

# **Efficient and reproducible measurements in FIJI**

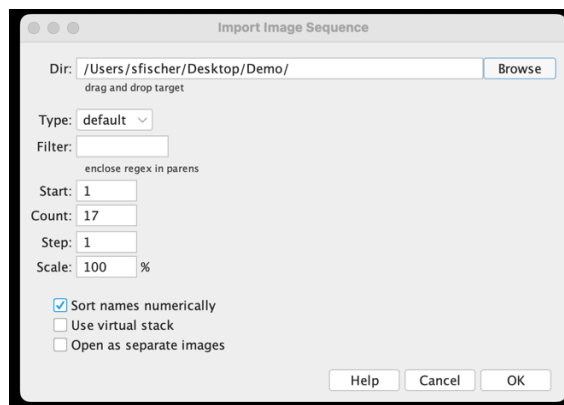
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All images in a folder can be measured more efficiently in an image stack, even if they have different dimensions, as long as the pixel size is identical. It makes no sense, if the images have different magnifications (= pixel sizes), as each individual image would have to be calibrated accordingly. However, this should generally be avoided for comparative measurements to ensure a uniform measurement error.

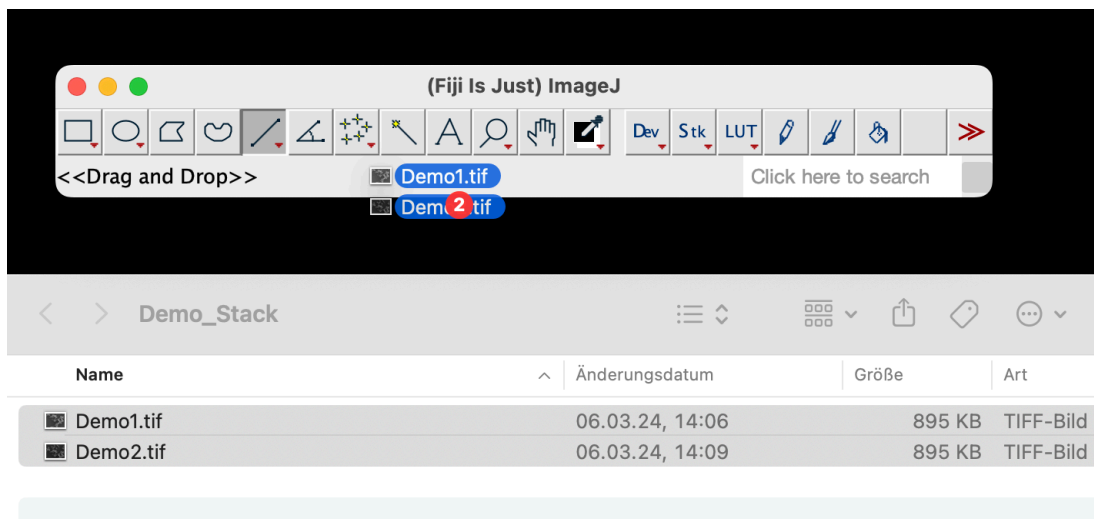
### ***Opening the images and creating an image stack***

Organize all images to be used in a single folder. Then drag and drop the folder to the bottom bar of the FIJI toolbar (see also image below under alternative method). In the pop-up window decide if the images should be sorted numerically (otherwise remove the tick) and close the window by hitting “OK”.

