Fluorescence quantification of protein reporters

This quantification pipeline is divided into 3 parts:

- 1st part: outlines determination.
- 2nd part: extraction of xy coordinates from outlines.
- 3rd part: image analysis.

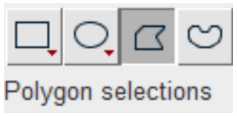
1st part: outlines determination

Requirements:

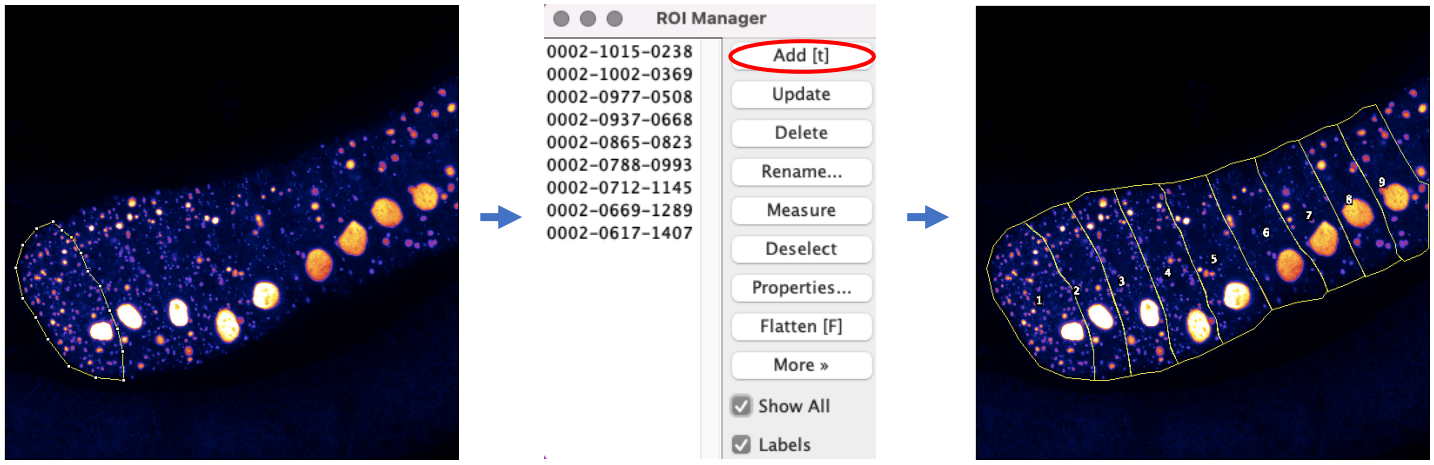
➢ Fiji (Image J)

Procedure:

1. Open Fiji (ImageJ).
2. Open *RIO Manager*
   - Analyze > Tools > RIO Manger…
3. Using Fiji, open the image you want to analyze.
4. Using the "polygon selections" tool manually draw outlines that delimit the oocyte borders.



- Each oocyte outline can be treated as an individual Region Of Interest (ROI).
- To delimit oocytes, a max projection could be useful as gonad dimensions are variable along the *z*-plane.
- Each oocyte outline (ROI) can be saved by clicking on the "Add [t]" bottom located in the *RIO Manager* window, as shown below:



#This image is located at ~/example_image/w11-1.tif

5. Once all outlines are added, select them and save them as a "*.zip*" file. **Important:** name the file with the same name as the image where the outlines are coming from.
   * In the case of only one outline, it will be saved as a "*.roi*" file.

2nd part: extraction of xy coordinates from outlines (required for image analysis in 3rd part)

Requirements:

- Fiji (Image J)
- R
- Optional: RStudio

Example data and description:

Example images and outlines for this guide can be found in the folder ~/example_image

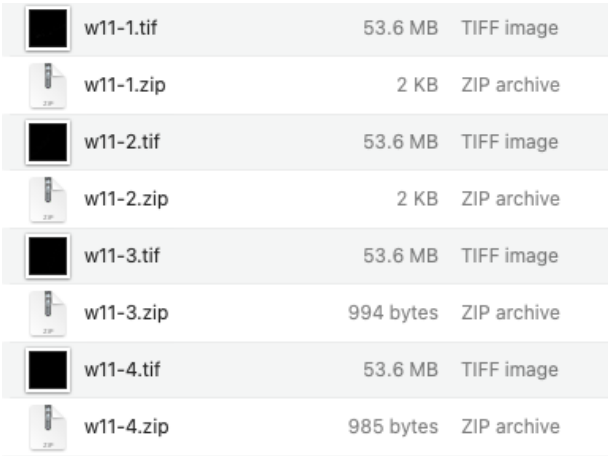
Images were obtained from **4** consecutive portions of a C. elegans gonad live imaged. Following a proximo-distal axis, they are labeled as *w11-**1*** to *w11-**4***.

