Quantification of Insulin Granules in Pancreatic Cells

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This protocol was created to quantify the number of insulin mature granules in pancreatic β-cells from electron micrographs. It quantifies the total number of mature granules as well as those which are "docked" (interacting with the cell membrane). If you have any doubts, find any bugs or want further information on how I created it, just send me an email at: carnopo@alumni.uv.es.

PREREQUISITES

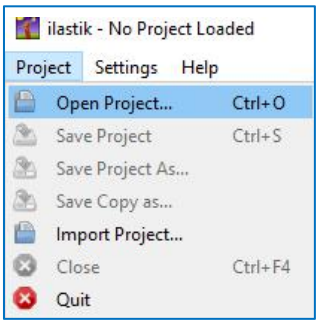
Before following the rest of the protocol, make sure you do the following:

- Download **Fiji** from *here*
- Download **Ilastik** from *here*
- Download the Ilastik project (trained_segmentation.ilp) and the Fiji macro (count_granules.ijm) from *here*
  (The easiest way to do this is by clicking on the green "*Code*" button and then selecting "*Download ZIP*")

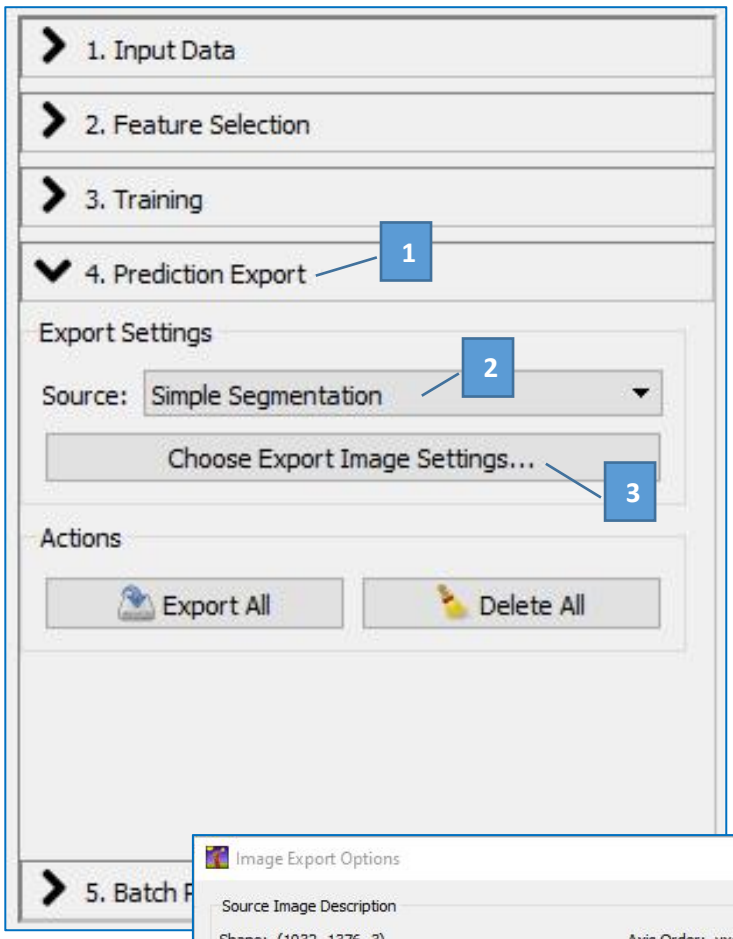
STEP 1: Granule Segmentation

This first part uses **Ilastik** to train a machine learning algorithm to segment the mature granules from the electron micrographs.

