Localization analysis with rainSTORM

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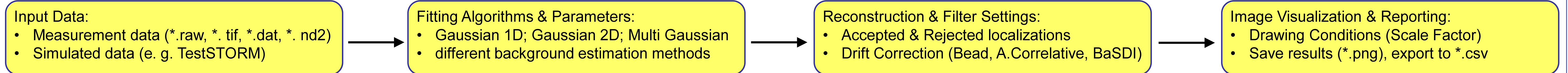4. MTA-SZTE Research Group on Photoacoustic Spectroscopy, Szeged, Hungary

Introduction

rainSTORM is a software written in MATLAB for evaluating single-molecule localization based microscopy measurement (PALM, (d)STORM etc.) or simulation (TestSTORM) data. The aim is to generate a sub-diffraction resolved image by identifying photoswitched single fluorophores in sequences of images. An image stack usually consists from 5 to 50 thousand frames, which is necessary for nanometer precision.

Main features of rainSTORM: **v3.1**
- ✓ Easy to use Graphical User Interface developed in Matlab.
- ✓ Multiple localization algorithms and reconstruction methods.
- ✓ Two different methods for automatic drift correction without marker.
- ✓ Fully adjustable fitting parameters and reconstruction filter settings.
- ✓ Extra utilities for batch processing measurement folders with pre-defined settings.
- ✓ Version controlled development, and available under the GPL and LGPL license.
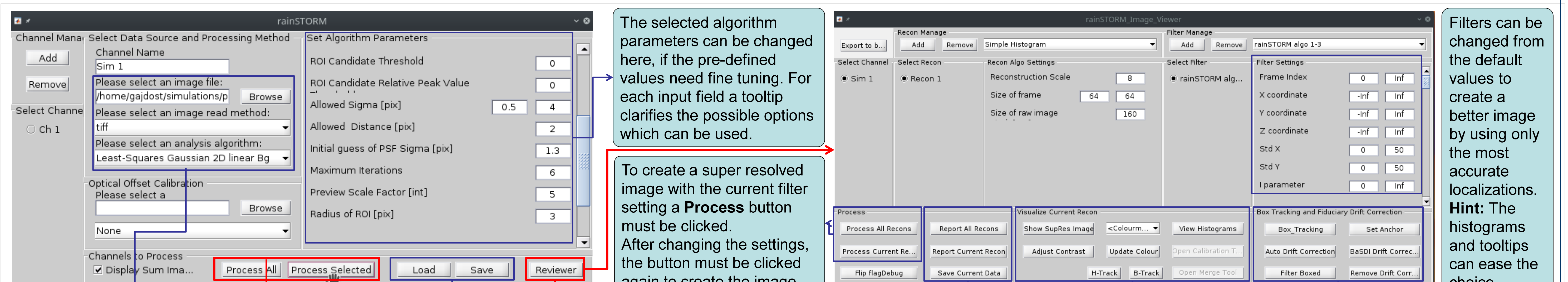
Input Data:
- Measurement data (*.raw, *. tif, *.dat, *. nd2)
- Simulated data (e. g. TestSTORM)

Fitting Algorithms & Parameters:
- Gaussian 1D; Gaussian 2D; Multi Gaussian
- different background estimation methods

Reconstruction & Filter Settings:
- Accepted & Rejected localizations
- Drift Correction (Bead, A.Correlative, BaSDI)

Image Visualization & Reporting:
- Drawing Conditions (Scale Factor)
- Save results (*.png), export to *.csv

rainSTORM: a software for localization based microscopy

Minimum Requirements: Hardware: 2 CPU cores, 8 GB of RAM; Operating System: Windows 7 or Ubuntu Linux 14.04 with Matlab 2014a (or newer).
Required Matlab Toolboxes: Image Processing Toolbox, Optimizations Toolbox , Parallel Computing Toolbox
Start rainSTORM in Matlab: startup; rainSTORM

Graphical User Interface of rainSTORM v3.x

The selected algorithm parameters can be changed here, if the pre-defined values need fine tuning. For each input field a tooltip clarifies the possible options which can be used.

To create a super resolved image with the current filter setting a **Process** button must be clicked.
After changing the settings, the button must be clicked again to create the image.

Filters can be changed from the default values to create a better image by using only the most accurate localizations. **Hint:** The histograms and tooltips can ease the choice.

