# The Role of Standards for Transmission Electron Microscopy

## Background

This report will present how standards play a role in both transmission electron microscopy and low voltage electron microscopy LVEM. Standards play an important role in ensuring the quality and trust of measurements and data reporting, underpinning the scientific method in research and enabling fair trade and commerce when purchasing specific quantities of materials. Every time we purchase a certain weight of fruit, vegetables, or meat, standards of weights and measures are supporting the fairness and trust of those transactions.

In general, standards provide increased confidence in many areas, including:

- Calibration of instruments
- Qualification of instrument performance
- Establishing metrological traceability
- Method development
- Validation of procedures and protocols
- Quality control measurements
- Proficiency testing of lab personnel
- Facilitating intercomparison of results

This report is inspired by portions of the webinar "Comparing Measurement Techniques: TEM, DLS, and AFM" delivered on March 24, 2021, available for replay in the linked reference. [1]

## Two Types of Standards – Method & Artifact

There are two general types of standards. Method standards are written protocols or procedures that describe how to perform a task. Artifact standards are physical objects that are often used to calibrate or verify the calibration of an instrument. Artifact standards are sold with certificates of measured parameters and the uncertainty of the measurement that is expected. Importantly, neither method or artifact standards can ever entirely eliminate measurement uncertainty; thus, all data reported using standards should always include reporting of the uncertainty associated with the measured data.

## Method Standards

Written procedures and protocols fall into the category of method standards. Method standards are sometimes called consensus standards, reflecting the work performed by standards committees to achieve broad agreement on the way an operation will be performed. Many international standards bodies exist, with just a few examples including:

- ISO – International Standards Organization
- ANSI – American National Standards Institute
- ASTM International – formerly the American Society for Testing and Materials
- IEEE – Institute of Electrical and Electronics Engineers

Typically filled with volunteers from stakeholders across academia, government, and industry, much time and care is taken to reach consensus on the current best and most practical practices available to implement. Standards are often proposed to serve a highly specific purpose. For example, Table 1 presents a sampling of some of the numerous specialized standards that exist which utilize Transmission Electron Microscopy.

*Table 1. Example ISO and ASTM Standards Utilizing TEM*

| STANDARD NUMBER | STANDARD TITLE |
|---|---|
| ISO 21363:2020 | Nanotechnologies — Measurements of particle size and shape distributions by transmission electron microscopy |
| ISO/TS 10797:2012 | Nanotechnologies — Characterization of single-wall carbon nanotubes using transmission electron microscopy |
| ISO 29301:2017 | Microbeam analysis — Analytical electron microscopy — Methods for calibrating image magnification by using reference materials with periodic structures |
| ISO/TS 11888:2017 | Nanotechnologies — Characterization of multiwall carbon nanotubes — Mesoscopic shape factors |
| ASTM D5755-09(2014) | Standard Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Structure Number Surface Loading |

| STANDARD NUMBER | STANDARD TITLE |
|---|---|
| ASTM D6281-15 | Standard Test Method for Airborne Asbestos Concentration in Ambient and Indoor Atmospheres as Determined by Transmission Electron Microscopy Direct Transfer (TEM) |
| ASTM D7201-06(2020) | Standard Practice for Sampling and Counting Airborne Fibers, Including Asbestos Fibers, in the Workplace, by Phase Contrast Microscopy (with an Option of Transmission Electron Microscopy) |
| ASTM E3143-18b | Standard Practice for Performing Cryo-Transmission Electron Microscopy of Liposomes |
| ASTM D6480-19 | Standard Test Method for Wipe Sampling of Surfaces, Indirect Preparation, and Analysis for Asbestos Structure Number Surface Loading by Transmission Electron Microscopy |
| ASTM D3849-14a | Standard Test Method for Carbon Black – Morphological Characterization of Carbon Black Using Electron Microscopy |

The concept of a "Standard Operating Procedure" in an organization's Quality Management Systems falls into a method standard, in other words a written procedure, or standard, that is followed every time the operation is performed or carried out. These company standards are similarly reviewed internally by committees, and often refer to international standards when available.

