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Pipeline for Image Processing and Quantification of Striatal Dopamine Innervation using Fiji (IF/IHC)

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We use this protocol and it's working

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Disclaimer

This is a method adapted from ImageJ available tools.

Abstract

Method for quantifying striatal innervation by measuring mean fluorescence intensity using ImageJ. This protocol outlines the image preprocessing steps required prior to analysis with ImageJ tools as well as the ImageJ pipeline to quantify striatal innervation. Here DAT and TH immunofluorescence coronal sections but this method can be adapted for other markers and XYZ planes.

Troubleshooting

Obtaining & Opening Image Files

1 Obtain single channel TIFF images for desired brain section and desired channels for analysis.

2 Obtain Allen Atlas TIFF image files that correspond with the Bregma distance of the brain section levels to be analyzed.

2.1 Open fully updated version of FIJI ImageJ2 (version 2.14.0 used for this analysis).

2.2 Scale the Atlas TIFF image to be just smaller than smallest brain section TIFF to be warped (path: Image>Adjust>Size...). This limits the amount of stretching that occurs from warping later on. (For example: if the smallest brain section image is 12500×13500 pixels, make the atlas TIFF image 12000×13000pixels.

3 Open Allen Atlas TIFF image and corresponding TIFF image of the fluorescent channel to be warped.

Processing & Warping Image Files

4 Using the Brightness/Contrast feature (path: Image>Adjust>Brightness/Contrast), adjust the brightness of the section such that key features are clearly visible, including background features. This often requires increasing the brightness.

4.1 Once adjusted, do not select “Apply” or “Set” as this will change the raw pixel values. Instead, click off the Brightness/Contrast window.

5 Navigate to the BigWarp function (path: Plugins>BigDataViewer>Big Warp). This will open up a “Big Warp Setup” window.

5.1 Select the previously brightened TIFF image of the brain to be warped as the “moving image.”

5.2 Select the scaled TIFF file of the Allen Atlas as the “target image.”

5.3 Drag and drop a .csv file into the “Landmarks file” box if a landmarks file has been previously generated. Leave blank to create landmarks as described in step 7.

5.4 Uncheck the “apply transform from landmarks” box.

6 Enable “landmark mode” by pressing the spacebar. Place landmarks on key anatomical structures in the Atlas image window.

6.1 Once placed export the landmark files if repeated and consistent warping is intended (path: File>Export Landmarks).

7 After placing all the landmark points on the Atlas image, open the “Landmarks” window (which opens automatically along with the two target and moving images). Select the first point, this should highlight the first point placed on the target (Atlas) image. Next, place a point in the moving image (the brain section) at the corresponding location. Repeat for all landmark points.

8 Warp the target image (the brain section TIFF) by pressing “T”.

9 Export the warped target image as a TIFF file (path: File>Export Moving Image).

10 Open the Brightness/Contrast feature (path: Image>Adjust>Brightness/Contrast) and select “Reset” to adjust the brightness settings back to the original values. Save the image as a new TIFF file.

