

Condensate quantification - mRNA molecules within condensates (How to use)

Before start

- Download the repository or the content of this folder.
- For Github downloads: example smFISH image (large file) is provided as '**.tif.txt**'. A link to download the image is within the text file.
 - <https://doi.org/10.17632/8jvrnztdvc.1> (location: **smFISH images quantification > example_image**).
 - <https://doi.org/10.17632/8jvrnztdvc.2> (location: **condensate quantification > example-data**).
 - This guide uses the smiFISH image from the folder: **./code-image-analysis-c-elegans/smFISH-images-quantification/example_image**.
- **Important:** this script uses results from **smFISH images quantification** (**./code-image-analysis-c-elegans/smFISH-images-quantification**).
- **Important:** an average PSF of experimental single mRNA molecules is required.

Requirements and set up

Requirements:

- Tested in MATLAB v R2018b or higher
 - Required toolboxes:
Image Processing Toolbox
Statistics and Machine Learning Toolbox
- Additional requirements:
FISH-quant (1,2,#)
https://bitbucket.org/muellerflorian/fish_quant/src/master/

Set up:

- Copy the scripts provided (**./condensate-quantification-scripts**) to the MATLAB folder (usually located in Documents directory).
- Install FISH-quant:
 - Go to https://bitbucket.org/muellerflorian/fish_quant/src/master/ (1,2).
 - Click Downloads and then download the repository (1,2).
 - Unzip the file in the MATLAB folder (Documents directory) (1,2).
 - Go to **./Documents/MATLAB/FISH_quant/Documentation** (1,2).
 - Open **FISH-QUANT__Tutorials.pdf** (1,2).
 - Follow the section **1.1 Install FISH-quant for Matlab** (1,2).

This script performs the following analysis:

- Inputs:
 - **MATLAB workspace with condensate coordinates:** **.mat** file obtained after **smFISH images quantification**.
 - **smFISH image:** smFISH image used to run **smFISH images quantification**.
 - **Average PSF of experimental single mRNA molecules:** it can be obtained as described previously (1,2).
- Analysis:
 - Estimation of the number of mRNA molecules within condensates segmented in **smFISH images quantification**. Estimates are computed by integrated intensity and cumulative intensity (3).
- Outputs
 - **.csv** table file with columns:

```
"imageID": image name
"Cell": oocyte
"condensate": condensate index
"SigmaXY": estimated sigma in x and y of condensate
"SigmaZ": estimated sigma in z of condensate
"Amplitud" estimated amplitude of condensate
"BGD": estimated background of condensate
"MaxI": maximum intensity of condensate
"volume_dil": volume of condensate after morphological dilation
"volume": volume of condensate
"mean_intensity": average intensity of condensate
"cumulative_intensity": cumulative intensity of condensate
"median_intensity": median intensity of condensate
```

```
"n_molecules_intg": number of mRNA molecules within condensate, calculated by integrated intensity
"n_molecules_cum": number of mRNA molecules within condensate, calculated by cumulative intensity
"n_molecules_cum_ctrl": number of mRNA molecules within condensate, calculated by cumulative intensity
(computing control)
```

User guide

Data provided

Location: `./example-data`

- **MATLAB workspace with condensate coordinates:** `./example-data/Dil_Coor_FISH_GFP_w4_Spn4.mat`.
- **smFISH image:** `./example-data/w4_Spn4.tif`.
- **Average PSF of experimental single mRNA molecules:** `./example-data/experimental_single_mol_PSF_8x6_mRNA_AVG_ns.tif`.

Analysis (Procedure):

Pre-analysis

1. Make a folder with the **inputs** (as in `./example-data`).
 - `Dil_Coor_FISH_GFP_w4_Spn4.mat`
 - `w4_Spn4.tif`
 - `experimental_single_mol_PSF_8x6_mRNA_AVG_ns.tif`
2. The script will look for the key words in **bold** to identify each input. This can be modified as shown below.

Analysis

3. Open MATLAB.
4. In the command window type:
`condensates_quantification_script`
and then press enter.
5. Select the folder with the **inputs** (as in `./example-data`).

The default parameters of the script include:

```
...

%===== file ID indicators
files = struct; % do not modify
files.matlab_ws = '*Coor*'; % unique file identifier for MATLAB workspace
files.FISH_img = '*Spn4*'; % unique file identifier for smFISH image
files.mRNA_img = 'experimental_single_mol*'; % unique file identifier for mRNA image

%===== modify below only if you need to change the microscope parameters
define_microscope_parameters = 0; % yes = 1, no = 0

...
```

if `define_microscope_parameters = 1`, the script will ask for microscope parameters in the command window.

To change this:

- Type in the command window:
`edit condensates_quantification_script`
- Press enter.
- Then, default values can be manually modified (lines # 9 to # 15).

Outputs:

Provided output examples:

The quantification results, for the example data provided in this tutorial, are included (location: `./quantification-example-data`).

References

1. Mueller, F., Senecal, A., Tantale, K. et al. FISH-quant: automatic counting of transcripts in 3D FISH images. Nat Methods 10, 277-278 (2013).
2. Tsanov, N., Samacoits, A., Chouaib, R. et al. smiFISH and FISH-quant - a flexible single RNA detection approach with super-resolution capability, Nucleic Acids Research 44 (22), e165 (2016).

3. Cardona et al., Self-demixing of mRNA copies buffers mRNA:mRNA and mRNA:regulator stoichiometries, Cell (2023), <https://doi.org/10.1016/j.cell.2023.08.018>