Superresolution localization microscopy using multiple modalities

Ph.D. thesis synopsis

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1 Summary

1.1 Introduction

Florescence has been the subject and also a tool in scientific research for multiple centuries. The energy difference between the absorbed and emitted photon's energy (which is called Stokes-shift) can be used in the microscope as a contrast-enhancing technique. But this energy difference can also emerge in a negative relation. This process is called anti-Stokes fluorescence, and before completion the missing energy has to be provided to the fluorophore. This can be a second photon or thermal energy. In the latter case we call the process hot-band absorption.

In a microscope the image can be formed using point-scanning or widefield imaging. Using imaging techniques a problem arises, which is related to the wave nature of light, which renders the achievable resolution finite and restricts the discrimination of two neighbouring fluorophores. This minimal lateral distance is $\approx 300nm$, which is given by the Rayleigh criterion. To break this limit many tricks and realized technique had to be applied.

Resolution enhancing techniques which take advantage of optical tricks and the photophysical and photochemical properties of fluorescent dyes can be labelled as super-resolution. The first technique which satisfied these criterion was **STED** which uses the stimulated emission and a point-scanning imaging mode. In widefield multiple similar techniques were implemented in 2006 and later a common collective name was given: singe-molecule localization microscopy, or **SMLM** for short. The **PALM** technique is based on the use of fluorescently inactive,

but switchable proteins. A similar technique, the **GSDIM** takes advantage of the photophysics (triplet states) of some organic fluorescent dyes, while **dSTORM** takes advantage of the photochemistry (long dark states). The end result is the same for all three: spatially and temporally separated switching events to which the Rayleigh-criterion does not apply. By fitting the differentiated fluorophores with the point spread function, the location of the maximum value is obtainable within a pixel. This microscope technique is photon limited, thus the location determination's precision depends on the signal-to-noise ratio. The goodness of a fit can be calculated from the Thompson-formula [1], while the resolution is obtainable as a blind estimate from the the expected value of the standard deviation [2]. The final image can be reconstructed using software based solutions in a pointillist style, using only the coordinates with the highest localisation precision. We developed two software packages, TestSTORM[B4] and rainSTORM[S4], to simulate the switching mechanism, to analyse the recorded frame stack, and to reconstruct the super-resolved image.

The **SMLM** techniques can be expanded to obtain an excess information above the fluorophores **xy** coordinates. 3D astigmatism is the most widely used modality to extract the third spatial coordinate. The point spread function modification is introduced via cylindrical lens in the detector pathway. It is modified to be elliptical above and below the focal plane and also asymmetric to the focal plane. The **z** coordinate can be deducted using the ratio of the two axes of the fitted point spread function. A second method is the 3D bi-plane modality. Here the detector path is separated into two, with a slight length

difference between the paths. This creates two separate focal planes. The third coordinate can be deduced by measuring the size of the two images. In a similar manner the image can be splitted using a polarization beamsplitter cube, a Wollaston-prism or a birefringent-wedge [B3]. By determining the intensity ratio of the two images the fluorophores dipole orientation can be determined or it's uncertainty to calculate the anisotropy for each single source. The spectral separation of fluorescent dyes is achievable with the use of specially selected dichroic mirror and emission filters. Following the previous theme, the fluorescent dyes excited with the same laser source but with a different Stokes-shift can be separated by measuring the intensity in the two spectral channels. Using a dispersion element (prism or grating) the image can be spectrally resolved. This way the emission spectrum can be directly determined. The problem with these **PSF** modifications are, that each can degrade the achievable resolution by lowering the number of photons-per-pixel or by introducing **PSF** distortions.

1.2 Objectives and methods

To overcome this issue, my research goal was to design new experiment arrangements and to implement new measurement modalities in **SMLM** techniques, which preserve the lateral localization precision and resolution. These measurement modes should be easily applicable on an existing microscope system. The achieved new scientific results were published in three international, referred journals, and I summarize them in four thesis points.

