High-throughput Image Processing for Screening A Microbial Mutant Library

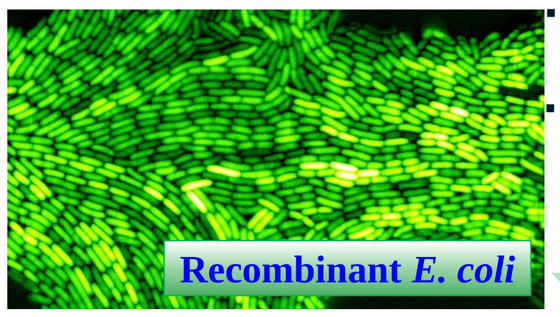
Jaemin Moon[1] Jiwon Lim[2*] and Taesung Kim[1*]

[1]School of Mechanical & Advanced Material Engineering, UNIST
[2]School of Nano Bio-Chemical Engineering, UNIST
Correspondence: bilermugle@gmail.com
tskim@unist.ac.kr

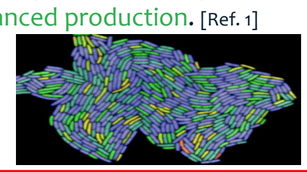
ABSTRACT

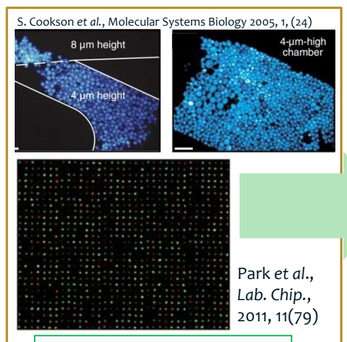
Currently, digital image processing techniques have become a popular approach for various research fields. Here, we propose a high-throughput digital image processing method for screening various microbial mutant libraries. Indeed, Synthetic Biology still requires compartmentalized cell-culture environments in an array format and sorting and extracting methods of a number of various engineered cell samples; in fact, each array of cells is analyzed manually and screening references are dynamic. As an application of our high-throughput image processing technique, we make it possible to analyze about 4000 images of cells obtained from a microfluidic device automatically with the same standard reference, showing a remarkable potential for rapid and accurate screening of microbial mutant libraries. It is highly believed that the technique would be broadly applied to other image processing areas.

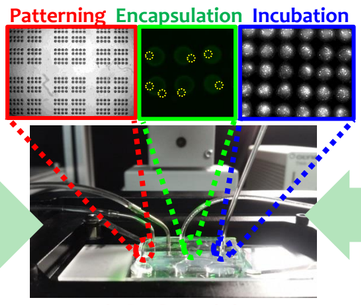
Introduction

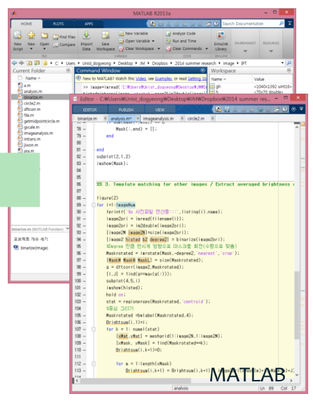


- *Escherichia coli* is a regular microorganism for many industrial purposes such as the mass production of chemicals.
- Directed mutation of a microorganism needs high-throughput screening method (>10^6) for enhanced production. [Ref. 1]