The input file and read method should be given first. To process multiple files, additional channels must be added first.
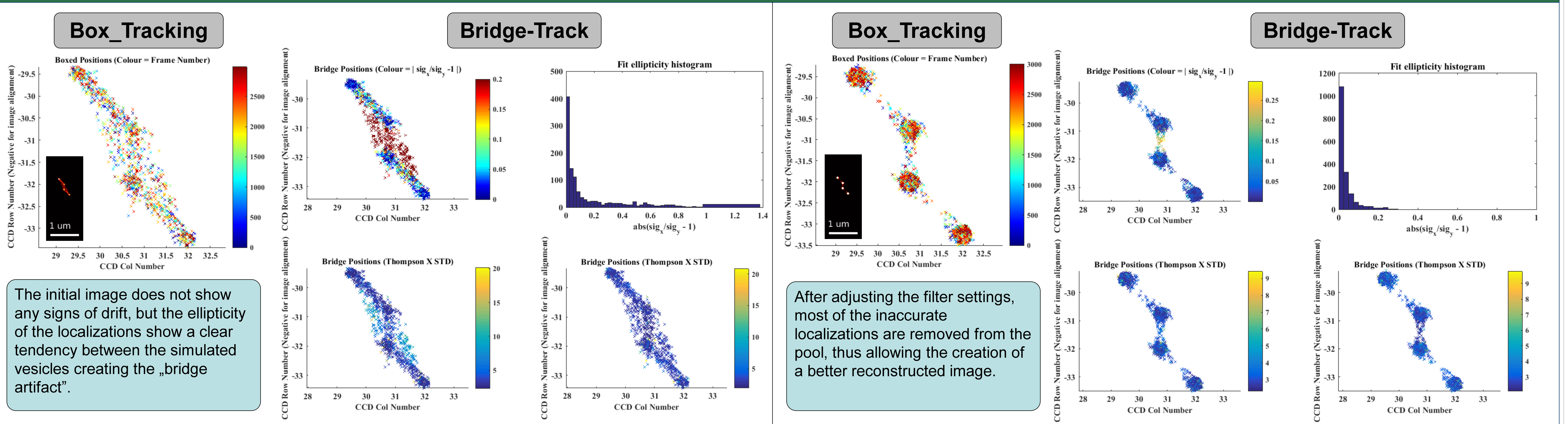The localization algorithm can also be changed by selecting a different one from the available options.

Each localization session can be saved and later restored, even to a different machine.

To start the input file analysis, a **Process** button must be clicked. Upon completion by clicking **Reviewer** the reconstruction will continue in a new window.

By reporting the reconstruction, the super resolved image, the localization histogram and the other info files are saved.
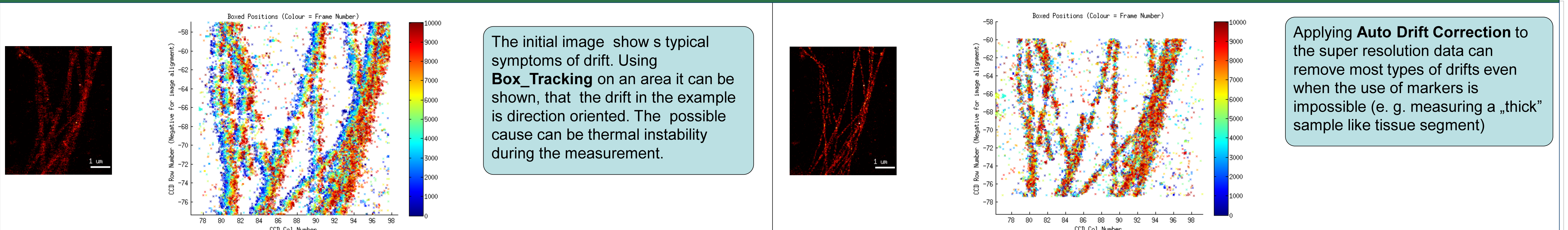It is also possible to export the reviewed localization in **csv**.

The super resolved image and the localization histograms can be viewed here for the current reconstruction. Drawing conditions can be changed also.
Localizations can be validated by using the trackers on an area.

Box_Tracking can be used to check for drift on the super resolved image by selecting an area for analysis. Three methods (Bead, Auto Correlative, BaSDI[3]) can be applied to correct the localizations for drift.

Analyzing reconstructed results & filtering localizations

Box_Tracking

Bridge-Track

The initial image does not show any signs of drift, but the ellipticity of the localizations show a clear tendency between the simulated vesicles creating the „bridge artifact".

Box_Tracking

Bridge-Track

After adjusting the filter settings, most of the inaccurate localizations are removed from the pool, thus allowing the creation of a better reconstructed image.

Drift correcting localizations during reconstruction

The initial image show s typical symptoms of drift. Using **Box_Tracking** on an area it can be shown, that the drift in the example is direction oriented. The possible cause can be thermal instability during the measurement.

Applying **Auto Drift Correction** to the super resolution data can remove most types of drifts even when the use of markers is impossible (e. g. measuring a „thick" sample like tissue segment)

Conclusion

Comparison with other packages (RapidSTORM, QuickPALM, ThunderSTORM etc.) our software has a greater 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 was developed in collaboration with the Laser Analytics Group at the University of Cambridge and the Advanced Optical Imaging Group at the University of Szeged. The software can be downloaded from our web page[4].

References

[1]   Rees, E. J., Erdelyi, M., Schierle, G. S. K., Knight, A., & Kaminski, C. F. (2013) Journal of Optics, 15(9), 094012.
[2]   Sinkó, J., Kákonyi, R., Rees, E., Metcalf, D., Knight, A. E., Kaminski, C. F., ... & Erdélyi, M. (2014) Biomedical optics express, 5(3), 778-787.
[3]   https://github.com/jiyuuchc/BaSDI/releases/tag/1.0
[4]   http://titan.physx.u-szeged.hu/~adoptim/

Acknowledgment

Information

If you have any question, please contact Tamás Gajdos or Miklós Erdélyi (meerdelyi@gmail.com).