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, melano-lipofuscin, 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 – http://www.slicer.org
2) FIJI – https://fiji.sc
3) ITK-SNAP – http://www.itksnap.org

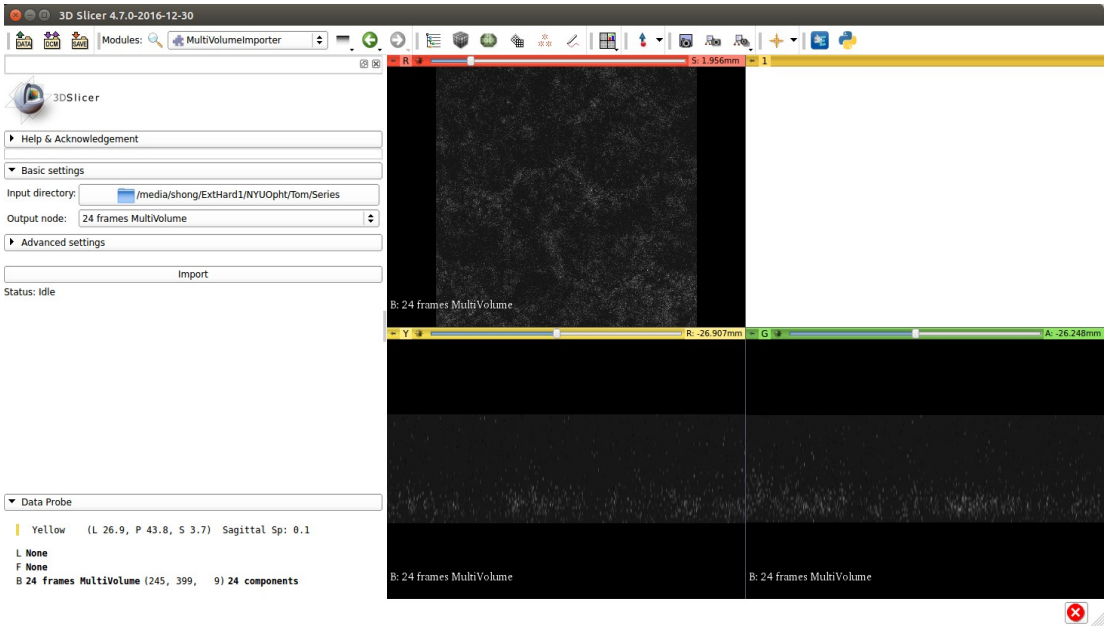
Workflow

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.

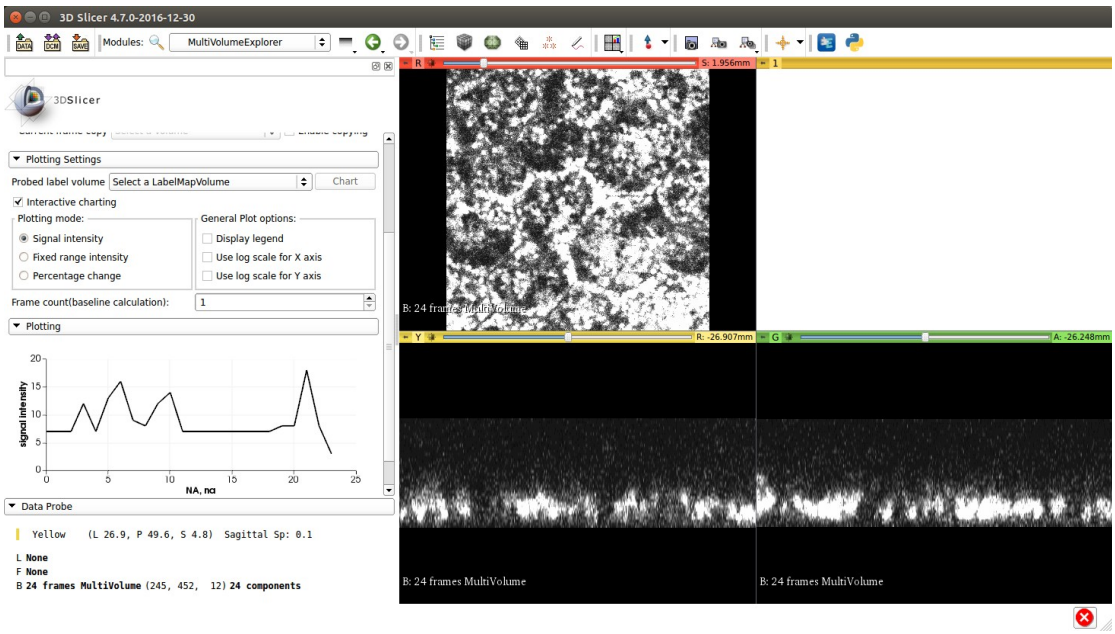
1. Read 4D LSM
2. Granule segmentation on 3D SIM
3. Co-Registration : SIM / LSM
Please see a previous tutorial for segmentation/registration processes using FIJI and ITK-SNAP

