

A wireless optogenetic stimulation system for long-term function evaluation of mice forelimb with sub-nerve resolution

Corresponding Author: Professor Su Jiang

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The MS provides evidence for long term implantation and selective stimulation of nerve fibers innervating different skeletal muscles and thus stimulation leads to distinct movements of the arms. The strength of the MS for sure is in the technical development and provides new chances for research. On the other side some more characterisation in terms of efficiency in regard to the biology of the light induced contractions would be enhancing the impact a lot.

1. What would happen if the device would be used during the phase between implantation and final experiments?
2. How strong is the light-induced contraction? The authors analyzed movements without any resistance. Thus an indication how strong the muscle contraction is, is missing.
3. How does emitted light power translates into contraction in terms of movement but also strength? Are different pulse frequencies more or less efficient? Analysing this will indicate whether it is possible to induce single twitches or also tetanic contractions.
4. If increasing the light energy and/or frequency and/or pulse duration, does this led to more unspecific stimulation?
5. Is there any kind of immune response against the implant: encapsulation, fibrosis, destruction of nerves, etc.?

Minor:

1. How is the newly developed device compared to other implantable optogenetic devices such as for cochlear implants, bladder stimulation and the heart in terms of efficiency in energy transfer, emitted brightness, cooling/preventing heating, endurance etc.
2. It would be nice to have a comparison between direct skeletal muscle stimulation and the herein presented indirect stimulation via the motor neurons and how the presented device can change the foreseen potential and advantages.

Reviewer #2

(Remarks to the Author)

Zhou et al. develop an optogenetic stimulation device (FIMOSS), which can be surgically implanted into transgenic mice to optically stimulate peripheral nerves. They use the Thy1-mhChr2-YFP mouse line, which expresses channelrhodopsin-2 protein in neurons under the Thy1 promoter, so that motor neuron action potential firing can be stimulated with blue light. The FIMOSS device builds on their prior MOSD device (Zheng et al Nature Comm 2019), with new wireless stimulation capabilities and updated geometry and design. They use this device for more detailed quantification of the extent of nerve regeneration following the C7 nerve transfer surgery following traumatic brain injury (TBI).

Overall, the manuscript is clearly motivated and shows impressive use of optogenetics for stimulation of peripheral nerves in live mice. The authors provide a very detailed description of the FIMOSS device design considerations, thorough quality control, and clear rationale for experimental design and motivation. The durability, spatial and temporal resolution, and heating of the device are all thoroughly discussed and addressed. However, a few major comments remain. I have focused my review on the optogenetic technical and experimental design aspects of the study, as I do not have the physiological and medical expertise to comment on the quality and novelty of the in-vivo findings.

Major comments:

1. The authors motivate the study in the Introduction by stating that peripheral nerve stimulation following nerve transfer can substantially benefit the regenerative process. This would imply that the authors plan to use the FIMOSS device as a treatment method and to test whether stimulation post-surgery can improve regeneration. However, the authors rather use the device to measure the progress of regeneration, in combination with CMAP readout. This leads to some confusion: is the device intended as a potential therapeutic strategy (and this is why biocompatibility, longevity, etc is critical) or as a monitoring research tool for the regeneration progress? If the latter, it raises the question why optogenetic stimulation is necessary, and why traditional needle electrode-based stimulation is not sufficient (in particular, the spatial resolution limits of such traditional approaches need to be specifically stated and discussed in comparison with the FIMOSS device, to clearly motivate the study).
2. The difference between the new FIMOSS/MOSDII device and the previous MOSD model needs to be clarified. What kind of experiments are now possible that were not with MOSD? I understand that the new device is wireless, smaller, and more durable, but the experiments of the current study are all performed under anesthesia and so the benefit of wireless stimulation is not utilized.

Specific comments:

3. How is the radial orientation of the LEDs around the nerve bundle specified? Can the MOSDII loop rotate due to movement of the mouse? Even if the spatial resolution of the device is sufficient, if its position can change over time the precision of stimulation can be compromised.
4. How sensitive is the FIMOSS device to ambient 2 MHz RF signal and how is specificity to the device encoded? Can the device be unintentionally activated by ambient (e.g. AM radio waves), and is it possible to independently control two neighboring devices?
5. I did not understand at which point is the FIMOSS device implanted. Is it implanted at the same time as the C7 transfer surgery? If so, can the presence of the MOSDII around the nerve interfere with the healing process?
6. Why was the 20 ms-on / 2 s-off stimulation pattern chosen for optogenetic stimulation in the brachial plexus? Is this sufficient for maximal Chr2 activation, and could longer on-times induce stronger action potentials?
7. Figure 2f,g,h, add label for the LED in the schematic (blue squares), and label each f,g,h with a title that states the different nerve diameters accommodated.
8. Figure 3g: what can be an explanation for the reduced temperature changes in vivo (3g) vs. in vitro (3e)? Is it possible that in the aqueous in vivo environment the heat is absorbed by the tissue? After long-term stimulation have you observed any evidence for heat-induced damage to the tissue?
9. Figure 3j,k,l: what are units and values for the rainbow intensity scale, and what is physical length of the white scale bar? Are the intensity units absolute or relative? Also please label what you mean by "corner sector" within the figures for clarity.
10. Related to point 9 above: the authors often refer to the high spatial resolution of the LED illumination, but do not provide exact values; please provide these values.
11. Related to point 9 and 7 above: the measured intensity of illumination at the nerve is not clearly provided. Can the authors provide an estimated measure of intensity (e.g. power/unit area) at the nerve surface? How does this compare to typical Chr2 activation intensities?
12. Figure 4h, i, j: what is approximately the expected extent of movement upon stimulation? For example when compared to electrical stimulation. Please also describe figure more clearly in caption – what are the units for the blue and red numbered axes?
13. Figure 5d-f: the legend text is not legible. In the text, a "control group" is referenced; what is the control here? No TBI, or no C7 transfer? What is "sham"?
14. Figure 5g heatmap can benefit from a different color scale, and perhaps normalization to control, to more clearly visualize effect of TBI and CC7. Please also clarify what is meant by "weighted analysis" and the Percentage scale.
15. Define OS acronym (I'm guessing optogenetic stimulation).
16. I do not follow the summary for Figure 5 in the text, as the figure does not measure wrist/hand/elbow function: "The electrophysiology changes of brachial plexus after CC7 transfer implied that although transferred C7 nerve participated in the innervation of various forelimb muscle groups, it played a more important role in the reinnervation of the wrist and hand muscles on the affected side, while upper and lower trunk compensate for the motor function of transferred C7 in elbow joint and showed a tendency to compensate for wrist and hand function simultaneously"
17. Why does elbow extension seem much larger in Fig 6b than in Fig 4?
18. The order of the last figure 7 seems out of place to me, perhaps it is best to combine it with earlier figures that characterize device durability.
19. Fig 7c: please clarify in caption whether the shown trajectory is for animals with or without FIMOSS implantation. The trajectory both with and without should be shown. Also please state clearly in caption what is meant by "control" condition in 7d-n. Is this without implant and no surgery?
20. The authors mention that the device can accommodate more than 4 LEDs. For which applications would this be useful – e.g. which nerves would have more than 4 fascicles that would be interesting to stimulate? How difficult would it be to modify the device (e.g. power requirements, size). Please add this to the Discussion section.
21. There are many small grammatical errors in the text that sometimes make the meaning difficult to understand. Also a few

typos, e.g. "which is is", Figure 3e "Tememprature". For clarity, some detailed descriptions can also be moved to supplementary, for example the current-limiting resistor choice.

Reviewer #3

(Remarks to the Author)

Manuscript ID: NCOMMS-25-04546-T

Zhou et al. present a fully implantable, wireless optogenetic stimulation system designed for long-term monitoring of upper limb function with sub-nerve resolution in vivo. The manuscript details the development of a miniaturized, battery-free device to drive four blue LEDs positioned on a nerve cuff. Power and control signals are delivered via magnetic induction, enabling battery-free operation and illumination parameter reconfiguration. The authors demonstrate the device's utility in acute upper limb optogenetic stimulation in Thy1-ChR2-EYFP mice and, importantly, its application in monitoring nerve function following traumatic brain injury and CC7 nerve transfer surgery. Validation is supported by physiological characterization of upper limb motion based on this device stimulation and other clinical stimulation standards.

The work is conceptually sound, and the presented biological results are compelling. The manuscript presents an interesting and potentially valuable contribution to the field of optogenetic stimulation for nerve injury/surgery evaluation. However, the manuscript lacks technical details regarding the device's engineering, raising concerns about the reproducibility and generalizability of the described system. Therefore, authors are strongly encouraged to address the comments outlined below, particularly regarding the engineering aspects of the device.

Major concerns

- An important design consideration for optogenetic devices is the irradiance at the region of interest. In this case, the LEDs illuminate the nerve tissue and, due to scattering and absorption, the light irradiance decreases at the site of need (the nerve fibers). Authors report optical power levels produced by the LEDs, but how much is the expected light irradiance at the nerve fiber of interest and how much is required to elicit functional optogenetic stimulation?
- Following on the previous comment. The authors present a strategy to identify the optimal electronic configuration to set the proper optical power, thermal performance, and device operation for UART Rx based on the choice of a resistor. The trial-and-error approach undertaken in this manuscript is too empirical. I would expect the authors to understand the total power budget of the device and the available power harvested by the device and based on this to propose design parameters that would be demonstrated experimentally. This is important since the loading effect of relatively high current demands lowers the power performance of the antenna. Thus, the characterization on a wired system (with external power supply with sufficient power) is not directly transferable to the wireless system where only a maximum electrical power is available. It is easy to establish LED operation parameters based on desired optical power which requires LIV LED characterization, but none of these are presented in this manuscript.
- In addition, the authors claim sub-nerve stimulating resolution, which is obviously demonstrated in the biology characterization in the second half of the manuscript. As a device engineering paper, I would have expected the authors to support this claim at least with more quantitative analysis, for example, via optical simulations. Authors do present optical simulation (Fig 3j-m), however, what quantitative insights do we gain from these results? How much is the irradiance thresholds used to establish the sector photon concentrations? What do the color bar even mean?

Minor concerns

- Confusing terminology: 1) The title delivers the message that the device will monitor upper limb function over long-term, which is not the case, it is just an optogenetic stimulator. Author should update the title accordingly. 2) The terminology used for describing optogenetic stimulation parameters is not standard. e.g. "on-Time(range 0.1~200ms, and special case of 2000ms),off-Time(range 0~199ms, and special case 2s)". Authors must reframe this terminology such as pulse widths (pw), frequency (f) and/or periods.
- There is a reference for eight channel operation as an option for future development, but this is not demonstrated in the manuscript. Therefore, this should be removed from the manuscript or include validation experiments that demonstrates it.
- It is surprising the device is 1.2 mm thick. Even when the polyamide substrate is somehow flexible, this thickness is quite unusual. What is the justification for such a 6-layer flexible PCB?
- Although it is true that the efficiency of WPT scales with the area of the planar coil and the number of turns, it is not validated how, for example the 6-layer coil system compares with the standard 2-layer counterpart. The resonance quality factor benefits for a larger turn count though. Is this the benefit the authors were looking for? If so, how does it change based on loading conditions?
- Authors describe and characterize thermal levels produced by the device. However, what about the LEDs? LEDs are inefficient, so, the electrical power delivered to the LEDs produce light and heat. Is the expected heat a parameter of concern?
- Authors argue that this device is compatible with commercial RF drivers such as those sold by the company Neurolux. However, operating the device introduced here within a large antenna lowers the wireless power transfer efficiency

considerably. So, authors are cautioned to claim operation of the device with the commercial RF hardware if the proper validation of illumination characteristics is not validated or confirmed.

- Furthermore, authors introduce here a mechanism for reprogramming in real-time. Since this is not even demonstrated in the manuscript, this should be removed from the claims of the paper or include demonstration of it.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The MS improved a lot especially in terms of biological characterisation. There are minor things which have to be corrected in wordings and explanations but I do not think that more and new experiments are needed:

1. Please compare the indirect optogenetic stimulation of skeletal muscles to direct optogenetic stimulation of skeletal muscles (Not only electrical). This is especially important for the muscle specific stimulation or cases in which neural input is completely lost.
2. Please specify further: This is a device (Or that's what has been shown) to test engraftment but not to improve engraftment. For the latter you would need optogenetic stimulation during the whole period and not only at one final experiment.
3. 2.5 mW/mm² is more than enough and rather supra supra threshold to evoke an action potential in neurons and this is also not matching the light efficiency curve shown in the graph and described as 1:1 match.
4. The explanation why 20 ms illumination is more efficient than shorter pulses is misleading and I am also not sure whether this is really still a single twitch contraction. Such prolonged illuminations can easily evoke several APs in motor neurons and thus induced rather short tetanic contractions by repetitive AP generation. This has to be discussed as alternative mechanism.

Reviewer #2

(Remarks to the Author)

This revised version of the manuscript fully addresses my concerns.

1. Thank you for your response. The added comparison between multisite optogenetic stimulation and electrical stimulation indeed showcases the increased nerve group specificity with the FIMOSS device. The wording changes in the Introduction also help with motivating the scope of the study more clearly.
2. Thank you for the clarification. I think this advantage of FIMOSS over MOSD can be emphasized more clearly in the manuscript (i.e. that repeated surgeries are not necessary).
3. Thank you for the clarification. I think that adding a statement similar to these lines to the main text of the manuscript will be useful: "So, the implanted MOSD II will not rotate during free moving behaviors in mice. This stability is one important advantage we considered when making decisions on the implant location of MOSD II."
4. Thank you for the clarification.
5. Thank you for your response, the updated timeline and immunofluorescence images are important additions to the manuscript.
6. The added experiments comparing different pulse width are good additions to the manuscript.
- 7-8. Thank you for the clarifications and changes.
- 9-11. Thank you for the nicely improved characterizations.
12. Thank you for adding the important angle change comparison to electrical stimulation, it is encouraging that both optogenetic and electrical stimulation give similar responses.
- 13-16. Thank you for addressing these concerns.
17. Thank you for the detailed explanation. Adding this explanation to the Discussion would benefit the manuscript, where you address the potential cause for the large observed anatomical variation.
- 18-21. Thank you for these added explanations and changes to the manuscript.

Reviewer #3

(Remarks to the Author)

This reviewer acknowledges the comprehensive manuscript revision the authors performed on. They have addressed my concerns and other reviewers' concerns. I do not have further comments or concerns.

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We would like to kindly remind the reviewer that the point-to-point reply below uses the following format:

Reviewer's Comments: *Times New Roman (black, italic)*

Author's reply: Times New Roman (blue)

Reference: Times New Roman (black)

Quote from the revised manuscript: Arial (red)

Reviewer #1 (Remarks to the Author):

The MS provides evidence for long term implantation and selective stimulation of nerve fibers innervating different skeletal muscles and thus stimulation leads to distinct movements of the arms. The strength of the MS for sure is in the technical development and provides new chances for research. On the other site some more characterization in terms of efficiency in regard to the biology of the light induced contractions would be enhancing the impact a lot.

Major concerns:

1. What would happen if the device would be used during the phase between implantation and final experiments?

Author's reply:

Thank you for your question. To elucidate the electrophysiology changes of different brachial plexus nerve trunks on the affected side after C7 transfer, FIMOSS implants were activated wirelessly with an external device at 4 weeks, 8 weeks and 12 weeks after implantation, in conjunction with recording CMAP from different muscle groups and tracking the movement trajectory of forelimbs (**Figure.6 and Figure.7 in revised manuscript**).

We have included these descriptions in the Methods of the revised manuscript.

Methods in revised manuscript, line 1195-1204

The FIMOSS implant was implanted on the brachial plexus in mice from all groups for 12 weeks. For mice from TBI or TBI+CC7 groups, the implantation surgery was performed 2 weeks after TBI surgery (when performing CC7 transfer for TBI+CC7 groups). The CMAP curves were recorded when the FIMOSS implant was activated (20ms pulse width, default wireless condition) to explore the change pattern of brachial plexus electrophysiological values and forelimb induced-behavioral angle

changes. For mice in control and TBI group, the experiments were conducted 12 weeks after implantation. For mice in TBI+CC7 group, the experiments were conducted 4, 8, 12 weeks after CC7 transfer/implantation surgery. And FIMOSS implants will not be surgically removed after the 4-week or 8-week postoperative evaluation after implantation surgery until the 12-week postoperative evaluation was finished.

2. How strong is the light-induced contraction? The authors analyzed movements without any resistance. Thus, an indication how strong the muscle contraction is, is missing.

Author's reply:

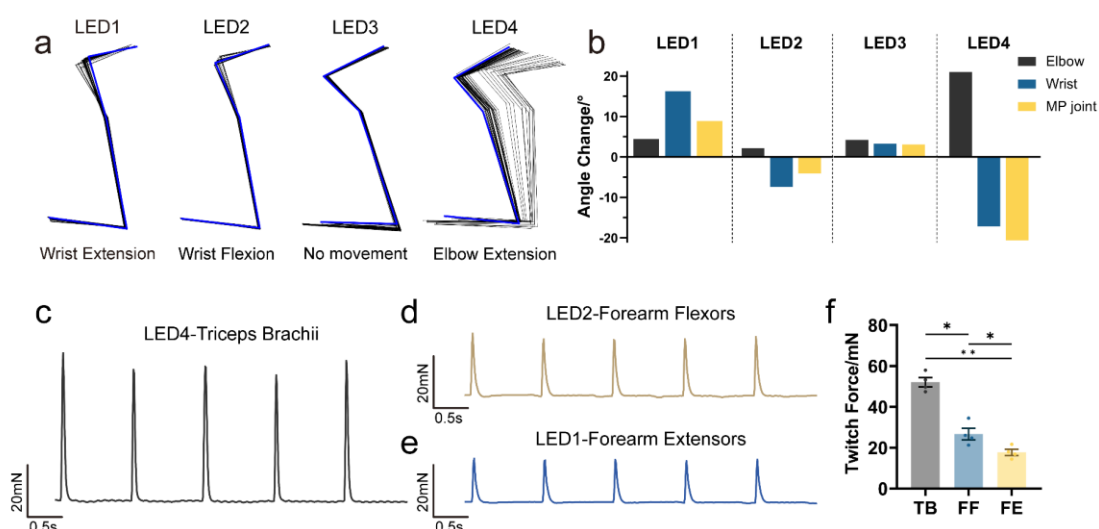
Thank you for this constructive comment.

We implanted FIMOSS implants on the lower trunk of Thy1-ChR2-EYFP mice and measured the contraction force of the innervated muscles. The twitch force of triceps brachii, forearm flexors, forearm extensors are $52.09 \pm 3.99 \text{ mN}$, $26.67 \pm 4.86 \text{ mN}$, $17.75 \pm 2.63 \text{ mN}$ respectively (**Supplementary Figure.12f**).

Below please see detailed description of the experiments:

4 μLEDs of MOSD II were sequentially illuminated after implantation. In a representative Thy1-ChR2-EYFP mouse, LED1 elicited wrist extension (induced by the contraction of forearm extensors), LED2 elicited wrist flexion (induced by the contraction of forearm flexors), and LED4 elicited elbow extension (induced by the contraction of triceps brachii), while LED3 elicited no obvious evoked movement. The movement trajectories and quantitative analysis of multi-joint angle changes induced by LED1, LED2, and LED4 are shown in **Supplementary Figure.12a** and **Supplementary Figure.12b**. Subsequently, we selected triceps brachii, forearm flexors and forearm extensors as representative target muscles innervated by the lower trunk. Using stimulation parameters of 1 Hz, pulses width = 20ms, default wireless condition, we continuously illuminate LED4, LED2, LED1 respectively to induce the contraction of triceps brachii, forearm flexors and forearm extensors. The contraction forces are shown in **Supplementary Figure.12c,d,e**. Different forelimb muscle groups show different twitch force (**Supplementary Figure.12f**).

