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Melanophore response assay in zebrafish

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Caio Maximino[1], Dainara Pereira dos Santos Souza[1]

[1]Universidade Federal do Sul e Sudeste do Pará

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Caio Maximino
Universidade Federal do Sul e Sudeste do Pará

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

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Keywords: Zebrafish, Melanophore response, Fish physiology, zebrafish melanophore, melanophore response, called melanocyte, anesthetized adult zebrafish, dark brown pigment granule, pigment, melanophore aggregation, chromatophore, rapid variations in color, cell, response to environmental stimuli, disperal response, color, dispersal of these cell

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.

Materials

MATERIALS

- 20 mg Eugenol **biorbyt Catalog #**orb104769
- Glass Petri dishes 90 × 15 cm
- Dissection microscope

STEP MATERIALS

- 20 mg Methyl eugenol **biorbyt Catalog #**orb105046
- Glass Petri dishes 90 × 15 cm
- Dissection microscope
- 20 mg Methyl eugenol **biorbyt Catalog #**orb105046
- Glass Petri dishes 90 × 15 cm
- Dissection microscope

Protocol materials

- 20 mg Methyl eugenol **biorbyt Catalog #**orb105046
- Glass Petri dishes 90 × 15 cm
- 20 mg Eugenol **biorbyt Catalog #**orb104769
- Dissection microscope

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

[M] 60 Mass Percent

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 × 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.

