Time for experiment

30-60 minutes

EGF Experiment

1. Set the cell culture dish on the stage of microscope.
   - Change the dish cover to a piece of flat cover glass.
   - Make sure the dish is securely settled on the rack of the stage.
   - Make sure the 40x objective touches the bottom of dish.

1. Start Metafluor

1. Open a CFP-YFP-FRET protocal

1. Use FRET-open to locate a good cell.

1. Starting focusing to adjust focusing and exposure time.

1. Configure-> Aquisition to set exposure time.

1. Aquire one image, define regions.
   - Enable background subtraction of a constant
   - The constants are chosen by image histograms.
   - Define regions for tracing change of FRET.
2. Aquire 5 images at 60 seconds intervals.

1. Pause aquiring.
   - Adding 20 ul x 20 ug/ml EGF to 2 ml dish (200ng/ml), to the edge of dish.
   - Pipet (big, 1000ul) up and down 3 times to mix well.
   - Be careful not to touch anything or cause the cell culture dish to move.
   - Add events
     - Events -> EGF -> mark events.

1. Start Aquiring at 30 seconds intervals for 20 cycles and the at 60 seconds intervals for 20 cycles.

1. Adjust Focus if necessary.

Note

The same protocol can be used for pervanadate experiments by changing EGF to pervanadate.