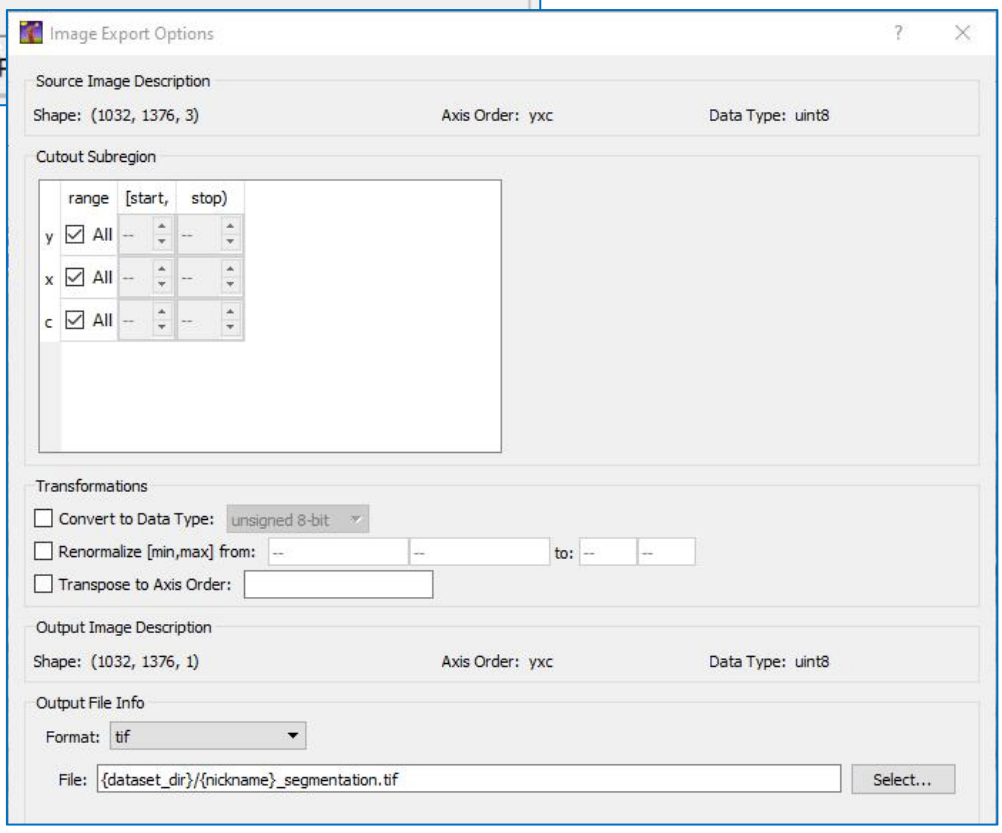
1. Open **Ilastik** and open the project you previously downloaded (trained_segmentation.ilp)



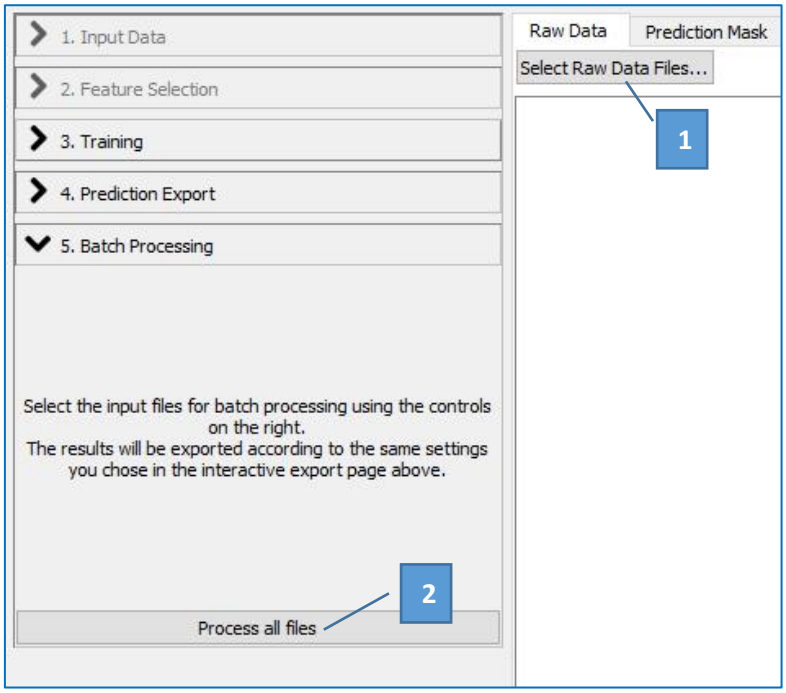
2. The model is already trained. So go to the "*4. Prediction Export*" section. Select "*Simple Segmentation*" from the drop-down menu labelled "*Source*:". Then click on "*Choose Export Image Settings…*".



