3.3 MATLAB® Toolbox Development

To analyse the data obtained through the usage of a TCSPC setup described in Section 3.2, a MATLAB® toolbox was developed during this Summer Internship. In this section, some of the most relevant aspects of how data is stored and accessed from a "flim" structure will be shown, as well as a brief instruction manual with the main guidelines on how to use the interface.

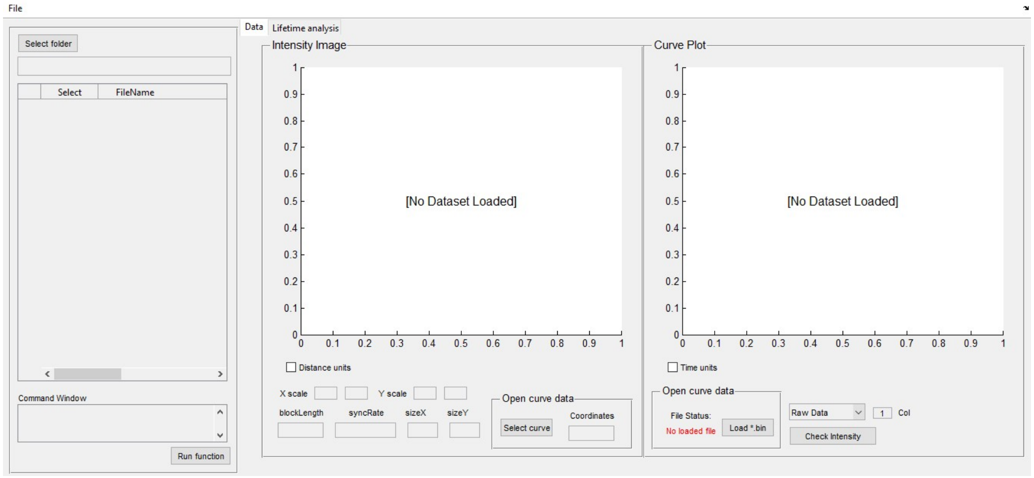
3.3.1 Main Features

The purpose of this platform is to allow the user the possibility to load and analyse FLIM data in a simple and practical way. It is important to note that in the setup described in Section 3.2, the measured data is stored in two types of file: *.hdf5 files and *.bin files, whose contents are described in table 5. Keeping that in mind, developed MATLAB® toolbox can thus be seen as a simpler way to manipulate these files and extract information from them.

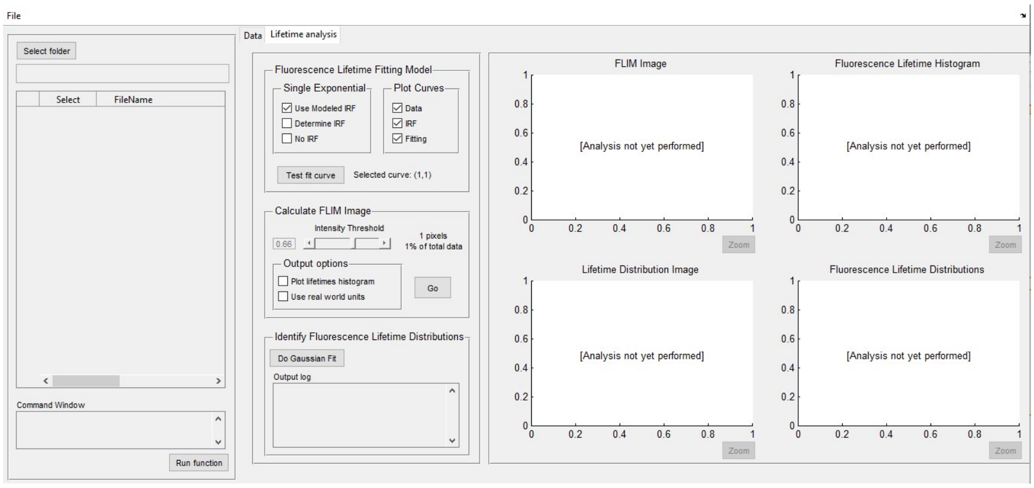
Table 5: The two types of file exported by the system described in Section 3.2.

File Extension	Contents
*.hdf5	Header information: Relevant experimental parameters Intensity Image: Each pixel contains the integral of a decay curve.
*.bin	Raw data: All the fluorescent curves of the scanning.

