White Paper

Regulation compliance in the nanometer to submicron size range with Opti-Count® certified number concentration particle reference materials

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1. Executive Summary

Accurate, traceable, and standardized particle characterization is essential for both regulatory compliance and scientific reliability. The introduction of tools such as the LUMiSpoc® particle counter and sizer and Opti-Count® particle reference materials addresses a longstanding gap by enabling validated measurements of number concentration and number-weighted size distribution in nanoscale materials. These developments represent a foundational step toward comparable methods and the broader adoption of metrologically sound practices in quality control and regulation compliance for nanoparticles.

Engineered nanomaterials (ENMs) are increasingly integrated into a wide array of commercial products — including pharmaceuticals, cosmetics, electronics, coatings, and agricultural technologies. As their applications expand, so does the need for accurate, standardized methods to measure key parameters like the particle number concentration and number-based size distribution.

The legal and regulatory landscape around number-based characterization of nanomaterials continues to evolve requiring accurate, traceable, and standardized particle analysis to be used for regulatory compliance, monitoring of quality, and ensuring scientific reproducibility. However, no such reference material (RM) exists to be used as the benchmark for nanoscale particle characterization less than a micrometer. The introduction of instruments such as the LUMiSpoc® — which provides high-resolution particle counting over a broad range of concentrations and sizes — together with the newly developed Opti-Count® RMs, addresses this problem by enabling validated measurements of both the particle number concentration as well as the number-weighted size distribution at the nanoscale. Opti-Count® RMs – developed in partnership between Dr. Lerche KG and Applied Microspheres GmbH – are a first-of-their-kind product; no other such RMs currently are available. They offer certified values for number concentrations and indicative values for the size distribution quantiles (D_{16}, D_{50}, D_{84}) at the nanoscale, and are manufactured following ISO 17034.

These developments represent a foundational step toward comparable method and the broader adoption of metrologically sound practices in quality control and regulation compliance for nanoparticles. Together, the LUMiSpoc® and Opti-Count® establish a new gold standard for number-based particle quantification. They enable inter-method comparison, support regulatory harmonization, and lay the groundwork for future RMs.

2. Contents

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3. Introduction

Submicron and nanomaterials are very important classes of materials that are paramount in the development of smart technologies. Although they have existed – due to natural processes – in abundance on Earth since its origin, it is only since the onset of the Industrial Revolution that the boom in the creation and exposure of so-called incidental nanomaterials – as a by-product of man-made processes (e.g. burning of fossil fuels) – has resulted in a new threat to health and the environment. In the last few decades emergence of engineered nano- and submicron-materials (ENMs) – due to new technologies – has led to a growth in the prevalence of nanoscale materials on the market and subsequently in the environment. ENMs are designed specifically for use in/as commercial products, although incidental nanomaterials may also be used (e.g. carbon black). Their small sizes and subsequent large surface area-to-volume ratios impart new and improved properties on commercial products drastically increasing their use.

Thousands of nanomaterials-containing consumer products are currently available on the market, worth over $25 billion (EUR 21.5 billion) as of 2025 and continuing to grow. Commercially available products incorporating ENMs span a wide range of industries, including battery materials, coatings, healthcare, functionalized inks, polishing slurries, food products, personal care items, and pharmaceuticals.

Global ENMs Market Value

25 billion USD in 2025

20% annual growth rate

The scale of nanomaterial integration, their inherent properties, and the fact that ENMs – such as nanoplastics – have never yet been dealt with by living organisms raises the concern that there are environmental and health risks associated with their exposure and accumulation.

In order to address these risks, laws and regulations are put in place that accurately define under what criteria must commercial and industrial use of nanomaterials be monitored and reported. Therefore, legal authorities have been tasked with defining nanomaterials and their monitoring. One recommendation being adopted by many international organizations binds the definition of nanomaterial not only to size, but additionally to the number of particles in a sample: the so-called number concentration. Such regulations require that reported values for compliance are compared to benchmark values, which are provided by reference materials (RMs). Unfortunately, currently there exists no RMs at the nanoscale for the purpose of number-based characterization techniques.

This white paper outlines an overview of the current regulatory landscape, as well as the challenges and future directions for developing and improving nanoscale particle RMs. Additionally, it introduces Opti-Count® particle RMs: first-of-their-kind nanosized monomodal number concentration RMs for liquid laser-based particle counting techniques.

