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Thermo-Plasmonic Optical Fiber for Localized Neural Stimulation

Hongki Kang^{1,2,3}, Woongki Hong², Yujin An¹, Sangjin Yoo^{1,5}, Hyuk-Jun Kwon², and Yoonkey Nam^{1,4}*

¹Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Republic of Korea

²Department of Information and Communication Engineering, DGIST, Daegu 42988, Republic of Korea

³Information & Electronics Research Institute, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, 34141, Republic of Korea.

⁴KAIST Institute for Health and Technology, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Republic of Korea.

⁵Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125, United States.

*Correspondence to: ynam@kaist.ac.kr

ABSTRACT

Thermo-plasmonic effect based neural stimulation has been suggested as an alternative optical neural stimulation technology without genetic modification. Integration of near infrared light with plasmonic gold nanoparticles has been demonstrated as a neuromodulation tool on *in vitro* neuronal network models. In order to further test the validity of the thermo-plasmonic neural stimulation across multiple biological models (*in vitro*, *ex vivo*, and *in vivo*) avoiding genetic modification in optical neuromodulation, versatile engineering approaches to apply the thermo-plasmonic effect would be required. In this work, we developed a gold nanorod attached optical fiber technology for the localized neural stimulation based on thermo-plasmonic effect. A simple fabrication process was developed for efficient nanoparticle coating on commercial optical fibers. The thermo-plasmonic optical fiber proved that it can locally modulate the neural activity *in vitro*. Lastly, we simulated the spatiotemporal temperature change by the thermo-plasmonic optical fiber and analyzed its applicability to *in vivo* animal models.

KEYWORDS (5-7 keywords)

optical neural stimulation, neuromodulation, thermo-plasmonics, photothermal effect, optical fiber, gold nanorod, microelectrode array

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Neural circuits in brain govern many different behaviors in our body including sensation, movement of limbs, characters, reasoning, memory, and so on. Investigation of the neural circuits is therefore crucial for understanding our behaviors, or the treatment of neurological disorders. For the control of neural activities in the neural circuits, optogenetics has been developed for more than a decade, and has become an essential research tool for versatile neurostimulation (*i.e.* both excitation and inhibition with millisecond precision).¹⁻³ Despite its successful adoption in many different areas of neuroscientific research, the need of genetic modification limits the biological models that optogenetics can be applied to; *e.g.* long-term toxicity and stability of virus transfection.⁴

In order to avoid any need of genetic modification, various optical neuromodulation technologies using light sensitive materials (*e.g.* plasmonic gold nanoparticles) have been also developed to implement certain functionalities of optogenetics.^{5,6} For example, plasmonic gold nanoparticles have been used to stimulate neurons *via* thermo-plasmonic effect, and both induction and suppression of action potentials were successfully demonstrated.⁷⁻¹⁰ It has been suggested that thermally induced membrane capacitance change is involved with the neural stimulation effects, while an endogenous thermo-sensitive potassium channel is involved with the neural suppression effects.^{7,9,11} The combination of plasmonic nanoparticles with tissue-transparent near infrared (NIR) light is advantageous as it minimizes off-target stimulation.^{12,13} Despite the need for more maturity and understanding of the underlying mechanism, it is promising that the photothermal stimulation approach could be an alternative technology of optogenetics while avoiding aforementioned concerns.

After the initial attempts of the photothermal stimulation using plasmonic nanoparticles, many engineering approaches have been developed to advance the neurotechnology: efficient

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3 application to *in vitro* neuronal chips down to a single cell resolution; integration with light
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5 patterning or nanoparticle patterning for selective photothermal stimulation; alternative thermo-
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7 plasmonic nanotransducers for higher photothermal efficiency.^{7,10,14–17} Despite the consistent
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9 experimental confirmation of the photothermal stimulation by multiple stimulation approaches,
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11 the application of the technology has been mostly limited to *in vitro* neuronal networks with a few
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13 trials in *ex vivo* brain slices and *in vivo* nerve cells.^{7,8} In order to further test the feasibility of the
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15 localized photothermal neural stimulation across different brain models, a versatile photothermal
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17 stimulation device applicable to all the brain models is needed.
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23 Photothermal stimulation technology across multiple animal brain models should satisfy
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25 the following characteristics. First, the technology should be able to generate sufficient heat by
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27 light while the light illumination by itself does not directly affect the cellular behaviors. Second,
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29 it is desirable that the photothermal stimulation neurotechnology be compatible with currently used
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31 neuroscientific tools and experimental protocols (*e.g.*, optogenetics) so that immediate validation
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33 of the photothermal stimulation is possible without significant modification from well-established
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35 experimental procedures and studied animal behaviors. Here, we integrated gold nanorods
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37 absorbing NIR light with a commercial optical fiber cannula. It is the most commonly used light
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39 delivery tool among many optogenetics technologies. We attached gold nanorod particles onto the
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41 tip of the optical fiber cannulas such that NIR light delivered through the core of the optical fiber
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43 can directly interact with the adhered gold nanorods. By doing so, we expect plasmonically
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45 generated heat only at the tip of the fiber. Owing to the high transmittance of the NIR light (around
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47 800 nm) by tissues, the rest of light passing through the nanoparticles will not be significantly
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49 absorbed by surrounding neural tissues or cells.¹² With this approach, minimal introduction of
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51 nanoparticles and light interaction is possible while implementing strong thermo-plasmonic effect
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and causing minimal photodamage. Using this thermo-plasmonic optical fiber, we successfully demonstrated that we can significantly modulate the local activity of neuronal network *in vitro* (**Figure 1a**). Lastly, we simulated the expected spatiotemporal temperature changes on the stimulated neurons by the thermo-plasmonic optical fibers both *in vitro* and *in vivo*.

RESULTS AND DISCUSSION

Fabrication of thermo-plasmonic (TP) optical fiber

To develop a nanorod-coated optical fiber, a biocompatible nanoparticle coating process was developed. We applied multilayer polyelectrolyte layer-by-layer (LbL) coating to attach high density of gold nanorods on the optical fiber. The multilayer polyelectrolyte LbL coating is known to implement homogeneous electrostatic force on various surfaces with good biocompatibility.¹⁸ It is also compatible with batch processes such that many optical fibers can be coated with the polyelectrolyte layers at high fabrication throughput.^{16,18} Although the electrostatic coating of plasmonic gold nanoparticles on optical fiber surfaces has been adopted in multiple surface plasmon resonance (SPR) biosensors, a positively charged single layer coating was used as the SPR sensors typically require low density of the nanoparticles for less plasmonic coupling.^{19–21} In our application, however, it was desired to have higher density for higher absorption of the NIR light and thus more heat generation.

