A compact, turn-key platform for multiplex stimulated Raman scattering microscopy

Francesco Crisafi[a], Benedetta Talone[a], Andrea Ragni[a], Gabriele Di Noia[a], Mujeeb Rahman[b], Jing He[c], Jeremiah Marcellino[c], Goutam Kar[c], Yarjan Samad[c], Boyang Mao[c], Renzo Vanna[d], Franziska Hoffmann[e], Orlando Guntinas-Lichius[e], Sze Yun Set[c], Andrea C. Ferrari[c], Giulio Cerullo[f], and Matteo Negro[g]

[a]Cambridge Raman Imaging Srl, Via Durando 39, 20158, Milan, Italy
[b]Cambridge Raman Imaging Ltd, Botanic House, 100 Hills Road, Cambridge, Cambridgeshire, CB2 1PH, United Kingdom
[c]Cambridge Graphene Centre, University of Cambridge, Cambridge, CB3 0FA, UK
[d]CNR IFN, P.zza Leonardo da Vinci 32, 20133, Milan, Italy
[e]Jena University Hospital, Am Klinikum 1, 07747 Jena, Germany
[f]Politecnico di Milano, Dipartimento di Fisica, P.zza Leonardo da Vinci 32, 20133, Milan, Italy
[g]Cambridge Raman Imaging Ltd, c/o Via Durando 39, 20158, Milan, Italy

ABSTRACT

We combine an all-fiber dual wavelength, self-synchronized laser and a dedicated multi-channel detection unit to perform state-of-the-art multiplex Stimulated Raman Scattering (SRS) microscopy. The system covers the full CH spectrum in 1 μs reaching shot-noise limited performances with 25 μW per detection channel. This all-in-one solution is based on a passively synchronized dual-wavelength laser source with shot-noise limited relative intensity noise from 600 kHz and a modular multi-channel lock-in detection unit. The synergistic design between laser source and detection system simplifies multiplex SRS implementation for real-time full-chemical imaging.

Keywords: Multiplex stimulated Raman scattering microscopy, fiber laser, multichannel lockin amplifier

1. INTRODUCTION

In the realm of label-free imaging techniques, coherent Raman imaging (CRI) emerges as a powerful tool, offering sub-cellular spatial resolution, molecular-specific contrast, and addressing the unmet need in life sciences for label-free chemically specific imaging by detecting the intrinsic vibrational fingerprints of cells and tissues.[1] Multiplex SRS microscopy,[2] combining single-shot detection of broad vibrational spectra and high spectral resolution, fully exploits the innovative potential of CRI tools. State of the art implementations of multiplex SRS systems are based on custom and complex solutions, rendering them completely inaccessible to non-specialists in the field.[2,3]

We present a platform designed to reach state-of-the-art performances in multiplex SRS with an unprecedented ease of use and long-term reproducibility. The core of the presented system are STRALE, an all-fiber dual wavelength self-synchronized laser, and CHAMP, a detection unit based on a compact high-frequency multi-channel lock-in amplifier. The laser delivers a two-color synchronized output for shot-noise limited broadband SRS in CH stretching region (2800-3100 cm^{-1}). The detection unit minimizes the electronic noise between 1 and 10 MHz, reaching the shot-noise limit at a power per channel as low as 25μW. This ensures state-of-the-art SRS performances over the entire CH spectrum, parallelizing the detection of up to 38 channels in 1 μs.

Corresponding author: Benedetta Talone
E-mail: benedetta.talone@cambridgeramanimaging.com

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2. STRALE - STIMULATED RAMAN LASER

The STRALE (STimulated RAman LasEr) system, shown in Fig. 1, has been thoroughly designed to fully exploit the capabilities of broadband SRS across diverse applications, without compromises. It achieves the complete detection of the CH-stretching region in a single shot, eliminating the need for time-consuming sequential tuning of the emitted wavelengths, while preserving a high level of spectral resolution. This system is based on a patented passively synchronized dual-output fiber laser, delivering two trains of pulses generated through a carefully designed master oscillator power amplifier (MOPA) structure enabling shot-noise limited performances and tens of nanojoule energy per pulse.

Figure 1. STRALE system.

