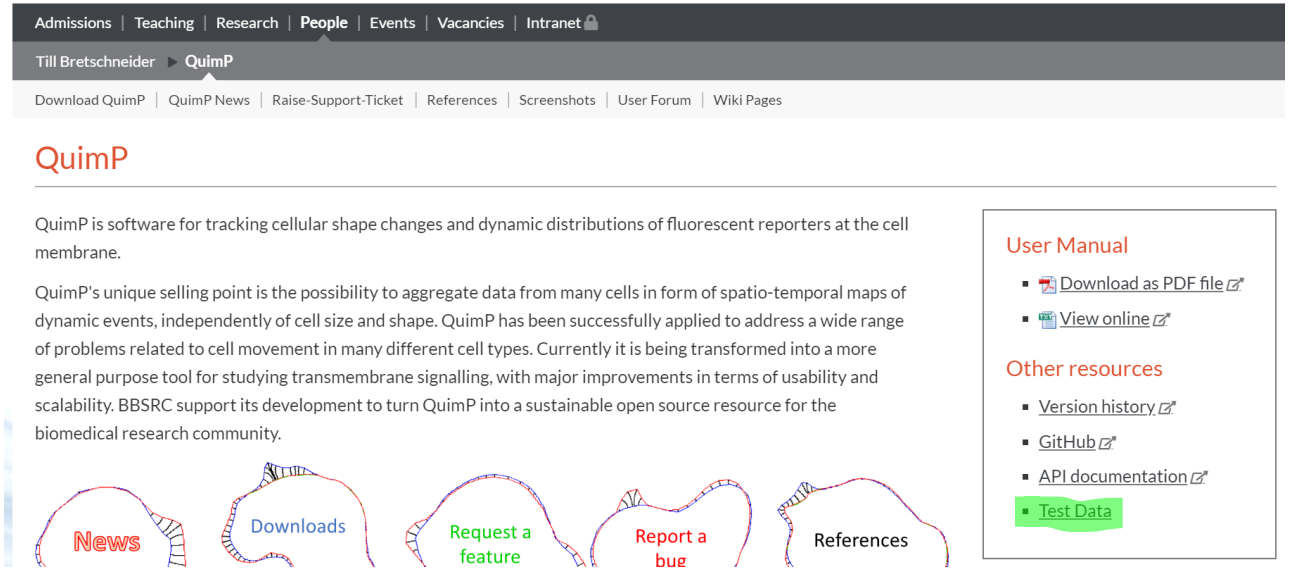
warwick.ac.uk/quimp

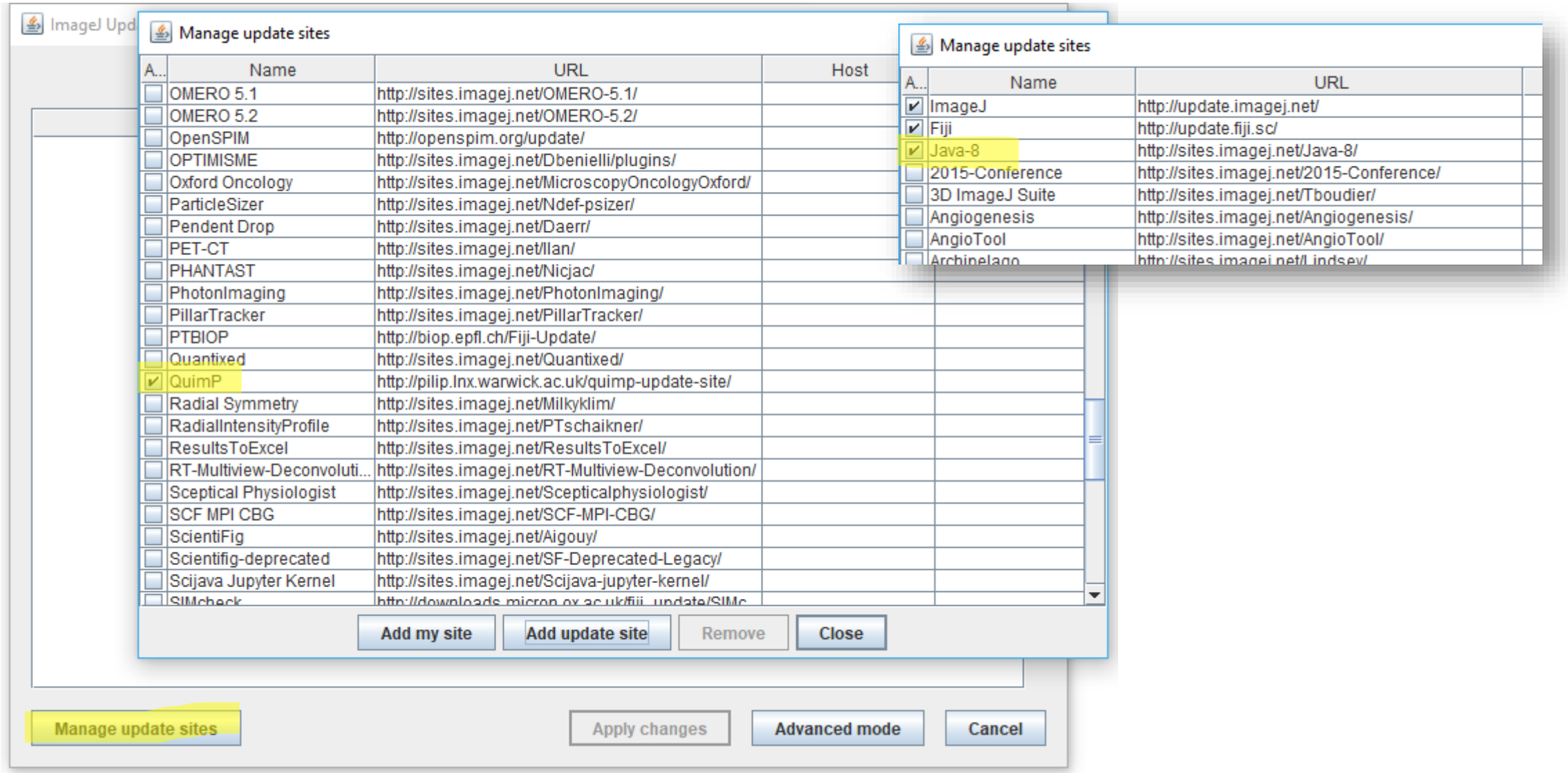
- https://github.com/CellDynamics/QuimP - issue tracker, source code
- https://github.com/CellDynamics/QuimP-Python - Python notebook with integration examples

Workshop files

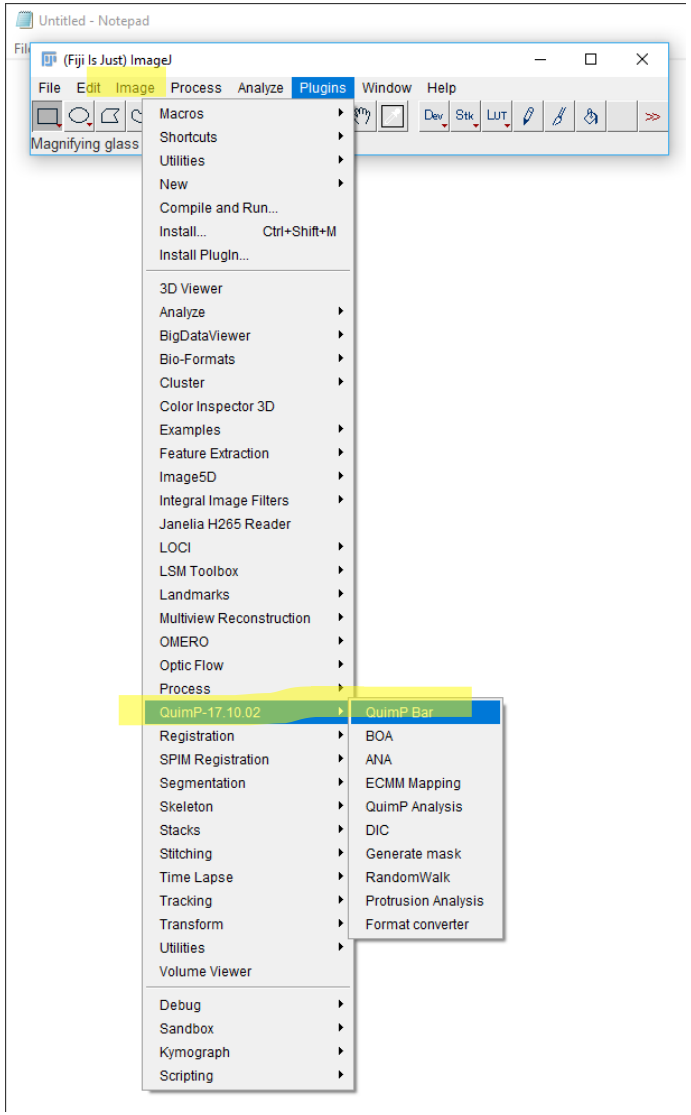
- warwick.ac.uk/quimp/test_data
- Download:
  - Example Analysis (single cell)

