

# 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  $\beta$ -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](mailto:carnopo@alumni.uv.es).

## PREREQUISITES

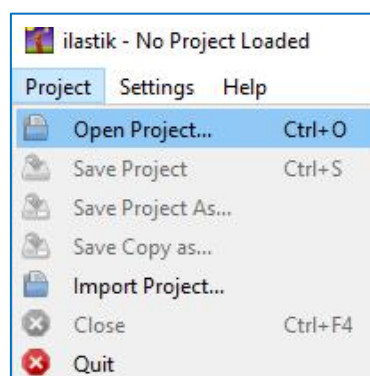
Before following the rest of the protocol, make sure you do the following:

- Download Fiji from [here](#)
- Download Ilastik from [here](#)
- Download the Ilastik project (trained\_segmentation.ilp) from [here](#)
- Download the Fiji macro (count\_granules.ijm) from [here](#)

## 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)



- The model is already trained. So go to the “4. *Prediction Export*” section, Select “*Simple Segmentation*” from the drop-down menu and click on “*Choose Export Image Settings...*”. Make sure the settings are the same as follow:

Image Export Options

Source Image Description  
Shape: (1032, 1376, 3) Axis Order: yxc Data Type: uint8

Cutout Subregion

	range	[start, stop]
y	<input checked="" type="checkbox"/> All	-- --
x	<input checked="" type="checkbox"/> All	-- --
c	<input checked="" type="checkbox"/> All	-- --

Transformations

☐ Convert to Data Type: unsigned 8-bit

☐ Renormalize [min,max] from: -- -- to: -- --

☐ Transpose to Axis Order: --

Output Image Description  
Shape: (1032, 1376, 1) Axis Order: yxc Data Type: uint8

Output File Info  
Format: tif

File: {dataset\_dir}/{nickname}\_segmentation.tif Select...

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

1. Input Data

2. Feature Selection

3. Training

4. Prediction Export

5. Batch Processing

Raw Data Prediction Mask

Select Raw Data Files...

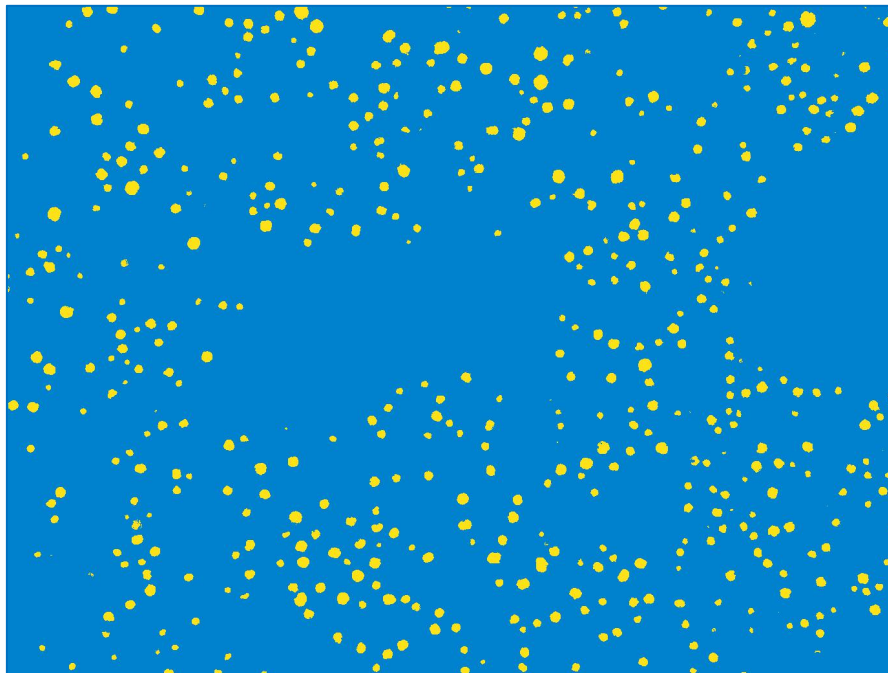
1

Select the input files for batch processing using the controls on the right. The results will be exported according to the same settings you chose in the interactive export page above.

