Supplementary Information for

Preparation of samples for large-scale automated electron microscopy of tissue and cell ultrastructure

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Supplementary Figures 1-5 with legends and Supplementary Tables 1-4

Separate files (merged)

02-07 pdf files of step-by-step protocols: Separate pdf files demonstrating data processing steps for 1) Stitching and lens correction of overlapping TEM image tiles to a coherent dataset with TrakEM2, including export to non-overlapping tif tiles as a basis for bigtif file generation; 2A) Semiautomated generation of a text file containing coordinates (X,Y and Z) of overlapping STEM image tiles from two large datasets for import into TrakEM2¹ using Excel; 2B) import and stitching of these tiles to coherent datasets with TrakEM2; 2C) export to non-overlapping tiles; 2D) bigtif generation using nip2; 2E) import in QuPath as a basis for in-depth analysis using measurements and annotations

08_Supplementary_Information_ExcelTemplate: Excel file for calculation of image tile coordinates, filled with exemplary data and a brief documentation how to use the file
09_Supplementary_Information_STEM_shower_protocol: Screenshot of the protocol for automated pre-irradiation (electron beam shower) for 12 grids using SmartSEM Software
10_Supplementary_Information_zoominvideo_hippocampus: A zoom-in video to the large-scale dataset of the dentate gyrus, prepared with SEM-STEM

Online repository datasets: Selected large-scale datasets, as indicated in Supplementary Table 2, are accessible for online pan-and-zoom analysis via <u>www.nanotomy.org</u>

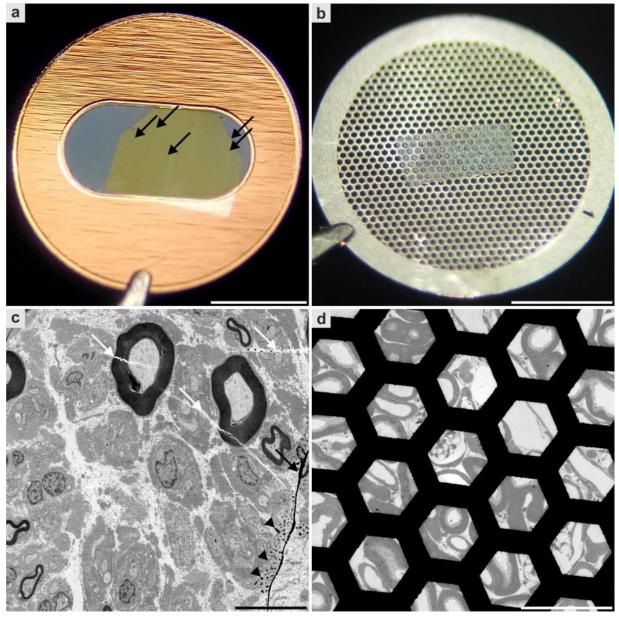
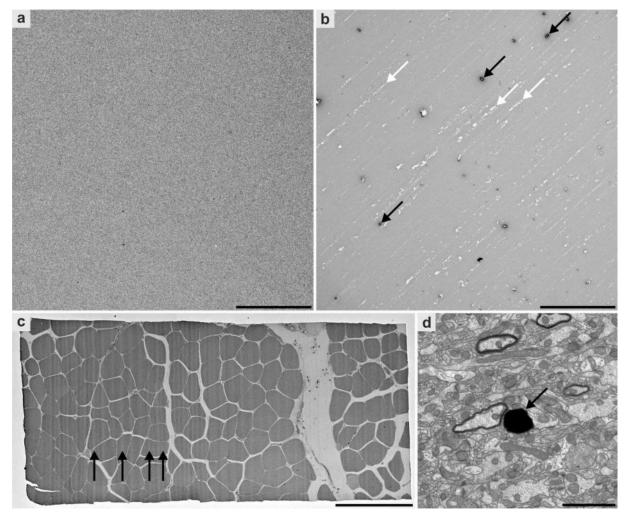
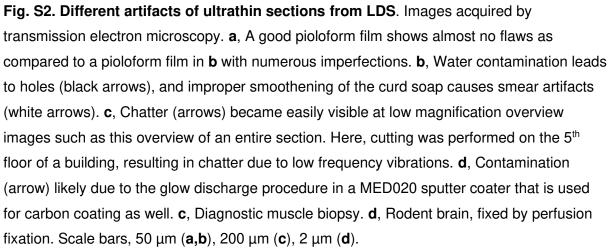


Fig. S1. Common limitations of slot and mesh grids. **a**,**c**, Slot grid provide a large observable area, allowing entire ultrathin sections to be viewed without limiting mesh bars. However, the delicate film is vulnerable to film imperfections, wrinkles (black arrows), stain precipitates (arrowheads in **c**), other contamination and section cracks (white arrows in **c**). **b**,**d**, The use of mesh grids on the other hand, allows preparation without additional film artifacts. **d**, This often results in cleaner samples, but viewing is impaired by mesh bars as well as decreased section stability. **c**, Biopsy of a peripheral nerve; hereditary neuropathy. **d**, Perfusion-fixed mouse kidney. Scale bars, 1 mm (**a**,**b**), 10 µm (**c**), 100 µm (**d**).





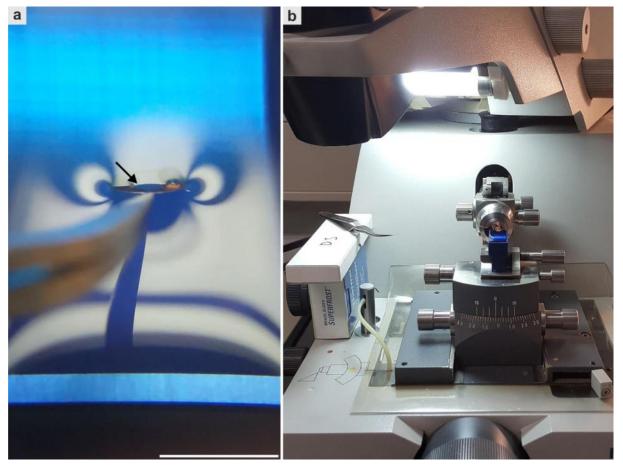


Fig. S3. Preparing LDS; collection and drying of a section using a glow-discharged and ethanol-smoothened pioloform-coated slot grid. a, The grid is inserted at the "6 o'clock position" to follow the section during the collection process. Ideal hydrophilicity of grids is indicated by a sharp and smooth water-grid borderline (arrow) which facilitates attachment of a section at the borderline and allows to collect the section by raising the grid out of the water. Note that the borderline is crossing the pioloform film; alternatively, the borderline may be placed on the metal surface directly underneath the forceps to compensate for mildly insufficient hydrophilicity (see Supplementary Table 3 for troubleshooting). **b**, After collection of a section, the self-closing forceps is placed next to the diamond knife, allowing examination of the attachment and drying processes. Scale bar, 5 mm (**a**).

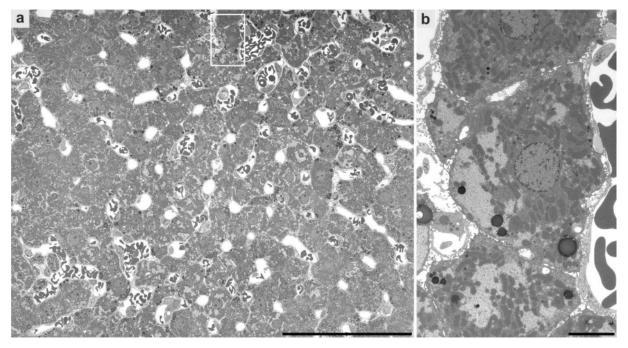


Fig. S4. Large-scale digitization of a medium-sized region of interest within a section of rodent liver using transmission electron microscopy. An intermediate magnification of 4646x was used, resulting in a pixel size of 9.3 nm. **a**, Microanatomical overview of parenchyma. **b**, Digitally magnified region from **a** shows hepatocytes with well-resolved glycogen, mitochondria, lysosomes, perisinusoidal space and intercellular bile ducts. Scale bars, 100 μ m (**a**), 5 μ m (**b**). See also <u>www.nanotomy.org</u> for download of the full resolution dataset.

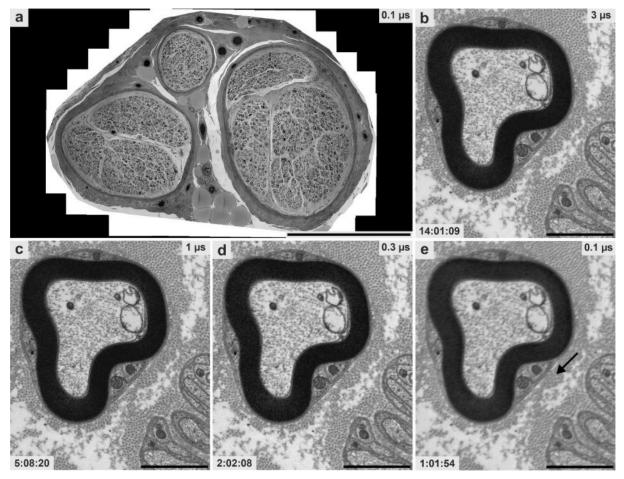


Fig. S5. Large-scale digitization of a diagnostic nerve sample using scanning transmission electron microscopy. **a**, An entire ultrathin section was digitized at 9 nm pixel size using different dwell times to analyze imaging time and quality. **b** to **d**, Image quality only moderately decreases with increased imaging speed using 3 μ s, 1 μ s and 0.3 μ s dwell time, while imaging time is drastically decreasing from 14 to 2 h. Quality in **d** allows to clearly resolve diagnostically relevant structures such as collagen pockets (see step-by-step protocol 2E; QuPath analysis). **e**, At 0.1 μ s dwell time, still individual collagen fibrils are resolved (arrow), but the image quality clearly appears blurred. Scale bars, 500 μ m (**a**), 2 μ m (**b**-**e**). See also <u>www.nanotomy.org</u> for internet browser-based pan-and-zoom analysis of the full resolution datasets of **c,d** and **e**.

Supplementary	v Table 1 Informati	ion on tissue blocks i	Ilustrated in this work*
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Fig.	Tissue	Pathology	Species	Resin	Osmication	UA en bloc staining
2,6	Kidney	Chronic hypokalemic nephropathy	Human	Epon	30 min 1% in 0.1 M phosphate buffer	-
3	Kidney	Lupus nephritis	Human	Epon	30 min 1% in 0.1 M phosphate buffer	-
4	Brain	Alzheimer's disease	Mouse	Renlam	Overnight 1% in 0.5 M cacodylate buffer	+
ga,5	Muscle	Tubular aggregates	Human	Renlam	Overnight 1% in 0.5 M cacodylate buffer	+
S4	Liver	-	Rat	Epon	2 h 2% in distilled water	-
S5	Nerve	Neuropathy	Human	Renlam	Overnight 1% in 0.5 M cacodylate buffer	+

*In total, we prepared ultrathin sections of 100 different tissue blocks from different research projects to establish the LDS protocol. However, early preparations were less sophisticated and demonstrated higher amounts of preparation flaws so that more sections were needed to prepare one high-quality section ^{2, 3}. ga, graphical abstract; UA uranyl acetate.

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Supplementary	Table 2.	Information o	on large-scale	datasets	illustrated in this work*	

Fig.	Imaging	Pixel	Dwell	Tile	Mosaic	Tile	Acquisition	Repository
	system	size	time	size, p	size	number	time	number
2a,d	TEM	7.3 nm	-	2,048	254 x 254 μm	400	1:06:40	2
2b,e	SEM-STEM	7.3 nm	3 µs	12,288	245 x 229 μm	9	01:09:37	3
2c,f	SEM-BSD	7.3 nm	3 µs	12,288	269 x 269 µm	16	01:35:32	4
3a-d	SEM-STEM	7.3 nm	1 µs	8,192	239 x 239 µm	16	00:24:51	5
4a-c	SEM-STEM	7.3 nm	1 µs	8,192	1,794 x 1,017 μm	510	12:32:09	6
5a**	SEM-STEM	9 nm	1 µs	10,240	1,383 x 760 μm	127	04:40:54	1
5b	SEM-STEM	100 nm	1 µs	2,048	1,236 x 432 μm	21	00:02:25	
5c-f	SEM-STEM	4 nm	3 µs***	24,576	94 x 49 μm	1	00:50:03****	7
6a	SEM-STEM	7.3 nm	0.3 µs	12,288	245 x 229 μm	9	00:09:18	9
6b	SEM-STEM	7.3 nm	0.2 μs	20,480	245 x 229 μm	9	00:07:07	10
6c	SEM-STEM	7.3 nm	0.1 μs	12,288	245 x 229 μm	9	00:03:21	11
6d	SEM-BSD	7.3 nm	3.0 µs					12
6e	SEM-BSD	7.3 nm	1.0 μs					13
6f	SEM-BSD	7.3 nm	0.1 μs					14
S4a,b	TEM	9.3 nm	-	2,048	380 x 240 μm	360	01:00:00	8
S5a,e	SEM-STEM	9 nm	0.1 μs	10,240	1,347 x 917 μm	155	01:01:54	15
S5b	SEM-STEM	9 nm	3.0 µs	10,240	1,347 x 917 μm	155	14:01:09	
S5c	SEM-STEM	9 nm	1.0 µs	10,240	1,347 x 917 μm	155	05:08:20	17
S5d	SEM-STEM	9 nm	0.3 µs	10,240	1,347 x 917 μm	155	02:02:08	16

*In total, we digitized large areas (entire ultrathin sections) from 92 different samples; 85 were diagnostic (76x skeletal muscle, 4x brain, 2x lung, 2x tumor, 1x nerve) and 10 experimental samples (5x kidney, 1x liver, 2x brain, 1x lung, 1x cell culture). Medium sized ROIs came from 5 different blocks of diagnostic samples (5x kidney). ** Same dataset as in graphical abstract; *** including line averaging of 3 x 3 µs; **** for each of the 6 regions of interest. p, pixels.

Supplementary Table 3. Critical steps in preparation of large-scale digitization samples

(troubleshooting)

Category	Artifact pattern	How to avoid them
Support film and grids		
Cleaning	Large spots on pioloform film	Cleaning sequence of first using acetone, then ethanol,
		then distilled water must be respected
Curd soap coating	Smears on pioloform film	Ensure homogeneous distribution of curd soap
Pioloform	Small dots and holes of pioloform film	Open the bottle with pioloform solution warmed to room
		temperature to avoid water contamination
Hydrophilization		
Glow discharging	Pioloform film destroyed in vacuum	Transfer grids to a separate parafilm strip to allow air
		circulation, thus avoiding damage of the pioloform film
Glow discharging	Overhydration; no sharp water-pioloform	Grids should only be moderately hydrophilic; during
	borderline during collection of sections	collection of sections from the knife's water trough, the
	Underhydration; sharp water-pioloform	water-grid borderline has to be sharp
	borderline, but wrinkles appear	When overhydrated, reduce glow discharging time (Fig.
		S3)
		Conversely, sections may be attached on the metal surface
		next to the forceps to stabilize water within the slot area in
		case of underhydration
Glow discharging	Electron-dense contaminations on pioloform	Avoid e.g. carbon contamination in the sputter coater, if it is
	film	also used for carbon coating (Fig. S2)
Glow discharging	Underhydration	Ideally, use glow-discharged grids within 1 to 2 h
Grid storage	Inhomogeneous wetting or wrinkles	Let freshly filmed grids dry for 2 d to ensure homogeneous
		wetting when collecting the sections and avoid use of grids
		older than 2 to 3 months to ensure adequate tension of the
		film
Ultramicrotomy		
Water borderline	Overhydration of pioloform film (no sharp	Place the forceps as far as possible at the grid periphery
	borderline)	After ethanol smoothening, insert grid only to 1/3 to 1/2 into
		the water of the knife's trough and wait for 10 s to generate
		a sharp and stable water-grid borderline
		Do not fully submerge the grid when inserting it into the
		water trough
Water borderline	Underhydration of pioloform film	Prepare another batch of glow-discharged grids; do not
		perform additional glow discharging of the same grids since
		this may result in overhydration
Drying process	No or displaced Newton ring formation,	Ensure a horizontal position of the grids held within forceps
, ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	wrinkles, section movement	Avoid or remove compression or folds in the sections by
	,	adjusting sectioning parameters and using xylene or
		chloroform vapor to ensure smoothness
		Prepare straight block edges to fix the section onto the
		water-grid borderline during collection
Staining		
Embedding pepper	Electron dense contamination	Treat grids with 1% EDTA in distilled water prior to staining
Precipitates	Needle-like (uranyl acetate) or spherical (lead	Use aqueous instead of ethanolic uranyl acetate solution
1 roopnatoo	citrate) contamination	Place NaOH pellets next to lead citrate in closed Petri dish
Other contamination	Amorphous electron-dense contamination	Rinse intensely after staining with uranyl acetate to avoid
		excess contamination and let the sections dry in a
		horizontal position for adequate Newton ring formation
Detachment artifacts	Small or medium sized, ring- or comma-	Trim block areas of pure resin or overfixed areas away
	shaped wrinkles, probably due to water-	Ensure adequate drying of the sections after collection at
	induced swelling of the sections	room temperature for 48 h
	Some areas, e.g. strongly fixed structures or	Stabilize sections, fixed individually by forceps, at 120°C ir
	areas with pure resin, e.g. cell culture with low	an oven for 15-30 min prior to staining
	cell density, seem to be prone to these	
	artifacts	

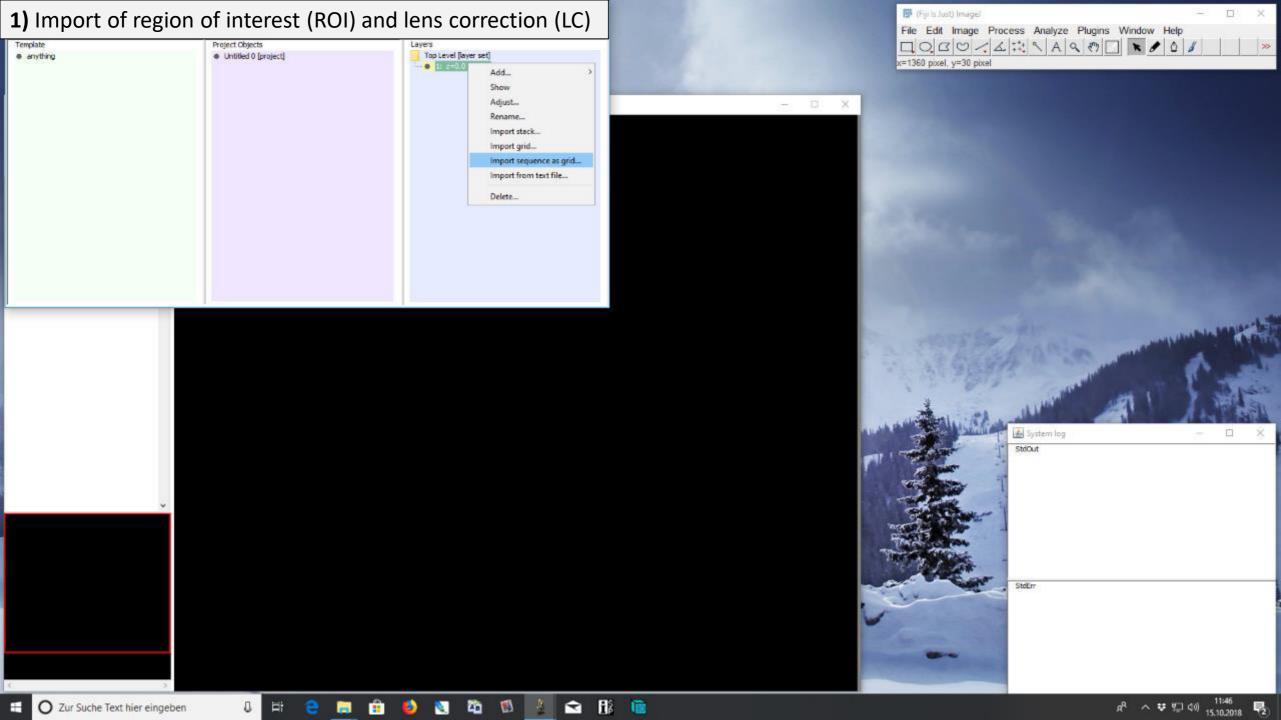
	TEM	SEM-STEM	SEM-BSD			
Substrate	Slot grid	Slot grid	Silicon wafer			
Imaging speed	Fixed, about 3 µs*	Variable, about 1 µs	Variable, 6 to 12 µs			
Image tiles (field dimensions)	2,048 or 4,096 pixels per side	Variable, up to 32,768 pixels per side				
Field size	15 μm or 30 μm (at 7.3 nm pixel size)) Variable, 50 to 100 μm				
Autofocus	Not available with our systems	Autofocus/ autostigmation in	center of image tiles			
Stitching	TrakEM2	Atlas 5 or TrakEM2				
Zoomable dataset	bigtif	Atlas 5 or bigtif				
Limitations	Fixed imaging speed,	Beam damage (support film	Slow imaging speed			
	Small-size image tiles**), pre-	required)	Special preparation for			
	irradiation recommended	Pre-irradiation required	improved results			
	Vulnerable	sections	Pre-irradiation sometimes			
			necessary			
Advantages	Good resolution and SNR,	Good resolution and SNR, high	Stable preparation			
	compatible with many old or	imaging speed. Up to 12 grids in				
	conventional TEM systems	the sample holder				
		meters such as dwell time,				
		tile and pixel size, averaging, autofocus and autostigr				
		Low-grade lens distortions, hig	h degree of automation.			
	Grids interchangeable (TEM, SI	EM-STEM; shuttle workflow)				

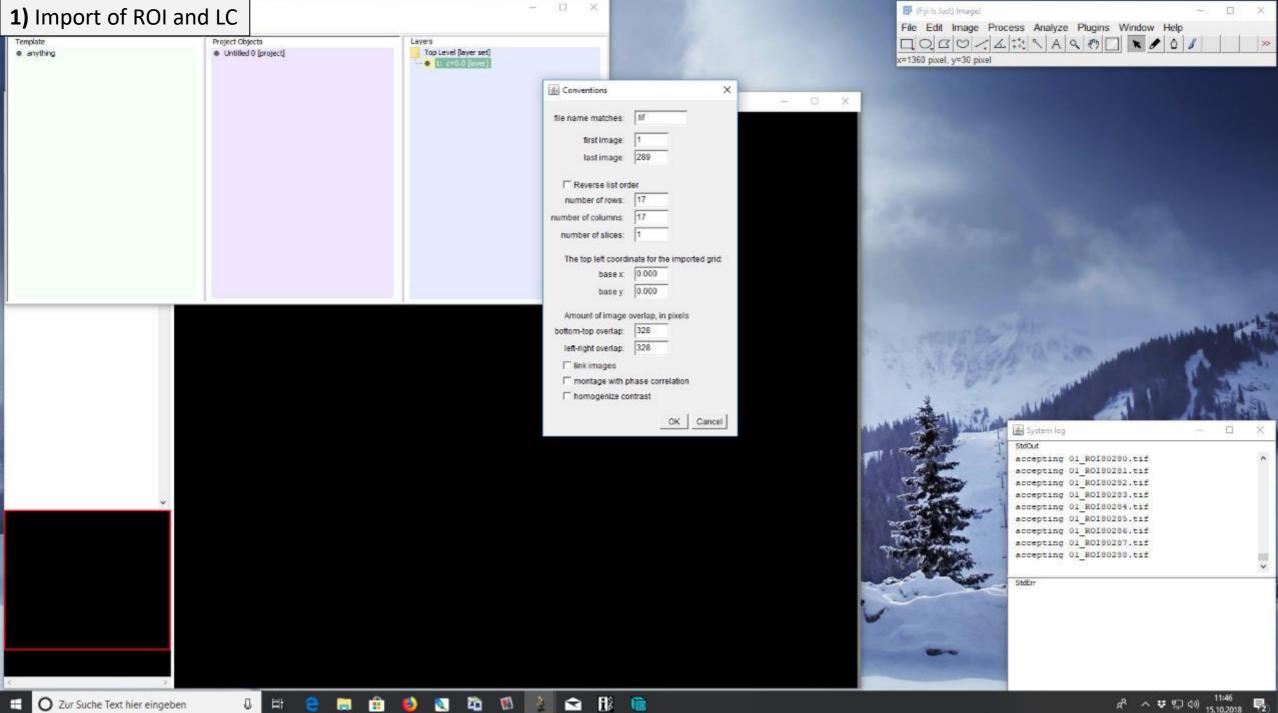
Supplementary Table 4. Comparison of large-scale digitization using TEM, STEM and BSD

* µs per pixel, 2k CCD camera. TEM, transmission electron microscopy; SEM, scanning electron microscope; STEM, scanning transmission electron microscopy; BSD, backscattered electron detection.

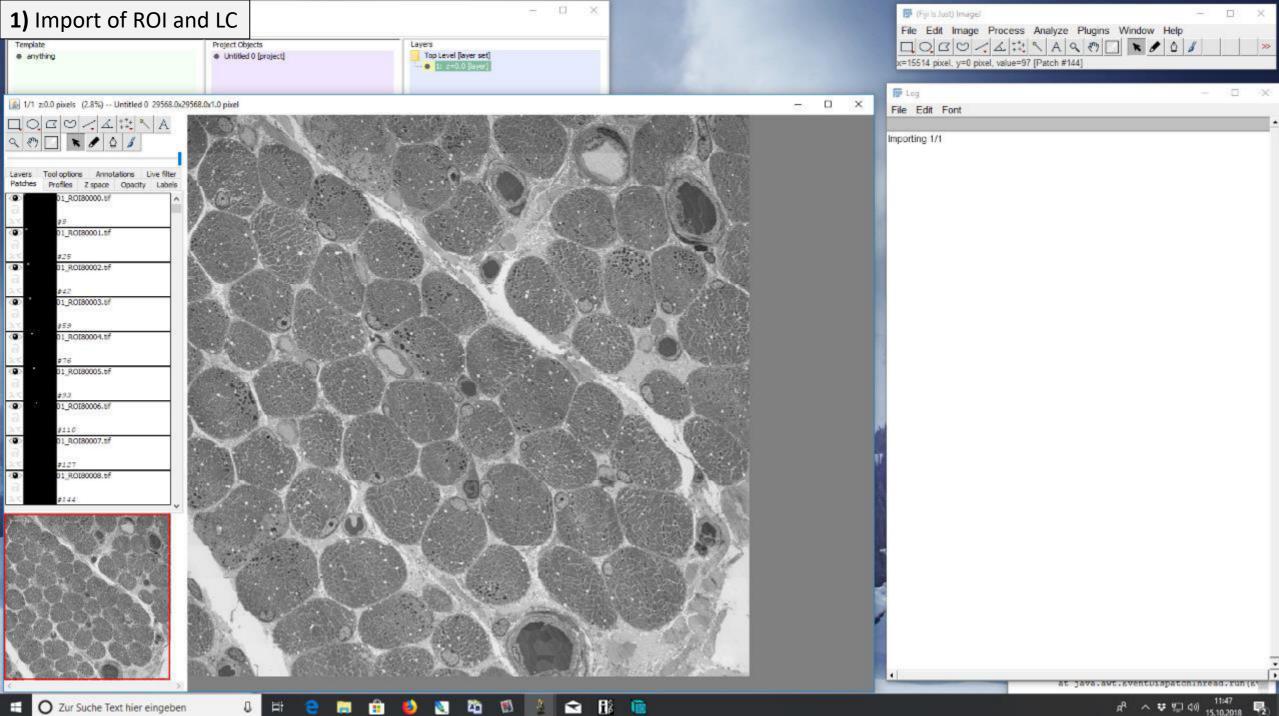
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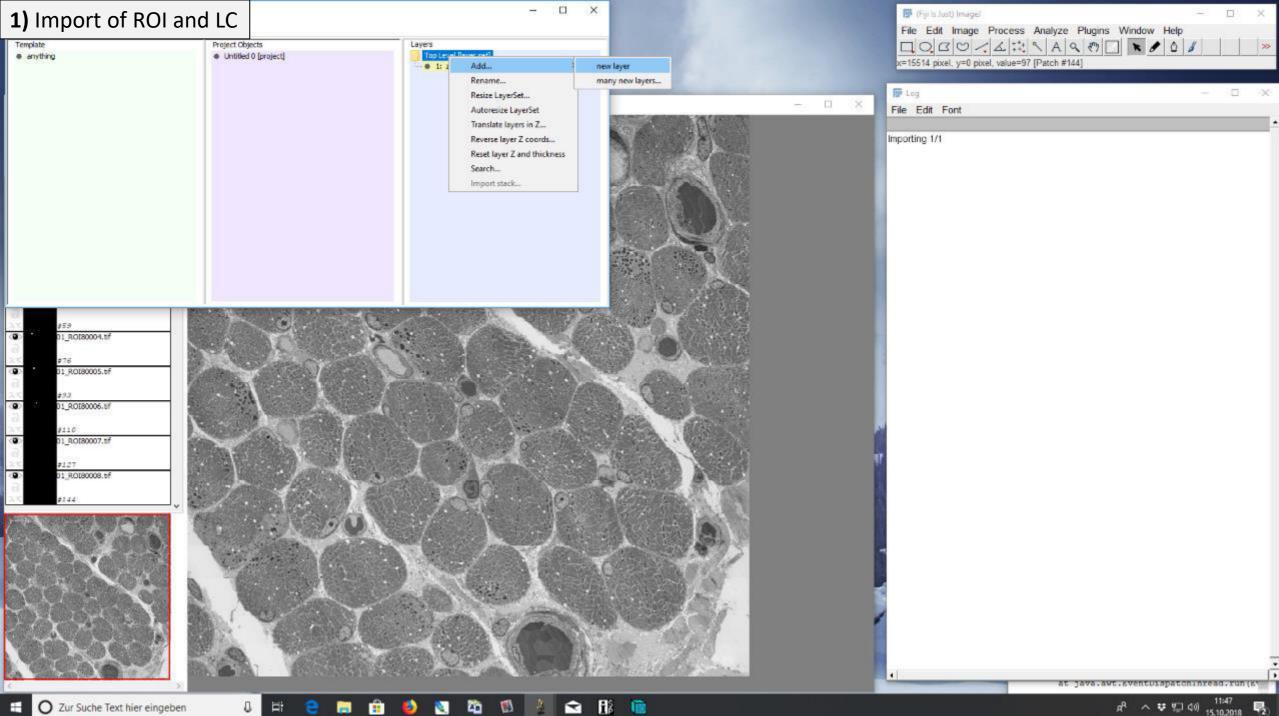
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- 2. Dittmayer, C. et al. Morphological characteristics of the transition from juvenile to adult dermatomyositis. *Neuropathol. Appl. Neurobiol.* **46**, 790–794 (2020).
- 3. Dittmayer, C. Optimierte Verfahren zur morphologischen und histochemischen Auswertung der Niere. *Medical thesis* (2019).

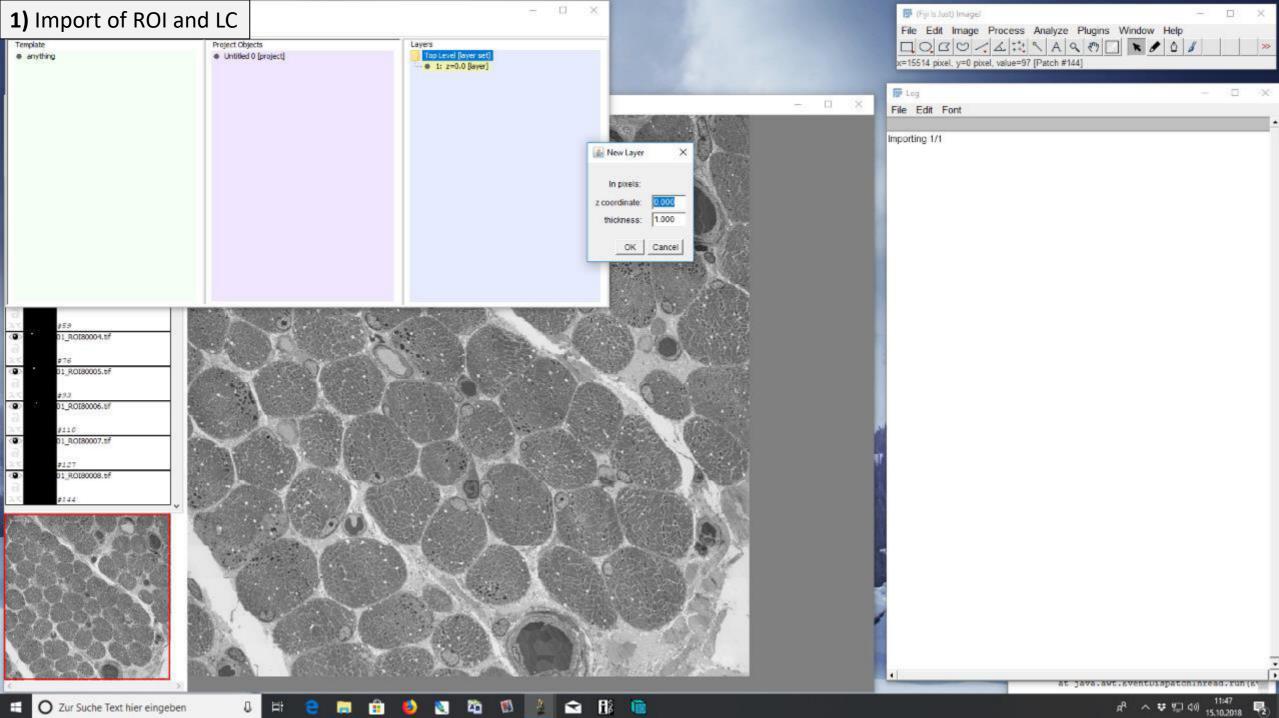


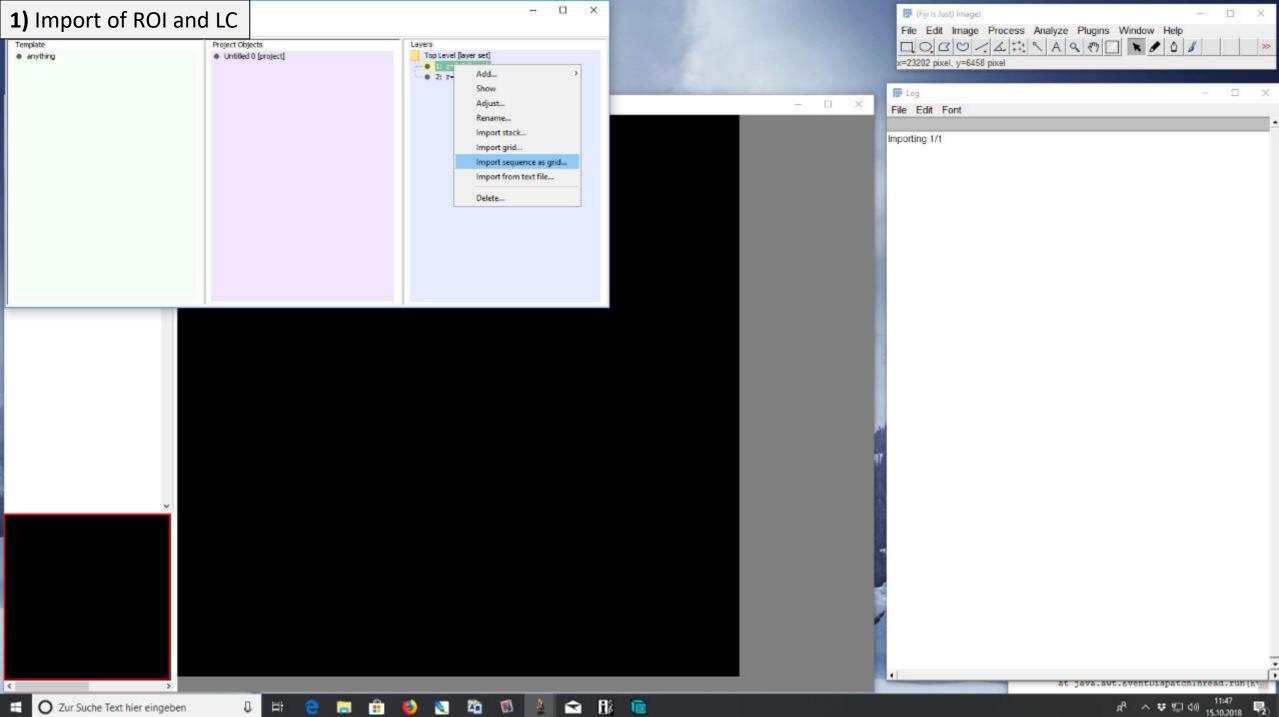


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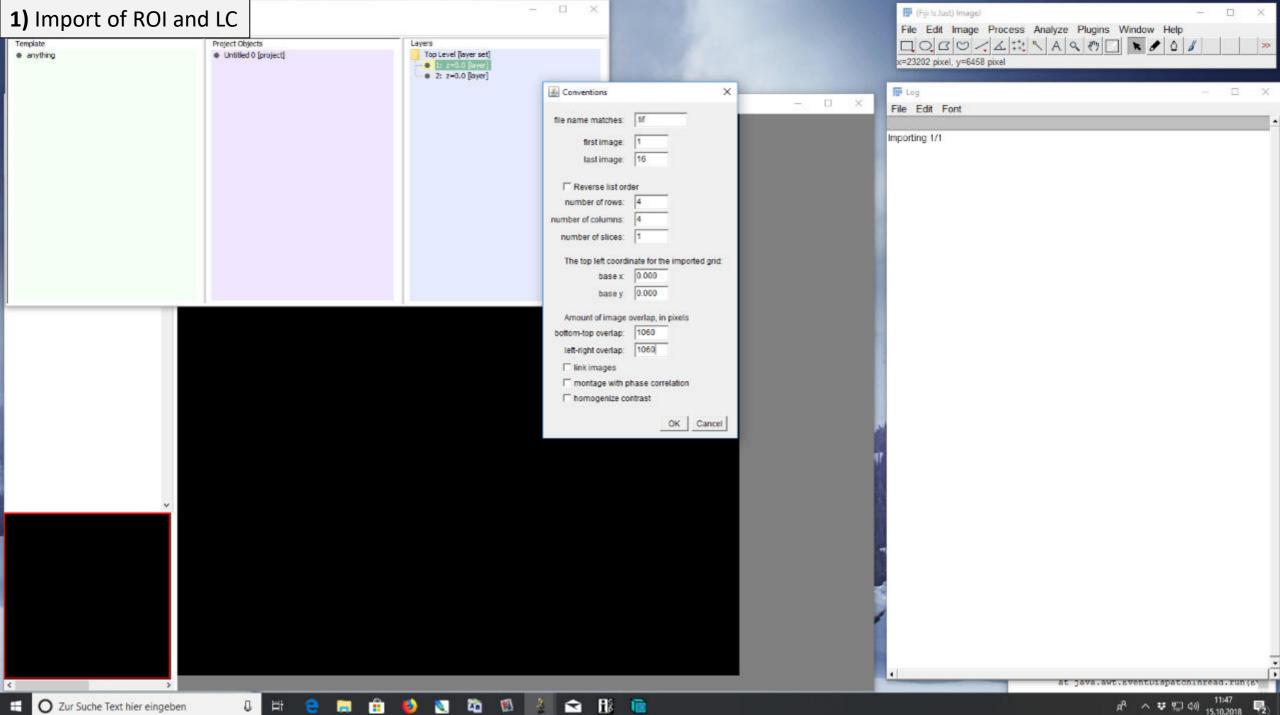


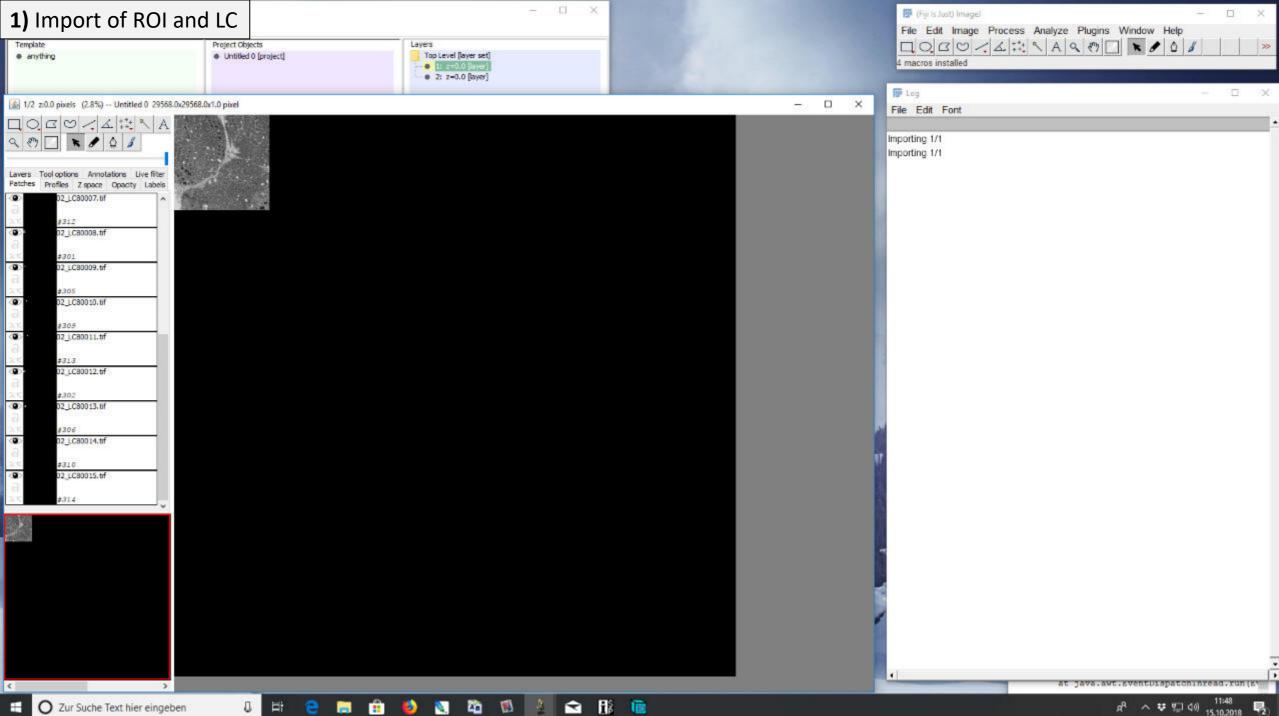




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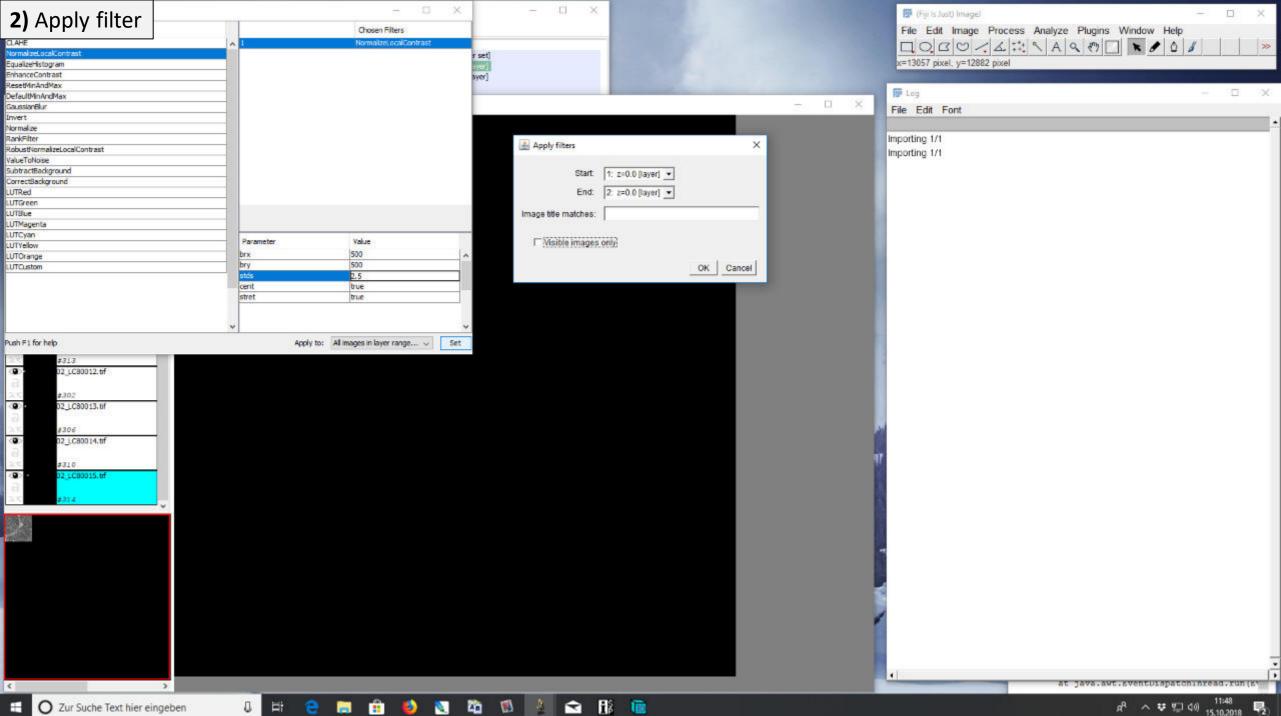
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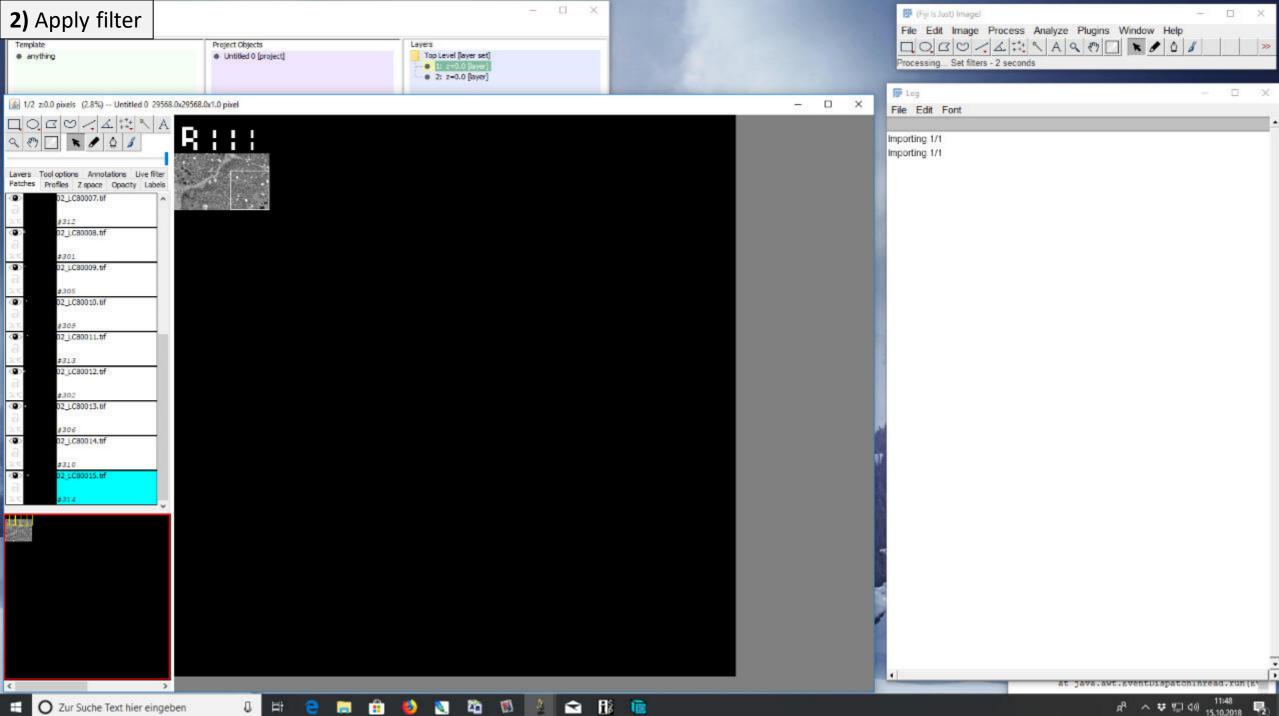
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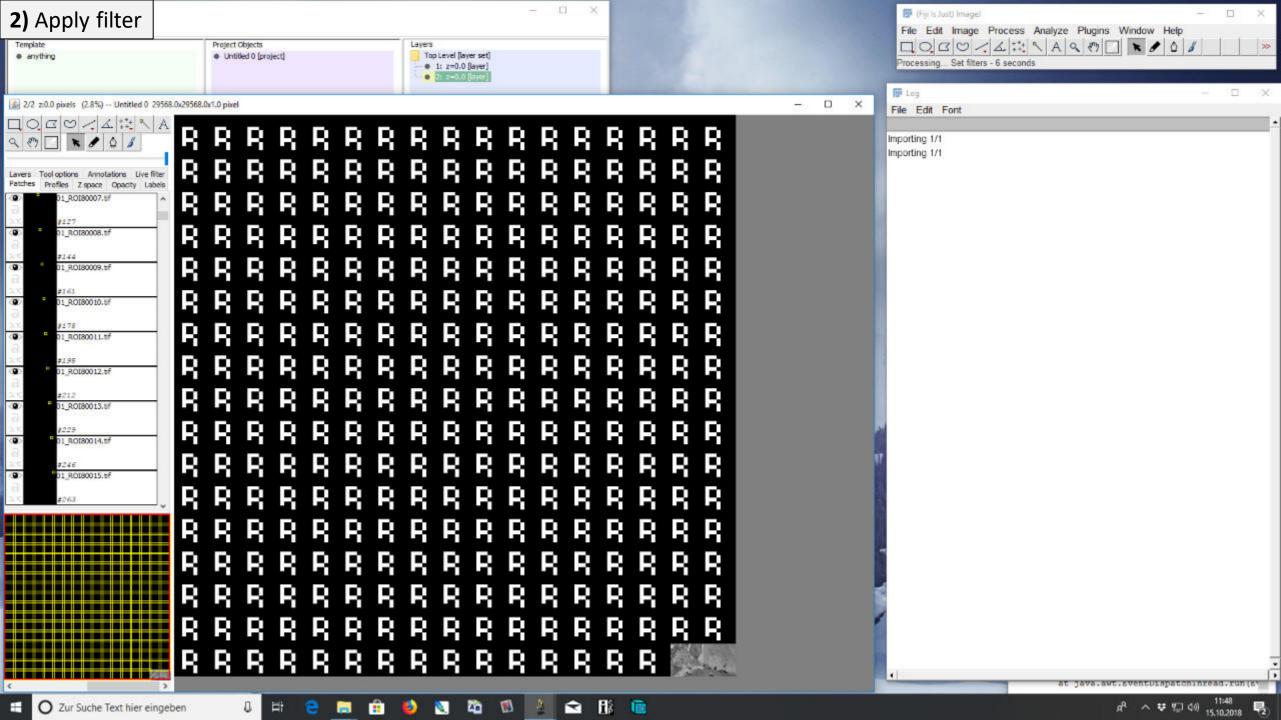
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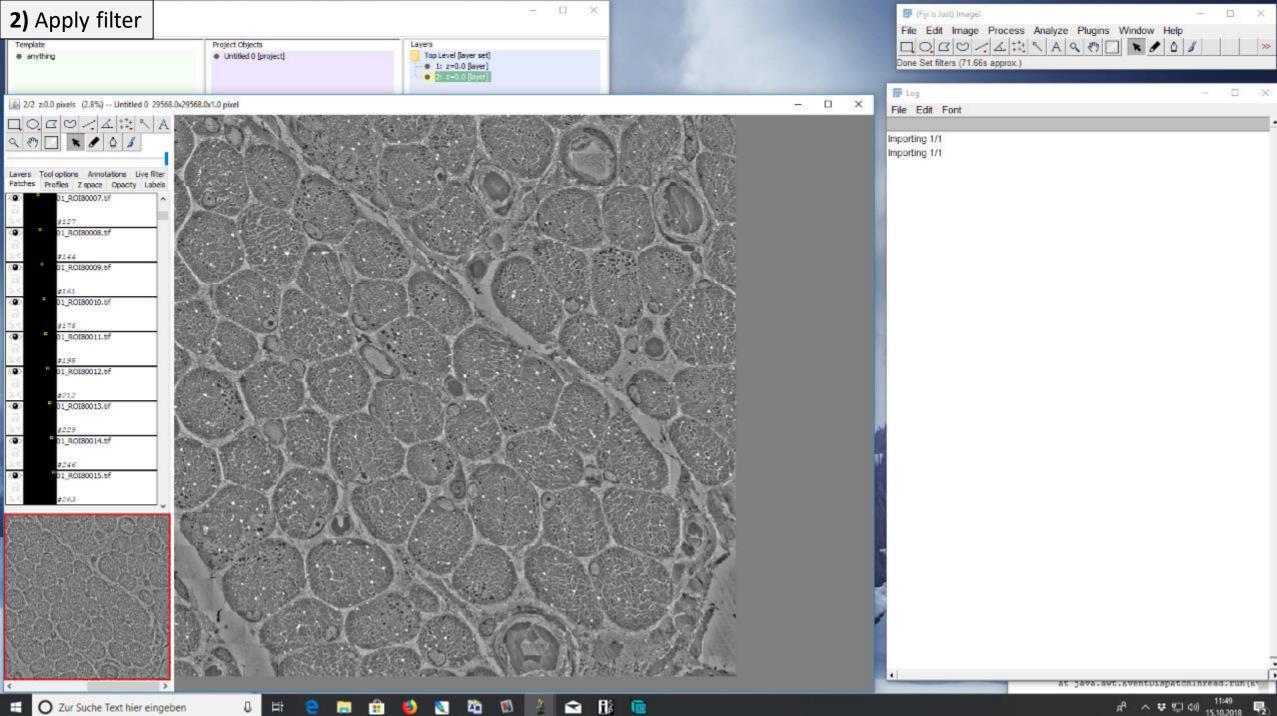
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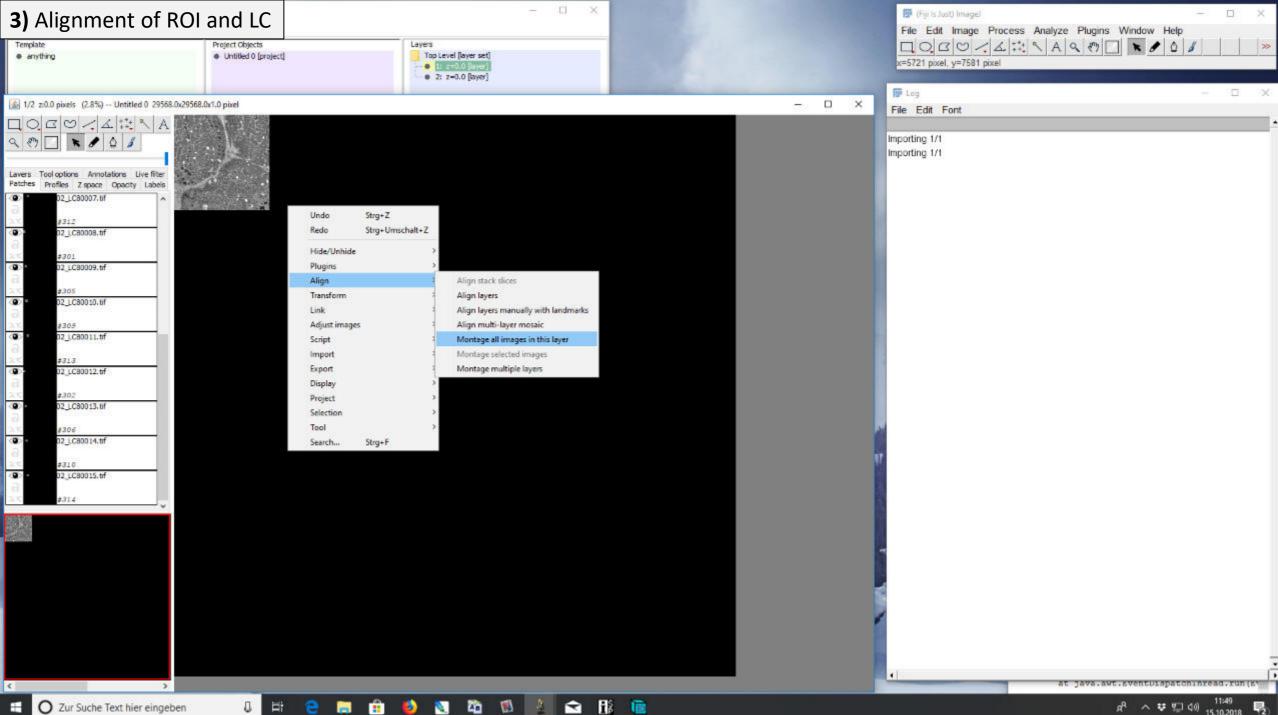
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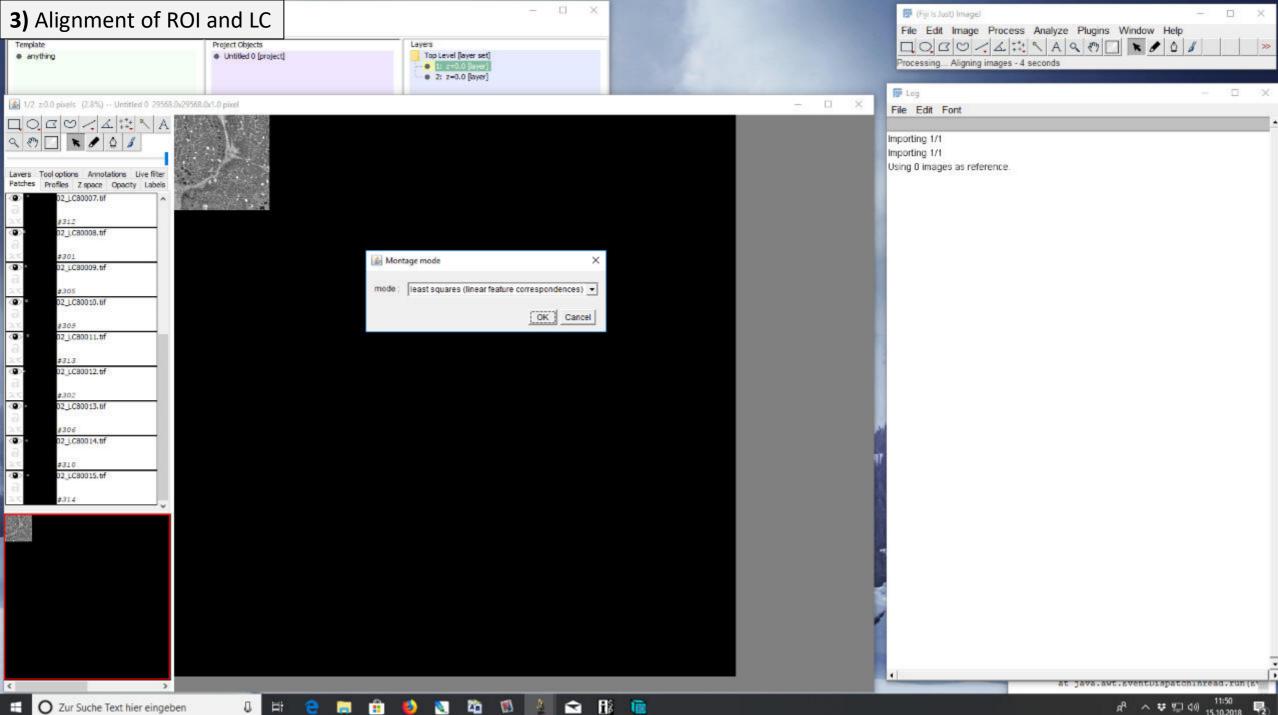






