sFLIM Matlab Pattern Matching Software Short Description

Requirements

Matlab 2019b mit folgenden Toolboxen:

- Image Processing Toolbox
- Parallel Computing Toolbox

A PC with good RAM memory (64 GB or more) and many cores. As more cores the PC has, as faster the processing and calculation time will be.

Program description

The program consists of three parts:

- Preprocessing of the files (run TheProcessor.m)
- Pattern Maching analysis (run sFLIM.m)
- Reload of saved results (Plot_UnmixingResults.m)

Mark the corresponding m-file in Matlab and click F9

For the linear unmixing step, the Pattern Matching analysis, there are two algoritms:

- matrix inversion with non-negative matrix factorization (NNMF). This algoritm is fast but not so precise. The m-files can be found here:
  sFLIM_Pattern_Matching_NNMF_Date

- Linear unmixing using a fitting algoritm. It is more precise.
  The m-files can be found here:
  sFLIM_Pattern_Matching_Fitting_Date

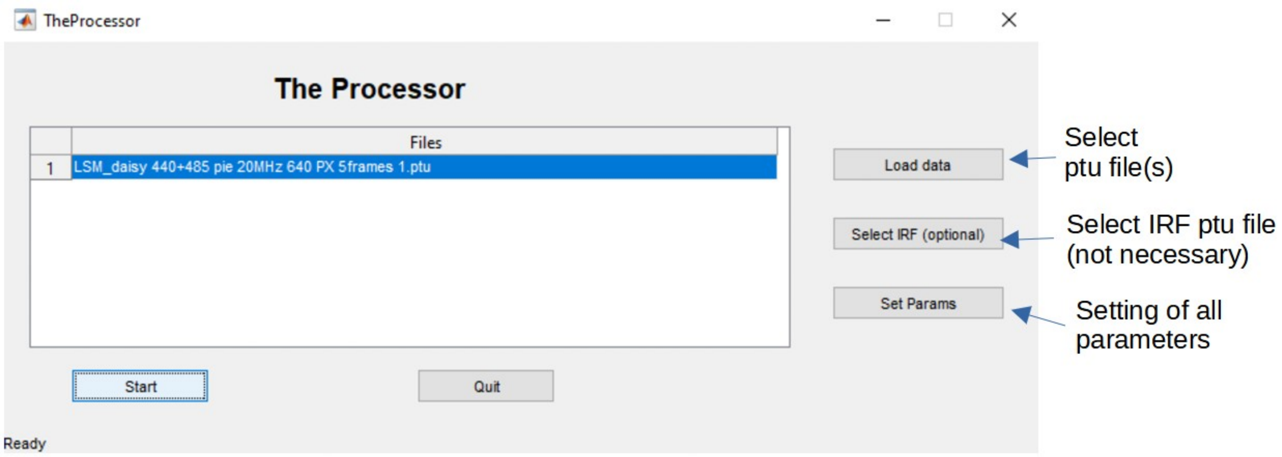
Example Data can be found here:
https://nc.picoquant.com/index.php/s/jxCmkHQWKrcZMXg

Processing of the files

The preprocessing of files generates TCSPC histogramms for each laser pulse and spectral detection channel and for each pixel.

