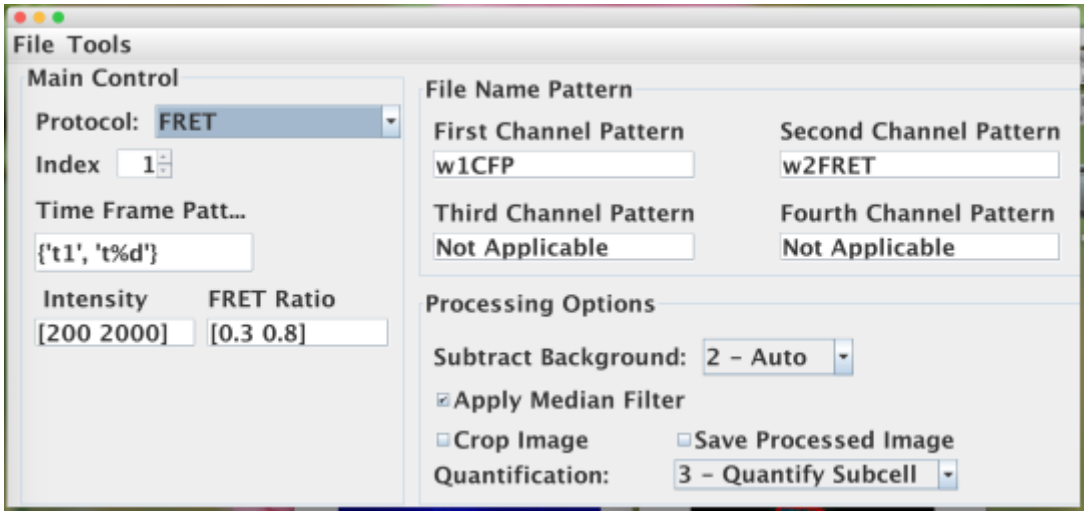
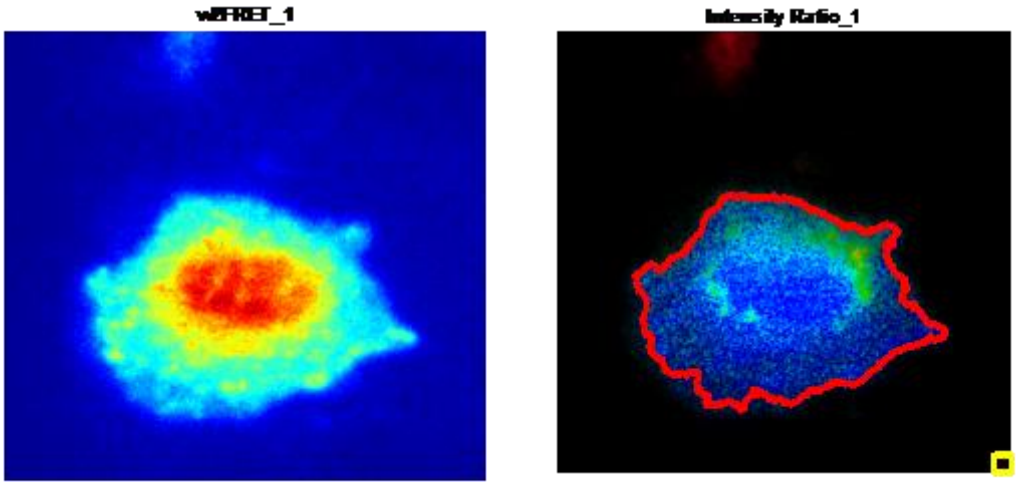
Fluocell – Quanty Module User's Guide
Automatic Quantification of Multiple-position Image Sequences

Step 1, Download the quanty model and related dataset

Download the quanty module from github (http://github.com/lu6007/quanty) and save to the "quant/" folder. Then download the sample data (http://wang.ucsd.edu/~kalu → Download Quanty Sample Data) and save to the folders "quanty/" and "data/quanty_sample/". Install Quanty by adding the subfolders "src/", "app/group/", "app/test/", and "contrib/" into the MATLAB searching path.

