ThunderSTORM: a comprehensive ImageJ plug-in for PALM and STORM data analysis and super-resolution imaging

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ABSTRACT

Summary: ThunderSTORM is an open-source, interactive and modular plug-in for ImageJ designed for automated processing, analysis and visualization of data acquired by single-molecule localization microscopy methods such as photo-activated localization microscopy and stochastic optical reconstruction microscopy. ThunderSTORM offers an extensive collection of processing and post-processing methods so that users can easily adapt the process of analysis to their data. ThunderSTORM also offers a set of tools for creation of simulated data and quantitative performance evaluation of localization algorithms using Monte Carlo simulations.

Availability and implementation: ThunderSTORM and the online documentation are both freely accessible at https://code.google.com/p/thunder-storm/

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Supplementary information: Supplementary data are available at Bioinformatics online.

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1 INTRODUCTION

Single-molecule localization microscopy (SMLM) methods such as stochastic optical reconstruction microscopy (STORM; Rust et al., 2006) and photo-activated localization microscopy (PALM; Betzig et al., 2006) have recently emerged to overcome the diffraction barrier, offering ~10 times higher lateral resolution and the possibility of 3D imaging by various approaches. In SMLM, a super-resolution image is reconstructed from a sequence of diffraction-limited images of sparsely distributed single photoswitchable molecules. As the sequence is usually long (thousands of images) and the positions of the molecules have to be estimated systematically with sub-diffraction precision, it is crucial to use specialized software for processing the data.

We present ThunderSTORM, an open-source, interactive, modular and platform-independent software, which provides a complete set of tools for automated processing, analysis and visualization of data acquired by SMLM methods. Our philosophy in developing ThunderSTORM has been to offer an extensive collection of processing and post-processing methods, which were developed based on extensive testing with both real and simulated data. We also provide a detailed description of the implemented methods and algorithms (Supplementary Note), as well as a user’s guide.

2 FEATURES AND METHODS

2.1 Raw data processing

Approximate molecular positions can be determined, in combination with a variety of feature-enhancing low-pass and band-pass filters (Křížek et al., 2011; Izeddin et al., 2012), by detection of local maxima, non-maximum suppression or calculation of the centroid of connected components of segmented objects. A feature exclusively unique to ThunderSTORM is the possibility of specifying the threshold for detection of molecules using a mathematical expression with quantities based on raw or filtered images. This allows computing the threshold value systematically for unknown input images with, for example, low signal to noise ratio, or where the global intensity slowly fluctuates. ThunderSTORM also offers a preview function to help visualize the detected molecules with the chosen combination of data processing settings.

Sub-diffraction localization of molecules is accomplished by computing the centroid of local neighborhoods, by a radial symmetry approach (Parthasarathy, 2012), or by fitting a suitable PSF model using standard or weighted non-linear least-squares methods, or using maximum-likelihood estimation (Mortensen et al., 2010). Users may also choose not to use any of the methods, thereby using the approximate localizations from the previous step. The uncertainty of the localization of molecules is calculated according to Thompson et al. (2002), or according to Quan et al. (2010) if EMCCD cameras are used.

Super-resolution 3D imaging is accomplished by an astigmatism approach (Huang et al., 2008). An integral part of this feature is the software’s calibration tool, in which a Z-stack of astigmatic images of sub-diffraction fluorescent beads is used to establish parameters for determining the axial position of each molecule.

Efforts to accelerate the acquisition process in SMLM have involved increasing the density of photoswitchable fluorophores. In this case, ThunderSTORM uses an algorithm based on fitting of multiple emitters (Huang et al., 2011).

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3 SUMMARY

ThunderSTORM introduces several new features and concepts for 2D and 3D SMLM data analysis. The software combines several algorithms for SMLM analysis into one comprehensive environment. One of the main features is the ability to process the data using any combination of the implemented feature-enhancing, spot detection and fitting methods. An important feature in ThunderSTORM is the possibility of specifying the threshold for detection of molecules using mathematical expressions. This allows users to systematically maximize the efficiency of molecule detection in the raw data by searching for the optimum combination, which may vary from experiment to experiment. ThunderSTORM also offers a much higher degree of user interactivity during data post-processing compared with other SMLM software packages, and introduces a new and fast visualization method that creates high-quality results. A realistic data generator within ThunderSTORM allows users to run multidimensional Monte Carlo simulations to evaluate the performance of localization methods. We have found ThunderSTORM’s flexibility and performance to be of critical importance when analyzing data with low molecular brightness, which we encountered when imaging A431 cells expressing mCitrine-erbB33 (Křízek et al., 2011) [Fig. 1(d–f)].

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