

## Hybrid Unmixing (HyU) on HySP

### Overview

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 Poisson noise.

### System Requirements

We currently have 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 are 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:

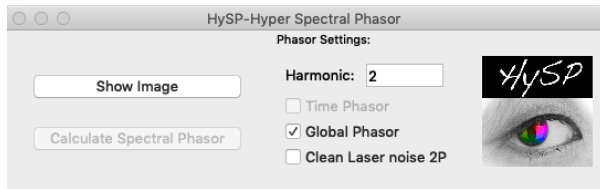
Step 1: Download and install HySP ( latest version is always on our [webpage](#))

[MacOS version](#)

[Win64 version](#)).

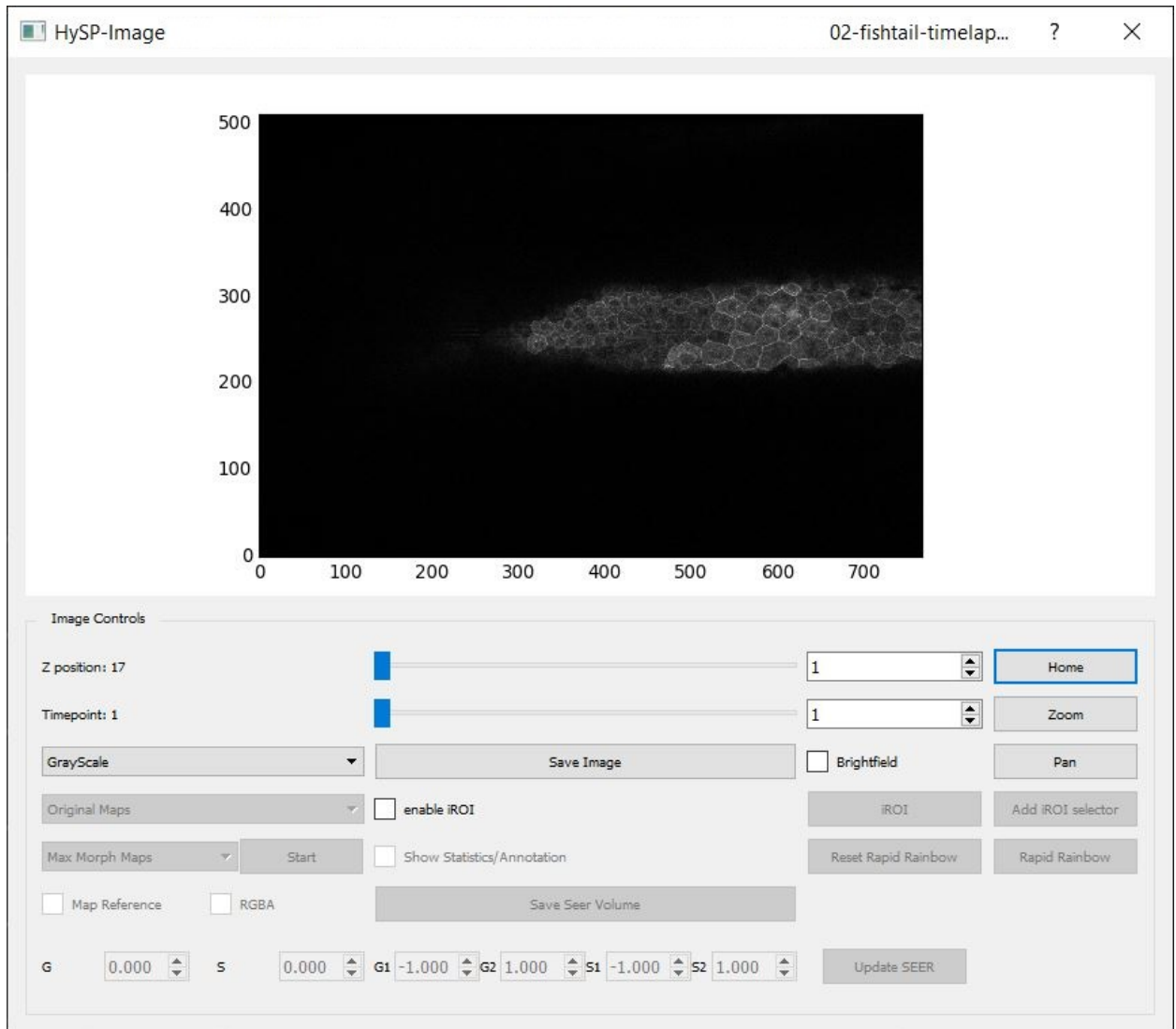
Step 2: Datasets in our publications are available on our webpage (<https://bioimaging.usc.edu/software.html#sampledatasets>), download datasets. Software currently accepts .lsm files and Tiff files. .lsm files are acquired in lambda mode with multiple channels. If Tiff files are used, they should be stored in a folder. Simulation data might be 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 and ../Applications/HySP for MacOS) and click on “Show Image”.

