MICROFLUIDIC CELL SORTER AIDED LIVE CELL SCREENING FOR IMPROVED FLUORESCENT PROTEIN

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ABSTRACT

We demonstrate a simple and low cost microFACS platform with an integrated piezoelectric actuator for directed evolution of fluorescence protein (FP) and FP-based indicators with high throughput up to 500 cells/s. The system was first employed to screen a 10^6 member library of a novel yellow FP. Compared to the manual on-plate screening method, the average brightness of the top 10 variants identified by our system was 50% brighter. The system also achieved 6-fold enrichment in isolating cells expressing superior FP-based indicator G-GECO1.0 from flash-pericam, showing the capability of screening FP-based indicators for brighter fluorescence and better functions.

KEYWORDS

Cell sorting, Directed evolution, Fluorescent protein

INTRODUCTION

Directed evolution is a powerful method to produce improved proteins with desirable properties. For example, many fluorescent proteins have undergone laboratory-based evolution for new colors, improved brightness, enhanced photostability and even calcium-sensing properties [1, 2]. These directed evolution efforts have relied on traditional on-plate screening of colony fluorescence or Fluorescence Activated Cell Sorting (FACS). Manual on-plate screening strategies can screen up to 10^6 variants and provides flexibility for screening of specific properties such as photoconversion and analyte-binding. However, the approach is labor intensive and time consuming. FACS can achieve much higher throughput (10^7 to 10^8 variants per hour), requiring costly, complex equipment. Here, we apply microfluidic technology to build a cost-effective high-throughput platform for directed evolution of FP and FP-based indicators. In pursuit of similar goals, Lubbeck and coworkers have recently reported a microfluidic system for screening photoactivation and photostability properties of FP, but with low throughput (20 cells/s) and a complex optical setup [3].

EXPERIMENT

Figure 1 illustrates a simple and low cost microFACS platform for directed evolution of FP and FP-based indicators with high throughput. PDMS microchips were fabricated using a standard soft lithography technique. A piezoelectric actuator is integrated on the PDMS chip for sorting, based on the work of Chen et al. [4] (Figure 2). The chip uses a 5-cm long channel for substrate mixing, to enable detection of the fluorescence change exhibited by variants with and without an analyte present, as well as screening for brightness. The gene libraries were constructed by error-prone polymerase chain reaction, inserted in a modified pBAD bacterial expression vector [2], then transformed into *E. coli* cells for protein expression using standard molecular cloning protocol. *E. coli* strain DH10B was used as the carrier of gene libraries. Confocal epifluorescence provided fluorescence detection of cells expressing FPs (Figure 3). The signals were processed in real time with a custom LabVIEW program, which then triggered sorting based on piezoelectric actuation.

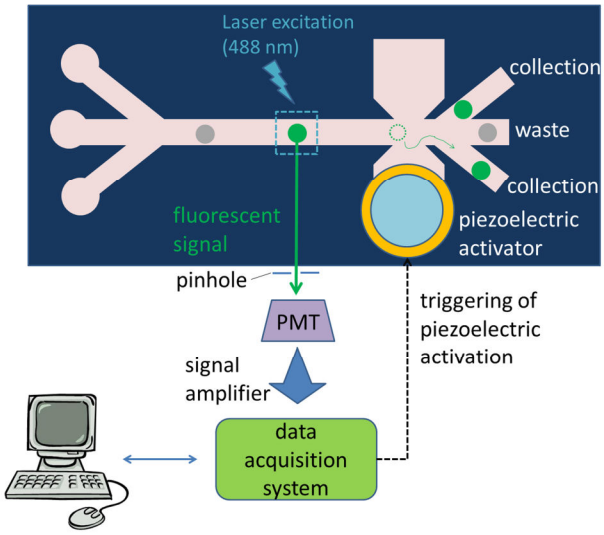

Figure 1. microFACS platform using on chip laser illumination and PZT actuator, off chip confocal epifluorescence detection, PMT signal amplification and acquisition system and electronic control system.

