Readout of image metadata & adding a scale bar

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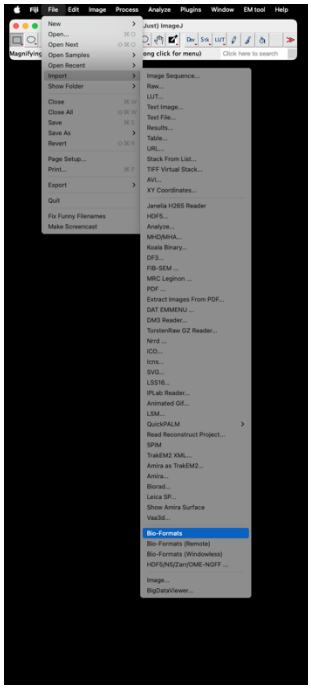
All important image and machine metadata are stored in the tiff header of the image files and can be read out in two different ways: via a regular text editor or via the Bio-Formats Import Tool in Fiji / Image J.

1) Readout via text editor (works for tif files)

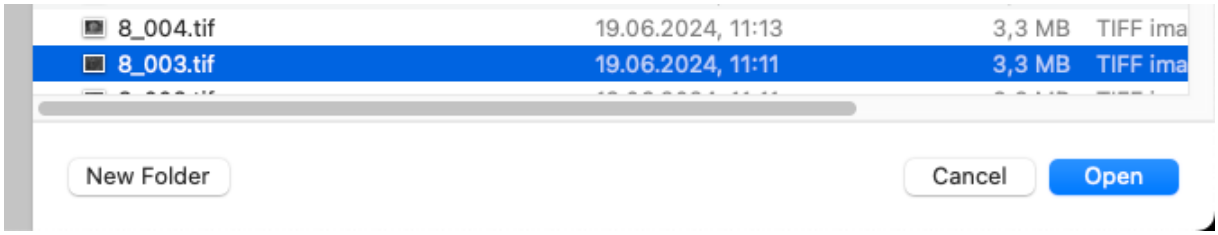
The metadata of the tif image files can be read out by opening the image file in a text editor of your choice (e.g. TextEdit (Mac) or Notepad (PC)). Scroll down & search for the terms such as “image pixel size”, “height”, “width” (in case of Zeiss SEM files, such as from the Crossbeam 550).

2) via Bio-Formats import tool in Fiji / ImageJ

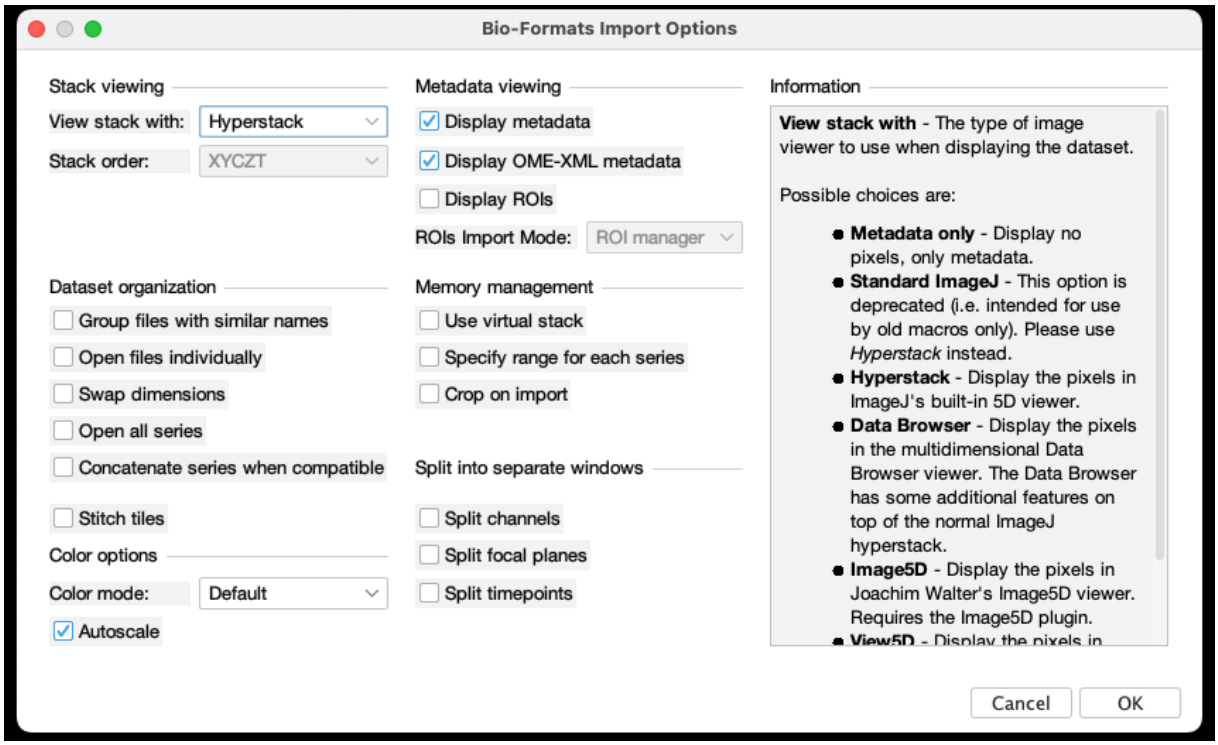
This methodology does not only work for tif files, but also for many other image formats. In FIJI open the Bio-Formats importer via → File/Import/Bio-Formats.



