

Type of presentation: Poster

**IT-3-P-5777 ThunderSTORM: a comprehensive ImageJ plugin for PALM and STORM data analysis and super-resolution imaging**

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We present ThunderSTORM, an open-source, interactive, and modular software designed for automated processing, analysis, and visualization of data acquired by single molecule localization microscopy (SMLM) methods including STORM, dSTORM, SPDM, PALM, and FPALM. ThunderSTORM was developed using a home-built SMLM system, but the software has been tested, and works well with data acquired using commercially available Nikon N-STORM and Zeiss Elyra systems. 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 very detailed description of the implemented methods and algorithms as well as a detailed user's guide. ThunderSTORM is written in Java and distributed as a plug-in for ImageJ. This enables users to run the software on computers with different operating systems, and to use all of the advantages of ImageJ including its rich collection of plug-ins. The latest version of ThunderSTORM and the source code are freely available at <https://code.google.com/p/thunder-storm/>.

ThunderSTORM can process data for both 2D and 3D SMLM imaging, including data with high spatial molecular density, which is known as the "crowded field" problem. The steps involved in SMLM data processing are shown in Figure 1. Several algorithms for each of the processing steps have been implemented so experienced users have many options to adapt the processing to their data. However, the default settings perform very well on many of the SMLM data sets we have experimented with.

ThunderSTORM is also capable of generating simulated SMLM data and of evaluation of the performance of localization algorithms based on the ground-truth positions of the molecules. This allows users to perform Monte Carlo simulations and to quantitatively evaluate the performance of the applied algorithms. Localization results, as well as the ground-truth positions of molecules, can be imported/exported to/from ThunderSTORM in a variety of data formats, allowing compatibility with other SMLM localization software.

We also present our preliminary data of replication and transcription processes in the cell nucleus of HeLa cells, see Figure 2. Our labeling strategy results in brightly stained cells revealing two distinct nuclear activities. Investigating how these processes are organized within the cell nucleus relates to the larger issue of understanding how DNA replication is regulated on a cellular level. The super-resolution data were processed by ThunderSTORM.

Acknowledgement: This work was supported by the Czech Science Foundation [P304/09/1047, P205/12/P392, P302/12/G157, 14-15272P]; by European Union Funds for Regional Development [OPPK CZ.2.16/3.1.00/24010]; and by Charles University in Prague [Prvok/1LF/1, UNCE 204022].

