MANTA INSTRUMENTS
Better Nanoparticle Characterization
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There Must be a Better Way… to characterize nanoparticles

MANTA's founding scientists, Professor Dariusz Stramski, leader of the Ocean Optics Research Lab at the Scripps Institution of Oceanography (SIO), University of California San Diego (UCSD), and his fellow SIO researchers Dr. Kuba Tatarkiewicz, Dr. Rick Reynolds and Monette Karr have decades of combined experience studying particles in seawater. This experience included many failed attempts with the legacy instruments to get good characterization data of size and concentration of nanoparticles in various samples, including highly challenging heterogeneous polydisperse samples from natural aquatic environments. They concluded "there must be a better way" and set about inventing MANTA's Most Advanced Nanoparticle Tracking Analysis technology which performs individual particle analysis simultaneously on wide ranging nanoparticle sizes co-existing in a liquid sample. An introduction to MANTA's technology and comparisons with the other most commonly used technologies are provided in this paper.

1. Introduction

There are various techniques for measuring and characterizing nanoparticles – each technique providing different information and accuracy. Some techniques may simply detect nanoparticles, and others may provide quantitative information about concentration, size distribution, chemical content or other properties of a nanoparticle sample. One of the most important characteristics of a sample consisting of a collection of nanoparticles is particle size distribution (PSD). A *monodisperse* sample consists only of particles of the same size and shape. Real-world samples are, however, always *polydisperse* to a certain degree, as they contain particles of various sizes. PSD describes how the sizes of individual particles vary within the sample. The most direct way for determining PSD is to measure how many particles have specific sizes or fall within specific "size bins." Depending on the size measuring method, PSDs also can be based on another type of quantity such as the volume, surface area, or mass of all particles in each size bin, or light-scattering intensity produced by particles in each size bin. From the number distribution, the particle volume and surface area distributions can be obtained if the shape of particles is known or assumed. The particle mass distribution can also be obtained if the density of particles is known or assumed. The primary interest of this paper is the capability to measure particle number size distribution and the related capability to visualize a wide range of particle sizes simultaneously.

The methods for measuring PSD of nanoparticles include two main categories: (i) methods that measure a large number of particles simultaneously (*ensemble methods*), and (ii) methods that measure particles individually (*individual-particle methods*). The main technologies in the individual-particle category include transmission electron microscopy (TEM), scanning electron microscopy (SEM), nanoparticle tracking analysis (NTA), and electric resistive pulse sensing. Within the ensemble methods the main technologies are dynamic light scattering (DLS) and static light scattering (SLS) which includes multi-angle light scattering (MALS). The ensemble methods can also involve fractionation of the sample such as field-flow fractionation (FFF), differential centrifugal sedimentation (DCS), and size-exclusion chromatography (SEC). However, in order to provide a measurement of PSD the fractionation techniques must be combined with detection techniques, such as optical detection of light attenuation or scattering. The commonly used technologies for measuring PSD of nanoparticles are DLS, FFF-MALS, NTA, and TEM and this paper is focused on how MANTA compares with those technologies.

2. MANTA's novel technology

MANTA's technology is an individual-particle method which employs a multi-spectral optical approach of NTA for characterizing nanoparticles in liquid samples by tracking and analyzing their Brownian motion. It can also analyze larger, micron-sized, particles by tracking their settling motion (driven by gravity). The invention leverages innovative illumination and detection techniques that enable video recording of scattered light from wide-ranging sizes of individual particles simultaneously. A key advancement of this system is its ability to work with the very large dynamic range of scattered light intensity produced by differently-sized nanoparticles co-existing in a polydisperse sample. The results in Figure 1 were obtained from Mie scattering calculations for three light wavelengths (in the blue, green, and red spectral bands) for spherical polystyrene particles suspended in water. In this example the scattering intensity at a scattering angle of 90^o is more than 10^8 or even 10^9 times higher (depending on light wavelength) for the 1,000 nm size compared with the 10 nm size.

