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

Templates for nanomaterial characterisation of tier 1 and tier 2 measurement methods

NanoDefine Technical Report D7.2

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The NanoDefine Consortium 2014

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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

AUC	Analytical Disk- and Ultra-Centrifugation
BET	Brunauer–Emmett–Teller Method
CLS	Centrifugal Liquid Sedimentation
CM	Characterisation Method
CPC	Condensation Particle Counter
CRM	Certified Reference Material
DLS	Dynamic Light Scattering
DMA	Differential Mobility Analyser
DMAS	Differential Mobility Analysing System
EC	European Commission
EM	Electron Microscopy
ESZ	Electrical Sensing Zone
FFF	Field Flow Fractionation
FTIR	Fourier Transform Infrared Spectroscopy
NP	Nanoparticle(s)
NTA	Nanoparticle Tracking Analysis
PSD	Particle Size Distribution
SAXS	Small-angle X-Ray Scattering
SEM	Scanning Electron Microscopy
SLS	Static Light Scattering
SMPS	Scanning Mobility Particle Sizer
SOP	Standard Operating Procedure
sp ICP-MS	Single Particle Inductively Coupled Plasma Mass Spectrometry
TEM	Transmission Electron Microscopy
TRPS	Tunable Resistive Pulse Sensing
TSEM	Scanning Electron Microscopy in Transmission Mode
USSp	Ultrasonic Spectroscopy
UV-Vis	Ultraviolet-Visible Spectroscopy
VSSA	Volume Specific Surface Area

2 Summary

This report constitutes the result of the evaluation of those characterisation methods (CMs) which may be considered for the reliable analysis of the size distribution of a nanomaterial according to the EC recommendation for a definition of nanomaterial.

Based on both material related and performance criteria already established in the NanoDefine Technical Report D7.1 the potential CMs are evaluated according to studies available in the literature as well as following the expertise of the NanoDefine consortium partners. The table template from the NanoDefine Technical Report D7.1 has been filled in the “Characterisation” column, for each CM in part, in the specific materials related section (blue) and in the specific CM performance related section (yellow), with the corresponding score (“Yes/No”, “good/average/poor”, or “1 nm to 10 nm”, etc). Furthermore, a “Notes” column has been added to this table for cases where the simple score characterisation in the predefined format is not unambiguously enough. Note that a ranking of the suitable CMs according to the criteria as in the NanoDefine Technical Report D7.1 and D7.2 and grouping in screening CMs (tier 1) and confirmatory CMs (tier 2) will be achieved in the project where the potential CMs are systematically evaluated and pre-selected for further development and validation in the NanoDefine project.

An empty template that specifies which parameters have to be determined in the practical evaluation with selected materials for the project (“real-world performance data”) is presented at the end of the completed NanoDefine Technical Report D7.1 tables. To the pure CM performance criteria (yellow) are added economic criteria such as instrument time cost, duration of sample preparation, analysis, evaluation, etc. (green). This template should be used by the partners to fill in the results of the practical evaluation, so that in the end there will be a filled template for each material/method combination.

The actual method characterisation tables from NanoDefine Technical Report D7.2 will be reviewed later for overall consistency, if necessary provided with more in-depth specific information and finally harmonized so that the NanoDefine Technical Report D7.6 Methods Manual can be accordingly created.

3 Introduction

The task of the NanoDefine Technical Report D7.2 consists in developing CMs templates which specify the technical performance criteria targeted at measurement requirements resulting from the EC definition of nanomaterial. To the material related criteria (blue) and the pure CM performance criteria (yellow) as defined in NanoDefine Technical Report D7.1, economic criteria such as instrument time cost, duration of sample preparation, analysis, evaluation, etc. are added (green).

All potentially suitable characterisation methods will be quantitatively evaluated against these criteria and ranked against each other.

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

- Counting techniques (measuring particle properties on 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), and
- Integral sizing methods (which solely measure an integral (effective/mean) property of the particle systems such as the specific surface area (SV or Sm) or the turbidity of a suspension).

4 Material and technical method related performance data

CMs can be evaluated based on the table template already generated in the NanoDefine Technical Report D7.1. The performance criteria templates provided within this document have to be filled out for every relevant CM. They are structured into two sections:

- The first (blue) section covers the properties of the material to be analysed needs to possess in order to be measured by the CM,
- The second (yellow) section covers the technical performance parameters of the CM provided that the CM is applied to a suitable sample.

There are different types of questions, some requiring a Yes/No answer, others requiring numerical values and others requiring a pseudo-quantitative rating (bad, average, good). In case that a Yes/No answer cannot be answered unambiguously, the question shall be answered as would be expected for the standard case and a comment shall be added, describing when the given answer does not apply. An example for such an ambiguous situation would be the question: “Can an EM measure (water) ice particles?” The answer to be given in this case would be “No” as most EMs operate at room temperature only and the ice particles would melt. However, a cryo-stage can be added to the EM, allowing to measure at reduced temperatures. Therefore, a comment needs to be added in the corresponding column, e.g. “yes, if cryo-stage availa-

ble". For all other cells with Sensitivity above 25°C and higher the answer should be "Yes", because EM typically operating at room temperature is suitable to measure materials which are sensitive above 25°C. The same hold true for materials which are sensitive to below 0°C.

There are cases where the answer is not known. It was agreed that "N/A" may be introduced with the aim that these cells are completed at a later stage.

In the following, some instructions are given about how to fill out the material related (blue) part, in most cases accompanied by illustrative examples. The questions to be answered shall clarify, whether a material with a certain property can be investigated with the CM at hand.

- Nanoparticles in powder or liquid suspensions or embedded in a matrix: Here the aim is to determine if the method is suitable for particles which are:
    - Dispersed in liquids
    - In solid particulate form (powder...)
    - Dispersed or embedded in different kinds of matrices

  For example, if the method cannot be applied to particles which are embedded in a matrix, please specify **No.**

- Dispersibility by dispersion protocols: The purpose here is to know whether the method could be applied if the sample is
    - Dispersed in aqueous media
    - Dispersed in non-polar liquids
    - Dispersed in polar liquids
    - Dispersed in material-specific media
    - is aerosolized

  It is obvious that water is a polar liquid, but the option "dispersed in polar liquids" is to be used for other kinds of polar liquid as DCM, DMF, etc... For example, if the characterisation can be achieved only in water or needs rare equipment or complicated calculations, please fill in **Yes** for the aqueous media and **No** for the other possibilities.

- Substance Nature: The aim here is to know if the technique can be used with different materials of various "nature".
    - Inorganic
    - Size-dependent absorption / fluorescence
    - Carbon based
    - Organic, particulate
    - Organic, non-particulate
    - Biological
    - Composite
    - Other

For example, if the method can characterise only inorganic and carbon based materials, please fill in **Yes** for these two boxes, or **No** for the other ones. If the method is suitable for some inorganic material but not for all, try to specify it if possible.

Size-dependent absorption / fluorescence: The question is whether the CM can accurately measure the size of NPs, which change their absorption / fluorescence properties depending on their size. Examples for such particles would be gold NPs or quantum dots, which change their absorption / fluorescence wavelength depending on their size, or small metal or semiconducting particles (<10 nm) which show a size dependent real and imaginary part of the refractive index.

- Composite: The purpose here is to determine if the method can be used for different types of composite material. This sort of material is described as follows :
    - Core/shell
    - Multiple coatings
    - A mix of two or more different materials

  For example, if the characterisation method can be used to measure the outer diameter (which is the relevant measure according to the EC definition of a nanomaterial) of a core/shell particle but not for multiple coatings then the corresponding box has to be filled with yes or no. If the methods cannot be used with a certain kind of material, please specify this. This may depend for example on the thickness of the shell. In this case, specify a range. It does not matter if the range is not precise, but it is necessary to have an idea of which kind of material can be characterised with the technique you describe.

- Number of nanoscaled dimensions: The question shall find out whether the CM can accurately measure particles having one, two or three dimensions on the nano scale:
    - 1 nanoscaled dimension (typically thin plates)
    - 2 nanoscaled dimensions (typically nanotubes, fibers, …)
    - 3 nanoscaled dimensions (typically nanospheres…)

  According to the EC definition of a nanomaterial, it is important to know whether the material under investigation has at least one dimension in the nanoscale. If the method you want to describe in this template will not correctly classify a particle with the given number of dimensions in the nanoscale, fill **No** for the corresponding dimensions.

- Shape of nanoparticles: The aim of this question is to know if the method yields accurate sizes (with respect to the EC definition of a nanomaterial) for particles in a certain category of shapes
    - Sphere or similar
    - Equiaxial (Prismatic, Cubic, Tetrahedral)
    - Tubes, fibres, rods

- Flakes and discs
- Other

If the method you describe yields reliable results only for nanospheres, please fill **Yes** in the corresponding box, and **No** in the other ones. If the method can be applied for some categories but needs rare equipment or complicated calculations, please specify **No**. "Other" refers to any kind of irregular particles that can be measured. If it is the case then specify it.

- Thermal degradation sensitivity: The question here is to determine if this method is suitable for materials which are sensitive to thermal degradation. It means if during the characterisation the sample would be heated it is necessary to know the temperature range.
  For example, if the analysis will be performed around 100 C, fill **No** for the boxes corresponding to "Sensitivity above 50, 37, 25, 0°C" and **Yes** for the other boxes. If the analysis is typically carried out at room temperature (25°C), fill out **No** in the field “Above 0°C” and **Yes** in all other boxes.

- Cooling degradation sensitivity: The question here is to determine if this method is suitable for materials which are sensitive to cooling degradation. It means if during the characterisation, the sample would be cooled, it is necessary to know the temperature range.
  For example, if the analysis will be performed around -50°C, fill **No** for the box corresponding to "Sensitivity below 25, 0, -18, -35°C" and **Yes** for the other boxes (-78°C and -195 °C). It is to know if a material which is sensitive to a certain cooling point temperature can be characterised by this technique or not.

- e-beam sensitivity: The question here is to determine if this method is suitable for materials which are sensitive to an electron beam. If the technique you describe uses an e-beam, please fill **No** for the box corresponding to "e- beam sensitive" and **Yes** for "Not e-beam sensitive".

- Sample dispersity and modality: The question here is to determine if this method can be applied to monodisperse and polydisperse samples on one hand and monomodal and polymodal samples on the other hand.

  So if the characterisation method you described is not suitable for polydisperse samples, please write **No** in the corresponding box. The same logic is to be applied to the sample modality.

- Conductivity properties: The aim here is to know if the technique can be used with material which has some specific conductivity specificity:

- Conductive
- Semi-conductive
- Insulator

If the method you describe can only characterise conductive materials. Please fill **Yes** in the "conductive" box and **No** for the two other ones.

- Magnetic properties: The aim here is to know if the technique can be used with a material having some magnetic properties.

  If the method you describe cannot be used with magnetic materials, please fill **No** in the "magnetic" box and **Yes** for the "non-magnetic" ones.

- Functionalization / no functionalisation: The aim here is to know if the technique can be used with materials which are functionalised, and **not to know if the material is functionalised**.

  In the EC definition of a nanomaterial, the outer size of the particle is the quantity that needs to be measured. If the fact that the particles are functionalised makes this measurement inefficient, or give some incorrect results, please write **No** in the "Functionalised" box, and **Yes** in "Not Functionalised" box. It is not possible to write **No** in the two boxes.