We have added the results in the revised manuscript and [Supplementary Figure.12 in revised manuscript.](#)



Supplementary Figure.12 in revised manuscript. Distinct forelimb muscle contractions induced by multisite optogenetic stimulation. **a** Representative trajectories of distinct forelimb movements induced by different μ LEDs. **b** Quantitative analysis of angle changes from different forelimb joints induced by FIMOSS implant. **c~e** Schematic images showing muscle force recordings of triceps brachii (c), forearm flexors (d) and forearm extensors (e) twitch induced by LED4, LED2 and LED1 respectively. (1Hz, pulse width=20ms, default wireless condition) (scale bar=0.5s/20mN) **f** Comparison of twitch force between different muscle groups innervated by lower trunk. TB, triceps brachii. FF, forearm flexors. FE, forearm extensors. One-way ANOVA and multiple comparison tests with Dunnett's correct. Data are presented as mean $\pm$ s.e.m. (n=4, *P < 0.05, **P < 0.01)

Results in revised manuscript, line 633-652

In previous study, muscle contraction of distinct hindlimb can be induced by optogenetic stimulation at stem cell-derived motor neurons engrafted on the sciatic nerve³³ or nerve branches from sciatic nerve³⁴. And yellow-green laser (561 nm) at sciatic nerve can reversibly inhibit twitch and tetanic muscle force induced by electrical stimulation in Thy1::eNpHR2.0 mice³⁵. To test whether multisite optogenetic stimulation of FIMOSS implants on brachii plexus can induce twitch or tetanic muscle contraction of different forelimb muscle groups, we conducted the isometric muscle tension physiology experiment.

We used the lower trunk as a representative nerve. A FIMOSS implant was implanted on the trunk and activated by an external device. Firstly, we conducted distinct movement induction to clarify the correspondence between different μ LEDs and muscle groups innervated by lower trunk. In a representative Thy1-ChR2-EYFP mouse, wrist extension, wrist flexion and elbow extension were respectively induced

by the illumination of μ LED1, μ LED2, μ LED4. No obvious movement can be observed when illuminating μ LED3 (Supplementary Figure.12a,b). Then the distal tendons of the triceps brachii, forearm flexors and forearm extensors were severed and connected to a force transducer. μ LED4 was continuously (1Hz, 20ms pulse width) illuminated to induce twitch contractions in the triceps brachii (Supplementary Figure.12c), while μ LED2 and μ LED1 was used to induce twitch contractions of the forearm flexors (Supplementary Figure.12d) and forearm extensors (Supplementary Figure.12e) respectively. Distinct forelimb muscle groups showed different twitch force when illuminating lower trunks with different μ LEDs (Supplementary Figure.12f).

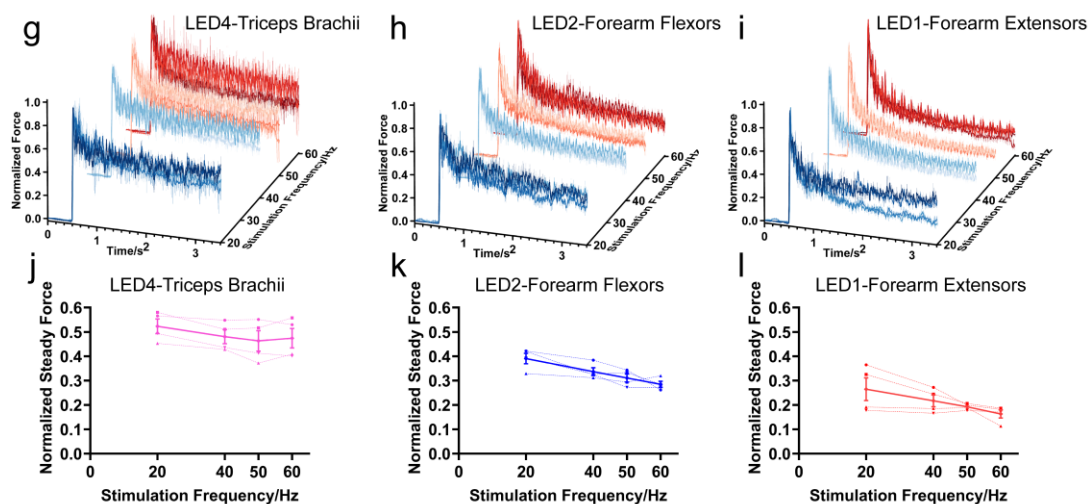
3. How does emitted light power translates into contraction in terms of movement but also strength? Are different pulse frequencies more or less efficient? Analyzing this will indicate whether it is possible to induce single twitches or also tetanic contractions.

Author's reply:

Thank you for your valuable comments.

Results for single twitch of triceps brachii, forearm flexors and forearm extensors, induced by multisite optogenetic stimulation at lower trunk, were shown in **Supplementary Figure.12d,e,f in the revised manuscript.**

Previous studies have reported fatigue-resistant tetanic contraction in hindlimb muscles induced by optogenetic stimulation with close-loop optogenetic neuromodulation¹. We conducted optogenetic stimulation experiments in forelimb by referring to the stimulation parameters used in this article (20Hz/40Hz/50Hz/60Hz stimulation frequency, 0.83ms pulse width). FIMOSS implant could also induce tetanic contraction with different steady state forces in different muscle groups in forelimb (**Supplementary Figure.12h,i,j in revised manuscript**). And the normalized steady force showed a tendency to decrease with increasing of stimulation frequency over 40Hz (**Supplementary Figure. 12k,l,m in revised manuscript**). Therefore, stimulations with frequencies under 40 Hz are more efficient in inducing contraction in forelimb muscles.



Supplementary Figure.12 in revised manuscript. Distinct forelimb muscle contractions induced by multisite optogenetic stimulation. g~i Normalized force of triceps brachii (g), forearm flexors (h) and forearm extensors (i) tetanic contraction induced by multisite optogenetic stimulation of lower trunk with different stimulation frequency (20Hz/40Hz/50Hz/60Hz stimulation frequency, 0.83ms pulse width, default wireless condition) (n=4). **j~l** Normalized steady force of triceps brachii (j), forearm flexors (k) and forearm extensors (l) with the increase of stimulation frequency. (pulse width=0.83ms, default wireless condition) The curve of each individual mouse is represented by a dashed line, and mean curve of all mice is represented by a solid line. (n=4, mean $\pm$ s.e.m.)

Reference:

1. Herrera-Arcos, G. *et al.* Closed-loop optogenetic neuromodulation enables high-fidelity fatigue-resistant muscle control. *Sci. Robot.* **9**, eadi8995 (2024).

Results in revised manuscript, line 653-666

Afterwards, optogenetic stimulation with higher frequency (20Hz, 40Hz, 50Hz, 60Hz) was applied in 4 mice (0.83ms pulse width, default wireless condition). We observed that tetanic contractions can also be induced with a steady state force within the 3s stimulation period (Supplementary Figure.12g,h,i). Moreover, triceps brachii exhibited relatively high normalized steady force compared with forearm flexors and extensors. When the stimulation frequency is over 40Hz, the normalized steady force began to decrease obviously (Supplementary Figure.12j,k,l).

In conclusion, both twitch or tetanic contraction of distinct forelimb muscle groups can be induced by FIMOSS implants. Among different forelimb muscles, the twitch force and normalized steady force profiles exhibited variations both in the magnitude of muscle force and the rate/degree of muscle fatigue. Moreover, when the stimulation frequency rose from 0 to 60 Hz, the normalized steady force demonstrated an obvious

decrease over 40Hz. These results aligned with previous studies. The frequency limit can be attributed to the desensitization of ChR2^{34,36}.

4. If increasing the light energy and/or frequency and/or pulse duration, does this led to more unspecific stimulation?

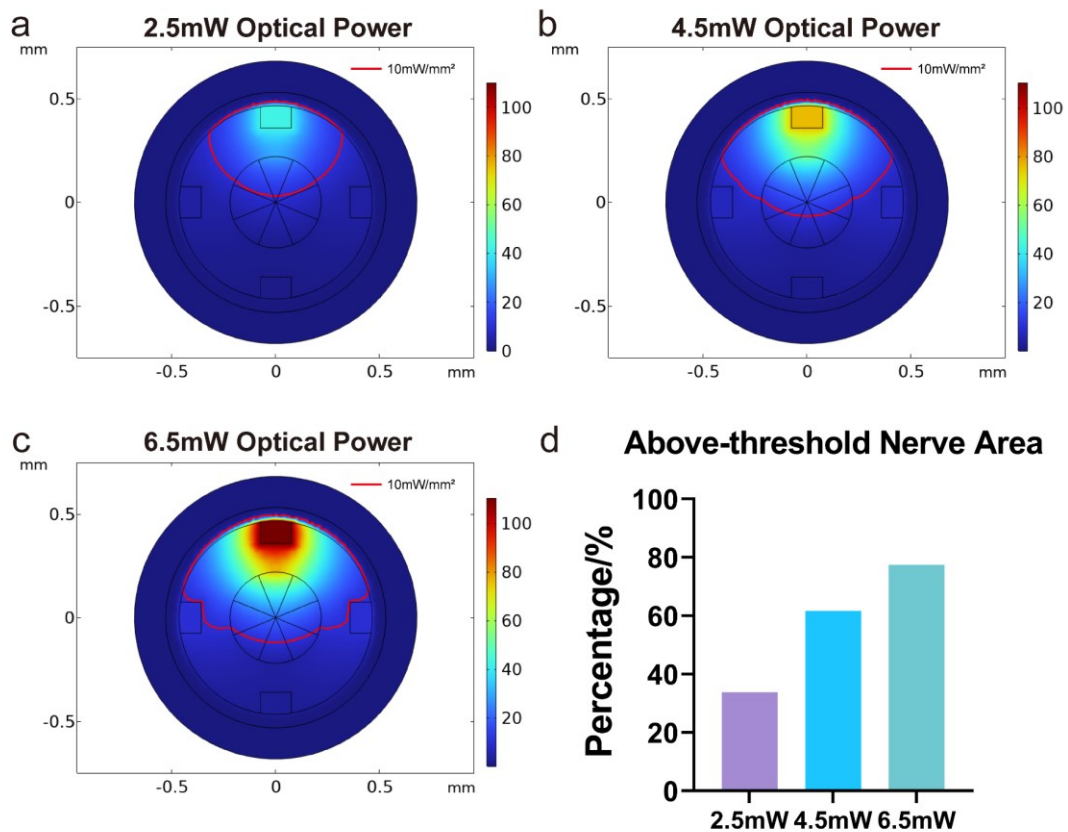
Author's reply:

Thank you very much for your comment. Your concerns are indeed valuable.

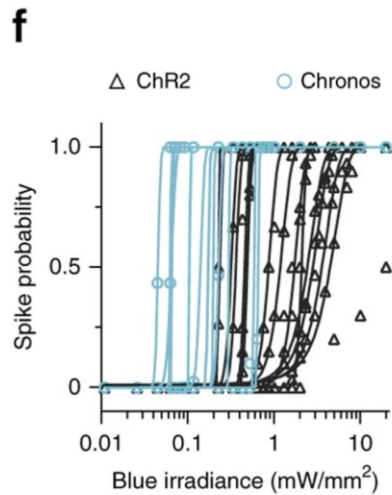
Our point-by-point responses are as follows:

- (1) **Light energy:** Increased optical power will lead to more unspecific stimulation, as a larger percentage of the nerve (encompassing more fascicles) receives above-threshold irradiance. We added new optical simulation of irradiance distribution across the target nerve when one μ LED was illuminated at an optical power of 2.5mW, 4.5mW and 6.5mW (**Supplementary Figure.8 in revised manuscript**). Previous studies showed that the spike probability of ChR2-expressing neurons ramps up exponentially from 0 to 1 when the received blue irradiance increased from 1mW/mm² to 10mW/mm² (**Response Figure.1**)². The percentage of nerve area that received irradiance above the 10mW/mm² threshold increased from 33.75% to 61.66% and to 77.45% under these three optical power conditions. This demonstrated that increasing optical power would lead to more fascicles within the nerve being activated, thus more unspecific stimulation.
- (2) **Frequency:** Increased frequency will not change the selectivity of optogenetic stimulation, because the amount of the activated nerve fascicles remained unchanged. However, according to the results of the tetanic contraction experiments, increased frequency will result in less efficiency, which may be attributed to the desensitization of ChR2 (**Supplementary Figure.12g~l in revised manuscript**).
- (3) **Pulse width:** We perform the electrophysiological and behavioral evaluation of middle with different pulse widths of 5ms, 10ms, 20ms, 30ms, and 40ms (**Supplementary Figure.10 in revised manuscript**). We found that both the induced CMAP area and angle change of forelimb movement exhibited an initial increase followed by a decrease. We hypothesize that when the pulse width was

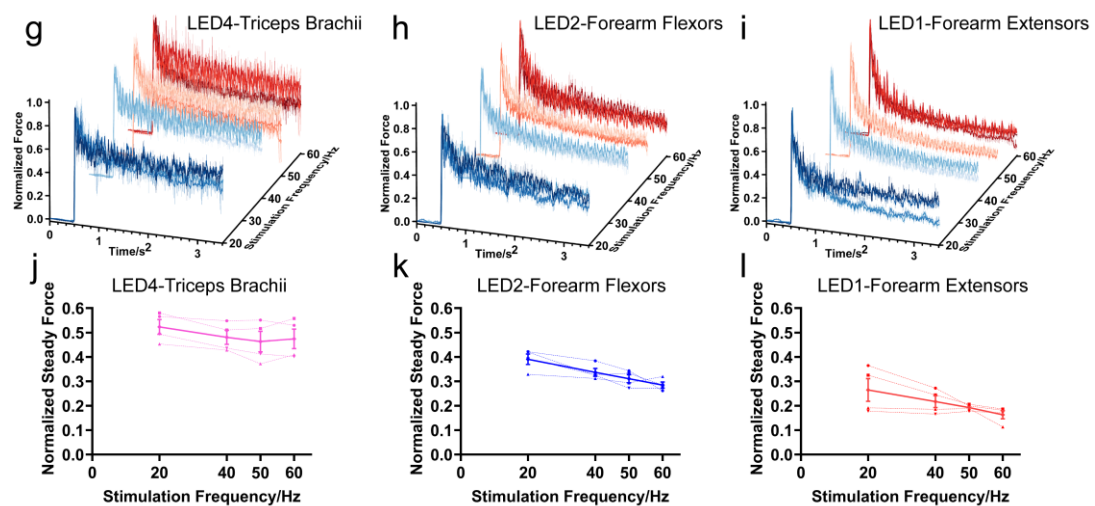
shorter than 20ms with insufficient irradiance, the ChR2 activation may be suboptimal due to incomplete channel opening. However, since the photocurrent kinetics of ChR2 show a fast peak ($\tau_{on} < 20\text{ms}$) followed by steady-state relaxation, while the deactivation time constant ranges around 10-20ms depending on voltage and irradiance. Prolonged illumination beyond this range could promote transition to the light-adapted low-conductance state, resulting in a prolonged but less efficient depolarization plateau phase³.



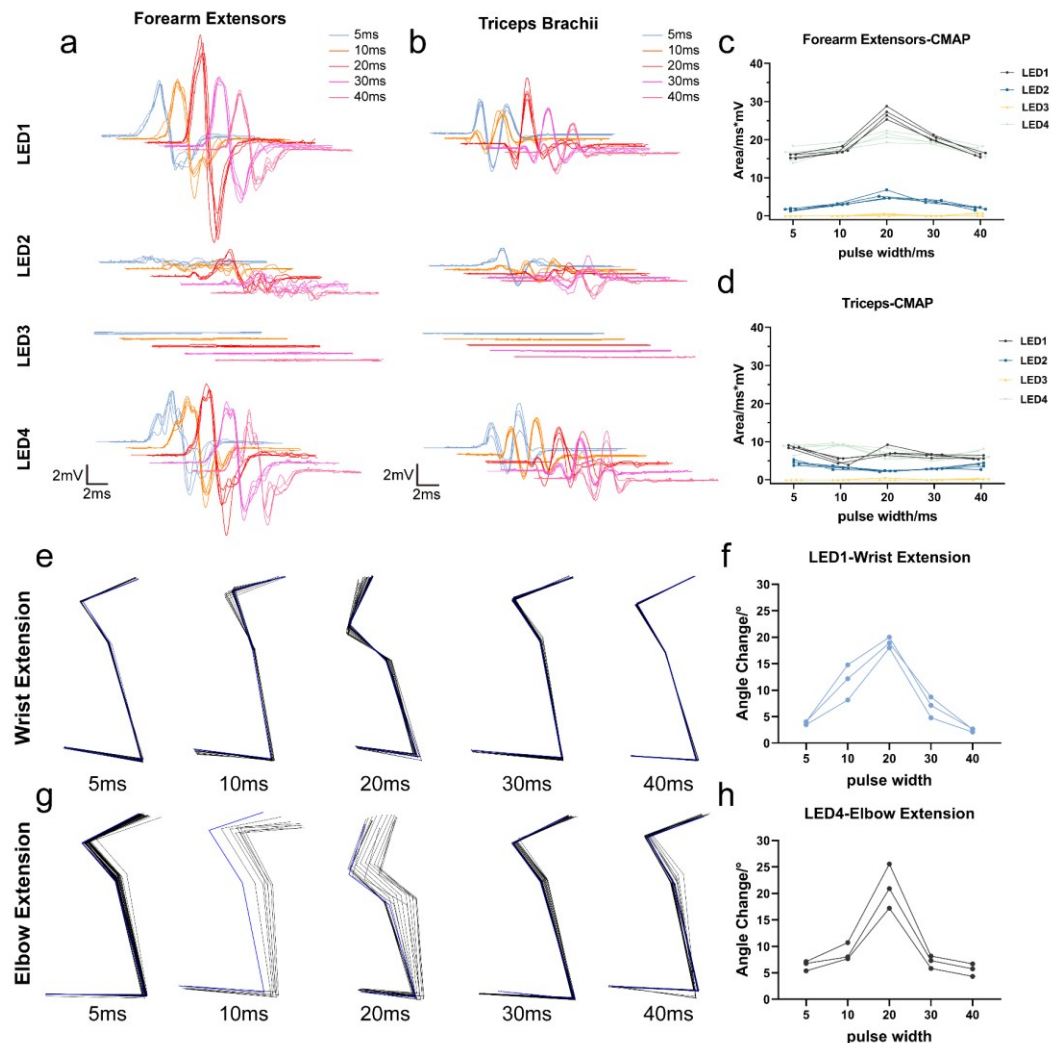
Supplementary Figure.8 Optical simulation of light irradiance distribution in the target nerve when illuminated by different optical power of the μLED . a,b Heat map of light irradiance in a representative 2D slice. One μLED was illuminated at optical power of 2.5mW(a), 4.5mW(b) or 6.5mW(c) respectively. The color bar represents light irradiance in mW/mm². The contour lines of 10mW/mm² were depicted in red. d Comparison between percentage of nerve cross-sectional area where light irradiance exceeds 10mW/mm², with optical power of 2.5mW, 4.5mW and 6.5mW.



Response Figure.1 Spike probability vs. blue light irradiance, plotted for individual Chronos- or ChR2-expressing neurons. From Nathan C. Klapoetke et. al. 2014, Figure.4f.



Supplementary Figure.12 in revised manuscript. Distinct forelimb muscle contractions induced by multisite optogenetic stimulation. **g~i** Normalized force of triceps brachii (g), forearm flexors (h) and forearm extensors (i) tetanic contraction induced by multisite optogenetic stimulation of lower trunk with different stimulation frequency (20Hz/40Hz/50Hz/60Hz stimulation frequency, 0.83ms pulse width, default wireless condition) (n=4). **j~l** Normalized steady force of triceps brachii (j), forearm flexors (k) and forearm extensors (l) with the increase of stimulation frequency. (pulse width=0.83ms, default wireless condition) The curve of each individual mouse is represented by a dashed line, and mean curve of all mice is represented by a solid line. (n=4, mean $\pm$ s.e.m.)



