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Micro-compartments

# Oil Microsealing: A Robust Micro-compartmentalization Method for On-Chip Chemical and Biological Assays

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*A simple and robust method to compartmentalize aqueous solutions into an array of independent microchambers is presented. The array of microchambers fabricated in poly(dimethylsiloxane) are filled with the sample solution through a microfluidic channel and then sealed with oil to isolate the microchambers from each other. A water reservoir close to the microchambers allows the maintainance and incubation of sub-nanoliter solutions (e.g., at 37 °C) within the chambers for hours without any problem of evaporation. Once assembled, the device is self-sustainable and can be used for different application purposes. As a demonstration, the device configuration is shown to be suitable for spatiotemporal control of the inner solution conditions by light stimulation through a photomask. This method was applied for the generation of regular EmGFP (emerald green fluorescent protein) expression arrays, selective photobleaching, photopatterning of calcium concentration, and cell culture in independent microchambers.*

## 1. Introduction

Microfluidics and lab-on-a-chip technologies offer revolutionary tools for cell and systems biology.[1] The high potentiality of microfluidics for biology relies on dynamic and precise control of the microenvironment at temporal and spatial scales relevant for biological systems[2] and on parallelization and integration for high-throughput screening and on-chip systems biology.[1c,3] One of the challenges of microfluidics for biology is to provide simple and robust methods to generate arrays of biocompatible micro-compartments that can be individually addressed in a dynamic and controlled way. Two main strategies for micro-compartmentalization have been proposed. The first one is based on the use of highly integrated microfluidic channels and valve networks. Such approaches allow controlling the composition of a large number of individual micro-compartments in a dynamic way but remain technically challenging.[3,4] The second strategy is to employ water-in-oil microdroplets as micro-compartments.[5] Microfluidic techniques (T-junction, flow focusing)[6] allow one to obtain highly monodisperse microdroplets, which can be stored, fused, sorted, and collected in the same device.[7] For the purpose of long term observation, microdroplets have to be trapped and immobilized, and several methods have been proposed.[8] Microdroplets have been successfully applied for various biological studies (e.g., in vitro evolution,[9] high-throughput cytotoxicity screening,[7] polymerase chain reaction (PCR)[10]), but the requirement of a surfactant for interface stabilization,[11] the curvature of droplet interface, and the low gas exchange with the outside medium can present some limitations for biological applications. Compared to the technologies described above, we propose here a much simpler and robust way to prepare an array of micro-compartments. It allows the confinement of a small sample solution (5 μL) into an array of sub-nanoliter chambers in a short time (ca. 5 min) and does not require any valve integration, multilayer microfabrication, or use of surfactant molecules. Our device is fabricated in poly(dimethylsiloxane) (PDMS) by standard soft lithography and consists of lateral chambers connected to a main canal. Our concept, called 'oil microsealing,' consists of filling microchambers with the sample solution by removing air through PDMS prior to replacing the solution in the main canal with

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mineral oil, thus creating a microseal and isolating microchambers from each other. Once the microseal has been created, the device can be transported and manipulated for further procedures such as incubation or photostimulation. To get rid of evaporation through PDMS,[12] a water reservoir[13] is simply added above the microchambers. The advantage of our system also relies on the fact that the solution is mainly in contact with PDMS, which is biocompatible and allows gas exchange, and with the flat glass substrate, which facilitates optical detection and light stimulation. The well-defined position of the chambers and the flat interface can be combined with the use of photosensitive compounds and light stimulation to individually address chamber composition. To demonstrate the versatility and robustness of this new concept of compartmentalization, our method was applied for the in situ gene expression and realization of protein arrays, selective photobleaching, and photopatterning of calcium concentration.

