User guide for TestSTORM program

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Version: 1.0

Introduction

TestSTORM program was developed for modeling the whole imaging procedure in an optical fluorescence localization microscope. Four types of sample are provided with different geometry: star, array, vesicles and lines. The algorithm creates the structure of the sample, generates a temporal trajectory of photoswitching fluorescent states to each dye molecule according to the three-state model and simulates the image acquisition process.

Download

- TestSTORM code with its user guide can be freely downloaded.
- Homepage of the Advanced Optical Imaging (AdOptIm) Research Group is http://titan.physx.u-szeged.hu/~adoptim/
- For further assistance, please contact Miklós Erdélyi via email.

System requirements

- MatLab 2011a or more recent version.

File history

- 2013.12.03: TestSTORM version 1.0 and User Guide 1.0

References

- Please refer and cite to the following publication(s):

József Sinkó, Róbert Kákonyi, Eric Rees, Daniel Metcalf, Alex E. Knight, Clemens F. Kaminski, Gábor Szabó, and Miklós Erdélyi, "TestSTORM: Simulator for optimizing sample labeling and image acquisition in localization based super-resolution microscopy," Biomed. Opt. Express 5, 778-787 (2014)

Related codes

- The suggested localization software for analysis of image stacks generated by TestSTORM is rainSTORM can be also freely available at http://laser.cheng.cam.ac.uk/wiki/index.php/Resources

1. First steps

1.1. TestSTORM can be run in Matlab or as an application .exe file

Running in Matlab

Download the TestSTORM_ver1_0.zip from the website http://titan.physx.u-szeged.hu/~adoptim/

Unzip the downloaded file.

Open your Matlab. (required 2011a or newer version)

Search the "testSTORM.m" file in the window "Current folder".

Run it (F9).

Please, make sure that your Windows Explorer has been closed to avoid the error in the image file writing (see http://www.mathworks.com/support/solutions/en/data/1-DQPRHC/?solution=1-DQPRHC)

Running as an application .exe

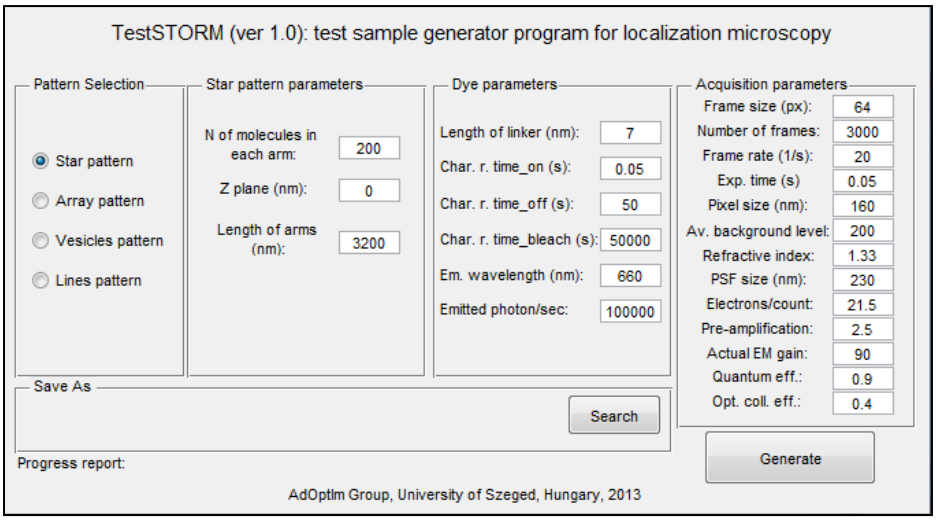
Download the TestSTORM_ver1_0pkg.zip from the website http://titan.physx.u-szeged.hu/~adoptim/

Unzip the downloaded file.

Please, make sure that MATLAB Compiler Runtime (MCR) (http://www.mathworks.com/products/compiler/mcr/) is installed on your computer.

Open TestSTORM_ver1_0.exe.

