FRETnorm

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FRETnorm - Signal Analysis with MATLAB

FRETnorm is a matlab program for analyzing FRET signal data captured by the imaging system at Wolf Frommer‘s group at the Carnegie Institution for Science, Department of Plant Biology, Stanford.

The Excel output file from the imaging system must be converted to csv and can be used as input file for FRETnorm. The format of the input file is described later. One input file contains the time values and the measured values for several evaluated regions in the image. In addition the file external-load.csv must be customized if the timing programmed in the perfusion system changes.

FRETnorm is started by running the file FRETnorm.m in MATLAB. The graphical user interface (GUI) comes up and the input file can be chosen by clicking one of the „Select csv file“ buttons.

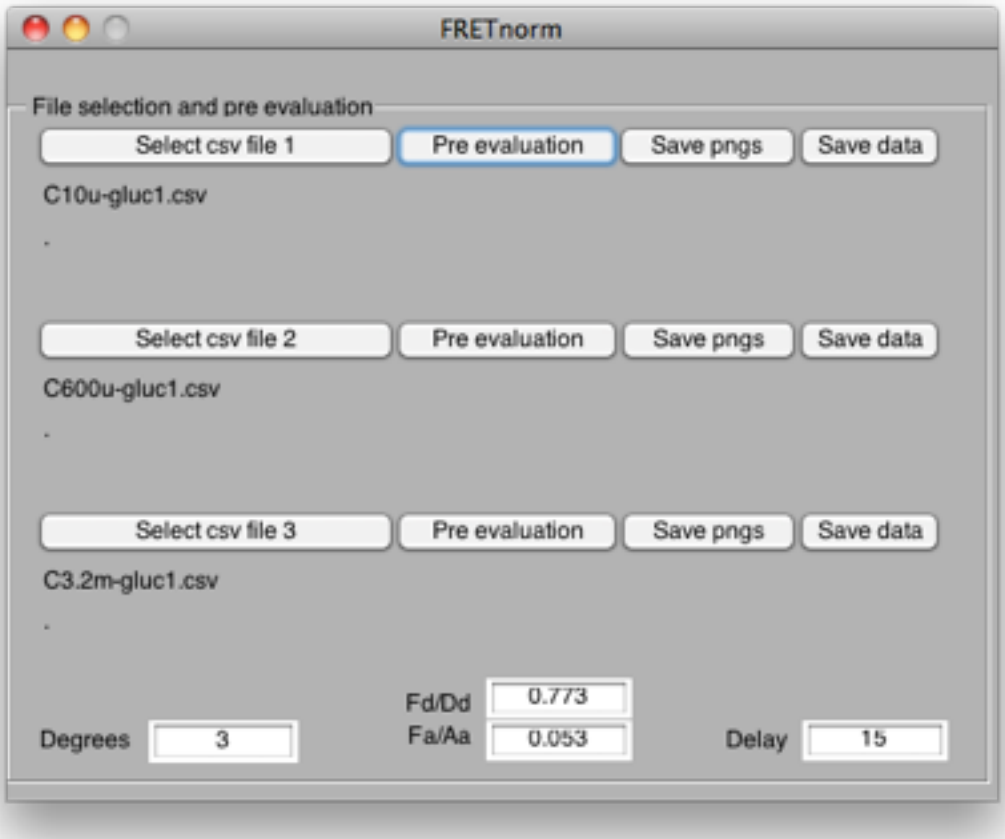


Image 1: FRETnorm main GUI

The *degrees* parameter is the degree of the polynomial used for the polynomial baseline correction. Parameters *Fd/Dd* and *Fa/Ad* are the constants for the bleed through correction. Parameter *delay* is used to decide which values are used for the baseline correction.

After setting the parameters or checking the defaults the button „Pre evaluation“ starts the signal analysis. Four figures allows to check the results for the main steps before saving the data and the final figures for further use.

