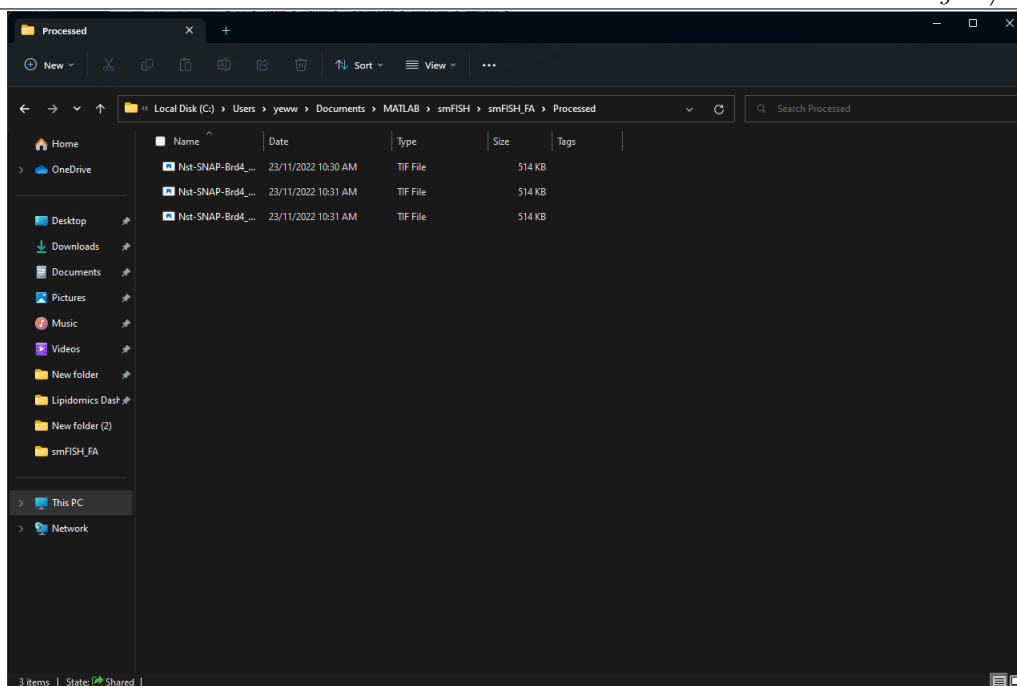


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## 1 Getting the necessary software

1. Download Fiji's distribution of the ImageJ from  
<https://imagej.net/software/fiji/downloads>.
2. Download Ilastik from  
<https://www.ilastik.org/download.html>.
3. Download MATLAB from  
[https://au.mathworks.com/login?uri=%2Fdownloads%2Fweb\\_downloads](https://au.mathworks.com/login?uri=%2Fdownloads%2Fweb_downloads).