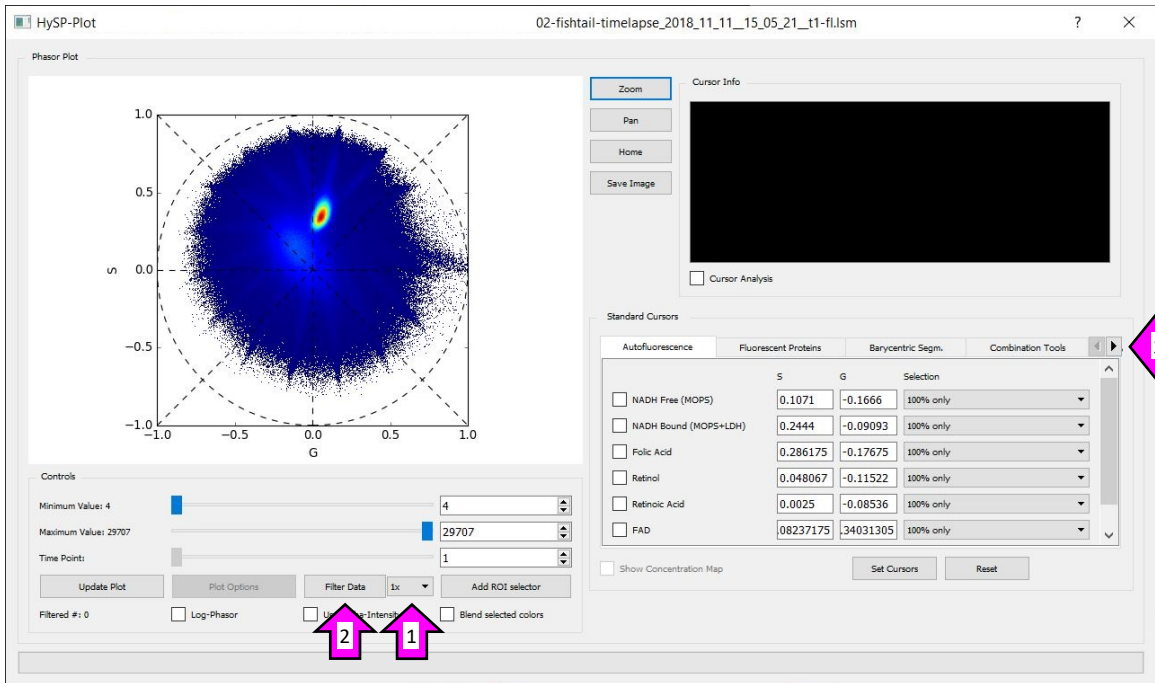


Navigate to the folder with the dataset and select the multispectral LSM file.