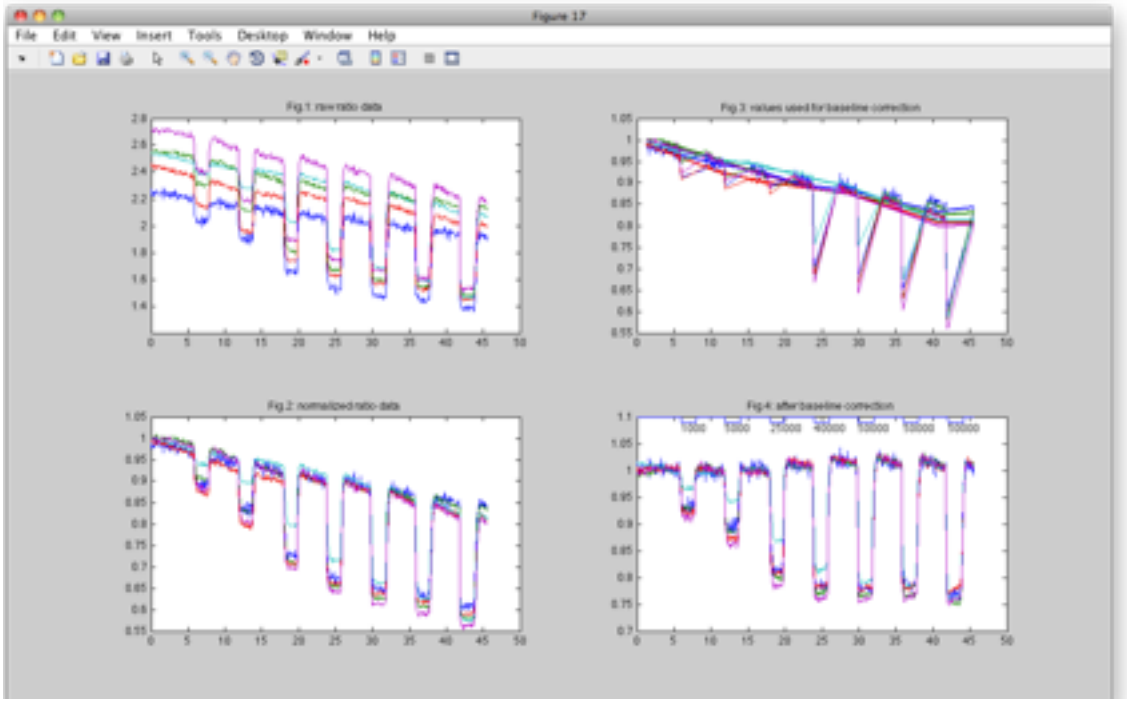


Image 2: Four figures from pre evaluation

The first step is to compute the ratios for all data columns in the input file. The background values are already subtracted from all values in the input file. The bleed through corrected FRET signal (*Fc*) is computed by equation:

$Fc = Ff - Df * Fd/Dd - Af * Fa/Aa$

The ration then is:

$r = Fc / Df$

All values except for the constants *Fd/Dd* and *Fa/Aa* are from the input file. Image 2, figure 1 shows the ratios of all data columns against time.

The second step is to normalize the ratios. This is obtained by dividing every value by the value at time zero:

$r_{norm} = r / r_{t0}$

The second figure (Image 2, figure 2) shows r_{norm} against time.

The baseline, which means the values at times without external glucose supply, has to be

corrected to have the same ratio *r* at all times without external glucose supply. The timing of the external supply is defined by the programable perfusion system. The file external-load.csv has one column with the external glucose concentrations for all times a measurement (image capture) has taken place. Only normalized ratios r_{norm} at times *t* with external concentration zero and external concentration zero at t_{d1}=*t-delay/2* and t_{d2}=*t-delay* are considered for baseline correction polynomial fit. This is due to the slow decay of internal concentration and ratio after the external concentration becomes zero. To check the parameter *delay* the ratios used for baseline correction are shown in image 2, figure 3. The ratios between two peaks in external concentration and of one data column are grouped and the mean value is calculated. The mean values are also shown in image 2, figure 3 with a thicker line.

Now, a polynomial of degree *degrees* is fitted to the mean values by MATLAB function polyfit for each ratio column and the ratios are corrected by the result of the polynomial at the corresponding point in time. The baseline corrected ratios are shown in image 2, figure 4.

