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Intracellular neuromelanin quantification

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for quantifying intracellular neuromelanin

- 1 Scan midbrain section using the Panoramic Midi II FL, HQ Scientific 60x
- 2 Acquire SNpc images at 63x with CaseViewer
- 3 Upload individual images at Image J software
- 4 Click on Adjust canvas size and adjust it at 1596×1198
- 5 Click on Invert image
- 6 With the free hand selections tool, draft a neuromelanin-pigmented neuron (excluding the nucleus) and measure the optical density (pixel brightness) and the area
- 7 With the free hand selections tool, draft the neuromelanin pigment of the neuron and measure the optical density (pixel brightness) and the area
- 8 With the free hand selections tool, draft 15-25 non-pigmented neurons (excluding the nucleus), measure the optical density and calculate the mean value
- 9 Normalize (i.e., subtract) the values of the neuromelanin-pigmented neuron's optical density with the mean value of the optical density of the non-pigmented neurons
- 10 Extra measurement: calculate the percentage of the neuronal area occupied by the pigment dividing the neuromelanin pigment area by the neuron's area