

Nanoparticle Size Measurement Techniques

Comparison of LVEM, TEM, SEM, AFM, DLS, spICP-MS, and AF4

Background

This report will present some of the commonly used measurement techniques for measuring the size of particles, including nanoparticles, colloidal particles, and nanoparticles. Highlights of the strengths of each instrumentation technique and the best approaches for sample preparation methods will be presented. The metrology, or fundamental measurement science, behind each technique will be discussed to inform on the strengths and limitations of what is actually being measured.

Selection of appropriate metrology tools requires contemplation of the intended purposes of the knowledge gained, and practical considerations such as the financial and time costs involved in collecting the particle characterization data (Matyi, 2020). Previous reports in this series have compared LVEM and TEM, and have compared TEM, DLS, and AFM. This report will focus on a broader comparison of techniques including:

- Low Voltage Electron Microscopy (LVEM)
- Transmission Electron Microscopy (TEM)
- Scanning Electron Microscopy (SEM)
- Atomic Force Microscopy (AFM)
- Dynamic Light Scattering (DLS)
- Single Particle Inductively Coupled Plasma Mass Spectroscopy (spICP-MS)
- Asymmetric field flow field fractionation (AF4)

Survey of Measurement Techniques

There are many instrumental techniques that can determine morphology (i.e., size and shape) at the nanoscale. Morphology measurements can be broken down into both the shape and the size of a nanostructure. Each of these techniques offers varying abilities to determine size and shape information, as shown with the non-exhaustive list in Table 1.

Conventionally microscopies are used to determine the shape and size, while other techniques typically offer benefits of either in situ measurements, faster data collection, or additional types of analysis data. Examples include X-Ray Diffraction (XRD) which can offer crystallographic information (as can high resolution TEM), Coulter-counter type particle analyzers, Laser Diffraction techniques (LDS), Quartz Crystal Microbalance (QCM), Microfluid Cantilevers, and aerosol particle sizing techniques including Electro-Spray Differential Mobility Analysis (ES-DMA), Optical Particle Counters (OPC) and Scanning Mobility Particle Sizing (SMPS).

Table 1. Examples of Morphology Characterization Techniques

TECHNIQUE	SHAPE?	SIZE?
Low Voltage Electron Microscopy (LVEM)	Yes	X & Y
Transmission Electron Microscopy (TEM)	Yes	X & Y
Scanning Electron Microscopy (SEM)	Yes	X & Y
Atomic Force Microscopy (AFM)	Yes	Z
Dynamic Light Scattering (DLS)	No	Z-average (equivalent sphere diameter)
Single Particle Inductively Coupled Plasma Mass Spectroscopy (spICP-MS)	No	Z-average (equivalent sphere diameter)
Asymmetric Flow-Field Flow Fractionation (AF4)	No*	Z-average (equivalent sphere diameter)
Static Light Scattering (SLS)	No	Z-average (equivalent sphere diameter)
Small Angle X-ray Scattering (SAXS)	Yes (based on model applied)	Yes (based on model applied)

TECHNIQUE	SHAPE?	SIZE?
Nanoparticle Tracking Analysis (NTA)	No	Z-average (equivalent sphere diameter)
Laser Diffraction Sizing (LDS)	No	Z-average (equivalent sphere diameter)
Centrifugal Particle Sizing (CPS) & Analytical Ultra-Centrifugation (AUC)	No	Z-average (equivalent sphere diameter)
Scanning Tunneling Microscopy (STM)	Yes	Z

TEM

TEM is considered the gold standard technique for nanoparticle sizing. Often, a TEM image is provided for the most convincing single characterization, and is considered the “gold standard” technique. Evidence of this includes guidelines for minimum characterization of nanomaterials for peer-reviewed publications, and the European Food Safety Administration (EFSA) requiring the characterization of a nanomaterial’s size and morphology by two methods, one of which must be TEM and one chosen by the submitter.

