From measurement planning to the final image: software solutions for SMLM image processing

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Introduction

To achieve high quality final images with super-resolution via SMLM image processing requires aware planning and precise implementation. Software solutions such as simulator, reconstruction and image processing programs play key role in the SMLM technique. Here we present our software package: TestSTORM a simulator program [1,*] which models the whole localization based imaging (sample structure, labeling strategy, photochemical processes of dye molecules and image acquisition) and rainSTORM [2,*] a localization and reconstruction software.

Simulation of localization based imaging (TestSTORM) → Image processing: localization and reconstruction (rainSTORM) → Further image analyses (rainSTORM)

Main features of testSTORM:
- ✓ Easy to use GUI developed in Matlab.
- ✓ Advanced PSF models
- ✓ Fully adjustable parameters
- ✓ Predefined and arbitrary structures

v2.0

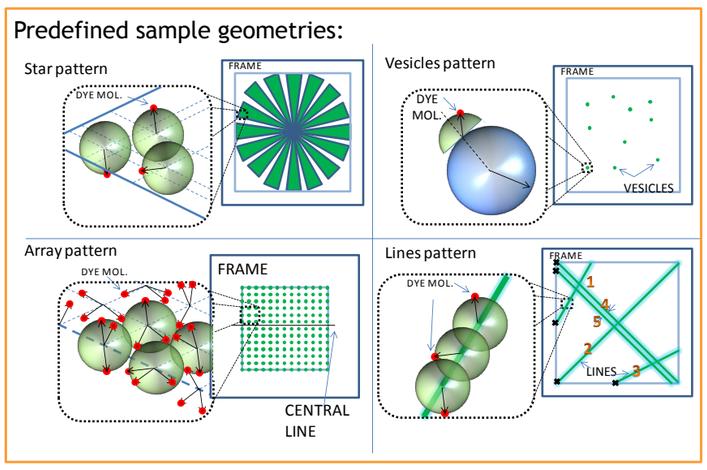
Main features of rainSTORM:
- ✓ New easy to use GUI developed in Matlab.
- ✓ Multiple localization algorithms
- ✓ Automatic drift correction
- ✓ Fully adjustable parameters

v3.1

TestSTORM: test sample generator program for localization based microscopy

Modeled sample structure and temporal behavior

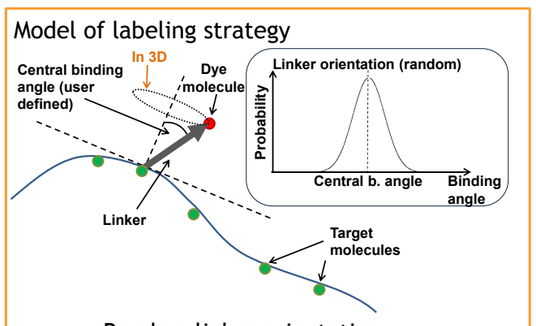
Predefined sample geometries:



Arbitrary, user defined sample geometry

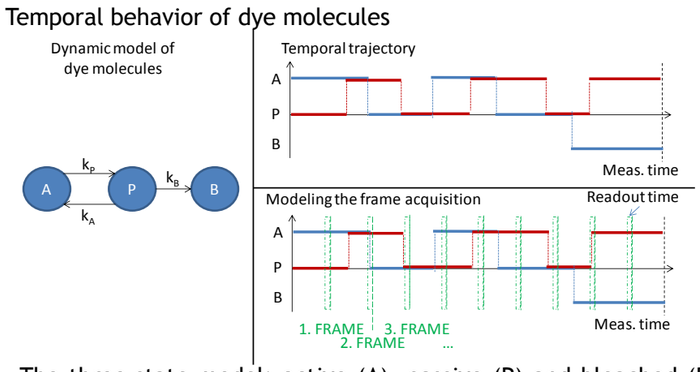
Input file: data file with coordinates:
- Lines (generated by Jfilament plug-in in ImageJ)
- Surfaces (stl text file format)

Model of labeling strategy



- Random linker orientation
- Predefined central binding angle
- Multiple labeling
- Adjustable dye parameters

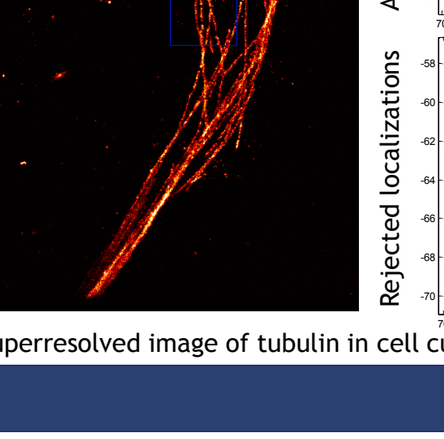
Temporal behavior of dye molecules



The three-state model: active (A), passive (P) and bleached (B) states. A random temporal trajectory (transitions between these states) is generated for each molecule.

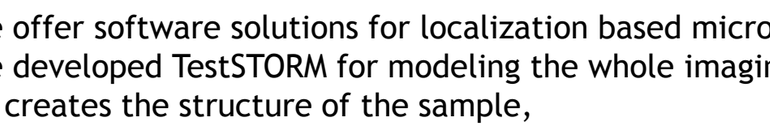
Modeled image acquisition

Advanced Point Spread Function (PSF) models



- Gaussian PSF, scalar and vector diffraction
- 3D astigmatism and other optical aberrations
- dipole orientation

Model of mechanical and thermal drift (3D random walking)

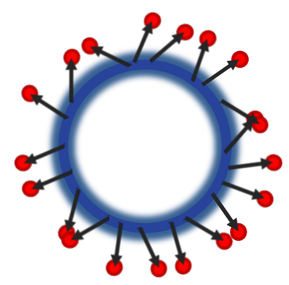


Model of camera image acquisition: amplification, pixel size, noise, etc.