## 2. Results and Discussion

### 2.1. Concept and Method

**Figure 1** shows the concept and procedure of oil microsealing. Our microfluidic chip consisted of two layers of PDMS; a lower layer (ca. 1 mm thick) with a microfluidic channel at the bottom, which was bond to a thin cover glass slide (0.13–0.16 mm thick), and an upper layer (ca. 5 mm thick) with a hole of 6 mm diameter to serve as a water pool, which was bond onto the lower layer of PDMS so that the hole covers all the microchambers in the microfluidic channel. The channel (50 μm height) consisted of 70 circular microchambers of 100 μm diameter, which were connected[14] to a zigzag main canal of 50 μm width with a neck of 15 μm width and 30 μm length (Figure 1A). For easier manipulation, the chip was mounted using scotch tapes on a thick glass slide (1.0 mm thick), which was removed for incubation and fluorescence microscopy observations. 15–30 min before the introduction of a sample solution, the pool in the upper PDMS layer was filled with water. The sample solution was introduced into the channel while the output was closed with a plug. Since PDMS is permeable to gas,[4,15] the air in the channel was gradually pushed out from the channel through PDMS, and totally replaced by the sample solution typically in 1–3 min (Figure 1B). During the process, about 300 mbar gauge pressure was applied to push the sample solution through the channel. Once the channel had been filled, the applied pressure was released, the plug at the outlet was replaced with open tubing, and mineral oil was introduced from the inlet. With a few tens mbar gauge pressure or eventually simply by hydrostatic pressure, the solution was replaced with oil in the main

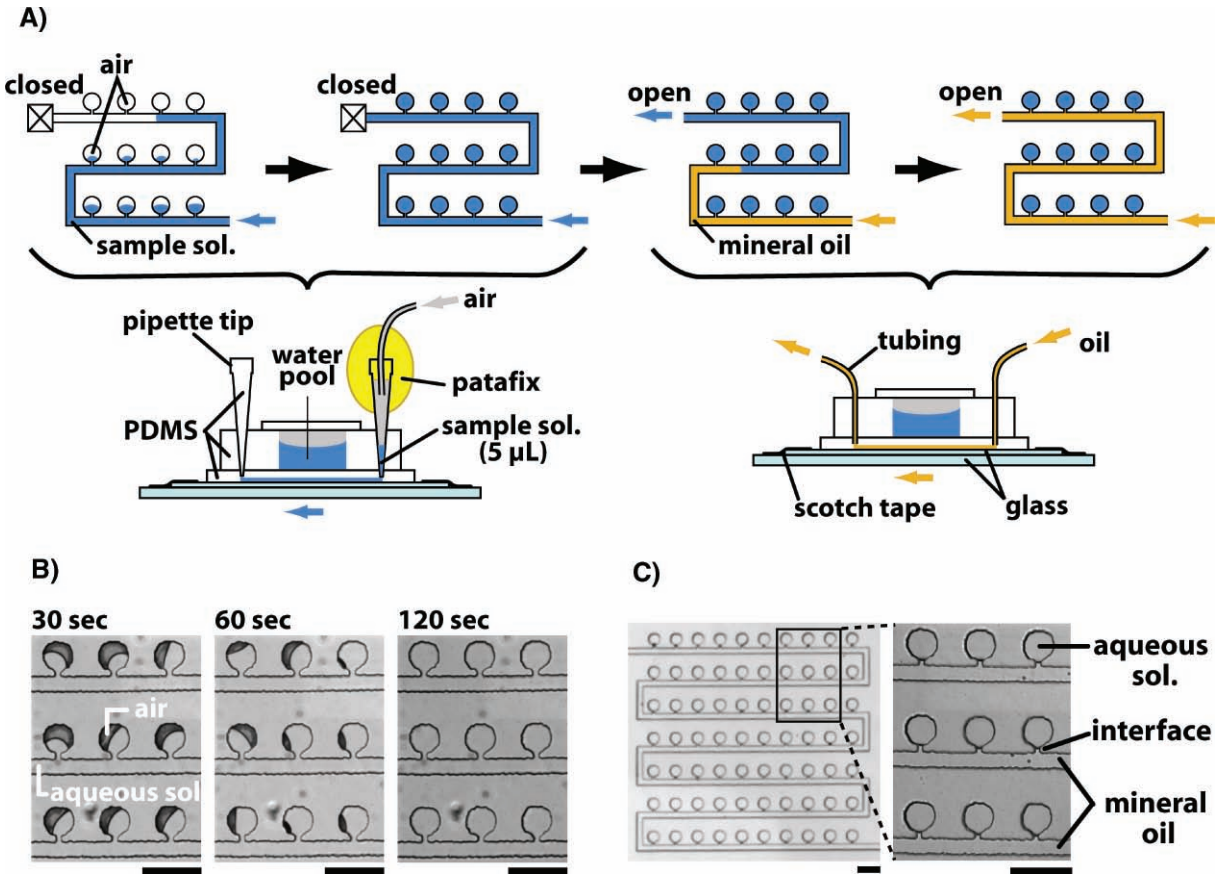


**Figure 1. Concept of oil microsealing.** A) Procedure to confine a sample solution into an array of microchambers. Upper figures illustrate the microfluidic channel and lower figures show the chip configuration. B) Time-lapse observation of the filling of the chambers by the sample solution. Scale bars are 200 μm. C) Configuration of the chambers after oil microsealing. Scale bars are 200 μm.