2

Process all files

4. You will have an output similar to this but in shades of grey.





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**

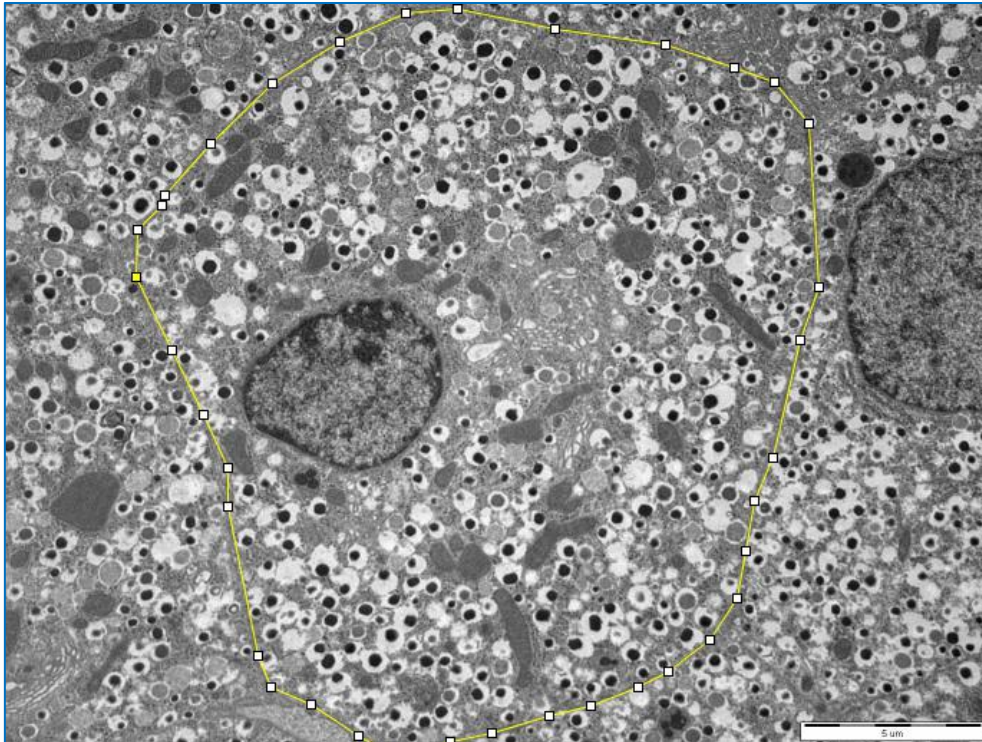
This second part uses a macro made for **Fiji** together with some user input to quantify the total granules per cell area and the “docked” granules.

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...

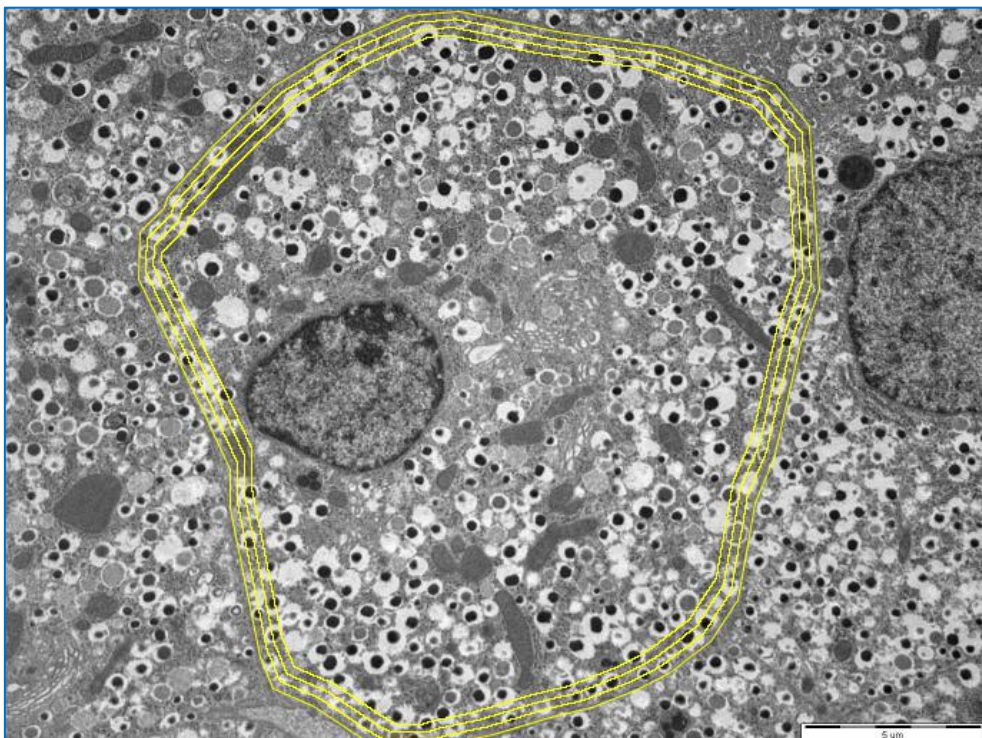
2. Open the .tif file produced previously in the segmentation step. Open the corresponding electron micrograph.
3. Over the micrograph, draw over the cell membrane with the ROI selection tools (polygon  or freehand  )

