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

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Active zone protein clusters and dopamine axons in striatal slices

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ABSTRACT

This protocol detail the procedures for measuring the size and density of active zone proteins and axon terminals with Imaris software.

Visualization of active zoon proteins and dopamine axons

1 Stain and amplify fluorescent labeled axons with GFP, cherry, and bassoon antibodies.

2 Capture z-stack images with 100x oil lens (Nikon N-SIM Structured Illumination Super-Resolution Microscope), 4 µm (0.1 µm steps)

3 9 images per section and 5 sections per mouse are used for analysis.

Quantification and colocalization analysis with Imaris 10.0

4 Open the 3D-reconstructed .nd2 files and convert to .ims files

5 Initiate the surface creation with surface module

6 **Axon terminal**

6.1 Rename the surface module to GFP/mch.

6.2 Select object-object statistics for algorithm settings.

6.3 Select smooth and set the surface detail at 0.0643 µm. Background subtraction set the diameter of the largest sphere which fit into 0.421 µm

6.4 Threshold set to auto for minimal value and none for max value, adject the value manually if needed.

6.5 Select “number of voxels of Img=1” for surface filter and set the min value to auto, adject the value manually if needed

7 Active zone protein

7.1 Select classify surfaces and object-object statistics for algorithm settings.

7.2 Select smooth and set the surface detail at 0.06 µm. Background subtraction set the diameter of the largest sphere which fit into 0.15 µm

7.3 Threshold set to auto for minimal value and none for max value, adject the value manually if needed. Select split touching objects with intensity based and enable seep points diameter set to 0.1 µm

7.4 Filter seed points with “Quality” and set the min and max value with auto.

Filter surfaces with “volume” and set the min at 0.003 µm^3 and max at 0.04 µm^3.

7.5

7.6 Filter colocalized active protein and axon terminal by select “overlapped volume ratio to surface surface=GFP/mch” and set the min=1 (100% volume of active zone protein overlapped with anxon).

7.7 Duplicate selection to new surface for statistic data of active zone protein within axon.

8 **Measure the axon length with filament module.**

8.1 Skip automatic creation, edit manually to 100% capture the axon surface.

8.2 Select source channel to GFP or mcherry. And use “AutoPath” to trace the generated GFP/mch surface.

9 Export all the statistical data to Excel for further analysis with GraphPad Prism9.