Melanophore response assay in zebrafish

Caio Maximino,Dainara Pereira dos Santos Souza

Abstract

Melanophores (also called melanocytes) are chromatophores (pigment-containing epithelial cells) that contain black or dark brown pigment granules. The aggregation or dispersal of these cells is under hormonal or nervous system control; these responses permit the animal to display rapid variations in color in response to environmental stimuli, including background color and stressful stimuli. This protocol was developed at Laboratório de Neurociências e Comportamento "Frederico Guilherme Graeff", at the Universidade Federal do Sul e Sudeste do Pará (UNIFESSPA), Marabá/PA, Brazil, to assess melanophore aggregation or disperal responses in live, anesthetized adult zebrafish. The protocol is intended to be easily applied, reproducible, and of low cost.

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Materials

20 mg Eugenol orb104769 by biorbyt

- Glass Petri dishes 90 x 15 cm by Contributed by users
- Dissection microscope by Contributed by users

Protocol

Step 1.
Anesthetize fish by immersion in eugenol (60-100 ppm) in a 50 ml beaker.

CONCENTRATION
60 Parts per Million (PPM) Additional info:

REAGENTS
20 mg Methyl eugenol orb105046 by biorbyt

Step 2.
After anesthesia, quickly remove the animal from the solution and transfer it to a glass Petri dish (90 x 15 cm) under a dissection microscope. The Petri dish should contain enough eugenol solution to cover the animal's body; the eugenol solution will keep the animal alive.

REAGENTS
- Glass Petri dishes 90 x 15 cm by Contributed by users
- Dissection microscope by Contributed by users

Step 3.
Using tweezers, position the animal so that it lies on its side, and the dorsal portion of its body is

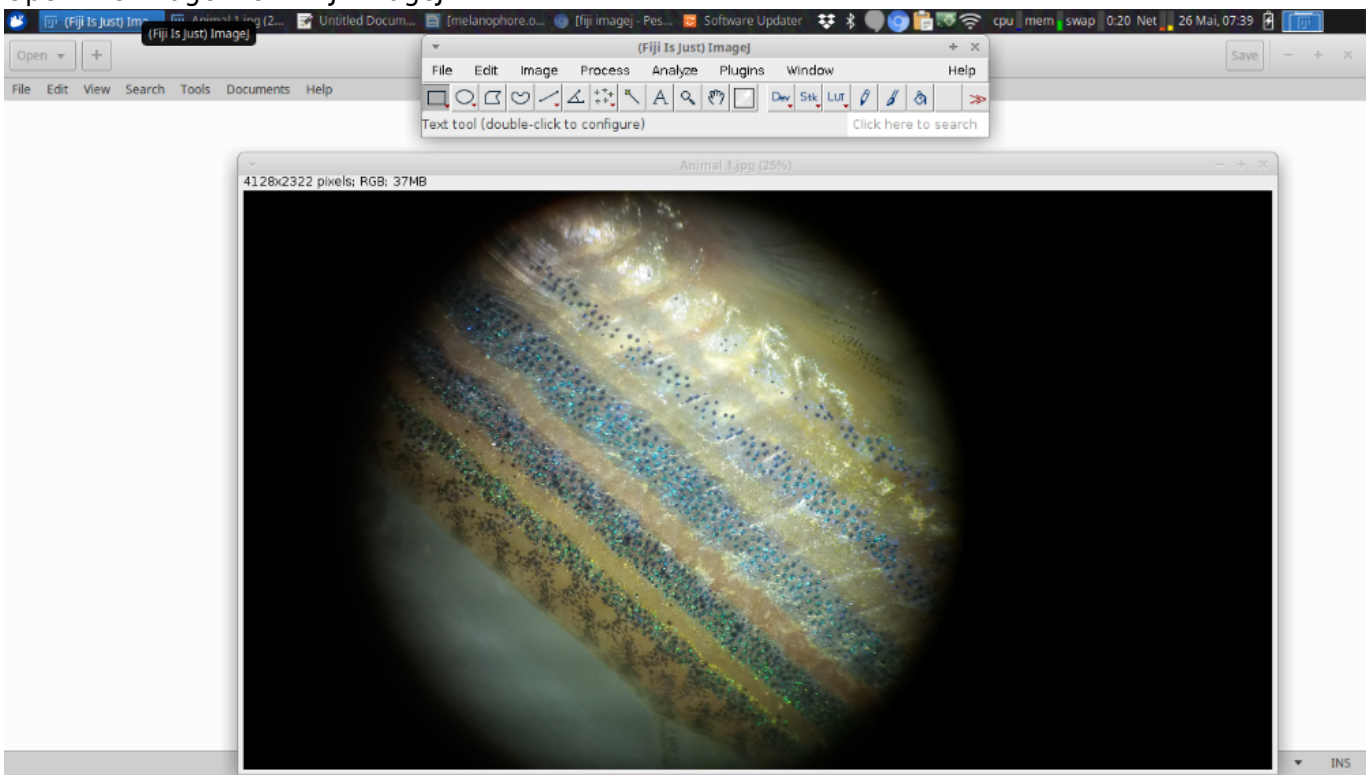
visible under the microscope.

