

DEC 07, 2023

OPEN ACCESS



DOI:
dx.doi.org/10.17504/protocols.io.dm6gp3j9pvzp/v1

Protocol Citation: Hemanth Ramesh Nelvagal, Toby J Curless, Zane Jaunmuktane 2023. CD3 Cell Density in Substantia Nigra and Cerebral Peduncle Image Analysis. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.dm6gp3j9pvzp/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

Created: Aug 07, 2023

CD3 Cell Density in Substantia Nigra and Cerebral Peduncle Image Analysis

Hemanth Ramesh

Nelvagal^{1,2},

Zane

Jaunmuktane^{1,3,2}

Toby J Curless¹,

¹Department of Clinical and Movement Neurosciences, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK;

²Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20 815, USA;

³Queen Square Brain Bank for Neurological Disorders (QSBB), 1 Wakefield Street, London WC1N 1PJ, UK

ASAP Collaborative Research Network



Hemanth Ramesh Nelvagal

University College London, University of London

ABSTRACT

QuPath is a bioimage analysis software designed for digital pathology and whole slide image analysis. This protocol describes how to measure CD3 density in the substantia nigra and cerebral peduncle using haematoxylin and DAB-stained brain sections.

MATERIALS

- CD3 IHC-stained sections
- NZConnect (Hamamatsu)
- StarDist
- QuPath

Last Modified: Dec 07, 2023

PROTOCOL integer ID:
86037

Keywords: ASAPCRN, Human Brain, Substantia Nigra, Cerebral Peduncle, CD3, Parkinson's disease, Parkinson's disease, QuPath, NZConnect, Image Analysis, Pathology

Funders

Acknowledgement:


The Michael J. Fox Foundation for Parkinson's Research (MJFF) and the Aligning Science Across Parkinson's (ASAP) Initiative
Grant ID: ASAP-000478

Annotation

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

QuPath Deconvolution and CD3 Density Measurement

- 3 In QuPath, set the colour deconvolution to facilitate the detection of haematoxylin and DAB staining on CD3 IHC-stained sections.
- 4 Segment all cell nuclei using StarDist [2] via the QuPath StarDist extension [3]; follow with an object classifier to classify CD3-positive cells.
- 5 Calculate CD3-positive cell density by the number of CD3-positive cells divided by the area of the region



of interest (CD3-positive cells per mm²).