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Fluorescence intensity analyses

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Protocol status: Working

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 $120\mu\text{m}^2$ size exclusion filter
- 7 Measure Syn-O2 intensity within these cells using the measure tool.