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Live-cell imaging

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We use this protocol and it's working

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Abstract

Live-cell imaging is a technique to visualize dynamic cellular processes in living biological samples.

1. Cell Preparation

1 Wash cells 1x with OptiMEM (Gibco).

2 Incubate cells with 100nM MitoTracker red CMH2Xros (Thermo Fisher Scientific) in OptiMEM at 37 °C for 30 min

3 Wash cells with OptiMEM once, and keep cells in the same medium for imaging

2. Imaging

4 Images were acquired using a Leica TCS SP8 confocal microscope (Leica, Germany) with a 100×/1.4 numerical aperture oil-immersion objective.

5 Analyze images using Diffraction PSF 3D and DeconvolutionLab2 plugins in Fiji-ImageJ version 2.3.0/1.53q (https://fiji.sc; RRID:SCR_002285)

Procedure for labelled alpha-synuclein Pre-formed Fibrils Experiments

6 Treat iPSC-derived neurons with 0.25 µM Alexa Fluor 594-labeled PFFs (594-PFF) with or without cotreatment with 1 µM CDDO-Me (Cayman Chemical)

7 After 24 h, incubate cells with 100 nM MitoTracker Green (Invitrogen, MA, USA) in a neuronal medium for 30 min at 37 °C

8 Use ACellBrite™ Steady 488 Membrane Staining Kit (Biotium) to visualize cell membranes following the manufacturer's instructions

9 Images were acquired using a Leica TCS SP8 confocal microscope with a 63 × /1.4 numerical aperture oil-immersion objective

10 Acquire Z-stacks for the calculation of the PFF particle area and fluorescence intensity

10.1 For each condition, 5-8 images were acquired from at least four independent experiments

Image processing

11 For the quantification of colocalization and image processing, images were analyzed using the “Analyze particles” and “EzColocalization” plugins in Fiji-ImageJ