S1: Step-by-step-guide using image J in the work flow.

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How to use ImageJ in our 3D-workflow from data manipulation, import, segmentation and export as OBJ-file.

DOI

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Dec 16, 2021

Apr 28, 2022

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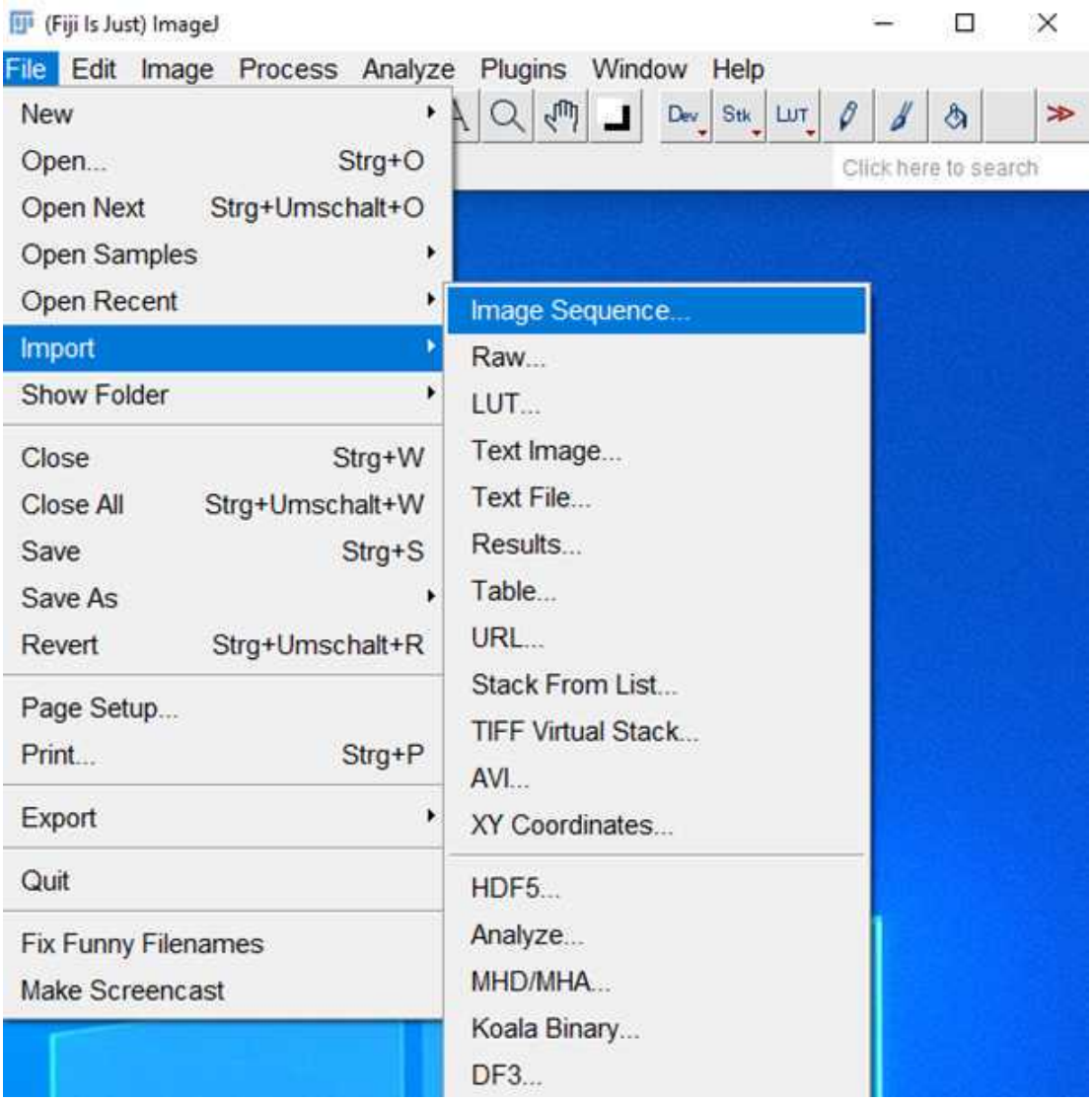
1



•Open Image J and import your *Image Sequence*
•It is possible in Image J to transform stacks into Images Sequences and vice versa

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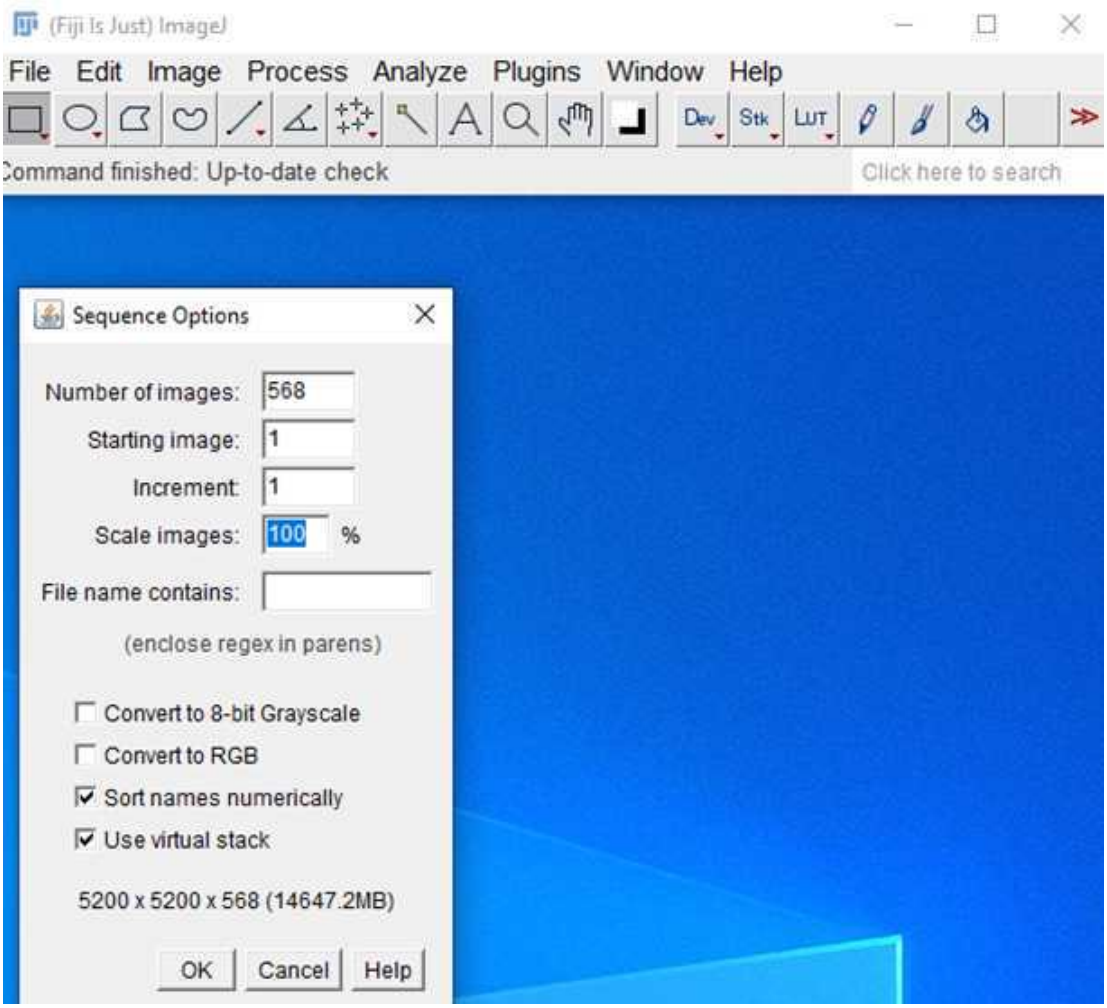
2