Step 4.

Take a still photo of the image. Export the file in a format that is readable by ImageJ (https://imagej.net/Importing_Image_Files)

Step 5.

Open the image file in FIJI ImageJ



SOFTWARE PACKAGE (Xubuntu -)

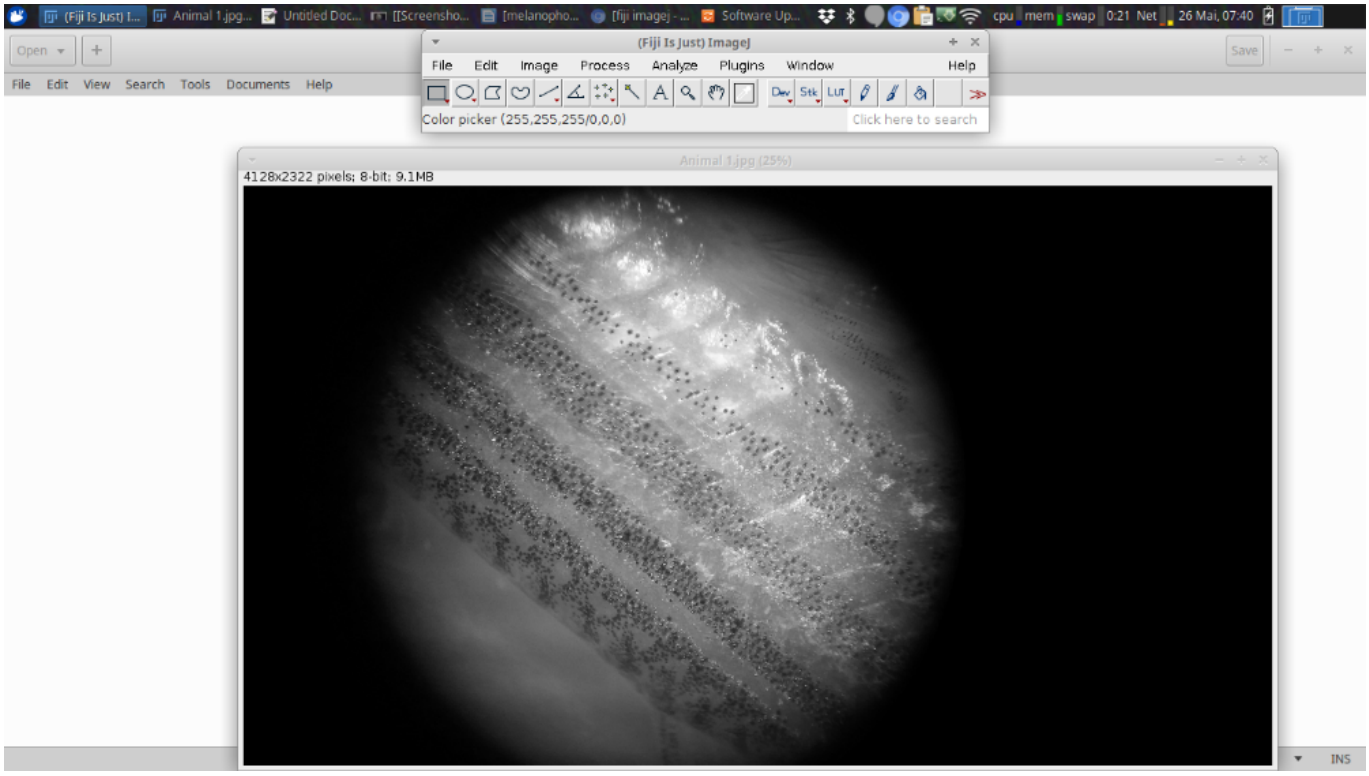
(FIJI Is Just) ImageJ, 2.0.0

Curtis Rueden

https://imagej.net/Fiji

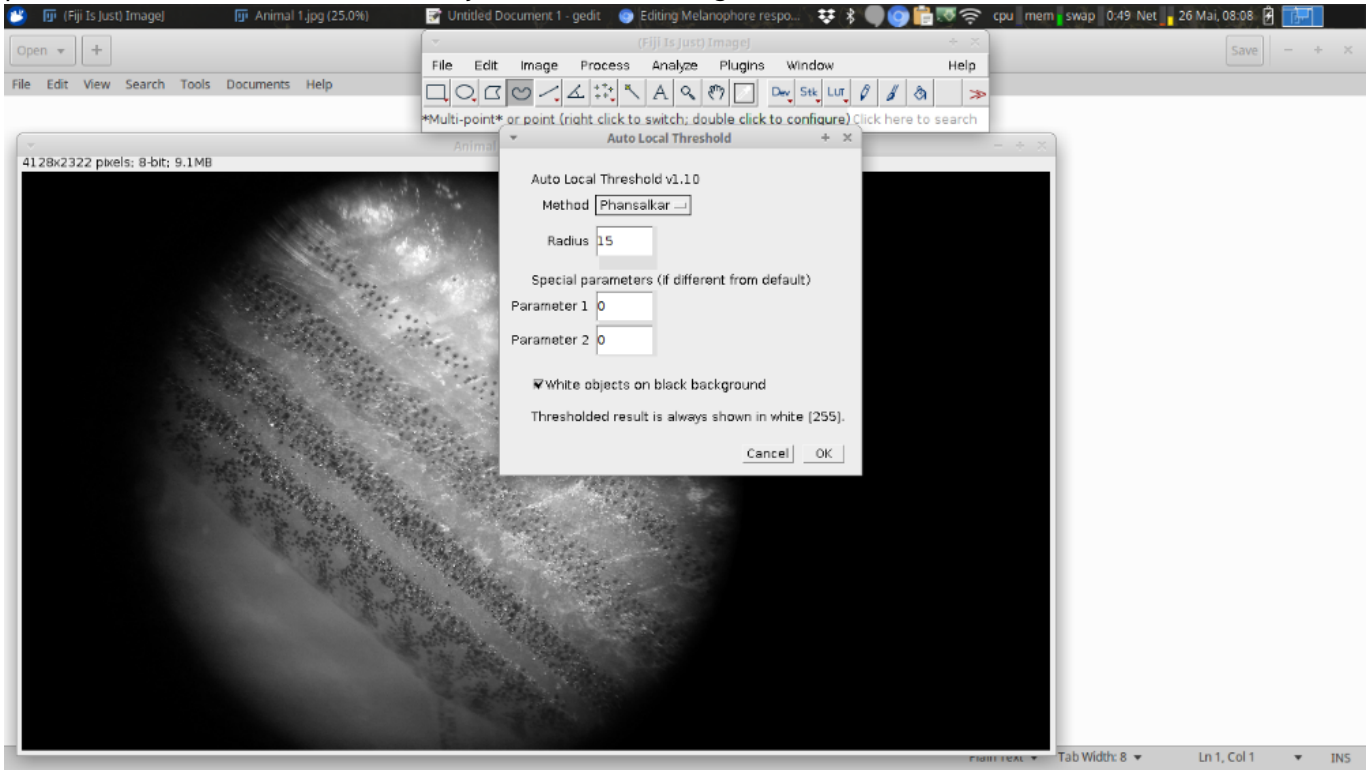
Step 6.

