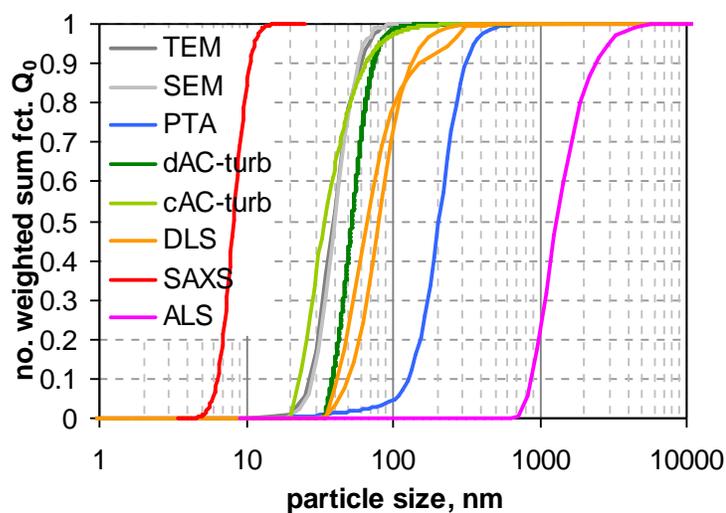
**Figure S-13:** RTM4 ( $\text{CaCO}_3$ ), number weighted sum functions from measurements with TEM (2 $\times$ ), SEM, PTA, spray-DEMA, discAC-turb, cuvAC-turb, cuvAC-RI, ALS (2 $\times$ ), and USSP



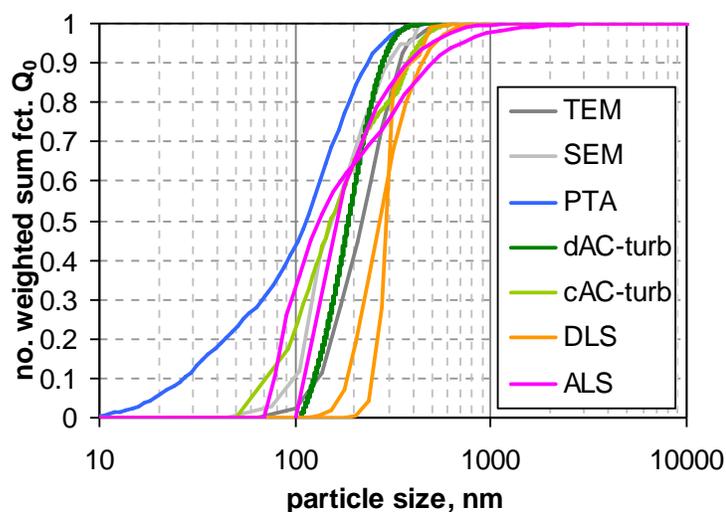
**Figure S-14:** RTM5 (kaolin), number weighted sum functions from measurements with TEM, SEM, PTA, spray-DEMA, cuvAC-turb, cuvAC-RI, DLS, SAXS, and ALS (2×)



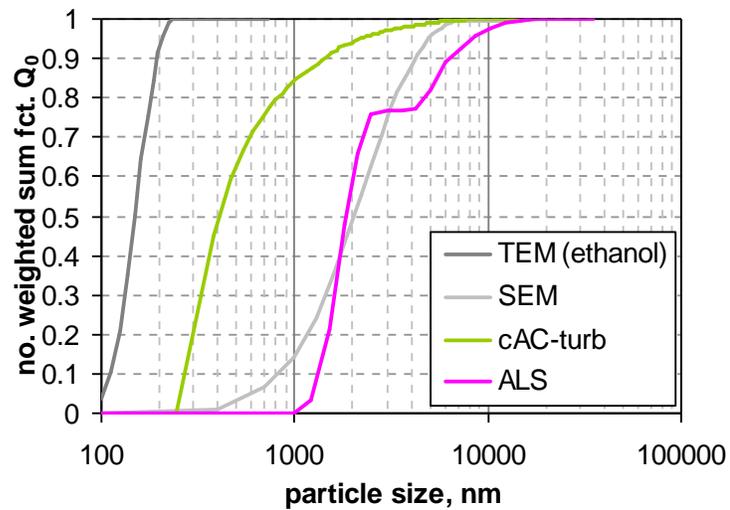
**Figure S-15:** RTM6 (fumed  $\text{SiO}_2$ ), number weighted sum functions from measurements with SEM, PTA (2×), spray-DEMA (2×), discAC-turb, cuvAC-RI, AF4-LS, DLS (2×), SAXS, ALS, and USSP (2×)