STRALE enables real-time comprehensive chemical mapping of specimens under observation, providing wide spectral coverage from 2800 cm^{-1} to 3100 cm^{-1} simultaneously. The pump beam is generated using an Erbium-doped fiber laser, which is amplitude modulated and then amplified, before a second harmonic generation stage. Its central wavelength is at 792±1 nm, with full width at half maximum (FWHM) 1 nm and pulse width of 1.1 ps. For the Stokes beam, an Ytterbium-doped fiber laser has been implemented, with central wavelength at 1030 nm, FWHM ≥ 30 nm and pulse width of 110 fs. Thanks to these features, summarized in Table 1 and depicted in Fig. 2, it can be directly integrated in various non-linear optical microscopy setups, such as two-photon excitation fluorescence (TPEF), second harmonic generation (SHG) microscope and pump-probe systems. The device is compact (610mm x 390 mm x 395 mm) and with stable day-to-day performances thanks to an air-cooled all-fiber architecture. 60-minutes-log measurements of pump and Stokes beams are reported in Fig. 3. Long-term femtosecond-scale jitter (see Fig. 4) and shot-noise limited performance open the way to microsecond pixel dwell times and high Signal-to-Noise Ratios (SNRs). With its simple and user-friendly software, STRALE supports fast, non-destructive, time-lapse experiments on live biological samples.

Table 1. STRALE specifications.

	Pump	**Stokes**
Central WL [nm]	792±1	1030
FWHM [nm]	≤ 1	≥ 30
Power [mW]	> 70@1-10 MHz mod. freq.	> 200@20 MHz rep. rate
Energy [nJ]	> 7	> 10
Pulse width [ps]	< 2	0.1-1
WN coverage [cm^{-1}]	2800-3100	
WN resolution [cm^{-1}]	< 16	