Step 2, Quantification for multiple positions:



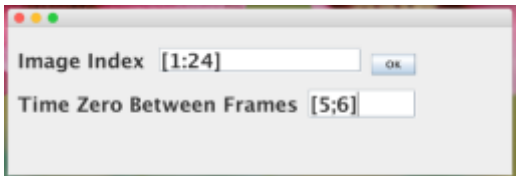


1. In the sample data, the multiple-position images have already been sorted into subfolders for each position. New imaging data will need to be sorted into subfolders following the instructions in **Part 5, Sort Files** in this guide.
2. Start Fluocell and following instructions in sections I-IV and VIII, to visualize and quantify the imaging results in data/quanty_sample/group1/p1" by choosing the options

shown above in the menue, and open the first CFP iamge "6_w1CFP_s1_t1.TIF". Here the intensity ratio is computed as w1CFP/w2FRET. The choice of channel patterns depends on how intensity ratio is computed. For certain biosensors, ratio = FRET/CFP, then we set the first and second channel patterns to match the file names of the FRET and CFP images respectively.

3. Select "Subtract Background → 2 - Auto", "Apply Median Filter", and "quantification: → 3 – Quantify Subcell". If you want to check ratio images later, select "Save Processed Image". The last selection will slow down the analysis process.
4. In command window,
   >> fluocell_data.num_layer = 3;

5. Go to "Tools" in Fluocell, and input "BrightnessFactor" value to be 0.7 and check "Show Detected Boundary".
6. Use Fluocell to open the FRET Ratio image file in p1/. Carefully check how many time points you have. Go to "Tools" in Fluocell and choose "Batch Update Image", input the index as format: [1:24], say 24 is the number of time points (frames) you have in your file (No need to click the "OK" button).



7. Check the pdgf information. In the "Batch Update Image" window, set "Time Zero Between Frames" to [5;6]. This means the stimulation (pdgf) is added at the images frames 5 and 6. And we will shift the imaging time to be zero at the estimated time of pdgf addition.
8. Pass the information in fluocell_data to the group data structure:

>> group = g2p_init_data(fluocell_data, 'group_data',[]);

In MATLAB, you will see the following output.

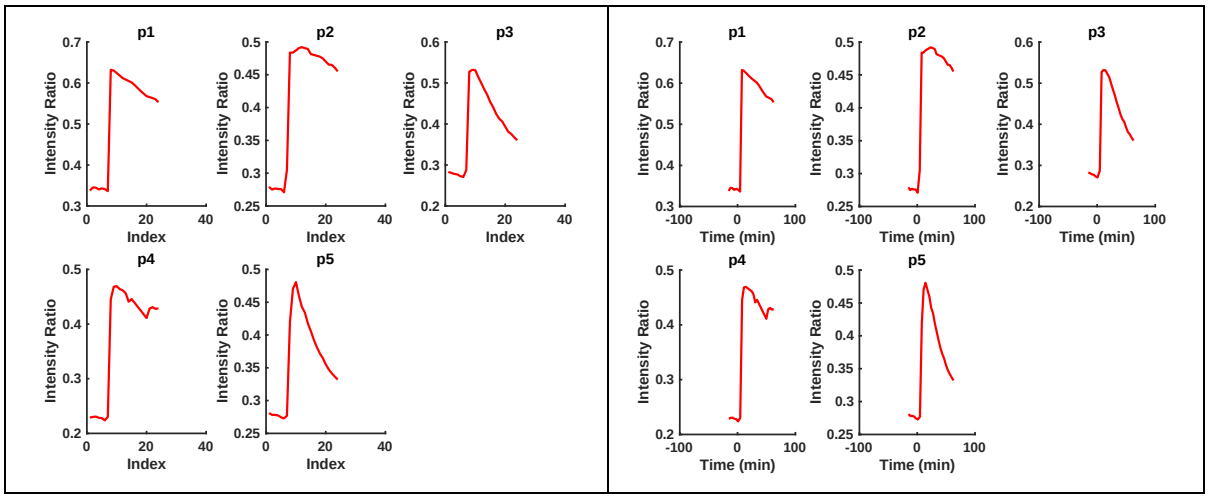
g2p_init_data: Update from fluocell_data since there is no input of group data or the data file.

g2p_init_data: Please make sure that fluocell is reading images from the p1 position.