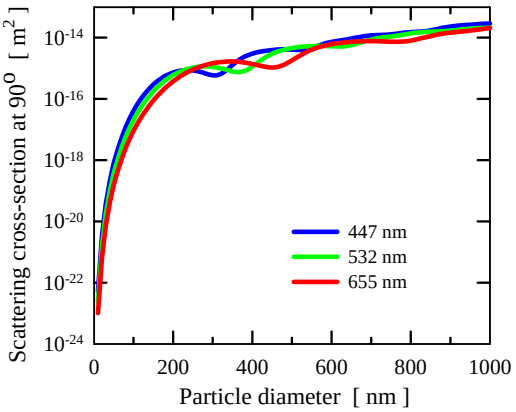

Figure 1. Variation in scattering cross-section of nanoparticles as a function of nanoparticle size for three selected wavelengths of light, 447, 532, and 655 nm. The calculations were made using the Mie scattering code for homogeneous spherical particles (Bohren, C. F. and D. R. Huffman, 1983, Absorption and Scattering of Light by Small Particles, John Wiley & Sons, New York) assuming the refractive index of polystyrene. The polystyrene particles were assumed to be suspended in water. The values of scattering cross-section were calculated as an integral within the range of scattering angles between 75^o and 105^o which is centered at 90^o. This range corresponds approximately to the light-scattering detection with MANTA's technology.

Results from other light scattering techniques typically have significant artifacts and uncertainties caused by this massively disproportionate scattered light intensity across the nanoparticle size range. For example, in DLS the main issue is that very high intensity of scattered light from even a small number of larger particles in the sample overwhelms the typical detection systems and obscures the analysis of other particles co-existing in the sample. Similarly, the conventional NTA technique is inadequate for measuring co-existing nanoparticles that produce very different scattered light intensities.

MANTA's breakthrough technology for addressing this huge range of scattered light intensity includes: (i) a plurality of lasers; (ii) a detection system that can simultaneously, and individually, track the light scattered from particles; and (iii) software to control the system and analyze the light scattered from particles on a particle-by-particle basis. With these innovations, MANTA divides the previously unmanageable dynamic range of scattered light intensity from sub-micron particles into discrete and manageable segments.

With this novel technology, MANTA's customers are able, for the first time, to visualize nanoparticles and measure the particle number concentration and particle number size distribution for nanoparticle samples containing a wide range of particle sizes within the submicron range. MANTA's capabilities also provide additional benefits, such as real-time measurement of particle kinetic processes.

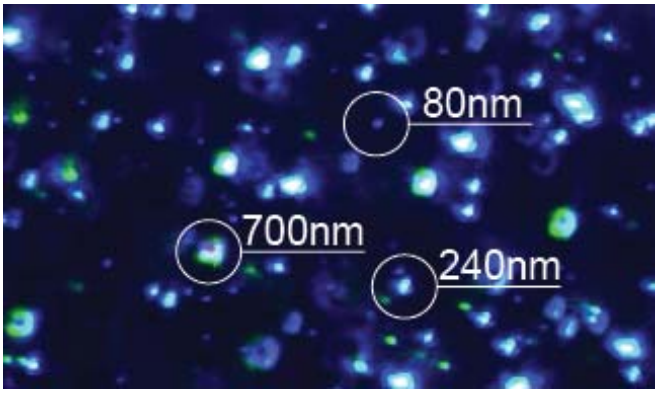

Figure 2. Single frame from video taken by MANTA's product the ViewSizer 3000, showing the ability to visualize a wide range of nanoparticle sizes simultaneously. This precise particle-by-particle analysis results in accurate and reproducible particle number size distribution measurements even for highly polydisperse samples.

Below in section 3 we compare MANTA's technology with the most commonly used methods for visualization, size measurements, and concentration measurements on nanoparticles.