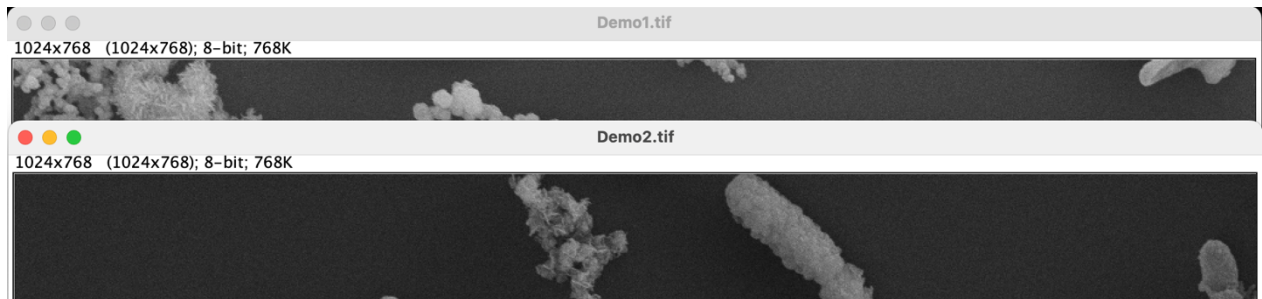


### ***Alternative method:***

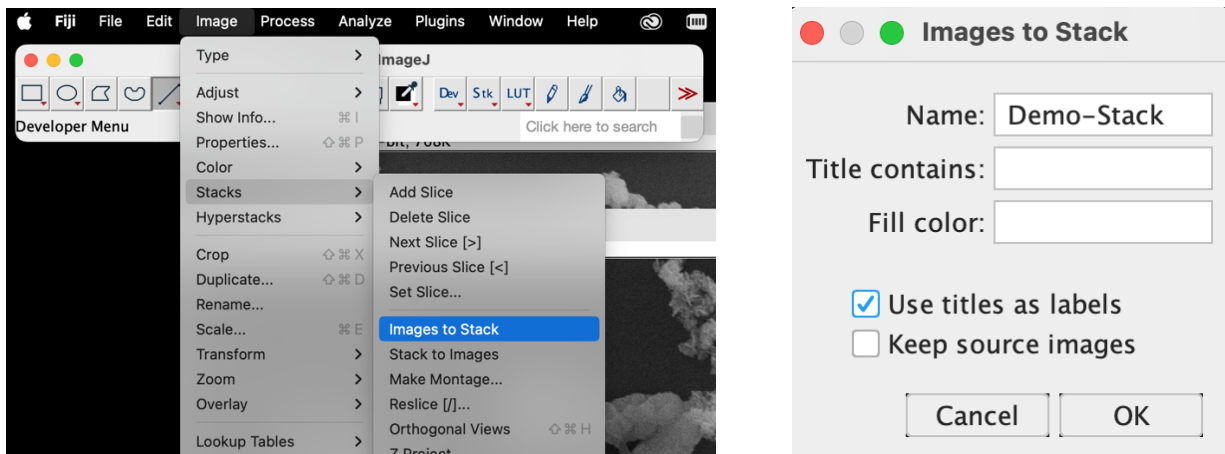
Open the folder with the images: select all the desired images in a folder and drag them to the bottom bar of the FIJI toolbar.



As soon as you move over it with the mouse, ">> Drag and Drop>>" is displayed in the bar. FIJI then opens all images.

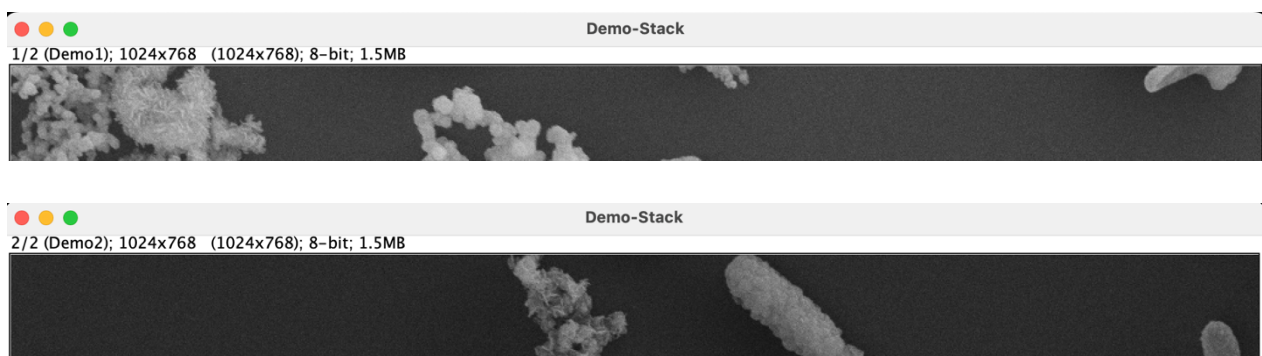


The individual images can now be combined into a stack (→ Image / Stacks / Images to stacks).

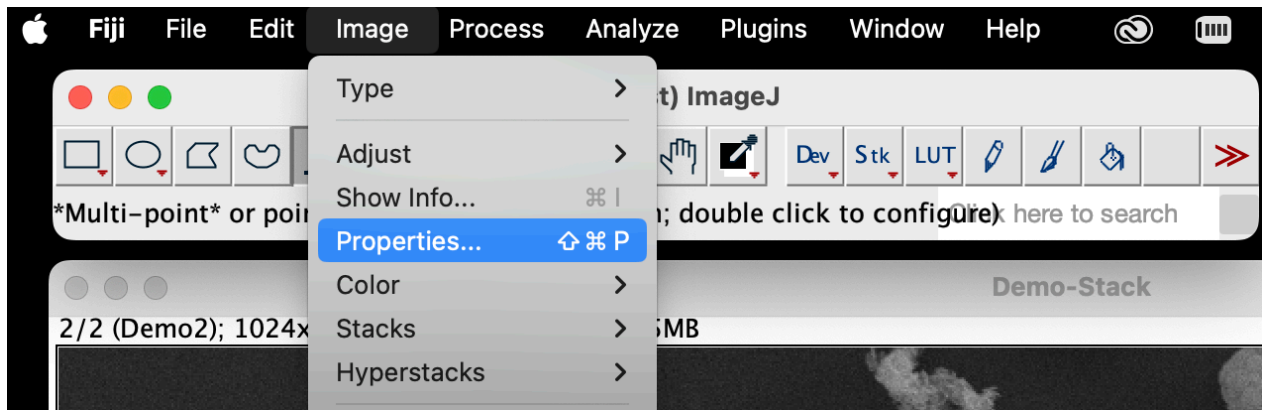


Give the stack a name and tick "Use titles as labels" & click Ok. A singular stack is generated.