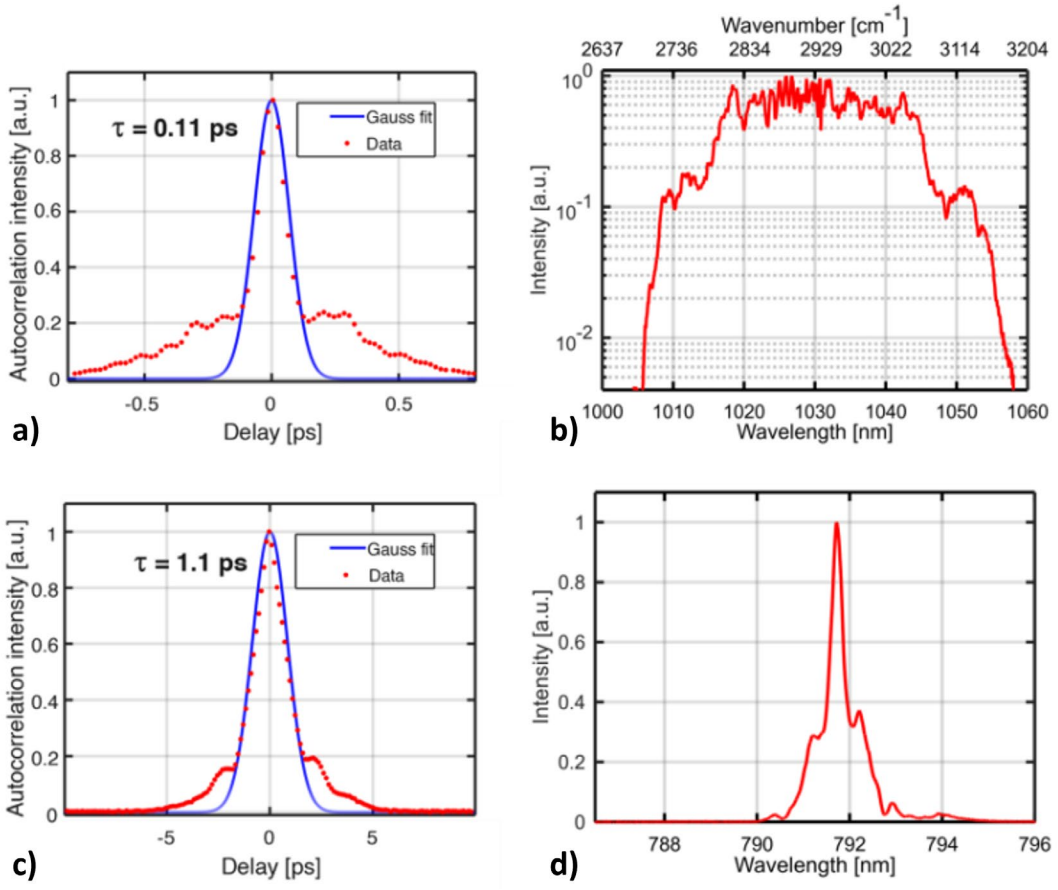

Figure 2. STRALE dual output characterization: a) Stokes beam pulse duration and b) spectral shape. c) Pump beam pulse duration and c) spectral shape.

3. CHAMP - MULTICHANNEL LOCK-IN AMPLIFIER

CHAMP (multiCHannel lock-in AMPlifier spectrometer), depicted in Fig. 5, is an advanced optical spectrometer that employs pioneering multi-channel lock-in technology, specifically designed for broadband SRS microscopy. This innovative device is designed for precise modulation-transfer measurements and seamlessly integrates into research microscope structures. It operates on a modular architecture, streamlining the expansion of detector channels and facilitating data transfer to the computer.

This spectrometer shows a compact design with 38 spectral channels and independent lock-in amplifiers. It allows for the simultaneous real-time extraction of both average power (DC) and demodulated signals (AC) on each channel. Engineered specifically for CH-stretching detection from 2800 cm^{-1} to 3100 cm^{-1} in a single shot, the spectrometer achieves a remarkable spectral resolution of better than 9 cm^{-1}. With a triggerable integration time averaging from 1 μs to 200 ms and user-controllable channel-independent demodulation phase between 0-360 degrees in 16 steps, it ensures precise and flexible operation. The spectrometer is equipped with a USB 3.0 connection for swift data transfer to the PC. With an extensive input shot-noise limited dynamic range of 22 dB and a mere 25 μW optical power per channel, it can achieve the ultimate noise limit, ensuring the highest Signal-to-Noise Ratio (SNR), as shown in Fig. 6. Technical specifications are presented in Table 2.

4. RESULTS

The platform has been tested on different kind of samples. Preliminary measurements have been done using reference samples of plastic microspheres. Fig. 7 shows an image of polymethyl methacrylate (PMMA) and

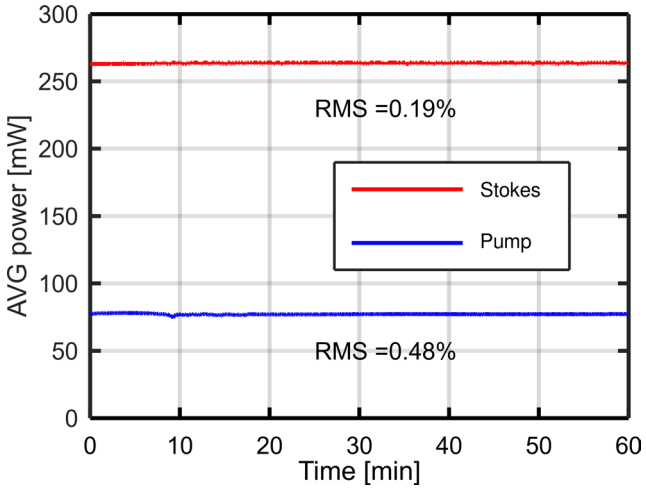

Figure 3. One hour power fluctations of pump and Stokes beam.

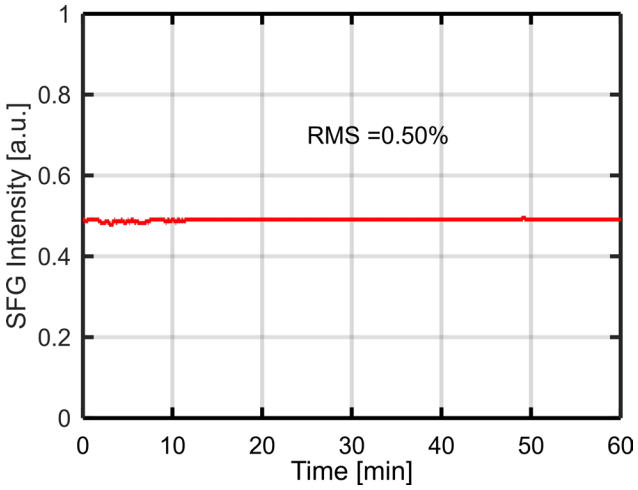

Figure 4. Pump-Stokes jitter evaluation through sum frequency generation (SFG) signal flactuation over one hour.