To conduct the experiments, I used the *AdOptIm* researchgroup's

dSTORM–CLSM–FLIM microscope. I also participated in the construction and in multiple reconstruction of the said microscope. The required measurement were carried out using these three techniques.

1.3 New scientific results

T1 To demonstrate the hot-band absorption properties of Alexa Fluor 568; wavelength dependence, spectral properties, temperature dependence, and fluorescent lifetime was measured. The measurements were preformed in a comparative style on the well-known Rhodamine 101 and on Alexa Fluor 568, which is a widely used organic dye in single-molecule localization microscopy. Based on these results I concluded that the Alexa Fluor 568 is a hot-band absorbing dye, which was previously not known in the scientific literature. Finally I proposed possible applications regarding the use of the anti-Stokes fluorescence in **dSTORM** and **EPI** measurements (autofluorescence-free imaging, structure marker, lateral drift estimation and local temperature sensing). [A3, S5]

T2 A new dual-objective optical imaging system with a Porro-prism was designed and modelled using optical ray-tracing software. The proposed optical design collects the photons which were not used in image formation, and makes them usable in the form of a real object for the microscope. In OSLO optical simulation software I prepared the two imaging arm model to simulate the formation of the two images, furthermore the obtained point spread functions were compared in the focal plane and in axial projection. I showed, that the proposed

layout is suitable for to be used in single molecule detection based measurement techniques. [A1,P3-5]

T3 I upgraded a single-molecule localization microscope with my self-designed dual-objective imaging system with Porro-prism to enhance the photon collection from the sample and to facilitate the application of modalities during measurements. I worked out the pair-finding for the dual-objective arrangement and built two 3D-, one dipole orientation- and one multicolour modality. With the application of each modality I made **dSTORM** measurements on fruitfly indirect flight muscle myofibrill samples and fluorescent beads to test the arrangement's usability and to prove the preservation of the localization precision. [A1, C1, P6-7, S2, S6]

T4 I performed **dSTORM** measurements to characterize fruitfly indirect flight muscle protein structure. To speed up the data processing I automatized the localization and the reconstruction process. In the myofibrill three distribution type were classified ("double line", "single line", "gap") to group the 35 examined protein structure. To evaluate these a new classifier software was developed, which extracts the characteristic parameters from the raw data. [A2, S3-4]

List of publications

MTMT ID: 10055222

Related publicationsin peer reviewed journals

[A1] T. Gajdos, Zs. Cserteg, Sz. Szikora, T. Novák, B. B. H. Kovács, G. Szabó, J. Mihály, M. Erdélyi (2019). *mmSTORM: Multimodal localization based super-resolution microscopy.* Scientific reports, **9(1)**, 798. **Q1** IF: 4,011 (2018); doi:10.1038/s41598-018-37341-9

[A2] Sz. Szikora, T. Gajdos, T. Novák, D. Farkas, I. Földi, P. Lenart, M. Erdélyi, J. Mihály (2020). *Nanoscopy reveals the layered organization of the sarcomeric H-zone and I-band complexes.* Journal of Cell Biology, **219(1)**. **Q1** IF: 8,891 (2018); doi:10.1083/jcb.201907026

[A3] T. Gajdos, B. Hopp, M. Erdélyi (2020) *Hot-band anti-Stokes fluorescence properties of Alexa Fluor 568.* Journal of Fluorescence, **x(x)**, xxx. **Q3** IF: 1,913 (2018); doi:10.1007/s10895-020-02496-0

Other publications in peer-reviewed journals

[B1] Zs. Farkas, T. Gajdos, B. Major, A. Nagy (2011). *Korok és tudósok-a színpadon Arkhimédész, Galilei és Newton: a szegedi Kutatók Éjszakájától a koppenhágai Science on Stage-ig.* FIZIKAI SZEMLE, **2011(7-8)**, 267-272.; http://fizikaiszemle.hu/archivum/fsz110708/Farkas-Zsuzsanna.pdf