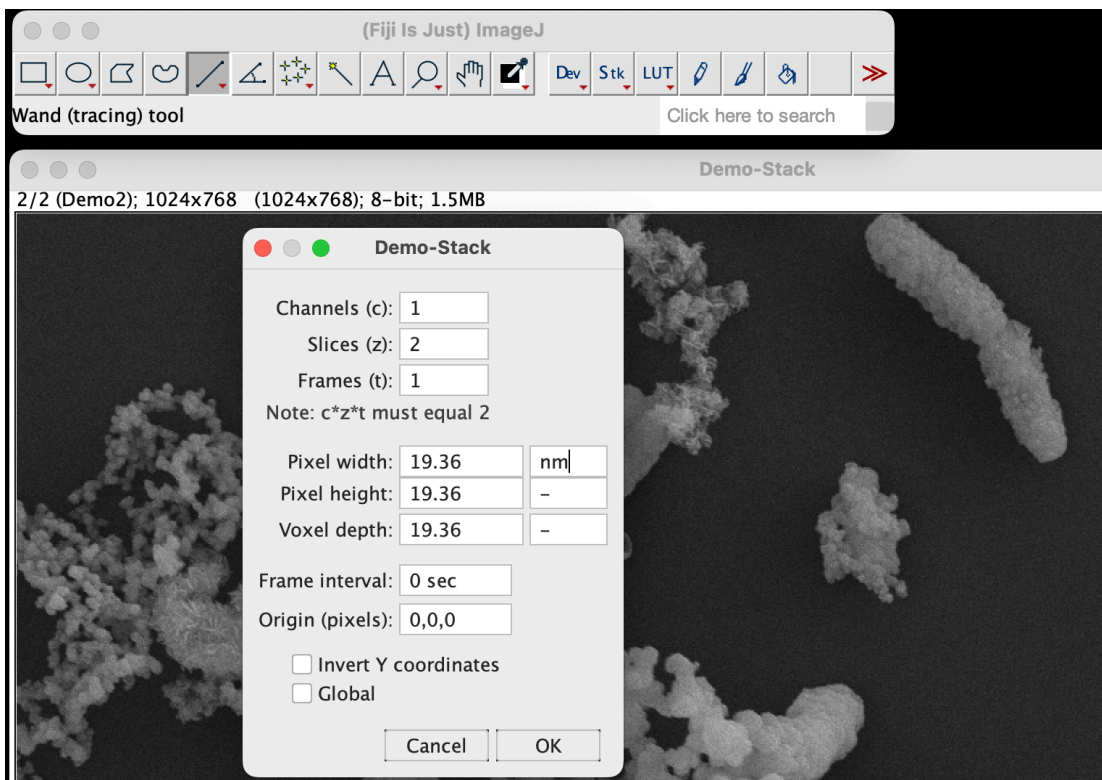
You can now scroll through the stack, the respective image names are displayed next to the pixel dimensions of these images.



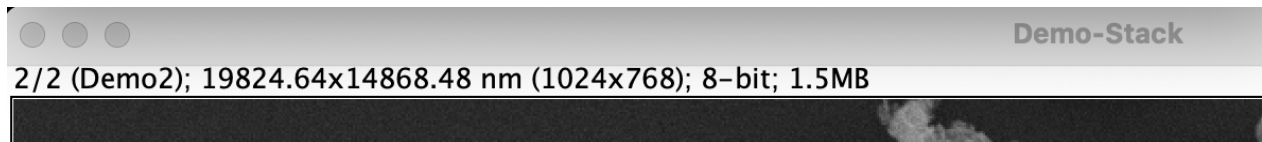
In the next step, the pixel size of the images must be calibrated. This is done via → Image / Properties.



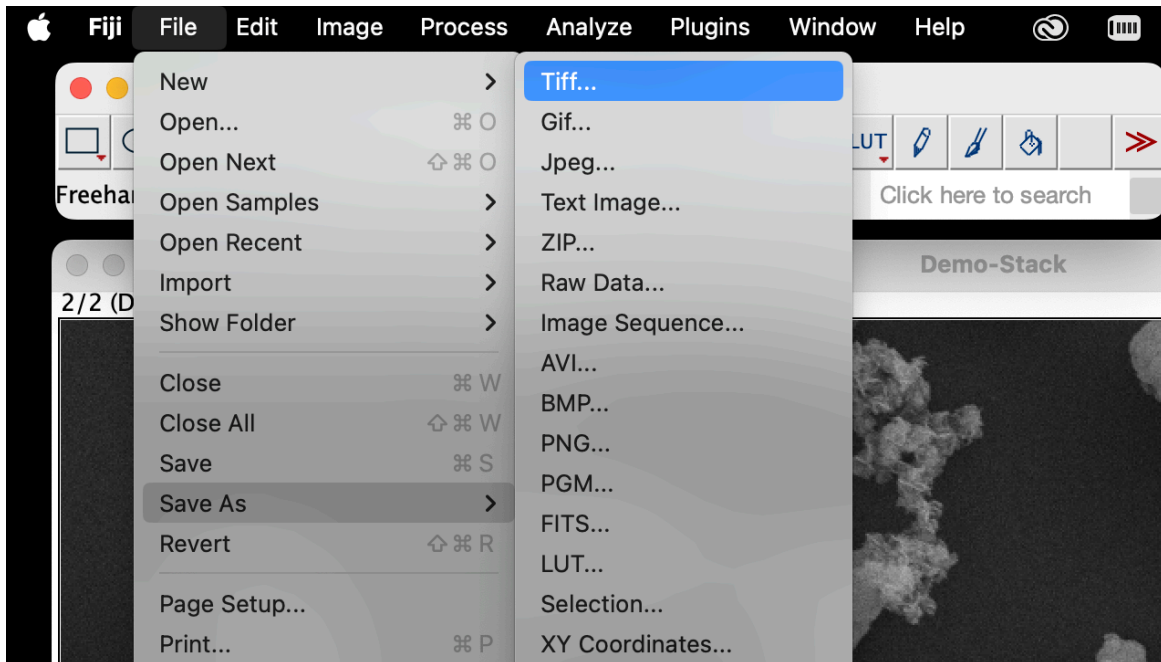
Enter the corresponding information under "Pixel width" and "Pixel height" and add the order of magnitude (nm,  $\mu$ m, ...) in the second field under "Pixel width" and click ok.