By applying the polyelectrolyte LbL coating as described in **Figure 1b** (See **Figure S1** and Methods for details), we were able to modify the surface to have wide contact angle hysteresis (**Figure S2**). The wide contact angle hysteresis indicates that the polyelectrolyte coating is well established according to our previous studies.^{16,22} Electrostatic force will be exerted between the optical fiber tip surface (*e.g.* positive) and oppositely charged gold nanoparticles (*e.g.* negative; –24.5 mV of zeta potential). Dipping the LbL coated optical fiber in concentrated gold nanorod solution for more than a day (up to 76 hours), the colloidal gold nanorods were successfully attached to the surface of the optical fiber by diffusion (**Figure 1c**, **Figure S3**). In addition, we synthesized the gold nanorods to have longitudinal absorption peak at NIR (800 nm), and thus the

nanorod coated optical fiber showed strong light absorption in the range of NIR (**Figure 1d**, **Figure S4**). As shown in **Figure 1e**, the gold nanorod attached optical fiber can generate heat only at the tip of the fiber, and the temperature change in air induced by thermo-plasmonic effect was quite rapid during heating and cooling (at least $\pm 30 - 80$ °C/sec) (**Figure S5**). Fundamentally, nanosecond resolution temperature change of the nanoparticles upon light illumination is a key benefit of the thermo-plasmonic effect as it enables millisecond scale temperature change of the surrounding environment.²³ On the other hand, non-plasmonic light absorption based heat generation (for example, NIR illumination on black drapes; **Figure S6**) or joule heating shows much slower responses (tens of seconds) during the heating and cooling processes.²⁴

Next, we optimized the geometry of the optical fiber such that we can maximize the photothermal efficiency of the nanorod coated optical fiber. Despite the successful local heat generation at the tip of the implantable optical fiber upon NIR light, the flat termination surface type (FLT) was not the best design in terms of photothermal efficiency. While in optogenetics the flat termination leads to more direct and localized light illumination, potential photo-damage onto the cells through directly illuminated light can be a disadvantage in plasmonic nanomaterial mediated photothermal stimulation. In order to induce stronger photothermal effect, larger fiber tip surface area would be beneficial as it increases the chance of light and nanoparticle interaction by having more attached nanoparticles over the larger surface area. We therefore chose 3-dimensional geometries of optical fiber termination (C45: cone shape with 45° slope, DFL: hemisphere shape for isotropic light diffusion) in **Figure 2a** (**Figure S7**). The 3-dimensional geometry can hold more nanoparticles attached on the surface despite the same fiber diameter. It can also spread the light over wider area than the directional flat type (FLT). Therefore, the illuminated laser areal power density—defined as the illuminated laser power divided by the given

size of a power meter—was reduced. Hemisphere shaped optical fiber (DFL) has the largest surface area of the terminated optical fiber. Therefore, upon the same NIR laser intensity, the diffusive type (DFL) fiber termination showed the highest photothermal efficiency—defined as the maximum temperature change (°C) at the optical fiber per delivered laser power (mW)—while the illuminated laser areal power density significantly decreased (**Figure 2b** and **c**). With the geometric optimization of the DFL type fiber and further nanoparticle coating duration optimization (up to 76 hours), the DFL type thermo-plasmonic optical fiber showed linearly proportional peak temperature change with respect to the delivered NIR light power (measured in air, **Figure 2d** and **Figure S8**). The DFL type thermo-plasmonic optical fibers herein were used in the following neural stimulation experiments.

Photothermal stimulation on *in vitro* neuronal network

Next, we tested the capability of photothermal stimulation using the TP optical fiber on neurons. Instead of using previously reported various gold nano-transducers deposited onto cell culture substrates prior to the neuronal cell culture, we used the TP optical fiber closely placed near the neuronal cell culture on microelectrode array (MEA) for local stimulation (as described in **Figure 1a**; approximately 100 μm above the cells in order to avoid physical damage).^{14,15,17,25} We then observed the photothermal stimulation response of the neuronal spike activity by the microelectrodes. It is important to note that in this approach, the thermo-plasmonic effect is expected to heat up the local micro-scale environment through water as heat transfer medium to induce the stimulation effect rather than the nanoparticles directly attached to the cell membrane within nanoscale distance for photothermal stimulation as initially developed.⁹ In our stimulation experiments, therefore, we aim to see if this indirect microscale thermo-plasmonic heating can

cause the same neural stimulation effect as it has been reported in the previous works.^{14,15,17,25} Ultimately, the heat transfer by the photothermal neural stimulation for other brain models such as *in vivo* using the TP optical fiber will be fundamentally identical to this approach except the fact that the heat transfer medium will be mostly brain tissue which has slightly different thermal properties from water (**Table 1**).

Another main difference in this experiment from previous works is the maturation of neuronal cell culture and thus different baseline spike firing characteristics. Previously, photothermal stimulation through substrate-coated gold nano-transducers was conducted for the hippocampal primary culture mostly from 11 to 18 days *in vitro* (DIV) when the spontaneous spikes are mostly tonic.^{14,16,25} On the other hand, in this work, we stimulated the primary hippocampal culture from as early as 22 DIV to as late as 41 DIV. Therefore, this neural stimulation experiment will also confirm the applicability of the photothermal stimulation using the thermo-plasmonic optical fiber technology to broader conditions of primary neuronal culture models and their network characteristics. For the different cell cultures, we quantified the network-wide firing characteristics by burstiness index (BI), and mean cross-correlation coefficients.²⁶ (See Method for the definition of *BI* and calculation of the mean correlation coefficient). BI measures how much burst firing is dominant among overall recorded spikes. Prepared samples for stimulation covered wide range of BI, from tonic firing (BI: 0.33 ± 0.06 , n=4 sections of baseline recordings with each section 5-minute long) to burst dominated firing (BI: $0.45 \pm 0.02 \sim 0.69 \pm 0.06$, n=4 baseline recordings) (**Figure S9** in Supporting Information). The mean cross-correlation coefficients, as a measure of synchrony (0: no synchrony, 1: complete synchrony), were from as low as 0.06 ± 0.02 (n=13 channels) to as high as 0.60 ± 0.09 (n=34 channels). We therefore tested the generality of the application of photothermal neural stimulation using the TP optical fiber.

In case of the tonic firing network (**Figure 3**, MEA #1, 22 DIV, BI: 0.329 ± 0.052 , $n=4$ baseline recordings; cross-correlation coefficient: 0.055 ± 0.024 , $n=13$ active channels), the thermo-plasmonic optical fiber served as an effective localized inhibitory stimulus. Based on the representative spike activity resulted from multiple channels, only the directly stimulated channel (Ch 51), located right underneath the TP optical fiber, showed strong inhibition of neural spikes upon light illumination, whereas the rest of channels away from the fiber showed not as strong spike rate (SR) changes upon the stimulation (Ch 21, Ch 25) as shown in **Figure 3**. With further statistical analysis of the change of average SR of the stimulated channel, 13 mW NIR laser was able to partially inhibit the firing (Ch 51, -57.1 ± 3.1 % of average SR change upon stimulation, mean $\pm$ SEM, $n = 32$ stimulation trials), and 24.4 mW power almost completely silenced the firing activity of the target channel (Ch 51, -92.5 ± 1.4 % of average SR change, mean $\pm$ SEM, $n = 20$ stimulation trials) (**Figure 3d**). The inhibition effect on the fiber-placed channel (Ch 51) at 24.4 mW was significantly stronger than the other two representative channels away from the fiber (**Figure 3e**). The channels away from the fiber showed either statistically different from baseline (0 mW) but only small SR change (Ch 21, 600- μ m away from the TP fiber, -21.6 ± 2.8 %, mean $\pm$ SEM, $n = 20$ trials), or no statistical difference from the baseline (0 mW) (Ch 25, 1 mm away, -19.1 ± 2.3 %, mean $\pm$ SEM, $n=20$ trials) (**Figure 3d**). As this neuronal network was not synchronized (**Figure 3f**, **Figure S9** correlation coefficient 0.055 ± 0.024), the slight change of SR in Ch 21 at 24.4 mW was not because of the network effect of the suppression of Ch 51, but possibly because of slight heat transfer from the fiber to the channel. However, only small change in SR confirms that the heat generation is localized in a few hundreds of μ m from the center of the fiber. Computational analysis conducted in a latter section shows 50% attenuation of the temperature change at 250 μ m from the center, and 75% at 440 μ m (**Figure S10**). Furthermore, no