4. The State of Nanomaterial Regulation

Defining Nano- and Sub-micrometer Materials

Nanomaterials are a class of material defined by their size. The increase in surface area, as a result of their small size, means that a large number of atoms are free at the surface providing novel properties. Upon an initial glance, the term nanomaterial should be self-defining; with the definition set by the units (nanometer is an SI unit defined as 10^{-9} m). Unfortunately, this is far from the case and can lead at the very least to confusion and at the most in failure to comply with or result in misunderstanding of regulations. A single regulatory definition of nanomaterials is difficult to find, as within and between different countries various official definitions for nanomaterials exist. Ramussen, et. al. have recently published an extensive list of governing bodies and regulatory agencies around the world and their definition of what constitutes a nanomaterial. They nicely show that although some definitions are shared between agencies, there is no single agreed upon definition that has been adopted internationally. Deeper analysis makes it clear that the agenda of the regulatory agencies themselves, strongly influences which of the definitions are adopted.

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A good starting point for defining nanomaterials is to look towards international standards. ISO 80004-1:2023 defines nanomaterial as having any external dimension in the nanoscale or having an internal structure or surface structure in the nanoscale, which is itself defined as between 1 nm and 100 nm. This definition for nanomaterial has been generally accepted internationally,

A size cutoff of 100 nm while theoretically clear, is in practice artificial. For example, regulators that are concerned with health and environmental impacts of nanomaterials, such as the FDA in the USA, have observed that health risks are seen up to 1 µm and therefore move their cutoff to 1000 nm. Other scientists question the value of strict cutoffs as they do not indicate the true behavior of the particles nor all properties that may lead to their behavior and therefore do not fully address the size-risk relationship. This lends credence to flexibility in definitions so that they are based on the practical needs of specific industries, while still having governing guidelines as a foundation.

Recommended Definition Based on the Number Concentration

In the review article from Ramuessen, et. al. it is stated that some regulations and laws, e.g. the Toxic Controlled Substances Act from the USA, define nanomaterials not just by their size, but link them to also to an additional property – in this case mass percent; nanomaterials are any substance comprised of greater than 1 wt % particulates under 100 nm.

In comparison, the European Commission (EC) has issued guidelines that provide a regulatory definition of nanomaterials to ensure consistency across legislative instruments that is growing quickly in its adoption globally. First introduced in 2011 and revised in 2022, the definition is quantitative and applies to materials composed of solid particles. Nanomaterials are distinguished from conventional materials based on their number-weighted particle size distribution, with a material classified as a nanomaterial if 50% or more of its constituent particles, by number, have at least one external dimension between 1 nm and 100 nm. Importantly, the revised 2022 definition expands the scope to include not only individual particles, but also those found in aggregates and agglomerates, which must also be counted in the number-based particle size distribution. Compared to definitions used in other regions, the EC's definition is notably more quantitative and measurement-driven.

The European Union (EU) has amended several regulatory frameworks to specifically include this definition of nanomaterials — including the main chemical legislation (REACH), as well as product-specific regulations.

This shifts particle characterization drastically to requiring accurate determinization of both the number concentration and the number-based size distribution of nanomaterials.

While the EC definition is popular and being adopted in new regulations globally, no RM exists that can be used to validate nanoscale number-based method in order to standardize values. This leads to a lag between regulations being adopted and the ability for accredited methods to provide comparable results using RMs.

There is then a need for a range of RMs that are at the nanoscale and are number-weighted. Additionally, these RMs should allow for flexibility in definitions. It is necessary therefore to develop a range of nanoscale RMs at sizes below 1 µm down to 1 nm that can be detected for various counting techniques.

Defining What Should be Counted

Efforts to regulate nanomaterials face a recurring challenge: how to define what exactly counts as a nanomaterial. The EC and other standardization bodies such as ISO have emphasized the importance of number

concentration and particle size distribution. However, this immediately raises a practical question: *what exactly are we counting?,* with the revision in the definition of the EC requiring agglomerates and aggregates to be included in the analysis.

