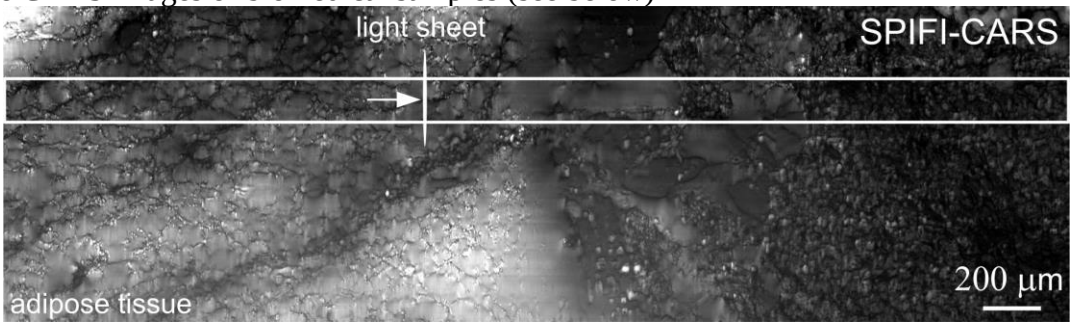
Multi-modulations schemes in coherent Raman microscopy (SRS and CARS) for spectral and spatial multiplexing image acquisition.

Sandro Heuke[1], Barbara Sarri[1], Randy A. Bartels[2], Hervé Rigneault[1]

Aix Marseille Univ, CNRS, Centrale Marseille, Institut Fresnel, Marseille, France.
School of Biomedical Engineering, Colorado State Univ, Fort Collins, Colorado 80523, USA.
* Corresponding author: herve.rigneault@fresnel.fr

To access the same information content as conventional histopathology, it was recently demonstrated that coherent Raman scattering (CRS) microscopy can be translated into virtual hematoxylin and eosin (H&E) images without any staining [1]. For this purpose, two stimulated Raman scattering (SRS) images at 2850 cm^{-1} and 2930 cm^{-1} are required which are ideally acquired within a single scan. We address here our latest innovations to speed up CRS image acquisition, still providing label free imaging with similar quality than gold standard H&E.
We present 3 approaches using the modulation of the CRS excitation fields to either allow for the simultaneous acquisition of SRS images at distinct Raman shifts or to multiplex the acquisition to 2 or more positions (CARS).
(1) Within the first approach, a novel three-color picosecond laser system where two colors, serving as the pump, are modulated at distinct RF frequencies allows to acquire simultaneously two wavenumbers within the 500 cm^{-1} - 5000 cm^{-1} Raman range.
(2) For the second approach the 2 colors modulated at different RF frequencies are each superimposed by with the third color (Stokes beam) and coupled into the laser scanning microscope with two angular directions. The latter translates into different lateral positions at the sample plane providing two distinct SRS focus, each addressing a different wavenumber. This dual-focus SRS approach allows to image a sample at distinct lateral or axial positions, i.e. sample planes. Further, it enhances the imaging speed by a factor of 4 while maintaining the signal-to-noise ratio of a dual-color single focus approach.
(3) The last approach uses a spatially frequency modulated illumination scheme known as SPIFI [2] to perform line scanning CARS (SPIFI-CARS). We show how the modulation pattern imprinted onto a spinning disk allows to retrieve simultaneously the signal of 40 foci/pixel across a 1D-excitation line profile (length 100 µm). The approach has the ability to acquire extended, label free CARS images of biomedical samples (see below)



REFERENCE
[1] B. Sarri, et al. "Stimulated Raman histology: one to one comparison with standard hematoxylin and eosin staining," Biomed. Opt. Express 10, 5378-5384 (2019).
[2] G. Futia, et al., "Spatially-chirped modulation imaging of absorbtion and fluorescent objects on single-element optical detector," Optics Express **19**, 1626-1640 (2011).