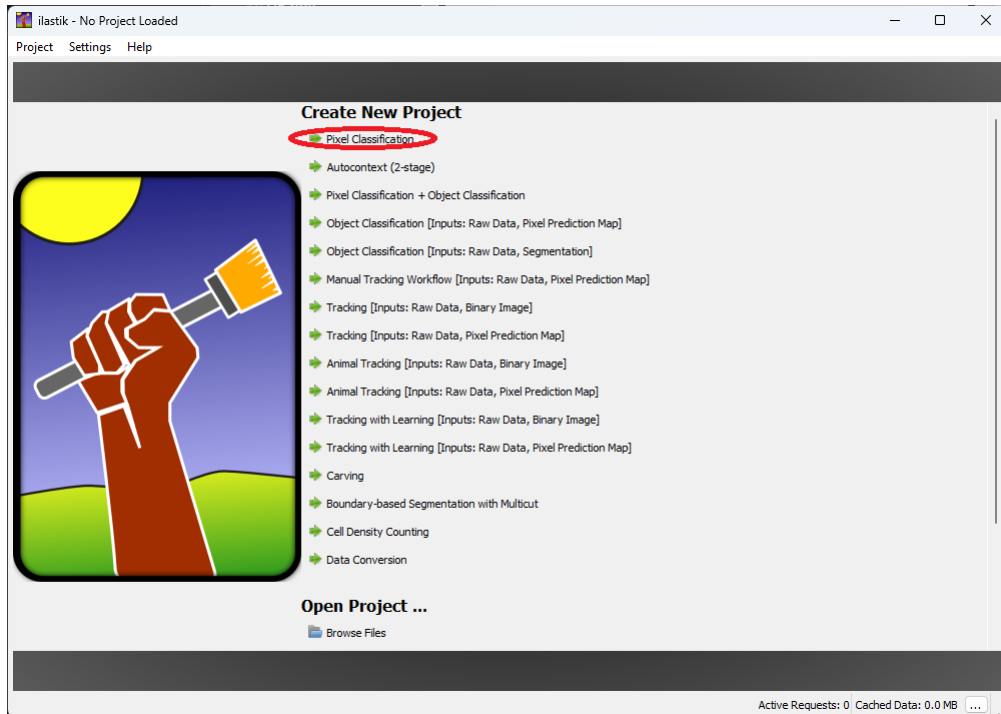




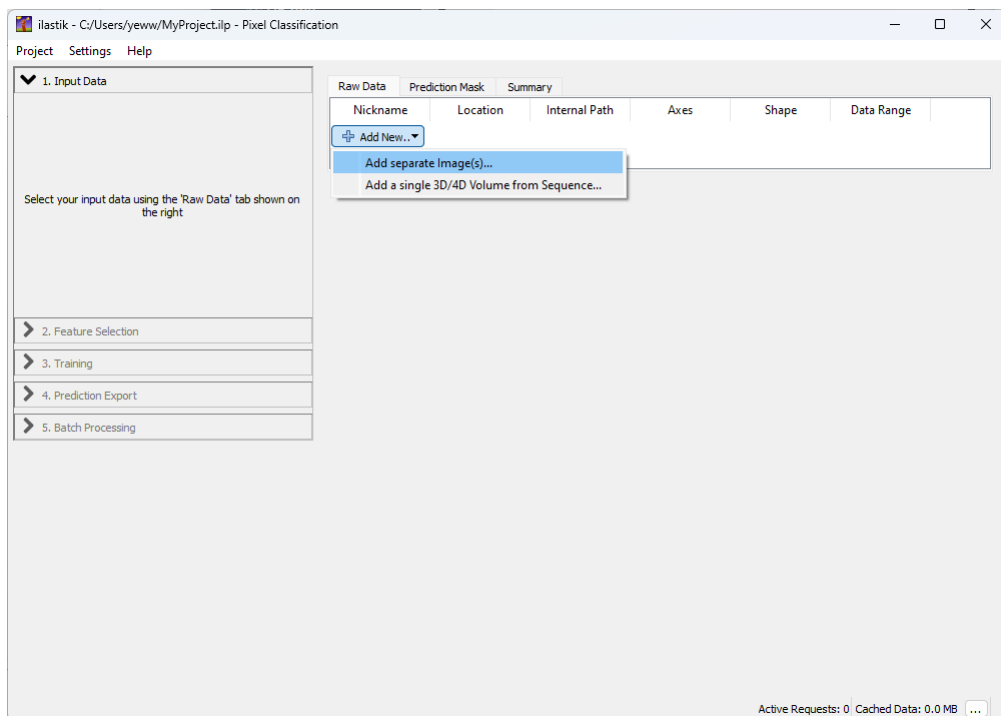
11. You may now exit Fiji.

