

Update on PFRET Software Version 2.1

1. Estimation of efficiency (E) and distance (r) between donor and acceptor molecules.

In order to improve the sensitivity of estimation of the efficiency (E) and distance (r) between donor and acceptor molecules, we have now included the quantum yield of the acceptor (Q_a) in our calculation. The revised equation is :

$$E_n = 1 - I_{DA} / (I_{DA} + \text{PFRET} \times (\psi_{dd} / \psi_{aa}) \times (Q_d / Q_a))$$

Compare with the old equation :

$$E_n = 1 - I_{DA} / (I_{DA} + \text{PFRET} \times (\psi_{dd} / \psi_{aa}) \times Q_d)$$

(see book chapter7 and Erratum-Method)

The corresponding change in PFRET software is in **Efficiency and Distance Analysis (ROI based) window** and **Efficiency and Distance Analysis (Time-Series)window**.

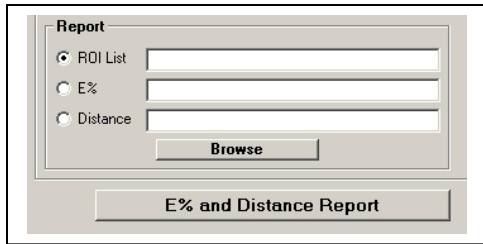
If you enable advanced parameters, you will see one more parameter the quantum yield of the acceptor (Q_a) is added. See the right side Figure where the red arrow points. The upper window is part of **Efficiency and Distance Analysis (ROI based) window**, the lower window is part of **Efficiency and Distance Analysis (Time-Series)window**.

The figure displays two screenshots of the PFRET software interface, illustrating the addition of the 'Acceptor Quantum Yield' parameter in the advanced settings.

The top screenshot shows the 'Parameter For Advanced Efficiency and Distance' window in the 'Efficiency and Distance Analysis (ROI based) window'. The window contains several input fields: Donor Quantum Yield (1), Acceptor Quantum Yield (1), PMT gain donor channel (1), PMT gain acceptor channel (1), Spectral sensitivity of the PMT for donor emission wavelength (1), and Spectral sensitivity of the PMT for acceptor emission wavelength (1). A red arrow points to the 'Acceptor Quantum Yield' field.

The bottom screenshot shows the 'Parameter For Advanced Efficiency and Distance' window in the 'Efficiency and Distance Analysis (Time-Series)window'. The window contains the same input fields as the top screenshot, but the 'Acceptor Quantum Yield' field is highlighted with a red arrow.

2. More reports produced from Efficiency and Distance Analysis (Time-Series) Window



Previously, Efficiency and Distance Analysis (Time-Series) only provided E% and Distance image. We added one more option to produce report based on ROI analysis which is similar as that in Efficiency and Distance Analysis (ROI based) window. Look at the figure below, this is the part of E% and Distance Analysis (Time-Series) Window. In order to get the report, you have to choose ROIs from image and copy it to clipboard. Then give the file name for ROIs, E% and distance shown in the figure. After processing, the information in ROI, E% and distance files will be like this:

series	1	2	3	4
101	0.10	0.08	0.07	0.05
102	0.11	0.09	0.07	0.05
103	0.13	0.09	0.08	0.06
104	0.15	0.12	0.09	0.08
105	0.15	0.13	0.11	0.09

E% File

series	1	2	3	4
101	80.10	81.00	81.20	82.00
102	79.80	80.90	81.20	82.00
103	79.00	80.90	81.00	81.90
104	76.00	79.40	80.90	81.00
105	76.00	79.00	79.80	80.90

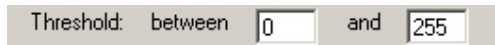
Distance File

ROIs	X	Y	Width	Height
1	215	234	23	21
2	145	225	25	20
3	196	389	35	29
4	574	212	18	17

