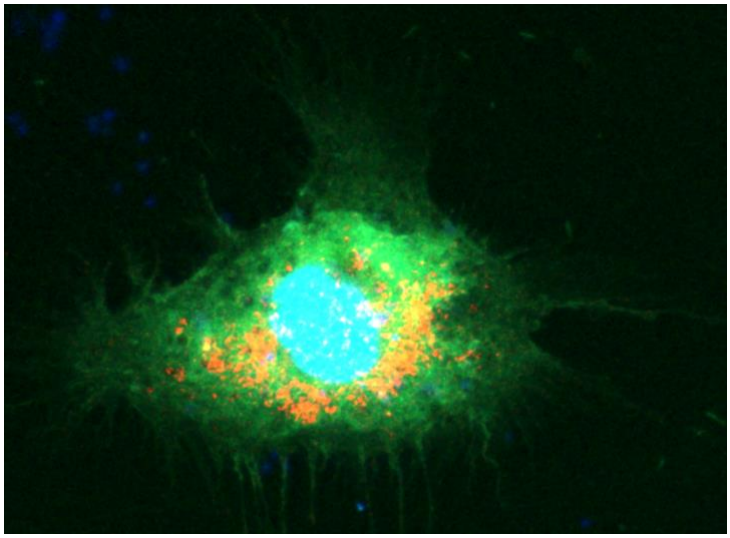
IntensityDistributionIsolines.ijm

This macro divides the cell into rings of identical area that converge towards the nucleus center. The intensity density of the interest signal is then measured per ring and normalized to the total intensity density of the cell. In this manner, the intensity distribution of a target protein can be compared between cells of different shapes and sizes. The macro needs an image containing the signal of interest, and two regions of interest, the cell and the nucleus boundaries, uploaded to the ROI Manager. This script can also be incorporated into any other macro specifically tailored for a particular set of images, where the previous steps have been automated accordingly.

We here use as example a three-channel image (Fig. below; courtesy from A. Aragay, IBMB) containing the signal of interest (red; lysosomal marker), a cell tracer (green) and a nuclear marker (blue).



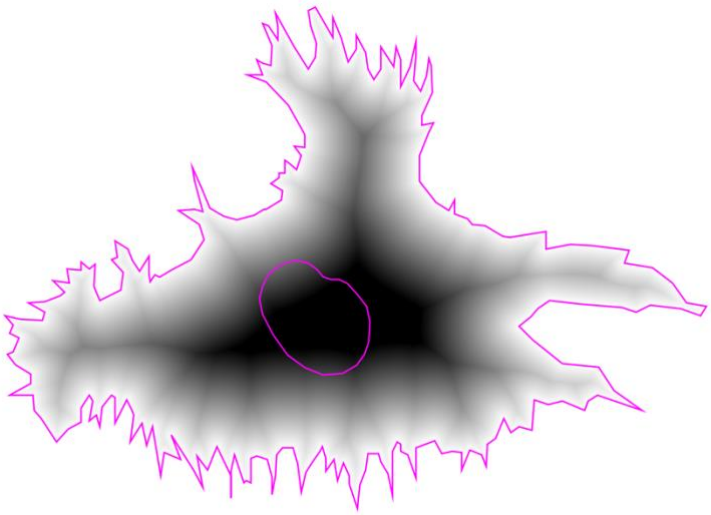
Before using the macro, the cell boundary (B) and the nuclear boundary (C) have to be extracted from their corresponding channels, either manually or using standard segmentation procedures adapted to the particular images (see Fig. below). Next, the channel containing the interest signal has to be preprocessed (Zs projected, BG subtracted, filtering, etc.) in order to be used for quantification (not shown).

From this point on:

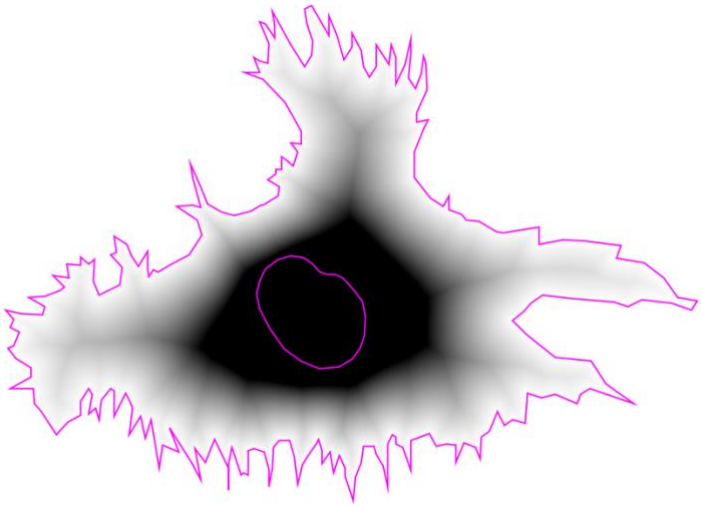
1. Open the macro *IntensityDistributionIsolines.ijm* by drag and drop into the Fiji bar.
2. Open the image to be analyzed.
3. Open the RoiSet.zip containing the cell (ROI01) and nucleus (ROI02) selections in this particular order.
4. Hit *Run* in the script editor window.