- Agglomeration/ aggregation state: The purpose of this crucial criterion with direct relation to the EC definition is to know if the method can adequately measure the dimensions of the primary nanoparticles if the nanoparticles are aggregated or agglomerated, and **not to have information on the agglomeration or the aggregation of particles.**
  - Nanoparticles are aggregated
  - Nanoparticles are not aggregated
  - Nanoparticles are agglomerated
  - Nanoparticles are not agglomerated

  If the fact that the particles are aggregated or agglomerated makes the characterisation inefficient, or give some incorrect results (e.g. the outer dimensions of the agglomerate/aggregate), please write **No** in the "Agglomerated" and "Aggregated" boxes, and **Yes** in "Not Agglomerated" and "Not Aggregated" boxes. It is not possible to write **No** in the two boxes.

Also for the method-related (yellow) part, some of the criteria are described in more detail and are illustrated by instructive examples:

- Limits of detection/quantification:
  - What is the lower limit to detect: What is the size of the smallest particles which can be measured reliably with the CM?

- Trueness
  - Indicate the trueness of this CM: Specify the trueness with respect to the measured particle dimensions

- Trueness in weighting the size fractions
  - Specify the trueness in weighting the size fractions of this CM: Specify the trueness for the weighting of the individual size fractions

- Selectivity
  - Discrimination from non-nanoparticles of the same composition: Can the CM distinguish between nanoparticles and non-nanoparticles of the same composition?
  - Discrimination from non-nanoparticles of another composition (matrix particles): Can the CM distinguish nanoparticles from non-nanoparticles of a different composition?
  - Discrimination from nanoparticles of another composition: Can the CM distinguish between nanoparticles of different compositions?
  - Impurities: Does the CM yield accurate results for the PSD according to the EC definition if impurities are present in the sample to be analysed?

    Impurities are defined in Guidance for identification and naming of substances under REACH and CLP, ECHA-11-G-10.2-EN as: “An unintended constituent present in a substance as manufactured. It may originate from the starting materials or be the result of secondary or incomplete reactions during the manufacture process. While it is present in the final substance it was not intentionally added.“

    A constituent is defined in Guidance for identification and naming of substances under REACH and CLP, ECHA-11-G-10.2-EN as: “Any single species present in a substance that can be characterized by its unique chemical identity.”

    Accordingly, impurities can be molecules or particles (small or large). It is important to keep in mind that also the impurity particles need to be included in the measured PSD in order to classify the material as nano/non-nano according to the EC definition.

- Measure aggregation
    - Is it possible to measure aggregation or agglomeration of particles?: The question to be answered here is whether the CM can obtain information on the agglomeration/aggregation state of the nanoparticles, i.e. whether the primary particles are agglomerated/aggregated or not. For example if the CM measures the size of the individual particles and also shows that they predominantly come in agglomerates, answer **Yes** to this question.

- Destructive method or not
    - Is it a destructive method?: This question needs to be answered in the following sense: if the supplied sample material needs to be changed in any sense in order to be measurable with the CM, including deposition on a substrate, dissolving, etc. The method is considered as destructive even if the individual particles stay unharmed during the measurement process. For example, if a method using X-rays can be performed in a lab or at a synchrotron, using a vial of the sample as delivered, the typical answer would be **No** because the sample is unchanged and in a lab, the X-ray flux is usually not sufficient to damage the individual particles, however, a comment should be added stating "**Yes** for very high flux (synchrotron radiation)" if the particles can be damaged in this manner.

4.1 Counting methods

Counting methods inherently yield 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. 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. The probed particle property may be either (i) geometric (in particular for image analysis), (ii) optical (e. g. scattering cross section), or (iii) related to mobility (diffusion coefficient).

4.1.1 Imaging methods

4.1.1.1 Electron microscopy (SEM, TEM and TSEM)

Table 1: Template for characterisation of EM (including SEM, TEM and TSEM)

Criteria (generally)	**Criteria (more specific)**	**Characterisation** (Yes/ No)	**Notes** 4.1.1.2
Nanoparticles in powder or liquid suspensions or embedded in a matrix	Dispersed in liquids	No	Possible if a cryo-stage is available
	Solid particulate form	Yes	
	Dispersed or embedded in different kinds of matrices	Yes	Only at the surface, in thin films or in ultramicrotomed sections
Dispersibility by dispersion protocols	Dispersible in aqueous media	No	Yes after successful deposition on substrates
	Dispersible in non-polar liquids	No	Yes after successful deposition on substrates
	Dispersible in polar liquids	No	Yes after successful deposition on substrates
	Dispersible in material-specific media	No	Yes after successful deposition on substrates
	Can be aerosolized	No	Yes after successful deposition on substrates
Substance Nature	Inorganic	Yes	
	Size-dependent absorption / fluorescence	Yes	
	Carbon based	Yes	
	Organic, particulate	Yes	
	Organic, non-particulate	Yes	
	Biological	Yes	

	Composite	Yes	
	Other	Yes	
Composite	Core/shell	Yes	
	Multiple coatings	Yes	Difficult
	A mix of two or more different materials	Yes	
Number of nanoscaled dimensions	1	No	Sometimes, the thickness can be measured when tilting the sample
	2	Yes	
	3	Yes	
Shape of nanoparticles	Sphere or similar	Yes	
	Equiaxial	Yes	
	Tubes, fibres, rods	Yes	
	Flakes and discs	No	
	Other	Yes	
Thermal degradation sensitivity	Above 0°C	No	Yes, with cryo stage
	Sensitivity above 25°C	Yes	
	Sensitivity above 37°C	Yes	
	Sensitivity above 50°C	Yes	
	Sensitivity above 100°C	Yes	
	Sensitivity above 150°C	Yes	
	Sensitivity above 500°C	Yes	
	Sensitivity above 1000°C	Yes	
Cooling degradation sensitivity	Sensitive below 25 °C	Yes	
	Sensitive below 0 °C	Yes	
	Sensitive below -18 °C	Yes	
	Sensitive below -35 °C	Yes	
	Sensitive below -78 °C	Yes	
	Sensitive below -195 °C	Yes	
E- beam sensitivity	e- beam sensitive	No	
	Not e-beam sensitive	Yes	
Sample dispersity and modality	Monodisperse sample	Yes	
	Polydisperse sample	Yes	
	Monomodal sample	Yes	
	Multimodal sample	Yes	
Conductivity properties	Conductive	Yes	
	Semiconductive	Yes	With altered image quality

	Insulator	Yes	SEM: low-voltage option or conductive high resolution sputter-coating needed
Magnetic properties	Magnetic	Yes	Depending on strength of magnetic field and worsening the CM figures of merit
	Non magnetic	Yes	
Functionalization / no functionalisation	Functionalised	Yes	
	Not functionalised	Yes	
Agglomeration/ aggregation state	Nanoparticles are aggregated	Yes	
	Nanoparticles are not aggregated	Yes	
	Nanoparticles are agglomerated	Yes	
	Nanoparticles are not agglomerated	Yes	
counting, separative or ensemble techniques	Single particle counting	Yes	
	Calculate number or concentration from ensemble methods	No	
	Method combination (hyphenated methods)	Yes	
Working range	Size range	> 1 nm	Lower range varies in dependence on instrument type, sample type and preparation
	Concentration range	"mono-layer"	EM measures accurately only "dry" particles deposited on a substrate. Droplet of 0.1-1 µL at 0.1%-vol. conc. typically sufficient.
	Minimum needed sample amount	0.1 µL	Minimum 500 NPs for a monodisperse/ monomodal sample (Motzkus et al., 2013)
	Linearity/proportionality	Yes	
	Limits of detection/quantification	1 nm to 10 nm	Depending on instrument, sample type and preparation, etc.
	Sensitivity (Counting efficiency) as a function of size	good	
Limits of detection/quantification	What is the lower limit to detect	1 nm to 10 nm	Depending on instrument, sample type and preparation, etc.
Trueness	Indicate the trueness of this CM	good	Based on traceability of reference materials; critical for NPs below 10-20 nm with SEM
Trueness in weighting the size fractions	Specify the trueness in weighting the size fractions of this CM	average	To be evaluated for specific cases
Robustness	Specify the robustness of this CM	average	Strong dependency on sample preparation

			To be evaluated for specific cases
Precision	Specify the precision of the CM	1 nm to 10 nm	Depending on many parameters, mainly preparation; better for TEM and poorer for SEM. To be evaluated for specific cases
Resolution	Specify the resolution of this CM	1 nm to 10 nm	Depending on many parameters, mainly preparation; better for TEM and poorer for SEM. To be evaluated for specific cases
Size distribution	Is it possible to measure size distribution?	Yes	
Selectivity	discrimination from non-nanoparticles of the same composition	Yes	
	discrimination from non-nanoparticles of another composition (matrix particles)	Yes	In cases when image contrast is high enough
	discrimination from nanoparticles of another composition	Yes	In cases when image contrast is high enough
	Impurities	Yes	TEM: Very large particles, in the µm range and above cannot be measured accurately SEM: Very small particles (nm range) may not be detectable
Measures aggregation	Is it possible to measure aggregation or agglomeration of particles?	Yes	Difficult and possible only after sampling on substrates and in vacuum
Measures individual particles	Does this CM measure individual particles?	Yes	
Counting constituent particles in aggregations	Is the method able to count constituent particles in aggregates?	Yes	Depending on contrast and size of aggregate
Composition	Does this CM analyse composition?	No	However, Z contrast sometimes possible and EDX with poorer spatial resolution is almost always available at a SEM
Specification of the type of size (diameter)	Specify: for example hydrodynamic…	"Diameter", "Ferret diameter"	
Destructive method or	Is it a destructive method?	Yes	Sample must be prepared on

not			substrates or as thin films, etc.
Other Specificity			
Vacuum	Does the method operate under vacuum?	Yes	
Sample support	Does this CM need preparation on suited supports?	Yes	

4.1.1.2 Atomic force microscopy (AFM)

Table 2: Template for characterisation of AFM

Criteria (generally)	Criteria (more specific)	Characterisation (Yes/ No)	Notes
Nanoparticles in powder or liquid suspensions or embedded in a matrix	Dispersed in liquids	No	Yes after successful immobilization on substrates
	Solid particulate form	Yes	After successful immobilization on substrates, generally from a liquid dispersion
	Dispersed or embedded in different kinds of matrices	Yes	Only at the surface or in thin films or in ultramicrotomed sections, as long as the surface is smooth enough with respect to the particle size and there is enough contrast with the matrix; possible bias due to random sectioning at non-controlled distance from the diameter plane (for spheres)
Dispersibility by dispersion protocols	Dispersible in aqueous media	No	Yes after successful immobilization on substrates
	Dispersible in non-polar liquids	No	Yes after successful immobilization on substrates
	Dispersible in polar liquids	No	Yes after successful immobilization on substrates
	Dispersible in material-specific media	No	Yes after successful immobilization on substrates
	Can be aerosolized	No	Yes after successful immobilization on substrates
Substance Nature	Inorganic	Yes	
	Size-dependent absorption / fluorescence	Yes	
	Carbon based	Yes	
	Organic, particulate	Yes	
	Organic, non-particulate	Yes	
	Biological	Yes	
	Composite	Yes	
	Other	Yes	
Composite	Core/shell	Yes	Only external size
	Multiple coatings	Yes	Only external size
	A mix of two or more different materials	Yes	If the difference in size is not too big and if the maximum size remains lower than the scanner

			range; other limitations, such as differences in hardness, might apply; additional info is required for identification of the different materials
Number of nanoscaled dimensions	1	Yes	
	2	Yes	
	3	Yes	
Shape of nanoparticles	Sphere or similar	Yes	
	Equiaxial	Yes	
	Tubes, fibres, rods	Yes	
	Flakes and discs	Yes	
	Other	Yes	
Thermal degradation sensitivity	Above 0°C	No	Yes, with cryo stage
	Sensitivity above 25°C	Yes	
	Sensitivity above 37°C	Yes	
	Sensitivity above 50°C	Yes	
	Sensitivity above 100°C	Yes	
	Sensitivity above 150°C	Yes	
	Sensitivity above 500°C	Yes	
	Sensitivity above 1000°C	Yes	
Cooling degradation sensitivity	Sensitive below 25 °C	Yes	
	Sensitive below 0 °C	Yes	
	Sensitive below -18 °C	Yes	
	Sensitive below -35 °C	Yes	
	Sensitive below -78 °C	Yes	
	Sensitive below -195 °C	Yes	
E- beam sensitivity	e- beam sensitive	Yes	
	Not e-beam sensitive	Yes	
Sample dispersity and modality	Monodisperse sample	Yes	
	Polydisperse sample	Yes	
	Monomodal sample	Yes	
	Multimodal sample	Yes	
Conductivity properties	Conductive	Yes	
	Semiconductive	Yes	
	Insulator	Yes	
Magnetic properties	Magnetic	Yes	