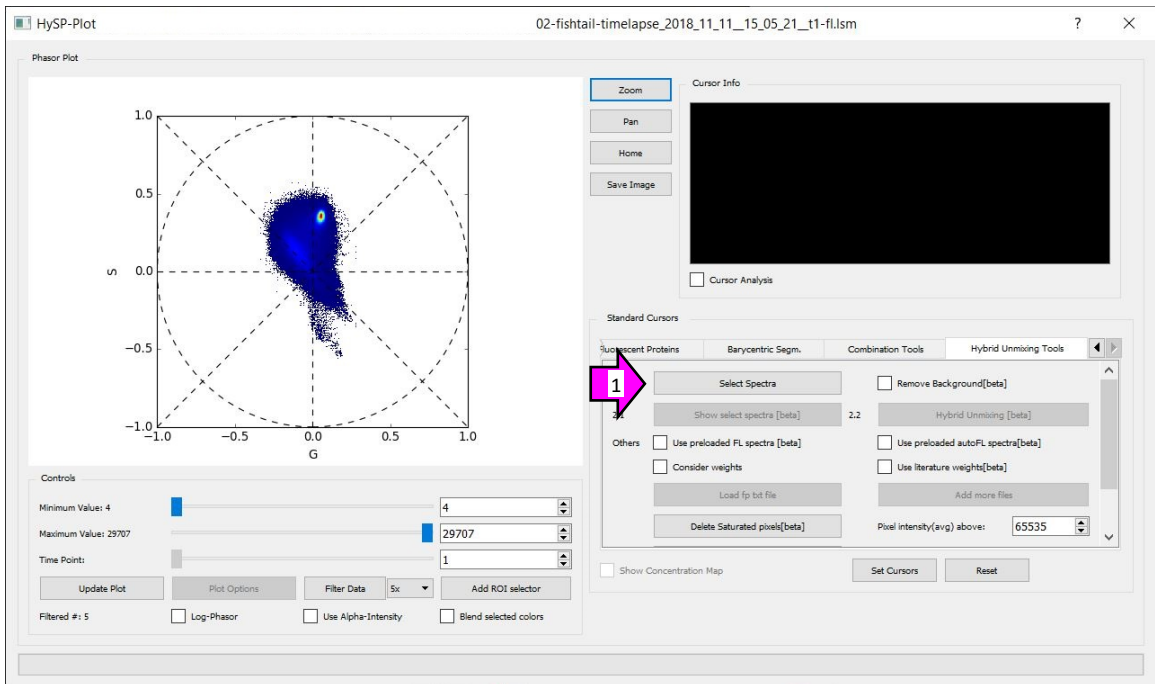
A new Image window showing the average grayscale image should appear.



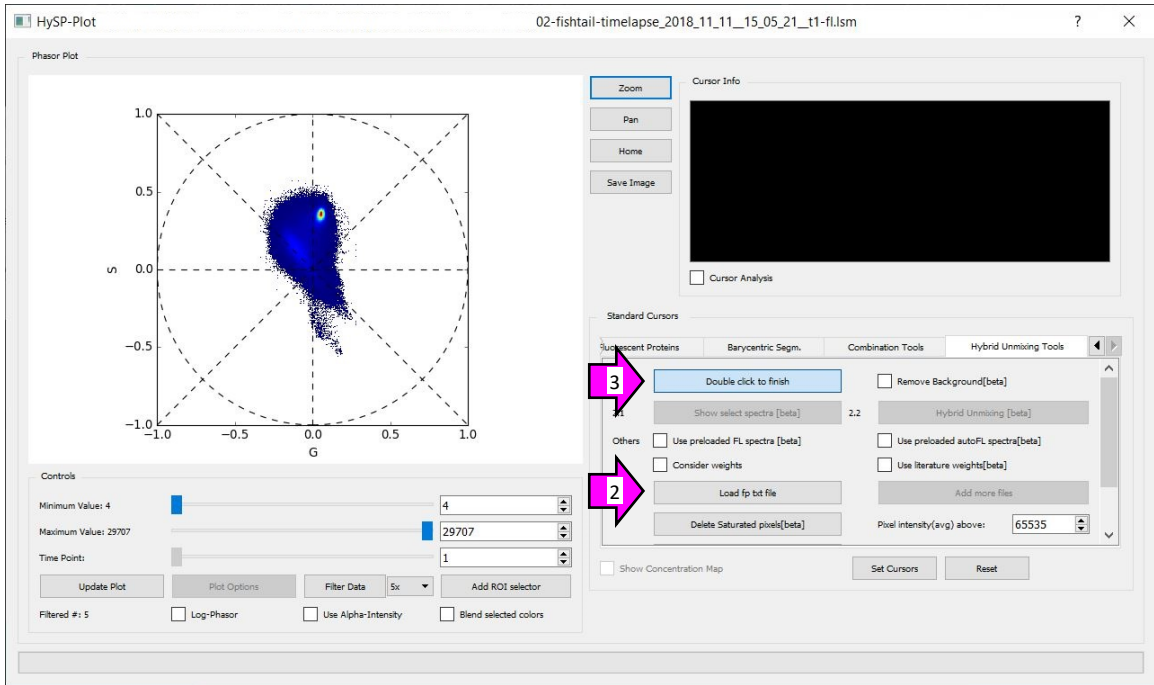
Step 4: Calculate Phasor. Click on the “Calculate Spectral Phasor” button which is now active in the main initial window. A new window with the Phasor plot will appear.