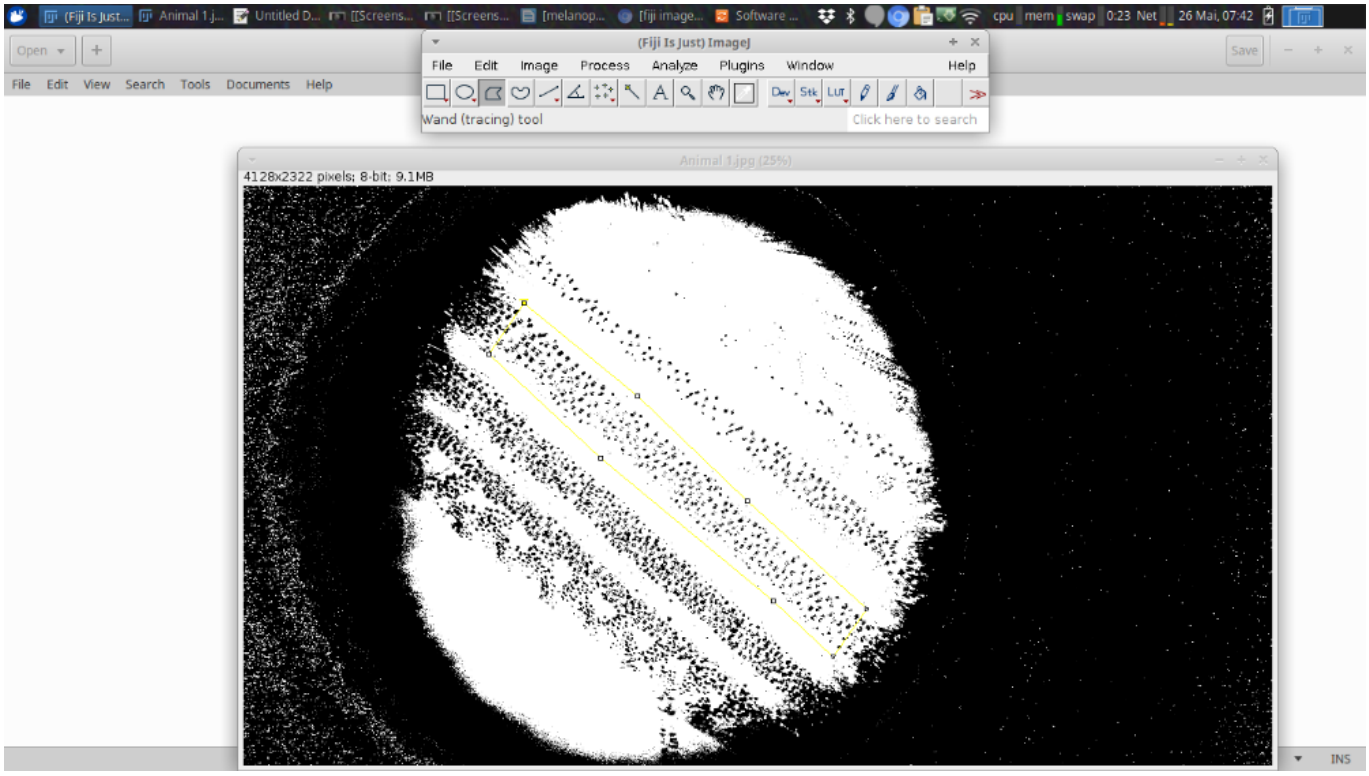
Convert the file to 8-bit (Image > Type > 8-bit)



Step 7.

Use the Auto Local Threshold tool to transform the image into black and white (Image > Adjust > Auto Local Threshold). The Phansalkar method for thresholding should be used, with Radius 15, both parameters set to 0; the “White objects of black background” box should be ticked.





