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# Optical Density Analysis for DAB Staining of Mouse Brain Sections

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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)

OPEN ACCESS



## DOI:

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

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- 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.