Microfluidic Approaches

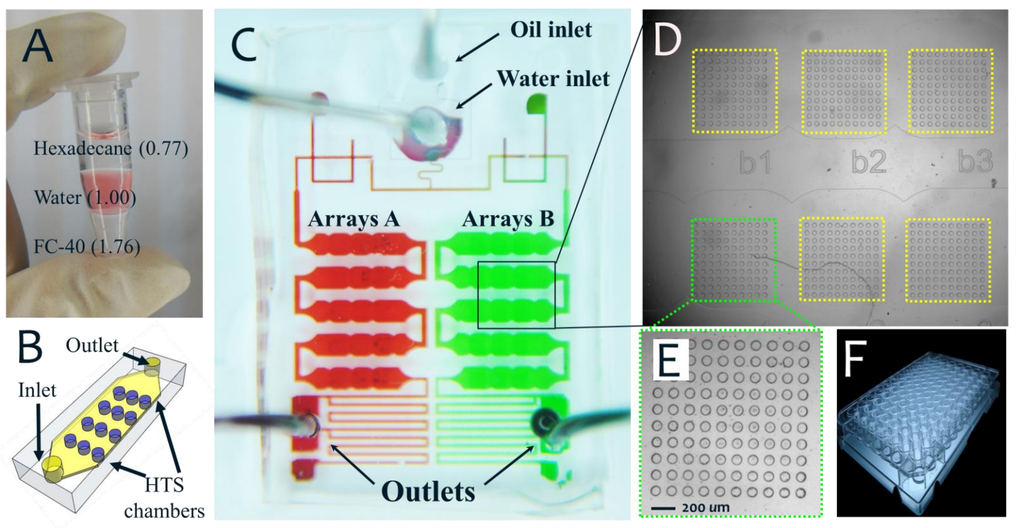
- Microfluidics enables the whole new biological applications not available in the past.
- Microdroplet-platforms have great advantages such as compartmentalization, miniaturization, and high-throughput screening. [Ref. 2]

Computational Approach

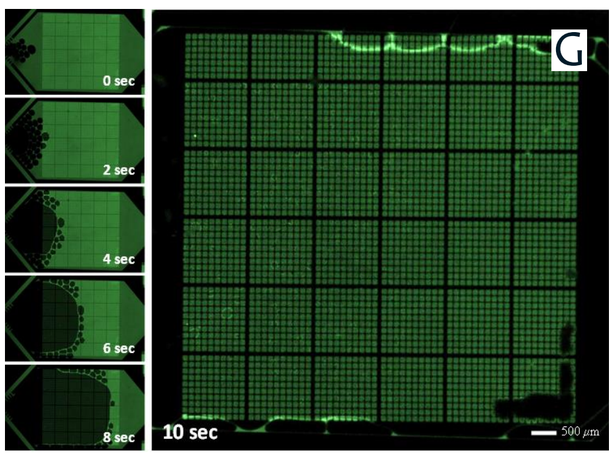
- Computational approach for analyzing samples has objective reference.
- Rapid automation is possible for analyzing thousands of results from experiment.

Working Principle of the Microfluidic Device

Fluid Patterning



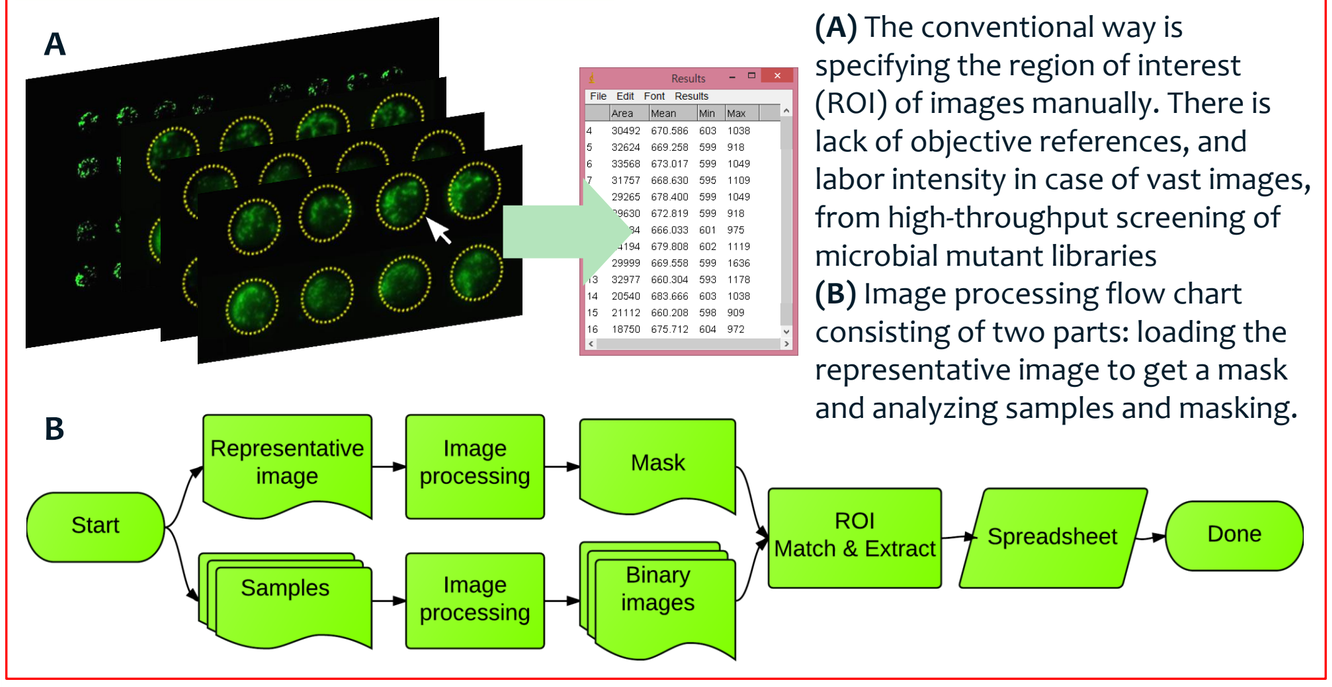
(A) Difference in the specific gravities of various oils against water **(B)** Schematic of the fluid patterning device **(C)** Real figure of the two layered microfluidic device **(D),(E)** More than 3,000 patterned arrays in a microfluidic device(100 cavities x 30 compartments) **(F)** Conventional 96 well bioreactor (Microplate reader)



