



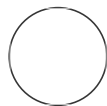
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🌐 Live-cell imaging

📁 In 2 collections

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ABSTRACT

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

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

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 79918

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 E...

- 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

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



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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

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