



## **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

# **Techniques evaluation report for selection of characterisation methods**

## **NanoDefine Technical Report D3.1**

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## **The NanoDefine Consortium 2015**

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## **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.

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## 1 Abbreviations and acronyms

AC	Analytical Centrifugation
AFM	Atomic Force Microscopy
ALS	Angular Light Scattering
AUC	Analytical Disk- and Ultra-Centrifugation
BAM	Federal Institute for Materials Research and Testing
BET	Brunauer–Emmett–Teller method
BSE	Backscatter Electrons
CLS	Centrifugal Liquid Sedimentation
CM	Characterisation Method
CPC	Condensation Particle Counter
CRM	Certified Reference Material
DLS	Dynamic Light Scattering
DMA	Differential Mobility Analyzer
DMAS	Differential Mobility Analyzing System
DMPS	Differential Mobility Particle Sizer
DUM	Dynamic Ultramicroscopy
ECD	Equivalent circle diameter
EDX	Energy Dispersive X-Ray Spectroscopy
EM	Electron Microscopy
ES-DMA	Electrospray - Differential Mobility Analysis
ESZ	Electrical Sensing Zone
FCS	X-Ray Fluorescence Correlation Spectroscopy
FFF	Field Flow Fractionation
FWHM	Full Width at Half-Maximum
HAADF-STEM	High-Angle Annular Dark Field Scanning Transmission Electron Microscopy
LD	Laser Diffraction
m	ion mass
MPPS	Mobility Particle Size Spectrometer
NIR	Near Infrared
NIST	National Institute of Standards and Technology
NTA	Nanoparticle Tracking Analysis
OS	Optical Spectroscopy

PSD	Particle Size Distribution
PTA	Particle Tracking Analysis
SAXS	Small-angle X-Ray Scattering
SE	Secondary Electrons
SEC	Size Exclusion Chromatography
SEM	Scanning Electron Microscopy
SFM	Scanning Force Microscopy
SLS	Static Light Scattering
SMPS	Scanning Mobility Particle Sizer
sp ICP-MS	Single Particle Inductively Coupled Plasma Mass Spectrometry
STEM	Scanning Transmission Electron Microscopy
TEM	Transmission Electron Microscopy
TRPS	Tunable Resistive Pulse Sensing
TSEM	Scanning Electron Microscopy in Transmission Mode
USSp	Ultrasonic Spectroscopy
UV	Ultraviolet
VSSA	Volume Specific Surface Area
WP	Work Package
XRD	X-Ray Diffraction
z	charge number

## **2 Summary**

This report is the result of a comprehensive study on the available CMs which come potentially in question for the reliable analysis of the number based size distribution of a nanomaterial according to the EC recommendation for a definition of nanomaterial.

Based on the performance criteria already established in NanoDefine the potential CMs are evaluated according to studies available in the literature as well as following the expertise of the NanoDefine consortium partners. The specific advantages and disadvantages of each method with respect to its applicability to the scope of NanoDefine are particularly highlighted.

An CM evaluation table is produced so that the mostly suited CMs with respect to the EC definition can be grouped and recommended to the corresponding NanoDefine work packages for further specific development (improvement and adaption), or for direct validation and standardisation, respectively.

The actual evaluation report including the recommended CMs will be revised and, if necessary, eventually updated at the mid time of the project. The update will be jointly discussed in the NanoDefine consortium on the basis of the results of testing the methods on the NanoDefine real world materials.



### 3 Scope

One of the NanoDefine tasks consists of a systematic critical review of those characterisation methods (CMs) currently presumed as reference methods, or widely used or with high-potential to be applied for the implementation of the EC definition of nanomaterial. It should be noted that all potentially suitable CMs are quantitatively evaluated in form of templates already developed in NanoDefine with respect to their applicability to specific materials and performance criteria.

The present study reviews and documents the potential CMs with respect to the most relevant performance criteria, which facilitate an evaluation of their application for the implementation of the EC definition of nanomaterial. Such performance criteria are type of samples, type of sizing, particle property measured, type of quantity, size range, concentration range, resolution of particle size distribution, information content, limits of application, analytical figures of merit, data analysis etc.; they are grouped into a concise table. Furthermore, this performance table contains the main specific advantages and disadvantages of each method with respect to its application to NanoDefine.

Based on the evaluation of the potential CMs according to the scheme as described above the CMs are grouped either to be passed over to corresponding NanoDefine work packages for further specific improvements and adaption or to be practically passed for direct validation and standardisation.

## **4 Evaluation of the characterization methods**

There are several means and physical phenomena that allow for the determination of size distributions (e. g. imaging, sedimentation, extinction). A more general distinction of particle sizing techniques is based on how the weights of the individual size fractions are determined (cf. Stintz 2005, Stintz et al. 2010):

- counting techniques (measuring particle properties at individual particles)
- fractionating techniques (measuring the amount or concentration of size/property classes after fractionating the particle system)
- ensemble techniques (measuring the spectral or parametric response of a representative particle ensemble of the total particle system)
- integral methods.

## 5.1 Counting methods

Counting methods inherently yield particle number weighted distributions ( $Q_0$ ) of a certain particle property or of a physical quantity that is related to a certain particle property (e. g. particle size, or the average displacement as a measure of the diffusion coefficient). They rely on the individualisation of the particle sample, which can be either achieved by analysing microscopy images (e. g. from electron microscopes) or by sufficient sample dilution or by reduction of sample or measurement volume. The probed particle property may be either geometric (in particular for image analysis), optical (e. g. scattering cross section), or related to mobility (diffusion coefficient).

### 5.1.1 Imaging methods

#### 5.1.1.1 Electron microscopy (SEM, TEM and TSEM)

- **Measuring principle**

One analytical method widely used for sample visualization down to the nm scale is the Electron Microscopy.

For the case of Scanning Electron Microscopy (SEM) the sample to be inspected is bombarded with a finely focused electron beam which is scanned over a defined field. Low-energy secondary electrons (SE) are released after inelastic collisions with the atoms in the specimen as well as high-energy backscattered electrons (BSE) after elastic collisions. Depending on their kinetic energy the information range carried by the released electrons varies from micrometer range (typically for BSE) down to nanometer (typically for SE). Hence, the SE are suited for high-resolution morphological characterization of the specimen surface at nanometer scale and even individual nanoparticles may be visualized and lateral dimensions measured in an SEM. Depending on the instrument used but also strongly on the challenging sample preparation onto typical sample substrates/holders accurate size characterization of nanoparticles with sizes down to several tens of nm is possible (Motzkus et al., 2013).

The preparation of the nanoparticles as a sample to be investigated by EM is key to successful analysis of the NP size distribution. Ideally, NPs which are well separated, free of preparation artefacts, and are distributed on the proper support not too far away from each other shall be accurately imaged. Care must be taken of possible beam or vacuum influence onto the size of NPs. Once such an accurate image is taken the post-measurement of the NP size can be performed with appropriate software packages offered either by the electron microscope manufacturers or for free available on the www. Decisive steps in the evaluation of the accurate size of the NPs determined with an electron microscope are the calibration of the magnification, i. e. of the pixel size including its re-calibration in the processing software, and the setting of threshold in the image histogram corresponding to the real position of the particle border.

This CM is able to count individual particles. Depending on the number of NPs acquired in an image, mostly several images are necessary to reach a good counting statistics. The automation tools such as motorized stage and sequential image acquisition should be available in order to speed up the whole measurement process. Also automatic image processing of batch images speeds up the determination of the size

distribution.

Whereas in the case of an SEM typical beam voltages up to 30 kV may be applied and SE/BSE are collected by various detectors in the case of TEM the beam voltage is up to 300 kV. The samples to be analyzed must be in the range of the electron transparency so that the electrons transmitted through the thin sample are collected. The highly energetic electron beam is even finer than in the SEM case (more sophisticated aberration correctors being typically also available), so that spatial resolution well below 1 nm can be attained. Similar requirements as in the SEM case for NPs regarding sample preparation, suitable substrate (typically the so-called TEM grids), vacuum suitability (Jung et al. 2002), calibration of the pixel size and automation of both acquisition and image processing hold true for TEM, too. By combining TEM imaging and semi-automatic image analysis, TEM allows characterizing the size, shape, and surface topology of colloidal nanomaterials (De Temmerman et al., 2013), aggregated nanomaterials (De Temmerman et al., 2012; Verleysen et al., 2014) and primary particles in aggregates (De Temmerman et al., 2014). A characterization methodology which includes a systematic selection procedure for unbiased random image collection, semi-automatic image analysis and data processing has been validated for size, shape and surface topology measurements of silica nanoparticles (De Temmerman, 2013). The expanded uncertainty of size measurements of two colloidal silica certified reference materials was estimated to be about 3 %.

TEM can be operated in the scanning mode, i.e. STEM, when the electron beam is focused into a narrow spot which is scanned over the sample. According to the range of angles by which they are scattered in the sample the transmitted electrons can be differentiated: bright-field electrons are those electrons slightly or not at all scattered and dark-field electrons are those collected concentrically to the optical microscope axis. The so-called HAADF-STEM (High-Angle Annular Dark-Field STEM) imaging mode results when only the strongly scattered [electrons](#) are collected with an annular dark-field detector. The contrast of this type of imaging with atomic resolution is directly related to the [atomic number](#) (Z-contrast image).

One hybrid type of electron microscopy is constituted by the SEM able to work in the transmission mode, i. e. TSEM, T-SEM or STEM in SEM etc. This means that by using TEM grids as supports for NPs the transmitted electrons (of lower energies than in the TEM case) are more or less absorbed by the NPs and a so called STEM detector placed under the sample holder detects them. The alternative is to use a special mount, i.e. single-unit transmission setup, which enables to perform TSEM with the available SE/BSE detector and not having necessary an additional STEM detector. It was recently demonstrated that both types are well suited for metrological measurement of NP size and size distribution down to a size of about 10 nm (Buhr et al., 2009, Klein et al., 2011; Hodoroaba et al., 2014).