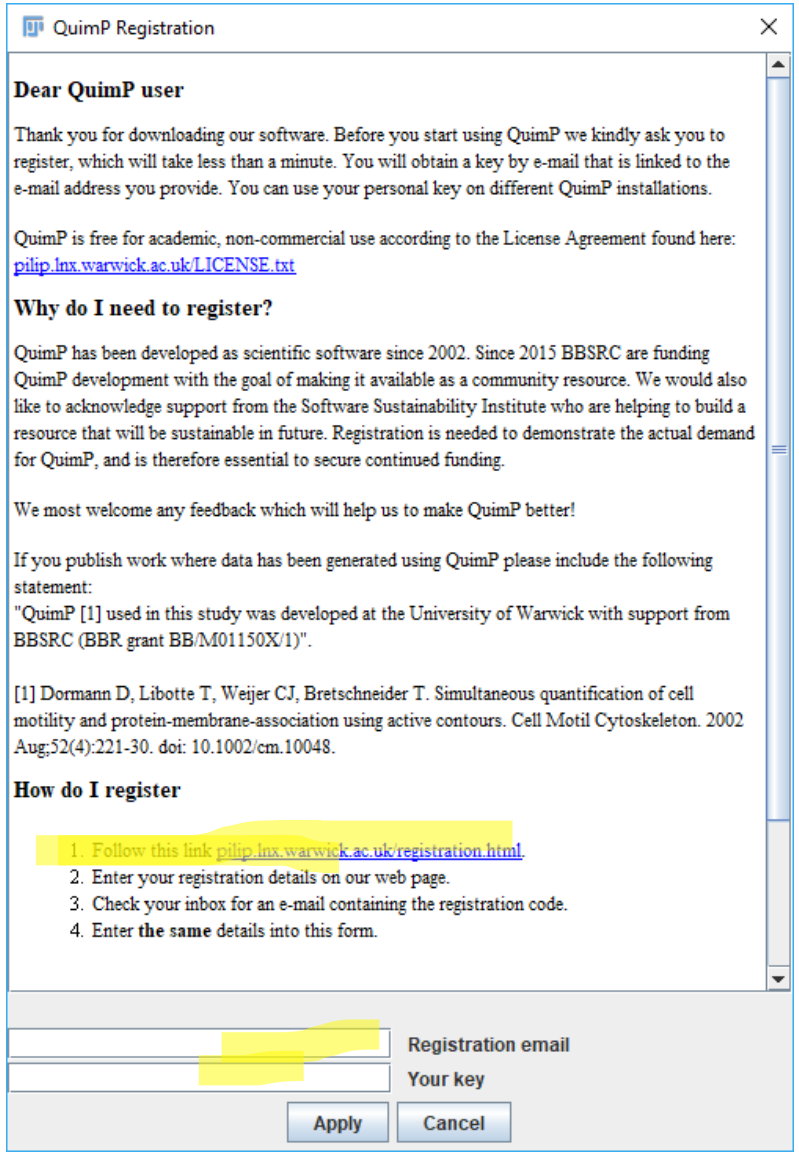


Installation

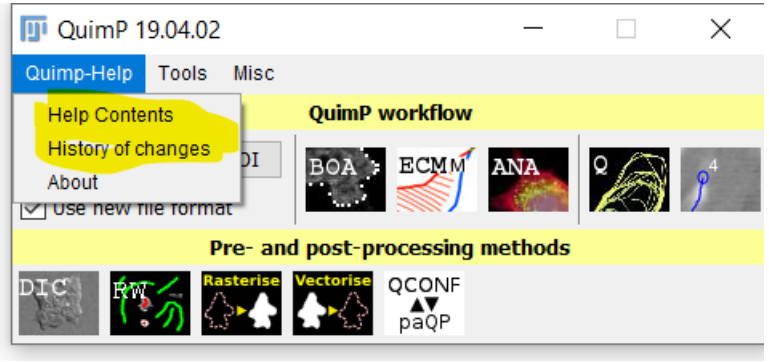


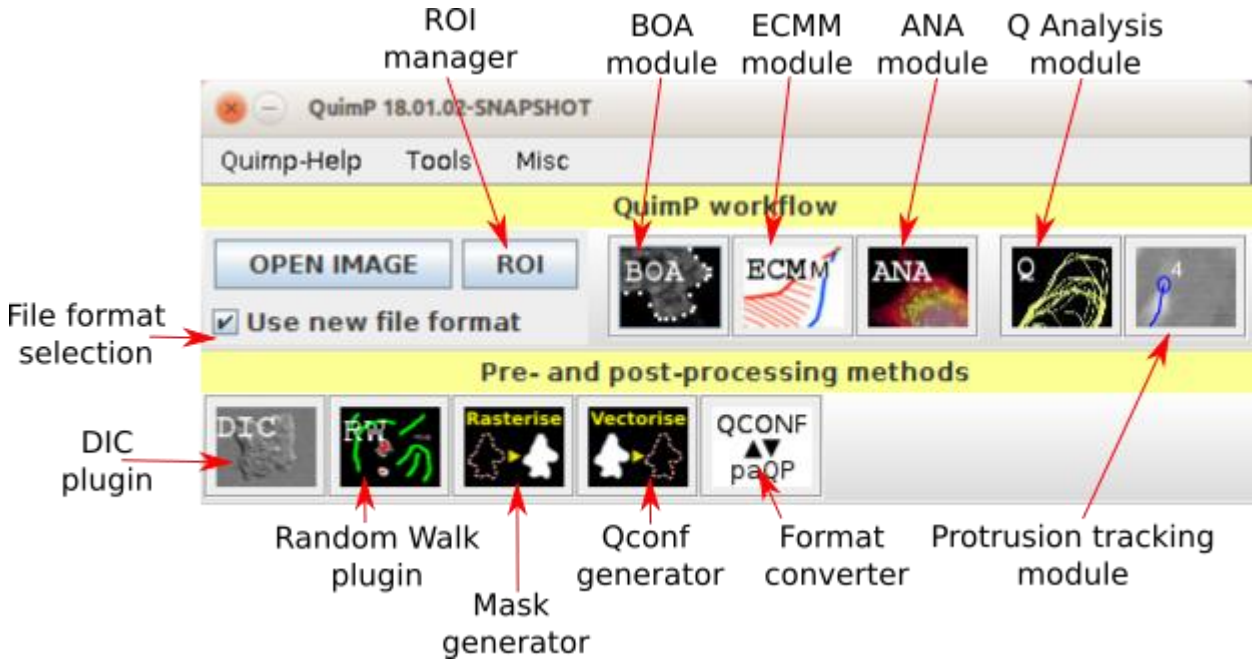
First run


Untitled - Notepad
(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
Magnifying glass
Macros
Shortcuts
Utilities
New
Compile and Run...
Install... Ctrl+Shift+M
Install PlugIn...
3D Viewer
Analyze
BigDataViewer
Bio-Formats
Cluster
Color Inspector 3D
Examples
Feature Extraction
Image5D
Integral Image Filters
Janelia H265 Reader
LOCI
LSM Toolbox
Landmarks
Multiview Reconstruction
OMERO
Optic Flow
Process
QuimP-17.10.02
Registration
SPIM Registration
Segmentation
Skeleton
Stacks
Stitching
Time Lapse
Tracking
Transform
Utilities
Volume Viewer
Debug
Sandbox
Kymograph
Scripting
QuimP Bar
BOA
ANA
ECMM Mapping
QuimP Analysis
DIC
Generate mask
RandomWalk
Protrusion Analysis
Format converter

