
SEM Segmentation

A brief tutorial

NanoSolveIT H2020 Nanoinformatics Project

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Version, November 2022

Overview

The tool can be easily accessed through the link:

<https://deepsegm.cloud.nanosolveit.eu/>

Instructions

Overview

SEM Segmentation is a web implementation of the deep learning model, which has been presented in the publication: **Rühle, B., Krumrey, J.F. & Hodoroba, V.D. Workflow towards automated segmentation of agglomerated, non-spherical particles from electron microscopy images using artificial neural networks. Sci Rep 11, 4942 (2021). <https://doi.org/10.1038/s41598-021-84287-6>**, for Scanning Electron Microscopy (SEM) images nano-particles segmentation and size calculation. The image segmentation based on GAN and MultiRes Unet deep learning methods, while the size of each nano-particle is calculated accordingly to its pixel size. The **Image 1** shows the initial SEM Segmentation page.

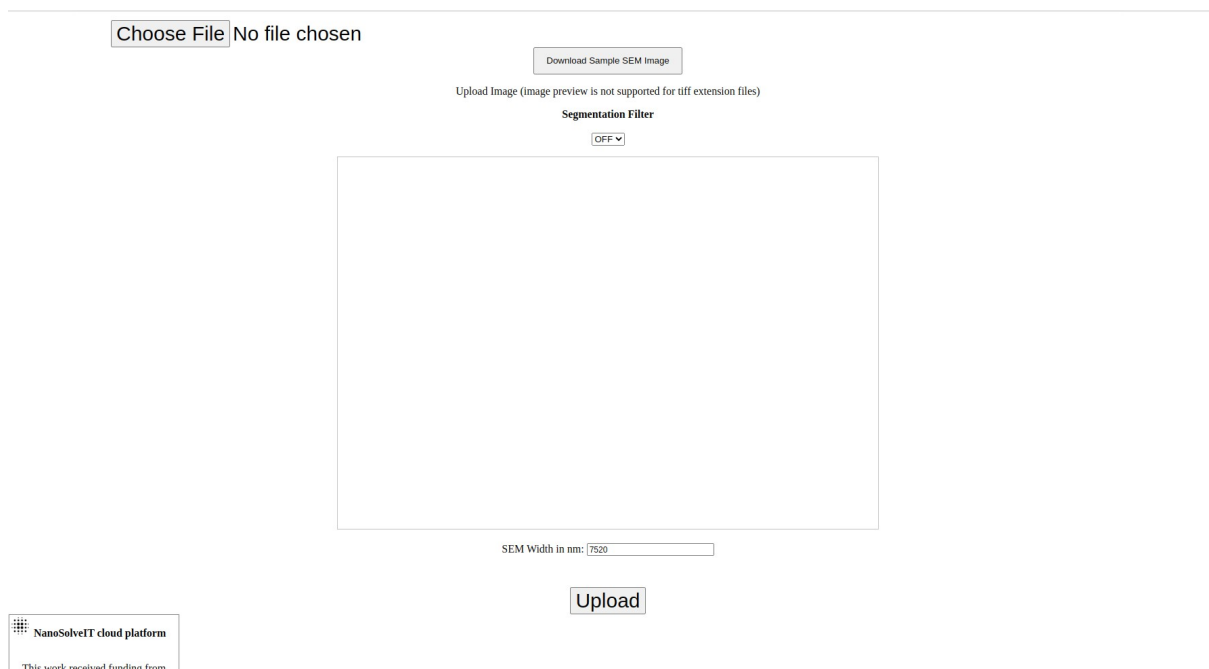


Image 1. Initial SEM Segmentation page.

File Selection

For the SEM image file selection, the button **Choose File** is used. In order to preview the image file, a file different than tiff extension must be chosen. SEM Segmentation includes also a **Download Sample SEM Image** button, to download a sample SEM image.

SEM Image file pre-processing

The neural network that is used for image segmentation has been trained on TiO₂ nanoparticles **Image 2** and will perform best when used with images that are “similar” to its training data. “Similar” in this context does not necessarily mean that the images also need to show TiO₂ nanoparticles – in fact, the network is not aware of the material or the absolute size during segmentation, but it is

aware of the contrast, texture, and relative size (in pixels) of the objects in the image. As long as these three parameters are similar, the network has a good chance of being able to extrapolate from its training data to the new data.

