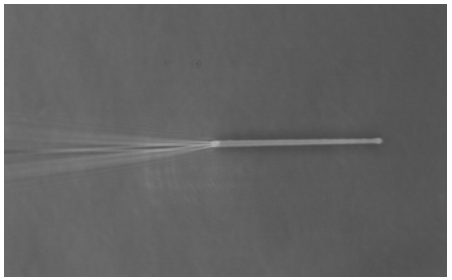
Yuhei Tanaka [1,*], Haruki Watanabe[1], Kenji Shimoda[1] and Kenji Yasuda[1]

[1] Department of Pure and Applied Physics, Graduate School of Advanced Science and Engineering, Waseda University, 3-4-1 Okubo, Shinjuku, Tokyo, 169-8555, Japan.

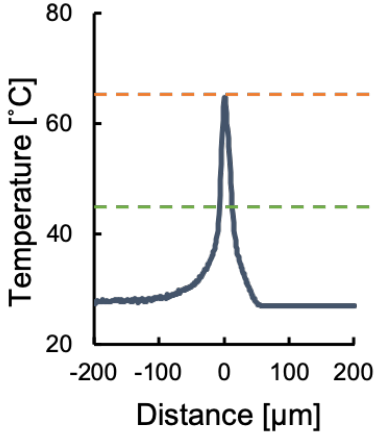
* Corresponding author: rmirjaa5613@fuji.waseda.jp

Flexible microfabrication on a chip during cultivation for a neuronal network direction control using stepwise photo-thermal etching of an agarose architecture

