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プロジェクト名： 基本システム開発

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実 施 状 況 報 告 書（成果）

平成２７年度

研究開発課題名：

Development of a microfluidic technology that focuses and orders single cells in a microchannel with high throughput

研究開発機関名：

University of California, Los Angeles

研究開発責任者

Dino Di Carlo

Abstract

1. Activities, Accomplishment and Findings

This project aims to develop a microfluidic technology that enables three-dimensional (3D) single-stream, single-focal plane focusing of cells varying in shape and size. By taking advantage of radiofrequency-tagged emission (FIRE) microscopy and microfluidic flow cells, *Euglena gracilis* (*E. gracilis)* cells, a desirable source for biofuel production, are focused, monitored, and screened in a high-throughput and high-efficiency manner to evaluate lipid productivity per biomass. During this reporting year, we engaged in the aforementioned overall aim along with accompanying activities, accomplishments, and findings as shown below:

1.1 Activities

1.1.1 Design, fabrication and characterization of microfluidic devices

We utilize a stepped microchannel device consisting of a low-aspect-ratio (i.e. 0.25) straight microchannel and a series (i.e. 30) expansion regions in channel height to focus *E. gracilis* in a high-throughput manner. By making use of inertial focusing and geometry-induced local secondary flows, *E. gracilis* having diverse shapes are directed to a single equilibrium position in a single focal stream. As shown in Fig. 1A, at finite Reynolds number, the movement and position of target *E. gracilis* cells in the four regions indicated by sequence numbers i, ii, iii and iv, from left to right are distinct: (i) cells are randomly distributed close to the inlet; (ii) cells migrate to mainly two positions at the long surface of the microchannel due to the balance of shear gradient lift force and wall-effect lift force; (iii) cells migrate laterally in response to the pair of helical secondary flow induced by stepped channels; (iv) *E. gracilis* cells are directed to a final single equilibrium position in a single focal plane. The device is fabricated by standard two-step photolithography and polydimethylsiloxane (PDMS) replica molding techniques.

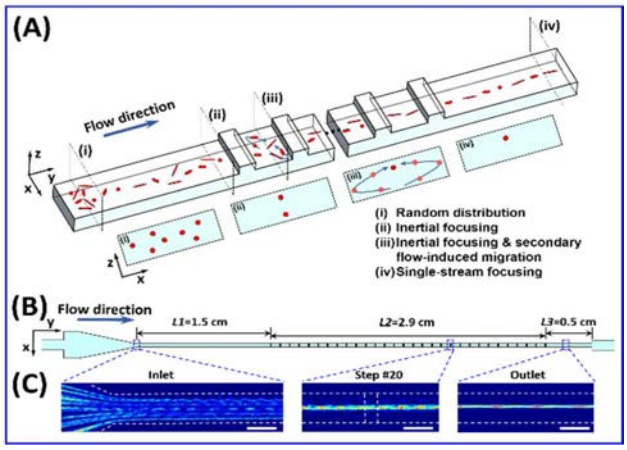

Figure 1 Schematic view of the device design and focusing principles.

The channel width and height is 120 and 30 μm, respectively, and there are a total of 30 stepped channels spaced out 1 mm apart with a height of 30 μm and a length of 40 μm.

1.1.2 Analysis of the performance of developed devices

We examined how the channel Reynolds number (*Re*) and cell aspect ratio (AR) affect the dynamic equilibrium position, *preferential orientation*, rotational and translational motions of *E. gracilis* cells flowing through the microchannel (see Fig. 2) as these motions are critical in developing cytometry systems. The key finding is that the complex and variable shapes in a population of *E. gracilis* makes it difficult to define one operating condition. Our recommendation is to use methods that modulate the shape of *E. gracilis* to a spherical shape to enhance uniformity of behavior.

In our experiments the microfluidic device was operated by a syringe pump. The flow rate in the experiment was varied from 50 to 1000 μl/min, which corresponds to an average fluid velocity from 0.23 to 4.63 m/s. The motion and behaviors of *E. gracilis* cells were monitored by an inverted microscope, and recorded by a high-speed camera. The exposure time was set to 1 μs. A custom MATALB routine and ImageJ were applied to post-process images. As can be seen in figure 2 the AR of euglena cells greatly affects their orientation and positioning in the z-drection, which both affects the downstream velocity. Variable downstream velocity leads to image stretching in line scan based optical analysis techniques, and difficulty in timing sorting decisions. As discussed in a section below, methods for transforming the shape of euglena to a sphere are promising to solve these challenges.