•Make sure your *Image Sequenc*e starts with 1 and choose Use *virtual stack* to save RAM
•You can scale your data set down too

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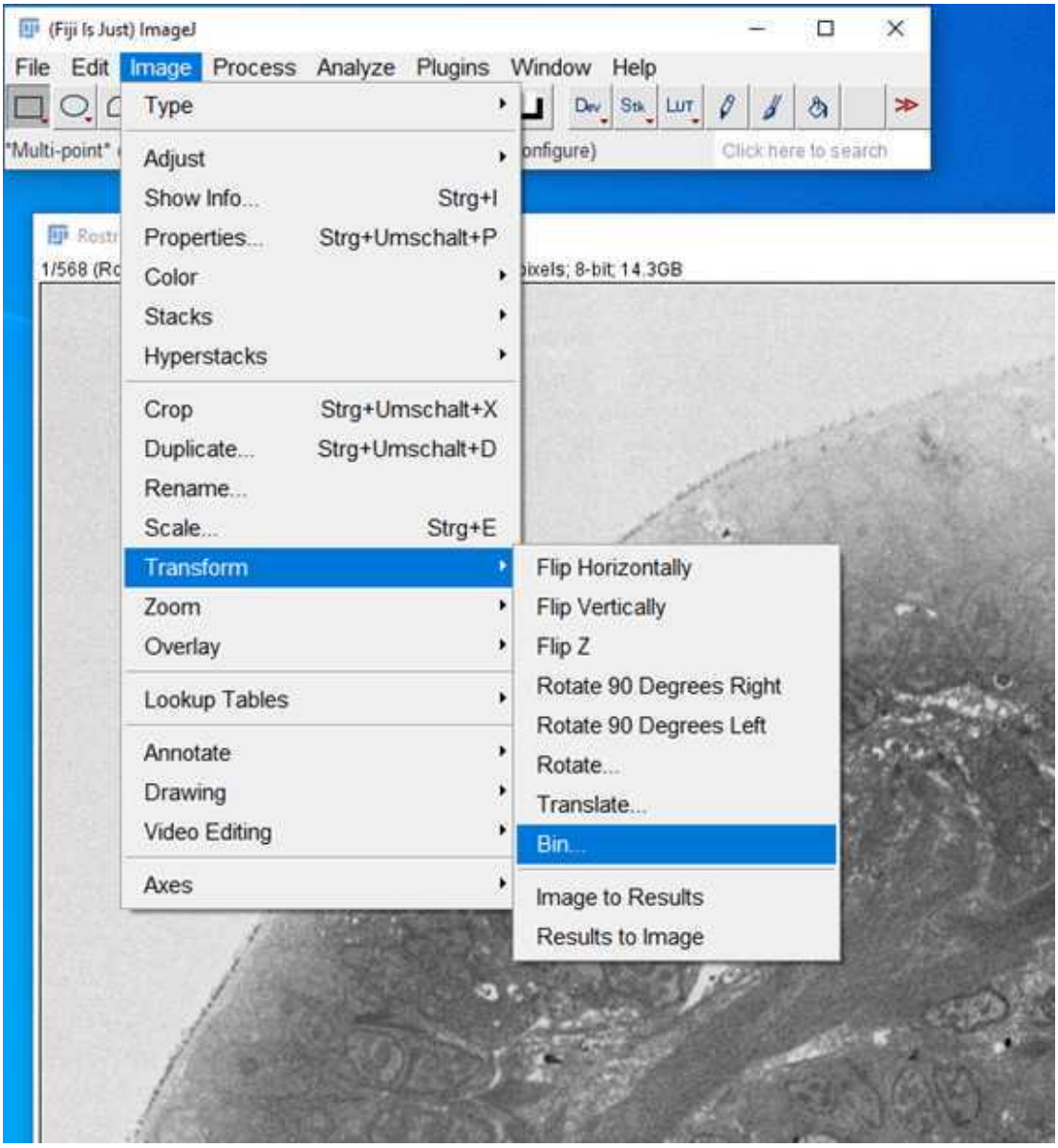
3



•Make sure your data set is processablefor your computer
•Binning is easy and suitable for reducing data size, here *bin2* from 14.3 to 3.4 GB
•Only bin x and y not z
•Save binned data set

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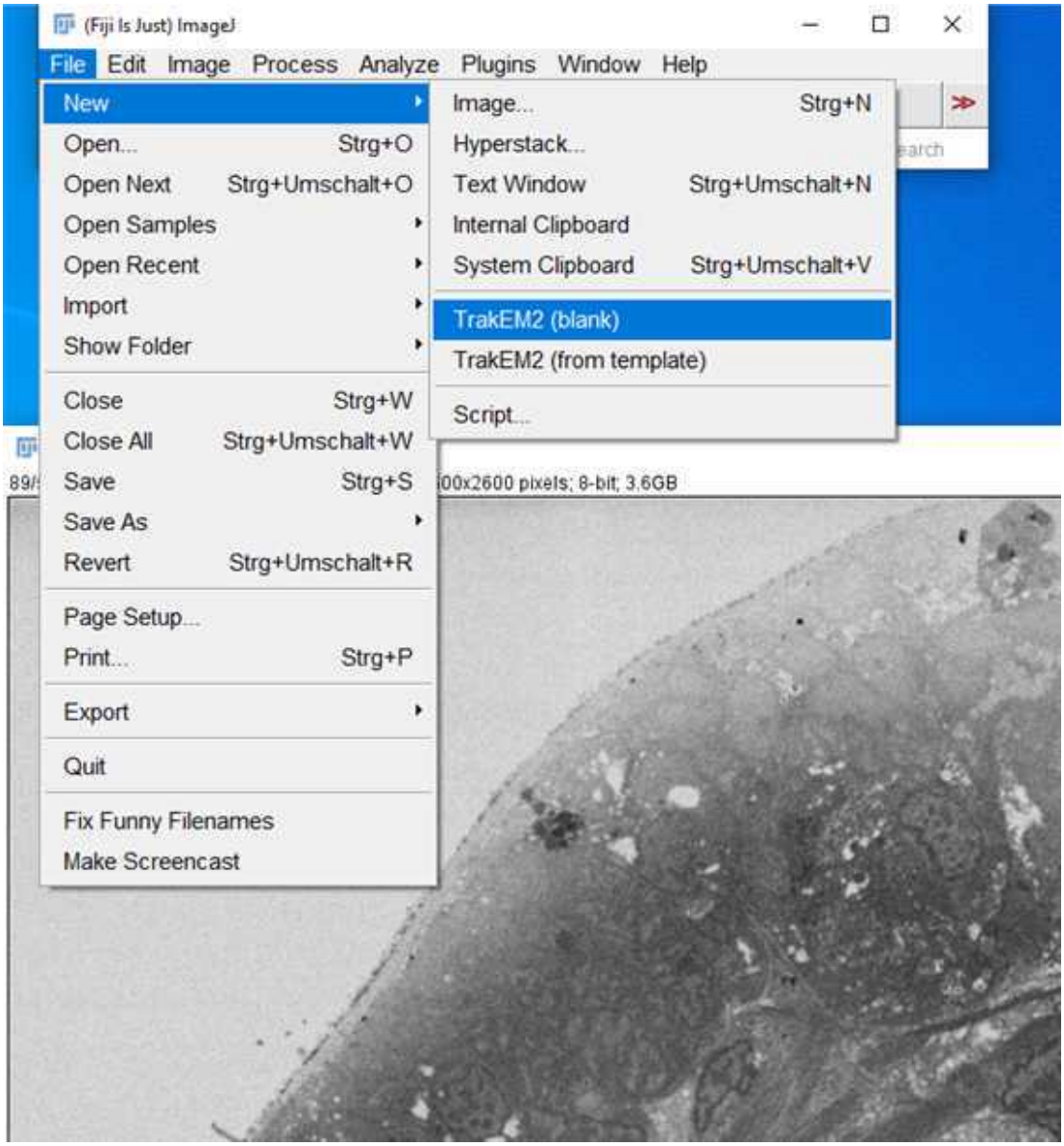
4



