Optica Biophotonics Congress 2026 (Translational, Microscopy, OCT, OTS, BRAIN) © Optica Publishing Group 2026

Broadband Stimulated Raman Scattering for Virtual Histopathology and High-Speed Microplastics Detection

Eric Michele Fantuzzi[1], Federica Padelli[1,2], Tiago Azevedo[3], Rebeca Martínez-Vázquez[4], Alessandra Nardini[4], Gabriele Di Noia[1], Federico Monti[1], Sara Pizzurro[1], Matteo Corti[1], Mujeeb Rahman[1,2], Andrea Ragni[1], Moe Vali[3], Chris Tomy[3], Renzo Vanna[4], Anna Mühlig[5], Orlando Guntinas-Lichius[5], Andrea C. Ferrari[3], Pietro Liò[3], Francesco Crisafi[1], and Giulio Cerullo[1,2,4]*

[1]Cambridge Raman Imaging S.r.l., Via Durando 39, 20158 Milano, Italy
[2]Politecnico di Milano, Piazza Leonardo da Vinci 32, 20133 Milano, Italy
[3]University of Cambridge, Cambridge CB2 1TN, United Kingdom
[4]CNR–Istituto di Fotonica e Nanotecnologie, Piazza Leonardo da Vinci 32, 20133 Milano, Italy
[5]Universitätsklinikum Jena, Am Klinikum 1, 07747 Jena, Germany
** giulio.cerullo@polimi.it*

Abstract: We present a broadband stimulated Raman scattering platform for virtual histopathology and rapid microplastics detection. Single-shot CH-range hyperspectral imaging enables label-free diagnostics and high-speed polymer identification in complex biological matrices.

Digital pathology continues to rely on chemical staining workflows that introduce delays, variability, and constraints on automation. Stimulated Raman Histology (SRH) offers label-free molecular contrast [1,2], yet conventional two-frequency systems provide limited spectral information and are mostly restricted to fresh or frozen samples. To make SRH compatible with routine clinical practice, especially processing of formalin fixed paraffin embedded (FFPE) samples, we present a multiplex SRH platform paired with deep-learning tools for virtual staining and automated tissue analysis.

The system acquires single-shot hyperspectral data across the full CH stretching region (2800–3100 cm^{-1}) using an all-fiber dual-wavelength laser and a compact multichannel lock-in detector that collects 38 spectral channels in parallel [3,4]. This broadband acquisition allows reliable discrimination of paraffin residues, lipid- and protein-dominated regions, nucleic-acid–rich structures, and other features that cannot be resolved with traditional two-frequency SRH, as shown in Fig.1.

