Second Year PhD in Physics and Bio-Nanoscience Report

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Cycle: **XXXIV**

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1. Research Summary

Project: Non-Linear Optical Process for Label-Free Microscope

1.1 State of the art and innovation

In recent years, super-resolution optical microscopy has become a hot research topic that typically has been developed based on fluorescent labelling, that becomes the essential tool for breaking diffraction-limited in biological imaging. Moreover, deep tissue and label-free imaging remain challenging, especially for tick and highly scattering biological specimen[1]. With the development of ultrashort pulse laser sources, non-linear optical (NLO) light-matter interaction has acquired a central role in optical microscopy for label -free imaging due to efficient signal generation in the non-linear process. To reach such an efficiency, ultrashort laser pulses with high peak power are needed [2]. The advantages of NLO approaches include the reduction of scattering due to the use of near-infrared radiation, which enables high penetration depth and reduces the aberrations introduced by the sample. Non-linear interaction comprise the generation of sum frequencies, high-harmonic generation (SHG/ THG), Raman scattering, two-photon fluorescence and others[3]. Transient absorption, also known as pump-probe, microscopy is a non-linear optical imaging technique that can probe the dynamic of the excited states. It is related to third order of non-linearity[4]. Two types of non-linear interaction, including transient absorption (TPA) and stimulated Raman scattering (SRS), can be exploited with a pump-probe approach to provide novel contrast mechanisms for label-free sample imaging. The development of a multimode-microscope and the combination of the experimental and computational approaches allow exploring non-linear processes to reach label-free super-resolution, which is the aim of this work.

1.2 Methodology, work-plan and Achieved results:

1.2.1 Absorption transition

We developed a custom-built femtosecond-pulsed near-infrared pump-probe. Two femtosecond pulsed laser beams, generated by an OPO pumped by a Ti:sapphire laser (Chameleon Ultra II and compact OPO Coherent) are coupled with a commercial laser scanning confocal microscope

(Nikon A1 MP). With the aim of exploring the saturation of transition absorption [5], and stimulated Raman scattering in order to reach super-resolution, we also used a STED-like approach where a doughnut-shaped beam is used to saturate the absorption. This development was achieved through following progression. First, optimization of both spatial and temporal alignment of system has been done. The spatial alignment is done by superimposing of three beams using gold-beads imaging while temporal performed is performed by detecting sum-frequency generation by means of a BBO crystal. Second, we customized the A1 commercial microscope to perform imaging scanning microscopy (ISM). We got Z-Stack at both wavelength 760 and 488 nm by a SPAD array detector with the aim of improving Z-resolution investigating the concept of focal modulation. In this experiment, first the position of SPAD array was changed along optical axis and doing ISM, and then moving the sample while SPAD was constant. Nevertheless, we cared about label-free non-linear signal components as forward SHG, for instance when imaging of collagen (generated myosin & muscle at high lateral resolution. Third, we characterized single layer graphene under pump-probe microscope with a Lock-in amplifier scheme, looking at the dependencies on applied powers. Forth, we realized a doughnut-shaped absorption saturation beam by passing the beam through a vortex phase plate, and we coupled it with the other beams by a polarizing beam splitter for exploiting transient absorption saturation in imaging single layer graphene. Results was in good agreement with images was obtained previously with another architecture [6], and shows that saturation pump-probe can be a promising technique in reaching label-free high-resolution image, and saturation absorption via an unmodulated pump beam can reduce the point spread function by suppressing pump-probe signals like in a STED approach. Fifth, we introduced an appropriate combination of band-pass filters and beam splitters in the detection path of the microscope to investigate backward SHG and sum frequency generation (SFG) processes. We are also going to investigate the capability of this optical setup to reach saturation stimulated Raman scattering (SSRS) since optical requirements are cooperatively similar to our actual setup.

1.2.2 Saturation Stimulated Raman Scattering computational approach

Energy-level diagram of Stimulated Raman Scattering (SRS) process have been studied in order to compute temporal evolution of molecules populated in different energy levels. Typically, a Raman process involves a ground state; a vibrational state and an electronic state. In a real molecular system under intense pulse laser excitation, the overtone states above the vibrational state can also be involved in molecular transitions via non-linear optical processes[7]. This makes SRS process difficult to saturate, and the overtone states cannot be neglected in saturation stimulated Raman scattering. To achieve Raman scattering, the electronic dipole moments should be nonzero in the electronic state[1]. The status of three level system is described by density matrix $\rho_{mn}$where $m, n = g, v, e$. The light interaction is described semiclassically by the Liouville-von Neumann equation as shown in following, which this equation cab be solved numerically by using forth-order of Runge-Kutta method to quantitatively calculateρ_{vv}. Since we need to find out how ρ_{vv} is saturated depending pump-probe electric field amplitude. Thus, finding the saturation condition is the key to designing super-resolution SRS microscopy. We are going to adjust and develop this code correspondent our setup in order to be able to compare the results in both theoretical and experimental framework.

$$\frac{\partial \rho_{nm}}{\partial t} = -i\omega_{nm}\rho_{nm} - \frac{i}{\hbar}\left[\hat{H}_{int}, \hat{\rho}\right] - \gamma_{nm}\rho_{nm} \qquad (n \neq m)$$

$$\frac{\partial \rho_{nm}}{\partial t} = -\frac{i}{\hbar}\left[\hat{H}_{int}, \hat{\rho}\right] + \sum_{E_{m} > E_{n}} \Gamma_{nm}\rho_{mm} - \sum_{E_{m} < E_{n}} \Gamma_{nm}\rho_{nn}$$

$\omega_{nm} = \omega_{\boldsymbol{n}} - \omega_{\boldsymbol{m}}$ is the frequency difference between two states, and γ_{nm} and Γ_{nm} are the decoherence and delay rate, respectively. I have solved these equations numerically by using an algorithm based on the forth-order Runge-Kutta (RK4) method in order to obtain temporal evolution of population in different energy level. In this modeling, I considered pulse-width 2 ps and 500 fs, respectively. I also adjusted other parameters depends on samples. This algorithm computed population probability from 0 to 4 ps with step size control (0.035 fs).