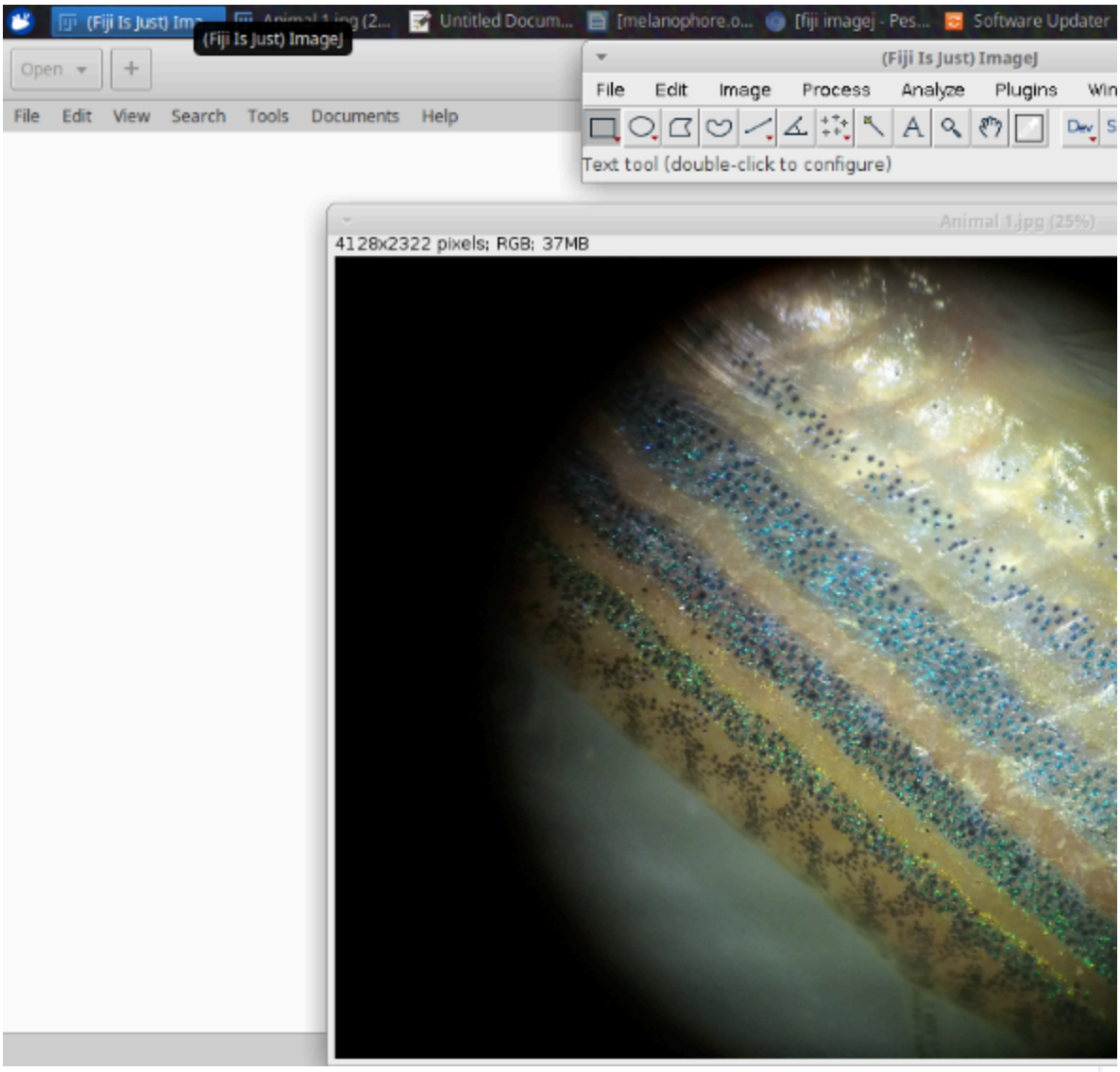
Glass Petri dishes 90 × 15 cm

Dissection microscope

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.

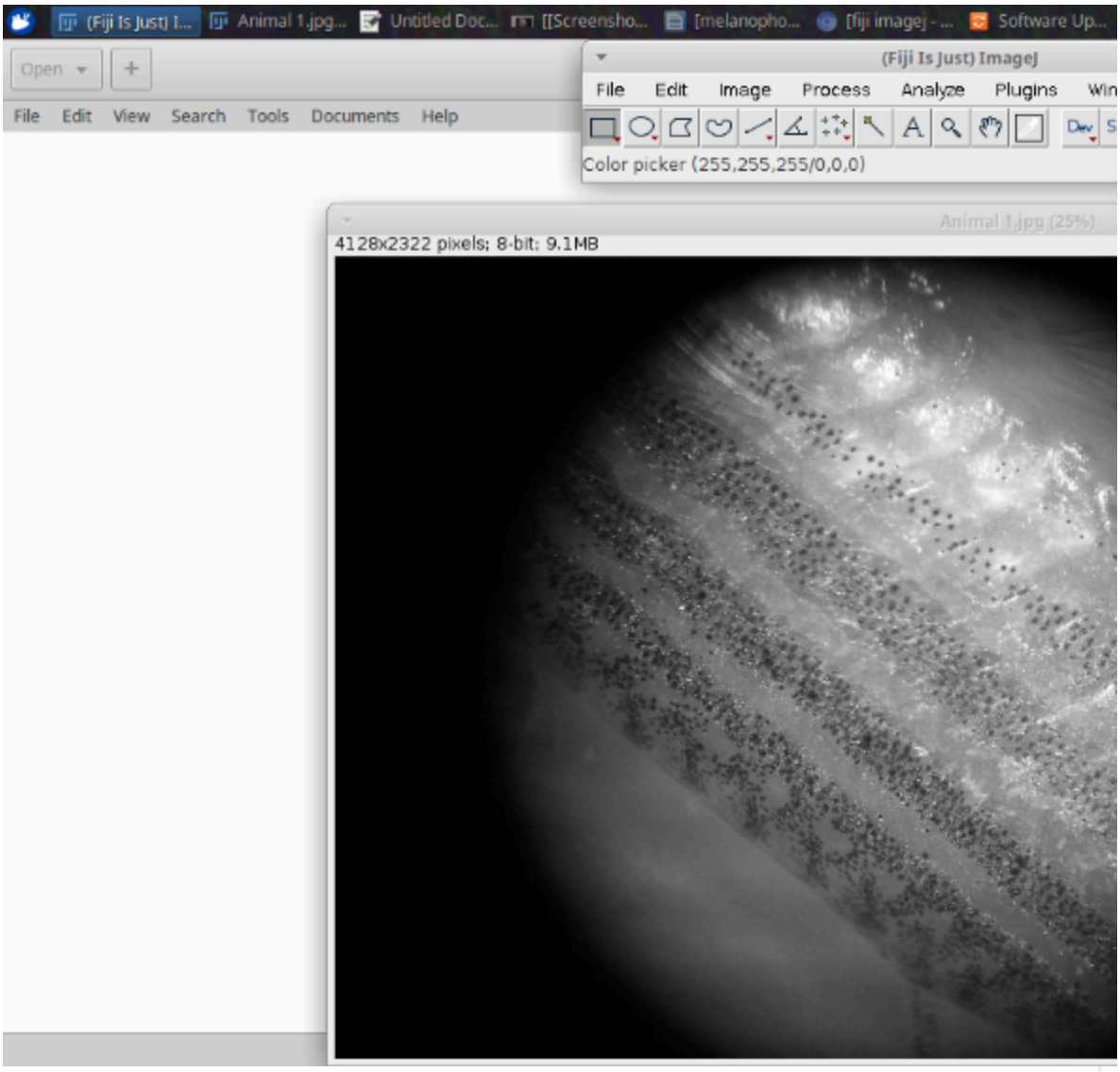
4 Take a still photo of the image. Export the file in a format that is readable by ImageJ (https://imagej.net/Importing_Image_Files)

