VERSION 1

APR 21, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.261ge397jl47/v1

Protocol Citation: Quyen Do 2023. Visualisation and quantification of dendritic spines in cultured human Medium Spiny Neurons (MSNs). **protocols.io** https://dx.doi.org/10.17504/protocols.io.261ge397jl47/v1Version created by Cláudia C. Mendes

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

Created: Apr 12, 2023

Last Modified: Apr 21, 2023

PROTOCOL integer ID:
80391

Visualisation and quantification of dendritic spines in cultured human Medium Spiny Neurons (MSNs) V.1

Quyen Do[1,2,3]

[1]Oxford Parkinson's Disease Centre and Department of Physiology, Anatomy and Genetics, University of Oxford, South Park Road, Oxford OX1 3QU, United Kingdom;
[2]Kavli Institute for Neuroscience Discovery, University of Oxford, Dorothy Crowfoot Hodgkin Building, South Park Road, Oxford OX1 3QU, United Kingdom;
[3]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815, USA

Cláudia C. Mendes

ABSTRACT

This protocol describes the visualisation of dendritic spines of human neurons cultured on coverslips *in vitro* and subsequent quantification using the software Imaris.

MATERIALS

Reagents:

- SlowFade™ Diamond Antifade Mountant (ThermoFisher Scientific, CAT# S36967)

Equipment:

- Olympus FluoView™ FV1000 Confocal Microscope

Software:

- Imaris v9.6.0 (Bitplane, South Windsor, CT, USA)

Keywords: Dendritic spine, Imaris, fluorescent, immunocytochemistry

Visualisation of fluorescent dendritic spines in MSNs

1 Mount a coverslip of Medium Spiny Neurons (MSNs) immunolabelled for DARP32 and neurobiotin onto SlowFade™ Diamond Antifade mountant (follow **Protocol: Immunocytochemistry of cultured human Medium Spiny Neurons (MSNs)**).

2 Place slide under under Olympus FluoView FV1000 confocal microscope with argon and solid-state laser with 488 nm and 559 nm excitation, respectively.

3 Use the 60x oil-immersion objective (NA = 1.40) to image and capture MSNs coexpressing Darpp32 and Neurobiotin as Z-stacks, sampling sequentially at resolution of 1024 * 1024 pixels and at 1.05 µm steps, as optimised by Nyquist sampling theorem.

4 Use the zoom function (3x) in the Olympus Dendritic FluoView FV1000 software to capture dendritic branches of biotinylated neurons.

Note

Approximately 2-4 images are captured per neurons, depending on image quality.

Quantification of dendritic spines

5 Use the 'Surface' module to threshold and segment the confocal images.

6 Use the 'Filament' module to automatically render the dendrites from images obtained after **step 5**.

7 Choose the spine detection function in the 'Filament' module to detect spines as protrusions from the previously-identified dendritic filament.

8 Manually exclude putative spines at branch points or disconnected dots.