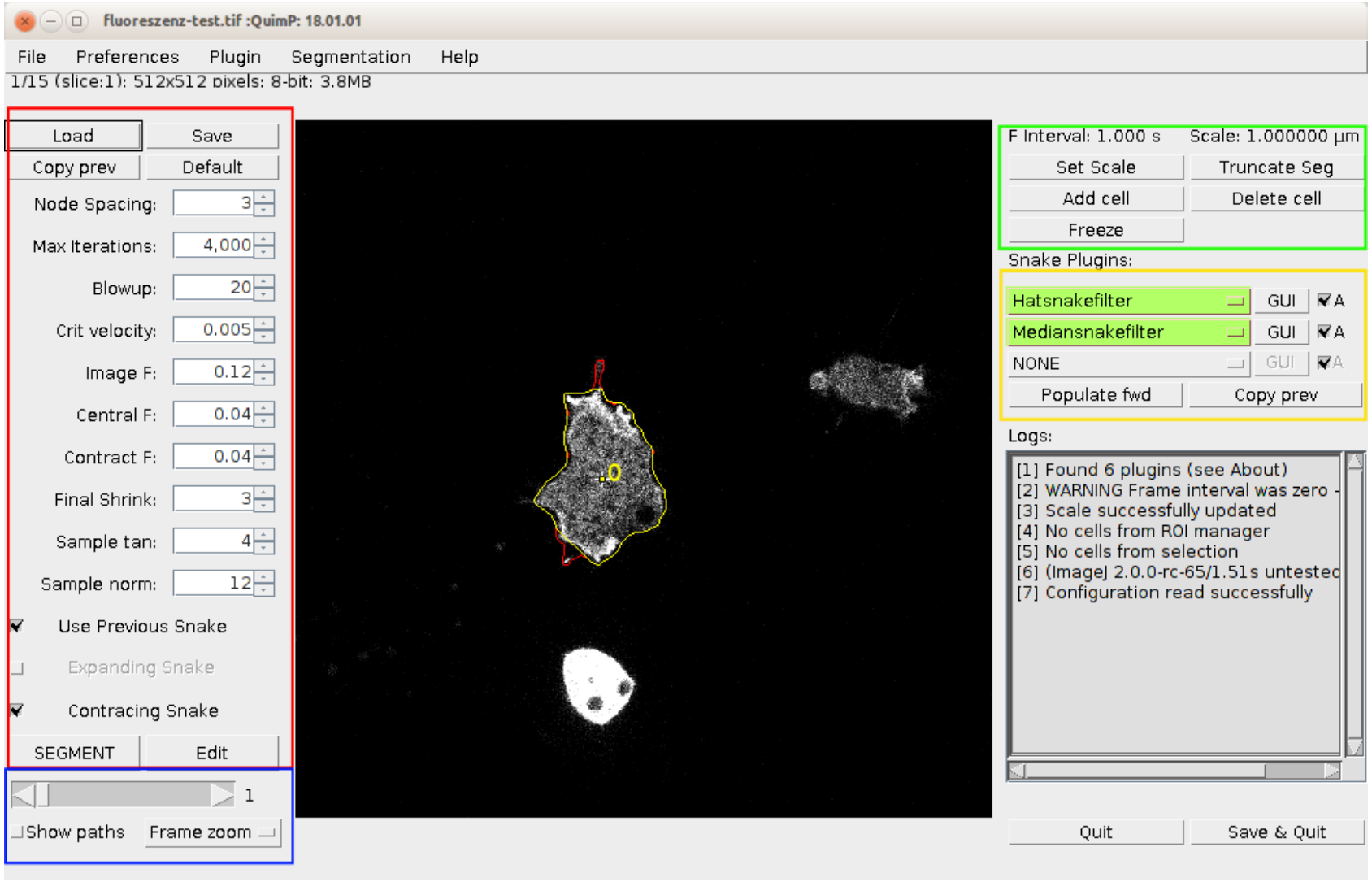

QuimP Registration
Dear QuimP user
Thank you for downloading our software. Before you start using QuimP we kindly ask you to register, which will take less than a minute. You will obtain a key by e-mail that is linked to the e-mail address you provide. You can use your personal key on different QuimP installations.
QuimP is free for academic, non-commercial use according to the License Agreement found here: pilip.lnx.warwick.ac.uk/LICENSE.txt
Why do I need to register?
QuimP has been developed as scientific software since 2002. Since 2015 BBSRC are funding QuimP development with the goal of making it available as a community resource. We would also like to acknowledge support from the Software Sustainability Institute who are helping to build a resource that will be sustainable in future. Registration is needed to demonstrate the actual demand for QuimP, and is therefore essential to secure continued funding.
We most welcome any feedback which will help us to make QuimP better!
If you publish work where data has been generated using QuimP please include the following statement:
"QuimP [1] used in this study was developed at the University of Warwick with support from BBSRC (BBR grant BB/M01150X/1)".
[1] Dormann D, Libotte T, Weijer CJ, Bretschneider T. Simultaneous quantification of cell motility and protein-membrane-association using active contours. Cell Motil Cytoskeleton. 2002 Aug;52(4):221-30. doi: 10.1002/cm.10048.
How do I register
1. Follow this link pilip.lnx.warwick.ac.uk/registration.html.
2. Enter your registration details on our web page.
3. Check your inbox for an e-mail containing the registration code.
4. Enter the same details into this form.
Registration email
Your key
Apply
Cancel

Looking around





BOA - Basic segmentation



- Red: segmentation parameters
- Blue: controls for navigating through frames in a time series and for zooming cells visible in the current frame.
- Green: tools for manual editing of contours.
- Yellow: access to cell contour postprocessing filters (described in section 7.3).

(User Manual, chapter 7)

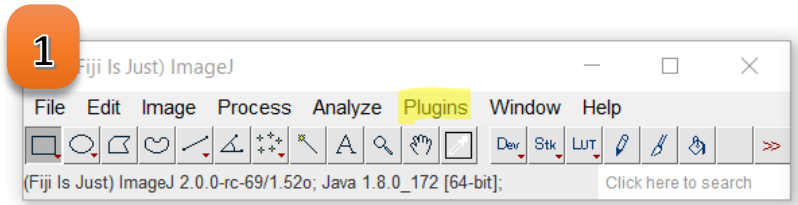
Walkthrough example

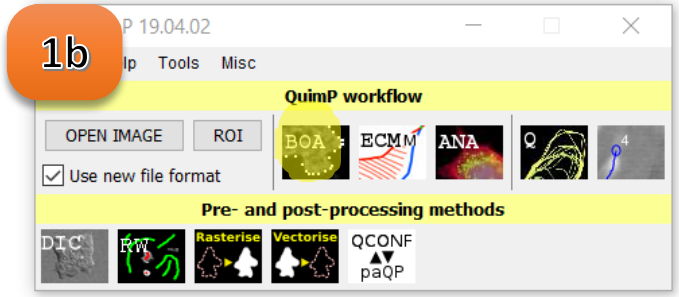
- The folder *QuimP_walkthrough* contains 4 tiff image sequences which you can analyse [1].
  - *QW_channel_1_actin.tif* - Actin label. Image and has been background corrected and contrast enhanced.
  - *QW_channel_2_neg.tif* - Negative stain. The cell appears as a shadow on a bright background, which has then been inverted.
  - *QW_channel_2_seg.tif* - For segmentation we shall use the negative stain channel. A 1 pixel Gaussian blur has been applied, the background removed, and contrast enhanced.
  - *QW_channel_2_talA.tif* – simulated talA label from corresponding binary mask by GAN network
  - *Segmentation.tif* - Already segmented image - will not be used in this tutorial.

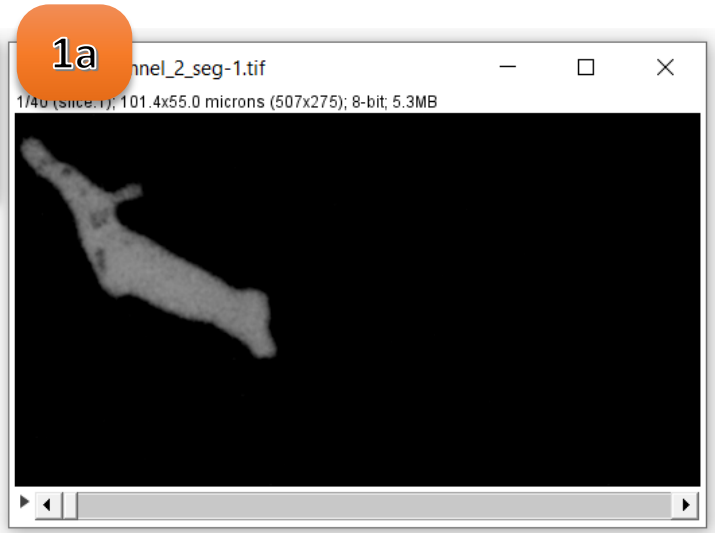
[1] Images are courtesy of Evgeny Zatulovskiy, Rob Kay Group, MRC Laboratory of Molecular Biology

