プログラム名：　セレンディピティの計画的創出による新価値創造
PM 名：　合田　圭介
プロジェクト名：　基本システム開発

委　託　研　究　開　発

実 施 状 況 報 告 書（成果）

平成 28 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：

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研究開発責任者

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Abstract

1. Activities, Accomplishment and Findings

1.1 Activities

1.1.1 Development of microfluidic devices for shape-based cell sorting

We developed inertial microfluidic devices to sort algae cells, such as *Euglena gracilis* based on their shape for the first time. We use a microfluidic device consisting of an inlet, a high aspect ratio (AR, height/width >2) straight microchannel, a gradually expanding region, and five branched outlets each terminated with fluidic resistors (i.e. serpentine channels). At finite Reynolds number, the movement and position of *E. gracilis* cells in the three regions indicated by sequence numbers i, ii, and iii, are distinct: (i) *E. gracilis* cells are randomly distributed close to the inlet; (ii) *E. gracilis* cells with various shapes generally migrate to two equilibrium positions along the long faces of the microchannel due to the balance between a shear-gradient lift force and a wall-effect lift force, and long rod-shaped cells migrate to positions closer to the channel centerline than spherical ones; (iii) the differences in shape-dependent lateral equilibrium positions are enhanced while cells continue along their focusing streamlines (Fig. 1). Finally, *E. gracilis* cells with variable shapes are directed to different outlets: spherical cells exit from the outlets closest to the channel sidewalls, while longer rod-shaped cells are collected from the outlet closer to the channel centerline. The whole microchannel has a uniform height of 90 μm. The width and length of the straight channel is 40 μm and 4.2 cm, respectively. The expansion region was designed by increasing the angle between the channel wall and flow direction by 2° per 100 μm. The serpentine microchannels serving as fluidic resistors at the outlets are composed of 20 U-shaped periods with a channel width of 40 μm and a total path length of around 2.5 cm.

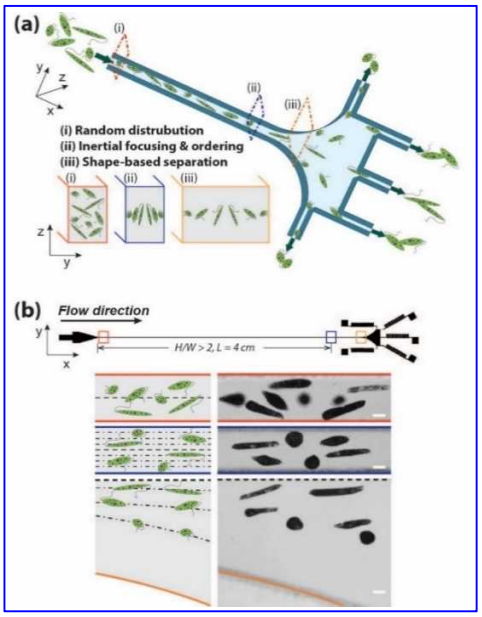

Figure 1 Schematic (a) and experimental (b) illustration of the device design and sorting principles for shape-based algae sorting.

1.1.2 Analysis of the performance of the developed microfluidic sorter

We study the behaviors of *E. gracilis* cells with different ARs ranging from 1 to 7 under inertial effects in a straight microchannel region, an expansion region, and outlets (Fig. 2). The dynamic inertial focusing equilibrium positions are dependent on *E. gracilis* cell AR as discussed above. *E. gracilis* with particular ARs migrate to distinct lateral equilibrium positions, and exit from

different outlets. *E gracilis* suspensions with a concentration of around 10^5 cells/mL were injected into the microchannel at flow rates ranging from 300 to 800 µL/min (channel Reynolds number, Re=77-205) using a syringe pump (Harvard Apparatus, MA, USA). The motion and behaviors of *E. gracilis* cells were monitored and recorded using an inverted microscope (Nikon Ti, Japan) equipped with a high-speed camera (Vision Research, NJ, USA). The high-speed images were captured with an exposure time of 1 µs, and frame rates were varied according to the channel region for measurement. Recorded videos and image sequences were processed using Phantom Camera Control Software (PCC) and a custom-built MATALB routine.

