PhD Program in Physics and Bio-Science

Student: Alberta Trianni
Cycle: XXXIV
Tutor(s): Alberto Diaspro, Nicholas Anthony

ANNUAL REPORT

1. Introduction

This PhD project regards the combination of ptychography (a label-free approach to study biological processes without the need of staining) and structured illumination microscopy (SIM). Ptychography is part of Quantitative Phase Microscopy technique, this means that it is able to quantify the phase shift that occurs when the light passes through a more optically dense object. It s a computational approach to phase microscopy, which provides mathematically derived information about a specimens phase-modulating characteristics. The phase map obtained with ptychography contains information about cell thickness and refractive index and can allow quantification of cellular morphology[1]. To retrieve the refractive index distribution (Δn) and thickness (d) of a sample the experimental phase (θ) and wavelength (λ) are used, following the equation:

$$d\Delta n = \frac{\theta\lambda}{2\pi}$$

If the thickness of the sample is known, then Δn is calculable[2]. The SIM-Ptychography correlative approach will be used to study, with high resolution, the refractive index distribution inside the distorted nuclear membrane in Hutchinson–Gilford progeria syndrome (HGPS) cells compared to normal cells.

2. Research Activity

During the second year, the main goal was to mount the ptychography setup onto a Nikon SIM microscope and measure the refractive index distribution of the nuclear membrane of HGPS cells compared to normal cells both with this correlative approach, and with conventional ptychography, to demonstrate the achievable resolution increase. The first step was to verify the effective possibility of mounting the setup on SIM, so I built and then tested a proper structure to support it.

To improve the frequency content of the image, a widespread solution consists of illuminating the sample with a non-uniform light pattern, like the striped pattern of SIM. However, various structured pattern can be realized, such as random speckles. To test the effectiveness of a random pattern I detected a wide field image of a random speckled probe made with several layers of scotch

tape. After that I run some simulation on Python to test which probe gives the best reconstruction accuracy, using three different probes: a normal Gaussian beam, the experimental random speckle pattern and the structured illumination pattern of the SIM. The results indicated that the SIM pattern an improvement in resolution can be appreciated[3].

With the aim to see more details of cellular structures, and since during my first year I performed measurements using a low magnification objective (20X), I performed several optical ptychography measurements with higher magnification objectives (60X and 100X) on different test samples, such as polystyrene beads and vegetable cells. Polystyrene beads (with a diameter of 4μm) represent uniform phase samples and have a known and constant refractive index. Vegetable cells (in this case I used onion epidermis cells) are much bigger than beads (width = 250μm, height = 50μm, thickness = 50μm) and mainly full of water but exhibit a good phase shift the same, due to specific cellular structure such as the cell wall, vacuoles, nucleus. In addition, the auto fluorescence signal of these cells has been detected and qualitatively correlated with the phase images. I started also to work on the preparation of samples such as HeLa cells transfected with progeria plasmid and HeLa cells transfected with a plasmid encoding for the stained wild type protein which is mutated in progeria cells. I started the imaging of normal HeLa cells with conventional ptychography, comparing the label-free images with fluorescence images (the samples were stained using a Hoechst for the nucleus and CellMask for the membrane). I observed that HeLa cells have a very low phase signal, so more test are required (ongoing experiment).

3. Exams

- Tecniche microscopiche e spettroscopiche per l'analisi di superfici e interfacce (Buzio, Gerbi, Savio)
- Report su ESRIC Super Resolution Summer School, Heriot-Watt University, Edinburgh, 15-19 July 2019
- Metodi ottici e spettroscopici per lo studio dei materiali (Maurizio Canepa)

4. Schools and Conferences

- Biophysical Society 64th Annual Meeting, San Diego, California, 15-19 February 2020

5. Posters

- A. Trianni, N. Anthony, I. Cainero, A. Diaspro, "*SIM-Enhanced Ptychography Imaging of HeLa Cells*", Biophysical Society 64th Annual Meeting, San Diego, California, 15-19 February 2020

References

1. Curl, C. L. *et al.* Quantitative phase microscopy: a new tool for investigating the structure and function of unstained live cells. *Clinical and experimental pharmacology & physiology* **31**, 896–901 (2004).
2. Anthony, N., Trianni, A., Bianchini, P., & Diaspro, A. *Label Free Quantitatitve Phase Imaging of Cellular Structures*. Biophysical Journal, 118(3), 136a (2020).
3. Trianni, A., Anthony, N., Cainero, I., & Diaspro, A. *SIM-Enhanced Ptychography Imaging of Hela Cells*. Biophysical Journal, 118(3), 312a (2020).