PhD program in Physics and Bio-Nanoscience

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Cycle: **XXXIV**

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REPORT OF FIRST YEAR PhD

1. RESEARCH ACTIVITY

1.1 State of the art and innovation:

Non-linear optical microscopy offers a series of advantages over linear optical microscopy. Non-linear optical processes have opened new avenues in biology and life sciences for development of new super- resolved microscopy techniques. In the last two decades, non-linear optical microscopy has been established as the new paradigm of optical 3D-vivo imaging, e.g., the use of near infrared radiation reduces the scattering, enables high penetration depth and limit the aberrations introduced by the sample [1-2]. It has been mostly based on fluorescence also because of the parallel development of Fluorescent Proteins. However, there is a need for innovative contrast mechanism to study non-fluorescent samples, for instance exploiting transient absorption. In such case, the combination of excited state absorption with ground state depletion saturation and differential techniques can help in reaching high- and super-resolution label-free microscopy [3-4]. The perspective of this project is to study how to design and develop a multimode microscope by exploring non-linear processes and exploiting their features to reach super-resolution.

Pump-probe: one type of non-linear process in light-matter interaction used in spectroscopy and microscopy is transient absorption, the associated methods are known as pump-probe. Pump-Probe is an absorption-based technique that provides contrast mechanisms observing the changes occurring to a probe beam after a pump beam has interacted with the sample [5]. Since this technique does not require observing any emission from the specimen, fluorescence is not necessary.

Stimulated Raman Scattering (SRS): In 1962, for the first time E. J. Woodby and W. K. Ng observed SRS when they were introducing a nitrobenzene cell within a ruby laser cavity that they realized. SRS is a Coherent Raman Scattering technique based on the inelastic scattering of photons by molecular vibrations [6].The difference between Coherent Raman Scattering technique (CARS) and SRS are in the number and location of intermediate levels and in the detected signal. SRS is obtained when the intermediate levels are degenerate, and as a result the pump beam experiences an intensity loss (stimulated raman loss, SRL) while the stokes beam experiences an

intensity gain (stimulated raman gain, SRG) [7]. SRS microscopy has powerful potentials for its high sensitivity and high contrast due to the reduction of non-resonant background.

Photoacoustic: The production of acoustic waves by a source of light was first demonstrated by A.G. Bell in 1880, which came to be known as photoacoustic or optoacoustic effect. The process consists in the formation of a sound wave following the absorption of light in a material sample. Both photoacoustic and optoacoustic effects involve the formation of mechanical waves [8]. Photoacoustic imaging has experienced exponential growth over the past decade, with many applications in biomedicine [9].

1.2 Scientific and Technological Objectives:

This project envisions the integration and combination of microscopy methods based on near-infrared light and non-linear interaction, i.e., pump-probe, SRS and Photoacoustic methods. Since, different imaging modalities extract complementary morphological functional and molecular information, multimodal-imaging technology can provide a comprehensive understanding of the physiological process in biological system. By implementing pump-probe concept on a laser-scanning microscope, we can obtain an image of non-fluorescent specimen. In addition, by implementing modulated excitation and lock-in detection, we can reach high sensitivity. SRS has emerged as an alternative to Coherent Anti-stoke Raman scattering (CARS) as a powerful non-destructive and label-free chemical imaging technique. Furthermore, the confinement of interaction due to non-linearity of the SRS process is helpful in achieving signal saturation, and in contrast with fluorescence microscopy, saturation power will not cause photo-bleaching and signal degradation. Designing an optical/acoustic system by attaching an ultrasound transducer on a pump-probe set-up enables to achieve high imaging sensitivity for photoacoustic modality, on the other hand the lateral resolution of the system can be further improved by using an optical objective with high NA (numerical aperture)[10]. By using a tunable laser source (e.g OPO), can be enhanced to produce detailed high-resolution images of single cell with contrast dependents primarily on the optical absorption properties of the cell.

