

# Installation

To run **GSLab.mlapp**, ensure the scripts folder is located in the same directory as the .mlapp file. Follow these steps to set up and launch the program for the first time:

1. Open MATLAB and navigate to the **Home** tab.
2. In the **Environment** section, click **Set Path**.
3. In the **Set Path** window, click **Add with Subfolders**.
4. Browse to the directory containing GSLab.mlapp and the scripts folder, then click **Select Folder**.
5. Click **Save** in the **Set Path** window and then click **Close**.

To launch the program, type GSLab in the MATLAB command window.

Once the path has been saved in MATLAB, simply type GSLab in the command window to run the program in subsequent sessions.

## User Manual

The program uses two windows, the main control GSLab window and the Image window. The GSLab window features a top panel displaying the phasor plot and a bottom panel organized into seven tabs, each providing access to specific functionalities. The Image Window is dedicated to rendering all images loaded into the software.

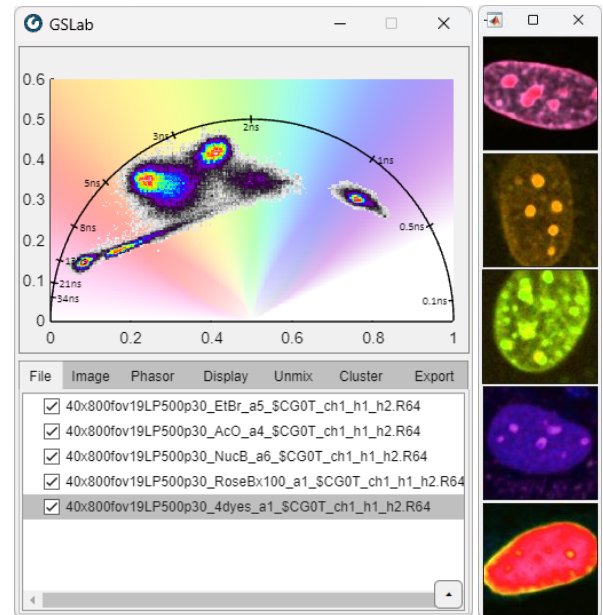
### Phasor Plot panel

This is the main panel where phasor plots are displayed. Hovering the mouse over the phasor space will show a circular cursor the size of which is controlled by the mouse scroll. The central coordinates of the current cursor location are displayed on the top left. If a file is loaded, the pixels whose phasor transform is within the cursor limits will be rendered in the color of the cursor. If the user clicks on the phasor space the cursor position is fixed and a new cursor with a different color is used as mouse pointer. If one or more cursors are on the phasor plot a button with a red X appears in the top-right corner that clears all cursors on the phasor plot.

### File tab

Loaded files are listed in the main panel. The checkboxes in front of each file name allow the user to include/exclude each loaded file from the representation and analysis.

- Contextual menu options (right-click)
  - Load menu: load files into the program.
    - Tif sequence : prompts user to select tiff file(s).
    - Falcon SnG: prompts user to select folder containing trios or quartets of tifs exported from the Leica Falcon as S and G coordinates using 'Save phasor GS - Fixed Range' in LASX.
    - Zeiss lsm: prompts user to select lsm file(s).
    - SimFCS ref: prompts user to select ref or r64 file(s).
    - Saved Session: loads a previously saved session including files and settings
  - Save as SimFCS ref: generates a phasor coordinates file with the first two harmonics.
  - Save current session: prompts user to browse location and input file name to save current settings and paths to loaded files.
  - Move top/up/down/bot: Moves the selected file to the desired position in the list to change the arrangement in the display window and in exported images.
  - Check All/None/Invert: Checks all or no or inverts current selection of files from the list for their display and analysis.
  - Calibration File: Sets the selected file as calibration. Will obtain the photon weighted average coordinates of the phasor distribution and assign it the lifetime in the "Calibration Lifetime" box in the Calibration Panel.
- Calibration Panel button: folds/unfolds the calibration panel.
  - Apply checkbox: apply the current calibration to the loaded files or not.
  - File/Manual radio button: allows the user to set calibration based on selected files or on manual input of phase shift and modulation factor.
  - File mode: the calibration panel table appears with three columns.
    - The first column is the text tag of the calibration file. By default it is 'All' meaning all files, but if user changes that to a specific text string, only files who's name contains the specified string will be calibrated using the selected calibration file.
    - The second is the lifetime associated with the file. This value can be changed by the user at any time.
    - The third is the filename that is used for the calibration.
  - Clear button: clears all calibration files.
  - Manual mode: Two boxes appear:
    - Phase shift: applies the inputted phase shift to the data (in radians).
    - Modulation factor: applies the inputted modulation factor to the data (normalized to unity).
  - Calibration Lifetime box: User can input the lifetime of the next file that is to be set for calibration.



