“Automatic” determination of particle size distribution

ImageJ is a free, very versatile program package for image processing and manipulation which runs under Java. It can be downloaded from:
http://rsb.info.nih.gov/ij/download.html.

One of its options is the automatic determination of particle size distributions. To gain reliable results, several steps must be followed:

1. Calibration of image magnification

In the name of STEM images (e.g. AuTiO2_STEM_84nm-3), the designation xyznm (84nm) gives the width of the image instead of a scale bar. Draw a straight line (selection in the tool bar of ImageJ, Fig. 1) from the left to the right side of the image. Go to drop down menu ***Analyze*** > *Set Scale*. A window opens (Fig. 2). The Distance in Pixels should be close to 2^n, typically 512 or 1024. Then type in the width of the image in Known Distance and set the Unit of Length to nm. Attention: If you select Global, the selected magnification is assigned to all images opened till ImageJ is closed.

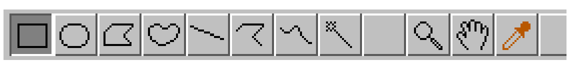

Fig 1. Tool bar of ImageJ

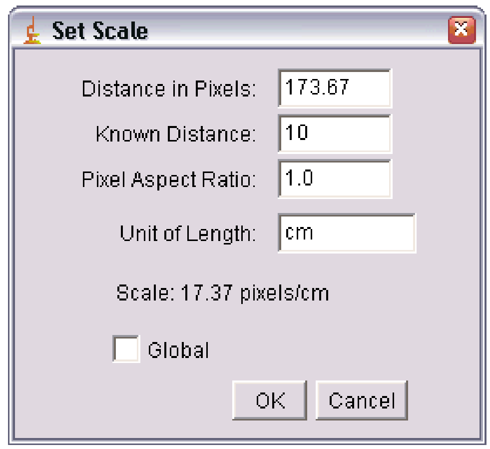

Fig. 2. Set Scale window

If you like, a scale bar can be added to the image (Fig. 3) by ***Analyze*** > *Tools* > *Scale Bar*. However, better do this after particle analysis since the scale bar might be treated as a particle.

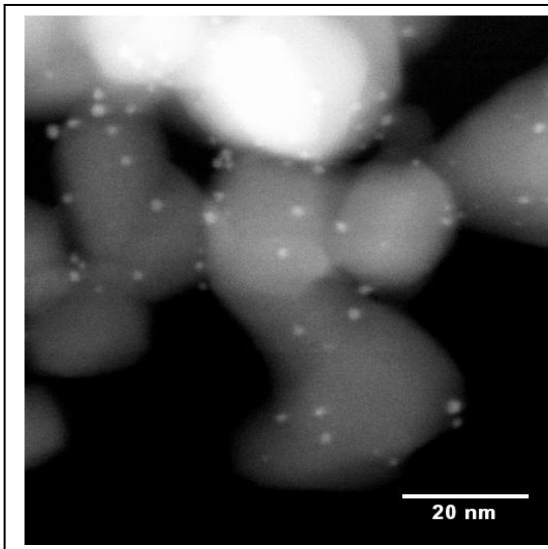

Fig. 3. HAADF-STEM image of Au particles on titania with scale bar

2. **Find a threshold for the image**

This is a quite straightforward step if the contrast between particles and background is strong. Using ***Image*** *> Adjust > Threshold,* it is easy to mark the looked-for features (here: bright particles), which can afterwards be shown in different forms (Fig. 4).