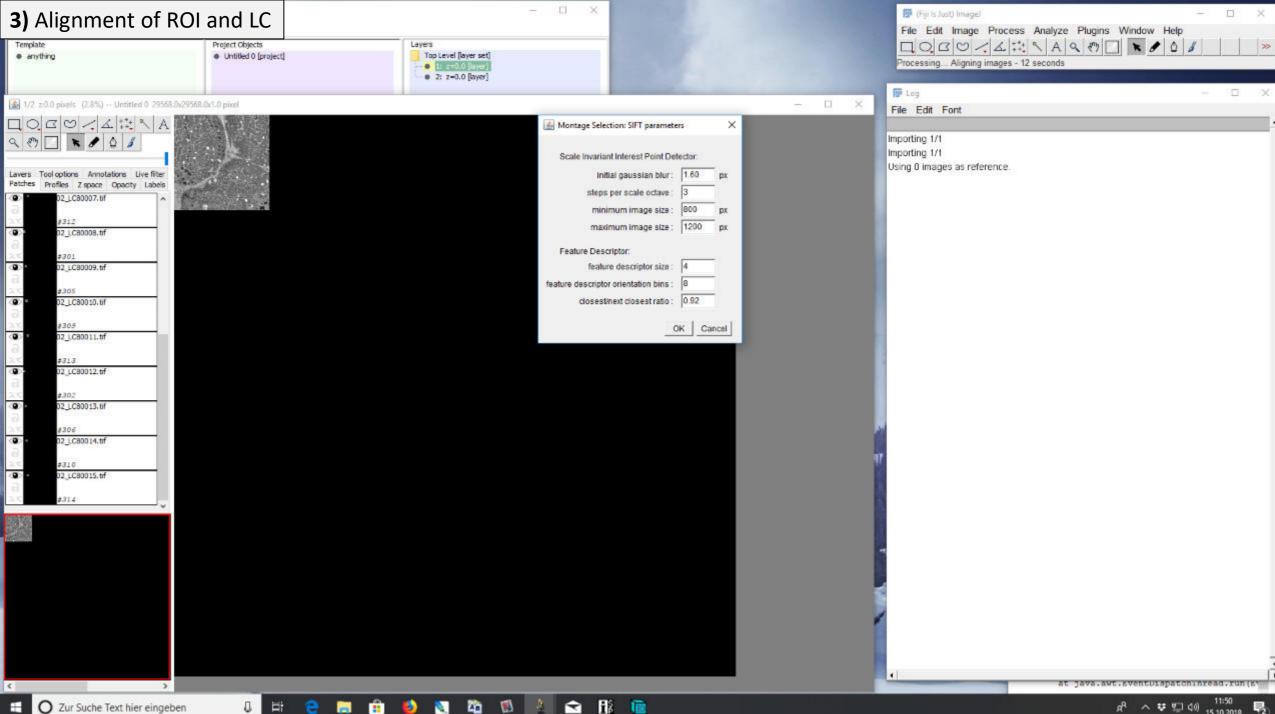


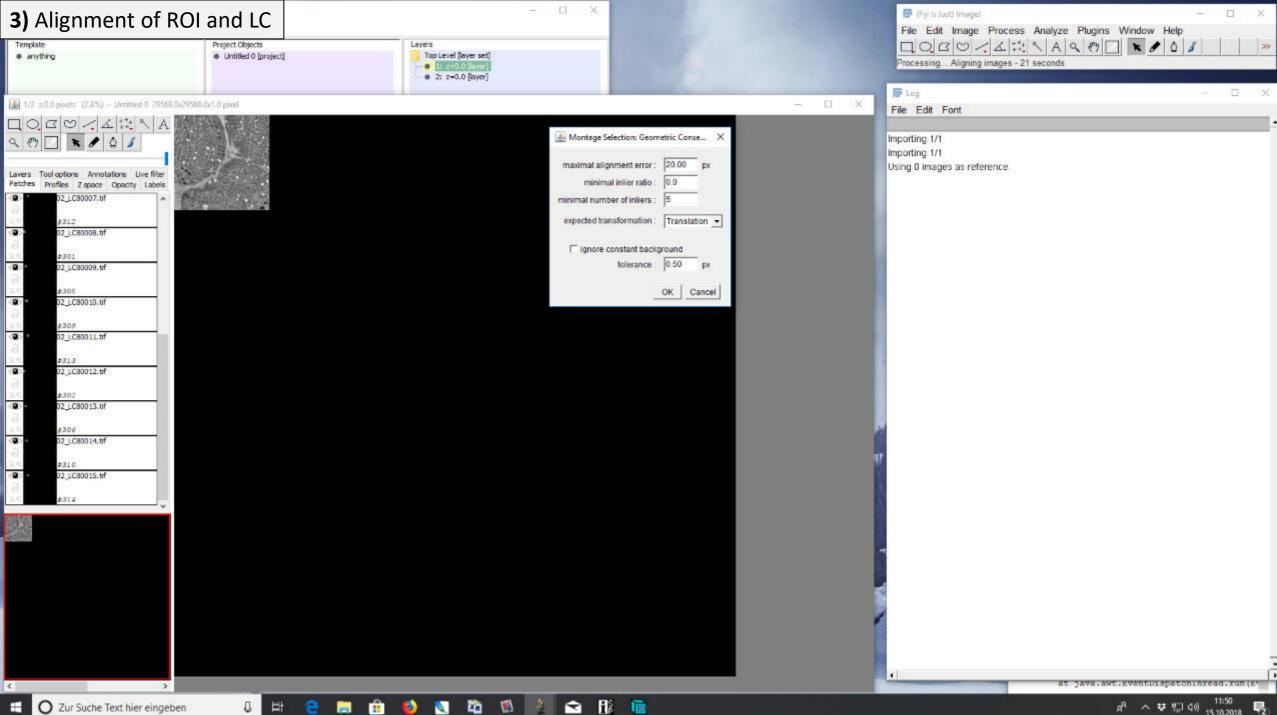
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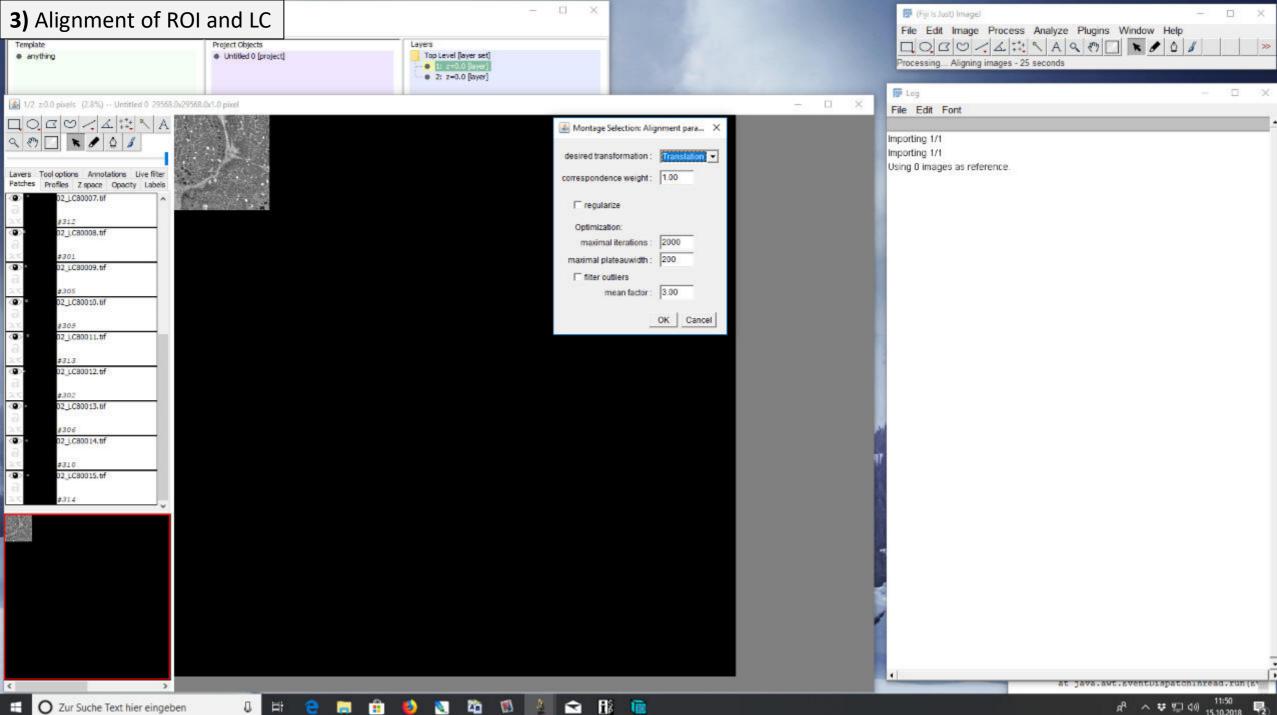
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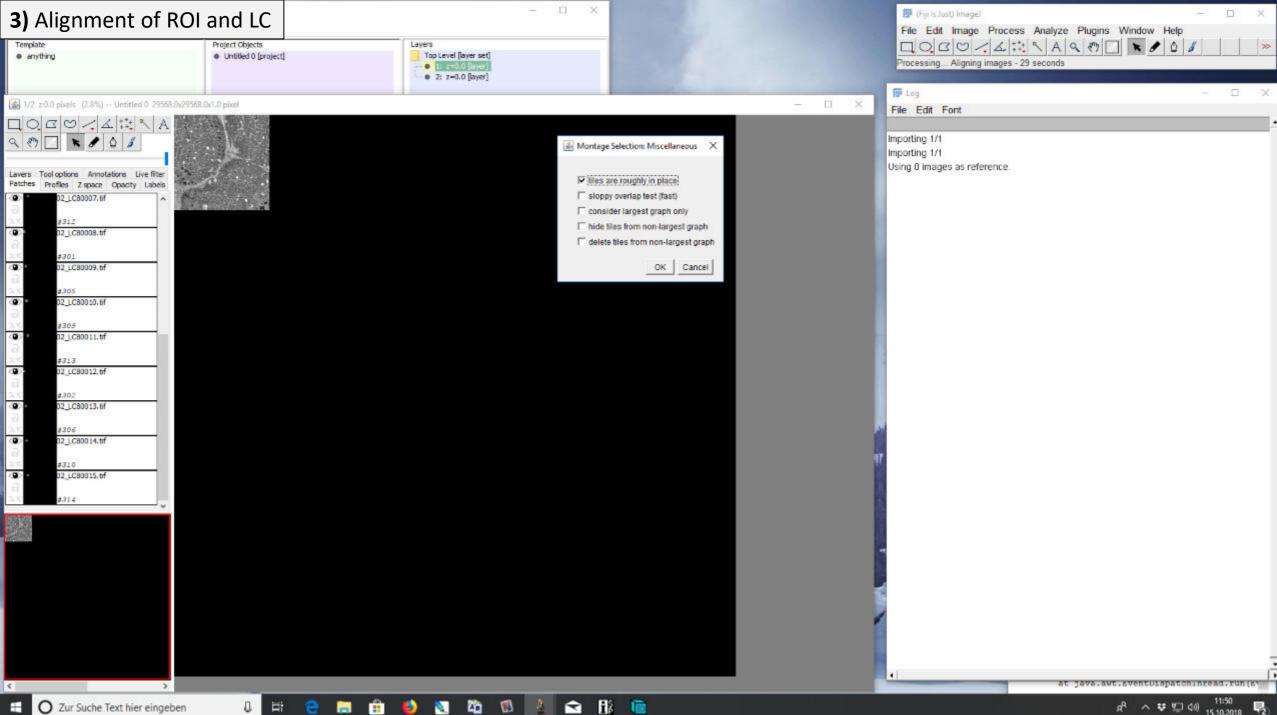


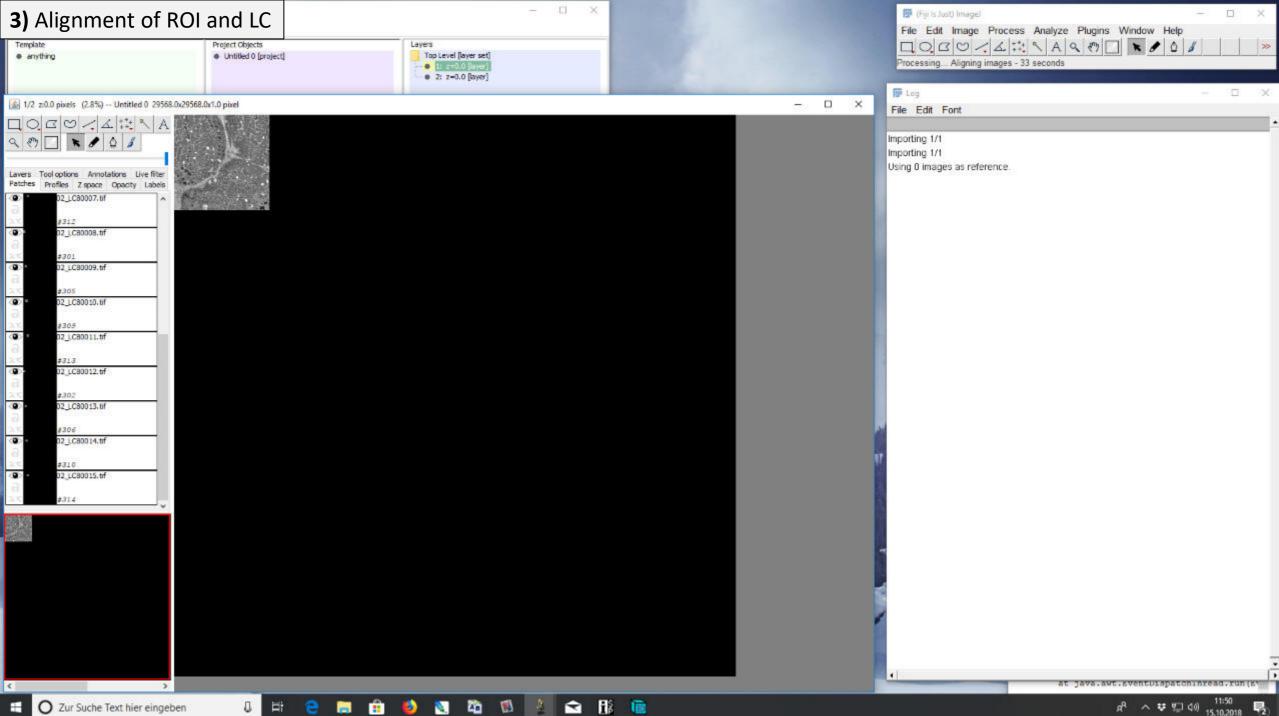


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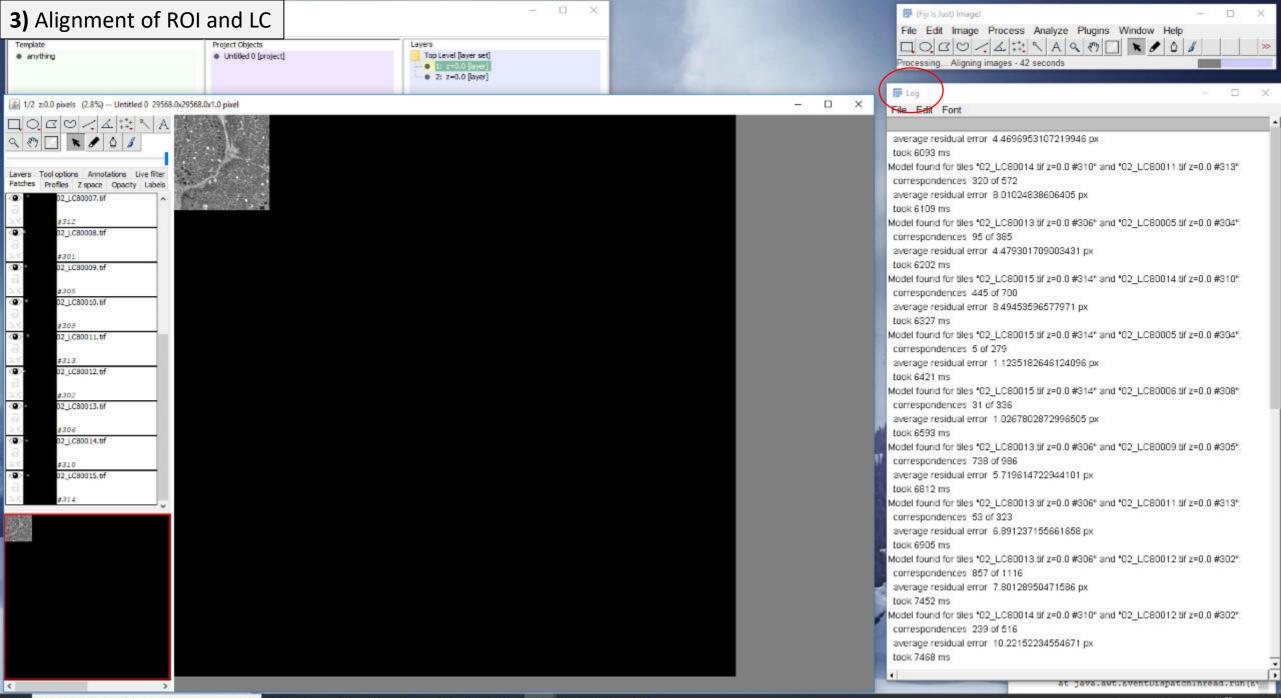
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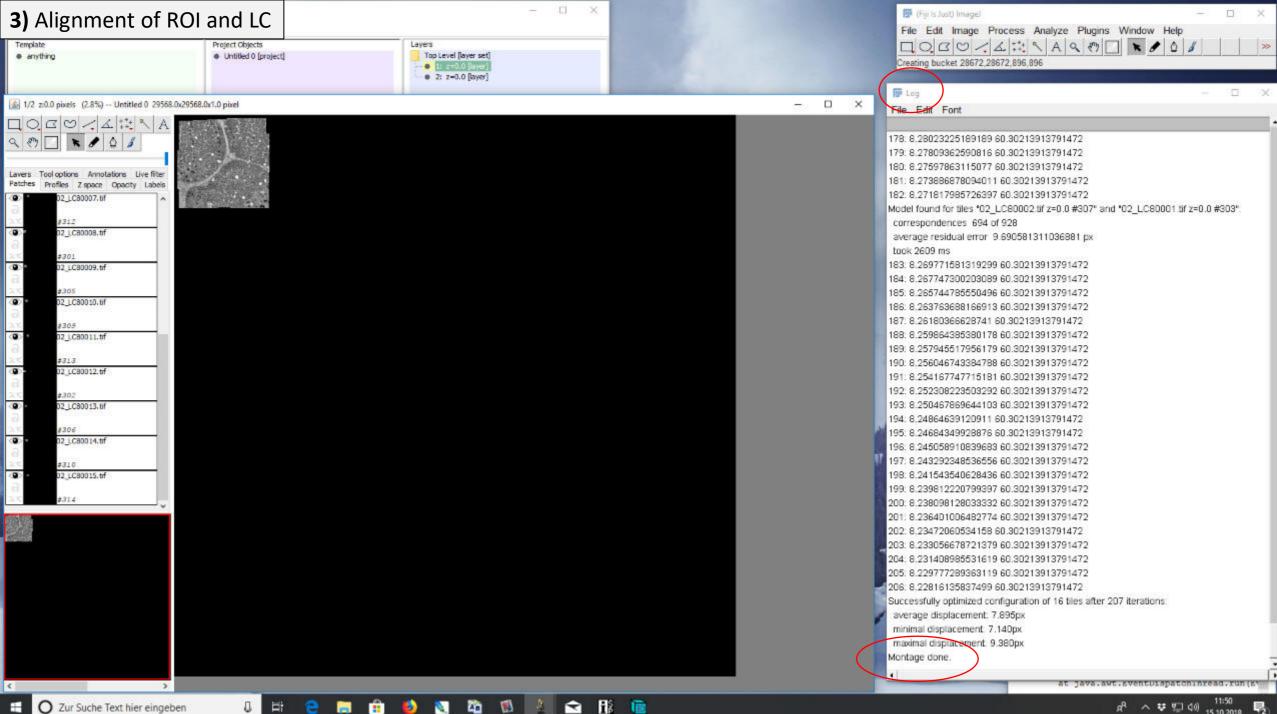
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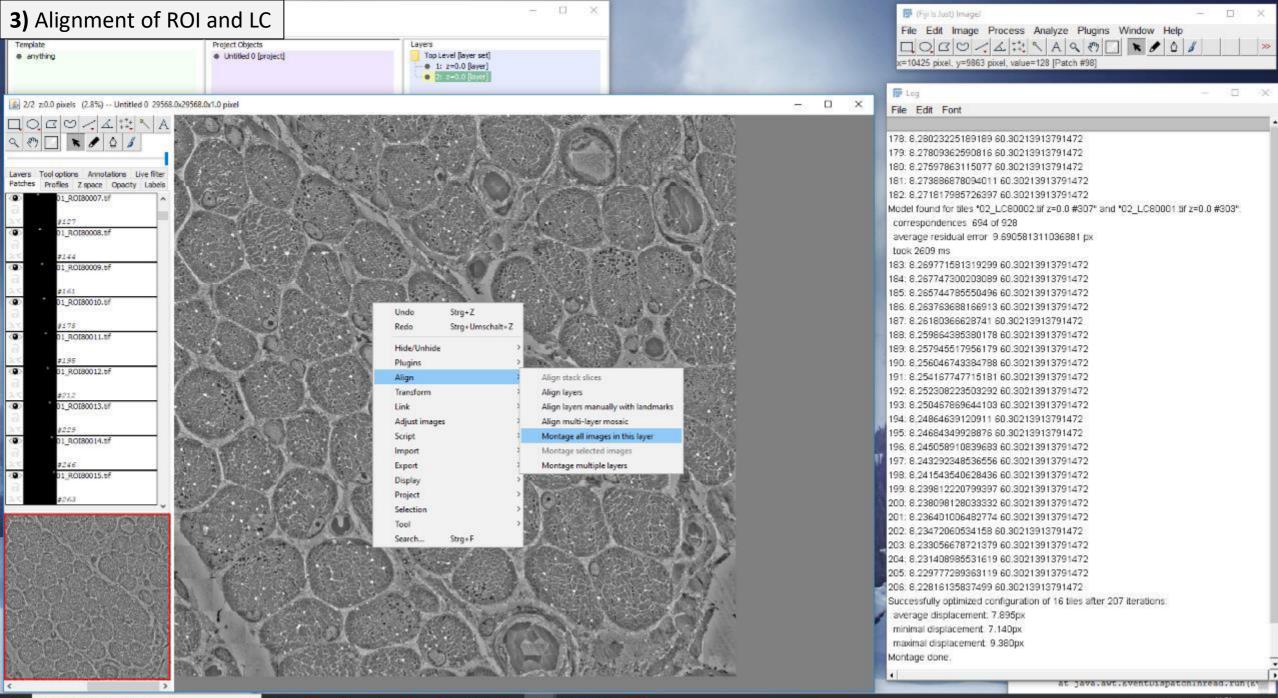
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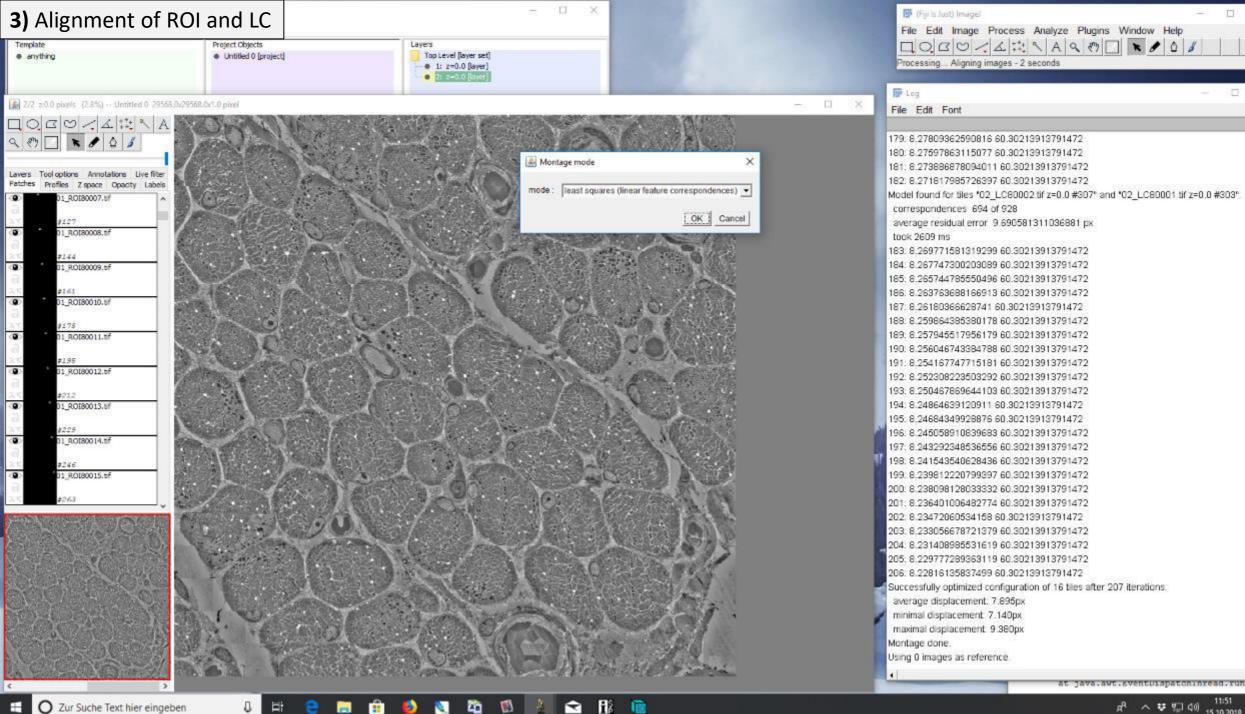
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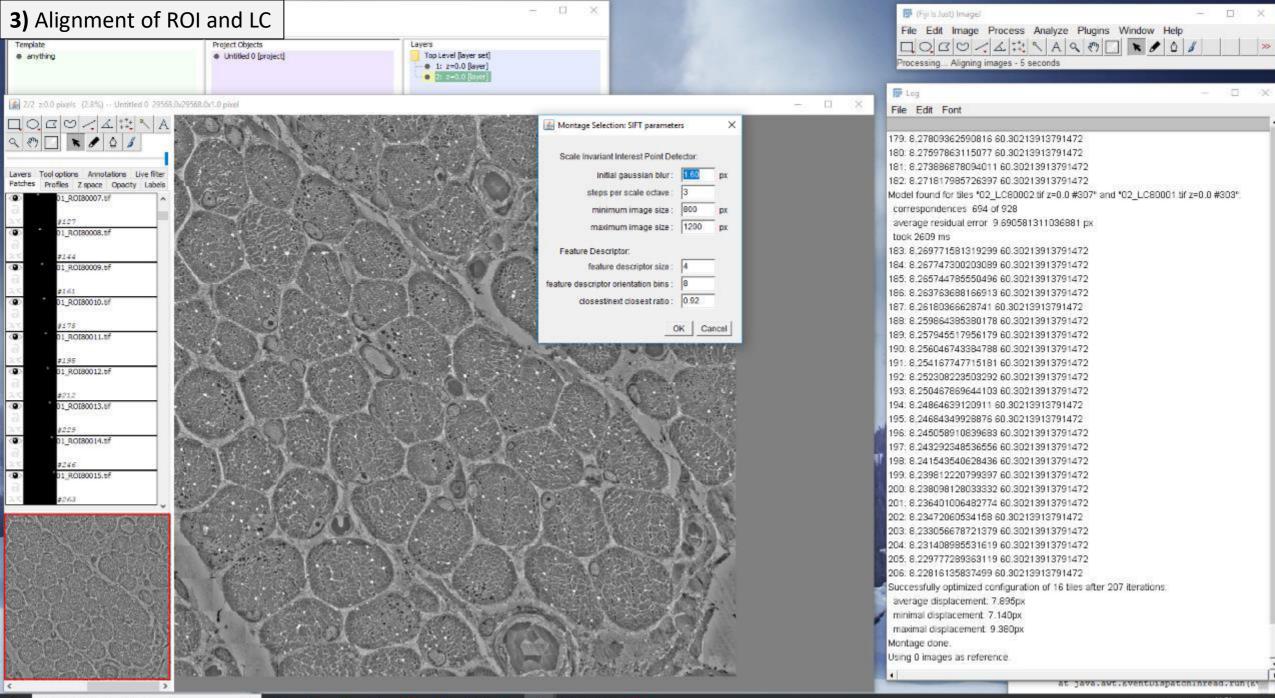
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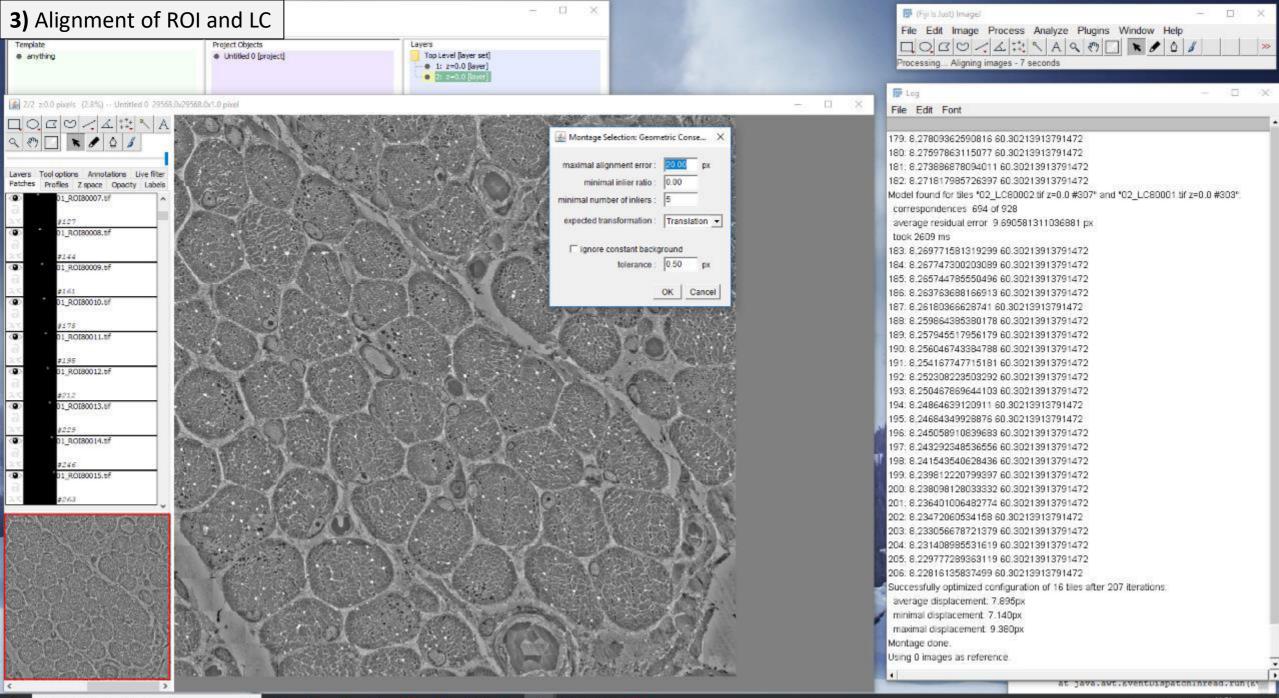
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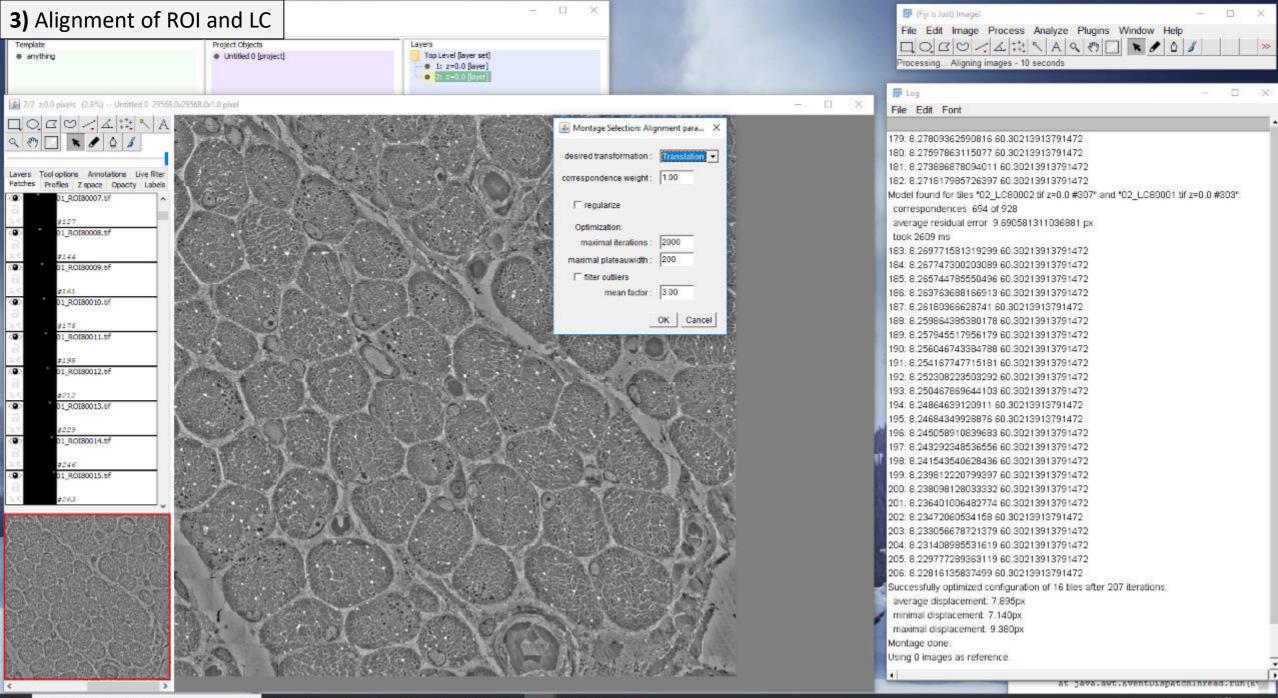
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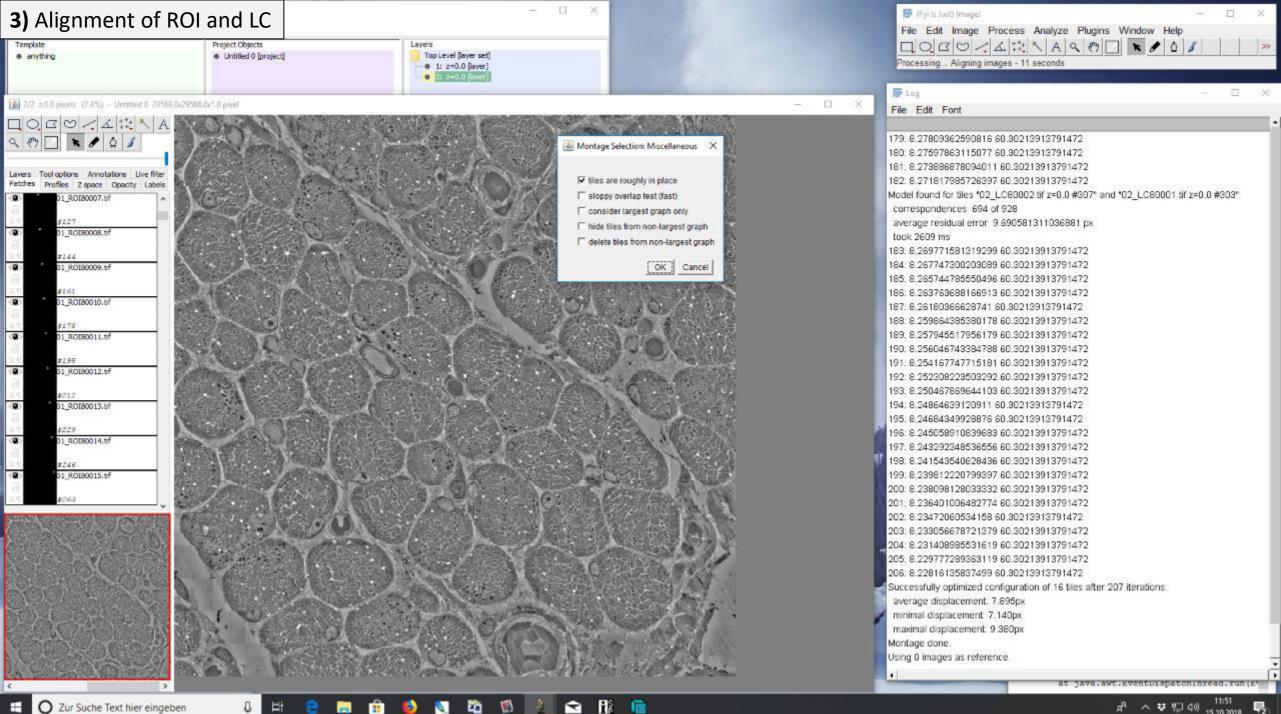
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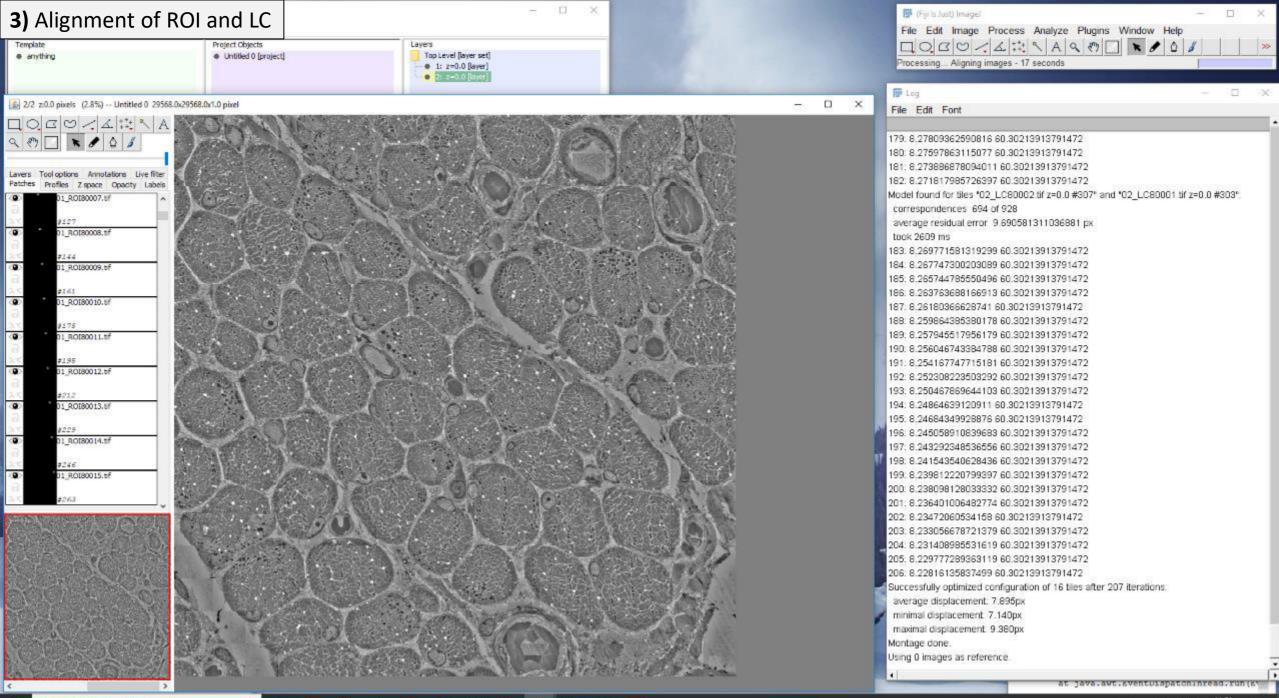
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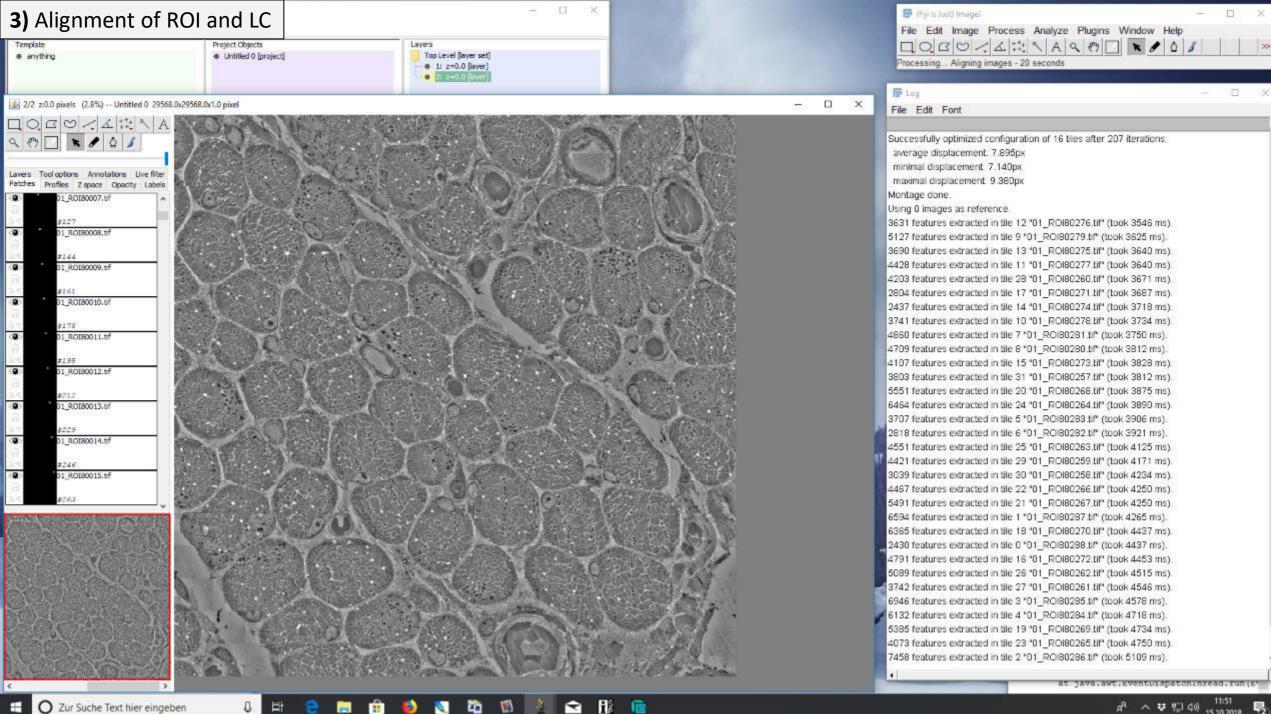
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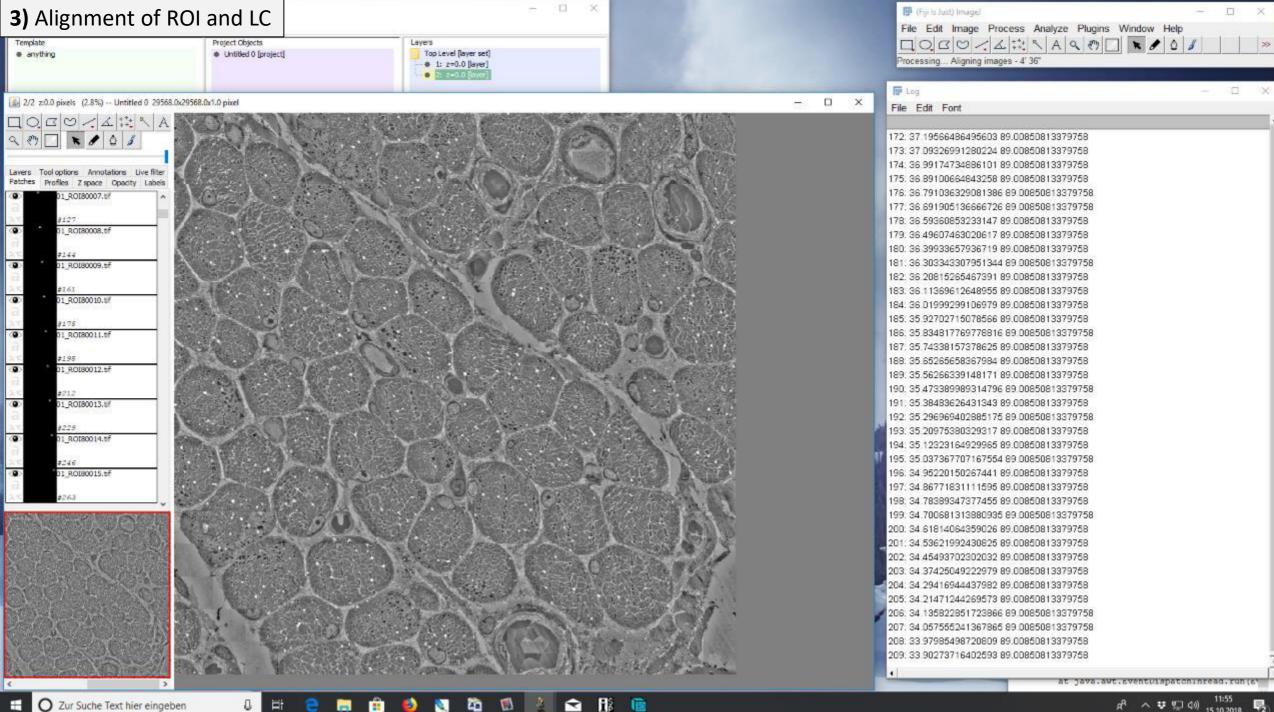