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canal but remained in the chambers due to the high hydrodynamic resistance. As a result, all microchambers were successfully sealed with oil (Figure 1C). Several parameters are particularly important for the success of this method. First, the PDMS has to be rendered hydrophobic (e.g., by baking) to avoid the trapping of the aqueous solution on the PDMS walls in the main canal. Second, the pressure to introduce the sample solution should be high enough to enable a complete filling in a short time but not too high to avoid channel deformation, which would result in excess trapping of the sample solution in the chamber during the microsealing by oil. Lastly, PDMS has to be saturated by vapor prior to sample introduction to prevent from sample evaporation. In our configuration, when the water pool was filled less than 15 min before the introduction of the sample solution, we observed the invasion of oil into the microchambers. When these three conditions were maintained, over 90% of trials resulted in the successful compartmentalization of the sample solution in all of the microchambers.

### 2.2. Gene Expression Array

First, our method was applied for the realization of a gene expression array. A cell-free gene expression medium was assembled at 4 °C using PURExpress, a commercially available transcription and translation system made of purified protein factors,[16] and a template plasmid DNA coding for EmGFP (emerald green fluorescent protein). 5 μL of the reconstituted gene expression solution was then distributed into the microchambers prior to oil microsealing using the method described before. All chambers were filled by the solution and no fluorescence could be detected. Then, the device was incubated at 37 °C. After 20 min., we could detect a weak EmGFP fluorescence. EmGFP fluorescence increased with time and after 2 h of incubation, we observed a strong EmGFP fluorescence in all chambers (**Figure 2**, top). Gene expression was successfully conducted without any problem of evaporation and the water/oil interface in the neck of each chamber did not move during incubation. The lower graphs in Figure 2 show the fluorescence intensity profile measured along the two dashed lines in the upper left image. Such results were reproducibly obtained in several replicates. Notably, the fluorescence image and the plot profiles showed that the fluorescence intensity (i.e., protein concentration) is homogeneously distributed with only small variations from one chamber to the other. This contrasts with the results obtained on protein expression performed in giant liposomes where a strong variability in protein concentration has been observed between liposomes encapsulating a similar gene expression material.[17] The regularity observed in our system is attributed to the defined configuration of the microchambers while liposomes can present inherent variability in size and shape. Moreover, in microconfined systems, the surface/volume ratio is particularly large and the role of the surface in contact with the solution is crucial. In our system, the gene expression solution is mainly in contact with PDMS and glass, which are well-defined biocompatible materials allowing gas exchange (through PDMS). On the contrary, in the case of microdroplets, the large contact area between the gene expression solution and oil and/or surfactants might have an impact on the yield of the expression reaction in each microdroplet, making the choice of oil and surfactant composition particularly delicate. The limited gas exchange might significantly affect the yield of expression as well.[18]

### 2.3. Selective Photobleaching

Similarly to the case of microdroplets, the microsealed chambers are isolated from the outside environment.

