The ImageJ IPLaminator Plugin

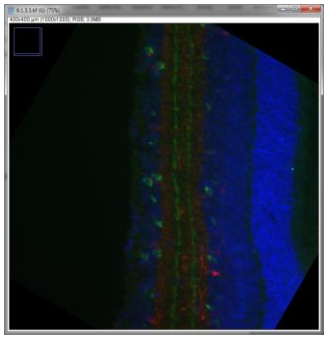
Initial Setup

The first time you run the program you will need to set a default output directory, to do so click the settings button and in the dialog that pops up click the Set Default Output Directory button. A file browser will open allowing you to select a folder for output to be saved in, after you have chosen a folder click select in the file browser and OK in the settings dialog.

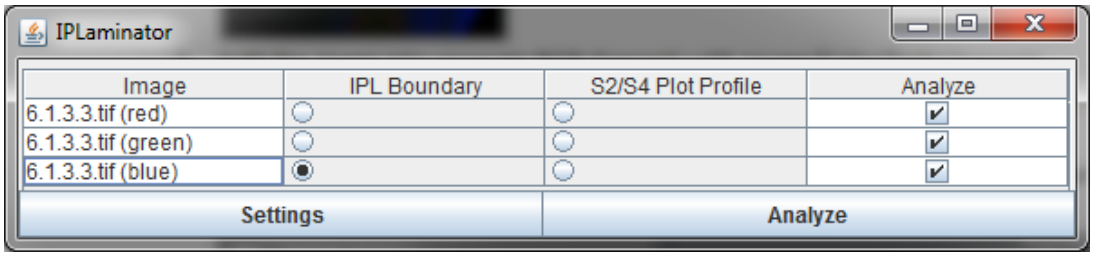
Step By Step Instructions

To follow along with these instructions ensure that none of the checkboxes are checked in the settings dialog and the boundary calculation method is set to the default value ("Use biological markers").

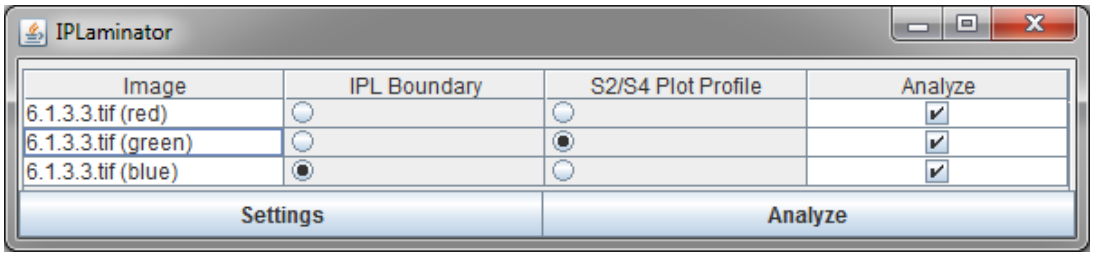
1. Open the image you wish to do the analysis on with ImageJ/FIJI (henceforth referred to as FIJI).
2. Rotate the image using Image > Transform > Rotate so that the neurite stripes are vertical and the retinal ganglion cell layer is on the left.



3. Split the image into separate RGB channels with Image/Color/Split Channels.
4. If you haven't done so already open the plugin by clicking Plugins > IPLaminator from the FIJI menu bar.
5. In the plugin under the column titled "IPL Boundary" select the radio button that corresponds to the color channel containing the cell body.

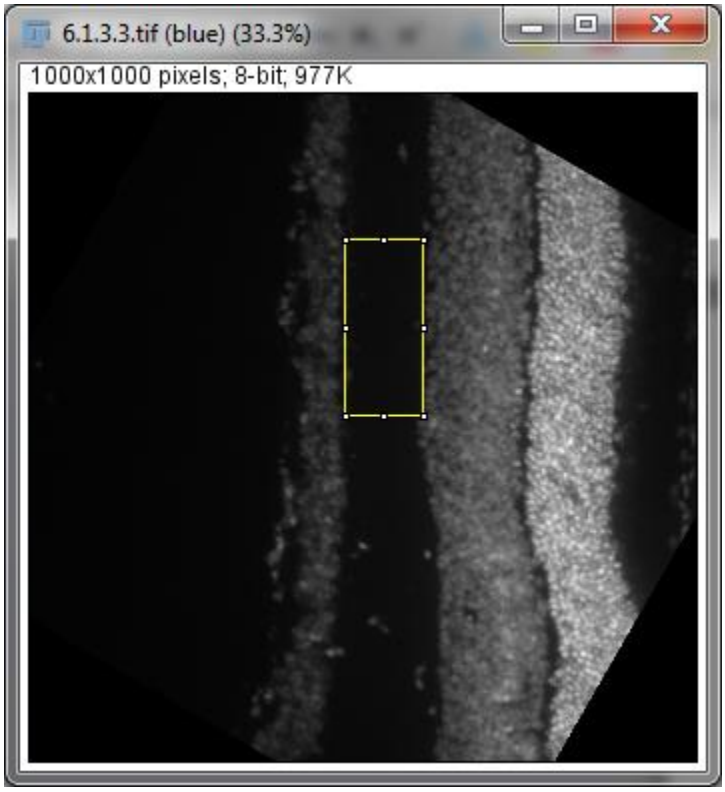


6. In the column titled “S2/S4 Plot Profile” select the radio box that corresponds to the color channel containing the neurite stripes (green in this example).



