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License and support

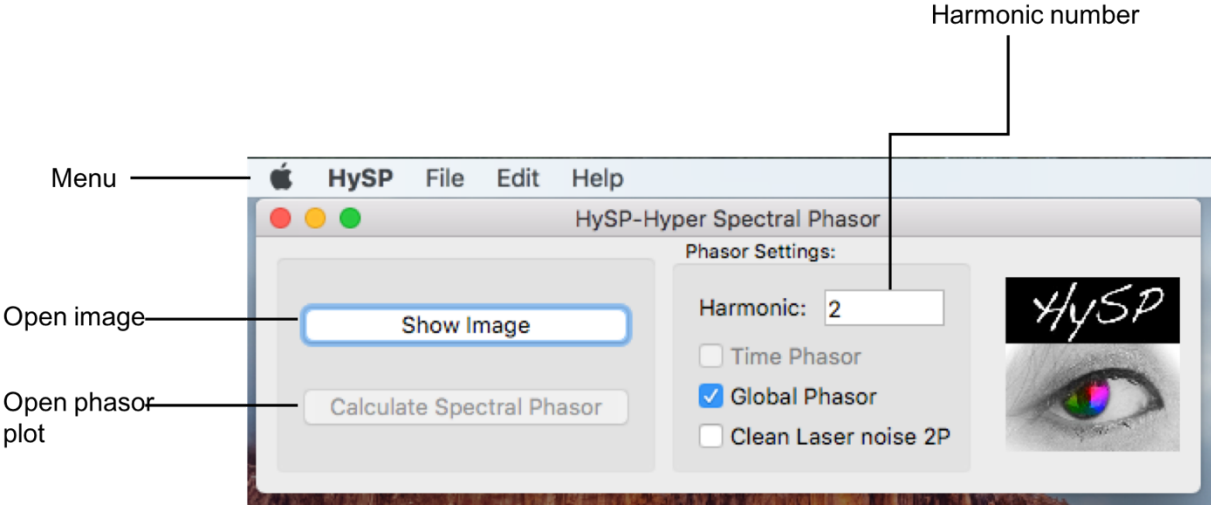
These instructions were written by Wen Shi, Daniel Koo, Rose Chiang and Francesco Cutrale at University of Southern California on 2021-12-21.

Introduction

HySP is a multi-platform software for analyzing multi-dimensional data. The application here uses hyper- and multi-spectral datasets. The software calculates a 2D phasor plot which can be analyzed interactively with ROI selectors, both phasor-to-image and image-to-phasor. The denoising algorithm improves the quality of data by reducing the poissonian noise. The SEER module provides a robust way to spectrally interpret and visualize lengthy datasets.

Command window

After opening HySP software, the command window shows as below.



Menu

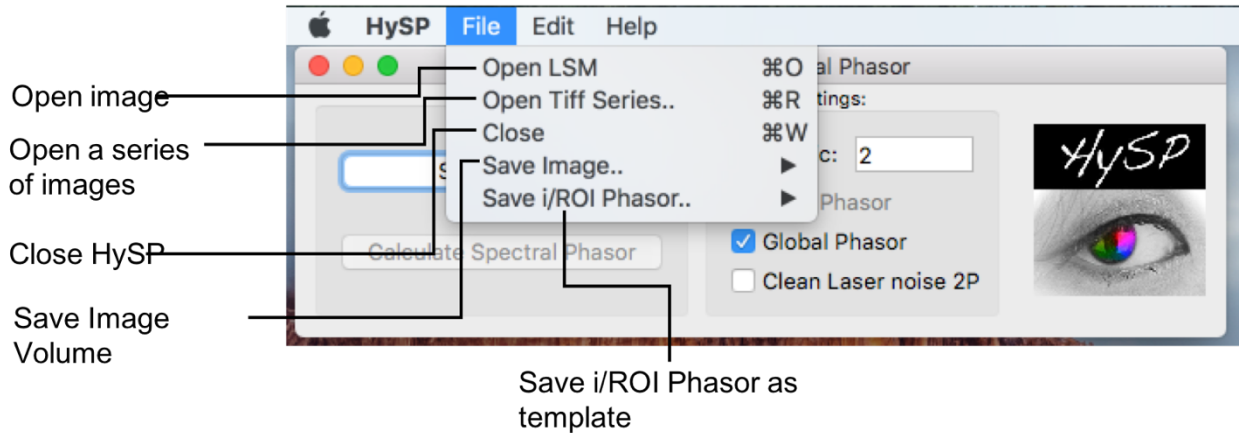
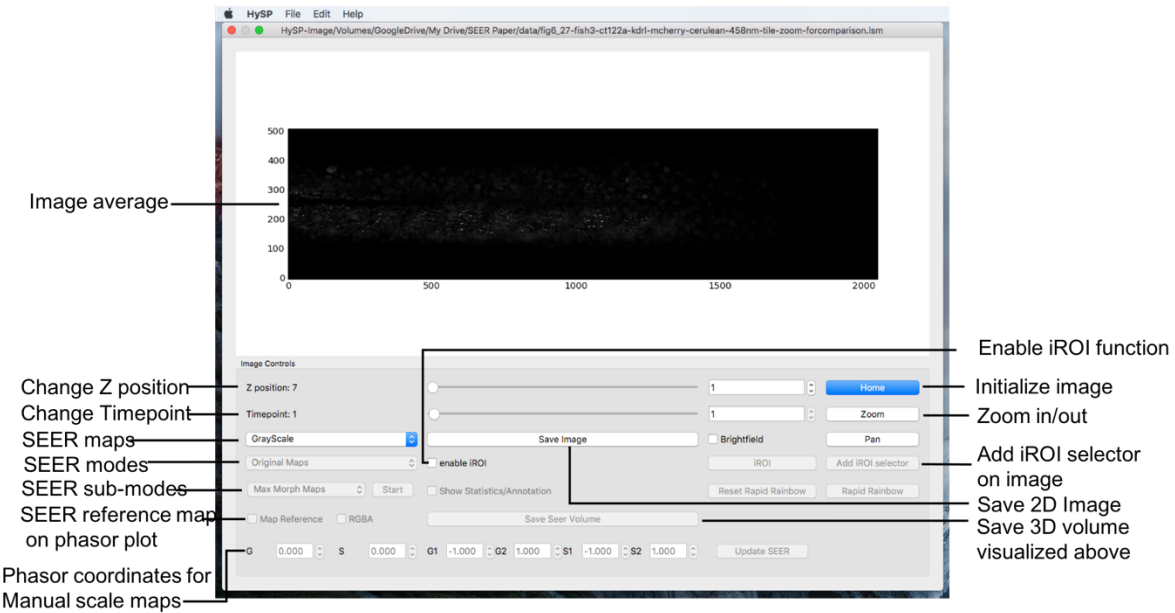
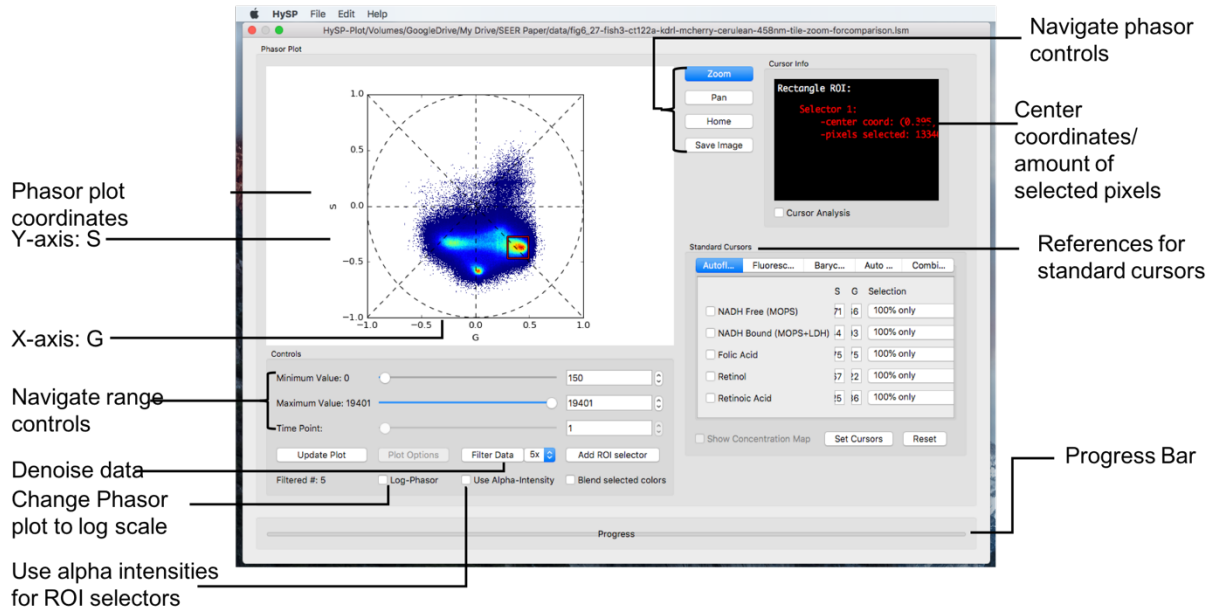


Image window



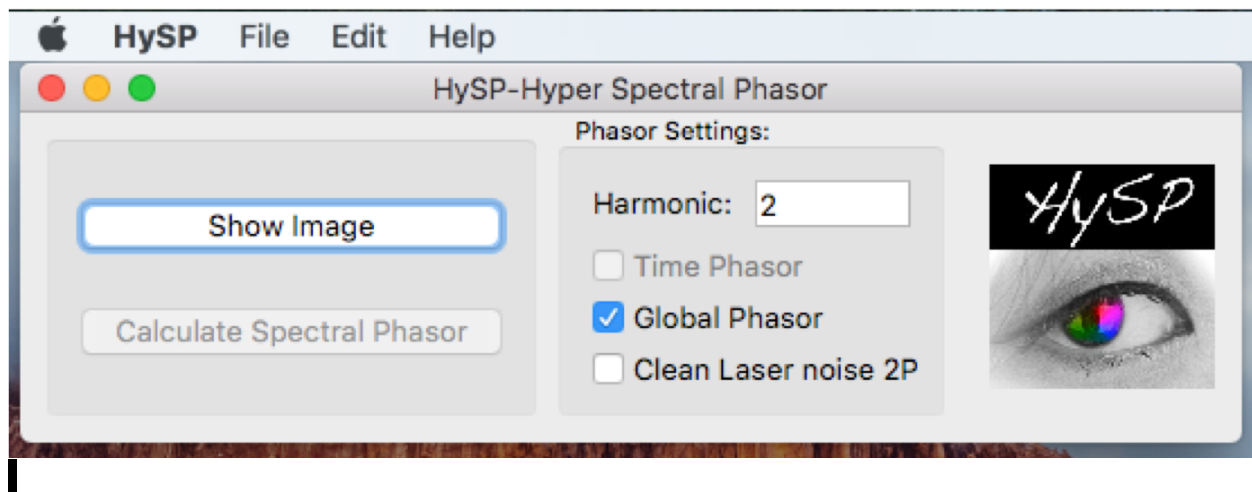
Phasor window



Set up HySP software

Harmonic

In the command window, under Phasor Settings the harmonic number can be typed. (i.e. harmonic:1; default configuration is 2)



