Region-specific tau analysis

Half-automatized pipeline

INFO: can be used for any other staining within neurons.

Preprocessing

- Use the preProcessingTau.ijm tool

```
159     if (channels == 3) {
160     //split channels
161         run("Split Channels");
162         selectWindow ("C1-MAX_" + picture);
163         rename("totalTau");
164         selectWindow ("C2-MAX_" + picture);
165         rename("AT8");
166         selectWindow ("C3-MAX_" + picture);
167         rename("Neuron");
```

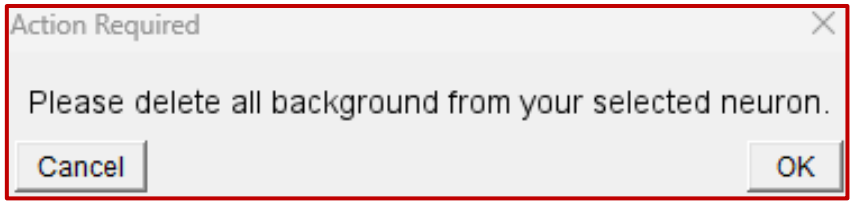
```
22      if (channels == 2) {
23      //split channels
24          run("Split Channels");
25          selectWindow ("C1-MAX_" + picture);
26          rename("AT8");
27          selectWindow ("C2-MAX_" + picture);
28          rename("Neuron");
```

Please make sure that the channels are recognized correctly.
Info: Instead of totalTau or AT8, you can use any other staining.

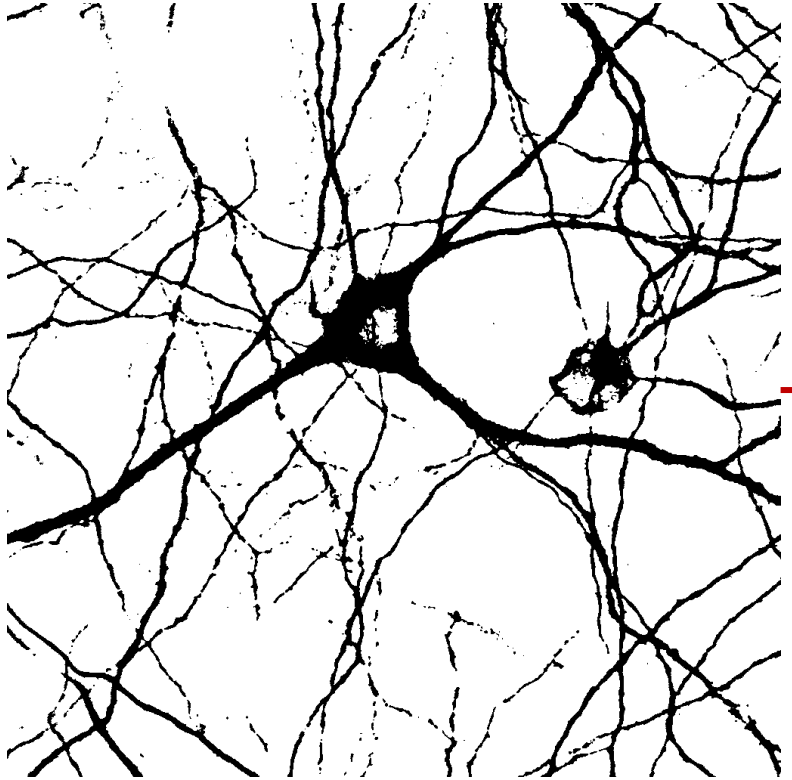
```
//Tresholding of the neuron
selectWindow("Neuron");
run("Duplicate...", "title=Neuron-1");
selectWindow("Neuron-1");
run("Enhance Contrast", "saturated=0.35");
run("Despeckle");
setAutoThreshold("Li dark");
setOption("BlackBackground", false);
run("Convert to Mask");

waitForUser("Please delete all background from your selected neuron.");
```

If the threshold is not good, please improve it here and may change the tresholding algorithm.