1.3 Methodology and Work-plan:

During this first year, I devoted a great part of my time studying and learning about optics, including advanced and non-linear optical microscopy methods such as Pump-Probe, Stimulated Raman scattering and Photoacoustic. In order to organize a comprehensive bibliography and theoretical framework for my Ph-D, I focused my attention on the state of the art and the new-achievements in the field [4-10]. Therefore, I practiced in the lab learning setting-up custom optical system on the optical table and using the microscopes available in the lab, e.g., pump-probe confocal and multiphoton customized Nikon microscope. In addition, I learned how to align such systems and how to acquire images and I helped in the experiments of an already ongoing project [11]. This work helped me in discerning which factors should be considered in building a super-resolution microscope setup so that I could design my own optical setup as aimed by the PhD project.

1.4 Achieved results, dissemination, deviation from the original plan

Since during this year the Nanoscopy lab moved from the Center of Converging Technologies (CCT) to Center for Human Technologies (CHT) of Istituto Italiano di Tecnologia, I display the activities performed in the two locations respectively. In CCT, I joined the last steps of the ongoing project by one of the senior PhD candidates (Giulia Zanini) titled "Label-free optical Nanoscopy of single-layer Geraphene" [10]. In this project, a custom-made NIR transient absorption microscope was used in imaging single and multi-layer graphene in order to obtain the optimal imaging parameters and achieve maximum resolution. I learned and helped changing and optimizing the alignment of the three beams since the precise temporal and spatial overlap is a critical point for achieving maximum efficiency in this system.

Spatial alignment: superimposition of pump and probe beams at the focus is a fundamental point for the confinement of the non-linear interaction volume and the optimization of pump-probe signal. The spatial alignment was done by using 250nm Polyester beads as sample where fluorescent images were acquired with pump beam tuned to 800nm, and probe beam to 1067nm. Fig.1

Temporal alignment: Such a synchronization will be even more important when it will be necessary to image samples with low damage threshold like biological specimens. The temporal alignment is done by adjusting the pulse delay by a delay line placed in the probe optical path. Figure legend: the superimposition of three beams: pump, probe and saturation at time zero. Fig.2

When the lab moved to CHT, I have been in charge to build again the custom NIR pump-probe optical setup. It consists of a tunable mode locked femtosecond pulsed Ti:sapphire laser (680-1080 nm, 80MHz, 140 fs, Chameleon Ultra II, coherent) a laser scanning confocal Nikon A1 MP microscopes, and all necessary optical items that allow to realize the three beams optical configuration. Fig.3

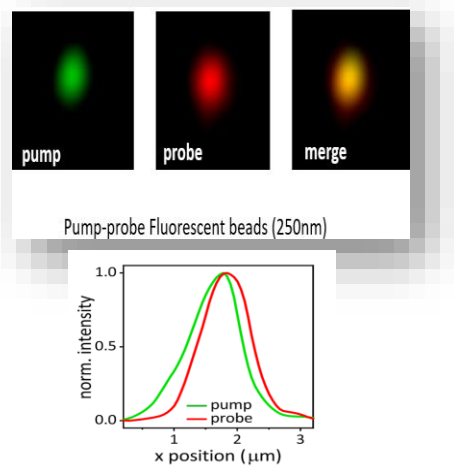

Fig.1 Spatial alignment

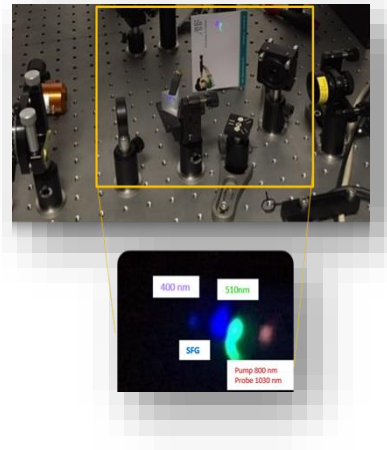

Fig. 2 Temporal alignment

Fig.3 Custom NIR pump-probe setup in CHT

Future Framework

The expected results can be organized in three levels. A first major goal will be to investigate dynamic properties of unstained biological samples with high spatial and temporal resolution by employing Stimulated Raman Scattering (SRS) and Saturated Stimulated Raman Scattering (SSRS) methods. The second goal will be the development of a multimodal imaging technology by implementing a transducer on the pump-probe setup to do photoacoustic imaging. A third and final step will be devoted to applying the realized system to the biological samples and refining the setup to achieve the best results.

