

Using QuickFluo

You can use the QuickFluo module immediately after the installation of the software. The data to be analyzed must be in “TIFF” format. **New in version 1.1** the software can also analyse stack of “JPG” format images. The images of a same stack must have the same name followed by the number of the images. For example for a stack of 10 images you will have: name1.jpg, name2.jpg...name10.jpg.

QuickFluo is available as an executable file or as an open-source Matlab module. The user interface is shown in Fig.1 and the various routines used by QuickFluo are illustrated in Fig. 2.

How to use QuickFluo

SELECTION OF PARAMETERS

- Select the dye type: ratiometric or non ratiometric (Fig. 1, area 1).
- Click on “Load TIFF” (Fig. 1, area 2) or “Load JPG” (Fig. 1, area 3) **in version 1.1** to open a browse window for selection of the file and the recording to be imported. The name of the recording appears in the next area (Fig. 1, area 4). In **version 1.1** select the first file of the stack which is called *name1.jpg*.
- Define the parameters:

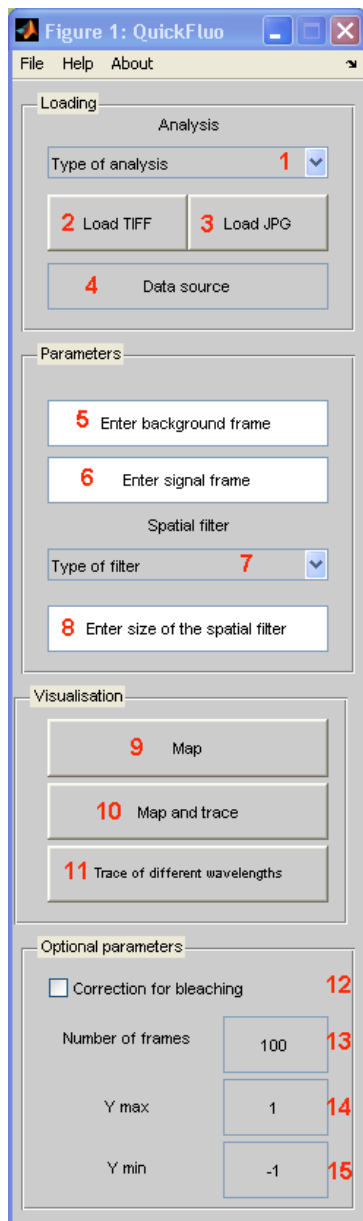


Figure 1: Graphic user interface of QuickFluo. Please refer to the text for explanation of the various areas

- Background frame (Fig. 1, area 5): a frame before stimulation, selected for use as the background F_{bi} for the calculation of $\Delta F/F = (F_{si} - F_{bi})/F_{bi}$.
- Signal frame (Fig. 1, area 6): the frame to be used as the F_{si} for the calculation of $\Delta F/F = (F_{psi} - F_{pbi})/F_{pbi}$ and for the construction of the map. The map shows variation in fluorescence at the selected moment in the recorded zone.
- Choose the type of filter (Fig. 1, area 7) to be applied: average, Gaussian, median, none.

- Size of the spatial filter (Fig. 1, area 8): a spatial filter is used to calculate the new value of a pixel, by applying a particular function to the pixels around the main pixel. The size of the spatial filter is the number of pixels used to calculate the new value. For example, an average filter of size 5 calculates the average over an area of 5 x 5 pixels.

- To visualize the results of the analysis:
 - **Map** (Fig. 1, area 9) builds a false-colour map of changes in fluorescence in a new window.
 - **Map and trace** (Fig. 1, area 10) builds the same map except that when you click on the map, it traces the curve of changes in fluorescence for the area around the pixel selected (by the mouse click) in a new window.
 - **Trace of different wavelengths** (Fig. 1, area 11) builds a map and the curves corresponding to the two wavelengths when ratiometric dyes are used. As in “map and trace”, the curves are obtained by clicking on the map. The curves show changes in fluorescence for the area around the pixel selected by the mouse click.

- Some additional parameters can be changed
 - Checking “Correction for bleaching” (Fig. 1, area 12) applies a correction for the decrease in fluorescence due to bleaching. This correction can be applied only if non ratiometric dyes are used.
 - “Number of frames” (Fig. 1, area 13) is the number of frames over which your data are recorded.