Finally the figures and the corresponding data can be saved by clicking the buttons „Save png“ and „Save data“ in the main GUI. The figures are saved as png images with filenames:

Name-input-file-r-raw.png

Name-input-file-r-norm.png

Name-input-file-r-polyfit.png

Name-input-file-r-final.png

The corresponding data are saved in csv format as:

Name-input-file-r.csv (normalized ratios)

Name-input-file-p.csv (ratios after baseline correction)

Format of data file from imaging system

There are no headlines for columns and rows.

The first column is the time in milliseconds.

Each row contains the measured values at one point in time.

The columns *2* to *n+1* are the *Ff* values for different regions in the image evaluated by the imaging system. Each column contains the values for the different points in time (column

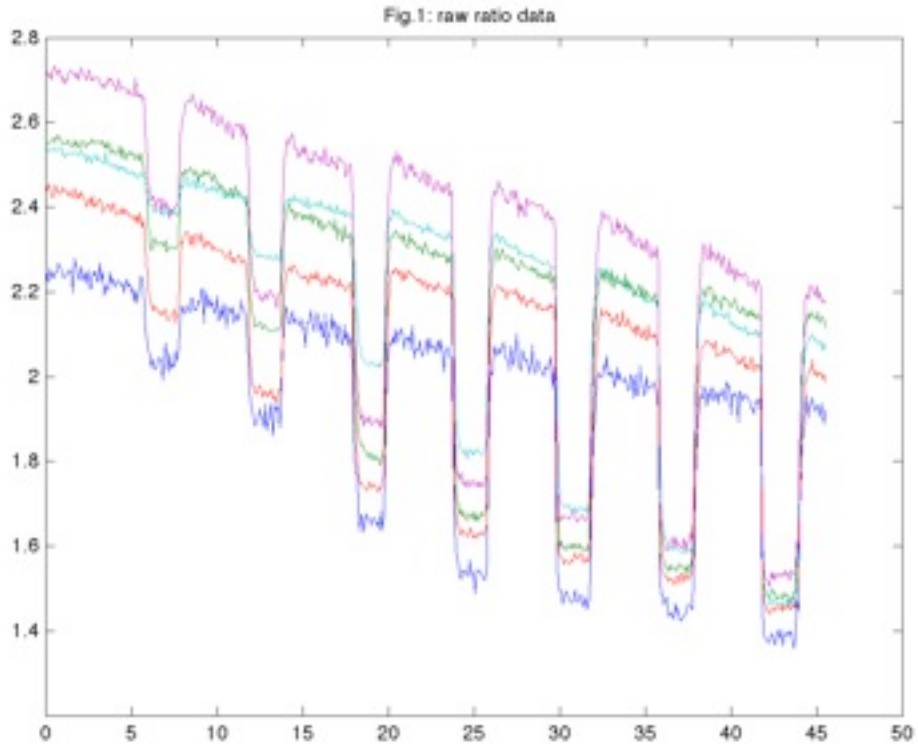
one).

The columns *n+2* to *2*n+1* are the *Df* values for different regions in the image evaluated by the imaging system. Each column contains the values for the different points in time (column one).

The columns *2*n+2* to *3*n+1* are the *Af* values for different regions in the image evaluated by the imaging system. Each column contains the values for the different points in time (column one).

Example files are distributed within the download file.

