Jun 12, 2025

Live-cell Imaging of Mitochondrial Membrane Proteins in Cultured Mammalian Cells by Airyscan Laser Scanning Confocal microscopy

DOI
https://dx.doi.org/10.17504/protocols.io.q26g79kx3vwz/v1

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.q26g79kx3vwz/v1

Protocol Citation: Eve Kakudji, Samantha C Lewis 2025. Live-cell Imaging of Mitochondrial Membrane Proteins in Cultured Mammalian Cells by Airyscan Laser Scanning Confocal microscopy. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g79kx3vwz/v1

Manuscript citation:
In situ cryo-ET visualization of mitochondrial depolarization and mitophagic engulfment
Kevin Rose, Eric Herrmann, Eve Kakudji, Javier Lizarrondo, A. Yasemin Celebi, Florian Wilfling, Samantha C. Lewis, James H. Hurley
bioRxiv 2025.03.24.645001; doi: https://doi.org/10.1101/2025.03.24.645001

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

Created: May 19, 2025

Last Modified: June 12, 2025

Protocol Integer ID: 218555

Keywords: Plasmids, Fluorophores, Photobleaching, live imaging, mitochondria, cell imaging of mitochondrial membrane protein, imaging of cultured mammalian cell, mitochondrial membrane protein, tagged mitochondrial protein, airyscan laser scanning confocal microscopy, cell imaging, mitochondrial protein, cultured mammalian cell, imaging, cell, mammalian cell

Funders Acknowledgements:
Michael J. Fox Foundation for Parkinson's Research
Grant ID: ASAP-000350

Abstract

This is a protocol for live-imaging of cultured mammalian cells transfected with plasmids encoding fluorescently-tagged mitochondrial proteins.

Attachments

PDF

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157KB

Materials

Equipment:

- LSM980 with Airyscan 2 (section 2: imaging)
- ZEISS ZEN 3.7 (section 2: imaging)
- Incubator (section 1: cell preparation)
- Tissue Culture Hood (section 1: cell preparation)

Reagents:

- Complete DMEM media (section 1: cell preparation)

1. 500 mL DMEM, high glucose, no glutamine **Thermo Fisher Scientific Catalog #**11960-044
2. 55mL Heat-inactivated fetal bovine serum **Thermofisher Catalog #**A3840101
3. 5.5 mL L-Glutamine (200 mM) **Thermo Fisher Catalog #**25030081
4. 5.5mL Penicillin/Streptomycin **Thermo Fisher Scientific Catalog #**Invitrogen 15140-122

- 500 mL Opti-MEM™ I Reduced Serum Medium **Thermo Fisher Scientific Catalog #**31985070 (section 1: cell preparation)
- Lipofectamine™ 2000 Transfection Reagent **Thermo Fisher Scientific Catalog #**11668030 (section 1: cell preparation)
- Plasmids (section 1: cell preparation)

1. mCherry-TOMM20-N-10 **addgene Catalog #**55146
2. ATP5F1B (NM_001686) Human Tagged ORF Clone **OriGene Catalog #**RG201638

- 35-nm glass bottom dishes (Mattek, P35GC-1.5-14-C) (section 1: cell preparation)
- Pipettes (section 1: cell preparation)
- Pipette tips (section 1: cell preparation)
- 1.5mL or 2mL microtubes (section 1: cell preparation)
- Immersol 518 F/ 37 °C (ZEISS Immersion Oil 518F 20ml for 37 degrees C, SKU: 444970-9010-000) (section 2: Imaging)

Cell Preparation

1 Cell Preparation (see transfection protocol: http://dx.doi.org/10.17504/protocols.io.ewov1dr82vr2/v1)).

1.1 Split cells onto 35nm dishes two days prior to imaging (~ 20-30% confluency).

1.2 Transfect the cells with your desired construct(s) one day prior to imaging.

1.3 Day of imaging (optional): Treat the cells with your desired drugs (e.g., 3uM:3uM oligomycin A: antimycin A mixture).

Imaging

2 Perform imaging using the LSM980 with Airyscan 2 laser scanning confocal microscope, possessing 405, 588, 561, and 639 nanometer laser lines, in a temperature-controlled chamber set at 37 °C and 5% CO_2.

3 Add a single drop of Immersol oil of refractive index appropriate for imaging at 37 °C onto the top of the objective lens.

> Note
>
> Do not let the applicator touch the objective lens.

4 Place imaging dish on the microscope stage.

5 Raise the 63X/1.4 NA oil objective until there is an oil interface between the objective and the bottom of the dish.

6 Find the focal plane using transmitted light.

7 Select your specific fluorophores in Smart Setup.

8 Go in live or continuous to determine your laser power (ideally, use a laser power < 1% to avoid photobleaching).

9 Select your acquisition parameters.

9.1 Use 1x or 2x to find field of view and 16x to zoom in.

9.2 Set sampling to SR-4Y and 2.0 multiplex acquisition.

9.3 Select the fps that will result in a pixel dwell time of approximately 1.

9.4 Set the scan to be bidirectional.

9.5 Select 8x line averaging.

10 Check the z-stack box and then go in live to determine the center of the z-stack you will take in your experiment.

11 In the Z-stack tab, select preferred capture parameters.

11.1 Use the scroll button on the system mouse to find the center of the z-stack.

11.2 Select a z-range of 1.00um.

11.3 Set your desired step size (e.g. 0.17 um) or click Optimal to set automatically.

11.4 Click on Center.

Experiment

12 Start the experiment.

Protocol references

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Acknowledgements

Development of this protocol was funded by Aligning Science Across Parkinson's through the Michael J. Fox Foundation for Parkinson's Research.