[B2] M. Erdélyi, J. Sinkó, T. Gajdos, T. Novák (2017, February). *Enhanced simulator software for image validation and interpretation for multimodal localization super-resolution fluorescence microscopy.* In Single Molecule Spectroscopy and Superresolution Imaging X (**Vol. 10071**, p. 100710X). International Society for Optics and Photonics.; doi:10.1117/12.2250116

[B3] Sinkó, J., T. Gajdos, E. Czvik, G. Szabó, M. Erdélyi (2017). *Polarization sensitive localization based super-resolution microscopy with a birefringent wedge.* Methods and applications in fluorescence, **5(1)**, 017001. **Q1** IF: 2,209 (2017); doi:10.1088/2050-6120/aa6260

[B4] T. Novák, T. Gajdos, J. Sinkó, G. Szabó, M. Erdélyi (2017). *TestSTORM: Versatile simulator software for multimodal super-resolution localization fluorescence microscopy.* Scientific reports, **7(1)**, 951. **Q1** IF: 4,445 (2017); doi:10.1038/s41598-017-01122-7

Conference presentations and posters

[E1] T. Gajdos, Zs. Cserteg, Sz. Szikora, T. Novák, B. B. H. Kovács, G. Szabó, J. Mihály, M. Erdélyi (2019) *Lokalizációs mikroszkópia több modalitásban a laterális pontosság megőrzése mellett.* MMT Conference, **2019.05.23-25.**, Siófok, Hungary

[P1] Zs. Farkas, T. Gajdos, B. Major, A. Nagy (2011). *Ages and Scientists.* Science on Stage Festival, **2011.04.16-19.**, Copenhagen, Denmark

[P2] T. Gajdos, J. Németh, J. Sinkó, D. Varga, E. J. Rees, G. Szabó, M. Erdélyi (2016) *Localization analysis with rainSTORM.* 16th international ELMI meeting, **2016.05.24-27.**, Debrecen, Hungary

[P3] T. Gajdos, J. Németh, J. Sinkó, D. Varga, E. J. Rees, G. Szabó, M. Erdélyi (2016) *Localization analysis with rainSTORM.* 6th Single Molecule Localization Microscopy Symposium, **2016.08.28-30.**, Lausanne, Switzerland

[P4] 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, United Kingdom

[P5] 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, Hungary

[P6] T. Gajdos, T. Novák, Zs. Cserteg, M. Erdélyi (2018) *Multimodális lokalizációs mikroszkóp effektív fotongyűjtéssel.* Kvantumelektronika 2018, **2018.06.15.**, Budapest, Hungary

[P7] 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, United Kingdom

List of developed software

[S1] *Arduino Laser Driver* (C, LabView), own, individual work.
`https://github.com/gajdipajti/thorlabs-ldc-arduino`

[S2] *Arduino Stepper Control* (C, LabView), own, individual work.
`https://github.com/gajdipajti/arduino_stepper_control`

[S3] *IFM Analyser*, (Mathworks Matlab), shared work.
`http://titan.physx.u-szeged.hu/~adoptim/?page_id=1246`

[S4] *rainSTORM* (Mathworks Matlab), minor improvements, subtasks.
`http://titan.physx.u-szeged.hu/~adoptim/?page_id=582`

[S5] *Beam Profiler* (Python), minor improvements.
`https://gist.github.com/gajdipajti/0ff1625115137866ca10f5cec136bed`

[S6] *mmSTORM-ast-lense* (LabView), work of my student.
`https://gitlab.com/adoptim/mmSTORM-ast-lense`

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[1] R.E. Thompson, D.R. Larson, W.W. Webb, *Precise Nanometer Localization Analysis for Individual Fluorescent Probes*, Biophysical Journal **82**(5), 2775 (2002). doi:10.1016/s0006-3495(02)75618-x

[2] E.J. Rees, M. Erdelyi, D. Pinotsi, A. Knight, D. Metcalf, C.F. Kaminski, *Blind assessment of localisation microscope image resolution*, Optical Nanoscopy **1**(1), 12 (2012). doi:10.1186/2192-2853-1-12