- **Performance – general remarks**

- Limits of application*

- Strongly dependent on sample preparation (incl. suitable substrate)
    - Vacuum suitability
    - SEM not able to measure accurately NPs below several tens of nm (depending on

instrument)

- Automation (batch image acquisition and batch image processing)
- Time-consuming depending on the complexity of sample preparation and degree of automation
- Costly, but in the same order of magnitude as ICP-MS
- Accurate delimitation of the NPs in the image processing software

*Analytical figures of merit*

- SEM minimal NP size to be measured accurately: several 10s of nm depending on instrument
- TEM minimal NP size to be measured accurately: below 1 nm depending on contrast and on instrument
- TSEM minimal NP size to be measured accurately: about 10 nm depending on instrument

*Data analysis, resolution of particle size distribution etc.*

Systematic results of metrological measurement of NP size and size distribution by SEM, TEM and TSEM were carried out recently in the frame of various round robin exercises specially dedicated to this purpose (Meli et al, 2012; Motzkus et al., 2013). According to them EM provides traceable results which are also consistent, i.e. comparable, with those obtained by AFM, SAXS and SMPS, but not with DLS.

● **Performance – table**

<b>Main features</b>	
<b>Type of samples</b>	particles properly deposited onto substrates
<b>Type of sizing</b>	counting technique (by identifying individual objects in images)
<b>Particle property measured</b>	Feret diameter, Equivalent circle diameter (ECD), wide range of 1D and 2D size, shape and surface measurands
<b>Type of quantity</b>	particle number
<b>size range</b>	<ul style="list-style-type: none"> <li>• SEM: 30 nm - 100 µm</li> <li>• TEM (incl. HAADF-STEM): &lt;1 nm - 10 µm</li> <li>• TSEM: 10 nm - 10 µm</li> </ul>
<b>concentration range</b>	"0" (individual particles) ... monolayer (immobilised particles)
<b>information content</b>	<ul style="list-style-type: none"> <li>• Good in x-y direction (parallel to the substrate);</li> </ul>
<b>Main advantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• yields well-resolved number weighted size distributions from measurement of individual particles.</li> <li>• Size, shape and surface measurands can be measured on 2D images</li> </ul>	

<ul style="list-style-type: none"> <li>• Sub-nm resolution for TEM, nm resolution for SEM</li> <li>• able to distinguish and measure aggregates and isolated particles. Classification between single and agglomerated particles is possible</li> <li>• primary particles in aggregates can be detected and their size can be measured</li> <li>• access to smallest dimension of particles in X-Y plane (TEM)</li> <li>• capable of chemical specificity of single particles by the attached EDX</li> <li>• crystallographic information is available by electron diffraction</li> <li>• significant instrumental developments (spatial resolution, automation, EDX detector sensitivity, table-top instruments, etc.)</li> </ul>
<p><b>Main disadvantages with regard to NanoDefine</b></p>
<ul style="list-style-type: none"> <li>• strongly dependent on sample preparation</li> <li>• needs vacuum and expensive instrumentation</li> <li>• Automation in image processing: in progress</li> <li>• Limited dynamic range (highest size/lowest size &lt; 40) based on one image only</li> </ul>
<p><b>Type of potential improvement within the NanoDefine project</b></p>
<ul style="list-style-type: none"> <li>• Sample preparation</li> <li>• Combination of results over different size ranges</li> <li>• Automation in image acquisition and processing</li> </ul>

**Table 1:** Performance table EM

## 5.1.1.2 Scanning force microscopy (SFM), or atomic force microscopy (AFM)

A further technique able to image colloidal particles or powders is the scanning force microscopy (SFM), also called atomic force microscopy (AFM), which was developed by Binnig and his co-workers in the 1980s (e. g. Binnig et al. 1986). SFM (or AFM) is the most used type of scanning probe microscopy (SPM). The scanning force microscopy (or: atomic force microscopy) and its related techniques are based on the interaction between a very fine probe tip with the atoms or molecules at the surface of the sample (Giessibl 2003, Danzebrink et al. 2006; cf. Figure). This can be used to resolve surface morphologies or particles on a substrate with vertical/out-of-plane resolution of 0.1 nm to 10 nm (lateral/in-plane resolution: 10 nm). The SFM is usually employed for the characterisation of films and surfaces (e. g. roughness), whereas the morphological characterisation of particles is of minor relevance. Its real strength is the sensitivity to the forces between probe and sample, which allows an evaluation of surface chemistry (e. g. functional groups, hydrophobicity) and the quantification of particle interactions, or interactions between particles and surfaces (e. g. adhesion, friction; Heim et al. 1999, Butt et al. 2007). Depending on the situation, forces that are measured in SFM include mechanical contact force, van der Waals forces, capillary forces, chemical bonding, electrostatic forces, magnetic forces, etc. As well as force, additional quantities may simultaneously be measured through the use of specialised types of probe. Samples in air or in liquid can be analyzed, but the sample must adhere to a substrate and be rigid and well dispersed on it. The roughness of the substrate must be less than the size of the nanoparticles being measured. The SFM can be operated in several modes. In general, imaging modes are divided into static (also called contact) modes and a variety of dynamic (or non-contact) modes where the cantilever is vibrated (Figure 1). The use of the AFM in biology, biochemistry and bionanotechnology, also for the characterization of nanomaterials (size, shape), are reviewed in an article by Kada et al., 2008.

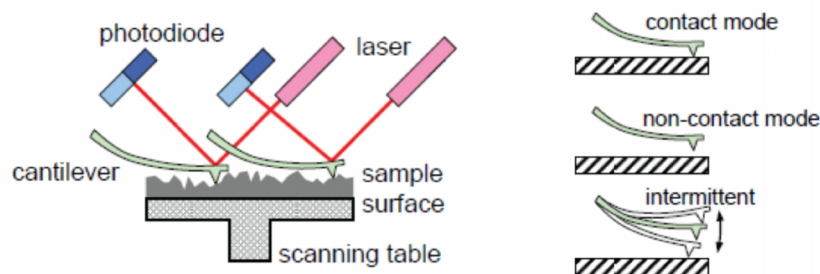


Figure 1: Measuring principle of SFM

The results of imaging methods are (mainly) particle number weighted particle size distributions (PSDs). That means that the sample size (number of probed particles) should be sufficiently high for ensuring low uncertainty in class frequencies. Moreover, the sample size required to achieve a certain confidence level increases with polydispersity. The accuracy of the measured particle properties depends on a variety of factors (e. g. magnification or spatial resolution of the scanning mode, or image processing). Most crucial, however, is the representativity of the imaged particles for the whole particle system. That requires that the particle deposition on the substrate is neither size-selective nor inhomogeneous (Fiala et al. 2011). In general, sample prep-

aration is a key issue for imaging methods.

• **Performance – table**

<b>Main features</b>	
<b>Type of samples</b>	Particles of (almost) any material, in vacuum, air or liquid, but they must be immobilized on a substrate
<b>Type of sizing</b>	Counting technique
<b>Particle property measured</b>	Particle height above the level of a substrate
<b>Type of quantity</b>	Particle number, size of individual particles
<b>size range</b>	<ul style="list-style-type: none"> <li>• 1 nm ... &gt;&gt; 1 μm (z-size, "height", most reliable), lateral size not recommended, depends on tip geometry)</li> </ul>
<b>concentration range</b>	"0" (individual particles) ... monolayer (immobilised particles)
<b>information content</b>	<ul style="list-style-type: none"> <li>• Good in z-direction ("height");</li> <li>• not reliable in x-y direction (laterally, parallel to the substrate)</li> </ul>
<b>Main advantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• Measures individual particles, no conversion necessary</li> <li>• Access to the minimum dimension of a particle</li> <li>• Measures a wide range of materials</li> <li>• Instruments are widely available and not expensive</li> </ul>	
<b>Main disadvantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• needs immobilization of particles on suitable substrates</li> <li>• lateral size measurements are not reliable, depend on tip conditions</li> <li>• low sample throughput, slow</li> <li>• Limited dynamic range</li> </ul>	
<b>Type of potential improvement within the NanoDefine project</b>	
<ul style="list-style-type: none"> <li>• Sample preparation</li> <li>• Combination of results over different size ranges</li> </ul>	

**Table 2:** Performance table SFM



## 5.1.2 Particle tracking analysis (PTA), Dynamic ultramicroscopy (DUM)

### 5.1.2.1 Measuring principle

Fine colloidal particles are usually smaller than the spatial resolution of an ordinary light microscope, which means that they are invisible with regard to an affine projection. However, when they are laterally illuminated by very intense light against a dark background (dark field microscopy), it is possible to see the scattering patterns with an optical microscope. Such an instrument is named ultramicroscope. When ultramicroscopy is used for particle sizing, one evaluates the Brownian motion of the scattering centres (i. e. particles), because of which this type of sizing is called particle tracking analysis (PTA) or dynamic ultramicroscopy (DUM).

In contrast to the majority of sizing techniques for NP suspensions PTA is in principle capable of measuring the particle number concentration. However, the reliability of such a measurement depends very much on material properties and distribution width. Taking the current state-of-the-art the concentration measurement is not very reliable in the general case and needs further investigations (Hole et al., 2013).

### 5.1.2.2 Performance – general remarks

#### *Limits of application*

The application limits of ultramicroscopy result from the requirements that the particle distances should be much larger than the optical resolution limit of the microscope and that the scattered light of all individual particles is sufficiently strong for detection. While the former can be achieved by appropriate dilution, the latter requirement is missed for particles below a material-specific size limit.

Additionally, there are principal difficulties in detecting weak scatterers in the presence of strong scatterers. That means, though providing number weighted PSD (similar to ordinary microscopy), the dynamic ultramicroscopy has a bias to strongly scattering particles (similar to dynamic light scattering; Domingos et al., 2009). This concerns very broad size distributions as well as multi-component particle systems.