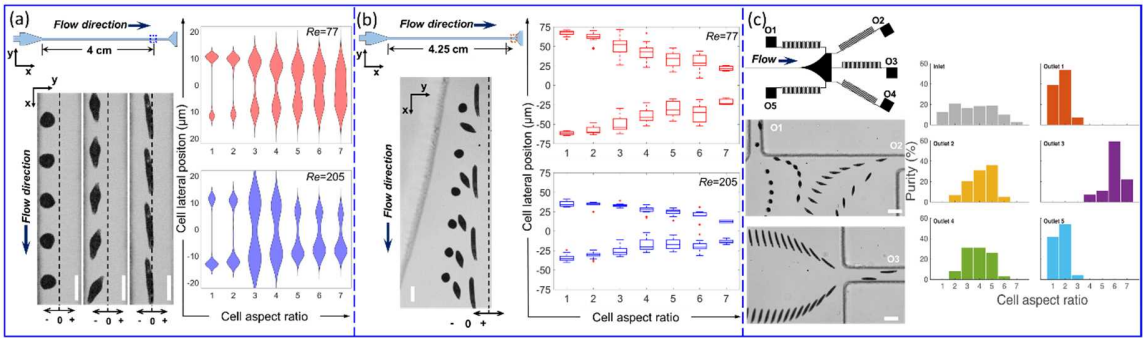

Figure 2 Shape-activated differences in lateral positions of E. gracilis cells with different aspect ratios ranging from 1 to 7 at a straight region 4 cm downstream from the inlet (a), an expansion region (b), and the collection outlets (c). High aspect ratio cells, AR>4 are enriched in outlet 3, while lower aspect ratio cells, AR<4 are enrichment in outlets 1 and 5, as can be seen in the histograms of the collected cells.

1.1.3 Investigation of metabolic and morphological heterogeneity in *E. gracilis*

We have been focusing on methods to analyze the lipid content within *E. gracilis* using fluorescence imaging and image analysis algorithms that can normalize for physical size and chlorophyll content within the cells. We utilize high-throughput fluorescence imaging flow cytometry based on radiofrequency-tagged emission (FIRE) and automated imaging analysis to visualize morphology, and intracellular lipid and chlorophyll content of single *E. gracilis* cells. At a speed as high as 1 m/s, we are able to acquire blur-free images in three channels: bright-field, FITC and Cy5. These channels correspond to morphology (area and shape), lipid stained with BODIPY and chlorophyll auto-fluorescence, respectively (Fig 3a). Moreover,

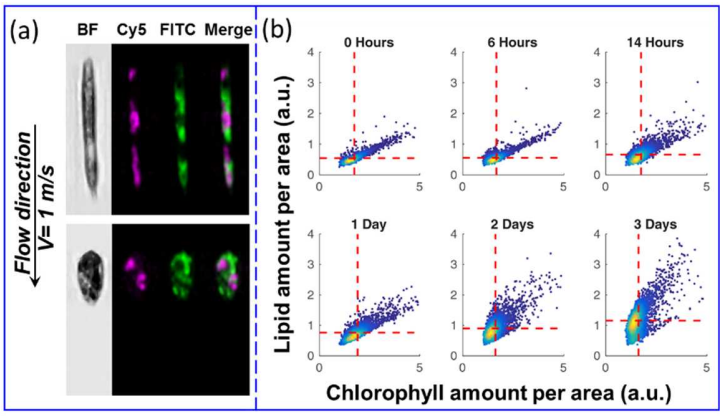

Figure 3 Representative images (a) and scatter plots (b) indicating the heterogeneity in E. gracilis subjected to fermentation for up to 3 days.

we examine the heterogeneity of morphology, lipid, and chlorophyll amount for *E. gracilis* at both single-cell and population levels under various environmental conditions, i.e. nitrogen and oxygen deficiency (Fig. 3b). We also compare the image-based analysis results to the ones based on fluorescence intensity, and to the lipid production in bulk measured by a gravimetric method.