5 Open the image file in FIJI ImageJ

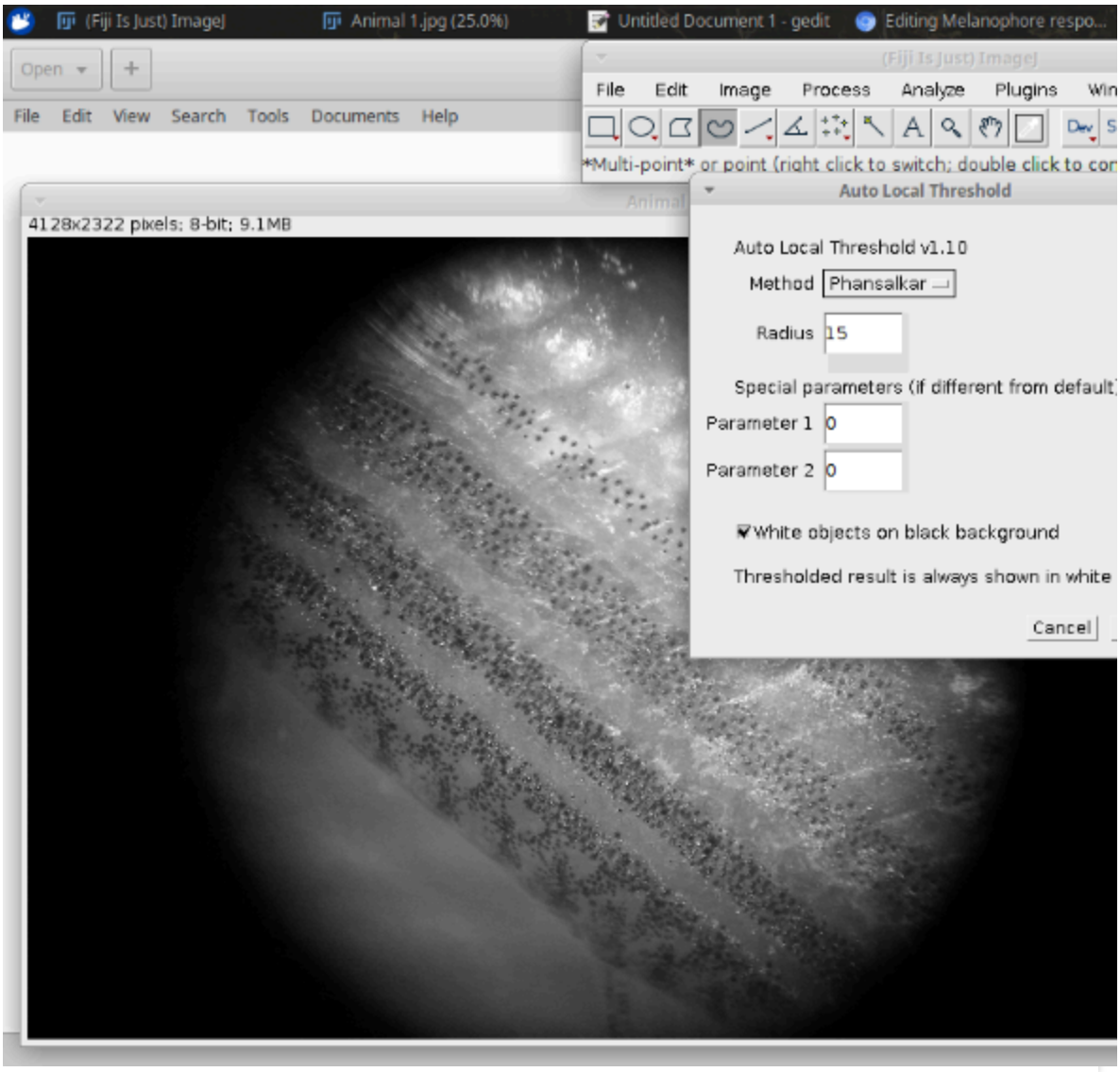


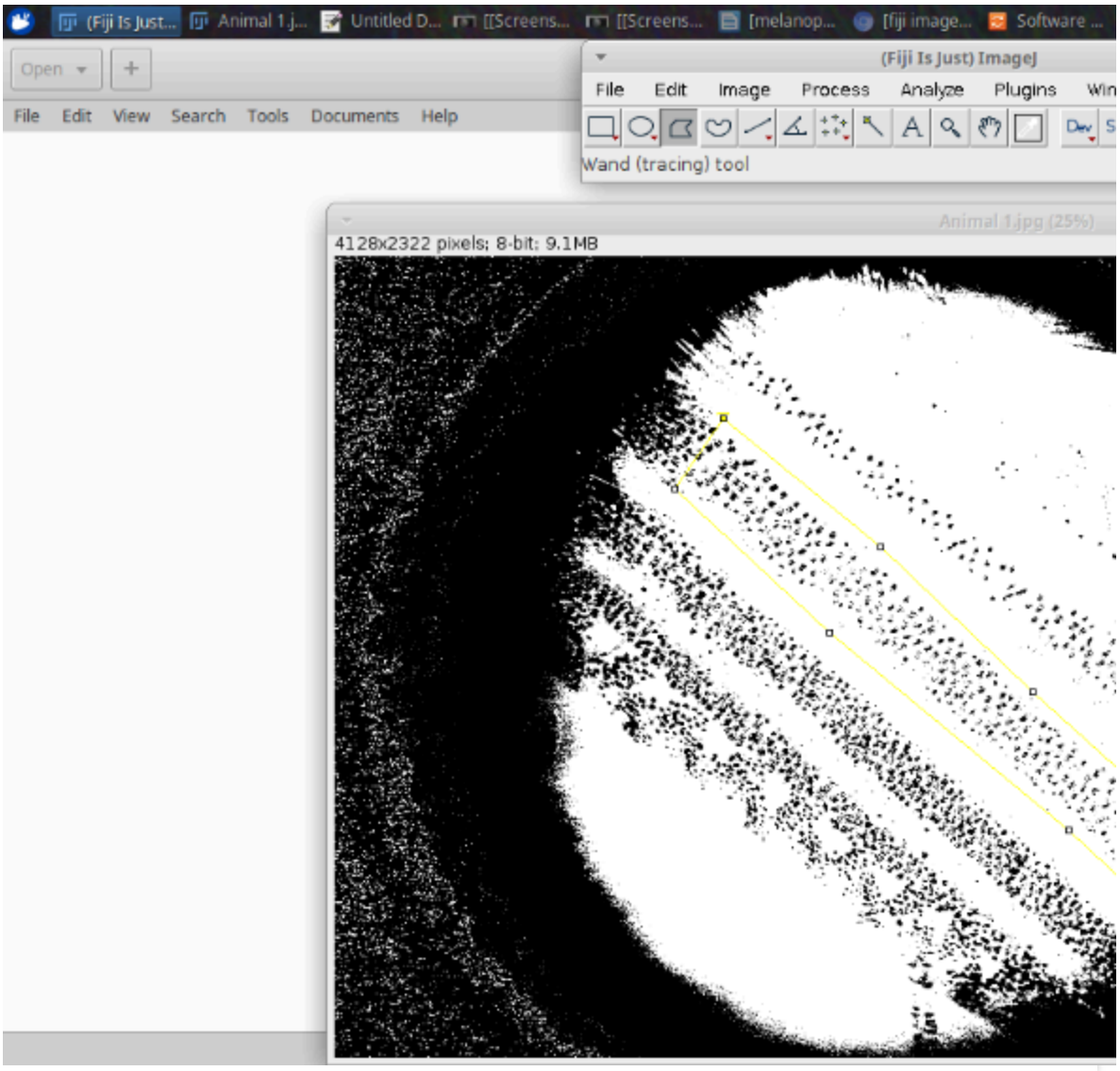
Software	
(FIJI Is Just) ImageJ	NAME
Xubuntu 16.04	OS
Curtis Rueden	DEVELOPER
https://imagej.net/Fiji	REPOSITORY

6 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.


