Candidate: Riccardo Marongiu

Exams given:

During the second year, I have passed the last two exams of my Study Plan (after passing the previous four last years):

- Statistica per Analisi Dati (Prof. Passaggio, Prof. Parodi)
- Tecniche Microscopiche e Spettroscopiche per l'analisi di Superfici e Interfacce (Prof. Savio, Prof. Buzio, Prof. Gerbi)

Schools and Workshops:

I have followed a very interesting Workshop on fluorescence techniques for microscopy and spectroscopy on biological samples:

- 6th European Workshop on Advanced Fluorescence Methods – Munich, 10-14/12/2018

Conferences attended:

I attended the Biophysical Society Annual Meeting in Baltimore, where I presented a poster for which I won the Student Research Achievement Award (SRAA)

- Biophysical Society Annual Meeting – Baltimore, Maryland (USA), 2-6 March 2019
- **Poster**: Label-free Chromatin-DNA imaging by circular polarized light scattering scanning microscopy, A. Le Gratiet, **R. Marongiu**, L. Pesce, M. Oneto, G. Zanini, P. Bianchini, A. Diaspro

Articles published:

- "Circular intensity differential scattering (CIDS) scanning microscopy to image chromatin-DNA nuclear organization" - A. Le Gratiet, L. Pesce, M. Oneto, **R. Marongiu**, G. Zanini, P. Bianchini and A. Diaspro, OSA Continuum (2018)
- ExCIDS: a combined approach coupling Expansion Microscopy (ExM) and Circular Intensity Differential Scattering (CIDS) for chromatin-DNA imaging – A. Le Gratiet, **R. Marongiu**, L. Pesce, P. Bianchini and A. Diaspro (ACS Photonics, under review)

Work:

My work this year has been focused on the continuation of my project about development and applications of polarized light microscopy setups to study chromatin conformation in a label-free way. In biophysics, the most common imaging approach is fluorescence microscopy, which consists in marking the target with fluorescent tag molecules then excited with light of an appropriate wavelength and observed at the fluorescence wavelength. This approach has a few problems however, including the required work and time required to properly mark the sample and the possible perturbations of the molecular processes. For this reason, label-free microscopy is an alternative easy and cheap method that reduces the operator dependence and doesn't require a priori knowledge. The method we chose is based on polarized light control, utilizing the Mueller Matrix formalism. In particular we have focused on circularly polarized light (right and left), well-known to present a high sensitivity to chiral molecular organization (a phenomenon called Circular Intensity Differential Scattering, CIDS), like the ones present in chromatin-DNA. Thanks to the use of a Photoelastic Modulator (PEM), we were able to quickly generate all polarization states (including the circular ones) to shine on the sample at 50 kHz without moving parts. This allowed us to be fast enough to couple the polarization control with the speed of a scanning microscope. A lock-in detector is then used to recover the signal at 50 kHz, deriving only from circular polarization.

In the first year of my PhD, the CIDS microscopy setup was built and I performed experiments to validate it on isolated cellular nuclei, resulting in a peer-reviewed journal article. This year, I started by combining the technique with expansion microscopy. Expansion microscopy consists in a method to enhance resolution by simply expanding the features of the sample to observe. The sample is encased in a gel that is then put into water, which it absorbs, expanding. The sample, cross linked with the gel structure, expands in turn. The expansion process also requires a first step of digestion of the lipids and other biological material present in the sample before the expansion. The coupling with our technique leads to an improvement of the quality of the signal due to the digestion process eliminating the superfluous biological material, as well as the improvement of resolution due to the expansion. An article featuring these experiments and results has been written and is currently under review on ACS Photonics. Preliminary results have also been collected on nuclei of cells affected by progeria, an illness that causes a change in nuclear conformation, with the objective of identifying changes in the conformation of chromatin in different healthy and pathological states.

Finally, in this year I have personally participated in building a new microscopic and spectroscopic setup for the study of light scattering in biological samples with polarized light. I have taken part both in the design and the actual construction of this setup, and I have performed some validation experiments using control samples. The setup presents a diode laser source at 633nm wavelength. The beam is expanded in a beam expander to fulfill the objective and then passes through a Polarization Stage Generation (PSG), composed of a linear polarizer and a $\lambda/4$ waveplate, to generate the circular polarization states. After this, the light reaches the objective by which it's focused on the sample and collected by the condenser in direct transmission configuration. The camera is put at the right distance from the subsequent lenses to image the fourier plane. In this way we have an angular image in Θ and ϕ of the scattered light from the sample.

First, a diffraction grid array was used, and in a second step we utilized silica microspheres. I compared the results of the experiments with theoretical simulations, finding a good accordance. The next step will

be to study nuclei samples and find their polarimetric signature when compared with microspheres, with the objective to eventually distinguish different types of nuclei by their polarized light scattering signature.