Pure fundamental researchers often feel standards are unsuitable for their research, because they lag behind the cutting edge of the science they are working on, or they may know of a truly better way to perform a procedure for their advanced research. Indeed, many peer reviewed articles will discuss new and emerging measurement challenges before, during, and after standards are developed. (MacCuspie, Rogers, et al., 2011; MacCuspie, 2018; Matyi & MacCuspie, 2020) Yet consensus standards have a pivotal role to play in communicating cutting edge research results. Confirming instruments are in proper calibration and ensuring measurements are made according to consensus best practices improves the rigor and stature of the findings. For example, a researcher may invent a new processing technique for a nanomaterial where standards appropriately do not yet exist. However, reporting measurements of the nanomaterial at each stage of the process is critical to advancing research quickly and effectively. [2]

## Artifact Standards

Of the two types of standards, artifact standards are perhaps more relatable due to the fact one can physically hold them and manipulate them.

The value comes not from the artifact itself being precisely crafted, rather the value comes from the certificate accompanying the artifact and the trust of the brand of the laboratory providing the certification.

Standards can be sold by national metrology institutes (NMIs) that are typically government run national laboratories focused on ensuring the quality of measurements. Some examples of NMIs include:

- NIST – National Institute of Standards and Technology (USA)
- NRCC – National Research Council Canada (Canada)
- IRMM – Institute for Reference Materials and Measurements (EU)
- BAM – Federal Institute for Materials Research and Testing (Germany)
- NPL – National Physical Laboratory (UK)
- CMI – Czech Metrological Institute (Czech Republic)
- KRISS – Korea Research Institute of Standards and Science (S. Korea)
- AIST – National Institute of Advanced Industrial Science and Technology (Japan)
- Longer lists of national metrology institutes can be found in online compilations. [3]

Numerous standard artifacts exist with dimensions at the nanoscale. A sample of some of the standards available from the NIST (the US NMI) available at www.nist.gov/srm is presented in Table 2. [4]

*Table 2. Example Nanoscale Reference Materials available from NIST.*

| NIST REFERENCE MATERIAL | DESCRIPTION |
|---|---|
| RM8011 | Gold Nanoparticles, 10 nm diameter |
| RM8012 | Gold Nanoparticles, 30 nm diameter |
| RM8013 | Gold Nanoparticles, 60 nm diameter |
| RM8017 | Silver Nanoparticles, 75 nm diameter |
| RM2483 | Carbon Nanotubes, SWCNT Raw Soot |
| RM8281 | Carbon Nanotubes, Length separated fractions |
| SRM1898 | $TiO_2$ Nanoparticles, Specific surface area |
| RM8027 | Silicon Nanoparticles, 2 nm diameter |

## Standard Traceability

When manufacturing many copies of an artifact, where the value is coming from the certificate of the measured values on that standard, being able to trace the measurement uncertainties of the instruments used is important to assuring the end user of what is or is not an acceptable calibration for their instrument.

Strictly speaking, only a measurement or series of measurements can be NIST-traceable, not a physical standard object itself. [5] Being able to trace a series of measurements back to a reference material through a documented and unbroken series of calibrations provides confidence in the uncertainty associated with a standard.

## LVEM & Standards

The LVEM 5 & LVEM 25 instruments offer both Transmission Electron Microscopy and Scanning Transmission Electron Microscopy (STEM) modes of operation, and the LVEM 5 also offers a Scanning Electron Microscopy (SEM) mode. Therefore, all of the standards useful for TEM and SEM are applicable to LVEM. Standards leverage the business and operational advantages that LVEM offers, which are listed in Table 3, including a smaller footprint as illustrated in Figure 1.