•For segmentation, use *TrakEM2*
•Open *blank*

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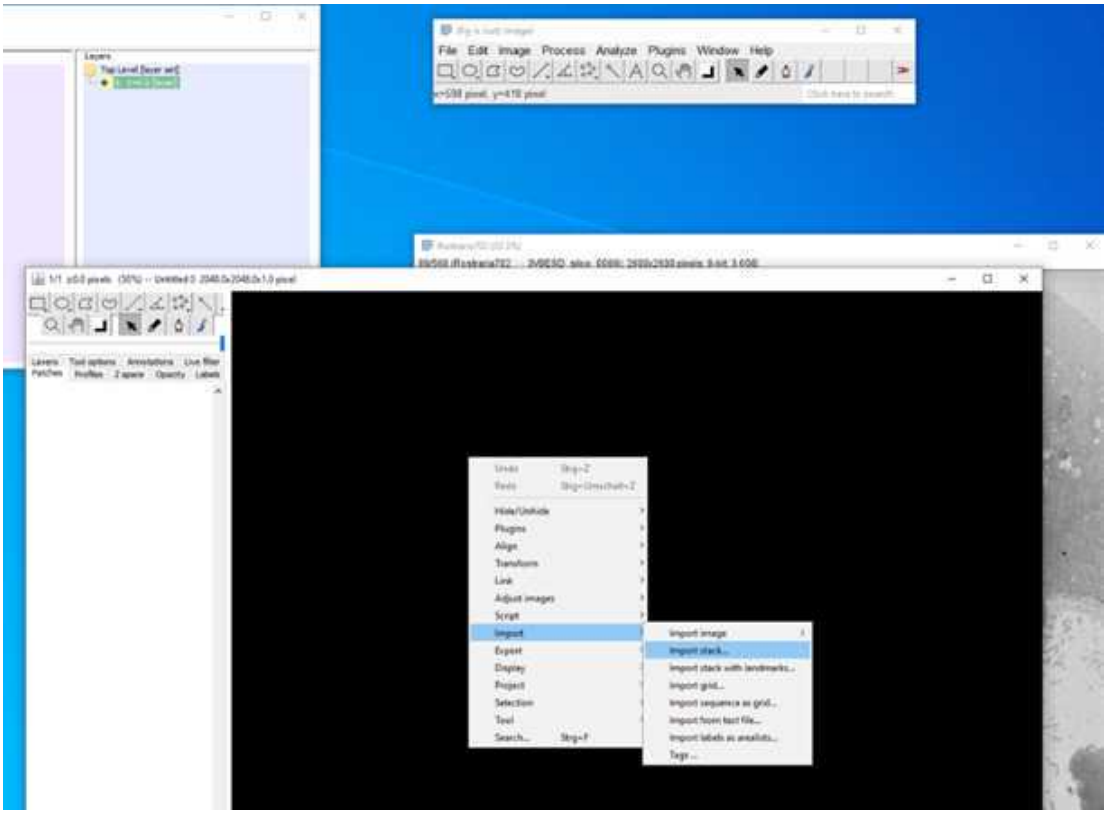
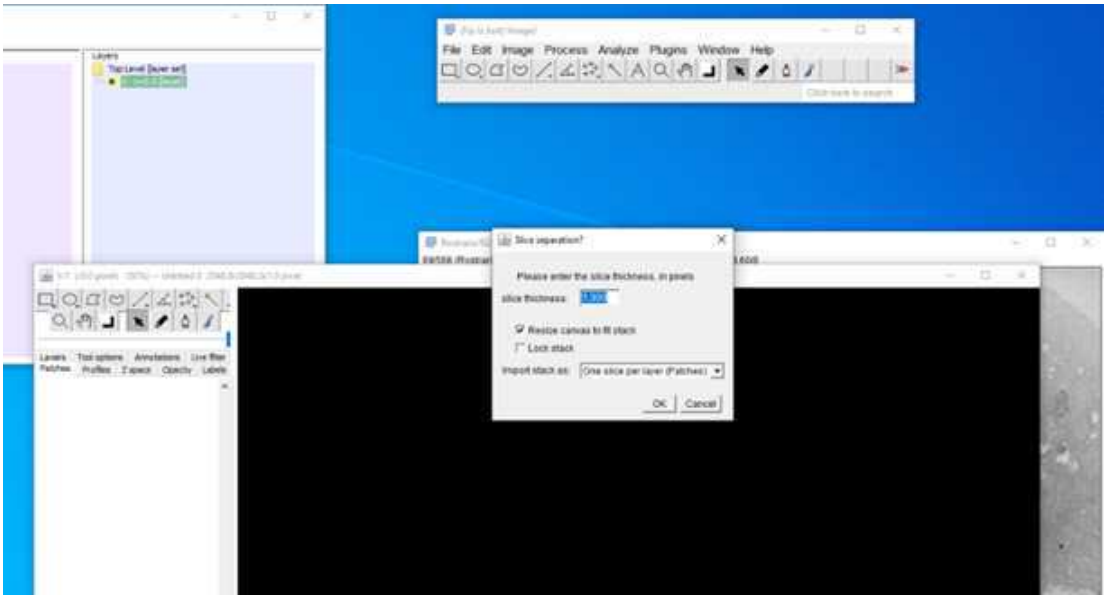
5



•Import your binned data set by right click in blank (black window) and select folder of binned data set

6



•Choose *slice thickness* depending on your data set, adjustments are possible in all programs

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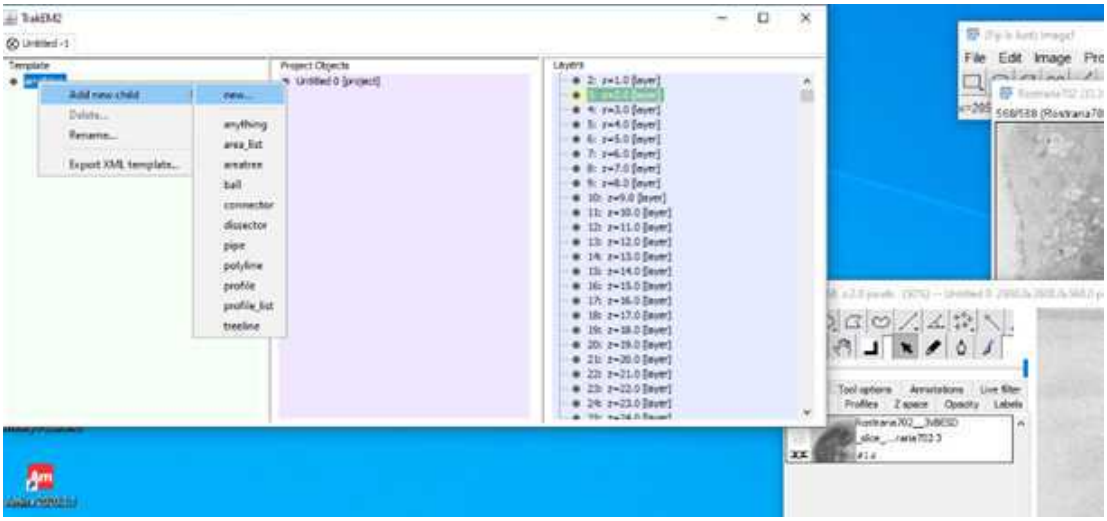
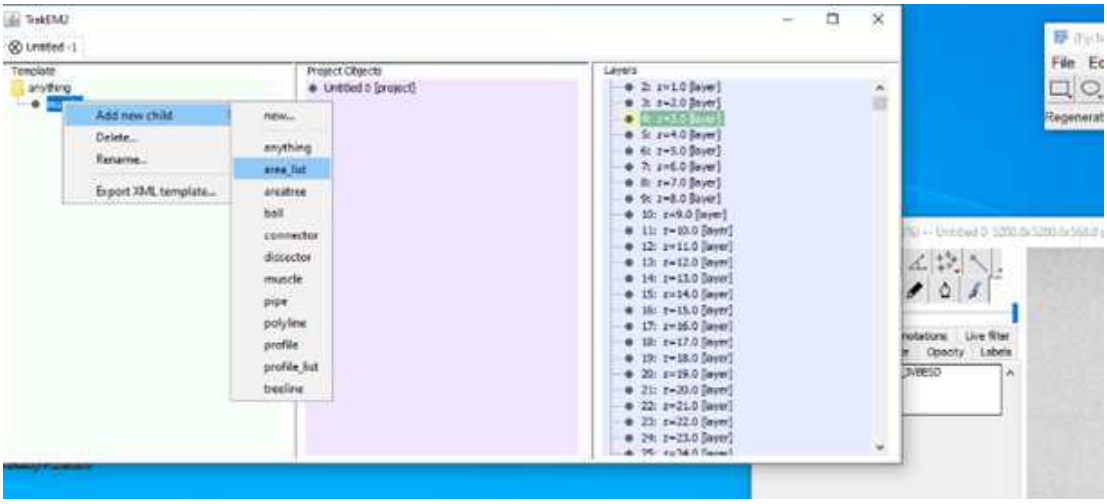
7