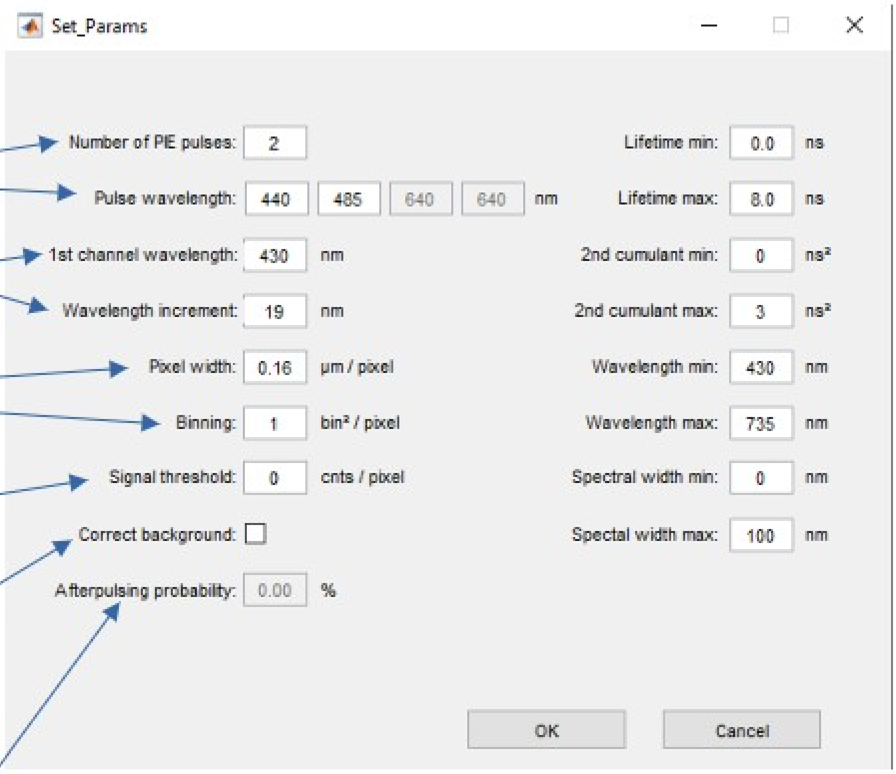
The result are ...DATA.mat and ...FLIM.mat files which can be analyzed with the sFLIM Pattern Matching analysis.

- Several ptu files can be processed in parallel using all cores of the PC
- Optimize the number of files so that they can be distributed on your PC cores
- Each file needs RAM memory, too much file could lead to lower processing speed if RAM memory limit is exceeded
- run TheProcessor.m (mark the m file and click F9)



- select the ptu files
- select the IRF ptu files (this is not mandatory since the program is estimating the IRF from the measured files)
- click on „Set Params“ in case the parameters are not set beforehand (see parameter setting below) and enter all needed parameter
- mark the files in the list which you want to process
- click „Start“
- Once all files are processed, the status „Ready“ on the left lower corner will change to „Done“
  - Hint: you can use the task manager to observe the working of the PC on the files since there is no progress indicator

Setting of parameters



For multi color excitation in Pulsed Interleaved Excitation mode

Spectrograph settings

Image settings
Binning can enhance the number of photons per pixel

Photons per pixel below the entered number will be disregarded → faster calculation

If a substantial background is there it can be removed in the analysis

If the afterpulsing probability is known of your detector you can enter the number. Background created by afterpulsing will then be automatically removed.