GSLab window and Image window.

## Image tab

This tab contains the image manipulation parameters and settings for rendering the intensity images prior to phasor transform.

- Numeric input boxes set intensity lower and upper bounds for image rendering.
- Slide bar also sets the lower and upper bounds for image rendering.
- Relative checkbox: indicates if the intensity values are relative to the dynamic range of each file or are the thresholds are to be set in absolute intensity units.
- Intensity histogram panel shows the intensity histogram of each loaded file.
- Log plot checkbox: plot the intensity histogram in logarithmic scale. This option is useful when you have pixels with low counts and others with very high counts.
- Log intensity checkbox: Render images in logarithmic intensity to compensate high dynamic range images.
- HDR intensity checkbox: Render images using a pixel-wise local dynamic range to compensate high dynamic range images.
- Excess to zero checkbox: Excludes pixels that are being saturated by the upper bound of the intensity thresholding.
- Threshold/cursors dropdown:
  - Threshold: masks will be created based on intensity thresholds.
  - Cursors: masks will be created based on cursors set on the phasor plot.
- To mask: Create a mask using the dropdown selection.
- Save mask button: Save current masks to file (png if single file or stacked tiff if multiple files).
- Load mask button: Load binary image to use as mask. If a single image is loaded it will be applied to all files in memory, if a stack of tiffs is loaded, each slice will be sequentially applied to each loaded image. Images created in other softwares can be uploaded and used to segment the images for phasor analysis.
- Apply mask checkbox: Apply or not the current mask to the loaded data.
- Invert checkbox: Invert the current mask.
- Generate RGB: Options only available for FLAME files.
  - 3Chan button: generate a 3 chan image taking the first 4/32 bins for red, the rest for green and second channel for blue.
  - 4Chan button: generate a 4 chan image taking the first 4/32 bins for red, the rest for green and the first 1/32 for blue and the rest for cyan.

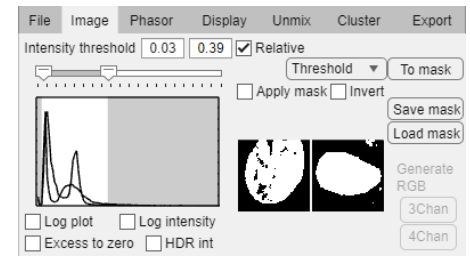
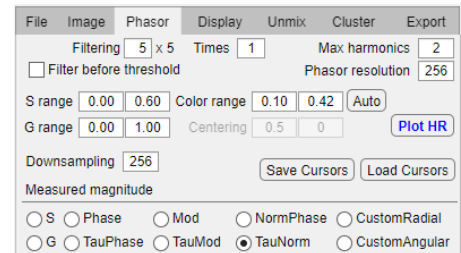


Image tab

## Phasor tab

The phasor tab includes all parameters affecting the phasor transform computation and the associated measurements.