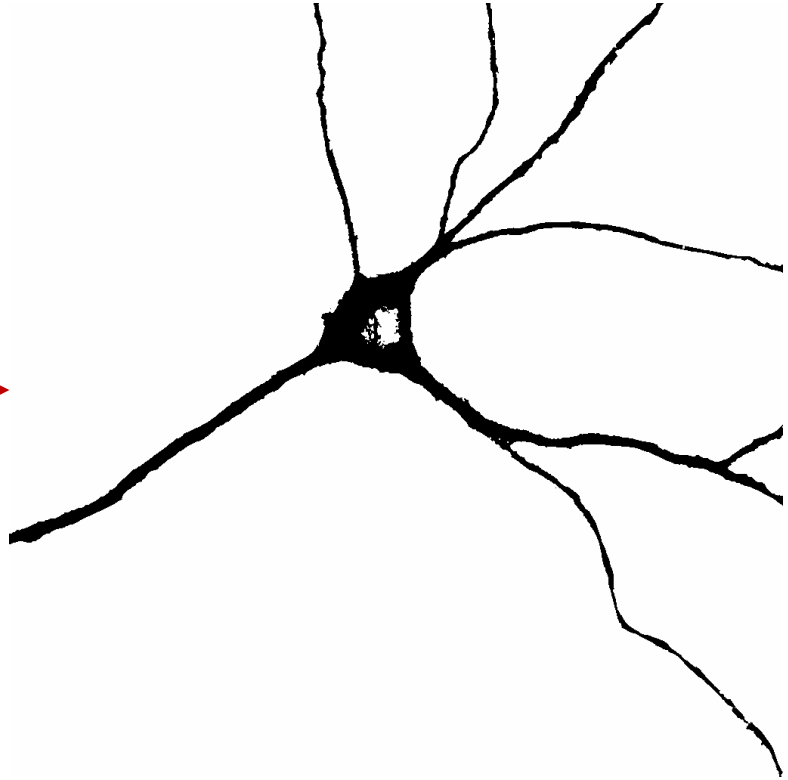
Preprocessing: Workflow



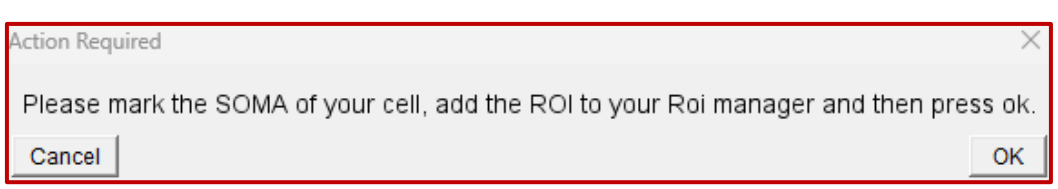
Binarized Image

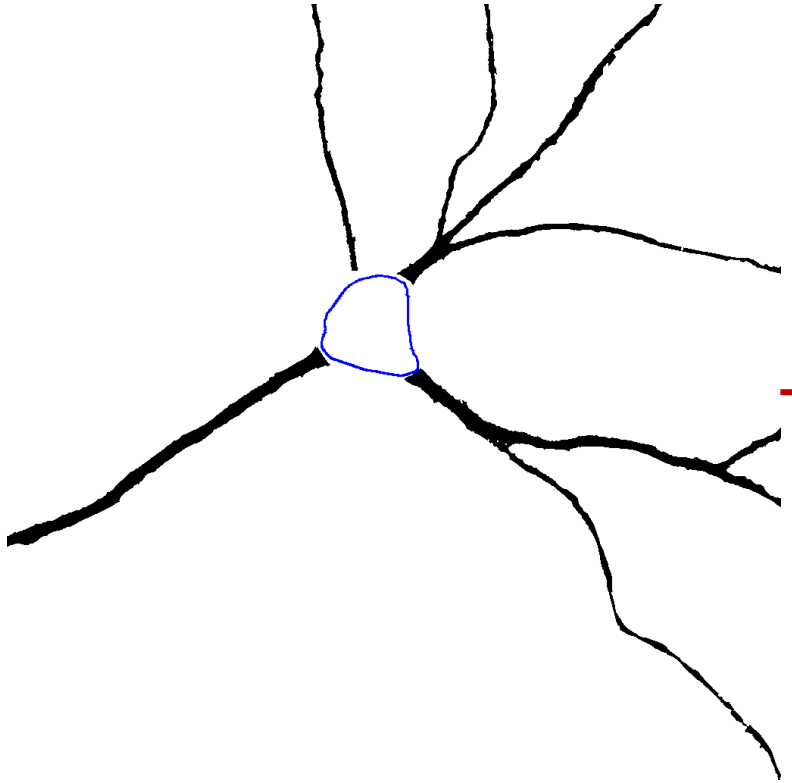


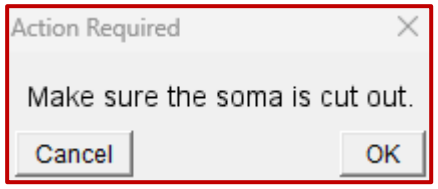
Cleaned Binarized Image



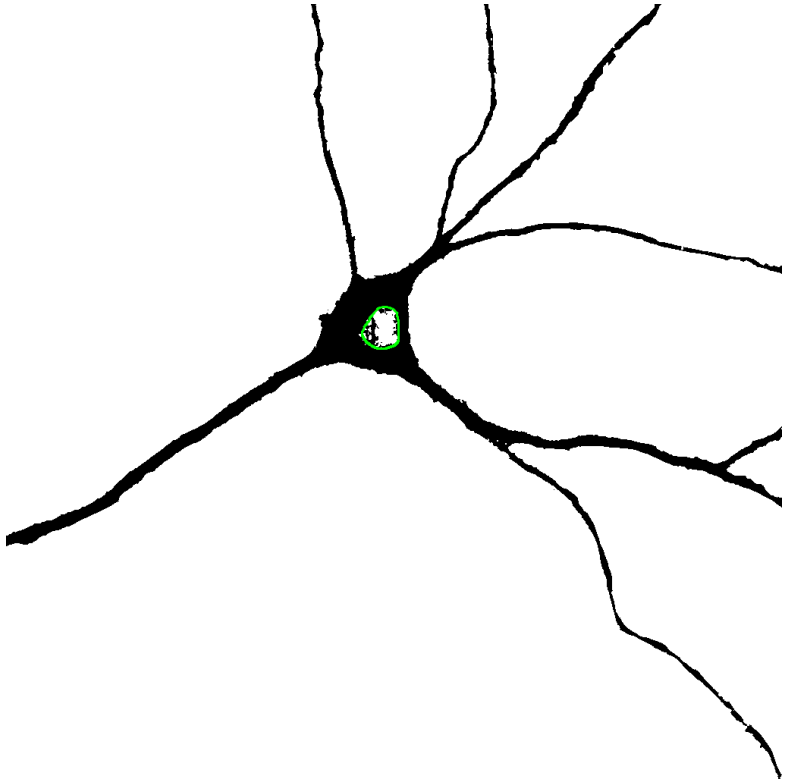
Preprocessing: Workflow

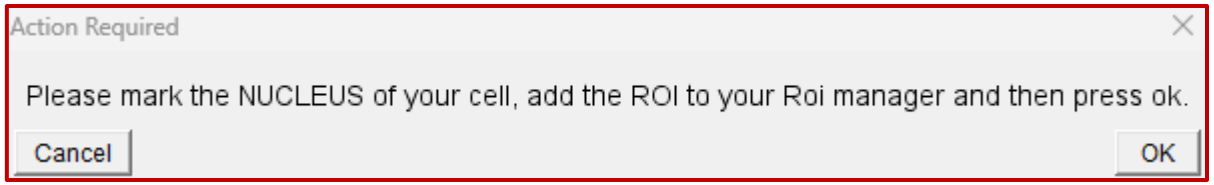




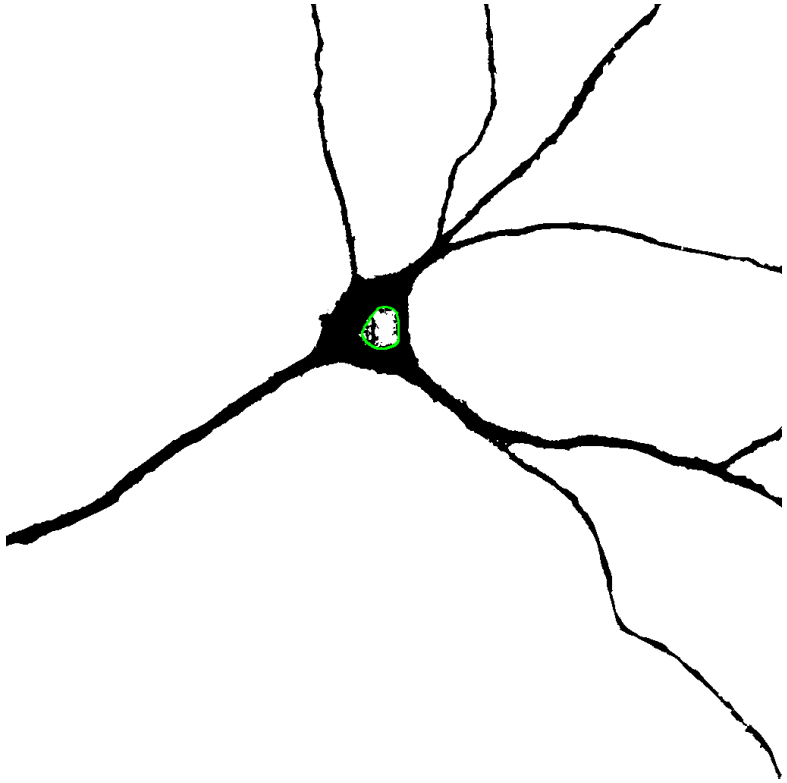


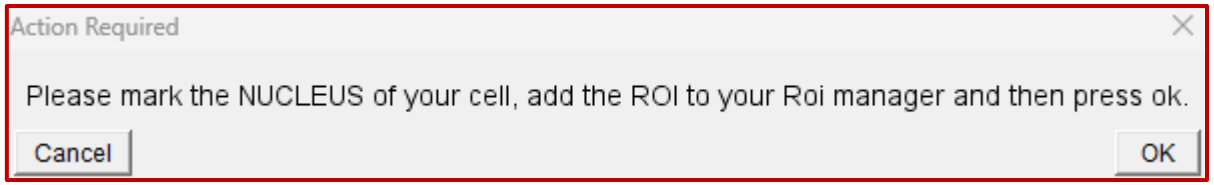
Preprocessing: Workflow



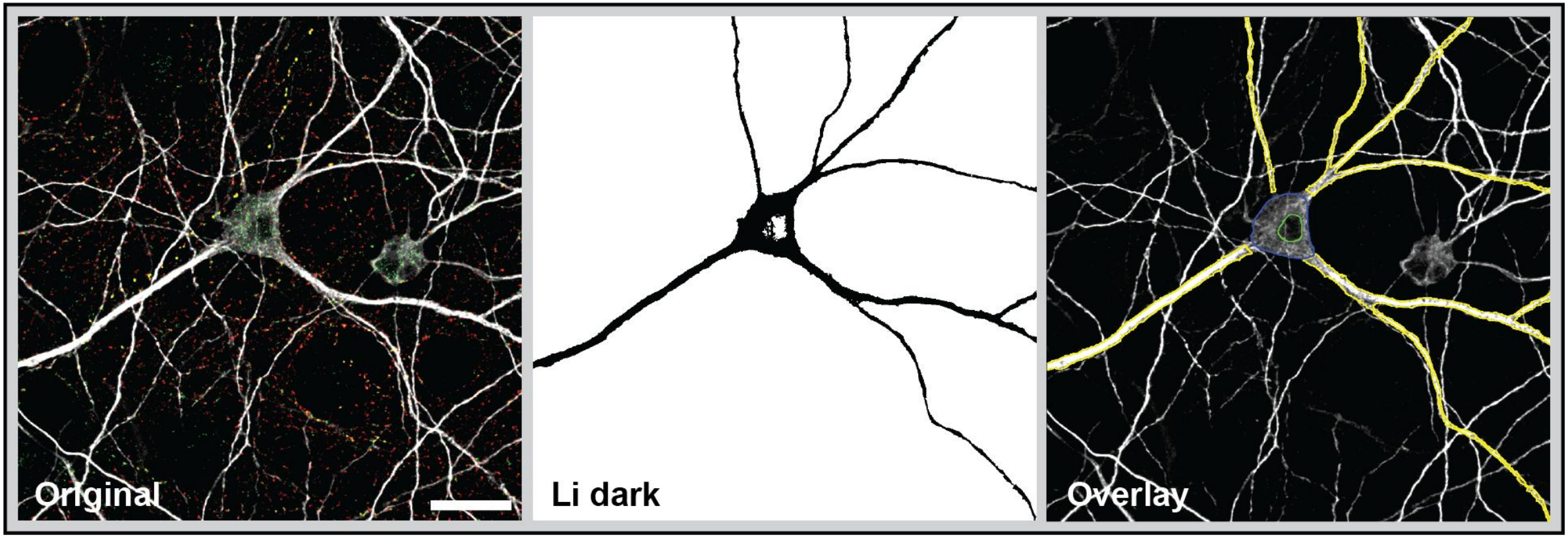


Preprocessing: Workflow



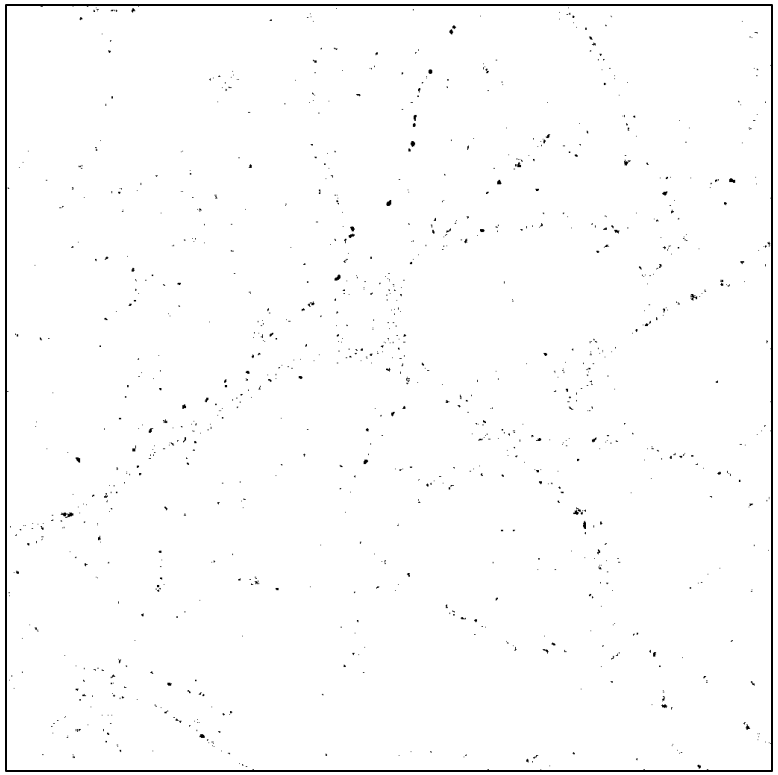


Preprocessing: Workflow final overview