Polystyrene (PS) beads, of 8 μm and 10 μm diameter respectively. Given the different chemical compositions, the two species are perfectly discernible collecting the SRS signal at their characteristic Raman shift: 2950 cm^{-1} for PMMA and 3050 cm^{-1} for PS.

The system has been tested also with biologically relevant samples. A multimodal image of human oral squamous cell carcinoma tissue slice is presented in Fig. 8. The sample is a fresh frozen unstained tissue slice of 12 μm thickness, mounted on a standard BK7 glass slide for microscopy. The image is in false-colour and it has been obtained merging SRS signals collected at 2930 cm^{-1}, colour-coded in red, and at 2845 cm^{-1}, colour-coded in green, and the two-photon autofluorescence signal, colour-coded in blue. These three channels respectively

Figure 5. CHAMP system.

Table 2. CHAMP specifications. *Input wavelength range: 1015 - 1050 nm.

	Performance
Frequency Range [MHz]	1 - 10
NEP [pW/$\sqrt{Hz}$]	< 20
Detector	38-channel Si array
Fixed filter time constant [μs]	0.5
Vertical resolution [bit]	16
Max. input power [mW/ch]	5*
Spectral resolution [cm^{-1}]	< 9*

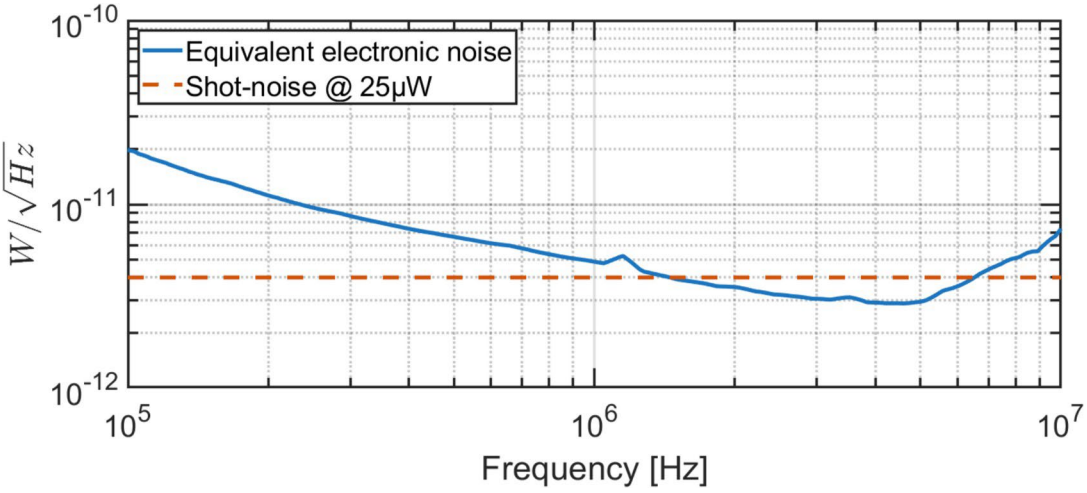

Figure 6. Noise performance of CHAMP coupled to STRALE laser.

allow to highlight the spatial distribution of proteins, lipids and collagen/elastin, i.e. the main constituents of

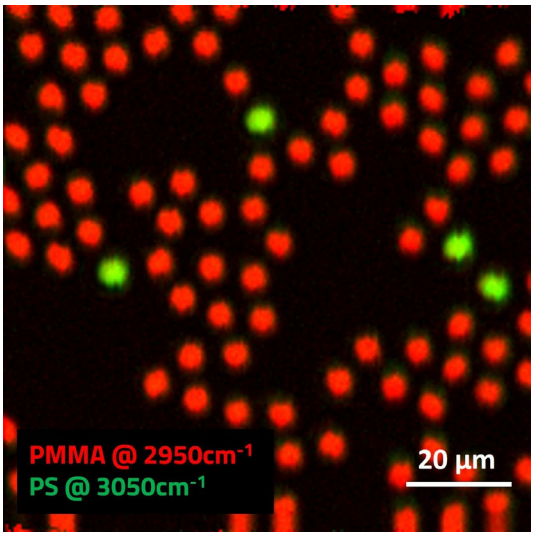

Figure 7. False-colour image of PMMA and PS microspheres, obtained combining signals collected from two channels of STRALE detector. Pixel dimension: 1μm^2, pixel dwell time: 1 ms.