	Non magnetic	Yes	
Functionalization / no functionalisation	Functionalised	Yes	
	Not functionalised	Yes	
Agglomeration/ aggregation state	Nanoparticles are aggregated	Yes	Only aggregate size, with limitations (size and roughness)
	Nanoparticles are not aggregated	Yes	
	Nanoparticles are agglomerated	Yes	Only agglomerate size, with limitations (size, roughness and hardness)
	Nanoparticles are not agglomerated	Yes	
counting, separative or ensemble techniques	Single particle counting	Yes	
	Calculate number or concentration from ensemble methods	No	
	Method combination (hyphenated methods)	Yes	Example: collecting fractions from FFF and redispersing them on adequate substrates for AFM
Working range	Size range	> about 1 nm for height; < 100 µm for lateral size	Range varies in dependence on instrument type, sample type and preparation
	Concentration range	"monolayer"	Droplet of 10 µL at 0.1% vol. conc. typically sufficient
	Minimum needed sample amount	0.1 µL for liquid suspension 1 mg for powder	Droplet of 10 µL at 0.1% vol. conc. typically sufficient
	Linearity/proportionality	Yes	
	Limits of detection/quantification	1 nm / several tens of nm	About 1 nm for height and several tens of nm for lateral dimensions (depending on tip convolution)
	Sensitivity (Counting efficiency) as a function of size	good	Low throughput
Limits of detection/quantification	What is the lower limit to detect	1 nm / several tens of nm	Individual particles can be detected (but statistics!) For individual particles: About 1 nm for height and a few tens of nm for lateral dimensions (depending on tip convolution)
Trueness	Indicate the trueness of this CM	good	only for height, as convolution

			with the tip geometry leads to a bias in lateral dimensions
Trueness in weighting the size fractions	Specify the trueness in weighting the size fractions of this CM	average	Depending on polydispersity and sample preparation; to be evaluated for specific cases
Robustness	Specify the robustness of this CM	average	Strong dependency on sample preparation; to be evaluated for specific cases
Precision	Specify the precision of the CM	< 1 nm for height	Depending on many parameters; precision on lateral size depends, among others, on tip convolution; to be evaluated for specific cases. Depends also on the type of material.
Resolution	Specify the resolution of this CM	1 nm to a few nm	Depending on dimension (height versus lateral), instrument type and imaging conditions and material type
Size distribution	Is it possible to measure size distribution?	Yes	if the size difference is not too big
Selectivity	discrimination from non-nanoparticles of the same composition	Yes	if the size difference is not too big
	discrimination from non-nanoparticles of another composition (matrix particles)	Yes	In cases when image contrast is high enough
	discrimination from nanoparticles of another composition	average	In cases when hardness properties are significantly different (in intermittent contact mode); modes based on other properties (electrical, magnetic, thermal…) could be helpful, but need to be evaluated
	Impurities	N/A	
Measures aggregation	Is it possible to measure aggregation or agglomeration of particles?	average	Only small aggregates if well separated on the substrate; agglomerates are expected to be more difficult to image due to their bigger size and poor mechanical properties
Measures individual particles	Does this CM measure individual particles?	Yes	
Counting constituent particles in aggregations	Is the method able to count constituent particles in aggregates?	No	

Composition	Does this CM analyse composition?	No	Although coupling with Raman or FTIR does exist, but remains limited (in resolution, among others) and not widespread
Specification of the type of size (diameter)	Specify: for example hydrodynamic…	height / lateral size	Bias on lateral size due to tip convolution.
Destructive method or not	Is it a destructive method?	Yes	Sample must be immobilised on substrates or as thin films, etc.
Other Specificity			
Vacuum	Does the method operate under vacuum?	No	Also possible, but not required.
Sample support	Does this CM need preparation on suited supports?	Yes	

4.1.2 Particle tracking analysis (PTA) / Dynamic ultramicroscopy (DUM)

Table 3: Template for characterisation of PTA/DUM

Criteria (generally)	Criteria (more specific)	Characterisation (Yes/No)	Notes
Nanoparticles in powder or liquid suspensions or embedded in a matrix	Dispersed in liquids	Yes	
	Solid particulate form	No	
	Dispersed or embedded in different kinds of matrices	No	
Dispersibility by dispersion protocols	Dispersible in aqueous media	Yes	
	Dispersible in non-polar liquids	Yes	
	Dispersible in polar liquids	Yes	
	Dispersible in material-specific media	No	
	Can be aerosolized	No	
Substance Nature	Inorganic	Yes	
	Size-dependent absorption / fluorescence	Yes	
	Carbon based	Yes	
	Organic, particulate	Yes	If scattering is sufficiently strong
	Organic, non-particulate	Yes	If scattering is sufficiently strong
	Biological	Yes	But shall not move itself
	Composite	No	Because matrix phase is solid
	Other		
Composite	Core/shell	Yes	Only outer size
	Multiple coatings	Yes	Only outer size
	A mix of two or more different materials	Yes	Only outer size
Number of nanoscaled dimensions	1	No	Does not resolve shape
	2	No	Does not resolve shape
	3	Yes	
Shape of nanoparticles	Sphere or similar	Yes	
	Equiaxial	Yes	
	Tubes, fibres, rods	No	
	Flakes and discs	No	
	Other		

Thermal degradation sensitivity	Above 0°C	No	
	Sensitivity above 25°C	Yes	
	Sensitivity above 37°C	Yes	
	Sensitivity above 50°C	Yes	
	Sensitivity above 100°C	Yes	
	Sensitivity above 150°C	Yes	
	Sensitivity above 500°C	Yes	
	Sensitivity above 1000°C	Yes	
Cooling degradation sensitivity	Sensitive below 25 °C	Yes	
	Sensitive below 0 °C	Yes	
	Sensitive below -18 °C	Yes	
	Sensitive below -35 °C	Yes	
	Sensitive below -78 °C	Yes	
	Sensitive below -195 °C	Yes	
E- beam sensitivity	e- beam sensitive	Yes	
	Not e-beam sensitive	Yes	
Sample dispersity and modality	Monodisperse sample	Yes	
	Polydisperse sample	Yes	Insensitive to fine NPs for very broad PSDs
	Monomodal sample	Yes	
	Multimodal sample	Yes	Insensitive to fine NPs for very broad PSDs
Conductivity properties	Conductive	Yes	
	Semiconductive	Yes	
	Insulator	Yes	
Magnetic properties	Magnetic	Yes	
	Non magnetic	Yes	
Functionalization / no functionalisation	Functionalised	Yes	
	Not functionalised	Yes	
Agglomeration/ aggregation state	Nanoparticles are aggregated	No	
	Nanoparticles are not aggregated	Yes	
	Nanoparticles are agglomerated	No	
	Nanoparticles are not agglomerated	Yes	
counting, separative or ensemble techniques	Single particle counting	Yes	
	Calculate number or concentration from ensemble methods	No	

	Method combination (hyphenated methods)	No	
Working range	Size range	10 nm – 1 µm	Lower size material dependent
	Concentration range	<< 1 vol.-%	
	Minimum needed sample amount	10 mL	
	Linearity/proportionality	Yes No	yes with regard to diffusion velocity, i.e. size Not really, with regard to concentration
	Limits of detection/quantification	N/A	Depend on optical contrast and size
	Sensitivity (Counting efficiency) as a function of size	good	Poor for very fine NPs in the presence of large particles
Limits of detection/quantification	What is the lower limit to detect	N/A	See size range
Trueness	Indicate the trueness of this CM	N/A	Size: “falseness” if wrong calibration of microscope and wrong model parameters (e. g. viscosity)
Trueness in weighting the size fractions	Specify the trueness in weighting the size fractions of this CM	N/A	Number/frequency: falseness if inappropriate illumination and image analysis or if too a high particle concentration
Robustness	Specify the robustness of this CM	poor	
Precision	Specify the precision of the CM	N/A	PSD parameters to be defined (PSD means, width, etc.)
Resolution	Specify the resolution of this CM	good	
Size distribution	Is it possible to measure size distribution?	Yes	
Selectivity	discrimination from non-nanoparticles of the same composition	Yes	
	discrimination from non-nanoparticles of another composition (matrix particles)	No	unless similar optical properties
	discrimination from nanoparticles of another composition	No	
	Impurities	N/A	Yes, dissolved impurities are ignored. No, particulate impurities (in the right size range and with suffi-

			cient contrast) are detected
Measures aggregation	Is it possible to measure aggregation or agglomeration of particles?	Yes	Size vs. time However, no identification of agglomerates/ aggregates below 1 µm, and for larger agglomerates/ aggregates no meaningful data analysis
Measures individual particles	Does this CM measure individual particles?	Yes	
Counting constituent particles in aggregations	Is the method able to count constituent particles in aggregates?	No	
Composition	Does this CM analyse composition?	No	
Specification of the type of size (diameter)	Specify: for example hydrodynamic…	"translational hydrodynamic diameter"	(long time self-)diffusion coefficient of particles
Destructive method or not	Is it a destructive method?	Yes	Usually, because of dilution
Other Specificity			
Vacuum	Does the method operate under vacuum?	No	
Sample support	Does this CM need preparation on suited supports?	No	

4.1.3 Tunable Resistive Pulse Sensing (TRPS)[1]

Table 4: Template for characterisation of TRPS

Criteria (generally)	Criteria (more specific)	Characterisation (Yes/ No)	Notes
Nanoparticles in powder or liquid suspensions or embedded in a matrix	Dispersed in liquids	Yes	
	Solid particulate form	No	
	Dispersed or embedded in different kinds of matrices	No	
Dispersibility by dispersion protocols	Dispersible in aqueous media	Yes	
	Dispersible in non-polar liquids	No	
	Dispersible in polar liquids	No	
	Dispersible in material-specific media	Yes	Needs to be aqueous based
	Can be aerosolized	No	
Substance Nature	Inorganic	Yes	
	Size-dependent absorption / fluorescence	No	
	Carbon based	Yes	
	Organic, particulate	Yes	
	Organic, non-particulate	No	
	Biological	Yes	
	Composite	Yes	only outer size
	Other		
Composite	Core/shell	Yes	only outer size
	Multiple coatings	Yes	only outer size
	A mix of two or more different materials	Yes	Possible in certain cases
Number of nanoscaled dimensions	1	No	
	2	No	Volume correct, if L/D<3

[1] TRPS (tunable resistive pulse sensing) is the preferred designation of the manufacturer (izon) 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.

