Lab on a chip

Supplementary Information

Title: One-photon and two-photon stimulation of neurons in a microfluidic culture system

Authors: Jae Myung Jang, Jeonghyueon Lee, Hyeongeun Kim, Noo Li Jeon, Woonggyu Jung
S.1 Long-term culture in the microfluidic system

Figure S1 shows the spontaneous activity of a single neuron and its axon in the microfluidic system on the microscope for 12 days that was measured continuously. When neurons cultured in the microfluidic device are placed in a larger CO$_2$ incubator, they can be cultured for longer than 3 weeks. However, when placed in a small microenvironmental chamber and placed on a microscope stage, where we could continuously measure neural activity, the cultures lasted for 12 days with medium changed once a week. Such continuous measurement of neuronal activity for extended periods with simultaneous optical imaging is an extremely difficult experiment to perform.

**Fig. S1** The microfluidic system could maintain a neuron in culture on an inverted microscope for 12 days. The cultures lasted for 12 days with medium changed once a week. The spontaneous activity was continuously recorded in both of soma and axon. The neural signals for each day were analyzed into the average of spike numbers per a second (a) and the length of inter-spike intervals (b). These graphs show that the neurons are active in the microfluidic system for 12 days at least.