

## SUPPLEMENTARY MATERIAL

### Development of an automated method to perform a quantitative study of particle size distribution and the effect of a conductive layer in Scanning Electron Microscopy

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### TUTORIAL OF PARTICLE SIZE DISTRIBUTION DETERMINATION USING IMAGEJ SOFTWARE, VERSION 1.49.

#### Introduction

The knowledge about the particle size diameter (PSD) is important in materials science since it has influence on the final properties of materials.

This tutorial was developed to support electron microscopy users in the task of producing a histogram of particle size using an automated method. Although it was developed and tested for electron microscopy images, it can also work for images taken by other techniques, such as atomic force microscopy and optical microscopy, since the scale of equipment is calibrated and a good sample was prepared.

#### Objective

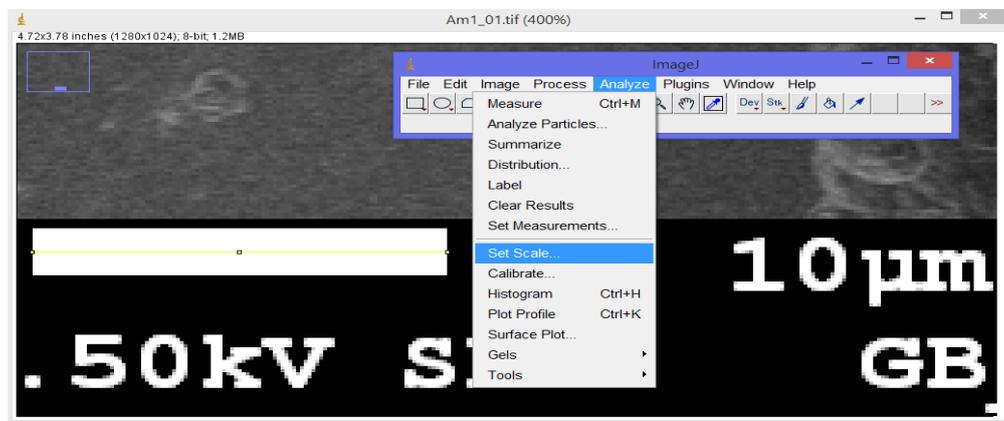
The main objective of this tutorial is to determine the average particle diameter using an automated method. In the present case, we decided to use the *ImageJ* software, and the main reason is that this software is free and very powerful. Then, it minimizes the user influence on the histogram.

## Procedure

We will assume you have already the *ImageJ* software (<https://imagej.nih.gov/ij/>) installed at your computer.

On the tab **File**, click **Open** and select the electron microscopy image you want to work on.

Select the **Straight** tool from the menu bar and draw a straight line fitting the scale bar, as shown in Figure 1S. Then, on the tab **Analyze**, click at **Set Scale** and pop-up window will be opened presenting the line size in pixels.



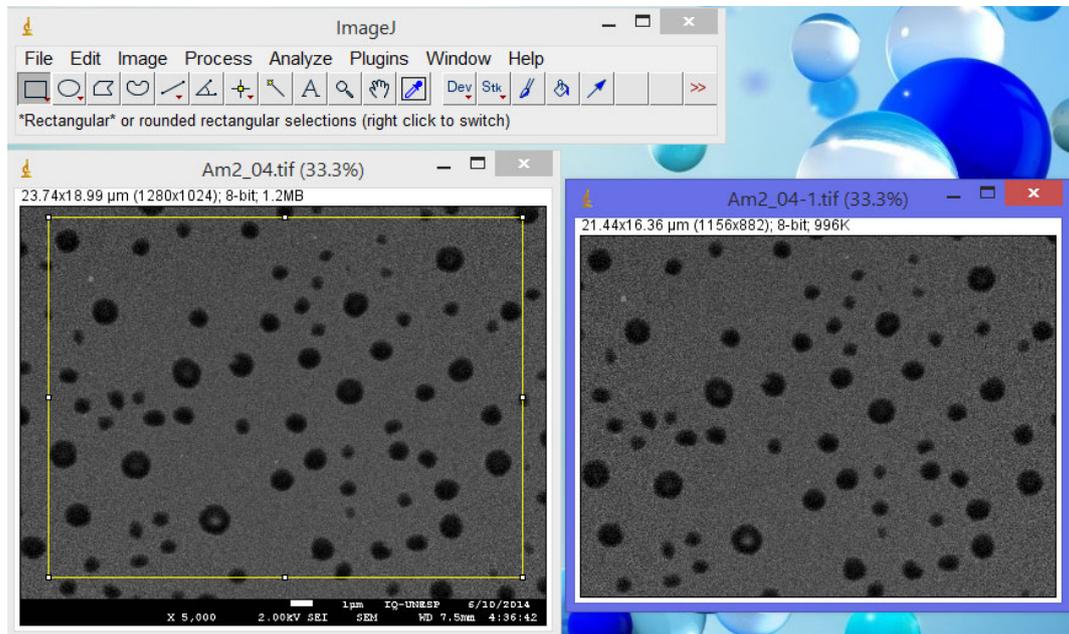
*Figure 1S. SEM image with the scale bar fitted by a straight line*