	3	Yes	Measures particle volume, replacing liquid volume
Shape of nanoparticles	Sphere or similar	Yes	Does not resolve shape
	Equiaxial	Yes	
	Tubes, fibres, rods	No	Volume correct, if L/D<3
	Flakes and discs	No	
	Other	No	
Thermal degradation sensitivity	Above 0°C	No	
	Sensitivity above 25°C	Yes	
	Sensitivity above 37°C	Yes	
	Sensitivity above 50°C	Yes	
	Sensitivity above 100°C	Yes	
	Sensitivity above 150°C	Yes	
	Sensitivity above 500°C	Yes	
	Sensitivity above 1000°C	Yes	
Cooling degradation sensitivity	Sensitive below 25 °C	Yes	
	Sensitive below 0 °C	Yes	
	Sensitive below -18 °C	Yes	
	Sensitive below -35 °C	Yes	
	Sensitive below -78 °C	Yes	
	Sensitive below -195 °C	Yes	
E- beam sensitivity	e- beam sensitive	Yes	
	Not e-beam sensitive	Yes	
Sample dispersity and modality	Monodisperse sample	Yes	
	Polydisperse sample	Yes	
	Monomodal sample	Yes	
	Multimodal sample	Yes	
Conductivity properties	Conductive	Yes	
	Semiconductive	Yes	
	Insulator	Yes	
Magnetic properties	Magnetic	Yes	
	Non magnetic	Yes	
Functionalization / no functionalisation	Functionalised	Yes	
	Not functionalised	Yes	
Agglomeration/ aggregation state	Nanoparticles are aggregated	No	
	Nanoparticles are not aggregated	Yes	But lower size limit depends on

			sensing pore size, which may be blocked
	Nanoparticles are agglomerated	No	
	Nanoparticles are not agglomerated	Yes	But lower size limit depends on sensing pore size, which may be blocked
counting, separative or ensemble techniques	Single particle counting	Yes	
	Calculate number or concentration from ensemble methods	No	
	Method combination (hyphenated methods)	No	
Working range	Size range	50 nm - 10 µm	But lower size limit depends on sensing pore size, which may be blocked
	Concentration range	10^5 - 10^{12} particles /mL	
	Minimum needed sample amount	500 µL	
	Linearity/proportionality	Yes	Pulse amplitude linear to displaced electrolyte volume
	Limits of detection/quantification	10^5 particles/mL	
	Sensitivity (Counting efficiency) as a function of size	Yes	
Limits of detection/quantification	What is the lower limit to detect	10^5 particles/mL	
Trueness	Indicate the trueness of this CM	N/A	
Trueness in weighting the size fractions	Specify the trueness in weighting the size fractions of this CM	N/A	
Robustness	Specify the robustness of this CM	N/A	
Precision	Specify the precision of the CM	N/A	
Resolution	Specify the resolution of this CM	N/A	
Size distribution	Is it possible to measure size distribution?	Yes	
Selectivity	discrimination from non-nanoparticles of the same composition	No	
	discrimination from non-nanoparticles of another composition (matrix particles)	No	

	discrimination from nanoparticles of another composition	Yes	Limited discrimination reported to be possible
	Impurities	N/A	Yes, dissolved impurities are ignored. No, particulate impurities (in the right size range and with sufficient contrast) are detected
Measures aggregation	Is it possible to measure aggregation or agglomeration of particles?	No	
Measures individual particles	Does this CM measure individual particles?	Yes	
Counting constituent particles in aggregations	Is the method able to count constituent particles in aggregates?	No	
Composition	Does this CM analyse composition?	No	
Specification of the type of size (diameter)	Specify: for example hydrodynamic…	volume (or equivalent diameter)	
Destructive method or not	Is it a destructive method?	Yes	
Other Specificity			
Vacuum	Does the method operate under vacuum?	No	
Sample support	Does this CM need preparation on suited supports?	No	

4.1.4 Single particle ICP-MS (sp-ICP-MS)

Table 5: Template for characterisation of sp-ICP-MS

Criteria (generally)	Criteria (more specific)	Characterisation (Yes/ No)	Notes
Nanoparticles in powder or liquid suspensions or embedded in a matrix	Dispersed in liquids	Yes	only aqueous liquids
	Solid particulate form	Yes	after dispersion in water
	Dispersed or embedded in different kinds of matrices	Yes	after appropriate sample preparation (digestion, extraction, clean-up etc.)
Dispersibility by dispersion protocols	Dispersible in aqueous media	Yes	
	Dispersible in non-polar liquids	No	
	Dispersible in polar liquids other than water	No	
	Dispersible in material-specific media	No	only aqueous media (which can be modified with e.g. dispersants, buffers, low percentage of organic solvents)
	Can be aerosolized	No	
Substance Nature	Inorganic	Yes	sensitivity and interferences depending on element
	Size-dependent absorption / fluorescence	Yes	
	Carbon based	No	
	Organic, particulate	No	
	Organic, non-particulate	No	
	Biological	No	
	Composite	Yes	if particle contains detectable elements (of inorganic nature)
	Other		
Composite	Core/shell	Yes	if particle contains detectable elements
	Multiple coatings	Yes	if particle contains detectable elements
	A mix of two or more different materials	Yes	if particle contains detectable elements
Number of nanoscaled dimensions	1	No	if overall particle mass does not exceed linear range of method; theoretical max. 10 µm in one dimension (droplet size; needs

			practical verification)
	2	No	if overall particle mass does not exceed linear range of method; theoretical max. 10 µm in one dimension (droplet size; needs practical verification)
	3	Yes	
Shape of nanoparticles	Sphere or similar	Yes	method can measure objects of different shapes, but does not provide shape information
	Equiaxial	Yes	method can measure objects of different shapes, but does not provide shape information
	Tubes, fibres, rods	No	method can measure objects of different shapes, but does not provide shape information
	Flakes and discs	No	method can measure objects of different shapes, but does not provide shape information
	Other	No	method can measure objects of different shapes, but does not provide shape information
Thermal degradation sensitivity	Above 0°C	No	
	Sensitivity above 25°C	Yes	
	Sensitivity above 37°C	Yes	
	Sensitivity above 50°C	Yes	
	Sensitivity above 100°C	Yes	
	Sensitivity above 150°C	Yes	
	Sensitivity above 500°C	Yes	
	Sensitivity above 1000°C	Yes	
Cooling degradation sensitivity	Sensitive below 25 °C	Yes	
	Sensitive below 0 °C	Yes	
	Sensitive below -18 °C	Yes	
	Sensitive below -35 °C	Yes	
	Sensitive below -78 °C	Yes	
	Sensitive below -195 °C	Yes	
E- beam sensitivity	e- beam sensitive	Yes	
	Not e-beam sensitive	Yes	
Sample dispersity and modality	Monodisperse sample	Yes	
	Polydisperse sample	Yes	
	Monomodal sample	Yes	
	Multimodal sample	Yes	

Conductivity properties	Conductive	Yes	
	Semiconductive	Yes	
	Insulator	Yes	
Magnetic properties	Magnetic	Yes	
	Non magnetic	Yes	
Functionalization / no functionalisation	Functionalised	Yes	gives no information on type of functionalisation
	Not functionalised	Yes	
Agglomeration/ aggregation state	Nanoparticles are aggregated	No	
	Nanoparticles are not aggregated	Yes	
	Nanoparticles are agglomerated	No	
	Nanoparticles are not agglomerated	Yes	
counting, separative or ensemble techniques	Single particle counting	Yes	
	Calculate number or concentration from ensemble methods	No	
	Method combination (hyphenated methods)	No	
Working range	Size range	N/A	depending on element (e.g. Ag: ca. 20 – 1000 nm)
	Concentration range	N/A	depending on element, particle size, instrument (e.g. Ag 60 nm: 5 – 500 ng/L)
	Minimum needed sample amount	5 mL of injection dispersion	usually not an issue due to the high dilution factors (1000 – 100000) of the original sample
	Linearity/proportionality	N/A	linear range depending on element, particle size, instrument
	Limits of detection/quantification	N/A	depending on element (e.g. Ag: ca. 20 nm)
	Sensitivity (Counting efficiency) as a function of size	N/A	see LoD
Limits of detection/quantification	What is the lower limit to detect		depending on element (e.g. Ag: ca. 20 nm)
Trueness	Indicate the trueness of this CM	good	depending on analyte, matrix, laboratory (e.g. own results for Ag in meat trueness for size, number concentration and mass concentration > 90%)
Trueness in weighting the size fractions	Specify the trueness in weighting the size fractions of this CM	good	starting from 2 x LoD

Robustness	Specify the robustness of this CM	good	
Precision	Specify the precision of the CM	good	good precision can be achieved, but in first interlab study (including less experienced labs) some deviations were observed
Resolution	Specify the resolution of this CM	good	
Size distribution	Is it possible to measure size distribution?	Yes	
Selectivity	discrimination from non-nanoparticles of the same composition	Yes	
	discrimination from non-nanoparticles of another composition (matrix particles)	Yes	
	discrimination from nanoparticles of another composition	Yes	
	Impurities	N/A	
Measures aggregation	Is it possible to measure aggregation or agglomeration of particles?	No	
Measures individual particles	Does this CM measure individual particles?	Yes	
Counting constituent particles in aggregations	Is the method able to count constituent particles in aggregates?	No	
Composition	Does this CM analyse composition?	Yes	at the moment only one m/z
Specification of the type of size (diameter)	Specify: for example hydrodynamic…	mass	can be used to calculate diameter for spherical, non-porous particles
Destructive method or not	Is it a destructive method?	Yes	
Other Specificity			
Vacuum	Does the method operate under vacuum?	No	
Sample support	Does this CM need preparation on suited supports?	No	

4.2 Fractionating methods

Fractionating (ensemble) methods include the two steps of fractionation and detection, respectively. 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.

4.2.1 Field-Flow-Fractionation (FFF)

Table 6: Template for characterisation of FFF

Criteria (generally)	**Criteria (more specific)**	**Characterisation** (Yes/ No)	**Notes**
Nanoparticles in powder or liquid suspensions or embedded in a matrix	Dispersed in liquids	Yes	
	Solid particulate form	No	
	Dispersed or embedded in different kinds of matrices	No	
Dispersibility by dispersion protocols	Dispersible in aqueous media	Yes	
	Dispersible in non-polar liquids	Yes	Channels for organic liquids available. Not routinely used
	Dispersible in polar liquids	Yes	
	Dispersible in material-specific media	Yes	Case by case decision
	Can be aerosolized	No	
Substance Nature	Inorganic	Yes	
	Size-dependent absorption / fluorescence	Yes	
	Carbon based	Yes	
	Organic, particulate	Yes	
	Organic, non-particulate	Yes	Macromolecules and similar are possible
	Biological	Yes	
	Composite	Yes	
	Other		
Composite	Core/shell	Yes	