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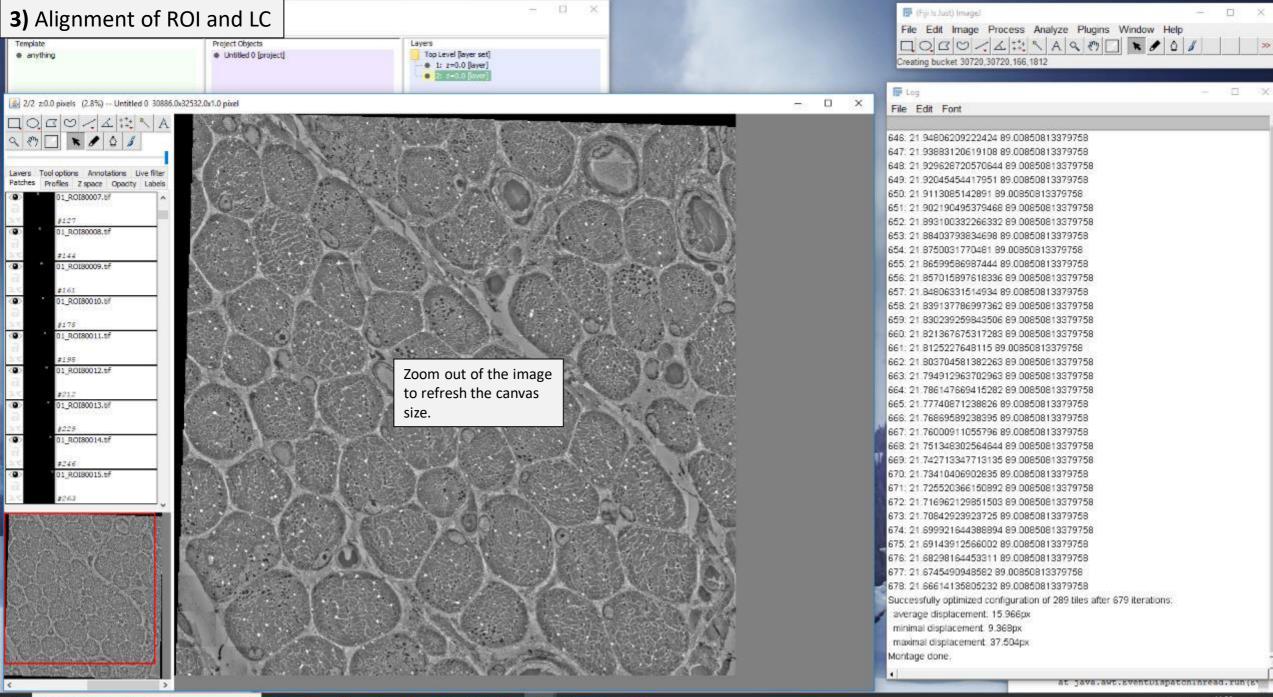
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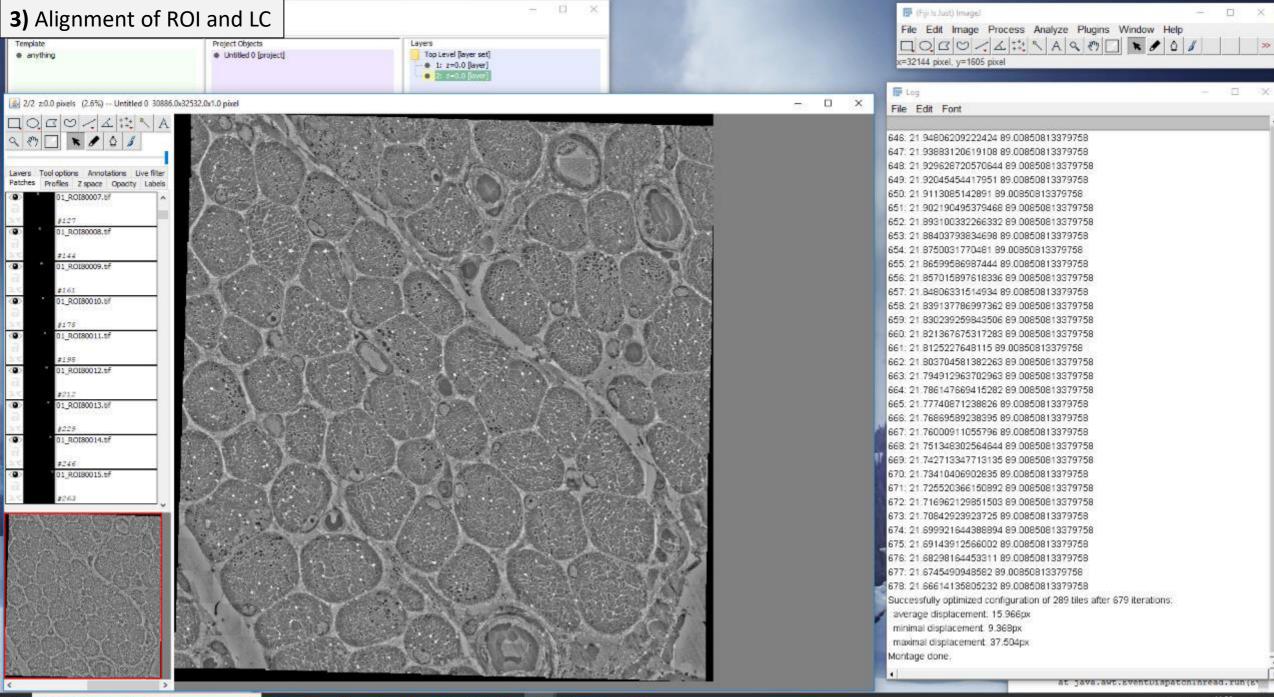
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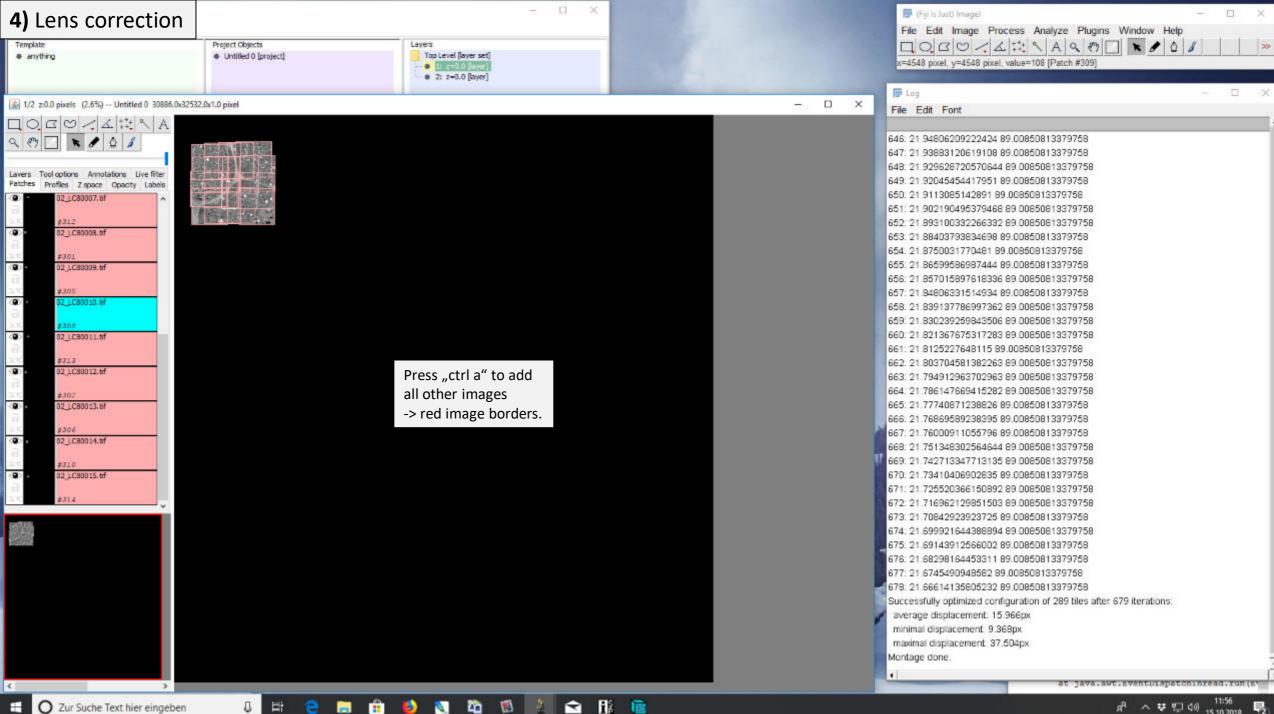
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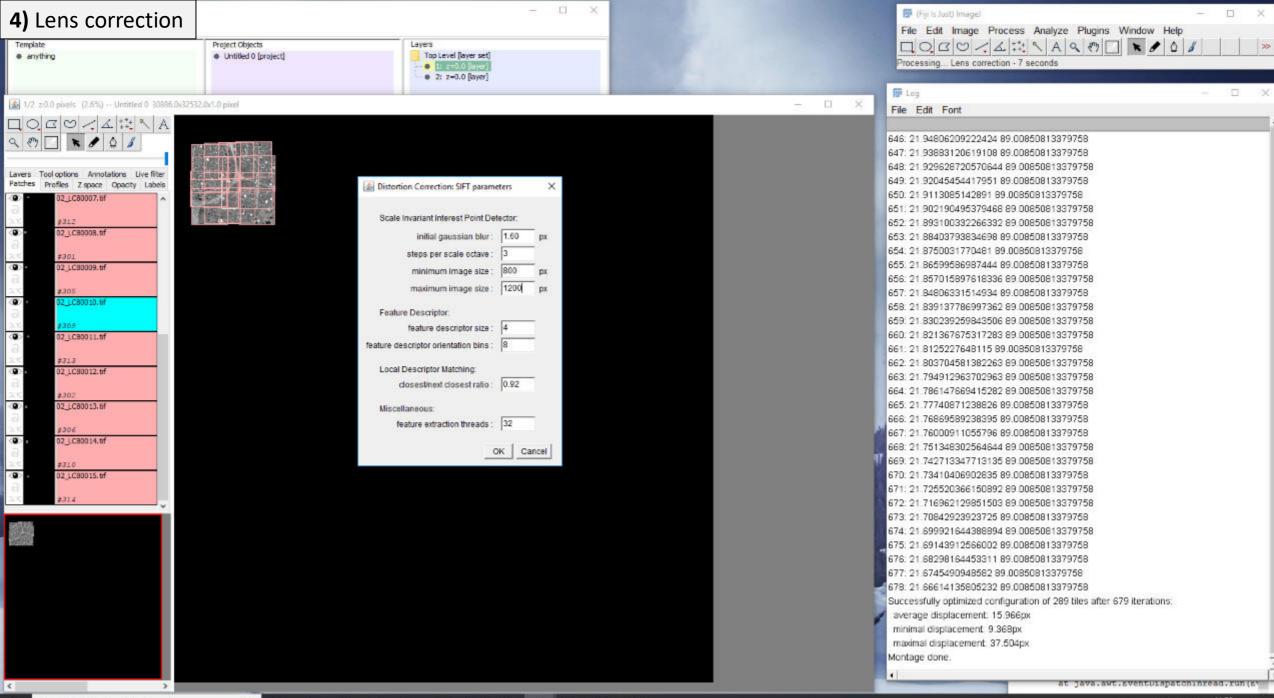
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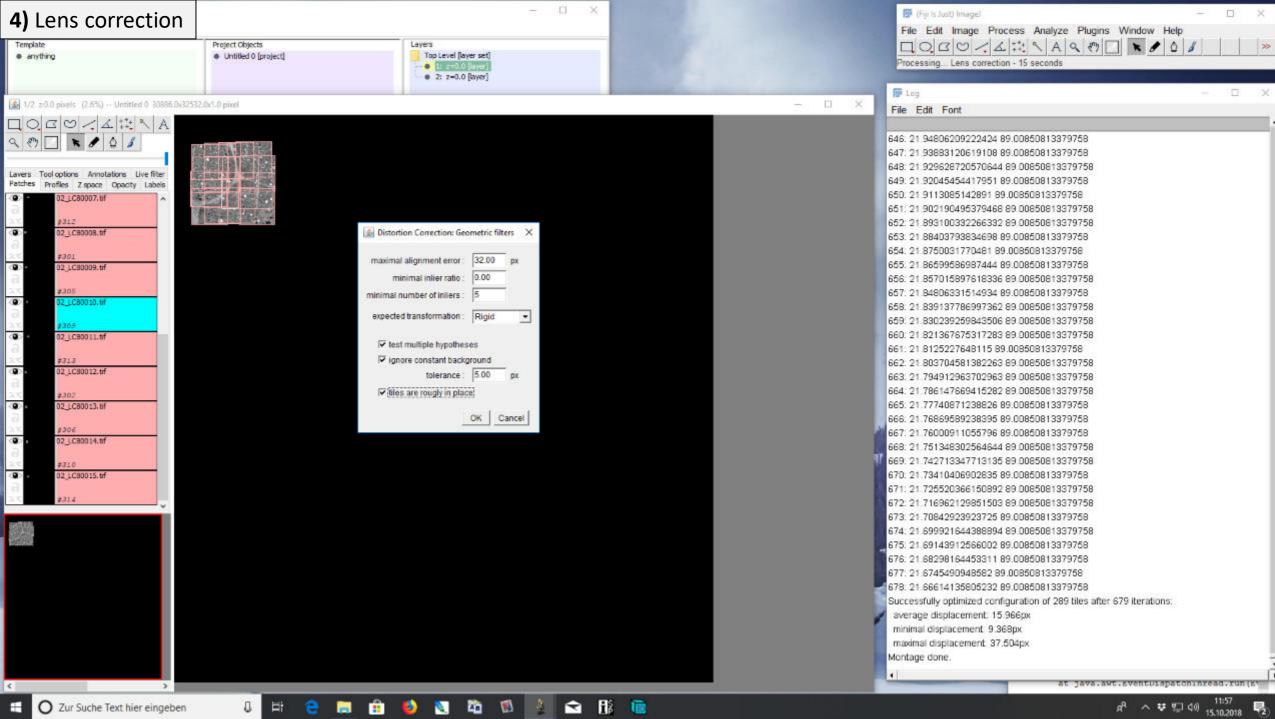
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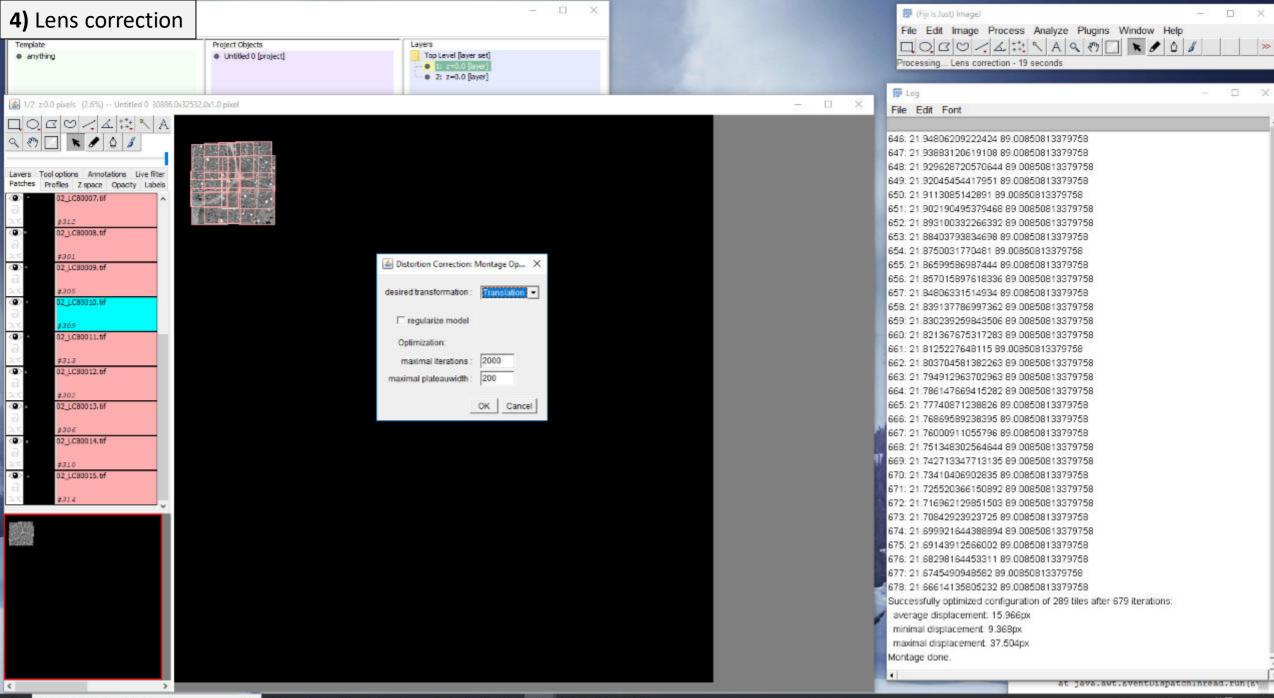
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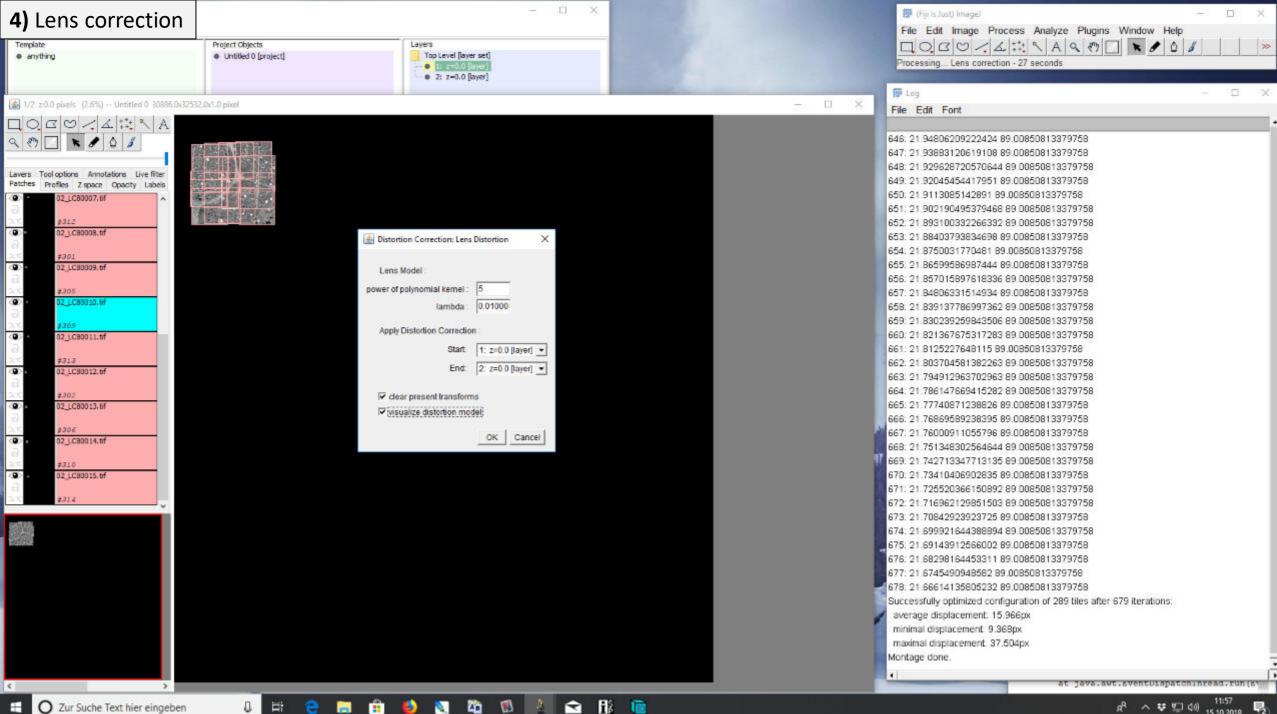
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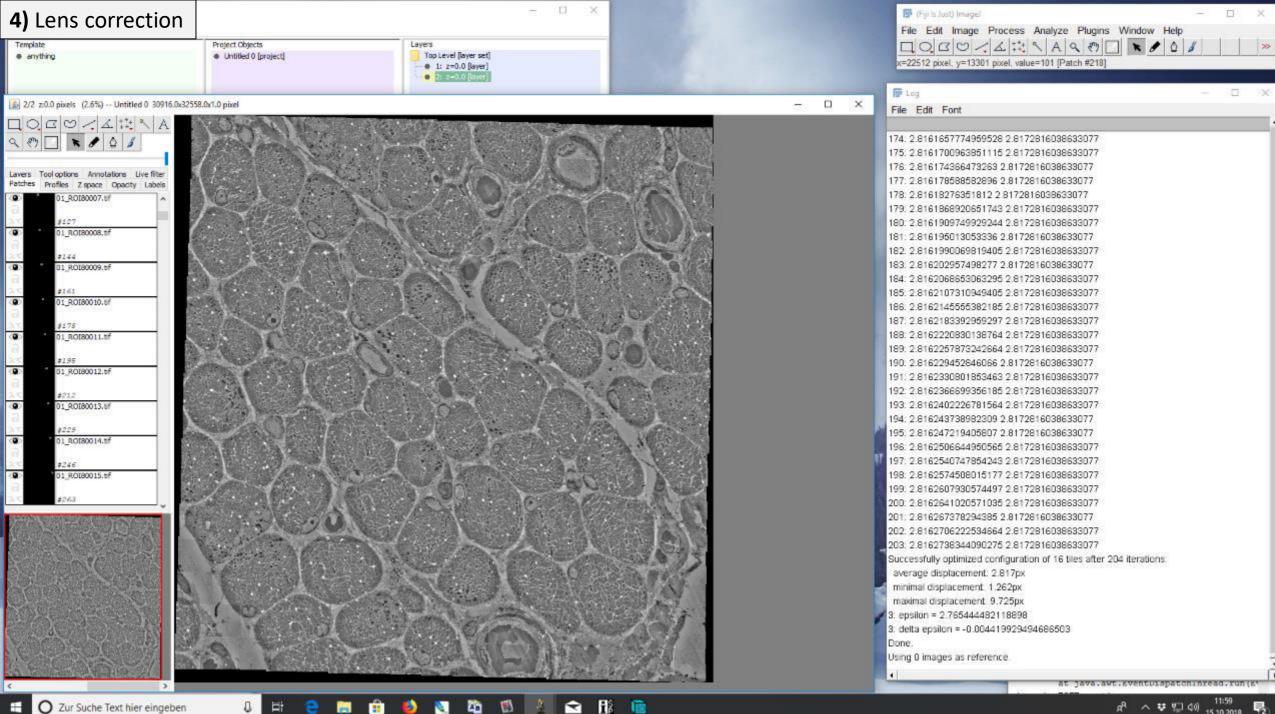


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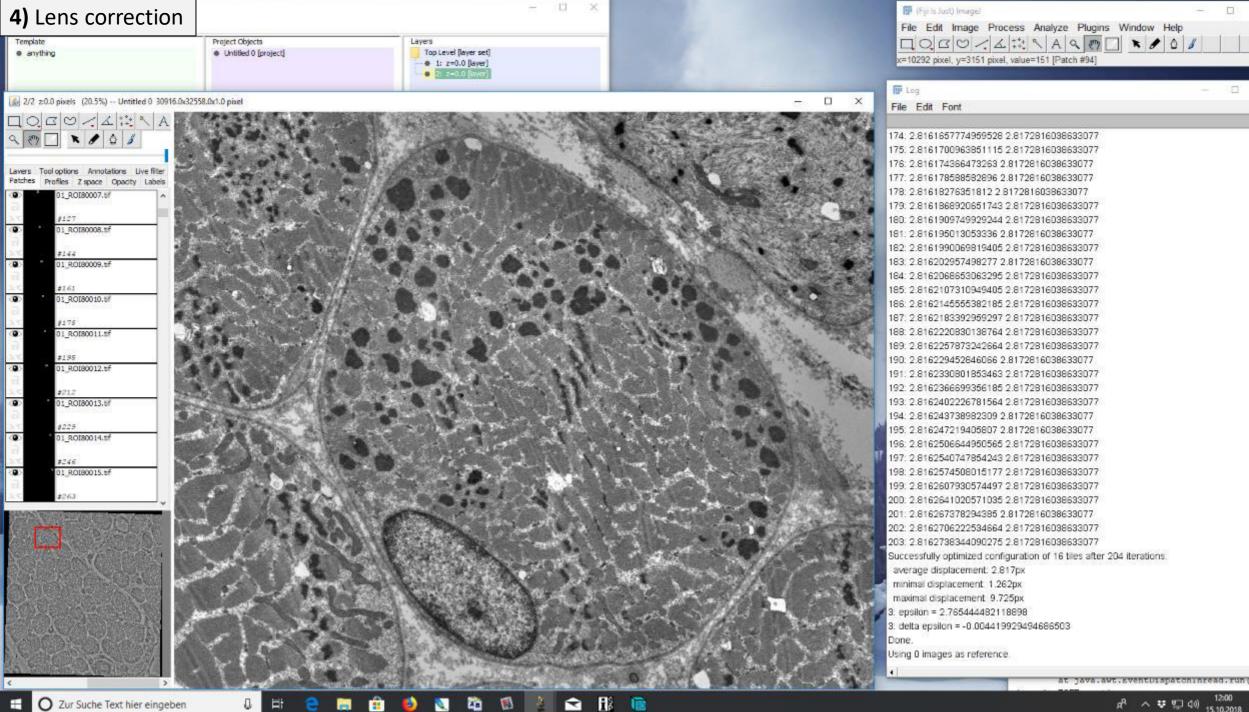
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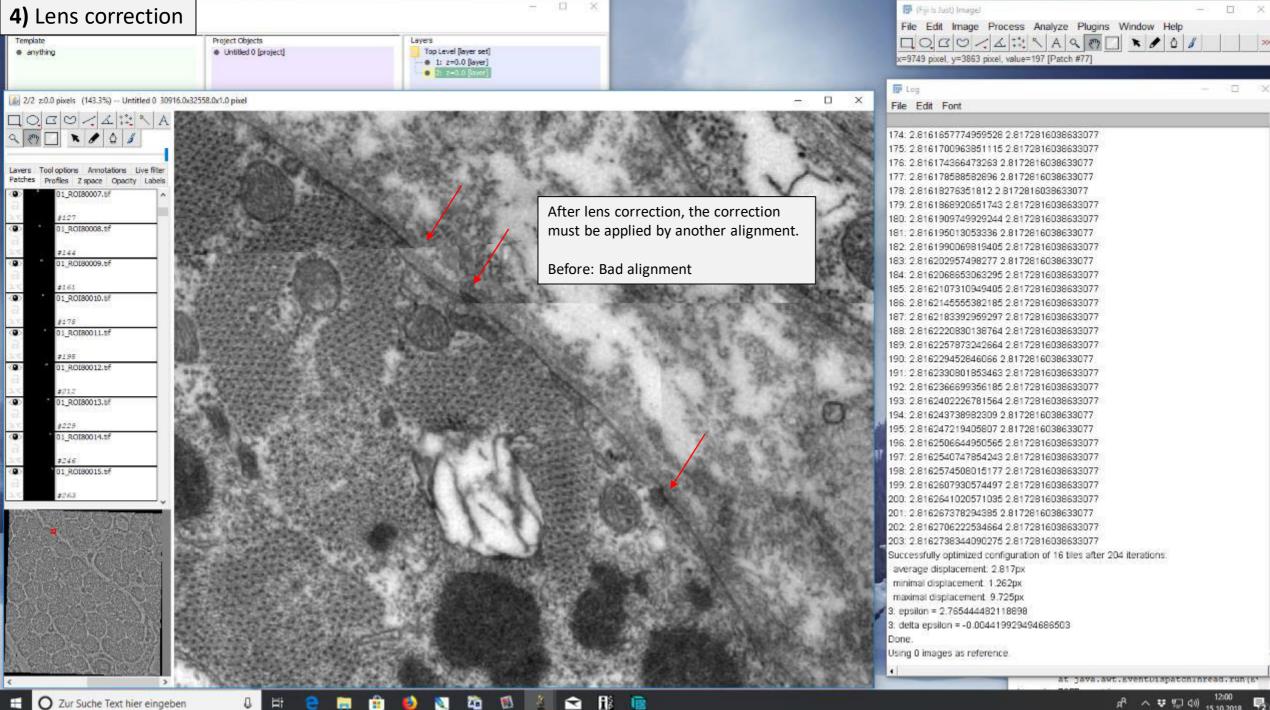
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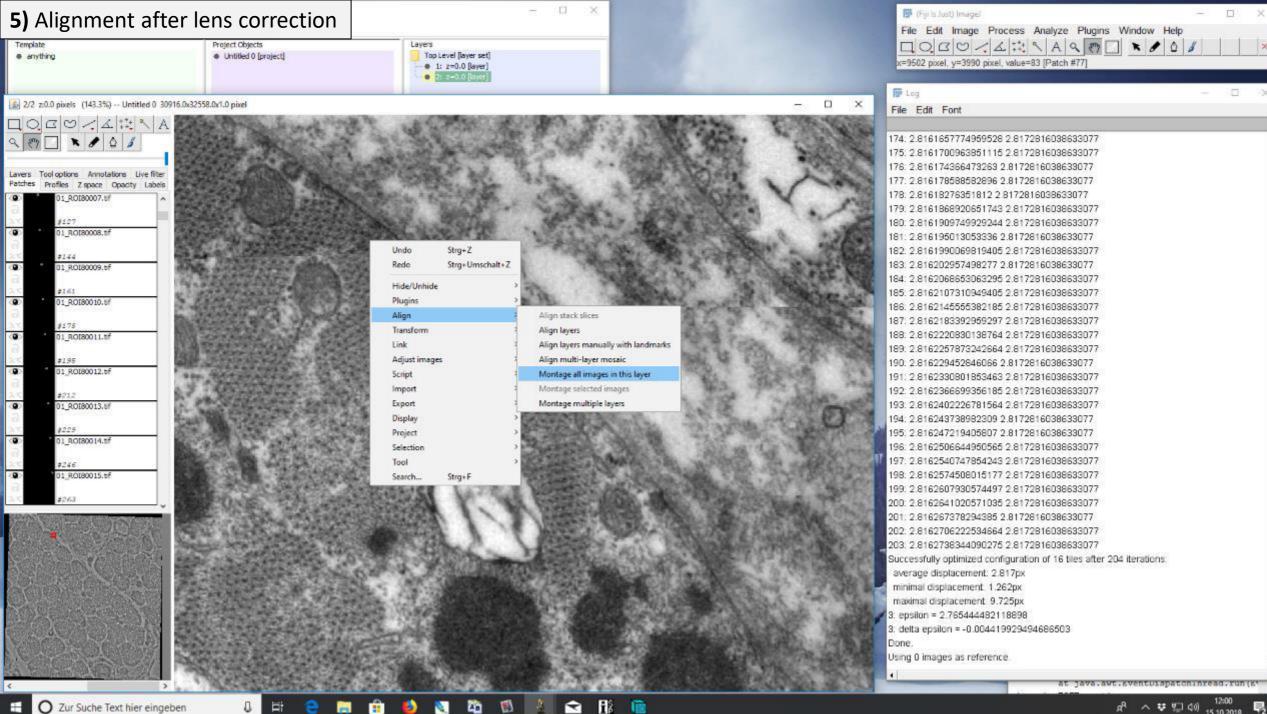
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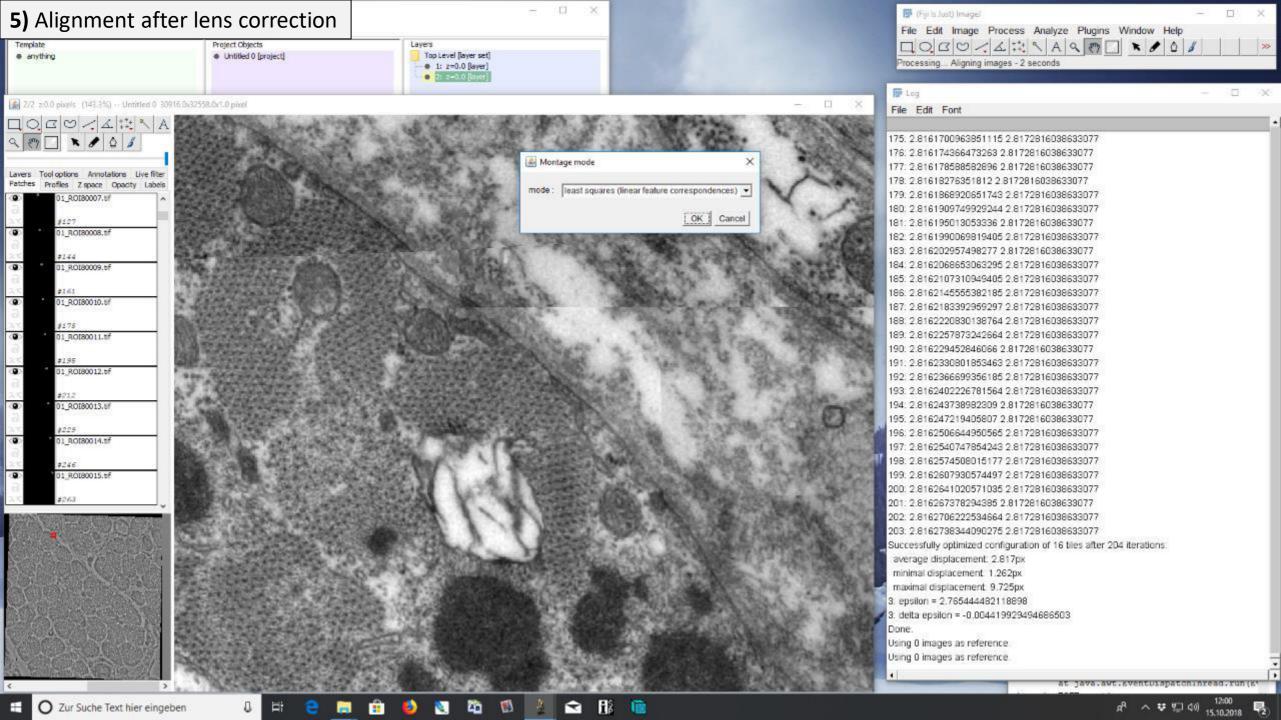


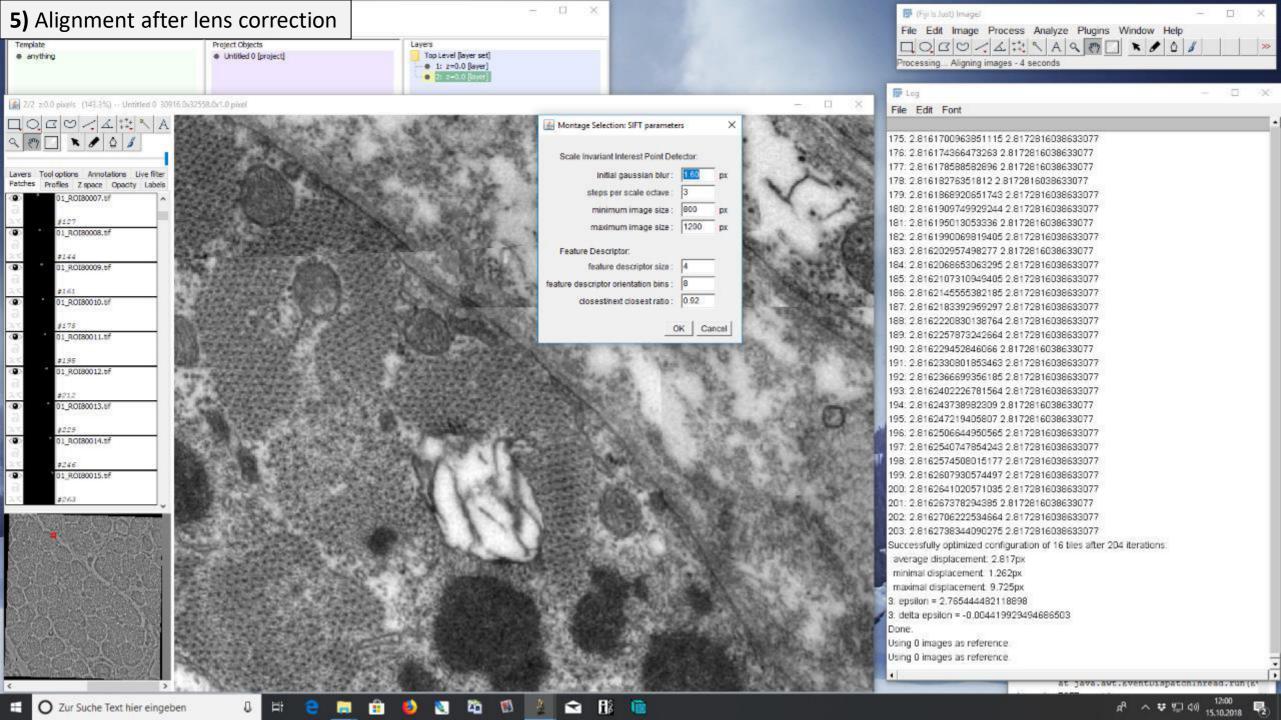


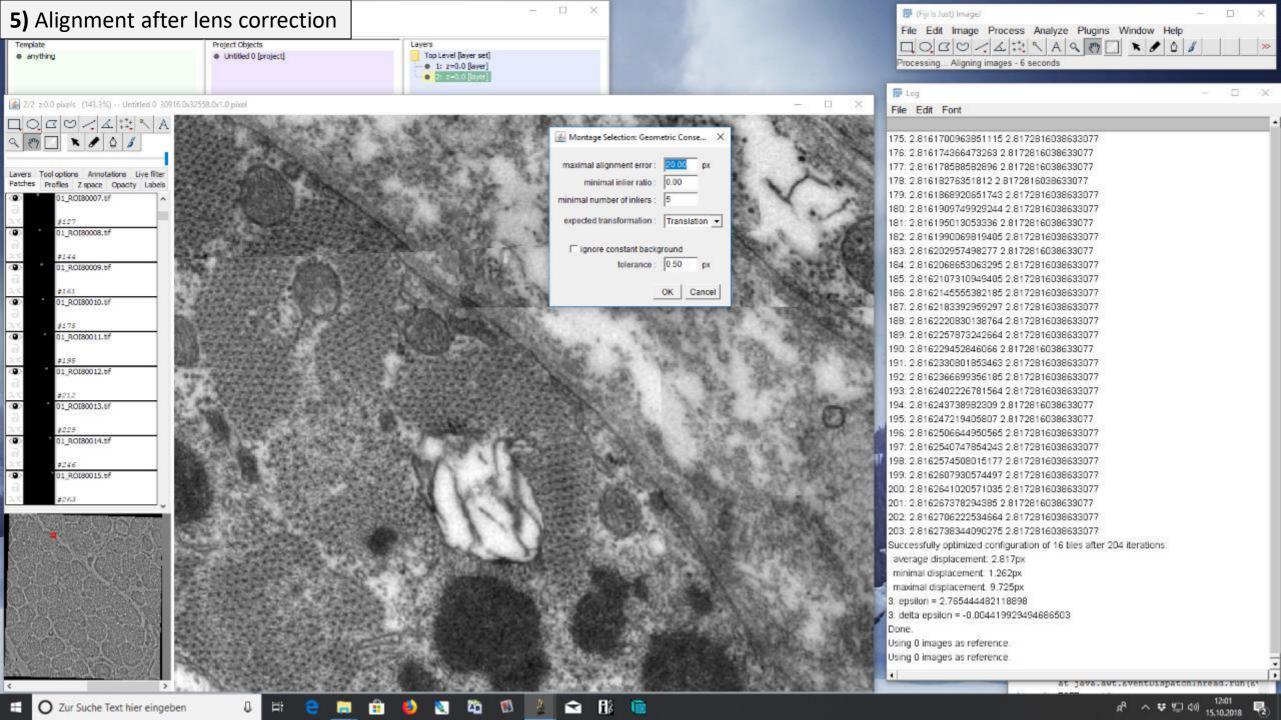
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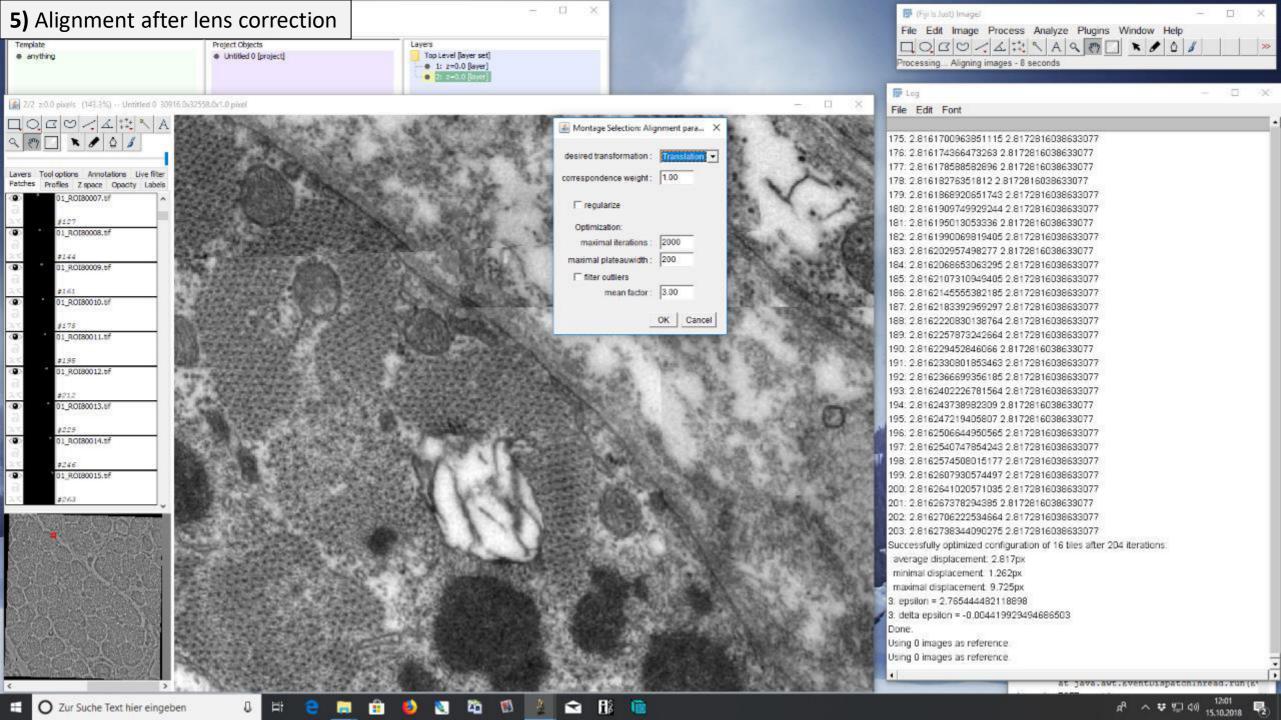
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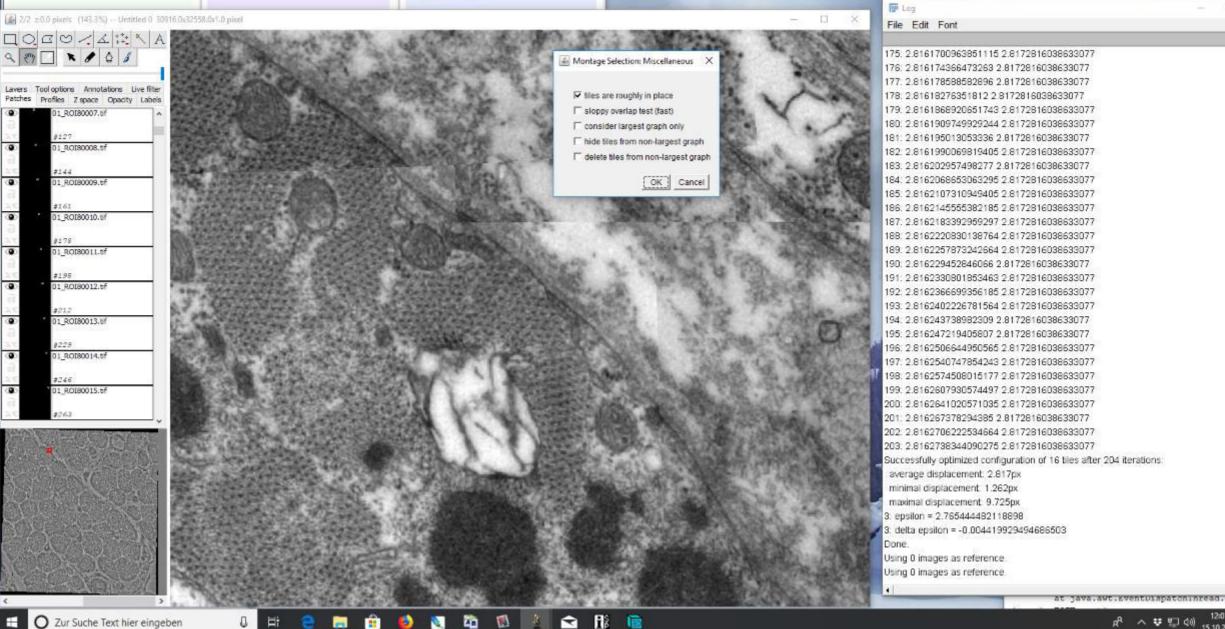




5) Alignment after lens correction

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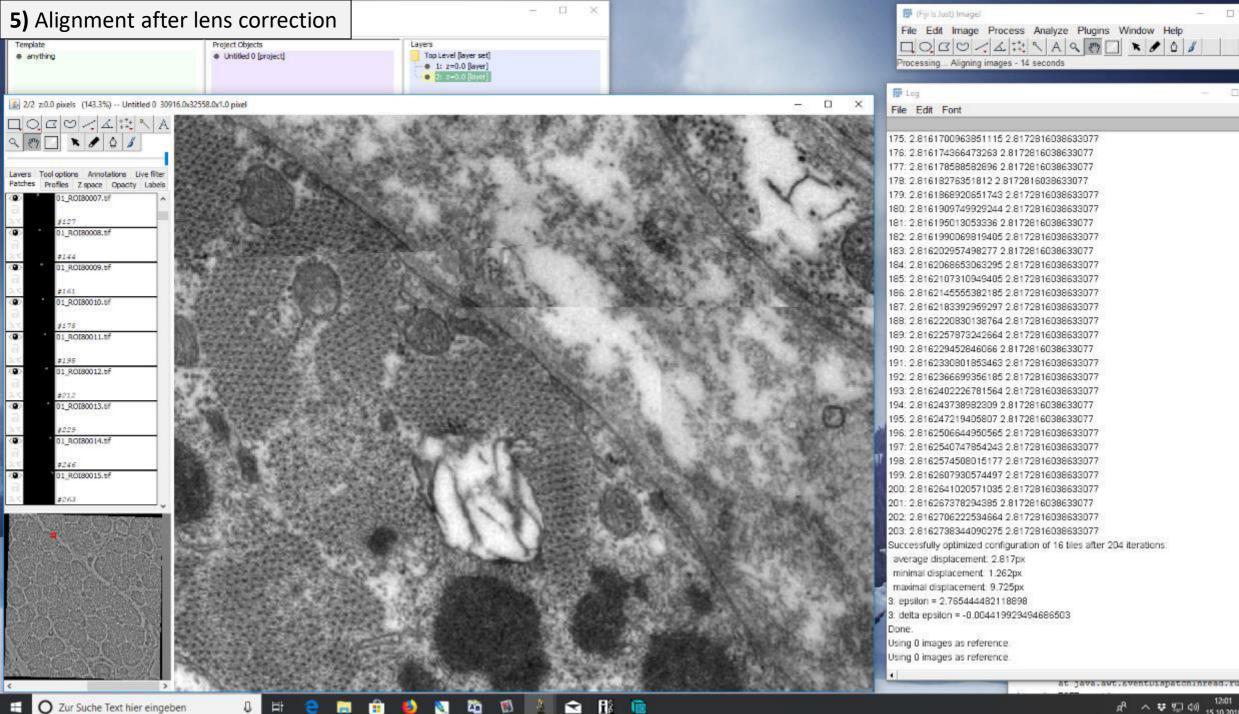
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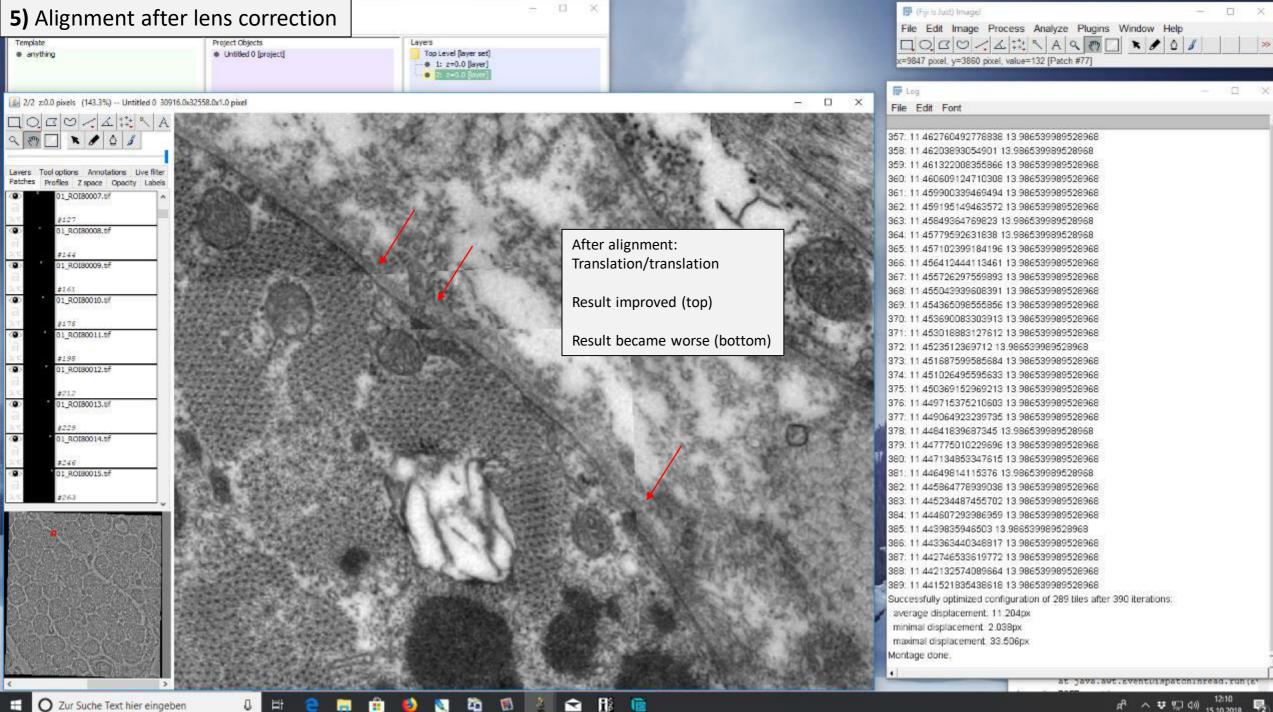
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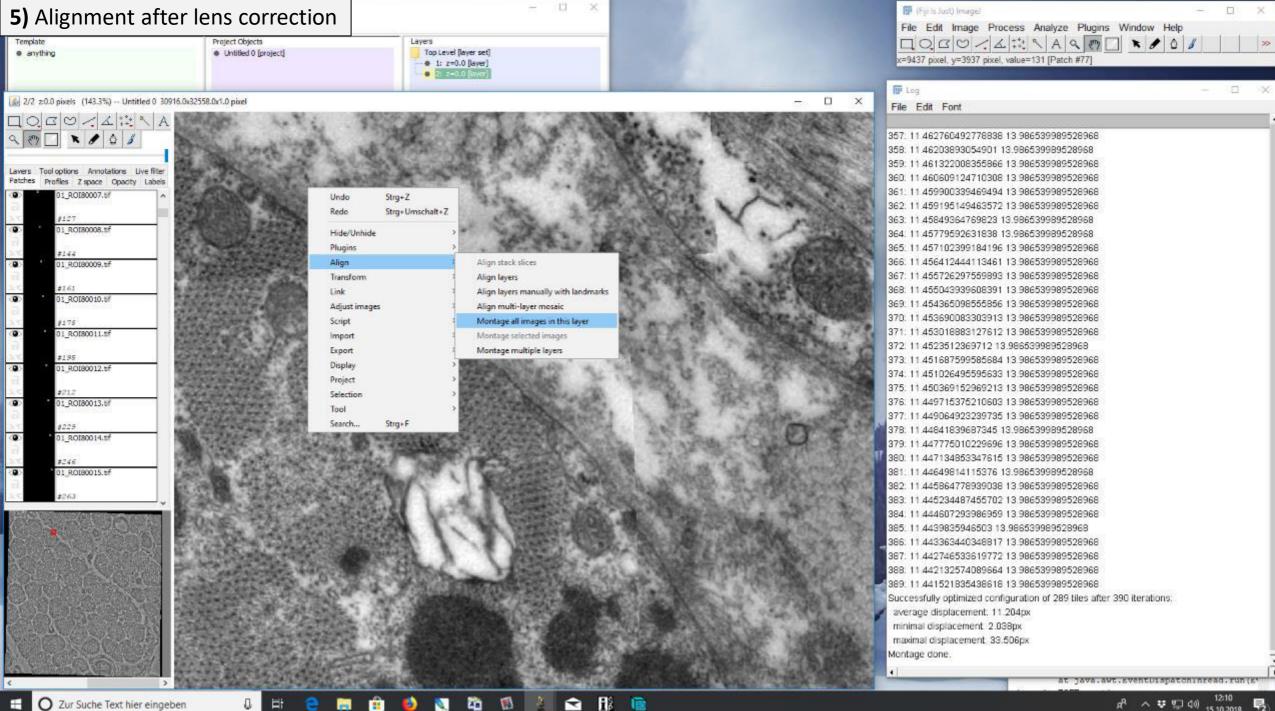
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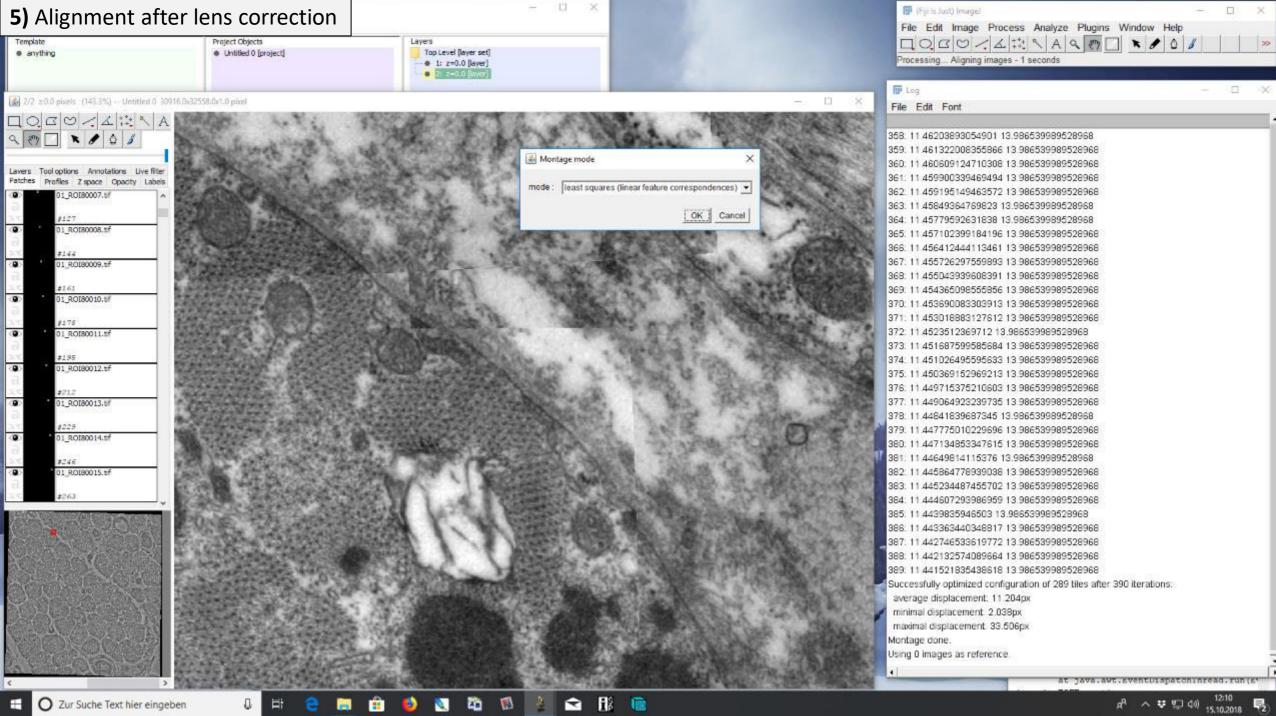
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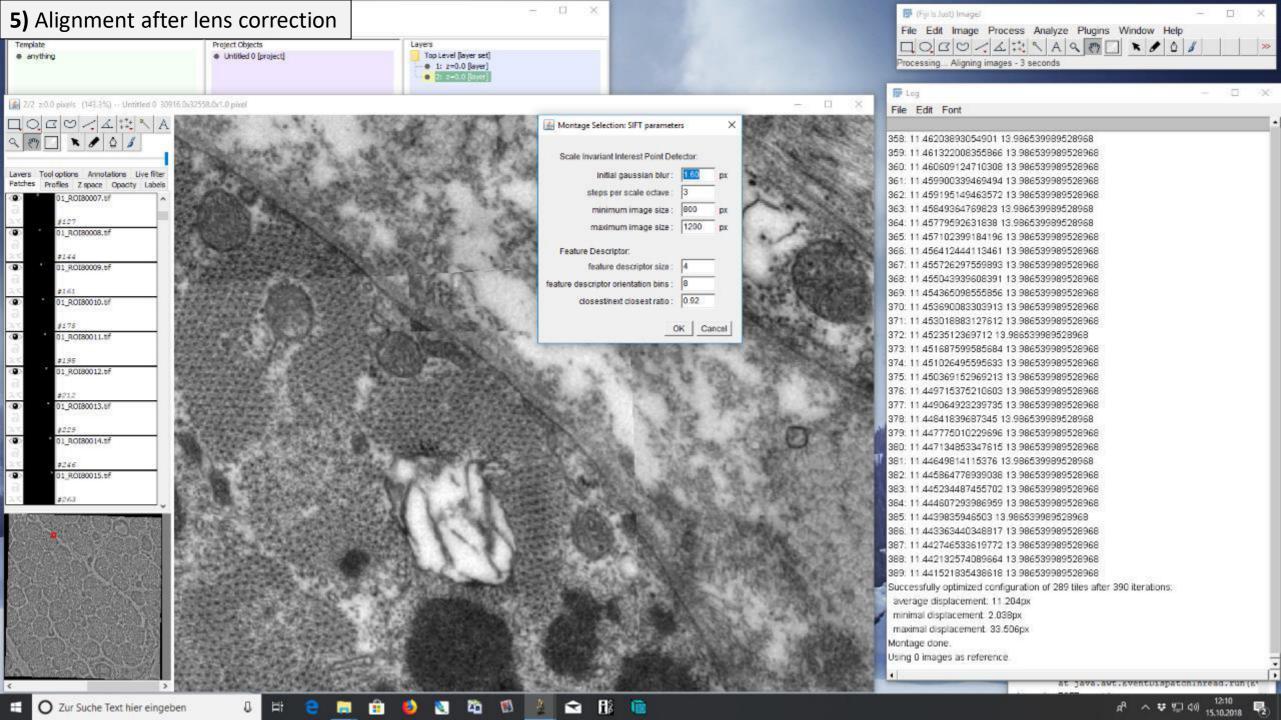


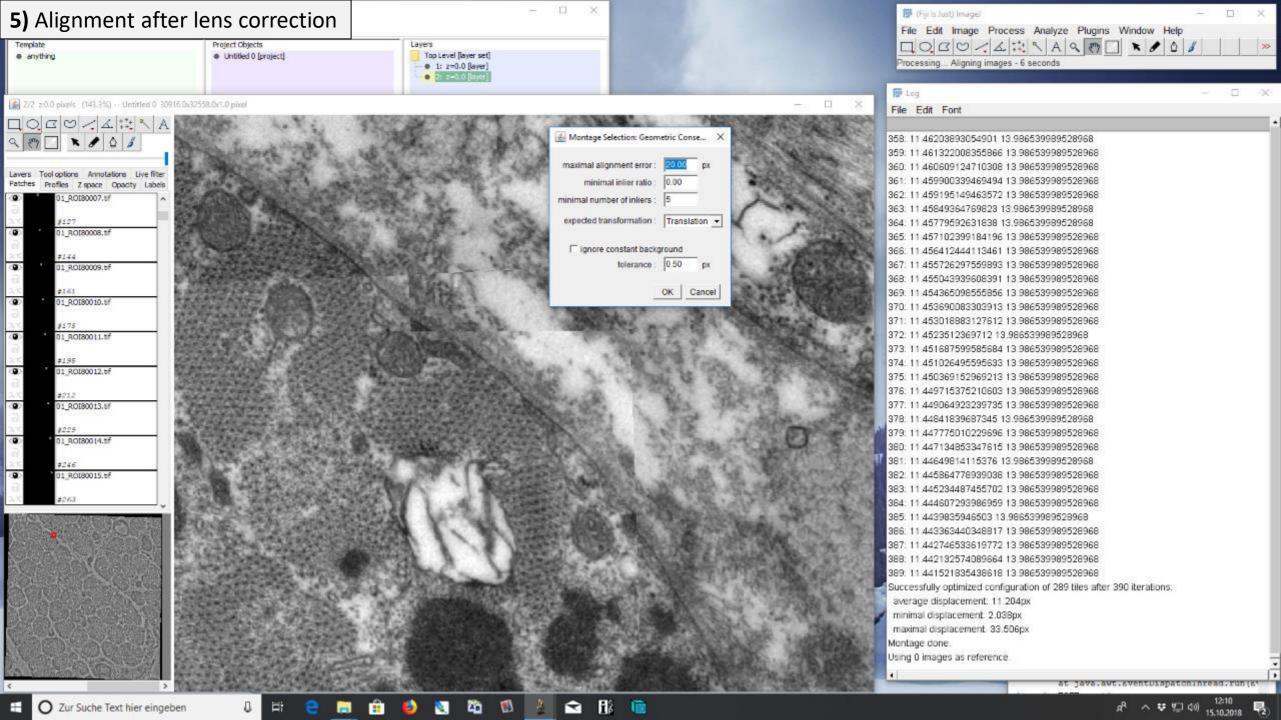
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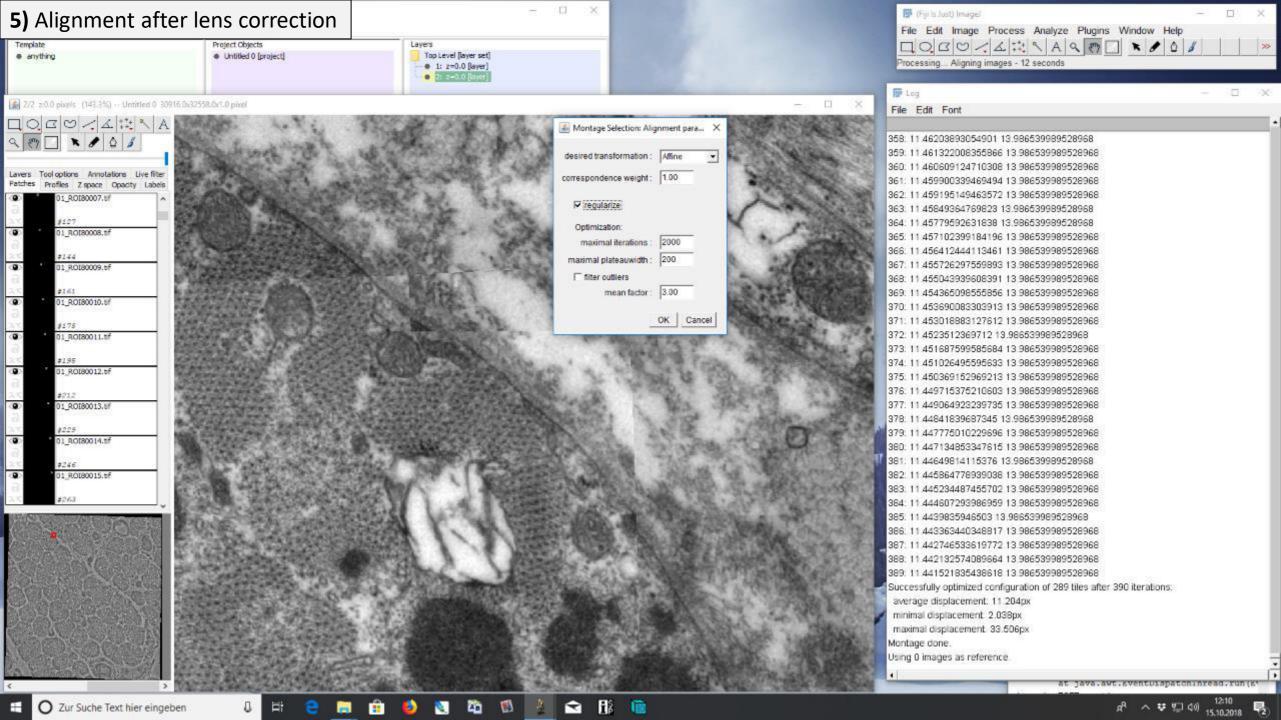