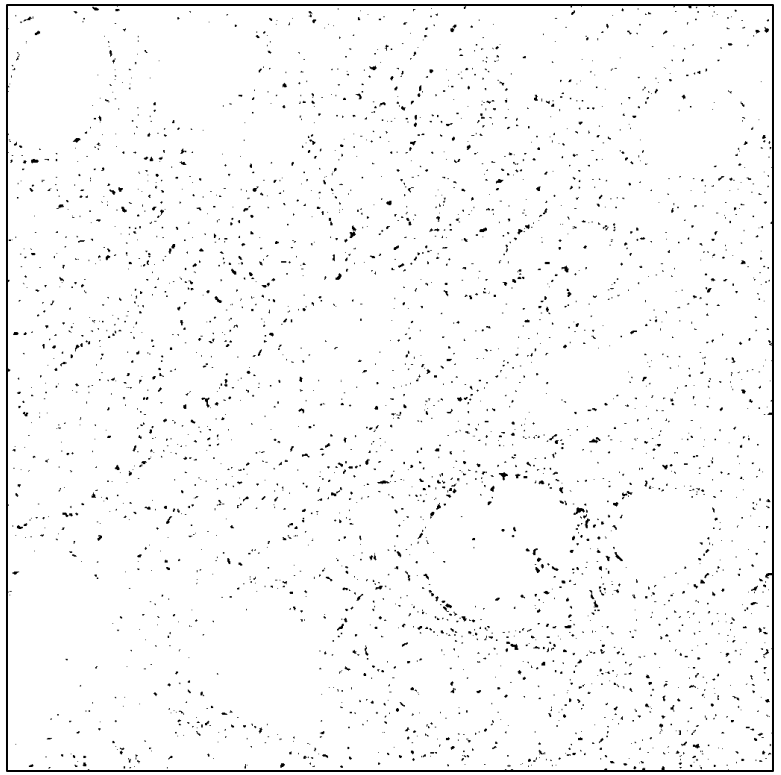


Preprocessing: Additonal

- Additionally, you generate thresholded images of your dot-like staining and an improvement of those via substraction of *gaussian blur* filtered image from *unsharped mask* filtered image via the Image Calculator



AT8 (pTau staining)



Total Tau

Please enter your used resolution and set the channel!

Postprocessing

- For two dot-like stainings use: *Tau_v003.py*
- For one dot-like staining use: *HyperphosTau_v003.py*

```
##############################################
#
# Please enter your resolution!
#
##############################################