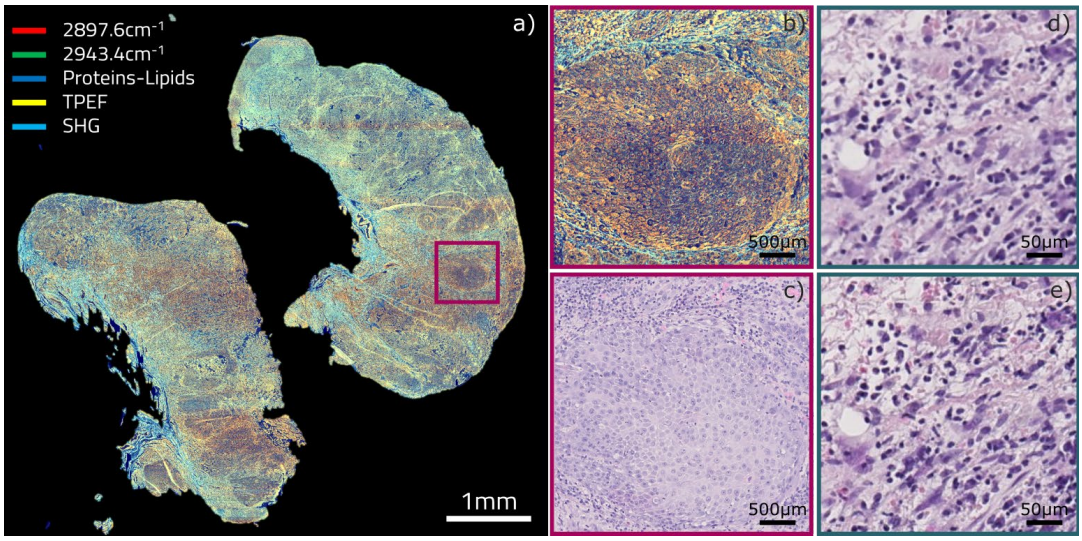

Figure 1: (a) Overlay of three stimulated Raman scattering (SRS) signals: lipids at 2897.6 cm^{-1} (red), proteins at 2937 cm^{-1} (green), and the difference between protein and lipid signals (blue), combined with two-photon excited fluorescence (yellow) and second harmonic generation (light blue). (b, c) Enlarged insets of the overlay image in (a) and the corresponding area after H&E staining, respectively. (d, e) virtually stained and H&E stained images of the same sample, respectively.

Paired multiplex-SRH and H&E images from the same FFPE sections are used to train a convolutional network that generates virtual H&E slides with high morphological and tinctorial fidelity (Fig.1d). A second model performs semantic segmentation, enabling automated identification of tumour regions and supporting structures. Initial validation across multiple tissue types shows strong correspondence with standard histology, confirmed by expert review.

The same broadband platform also supports rapid chemical imaging beyond histopathology. As an example, we demonstrate fast microplastics identification through CH-band spectral fingerprints, using a reference library of common polymers and measurements on glass slides, filtration membranes, and in-flow aqueous environments via silica microfluidic cells, as shown in Fig.2.

Taken together, multiplex SRH and deep learning provide a fully digital, reagent-free workflow compatible with standard FFPE pipelines while extending to applications such as microplastics analysis. This approach improves reproducibility, increases throughput, and broadens the scope of label-free vibrational imaging in both clinical and environmental contexts.

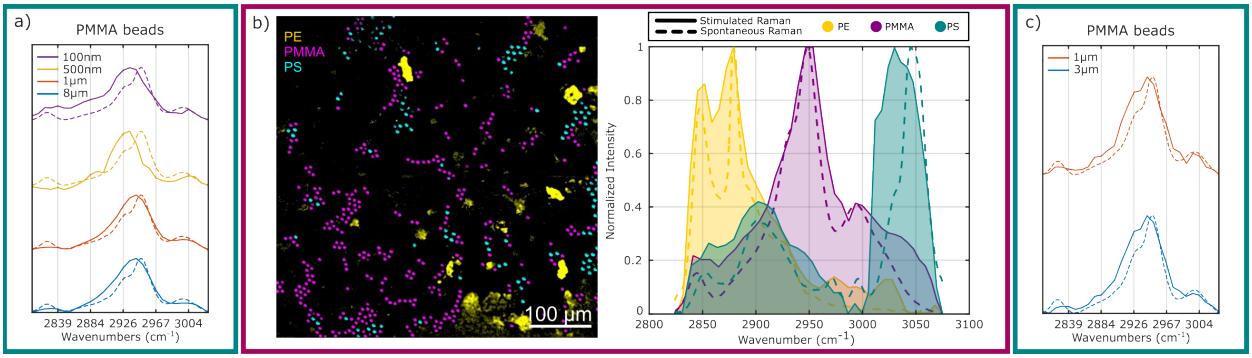

Figure 2: Micro- and Nanoplastic Analysis with CORAL. (a) Broadband SRS spectra of PMMA beads of various diameters, measured on a static glass slide. (b) Chemical identification and classification of a mixed microplastic sample, differentiating PMMA, PE, and PS particles. (c) In-flow SRS measurement of PMMA beads, with automatic size-based grouping.

References

[1] Minbiao Ji, Daniel A. Orringer, et al., "Rapid, label-free detection of brain tumors with stimulated Raman scattering microscopy," Science Translational Medicine 5, 2013.

[2] Daniel A. Orringer, Minbiao Ji, et al., "Rapid intraoperative histology of unprocessed surgical specimens via fiber-laser-based stimulated Raman scattering microscopy," Nature Biomedical Engineering 1, 2017.

[3] Dawei Fu, Wenlong Min, et al., "Hyperspectral imaging with stimulated Raman scattering," Journal of Physical Chemistry B 117, 2013.

[4] Liang Shi, Yufeng Wang, et al., "Advances in stimulated Raman scattering imaging for tissues: review and perspectives," Progress in Biomedical Optics and Imaging (2021).