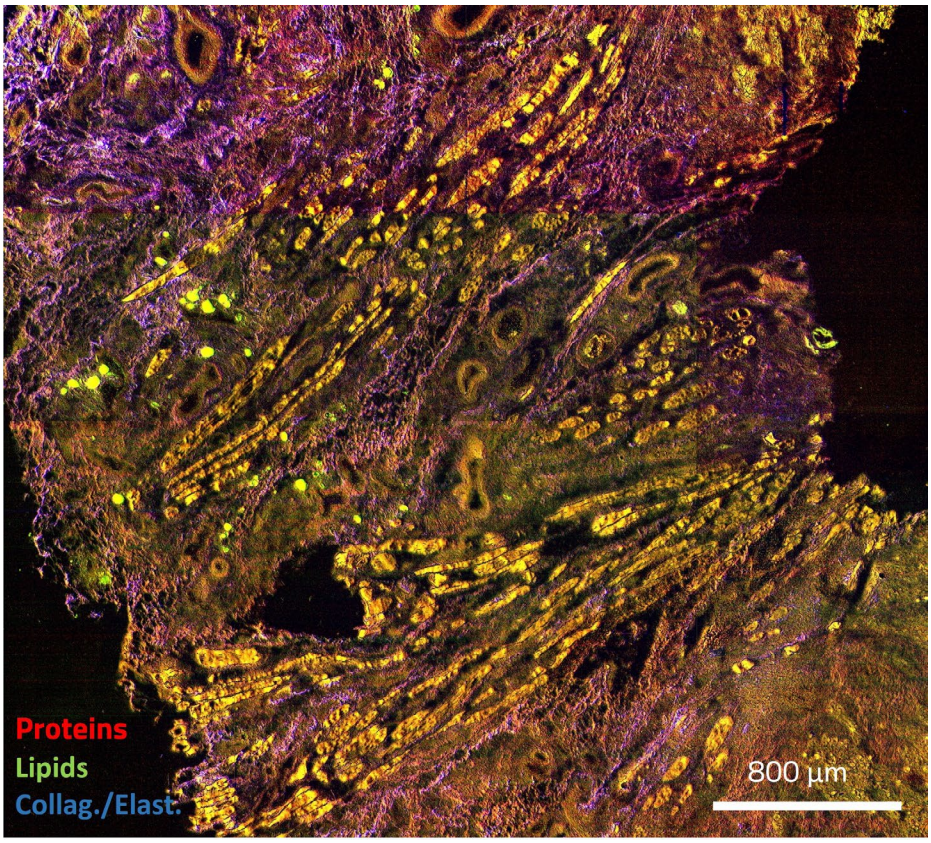

Figure 8. Multimodal image of human oral squamous cell carcinoma tissue slice. Proteins signal in red: SRS @2930 cm^{-1}; lipids signals in green: SRS @2845 cm^{-1}, collagen/elastin in blue: TPEF 390-600 nm. Pixel dimension: 1μm^2, pixel dwell time: 0.5 ms.

any biological specimens.

5. CONCLUSION

This platform represents the first commercially available instrument capable of bringing this technology out of research laboratories, finally making it accessible to end-users without expertise in technological development. The performance achieved by the two core components of the system, STRALE and CHAMP, makes this technology interesting even to users involved in development of custom solutions. Combining the broadband label-free approach unlocked by this technology with artificial intelligence tools, the full power of hyperspectral data for chemometric analysis of biological specimens is finally unleashed. Such a system finds broad application in biomedical sectors where traditional exogenous labeling is a limiting factor, such as in live cell imaging, metabolomics, and histopathology.

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