NOTES

Caio Maximino 26 May 2018

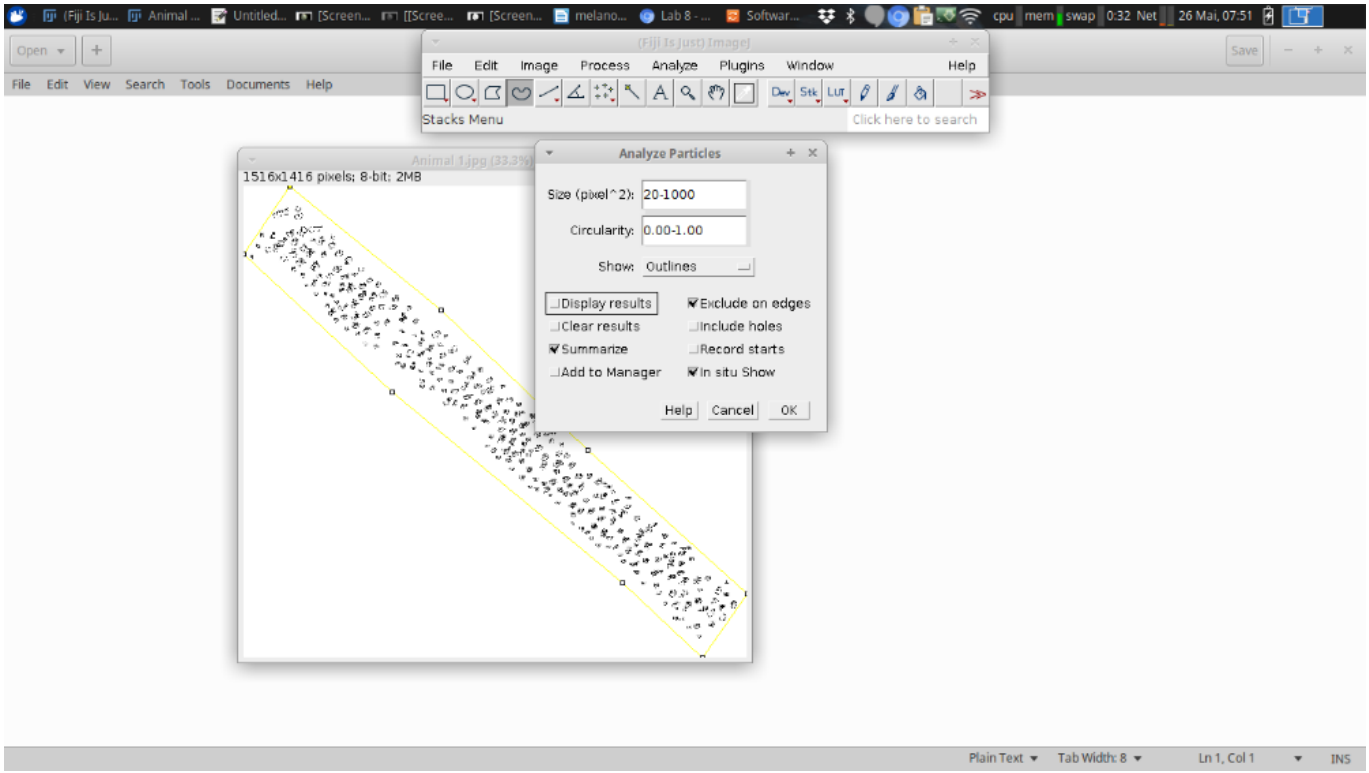
This step is crucial for reproducibility; thresholding images by using the "Threshold" tool and subjective parameters can lead to irreproducible results.

Step 8.

Using the polygon tool, make a selection involving the majority of melanophores in the third strip (from most dorsal to most ventral). Crop the image (Image > Crop)

Step 9.

Apply the Analyze Particles plugin (Analyze > Analyze Particles), restricting particles with sizes between 20-1000 pixels², and circularity from 0.0 to 1.0. In the dropdown menu “Show”, select “Outlines”. Tick the boxes “Summarize”, “Exclude on edges”, and “In situ show”; untick the other boxes, and click OK.



Step 10.
The summary output will display the number of particles (melanophores) detected, total area covered by melanophores (in pixels²), average melanophore size, and percentage of the image area that is covered by melanophores (in %).

Step 11.
Repeat the protocol for each image. If using still photos taken from the same animal, data should be summarized as average value per animal before statistical analysis to prevent pseudoreplication.