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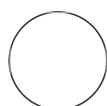
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**Protocol status:** Working  
We use this protocol and it's working

## Fluorescence intensity analyses

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### ABSTRACT

Fluorescence intensity analyses

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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  $\mu\text{m}$ -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.