A Droplet Microfluidic Platform for Lab Automation

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Project Goals: The JBEI mission is to establish the scientific knowledge and new technologies to transform the maximum amount of carbon available in bioenergy crops into biofuels and bioproducts. The goal of this project, performed in the Microfluidic Assays group in the Technology Division at JBEI, is to develop a robust and easy-to-use droplet microfluidic platform to automate the steps involved in engineering of metabolic pathways to produce biofuel molecules.

Synthetic biology offers a promising approach to produce biofuel and other chemicals. Optimization of metabolic pathways however, requires conducting a large number of experiments that are labor-intensive with repetitive pipetting and plating and require large amounts of expensive reagents. Robotic liquid handling stations represent a solution to automate genetic engineering processes however, they still require large volume of reagents and their high equipment and maintenance cost can be prohibitive to many users. Microfluidic platforms have attracted a significant attention for performing biochemical reactions and analysis as they provide improvement over their macroscale counterparts in cost, amounts of reagents required, speed, and integration.

We are developing microfluidic devices for many biofuel research applications including enzyme screening, enzyme evolution, and synthetic biology. Our droplet-based microfluidic platforms use digital microfluidic (DMF) format where tiny (nL) aqueous droplets suspended in oil are manipulated on an electrode array using electrowetting on dielectric concept (references). The systems can handle large numbers of droplets at once as well as actively manipulate droplets in a programmable manner, and are capable of multiple steps of droplet manipulation including formation of aqueous droplets and encapsulation of reagents and cells, electric-field driven merge and split of the droplets to add or remove liquid, on-chip electroporation, and incubation steps with localized temperature control. Electroporation is achieved by placing pairs of electrodes in each chamber to apply voltages to the arrayed droplets. This configuration allows us to customize the electroporation condition at each droplet and scale-up the numbers of

reactions by making high-density electrode arrays. We integrate optical fibers in the microchannels for fluorescence-based detection of encapsulated cells and enzymatic activities in the discrete droplets, and for triggering sorting of droplets. We used the microfluidic platform for automating CRISPR-based MAGE recombineering in *E. coli* to optimize the biosynthetic pathway of an example molecule, indigoidine.

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