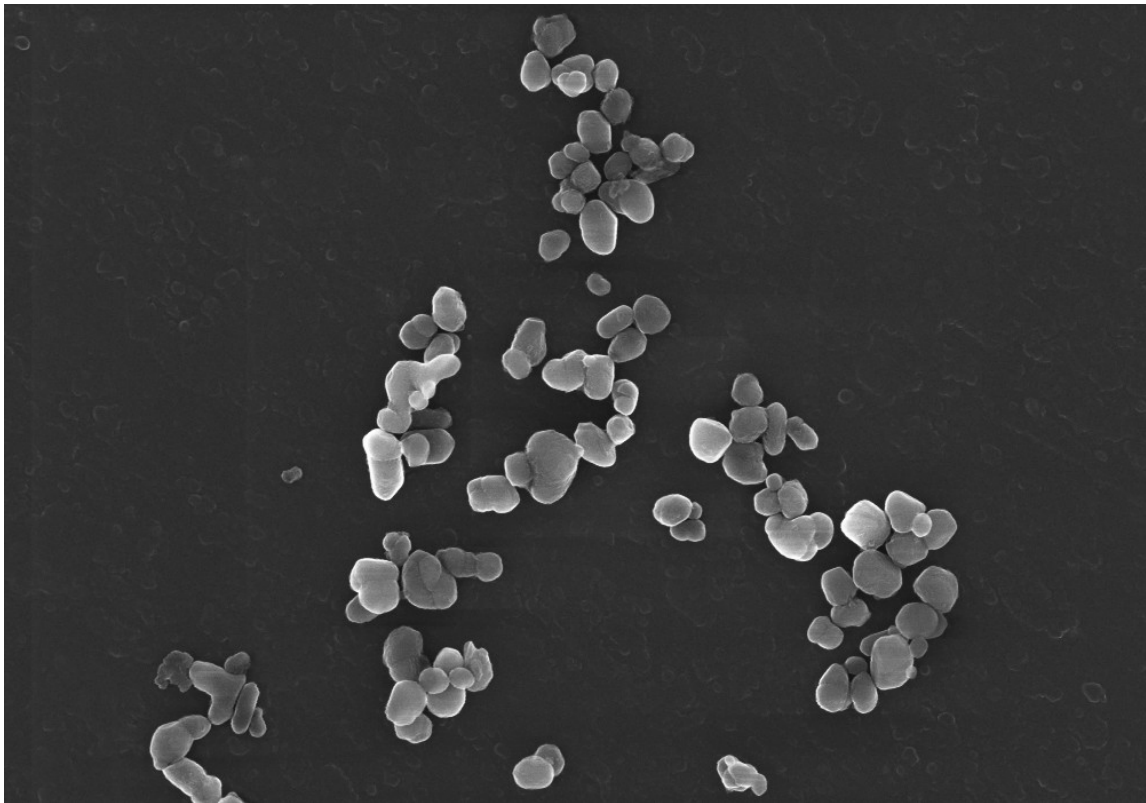


Image 2. TiO₂ SEM image example.

In case of hot or cold pixels presence in the image, possibly due to a SEM labels or text, it is necessary to remove them before uploading it because it will cause problems to segmentation and the contrast adjustment
Image 3.

Image 3. Label with hot and cold pixels image example.

If the particles in the SEM images are much smaller or much larger than the particles the network was trained on, a resampling/rescaling of the images before inference may be needed. One reason why the particles image might differ in size from the training images could be that they were recorded with a detector that has a higher or lower resolution (e.g., 2048 x 2048 or 640 x 480, while the network was trained on images with a size of 1024 x 768), another reason could just be personal preference or a different analytic goal of the microscope operator who acquired the images (the operator might opt for a lower magnification to have more particles per image, or they might want to highlight small features and opt for acquiring images at a higher magnification which will then show only a few particles). This can be “corrected” to some extent by measuring the sizes of a few particles in pixels and rescaling your images accordingly. As an example, let us assume images that acquired at a resolution of 2048 x 2048 pixels and measured particle sizes of approximately 100 pixels. The particles in the training images were typically somewhere between 30-50 pixels in size, so you could try downscaling your images by a factor of $40/100 = 0.4$, i.e., to a size of $2048 * 0.4 \times 2048 * 0.4 = 819 \times 819$ pixels.

Filtering

A possibly mis-segmented particle filter can be applied as an option to the segmentation that considers mis-segmented particles with small pixel size, for this a toggle button with option is present in the application **Image 4**.

Segmentation Filter

OFF ▼

Image 4. Segmentation filter applied toggle button.

Image Width

To apply a particle size measurement to the SEM image, the image width must be defined in the application, therefore a field with the image width in nm is present with a default value of 7520 nm

Image 5.

SEM Width in nm:

Image 5. Image width size field.

Upload File

In order to upload the file, use the **Upload** button below the image preview.

Segmentation and Size Histogram Figure

When the file is uploaded, the deep learning and size measurements models are processing the image and finally a figure that includes the initial image, the segmented image, an image that shows possibly wrong segmented particles with red and the size frequencies histogram is produced **Image 6**.

