Jul 11, 2024

Assessing Astrocyte Territory Volume and 3D Sholl Analysis

DOI
dx.doi.org/10.17504/protocols.io.rm7vzj734lx1/v1

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.rm7vzj734lx1/v1

Protocol Citation: Shiyi Wang 2024. Assessing Astrocyte Territory Volume and 3D Sholl Analysis. **protocols.io** https://dx.doi.org/10.17504/protocols.io.rm7vzj734lx1/v1

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

Created: July 11, 2024

Last Modified: July 11, 2024

Protocol Integer ID: 103198

Keywords: ASAPCRN

Funders Acknowledgement:
Aligning Science Across Parkinson's (ASAP) initiative
Grant ID: ASAP-020607

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Abstract

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1 **Section Collection**

1.1 - Collect 100 µm-thick floating sections containing the anterior cingulate cortex (ACC) and primary motor cortex (MOp) from mice.

1.2 - Ensure astrocytes are sparsely labeled via PALE with mCherry-CAAX.

2 **Imaging**

2.1 - Use an Olympus FV 3000 microscope with a 60x objective to acquire high-magnification images (50-60 µm z-stack) of individual astrocytes.

2.2 - Criteria for inclusion: Capture the entire astrocyte within a single brain section, located in layer 2/3 of ACC or MOp.

3 **Surface Reconstructions**

3.1 - Analyze imaged astrocytes using Imaris Bitplane software.

3.2 - Generate surface reconstructions of the astrocytes.

4 **Territory Volume Measurement**

4.1 - Use Imaris Xtensions “Visualize Surface Spots” and “Convex Hull” to create additional surface renders representing the territory of each astrocyte.

4.2 - Record the volume of each astrocyte territory.

5 **Statistical Testing**

5.1 - Analyze astrocyte territory sizes from biological replicates across experimental conditions.

5.2 - Use a nested two-way ANOVA followed by the Bonferroni posthoc test for statistical comparisons.

6 **Surface and Filament Creation**

6.1 - Load astrocyte images onto Imaris and create a surface representation for each astrocyte.

7 **Filament Generation**

7.1 - Create filaments using ‘Add new filament (leaf icon)’ in Imaris.

8 **Quantification of Complexity**

8.1 - Use the gear tool on Imaris to display Sholl intersections for each astrocyte.

8.2 - Quantify the complexity of astrocytes based on Sholl intersections.

9 #### Experimental Details

10 ### Notes:

11 - Maintain consistency in imaging and analysis methodologies across experimental conditions.

12 - Consider biological variability and replicate experiments to ensure robust conclusions.