A single TEM image alone is insufficient nanomaterial characterization. At minimum, an accompanying size distribution histogram should be reported with the representative image. Histograms should be generated from several hundred particles (typically $N > 200$) for average size determinations, and several thousand particles (typically $N > 3,000$) for width of the size distribution determinations such as full width half maximum. (NIST SP 960). Other examples include the NIOSH 7402 TEM analysis which requires the examination of numerous (no less than 40) grid squares to ensure representative surveying and data collection.

TEM has traditional high voltage and modern low voltage instrumentation approaches. In both cases, an electron beam is generated, is passed through the sample to a detector. This occurs in a vacuum column, as electrons cannot travel far in air. One can imagine this as a beam of light, passing through a series of lenses to focus the light through the sample and onto the camera’s detector. Where a beam of light is focused with glass lenses, for a beam of electrons the lenses typically are coil-shaped electromagnetics. A series of lenses concentrate the beam of electrons into a small spot, focus the beam onto the plane of the sample, and magnifies the image before it arrives at the detector.

LVEM

LVEM is widely utilized in nanoparticle studies. The lower accelerating voltage provides a better contrast with lower atomic number (Z) elements. This is a strong advantage for carbon-based polymer nanoparticles and provides sharper images even for metal oxide nanoparticles. While the contrast difference is less for metals, when organic surface coatings are applied to the metal nanoparticle core, it allows for easier imaging of more complex nanostructures.

Direct comparisons of TEM and LVEM for nanoparticle sizing have revealed the incredibly strong consistency across techniques, with agreement of 2.5% to 15% reported in the literature (Dazon, 2019). There are several well-established operational and business advantages to LVEM compared to traditional TEM instruments:

- Lower initial cost
- Lower operating cost
- Easier operation
- Easier maintenance
- Smaller laboratory footprint
- No specialized site prep required

The significantly lower initial cost of a new LVEM instrument compared to even a used TEM is a tremendous advantage, allowing routine access to electron microscopy images when otherwise unobtainable and freeing up larger budgets for other critical tasks.

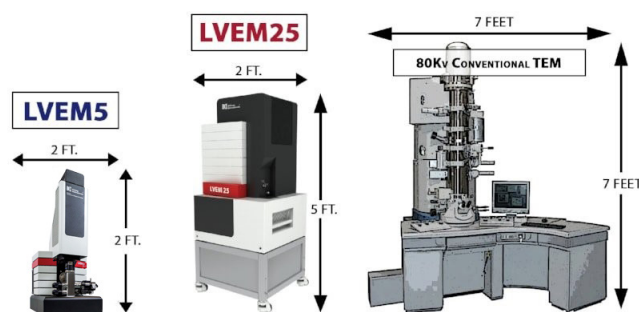


Figure 1. Comparison of the significantly smaller footprint of LVEM instruments vs. traditional TEM instruments

Additionally, placement of an LVEM is possible in many laboratories, making for much more efficient collection of routine characterization data. Much as low-cost instruments are ubiquitous in synthesis labs for initial screening characterization, LVEM enables electron microscopy to now become a rapid, affordable and easy screening tool for nanoparticle size characterization, eliminating the need for costly core user facilities.

SEM

In electron microscopy, there are many potential interactions between the electron beam and the solid material being imaged, as illustrated in Figure 2. In the SEM, imaging is most commonly achieved by monitoring the intensities of either backscattered electrons (BSE) or secondary electrons (SE) that are generated when the incident electron strikes the sample. In the SEM, the electrons that are ejected from the sample (SE or BSE) and travel to detectors without modification. This is in contrast to TEM where the signal (transmitted electrons) passes through the sample and must be processed by the objective lens. A comparison between SEM and TEM imaging is illustrated in Figure 3.