Left side parameters are for calculation

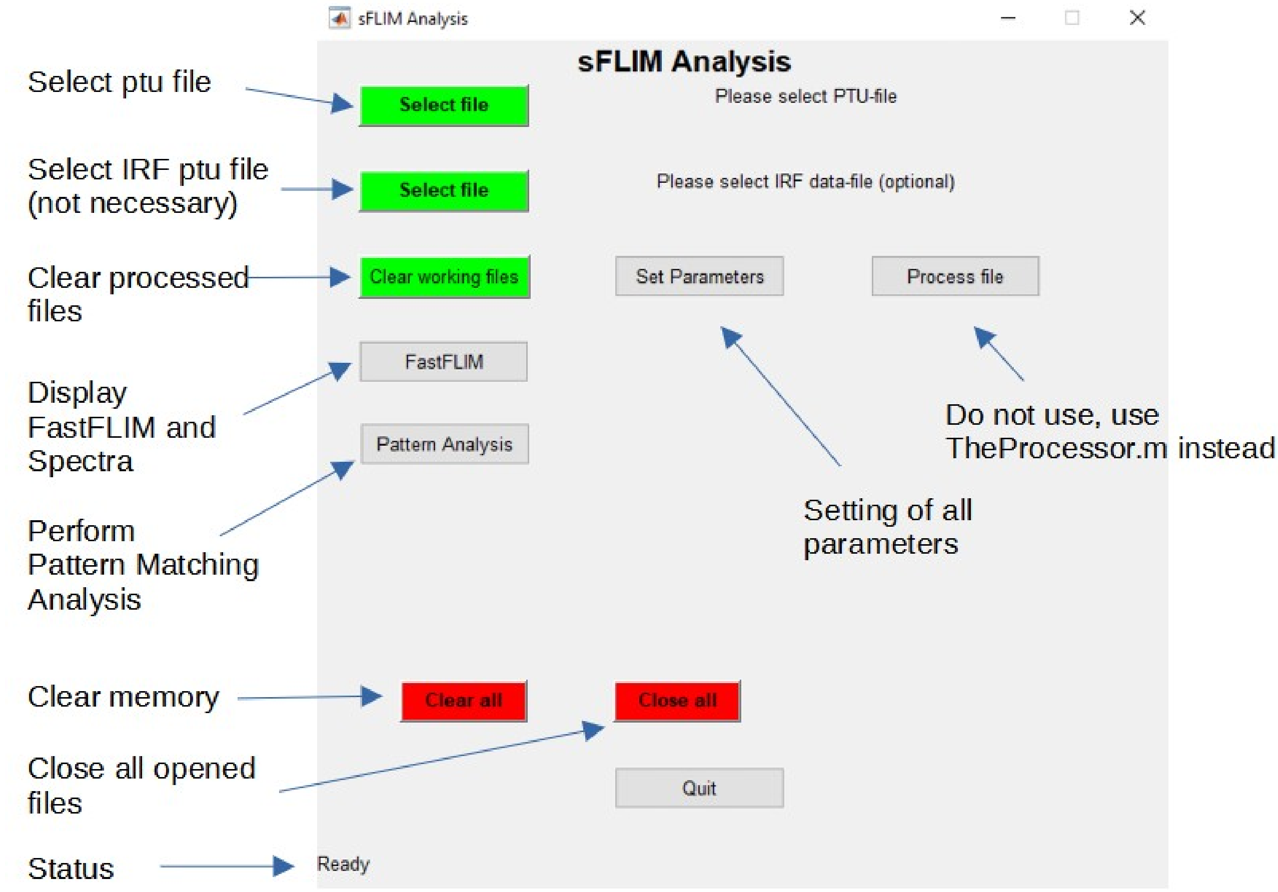
Right side parameters are for the display of images and graphs

Background will be automatically esimated by the mean photon numbers in the first nanosecond of the TCSPC decay of the correponding channels.

In case the afterpulsing probability is given, background will be calculated using this number.