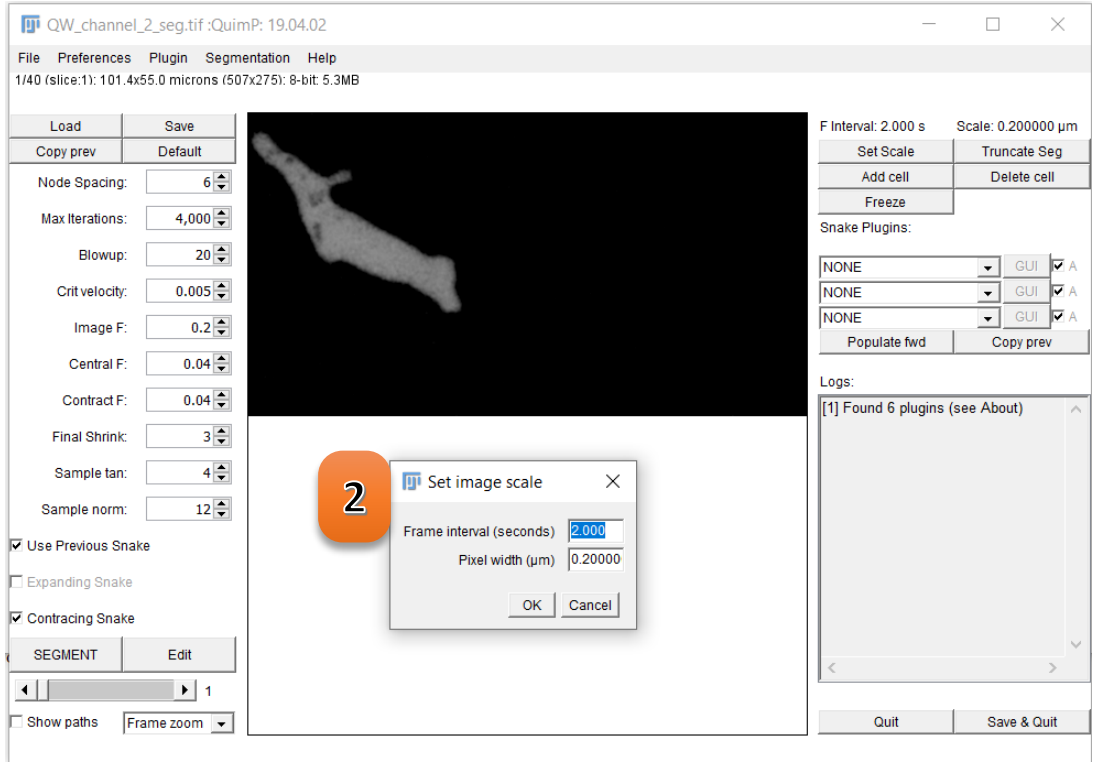
BOA

1. Open ImageJ and launch the QuimP bar [*Plugins → QuimP → QuimPBar*].
   a) Open the image *QW_channel_2_seg.tif*.
   b) Launch BOA from the QuimP bar.
2. At the prompt check the scale is correct (2 second frame interval, pixel width 0.2 microns).



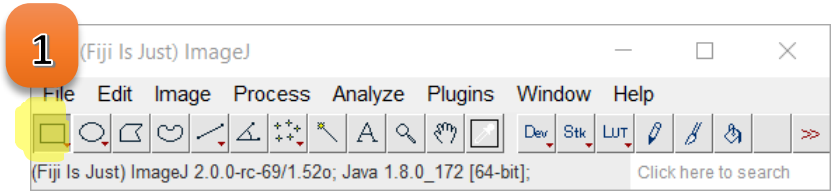


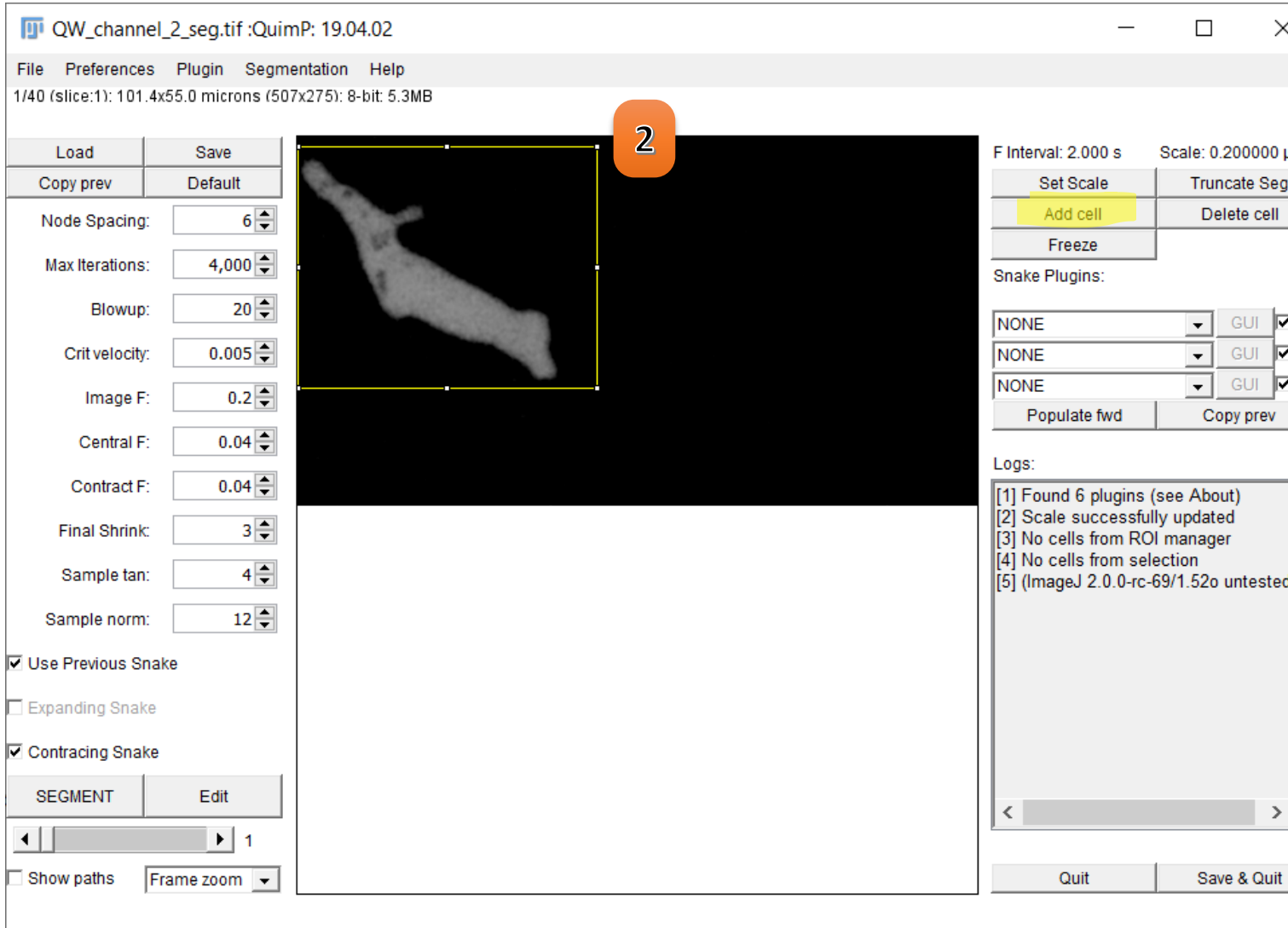




BOA

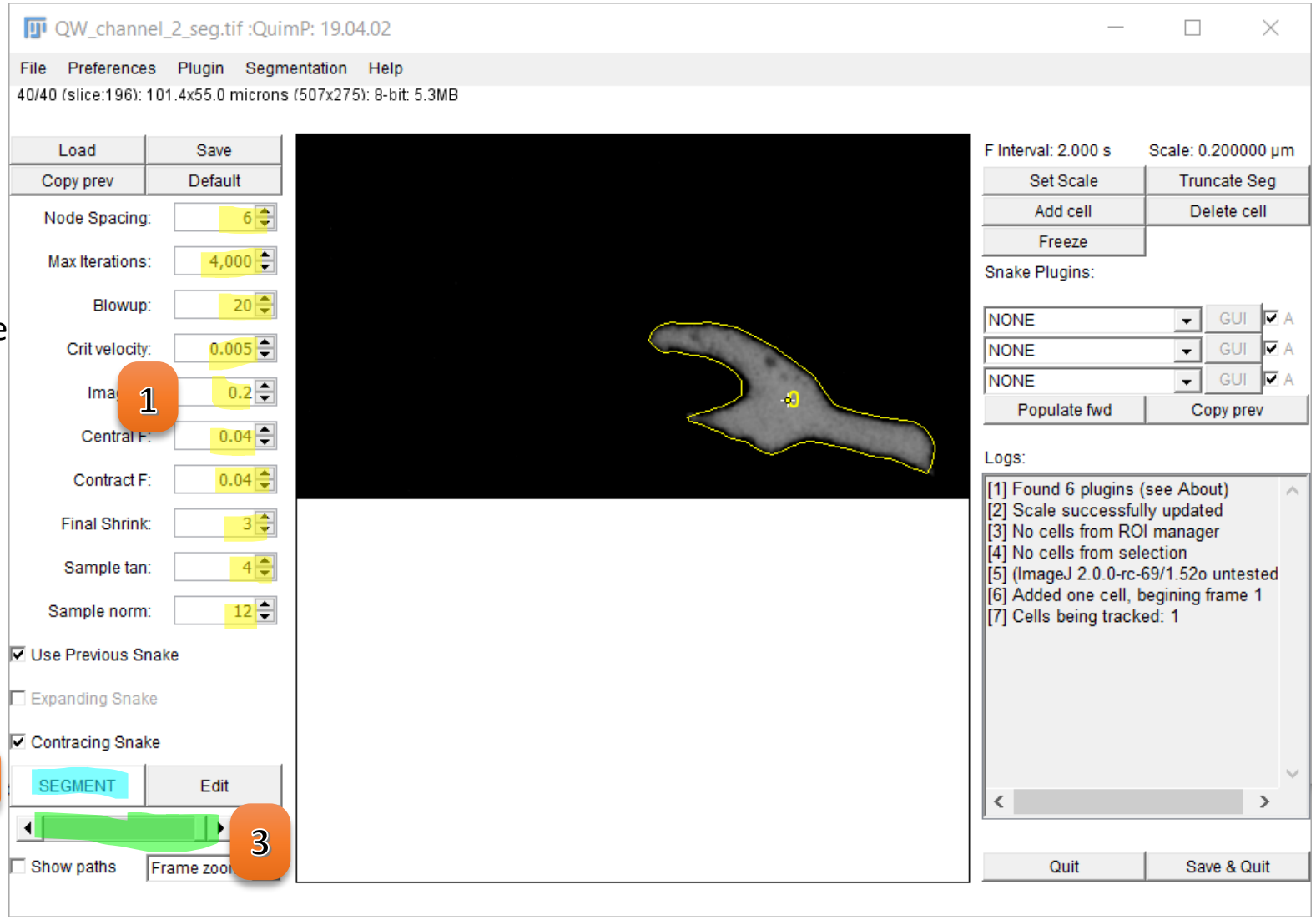
1. Using the polygon selection tool,
2. create a selection encompassing the cell. This can be very rough. Click *Add cell*.





