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).

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	* 8_003.tir		8_003.tif
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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 \rightarrow File/Import/Bio-Formats.

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 FU 	File Edit Image	Process	Analyze Plugins Window EM tool Help
	New	>	Just) ImageJ
	Open	200	D 🖓 🖬 Dev Six LUT Ø 🖌 👌 🔉 🗩
Magnifying	Open Next	0.00	ong click for menu) Click here to search
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	0.0		AT COORDINATES
			Janelia H265 Reader
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			IPLab Reader
			Animated Gif
			LSM
			QuickPALM >
			SPIM
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			Amira
			Biorad
			Show Amira Surface
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			Bio-Formata (Remote)
			Bio-Formats (Windowless)
			HDF5/N5/Zan/OME-NGFF
			Image
			BigDataViewer

Choose the file of interest and click open.

8_004.tif	19.06.2024, 11:13	3,3 MB TIFF ima
8_003.tif	19.06.2024, 11:11	3,3 MB TIFF ima
New Folder		Cancel Open

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

Stack viewing		Metadata viewing	Information				
/iew stack with:	Hyperstack \vee	Display metadata	View stack with - The type of image				
Stack order:	XYCZT \lor	Display OME-XML metadata	viewer to use when displaying the dataset.				
		Display ROIs	Possible choices are:				
		ROIs Import Mode: ROI manager \vee	 Metadata only - Display no pixels, only metadata. 				
Dataset organization		Memory management	Standard ImageJ - This option is				
Group files wit	th similar names	Use virtual stack	by old macros only). Please use				
Open files ind	ividually	Specify range for each series	Hyperstack instead.				
Swap dimensi	ons	Crop on import	 Hyperstack - Display the pixels in ImageJ's built-in 5D viewer. 				
Open all series	5		Data Browser - Display the pixels				
Concatenate series when compatible		Split into separate windows	in the multidimensional Data Browser viewer. The Data Browser				
Stitch tiles		Split channels	has some additional features on top of the normal ImageJ				
Color options		Split focal planes	hyperstack.				
Color mode:	Default ~	Split timepoints	Joachim Walter's Image5D viewer.				
Autoscale			Requires the Image5D plugin.				

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.

8_003"; 142.91x107.18 microns (2048x1536); 8-bit; 3MB

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:

Original Metadata - 8_003.tif		Original Metadata -	8_003.tif	Original Metadata - 8_003.tif		
Key	Value	Key	Value	Key	Value	
Holder Diameter	50.000 mm	HRRU No.	0	Version	V07.05.00.00 : 27	
Holder Height	17.200 mm	HSync delay	1000.00 ms	Video Delay	0 ns	
Holder Length	0.000 mm	HT BSD Actual Position	0.000 mm	Volume BSD Gain	High	
Holder Width	0.000 mm	HT BSD Critical Temperature	50.0 �C	WD	5.0 mm	
Humidity	0.0 %	HT BSD In Cal	90.000 mm	WDX Gate Valve posn	OK	
Humidity Target	0.0 %	HT BSD Over Heating Tempera	atu576.0 �C	WDX Gate valve	Closed	
Humidity Valve	Closed	HT BSD Port	None	Water OK	Yes	
I Probe	100 pA	HT BSD Position	Out	White Threshold	100.0 %	
IGP Enabled	Yes	HT BSD Temp. Actual	0.0 �C	Width	142.9 �m	
Image	Frozen	HT BSD Temp. Status	Normal Temperat	Windowing	Off	
Image Detect	Black	Height	107.2 �m	XResolution	1.0	
Image Pixel Size	69.78 nm	HiTempBSD Fitted	No	Xtalk12	0.0000	
Image saved	No	High Current	Off	Xtalk21	0.0000	
ImageLength	1536	High Resolution Mode	On	YResolution	1.0	
ImageWidth	2048	Holder Diameter	50.000 mm	Zone	0	
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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):

fiji File Edit	Image	Process	Analyz	e Plug	•	8	_003.tif	
	Tune				(Channels (c):	1	
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LUT Menu	Droportio	-	1 94 D			Pixel height:	0.0697800	-
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000	Color		>		Fra	me interval:	0 sec	
- 8_003"; 142.91x107	Stacks		>	-bit; 3MB	Or	igin (pixels):	0,0	
and and	Hypersta	cks	>	2		Global	oordinates	
100	Crop	4	X#<				Cancel	ОК

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.