Investigating 3D resolution improvement by tilting 2D doughnut beams

In this study, a 2D stimulated emission depletion super-resolution microscope was modified with the main aim to improve Z-resolution. In general, the stimulated emission process through a doughnut-shaped depletion laser beam confine the fluorescence emission in its very center [8]. In this work, we to achieve 3D resolution we need tilting two-overlapped doughnut beams. We achieved their shapes and tilt by a wavefront engineering, applying a non-centered helical phase shift. Whereas the traditional 3D is achieved by two co-aligned beams with different shapes, i.e., vortex and bottle beams [8]. We characterized the performance of the tilted depletion beam in the context of 3D STED microscopy at the presence of defined optical aberrations introduced by spatial light modulator (SLM). Such a development although made for fluorescence microscopy can be translated to pump-probe nanoscopy. Moreover, the collaboration with other teams gave me the chance to learn many aspects about STED beam shaping by SLM and adaptive optics concepts.

[1] G. Li, Z. Wei, M. Ying, and H. Zhiwei, "Saturated Stimulated-Raman-Scattering Microscopy for Far-Field Superresolution Vibrational Imaging," vol. 034041, pp. 1–12, 2019, doi: 10.1103/PhysRevApplied.11.034041.

[2] T. Hellerer, A. M. K. Enejder, and A. Zumbusch, "Spectral focusing: High spectral resolution spectroscopy with broad-bandwidth laser pulses," *Appl. Phys. Lett.*, vol. 85, no. 1, pp. 25–27, 2004, doi: 10.1063/1.1768312.

[3] C. J. R. Sheppard, "Multiphoton microscopy: a personal historical review, with some future predictions," *J. Biomed. Opt.*, vol. 25, no. 01, p. 1, 2020, doi: 10.1117/1.jbo.25.1.014511.

[4] "Pump – Probe Microscopy : Theory , Instrumentation , and Applications," vol. 32, no. April, pp. 2–11, 2017.

[5] P. Bianchini, K. Korobchevskaya, G. Zanini, and A. Diaspro, "Pump-Probe Nanoscopy by Means of Transient Absorption Saturation," *Int. Conf. Transparent Opt. Networks*, vol. 2018-July, pp. 1–5, 2018, doi: 10.1109/ICTON.2018.8473625.

[6] G. Zanini, K. Korobchevskaya, T. Deguchi, A. Diaspro, and P. Bianchini, "Label-Free Optical Nanoscopy of Single-Layer Graphene," *ACS Nano*, vol. 13, no. 8, pp. 9673–9681, 2019, doi:

10.1021/acsnano.9b05054.

[7] L. Gong and H. Wang, “Breaking the diffraction limit by saturation in stimulated-Raman-scattering microscopy: A theoretical study,” *Phys. Rev. A - At. Mol. Opt. Phys.*, vol. 90, no. 1, pp. 1–10, 2014, doi: 10.1103/PhysRevA.90.013818.

[8] G. Vicidomini, P. Bianchini, and A. Diaspro, “STED super-resolved microscopy,” *Nat. Methods*, vol. 15, no. 3, pp. 173–182, 2018, doi: 10.1038/nmeth.4593.

2. List Courses followed

2.1 Advanced Crystallography: theory and experiments was taught by **Alberto Martinelli,** date of exam **(**22nd July)/ PASS

3. Conference and Webinar

3.1 Webinar Summer Symposium

- MINFLUX-superresolution post Nobel of Stefan W. Hell **,** 7th July
- STELLARIS Confocal Workshop, 27th September
- Multi-dimensional image visualization in Python using napri of Nicholas Sofroniew, 4th June, NEUBIAS academy.

3.2 Conference Communications

- **Oral presentation**: FOM 2020, OSAKA, JAPAN

B.S.Kariman, T. Deguchi, R. Ranjan, G.zanini, A. Diaspro, and P. Bianchini

"Exploring Satuartion Process in Stimulated Raman Scattering"

http://www.focusonmicroscopy.org/

- **Proceeding paper:** Multiphoton Microscopy in the Biomedical Sciences, SPIE 2020

P. Bianchini, B. Kariman, F. Garzella, E. Uriati, G. Zanini, T. Deguchi and A. Diaspro

"Improving the resolution in multiphoton microscopy"

https://lux.spie.org/PW20B/conferencedetails/multiphoton-microscopy

3.3 Publication

- **In proceeding paper:** "Pump-probe Nanoscopy: Principle, implementation and application in Label-Free imaging"
  B.S.Kariman, G.Zanini, A. Diaspro, and P. Bianchini