978-0-9798064-5-2/μTAS 2012/$20©12CBMS-0001

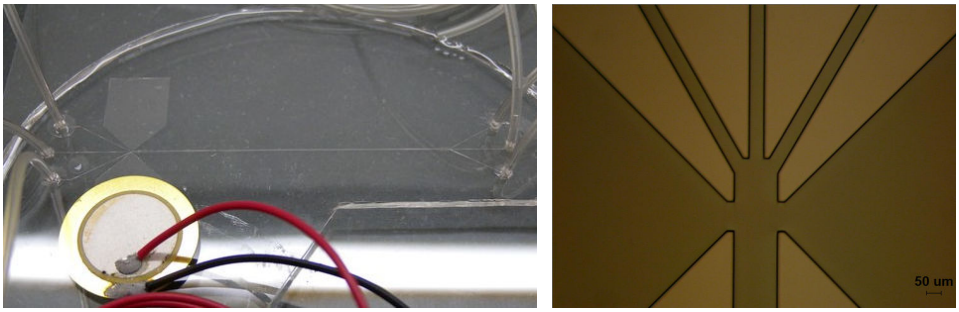

Figure 2. View of microfluidic cell sorter(upper) and microscope image of channels in sorting junction (lower, channel depth: 50 μm)

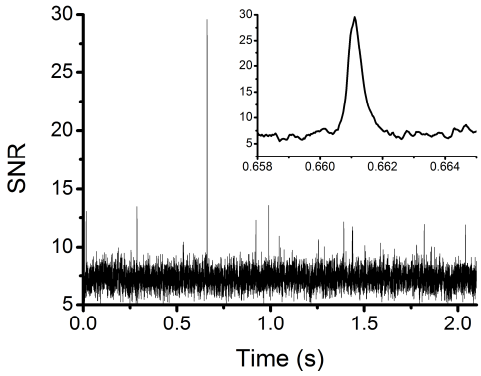

Figure 3. Signals of E. coli cells expressing YFP libraries on chip acquired by PMT detector. Inset shows the enlarged signal peak from a bright variant.

RESULT AND DISCUSSION

We first employed this system to screen a 10^6 member library of a novel yellow FP from *Zoanthus sp.* to improve brightness, with throughput of 500 cells/s. Figure 4A and B are the result of cytometric analysis before and after sorting using our microFACS system. The region of interest (ROI) indicates the distribution of the fluorescence of YFP variants and the mean fluorescence of the library increased by 77% after sorting using microFACS (Figure 4C). The sorted samples were grown on an agar plate and the 10 brightest colonies were picked up and compared to the 10 brightest colonies picked from 10^5 colonies of the same library using a manual screening method. On average, the variants identified by microFACS were 50% brighter than the ones identified by traditional methods, owing to the enrichment provided by the high throughput sorting system (Figure 4D).

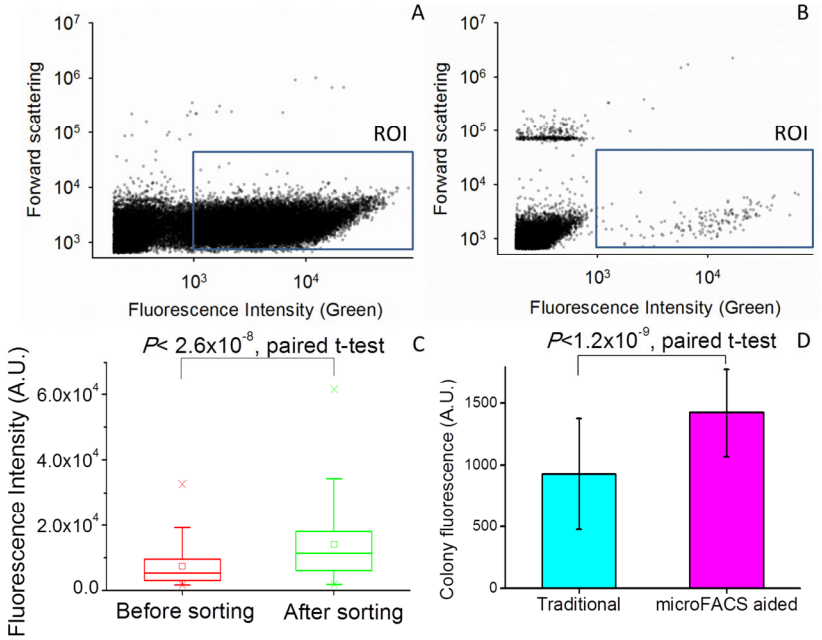

Figure 4. Cytometric analysis plots with identical gating condition for YFP library of initial(A) and sorted by microFACS system(B). A boxplot comparing cell fluorescence intensities of ROI in initial and sorted YFP library.(C, small squares indicate the averages). Comparison of colony brightness of top 10 brightest variants (n=60, six replicates for each variants) from traditional screening and microFACS screening (D).