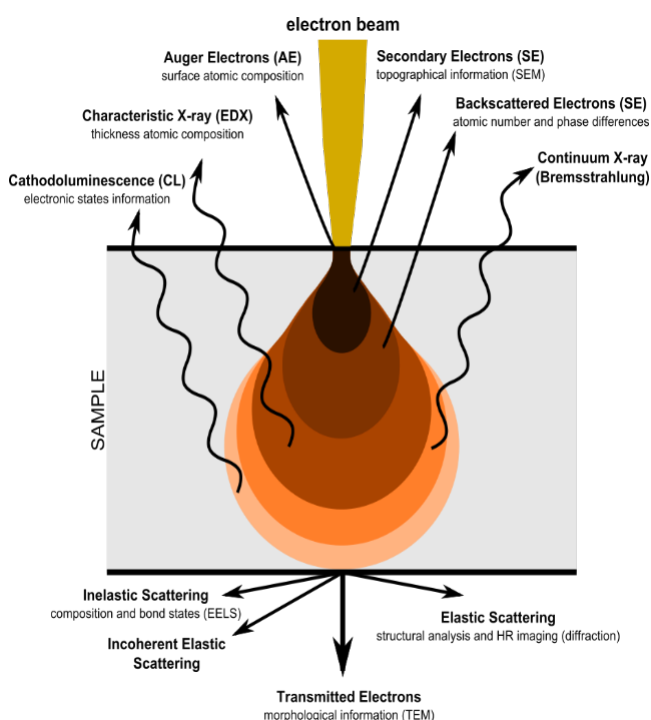


Figure 2. Schematic illustration of the typical electron-solid interactions that occur in electron microscopy. [1]

Comparing SEM and TEM, several differences arise. The spatial resolution of a typical lab-based SEM is inferior to a corresponding TEM. Additionally, the SEM cannot offer the crystallographic details of a TEM analysis. Nevertheless, SEM carries a number of advantages. Most SEMs can be operated by competently trained individuals, and SEM sample preparation is often less complicated. Environmental SEMs can operate in lower vacuum conditions, making sample prep requirements even easier.

When specifically comparing these techniques for nanoparticle analysis, the more serious of an analysis,

the more likely that an electron beam microscopy imaging process will be involved. (Matyi, 2020).

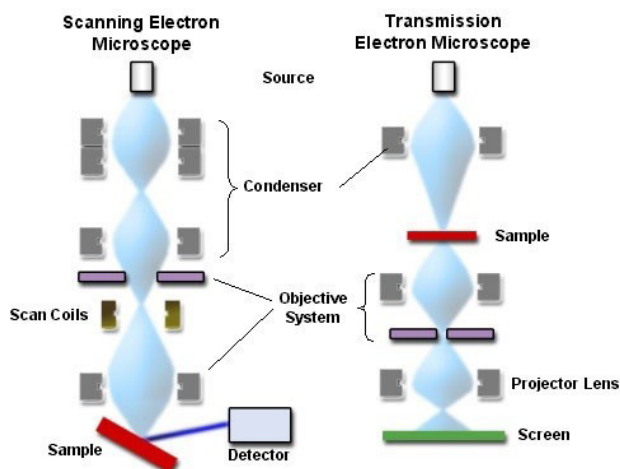


Figure 3. Schematic illustrations of the SEM (left) and TEM (right) [2].

AFM

AFM uses a sharp tip to probe or interact with the sample surface and generate a topographical map. The resulting topography data can be used to precisely measure the height of nanostructures deposited onto an atomically smooth surface. AFM instruments are typically operated in either a contact mode with a constant physical deflection of the probe, an intermittent contact mode often imagined as tapping on a surface, or a non-contact mode driven by probe-surface forces.

One of AFM's great strengths is the ability to gather extremely precise z-axis or height data, often with sub nanometer height resolutions. Yet as a probe-based technique, the lateral resolution in the x-y plane is limited by how close to atomically sharp the AFM probe's tip is during the image. The so-called "tip broadening effect" arises from the relationship of the larger the radius of curvature at the end of the tip interacting with the sample, the more "broadening" in the x-y plane will occur resulting in topography image artifacts, as illustrated in Figure 4.