- Y max (Fig. 1, area 14) is the maximum value of the ordinate on the time-course graphs (corresponding to $\Delta F/F$ for a single-wavelength analysis or to $\Delta Fw1/ \Delta Fw2$ when two different wavelengths, $w1a$ and $w2$, are used).
- Y min (Fig. 1, area 15) is the minimum value of the ordinate on time-course graphs (corresponding to $\Delta F/F$ for a single-wavelength analysis or to $\Delta Fw1/ \Delta Fw2$ when two different wavelengths, $w1a$ and $w2$, are used).

How to change default values

The default values of some parameters can be modified in the open-source Matlab module version of QuickFluo

- You can change the size of the area of the map used to calculate changes in fluorescence on time-course curves
- You can also change the default values of the number of frames, Y max and Y min (these can also be changed in the interface)

To make changes:

- The time curve is calculated using the values of a square of 5 pixels around the point selected. You can change the size of this area in the .mat file named “trace,” at line 15.
- Open the *QuickFluo.m* file using Matlab and change the value in line 41 for frames, line 65 for Y max and line 68 for Y min.

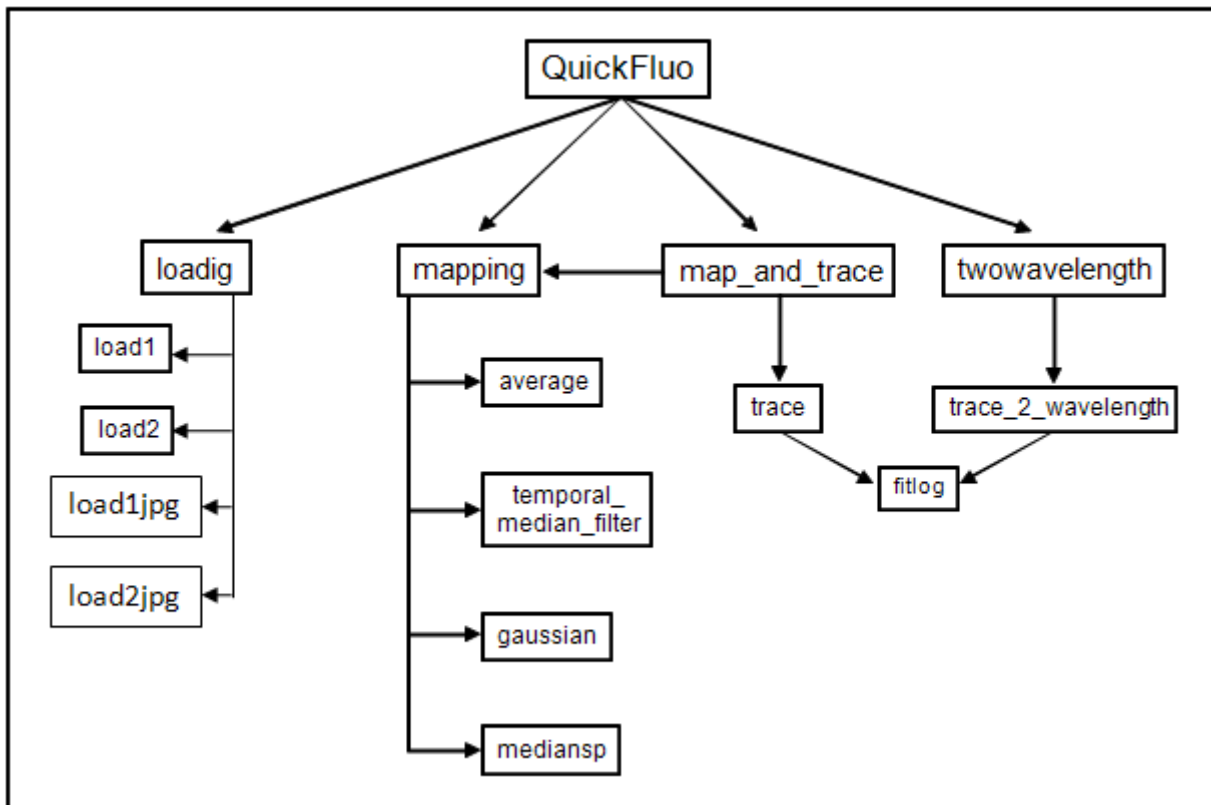


Figure 2: Diagram of the subroutines used by QuickFluo