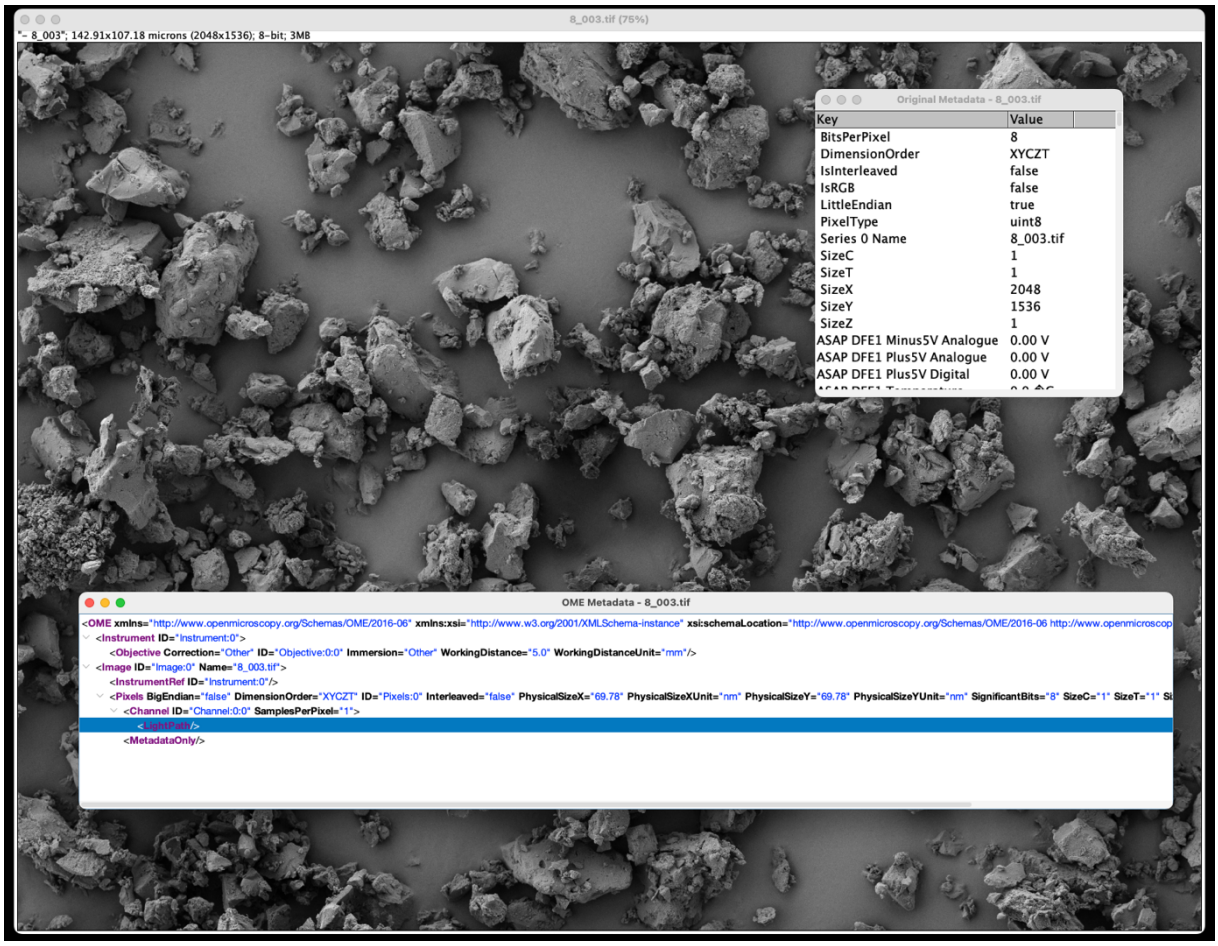
Choose the file of interest and click open.



