## **FRET Calculations in Elements 3.0**

This summary demonstrates how to calculate FRET crosstalk between samples and how to apply these corrections to a recently acquired FRET sample.

## **Calculation**

1. Calibrate FRET Images using samples loaded ONLY with Donor and then ONLY with Acceptor.

2. Open the "Create FRET Image" Window:

The 2-Image Acquisition Method requires ONLY the CFP-YFP and YFP-YFP image sets.

| Donor - acceptor (Da):                 | Donor - donor (Dd): | Acceptor - acceptor (Aa): | Acceptor - donor (Ad): |  |
|--|---------------------|---------------------------|------------------------|--|
| <none></none>                          | <none></none>       | <none></none>             | <none></none>          |  |
| Thumbnail                              |                     | Thumbnail                 | _ Thumbnail            |  |
| No Image                               | No Image            | No Image                  | No Image               |  |
| FRET Method Method Sensitized emission |                     |                           | Calibrate OK           |  |
|  |                     |                           |                        |  |
| Acceptor in FF                         | RET (CoA) 0.02      | Donor in Acceptor         |                        |  |
|  |                     | Occeptor in Dopor         | Save • Hei             |  |

Note this calibration is saved and is performed by TI during the training on the instrument.

3. Load Sequences of acquired data (can be single images, time-lapse image sets or 3-D image stacks).

| Create FRET Image                                      |                        |                           |                        | ×      |
|--|------------------------|---------------------------|------------------------|--------|
| Donor - acceptor (Da):                                 | Donor - donor (Dd):    | Acceptor - acceptor (Aa): | Acceptor - donor (Ad): |        |
| C:\Documents and Set 💌                                 | C:\Documents and Set 💌 | <none></none>             | <none></none>          | •      |
| Thumbnail  | Thumbnail              | Thumbnail                 | Thumbnail              |        |
| 000  | 000                    | No Image                  | No Ir                  | nage   |
| FRET Method  |                        |                           | Calibrate              | ОК     |
| Method Sensitized emission                             |                        |                           | Load 👻                 | Cancel |
| Acceptor in FR   | ET (CoA) 0.02 Dono     | or in Acceptor            | Save 💌                 | Help   |
| Donor in FRET (CoB) 0.57 Acceptor in Donor 9e-4 Remove |                        |                           |                        |        |

## 2 Images Loaded - No other settings to adjust

## 3: View FRET Image



4. Example of image sequence with dropping FRET efficiency. Change in FRET is graphed using Time Measurement:

