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Webinar Q&A Report: **Automated Particle Size Analysis for TEM**

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How can I keep the sample in focus when screening large areas across a sample?

There are auto-focusing routines and auto-eucentric routines that can be used to keep the sample in focus. When screening large areas it is a good strategy to use very parallel beams like in microprobe stem to screen large areas without the need to refocus.

EDX data is typically very noisy and low resolution. How can you measure particle size based on EDX data?

We can combine the information of imaging and edx in 1 analysis. A good strategy is to create a mask based on the filtered EDX data to mask out the particles of a certain composition from the image data. Then the particle size can be measured based on the masked image data.

What is the particle size range?

The particle sizes that can be determined depends on the resolution of the microscope. Typically, one could measure particle sizes in the TEM from 10000nm to 0.1nm

Can I use machine learning to recognize my samples?

Yes, currently there is the possibility to train an AI to recognize a certain type of particles and use that AI to identify those particles. This allows users to identify particles in very challenging samples and is very suitable if there is a need to analyze the same type of sample repetitively.

How does the software deal with particle clusters?

There are several options to tackle this problem. As an example, there are advanced segmentation methods which we use to identify the particles and then one could use ellipse fitting to handle the possible overlap between particles in an aggregate. Another option is to train an AI to pick the individual particles from an aggregate. Yet another approach is to cross correlate between EDX and imaging to separate particles from a larger aggregate when there are compositions differences.

Is APW able to handle non-uniform backgrounds?

Thanks to the ability to cross correlate between imaging and EDX data it makes it easier to handle non-uniform backgrounds. EDX data typically doesn't suffer from a non-uniform background because where a certain element is not present the background is black. When there is enough statistics in EDX the particle size could be determined on the EDX data.

Is APW compatible for retrofitting on Titan (either Themis or Krios) systems?

Retrofitting is possible for Thermo Fisher TEMs on Windows 10.

Could APW be used for biological nano particles?

APW analysis approach is very generic. A recipe can be made in Avizo2D to specifically target biological particles.

Fiber or rod: does it make a difference?

Different shapes of particles can be analyzed and different parameters like long and short diameter can be defined in a measurement group and automatically extracted from the data.

For the example n Slide 17, how long did the acquisition take?

This dataset took 1 hour to acquire. Note that this is a dataset containing EDX data and that I chose to acquire ~2 mins per tile and with 25 images that is about 1 hour. When doing only imaging and no EDX the acquisition can go much faster and this whole are could be screened in 5 minutes.

One of the biggest issues in particle size analysis is the overlap between particles. For example when two particles have some overlap depending on how you process the data, you can say that you have one (but bigger) particle, or you can say I have 2 (but smaller) particles. How does your automatic software package addresses this? Is there any way the operator can intervene if needed?

There is the ellipse fitting feature which allows ellipses to be fitted on the edges of the particle that do not overlap. The ellipses can overlap and then the measures of the ellipses are reported out. This is a possible way of dealing with overlap. Advanced segmentation methods/AI can be used in combination with ellipse fitting.

When a user is not happy with the analysis the analysis recipe can be adjusted offline and reapplied to an already acquired dataset to re-evaluate the result.

There are many selection criteria available to distinguish between what to measure and what not to measure. Manual intervention in the automated workflow is not possible, but offline any fine tuning can be done.

Can we run analysis on TEM negatively stained particles?

Yes, a recipe can be made to deal with the inverted contrast of negatively stained particles. One could just add a step to invert the contrast in the recipe and target the bright regions instead of the dark ones.

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