Welcome to the first NanoDefine news bulletin

In response to a specific EU FP7 Call, the NanoDefine project was launched in November 2013 to develop the analytical methodology required to support the implementation of the EU recommendation for a definition of nanomaterial (2011/696/EU).

This first NanoDefine bulletin will inform key stakeholders, such as industry, regulatory bodies and the scientific community on the progress of work and highlight latest project developments achieved so far within the seven technical workpackages addressing the following main areas:

- techniques and methods review and evaluation
- new instrument and software innovations
- development of new standards and reference materials
- guidance for implementing the EU definition
- limitations and bottlenecks

Analytical challenges

The practical implementation of the EC recommendation for a definition of nanomaterial (NM) will be a tremendous analytical challenge especially for small and big industries that need to comply with this new provision, but also for regulatory bodies, as the definition includes all particulate materials with a size distribution below 100 nm, which in turn means that a lot of conventional materials, such as pigments, fillers, additives etc., need to be classified accordingly.

To support the implementation of the proposed definition, NanoDefine is developing integrated approach consisting of a continuous performance evaluation of relevant techniques, improvement of available software and instruments, and of proper sample preparation methods, and the design of a new 2-tiered measurement strategy based on a decision-support system in the form of a method manual and an eTool to easily select the most appropriate (or combination of) methods to classify materials according to the EC definition. The measurement concept includes techniques of increasing complexity that complement and support each other by adequate procedures for sampling, sample preparation, instrument calibration and data analysis as well as plausibility checks with minimum performance requirements.
Technique evaluation and method development

Sample preparation, dispersion and collection has been realised as an utmost important first step to ensure the correct and reproducible measurement of the size of nanoparticles. This was the main goal of WP2, where representative sampling strategies have been identified and methods developed, tested and improved to disperse nanoparticles and to produce stable dispersions with a maximum of mainly primary constituent particles. Electron microscopy (EM) has been used as “gold standard” to distinguish between large primary constituent particles and non-dispersible aggregates.

The main achievements in WP2 of the first two years of the project can be summarised:

- new sample preparation protocols (SOPs) for EM have been developed by analysing Fe₂O₃ nanoparticles in polyethylene (PE), and TiO₂ nanoparticles in sunscreen
- new dispersion protocols (SOPs) have been established for 11 substances and their performance evaluated by detailed qualitative and quantitative TEM analysis

In WP3, “the methods evaluation hub”, the applicability and performance of relevant techniques and methodologies have been evaluated to:

- recommend the most powerful techniques to be further developed in WP4 and WP5, and for standardisation in WP6 (Table 1);
- continuously benchmark methods under development against a set of quality criteria, taking into account purpose, sample complexity and acceptable limitations
- assess the relationship between the volume specific surface area and the number based particle size distribution based on well characterized NanoDefine materials;
- compare with new analytical developments for further improvement of the newly developed techniques.

Table 1: Evaluation, ranking and selection of techniques in NanoDefine

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<tr>
<th>Recommended to WP4 (Screening methods)</th>
<th>Recommended to WP5 (Confirmatory methods)</th>
<th>Recommended to WP6 (Methods ready for direct validation or standardisation)</th>
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- In addition, the performance of available particle sizing techniques to all NanoDefine materials including real-world samples is assessed and ranges of applicability established.
In **WP4** the most promising screening (tier 1) methods will be tested, such as:

- Analytical centrifugation (AC)
- Single particle ICP-MS (sp ICP-MS)
- Electric mobility spectroscopy (EMS)
- Particle tracking analysis (PTA)

The aim is to:

- establish a set of validated, cost-efficient, robust and easily implementable methods for rapid distinction between nano/non-nano according to the EC definition;
- improve instruments and related methods to increase their applicability and cost efficiency;
- determine their limitations and obtain maximum application ranges not only for ideal calibration materials but also real world materials.

Selected methods are validated within each partner lab according to international guidelines / standards and the default approach developed in **WP6** (see below).

Where methods developed and tested in WP4 do not work anymore, because the materials to be analysed and classified are too complex, NanoDefine is testing and establishing confirmatory (tier 2) methods in WP5. These more in-depth approaches will in particular help to classify "real-world" samples, such as industrial materials with different shapes and wide size distributions, non-dispersible powders, or complex products or matrices, with characteristics outside the applicability of rapid screening methods developed in WP4. Also new software will be developed, e.g. for automated image analysis, spICP-MS data evaluation, or to generate reliable number based size distributions from non-counting methods (such as BET).

### Instrument and software innovations

NanoDefine will develop new measurement instruments and software to:

- analyse samples that cannot be classified based on the tier 1 methods (e.g. too poly-disperse, very complex shapes etc.)
- measure particles in the lower nm size range (1 – 20 nm)
- reduce costs per analysis by simplification of procedures

New measurement techniques approaches will include:

1. New EM sample preparation by electrospray deposition to improve sample deposition from aqueous suspensions on carriers (grids) for TEM analysis (Figure 1).
Instrument and software innovations (ii)

2. New high vacuum baking station for TEM grids pre-treatment to improve imaging of nanomaterials (Figure 2).

3. Fully automated image analysis for mono- and poly-modal distribution (complex shapes need expert knowledge and manual removal of selected particles (Figure 3). Automated operation by means of an “auto EM toolbox” combining pre-set particle recognition, measurement, counting and image analysis.
4. Developing specific new software (the “NanoDefine ParticleSizer”) for automated particle size analysis of recorded EM images.

