

Spectrally Encoded Enhanced Representations (SEER) on HySP

Overview

SEER is a pre-processing visualization approach for hyper- and multi-spectral fluorescent images. The technique is incorporated in the HySP software platform, a multi-platform software for analysis of multi-dimensional spectral data. SEER provide rapid enhanced visualization of spectral datasets, starting from fluorescent data. 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 system noise.

System Requirements

Currently SEER-HySP are packaged in Windows7 64bit or later and MacOSX 64bit versions.

The software has been tested on Windows 7, Windows 10, MacOS 10.11, MacOS 10.13, MacOS 10.14.

RAM requirement depends on the size of data. Performance will be dependent on the number of CPU cores available on the system. There is no further non-standard hardware requirements.

Installation Guide

Win64:

Double click .msi installer and follow wizard instructions
(executable should be in ../Program Files/HySP/HySP.exe)

MacOS:

Double click on .dmg will load an installation volume. Drag-drop the .app to the application folder.
(.app should be in ../Applications/HySP-x.y.z.app)

Typical installation time for a “normal” desktop computer should be less than a minute.

Demo

Step 1: Download and install HySP (latest version is always on our [webpage](#))

[MacOS version](#)

[Win64 version](#)).

Step 2: Datasets from our publications are available on our webpage in the Sample Datasets section (<http://bioimaging.usc.edu/software.html#sampledatasets>). Download any dataset.

Software currently accepts .lsm files and Tiff files.

.lsm files need to contain a lambda-mode acquisition with multiple channels.

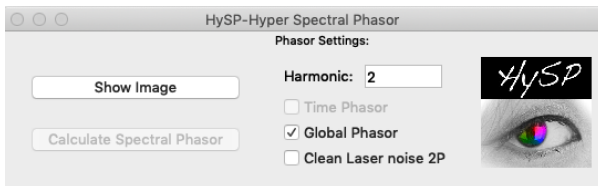
Tiff files can be single or multi-layer tiff, with at least 3 channels. If Tiff files are used, they should be stored in the same folder. Our simulation data [[LINK](#)] is compressed in a zip file, in this case extract zip file before loading tiffs in software.

Step 3: Open the HySP application

(../Program Files/HySP/HySP.exe for windows

or

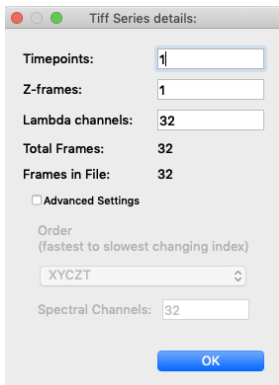
../Applications/HySP for MacOS) and click on “Show Image”.



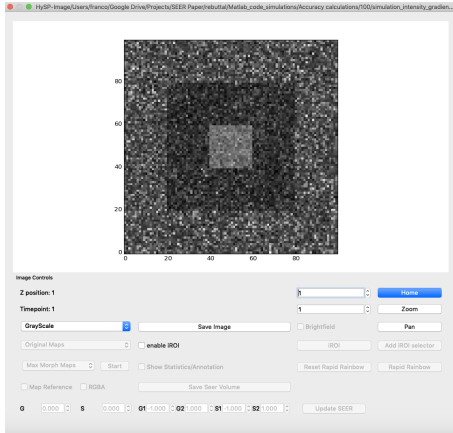
Navigate to the folder with the datasets and select one of the sample datasets.

If file is .lsm, the dataset will be loaded directly.

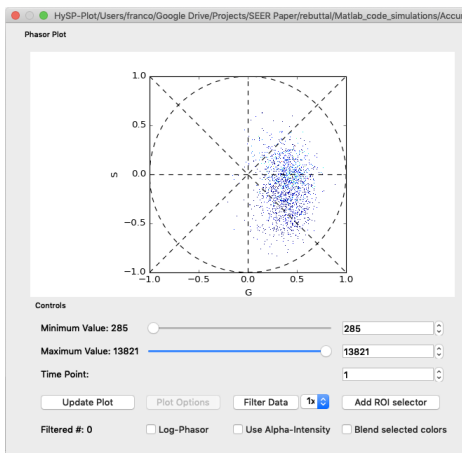
If files are in tiff format, a secondary window will appear. Here we show an example with multilayer tiffs with 32 channels. After selecting one (in this case) multilayer tiff file, this prompt will appear:



this is the importer for tiff files and should list a good guess of the data shape. In this case it's a single multilayer tiff file with 32 channels inside so it is correct. Click OK and a new Image window showing the average grayscale image should appear.

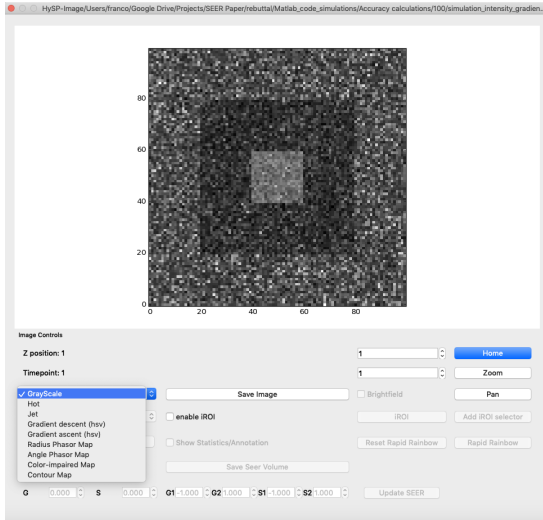


Step 4: Calculate Phasor. Click on the “Calculate Spectral Phasor” button which is now active in the main initial window. A new window with the Phasor plot will appear.

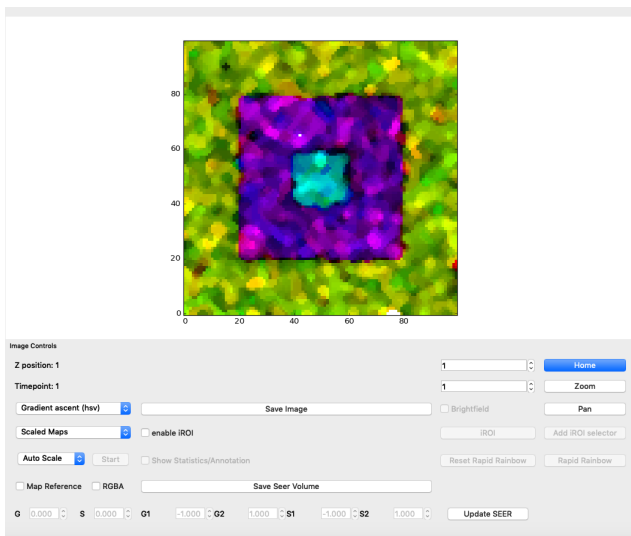


Select the multiplier next to “Filter Data” and set it to 5x. Click on “Filter Data”.

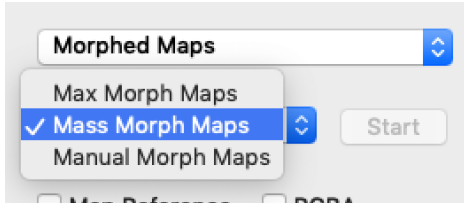
Step 5: Visualize SEER. Return to the Image window and on the bottom left click on the dropdown menu labeled “Grayscale” and the different SEER options of Gradient ascent, descent, radius, angle, and contour will appear. Select “Gradient descent (hsv)”.



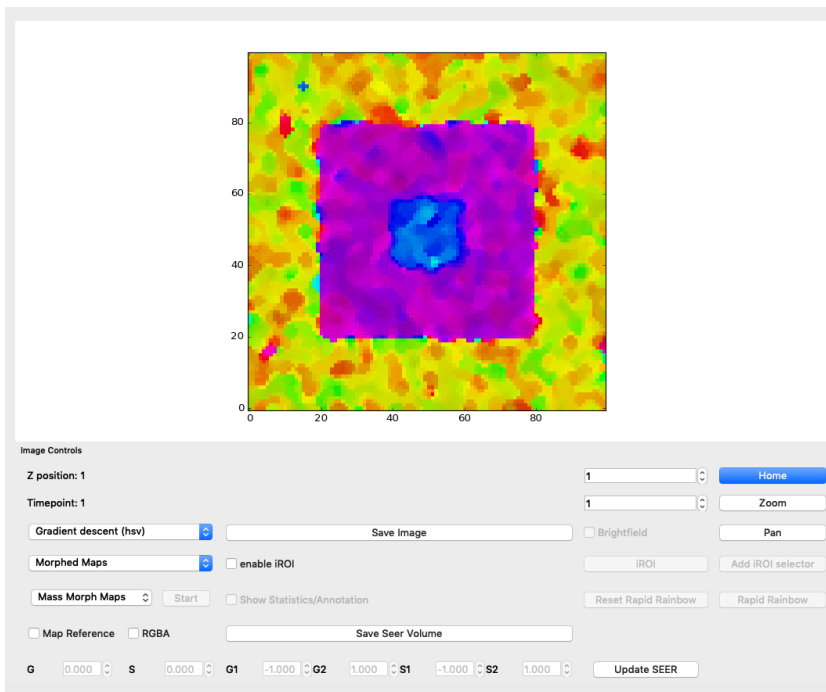
The “Mode” dropdown menu is right below the SEER map menu. The initial value is set as “Original Maps”. Click on it and select “Scaled”. The image will update.



Selecting Morphed Maps in the “Mode” dropdown menu will activate the different Morph modes in the drop down right below.



Expected output: Selecting Mass Morph will yield the result below:



Expected run time for this demo should be a few seconds.

Detailed Instructions for General Use

More detailed instructions on HySP can be accessed on our webpage in the Instructions section (<http://bioimaging.usc.edu/software.html#instructions>).

License

Our software follows the University of Southern California and Translational Imaging Center Software License is available here <http://bioimaging.usc.edu/license.html>