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Confocal imaging and digital image analysis

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

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Abstract

This protocol describes confocal imaging on a Nikon A1R confocal microscope and digital image analysis.

Attachments

DOCX

727-1838.docx

15KB

Materials

Apparatus

- Nikon A1R confocal microscope

Confocal imaging on a Nikon A1R confocal microscope

1 Turn on the power of laser, microscope, and computer.

2 Open the NIS-Elements AR software.

3 Set lightpath to Eyepiece to locate brain slice under 10x lens.

4 Once the brain slice is located, switch the lighpath to camera.

5 Check the pinhole size and make sure it is set to 1.2.

6 Check for lasers of interest and make sure they are in use.

7 For scanning, set resolution to 256×256 so that you can find the target regions as fast as possible.

8 Adjust Z using knob to find slices.

9 For the imaging of vGluT2, the immunostaining signals were taken between 5 and 8 µm below the surface of slice using a 100x objective lens with a 0.15 µm interval.

10 For the imaging of neuron density, NeuN-ir signals are collected using a 40x objective lens (1024 X1024 pixels; z step = 1 µm).

Digital image analysis: Spine density analysis using Imaris

11 Convert the confocal images (.ND2) to imaris images (.ims) using the convert function of imairs.

12 Open the images in imaris.

13 Add a new filament and skip the automatic creation.

14 Select “AutoPath” algorithm method and put your pointer in the “Select” mode.

15 Press shift and right click at the beginning of the dendrite of interests.

16 Let the algorithm starts quantified in a starting point.

17 Move the cursor from the beginning to the end of the dendrite.

18 When reach the end of the dendrite, press shift and left click to specify the end of the dendrite, make sure the length of the dendrite is around 20-30 microns.

19 Re-construct and quantify the spines manually.

20 The spine density = amount of spines/length of dendrite.