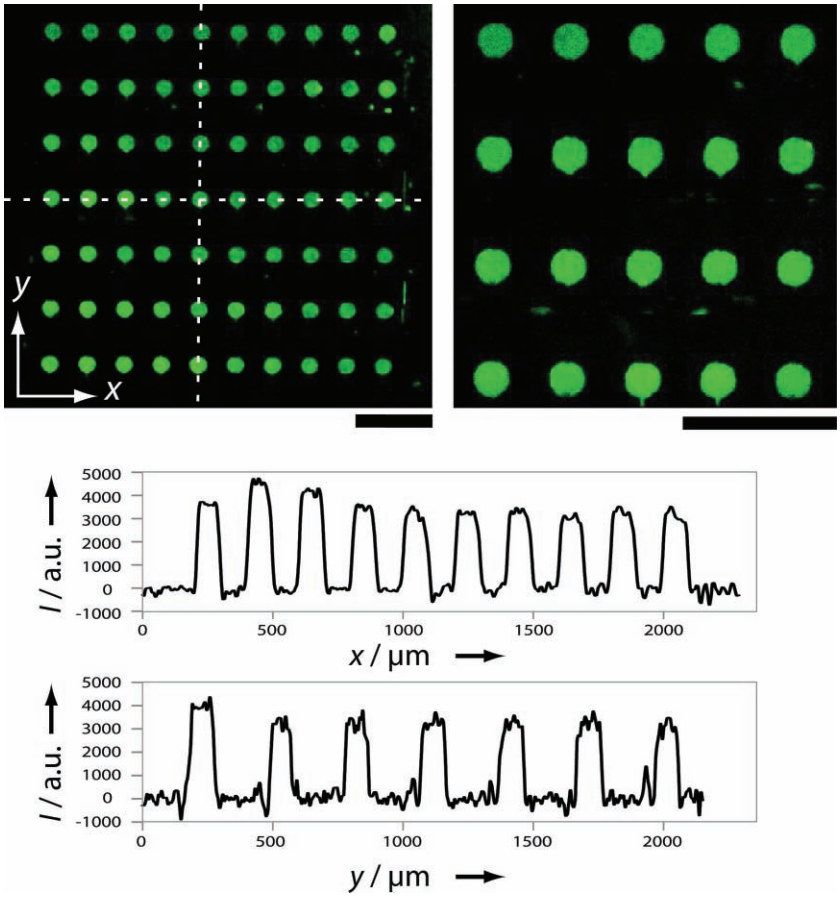


**Figure 2. Obtention of regular and stable gene expression arrays.** Top: Fluorescence microscopy images at two magnifications of an EmGFP (emerald green fluorescence protein) expression array obtained after 2 h of incubation at 37 °C of a microsealed chamber array initially containing the reconstituted gene expression solution. Bottom: EmGFP fluorescence intensity (*I*) profiles along the dashed lines shown in the upper left image. Scale bars are 400 μm.

To change their composition at will, we can use an outside stimulus able to address the composition of each individual chamber. As an external stimulus to control biochemical reactions, light is an ideal external trigger as it offers several advantages such as high spatiotemporal resolution of the excitation, tunability of the intensity, and high potentiality for biotechnological applications.[19] Moreover, the well-defined position of the chambers and the flat glass interface of the microchamber array make our method particularly suitable for selective photostimulation. As a first demonstration, we performed selective photobleaching on an array of microchambers containing the same fluorescein solution. Fluorescein was chosen because it is a common fluorescent dye, which is particularly sensitive to photobleaching. We used an excitation of 455–495 nm provided by the microscope at low and high intensity for fluorescence observations and photobleaching, respectively. First, a fluorescein solution (10 μM in 10 mM Tris-HCl buffer, pH = 7.4) was distributed in the microchambers prior to oil microsealing. Fluorescence microscopy observations using a low intensity excitation indicated a very homogeneous distribution of fluorescence intensity in all individual microchambers. Then, we applied an intense illumination through a photomask inserted between the thin cover glass (bottom part of the device) and the thick glass support (**Figure 3**, top). With an increase in illumination time, we observed a decrease in fluorescence intensity in the exposed chambers. After 30 minutes of illumination, the mask was removed and we observed by fluorescence microscopy the array of microchambers using again the low intensity excitation (Figure 3, bottom). Regardless of photomask configuration, all microchambers exposed to the intense illumination through the transparent parts of the mask exhibit a very weak fluorescence while chambers protected by the dark parts of the mask exhibit fluorescence intensity similar to that before photobleaching. These results show that we can easily combine a selective photo-illumination (e.g., using illumination through a photomask) with our micro-compartmentalization method to address specifically each individual chamber and obtain well-defined patterns of desired composition. Such approach could be extended for protein patterning using the laser-assisted protein adsorption by photobleaching (LAPAP) approach.[20]

