README — Perisomatic particle analysis in Fiji

MACRO FILE

DN_perisomatic_particle_analysis.ijm

PURPOSE

This macro automates particle analysis within a perisomatic region surrounding a manually defined dysmorphic neuron soma. It uses existing Fiji/ImageJ commands for thresholding, region-of-interest (ROI) processing and particle analysis.

REQUIREMENTS

- Fiji/ImageJ with the ROI Manager.
- An active image containing the signal to be analysed.
- Exactly one ROI in the ROI Manager before execution: the manually delineated soma ROI.
- Correct image calibration and appropriate measurement settings.

Fiji/ImageJ version used in the study: 2.16.0/1.54p.

INPUT PREPARATION

1 Open the image in Fiji and select the channel intended for particle analysis.

2 Verify the spatial calibration using Analyze > Set Scale.

3 Delineate the soma and add this ROI to the ROI Manager.

4 Ensure that the ROI Manager contains only this soma ROI.

5 Configure the required measurements using Analyze > Set Measurements.

6 Work on a duplicate image, as the macro converts the active image to an 8-bit binary mask.

EXECUTION

Open DN_perisomatic_particle_analysis.ijm in Fiji's macro editor and click Run.

PROCESSING STEPS

1 Duplicate the soma ROI and measure it on the input image.

2 Set the BlackBackground option to false.

3 Convert the image to 8-bit.

4 Apply ImageJ's Default automatic threshold using the “dark no-reset” options.

5 Convert the image to a binary mask.

6 Enlarge the duplicated soma ROI by 3 μm, as specified by the Enlarge command.

7 Use an exclusive OR (XOR) operation between the original and enlarged soma ROIs to generate a perisomatic ring.

8 Analyse particles within this ring.

9 Measure the perisomatic ring on the binary image.

ANALYSIS PARAMETERS

- Threshold method: Default.
- Threshold options: dark no-reset.
- ROI enlargement: 3 μm.
- Particle size range: 0.10–1.50 μm^2.
- Particle circularity range: 0.20–1.00.
- Particle output: outlines, individual results, summary and overlay.
- Particle-start recording: enabled.

ROI MANAGER OUTPUT

With exactly one soma ROI present at the start, the resulting ROI order is:

0: Original soma ROI.1: Duplicate soma ROI.2: Enlarged soma ROI, named “Perisomatic” by the macro.3: Perisomatic ring, named “Soma_expanded_3” by the macro.

Despite these names, ROI 2 contains the expanded soma, whereas ROI 3 is the ring used for particle analysis.

RESULTS

The macro generates particle measurements, a summary, particle outlines and an overlay. It also adds soma and perisomatic-ring measurements according to the current ImageJ measurement settings.

The initial soma measurement is performed before conversion to a binary mask. The final ring measurement is performed on the binary image and therefore does not represent fluorescence intensity in the original image.

SAVING OUTPUT

The macro does not save files automatically. Save the required results tables, ROI set, outlines and overlays manually after execution.

QUALITY CONTROL AND LIMITATIONS

Inspect the soma boundary, perisomatic ring and thresholded particles for every analysed image. Confirm that the ring does not include neighbouring somata or unrelated structures.

The macro depends on ROI order and pre-existing image calibration and measurement settings. It does not perform channel selection, background subtraction or manual verification of particle identity. Thresholding is automatic and should be checked against the original fluorescence image.

MACRO DN_perisomatic_particle_analysis.ijm

```
roiManager("Select", 0);
roiManager("Add");
roiManager("Select", 1);
roiManager("Measure");
setOption("BlackBackground", false);
run("8-bit");
setAutoThreshold("Default dark no-reset");
run("Convert to Mask");
roiManager("Select", 1);
run("Enlarge...", "enlarge=3");
roiManager("Add");
roiManager("Select", 2);
roiManager("Rename", "Perisomatic");
roiManager("Select", newArray(2,1));
roiManager("XOR");
roiManager("Add");
roiManager("Select", 3);
roiManager("Rename", "Soma_expanded_3");
run("Analyze Particles...", "size=0.10-1.50 circularity=0.20-1.00 show=Outlines display
summarize overlay record");
roiManager("Select", 3);
roiManager("Measure");
```