# PhD Program in Physics and Bio-Science

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**Cycle: XXXIV**
**Tutor(s): Alberto Diaspro, Nicholas Anthony**

## ANNUAL REPORT

## 1. Research Activity

This PhD project regards the implementation and application of ptychography as a label-free approach to study biological processes without the need of staining. The imaging of biological samples is commonly performed using fluorescent labels that offer molecular specificity and high-contrast. However, the labeling procedure is not always compatible with live-cell imaging due to fixation, photobleaching and phototoxicity effects[1]. For these reasons several optical contrast-enhancing imaging techniques have been developed under the name of label-free techniques. Phase Contrast techniques (PC) in particular provide contrast by measuring the phase shift of light caused by cells and intracellular features. One such method is ptychography, a quantitative phase imaging technique that uses an iterative algorithm to recover the amplitude and phase values of the sample and the probe. In this technique a coherent illuminating beam (probe) and the specimen are moved with respect to one another following a sequential or random array of overlapping illuminated areas. For each illuminated area, the light transmitted from the sample is captured as a diffraction pattern on the detector. The diffraction patterns are then processed using a ptychographic algorithm; the most common is the extended ptychographic iterative engine (ePIE).

During the 1st year of my PhD, my activity has focused on the study of the theory of ptychography. For this work, we used a modular setup that follows the "microscope approach"[2], that can be easily attached onto other microscopes. I used the setup mounted onto a Nikon A1 confocal laser microscope and I've tested the sensitivity of the technique using polystyrene beads samples of different diameter (from 15 to 1 micron) that represent uniform phase samples and have a known and constant refractive index. After that I tested some biological samples like fixed cheek and HeLa cells. We observed that cheek cells experience a larger phase shift than HeLa cells due to impurities within the sample.

Since one of the limitations of ptychography is the low achievable resolution, one of the goals of this project is to increase the lateral resolution by coupling this technique with structured illumination microscopy (SIM). The use of structured illumination allows high-frequency information to be retrieved by modulating diffraction patterns into the low-frequency range, thus achieving a higher resolution. For this reason in the second part of this year I studied the theory of SIM and I learned how to use the instrument and to prepare HeLa cells samples stained with Hoechst (for the nucleus) and CellMask (for the plasma membrane).

During the next year the main goal will be to focus on the combination of SIM and ptychography to increase the lateral resolution, and to test the technique on different kinds of fixed samples (animal and vegetal cells). The next step will be to use this approach to study phase-sensitive biological processes like mitosis and apoptosis on live samples.

## 2. Courses

- Advanced Optical Fluorescence Microscopy Methods (Luca Lanzanò, Paolo Bianchini)
- Metodi ottici e spettroscopici per lo studio dei materiali (Maurizio Canepa)
- The optical microscope: optics, image formation and resolution (Alberto Diaspro)
- Tecniche microscopiche e spettroscopiche per l'analisi di superfici e interfacce (Buzio, Gerbi, Savio)
- Elettronica e Acquisizione Dati (Fontanelli, Musico)

## 3. Exams

- Elettronica e Acquisizione Dati
- Advanced Optical Fluorescence Microscopy Methods
- The optical microscope: optics, image formation and resolution

## 4. Schools and Conferences

- International School on Nanoscale Optical Microscopy, Palazzo Loredan, Venice, 11-14 June 2019
- ESRIC Super Resolution Summer School, Heriot-Watt University, Edinburgh, 15-19 July 2019

## 5. Posters

- A. Trianni, N. Anthony, I. Cainero, P. Bianchini, A. Diaspro, "*Label Free Imaging with Super-Resolved Ptychography*", International School on Nanoscale Optical Microscopy, Palazzo Loredan, Venice, 11-14 June 2019

# References


1. Kasprowicz, R., Suman, R. & O'Toole, P. Characterising live cell behaviour: Traditional label-free and quantitative phase imaging approaches. *Int. J. Biochem. Cell Biol.* **84**, 89–95 (2017).
2. Li, P. & Maiden, A. M. Ten implementations of ptychography. *J. Microsc.* **269**, 187–194 (2018).