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

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AT8 Tau Pathology Image Analysis

Hemanth Ramesh Nelvagal[1,2], Toby J Curless[1,2], Zane Jaunmuktane[1,3,2]

[1]Department of Clinical and Movement Neurosciences, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK;
[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20 815, USA;
[3]Queen Square Brain Bank for Neurological Disorders (QSBB), 1 Wakefield Street, London WC1N 1PJ, UK

ASAP Collaborative Research Network

Hemanth Ramesh Nelvagal
University College London, University of London

ABSTRACT

QuPath is a bioimage analysis software designed for digital pathology and whole slide image analysis. This protocol describes how to analyse AT8 tau pathology in human brain tissue (FFPE sections with IHC).

MATERIALS

- QuPath
- NZConnect (Hamamatsu), a web-based whole-slide image (WSI) viewer: https://www.hamamatsu.com/us/en/product/life-science-and-medical-systems/digital-slide-scanner/U16179-01.html.
- Stained slides

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Annotation

1 Manually annotate regions of interest on NZConnect (Hamamatsu), a web-based whole-slide image (WSI) viewer:https://www.hamamatsu.com/us/en/product/life-science-and-medical-systems/digital-slide-scanner/U16179-01.html.

2 Download annotations using a Python script.

QuPath De-Convolution and Measurements

3 Import into QuPath using a Groovy script. Refer to: Bankhead, P., Loughrey, M.B., Fernández, J.A. *et al.* QuPath: Open source software for digital pathology image analysis. *Sci Rep* **7**, 16878 (2017). https://doi.org/10.1038/s41598-017-17204-5

4 In QuPath, apply colour deconvolution to distinguish DAB from the haematoxylin counterstain.

5 Measure the area of positive DAB staining for tau pathology using a fixed threshold value of 0.2 on the DAB deconvolved channel.

6 Calculate the percentage of positive DAB staining within the ROI by calculating the area of positive DAB staining divided by the area of the ROI and multiplied by 100.

$$\%\ positive\ stain = \frac{Area\ of\ positive\ DAB\ staining\ (\mu m^2)}{Area\ of\ ROI\ (\mu m^2)} \times 100$$