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Statistical Analysis of Three-Dimensional Particles Using Focused Ion Beam Scanning Electron Microscopy Tomography and Image Processing

FIB-SEM tomography and image processing enable us to extract statistical analysis of particles from three-dimensional and high-resolution imaging data.

Introduction

Particle characteristics such as size distribution and shape are an important quality criterion in many fields. These characteristics significantly affect material flowability, reactivity, solubility and so on. Imaging is a good technique to acquire data on a sample consisting of particles with a range of sizes and shapes, since it can provide both the general and the local information. Focused ion beam scanning electron microscopy (FIB-SEM) is a powerful tool that can be used to collect tomography information about a sample. The SEM can capture high-resolution images of features ranging from hundreds of microns to nanometers in size. When paired with FIB, the SEM is also able to collect images from the sample's interior. This is done by using the FIB to mill away small volumes of material to reveal a local cross-section. By this method, nm-to-micron thick slices of material can be serially milled away to create a stack of SEM images. This stack of two-dimensional (2D) images can then be used to construct a three-dimensional (3D) dataset. Once the 3D volume is generated, image processing methods can be applied to further gather the particle statistical information. In this application note, we demonstrate our capability to quantitatively analyze particle morphology with the conventional image processing methods.

Data Collection and Processing

A commercial metal powder was ordered and dispersed inside a droplet of spin-on-polymer (SOP). A typical SEM cross sectional image of the sample is shown in Figure 1a. The bright areas are metal spheres of different sizes. The dark area is the SOP. We used a ThermoFisher FEI Helios G4 gallium FIB/SEM to collect a stack of 225 cross-sectional SEM images. The step size between SEM slices is 20 nm. To extract 3D information, pre-processing is applied to these 225 images. Figure 1 demonstrates a cross-sectional scanning electron microscope (SEM) image of the particles before (Fig.1a) and after (Fig.1b) pre-processing. The pre-processing steps include tilt correction, noise filtering, region of interest selection, image alignment, and intensity correction. The pixel size

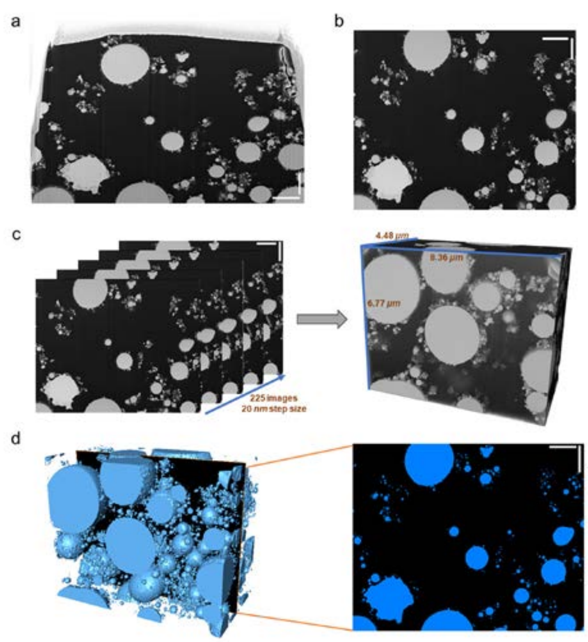

Figure 1

a. Example of cross section image before pre-processing. Scale bar size: 1000 nm.
b. Example of cross section image after pre-processing. Scale bar size: 1000 nm.
c. 3D structure created by stacking FIB-SEM images together. Scale bar size: 1000 nm.
d. Particles extracted as a whole section by a threshold step. The particle section is shown in blue. Scale bar size: 1000 nm.

of the 2D SEM image is 6.52 nm × 8.16 nm. Stacking the images yields a 3D structure with a physical volume of 8.36 µm x 6.77 µm x 4.48 µm as shown in Figure 1c. The essential step to analyze the particle characteristics is to separate all the particles as a whole section out from the dataset. The metal particles appear brighter than the polymer matrix in the SEM images, so a thresholding step was used to segment the particle pixels out to a series of binary images. After this step, all the particles are extracted as one, and the resulting segmented data is shown in Figure 1d.

As shown in Figure 1, many particles touch and form agglomerations. Thus, the second step is to separate the particles from each other to obtain their morphology. To achieve this goal, we first computed the distance map of the segmented data. As shown in Figure 2a, the distance map highlights the distance of the segmented objects to the closest boundary. From the distance map, we can then take advantage of a watershed algorithm to separate the particles. The watershed algorithm enables an automatic object separation operation. It can provide reasonable results in cases when particles are not cracked or disintegrated. The result of the particle separation is shown in Figure 2b. The particle separations are based on their connectivity.