In the following menu make sure to activate “Display metadata” and “Display OME-XML metadata” & click ok.



This opens three windows: the image file, an “Original metadata window” and the “OME metadata” window.

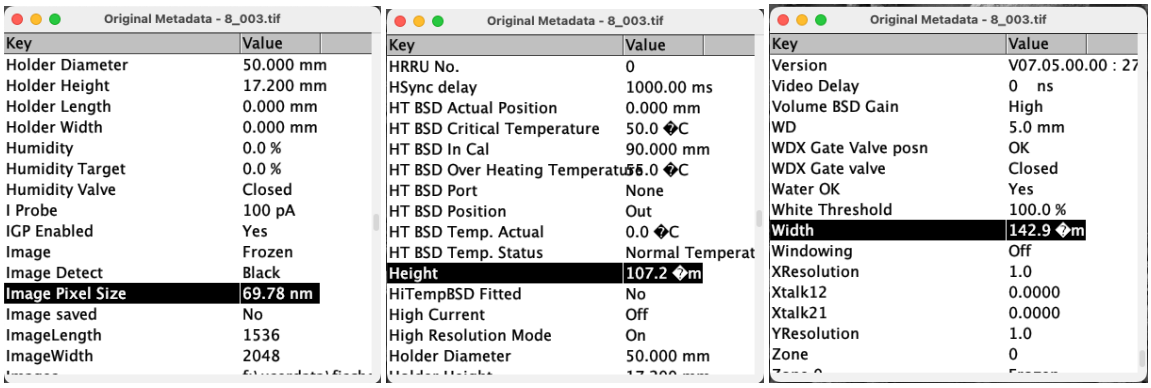


Please notice, that the image file was automatically calibrated (pixel size value) during this import. Not only the pixel dimensions of the file are shown (2048x1536px), but also the dimensions of the image height and width in microns.

The pixel and image dimensions can be read out from within the Metadata windows: The OME Metadata window only reveals the pixel size and the size unit:

PhysicalSizeX="69.78" PhysicalSizeXUnit="nm" PhysicalSizeY="69.78" PhysicalSizeYUnit="nm"

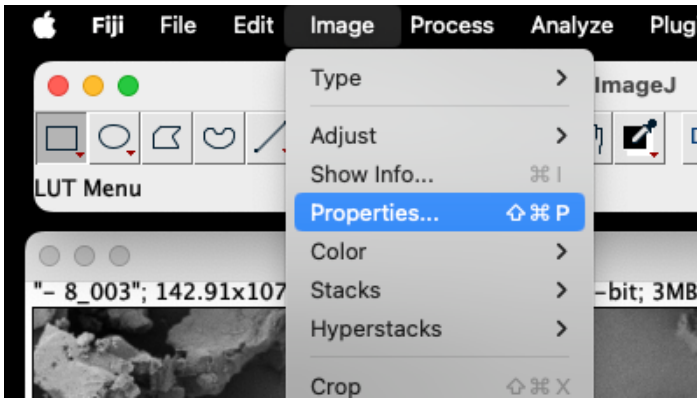
The original Metadata window on the other side contains all stored technical metadata from the SEM and in addition to the pixel size, also image height and width can be read out:

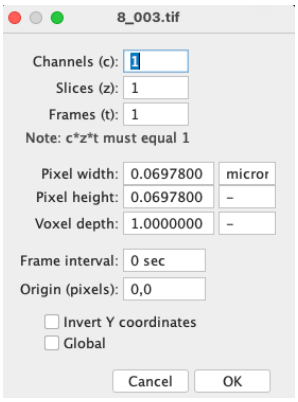


Please notice, that image width and height values are rounded to the second decimal.

The image width value is of interest also during the preparation of figure plates (in case scale bars are not inserted via Fiji (see below), but by graphics/vector graphic software.

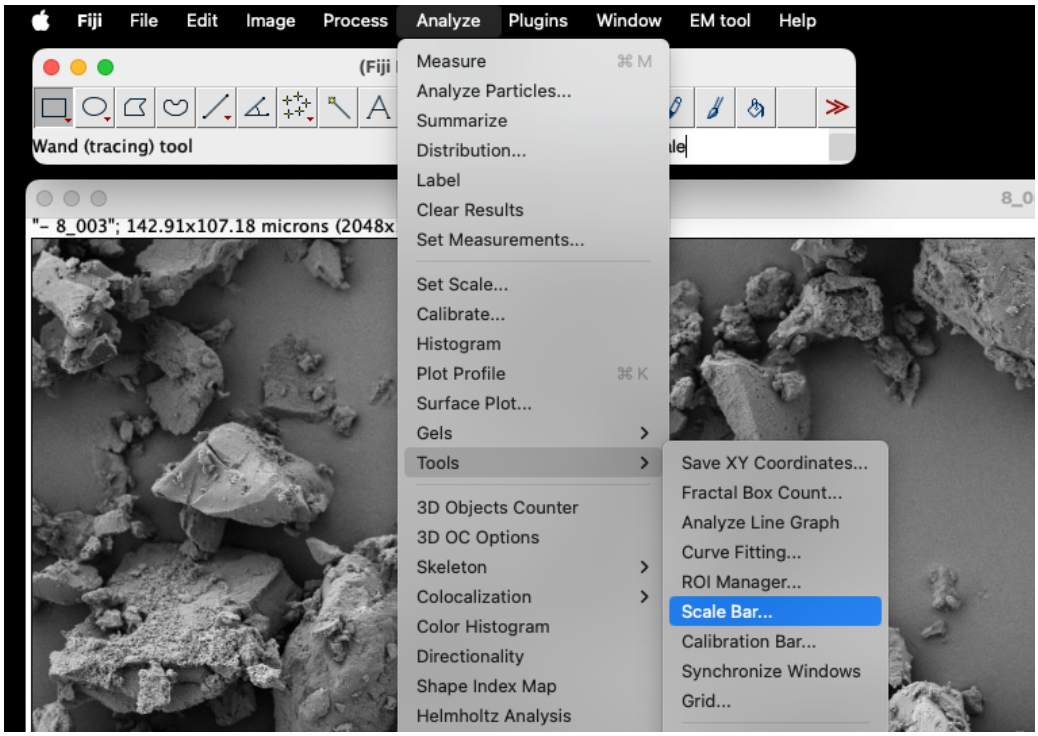
We can crosscheck, that the image is calibrated by checking the image properties (Image/Properties):