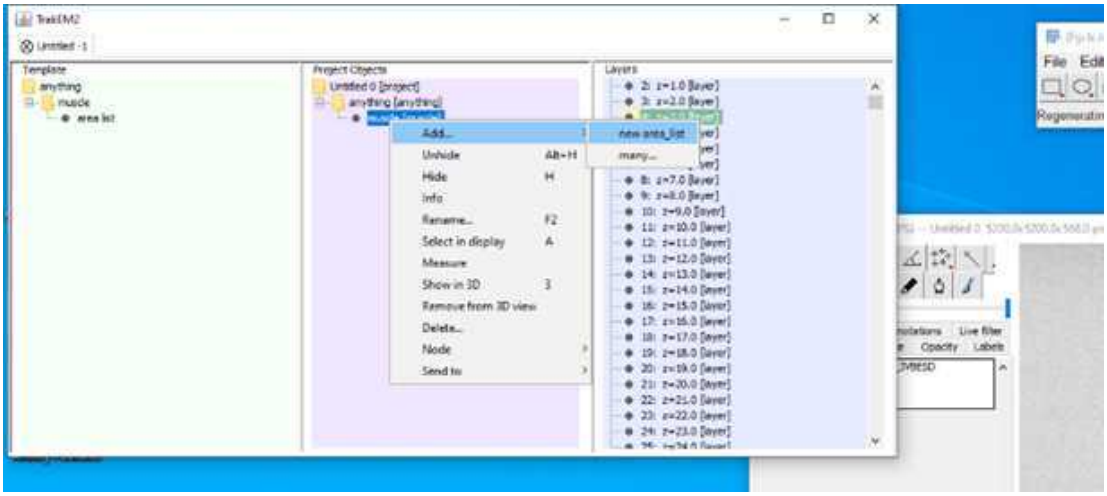
•To create different labels, each for a structure you want to reconstruct, you need a *new child* and give it the name of your label

8



•Then right click on the *new child* and create an *area_list*

9



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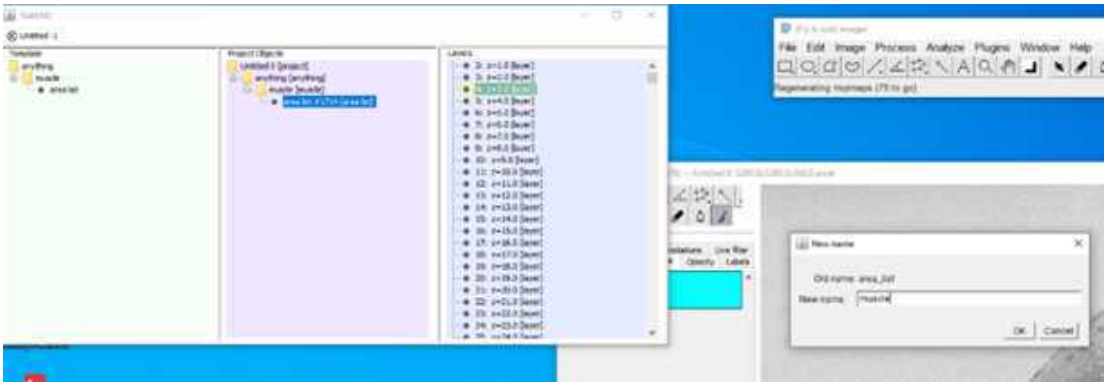
•Drag `n`drop the named folder (child) together with the *area_list*
•Right click on the *area_list* and add a *new area_list*

10



•Name the *new area_list* like the child in the beginning
•save your Trak EM2 file by press save in main tool bar (.xml-file)

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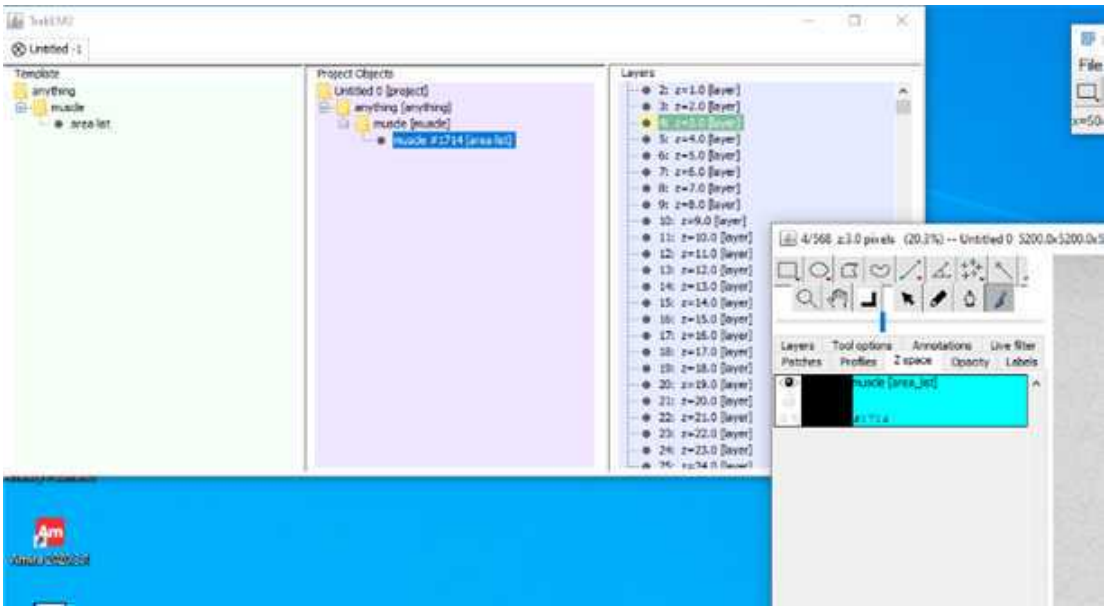