5. Develop Field Flow Fractionation (FFF) technique coupled to particle counting detectors (liquid and aerosol based) that can be applied to difficult product samples, such as sunscreen and various food products, such as SiO2 particles extracted from powdered tomato soup (Figure 4). This method obtains true number based size distributions after high-resolution separation.

6. Particle counting methods improved and developed include:

- HRMS (High Resolution Mobility Sizer) prototype for the analysis of particles in the lowest 1-20 nm size range;
- evaluation of the performance of sp-ICPMS;
- software and hardware for NTA (Nanoparticle Tracking Analysis) was improved regarding particle measurements.
Development of new standards and reference materials

To cover a broad range of representative materials, with different shapes, size ranges and chemical compositions available in reasonable amount samples, including most relevant industries and applications, like pigments, fillers, polymers, cosmetics, food and active materials, 16 materials have been made available (see Figure 5).

![NanoDefine test materials reflecting different shapes, sizes, compositions and applications](image)

Figure 5: NanoDefine test materials reflecting different shapes, sizes, compositions and applications

Many samples require the development of dispersion protocols and new characterization strategies. Therefore, unified mono- and poly-disperse calibrants were provided for all NanoDefine labs (Figure 6). In the second half of the project, a panel of appropriate test substances and products will be further developed into reference materials.

![SEM images of NanoDefine substances and products](image)

Figure 6: SEM images of NanoDefine substances and products

**WP6** is providing a platform for intra- and inter-laboratory method harmonisation, validation and standardisation to ensure a high measurement and data quality and comparable performances with the aim of developing validated methods further to New Work Item Proposals (NWIP) for international standardisation. A project liaison with CEN/TC 352 has been established.
Guidance for the implementation of the EU definition

To support end-users such as industries or regulatory bodies in deciding whether a material is nano or not according to the EC definition, NanoDefine will create an intelligent, decision support tool in WP7: the NanoDefiner eTool and Manual (Figure 7).

The “NanoDefiner” will integrate the materials classification system, the decision criteria and ranking system, and the technical performance criteria including method applicability to different material groups, to polydisperse samples, measurement range and medium, and the capacity to measure aggregates, primary particles and/or non-spherical particles.

A specific software will pool together results and conclusions from method evaluation and development and from findings obtained from validation and case studies. This eTool will consider material type, purpose, required data quality (including confidence level) and economic parameters, and guide the user to the most reliable and cost-efficient measurement method to identify/classify any substance or mixture according to the definition. It will allow the user to extend the measurement to the widest possible range of substances, mixtures, including also complex products and matrices.

A "Methods Manual" will support the eTool and provide detailed information on capabilities, strengths and weaknesses of each suggested method. Both the eTool and manual will be available as software and printed guidance documents, and integrated into the web platform on nanomaterials and nanotechnology as announced by the European Commission. Case studies will finally demonstrate the real-world applicability of the developed methods.
Limitations and bottlenecks

Even though there are measurement techniques (MTs) available for determining particle sizes in the relevant range of 1 nm to 10 μm, only a few of them have been validated. In particular, validated and standardised methods for counting particles in suspensions are lacking.

PTA (particle tracking analysis) does not cover the whole range and is not fully validated, as is the case for spray-DEMA (differential electrical mobility analysis) and TRPS (tunable resistive pulse sensing). Hence, the only way to reliably count particles are imaging techniques, such as SEM (scanning electron microscopy) or TEM (transmission electron microscopy), which nevertheless require the deposition of particles on a suitable substrate to ensure the representativeness of the deposited particles. Imaging techniques have the further advantage of detecting constituent particles within aggregates and agglomerates.

Other suitable MTs include SAXS (small-angle X-ray scattering), XRD (X-ray diffraction) and BET (Brunauer–Emmett–Teller Method) and MTs based on a particle’s mobility, such as DLS (dynamic light scattering), and AC (analytical disc- and cuvette centrifugation), but they typically yield equivalent diameters that are similar to the external (aggregate) dimension or the dimension of large “pores”, and in any case are larger than the constituent particles.

NanoDefine is evaluating the performance of all these measurement techniques (MTs) for real-world particulate materials. As the intrinsically measured size distributions are typically non-number-weighted, they are expected to perform well for low and moderate poly-dispersity. Moreover, most techniques relevant for tier 1 (suspension) cannot resolve the internal structure of aggregates or probe aggregate properties. Their ability to reliably assess particulates according to the EC definition is restricted to materials that consist of individual particles or of highly-dispersible aggregates.

Based on our data, matching nano / non-nano classification by Tier 1 (powder) indicates that this validity criterion is fulfilled. Otherwise, Tier 2 (imaging) can help. But although the tested, further developed and improved analytical tools already allow the practical implementation of the EC definition, the following still significant gaps remain:

✓ standardised procedures for preparing representative samples for imaging techniques

✓ validation of promising MTs (not only imaging techniques and including high standards for sample handling and data evaluation, with different kinds of reference materials (RMs)

✓ further development of RM for x < 10 nm, of multi-disperse RMs with defined number-based mixing ratio and of RMs for particle number concentration
NanoDefine Project Partners