7. Ensure that the images that you want to analyze have their corresponding checkboxes checked in the Analyze column. Click Analyze.

8. You should see the image window you selected in the “IPL Boundary” column brought to the front of the screen along with a dialogue instructing you on how to place a rectangular ROI (region of interest) with the rectangular selection tool.



Place the rectangular ROI so that it covers the IPL without overlapping either of the adjacent layers.

After the ROI has been correctly placed click OK in the dialog. If you have made a mistake during this step or wish to go back click Cancel instead.

9. After clicking OK the images you have selected will be analyzed and the results will be saved to a text file named Results_<date and time>.txt where <data and time> is a string representing the date and time the analysis took place. These text files can be found in the default output directory you selected when setting up the plugin for the first time.

Settings

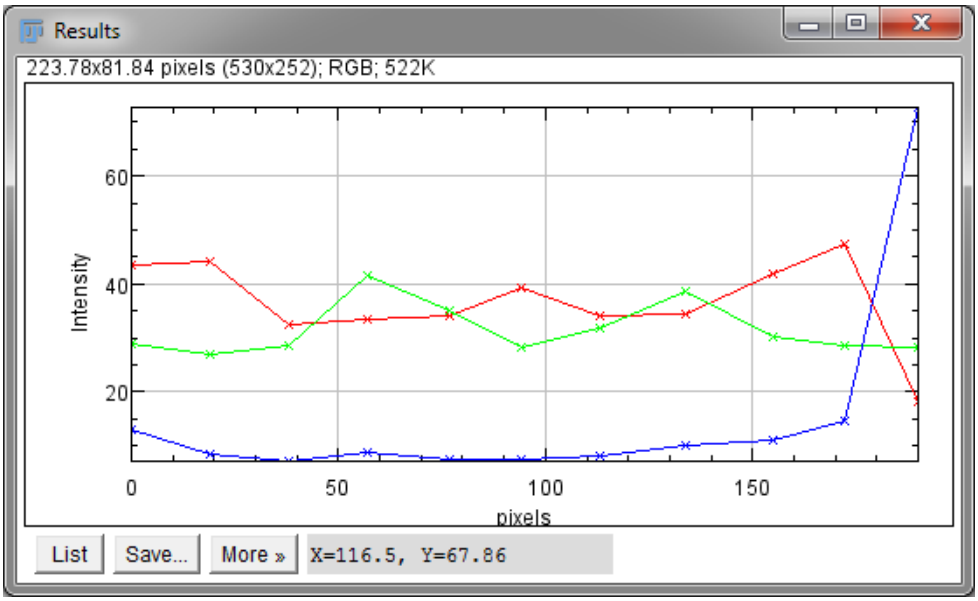
Below each of the options found in the settings dialog are described.

Set Default Output Directory

This option is described in the Initial Setup section at the top of this guide.

