



Third Year Annual Report



Candidate: Riccardo Marongiu

Tutor: Aymeric Le Gratiet

Supervisor: Alberto Diaspro

Work:

This year I have continued my work on polarization based techniques to study biological structures and in particular the organization of chromatin-DNA. My attention has been divided on different setups, belonging to two main parallel projects of my PhD work.

The first project is the continuation of my previous work on polarization based label-free microscopy and has been divided on two different setups. One setup is the Circular Intensity Differential Scattering custom microscope that we have built in the first year, starting from a commercial Nikon confocal fluorescence microscope. Due to a relocation of the laboratory where I work, the setup has been moved and I contributed to operations to rebuild it, after which I have collected more data. The setup has been mounted in a completely custom configuration without the use of the previous commercial microscope body and is destined to house multiple imaging techniques at the same time, in what is to become a setup that is to be named Multimessenger Microscope. Currently, the supported techniques are CIDS and fluorescence, but more (for example Second Harmonic Generation Microscopy, STED, phase imaging etc.) are planned to be added with time. I have performed multiple experiments to characterize the performance of the new setup, including the polarization analysis, the optical alignment and finding the source of systematic errors. I have also applied new analysis techniques to old unpublished data, making progress in creating a completely automated data treatment protocol with the use of ImageJ[®] macros.

The second setup of the first project is a complete Mueller Matrix microscope. I have performed some experiments by imaging HEK and HeLa cells, obtaining a complete Mueller matrix image with heavy magnification (up to 60x objective) on biological samples, something rarely attempted before. I have also imaged cancer cell spheroids with this modality, with the future intent to cut them in slices to image individually to reconstruct a 3D polarization map of the sample.

Finally, my minor project regards the use of Polarization-Resolved Light Scattering Spectroscopy (pLSS) to study chromatin-DNA organization. I built a custom setup that allows for fourier imaging by scattering and tested its functionality by using grid and microspheres samples and by comparing the results with theory. The objective of this setup is to integrate polarization control to measure CIDS signal and take advantage of the scattering configuration to extract more precise angular information from cells and cell nuclei and compare them with non-chiral samples like microspheres.

Exams:

I have already completed all my required exams in the previous years.

Conferences attended:

I attended the Biophysical Society Annual Meeting in Baltimore with a poster presentation

- 64th Biophysical Society Annual Meeting – San Diego, California (USA), 15-19 February 2020
Poster: Polarization-Resolved Light Scattering Spectroscopy (pLSS) to study chromatin-DNA organization, **R. Marongiu**, A. Le Gratiet, M. W. Ashraf, A. Diaspro

I also attended the following online (due to coronavirus concerns) conferences with oral presentations:

- Italian Conference on Optics and Photonics (ICOP) 2020 – Online, 8-11 September 2020
Oral: A Combined Expansion Microscopy And CIDS Approach To Chromatin DNA Study, **R. Marongiu**, A. Le Gratiet, L. Pesce, P. Bianchini, A. Diaspro
- 106° Congresso Nazionale della Società Italiana di Fisica (SIF) – Online, 14-18 September 2020
Oral: ExCIDS: improvement of CIDS imaging of DNA organization by Expansion Microscopy coupling, R. Marongiu, A. Le Gratiet, L. Pesce, P. Bianchini, A. Diaspro

Articles published:

- “Zebrafish Structural Development In Mueller-Matrix Scanning Microscopy” – A. Le Gratiet, M. d’Amora, M. Duocastella, **R. Marongiu**, A. Bendandi, S. Giordani, P. Bianchini & A. Diaspro, Scientific Reports (2019).
- “Excids: A Combined Approach Coupling Expansion Microscopy (Exm) And Circular Intensity Differential Scattering (CIDS) For Chromatin-DNA Imaging” – **R. Marongiu**, A. le Gratiet, L. Pesce, P. Bianchini and A. Diaspro, OSA Continuum (2020).
- “Circular Intensity Differential Scattering (CIDS) for label-free chromatin-DNA characterization: a review for optical microscopy”, A. Le Gratiet, **R. Marongiu**, A. Diaspro, Polymers (2020) - under review.
- “Phasor approach of Mueller matrix optical scanning microscopy for label-free biological tissue orientation imaging”, A. Le Gratiet, L. Lanzano, R. Marongiu, A. Bendandi, P. Bianchini, C. Sheppard, A. Diaspro, Biophysical journal (2020) - under review.