Intracellular neuromelanin quantification

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ABSTRACT

Protocol for quantifying intracellular neuromelanin

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1 Scan midbrain section using the Pannoramic 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 1596x1198

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., substract) 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 diving the neuromelanin pigment area by the neuron's area

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