Coherent Raman Microscopy for Biological Applications

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Label-free imaging techniques are transforming the way we study biological systems, offering molecular specificity without the need for dyes or stains. Among them, Coherent Raman Microscopy has emerged as a powerful tool for biomedical research, enabling fast and chemically selective imaging based on vibrational contrast. This presentation will introduce the principles and applications of the two main Coherent Raman modalities: Coherent Anti-Stokes Raman Scattering (CARS) and Stimulated Raman Scattering (SRS). We will highlight their strengths—such as high acquisition speed, three-dimensional sectioning, and compatibility with live-cell imaging—while discussing their respective challenges, including non-resonant background (NRB) in CARS and the need for balanced detection in SRS. We will present the recent advances in this field by our group in Politecnico di Milano, including: (1) Ultra-broadband (400–3100 cm^{-1}) CARS microscopy at high speed (<3 ms pixel dwell time) coupled to deep-learning spectral denoising and NRB removal [1-2]. (2) Tunable wide-field broadband video-rate CARS microscopy in the fingerprint region over large fields of view. (3) Broadband SRS microscopy [3] equipped with a home-built differential multichannel lock-in detection board, delivering single-shot SRS spectra with 32 vibrational frequencies with ≈40 µs integration time.

Beyond Raman-based methods, we will explore complementary linear and nonlinear optical imaging modalities, including Brillouin microscopy for mechanical property mapping [4], Second Harmonic Generation (SHG) for imaging ordered biological structures like collagen, and Two-Photon Excited Fluorescence (TPEF) for endogenous fluorophores -such NADH and FAD- and holo-tomography for visualizing 3D morphology [5]. The integration of these techniques in multimodal platforms offers a more complete picture of the biological landscape, enabling simultaneous assessment of chemical, structural, mechanical, and metabolic properties. Through selected examples, we will demonstrate how combining these imaging modalities enhances our understanding of complex biological phenomena at the cellular and tissue level.

1. Fingerprint Multiplex CARS at High Speed Based on Supercontinuum Generation in Bulk Media and Deep Learning Spectral Denoising, Opt. Express 30, 30135 (2022). https://doi.org/10.1364/OE.463032
2. Full-Spectrum CARS Microscopy of Cells and Tissues with Ultrashort White-Light Continuum Pulses, J. Phys. Chem. B 127, 4733 (2023) https://doi.org/10.1021/acs.jpcb.3c01443
3. Broadband Stimulated Raman Imaging based on Multi channel Lockin Detection for Spectral Histopathology, APL Photonics 7, 076104 (2022) https://dx.doi.org/10.1063/5.0093946
4. Birefringence-induced phase delay enables Brillouin mechanical imaging in turbid media, Nature Communications 15, 5202 (2024). https://doi.org/10.1038/s41467-024-49419-2
5. Noninvasive morpho-molecular imaging reveals early therapy-induced senescence in human cancer cells, Science Advances 9, eadg6231 (2023) https://doi.org/10.1126/sciadv.adg6231