

In Silico simulation of smFISH images

This simulation pipeline is divided into 2 parts:

- 1st part: generation of experimental PSF/mRNA images repository.
- 2nd part: generation of synthetic smFISH images at different mRNA concentrations.

Requirements:

➤ MATLAB v R2018b or higher

* Required toolboxes:

Image Processing Toolbox and Statistics and Machine Learning Toolbox

Set up: Copy the scripts provided (~/scripts_simulation) into the MATLAB folder (Documents directory).

Important:

Here, two simulation approaches (app) are described:

➤ Approach 1:

Simulation is performed placing experimental mRNA images (PSF) cropped from smFISH images.

➤ Approach 2:

Simulation is performed placing an average experimental mRNA image (average PSF) with the intensities of experimental mRNA images.

- First, an average experimental PSF image is obtained and normalized.
- Second, the intensity value of experimental mRNA images is multiplied by the normalized PSF and then placed.

Data provided

Generation of experimental PSF/mRNA images repository

➤ Approach 1 (location: ~/simulation_inputs/mRNA_repositories/spots_for_approach1):

* mRNA positions table:

.csv file with columns: "Image", "Pos_Y", "Pos_X", "Pos_Z", "Y_det", "X_det", "Z_det" (i.e.: obtained using FISH-quant)

%Image: names should match images where mRNAs are extracted

% Pos_Y, _X, and _Z: mRNA positions in nm

% Y_, X_, and Z_det: mRNA position in px index

* smFISH Images

➤ Approach 2 (location: ~/simulation_inputs/mRNA_repositories/spots_for_approach2):

* mRNA positions table:

.csv file with columns: "Image", "Pos_Y", "Pos_X", "Pos_Z", "Y_det", "X_det", "Z_det" (i.e.: obtained using FISH-quant)

%Image: names should match images where mRNAs are extracted

% Pos_Y, _X, and _Z: mRNA positions in nm

% Y_, X_, and Z_det: mRNA position in px index

* smFISH Images

Generation of synthetic smFISH images at different mRNA concentrations

➤ Approach 1

- * Binary image of an oocyte where mRNAs will be placed: “*oocyte_to_model_16bits.tif*”
location: ~/simulation_inputs
- * Repository of mRNA images: “*mRNA_repository_approach1.mat*”
location: ~/simulation_inputs/mRNA_repositories

➤ Approach 2

- * Binary image of an oocyte where mRNAs will be placed: “*oocyte_to_model_16bits.tif*”
location: ~/simulation_inputs
- * Average experimental mRNA image (average PSF):
“*experimental_single_mol_PSF_8x6_mRNA_AVG_ns*”
location: ~/simulation_inputs
- * Repository of mRNA images: “*mRNA_repository_approach2.mat*”
location: ~/simulation_inputs/mRNA_repositories

1st part: generation of experimental PSF/mRNA images repository

Procedure:

1. Open MATLAB
2. In the command window:

Approach 1	Approach 2
2.1. Type * and then press enter: * <i>Script_to_generate_mRNA_repository_approach1</i>	2.2. Type * and then press enter: * <i>Script_to_generate_mRNA_repository_approach1</i>

3. Select the folder containing the mRNA positions table

Approach 1	Approach 2
3.1. The script will look for a .csv file with the identifier 'dissolved' in its file name. To change this: - Type in the command window: <i>edit Script_to_generate_mRNA_repository_approach1</i> - Press enter. - Then, in the line # 9 change: 'dissolved' for anything you want.	3.2. The script will look for a .csv file with the identifier 'L44440' in its file name. To change this: - Type in the command window: <i>edit Script_to_generate_mRNA_repository_approach2</i> - Press enter. - Then, in the line # 9 change: 'L44440' for anything you want.