In addition to the pixel dimensions of the image, the image dimensions are now also displayed in nm/ $\mu$ m in the title bar of the image stack.

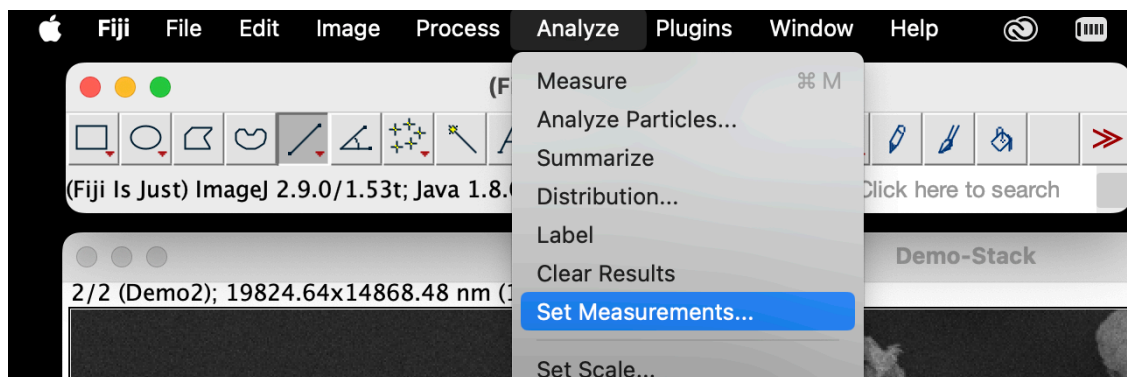


The calibrated stack should be saved at this point ( → File / Save as... / tiff).

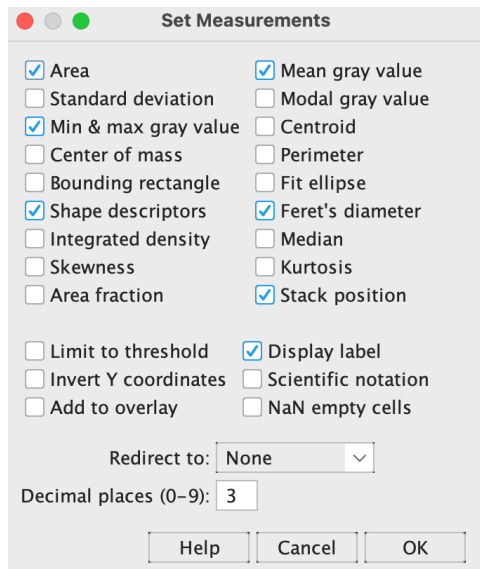


### ***Take measurements***

If you have not already done so, additional settings should be made under → Analyze / Set Measurements.... (FIJI saves these settings for the future)



Please tick "Stack position" and "Display label" here and confirm the window with OK. This information is automatically listed in a table during measurements, which is very helpful for further analyses.



Measurements can be carried out now in the following two ways, described in detail below:

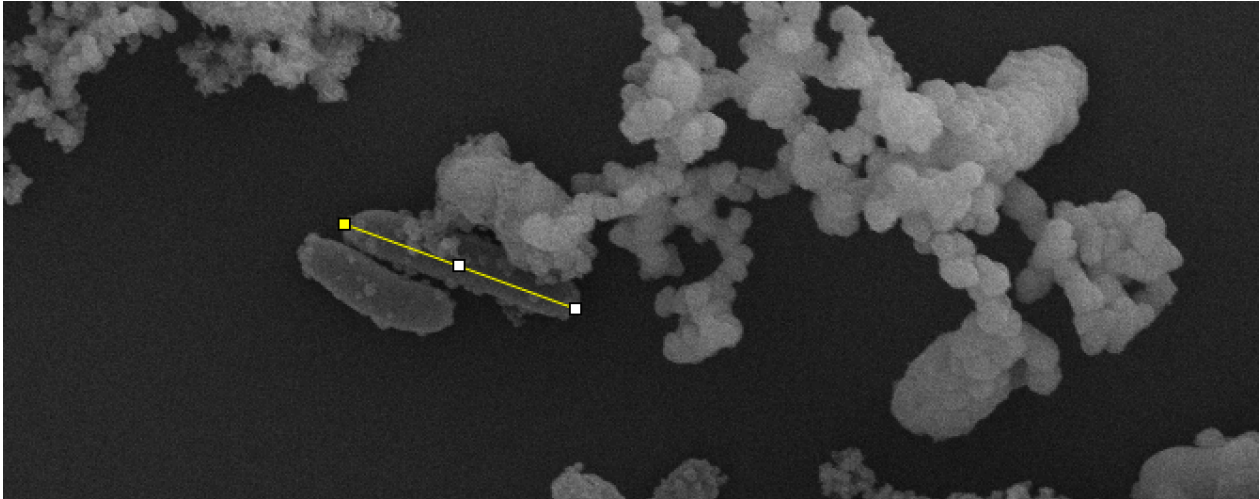
A) the quick way & if the results are not to be cross-checked again (reproducibly) or the measurement points are not to be saved as ROIs (**R**egions **O**f **I**nterest). Export of the measurements is performed as a .csv file.

