Development and application of lacunarity analysis, astigmatic 3D and multicolor image merging algorithms and methods for the quantitative analysis of single molecule localization microscopy images

Ph.D. thesis synopsis

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1. Summary

1.1. Introduction

The study and understanding of structures smaller than the resolution of the human eye is made possible by microscopes and the discipline of microscopy, which is concerned with their development. The most common way to magnify structures in the micro-world is with optical microscopes, which use light that is visible to the human eye. Due to the wave properties of light, the size of the smallest structure that can be resolved is limited by diffraction. Utilizing a high numeric aperture optical microscope, it is typically 250 nm. Further improvements in resolution can be achieved using light or particles of smaller wavelengths. These methods, however require a complete re-design of the optical system. If a higher resolution is to be achieved with an optical system optimized for visible light, another solution is needed. The term optical superresolution is the general name for the techniques that address or solve this problem [1]. The different superresolution techniques allow to increase the resolution while using visible light. Of these techniques, the so-called single molecule localization microscopy (SMLM) provides the best resolution (20 nm) [2], [3], [4]. This resolution allows the observation of intracellular organelles and biological processes, making the technique very popular in the fields of biology and medicine. Some subsets of localization techniques can even be applied to living cells. Of course, like all techniques, localization microscopy has its drawbacks. The localization measurement data evaluated are point sets, which makes it difficult to compare the results with previous results that consist of images. To remedy this, algorithms and procedures have been developed that can extract additional information from point sets of measurements specific to the localization technique [5]. Such algorithms have greatly facilitated the consistent comparison of localization results with previous conventional fluorescence or confocal results, but there remains a significant need to develop new quantitative evaluation methods, to accelerate previous methods and to extract additional information from localization data sets.

1.2. Objectives and methods

The main objective of my research was to complement and further develop the localization microscope technique with methods and evaluation algorithms that allow the extraction of information beyond the lateral position of the fluorescent molecules under investigation. I also aimed to apply the new techniques to the investigation of cell biological systems. I planned to achieve this goal along two strands. One is through hardware, where I use the localization

microscope with new modalities or in combination with other techniques to extract more information. The other is through software with the quantitative evaluation of existing data using new algorithms. My specific goals were:

Implementation of an astigmatism-based 3D localization procedure in our dSTORM system. Development of a measurement protocol for this procedure. Exploration of 3D structures of biological systems using the 3D superresolution system. Modeling the astigmatic 3D modality of the multimodal microscope system developed in our team.

Optimization of our microscope system for multicolor dSTORM and confocal imaging of the same sample area. Development of a procedure to merge and analyze dSTORM and confocal images. Structural analysis of multi-component complex biological samples using multicolor imaging.

Introduction of lacunarity for quantitative evaluation of data sets from localization microscopy techniques based on single molecule detection. Development of a procedure and software for the evaluation of lacunarity of localization data sets. Validation of the method using results and simulations on previous biological samples.

Optimization of the calculation of lacunarity values. Collection of different high-precision lacunarity calculation algorithms. Development of new lacunarity calculation algorithms. Comparison of lacunarity computing algorithms on test data sets. Determination of a dataset specific optimal lacunarity computing algorithm.

1.3. New scientific results

T1: I have developed a matrix optics-based method for modelling astigmatic 3D superresolution systems with cylindrical lenses in the LabView programming environment. By modelling our system, I investigated how the magnitude of astigmatism depends on the focal length and position of the cylindrical lens. I have shown the advantages and disadvantages of each cylinder lens position range in terms of reproducibility and adjustability of astigmatism. The model was supported by OSLO simulations. I supplemented our system with a cylinder lynch chosen from the model and validated the simulation results by measurements on test samples. The optimized 3D system has been used in several collaborations. Among other things, the inclusion of 3D images allowed a more accurate analysis of phase-separated ribosome clusters. Furthermore, I used the implemented procedure to model the astigmatic 3D modality of the mmSTORM localization microscope. I determined the

nature of the dependence of the focal length and position of the astigmatic cylinder lens in the multimodal system. [A1, A2]

T2: I have optimized our microscope system for the comparative evaluation of multicolor confocal and dSTORM images and for the gathering of colocalization information. I have written a LabView code for the merging of confocal and dSTORM images. I have taken dual-color confocal and dSTORM images of the HCN1, HCN2 and Kv3.1 ion channels of interneurons found in human and mouse brain samples. On the dual-color confocal images in one channel we visualized the cell membrane with labeling parvalbumin, while in the other one the ion channels were labeled enabling the confocal-dSTORM comparison. Using the high resolution dSTORM measurements we localized the positions of the ion channels with a precision of 20 nms. Using the image merging algorithm I made and the visual comparison of the dSTORM and confocal images we have shown the different behavior of HCN1 and HCN2 channels in human and mouse interneurons. [A3]

T3: I have developed a lacunarity-based method for quantitative evaluation of the geometry of localization microscopy data sets. I introduced the lacunarity divergence curve, which can efficiently visualize the homogeneity of 2D and 3D structures at different scales by representing normalized lacunarity values. Using synthetic data sets generated using TestSTORM simulation software, I showed how the lacunarity divergence curves can be used to infer different clustering processes and cluster parameters (e.g. cluster size cluster number cluster density etc.). Using dSTORM recordings of DNA double-strand break repair proteins, I have shown that the results obtained with the lacunarity divergence curves are in agreement with previous DBSCAN cluster analysis results. I have shown that lacunarity-based evaluations can be performed five times faster than DBSCAN. [A4]