### 2.4. Photopatterning of Calcium Ion Concentration

Although photobleaching of probes is widely used to study diffusion properties, it is not well adapted for controlling the composition or activity of molecules of biological interest. Considerable efforts have been devoted in the past ten years to develop biocompatible systems that can potentially be implemented in living organisms and activated in response to a light stimulus. For instance, DNA modification with photo-activable groups,[21] light-switchable gene promoter systems,[22] and photo-induced DNA conformational changes[23] have been proposed to control gene expression using light. Among various other approaches, the use of photocaged molecules, which can release a desired biologically active compound in response to a light stimulus, has emerged as a particularly efficient and versatile strategy.[19] Our next purpose was thus to demonstrate the possibility to combine our micro-compartmentalization method with photocaged molecules to selectively activate by light and control the concentration of a molecule of biological interest in individual microchambers. As a demonstration, we performed selective photorelease of calcium ion $Ca^{2+}$ in an array of microchambers containing the same caged calcium solution. We employed 1-(4,5-dimethoxy-2-nitrophenyl) ethylenediaminetetraacetic acid (DMNP-EDTA) originally developed by Kaplan et al. as a photolabile calcium ion chelator.[24] Upon UV illumination (365 nm), the affinity of DMNP-EDTA for $Ca^{2+}$ decreases about 600 000 fold and the dissociation constant $K_d$ for calcium ion increases from 5 nM to 3 mM. To follow the $Ca^{2+}$ concentration, we used Fluo-3 as a fluorescent $Ca^{2+}$ indicator. A solution containing $CaCl_2$ (10 μM), DMNP-EDTA (10 μM), and Fluo-3 (5 μM) was distributed in the microchamber array prior to oil microsealing. In the absence of UV illumination, all chambers exhibit a weak fluorescence indicating a low concentration of free $Ca^{2+}$. After UV (365 nm) exposure for 30 min through the photomask shown in **Figure 4**B and removal of the mask, we observed a much higher fluorescence intensity in the chambers which were specifically exposed to UV, while non-exposed chambers remained at a low fluorescence level (Figure 4A). This indicates that $Ca^{2+}$ has been specifically released in the illuminated chambers while $Ca^{2+}$ remains complexed to the chelator in the non-illuminated chambers. Our method thus allows one to control the amount of free $Ca^{2+}$ concentration in each individual chamber. We then followed the kinetics of the release of calcium ions in the eight chambers corresponding to the eyes of the 'smiley' as a function of

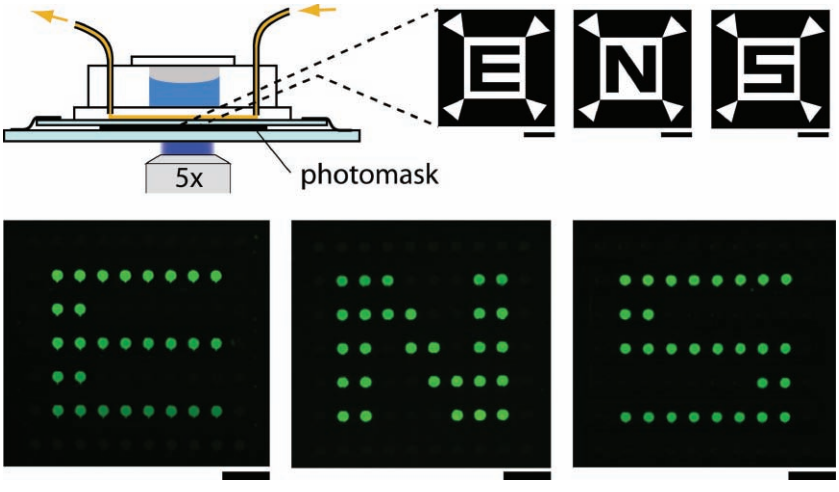


**Figure 3. Selective photobleaching.** Top: A microsealed chamber array containing fluorescein is exposed to high-intensity light through a photomask for selective photobleaching. Scale bars are 1 mm. Bottom: Fluorescence microscopy images at a low-intensity excitation for the different masks after 30 min of photobleaching and removal of the photomask. Scale bars are 400 μm.