Real-world materials are rarely made up of isolated primary particles. Instead, they often exist as aggregates (strongly bound clusters), agglomerates (weakly bound particles), or mixtures of both. To ensure consistency, regulations must specify whether counting refers to the constituent parts (primary particles) or the entire morphological unit (aggregate/agglomerate). ISO provides helpful terminology here: *constituent parts* are any identifiable particulate within a larger particle. Thus, when reporting measurements, it is essential to state how particles were counted, what morphologies were included, and which constituent parts were considered.

Another regulatory challenge is distinguishing between different sources of nanomaterials. Not all nanoscale particles are ENMs. Many occur naturally, through geochemical or biological processes such as erosion, organic matter degradation, or biosynthesis. For example, viruses (20–500 nm) are technically nanoparticles and have even be harnessed as nanomaterial products, while organisms such as magnetotactic bacteria, coccolithophores, and diatoms all produce biogenic nanomaterials.

In contrast, anthropogenic nanomaterials include intentionally engineered ENMs and incidental particles generated as by-products of human activity. Properly distinguishing among natural, incidental, and ENMs is essential for environmental monitoring, risk assessment, and regulatory development and like the definition for nanomaterials may not be a “one-size-fits-all” requirement.

A related issue that has recently gained widespread attention is the prevalence of microplastics and nanoplastics in the environment. Although their definitions remain under international discussion, microplastics are generally considered to be 1 µm–5 mm in size, while nanoplastics fall within (1–1000) nm. These materials may be engineered or incidentally generated from the degradation and wear of larger plastics. Unlike engineered polymer nanoparticles, which are often spherical, environmentally derived nanoplastics may have irregular morphologies. Evidence suggests that microplastics and nanoplastics are pervasive. Nanoplastics have recently been identified as being easily formed through wear or even during aging of polymeric materials. The lack of methods to quickly identify nanoplastics additionally explains why primarily microplastics have been observed in the oceans that are significantly lower than the total concentration expected based on modelling. Nanoplastics existence may account for this “missing plastics” in the oceans.

Recent studies by our laboratory, using the liquid laser particle counting system the LUMiSpoc® (LUM GmbH), highlights the need for monitoring and regulation. An evaluation of water from laboratory, municipal, and commercial bottled sources found microparticles and nanoparticles in all samples, including treated drinking water. Such findings emphasize both the ubiquity of nanoscale particulates in the environment and the need for harmonized definitions and RMs to guide risk assessments and policy decisions.

Given this complexity, a range of RMs are urgently needed to cover all bases. Without standardized definitions, agreed counting methods, and benchmark materials – comprised of different sizes, materials, shapes, concentrations, etc. – comparisons between studies or across regulations are inconsistent. RMs would serve as a goal post against which particle size, morphology, and distribution can be measured, ensuring that reported data are both reproducible and comparable.

Health and Environmental Risks Leading to Regulation

Nanomaterials pose significant challenges to human health and the environment due to their small size and unique properties. Unlike natural materials, ENMs are synthetic and often not biodegradable, meaning ecosystems and biological systems lack effective mechanisms to process or adapt to them. Their nanoscale dimensions enable them to become airborne, travel long distances, and

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penetrate human, animal, and plant cells, where they may trigger respiratory and cardiovascular issues, cellular stress, genetic damage, or other toxic effects.

Plastic-based ENMs, particularly nanoplastics, are of special concern because they persist and accumulate in organisms, aggregate unpredictably, and resist natural degradation. They can also act as substrates for bacterial biofilms, potentially contributing to antimicrobial resistance — studies have shown that *E. coli* exposed to common microplastics formed durable biofilms that remained even after the plastics were removed. Metallic nanoparticles add another layer of complexity: copper nanoparticles, for example, can dissolve within cells, releasing highly toxic ionic species. These transformations highlight how the long-term behavior and functionality of nanoparticles are critical to understanding their risks, and why one-time studies are often insufficient.

Environmental exposure is substantial; an estimated 42,000 tonnes of microplastics are released annually from product use alone. Major sources include tribological wear, such as micro- and nanoplastic release from clothing during washing, which produces particles with irregular shapes, varied sizes, and mixed compositions. The shape, surface properties, solubility, charge, and aggregation state of these particles all strongly influence toxicity and environmental impact. To assess risk, it is essential to measure and track these parameters across the life cycle of nanomaterials, not just at a single point in time.

