

Segmentation in Ilastik

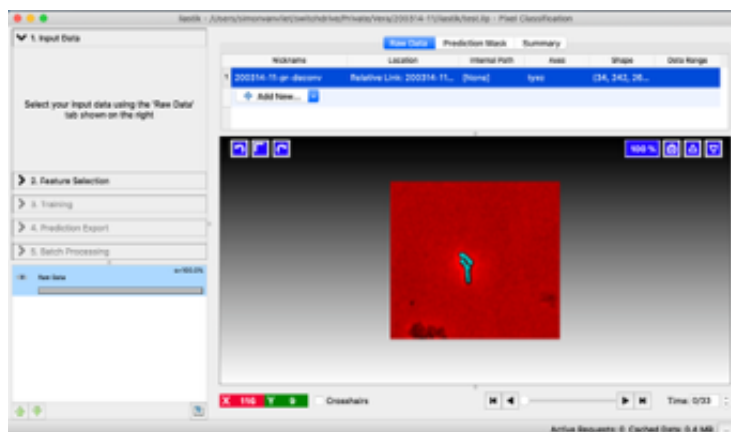
Export data to Ilastik for segmentation. Once export is done, segment data in Ilastik and export result.

Open Ilastik, you have two options:

1. Option 1: Create a new Pixel Classification Project
2. Option 2: Copy an existing Pixel Classification Project and open this

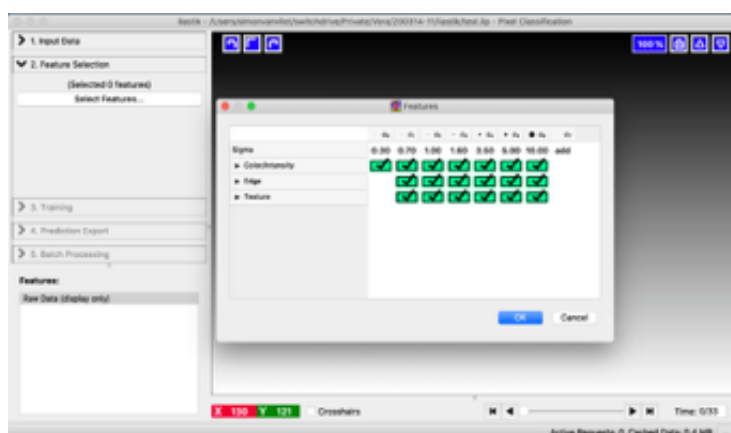
For option 1:

1. Go to tab "1. Input data"
 1. Go to "Add New"
 2. Select "Add Separate Images"
 3. Select the file indicated in the output window in ColonyWorkflow



Input data interface.

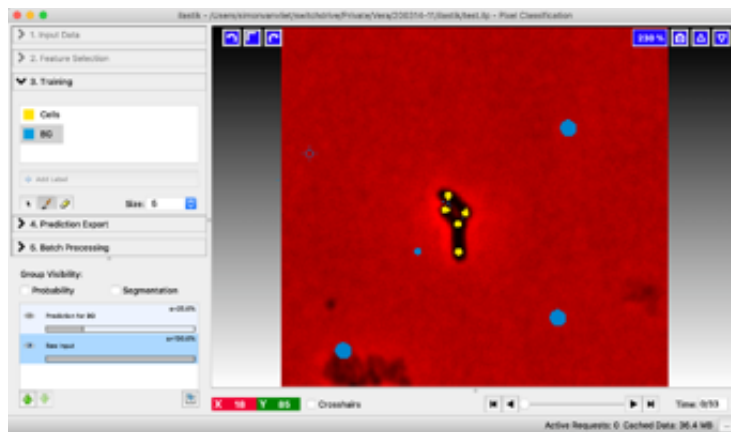
2. Go to tab "2 Feature Selection"
 1. Go to "Select Features"
 2. Select all
 3. Click OK



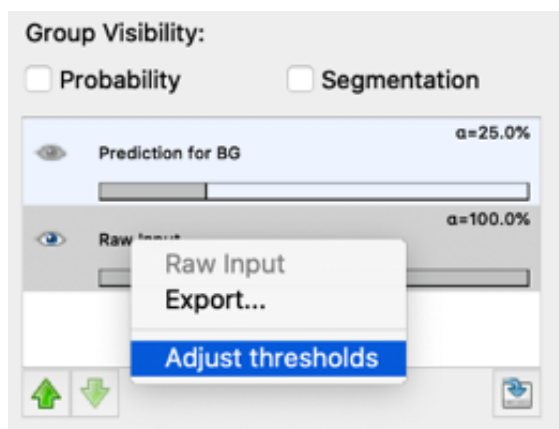
Recommended selection.

