Supplementary file 2
Cell toxicity of the proposed method

In this supplementary data, we confirmed the cell toxicity of the proposed drive method.

1. Cell toxicity of materials

1.1 Method

First, we evaluated viability of cells cultured with four different materials that were used for our devices, gold (Au), chromium (Cr), PNIPAAm, and glass. Au, Cr and PNIPAAm were patterned on glass substrates with actual patterns that were used for experiments in main manuscripts. Here, we note that the PNIPAAm samples were fabricated with Cr patterns exactly same as the devices used in main manuscripts. For evaluations of glass substrate, we used bare cover glass as a sample. To count the live and dead cells, we bonded culture chambers made of PDMS on the fabricated samples, as shown in Fig. S1. We made hole with diameter of 1.5 mm on the PDMS to cover the gel actuator array of approximately 1.5 mm x 1.5 mm sizes.

We used Jurkat cells for this evaluation. We prepared cell suspension of the target cell in culture medium (RPMI 1640, 11875-093, Thermo Fisher, Tokyo, Japan). We applied the cell suspension onto the PDMS chamber. After the applications of samples, we cultured the samples for one day and two days in a CO_2 incubator. Then, we added Live/Dead viability kit (Cellstain Double Staining Kit, CS01, Dojindo Laboratories, Kumamoto, Japan) to culture medium and incubate it for 15min. After that, we counted the number of live/ dead cells in the chamber by using fluorescent microscope. The counts of the live/ dead cells were performed for three different samples for each condition.

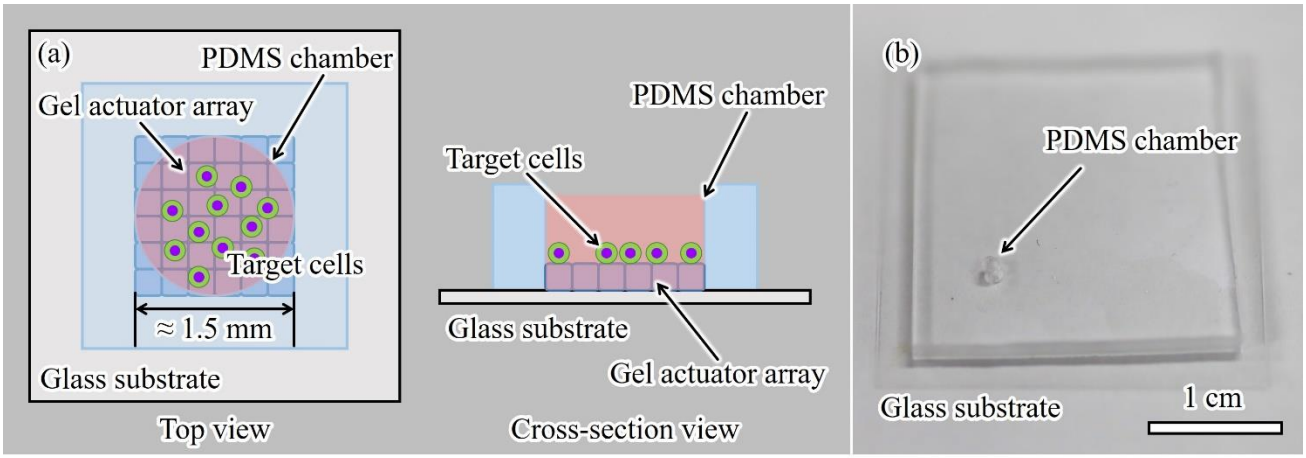


Figure S1. Experimental setup for cell toxicity
(a) schematic of chamber structure, (b) picture of fabricated sample with PDMS chamber.

1.2 Results

The results of cell viability with respective materials are shown in Fig. S2. Additionally, typical fluorescent images of live cells (green) and dead cells (red) on each material were shown in Table. S1.