Supplementary Figure.10 in revised manuscript. Comparison of CMAP area and distinct forelimb movement induced by different pulse widths. a,b Representative CMAP curve recorded from forearm extensors (a) and triceps brachii (b) with different stimulation pulse width when illuminating different LEDs. Rows 1 to row 4 display CMAP curves induced by LED1-LED4 respectively. 5ms, blue; 10ms, orange; 20ms, red; 30ms, magenta; 40ms, pink. **c,d** Comparison of CMAP area recorded from forearm extensors (c) and triceps brachii (d) when illuminating LED1 and LED4 respectively with different pulse widths. **e~h** Representative trajectories of wrist extension (e) and elbow extension (g) with different pulse-width. Column 1 to column 5 display representative induced forelimb trajectories with stimulation pulse width of 5ms, 10ms, 20ms, 30ms and 40ms respectively. Quantitative analysis of angle changes in wrist extension (f) and elbow extension (h) induced by LED1 and LED4 respectively with different pulse widths.

Reference:

- Klapoetke, N. C. *et al.* Independent optical excitation of distinct neural populations. *Nat Methods* **11**, 338–346 (2014).

3. Entcheva, E. Cardiac optogenetics. *Am J Physiol Heart Circ Physiol* **304**, H1179-1191 (2013).

Results in revised manuscript, line 412-421

When one μ LED (μ LED2 in this simulation) was activated at the standard optical power of 2.5mW, sector 3 that directly faced it received an average irradiance of 21.9mW/mm², where sector 7 that was further away from it only received an average irradiance of 2.5mW/mm² (Figure.3l,n). Despite the two sectors are only $\sim$ 300 μ m (centroid-to-centroid distance) apart, a fascicle in sector 7 would elicit 90% less force than a fascicle in sector 3 due to the irradiance differences³⁰. Therefore, we estimate the spatial resolution of FIMOSS's optogenetic stimulation to be around 300 μ m. This demonstrated irradiance gradient was expected to allow for selective activation of a sub-section of the target nerve. By illuminating different μ LEDs, fascicles across different sections of the nerve can be targeted, thus triggering distinct downstream muscle movements.

Results in revised manuscript, line 548-554

Firstly, we stimulated middle trunk as a representative nerve with different pulse width (5ms, 10ms, 20ms, 30ms, 40ms) to determine appropriate parameter to induce forelimb movement and record CMAP curve. The results indicated that the pulse width of 20ms was sufficient to induce obvious CMAP curve recorded from forearm extensors (Supplementary Figure.10a,c) and triceps brachii (Supplementary Figure.10b,d), with obvious wrist extension (Supplementary Figure.10e,g) and elbow extension (Supplementary Figure.10f,h) being induced at the same time. So, a pulse width of 20ms was selected for further experiments.

Results in revised manuscript, line 653-659

Afterwards, optogenetic stimulation with higher frequency (20Hz, 40Hz, 50Hz, 60Hz) was applied in 4 mice (0.83ms pulse width, default wireless condition). We observed that tetanic contractions can also be induced with a steady state force within the 3s stimulation period (Supplementary Figure.12g,h,i). Moreover, triceps brachii exhibited relatively high normalized steady force compared with forearm flexors and extensors. When the stimulation frequency is over 40Hz, the normalized steady force began to decrease obviously (Supplementary Figure.12j,k,l).

5. Is there any kind of immune response against the implant: encapsulation, fibrosis, destruction of nerves, etc.?

Author's reply:

Thank you for your insightful comments. Ensuring that both the device itself and the implantation procedure cause no neural damage is indeed a fundamental priority in our study.

We observed thin layer of connective tissue encapsulating the driver, interconnect, and MOSD II of FIMOSS implant (**Figure.4b.c in revised manuscript**), which was normal aseptic tissue response. After removing the connective tissue, we observed that the PDMS encapsulation of the device remained intact. Under visual inspection no significant damage was observed on the pectoralis major. The continuity and texture of the brachial plexus nerves (including the implanted and adjacent nerves) showed no apparent damage.

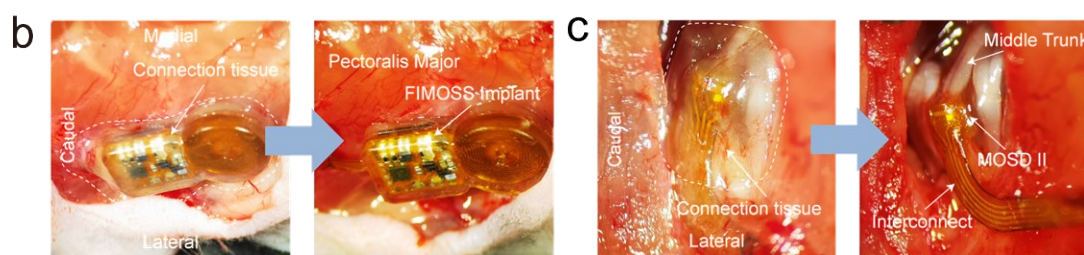


Figure.4 in revised manuscript. Biocompatibility of long-term FIMOSS implantation in brachial plexus after 12 weeks. b Intraoperative images of the FIMOSS implant 12 weeks after implantation surgery. The PDMS encapsulation was observed to be intact after removing the thin layer of connective tissue encapsulation. **c** Intraoperative images of MOSD II and brachial plexus 12 weeks after implantation surgery. The texture and continuity of middle trunks and adjacent upper and lower trunks were not affected by the implantation of MOSD II.

Subsequently, using the middle trunk implantation group as an example, we performed immunofluorescence staining for myelin (MBP) and inflammatory cells (Iba-1) on the implanted site and distal site of middle trunk, and adjacent upper/lower trunk nerves (**a schematic image was shown in Supplementary Figure.9e in revised manuscript**). The results showed that almost no obvious iba-1-positive inflammatory cells can be observed between the perineurium of the implanted middle trunk (**Figure. 4d in revised manuscript**). Only a few iba-1-positive inflammatory cells can be observed in the connective tissue around the nerve trunks of upper trunks and lower trunks (**Supplementary Figure.9a-d in revised manuscript**). This

revealed no significant inflammatory response in the implanted group compared to the control group.

Furthermore, the myelin of the nerve trunks was still intact 12 weeks after implantation. There were no significant changes in myelin structure or nerve compression at the implantation site or distal nerves. Statistical analysis confirmed no significant difference in myelin thickness at the implanted site of middle trunk between the control group and the middle trunk implantation group ([Supplementary Figure.9f in revised manuscript](#)).

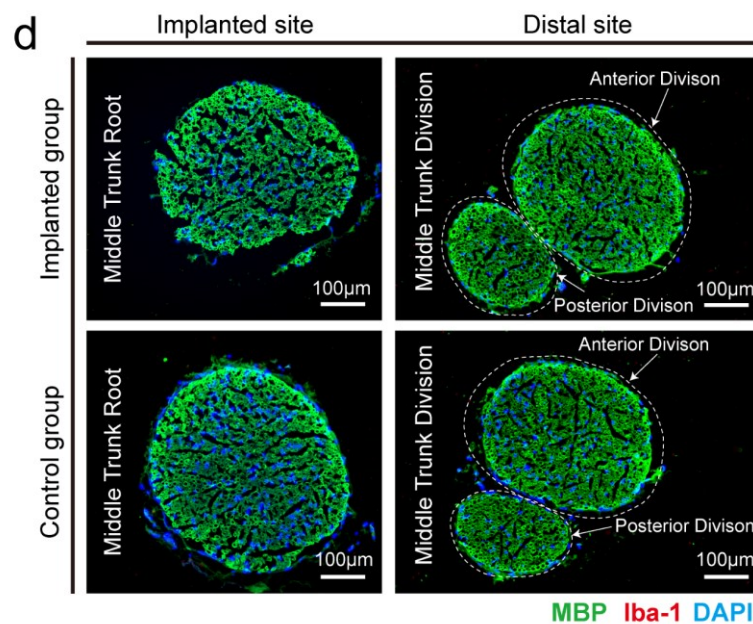
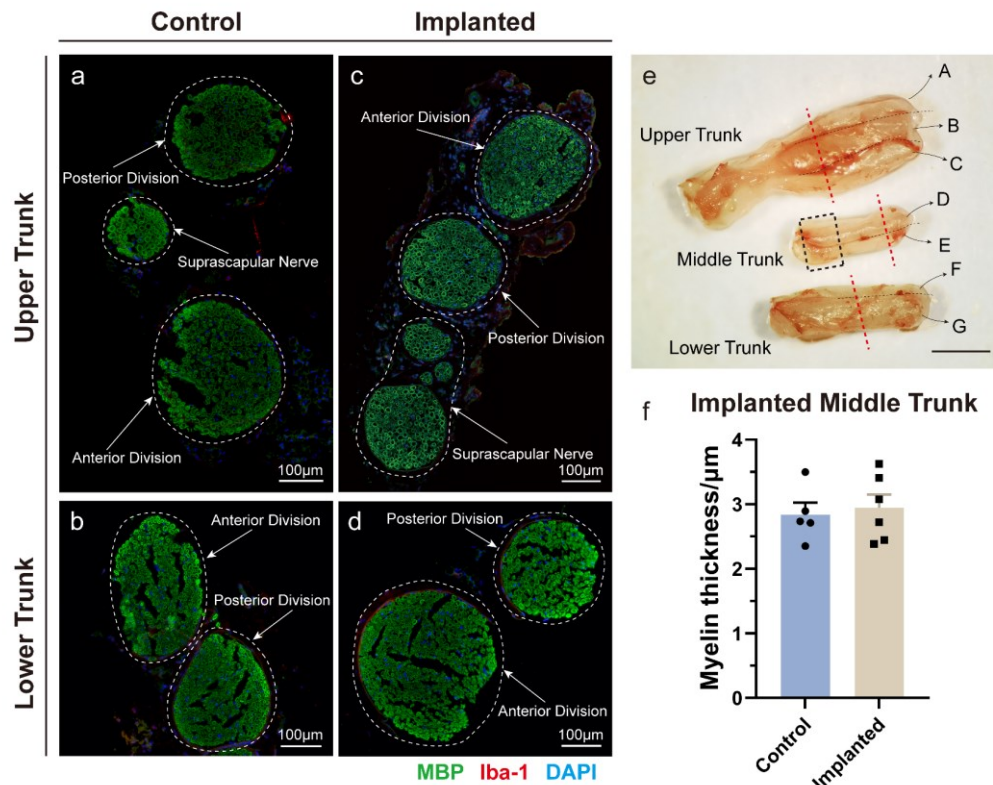


Figure.4 in revised manuscript. Biocompatibility of long-term FIMOSS implantation in brachial plexus after 12 weeks. d Immunofluorescent image of middle trunks at the implantation (main trunk) and distal site (nerve branches) of MOSD II. No obvious inflammation response can be observed. (scale bar=100µm)



Supplementary Figure.9 in revised manuscript. Immunofluorescence staining of upper and lower trunk and myelin thickness analysis of implanted middle trunk. a~d Representative immunofluorescence staining image of adjacent upper trunk and lower trunk of mouse from control group (a,b) and middle trunk implantation group (c,d). (scale bar = 100 μm). e A photograph of the harvested brachial plexus nerve trunks. The black dashed box indicates the implantation site of MOSD II, while the 3 red dashed lines represent: (1) the position adjacent to MOSD II on the upper trunk; (2) the distal site of MOSD II implantation position on the middle trunk; (3) the position adjacent to MOSD II on the lower trunk. Cross sections of neural tissues from all 4 positions were prepared for immunofluorescence staining. A, suprascapular nerve. B, posterior division of upper trunk. C, anterior division of upper trunk. D, anterior division of middle trunk. E, posterior division of middle trunk. F, posterior division of lower trunk. G, anterior division of lower trunk. (scale bar = 500 μm) f Comparison between myelin thickness of middle trunk at the implantation site from control group (n=5) and implanted group (n=6).

Results in revised manuscript, line 507-529

After the behavioral tests, we reopened the original incision as the implantation surgery. A thin layer of connective tissue could be observed encapsulating the FIMOSS Implant, with no significant abnormalities in the texture or structure of the pectoralis major muscle (Figure.4b). The PDMS encapsulation of FIMOSS implant was intact. Around the brachial plexus, scar tissue was seen encapsulating the MOSD II, without noticeable inflammatory exudate or infectious tissue. Following the removal of

MOSD II and the transection of scar tissue, the continuity and texture of the nerve appeared normal without any evident abnormalities (Figure.4c).

Then we performed immunofluorescence staining on the middle trunks at implantation site and distal site of MOSD II along with upper trunks and lower trunks adjacent to the MOSD II (Supplementary Figure.9e). And the myelin sheath at the implantation site was calculated to evaluate whether nerve compression had happened. As a result, the myelin sheath at the implantation site of MOSD II was normal, orderly distributed (Figure.4d). And there is no significant difference in myelin sheath thickness compared with control group (Supplementary Figure.9f). And there were almost no obvious inflammatory cells between the perineurium. Moreover, myelin sheath of the middle trunks distal to MOSD II implantation site and the adjacent upper and lower trunks (Supplementary Figure.9a~d) were also without obvious abnormalities. Only a few inflammatory cells could be seen in the connective tissue surrounding the epineurium.

In conclusion, our results showed that the function of forelimb on the implanted side was not significantly different from that of mice in control group, indicating that FIMOSS implants have good biocompatibility because the free moving of the implanted mice and the normal function of the implanted brachial plexus were not affected.

Minor concerns:

1. How is the newly developed device compared to other implantable optogenetic devices such as for cochlear implants, bladder stimulation and the heart in terms of efficiency in energy transfer, emitted brightness, cooling/preventing heating, endurance etc.

Author's reply:

Thank you for your constructive comment. We conducted further literature research on the existing optogenetic devices, and tabulated the comparisons of characteristics in **Response Table 1**.

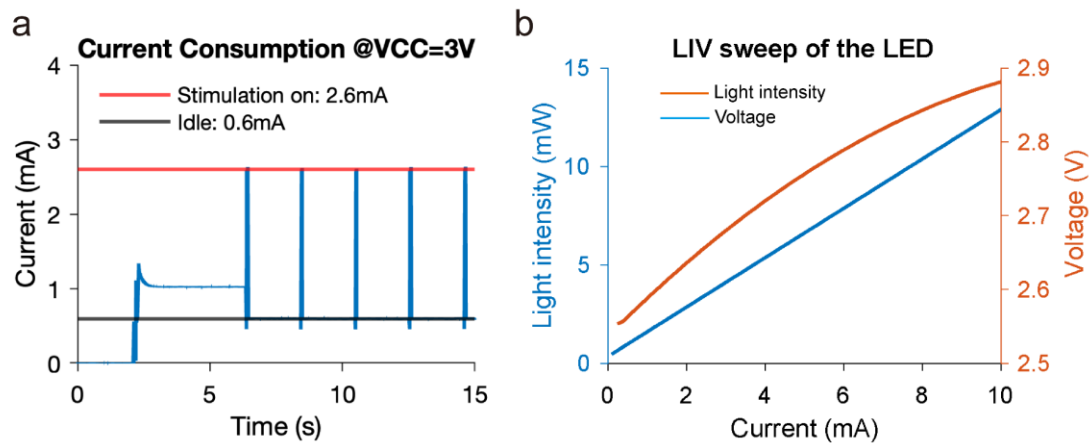
Endurance: Overall, not many devices reported on their endurance result in long-term *in vivo* operation, where some devices estimated it through accelerated aging in 60°C PBS solution. We tested the *in vivo* endurance of FIMOSS implants up until 12 weeks after implantation.

Emitted brightness: Devices were designed to provide appropriate optical power outputs for its application, for example the transcranial optogenetic stimulation in mice⁴ required a strong optical power (13mW, red light) to penetrate the skull and brain tissue, whereas the intracranial stimulation⁵ (0.91 or 1.93mW, blue light) and FIMOSS implants (2.5mW, blue light) employed a rather modest optical power to localize the effect of optogenetic stimulation.

Thermal performances: Our FIMOSS implants and other devices all had a temperature increase below 2°C, which is compliant with thermal safety regulation of implantable devices. Apart from the thermal performance of the device, we further characterized that the μ LED on our nerve cuff is also thermally safe (**Figure.3h in revised manuscript**).

Power consumption: As we are comparing among wireless and battery-free optogenetic implants, we characterized the available power harvested by various implants, instead of the efficiency of energy transfer (defined as the ratio between the received power on the implant versus the emitted power by the external device), because the available power is more meaningful to determine if the WPT is sufficient to support the device's power consumption under typical wireless condition. We have conducted further experiments that validated the FIMOSS implant's power consumption (0.6-2.6mA at 3V, **Supplementary Figure.6a in revised manuscript**) is within the limit of its available power gathered from WPT (maximum 4mA at 3V, **Figure.3d in revised manuscript**).

Lastly, we have researched articles regarding optogenetic cochlear implants^{6,7}, which used wave-guide coupled with laser to deliver optical stimulation to the cochlea. However, the publicly-available reports have not yet showcased a completed optogenetic cochlear implant, and only have described acute stimulation under surgical condition. Therefore, we have excluded optogenetic cochlear implant from **Response Table.1**.



Supplementary Figure.6 in revised manuscript. Power consumption of FIMOSS implant and LIV characterization of LED. a Power consumption of the FIMOSS implant in a typical stimulation event. **b** Luminous intensity, current and voltage across the LED were measured simultaneously to obtain the LED's LIV characterization.

Device	Y. Yang et. al. 2021 ⁵	J. Ausra et. al. 2021 ⁴	Y. Zhang et. al. 2019 ⁸	W. Ouyang et. al. 2023 ⁹	V. Samineni et. al. 2017 ¹⁰	J. Grajales-Reyes et. al. 2021 ¹¹	J. Ausra et. al. 2022 ¹²	FIMOSS (This work)
Stimulation Target	Intracranial	Transcranial	Sciatic nerve	Intracranial	Bladder	Spinal cord	Heart	Brachial plexus
Wireless power harvest limit	N/A	N/A	~15mW	>11mW	N/A	N/A	32mW	~15mW
Device power Consumption	N/A	56mW	5.5mW	2.8mW	N/A	N/A	33mW	7.8mW
LED Emitted brightness	0.91mW, 1.93 mW (blue)	13mW (red)	N/A	N/A	20, 50, 100 mW/mm ² (green)	1-30 mW/mm ² (blue)	101mW/mm ² (blue)	2.5mW (42mW/mm ²) (blue)
Thermal increase	<2°C	<2°C	<2°C	<2°C	<2°C	N/A	<2°C	<2°C
<i>in vivo</i> Lifetime	N/A	N/A	N/A	40% survival at 10weeks	N/A	N/A	> 12 days (Estimated to be 205 days)	100% at 12 weeks

Response Table.1 Comparison with existing implantable optogenetic devices

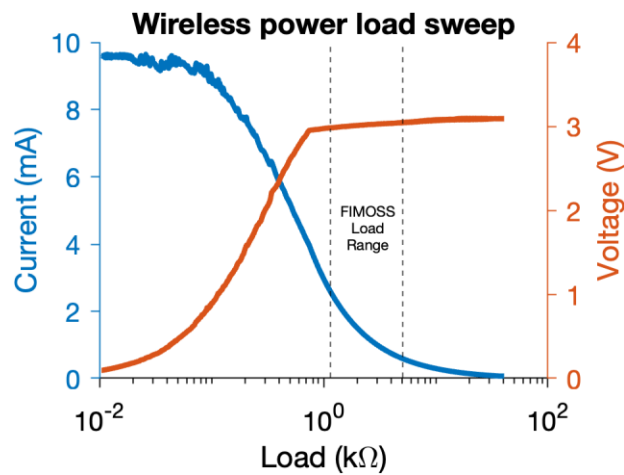


Figure.3 in revised manuscript. Characterization of FIMOSS implant. d Current and voltage across a variable load resistor while it sourced power from the FIMOSS implant that was wirelessly powered under the default wireless condition. The two vertical lines corresponds to the minimum (0.6mA) and maximum (2.6mA) current consumption of the FIMOSS implant at 3V.

