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High-speed Multicolor Stimulated Raman Imaging Enabled by a Compact and Robust Light Source

We present our recent developments in utilizing a compact and portable light source for high-speed multicolor stimulated Raman scattering imaging (SRS) in biomedical and medical environments.

For visualizing cells and tissue in SRS with high chemical specificity, successive images at multiple vibrational resonances must be acquired at video-rate speed. Recent approaches to video-rate multicolor SRS, based on parallel laser amplifiers or spectral focusing techniques [1,2,3], indeed allowed wavelength switching on a timescale of (sub)milliseconds, but restricted the tuning-bandwidth to below 300 cm^{-1} at maximum, significantly limiting the chemical specificity. In contrast, the here presented all-fiber light source is tunable within milliseconds across the broad spectral range between 700 and 3300 cm^{-1}, spanning the fingerprint, silent and high-wavenumber regions. To demonstrate the capabilities of the light source, it was applied in a proof-of-principle experiment for the differentiation of lipids and deuterated components with video-rate multicolor SRS imaging [4]. Successive images of the same sample were acquired with an acquisition time of 125 ms for each image (pixel dwell time of 750 ns, 100 x 100 pixel), limited only by the scanning speed and the sampling rate of the used home-built microscope. The excitation was switched in a frame-by-frame manner between 2150 and 2850 cm^{-1} in only milliseconds, a negligible time span compared to the acquisition time. As a result, a multicolor video of the sample with a frame rate of 8 Hz, i.e., a sub-framerate of 4 Hz for each wavenumber was recorded. Differentiating lipids and deuterated components with high specificity is a key requirement of several biomedical investigations, such as drug screening or imaging of metabolic changes in organisms and has not been shown with video-rate speed before. In order to evaluate the performance of the presented system for organism study, it was recently used by Hongli Ni et al. to image C.elegans in both fingerprint and C-H region [5]. Three characteristic vibrations were imaged for a quantitative analysis of the organism's lipid metabolism: the 1650 cm^{-1} vibration (acyl C=C bond) shows the distribution of unsaturated fatty acids; the 1670 cm^{-1} vibration (sterol C=C bond) shows cholesterol distribution; the 2845 cm^{-1} vibration in the C-H region shows the overall lipid storage. Furthermore, by implementing active monitoring and closed-loop regulators, we have achieved long-term power stability with a standard deviation of less than 0.3 % and wavelength stability better than 5 pm throughout 100 h. These developments constitute necessary steps for advancing SRS imaging in terms of reliability, ease-of-use, and specificity for applications in biomedical and medical environments.

References

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