The macro will perform the following steps:

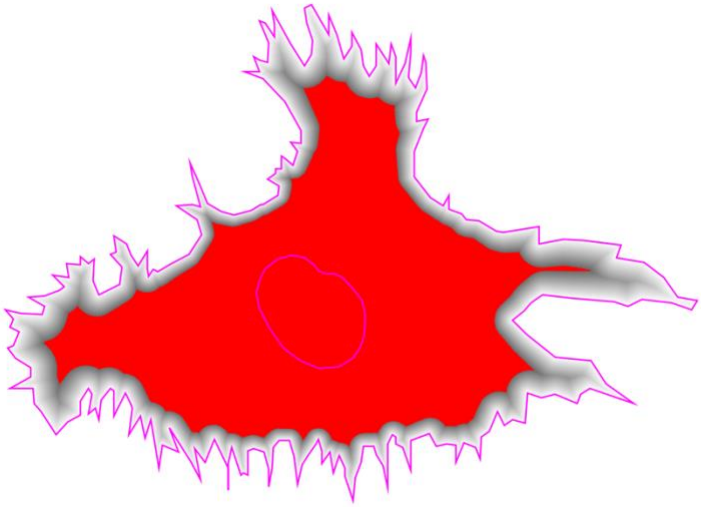
1. A distance map (below) will be created inside the cell ROI. The values in this map will converge towards the ROI´s center of mass.



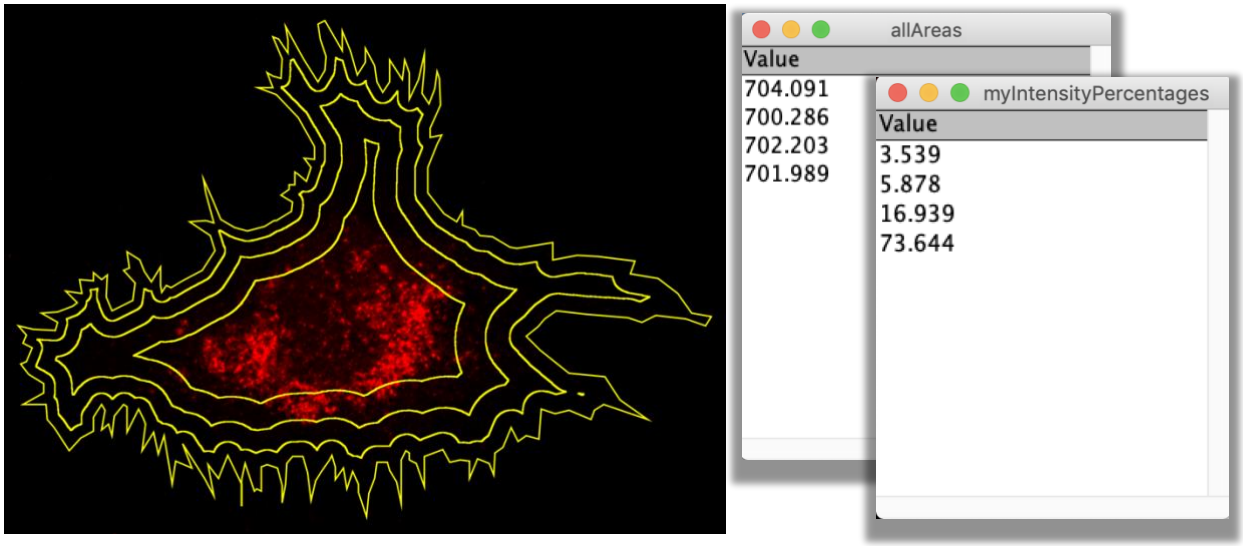
2. A new distance map (below) will be created, where the corrected values will now converge towards the centroid of the nuclear ROI.



3. Thresholding (below) of the corrected distance map is then applied iteratively, changing one cutoff value at a time, until the region comprised by the thresholded pixels meets an area criteria. Such criteria is based on the number of regions in which the cell will be divided, i.e. if the cell will be divided in 4 rings, the iterative thresholding will run until it has rendered regions that comprise the 75%, 50% and 25% of the total cell area.



4. Each resulting new region is added to the ROI Manager as a new selection which, together with the original cell boundary, delimit the final cytoplasmic rings (below) of equivalent area.



5. Finally, the intensity density is measure for each selection; the values per ring are calculated by subtracting from each ROI measurement the value obtained for the subsequent ROI. The ring measurements are normalized to the total intensity density of the cell, thus obtaining the intensity percentage per ring (figure above).