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We use this protocol and it's working

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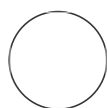
# Visualisation and quantification of dendritic spines in cultured human Medium Spiny Neurons (MSNs) V.1

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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™ 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 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**.

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- 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.