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Title: Documentation of operational protocol for the use of MAMA software

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Documentation of operational protocol for the use of MAMA software

Task 2b. Documentation of operational protocol/procedure for the use of MAMA software in image analysis applications

Image analysis of Scanning Electron Microscope (SEM) micrographs is a complex process that can vary significantly between analysts. The factors causing the variation are numerous, and the purpose of Task 2b is to develop and test a set of protocols designed to minimize variation in image analysis between different analysts and laboratories, specifically using the MAMA software package, Version 2.1. The protocols were designed to be "minimally invasive", so that expert SEM operators will not be overly constrained in the way they analyze particle samples.

The protocols will be tested using a round-robin approach where results from expert SEM users at Los Alamos National Laboratory, Lawrence Livermore National Laboratory, Pacific Northwest National Laboratory, Savannah River National Laboratory, and the National Institute of Standards and Testing will be compared. The variation of the results will be used to quantify uncertainty in the particle image analysis process. The round-robin exercise will proceed with 3 levels of rigor, each with their own set of protocols, as described below in Tasks 2b.1, 2b.2, and 2b.3.

The uncertainty will be developed using NIST standard reference material SRM 1984 "Thermal Spray Powder – Particle Size Distribution, Tungsten Carbide/Cobalt (Acicular)" [Reference 1]. Full details are available in the Certificate of Analysis, posted on the NIST website (http://www.nist.gov/srm/).

Task 2.b.1. Protocols for optimizing analysis using MAMA software, Version 2.1

This task is designed to be a pure test of the variation between users analyses due to the MAMA software (Version 2.1) alone. A series of SEM images of SRM 1984 taken at LANL will be sent to all participants in the round-robin, who will use the MAMA software to measure 400-500 particles (an example image is shown in figure 1). The resulting datasets will be compared for all the parameters determined by MAMA, and variation will be quantified and assessed. The following set of protocols will be used.

1. The magnifications for each image will be determined by using the size bar in the lower right of each image and the RULER function in the MAMA software. Users will report the magnification that the RULER tool calculates in pixels/µm for each image.

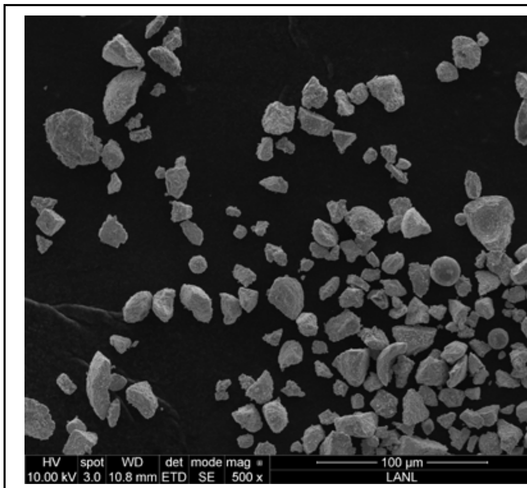


Figure 1. Typical SEM image of SRM 1984 for use in round-robin morphological analysis.

2. Users will include a total of 400-500 particles in their measurements. This number gives a good, well-understood statistical sample [Appendix A].
3. All particles overlapping or touching any edge of the images shall be excluded from the measurements. MAMA has multiple ways to exclude particles (figure 2).
4. Overlapping particles shall be excluded from the measurements (figure 3).
    a. The foreground particle in an overlap may be included if its boundary is well-defined.
    b. If particles are simply touching, they may be separated using the SPLIT SEGMENT tool. Some user judgement will be required to decide when to split particles instead of excluding them. However, error due to a few percent overlap will be much smaller than the confidence band associated with the 400-500 particle population. The round-robin exercise will give us a quantitative assessment of how large the uncertainty due to overlap is.
5. Prior to finalizing the data set, analysts shall review their segmented images for any obvious mis-segmentations. In general, it is better to exclude a questionable particle even though this will make it more difficult to get to the 400-500 particle goal.

