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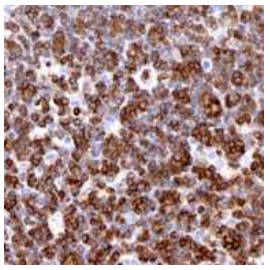
CD3 Cell Density in Substantia Nigra and Cerebral Peduncle Image Analysis



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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 measure CD3 density in the substantia nigra and cerebral peduncle using haematoxylin and DAB-stained brain sections.

Materials

- CD3 IHC-stained sections
- NZConnect (Hamamatsu)
- StarDist
- QuPath

Troubleshooting

Annotation

1 Manually annotate the substantia nigra and cerebral peduncle on NZConnect (Hamamatsu), a web-based whole-slide image (WSI) viewer.

2 Download the annotations using a Python script then import into QuPath **[1]** using a Groovy script.

QuPath Deconvolution and CD3 Density Measurement

3 In QuPath, set the colour deconvolution to facilitate the detection of haematoxylin and DAB staining on CD3 IHC-stained sections.

4 Segment all cell nuclei using StarDist [2] via the QuPath StarDist extension [3];followy with an object classifier to classify CD3-positive cells.

5 Calculate CD3-positive cell density by the number of CD3-positive cells divided by the area of the region of interest (CD3-positive cells per mm^2).

Protocol references

[1] 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

[2] Schmidt, U., Weigert, M., Broaddus, C., & Myers, G. (2018, September). Cell detection with star-convex polygons. In *International Conference on Medical Image Computing and Computer-Assisted Intervention* (pp. 265-273). Springer, Cham. https://arxiv.org/abs/1806.03535

[3] StarDist extension for **QuPath**
https://github.com/qupath/qupath-extension-stardist