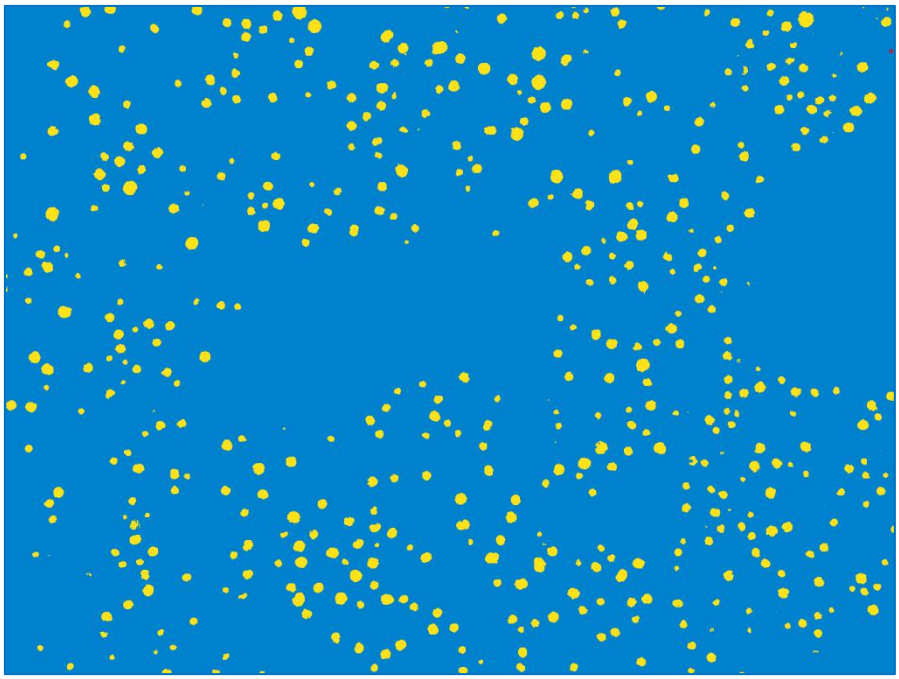
Make sure the settings are the same as follow:



3. Now go to the "*5. Batch Processing*" section, select all the images you want to segment and then click "*Process all files*"



4. You will have an output similar to this but in shades of grey (so when you open it in **Fiji** later it will look completely black, don't worry) .



5. If you wish to train the algorithm further you can always go to the "*1. Input Data*" section, add more images and add more labels in the "*3. Training*" section. You can read more on how to do this in Ilastik's provided documentation (*here*).

STEP 2: Quantification

This second part uses a macro made for **Fiji** together with some user input to quantify the total granules per cell area and the "docked" granules.

1. Open **Fiji**

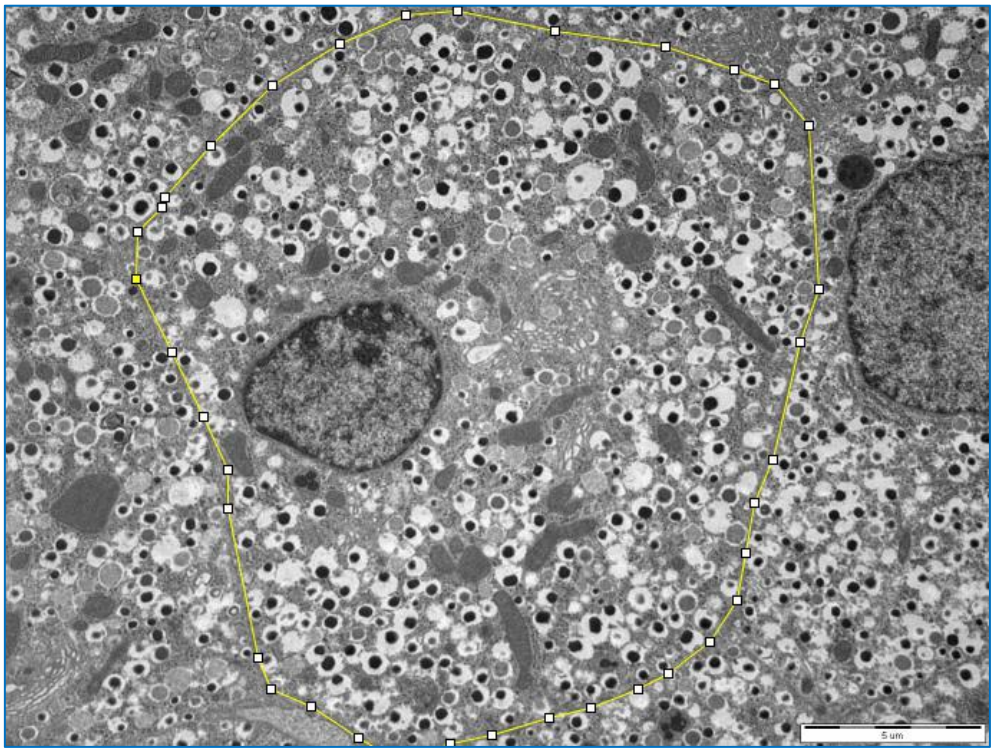
   Note: I recommend you to install the macro downloaded previously (count_granules.ijm) through `Plugins > Install...` and then selecting the "*Macro*" folder. You'll have to restart **Fiji**. For ease of use you can even add a keyboard shortcut to run the macro by going to `Plugins > Shortcuts > Add Shortcut...`