### 3 Identify the objects in each channel

12. Run Ilastik.
13. A new window should up, create new *Pixel Classification* project.

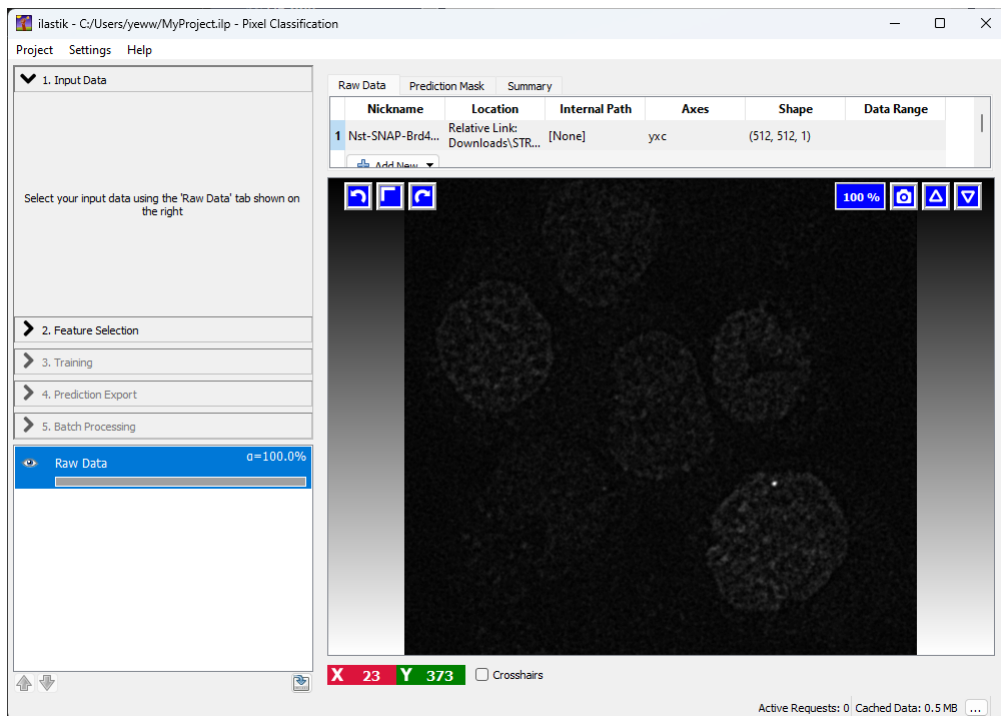


14. Save the project file at a location of convenient.
15. Click the "Add New.. - Add separate Image(s)..." as shown in the figure below.



16. Select a few of the files created in from the Fiji earlier (all the files need to be from the same channel,

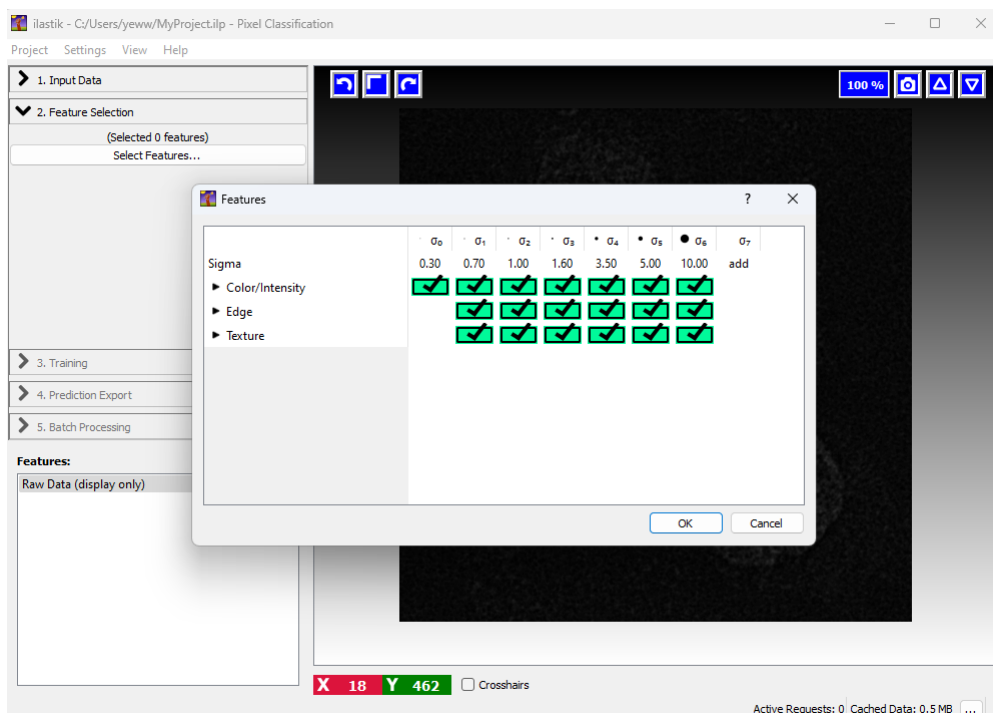
it is recommended to make use of the *mNG* channel). You should see the image successfully loaded as below.



