3rd Year Report

Isotta Cainero, PhD student in Physics XXXIII cycle
Supervisors: ***Alberto Diaspro***; ***Luca Lanzanò***

Short description of my third year research

Since the beginning of my Ph.D., my project has been focused on applying Structured Illumination Microscopy (SIM) to investigate chromatin organization at the nanoscale. Among all super-resolution optical microscopy techniques, we choose SIM, because of its low photo-toxicity, for multicolor channel acquisition and its capability to perform optical sectioning (3D-SIM) [1,2]. In SIM, to collect all the sub-diffraction features of the sample, the illumination is performed with a high modulation frequency pattern generated by laser beam interference. To illuminate all the sample, the pattern is shifted multiple times in three angles orientation. The imposition of this striped pattern onto the sample causes the Moiré fringes effect. Thus, the acquired raw images, contain the information from the pattern, the sample, and the Moiré fringes. All these information can be visualized and converted into the frequency domain, the Fourier space. Considering the frequency domain, the resolution of a wide-field microscope corresponds to a circumference whose radius length is the inverse of the maximum achievable resolution. Instead, in SIM, the formation of Moiré Fringes causes a shift of the frequency central zero in two new offsets for each orientation, increasing the observable region and thus accessing super-resolution, i.e. high-frequency, information, below the Diffraction Limit of Light (200-250 nm). As briefly introduced before, in this last 3rd year, I have worked on two main projects: the development and application of a new algorithm to analyze SIM data and the application of SIM to investigate oncogene-induced alteration in chromatin organization.

For the first project, I developed a new algorithm to reconstruct SIM data, based on the principle of Separation of Photons by Lifetime Tuning (SPLIT)[3,4]. The SPLIT algorithm can be applied to different Super-Resolution techniques. The super-resolution spatial information is encoded in an “additional channel” of the microscope. In SIM, this “additional channel” consists of the different information acquired thanks to the different illumination patterns. To reconstruct the final image, the algorithm analyzes the intensity at each pixel in relation to the phase illumination, in terms of phase and modulation. The algorithm then uses the information of phase and modulation in each pixel to construct the phasor plot from which it is possible to separate the super-resolved information from the diffraction-limited ones, in relation to their localization in the phasor plot. We tested our algorithm on MATLAB simulations, then on 100nm fluorescent beads samples, and then onto a biological sample. We performed some acquisitions of two color biological samples reconstructing the images with the SPLIT-SIM approach and we aim to reach 4 color acquisitions to study DNA-chromatin and chromatin-related structures. To validate multicolor reconstructions I’m working on analyzing two-color measurements of GATTAquant Nanorulers. These are designed samples whose fluorophores are precisely outdistanced. In our case, we choose to use 160nm nanoruler in which three fluorophores are 80nm far. During the lockdown, due to the Covid-19 pandemic, I focused my work on analyzing all the data previously acquired and I wrote and submitted my first manuscript. Now I’m finishing the nanoruler analysis and I plan to start writing my second manuscript about this work.

In the second project, I focused my attention on studying oncogene-induced alterations in chromatin organization. Oncogenes activation may cause genomic instability, one of the hallmarks of cancer, by altering fundamental processes such as the DNA Damage Response (DDR), DNA transcription, and DNA replication [5] and thus altering chromatin structure and organization.

To study this biological issue, I used an engineered cell line, U937-PR9, derived from Acute Promyelocytic Leukemia(APL) patients as a model of oncogene activation. In APL, 70% of the patients present a mutation that causes the formation of a fusion protein PML-RARalpha. This engineered cell line can be treated with a zinc solution that activates the transcription of the fusion protein, thus simulating the cancer cell situation. This cell line allows us to study DNA transcription and DNA replication both in the activated and inactivated samples. Having this comparison allows comparing the relative spatial distributions of replication and

transcription foci in the context of the expression of the oncoprotein. To quantify the colocalization I used two different analysis methods. One in an object-based algorithm available on ImageJ and the other one is an algorithm of Image Cross-Correlation Spectroscopy (ICCS) method.Gustaffson MGL., et al. Biophysical Journal (2008)

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

Oral Presentations

I. Cainero , E. Cerutti , S. Pelicci , M. Faretta , G.I. Dellino , P.G. Pelicci, A. Diaspro, L. Lanzanò. *"Structured Illumination microscopy as a tool to investigate oncogene-induced alterations in chromatin organization."* 64th Biophysical Society Meeting, San Diego (February 15th-19th, 2020).

I. Cainero, A. Diaspro, L. Lanzanò. *"SPLIT-SIM: an innovative approach to analyze Structured Illumination Microscopy (SIM) data"*. FOM, Osaka (April 5th-9th, 2020). Conference canceled due to Covid-19 Pandemic.

Poster Presentations

I. Cainero, E. Cerutti, A. Diaspro, L. Lanzanò. **"***IMAGE reconstruction in structured illumination microscopy (SIM) by SPLIT-SIM".* 106° Congresso della Società Italiana di Fisica, (September 14th-18th, 2020)

Publications

I. **Cainero**, E. Cerutti, S. Pelicci, M. Faretta, G.I. Dellino, P.G. Pelicci, A. Diaspro, L. Lanzano. *"Structured Illumination Microscopy as a Tool to Investigate Oncogene-Induced Alterations in Chromatin Organization"*. Biophysical Journal 118 (3), 166a-167°
II. M. Oneto, L. Scipioni, M. Sarmento, **I. Cainero**, E. Cerutti, S.Pelicci, L. Furia, P.G. Pelicci, G.I. Dellino, P. Bianchini, M. Faretta, E. Gratton, A. Diaspro, L. Lanzano. "*Nanoscale Distribution of Nuclear Sites Analyzed by Superresolution STED Image Cross-Correlation Spectroscopy"*. Biophysical Journal 118 (3), 20a
III. A. Trianni, N. Anthony, **I. Cainero**, A. Diaspro. *"SIM-Enhanced Ptychography Imaging of Hela Cells"*. Biophysical Journal 118 (3), 312°
IV. E. Cerutti, **I. Cainero**, G.I. Dellino, M. Faretta, P.G. Pelicci, A. Diaspro, L. Lanzano. *"An Image-Based Approach to the Evaluation of Oncogene Activation Effects on Cell's Genomic Stability"*. Biophysical Journal 118 (3), 65a

Submitted Paper

I. Cainero, E. Cerutti, M. Faretta, G.I. Dellino, P.G. Pelicci, A. Diaspro, L. Lanzanò..*"Decoding sub-diffraction spatial information in structured illumination microscopy by the SPLIT method.*"

Paper in preparation

I. Cainero et al. "*Measuring nanoscale distances by multicolor structured illumination microscopy*"