B) Reproducible measurements by saving the ROIs and exporting all measurements as a .csv file. ***We recommend this method.***

### **Method A**

Measurements can be taken on the images using the line tool. To do this, click on the tool and mark the structure to be measured by dragging the pressed left mouse button between beginning and end point of the measurement.

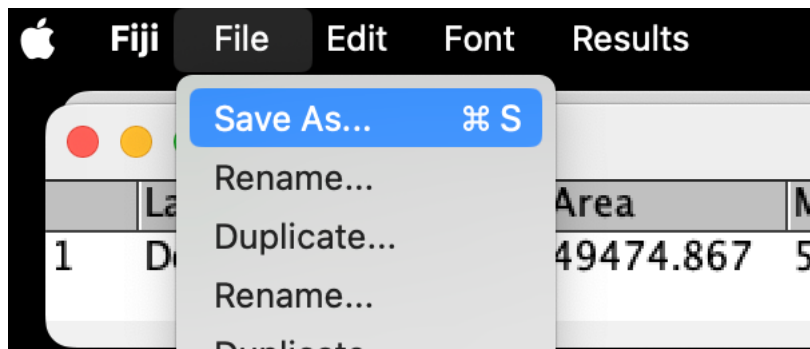




Pressing the "m" key saves the measurement in a table, which now also opens as an additional "Results" window.

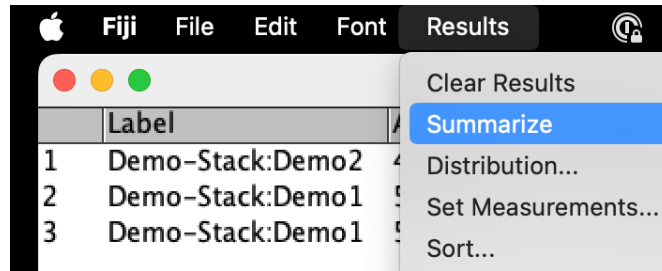
Results																
Label	Area	Mean	Min	Max	Angle	Circ.	Feret	Slice	FeretX	FeretY	FeretAngle	MinFerret	AR	Round	Solidity	Length
1 Demo-Stack:Demo2	49474.867	57.593	41	101.318	-20.095	0.097	2535.643	2	189	419	159.905	0	0.000	0.000	NaN	2535.643

This way, all planned measurements can now be continued across all images in the stack (save each measurement with "m"). The table reveals that the name of the original image file is also listed. This means that it is possible to later analyse how many measurements were taken per image, etc. The results can finally be saved as a .csv file via → File / Save as (make sure that the results window is activated beforehand).



If it is a list of measurements from which the mean value, SD & Min & Max are to be determined, this can also be carried out via → Results/Summarize before saving.





Results																	
Label	Area	Mean	Min	Max	Angle	Circ.	Feret	Slice	FeretX	FeretY	FeretAngle	MinFeret	AR	Round	Solidity	Length	
1	Demo-Stack:Demo2	49474.867	57.593	41.000	101.318	-20.095	0.097	2535.643	2.000	189.000	419.000	159.905	0.000	0.000	0.000	NaN	2535.643
2	Demo-Stack:Demo1	57345.869	131.348	97.961	173.019	-141.927	0.083	2951.114	1.000	468.000	432.000	38.073	2.274E-13	0.000	0.000	NaN	2951.114
3	Demo-Stack:Demo1	52473.344	117.067	88.000	148.639	16.343	0.092	2683.299	1.000	464.000	673.000	16.343	0.000	0.000	0.000	NaN	2683.299
4	Mean	53098.027	102.003	75.654	140.992	-48.560	0.090	2723.352	1.333	373.667	508.000	71.440	7.579E-14	0.000	0.000	NaN	2723.352
5	SD	3972.510	39.117	30.422	36.457	82.886	0.007	210.612	0.577	159.939	143.042	77.379	1.313E-13	0.000	0.000	NaN	210.612
6	Min	49474.867	57.593	41.000	101.318	-141.927	0.083	2535.643	1.000	189.000	419.000	16.343	0.000	0.000	0.000	1.798E308	2535.643
7	Max	57345.869	131.348	97.961	173.019	16.343	0.097	2951.114	2.000	468.000	673.000	159.905	2.274E-13	0.000	0.000	-1.798E308	2951.114

If these added lines (or certain previously performed measurements) are not to be finally saved, the lines can be marked in the list with the mouse (they are then highlighted in black) and deleted using the delete button.