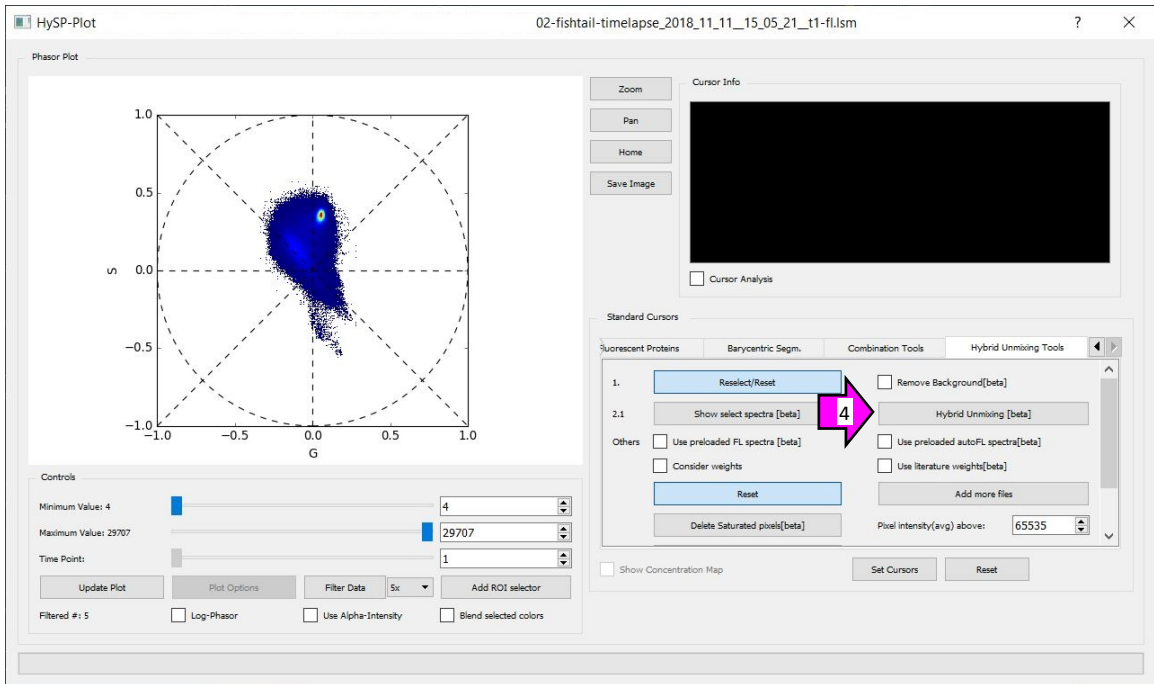
Step 5: Filter Phasor and go to Hybrid Unmixing tab. (1) Select the multiplier next to “Filter Data” and set it to 5x. (2) Click on “Filter Data”. (3) Click on the rightward arrow next to the “Combination Tools” tab twice until the Hybrid Unmixing tab appears. Then select the Hybrid Unmixing tab.



Step 6: 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.

**Expected run time for this demo should be a few minutes.**

### **Instructions for Use**

More detailed instructions on HySP can be accessed on our [webpage](#).

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Publications and conference proceedings that use the software shall reference it as “Hyper-spectral phasor analysis enables multiplexed 5D in vivo imaging, Cutrale, Trivedi et al, Nature Methods 2017” and explicitly reference use of the software in the publication.

### **Citation**

If you use HySP for your work or publication, please cite it as:

Hyperspectral phasor analysis enables multiplexed 5D in vivo imaging

Francesco Cutrale, Vikas Trivedi, Le A Trinh, Chi-Li Chiu, John M Choi, Marcela S Artiga & Scott E Fraser

Nature Methods 14, 149–152 (2017) doi:10.1038/nmeth.4134

If you use SEER for your work or publication, please cite it as:

Pre-processing visualization of hyperspectral fluorescent data with Spectrally Encoded Enhanced Representations

Wen Shi, Daniel E. S. Koo, Masahiro Kitano, Hsiao J. Chiang, Le A. Trinh, Gianluca Turcatel, Benjamin Steventon, Cosimo Arnesano, David Warburton, Scott E. Fraser & Francesco Cutrale

Nature Communications Vol 11, Article num: 726 (2020) doi: 10.1038/s41467-020-14486-8