# Comparison of Nanoparticle Sizing Techniques: TEM vs. DLS vs. AFM

## Background

This report will present some of the commonly used measurement techniques for measuring the size of 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.

Techniques to be analyzed in detail include transmission electron microscopy (TEM), dynamic light scattering (DLS), and atomic force microscopy (AFM).

Conventionally microscopies are used to determine the shape and size, while other techniques typically offer benefits of either *in situ* measurements or faster data collection.

## 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, as well as 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 of which may be chosen by the submitter. Many nanoparticle manufacturers verify their size with TEM (MacCuspie, et al., 2011).

However, a single TEM image alone is grossly insufficient nanomaterial characterization; at a 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.

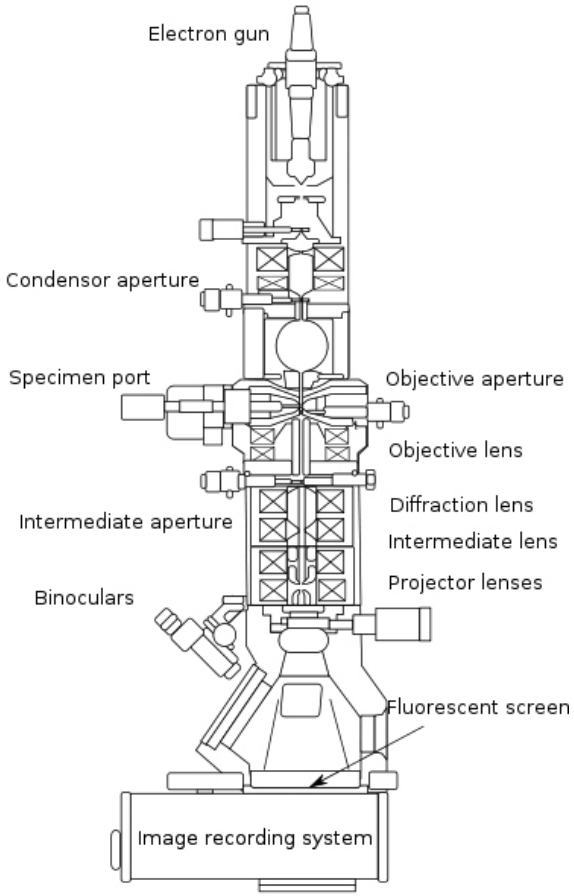



*Figure 1. Illustration of how a TEM is constructed [1].*

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 (Figure 1). 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.

Images appear darker where more material is deposited, and lighter where there is no material. Materials containing atoms with higher atomic number (Z) elements will appear darker than the same thickness of material containing lower atomic number elements. For example, if three identically sized nanoparticles were imaged, a particle made entirely of gold (Z = 79) will be darker than a particle made entirely of iron (Z = 26) which will be darker than a particle made entirely of carbon (Z = 6).

## LVEM

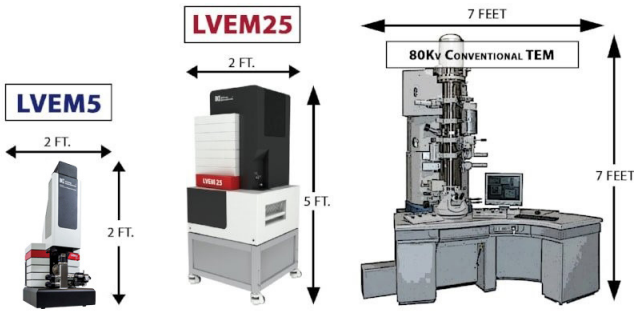
LVEM is widely utilized in nanoparticle studies. The lower accelerating voltage provides a darker contrast with lower atomic number (Z) elements. This is a strong advantage for carbon-based polymer nanoparticles and provides sharper images even for $SiO_2$ and $TiO_2$ nanoparticles. While this contrast difference becomes less noticeable for metals nanoparticles, when thick organic surface coatings are applied to the metal nanoparticle core, it allows for easier imaging of these 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. 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.