1.2 Accomplishment

1.2.1 **Developed an inertial microfluidic platform that is able to separate *E. gracilis* by a key shape parameter-cell AR.** The platform can be operated at high flow rates, without sheath fluid and external force. Since shape is an important biomarker for *E. gracilis* in terms of day-night cycle and fermentation activity the proposed ability to prepare *E. gracilis* of uniform shape at high purities has significant implications for various applications in biological research and industrial processes.

1.2.2 **Characterized the effects of channel *Re* and cellular AR on the dynamics and sorting of *E. gracilis* cells.** With this understanding, it would be helpful to design microfluidic devices for other applications or algae with complex shapes, where shape-synchronized cell populations are important. High-efficiency shape-based separation of cells prior to cytometry could address uncertainty in cytometry caused by the deviation from spherical symmetry, and potentially produce more reliable and stable measurements.

1.2.3 **Explored the use of FIRE fluorescence imaging flow cytometry and image analysis algorithms as an information-rich method to evaluate algal metabolite productivity per biomass**. Bright-field images provide information about the morphological features of the cells, such as area and shape, and fluorescence images in FITC and CY5 channel are effective for probing the lipid and chlorophyll productivity, respectively. We have developed algorithms focusing on extracting various metric from these combined brightfield and fluorescence images of cells, such as lipid to cell area ratio (LCAR). We also have begun to characterize how other more easily computed metrics can correlate to LCAR. These metrics can help to explain morphological and metabolic differences at the levels of both single cells and cell populations, to identify high-achieving individual cells, and to screen *E. gracilis* and potentially other microalgae species for biofuel and biomass production applications, once combined with sorting technologies being developed in the ImPACT program.

1.3 Findings

- When *E. gracilis* cells with various ARs flowing through a rectangular microchannel (channel AR>2) under inertial effects, the distance to the channel centerline decreases with increasing cellular AR.

- The expansion region at a location downstream of the straight microchannel enhances the shape-activated differences in dynamic equilibrium positions, while maintaining cells in focusing streamlines.
- The fluidic resistors located in the respective outlets could be tuned to capture enriched fractions of cells having particular particle shapes, which is achieved by adjusting the amount of the fluid that splits into each outlet.
- *E. gracilis* changes its shape in response to the environment (i.e. light and oxygen levels).
- *E. gracilis* accumulates higher lipid content but lower chlorophyll content per cell area under nitrogen deficiency conditions.

2. Outreach, Events and Other Activities

2.1 Outreach

Agarose droplets for single microalgae cell analysis and sorting

In order to select algae based on growth rate, one methodology uses encapsulation in a compartment in order to track the amount of biomass accumulating from an initial known starting amount (i.e. a single cell). In order to make this type of approach compatible with traditional sorting instruments we use a liquefied agarose drop that algae are initially encapsulated into. Once encapsulated and grown for a time, the algae can be cooled to solidify and trap the algae biomass accumulated. This solidified agarose drop can then be sorted and re-melted to extract the algae for future culture. We developed a protocol interfacing agarose droplet microfluidics with fluorescence-activated cell sorter that could allow for the selection of fast growing and high lipid yielding microalgae. The actual procedures of the protocol are as follows:

a) Encapsulation. The agarose droplets in oil encapsulating single microalgae cells are generated using a microfluidic device.
b) Off-chip cell culture. The droplets are collected in a centrifuge tube, and microalgae grow within the droplets at a temperature of 29 °C and a light intensity of 100 µmol photons m^{-2} s^{-1}.
c) Phase transfer. The droplets are cooled to room temperature to form agarose beads, and then transferred to aqueous solution after washing.
d) Selection. The selection of microalgal cells with desirable properties, i.e. fast growth rate and high lipid content per volume is performed using a FACS or large particle Biosorter system.
e) Cell Recovery. The microalgae cells are released from the agarose beads after melting, further cultivated and analyzed.