4. Convert concatenated 4D LSM to an image series
5. Hyperspectral Analysis

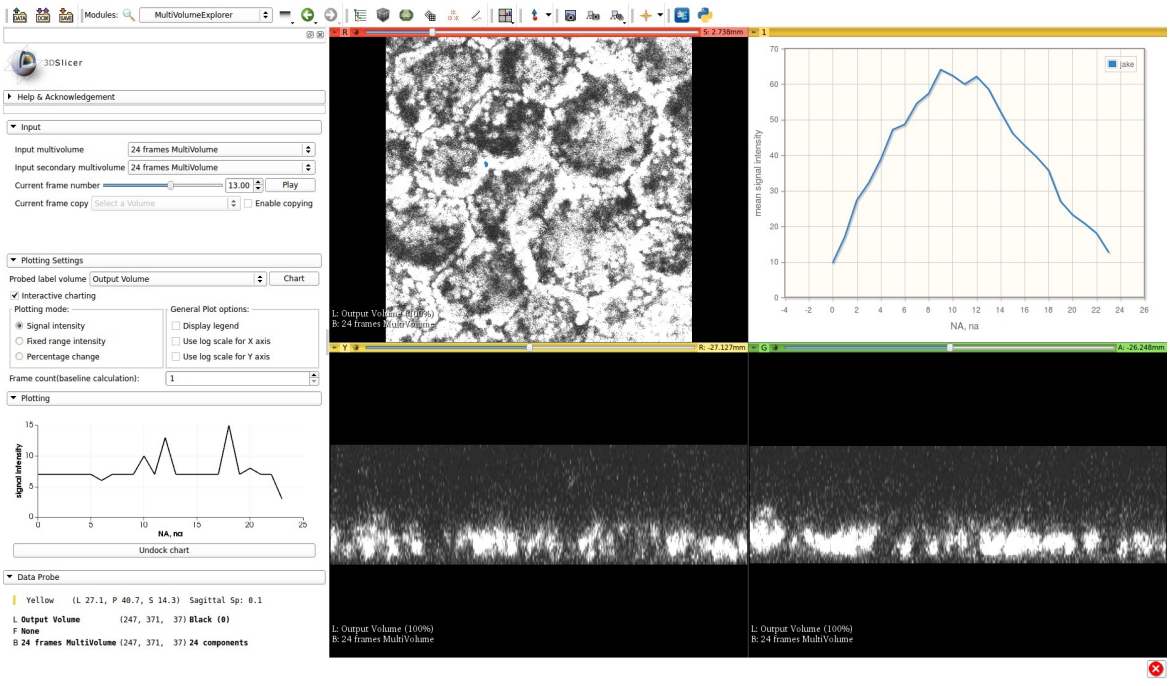
1) Open 3D Slicer
2) Go to Extension ⟶ MultiVolume Support ⟶ Multivolume Importer
3) Set Input Directoriy to the folder of an image series
4) Create new MRMLMultiVolume at Output node
5) Import (May take a few minutes)
6) Imported volume will be displayed as # frames MultiVolume (e.g. 24 frames MultiVolume)

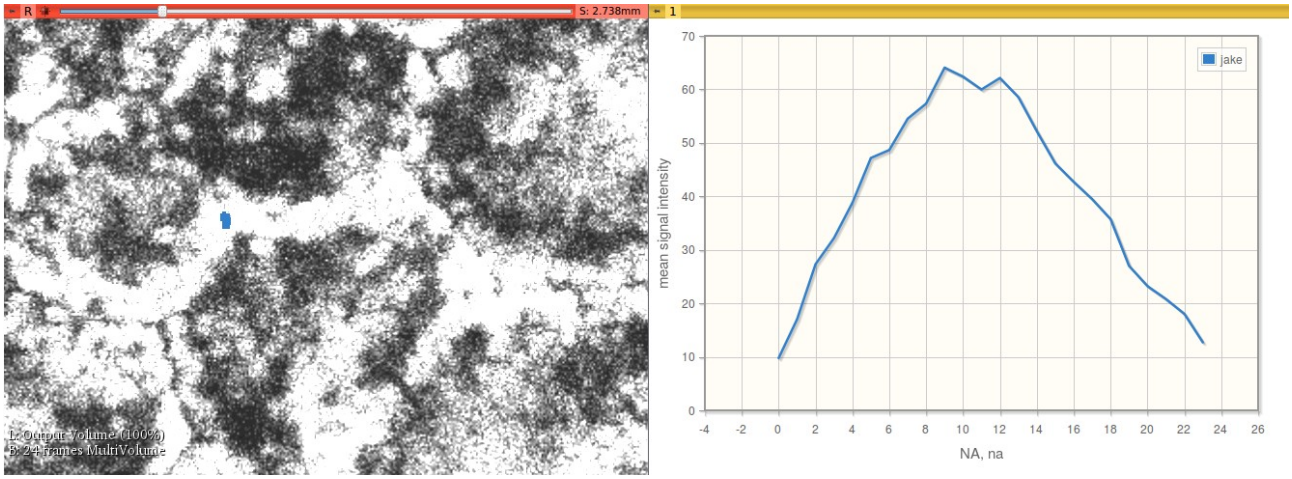


7) Go to Extension → MultiVolume Support → Multivolume Explorer
8) Adjust Window/Level by drag-n-drop on one of viewports
9) Set Input multiVolume to # frames MultiVolume (imported from Step 5))
10) Click Play next to Current frame number to play a video to display a sequence of image channels
11) The 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 → Probed label Volume”
13) Click Chart next to “Probed label Volume”




R
S: 2.738mm
1
1: Output Volume (100%)
2: 24 frames MultiVolume
jake
mean signal intensity
NA, na
0
10
20
30
40
50
60
70
-4
-2
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