easyFRAP: Quick Start Guide

Installation and step-by-step example

Installation Guidelines

1. Standalone version (if you don't have MATLAB installed on your computer):

- Download and install MATLAB Compiler Runtime. This is required for standalone applications to run outside MATLAB.
- Download easyFRAPXX.zip and run (double-click) easyFRAPXX.exe.

2. If you do have MATLAB installed:

- Download the appropriate source files and run the code. It is advised to save the files in the MATLAB root directory.

Supported File Formats

- **Leica LAS AF:** .csv files (comma-separated)
- **Olympus:** .xls files
- **Zeiss:** .txt (tab-delimited) or .xls files
- **ImageJ:** .txt (tab-delimited) or .xls files

	A	B	C	D
1	Channel 1			
2	Axis [s]	ROI1 []	ROI2 []	ROI3 []
3	0	190.8138	175.8727	6.606061
4	0.067	176.9931	169.9662	7.121212
5	0.134	171.6276	166.3296	5.939394
6	0.201	174.8	164.1675	6.373737
7	0.269	171.5931	161.406	5.626263
8	0.341	170.5931	160.8813	7.434343
9	0.408	166.5103	159.701	5.888889
10	0.475	167.1103	158.4837	7
11	0.544	171.0759	156.8855	6.828283
12	0.611	163.4345	156.9576	8.323232
13	0.678	168.469	156.2061	5.878788
14	0.748	168.2276	156.3602	7.020202

.csv file, 2 header lines

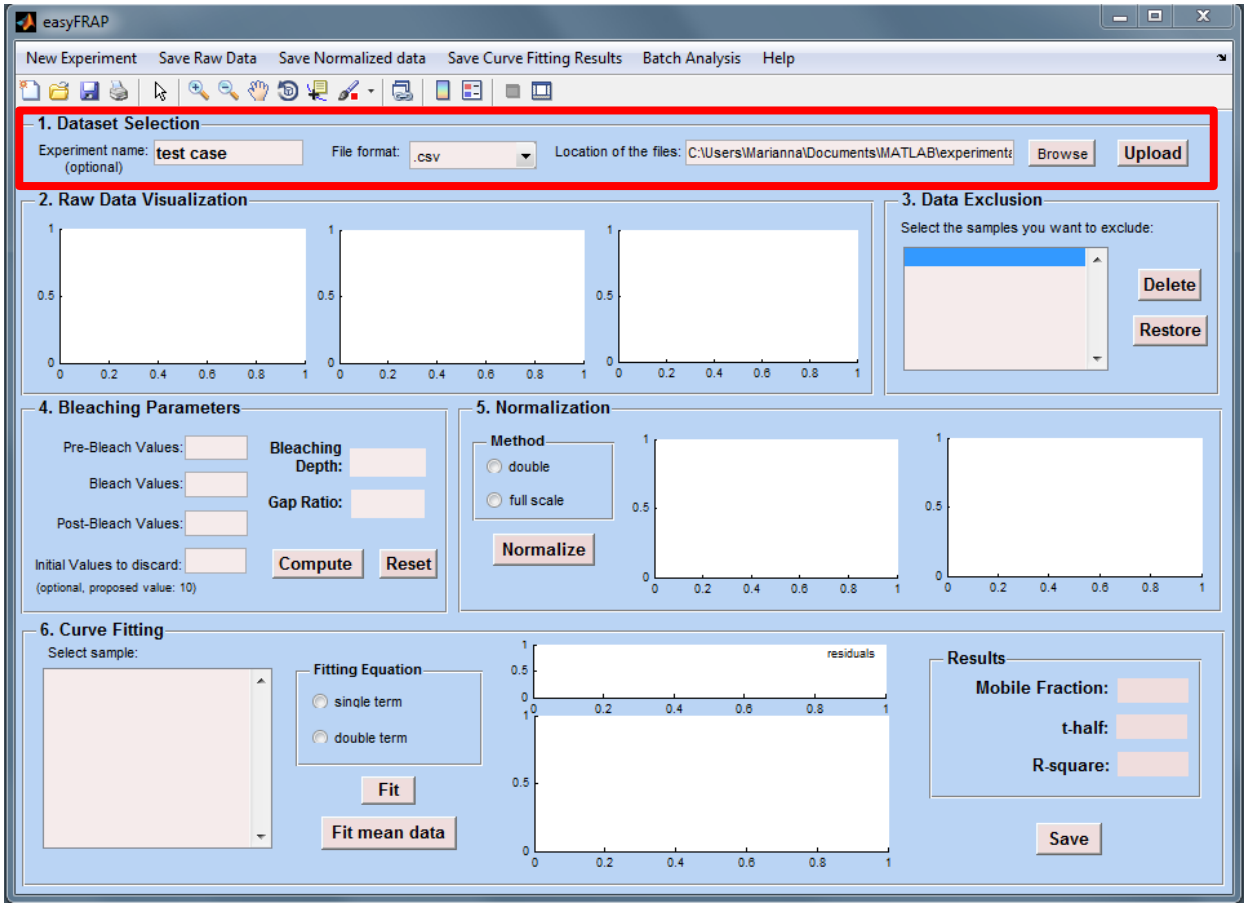
```
Channel 1
Axis [s]        ROI1 []  ROI2 []  ROI3 []
0       190.8137931      175.8727196      6.606060606
0.067   176.9931034      169.9662403      7.121212121
0.134   171.6275862      166.3296289      5.939393939
0.201   174.8    164.1675404      6.373737374
0.269   171.5931034      161.4059551      5.626262626
0.341   170.5931034      160.8813168      7.434343434
0.408   166.5103448      159.7009855      5.888888889
0.475   167.1103448      158.4837492      7
0.544   171.0758621      156.8855106      6.828282828
0.611   163.4344828      156.9576431      8.323232323
0.678   168.4689655      156.2061229      5.878787879
0.748   168.2275862      156.3602432      7.02020202
0.815   172.0827586      155.2759488      5.636363636
0.882   171.6896552      155.4887817      7.161616162
0.953   167.6    155.451038      6.04040404
1.02    168.9793103      155.9178025      6.838383838
1.087   168.4758621      154.2421891      5.444444444
1.154   173.2965517      156.5944642      6.161616162
1.221   173.6344828      156.0232753      7.181818182
1.289   171.3172414      155.638918       5.95959596
1.361   165.8137931      156.036905       6.03030303
```

.txt file, tab delimited, 2 header lines

	A	B	C	D	E
1	Image0068				
2	Records Bleach Event Markers				
3	Time (s)	Label			
4	3.9	BleachStart			
5	4.2	BleachEnd			
6					
7	Series	Color			
8	Ch1 Regio	RGB:0	255	255	
9					
10	No	Time :0.0(	ROI1	ROI2	ROI3
11	0	0	190.8138	175.8727	6.606061
12	1	0.067	176.9931	169.9662	7.121212
13	2	0.134	171.6276	166.3296	5.939394
14	3	0.201	174.8	164.1675	6.373737
15	4	0.269	171.5931	161.406	5.626263
16	5	0.341	170.5931	160.8813	7.434343
17	6	0.408	166.5103	159.701	5.888889
18	7	0.475	167.1103	158.4837	7
19	8	0.544	171.0759	156.8855	6.828283
20	9	0.611	163.4345	156.9576	8.323232
21	10	0.678	168.469	156.2061	5.878788
22	11	0.748	168.2276	156.3602	7.020202
23	12	0.815	172.0828	155.2759	5.636364
24	13	0.882	171.6897	155.4888	7.161616
25	14	0.953	167.6	155.451	6.040404
26	15	1.02	168.9793	155.9178	6.838384

