AIC FRAP UTILITY USER MANUAL

Installation and Starting the Utility

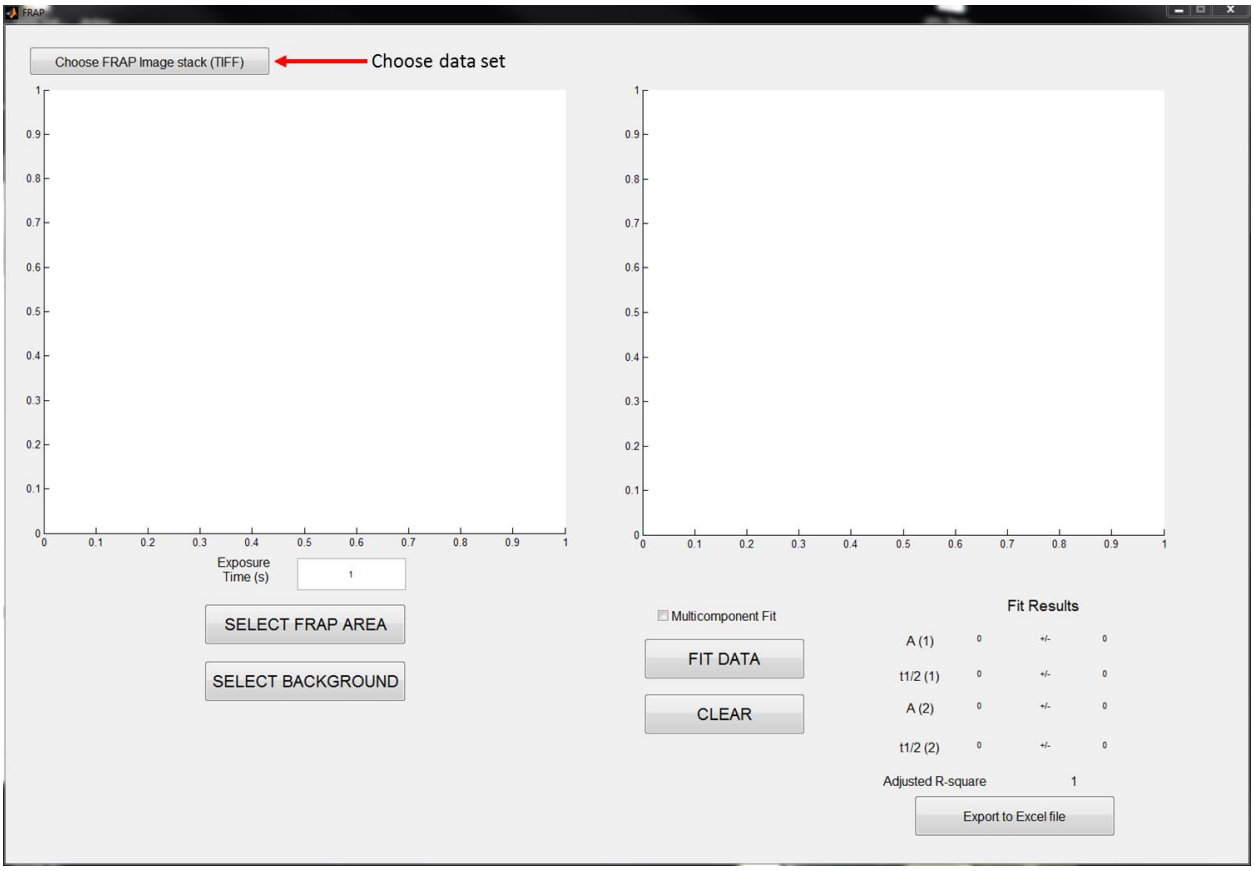
1. Unzip the contents of the **FRAP.zip** file into your Matlab working directory. This .zip file contains two Matlab files (FRAP.m and FRAP.fig). It also contains a sample data set to test the utility (FRAP.tif). You can copy the FRAP.tif file to any location on your computer.

2. From the Matlab prompt, type **FRAP**.

3. The utility window should appear, and look like the image below.

Loading Image Data

4. Click "**Choose FRAP Image stack (TIFF)**", and use the dialog box to navigate to the location of the sample data set **FRAP.tif**, or any FRAP image data you want to analyze.