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3 statistical difference between baseline conditions (0 mW) before and after the repeated
4 photothermal stimulation confirms that the stimulation effect is only temporary. It therefore
5 confirms that the TP optical fiber based photothermal stimulation can induce similar localized
6 inhibitory effect as it has been reported previously.^{10,16,17}
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13 Next, we applied the localized photothermal stimulation to a strongly synchronized
14 bursting network (**Figure 4**, MEA #2, 41 DIV, BI: 0.448 ± 0.028 , $n = 4$ baseline recordings; cross-
15 correlation coefficient: 0.596 ± 0.091 , $n = 34$ active channels). Bursting networks are known to
16 have strong connections among neurons, and they have persistent firing patterns. In this network,
17 all of the active channels were highly synchronized (cross-correlation coefficient: 0.596 ± 0.182 ,
18 **Figure S9b**), and the average burst duration was 62.8 ± 25.0 sec ($n=370$ bursts from 30 active
19 channels). To apply photothermal stimuli during a burst spiking activity, we initiated a
20 programmed stimulation (10 sec delay followed by 20-sec long NIR illumination, **Figure S11b**)
21 manually as soon as the burst firing was visually detected. We then investigated the localized
22 stimulation effect on the burst firing network by the 20-sec long photothermal stimulation on a
23 target channel (Ch 35). Similar to the tonic firing network, the target stimulation channel in this
24 bursting network was also strongly inhibited. Despite strong bursts in long-lasting spike trains,
25 upon the 20-sec long stimulation, we were able to completely silence the spikes at 50 mW (**Figure**
26 **4a, b**). The inhibition effect occurred immediately after the laser was turned on, and the spikes
27 reappeared hundreds of msec (median: 528 msec, min: 68 msec, max: 2297 msec, $n=7$ trials) after
28 the laser was turned off (**Figure 4a** 50 mW (w/GNR), **Figure S12**). The same laser power
29 illumination without gold nanorod did not show the inhibition effect at all.
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53 The rest of the active channels of the bursting network, however, showed different
54 responses. As shown in **Figure 5**, the effect of the inhibition of Ch 35 on neighboring channels
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was significantly different between the channels. One group was partially inhibited, while others were excited upon the stimulation on the target channel. Based on in-depth network analysis, the different responses were related with the burst firing order (**Figure 6**). We grouped the burst signals for each channel, compared the timestamps of the first spike of each burst signal in each channel, and sorted the order of propagation through channels based on the mean delay with respect to the earliest spike signal between channels (**Figure 6a, b**; see the details in Methods). We then identified a couple of early-firing channels (Ch 16, 24, 27; positioned left bottom of the stimulated Ch 35 in **Figure 4c**, and grouped as *L.B.*) compared with the stimulated Ch 35. With the stimulated Ch 35 located at relatively early stage of the propagation path in **Figure 6b**, the rest of the channels that fired after the Ch 35 (*e.g.* Ch 63 in **Figure 4c**, grouped as *The rest* in **Figure 6d**) were mostly partially inhibited. In addition to the average spike rate change, there were some time delay in partially inhibited channels before the inhibition occurred from the onset of stimulation (Ch 63 in **Figure 5b**, other channels for **Figure S13**). On the other hand, several early firing channels (*L.B.*) showed increased spike rate changes upon the photothermal stimulation (*e.g.* Ch 27 in **Figure 5**). The increase of the activity in upper stream channels implies that the local inhibition at Ch35 might have increased the excitatory feedback loops to modulate network excitability. These results suggest that the TP optical fiber based neural stimulation may be used for modulating local neural circuits even when the multiple channels are highly synchronized and show long-lasting burst firings. Moreover, the resulting response of other neighboring channels may be closely related to the position in the neural circuit and the location of the local stimulation.

Both cases of stimulation experiments (smaller BI samples; $BI < 0.5$; MEA #1, #2) so far showed complete suppression on the directly stimulated channels. However, we also observed that, in an even higher BI sample with strong synchronization ($BI: 0.689 \pm 0.008$; MEA #3, 29 DIV,

cross-correlation coefficient: 0.504 ± 0.120 , $n = 43$ channels), there has been only partial suppression observed on the stimulated channel ($< 66\%$) even at strong stimulation power. The photothermal stimulation results are provided in **Figure 7**. **Figure 7a** shows spike raster plots of the stimulated channel (Ch 61) by the TP optical fiber at different laser power conditions. **Figure 7b, c** show that average spike rate changes upon the 20-sec photothermal stimulation for directly stimulated channel (Ch 61), and representative non-stimulated channels (Ch 63, Ch 78; $400\ \mu\text{m}$ and $1414\ \mu\text{m}$ away from Ch 61, respectively). Compared to the non-stimulated channels (Ch 63, 78) showing no significant changes, photothermal stimulation at 30 mW of NIR laser power led to $-64.7 \pm 2.2\%$ (mean $\pm$ SEM) of spike rate change for the stimulated channel (Ch 61), which is similar to what we observed in the previous two experiments. However, as shown in **Figure 7a, b**, laser power beyond 30 mW did not lead to stronger suppression of the extracellular spikes. Instead, the same rate of suppression was maintained. When we analyzed the observation based on the recording data during 50 mW stimulation in **Figure 7d**, we noticed that the spike signals that were globally synchronized (marked with asterisks on **Figure 7d**) were not significantly affected even at stronger stimulation power. This may indicate that there could be different types of firings that can be either turned off or not by photothermal stimulation in this condition of neuronal culture. It, however, needs further investigation to understand the reasons for the observed responses.

Photothermal stimulation experiments in this section conclude the followings: localized photothermal stimulation can be implemented using the thermo-plasmonic optical fiber developed in this work. Despite broad range of network maturation conditions, the local photothermal stimulation was able to significantly modulate the spiking activities of neurons within a range of the illuminated NIR laser power, 25 – 50 mW. The resultant responses of the rest of network vary

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3 depending on the network conditions. The inhibitory responses of the stimulation showed
4 characteristics similar to the previously reported photothermal neuromodulation platforms.
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10 11 **Computational analysis of the spatiotemporal temperature change during photothermal** 12 **stimulation** 13 14 15

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17 With the successful influence of the thermo-plasmonic optical fiber based neural
18 stimulation, details on the spatiotemporal temperature change around the stimulated neurons need
19 to be understood: how much the temperature change varies over area during stimulation; how
20 quickly the temperature changes (heat up and cool down); and how the same thermo-plasmonic
21 effect would appear *in vivo*. We used a multiphysics finite element method (FEM) simulation to
22 investigate these questions. For higher accuracy, we built a simulation model of the thermo-
23 plasmonic optical fiber (**Figure 8a**), and verified its performance based on the experimental
24 temperature measurement data in air. Then, we used the optical fiber model in different simulation
25 environments (*in vitro* and *in vivo*; **Figure 8b**, **Figure S14**).
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39 Despite the design flexibility in the computational simulation, precise geometrical
40 modeling of nanoparticles in nanoscale over several millimeter scale of overall environment was
41 not feasible due to the significantly different physical dimension that leads to simulation
42 convergence failures. Therefore, instead of simulating the exact light to heat plasmonic conversion
43 at the nanoscale in the FEM simulator, we have taken a simplified approach. For densely packed
44 2D nanoparticles, an assembly of nanoparticles can be treated as a continuous heat source owing
45 to collective heating.^{27,28} We directly defined heat rate (mW) generated at the tip of the optical
46 fiber, and calibrated the defined heat rate such that the simulated optical fiber model can accurately
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mimic both the maximum amount and transient characteristics of the temperature change that we experimentally observed from the TP optical fiber in air upon NIR light illumination (see the applied heat rate labeled as simulation input in **Figure 8a**). In this simpler approach, we can accurately simulate the heat transfer characteristics in the neural stimulation experiments at high spatiotemporal accuracy. We can also attempt to estimate light to heat conversion ratio (%) by comparing experimental data input (NIR light in mW) with simulation data input (heat rate in mW), with which identical temperature changes are observed. For the simplified thermo-plasmonic optical fiber simulation model, we defined a thin gold layer surrounding the fiber tip, which is where we applied the heat rate as a boundary heat source (the surface of gold layer exposed to water or brain). When we compared with the thermo-plasmonic heating which we measured with an infrared thermographic camera, we confirmed that the model we designed was able to accurately reproduce the transient characteristics of the thermo-plasmonic heating we experimentally measured as the center surface of the fiber tip in air (**Figure 8c**). With this confirmation, we used this simulation model throughout the study. Based on the required input heat rate to the gold layer in simulation to generate the same measured temperature increase in air, we calculated the approximate light to heat conversion percentage to be 15.8 % (*i.e.*, if 10 mW laser is passed through the fiber, 1.58 mW of heat rate is converted to induce temperature change; **Figure S15c**). As the peak absorbance (A) was measured to be ~ 0.1 (**Figure 1d**), the absorption ($1-10^{-A}$) was ~ 21 %, which would be the upper limit of the light-to-heat conversion percentage.