	Multiple coatings	Yes	
	A mix of two or more different materials	Yes	
Number of nanoscaled dimensions	1	No	
	2	No	Yes for small aspect ratios
	3	Yes	
Shape of nanoparticles	Sphere or similar	Yes	
	Equiaxial	Yes	
	Tubes, fibres, rods	No	Yes in certain cases. Separation can be achieved; sizing would require sample-similar standards or external sizing methods.
	Flakes and discs	No	Yes in certain cases. Separation can be achieved; sizing would require sample-similar standards or external sizing methods.
	Other		
Thermal degradation sensitivity	Above 0°C	No	
	Sensitivity above 25°C	Yes	
	Sensitivity above 37°C	Yes	
	Sensitivity above 50°C	Yes	
	Sensitivity above 100°C	Yes	
	Sensitivity above 150°C	Yes	
	Sensitivity above 500°C	Yes	
	Sensitivity above 1000°C	Yes	
Cooling degradation sensitivity	Sensitive below 25 °C	Yes	
	Sensitive below 0 °C	Yes	
	Sensitive below -18 °C	Yes	
	Sensitive below -35 °C	Yes	
	Sensitive below -78 °C	Yes	
	Sensitive below -195 °C	Yes	
E- beam sensitivity	e- beam sensitive	Yes	
	Not e-beam sensitive	Yes	
Sample dispersity and modality	Monodisperse sample	Yes	
	Polydisperse sample	Yes	
	Monomodal sample	Yes	
	Multimodal sample	Yes	
Conductivity proper-	Conductive	Yes	

ties	Semiconductive	Yes	
	Insulator	Yes	
Magnetic properties	Magnetic	Yes	
	Non magnetic	Yes	
Functionalization / no functionalisation	Functionalised	Yes	
	Not functionalised	Yes	
Agglomeration/ aggregation state	Nanoparticles are aggregated	No	
	Nanoparticles are not aggregated	Yes	
	Nanoparticles are agglomerated	No	
	Nanoparticles are not agglomerated	Yes	
counting, separative or ensemble techniques	Single particle counting	N/A	Depends on detection system. If detector counts particles, particle number based distribution can be achieved.
	Calculate number or concentration from ensemble methods	Yes	
	Method combination (hyphenated methods)	Yes	
Working range	Size range	1 - 1000 nm	
	Concentration range	20 µg/L – 500 mg/L	Detector dependent
	Minimum needed sample amount	10 µL	
	Linearity/proportionality	Yes	Detector dependent
	Limits of detection/quantification	> 1 nm	Detector dependent
	Sensitivity (Counting efficiency) as a function of size	good	Detector dependent
Limits of detection/quantification	What is the lower limit to detect	1 nm to 10 nm	Membrane and detector dependent
Trueness	Indicate the trueness of this CM	good	If reference materials available
Trueness in weighting the size fractions	Specify the trueness in weighting the size fractions of this CM	good	Mass quantification if performed by mass specific detector
Robustness	Specify the robustness of this CM	average	Important parameter is the membrane quality
Precision	Specify the precision of the CM	1 nm to 10 nm	Can be tuned to needs
Resolution	Specify the resolution of this CM	1 nm to 10 nm	determined by size standards
Size distribution	Is it possible to measure size dis-	Yes	

	tribution?		
Selectivity	discrimination from non-nanoparticles of the same composition	No	Pre-treatment of the sample is necessary.
	discrimination from non-nanoparticles of another composition (matrix particles)	No	Pre-treatment of the sample is necessary. Depends on detection technique.
	discrimination from nanoparticles of another composition	Yes	Depends on detection technique.
	Impurities	N/A	
Measures aggregation	Is it possible to measure aggregation or agglomeration of particles?	No	
Measures individual particles	Does this CM measure individual particles?	No	
Counting constituent particles in aggregations	Is the method able to count constituent particles in aggregates?	No	
Composition	Does this CM analyse composition?	Yes	Depending on constituents and applied detection technique.
Specification of the type of size (diameter)	Specify: for example hydrodynamic…	"Hydrodynamic diameter	Diffusion coefficient (FlowFFF) hydrodynamic diameter can be derived, volumetric diameter (SedFFF or CFFF). In few cases when MALS is applicable also rms and geometrical diameter, respectively.
Destructive method or not	Is it a destructive method?	No	Fractions can be collected; sample will be diluted.
Other Specificity			
Vacuum	Does the method operate under vacuum?	No	
Sample support	Does this CM need preparation on suited supports?	No	

4.2.2 Analytical centrifugation / Centrifugation analysis - including Centrifugal Liquid Sedimentation (CLS) and Analytical Ultra Centrifugation (AUC)

Table 7: Template for characterisation of Analytical centrifugation / Centrifugation analysis - including Centrifugal Liquid Sedimentation (CLS) and Analytical Ultra Centrifugation (AUC)

Criteria (generally)	Criteria (more specific)	Characterisation (Yes/ No)	Notes
Nanoparticles in powder or liquid suspensions or embedded in a matrix	Dispersed in liquids	Yes	
	Solid particulate form	No	
	Dispersed or embedded in different kinds of matrices	No	
Dispersibility by dispersion protocols	Dispersible in aqueous media	Yes	
	Dispersible in non-polar liquids	Yes	Depending on liquid compatibility of instrument
	Dispersible in polar liquids	Yes	Depending on liquid compatibility of instrument
	Dispersible in material-specific media	Yes	Depending on liquid compatibility of instrument
	Can be aerosolized	No	
Substance Nature	Inorganic	Yes	
	Size-dependent absorption / fluorescence	Yes	Size-dependent scattering relevant to algorithm converting detector signal to mass value.
	Carbon based	Yes	
	Organic, particulate	Yes	
	Organic, non-particulate	No	No for CLS, but yes for AUC
	Biological	Yes	Need to know material density- not always possible in biological samples
	Composite	No	
	Other		
Composite	Core/shell	No	Yes if density values are known and one of either core or shell thickness are known
	Multiple coatings	No	Theoretical possible but in practice very complex
	A mix of two or more different materials	No	Not normally possible with CLS instrument (single-detector operation), but possible by combination of different optical (turbidity, RI,

			UV) and X-ray detectors.
Number of nanoscaled dimensions	1	No	
	2	No	
	3	Yes	
Shape of nanoparticles	Sphere or similar	Yes	
	Equiaxial	Yes	Approximations may be possible by use of aspect-ratio coefficients in software
	Tubes, fibres, rods	No	Approximations may be possible by use of aspect-ratio coefficients in software
	Flakes and discs	No	If the lateral extension is known, the thickness distribution can be measured from below 1nm to above 100nm, and vice-versa.
	Other		
Thermal degradation sensitivity	Above 0°C	No	
	Sensitivity above 25°C	Yes	
	Sensitivity above 37°C	Yes	
	Sensitivity above 50°C	Yes	
	Sensitivity above 100°C	Yes	
	Sensitivity above 150°C	Yes	
	Sensitivity above 500°C	Yes	
	Sensitivity above 1000°C	Yes	
Cooling degradation sensitivity	Sensitive below 25 °C	Yes	
	Sensitive below 0 °C	Yes	
	Sensitive below -18 °C	Yes	
	Sensitive below -35 °C	Yes	
	Sensitive below -78 °C	Yes	
	Sensitive below -195 °C	Yes	
E- beam sensitivity	e- beam sensitive	Yes	
	Not e-beam sensitive	Yes	
Sample dispersity and modality	Monodisperse sample	Yes	
	Polydisperse sample	Yes	
	Monomodal sample	Yes	
	Multimodal sample	Yes	
Conductivity properties	Conductive	Yes	
	Semiconductive	Yes	

	Insulator	Yes	
Magnetic properties	Magnetic	Yes	
	Non magnetic	Yes	
Functionalization / no functionalisation	Functionalised	N/A	
	Not functionalised	Yes	
Agglomeration/ aggregation state	Nanoparticles are aggregated	No	
	Nanoparticles are not aggregated	Yes	
	Nanoparticles are agglomerated	No	
	Nanoparticles are not agglomerated	Yes	
counting, separative or ensemble techniques	Single particle counting	No	
	Calculate number or concentration from ensemble methods	No	
	Method combination (hyphenated methods)	Yes	Not with CLS (single detector). With AUC, multiple detectors acquire differently weighted size distributions during the same fractionation.
Working range	Size range	5 nm-70 µm	Range depends on instrument type, sample type and preparation. Maximum and minimum may not be possible in single run. From 0.5nm for AUC.
	Concentration range	>50 ppm	
	Minimum needed sample amount	100 ng	Sample volume is typically around 100 µl
	Linearity/proportionality	No	Non-linear
	Limits of detection/quantification	10 ppm / 50 ppm	Highly dependent on material, size and detector type
	Sensitivity (Counting efficiency) as a function of size	Yes	Decreasing sensitivity with size for optical detection.
Limits of detection/quantification	What is the lower limit to detect	10 ppm / 50 ppm	Highly dependent on material, size and detector type
Trueness	Indicate the trueness of this CM	good	
Trueness in weighting the size fractions	Specify the trueness in weighting the size fractions of this CM	N/A	Depends on material and the algorithms used to convert measured signal to weight% and then to number%
Robustness	Specify the robustness of this CM	good	Particle density must be reliably known
Precision	Specify the precision of the CM	good	Depends on the accuracy of the calibration standards used
Resolution	Specify the resolution of this CM	2% of	For non-aggregated materials

		nominal size	
Size distribution	Is it possible to measure size distribution?	Yes	Produces mass based size distribution
Selectivity	discrimination from non-nanoparticles of the same composition	Yes	
	discrimination from non-nanoparticles of another composition (matrix particles)	No	
	discrimination from nanoparticles of another composition	No	
	Impurities	N/A	
Measures aggregation	Is it possible to measure aggregation or agglomeration of particles?	No	
Measures individual particles	Does this CM measure individual particles?	No	
Counting constituent particles in aggregations	Is the method able to count constituent particles in aggregates?	No	
Composition	Does this CM analyse composition?	No	
Specification of the type of size (diameter)	Specify: for example hydrodynamic…	"Stokes diameter"	
Destructive method or not	Is it a destructive method?	Yes	
Other Specificity			
Vacuum	Does the method operate under vacuum?	No	
Sample support	Does this CM need preparation on suited supports?	No	

4.2.3 Differential electrical mobility analysis (DMA)

Table 8: Template for characterisation of DMA

Criteria (generally)	Criteria (more specific)	Characterisation (Yes/ No)	Notes
Nanoparticles in powder or liquid suspensions or embedded in a matrix	Dispersed in liquids	No	measures only airborne NPs
	Solid particulate form	No	measures only airborne NPs
	Dispersed or embedded in different kinds of matrices	No	measures only airborne NPs
Dispersibility by dispersion protocols	Dispersible in aqueous media	No	
	Dispersible in non-polar liquids	No	
	Dispersible in polar liquids	No	
	Dispersible in material-specific media	No	
	Can be aerosolized	Yes	measures only particles aerosolized
Substance Nature	Inorganic	Yes	
	Size-dependent absorption / fluorescence	Yes	
	Carbon based	Yes	
	Organic, particulate	Yes	
	Organic, non-particulate	Yes	
	Biological	Yes	
	Composite	Yes	
	Other	Yes	
Composite	Core/shell	Yes	Only outer size
	Multiple coatings	Yes	Only outer size
	A mix of two or more different materials	Yes	Only outer size
Number of nanoscaled dimensions	1	No	measures an equivalent diameter called electrical mobility diameter
	2	No	measures an equivalent diameter called electrical mobility diameter
	3	Yes	measures an equivalent diameter called electrical mobility diameter
Shape of nanoparticles	Sphere or similar	Yes	The diameter obtained can be compared with other technique if

