Live-cell imaging of *E. coli* biofuel synthesis by spectroscopic stimulated Raman scattering microscopy

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Project Goal: Fatty acids are essential biofuel precursors that can be synthesized in microbes such as *E. coli.* Currently, quantitation of fatty acid production levels mainly relies on ensemble measurements such as gas chromatography-mass spectrometry (GC-MS), which ignores the potential for genetic or phenotypic variation among cells. Stimulated Raman scattering (SRS) is a high-speed imaging modality that produces label-free chemical maps of molecules, allowing tracking of the production process at the single-cell level. To perform live-cell imaging, we built a microscope incubator and recorded both wide-field transmission images and SRS. The biologically safe SRS laser power enables multiple SRS acquisitions without perturbing cell growth. We performed spectral unmixing on spectroscopic SRS images to determine the concentration of fatty acids within each cell. With the platform, we can obtain information on the potential genetic or phenotypic variation among cells to improve the biofuel production yield.

Abstract

Stimulated Raman scattering (SRS) is a label-free imaging modality that offers chemical contrast based on intrinsic molecular vibrations. To improve chemical specificity in a complex biological system, spectroscopic SRS has been developed to provide a Raman spectrum at each pixel, which can be further decomposed into maps of chemical species such as protein and fatty acid. SRS has seen a wide range of biomedical applications, including cell metabolism, drug delivery and neuron voltage tracking. Fatty acid synthesis by *E. coli* is an ideal application for SRS given the strong signal of fatty acids in the CH region. Due to its high imaging speed, SRS enables imaging the dynamics of cell division and biofuel synthesis, which provides insights into genetic or phenotypic variations among cells. Here, we report our work towards the live tracking of single-cell biofuel synthesis.

To achieve live-cell imaging, we installed a microscope incubator to control the temperature. An eyepiece camera is installed to capture the wide-field of view with white light lamp illumination. We selected 15 mW for 800 nm pump and 25 mW for 1040 nm Stokes on the sample to avoid laser damage on cell growth. As shown in Fig. 1, we prepared a sample slide with an agarose gel pad to trap the cells and provide nutrition for cell growth. We performed a time-series imaging of the same field of view for a period of 19 hours. Two example time points (7 and 19 hours) are shown in **Fig. 1**, each of which consists of

a wide-field image, the raw spectroscopic SRS image and chemical maps of protein and fatty acid after spectral unmixing using the references shown in **Fig. 1c**. We observed that in the 7-hour image, cells were predominately focused on growing instead of fatty acid production, as no signal is detected in the fatty acid channel. In the 19-hour image, fatty acid signals appear in the form of aggregated droplets. Heterogeneity of fatty acid concentrations among different cell colonies can be observed in the fatty acid map. In the future, we will perform the study on *E. coli* strains with various fatty acid production levels, with a focus on the dynamics of the fatty acid synthesis process and cellular heterogeneity.

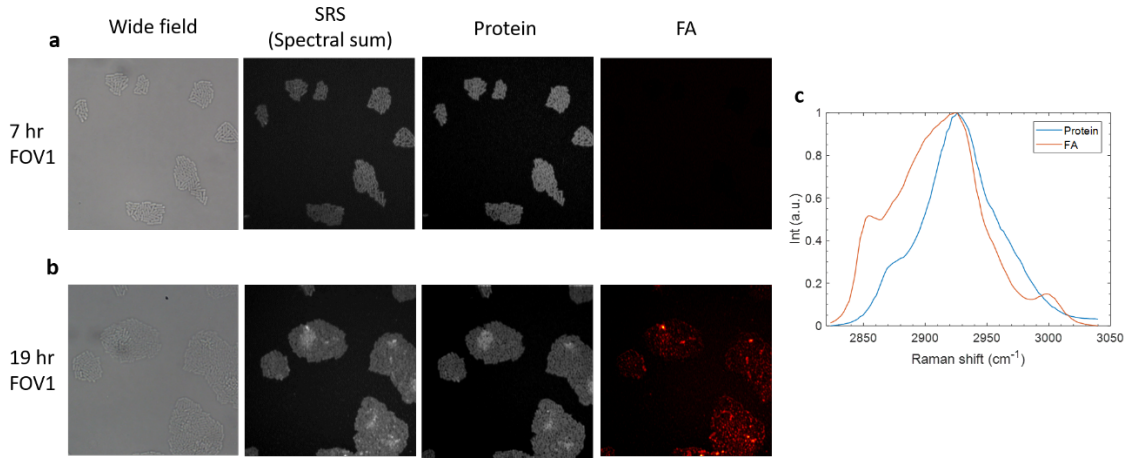

Figure 1. Live-cell imaging of *E. coli* with spectroscopic SRS imaging. (a-b) 7-hour and 19-hour imaging of the same field of view. From left to right: wide-field transmission image, SRS (spectral sum), protein concentration map, fatty acid, FA concentration map. (c) Spectral profiles of protein and fatty acid, FA.

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