Applying the thermo-plasmonic optical fiber simulation model on a glass substrate placed in water, we simulated the spatiotemporal temperature change around the fiber and above the cell culture substrate. This simulation condition mimics the *in vitro* neural stimulation experimental setup in the previous section. We assumed that the fiber tip location is 100 μm above the substrate

surface (**Figure 8a**); indeed, in the neural stimulation experiments, we tried to keep the fiber tip as close as possible to the substrate (within 100 μm) (**Figure S17**). Heat transfer from the optical fiber through environment is determined by two important parameters: heat capacity and thermal conductivity (**Table 1**). Switching the environment from air to water, due to the larger thermal conductivity of water, the overall temperature change given the same laser power is much reduced (9.68 % of 'air' condition) (**Figure 8d, e**). With high thermal conductivity of water, the thermal transient response time was 440 – 500 msec for light on/off (during heat up, reaching 50% of the maximum saturated temperature change in 20 msec, 90% in 439 msec; during cool down, reaching 50% in 19 msec, 10% in 501 msec) (**Figure 8d**), which would limit the millisecond timescale response of the neuronal activity changes upon the photothermal stimulation.

Considering the simulated effect of surrounding thermal environment to the temperature change (*i.e.* water *vs.* air), the induced temperature change at the fiber core tip is estimated to be based on the following ratio:

$$\begin{aligned} \frac{\Delta T_{core\ tip}(\text{°C})}{\Delta P_{light}(\text{mW})}(\text{in water}) \\ &= \frac{\Delta T_{core\ tip}(\text{°C})}{\Delta P_{light}(\text{mW})}(\text{in air, measured}) \times \left(\frac{\Delta T_{water}}{\Delta T_{air}} \right) = 2.28 \pm 0.90 \text{ °C/mW} \times 9.68 \% \\ &= 0.22 \pm 0.09 \text{ °C/mW}, \end{aligned}$$

where the variation comes from the attached gold nanorod concentration variation (**Figure 2d**). Therefore, the range of light power condition (24.4 – 50 mW) that showed strong photothermal stimulation effect in the previous sections is anticipated to induce peak temperature change, $5.38 \pm 2.12 \text{ °C}$ for 24.4 mW, $6.62 \pm 2.61 \text{ °C}$ for 30 mW, and $11.03 \pm 4.35 \text{ °C}$ for 50 mW at the optical fiber core tip. As shown in **Figure 8f, g**, the temperature gradually decreased with increasing the distance from the tip of the optical fiber. Toward glass substrate, 39% decrease from maximum at

40 μm distance, and 62% decrease at 85 μm distance were expected. Laterally, 50% decrease from peak (full width of half maximum) is expected at 270 μm from the center of optical fiber (**Table S1**). Precise micrometer-scale distance between the fiber and neuronal cells could not be recorded due to the incapability of precision height positioning. However, based on the simulation model, we can conclude that the cultured neuronal cells would not be affected more than the maximum temperature change of the fiber core tip (*i.e.* 5.5 – 11.0 $^{\circ}\text{C}$ increase from baseline temperature of 37 $^{\circ}\text{C}$ for 25 – 50 mW of NIR light). The temperature of the MEA surface where the cells are located (100 μm from the tip) would be 1.7 – 3.5 $^{\circ}\text{C}$, and the surface temperature gradient profile estimated 75% attenuation of the temperature change at 440 μm (**Figure S10**).

With this simulation approach, we could estimate the spatiotemporal temperature change in a brain tissue for *in vivo* photothermal stimulation. Modeling the surrounding of the optical fiber as a brain tissue instead of water, we further simulated how *in vivo* photothermal stimulation would be different from *in vitro* conditions (**Figure 8b**). Because of slightly smaller heat capacity and higher thermal resistance of the brain compared to water (**Table 1**), for a given laser power, higher temperature change (22 % higher) and more spatial localization (55 % narrower) were expected for *in vivo* photothermal stimulation using the thermo-plasmonic optical fiber (0.27 $^{\circ}\text{C}/\text{mW}$, **Figure 8e**). It indicates that the *in vivo* photothermal stimulation using the gold nanorods attached optical fiber can be applied with higher efficiency than *in vitro*; *i.e.* 18% less NIR laser power would be required for the same amount of temperature change.

Discussion

In this work, we developed a photothermal neural stimulation method using thermo-plasmonic gold nanoparticle coated optical fiber with near infrared light. With this approach, we demonstrated that localized photothermal neuromodulation is achievable on primary cultured neuronal network models. The results of experiments have the following implications.

First, the photothermal effect – how efficiently this technology can produce localized heat for given light power – is as strong as what has been previously demonstrated in thermo-plasmonic neural interfaces. So far, photothermal neural stimulation method using gold nanorods and NIR light have been demonstrated on *in vitro* neuronal cultures (mostly gold nanotransducers attached onto the cell culture substrates) over wide range of stimulation area (20 μm – 5 mm). Depending on the thermo-plasmonic layer conditions (nanoparticle density, size of heat sources) and thermal environment (distance between the heat sources and neurons), the required laser power would vary. While the reported power areal density is often reported for comparison, the correlation between the power density and anticipated temperature change is not straightforward because of the different size of illumination area and different boundary conditions of thermal environment. As examples, larger area (5 mm diameter) illumination requires 200 – 300 mW (15 – 21 mW/mm^2), and smaller area (20 – 30 μm diameter) requires 10 – 80 mW of continuous wave (CW) NIR laser power illumination (32 – 116 W/mm^2) for strong inhibition effects.^{10,14,17} Considering the 200- μm diameter of optical fiber used in this work, previous works with 265–330 μm diameter illumination requiring 21–41 mW laser power for strong inhibition (*i.e.* 380 – 480 mW/mm^2) are the most similar to this experiment.²⁹ Therefore, similar range of 24 – 50 mW of laser power utilized in this work with similar effective stimulation area has comparable photothermal effect as previous works with the gold nanorods coated onto the cell culture substrates. In other words, the nanoparticle

