Computational methods in single molecule localization microscopy

Abstract

Fluorescence microscopy is one of the chief tools used in biomedical research as it is a non invasive, non destructive, and highly specific imaging method. Unfortunately, an optical microscope is a diffraction limited system. Maximum achievable spatial resolution is approximately 250 nm laterally and 500 nm axially. Since most of the structures in cells researchers are interested in are smaller than that, increasing resolution is of prime importance. In recent years, several methods for imaging beyond the diffraction barrier have been developed. One of them is single molecule localization microscopy, a powerful method reported to resolve details as small as 5 nm. This approach to fluorescence microscopy is very computationally intensive. Developing methods to analyze single molecule data and to obtain super-resolution images are the topics of this thesis.

In localization microscopy, a super-resolution image is reconstructed from a long sequence of conventional images of sparsely distributed single photoswitchable molecules that need to be systematically localized with sub-diffraction precision. We designed, implemented, and experimentally verified a set of methods for automated processing, analysis and visualization of data acquired by single molecule localization microscopy and we "packaged" them as an open source software called ThunderSTORM. ThunderSTORM has become one of the top softwares in the field.

Next, we introduce our design for a novel dual-objective super-resolution microscope, which roughly doubles the count of collected photons. This further improves achievable resolution by a factor of $\sqrt{2}$. We built a working prototype of the microscope and developed and experimentally verified methods for calibration of the microscope and for image analysis.

In the last part of the thesis we address the issue that despite the high spatial resolution of localization microscopy it is not always suitable for live cell imaging due to its limited temporal resolution. One strategy is to increase the density of photoactivated molecules present in each image. Such an approach poses a challenge for the image analysis. We present 3denseSTORM, a new algorithm which is able to recover 2D or 3D super-resolution images from a sequence of diffraction limited images with high densities of photoactivated molecules. The algorithm utilizes methods from compressed sensing and uses a Poisson noise model, which becomes critical in low-light conditions. We derive the theoretical resolution limits of the method and show examples of image reconstructions in 2D and 3D simulations and in real data of biological samples. The method is suitable for fast image acquisition in densely labeled samples and helps facilitate live cell studies with single molecule localization microscopy.

Keywords

Super-resolution microscopy, single-molecule localization microscopy, image processing, numerical optimization, sparse representations.