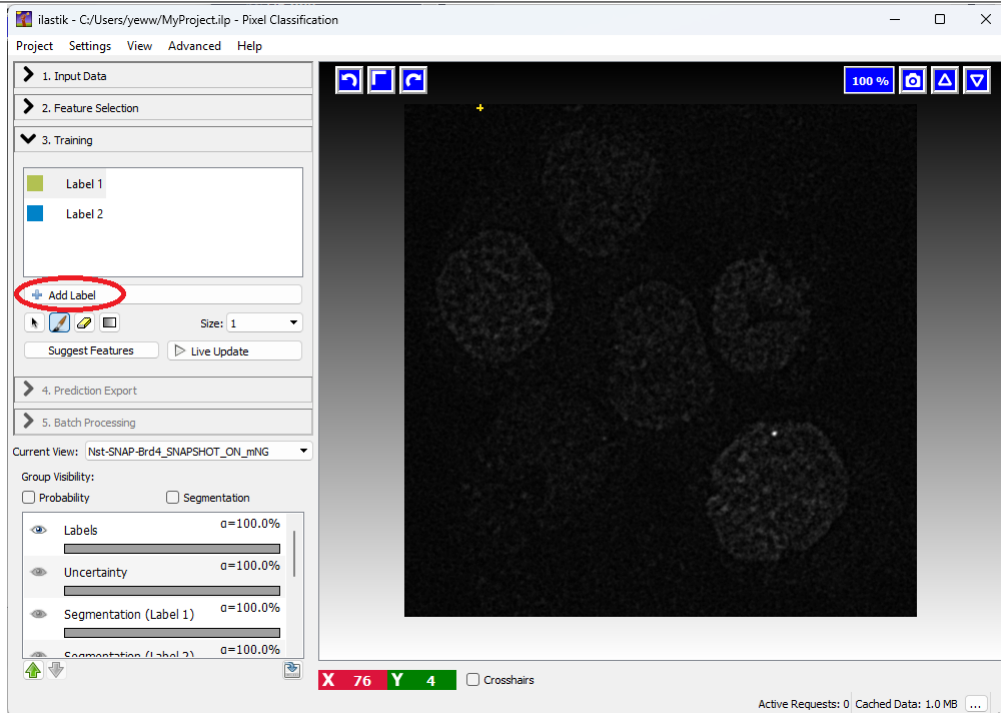
17. Click on "2. Feature Selection" on the left hand side.

18. Click "Select Features..."

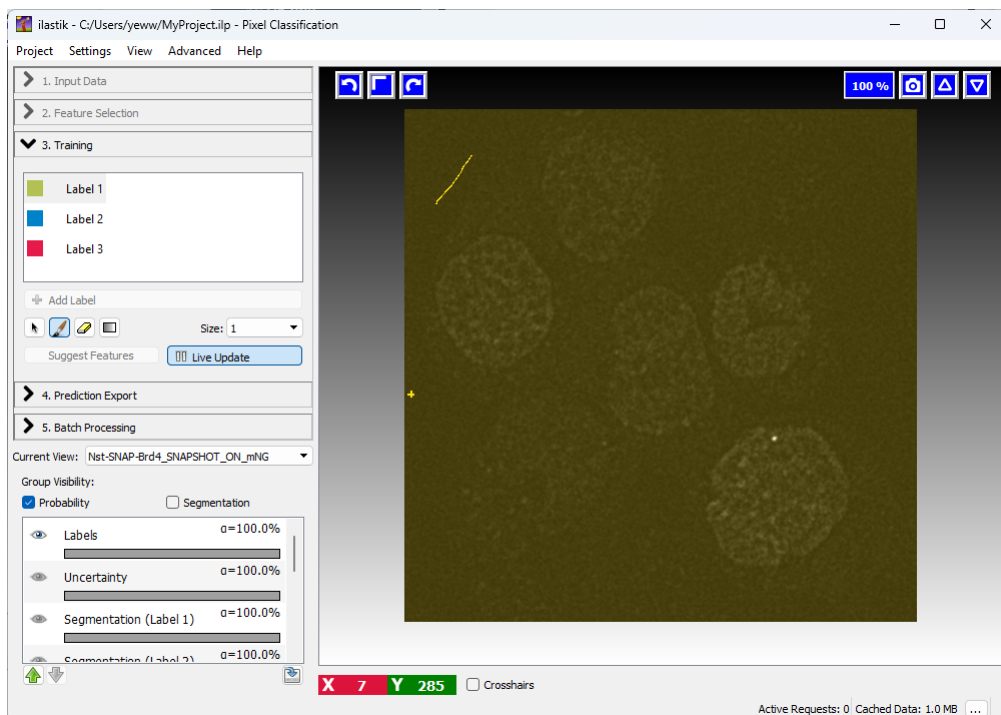
19. Select the number of features to use for the AI training to use for detection.