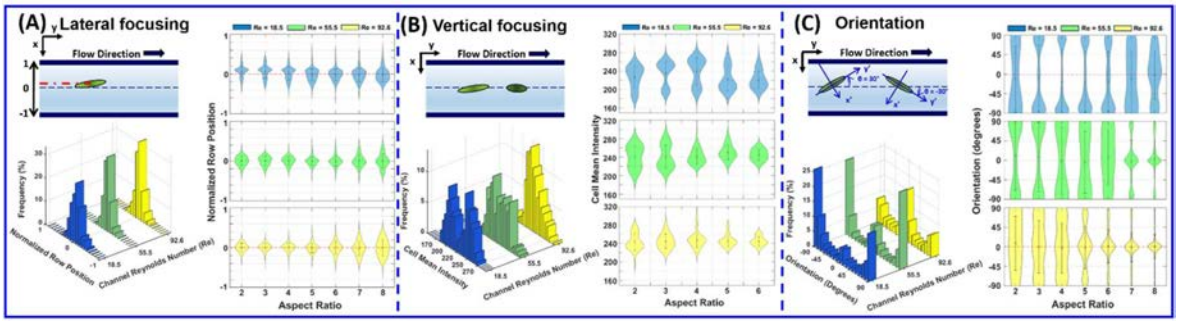

Figure 2 The effects of Re and AR on lateral focusing (A), vertical focusing (B) and orientation (C) of ellipsoid E. gracilis.

1.1.3 Evaluation of lipid production using FIRE fluorescence imaging flow cytometry

We quantify intracellular lipid droplets (LDs) in *E. gracilis* via high-throughput fluorescence imaging flow cytometry based on radiofrequency-tagged emission (FIRE), and automated image analysis. As a proof of concept, LDs present in *E. gracilis* that were grown under different conditions were analyzed, characterized, and compared. As shown in Fig. 3, images in three channels, bright-field (morphology and size), FITC (lipid-BODIPY 505/515 and chlorophyll-autofluorescence), and Cy5 (chlorophyll), are first measured. Then a Laplacian of Gaussian (LoG) filter is applied, and a watershed algorithm is used to segment the LDs. Also, Normalized Mutual Information (NMI) and Normalized Cross-Correlation (NCC) maps are computed to exclude regions of high similarity between FITC and Cy5 channels and compensate for the high autofluorescence of chlorophyll. Finally, the value of LDs to cell area ratio (LCAR), the parameter for evaluating lipid productivity per biomass, is calculated.

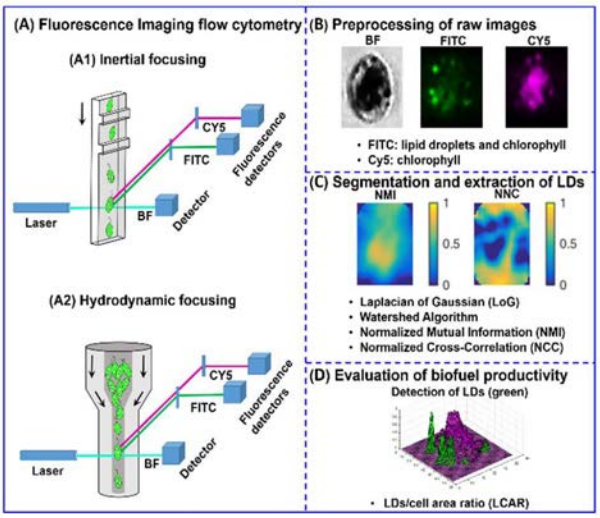

Figure 3 The workflow of FIRE imaging flow cytometry for E. gracilis lipid production evaluation.

1.2 Accomplishment

The accomplishments are divided into three main phases described below, which are also according to the activities outlined above.

1.2.1 **Developed an inertial microfluidic platform that is able to focus *E. gracilis* cells with varied sizes and shapes in a single focal stream.** The proposed design features high throughput, high focusing efficiency and purity, elimination of sheath fluid and external force, simplicity in operation, and easy integration. It can be potentially applied to flow cytometry, imaging flow cytometry, and integrated with other on-chip functional components for more applications. The approach works extremely well for spherical objects such as cells in blood, or sphered euglena cells.

1.2.2 **Clarified the effects of *Re* and AR on the focusing and dynamics of ellipsoid *E. gracilis* cells in a step microchannel.** With this understanding, it would help to predict the position and alignment of shaped cells by inertial focusing and secondary flow, to address uncertainty in cytometry caused by the deviation from spherical symmetry, and to produce more reliable and stable measurements.

