Dipartimento di Fisica
Università degli Studi di Genova

ISTITUTO ITALIANO
DI TECNOLOGIA

PhD program in Physics and Bio-Nanoscience

Cycle: XXXV

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Third year PhD Annual Report

Introduction

In order to take the advantage of various optical resolution and image enhancement schemes, a versatile approach is to use optical scanning microscope by scanning a diffraction-limited spot of light relative to a specimen in a raster way so that the image is built up point by point. To enhance contrasts discerning polarimetric characteristics of anisotropic structures and chiral organization of macromolecules, conventional microscopes can be equipped with additional features that permit observation under polarized light. The idea behind this study is to add up polarimetric techniques and data analysis for yielding polarization based contrast out of nonlinear phenomena to enhance image data, and discriminate anomalies from control organelle species. Here we propose a polarization-resolved fluorescence imaging for a better understanding of the bio-system.

I aligned and implemented a homemade two-photon excitation fluorescence (TPEF) microscopy setup aiming to quantitatively investigate the polarimetric anisotropy of sample using linear polarizer (LP), Half Wave-Plate (HWP) and Quarter Wave-Plate (QWP) as polarization state generator (PSG) to generate right circular polarized input light in order to illuminate the sample in a non-preferential direction. I also substituted CP with a rotating LP light to excite in different directions to compare the image results. A rotational LP is then located before the detectors to decode the anisotropic nonlinear signal at different angles as polarization state analyzer (PSA) in reflective configuration. In this way, nonlinear signal detected based on polarization resolved images using specified band pass filter and PMT in non-descanned way. The sample is scanned by right circular polarized light (RCP) and the anisotropic emission of the non-linear signal captured from a scanned image (average depending on signal to noise ratio) angle-by-angle. Consequently, at each orienting angle of rotating LP, the signal measurement is performed pixel-by-pixel to form an image. Accordingly, the output of the measurements is a stack of polarization-resolved images at different decoded angles. Therefore, we are able to trace the polarization intensity nonlinear signal in terms of rotating LP (angles θ) pixel-by-pixel. In this way, I convert the collected polarization-resolved images into phasor map. After calibration of the setup and PSF measurements, I examined colorectal cancer cells in 2D spheroid and tissue to determine biomarkers based on polarization mechanism at sub-cellular

level to provide a preliminary diagnosis under phasor map. The results compared to that of normal healthy colon cells in tissue (Fig 1). This work has been a part of my PhD project as invited research in the biophotonic imaging group in department of health technology at Technical University of Denmark (DTU).

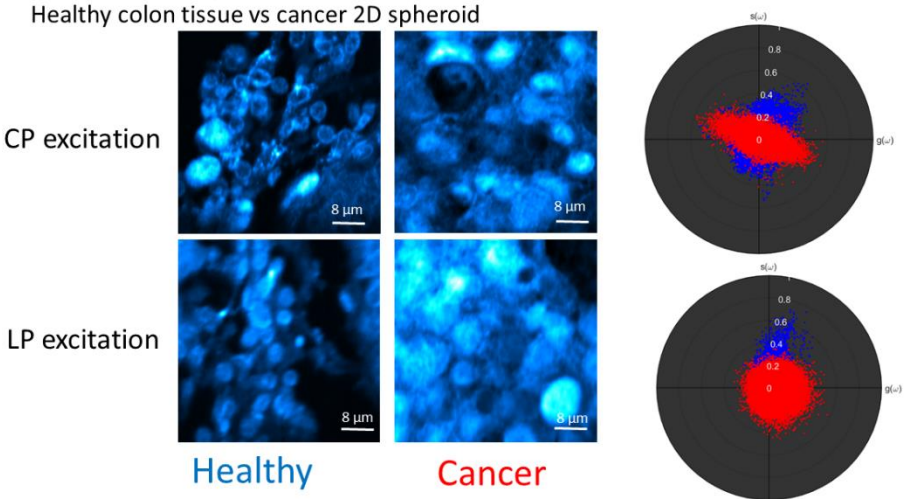

Figure 1. Colorectal cancer cells in 2D spheroid compared to healthy cells in tissue using linear and circular polarized light for excitation and corresponding phasor map analysis.

The outcome of my work is being written as a manuscript to be submitted entitled: "*Phasor map analysis of polarimetric multiphoton laser scanning microscopy of colon cancer cells".*

Featured publications:

Ali Mohebi, Aymeric Le Gratiet, Michele Oneto, Fabio Callegari, Paolo Bianchini and Alberto Diaspro, "***Cellular identification between different cell types using Circular Intensity Differential Scattering Microscopy Imaging***," Under review.

Ali Mohebi, Aymeric Le Gratiet, Alberta Trianni, Fabio Callegari, Paolo Bianchini and Alberto Diaspro, "***Phasor map analysis to investigate Hutchinson-Gilford progeria cell under polarization-resolved optical scanning microscopy***," Scientific Reports, Vol. 12(1679), DOI: 10.1038/s41598-022-05755-1, January 2022.

Ali Mohebi, Aymeric Le Gratiet, Fabio Callegari, Paolo Bianchini and Alberto Diaspro, "***Multimodal polarization-resolved/ uorescence optical scanning microscopy for chromatin organization imaging***," Proc. (ECBO) OSA-SPIE Vol. 11922, Advances in Microscopic Imaging III; 119220V, DOI: 10.1117/12.2615731, December 2021.

Schools and conferences attended:

BPS 2022, 66th Biophysical Society Annual Meeting, 22-26 February 2022; online

Image phasor analysis in polarization-resolved optical scanning microscopy of neuroblastoma cells

Focus On Microscopy (FOM 2022), Image Analysis, 10 - 13 April 2022; online

Phasor map analysis in polarization-resolved optical scanning microscopy of Hutchinson-Gilford progeria cell

The 10th International Graduate summer school Biophotonics '22 in Ven Iland, Sweden, 11-18 Jun 2022. ; on-site in Sweden

Oral presentation; "Phasor analysis in circular polarization-resolved optical scanning microscopy for biological organization imaging"