Loading images

As of now HySP supports .ism and .tiff files. Tiff files should be in a single tiff sequence or multiple multi-layer tiff files. NOT all layers in a single tiff file. Example: if dataset has shape [time, z-stack, channel, x, y] then it should be saved as one multi-layer tiff for each channel or each z-stack [t, :, ch, :,:], or [t, z, :, ::]

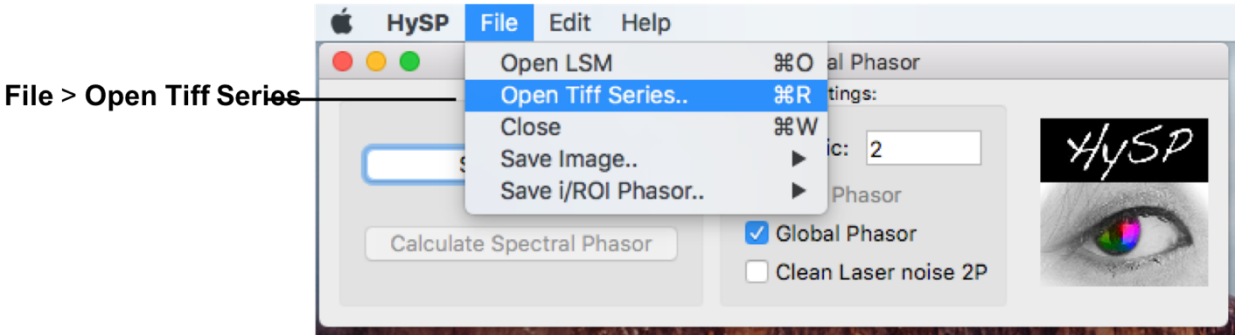
1. Open .ism file

Click Show Image > choose the file (file in .ism format) > click Open .

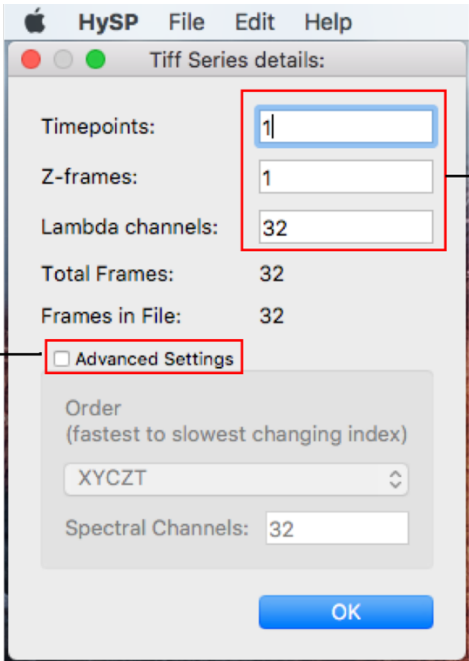
File > Open LSM > choose the file (file in .ism format) > click Open .

2. Open generic .tiff file File > Open Tiff Series > set up Tiff Series Details window.

Detailed info on dataset shape can be set in the window as below, parameters can be edited according to user's data.

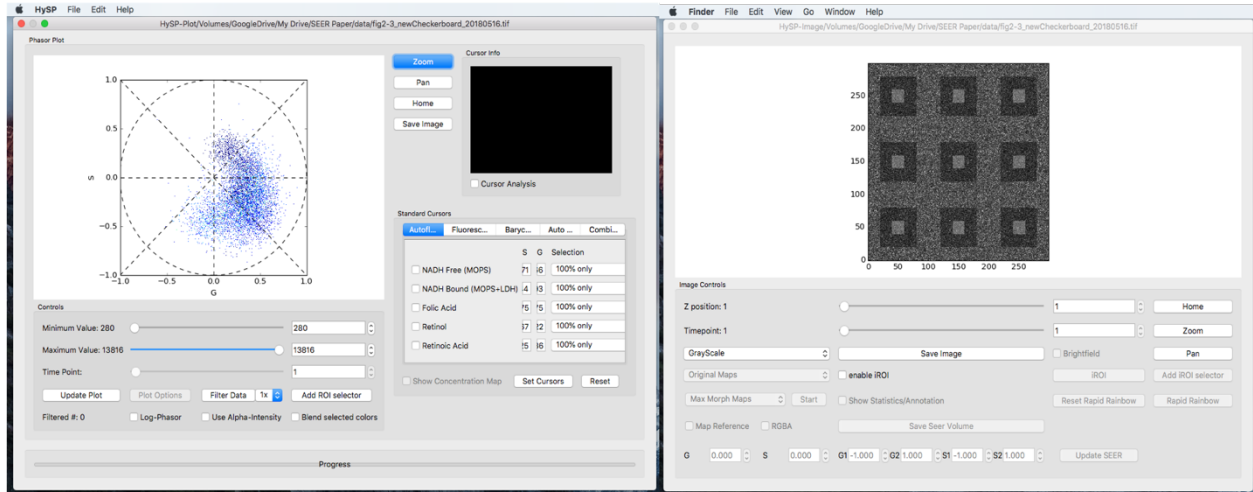


File > Open Tiff Series



Adjust to your TIFF dataset

Check **Advanced Setting** if single image has multiple Z-frames/λ channels

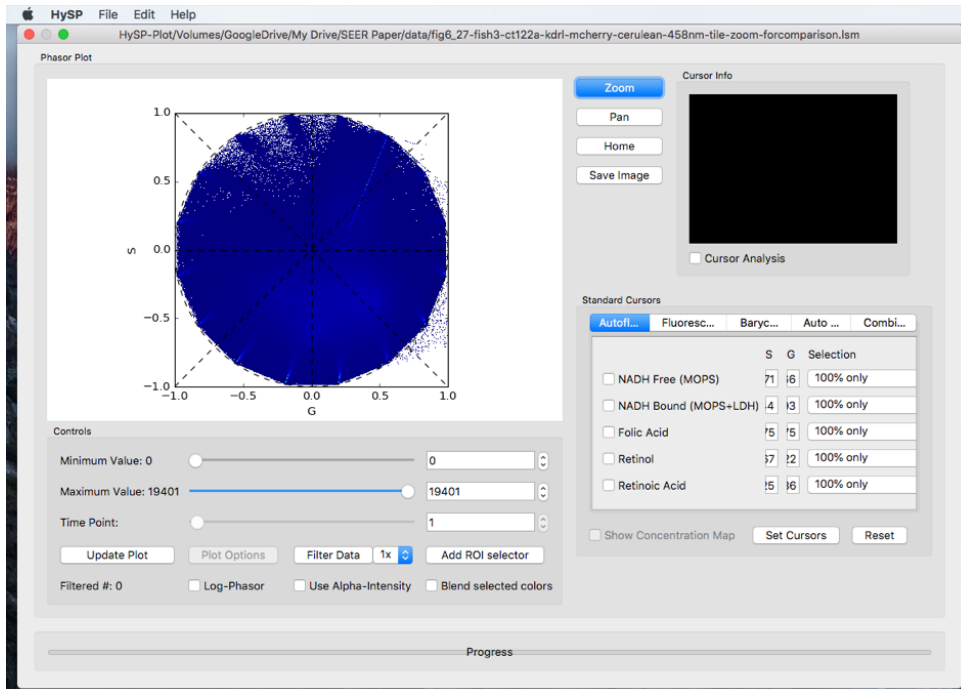


Phasor Window

Image Window

Open Spectral Phasor window

On to the command window > Click Calculate Spectral Phasor.
The phasor window shows as below.



Working with Phasor

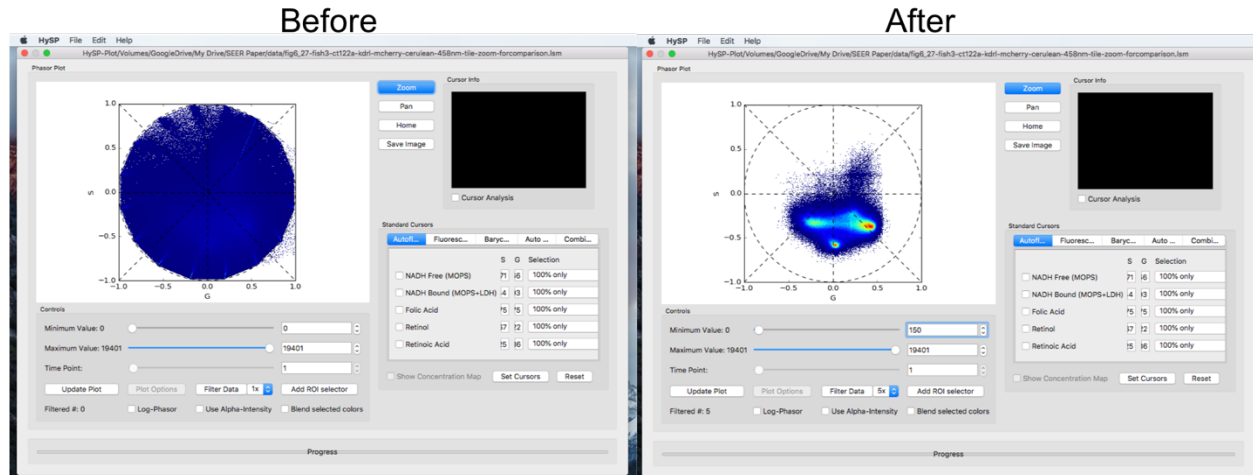
Filter

Click Filter Data/SDnoise to reduce noise in phasor plot. This step can be repeated up to 10 times to improve phasor quality.