Pt-coated needle

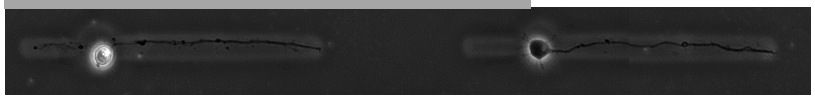


Localized heating



Processing while culturing

3DIV : Before processing

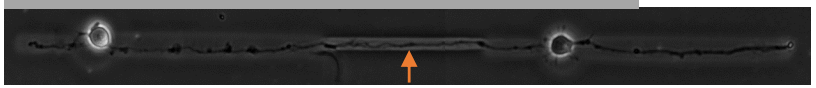


▼ Additional patterning during cultivation

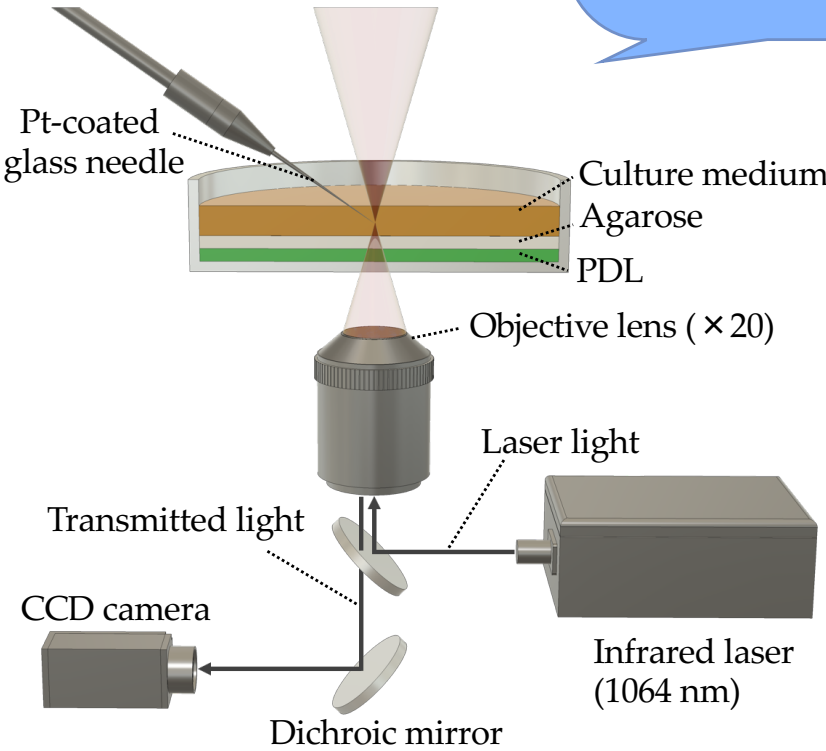
3DIV : 3 hours after processing

▼ The neurite started to elongate and reached to another neurite

4DIV : Elongation of the neurite

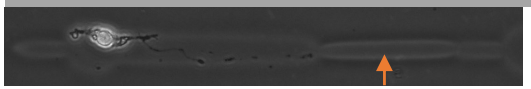


50 μm



Another technology

3DIV : 3 hours after processing



50 μm

ICMA 2021

Abstract

Abstract: Control over spatial distributions and patterns of individual neurons and their neurites provides an essential tool for studying the meaning of neuronal network patterns. Moreover, the complete direction control of synaptic connections between cells in each neuronal network is also essential to investigate the detailed information on the relationship between the forward and feedback signaling among the cells. Here, we have developed a method for topographical control of the direction of synaptic connections within a living neuronal network using a new type of individual-cell-based on-chip cell-cultivation system with an agarose microfabrication technology. The advantages of this system include the ability to control positions and number of cultured cells as well as flexible control of the direction of elongation of axons and dendrites with stepwise melting of thin agarose layer coated on the cultivation chip with a focused infrared laser beam even during cultivation without any destructive damage on cells. Using this system, we succeeded in forming fully-direction controlled single-cell-based neuronal network from individual Rat hippocampal cells. In this meeting, we discuss the potential damage of heat to cells while stepwise melting of agarose and demonstrate the ability of our on-chip agarose microfabrication method for individual cell-based neural networks.

Keywords: Neuronal network; agarose microfabrication technology; non-destructive stepwise photo-thermal etching; neurite direction control.

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Introduction

How to create the fully direction controlled neuronal network on a chip?

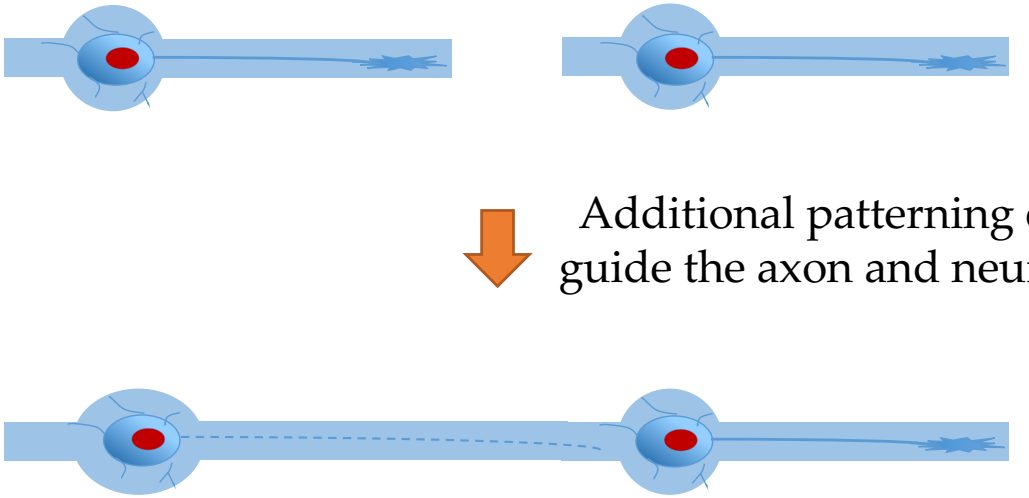

Fig.1. A method to guide each neurite with additional patterning.

Results and Discussions

An agarose microfabrication technology: Melting a portion of agarose with a focused infrared laser absorbance.