			the particle shape is spherical
	Equiaxial	Yes	does not resolve shape
	Tubes, fibres, rods	No	does not resolve shape
	Flakes and discs	No	does not resolve shape
	Other		
Thermal degradation sensitivity	Above 0°C	No	
	Sensitivity above 25°C	Yes	
	Sensitivity above 37°C	Yes	
	Sensitivity above 50°C	Yes	
	Sensitivity above 100°C	Yes	
	Sensitivity above 150°C	Yes	
	Sensitivity above 500°C	Yes	
	Sensitivity above 1000°C	Yes	
Cooling degradation sensitivity	Sensitive below 25 °C	Yes	
	Sensitive below 0 °C	Yes	
	Sensitive below -18 °C	Yes	
	Sensitive below -35 °C	Yes	
	Sensitive below -78 °C	Yes	
	Sensitive below -195 °C	Yes	
E- beam sensitivity	e- beam sensitive	Yes	
	Not e-beam sensitive	Yes	
Sample dispersity and modality	Monodisperse sample	Yes	
	Polydisperse sample	Yes	
	Monomodal sample	Yes	
	Multimodal sample	Yes	
Conductivity properties	Conductive	Yes	
	Semiconductive	Yes	
	Insulator	Yes	
Magnetic properties	Magnetic	Yes	
	Non magnetic	Yes	
Functionalization / no functionalisation	Functionalised	Yes	
	Not functionalised	Yes	
Agglomeration/ aggregation state	Nanoparticles are aggregated	No	
	Nanoparticles are not aggregated	Yes	
	Nanoparticles are agglomerated	No	
	Nanoparticles are not agglomerat-	Yes	

	ed		
counting, separative or ensemble techniques	Single particle counting	Yes	measures the number concentration in aerosol phase
	Calculate number or concentration from ensemble methods	No	Is not an ensemble method
	Method combination (hyphenated methods)	No	
Working range	Size range	2 nm to 1 µm	Range varies in dependence on instrument type like DMA and CPC and the parameter used (flow rate,…)
	Concentration range	$<10^6$ part/cm^3	For CPC
	Minimum needed sample amount	10^4 part/cm^3	
	Linearity/proportionality	Yes No	With regard to concentration With regard to particle size (for low particle diameters the fraction of charged particles is very low)
	Limits of detection/quantification	> 2 nm	depends on the instrument type (3-5 nm)
	Sensitivity (Counting efficiency) as a function of size	good	There is a strong impact of the size particle < 10 nm for the counting efficiency (CPC)
Limits of detection/quantification	What is the lower limit to detect	2 nm to 10 nm	Dependent on instrument type, and parameters used (flow rate,…)
Trueness	Indicate the trueness of this CM	good	
Trueness in weighting the size fractions	Specify the trueness in weighting the size fractions of this CM	good	
Robustness	Specify the robustness of this CM	good	
Precision	Specify the precision of the CM	2 channels	depends on width of channel used, particle diameter and instrument type
Resolution	Specify the resolution of this CM	0.5 nm to 3.6 nm	for d_p < 100 nm Resolution depends on the size of particle and instrument type
Size distribution	Is it possible to measure size distribution?	Yes	
Selectivity	discrimination from non-nanoparticles of the same composition	Yes	

	discrimination from non-nanoparticles of another composition (matrix particles)	Yes	
	discrimination from nanoparticles of another composition	No	
	Impurities	N/A	• "impurities" only refer to residual particles (salt, polymers) after aersolisation from suspension; this entry is irrelevant for aerosol characterisation
Measures aggregation	Is it possible to measure aggregation or agglomeration of particles?	No	SMPS measures all size of particles (primary, agglomerate and aggregate) without knowing the state of agglomeration. It is necessary to use other method like SEM, TEM,…
Measures individual particles	Does this CM measure individual particles?	Yes	
Counting constituent particles in aggregations	Is the method able to count constituent particles in aggregates?	No	in rare cases it is possible to identify dublets, triplets and quartets
Composition	Does this CM analyse composition?	No	
Specification of the type of size (diameter)	Specify: for example hydrodynamic…	"electrical mobility diameter"	an equivalent diameter
Destructive method or not	Is it a destructive method?	Yes	
Other Specificity			
Vacuum	Does the method operate under vacuum?	No	
Sample support	Does this CM need preparation on suited supports?	No	

4.3 Ensemble methods

The immediate result of an ensemble method is the variation of the measured signal *g* over the (spectral) parameter *s* (time, space or frequency). Each size fraction *x* possesses a characteristic "spectrum" $k_r(s,x)$, which in general covers the whole spectral 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).

4.3.1 Dynamic light scattering (DLS)

Table 9: Template for characterisation of DLS

Criteria (generally)	Criteria (more specific)	Characterisation (Yes/No)	Notes
Nanoparticles in powder or liquid suspensions or embedded in a matrix	Dispersed in liquids	Yes	
	Solid particulate form	No	
	Dispersed or embedded in different kinds of matrices	No	
Dispersibility by dispersion protocols	Dispersible in aqueous media	Yes	
	Dispersible in non-polar liquids	Yes	
	Dispersible in polar liquids	Yes	
	Dispersible in material-specific media	No	
	Can be aerosolized	No	
Substance Nature	Inorganic	Yes	
	Size-dependent absorption / fluorescence	Yes	see size criteria
	Carbon based	Yes	
	Organic, particulate	Yes	
	Organic, non-particulate	Yes	
	Biological	Yes	
	Composite	No	because of solid matrix
	Other	No	
Composite	Core/shell	Yes	Outer particle size
	Multiple coatings	Yes	Outer particle size
	A mix of two or more different ma-	Yes	Outer particle size

	terials		
Number of nanoscaled dimensions	1	No	
	2	No	
	3	Yes	
Shape of nanoparticles	Sphere or similar	Yes	
	Equiaxial	Yes	Conventional DLS measurement and analysis do not resolve shape
	Tubes, fibres, rods	No	Conventional DLS measurement and analysis do not resolve shape
	Flakes and discs	No	Conventional DLS measurement and analysis do not resolve shape
	Other		
Thermal degradation sensitivity	Above 0°C	No	
	Sensitivity above 25°C	Yes	
	Sensitivity above 37°C	Yes	
	Sensitivity above 50°C	Yes	
	Sensitivity above 100°C	Yes	
	Sensitivity above 150°C	Yes	
	Sensitivity above 500°C	Yes	
	Sensitivity above 1000°C	Yes	
Cooling degradation sensitivity	Sensitive below 25 °C	Yes	
	Sensitive below 0 °C	Yes	
	Sensitive below -18 °C	Yes	
	Sensitive below -35 °C	Yes	
	Sensitive below -78 °C	Yes	
	Sensitive below -195 °C	Yes	
E- beam sensitivity	e- beam sensitive	Yes	
	Not e-beam sensitive	Yes	
Sample dispersity and modality	Monodisperse sample	Yes	
	Polydisperse sample	Yes	Insensitive to fine NPs for very broad PSDs
	Monomodal sample	Yes	
	Multimodal sample	Yes	Insensitive to fine NPs for very broad PSDs
Conductivity properties	Conductive	Yes	
	Semiconductive	Yes	

	Insulator	Yes	
Magnetic properties	Magnetic	Yes	
	Non magnetic	Yes	
Functionalization / no functionalisation	Functionalised	Yes	
	Not functionalised	Yes	
Agglomeration/ aggregation state	Nanoparticles are aggregated	No	
	Nanoparticles are not aggregated	Yes	
	Nanoparticles are agglomerated	No	
	Nanoparticles are not agglomerated	Yes	
counting, separative or ensemble techniques	Single particle counting	No	
	Calculate number or concentration from ensemble methods	Yes	only estimates
	Method combination (hyphenated methods)	No	
Working range	Size range	1 nm – 1 µm	
	Concentration range	<0.1 vol.-%	
	Minimum needed sample amount	2 mL	
	Linearity/proportionality	No Yes	No, with regard to size (decay of acf or spectral shift) Yes with regard to concentration (total intensity) for diluted systems (where linear dependence on concentration)
	Limits of detection/quantification	N/A	Depends on optical contrast and size
	Sensitivity (Counting efficiency) as a function of size	N/A	Insensitive to very fine particles (weak scattering signal) and very coarse particles (do not contribute to signal fluctuation and disappear from measurement zone)
Limits of detection/quantification	What is the lower limit to detect	N/A	
Trueness	Indicate the trueness of this CM	N/A	Size: “falseness” if wrong model parameters (e.g. viscosity, wavelength) or if too a high concentration (multiple scattering, hydrodyn. interaction)
Trueness in weighting the size fractions	Specify the trueness in weighting the size fractions of this CM	N/A	Intensity weights: “falseness” if multiple scattering or if too high laser intensities
Robustness	Specify the robustness of this CM	good	

Precision	Specify the precision of the CM	N/A	PSD average (mode, median, (power) means): high PSD width: satisfactory
Resolution	Specify the resolution of this CM	poor	
Size distribution	Is it possible to measure size distribution?	Yes	
Selectivity	discrimination from non-nanoparticles of the same composition	Yes	If NP dominate
	discrimination from non-nanoparticles of another composition (matrix particles)	No	Yes, only if NP dominate and have higher refractive index
	discrimination from nanoparticles of another composition	No	
	Impurities	N/A	Yes, dissolved impurities are ignored. No, particulate impurities (in the right size range and with sufficient contrast) are detected
Measures aggregation	Is it possible to measure aggregation or agglomeration of particles?	Yes	(size vs time) However, no identification of aggregates/ agglomerates
Measures individual particles	Does this CM measure individual particles?	No	
Counting constituent particles in aggregations	Is the method able to count constituent particles in aggregates?	No	
Composition	Does this CM analyse composition?	No	
Specification of the type of size (diameter)	Specify: for example hydrodynamic…	"Effective hydrodynamic diameter"	Short time self-diffusion: translation affected by rotation for non-spherical objects
Destructive method or not	Is it a destructive method?	Yes	If dilution is required; otherwise not
Other Specificity			
Vacuum	Does the method operate under vacuum?	No	
Sample support	Does this CM need preparation on suited supports?	No	

4.3.2 Small-angle X-ray scattering (SAXS)

Table 10: Template for characterisation of SAXS

Criteria (generally)	Criteria (more specific)	Characterisation (Yes/ No)	Notes
Nanoparticles in powder or liquid suspensions or embedded in a matrix	Dispersed in liquids	Yes	
	Solid particulate form	Yes	
	Dispersed or embedded in different kinds of matrices	Yes	as long as the X-rays transmit through the material
Dispersibility by dispersion protocols	Dispersible in aqueous media	Yes	In-situ measurement
	Dispersible in non-polar liquids	Yes	In-situ measurement
	Dispersible in polar liquids	Yes	In-situ measurement
	Dispersible in material-specific media	Yes	In-situ measurement
	Can be aerosolized	No	aerosols can be measured with special set-ups (Shyjumon et al., 2008)
Substance Nature	Inorganic	Yes	
	Size-dependent absorption / fluorescence	Yes	size dependent absorption or fluorescence in the UV-Vis range does not affect the SAXS results
	Carbon based	Yes	
	Organic, particulate	Yes	
	Organic, non-particulate	Yes	
	Biological	Yes	
	Composite	Yes	
	Other		
Composite	Core/shell	Yes	core and shell must have different electron densities
	Multiple coatings	Yes	multiple coatings must have different electron densities
	A mix of two or more different materials	Yes	
Number of nanoscaled dimensions	1	No	
	2	No	
	3	Yes	
Shape of nanoparti-	Sphere or similar	Yes	