**Figure S-16:** RTM7 (pigment Y83, transparent), number weighted sum functions from measurements with TEM, SEM, PTA, discAC-turb, cuvAC-turb, DLS (2×), SAXS, and ALS

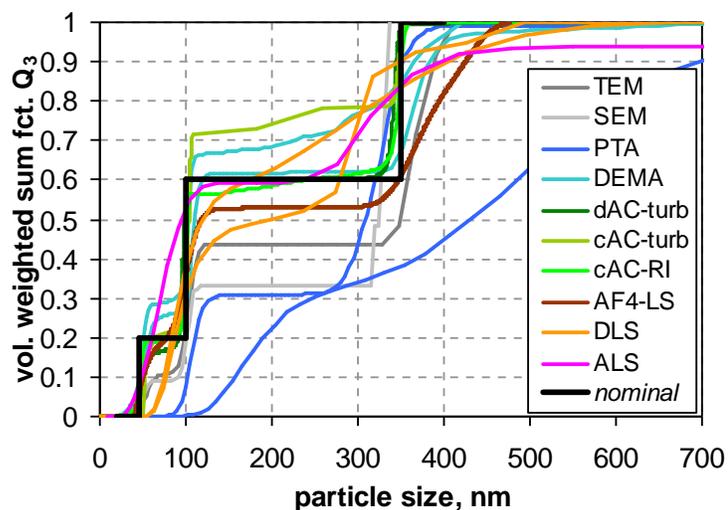


**Figure S-17:** RTM8 (pigment Y83, opaque), number weighted sum functions from measurements with TEM, SEM, PTA, discAC-turb, cuvAC-turb, DLS (2×), and ALS (2×)



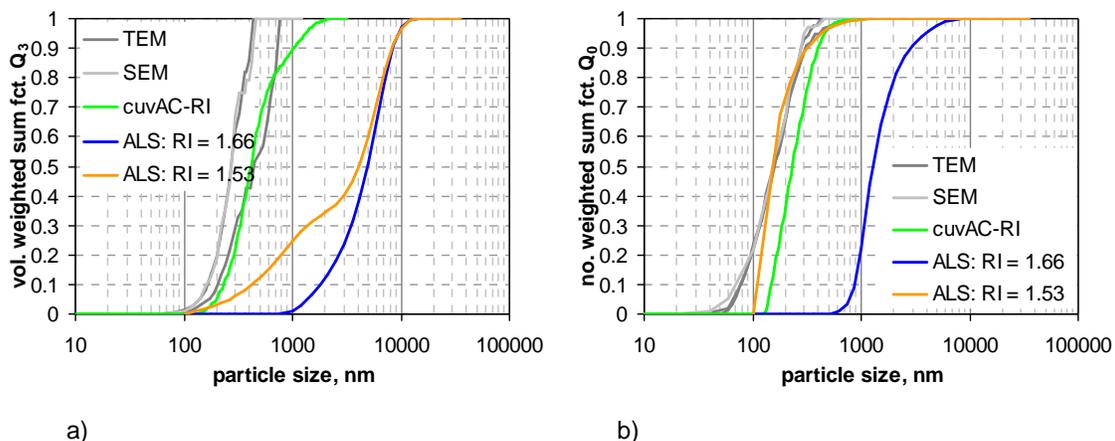
**Figure S-18:** RTM9 (BMC), number weighted sum functions from measurements with SEM, cAC-turb, and ALS

