

# Celluloid Microenclosure and Microlens Array Fabricated by Suzuki's Universal Microprinting Method and XeF<sub>2</sub> Vapor Etching for Microbial Analysis

Akihiro Matsutani<sup>1\*</sup> and Ayako Takada<sup>2</sup>

<sup>1</sup>Semiconductor and MEMS Processing Division, Technical Department, Tokyo Institute of Technology,  
4259 Nagatsuta, Yokohama, Kanagawa 226-8503, Japan

<sup>2</sup>Biomaterials Analysis Division, Technical Department, Tokyo Institute of Technology,  
4259 Nagatsuta, Yokohama, Kanagawa 226-8501, Japan

(Received August 22, 2017; accepted October 17, 2017)

**Keywords:** single-cell isolation, microenclosure, microlens, XeF<sub>2</sub>, SUMP method

We demonstrate the celluloid-based biochip for cell trapping, prepared by Suzuki's universal microprinting (SUMP) method. The structure of  $8 \times 8 \mu\text{m}^2$  microenclosures consists of a micropillar array with  $2 \mu\text{m}$  spaces between  $2\text{-}\mu\text{m}$ -diameter pillars. We succeeded in fabricating the single-cell trap for yeast cells by using the celluloid microenclosure array chip. In addition, we demonstrated the fabrication process of the celluloid microlens array by the SUMP method and XeF<sub>2</sub> vapor etching. By using this celluloid microlens array, we carried out experiments on collecting laser light and observed the focal spots for each microlens. We consider that this simple technique will be very useful for the single-cell isolation and analysis of microbial cells.

## 1. Introduction

Single-cell analysis techniques are required to clarify the functions of cells and for medical applications. Conventional biochemical techniques, i.e., bulk analyses, can provide only average values obtained from large numbers of cells. We previously demonstrated the single-cell isolation and positioning of bacteria using microenclosure arrays with structures composed of many micropillars.<sup>(1)</sup> This structure enables the positioning of cells. Therefore, by using this microenclosure structure, cell analysis can be performed while identifying individual cells. We demonstrated, in the previous work, that this microenclosure can be used to measure the size change of *Escherichia coli* cells using oxygen plasma at each individual cell.<sup>(2)</sup> However, this microenclosure was fabricated on semiconductor wafers such as GaAs and InP by semiconductor processing technologies such as electron beam (EB) lithography and dry etching. A simpler fabrication process for microenclosure array structures is needed for the application of microenclosures to the field of microbial analysis. We regarded Suzuki's universal microprinting (SUMP) method<sup>(3–6)</sup> to be a simple microenclosure

---

\*Corresponding author: e-mail: matsutani.a.aa@m.titech.ac.jp  
<http://dx.doi.org/10.18494/SAM.2018.1729>

manufacturing method. The SUMP method is a technique that forms a replica of a sample on a celluloid plate. Although this method was invented in 1930, it is very similar to current nanoimprinting technology. The SUMP method is also similar to the replica fabrication process using poly(dimethylsiloxane) (PDMS). However, the SUMP method is easy to handle and inexpensive. In the process of PDMS, debubbling and curing are required, which take a long time. On the other hand, in the SUMP method, the steps of debubbling and curing are unnecessary, and the processing time is very short. These points are an advantage of the SUMP method compared with the PDMS process. Therefore, the throughput of the fabrication by the SUMP method is very high. If researchers have molds, they can easily fabricate their desired biochips by themselves. We confirmed the transfer of 200 nm patterns to a celluloid plate by the SUMP method using a nanoimprint test mold made of quartz. In other words, the accuracy of the SUMP method is very good. Figures 1(a) and 1(b) show optical microscopy images of specimens of *Tradescantia ohiensis* stoma and a cross section of the ovary of a *Rhododendron* sp. prepared by the authors by the SUMP method 40 years ago. It is understood that the specimens produced by the SUMP method have excellent durability. Therefore, the SUMP method can be used as a microscopically precise replica molding method for forming a microenclosure array chip.