Range controls

Type in Minimum Value, Maximum Value > Click Update Plot to view fluorescent pixels in interested range.

Here is a comparison of filtering and range controlling



Scale controls

Select Log-Phasor checkbox to visualize the phasor in log-scale. Click Update Plot to update window.

ROI controls

Select Use Alpha-Intensity checkbox to represent ROI phasor selection as an alpha-overlay on top of the image in Image Window. This visualization mode creates a colormap black-to-[color of ROI] and maintains the intensity of the underlying pixel. Generally useful for visual representation/rendering. Use Alpha-Intensity applies to all the ROIs added after the checkbox selection. Default mode (unchecked Use Alpha-Intensity) is a solid color representation.

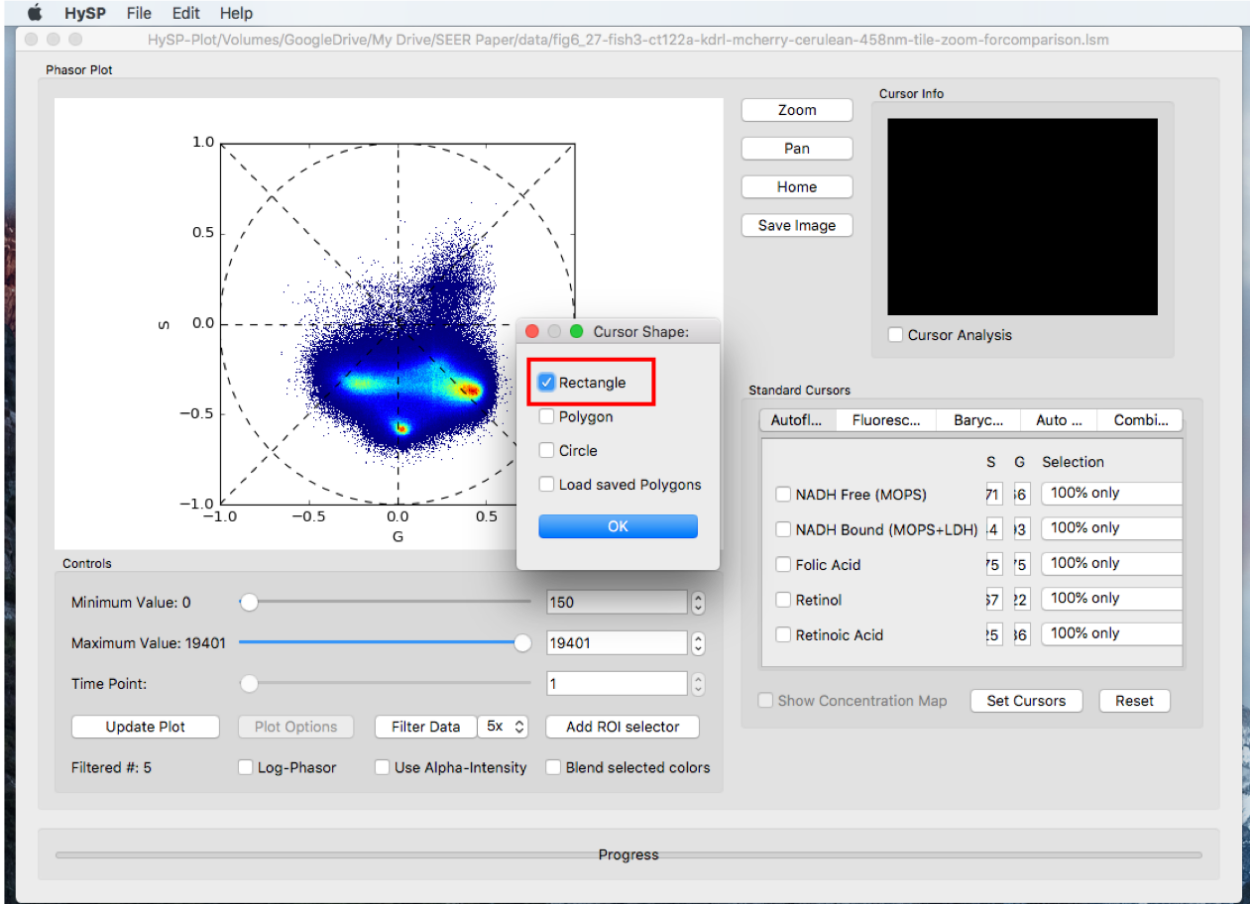
ROI selector

Rectangle

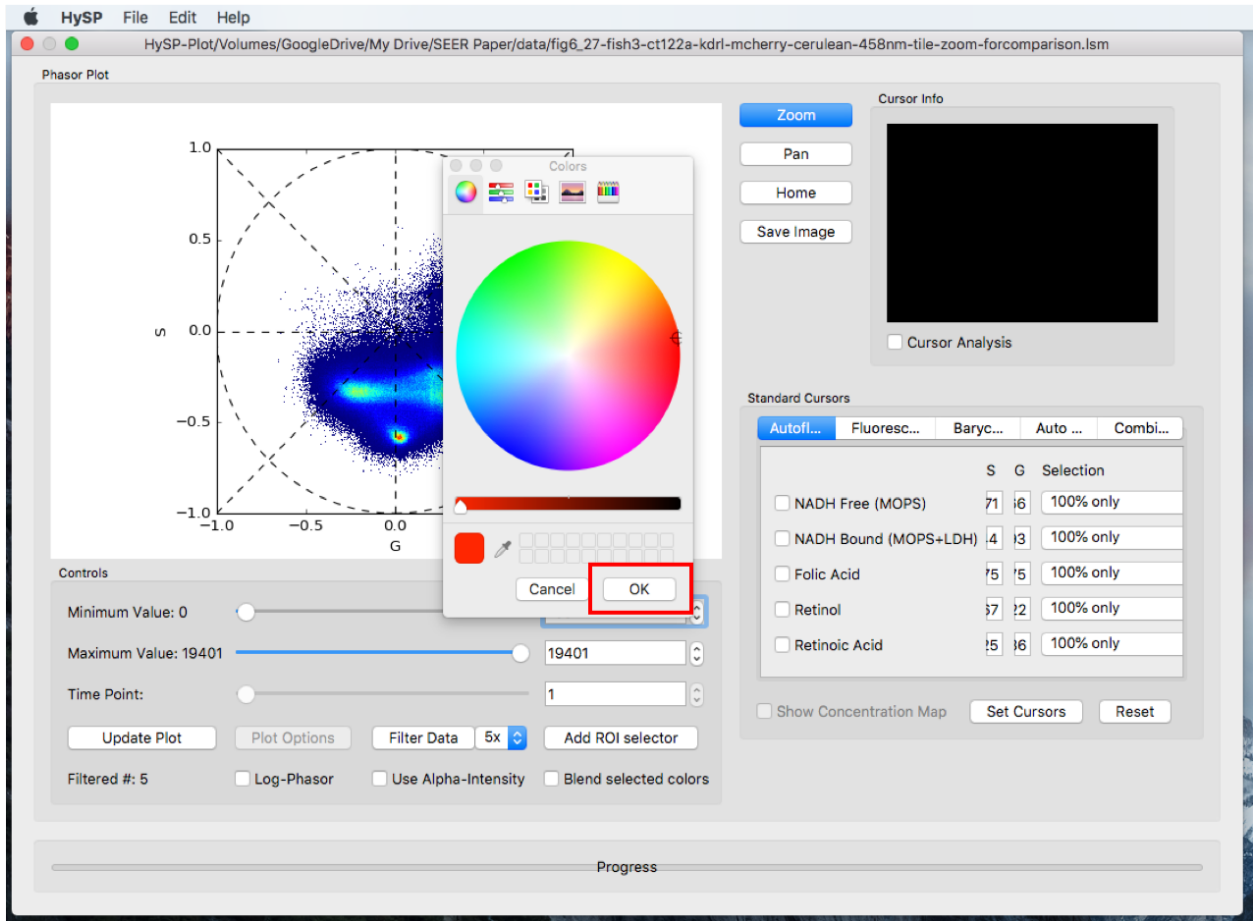
Rectangle ROI selector can acquire the coordinates in Cursor Info window; Click Add ROI selector > Choose the options, i.e. Rectangle > Click OK > Choose the color > Click OK > Move the rectangle on phasor.

Note: The phasor window and the image window can be viewed the same time to see the phasor chosen by rectangle is in which part of specimen in image. The specimen in image will be marked the same color user has chosen for rectangle in previous step.

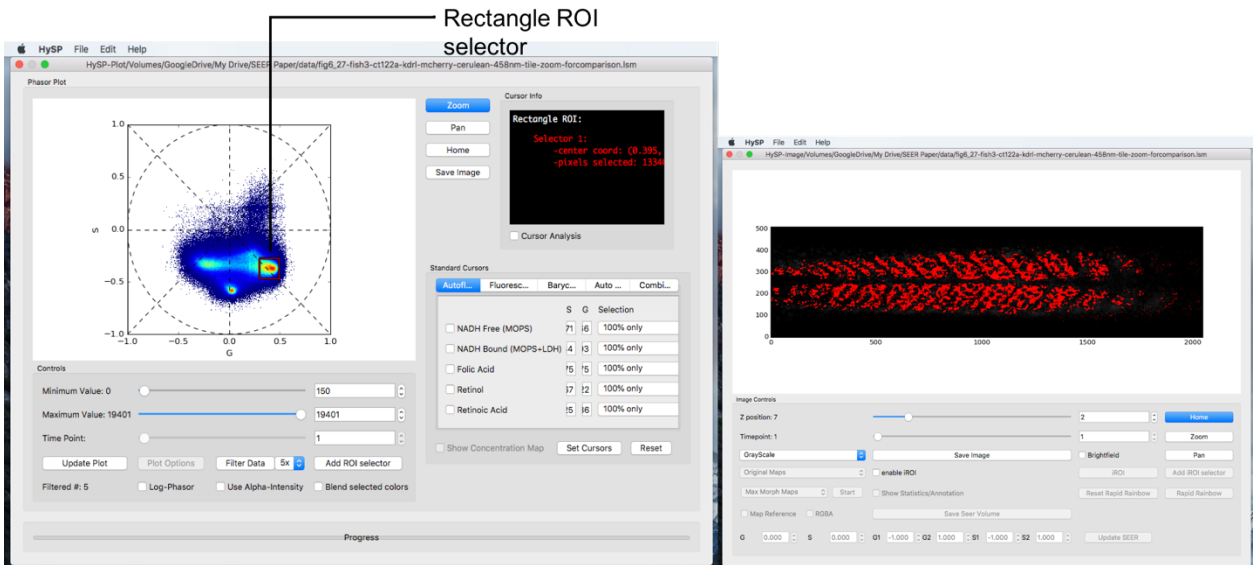
Step1. Add ROI Selector



Step2. Selector color



Step3.