At the **Set Scale** dialog box click the **Click to Remove Scale** button (Figure 2S). Following, fill the **Known distance** and the **Unit of length** boxes (10 and  $\mu\text{m}$  for Figure 2S) and press **OK**.



*Figure 2S. Set Scale dialog box showing the parameters to be entered*

Select the **Rectangle** tool and draw a rectangle at the region of interest (ROI) of image. Press **Ctrl+Shift+d** to produce a new window having the ROI (Figure 3S).

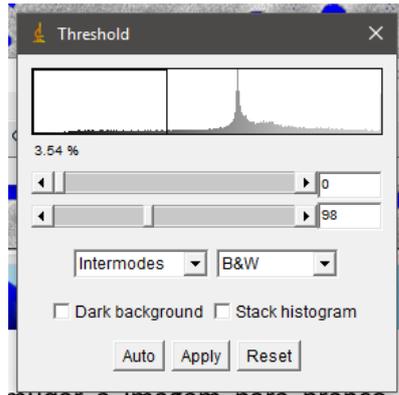


*Figure 3S. Duplicate image step. The blue window shows the new area*

Note 1: Although we avoid using filters and image changes before using the threshold, in some cases it is essential due to the low quality of image. If this is your case, first try to improve image using **Brightness** and/or **Contrast** controls. If a filter is really necessary, **Bandpass Filter** is a good option because it can be less aggressive to the filtered image.

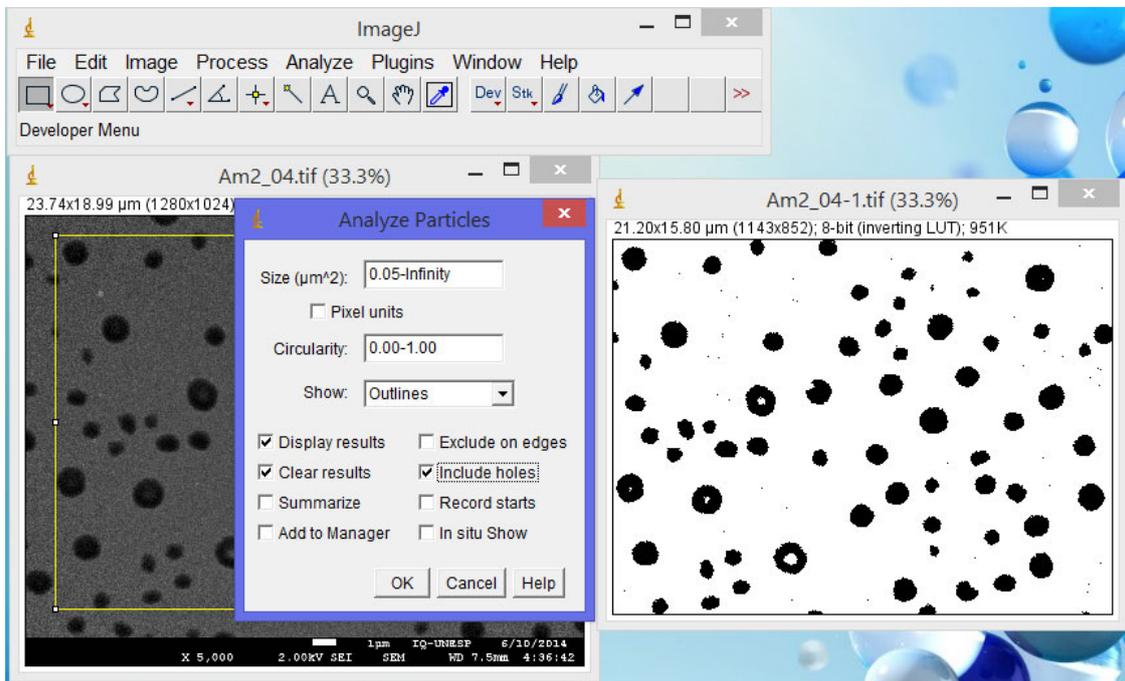
Note 2: Please realize that when using filters the method can produce divergence on particle size diameter calculated by different users due to this additional step.