On the other hand, techniques for trapping biological cells are also important. The optical tweezers technique has been used for this purpose.<sup>(7–10)</sup> For the transport of parallel biological cells, vertical-cavity surface-emitting laser (VCSEL) arrays are used as individually addressable optical tweezers.<sup>(11)</sup> However, we consider that light focused by a microlens array can also be used for total transport or as a trap. A mold is needed for replica microlens fabrication. When Si wafer is used as a lens mold, it is necessary to etch a hemisphere profile. Isotropic etching is desirable to etch the hemisphere. In general, chemical etching is suitable for etching such a profile. For accurate hemispherical shape formation, wet etching through a submicron opening is necessary. However, it is not easy to fabricate the structure because of the difficulty of exchanging the etchant through a small opening. On the other hand, the controllability of dry etching is better than that of wet

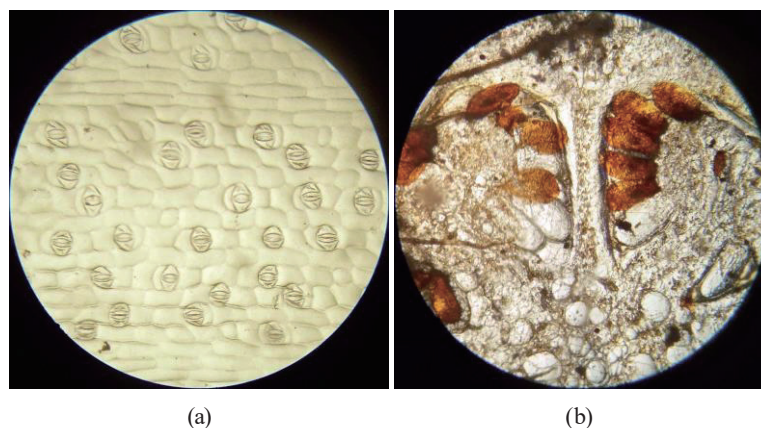


Fig. 1. (Color online) Optical microscopy images of specimens prepared by SUMP method 40 years ago. (a) Stoma of *T. ohiensis* and (b) cross section of ovary of *R. sp.*

etching, in etching through a small opening. Vapor etching satisfies these requirements. However, there are few reports on  $\text{XeF}_2$  vapor etching for the fabrication of microlens array molds.

In this paper, we demonstrate single-cell trapping of yeast cells using a microenclosure array formed by the SUMP method. In addition, we describe the fabrication of a celluloid microlens array by  $\text{XeF}_2$  vapor etching and by the SUMP method.

## 2. Single-cell Trap Using Microenclosure Array

Figure 2 shows the process flow for the fabrication of a microenclosure array by the SUMP method. A Si (100) substrate was used as the mold material. First, after a Cr film was deposited by sputtering, the microenclosure array pattern was formed in a photoresist by lithography, as shown in Fig. 2(a). The size of one microenclosure was  $8 \times 8 \mu\text{m}^2$  consisting of  $2 \mu\text{m}$  spaces and  $2\text{-}\mu\text{m}$ -diameter pillars. Next, the holes of the microenclosure were etched by reactive ion etching (RIE) using a RIE system (Samco, RIE-1) with  $\text{CF}_4$  plasma, as shown in