It should look something like this:



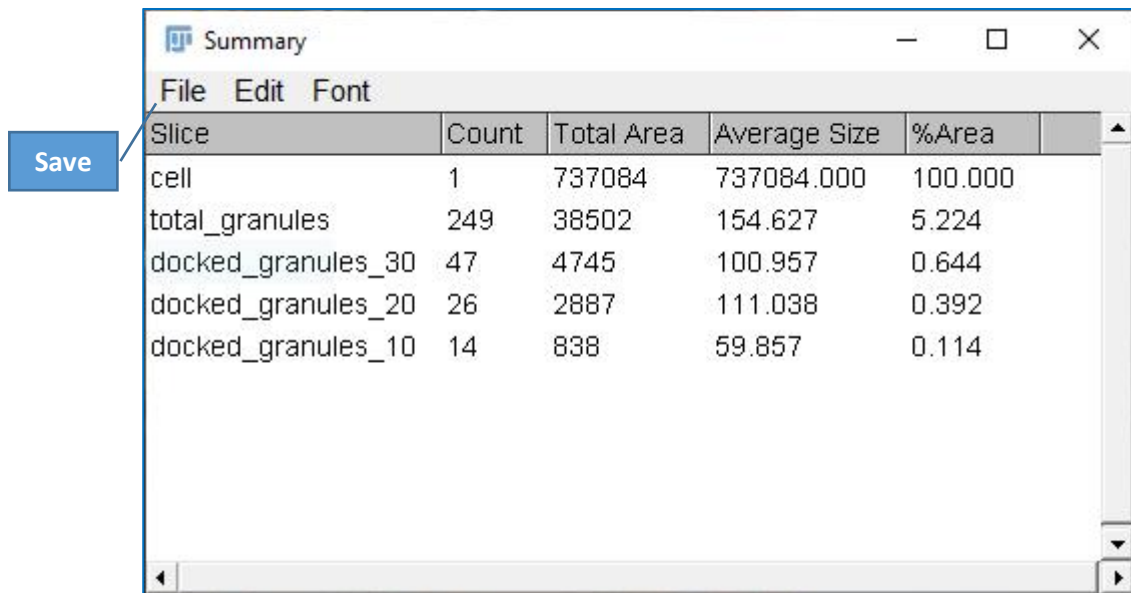
4. Now run the macro through the keyboard shortcut you created earlier or through Plugins > Macros > Run...

It will automatically select that same region 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:



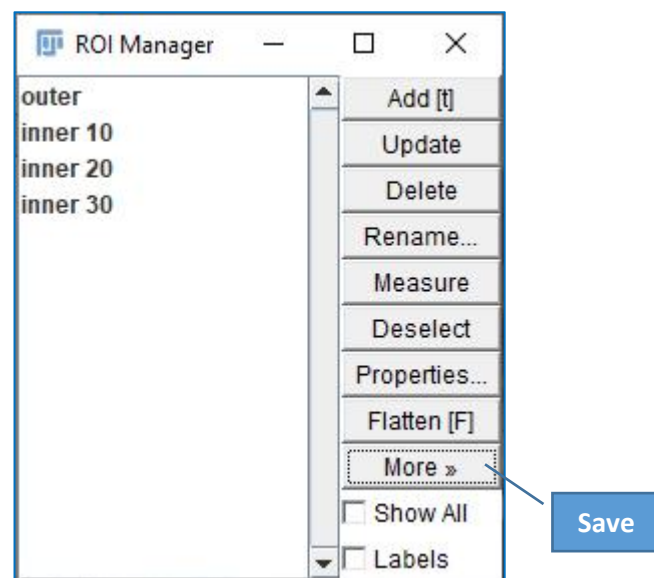


- After the quantification has taken place, there will be two windows remaining. The *Summary* window displaying all the metrics calculated for the several regions of interest and the *ROI Manager* window containing the outlines of all these regions. You can choose to save this information if you wish.



The screenshot shows the 'Summary' window with a menu bar (File, Edit, Font) and a table of data. A blue 'Save' button is positioned to the left of the 'File' menu, with a line pointing to it.

Slice	Count	Total Area	Average Size	%Area
cell	1	737084	737084.000	100.000
total_granules	249	38502	154.627	5.224
docked_granules_30	47	4745	100.957	0.644
docked_granules_20	26	2887	111.038	0.392
docked_granules_10	14	838	59.857	0.114



- Before running the macro again, make sure to close both of these windows.