ROI file

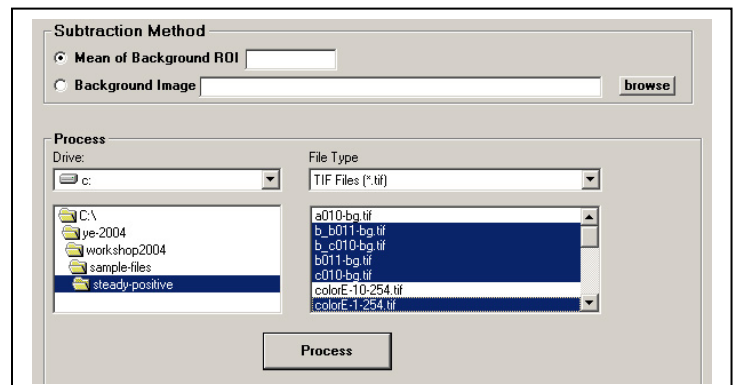
3. Spectral Bleedthrough (SBT) and Fluorophore Expression Level (FEL) correction.

In Image Correction window (Single and Timeseries), we add one more option threshold, by default it is between 0 to 255 which keeps everything same as before. If you change the value, for example the threshold is 10 to 255, any pixels in image 'e' below intensity level 10 will be crossed out, the corresponding pixels in corrected image PFRET image will be 0.



4. Batch Background Subtraction

We made change on Batch Background Subtraction to make it more flexible. In previous method, it only allowed you to do batch process for time series images which have special file name format like image1, image2, image3... that require consecutive numbers after file name. The updated one doesn't have the



limitation. See the right side figure which is new Batch Background Subtraction Window. You just need to highlight all the files you want to process, click process button, the background subtracted images will be saved into the same directory as the source files, the file name will be the source file name plus Prefix "b_".

5. Arithmetic Function

We add more functions under Image-Process-Arithmetic. Previously, we only have add image and subtract image functions. We rename add image function to overlay image in the updated versions. The new functions are: (1) add constant to an image (2) subtract constant from an image (3) ratio image (4) divide image by a constant (5) multiply image by a constant.

6. Histogram

In the updated version, except to save the histogram figure, you can export the intensity distribution raw data to a txt file.

7. Export ROIs to a file and Import ROIs

In old version, you can choose several ROIs on one image, copy them to a clipboard, then you can paste the same ROIs to other images. However if you close the software, the ROI information can not be saved. In the updated version, you can export ROIs to a file. Whenever you need to apply the same ROIs to an image, you just use Import ROIs function to import those ROIs to clipboard then paste the ROIs to the image.

8. Batch process images to get statistic data

In image detail window, you can check statistic value for special ROI like min value, max value, mean value, standard deviation of this ROI, however you cannot save the information to a file. In the updated

version, under menu Image-Process, submenu Batch Process allows you to Import ROIs from a file, process several images same time for those ROIs and save the result in a text file. The result in a file shows in the figure. It has two ROIs and 6 processed images. You can use excel to open the file and plot the data based on your application.

	170	219	33	39	
file name	minimum	max	mean	std	
C:\ye-2004\michelle\test\b_CFP101.tif	54	111	82	9	
C:\ye-2004\michelle\test\b_CFP102.tif	49	100	77	8	
C:\ye-2004\michelle\test\CFP101.tif	76	133	104	9	
C:\ye-2004\michelle\test\CFP102.tif	71	122	99	8	
C:\ye-2004\michelle\test\FRET101.tif	68	101	82	5	
C:\ye-2004\michelle\test\FRET102.tif	66	94	80	5	
	179	318	47	45	
file name	minimum	max	mean	std	
C:\ye-2004\michelle\test\b_CFP101.tif	11	110	67	20	
C:\ye-2004\michelle\test\b_CFP102.tif	15	107	66	14	
C:\ye-2004\michelle\test\CFP101.tif	33	132	89	20	
C:\ye-2004\michelle\test\CFP102.tif	37	129	88	14	
C:\ye-2004\michelle\test\FRET101.tif	41	100	73	11	
C:\ye-2004\michelle\test\FRET102.tif	44	94	73	8	