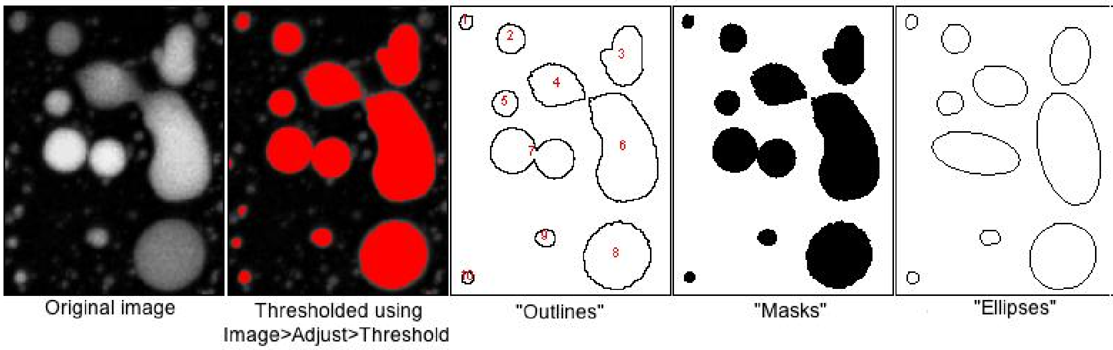

Fig. 4: Threshold for an image with bright particles

Unfortunately, this essential step is quite difficult in the case of STEM images of small particles. Even if the image shows a large Z contrast between the metal particles and the support (Fig. 3), the particles frequently appear with similar or even less brightness as thick areas of the supporting material. To take care of that, the intensity of the background has to be determined and subtracted.
(i) Duplicate the image: ***Image*** *> Duplicate*
(ii) Apply a Gaussian blur filter with a high value for the radius (here: 25 pixels): ***Process*** *> Filters > Gaussian Blurr*, generating an image of the background (Fig. 5).

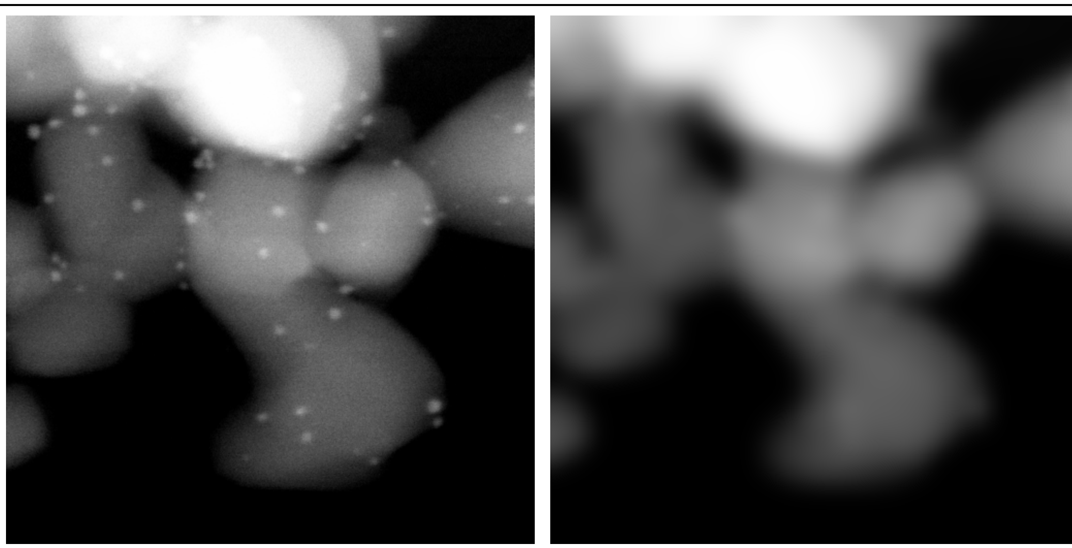

Fig. 5: Original STEM (left) and background image (right, after applying Gaussian blurr)

(iii) The filtered image is now subtracted from the original one using ***Process*** > *Image Calculator* (Fig. 6). Select Substract and tick Create New Image. Select the original image as 1 and the background image as 2.

(iv) The resulting image shows the particles clearly, especially after applying ***Image*** > *Adjust* > *Brightness/Contrast*, e.g. selecting Automatic (Fig. 7).

One can try to use the button ***Process*** > *Subtract Background*, which works similar on the original image.