global resolution
resolution = 0.1221896

##############################################
#
# Please ensure the channel are correctly set
#
##############################################

AT8_channel = 1
totalTau_channel = 0
```

- For running the code correctly and efficently (for all your data together), please ensure the following folder structure:
- Folder containing all data
  - ConditionA
    - Round1
      - Data obtained via preprocessing
    - Round2
      - Data obtained via preprocessing
    - …
  - ConditionB
    - Round1
      - …

What you get

neuron_data_AT8

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	file	hyperphosPuncta_nucleus	hyperphosPuncta_soma	hyperphosPuncta_dendrite	hyperphos_area_nucleus	hyperphos_area_soma	hyperphos_area_dendrite	area_nucleus	area_soma	area_dendrite	total_area	totalhyperphosPuncta_norm	hyperphosPuncta_nucleus_norm	hyperphosPuncta_	hyperphosPuncta_dendrite	totalhyperphosArea_norm	hyperphosArea_nucleus_norm
2	A6_1.tif	3	29	129	0.238884774	3.448898918	13.21331404	49.03524414	164.650918	551.9880602	765.674223	0.210272196	0.061180485	0.176130205	0.233700707	0.022073484	0.004871695
3	A6_10.tif	6	55	335	0.298605967	6.225934411	52.00222915	55.47492323	330.338916	1078.624474	1464.43831	0.270410844	0.108156977	0.166495672	0.310580752	0.039965336	0.00538272
4	A6_11.tif	28	58	238	1.926008487	4.240204731	28.24812447	114.1421381	146.686753	810.8197124	1071.6486	0.302337911	0.245308179	0.395400395	0.293530111	0.032113454	0.016873773
5	A6_12.tif	8	21	211	0.388187757	1.702054012	40.43124793	68.52674378	106.073764	496.9549805	671.555488	0.357379255	0.116742742	0.197975439	0.424585744	0.06331791	0.005664763
6	A6_13.tif	13	60	114	1.149632973	5.927328444	16.93095833	62.4658831	211.312864	517.1855348	790.964282	0.236420284	0.208113603	0.283939174	0.220423798	0.030352723	0.018404174