G) Rapid and sequential fluid patterning of 3,000 array in 10 *sec.*

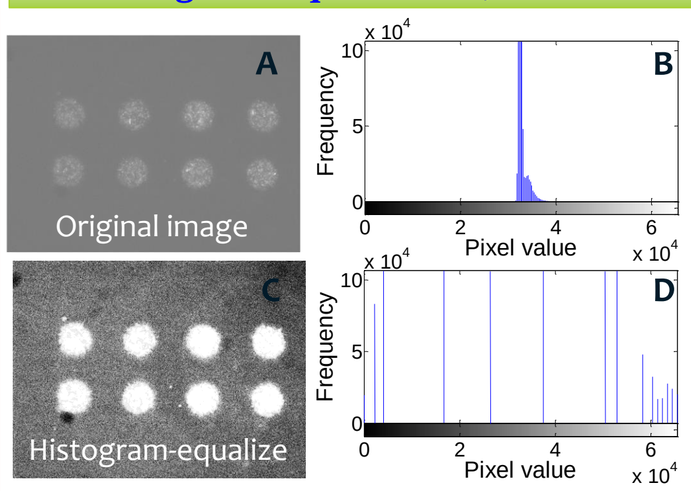
Image Processing Algorithm

Image processing flow chart



(A) The conventional way is specifying the region of interest (ROI) of images manually. There is lack of objective references, and labor intensity in case of vast images, from high-throughput screening of microbial mutant libraries
(B) Image processing flow chart consisting of two parts: loading the representative image to get a mask and analyzing samples and masking.

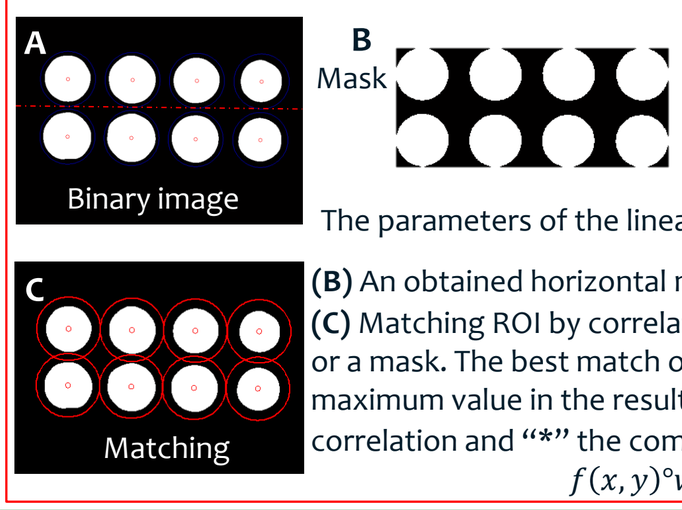
Histogram Equalization, and Noise Filtering