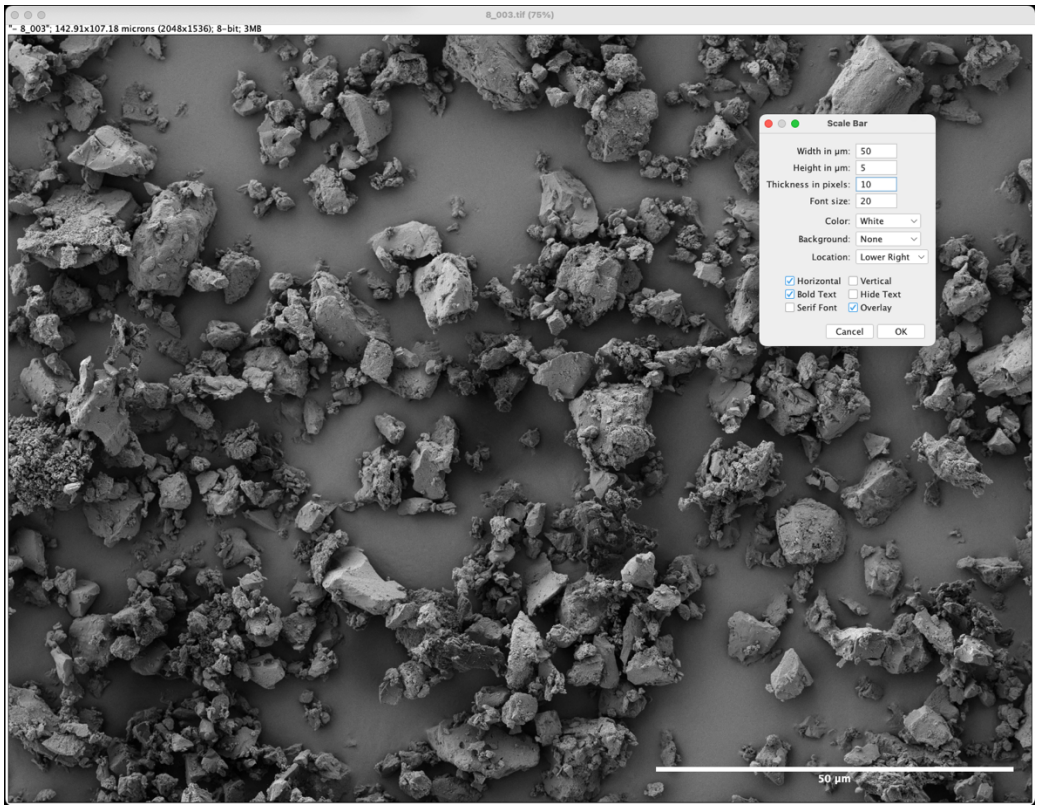




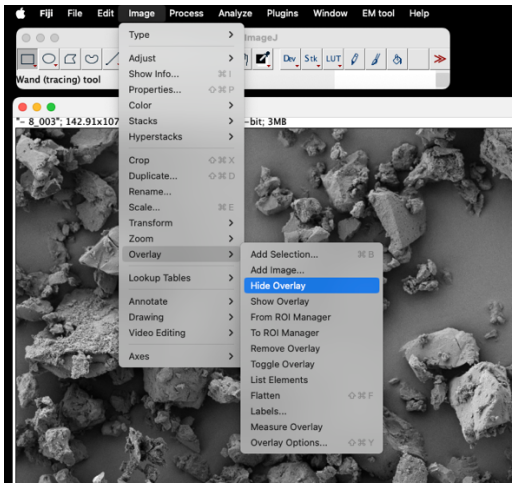
Adding a scale bar

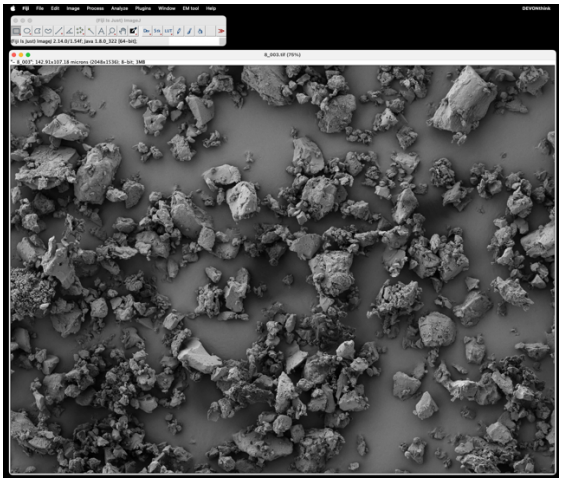
Under Analyze/Tools/Scale bar the scale bar tool can be found. Adjust the values according to your liking.