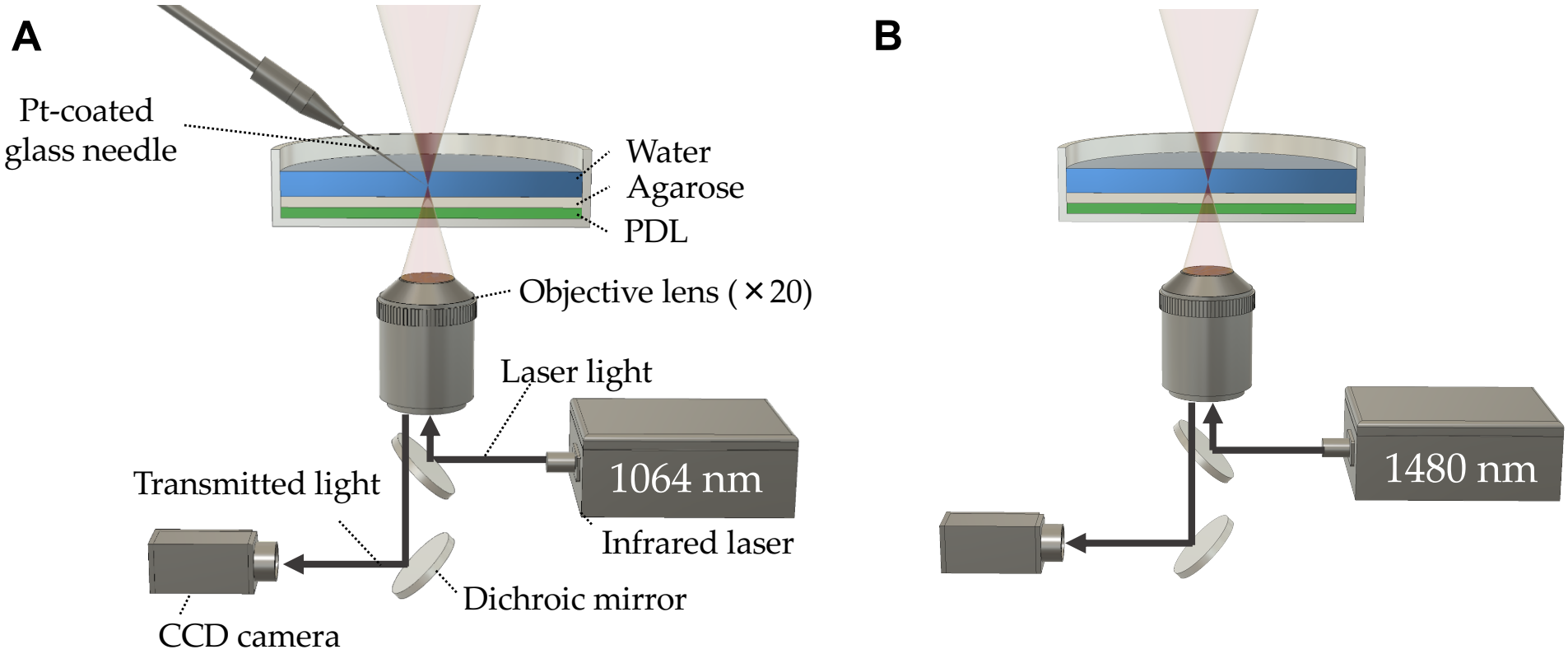

Fig.2. Two ways of system design: (A) Microneedle absorbance technology, (B) direct absorbance technology.

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Results and Discussions

Microneedle absorbance technology: Melting a one-micrometer portion of agarose layer with a heat of 0.7 micrometer microneedle tip by absorbance of 1064-nm IR laser.