(Fiji Is Just) ImageJ
Animal 1.jpg (25.0%)
Untitled Document 1 - gedit
Editing Melanophore respo...
Open
File Edit View Search Tools Documents Help
(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Win
Multi-point or point (right click to switch; double click to cor
4128x2322 pixels; 8-bit; 9.1MB
Auto Local Threshold
Auto Local Threshold v1.10
Method Phansalkar
Radius 15
Special parameters (if different from default)
Parameter 1 0
Parameter 2 0
White objects on black background
Thresholded result is always shown in white
Cancel

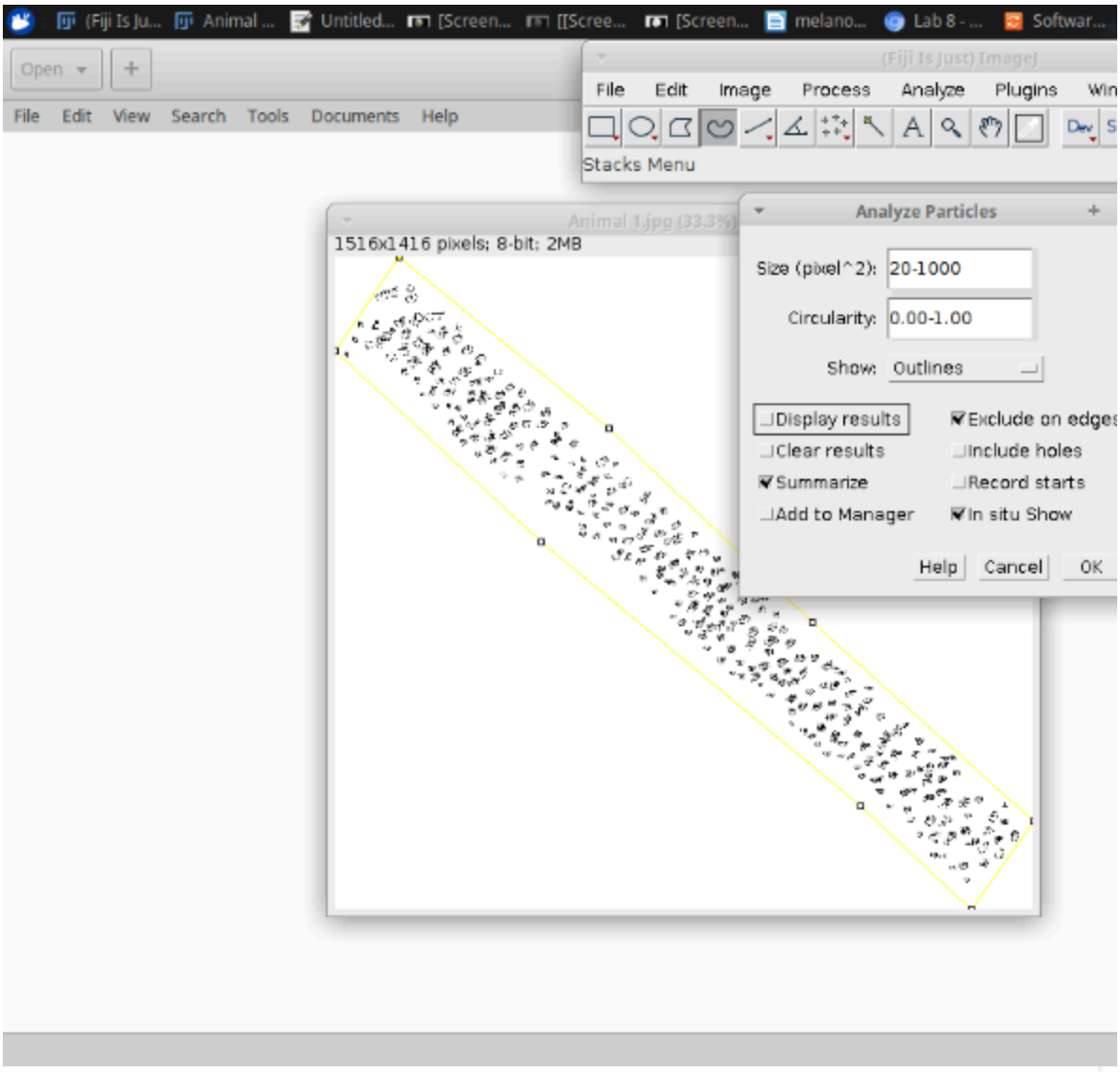


Note

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 pixels^2), 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.