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Quantification of area and optical density of intracellular neuromelanin with Image J

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Camille Guillard-Sirieix[1,2], Thais Cuadros[1,2], Miquel Vila[1,2,3,4]

[1]Neurodegenerative Diseases Research Group, Vall d'Hebron Research Institute (VHIR)-Network Center for Biomedical Research in Neurodegenerative Diseases (CIBERNED), 08035 Barcelona, Spain;
[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815;
[3]Institute of Neurosciences, Autonomous University of Barcelona (INc-UAB), Bellaterra, Barcelona, Spain;
[4]Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain

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Miquel Vila
VHIR-CIBERNED-ASAP

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We use this protocol and it's working

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Abstract

Quantification of area and optical density of intracellular neuromelanin with Image J

Image Acquisition

1 Scan sections using 20x objective (NA=0.8) with pre-set focusing and exposure parameters for optimal NM signal quality with an automated Slide Scanner Olympus (SLIDEVIEW VS200, Tokyo, Japan).

2 Acquire SNpc images with Qupath v0.5.0 software

Neuromelanin Quantification

3 Upload images at Image J software

4 Adjust canvas size at 1596×1198

5 Invert image

6 With the *free hand* selections, draft a neuromelanin-pigmented neuron (excluding the nucleus) and measure the optical density (pixel brightness) and the cell area

7 With the *free hand* selections, draft the neuromelanin pigment of the neuron and measure the optical density (pixel brightness) and the neuromelanin-occupied area

8 With the *free hand* selections, draft 15-25 non-pigmented neurons (excluding the nucleus) and measure the optical density and calculate mean

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 Additional: calculate the percentage of occupied area diving the neuromelanin pigment area by the neuron's area