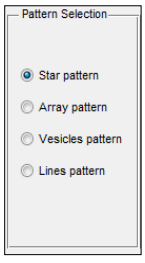
1.2. The following GUI window will open:



2. Detailed description

2.1. Input parameters

2.1.1. "Pattern selection" panel

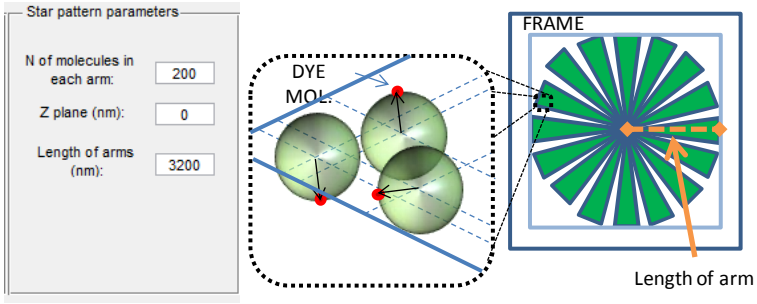


The user can select from among four types of pattern with different geometrical parameters. In the star pattern the molecules form a star with 16 arms within the frame. In the array pattern the molecules are linked to the central points of pixels form a matrix. The vesicles pattern consists of labeled spheres. In the case of lines pattern five fixed line are labeled.

2.1.2. “Pattern parameters” panel

a. Star

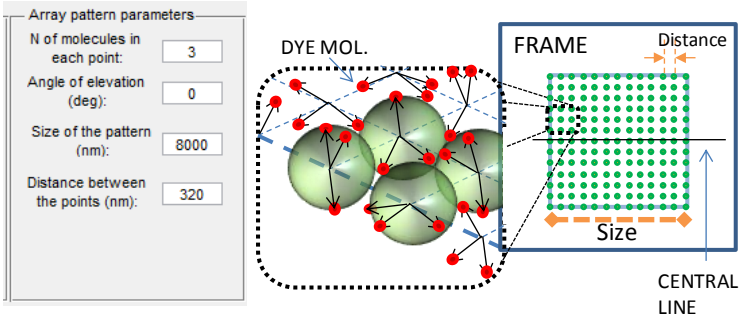
The “star” pattern forms a centered star on the frame with 16 arms. The linkers are randomly distributed on the surface of each arm. The number of the dye molecules linked to the arms, the length of the arms and the plane of the pattern in z direction can be set (the focal plane corresponds to z=0 nm).



Star pattern

b. Array

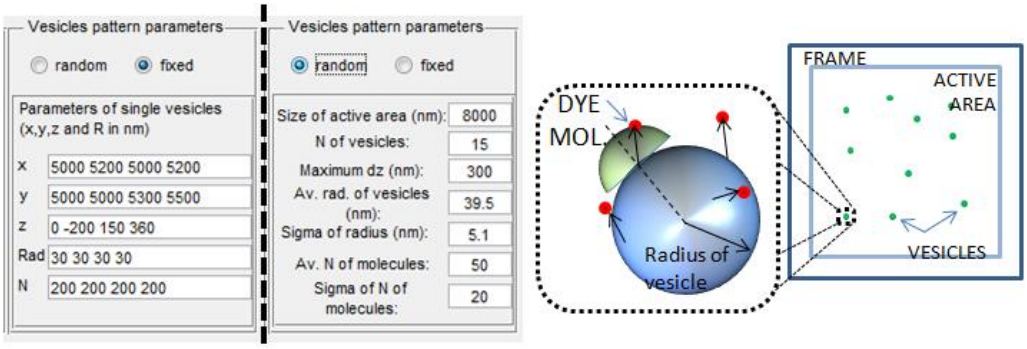
The “array” pattern is a centered grid on the frame. The linkers are located in the points of the grid. The number of dye molecules linked to one point, the plane of the central line of the pattern in z position and the angle between horizontal plane and the plane of the pattern (elevation angle) are variable. The distance between the grid points and the size of the array can be modified by the user too.



Array pattern

c. Vesicles

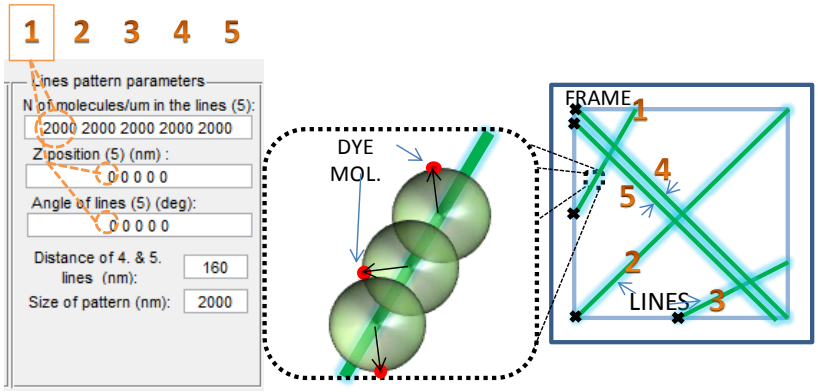
The “vesicles” pattern consists of labeled spheres. The position of the centers of modeled vesicles could be defined by the user or randomly distributed within an active area. The linkers are randomly placed on the surface of the sphere. The possible positions of the molecules form a half sphere. The input parameters are in the case of random sample: the average radius of vesicles, the standard deviation of radius of vesicles, the average number of molecules connected to one vesicle, the standard deviation of the number of molecules linked to one vesicle, the size of active area, the number of vesicles and the maximum distance between the centre of the vesicles and the focal plane in z direction. The radius of vesicles and the number of molecules linked to one vesicle follow normal distribution. The input parameters in the case of fixed sample: x, y and z coordinates of the centre, the radius of each vesicle and the number of molecules linked to the vesicles.