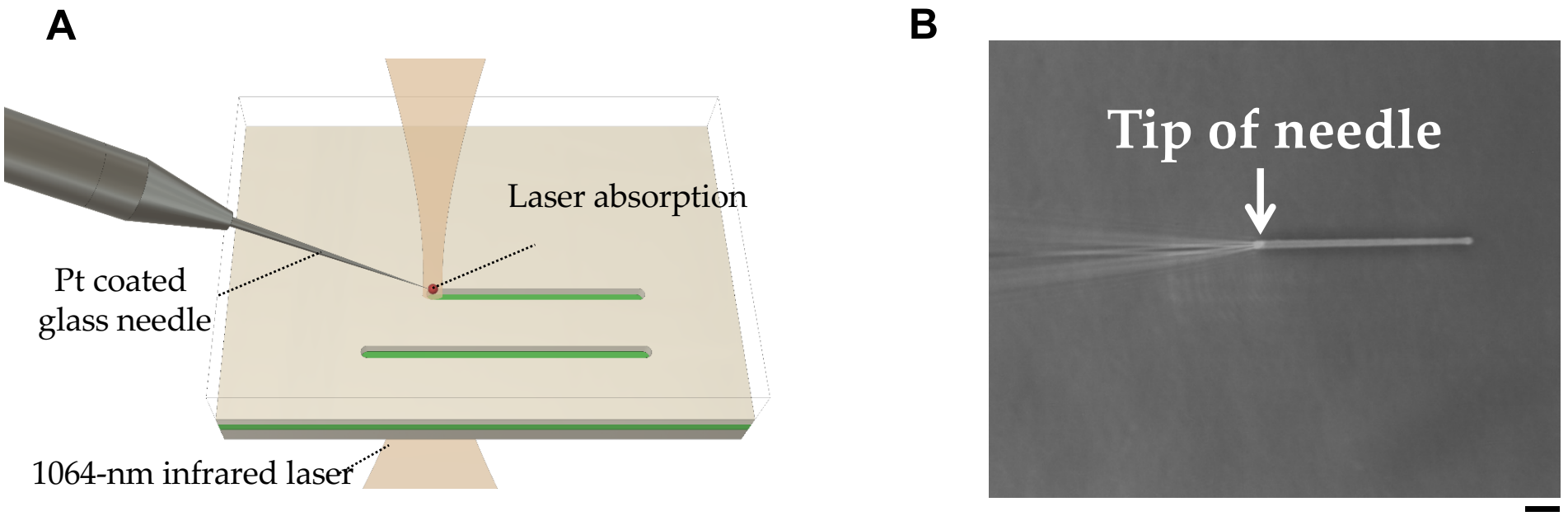
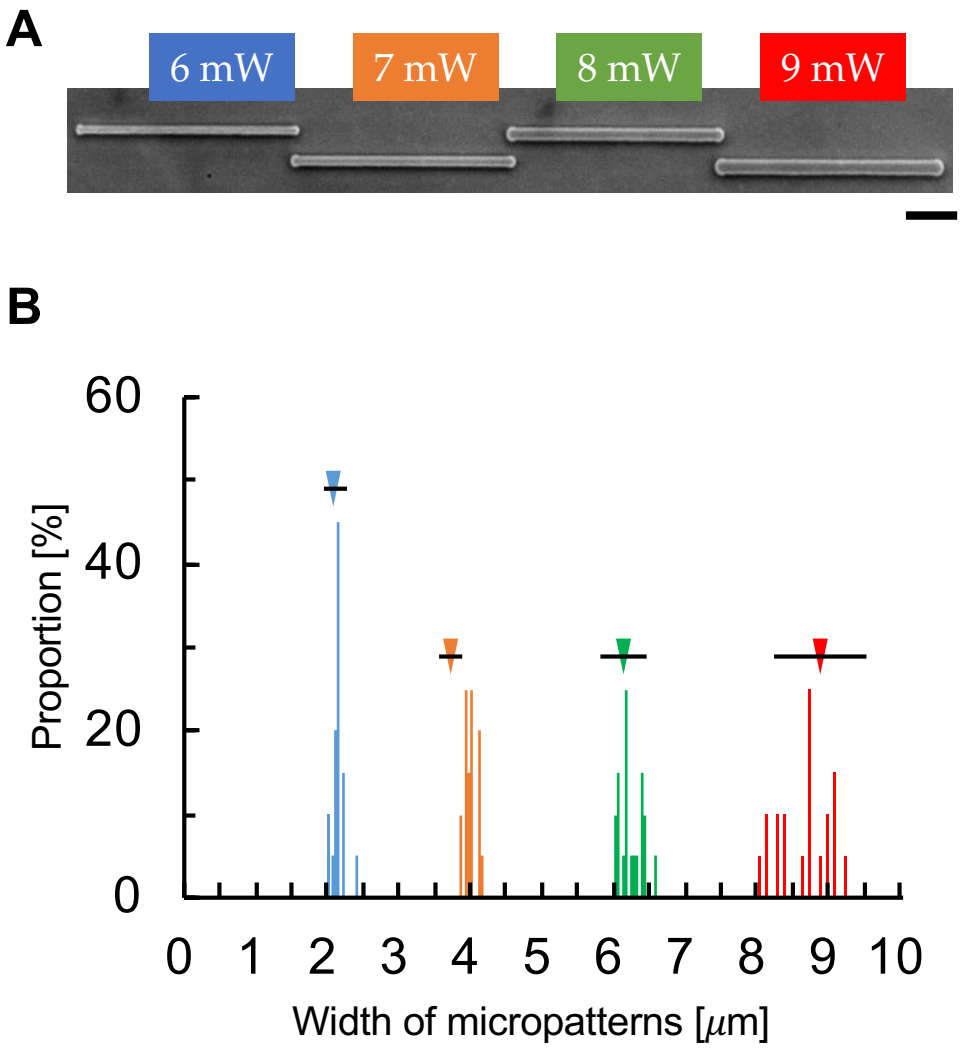