- Phasor filtering box: Set the size of the median filter kernel.
- Times box: Number of times the filter should be applied.
- Filter before threshold checkbox: Apply filter before or after thresholding the image.
- S range boxes: Lower and upper limits of the S axis for displaying the phasor plot.
- G range boxes: Lower and upper limits of the G axis for displaying the phasor plot.
- Color range boxes: Lower and upper limits for the color range (in units of a whole turn for angular quantities and in phasor units for radial quantities).
- Centering boxes: G and S coordinates of the center of a custom measuring magnitude.
- Auto button: Automatically set the color range based on the contents of the phasor plot.
- Max harmonics box: Set the number of harmonics to compute when loading data.
- Phasor resolution box: Number of bins to use in the phasor space for computing the phasor plot.
- Downsampling box: Size of low-resolution images. Set to 0 for no downsampling.
- Plot HR button: Compute phasor plot for High resolution images.
- Save Cursors button: Save the current cursor position, dimensions and colors in a file.
- Load Cursors button: Load a file containing cursor positions, dimensions and colors.
- Measured Magnitude radio buttons:
  - S: Generate a color gradient based on S coordinate.
  - G: Generate a color gradient based on S coordinate.
  - Phase: Generate a color gradient based on polar phase.
  - TauPhase: Generate a color gradient based on polar phase and compute the associated lifetime.
  - Mod: Generate a color gradient based on polar modulation.
  - TauMod: Generate a color gradient based on polar modulation and compute the associated lifetime.
  - NormPhase: Generate a color gradient based on pseudo-phase.
  - TauNorm: Generate a color gradient based on pseudo-phase and compute the associated lifetime.
  - CustomRadial: Generate a custom radial color gradient (center coordinates need to be specified).
  - CustomAngular: Generate a custom angular color gradient (center coordinates need to be specified).

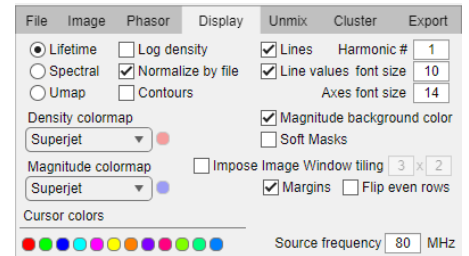


Phasor tab

## Display Tab

The display tab includes all options that are related to phasor plot style and formatting.

- Phasor space radio button:
  - Lifetime: Sets the S and G ranges appropriate for lifetime phasor and adds universal circle lines.
  - Spectral: Sets the S and G ranges appropriate for spectral phasor and removes universal circle lines.
- Log density checkbox: Plot phasor density in log scale enhancing bins with low counts.
- Normalize by file checkbox: The phasor distribution density range of each loaded file is normalized so all files appear in similar color range regardless of the spreading of the data.
- Contours checkbox: Adds contour lines for lower counts in the phasor plot.
- Density colormap dropdown: Allows choices of different color schemes for the phasor density heatmap (if last option "single color" is selected the button to select color is enabled).
- Magnitude colormap: Allows choices of different color schemes for the phasor-based measurement (if last option "single color" is selected the button to select color is enabled).
- Cursor colors panel: Each colored button corresponds to the successive cursors that can be used, click on the buttons to open up the color palette and change the color of the cursor.
- Lines checkbox: Draws the universal semicircle for lifetime or the unity circle for spectral.
- Line values checkbox: Adds ticks of selected lifetimes on the universal semicircle depending on excitation source frequency.
- Line values font size box: Choose the font size for lifetime values displayed.
- Axes font size box: Choose the font size for the S and G axes.
- Harmonic # numeric box: Plot the phasor in the corresponding harmonic number (if higher than 1 it will show in red as a warning).
- Magnitude background color checkbox: Plot the color gradient according to the measured magnitude selected in the Phasor tab.
- Soft masks checkbox: Overlays cursor/magnitude colors on the intensity window as a soft mask (colored intensity image) or hard mask (just the colors regardless of intensity).
- Impose image window tiling checkbox: Force tile arrangement in the intensity window.
- Impose image window tiling numeric boxes: Set the number of rows and columns for displaying the loaded files.
- Margins checkbox: Add/remove margin between images in the intensity window.
- Flip even rows checkbox: Arranges tiles in zig-zag to account from some mosaic stage paths.
- Source frequency numeric box: Set the lifetime excitation laser frequency for the phasor transform trigonometric period.