1.5 References:

[1] K. Korobchevskaya, P. Bianchini, A. Diaspro et al. "*Intensity Weighted Subtraction Microscopy Approach for Image Contrast and Resolution Enhancement",* Nature Scientific Reports, (2016).

[2] M. Bouzin, G. Chirico, L. D'Alfonso, L. Sironi et al. "*Stimulated Emission Properties of Fluorophores by CW-STED Single Molecule Spectroscopy*" Physical Chemistry, (2013).

[3] Barry R.Masters and Peter T.C.So "*Biomedical Nonlinear Optical Microscopy*" Handbook, OXFORED University Press, p.15-28, (2008).

[4] Cheng, J. X., Yue, S. H., "*Slipchenko, M. N. Mutimodal Nonlinear Optical Microscopy*", Laser Photon. Rev. **5**, 496-512, (2011).

[5] T. Kobayashi, K. Kawasumi, J. Miyazaki and K. Nakata "*Resolution Enhancement of Pump-Probe Microscope with an Inverse Annular Filter*", Optical Review, (2018).

[6] E.J.Woodbury and W.K.Ng, "Ruby Laser Operation in the Near IR", in Proc. Inst. Radio Eng. 50, p. 2367, (1962).

[7] J.X.Cheng and X.S.Xie, " Coherent Raman Scattering Microscopy", (2013).

[8] E.M. Strohm, M.J. Moore, and M. C. Kolios, "*Single Cell Photoacoustic Microscopy: A Review", IEEE JOURNAL OF SELECTED TOPICS IN QUANTUM ELECTRONICS*, (2016).

[9] J. Yao, L. V. Wang, "*Sensitivity of Photoacoustic Microscopy*", Photoacoustic, (2014).

[10] C. Liu, J. Liao, L. Chen, R. Ding, X.Gong, C. Cui, Z. Pang, W. Zheng and L. Song, " *The Integrated High-Resolution Reflection-Mode Photoacoustic and Fluorescence confocal Microscopy",* Photoacoustic 14, p. 12-18 (2019).

[11] G.Zanini, K. Korobchevskaya, T. Deguchi, A. Diaspro, and P.Bianchini, "*Label-Free Optical Nanoscopy of Single-Layer Graphene*", ACS Nano, (2019).

2. TRAINING RELEATED TO PhD PROGRAM

2.1 Courses

1. **Quantum Optics** was taught by **Dario Ferraro,** date of exam **(**27th May)/ PASS
2. **Microscopic and spectroscopic techniques for the analysis of surfaces and interfaces,** was taught by **Buzio, Gerbi &Savio**, date of exam (27th June)/PASS
3. **Lasers and applications** was taught by **Marti Duocastella,** date of exam**(**30th April)/ PASS
4. **The optical microscope: optics, image formation and resolution** was tough by **Alberto Diaspro,** date of exam **(**5th August)/ PASS
5. **Fluorescence Super-Resolution Microscopy: Basis, Applications and Perspectives** was tough by **Giuseppe Vicidomini,** date of exam (7th June)/ PASS
6. **Advanced Optical Fluorescence Microscopy Methods** was taught by Paolo Bianchini and **Luca Lanzanò,** date of exam (24th September)/ PASS
7. **Specific risk training CHEM - CHEBIO NANO – PHYS (at IIT),** by **Massimo Sola,** date of exam**(**19th December)/PASS
8. **General training on safety (at IIT), by Massimo Sola,** date of exam (27th November)/ PASS

2.2 School:

1. International School ON Nanoscale Optical Microscopy, June 11th to 14th, 2019, Venice, Italy. https://mix.iit.it/events/ivsla-2019/55-ivsla-international-school-on-nanoscale-optical-microscopy

3. OTHER ACTIVITIES

3.1 Poster Publication

1. B. Kariman, T. Deguchi , F. Garzella[1,3] , E. Uriati , M. Cozzolino, A.Diaspro P. Bianchini[1] , "***Non-Linear Process for Label Free Microscopy"***, International School ON Nanoscale Optical Microscopy June 11th to 14th, 2019, Venice.