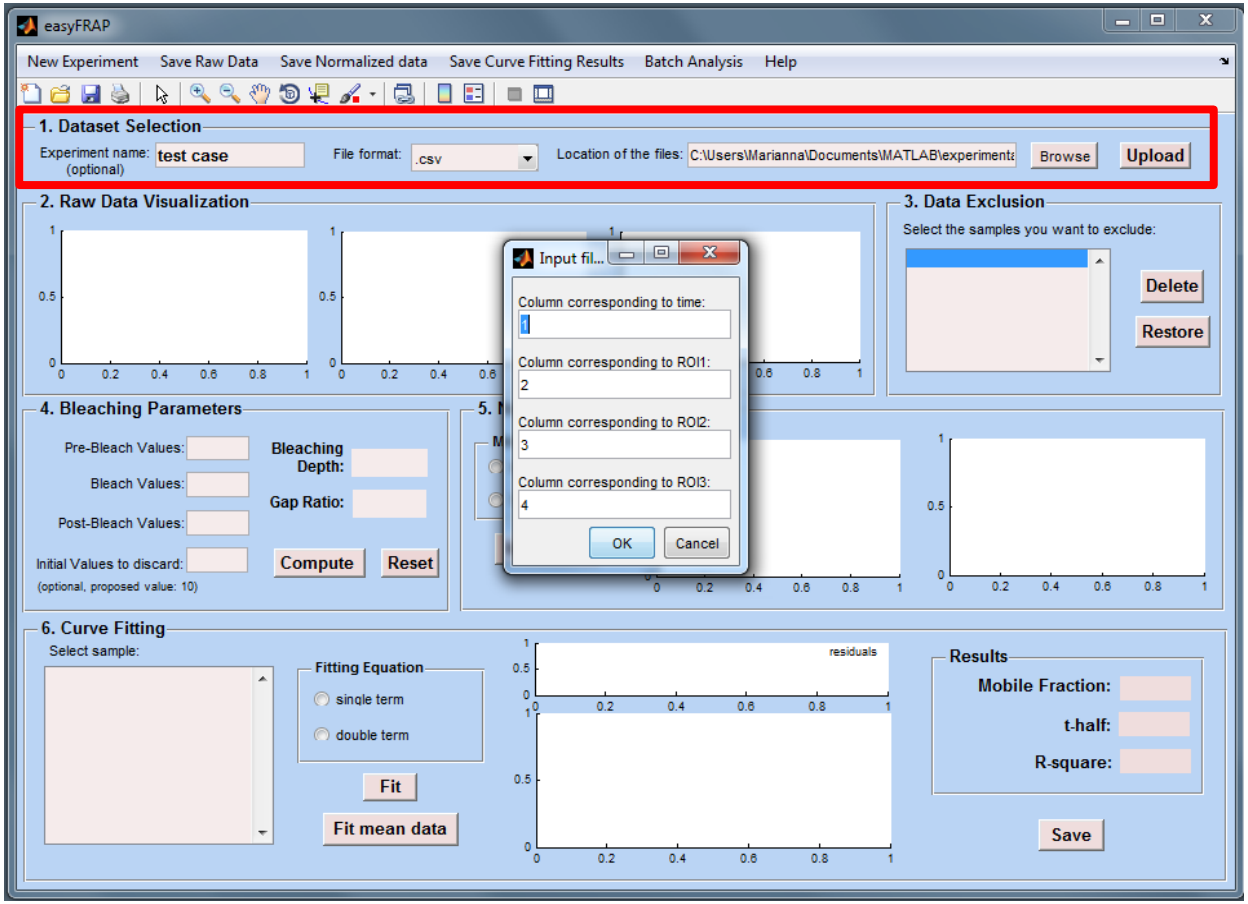
.xls file, 10 header lines, 5 columns

Dataset Selection



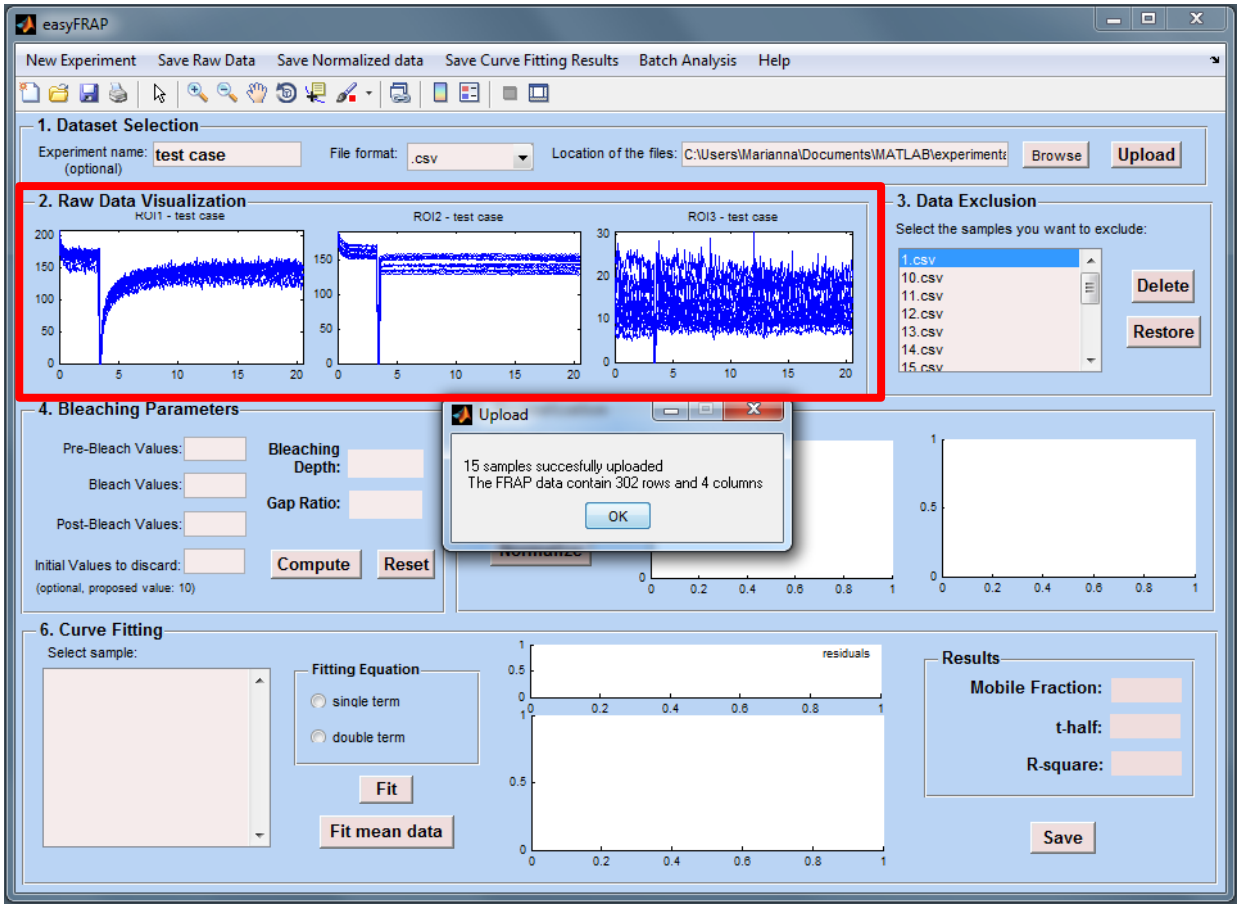
1. Name the experiment (*optional*)
2. Define file format (.csv, .txt or .xls)
3. Choose folder containing the files
4. Press **Upload**