Fig.3. Detailed drawings of agarose microfabrication with Pt-coated microneedle (A) Schematic figure. (B) Phase contrast image of agarose microfabrication. Bar, 10 μm.

Results and Discussions

Precise control of agarose microfabrication with micrometer resolution.



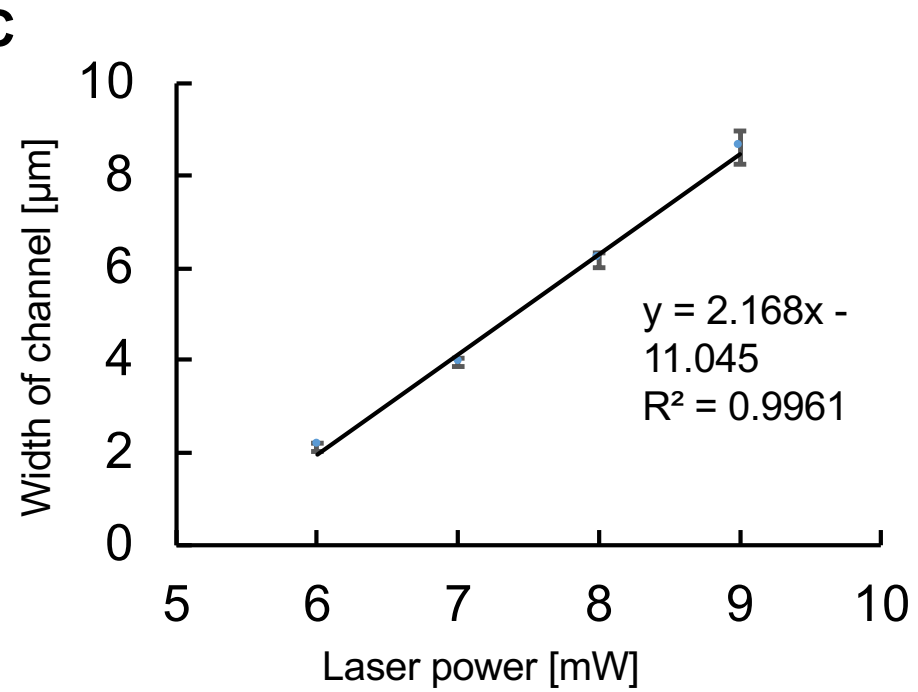


Fig.4. Comparison of processing width by laser power. (A) Processed chanel. Bar, 50 μm. (B) Width distribution. (C) Relationship between laser power and width.