Analysis of Warped Images

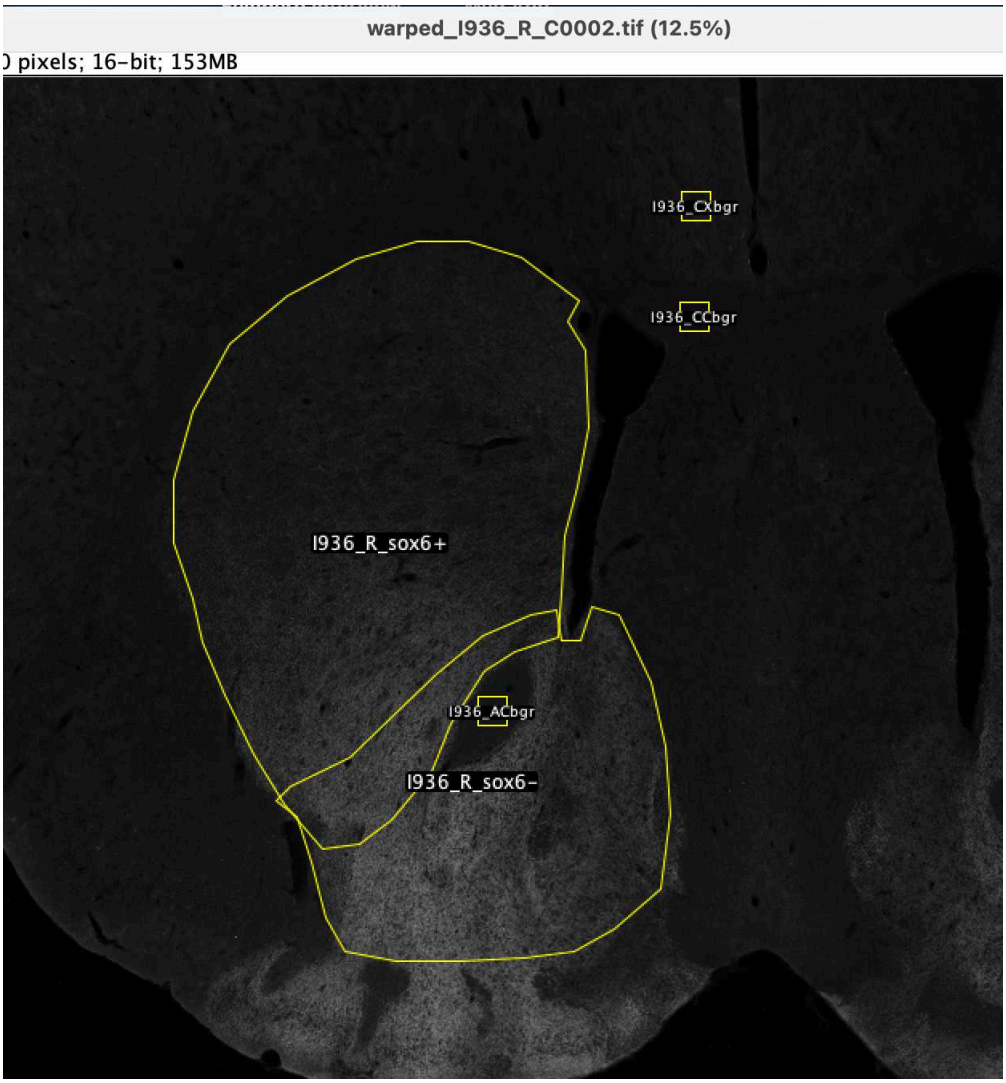
11 Open the scaled TIFF file of the Allen Atlas image corresponding with the brain section to be analyzed

12 Draw desired Region(s) Of Interest (abbreviated: “ROI”) on the Atlas image, add them to the ROI Manager (path for manager: Analyze>Tools>ROI Manager).

12.1 Include square ROI(s) of same size for background sampling and subtraction in data analysis. (For example: 150×150 pixels, total area of 22500 pixels)