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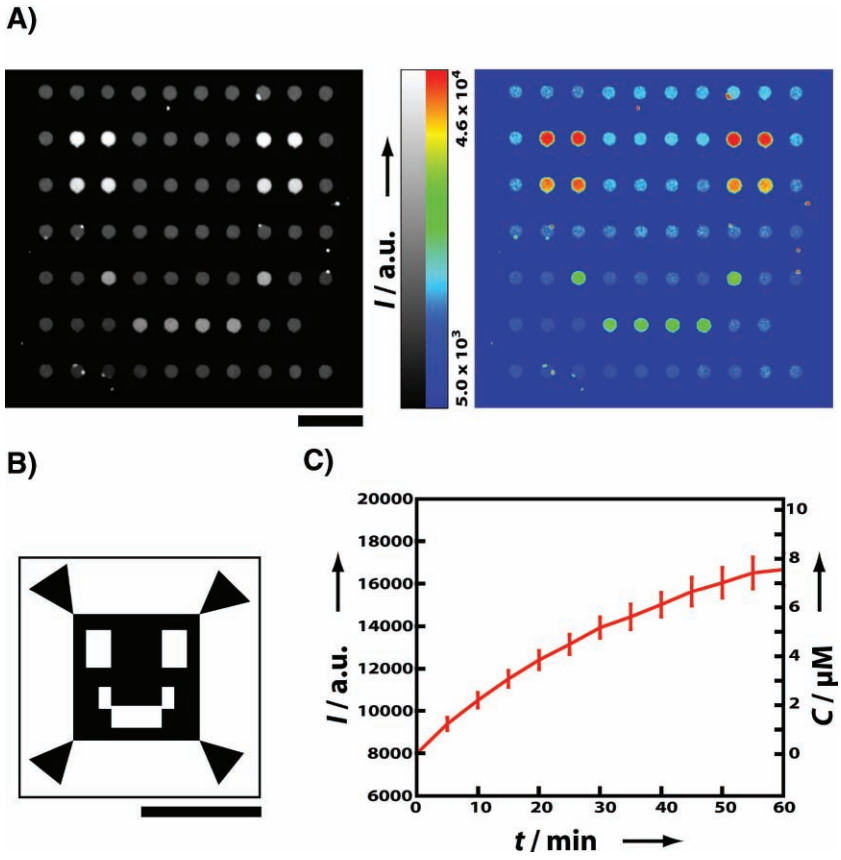


**Figure 4. Dynamic photopatterning of calcium ion concentration.** A) Fluorescence microscopy images (left grayscale, right false colors) of a microsealed array containing $CaCl_2$ (10 μM), the photolabile calcium chelator DMNP-EDTA (10 μM), and the fluorescent $Ca^{2+}$ indicator Fluo-3 (5 μM) after 30 min of UV illumination (365 nm) through and removal of the photomask shown in B. Scale bar is 400 μm. B) Photomask. Scale bar is 2 mm. C) Average fluorescence intensity (*I*) and corresponding $Ca^{2+}$ concentration (*C*) as a function of UV exposure time (*t*) in the 8 chambers corresponding to the eyes of the 'smiley' pattern.

UV illumination time while keeping the photomask. Figure 4C shows that the average Fluo-3 fluorescence intensity gradually increases with an increase in UV exposure time. The fluorescence intensity was converted into free $Ca^{2+}$ concentration by using a calibration curve between fluorescence intensity and various known concentrations of free calcium confined in the chambers in the same configuration. It indicates that free $Ca^{2+}$ increased from 0 to about 7.5 μM in 60 min of UV exposure. Such slow release is attributed to the low power of UV excitation provided by the microscope lamp and might be accelerated by using another UV light source such as a laser. Although the dynamics and the efficiency of the release should be improved for future applications, these results are, to our knowledge, the first demonstration of a control of the spatiotemporal concentration profile of a molecule of interest in a microarray without any valve, pump, or micro injection device.

## 3. Conclusion

We described a new way to distribute a sample solution into an array of independent sub-nanoliter micro-compartments. This simple, robust, and cost-effective method does not involve any active elements such as pumps and valves nor the use of surfactant molecules. Mainly in PDMS, the device is biocompatible, gas-permeable and suitable for optical detection and photostimulation. We demonstrated the possibility of conducting biochemical reactions, such as gene expression in the microchambers, which resulted in regular gene expression arrays. By illuminating an array of microchambers containing a caged calcium solution through a photomask, we selectively controlled the release of free $Ca^{2+}$ in individual chambers in a dynamic way. Extended to other photosensitive compounds, this method should open up the possibility of using light to control the concentration of various molecules of biological interest along any desired spatiotemporal distribution. Finally, the method can be applied for the realization of arrays of living single-cells, which can be further stimulated, cultured, and/or analyzed with high precision (see the Supporting information). By replacing the oil with an aqueous solution containing active water-soluble molecules, the method can be applied to study the response of entrapped cells or compounds to various chemical stimuli. Easily implementable in standard biological and chemical laboratories, this method will be useful for various fundamental investigations and practical applications requiring the use of well-defined, addressable micro arrays, such as single-cell PCR, phenotypic variability screening, or gene expression dynamic studies.