3. Other techniques and their comparison with MANTA's technology

3.1. Conventional Nanoparticle Tracking Analysis (NTA)

NTA is a method for visualizing and analyzing individual nanoparticles in liquids, which has been in use for more than a decade. The conventional NTA is similar to MANTA's technology in the following ways: (i) it is an individual-particle counting and sizing method that produces a size value for each particle analyzed and from this information the required particle number size distribution is constructed, (ii) size determinations are not influenced by particle density or refractive index and only depend on the liquid's viscosity and temperature, (iii) the technique uses a laser for illumination of sample and microscope/camera system for detecting light scattered by nanoparticles, which together allow individual nanoparticles in liquid suspension to be visualized, (iv) the light scattered by the particles is captured on video recordings using a CCD and software is then used to track the Brownian motion of each particle, (v) the hydrodynamic diameter of nanoparticles is calculated from the Stokes–Einstein equation, (vi) the technique calculates particle size on a particle-by-particle basis, overcoming inherent weaknesses in ensemble techniques such as DLS or SLS, (vii) because video clips form the basis of the analysis, characterization of real time kinetic events such as aggregation and dissolution are possible (but only in a limited manner with conventional NTA), and (viii) samples require minimal preparation.

NTA is applicable for particles from about 10 to 1,000 nm in diameter. Analysis of particles at the lowest end of this range is possible only for particles composed of materials with a high refractive index relative to liquid medium in which the particles are suspended. The upper size limit is restricted primarily by the limit of detectability of Brownian motion of large particles which

move very slowly. The liquid viscosity also influences particle movement, so it also has an effect on the upper size limit for a specific system.

The NTA technique pioneered a new field of nanoparticle visualization and analysis. However, the conventional NTA suffers from significant limitations related to the large dynamic range of light scattering intensity from submicron particles mentioned above in Section 2. The following table describes various benefits of MANTA's technology relative to the conventional NTA.

	Conventional NTA	**MANTA**
Particle Visualization	**Yes.** Image from scattered light seen but may include artifacts.	**Yes.** Image from scattered light seen.
Particles in Native State	**Yes.**	**Yes.**
Particle Number Concentration	**Limited.** Prone to significant error due to inadequate measurement of the full dynamic range of scattered light intensity.	**Yes.** Accurate measurements made by counting all particles in sample.
Particle Number Size Distribution	**Limited.** Error prone due to large dynamic range of scattered light intensity. Curve fitting can obscure underlying data.	**Yes.** Accurate measurements for particles in liquid samples including polydisperse samples.
Kinetics	**Limited.** Inadequate for polydisperse samples or samples where kinetic processes result in large changes in particle size.	**Yes.** Routine and real time.
Ease of Use	**Yes.** Routine, timely, simple and cost effective however, results can be strongly dependent on operator through choice of settings for imaging and analysis.	**Yes.** Routine, timely, simple and cost effective.

3.2. Transmission Electron Microscopy (TEM)

TEM is a commonly used variant of electron microscopy technology that uses electron beams to visualize structures that are not visible to the naked eye, or even with optical microscopy. In TEM, images of individual nanoparticles are constructed based on a beam of electrons that are transmitted through an ultra-thin specimen. The interaction of the electrons with the specimen forms an image and an imaging device, such as a CCD, captures the magnified image.

TEM images have very high resolution due the very small de Broglie wavelength of electrons. This enables examination of very fine detail as small as a single column of atoms, which is thousands of times smaller than the smallest resolvable object in a light microscope. From TEM images of individual nanoparticles, a multitude of particle size values can be obtained, and from this information the required number size distribution of nanoparticles can be constructed. TEM images also provide information about particle shape and enable visualization of the degree of particle aggregation/agglomeration. In addition to imaging, TEM can observe chemical identity, crystal orientation, electronic structure, and sample induced electron phase shift.