- pTau punta in nucleus (count)
- pTau puncta in soma (count)
- pTau puncta in dendrite (count)
- pTau area in nucleus (µm²)
- pTau area in soma (µm²)
- pTau area in dendrite (µm²)
- Area soma (µm²)
- Area dendrite (µm²)
- Total area of neuron (µm²)
- pTau total frame per total area
- pTau puncta in nucleus per nucleus area
- pTau puncta in soma per soma area
- pTau puncta in dendrite per dendrite area
- pTau puncta in whole area per total area
- pTau area in nucleus per nucleus area

What you get

neuron_data_AT8

R	S	T	U	V	W	X	Y	Z	AA	AB	AC
hyperphosArea_soma_norm	hyperphosArea_dendrite_norm	mean_Int_nucleus_AT8	mean_Int_soma_AT8	mean_Int_dendrite_AT8	IntDen_nucleus_AT8	IntDen_soma_AT8	IntDen_dendrite_AT8	hyperphos_area_whole	hyperphos_area_whole_norm	mean_Int_whole_AT8	IntDen_whole_AT8
0.020946734	0.023937681	3.008466604	3.290487714	1.599276874	147.5208944	541.7818241	882.7817396	15107	0.964961152	1.29183197	20224.34326
0.018847112	0.048211616	2.287823906	2.787432971	2.661239151	126.9168556	920.7975872	2870.477679	13713	0.875919261	0.91693306	14355.09367
0.028906528	0.034838971	2.024083489	2.712061037	1.594692383	231.0332171	397.8234272	1293.008019	5903	0.377054722	0.816482544	12782.48535
0.016045947	0.081357969	2.245266005	3.319633353	3.811304624	153.8607683	352.1260048	1894.046815	5939	0.379354225	0.83829689	13124.00099
0.028050012	0.032736721	3.43654321	3.276026951	1.521683925	214.6667064	692.2666375	786.9929144	5024	0.320908508	0.539797783	8450.832541

- pTau area in soma per soma area
- pTau area in dendrites per dendrites area
- pTau mean intensity in nucleus
- pTau mean intensity in soma
- pTau mean intensity in dendrites
- Integrated density in nuclues
- Integrated density in soma
- Integrated density in dendrites
- Whole pTau area (whole frame)
- Whole pTau area per total area (whole frame)
- Whole pTau mean intensity (whole frame)
- Whole pTau integrated density (whole frame)

What you get

neuron_data_tau

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	file	tauPuncta_nucleus	tauPuncta_soma	tauPuncta_dendrite	tau_area_nucleus	tau_area_soma	tau_area_dendrite	area_nucleus	area_soma	area_dendrite	total_area	totaltauPuncta	tauPuncta_nucleus_norm	tauPuncta_soma_	tauPuncta_dendrite_norm	totaltauArea_norm	tauArea_nucleus
2	A6_1.tif	1	18	88	0.029860597	2.851686984	8.196733793	49.03524414	164.650918	551.9880602	765.67422	0.139746118	0.020393495	0.109322196	0.159423738	0.014468662	0.000608962
3	A6_10.tif	2	57	348	0.209024177	8.032500511	53.65949226	55.47492323	330.338916	1078.624474	1464.4383	0.277922256	0.036052326	0.17255006	0.322633139	0.04226946	0.003767904
4	A6_11.tif	10	31	247	0.731584619	2.971129371	33.4587986	114.1421381	146.686753	810.8197124	1071.6486	0.26874481	0.087610064	0.211334694	0.304629989	0.034676957	0.006409418
5	A6_12.tif	3	20	130	0.104512088	2.344056841	16.2889555	68.52674378	106.073764	496.9549805	671.55549	0.227829275	0.043778528	0.188548037	0.261593112	0.027901677	0.001525128