T4: I have developed two new lacunarity computation algorithms one based on the convolution theorem and one based on the inversion of the original method, both of which are capable of computing gliding-box lacunarity values orders of magnitude faster than the original gliding-box algorithm. The new algorithms have also been implemented for the evaluation of 2D and 3D data sets. I further improved the original gliding-box and two optimized 2D algorithms for the evaluation of 3D data sets. I compared these three algorithms known from the literature and the two new algorithms I implemented with the evaluation of 2D and 3D synthetic data sets in terms of runtime, memory requirements and code complexity. I have shown the advantages and disadvantages of each algorithm and determined the optimal lacunarity computing algorithm for given samples and hardware configurations. [A5]

2. Publication list

MTMT ID: 10071753

Publications related to the thesis:

[A1] Tamás Gajdos, Zsófia Cserteg, Szilárd Szikora, Tibor Novák, Bálint Barna H. Kovács, Gábor Szabó, József Mihály, and Miklós Erdélyi. "mmSTORM: Multimodal localization based super-resolution microscopy." Scientific Reports 9,1 (2019): 798. **Q1**, IF: **4,6**; doi: 10.1038/s41598-018-37341-9

[A2] Orsolya Németh-Szatmári, Ádám Györkei, Dániel Varga, Bálint Barna H. Kovács, Nóra Igaz, Kristóf Német, Nikolett Bagi et al. "Phase separated ribosome nascent chain complexes paused in translation are capable to continue expression of proteins playing role in genotoxic stress response upon DNA damage" bioRxiv. **Q-**, IF: **0**; doi: 10.1101/2022.03.16.484567

[A3] Viktor Szegedi, Emőke Bakos, Szabina Furdan, Bálint H. Kovács, Dániel Varga, Miklós Erdélyi, Pál Barzó, Attila Szücs, Gábor Tamás, and Karri Lamsa. "HCN channels at the cell soma ensure the rapid electrical reactivity of fast-spiking interneurons in human neocortex." Plos Biology 21,2 (2023): e3002001. **Q1**, IF: **9,8**; doi: 10.1371/journal.pbio.3002001

[A4] Bálint Barna H. Kovács, Dániel Varga, Dániel Sebők, Hajnalka Majoros, Róbert Polanek, Tibor Pankotai, Katalin Hideghéty, Ákos Kukovecz, and Miklós Erdélyi. "Application of Lacunarity for Quantification of Single Molecule Localization Microscopy Images." Cells 11,19 (2022): 3105. **Q1**, IF: **7,666**; doi: 10.3390/cells11193105

[A5] Bálint Barna H. Kovács, and Miklós Erdélyi. "Methods for calculating gliding-box lacunarity efficiently on large datasets." Expert Systems With Applications, BÍRÁLAT ALATT **Q1**, IF: **8,5**; ssrn: https://ssrn.com/abstract=4516313 or http://dx.doi.org/10.2139/ssrn.4516313

Other publications:

[A6] Tibor Novák, Dániel Varga, Péter Bíró, Bálint Barna H. Kovács, Hajnalka Majoros, Tibor Pankotai, Szilárd Szikora, József Mihály, and Miklós Erdélyi. "Quantitative dSTORM superresolution microscopy." Resolution and Discovery 6,1 (2022): 25-31. **Q-**, IF: **0**; doi: 10.1556/2051.2022.00093

[A7] Orsolya Németh-Szatmári, Bence Nagy-Mikó, Ádám Györkei, Dániel Varga, Bálint Barna H. Kovács, Nóra Igaz, Bence Bognár et al. "Phase-separated ribosome-nascent chain complexes in genotoxic stress response." RNA 29,10 (2023): 1557-1574. **Q1**, IF: **4,5**; doi: 10.1261/rna.079755.123

Conferences:

[K1] Bálint Barna H. K., D. Varga, D. Sebők, H. Majoros, R. Polanek, T. Pankotai, K. Hideghéty, Á. Kukovecz, M. Erdélyi, P. Bíró és N. Tibor. "Application of Lacunarity for Quantification of Single Molecule Localization Microscopy Images." FOM, 2023.04.02.-05., Porto, Portugal

3. References

[1] B. Huang, H. Babcock, and X. Zhuang, “Breaking the Diffraction Barrier: Super-Resolution Imaging of Cells,” *Cell*, vol. 143, no. 7, pp. 1047–1058, Dec. 2010, doi: 10.1016/j.cell.2010.12.002.

[2] E. Betzig *et al.*, “Imaging Intracellular Fluorescent Proteins at Nanometer Resolution,” *Science*, vol. 313, no. 5793, pp. 1642–1645, Sep. 2006, doi: 10.1126/science.1127344.

[3] M. J. Rust, M. Bates, and X. Zhuang, “Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM),” *Nat Methods*, vol. 3, no. 10, pp. 793–796, Oct. 2006, doi: 10.1038/nmeth929.

[4] M. Heilemann *et al.*, “Subdiffraction-Resolution Fluorescence Imaging with Conventional Fluorescent Probes,” *Angew Chem Int Ed*, vol. 47, no. 33, pp. 6172–6176, Aug. 2008, doi: 10.1002/anie.200802376.

[5] Y.-L. Wu, A. Tschanz, L. Krupnik, and J. Ries, “Quantitative Data Analysis in Single-Molecule Localization Microscopy,” *Trends in Cell Biology*, vol. 30, no. 11, pp. 837–851, Nov. 2020, doi: 10.1016/j.tcb.2020.07.005.