Given these risks, effective regulation is increasingly urgent as well as the use of RMs at the size-range. Together they will guide industry and ensure product safety and compliance. Robust monitoring and regulatory frameworks are necessary.

A compelling example of the importance of precise definitions and accurate characterization on regulation is the 2022 Judgment of the General Court Ninth Chamber, Extended Composition that involved the classification of titanium dioxide particles. Authorities had initially classified agglomerated particles as hazardous, based on the density of pure titanium dioxide. However, the court recognized that these agglomerates contained significant internal voids, resulting in much lower actual density and therefore substituting this new value into regulatory limits indicated that the exposure risk was under the critical limit. This case highlighted the critical interplay between accurate material definitions, property assessment, and risk classification.

Such legal precedents show how misclassification can result from unclear definitions, potentially leading to inappropriate risk mitigation strategies. Both commercial developers and regulators must have a comprehensive understanding of nanomaterials and their properties to ensure accurate risk assessment.

Regulatory compliance is essential for the commercialization of nanomaterial-based products. Several key regulations govern the use of nanomaterials, e.g.:

REACH Regulation (EC) No 1907/2006: Since January 2020, REACH includes specific provisions for nanomaterials, requiring manufacturers and importers to provide detailed chemical property and safety information.

EU Regulation (EU) 2023/2055: Restricts the intentional addition of synthetic polymer microparticles (including nanoplastics) to products.

Medical Devices Regulation (EU) 2017/745 and In Vitro Diagnostic Medical Devices Regulation (EU) 2017/746: Address the use of nanomaterials in medical devices, with stringent safety and performance requirements.

Cosmetic Products Regulation (EC) No 1223/2009: Covers cosmetic products containing nanomaterials, requiring notification and safety assessments.

Drinking Water Directive (EU) 2020/2184: Though not exclusive to nanomaterials, this directive includes monitoring microplastic pollutants that may degrade into nanoscale particles. Microplastics monitoring is now legally required. Public sector bodies, including environmental monitoring agencies and accredited laboratories, increasingly depend on reliable characterization techniques to comply with such regulations

Compliance with these frameworks increasingly depends on number-based characterization, particularly for assessing particle number concentration, an essential metric for dosage, exposure risk, and regulatory thresholds. This aligns with the EU

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Recommendation 2022/C 229/01, which emphasizes the importance of determining the amount of nanoscale particles in materials. Despite increasing research, there remains a critical lack of validated analytical methods for sampling, identifying, and quantifying micro- and nanoplastics. Moreover, comprehensive hazard and fate data are still insufficient for robust risk assessment.

The European Metrology Network for Pollution Monitoring is one such consortium addressing these challenges globally. Prioritizing and harmonizing limit-of-detection and quantification methods — including number-based concentration techniques — for effective monitoring of these particles in aquatic environments.

To meet these complex regulatory challenges, collaborative initiatives are gaining traction. One such effort is the NanoDatabase (nanodb.dk), developed by DTU Environment in collaboration with the Danish Ecological Council and Danish Consumer Council. This dynamic database compiles data on nanomaterials in consumer products and provides NanoRiskCat categorization, serving as both a scientific tool and consumer resource.

Despite progress, significant challenges remain. The unique properties of nanomaterials, combined with evolving understanding of their long-term impacts, demand continued research, standardization, and international coordination. Regulatory frameworks must keep pace with scientific developments to ensure the safe use, monitoring, and disposal of nanomaterials across industries.

Methods for Determining the Number Concentration of Nanomaterials

Number concentrations can be determined by 2 classes of measurement techniques: absolute counting methods which count individual particulates and ensemble methods which evaluate data based on a determined measured volume and through theory can calculate the number concentration. A specific form of ensemble method relies on fractionation such as in sedimentation techniques in which concentration of particles of particular sizes and densities separate from the original ensemble of particles, resulting in chromatographic analysis with higher resolution than in most ensemble methods. Such techniques are available in device by LUM GmbH such as the LUMiSizer® analytical centrifuge and the LUMiReader® PSA or X-ray a gravitational sedimentation analyzer possessing either VIS-NIR or X-ray radiation as a light source. Other

Injecting Opti-Count® reference material particles into a LUMiSpoc® for method validation. ©LUM GmbH

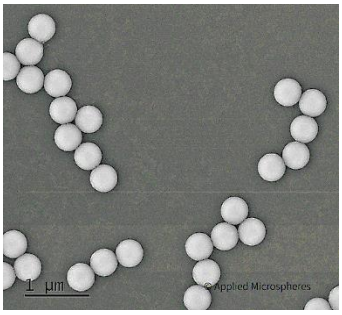
fractionation methods include such techniques as field flow fractionation and sieve analysis.