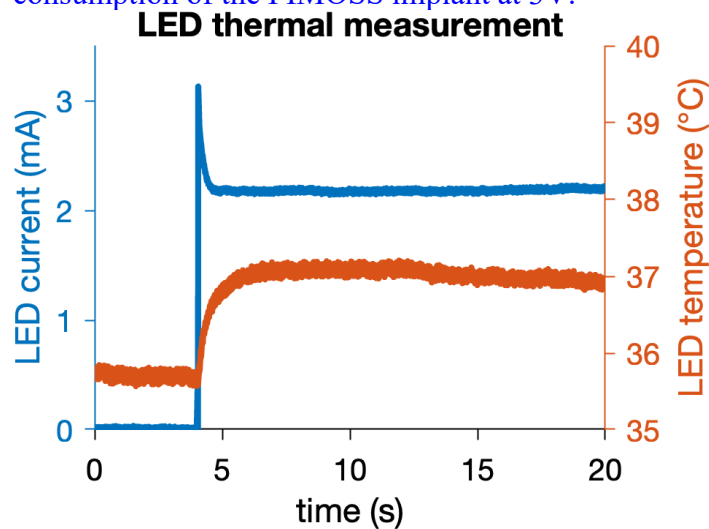


Figure.3 in revised manuscript. Characterization of FIMOSS implant. h Temperature change of the μ LED in vivo during a 20s test period, where the current through the μ LED was simultaneously recorded. The μ LED was powered by a constant current source that was set to 2.1mA.

Reference:

4. Ausra, J. *et al.* Wireless, battery-free, subdermally implantable platforms for transcranial and long-range optogenetics in freely moving animals. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2025775118 (2021).
5. Yang, Y. *et al.* Wireless multilateral devices for optogenetic studies of individual and social behaviors. *Nat Neurosci* **24**, 1035–1045 (2021).
6. Ajay, E. A. *et al.* Combined-electrical optogenetic stimulation but not channelrhodopsin kinetics improves the fidelity of high rate stimulation in the auditory pathway in mice. *Sci Rep* **14**, 21028 (2024).

7. Helke, C. *et al.* On the Fabrication and Characterization of Polymer-Based Waveguide Probes for Use in Future Optical Cochlear Implants. *Materials* **16**, 106 (2022).
8. Zhang, Y. *et al.* Battery-free, fully implantable optofluidic cuff system for wireless optogenetic and pharmacological neuromodulation of peripheral nerves. *Sci. Adv.* **5**, eaaw5296 (2019).
9. Ouyang, W. *et al.* A wireless and battery-less implant for multimodal closed-loop neuromodulation in small animals. *Nat. Biomed. Eng* (2023) doi:10.1038/s41551-023-01029-x.
10. Samineni, V. K. *et al.* Optogenetic silencing of nociceptive primary afferents reduces evoked and ongoing bladder pain. *Sci Rep* **7**, 15865 (2017).
11. Grajales-Reyes, J. G. *et al.* Surgical implantation of wireless, battery-free optoelectronic epidural implants for optogenetic manipulation of spinal cord circuits in mice. *Nat Protoc* **16**, 3072–3088 (2021).
12. Ausra, J. *et al.* Wireless, fully implantable cardiac stimulation and recording with on-device computation for closed-loop pacing and defibrillation. *Sci. Adv.* **8**, eabq7469 (2022).

Results in revised manuscript, line 302-310

The wireless power transfer (WPT) capability of the FIMOSS implant was evaluated by a load sweep sourcing power from the implant while it was wirelessly powered by the external device under the default wireless condition (external device set at 3.3V, 60~80mA; the emitting antenna and receiver antenna were concentrically aligned, in parallel and 1mm apart). The load sweep showed that the implant was able to maintain the target voltage output of 3V when the load is sourcing 4mA or less (Figure.3d). The current consumption of the implant (when operating at 3V) was characterized to be between 0.6mA when idle to 2.6mA when stimulation was on (Supplementary Figure.6a). Thus, we confirmed that the default wireless condition was sufficient to support the power budget of the implant.

Results in revised manuscript, line 388-398

The temperature change within the μ LED nerve cuff was also measured *in vivo* by replacing the nerve with a thermistor of the same size. Under the extreme case of constant illumination at 2.1mA, the μ LED temperature increased from 36°C (body temperature) and stabilized to 37°C during the 20s testing period (Figure.3h). The realistic operating condition of the μ LEDs used in our animal experiments was far less

demanding, where the μ LED was activated using 2.0mA of current (Supplementary Figure.6a), and with a low duty cycle (~1%) in the typical stimulation parameter of 20ms pulse width and 2000ms inter-pulse interval. Hence, both the μ LED and the driver were deemed to be thermally safe for animal experiments, and further confirmed by intraoperative observation and immunofluorescence staining of the nerves and tissues near the implantation site that showed no signs of heat damage (Figure.4b.c).

2. It would be nice to have a comparison between direct skeletal muscle stimulation and the herein presented indirect stimulation via the motor neurons and how the presented device can change the foreseen potential and advantages.

Author's reply:

Thank you very much for this intriguing comment.

Direct electrical stimulation of skeletal muscles, achieved by inserting electrodes directly into the muscle, enables precise stimulation of targeted muscles^{13,14}. Moreover, adjusting the stimulation intensity of electrical muscle stimulation rarely affects muscles other than the target muscle. Consequently, this method can achieve exceptionally precise control over muscle contraction¹⁵. So direct skeletal muscle stimulation is a promising technique when applied for control of a small number of muscles in rat or other bigger animals.

However, there are numerous groups of muscles controlling the shoulder, elbow, wrist and hand. In order to stimulate each muscle, a system with a significant large number of stimulation channels needs to be designed and produced. Operational space to implant such a multichannel system will be extremely small, making it impractical to be applicable in animal study or clinical settings.

The advantage of our device lies in its ability to decode the functions of distinct motor axon fascicles within a nerve trunk using MOSD II. The forelimbs/upper limbs of mice/humans are controlled by the brachial plexus, which forms 3 nerve trunks from 5 nerve roots at area caudal to the collarbone. Performing neuromodulation at this position is the most efficient and space-saving approach. However, compared to direct muscle stimulation, the anatomical position of the brachial plexus at the nerve trunk level is much deeper, and each nerve trunk contains many different nerve

fascicles, thus placing higher demands on the resolution of device stimulation and the structure of the device itself. Our device was developed to address these challenges.

Reference:

13. Lee, Y. *et al.* A low-power stretchable neuromorphic nerve with proprioceptive feedback. *Nat. Biomed. Eng* (2022) doi:10.1038/s41551-022-00918-x.
14. Zhao, Q. *et al.* Highly stretchable and customizable microneedle electrode arrays for intramuscular electromyography. *Sci Adv* **10**, eadn7202 (2024).
15. Burton, A. *et al.* Fully implanted battery-free high power platform for chronic spinal and muscular functional electrical stimulation. *Nat Commun* **14**, 7887 (2023).

Discussion in revised manuscript, line 832-839

Some previous studies have also proposed devices for precise motor control through electrical muscle stimulation^{60,61}. Adjusting the stimulation intensity of electrical muscle stimulation rarely affects the untargeted muscles. Consequently, this method can achieve exceptionally precise control over muscle contraction. However, multiple electrodes are required when targeting distinct muscle groups using this method, which may demand a much larger system and cause considerable damage during the implantation process in small animals. Our system offers a reliable and efficient method for spatially selective control of different nerve fascicles in mice.

Reviewer #2 (Remarks to the Author):

Zhou et al. develop an optogenetic stimulation device (FIMOSS), which can be surgically implanted into transgenic mice to optically stimulate peripheral nerves. They use the Thy1-mhChr2-YFP mouse line, which expresses channelrhodopsin-2 protein in neurons under the Thy1 promoter, so that motor neuron action potential firing can be stimulated with blue light. The FIMOSS device builds on their prior MOSD device (Zheng et al Nature Comm 2019), with new wireless stimulation capabilities and updated geometry and design. They use this device for more detailed quantification of the extent of nerve regeneration following the C7 nerve transfer surgery following traumatic brain injury (TBI).

Overall, the manuscript is clearly motivated and shows impressive use of optogenetics for stimulation of peripheral nerves in live mice. The authors provide a very detailed description of the FIMOSS device design considerations, thorough quality control, and clear rationale for experimental design and motivation. The durability, spatial

and temporal resolution, and heating of the device are all thoroughly discussed and addressed. However, a few major comments remain. I have focused my review on the optogenetic technical and experimental design aspects of the study, as I do not have the physiological and medical expertise to comment on the quality and novelty of the in-vivo findings.

Major concerns:

1. The authors motivate the study in the Introduction by stating that peripheral nerve stimulation following nerve transfer can substantially benefit the regenerative process. This would imply that the authors plan to use the FIMOSS device as a treatment method and to test whether stimulation post-surgery can improve regeneration. However, the authors rather use the device to measure the progress of regeneration, in combination with CMAP readout. This leads to some confusion: is the device intended as a potential therapeutic strategy (and this is why biocompatibility, longevity, etc is critical) or as a monitoring research tool for the regeneration progress? If the latter, it raises the question why optogenetic stimulation is necessary, and why traditional needle electrode-based stimulation is not sufficient (in particular, the spatial resolution limits of such traditional approaches need to be specifically stated and discussed in comparison with the FIMOSS device, to clearly motivate the study).

Author's reply:

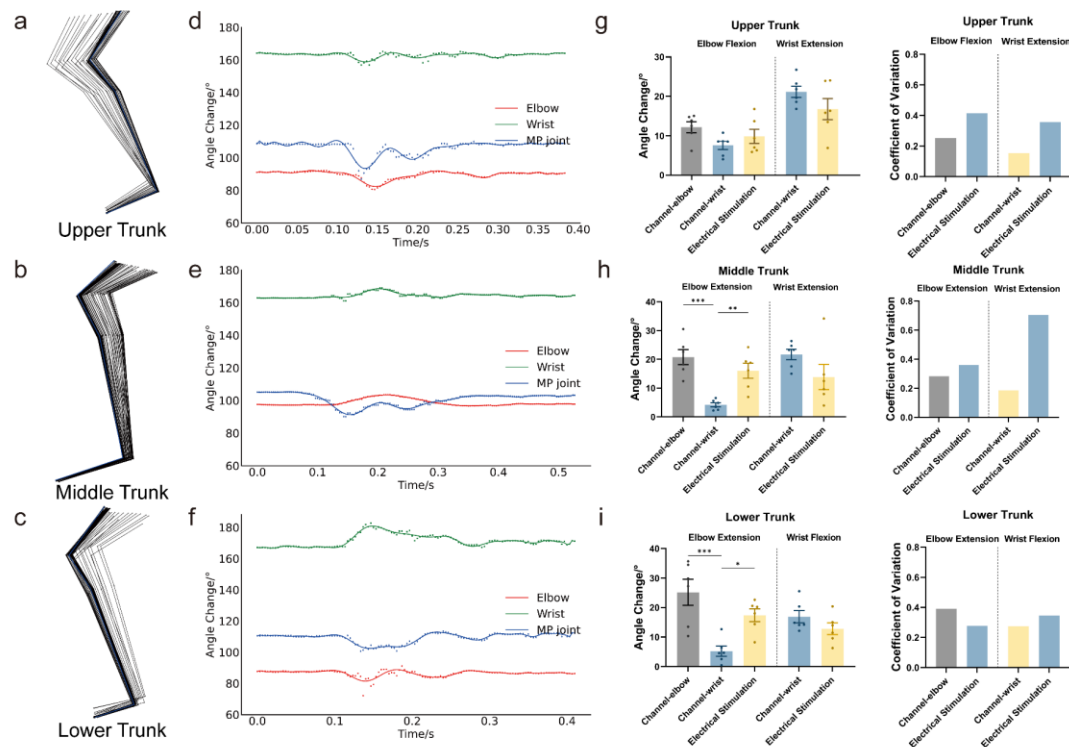
Thank you for your valuable comments.

The immediate application of our FIMOSS device was to evaluate functional changes in the brachial plexus of mice during disease progression or regeneration after nerve transfer (the latter point you mentioned). Our previous study indicated that the regeneration process lasted 12 weeks¹⁶. Fully implantable optogenetic stimulation avoids repeated nerve exposure, enabling non-invasive, high-resolution stimulation on different nerve fascicles within a single nerve trunk.

In contrast, since traditional needle electrode-based stimulation can sufficiently activate the whole nerve, in which case the contraction of all the innervated muscle groups can be induced at the same time. As a result, the induced movement of wrist and MP joints were interfered by the elbow movements (**Supplementary Figure.11a~f in revised manuscript**), showing lower spatial resolution in inducing

different forelimb movements controlled by brachii plexus nerves. Moreover, fully-implantable electrical stimulation devices require direct contact between the needle electrodes and the nerve tissue, inevitably exacerbating local immune reactions.

These new results have been added into the revised manuscript.



Supplementary Figure 11. Comparison between multisite optogenetic stimulation/electrical stimulation-induced forelimb movements of different nerve trunks in brachial plexus. a~c Representative trajectories induced by electrical stimulation on upper (a), middle (b) and lower trunk (c). d~f The angle-time curves of elbow, wrist and MP joint movements induced by electrical stimulation of the upper (d), middle (e) and lower trunk (f). g~i Comparison between angle changes (left) and coefficient of variation (right) of multisite optogenetic stimulation (n=6)/electrical stimulation-induced (n=6) forelimb movements at different brachial plexus nerve trunks. Channel elbow/wrist represent the μ LED which can induce elbow/wrist extension or flexion with the largest angle changes among the 4 μ LEDs of MOSD II.

Reference:

16. Gao, Z. *et al.* Crossing nerve transfer drives sensory input-dependent plasticity for motor recovery after brain injury. *Sci. Adv.* **8**, eabn5899 (2022).

Results in revised manuscript, line 585-599

Furthermore, to clarify the characterization of optogenetic stimulation by FIMOSS, we systematically compared the induced movements between multisite optogenetic (20ms pulse width, default wireless condition, n=6) and electrical stimulation (single-site, 0.2ms pulse width, 0.5mA current intensity, n=6). As a result, both electrical stimulation and multisite optogenetic stimulation at brachial plexus could effectively induce obvious elbow joint movement (Supplementary Figure.11a,b,c). However, the relatively low spatial resolution of electrical stimulation limited its function in selective activation of distal forelimb joints (Supplementary Figure.11g,h,i). The angle changes of wrist and metacarpophalangeal joints were often obscured by concomitant elbow movement, manifesting as low-amplitude oscillations in joint angle measurements in these joints (Supplementary Figure.11d,e,f). These elbow motion-associated wrist/hand movements cannot serve as valid metrics for evaluating wrist and hand motor movements. In contrast, multisite optical stimulation induced selective activation of wrist and metacarpophalangeal joints with minimal elbow involvement, which indicated that the observed joint movements resulted from direct muscle contractions rather than being kinematic artifacts of proximal joint movement.

2. The difference between the new FIMOSS/MOSDII device and the previous MOSD model needs to be clarified. What kind of experiments are now possible that were not with MOSD? I understand that the new device is wireless, smaller, and more durable, but the experiments of the current study are all performed under anesthesia and so the benefit of wireless stimulation is not utilized.

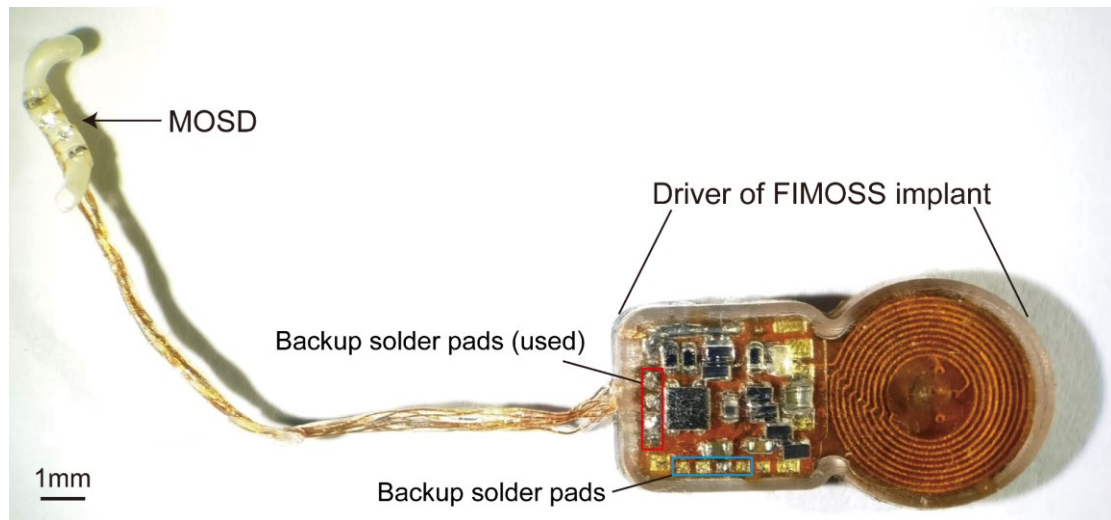
Author's reply:

Thank you for your constructive comments.

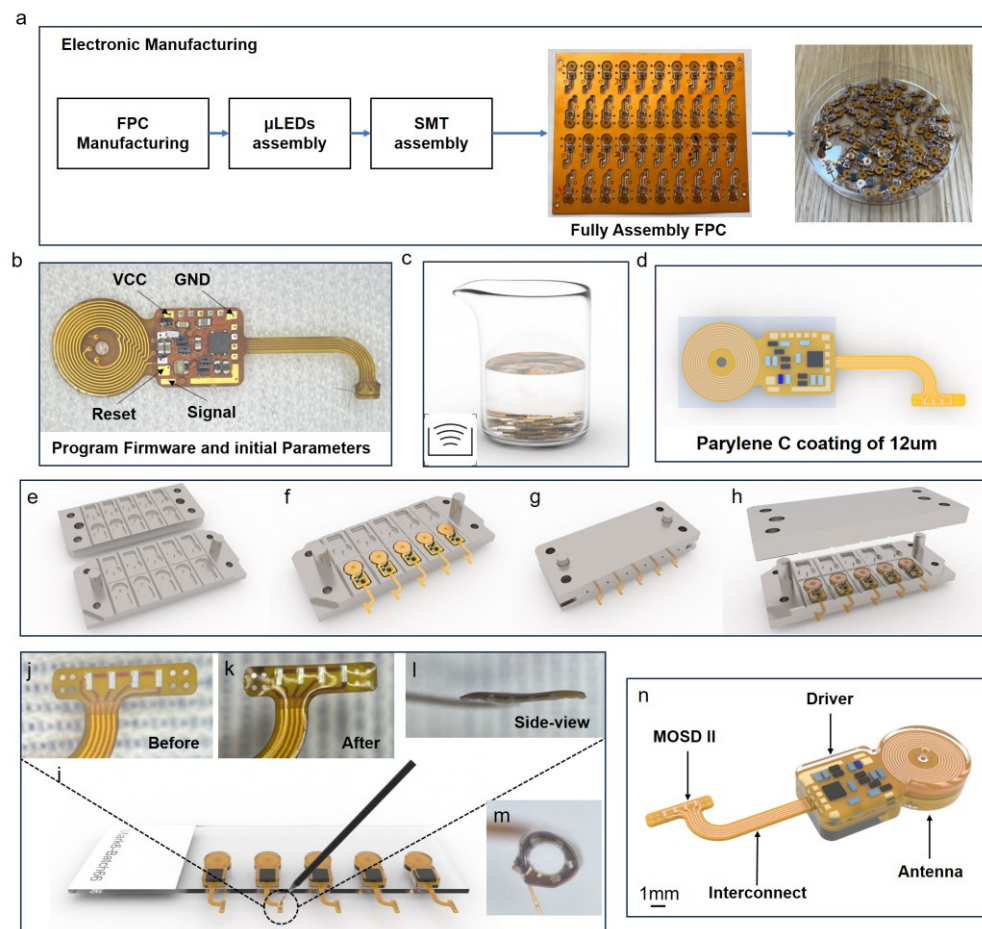
First, MOSD is consisted of multiple LEDs (**Supplementary Fig.2 in revised manuscript**), whereas FIMOSS is a fully implantable device that is consisted of MOSD II (**Supplementary Figure.13 in revised manuscript**). Moreover, MOSD was fabricated manually, whereas FIMOSS/MOSD II was manufactured in a semi-automatic process (**details in the revised Manuscript Method.1~4**).