### 5.1.2.3 Performance – general remarks

<b>Main features</b>	
<b>Type of samples</b>	Suspended particles
<b>Type of sizing</b>	counting technique (by identifying individual objects in video images)
<b>Particle property measured</b>	translational hydrodynamic diameter
<b>Type of quantity</b>	particle number
<b>size range</b>	10 nm ... 1 µm (depending on the scattering properties of the material)
<b>concentration range</b>	<< 1 vol.-%
<b>information content</b>	good, yet not perfect resolution (widening of PSD due to stochastic nature of Brownian motion, statistic uncertainty for rare particle sizes, insensitivity for very fine particles).
<b>Main advantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• yields number weighted size distributions <math>Q_0</math>.</li> </ul>	

<b>Main disadvantages with regard to NanoDefine</b>
<ul style="list-style-type: none"><li>• measures aggregate size rather than size of the constituent particles.</li><li>• poor sensitivity in the fine particle range</li><li>• Lower size limit depends on scattering properties of particles</li><li>• Limited dynamic range</li></ul>

**Table 3:** Performance table PTA/DUM

### 5.1.3 Tunable Resistive Pulse Sensing (TRPS)<sup>†</sup> / Electrical sensing zone (ESZ) / nano Coulter counter

#### 5.1.3.1 Measuring principle

A Coulter counter is an apparatus for counting and sizing particles suspended in electrolytes (Coulter 1949); the measuring technique is also called electrical sensing zone. It is used for cells, bacteria, prokaryotic cells and virus particles and more recently for fine particles. A typical Coulter counter has one or more micro-channels that separate two chambers containing electrolyte solutions. As fluid containing particles or cells is drawn through each micro channel, each particle causes a brief change to the electrical resistance of the liquid. The counter detects these changes in electrical resistance. The Coulter principle relies on the fact that particles moving in an electric field cause measurable disturbances in that field. The magnitudes of these disturbances are proportional to the volume of the particles in the field (Scarlett 1979, Hartfield et al. 1984, Lines 1992). First, the particles should be suspended in a conducting liquid. Second, the electrical field should be physically constricted so that the movement of particles in the field causes detectable changes in the current. Finally, the particles should be dilute enough so that only one at a time passes through the physical constriction, preventing an artefact known as coincidence.

#### 5.1.3.2 Performance – general remarks

The Coulter counter needs calibration (usually with spherical polymer latex). This calibration holds true for any other non-conducting material with particles that do not deviate considerably from spherical shape. The calibration constants should be changed for non-spherical particles (even though the signal is still proportional to the particle volume). Conducting particles require a defined adjustment of the applied voltage. Porous particles or aggregates need appropriate models or calibration, which means that the morphology of such particles has to be known.

The electrical sensing zone technique principally allows the measurement of number weighted size distributions with high resolution. In addition, it can be used to measure the particle number concentration and their volume concentration (because the signal is volume proportional).

#### 5.1.3.3 Performance table

Main features	
Type of samples	Suspended particles
Type of sizing	single particle counting and sizing
Particle property measured	particle volume, equivalent sphere diameter
Type of quantity	particle number

<sup>†</sup> TRPS (tunable resistive pulse sensing) is the preferred designation of the manufacturer (*ison*) for their newly introduced instrument (*qNano*): particles dispersed in water with dissolved salt move through the single pore of an elastic separator (hence the 'tunable' detection interval) which separates two electrodes that detect the ion current. Whenever a single particle blocks the pore, the current reduces, and the duration and depth of this 'pulse' provide information on size. The sequential detection of blockade events constitutes a size distribution in number metrics without further conversion.

This detection principle is related, but not identical to the conventional ESZ (electrical sensing zone), and hence the designation 'Nano Coulter counter' for TRPS is not preferred.

<b>size range</b>	<ul style="list-style-type: none"> <li>• minimum size &gt;70 nm</li> <li>• maximum is in the range 1-10 µm</li> </ul>
<b>concentration range</b>	10 <sup>5</sup> - 10 <sup>12</sup> particles / mL
<b>information content</b>	Good as it gives a particle number based PSD based on direct counting. Particle number concentration can also be determined. Information on particle charge is also possible in some instruments.
<b>Main advantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• very sensitive,</li> <li>• true single particle counting</li> </ul>	
<b>Main disadvantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• cannot distinguish single particles from agglomerate/aggregates,</li> <li>• lower size limit (70 nm) does not allow access to the whole range necessary for the definition</li> <li>• Non-spherical particle shape or particle aggregates will introduce errors</li> <li>• Limited dynamic range because of clogging</li> </ul>	

**Table 4:** Performance table TRPS / ESZ / nano Coulter counter

## 5.1.4 Single particle ICP-MS (sp ICP-MS)

### 5.1.4.1 Measuring principle

Sp ICP-MS is based on the measurement of highly diluted nanoparticle dispersions by ICP-MS operated in time resolved mode for a pre-selected mass-to-charge ratio ( $m/z$ ) value (Degueldre et al. 2003, Pace H E et al. 2011, Laborda F et al. 2011, Pace H E et al. 2012, Olesik J W et al. 2012, Laborda F et al. 2013, Tuoriniemi J et al. 2014, Laborda F et al. 2014). Ideally, individual particles enter the ion source and are atomised and ionised in the plasma torch to produce a plume of element ions that is transferred to the mass spectrometric detector. The discrete measurement intervals (dwell times) of the MS are set to a value ( $\leq 10$  ms) that allows the registration of the signal of the ion plume from only one particle. A prerequisite to operate in the single particle mode is (besides the short dwell times) that the concentration of particles is small enough to avoid simultaneous ionisation of more than one particle or the generation of overlapping ion plumes per dwell time. When these requirements are met the signal intensity is proportional to the mass of the respective element in the particle. The diameter of spherical particles can then be calculated from the measured mass based on the known or assumed stoichiometry and density of the target analyte. The number concentration of the particles in the measured dispersion can be inferred from the number of signals, the infusion rate, nebulisation efficiency and the acquisition time.

### 5.1.4.2 Performance – general remarks

This CM has a number of unique features. It is a relatively robust technique and can be run on conventional ICP-MS instruments that are widely available in both commercial and official control laboratories. Sample preparation is simple (often only dilution) and the measurement time per sample very short (1 min.) which allows high throughput analysis. Furthermore, it is chemically specific and provides actual number based size distributions.

#### *Limits of application*

Current application limits include:

- The detection limits in terms of size are limited by (i) the sensitivity of the detector for the target element, and (ii) isobaric interferences/background for the target isotope. In general, sensitivity is better for heavier elements.
- The correct size determination is limited to spherical particles of known density.
- Current instruments mainly only allow mono-isotopic detection, i.e. different particles that carry the same target element cannot be distinguished (e.g. Ag NP from Ag/Au NP). New instruments are on the edge to allow bi-isotopic detection (at the cost of compromising correct quantification and thus size determination)
- Constituent particles in aggregates are not resolved, in agglomerates only by appropriate dispersion in the sample preparation step (not in the instrument).

### 5.1.4.3 Performance table

Main features	
Type of samples	Suspended particles
Type of sizing	Calculated from mass

<b>Particle property measured</b>	mass
<b>Type of quantity</b>	particle number concentration, mass concentration
<b>size range</b>	depending on target element, e.g.: <ul style="list-style-type: none"> <li>• Au 15 – 1000 nm,</li> <li>• Ag 20 - 1000 nm,</li> <li>• TiO<sub>2</sub> 50 – 1000 nm,</li> <li>• SiO<sub>2</sub> 200 – 1000 nm</li> </ul>
<b>concentration range</b>	depending on element, particle size, instrument (e.g. Ag 60 nm: 5 – 500 ng/L)
<b>information content</b>	good (chemical composition, particle size, particle number concentration, mass concentration)
<b>Main advantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• measures individual particles,</li> <li>• chemically specific,</li> <li>• rapid,</li> <li>• cost-efficient</li> </ul>	
<b>Main disadvantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• particle size limits do not go down to 1 nm,</li> <li>• accurate size determination limited to spherical particles,</li> <li>• does not resolve particles in aggregates and agglomerates (if not deagglomerated in sample preparation)</li> </ul>	
<b>Type of potential improvement within the NanoDefine project</b>	
<ul style="list-style-type: none"> <li>• Lower size limit</li> </ul>	

**Table 5:** Performance table sp ICP-MS

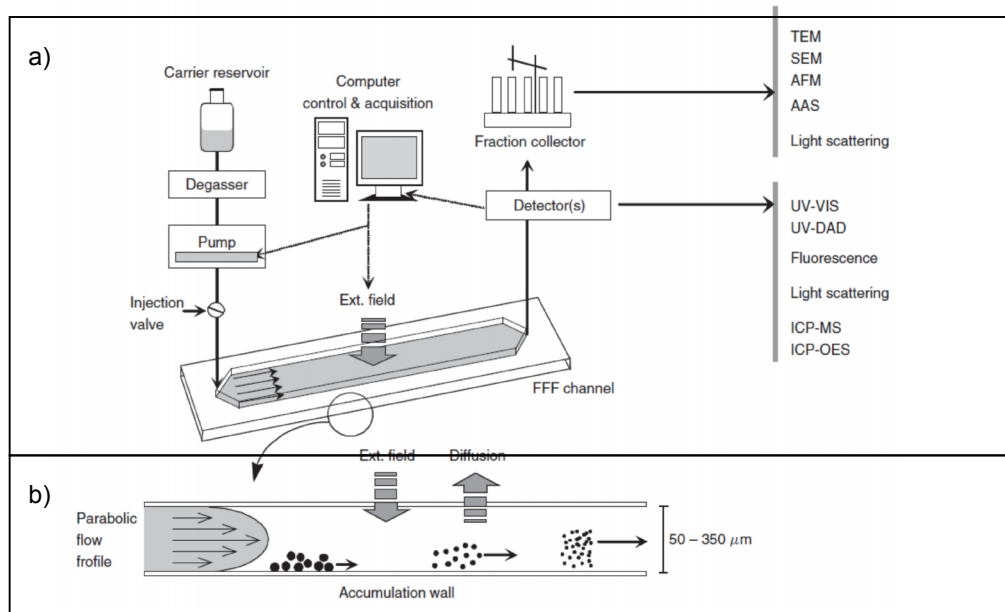
## 5.2 Fractionating methods

Fractionating (ensemble) methods include the two steps of fractionation and detection. The former can either result in a physical separation of the different size classes or in the depletion of coarse or fine particles in the measurement zone. In the case of colloidal suspensions, the fractionating effect is usually related to the mobility of the particles (e. g. settling velocity). The detection system monitors the fractionation process and, thus, serves for evaluating the class frequencies. It frequently employs the phase shift, extinction, or scattering of some radiation (e. g. X-rays). The applied detection system determines the type of quantity in which the size fractions are intrinsically weighted (e. g. extinction of X-rays is mass proportional –  $Q_3$ ).

