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Version 1

AT8 Tau Pathology Image Analysis V.1

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Protocol status: Working

We use this protocol and it's working

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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

Troubleshooting

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.

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



Protocol references

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>