1.2.3 **Demonstrated the use of FIRE fluorescence imaging flow cytometry as an information-rich method to evaluate algae lipid productivity per biomass**. We have initially evaluated the parameter LCAR that quantifies the lipid drops (LDs) per cell area. This metric correlates with lipid production in bulk measured by gravimetric method, and may be less sensitive to lipid dye differences or other intensity differences between experiments. These metrics can help identify high-achieving individual cells, and explain differences between cell populations. Moreover, this technique may be useful for screening, monitoring and evaluating *E. gracilis* and potentially other microalgae species for biofuel production applications.

1.3 Findings

- The combined effects of inertial focusing and secondary flow can be utilized to focus ellipsoid cells into a single focal stream, although AR has a large effect on downstream velocity.
- The focusing of ellipsoid *E. gracilis* cells is controllable with microchannel geometry, flow rates, and cell aspect ratio. Lateral focusing, vertical focusing, and non-rotational behavior frequency increase with flow rate, and elongated cells prefer the horizontal orientation and non-rotational behavior.
- Cellular shape is a parameter indicating lipid content in single *E. gracilis* cells: spherical *E. gracilis* cells were found to contain more lipid than elongated ones using the FIRE cytometry approach.

2. Outreach, Events and Other Activities

2.1 Encapsulation of *E. gracilis* for directed evolution

Encapsulation of *E. gracilis* in aqueous droplets is attractive as an alternative way to eliminate velocity fluctuation in microfluidic chips caused by the variation in shape and size of *E. gracilis* cells. Moreover, it allows compartmentalization for selecting desirable species or individual cells based on biomass accumulation and growth in parallel arrays of conditions and/or for separate mutants. In addition, droplet manipulation such as addition (merging) and subtraction (dividing) and sorting is possible. We encapsulate cells using a 3D-printed microfluidic droplet generator using fluorinated oil and surfactants for gas exchange. The viability of the encapsulated *E. gracilis* is assessed by incubating and monitoring cell growth.

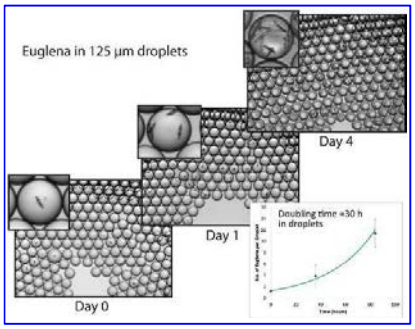

Figure 4 The growth of E. gracilis encapsulated in 125 µm droplets.

It shows that *E. gracilis* survive well the encapsulation process without any visible side effects, and the doubling time is around 30 hours in 125 μm droplets (see Fig. 4). We saw reduced or no growth in smaller droplets.

2.2 Regulation of *E. gracilis* shape under the synchronizing effect of a daily light/dark cycle

The results of our studies indicated that an effective method to make *E. gracilis* shape and size more uniform can help improving focusing performance of the microfluidic platform, and facilitate cell sorting downstream. We demonstrated that the shape of *E. gracilis* cells changes in a rhythmic manner under the control of the biological clock, when grown under the synchronizing effect of a daily light/dark (14h/10h) cycle. As shown in Fig. 5, the values of cell length and aspect ratio increase to a maximum in the middle of light period (13.00 pm), and decrease for the remainder of the time in the cycle. While at the beginning of the light period (6.00 am), and end of dark period (6.00 am of next day), the population of cells largely become spherical in shape which can be more easily focused and sorted.

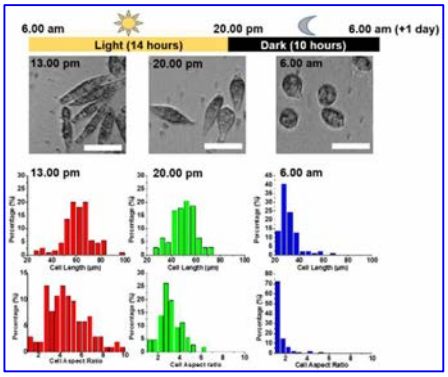

Figure 5 The variation of E. gracilis shape within a daily light/dark cycle.

Other Activities

- We participated in an elementary school's science slam by operating demonstrations of using a home-made microscope combined with cell phones such that children could take photos and movies of *E. gracilis* twisting, wiggling, and swimming movement.
- Postdoc (Carson T. Riche), and two development engineers (Edward Pao, and Alexander Schmidt), were recruited and started work in early 2016. Also, I supported a postdoc (Ming Li), and a graduate student (Hector Munoz) during this reporting year.