cles	Equiaxial	Yes	
	Tubes, fibres, rods	No	Yes if the shape is known
	Flakes and discs	No	Yes if the shape is known
	Other	No	If different forms are mixed in the same sample SAXS analysis is possible only with additional information like shape information from imaging methods
Thermal degradation sensitivity	Above 0°C	No	
	Sensitivity above 25°C	Yes	
	Sensitivity above 37°C	Yes	
	Sensitivity above 50°C	Yes	
	Sensitivity above 100°C	Yes	
	Sensitivity above 150°C	Yes	
	Sensitivity above 500°C	Yes	
	Sensitivity above 1000°C	Yes	
Cooling degradation sensitivity	Sensitive below 25 °C	Yes	
	Sensitive below 0 °C	Yes	
	Sensitive below -18 °C	Yes	
	Sensitive below -35 °C	Yes	
	Sensitive below -78 °C	Yes	
	Sensitive below -195 °C	Yes	
E- beam sensitivity	e- beam sensitive	Yes	
	Not e-beam sensitive	Yes	
Sample dispersity and modality	Monodisperse sample	Yes	
	Polydisperse sample	Yes	If polydispersity is above ~20 %, data evaluation may become ambiguous without further info
	Monomodal sample	Yes	
	Multimodal sample	Yes	if modes are too close to each other distinction of the modes is difficult
Conductivity properties	Conductive	Yes	
	Semiconductive	Yes	
	Insulator	Yes	
Magnetic properties	Magnetic	Yes	
	Non magnetic	Yes	
Functionalization / no	Functionalised	Yes	

functionalisation	Not functionalised	Yes	
Agglomeration/ aggregation state	Nanoparticles are aggregated	No	
	Nanoparticles are not aggregated	Yes	
	Nanoparticles are agglomerated	No	
	Nanoparticles are not agglomerated	Yes	
counting, separative or ensemble techniques	Single particle counting	No	
	Calculate number or concentration from ensemble methods	Yes	
	Method combination (hyphenated methods)	Yes	
Working range	Size range	> 1 nm	Range varies in dependence on instrument type
	Concentration range	0.01 to 100 vol. %	the concentration range depends strongly on the electron density of the particles. The higher the electron density is, the more sensitive SAXS is.
	Minimum needed sample amount	1 to 10 µL	depending on the instrument and sample holder used
	Linearity/proportionality	Yes	
	Limits of detection/quantification	> 1 nm	
	Sensitivity (Counting efficiency) as a function of size	good	sensitivity increases with size
Limits of detection/quantification	What is the lower limit to detect	1 nm to 100 nm	depends on instrument
Trueness	Indicate the trueness of this CM	good	SAXS is a metrologically traceable method (Meli et al., 2012)
Trueness in weighting the size fractions	Specify the trueness in weighting the size fractions of this CM	good	Can be quantified if uncertainties are considered properly (Pauw et al., 2013)
Robustness	Specify the robustness of this CM	good	Results are robust for defined SOPs of data processing
Precision	Specify the precision of the CM	0.1 nm to 1.0 nm	Depending on the particles size. The larger the particles the smaller the absolute precision
Resolution	Specify the resolution of this CM	0.1 nm to 5 nm	The larger the particles the smaller is the absolute resolution
Size distribution	Is it possible to measure size distribution?	Yes	
Selectivity	discrimination from non-nanoparticles of the same composition	Yes	

	discrimination from non-nanoparticles of another composition (matrix particles)	Yes	
	discrimination from nanoparticles of another composition	Yes	with contrast variation techniques
	Impurities	N/A	
Measures aggregation	Is it possible to measure aggregation or agglomeration of particles?	Yes	Restricted to small agglomerates/aggregates (typically <100nm)
Measures individual particles	Does this CM measure individual particles?	No	
Counting constituent particles in aggregations	Is the method able to count constituent particles in aggregates?	Yes	
Composition	Does this CM analyse composition?	No	
Specification of the type of size (diameter)	Specify: for example hydrodynamic…	physical length	based on density differences
Destructive method or not	Is it a destructive method?	No	only if a synchrotron is used as a high flux X-ray source
Other Specificity			
Vacuum	Does the method operate under vacuum?	No	Yes, if required
Sample support	Does this CM need preparation on suited supports?	No	

4.3.3 Ultrasonic spectroscopy (USSp)

Table 11: Template for characterisation of USSp

Criteria (generally)	Criteria (more specific)	Characterisation (Yes/No)	Notes
Nanoparticles in powder or liquid suspensions or embedded in a matrix	Dispersed in liquids	Yes	
	Solid particulate form	No	
	Dispersed or embedded in different kinds of matrices	No	
Dispersibility by dispersion protocols	Dispersible in aqueous media	Yes	
	Dispersible in non-polar liquids	Yes	If not too viscous
	Dispersible in polar liquids	Yes	If not too viscous
	Dispersible in material-specific media	No	
	Can be aerosolized	No	
Substance Nature	Inorganic	Yes	
	Size-dependent absorption / fluorescence	Yes	
	Carbon based	Yes	
	Organic, particulate	Yes	But not always
	Organic, non-particulate	No	
	Biological	No	
	Composite	No	because of solid matrix
	Other	No	
Composite	Core/shell	Yes	Particle outer size
	Multiple coatings	Yes	Particle outer size
	A mix of two or more different materials	No	Yes, particle outer size, if mixture is known
Number of nanoscaled dimensions	1	No	
	2	No	
	3	Yes	
Shape of nanoparticles	Sphere or similar	Yes	
	Equiaxial	Yes	
	Tubes, fibres, rods	No	
	Flakes and discs	No	
	Other		

Thermal degradation sensitivity	Above 0°C	No	
	Sensitivity above 25°C	Yes	
	Sensitivity above 37°C	Yes	
	Sensitivity above 50°C	Yes	
	Sensitivity above 100°C	Yes	
	Sensitivity above 150°C	Yes	
	Sensitivity above 500°C	Yes	
	Sensitivity above 1000°C	Yes	
Cooling degradation sensitivity	Sensitive below 25 °C	Yes	
	Sensitive below 0 °C	Yes	
	Sensitive below -18 °C	Yes	
	Sensitive below -35 °C	Yes	
	Sensitive below -78 °C	Yes	
	Sensitive below -195 °C	Yes	
E- beam sensitivity	e- beam sensitive	Yes	
	Not e-beam sensitive	Yes	
Sample dispersity and modality	Monodisperse sample	Yes	
	Polydisperse sample	Yes	Difficult for NPs if broad PSD
	Monomodal sample	Yes	
	Multimodal sample	Yes	Difficult for NPs if broad
Conductivity properties	Conductive	Yes	
	Semiconductive	Yes	
	Insulator	Yes	
Magnetic properties	Magnetic	Yes	
	Non magnetic	Yes	
Functionalization / no functionalisation	Functionalised	Yes	
	Not functionalised	Yes	
Agglomeration/ aggregation state	Nanoparticles are aggregated	Yes	Reflects internal lengths (e.g. pore size, inter-particle distance)
	Nanoparticles are not aggregated	Yes	
	Nanoparticles are agglomerated	Yes	Reflects internal lengths (e.g. pore size, inter-particle distance)
	Nanoparticles are not agglomerated	Yes	
counting, separative or ensemble techniques	Single particle counting	No	
	Calculate number or concentration from ensemble methods	Yes	

	Method combination (hyphenated methods)	No	
Working range	Size range	1 nm – 100 µm	
	Concentration range	>1 vol.-%	
	Minimum needed sample amount	100 mL	
	Linearity/proportionality	No Yes	No, with regard to size Yes, with regard to quantity (volume) for dilute suspensions
	Limits of detection/quantification	N/A	Depends on density/ thermo-acoustic contrast and size
	Sensitivity (Counting efficiency) as a function of size	N/A	
Limits of detection/quantification	What is the lower limit to detect	N/A	
Trueness	Indicate the trueness of this CM	N/A	Size: “falseness” if wrong model parameters (e.g. viscosity, sound speed) ot if too a high particle concentration (>> 10 vol.-%)
Trueness in weighting the size fractions	Specify the trueness in weighting the size fractions of this CM	N/A	Volume weights: “falseness” if frequency range too narrow or PSD too broad (low information content below 5 µm)
Robustness	Specify the robustness of this CM	good	
Precision	Specify the precision of the CM	N/A	PSD average (modes, median, (power) mean): high PSD width: high
Resolution	Specify the resolution of this CM	poor	For NPs
Size distribution	Is it possible to measure size distribution?	Yes	
Selectivity	discrimination from non-nanoparticles of the same composition	Yes	
	discrimination from non-nanoparticles of another composition (matrix particles)	Yes	If similar acoustic properties
	discrimination from nanoparticles of another composition	Yes	If similar acoustic properties

	Impurities	N/A	
Measures aggregation	Is it possible to measure aggregation or agglomeration of particles?	No	
Measures individual particles	Does this CM measure individual particles?	No	
Counting constituent particles in aggregations	Is the method able to count constituent particles in aggregates?	Yes	see “internal lengths” above
Composition	Does this CM analyse composition?	No	
Specification of the type of size (diameter)	Specify: for example hydrodynamic…	“acoustophoretic diameter”	For inorganic particles in water (i.e. high-frequency hydrodynamic diameter) which is approx. the specific surface area
Destructive method or not	Is it a destructive method?	No	
Other Specificity			
Vacuum	Does the method operate under vacuum?	No	
Sample support	Does this CM need preparation on suited supports?	No	

4.3.4 X-ray diffraction (XRD)

Table 12: Template for characterisation of XRD

Criteria (generally)	Criteria (more specific)	Characterisation (Yes/No)	Notes
Nanoparticles in powder or liquid suspensions or embedded in a matrix	Dispersed in liquids	No	Yes for high concentrations
	Solid particulate form	Yes	
	Dispersed or embedded in different kinds of matrices	Yes	If there is no interference from the matrix
Dispersibility by dispersion protocols	Dispersible in aqueous media	No	Yes after successful deposition on substrates
	Dispersible in non-polar liquids	No	Yes after successful deposition on substrates
	Dispersible in polar liquids	No	Yes after successful deposition on substrates
	Dispersible in material-specific media	No	Yes after successful deposition on substrates
	Can be aerosolized	No	Yes after successful deposition on substrates
Substance Nature	Inorganic	Yes	
	Size-dependent absorption / fluorescence	Yes	
	Carbon based	Yes	
	Organic, particulate	Yes	
	Organic, non-particulate	Yes	
	Biological	No	
	Composite	Yes	
	Other		
Composite	Core/shell	No	Only information on crystal size
	Multiple coatings	No	Only information on crystal size
	A mix of two or more different materials	No	Only information on crystal size
Number of nanoscaled dimensions	1	No	
	2	No	
	3	Yes	The material has to be crystalline
Shape of nanoparticles	Sphere or similar	Yes	If particle is single crystalline
	Equiaxial	Yes	If particle is single crystalline