Results																	
Label	Area	Mean	Min	Max	Angle	Circ.	Feret	Slice	FeretX	FeretY	FeretAngle	MinFeret	AR	Round	Solidity	Length	
1	Demo-Stack:Demo2	49474.867	57.593	41.000	101.318	-20.095	0.097	2535.643	2.000	189.000	419.000	159.905	0.000	0.000	0.000	NaN	2535.643
2	Demo-Stack:Demo1	57345.869	131.348	97.961	173.019	-141.927	0.083	2951.114	1.000	468.000	432.000	38.073	2.274E-13	0.000	0.000	NaN	2951.114
3	Demo-Stack:Demo1	52473.344	117.067	88.000	148.639	16.343	0.092	2683.299	1.000	464.000	673.000	16.343	0.000	0.000	0.000	NaN	2683.299
4	Mean	53098.027	102.003	75.654	140.992	-48.560	0.090	2723.352	1.333	373.667	508.000	71.440	7.579E-14	0.000	0.000	NaN	2723.352
5	SD	3972.510	39.117	30.422	36.457	82.886	0.007	210.612	0.577	159.939	143.042	77.379	1.313E-13	0.000	0.000	NaN	210.612
6	Min	49474.867	57.593	41.000	101.318	-141.927	0.083	2535.643	1.000	189.000	419.000	16.343	0.000	0.000	0.000	1.798E308	2535.643
7	Max	57345.869	131.348	97.961	173.019	16.343	0.097	2951.114	2.000	468.000	673.000	159.905	2.274E-13	0.000	0.000	-1.798E308	2951.114

Result.csv files can also be opened again in FIJI by simply dragging the files (like the images) onto the bar, which displays the result table again.

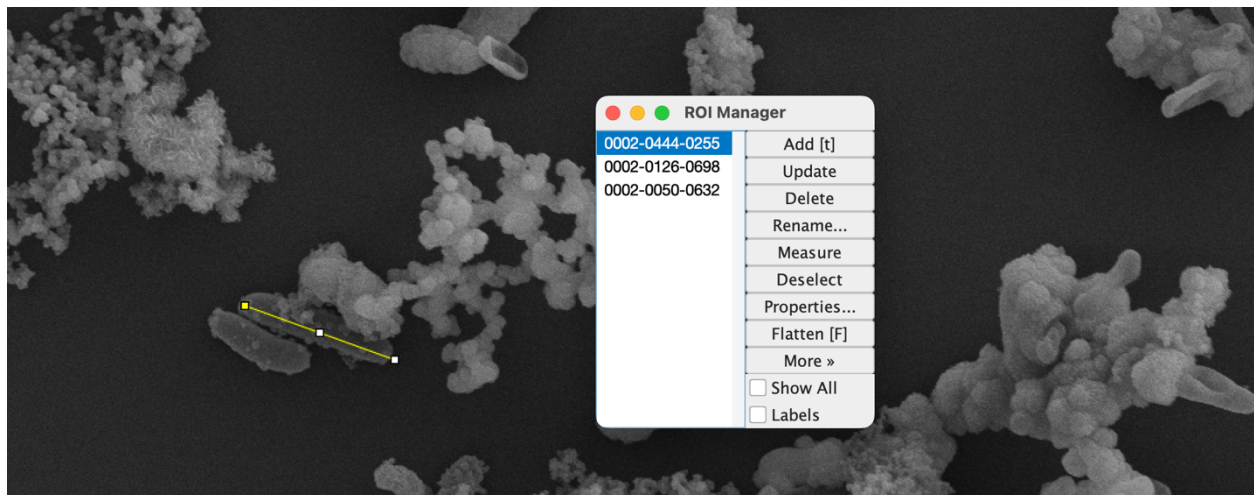
### **Exporting the data to Excel:**

The Result.csv file can be imported into a data sheet using the Excel data importer, but alternatively the results table can also be simply transferred to Excel via copy and paste. Simply select and copy all rows in the table. The header row of the table is automatically copied.

### **Method B**

Creating the measurements is very similar to method A, but instead of the "m" key, each measurement is confirmed using the "t" key. The "ROI Manager" opens for the first measurement and each measurement is given a unique ID number.

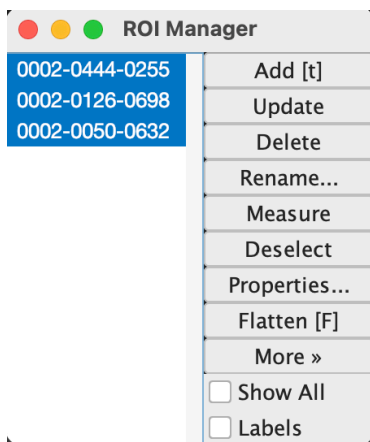




The measurements are now carried out as before and the list in the ROI Manager is extended with an entry for each measurement. Unlike in method A, where it is not possible to see where and how measurements were taken afterwards, this is possible via the ROI Manager. By clicking on an ID number, the measurement is displayed in the stack.