S.8 Volume weighted size distributions

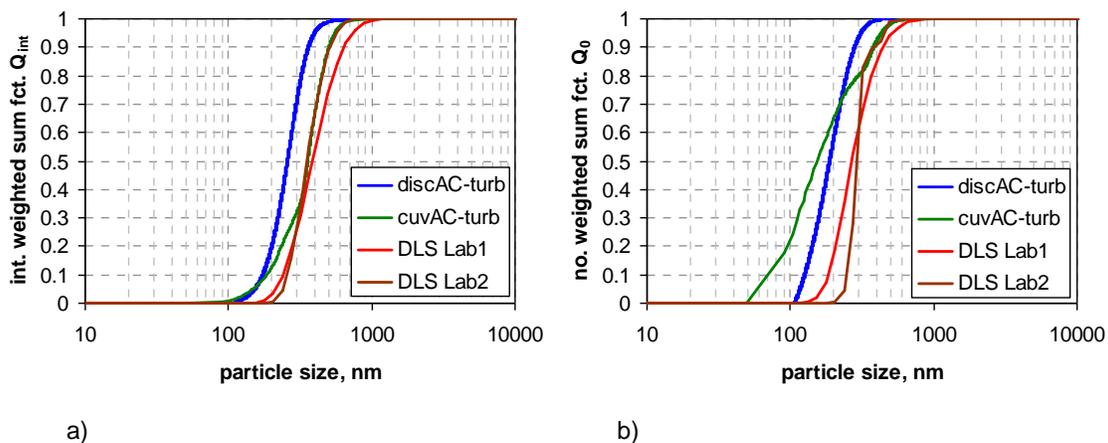


**Figure S-19:** QCM5 (trimodal PSL, left) volume weighted sum functions from measurements with TEM, SEM, PTA (2×), spray-DEMA (2×), discAC-turb, cuvAC-turb, cuvAC-RI, AF4-LS, DLS (2×), ALS

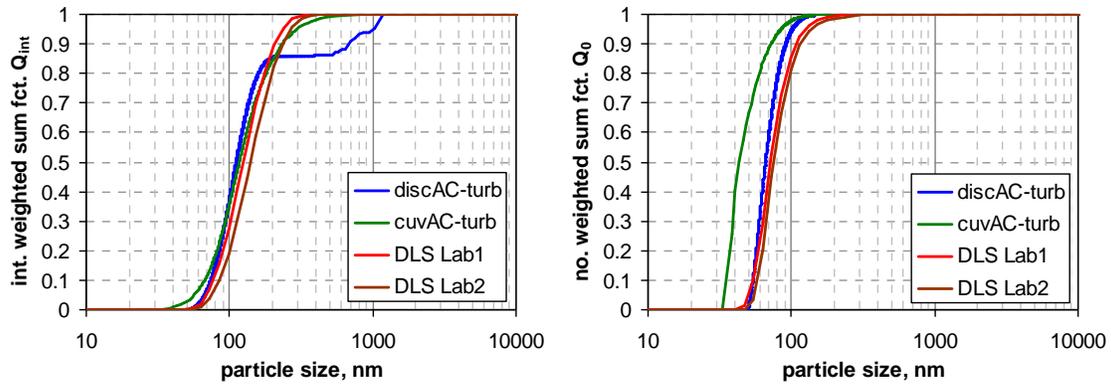
### S.9 Discussion on the quality of measurement data



**Figure S-20:** ALS result for RTM4, impact of RI values on a) volume and b) number weighted size distribution (additionally results of TEM, SEM & cuvAC-RI)



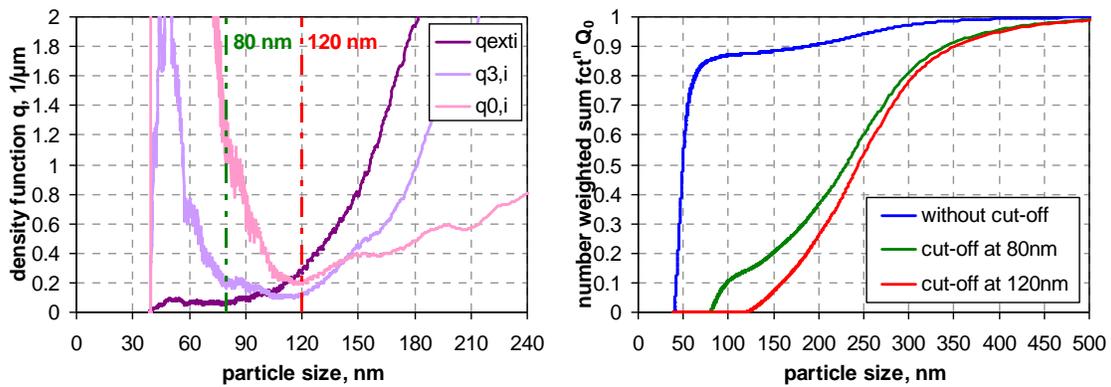
**Figure S-21:** RTM8 (PY83, opaque) results of mobility-based MTs with particle quantification by light extinction or light scattering; a) intrinsically measured intensity and extinction weighted and b) derived number weighted sum functions



a)

b)

