

Raman light sheet microscopy: a fast micro-spectroscopy imaging technique

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Raman spectroscopy provides information about the chemical composition of materials, cells and tissues. The Raman process is induced by scattering of the excitation light with the vibration of the molecules contained in the sample. This provides an intrinsic label-free contrast mechanism from the sample that is rich in biochemical content. Taking 2D information of the molecular distribution that conforms the sample is thus, very attractive to the biomedical science community. However, the efficiency of the Raman scattering process is very low and, therefore, long acquisition times (that goes from few minutes to several hours) are required to obtain a single 2D image. This makes practically impossible the use of Raman imaging for in vivo studies and/or volumetric chemical information. In this application note we present a recently published approach for fast 2D Raman imaging [1], based on a digital scanned light sheet microscopy (DSLMS) system that uses interferometric tunable filters and a compact Cobolt Laser operating at 638 nm.

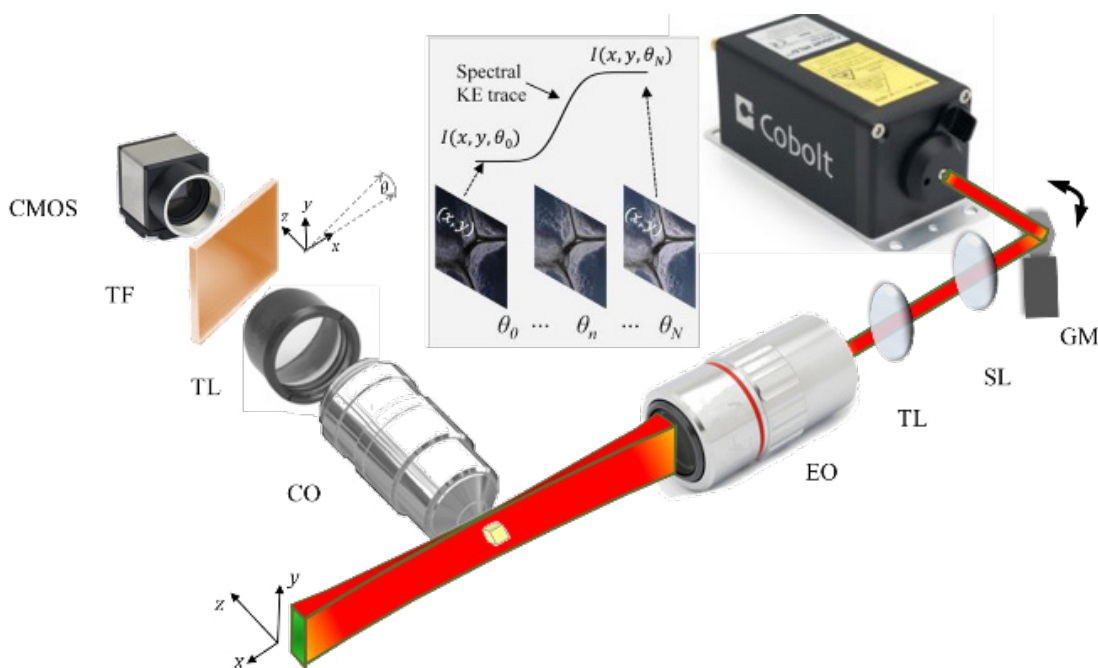


Figure 1. Raman LiShMS setup. GM: x-y Galvo-Mirror; EO: exciting objective; CO: collecting objective; SL: scan lens; TL: tube lens TF: tuning filter.

Raman imaging without using a spectrometer

A DSLM system can be adapted such that the digitally scanned plane of light (see Figure 1) is used to excite the Raman process at a single plane of the sample. The generated 2D Raman information is then imaged onto a CMOS camera through the collection arm of the microscope. Without any modification, such system will record an image where each pixel represents the integrated Raman information without any spectral resolution. To spectrally resolve the Raman information, an interferometric tunable filter (TF) is utilized. By changing the angular orientation of the TF, its sharp edged transition is continuously shifted.

