2nd Year Report

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Short description of my research

In my project, I'm applying Structured Illumination Microscopy (SIM) to investigate chromatin organization at the nanoscale.
SIM is an Optical Super-Resolution Microscopy (SRM) technique capable to perform optical sectioning (3D-SIM), multicolor channel acquisition, and it can be used on live samples because of its low phototoxicity [1,2]. SIM relies on the interference between two or more laser beams to create a high modulation frequency pattern to illuminate the specimen, instead of uniform widefield illumination. In SIM acquisition, several images of the sample are collected, corresponding to different orientations and phases of the striped illumination pattern. The multiplication of specimen features with the striped pattern causes the formation of Moiré fringes. All the information contained in the acquired raw image, pattern, sample and Moiré fringes, can be visualized and converted in the frequency domain. In this Fourier space, the observable region of a conventional microscope is delimited by a circumference whose radius is the maximum achievable resolution (200-250 nm). However, the formation of Moiré Fringes causes a shift of the Fourier central zero into new offsets, located in the direction of the three orientations, increasing the observable frequencies and thus the observable Super Resolution features. This means that SIM allows accessing high-frequency information, i.e. below the Diffraction Limit of Light (200-250 nm).
During the 2nd year of my PhD, I worked both on the development of a new method for the analysis of SIM data and on the application of SIM to investigate chromatin organization at the nanoscale.
In the first project, I explored the combination of SIM with Separation of Photons by LIfetime Tuning (SPLIT) [3]. SPLIT is a novel algorithm, recently introduced by our group, that can be used to reconstruct images acquired with different SRM techniques. In this method, the sub-diffraction spatial information is encoded into an additional channel of the microscope: in the case of SIM, it is possible to use the SPLIT algorithm to analyze the information encoded into the images acquired at different illumination patterns. For each pixel, we analyze the profile of the intensity as a function of the phase of the illumination pattern to generate the super-resolved image. The analysis of the intensity profile at each pixel is performed in frequency-domain and visualized using a tool called phasor plot. In particular, an important aspect of SPLIT is that the phasor plot provides a visual, intuitive and direct evaluation of the acquired data [4]. We believe that this property could be useful to minimize artifacts in the
SIM reconstruction process. We already demonstrated by simulated SIM data that knowledge of the illumination pattern is sufficient to perform SPLIT analysis and to reconstruct a super-resolved image bypassing the conventional Fourier reconstruction. We also tested the algorithm on model samples like fluorescent beads. We are currently testing the SPLIT-SIM approach to perform super-resolution imaging of chromatin-related structures. During the last few months, I also started working on another project that is the application of SIM to visualize alterations in chromatin organization in a model of oncogene activation. Recently it has been introduced the hypothesis that the activation of oncogenes may lead to genomic instability, which is one of the hallmarks of cancer, by altering fundamental processes such as the DNA Damage Response (DDR), DNA transcription and DNA replication [6]. For this reason, I aim to use SIM to investigate oncogene-induced alterations in the organization of cell nuclei. For this project, I will use a specific cell line, U937-PR9, engineered to activate an oncogene, which causes the formation of the PML-RARalpha fusion protein. With this model is possible to monitor the spatial distributions of DNA transcription and DNA replication and DDR before and after oncogene activation. I aim to observe, by single-channel imaging, alterations of the size and the number of replication and transcription foci in relation to the expression of the oncoprotein. I will use multicolor imaging to compare the relative spatial distributions of replication and transcription in the control versus the oncogene-activated sample. I will quantify the degree of colocalization between replication and transcription through different methods, including object-based algorithms available on the open-source software ImageJ and Image Cross-Correlation Spectroscopy (ICCS).

[1] Gustaffson MGL., et al. Biophysical Journal (2008)
[2] Schermelleh L., et al. Journal of Cell Biology (2010)
[3] Lanzanò L., et al. Nature Communications (2015)
[4] Sarmento M., et al. Nature Communications (2017)
[5] Negrini S., et al. Nature Reviews Molecular cell biology (2010)
[6] Helmrich A. et al. Nature Structure &molecular biology (2013)

Poster Presentations

I. **Cainero**, M. Oneto, L. Pesce, G. Zanini, L. Lanzanò, A. Diaspro, P. Bianchini (2017). Combining Expansion Microscopy and STED Nanoscopy for the Study of Cellular Organization. Biophysical Journal 112 (3), 140a. https://doi.org/10.1016/j.bpj.2016.11.775