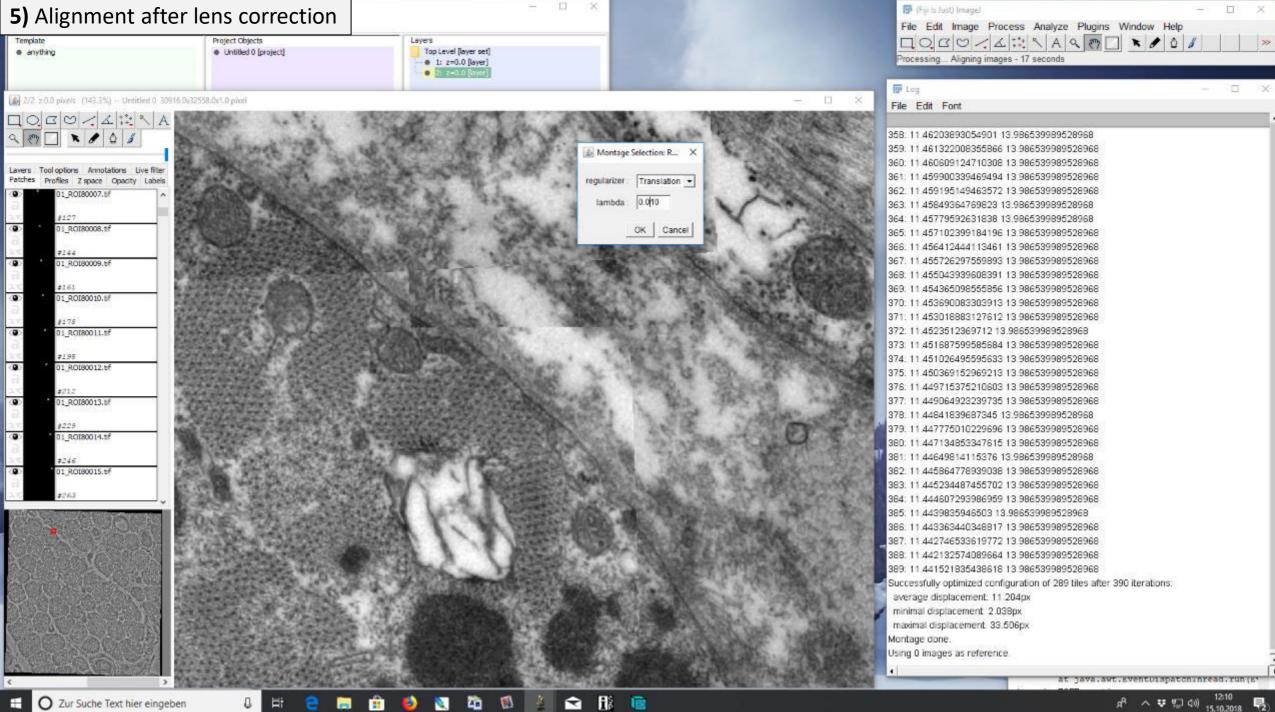


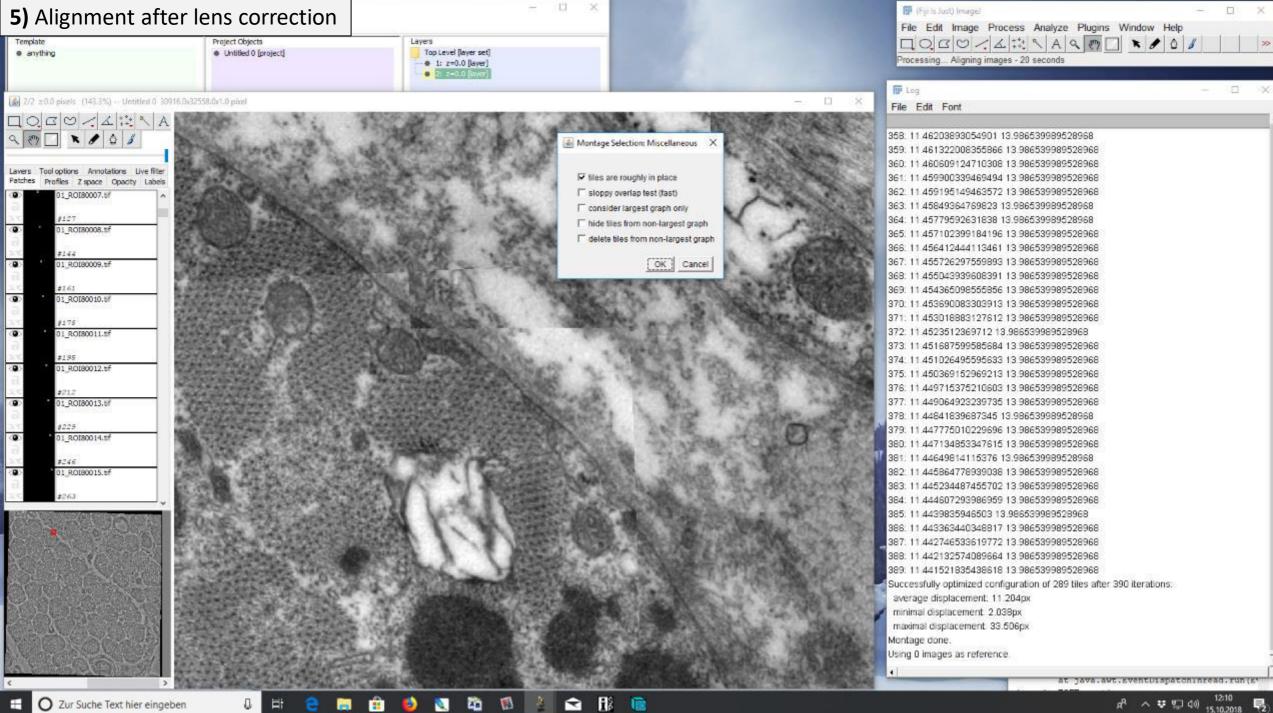


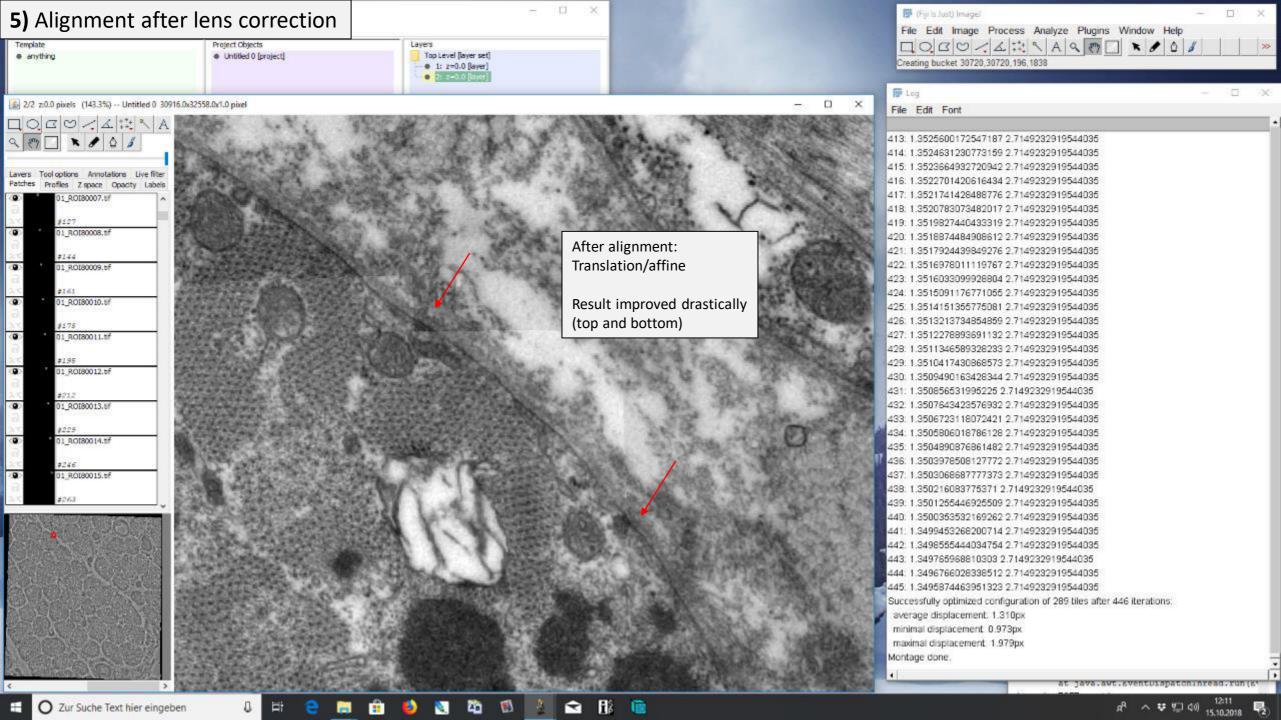


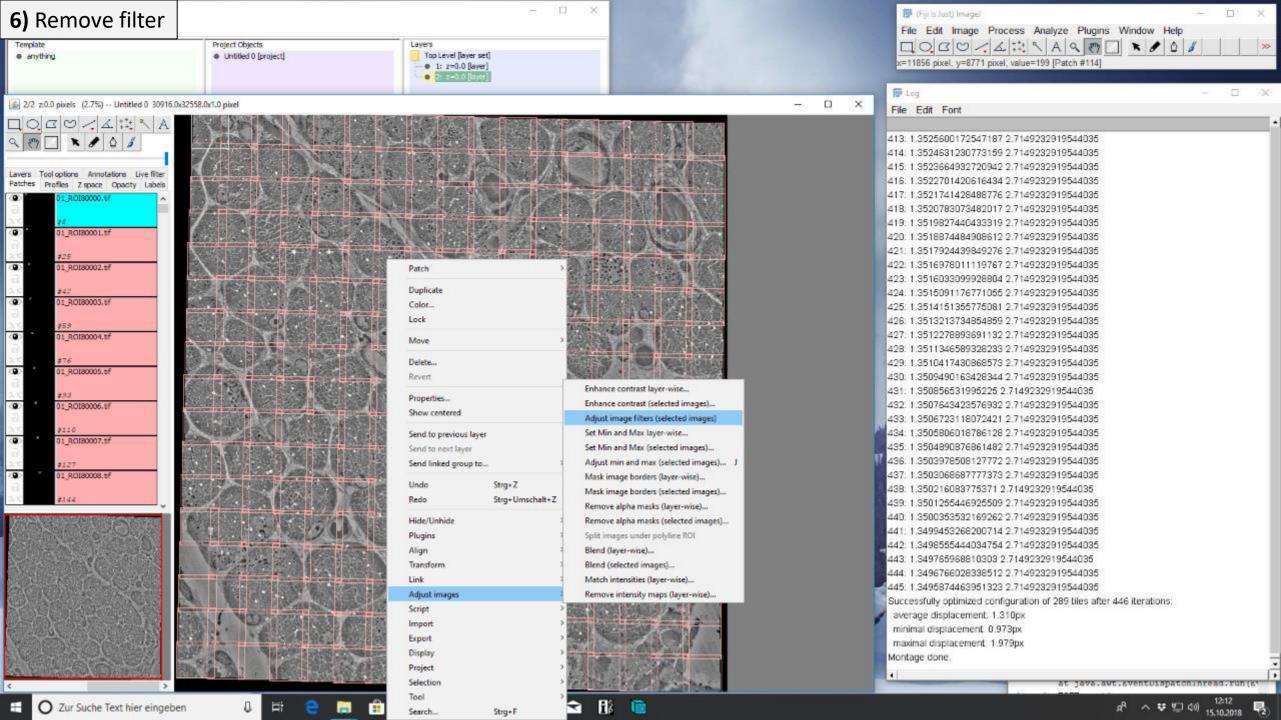


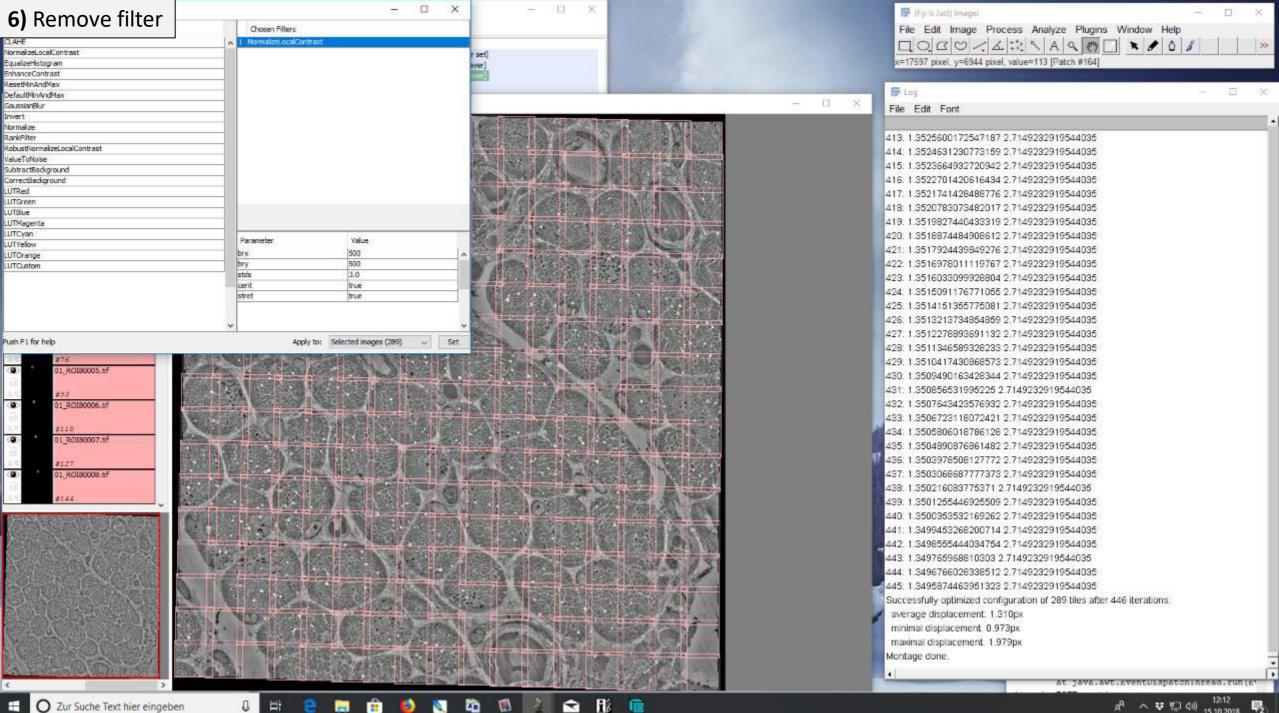




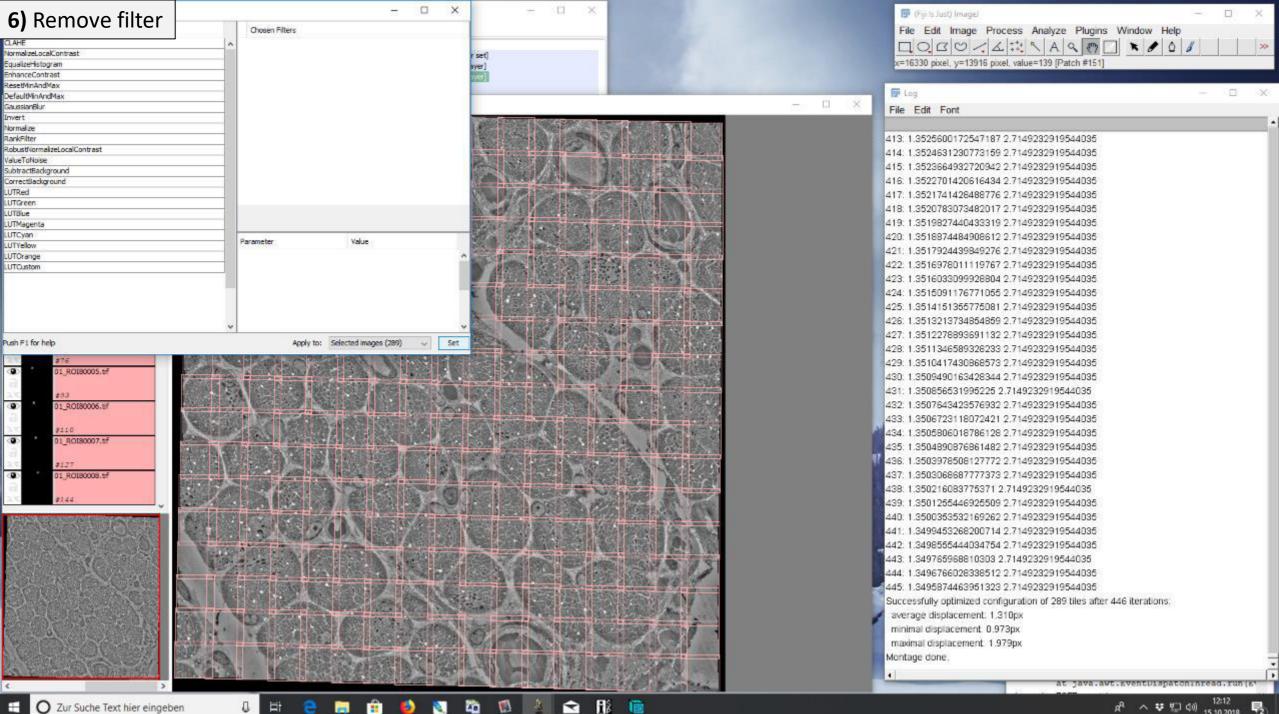




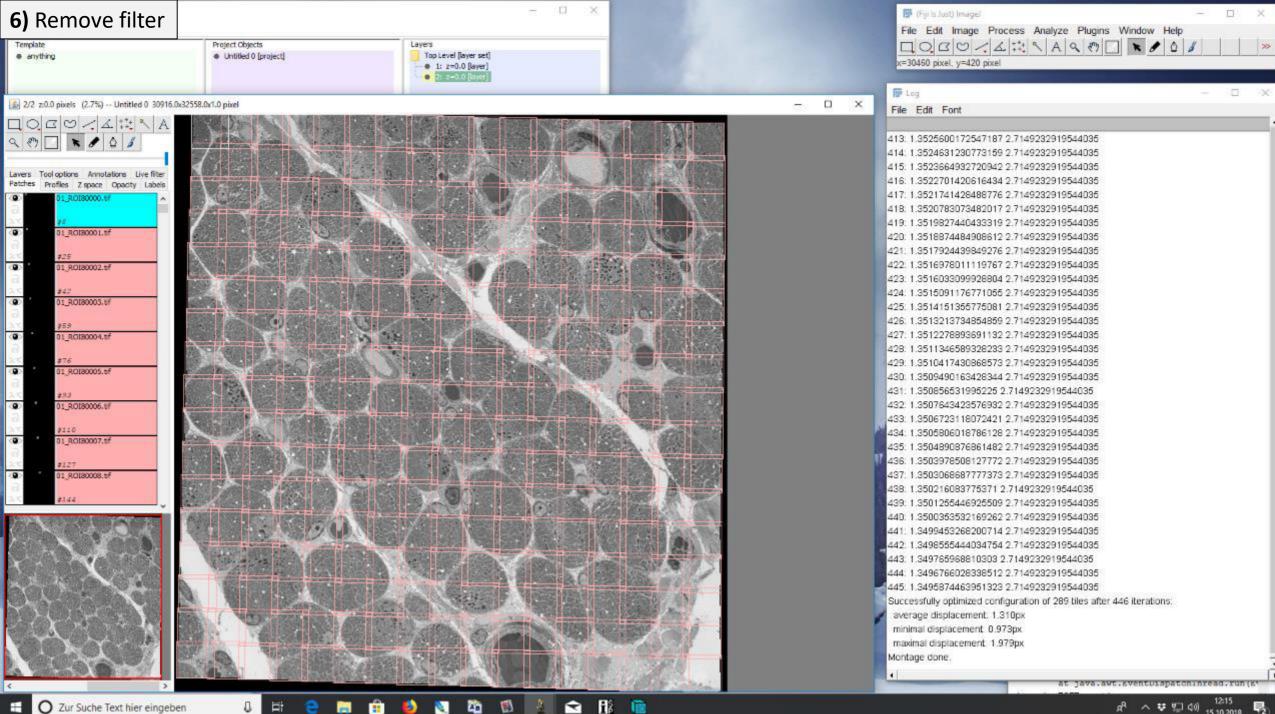


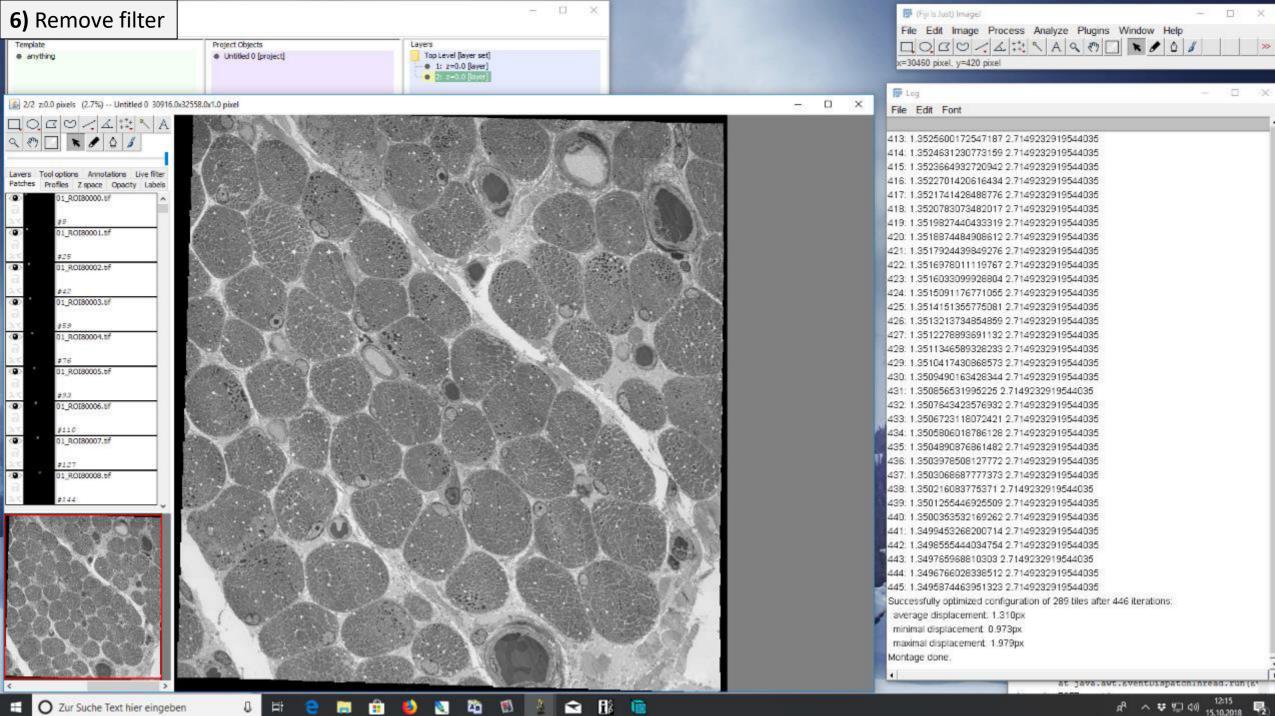


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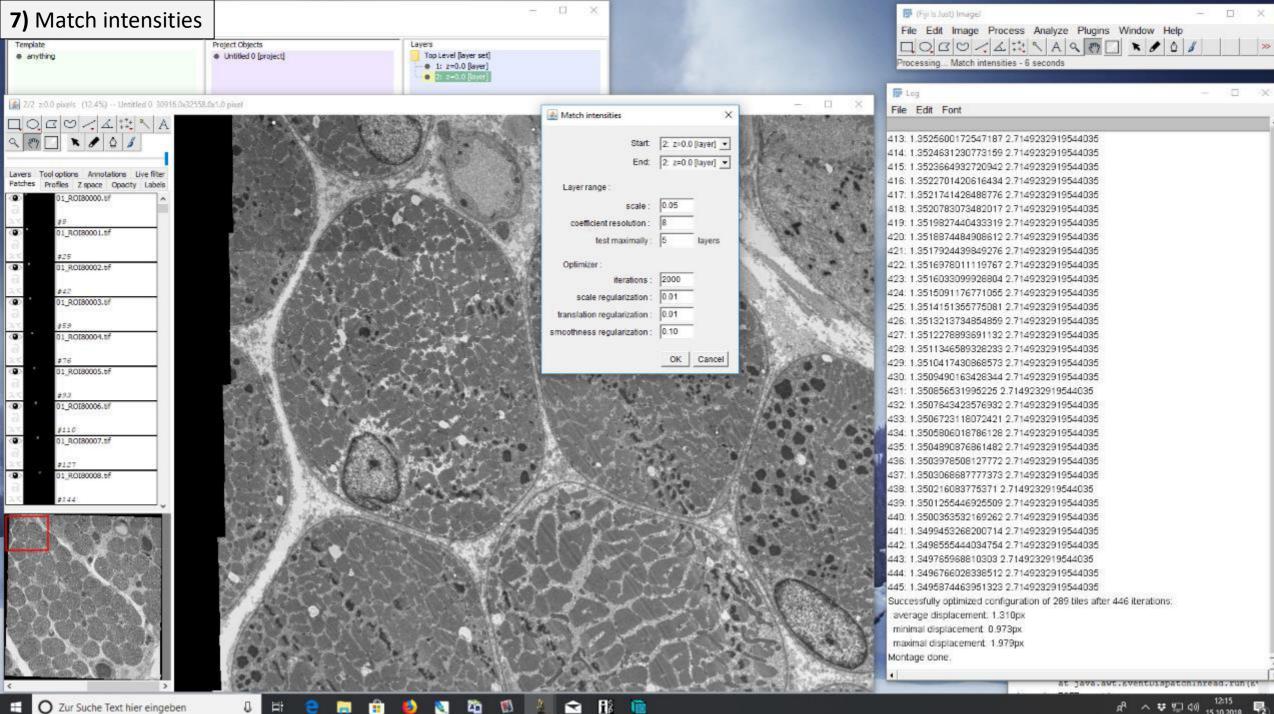
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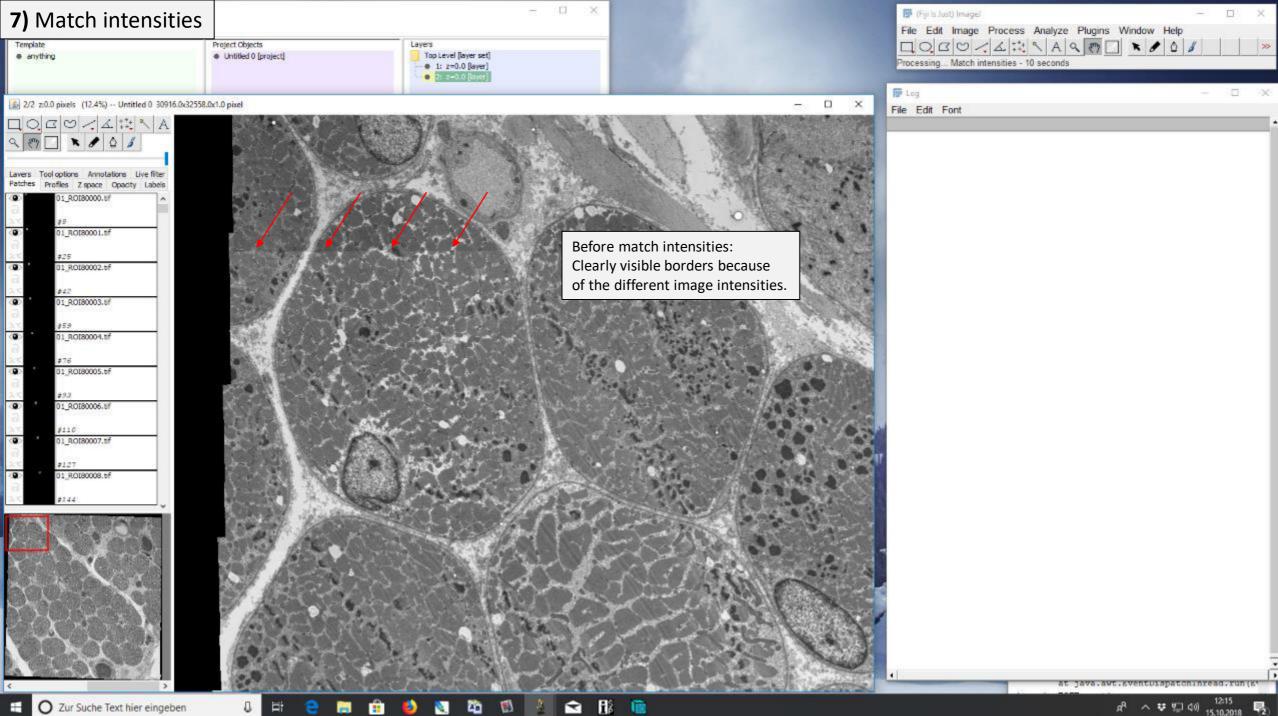
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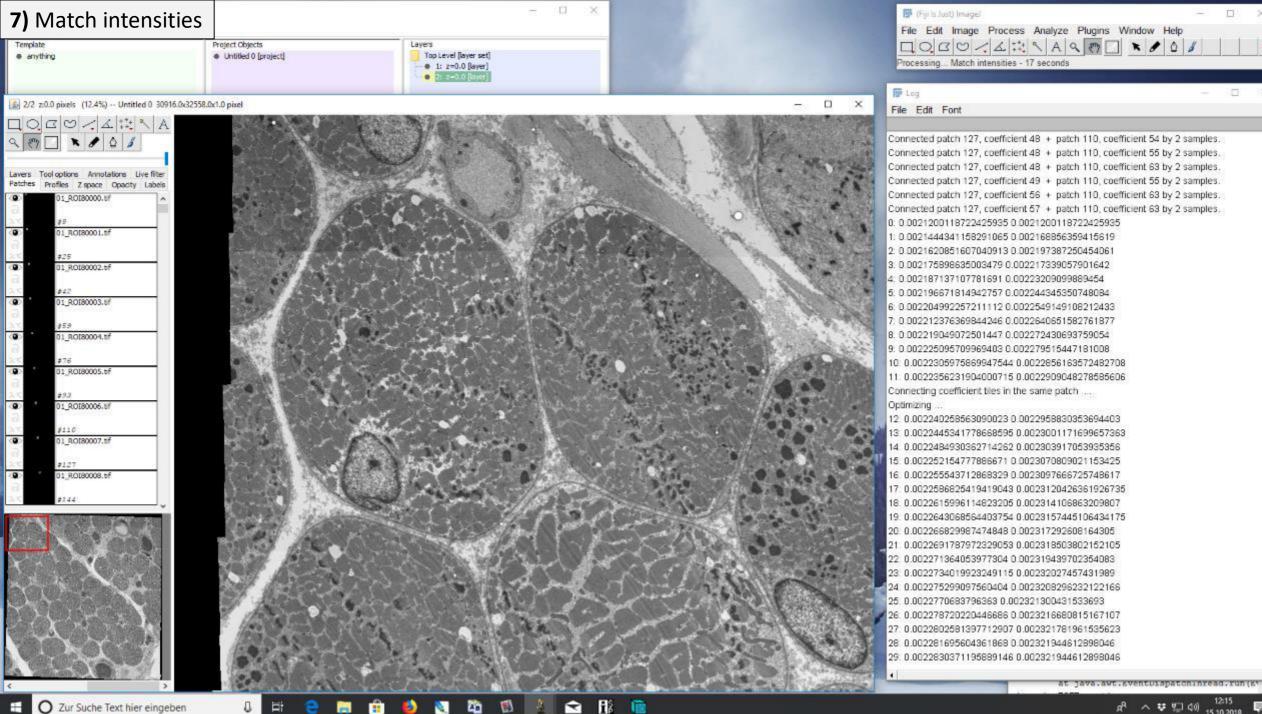
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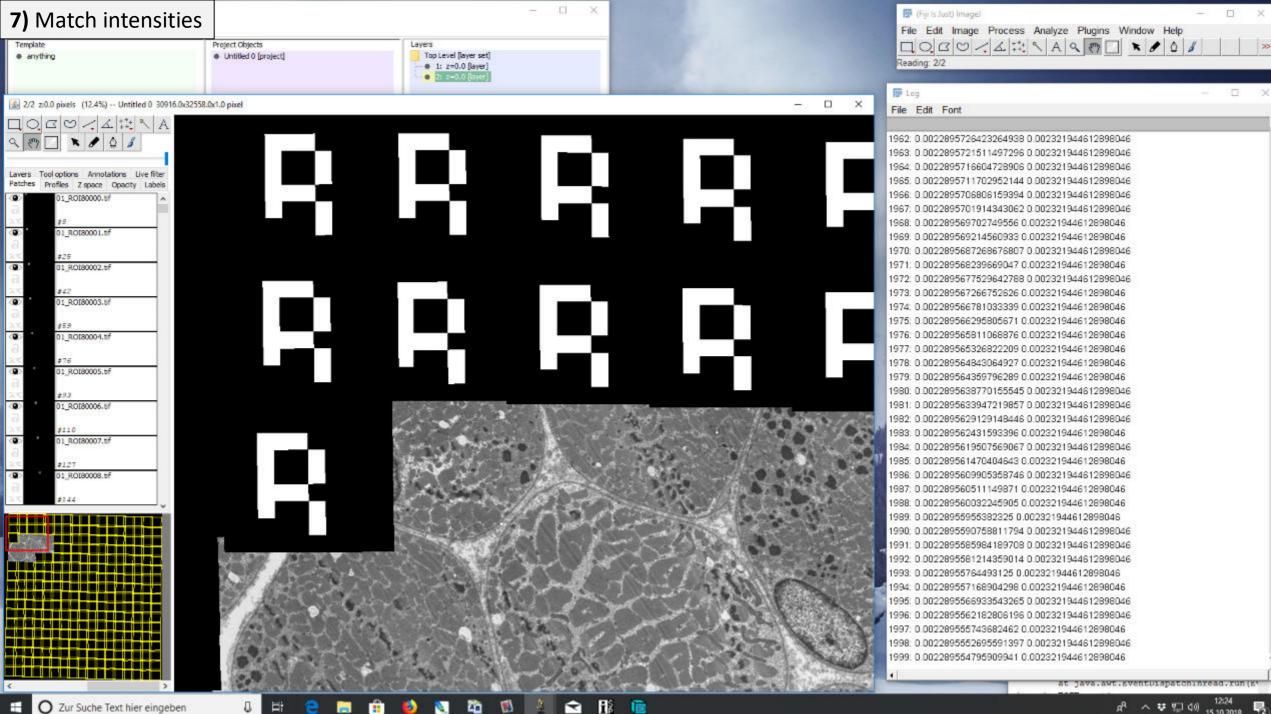


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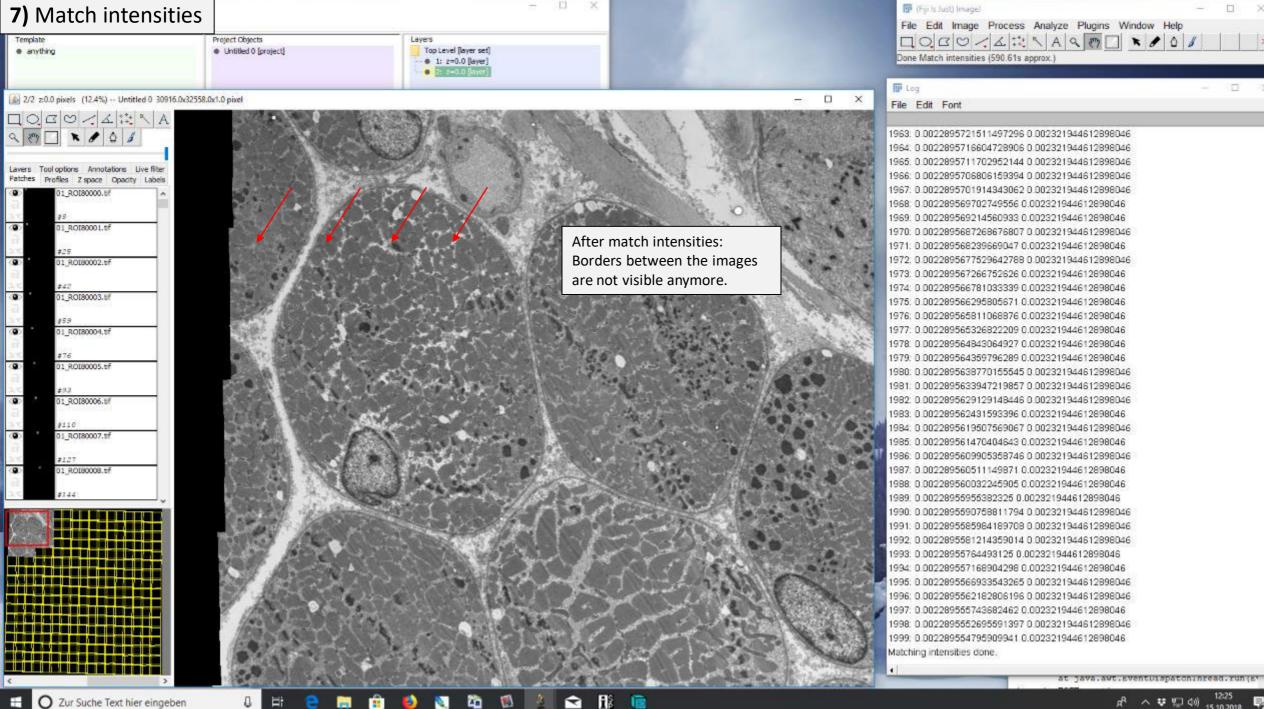


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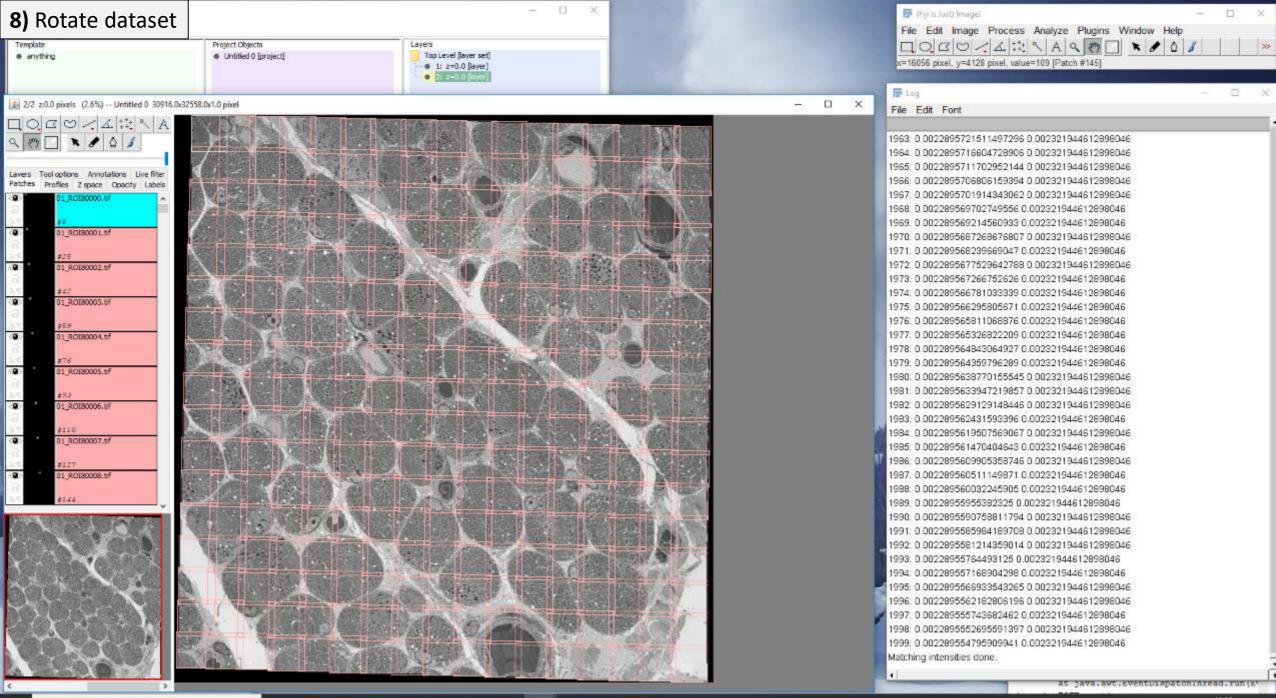
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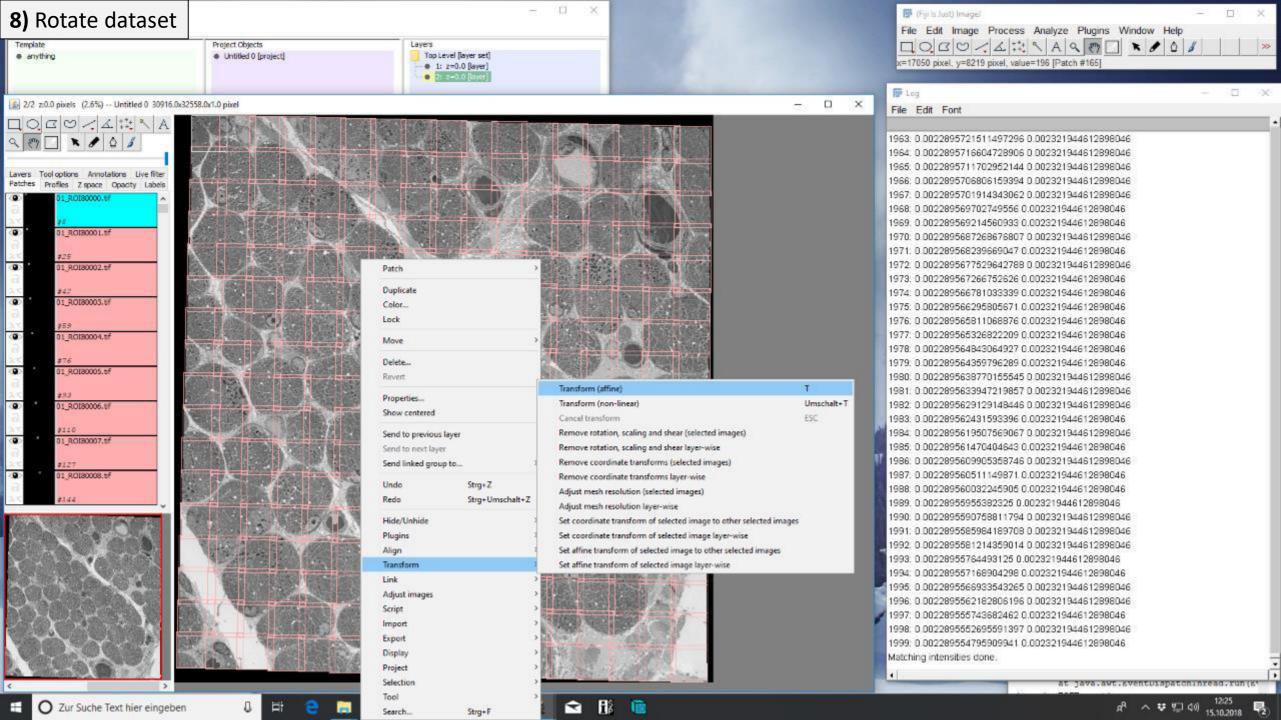
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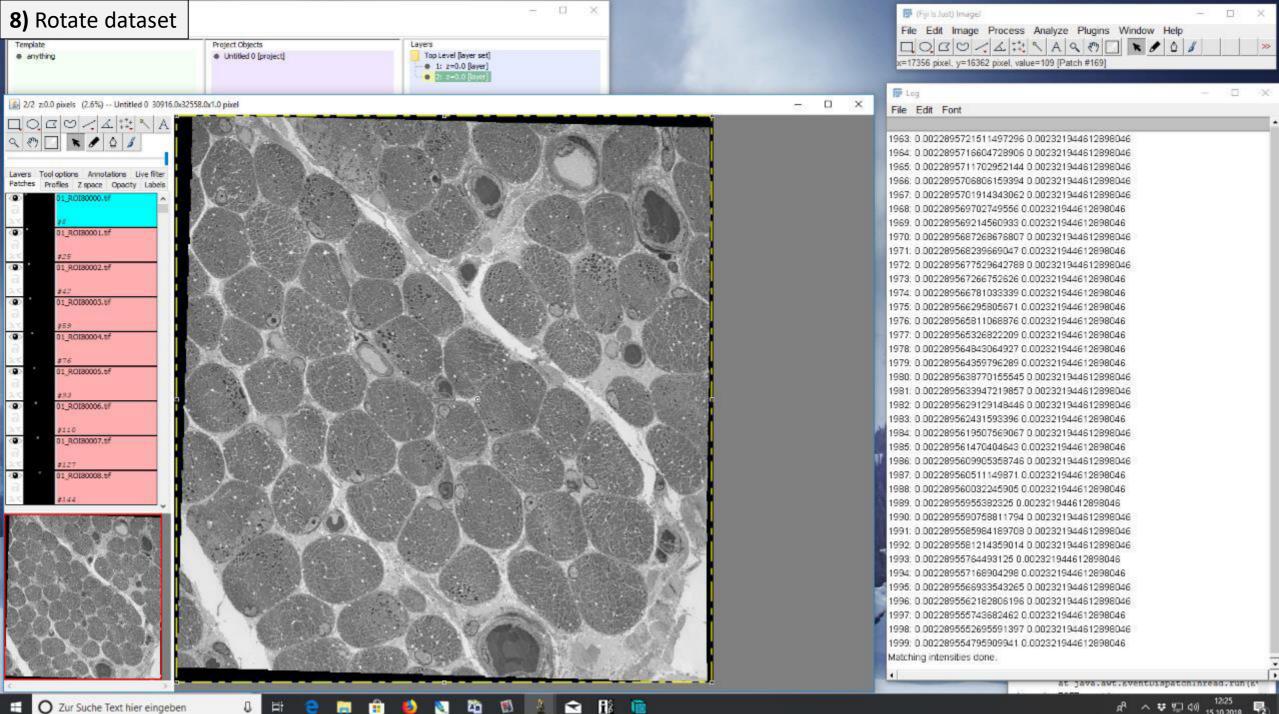
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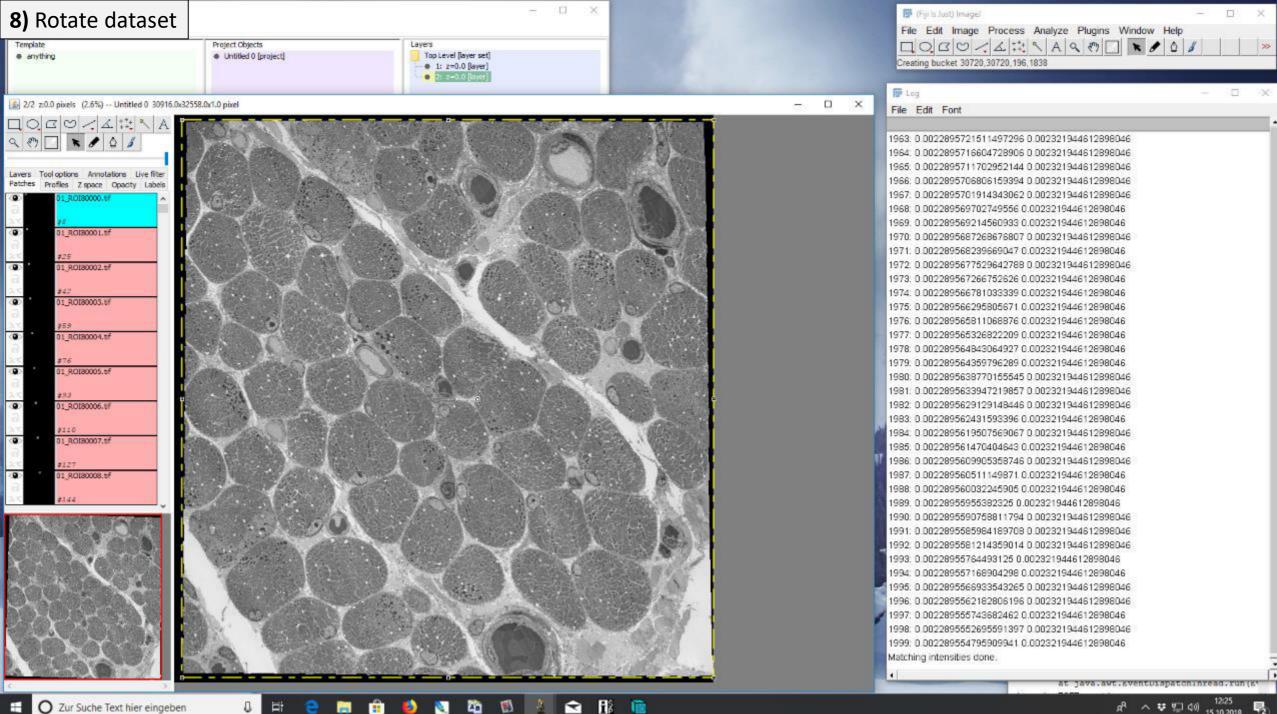




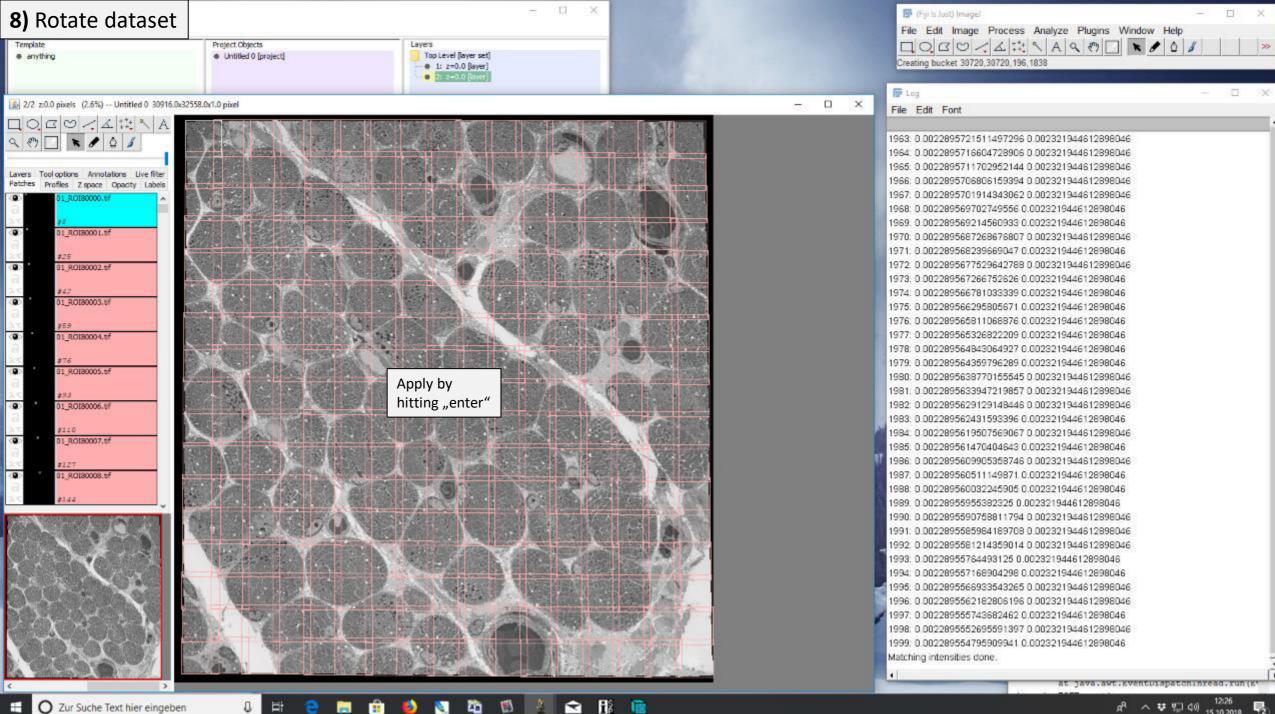
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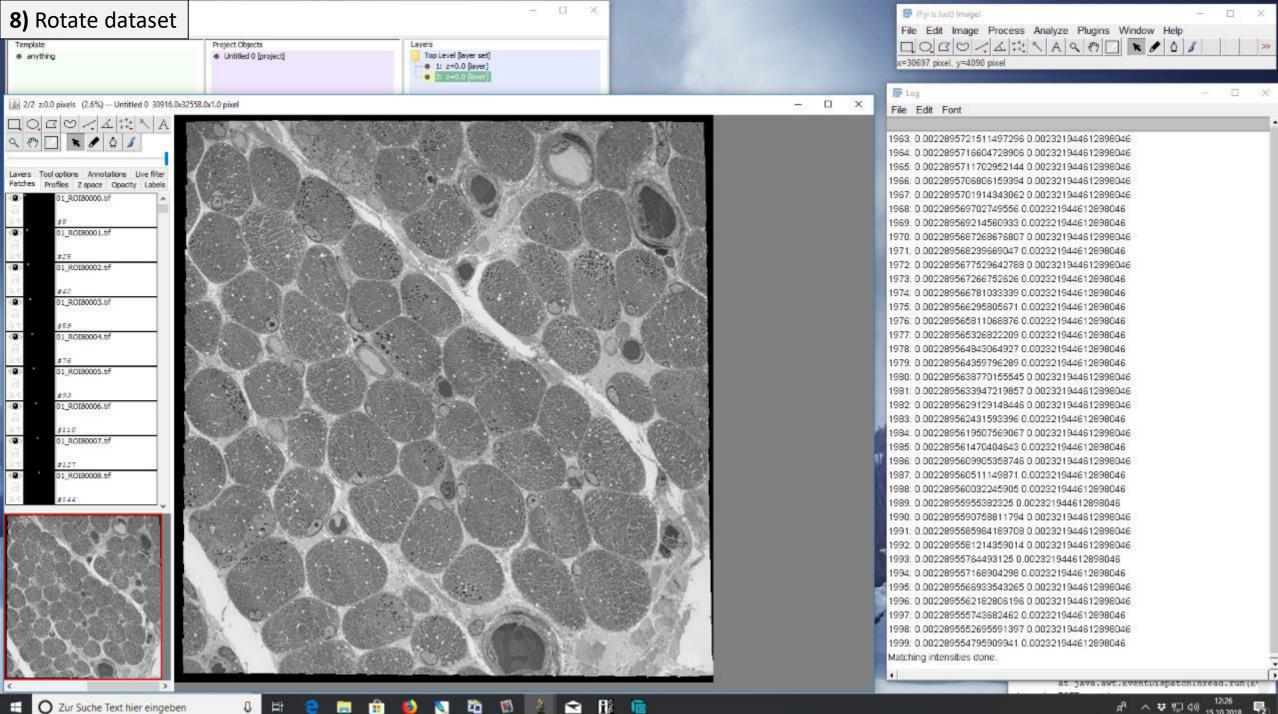