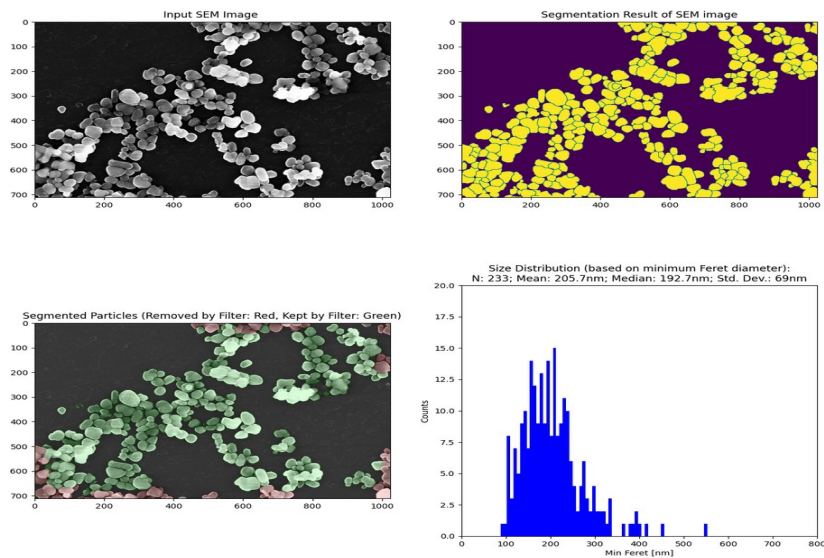


Image 6. Figure that includes the initial image, the segmented image, an image that shows possibly wrong segmented particles with red and the size frequencies histogram.

The software includes also two buttons **Image 7** for figure download and for a CSV file download, that includes the mean value of nano-particles sizes, the median, the respective standard deviation and the individual nano-particle size as well.

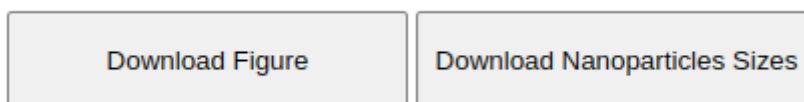
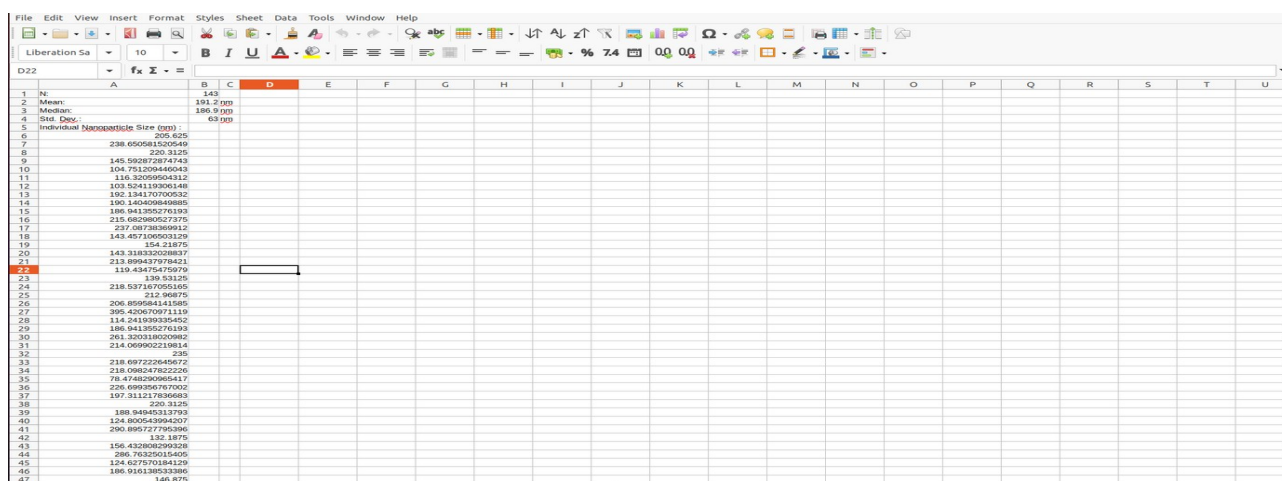


Image 7. Download Figure and Download Nano-particle Size File buttons

The nano-particle size CSV file is shown in **Image 8**.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	N																				
2	Mean:			143																	
3	Median:			186.9 DD																	
4	Std. Dev.:			69 DD																	
5	Individual Nanoparticle Size (nm)																				
6		238.650581520549																			
7		220.3125																			
8		145.592872874743																			
9		104.791209446043																			
10		116.32059504312																			
11		103.524110306148																			
12		182.134170700532																			
13		190.14040849885																			
14		186.941350576193																			
15		215.682980527375																			
16		237.68738369412																			
17		143.457106903129																			
18		154.21875																			
19		143.31832028837																			
20		213.899437978421																			
21		119.43475475979																			
22		139.53125																			
23		218.537167055165																			
24		912.94875																			
25		206.859584141585																			
26		395.420670971119																			
27		114.241539333452																			
28		186.941350576193																			
29		261.320318030982																			
30		214.069902219614																			
31		235																			
32		218.697225845672																			
33		218.08647822226																			
34		78.4748290965417																			
35		226.699256767602																			
36		197.311217836683																			
37		220.3125																			
38		189.94845313793																			
39		124.800543994207																			
40		290.895727795396																			
41		152.1875																			
42		156.432808299328																			
43		286.763250154405																			
44		124.627570184129																			
45		186.931138033386																			
46		146.875																			
47																					

Image 8. Nano-particle Size CSV file

Support