<p>Example data location: ~/simulation_inputs/mRNA_repositories/spots_for_approach1</p> <p><u>Important:</u> Image names should match between the table of positions and the image files.</p>	<p>Example data location: ~/simulation_inputs/mRNA_repositories/spots_for_approach2</p> <p><u>Important:</u> Image names should match between the table of positions and the image files.</p>
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4. Select the folder containing the smFISH image files.

Approach 1	Approach 2
<p>4.1. The script will look for images with the identifier 'spn4' in their file names.</p> <p>To change this:</p> <ul style="list-style-type: none"> - Type in the command window: <i>edit Script_to_generate_mRNA_repository_approach1</i> - Press enter. - Then, in the line # 10 change: 'spn4' for anything you want. <p>Example data location: ~/simulation_inputs/mRNA_repositories/spots_for_approach1</p> <p>Important: Image names should match between the table of positions and the image files.</p>	<p>4.2. The script will look for images with the identifier 'distal' in their file names.</p> <p>To change this:</p> <ul style="list-style-type: none"> - Type in the command window: <i>edit Script_to_generate_mRNA_repository_approach1</i> - Press enter. - Then, in the line # 10 change: 'distal' for anything you want. <p>Example data location: ~/simulation_inputs/mRNA_repositories/spots_for_approach2</p> <p>Important: Image names should match between the table of positions and the image files.</p>

The default parameters of the script include:

```
% ===== voxel size in nm (*)
pixel_size.xy = 49;
pixel_size.z = 250;
```

(*) This is the voxel size of the example images. It is used to compute the locations of the mRNAs spots from the positions table (see step 3) when the positions are given in nm.

To change this:

- Type in the command window:
edit Script_to_generate_mRNA_repository_approach1
- or
edit Script_to_generate_mRNA_repository_approach2
- Press enter.
- Then, in the line # 5 and # 6, default values can be changed.

5. Select the folder where to save the repositories

2nd part: generation of synthetic smFISH images at different mRNA concentrations

Procedure:

6. Open MATLAB

7. In the command window:

Approach 1	Approach 2
7.1. Type * and then press enter:	7.2. Type * and then press enter:
* <i>Script_to_generate_synthetic_images_approach1</i>	* <i>Script_to_generate_synthetic_images_approach2</i>

The default parameters of the script are:

```
% ===== voxel size
pixel_size.xy = 49;
pixel_size.z = 250;

%===== expected concentrations in mol/um3
concentration_desired = [0.01  0.025:0.025:0.1  0.25:0.25:1  1.25:0.25:5];
% number of replicates by concentration
replicates_simulation = 3;
% reduce PSF
additional_BGD_to_subtract_mRNA = 34;
```

To change default parameters:

- Type in the command window:

edit Script_to_generate_synthetic_images_approach1

or

edit Script_to_generate_synthetic_images_approach2

- Press enter. Then, modification can be included.

8. Select the file of binary image where random placement will be performed.

Approach 1	Approach 2
Provided image example: <i>oocyte_to_model_16bits.tif</i>	Provided image example: <i>oocyte_to_model_16bits.tif</i>
location: ~/simulation_inputs	location: ~/simulation_inputs

9. Select the file of average PSF image (**only for approach 2**)

Approach 1	Approach 2
Step not performed.	Provided image example: <i>experimental_single_mol_PSF_8x6_mRNA_AVG_ns</i>
	location: ~/simulation_inputs

10. Select the file of the mRNA images repository.

Approach 1	Approach 2
Provided example: <i>mRNA_repository_approach1.mat</i>	Provided example: <i>mRNA_repository_approach2.mat</i>
location: ~/simulation_inputs/mRNA_repositories	location: ~/simulation_inputs/mRNA_repositories

Outputs:

In Silico simulated images.

Provided output examples.

Simulations run with an expected concentration of $0.025 \text{ mol}/\mu\text{m}^3$

Location:

~/simulated_images_examples

Note: expected concentrations are used as inputs. However, molecules placed very close to the oocyte edges whose PSF does not fit within the oocyte area are removed. Therefore, the actual concentration will be lower when the number of molecules placed is very high.