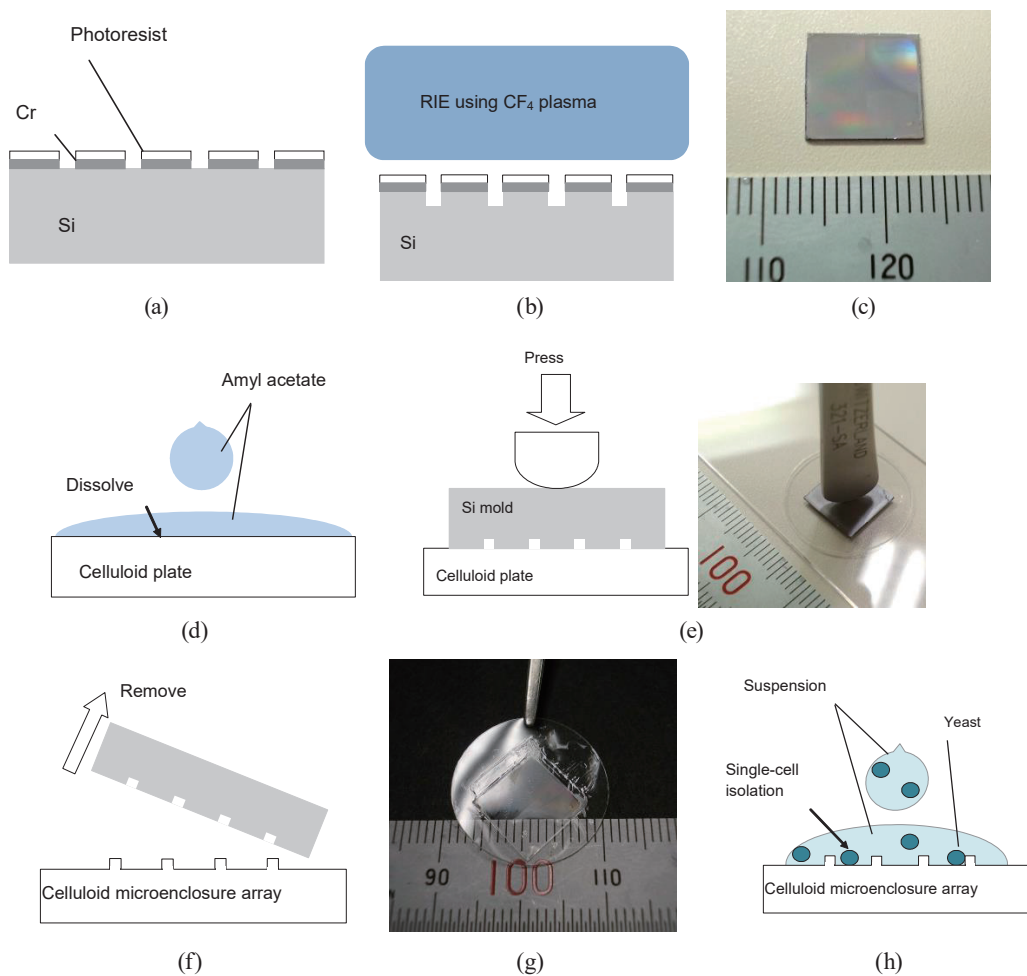


Fig. 2. (Color online) Process flow for the fabrication of microenclosure array by SUMP method.

Fig. 2(b).<sup>(12)</sup> The flow rate of  $\text{CF}_4$  was 25 sccm and the process pressure was 0.1 Torr. The RF input power was 100 W. The etched depth was approximately 2  $\mu\text{m}$ . Figure 2(c) shows a photograph of the mold fabricated by the above-described process. A drop of amyl acetate was placed on a thin plastic plate (celluloid, SUMP plate B) and allowed to slightly dissolve the surface, as shown in Fig. 2(d). The microenclosure array mold was pressed into the plastic plate, forming a replica of the microenclosure structure, as shown in Fig. 2(e). The pressure for this SUMP process, measured using a pressure measurement film (Fujifilm, Prescale), was 0.25 MPa. After drying for 3 min, the microenclosure mold was removed with tweezers, as shown in Figs. 2(f) and 2(g). Finally, the celluloid plate was mounted on a glass slide. Yeast cell suspensions were applied to the celluloid microenclosure array, as shown in Fig. 2(h). Each single cell captured in the celluloid microenclosure was observed by optical microscopy and scanning electron microscopy (SEM).