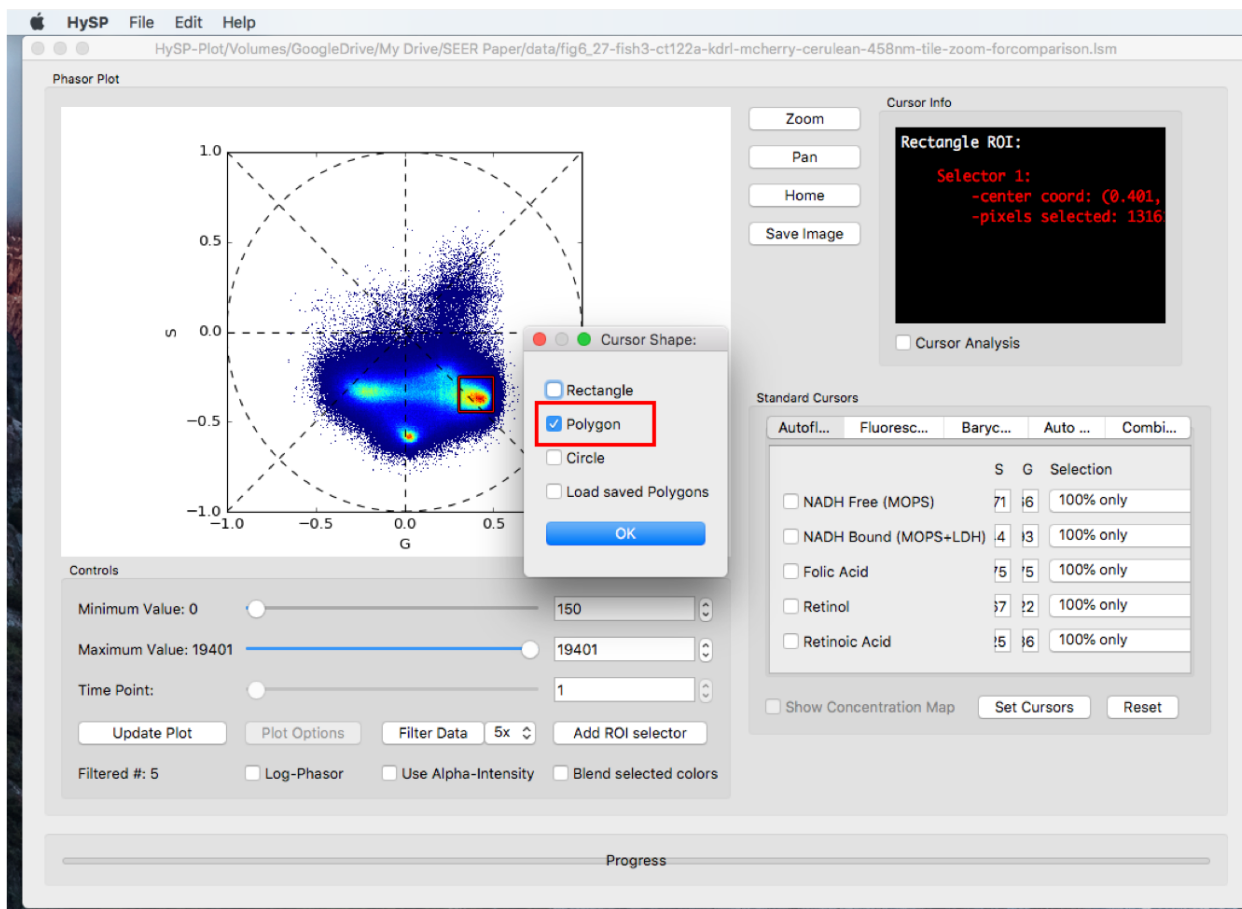


Phasor Window

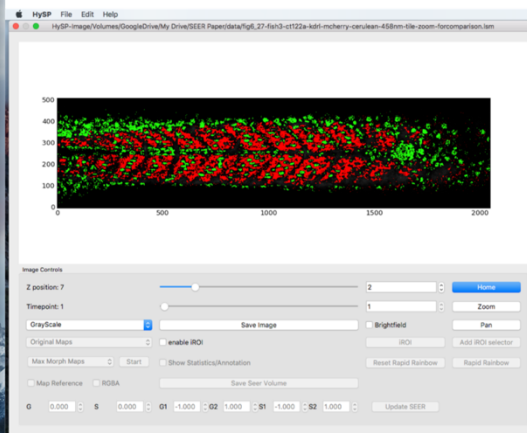
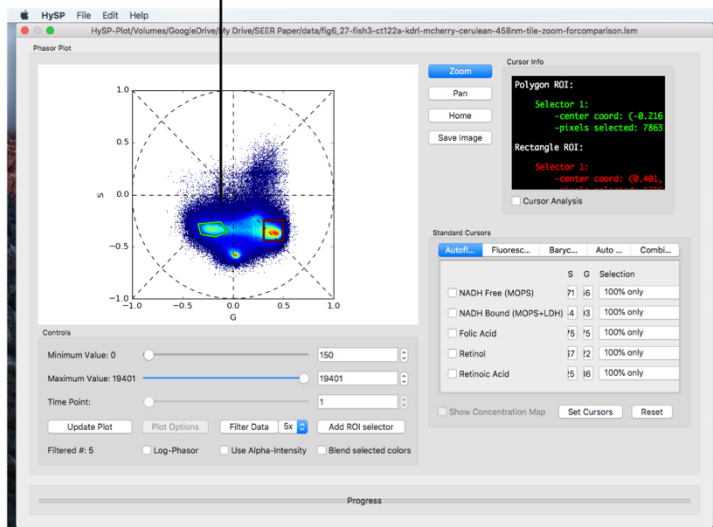
Image Window

Polygon

Click Add ROI selector > Choose the options, i.e. Polygon > Click OK > Choose color > Click OK > Right click to move the polygon; Left click inside polygon close to vertices to change the shape.

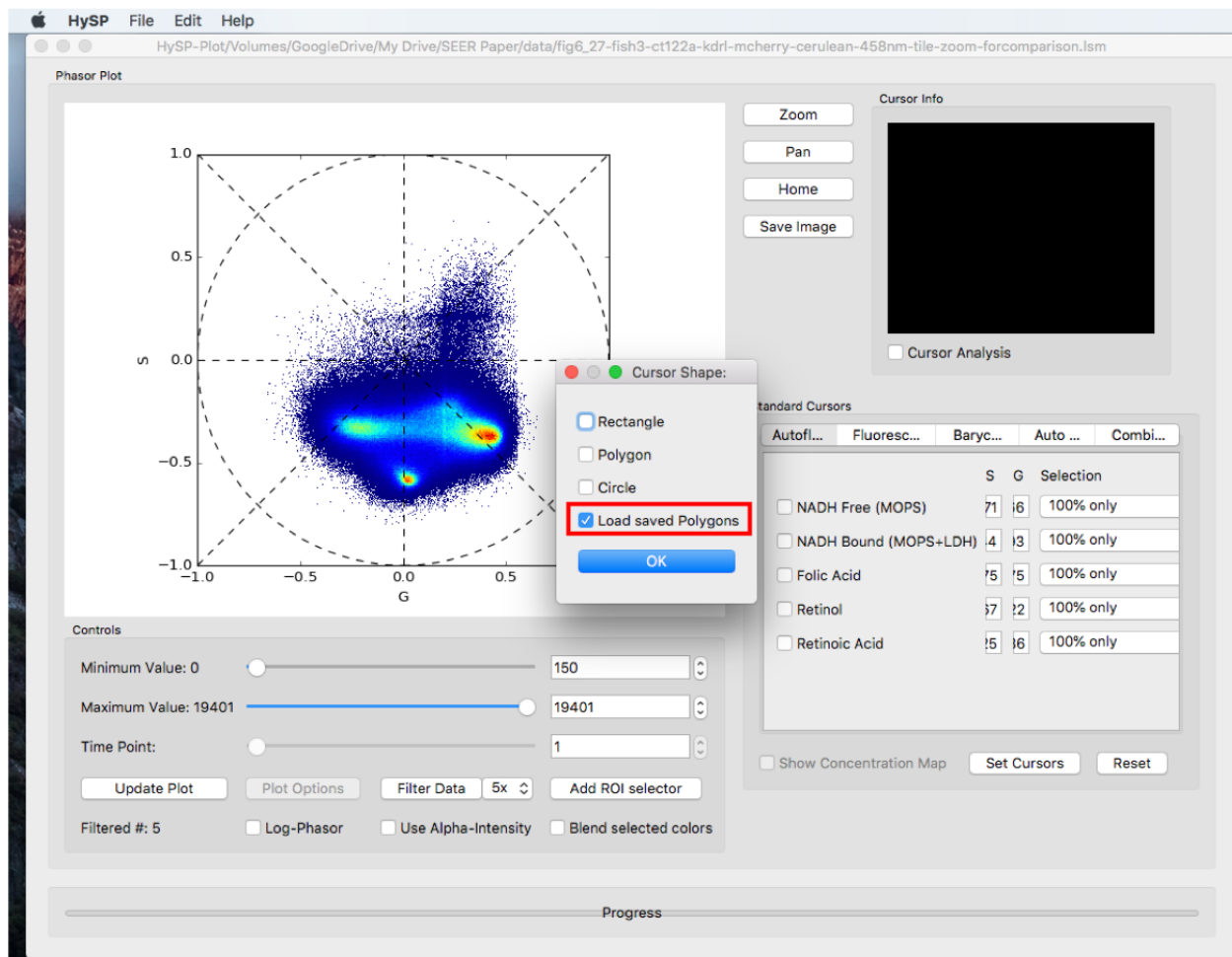


Polygon ROI selector 2



Load Saved Polygons

Click Load Saved Polygons and load previous polygons as template.



