***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 “quanty/” 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:

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Figure 1. *Fluocell* settings

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 follow instructions in “The Fluocell User’s Guide”, sections I-IV and VIII, to visualize and quantify the imaging results in data/quanty\_sample/group1/p1”. Choose the options shown in Fig. 1, and open the first CFP image “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, the ratio is 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.

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1. In the MATLAB command window,

>> fluocell\_data.num\_roi = 3;

% default is 1

1. Go to “Tools” in Fluocell to input 0.7 as the “Brightness Factor”, and check “Show Detected Boundary”.
2. 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 in the format: [1:24], if 24 is the number of time points (frames) you have in your file (No need to click the “OK” button).
3. 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. We will shift the imaging time to be zero at the estimated time of pdgf addition.
4. In MATLAB, pass the information in fluocell\_data to the group data structure:

>> fluocell\_data.multiple\_object = 1;

>> 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

1. To quantify multiple positions, input:

>> g2p\_quantify(group);

Output:

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1. To plot group results together, input:

>> group\_plot(group,'method',1, 'save\_excel\_file', 1, 'sheet\_name', 'Group1', 'y\_limit', [0.5 2.5]);

* This will output the time and both normalized and original ratio sequences for inspection.
* Save the time-ratio in 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').

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**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 directory (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 the MATLAB command window input:

>> group\_compare(group, 'group\_name', {'Group1', 'Group2'});



>> group\_image\_view(group, 'time\_point', [-5; 30], 'num\_col', 3);

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The following commands make a movie 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 a specific position after quantification  
We still use the “g2p\_quantify” function here but with more parameter inputs. 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, if you want to check position 3, use these options:

>> g2p\_quantify(group, 'show\_figure', 1, 'num\_roi', 3, 'name\_i', 'p3');

which is

>> g2p\_quantify(group, ‘show\_figure’, 1 (default value is 0 and you cannot see the tracking with 0), ‘num\_roi’, 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, so that *Fluocell* and MATLAB can recognize and process the images:

1. An example of the path of the data folders:

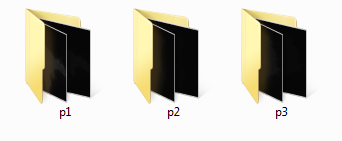
>> p = 'D:/sof/data/quanty\_sample/'; %You can copy the path directly. Remember to add a forward slash at the end of the path string.

1. 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.

1. 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.

The exp data structure contains the following fields:

(This 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

**Test Functions**

Quanty is distributed with a set of test functions in the folder app/test/ . Before running the tests, the location of data files needs to be specified in the *app/test/my\_function()* by the variable *my.root*.