Figures 3(a) and 3(b) show an optical microscopy image and an SEM image, respectively, of the single-cell trapping of yeast cells in a microenclosure array formed by the SUMP method. The yeast cell concentration was  $1 \times 10^4$  cells/ml. Prior to applying the suspension, the surface of the microenclosure was hydrophilized with oxygen plasma. These results demonstrated that the single-cell trapping of yeast cells was accomplished using the plastic microenclosure array formed by the SUMP method.

### 3. Fabrication of Celluloid Microlens Array by SUMP Method and $\text{XeF}_2$ Vapor Etching

Figures 4(a)–4(d) show schematic diagrams of the fabrication process of a celluloid microlens array by the SUMP method. A Si (100) substrate was used as the material. First, a pattern of small openings (20–500 nm diameter) was formed by direct writing by EB lithography on the EB resist (ZEP 520A; 160 nm thickness), as shown in Fig. 4(a). Next, the Si substrate was

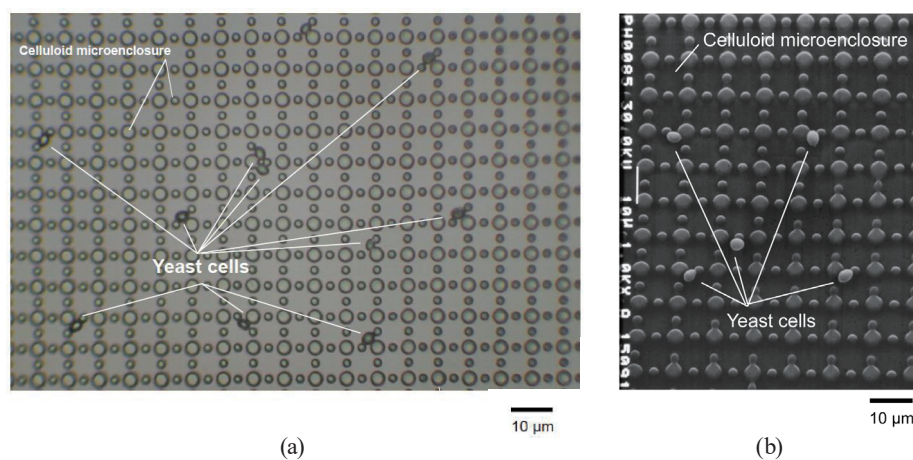


Fig. 3. (a) Optical microscopy and (b) SEM images of the single-cell trapping of yeast cells in microenclosure array formed by SUMP method.

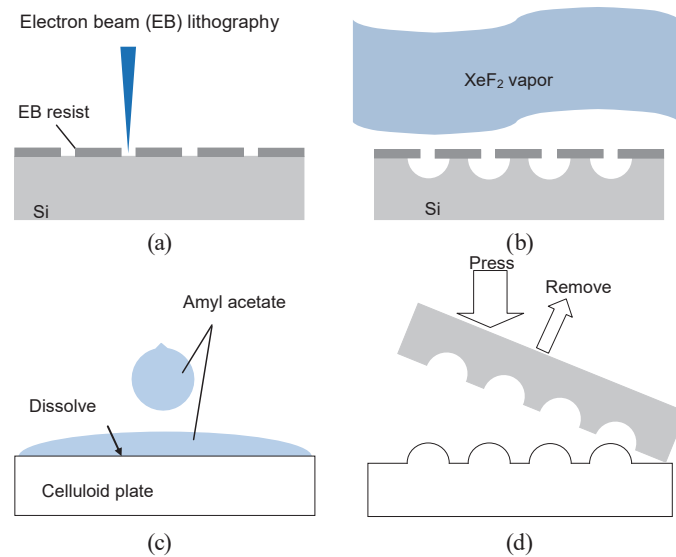


Fig. 4. (Color online) Process flow for fabrication of celluloid microlens array by SUMP method.

etched isotropically by supplying  $\text{XeF}_2$  vapor via the submicron openings of the etching mask, as shown in Fig. 4(b). The surface of the celluloid plate (SUMP plate B) was melted with amyl acetate and pressed against the mold, as shown in Fig. 4(c). After drying for 3 min, the mold was removed to leaving a celluloid microlens array, as shown in Fig. 4(d). The celluloid plate on which the microlens array was fabricated was mounted on a slide glass.

