

Droplet-Based Analog and Digital Microfluidic Platforms for High-Throughput Screening and Synthetic Biology Applications

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<https://www.jbei.org/research/divisions/technology/microfluidic-assays/>

Project Goals:

The JBEI mission is to conduct basic and applied research to enable economically-viable conversion of lignocellulosic biomass into biofuels to provide the nation with clean, renewable transportation fuels identical to gasoline, diesel and jet fuel. The goal of this project, performed in the Microfluidic Assays group in the Technology Division at JBEI, is to deliver the robust and easy-to-use microfluidic platforms to automate the synthetic biology process for advancing biofuels development.

In recent years, synthetic biology has dramatically grown and became significantly important for both of scientific researches and industrial applications such as biofuel and pharmaceutical applications. However, multiple genetic engineering steps required for synthetic biology are often time-consuming and labor-intensive with repetitive pipetting and plating. Therefore, automated and efficient processes to perform molecular biology assays have been long desired. Microfluidic assays and devices with aqueous droplets (microliter to picoliter in volume) suspended in oil phase as compartmentalized reaction chambers have attracted a significant attention for performing biochemical reactions and analysis as they provide drastic improvements over their macroscale counterparts with various benefits such as faster reaction time, less volume of reagent consumption required, better control of experimental environment, and higher throughput with multiplexed processes.

We are involved in developing innovative microfluidic assays and integrated devices for many biofuel research applications including enzyme screening, enzyme evolution and synthetic biology. Our hybrid microfluidic platforms utilize continuous-flow (analog) microfluidics that manipulate the droplets by controlling the hydrodynamic force, and digital microfluidics (DMF) that utilize surface tension from electrowetting on dielectric with arrayed electrodes. The systems can handle large numbers of droplets at once as well as actively manipulate target 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, hydrodynamic capture and array of the droplets, electric-field driven merge and split of the droplets to add specific amount and concentrations of various reagents, and incubation process with localized temperature control. In addition, we integrate optical fibers in the microchannels to add on-chip capability for fluorescence-based detection of encapsulated cells and enzymatic activities in the discrete droplets, and for triggering sorting of droplets. We are also integrating this platform with mass spectrometry to allow sensitive, label-free detection of chemicals and biofuels produced by the engineered cells.

One example application of our system is automation of synthetic biology experiments. Optimization of pathways can involve very large number of experiments as multiple variants are available for each gene. Our platform can integrate and automate the processes of DNA assembly, transformation, and cell culture in one device. We show that the platform is capable of accurate DNA assembly, efficient transformation, and cell culture and is compatible with many cloning methods (e.g., Golden Gate and Gibson) and chassis organisms (e.g., bacteria, yeast and fungus). We additionally demonstrate capability of the system for on-chip gene editing of *Saccharomyces cerevisiae* utilizing the CRISPR-Cas9 based cloning-free toolkit.

Unlike conventional microtiter plate based reactions, our analog-digital microfluidic platforms with on-chip fluorescence detection allow completely automated genetic engineering steps using 10-100-fold lower amounts of reagents and can be useful for application requiring high throughput screening and reactions, and integration with mass spectrometry enables higher sensitivity detection.

References

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