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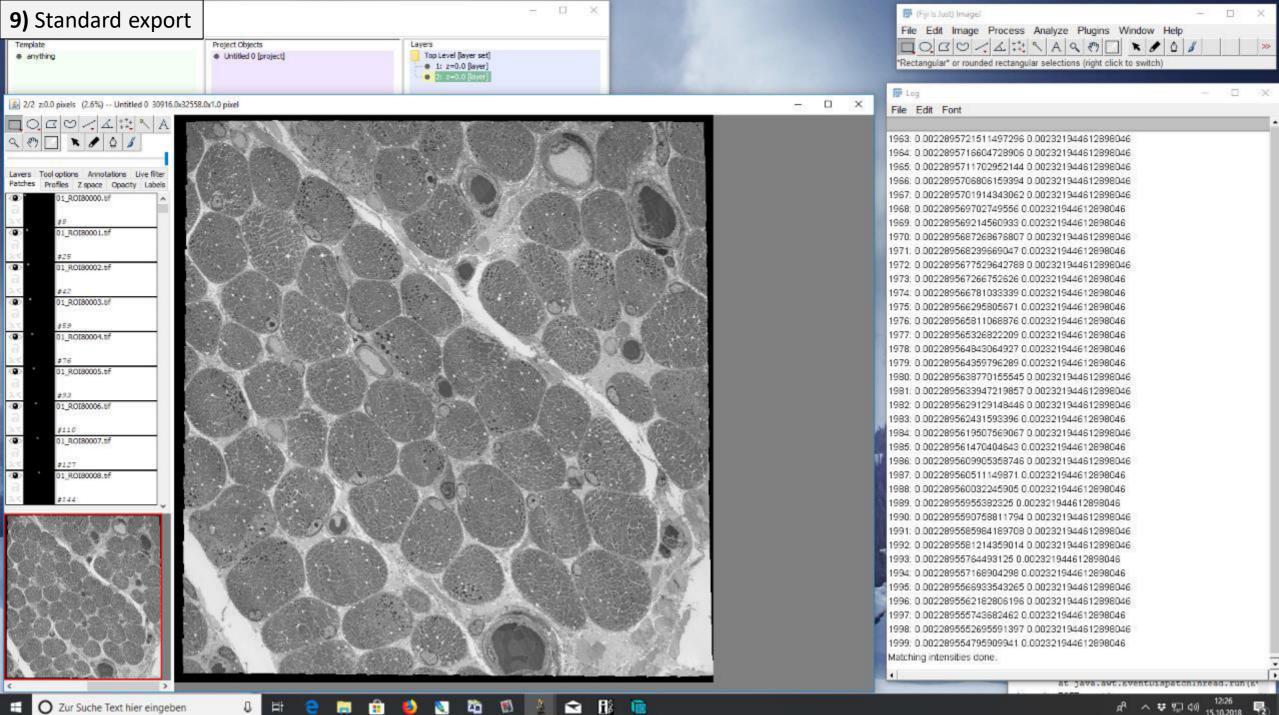
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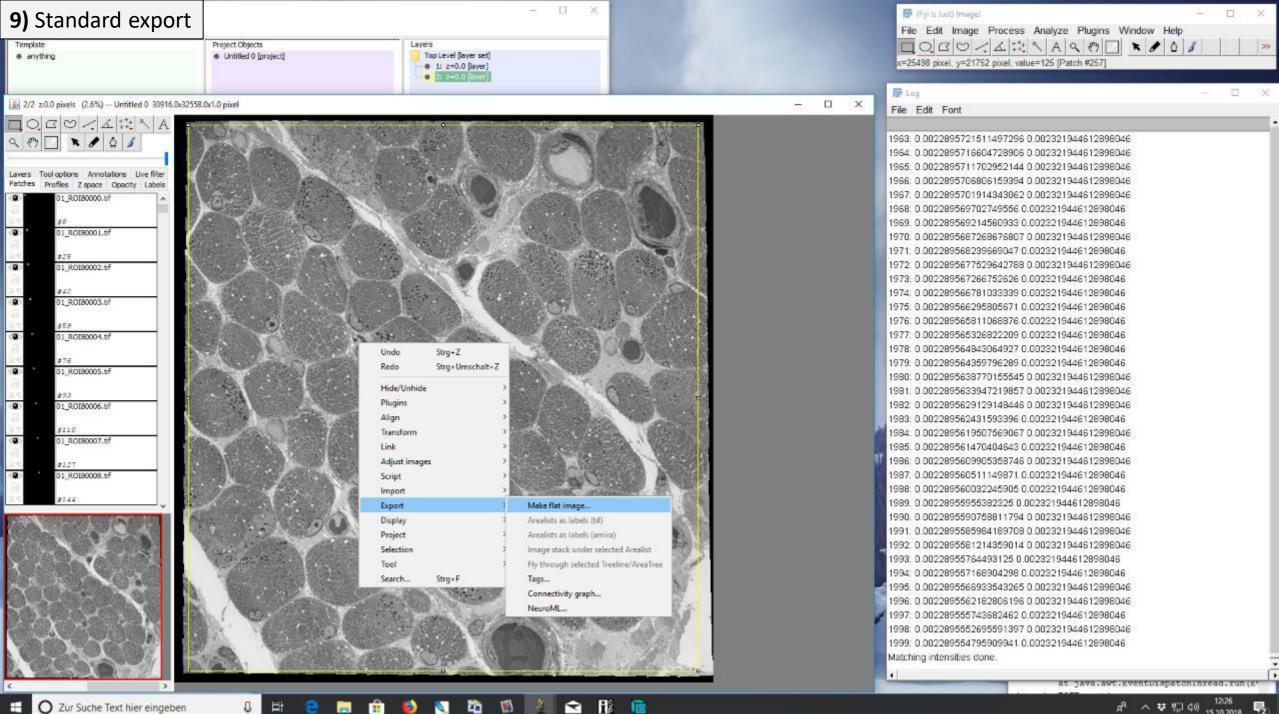
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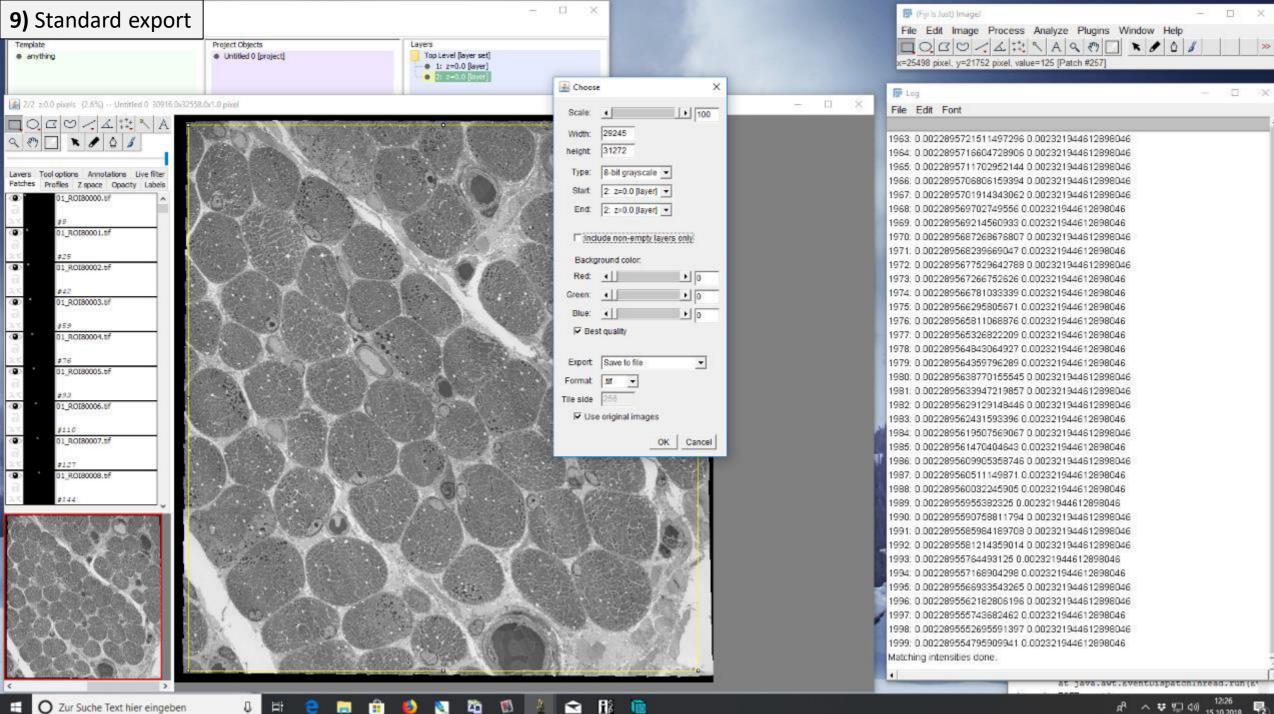
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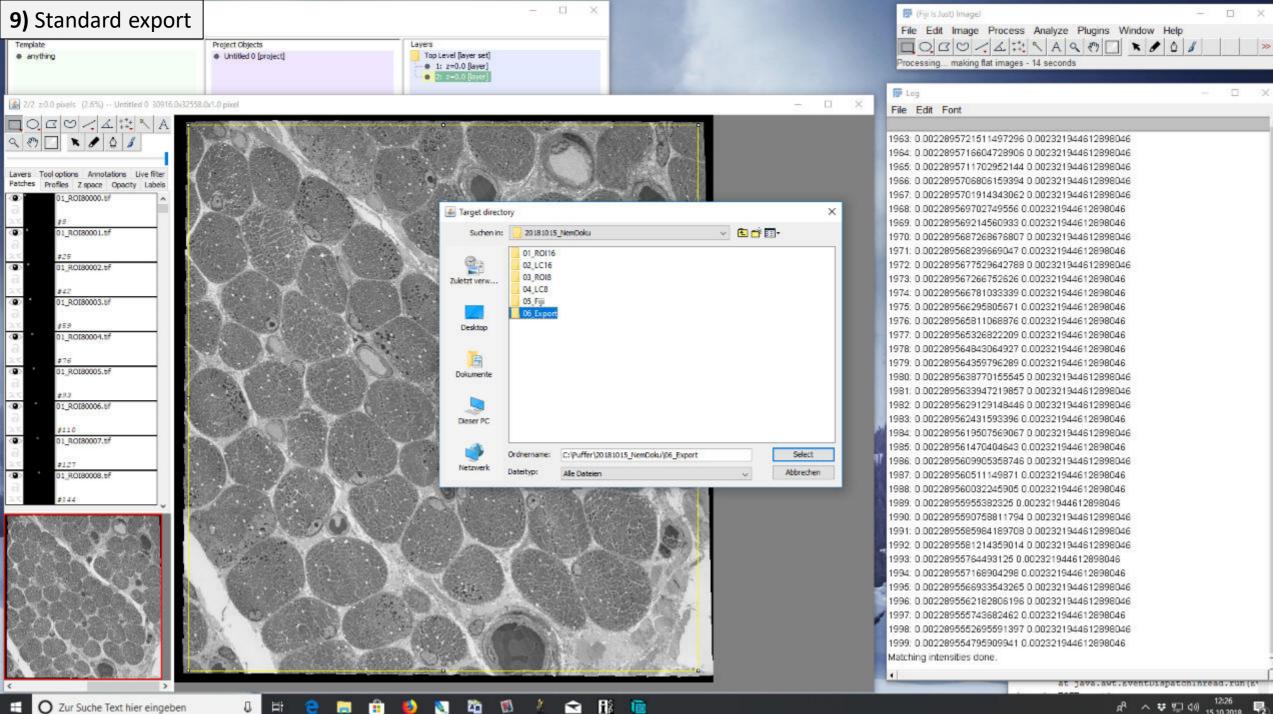
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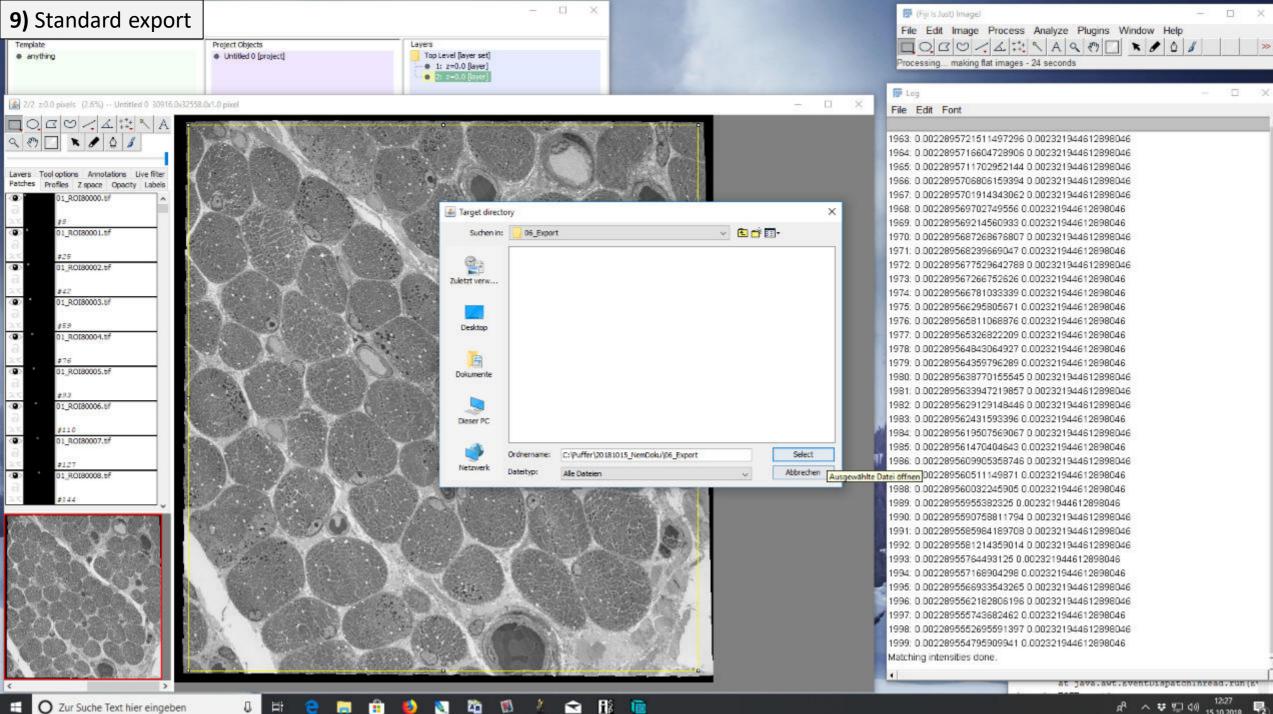
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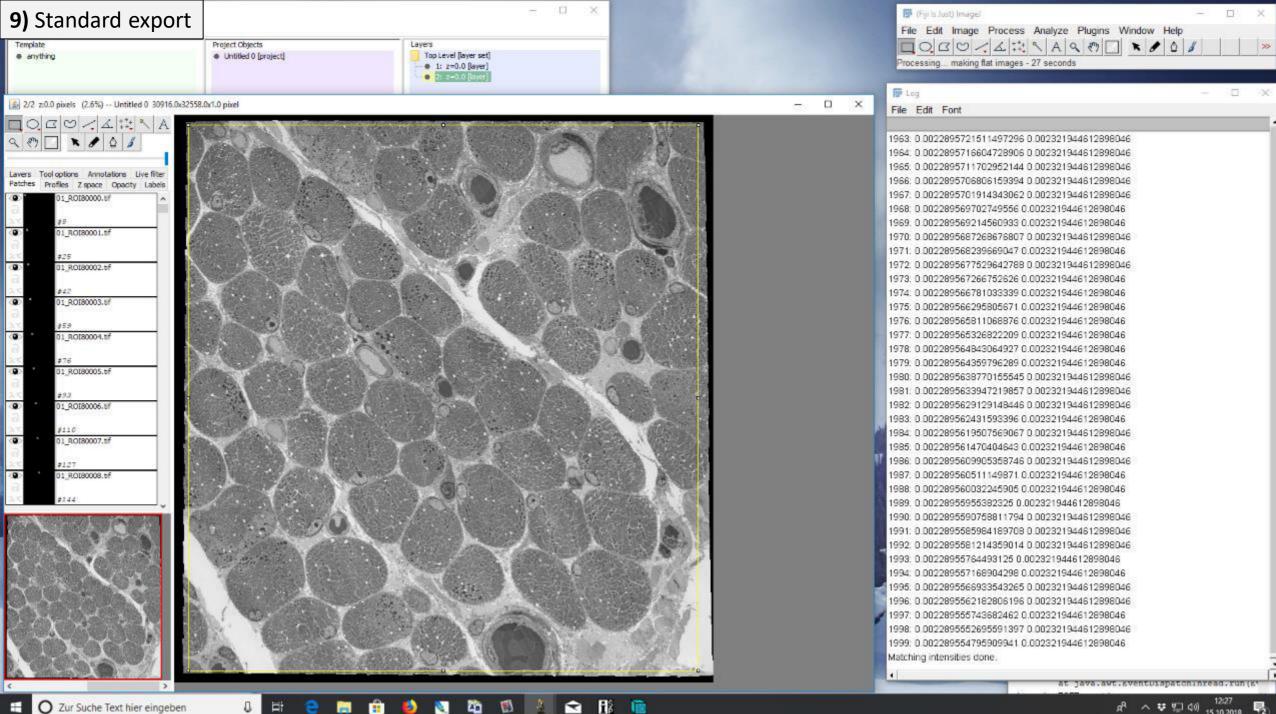
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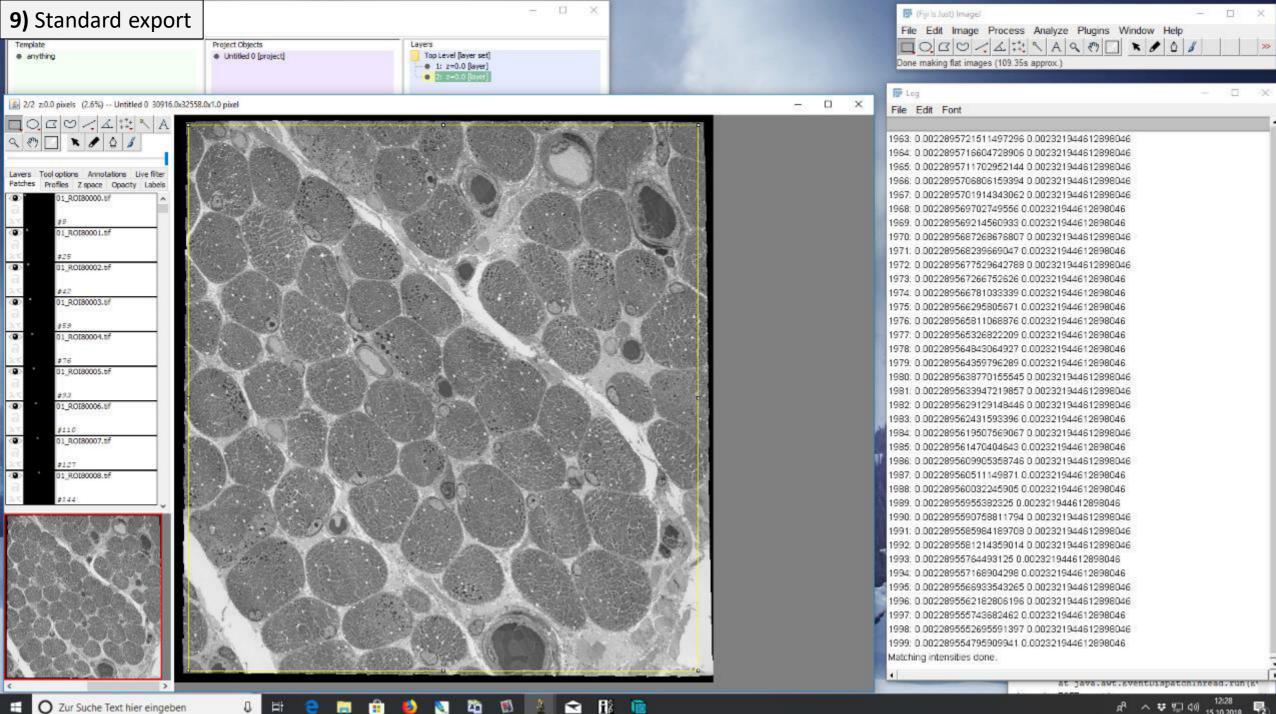


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10) CATMAID tile export

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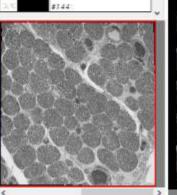
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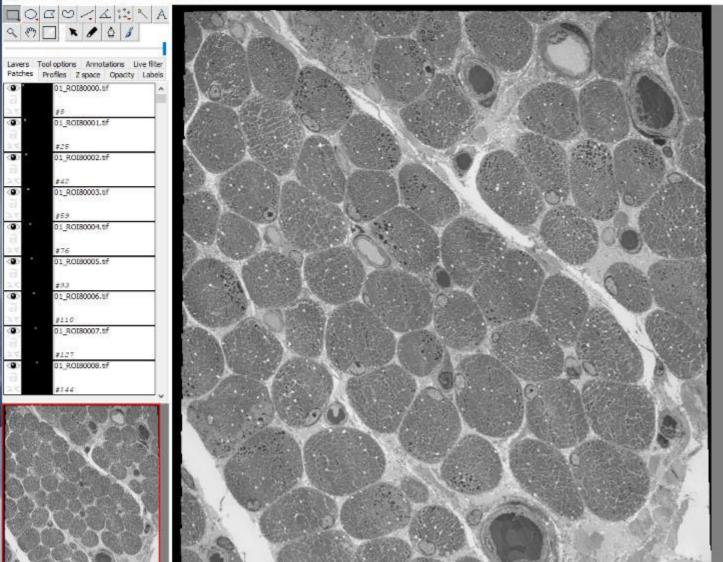
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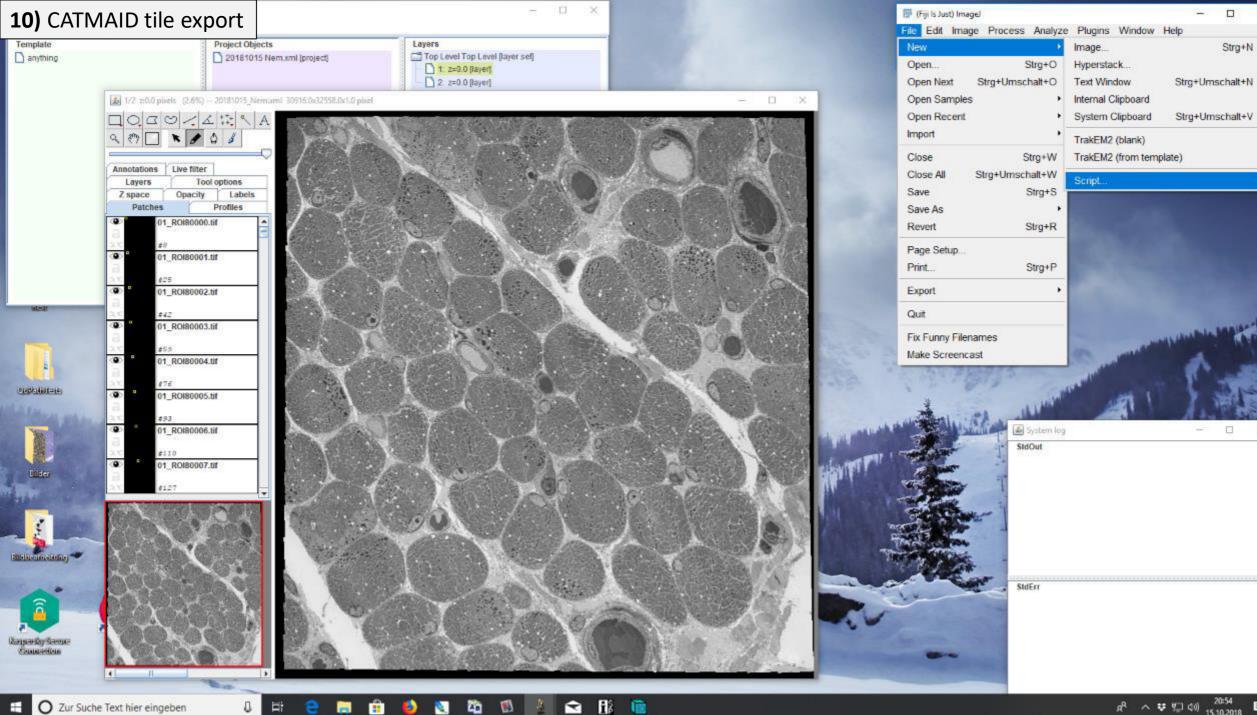
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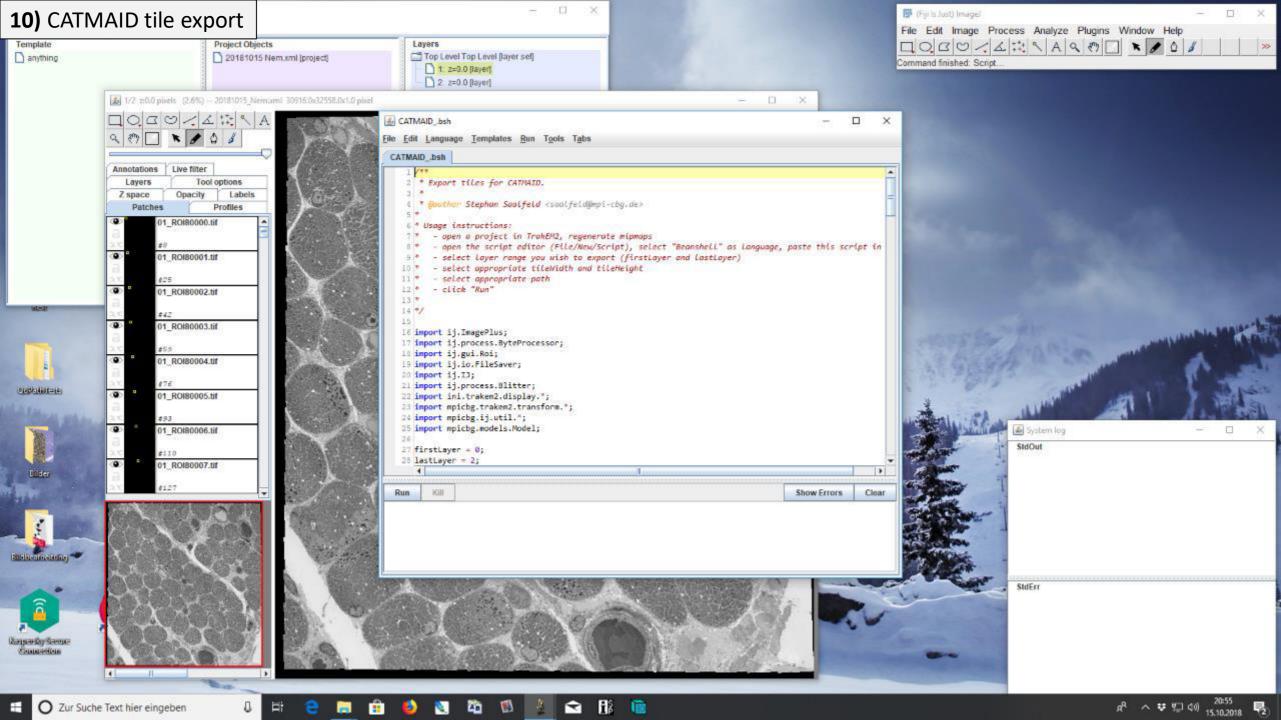
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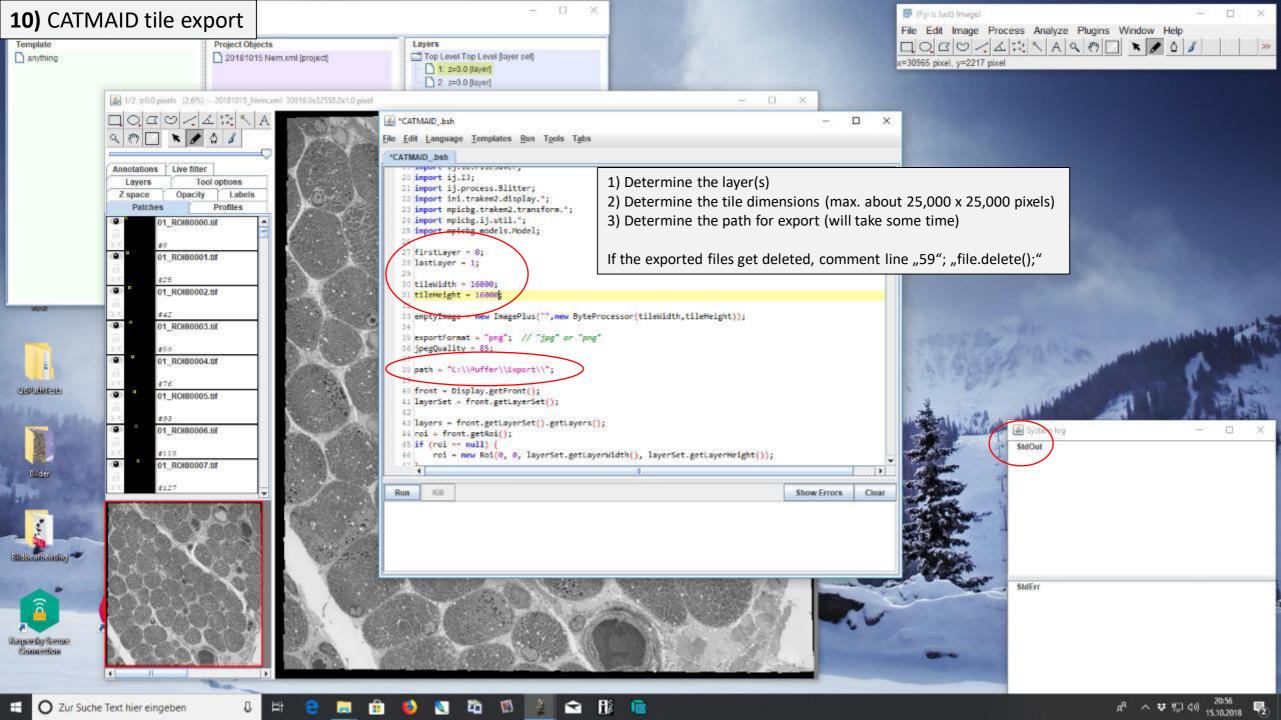
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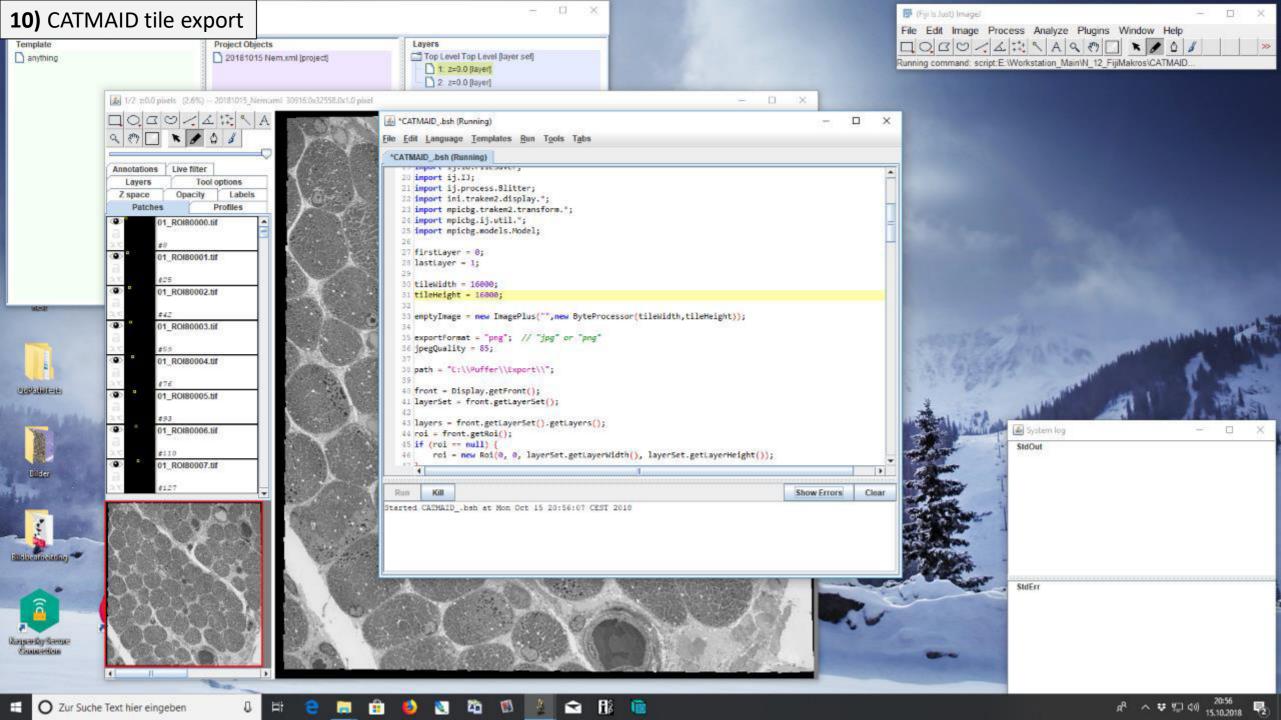


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step-by-step protocol 2 (pdf files A-E)

Processing of STEM image tiles; stitching and bigtif generation (the process is scalable for up to more than 10 large datasets)

A) Excel; semiautomated generation of a text file containing coordinates (X, Y and Z) of overlapping STEM image tiles

B) Fiji/ TrakEM2 plugin; import of overlapping STEM image tiles and stitching of these into coherent datasets

C) Fiji/ TrakEM2 plugin; export of stitched datasets into non-overlapping tif tiles

D) Nip2; import of non-overlapping tif tiles and export into a coherent bigtif file

E) QuPath; basic workflow for import, pan-and-zoom analysis, annotation, measurement, saving

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Tile_r11 c15_Regi

Dataset 1: Nerve, digitized with SEM-STEM at 0.1 μs dwell time and 9 nm pixel size (155 tiles) Dataset 2: Same nerve section, digitized with SEM-STEM at 0.3 μs dwell time and 9 nm pixel size (155 tiles)

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"Puffer"; main directory for data processing in this example with dataset 1 and dataset 2 folder ExcelTemplate as a basis for image coordinate calculation

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Prepare the image tile names of dataset 2 and copy these into the Template

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ile_r8-c6_Region5_143208460.tif	8-		8	c6 R	6					0)	0								9625	9625 0.0				_
ile_r8-c7_Region5_143208460.tif	8-		8	c7_R	7				1	0)	0								9625	9625 0.0				
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ile_r8-c9_Region5_143208460.tif	8-		8	c9_8	9					0)	0								9625	9625 0.0				
ile_r8-c10_Region5_143208460.tif	8-		8	c10_	10	1		-		0		0								9625	9625 0.0				_
ile_r8-c11_Region5_143208460.tif	8-		8	c11_	11			-		L	3	0							-	9625	9625 0.0				_
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ile_r8-c16_Region5_143208460.tif	8-		8	c16_	16					0		0								9625	9625 0.0				
ile_r9-c1_Region5_143208460.tif	9.		9	c1_R	1					0)	0								9625	9625 0.0				
ile_r9-c2_Region5_143208460.tif	9-	9	9	c2_R	2					C)	0								9625	9625 0.0				
Tile_r9-c3_Region5_143208460.tif	9.	3	9	c3_R	з					C)	0								9625	9625 0.0				
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file_r9-c5_Region5_143208460.tif	9-		9	c5_R	5			_		C	1	0							1	9625	9625 0.0				
ile_r9-c6_Region5_143208460.tif	9		9	c6_R	6					0)	0							_	9625	9625 0.0				
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ile r10-c5 Region5 143208460.tif ile r10-c6 Region5 143208460.tif	10		10	-c5	5			-		0	2	0								9625	9625 0 0 9625 0 0				
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ile r10-c8 Region5 143208460.tif	10		10	-c8	8				10 I 10			0							1	9625	9625 0.0				_
ile r10-c9 Region5 143208460.tif	10		10	-c9	9		1			0		0							11	9625	9625 0.0	 			-
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le_r11-c12_Region5_143208460.tif	11	1	11	-c12	17					C)	0								9625	9625 0.0				
le_r11-c13_Region5_143208460.tif	11	1	11	-c13	13					C)	0								9625	9625 0.0				
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ile_r11-c15_Region5_143208460.tif	11	1	11	-c15	1.5					C	0	0								9625	9625 0.0				
le_r1-c5_Region5_143208460.tif	1-	3	1	c5_R	5					0	1	0								9625	9625 0.0				
ile_r1-c6_Region5_143208460.tif	1-		1	c6_R	6					0	1	0								9625	9625 0.0				
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Extract the numbers in column E to column F the same way

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le r8-c2 Region5 143208460.tif	8-	8	c2 R	2	8 2	19250	77000					9625	9625 19250 7700		
le_r8-c3_Region5_143208460.tif	8-	8	c3_R	3	8 3	28875	77000					9625	9625 28875 7700		
le_r8-c4_Region5_143208460.tif	8-	8	c4_R	4	8 1	38500	77000					9625	9625 38500 7700		
le_r8-c5_Region5_143208460.tif	8-	8	c5_R	5	8 5	48125	77000				1.2	9625	9625 48125 7700		
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e_r8-c14_Region5_143208460.tif	8-	8	c14	14	8 14	134750	77000					9625	9625 134750 770		
e_r8-c15_Region5_143208460.tif e_r8-c16_Region5_143208460.tif	8-	8	c15_	15	8 15	144375	77000					9625	9625 144375 770 9625 154000 770		
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File_r9-c13_Region5_143208460.til	9	9	c13_	13	9 13	125125	86625					9625	9625 125125 860		
file_r9-c14_Region5_143208460.til	9.	9	c14_	14	9 14	134750	86625					9625	9625 134750 866		
ile_r9-c15_Region5_143208460.til	9-	9	c15_	15	9 15	144375	86625					9625	9625 144375 866		
ile_r9-c16_Region5_143208460.til	9-	9	c16_	16	9 16	154000	86625					9625 9625	9625 154000 866		
ile_r10-c2_Region5_143208460.tif ile_r10-c3_Region5_143208460.tif	10	10	-c2_ -c3	2	10 2	19250 28875	96250					9625	9625 19250 9625 9625 28875 9625		
ile r10-c4 Region5 143208460.tif	10	10	-c4	4	10 4	38500	96250				100	9625	9625 38500 9625		
le r10-c5 Region5 143208460.tif	10	10	-c5	5	10 5	48125	96250					9625	9625 48125 9625		
ile r10-c6 Region5 143208460.tif	10	10	-c6	6	10 6	57750	96250				Q. 7	9625	9625 57750 9629		
ile r10-c7 Region5 143208460.tif	10	10	-c7	7	10 7	67375	96250					9625	9625 67375 9629		
ile r10-c8 Region5 143208460.tif	10	10	-c8	8	10 8	77000	96250					9625	9625 77000 9629		
ile r10-c9 Region5 143208460.tif	10	10	-c9	9	10 9	86625	96250					9625	9625 86625 9625		
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le_r10-c15_Region5_143208460.tif	10	10	-c15	15	10 15	144375	96250					9625	9625 144375 962		
le_r10-c16_Region5_143208460.tif	10	10	-c16	16	10 16	154000	96250					9625	9625 154000 962		
le_r11-c4_Region5_143208460.tif	11	11	-c4_	4	11 4	38500	105875					9625	9625 38500 1058		
e_r11-c5_Region5_143208460.tif	11	11	-c5_	5	11 5	48125	1058/5					9625	9625 48125 1058		
e_r11-c6_Region5_143208460.tif e_r11-c7_Region5_143208460.tif	11	11	-c6_	6	11 6 11 /	57750	1058/5					9625	9625 57750 1058 9625 67375 1058		
e_r11-c8_Region5_143208460.tif	11	11	-c/_ -c8_	8	11 7	7/000	105875					9625 9625	9625 77000 1058		
le_r11-c9_Region5_143208460.tif	11	11	-c9_	9	11 9	86625	105875					9625	9625 86625 1058		
le_r11-c10_Region5_143208460.tif	11	11	-c10	10	11 10	96250	105875					9625	9625 96250 1058	875	
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le_r11-c14_Region5_143208460.tif	11	11	-c14	14	11 14	134750	105875					9625	9625 134750 105		
le_r11-c15_Region5_143208460.tif	11	11	-c15	15	11 15	144375 48125	105875 9675					9625 9625	9625 144375 105 9625 48125 9625		
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le_r1_c8_Region5_143208460.tif	1	1	c8_R	8	1 8	77000	9625					9625	9625 77000 9625		
le_r1 c9_Region5_143208460.tif	1	1	c9_R	9	1 9	86625	9625					9625	9625 86625 9625		
ile_r1 c10_Region5_143208460.tif	1	1	c10_	10	1 10	96250	9625					9625	9625 96250 9625		

Copy all numbers of column C and column F to column H and column I

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ile_r4-c8_Region5_143208460.tif	4-	4	c8_R	8	4	8	77000	38500				3 1	9625	9625 77000 3	8500	
ile_r4-c9_Region5_143208460.tif	4-	4	c9_R	9	4	9	86625	38500					9625	9625 86625 3		
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Include the "z" coordinate manually in column N; "0" for dataset 1 and "1" for dataset 2

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Copy the names of the image tiles in column A to column O Note the values in column R,S; image tile size 10,240 pixels, overlap 6%; 10,240*0.94=9625,6

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Prepare the following folders;

"01_Tiles" with all image tiles of the different datasets (here dataset 1 and dataset 2)

"02_Fiji" for the Fiji/ TrakEM2 project to be saved

"03_TifExport" for the exported non-overlapping tif tiles (using TrakEM2)

"04_Bigtif" for the export of the bigtif file (using nip2)

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Open Fiji and adjust "Memory & Threads" settings according to the computer parameters, restart afterwards

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Netzwerk

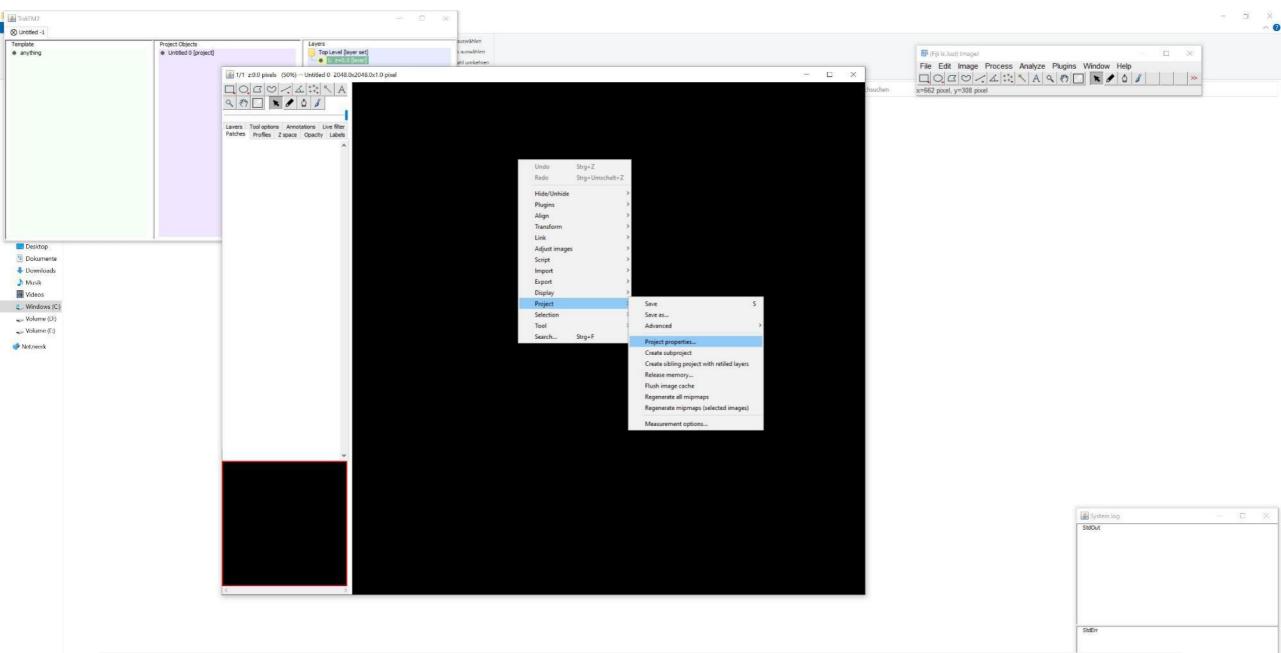
Open a new TrakEM2 project

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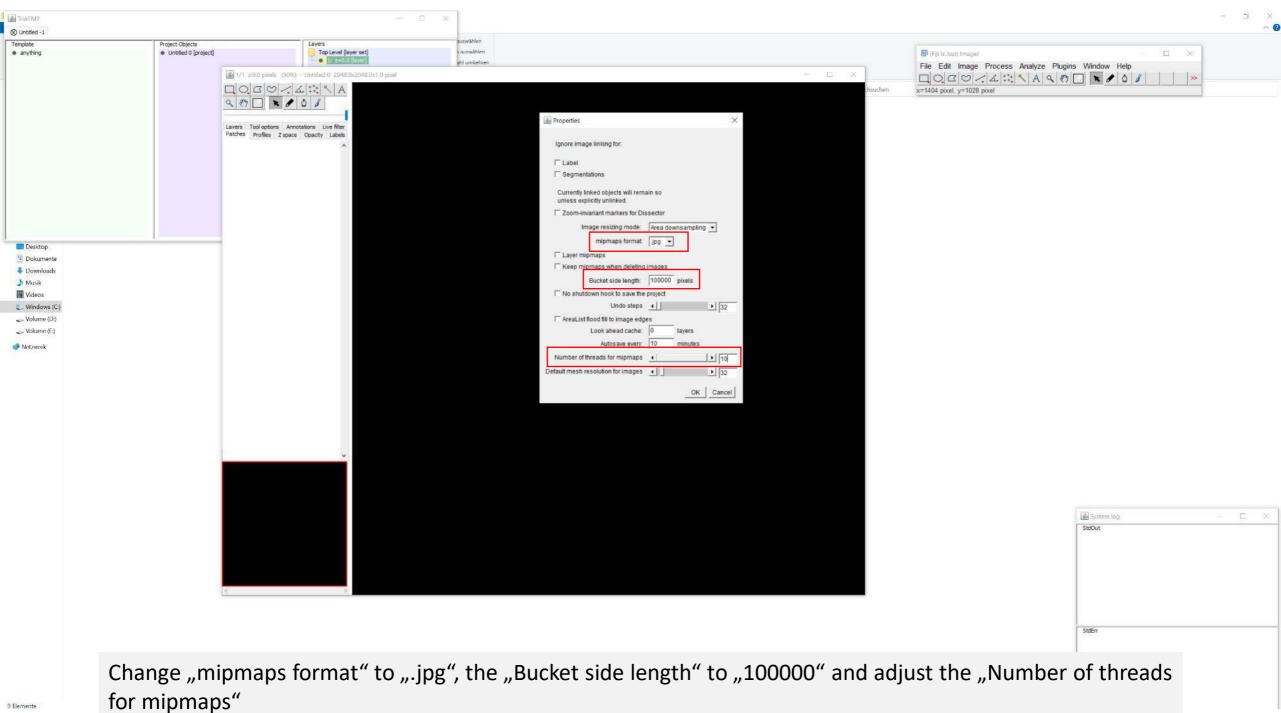
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Choose "02_Fiji" directory

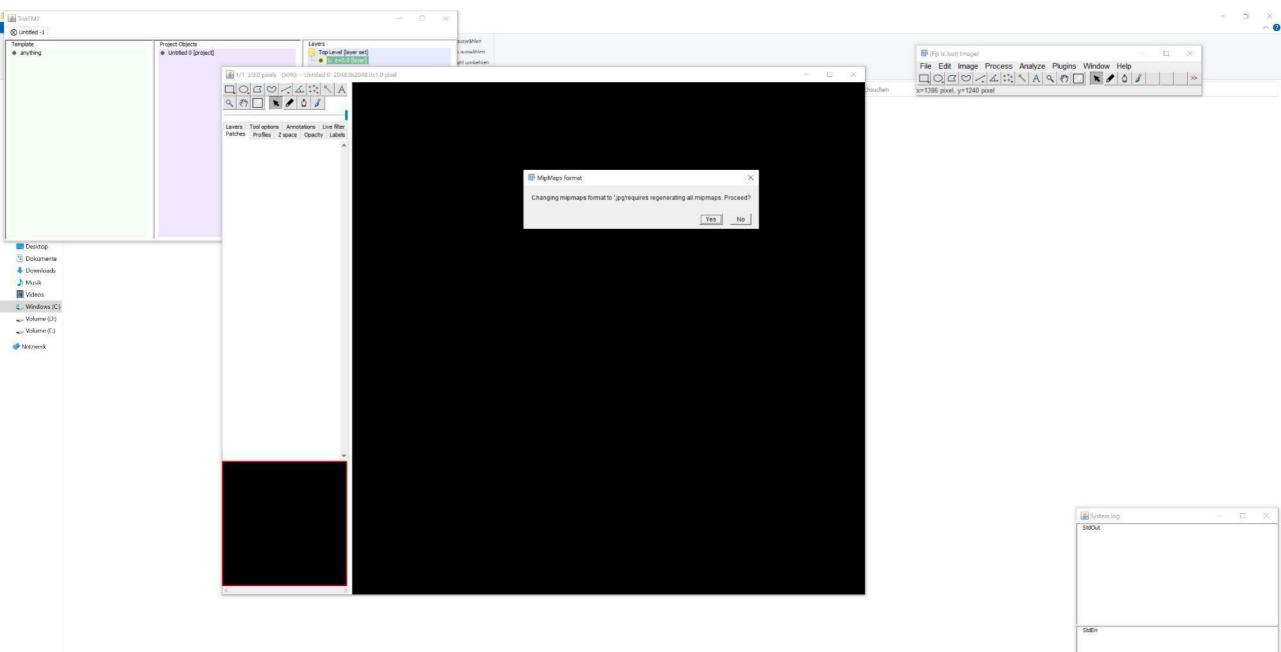
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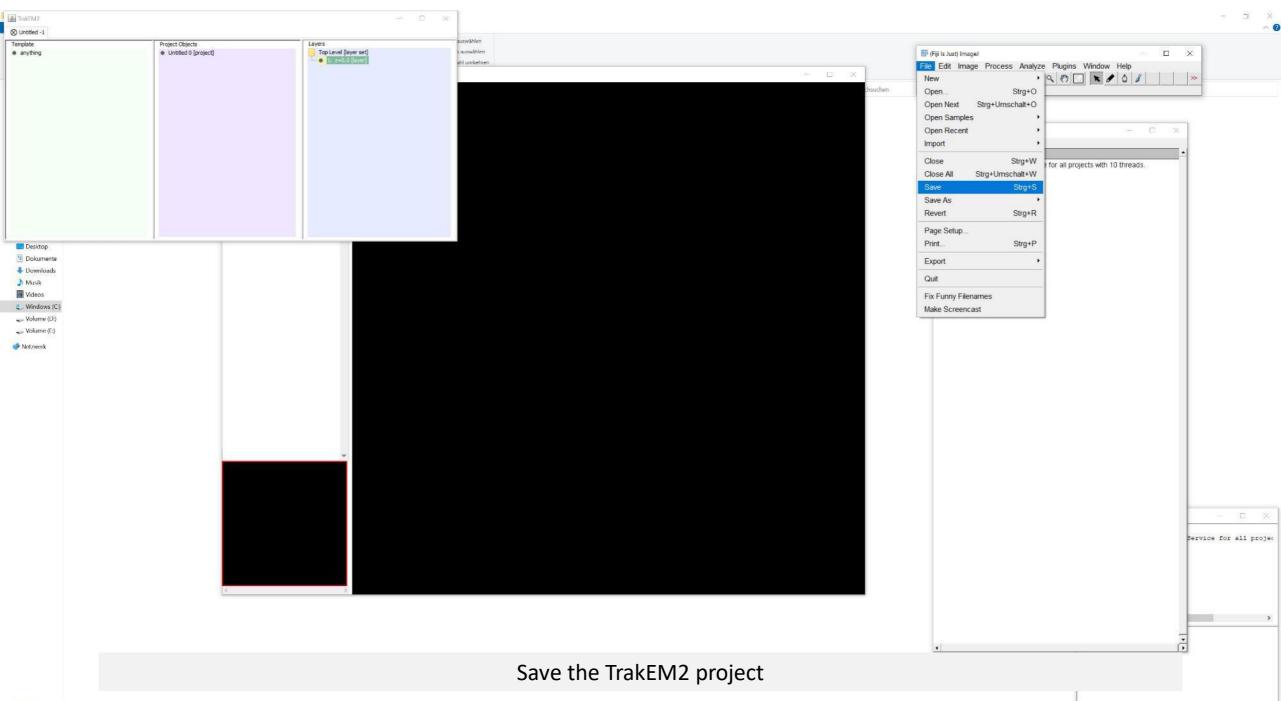


Right-click in the black canvas and adjust the "Project properties"



9 Elemente

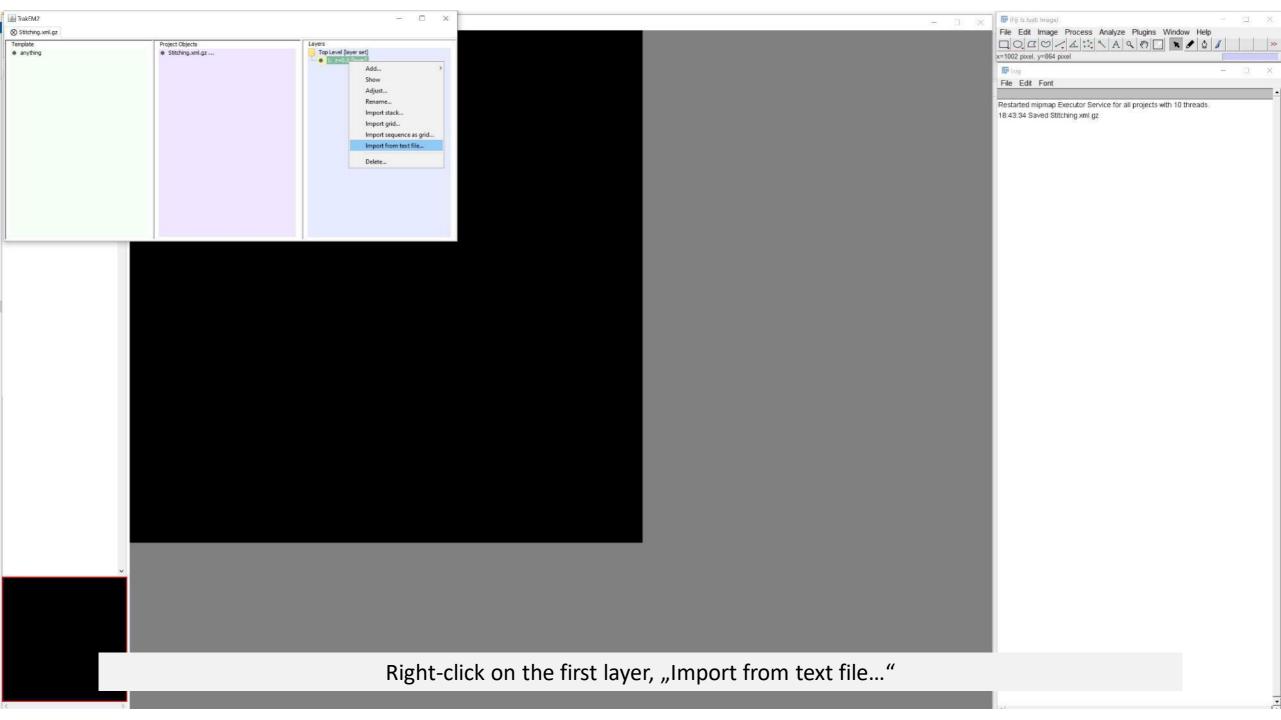




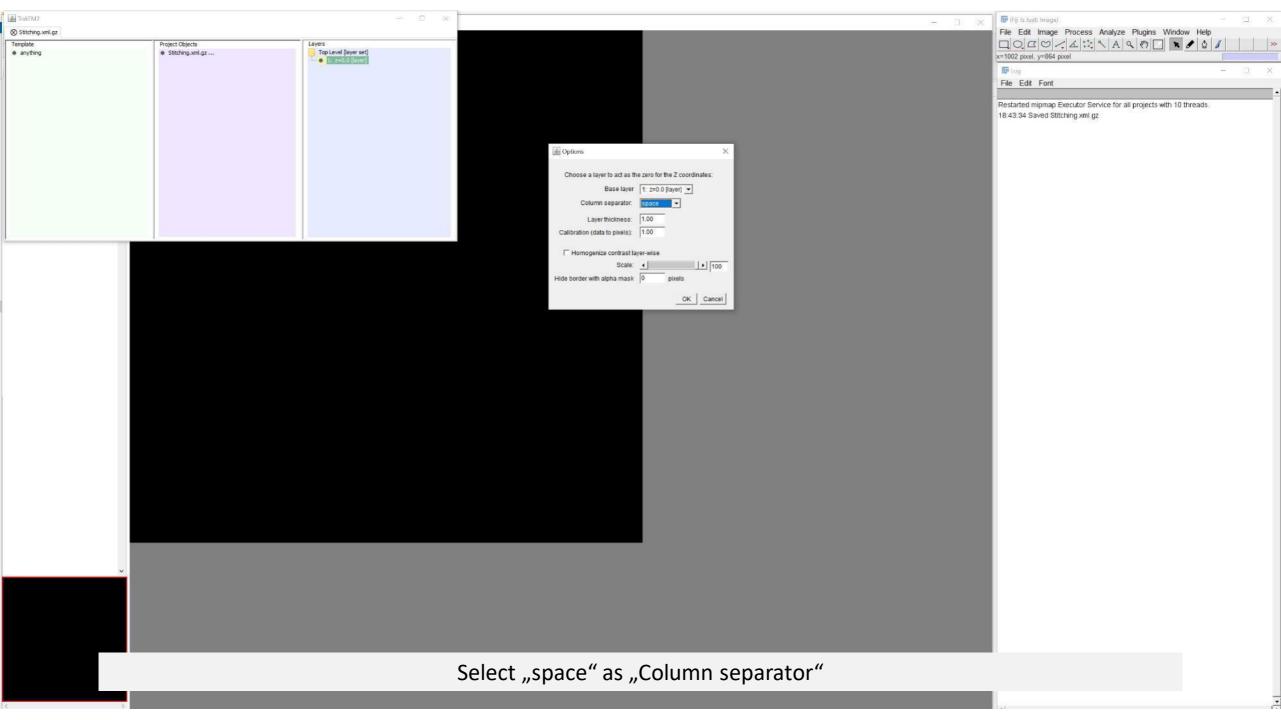


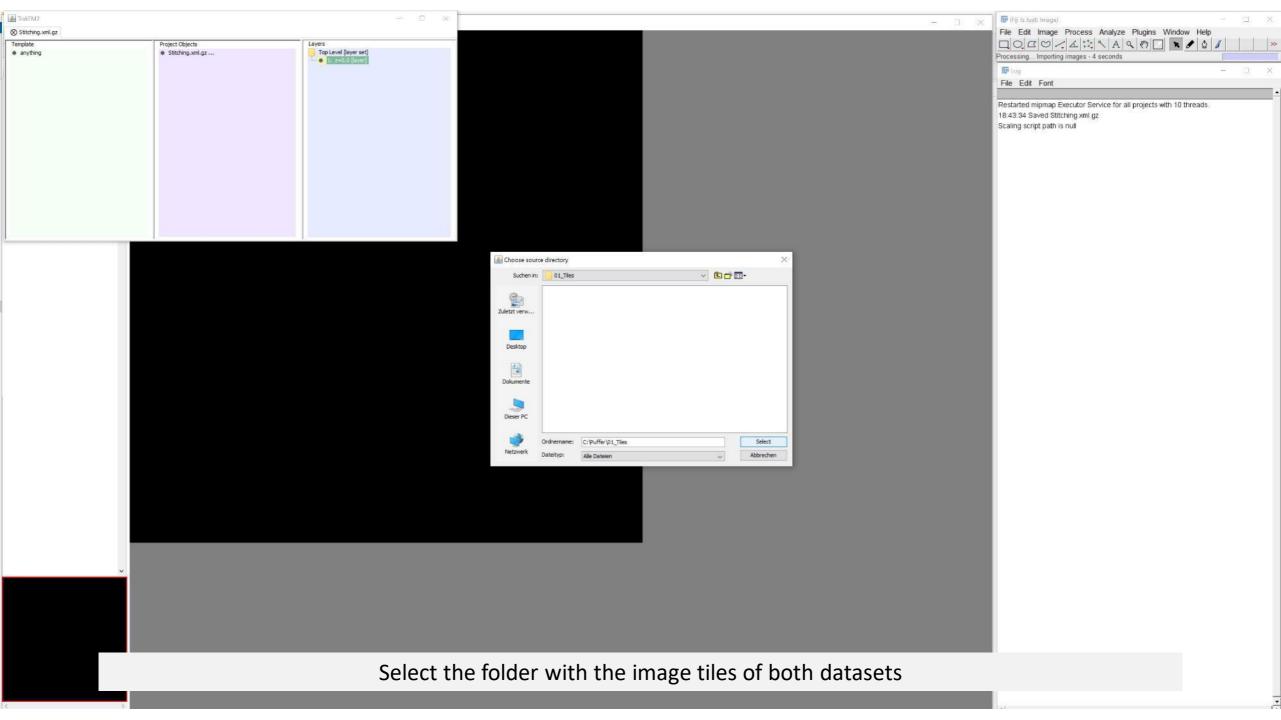
(The TrakEM2 project can be opened in a new session via drag&drop of this xml file on the small Fiji menu)