We have also tried to expose the nerves repeatedly with acutely implanted MOSD, which resulted in major adverse events at the second surgery (data not shown). Hence, even though we applied anesthesia during experiments using the FIMOSS/MOSD II (for accurate alignment of the emitting coil and the antenna during the stimulation),

our current device allowed the evaluation of the functional changes of brachial plexus at multiple time points, which is not possible using MOSD. We have added these considerations into the Discussion of the revised manuscript.



Supplementary Figure.2 in revised manuscript. A photo of the FIMOSS driver soldered with a 4-channel MOSD and with complete encapsulation. (scale bar=1mm)



Supplementary Figure.13 in revised manuscript. Fabrication process of FIMOSS implant. **a** Electronic manufacturing process of FIMOSS implants involving the FPC manufacturing, μ LEDs assembly and SMT assembly. **b** Program firmware and initial parameters into the MCU of the FIMOSS implant. **c** The ultrasonic cleaning of the device. **d~h** The antennas and drivers were encapsulated by 12 μ m Parylene C (d) and then encapsulated by PDMS using a stainless steel mold (e~h) **i~l** Encapsulate the interconnect and MOSD II of several FIMOSS implants by PDMS. **m** Fixation of MOSD II by a 11-0 prolene suture. **n** A schematic diagram of the completely prepared FIMOSS implant. (scale bar=1mm)

Results in revised manuscript, line 86-94

This new technology proceeded from our previously reported multisite optogenetic stimulation device (MOSD) which provided a machine-nerve interface for acute activation of different nerve fascicles in C7 nerve, effectively inducing various movements of forelimbs in mice^{22,23}. In FIMOSS, we advanced this method for chronic use by integrating wireless technology into an optogenetic implant that can fit comfortably on a mouse's chest. The implant consists of a cuff optrode with a similar structure as MOSD (we called it MOSD II), which can be wirelessly powered and controlled to execute various stimulation parameters after implantation in mice and maintained stable performance for at least 12 weeks.

Minor concerns:

3. How is the radial orientation of the LEDs around the nerve bundle specified? Can the MOSDII loop rotate due to movement of the mouse? Even if the spatial resolution of the device is sufficient, if its position can change over time the precision of stimulation can be compromised.

Author's reply:

Thank you very much for your important comment.

Despite that the diameters of upper, middle, and lower trunks differ from each other, the diameter of the same nerve trunk is similar in different mice. Based on this anatomical characteristic, we adopted a method in which No.2, No.3 μ LED and the interconnect are always located on the ventral side of the mouse, as shown in **Figure. 5e,f,g in revised manuscript**. This approach ensures that the positions of 4 μ LEDs for the same nerve trunk are similar among different mice. And MOSD II will maintain its original orientation throughout both the implantation procedure and wound closure, avoiding any rotational adjustments.

Additionally, the brachial plexus nerve trunks are located between the shoulder and neck, with no significant displacement during movements. So, the implanted MOSD II will not rotate during free moving behaviors in mice. This stability is one important advantage we considered when making decisions on the implant location of MOSD II.

Finally, in our experiments, the body position of the mice is standardized, so during testing, the positions of the μ LEDs are roughly the same across different mice, ensuring the consistency and reproducibility of the experiments.

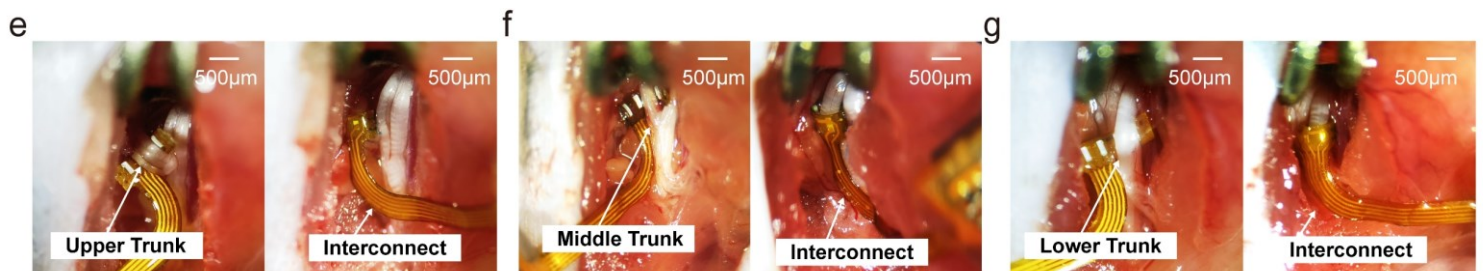


Figure.5 in revised manuscript. The application of FIMOSS on brachial plexus to induce distinct movements of forelimb in mice. e~g Intraoperative photographs of MOSD II interfacing with upper trunk (e), middle trunk (f), lower trunk (g). (scale bar=500 μ m)

4. How sensitive is the FIMOSS device to ambient 2 MHz RF signal and how is specificity to the device encoded? Can the device be unintentionally activated by ambient (e.g. AM radio waves), and is it possible to independently control two neighboring devices?

Author's reply:

Thank you for the constructive comment. The FIMOSS device is not sensitive to ambient-level 2MHz RF signal. We designed a customized wireless communication protocol for FIMOSS that it encodes bit 1 and 0 by presence and absence of wireless power respectively. The FIMOSS device needs to receive at least 1.8mW of power to register a bit 1, and registers a bit 0 when it receives 0 power. Ambient radio waves (e.g. the AM radio) are only transmitting signal, not power, thus their RF power are many orders of magnitudes weaker (in the range of nW to a few μ W), hence are not able to be perceived by the FIMOSS device.

The specificity of the device is not encoded in software, but rather distinguished by the physical separation between neighboring devices. In order to power and control a

FIMOSS implant, the experimenter needs to bring the emitting coil to within 1mm parallel distance from the receiving coil and align the two coils concentrically ([Supplementary Movie.2 in revised manuscript](#)). Therefore, two neighboring devices can be independently powered and controlled if they are more than 5mm apart.

Methods in revised manuscript, line 952-961

During a programming session, the user first switch on the DC power supply, thus activating the FIMOSS external device to emit wireless power. Then, the external antenna was brought close to the receiving antenna of the FIMOSS implant (the antenna pair should be concentric and parallel with each other, at a parallel distance of ~1mm) to power the implant. If implant was checked to be correctly powered, by observing the blue status LED on the driver region of the implant being illuminated, and within 2 s window starting from the boot up of the implant, the operator should click “send” on the serial communication software on the computer, to transmit the command string. The success of the wireless programming operation was confirmed by the blue status LED flashing in a rapid pattern ([Supplementary Movie.2](#)).

5. I did not understand at which point is the FIMOSS device implanted. Is it implanted at the same time as the C7 transfer surgery? If so, can the presence of the MOSDII around the nerve interfere with the healing process?

Author’s reply:

Thank you for your clarifying comment. We apologize for not explaining this issue clearly.

As shown in the modified timeline in [Figure.6c in revised manuscript](#), the CC7 surgery and device implantation are performed simultaneously. To avoid interfering with nerve regeneration at the coaptation site, we use a neck incision for the CC7 surgery and a supraclavicular incision for device implantation. In this way, the cuff electrode is located slightly distal to the coaptation site (as shown in [Figure. 6a.b in revised manuscript](#)), thereby avoiding potential impact on nerve regeneration. We also supplement gross images and immunofluorescence staining of the nerves after 12 weeks of device implantation to demonstrate that the implantation surgery did not

have a significant effect on the nerves ([Figure.4d and Supplementary Figure.9 in revised manuscript](#)).

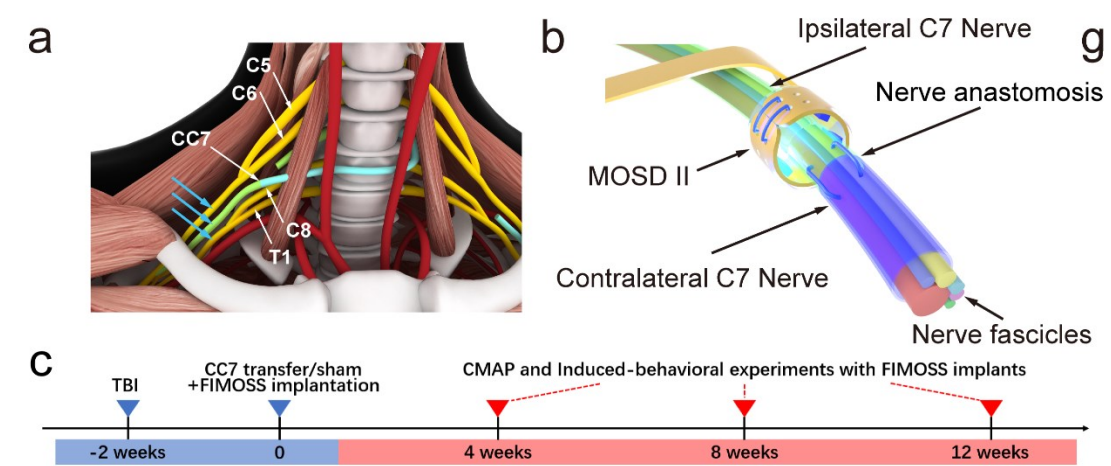


Figure.6 in revised manuscript. Electrophysiology change pattern of brachial plexus after CC7 transfer evaluated by multisite optogenetic stimulation method. **a** A schematic diagram of CC7 transfer surgery. Upper trunks and lower trunks were marked yellow. Middle Trunks of healthy side and impaired side were marked cyan and green respectively. The implantation positions of the MOSD II were pointed by blue arrows. **b** Schematic of the strategy of CMAP experiments by MOSD II. MOSD II was wrapped around the transferred C7 nerve and fixed on the distal(green) of nerve anastomosis by microsutures. Different nerve fascicles were illustrated in different colors. **c** Timeline of CMAP and induced-behavioral experiments after TBI and CC7 transfer.

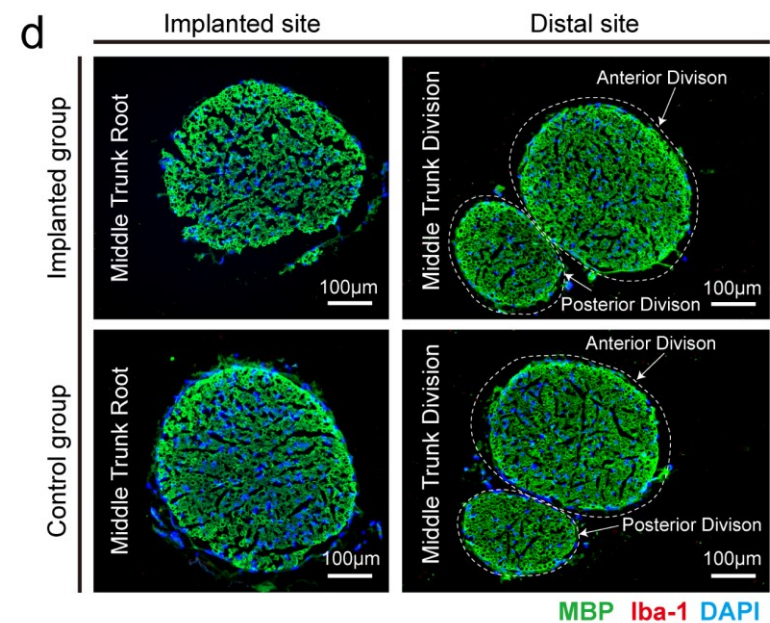
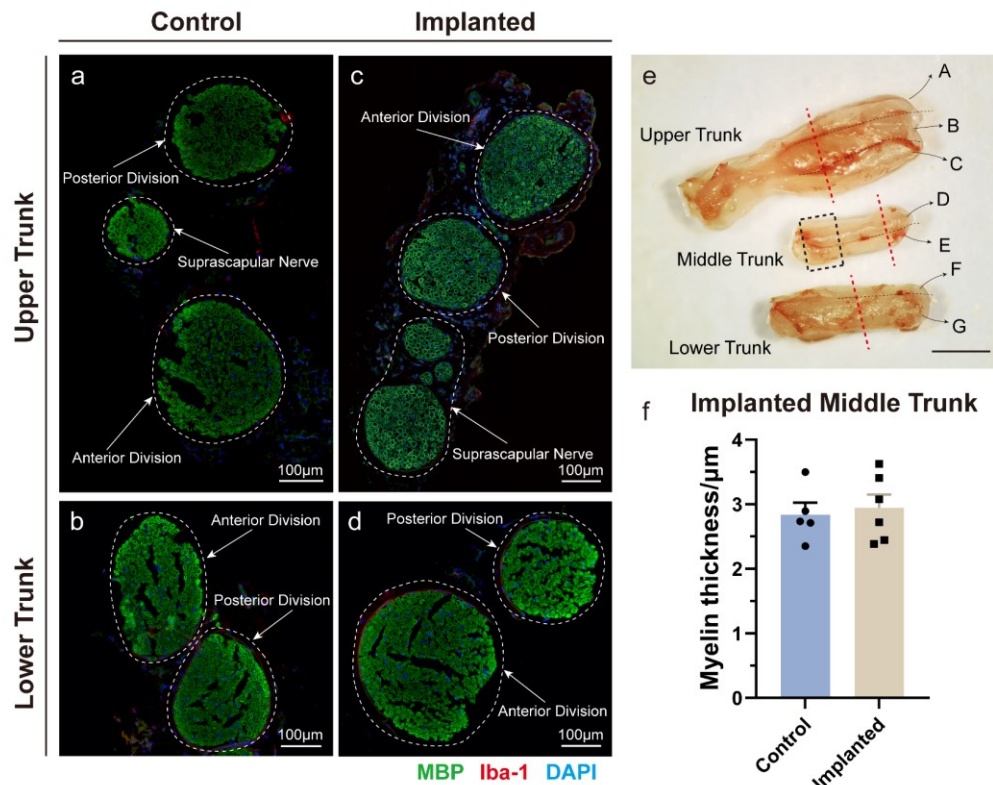


Figure.4 in revised manuscript. Biocompatibility of long-term FIMOSS implantation in brachial plexus after 12 weeks. **d** Immunofluorescent image of middle trunks at the implantation (main trunk) and distal site (nerve branches) of MOSD II. No obvious inflammation response can be observed. (scale bar = 100µm)



Supplementary Figure.9 in revised manuscript. Immunofluorescence staining of upper and lower trunk and myelin thickness analysis of implanted middle trunk. **a~d** Representative immunofluorescence staining image of adjacent upper trunk and lower trunk of mouse from control group (a,b) and middle trunk implantation group (c,d). (scale bar = 100 μm). **e** A photograph of the harvested brachial plexus nerve trunks. The black dashed box indicates the implantation site of MOSD II, while the 3 red dashed lines represent: (1) the position adjacent to MOSD II on the upper trunk; (2) the distal site of MOSD II implantation position on the middle trunk; (3) the position adjacent to MOSD II on the lower trunk. Cross sections of neural tissues from all 4 positions were prepared for immunofluorescence staining. A, suprascapular nerve. B, posterior division of upper trunk. C, anterior division of upper trunk. D, anterior division of middle trunk. E, posterior division of middle trunk. F, posterior division of lower trunk. G, anterior division of lower trunk. (scale bar = 500 μm) **f** Comparison between myelin thickness of middle trunk at the implantation site from control group (n=5) and implanted group (n=6).

Methods in revised manuscript, line 1195-1204

The FIMOSS implant was implanted on the brachial plexus in mice from all groups for 12 weeks. For mice from TBI or TBI+CC7 groups, the implantation surgery was performed 2 weeks after TBI surgery (when performing CC7 transfer for TBI+CC7 groups). The CMAP curves were recorded when the FIMOSS implant was activated (20ms pulse width, default wireless condition) to explore the change pattern of brachial plexus electrophysiological values and forelimb induced-behavioral angle changes. For mice in control and TBI group, the experiments were conducted 12 weeks after

implantation. For mice in TBI+CC7 group, the experiments were conducted 4, 8, 12 weeks after CC7 transfer/implantation surgery. And FIMOSS implants will not be surgically removed after the 4-week or 8-week postoperative evaluation after implantation surgery until the 12-week postoperative evaluation was finished.

Results in revised manuscript, line 507-525

After the behavioral tests, we reopened the original incision as the implantation surgery. A thin layer of connective tissue could be observed encapsulating the FIMOSS Implant, with no significant abnormalities in the texture or structure of the pectoralis major muscle (Figure.4b). The PDMS encapsulation of FIMOSS implant was intact. Around the brachial plexus, scar tissue was seen encapsulating the MOSD II, without noticeable inflammatory exudate or infectious tissue. Following the removal of MOSD II and the transection of scar tissue, the continuity and texture of the nerve appeared normal without any evident abnormalities (Figure.4c).

Then we performed immunofluorescence staining on the middle trunks at implantation site and distal site of MOSD II along with upper trunks and lower trunks adjacent to the MOSD II (Supplementary Figure.9e). And the myelin sheath at the implantation site was calculated to evaluate whether nerve compression had happened. As a result, the myelin sheath at the implantation site of MOSD II was normal, orderly distributed (Figure.4d). And there is no significant difference in myelin sheath thickness compared with control group (Supplementary Figure.9f). And there were almost no obvious inflammatory cells between the perineurium. Moreover, myelin sheath of the middle trunks distal to MOSD II implantation site and the adjacent upper and lower trunks (Supplementary Figure.9a~d) were also without obvious abnormalities. Only a few inflammatory cells could be seen in the connective tissue surrounding the epineurium.