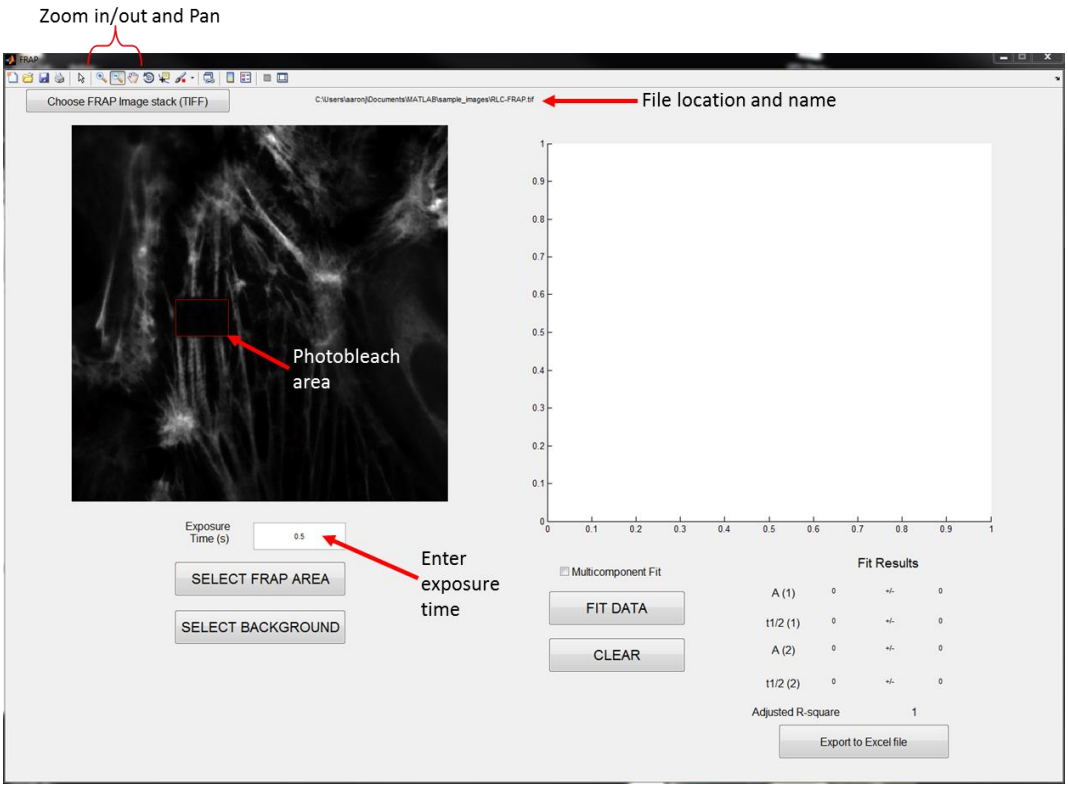


5. The left display should now show an image. Also, the location and name of the file you chose will be displayed to the right of the "**Choose FRAP Image stack (TIFF)**" button.

6. Under the image display, enter the exposure time (in seconds) that was used to acquire the data. For the test data, you can enter an arbitrary number.

7. The left display shows the *minimum intensity* projection of your data (i.e. the minimum signal over the whole image series), so the area that underwent targeted photobleaching should be apparent.

8. You can use the menu bar above the displayed image to zoom in/out, and pan in any direction to better find the area that underwent targeted photobleaching.



Selecting Areas for Analysis

9. Click the "SELECT FRAP AREA" button. The mouse cursor should change from an arrow to crosshairs. With the image zoomed in (if needed), draw a box around the area that underwent photobleaching. The area you chose will be indicated by a blue box.

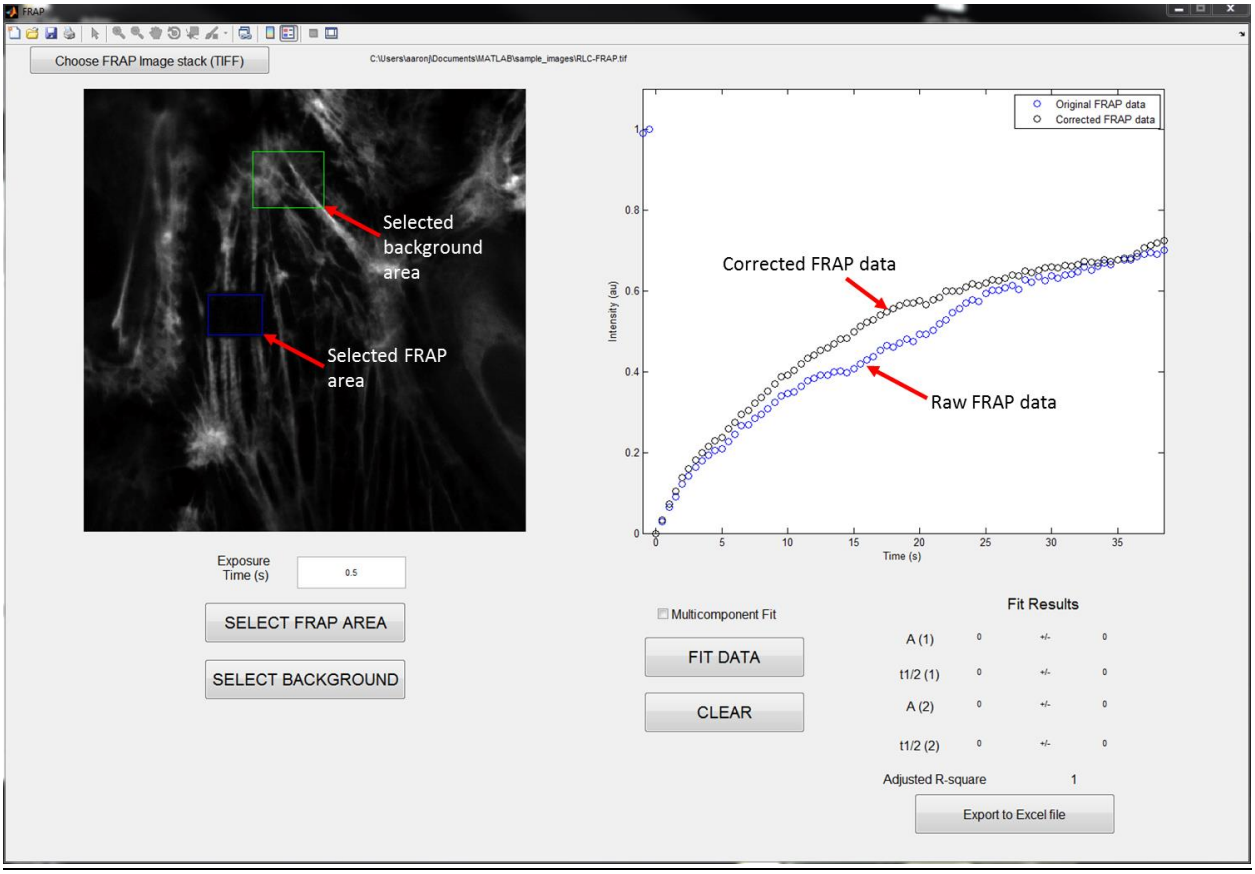
10. If you want to re-do your selection, you can. Just click the "SELECT FRAP AREA" button again, and re-draw the blue box.

