

## FRET in MetaMorph® Software

### Overview

The FRET application within MetaMorph software is a post acquisition process producing a correct FRET image that will need some further processing for measurements. The appropriate images will need to be acquired before using the FRET dialog. The dialog does not include any background or shading correction, which should be done at acquisition or post acquisition with the Background Subtraction and Shading Correction functions in the Process Menu. The number of images needed for the FRET dialog depends on the method of correction done and they are listed in the charts below. For Sensitized Emission seven images are needed. For Specified Bleed Through, nine images are needed.

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### Correction Factors

The correction factors are calculated by taking the average intensity values of the specified images and doing the ratio (division) calculations indicated. The average intensity values can be measured by a couple different methods. First, placing several Regions of Interest on the fluorescent areas of the image, use Region Measurements to log the average intensity values to a spread sheet. The average intensity for each region can be logged and then the mean can be calculated in the spreadsheet or logging the Summary average of all the regions in Region Measurement will accomplish the same thing. The charts below show which images are used in the different correction factors and whether they are use in the numerator or denominator of the calculation. Using FRET pairs of CFP and YFP as an example, the definitions for Filter Sets and Samples are as follows:

#### Filter Set

Donor Filter set:  $CFP_{ex} / CFP_{em}$

Acceptor Filter set:  $YFP_{ex} / YFP_{em}$

FRET Filter set:  $CFP_{ex} / YFP_{em}$

#### Sample (Cells)

Donor Only: cells labeled with the CFP tagged compound only.

Acceptor Only: cells labeled with the YFP tagged compound only.

FRET: cells labeled with both CFP tagged and YFP tagged compounds.

A typical experiment will include:

1 slide of Donor Only sample

1 slide of Acceptor Only sample

1 slide of negative FRET control

1 slide of Positive FRET control

Several slides of the FRET experimental cells

Sensitized Emission		Filter Set		
		Donor	Acceptor	FR ET
Sample (Cells)	Donor Only	Coeff. B Denom.	Not Acquired	Coeff. B Num.
	Acceptor Only	Not Acquired	Coeff. A Denom.	Coeff. A Num.
	FR ET	Donor Image	Acceptor Image	rawFRET Image

Sensitized Emission Formula:

$$\text{Corrected FRET} = \text{rawFRET} - (A * \text{Acceptor}) - (B * \text{Donor})$$

$$\text{Coefficient A} = \frac{\text{Acceptor Only Sample in FRET filter set}}{\text{Acceptor Only Sample in Acceptor filter set}}$$

$$\text{Coefficient B} = \frac{\text{Donor Only Sample in FRET filter set}}{\text{Donor Only Sample in Donor filter set}}$$

Specified Bleed Through		Filter Set		
		Donor	Acceptor	FR ET
Sample (Cells)	Donor Only	AiD Denom. DIF Denom.	AiD Num.	DiF Num.
	Acceptor Only	DiA Num.	DiA Denom. AiF Denom.	AiF Num.
	FR ET	Donor Image	Acceptor Image	rawFRET Image

Specified Bleed Through Formula:

$$\text{Corrected FRET} = \text{rawFRET} - [(\text{Acceptor} - (\text{Donor in Acceptor} * \text{Donor})) * (\text{Acceptor in FRET})] - [(\text{Donor} - (\text{Acceptor in Donor} * \text{Acceptor})) - (\text{Donor in FRET})]$$

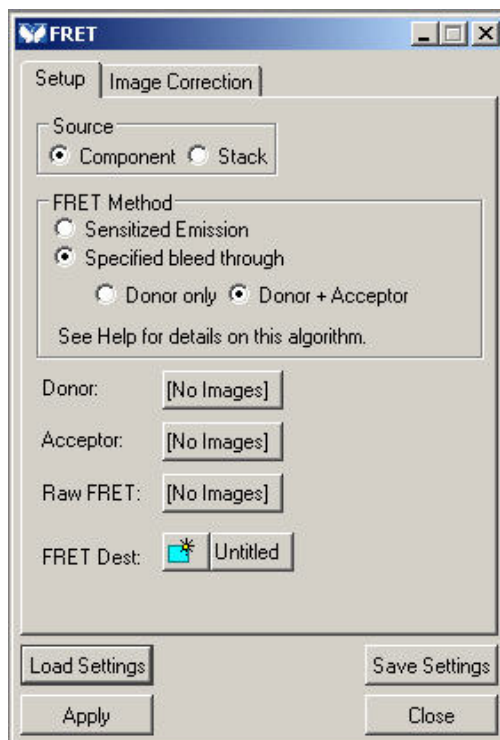
$$\text{Donor in Acceptor (DiA)} = \frac{\text{Acceptor Only Sample in Donor filter set}}{\text{Acceptor Only Sample in Acceptor filter set}}$$

$$\text{Acceptor in Donor (AiD)} = \frac{\text{Donor Only Sample in Acceptor filter set}}{\text{Donor Only Sample in Donor filter set}}$$

$$\text{Donor in FRET (DiF)} = \frac{\text{Donor Only Sample in FRET filter set}}{\text{Donor Only Sample in Donor filter set}}$$

$$\text{Acceptor in FRET (AiF)} = \frac{\text{Acceptor Only Sample in FRET filter set}}{\text{Acceptor Only Sample in Acceptor filter set}}$$

## Dialog Settings



The Donor, Acceptor and Raw FRET image selectors in the FRET dialog refer to the Donor, Acceptor and rawFRET images respectively in the charts above.

## FRET Efficiency

The result image from the FRET dialog is corrected FRET image not a FRET Efficiency image. One standard FRET Efficiency calculation is to take the corrected FRET image and divide it by the Donor image. This can be done with the Arithmetic dialog. Source Image 1 is the FRET result image, Source 2 in Image and select the same Donor image used in the FRET dialog. Set the bit depth to 16. Operation is set to Divide. Set Constant Values: Numerator to 1000. Without doing the multiplication by 1000, the result image of the division will be intensity values below 1 (FRET Efficiency is a percentage). Since the intensity values for all images in MetaMorph are integer values, doing the multiplication will shift the decimal point 3 places to the right allowing FRET Efficiency values to be calculated as follows:

Measuring the average intensity values of specific regions with the cells, an intensity value of 300 will have a FRET Efficiency of 30.0%. An intensity value of 265 will have a FRET Efficiency of 26.5%

There are several other methods for calculating the FRET Efficiency listed in the literature. Similar techniques as above can be used for those methods as well.

If there are any further issues, please contact Technical support at 800-635-5577 x1820 or [support.dtn@moldev.com](mailto:support.dtn@moldev.com).