sFLIM Analysis graphical user interface

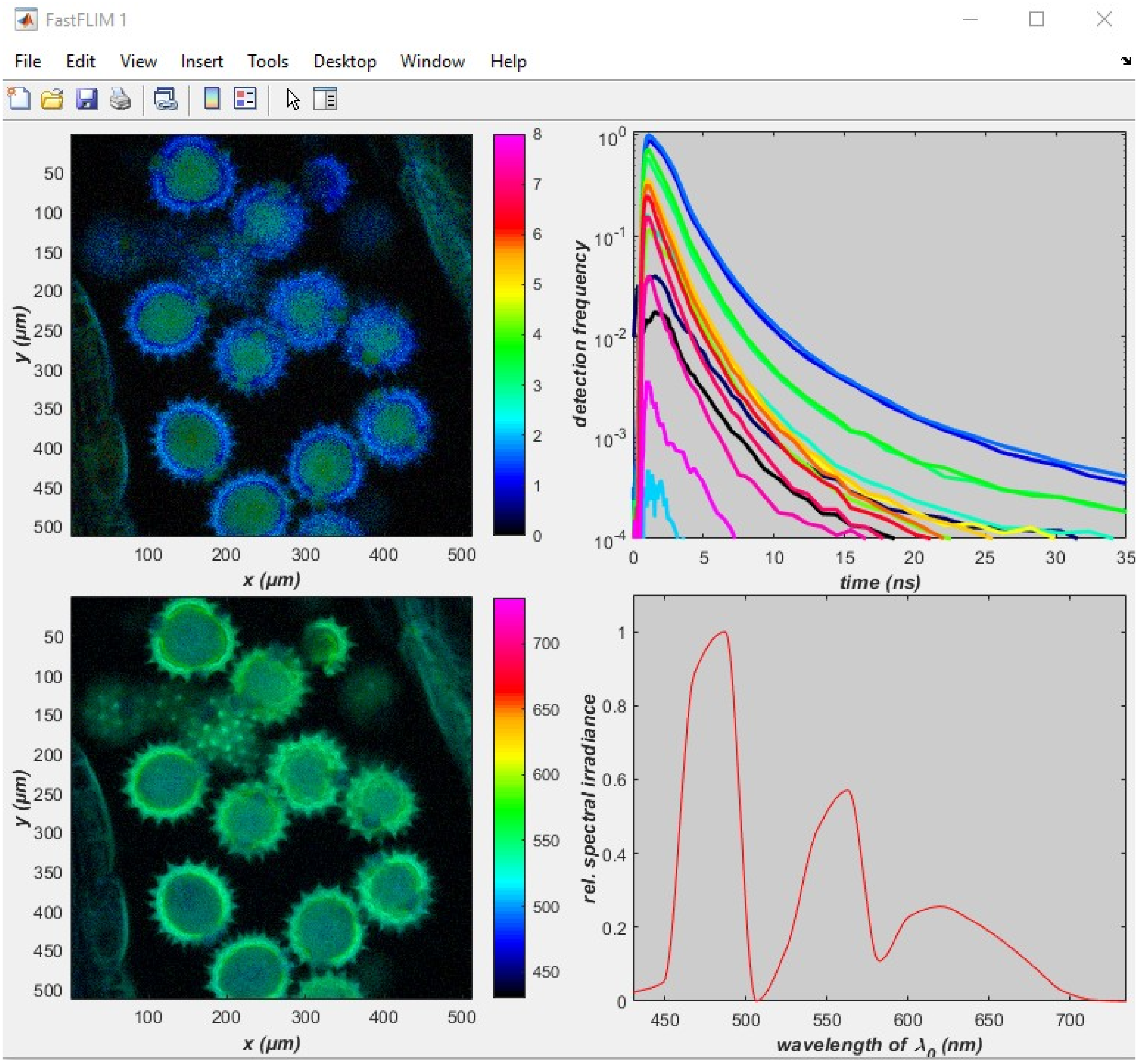
- Process your files using „TheProcessor.m“
- In order to start the sFLIM analysis run „sFLIM.m“ file (marking the file and click on F9)
- Overview of the graphical user interface:



FastFLIM Display

- Select file
- If necessary set parameters
- Click on „FastFLIM“

The FastFLIM display opens the following display for each exciation wavelengths:



Upper left graph displays an average lifetime (in ns) of all spectral channels summed together

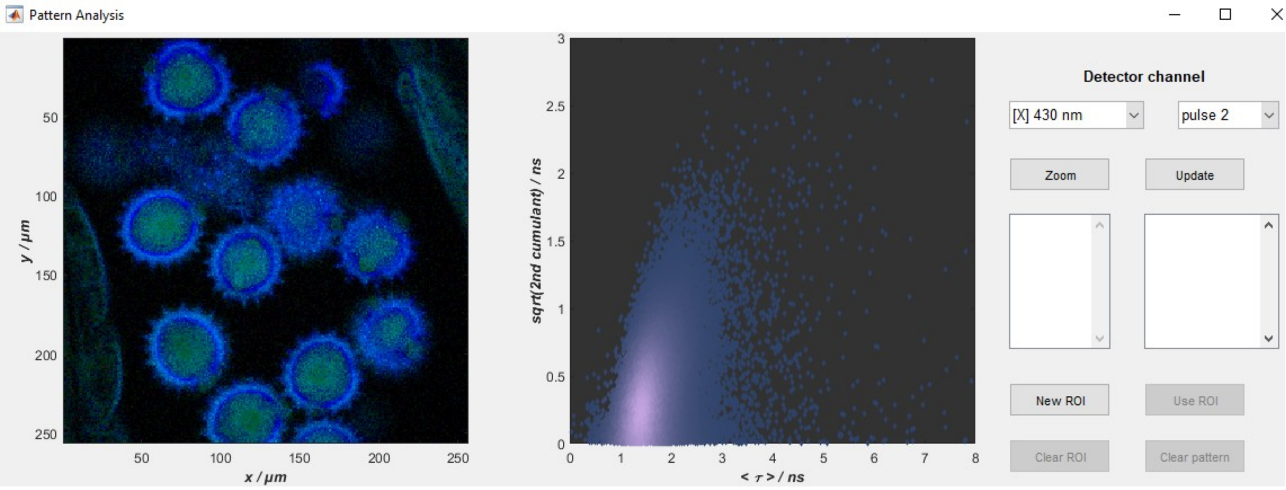
Lower left graph displays the average wavelength (in nm) of all time channels summed together

Upper right graph shows the TCSPC histograms of all pixels. The spectral channels are color coded.

