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

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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
University of Oxford

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We use this protocol and it's working

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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^{TM} FV1000 Confocal Microscope

Software:

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

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.