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3 attachment method and the resulting indirect microscale thermo-plasmonic heating developed in
4 this work is as effective as previously reported fabrication methods (nanoparticles attached to the
5 cell membrane, or cells grown on top of the nanoparticle attached surfaces). Despite the
6 comparable range of laser power we used, isotropically diffused light by the spherical diffuser
7 structure (DFL type geometry) significantly lowers the laser power delivered to the neighboring
8 cells (*i.e.* lower laser power per area).
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18 We also observed interesting neural stimulation behaviors that have not been reported
19 previously. In addition to nearly perfect silencing of tonic spiking activity that has been repeatedly
20 studied, we have observed that the inhibitory stimulation was effective during synchronized burst
21 firing. By localized inhibitory photothermal stimulation using TP optical fiber, we observed that
22 the strongly synchronized burst firing neural circuits can be modulated depending on the burst
23 propagating order. Similarly, inhibitory optogenetic stimulation previously demonstrated that it
24 can block burst propagation in spinal cord slices.³⁰ With further maturation of the culture of higher
25 burstiness and synchronization index (*i.e.* synchronized burst dominated), we have also observed
26 that some portion of spikes were not silenced despite strong stimulation. Due to the lack of precise
27 control of neuronal network culture parameters in this work, we can only classify the burstiness
28 output behavior by BI and cross-correlation coefficients, rather than the origin of the burst signals
29 in each sample. Further analysis such as transmembrane potential change upon the stimulation,
30 subcellular behaviors including the roles of various ion channels, and the roles of different types
31 of neuronal cells and synaptic connections (*e.g.* ratio of excitatory to inhibitory) remain to be
32 conducted for more accurate understanding of underlying mechanisms. The most important lesson
33 we learned, however, is that the thermo-plasmonic optical fiber in this work can significantly alter
34 the strongly correlated neuronal cell culture network compared to control experiments (either no
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light with gold nanorod, or light without gold nanorod). Therefore, in order to conduct aforementioned studies especially over multiple neuronal network models (*in vitro*, *ex vivo*, and *in vivo*) and correlate the characteristics across the models, the thermo-plasmonic optical fiber method developed in this work can be used as a universal local stimulation tool.

The thermo-plasmonic optical fiber offered multiple advantages. First, plasmonic nanoparticles provided wavelength specific localized heat generation. Since 808 nm wavelength had minimal absorption in water, the heat source was well-localized at the fiber tip, and thermal stimulation was mediated by heat diffusion. Second, the thermo-plasmonic layer provided heat generation and diffusion mechanism for high spatial resolution. Comparing with a direct laser induced heating by absorption in the liquid medium from a longer wavelength (*e.g.* 1800 ~ 1900 nm) which is used in infrared neural stimulations (*e.g.* INS), light propagation along the absorbing medium generates a volume heat source. Comparing with a recent study using a continuous wave laser through an optical fiber (1550 nm, 170- μm wide), the temperature change by thermo-plasmonic effect can be 5 – 9 times more localized than the infrared induced heating (full width half maximum, 270 μm *vs.* 1020 μm along the fiber/probe; **Figure 8f**).^{31–34} Similar broad temperature distribution could be also found in visible light induced heat generation in optogenetics.³⁵ Third, plasmonic nanoparticles made it relatively easy to functionalize various types of optical fibers by a simple dip-coating method. Using this fabrication approach, it would be possible to add multiple modalities on the same fibers. As a specific wavelength of light is absorbed by the thermo-plasmonic layer to generate heat, the same optical fiber can be used to deliver other range of wavelengths of light without significant heat generation. For example, while NIR can be used for the thermo-plasmonic neural stimulation, visible range of light can be used

for either optogenetics, SPR biosensors, or surface-enhanced Raman spectroscopy when multiple types of plasmonic nanoparticles are incorporated.^{19–21, 36}

The thermo-plasmonic optical fiber developed in this work is expected to be applicable to *in vivo* brain models similarly as the optogenetics stimulation methods. Compared to optogenetics, NIR laser in this thermo-plasmonic optical fiber technology required slightly higher power. However, because of much weaker absorption of NIR wavelength by cells and tissues compared to visible light wavelengths of optogenetics, the slightly higher power NIR illumination can be tolerable even for *in vivo* neural stimulation.¹² In some representative cases of optogenetics protocol with Channelrhodopsin (ChR2) and Halorhodopsin (NpHR), smaller range of laser power is often used: for examples, conservatively up to 2.35 mW (75 mW/mm²; long pulses) of 470 nm for ChR2, and 16 mW of 532 nm for eNpHR3.0.^{37–39} Absorption coefficient of gray matter for NIR is much smaller than visible lights (0.37 for 480 nm, 0.19 for 580 nm, and 0.02 for 780 nm). Approximately ten times smaller absorbance for NIR than blue light (480 nm) is anticipated.⁴⁰ Taking the weaker absorption of NIR and larger surface area of diffuser-type fiber termination into consideration, NIR laser power up to 47 mW (750 mW/mm²) would be safe enough not to directly affect the cellular behaviors. Only localized heat generation at the tip of the fiber, but no significant heat generation within the brain tissues by light absorption would be expected. As a reference, transcranial NIR based *in vivo* deep brain stimulation through upconversion nanoparticles operated without direct photo effect at up to 700 mW peak power millisecond pulsed illumination with average power of 15 – 22 mW.¹² In addition, based on our *in vivo* photothermal effect simulation result, approximately 18% less laser power (*i.e.* 20 – 41 mW range) than *in vitro* environment would be required to induce similar temperature change. Compared to an optogenetic silencer,

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3 eNpHR3.0 (532 nm), only 25 – 150 % more power can be used with much less light scattering
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5 attenuation.³⁹
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9 Although in this work we have not applied the photothermal neural stimulation *in vivo*, the
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11 design consideration of the fiber is suitable for immediate replacement of well-documented
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13 optogenetics optical stimulation protocols without transfection and recovery steps.^{37,41} As we
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15 verified the suitability of the photothermal stimulation *in vivo* compared to optogenetics protocol,
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17 we expect to apply this technology to *in vivo* models in the foreseeable future. However, it is
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19 important to note that cell-type specific targeting will not be possible in the photothermal
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21 stimulation due to the non-selective heat generation mechanism. With growing interests in the
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23 thermal effect of optogenetics method on neural activities, we believe the photothermal stimulation
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25 using the TP optical fiber technology developed in this work can be a powerful local neural
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27 stimulation tool without any need of genetic modification.^{35,42}
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CONCLUSIONS

We developed a plasmonic gold nanorod coating process on optical fiber for localized neural stimulation. Electrostatic attachment of the nanoparticles by polyelectrolyte layer-by-layer coating on optical fibers is introduced for reliable device fabrication. Geometric optimization of the optical fiber termination surface is done for highly efficient thermo-plasmonic effect and reduction of direct photodamage. Using the thermo-plasmonic optical fiber approach, we showed that we can significantly modulate the spike activities of *in vitro* primary cultured hippocampal neuronal networks at different maturation stages. Furthermore, computational simulation of spatiotemporal temperature change around the fiber allowed more detailed investigation of the correlation between temperature and spike activity change. Based on the experimental and simulation results in this work, we anticipate that the thermo-plasmonic optical fiber based *in vivo* neural stimulation is applicable within similar optical damage tolerance to optogenetics but without genetic modification.

METHODS

Preparation of gold nanorod and polyelectrolyte coating solutions

The gold nanorod coating solution was prepared following a seed mediated gold nanorod synthesis procedure without modification.¹⁶ The synthesized gold nanorod particles were optimized to achieve longitudinal absorption peak in near infrared wavelength (785 nm). The nanorods were finally coated with methoxyl polyethylene glycol thiol (mPEG-SH) (PG1-TH-5k, Nanocs) for negative electrostatic surface charge. As the main mechanism of gold nanorod coating is the electrostatic attraction in the nanoparticle colloidal solution, the higher the nanorod concentration is, the higher the chance of nanorod attachment would be. Therefore, after the mPEG-SH coating, the gold nanorod solution was concentrated using a centrifuge. See **Figure S1b** for the concentrated gold nanorod solution (120 optical density (OD) as defined in the previous work).¹⁶ The concentrated gold nanorod solution used in this work showed maximum absorbance peak around 785 nm in water and zeta potential of -24.5 mV (Zetasizer Nano ZS, Malvern).