- Total tau punta in nucleus (count)
- Total tau puncta in soma (count)
- Total tau puncta in dendrite (count)
- Total tau area in nucleus (µm²)
- Total tau area in soma (µm²)
- Total tau area in dendrite (µm²)
- Area soma (µm²)
- Area dendrite (µm²)
- Total area of neuron (µm²)
- Total tau total frame per total area
- Total tau puncta in nucleus per nucleus area
- Total tau puncta in soma per soma area
- Total tau puncta in dendrite per dendrite area
- Total tau puncta in whole area per total area
- Total tau area in nucleus per nucleus area

What you get

neuron_data_tau

R	S	T	U	V	W	X	Y	Z	AA	AB	AC
tauArea_soma_norm	tauArea_dendrite_norm	mean_Int_nucleus_tau	mean_Int_soma_tau	mean_Int_dendrite_tau	IntDen_nucleus_tau	IntDen_soma_tau	IntDen_dendrite_tau	tau_area_whole	tau_area_whole_norm	mean_Int_whole_tau	IntDen_whole_tau
0.017319594	0.014849477	3.613044842	6.874162323	6.552388371	177.1665359	1131.83714	3616.840147	20353	1.300049933	5.110275269	80004.18286
0.024315938	0.049748076	6.466425188	10.89348733	14.56793049	358.7244409	3598.542799	15713.32636	15359	0.981057678	4.696550369	73527.09097
0.020254926	0.041265399	5.082954241	7.648097826	7.773335969	580.1792649	1121.874636	6302.774035	22584	1.442555283	4.154378891	65039.09692
0.022098366	0.032777527	4.161406673	8.139414548	10.54948593	285.1676488	863.3783376	5242.619574	20966	1.33920537	4.132873535	64702.41869

- Total tau area in soma per soma area
- Total tau area in dendrites per dendrites area
- Total tau mean intensity in nucleus
- Total tau mean intensity in soma
- Total tau mean intensity in dendrites
- Integrated density in nuclues
- Integrated density in soma
- Integrated density in dendrites
- Whole Total tau area (whole frame)
- Whole Total tau area per total area (whole frame)
- Whole Total tau mean intensity (whole frame)
- Whole Total tau integrated density (whole frame)

What you get

neuron_data_ratios

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	file	ratio_Puncta_nucleus	ratio_Puncta_soma	ratio_Puncta_d	ratio_area_nucleus	ratio_area_som	ratio_area_dendrite	ratio_mean_int_nucleus	ratio_mean_int_soma	ratio_mean_int_dendrite	ratio_IntDen_nucleus	ratio_IntDen_soma	ratio_IntDen_dendrite	ratio_area_whole	ratio_mean_Int_whole	ratio_IntDen_whole	
2	A1_1.tif		2.692307692	2.5		2.580357143	3.476047904	0.621871254	0.519060123	0.302780305	0.621871254	0.519060123	0.302780305	0.572573581	0.234696786	0.23469679	
3	A1_10.tif		3.444444444	1.375565611		2.129032258	1.329606223	0.650205339	0.394449198	0.221000799	0.650205339	0.394449198	0.221000799	0.709749127	0.210245424	0.21024542	
4	A1_11.tif		1.628571429	1.047008547		1.255813953	0.71112311	0.462120738	0.233823518	0.178158596	0.462120738	0.233823518	0.178158596	0.574263719	0.17366134	0.17366134	

- Total tau area in soma per soma area
- Total tau area in dendrites per dendrites area
- Total tau mean intensity in nucleus
- Total tau mean intensity in soma
- Total tau mean intensity in dendrites
- Integrated density in nuclues
- Integrated density in soma
- Integrated density in dendrites
- Whole Total tau area (whole frame)
- Whole Total tau area per total area (whole frame)
- Whole Total tau mean intensity (whole frame)
- Whole Total tau integrated density (whole frame)