II. **Cainero**, A. Le Gratiet, P. Bianchini, L. Lanzanò, A. Diaspro (2018). Combining Structured Illumination Microscopy (SIM) and Separation of Photons by Lifetime Tuning (SPLIT) in a multimodal imaging architecture to study in-situ chromatin organization. Biophysical Society Meeting, Late Abstract (18-L-5613-BPS)

III. A Novel Viewpoint to Analyze Structured Illumination Microscopy (Sim) Data (2019) **I. Cainero**, S. Pelicci, M. Di Bona, A. Diaspro, L.Lanzano (2019). Biophysical Houtnal 116 (3) 280a. https://doi.org/10.1016/j.bpj.2018.11.2361

Publications

I. Diaspro, N. Anthony, P. Bianchini, **I. Cainero**, M. Di Bona, L. Lanzanò, A. Le Gratiet, R. Marongiu, M. Oneto, S. Pelicci, L. Pesce (2018). LIQUITOPY®: A Liquid Tunable Microscope to Study Chromatin Organization in the Cell Nucleus. *Microscopy and Microanalysis* 24 (S1), 1368-1369. https://doi.org/10.1017/S1431927618007328

II. S. Pelicci, M. Oneto, M. Di Bona, **I. Cainero**, P. Barboro, A. Diaspro, L. Lanzano (2019). Chromatin Nanoscale Organization Investigated by FLIM-FRET and STED Superresolution Microscopy. *Biophysical Journal* 116 (3), 174a. https://doi.org/10.1016/j.bpj.2018.11.965

III. L. Lanzano, M. Oneto, **I. Cainero**, S. Pelicci, M. Sarmento, L. Scipioni, M. Faretta, L. Furia, G. I. Dellino, P. G. Pelicci, P. Bianchini, A. Diaspro (2019). Chromatin Alterations in a Model of Oncogene Activation Studied by Advanced Fluorescence Microscopy. *Biophysical Journal* 116 (3), 280a. https://doi.org/10.1016/j.bpj.2018.11.1515

IV. M. Di Bona, S. Pelicci, **I. Cainero**, G. Vicidomini, D. Mazza, M. A. Mancini, A. Diaspro (2019). Intensity Sorted Fluorescence Correlation Spectroscopy: A Novel Method to Probe Nuclear Dynamics and Chromatin Organization in Living Cells. *Biophysical Journal* 116 (3) 72a. https://doi.org/10.1016/j.bpj.2018.11.429

V. M. Oneto, L. Scipioni, M. J Sarmento, **I. Cainero**, S. Pelicci, L. Furia, P.G. Pelicci, G. I. Dellino, P. Bianchini, M. Faretta, E. Gratton, A.Diaspro, L. Lanzanò (2019). Nanoscale distribution of nuclear sites analyzed by superresolution STED-ICCS. *BioRxiv*, 753228. https://doi.org/10.1101/753228

VI. Diaspro, **I. Cainero**, L. Lanzanò, P. Bianchini, G. Vicidomini, F. Cella Zanacchi, L. Pesce, S. Pelicci, M. Oneto, M. Di Bona, M. Faretta, P.Barboro, A. Le Gratiet (2018). A Liquid Tunable Microscope as a New Paradigm in Optical Microscopy to Paint 4D Chromatin Organisation in the Cell Nucleus. *Biophysical Journal* 116 (3), 347a. https://doi.org/10.1016/j.bpj.2017.11.1937

Book chapter

Springer Handbook of microscopy. P. Hawkes, J. Spence. (2019) Chapter 21 "Fluorescence Microscopy" P. Bianchini, F. Cella Zanacchi, L. Lanzanò, G. Vicidomini, M. Oneto, L. Pesce, **I. Cainero**. https://www.springer.com/gp/book/9783030000684

In Preparation

I. Cainero, A. Diaspro, L.Lanzanò. "*SPLIT-SIM: a novel viewpoint to analyze Structured Illumination Microscopy data through Separation of Photons by LIfetime Tuning algorithm*."

Schools And Workshops 2019

I. 5th IIT Advanced Microscopy Workshop. December 3rd - December 6th, 2018 at the IIT Nikon Imaging Centre (NIC@iit). Paolo Bianchini, Alberto Diaspro. Istituto Italiano di Tecnologia, Genova, Italy.

II. IVSLA International School on Nanoscale Optical Microscopy; 12 - 15 June 2018, Venice, Italy

2nd year Exams

Statistica per analisi dati (Prof. Parodi e Passaggio)