11. The right display window will show a plot of the average signal intensity over time, indicated by blue circles. This is the raw data, and is not corrected for any additional photobleaching that occurred while imaging.

12. Once you are happy with your selection of the FRAP area, click the "SELECT BACKGROUND" button. For best results, choose an area with lots of visible sample structure to get an accurate measure of the background photobleaching rate. Again, you can use the zoom and pan buttons above the image to help select a good area.

13. The background area will be indicated with a green box. If you want to change your selection, just click the "SELECT BACKGROUND" button again, and re-draw the green box.

14. The right display window will now show both the raw FRAP data (blue circles), and FRAP data that has been corrected for background photobleaching (black circles).



Analyzing the Data

15. Now we are ready to fit the data. The default setting assumes a single mobile fraction (***A***) with a single exponential time constant (τ), such that the image intensity over time ***I(t)*** given by:

$$I(t) = A(1 - e^{-\tau t})$$

16. Click the "**FIT DATA**" button. A red line should now be visible in the right display window. If the red line does not appear after a few seconds, this indicates that the fitting algorithm did not converge to an appropriate answer. If this is the case, press the "**CLEAR**" button to remove the plotted data, and try re-selecting the FRAP and BACKGROUND area, as outlined in steps 9-14.

17. If a fitted curve still fails to be generated after several attempts, consider re-acquiring data with higher signal to noise ratio (SNR) and/or with more complete targeted photobleaching.

18. The results of the data fit will be shown under the **FIT RESULTS** heading, beneath the right display window. For the case of a single mobile fraction, the top two parameters "**A(1)**" and "**t1/2 (1)**" will now have values associated with them. In addition, the 95% confidence interval (in terms of +/- the fitted value) will also be displayed. The "**A(1)"** value corresponds to ***A*** in the above equation, while "**t1/2 (1)**" corresponds to the time (in seconds) that is required to recover half of the equilibrium intensity value, and is represented by $t_{1/2} = \ln(2)/\tau$.

19. In addition, an "**Adjusted R-squared**" value is also displayed, and corresponds to the R^2 value (adjusted for non-linear fitting) of the data fit. It will give a general idea of the goodness-of-fit to the data, with values near unity indicating a "good" fit.

20. There is also an option to model your data with the assumption of two mobile fractions, such that:

    $$I(t) = A_1(1 - e^{-\tau_1 t}) + A_2(1 - e^{-\tau_2 t})$$

    Where the subscripts ***1*** and ***2*** indicate the first and second mobile fractions, respectively. To enable this option, click the "**Multicomponent Fit**" check box, above the "**FIT DATA**" button.

21. You can re-click the "**FIT DATA**" button to try fitting your data with the "**Multicomponent Fit**" option selected. Caution, however, should be taken. Although it may result in a higher R^2 value, this may not be biologically relevant. Rather you can simply be "fitting the noise" in your data.

22. You can click the "**CLEAR**" button to remove all data from the right display window. You will have to re-select your FRAP and BACKGROUND area, as outlined in steps 9-14 before you re-fit the data.

23. Once you are happy with your results, you can export your analysis to an Excel file (.xls). Simply click the "**Export to Excel**" file button in the far lower-right corner of the utility window. A dialog box will appear for you to choose a directory and name in which to save the Excel file.

24. The resulting Excel file will contain several parameters, including:

a. The original image (TIFF) file name
b. The time points corresponding to each frame in the image data
c. The "raw" FRAP data
d. The corrected FRAP data
e. The fitted parameters displayed under the "**Fit Results**" heading in the utility window, including the 95% confidence intervals (+/-), and R^2 value.