Lower right graph shows the spectra of all pixels and all TCSPC channels together

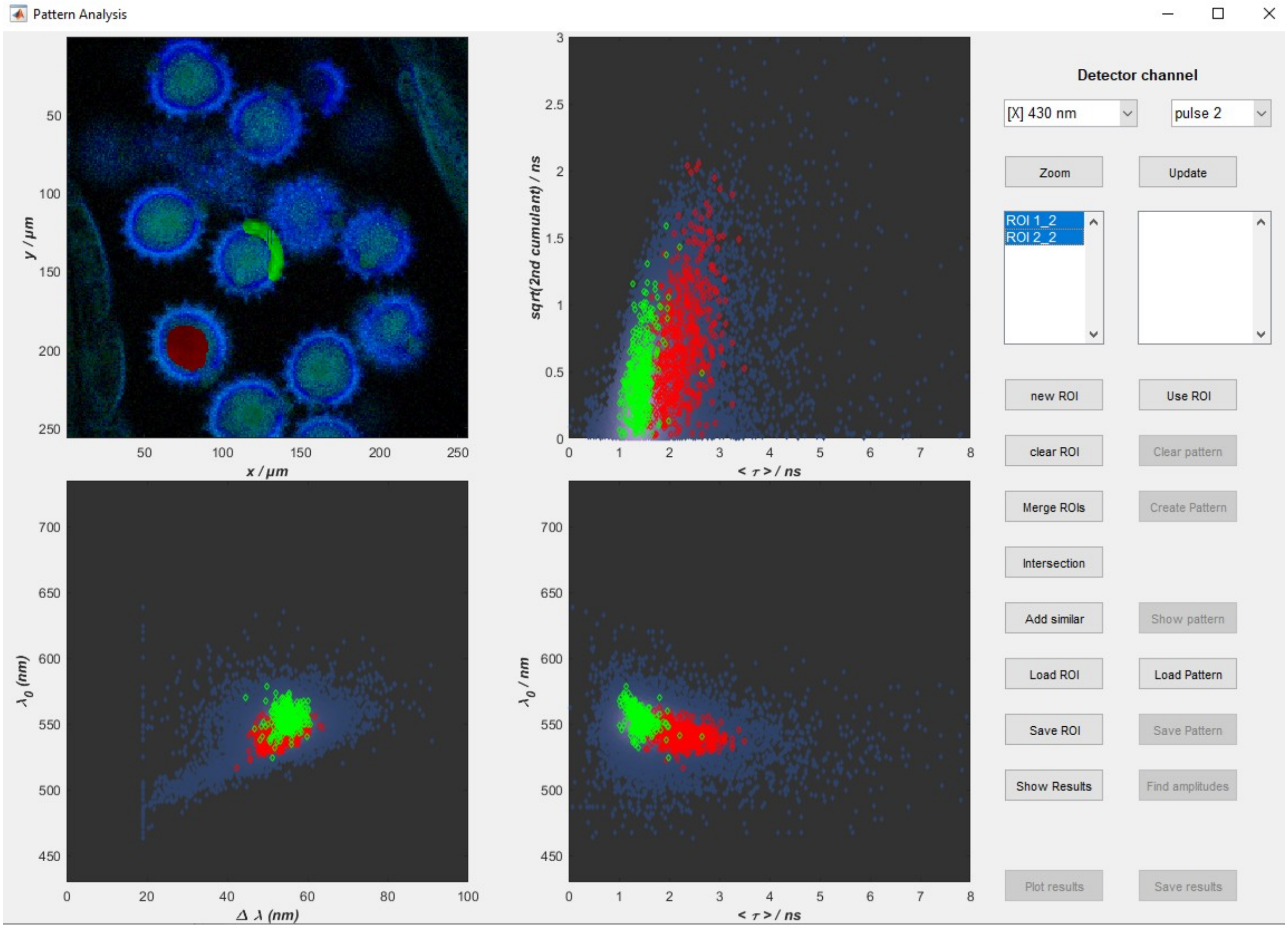
Pattern Matching

- Select file
- If necessary set parameters
- Click on „Pattern Analysis“
- The FastFLIM display opens the following display for each exciation wavelengths
- Using the drop down menu „Detector channel“ the wavelengths included in the analysis can be selected. After changing the selection click on „Update“
- Using the drop down menu „pulse“ the excitation pulse corresponding to the excitation wavelength can be selected. For example using 440 nm and 485 nm excitation in PIE mode, „pulse 1“ refers to 440 nm and „pulse 2“ refers to 485 nm. All 4 graphs visible are displaying the result after excitation with the selected pulse. Do not forget to click on „Update“ after changing the pulse number.
- With Zoom parts of the images can be enlarged
- The upper left image is the FastFLIM image displaying the lifetime color coded
- The upper right image is a scatter plot indicating the frequency of pixels with certain properties, here displayed on the x – axis the mean lifetime and on the y – axis the square root of the second cumulant. It is in indication for the multiexponentiality of the lifetime decay. The value is low for mono-exponential decay and higher for a multi-exponentaily decaying samples.



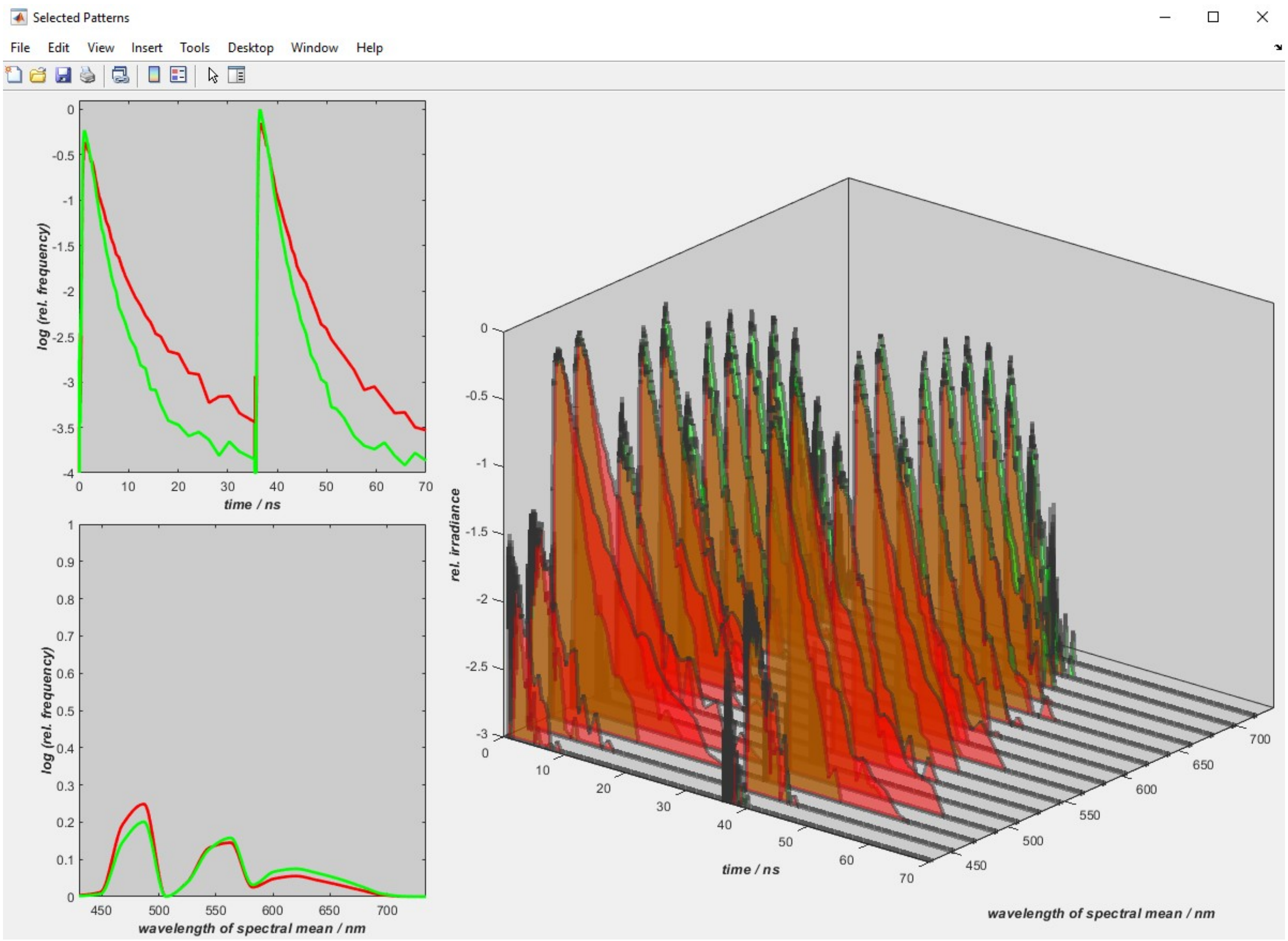
- In order to create patterns the first step is to define ROIs
- ROIs can be defined in all scatter plots and the image of the Pattern Analysis window
- ROIs can be defined separately for the different excitation wavelengths (pulse 1, 2 …)
- In order to create a ROI click on „New ROI“
- Now click in the graphs or image with left mouse button. Right mouse button will close the ROI.