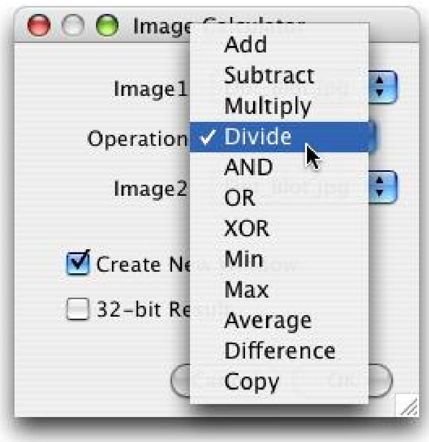

Fig. 6: Image Calculator page

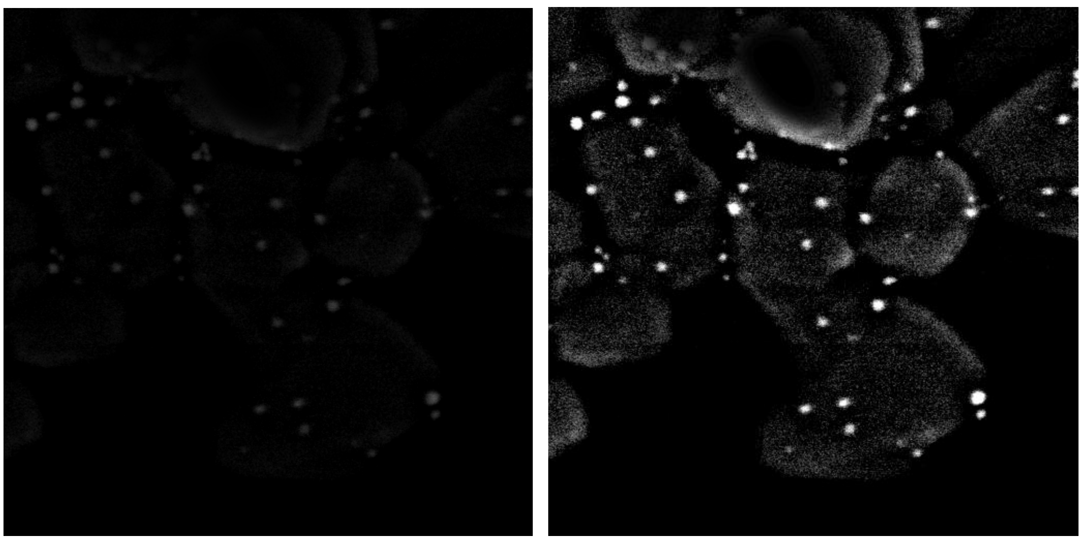

Fig. 7: Background-subtracted STEM image (left) and the same with increased brightness/contrast (right)

(v) On the background-subtracted STEM image, the threshold operation is applied now: ***Image*** > *Adjust* > *Threshold*. The sliders have to be adjusted in such a way that the particles appear as dark spots in the black&white mode (Fig. 9). Some features at the rim of the very bright areas in the original image appear dark as well, illustrated the difficulty of “automatic” particle determination in such images.