Form these results, approximately 80% of cells were alive after one day culture with all materials. After two days culture, approximately 73 %, 59 %, 65 % and 64 % of cells were alive for Au, Cr, Glass and PNIPAAm, respectively. For results of Cr, cell viability was slightly low comparing to other materials. It is thought to be due to the cell toxicity of Cr [1]. However, cell viability of PNIPAAm with Cr light absorber was little different from that of other materials. This result indicates that Cr covered by PNIPAAm gives little damage to cells. Thus, it is thought that the proposed drive method has low cell toxicity caused by materials used for the device.

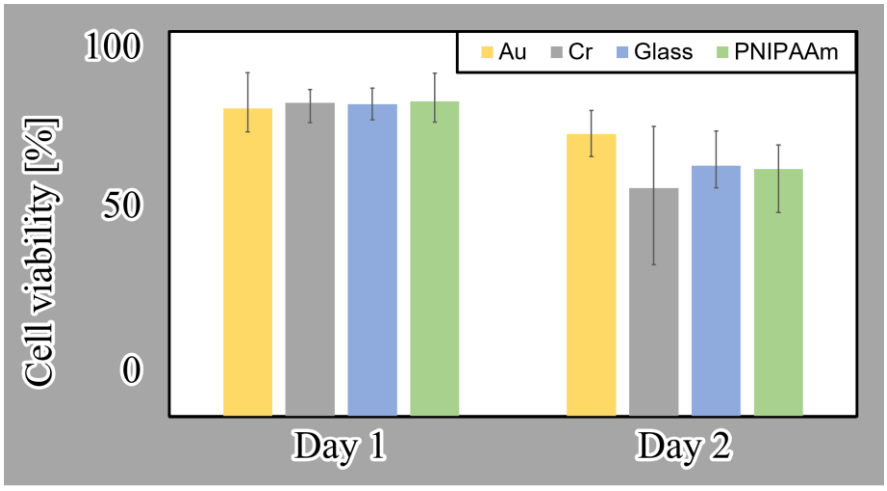


Figure S2. Cell viability with materials used for proposed drive method. Sample numbers were followings. Au day 1: 155, Cr day 1: 208, glass day 1: 300, PNIPAAm day 1: 338. Au day 2: 167, Cr day 2: 221, glass day 2: 291, PNIPAAm day 2: 260.