### 5.2.1 Field-Flow-Fractionation (FFF)

#### 5.2.1.1 Measuring principle

FFF is a continuous analytical separation technique for macromolecules and colloidal suspensions. Until now several FFF techniques have been developed. They are based on various separation principles such as particle diffusion (e.g. asymmetric flow field-flow-fractionation, AF4) or buoyant mass (e.g. sedimentation field-flow-fractionation, SedFFF). In combination with suitable detection system FFF techniques enables us to derive particle size or molecular mass from sample specific properties, such as particle diffusion. The separation concept of all FFF techniques is similar. Particles are transported by laminar flow profile (flow field) through a channel. Separation is achieved by interactions of the particles with a concurrent second field force. The field force can be e.g. a perpendicular flow field (AF4) or centrifugal force (SedFFF). This interaction results in a heterogeneous distribution of the sample in the channel. For example during AF4 separation a forces drives the sample (i.e. dissolved and particulate components) towards the accumulation wall which is covered by ultrafiltration membrane which is permeable for components small than the cut-off of the membrane. Components which are retained in the channel will be distributed in the channel profile according to their diffusional properties. Depending on the diffusion properties (i.e. size) particle will experience different laminar flows resulting in a separation according to their hydrodynamic size. The separated sample is detected online by a suitable detector.



**Figure 2:** a) schematic diagram of a FFF-System including commonly used detection systems; b) cross-section of the channel illustrating the separation mechanism (adapted from v. d. Kammer 2005)

### 5.2.1.2 Performance – general remarks

FFF separation methods provide a robust technique to separate particles according to their size or molecular mass. Together with a subsequent detection technique FFF can provide distributions of both physical and chemical properties.

#### *Limits of application*

Particle size determined by FFF is always an equivalent spherical particle diameter. Thus values obtained for non-spherical particles from different sub-techniques will differ from each other.

In case of ideal conditions in AF4 only diffusional properties of the sample affect the separation. However, in practice completely ideal conditions cannot be achieved. Therefore particle sizing might be affected by a number of potentially interfering instrumental factors. It has to be emphasized that optimization of the run conditions for both size standard and sample have to be performed rigorously until close to ideal elution behaviour is achieved.

For SedFFF no size calibration is required. For size determination the buoyant mass of the particle sample has to know.

### 5.2.1.3 Performance table

Main features	
Type of samples	Suspended particles
Type of sizing	derived from calibration of the elution time with known size standards or by application of FFF theory or by coupled particle sizer.
Particle property measured	FlowFFF: particle diffusion coefficient (derived hydrodynamic diameter)
Type of quantity	detector dependent:



	<p>UV/VIS: particle mass concentration if particles absorb light in the operation range of the detector,</p> <p>fluorescence: particle mass concentration if particle is fluorescing,</p> <p>MALLS → intensity of scattered light;</p> <p>ICPMS → element mass concentration (element must be constituent of the particle and stoichiometry known);</p> <p>if counting detectors are used, particle number concentrations can be determined (e.g. sp ICP-MS)</p>
<b>size range</b>	in general 1-1000 nm. Instrumental settings need to be tuned for the size range of interest, dynamic range is typically 20 – 40-fold the smaller diameter
<b>concentration range</b>	adjustable, case-specific, the minimum and maximum acceptable concentration depends strongly on the sample characteristics, the size range and applied detector
<b>information content</b>	<ul style="list-style-type: none"> <li>• for AF4: diffusion coefficient, hydrodynamic radius;</li> <li>• for Sedimentation FFF: volumetric radius</li> <li>• further information depending on coupled detector e.g.             <ul style="list-style-type: none"> <li>• light scattering (SLS): rms-radius and geometric radius, intensity weighted size distribution</li> <li>• ICP-MS: chemical composition, mass concentration, mass based size distribution)</li> <li>• UV/DAD: indicator for chemical composition (element specific wave length absorption and surface plasmon resonance)</li> </ul> </li> </ul>
<b>Main advantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• Physical separation of particles: can operate on complex mixtures and matrices</li> <li>• Element specific: can operate on complex mixtures and matrices</li> <li>• The technique provides mass-based size distributions even for particles composed of multiple elements.</li> <li>• Number based size distributions can be either determined by mathematical transformation of the mass signal or in the course of the project potentially by AF4/SedFFF coupling to single particle counting techniques</li> </ul>	
<b>Main disadvantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• Number-based size distribution cannot directly be measured.</li> <li>• It does not resolve particles in aggregates and agglomerates.</li> <li>• Limited dynamic range (different elution regimes for different particle sizes)</li> </ul>	
<b>Type of potential improvement within the NanoDefine project</b>	
<ul style="list-style-type: none"> <li>• Sample-membrane interaction</li> </ul>	

**Table 6:** Performance table FFF

## 5.2.2 Analytical centrifugation<sup>‡</sup> (AC)

### 5.2.2.1 Measuring principle

Analytical centrifugation operates on the principle of separating particles by size using centrifugal sedimentation in a liquid medium. This leads to variations in the local particle concentration when measured along the settling path and/or over time. Settling distance and settling time correspond to the terminal settling velocity, which in the case of isolated particles solely depends on their individual size, shape, and density. Hence, concentration profiles or time curves reflect the size distribution of the particle system.

This basic concept can be varied with respect to the particle detection (i.e. concentration measurement) or the mode of operation. Nowadays, two fundamental types of centrifuges are distinguished: disc centrifuges and cuvette centrifuges:

Disc centrifuges consist of a hollow disc which contains the suspension medium. Upon rotation, the liquid forms a stagnant layer on which a thin layer of the particle system is injected (line-start technique). The particles migrate according to their settling velocity to the bottom of the disc. All particles of a certain size (or settling velocity) move in a narrow band with growing distance from the initial position. In the case of multidisperse particle systems, one can observe several of such bands in analogy to chromatographic techniques. The radial concentration profile is, hence, a distorted projection of the density function of the size distribution ( $q(x_{Stokes})$ ). The line start technique requires a density gradient in the suspension medium (e. g. by sugar) before the particles are injected; otherwise there was a convective transport of particles within strands of the (heavy) suspension layer. The density gradient sets some practical limits to the measurement (e. g. duration) and has to be calibrated before conducting size measurements.

A different set-up and even mode of operation is found in cuvette centrifuges, where the particle sedimentation is observed in small cuvettes that are fixed on a rotating table. In this case, the particles are homogeneously suspended in the continuous phase before the centrifugation starts (homogeneous technique). During the centrifugation, all particles migrate towards the bottom of the cuvette, which results in the formation of a sediment, in a steady decline of local particle concentrations above the sediment, and in a monotone decrease of particle concentration in the direction from the sediment to the meniscus. The two types of variation in particle concentration, the temporal evolution, and the radial profile, can be considered as distorted projections of the cumulative function of the particle size distribution ( $Q(x_{Stokes})$ ). The cuvette centrifuge was introduced by Svedberg and co-workers (Svedberg & Nichols 1923, Svedberg & Rinde 1924). They called the instrument "ultra-centrifuge". Nowadays the term "Analytical Ultracentrifugation" (AUC) is only used for centrifugal accelerations above 100,000xg.

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<sup>‡</sup> general term = Centrifugal Liquid Sedimentation methods (ISO 13318:2001) - available for different centrifuge types and different detection systems photo and X-ray;

According to ISO 13318-2:2007 "photocentrifuge method" including disc and cuvette centrifuges (the description partly covers the AUC, but it is intended to normal cuvette type centrifuges);

"Centrifugal Liquid Sedimentation (CLS)" is not known in ISO 13318, moreover: CLS is sometimes/frequently employed for both, cuvette and disc centrifuges.

Apart from the centrifuge type instruments differ with regard to the measurement of particle concentration. The most important principles are

- optical extinction (photocentrifuge), which yields volume weighted size distributions ( $q_3$ ) for light-absorbing nanoparticles and intensity weighted size distribution for non-absorbing nanoparticles ( $q_6$ );
- X-ray absorption (X-Ray centrifuge), which always yields volume weighted size distributions ( $q_3$ );
- refractive index determination by interferometry, which (approximately) yields volume weighted size distributions for nanoparticles.

Last not least it should be mentioned that the AUC usually employs a set of different detection systems.

### 5.2.2.2 Performance table

<b>Main features</b>	
<b>Type of samples</b>	Suspended particles
<b>Type of sizing</b>	fractionating ensemble technique
<b>Particle property measured</b>	<ul style="list-style-type: none"> <li>• Stokes diameter determined from settling velocity and mass density, or</li> <li>• hydrodynamic diameter from settling velocity and the effective particle density (used for compact aggregates)</li> </ul>
<b>Type of quantity</b>	<ul style="list-style-type: none"> <li>• solid volume by X-ray, refractive index or turbidity measurement of light-absorbing and -scattering nano-particles (<math>q_3</math>)</li> <li>• squared solid volume by turbidity measurement non-absorbing nano-particles (<math>q_6</math>)</li> <li>• for non-nanoparticles the physical quantity should be stated (e.g. extinction)</li> </ul>
<b>size range</b>	< 5 nm - 10 $\mu$ m with minimum depending on particle density
<b>concentration range</b>	$\leq$ 0.1 wt.%
<b>information content</b>	rel. high for monodispersed and polydispersed non-aggregated materials
<b>Main advantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• rapid screening</li> <li>• high resolution in size</li> <li>• and applicable to polydisperse materials</li> </ul>	
<b>Main disadvantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• cannot distinguish single particles from agglomer-ate/aggregates,</li> <li>• Accuracy of size measurement is influenced by any uncertainty in the assumed values of materials density,</li> <li>• Non-spherical particle geometry will introduce deviations to other equivalent diameters (e.g. to <math>x_V</math>)</li> <li>• Limited dynamic range - needs a balance between sedimentation rate (depending on particle size), density of gradient and centrifugal speed.</li> </ul>	
<b>Type of potential improvement within the NanoDefine project</b>	
<ul style="list-style-type: none"> <li>• Calibration</li> <li>• Densitometry</li> </ul>	

**Table 7:** Performance table AC

## 5.2.3 Differential electrical mobility analysis (DMAS)

### 5.2.3.1 Measuring principle

There are different terminologies for aerosol particle size spectrometers based on electrical mobility: MPPS for mobility particle size spectrometer, SMPS for scanning mobility particle sizer, DMPS for differential mobility particle sizer and DMAS for differential mobility analyzing system.