Development of Opti-Count® number concentration RMs was possible via analysis by the recently developed LUMiSpoc® by LUM GmbH; a counting method based on single particle light scattering and used for determining the number concentration and number-weighted size distribution of the particle system. Other particle counting techniques include extinction-based particle counters widely used, particularly in pharmaceutical and biomedical sectors, they typically operate within a different size and concentration range and may be more suited to low-concentration applications (<10,000 particles·ml^{-1}), Resistive pulse sensing (RPS) is used to count particles in electrolyte suspensions and measures electrical current or voltage drops as particles pass through a membrane pore. However, this method, like the Coulter Counter, may lead to destabilization and aggregation of nanoparticles, due to the electrolyte. More recently, these methods have been adapted for submicron-scale particles, such as TRPS and MRPS. Additionally, single-particle ICP-MS is increasingly used for particle number concentration determination. ICP-MS necessitates that particles or their markers be soluble in acids, meaning reference materials must be made from inorganic substances or must be labelled with chemicals that can be detected.

Recognizing the growing need for standardized number concentration methods, standardization bodies and metrology institutes have recently introduced several new technical standards and guidelines for additional particle characterization methods. These include ISO 23484:2023 for small-angle X-ray scattering (SAXS), ISO

19430:2024 for particle tracking analysis (PTA), and ISO/TS 24672:2023, which offers broader guidance on nanoparticle number concentration measurements. These efforts underscore the global momentum behind developing robust, validated methods for this critical parameter.

Electron microscopy has historically been a cornerstone for absolute particle counting, especially in nanomaterials metrology. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) allow direct visualization and enumeration of particles. Their resolution allows for the determination of particle shape and relatively easy separation of primary particles from agglomerates and aggregates for enhanced analysis. However, they are often limited by time-intensive sample preparation, low statistical throughput, and the requirement for vacuum conditions, which may alter the native (wet) state of the particles under study. Despite these drawbacks, electron microscopy remains an essential benchmark for validation and comparison.

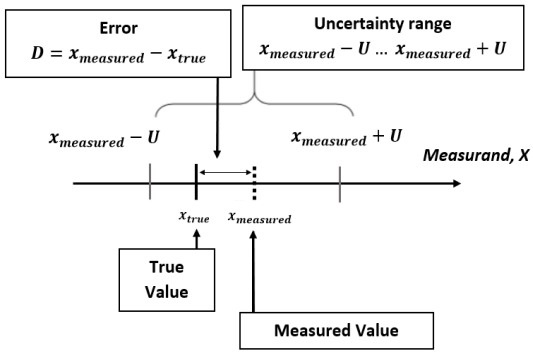


Color inverted transmission electron microscopy image of Opti-Count® particles. ©Applied Microspheres

It is important to recognize that each measurement technique has its strengths and limitations, and the accuracy, reproducibility, and comparability of results depend heavily on the availability and use of well-characterized RMs. The development and adoption of standardized RMs for nanoparticle number concentration are thus essential to support regulatory compliance, inter-laboratory consistency, and technological innovation across industries.

5. What are Reference Materials?

RMs are essential tools that play a critical role across academic research, industrial manufacturing, and governmental oversight. They enable reliable, comparable, and traceable measurements. In scientific research, RMs support reproducibility and comparability of results, allowing studies from different laboratories and regions to be meaningfully aligned.



RMs and CRMs provide with highest statistical precision a certified measurand value (x) with a well-defined uncertainty (U) in which the true value of a system is likely contained. ©

In industry, RMs play a key role in quality assurance, process optimization, and efficiency improvement. They enable manufacturers to monitor the use of ENMs precisely while ensuring that final products consistently meet required performance and safety standards.

