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Fluorescence intensity analyses

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We use this protocol and it's working

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Abstract

Fluorescence intensity analyses

1 Collect confocal z-stack images from fluorescent-labeled tissue samples. Here we used DHE-treated mice tissue counter-stained with human alpha-synuclein or tissue co-immunolabeled with a human alpha-synuclein and SynO2 antibodies.

Ox-DHE fluorescent signal measurement

2 Create a 3D surface rendering model of h-alpha-synuclein–immunoreactive DMnX neurons using the Imaris software.

3 By applying a constant intensity threshold select ox-DHE puncta and filter through h-alpha-synuclein–immunoreactive neuronal surfaces. This will allow for specific detection of ox-DHE puncta within immunoreactive neurons

4 Quantify puncta on a per-cell basis using the quantification tools.

Syn-O2 fluorescence intensity measurement

5 Generate 2D images that from 5 µL -thick z-stack images using maximum intensity projection function of the Zen software (Carl Zeiss).

6 Select human alpha-synuclein labeled neurons by applying a 120µm^2 size exclusion filter

7 Measure Syn-O2 intensity within these cells using the measure tool.