DMAS combines a particle classifier (Differential Mobility Analyser DMA or DEMC for differential electrical mobility classifier) that transmits particles within a narrow interval of sizes from an initially polydisperse aerosol, and a detector (for example, a Condensation Particle Counter CPC) that counts the particles within that differential size interval. First, the aerosol passes through an inertial impactor (to remove the largest particles  $> 1\mu\text{m}$ ) to avoid the largest particles to enter the DMA column, then the aerosol enters a particle charge conditioner like a charge neutraliser to be conditioned, so, particles that carry several charges lose their charge excess. Once the aerosol is well conditioned particles are selected using electrical classification inside DMA column: an electric field is created and the airborne particles drift along the DMA according to their electrical mobility  $Z_d$ . It is related to the particle diameter  $d_p$  via the expression:

$$Z_d(p, d_p) = \frac{peC_c(d_p)}{3\pi\mu_g d_p},$$

where  $e$  is the charge of the electron,  $\mu_g$  the dynamic gas viscosity and  $C_c$  the slip correction factor de-fined as:

$$C_c(d_p) = 1 + K_n(d_p) \left[ \alpha + \beta \exp\left(\frac{\gamma}{K_n(d_p)}\right) \right],$$

$$K_n(d_p) = \frac{2\lambda_m}{d_p},$$

where  $K_n$  is the Knudsen number and  $\lambda_m$  is the mean free path of a particle. ( $\alpha$ ;  $\beta$ ;  $\gamma$ ) are taken from experiments.

Figure 1 presents the fundamental components of DMAS (ISO 15900: 2009). The preconditioner indicated in this figure serves generally two goals: removing the large particles with impactor the most used and, if necessary, reducing the sample humidity using a dryer. Concerning the aerosol detector, there are two type of detector: a CPC and an aerosol electrometer. Concerning the particle charge conditioner, a bipolar diffusion particle charger (also called an aerosol neutralizer) is often used in SMPS. This is often done using a radioactive source like  $^{85}\text{Kr}$  or a bipolar ion generator. These chargers establish the equilibrium charge distribution on the aerosols particles. Unipolar Corona chargers may also be used in a DMAS. The DMAS is operated by software controlling the sheath air flow, reading the aerosol flow, reading other system parameters such as  $T$ ,  $p$ , setting the voltage, and reading the CPC output.

$$K_n(d_p) = \frac{2\lambda_m}{d_p},$$

where  $K_n$  is the Knudsen number and  $\lambda_m$  is the mean free path of a particle. ( $\alpha$ ;  $\beta$ ;  $\gamma$ )

are taken from experiments.

Figure 3 presents the fundamental components of DMAS (ISO 15900: 2009). The pre-conditioner indicated in this figure serves generally two goals: removing the large particles with impactor the most used and, if necessary, reducing the sample humidity using a dryer. Concerning the aerosol detector, there are two type of detector: a CPC and an aerosol electrometer. Concerning the particle charge conditioner, a bipolar diffusion particle charger (also called an aerosol neutralizer) is often used in SMPS. This is often done using a radioactive source like 85Kr or a bipolar ion generator. These chargers establish the equilibrium charge distribution on the aerosols particles. Unipolar Corona chargers may also be used in a DMAS. The DMAS is operated by software controlling the sheath air flow, reading the aerosol flow, reading other system parameters such as T, p, setting the voltage, and reading the CPC output.

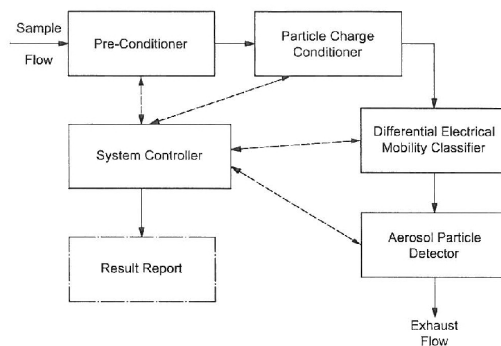


Figure 3: Fundamental components of the differential mobility analysing system (DMAS) (ISO 15900: 2009)

### 5.2.3.2 Performance - general remarks

#### *Limits of application*

- Stability of the aerosol:

The system (DMA + CPC) can only be used when the aerosol is stable during the time of scan. If the aerosol (number size distribution) is unstable below the specified scan time, other systems must be used (DMA + Electrometers detectors like commercial instrument DMS 500, FMPS, EEPS). Nevertheless it has to be noted that the size resolution for such system is lower compared to the coupled system DMA and CPC.

- Strongly dependent on the physical model used to retrieve the size distribution (charge distribution function, transfer function, etc)
- When using the system (DMA + CPC), the estimation of the size distribution strongly depends on the type of inversion being implemented in the commercial software.
- System optimized for particles with spherical shape.

#### *Data analysis, resolution of particle size distribution etc*

The measured electrical mobility distribution is converted to a particle number size distribution employing the charge distribution (see also ISO 15900) and the DMA-transfer probability. Additional corrections could be done for internal particle losses due to diffusion and the size-dependent CPC counting efficiency.

The performances of four SMPS were evaluated by Fissan et al. (1996) under the same conditions for flow rates, flow ratio, input monodisperse aerosols, and transport-line

lengths in the 6–50 nm size range. Their results provide a quantitative comparison of the mobility resolution and diffusion loss of the nanometer aerosols in such systems. Moreover, the performance assessment of Fast MPS (FMPS) and Ultrafine Water-based Condensation Particle Counter (UWCPC) equipped SMPS was performed by Jeong and Evans (2009) under various conditions on urban ambient particles, urban indoor particles, rural ambient particles, and laboratory-generated particles. Asbach et al. (2009) tested four different mobility particle sizers on NaCl and diesel soot particles measurements. Recently the paper of Wiedensohler et al. (2012) talks about harmonization of measurement procedures to facilitate high quality long-term observations of atmospheric particle size number distributions obtained by SMPS. Some results of metrological measurement of NP size and size distribution by SMPS have been carried out recently in the frame of various interlaboratory comparison specially dedicated to this purpose (Motzkus et al., 2013). Electro-spray-differential mobility analysis (ES-DMA), a technique that exerts electrical and drag forces on clusters, can be used to determine the size and packing of colloidal small clusters (and aggregates more generally) of nanoparticles (Pease et al., 2010).

### 5.2.3.3 Performance table

<b>Main features</b>	
<b>Type of samples</b>	airborne submicrometer particles
<b>Type of sizing</b>	Fractionation ensemble technique
<b>Particle property measured</b>	number size distribution (or Count size distribution)
<b>Type of quantity</b>	an equivalent diameter called electrical mobility diameter
<b>size range</b>	2.5 nm to 1 µm (Range varies in dependence on instrument type like DMA and CPC and the parameter used (flow rate,...))
<b>concentration range</b>	1 to 10 <sup>8</sup> particles/cm <sup>3</sup> (Maximum concentration of CPC is 10 <sup>7</sup> part/cm <sup>3</sup> )
<b>information content</b>	2 modes of measurement: <ul style="list-style-type: none"> <li>• Scanning mode: SMPS (scanning mobility particle sizer); often described for a continuous voltage scan. Up scan: 20 to 300 seconds</li> <li>• Stepping mode: DMPS (differential mobility particle sizer); often described for a stepwise voltage scan</li> </ul>
<b>Main advantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• well-resolved number size distributions</li> <li>• adapted to measure airborne submicrometer particles</li> <li>• quick measurement (a few min)</li> <li>• applicable to polydisperse population between 3 nm to 1 µm</li> <li>• non-destructive method</li> <li>• Physical separation of particles with the electrical mobility</li> </ul>	
<b>Main disadvantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• Non-spherical particle shape or particle aggregates will introduce errors</li> <li>• Primary particles in aggregates/agglomerates cannot be resolved</li> </ul>	

**Table 8:** Performance table DMAS

#### **5.2.4 Size exclusion chromatography (SEC)**

Any chromatographic technique is based on the flow through a porous medium – the stationary phase. The flow in the pores is very slow and the transport of solutes and particles is mainly diffusive. Adsorption/desorption, hydrodynamic or steric effects specifically influence the residence time of the different species and, thus, facilitate their separation.

Size-exclusion chromatography (SEC) is based on the flow through a porous medium – the stationary phase – with very broad pore size distributions. Since the particles can only move into those pores that exceed their geometric dimensions, the penetrable pore volume decreases with increasing particle size. Coarse particles, therefore, pass the column more quickly than fine ones (Fedotov et al. 2011). SEC was originally developed for the separation of polymer solution, yet could be successfully applied to the characterisation of solid nanoparticles, in particular to gold nanoparticles (Wei et al. 1999, Liu 2009). Unlike FFF, the classification is related to geometric and not hydrodynamic particles properties, whereas the quantification can be conducted with the same methods.

Ideally, the particles should not interact with the stationary phase in SEC. In reality, however, it is not possible to avoid material-specific interaction. For that reason, it is highly recommended to supplement the classical SEC set-up with a particle sizing technique (e. g. DLS, Yamaguchi et al. 2006). Currently, SEC cannot be considered a mature technique for nanoparticle characterisation.