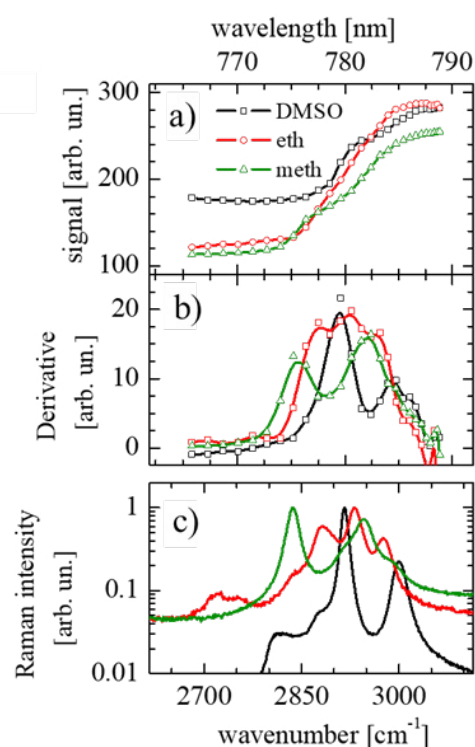


Figure 2. Raman LiShMS imaging in different organic solvents using a 638 nm Cobolt Laser at 20 mW, a) spectral knife-edge traces, b) Raman spectra after the derivation process, and, c) Corresponding Raman spectra of the same solvents, taken with a confocal micro-Raman system. Reproduced from Rocha-Mendoza et al., *Biomed. Opt. Express* 6(9), 3449-3461 (2015).

This allows recording on every pixel a cumulative intensity distribution of the vibrational Raman bands as the TF

angular position is changed (see inset of Fig. 1 and Fig. 2a). Afterwards, a simple differentiation of the images is used to recover the Raman spectrum (see Fig. 2b). Since this procedure is performed pixel-by-pixel on the entire image, a 3D spectrally resolved Raman map is obtained for each illuminated plane. The obtained Raman spectra are in good agreement with the spectra taken under a confocal micro Raman system (Fig. 2c).

Getting faster 3D hyperspectral Raman images

The feasibility of the technique for imaging solid 3D samples was demonstrated using a compact CW Cobolt laser at 638nm to resolve the Raman spectra of polystyrene beads (PS) and lipid droplets (LF) immersed in agar at the C-H region as shown in figure 3 (see figure caption). Additionally, the potential use of the technique for studying biological living samples is also demonstrated using *C. elegans* (Figure 4). It is worth mentioning that the required time to extract all this 3D-Raman information ($300 \times 300 \times 180 \mu\text{m}^3$) was about 5 minutes.

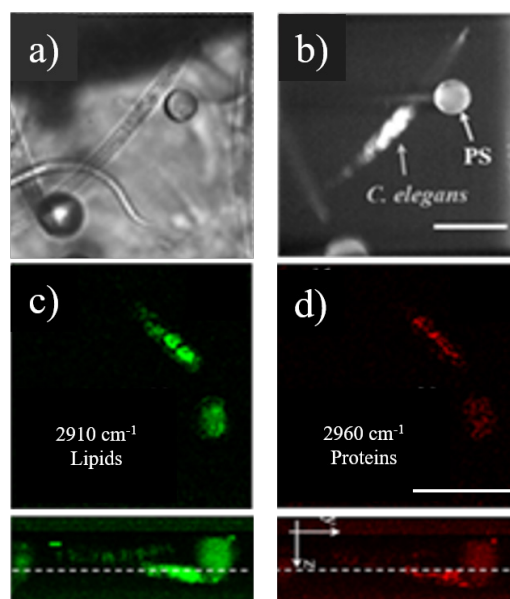


Figure 3. 3D Raman Light Sheet micro-spectroscopy of *C. elegans* nematodes. a) Bright-field and b) integrated Raman images of a sample with polystyrene (PS) beads as reference Raman markers. 3D orthogonal views of spectrally resolved Raman images of a *C. elegans* at c) 2910cm⁻¹ and d) 2960 cm⁻¹, respectively. Scale bars: 100 μm. Adapted from Rocha-Mendoza et al., *Biomed. Opt. Express* 6(9), 3449-3461 (2015).

The feasibility of the technique for imaging solid 3D samples was demonstrated using a compact CW Cobolt laser at 638nm for studying the contents of lipids and proteins in living biological samples, such as *C. elegans* nematodes (Figure 3). It is worth mentioning that the required time to extract all this 3D-Raman information ($300 \times 300 \times 180 \mu\text{m}^3$) was about 5 minutes.

Summary

A compact Cobolt laser was utilized in a light sheet scheme to perform rapid 2D and 3D Raman imaging spectrally resolved in the C-H region. The image acquisition speed is up to 4 orders of magnitude faster than the acquisition speed of confocal micro-Raman micro-spectroscopy [1].

References

1. I. Rocha-Mendoza, J. Licea-Rodriguez, M. Marro, O. E. Olarte, M. Plata-Sanchez, and P. Loza-Alvarez. "Spontaneous Raman light sheet microscopy using cw-lasers and tunable filters", *Bio-med. Opt. Express*. 6(9), 3449-3461 (2015).