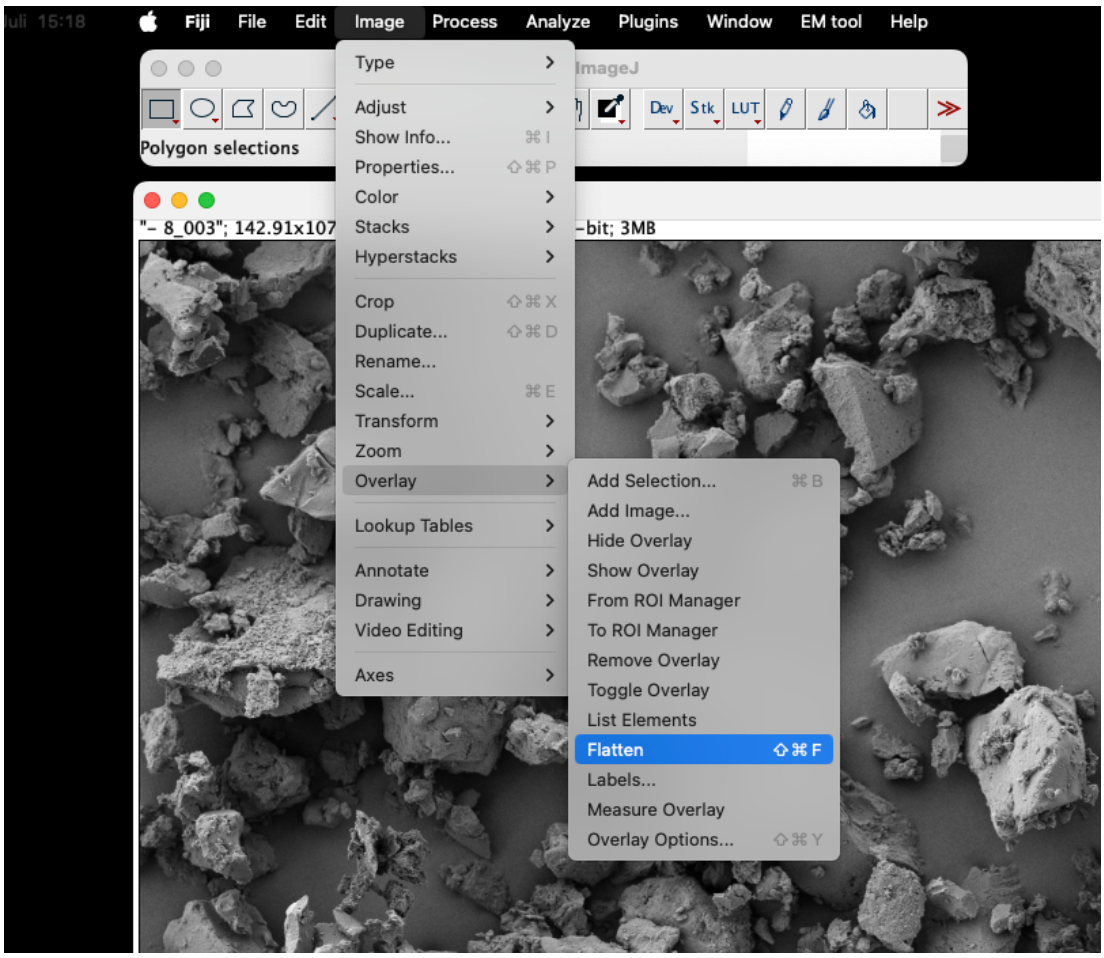


Before hitting ok, please decide if the scale bar should be printed as pixels into the image (not reversable) or if it should be saved as an overlay (for the latter, set the tick at “Overlay”). An overlay is saved in an image (in tiff format, for saving of the file: File/Save as /Tiff…) and can also be hidden from the view.





By hitting “Flatten” a copy of the image will be generated in which the scale bar is printed as pixels into the image.



Please notice the difference: clear edges of numbers and letters (Overlay)

In the flattened copy one clearly can identify single pixels at the edges of numbers and letters, if we zoom in.