•Now your named label is available forsegmentation under *z space*

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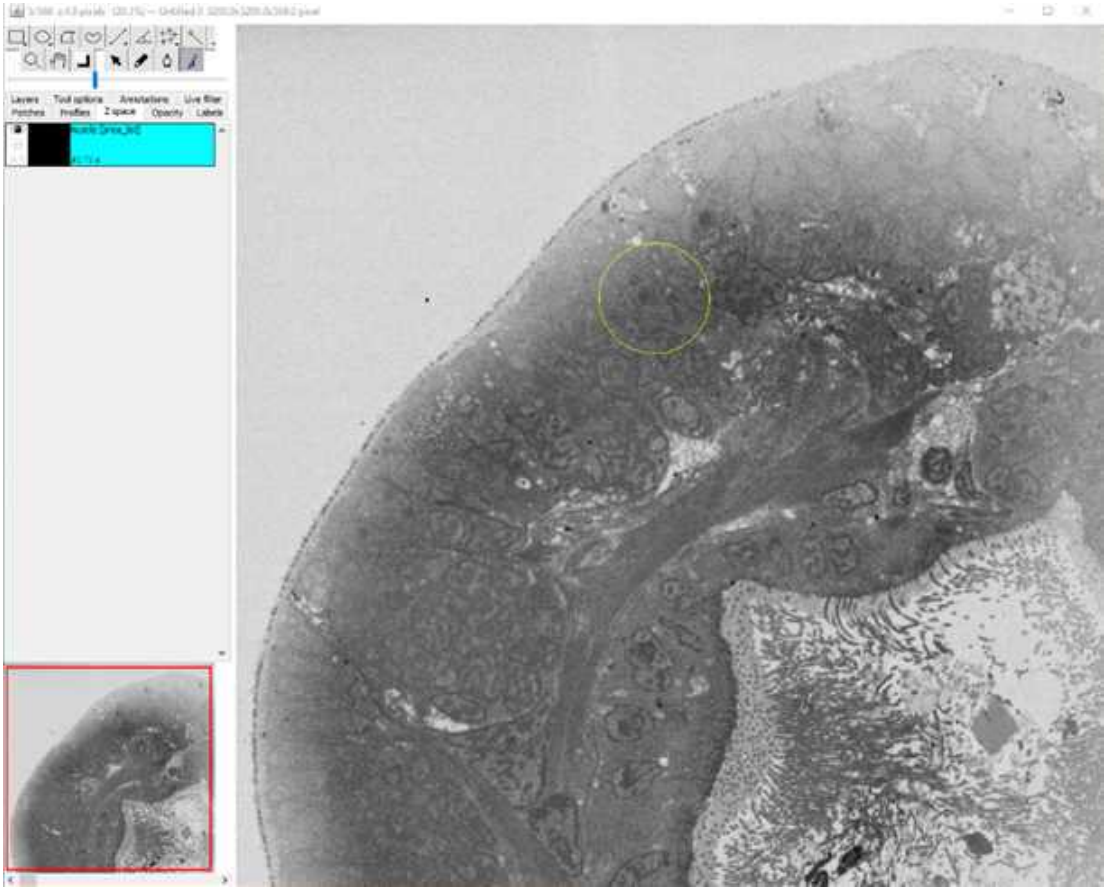
12



•Now start to segment your structure
•Make sure the brush tool in the main tool bar is checked
•Hot keys and how to reconstruct:
-Mouse wheel (mw); , and . : scrolling through data set
-Ctrl + mw; + and - : zoom in and out
-Shift + mw: size of pen
-Left mouse button (l mb): mark
-Shift + l mb: fill
-Alt + l mb: erase
-Alt + shift + l mb: delete all on slice
-Right mouse button (r mb) – *Area:* for all kind of interpolations

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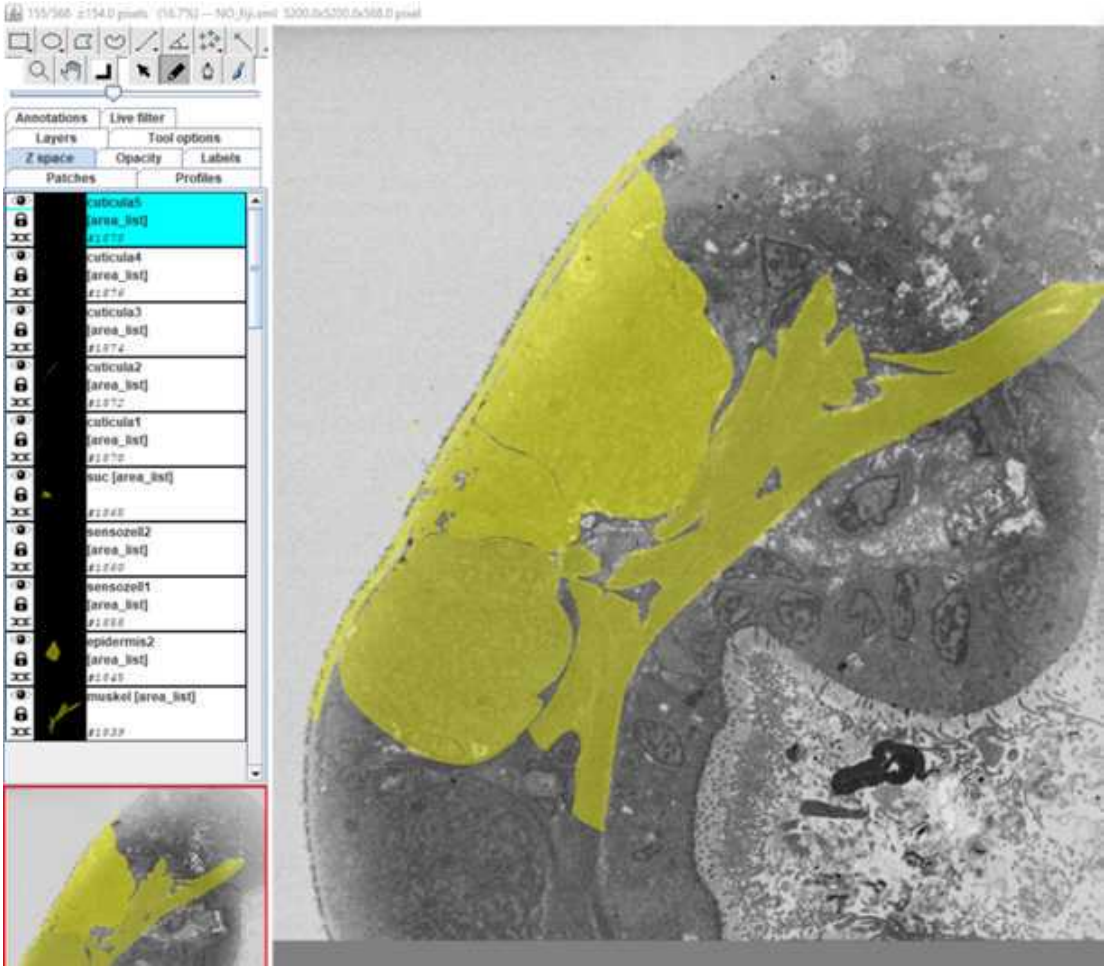
13



•after finishing your segmentation it is time to export your labels
•it is possible to export different labels solely, or together, but then they work like one
•e.g. in this example it is smart to export all parts of the cuticle as one label