Figure 7 shows the overall appearance of the designed interface, which can be divided in three sections: The folder explorer panel (on the left side), the Data tab and the Lifetime analysis tab. The folder explorer panel allows the user to dynamically browse through multiple datasets in a folder. In the Data tab, the user can visualise an intensity image, which is extracted by the program from the selected *.hdf5 file and the decay curve (which is extracted by the program from the correspondent *.bin file) from a specific pixel selected by the user in the intensity image. In the Lifetime analysis tab, the user can test several single exponential decay models to the decay curves and use one of them to fit multiple curves and form a fluorescent lifetime image. Furthermore, it is possible to plot the lifetime histogram of that image and perform a Gaussian fit to that histogram, so as to determine the lifetime distribution in that image.



(a) Data viewer tab



(b) Lifetime analysis tab

Figure 7: Screen shot of the implemented MATLAB® toolbox interface.

3.3.2 Loading data

When working with multiple data sets, an efficient method of browsing through data can be very useful. The folder browsing tool shown in Figure 8 allows the user to select a directory where data is stored by clicking in the "Select folder" button. By performing this action, the names of all the files with file extension *.hdf5 in that directory will be loaded into the table also shown in Figure 8. To open one file, the user needs only to click in the corresponding checkbox in the "Select" column.

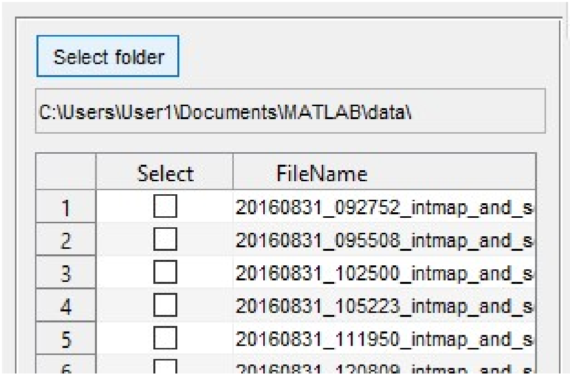


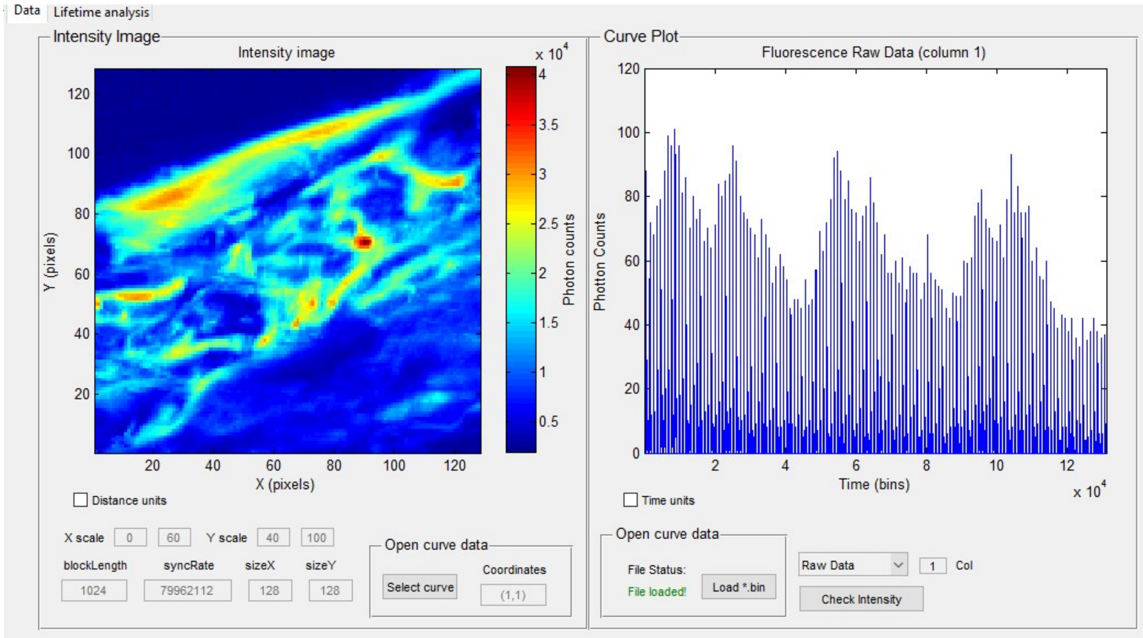
Figure 8: Folder browsing tool. The button "Select folder" allows the user to open a directory. The user can then select which file to open in the "Select" column.

