

Lab 7. Advanced Technique: FRET Using the Confocal Microscope

Objective: Examine FRET in a fixed sample. Measure the ratio and compare with controls. Observe the effect of bleaching the acceptor fluorescence.

Samples: Cos-7 cells labeled with Cy3 & Cy5 cholera toxin

Procedure: You will have 2 slides: Slide 1 with two fields containing Cy3 or Cy5 single labeled cells, and slide 2 containing double labeled cells (the slide where you should detect FRET).

1. Optimize the signal and store and image for the Cy3-only sample.
2. Optimize the signal and store and image for the Cy5-only sample.
3. While using the Cy5-only sample, sequentially acquire both Cy3 and Cy5 channels.
Do you see Cy3 signal in the Cy5 channel? Why or why not?
4. While using the Cy3-only sample, sequentially acquire both Cy3 and Cy5 channels.
Do you see any Cy5 signal? Why or why not?
5. Switch to the FRET (Cy3+Cy5) slide and optimize the FRET channel (using the gain only, i.e., keep the 543 laser setting that is used on the Cy3-only sample).
6. Acquire all three tracks, Cy3, Cy5, and FRET. Save the image.
7. Zoom the image, open the "Bleach" control window, select a small ROI and set the "bleach after n scans" to 2. Set the 633 laser to 100% and iterations to about 400.
8. Open the time series window and set number of scans to 5, interval to 0, and then "start bleach" while all 3 tracks are active.
9. Save the time sequence and observe how the ROI signal changes between the 3 channels. Plot the ROI as a function of time.
10. Do you see "donor dequenching?"