Figure 5 shows a schematic diagram of our original  $\text{XeF}_2$  vapor etching system used in our experiment. We used a stainless-steel chamber to avoid chemical reactions between the chamber wall material and the etching species. Borosilicate glass was used for the viewing window. We used  $\text{XeF}_2$  as the etching gas, where the temperature of the  $\text{XeF}_2$  cylinder was 65 °C. The etching gas was supplied to the chamber through a mass flow controller. The substrate was at room temperature. Prior to the etching, the process chamber was depressurized to 2 Pa with a rotary pump using 10  $\text{N}_2$  purge cycles. The process pressure was 120 Pa. The etching rate of Si was approximately 2  $\mu\text{m}/\text{min}$  at the opening width of 500 nm. The depth and curvature radius of etching depended on the opening width.

Figures 6(a) and 6(b) show an optical microscopy image and an SEM image of the celluloid microlens array fabricated by SUMP method. The profile of the celluloid microlens was hemispherical and the surface was smooth.

Figure 6(c) shows the optical microscopy image of the focal point of the celluloid microlens array irradiated by collimated semiconductor laser ( $\lambda = 670 \text{ nm}$ , 0.9 mW) as the light source. The beam profile was circular. It is found that a focal point is confirmed for each lens and that the structure functions as a microlens array. The distance from the flat surface of the lens with a diameter of approximately 5  $\mu\text{m}$  to the focal point was 6  $\mu\text{m}$  in air and about 11  $\mu\text{m}$  in water. Figure 6(d) shows the result of a numerical ray trace simulation of the celluloid microlens. The diameter of the lens was 4  $\mu\text{m}$  and the curvature of the lens



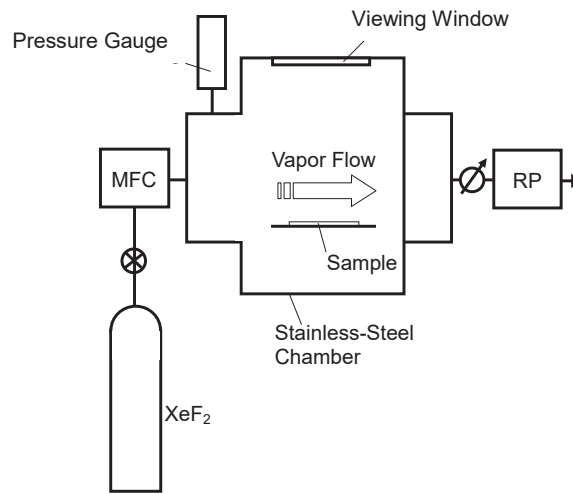


Fig. 5. Etching system using  $\text{XeF}_2$  vapor.