**Generate the measurement table:**

To do this, mark all ID numbers in the ROI Manager (click on the top ID, scroll down and mark the last ID number with the shift key and mouse click) and then click on "Measure" in the ROI Manager.



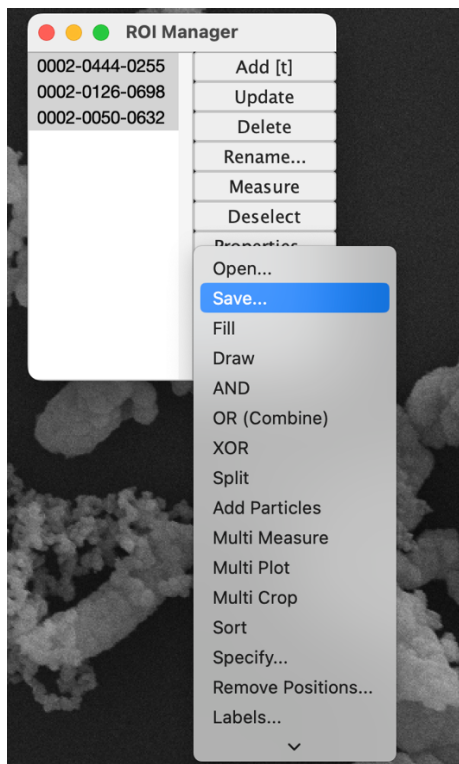
This creates a result table again. In addition to the original image names, the ID numbers of the measurements are also noted here.

Results																
Label	Area	Mean	Min	Max	Angle	Circ.	Feret	Slice	FeretX	FeretY	FeretAngle	MinFeret	AR	Round	Solidity	Length
1 Demo-Stack:0002-0444-0255:Demo2	49100.058	57.383	25.821	102.509	-19.832	0.098	2510.836	2	194	422	160.168	0	0.000	0.000	NaN	2510.836
2 Demo-Stack:0002-0126-0698:Demo2	37106.150	115.173	33.000	150.000	-64.486	0.131	1887.774	2	677	82	115.514	1.137E-13	0.000	0.000	NaN	1887.774
3 Demo-Stack:0002-0050-0632:Demo2	44977.152	108.883	85.010	126.901	-40.914	0.106	2305.710	2	587	11	139.086	0.000	0.000	0.000	NaN	2305.710

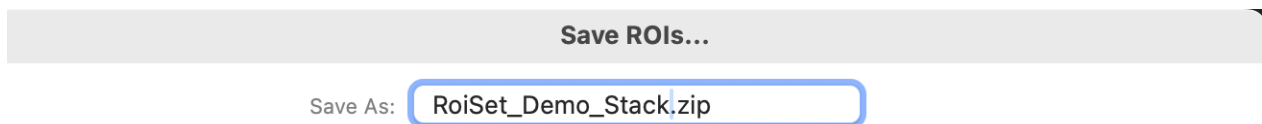
Exporting the table as a csv file is again possible via → File / Save as.

### Exporting the measurements as ROIs

When all measurements (ID numbers) are marked in the ROI Manager, click on "More>>" and then on "Save".



The measurements can be saved as a .zip file in the following window.



## ***Importing measurements / ROIs***

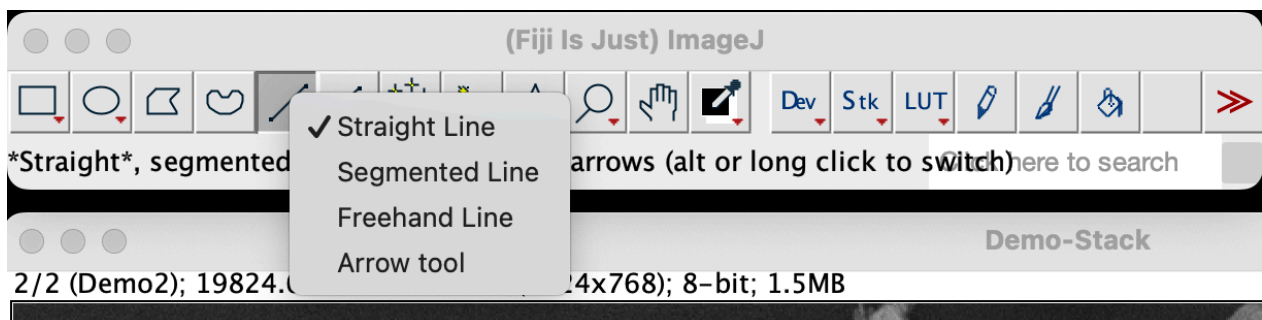
The measurements can be displayed again in the stack via the ROI Manager (e.g. after a restart, or also on another computer / by a cooperation partner / supervisor etc.) by first opening the image stack again. In the second step, the zip file can also be just dragged onto the bar of the FIJI toolbar, which automatically opens the ROI Manager with the measurements and displays the measurements in the image stack.