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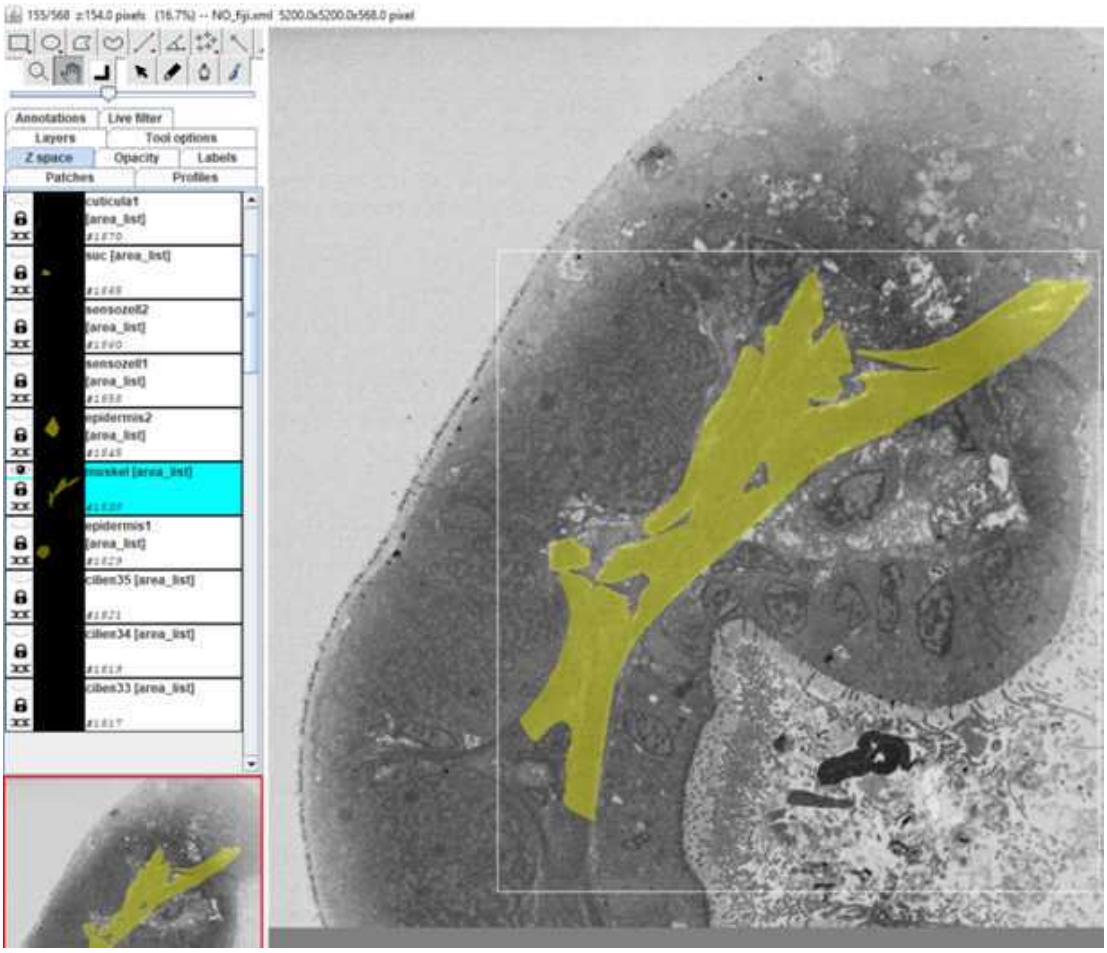
14



•hide all labels you do not want to export in this turn by unchecking the eye button in the Z space

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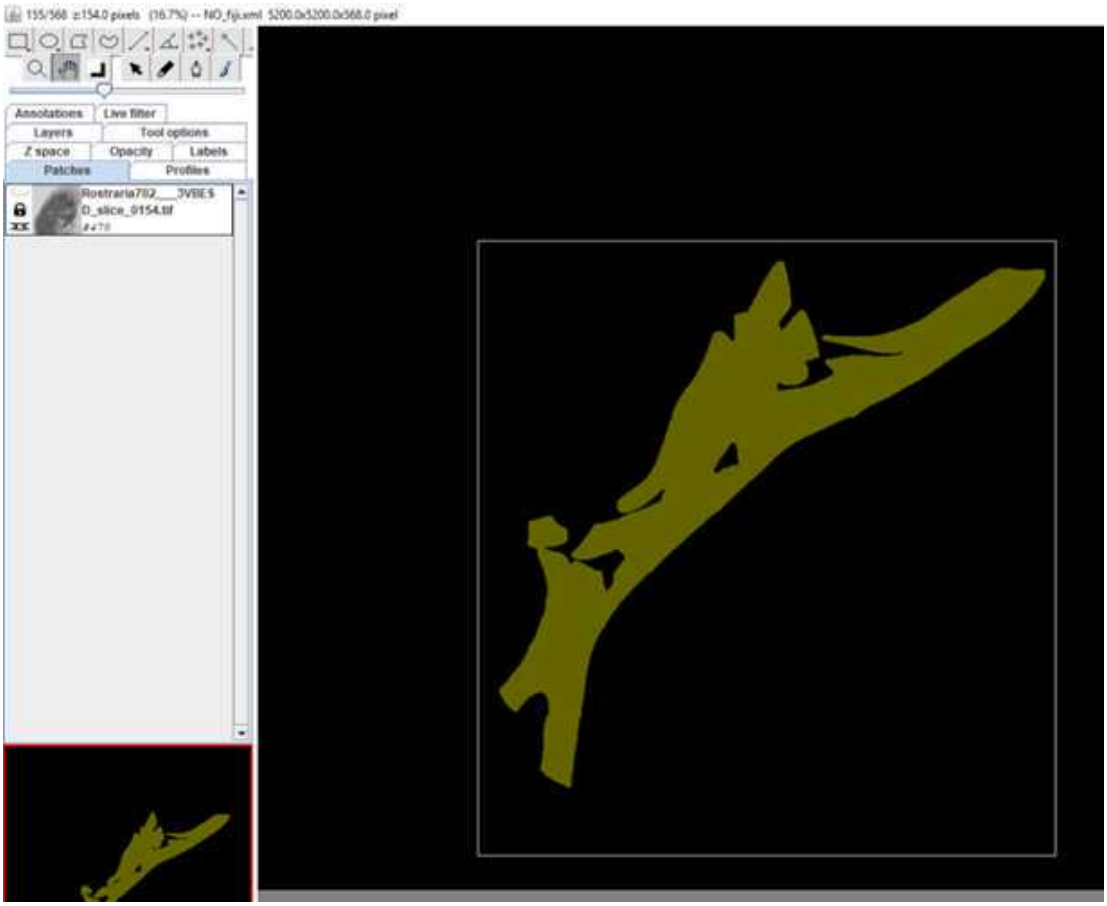
15



•now turn the "background" black, go to patches and push the fader on top to the left
•now you should only see your marked label, check the whole stack

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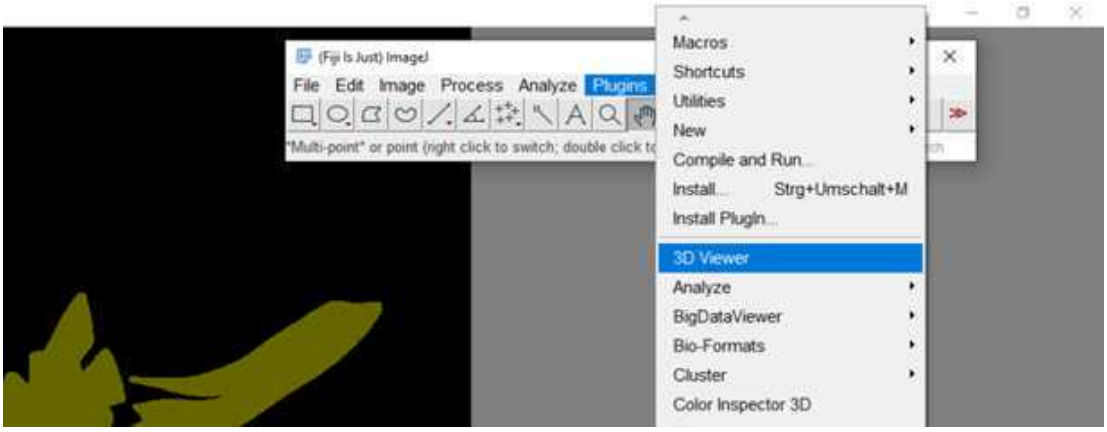

