

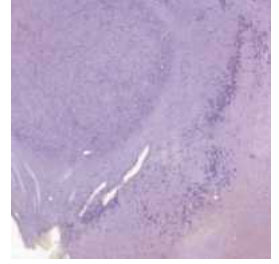


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## Neuromelanin-positive Neuron Density in Substantia Nigra Image Analysis

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**We use this protocol and it's working**

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## Abstract


The protocol covers the steps to measure neuromelanin-positive neuron density in substantia nigra using image analysis tools including NZConnect (Hamamatsu), a web-based whole-slide image (WSI) viewer, Cellpose and QuPath.

## Troubleshooting

## Annotation and Deconvolution

- 1 Manually annotate the substantia nigra on **NZConnect** (Hamamatsu), a web-based whole-slide image (WSI) viewer. 
- 2 Download the annotations using a Python script, and then import into QuPath [1] using a **Groovy script**

## Segmentation and Calculating Neuromelanin-positive Cell Density

- 3 Segment neuromelanin cells using Cellpose [2,3] via the QuPath Cellpose extension [4], followed by an object classifier to filter out non-specific detections. 
- 4 Calculate neuromelanin-positive cell density by the number of neuromelanin-positive cells divided by the area of the region of interest (neuromelanin-positive cells per mm<sup>2</sup>).

### Note

#### 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] Stringer, C., Wang, T., Michaelos, M. *et al.* Cellpose: a generalist algorithm for cellular segmentation. *Nat Methods* 18, 100–106 (2021). <https://doi.org/10.1038/s41592-020-01018-x>
- [3] Pachitariu, M., Stringer, C. Cellpose 2.0: how to train your own model. *Nat Methods* 19, 1634–1641 (2022). <https://doi.org/10.1038/s41592-022-01663-4>
- [4] BIO/Pqupath-extension-cellpose  
<https://github.com/BIOPath/qupath-extension-cellpose>



## Protocol references

### 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>
  
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- [4] BIO/Pqupath-extension-cellpose  
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