3. Go to tab "3. Training"

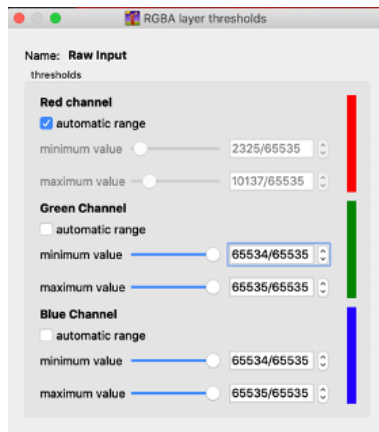
1. Relabel Label 1 to "Cells"
2. Relabel Label 2 to "BG"
3. Turn on "Live Update"
4. In frame 1, add training points for cells and background. Use sparse (small) points, this works better (see example).
5. Especially add BG points in the space between cells to separate them
6. Play with visibility of layers to check your results. Especially handy are
 1. "Segmentation Cells", turn to $\alpha=100\%$ to see preview of segmentation
 2. "Uncertainty", shows pixels where software is uncertain whether it is cell or BG, adding training points here helps the most
7. Once, happy, go to last frame and repeat
8. Once happy, go through all frames and keep adding training points till segmentation is $>95\%$ correct. Training Ilastik is faster than manual correcting.
9. Some hints:
 1. You can save time by copying a classifier from another colony. This way you only need to tune segmentation.
 2. Right click on "Raw Input" allows you to adjust color channels, of raw data.
 3. Toggle "Live update" off to increase speed. Toggle back on to check results.



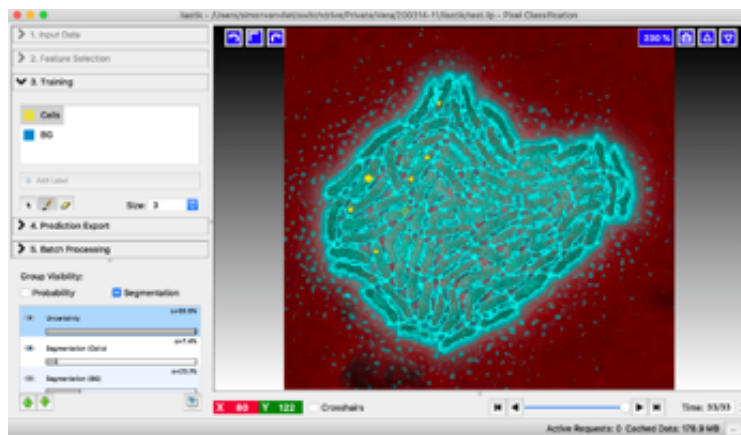
Add sparse training points.



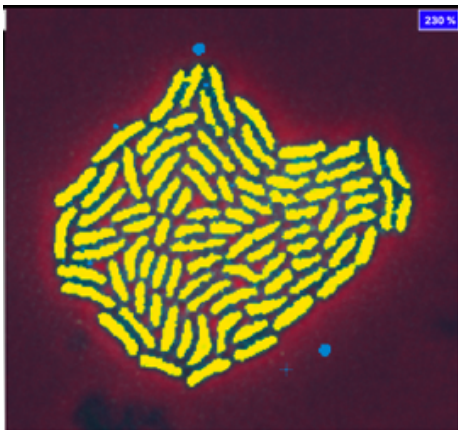
Adjust colors of raw data.



Adjust colors of raw data.

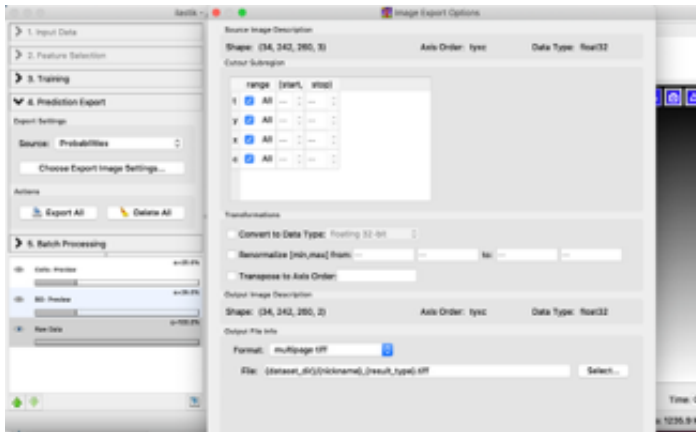


Using Uncertainty layer on last frame to help classifier. Put training points in bright blue regions.



Good end point for Ilastik segmentation: most cells are separated, only some small clusters need to be cut by hand.

4. Go to Tab "4 Prediction export"
 1. Under Source select "Probabilities"
 2. Select "Choose Export Image Settings"
 1. Under format select "Multipage Tiff"
 2. Click Ok
 3. Click Export All



Export dialog with required settings.

For option 2: add your new data to the existing Ilastik classifier as in point 1 above. Check the segmentation, and add extra training points as in in point 2 above. Export data as in point 4 above.