We used the same recipe for polyelectrolyte layer-by-layer (LbL) coating solution as we previously introduced.⁴³ The 10 mg/mL of positive polyelectrolyte, poly(allylamine hydrochloride) (PAH) (652407, Aldrich), and negative polyelectrolyte poly(4-styrenesulfonic acid) (PSS) (561258, Aldrich), were dissolved in 10 mM NaCl aqueous solution, respectively (**Figure S1a**).

Thermo-plasmonic optical fiber fabrication and characterization

Three different termination types of mono fiber optic cannulas with 4-mm long core were used in this work (diameter 200 μm , NA 0.48, MFC_200/230-0.48_4mm_ZF2.5(G)_FLT/C45/DFL, Doric Lenses Inc., Quebec, Canada). The optical fiber samples were used only after rinsing with DI water, acetone and isopropyl alcohol. First, we coated the fiber with 10 bi-layers of polyelectrolyte pairs with the final layer to be positively charged (PAH). Multiple cannulas were immersion-coated with the polyelectrolytes at once as described in the experimental setup in **Figure S1a**. Starting with the coating in the positive polyelectrolyte (PAH) solution for 5 min, alternative polarity coating was performed for up to 10-bilayers. Between the two polyelectrolyte solutions, DI water wash was conducted for at least 1 min. The last coating was done with PAH solution to ensure positively charged optical fiber surface. After the LbL coating, the fibers were rinsed with DI water and dried. Contact angle hysteresis change after the LbL coating was monitored using a contact angle measurement system (EasyDrop FM40, Kruss, Germany).

The LbL coated fiber optic cannulas were coated with gold nanorods by immersion in the concentrated gold nanorod solution (**Figure S1b**). It was performed at room temperature for up to 76 hours. The nanorod coating was confirmed using scanning electron microscopy as in **Figure S3**. After the nanorod coating, the optical fiber was rinsed with DI water and dried. Before the neural stimulation, the gold nanorod coated optical fiber was sterilized by 70% ethanol.

The absorbance of the gold nanorod coating layer with the LbL polyelectrolyte layer was characterized using a spectrometer covering both visible and near-IR range (350 – 1000 nm; USB4000-VIS-NIR-ES with halogen light source HL-2000, Ocean Optics). Flat type fibers termination (FLT) were used. Fresh FLT type fibers without GNRs were used as reference.

Temperature change upon near infrared light illumination in air was measured by a handheld infrared camera (E40, FLIR Systems Inc., Oregon, USA). Average values were calculated after the temperature change reaches steady state (**Figure S8**).

Near infrared laser system for photothermal stimulation

Two different NIR laser systems were used in this work. One was 785-nm continuous wave laser (300 mW, MDL-III-785-300mW, CNI Tech., Changchun, China) connected to a fiber optic cable (NA: 0.39, 200 μm), and the other was 808-nm continuous wave mode laser (4 W, Laserlab, Anyang, Korea) connected to a fiber optic cable (NA: 0.50, 600 μm). The laser power was controlled either by manually or by analog modulation, and the timing of laser on/off was controlled by TTL pulses. Before experiments, the power of emitted laser through the FLT-type fiber optic cannula was measured for calibration.

As shown in **Figure S16**, during the photothermal stimulation experiments, the MEA was kept with a transparent plastic cover case. CO_2 gas was supplied under the cover case, and the MEA was placed on a metal plate heater which was heated at 37 $^\circ\text{C}$. The TP optical fiber was held by a manipulator for x/y/z axis movement. The MEA, the plate heater and recording amplifiers were placed on an inverted microscope. The imaging through the microscope was used to control the position of the TP optical fiber with respect to the cultured cells and microelectrodes. NIR laser was connected to the TP optical fiber, and its ON/OFF was controlled by TTL pulses.

Primary hippocampal neuronal culture and neural recording

In order to avoid light induced artifacts, we used transparent electrode based microelectrode array (MEA) chips, ITO conductive lines and 30- μm TiN electrodes with 200- μm

spacing, (60MEA200/30IR-ITO-w/o, Multi Channel Systems). On the MEAs, we cultivated neuronal cells, and recorded extracellular neural spikes as we previously conducted.¹⁶ As-received MEA chips were cleaned with acetone, isopropyl alcohol, and deionized water in sonication for at least 5 min each. For the adhesion promotion for the cultured neurons, the MEA chip surface was coated with poly-D-lysine (0.1 mg/mL in Trizma buffer of pH 8.5, Sigma Aldrich) for 12 hours in a humidified incubator (37 °C, 5% CO₂). Before cell seeding, we rinsed the MEA with sterilized deionized water three times, and with 70% of ethanol for 1 min. We used a custom-built multichannel voltage amplifier system (gain: 1000 V/V, bandwidth: 150 Hz to 4 kHz) for extracellular spike recording was carried out using. The amplified analog voltage signals were digitized by MC card (12 bit, 25 kS/s, Multi Channel Systems) and further digitally low-pass filtered at 200 Hz for analysis.

The cultured hippocampal neurons were dissected from the embryonic day 18 Sprague–Dawley rats. We dissected the tissue and dissociated in Hank’s balanced salt solution (HBSS, WelGENE, Korea) by multiple micro-pipetting. The dissociated neuronal cells were centrifuged at 1000 rpm, and resuspended in a plating medium (Neurobasal medium with 2% of B27 (Invitrogen), 2 mM of glutamax (Invitrogen), 12.5 μM of glutamate (Sigma Aldrich) and 1% of penicillin-streptomycine (Invitrogen)). The cell seeding and growth area was 6 mm in diameter covering the 8 × 8 microelectrode layout (1.6 mm square). The cells were plated at density of 1000 cells/mm² and maintained in a humidified incubator (37 °C, 5% CO₂). 50% of medium was replaced every three or four days with fresh maintenance medium. The maintenance medium was prepared by mixing neurobasal medium with 2% of B27 (Invitrogen), 2 mM of glutamax (Invitrogen), and 1% of penicillin-streptomycine (Invitrogen). It must be noted that despite the different maturation and days *in vitro*, the different cultures used in this experiment were all freshly

stimulated. So, no pre-stimulation history affected the stimulation experiment results. All experiments were performed in accordance with the guidance of the Institutional Animal Care and Use Committee (IACUC) of Korea Advanced Institute of Science and Technology (KAIST), and all experimental protocols were approved by IACUC of KAIST.

Neural data analysis

From the recorded data, the extracellular spikes were detected using a threshold method ($-6 \times$ background rms noise level) in MC Rack program (Multi Channel Systems). We then conducted the spike train analysis using a neurophysiological data analysis software (Neuroexplorer, Nex Technologies). Spike rate change upon stimulation was defined as $([\text{spike rate during stimulation}] - [\text{spike rate before stimulation}]) / [\text{spike rate before stimulation}]$. To calculate the spike rate (spikes/sec), time bin of the duration of the photothermal stimulation (either 20 or 30 sec depending on experiments) was used. Hence, -100% of spike rate change during the stimulation refers that there is no detected spike during the stimulation. Light-induced recording artefacts due to photoelectric effect were removed from the recorded spike data set by not detecting any signal around the laser turn on and off events (± 3 msec). We quantified the network burst characteristics by burstiness index (BI) as previously defined by Wagenaar *et al.*²⁶ 20-min long baseline recording of each cultured network was divided into four 5-min long sub-recording sections. We divided each 5-min recording into 300 time bins and counted the number of spikes for each 1-sec bin. After the data are sorted in descending order, the ratio of the summation of the first 15% of bins to the sum of the total 300 bins was defined as f_{15} . The burstiness index was defined as $BI = (f_{15} - 0.15)/0.85$. This calculation was done for all the active channels individually for each 5-min sub-recordings. Finally, all the BI values from each MEA was averaged to represent the network burstiness characteristics.