pdgf time = 643.100000 sec

9. Quantification of multiple positions:

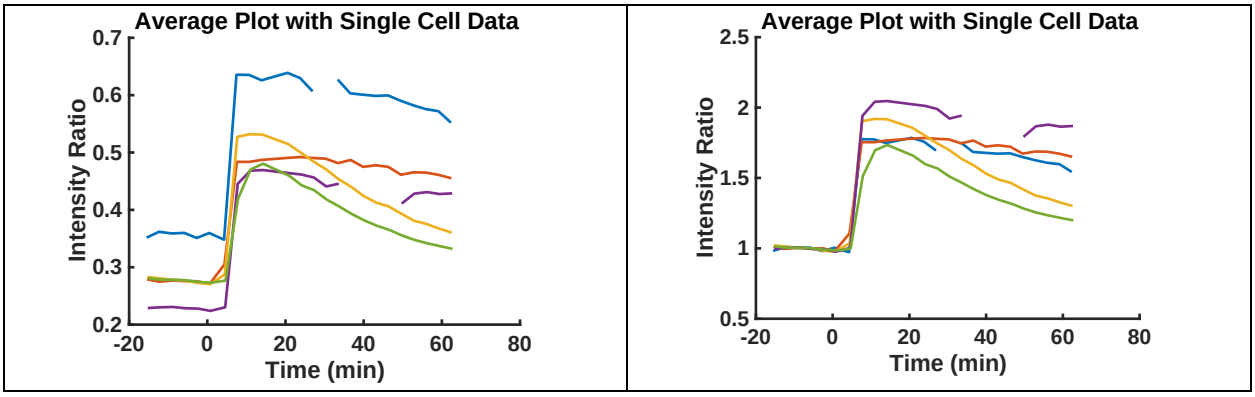
>> g2p_quantify(group);



10. Plot group result together:

>> group_plot(group,'method',1, 'save_excel_file', 1, 'sheet_name', 'Group1', 'y_limit', [0.5 2.5]);

- Output time and ratio sequence for inspection (Both normalized and orginal ratio);
- Save the time-ration excel for statistics;
- The options “y_limit” and “t_limit” specify the y- and x-axis range, respectively;
- The option “sheet_name” can be selected to name the experimental data, for example ('sheet_name', 'MEF_src').



Note: on Mac computer, the excel file server cannot be started, so the excel files will be saved in the csv file, but they can still be read from the group_compare function in the “xlsx” format.

Step 3, group comparison, view images, and make movies.

How to use the excel file generated in part 2 to compare groups:

First, we need to add another group to the excel file by repeating step 2 for another group called “dish2”. Start at the quanty root direction (by >> cd quanty)

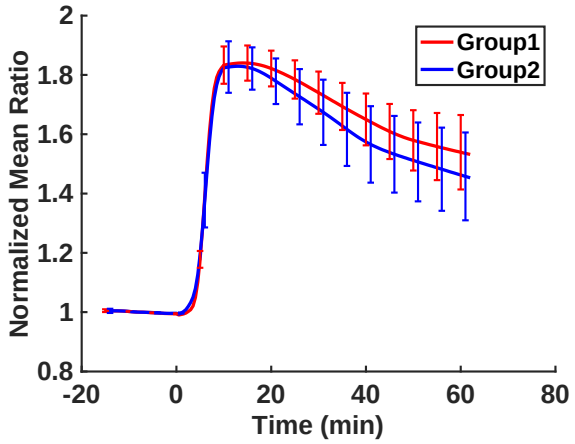
>> p = '../data/quanty_sample/group2/p1/';

```
>> data_file = strcat(p, '../output/data.mat');
>> test = load(data_file);
>> data = test.data;
>> data.path = p;
>> data.first_file = strcat(p, '6_w1CFP_s1_t1.TIF');
>> data.show_detect_boundary = 1;
>> save(data_file, 'data');
>> group2 = g2p_init_data(data, 'load_file', 1); % load from saved file
>> g2p_quantify(group2);
>> group_plot(group2,'method',1, 'save_excel_file', 1, 'sheet_name', 'Group2', 'y_limit', [0.5
2.5]);
```