BOA

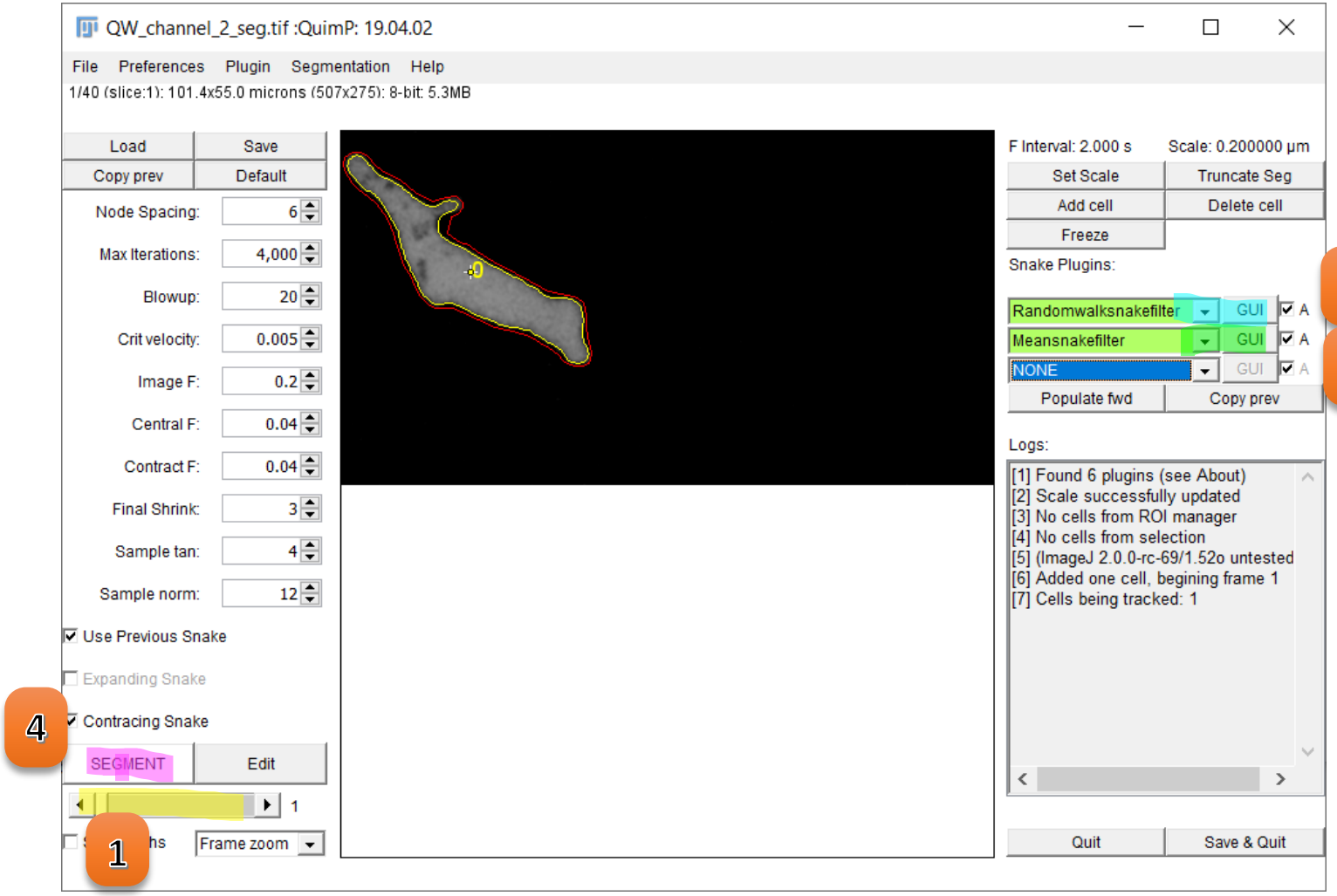
1. Adjust the parameters to get a good segmentation (default are fine).
2. Click *SEGMENT* and wait for completion.
3. Scroll through the sequence to check the segmentation result using either the scroll bar or mouse wheel. The segmentation should have completed to the last frame.



BOA

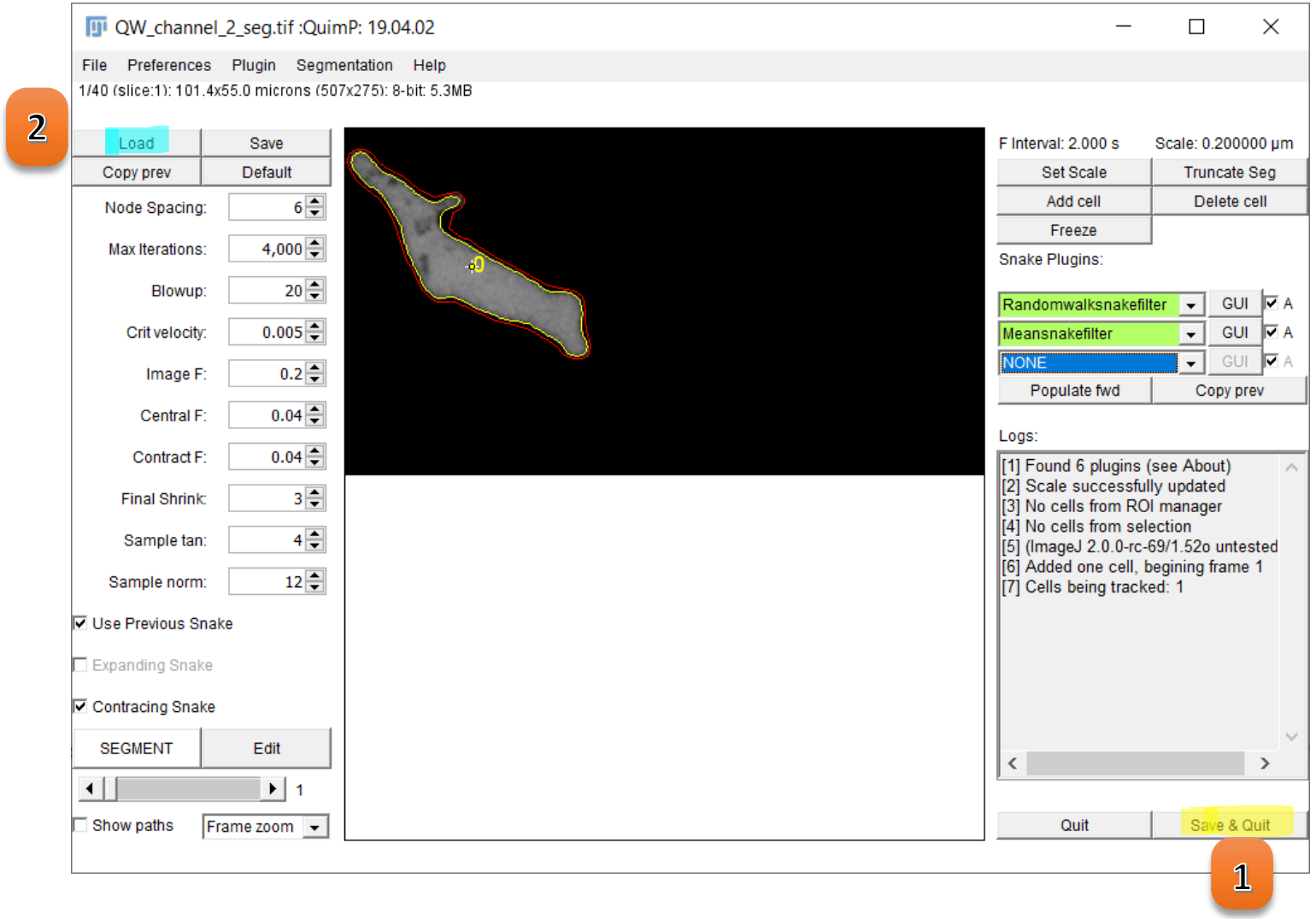
We can try to improve segmentation in concave regions by using Random Walk plugin.

