Z-directional motion control of microorganisms using electrofluidic devices fabricated by femtosecond laser

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Dynamic observation and analysis of microorganism motion are of importance to understand the functions of specific body parts of microorganisms such as flagella. For detailed and in-depth analysis of the motion, observation from multiple angles is necessary. Observation in the direction perpendicular to the body movement has been typically carried out, while there were few reports observing in the parallel direction. Introduction of microorganism suspensions in 3D microfluidic devices is capable of increasing the possibility for such observation due to the spatial confinement of biological fluids [1]. The use of 3D microfluidic devices can also significantly reduce the observation time, which is beneficial to well maintain the conditions of microorganisms. However, for continuous observation of microorganisms in the direction parallel to the body movement, the observation efficiency even when using the microfluidic devices is still not high due to random swimming behavior of microorganisms. To achieve the observation from any angles with high efficiency, 3D nondestructive control of microorganism motion in microfluidic environments will be highly desirable. Previously we reported flexible electrode patterning in microchannels based on hybrid femtosecond (fs) laser microfabrication and its application to fabrication of electrofluidics for 3D orientation of microorganism motion [2]. When a proper electric field was applied in the microfluidic environment, *Euglena* cells aligned their bodies and bidirectionally swam along the electric-field direction. In this work, we demonstrate *z*-directional motion control of microorganisms using electrofluidic devices fabricated by fs laser for high-efficiency continuous observation of microorganisms in the direction parallel to the movement. To prepare a 3D electrofluidic chip shown in Fig. 1a, metal microelectrodes with outlined square shape configuration (side length of outer square ~1 mm, side length of inner square ~0.5 mm, electrode width ~0.25 mm) were formed on the top and bottom surfaces of a microfluidic channel (~1.1 mm wide and ~0.5 mm high) using water-assisted fs laser ablation followed by electroless plating. Using the fabricated device, continuous observation of the motion of about 45 *Euglena* cells swimming along the *z*-direction was performed for a minute in an imaging area of about 160 × 120 μm^2. Due to the electro-orientation effect, the required average time for continuous observation of five *Euglena* cells swimming along the *z*-direction was shortened by a factor of ~43 (Fig. 1b). Additionally, influence of laser processing parameters and electroless plating process on the performance of fabricated devices are discussed.

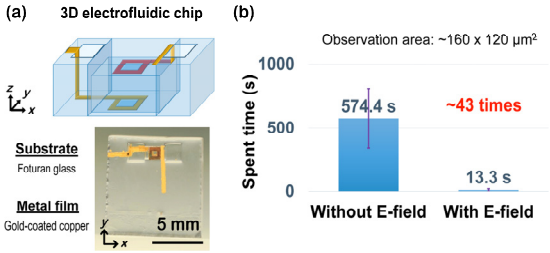

Fig. 1 (a) Schematic and photo of 3D electrofluidic chip; (b) Comparison of average time required for capturing images of five *Euglena* cells swimming along the *z*-direction in a microfluidic channel without/with an electric field (0.8 MHz, 10 Vp-p). Observations were repeated 10 times for each scheme. Observation area is about 160 × 120 μm^2. The conductivity of *Euglena* cell solution is about 0.96 mS/cm.

[1] Y. Hanada, *et al., Biomed. Microdevices* 10, 403 (2008); [2] J. Xu, *et al.*, The 62nd JSAP Spring Meeting, 12p-B6-6, March (2015).