2. Open the .tif file produced previously in the segmentation step

3. Open the corresponding electron micrograph

4. Over the micrograph, draw over the cell membrane with the ROI selection tools (polygon or freehand)

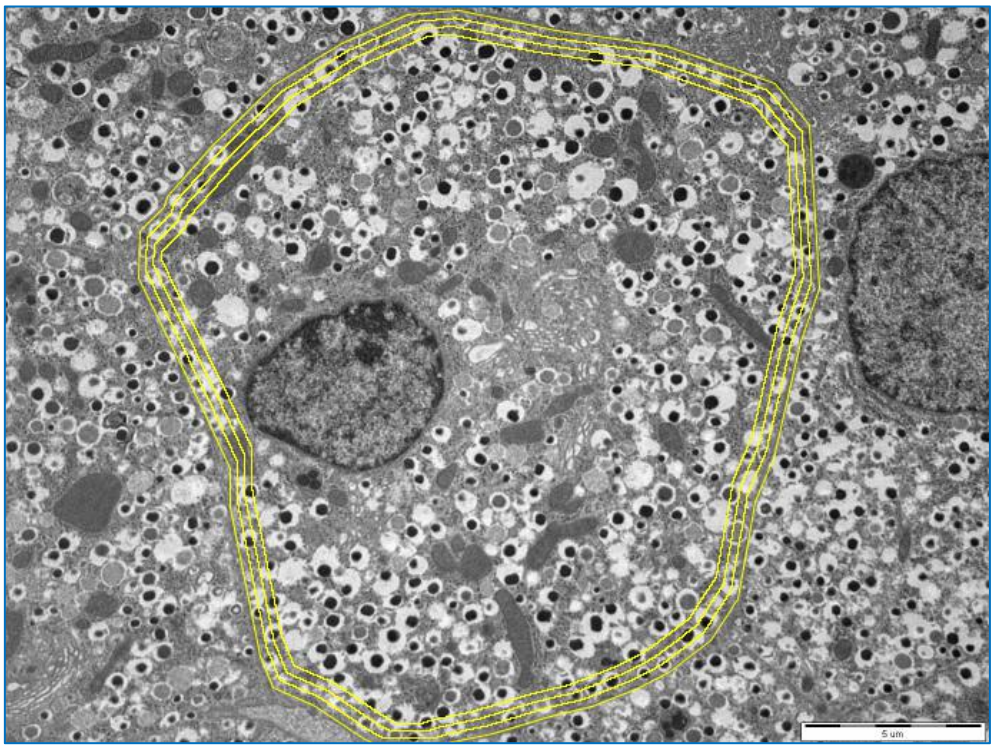
   It should look something like this:



5. Now run the macro through the keyboard shortcut you created earlier or through `Plugins > Macros > Run...`

   A dialogue window will appear where you can choose the directory where the results will be saved.

The macro will automatically select that same region of interest in the segmentation image and perform the whole cell analysis. It will then create three concentric rings which will be used to calculate the metrics for the “docked” granules at the different levels. This is what those three regions look like:



6. After the quantification has taken place, there will be two files produced. A .csv file containing a summary of all the metrics calculated for the several regions of interest and a .zip file containing the outlines of all these regions.

   You can find these files in the folder you selected when you ran the macro