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Fluorescent image acquisition and processing using Axiovert 200M microscope and ImageJ software

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We use this protocol and it's working

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Abstract

Fluorescent image acquisition and processing using Axiovert 200M microscope and ImageJ software to analyze morphological and dynamic changes of primary fluorescent microglia.

Troubleshooting

Step 1: Acquire live microglia images using Axiovert 200M microscope with dedicated software (AxioVision Rel 4.9, Zeiss)

1 Insert the cell culture plate in the microscope holder, and set chamber parameters: T 37°C and CO2 5%.

2 Using X20 magnification chose 20 random fields;

3 Set exposition of the fluorescent channel to have a sharp image of microglia bodies and branches;

4 Records the live fluorescent microglia for 2 h taking a picture every 5 min.

5 Save the recorded file as a “.zvi”.

Step 2: Elaborate the acquired images using Fiji software (ImageJ, NIH, version 2.0.0)

6 Open ".zvi" file with Fiji software as hyperstack and tick the “split channel” option;

7 close the bright field channel and start to elaborate the fluorescent channel (microglia);

8 subtract the background using “Process › Subtract Background”(identifier: legacy:ij.plugin.filter.BackgroundSubtracter);

9 defined threshold (foreground) that corresponds to green fluorescent objects using “Image › Adjust › Threshold” (identifier: legacy:ij.plugin.frame.ThresholdAdjuster). Keep the threshold consistent between acquisitions;

10 apply despeckle function “Process › Noise › Despeckle” (identifier:legacy:ij.plugin.filter.RankFilters("despeckle"));

11 apply smoothing function “Process › Smooth” (identifier: legacy:ij.plugin.filter.Filters("smooth"));

12 set the measurement: “Analyze › Set Measurements” (identifier: legacy:ij.plugin.filter.Analyzer("set")), and tick “Area”, “Center of Mass”, “Feret’s Diameter” and “Shape Descriptors”;

13 for each microglia select the area that contains the microglia in each time-frames using “Edit › Options › Roi Defaults” (identifier:legacy:ij.gui.RoiDefaultsDialog);

14 run analyze particles “Analyze › Analyze Particles” (identifier:legacy:ij.plugin.filter.ParticleAnalyzer), set size (micron^2):130-infinity, circularity: 0.00-1.00; tick “display results”;

15 process “all images”;

16 copy the data in an Excell file;

17 repeat the steps from 13 to 16 for each microglia.

18 Among the "Shape Descriptors", keep the values of “Area”, “Solidity”, “FeretAngle”, “XM” and “YM”;

19 to calculate the distance covered by the cell during the time-lapse use the coordinates of the center of mass and sum the distance covered in each time frame assuming that the distance between frames corresponds to the cathetus of a right triangle made by X-axis and Y-axis displacement;

20 to calculate the rotation sum the “FeretAngle” taking into account that it is the angle among Ferret’s diameter and parallel line to the cell contour only on x-axis;

21 calculate the median and the CV% of "Solidity" and "Area";

22 to perform the cluster analysis use each parameter obtained from the analysis; use the values of the vehicle and treated cells and identify the median parameter for the experiment;

23 use the identified median as a threshold to cluster the cells in two groups (over or under the median);

24 combine two parameters to generate four different clusters.