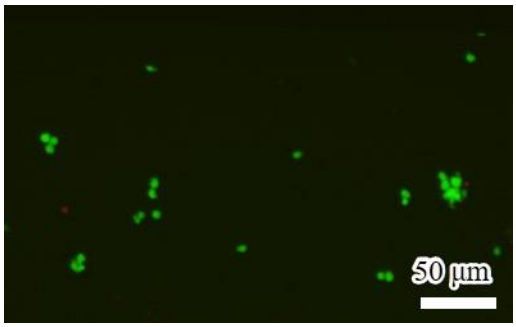
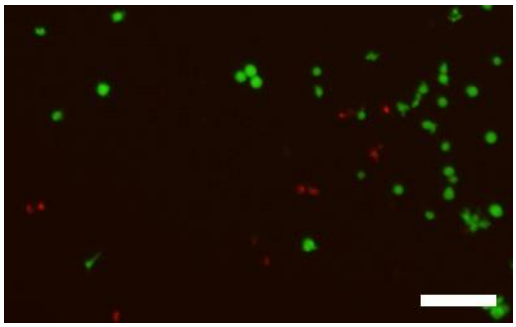
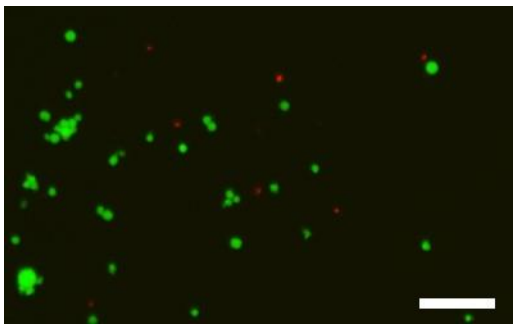
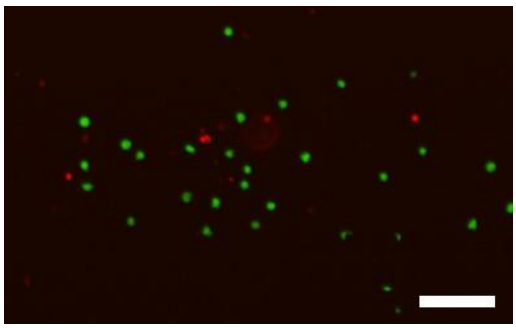
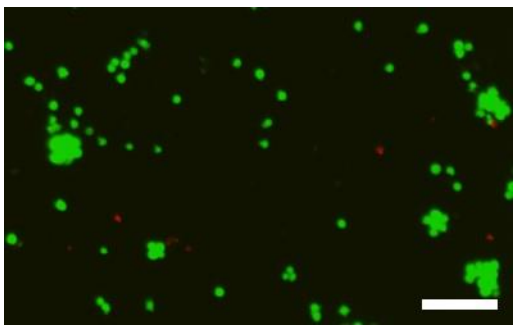
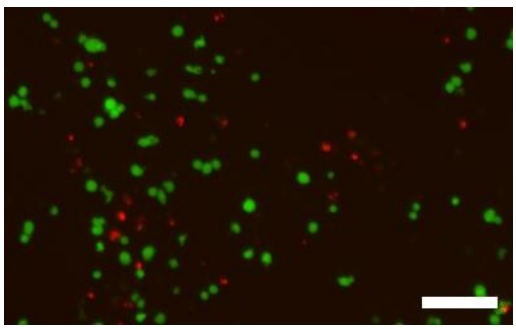
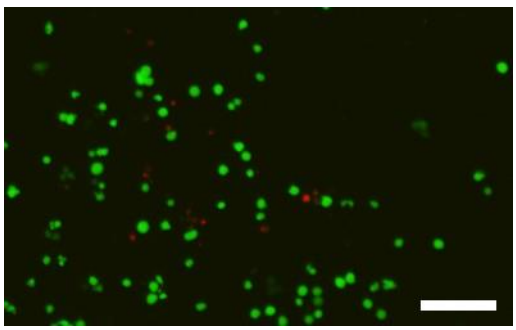
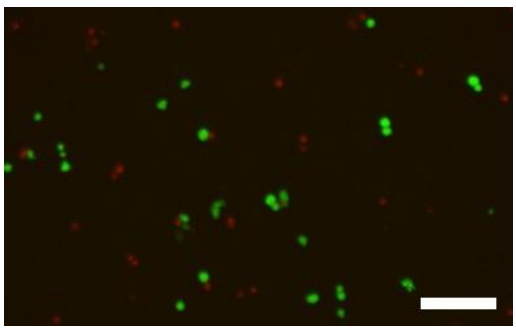
	Day 1	Day 2
Au		
Cr		
Glass		
PNIAPAAm		

Table S1. Typical fluorescent images of cell viability check with respective materials

2. Thermal damage to the cells with laser irradiation

2.1 Method

Second, we evaluated cell damage with laser irradiation with various laser powers. IR laser with wavelength of 1064 nm is known to have low cell damage because of low absorbance to water [2, 3]. According to ref. [3], direct irradiation of IR laser to living cells with 176 mW for shorter than 1 min has little damage to the cells. This laser power is larger than laser power we used in the main manuscript; 150 mW. Thus, we mainly focus on thermal damage generated by irradiation of the laser to Cr pattern with proposed drive method.

We prepared samples of gel actuator arrays with fabrication process explained in the main manuscript. For evaluations of cell viability, we bonded observation chamber made of PDMS on the fabricated samples, similar to evaluations in Section 1. For cell samples, we prepared Jurkat cell suspension as same way as section 1 and applied it for the fabricated samples.

In this evaluation, we irradiated IR laser to the samples with laser powers of 50, 100 and 150 mW. With respective laser powers, we irradiated laser to integrated gel actuators with same conditions as experiments in main manuscript. We scanned the actuator array with laser with irradiation time of 1 s and 10 s for all actuators in the array. After scanning the actuators with the laser, we confirmed the viability of the cells with fluorescent microscope after live/dead staining as same as section 1. Experimental procedure is summarized in Fig. S3.

