Imaris Coloc Protocol

Hong-Yuan Chu[1,2]

[1]Van Andel Institute; [2]ASAP

Jun 29, 2022

1 *Works for me* Share

dx.doi.org/10.17504/protocols.io.n2bvj61bblk5/v1

johnson.agniswamy

ABSTRACT

This protocol details the procedure of the imaris coloc protocol.

ATTACHMENTS

h78gbu5cf.docx

DOI

dx.doi.org/10.17504/protocols.io.n2bvj61bblk5/v1

PROTOCOL CITATION

Hong-Yuan Chu 2022. Imaris Coloc Protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n2bvj61bblk5/v1

FUNDERS ACKNOWLEDGEMENT

ASAP
Grant ID: ASAP-020572

Department of Defense
Grant ID: W81XWH2110943

KEYWORDS

Imaris, Coloc, Surface Function

LICENSE

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Jun 27, 2022

Citation: Hong-Yuan Chu Imaris Coloc Protocol https://dx.doi.org/10.17504/protocols.io.n2bvj61bblk5/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

LAST MODIFIED

Jun 29, 2022

OWNERSHIP HISTORY

Jun 27, 2022 madhavi.d

Jun 29, 2022 johnson.agniswamy

PROTOCOL INTEGER ID

65463

Background Subtraction

1

Open confocal files (.ND2) in ImageJ.

2 Perform background subtraction on z-stack of images (process -> subtract background).

2.1 Rolling ball radius: 50.0 pixels.

3

Save z-stack as a .TIFF file.

4

To select the .TIFF files containing folder open Imaris and press the “Choose Folder” icon.

5

Double click the desired .TIFF files and they will automatically convert to .IMS format in the Arena.

6 Open the new .IMS file.

Citation: Hong-Yuan Chu Imaris Coloc Protocol https://dx.doi.org/10.17504/protocols.io.n2bvj61bblk5/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Region of Interest using Surface Function

7

To create a region of interest, click on the blue “add new surface” button to initiate the surface creation.

8

In the first step, select “skip automatic creation, edit manually”.

9

Next, select the contour tab that is in the “Draw” setting.

10 Slide the slice position to the bottom of the image.

11

In the resolution settings, select manual and preserve features and increase size to highest possible option.

11.1 This will maintain the size and dimension of the surface throughout the image and is important to keep uniformity.

12

Change from the “Board” display to “Mode”.

13

Select the “Circle” option under “Drawing Mode”, and set the parameters to the following: radius = 15 µm; number of vertices = 30.

14

Select the “draw” button in the bottom right corner While on the bottom slice.

Citation: Hong-Yuan Chu Imaris Coloc Protocol https://dx.doi.org/10.17504/protocols.io.n2bvj61bblk5/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

14.1

The pointer must be set to “Select” and will not work under the “Navigate” option.

14.2 This can be changed by pressing the “Esc” button on the keyboard, or in the top right corner of the Imaris Arena.

15 Place the circle over the desired region in the image.

16

Change back to the “Board” display and under “Selection” press “Copy”.

17

Move the slice position to the top of the image and press “Paste”.

18

Press the “Create Surface” button to generate the surface construction.

Note: Make sure you did step 11 and that it is still set to manual and preserve features!

19

To mask each channel based on the generated surface, click on the “Edit” tab and under mask properties select “Mask All”.

20

A pop up will appear and select the channel to mask.

20.1 Set voxels outside surface to: 0.00.

Citation: Hong-Yuan Chu Imaris Coloc Protocol https://dx.doi.org/10.17504/protocols.io.n2bvj61bblk5/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

21

A new channel will be created that only contains image data within the region of interest created (where the surface was created).

22 Repeat steps 19 & 20 for each channel necessary.

23

Open “Display Adjustments” in the top “Edit” button and turn off the original channels so just the masked channels are on.

Colocalization

24

Press the “Coloc” button to begin colocalization.

25

Set Channel A to the first masked channel (either vGluT1 or vGluT2).

26

Set Channel B to the other masked channel (aSyn) that is being measured against Channel A.

27 Set the selection mode to threshold and use the automatic thresholding function (“Calculate Thresholds”) to set thresholding parameters.

28

Click “Build Coloc Channel” to save the results as a new channel.

29 Repeat steps 25-28 for each combination of channels that are needed (example: vGluT1/aSyn & vGluT2/aSyn).

30

Citation: Hong-Yuan Chu Imaris Coloc Protocol https://dx.doi.org/10.17504/protocols.io.n2bvj61bblk5/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

To export colocalization results, go to “Image Properties” under the edit tab and select the channel equating to the colocalization channel.

31

Press the export button to export all colocalization results and repeat with each necessary channel.

Note: Repeat steps 7-31 for a second region of interest if multiple regions are needed per image.

Citation: Hong-Yuan Chu Imaris Coloc Protocol https://dx.doi.org/10.17504/protocols.io.n2bvj61bblk5/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited