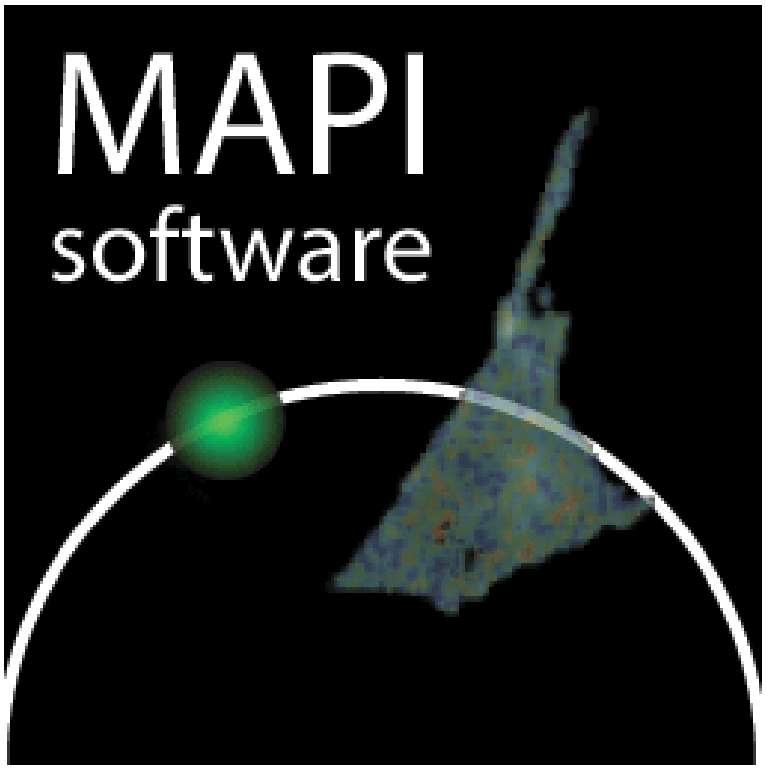
MAPI software manual

Multispectral Analysis of

Polar Images

version 4.4



https ://github.com/ayleray/MAPI-software

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1 Introduction

This manual describes the installation and operation of MAPI software. This software gives you access to several tools for analyzing data acquired with fluorescence lifetime imaging microscopy (FLIM). For instance, with this software, you can easily calculate the polar representation of classical FLIM images and also multispectral FLIM acquisitions.

MAPI software is under constant development. We appreciate any feedback on bugs, missing features or other inconveniences. We hope you will enjoy analyzing your lifetime data with this software.

2 Installation

When you install MAPI software for the first time, you have to install first the Matlab Runtime and then the MAPI software.

2.1 System requirements

Before installing MAPI software, please make sure that your PC meets the following requirements :

— Windows11 or Windows 10 (version 21H2 or higher) operating system

— Any Intel or AMD x86-64 processor with two or more cores

— 8 GB internal memory (RAM). 16 GB is recommended

— 4-6 GB for storage for a typical installation

2.2 Matlab Component Runtime

The Matlab Component Runtime should be installed before installing the MAPI software for the first time. It can be find here :

https://fr.mathworks.com/products/compiler/matlab-runtime.html

Please download, extract and install the Matlab Runtime by following the instructions. Once it is done, you can launch the MAPI software.

2.3 MAPIsoftware

To launch the MAPI software, double click the `MAPIsoftware.exe` file located in the same folder.

The following window will appear (Figure 1) :

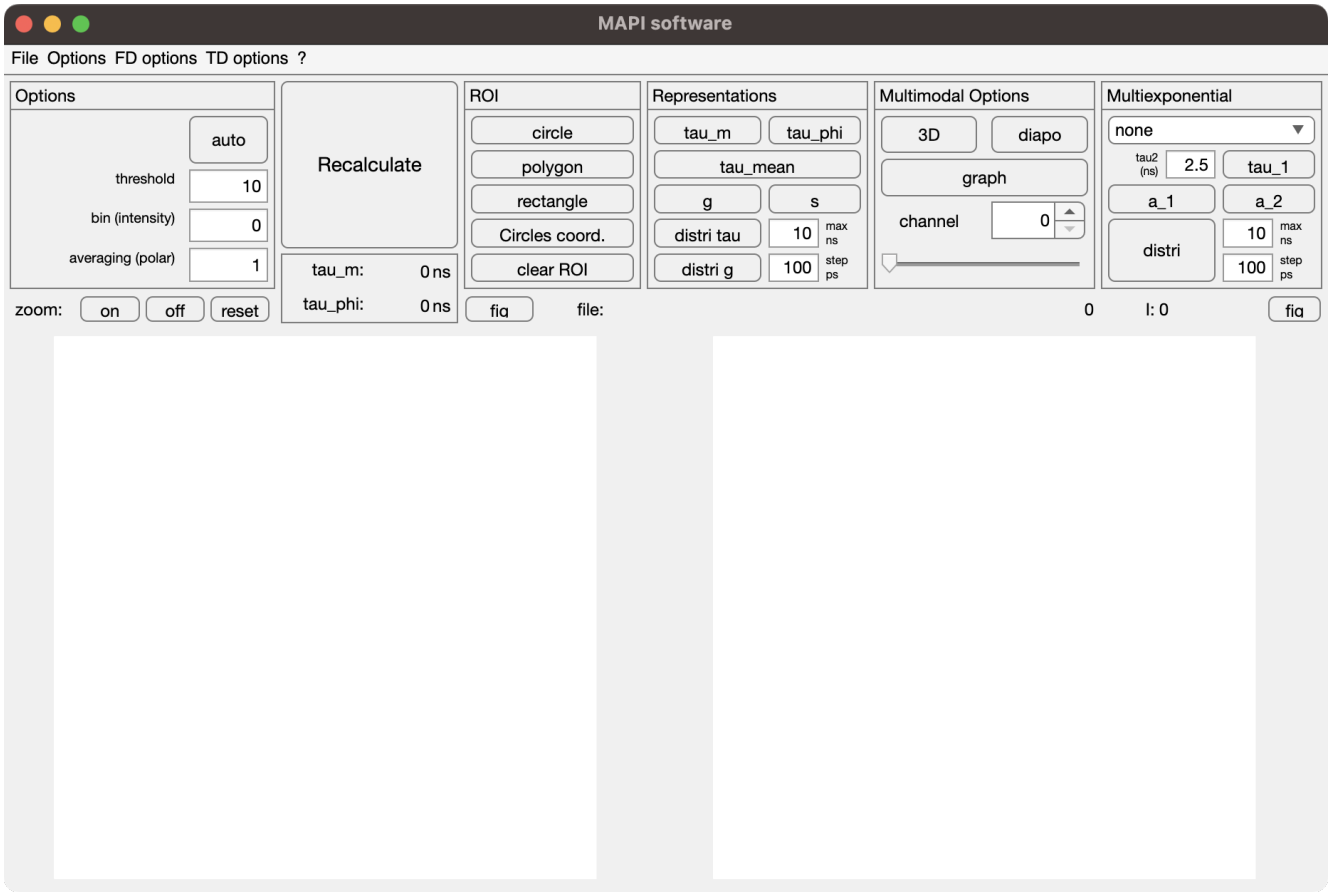

Figure 1: *The Main MAPI software window*

For having access to the documentation file with MAPI software, you just need to click on the help menu (" ?") and select "Documentation".

You are now ready to use the MAPI software.

3 Quick start guide

This tour will guide you through the process of analyzing lifetime images with MAPI software. The purpose of this chapter is to introduce you the most common tools that you can do with MAPI software : opening a file and using the interactive interface.

If you come across some menu options or toolbar buttons in MAPI software, that are not explained in this quick start guide, please refer to the section 4 for a complete reference of all options, features and functionality of MAPI software.

If you want to know more details about the mathematical equations enabling the fluorescence lifetime data analysis, you should read the section 5.

3.1 Opening a file

To launch the MAPI software, double click the `MAPIsoftware.exe` file. Then, you will see the main window (Figure 1). To open a lifetime data file, click on the “File” menu and the “Open...” menu item. The following window will appear (Figure 2) :

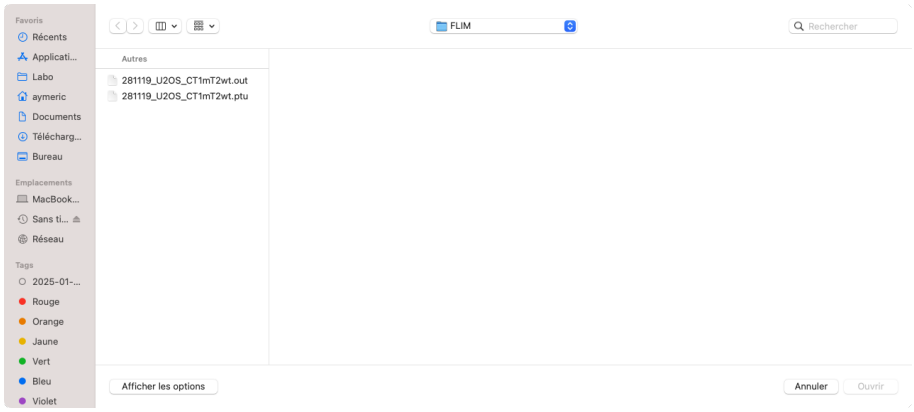

Figure 2: *The “Select the File” window*

Then, select a data file and click on the “Open” button. The following window will then pop up (Figure 3) :

If you want to open all files in your folder to see the evolution of lifetimes values, click "Yes". If you only want to open one file, click "No".

The following interface will then pop up (Figure 4) :

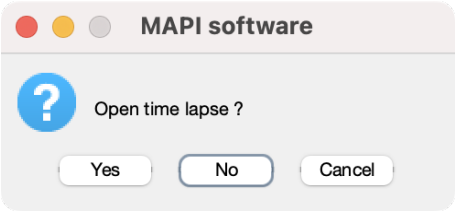

Figure 3: *The "Time Lapse" option window*

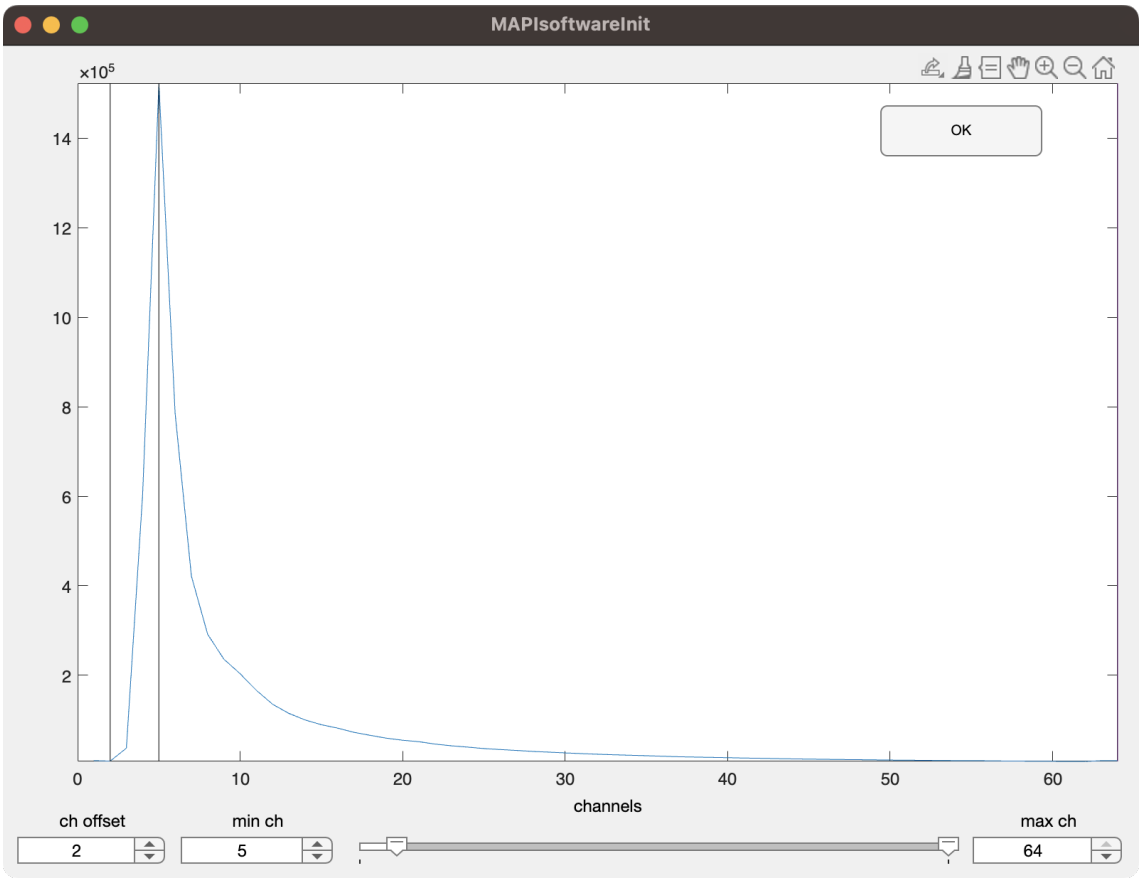

Figure 4: *The "exponential decay" interface*

To calculate correctly the polar representation, you need to adjust the start time and the stop time of your lifetime data. Generally, this start time is set to the maximum of the exponential decay curve. To select the desired starting time, you can move the slider or change the value of "min ch". For selecting the desired stop time, you can also move the slider or change the value of the "max ch". The value of "ch offset" corresponds to the channels used for calculating the offset.

When all the values are fixed, click on the "OK" button, the following window will then pop up (Figure 5) :

If you want that the offset is calculated automatically, click "Yes". Otherwise, click "No" and an

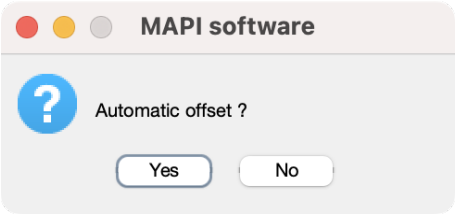

Figure 5: *The "Automatic offset" option window*

other window will pop up asking for entering the value of the offset. After this, the main window of MAPI software becomes as indicated in Figure 6).

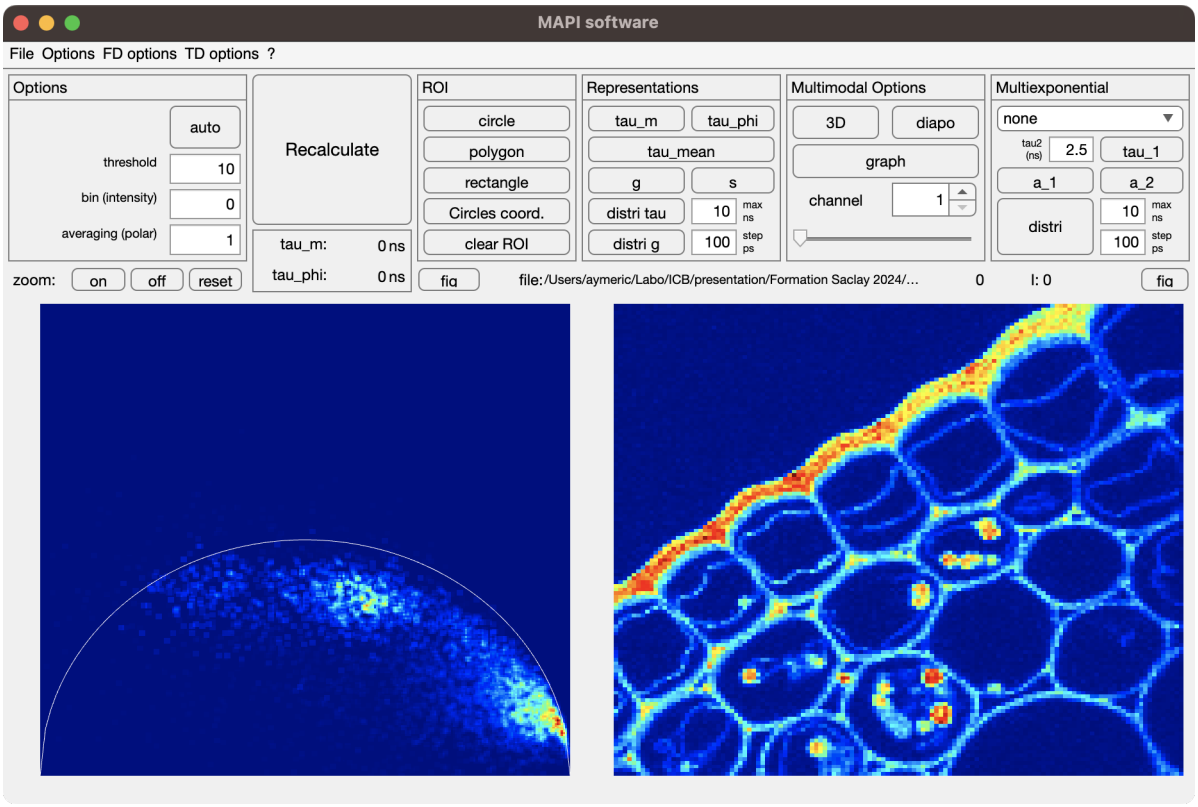

Figure 6: *The Main window of MAPI software*

This main window consists of numerous buttons and options (Figure 6). If you want more detail about all of them, please refer to the section 4. In this section, we will just briefly describe the images and accomplish some basic tasks.

The right image is the intensity image of your acquisition. If you want to know the intensity value in a pixel, just left click on the image and the intensity value will be displayed in the intensity part above the image (called "I").

The left image is the polar representation of your lifetime data. For this image, you can know

the phase and modulation lifetime for each pixel (called “tau_m” and “tau_phi”). To do this, left click on the image and the lifetime values will be displayed in nanosecond in the lifetime part above the polar image.

Above each image, you have a “fig” button which allow to plot the corresponding image in a new figure window (which is a standard Matlab figure environment). Before closing each new figure, you have the possibility to save the data in different format (see section 4).

Finally, you have also access to some information about the data file and the experimental parameters in the “file” part above the images. You can see the name of the data file, the time resolution, the channel number and the pixel numbers of your acquisition.

To improve the polar representation of your experimental data, you can change the intensity bin (called “bin(intensity)” in the MAPI software) and also the averaging of the polar representation (called “averaging (polar)”) of the “Options” group. By fixing both parameters to 2 and clicking on the “Recalculate” button, you then will see the next main window (Figure 7) :

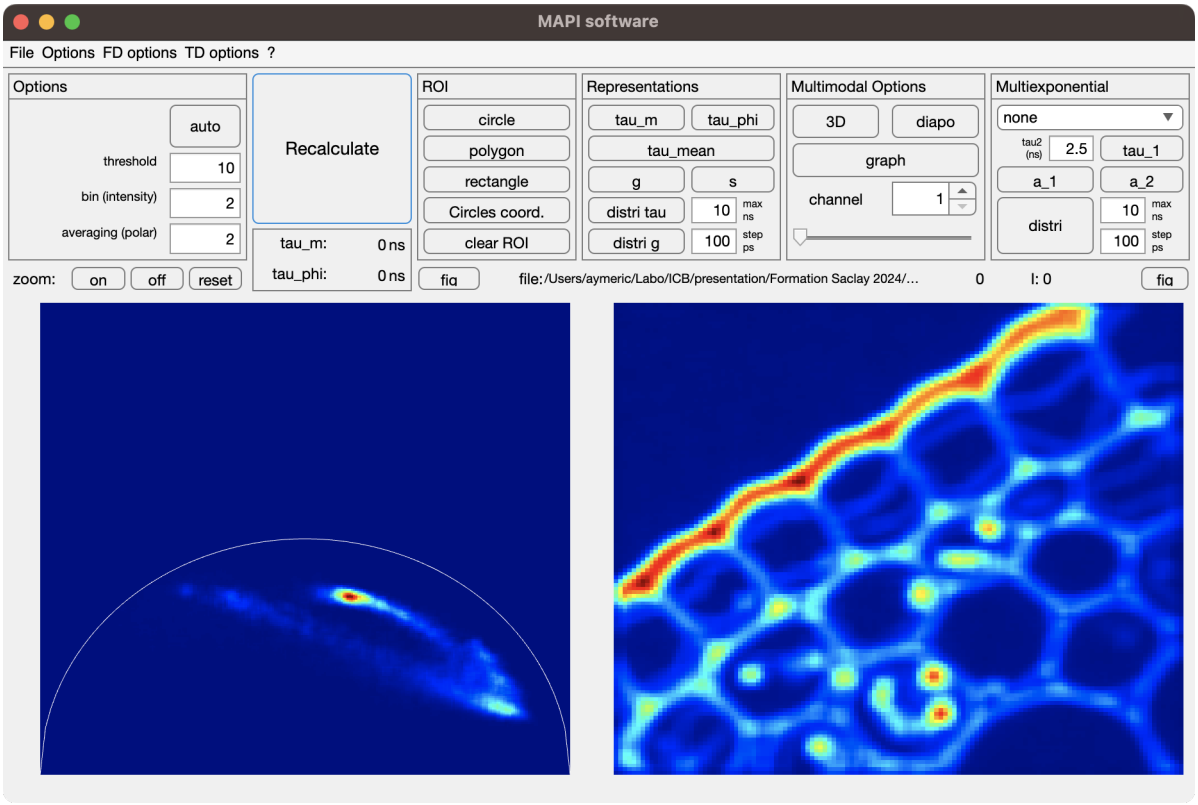

Figure 7: *The main window of MAPI software after modifying the options*

Each time that you want to modify the options of the “Options” group, do not forget to click

on the “Recalculate” button to take into account your new parameters.

3.2 Creating a Region Of Interest

In this section, we will describe how to create a circular region of interest (ROI) on the polar image and describe some functionalities that you will have access with this ROI option. Even if we describe only the circle ROI, these functionalities are still compatible with all ROIs. If you want to create an other ROI, please refer to the section 4).

To create a circle ROI, click on the “circle” button. Then you will see the following window which explains you how to do (Figure 8) :

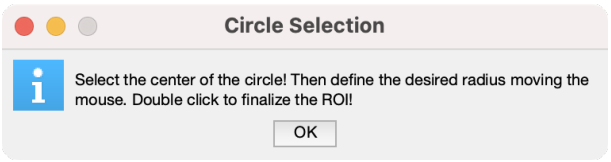

Figure 8: *The circle ROI selection window*

First, on the polar image (left image), you have to move the mouse cursor to the desired center of the circle and just left click to validate your choice. Once it is done, move the cursor to the desired radius of the circle and then, double click to finalize the ROI. After, the following dialog box appears (cf. Figure 9). Click on the “inside” button for selecting the pixels inside the ROI.

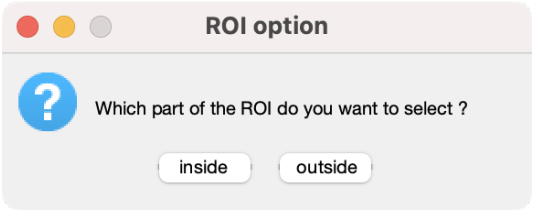

Figure 9: *The ROI option window*

After calculation, the following window appears (Figure 10) :

Now, on the intensity image of the main window (on the right part of the main window), the white pixels corresponds to the selected pixels of the circle ROI. This is called the “Intensity Mask”. By selecting a ROI, you can easily discriminate the lifetimes of each pixel. When you select a ROI, all the button groups (which means : “Representations”, “MultimodalM options”

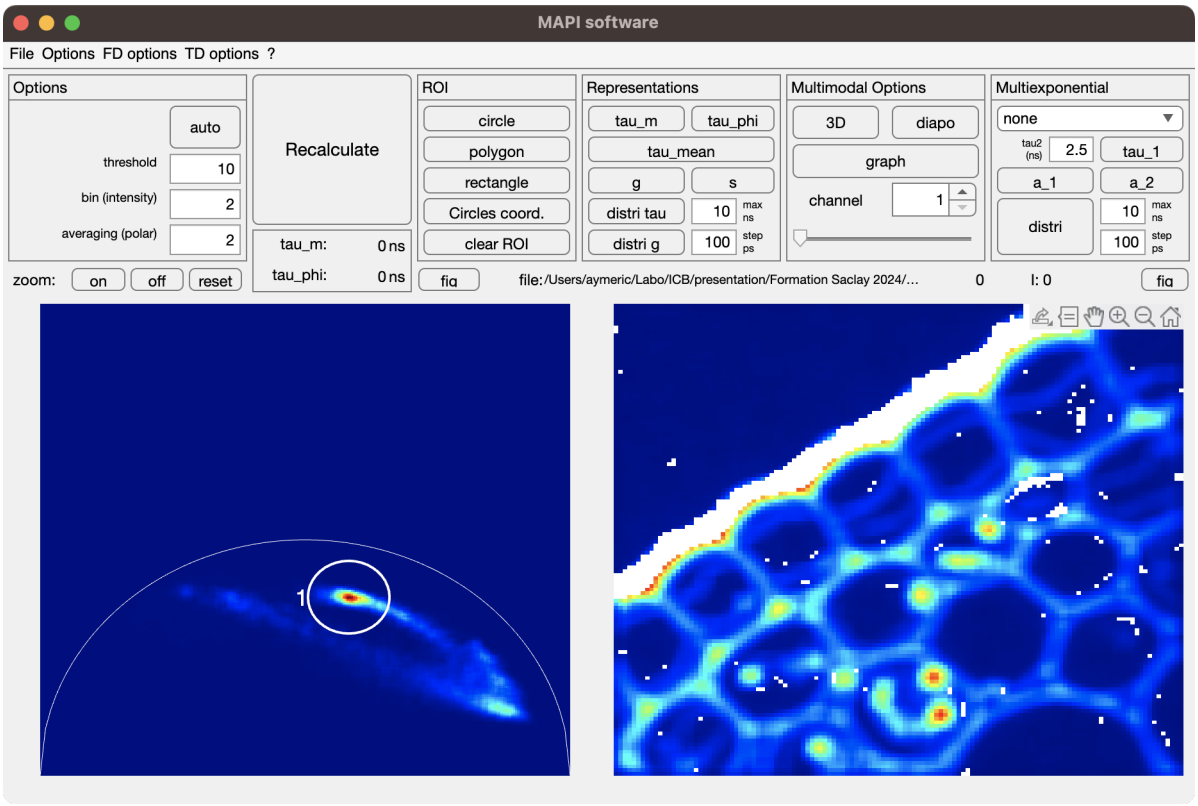

Figure 10: *The main window after creating a circle ROI*

and "Multiexponential options") will represent the lifetime results for the selected pixel of the ROI only. If you want to reset the ROI, just click on the "Recalculate" button.

You can also draw a circle ROI in the intensity image (see Figure 11). In this case, the polar image is calculated only for the pixels including in the selected ROI.

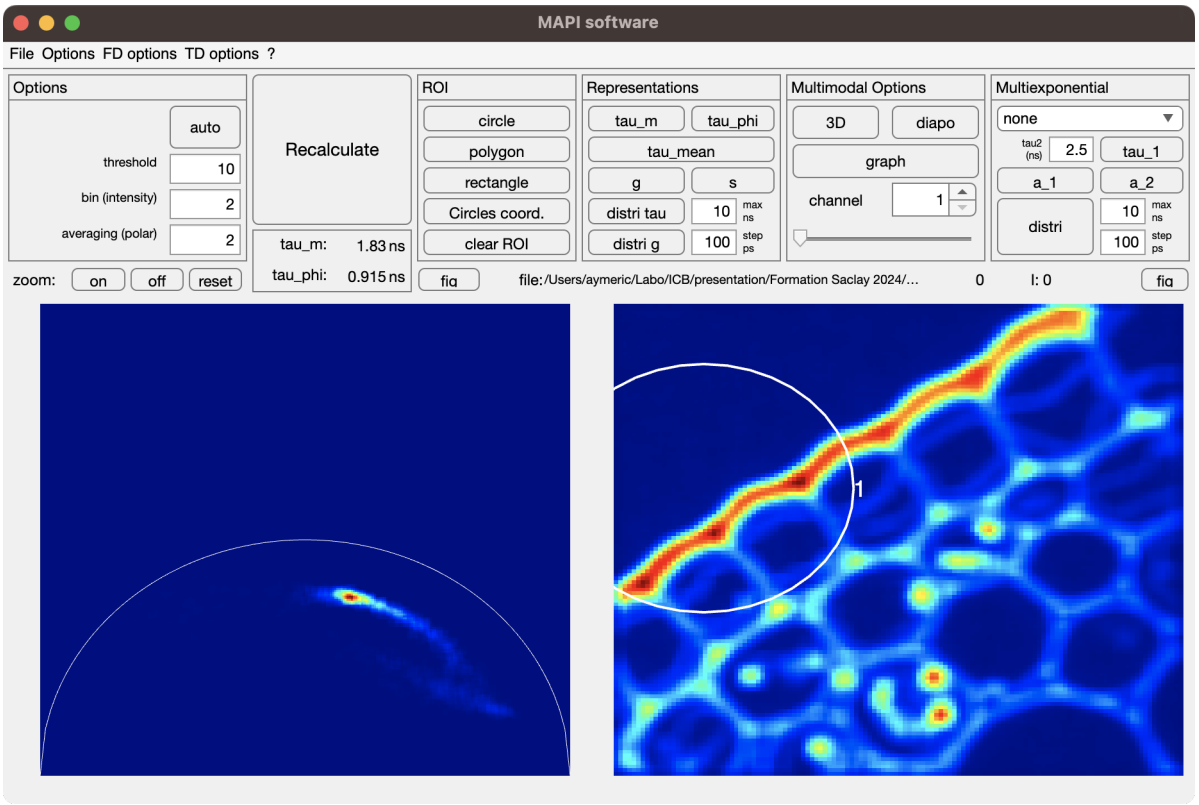

Figure 11: *The main window after creating a circle ROI in the intensity image*

4 Reference guide

This section describes the main menu options and the different buttons groups of the main window of the MAPI software. All buttons and other functional items which are not described here have been described in the section 3.

4.1 The Main menu

The main menu bar (Figure 12) sits at the top of the main window.

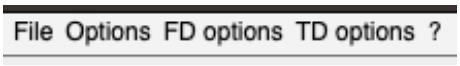

Figure 12: *The Main menu*

4.1.1 The File menu

The "File" menu is illustrated in Figure 13 and it is constituted of several items.

Figure 13: *The "File" menu*

- The "Open..." menu item allows you to open several types of data files : ACS, multiTIF, FLI, SDT and PTU formats.

  The ACS file format is a standard ascii format with a space as separator. When you open a ACS file, you will see the following dialog box (Figure 14) :

  By clicking on "No", you will just open one FLIM image. If you want to open a stack of 16 FLIM data (called a "SLIM image"), click on the "Yes" button. Note that the name of the different files has to be identical (only the incrementation number at the end of the file is

Figure 14: *The "Open SLiM file ?" dialog box*

changed). If the files are not compatible with MAPI software, an error message is displayed. In all case, you will see after the following window appearing (Figure 15) :

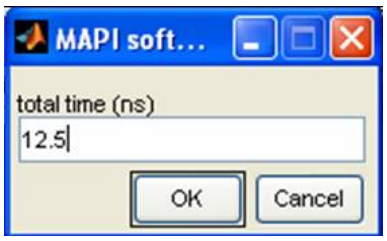

Figure 15: *The "total time" window*

This window enables you to enter the exact total time of your TCSPC set up. Generally it is set to 12.5ns but it can differs. Once you type the total time of your set up and click on the "OK" button, you will see the window enabling you to adjust the starting time and the end time of your lifetime data (Figure 4). Generally, this starting time is set to the maximum of the exponential decay curve.

The multiTIF format is a common image format. After opening a multiTIF file, you will see the following dialog box (Figure 16) : If you click on the "Yes" button, the following window

Figure 16: *The "Open Streak Camera file ?" dialog box*

appears (cf. Figure 17). After entering the total time of each temporal decay (in ns) and the number of spectral channels, click on the "OK" button. Then you will see the window enabling you to adjust the starting time and the end time of your lifetime data (Figure 4). If you click on the "No" button, the following window appears (cf. Figure 18). After entering

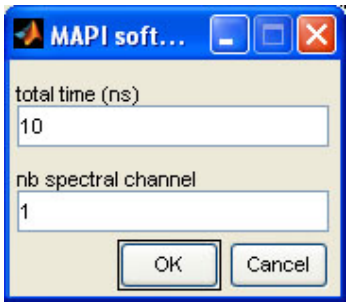

Figure 17: *The "Streak Options" dialog box*

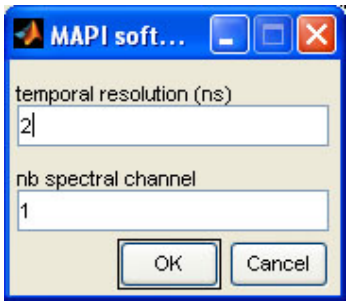

Figure 18: *The "TIF Options" dialog box*

the temporal resolution (in ns) between two images and the number of spectral channels, click on the "OK" button. Then you will see the window enabling you to adjust the starting time and the end time of your lifetime data (Figure 4).

The FLI format is the Lambert Instrument file format. To use this format, you need to open the LIFA software and load a FLIM image on it. If it is not the case, an error message is displayed. After opening a correct file, a new dialog box allowing to enter the reference lifetime is popped up (Figure 19) :

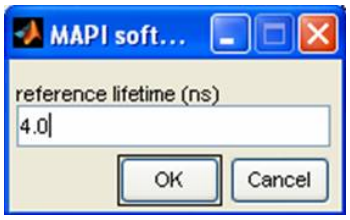

Figure 19: *The "Reference Lifetime" window*

The SDT format is the Becker & Hickl file format. With this format, all the experimental parameters are saved and consequently you will directly see the Figure 4 permitting to choose the start time and stop time of your data.

The PTU format is the PicoQuant file format. With this format, all the experimental para-

meters are saved and consequently you will directly see the Figure 4 permitting to choose the start time and stop time of your data.

After loading an image or a stack, MAPI software calculates the polar representation of the lifetime image and display the results on the main window.

- The "Open Batch..." menu item will open a window to select one file in the requested folder. All the files in the folder will be treated automatically by MAPi software. The procedure is identical to the procedure for opening one file. The only difference is that an other file window will pop up for saving the data. One text file will be saved containing the mean lifetimes for each file. The phasor and intensity images will also be saved automatically.
- The "Save..." menu item will save the data that are currently opened. It will create a new folder whose name is chosen by the user. In this new folder, a text file (called `info.txt`) with both acquisition and image processing parameters are saved. If you opened a single image, then you will save also in this new folder the intensity image and the polar image for this single lifetime image in TIF format with no compression. If you opened a stack of images, you will save the intensity and polar images in a TIF format (with no compression) for each stack.
- The "Export..." menu item will also create a new folder whose name is chosen by the user and will save the same information (text file with information, intensity and polar images) in a different format file. You can choose among different format : TXT, EPS, FIG, JPG or TIF. The TXT format allows you to open your images with almost all the scientific image processing software (like ImageJ for example). The EPS format is the EPS level 1, FIG is the standard Matlab figure format, JPG is the JPEG image format and TIF is the TIFF format with no compression. Like previously, the `info.txt` file stores the acquisition settings and the image processing parameters.
- The "Close" menu item closes MAPI software. Note that it does NOT warn you if you have unsaved images opened in the main window.

4.1.2 The "Options" menu

The "Options" menu represented in Figure 20 gives you access to several options.

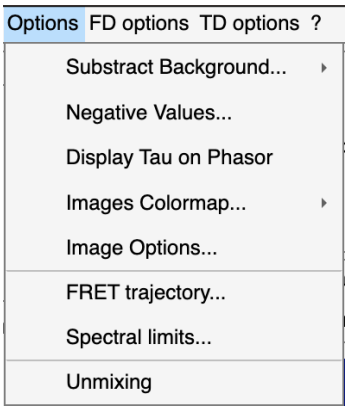

Figure 20: *The "Options" menu*

- The "Substract Background..." menu item enables to substract a background on each temporal decay (cf. Figure 21). By selecting the "Value..." menu item, you can enter an offset value that will be substracted on each temporal decay of the FLIM image. The offset value corresponds to the total background on each temporal decay. If you select the "Image..."

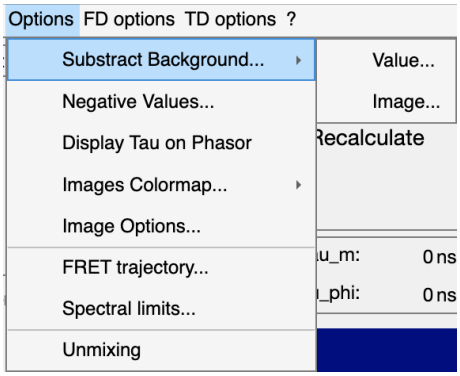

Figure 21: *The "Substract Background..." menu*

  menu item, you will need to open a background image. This background image will be substracted pixel per pixel from your FLIM image and the corresponding polar representation will be recalculated.

- The "Negative Values..." menu item allows you to choose if you want to display the negative values of lifetimes. An other window will pop up asking if you want to allow the negative vales.

- The “Display Tau on Phasor” menu item enables to display some values on the phasor image as you can see in Figure 22.

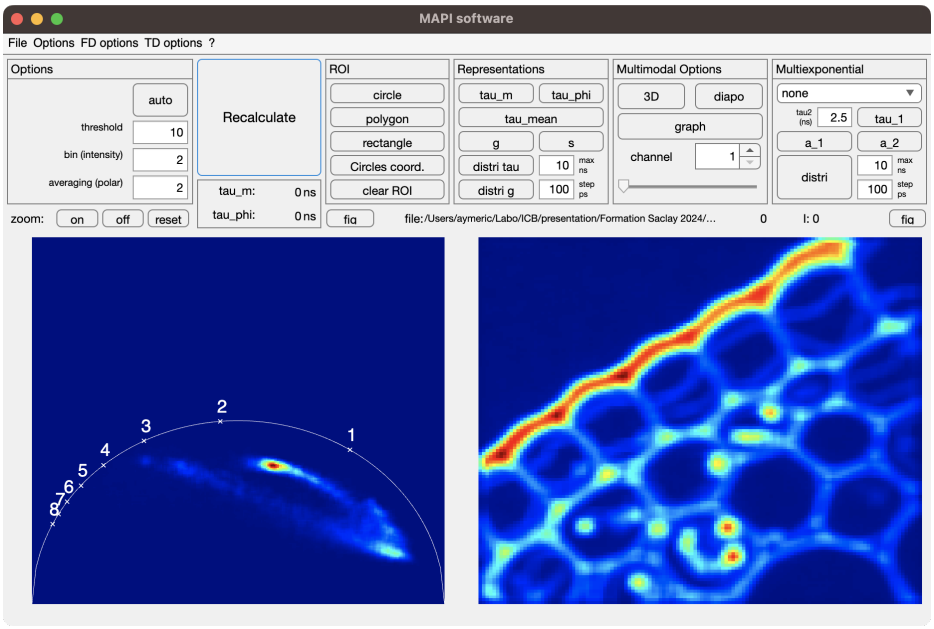

Figure 22: *The main window after selecting the option*

To remove the values, return in Options and select the “Hide Tau on Phasor” menu item.

- The “Images Colormap...” menu item allows you to change the current look up table (or colormap) of the polar and intensity images. By default, it is fixed to “jet” but you can modify it and all the possible look up tables are represented in Figure 23 :

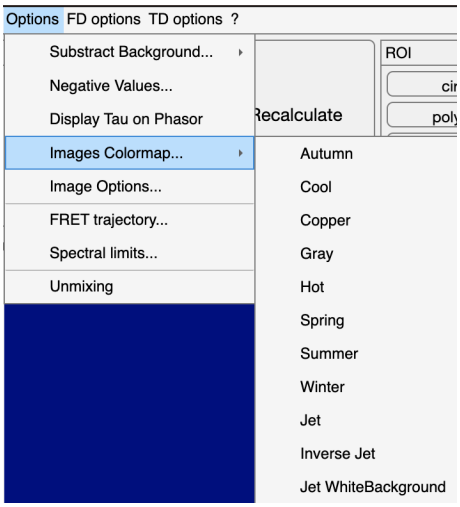

Figure 23: *The “Images Colormap...” menu*

For example, if you prefer representing the images in grayscale, choose the “gray” colormap.

- The “Image Options...” menu item allows you to modify the minimal and maximal values of the colormap of the intensity image and the number of pixels for the phasor image (cf. Figure 24).

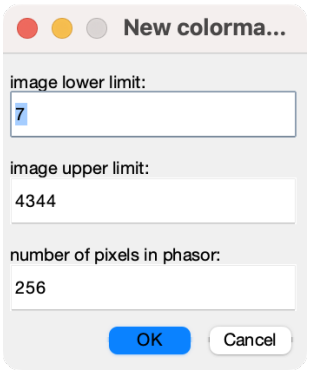

Figure 24: *The “Image Options” dialog box*

- The “FRET trajectory” dialog box of Figure 25 appears when you click on the “FRET trajectory” menu item.

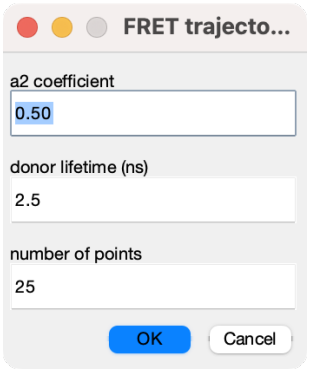

Figure 25: *The “FRET trajectory” dialog box*

After entering all the requested parameters of this dialog box, the FRET trajectory will be displayed on the polar image.

- The “Spectral limits...” menu item requires a stack of several FLIM images. Otherwise, an error message appears. By selecting this ‘Spectral limits...” menu item, the following dialog box will pop up (Figure 26) :

  This option allows you to modify the limits of your stack of images. By modifying these limits, all the “SLIM options” functions will be affected (see the section 4.2.5).

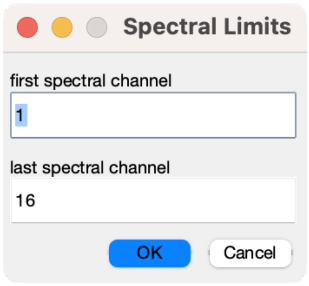

Figure 26: *The Spectral Limits dialog box*

- Finally, the last menu item of the “Options” menu is the “Unmixing” menu item. After selecting it, the following window will pop up (Figure 27) :

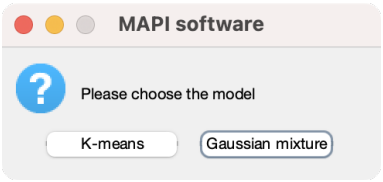

Figure 27: *The dialog box for selecting the Unmixing algorithm*

You can choose between two algorithms : the "K-means" or the "Gaussian Mixture". Then an other window will pop asking for entering the size of the filter (Figure 28) :

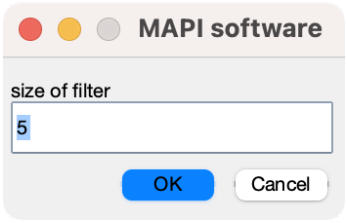

Figure 28: *The dialog box for entering the filter size*

The unmixing will start automatically and the ROI will be displayed in the main window. An example of unmixing is shown in the Figure 29.

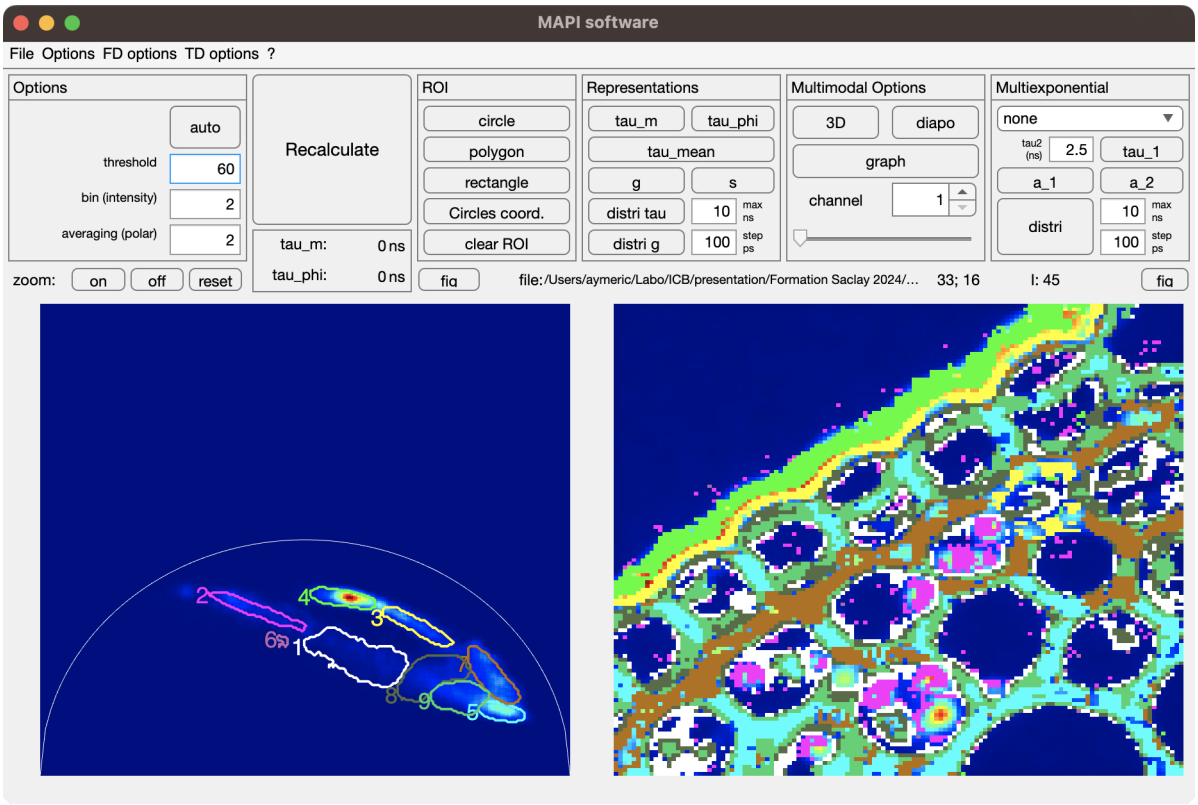

Figure 29: *The main window after the unmixing process*

4.1.3 The "FD options" menu

The "FD Options" menu is illustrated in Figure 30. It gives you access to specific options accessible only if the FLIM data have been acquired with the frequency domain method. If it is not the case, an error message appears.

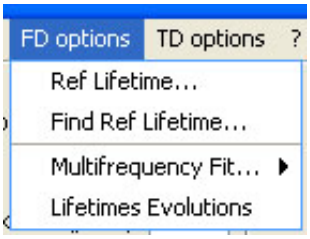

Figure 30: *The "FD Options" menu*

- The "Ref Lifetime..." menu item allows you to change the reference lifetime used to calculate the FLIM images. By selecting it, the dialog box shown in Figure 19 will be opened.
- If you do not know the reference lifetime of you experiment or if you want to verify that you reference lifetime is correct, you can select the "Find Ref Lifetime..." menu item. It is important to keep in mind that the fluorescent reference sample must have a single lifetime component. In other word, the phase and modulation lifetimes are identical. In order to calculate the reference lifetime, MAPI software minimizes the difference between the phase and modulation lifetimes with a Levenberg-Marquardt algorithm. The fitting options accessible are displayed on Figure 31 :

  After entering the initial lifetime guess and the number of iteration, MAPI software will minimize the phase and modulation lifetime difference with a Levenberg-Marquardt algorithm ; the calculated results (reference lifetimes and modulation frequency) are displayed on the following window (Figure 32) :

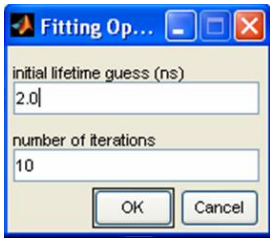

Figure 31: *The "Find Ref Lifetime Options" window*

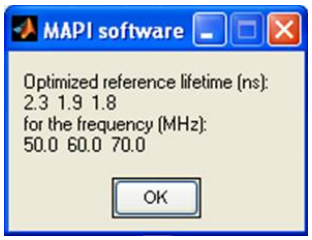

Figure 32: *The "Find Ref Lifetime Results" window*

- Finally, the "Multifrequency Fit..." menu requires that a multifrequency FD file is opened (Otherwise an error message is displayed). You can choose to fit your experimental data with a single or a bi exponential intensity decay (Figure 33).

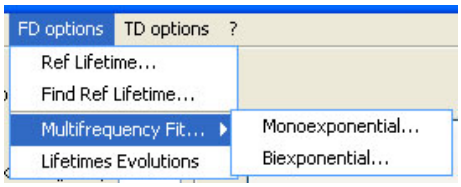

Figure 33: *The "Multifrequency Fit..." menu*

When you choose the "monoexponential..." menu item, MAPI software will adjust the experimental points (averaged on all image) with a monoexponential decay by minimizing the χ^2 with a Levenberg-Marquardt algorithm. The fitting options accessible (initial lifetime guess and number of iteration) will be displayed on the same dialog box as previous (Figure 31). After computation, the experimental points and the fitting results are displayed on a standard Matlab figure environment (Figure 34) :

On this figure, both phase (phi) and modulation (m) are plotted as a function of frequency. Note that the lifetime and the χ^2 values are displayed in the title of the figure window.

If you want to adjust your experimental points (averaged on all image) with a biexponential decay by minimizing the χ^2 with a Levenberg-Marquardt algorithm, you will see the following fitting options dialog box appearing (Figure 35).

After entering the initial a1 guess, the initial tau1 and tau2 guess and the number of iterations, an other fitting options dialog box appears (Figure 36) :

This dialog box enables you to fix (or not) one or several lifetime components. As explicitly mentioned in this dialog box, if you want free lifetime component, enter "NaN". Otherwise,

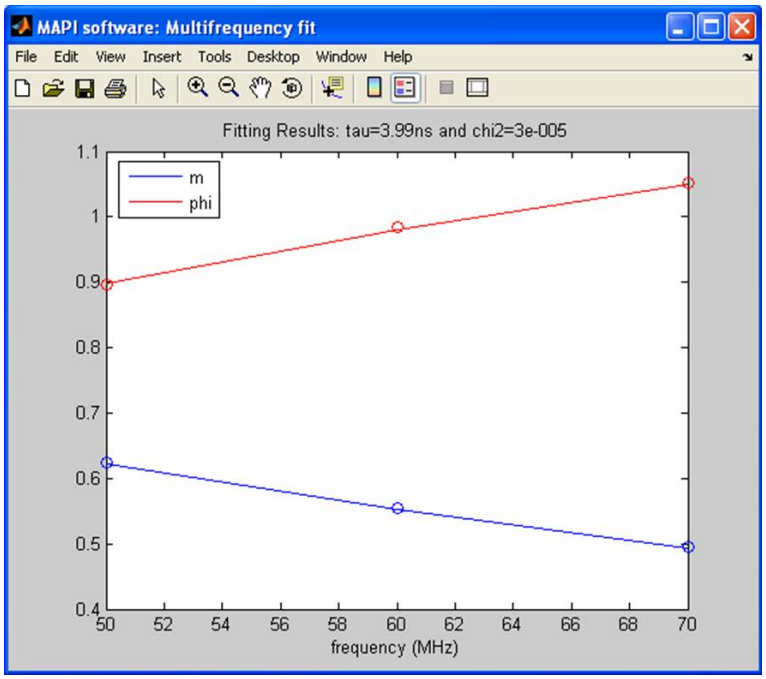

Figure 34: *Results of Mono Exponential fit*

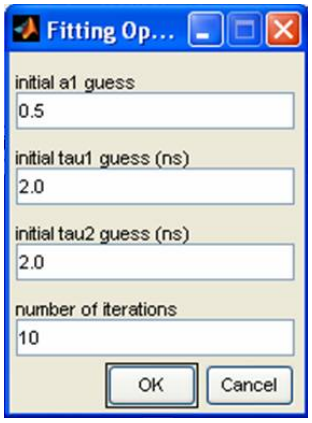

Figure 35: *Fitting options dialog box with Biexponential fit*

enter a positive number. After computation, the experimental points and the fitting results are displayed on a standard Matlab figure environment (Figure 37). On this figure, both phase (phi) and modulation (m) are plotted as a function of frequency.

Note that the lifetime components (a1, tau1 and tau2) and the χ^2 values are displayed in the title of the figure window.

When you shut down a Multifrequency fit figure (mono or biexponential), a warning dialog box appears for saving the current phase and modulation data. By clicking on "Yes", you will have the possibility to save it on a new folder. With this action, you will save the current

Figure 36: *Other Fitting options dialog box with Biexponential fit*

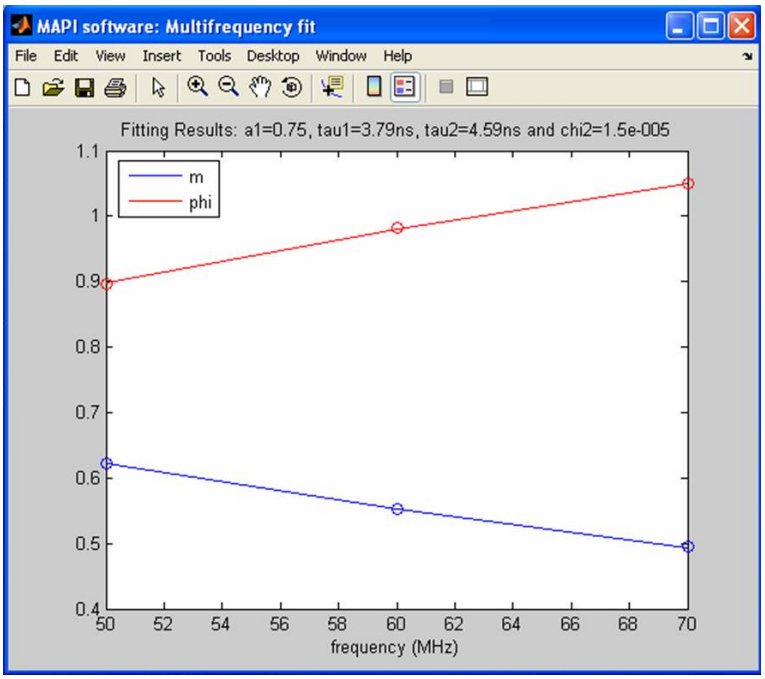

Figure 37: *Results of Bi Exponential fit*

phase and modulation data, the fitting results and the `info.txt` file.

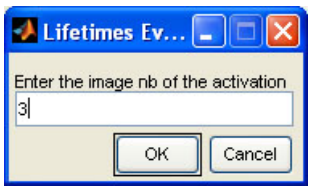

Figure 38: *The "Lifetime Evolution Option" dialog box*

- The "Lifetimes Evolutions" menu item is adapted for biosensor experiments. It enables to plot the quantity DeltaTau/Tau0 as a funcion of time. This quantity is equal to (Tau-Tau0)/Tau0 where Tau0 is the lifetime before activation. After selecting the "Lifetimes Evolutions" menu item, the following dialog box appears (Figure 38).

  For calculating Tau0, you need to enter the number of the image when the activation is

performed. Once it is done, the calculated results for the phase lifetime, the modulation lifetime and the mean lifetime are displayed in a new figure (cf. Figure 39).

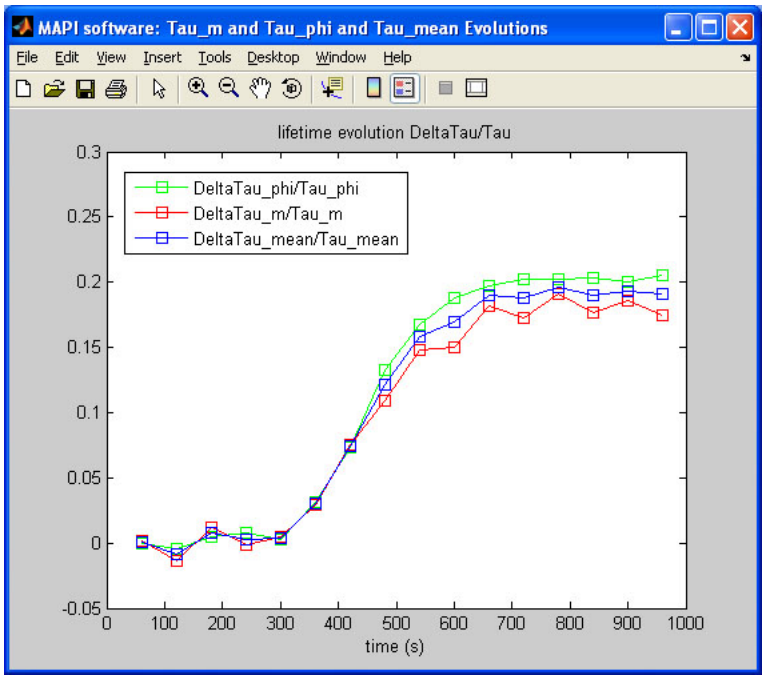

Figure 39: *Lifetime evolution figure*

4.1.4 The "TD options" menu

The "TD options" menu is represented in Figure 40. It gives you access to specific options accessible only if the FLIM data have been acquired with the time domain method. If it is not the case, an error message appears.

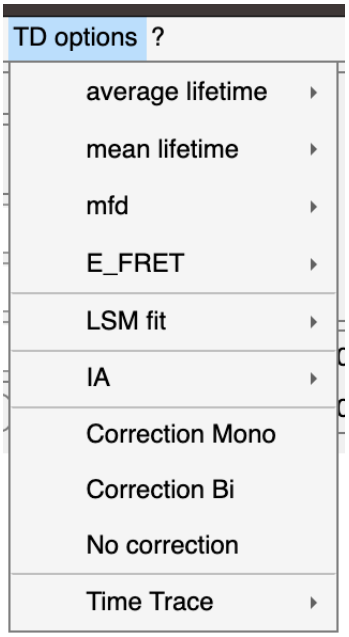

Figure 40: *The "TD options" Menu*

- The "average lifetime..." menu item enables to calculate the weighted average lifetime defined by :

$$\tau_{average} = \sum_i a_i \tau_i \tag{1}$$

By selecting it, the dialog box shown in Figure 41 is opened allowing displaying either the average lifetime image or the average lifetime distribution.

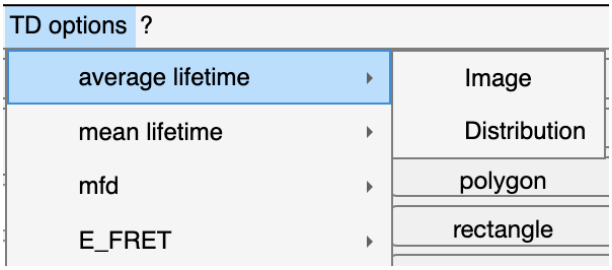

Figure 41: *The "average lifetime" menu*

- The "mean lifetime" menu item enables to calculate the mathematical mean lifetime defined by :

$$\tau_{mean} = \frac{\sum_i a_i \tau_i^2}{\sum_i a_i \tau_i} \tag{2}$$

When you select it, you have the possibility to display either the mean lifetime image or the mean lifetime distribution (Figure 42).

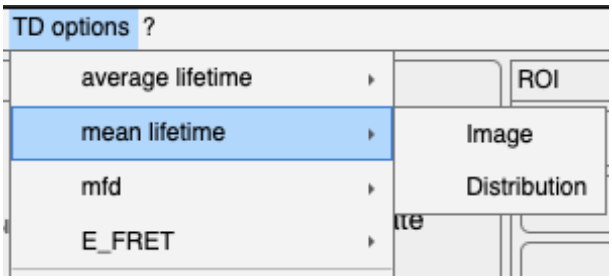

Figure 42: *The "mean lifetime" menu*

- The "mfd" menu item enables to calculate the minimal fraction of donor defined by :

$$mfd = \left(1 - \frac{\tau_{mean}}{\tau_D}\right) / \left(\frac{\tau_{mean}}{2\tau_D} - 1\right)^2 \tag{3}$$

where τ_{mean} was defined previsouly in Equation 2 and τ_D is the donor lifetime that will be asked after.

You can choose to display either the mfd image or the mfd distribution (Figure 43).

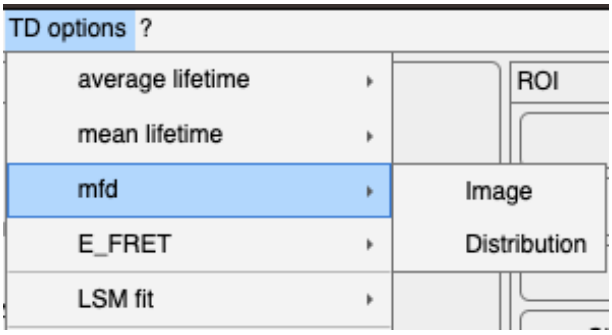

Figure 43: *The "minimal fraction donor" menu*

- The "E_FRET" menu item enables to calculate the FRET efficiency defined by :

$$E_{FRET} = \left(1 - \frac{\tau_{average}}{\tau_D}\right) \tag{4}$$

where $\tau_{average}$ was defined previsouly in Equation 1 and τ_D is the donor lifetime that will be asked after.

You can choose to display either the E_{FRET} image or the E_{FRET} distribution (Figure 44).

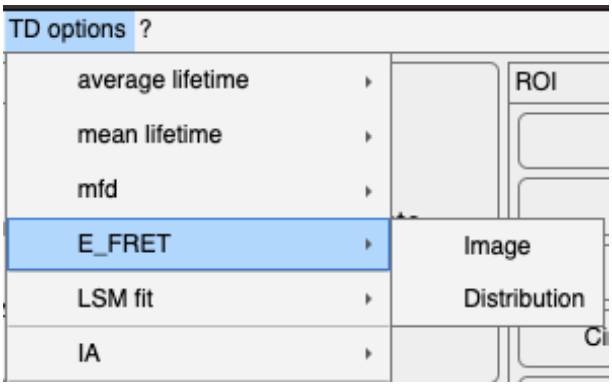

Figure 44: *The "FRET efficiency" menu*

In both cases ("mfd" menu item or "E_FRET" menu item), the window represented in Figure 45 will appear and you will need to enter the donor lifetime value τ_D. The mfd (or E_{FRET}) image or the mfd (or E_{FRET}) distribution will be displayed after.

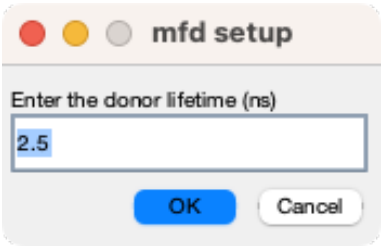

Figure 45: *The "donor' lifetime" window*

- The "LSM fit" menu item enables to perform non linear least square fitting with a trust region reflective algorithm. This function is not optimized and the process can be long according to the size of your image. You have the choice between two simple models : mono-exponential or bi-exponential intensity decays (cf. Figure 46).

  Once you select a model, a pop up window asking to enter the maximum value of lifetime (called "max lifetime") as well as the step between two points (called "steps") will appear. You need to click on the "OK" button. The calculation will then start. When the fitting process is finished, the results will be displayed.

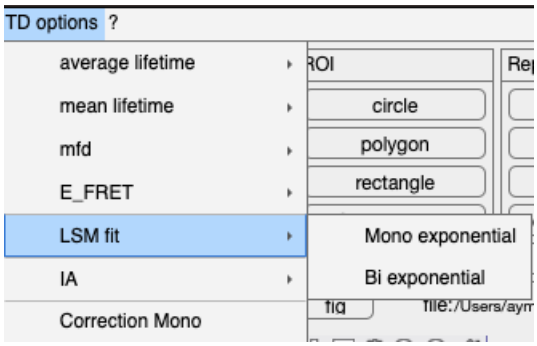

Figure 46: *The "LSM fit" menu*

If you select the mono-exponential model, two figures will appear : one figure with the lifetime histogram (cf. Figure 47-A) and a second figure with the lifetime image (cf. Figure 47-B)

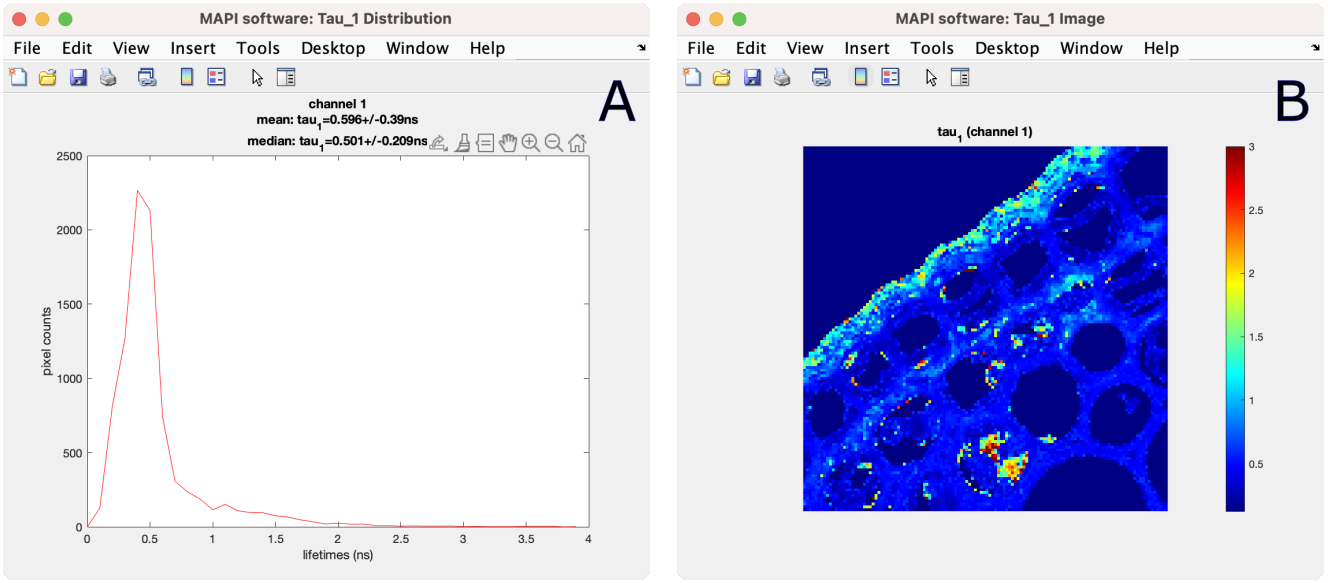

Figure 47: *(A) The lifetime histogram. (B) The lifetime image.*

When you shut down a figure, a warning dialog box appears for saving the current values. By clicking on "Yes", you will have the possibility to save the lifetime values in a new folder. In this new folder, the `info.txt` file is created and the distribution values are stored in three text files in standard ascii format with tab separator. If you opened a stack of images, you will have the possibility to save either the distributions of one stack or the distributions of each stack in different files.

If you select the bi-exponential model, three figures will appear : one figure with the first

lifetime, the mean lifetime (indicated in Eaquation 1) and the proportion a_2 histograms, one second figure with the first lifetime τ_1 image and a third figure showing the proportion a_2 image.

- The "IA" menu item enables to use deep learning algorithm for analyzing the data. The principle of this method is detailed in this publication [Leray2021].
- The "Correction Mono" menu item improves the analyses of FLIM data if the number of channels is low (less than 16). This correction is not necessary when the number of channels is large. The principle of this method is detailed in this publication [Leray2012].
- The "Correction Bi" menu item improves the "Correction Mono" but it is valid only for FLIM data with 5 gates.
- The "No Correction" menu item removes any correction applied.
- The "Time trace" menu item gives access to the time trace of the fluorescence. You can choose to display the time trace of one pixel or of all pixels (cf. Figure 48). This option is accessible only for ptu image (PicoQuant format) because the information is not stored in other formats.

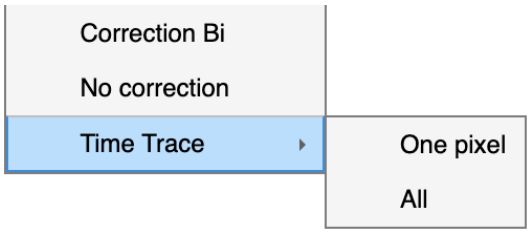

Figure 48: *The "Time Trace" menu*

If you select the "One pixel" option, a window will pop up asking to enter the (X,Y) coordinates of the requested pixel. After selecting them, two windows will appear : one showing the photon count as a function of time (cf. Figure 49-A) and a second showing the evolution of lifetime as a function of time (cf. Figure 49-B).

If you select the "All" option, the calculation will start and it can take several minutes according to the size of your data. When the calculation is done, two windows will appear : one showing the photon count as a function of time and a second showing the evolution of

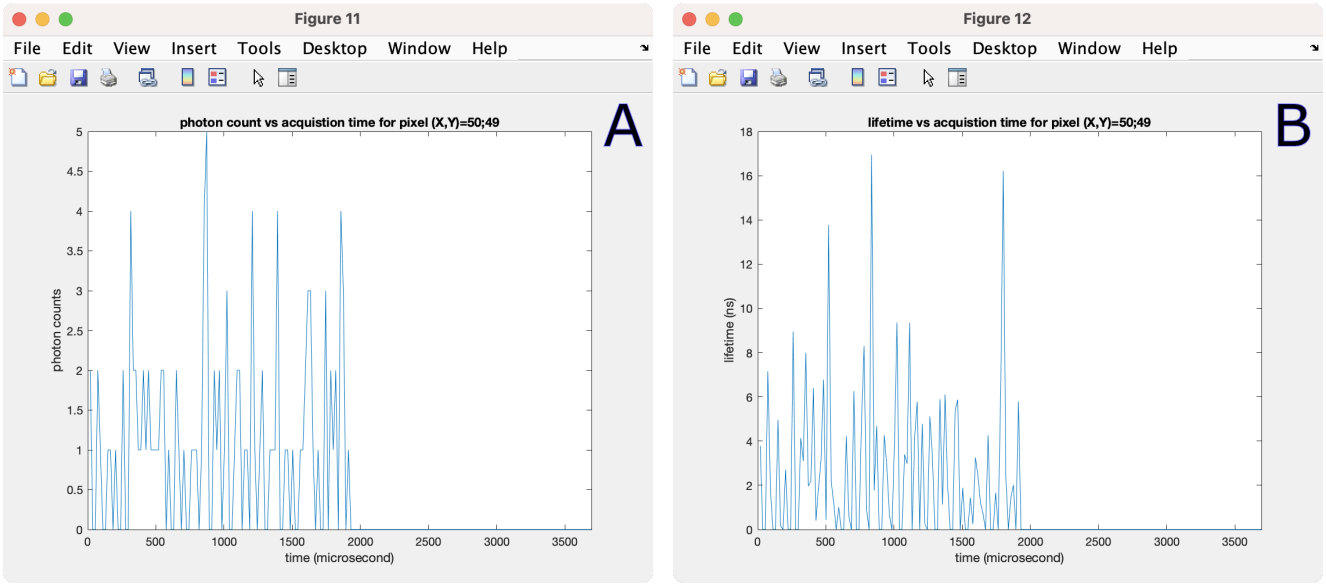

Figure 49: *(A) The photon count as a function of time. (B) The lifetime as a function of time.*

lifetime as a function of time. Due to the large number of pixels, the displaying of the data may be difficult.

When you shut down a figure, a warning dialog box appears for saving the current values. By clicking on "Yes", you will have the possibility to save the lifetime values in a new folder. In this new folder, the `info.txt` file is created and the distribution values are stored in three text files in standard ascii format with tab separator. If you opened a stack of images, you will have the possibility to save either the distributions of one stack or the distributions of each stack in different files.

4.1.5 The " ?" menu

The Help menu which is constituted of 2 items is shown in Figure 50 :

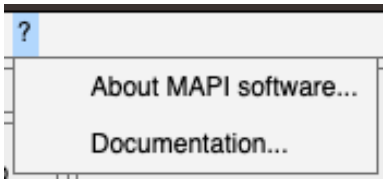

Figure 50: *The " ?" Menu*

- To display information about the MAPI software, click on the "About MAPI software..." menu item. The following window will pop up (Figure 51) :

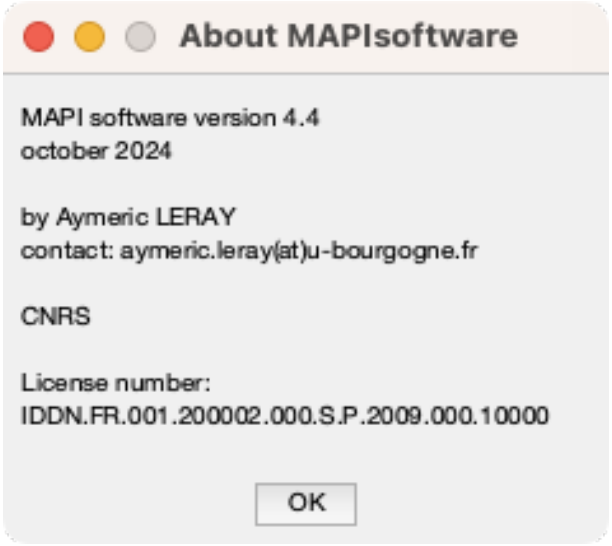

Figure 51: *The "About MAPI software..." dialog box*

  In this window, you can see the version of MAPI that you are currently using. If you need further information, you can contact the team work by visiting the website : https://github.com/ayleray/MAPI-software.

- The "Documentation..." menu item should launch the Adobe Acrobat Reader and display the manual that you are looking now. If you do not have the Acrobat Reader installed, this option will not work. To download and install the latest version of the free reader, please visit the website of Adobe : https://www.adobe.com/.

4.2 The MAPI main window

The main window of MAPI software is represented in Figure 52 :

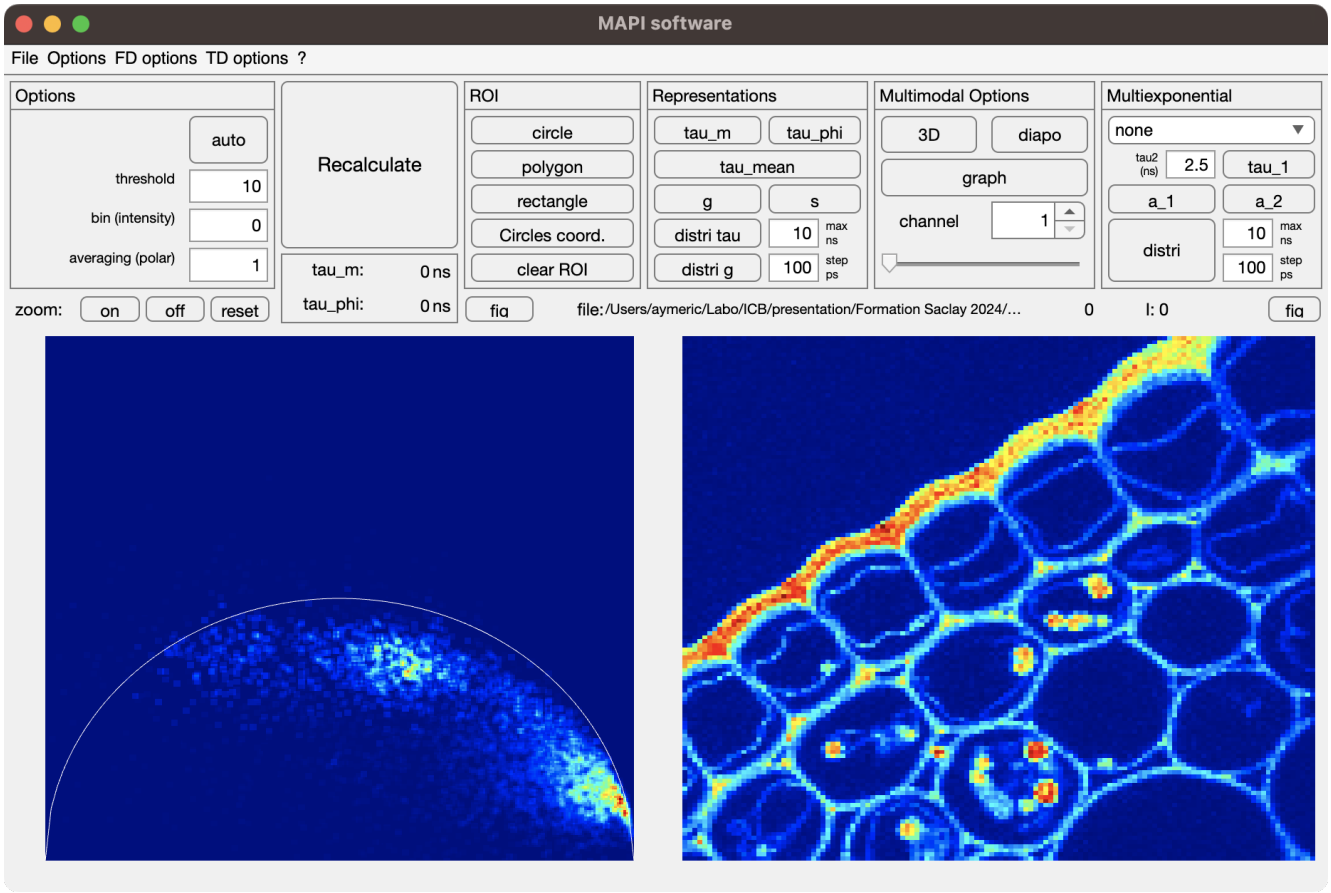

Figure 52: *The Main window of MAPI software*

This main window consists of numerous buttons and options (Figure 52).

The right image is the intensity image of your acquisition. If you want to know the intensity value in a pixel, just left click on the image and the intensity value will be displayed in the intensity part above the image (called “I”).

The left image is the polar representation of your lifetime data. For this image, you can know the phase and modulation lifetime for each pixel (called “tau_m” and “tau_phi”). To do this, left click on the image and the lifetime values will be displayed in nanoseconds in the lifetime part above the polar image.

Above each image, you have a “fig” button which allow to plot the corresponding image in a new figure window (which is a standard Matlab figure environment). Before closing each new

figure, you have the possibility to save the image into a new folder. All the format files describe in the "Export..." menu item are accessible (see section 4.1.1). With this action, you will only save the current image and the `info.txt` file. If you opened a stack of images, a question dialog box allows you to save either one image or the total stack of images in different files.

You have also access to some information about the data file and the experimental parameters in the "file" part above the images. You can see the name of the data file, the time resolution, the channel number and the pixel numbers of your acquisition.

All the other groups of the main window will be described in the next sections of this documentation.

4.2.1 The "Options" group

The "Options" group (illustrated in Figure 53) is located on the left part of the MAPI main window. This group allows you to modify four image processing parameters.

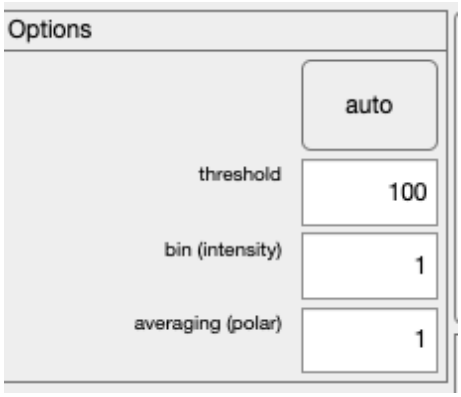

Figure 53: *The "Options" group*

- The "auto button" will calculate the level of the intensity threshold automatically.
- The "threshold" corresponds to the intensity threshold. By default it is fixed to 10. In brief, only the intensity pixels whose the total photon number is superior to the threshold will be take into account in the calculation of the polar representation. This parameter is a well known and simple segmentation method in image processing.
- The "bin (intensity)" is the standard pixel binning parameter n of the intensity image. It refers to the addition of the information of a $2n+1 \times 2n+1$ matrix pixels into the single center

pixel and it is calculated for all pixels of the intensity image. For instance, by applying an intensity bin of 1, the total photon number of a 3×3 pixels matrix is attributed to the centered pixel and this operation is realized for each pixel. By default this parameter is fixed to 0 and can vary from 0 to 10.

- The "averaging (polar)" parameter is the size of the standard averaging filter. By default it is fixed to 1 which represents a 3×3 pixels matrix. It can vary from 0 to 10.

4.2.2 The "Zoom" button group

The "Zoom" button group is also located on the left part of the MAPI main window. It is composed of three buttons (Figure 54) :

Figure 54: *The Zoom button group*

- The "on" button gives you access to the standard Matlab zoom environment. By left clicking, you zoom in and by right clicking you zoom out. The zoom option can be used for both polar and intensity images. However, when the zoom environment is "on", you can not anymore display the phase and modulation lifetime values of the polar image (called "tau_m" and "tau_phi") nor the intensity value (called "I") of the intensity image by directly clicking on the selected image.
- To redisplay the lifetimes values or the intensity, just click on the "off" button of the "Zoom" button group.
- The "reset" button enables to reset the zoomed image(s) to the(ir) original view(s) and put the zoom environment "off".

4.2.3 The "ROI" button group

The "ROI" button group is placed on the center of the MAPI main window and it is represented in Figure 55 :

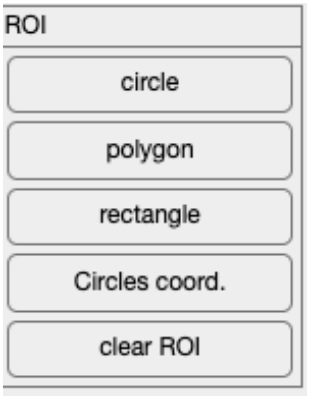

Figure 55: *The "ROI" button group*

This option allows to create a Region Of Interest (ROI) on the polar image or the intensity image in order to select only the desired pixels. You can choose to create a "circle", a "polygon", a "rectangle" or multiple ROI with "circle coordinates" according to your data.

- The "circle" ROI creation was explained in the section 3.2.
- By clicking on the "polygon" button, you will see the following window (Figure 56) :

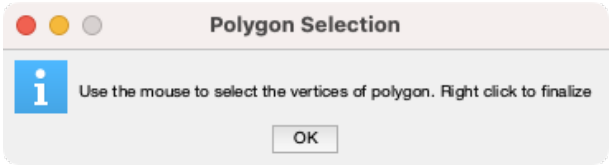

Figure 56: *The polygon ROI selection window*

  To create a "polygon" ROI on the polar image (left image), you have first to move the mouse cursor to the desired first point and left click then move the cursor to the desired second point and left click. You will see a line linking these two points. Do it until you obtain the desired region and right click to finalize the ROI. After this, the intensity mask is calculated and displayed on the intensity image if you performed the ROI on the polar image. Otherwise, the polar representation is calculated only for the corresponding ROI in the intensity image.

- When you press the "rectangle" button, the following window will pop up to explain you the creation of the ROI (Figure 57) :

  To create a "rectangle" ROI on the polar image (left image), you have first to move the mouse cursor to the desired up left point of the rectangle and left click. Then move the cursor to

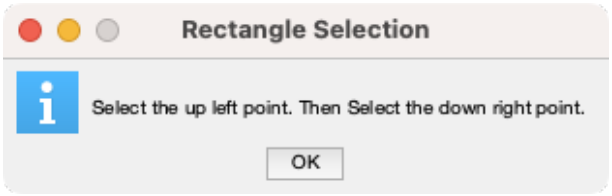

Figure 57: *The rectangle ROI selection window*

the down right desired point of the rectangle and left click. After this, the rectangle ROI is finalize and the intensity mask is calculated and displayed on the intensity image if you draw the ROI in the polar image. Otherwise, the polar representation is calculated only for the corresponding ROI in the intensity image.

- When you click on the "Circles coord." button, you will se the following pop up (Figure 58) :

Figure 58: *The circles coordinates selection window*

You need to enter the number of ROI that you want, the coordinates (X,Y) of their centers and their radii. After this, the circles ROI are calculated and displayed for the polar image only (it does not work for the intensity image).

- Finally, when you press the "Clear ROI" button, all the ROIs are removed.

4.2.4 The "Representations" button group

The "Representations" button group (illustrated in Figure 59) allows you to display the lifetime images or the lifetimes distributions.

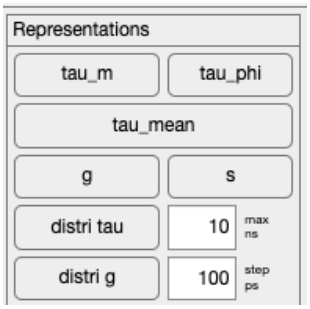

Figure 59: *The "Representations" button group*

- By pressing the "tau_m" button and displaying the look up table of the image, you will see the Figure 60.

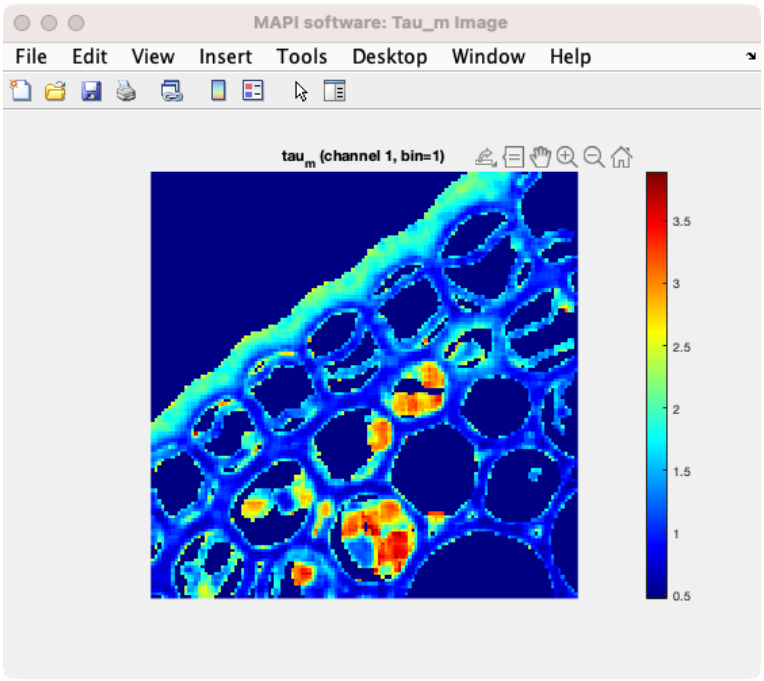

Figure 60: *The modulation lifetime image*

On this figure environment, you can see the modulation lifetime image of your sample in nanosecond. In other words, the look up table represented on the right of the image corresponds to the modulation lifetime values in nanosecond. You can also have access to some

figure options by clicking on the right button of the mouse. In this case, the menu indicated in Figure 61 will appear. Note that this option is accessible in all figure environment.

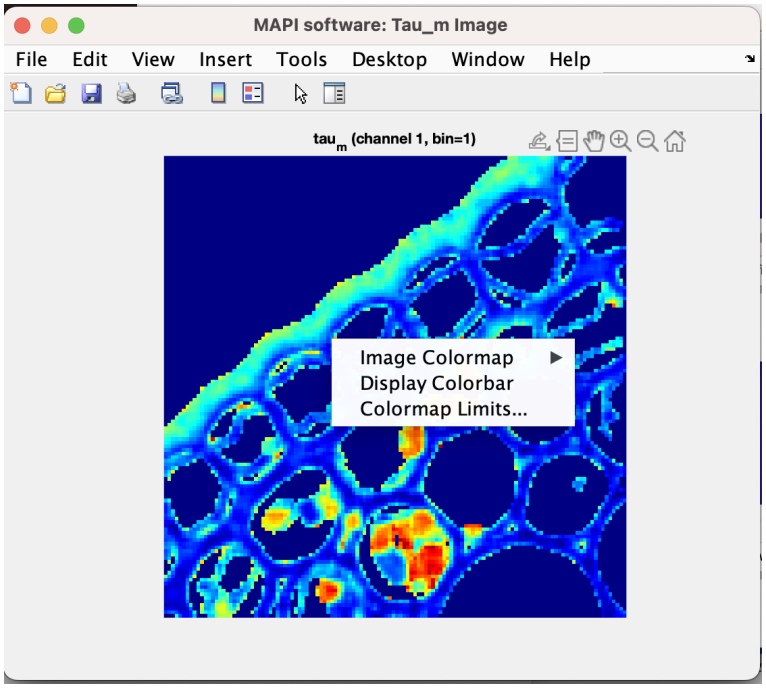

Figure 61: *image menu*

— The "image colormap" menu item allows you to change the current look up table (or colormap) of the image. By default, it is fixed to "jet" (more details in section 4.1.2).

— The "display colorbar" menu item enables to display or not the current look up table on the right of the image.

— The "colormap limits..." menu item allows you to modify the minimal and maximal values of the colormap.

- If you press the "tau_phi" button, the phase lifetime image will be displayed and when you click on the "tau_mean" button, you will see the mean lifetime image of your sample (the mean of the phase and modulation lifetimes). All these lifetime values are also expressed in nanoseconds.

  When you shut down a lifetime figure (modulation, phase or mean), a warning dialog box appears for saving the current image. By clicking on "Yes", you will have the possibility to save it on a new folder. All the format files describe in the "Export..." menu item are

accessible (see section 4.1.1). With this action, you will only save the current lifetime image and the `info.txt` file. If you opened a stack of images, a question dialog box allows you to save either one image or the total stack of images in different files.

- The "distri tau" button enables to display the distributions of the modulation (tau_m), phase (tau_phi) and mean lifetime (tau_mean) values on a same new window (see Figure 62). You can choose the maximum value of the x-axis (called "max") as well as the step between two points (called "step").

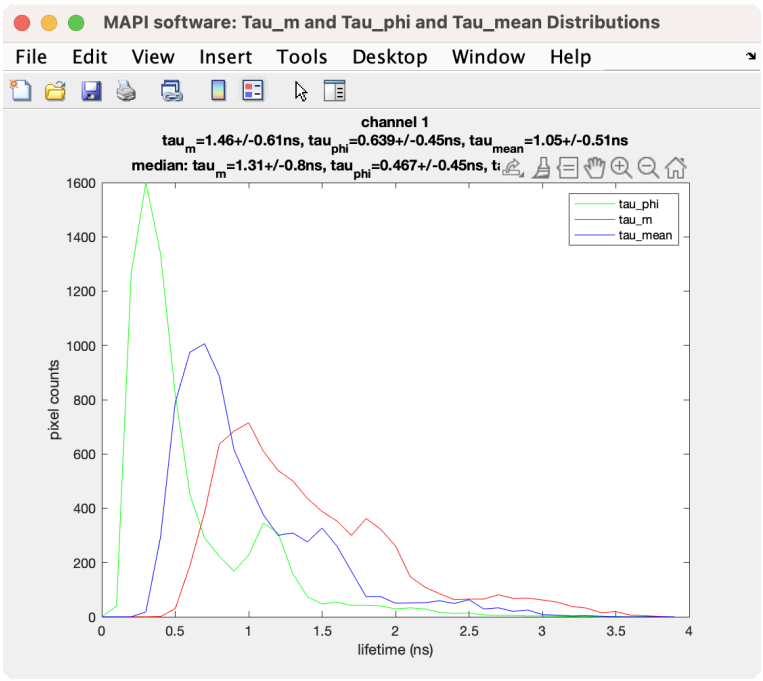

Figure 62: *Lifetime Distribution*

The x-axis is the lifetime value in nanoseconds and the y-axis corresponds to the pixel count in pixel number. On this new window, in the title, you can also know the mean value and the median of all lifetimes.

When you shut down a distribution figure, a warning dialog box appears for saving the current values. By clicking on "Yes", you will have the possibility to save the lifetime values in a new folder. In this new folder, the `info.txt` file is created and the distribution values are stored in three text files in standard ascii format with tab separator. If you opened a stack of images, you will have the possibility to save either the distributions of one stack or the distributions of each stack in different files.

Last important remark : if you create a ROI on the polar image, the lifetime images display only the pixels located inside the ROI and the lifetime distributions are calculated only for the pixels located inside the ROI.

- when you click on the “g” button, , you will see the Figure 63.

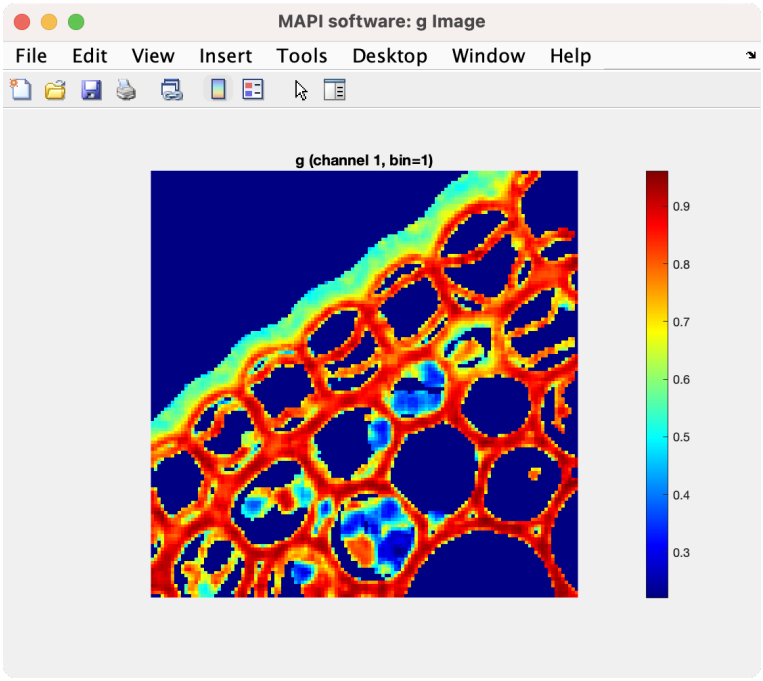

Figure 63: *The g coordinate image*

- If you press on the “s” button, , you will see the s coordinate image. When you shut down a g or s coordinates figure, a warning dialog box appears for saving the current image. By clicking on “Yes”, you will have the possibility to save it on a new folder. All the format files describe in the “Export...” menu item are accessible (see section 4.1.1). With this action, you will only save the current lifetime image and the `info.txt` file. If you opened a stack of images, a question dialog box allows you to save either one image or the total stack of images in different files.
- The “distri g” button enables to display the distributions of the g and s coordinates on a same new window (see Figure 64). You can choose the maximum value of the x-axis (called “max”) as well as the step between two points (called “step”).

  The x-axis is the g or s coordinates values (comprised between 0 and 1) and the y-axis

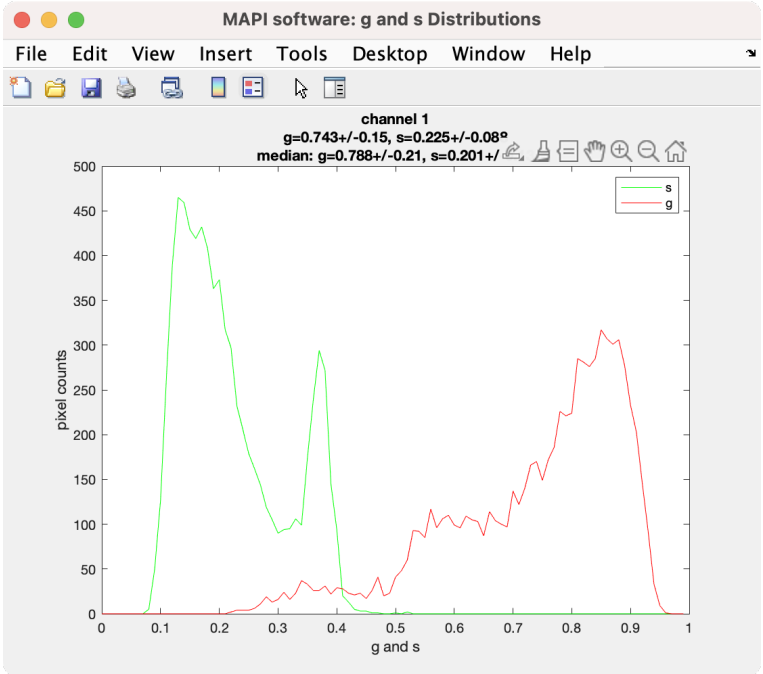

Figure 64: *g and s coordinates distributions*

corresponds to the pixel count in pixel number. On this new window, in the title, you can also know the mean value and the median of the coordinates.

When you shut down a distribution figure, a warning dialog box appears for saving the current values. By clicking on "Yes", you will have the possibility to save the lifetime values in a new folder. In this new folder, the `info.txt` file is created and the distribution values are stored in three text files in standard ascii format with tab separator. If you opened a stack of images, you will have the possibility to save either the distributions of one stack or the distributions of each stack in different files.

4.2.5 The "Multimodal options" group

The "Multimodal options" group (represented in Figure 65) is accessible only if you have opened a stack of FLIM images.

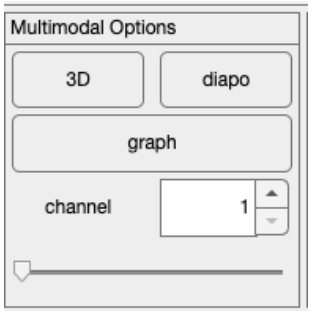

Figure 65: *The "Multimodal options" group*

- The "3D" button enables you to display the three dimensional polar representation of your stack of FLIM images in a new Matlab figure environment (see Figure 66). By pressing the "Rotate 3D" button you can modify the point of view of you 3D representation.

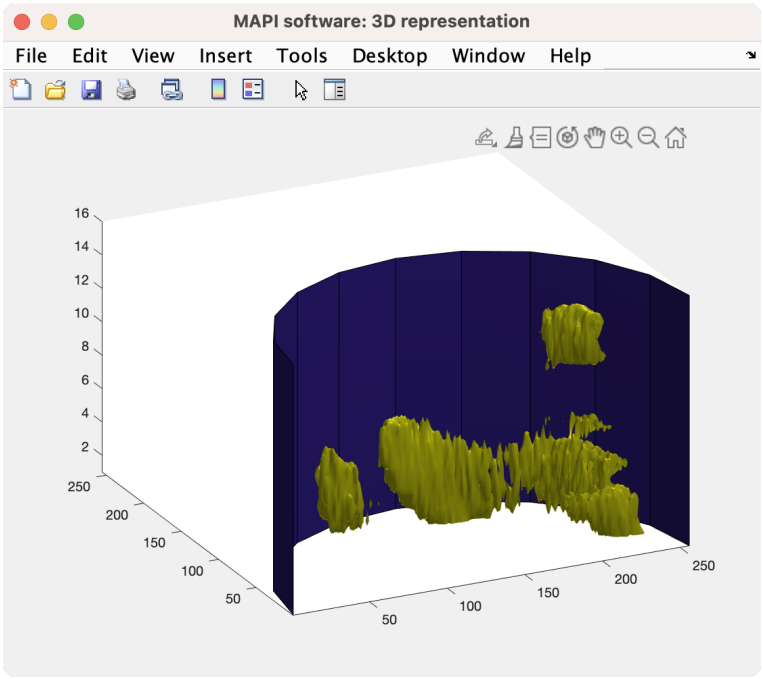

Figure 66: *The three dimensional polar representation window*

- The "diapo" button will also pop up a new Matlab figure environment. This time, it will display the polar image of each stack on a same window. Note that the polar representation are normalized on the maximum of all channels.

- The “graph” button allows displaying a new Matlab figure environment that represents the mean phase lifetime, modulation lifetime and mean lifetime as a function of the spectral channel (or time depending on your experiment), as indicated in Figure 67.

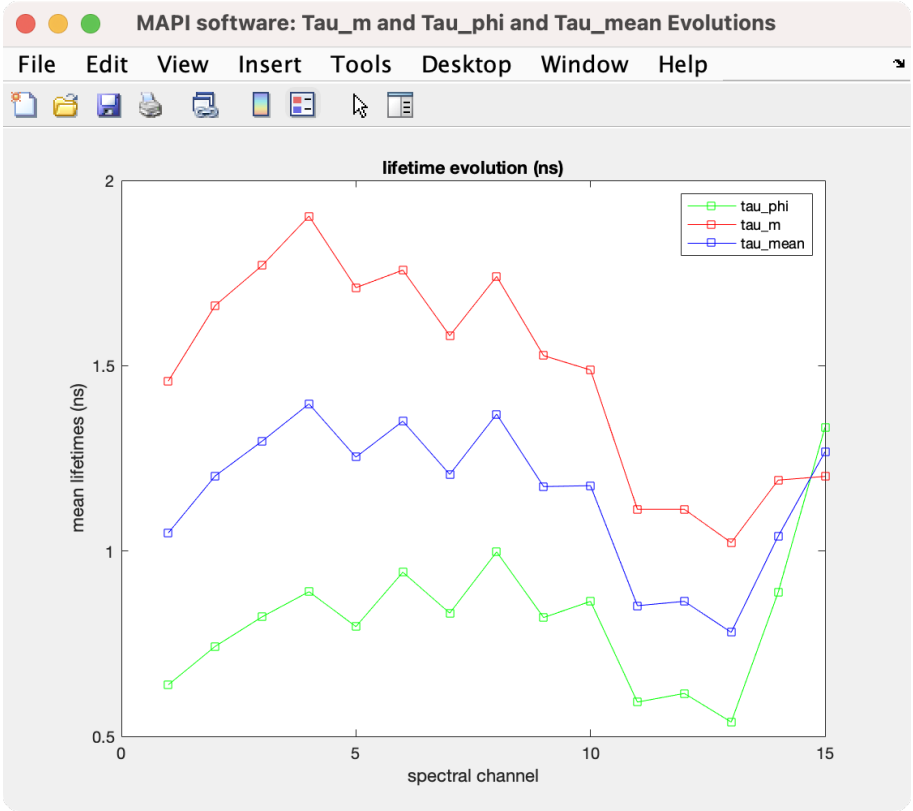

Figure 67: *The evolution of the lifetime window*

- Finally, the “channel” slider permits to modify the current stack which is displayed on the MAPI main window. It is important to remark that all functionalities of the “Representations” button group and the “FRET options” group are calculated only for the current stack channel.

4.2.6 The "Multiexponential" group

The "Multiexponential" group (see Figure 68) allows you to calculate and display the lifetime components in FRET experiments.

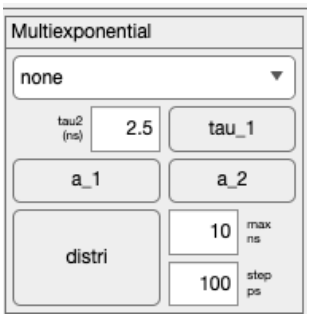

Figure 68: *The "Multiexponential" group*

- You need first to select an algorithm. You have to choose between three different algorithms : "polar", "moment" and "RLD".

  The polar algorithm is described in this publication : [Leray2009].

  The moment algorithm is detailed here : [Leray2013].

  The RLD algorithm is described here : [Sharman1999].

- Once you have selected an algorithm, in the "tau2" edit box, you can enter the lifetime value of the donor in nanoseconds.

- When you click on the "a_2" button, a new standard Matlab environment figure appears (cf. Figure 69). On this figure, you will see the donor proportion (called a_2) image. The donor proportion values are comprised between 0 and 1.

- The "tau_1" button displays a new figure representing the acceptor lifetime image of your sample in nanoseconds. In other words, the look up table corresponds to the acceptor lifetime values in nanosecond.

  When you shut down a figure (donor proportion a_2 or acceptor lifetime tau_1), a warning dialog box appears for saving the current image. By clicking on "Yes", you will have the possibility to save it on a new folder. All the format files describe in the "Export..." menu item are accessible (see section 4.1.1). With this action, you will only save the current image

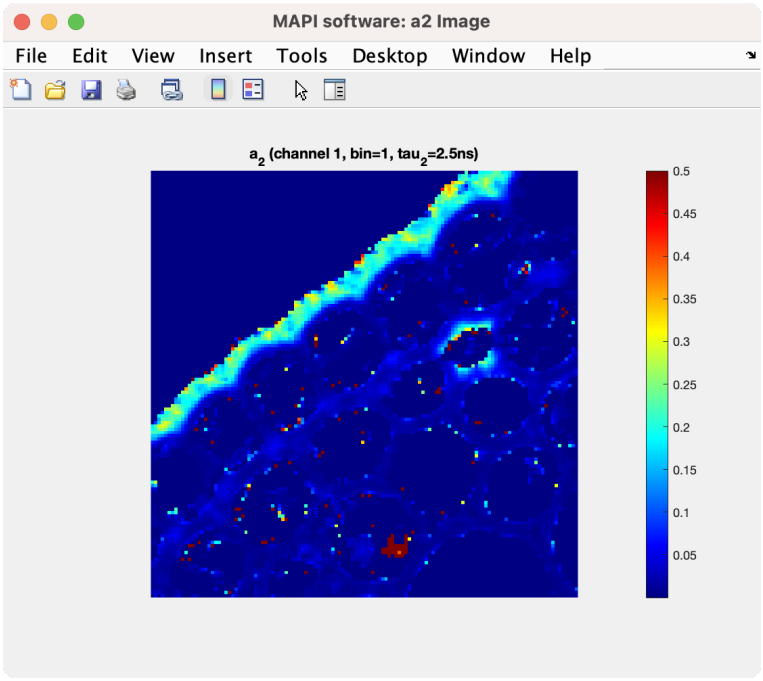

Figure 69: *The a_2 image*

and the `info.txt` file. If you opened a stack of images, a question dialog box allows you to save either one image or the total stack of images in different files.

- The "distri" button display on a same new window the distributions of : the acceptor lifetime (tau_1), the donor proportion (a_2) and the mean lifetime (tau_mean). You can choose the maximum value of the x-axis (called "max") as well as the step between two points (called "step").

  The x-axis is the lifetime value in nanoseconds (or the donor proportion) and the y-axis corresponds to the pixel count in pixel number. On this new window, in the title, you can also know the mean value and the median of all parameters.

  When you shut down a distribution figure, a warning dialog box appears for saving the current values. By clicking on "Yes", you will have the possibility to save the lifetime values in a new folder. In this new folder, the `info.txt` file is created and the distribution values are stored in three text files in standard ascii format with tab separator. If you opened a stack of images, you will have the possibility to save either the distributions of one stack or the distributions of each stack in different files.

  Last important remark, like for the "Representations" button group : if you create a ROI

on the polar image, the proportion or lifetime images display only the pixels located inside the ROI and the distributions are calculated only for the pixels located inside the ROI.

5 Theory

To be implemented

Références

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[Leray2012] A. Leray, C. Spriet, D. Trinel, Y. Usson et L. Héliot, « Generalization of the Polar Representation in Time Domain Fluorescence Lifetime Imaging Microscopy for Biological Applications : practical implementation », *J. Microsc.*, **248**, 66–76 (2012). p.30

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