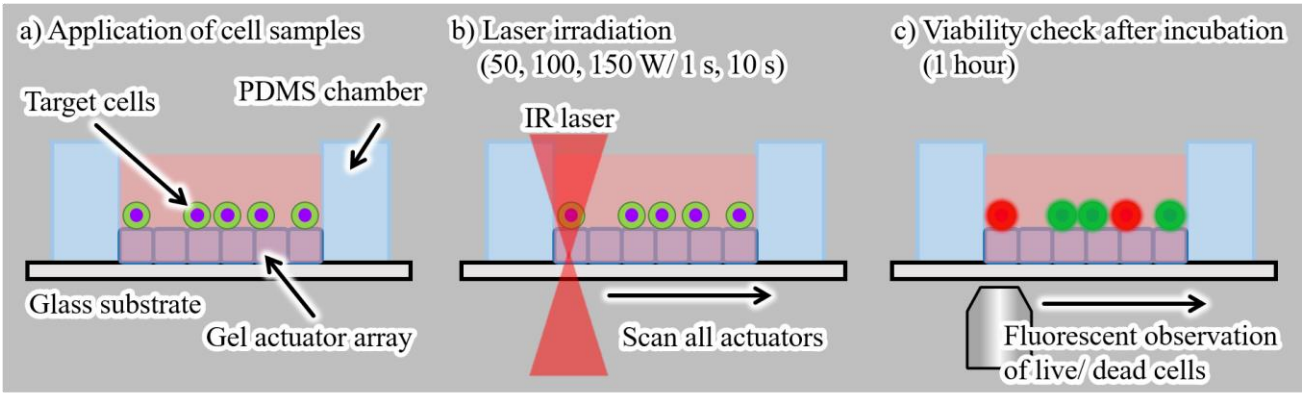


Figure S3. Experimental procedure of evaluation of thermal damage

2.2 Results

The results of cell viability with respective laser powers are shown in Fig. S4. Additionally, we show the typical fluorescent images of cells in Table S2 and S3. From these results, approximately 90 % of cells were alive after one hour with all conditions, as shown in Fig S3 (a). Thus, it is thought that there is a little thermal damage for cells with the proposed drive method.