3.3.3 Observing data

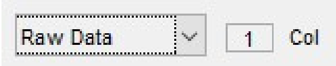
After selecting a folder and specifying an *hdf5 file to be opened, its intensity image will be represented on the "Intensity Image" axes and the raw data extracted from the correspondent *.bin file will be represented in the "Curve Plot" axes. This tool allows the user to visualize the data as it is stored in the *.bin file, which means that it will display the decay curves from one entire column. To select which column to plot, insert the column value in the "Col" text box from "Curve Plot" panel (see Figure 9b).

Because each pixel in the intensity image stored in an *.hdf5 file represents the integral of the fluorescent decay curve acquired in that position, in can be interesting to plot this curve for a specified pixel in the intensity image. To do so, the user can either select the option "Decay Curves" in the pop-up menu show in Figure 9c or use the "Open curve data" tool (see Figure 10a). By clicking in the "Select curve" button, the pointer is replaced by a cross that allows the user to select a pixel from the intensity image, whose decay curve is to be plotted in the "Curve Plot" axes (see Figure 10b).

Because acquisition and/or loading errors may eventually occur, a fast way to evaluate the correspondence between the *.hdf5 file and the *.bin file is to click the "Check intensity button". This tool will generate an intensity image by calculating the integrals of each decay curve and then compare it to the original intensity image stored in the *.hdf5 file. A data set with no errors should generate a comparison image that is exclusively composed of zeros, just like the one showed in Figure 11a.If however there is a missmatch between the information stored in the *.hdf5 and *.bin files, it will be displayed as non zero values in the comparison image generated by this tool (see the example in Figure 11b).



(a) Once a file is loaded, its intensity image is loaded into the "Intensity Image" axes.



(b) When in "Raw Data" mode, insert the column index in the "Col" textbox.

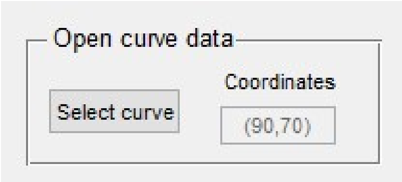
(c) When in "Decay Curves" mode, a decay curve from a single pixel is plotted.

Figure 9: Data visualization tab. The intensity image The "Curve Plot" axes can be used to represent a full column (b) of raw data or the decay curve of a specified pixel (c).

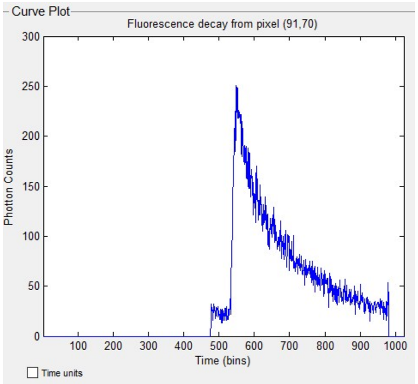
3.3.4 Calculating a FLIM image

To calculate the Fluorescence Lifetime image, the software will run the previously selected fitting function through the data matrix and build an image with the calculated lifetime values. In this analysis, the number of pixels to be evaluated is defined by the "Intensity Threshold", which can be defined with the slider shown in Figure 12. When the "Go" button is pressed, every pixel whose value is above the selected intensity threshold will be evaluated. This functionality can be used not only for a faster analysis of the most relevant data, but also for eliminating background pixels whose intensity may sometimes be too low to be fitted, which could lead to critical MATLAB® errors.

By selecting the "Plot lifetimes histogram" checkbox, a histogram of the lifetime values obtained in the Lifetime image will be plotted in the "Fluorescence Lifetime Histogram" axes.



(a) Curve Selection tool.

