# HIGH-THROUGHPUT MUTAGENIZED CELL SCREENING SYSTEM CAPABLE OF SELECTIVE SINGLE CELL EXTRACTION

**Hyun Soo Kim[1], Taylor L. Weiss[2], Timothy P. Devarrenne[2], Arum Han[1, 3*]**

*[1]Department of Electrical and Computer Engineering, [2]Department of Biochemistry and Biophysics, and [3]Department of Biomedical Engineering, Texas A&M University, College Station, Texas, USA*

## ABSTRACT

We have developed a high-throughput microfluidic screening platform capable of culturing, analyzing, and selectively extracting mutagenized cells to investigate genetic variants. Combination of two pneumatic control layers, each comprising 32 microchannels, enables individually addressing and controlling each of the 1024 trapping sites in the cell analysis layer. By pressurizing either of the two pneumatic control channels or releasing pressure from both channels, the underlying trapping site was opened or closed, respectively. Cells inside a particular trapping site were extracted with backflow by selectively opening only this site. The performance of this screening platform was characterized using green microalga *Tetraslimis suecica*.

## KEYWORDS

Mutagenesis, Selective single cell extraction, Microfluidic high-throughput screening platform, Microalgae culture microsystem, Photosynthetic microalgae

## INTRODUCTION

Mutagenesis is a powerful technique to produce proteins, enzymes, genomes, or metabolic pathways with desirable properties. Coupled with screening or selection systems, this method has been successfully employed in various fields [1]. Conventionally, screening and selection are conducted by culturing mutagenized cell populations at low dilution on culture plates and manually picking cells showing desired trait [2]. Although this process is useful and widely used, it is time-consuming, quite labor-intensive, and requiring long culture periods. Also, the large numbers of mutants ($10^3$–$10^6$) that need to be screened to find genetic variants showing desired properties make this strategy costly and challenging. Here, we present a high-throughput mutagenized cell screening platform that enables easy monitoring of mutagenized cell properties through light/fluorescence microscopy followed by selective extraction of a particular genetic variant of interest ("hit") for further off-chip analysis and sampling. We are particularly interested in applying this platform toward high-throughput screening of mutagenized microalgae.

## EXPERIMENT

The high-throughput screening platform is made of a poly(dimethylsiloxane) (PDMS) assembly consisting of two functional layers; a microfluidic cell analysis layer and a microfluidic control layer (Figure 1). The cell analysis

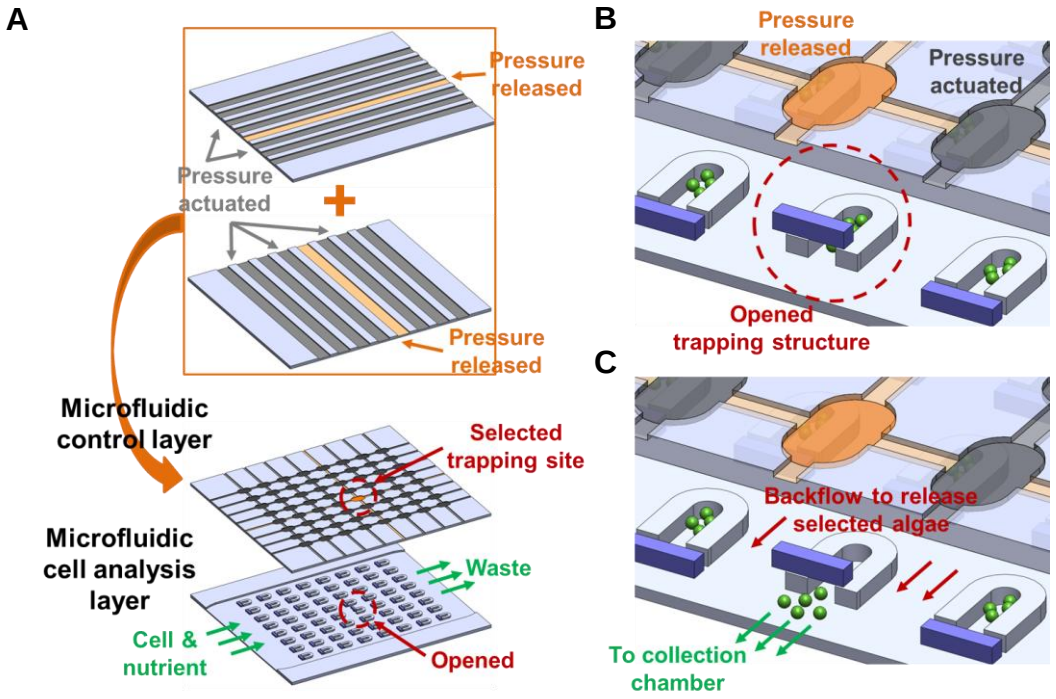


*Figure 1. Illustration of a high-throughput mutagenized cell screening platform. (A) Two PDMS functional layers – a microfluidic control layer and a microfluidic cell analysis layer. (B-C) Enlarged view of a selected trapping site where both row and column channels in the control layer are released simultaneously, resulting in opened trapping site. Trapped cells can be extracted with backflow.*