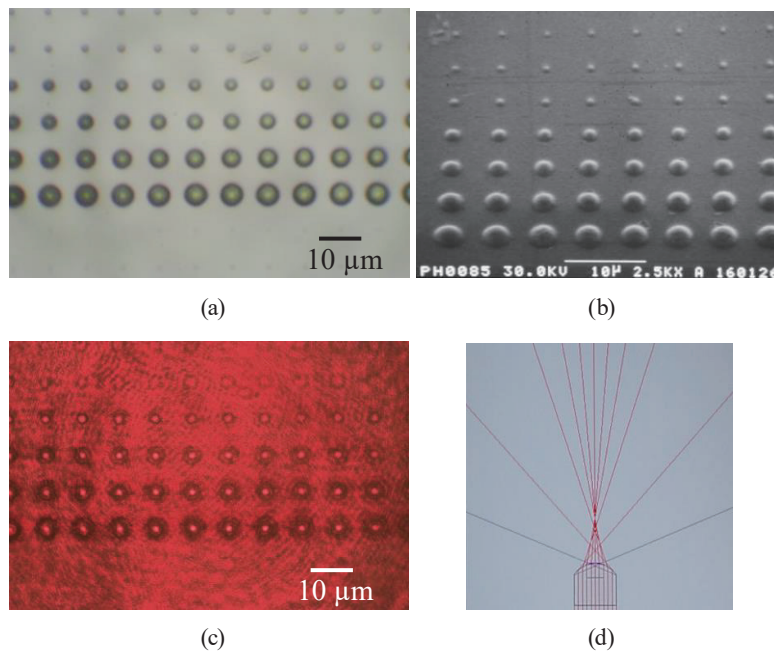


Fig. 6. (Color online) (a) Optical microscopy and (b) SEM images of celluloid microlens array formed by SUMP method. (c) Optical microscopy image of focal point of celluloid microlens array irradiated by collimated semiconductor laser light and (d) ray trace simulation of convex microlens.

was assumed to be  $10\ \mu\text{m}$ . The lens was assumed to be planoconvex though the actual profile was elliptical. The refractive index of the celluloid lens was assumed to be 1.49, which is the same as that of polymethyl methacrylate (PMMA). In this calculation, the focal length was approximately  $6\ \mu\text{m}$ . Its spherical aberration was large. It is necessary to optimize the lens shape for efficient collection of light.

As a preliminary experiment for our future prospects, we observed yeast cells which were kept at the focal point of microlens (data not shown). In the video image, this yeast cell was swaying on the microlens in convection. On the other hand, yeast cells adhered to the celluloid surface did not move. We believe that trapping a single cell in liquid using a celluloid microlens fabricated by the SUMP method will be possible in combination with a VCSEL array, using optimized parameters such as the power of the laser light, the diameter of the microlens and the focal length.

#### 4. Conclusions

We demonstrated the single-cell trapping of yeast cells using a celluloid microenclosure array formed by the SUMP method. In addition, we demonstrated the celluloid microlens array fabricated by the SUMP method and  $\text{XeF}_2$  vapor etching. By using this celluloid microlens array, we conducted experiments on collecting laser light and observed focal spots for each microlens. We consider that this simple technique will be very useful for the single-cell trapping and analysis of bacterial cells.

#### Acknowledgments

The authors would like to thank Elionix Inc. for their assistance with the of EB lithography process. This work was supported by JSPS KAKENHI Grant Number JP17K05020.

#### References

- 1 A. Matsutani and A. Takada: Jpn. J. Appl. Phys. **49** (2010) 127201.
- 2 A. Matsutani and A. Takada: Jpn. J. Appl. Phys. **51** (2012) 087001.
- 3 J. Suzuki: Japan Patent No. 88353 (1930).
- 4 J. Suzuki: Oyo Buturi **5** (1936) 279 (in Japanese).
- 5 K. Kurata: Oyo Buturi **6** (1937) 460 (in Japanese).
- 6 H. R. Bullock: Trans. Am. Microsc. Soc. **79** (1960) 167.
- 7 A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and S. Chu: Opt. Lett. **11** (1986) 288.
- 8 H. Liang, W. H. Wright, S. Cheng, W. He, and M. W. Berns: Exp. Cell Res. **204** (1993) 110.
- 9 A. Askin: Proc. Natl. Acad. Sci. U.S.A. **94** (1997) 4853.
- 10 H. Zhang and K. K. Liu: J. R. Soc. Interface **5** (2008) 671.
- 11 R. A. Flynn, A. L. Birkbeck, M. Gross, M. Ozkan, B. Shao, M. M. Wang, and S. C. Esener: Sens. Actuators, B **87** (2002) 239.
- 12 A. Matsutani, F. Koyama, and K. Iga: Jpn. J. Appl. Phys. **30** (1991) 428.