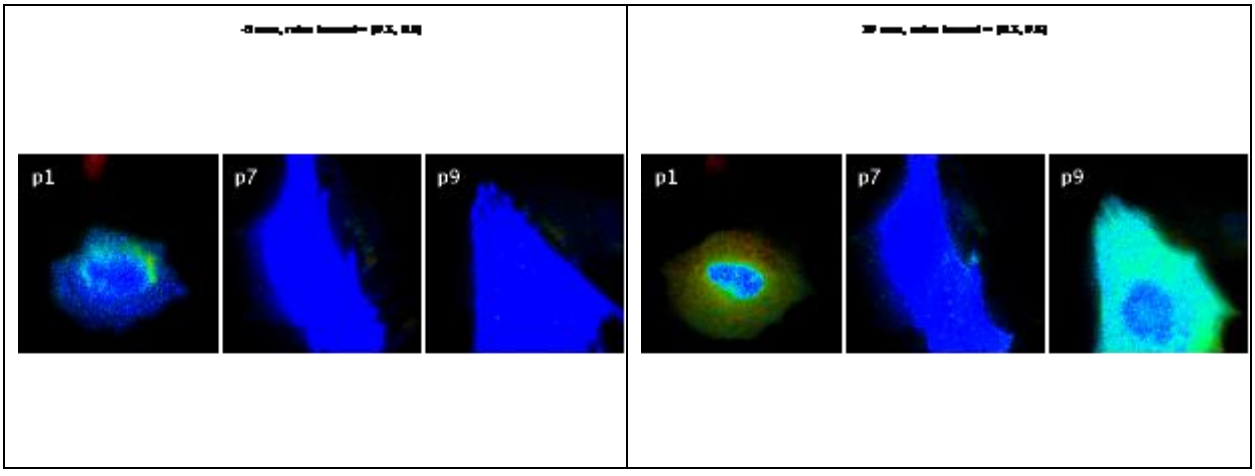
Open the result.xls file in the data folder to confirm that there are two sheets “dish1” and “dish2”. Remove all other sheets from the exel file, save and exit.

In MATLAB command window,

```
>> group_compare(group, 'group_name', {'Group1', 'Group2'});
```




```
>> group_image_view(group, 'time_point', [-5; 30], 'num_col', 3);
```



The following commands make a move saved in the file “dish2\p1\output\FRET.avi”.
>> group.data.intensity_bound = [1 1000];
>> group_make_movie(group);

Part 4, special handling

How to inspect specific position after quantification

we still use “g2p_quantify” function here but with more parameter input. If the positions 1 and 2 are not in the data folder, and the quantification starts with another position, p3.
>> group = g2p_init_data(fluocell_data, 'group_data', [], 'name', 'p3');

If you want to check whether the tracking and detection are correct for one specific position, for example you want to check position 3, use these options:
>> g2p_quantify(group, 'show_figure', 1, 'num_layers', 3, 'name_i', 'p3');
or
>> g2p_quantify(group, ‘show_figure’, 1 (default value is 0 and you cannot see the tracking with 0), ‘num_layers’, 3, (decreased the influence from nucleus, 3 layers) ’name_i’, ‘p3’ (detecting the specific position we are interested in));

Part 5, Sort files

Before processing new imaging data, you need to sort the image files for each position into separated folders and so that Fluocell and MATLAB can recognize and process the images:

1. Path of the data folders:

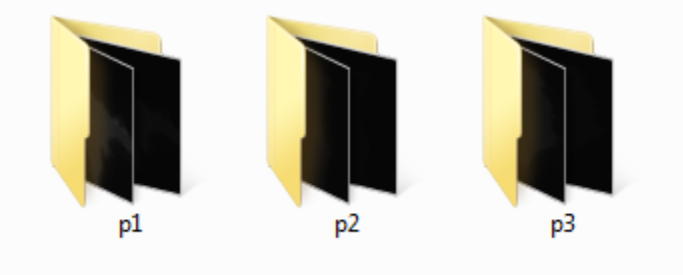
>> p = 'D:/sof/data/quanty_sample/'; % You can copy the path directly. Remember to add a forward slash at the end of the path string.

2. Pass subfolder name to MATLAB parameter, make the folder names as simple as possible for MATLAB recognitions:

>> sub_dir = {'dish1/', 'other_subfolder_name/'}; % Remember to add a forward slash after subfolder name.

3. Run the function in MATLAB:

>> batch_sort_file_multiple_position(p, sub_dir);



Data Structures

In `group_plot()`, the data structure `exp` is created and used when the function is run. Data for only one ROI is contained in the structure. Different ROIs can be selected with the function parameter `i_layer`. i.e. `i_layer = 1` or `i_layer = 2`.

It contains the following fields:
(Example shows `exp` at the end of the `group_plot()` function. Sample data had 30 time frames.)

```
exp{1}.cell(1) =
          time: [30x1 double]
         value: [30x1 double]
    norm_value: [30x1 double]
```

`exp{n}.cell{k}`
`n` – experiment number
`k` – object (objects from all the positions are included)

`exp{n}.cell{k}.time` – time only

`exp{n}.cell{k}.value` – raw value of intensity ratio

`exp{n}.cell{k}.norm_value` – normalized value of intensity ratio