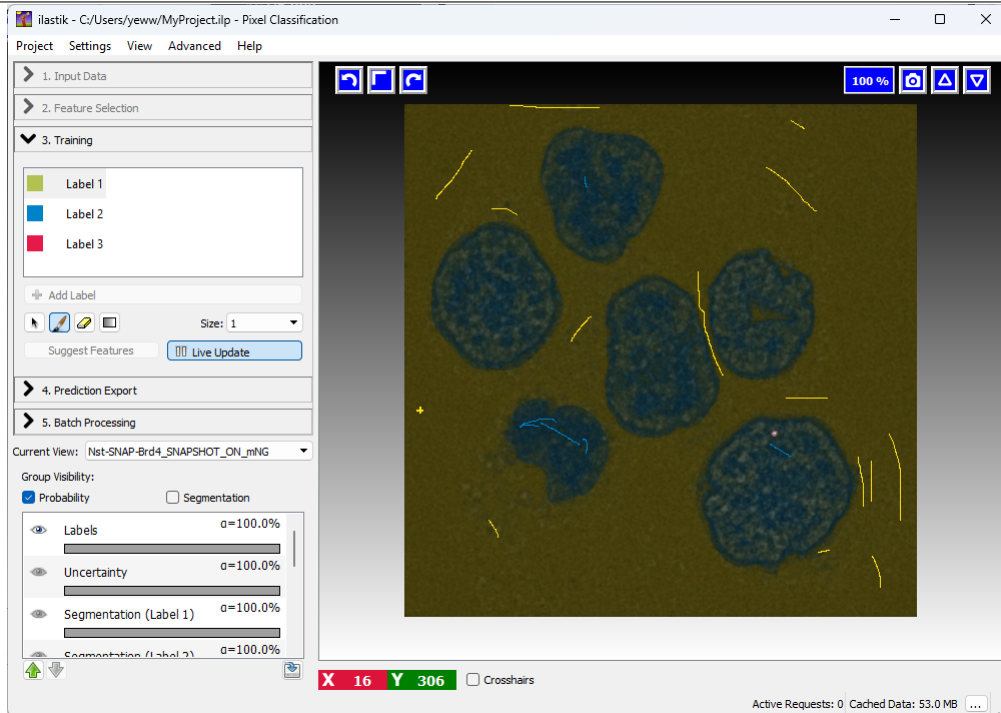
20. Click "Okay, and click "3. Training", then "Add Label".