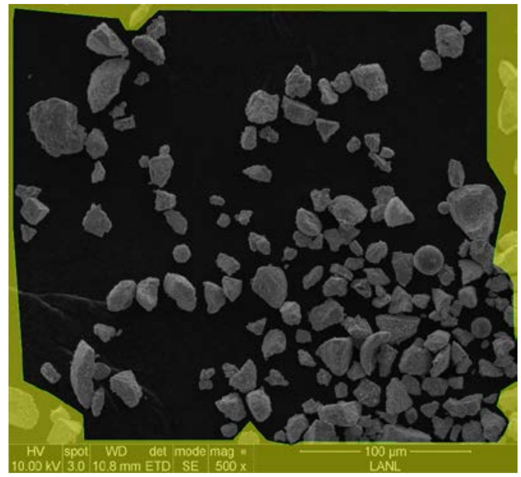


Figure 2. Particles overlapping the image edge are eliminated from the analysis.

Task 2b.2 Protocols for SEM imaging

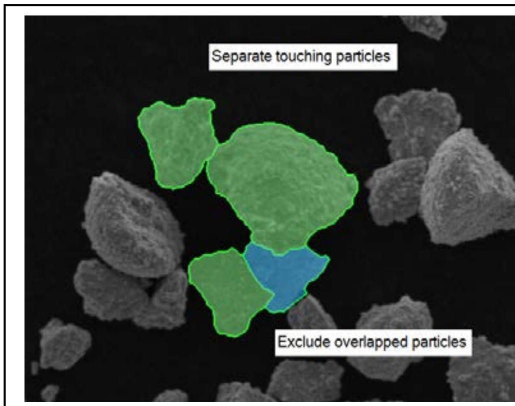


Figure 3. Separate touching particles, and remove overlapped particles from analysis.

After assessment of the variability in the use of the MAMA software in Task 2b.1, round-robin participants will proceed to testing variability in the SEM imaging process. Five SEM mounts will be prepared at LANL using the SRM 1984 material. From this set, two will be chosen as the primary specimens and the remainder will be held as back-ups. Approximately 20 SEM images will be made of the two primary specimens. Using these images, analysts will quantify the particle morphology using the MAMA software. The images will be compared qualitatively and the datasets from the MAMA analysis will be compared quantitatively. We will test that the parameters independently measured by round-robin participants overlap within a 95% confidence interval (k=2). This process will give us an understanding of the variability that results from normal differences in SEM instruments and the way SEM experts operate the instruments. The following set of protocols will be used:

1. SEM operators will obtain at least 20 images using the two primary specimens at a variety of magnifications in the range they would normally use for particles like SRM 1984. The images shall be approximately 2000 x 2000 pixels (SEMs manufactured by FEI have several image sizes to choose from, one of which is 2048 x 1887 pixels).
2. SEM operators will calibrate the magnifications of their instruments using a calibration standard, such as the Geller MRS-4 magnification reference standard. Operators will report the calibration in the form of a calibration curve or table (pixels/micron for each magnification). Magnification in both X and Y directions shall be reported.
3. SEM operators shall use a standard specimen height (objective-to-specimen focal plane distance) for their images. The magnification is influenced by this parameter, so the calibrations done in step 2 should be done at this standard specimen height. The specimen height shall be chosen at the discretion of the SEM operator and shall be reported for each image.
4. SEM parameters such as voltage, spot size, and image mode (backscatter vs. secondary electron) shall be left to the discretion of the analysts. Analysts shall use their expertise to capture sharp particle images with good edge contrast.
5. Analysts shall use MAMA to measure the particles in their images, following the protocols in Task 2b.1.
6. When analysts at each laboratory are finished, they shall pass the specimens to the next laboratory in series. The *same* two specimens will be examined by each laboratory, so participants must be careful to preserve the mounts.

<u>Task 2b.3 Protocols for full SEM analysis of powder sets</u>

The round-robin effort for this task is designed to realistically simulate the entire process of analyzing powder sets produced by the NTNFC Pu-oxide manufacturing study. NIST SRM 1984 is similar in size and shape to some types of Pu-oxides and is a suitable non-radioactive surrogate for developing an understanding of uncertainties in quantifying morphology.