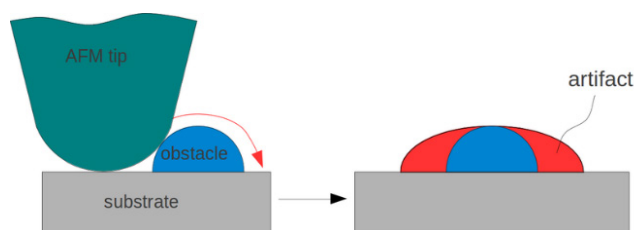


Figure 4. Illustration of the "tip broadening effect" in AFM creating artifacts of wider particle dimensions than truly exist. [3]

DLS

DLS measures the hydrodynamic diameter of an equivalent sphere of the nanoparticles. Included are the metal core, organic surface coatings, and any solvent molecules tightly associated with the surface coating.

The principle of the measurement arises from the natural Brownian motion of all particles above absolute zero temperature. At the same temperature, larger particles will move slower than smaller particles. When a laser illuminates a suspension of particles, a speckle pattern is created on the detector. By comparing the how the intensity of light at each point in this speckle pattern changes over time, the instrument's software is able to generate an autocorrelation function (Figure 5) allowing the subsequent fitting of what size distribution of particles would result in the autocorrelation function observed.

DLS observations rely upon Rayleigh scattering. The intensity of the light hitting the photodetector is proportional to the radius of the particle raised to the sixth power.

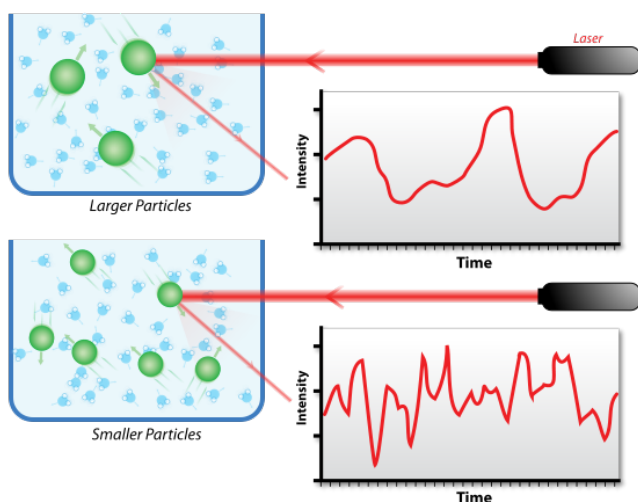


Figure 5. Basic structure of a DLS measurement and example of how particle size influences data observed. [4]

spICP-MS

Single nanoparticle inductively coupled plasma mass spectroscopy (spICP-MS) is a technique that arises from advancements in the chemical analysis technique of ICP-MS first reported by Degueldre et al (2003). In traditional ICP-MS, a solution is drawn into the instrument, introduced into a plasma torch which atomizes and ionizes the elements before precise quantification of the mass of elements present. In the case

of spICP-MS, sufficiently dilute suspensions allow the introduction of a single nanoparticle at a time into the plasma, which produces a momentary burst of ions. (Murphy, 2015) Figure 6 illustrates this concept.

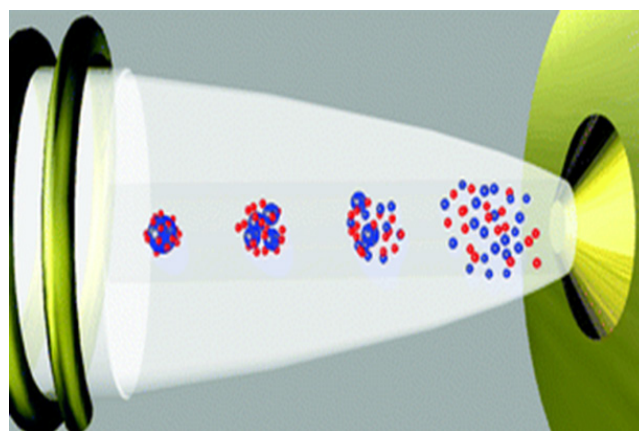


Figure 6. Illustration of a nanoparticle undergoing atomization and ionization. [5]