### 5.3 Ensemble methods

The immediate result of an ensemble method is the variation of the measured signal  $g$  over the parameter  $s$  (time, space or frequency). Each size fraction  $x$  possesses a characteristic part  $kr(s,x)$ , which in general covers the whole range. Assuming that each size fraction contributes independently and linearly to the measured signal, the determination of the size distribution requires the inversion of a linear integral equation (Fredholm type). The intrinsic type of quantity is not necessarily obvious; it refers to the impact of a single particle to the integrated signal. The probed particle property of an ensemble method frequently relates to the particle mobility (diffusion) or to its interaction with external fields (scattering, extinction).

#### 5.3.1 Dynamic light scattering (DLS)

##### 5.3.1.1 Measuring principle

Dynamic light scattering (DLS) CMs evaluate the fluctuations in scattered light (Figure 4). These fluctuations may be caused by any changes in the microstructure of the suspension, e. g. by particle motion or vibrations of particle networks. For this reason, there are manifold applications for DLS, e. g. particle sizing, molecular weight determination, studying particle aggregation, monitoring phase transition in colloidal suspensions, or measuring the strength of colloidal gels.

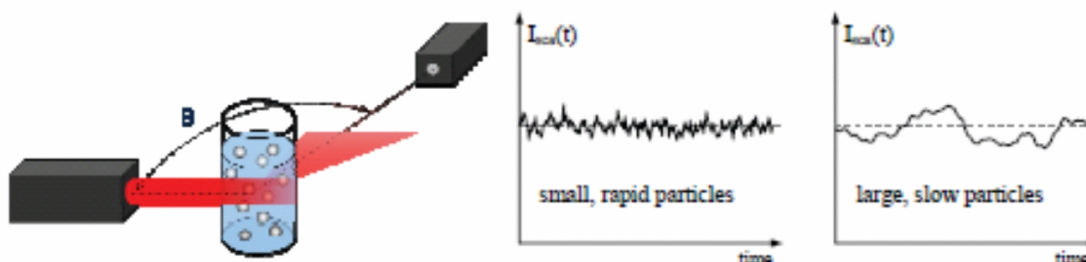


Figure 4: Measuring principle of DLS

The intensity fluctuations of DLS experiments can be analysed in terms of (ISO 22412, Xu 2000):

- a frequency spectrum (frequency analysis – FA), or
- a time correlation function (photon correlation spectroscopy – PCS).

PCS requires a different hardware than FA, but it can be shown that the results of both techniques are equivalent (Jakeman 1970; Xu 2000, pp. 86-89). Today, a large variety of commercial or “self-made” DLS instruments are used. Apart from data processing (FA or PCS), they can be distinguished with regard to laser optics and signal modulation.

In quiescent, dilute suspensions, the light fluctuations result essentially from the Brownian displacement of the single particles and thus reflect the particles’ translational diffusion coefficient  $D_t$ . For spherical particles, this parameter ( $D_t$ ) is inversely proportional to the sphere diameter (Stokes-Einstein equation).



### 5.3.1.2 Performance – general remarks

#### *Limits of application*

Limits for the applicability of DLS are mainly set by size and concentration of particles. First of all, the concentration should be low enough to avoid strong multiple scattering. For particle sizing the concentration should be even low enough to ensure measurement of short time self-diffusion of particles. As a rule of thumb, this may be achieved for concentrations below 0.01...0.1 vol.-%, but lower concentration values may be necessary for very fine nanoparticles.

Apart from upper concentration limits, there is a further one at the lower edge, where the intensity fluctuations start to become affected by the statistic variation of particle number in the measurement zone. According to Willemse et al. (1997), a minimum of 100 particles should stay in the measurement zone. This is fulfilled for most colloidal suspensions; problems may arise for micrometre particles (i.e.  $\geq 1 \mu\text{m}$ ).

Sedimentation sets a limit to the detection of coarse particles because the particle displacement of micrometre particles is governed by sedimentation rather than by diffusion. This is of particular importance for polydisperse particle systems, where diffusion and sedimentation are coupled (Batchelor 1982, Batchelor & Wen 1982). Moreover, sedimentation may affect the size distribution in the measurement zone. However, in the colloidal size range ( $x \leq 1 \mu\text{m}$ ) there is virtually no impact of sedimentation on DLS results (e. g. Paul & Pusey 1981). A lower size limit exists only as much as the scattering intensity of the particles should considerably exceed that of the fluid molecules. Xu (2000, p. 241) proposes a minimum factor of 2.5; yet, for highly reliable DLS this value should be multiplied by 10.

#### *Accuracy etc.*

During the last two decades, dynamic light scattering has evolved into a major characterisation technique for colloidal suspensions. A recent interlaboratory study into the characterisation of a monomodal colloidal silica (Braun et al. 2011) showed that state-of-the-art DLS instrumentation facilitate a highly reproducible and very reliable acquisition of correlation function and corresponding mean particle size  $x_{cum}$ . The study involved 17 participants from EU and USA, which provided 19 independent data sets from 6 different commercial instruments covering sideward scattering ( $90^\circ$ ) and backscattering. An earlier study with a different test material already indicated the high interlaboratory comparability (Lamberty et al. 2011).

#### *Data analysis, resolution of particle size distribution etc.*

The reliable, meaningful, and robust inversion of correlation functions into PSDs has been tackled by several authors (e. g. Stock et al., 1985, Finsy et al. 1989). Most often, regularisation approaches (Provencher 1982, Maier et al. 1999) with a non-negative constraint on the weights  $dQ_{int}$  (Lawson & Hanson 1995) are employed.

### 5.3.1.3 Performance table

<b>Main features</b>	
<b>Type of samples</b>	Suspended particles
<b>Type of sizing</b>	spectroscopic ensemble technique
<b>Particle property measured</b>	(apparent = translation affected by rotation) hydrodynamic diameter
<b>Type of quantity</b>	intensity of scattered light; for NPs: $I_{sca} \propto \chi^6$
<b>size range</b>	1 nm ... 1 $\mu$ m
<b>concentration range</b>	$\leq$ 1 vol.-% (depends on the material)
<b>information content</b>	relatively low, i.e. weak ability to accurately resolve PSD details
<b>Main advantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• when DLS finds NPs then there are NPs,</li> <li>• rapid screening</li> </ul>	
<b>Main disadvantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• in polydisperse or multi-component particle systems DLS will severely underestimate the amount of weakly scattering NPs</li> </ul>	

**Table 9:** Performance table DLS

### 5.3.2 Small-angle X-ray scattering (SAXS)

#### 5.3.2.1 Measuring principle

SAXS is an analytical technique where the elastic scattering of X-rays by inhomogeneities in the range of 1 to 100 nm is recorded at low scattering angles (typically  $\theta = 0.1 - 10^\circ$ , see figure below). The resulting scattering patterns display the scattering intensities  $I(q)$  as a function of the scattering vector  $q$ , which is given by the scattering angles and the X-ray wave length  $\lambda$  as  $q = 4\pi/\lambda \sin \theta$ . The shape of the scattering pattern is highly sensitive to size and shape of nanostructures. Therefore, two main applications of SAXS are characterization of nanoparticles and determination of large surface areas. The SAXS theory is complete and based on fundamental physical laws (Glatter and Kratky, 1982). A study of six European metrology institutes proved recently that SAXS allows traceable size determination of monomodal, spherical nanoparticles (Meli et al., 2012).

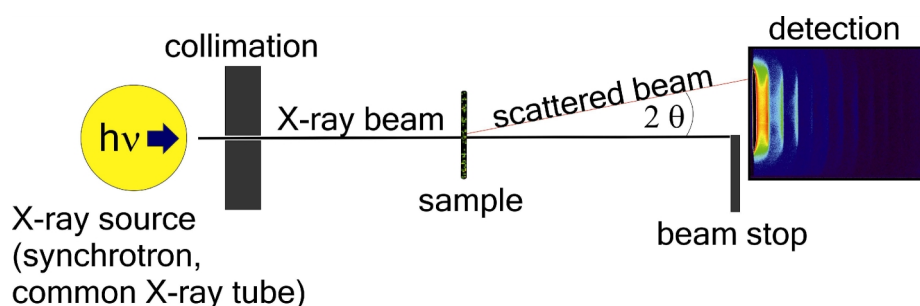


Figure 5: Measuring principle of SAXS

#### 5.3.2.2 Performance – general remarks

The method is accurate, non-destructive and requires a minimum of sample preparation. SAXS covers the whole range of interest from 1 to 100 nm. The experimental devices at synchrotrons and common laboratories are well developed. Numerous technical improvements have been made during the last five years. High throughput instruments were realized by using sample changing robots (Round, 2008) and new X-ray detectors for low noise data recording are available (Pauw, 2013) for synchrotrons and normal SAXS laboratories. Currently numerous SAXS manufacturers in Europe released improved SAXS instruments. These instruments are suitable for routine and standardized measurements in accordance with ISO standards.

### 5.3.2.3 Performance table

<b>Main features</b>	
<b>Type of samples</b>	nano-particles suspended in liquids, nanoparticles in viscous and solid matrices, nanoparticles in powders etc.
<b>Type of sizing</b>	scattering ensemble averaging technique
<b>Particle property measured</b>	density of particles must differ from that of their surroundings (e.g. water)
<b>Type of quantity</b>	size (metrological traceable), shape and concentration
<b>size range</b>	1 to 100 nm (upper limit depends on specific instrument)
<b>concentration range</b>	10 <sup>-4</sup> to 100 vol.-% (lower limit depends strongly on size and density of particles, as well as on the quality of the instrument)
<b>information content</b>	Size, shape, size distribution (intensity, volume and number-weighted)
<b>Main advantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• Particles can be detected with minimal sample preparation. No or little danger exists for sample preparation artifacts.</li> </ul>	
<b>Main disadvantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• Larger Particles produce higher signals than smaller particles. Therefore, the determination of number-weighted size distributions makes a detailed analysis of the measuring uncertainties necessary. Procedures in determination of complete error budgets in SAXS data analysis is not yet established in the SAXS data evaluation.</li> <li>• Upper size limit.</li> </ul>	

**Table 10:** Performance table SAXS

### 5.3.3 Ultrasonic spectroscopy (USSp)

#### 5.3.3.1 Measuring principle

Ultrasonic spectroscopy is the generic term for all particle sizing techniques that are based on the frequency dependent measurement of sound velocity or attenuation in the ultrasonic domain (mostly with-in 100 kHz to 200 MHz). While velocity spectroscopy is mainly used for the study of inter- and intramolecular processes, attenuation spectroscopy has found its major application in particle sizing (McClements 1996, Kachanovskaya et al. 1996). The most promising feature of acoustic characterisation techniques is their applicability to highly concentrated particle systems (up to 70 vol.-%) under non-equilibrium conditions (McClements 1991). That means it offers the opportunity to monitor the state of dispersion of dense product streams, to control the deagglomeration of suspensions or the homogenisation of emulsions, and to study polymerisation or crystallisation processes

#### 5.3.3.2 Performance – general remarks

##### *Limits of application*

In principle, ultrasonic spectroscopy can be used for the characterisation of particles in the colloidal and micrometre size range, provided that the particle concentration is sufficiently high (at least 1 vol.-%) and that the signal contribution by the particles is significant compared to those by the continuous phase (may be a problem for attenuation measurements in highly viscous solvents).