For the network-wide analysis of MEA #2 in **Figure 6**, the following methodology was used. The active channels with average spike rate higher than 0.3 spikes/sec were selected. Spikes and the signal of laser triggering were analyzed by porting them to MATLAB (Mathworks, Inc.) (**Figure S20a**). After the burst detection, we grouped the spikes into disjointed subsets that are sequentially related to each other.⁴⁴ If the interval between successive spikes is greater than a certain period of time (*e.g.* 10 sec), they were classified into different groups (see the blue lines in **Figure S20b** as group separating boundaries). Subsets having at least 100 spikes/sec were defined as bursts. Within the propagation pattern of the network burst, we sorted the channel order of the propagation by the time of the first spike of the burst in each channel.^{45,46} The burst propagation delay in **Figure 6** was calculated by the time delay of the first spike of a burst in a given channel with respect to the first spike of the same subset group. Among eight burst events, mean values were plotted in **Figure 6**.

For network analysis, cross-correlation matrix and the mean value of the cross-correlation coefficients from the cross-correlation matrix were obtained by following the literature.^{16,47,48} Spike rate histogram was obtained with 10 msec long bin and gaussian smoothing (size 5). We calculated the cross-correlation (xcorr () normalized by 'coeff', MATLAB) using the smoothed rate histograms, and we took the maximum values as the correlation coefficient for the matrix. To quantify the synchrony, the mean value of the cross-correlation matrix was calculated.

COMSOL simulation

We used heat transfer module of COMSOL v5.3a (COMSOL Inc., Burlington, MA) for three-dimensional numerical simulation of thermal transport around the DFL TP optical fiber. The DFL optical fiber with the radius of 200 μm and length of 10 mm was modeled. The materials for

the core (200 μm diameter) and the cladding (30 μm thickness) of the optical fiber were glass and a polymer, defined as silica glass and poly(methyl methacrylate) (PMMA), respectively. A 500-nm thick gold layer was coated on the hemisphere shaped core tip of the DFL optical fiber. The gold nanofilm coated optical fiber was placed in a block of different surrounding materials (air, water, and brain), which has the dimension of $20 \times 20 \times 10 \text{ mm}^3$ (**Figure 8b**). We used this setup to analyze the spatial and temporal characteristics of the heat transfer around the optical fiber. For air simulation, the initial temperature of the air environment was set to 24 $^{\circ}\text{C}$. For *in vitro* simulation, we set the initial temperature of water (culture medium) at 37 $^{\circ}\text{C}$. The MEA chip, defined as quartz glass material with a volume of $20 \times 20 \times 0.95 \text{ mm}^3$, was placed right under the bottom of the water block, and its initial temperature was set to 37 $^{\circ}\text{C}$. The optical fiber was placed at 100 μm above the MEA top surface (**Figure 8a**). For *in vivo* simulation, we also set the initial temperature of surrounding block (brain) at 37 $^{\circ}$, and the optical fiber was inserted at 5 mm depth. For both *in vitro* and *in vivo* simulation, only the top surface of the water and brain were defined to be interfacing with air of fixed temperature at 24 $^{\circ}\text{C}$. These represent possible cooling of the top area of water and brain in experiments. The initial temperature of optical fiber was defined to be identical to the surrounding initial temperature.

To simulate the heat generated by the gold layer, a periodic boundary heat source was applied to the bottom surface of the Au film layer in **Figure 8a**. The heat transfer equation for the simulation was,

$$\rho C_p \frac{\partial T}{\partial t} + \rho C_p \mathbf{u} \cdot \nabla T = Q + k \nabla^2 T$$

where ρ , C_p , $T(x, y)$, $\mathbf{u}$, k and $Q(T, x, y)$ are mass density (kg/m^3), heat capacity ($\text{J}/\text{kg}\cdot\text{K}$), temperature ($^{\circ}\text{C}$), fluid velocity vector, thermal conductivity ($\text{W}/\text{m}\cdot\text{K}$), and heat source per volume (W/m^3), respectively. The parameters for different materials used in the simulation are given in

Table 1. The boundary heat source (Q_b) – local heat flux density (W/m^2) – representing the thermo-plasmonic heating on the gold layer was defined by P_b/A where P_b is the heat rate (mW) we applied as an input of simulation (**Figure 8a**), and A is the gold layer boundary area (m^2). The conduction of the boundary heat source is governed by the following equation

$$-n \cdot (-k\nabla T) = Q_b.$$

In addition to the heat conduction, we only considered the heat convection in air. For the TP optical fiber simulation in air, we considered heat convection and resulting mass transport of air ($u = 0.001$ m/s). For other materials including water and brain, we did not consider heat convection or mass transport in our simulation. We expected that the heat conduction in those materials is much stronger than heat convection, and that no significant water flow is expected in the contained culture medium due to small temperature change (less than 11 °C) and small volume for heating. However, for the simulation of *in vitro* (water) and *in vivo* (brain), at the top surface of water and brain interfacing with air at 24 °C, we simulated free convection into air. The free convection represents the cooling of brain or culture medium by air. It is governed by the following equation,

$$n \cdot (k\nabla T) = h(T_{\text{ext}} - T)$$

where n , h , and T_{ext} are, outward normal vector, convective heat transfer coefficient ($W/m^2 \cdot K$), and external temperature (°C), respectively. In air, the external temperature T_{ext} and heat transfer coefficient h were assumed to be 24 °C and 5 $W/m^2 \cdot K$, respectively.

Statistical analysis

Unless otherwise noted, mean value $\pm$ standard deviation (SD) were reported. Statistical significance analysis methods we used for all the data sets were noted along with the p -values.

Figure 1. Thermo-Plasmonic (TP) optical fiber. **a**, Schematic illustration of the localized photothermal neural stimulation of *in vitro* neuronal network on microelectrode array (MEA) chips using the TP optical fiber. When near infrared (NIR) light is delivered through the optical fiber (red bar), neurons (red colored) underneath the fiber receives localized heat stimulation directly and spiking activity are modulated (*i.e.*, inhibition). For neurons (blue colored) that are far from the fiber are not affected directly by the stimulation. The modulation effect is illustrated by the action potential (AP) spikes of the neurons. **b**, Fabrication processes for the TP optical fiber. **c**, Scanning electron microscopic images of GNR coated DFL type optical fiber. (Find more images in Figure S4 in Supporting Information) **d**, Extinction spectrum of the GNR coated optical fiber **e**, Infrared thermographic image of the TP optical fiber with NIR laser turned on (red circle pointed by an arrow indicates the localized temperature change at the tip of the optical fiber).

Figure 2. Optimization of thermo-plasmonic effect on the TP optical fiber by geometric modification. **a**, Both microscopic and macroscopic laser beam paths (side view) for different geometric types of optical fiber termination (FLT, C45, DFL). The diameter of all optical fibers was 230 μm (200 μm for core, and 30 μm for cladding). **b**, Thermo-plasmonic effect comparison between the different types of optical fibers upon NIR laser illumination. Saturated maximum temperature change from room temperature in air upon 16 mW of 785 nm laser illumination. **c**, Illuminated light areal power density and photothermal efficiency comparison between the fiber geometries. The same laser power meter was used in front of the three types of optical fibers with the same distance. Detected laser power per area of the power meter is defined as the light density (mW per size of the power meter). Photothermal efficiency ($^{\circ}\text{C}/\text{mW}$) is defined as the maximum temperature change ($^{\circ}\text{C}$) at the optical fiber per delivered laser power (mW). The relative light density and photothermal efficiency of FLT type fiber were defined as 100% for comparison. **d**, Thermo-plasmonic effect of optimized TP optical fiber (DFL type) used in the neuromodulation experiments in this work.

