Quantitative analysis of superresolution localization microscopy measurements

Ph.D. thesis synopsis

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1 Summary

1.1 Introduction

The appearance of fluorescent microscopy has made possible the investigation of biological samples with high contrast in a non-invasive way. However, the resolution was limited by diffraction, therefore structures below a few hundred nanometers remained hidden. Super-resolution microscopy techniques in turn opened new ways to research, because these methods have made it possible to analyze structures of fluorescent samples below the diffraction limit [1]. Among these techniques localization microscopy (SMLM) has become popular. In SMLM, the positions of individual molecules are determined with a resolution of $10-20\ nm$ [2–4]. As a result of this approach the raw localization file, unlike the pixelated nature of the conventional microscopical images, is a data set, which contains the localization coordinates of the fluorophores. The analysis of such point clouds requires new approaches [5].

Since a localization corresponds to the coordinates of a single fluorophore, the SMLM technique aroused interest in terms of the quantitative characterization of target molecules. The determination of the number of molecules is extremely relevant, but the stochastic photophysical behavior of the fluorophores and the often unknown labeling stoichiometry makes this task difficult. The point clouds generated by SMLM also gave new opportunities to cluster analysis. Since cell functions are often regulated by the spatial and temporal organization of proteins, studying these organizations could help answering important

questions. Detecting clusterization is an important task on its own, but the quantitative characterization of the clusters can lead to further findings. The best way to describe different cluster properties is to determine the distribution of the properties which can have a spatial and a temporal dependence. The spatial structure of the clusters formed by the target proteins and the shape factor of these clusters could help answering relevant biological questions. In case of localization data sets, structural investigations require new approaches. Determination of physical dimensions usually happens through a theoretical curve fitting to the cross sectional profile of the examined structure. There are multiple ways to produce this profile. On the one hand a super-resolution image can be created based on the localization coordinates on which the familiar methods of conventional image analysis can be used. While on the other hand the generation of such an image can result in information loss, so it is advisable to use the localization coordinates directly.

1.2 Objectives and methods

My goal was to interpret the point clouds generated by the SMLM technique and obtain quantitative information from them, in particular the number of labeled molecules, the detection of clusterization as well as the characterization of the clusters and the geometrical parameters of the sample. It was among my plans to compare these results with results obtained form the analysis of confocal images to explore the possibilities of correlative measurements. It was relevant, because confocal laser scanning microscopy (CLSM) is a prevalent technique in

biological research. Performing the necessary measurements was also among my tasks. It was my purpose to not complicate the measurement protocol and to not increase the measurement time with the employed analytical methods.

My objectives were motivated by important biological problems, such as estimating the number of γH2AX histones (which are signaling proteins of DNA double-strand breaks), the time resolved analysis of γH2AX clusters after X-ray irradiation, the changes in the structure of actin filaments or the quantitatve characterization of RNC clusters after a genotoxic stress. I used the dSTORM and CLSM system of AdOptIm research group to conduct the measurements and I mainly used MATLAB and Python to analyze measurement data.

1.3 New scientific results

T1: I determined the probability mass function of the localization numbers corresponding to a single target molecule in case of any labeling ratio during dSTORM measurements by modeling the fluorescent molecules with a three state system. I developed a computer algorithm for the determination of the response function of the used microscope system directly from the localization data set. I performed super-resolution dSTORM measurements on U2OS and DIvA cell nuclei and determined the response function corresponding to the subunit of the chromatin structure. [A1, A2]

T2: I developed an algorithm based on the DBSCAN method to cluster analyze localization data sets. I quantitatively characterized the

properties of the clusters and determined the distributions of these parameters within the region of interest. I performed super-resolution dSTORM and confocal (CLSM) measurements on U251 human glioblastoma cells after X-ray irradiation and performed a time resolved investigation on the DNA double-strand breaks signaling γH2AX clusters. I compared the results obtained with the two techniques and characterized the possibilities of correlative measurements. I explored the limits of the applicability of $2D$ and $3D$ analysis via TestSTORM simulations and validated my $2D$ dSTORM measurement results. [A3]

T3: I developed a computer algorithm based on skeletonization to quantitatively characterize filamental networks of fixed cells based on the localization data set. Using the localization coordinates, the thickness of the filamentary structure can also be determined. The methods have been successfully applied to the evaluation of cell biological studies using confocal (CLSM) and high-resolution dSTORM techniques. [A4]

T4: By analyzing the localization measurements I showed that the density of Rpt1-RNC clusters is increasing toward the cell membrane of A549 adenocarcinomic human cells, while the same phenomenon was not observable in yeast cells. Using TestSTORM test sample generator, I have developed a method to set the optimal DBSCAN parameters to analyze clusters. I found that the ribosome-nascent chain complexes, which are paused during the translation of Rpt1 and Sgs1 proteins, show clustering in yeast cells and this clustering is more significant in

Sgs1-RNC expressing cells. I characterized the effect of UV treatment on Not1 containing clusters with DBSCAN algorithm. I developed a cluster finding algorithm based on cross-correlation and found that a significant portion of the clusters have a hollowed structure. [A5]

List of publications

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Publications related to the thesis

[A1] D. Varga, H. Majoros, Zs. Újfaludi, M. Erdélyi, T. Pankotai (2019). *Quantification of DNA damage induced repairfocus formation via super-resolution dSTORM localization microscopy.* Nanoscale, **11(30)**, 14226-14236. **Q1** IF: 7,79 (2020); doi:10.1039/C9NR03696B

[A2] D. Varga, H. Majoros, Zs. Újfaludi, T. Pankotai, M. Erdélyi (2020, február) *Quantification of labelled target molecules via super-resolution dSTORM localization microscopy.* Single Molecule Spectroscopy and Superresolution Imaging XIII (11246. kötet, 1124612. oldal). International Society for Optics and Photonics. doi:10.1117/12.2545099