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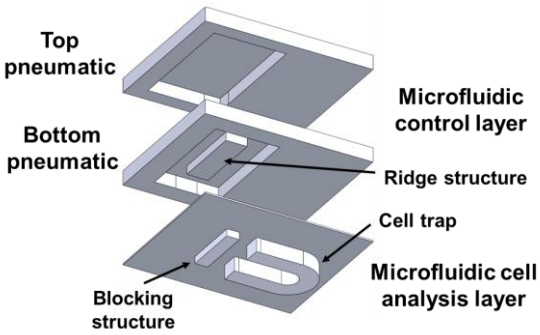


*Figure 2. A schematic view of a single trapping site integrated with pneumatic control layers. Each trapping site consists of a cell trap where a single cell is captured and a blocking structure, which can be selectively opened and closed during the cell extraction process by actuating the control layers with pressure.*

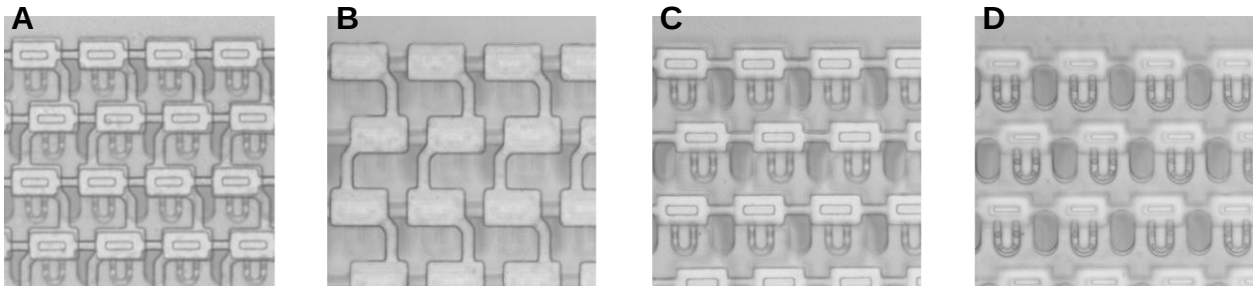


*Figure 3. Microscopic images of the fabricated device. (A) An assembly showing all three layers – top and bottom pneumatic layers and a cell analysis layer. (B) Top pneumatic layer. (C) Bottom pneumatic layer. (D) Cell analysis layer.*

layer (15 µm high) has 1024 cell trapping structures (32 x 32 array), where either single or multiple cells can be captured, cultured, and analyzed. Each trapping site consists of top-hanging structures having two different height (Figure 2); a backside semi-circular structure (12 µm high, cell trap) is utilized to capture cells while a front bar structure (8 µm high, blocking structure) is open during cell loading, culturing, and analysis, but pushed down and closed during the cell extraction process. This front blocking structure can be selectively opened and closed to extract cells of particular interest from a specific trapping site. The control layer is composed of two microfluidic pneumatic layers, each having 32 columns or rows of pneumatic control channels, respectively. This control layer is utilized to individually address and control each of the 1024 trapping sites in the analysis layer for selective cell extraction. To extract cells from a particular trapping site, first, pressure is applied to all pneumatic control channels to close all trapping sites. Then, only the row and the column covering the desired trapping sites are released by removing the applied pressure from the channels. Among trapping sites under these pressure-released regions, only the desired trapping site is open since all trapping sites can be successfully blocked with only either a column or a row control channel actuated by pressure (Figure 4). Cells inside this particular site can be extracted and collected to off-chip reservoirs with backflow for further analysis. All microchannels in the pneumatic control layer was controlled individually by integrating a binary multiplexer scheme, which allowed each of the 1024 trapping sites to be regulated with only 22 actuation sources (20 for binary multiplexer control lines + 2 for input source).

*Tetraslimis suecica* is a marine and green unicellular microalga, suitable as a model organism for genetic studies [3]. The functionality of the developed platform was tested by capturing and selectively releasing this microalga.

**RESULT AND DISSCUSSION**

In the screening platform, each of the 1024 trapping sites can be opened or closed independently by controlling the front bar-shape blocking structure, with actuating the top or the bottom pneumatic channels. Prior to experiments with cells, the working principle of this scheme was tested and characterized with an incremental actuation pressure (Figure 4). When only the bottom pneumatic control channel was actuated, the blocking structure could partially block the trapping site under 15 ~ 18 psi of pressure and could completely block the site at pressure of 18 psi or higher (Figure 4B). However, to fully close the blocking structures only with the top pneumatic layer actuation, a fairly high pressure of more than 50 psi was required since the top layer should be bent sufficiently to deform the bottom pneumatic layer, which then pushed down the blocking structure. To reduce the required pressure for the top pneumatic actuation, a top-hanging ridge structure positioned approximately 3 µm above the bottom surface (Figure 2) was introduced in the middle of the bottom pneumatic channel. When the top pneumatic channel was actuated and deformed to a certain degree, this ridge structure made contact with the blocking structure in the analysis layer and began to push it down for closure. By utilizing this ridge structure, the blocking structure was able to completely close the trapping site with significantly lower pressure. When only the top pneumatic part was pressurized, the site was partially blocked under 25 ~ 29 psi of pressure, but then was completely blocked when applying pressure of 30 psi or higher (Figure 4C).

