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Protocol status: Working
 We use this protocol and it's working

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Melanophore response assay in zebrafish

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Fish behavior and physiology

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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 dispersal responses in live, anesthetized adult zebrafish. The protocol is intended to be easily applied, reproducible, and of low cost.

MATERIALS

MATERIALS

- 20 mg Eugenol **biorbyt Catalog #orb104769**
 - Glass Petri dishes 90 x 15 cm **Contributed by users**
 - Dissection microscope **Contributed by users**
- #### STEP MATERIALS
- 20 mg Methyl eugenol **biorbyt Catalog #orb105046**
 - Glass Petri dishes 90 x 15 cm **Contributed by users**
 - Dissection microscope **Contributed by users**
 - 20 mg Methyl eugenol **biorbyt Catalog #orb105046**
 - Glass Petri dishes 90 x 15 cm **Contributed by users**
 - Dissection microscope **Contributed by users**

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

[M] 60 Parts per Million (PPM)

20 mg Methyl eugenol **biorbyt Catalog #orb105046**
- 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.

Glass Petri dishes 90 x 15 cm **Contributed by users**

Dissection microscope Contributed by users

- Using tweezers, position the animal so that it lies on its side, and the dorsal portion of its body is visible under the microscope.
- Take a still photo of the image. Export the file in a format that is readable by ImageJ (https://imagej.net/Importing_Image_Files)
- Open the image file in FIJI ImageJ

Software

(FIJI Is Just) ImageJ

Xubuntu 16.04

Curtis Rueden

<https://imagej.net/Fiji>

NAME

OS

DEVELOPER

SOURCE LINK

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

- 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.

Note

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

- 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)
- 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.

- 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 %).
- 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.