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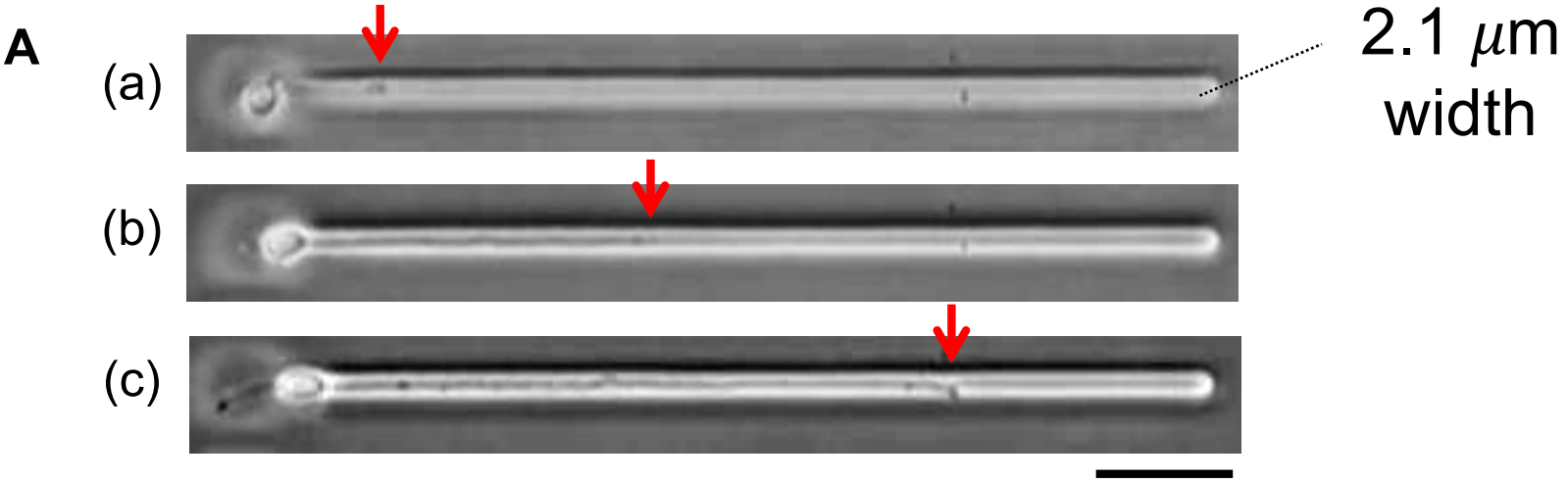
Results and Discussions

Single neurite was elongated in the two-micrometer- width agarose microchannel.



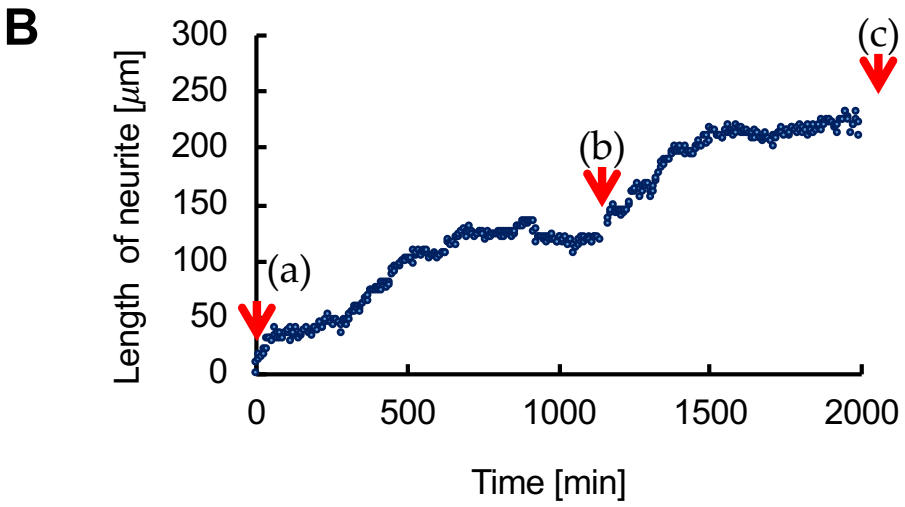


Fig.5. Observation of neurite elongation. (A) Elongation of the two neurites. Bar, 50 μm. (B) Time course of neurite length.

Results and Discussions

Heat distribution of our two agarose microfabrication technologies: Microneedle and direct absorbance methods.