(A) Original image **(B)** its histogram **(C)** Histogram-equalization, and logarithmic contrast-stretching transformation, and **(D)** its histogram

(E) Pepper and salt noise filtering, linear spatial filtered, Gaussian of filter size 50x50 and standard deviation 15.

Binarization and Template-matching



(A) Binarize images with an adaptive threshold and find the gradient of the centroids by using least-square fitting:

$$a + b\bar{x} = \bar{y}$$

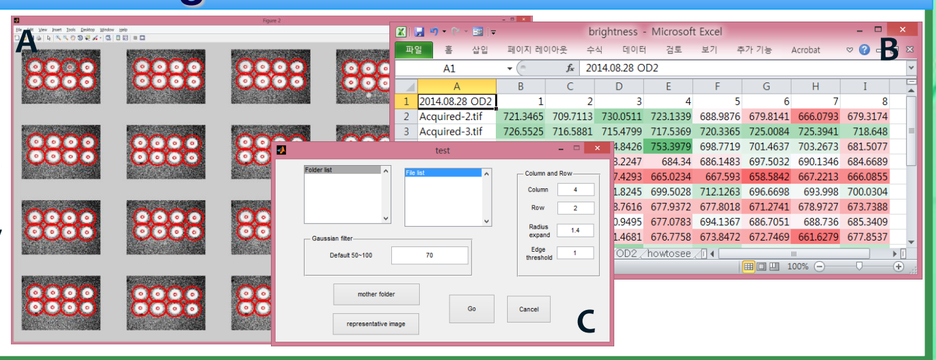
The parameters of the linear form [Ref. 3] $b = \frac{\sum y_i(x_i - \bar{x})}{\sum x_i(x_i - \bar{x})}$ $a = \bar{y} - \bar{x}b$

(B) An obtained horizontal mask with uniform intervals and averaged areas
(C) Matching ROI by correlation, *f* is the original image, *w* is a sub-image or a mask. The best match of $w(x,y)$ in $f(x,y)$ is the location of the maximum value in the resulting correlation image. Let "$\circ$" denote correlation and "*" the complex conjugate: [Ref. 4]

$$f(x,y) \circ w(x,y) \Rightarrow F(u,v) * H(u,v)$$

Developed Software Using MATLAB

(A),(B) ROI matching results and exporting masked image data to a spreadsheet for each sample and its cells.
(C) GUI for adjustable parameters for compatibility in cases of environmental changes.



REFERENCES

1. J. A. Dietrich, A. E. McKee, J. D. Keasling, High-Throughput Metabolic Engineering: Advances in Small-Molecule Screening and Selection, ***Annual Review of Biochemistry***, 2010, 79, 563-590
2. M. C. Park, J. Y. Hur, H. S. Cho, S. Park, K. Y. Suh*, High-throughput single-cell quantification using simple microwell-based cell docking and programmable time-course live-cell imaging, ***Lab Chip.***, 2011, 11, (79)
3. Jaan Kiusalaas, Numerical methods in engineering using MATLAB, Cambridge university press, 2009, 3, ISBN-13 978-0521191333 .
4. Rafael C. Gonzalez, Richard E. Woods, Steven L. Eddins, Digital Image Processing, 2004, PEARSON Prentice Hall, 10-11, ISBN 0-13-008519-7

CONCLUSIONS

I. We developed a high-throughput image processing technique (software) that analyzes a number of images obtained automatically from a microfluidic device.

II. We showed the potential for rapid and accurate screening by demonstrating that it can analyze various images of cells obtained from a microfluidic device automatically, with the same standard reference.

III. There are more potential applications for experimental analysis with the principles.

Mechanical and Advanced Materials Eng. (MAME), Ulsan National Institue of Science Technology(UNIST)