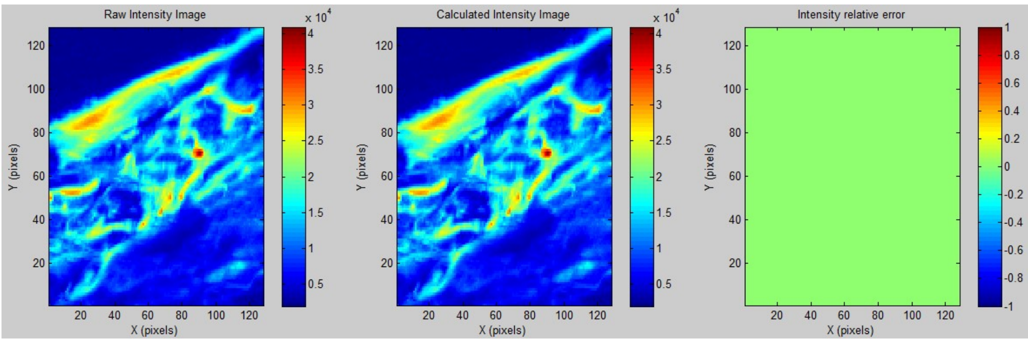


(b) Decay curve from a specific pixel

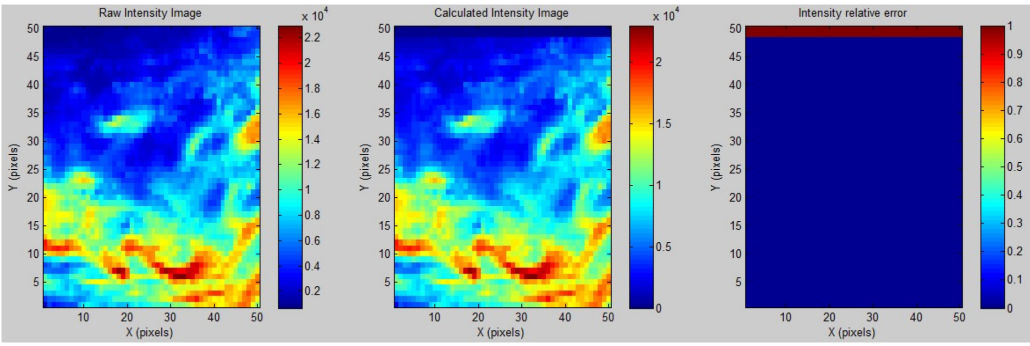
Figure 10: Plotting the curve of a specified pixel. The "Open curve data" tool allows the user to select a pixel from the intensity image - coordinates (90,70) in this example, so that it can be plotted in the "Curve Plot" axes.

3.3.5 Calculate lifetime distributions

bla bla



(a) Acquisition with no error.



(b) acquisition with and error in the last to rows.

Figure 11: The "Check intensity" tool calculates an intensity image by integrating all the curves contained in the *.bin file and compares it with the original intensity image. If everything is correct, the relative error image should only contain zeros.

4 RESULTS

5 CONCLUSION

0

test [1] bla [2]

REFERENCES

[1] Martin A Bopp, Yiwei Jia, Liangquan Li, Richard J Cogdell, and Robin M Hochstrasser. Fluorescence and photobleaching dynamics of single light-harvesting complexes. *Proceedings of the National Academy of Sciences*, 94(20):10630–10635, 1997.

[2] Alberto Diaspro, Giuseppe Chirico, Cesare Usai, Paola Ramoino, and Jurek Do-

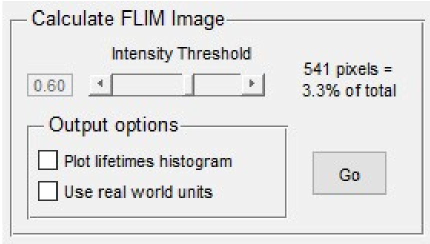
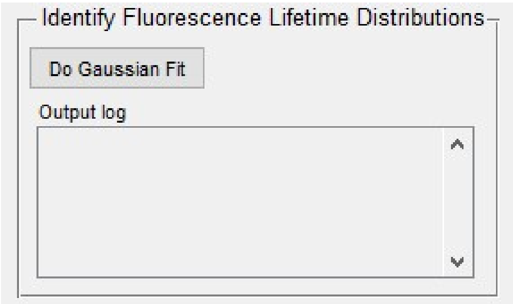
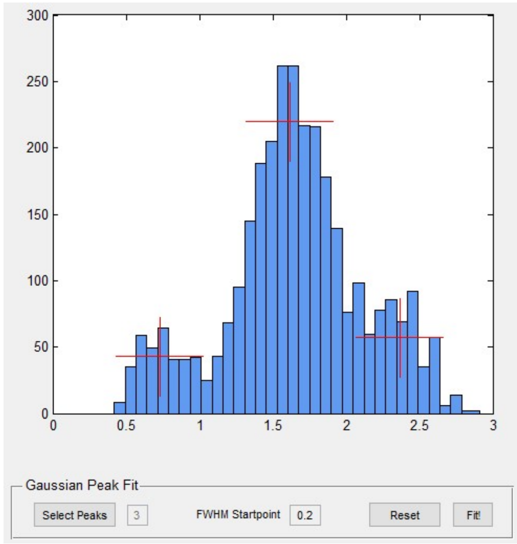


Figure 12: Lifetime calculation panel. In this tool, the "Intensity Threshold" defines the minimum intensity value that a pixel must have to be evaluated.



(a) Acquisition with no error.



(b) acquisition with and error in the last to rows.

Figure 13: The "Identify Dist...."

brucki. Photobleaching. In *Handbook of biological confocal microscopy*, pages 690–702. Springer, 2006.