Navigating Phasor plot

Zoom

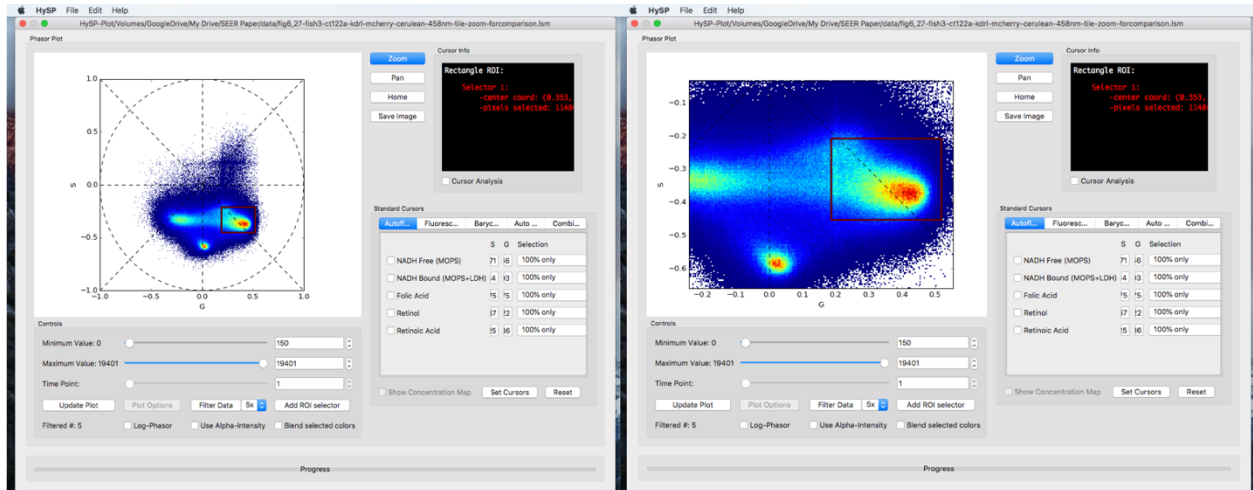
Click Zoom, frame the region surrounding the area of interest and zoom into it.
Click Zoom again to lock the region in case the phasor plot moves.

Home

Click Home to get the original zoom factor.

Pan

Click Pan to translate S and G direction on the phasors.



3D datasets $[z, \lambda, x, y]$ and 4D $[t, z, \lambda, x, y]$

Scroll Z-position in Image Window to visualize the chosen volume slice in dataset.
 Scroll Timepoint in Image Window to change time position in dataset.

Cursor info

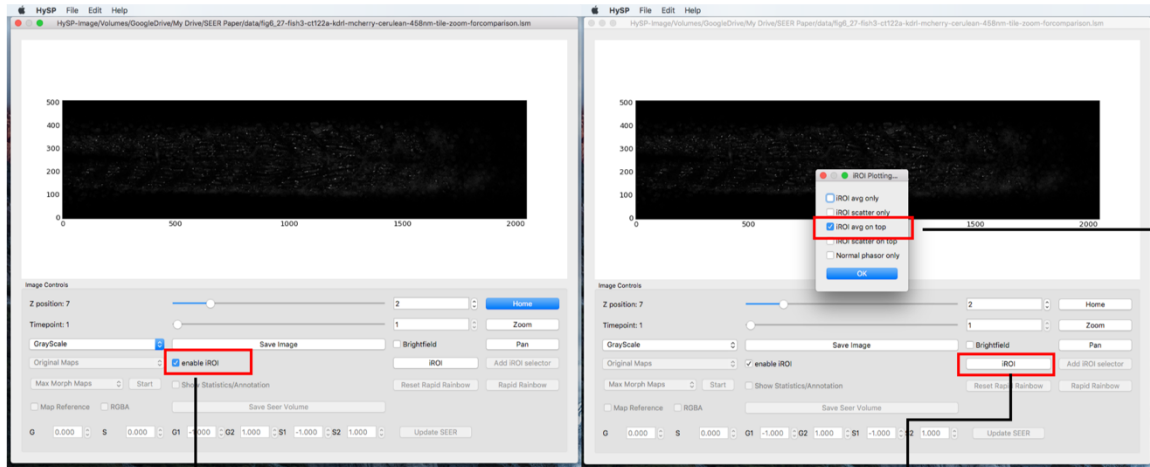
For rectangle ROI and iROI, center coordinates are reported in >Cursor Window.

iROI selector

This option enables an ROI selector on the Image (rather than on the phasor). The (S,G) values of each pixel selected on the image are then reported on the phasor according to the modality selected. E.g. average (S,G) with std for G and S axis.

In Image Window Choose enable iROI > Click iROI options and choose (i.e. iROI avg on top) > Click on iROI > Choose cursor options (i.e. Polygon) > Select color (red as default) > Click OK > Move polygon on interested area > Statistics are reported on Cursor Info tab and in Phasor plot in Phasor window.

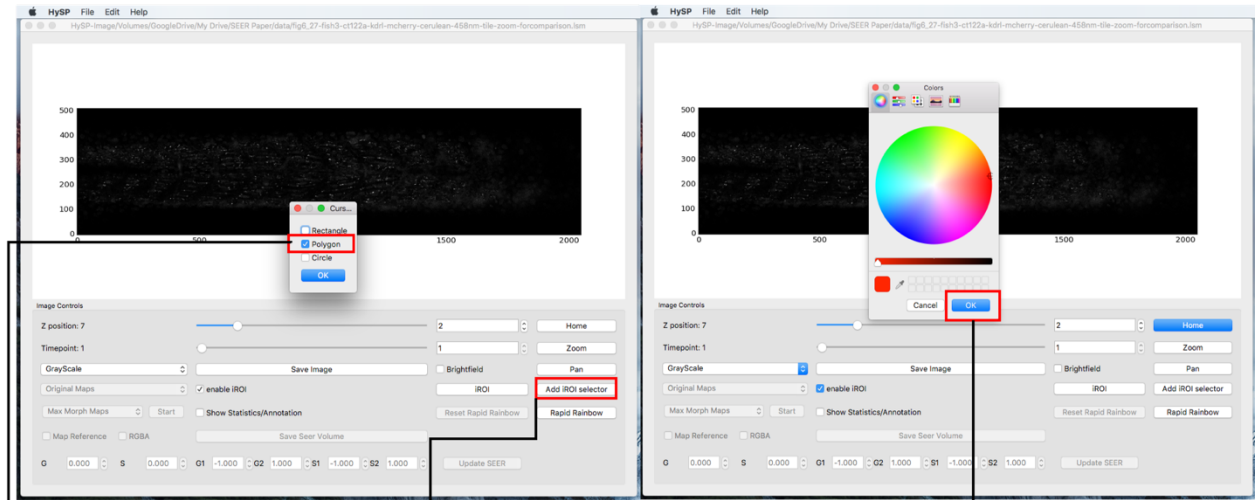
Note: Multiple polygons can be added on image with different colors, the area selected is labelled the same color in Phasor plot.



Step 1: Click **enable iROI**

Step 2: Click **iROI**

default setting is **iROI avg on top**

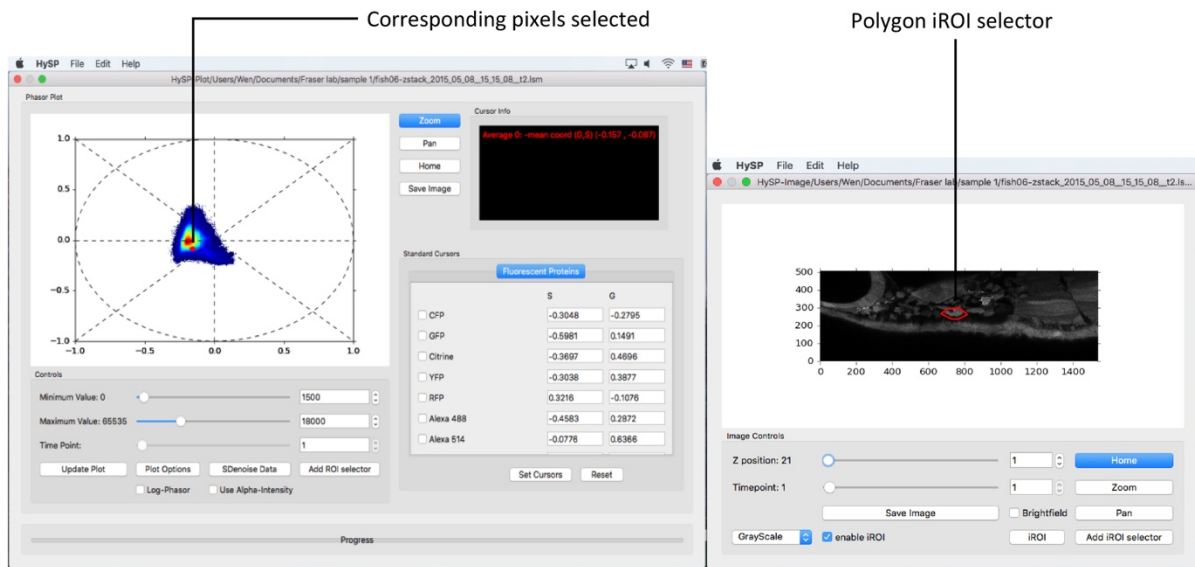


i.e. Click **Polygon**

Step 3: Click **Add iROI selector**

Step 4: Click **OK**

The iROI selector is represented with the same color on top of the phasor plot



Note: To load a new image and acquire the phasor of that image, you need to close all the windows and reopen HySP

Saving Data, ROIs and Images

Save Data

Processed data can be saved as .TIFF multilayer files by pressing File > Save Volume.. > Save Image Volume..

Each channel will be saved as a separate file. Each file will contain an entire z-stack if present. Multiple timepoint will contain t__ in filename

Save ROIs

ROIs selections on phasor can be saved by pressing File > Save i/ROI Phasor.. > Save ROI Phasor as..