A major difficulty for the particle sizing by USSp is that the acoustic behaviour depends on a variety of material parameters. This is of particular relevance for emulsions (14 properties), whereas for aqueous suspensions only the viscosity and sound speed of liquid and the density contrast have to be known (Babick et al. 2000).

##### *Non-spherical particles*

In colloidal suspensions, the sound propagation is typically governed by the acoustophoretic motion of particles. For monodisperse spheroids that do not deviate too much from spherical shape (aspect ratio < 10/1), the attenuation spectrum essentially reflects the volume specific surface area of the particles (Babick & Richter 2006). Similar results would probably be obtained for any convex particle shape. For particle aggregates, the inner structure is decisive. Regarding the type of quantity, acoustically measured size distributions are ideally volume weighted distributions.

##### *Accuracy and resolution of particle size distribution etc.*

It could be shown that the results of ultrasonic spectroscopy agree fairly well with those of other characterisation methods and are hardly affected by the extent of sample dilution (Dukhin & Goetz 1996, Knösche et al. 1997, Babick et al. 1998). Interlaboratory comparisons of ultrasonic spectroscopy measurements on suspensions of inorganic particles also showed good agreement (Steinborn et al. 2010, Dukhin et al. 2012). That is why ultrasonic spectroscopy is considered as a powerful tool for monitoring colloidal processes. However, the method does not allow for a very sharp resolution of size distributions in the colloidal size range (Babick & Ripperger 2002).

### 5.3.3.3 Performance table

<b>Main features</b>	
<b>Type of samples</b>	Suspended particles
<b>Type of sizing</b>	spectroscopic ensemble technique
<b>Particle property measured</b>	acoustophoretic diameter
<b>Type of quantity</b>	particle volume
<b>size range</b>	10 nm - 100 µm
<b>concentration range</b>	> 1 vol.-%
<b>information content</b>	<ul style="list-style-type: none"> <li>• relatively low for <math>x \leq 1 \mu\text{m}</math>,</li> <li>• relatively high for <math>x \geq 10 \mu\text{m}</math></li> </ul>
<b>Main advantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• for aggregates of NPs USSP detects the internal aggregate structure rather than the outer proportions,</li> <li>• does not require dilution for dense suspensions (which may affect the state of dispersion)</li> </ul>	
<b>Main disadvantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• does not yield particle number-weighted distributions</li> <li>• does not allow the characterisation of dilute suspensions, i.e. requires a lot of substance</li> </ul>	

**Table 11:** Performance table USSp

### 5.3.4 X-ray diffraction (XRD)

#### 5.3.4.1 Measuring principle

X-ray diffraction (XRD) is usually applied to study crystalline materials. Their three-dimensional structure is defined by regular, repeating planes of atoms that form a crystal lattice. When a focused X-ray beam interacts with these planes of atoms, a part of the incoming beam is diffracted. The distances between the planes of the atoms that constitute the sample can be calculated applying Bragg's Law:

$$n\lambda = 2d \sin\theta,$$

where the integer  $n$  is the order of the diffracted beam,  $\lambda$  is the wavelength of the incident X-ray beam,  $d$  is the distance between adjacent planes of atoms, and  $\theta$  is the angle of incidence of the X-ray beam. The characteristic set of  $d$ -spacings and their intensity generated in a typical X-ray pattern provides a unique 'fingerprint' of the crystalline phases present in the sample.

#### 5.3.4.2 Performance – general remarks

XRD methods for crystallite size determination are applicable to crystallites in the range of 2-100 nm. The reflections are very broad for crystallites below 2-3 nm, while for crystallites with size above 100 nm the peak broadening is too small. Given that the analysed crystals are free from microstrains and defects, the peak broadening depends only on the crystallite size and diffractometer characteristics. The classical Scherrer equation is typically used for crystallite size determination:

$$d = (K \times \lambda) / (b \times \cos\theta),$$

where  $d$  is the crystallite size,  $\lambda$  is the X-ray wavelength,  $b$  is the width of the peak (full width at half maximum (FWHM)) after correcting for instrumental peak broadening ( $b$  expressed in radians),  $\theta$  is the Bragg angle and  $K$  is the Scherrer constant. The Scherrer equation provides volume-weighted mean column length (often regarded as the apparent size of the crystallites). The  $d$  value calculated for (hkl) peak should be understood as mean crystallite size in the direction that is perpendicular to the (hkl) plane.

### 5.3.4.3 Performance table

<b>Main features</b>	
<b>Type of samples</b>	powder, dense suspensions
<b>Type of sizing</b>	scattering ensemble technique
<b>Particle property measured</b>	crystalline size
<b>Type of quantity</b>	size
<b>size range</b>	2 nm - 100 nm
<b>concentration range</b>	> 2 wt.-%
<b>information content</b>	relatively low, limited on the crystalline size
<b>Main advantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• non-destructive method,</li> <li>• information on the phases of the crystalline phases in the sample and their crystalline size</li> <li>• does not require dilution for dense suspensions (which may affect the state of dispersion)</li> </ul>	
<b>Main disadvantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• does not allow the characterisation of dilute suspensions, i.e. requires a lot of substance</li> <li>• use of Scherrer equation does not provide information on the size distribution</li> </ul>	
<b>Type of potential improvement within the NanoDefine project</b>	
<ul style="list-style-type: none"> <li>• Standardisation of spectrum analysis methods</li> </ul>	

**Table 12:** Performance table XRD



### 5.3.5 Angular light scattering (ALS) – static light scattering (SLS) and laser diffraction (LD)

Angular light scattering (ALS) techniques measure the spatial distribution of scattered light. Historically, this has been realised by two different concepts of instrumentation which cover distinct size ranges. These are the static light scattering (SLS), which is conventionally employed for fine colloids, and the laser diffraction (LD), which was originally used for micrometre particles only. Even though the measurement ranges of both techniques have actually converged in the recent past, there still remain qualitative differences in the sensor set-up and in data analysis, which justify their separate treatment.

SLS is operated for a wide range of scattering angles (typically of 10° to 150°). The time averaged angular distribution of scattered light is then commonly employed for the characterisation of macromolecules (molecular weight, radius of gyration, the second virial coefficients), but can be used to study suspensions of inorganic colloids as well (e. g. Heimer & Težak 2002). However, the angular distribution of scattered light is insensitive to particle size for nanoparticles, in which case only an average particle size can be determined. For very fine nanoparticles (< 10 nm) the size information may be even completely lost. It could be shown that above this critical size, SLS measures the average particle size with fairly good reproducibility (Just & Werthmann 1999).

The term laser diffraction (LD) comprises angular light scattering techniques, which are primarily de-signed to resolve the scattering pattern at small scattering angles. Historically, LD instruments and software were restricted to the characterisation of micrometre objects for which the scattering pattern is mainly caused by diffraction and can be explained by Fraunhofer's theory (1821). In the micrometre range, which is diffraction dominated, size distributions can be determined with high accuracy and good resolution (Mori et al. 2007, Witt et al 2012). In order to extend the instrument applicability to colloidal particle systems, several modifications have been realised, e. g. variation of wavelength and polarisation or inclusion of wide angle scattering (Xu 2000, pp. 111-181; ISO13320). These modifications have evidently enhanced the sensitivity to colloidal particles far below 1 µm, but not in a uniform, reproducible way as interlaboratory comparisons prove (e. g. Kuchenbecker et al. 2012). Even so, laser diffraction may serve as a useful tool for the characterisation of colloidal suspensions, in particular for monitoring dispersion procedures or for evaluating the coarse particle content (≥1 µm). Hence, this CM plays a significant role in NanoDefine in the combination of various methods for different size ranges.

### **5.3.6 Optical spectroscopy (OS)**

Optical spectrometers deliver transmission or turbidity spectra, which commonly range from the ultra-violet (UV) to the near infrared (NIR) domain. They are standard analytical tools which are mainly used to identify solutes and to determine their concentration.

The optical spectra of colloidal particles have usually a smooth and monotone shape, from which only a few details of the size distribution can be deduced. Yet, for metals with a surface plasmon resonance in the optical domain (e. g. Ag or Au), one can observe a distinct, size dependent maximum in the turbidity spectra of nanoparticles (Njoki et al. 2007).

Although several studies have demonstrated that optical spectroscopy reliably measures the size and concentration of suspended nanoparticles, the method is only of minor relevance for particle sizing. Recent developments try to considerably increase the method's sensitivity, precision, and resolution by employing pulsed laser light with tuneable wavelength (Li et al. 2010).

### **5.3.7 X-ray fluorescence correlation spectroscopy (FCS)**

X-ray fluorescence correlation spectroscopy belongs to the group of dynamic scattering techniques, which basically probe the dynamics of colloidal systems (e. g. the particle diffusion). It has the advantage of providing element specificity and high sensitivity, which offers a way to study the motion of nanoparticles in complex media. However, the technique requires synchrotron radiation and is currently still in the stage of development (Wang et al. 1998, Leupold et al. 2007).