For regulators and governmental agencies, RMs provide the measurement foundation necessary for effective policy implementation, compliance monitoring, and enforcement. They enable consistent interpretation of technical standards and ensure that regulated thresholds are applied uniformly.

According to ISO Guide 30 and ISO 17034:2017, the primary objective of any RM is to enable consistent and reliable characterization by providing a well-defined, stable benchmark. ISO defines the highest two levels of products to attain these reference values as RMs and certified reference materials (CRMs).

Definition of a RM

> *"A material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process."*

Definition of a CRM

> *"A reference material characterized by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its*

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associated uncertainty, and a statement of metrological traceability."

In the context of nanoparticle analysis — particularly for number concentration and number-weighted size distribution — RMs are essential for achieving the level of analytical accuracy demanded by regulations.

Measured measurand values during typical characterization are approximations of the true measurand value of the system. There are many sources of uncertainty (u or U) that exist and influence the measured value; altering it from the true value. Bias due to differences in the device, the analytical method, and the users tendencies are systematically applied to all values measured under the same conditions. On the other hand, random errors due to factors that are temporally present only effects the measured values under local conditions and therefore may change with time. These uncertainties are quantified during the development of CRMs and RMs. Certified values that are provided with RMs will therefore come with an uncertainty budget that indicates an estimate of the statistical range from the certified value in which true values likely exists. Full uncertainty budgets should therefore cover all known sources of uncertainty and include estimates of the batch inhomogeneity, stability during transport, and stability during storage and can take years to develop.

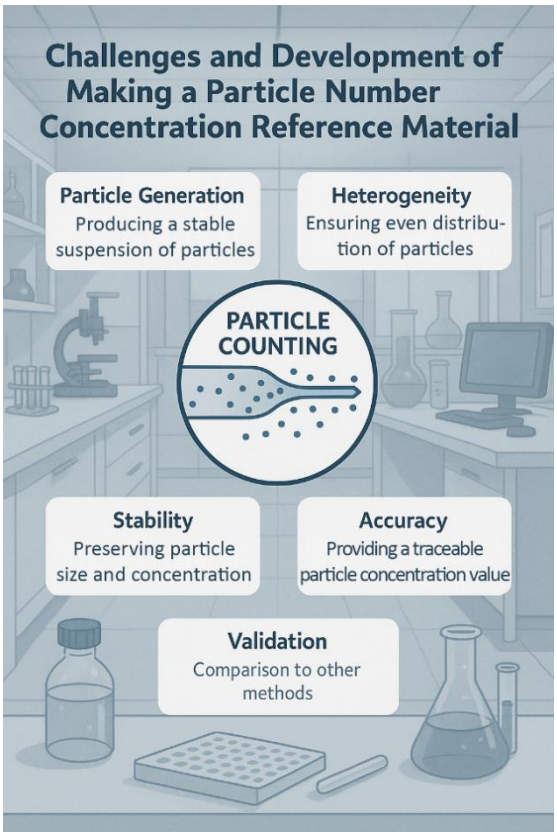
Count Statistics in Number Concentration Measurements

An often underemphasized but critical aspect of number concentration measurements is determining the statistically valid number of particles to count in order to meet regulatory requirements and ensure reliable data. Accurate particle counting is inherently governed by Poisson statistics, which define the relative count uncertainty based on the number of observed events (particles) – the relative uncertainty scales with the inverse of the square root of the count events.

To achieve a 95% confidence interval, a minimum of approximately 1,500 particles must be counted. For a 99% confidence interval, this number increases substantially to around 40,000 particles. In most practical applications, the required uncertainty typically falls somewhere between these two benchmarks.

However, these values assume ideal conditions where the counting strictly follows Poisson-distributed random sampling and does not include other sources of uncertainty. By keeping the error due to counting low the method uncertainty can be reduced. Many commonly used techniques that rely on image-based methods do not easily adhere to these values and generally count between 300 – 500 particles. To ensure reproducible and reliable count statistics, it is generally recommended to count at least 1,500 particles per sample. However, achieving this level of statistical robustness is time-consuming and challenging for many image analysis systems.

During the development of Opti-Count® reference materials, we have consistently aimed to count 40,000 particles or more per injection. Using the LUMiSpoc®, this process is both automated and efficient, providing the high reproducibility and precision required for dependable number concentration measurements.