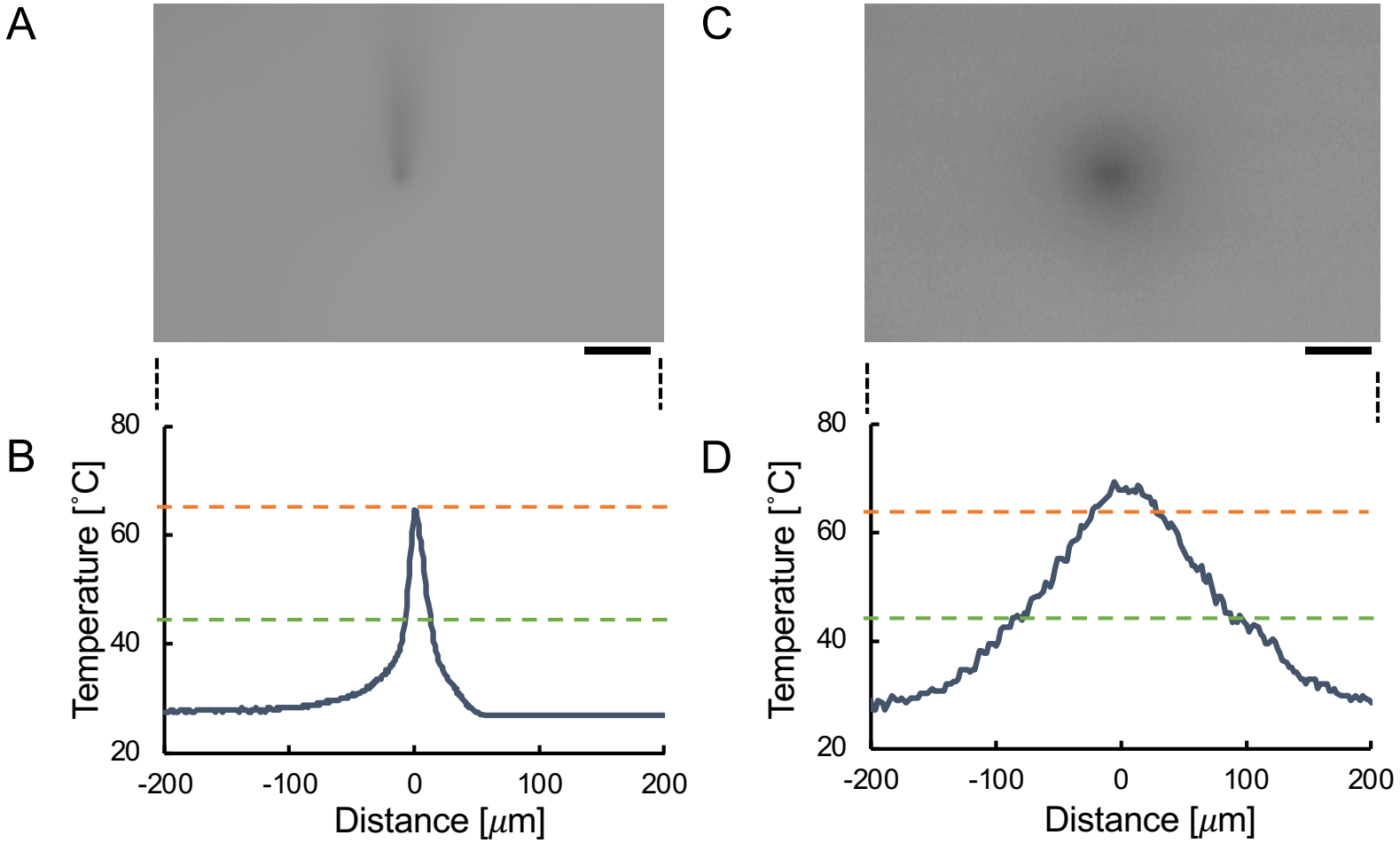


Fig.6. Comparison of the heating ranges of agarose microfabrication technologies. (A)(B) Microneedle method. (C)(D) Direct absorbance method. Bars, 50 μm.

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Stepwise additional agarose microfabrication guided an axon to another neuron.