•open the 3D viewer

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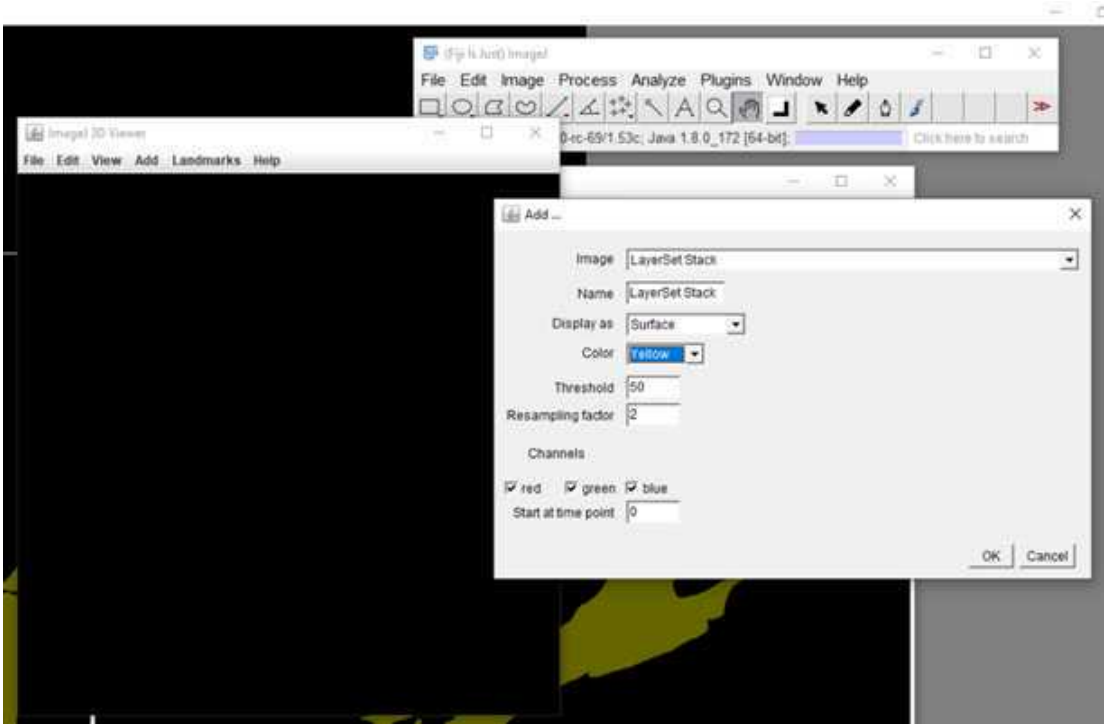
17



•you can choose in *Display as* between *surface or volume*
•for later work with MeshLlaband Blender it doesn´t matter
•if you are not familiar with ImageJ keep the other parameters as in the shown image

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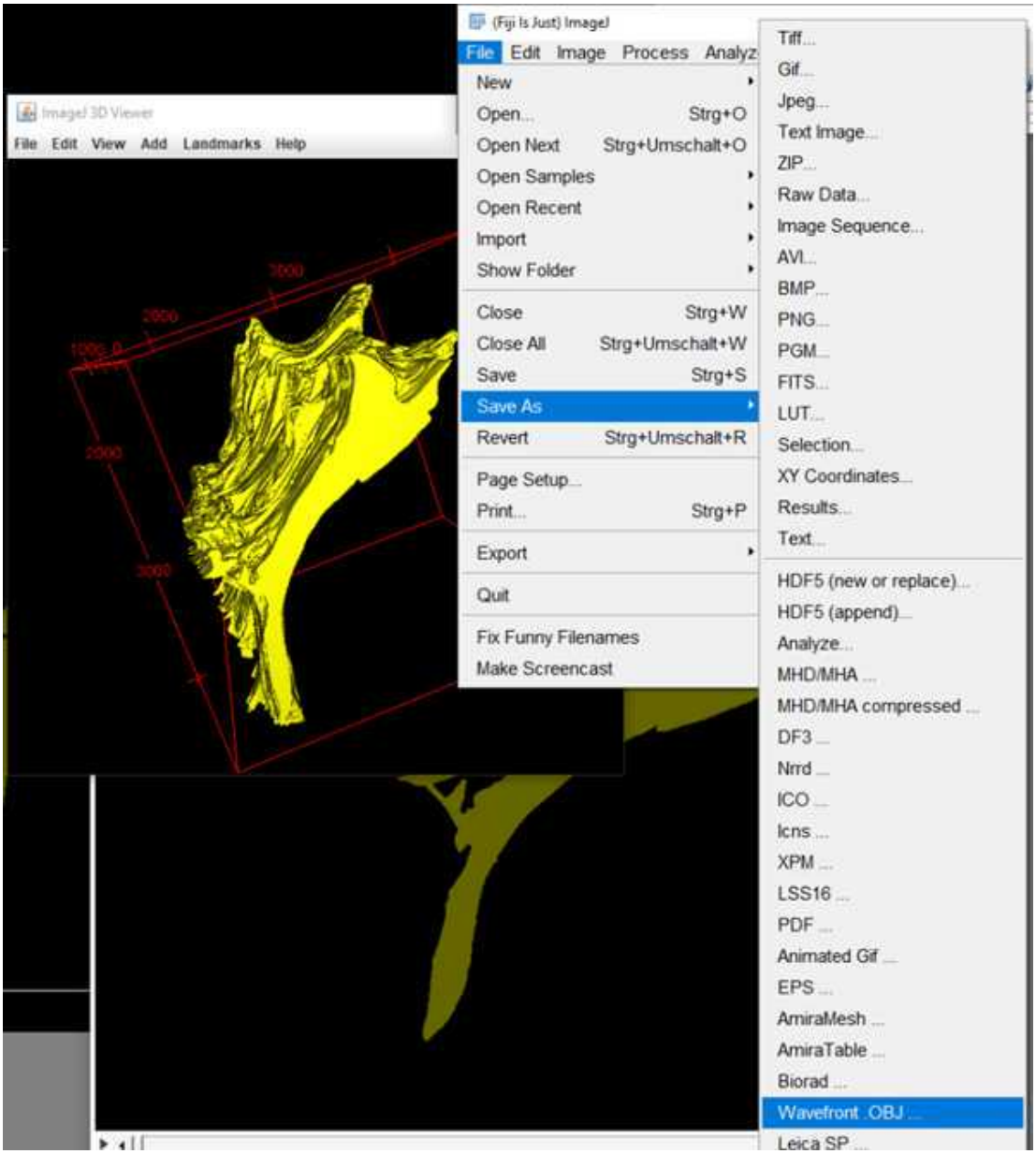
18



•press ok and wait for the result
•if it is stretched or clinched don´t bother, you can change later or go back to the *TrakEM2* window (see last step)
•save label as *wavefront*-file (.OBJ)

