Simulative and experimental examination of localization microscopy modalities

PhD Thesis Synopsis

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2022
Szeged

Summary

Introduction

Microscopes have been indispensable tools for the study of the microscopic world for centuries. Since then they have gained a wide variety of applications in many scientific fields, for which many specialized techniques have been developed. One such optical method is the fluorescence microscopy which gained widespread adoption in biology half a century ago. Its popularity is mainly due to its ability to examine the cell components with great specificity. However, the applicability of the optical microscopes is hindered by the resolution due to the diffraction of the optical system. This was only recently overcome with different measurement solutions collectively termed as super resolution microscopy. Among these, a popular branch is the localization microscopy, which is based on temporal separation of the signals of individual fluorophores. For example, in the dSTORM technique, the fluorophores are placed in a special buffer solution which induces photoblinking. In this way, localizing the dyes separately, $\sim$20 nm resolution can be reached. However, the increased resolution does not come without drawbacks, these methods require elaborate data evaluation and result interpretation.

In localization microscopy, a very important measure is the precision and accuracy of the blinking events, which describe stochastic and systematic errors, respectively. It is equally important that the labels are detected at a sufficient density, so the structure under examination emerges in details. The aforementioned quantities can be quantified, but in themselves are inadequate to fully characterise image quality. In every non-ideal experimental condition, that is not only the signals of individual molecules are detected, image reconstruction artifacts are introduced. This happens when the random photoblinking occurs at high density, the images of the molecules overlap and the algorithm cannot separate them. This typically results in multiple blinking events being identified as one with a false center, or being missed altogether. Moreover, other factors such as the drift of the sample or the autofluorescence background must be taken into account. As an example, the latter results in false localizations or their centers being shifted.

The imaging artifact are difficult to investigate both theoretically and experimentally. This is best done by software simulation, where the parameters can be freely varied and it is easy to observe the deviation of the reconstructed structure from the ground-truth. In this way, it is possible to determine what image quality can be expected for the given measurement modality and

sample type in case of different measurement parameters. In the localization microscopy, beside the lateral position, many other information can be inferred from the single molecule signals. We obtain statistics of single events instead of ensemble information keeping the high resolution. One such information is the axial position which is required for 3D reconstruction. This is perhaps most easily achieved by placing a cylindrical lens into the imaging system. This causes the image of the molecule to be elongated along two perpendicular axes on either side of the focal plane, whose shape encodes its axial position. By applying several fluorophores with distinct spectral properties, multi-color measurements can be performed, obtaining richer structural information from the same sample. Exploiting the different spectral properties of the fluorophores on the excitation or emission side, their signals can be separated into several channels. Then we can identify the fluorophores based on the signal ratios measured in the channels. It is also possible to obtain information on the orientation of fluorophores acting as dipole emitters. This can only be done in cases where the fluorescent labels are well embedded in the sample and consequently their rotation is limited or even fixed. In this case, information about the molecular organization of the sample can be obtained by measuring the orientation. The fluorophores can engage into intense interac-

tion with plasmonic structures in their vicinity. This can also be investigated at single molecule level. The interaction manifests in several emission properties, but is most directly measured through the emission brightness of blinking events. Thus, inferences can be made about the properties of plasmonic structures, or theoretical predictions can be verified experimentally.

Each measurement modality requires a different experimental setup and data evaluation procedure and, accordingly, different imaging artifacts can be expected.

Objectives and methods

One of my goals was to extend the functionality of the TestSTORM software tool used for simulating single molecule localization measurements, and thus to enable the simulation of a wide range of localization measurements, as realistically as possible. I aimed to investigate the effect of defocus and dipole orientation on the reconstructed image using realistic point spread functions. I set the goal of characterising the image reconstruction artifacts arising for astigmatic 3D and two-colour measurement modalities, and to interpret a previous polarisation sensitive measurement of the AdOptIm group. My task was to develop an evaluation procedure to extract information on the brightness of the blinking events from single molecule signals and

apply the procedure to analyse the measurement results.

The development of the TestSTORM simulation software and the evaluation of the localization measurements were carried out in MATLAB. The source code for TestSTORM is freely available on the AdOptIm group website (`http://titan.physx.u-szeged.hu/~adoptim/`). The frame count calculations for the trajectories were written in Julia. The dSTORM and white-light transmission measurements were performed on the AdOptIm group's custom built microscope system. The instrument is based on a Nikon Eclipse Ti-E microscope frame. For dSTORM measurements, I used a 647 nm laser from MPB Communications Inc. and the images were acquired using the electron multiplier readout mode of an Andor iXon3 897 camera.