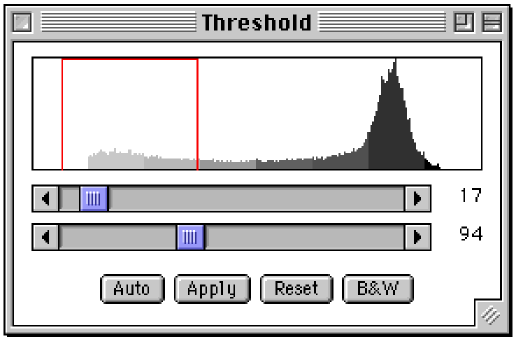

Fig. 8: Threshold window

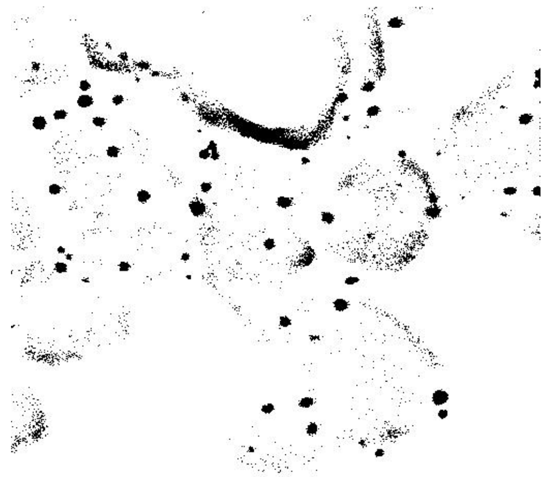

Fig. 9: “Particles” found by thresholding

3. **Analyzing particles**

Now, the image is ready for analyzing the particles and their distribution automatically. Some parameters must be set (Fig. 10). Most important is the particle size. Try to select the values in such a way that only the looked.for particles appear in the final image (Fig. 11). Do this by comparison with the original image. This step is very critical since too small values might include other feature whereas too large vales might exclude the smaller particles. Optionally, one can get a list of particles analyzed (see below, particle number corresponds to that in the figure (Fig. 11)) and a histogram of particle size distribution.
The average particle size is calculated and listed in the summary:

```
Threshold: 0-128
Count: 61
Total Area: 110.196 nm^2
Average Size: 1.806 nm^2
Area Fraction: 1.6%
```

From the average size F, the average diameter can be calculated from

$$d = 2\sqrt{F/\pi}$$

assuming round particles.

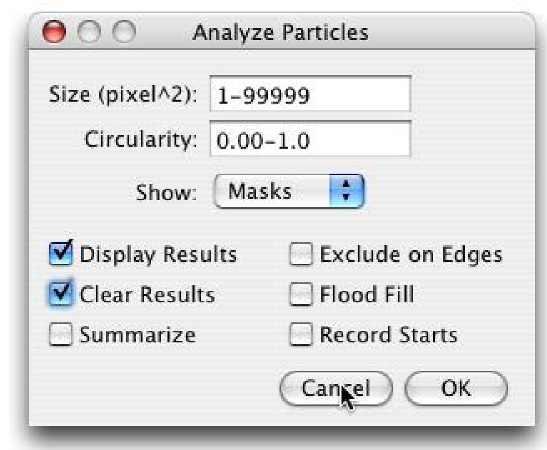

Fig. 10: Analyze particles window

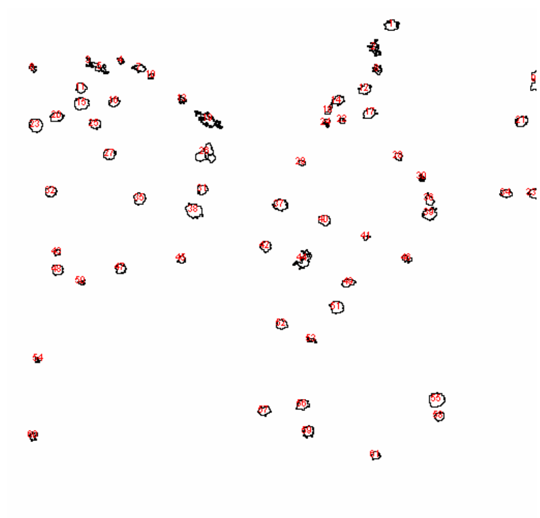

Fig. 11: Analyzed particles outlined.