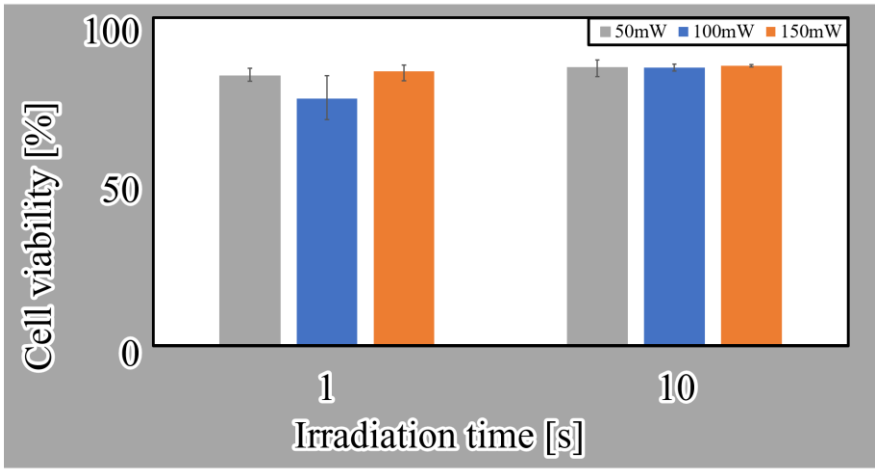


Figure S4. Cell viability with various laser powers

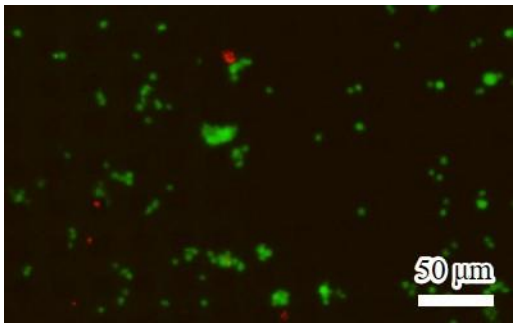
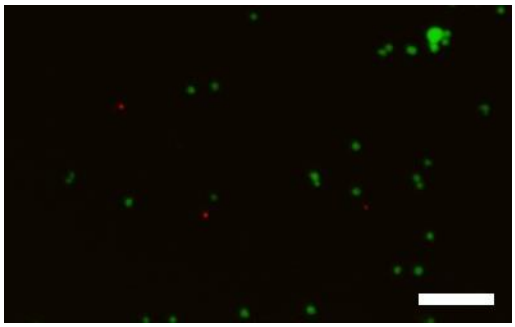
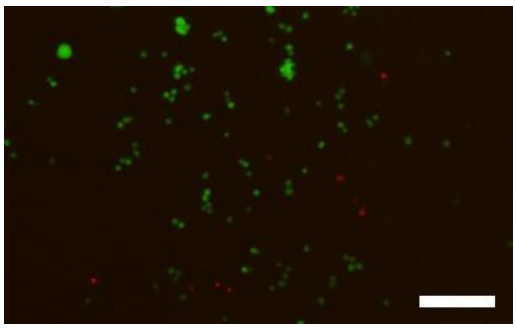
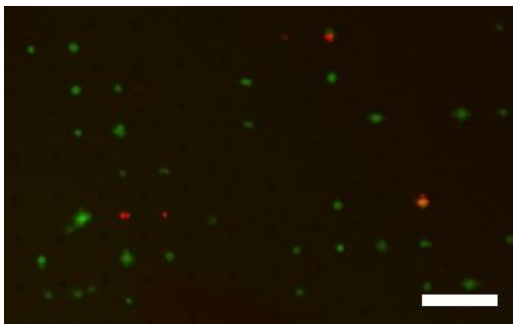
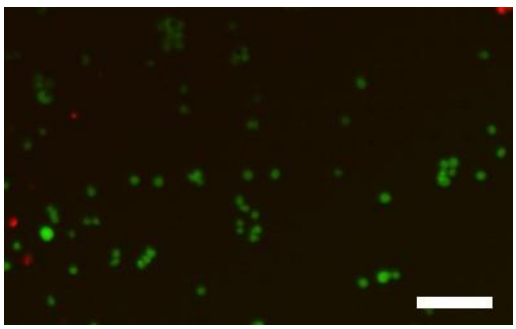
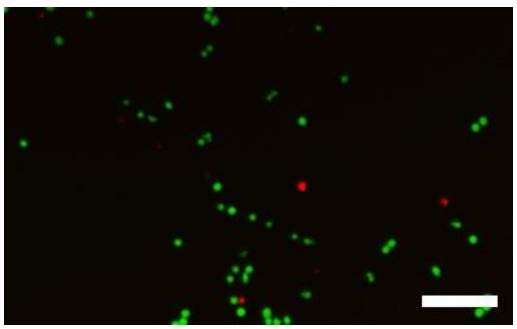
	1 s	10 s
50 mW		
100 mW		
150 mW		

Table S2 Typical fluorescent images of cell viability check with laser irradiation for 1 s

References

[1] Mehari, Tsdale F, et al. “Influence of arsenic (III), cadmium (II), chromium (IV), Mercury (II), and Lead (II) ions on human triple negative breast cancer (HCC1806) cell cytotoxicity and cell viability” Journal of chemical health risks, 7(1), 1-17, 2017.

[2] Schneckenburger, Herbert, et al. “Cell viability in optical tweezers: high power red laser diode versus Nd: YAG laser” Journal of biomedical optics, 5.1: 40-44, 2000.

[3] Hong Liang et al, “Wavelength Dependence of Cell Cloning Efficiency after Optical Trapping,” Biophysical Journal, 70,