TEM is an important analysis method in both physical and biological sciences with application in many fields including cancer research, virology, materials science as well as pollution, nanotechnology, and semiconductor research. While useful, there are a number of drawbacks to the TEM technique. The instruments are expensive and large. Many materials require extensive and often challenging sample preparation to produce an electron transparent specimen. This makes TEM analysis a time consuming process that requires high skill levels and has low throughput. The structure and properties of the sample may also be changed during the preparation process. In addition, the field of view is relatively small, which means the region analyzed may not be characteristic of the whole sample, resulting in poor statistical representation of a sample. There is also potential that the sample may be affected or damaged by the combined action of high vacuum and electron beam, in particular for biological samples. The following table describes various benefits of MANTA's technology relative to TEM.

	TEM	**MANTA**
Particle Visualization	**Yes.** Actual images of particles seen.	**Yes.** Image from scattered light seen
Particles in Native State	**No.**	**Yes.**
Particle Number Concentration	**Limited.** Error prone based on sample preparation.	**Yes.** Accurate measurements made by counting all particles in sample.
Particle Number Size Distribution	**Limited.** Error prone due to sample preparation.	**Yes.** Accurate measurements for particles in liquid samples including polydisperse samples.
Kinetics	**Limited.** Laborious sample preparation in discrete time slices.	**Yes.** Routine and real time.
Ease of Use	**No.** Complex, slow, high skill required, costly.	**Yes.** Routine, timely, simple and cost effective.

3.3. Dynamic Light Scattering (DLS)

DLS is an ensemble method which uses a monochromatic, coherent laser beam to illuminate a collection of particles suspended in liquid and then measures fluctuations in the scattered light produced by the sample. The fluctuations around the mean intensity originate from Brownian motion of the particles because neighboring particles can produce either constructive or destructive interference of the scattered light intensity in a given direction of observation. An autocorrelation function allows comparison (correlation) of the intensity signals over time periods ranging from microseconds to milliseconds. The autocorrelation function contains information about the size of particles because smaller particles with faster Brownian diffusion lose the correlation more rapidly than larger particles with slower diffusion. The autocorrelation function can be converted to a scattered-light intensity-weighted (z-average) diffusion coefficient, which then can be used to calculate an intensity-weighted average size (hydrodynamic diameter) of the particles via the Stokes-Einstein relationship.

Whereas this method performs reasonably well for monodisperse or nearly-monodisperse samples, the performance and size resolution for polydisperse samples is poor. This is because for polydisperse samples the conversion of the autocorrelation function to information on diffusion coefficients is an ill-posed mathematical problem that contains more unknowns than equations. Small variations in the data can give large deviations in the output and the results are

strongly dependent on the algorithm. In addition, a major difficulty of DLS is that the signal from larger particles dominates over smaller particles so the results can be strongly biased in the presence of a small fraction of larger particles. Sample preparation either by filtration or centrifugation is often critical to remove dust and artifacts from the sample. Overall, accurate results become exceedingly difficult for polydisperse particle samples, especially those containing larger particles. In addition, as DLS provides scattering-intensity-weighted results, the conversion to number size distribution requires strong assumptions about the particle shape, refractive index, and dispersity, which are usually not fulfilled or known for real-world samples. The following table describes how MANTA's technology provides various benefits relative to DLS.

	DLS	**MANTA**
Particle Visualization	**No.**	**Yes.** Image from scattered light seen.
Particles in Native State	**Yes.**	**Yes.**
Particle Number Concentration	**No.** Not capable of counting particles.	**Yes.** Accurate measurements made by counting all particles in sample.
Particle Number Size Distribution	**No.** Provides only scattering-intensity-weighted distributions, not suitable for polydisperse samples.	**Yes.** Accurate measurements for particles in liquid samples including polydisperse samples.
Kinetics	**Limited.** Not quantitative but may be suitable for qualitative monitoring of changes in some samples.	Yes. Routine and real time.
Ease of Use	**Yes.** Routine, timely, simple and cost effective.	**Yes.** Routine, timely, simple and cost effective.

