Stimulated Raman Scattering Microscopy for Label-Free Chemical Imaging

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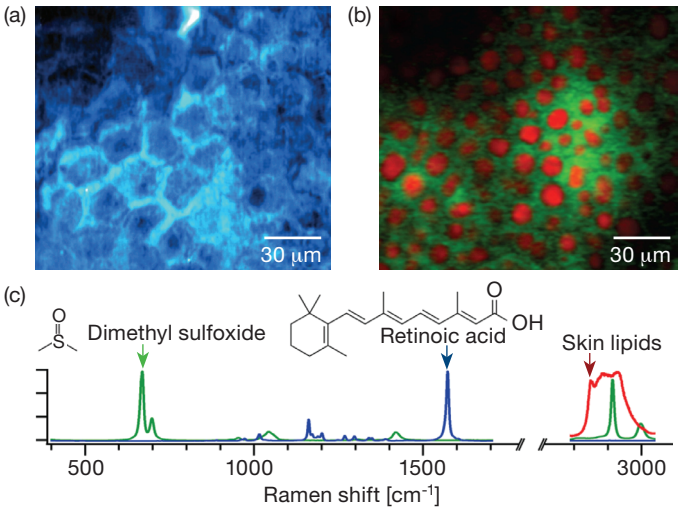
We recently developed a multiphoton imaging technique, stimulated Raman scattering (SRS) microscopy, which allows chemical imaging of biological samples without the need for staining or fluorescent labeling.[1] Such label-free imaging is desirable in biomedical research, because labeling often perturbs the function of small metabolites and drug molecules and may be too toxic to use *in vivo*.

The contrast of the technique originates from molecular vibrational frequencies intrinsic to specific chemical compounds. Imaging based on vibrational spectroscopy has traditionally relied on infrared absorption or spontaneous Raman scattering, which have limited utility for biomedical imaging because of low spatial resolution and slow imaging speed, respectively. In SRS, however, the Raman signal is enhanced by orders of magnitude by virtue of the stimulated excitation of molecular vibrational transitions, allowing for fast image acquisition. As in multiphoton-excited fluorescence, the nonlinear intensity dependence of SRS also allows intrinsic 3-D sectioning capability.

Although Ploetz et al.[2] reported SRS microscopy, the high peak power lasers and low sensitivity were incompatible with biological applications. We developed a different approach: Instead of boosting the nonlinear signal by using very high peak powers, we implemented a high-frequency phase-sensitive detection scheme to extract the week SRS signal that would otherwise be buried in laser noise of orders of magnitude higher amplitude.[3] This approach allows for superb sensitivity at biocompatible excitation power levels.

Unlike coherent anti-Stokes Raman scattering,[4] SRS microscopy does not suffer from an unwanted nonresonant background signal that limits sensitivity, distorts vibrational spectra and introduces imaging artifacts. As such, SRS is no longer limited to imaging only the strongest Raman bands; it allows access to vibrations in the crowded fingerprint region of Raman spectra. The SRS signal is also linear in analyte concentration and free from complications related to phase-matching conditions. This allows for a more easily interpreted image contrast and makes coherent Raman imaging more accessible to the broader biomedical community.



Label-free imaging of drug delivery. Lateral profile in mouse skin of (a) topically applied retinoic acid (RA) and (b) dimethyl sulfoxide (DMSO). Images were acquired with SRS microscopy tuned into the Raman shifts (c) of RA at 1570 cm^{-1} (blue) and DMSO at 670 cm^{-1} (green). Skin structures highlighted by tuning into the CH_2 stretching vibration of typical skin lipids at 2845 cm^{-1} (red). The hydrophobic RA penetrates through the lipid-rich intercellular space of the stratum corneum in (a), while the hydrophilic DMSO avoids lipid-rich skin structures such as the adipocytes in the subcutaneous fat layer (b).

We have demonstrated a variety of biomedical applications, including following the uptake of omega-3 fatty acids by living cancer cells and monitoring the transdermal delivery of topically applied drugs into mouse skin.[1] The figure shows SRS images of retinoic acid and dimethyl sulfoxide. We visualized that the different compounds followed distinct penetration pathways into the skin, highlighting the potential of SRS microscopy to study pharamacokinetics.

The strongest SRS signals in biological samples originate from CH_2 stretching vibration from lipids. They can be utilized for rapid imaging of tissue morphology without staining.[5] As such, coherent Raman imaging can be used for *in vivo* virtual histology of fresh and unfixed tissue and for distinguishing healthy tissue from diseased or tumorous tissue. This will allow for minimally invasive optical biopsies based. ◭

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