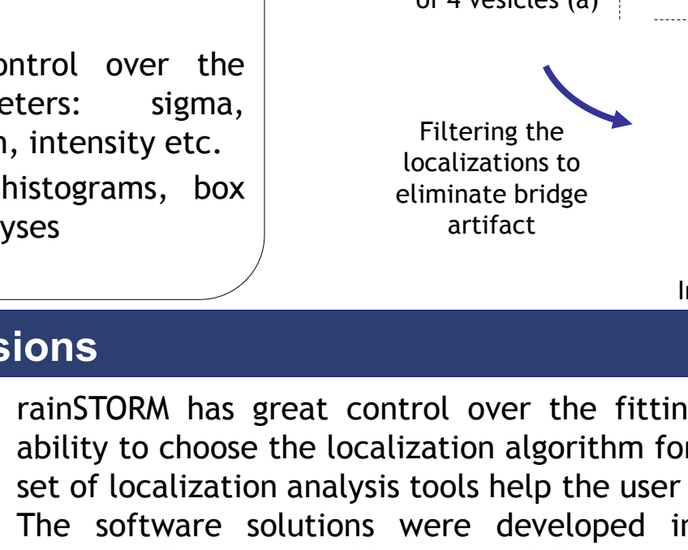
Simulation of actin ring structure on axon initial segment

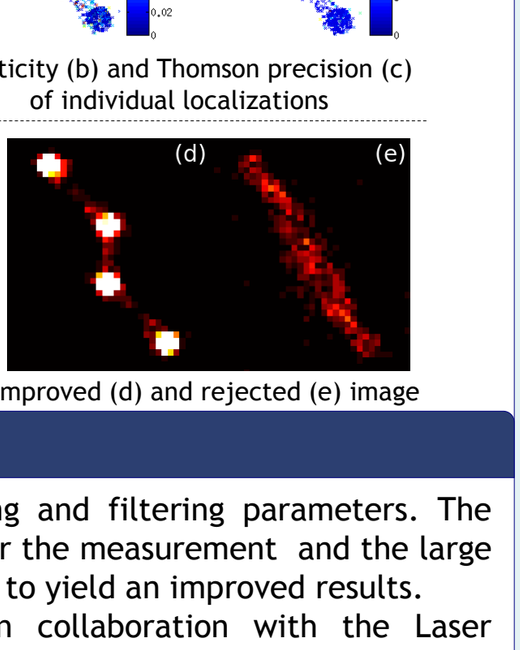
It has been observed recently that actin forms ring-like structure at the initial segment of the axon.

Model of single actin ring



Axon initial segment with actin ring structure in 3D





Reconstructed superresolved image

Simulated widefield image

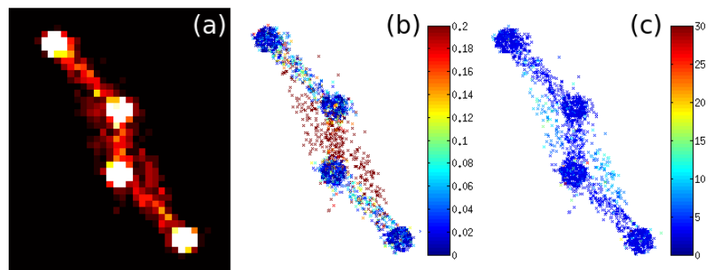
rainSTORM: localization and image reconstruction software

Structure validation based on the rejected localization

Superresolved image of tubulin in cell culture

rainSTORM feature list:
- Input file: several supported file formats due to the BioFormats package
- Localization algorithms: Levenberg-Marquardt fitting procedure, 1D and 2D Gaussian fitting, multiple emitter fitting
- Reconstruction: control over the filtering parameters: sigma, Thompson precision, intensity etc.
- Visualization: 2D histograms, box tracking, local analyses
- Reporting

Image quality improvement through effective filtering of localizations



Unfiltered image of 4 vesicles (a)

Ellipticity (b) and Thomson precision (c) of individual localizations

Filtering the localizations to eliminate bridge artifact

Improved (d) and rejected (e) image

Conclusions

We offer software solutions for localization based microscopy method.
We developed TestSTORM for modeling the whole imaging procedure:
- creates the structure of the sample,
- generates a temporal trajectory of photoswitching fluorescent states to each dye molecule according to the three-state model
- simulates the image acquisition process.

rainSTORM has great control over the fitting and filtering parameters. The ability to choose the localization algorithm for the measurement and the large set of localization analysis tools help the user to yield an improved results.
The software solutions were developed in collaboration with the Laser Analytics Group at the University of Cambridge.
The software can be downloaded from our web page [*].

References

[1] József Sinkó et al, "TestSTORM: Simulator for optimizing sample labeling and image acquisition in localization based super-resolution microscopy," Biomed. Opt. Express 5, 778-787 (2014)

[2] E. J. Rees et al, "Blind assessment of localization microscopy image resolution," Opt. Nanoscopy 1(1), 12 (2012)

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Information

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