How to quantify structural complexity using MorphoScope

Introduction

Protocol to use MorphoScope, the graphical user interface to quantify neuronal structural complexity.

Materials

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› Download the standalone executable from: https://github.com/FranTassara/MorphoScope?tab=readme-ov-file

Procedure

Quantification

1. Click “**Load images**”. The document explorer will open. Select your fluorescence images (.tiff, .czi, or .lsm). You can select the number of images you want; however, we recommend not opening more than 20 at a time.

2. A window titled “Output file name” will open. Enter the name of the CSV that will be generated with the quantification results. This CSV will be saved in the images folder.

3. Select the first image you want to quantify in the upper-left panel. The image will be displayed on the right.

4. In the “**Visualizer**" module you can choose:

   Channel: which channel you want to visualize in multi-channel images.
   Visualization mode: whether you see the Z-projection or the Z-stack. If you choose the Z-stack, you can navigate it with the slider located below the terminal image.
   On the right side of the window, there is an additional vertical slider. It adjusts the display range of the signal to facilitate visualization. It does not affect the signal itself; it is for visualization only.

5. In "**Channels for quantification**", select which channel will be used for structural plasticity quantification and which one will be used for fluorescence intensity quantification. If you do not want to quantify fluorescence intensity, select “No channel”. At this step, you can apply a filter to your signal if it has too much background. The GUI offers several filter options. Filter application can be undone, so you can test different ones.

6. You can write an observation in the “**Observation**” panel. This will appear as a column in the final CSV. This observation is not cleared automatically after quantification, so you should delete it before quantifying the next image.

7. Click “**Create ROI**” in the “**Region of interest (ROI) selection**” panel to draw a polygon that defines the terminal region to be quantified. To delete a point, right-click, press “Del” (“Supr”), or the space bar. This may vary between computers.

8. Once you finish defining the ROI, click “**Apply ROI**”.

9. After applying it, define the slices of the Z-stack in the “**Slice selection**” panel using the slider below the image.

10. Once the slices are defined, click “**Process image and save**”. The quantification results will be automatically saved in the CSV file.

    Results are exported to CSV with the following columns:

Resulting CSV

	A	B	C	D
1	Column	Description	Units	
2	Image filename	Source filename	—	
3	Spread x [pixel]	X-axis spread (horizontal, maximum length direction)	pixels	
4	Spread y [pixel]	Y-axis spread (vertical, perpendicular to X)	pixels	
5	Spread z [pixel]	Z-axis spread (depth, dorsal-ventral)	pixels	
6	Spread x*y [pixel^2]	Product of X and Y spreads	pixels^2	
7	Spread xyz [pixel^3]	3D spread (product of all three)	pixels^3	
8	Spread x [µm]	X-axis spread in physical units	µm	
9	Spread y [µm]	Y-axis spread in physical units	µm	
10	Spread z [µm]	Z-axis spread in physical units	µm	
11	Spread x*y [µm^2]	Product of X and Y spreads	µm^2	
12	Spread xyz [µm^3]	3D spread in physical units	µm^3	
13	Axonal Volume (integrated intensity)	Sum of all intensity values × voxel volume	AU × µm^3	
14	Geometric volume [µm^3]*	Physical volume of occupied voxels	µm^3	
15	Fluorescence_ px [AU/pixel]	Mean intensity per pixel	AU/pixel	
16	Fluorescence_ um [AU/µm^2]	Mean intensity per physical area	AU/µm^2	
17	Observation	The notes you wrote in the "observation" box	—	

*NOTE: to quantify **geometric volume**, it is important to apply a filter to the signal used to quantify plasticity (e.g., a 3% threshold), since volume is calculated from voxels with signal intensity greater than 0. In all cases, even when the background appears clean, it still contains some intensity values. A recommended approach is to open the images in FIJI, measure the background intensity, and determine what percentage it represents relative to the maximum signal, in order to apply an appropriate threshold.