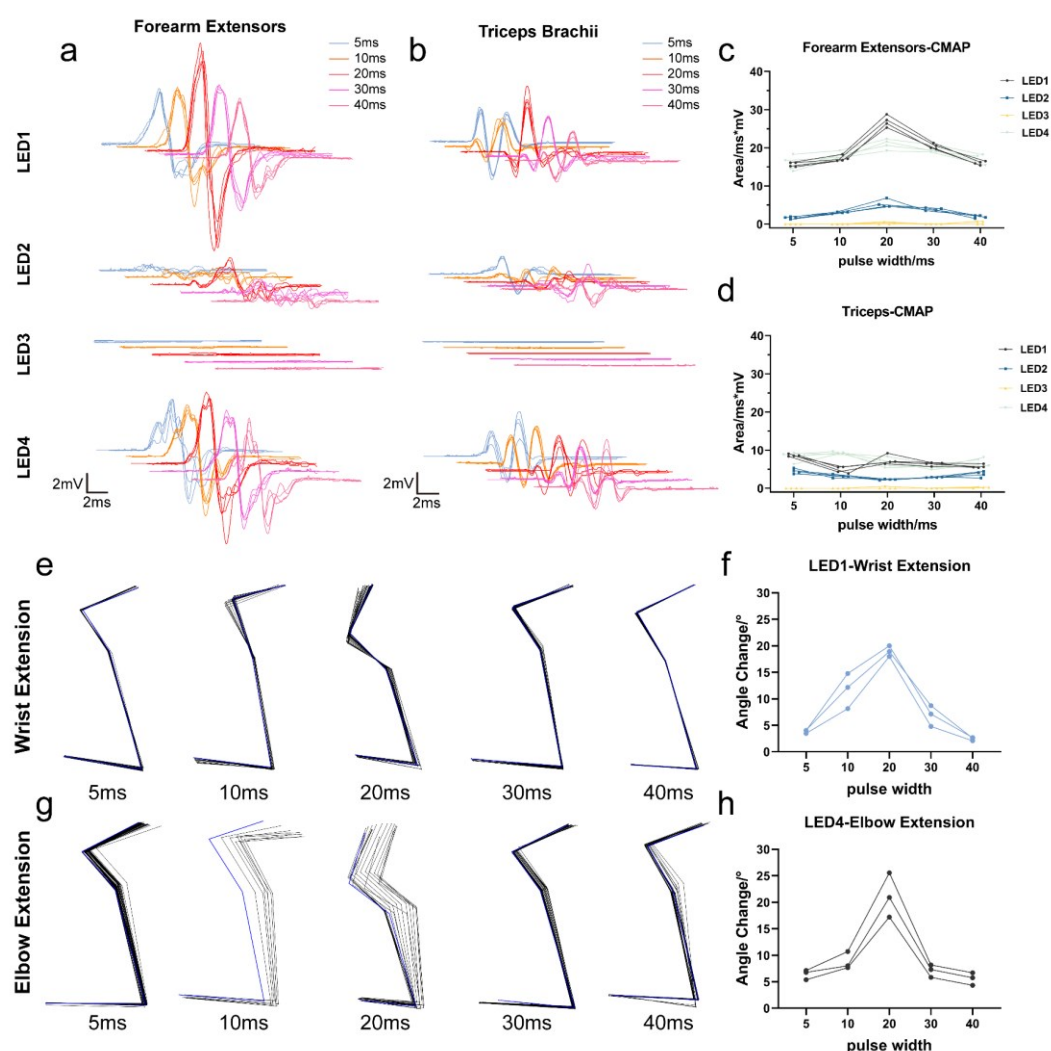
6. Why was the 20 ms-on / 2 s-off stimulation pattern chosen for optogenetic stimulation in the brachial plexus? Is this sufficient for maximal Chr2 activation, and could longer on-times induce stronger action potentials?

Author's reply:

Thank you for your question. Indeed, appropriate stimulation parameters are fundamental for electrophysiological experiments and induced-behavioral studies. On one hand, we referred to our previous study of MOSD and chose the 20ms pulse width stimulation parameter¹⁷, which has proved to be effective for

electrophysiological and induced-behavioral experiments. On the other hand, single-channel open duration of ChR2 is approximately 10ms, while the deactivation time constant (the time required to transition from the open state back to the resting state) is approximately 20ms. Exceeding these time ranges may lead to a prolonged depolarization plateau phase but with reduced efficiency^{3,18,19}.

To test the effect of different pulse widths on optogenetic stimulation, we conducted new experiments with 5ms/10ms/20ms/30ms/40ms pulse widths. The results are shown in the **Supplementary Figure.10 in revised manuscript**. Both the evoked CMAP area and angle change of forelimb movement exhibited an initial increase followed by a decrease. Notably, 20ms proved to be an effective stimulation duration for inducing forelimb muscle CMAPs and triggering limb movements.



Supplementary Figure.10 in revised manuscript. Comparison of CMAP area and distinct forelimb movement induced by different pulse widths. a,b Representative CMAP curve recorded from forearm extensors (a) and triceps brachii (b) with different stimulation pulse width when illuminating different LEDs. Rows 1 to row 4

display CMAP curves induced by LED1-LED4 respectively. 5ms, blue; 10ms, orange; 20ms, red; 30ms, magenta; 40ms, pink. **c,d** Comparison of CMAP area recorded from forearm extensors (c) and triceps brachii (d) when illuminating LED1 and LED4 respectively with different pulse widths. **e~h** Representative trajectories of wrist extension (e) and elbow extension (g) with different pulse-width. Column 1 to column 5 display representative induced forelimb trajectories with stimulation pulse width of 5ms, 10ms, 20ms, 30ms and 40ms respectively. Quantitative analysis of angle changes in wrist extension (f) and elbow extension (h) induced by LED1 and LED4 respectively with different pulse widths.

Reference:

3. Entcheva, E. Cardiac optogenetics. *Am J Physiol Heart Circ Physiol* **304**, H1179-1191 (2013).
17. Zheng, H. *et al.* A shape-memory and spiral light-emitting device for precise multisite stimulation of nerve bundles. *Nat Commun* **10**, 2790 (2019).
18. Han, X. In Vivo Application of Optogenetics for Neural Circuit Analysis. *ACS Chem. Neurosci.* **3**, 577–584 (2012).
19. Feldbauer, K. *et al.* Channelrhodopsin-2 is a leaky proton pump. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 12317–12322 (2009).

Results in revised manuscript, line 548-554

Firstly, we stimulated middle trunk as a representative nerve with different pulse width (5ms, 10ms, 20ms, 30ms, 40ms) to determine appropriate parameter to induce forelimb movement and record CMAP curve. The results indicated that the pulse width of 20ms was sufficient to induce obvious CMAP curve recorded from forearm extensors (Supplementary Figure.10a,c) and triceps brachii (Supplementary Figure.10b,d), with obvious wrist extension (Supplementary Figure.10e,g) and elbow extension (Supplementary Figure.10f,h) being induced at the same time. So, a pulse width of 20ms was selected for further experiments.

7. Figure 2f,g,h, add label for the LED in the schematic (blue squares), and label each f,g,h with a title that states the different nerve diameters accommodated.

Author's reply:

Thank you for your valuable suggestions. We have made the corresponding adjustments to the Figures.

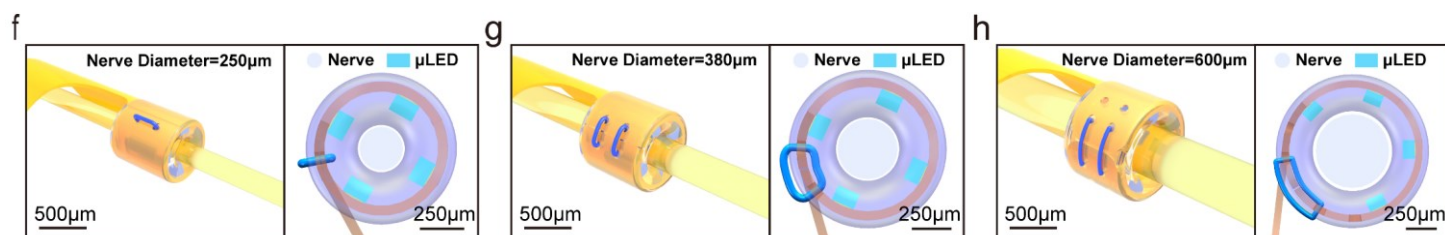


Figure.2 in revised manuscript. Design and structure of FIMOSS implant for long-term multisite optogenetic stimulation. f~h Different stitching and fixation method(left, scale bar=500μm) and cross section diagram(right, scale bar=250μm) of MOSD II adapted to nerves of different diameters.(f) The outer and inner suture eyelets were sacrificed and overlapped respectively to wrap around the nerve with a diameter of around 250μm.(g) The outer suture eyelets were sacrificed while the inner were sutured to adapt to a nerve with diameter of 380μm.(h) The outer suture eyelets were sutured to adapt to a nerve with diameter of 600μm.

8. Figure 3g: what can be an explanation for the reduced temperature changes *in vivo* (3g) vs. *in vitro* (3e)? Is it possible that in the aqueous *in vivo* environment the heat is absorbed by the tissue? After long-term stimulation have you observed any evidence for heat-induced damage to the tissue?

Author's reply:

Thank you for your constructive comment.

Muscle tissue exhibits superior heat dissipation capabilities. Indeed, when the FIMOSS implant works *in vivo*, the generated heat can be effectively dissipated through surrounding body fluids and muscle tissue, resulting in minimal temperature change during device operation (**Figure.3g in the revised manuscript**). In contrast, when tested with default wireless condition (external device set at 3.3V, 60~80mA; the emitting antenna and receiver antenna were concentrically aligned, in parallel and 1mm apart) *in vitro* (**Figure.3e in the revised manuscript**), FIMOSS exhibited larger temperature increase.

Furthermore, 12 weeks after implantation, we observed a thin layer of connective tissue encapsulation around the device, with no evidence of infection/inflammatory reactions, pectoralis major muscle damage, or scar hyperplasia (**Figure.4b in the revised manuscript**). These findings indicate that our device does not induce significant thermal injury.

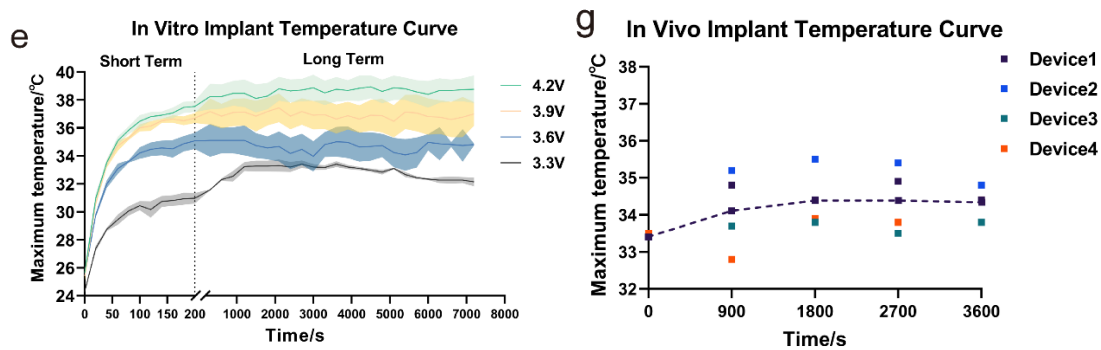


Figure.3 in revised manuscript. Characterization of FIMOSS implant. **e** The temperature changes in vitro during the 7200s' continuous working with 4 different input voltages. (n=4, 20ms pulse width, 1000ms inter-pulse interval) FIMOSS implants were placed on a 1mm-thick glass slide, and the external device antennas were fixed underneath the slide. Voltages of the DC power supply were adjusted to 3.3V, 3.6V, 3.9V, and 4.2V to power the external device, which wirelessly drive the FIMOSS implants. **g** The temperature changes of FIMOSS implants in vivo during the 3600s' continuous working. (n=4, input voltage=3.3V, 20ms pulse width, 1000ms inter-pulse interval) FIMOSS implants were implanted subcutaneously on the surface of pectoralis major, whose temperature were measured by an infrared thermometers

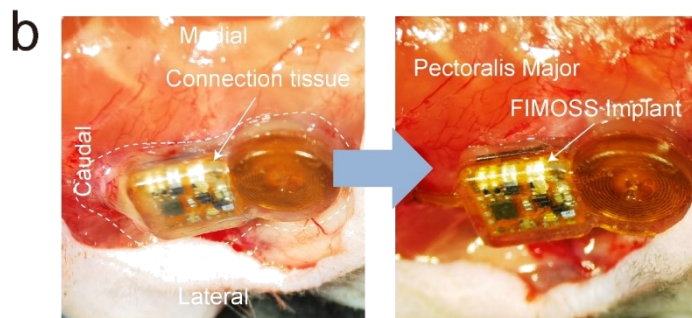


Figure.4 in revised manuscript. Biocompatibility of long-term FIMOSS implantation in brachial plexus after 12 weeks. **b** Intraoperative images of the FIMOSS implant 12 weeks after implantation surgery. The PDMS encapsulation was observed to be intact after removing the thin layer of connective tissue encapsulation.

Results in revised manuscript, line 507-510

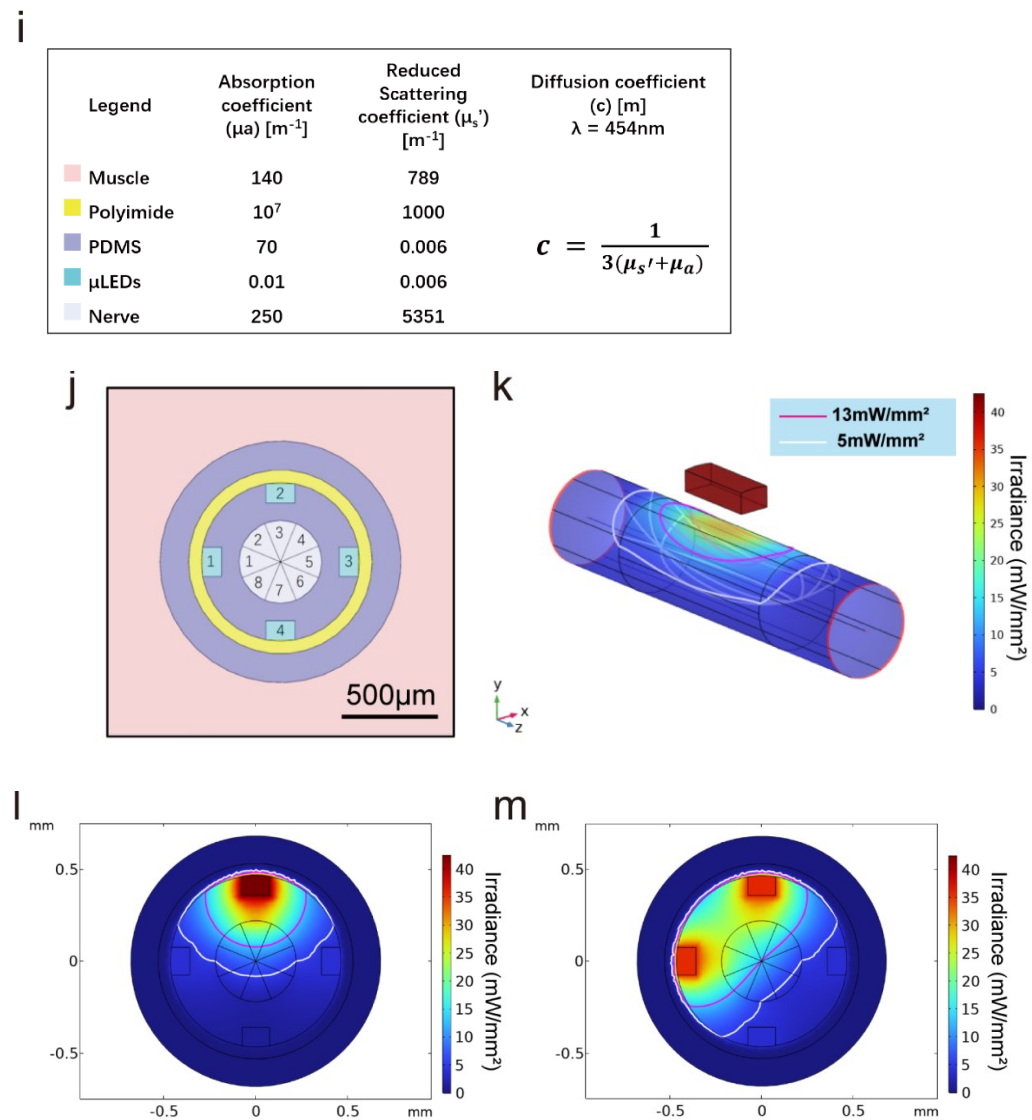
After the behavioral tests, we reopened the original incision as the implantation surgery. A thin layer of connective tissue could be observed encapsulating the FIMOSS Implant, with no significant abnormalities in the texture or structure of the pectoralis major muscle (Figure.4b). The PDMS encapsulation of FIMOSS implant was intact.

9. Figure 3j,k,l: what are units and values for the rainbow intensity scale, and what is physical length of the white scale bar? Are the intensity units absolute or relative? Also please label what you mean by “corner sector” within the Figures for clarity.

Author's reply:

Thank you for the constructive comment.

We have conducted new optical simulation experiments in response to your comment 9~11 and updated [Figure.3i~n in revised manuscript](#). We added the scale bar for the rainbow intensity scale in [Figure. 3k](#), as well as the scale bar for the physical length in [Figure. 3j in the revised manuscript](#). The intensity units are absolute (in mW/mm^2). We equally divided the nerve's cross-section into 8 sectors ([labeled in Figure.3j in revised manuscript](#)) to evaluate the average irradiance received by each nerve sector.



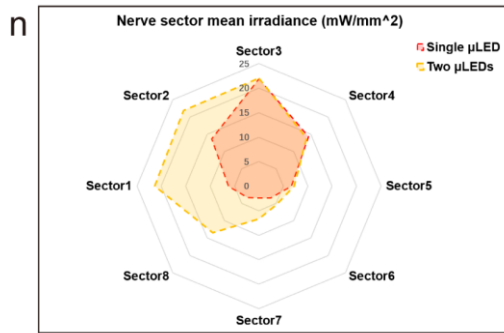


Figure.3 in revised manuscript. Characterization of FIMOSS implant. i The absorption coefficients and reduced scattering coefficients used for the optical simulation. **j** Cross-sectional view of the 3D model of a MOSD II cuffing on a typical target nerve (440μm diameter, scale bar=500 μ m). This 3D model was used in the optical simulation, and each domain was color-coded corresponding to the color legend in Figure.3i. The four μLEDs were numbered, and the nerve was equally divided to eight sectors and numbered as references for the quantitative analysis result in Figure.3n. **k** Heat map of the light irradiance distribution in 3D optical simulation, when one μLED was illuminated at 2.5mW of optical power. The color legend is the same as in Figure.3i,m. Domains except for the activated μLED and the target were hidden. The plot was set at 40% transparency to allow visualization of the contour lines of 13mW/mm² and 5mW/mm² depicted in magenta and white. **l,m** Heat map of light irradiance in a representative 2D slice out of the 3D optical simulation result. The 2D slice was defined as the XY-plane slicing down the middle (Z-direction) of the μLED in Figure.3k. One μLED (l) was illuminated at the standard optical power of 2.5mW. Two neighboring μLEDs (m) were illuminated at a reduced optical power of 2.0mW. The color bar represents light irradiance in mW/mm². **n** The average irradiance in each sector of the nerve computed by optical simulation of one-μLED (orange) and two-μLED (yellow) illumination in Figure.3l,m.

Results in revised manuscript, line 403-411

A set of 3D finite element analysis (FEA) studies were performed to simulate the propagation of light irradiance from the μLED (2.5mW optical power, 42mW/mm² irradiance at μLED's surface) in the implanted environment (Figure.3i,j,k). A 2D representative slice out of the 3D result was used to visualize the irradiance distribution in the cross-section of the nerve fiber, where contour lines of 13mW/mm² and 5mW/mm² irradiance were illustrated (Figure.3l,m). These two irradiance thresholds were chosen because the transgenic mice line Thy1-ChR2-EYFP has been previously characterized that the muscle force elicited by optogenetic stimulation of the peripheral nerve reaches 50% of the maximum force when light irradiance is 5mW/mm², and reaches the maximum force at 13mW/mm² or higher³⁰.

Results in revised manuscript, line 412-421

When one μ LED (μ LED2 in this simulation) was activated at the standard optical power of 2.5mW, sector 3 that directly faced it received an average irradiance of 21.9mW/mm², where sector 7 that was further away from it only received an average irradiance of 2.5mW/mm² (Figure.3l,n). Despite the two sectors are only ~300 μ m (centroid-to-centroid distance) apart, a fascicle in sector 7 would elicit 90% less force than a fascicle in sector 3 due to the irradiance differences³⁰. Therefore, we estimate the spatial resolution of FIMOSS's optogenetic stimulation to be around 300 μ m. This demonstrated irradiance gradient was expected to allow for selective activation of a sub-section of the target nerve. By illuminating different μ LEDs, fascicles across different sections of the nerve can be targeted, thus triggering distinct downstream muscle movements.