13 Open the warped TIFF file of the warped fluorescent channel for analysis.

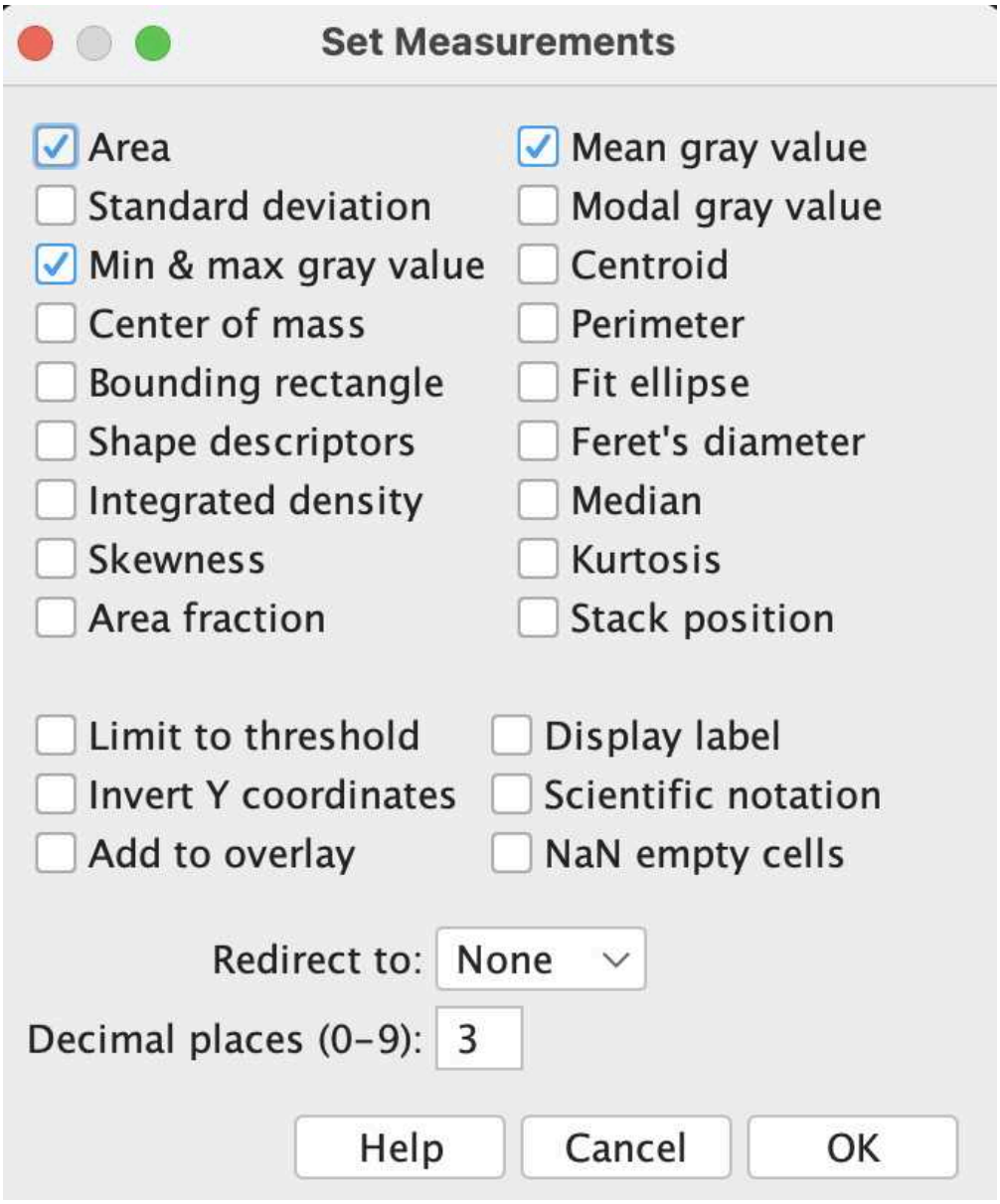
13.1 Adjust the ROIs drawn on the Atlas image to fit the exact borders of the brain section (if warped correctly, very minor or no adjustments should be needed).



Regions "I936_R_sox6+" and "I936_R_sox6-" were drawn first on Atlas image corresponding to the Bregma level of this section, then adjusted as needed to fit the proper outline of the warped image. "I936_ACbgr", "I936_CCbgr", and "I936_CXbgr" are 150x150 pixel square ROIs placed in the anterior commissure (AC), corpus callosum (CC), and cortex (CX) respectively in order to measure the background intensity at these locations.

14 Set the measurements to be taken by accessing the “Set Measurement...” feature (path: Analyze>Set Measurements...).

14.1 For example:



15 Using the ROI Manager with the warped fluorescent channel image selected, select the ROIs to be measured and click “measure” in the ROI Manager. Repeat for all ROIs to be measured. A “Results” window will be generated containing the resulting measurement data.

15.1 **Take note** of the order in which each ROI is measured as the resulting data will not include the name of the ROI in which it originates from but only a number corresponding to the order in which it was measured. (For example: for the image of mouse brain I936 included above, the ROIs were measured in the following order: Sox6+, Sox6-, ACbgr,

CCbgr, CXbgr. This order was maintained throughout the analysis of all images to simplify data analysis in later steps.)

16 Open the “Results” window, save the file as a .csv file (path: File>Save As...)

17 Open the saved .csv file and transfer data to a Microsoft Excel spreadsheet for data analysis.