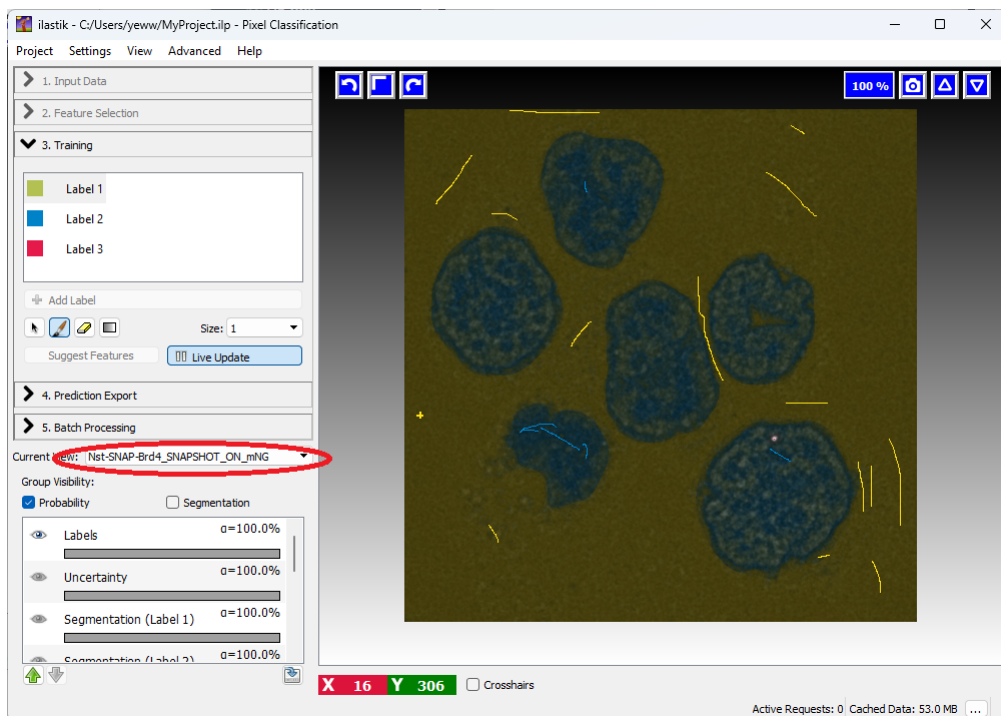
21. Click on "Label 1" and draw line to indicate the background on the image. Click on "Live Update" on the left hand side and your image should change to that colour as shown below.



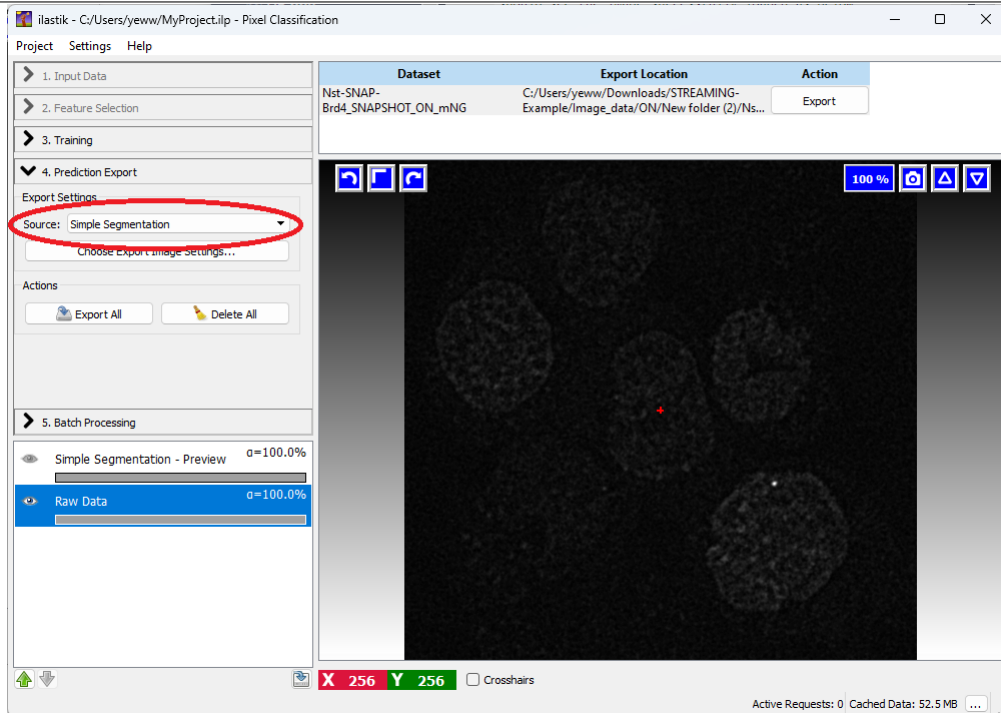
22. Click on "Label 2" to label the cell" and "Label 3" to label the spots of interest, continue brushing the image until you arrive at satisfactory segmentation.