- w11-1.tif: image of oocytes minus 1 to minus 9.
- w11-1.zip: outlines of oocytes minus 1 to minus 9.

- w11-2.tif: image of oocytes minus 10 to minus 17.
- w11-2.zip: outlines of oocytes minus 10 to minus 17.

- w11-3.tif: image of oocytes minus 18 to minus 21.
- w11-3.zip: outlines of oocytes minus 18 to minus 21.

- w11-4.tif: image of oocytes minus 22 to minus 25.
- w11-4.zip: outlines of oocytes minus 22 to minus 25.



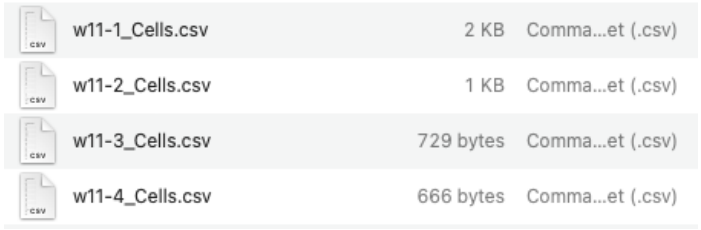
Procedure:

6. Data should be organized in a folder as shown just above:
   For each *"name".zip* outline file, a matching *"name".tif* image must be in the folder.
   - For this example, 4 images will be analyzed simultaneously.

7. Using Fiji, open the script "*BATCH_improved_outlines_oocyte_extraction.ijm*"
   Script location at ~/image_outlines

8. Run it and select the folder containing the images and oocyte outlines (ROIs) to analyze. This script extracts the xy coordinates from the ROIs delimited and saved using Fiji.

   Output: *"name"_Cells.csv* files with xy coordinates of ROIs.



9. Using R or RStudio, open the script "*BATCH_automatic_outlines_reorganization_by_columns.R* Script location at ~/image_outlines

10. Run the script and select any file within the folder containing the images and ROIs to analyze. This script reorganizes the outlines files, so they are readable in the image processing step.

    Output: *MOD_"name"_Cells.csv* files with reorganized xy coordinates of ROIs.

How to modify "*BATCH_improved_outlines_oocyte_extraction.ijm"*

Line 1 >

```
//... Modify if needed (File Identifiers)...
```

Line 2 >

```
file_ext = "zip"; // ROI file extension
```

Line…

Only line 2 should be modified. If needed replace "*zip*" by "*roi*".

3rd part: image analysis

Expected results:

- Condensates/granules segmentation and quantification: volumes, mean intensities, median intensities, among others descriptors.
- Oocyte analyses: volumes, soluble and condensed cumulative intensities.

Requirements:

- MATLAB v R2018b or higher
  - Required toolboxes:
    Image Processing Toolbox
    Signal Processing Toolbox
    Statistics and Machine Learning Toolbox

Set up:

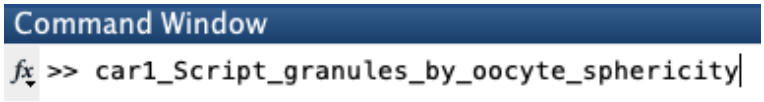
- Go to ~/image_analysis_scripts
- Copy the scripts into the MATLAB folder (usually located in the Documents directory).

Procedure:

