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Whole-Brain Axonal Projection Quantification Using ABBA and QuPath

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We use this protocol and it's working

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Abstract

This protocol describes whole-brain quantification of axonal projections using atlas-based registration with ABBA [51] followed by fluorescence intensity measurement in QuPath [52]. Brain sections are first aligned to the Allen Mouse Brain Atlas using ABBA to obtain standardized anatomical region boundaries. Fluorescence intensity is then quantified in QuPath and background-corrected. Data are aggregated per animal to avoid pseudoreplication.

Materials

- ABBA (Allen Brain Atlas Alignment) [51].
- Allen Mouse Brain Atlas reference.
- QuPath (fluorescence analysis mode) [52].
- Spreadsheet software for aggregation.

Procedure

1 Import section images into ABBA.

2 Align sections to the Allen Mouse Brain Atlas using the ABBA workflow.

3 Manually verify alignment quality.

4 Export atlas-defined region boundaries or masks for downstream quantification.

5 Open registered section images in QuPath.

6 Confirm correct pixel calibration.

7 Import atlas-derived ROI boundaries if exported from ABBA.

8 Use atlas-aligned boundaries to define anatomically consistent ROIs.

9 Treat hemispheres separately if hemispheric analysis is required.

10 Apply ROI boundaries consistently across animals.

11 Define a local background ROI on the same section.

12 Select an area without specific axonal labeling.

13 Maintain consistent background selection criteria across sections.

14 Extract mean fluorescence intensity for each ROI.

15 Extract mean intensity for corresponding background ROI.

16 Compute background-corrected intensity as: ROI mean - background mean.

17 Average corrected values per region per animal.

18 Retain hemisphere-specific values if required before final averaging.

19 Ensure each animal contributes one value per region.

Protocol references

[51] Chiaruttini, N., Castoldi, C., Requie, L., et al. ABBA+BraiAn, an integrated suite for whole-brain mapping, reveals brain-wide differences in immediate-early genes induction upon learning. Cell Reports (2025). https://doi.org/10.1016/j.celrep.2025.115876

[52] Bankhead, P., Loughrey, M. B., Fernández, J. A., et al. QuPath: Open source software for digital pathology image analysis. Scientific Reports 7, 16878 (2017). https://doi.org/10.1038/s41598-017-17204-5

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Outputs
- Background-corrected fluorescence intensity per region per animal.
- Hemisphere-specific values when applicable.
- Exportable dataset for statistical analysis.

Critical Steps
- Verify ABBA atlas alignment accuracy before quantification.
- Use identical imaging settings within datasets.
- Maintain consistent ROI and background selection criteria.
- Avoid treating multiple sections from the same animal as independent samples.