Images



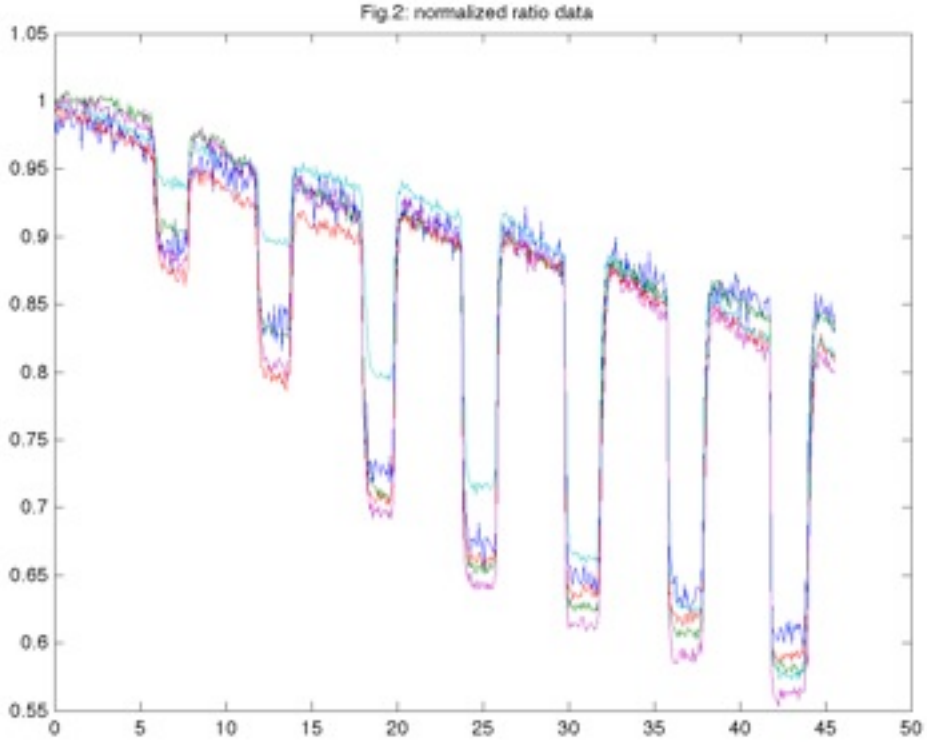


Fig.2: normalized ratio data
1.05
1
0.95
0.9
0.85
0.8
0.75
0.7
0.65
0.6
0.55
0
5
10
15
20
25
30
35
40
45
50

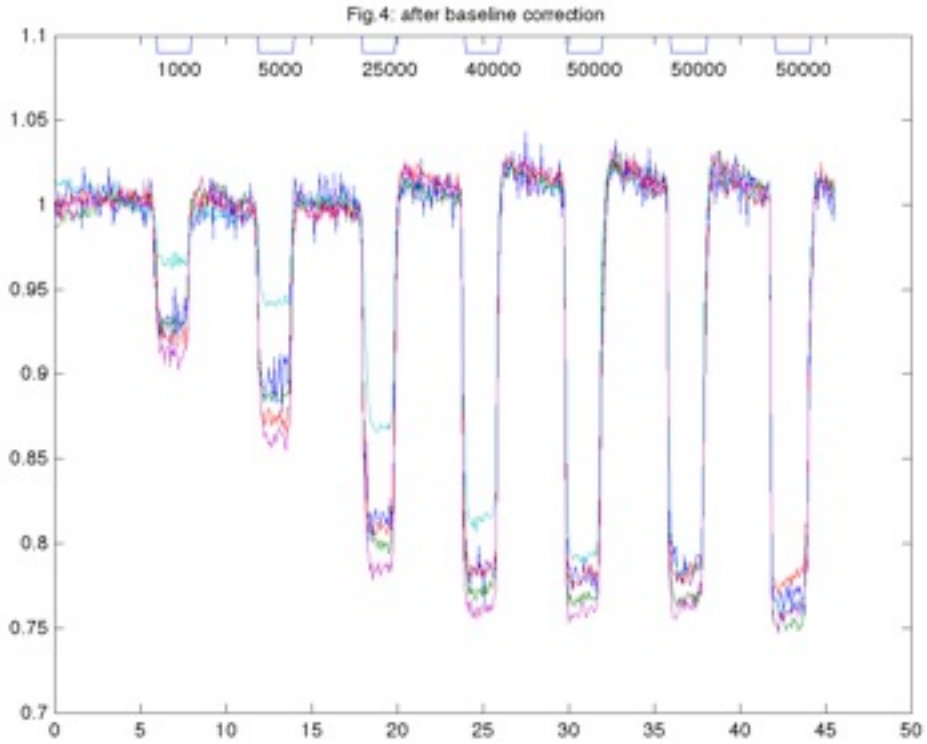


Fig.4: after baseline correction
1000
5000
25000
40000
50000
50000
50000
1.1
1.05
1
0.95
0.9
0.85
0.8
0.75
0.7
0
5
10
15
20
25
30
35
40
45
50