Analysis of Adipose Tissue Cellularity in Histological Sections

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This is a simple protocol on how to analyse adipose tissue in histological sections. It quantifies both the lipid droplets themselves as well as the inter-lipid droplet tissue. The analysis is centred around *Fiji* and the *Adiposoft* plugin. 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:

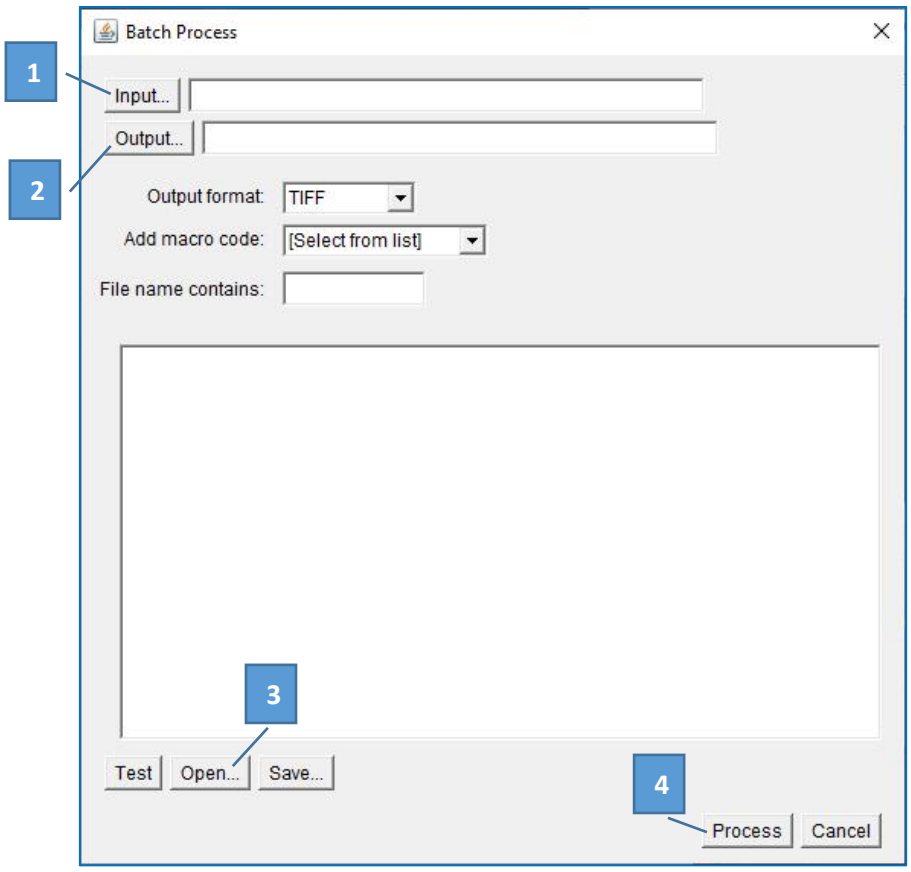
- Download Fiji from *here*
- Download the Adiposoft plug-in from *here*
  (Copy the Adiposoft jar file into the Plugins folder of Fiji application's main folder. The Adiposoft tool will appear in the plugins menu the next time you start Fiji)
- Download the macros (pre_proc.ijm and ratio_calc.ijm) from *here*
  (The easiest way to do this is by clicking on the green "*Code*" button and then selecting "*Download ZIP*")

STEP 1: Image Preprocessing

This first part is only needed in some cases. Through testing I found it was usually necessary when the lipid droplets were of significant size and the illumination was not homogeneous. If you are not completely sure, just check the analysis on a few images with and without this step and choose what is most appropriate for your dataset.