## ***Change / revision of measurements***

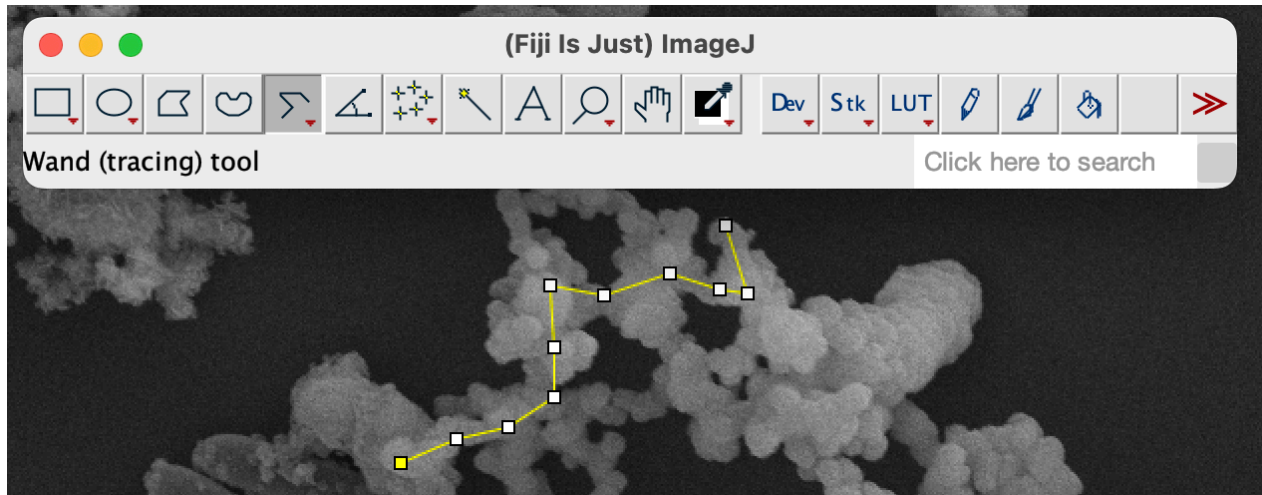
The ROIs in the ROI Manager can also be changed retrospectively if a methodological error is discovered, or a structure was not measured correctly. To do this, click on the corresponding measurement / ID number in the ROI Manager. The corresponding measurement is displayed in the image. If the mouse pointer passes over one of the squares at the end of a measurement line, the pointer changes to a hand. You can now move the square to adjust the measurement. If you click on the center square in the line, the entire line can be moved. Once the measurement has been adjusted, the change must be confirmed in the ROI Manager under "Update" (for each ROI after the change has been made). The ROIs can then be saved again as an updated zip file or the updated measurements can be exported.

## ***Further measurement tools***

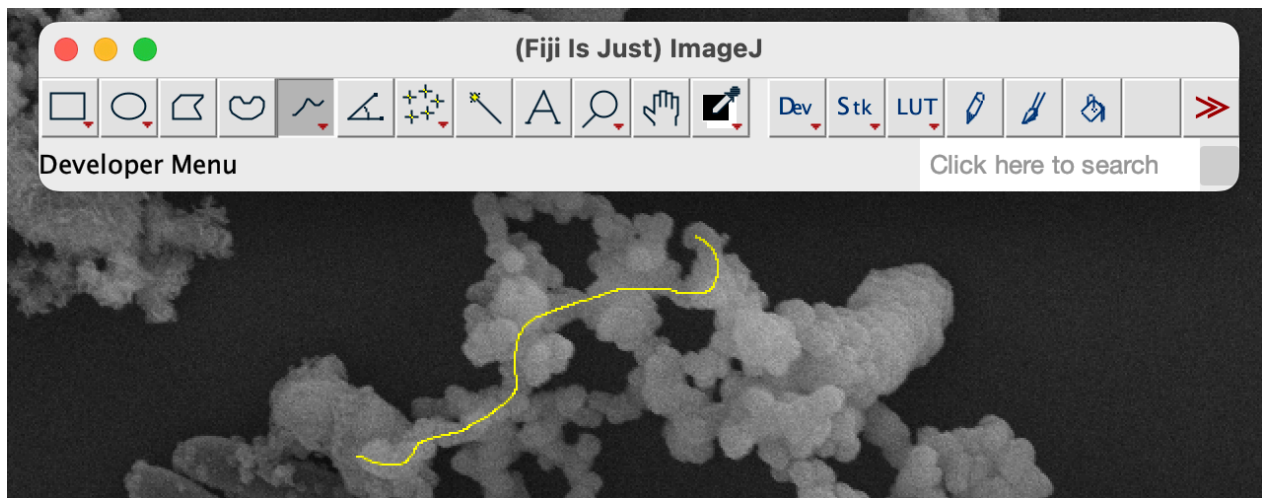
Right-clicking on the line tool opens a drop-down menu with further selection tools: "Straight Line, Segmented Line, Freehand Line, Arrow tool".



Except for the "Arrow tool", all others can be used for measurements.



"Segmented Line" allows curved structures to be measured by setting multiple points (which are connected by straight lines). The total length is specified in the measurement table. The individual points are set with the left mouse button. A line is ended by pressing the right mouse button.



A continuous line can be drawn with the mouse using the "Freehand Line" tool. The total length of the line is also saved in the measurement table here.