Figure 3. Photothermal neural stimulation on *in vitro* neuronal network using DFL type TP optical fiber (Results 1). **a**, Stimulation experiment procedure for MEA #1. TP optical fiber is located only on Ch 51 as illustrated. The location of chosen channels for analysis is described. **b**, Raster plots for the stimulated channel 51 over repeated stimulation trials at different laser power. **c**, Representative raw recording data for the localized neuromodulation on MEA #1. Black arrowheads represent light-induced artefacts. **d**, Average spike rate changes upon NIR laser stimulation with different power. All values represent mean $\pm$ SEM. One way ANOVA; $p < 0.0001$ (Ch 51), $p < 0.0001$ (Ch 21); $p = 0.0036$ (Ch 25), $p < 0.0001$ (24.4 mW); $n = 22$ trials (the 1st 0 mW), 32 (13 mW), 20 (24.4 mW) and 20 (the 2nd 0 mW); (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). **e**, Average spike rate changes upon 24.4 mW of NIR laser stimulation for the channels in **c**. All values represent mean $\pm$ SEM ($n = 20$; One way ANOVA; $p < 0.0001$); (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). **f**, Cross-correlogram of the channels away from the TP fiber in **a** with respect to the stimulated channel, Ch 51 (bin: 5 msec).

Figure 4. Photothermal neural stimulation on *in vitro* neuronal network using DFL type TP optical fiber (Results 2; MEA #2). **a**, Raster plots and representative neural stimulation extracellular spike data of stimulated channel (Ch 35) by TP fiber. Different levels of NIR laser power (mW) and the condition of gold nanorods (GNR) are specified for each condition. See more details for 50 mW w/ GNR condition in **Figure S12**. Black arrowheads represent light-induced artefacts. **b**, Average spike rate changes of the photothermally stimulated microelectrode. All values represent mean $\pm$ SEM. One way ANOVA; $p < 0.0001$, $n = 12$ trials (the 1st 0 mW), 10 (40 mW), 7 (50 mW), 9 (the 2nd 0 mW) and 12 (50 mW without GNR). **c**, Location of the stimulated microelectrode on the cultured neuronal network.

Figure 5. Photothermal neural stimulation on *in vitro* neuronal network using DFL type TP optical fiber (Results 2; MEA #2) (continued). **a,b** Raster plots and a representative spike recording data for non-directly stimulated channels Ch 27 (**a**) and Ch 63 (**b**) during 50 mW stimulation on Ch 35 by GNR coated TP optical fiber as described in **Figure 4c**. **c**, Average spike rate (SR) changes of the channels in **a, b** for different stimulation power conditions on Ch 35. All values represent mean $\pm$ SEM. Unpaired t-test, two-tailed p values ($p = 0.0962$ for 40 mW vs. 50 mW for Ch 27; $n = 12$ trials (0 mW), 10 (40 mW), 7 (50 mW)); (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Figure 6. Photothermal neural stimulation on *in vitro* neuronal network using DFL type TP optical fiber (Results 2; MEA #2) (continued). **a**, Network burst propagation order. (Green indicates Ch 35 and a channel with a similar delay; red shows a channel with a shorter delay than the stimulated channel; cyan for longer delay channels; numbers next to data points represent each channel number). **b**, The details of the latency of the colored channels in **a**. **c**, Graphical representation of the propagation delay of **a** on the array of channels in **Figure 4c**. (Colored channels correspond to the ones in **a**; color map in logarithmic scale). **d**, Average of spike rate changes upon 50 mW stimulation on Ch 35 by groups defined in **d**. All values represent mean $\pm$ SEM. Unpaired t-test, two-tailed p values ($p = 0.0039$ for *L.B.* vs. *Ch 35*; $p < 0.0001$ for *L.B.* vs. *The rest*; $p = 0.0007$ for *Ch 35* vs. *The rest*; $n = 7$ trials for *Ch 35*, $n = 36$ for *L.B.*, $n = 162$ for *The rest*); (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Figure 7. Photothermal neural stimulation on *in vitro* neuronal network using DFL type TP optical fiber (Results 3; MEA #3). **a**, Spike raster plots of the stimulated channel 61 upon with different laser power (20 sec stimulation; spike rate average for 10 sec). **b**, Average spike rate changes of Ch 61 in **a**. All values represent mean $\pm$ SEM. One-way ANOVA, $p < 0.0001$; N.S. between non-zero laser power stimulation conditions; $n = 9$ trials (0 mW), 12 (20 mW), 11 (30 mW), 12 (40 mW), 14 (50 mW); (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). **c**, Average spike rate change of representative non-stimulated channels (Ch 63, 78). All values represent mean $\pm$ SEM. One-way ANOVA, $p = 0.8816$ for Ch 63, and $p = 0.4891$ for Ch 78; $n = 9$ trials (0 mW), 12 (20 mW), 11 (30 mW), 12 (40 mW), 14 (50 mW). **d**, Representative extracellular spike recording data of multiple channels upon the 20-sec, 50-mW stimulation. Red asterisks represent the globally synchronized spike signals that were not suppressed during strong neural stimulation. Black arrowheads represent light-induced artefacts.

Figure 8. Computational analysis of the spatiotemporal temperature change by thermo-plasmonic optical fiber (DFL type). **a**, Graphical illustration of the simulated TP optical fiber model with heat transfer models at the interface of gold thermo-plasmonic layer (Au film, GNR layer). **b**, Different simulation environments (air, *in vitro*, or *in vivo*). **c**, Verification of the thermo-plasmonic effect of the computational fiber model (blue) in comparison with the experimental measurement data (red, 20.4 mW, 785 nm) by thermographic infrared camera. DFL type TP optical fiber was tested in air condition as described in **b**. **d**, Simulated maximum temperature change at the tip of the TP optical fiber for *in vitro* and *in vivo* conditions (light power: 13.4 mW). **e**, Peak temperature change of the TP optical fiber with respect to the laser power for different environments in **b**. **f**, Temperature change spatial distribution over time in lateral direction (x defined in **a**, $y=0 \mu\text{m}$; light power: 20.1 mW; laser turns on at $t=0$ sec; *in vitro*). **g**, Thermo-plasmonic effect attenuation over distance (y defined in **a**) from the tip of the fiber ($x=0 \mu\text{m}$).

Table 1. Physical properties of materials used in the thermal simulations. Parameters for brain were adopted from a literature⁴⁹, and the rest of parameters were found from the FEM software database.

Material	Density (kg/m ³)	Specific heat capacity (J/kg·K)	Thermal conductivity (W/m·K)
Air	1.188	1006	0.02617
Water	993.37	4180	0.623
Quartz glass	2210	730	1.4
Brain ⁴⁹	1041	3640	0.528
Gold	19320	125.604	314.0
Silica glass ¹⁾	2203	703	1.38
Poly(methyl methacrylate) ²⁾	1190	1420	0.17

1) optical fiber core, 2) optical fiber cladding

ASSOCIATED CONTENT

Supporting Information.

The Supporting Information is available free of charge.

Additional data on (1) the fabrication and characterization of thermo-plasmonic optical fiber: photos of fabrication processes, SEM images of attached nanorods, extinction spectrum measurement, thermographic infrared camera measurement data, (2) photothermal stimulation on *in vitro* neuronal network: experimental setup illustration and optical micrographs, cross-correlogram, computational analysis of the temperature profile, representative raw recording data and peri-event raster plots, (3) computational analysis of the spatiotemporal temperature change during photothermal stimulation: simulation results temperature profile, numerical graph, and simulation parameters.

AUTHOR INFORMATION

Corresponding Author

*E-mail: ynam@kaist.ac.kr

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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