Results in revised manuscript, line 431-438

Performing nerve stimulation using only one μ LED at a time can create blind spot at the corner sectors (sector 2,4,6,8 in Figure.3j), because there were no μ LEDs directly facing them. Therefore, further FEA study was done when two neighboring μ LEDs were simultaneously illuminated in a reduced optical power of 2.0mW each to explore resolution of the blind spot issue. The FEA result showed that in this manner, the corner sectors can receive the same level of mean irradiance as the sectors which have μ LEDs facing them (Figure.3m,n). Hence, this modality of illumination can ensure that all sectors of the nerve are accessible by optogenetic activation.

10. Related to point 9 above: the authors often refer to the high spatial resolution of the LED illumination, but do not provide exact values; please provide these values.

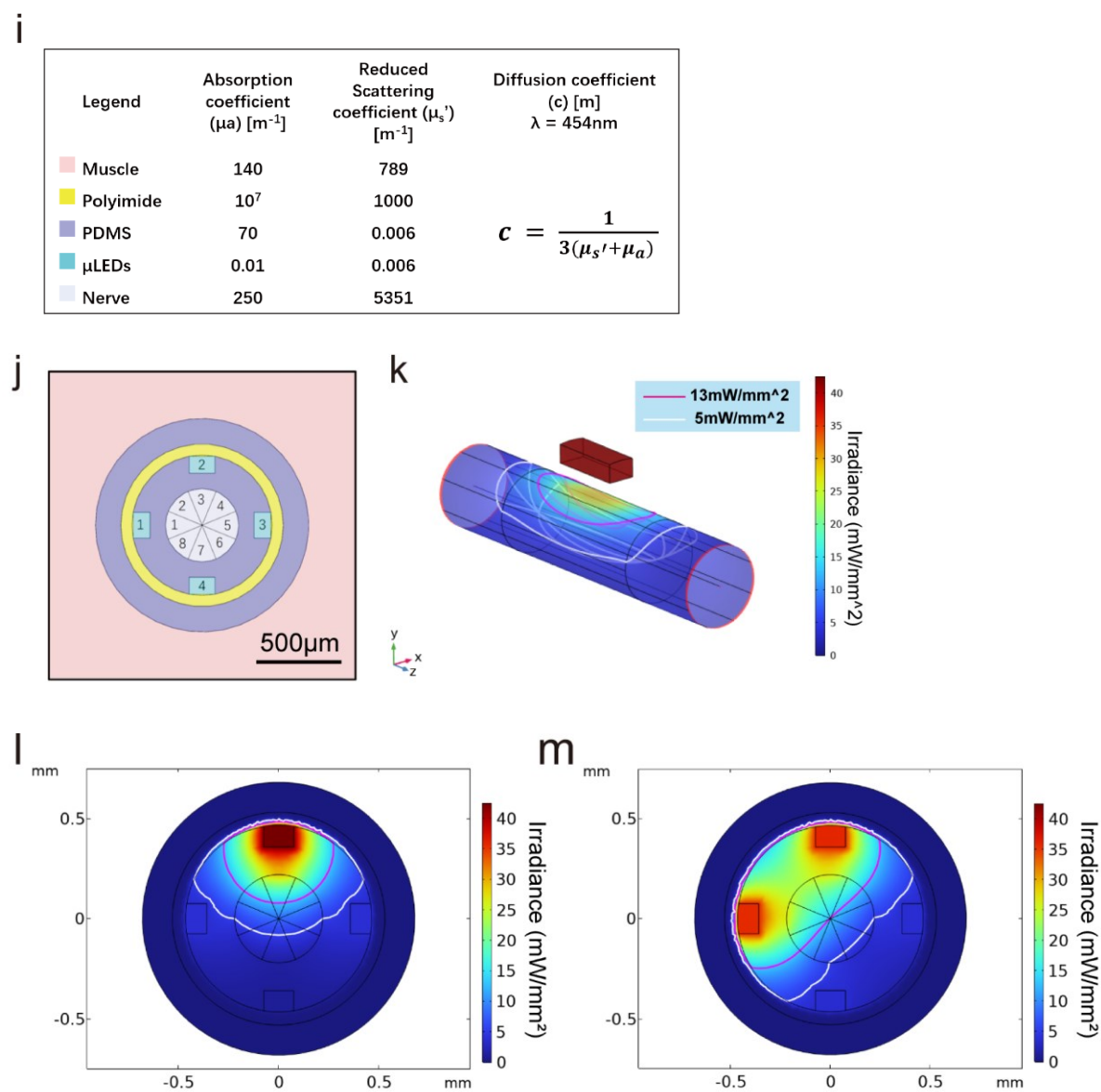
Author's reply:

Thank you for the constructive comment. We agree with the reviewer that we need to provide experimental evidences for the statement of high spatial resolution.

We first define the spatial resolution by the minimal distance with which μ LED stimulation can elicit differential responses. When one μ LED is illuminated at 2.5mW optical power, the target fascicles are expected to elicit 100% maximum force within 250 μ m distance from the surface of the μ LED, with irradiance greater than the 13mW/mm² threshold for optogenetic activation (**Figure.3l in revised manuscript. Response Figure.2**)²⁰. Beyond 250 μ m, the irradiance further decreases from 13mW/mm², where the elicited force is expected to decrease as well (**Response**

Figure.2)²⁰. This demonstrated that differential responses in muscle activity starts to occur when fascicles are more than 250μm from the μLED.

To further quantify the spatial resolution, we showed that nerve sector 3 that directly faced the illuminated μLED received an average irradiance of 21.9mW/mm², where sector 7 only received an average irradiance of 2.5mW/mm² **(Figure.3l,n in revised manuscript)**. Despite the two sector are only ~300μm (centroid-to-centroid distance) apart, a fascicle in sector 7 would elicit 90% less force than a fascicle in sector 3 **(Response Figure.2)**. Therefore, we estimate the spatial resolution of FIMOSS’s optogenetic stimulation to be around 300μm.



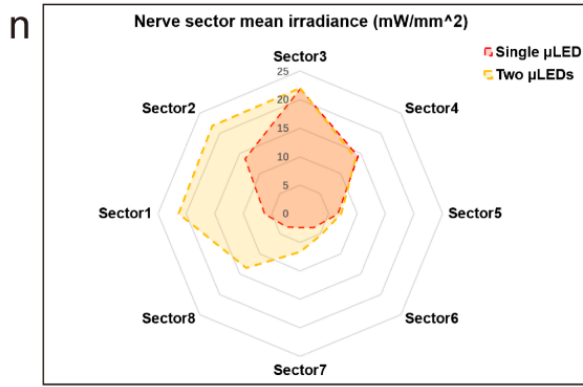
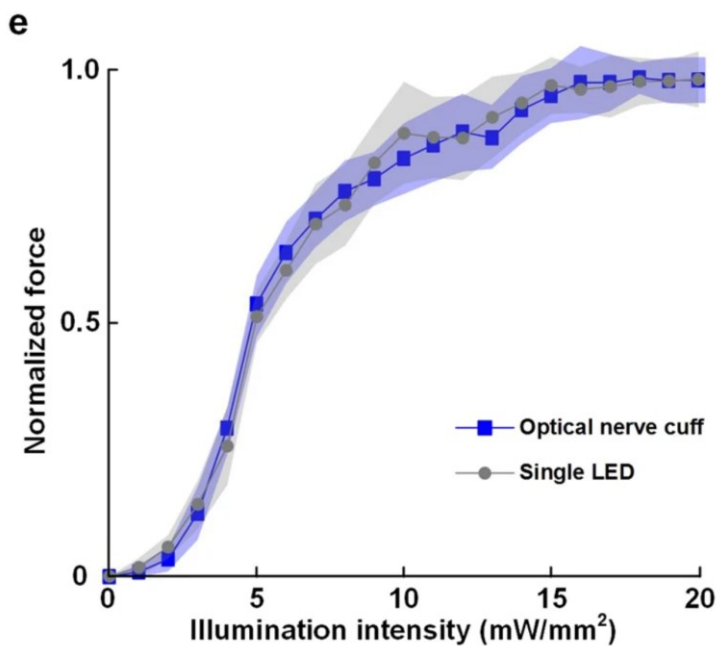


Figure.3 in revised manuscript. Characterization of FIMOSS implant. i The absorption coefficients and reduced scattering coefficients used for the optical simulation. **j** Cross-sectional view of the 3D model of a MOSD II cuffing on a typical target nerve (440 μm diameter). This 3D model was used in the optical simulation, and each domain was color-coded corresponding to the color legend in Figure.3i. The four μLEDs were numbered, and the nerve was equally divided to eight sectors and numbered as references for the quantitative analysis result in Figure.3n. **k** Heat map of the light irradiance distribution in 3D optical simulation, when one μLED was illuminated at 2.5mW of optical power. The color legend is the same as in Figure.3i,m. Domains except for the activated μLED and the target were hidden. The plot was set at 40% transparency to allow visualization of the contour lines of 13 mW/mm^2 and 5 mW/mm^2 depicted in magenta and white. **l,m** Heat map of light irradiance in a representative 2D slice out of the 3D optical simulation result. The 2D slice was defined as the XY-plane slicing down the middle (Z-direction) of the μLED in Figure.3k. One μLED (l) was illuminated at the standard optical power of 2.5mW. Two neighboring μLEDs (m) were illuminated at a reduced optical power of 2.0mW. The color bar represents light irradiance in mW/mm^2 . **n** The average irradiance in each sector of the nerve computed by optical simulation of one- μLED (orange) and two- μLED (yellow) illumination in Figure.3l,m



Response Figure.2 Muscle force measurements during the optogenetic stimulation using the optical nerve cuff electrode and using a single LED. From Kang-II Song et. al. 2018, Figure. 2e.

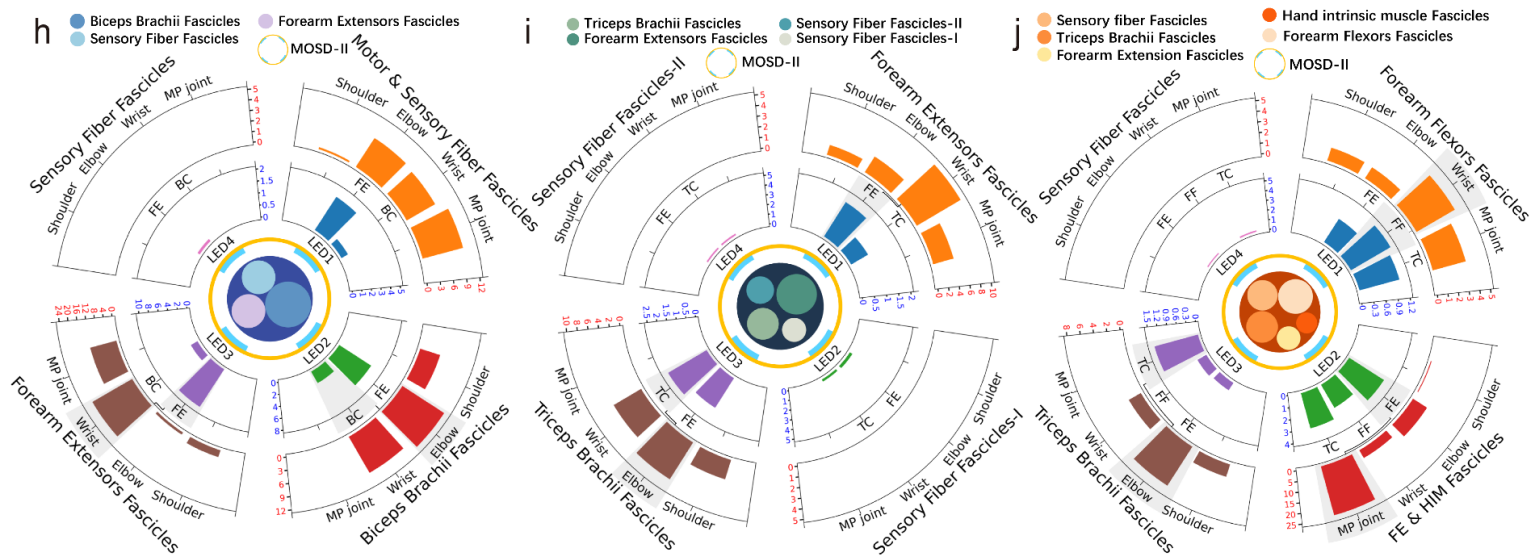


Figure.4 in revised manuscript. The application of FIMOSS on brachial plexus to induce distinct movements of forelimb in mice. h~j Circular statistical graph of representative CMAP area/mV*ms (blue axis) from target muscles and angle changes/° (red axis) of distinct forelimb movements induced by different μ LEDs (20ms pulse width, 2000ms inter-pulse interval, default wireless condition) during multisite optogenetic stimulation of upper trunk (h), middle trunk (i) and lower trunk (j). BC, biceps brachii. TC, triceps brachii. FE, forearm extensors. FF, forearm flexors. HIM, hand intrinsic muscles. MP joint, metacarpophalangeal joint.

Reference:

20. Song, K.-I. *et al.* Compact Optical Nerve Cuff Electrode for Simultaneous Neural Activity Monitoring and Optogenetic Stimulation of Peripheral Nerves. *Sci Rep* **8**, 15630 (2018).

Results in revised manuscript, line 403-421

A set of 3D finite element analysis (FEA) studies were performed to simulate the propagation of light irradiance from the μ LED (2.5mW optical power, 42mW/mm² irradiance at μ LED's surface) in the implanted environment (Figure.3i,j,k). A 2D representative slice out of the 3D result was used to visualize the irradiance distribution in the cross-section of the nerve fiber, where contour lines of 13mW/mm² and 5mW/mm² irradiance were illustrated (Figure.3l,m). These two irradiance thresholds were chosen because the transgenic mice line Thy1-ChR2-EYFP has been previously characterized that the muscle force elicited by optogenetic stimulation of the peripheral nerve reaches 50% of the maximum force when light irradiance is 5mW/mm², and reaches the maximum force at 13mW/mm² or higher³⁰.

When one μ LED (μ LED2 in this simulation) was activated at the standard optical power of 2.5mW, sector 3 that directly faced it received an average irradiance of 21.9mW/mm², where sector 7 that was further away from it only received an average irradiance of 2.5mW/mm² (Figure.3l,n). Despite the two sectors are only ~300 μ m (centroid-to-centroid distance) apart, a fascicle in sector 7 would elicit 90% less force than a fascicle in sector 3 due to the irradiance differences. Therefore, we estimate the spatial resolution of FIMOSS's optogenetic stimulation to be around 300 μ m. This demonstrated irradiance gradient was expected to allow for selective activation of a sub-section of the target nerve. By illuminating different μ LEDs, fascicles across different sections of the nerve can be targeted, thus triggering distinct downstream muscle movements.

Results in revised manuscript, line 431-438

Performing nerve stimulation using only one μ LED at a time can create blind spot at the corner sectors (sector 2,4,6,8 in Figure.3j), because there were no μ LEDs directly facing them. Therefore, further FEA study was done when two neighboring μ LEDs were simultaneously illuminated in a reduced optical power of 2.0mW each to explore resolution of the blind spot issue. The FEA result showed that in this manner, the corner sectors can receive the same level of mean irradiance as the sectors which have μ LEDs facing them (Figure.3m,n). Hence, this modality of illumination can ensure that all sectors of the nerve are accessible by optogenetic activation.

Results in revised manuscript, line 555-584

When MOSD II was implanted onto upper trunk, we observed that elbow flexion (Figure.5m) and wrist extension (Figure.5n) were respectively induced by different angles, which was mainly attributed to the activation of biceps brachii innervated by musculocutaneous nerve and brachioradialis as well as extensor carpi muscles innervated by partial branches of radial nerve derived from the upper trunk. Figure.5h showed the relationships between the induced forelimb movements, the CMAP area and the position of the μ LEDs. In detailed, LED2 elicited typical movements of elbow flexion with a significant larger CMAP area recorded from biceps brachii compared with LED1,3 and 4. While LED3 elicited wrist extension with a largest CMAP area recorded from forearm extensors. LED1 elicited elbow flexion with a relatively small angle and CMAP area recorded from biceps brachii. When LED4 were turned on, no typical movements were observed, and relatively small CMAP area were recorded from all the 4 muscle groups.

As for the middle trunk (C7 nerve), multisite optogenetic stimulation mainly induced the contraction of triceps brachii (Figure.5o) or forearm extensor muscles (Figure.5p). As illustrated in Figure.5i, LED1 exhibited a tendency to induce the extension of wrist and MP joints with the largest CMAP area recorded from forearm extensors. LED3 typically induced elbow extension, with a significant CMAP area from triceps brachii was recorded compared with those from forearm extensors, flexors and biceps brachii. Weak CMAP area were recorded from different muscle groups when LED2 and LED4 were turned on respectively. We assumed that an extremely small quantity of motor axons within middle trunk were directly against by these two LEDs resulting in no movements observed with limited CMAP area recorded.

Optogenetic stimulation of lower trunk could commonly induce elbow extension, flexion or extension of wrist and finger abduction (Figure.5q,r,s) respectively. However, under the same optical power of optogenetic stimulation, lower trunk stimulation exhibited a tendency to induce more obvious wrist and finger movement compared with middle trunk. Figure.5j showed the detailed results of electrophysiological and induced-behavioral tests. LED1 induced wrist and MP joint flexion with a larger CMAP area recorded from forearm flexors. LED2 induced typical movements of fingers abduction with MP joint extension. The lighting of LED3 selectively elicited elbow extension with an obvious CMAP amplitude recorded from triceps brachii. LED4 induced no obvious forelimb movements and CMAP area recorded.

11. Related to point 9 and 7 above: the measured intensity of illumination at the nerve is not clearly provided. Can the authors provide an estimated measure of intensity (e.g. power/unit area) at the nerve surface? How does this compare to typical Chr2 activation intensities?

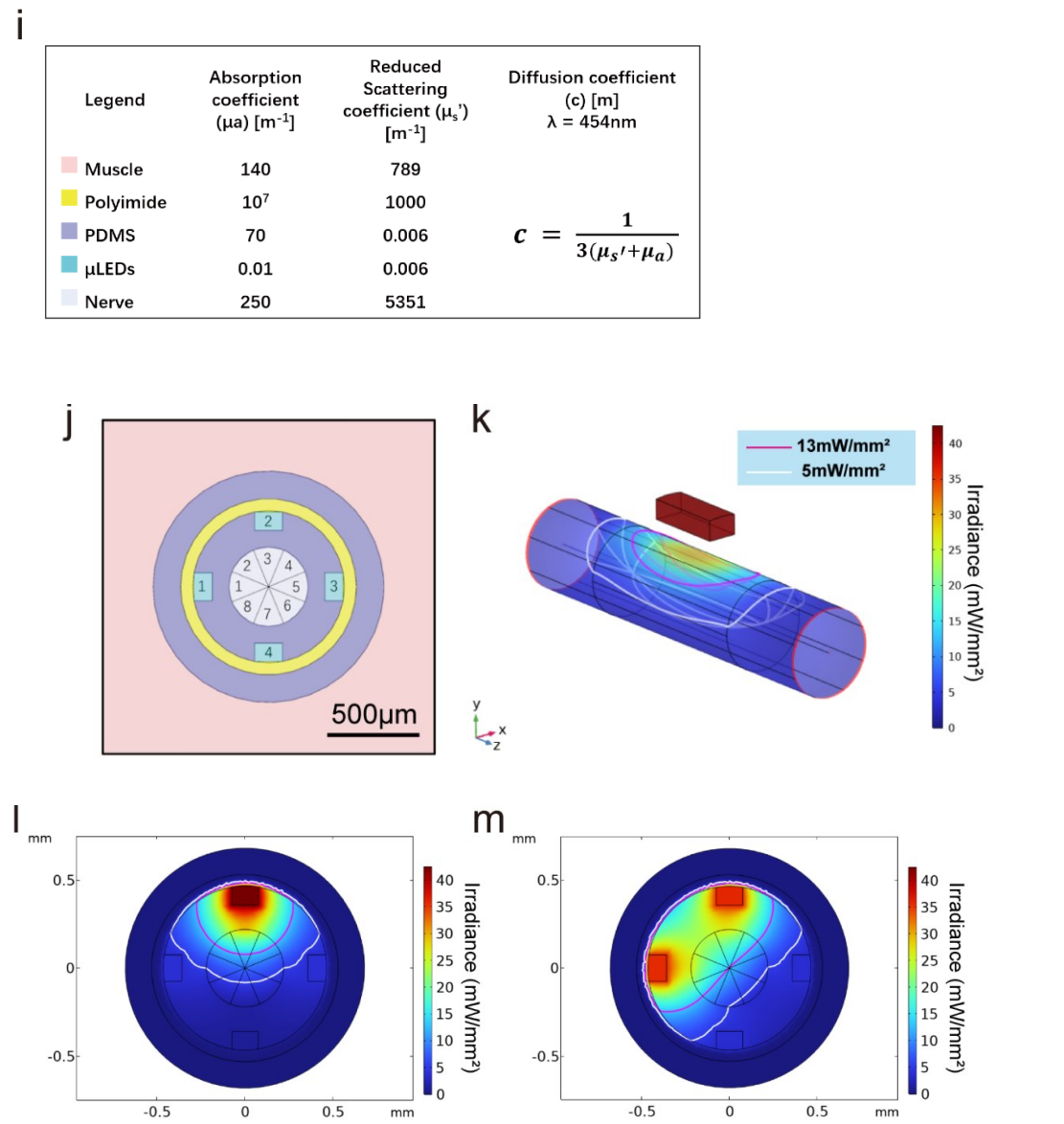
Author's reply:

Thank you for the constructive comment.