**Figure S-22:** RTM1 ( $\text{BaSO}_4$ , ultrafine) results of mobility-based MTs with particle quantification by light extinction or light scattering; a) intrinsically measured intensity or extinction weighted and b) derived number weighted sum functions



a)

b)

**Figure S-23:** impact of setting a lower size limit on  $Q_0$  for RTM3 (coated  $\text{TiO}_2$ ) when measured with discAC-turb; a) detail of the originally measured density functions with possible cut-off values for the lower size limit, b) sum functions of number weighted size distribution for different values of minimum particle size

S.10 Additional data on TEM and SEM results**Table S-3:** number of particles employed in size analysis with EM techniques and polydispersity according to SEM

code (material)	N(SEM)	N(TEM-1)	N(TEM-2)	$x_{99,0}/x_{1,0}$ ( $x_{max}/x_{min}$ )
QCM1 (polystyrene)	65	923		1.7 (1.8)
QCM2 (colloidal SiO <sub>2</sub> )	923	1666		3.0 (5.2)
QCM3 (colloidal Au)	1748	899	339	1.7 (2.4)
QCM4 (colloidal Ag)	2133	4177	388	3.8 (54)
QCM5 (3-mod PSL)	251	872		12 (14)
QCM6 (3-mod SiO <sub>2</sub> )	147	466		10 (13)
RTM1 (BaSO <sub>4</sub> , UF)	5816	259	691	7.1 (21)
RTM2 (BaSO <sub>4</sub> , fine)	234	1633	372	8.5 (16)
RTM3 (coated TiO <sub>2</sub> )	9456	319	58	4.5 (16)
RTM4 (CaCO <sub>3</sub> )	133	261	327	8.7 (15)
RTM5 (kaolin)	753			24 (90)
RTM6 (fumed SiO <sub>2</sub> )	147			2.9 (3.6)
RTM7 (pigment Y83)	129	366		3.5 (4.6)
RTM8 (pigment Y83)	75	353	225	6.1 (7.2)
RTM9 (BMC)	390			14 (40)

## S.11 Glossary

### particle size $x$

equivalent diameter or characteristic length

### equivalent diameter $x_z$

diameter of a sphere being equivalent to particle with respect to property  $Z$

### characteristic length

any length describing the outer dimensions of a particle (mostly in image analysis), e. g. minimum Feret diameter, rod diameter, cord length

### measurement principle

(physical) effect that is employed for the quantification of the measurand (e. g. particle size, zeta-potential); the dependency of the effect on the measurand has to be well-defined; examples: i) settling velocity grows with particle size, ii) diffusion coefficient is inversely proportional to size, iii) 2D-images obtained by orthogonal projection allow the direct quantification of characteristic lengths and areas.

### measurement technique (MT)

specific way of employing a measurement principle for the quantification of a measurand (e. g. particle size); similar measurement techniques are sometimes grouped as one measurement method; examples: i) the settling velocity as a measure of size (= measurement principle) can be measured by gravitational sedimentation with an X-ray transmission detector (= measurement technique) or by centrifugal sedimentation in homogeneously filled cuvettes with an optical transmission detector, ii) the diffusion coefficient can be measured with dynamic light scattering by means of a self-beating autocorrelation detector or by means of homodyne light detection and frequency analysis, iii) sphere diameters can be derived from images of a scanning electron microscope, which were obtained with or without using the emitted secondary electrons.

### characterisation method (CM)

specific procedure for the characterisation of a material which includes the steps of sampling, sample preparation, sample analysis with (a) certain measurement technique(s) and defined algorithms of signal interpretation, and final data interpretation

### quality control material (QCM) for particle size analysis

a particulate material relatively well defined with respect to particle shape (spherical or similar), state of aggregation (non-aggregated, non-agglomerated) and particle size distribution, which are supplied as ready-to-use suspension samples

### representative test materials (RTM) for particle size analysis

a particulate material from a single batch, which is sufficiently homogeneous and stable with respect to all properties that affect particle size analysis; can be provided as powder or suspension and requires defined sample preparation (e. g. to obtain sufficiently stable, well-dispersed suspension sample)