Dataset Selection



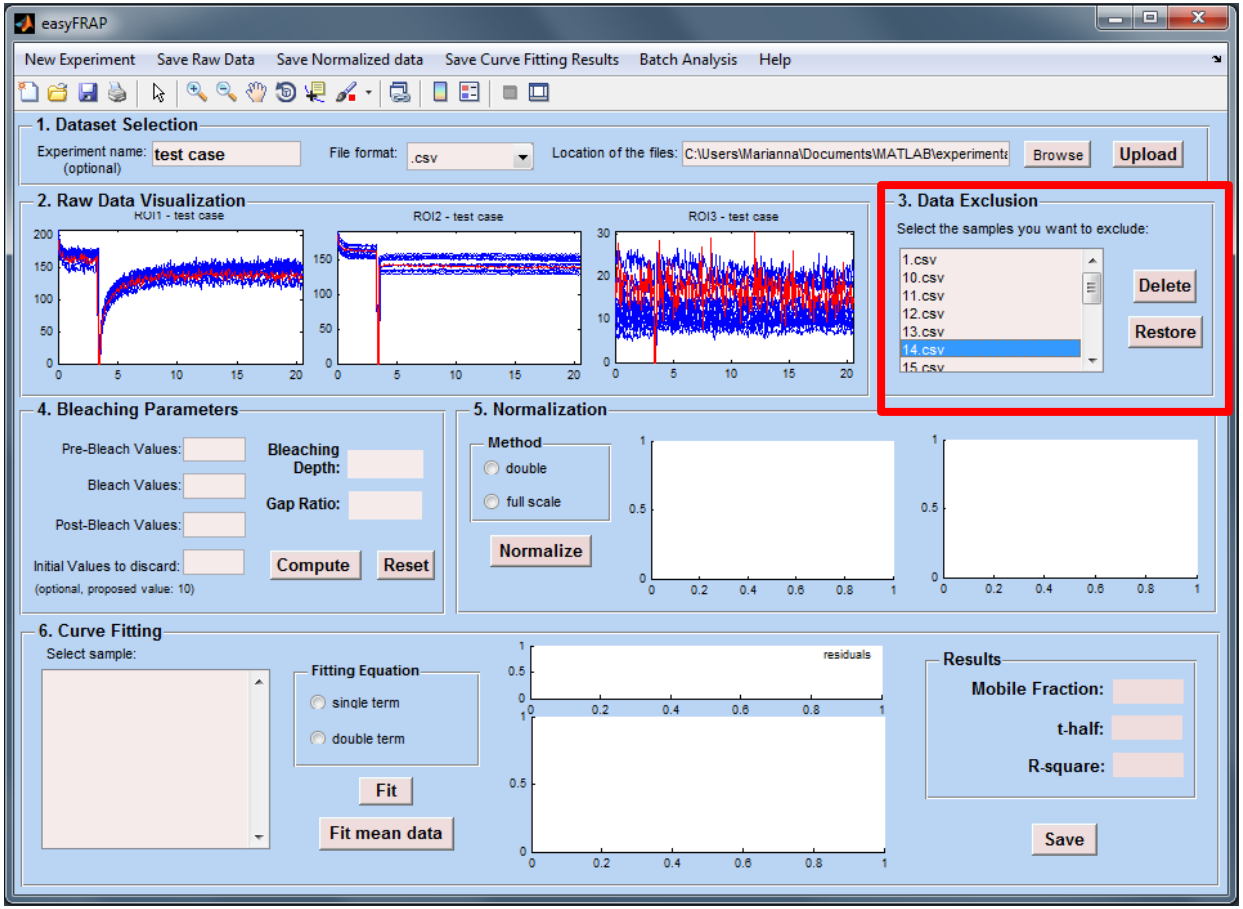
1. Define the correspondence of the measurements to columns in the files
2. Press **OK**

Raw Data Visualization



The raw recovery curves in ROI1, ROI2, ROI3 are automatically visualized and information about the files is returned

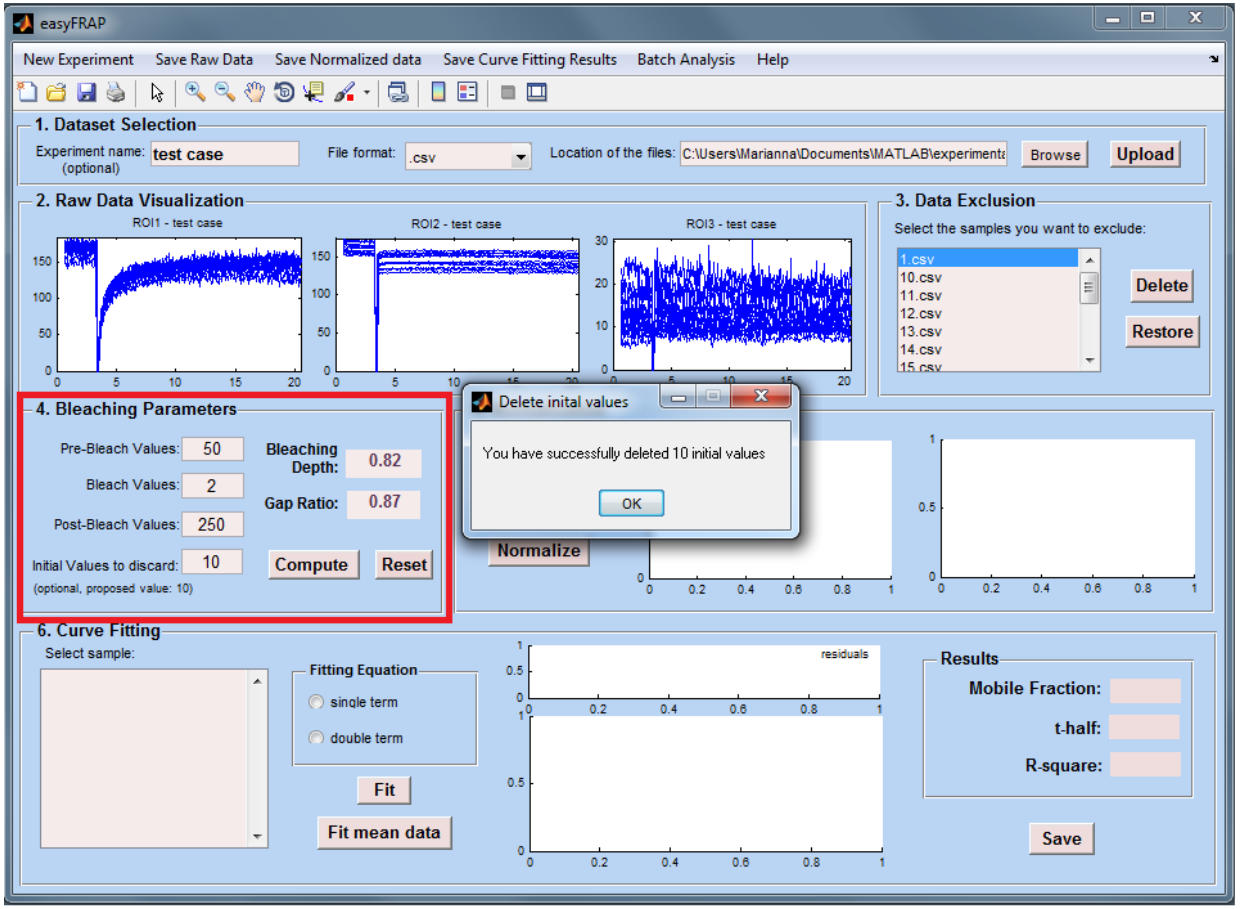
Data Exclusion



Optionally:

1. Select one or more samples from the list box
✓ Selected samples are automatically plotted in red
1. Press **Delete** to exclude them from the dataset
2. Press **Restore** to restore them
✓ The plots are automatically refreshed