[A3] Sz. Brunner, D. Varga, R. Bozó, R. Polanek, T. Tőkés, E. R. Szabó, R. Molnár, N. Gémes, G. Szebeni, L. G. Puskás, M. Erdélyi, K. Hideghéty (2021). *Analysis of ionizing radiation induced DNA damage by superresolution dSTORM microscopy.* Pathology and Oncology Research, **27**, 13 p. **Q2** IF: 3,201 (2020); doi:10.3389/pore.2021.1609971

[A4] K. Szabó, D. Varga, A. G. Vegh, N. Liu, X. Xiao, L. Xu, L. Rovo, L. Dux, M. Erdelyi, A. Keller-Pinter (2022). *Syndecan-4 affects myogenesis via Rac1-mediated actin remodeling and exhibits copy-number amplification and increased expression in human rhabdomyosarcoma tumors.* Cellular and Molecular Life Sciences, **79(2)**, 1-21. **Q1** IF: 9,261 (2020); doi:10.1007/s00018-021-04121-0

[A5] O. Szatmári, Á. Györkei, D. Varga, B. H. Kovács, N. Igaz, K. Német, N. Bagi, B. Nagy-Mikó, D. Balogh, Zs. Rázga, M. Erdélyi, B. Papp, M. Kiricsi, A. Blastyák, M. A. Collart, I. M. Boros, Z. Villányi (2022). *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, doi:10.1101/2022.03.16.484567

Other publications

[B1] M. Erdélyi, R. Kákonyi, A. Kelemen, E. Rees, D. Varga, G. Szabó (2015). *Origin and compensation of imaging artefacts in localization-based super-resolution microscopy.* Methods, **88**, 122-132. **Q1** IF: 3.608 (2020); doi:10.1016/j.ymeth.2015.05.025

[B2] O. Szatmári, Á. Györkei, D. Varga, B. H. Kovács, N. Igaz, K. Német, N. Bagi, B. Nagy-Mikó, D. Balogh, Zs. Rázga, M. Erdélyi, B. Papp, M. Kiricsi, A. Blastyák, M. A. Collart, I. M. Boros, Z. Villányi (2022). *Validation of an in silico approach to identify new components of assemblysomes.* submitted to RNA. **Q1** IF: 4,389 (2020);

[B3] V. Szegedi, E. Bakos, S. Furdan, D. Varga, M. Erdélyi, P. Barzo, A. Szücs, T. Gabor, K. Lamsa (2022). *Somatic HCN channels accelerate the input–output kinetics of human cortical fast-spiking interneurons.* submitted to PLOS Biology, **Q1** IF: 7.494 (2020);

Conference presentations and posters

[E1] D. Varga (2022) *Quantitative analysis of SMLM data.* Second Symposium on Super-resolution and Advanced Fluorescence Microscopy and István Ábrahám Memorial Workshop, **2022.04.01-02.**, Pécs, Hungary

[E2] D. Varga, T. Novák, P. Bíró, S. Szikora, J. Mihály, M. Erdélyi (2022) *Segmentation of sarcomeric structures in SMLM with machine learning.* FOM2022, **2022.04.10-13.**, Online

[E3] D. Varga (2021) *Quantification of DNA Damage Induced Repair Focus Formation via dSTORM Localization Microscopy.* 7th NANO Boston Conference, **2021.10.18-20.**, Boston, MA, USA

[E4] D. Varga, H. Majoros, Zs. Újfaludi, T. Pankotai, M. Erdélyi (2019) *Quantification of DNA double-strand breaks via dSTORM localization microscopy.* MMT Konferencia, **2019.05.23-25.**, Siófok, Hungary

[P1] H. Majoros, D. Varga, Zs. Újfaludi, M. Erdélyi, T. Pankotai (2020) *Quantification of DNA damage induced repair focus for-*

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[P2] D. Varga, H. Majoros, Zs. Újfaludi, T. Pankotai, M. Erdélyi (2019) *Quantification of DNA damage induced repair focus formation via super-resolution dSTORM localization microscopy.* III. Sejt-, Fejlődés-, és Őssejtbiológusok Éves találkozója, **2019.10.30.**, Gödöllő, Hungary

[P3] D. Varga, H. Majoros, Zs. Újfaludi, T. Pankotai, M. Erdélyi (2019) *Quantification of DNA double-strand breaks via storm localization microscopy.* FOM2019, **2019.04.14-17.**, London, United Kingdom

[P4] D. Varga, H. Majoros, Zs. Újfaludi, T. Pankotai, M. Erdélyi (2019) *Protein counting in localisation microscopy.* QBI2019, **2019.01.9-11.**, Rennes, France

[P5] D. Varga, H. Majoros, Zs. Újfaludi, T. Pankotai, M. Erdélyi (2018) *Dissolving single DNA repair foci by super-resolution STORM microscopy.* FEBS3+ Meeting; From molecules to living systems, **2018.11.2-5.**, Siófok, Hungary

[P6] D. Varga, H. Majoros, Zs. Újfaludi, T. Pankotai, M. Erdélyi (2018) *Kvantitatív lokalizációs mikroszkópia.* Kvantumelektronika 2018, **2018.06.15.**, Budapest, Hungary

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[P8] 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

[P9] M. Erdélyi, R. Kákonyi, A. Kelemen, E. Rees, D. Varga, G. Szabó (2016) *Artifacts analysis in localization based microscopy.* 16th international ELMI meeting, **2016.05.24-27.**, Debrecen, Hungary

[P10] 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

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[5] Yu-Le Wu et al. “Quantitative data analysis in single-molecule localization microscopy”. In: *Trends in Cell Biology* 30.11 (2020), pp. 837–851.