	Tubes, fibres, rods	No	
	Flakes and discs	No	
	Other	No	
Thermal degradation sensitivity	Above 0°C	No	
	Sensitivity above 25°C	Yes	
	Sensitivity above 37°C	Yes	
	Sensitivity above 50°C	Yes	
	Sensitivity above 100°C	Yes	
	Sensitivity above 150°C	Yes	
	Sensitivity above 500°C	Yes	
	Sensitivity above 1000°C	Yes	
Cooling degradation sensitivity	Sensitive below 25 °C	Yes	
	Sensitive below 0 °C	Yes	
	Sensitive below -18 °C	Yes	
	Sensitive below -35 °C	Yes	
	Sensitive below -78 °C	Yes	
	Sensitive below -195 °C	Yes	
E- beam sensitivity	e- beam sensitive	Yes	
	Not e-beam sensitive	Yes	
Sample dispersity and modality	Monodisperse sample	Yes	
	Polydisperse sample	Yes	
	Monomodal sample	Yes	
	Multimodal sample	Yes	
Conductivity properties	Conductive	Yes	
	Semiconductive	Yes	
	Insulator	Yes	
Magnetic properties	Magnetic	Yes	
	Non magnetic	Yes	
Functionalization / no functionalisation	Functionalised	Yes	
	Not functionalised	Yes	
Agglomeration/ aggregation state	Nanoparticles are aggregated	Yes	If particle is single crystalline
	Nanoparticles are not aggregated	Yes	If particle is single crystalline
	Nanoparticles are agglomerated	Yes	If particle is single crystalline
	Nanoparticles are not agglomerated	Yes	If particle is single crystalline
counting, separative	Single particle counting	No	

or ensemble techniques	Calculate number or concentration from ensemble methods	No	
	Method combination (hyphenated methods)	No	
Working range	Size range	> 5 nm	Range dependent on instrument type, sample type and preparation
	Concentration range	N/A	Depends on chemical composition.
	Minimum needed sample amount	100 mg	
	Linearity/proportionality	Yes	
	Limits of detection/quantification	< 100 nm	
	Sensitivity (Counting efficiency) as a function of size	medium	
Limits of detection/quantification	What is the lower limit to detect	5 nm to 10 nm	Dependent on Z, instrument type, sample prep, etc.
Trueness	Indicate the trueness of this CM	good	
Trueness in weighting the size fractions	Specify the trueness in weighting the size fractions of this CM	good	
Robustness	Specify the robustness of this CM	average	Strong dependency on sample preparation
Precision	Specify the precision of the CM	good	
Resolution	Specify the resolution of this CM	5 nm to 10 nm	
Size distribution	Is it possible to measure size distribution?	No	
Selectivity	discrimination from non-nanoparticles of the same composition	Yes	
	discrimination from non-nanoparticles of another composition (matrix particles)	Yes	
	discrimination from nanoparticles of another composition	Yes	
	Impurities	No	If crystalline, sometimes Yes.
Measures aggregation	Is it possible to measure aggregation or agglomeration of particles?	No	

Measures individual particles	Does this CM measure individual particles?	No	
Counting constituent particles in aggregations	Is the method able to count constituent particles in aggregates?	No	
Composition	Does this CM analyse composition?	Yes	
Specification of the type of size (diameter)	Specify: for example hydrodynamic…	Equivalent diameter, crystalline size	
Destructive method or not	Is it a destructive method?	No	
Other Specificity			
Vacuum	Does the method operate under vacuum?	No	
Sample support	Does this CM need preparation on suited supports?	No	

4.4 Integral sizing methods

Additional to those methods that resolve the distribution of particle size there are a few methods (integral sizing 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.

4.4.1 BET for determination of specific surface area

Table 13: Template for characterisation of BET for VSSA

Criteria (generally)	Criteria (more specific)	Characterisation (Yes/No)	Notes
Nanoparticles in powder or liquid suspensions or embedded in a matrix	Dispersed in liquids	No	
	Solid particulate form	Yes	
	Dispersed or embedded in different kinds of matrices	No	
Dispersibility by dispersion protocols	Dispersible in aqueous media	No	
	Dispersible in non-polar liquids	No	
	Dispersible in polar liquids	No	
	Dispersible in material-specific media	No	
	Can be aerosolized	No	
Substance Nature	Inorganic	Yes	
	Size-dependent absorption / fluorescence	No	
	Carbon based	Yes	
	Organic, particulate	Yes	
	Organic, non-particulate	Yes	
	Biological	No	
	Composite	Yes	
	Other		
Composite	Core/shell	Yes	Particle outer size

	Multiple coatings	Yes	Particle outer size
	A mix of two or more different materials	Yes	Particle outer size
Number of nanoscaled dimensions	1	No	
	2	No	
	3	Yes	Size derived from measured surfaces as available for gas sorption
Shape of nanoparticles	Sphere or similar	Yes	Size derived from measured surfaces as available for gas sorption
	Equiaxial	Yes	Size derived from measured surfaces as available for gas sorption
	Tubes, fibres, rods	No	
	Flakes and discs	No	
	Other		
Thermal degradation sensitivity	Above 0°C	No	
	Sensitivity above 25°C	Yes	
	Sensitivity above 37°C	Yes	
	Sensitivity above 50°C	Yes	
	Sensitivity above 100°C	Yes	
	Sensitivity above 150°C	Yes	
	Sensitivity above 500°C	Yes	
	Sensitivity above 1000°C	Yes	
Cooling degradation sensitivity	Sensitive below 25 °C	Yes	
	Sensitive below 0 °C	Yes	
	Sensitive below -18 °C	Yes	
	Sensitive below -35 °C	Yes	
	Sensitive below -78 °C	Yes	
	Sensitive below -195 °C	Yes	
E- beam sensitivity	e- beam sensitive	Yes	
	Not e-beam sensitive	Yes	
Sample dispersity and modality	Monodisperse sample	Yes	
	Polydisperse sample	Yes	
	Monomodal sample	Yes	
	Multimodal sample	Yes	
Conductivity proper-	Conductive	Yes	

ties	Semiconductive	Yes	
	Insulator	Yes	
Magnetic properties	Magnetic	Yes	
	Non magnetic	Yes	
Functionalization / no functionalisation	Functionalised	Yes	
	Not functionalised	Yes	
Agglomeration/ aggregation state	Nanoparticles are aggregated	No	
	Nanoparticles are not aggregated	Yes	
	Nanoparticles are agglomerated	Yes	
	Nanoparticles are not agglomerated	Yes	
counting, separative or ensemble techniques	Single particle counting	No	
	Calculate number or concentration from ensemble methods	No	
	Method combination (hyphenated methods)	No	
Working range	Size range	1 nm to ~10 µm	
	Concentration range	only 100 %	pure dried material is needed, normally as powder
	Needed sample amount	~100 mg	
	Linearity/proportionality	Yes	
	Limits of detection/quantification	1 nm	
	Sensitivity (Counting efficiency) as a function of size	good	
Limits of detection/quantification	What is the lower limit to detect	1 nm	
Trueness	Indicate the trueness of this CM	mean	the values are often apparent BET surfaces. The surfaces are not real surfaces for large specific surfaces. The measured surface depends often on the type of gas used for measurement.
Trueness in weighting the size fractions	Specify the trueness in weighting the size fractions of this CM	poor	
Robustness	Specify the robustness of this CM	good	for a defined SOP or the ISO standard ISO 9277:2010.
Precision	Specify the precision of the CM	good	
Resolution	Specify the resolution of this CM	good	
Size distribution	Is it possible to measure size dis-	No	

	tribution?		
Selectivity	discrimination from non-nanoparticles of the same composition	No	
	discrimination from non-nanoparticles of another composition (matrix particles)	No	
	discrimination from nanoparticles of another composition	No	
	Impurities	N/A	
Measures aggregation	Is it possible to measure aggregation or agglomeration of particles?	No	
Measures individual particles	Does this CM measure individual particles?	No	
Counting constituent particles in aggregations	Is the method able to count constituent particles in aggregates?	Yes	surfaces are measured as long as the surface is available for gas sorption
Composition	Does this CM analyse composition?	No	
Specification of the type of size (diameter)	Specify: for example hydrodynamic…	specific surface areas	surface areas in square meters per gram of particles
Destructive method or not	Is it a destructive method?	No	as long as the particles are stable in high vacuum
Other Specificity			
Vacuum	Does the method operate under vacuum?	Yes	
Sample support	Does this CM need preparation on suited supports?	No	

4.5 Templates for real-world method performance criteria

An empty template that specifies which parameters have to be determined in the practical evaluation with the selected materials ("real-world performance data") is presented. To the pure CM performance criteria (yellow), economic criteria such as instrument time cost, duration of sample preparation, analysis, evaluation, etc. (green) are added. This template should be used by the partners to fill in the results of the practical evaluation, so that in the end there will be a filled template for each material/method combination.

Table 14: Real-world method performance criteria template for tier 1 and tier 2 methods

Criteria (generally)	**Criteria (more specific)**	**Characterisation (Yes/ No)**	**Notes**
counting, separative or ensemble techniques	Single particle counting		
	Calculate number or concentration from ensemble methods		
	Method combination (hyphenated methods)		
Working range	Size range		
	Concentration range		
	Needed sample amount		
	Linearity/proportionality		
	Limits of detection/quantification		
	Sensitivity (Counting efficiency) as a function of size		
Limits of detection/quantification	What is the lower limit to detect		
Trueness	Indicate the trueness of this CM		
Trueness in weighting the size fractions	Specify the trueness in weighting the size fractions of this CM		
Robustness	Specify the robustness of this CM		
Precision	Specify the precision of the CM		
Resolution	Specify the resolution of this CM		
Size distribution	Is it possible to measure size distribution?		
Selectivity	discrimination from non-nanoparticles of the same composition		

	discrimination from non-nanoparticles of another composition (matrix particles)		
	discrimination from nanoparticles of another composition		
	Impurities		
Measures aggregation	Is it possible to measure aggregation or agglomeration of particles?		
Measures individual particles	Does this CM measure individual particles?		
Counting constituent particles in aggregations	Is the method able to count constituent particles in aggregates?		
Composition	Does this CM analyse composition?		
Specification of the type of size (diameter)	Specify: for example hydrodynamic…		
Destructive method or not	Is it a destructive method?		
Other Specificity			
Direct counting CM	Does the CM yield directly number-based size distribution?		
Convertibility to number weighted size distribution	How good is the quality of the conversion algorithms applied?		
Full size range acc. to EC definition	Can the CM access the full size range acc. to EC definition (1 nm – 100 nm)?		
Upper size	What is the approximate upper size limit of the CM?		
Lower size	Can the CM measure particles close to 1 nm?		
Smallest particle dimension	Has the CM access to the smallest particle dimension?		
Primary particle access	Can the CM access the primary particles within agglomerates and aggregates?		
CM availability	Is the CM widely used?		
Standard availability	Is a standard (ISO, CEN/IEC?,…)		

	for use of the CM for size analysis available?		
Potential as a Reference CM	Can the CM potentially be developed into a reference CM?		
Expenditure of time	Time for sample preparation		
	Measurement time		
	Data reduction time		
	Total time until result		
Costs	Instrument costs		
	Analysis costs		
Cost-efficiency	How good is the quality of the result in relation to the analysis costs?		
CM hyphenation	Can the CM be hyphenated to other CMs?		
Vacuum	Does the method require vacuum?		
Sample support	Does the CM require preparation on suited supports?		
Chemical degradation	Can the measurement process of the CM lead to chemical changes in the particles? (Degradation, formation of an oxide layer, ...)		

5 Conclusions

All available CMs with potential to be selected after proper evaluation as CMs to be applied explicitly for the reliable analysis of the size distribution of a nanomaterial according to the EC recommendation for a definition of a nanomaterial are specified individually on the basis of the general CM template supplied by the NanoDefine Technical Report D7.1.

The table template with its materials related and technical CM performance criteria has been filled in the "Characterisation" column, for each CM in part, with the corresponding score, e.g. "Yes/No", "good/average/poor", or "1 nm to 10 nm", etc. Furthermore, a "Comments" column has been added to the table for cases where the simple score characterisation in the pre-defined format is not unambiguous enough.

The completed NanoDefine Technical Report D7.1 templates serve to the CM evaluation, ranking and pre-selection. A new template with real-world method performance criteria (including economic criteria) has been defined. Based on the unitary pre-characterisation of the selected materials this template has to be completed at a later stage. This will enable to tag the tier 1 and tier 2 methods according to their suitability for certain materials and the decision criteria and priorities developed in NanoDefine Technical Report D7.3. In turn, the template with real-world method performance criteria from NanoDefine Technical Report D7.2 will be further reviewed for overall consistency, if necessary provided with more in-depth specific information, and finally harmonized so that the *NanoDefine Methods Manual* (NanoDefine Technical Report D7.6) can be accordingly created.

6 References

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