Vesicles pattern

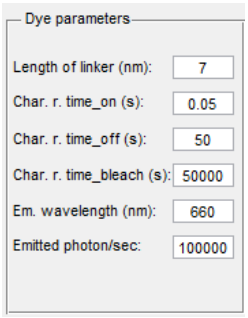
d. Lines

The "lines" pattern consists of five stained lines with fixed position. The contact point of the linkers is randomly distributed along each line. The following parameters can be modified by the users: The number of molecules/µm (labeling density) linked to the lines, the z position of the starting point of each line (sign by blond "x"), the elevation angle of the lines and the size of the pattern. The distance between the 4. and 5. line is also variable.



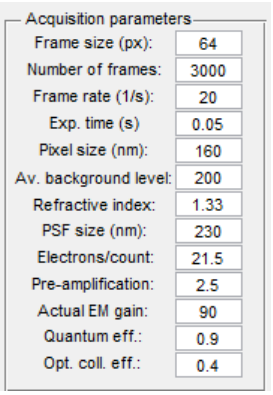
Lines pattern

2.1.3. "Dye parameters" panel



These parameters describe the general properties of the dye molecules. The length of linker parameter is for modeling the labeling, the distance between the contact point end the dye molecule (e.g. the size of the antibody). Three characteristic constant determine the behavior of the dye molecules in time: the characteristic rate time constant of ON, OFF and BLEACHED state. These define the probabilities of the transition between the states. The emission wavelength is the central wavelength of the emission spectra. This parameter is required in the 3D Gaussian PSF calculation. The emitted photon/sec parameter means the photon number which is emitted in 1 s from a fluorescent dye molecule in active state.

2.1.4. "Acquisition parameters" panel



The "Acquisition parameters" panel encompasses the mean parameters of the camera and optical system required in the image creating. Frame size is the number of pixels in one direction in a frame. Number of frame is the length of the simulated sequence. Frame rate describes how many frames can be acquired during 1 s. The exposure time is the time of the photon capturing (full frame time - readout time). The pixel size is the size of a pixel measured in the focal space of the objective (Real pixel size of the camera/magnification of the imaging system). Poisson noise added to each frame with an average background level. Refractive index of the medium is used in the 3D Gaussian PSF calculation. PSF size is the full width at half maximum of the PSF (measured in the focal region of the objective). The optical collection efficiency describes the portion of the emitted photon flux which can be collected by the microscope system and imaged onto the surface of the CCD camera. This parameter is determined by the total transmittance of the system and the numerical aperture (NA) of the microscope objective. Its typical value is ~0.3-0.4 in case of an oil immersion objective with high numerical aperture. The incoming photon-count conversion of the camera is calculated in the following way: the product of the incoming photon number calculated from the emitted photon/s, optical collection efficiency and the actual lifetime of the active state, the pre-amplification, the actual electron multiplying gain and the quantum efficiency of the camera is divided by the electron/count rate. Most of these parameters can be found in the camera manuals.

2.1.5. "Save as" panel

The generated image stack can be saved in TIF or RAW format. The destination and filename can be determined with a click on "Search" button. A data file is also provided with columns: framenumber, x, y and z coordinates in nm of the active molecules.

2.2. Generating the sample

The "testSTORM.m" calls firstly the "m_gen_mol_coord.m" which generates the positions of the molecules according to the chosen geometry. Then "m_gen_traj_gen.m" generates temporal trajectories to each molecule using the defined dye parameters. After that "m_gen_trajs_to_seq.m" produces the frame stack considering the acquisition parameters and molecule temporal trajectories. The "m_gen_noise_addition.m" program adds noise to the frame sequence and finally the "m_gen_seq_save.m" routine saves the frame stack in the appropriate file format.

3. More information

If you have any questions, reflections or suggestions, please, do not hesitate to contact Dr. Miklos Erdelyi (meerdelyi@gmail.com).

4. Please, refer our paper if you use this program in your work.