



JAN 24, 2024

OPEN ACCESS



DOI:

dx.doi.org/10.17504/protocols.io.81wgbxer1lpk/v1

Protocol Citation: Camille Guillard-Sirieix, Thais Cuadros, Miquel Vila 2024. Quantification of area and optical density of intracellular neuromelanin with Image J. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.81wgbxer1lpk/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

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

Camille Guillard-Sirieix^{1,2}, Thais Cuadros^{1,2}, Miquel Vila^{1,2,3,4}

¹Neurodegenerative Diseases Research Group, Vall d'Hebron Research Institute (VHIR)-Network Center for Biomedical Research in Neurodegenerative Diseases (CIBERNED), 08035 Barcelona, Spain;

²Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815;

³Institute of Neurosciences, Autonomous University of Barcelona (INc-UAB), Bellaterra, Barcelona, Spain;

⁴Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain

Vilalab Public



Miquel Vila

ABSTRACT

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

Created: Jan 24, 2024

Last Modified: Jan 24, 2024

PROTOCOL integer ID: 94081

Funders Acknowledgement:

Aligning Science Across

Parkinson's (ASAP)

Grant ID: ASAP-020505

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 1596x1198
- 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 dividing the neuromelanin pigment area by the neuron's area