By using time-resolved data acquisition with very short detector dwell times, typically on the order of microseconds, the concentration of element (i.e., atoms) per unit time bin can enable the determination of the mass of each particle introduced into the instrument. With sufficient knowledge of the shape of the particles, typically obtained through microscopy such as TEM or LVEM, the mass of atoms in each particle can be used to determine the size of each particle, allowing a number-based size distribution measurement to be obtained while also determining a number-based nanoparticle concentration measurement to be obtained knowing the flow rate of sample introduction.

Improvements to the data acquisition, signal processing, and time of flight mass analyzers have overcome many of the limitations of applying spICP-MS in routine measurement conditions, including the automation of data analysis. (Montaño, 2016) Particles must be of a sufficient size to be statistically significant from the background levels of the elements present in solution. This highlights a combined opportunity and challenge afforded by spICP-MS. For soluble nanoparticles, a measurement of both the concentration of soluble fraction and the concentration of the nanoparticle fraction is possible while simultaneously obtaining the particle size. (Bustos, 2018) However, this only is feasible when large particles are present, with minimum particle diameter cutoffs ranging from 20–40nm.

Sample preparation conditions and data processing can take significant time to learn and optimize for

a given set of samples. Beyond their initial capital outlay expense, ICP-MS instruments have significant site preparation requirements including exhaust, power and environmental conditions, and consumables requirements including gas and cooling water.

However, when combined with electron microscopy, as NIST researchers did in 2018, to ensure shape measurements and small nanoparticles are captured, the liquid-based sample analysis of spICP-MS giving combined number-based particle concentration and size distribution from a single measurement is a powerful tool.

AF4/FFF

Asymmetric field flow fractionation (AF4), or field flow fractionation (FFF), is a liquid phase technique that separates particles by their hydrodynamic diameter. A sample containing particles is introduced into a channel with a semi-permeable membrane on the bottom using a flow that is parallel to the direction of travel down the channel. This is sometimes called the sample introduction step. In FFF, a second flow is applied perpendicular to the channel, such that all of the particles are pushed downward against the semi-permeable membrane; in AF4, the cross flow is provided by the carrier liquid, as shown in Figure 7. This is sometimes referred to as the sample focusing step.

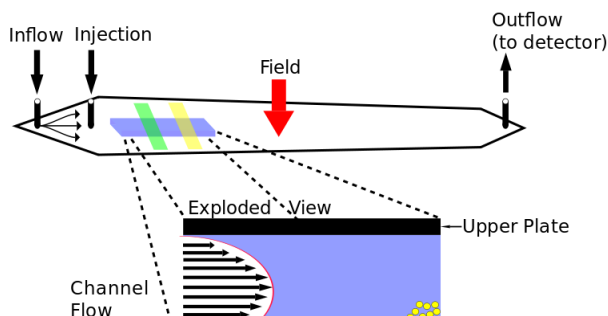


Figure 7. Illustration of AF4 particle separation. [6]

Next, the perpendicular flow is gradually reduced, so that first the particles with the smallest diameter and thus the greatest Brownian motion will return into the parallel flow and elute to the detectors. As the perpendicular flow is ramped down, larger and larger particles will enter the parallel flow and elute, allowing separation of particles by size. This is sometimes referred to as the sample fractionation step.

Through an understanding of the flow field properties of the channel, the hydrodynamic diameters of

particles in a mixture can be more precisely determined compared to a technique like dynamic light scattering.

Depending upon the nature of the detectors and the particles, hyphenated techniques can allow additional details to be elucidated; for example, optical DAD detectors can distinguish between the surface plasmon resonance absorbance bands of gold or silver nanoparticles versus polymer nanoparticles.