- The ROIs are named …_1 or ..._2 indicating the laser pulse / excitation wavelength
- ROIs can be selected (they appear in blue color). Once selected the ROIs can be merged (Merge ROIs) or intersected (Intersection, only the pixels which are in both ROIs will be selected)
- As a next step Patterns can be created from the selected ROIs (marked in blue) by clicking on „Use ROI“.
- The Pattern contain all spectral and lifetime information (complete decays for every spectral detection channel)



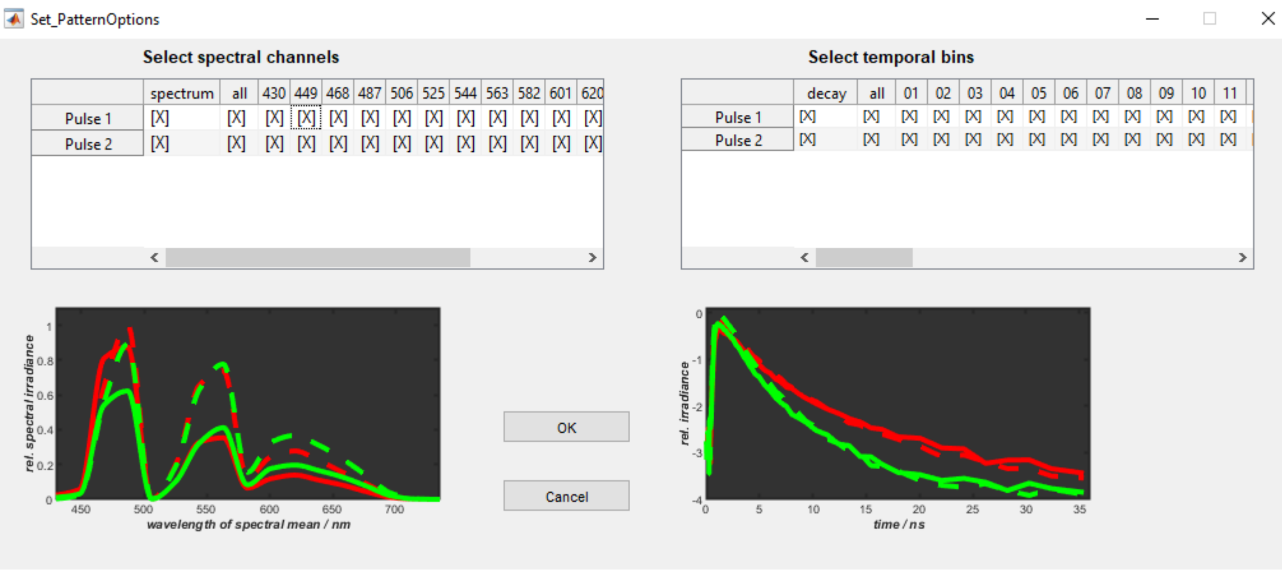
- The lower two panels displaying the mean wavelength over the width of the wavelengths distribution (left)
- The lower right panel displays the mean wavelength over the mean lifetime