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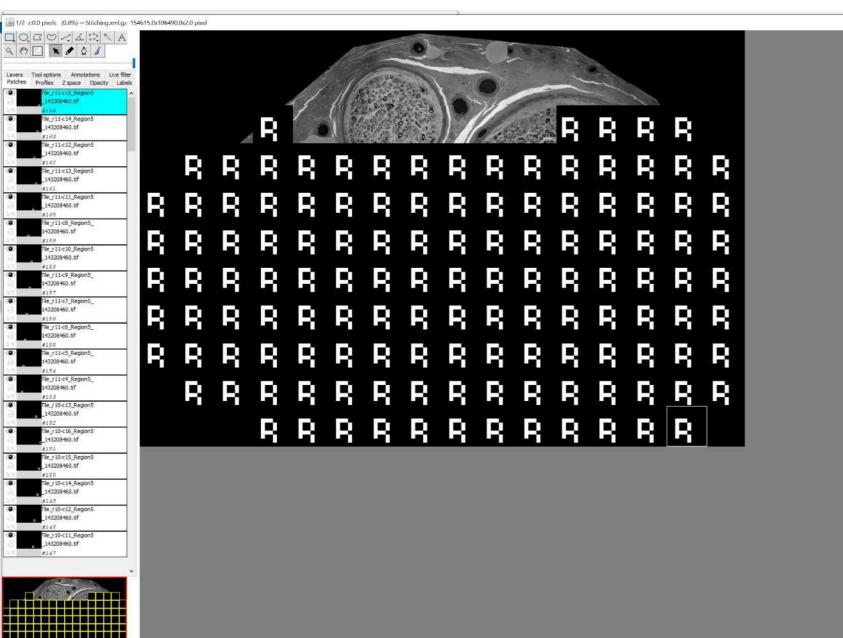
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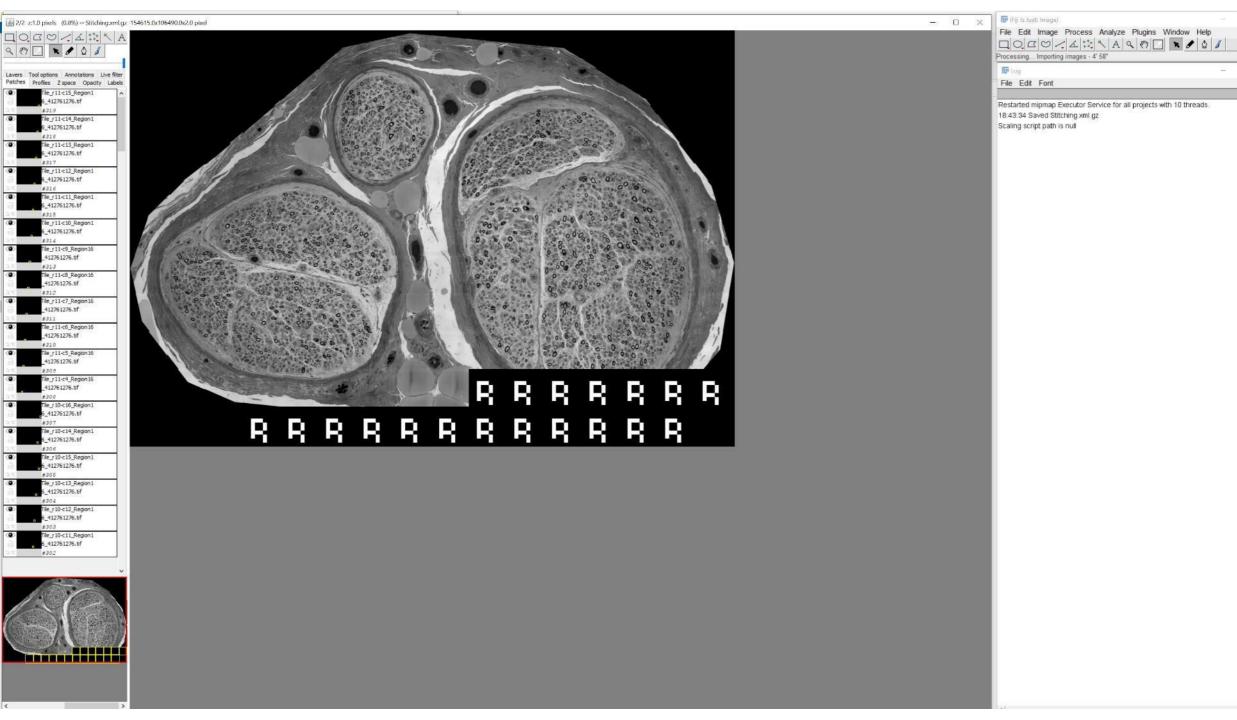
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All 310 image tiles will be imported based on the calculated coordinates See Task Manager for workstation performance



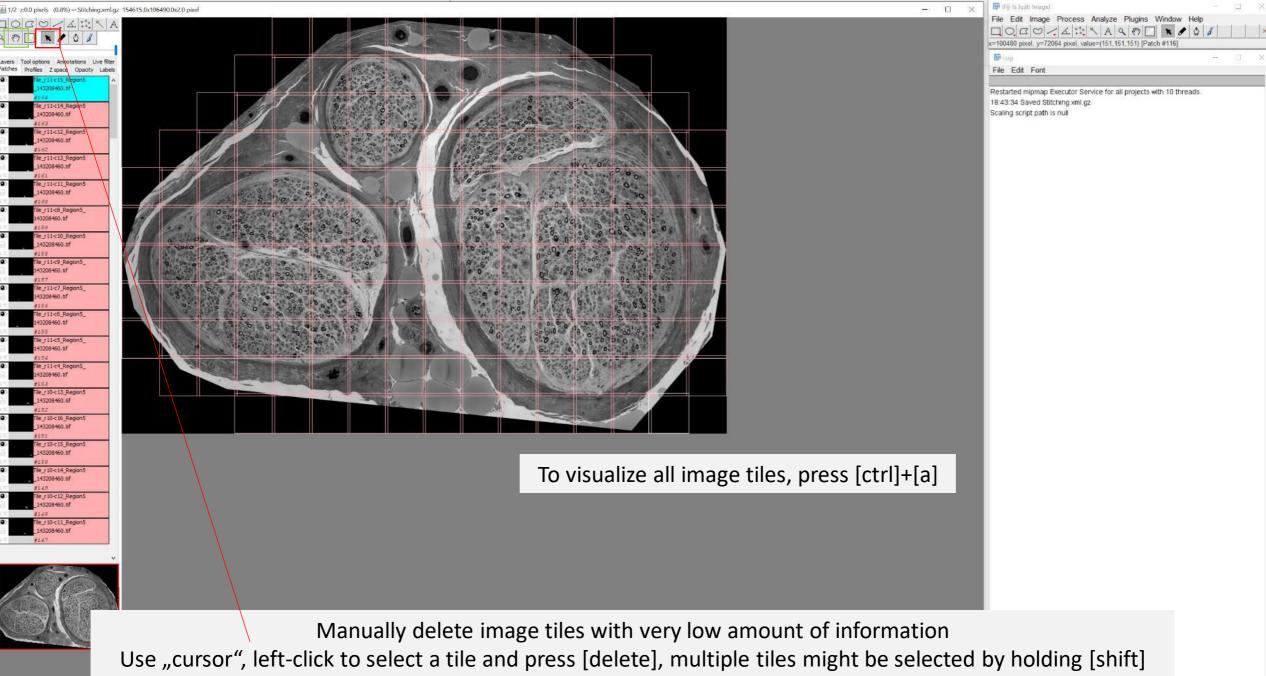
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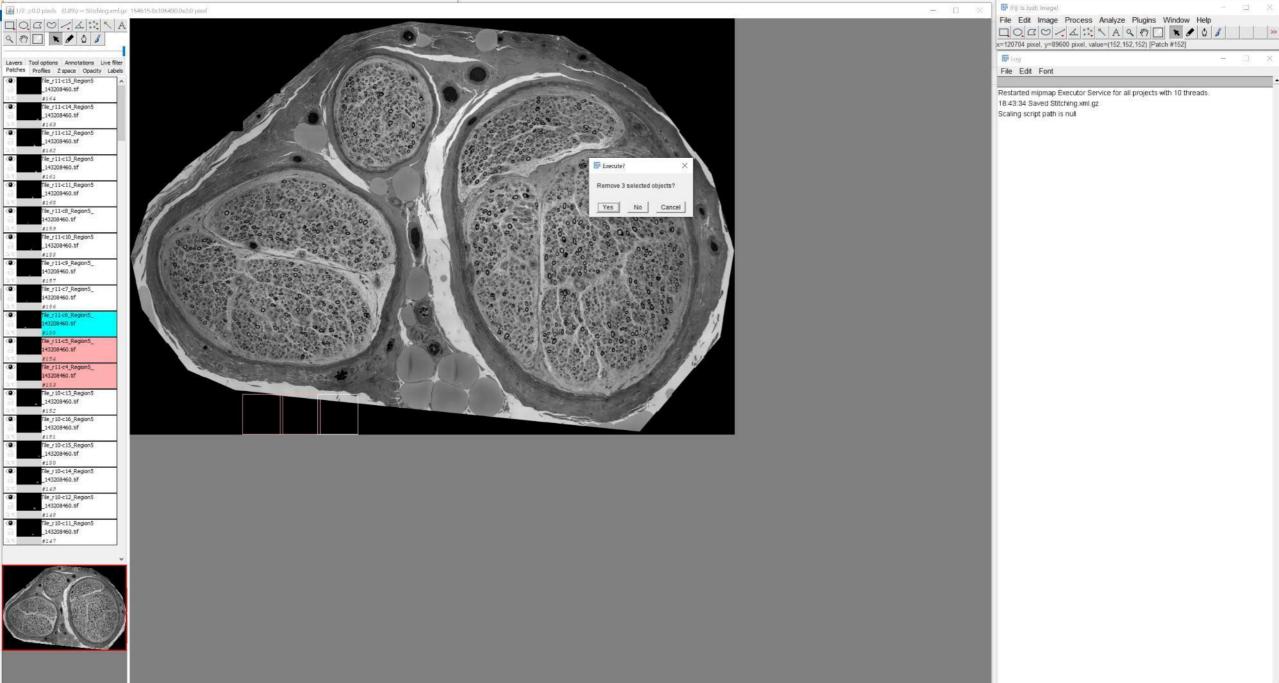


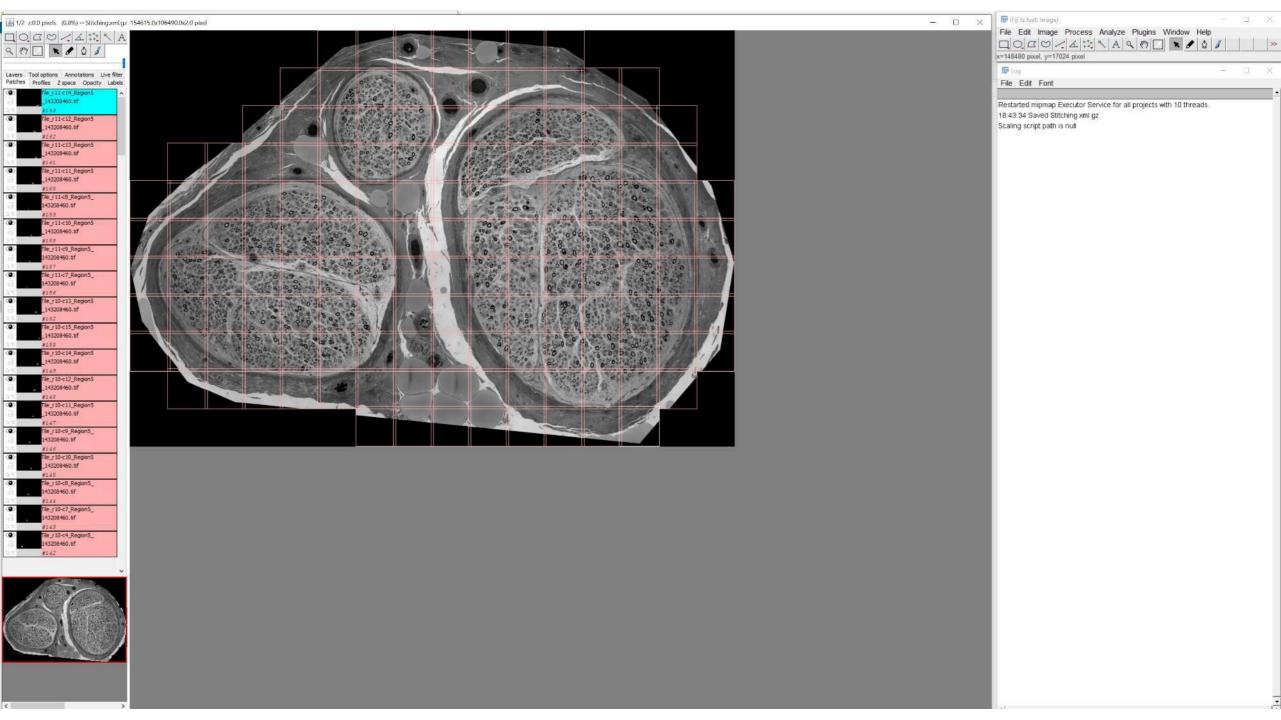


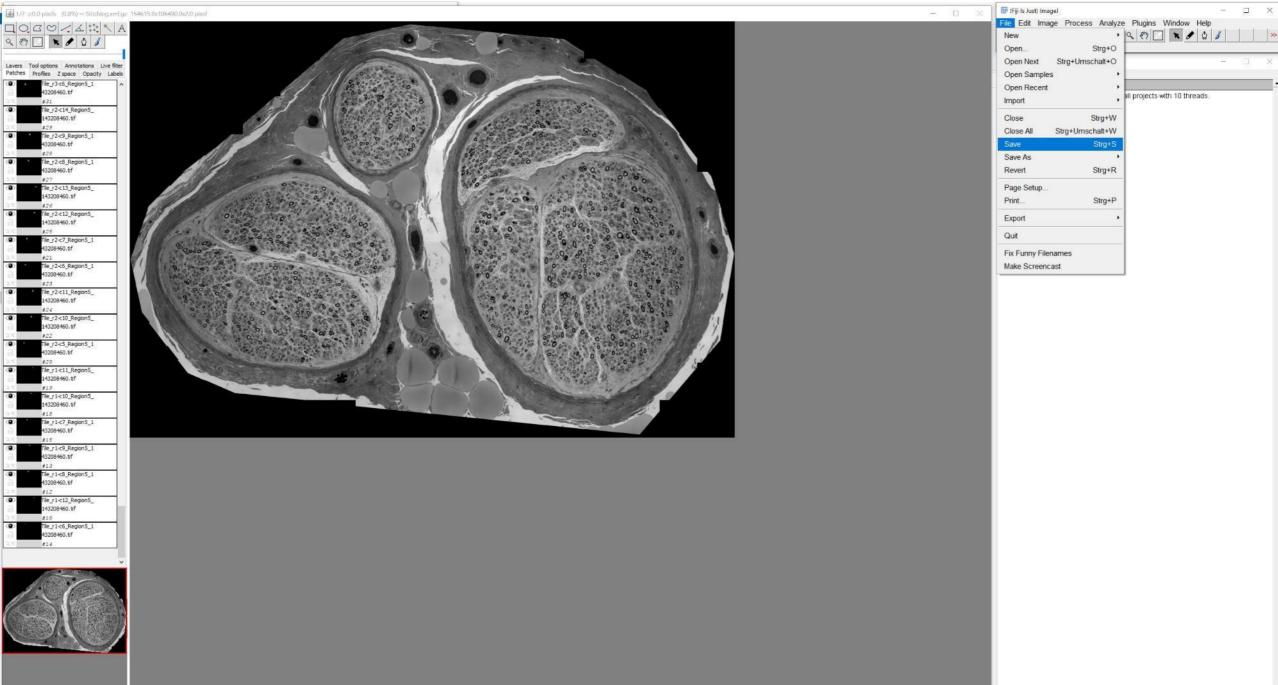
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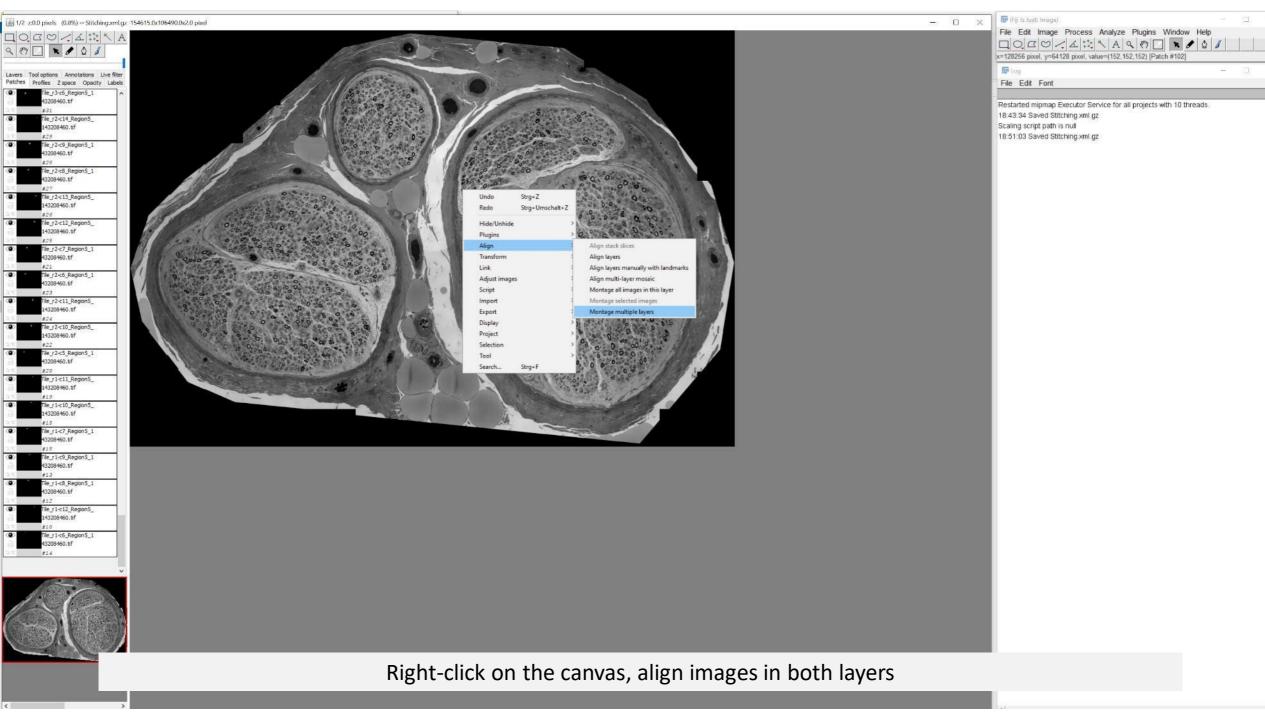


To avoid accidental deplacement of tiles while using the cursor afterwards, select the "hand" tool (green)

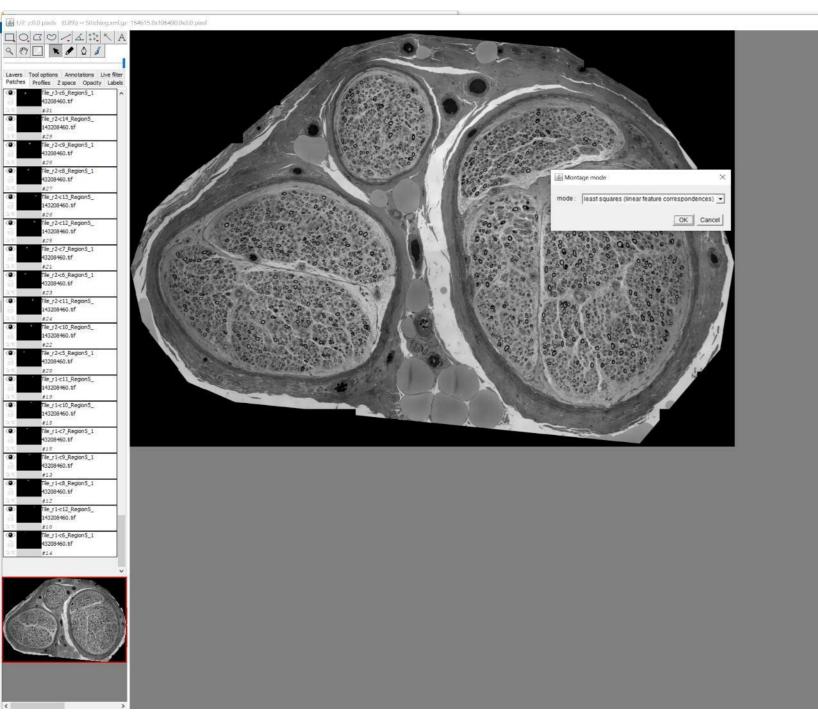






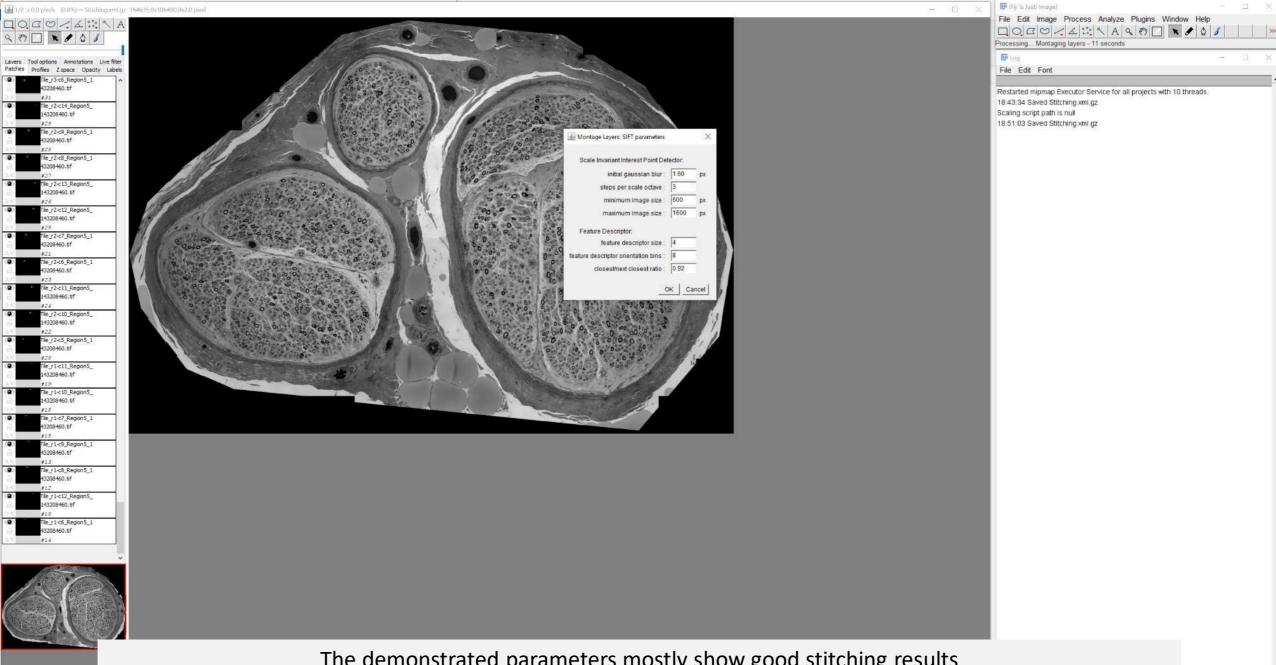




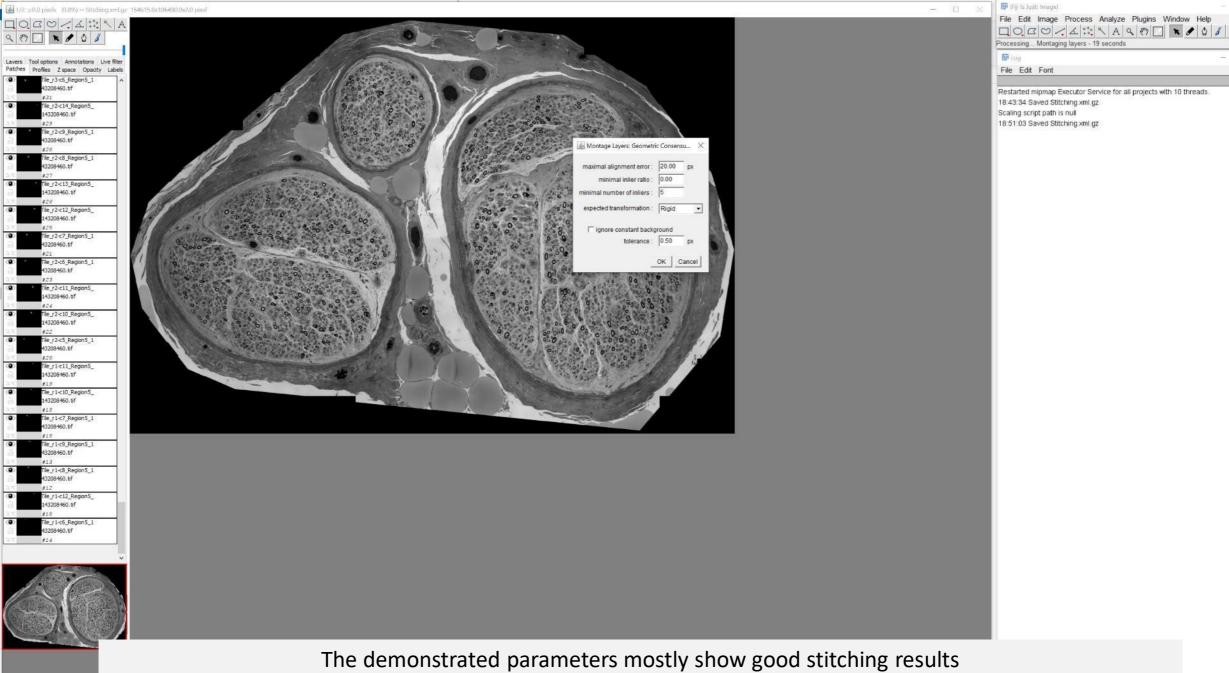


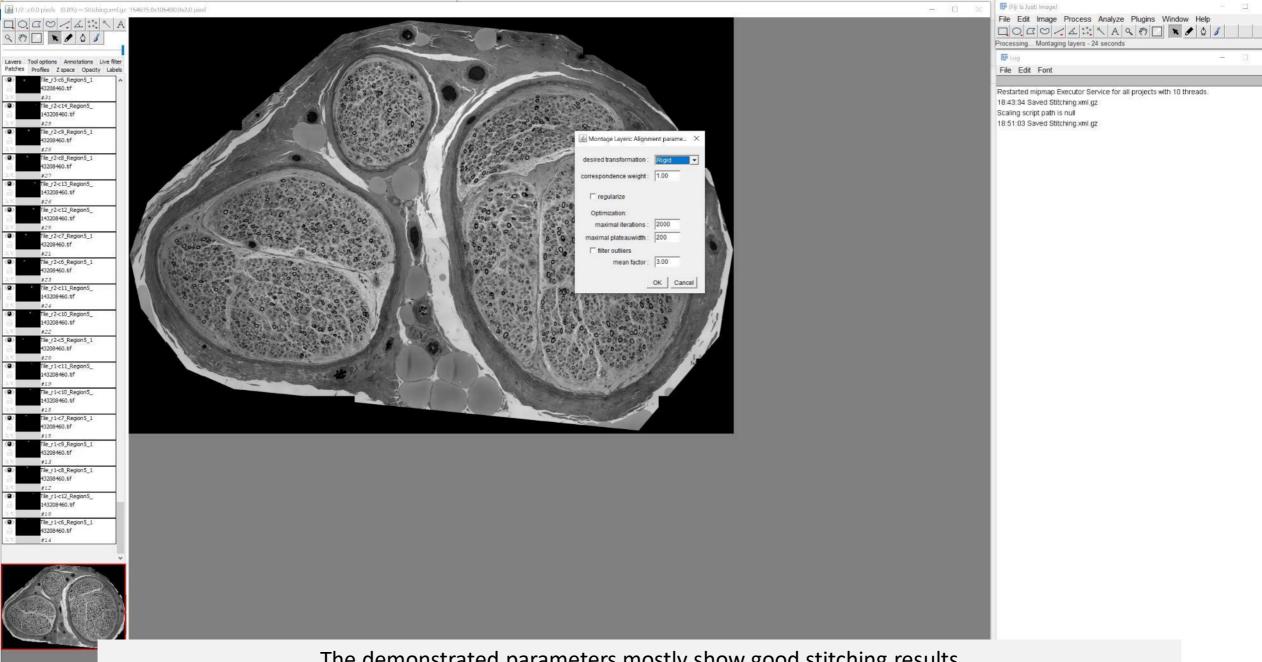
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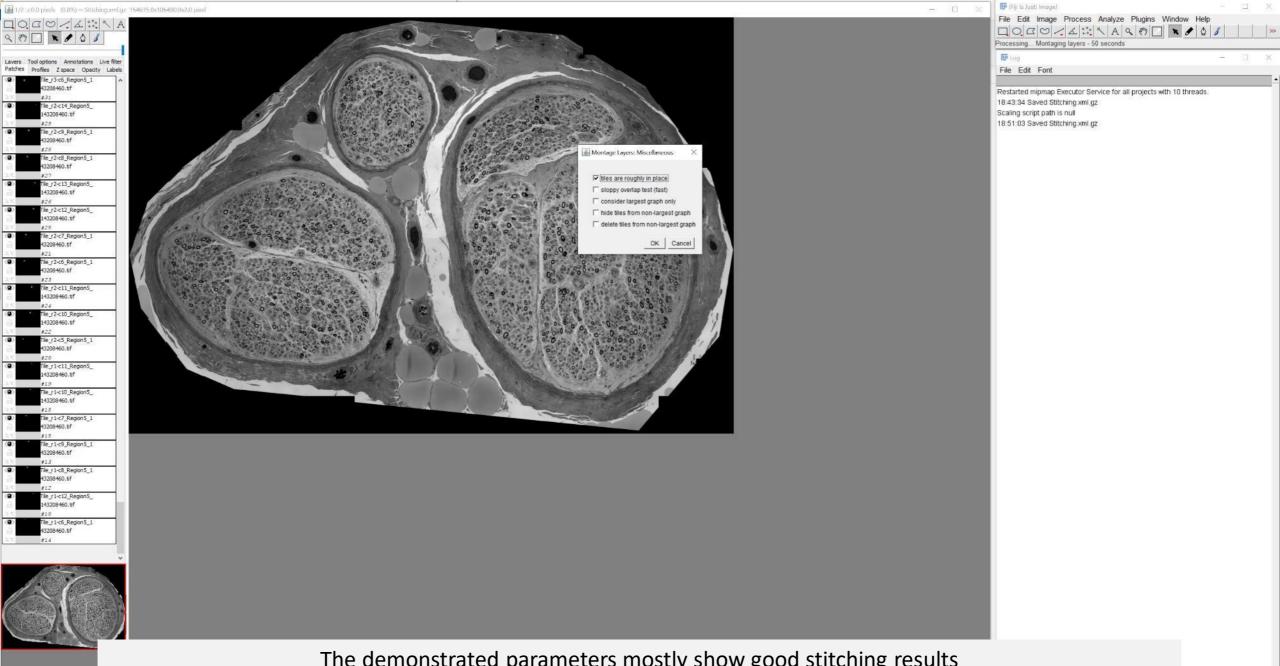


The demonstrated parameters mostly show good stitching results

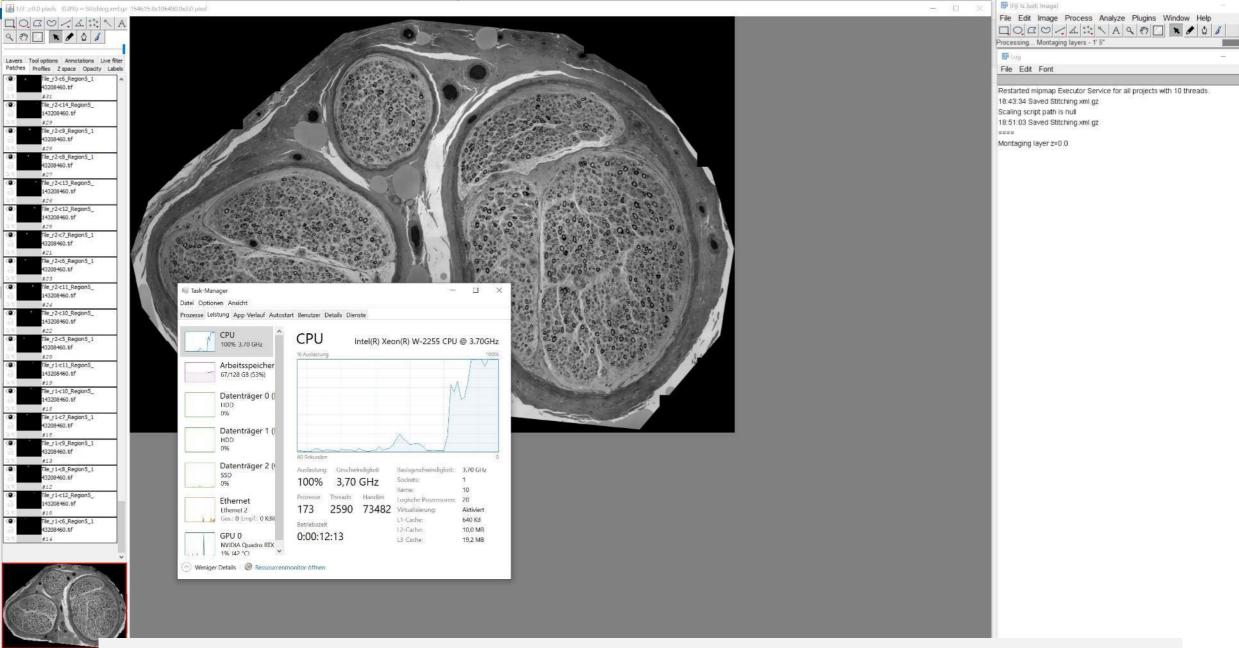




The demonstrated parameters mostly show good stitching results



The demonstrated parameters mostly show good stitching results



See information in Log

See Task Manager for workstation performance (multiple cores can be used for stitching)

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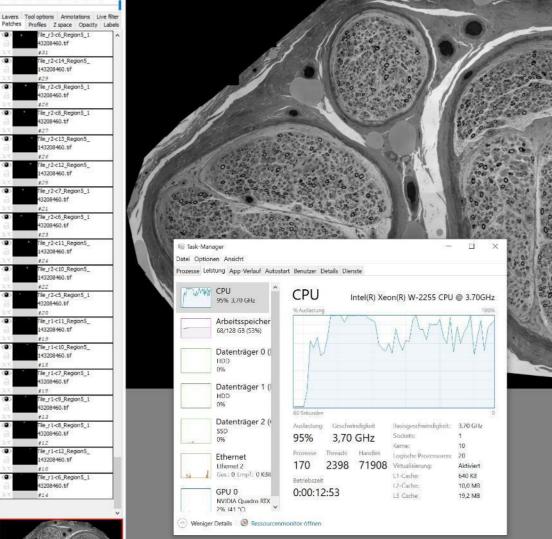
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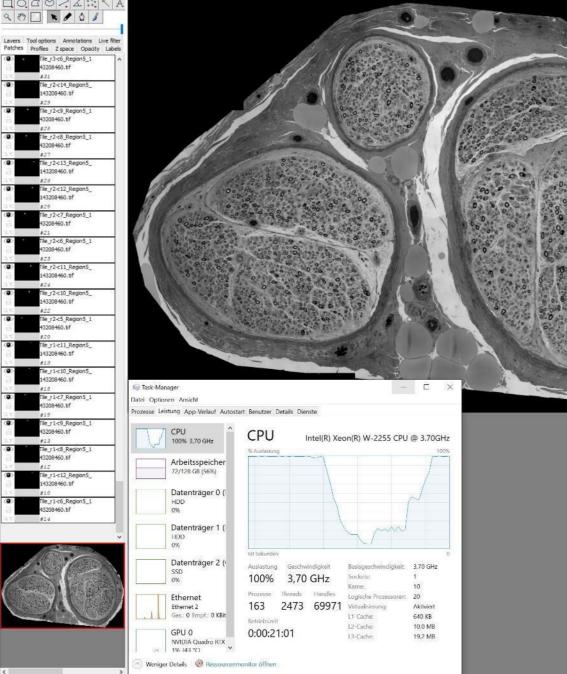
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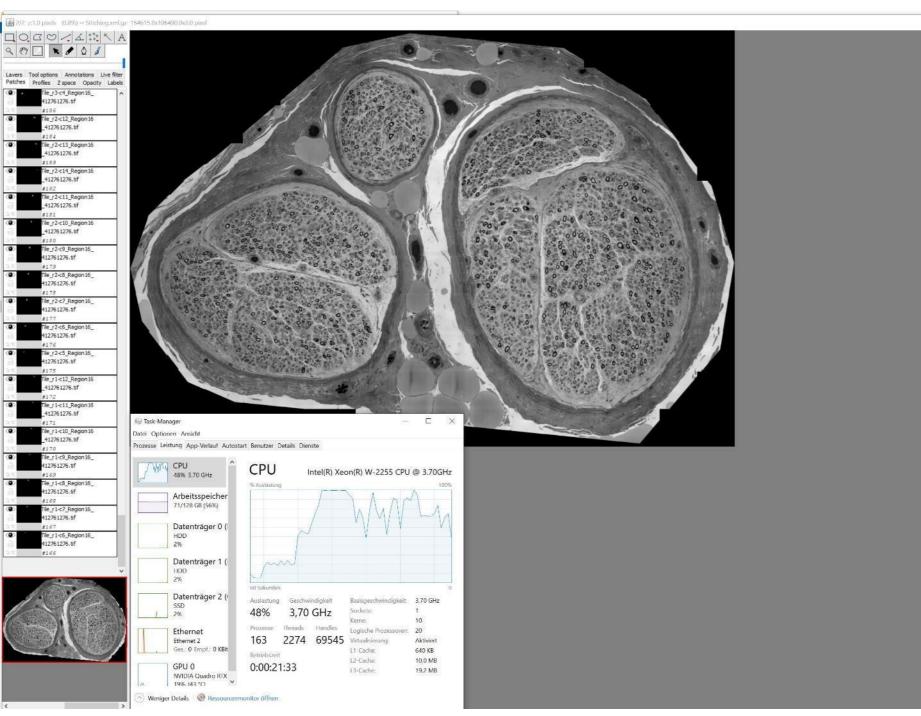
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3,70 GHz

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Downloads

👃 Musik

Videos

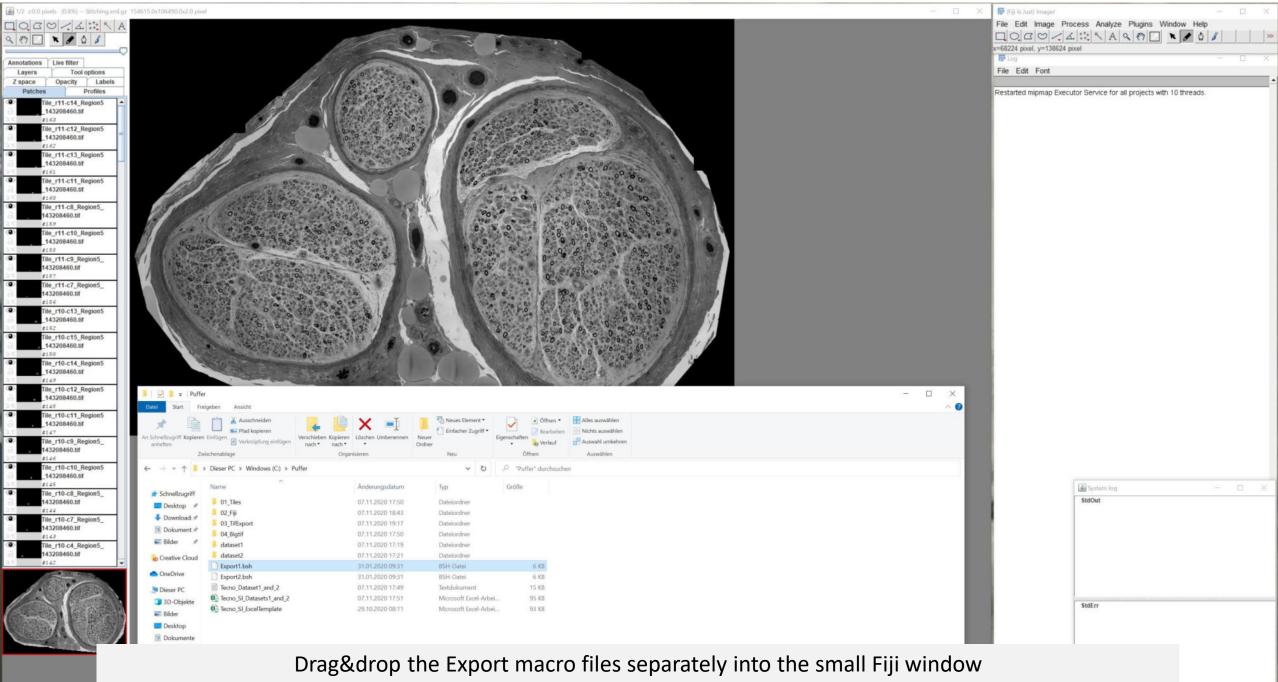
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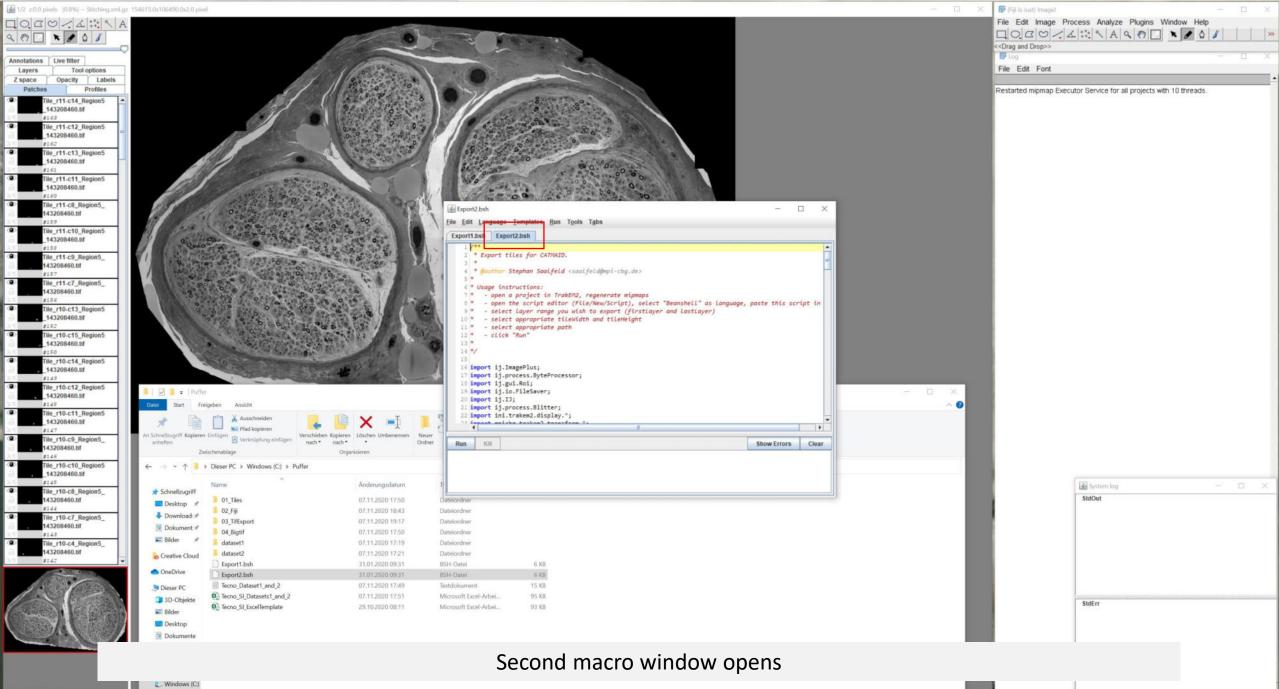
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Prepare Export.bsh (macros; see online methods for source) for each dataset to be exported



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The_r11-c9_Region5_ 14320840.tf 14320840.tf		
Tile_r11-c7_Region5 Usage instructions:		
143200400.01 7 - open a project in TrakEN2, regenerate mipmaps #156 7 - open the script editor (File/New/Script), select "Beanshell" as language, paste this script in • Tile_r10.c13_Region5 - select layer range you wish to export in (Firstlayer and lastlayer) • 143200460.01 - select appropriate tileWidth and tileWidth and tileWidth		
#152 11 * - select appropriate path Tile_r10-c15_Region5 12 * - click "Run"		
142008460.0f #150 The_rt0-ct4_Region5		
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● OneDrive Export2.bsh 31.012020 09:31 BSH-Datei 6 KB		
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Desktop		
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2. Windows (C)		
Volume (D:)		4



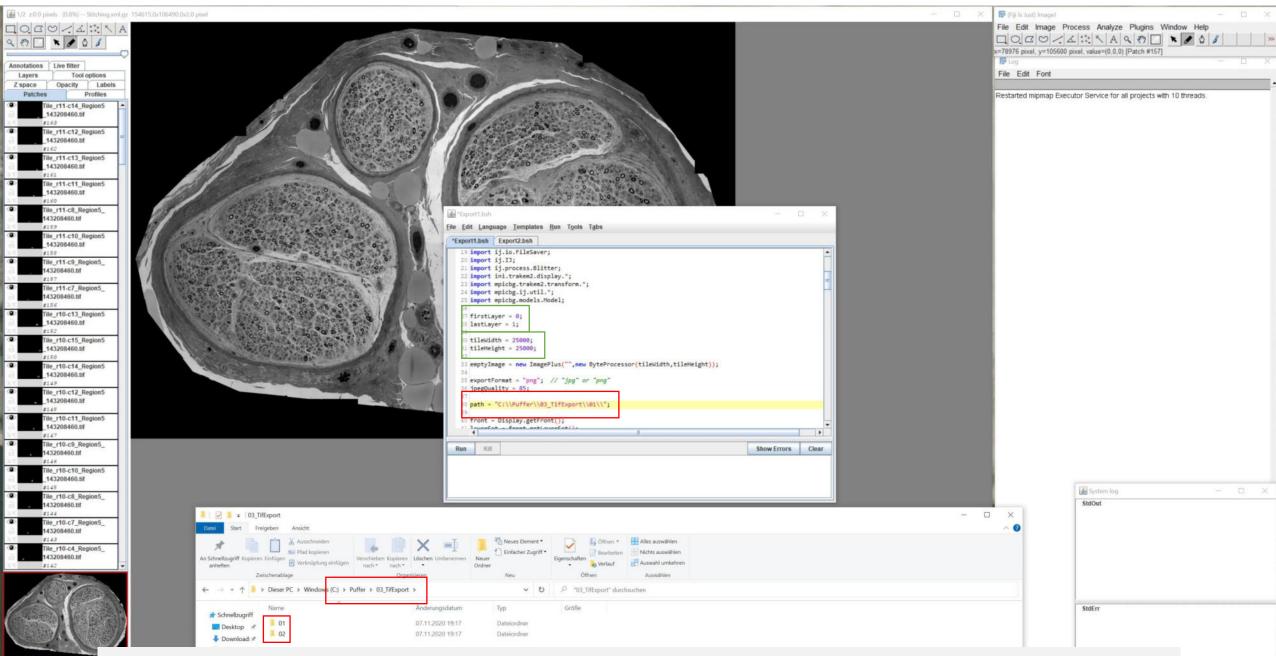
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		ge Process Analyze Plugins Window Help 	<i> </i> >>
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2 143 Tile_r11-c12_Region5 143208460.01 143208460.0			
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0 116_11.69_Region5_ 143208460.01 1 2 * Export tiles for CATMAID. 1 0 143208460.01 3 4 * Export tiles for CATMAID. 1 0 143208460.01 3 4 * Export tiles for CATMAID. 1			
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INE_110-C13_Region5 143208460.trt 152 INE_110-C15_Region5			
13 <i>i</i> ±80 TBe_r10-c14_Region5 143208460.uf 15 16 import ij.ImagePlus;			
#149 17 isport ij.process.ByteProcessor; 10 Tile_rflo.r12_Region5 143208460.iif 143208460.iif 143208460.iif 19 isport ij.process.ByteProcessor;			
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		StdErr	
Switch to Export1.bsh macro			