The file saved can be then reloaded on a different dataset by clicking on Add ROI in Phasor Window and selecting Load ROI.

Save Images

Each image can be saved as a color picture (*.png). On Phasor Window > Save Image.. will save the phasor canvas with ROIs as currently visualized.

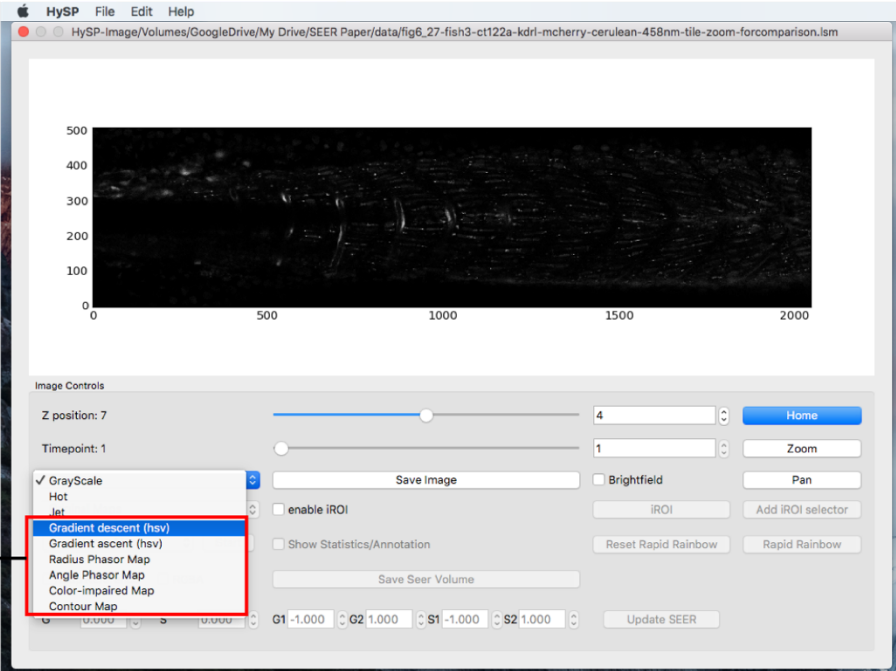
On Image Window > Save Image.. will save the image currently represented in the image canvas.

SEER Visualization

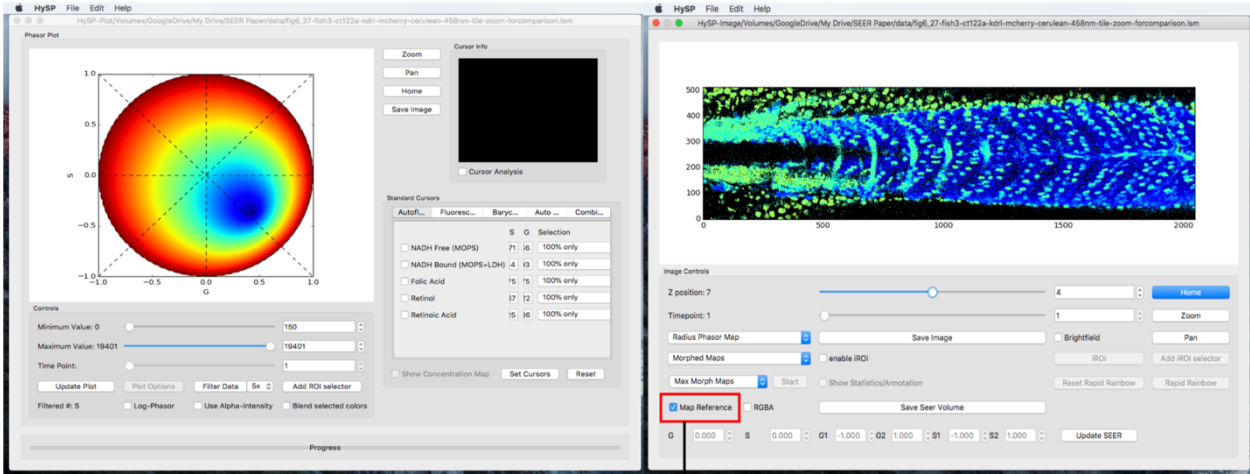
SEER Maps

Return to the Image window and on the bottom left click on the dropdown menu labeled “Grayscale” and the different SEER options of Gradient ascent, descent, radius, angle, and contour will appear.

SEER Maps



SEER Reference Maps
 Toggle the **Map Reference** checkbox, corresponding reference map will be updated on phasor window.

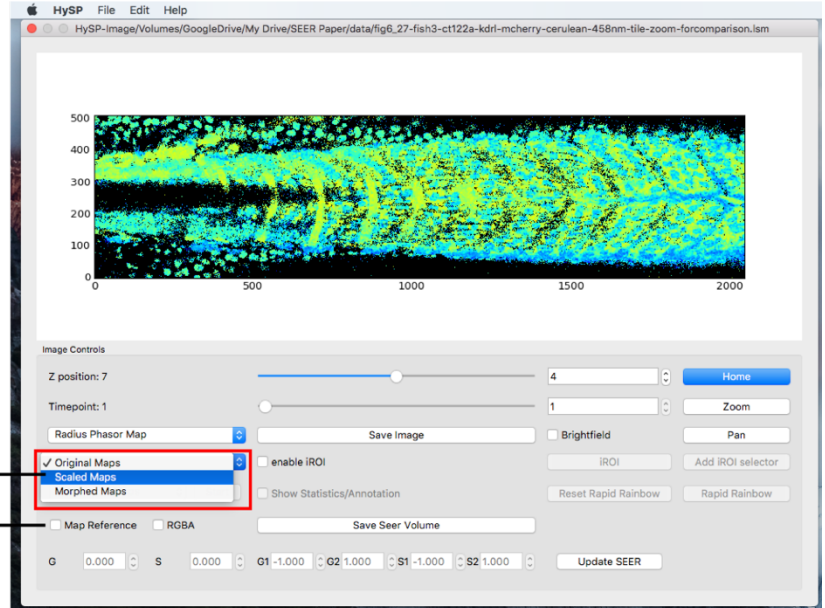


Apply SEER Reference Maps on Phasor Plot

Modes

The “Mode” dropdown menu is right below the SEER map menu. The initial value is set as “Original Maps” and there are “Scaled Mode” and “Morphed Mode” available.

SEER Modes
Apply SEER Reference
Maps on Phasor Plot



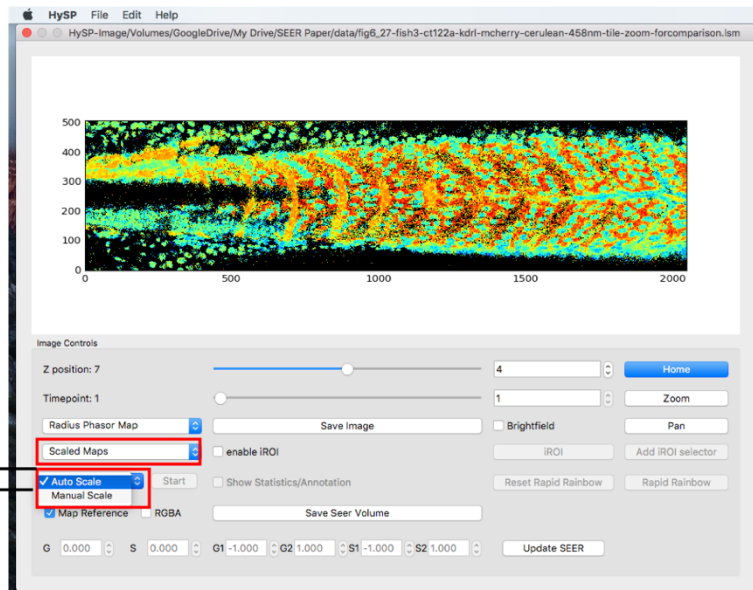
Scale Mode

Under Scaled mode there are auto-scale and manual scale mode.

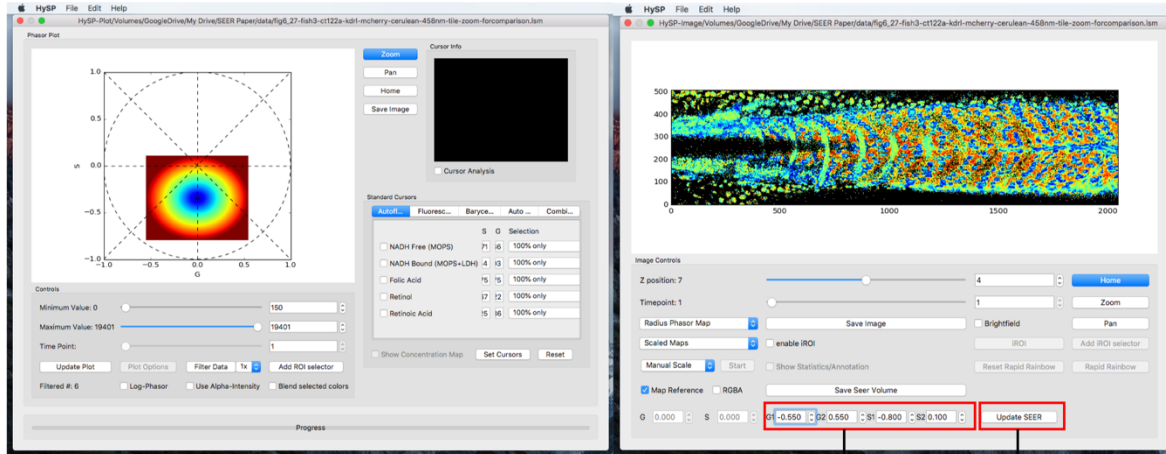
Manual Scaled mode:

Selecting “Scaled Maps” will enable the sub-modes for this mode.

Step 1: Select SEER
Scale Mode
Step 2: SEER sub-modes
for Scale Mode



One can type in the G/S values to specify the reference map position on phasor plot, click **Update SEER** to scale and relocate the map reference on phasor plot. See figure below.