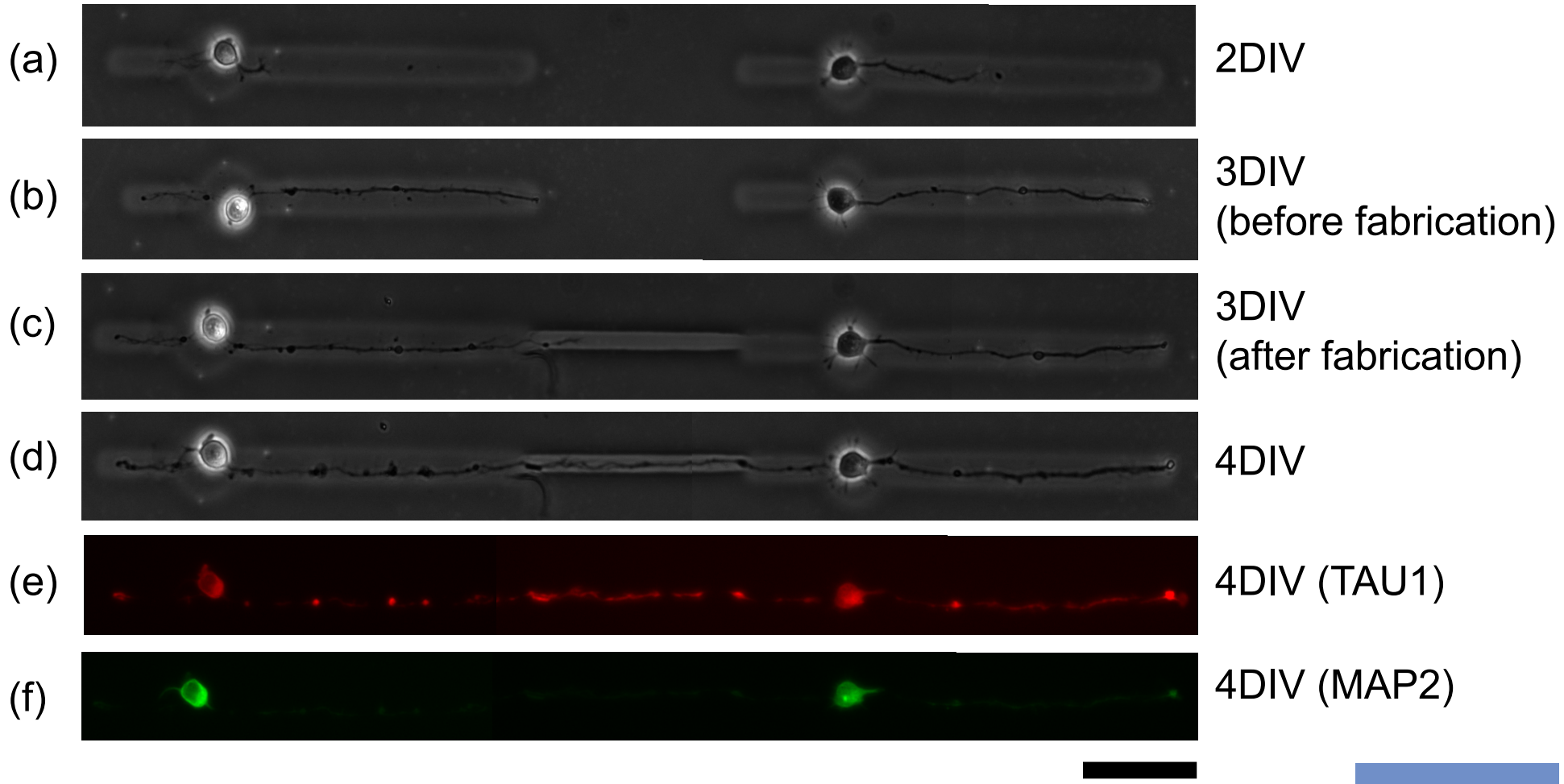

Fig.7. Additional processing during cell culture and subsequent staining. Bar, 50 μm.

Conclusions

1) We have developed two novel agarose microfabrication methods with infrared laser absorbance.

2) We have succeeded in fabricating 2 μm width microchannels even during cultivation and confirmed the neurites elongated in those agarose microchannels.

3) We have succeeded in guiding an axon to the desired neuron to form a direction controlled neuronal network without any damage while additional microfabrication.

Acknowledgements

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