Bleaching Parameters

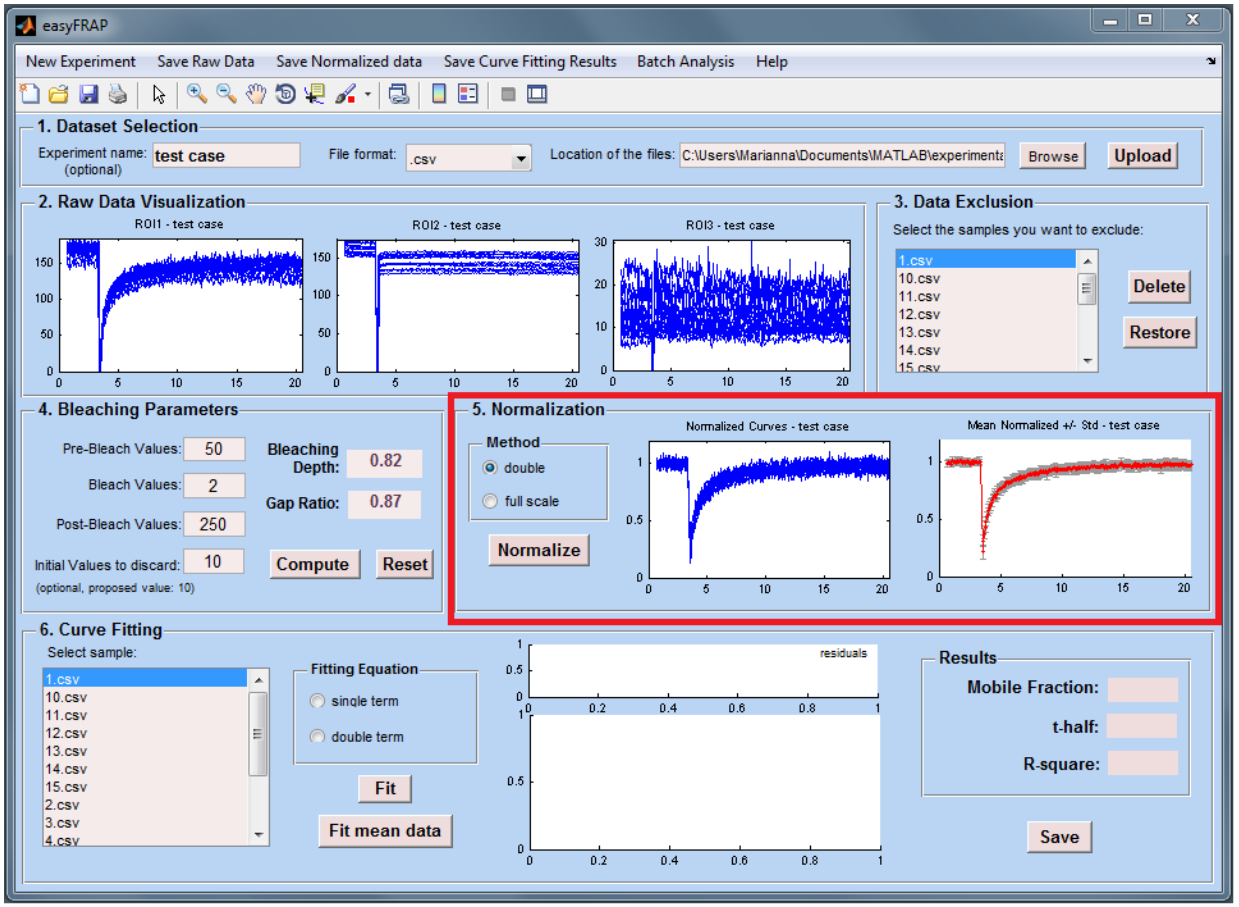


1. Insert number of prebleach, bleach and postbleach images
2. Insert initial values to discard (optional)
3. Press **Compute**

✓ The bleaching depth and gap ratio values are calculated

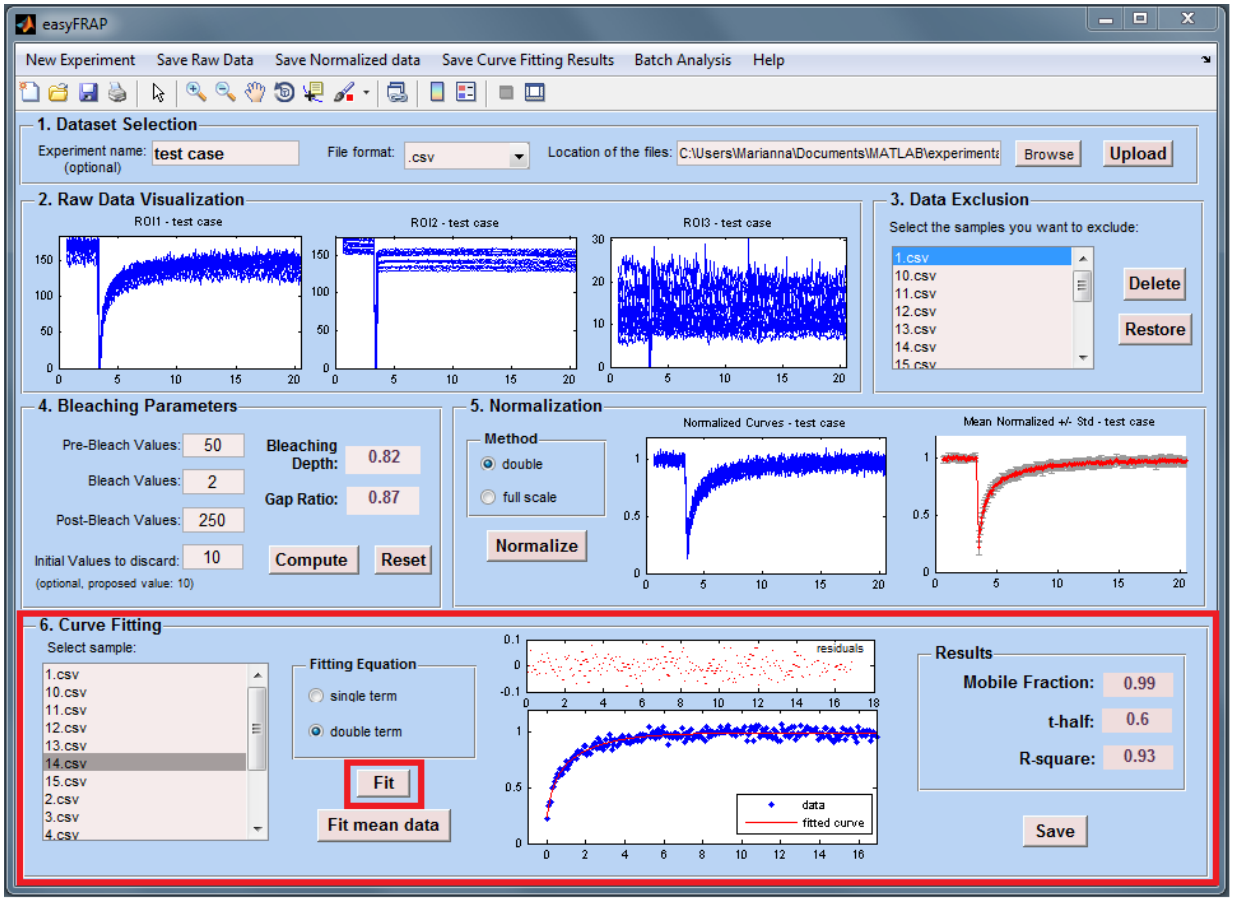
4. Press **Reset** to restore the initial values and repeat the computation

Normalization



1. Choose Normalization method
2. Press **Normalize**
- ✓ The Normalized plots (individual and mean) are visualized
- ✓ Click on the plots to export them for better examination