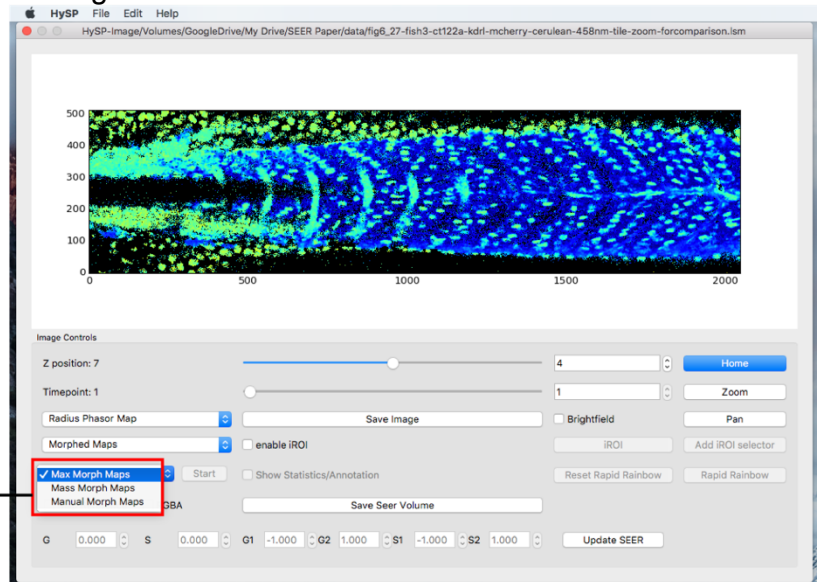
Step 3: Specify the boundary box coordinates for SEER reference map

Step 4: Click **Update SEER**

Morph Mode

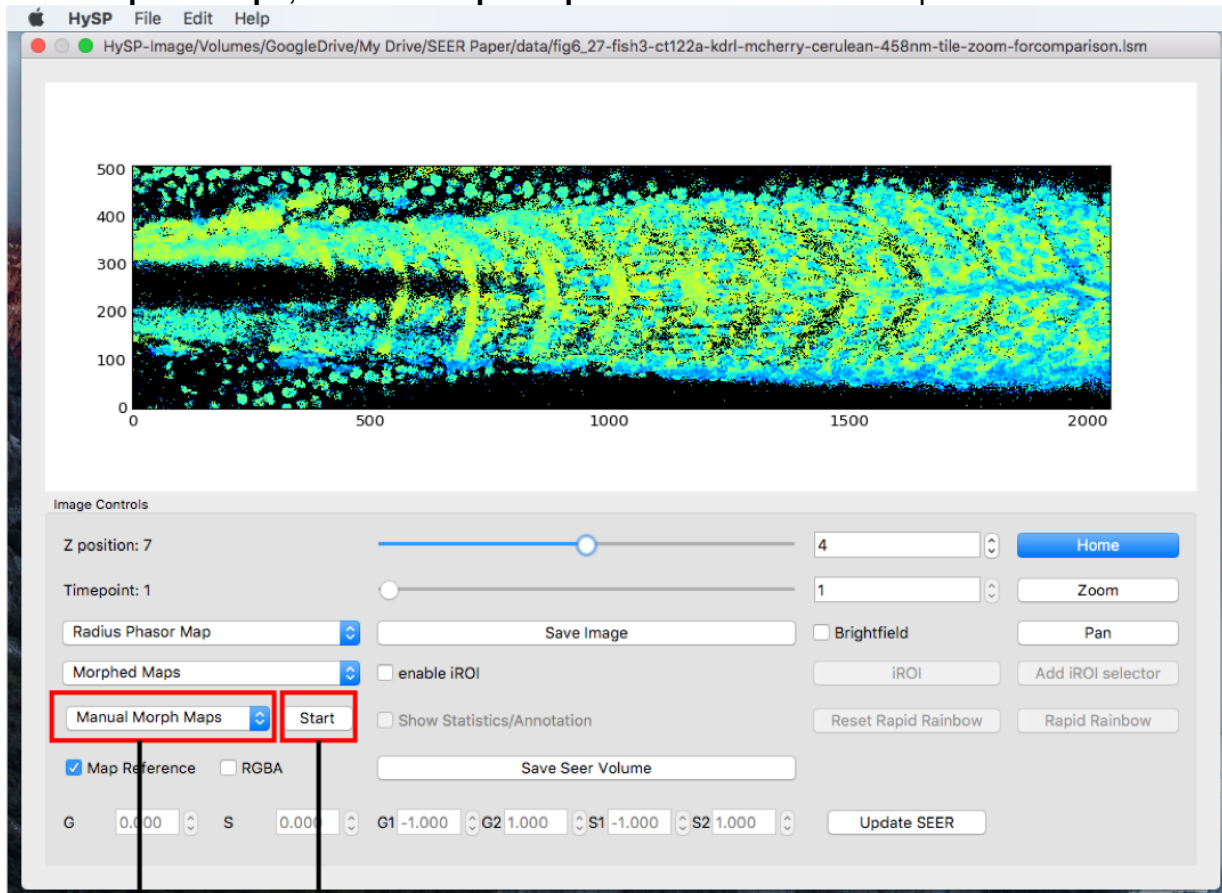
Under Morph mode, there are Max Morph mode, Mass Morph mode, and Manual morph mode. As an example, selecting **Morphed Maps** in the “Mode” dropdown menu will activate the different Morph modes in the drop down right below.

SEER sub-modes for Morph Mode



Manual Morph mode

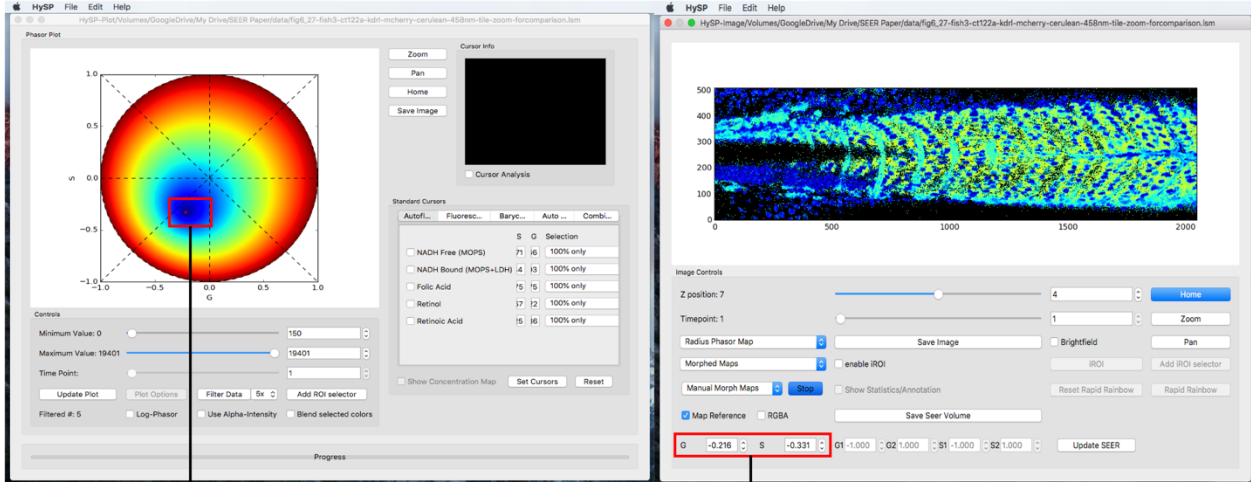
Select **Morphed Maps**, **Manual Morph Maps** to activate manual morph mode. Click **Start**.



Step 1: Select **Manual Morph Maps**

Step 2: Click **Start**

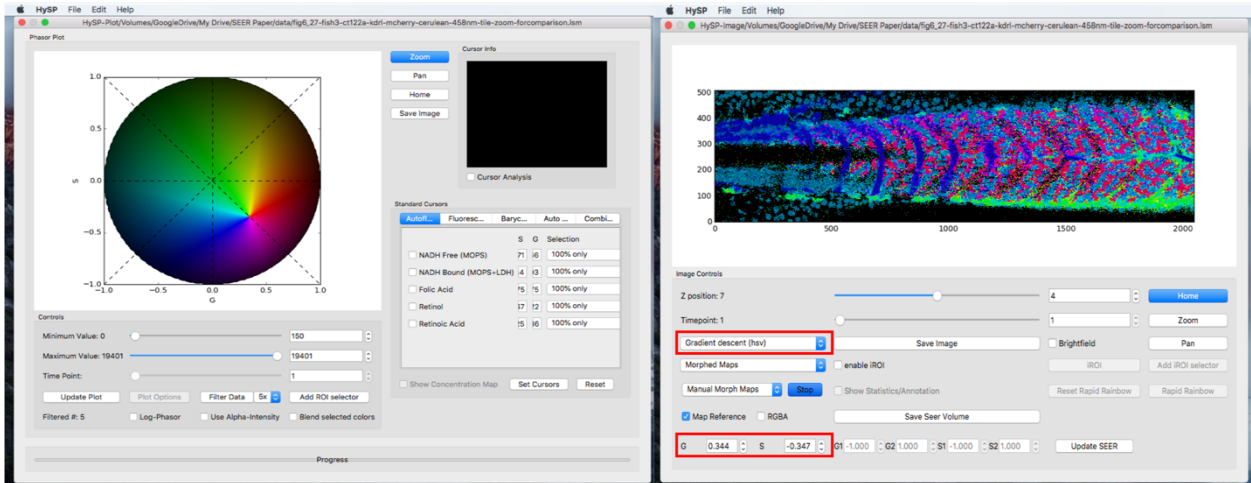
Mouse double click on phasor plot to morph the map reference to a new center, (G, S) coordinate of this new center is also updated when mouse released.