Current Availability of Reference Materials and Challenges and Considerations in Developing a Number Concentration Reference Materials

As of the writing this white paper, neither RMs nor CRMs are commercially available that provide certified values for the particle number concentration in the nanoscale size range.

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However, there do exist RMs for particle size distributions, although these tend not to be number-weighted. A useful resource for finding RMs is COMAR, the centralized reference material database maintained by BAM, accessible at www.comar.bam.de.

A step towards developing an RM for number concentration was recently made by LGC through their development of an approximately 30 nm gold particle quality control material (QCM) — LGCQC5050 — with an assigned particle number concentration value of approximately 10^{11} ml^{-1}. Although not an RM this is a valuable stepping stone towards creating a variety of possible nanoscale number concentration RMs and CRMs and has been an inspiration in the development of Opti-Count® particles.

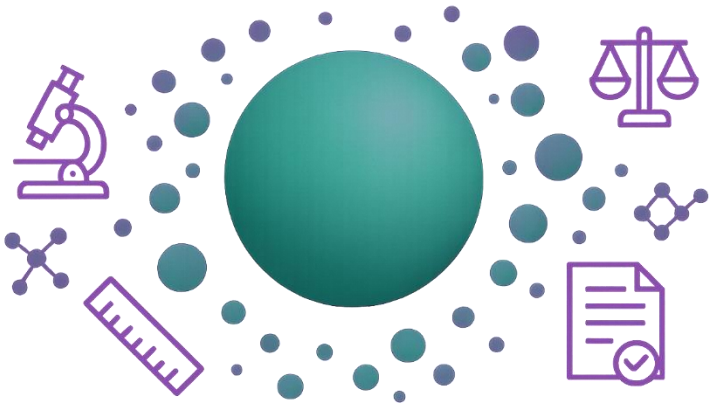
Development of Opti-Count® particles was undertaken to address the gap in available RMs, but such a high-quality nanoscale number concentration RM was fraught with unique challenges. At the core of the challenge lies the physical and chemical behavior of nanoparticles themselves. Nanoparticles tend to agglomerate/aggregate, dissolve, or undergo surface transformations such as oxidation and hydrolysis, particularly when suspended in aqueous media. These dynamic behaviors can significantly alter the particle number concentration over time as well as the number-weighted particles size distribution. Compounding the issue, particles can adsorb to container walls or degrade due to environmental exposure such as light, oxygen, or changes in pH. Experimental observations have shown that even short-term storage in plastic or glass containers can result in the appearance of contaminant particles ranging from less than 100 nm to 600 nm in otherwise pure water samples. The problem is particularly pronounced at lower concentrations, where the signal from such background contaminants may overwhelm the true particle count. Additionally, without the proper formulation biocontamination can occur due to the growth of bacteria in the medium; particularly when stored for long timeframes (i.e. months to years).

Various mitigation strategies were implemented, including the use of high-refractive-index particles to enhance detection fidelity and improve signal-to-noise ratios. Stabilizing the particles in buffered systems — Tris-based buffers with antimicrobial additives — proved effective in maintaining the chemical and biological stability of the dispersions over time.

While sealing RMs in blown glass ampules under inert atmospheres is often considered ideal for minimizing oxygen exchange with the continuous phase, this approach introduces new risks, such as contamination when opening the ampules. As a result, this method was not pursued. Instead, high-quality plastic vials with excellent sealing properties were selected to ensure both stability and practicality.

Each challenge encountered during development was carefully assessed, and tailored solutions were implemented and refined. These efforts were designed not only to enhance the reproducibility and accuracy of particle number measurements but also to support broader standardization and validation of emerging analytical techniques. The culmination of these efforts is Opti-Count® particle RMs.

Opti-Count® Certified
Number Concentration Particle Reference Materials



6. Introducing Opti-Count® Reference Materials for Determining Number Concentrations and Number-Weighted Particle Size Distributions

Opti-Count® particles are first-of-their-kind commercially available nanoscale number concentration RMs that set a new standard for accuracy and reliability in liquid, laser-based particle counting.