11. Open MATLAB

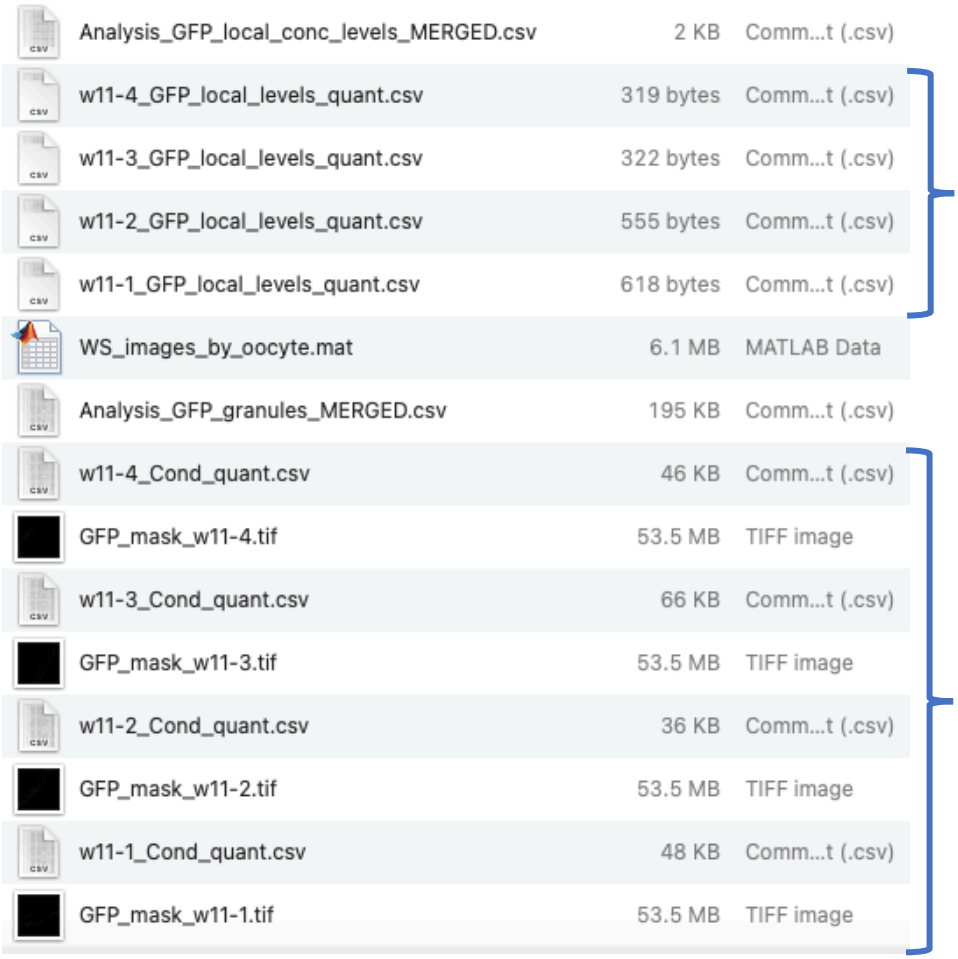
12. Run "*car1_Script_granules_by_oocyte_sphericity.m*"

- To run the script, type *car1_Script_granules_by_oocyte_sphericity* in the command window and press enter, as shown below:



13. Select the folder containing the images and organized oocyte outlines (from step 10; *MOD_"name"_Cells.csv* files).

Output: this image analysis script will yield the results shown below



…_local_levels_quant.csv files: tables (by image) with delimited oocytes and their respective measurements

- image_ID_name
- img
- cell
- CAR1_total: oocyte's cumulative intensity
- CAR1_cond: condensates' cumulative intensity
- CAR_dil: soluble cytosol's cumulative intensity
- vol_cell: oocyte volume
- vol_cond: condensates' cumulative volume
- vol_dil: soluble cytosol's cumulative intensity

GFP masks: images with detected condensates outlined

…_Cond_quant.csv files: tables (by image) with segmented condensates and their respective measurements

- image_ID_name
- Cell
- Granule
- Surface_Area
- Volume
- Sphericity
- MeanInt: mean intensity
- CumInt: cumulative intensity
- MedianInt: median intensity
- Volume_um3

- ***Analysis_GFP_granules_MERGED***:
  - …_Cond_quant.csv files merged in one file.
- ***Analysis_local_conc_levels_MERGED***:
  - …_local_levels_quant.csv files merged in one file.
- ***WS_images_by_oocyte***: MATLAB workspace file

How to modify: *"car1_Script_granules_by_oocyte_sphericity.m"*

```
%% script to analyzed CAR-1 in live worms

% file identifiers
image_file.GFP  = '*.tif';
image_file.ROIs = 'MOD*Cells.csv';


% Segmentation parameters
clear parameters
parameters.quantile = 0.95; % BGD approximation quantile for initial filtering,
recommended values: 0.9 to 0.95.
% thresholding
parameters.threshold_granules = 0.6; % values are between 0 and 1, one is likely to
be a pixel that corresponds to granules
% excluding small objects
parameters.small_objects_size = 32; % objects smaller than the value (pixels) will
be excluded

% Description_ of _ stack
% there possible setting for acquire z-stacks are:
% in focus: acquisition within the gonad's z-plane boundaries.
% out of focus: slides out of gonad focus
%     1. Whole gonad with frames out of focus
%     2. half gonad with initial frames out of focus
%     3. Z-Stack in focus
slides_out_of_focus = 0;    % yes = 1, No = 0
half_gonad          = 1;    % yes = 1, No = 0

pixel_size_in_x_and_y   = 0.0670922;    % micro meters
pixel_size_in_z         = 0.5;          % micro meters


% Flags for specific analysis
clear flags
flags.Gauss = 0; % 1 if BGD smoothing should be performed
```