New scientific results

T1: Using diffraction-based PSF models, I have investigated the impact of the stratified medium's phase aberration and the effect of static dipole moments on the localization image. I have shown, that the phase aberration induced asymmetry regarding the defocus appears on the reconstructed image, mainly as the consequence of filtering on the PSF standard deviation or on the residuum of the fit. Using the vectorial PSF model I

have examined the effect of the photoselection and the PSF shape on cylindrical spherical structures in case of different static dipole orientations. I have shown that under different excitation schemes and dipole orientations, significantly different imaging artifacts can be expected. [A1]

T2: I have implemented and applied the polarization sensitive, the dual-color and the astigmatic 3D simulation modalities in the TestSTORM software. Using the polarization sensitive simulation, I have interpreted a previous measurement result of the AdOptIm group and provided an estimate of the dipole moment confinement value. Using dual-color simulations, I have examined the effectiveness of the brightness-based filtering on the cross-talk removal. With the application of the astigmatic 3D simulations I have shown the effect of the defocus asymmetry, of the overlaps and of the structured background on the reconstructed image. I have shown the presence of various localization artifacts that cannot be corrected by localization data filtering. [A1, A2]

T3: I have developed a procedure for performing and evaluating measurements on plasmonic nanoparticles to extract the single molecule brightness in-

formation. The algorithm I developed determines the brightness of the photoblinking dyes. In order to extract the faithful single molecule information, I have made the algorithm capable of removing fluorescence background originating from the nanoparticles. Furthermore, the method is able to identify the individual nanoparticles and select the localization data belonging to them. I have performed dSTORM measurements on samples containing gold nanorods and I have examined the brightness of the blinking events occurring on them utilizing the developed procedure. [A3]

List of publications

My MTMT identifier: 10058546

Related publications

[A1] Novák, T., Gajdos, T., Sinkó, J., Szabó, G. and Erdélyi, M., 2017. TestSTORM: Versatile simulator software for multimodal super-resolution localization fluorescence microscopy. *Scientific reports*, 7(1), pp.1-8.

[A2] Erdélyi, M., Sinkó, J., Gajdos, T. and Novák, T., 2017, February. Enhanced simulator software for image validation and interpretation for multimodal localization super-resolution fluorescence microscopy. *In Single Molecule Spec-*

troscopy and Superresolution Imaging X (Vol. 10071, pp. 70-75). SPIE.

[A3] Tóth, E., Ungor, D., Novák, T., Ferenc, G., Bánhelyi, B., Csapó, E., Erdélyi, M. and Csete, M., 2020. Mapping fluorescence enhancement of plasmonic nanorod coupled dye molecules. *Nanomaterials*, 10(6), p.1048.

Other publications

[B1] Csizmadia, T., Erdélyi, M., Smausz, T., Novák, T. and Hopp, B., 2015. Simulation of the reflectivity properties of microstructured titanium surface by ray tracing method. *Journal of Laser Micro Nanoengineering*, 10(2), pp.210-215.

[B2] Gajdos, T., Cserteg, Z., Szikora, S., Novák, T., H Kovács, B.B., Szabó, G., Mihály, J. and Erdélyi, M., 2019. mmSTORM: Multimodal localization based super-resolution microscopy. *Scientific reports*, 9(1), pp.1-9.

[B3] Szikora, S., Gajdos, T., Novák, T., Farkas, D., Földi, I., Lenart, P., Erdélyi, M. and Mihály, J., 2020. Nanoscopy reveals the layered organization of the sarcomeric H-zone and I-band complexes. *Journal of Cell Biology*, 219(1).

[B4] Szikora, S., Novák, T., Gajdos, T., Erdélyi, M. and Mihály, J., 2020. Superresolution Microscopy of Drosophila Indirect Flight Muscle Sarcomeres. *Bio-protocol*, 10(12), pp.e3654-e3654.

Conference posters

[P1] T. Gajdos, T. Novák, Zs. Cserteg, M. Erdélyi (2017) *Multimodal localization based super-resolution microscopy with efficient photon collection.* MMC2017, 2017.07.3-6., Manchester, Egyesült Királyság

[P2] T. Gajdos, T. Novák, Zs. Cserteg, M. Erdélyi (2017) *Multimodal localization based super-resolution microscopy with efficient photon collection.* MBFT XXVI. Kongresszus, 2017.08.22-25., Szeged

[P3] T. Gajdos, T. Novák, Zs. Cserteg, M. Erdélyi (2018) *Multimodális lokalizációs mikroszkóp effektív fotongyűjtéssel.* Kvantumelektronika 2018, 2018.06.15., Budapest

[P4] T. Gajdos, Zs. Cserteg, Sz. Szikora, J. Mihály, B. B. H. Kovács, T. Novák, M. Erdélyi (2019) *Multimodal localization microscopy with efficient photon collection.*, FOM2019, 2019.04.14-17., London, Egyesült Királyság