Conclusion

A variety of nanoparticle sizing techniques are available to the scientific community. Careful selection of the measurement technique reveals strengths unique to each approach. Nevertheless, TEM remains the gold standard technique, and is often the first and preferred choice for measuring the size, shape, and size distribution of nanomaterials. LVEM is a powerful tool for TEM characterization of nanoparticles with great accuracy and fidelity. Compared to traditional high voltage TEM, LVEM offers benefits including lower costs, easier operation, and rapid results.

The world's best benchtop electron microscope, the DeLong LVEM5, continues to contribute to many scientific disciplines beyond nanotechnology, including cell biology, materials science, higher education, environmental toxicology, and energy research.

About the author:

Robert I. MacCuspie, Ph.D., has over twenty years of experience in nanotechnology and materials characterization. Career highlights include leading the team that developed the silver nanoparticle reference materials at the National Institute of Standards and Technology, the first faculty and Director of Nanotechnology and Multifunctional Materials Program at Florida Polytechnic University, and over five years of consulting at the business-science interface from MacCuspie Innovations, helping companies commercialize and educate on technologies to improve human health.

References:

[1] https://commons.wikimedia.org/wiki/File:Electron_Interaction_with_Matter.svg

Image used under Creative Commons Attribution-Share Alike 4.0 license Unported, by author Claudionico.

[2] <https://whynano.files.wordpress.com/2011/03/tem-and-sem.jpg>, accessed 12/02/20.

[3] Image used under Creative Commons Attribution-Share Alike 3.0 license Unported, by author Patrick21-TF.

[4] Image used under Creative Commons Attribution-Share Alike 3.0 license Unported, by author Mike Jones.

[5] https://www.wur.nl/upload_mm/5/5/2/39bd6771-8ad1-49ac-bff1-02f8af431b18_Single%20particle.png accessed 11/28/20.

[6] Image used under Creative Commons Attribution-Share Alike 3.0 license Unported, by author Lictuel.

Bustos ARM, Purushotham KP, Possolo A, Farkas N, Vladár AE, Murphy KE, Winchester MR. Validation of single particle ICP-MS for routine measurements of nanoparticle size and number size distribution. *Anal. Chem.* 2018 Nov 25;90:14376–86.

Dazon C, Maxit B, Witschger O. Comparison between a low-voltage benchtop electron microscope and conventional TEM for number size distribution of nearly spherical shape constituent particles of nanomaterial powders and colloids. *Micron.* 2019 Jan 1;116:124–9.

Degueldre C and Favarger PY, Colloid analysis by single particle inductively coupled plasmamass spectroscopy: a feasibility study. *Colloids Surf., A*, 2003, 217, 137–142.

MacCusprie RI, Rogers K, Patra M, Suo Z, Allen AJ, Martin MN, Hackley VA. Challenges for physical characterization of silver nanoparticles under pristine and environmentally relevant conditions. *Journal of Environmental Monitoring.* 2011;13(5):1212–26.

Matyi, RJ, MacCusprie, RI. Characterization of Nanoparticles for Nanomaterial Environmental Health and Safety Studies: The physics and metrology for several common approaches. *IEEE Nanotechnology Magazine.* 2020 Aug 31; 14(5):7–22. Doi: 10.1109/MNANO.2020.3009962

Montaño MD, Olesik JW, Barber AG, Challis K, Ranville JF. Single Particle ICP-MS: Advances toward routine analysis of nanomaterials. *Analytical and bio-analytical chemistry.* 2016 Jul 1;408(19):5053–74.

Murphy, KE, Montoro Bustos, AR, Johnson, ME, Winchester, MR, Liu, J. Characterization of nanoparticle suspensions using single particle inductively coupled plasma mass spectrometry. *NIST Special Publication 1200–21.* 2015 Dec. 1–29. Doi: 10.6028/NIST.SP.1200–21

Walczyk D, Bombelli FB, Monopoli MP, Lynch I, Dawson KA. What the cell “sees” in bionanoscience. *Journal of the American Chemical Society.* 2010 Apr 28;132(16):5761–8.