2020
2021

Annual PhD Report
PhD Course in Physics and Nanosciences
Curriculum Bio-Nanosciences

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Research Activity

I am carrying out my PhD research activity in the Nanoscopy & NIC Lab @ IIT and my research project concerns the development of light sources whose properties are tailored to the requirements of optical microscopy. This work is being developed in the context of the MOMIX project, consisting in the design and realization of a versatile multi-modal optical microscopy platform, having tunable contrast mechanism and performances both based on fluorescence and label-free approaches. Currently, I am dealing with a label-free microscopy method based on the generation and the analysis of polarized light, called Mueller Matrix Microscopy. Label-free contrast is obtained thanks to the great sensitivity of the polarization state to morphological properties of specimens, such as size, orientation, handedness and shape. The generation of different states of polarized light is mandatory to study the complete polarimetric signature of samples that can be summarized by a single 4x4 Mueller-matrix. Each element of such matrix is associated to different physical properties, such as dichroism or retardance, with the respect of both linearly and circularly polarized light. When dealing with Laser Scanning Microscopy, a good matching between the rate of the polarization generation and the pixel-dwell time during the scanning has to be achieved. So, I worked on a technique relying on the heterodyne detection of the dual-frequency, dual-polarization output of a Zeeman Laser. I developed a custom Zeeman Laser based on a Helium-Neon Laser tube: the custom light source requires an external magnetic field, provided by some magnetic bars, and a stabilization electronics with feedback action on the light source. Once the Zeeman Laser has been assembled and stabilized, it is able to emit two slightly separated longitudinal modes, whose distance in frequency is around 700 kHz, having Orthogonal Circular Polarization (OCP) states. The imaging technique exploits this output to detect the polarimetric properties of specimens. When the orthogonality between the two component emitted by the ZL is broken, as it may happen when the light travels through a linear polarizer, a beating signal coming from their heterodyne interference arise. From its analysis, it is possible to have access to six Mueller coefficients. This technique has been firstly validated through the characterizations of reference polarization optics. Then, the ZL source has been integrated in a Laser Scanning Microscope that is shown in Fig.1, and images of biological samples has been acquired.

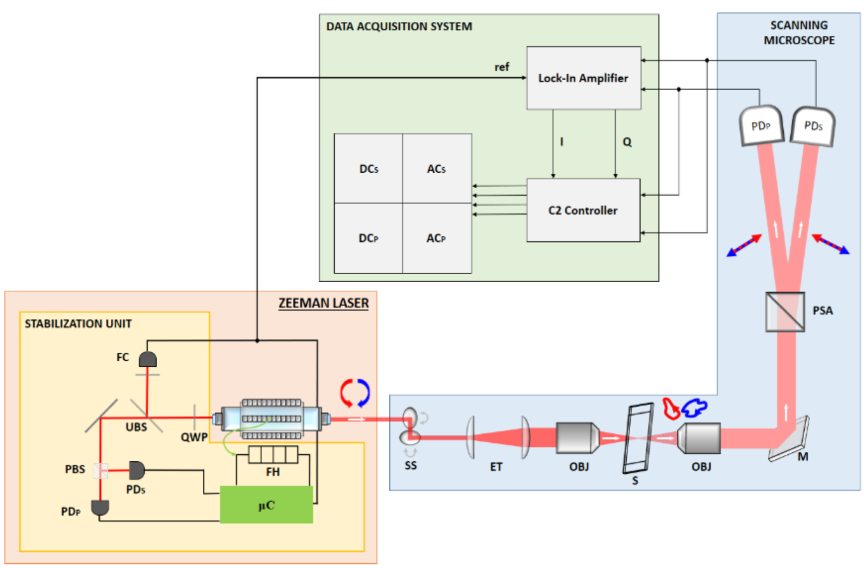

Figure 1 - The white arrows represent the direction of the beam, whereas the red and blue arrows are describing the polarization states. The Zeeman Laser needs a Stabilization Unit (SU) made up of Polarizing and Unpolarizing Beam Splitters (PBS/UBS), a Quarter-Wave Plate (QWP), a Linear Polarizer (LP) and three photodiodes, a foil heater (FH) and a microcontroller (uC). Then, the main output of the ZL travels through a Scanning Stage (SS), an Expanding Telescope (ET), excitation and detection objectives (obj) in between which the sample (S) is placed. Finally, the polarization is split by the Wollaston Prism acting as PSA and detected by means of two fast photodiodes, linked to a Lock-In Amplifier and to the Scanning Stage Controller, providing the images of both the continuos (DC) and oscillating (AC) components of the heterodyne signals.

Next steps in the development of this microscopy setup are the optimization of the current imaging performances and rigorous studies on biological specimens by using this label-free technique. Finally, I'm planning to extend the imaging capabilities to other Mueller Matrix elements by means of birefringent wave-plate that will be used to modify the output of the ZL from OCP to Orthogonal Linearly Polarized (OLP). Meanwhile also the analysis stage (PSA) can be further improved, in order to build a setup able to reconstruct a full Mueller Matrix of a sample without the need for additional active stages beyond the laser source itself.

Courses and Exams

2nd Year	**Course**	**Teacher**	**Hours**	**Department**	**State**
4-5	Ottica Applicata	L. Repetto	~ 60	LM - DIFI	DONE
6	OSA Innovation School	OSA	> 30 h	OSA	DONE
extra	Hybrid microfluidics systems for electronics, photonics, and sensors	S. Surdo	9 h	DIBRIS	DONE

Publications

- A. Le Gratiet, A. Mohebi, F. Callegari, P. Bianchini, A. Diaspro, *"Review on Complete Mueller Matrix Optical Scanning Microscopy Imaging"*, Appl. Sci. 2021, 11, 1632

- A. Mohebi, A. Le Gratiet, R. Marongiu, F. Callegari, P. Bianchini, A. Diaspro, *"Combined approach using circular intensity differential scattering microscopy under phasor map data analysis"* Appl. Opt. 60, 1558-1565 (2021)

Conferences and Workshops

- Focus On Microscopy 2021 (FOM 2021, March 28-31)
  I presented a poster presentation on my research project titled *"Application of He-Ne Zeeman Laser in polarization-based characterization of chiral structures"*

- Industrial Problem Solving with Physics @ Università di Trento (IPSP 2021, July 19-24)
  I participated to an intensive five days hackathon aimed to solve physics-related problems presented by companies in the context of their work.

- Società Italiana di Fisica Congress 2021 (SIF, September 13-17)
  I'll join this congress and I'll send a 10 min. video-slide communication on my current research work titled *"Label-free microscopy enhanced by the polarization emission of a Zeeman laser"*

- Frontiers in Optics and Laser Science 2021 (FiO+LS 2021, 31 October – 04 November)
  I'll present a poster describing my research project on *"Polarization label-free microscopy imaging of biological samples by exploiting the Zeeman Laser emission"*