FRAP experiment protocol

Before the experiment, prepare a dish of cells. Use the media inside the dish to wash away dead cells. Add 2 ml CO^2 independent media into each dish.

Using microscope and metafluo to record FRAP images. In Metafluo, open the FRAP protocol.

Protocal -> FRAP.FSF -> ok.

Use FRET_open light to search for a good cell, and use YFP light to image the cell.

1. Use eyepiece to spot a usable cell. (Note that this step has to be performed quickly to avoid photobleaching)
   - a)In the microscope, push in filter block (HQ bla bla).
   - b)In the top menu of Metafluo, click
     - Configure -> Illumination Control -> double click FRET_open
     - Click Shutter to open.
   - c)Move the stage and adjust focus to locate a good cell.
   - d)Pull down aperture (F) to check photobleaching area.
   - e)Pull out filter block.

1. Adjust focus in Metafluo.
   - a)In the side menu, click New, then click
     - Focus -> yfp (Focus window, may need to adjust exposure time ~1000ms)
     - -> Start Focusing
     - Adjust focusing on microscope,
   - b)Pull down aperture (F) to check photobleaching area.
   - c)When finish, click
     - Stop Focusing -> close.

1. Define region, perform photobleaching, acquire and save images.
   - a)Acquire one image with aperture down and another image with aperture up; adjust color setting based on the images; define three regions where the average fluorescent intensity is monitored. The first region lies in the

photobleaching area, the second region lies part of the cell farthest from the first region, and the third region lies in the image background. Define regions:

- In side menu, click
- Regions -> ok -> select a curve -> use mouse to define regions
- -> finish each region by double clicking left button
- -> done.

- b)Before photobleaching
  - Make sure that the filter block is pulled out and aperture is at up position.
  - In Experiment Control Panel, click
  - Set Timelapse -> 1 sec
  - ->Zero Clock
  - Check Log Data -> choose file name -> ok
  - Check Save Images -> choose file name -> ok
  - Acquire 15 images before photobleaching,
  - In Experiment Control Panel, click
  - Acquire -> pause.
  - -> Set Timelapse -> 10 sec. (This length of time interval depends on the diffusion rate of biosensor. For example, we choose 1 second for fast-diffusing cytosolic biosensor and 10 second for slow-diffusing membrane-tethered biosensors, respectivley).
- c)Photobleaching using yfp
  - In top menu, click Configure -> Illumination Control -> double click YFP_open,to switch to hight intensity light for photobleaching
  - Pull down aperture and open shutter for 15 sec to allow photobleaching.
  - Close shutter and pull up aperture
  - double click YFP to swich back to low intensity light for imaging.
- d)Acquire image after photobleaching
  - First, make sure the aperture is up.
  - In Experiment Control Panel, make sure time lapse is correct (10 sec or 1 sec).
  - Acquire image until fluorescence recovery stops.-> acquire
  - Stop.-> pause

- e)Close experiment without saving protocol. In the side menu, click Close -> No.

Close Metafluo and turn off microscope.

Some notes about using microscope

1. To switch between eyepiece and the camera, push the button located at the left hand side of the microscope body. The button is labeled with “Left/Right”.
2. Use 20x objective to locate the cell. Afterwards, switch to 100x object to imaging the cell. 40x and 100x objectives needs oil.
3. To avoid automatically scroll graph when plotting average fluorescent intensities in the regions of interest, click
   - Graph -> un-check “scroll graph to show new data”.