## 4. Experimental Section

*Fabrication of the Microfluidic Chip*: Photomasks for photolithography and photopatterning were drawn using LayoutEditor software and printed on transparencies. A mold for the microfluidic channel was fabricated in a 50 μm-thick SU-8 3050 photoresist (Kayaku Microchem Co., Ltd.) layer, which was spread by spin-coater WS-400E-6NPP-LITE (Laurell) at 3000 rpm on a silicon wafer (Siltronix) prior to soft bake, UV exposure with a spot light source (LC5, Hamamatsu) through a photomask, postbake, and development with SU-8 developer (Microchem) following the protocol described by the manufacturer. The SU-8 mold was then duplicated into epoxy molds according to a protocol described elsewhere.[25] To obtain the lower layer of the chip, poly(dimethylsiloxane) (PDMS) (RTV 615, GE Toshiba Silicones Co., Ltd., solutions A and B were mixed at the ratio of 10:1 (w/w)) was poured onto the mold to be ca. 1 mm thick, degassed, cured at 75 °C for more than 2 h, and peeled off from the mold. To obtain the upper layer of the chip, ca. 5 mm thick PDMS was degassed and cured in a plastic Petri dish prior to punching a 6 mm diameter

hole. The lower layer was laid on a glass slide with the channel-fabricated surface down, and the upper layer was glued with non-cured PDMS onto it so that the hole of the upper layer covered all of the microchambers in the lower layer. This two-layer PDMS block was baked on a hot plate at 95 °C for 5 min to cure the PDMS glue. Inlet and outlet holes for the microfluidic channel were punched in the PDMS block by using a syringe needle without a pointed end (threaded hub needle gauge 21, Kahnetics), then these holes were flushed with isopropanol. The channel-fabricated side was cleaned with isopropanol and a scotch tape (3M), and bonded to a cover glass slide (0.13–0.16 mm thick, Menzel-Gläser) by using a plasma cleaner (Harrick). Thus obtained microfluidic chip was baked on a hot plate (Heidolph) at 150 °C for 1 h to make the PDMS wall of the channel hydrophobic.

*Filling and Sealing of the Microchambers*: Several minutes (15–30 min) before injection of a sample solution to the chip, the hole in the upper PDMS layer of the chip was filled with water to saturate PDMS surrounding the microfluidic channel with vapor. The water pool was sealed with a piece of thin PDMS sheet for convenience. Then the chip was placed on a microscope glass slide (1.0 mm thick, Menzel-Gläser) and fixed with scotch tapes to protect the thin cover glass slide at the bottom of the chip. The channel outlet was closed with a plug, which was made of a micropipette tip filled with PDMS and cured prior to use. 5 μL of a sample solution was taken by micropipette and its tip was removed from the pipette. Then the tip was gently tapped so that the sample solution was settled down to the tip end, and inserted into the channel inlet. The tip was then connected to a digital manometer (LEO 2, Kelatron), a precision pressure regulator (IR402, SMC), and compressed air with flexible tubing (Tygon tubing, Saint-Gobain). The joint between the inlet tip and the tubing was sealed with patafix (UHU GmbH & Co. KG).[26] The sample solution was pushed into the channel with the compressed air at about 300 mbar gauge pressure under observation by microscope. Once all the microchambers were completely filled, the plug and the inlet tip were removed and oil was introduced into the channel as follows. A 1 mL plastic syringe (Codan) without plunger, containing 500 μL of mineral oil (Fluka), and placed at about 20 cm above the chip was used as an oil reservoir. The inlet and outlet of the syringe were connected by tubing to the air pressure control system and to the channel inlet, respectively.[26] The outlet of the channel was connected by tubing to a waste container placed lower than the oil reservoir. Oil spontaneously flowed by hydrostatic pressure or eventually pushed by the compressed air at a few tens mbar gauge pressure in the main canal of the channel. As a result, the microchambers were sealed and isolated. Then the pressure was released and all of the experiments were performed with this final configuration keeping the oil reservoir connected. The thick glass slide was removed during incubation and further observations.