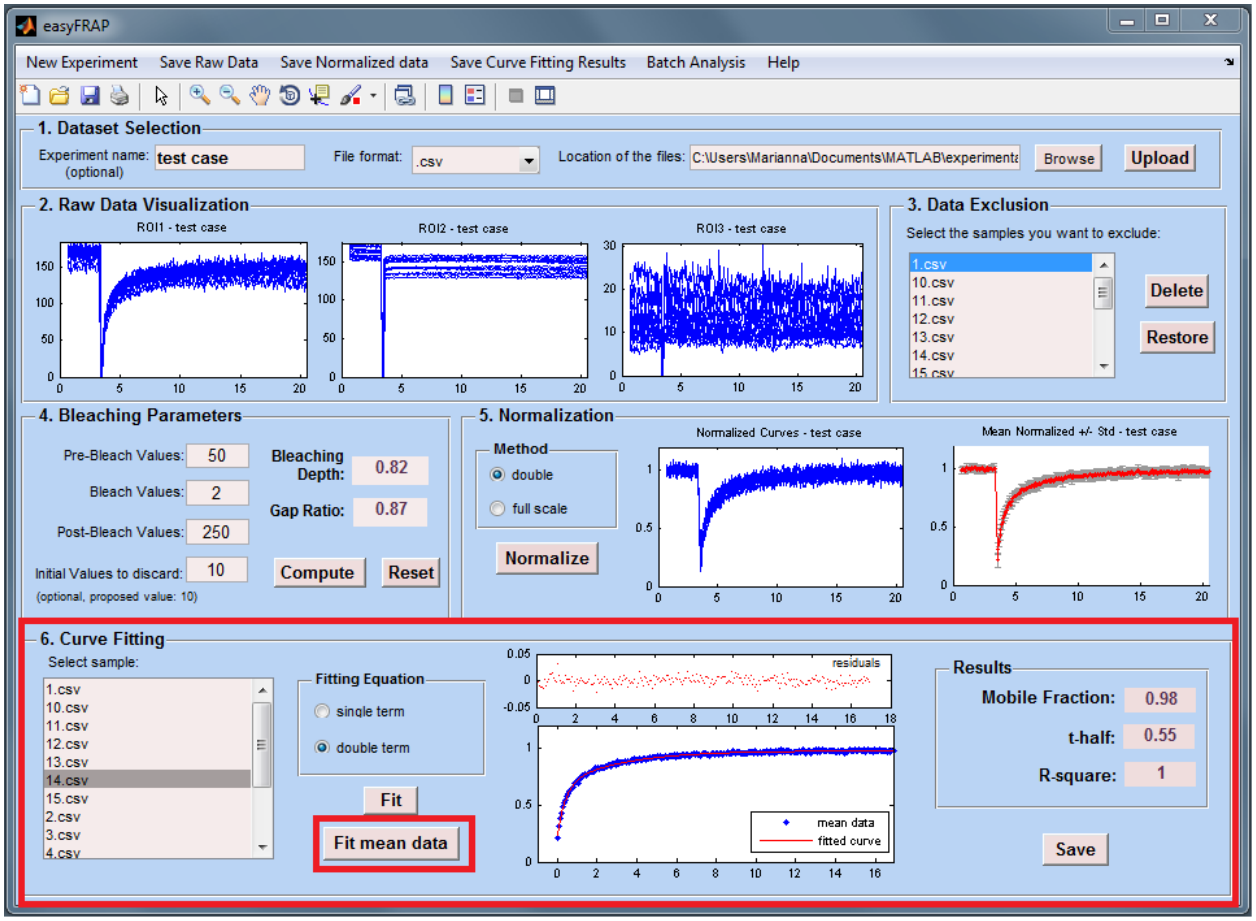
Curve Fitting



1. Choose a sample from the list box
✓ It is automatically plotted
2. Choose fitting equation
3. Press **Fit**
✓ The fitted curve and residuals are plotted
✓ The mobile fraction and t_{half} are calculated and shown on the right
✓ Goodness-of-fit statistics (R-square) are also provided

Attention: R-square must be close to 1, small values indicate bad fits and thus inaccurate estimates

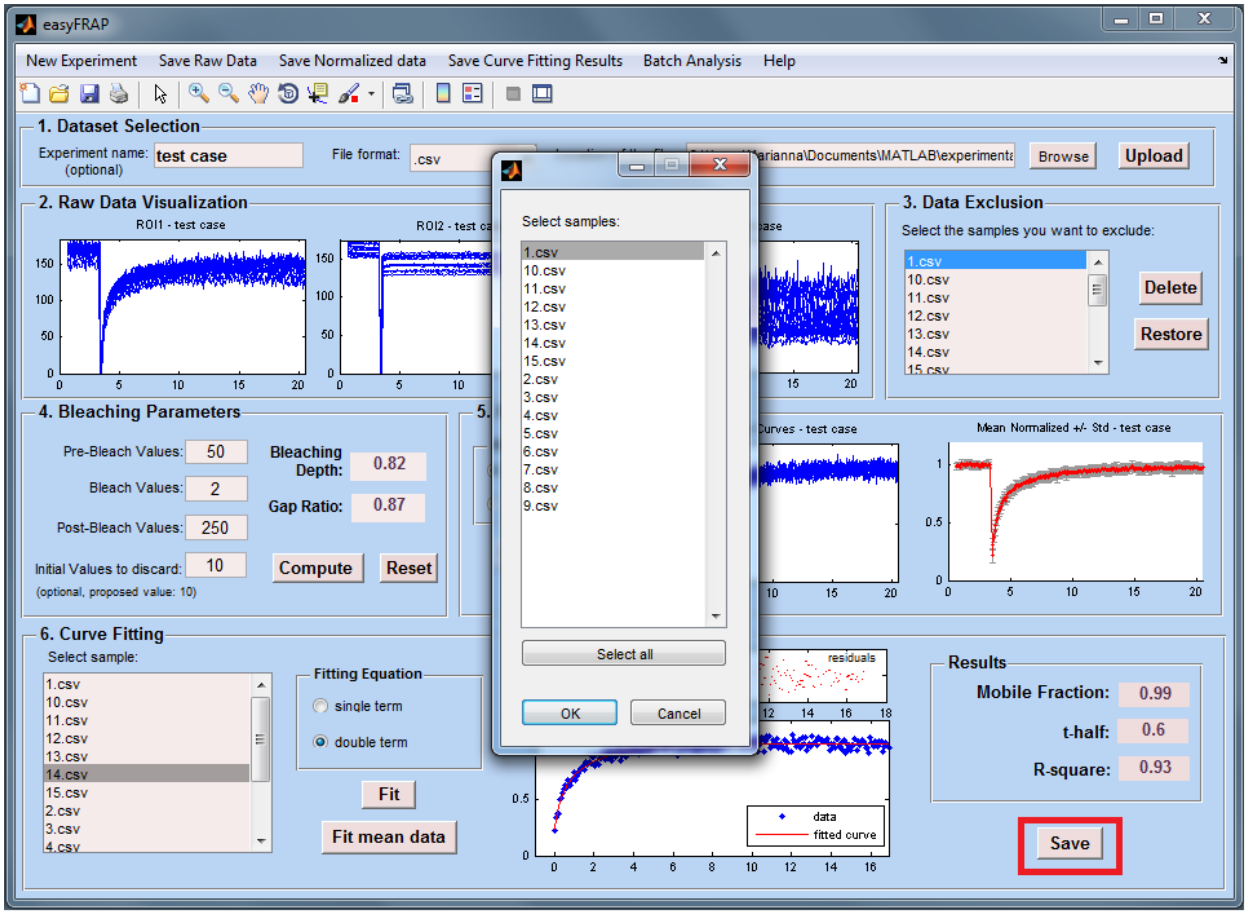
Curve Fitting



1. Press **Fit Mean Data**

- ✓ The fitted curve and residuals for the mean are plotted
- ✓ The mobile fraction and t-half concerning the mean are calculated and shown on the right
- ✓ Goodness-of-fit statistics (R-square) are also provided