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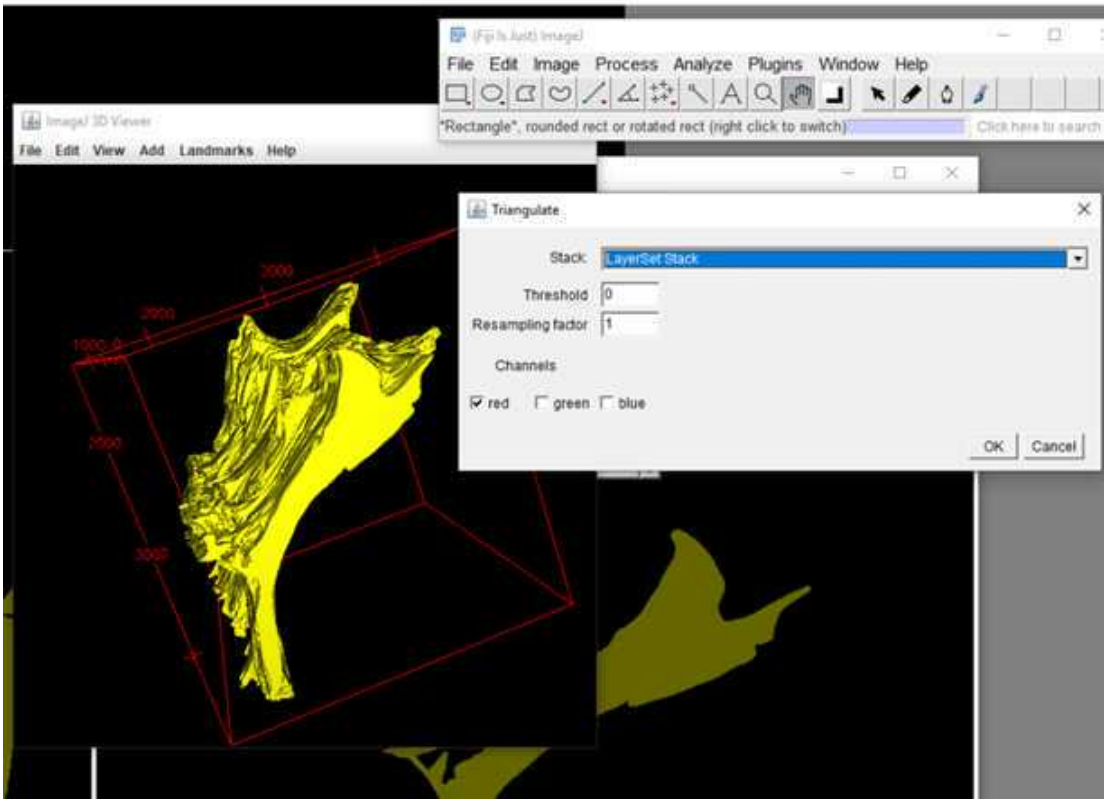
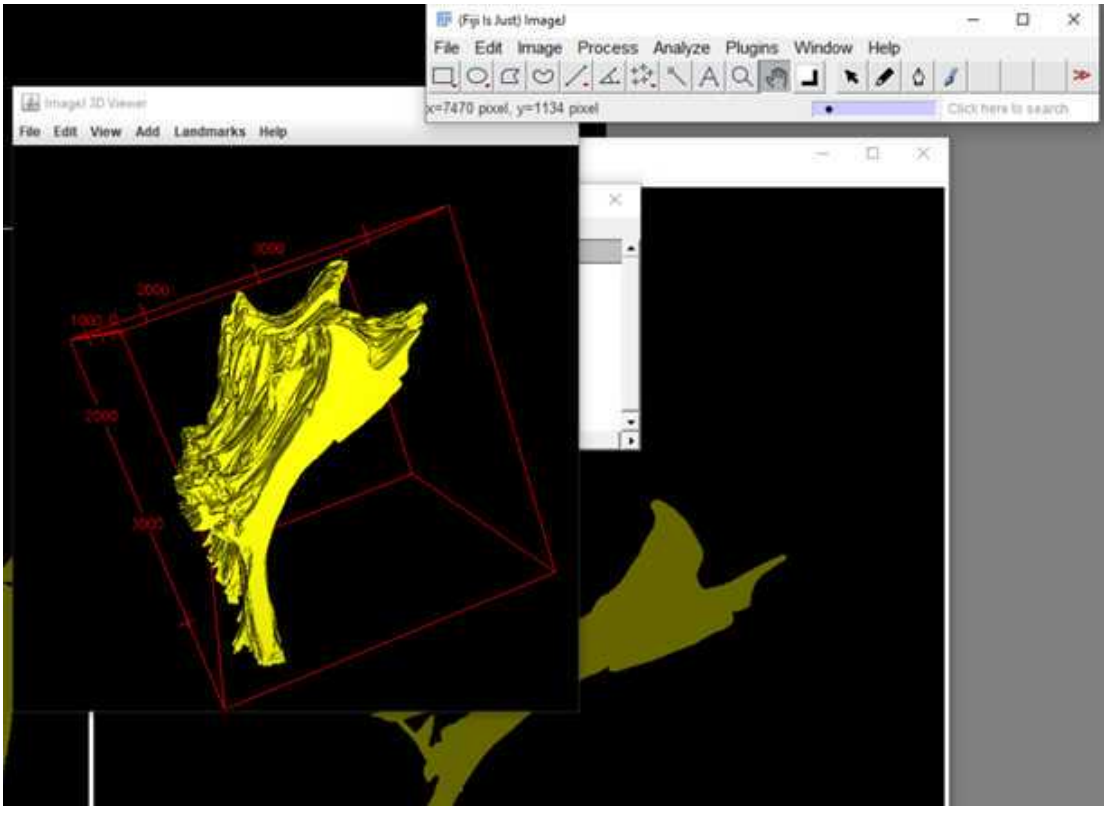
19



•use parameters as shown in image, press ok

20



•wait for the little black dot in the main tool bar before you export other labels

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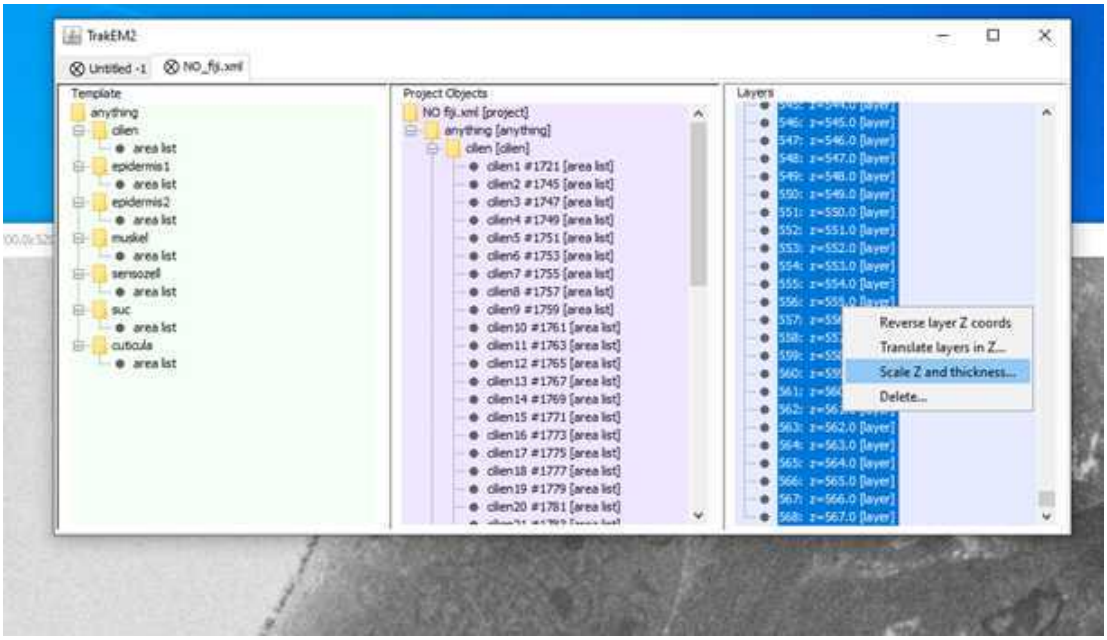
21



•if you want to change the slice thickness go to *Layers* of the *TrackEM2* window, select all slices and right click
•Choose a factor for all slices

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