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IMARIS Analyses of LAMP_CATD in vitro

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Chiara Pavan[1], Stefano Frausin[2], Clare Parish[1], Lachlan Thompson[2]

[1]Florey Institute for Neuroscience and Mental Health; [2]University of Sydney

courtney.wright Wright
University of Sydney

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We are still developing and optimizing this protocol

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Abstract

This protocol details how to analyse in-vitro LAMP and cathepsin D staining using Imaris software.

Materials

Software
Imaris Analysis Software (Oxford instruments Andor)

Before start

Select your image files folders. Drag the .LIF files into the Imaris Arena.
Double click. Do not open \LNG files

Creation of MAP2 surface

1

1. Add new surface.
2. Leave Creation Parameters as default
3. Selected segment only ROI.
4. Click next. Drag the yellow box to select the cell. Whole cell of interest.
5. Click *Next.* Select the channel (for your marker of interest). Smooth Surface detail. 0.0360. Machine learning segmentation.
6. Click *Next.* Draw thresholds; foreground and background w Shift-Click > 3 planes. Ensure the “pointer selection mode” is selected.
7. Click *Next.* twice.
8. Threshold to have only one mask included.
9. Click *Finish.*
10. Go to *Statistics, detailed tab, average values, save/export data.*
    - Save with suffix Cell #_MAP2

Create LAMP1 Surface

2

1. Add new surface.
2. Add LAMP1 parameters. Ensure *Select only a ROI* is ticked. Ensure object-object stats is checked.
3. Click *Next.*
4. Drag yellow box to the ROI. Click *Next.*
5. Select green (LAMP1) channel. Smooth 0.0361. Machine learning selected. Click *Next.*
6. Add Machine Learning Thresholds. Foreground (green) and background (purple) w Shift-Click > 3 planes. Click *Next.*
7. Check enable. Seed Point Diameter 0.181.
8. Click *Next.* Adjust slide bar to highlight LAMP1 included regions. Click*Next.*
9. Click *Next.* again and adjust.
10. Click Finish.
11. Remove LAMP1 regions by selecting the pencil (edit) and click the regions outside the cell and
    ‘*delete’*. Move through all the planes (z-axis).
12. Click *Statistics, detailed tab; Average Values* and *export data.*
    - Save with suffix Cell #_LAMP1

Create Cathepsin Spots

3

1. Create new ‘Spots’ for Cathepsin. Check the ‘different spot size (region growing). Click next.
2. Select ROI using the yellow box. Click *Next.*

3. Select the correct channel (red). Model XYZ diameter= 0.291, Estimated z diameter = 0.830. Check
   background subtraction. Click *Next.*.
4. Select threshold slider of Cat+ spots. For simplicity, select slice rendering for 3D spots. Click *Next.*
5. Check absolute intensity. Click *Next.*
6. Click *Finish.*
7. Remove Cathepsin dots outside the cell by selecting the pencil (edit) and click the regions outside the cell and 'delete'. Move through all the planes (z-axis).
8. Click *Statistics, detailed tab*; *Average Values* and *export data.*
    - Save with suffix Cell #_CatD

Determining CatD-LAMP1 coalescence

4

1. Select filer. Add Shortest distance to LAMP1 surface #.
2. Slide scale bar to remove spots that aren't touching.
3. Click *duplicate selection.*
4. Click *Statistics, detailed tab; Average Values* and *export data.*
    - Save with suffix Cell #_CatD_co