At the tab **Image** select **Adjust** and click on **Threshold**, which will open a new window (Figure 4S). From our exhaustive tests, intermodes and B&W modes are the best ones, but you can try other modes. Once the threshold is applied, move the two adjustment bars to fit all particles. Then, mark the **Dark background** box and press **Apply**. At this point we are ready to start counting the particles.



**Figure 4S.** FFT Threshold dialog box at the ImageJ software

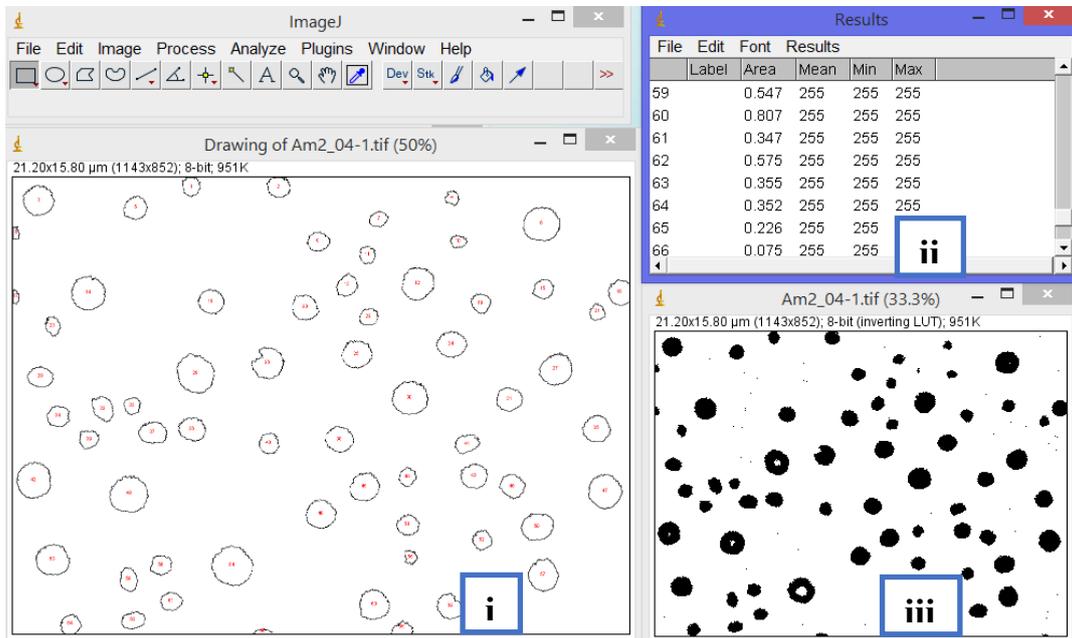
On the tab **Analyze**, click on **Analyze Particles**. Once you have an idea about the diameter size, you can exclude too small and too big pixels by defining the minimum and maximum area of particles (**Size  $\mu\text{m}^2$** ). You can also filter particles by **Circularity**, which can be important if you have spherical particles and rods mixed, but just want to count the particles. Select **Outlines** on the **Show** selecting dialog, and mark **Display results**, **Clear results** e **Include holes** (Figure 5S). Press **OK** and the particle counting will start.



**Figure 5S.** Analyze particles dialog box at ImageJ software

Two new windows will be opened, one showing the outlines and other exhibiting the results of particle area (Figure 6S). Save the **Results** window (**File, Save as...**), and this file can be used to calculate the diameter of particles and produce the diameter histogram. SciDavis is a free and

powerful software for this task, although you may want use other private software's, like Origin, Excel, etc.



**Figure 6S.** Print screen of analyze particles results. (i) Outline drawing image, (ii) table results, (iii) SEM image after applying the threshold process

The present method was tested and validated in our laboratory for near spherical particles, but users can test and adapt it for their own situations.