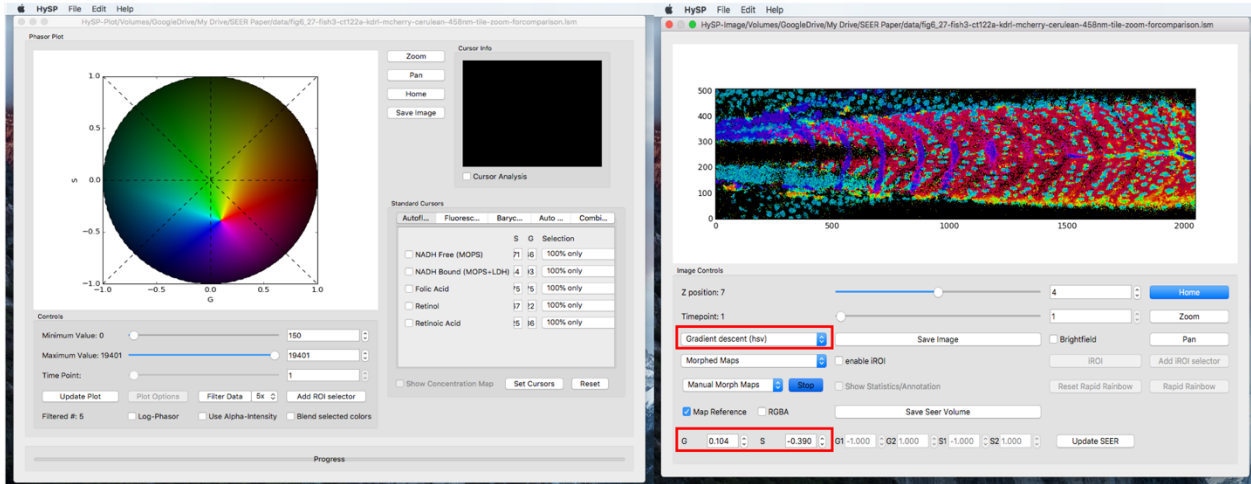


Step 3: Double Click red dot on phasor plot to shift the reference map to a new center

Phasor coordinates of the new center are updated accordingly

To explore the dataset, one can always switch to another map under any mode and sub-mode.



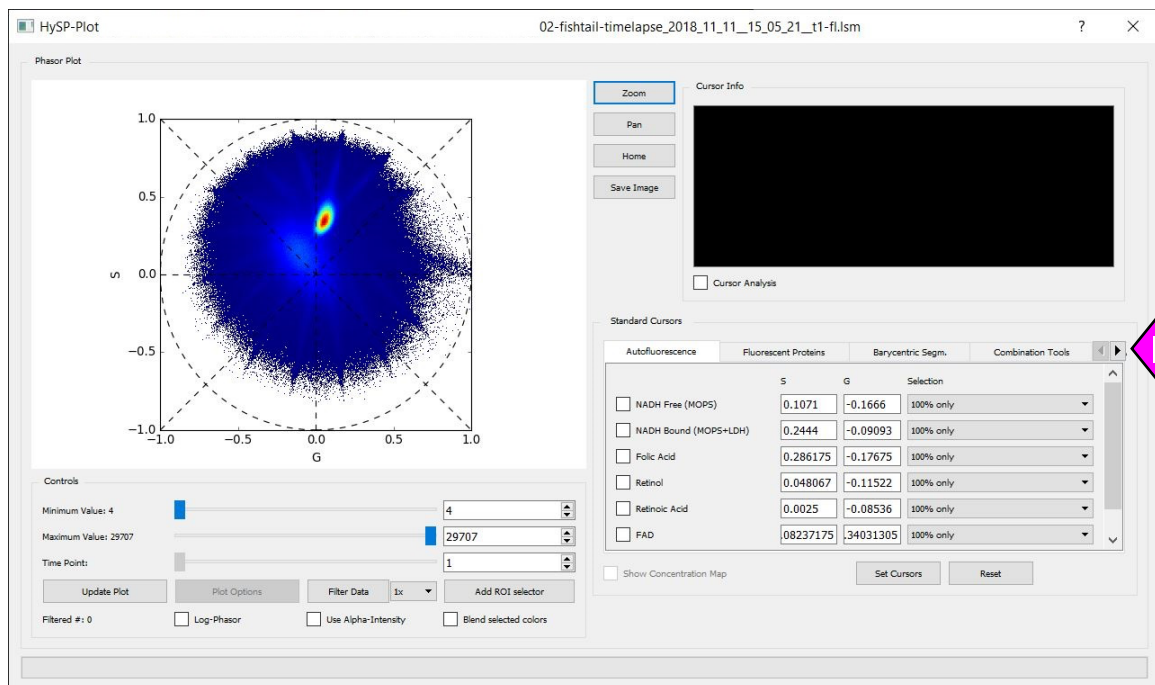


Save volume image

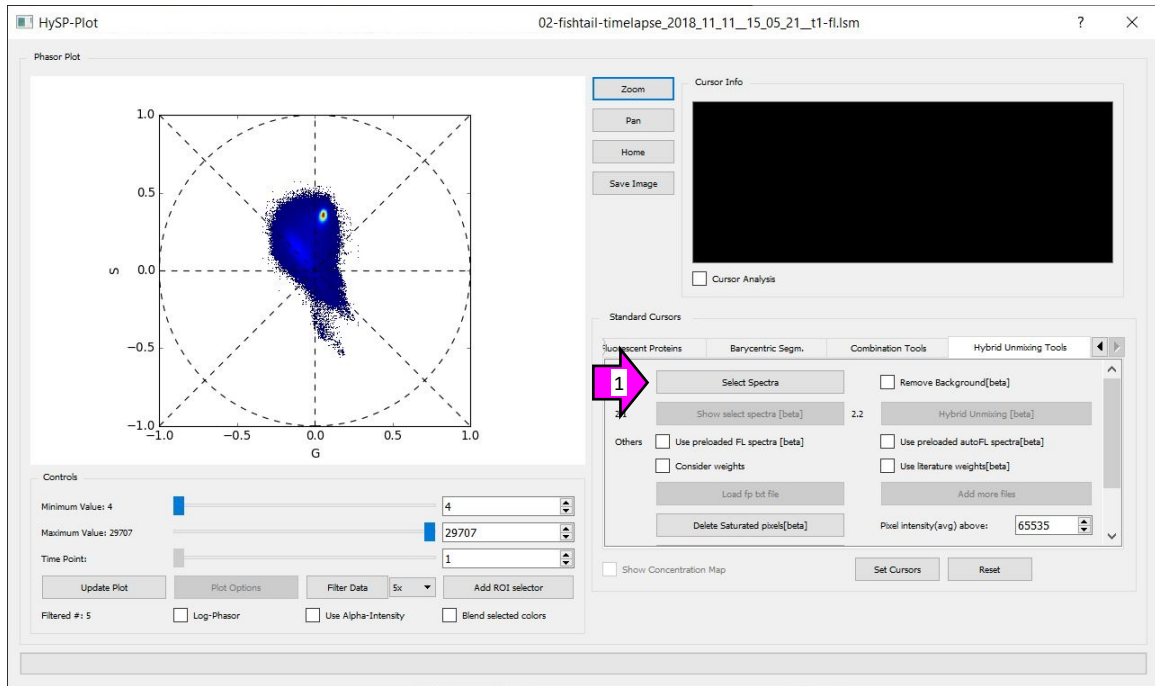
Click **Save Seer Volume** will yield a series of Tiff files in a name required folder for future processing, i.e. 3D rendering.

Hybrid Unmixing (HyU)

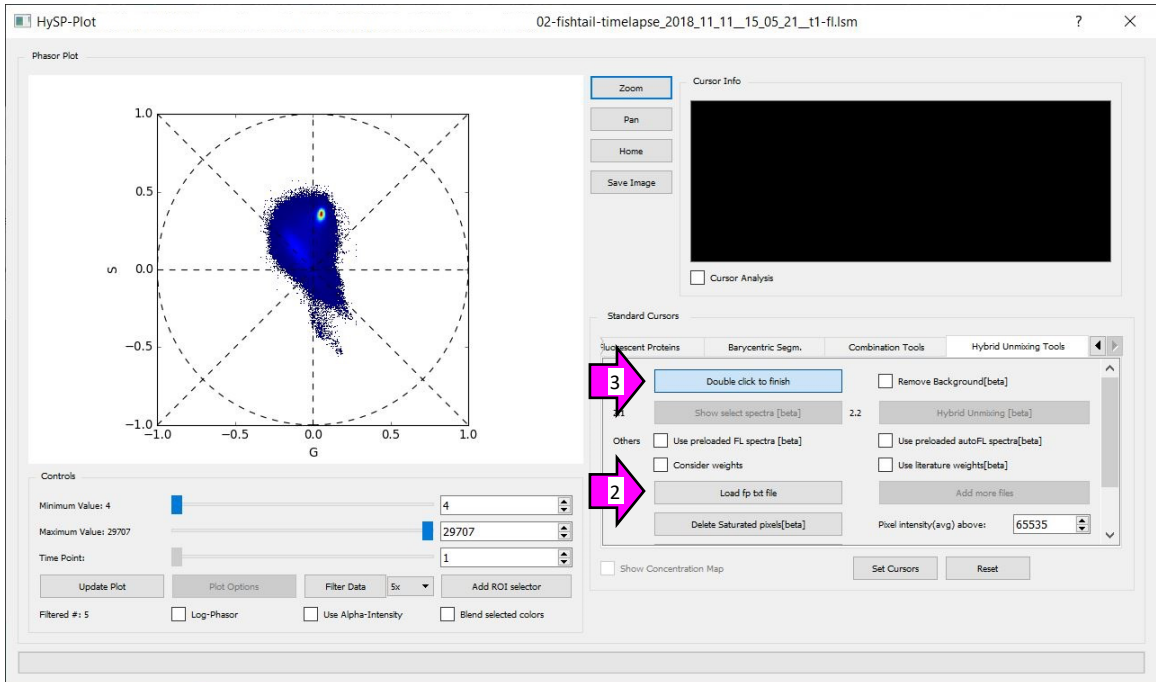
To use HyU, first navigate to the Hybrid Unmixing tab: **(1)** Click on the rightward arrow next to the “Combination Tools” tab twice until the Hybrid Unmixing tab appears. Then select the Hybrid Unmixing tab.



Unmix hyperspectral dataset. **(1)** In the Hybrid Unmixing tab, click the “Select Spectra” button and wait for the progress bar on the bottom of the window to finish; the “Select Spectra” should turn into a “Double click to finish” button.



(2) Then click the “Load fp txt file” button. Select the text file with the spectra values. **(3)** After selecting the text file, click the “Double click to finish” button; the “Hybrid Unmixing [beta]” button should become active.



(4) Finally, click the “Hybrid Unmixing [beta]” button. The unmixed files should be created in the same directory as the input file.