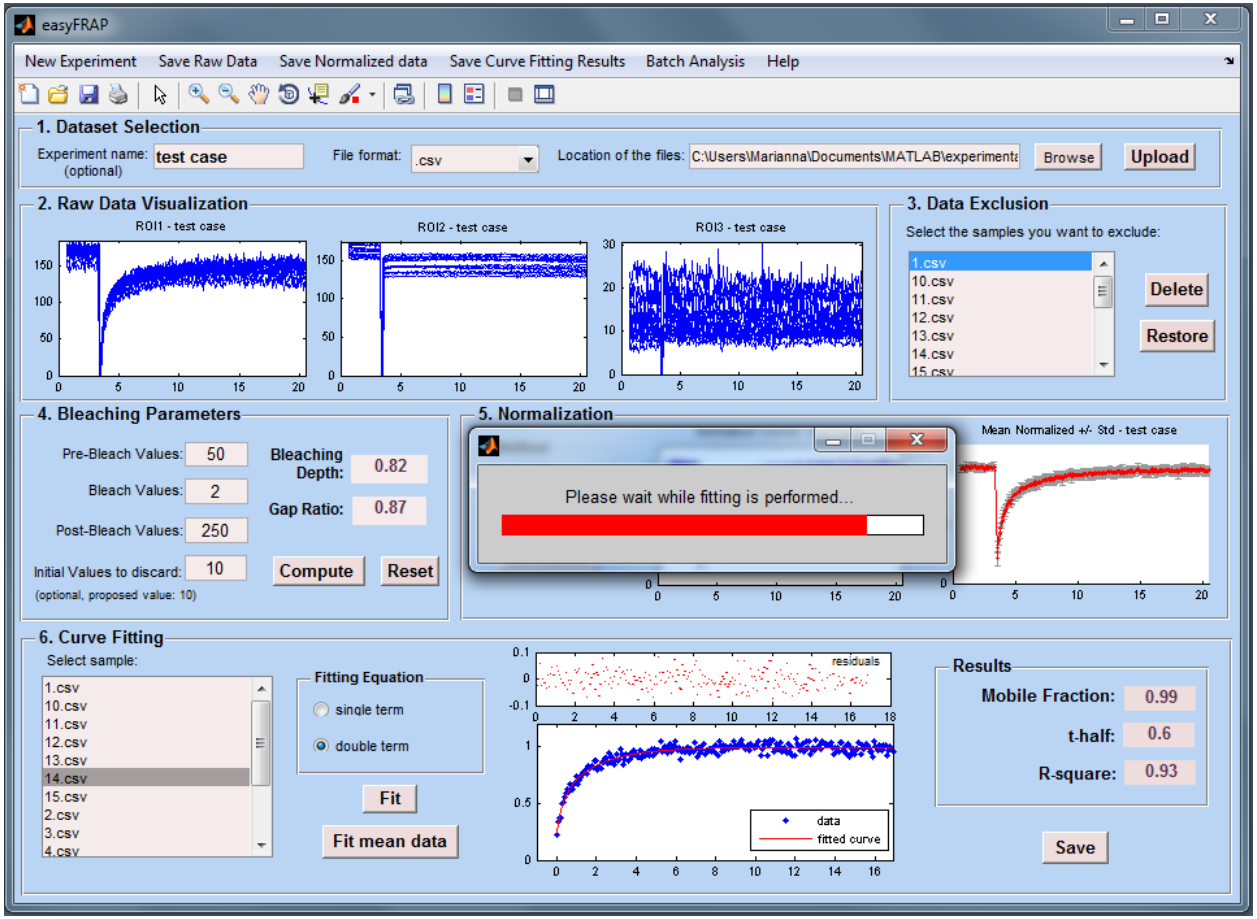
Curve Fitting



Press **Save** and:

1. Select file to save the results
2. Select sample(s)

Curve Fitting



- ✓ easyFRAP performs curve fitting to all selected samples and saves the results (individual and mean values) in a separate file

Exported fitting results

- On Windows platforms, the computed data are saved on an .xls file that contains all the necessary information (numerical values and header/sample information)

- On Mac platforms, 2 separate files are saved: a .csv file with all numerical values and a .txt file (with the same name) with all header/sample information

Exported files - Windows

Choice of normalization and fitting method

	A	B	C	D	E	F	G	H
1	double	double_exponential						
2	sample	t-half (sec	mean	std	mobile fra	mean	std	R-square
3	1.csv	0.52	0.55	0.09	0.97	0.95	0.04	0.96
4	10.csv	0.5			0.96			0.95
5	11.csv	0.68			0.99			0.94
6	12.csv	0.54			0.94			0.94
7	13.csv	0.47			0.88			0.93
8	14.csv	0.6			0.97			0.93
9	15.csv	0.78			1			0.94
10	2.csv	0.53			0.88			0.93
11	3.csv	0.51			0.88			0.92
12	4.csv	0.51			0.93			0.96
13	5.csv	0.48			0.95			0.94
14	6.csv	0.48			1			0.92
15	7.csv	0.47			0.96			0.95
16	8.csv	0.65			0.97			0.96
17	9.csv	0.5			0.98			0.92

Header

Samples

Exported files(Mac)

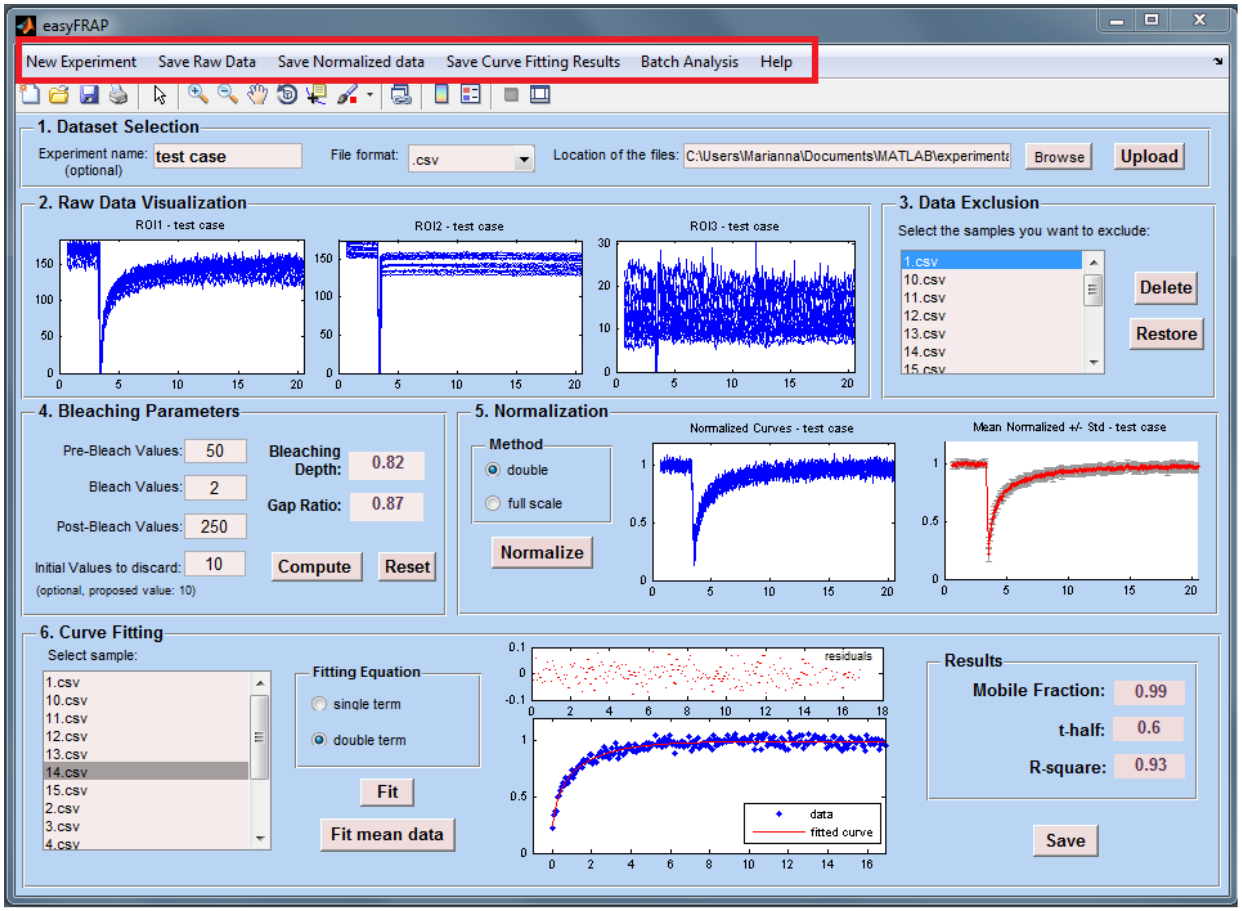
.csv file:

	A	B	C
1	0.52	0.97	0.96
2	0.5	0.96	0.95
3	0.68	0.99	0.94
4	0.54	0.94	0.94
5	0.47	0.88	0.93
6	0.6	0.97	0.93
7	0.78	1	0.94
8	0.53	0.88	0.93
9	0.51	0.88	0.92
10	0.51	0.93	0.96
11	0.48	0.95	0.94
12	0.48	1	0.92
13	0.47	0.96	0.95
14	0.65	0.97	0.96
15	0.5	0.98	0.92

.txt file:

```
double  double_exponential
sample  t-half  mobile_fraction R-square
1.csv
10.csv
11.csv
12.csv
13.csv
14.csv
15.csv
2.csv
3.csv
4.csv
5.csv
6.csv
7.csv
8.csv
9.csv
```

Top menu



1. Export images and save them in various formats
2. Save all resulting data (raw, normalized data and curve fitting results) in separate files
3. Perform Batch analysis

Exported Normalized data

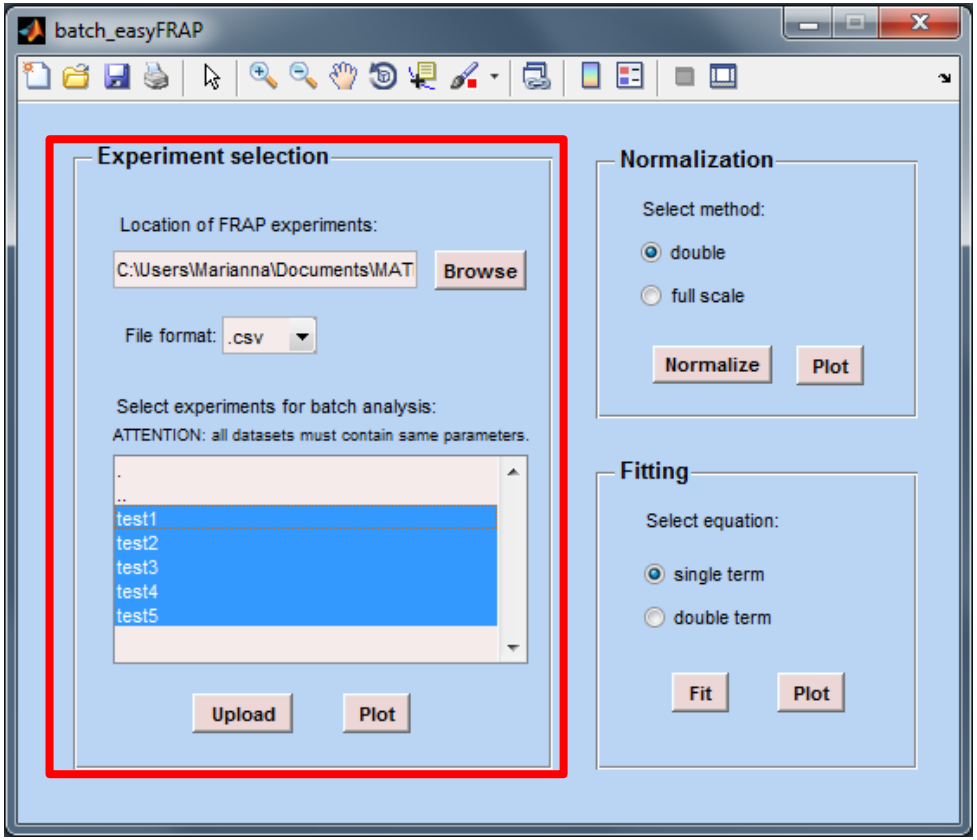
	A	B	C	D	E	F	G	H	I
1	time	mean	std	1.csv	10.csv	11.csv	12.csv	13.csv	14.csv
2	0	1.01388	0.016082	1.004213	0.996227	1.023791	1.008968	1.025692	1.032374
3	0.067	0.996663	0.025924	0.962579	1.025273	0.971183	1.010314	0.963116	0.985147
4	0.134	0.998529	0.023035	0.953242	1.022788	0.970539	1.013034	0.997192	1.009976
5	0.201	1.001795	0.016121	0.984939	0.994534	0.977204	0.976307	1.001692	1.012312
6	0.269	0.997897	0.021628	0.983105	0.999716	0.97781	0.983061	1.008291	0.986367
7	0.341	1.003843	0.029659	0.981164	1.013726	1.024661	0.950468	0.990912	1.030163
8	0.408	0.991059	0.023647	0.963613	1.000412	0.975952	0.937996	1.032571	1.011383
9	0.475	1.002244	0.020232	0.97531	1.027065	1.018167	0.99214	1.015583	0.990351
10	0.544	1.001113	0.023804	1.010023	0.981355	0.977065	1.02807	1.010072	0.973865
11	0.611	0.99822	0.022985	0.962971	1.003095	0.989159	0.992796	0.994772	0.987044
12	0.678	0.990953	0.02886	0.998035	0.976992	1.056163	0.994277	0.985168	0.973113
13	0.748	1.002236	0.021607	0.996089	1.015385	0.96038	1.00538	1.021091	0.985225
14	0.815	0.994455	0.019265	1.026402	0.999802	0.984635	1.017933	0.988049	1.028467
15	0.882	0.987491	0.028904	1.023549	1.028901	0.975105	0.983465	1.028477	0.96049
16	0.953	1.001631	0.019272	0.997793	0.969186	1.045555	1.006166	0.993749	0.993823
17	1.02	0.984236	0.026443	1.003609	0.937998	1.000892	0.965785	0.981935	0.986213
18	1.087	1.01095	0.033079	1.011031	1.003031	1.073355	0.965043	1.018909	0.963746
19	1.154	1.004298	0.017398	1.025213	0.967765	0.996957	0.998385	1.020849	1.019271
20	1.221	1.003483	0.020989	1.031944	1.011598	1.017346	1.029637	0.99802	1.001928
21	1.289	1.004085	0.037241	1.019417	1.010397	0.973636	0.962527	1.051247	0.955697
22	1.361	1.007712	0.022947	0.982904	0.986308	1.033464	1.054542	1.003359	1.030621
23	1.429	0.988146	0.022632	1.003884	0.997226	0.984471	0.961263	1.012445	1.010684
24	1.496	1.009602	0.027808	1.073146	1.02349	1.006782	1.028006	0.97422	1.019574

Individual normalized curves

Mean normalized curve and standard deviation

For Mac users: again 2 separate files are saved: a .csv file with all numerical values and a .txt file (with the same name) with the header information