1. Scroll to the first frame
2. Select RandomWalkFilter in plugin selector at first position. Current view will be updated.
3. Select MeansnakeFilter at second slot. Current view will be updated.
4. Click SEGMENT and wait until segmentation finishes.
5. Scroll through the sequence to check the segmentation result.



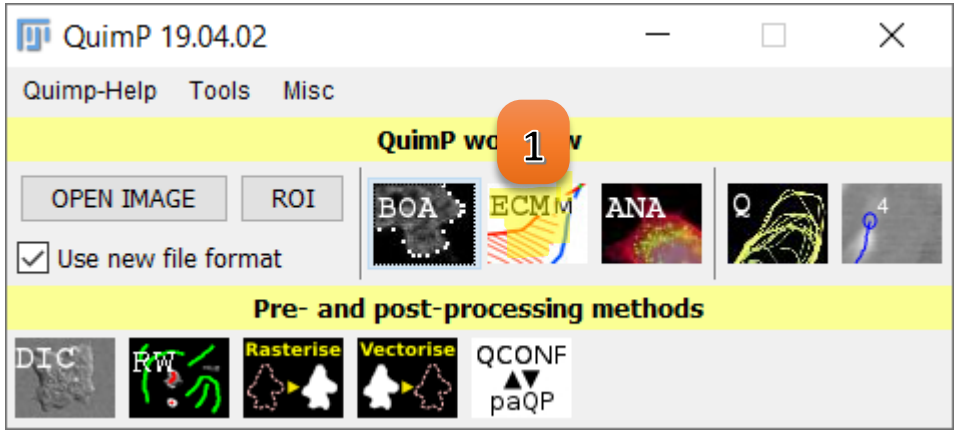
BOA

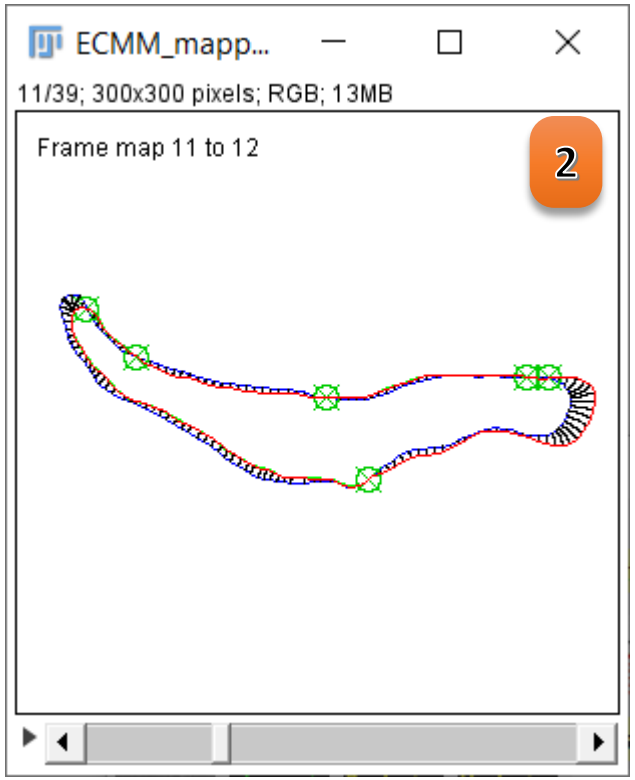
1. Click *Save&Quit* – the QCONF file will be created.
2. This file can be loaded back to BOA if needed



ECMM

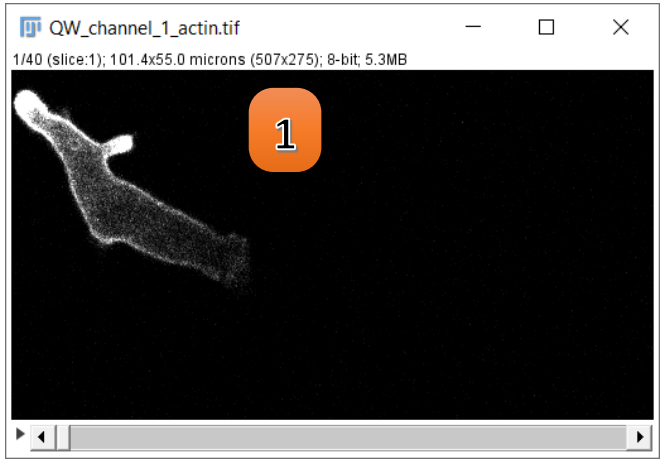
1. Launch the ECMM plugin from the QuimP bar. It does not require an image. When prompted, locate the QuimP parameter file (*QCONF*) you just created.
2. ECMM will run. You can view the result and close the image.

