Enhanced sensitivity in label-free live-cell imaging using multi-pass stimulated Raman scattering microscopy

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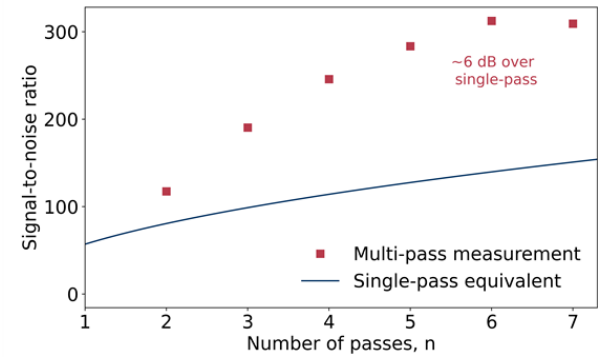
Background incl. aims
Stimulated Raman scattering (SRS) microscopy is a label-free imaging technique used for measuring chemical concentrations with high spatial resolution. The chemical sensitivity of SRS microscopy is limited by photostress or photodamage in sensitive specimens or by the timescales of fast dynamics. To increase the signal-to-noise and thus the sensitivity of SRS microscopy in live samples, we implement a multi-pass imaging protocol.

Methods
Two femtosecond near-infrared laser beams—a pump beam and a Stokes beam—are used to drive SRS. Pulses from both beams are chirped to gain spectral resolution in the measurements [1]. A Pockels cell is used to rotate the polarization of the Stokes pulses, first to initiate and then to end the multi-pass process. An intensity modulation on the pump beam results in a time-varying intensity gain in the multi-passed Stokes beam due to SRS, which is detected with a lock-in amplifier.

Results
We demonstrate an enhanced signal-to-noise ratio in SRS measurements at constant optical dose relative to equivalent single-pass measurements in both spectroscopy and imaging, with signal-to-noise increases currently of about 6 dB. We further characterize the performance of the microscope as a function of the number of interactions between the Stokes pulse and the sample and numerically study the tradeoffs and optimal operating conditions for multi-pass imaging. Using this microscope, we image live biological targets including plant roots and nematodes and use the chemical specificity of SRS to differentiate important biomolecules with subcellular spatial resolution.

Conclusion
Multi-pass protocols enable higher signal-to-noise measurements and thus increased sensitivity or imaging speed, as we demonstrate here for label-free live-cell imaging in an SRS microscope. Multi-pass techniques can be used to increase the sensitivity of many dose-limited measurements up to fundamental quantum limits [2, 3]. We show that multi-passing overcomes the shot-noise-limited signal-to-noise ratio of a standard single-pass experiment conducted with the same total optical dose. This increased sensitivity could enable new measurements of the spatial distribution of low-concentration or weakly scattering molecules in dose-sensitive biological specimens without the need for disruptive labels.

Graphic:



Keywords:

Label-free, stimulated Raman scattering

Reference:

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