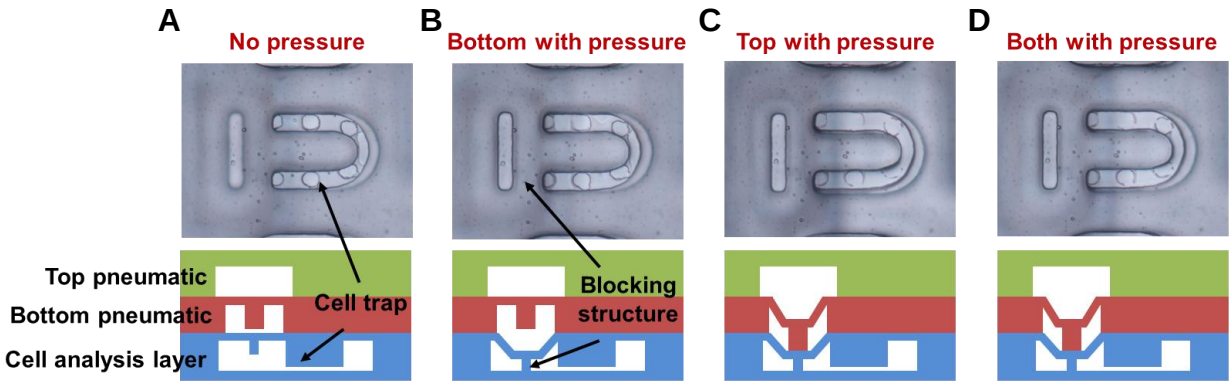


*Figure 4. Micrographs and schematics of the single trapping site, which show the operation of the top-hanging blocking structure. (A) During cell loading, culturing, and analysis, no pressure is applied and all blocking structures as well as all trapping sites are open. (B-D) During the extraction process, pressure is applied to one of the two pneumatic layers or to both, resulting in closure of the trapping sites. Only the particular trapping site, where both pneumatic layers are released from pressure simultaneously, stays open during this process, as shown in (A).*

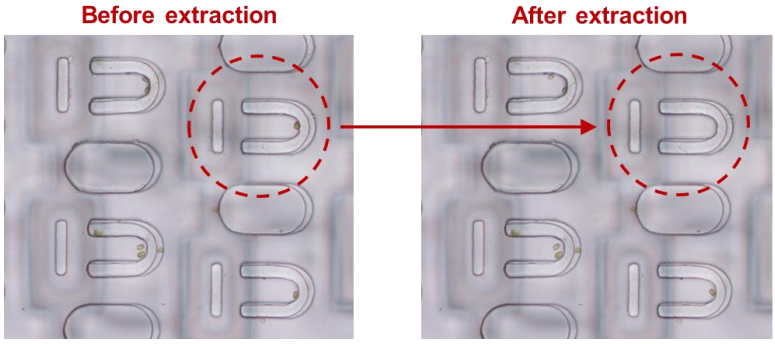


*Figure 5. Microscopic images showing selective extraction of cells from the platform. Tetraselmis suecica cell was successfully extracted from a particular trapping site (dashed circle) without affecting cells captured at other trapping sites.*

*Tetraslimis suecica* was used to test the functionality of the screening platform. First, *Tetraslimis suecica* cells were loaded into the platform with the flow rate of 4 ~ 6 µl/min, followed by a flushing step to remove uncaptured cells. As shown in Figure 5, a single cell or multiple cells were successfully captured at each trapping sites in the analysis layer. During the culture and analysis period, the blocking structures stayed open to provide nutrients with culture media flow. To extract the cells from a particular trapping site after analysis, all trapping sites were blocked by actuating all control channels with pressure (30 psi), and only a particular trapping site (dashed circle) was opened by selectively releasing pressure from control channels covering that site. Using backflow (10 ~ 15 µl/min), *Tetraslimis suecica* cells inside this particular trapping site were successfully extracted without affecting cells captured at other trapping sites.

## CONCLUSION

A high-throughput mutagenized cell screening platform has been developed and the functionality of the system was demonstrated by successfully capturing and selectively extracting cells. This platform is currently being used to examine mutagenized microalgae and to selectively extract them for further analysis. We expect that this system will serve as a powerful high-throughput analysis and screening tool in broad ranges of microbiology applications where investigating genetic variants is needed.

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## CONTACT

*A. Han, tel: +1-979-8459686; arum.han@ece.tamu.edu