- Once the Patterns are calculated clicking on „Use ROI“ they can be displayed by clicking on „Show Pattern“
- The following plot appears displaying the projection of the Pattern on the time axis (upper left plot, showing the lifetime decays) and on the wavelengths axis (upper lower plot, displaying the spectra). One Pattern is displayed in red, the other one in green.
- The complete infomation is displayed in a 3D graph on the right side. Visible are the two rows of decays belonging to the first excitation pulse (e.g. 440 nm) and the second excitation pulse (e.g. 485 nm) of the PIE excitation.



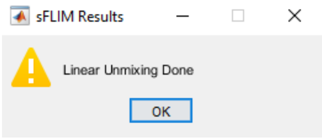
- Once the Pattern are created they can be saved for later usage clicking on „Save Pattern“. Each Pattern is saved as a separate file.

- The next step is the Pattern Matching step which can be started by clicking on „Find Amplitudes“
- The amplitude or intensity of each pattern in the image is calculated for every image pixel
- After clicking on „Find Amplitudes“ the following window appears

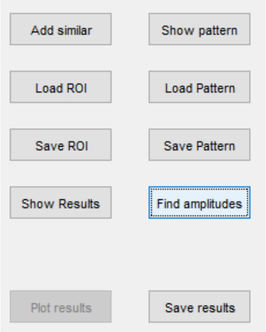


- Here all the spectral and timing channels can be used or the some of the channels can be selected / deselected
- For information the spectral and decay content is visualized in the graphs below
- Once the channel selection is optimized click on „OK“ to start the Pattern Matching process with is performed using a non-negative matrix inversion
- The non-negativity makes sure that all amplitudes / brightnesses of the resulting images remain with positive values

- Once the Pattern Matching / linear unmixing is done, click on OK on the following information:



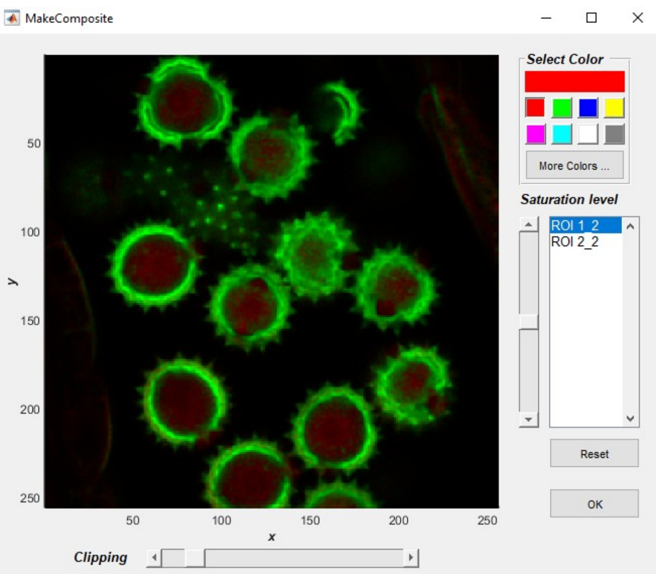
- Now you can click in the Pattern Analysis window on „Show Results“



The results appear with several images.

  - One composite image in which the color and brightness of each Pattern in the image can be adjusted
  - One black/white image for every Pattern
  - One image indication the residuals which can be informative if the selected Pattern have been sufficient to describe the spectral / lifetime properties of the sample

- Finally the results can be saved with „Save results“



- Composite Image of the unmixing result using the two selected Pattern
- The color of the Pattern display (here named according to the selected ROIs) can be selected as well as the brightness
- The saved results can be opened with the routine „Plot_UnmixingResults.m“