Area	Mean	Min	Max	
1	2.557	0	0	0
2	1.534	4.474	0	255
3	0.458	0	0	0
4	0.565	12.143	0	255
5	1.723	19.922	0	255
6	0.646	0	0	0
7	1.453	4.722	0	255
8	0.969	7.083	0	255
9	2.180	0	0	0
10	0.565	12.143	0	255
11	1.938	0	0	0
12	2.153	12.750	0	255
13	0.673	0	0	0
14	2.180	12.593	0	255
15	3.418	0	0	0
16	1.642	0	0	0
17	2.342	0	0	0
18	0.458	0	0	0
19	3.580	15.338	0	255
20	2.288	0	0	0
21	2.315	0	0	0
22	0.538	12.750	0	255
23	3.418	0	0	0
24	0.727	0	0	0
25	1.938	0	0	0
26	4.737	0	0	0
27	2.449	0	0	0
28	1.023	0	0	0
29	0.673	0	0	0
30	0.431	0	0	0
31	1.857	0	0	0
32	2.153	0	0	0
33	1.534	0	0	0
34	1.750	0	0	0
35	2.584	2.656	0	255
36	1.534	0	0	0
37	2.988	0	0	0
38	4.360	1.574	0	255
39	2.880	2.383	0	255
40	2.288	0	0	0
41	0.458	0	0	0
42	2.019	3.400	0	255
43	0.673	0	0	0
44	2.961	16.227	0	255
45	0.969	0	0	0
46	0.915	30	0	255
47	1.830	0	0	0
48	2.207	0	0	0
49	1.857	0	0	0
50	0.431	0	0	0
51	3.122	0	0	0
52	2.019	3.400	0	255
53	0.538	0	0	0
54	0.404	0	0	0
55	4.307	0	0	0
56	2.234	6.145	0	255
57	1.965	0	0	0
58	1.561	0	0	0
59	2.126	0	0	0
60	0.888	30.909	0	255
61	1.211	0	0	0

Problems

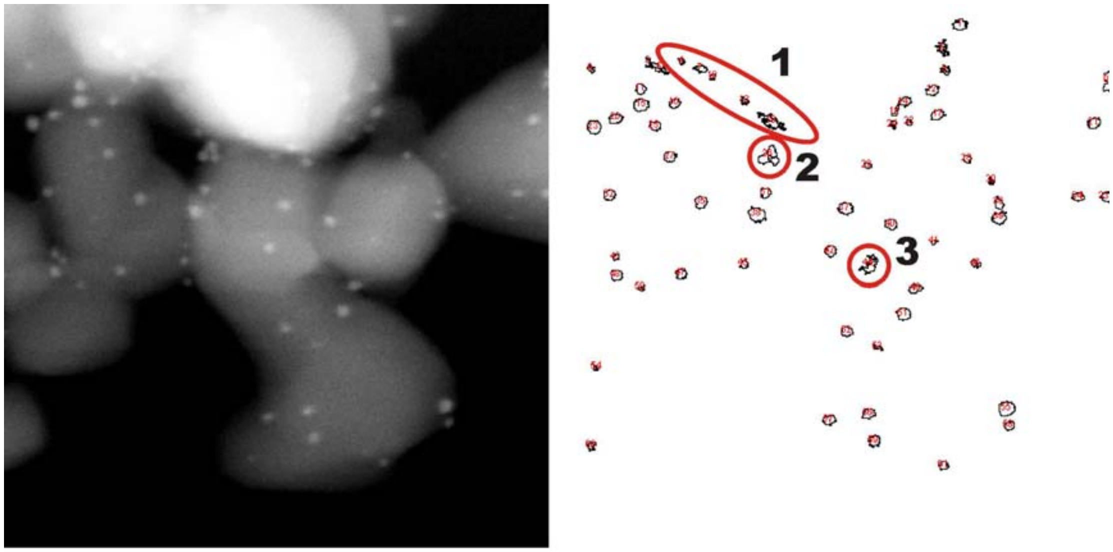

Fig. 12: Original STEM image (left) and the map of particles (outlined, right) found by the analysis described in the text

It is a problem with this method to determine all particles unambiguously. Some of the problems appear in this example (Fig. 12): the particles at the edge of thick areas are not clearly recognizable (1). Particles close together might not be resolved (2). Artifacts appear at some sites (3).