# In Situ Neurite Guidance Activated by Femtosecond Laser Processing in Microfluidic Device

°Dian Anggraini[1], Kazunori Okano[1], Yo Tanaka[2], Sohei Yamada[1],
Yaxiaer Yalikun[1,2], Yoichiroh Hosokawa[1]

[1] Div. Mat. Sci., NAIST, Japan, [2] Cent. BDR, RIKEN, Osaka, Japan

E-mail: dian.anggraini.cu7@ms.naist.jp

Cell transplantation is the current gold standard for the treatment of brain damage such as traumatic brain injury, spinal cord injury, and stroke. Neuronal integration post-transplantation between donor and host has always been the biggest challenge. Misalignment of neuronal integration site and timing could lead to severe side effects such as graft-induced-dyskinesia and epilepsy. Therefore, guided neurite growth, both spatially and temporally, is highly necessary for achieving functional brain recovery. Recently, femtosecond laser has been used for high precision spatial neurite guidance [1, 2]. The exclusive feature of femtosecond laser allows real-time manipulation during cell culture. Therefore, not only spatial but also temporal control of neuronal manipulation could be attained. In this work, we developed a novel real-time manipulation system utilizing femtosecond laser for guiding spatiotemporal arrangements of PC12 cells by nerve growth factor (NGF) stimulation in a microfluidic device.

We prepared microfluidic experimental setup for an NGF introduction, femtosecond laser irradiation system, and single-cell observation system. The microfluidic device consisted of four layers: PDMS chamber, thin-glass (thickness, 4 µm), PDMS channel, and glass substrate. PC12 cell line was cultured inside the PDMS chamber on the thin-glass. NGF (100 ng/ml) was introduced into the PDMS channel. After one day of culture, femtosecond Ti:Sapphire laser amplifier (800 nm, 150 fs, 1 kHz, 250 nJ/pulse) was focused through a 10x objective (NA = 0.25) on the thin-glass to fabricate the micro-holes. Live-cell imaging was performed to observe the neurite extension before and after the laser processing (**Fig. 1**).

The femtosecond laser irradiation within 2-8 s created micro-holes with diameter of 0.3-1.5 µm. From the micro-holes, NGF was released and induced the neurite growth of PC12 cells 1–16 h after the laser processing (**Fig. 2**). The neurite growth directed to the micro-hole position starting from 4 h after the laser processing, indicating spatial guidance of neurite growth (**Fig. 3**). Interestingly, two distinct neurite elongations were observed at different cells-to-micro-holes distances. Longer neurite extension was observed at cells positioned far (420–840 µm), rather than at close (0–420 µm) distance from the micro-holes (**Fig. 4**). Such phenomenon could be attributed to NGF accumulation, in which lower NGF accumulation at far distance (420-840 µm) was sufficient to induce the latent process of PC12 neurite elongation, compared to over-accumulation of NGF at short distance (0–420 µm) that suppress neurite growth. This result also suggests that two regions of NGF gradient were generated on the PDMS chamber, indicating the slow release of NGF through the micro-holes. Combined, these results demonstrate that our method allows control of multiple neuronal arrangements with directional neurite growth. Next, we will develop precised multidirectional control and age-dependent arrangements of neurons.

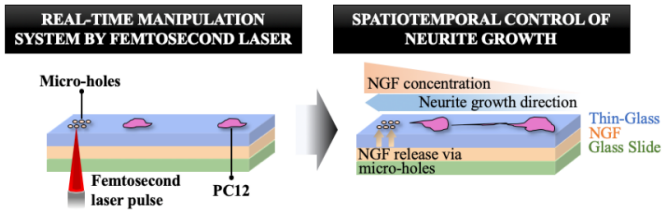


**Fig.** 1. Schematic diagram of real-time manipulation system to create femtosecond laser-fabricated micro-holes that release nerve growth factor (NGF) and induce neurite guidance.

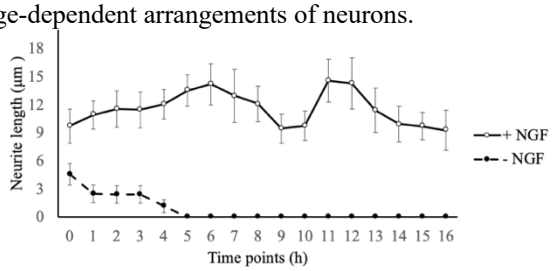


**Fig.** 2. Neurite length of PC12 cells with NGF stimulation (+ NGF) and without NGF stimulation (- NGF). Values are shown as means ± SEM ($n \geqq 40$). $p<0.001$, both experimental groups.

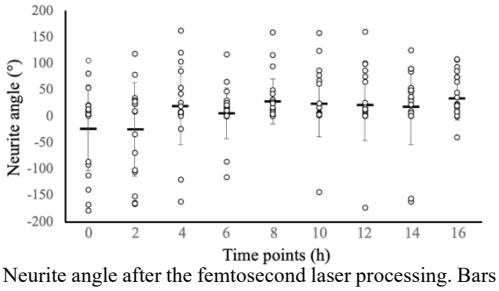


**Fig.** 3. Neurite angle after the femtosecond laser processing. Bars indicate standard deviation values ($n \geqq 20$). $p<0.05$, at 4-16 h time points.

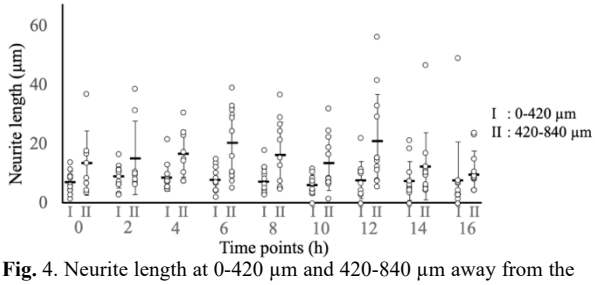


**Fig.** 4. Neurite length at 0-420 µm and 420-840 µm away from the micro-holes. Bars indicate standard deviation values ($n \geqq 20$). $p<0.05$, both experimental groups at 4-12 h time points.

**References**

[1] H. Yamamoto et al. Appl. Phys. Lett. **99**(16) (2011) 163701. [2] K. Okano et al. IEEE (2016) pp. 1-5.