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Imaging and quantification V.2

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Tony Hsiao[1,2], Felicia Xaveria Suteja[1,2], Barkha Kishor Goswami[1,2], Anahid Ansari Mahabadian[1,2], YuHong Fu[1,2], Glenda Halliday[1,2]

[1]Brain and Mind Centre & Faculty of Medicine and Health School of Medical Sciences, The University of Sydney, Sydney, NSW 2050, Australia;
[2]2Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815

Tony Hsiao
Usyd

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We use this protocol and it's working

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Abstract

Imaging using Olympus VS200 scanner and quantification by Qupath ver 3.8

Cropping (OlyVIA ver 3.8) and Quantification (QuPath ver 0.4.3)

1 Cropping and section selection

1.1 Images are cropped into groups following the Bregma sections (Bregma -2.69mm to -4.03mm):

1.2 a. Rostral

1.3 b. Intermediate

1.4 c. Caudal

2 Channel separation and Annotation

2.1 Channels are renamed based on their antibody (DAPI, TH, NeuN, mCherry, αSyn)

2.2 Annotations (categorized as SNC and VTA) is created surrounding TH+ cells clusters.

3 Cell detection

3.1 Cell detection is applied with the parameters as followed:
a. Detection channel: DAPI/TH/NeuN/mCherry/αSyn (depending on what channel we would like to detect)
b. Background radius: 8µm
c. Median filter radius: 0µm
d. Median filter radius: 0µm
e. Sigma: 1.5 to 2 µm (depending on the size of the targeted stain)
f. Minimum area: X μm^2 (depending on the size of the targeted stain)
g. Maximum area: 1000 μm^2 (depending on the size of the targeted stain)

h. Threshold: X (depending on the average targeted stain intensity on each detection)

3.2 Single Measurement Classifier

1. Add class for the targeted channels of the classifier:

a. TH+
b. TH-
c. NeuN+
d. NeuN-
e. mCherry+
f. mCherry-
g. αSyn+
h. αSyn-

3.3 After the necessary detections are made, a new 'Single measurement classifier' is created with the parameters as followed:

a. Object filler: Detections (all)
b. Channel filter: TH/NeuN/mCherry/αSyn (depending on the targeted stain, but was not detected using 'cell detection')
c. Measurement: Cell/Nucleus/Cytoplasm: DAPI/TH/NeuN/mCherry/αSyn mean (depending on where the targeted channel is concentrated)
Threshold: X (depending on the average targeted stain intensity on each detection)
d. Above Threshold: TH+/NeuN+/mCherry+/αSyn+
e. Below Threshold: TH-/NeuN-/mCherry-/αSyn-

3.4 The new classifier was saved after given a name according to the detected channel and threshold.

3.5 Export measurement

a. 'Annotation measurement' results of each image are then exported to excel and arranged accordingly.

3.6 DAPI count

a. Apply 'cell detection' using the channel: DAPI

3.7 NeuN+ with TH+/TH- and αSyn+/αSyn- count

a. Apply 'cell detection' using the channel: NeuN
b. Apply 'single measurement classifier' using the channel: TH
c. Apply 'single measurement classifier' using the channel: αSyn