Tutorial : Hyperspectral Analysis on 3D SIM/4D LSM Image Data

Introduction

This tutorial is to help users analyze spectral information of granules (such as, lipofuscin, melanolipofuscin, and etc.) with 3D high-resolution structured illumination microscopy (SIM) and 4D confocal multispectral laser scanning microscopy (LSM).

The required programs to follow the procedure in this tutorial are

1) 3D Slicer – <u>http://www.slicer.org</u> 2) FIJI – <u>https://fiji.sc</u> 3) ITK-SNAP - <u>http://www.itksnap.org</u>

Workflow

We will describe the overall workflow of hyperspectral analysis, starting from 1) reading 4D LSM image, 2) granule segmentation on 3D SIM data, 3) co-registration between 4D LSM and 3D SIM data, and 4) hyperspectral analysis of granules.

1. Read 4D LSM

- 2. Granule segmentation on 3D SIM
- 3. Co-Registration : SIM / LSM

Please see a previous tutorial for segmentation/registration processes using FIJI and ITK-SNAP

4. Convert concatenated 4D LSM to an image series

5. Hyperspectral Analysis

1) Open 3D Slicer

- 2) Go to Extension \rightarrow MultiVolume Support \rightarrow Multivolume Importer
- 3) Set Input Directoriy to the folder of an image series
- 4) Create new MRMLMultiVolume at Output node
- 5) Import (May take a few minutes)

6) Imported volume will be displayed as # frames MultiVolume (e.g. 24 frames MultiVolume)

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7) Go to Extension \rightarrow MultiVolume Support \rightarrow Multivolume Explorer

8) Adjust Window/Level by drag-n-drop on one of viewports

9) Set Input multiVolume to # frames MultiVolume (imported from Step 5))

10) Click Play next to Current frame number to play a video to display a sequence of image channels

11) The plot of hyperspectral information will be displayed on the left panel under "Plotting" while you navigate on the viewports.



12) Load a label map generated from the segmentation step in "Plotting Settings \rightarrow Probed label Volume"

13) Click Chart next to "Probed label Volume"