• 8

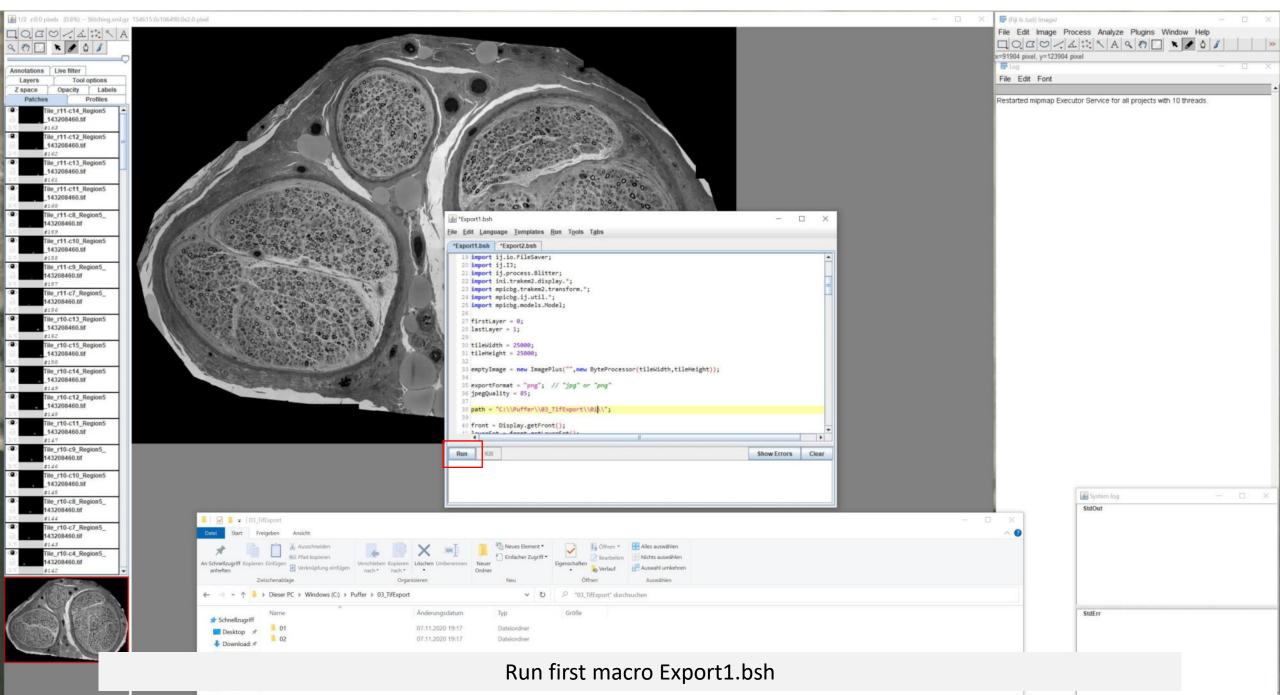
+

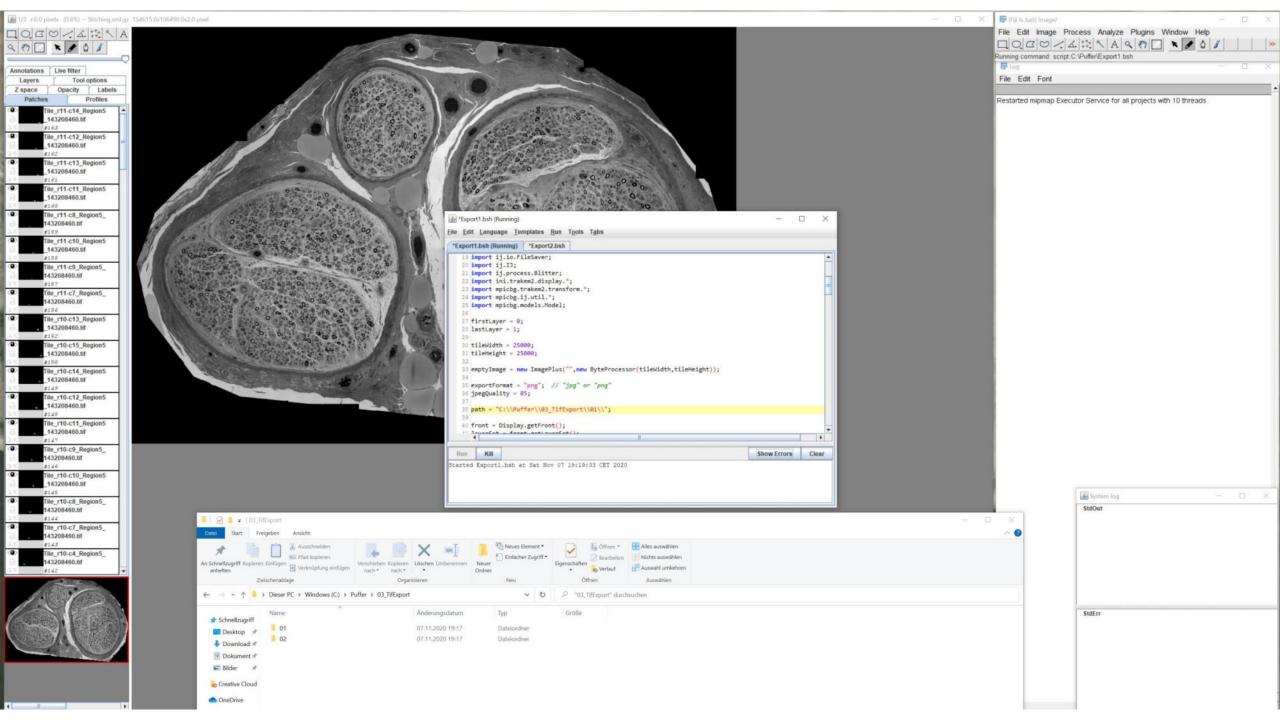


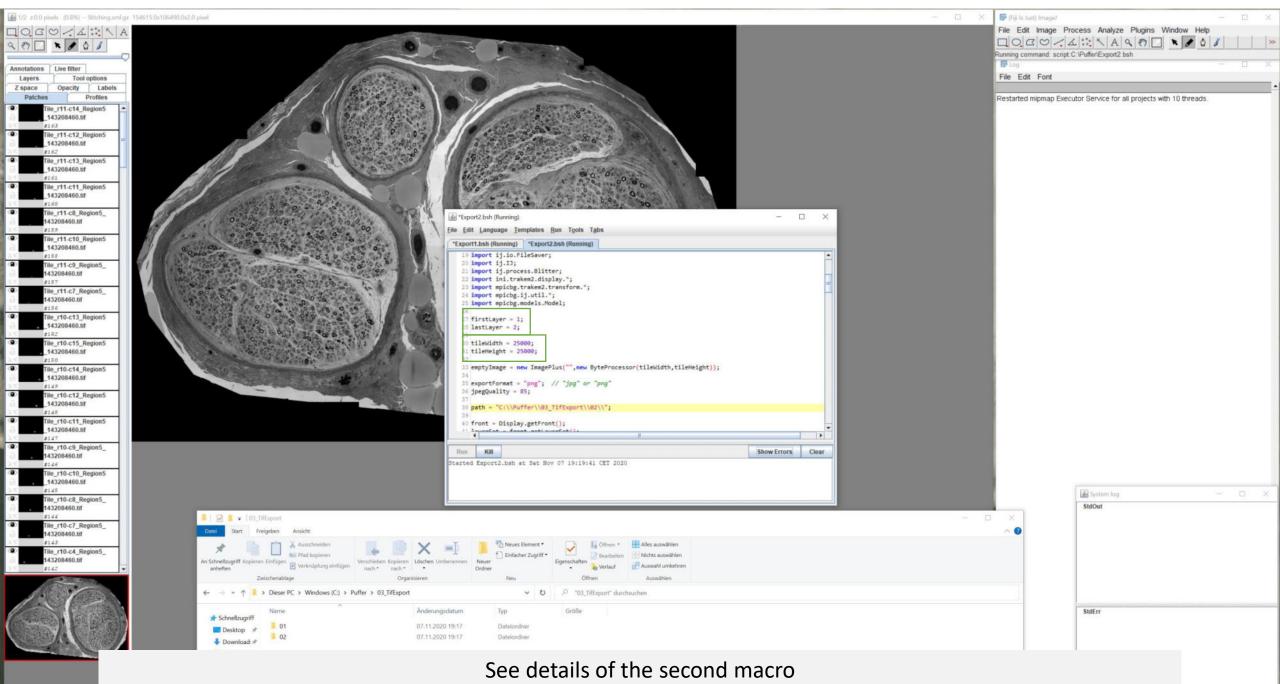
Prepare individual output folders for both datasets and select the respective path in the macros See details for export parameter (green; layer, tile dimensions)

🕌 *Export2.bsh (Running)	- 🗆	×	
ile <u>E</u> dit <u>L</u> anguage <u>T</u> emplates <u>R</u> un T <u>o</u> ols T <u>a</u> bs			<u>File Edit Language Templates Run Tools Tabs</u>
*Export1.bsh (Running) *Export2.bsh (Running)			*Export1.bsh (Running) *Export2.bsh (Running)
<pre>2 Experiment (uninny) Experiment (uninny) 47 } 48 left = roi.getBounds().x; 49 top = roi.getBounds().width; 50 w = roi.getBounds().height; 52 53 ImagePlus openAndDelete(path) 54 { 55 file = new File(path); 56 if (file.exists()) 57 { 58 imp = new ImagePlus(path); 59 // file.delete(); 60 return imp; 61 } 62 else 63 return emptyImage; 64 } 65 66 emptySections = new ArrayList(); 67 68 for (int l = firstLayer; l <= lastLayer; ++1) 4 Run Kill Started Export2.bah at Sat Nov 07 19:19:41 CET 2020</pre>		Þar	<pre> layerse layersecropec() gecome () gecome () gecome ge(</pre>

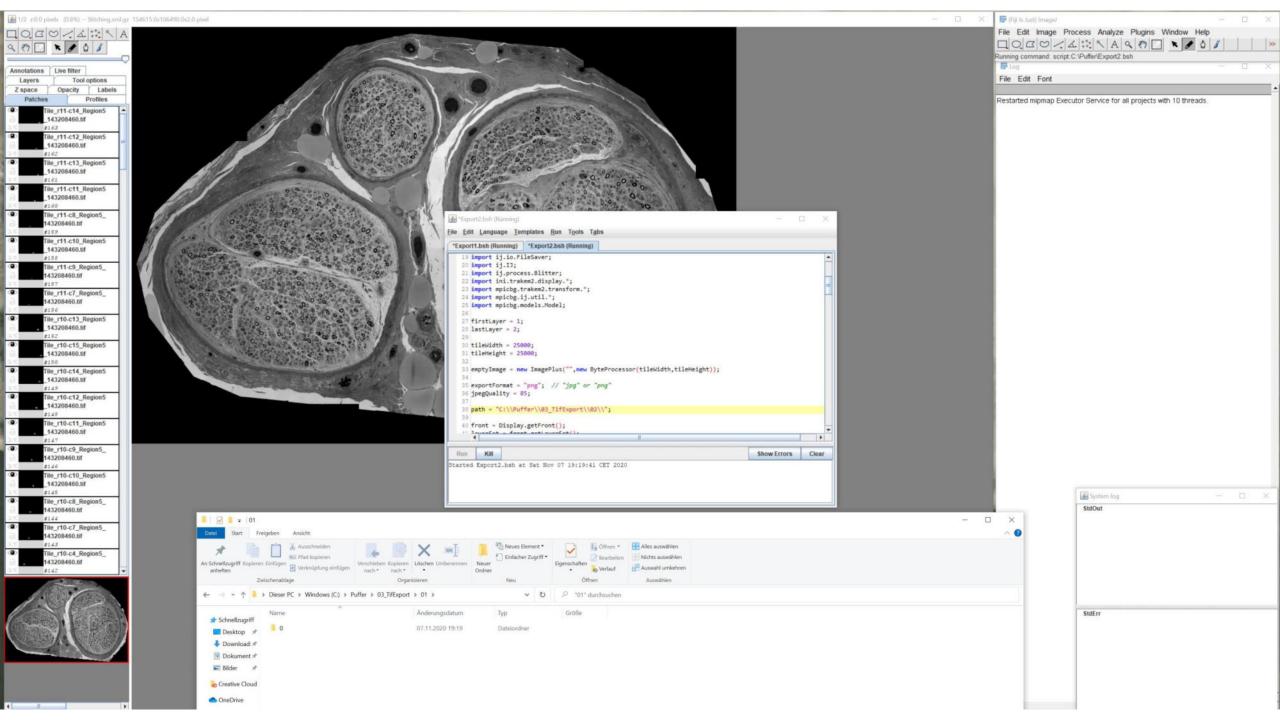
Modifications of the CATMAID macro to only export large tif tiles Left; line 59 commented to avoid that the large tiles are deleted Right; lines 101-108 commented to only export tif tiles







Run second macro Export2.bsh



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2 4145 23 import mpicbg.trakem2.transform.";	Uindows (C:)		
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143208460.uf 20 lastLayer = 1; 29	🧼 Netzwerk		
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143208460.mf 31 tlieheight = 25000; 22 2142 32 33 emptyImage = new ImagePlus("",new ByteProcessor(tileWidth,tileHeight));			
34	4_0_0 4_1_0	4_2_0 4_3_0	4_4_0 4_5_0
35 exportFormat = "png"; // "jpg" or "png" 36 jpegQuality = 85;			
37 30 path = "C:\\Puffer\\03_TifExport\\01\\";			
39 40 front = Display.getFront();			
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Non-overlapping tif tiles of both datasets were exported

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8 . 1	le_r10-c11_Region5 143208460.tif	Elie Edit Language Templates Run Tools Tabs	1
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C 18	le_r10-c10_Region5 143208460.tif	21 import ij.process.Blitter;	
2.5 #1	145	23 import mic traxema.usplay; 23 import mic traxema; 24 Windows (C)	
	le_r10-c8_Region5_ 43208460.tif	24 import mpicbg.ij.util."; 25 import mpicbg.models.Model;	
	l 44 le_r10-c7_Region5_	26 27 firstLayer = 1;	
8 . 14	13208460.tif	28 lastLayer = 2; Image: State of the state	
	l 43 le_r10-c4_Region5_	30 tileWidth = 25000;	
8 . 14	43208460.tif	31 tileHeight = 25000; 32	
1	A COLORADO AND A COLO	<pre>33 emptyImage = new ImagePlus("",new ByteProcessor(tileWidth,tileHeight)); 34</pre>	
15		35 exportFormat = "png"; // "jpg" or "png" 36 jpegQuality = 85;	
1		37 38 path = "C:\\Puffer\\03_TifExport\\02\\";	
Contraction of the		39 40 front = Display.getFront();	
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and the second		Run Kill Show Output Clear	
		Sourced file: null : Object constructor : at Line: 147 : in file: <unknown file=""> : new ByteFrocessor (tile</unknown>	
		Width * 2 , tileHeight * 2)	
		Target exception: java.lang.NegativeArraySizeException	
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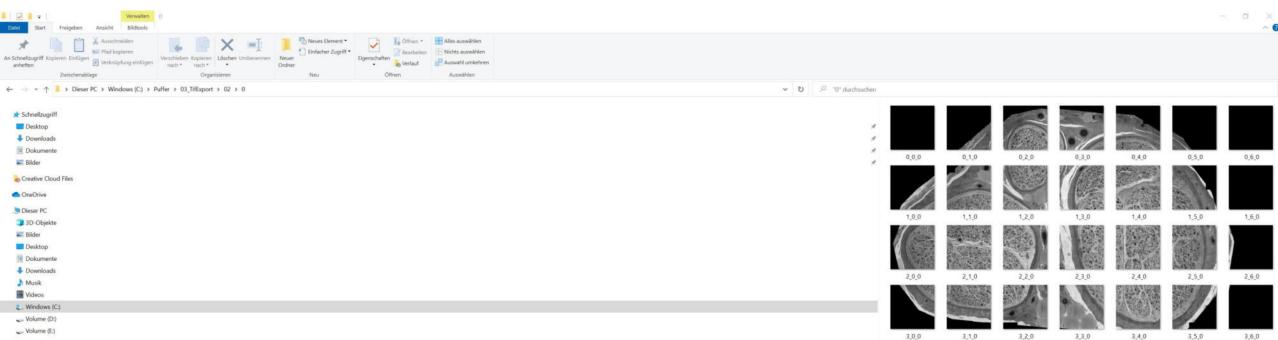
Netzwerk

Tiles with low amounts of structural details might be deleted

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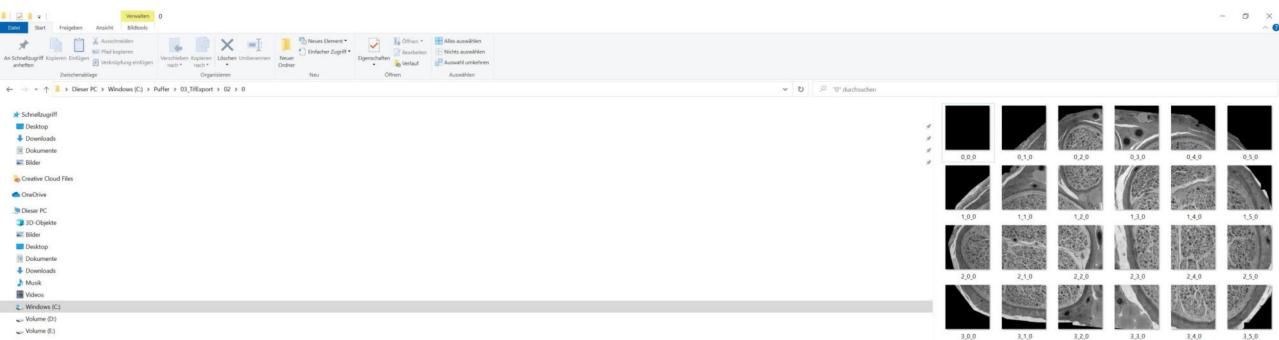
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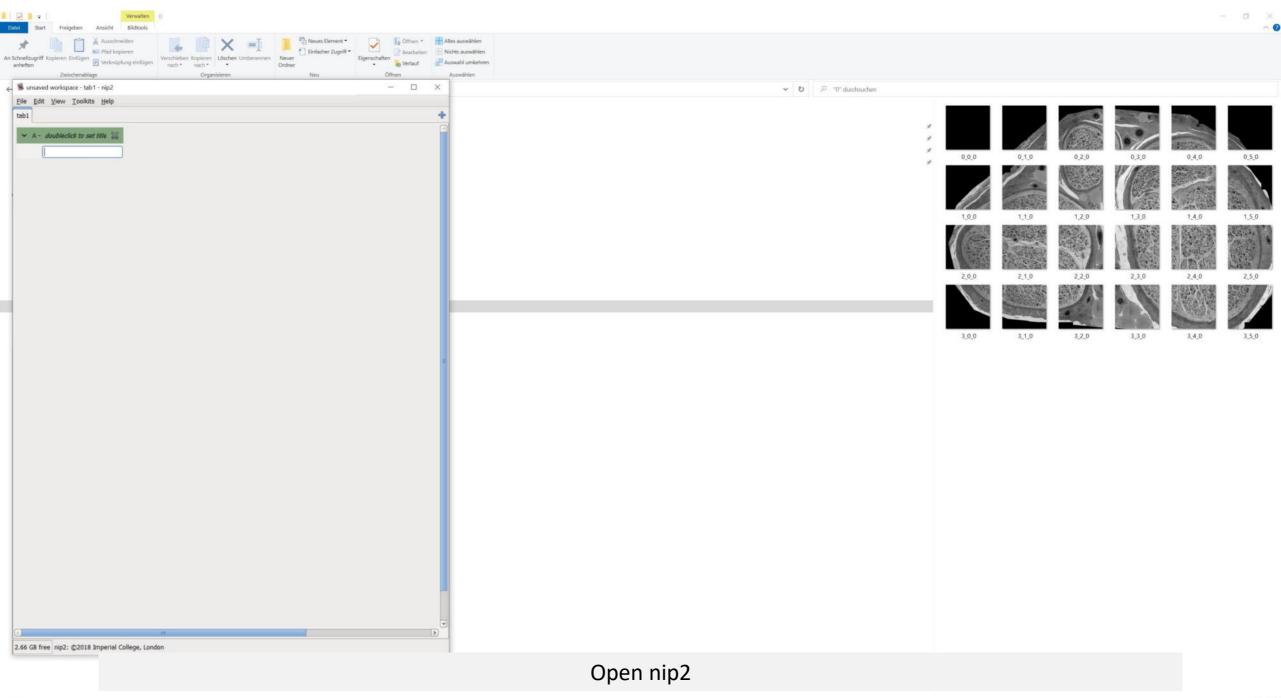
Netzwerk

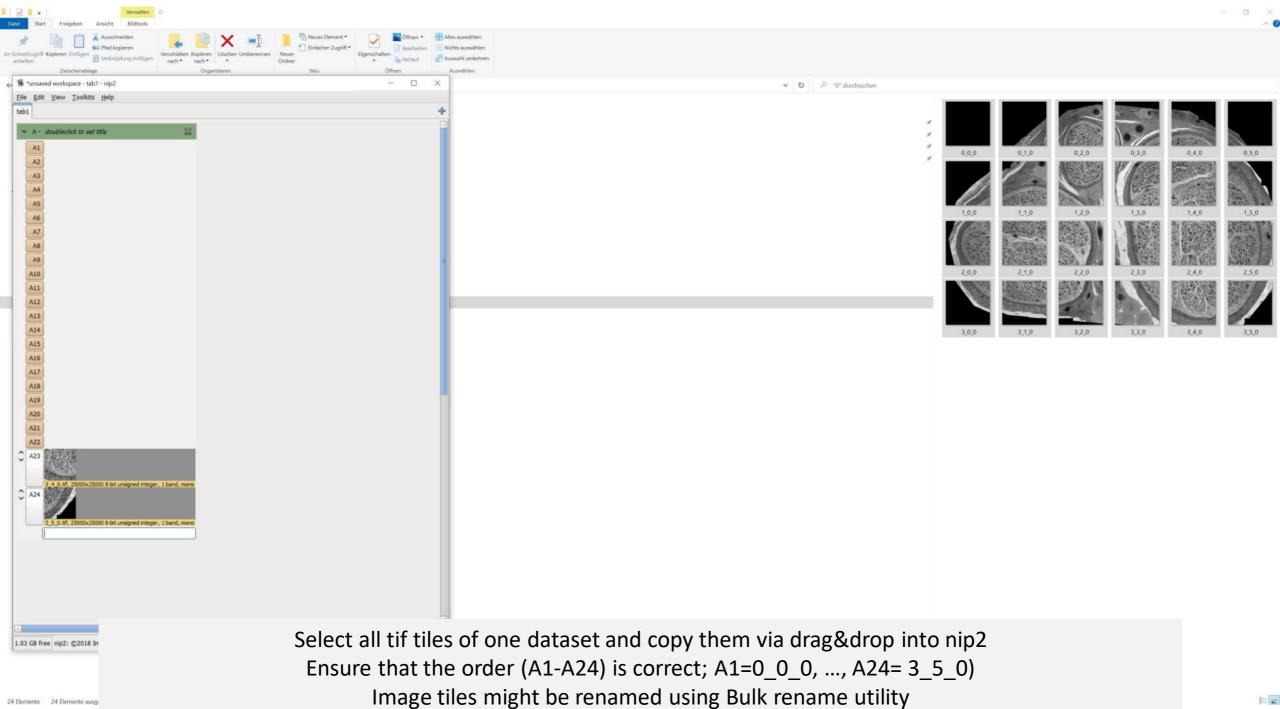
Tiles with low amounts of structural details might be deleted

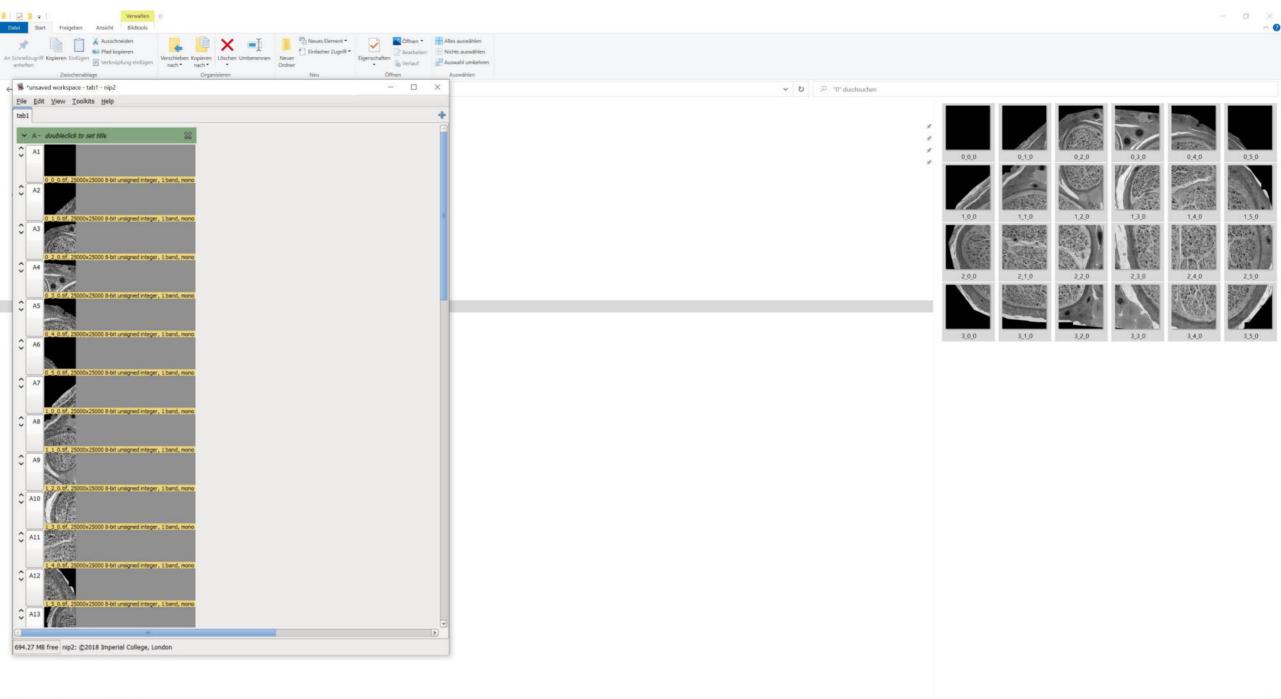


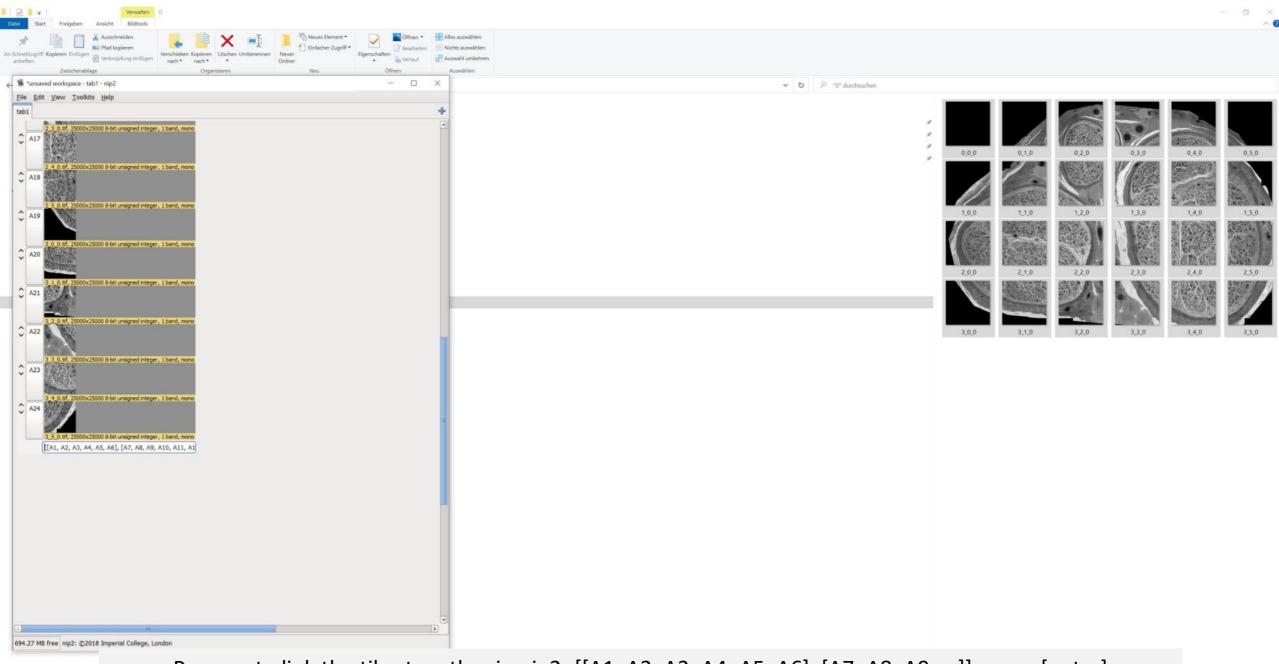
Netzwerk

Tiles with low amounts of structural details might be deleted

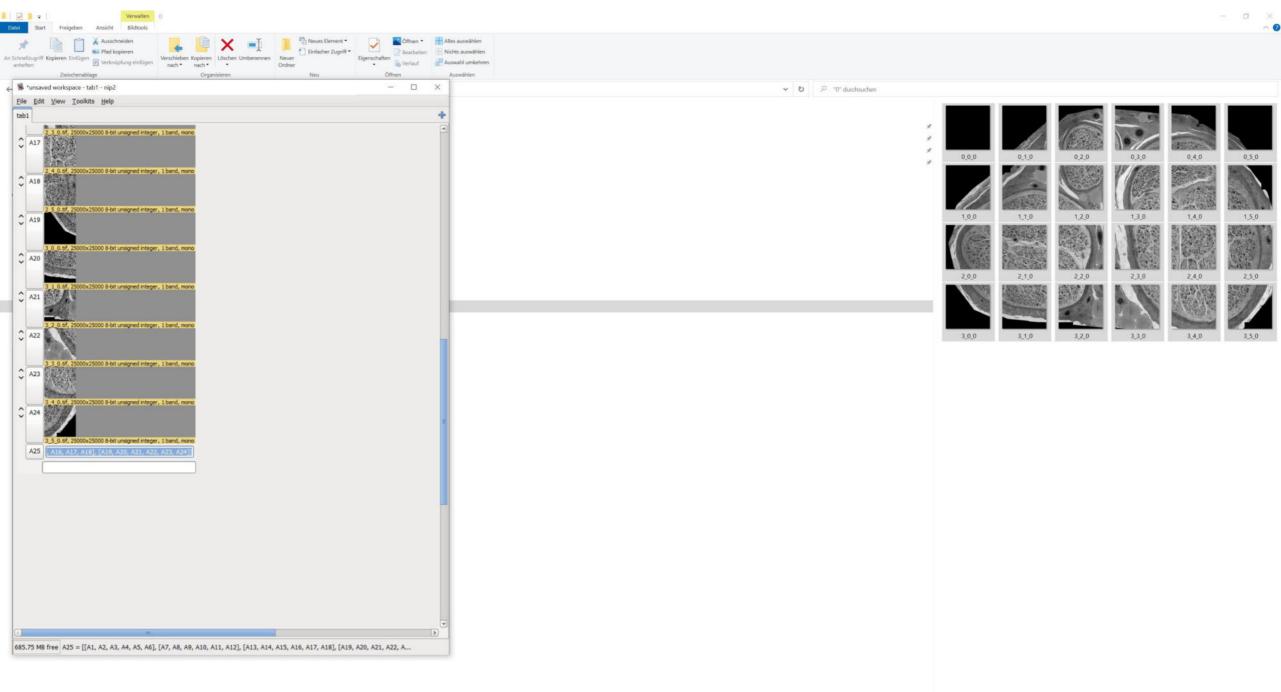


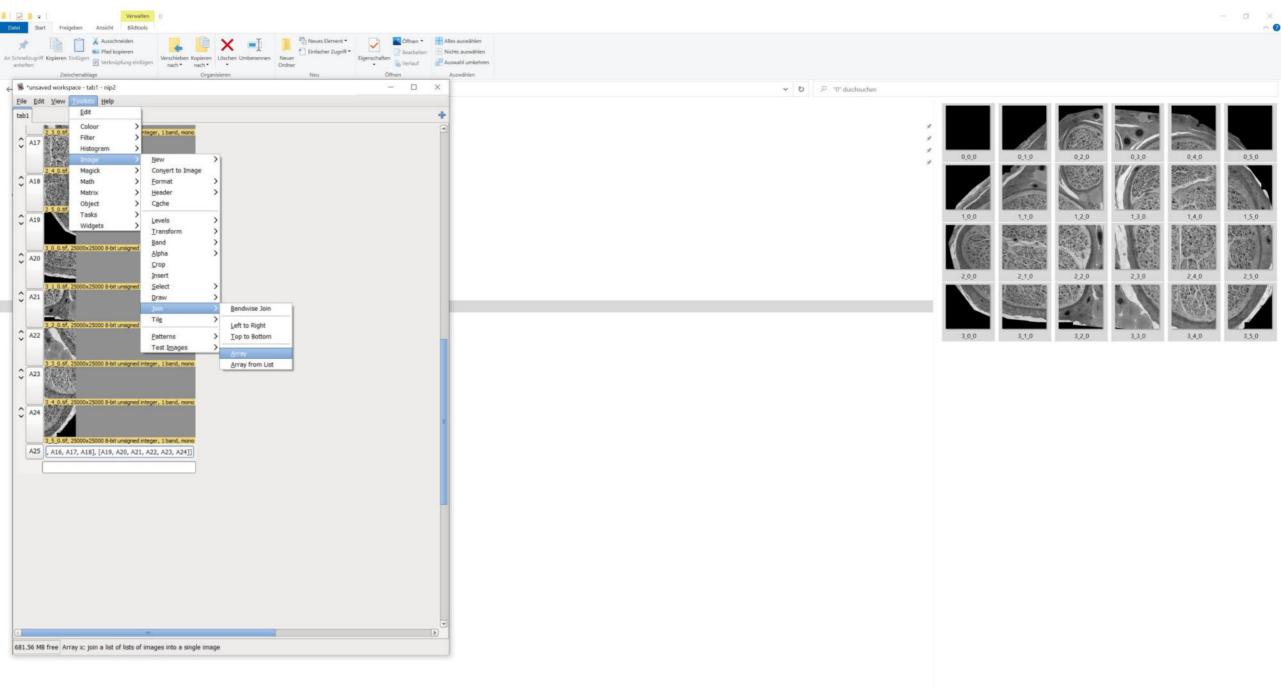


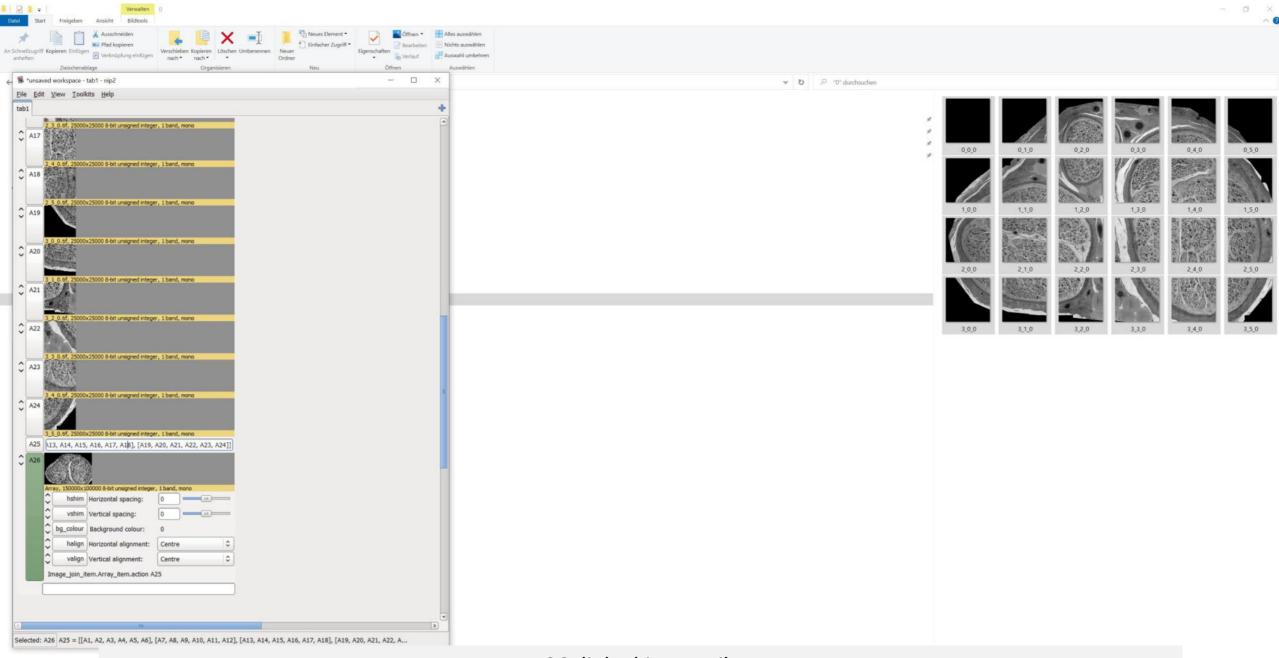




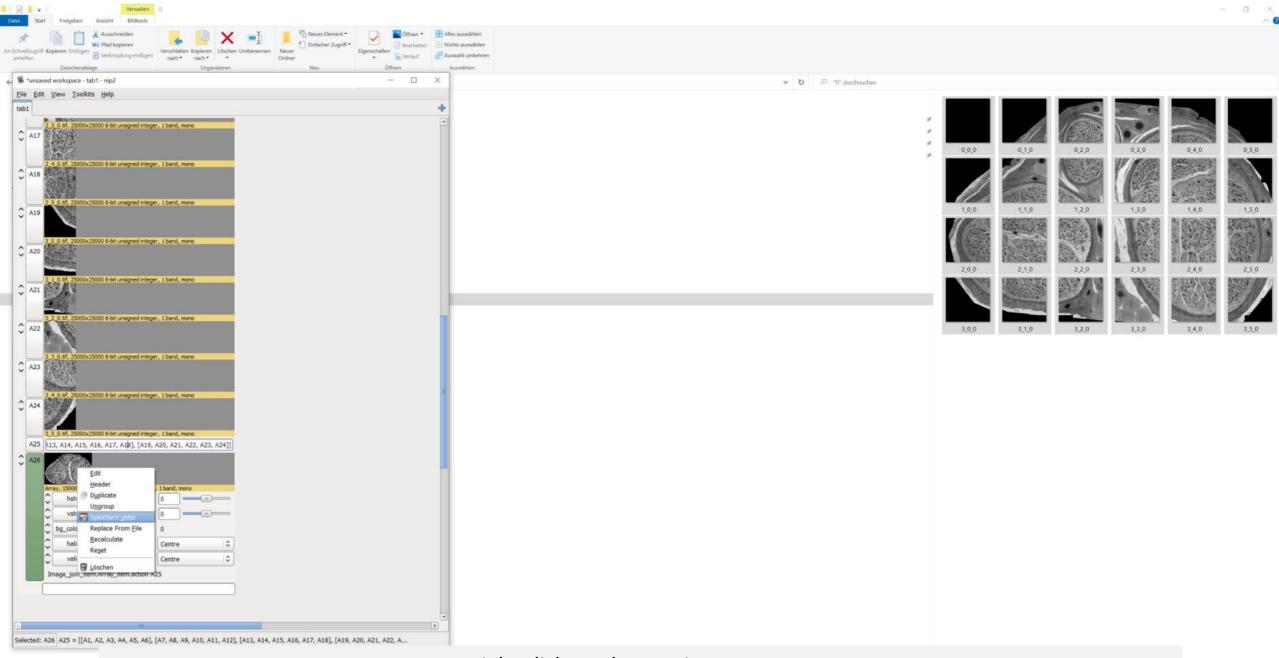
Prepare to link the tiles together in nip2; [[A1, A2, A3, A4, A5, A6], [A7, A8, A9, ...]], press [enter]



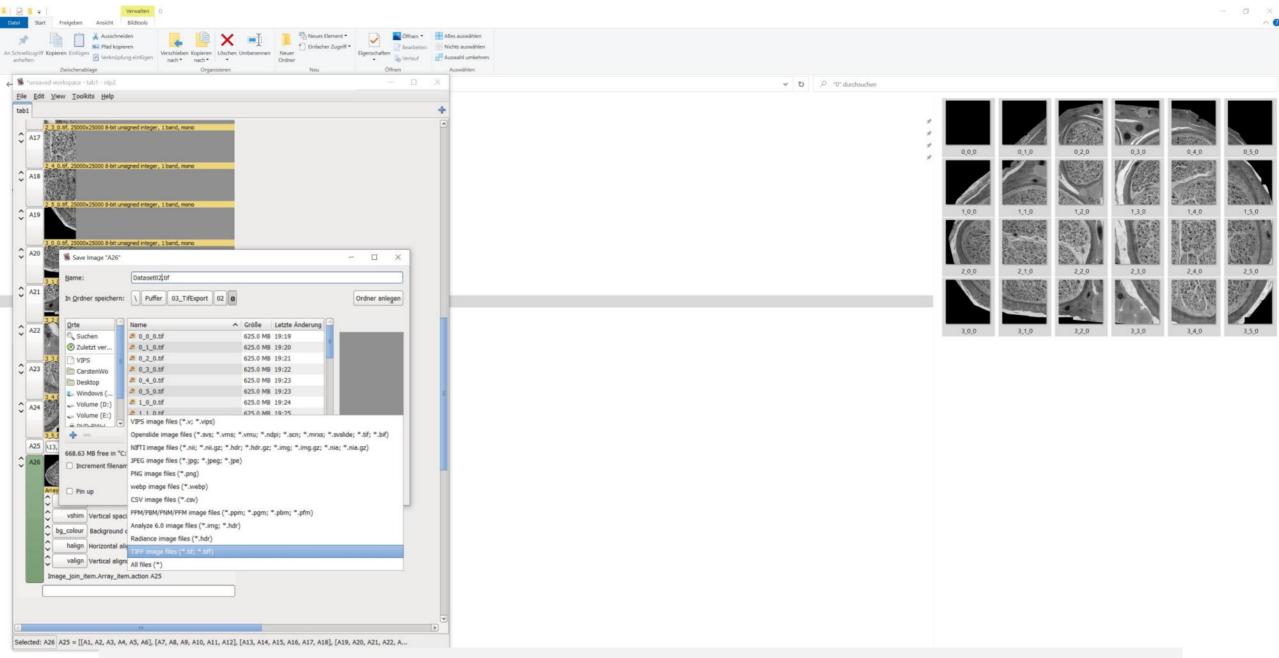




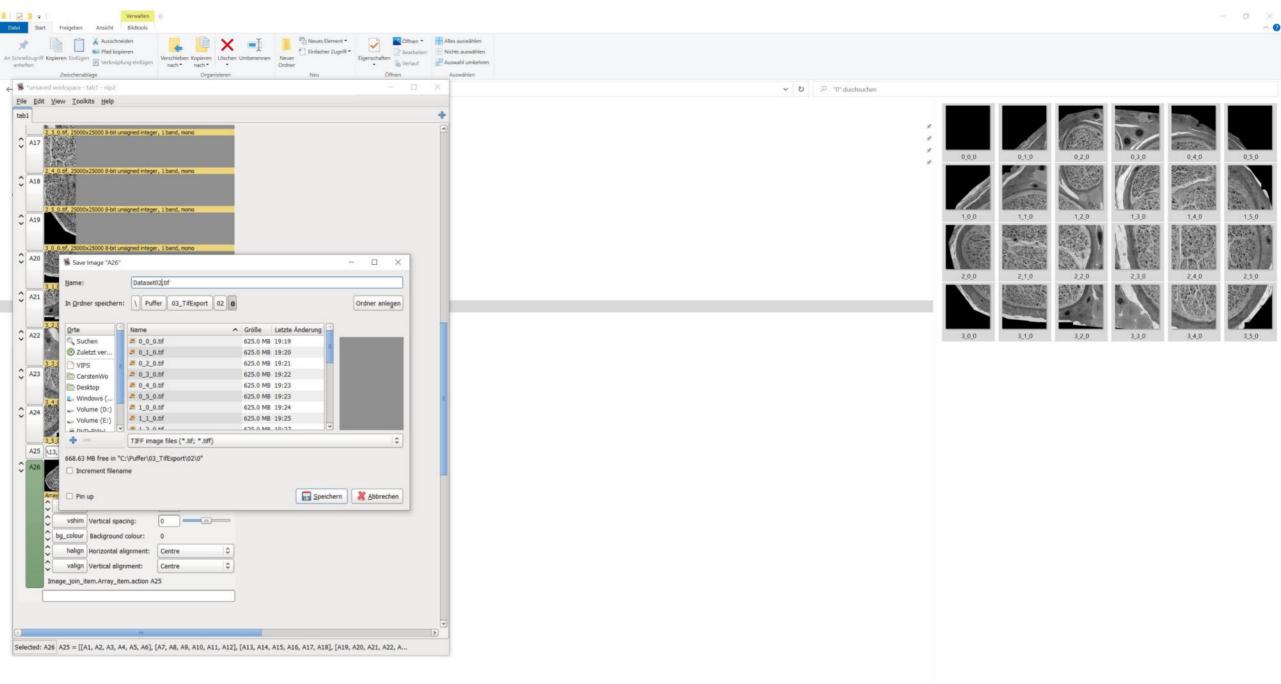
A26; linked image tiles

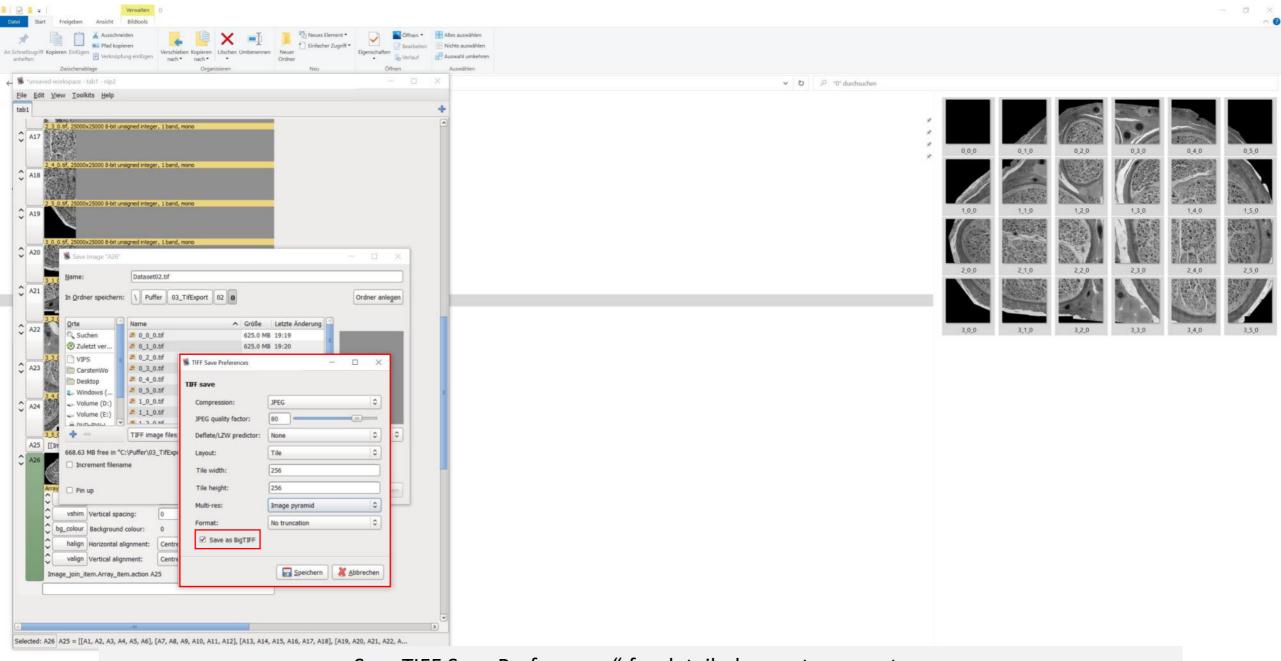


Right click on the preview-> save as



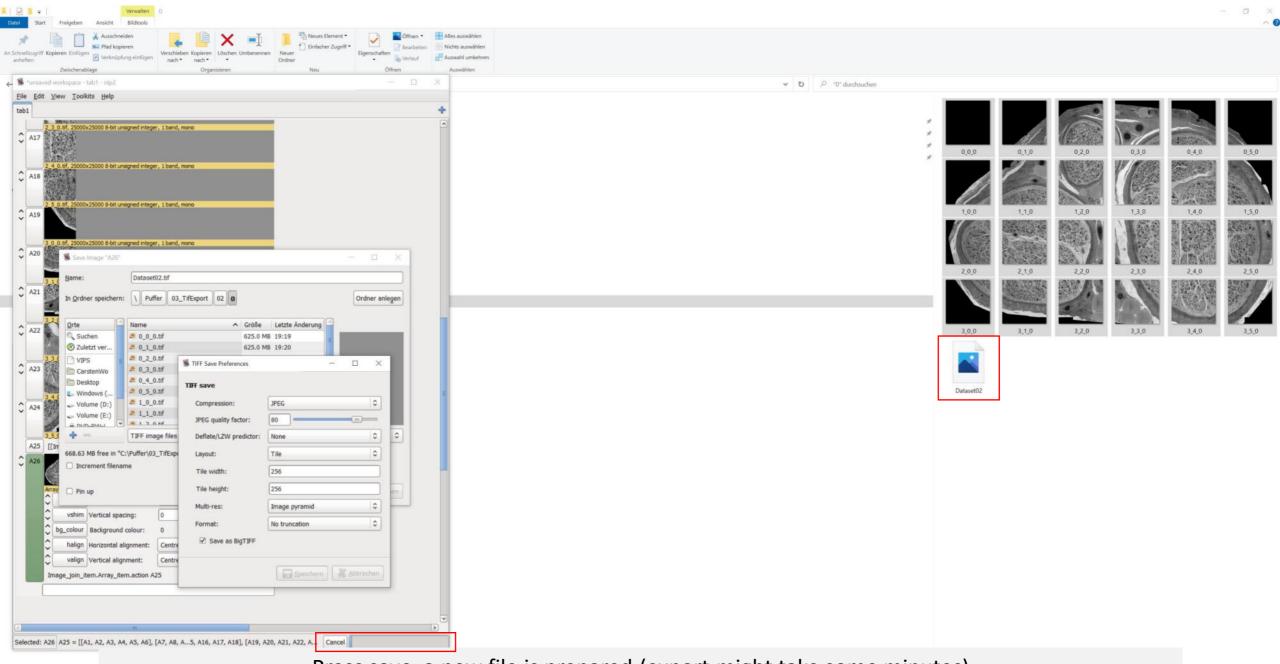
Save as TIFF



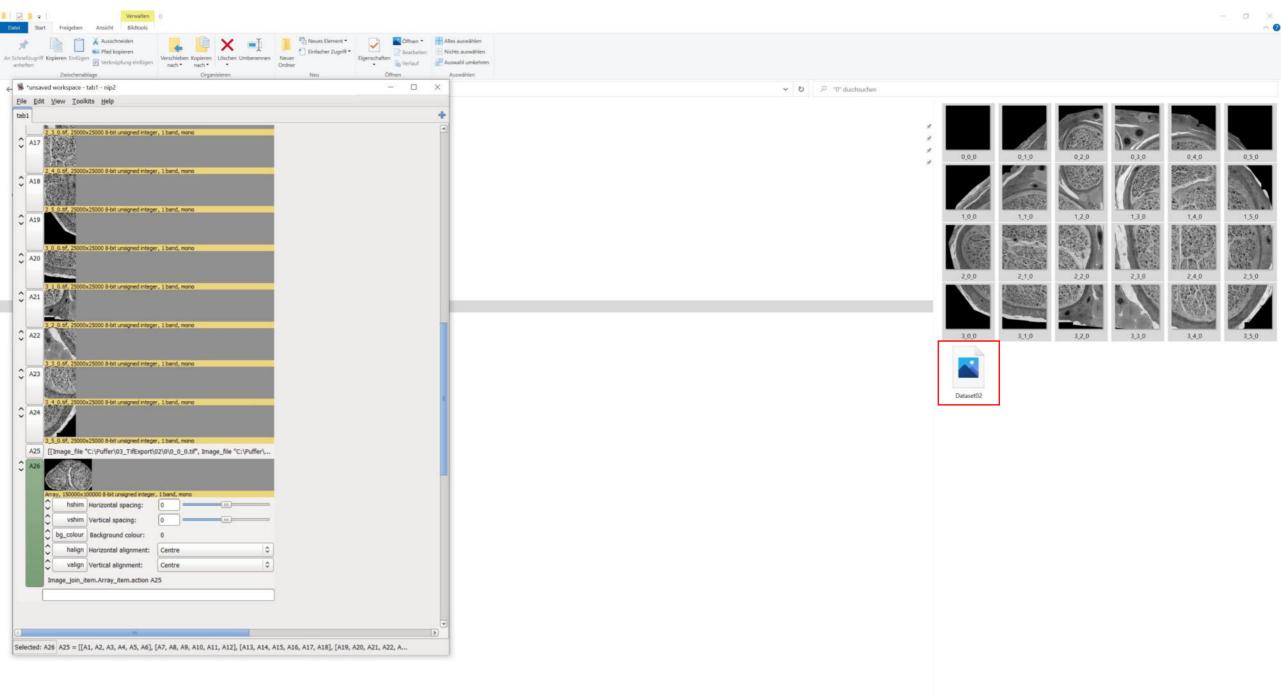


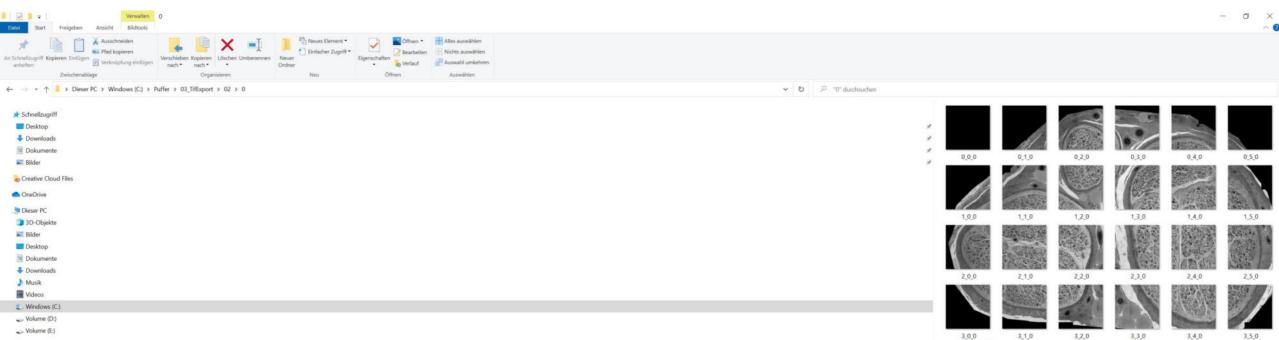
See "TIFF Save Preferences" for detailed export parameters

Check "Save as BigTIFF"



Press save; a new file is prepared (export might take some minutes)

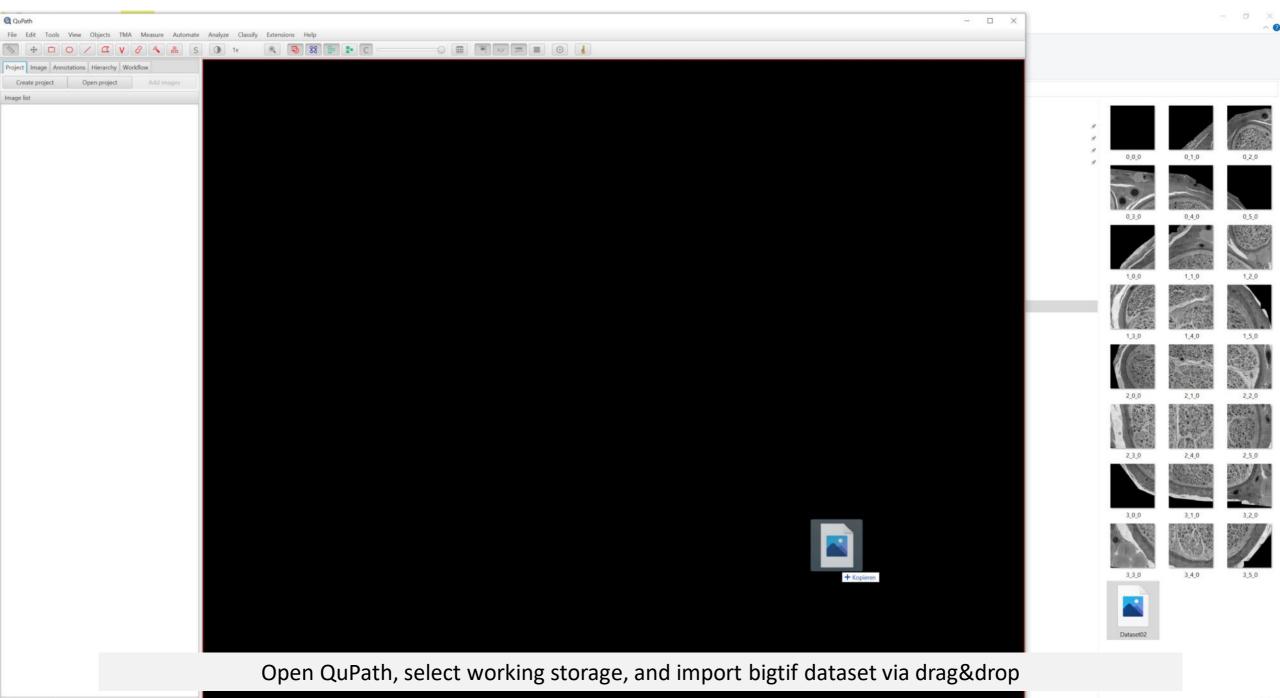




Dataset02

Netzwerk

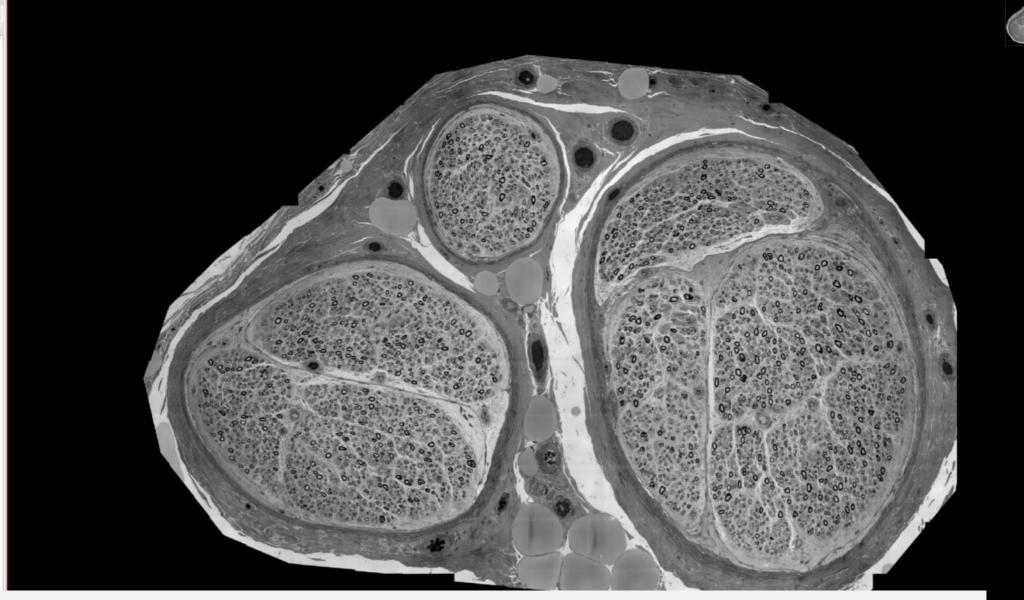
Size of the bigtif file; 3.35 GB





Project Image Annotations Hierarchy Workflow Create project Open project Add Images

Image list



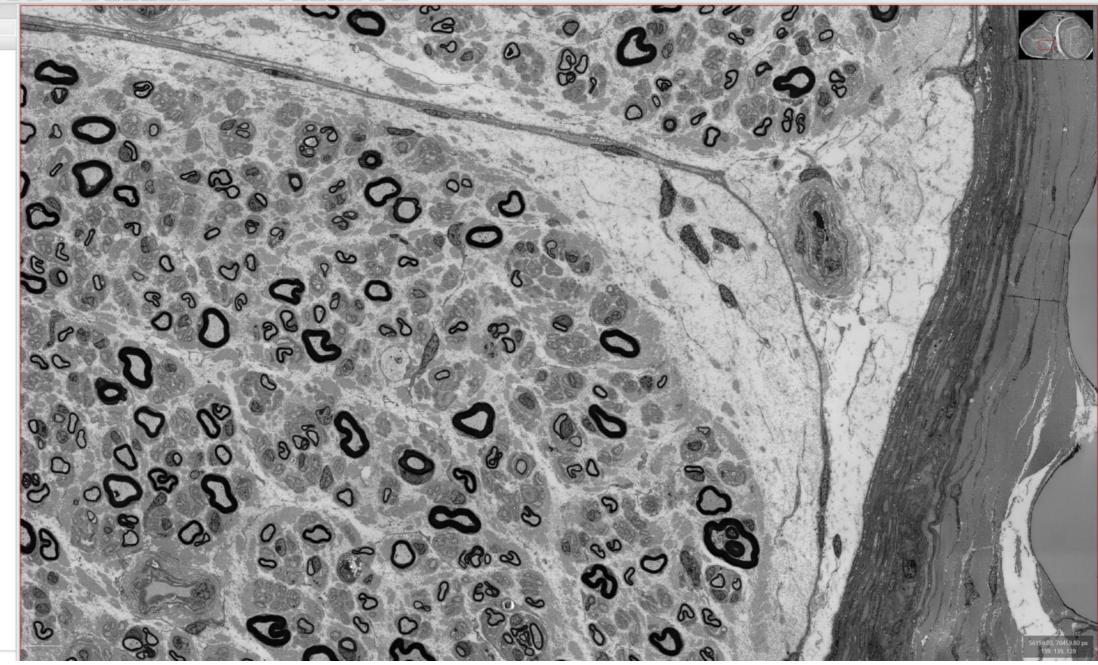
Pan-and-zoom analysis via mouse wheel

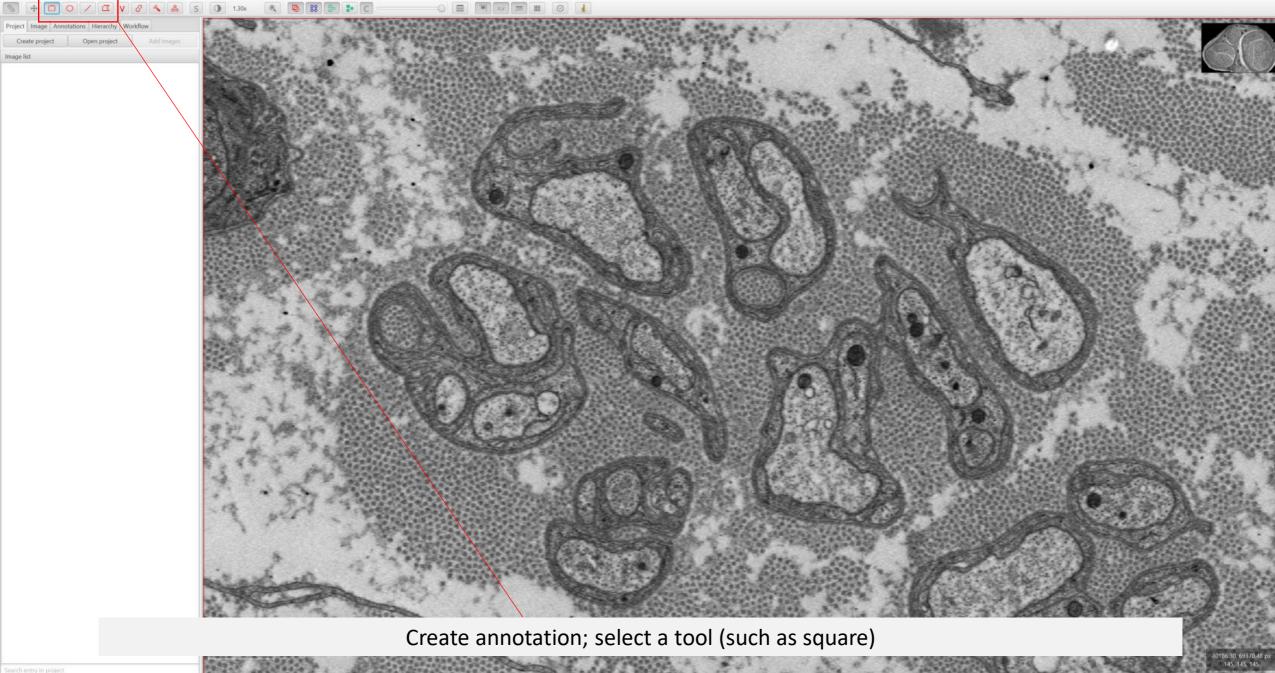
S+ C ----- E T x m C .

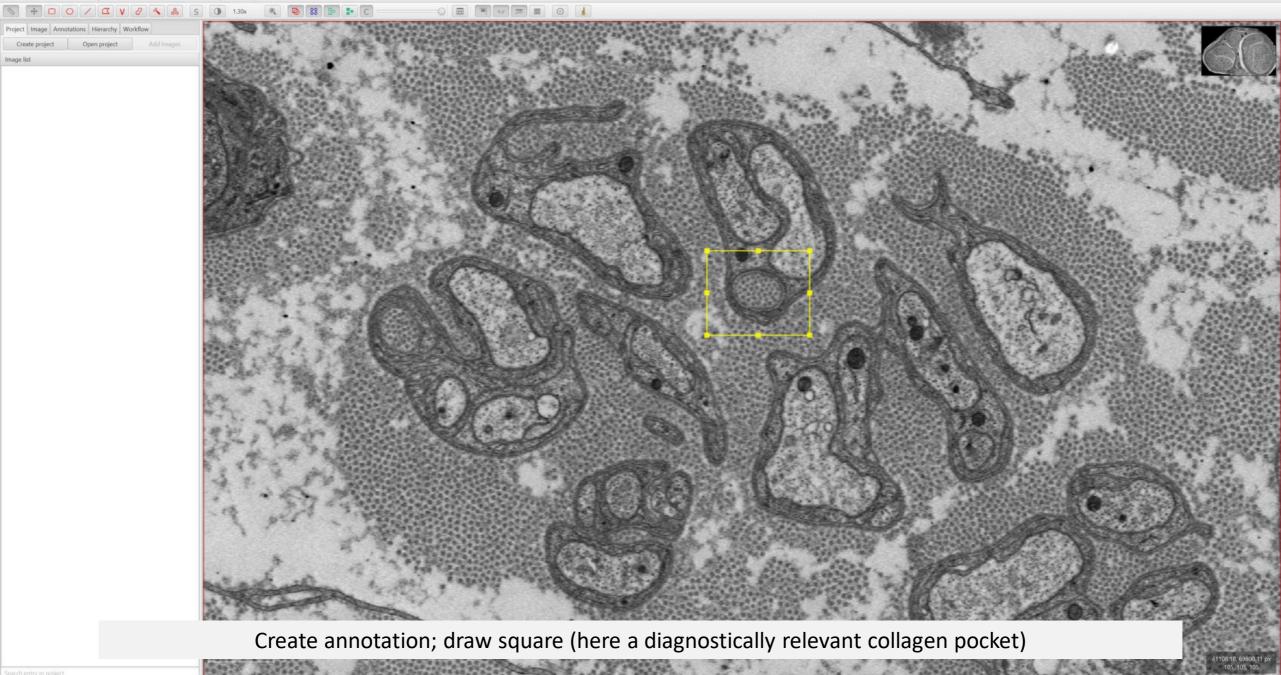
 Project
 Image
 Annotations
 Hierarchy
 Workflow

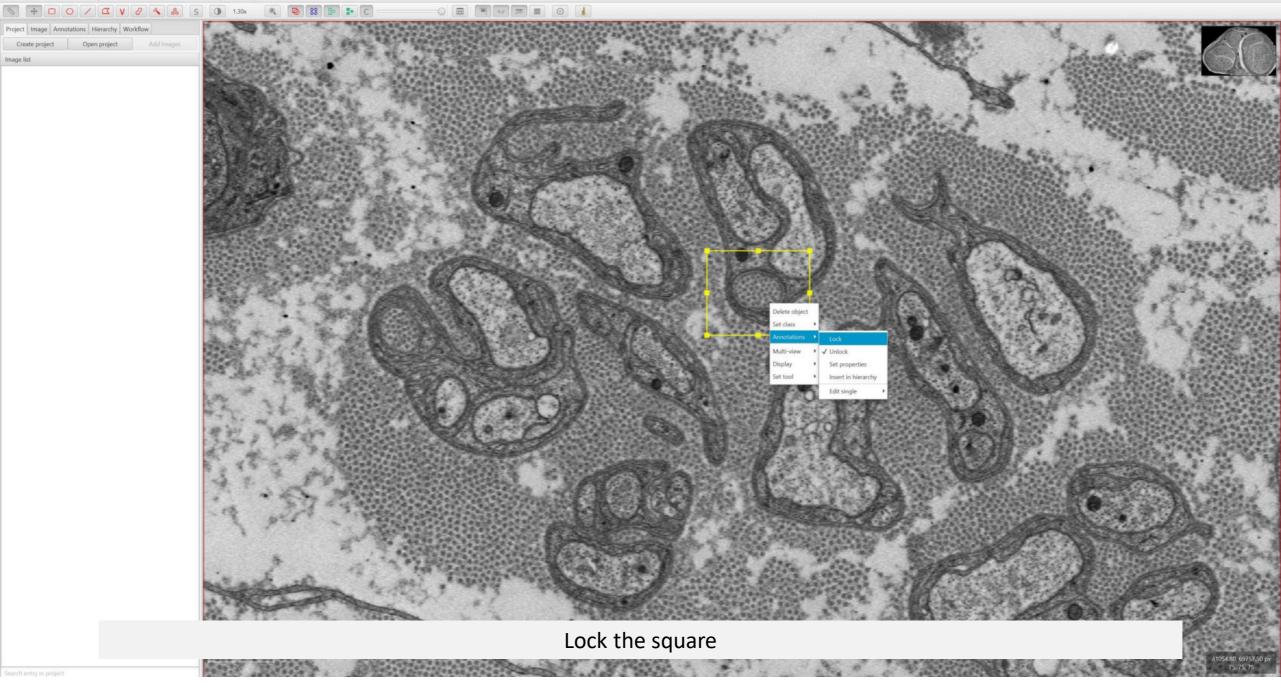
 Create project
 Open project
 Add images

 Image list
 Image list
 Image list





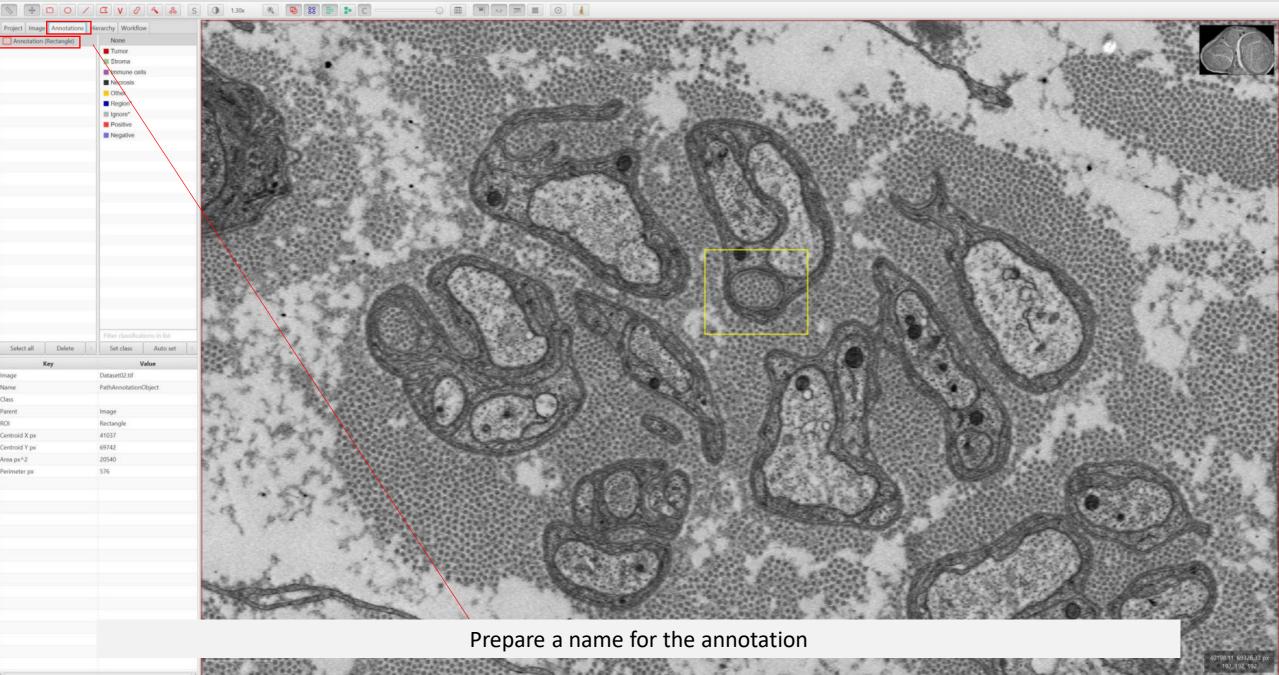




Image

Class Parent

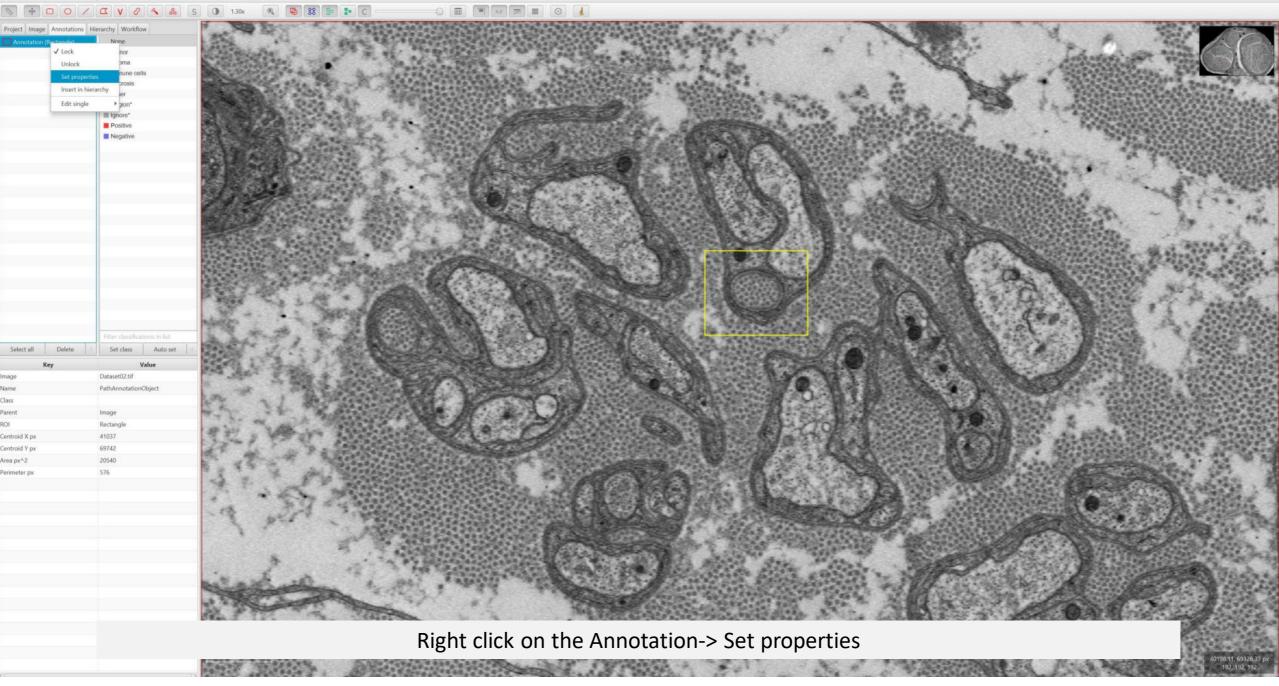
ROI



Image

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ROI



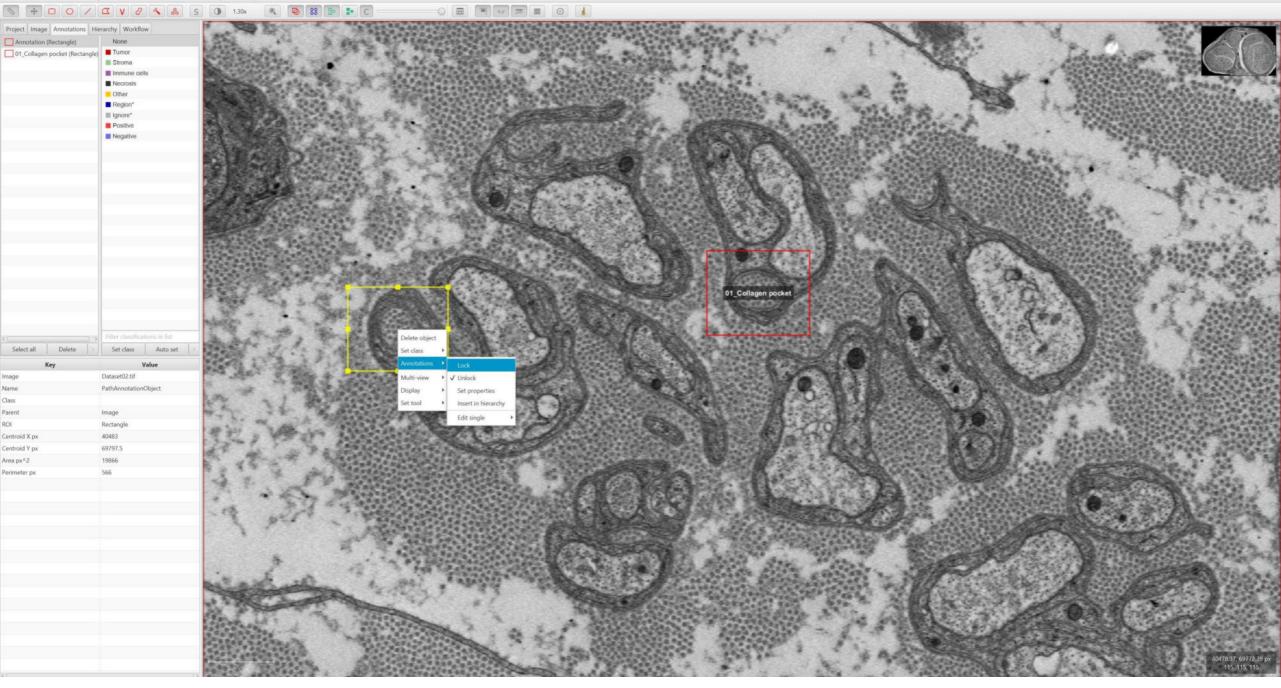
Class Parent

ROI

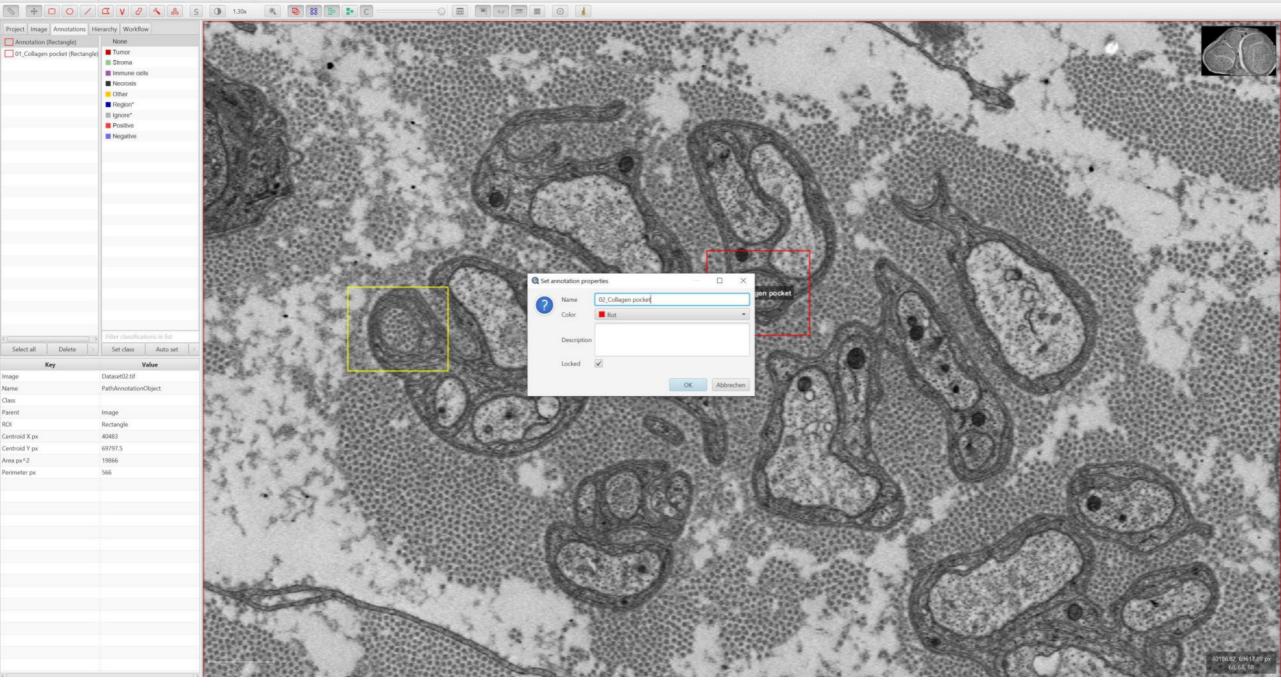


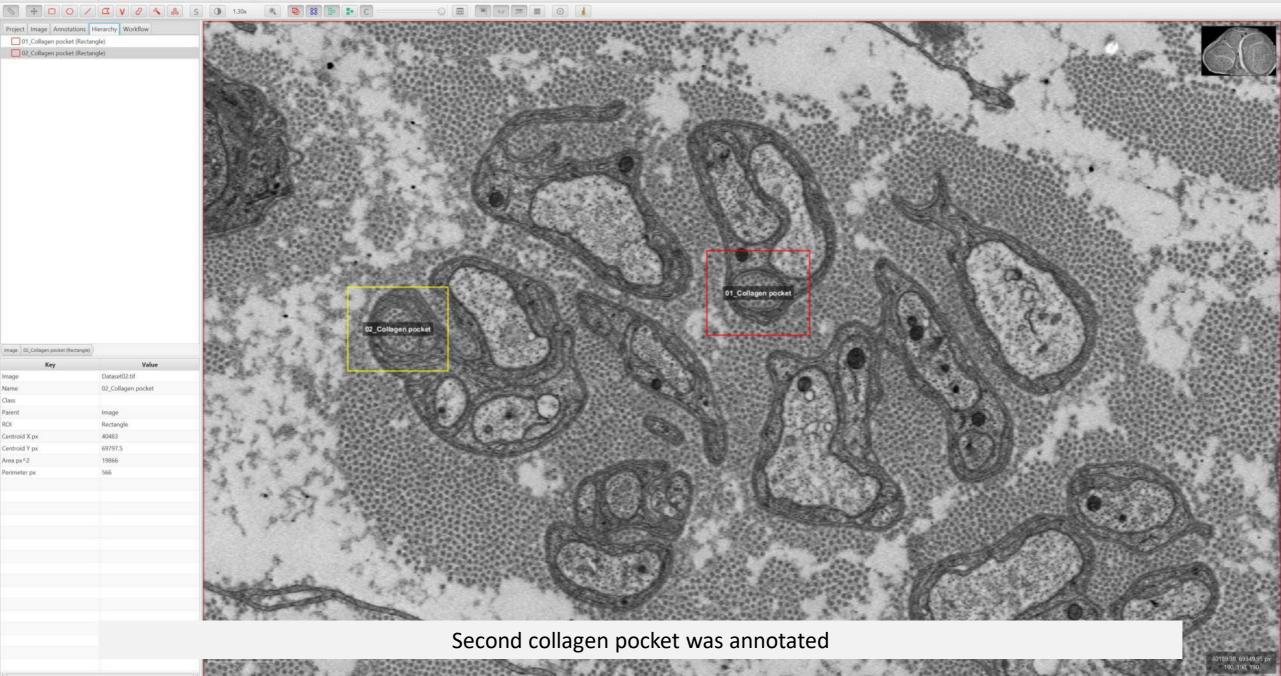


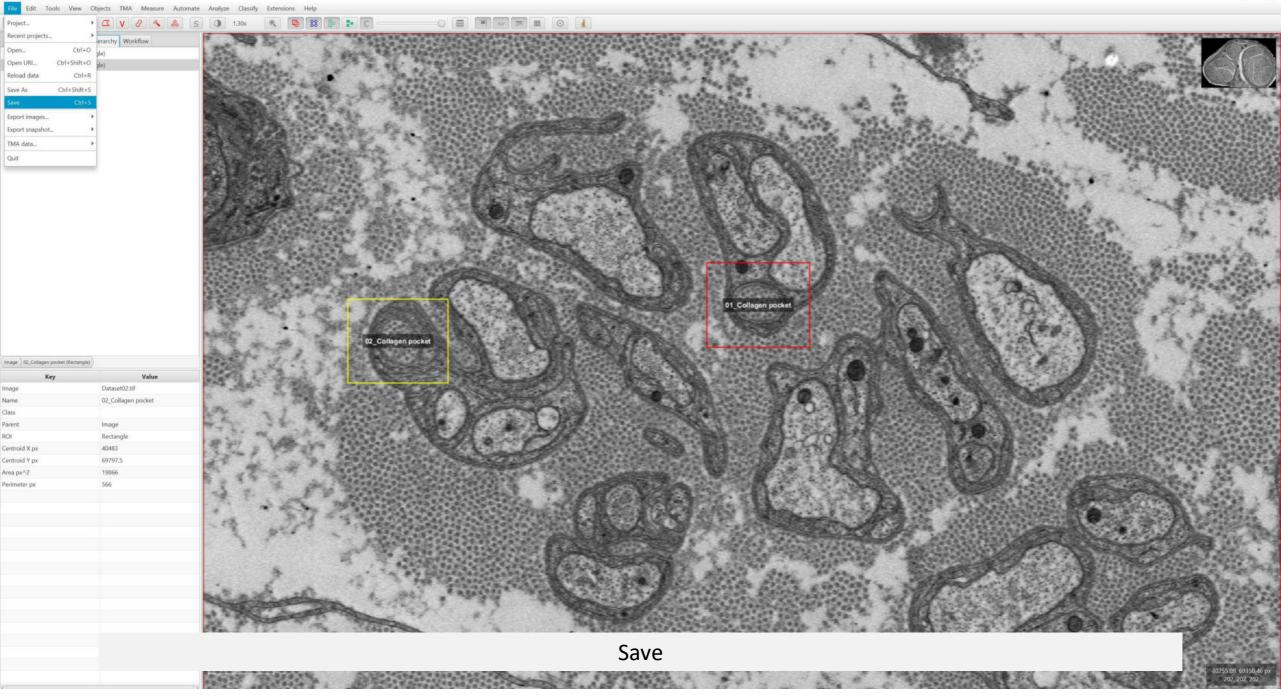










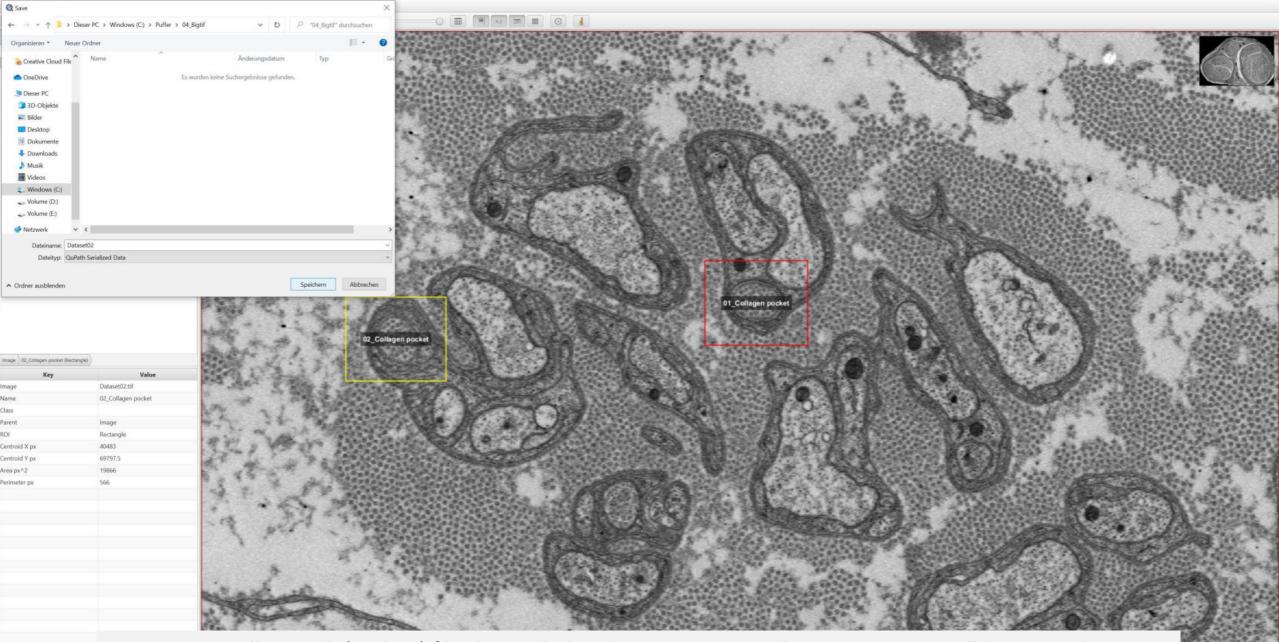


QuPath - Dataset02.tif

Class

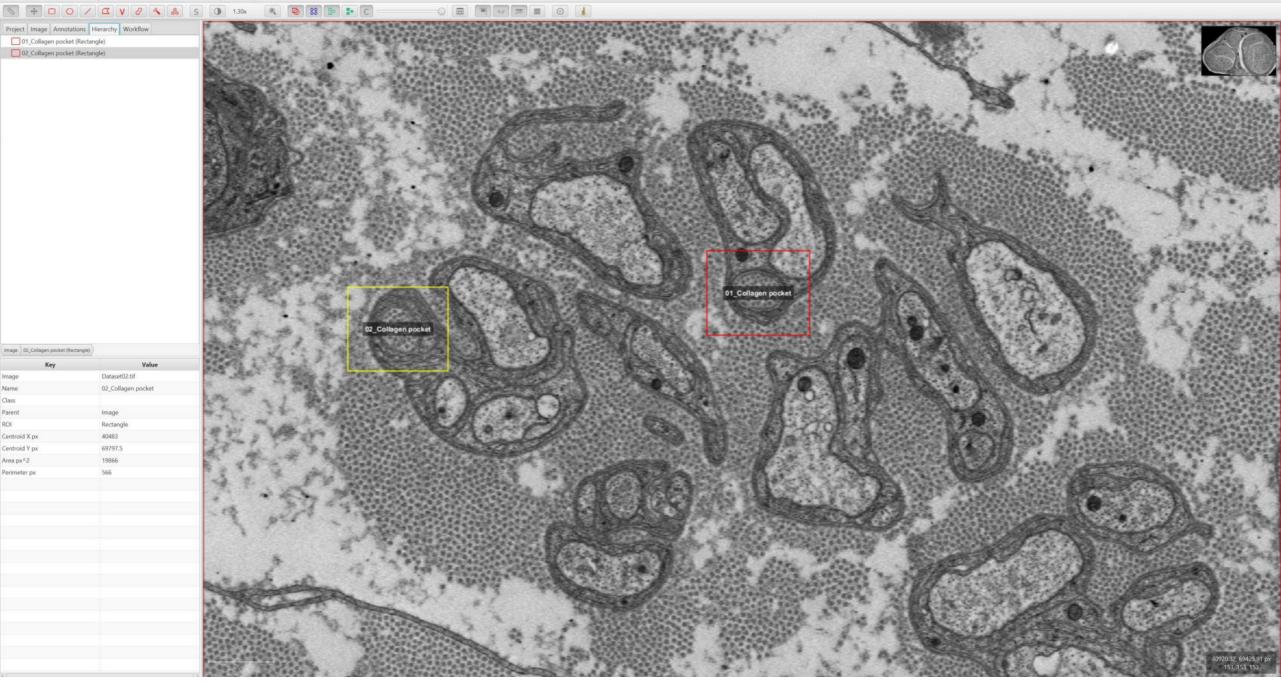
ROI

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A small QuPath (qpdata) file that includes the annotation and measurements will be created

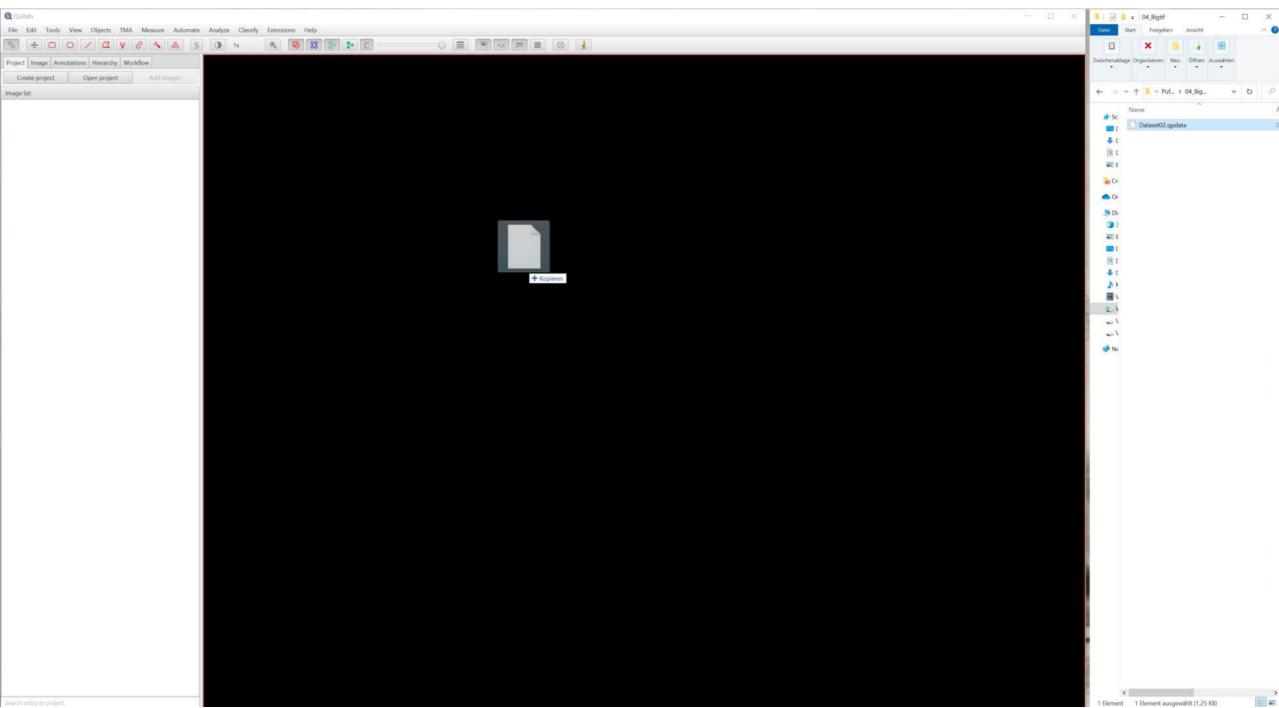
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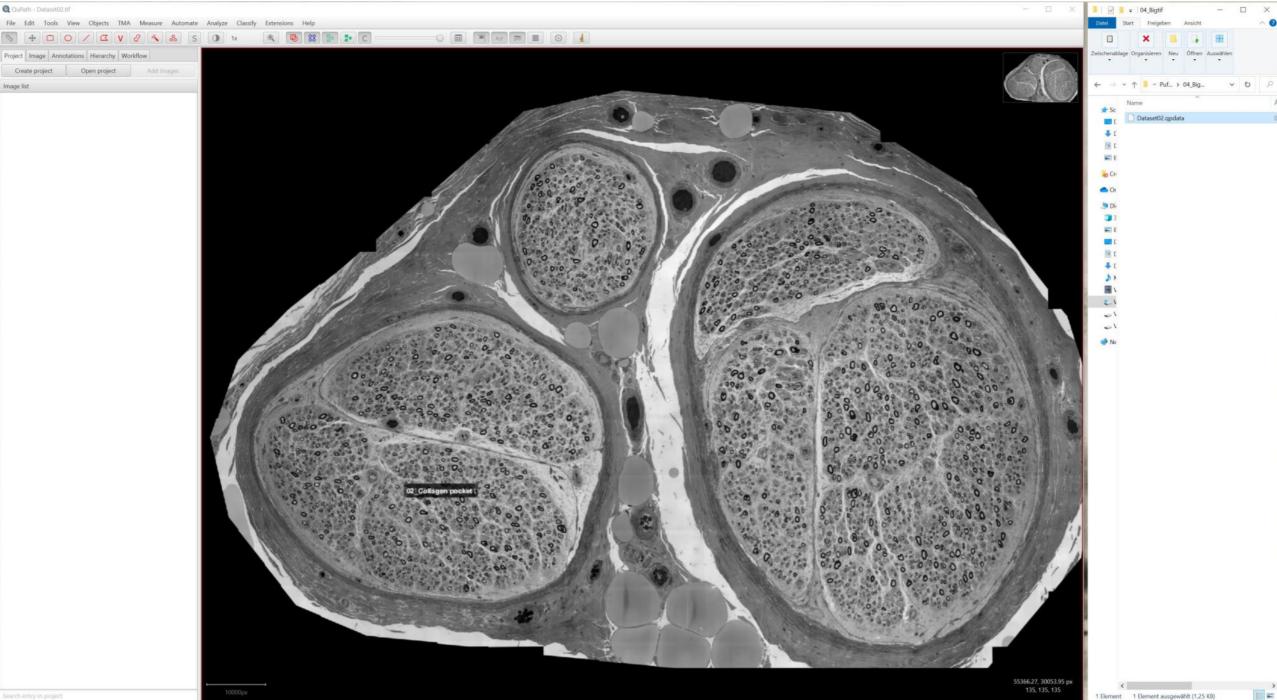


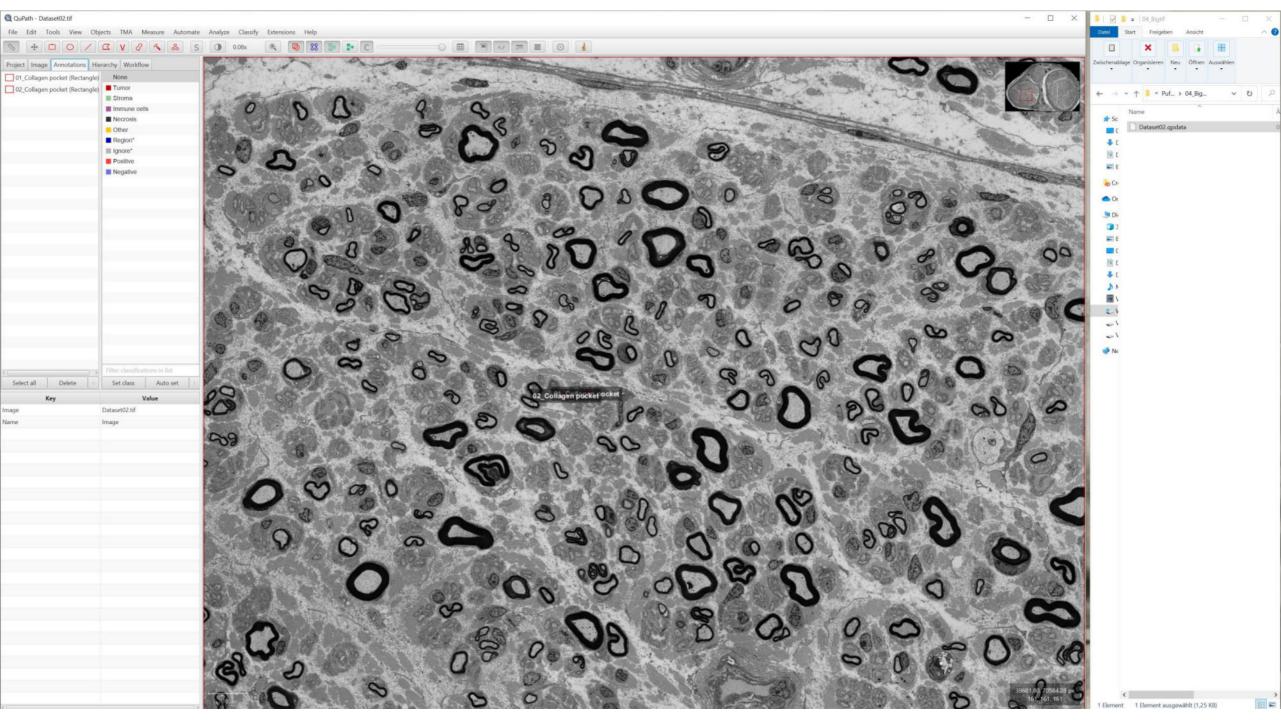
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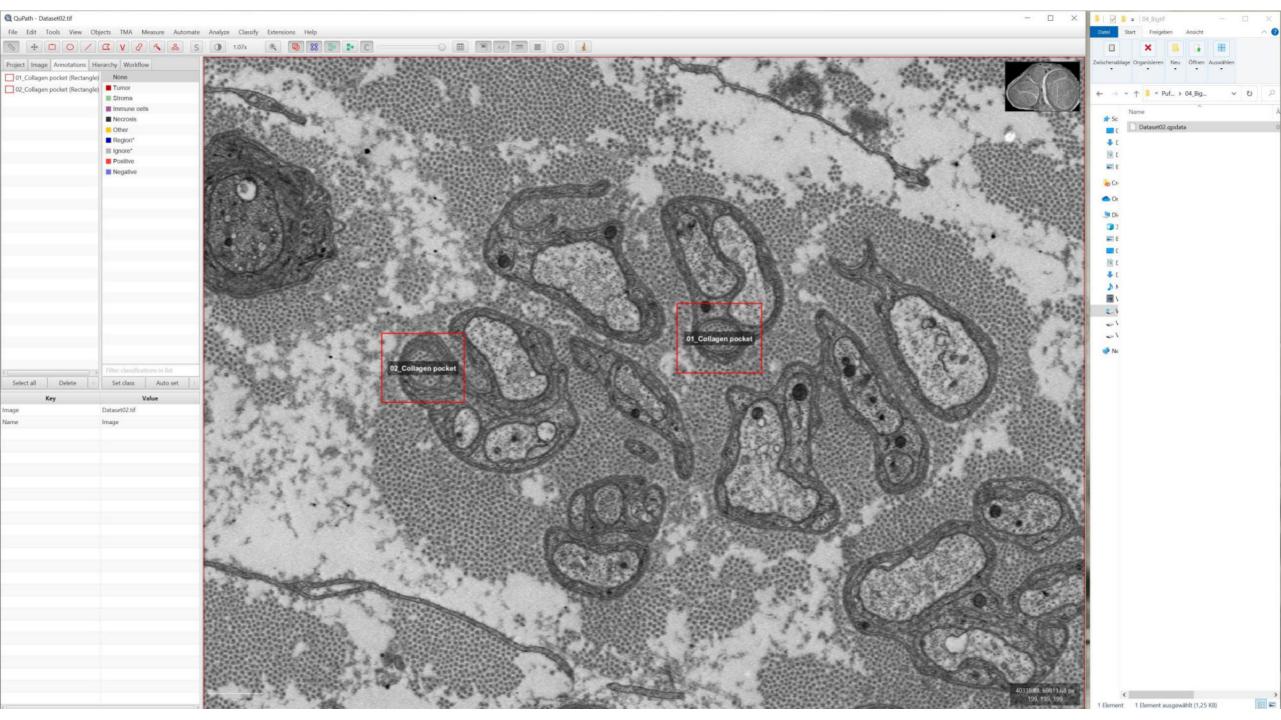
In a new session, the qpdata file can be opened via drag&drop, thus, the bigtif will be opened as well It might be required to update the path of the bigtif file

ahlt (1,25 KB)







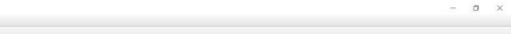


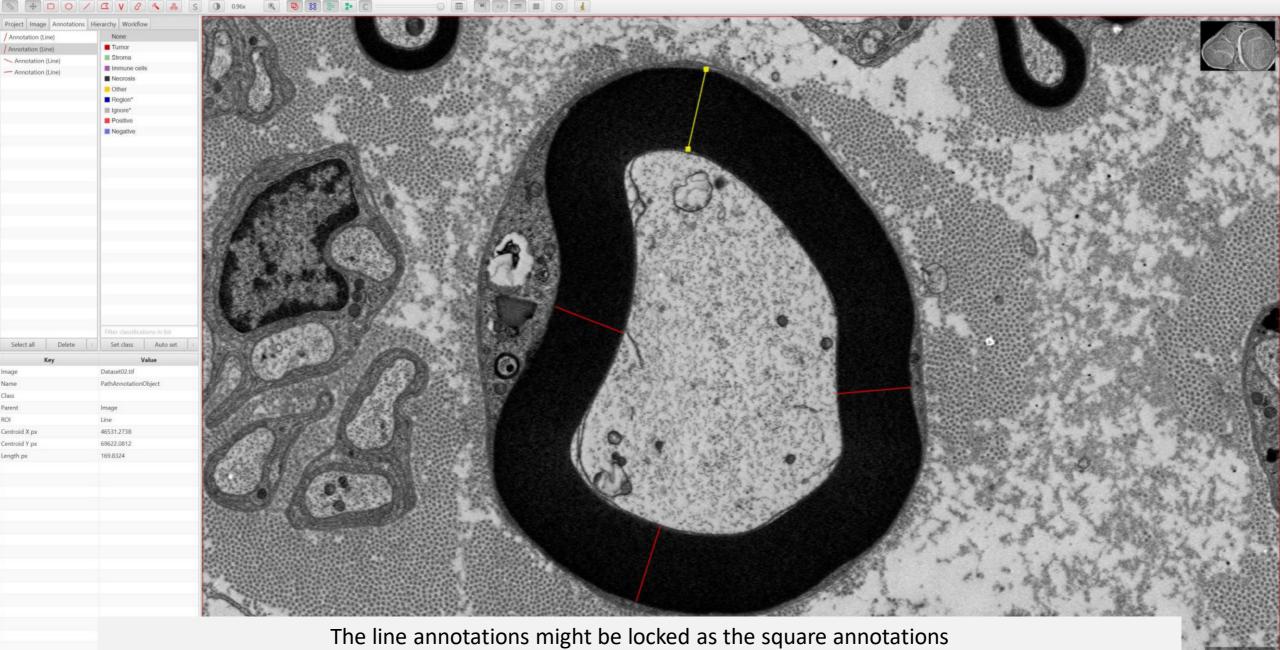
÷ ierarchy Workflow roject Image Annotation (Line) None Tumor Stroma Immune o Necrosi Other Region* Ignore* Positive Negative Select all Set class Auto set Dataset02.tif PathAnnotationObject Image Line entroid X px 46430.0777 70565.423 163.7431



A line can be used to measure distances; 163 pixels * 9 nm pixel size= 1,467 nm

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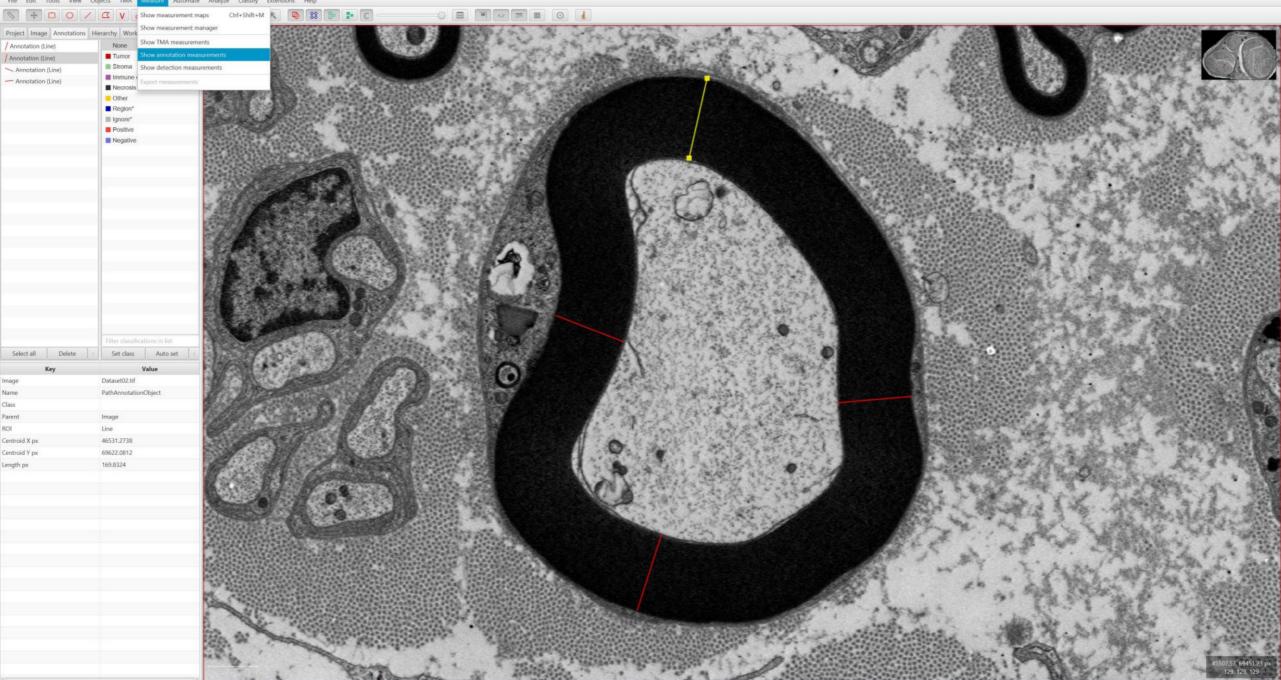
🔇 QuPath - Dataset02.tif

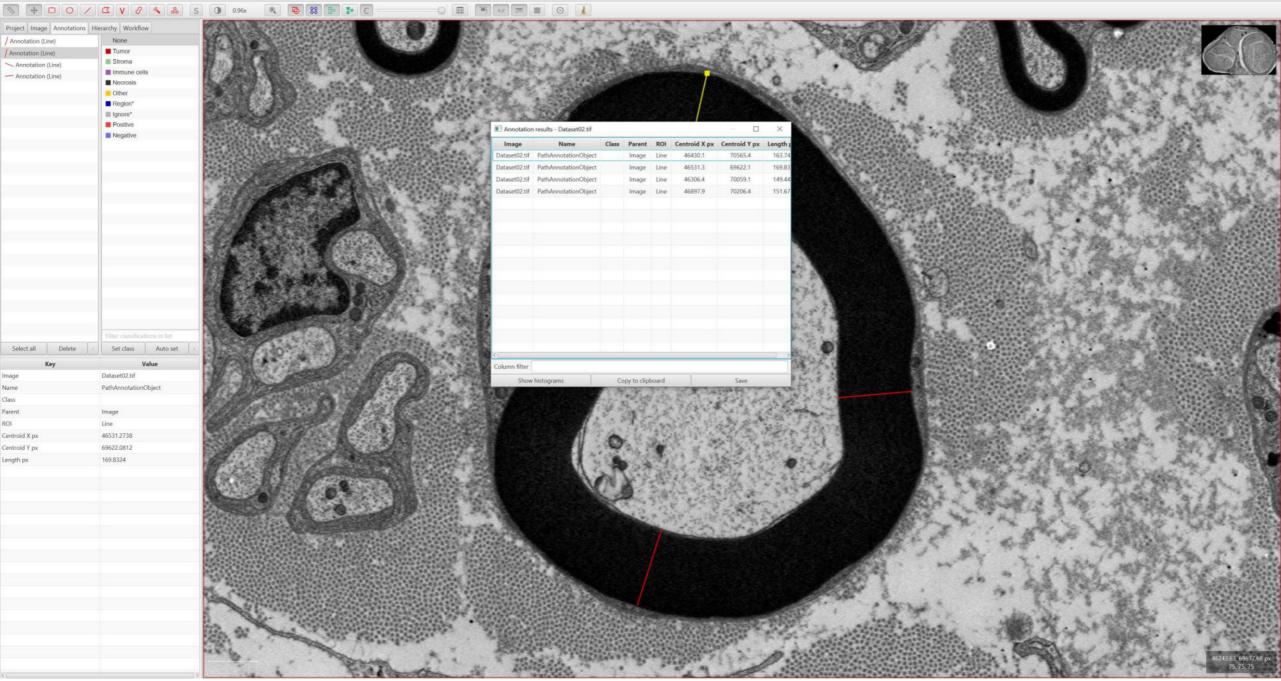
Image

Name Class

ROI

File Edit Tools View Objects TMA Measure Automate Analyze Classify Extensions Help





File Edit View Insert Debug Help

Macro Name : carsten_SK_III_30s_02

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Version	
Stage goto X= 61.471 mm	Stage goto Y= 45.453 mm
Stage goto X= 61.471 mm	Stage goto Y= 45.453 mm
Stage goto Z= 29.880 mm	
Stage goto R= 169.3 °	
Scan Rotation= 356.1 "	
Delay For 30 seconds	
Stage goto X= 61.803 mm	Stage goto Y= 45.538 mm
Stage goto X= 61.803 mm	Stage goto Y= 45.538 mm
Stage goto R= 138.2 °	
Scan Rotation= 358.6 "	
Delay For 30 seconds	
Stage goto X= 61.368 mm	Stage goto Y= 45.605 mm
Stage goto X= 61.368 mm	Stage goto Y= 45.605 mm
Stage goto R= 109.4 °	
Scan Rotation= 18.4 °	
Delay For 30 seconds	
Stage goto X= 61.666 mm	Stage goto Y= 45.530 mm
Stage goto X= 61.666 mm	Stage goto Y= 45.530 mm
Stage goto R= 78.1 °	
Scan Rotation= 4.2 °	
Delay For 30 seconds	
Stage goto X= 61.161 mm	Stage goto Y= 45.593 mm
Stage goto X= 61.161 mm	Stage goto Y= 45.593 mm
Stage goto R= 49.4 "	
Scan Rotation= 358.5 "	
Delay For 30 seconds	
Stage goto X= 61.433 mm	Stage goto Y= 45.462 mm
Stage goto X= 61.433 mm	Stage goto Y= 45.462 mm
Stage goto R= 17.8 °	
Scan Rotation= 10.3 °	
Delay For 30 seconds	B
Stage goto X= 61.319 mm	Stage goto Y= 45.468 mm
Stage goto X= 61.319 mm	Stage goto Y= 45.468 mm
Stage goto R= 348.2 °	
Scan Rotation= 348.7 *	
Delay For 30 seconds Store gots X= 61 214 mm	Stage gets V- 4E 462 mm
Stage goto X= 61.314 mm Stage goto X= 61.314 mm	Stage goto Y= 45.463 mm Stage goto Y= 45.463 mm
Stage goto R= 318.5 "	Stage you 1 - 45.465 mm
Scan Rotation= 356.5 °	
Delay For 30 seconds	
Stage goto X= 61.339 mm	Stage goto Y= 45.292 mm
Stage goto X= 61.339 mm	Stage goto Y= 45.292 mm
Stage goto R= 288.6 "	5/age goto 1 - 45.252 mm
Scan Rotation= 0.1 °	
Delay For 30 seconds	
Stage goto X= 61.852 mm	Stage goto Y= 45.276 mm
Stage goto X= 61 852 mm	Stade doto Y= 45 275 mm
Stage goto X= 61.852 mm Stage goto B= 257 8 °	Stage goto Y= 45.276 mm
Stage goto R= 257.8 °	Stage goto Y= 45.276 mm
Stage goto R= 257.8 ° Scan Rotation= 348.4 °	Stage goto Y= 45.276 mm
Stage goto R= 257.8 ° Scan Rotation= 348.4 ° Delay For 30 seconds	
Stage goto R= 257.8 ° Scan Rotation= 348.4 ° Delay For 30 seconds Stage goto X= 61.698 mm	Stage goto Y= 45.308 mm
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Stage goto R= 257.8 ° Scan Rotation= 348.4 ° Delay For 30 seconds Stage goto X= 61.698 mm Stage goto X= 61.698 mm Stage goto R= 228.2 °	Stage goto Y= 45.308 mm
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