3.4. Field-Flow Fractionation with Static Light Scattering (FFF-SLS)

Fractionation methods provide a means to separate particles based on their size or mass. Because fractionation is in reality a separation technique, not a size measurement technique, the fractionation method *per se* cannot be reliably translated to the particle number size distribution. To minimize these limitations the fractionation techniques are often coupled to a detection system that performs measurements of static or dynamic light scattering, light absorption, or refractive index.

FFF is based on laminar flow of particles in a suspension (or macromolecules in a solution) where high-resolution separation is achieved within a very thin flow channel against which a perpendicular force field is applied. The flow profile within the FFF channel is typically a parabolic pattern with the highest flow velocity at the center and decreasing velocity towards the channel walls. Particles present in the fluid separate depending on their differing mobility under the force exerted by the field. Under the normal mode of operation, smaller particles that on average spend more time further away from the FFF channel wall are transported out of the channel faster than larger particles that accumulate near the wall. The elution time can provide information about particle size based on calibration with particle size standards but is not sufficient for obtaining quantitative information on particle number size distribution.

The FFF technique is often combined with downstream detection by a form of SLS known as multi-angle light scattering (MALS). MALS is an ensemble method in which the sample of particle suspension is illuminated by a beam of light (typically nearly monochromatic laser beam) and the intensity of light scattered by all illuminated particles is measured as a function of scattering angle. In MALS the static light scattering is measured which represents time-average intensity of scattered light (as opposed to short-term fluctuations in scattered intensity measured by dynamic light scattering technique). Such enhanced information about angular scattering pattern can be exploited for the use in the submicron range of particle sizes. However, the measurement of MALS on bulk polydisperse heterogeneous samples of nanoparticles cannot be reliably converted to particle size distribution. The rationale for coupling FFF with MALS is that the determination of size distribution of particle ensembles from a scattering pattern is expected to be feasible or more reliable when all particles have exactly the same size. Accordingly, MALS measures the scattering patterns for various particle size fractions eluting from the FFF system, and from these measurements the number size distribution of the unfractionated ensemble is determined.

The major limitation of this method stems from the fact that the scattering pattern for a given size fraction (i.e., for a single size or very narrow distribution of sizes) is unique only if all particles are also identical in terms of the refractive index and shape. Therefore, a conversion of MALS measurements to particle number for different size fractions is prone to significant unknown errors if the sample includes particles with different refractive index and shapes, which is common for many real-world samples. In addition a limitation of FFF is that there is no "one size fits all" fractionation protocol for all applications and sample types. Although FFF is a versatile technique, development of relatively complex protocols is required to optimize particle separation. The following table describes how MANTA's technology provides various benefits relative to FFF-SLS.

	FFF-SLS	MANTA
Particle Visualization	**No.**	**Yes.** Image from scattered light seen.
Measures Particles in Native State	**Yes.**	**Yes.**
Particle Number Concentration	**Limited.** Applicable to homogeneous particle assemblages with known composition.	**Yes.** Accurate measurements made by counting all particles in sample.
Particle Number Size Distribution	**Limited.** Applicable to homogeneous particle assemblages with known composition.	**Yes.** Accurate measurements for particles in liquid samples including polydisperse samples.
Kinetics	**Limited.** Analysis is relatively long. Agitation from flow impacts kinetics.	**Yes.** Routine and real time.
Ease of Use	**No.** Requires complex method development for optimizing particle separation and long analysis time.	**Yes.** Routine, timely, simple and cost effective.

4. Summary

In most applications it is important to produce nanoparticles within a certain range of sizes. If you are not able to accurately measure particle size distributions, processes cannot be managed to make more of the desired particles and less of the out of spec particles. Similarly, it is hard to optimize process yields if particle concentrations cannot be measured. And if you want to know about particle kinetic processes you'll need accurate PSD measurements and / or particle visualization over time. These needs are important across the full range of nanoparticle commercialization efforts - from R&D to manufacturing. MANTA's breakthrough technology addresses all these needs with one, simple to use bench top instrument.

For comprehensive validation results from MANTA's commercially available product the *ViewSizer 3000*, please see our white paper "The Better Way to Characterize Nanoparticles."

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