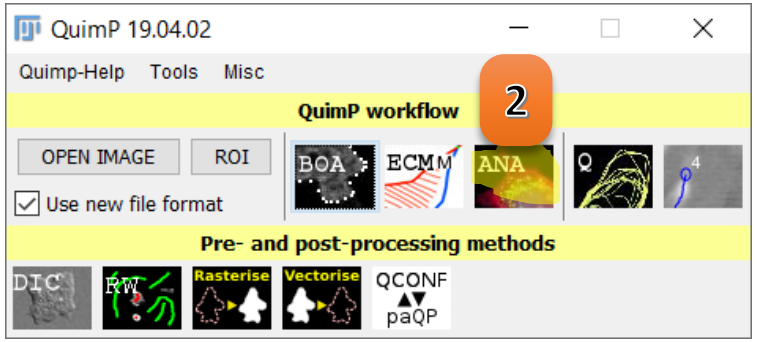


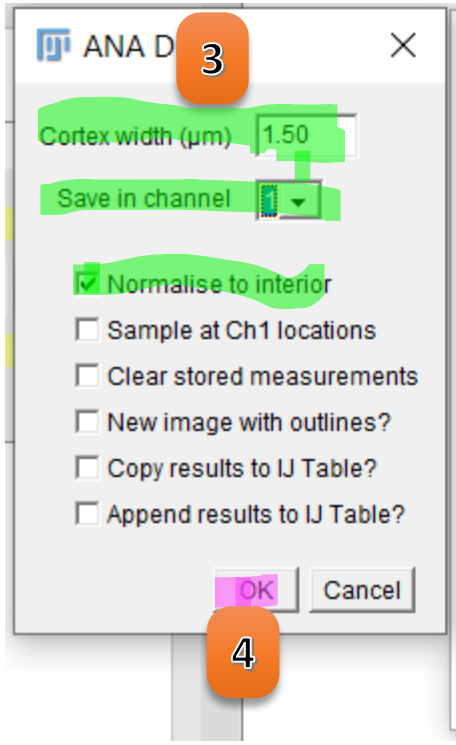


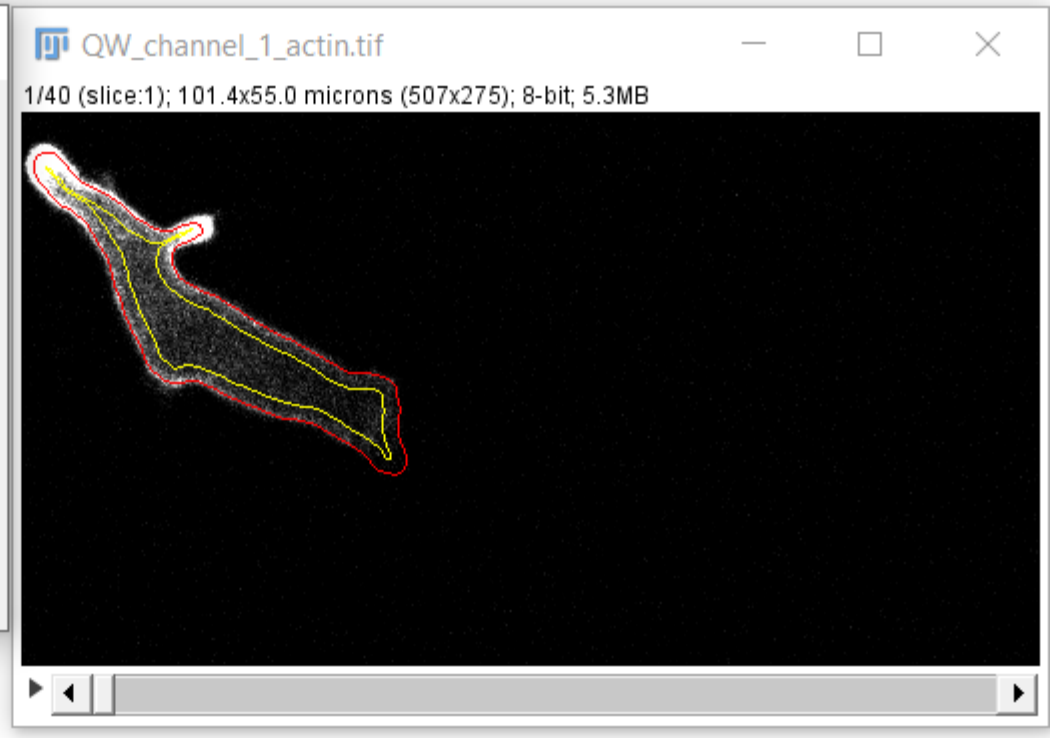
ANA

1. Open the image *QW_channel_1_actin.tif*.
2. With it in focus, launch the ANA plugin, and again select your parameter file.
3. Choose a sensible cortex width (e.g. 1.5 microns). Make sure ‘save in channel’ is set to 1, and normalise to interior is ticked.
4. Click *ok*. When complete, ANA will show the sample locations for the last frame.



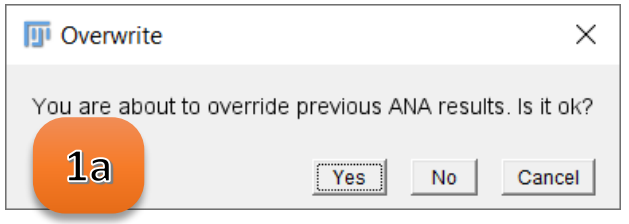


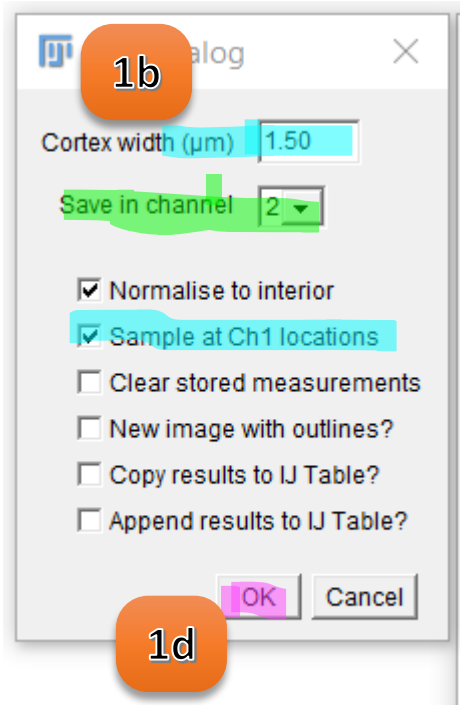


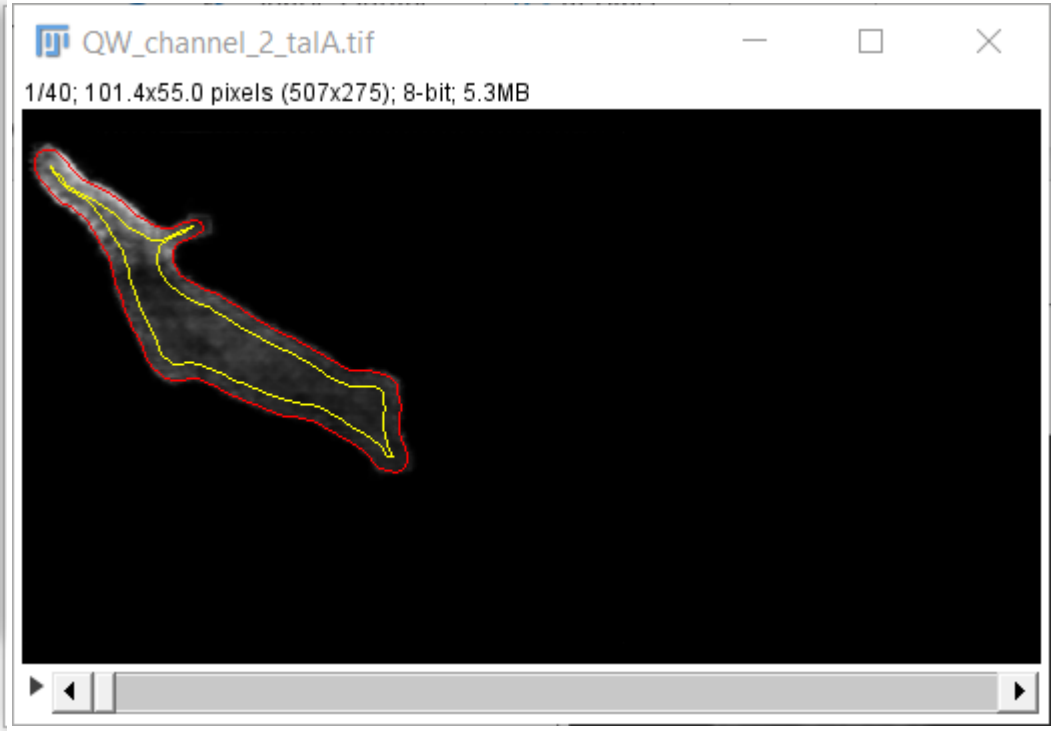


ANA

1. Close *QW_channel_1_actin.tif*, and open *QW_channel_2_talA.tif*.
2. We will record another channel for good measure. Repeat the last step, but with the channel 2 image, this time storing data in channel 2 (which is the default).
   a) Run ANA, select your parameter file and answer **Yes** on dialog box that appeared
   b) Tick *Sample at Ch1 locations*. ANA will now use the same sample points as computed for channel 1 (useful if you want to compute ratios between channels).
   c) Make sure that second channel is selected.
   d) Click *ok*. When complete, ANA will show the sample locations for the last frame.