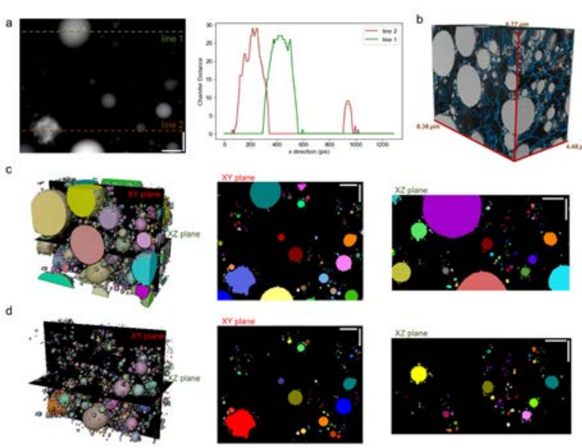

Figure 2
a. Example slice of the distance map. Two line-profiles are provided. Scale bar size: 1000 nm.
b. 3D rendering of the FIB-SEM dataset with the particle separation result. The watershed separation lines are shown in blue.
c. 3D rendering of particles labeled as different objects. Two example slices were provided. Scale bar size: 1000 nm.
d. 3D rendering of particles after filtering. Same example slices as in c were provided. Scale bar size: 1000 nm.

After the particles are separated, they can be labeled as different objects for further analysis, as shown in Figure 2c. For demonstration purposes, the largest 5,000 particles were chosen for analysis. After further removing particles not fully captured in the volume, 4,516 objects were extracted as particles shown in Figure 2d.

Statistical Analysis

The particle separation enables a series of statistical analysis, and we provide some examples as shown in Figure 3. The particle size is represented as equivalent diameter (the diameter of the sphere with the same volume that the object holds) and is shown in Figure 3a. In our example, the mean equivalent diameter of the particles is 92.27 nm. Around 51% of the particles are within the range of 41.04 nm to 68.52 nm. The maximum value of the equivalent diameters among the particles is 2,051.97 nm, meaning that the particle holds the volume of 4.52 µm^3 and occupies 4,243,004 voxels. The minimum value of the equivalent diameters in our example is 41.04 nm, indicating that the particle holds a volume of 36,197 nm^3 and occupies 34 voxels. The particles with largest and smallest equivalent diameters are shown in Figure 3b.

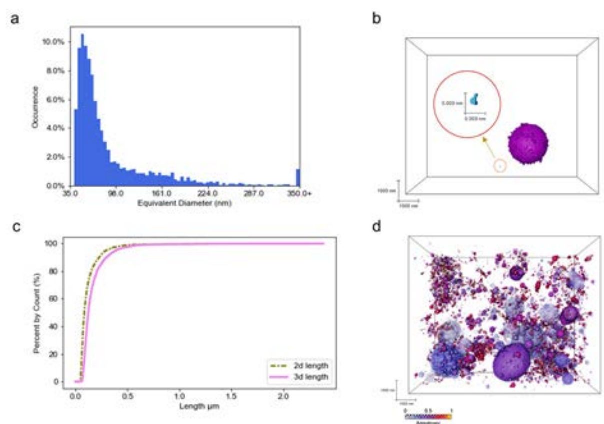

Figure 3
a. Histogram of the particle 3D equivalent diameters
b. 3D rendering of the particles with largest and smallest equivalent diameters. The black lines demonstrate the bounding box. The rendering is shown in projection view. The scale bar is for the plane located at the focal distance.
c. Particle length distribution measured by 3D and 2D
d. 3D rendering of the particles colored by anisotropy. The black lines demonstrate the bounding box. The rendering is shown in projection view. The scale bar is for the plane located at the focal distance.

The length distributions of the particles measured by 3D and 2D are shown in Figure 3c. Length measurement is based on the Feret diameter measurement. Feret diameter is a collection of the object size along specified directions, while length is the maximum in this collection. The results from 2D measurement are smaller than the ones from 3D measurement. As 2D measurements ignore the length through the direction out of the cross-sectional plane, the results are less accurate.

Particle anisotropy is also computed. Anisotropy is used to describe the shape of the particle. When a particle is spherical, its anisotropy should be zero. In Figure 3d, all the particles are colored based on their anisotropy.

Summary

In this application note, we demonstrate how conventional image processing can be used to perform statistical analysis of 3D particle morphology with FIB/SEM tomography. Particle morphological characteristics are important qualities in many different fields. Outside of the particle size distribution and anisotropy, additional properties of the bulk materials can be gained from the particle morphology including the packing density, porosity, and individual particle orientation. With the newly installed plasma-FIB/SEM at Eurofins EAG Laboratories, much larger volumes (hundreds of μm) are now accessible. Please look for future updates detailing how plasma-FIB/SEM can be combined with deep learning methods to extract information from complex structures. Eurofins EAG delivers high-quality data to our clients and provides customized image analysis services to fit our clients' needs. Contact us today to learn how we can help with your next project.