The capability of screening FP-based indicators was demonstrated in a preliminary baseline experiment, with a mixture of 12% (by number) of cells expressing G-GECO1.1 with 88% of flash-pericam mutant [2], the former having a much brighter response to mixing with calcium than the latter. After sorting, G-GECO1.1 expressing cells had been enriched to 77%, showing the system can isolate variants with brighter fluorescence and better function (Figure 5).

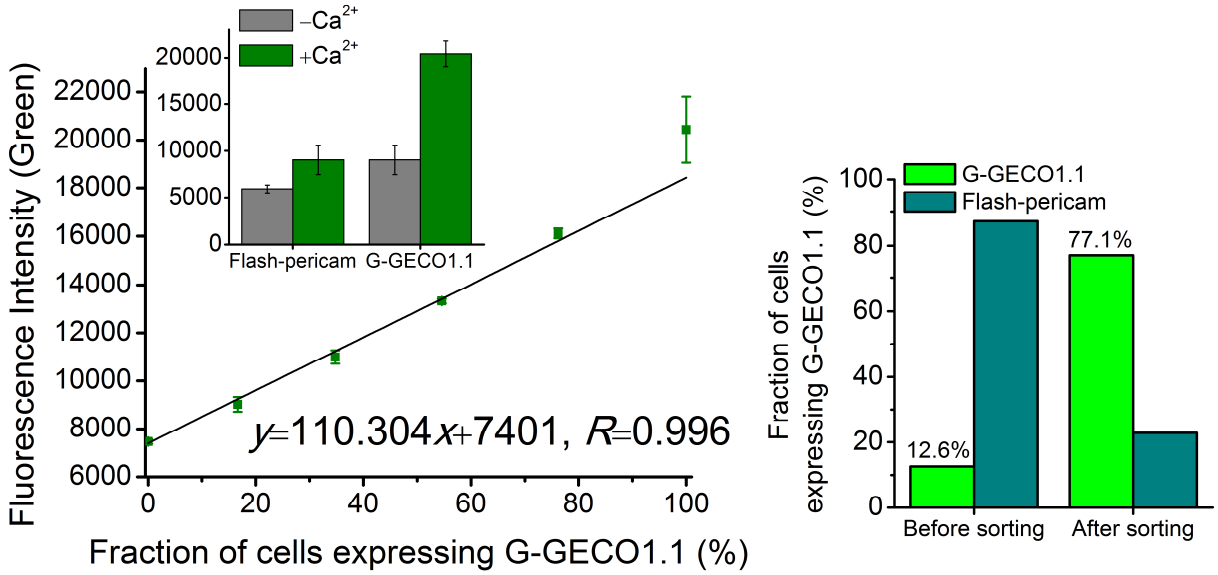

Figure 5. Calibration curve used for determining fraction of cells expressing G-GECO1.1 and a flash-pericam mutant. The inset shows brightness and dynamic range of each Ca^{2+} indicator measured by a cytometer (left). Screening result is shown on the right panel.

CONCLUSION

Our results demonstrate the capability of our microFACS system for discovery of improved FPs and FP-based Ca^{2+} indicators. Further optimization of operational parameters, such as volume flow rate, piezo activation timing, and channel geometry should increase the sorting efficiency, giving systems with significant throughput at relatively low cost.

ACKNOWLEDGEMENT

The authors thank T. Nagai for flash-pericam cDNA. Technical support was provided by the University of Alberta Nanofab and Molecular Biology Service Unit. This work was supported by Natural Sciences and Engineering Research Council of Canada (NSERC), Canadian Institutes of Health Research and Alberta Innovates Technology.

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