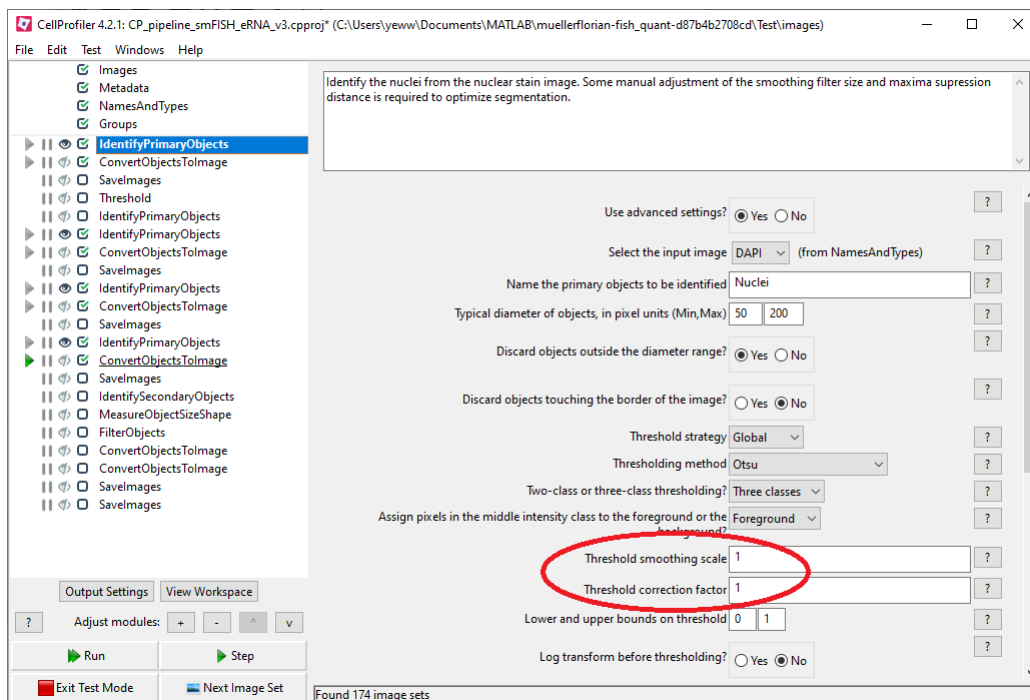
23. Change "Current View" on the left hand side and work on different images if you uploaded more than one.



24. When you're done, click on "4. Prediction Export" on left hand side and change the "Source" to "Simple Segmentation".