1. Open **Fiji**

2. Go to `Process > Batch > Macro...` Choose the Input folder containing all your raw images and the Output folder where you want to save the processed images in. As for the output format I recommend TIFF. Then click on `Open...` and select the *pre_proc.ijm* file you downloaded earlier. Finally click `Process`

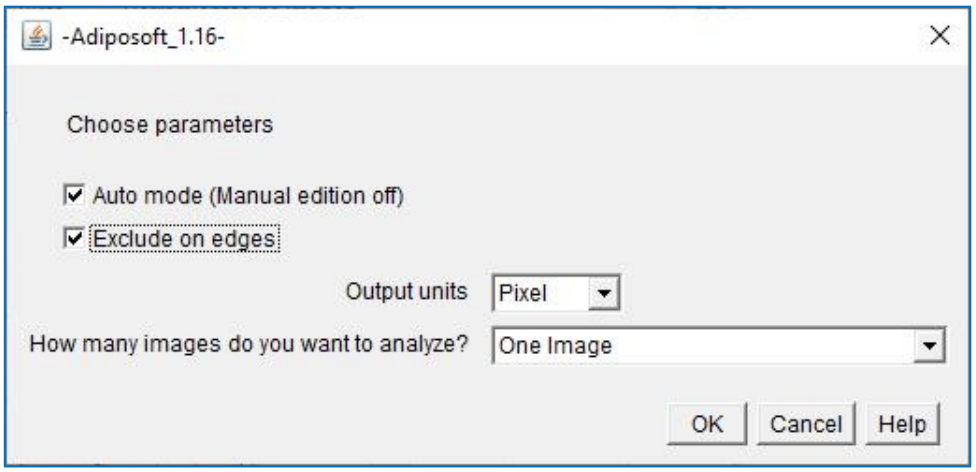


STEP 2: Lipid Droplet Analysis

This second part utilizes the **Adiposoft** plugin to analyse the lipid droplets in the histological section.

1. Open **Fiji**

2. Go to `Plugins > Adiposoft`, the main dialogue window will appear



a) I would recommend starting with the "*Auto mode*" on. If you then notice that the plugin is not segmenting the lipid droplets accurately, switch to "*Manual mode*" by checking off the box. This mode lets you manually edit the results by adding, deleting, splitting or merging lipid droplets. Note that this mode is only available when the images are analysed one by one and not in batch mode

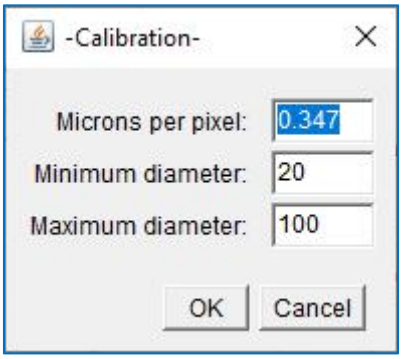
b) I would also recommend the "*Exclude on edges*" option if you want accurate measurements of the individual droplets

c) For the "*Output units*" selecting "Pixel" will always work. However, if the calibration of the images is known, you can select "Microns", but be ready to introduce the number of microns that correspond to one pixel of your images in the dialogue window that will appear when you press OK

d) In terms of "How many images you want to analyze?", you can choose to analyse one image at a time ("One Image"), a directory that contains all the images that you want to analyse ("A Directory") or a directory and all directories below ("A list of nested Directories – Batch Mode")

   If you preprocessed the images in STEP 1, make sure you select the images produced in this step rather than the original images

3. After selecting all the parameters and clicking "OK", you will be prompted with a "Calibration" window where you will have to introduce the minimum and maximum diameters the plugin should expect the lipid droplets to be



4. Finally you will be prompted to choose (or create) the output directory where you want to store the results of the analysis

   This analysis will produce 3 files:
   - The original image with the labelled outlines overlayed
   - An Excel file with the results of the Area for each lipid droplet
   - A zip file containing all the ROIs produced in the segmentation

STEP 3: Inter-Lipid Droplet Tissue Analysis

This final step is only needed if you want to also analyse the tissue in between the lipid droplets. Useful for quantifying browning for example. If you just want to analyse the lipid droplets, you can skip this part, STEPS 1 and 2 are enough.

1. In **Fiji**, open the original picture (or the preprocessed image if you went through with STEP 1)

2. Run the the *ratio_calc.ijm* macro by clicking `Plugins > Macros > Run...` and selecting the macro file downloaded previously

3. After running, a results table like the one shown below will appear. It will show the area calculations for both the inter-lipid droplet and the lipid droplets themselves. This last data will differ from the one you obtained in STEP 2 if you selected the "*Exclude on edges*" option