**High-throughput Chemical Imaging for Optimizing Biofuel Synthesis Using Synthetic Biology**

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**Project Goals: Develop a chemical imaging platform to provide a direct, high-throughput method for imaging biofuel production. Major goals include: (1) Establish a chemical imaging platform for rapid quantification of biofuel production. (2) High-throughput, multiplexed strain engineering enabled by chemical imaging. (3) Optimizing biofuel production using imaging for cell sorting and selection.**

Recent advances in the fields of synthetic biology and metabolic engineering have resulted in an unprecedented ability to engineer genomes and design and build gene circuits for improving biofuel production. However, the ability to design and build these genetic variants has far outpaced methods for assessing biofuel production. Current methods for quantifying production rely on low-to-medium throughput approaches such as GC-MS, or indirect measurements such as those using biosensors coupled with fluorescent reporters. A direct, high-throughput method for imaging biofuel production *in vivo* has the potential to greatly advance our ability to rapidly design, build, and test strains for enhanced biofuel synthesis. This project addresses this gap by introducing a technology for directly measuring synthesis of biofuels in living cells.

We use a chemical imaging method called stimulated Raman scattering (SRS) microscopy. SRS uses photons to produce a vibrational spectrum on the microsecond time scale. Such high speed allows real-time chemical mapping of a sample at sub-micron diffraction-limited spatial resolution. This capacity is significant for quantifying biofuel synthesis because it can directly reveal production levels and further distinguish between different structures of chemical bonds. In this project we focus on fatty acids, which are biodiesel candidates and can serve as precursors to high value oleochemicals. We are developing a high-throughput platform for chemical imaging of biofuel production, which will be used in concert with multiplexed genome engineering and gene circuit design strategies to improve *E. coli* fatty acid production. Overall, our goal is to develop SRS imaging as a new technology for directly measuring chemical signatures in *in vivo* samples for the engineering and optimization of biofuel production strains. Here, we present results towards these goals including SRS imaging of fatty acid biosynthesis for different engineered strain backgrounds, and a multiplexed CRISPRi-based approach to strain generation. In addition, we will present advances in the imaging methodology that dramatically increase image acquisition speeds, enabling the potential for high-throughput imaging.

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