*The LVEM 5 fits on a 2 ft wide benchtop, and the LVEM 25 footprint is about 2 ft by 3 ft, compared to 7 ft by 8 ft for conventional TEM.*

## 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.

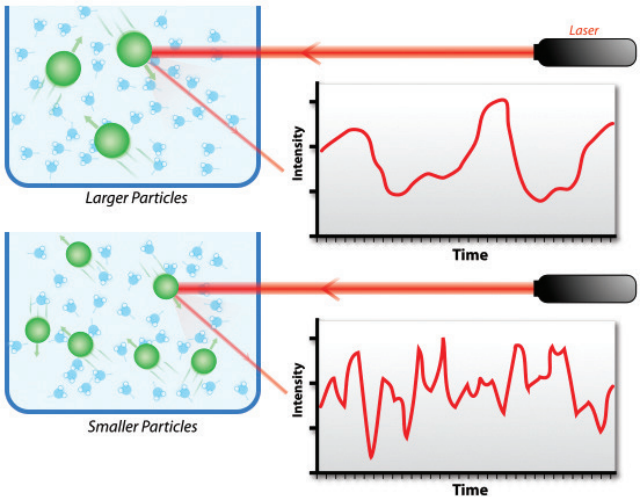



*Figure 2. Basic structure of a DLS measurement and example of how particle size influences data observed. [2]*

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 2) 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.

## 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.

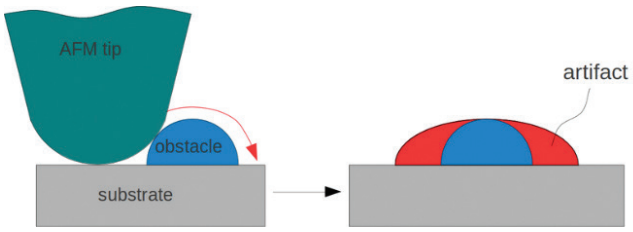



*Figure 3. Illustration of the "tip broadening effect" in AFM creating artifacts of wider particle dimensions than truly exist. [3]*

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 3.

## Comparison of TEM, DLS and AFM

Table 1 summarizes the capacity of TEM, DLS and AFM to measure the shape and size of nanoparticles, and which size measurands the technique utilizes (e.g. Z-height for AFM).

Because each of these techniques relies on slightly different measurands and measurement methods, it leads to strengths of what each technique will "see" more easily. Therefore, Table 2 compares these techniques.

*Table 1. Comparison of TEM, DLS and AFM Shape and Size Measurement*

| TECHNIQUE | SHAPE? | SIZE? |
|---|---|---|
| Transmission Electron Microscopy (TEM) | Yes | X & Y (plane of view dimensions) |
| Dynamic Light Scattering (DLS) | No* | Z-average (equivalent sphere diameter) |
| Atomic Force Microscopy (AFM) | Yes* | Z (height dimension) |

Microscopies like TEM and AFM rely on number-based particle measurements, leading to a strength of measuring heterogenous size distributions well, and measuring very small nanoparticle sizes well. DLS provides strong sensitivity to an extremely small number of aggregates, due to the volume-squared or intensity-based measurement.

*Table 2. Comparison of TEM, DLS and AFM Measurement Strengths*

| | TEM | DLS | AFM |
|---|---|---|---|
| Measurement basis | Number R | Volume$^2$ $R^6$ | Number R |
| Measures size of: | Metal core only | Metal core + hydrated coating + solvent | Metal core + dehydrated coating |
| Strength "sees" | Small NPs | Infrequent Large NPs | Very Small NPs |
| Weakness "misses" | Very Infrequent structures | Small NPs in mixtures | X–Y size & Infrequent structures |

Historically, multiple measurement techniques were needed to see multiple aspects of a sample, such as the metal nanoparticle core and its organic shell. Electron microscopy imaging of the shell around the nanoparticle core provides important context when comparing traditional TEM results with other techniques. For example, Dynamic Light Scattering results include the metal core, the surface coating molecules, and a sphere of hydration around the nanoparticles,

compared to typically just the metal core being reported by traditional TEM size measurements (MacCuspie, 2011). LVEM offers the potential capability to bridge this gap between techniques by distinguishing metal core and organic shell with better contrast resolution.

## Conclusion

A variety of nanoparticle sizing techniques are available to the scientific community. Some of the most commonly used are TEM, DLS and AFM. Each approach has a unique underlying scientific mechanism of making measurements, with TEM remaining 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] Image used under Creative Commons Attribution-Share Alike 3.0 license Unported, by author Granger.

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

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