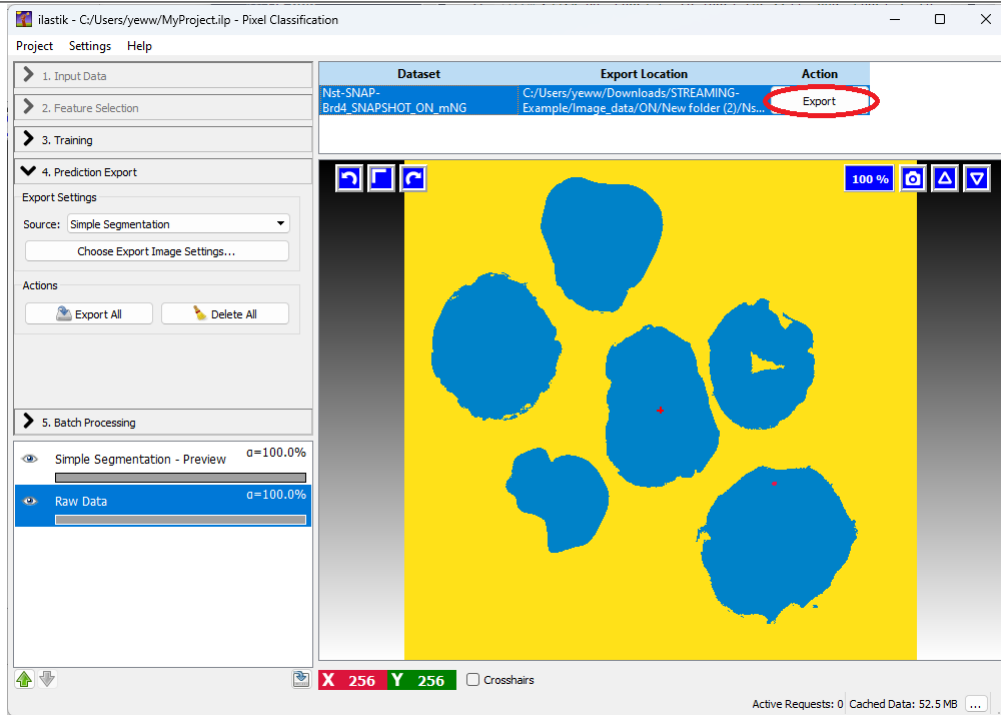


25. Click on the eye button next to "Simple Segmentation - Preview" on the bottom left to see the result.

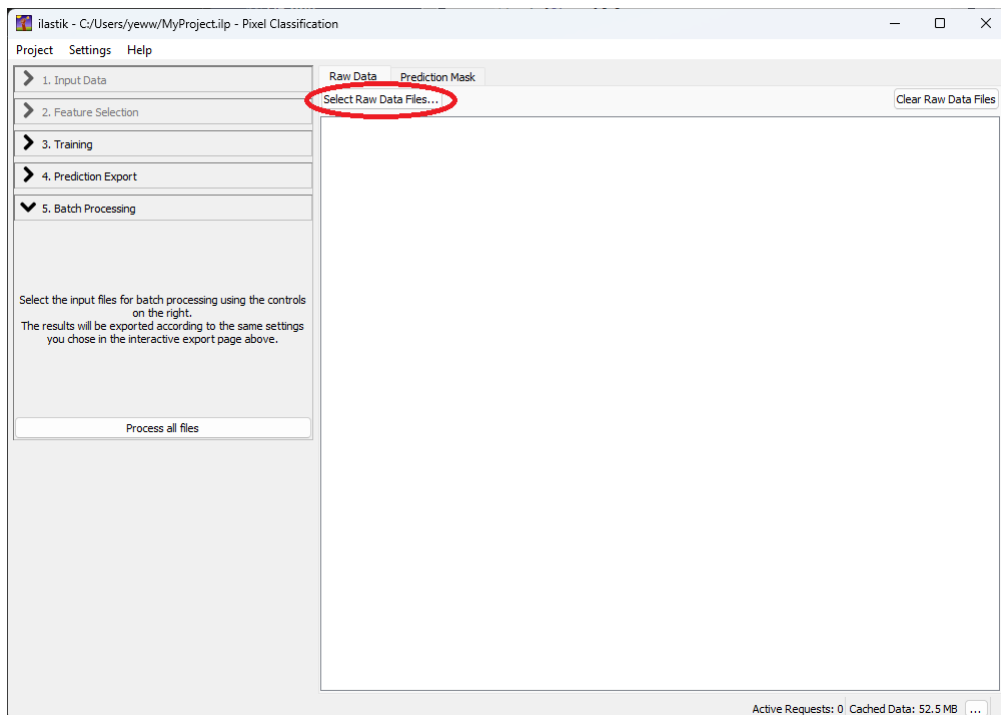


26. Click the "Export" button if you're happy with the result





27. Finally, we go to "5. Batch Processing" tab on the left hand side and "Select Raw Data Files..." and select all the remaining files produced by Fiji (they have to be the same channel).



28. There should be segmentation *h5* files in the same folder as your *tif* files generated by the Fiji earlier now.

## 4 Cell Quantification

29. Start up MATLAB by running the *smFISH-TA-Ilastik.m*. Define the parameters defined in line 3 - 7. Then click "Run" on the top bar.
30. A pop-up will appear and let you select the folder containing all the files from *Ilastik* and *Fiji*.
31. Select the folder and press "Select Folder".
32. Wait for MATLAB to finish running and a new "Solution.csv" file should appear in the folder.
33. The first column in that folder is the name of the file, follow up by whether the *RFP* and *mNG* is located within tolerance given in MATLAB, with 1 being yes and 0 being no. Finally, the third column is the minimum distance between the *SNAP* and *mNG*.