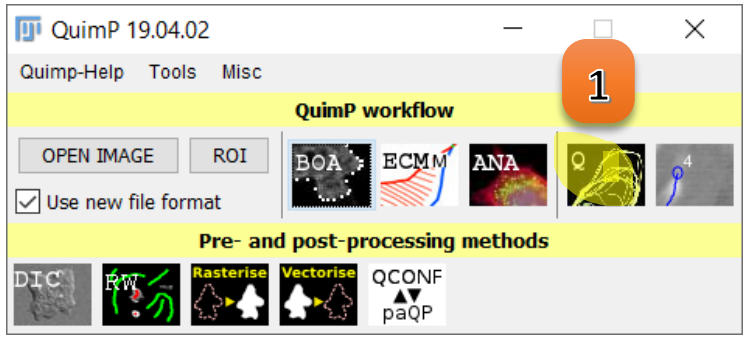


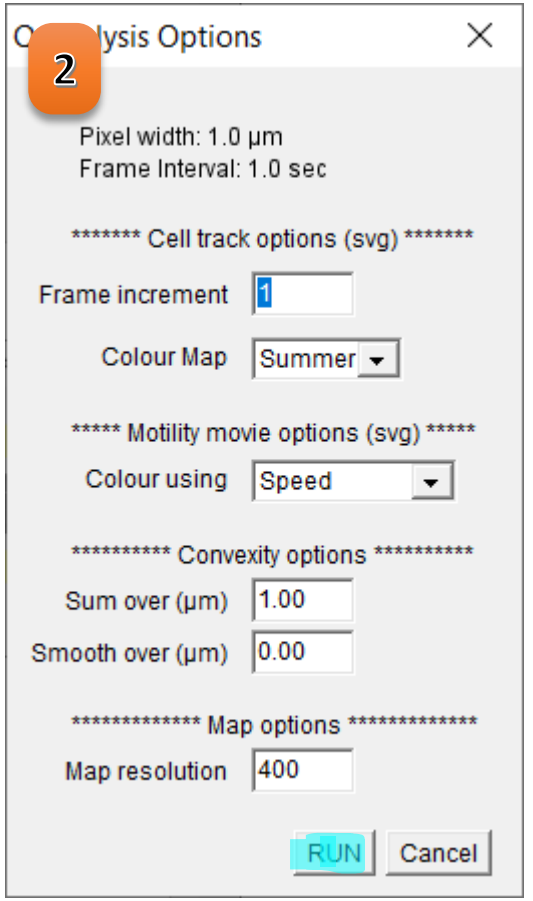


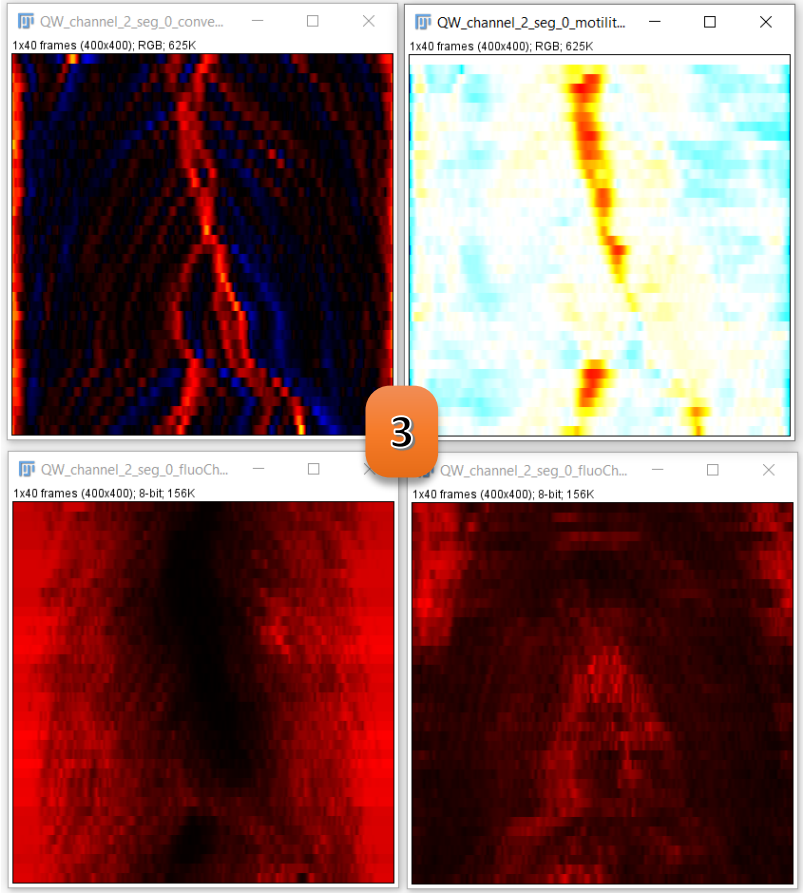


Q-Analysis

1. Launch the Q Analysis plugin
2. Check your scale is what you expect at the top of the parameter window. We will use all the current defaults, so click *RUN*. Inspect your maps, all of which are automatically saved to disk.
   a) again choose your parameter file.
3. The displayed images have been scaled to cover the colour space. The raw values have been saved as text file, with the extension *.maQP* (e.g. *QTest_0_fluoCh1.maQP*). You can view them in ImageJ by opening them via [*File- > Import- > TextImage...*].
   a) Note that latest versions of QuimP store these files inside *QCONF* configuration file. One can retrieve them by using Format Converter (see section 14 of User Manual).
   b) Investigate other files created in current folder

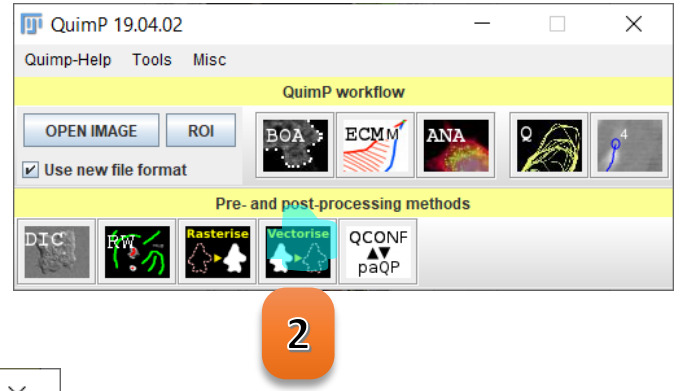


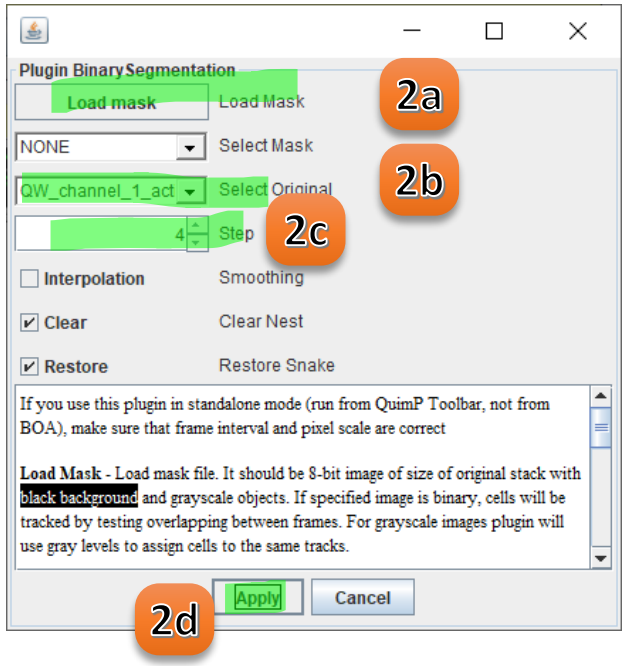




Advanced topics - external segmentation

- You can use your favourite segmentation package and still be able to work with QuimP workflow
  1. Open original image - *QW_channel_1_actin.tif*
  2. Select *Vectorise* plugin from QuimP
     a) Click *Load Mask* and select binary file *Segmentation.tif*
     b) Select original image in *Select Original*
     c) Set sampling to 4
     d) Click *Apply – new QCONF* file will be created that can be processed by ECMM and other modules.





Advanced topics - macros

- All modules can be called from macro apart from BOA and Protrusion Analysis.
- But if you use external segmentation, the full workflow can be scripted
- Have a look at *QuimP_walkthrough.ijm* example. You need to adjust paths in lines 11, 13, 15 and 29 to make is working on your computer.

QuimP_walkthrough.ijm

```
1  /**
2   * Exemplary macro running full QuimP analysis.
3   *
4   * Require already segmented image.
5   *
6   * Modify paths before use.
7   */
8
9  // define where to save main configuration file. It will be shared among QuimP modules.
10 // Any other file generated by QuimP will be saved in this folder as well
11 qconfOutput = "C:/Users/baniu/Downloads/experiment.QCONF"
12 // open segmented image, you can use any other segmentation software to obtain masks
13 open("C:/Users/baniu/OneDrive - University of Warwick/Documents/QuimP workshop/QuimP_walkthrough/Segmentation.tif")
14 // open original image
15 open("C:/Users/baniu/OneDrive - University of Warwick/Documents/QuimP workshop/QuimP_walkthrough/QW_channel_1_actin.tif")
16
17 // 1) perform conversion from mask to QCONF file. This step corresponds to saving segmentation in BOA
18 run("Generate Qconf", "opts={options:{" +
19         "select_mask:Segmentation.tif," +   // name of the mask image (nod ID)
20         "select_original:QW_channel_1_actin.tif," + // name of the image or path here
21         "step:4.0,smoothing:true}," +   // step stands for density of nodes, step=1 means each pixel of mask will be mapped to
22         "maskFileName:()," +                // alternatively path to mask file
23         "paramFile:("+qconfOutput+")}");
24
25 // 2) run ECMM analysis on configuration file
26 run("ECMM Mapping", "opts={paramFile:("+qconfOutput+")}");
27
28 // 3) run ANA analysis, we use only one channel
29 open("C:/Users/baniu/OneDrive - University of Warwick/Documents/QuimP workshop/QuimP_walkthrough/QW_channel_1_actin.tif"); // i
30 selectWindow("QW_channel_1_actin.tif");
31 run("ANA", "opts={plotOutlines:true,fluoResultTable:true,fluoResultTableAppend:false," + // configure displaying
32         "channel:0, userScale:5.0," + // set channel and cortex width (in um, pixel size from image will be used)
33         "normalise:true, sampleAtSame:false," +
34         "clearFlu:false," +
35         "paramFile:("+qconfOutput+")}");
36
37 // 4) run Q analysis
38 run("QuimP Analysis", "opts={trackColor:Summer," +
39         "outlinePlot:Speed," +
40         "sumCov:1.0,avgCov:0.0," +
41         "mapRes:400," +
42         "paramFile:("+qconfOutput+")}");
43
44 // 5) convert data to csv files and generate coordinates maps
45 run("Format converter", "opts={status:[ecmm:outlines,ecmm:centroid,map:coord,map:origin,map:ycoords,map:xcoords]," +
46     "areMultipleFiles:true," +
47     "paramFile:("+qconfOutput+")}");
```