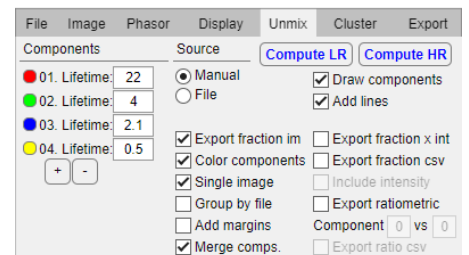


Display tab

## Unmix tab

This tab includes all options for defining pure components to run the multi-harmonic multi-component phasor unmixing routines.

- Compute LR button: Computes unmixing and generates the selected exporting options using the low resolution versions of the loaded images.
- Compute HR button: Computes unmixing and generates the selected exporting options using the high resolution versions of the loaded images.
- Source radio buttons:
  - Manual: Use the Components panel to manually typed in lifetimes of pure single-exponential components for the unmixing.
  - File: Select loaded files as empirical components for the unmixing.
- Components panel (Manual mode):
  - (+/-) buttons: Add/remove a component for the unmixing.
  - Colored circle button: Open up the color palette to select the color assigned to the component.
  - Lifetime numeric box: Input the lifetime of the component.
- Components panel (File mode): The loaded files list is shown and individual files can be checked to use as a empirical component. The photon-weighted average phasor coordinates is computed for each checked file and the color selected in the manual component panel is assigned to each file.
- Draw components checkbox: Plots the position of the unmixing components.
- Add lines checkbox: Draws lines between consecutive components in the phasor plot.
- Export fraction im. checkbox: When enabled exports each component as an image and raw data in different formats:
- Color components checkbox: Export fractional intensity images based on the color selection in the components panel.
- Single Image checkbox: Groups into a single image the output of the unmixing instead of individual images for each file and each component separately (uses tiling rows/columns in Display tab).
- Group by file checkbox: Creates a single image with all the components for each file or creates a single image for all the files for each component (uses tiling rows/columns in Display tab).
- Add margins checkbox: Adds/removes margin between the tiles in the composed images.
- Merge components checkbox: Creates a merge image based on the color selection of the components tab (option enabled when "Group by file" is unchecked).
- Export fractions x int: Export the photon fraction images as absolute number of photons assigned to each component (normalized to the dynamic range of the 8-bit output image) or as relative fractions over unity (again, normalized to the dynamic range of the 8-bit output image).
- Export fraction csv checkbox: The calculated fractional intensity values of all pixels surviving thresholds is exported as a spreadsheet, one column per component.



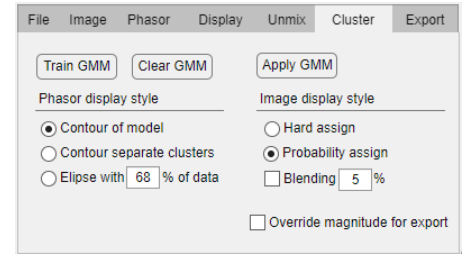
Unmix tab

- Include intensity checkbox: Adds the intensity values for each pixel as the first column of the exported csv file (only available when "Export fraction csv" is checked).
- Export ratiometric checkbox: Computes a ratiometric image using two components  $X/(X+Y)$ .
- Component numeric boxes: Select the component X and Y for ratiometric imaging.
- Export ratio csv checkbox: Export a spreadsheet of pixel calculated ratiometric values.

## Cluster tab

The clustering tab is used to create automated clustering of phasor distributions in the phasor space.