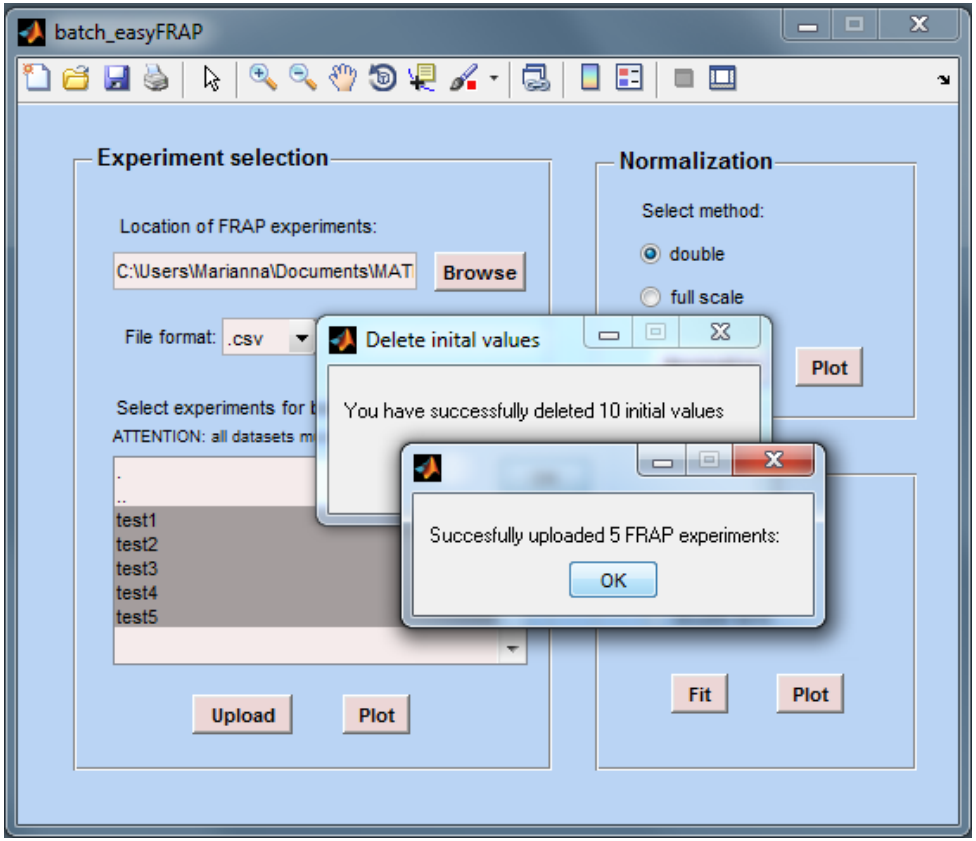
easyFRAP Batch analysis



Experiment selection:

1. Select parent directory that contains all your experiments
2. Select file format
3. Choose the experiments of interest from the list box and press **Upload**

easyFRAP Batch analysis

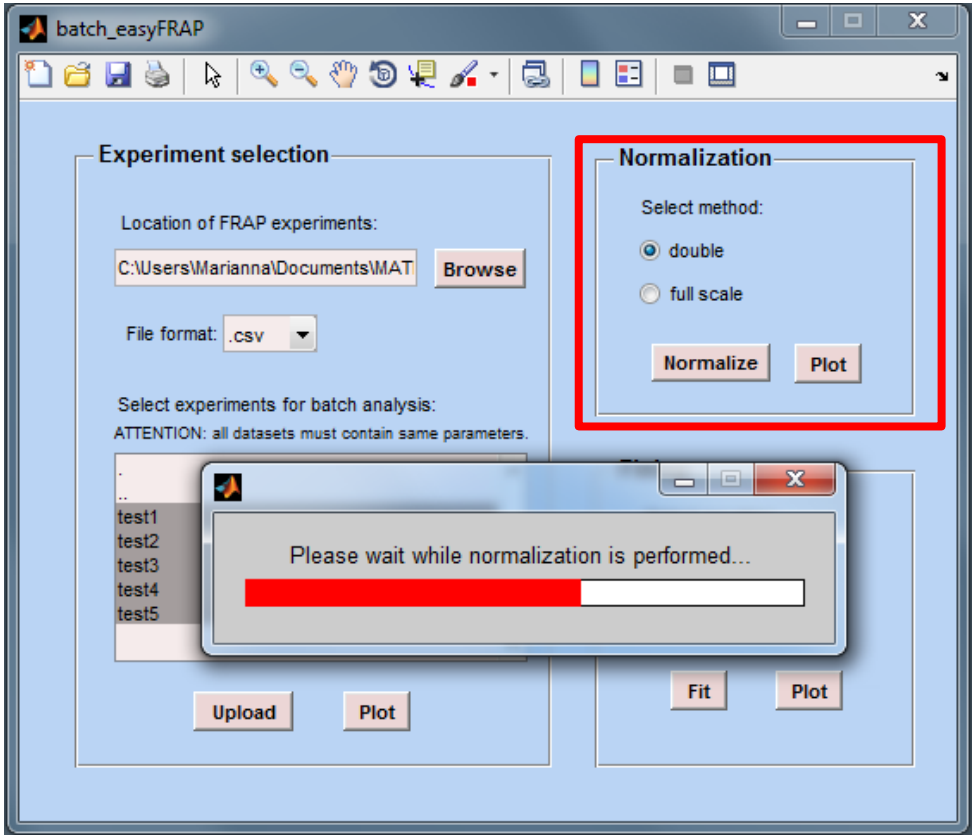


Experiment selection:

1. Provide number of header lines, column - measurement correspondence and bleaching parameters
2. ✓ The data are uploaded
3. Press **Plot** to plot all raw intensity values

Attention: when you provide the number of header lines, **do not count the empty ones**!

easyFRAP Batch analysis



Normalization:

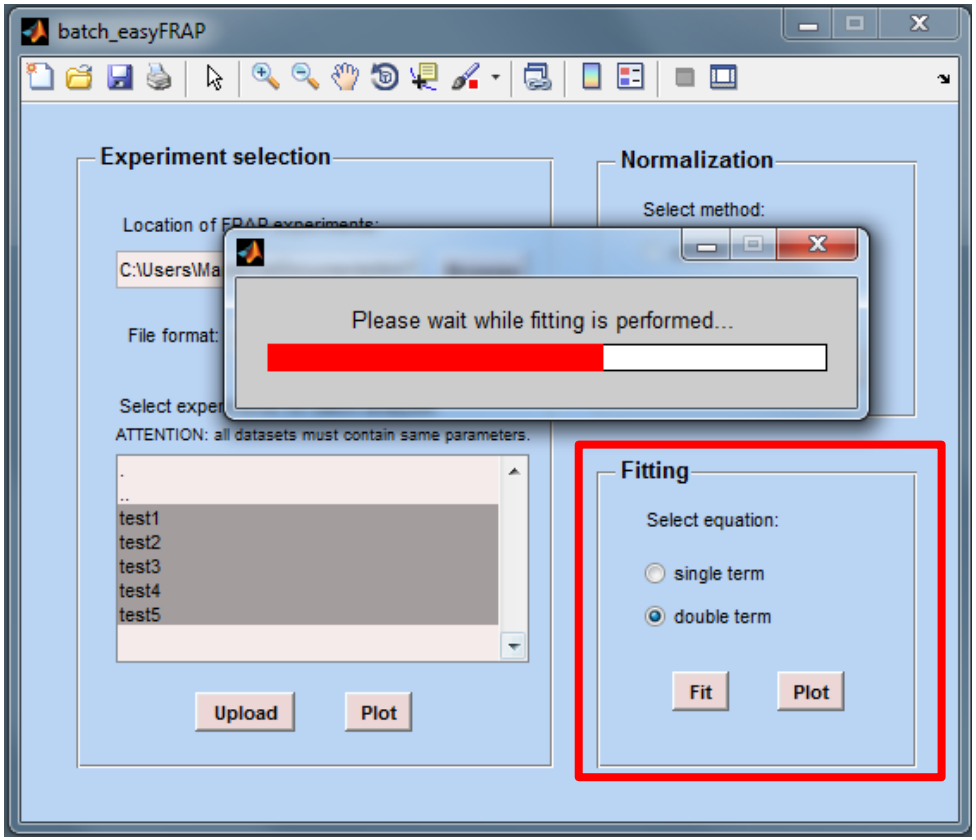
1. Select Normalization method
2. Press **Normalize**

✓ The data are normalized and saved to .xls files in the parent directory named after the experiments (e.g. *Normalized data - test1.xls*)

2. Press **Plot** to plot all normalized intensity values

For Mac users: header information is saved on a separate .txt file in the same directory

easyFRAP Batch analysis



Fitting:

1. Select fitting method
2. Press **Fit**

✓ The data are fitted and the results (mobile fraction, t-half, their means and standard deviations) are saved to .xls files in the parent directory named after the experiments (e.g. *Fitting results-test1.xls*)

2. Press **Plot** to plot all fitted curves and residuals

For Mac users: header information is saved on a separate .txt file in the same directory