Protocols developed under this task were designed to minimize variability between different analysts/laboratories for the entire process of sampling from a larger powder set, mounting the sample, imaging the particles, and measuring them using MAMA. Each participant in this part of the round-robin effort will procure their own bottle of SRM 1984 powder, sample and mount a subset for SEM analysis, and use MAMA software to analyze the SEM images. The resulting datasets will be quantitatively compared, and will give a realistic measurement of uncertainty for powder morphological analysis as a whole. The following set of protocols will be used:

1. Participants will procure a bottle of SRM 1984 from NIST [1].
2. All sampling from the original SRM 1984 bottle shall be done using a riffler. Note that riffling must be done on the entire bottle of SRM 1984 to avoid bias.
3. Final sub-sampling shall be done using a sharp-tipped spatula. If the round-robin participants jointly decide a different type of spatula is better, all participants should adopt the better spatula, so that sub-sampling is done with the same type of spatula at each laboratory.

4. SRM 1984 powder shall be delivered by sprinkling from the sharp-tipped spatula at very low density onto sticky tape mounted on an SEM stub.
5. Powder will be delivered at normal incidence (not onto a tipped SEM mount) to minimize particle rolling bias.
6. Sufficient SEM images will be captured so that 400-500 particles can be measured by MAMA. Magnification should be varied such that the micrograph set includes clear images of both the smallest and largest particles in the sample.
7. Protocols described in Task 2b.1 and 2b.2 shall be followed for making the SEM images and measuring the particles using the MAMA software.

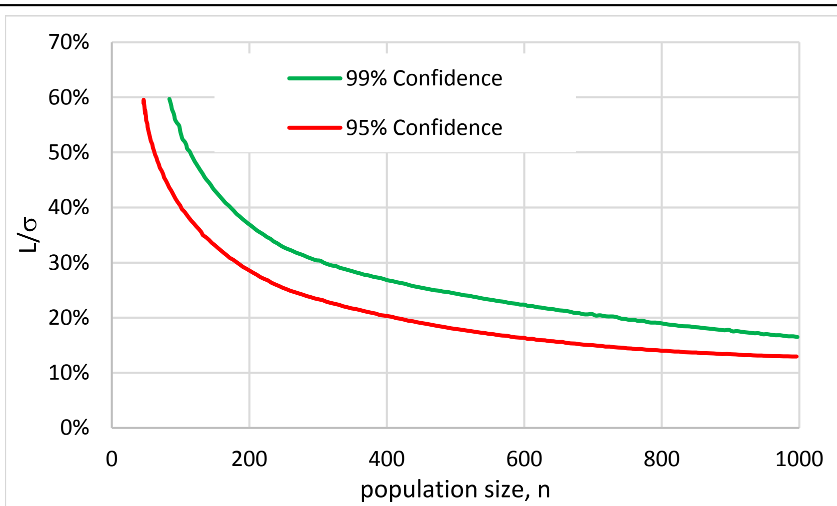
References

[1] National Institute of Standards & Technology Certificate of Analysis: Standard Reference Material® 1984, Thermal Spray Powder – Particle Size Distribution, Tungsten Carbide/Cobalt (Acicular)
This Standard Reference Material (SRM) is primarily intended for use in the calibration of equipment used to measure particle size distributions (PSD) in the 9 µm to 30 µm range. SRM 1984 consists of a single bottle containing approximately 14 g of tungsten carbide/cobalt powder.

Appendix A

The requirement for measuring 400-500 particles is based on Confidence Band analysis. At a confidence level of 95%, a population of 400-500 particles will yield a confidence interval (L) of 0.20σ , where σ is the standard deviation. At a 99% confidence level, 400-500 particles yields L ≈ 0.25σ.

Caveat: this analysis is based on the assumption of a normal population distribution. In general, this is a good assumption for particle samples, but certain particle sets may deviate significantly from normality (for example, sieved particles).



Confidence bands as a function of population size. L is the confidence interval, such that the measured mean using n particles is within ±L of the true mean (σ is the standard deviation).