- Train GMM: Taking the data on the phasor plot, a gaussian mixture models is trained using the expectation-maximization algorithm. The user must select the number of clusters to model by the starting locations of the clusters.
- Clear GMM: Clears the current trained model.
- Apply GMM: Re-applies the model to the current displayed data.
- Phasor display style:
  - Contour of model: Plots the agglomerated sum of gaussians as a single contour plot.
  - Contour separate clusters: Plots each clusters gaussian component as a separate contour plot.
  - Ellipse with percentage of data: Plots a single contour for each gaussian such that it contains the percentage of the data set by the text box.
- Image display style:
  - Hard assign: Each pixel is colored with the gaussian component that has a higher posterior probability.
  - Probability assign: Each pixel is colored with a linear combination colors corresponding to the linear combination weights of each gaussian component in the pixel.
- Blending percentage: Applies smoothing to the color masking image to blend regions.
- Override magnitude for export: If checked it will swap the magnitude-colored checkboxes in the export tab for GMM-colored checkboxes in order to allow user to export the resulting images of the clustering.

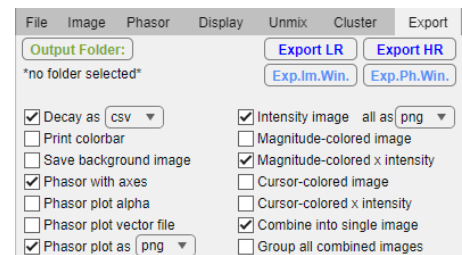


Cluster tab

## Export tab

Options regarding exporting of images and data files.

- Output Folder button: Prompts user to select a path to a folder where data should be saved. The folder name is displayed below.
- Export LR button: Export data using the low-resolution downsampled images for faster computational time.
- Export HR button: Export data using the original resolution images.
- Exp.Im.Win button: Export the current image window contents as an image.
- Exp.Ph.Win button: Export the current phasor plot panel as an image.
- Data export options:
  - Decay as checkbox: For each checked file generate a file with the average intensity in each spectral/lifetime channel.
  - Decay as dropdown: Select the file format.
  - Print colorbar checkbox: Render a colorbar of the measured magnitude color gradient
  - Save background image checkbox: Render a color gradient corresponding to the measured magnitude on the phasor plot.
  - Phasor with axes checkbox: Export the phasor plot with the axes numbers and ticks.
  - Phasor plot alpha checkbox: Export phasor plots as png files with transparency (only if exported separately).
  - Phasor plot vector file: If exporting phasor plots separately a vector file is generated.
  - Phasor plot as checkbox: For each checked file export its phasor plot.
  - Phasor plot as dropdown: Select the file format for phasor plots.
  - Intensity image checkbox: For each checked file export its intensity image in grayscale.
  - Intensity image all as dropdown: Select file format for image exports.
  - Magnitude values as checkbox: Export spreadsheet with pixel intensity and pixel magnitude (radio button in phasor tab)
  - Magnitude values as dropdown: Format for spreadsheet file
  - Magnitude-colored image checkbox: For each checked file export the magnitude-colored image (hard mask style).
  - Magnitude-colored x intensity image checkbox: For each checked file export the magnitude-colored image times the intensity image (soft mask style).
  - Cursor-colored image checkbox: For each checked file export the cursor-colored image (hard mask style).
  - Cursor colored x intensity image checkbox: For each checked file export the cursor-colored image times the intensity image (soft mask style).
  - Combine into single image checkbox: For each checked file join all selected exports into a single file (it will override the phasor plot file format if selected).
  - Group all combined images checkbox: Join all file exports into a single image using the Image Window tile disposition.



Export tab

## Image Window

The Image Window contains the rendered images. Hovering over an image in this window will select the respective file name in the file tab and will display a black cursor on the phasor plot marking the phasor coordinates of the pixel under the mouse pointer.