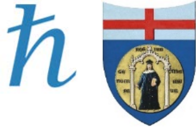

Third Year PhD in Physics and Bio-Nanoscience Report

Student: **Behjat S. Kariman**

Cycle: **XXXIV**

Tutor(s): **Prof. Alberto Diaspro and Dr. Paolo Bianchini**

Year: **2021**

1 Research Summary: Project and PhD abroad

1.1 Project: Non-Linear Optical Process for Label-Free Microscope

The growing demand for multimodal label-free imaging with a fast frame rate (>1 frame/s) and sub-micrometre spatial resolution in biological and chemistry application, calls for the development of a microscope with novel approaches that can enable fast, multi-contrast label-free, and noninvasive imaging. Today, a non-linear optical microscope can be a solution because it offers a multimodal multicontrast approach although the assemble and optimization of the optical setup remain a challenge. Among non-linear microscopy techniques, pump-probe microscopy can be considered a label-free and multicontrast imaging. In pump-probe microscopy, applying two pulsed laser beams with different frequencies enables exploring different non-linear processes through one platform, including sum-frequency generation, second-harmonic generation, multi-photon, and stimulated Raman scattering. We have constructed a multimodal microscope that allows for simultaneous image acquisition from multiple optical imaging modalities. The microscope consists primarily of pump-probe (PP), multi-photon (MP), sum-frequency generation (SFG), second harmonic generation (SHG), and saturated pump-probe (SPP) microscopy. The unique configuration of the integrated microscope allows for the acquisition of both scattered and transient absorption-based imaging information with particular emphasis in the fields of label-free super-resolution imaging through applying saturation absorption. During the third year of my PhD, I focused on the optimization of such custom-built femtosecond-pulsed near-infrared pump-probe microscope for imaging biological and material specimens. Specifically, I used the following contrast mechanisms in a multimodal simultaneous approach: autofluorescence, SFG, SHG, and pump-probe. I applied the imaging methods at different biological targets, i.e., collagen, myosin, and cells. In addition, I modified the setup to collect also stimulated-Raman scattering from tendon, and I studied how the effinciency changes at varying the pairs of wavelength and Raman shifts. Furthermore, I explored saturation of transient absorption process using a doughnut-shaped beam, in a STED-like approach, in the imaging of single-layer Graphene (SLG) and Polycarbonate (Pellet, Microplastic, Nanoparticles). The results showed that it is possible to obtain label-free super-resolution imaging of SLG and polycarbonate. Finally, I compared experimental findings with simulations to investigate and model the SLG states while it undergos saturation.

1.2 PhD abroad: Multi-plane phase retrieval, super-resolution optical fluctuation, and SRS imagings

In collaboration with the Laboratory of Nanoscale Biology (LBEN) EPFL directed by Prof. Radenovic

As the aim of my PhD research was multimodal imaging to reach label-free super-resolution. I studied label-free white light quantitative phase imaging with fluorescence to provide high-speed imaging and spatial super-resolution. I realized both phase imaging and super-resolution fluctuation imaging (SOFI) of fixed HeLa and HEK cells by a PRISM multi-plane custom optical microscope made in the nanoscale biology (LBEN) laboratory at EPFL. This microscope allowed me to collect 3D phase images and integrate SOFI imaging while acquiring eight plane images simultaneously. PRISM splits and directs the light or fluorescent signals into eight distinct images to perform fast 3D image acquisition. We sequentially retrieved the 3D quantitative phase from a stack of bright-field images using Fourier filtering and imaging cell samples with 3D SOFI. In this investigation, I have made progress in the following research challenges:

- I studied multi-plane 2D SOFI and phase imaging of HeLa cell labelled Vimentin using the fluorescent protein Dreiklang. The high-resolution 3D phase image was recovered from a stack of 50 bright-field images acquired with a 20 ms exposure time of the camera and displacement step of 200 nm.
- I investigated 3D-phase retrieval of unlabeled HEK 293 cells and then calculated the refractive index changes as a function of the quantitative phase.
- I have studied wide-field, bright-field, and phase imaging for Httex1-72Q and Httex1-72Q-GFP(HEK 293 cells expressing exon 1 of the Huntingtin protein with a polyQ repeat length of 72, fused to GFP).

The future plan is to apply stimulated-Raman scattering imaging at these samples to improve information content through multimodal imaging.

2 List of Publications and Conferences

[1] B. S. Kariman, G.Zanini, A. Diaspro, and Paolo Bianchini, " Pump-Probe Nanoscopy: Principle, implementation and application in Label-Free Imaging," in the proceeding.

[2] B. S. Kariman, M. Scotto, A. Diaspro, and Paolo Bianchini, " Multimodal and Label-Free Super-Resolution Imaging By means of Pump-Probe Microscopy," **ELMI** conference 23rd June 2021.
Oral presentation. Online
DOI http://dx.doi.org/10.22443/rms.elmi2021.55

[3] B. S. Kariman, T. Deguchi , M. Scotto, G.Zanini, A. Diaspro, and Paolo Bianchini, " Multimodal and Label-Free Super-Resolution Imaging," **FOM** conference 31st March 2021.
Oral presentation. Online

[4] B. S. Kariman, T. Deguchi , M. Scotto, G.Zanini, A. Diaspro, and Paolo Bianchini, " Label-Free Super-Resolution Microscopy By Means of Transient Absorption Saturation," Biophysical Journal, Vol.120(3), p.181 a, 2021.
DOI:10.1016/j.bpj.2020.11.1259

3 PhD abroad period

During the third year of my PhD, I have continued my research and activities in the context of label-free super-resolution imaging approach in the Laboratory of Nanoscale Biology (LBEN) is directed by Prof. Radenovic at EPFL, Laussane. (Jun-Aug 2021)