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Optical Density Analysis for DAB Staining of Mouse Brain Sections

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We use this protocol and it's working

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Abstract

This protocol describes steps for optical density analysis of immunohistochemical 3,3′-Diaminobenzidine (DAB) staining of mouse brain sections using ImageJ.

Materials

- FIJI/ImageJ Software (RRID:SCR_002285)

1 Open ImageJ, then calibrate for analysis.

1.1 See https://imagej.net/ij/docs/examples/calibration/ for calibration setup steps and files.

1.2 Select Analyze > Calibrate, then select the following:
i. Rodbard (NIH Image)
ii. Unit: O.D.
iii. Open the calibration.txt file
iv. Uncheck ‘show plot’
v. Check ‘Global calibration’

2 Open an image and outline the regions of interest, including a background area for subtraction.

3 Select ‘multi measure’ and copy the results to an Excel sheet for analysis. Repeat for all images.

4 Take the ‘mean OD’ value for each region and subtract the background area ‘mean OD’ value. Values can be plotted directly or normalized to control groups.