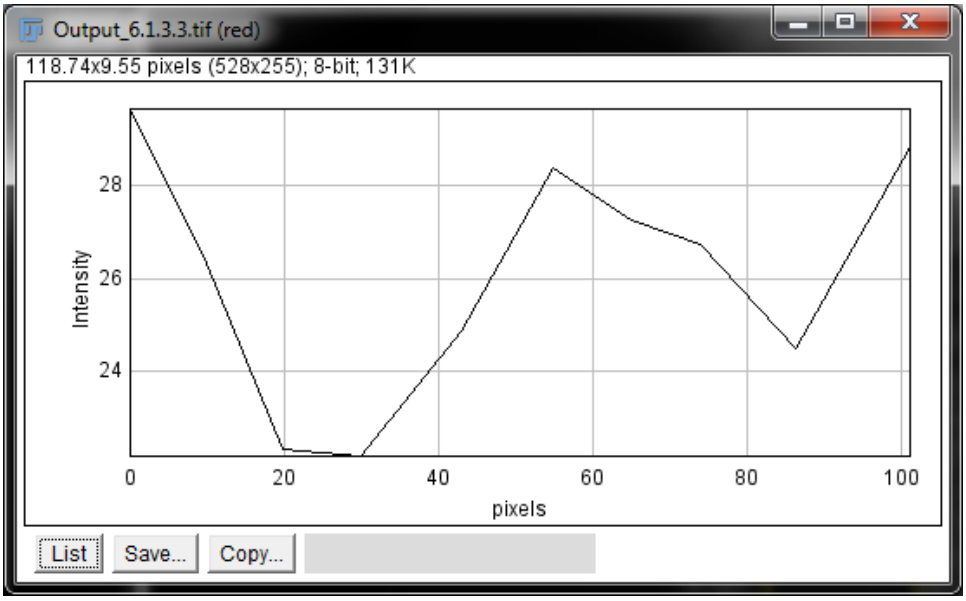
Display results histogram

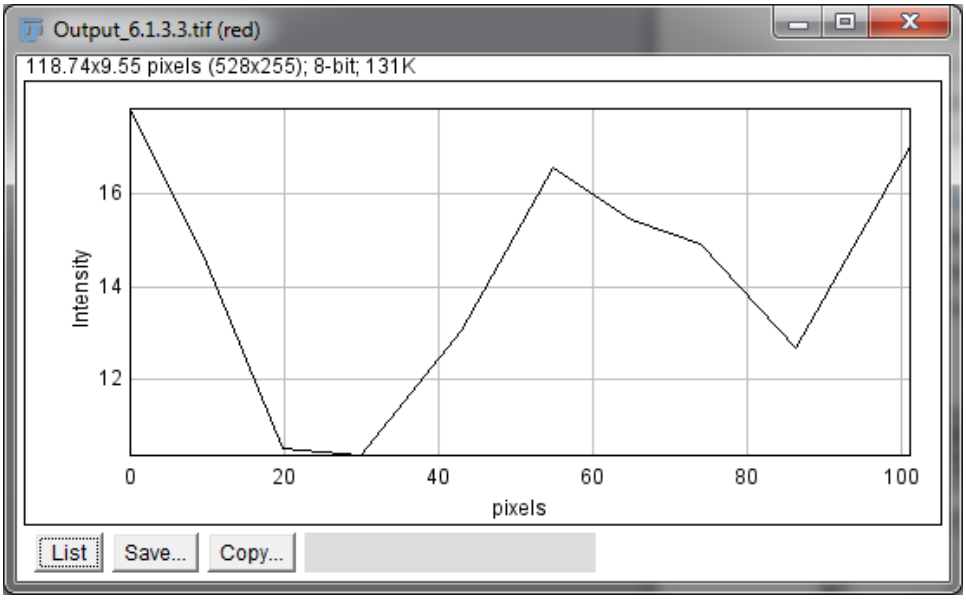
If this checkbox is checked a results histogram/plot profile will be displayed for each image selected for analysis.



Reduce background noise

If this checkbox is checked background noise will be removed from the output. Background noise is caused by background areas where intensity values are not zero. Here are example histograms without and with background noise subtracted respectively.





While the program is running the user will be prompted to select a pixel to represent the background noise. The intensity of a given pixel can be shown in the FIJI status bar by hovering over the pixel with the point picker tool. After a pixel is selected the user clicks OK in the dialog that asked them to select it (similar to how a ROI is chosen).

Note that if you do not wish to remove background noise you must start over and uncheck the checkbox.

Add additional analysis region outside IPL

If this checkbox is checked the user will be prompted to add an additional region for analysis outside of the IPL. This allows the user to add an additional data point to the output, for example, at the INL.

This region is selected by placing a point to the right of the already selected IPL ROI when prompted. The IPL will be analyzed as normal but another data point will be generated and appended to the output using intensity values between the right bound of the IPL and the user selected point.

Boundary Calculation Method

Use biological markers

The default boundary calculation method, if "Use biological markers" is selected the program will place layer boundaries based upon the location of the neurite stripes found within the IPL. Whether the program looks for two or three neurite stripes is determined by the S2 / S4 Stain Type dropdown.

Use percentile values

If this option is selected layer boundaries are placed at percentile positions in the IPL. The boundaries are as follows: 0%, 11.9%, 23.8%, 35.7%, 46.2%, 54.2%, 62.3%, 70.7%, 80%, 90%, 100%. These values were experimentally calculated and are designed to provide the best possible representation of the IPL when the biological markers necessary for calculating boundaries (neurite stripes) are missing.

n Equal boundaries (user defined n)

If this option is selected the IPL will be divided into n equal layers. The value of n is chosen by the user after the IPL is selected.

User Defined Boundaries

If this option is selected the user will be prompted to place points with the multipoint tool indicating the locations of layer boundaries. The left and right IPL boundaries are automatically set to the leftmost and rightmost boundaries respectively.

S2 / S4 Stain Type

This dropdown is used to choose the stain type applied to the neurite stripes found within the IPL. ChAT staining (left) is an example of a stain method that produces two neurite stripes. Calbindin and calretinin

staining (right) are examples of stains that show three neurite stripes in the IPL with the center stripe having an average intensity less than the others.