## 6 Integral methods

Additional to those methods that resolve the distribution of particle size there are a few methods which solely measure an integral (effective/mean) property of the particle systems such as the specific surface area ( $S_V$  or  $S_m$ ) or the turbidity of a suspension. These properties can be directly converted into mean values of PSD (e. g.  $S_V \rightarrow$  harmonic mean of the volume weighted PSD). Note that ensemble methods – in principle – also yield such integral properties (e. g. the mean decay of signal fluctuation in DLS which gives  $x_{cum}$ , i.e. the harmonic mean of the intensity weighted size distribution). The measurement of integral properties can be conducted with relatively high accuracy. Hence, it is widely used to detect changes in size distribution even though it does not provide any piece of information on the distribution width.

### 6.1.1 BET for determination of specific surface area

#### 6.1.1.1 Measuring principle

The Brunauer-Emmett-Teller (BET) theory was derived in 1938 to explain the physical adsorption of gas molecules on a solid surface (Brunauer et al., 1938). BET serves as the most often applied technique for the measurement of the specific surface of a material – typically porous. BET explains mono- and multilayer adsorption of gas molecules on a solid and dry material. Nitrogen and argon gas are widely used for measurements. BET is based on three hypotheses:

- 1.) gas molecules physically adsorb in infinite layers,
- 2.) no interactions exist between adsorbed layers, and
- 3.) the Langmuir theory is applicable for each layer of gas molecules.

The resulting BET equation is applied for fitting experimental gas adsorption isotherms and gives the adsorbed monolayer gas quantity. Knowledge of gas quantity, adsorption cross section of the adsorbing gas and the molar gas volume allows calculation of the specific surface area of the material (Dabrowski, 2001).

#### 6.1.1.2 Performance – general remarks

The BET method is widely used and accepted in Industry, academia and (governmental and regulatory) research institutes. For example, the National Institute of Standards and Technology (NIST, US) and BAM provide a practical guide for its application which is available without charge from NIST (Klobes et al., 2006). BET is also standardized by ISO (ISO 9277: 2010). BET can be applied easily. The BET theory is based on expansive assumptions (see above), and therefore, the results obtained by BET can be made traceable (Hackley, 2013). Also different values for the same material can be obtained if different gases are used. As a consequence the specific surface area values should be named BET surface area and must often be considered as apparent. Nevertheless, Round-Robin tests for the development of reference materials for BET as performed by BAM proved good accuracy of the BET method.

### 6.1.1.3 Performance table

<b>Main features</b>	
<b>Type of samples</b>	dry solid
<b>Type of sizing</b>	BET is not a sizing method; for non-porous nanoparticles of known density it is possible to calculate an average size
<b>Particle property measured</b>	surface area
<b>Type of quantity</b>	surface per volume ratio as integral value over all particles in a test sample
<b>size range</b>	all size ranges
<b>concentration range</b>	only 100 % (pure, dried material is needed, normally as powder)
<b>information content</b>	relatively low, requires dry samples
<b>Main advantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• Certified reference materials are available for a wide range of specific surfaces up to 1300 m<sup>2</sup>/g,</li> </ul>	
<b>Main disadvantages with regard to NanoDefine</b>	
<ul style="list-style-type: none"> <li>• Particles and non-particulate porous materials cannot be distinguished</li> <li>• Materials must be free of any volatile compounds, for example, water-free</li> <li>• Measurement times can be in the range of hours and increase with increasing surface area</li> </ul>	

**Table 13:** Performance table BET

## 7 Evaluation table

All the CMs described in the previous section are evaluated in an overview table. The criteria selected were jointly agreed by the NanoDefine consortium and are of various representative natures: direct relation to the EC recommendation for a definition of nanomaterial (red), analytical (yellow), economical (blue), method hyphenation and potential improvement (green).

Following scores were allowed to be assigned:

-- = very poor; - = poor; o = middle-rate; + = well; ++ = very well.

Brackets denote eventuality in case of e.g. advanced instrumentation. Where no score was conferred, an "N/A" is given.

The scores for every CM in every category were median averaged after removal of potential outliers and typing errors. When the median was between two subsequent scores, the mean value has been considered in the assumption that the "—" to "++" scores correspond to the numbers 1 to 5. For most CMs at least six to seven scores have been conferred by the NanoDefine experts.

		Size range						Direct counting CM	Convertibility to number weighted PSD / Quantitative accuracy	Access to the smallest dimension of each particle	Measurement of the material as it is	ISO standards available	Size Accuracy	Chemical selectivity	Access to primary particles?
		nm			µm										
		1-10	10-30	30-100	0.1-1	1-10	>10								
Counting	SEM	-	(+)	++	++	++	++	++		+	--	0	+	++	+
	EM TSEM	(+)	+	++	++	++	++	++		+	--	+	++	++	+
	TEM	++	++	++	++	++	+	++		+	--	+	++	++	++
	SFM	+	++	++	+	0	-	++		++	-	+	++	-	0
	PTA	--	0	+	++	-	--	+		--	-	+	+	0	--
	TRPS	--	--	0	+	+	--	++		--	-	++	++	-	--
	sp ICP-MS	-	0	+	++	-	--	++		--	-	0	+	++	-
Fractionating	FFF	+	++	++	++	0	--	-	-	-	-	-	+	+	-
	AC	0	+	++	++	+	-	-	0	--	-	++	++	-	--
	DMAS	+	++	++	++	--	--	+	+	-	-	++	+	-	-
	SEC	0	+	+	+	0	--	-	0	--	-	-	+	-	--
Ensemble	DLS	+	++	++	++	0	-	--	-	-	-	+	+	-	-
	SAXS	+	+	+	-	--	--	--	-	0	+	++	+	0	+
	USSp	-	+	++	+	0	0	--	-	-	-	++	+	-	--
	XRD	+	+	+	-	--	--	--	--	-	+	+	0	+	+
	ALS	-	0	+	++	++	++	--	-	-	-	++	+	-	-
	OS	+	+	+	0	-	--	--	--	-	-	-	+	+	--
	FCS	--	--	--	--	--	--	--	--	--	--	--	--	+	--
Integral	BET	+	+	+	+	+	0	--	--	-	0	++	-	-	0

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under Grant Agreement n° 604347

		Costs per sample	Analysis turn-around time	Widely used/ Availability	Hyphenation to other methods?	Potential for improvement in NanoDefine 2014-2017?	Recommended to WP4	Recommended to WP5	Recommended to WP6	
Counting	EM	SEM	-	-	++	-	+	-	++	+
		TSEM	-	-	+	-	+	-	++	++
		TEM	--	--	0	--	0	--	++	++
	SFM	-	--	+	-	0	-	+	+	
	PTA	+	+	0	-	+	+	-	+	
	TRPS	+	+	-	-	-	0	-	-	
	sp ICP-MS	+	+	-	+	+	+	+	+	
Fractionating	FFF	0	0	-	++	+	0	++	+	
	AC	+	+	+	-	+	+	-	+	
	DMAS	+	+	0	+	0	+	-	0	
	SEC	0	0	0	++	-	-	-	-	
Ensemble	DLS	++	++	++	+	0	+	-	0	
	SAXS	+	+	0	0	0	0	-	0	
	USSp	+	+	-	-	-	0	-	-	
	XRD	+	+	++	--	-	-	-	-	
	ALS	+	+	0	+	0	0	-	0	
	OS	+	+	+	-	-	-	--	-	
	FCS	N/A	N/A	--	--	--	--	--	--	
Integral	BET	+	0	++	-	-	0	-	-	

Table 14: Evaluation table for all considered characterisation methods



## 8 Pre-Selection

Based on the average scores conferred by NanoDefine consortium experts in the Evaluation table in section 6, recommendations of CMs to be taken over in other work packages can be done. The last three columns (purple) show the “NanoDefine” representative average score as direct recommendation; they do not result from averaging the individual parameters of each CM (red, yellow, blue and green). However, these scores are consistently supported by the evaluation of the individual parameters in Table 14, which show the particular strengths of each individual CM.

Recommended to further development as Screening methods		Recommended to further development as Confirmatory methods		Recommended as Methods ready for direct validation or standardisation	
Strongly recommended	recommended	Strongly recommended	recommended	Strongly recommended	recommended
<b>PTA/DUM</b>	TRPS	<b>EM</b>	SFM	<b>EM</b>	SFM
<b>sp ICP-MS</b>	FFF	<b>FFF</b>	sp ICP-MS <sup>§</sup>		PTA/DUM
<b>AC</b>	SAXS				sp ICP-MS
<b>DMAS</b>	USSp				FFF
<b>DLS</b>	ALS				AC
	BET**				

**Table 15:** Table with recommended characterisation methods as resulted from the evaluation table 14

<sup>§</sup> It should be noted that sp ICP-MS has been recommended by the NanoDefine experts in two categories (as screening as well as confirmatory methods).

\*\* BET has been recommended as Screening method. However, an evaluation report on the applicability ranges of the VSSA and the quantitative relation to number based particle size distribution for real world samples is fore-seen in NanoDefine.

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under Grant Agreement n° 604347

## 9 Conclusions

The most promising CMs with potential to be selected for recommendation to other NanoDefine work packages for further specific development (improvement and adaptation), or respectively for direct validation and standardisation were evaluated. The evaluation of the CMs at this early stage of the project was done by means of studies available in the literature as well as following the expertise of the NanoDefine consortium partners. The base for the evaluation process is offered via a dedicated evaluation table, resulting from the specific criteria required by the application of the individual CMs to the reliable analysis of the size distribution of a nanomaterial according to the EC recommendation for a definition of nanomaterial.

Main performance parameters of each pre-selected method with respect to the type of samples, type of sizing, particle property measured, type of quantity, size range, concentration range and information content led to an evaluation table of CMs so that the most suited CMs as evaluated against performance criteria could be finally grouped and recommended to other NanoDefine work packages for further development or respectively for direct validation and standardisation.

An open question is the combination of the results from different techniques, e.g. Anderson et al. (2013). It is noted that the many available particle size analysis methods have their advantages and disadvantages. The art of making a comprehensive particle size analysis is to combine the information, or, ideally, the different particle size distributions. The latter is needed because only few methods have a measurement range spanning the size range of interest, from 1 nm to 1 micrometre or even bigger.

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