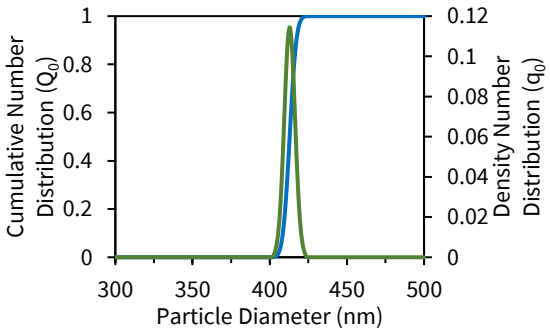
- **Certified Values:** Number concentration (<15% uncertainty).
- **Particle Size:** Monomodal nominal 400 nm polystyrene particle with indicative values for the number-weighted size distribution (<8% uncertainty).
- **Concentration:** 10^6 and 10^8 particles/ml available
- **Full Uncertainty Budget:** Includes an expanded uncertainty from characterization, homogeneity, storage and transport stability that covers a 95% confidence interval.
- **Ready-to-Use:** Single-use plastic vials (1 mL), requiring only gentle redispersion.
- **Surfactant-Free:** Electrostatic stabilization in an aqueous medium means low background noise — ideal for sensitive analytical methods.
- **Storage:** Room temperature, no refrigeration needed.

Designed specifically for the validation and calibration of analytical methods, Opti-Count® RMs enables precise measurement of the particle number concentration and support robust quality assurance protocols in liquid laser particle counting systems. They fill a critical gap in the market — empowering laboratories and manufacturers with the confidence and traceability they need in nanoparticle analysis. Precise monitoring of nanoparticle number concentration is essential for ensuring consistent product quality control, performance, and address all present and future regulatory needs.

Opti-Count® RMs are rigorously produced following ISO 17034 and ISO Guide 35 and represent the highest level of accuracy. Values are traceable to SI units by calibration at each step of the measurement process. Optimized formulations ensure reliable performance over the life-time of the certificate.

Opti-Count® RMs are the results of the collaboration between Dr. Lerche KG, an ISO 17205-accredited analytical laboratory with expertise in colloidal analysis, and Applied Microspheres GmbH, a leading manufacturer of particle standards and related products.

Number concentration and number-weighted size distributions have been optimized using the LUMiSpoc®, a liquid-borne single particle optical counter and sizer based on multi-angle laser light scattering and advanced cytometry.



Example of Opti-Count® number-weighted particle size distribution measured by the LUMiSpoc®.

Adoption of Opti-Count® RMs will significantly contribute to the development of safer and more efficacious products across industries.

More information about Opti-Count® particles can be found at:

www.opti-count.com

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7. Conclusion, Future Directions, and Recommendations

RMs play a pivotal role in ensuring regulatory compliance for nanomaterials by serving as standardized benchmarks for measurement, safety evaluation, and method validation. They are essential to scientific research, industrial quality assurance, and environmental health monitoring — enabling reproducible, accurate, and comparable data across sectors and disciplines.

With the development of Opti-Count® RMs a first, critical step has been reached in being able to accurately characterize the number concentration of particles in the nanometer to submicron size range in a manner befitting current and future definitions for nanomaterials that will be adopted by regulatory agencies.

Future Directions and Recommendations

Adoption of number concentration RMs such as Opti-Count® particles and validated instruments like the LUMiSpoc® to enable accurate and reproducible particle characterization are necessary in addressing the regulatory need for standardized methods and RMs.

Further efforts rely on coordinated international action to develop a wider range of nanoscale RMs that are stable, traceable to SI units, and applicable across different measurement platforms. Regulatory agencies, metrology institutes, and industrial stakeholders must work collaboratively to:

- Establish standardized protocols for RM use across platforms and laboratories.
- Expand the availability of RMs representative for mono- and multi-modal particles, those that mimic real-world materials, and more complex nanomaterials (e.g. core-shell particles, aspherical shapes, different materials).
- Encourage large-scale ILCs to validate and benchmark RMs and analytical techniques.
- Monitor number concentration and size of materials through their usage life-cycle. Such analysis ensures that measured properties remain the same, something seen not to be the case for nanomaterials.

8. Declaration

This document was written with the support of artificial intelligence in the restructuring of sentences. All factual information was written by the author and checked after AI editing for correctness. Additionally, some icons and images were developed using AI.

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