*Table 3: Business & Operational Advantages of LVEM*

| LVEM BUSINESS & OPERATIONAL ADVANTAGES: |
|---|
| Lower initial cost |
| Lower operating cost |
| Easier operation |
| Easier maintenance |
| Smaller laboratory footprint |
| No specialized site prep required |

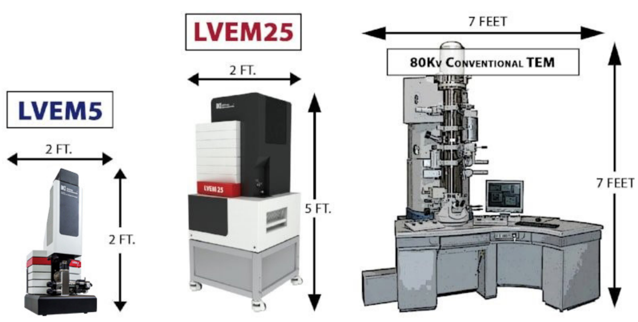


*Figure 1. Comparison of the significantly smaller footprint of LVEM instruments vs. traditional TEM instruments*

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.[6]

However, sometimes skeptics can wonder if such a low cost and high quality measurement from an instrument is "too good to be true." The next section discusses how standards can be used to help answer these valid concerns others may express.

## Standards Convince Skeptics & Build Confidence

Skepticism is justified in science, and provides a healthy improvement of trust and quality in reported results. Often times, skepticism is greater when long-held perceptions are shattered by new concepts. For example, how can a lower cost instrument be any good compared to a more expensive tool? These types of questions drive a healthy need for validation and verification experiments. Standards are one of the easiest ways to provide validation and verification of instrument and operator performance.

Demonstration that standard samples are measured correctly, by a consensus method, within the reported uncertainty, provides confidence that both the instruments and the operators are performing the desired measurement procedures correctly and accurately.

A good example of this process is provided by a recent peer-reviewed article comparing an LVEM 5 and a conventional TEM CM200. (Dazon, 2019). In this work, a direct comparison of results from both instruments is performed, using a range of nanoparticle standards spanning different sizes and materials of composition. The authors used an approach showing that a standard artifact was measured by a written procedure allows others to have full confidence in all aspects of the process. This allows the authors to conclude in the work "The results demonstrated that benchtop LVTEM is a suitable device for generating quality micrographs with a resolution comparable to its TEM equivalent." Having the written data available for review by others is not only the spirit of the peer-review process, it is also the spirit of quality management systems, and fosters a strong scientific culture of relying upon data-driven decisions and insights. Quality management systems allow everyone involved, from the operators to upper management to external reviewers, to be skeptical at first and be convinced by the data.

Industry professionals also use standards to reduce skepticism over human error. For example, laboratory managers often embed standards as samples in measurement matrices for this very reason. It is a useful management tool to build confidence in their team by showing newly trained staff that they indeed successfully measured standard samples as expected, and they are capable of performing those desired measurements with competence.

## Conclusion

Both method standards and standard artifacts play an important role science and industry, including in electron microscopy. There are many method standards for TEM, providing written procedures for ways of collecting data with the best consensus available. Additionally, numerous artifact standards are available to provide verification and validation of calibration of TEM measurements.

Compared to traditional high voltage TEM, LVEM offers benefits including lower costs, easier operation, and rapid results. TEM standards can provide the highest confidence of achieving the same top-quality measurements from LVEM that users have grown accustomed to expecting.

The world's best low voltage electron microscope, the Delong LVEM 25, and the world's most affordable low voltage electron microscope, the Delong LVEM 5, continue to contribute to many scientific disciplines beyond pathology, including nanotechnology, cell biology, materials science, higher education, environmental toxicology, and energy research.

## References:

[1] https://www.youtube.com/watch?v=0IAhYKpstAU

[2] https://www.lv-em.com/website_lvem/static/pdf/User_Profiles/LVEM_User_Profile-Kate_Plass.pdf, accessed 3/28/21.

[3] https://www.nist.gov/iaao/national-metrology-laboratories, accessed 3/28/21.

[4] https://www.nist.gov/srm, accessed 2/24/21.

[5] https://www.nist.gov/traceability/supplementary-materials-related-nist-policy-metrological-traceability#faq_general1, accessed 3/28/21.

[6] https://www.lv-em.com/website_lvem/static/pdf/LVEMApplicationNotes/LVEM_Application_Note-Comparison_of_LVEM_and_TEM_for_Nanoparticle_Sizing.pdf, accessed 4/14/21.

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## 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.