High-speed spectroscopic stimulated Raman scattering microscopy for measuring biofuel synthesis

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Project Goal: Spectroscopic stimulated Raman scattering (SRS) produces label-free chemical maps of molecules in living systems. However, the limited speed of the conventional SRS platform makes it challenging to study highly dynamic or large-scale samples. To resolve biofuels at the single-cell level in real time, we are developing a high-speed SRS system by incorporating an ultrafast polygon scanner as a delay tuner. Using the proposed method, we achieved an acquisition speed of up to 20 μs per SRS spectrum. Direct measurement of fatty acid production levels in hundreds of individual *E. coli* cells within 4 seconds is demonstrated. Such high-throughput single-cell measurements provide insights about production levels and phenotypic variation among cells. Understanding these properties can be exploited to improve the design of biofuel production strains.

Abstract

Stimulated Raman scattering (SRS) is an emerging imaging modality that produces label-free maps of chemical vibration bonds in living biological environments. Compared to spontaneous Raman, the SRS signal is generated by overlapping two lasers, termed pump and Stokes, to generate Raman signals with up to 10^6 orders of signal enhancement. By exciting a single Raman band, SRS can reach video-rate. However, most biomolecules have overlapping Raman signatures, which reduces its chemical specificity. To overcome the limitation, spectroscopic SRS has been developed, which can generate a series of SRS images at consecutive Raman excitation bands. Yet, the imaging speed of current spectroscopic SRS is limited to minutes per image stack, which is insufficient for rapid imaging of biofuel production for a large *E. coli* production library at the single-cell level.

We use spectral focusing to generate SRS spectrum at high speeds. The scheme of spectral focusing is shown in **Fig. 1a**. Two femtosecond pulses are chirped by glass rods such that different wavelength components are temporally separated, by changing the time delay of the chirped pulses, the beating frequency is changed to excite different Raman band. The setup for the SRS system is depicted in **Fig. 1b**. Two 80 MHz femtosecond pulses generated by the same laser source (Insight DeepSee+, Spectra Physics, CA) serve as pump and Stoke lasers. The Stokes beam is directed to a polygon scanner and is subsequently scanned to a blazed grating. The grating is set to Littrow configuration such that the first-order diffraction beam is retroflected to the same optical path. The scanning of the polygon mirror results in a path difference of a few millimeters for the

retroflected beam. The delay range of the Stokes beam is freely adjustable by rotating the blazed grating such that the system can cover ~200 cm^{-1} of the Raman spectrum range. The introduced delay is used to tune the beating frequency of the two beams after chirping both beams by high-dispersive glass rods (SF-57).

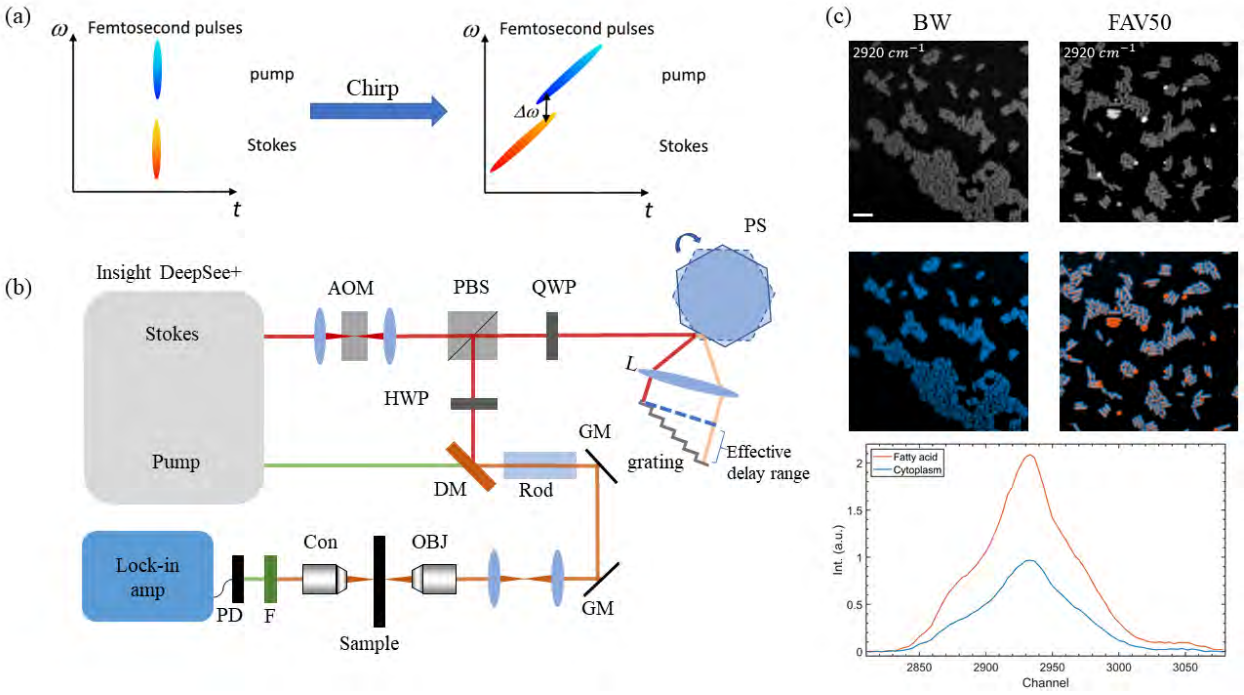

Figure 1. Experimental setup and application to imaging *E. coli* fatty acid production. (a) Concept of spectral focusing. Two femtosecond pulses are linearly chirped to separate different wavelength components in the time domain, by changing the time delay between the two pulses, the beating frequency for SRS excitation is changed. (b) Optical setup. AOM, acousto-optic modulator; C, condenser; F, filter; HWP, half-wave plate; L, lens; M, mirror; OBJ, objective; PBS, polarizing beam splitter; PD, photodiode; PS, polygon scanner; QWP, quarter-wave plate. (c) Experimental results for imaging wild-type (BW) and fatty acid producing (FAV50) strains. Single color SRS image at 2920 cm^{-1} and chemical maps for fatty acid and cytoplasm are shown, indicating a significant increase in fatty acid in the FAV50 strain. Scale bar, 10 μm.

Using the platform, we performed spectroscopic SRS imaging of *E. coli* fatty acid production strains. **Fig. 1c** shows the experimental results for wild type (BW) and fatty acid producing (FAV50) strains, where each spectroscopic image stack was acquired within 1.8 seconds. By performing linear unmixing of the spectroscopic image, we can separate fatty acid from the cytoplasm (protein) and generate chemical maps for two components. Comparing the two strains, we can observe from FAV50 an overall SRS intensity increase. In addition, the localized aggregation of fatty acid formed lipid droplets within the cell body. Cell-to-cell heterogeneity in fatty acid production levels in the FAV50 strain were also observed.

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