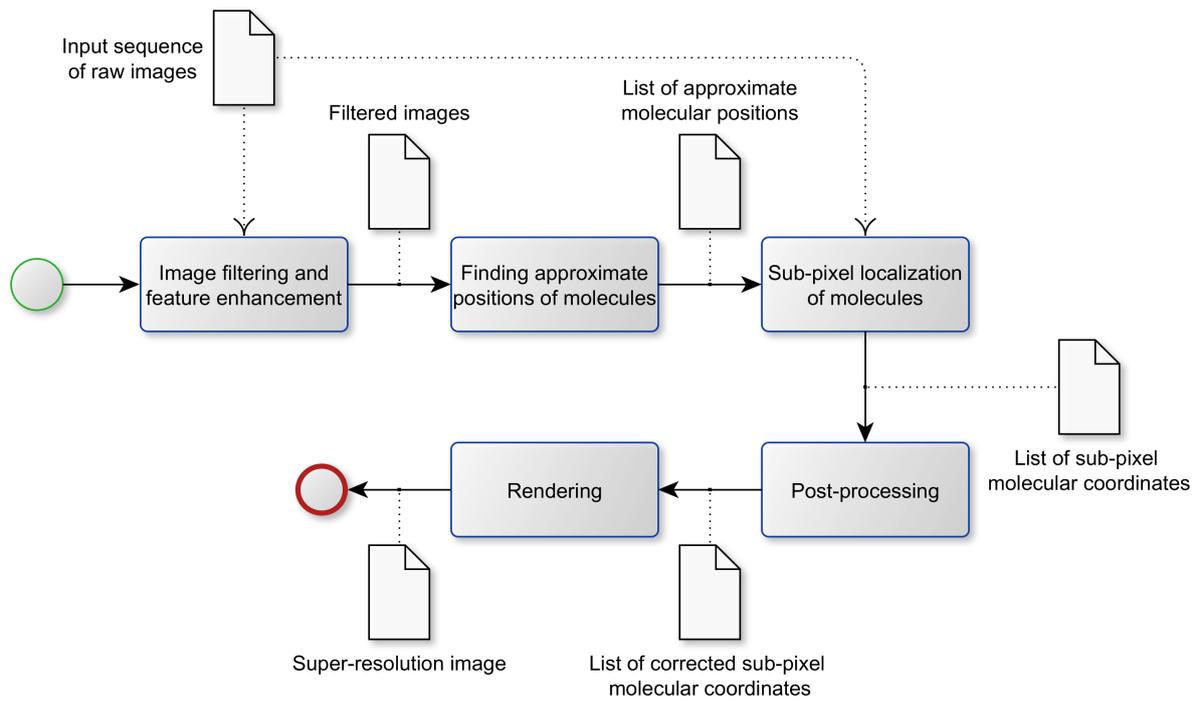


Fig. 1: Data processing pipeline for single molecule super-resolution imaging.

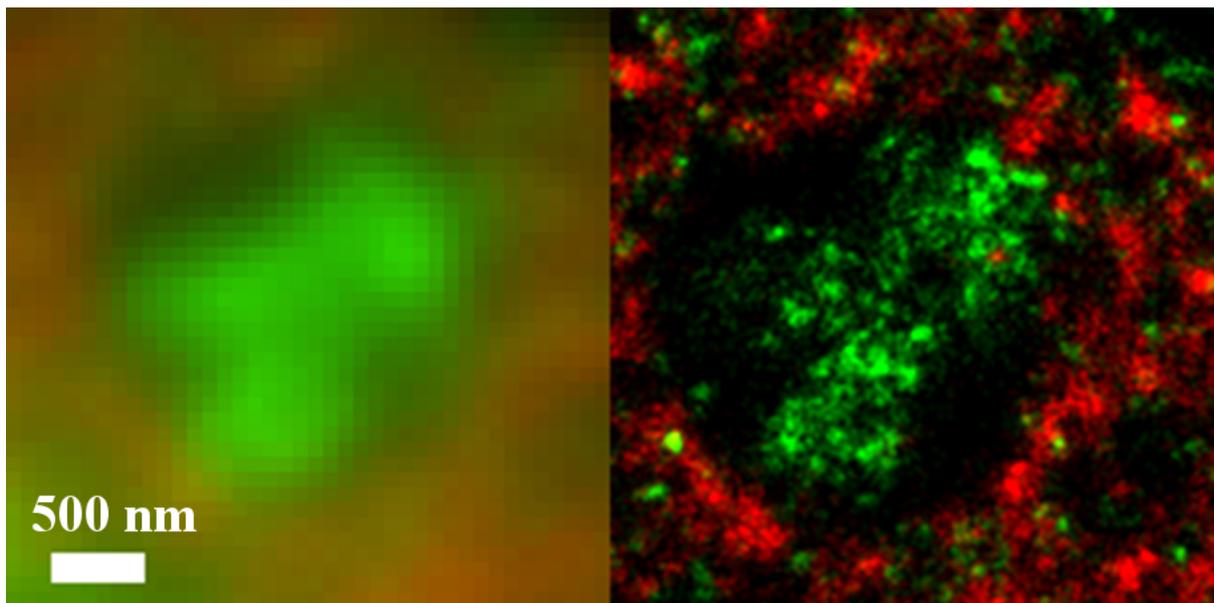


Fig. 2: Replication and transcription, detail of nucleolus. HT-1080 cells labeled with EdU-Alexa647 (a fluorescently tagged nucleotide that incorporates into newly replicated DNA, red) and fluorouridine (a synthetic nucleotide which is incorporated into active transcription sites, green).