*Fluorescence Microscopy*: We used an Axio Observer inverted microscope (Zeiss) equipped with an EM-CCD camera, PhotonMAX (Princeton Instruments/Acton). EC Plan-Neofluar 2.5x/0.075, N-Achroplan 5x/0.13, and Achroplan 20x/0.45 objectives (Zeiss) were used to obtain images of this article. N-Achroplan 5x/0.13 lens was used for light illumination in the photobleaching and calcium photopatterning experiments. An HBO 100 W mercury lamp (Osram) was used as a light source.

*Image Analysis*: All image analyses were performed by using ImageJ software.

*GFP Expression in the Microchambers*: This experiment was performed under RNase-free condition. A cell-free gene expression medium, PURExpress was purchased from New England Biolabs. Inc. pRSET/EmGFP plasmid DNA carrying a T7 promoter, a ribosome binding site, and a gene coding for EmGFP was obtained from Invitrogen. RNase free water was from Sigma. Water, PURExpress solution A, plasmid DNA, and PURExpress solution B were assembled in this order on ice to a final volume of 20 μL. The final concentration of plasmid DNA was 2 μg $mL^{-1}$. The mixed solution was introduced to the microchamber array under observation by microscope as described above. After sealing with oil, the chip was protected from light and incubated on a heat block (Fisher scientific) at 37 °C.

*Selective Photobleaching*: Deionized water (Millipore, 18 MΩcm$^{-1}$) was used for this experiment. Fluorescein and Tris-HCl were obtained from Sigma. The mixed solution containing 10 μM Fluorescein and 10 mM Tris-HCl (pH 7.4) was used for the experiment. The photomask was stacked onto the thick glass slide by capillary force with 5 μL of water. Then the chip filled with the sample solution and oil was put on the photomask and aligned on the microscope so that the proper chambers were exposed to the excitation light. The chip was fixed on the thick cover glass slide with scotch tapes. We exposed a light of 455–495 nm wavelength (filter set 44, Zeiss) at maximum intensity to selectively bleach fluorescein in the exposed chambers. It took 30 min to get complete disappearance of the fluorescence from the exposed chambers. After removing the thick glass slide and the photomask, fluorescence microscopy images were recorded with a low intensity excitation.

*Photopatterning of Calcium Concentration*: DMNP-EDTA and Fluo-3 were purchased from Invitrogen. Calcium chloride was from Sigma. Deionized water was used for the experiment. Calcium chloride, DMNP-EDTA, and Fluo-3 were mixed in this order and confined in microchambers. The final concentrations of these compounds were 10, 10, and 5 μM, respectively. After the alignment of the chip and the photomask in the same way as described above, we exposed UV light (365 nm, filter set 49, Zeiss). Then the fluorescence from Fluo-3 was observed at 455–495 nm excitation (filter set 44, Zeiss). The fluorescence intensities of 8 microchambers were measured every 5 min to follow the kinetics of calcium ion release. The calibration curve for the free calcium ion concentration was obtained by measuring the Fluo-3 fluorescence intensities of the same 8 microchambers with calcium chloride of defined concentrations. The measurement for calibration was done under exactly the same condition; that is, the solution was confined in microchambers, with the photomask, and with the same configuration for detection. The fluorescence microscopy image of Figure 4A is obtained after removal of the thick glass slide and the photomask.

*Cell Culture and Loading*: Cell trapping has been performed using human cervical cancer cell line (HeLa cells) at P7. Cells from cryogenized stocks have been thawed quickly and cultured to confluence in a standard T25 flask (Techno Plastic Products AG) at 37 °C, 5% $CO_2$. Growth medium used was DMEM (Dulbecco's Modified Eagle Medium, Invitrogen) with 10% v/v FBS (fetal bovine serum, Invitrogen). Cells have been rinsed once with 1 × PBS (Phosphate-Buffered Saline, Invitrogen) and harvested by 3 min incubation with 1 mL commercial Trypsine-EDTA solution (Trypsin 0.05% in 0.53 mM EDTA, Invitrogen). Trypsine digestion has been

  

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stopped by addition of 9 mL growth medium and cells have been counted using disposable counting chambers (Kova slide, CML), centrifuged at 1000 rpm for 5 min and resuspended in growth medium to a final concentration of $10^7$ cells $mL^{-1}$. Dilutions for experiments have been obtained by simply adding the proper quantity of medium to this initial suspension. Cell solution has been loaded into the device as described before.

## Supporting Information

*Supporting Information is available from the Wiley Online Library or from the author.*

## Acknowledgements

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