Supplementary Materials:

Nanofluidic analytical system integrated with nanochannel open/close valves for enzyme-linked immunosorbent assay

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Tolerable position error of pressing pin and valves

Although the position of the actuator and the valve is designed to coincide each other, alignment of the actuator and the center of the valve will not automatically be achieved and an alignment error it inevitable when manually fixing the device to the lower jig and covering the upper jig, which holds the actuators inside. Therefore, an experiment was carried out to investigate the tolerable alignment error. Figure S1 (a) shows the experimental setup. For this experiment, we used a device with one nanochannel open/close valve and an actuator with a micrometer stage that can adjust the XY position of the actuator within a um precision. The actuator was initially aligned to the center of the valve, and then the position was intentionally shifted by a certain distance d ($d = 5, 10, 15, 20, 30, 40, 60, 80 \mu$ m). The fluorescence intensity of the valve chamber I was compared to the fluorescence intensity of the valve chamber when fully opened (I_0) and the ratio (I/I_0) was used to estimate the open/close value. The results are shown in Fig. S1 (b). When the actuator was ideally aligned (d = 0) and pressed the center of the valve, the intensity ratio I/I_0 was 4.5 %. While d was below 20 µm, the intensity ratio remained below 5 %. However, the ratio greatly increased after d was greater than the radius of the valve (37.5 μ m). These results imply that while pressing the inside region of the valve, the deformation distribution would not change greatly and sufficiently closes the valve. On the other hand, once the actuator is aligned outside of the valve, the valve remains more than half open. From these results, we concluded that the tolerable position error of the actuators and the valves is up to 20 µm.

Measurement of coordinates of jig holes

The position of the jig holes to insert the actuators are designed at the same position as that of the nanochannel open/close valves on the device. However, because the jig holes are mechanically fabricated, the designed position and the actual position of the holes will be slightly different ($\sim 100 \text{ }\mu\text{m}$). Although this error is negligible in the macroscopic level, this would greatly affect the valve operation performance where the actuators should press the inner part of the 75 µm-diameter valve. The valves, on the other hand, are fabricated by top-down nanofabrication, which means the precision of the position can be controlled within um scale. Therefore, in order to fabricate the jig holes and the valves at the same position corresponding with each other, the position of the valves and the jig holes should be designed separately: The jig holes were fabricated first, and then, based on the actual position of the holes, the position of the valves are determined. This process was carried out as follows. First, a photoresist (THB-111N, JSR Corp., Tokyo, Japan) was spin-coated on the substrate with the thickness of ~10 µm. Then, the actuators were inserted in each hole and pressed the substrate for more than five times. During this process, the tip of the pressing pin left a mark on the photoresist (Fig. S2). The center position of the jig holes was obtained as the average coordinates or the marks. The surface of the substrate was observed using scanning electron microscope (SEM) (ELS-7800K, Elionix, Japan). Before this observation, a conductive polymer (Espacer 300, Showa Denko, Tokyo, Japan) was spin-coated over the photoresist to prevent charge-up. The valve closest to the center of the device was used as the base position and the relative coordinates of the other valves were calculated. The results are shown in Table S1.

Supplementary Figures



Figure S1. (a) The experimental setup to investigate the tolerable alignment error of the pressing pin and the valve. The distance d between the pressing pin and the center of the valve was varied and the fluorescence intensity of the valve chamber was measured with a fluorescence microscope. (b) The results when changing d from 0 to 80 μ m. The fluorescence intensity greatly rose when d was larger than the valve radius. On the other hand, the intensity ratio remained under 5 % while d was under 20 μ m.



Figure S2. Procedure of the experiment to obtain the coordinates of the jig holes. (a) Spin-coating photoresist on the surface. (b) Inserting actuators and pressing the surface, leaving a mark on the photoresist. (c) Observation with SEM and obtaining the coordinates.



Figure S3. The alignment system developed and used for aligning the position of the actuators and the valves. The lower jig holding the device is fixed on the stage. The upper jig containing the actuators are clamped by an arm and the XY position and angle θ in the XY plane is adjustable.

Supplementary Tables

	Coordinates (mm)	Error from design (µm)
1	(34.0086, 32.9933)	9
2	(34.0165, 26.9828)	17
3	(35.7265, 36.0018)	38
4	(35.7650, 30.0000)	-
5	(35.7837, 23.9896)	21
6	(37.4676, 39.0089)	48
7	(37.4950, 33.0034)	20
8	(37.5107, 27.0032)	5
9	(37.5474, 20.9969)	32
10	(39.2102, 35.9894)	56
11	(39.2625, 30.0054)	6
12	(39.2994, 24.0089)	35

Table S1. Measured coordinates of the jig holes and the error from the designed position.