

Label-Free Biomedical Imaging: Stimulated Raman Scattering and Stimulated Emission Microscopy

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Label-free chemical contrast is highly desirable in biomedical imaging. Spontaneous Raman microscopy provides specific vibrational signatures of chemical bonds, but is often hindered by low sensitivity. We report a three-dimensional multiphoton vibrational imaging technique based on stimulated Raman scattering (SRS). The sensitivity of SRS imaging is significantly greater than that of spontaneous Raman microscopy, which is achieved by implementing high-frequency (megahertz) phase-sensitive detection. SRS microscopy has a major advantage over previous coherent Raman techniques in that it offers background-free and readily interpretable chemical contrast. We show a variety of biomedical applications, such as differentiating distributions of omega-3 fatty acids and saturated lipids in living cells, imaging of brain and skin tissues based on intrinsic lipid contrast, and monitoring drug delivery through the epidermis.

Many chromophores absorb but have undetectable fluorescence because the spontaneous emission is dominated by their fast non-radiative decay. Yet the detection of their absorption is difficult under a microscope. We use stimulated emission, which competes effectively with the nonradiative decay, to make the chromophores detectable, and report a new contrast mechanism for optical microscopy. In a pump-probe experiment, on photoexcitation by a pump pulse, the sample is stimulated down to the ground state by a time-delayed probe pulse, the intensity of which is concurrently increased. We extract the miniscule intensity increase with shot-noise-limited sensitivity by using the same high-frequency (megahertz) phase-sensitive detection as SRS. The signal is generated only at the laser foci owing to the nonlinear dependence on the input intensities, providing intrinsic three-dimensional optical sectioning capability. In contrast, conventional one-beam absorption measurement exhibits low sensitivity, lack of three-dimensional sectioning capability, and complication by linear scattering of heterogeneous samples. We demonstrate a variety of applications of stimulated emission microscopy, such as visualizing chromoproteins, non-fluorescent variants of the green fluorescent protein, mapping transdermal drug distributions without histological sectioning, and label-free microvascular imaging based on endogenous contrast of hemoglobin.

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