Our new optical simulation used the measured optical power emitted from the μ LED (2.5mW, $\sim 42\text{mW/mm}^2$ irradiance at the μ LED's surface) as source and estimated the expected irradiance (mW/mm^2) across the nerve (**Figure.3k.l.m in revised manuscript**). In single- μ LED-illumination, the most and the least illuminated nerve sectors were estimated to receive an average irradiance of 21.9mW/mm^2 (sector 3)

and 2.5mW/mm² (sector 7), respectively ([Figure.3n in revised manuscript](#)). The average irradiance of a sector was computed by evaluating the surface average of irradiance values within the sector.

Previous studies showed that the spike probability of ChR2-expressing neurons ramps up exponentially from 0 to 1 when the received blue irradiance increased from 1mW/mm² to 10mW/mm² (**Response Figure.1**)². In our study, the least illuminated nerve sector with 2.5mW/mm² (sector 7) of irradiance was expected to have significantly lower activation probability than the most illuminated sector with more than 10mW/mm² of irradiance.



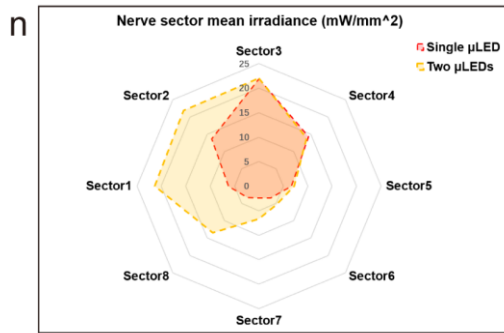
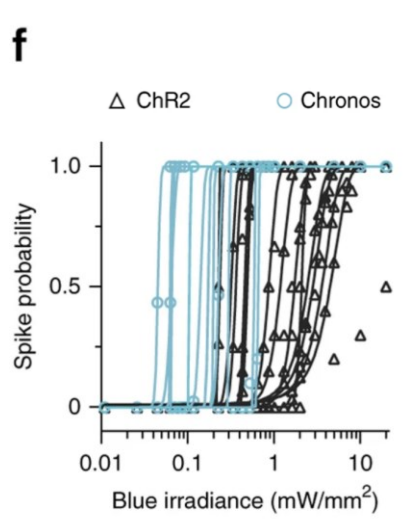


Figure.3 in revised manuscript. Characterization of FIMOSS implant. **i** The absorption coefficients and reduced scattering coefficients used for the optical simulation. **j** Cross-sectional view of the 3D model of a MOSD II cuffing on a typical target nerve (440μm diameter, scale bar=500 μ m). This 3D model was used in the optical simulation, and each domain was color-coded corresponding to the color legend in Figure.3i. The four μLEDs were numbered, and the nerve was equally divided to eight sectors and numbered as references for the quantitative analysis result in Figure.3n. **k** Heat map of the light irradiance distribution in 3D optical simulation, when one μLED was illuminated at 2.5mW of optical power. The color legend is the same as in Figure.3i,m. Domains except for the activated μLED and the target were hidden. The plot was set at 40% transparency to allow visualization of the contour lines of 13mW/mm² and 5mW/mm² depicted in magenta and white. **l,m** Heat map of light irradiance in a representative 2D slice out of the 3D optical simulation result. The 2D slice was defined as the XY-plane slicing down the middle (Z-direction) of the μLED in Figure.3k. One μLED (**l**) was illuminated at the standard optical power of 2.5mW. Two neighboring μLEDs (**m**) were illuminated at a reduced optical power of 2.0mW. The color bar represents light irradiance in mW/mm². **n** The average irradiance in each sector of the nerve computed by optical simulation of one-μLED (orange) and two-μLED (yellow) illumination in Figure.3l,m.



Response Figure.1 Spike probability vs. blue light irradiance, plotted for individual Chronos- or ChR2-expressing neurons. From Nathan C. Klapoetke et. al. 2014, Figure.4f.

Reference:

2. Klapoetke, N. C. *et al.* Independent optical excitation of distinct neural populations. *Nat Methods* **11**, 338–346 (2014).

Results in revised manuscript, line 403-411

A set of 3D finite element analysis (FEA) studies were performed to simulate the propagation of light irradiance from the μ LED (2.5mW optical power, 42mW/mm² irradiance at μ LED's surface) in the implanted environment (Figure.3i,j,k). A 2D representative slice out of the 3D result was used to visualize the irradiance distribution in the cross-section of the nerve fiber, where contour lines of 13mW/mm² and 5mW/mm² irradiance were illustrated (Figure.3l,m). These two irradiance thresholds were chosen because the transgenic mice line Thy1-ChR2-EYFP has been previously characterized that the muscle force elicited by optogenetic stimulation of the peripheral nerve reaches 50% of the maximum force when light irradiance is 5mW/mm², and reaches the maximum force at 13mW/mm² or higher³⁰.

Results in revised manuscript, line 412-421

When one μ LED (μ LED2 in this simulation) was activated at the standard optical power of 2.5mW, sector 3 that directly faced it received an average irradiance of 21.9mW/mm², where sector 7 that was further away from it only received an average irradiance of 2.5mW/mm² (Figure.3l,n). This demonstrated irradiance gradient was expected to allow for selective activation of a sub-section of the target nerve. By illuminating different μ LEDs, fascicles across different sections of the nerve can be targeted, thus triggering distinct downstream muscle movements.

Results in revised manuscript, line 431-438

Performing nerve stimulation using only one μ LED at a time can create blind spot at the corner sectors (sector 2,4,6,8 in Figure.3j), because there were no μ LEDs directly facing them. Therefore, further FEA study was done when two neighboring μ LEDs were simultaneously illuminated in a reduced optical power of 2.0mW each to explore resolution of the blind spot issue. The FEA result showed that in this manner, the corner sectors can receive the same level of mean irradiance as the sectors which have μ LEDs facing them (Figure.3m,n). Hence, this modality of illumination can ensure that all sectors of the nerve are accessible by optogenetic activation.

12. Figure 4h, i, j: what is approximately the expected extent of movement upon stimulation? For example, when compared to electrical stimulation. Please also describe Figure more clearly in caption – what are the units for the blue and red numbered axes?

Author's reply:

We sincerely appreciate your valuable suggestions.

The angle change extents of distinct movements are listed in the **Response Table.2** and illustrated in **Supplementary Figure.11g,h,i in revised manuscript.**

	Elbow/°	Wrist/°	MP joint/°
Upper Trunk	-Elbow Flexion 25.19 ± 9.83	-Wrist Extension 16.87 ± 4.63	—
Middle Trunk	-Elbow Extension 20.76 ± 5.85	-Wrist Extension 21.69 ± 4.04	-Finger Extension 11.32 ± 5.15
Lower Trunk	-Elbow Extension 12.17 ± 3.08	-Wrist Flexion 21.14 ± 3.26	-Finger Flexion 18.89 ± 6.15

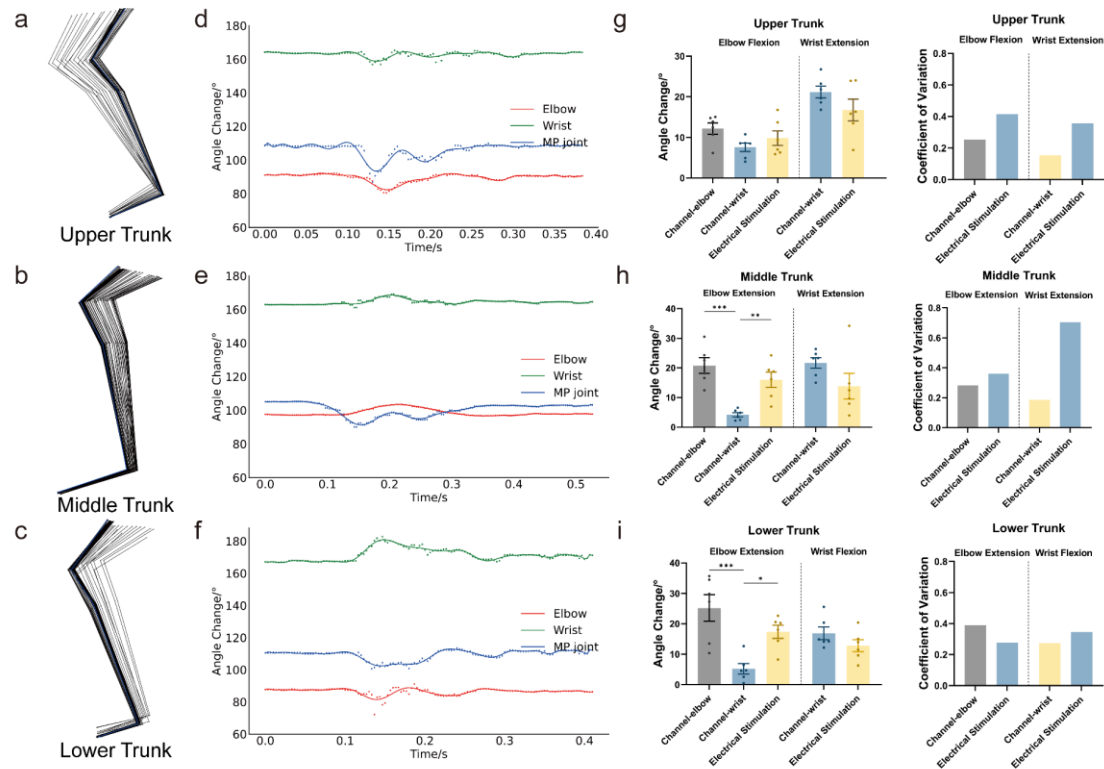
Response Table.2 Angle changes of distinct forelimb movements when stimulating different brachial plexus nerve trunks. (mean $\pm$ SD, n=6)

Experiments were conducted to compare electrical stimulation-induced movements with multisite optogenetic stimulation-induced movements. Electrical stimulation effectively induced a similar level of angle change compared to optical stimulation in both upper, middle and lower trunk (**Supplementary Figure 11g, h, i in revised manuscript**). However, electrical stimulation could not selectively activate smaller joints such as the wrist or hand. The angle change calculation of the wrist and metacarpophalangeal joints (MP joints) were often affected by elbow joint movement, as is shown in **Supplementary Figure.11d,e,f in revised manuscript**. Such wrist and metacarpophalangeal joint movements driven by elbow motion cannot serve as reliable evaluation indicators for assessing wrist and hand motor function in mice. In contrast, multisite optical stimulation could selectively induce wrist and metacarpophalangeal joint movements while causing minimal elbow movement (**Supplementary Figure 11g, h, i in revised manuscript**). Under these conditions, we can confirm that the observed joint movements were indeed caused by corresponding muscle contractions rather than being secondary to elbow joint motion.

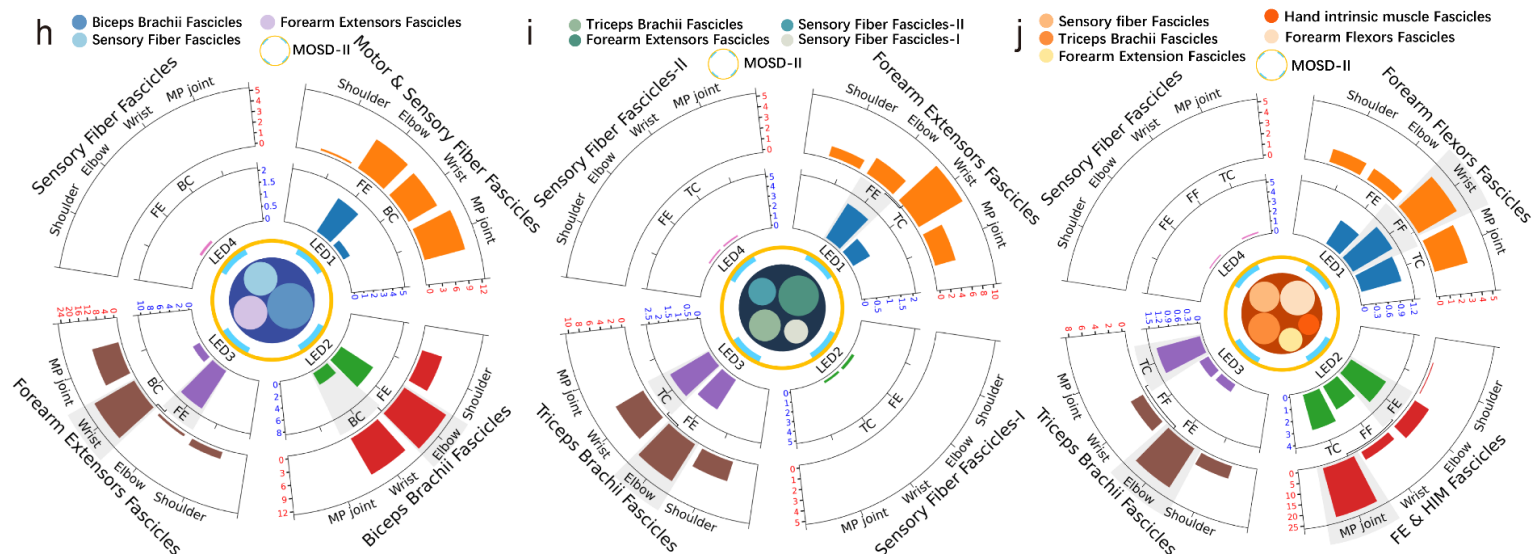
In conclusion, multisite optogenetic stimulation was able to achieve the correspondence between different nerve bundles and induced forelimb movements of

various muscle groups more precisely, which is not possible with electrical stimulation.

And we have made corresponding change to the captions of [Figure.5h.i.j](#) in revised [manuscript](#). Thank you for your suggestion.



Supplementary Figure 11. Comparison between multisite optogenetic stimulation/electrical stimulation-induced forelimb movements of different nerve trunks in brachial plexus. a~c Representative trajectories induced by electrical stimulation on upper (a), middle (b) and lower trunk (c). **d~f** The angle-time curves of elbow, wrist and MP joint movements induced by electrical stimulation of the upper



(d), middle (e) and lower trunk (f). **g~i** Comparison between angle changes (left) and coefficient of variation (right) of multisite optogenetic stimulation (n=6)/electrical stimulation-induced (n=6) forelimb movements at different brachial plexus nerve trunks. Channel elbow/wrist represent the μ LED which can induce elbow/wrist extension or flexion with the largest angle changes among the 4 μ LEDs of MOSD II.

Figure.5 in revised manuscript. The application of FIMOSS on brachial plexus to induce distinct movements of forelimb in mice. h~j Circular statistical graph of representative CMAP area/mV*ms (blue axis) from target muscles and angle changes/ $^{\circ}$ (red axis) of distinct forelimb movements induced by different μ LEDs (20ms pulse width, 2000ms inter-pulse interval, default wireless condition) during multisite optogenetic stimulation of upper trunk (h), middle trunk (i) and lower trunk (j). BC, biceps brachii. TC, triceps brachii. FE, forearm extensors. FF, forearm flexors. HIM, hand intrinsic muscles. MP joint, metacarpophalangeal joint.

Results in revised manuscript, line 567-599

As for the middle trunk (C7 nerve), multisite optogenetic stimulation mainly induced the contraction of triceps brachii (Figure.5o) or forearm extensor muscles (Figure.5p). As illustrated in Figure.5i, LED1 exhibited a tendency to induce the extension of wrist and MP joints with the largest CMAP area recorded from forearm extensors. LED3 typically induced elbow extension, with a significant CMAP area from triceps brachii was recorded compared with those from forearm extensors, flexors and biceps brachii. Weak CMAP area were recorded from different muscle groups when LED2 and LED4 were turned on respectively. We assumed that an extremely small quantity of motor axons within middle trunk were directly against by these two LEDs resulting in no movements observed with limited CMAP area recorded.

Optogenetic stimulation of lower trunk could commonly induce elbow extension, flexion or extension of wrist and finger abduction (Figure.5q,r,s) respectively. However, under the same optical power of optogenetic stimulation, lower trunk stimulation exhibited a tendency to induce more obvious wrist and finger movement compared with middle trunk. Figure.5j showed the detailed results of electrophysiological and induced-behavioral tests. LED1 induced wrist and MP joint flexion with a larger CMAP area recorded from forearm flexors. LED2 induced typical movements of fingers abduction with MP joint extension. The lighting of LED3 selectively elicited elbow extension with an obvious CMAP amplitude recorded from triceps brachii. LED4 induced no obvious forelimb movements and CMAP area recorded.

Furthermore, to clarify the characterization of optogenetic stimulation by FIMOSS, we systematically compared the induced movements between multisite

optogenetic (20ms pulse width, default wireless condition, n=6) and electrical stimulation (single-site, 0.2ms pulse width, 0.5mA current intensity, n=6). As a result, both electrical stimulation and multisite optogenetic stimulation at brachial plexus could effectively induce obvious elbow joint movement (Supplementary Figure.11a,b,c). However, the relatively low spatial resolution of electrical stimulation limited its function in selective activation of distal forelimb joints (Supplementary Figure.11g,h,i). The angle changes of wrist and metacarpophalangeal joints were often obscured by concomitant elbow movement, manifesting as low-amplitude oscillations in joint angle measurements in these joints (Supplementary Figure.11d,e,f). These elbow motion-associated wrist/hand movements cannot serve as valid metrics for evaluating wrist and hand motor movements. In contrast, multisite optical stimulation induced selective activation of wrist and metacarpophalangeal joints with minimal elbow involvement, which indicated that the observed joint movements resulted from direct muscle contractions rather than being kinematic artifacts of proximal joint movement.

13. Figure 5d-f: the legend text is not legible. In the text, a “control group” is referenced; what is the control here? No TBI, or no C7 transfer? What is “sham”?

Author’s reply:

Thank you very much for your suggestions. We apologize for the confusion in the description of experimental groups.

In this study, control group in Figure.6 and Figure.7 refers to the mice which only experienced nerve exposure (sham surgery), no TBI and CC7 transfer surgery were performed.

Only the CMAP curves of TBI group and TBI+CC7 group are presented in **Figure. 6d,e,f in revised manuscript** to demonstrate the improvement effect of CC7 surgery on EMG indicators in TBI model mice.

CMAP area values of control group are illustrated in the heatmap in **Figure. 6g in revised manuscript**.

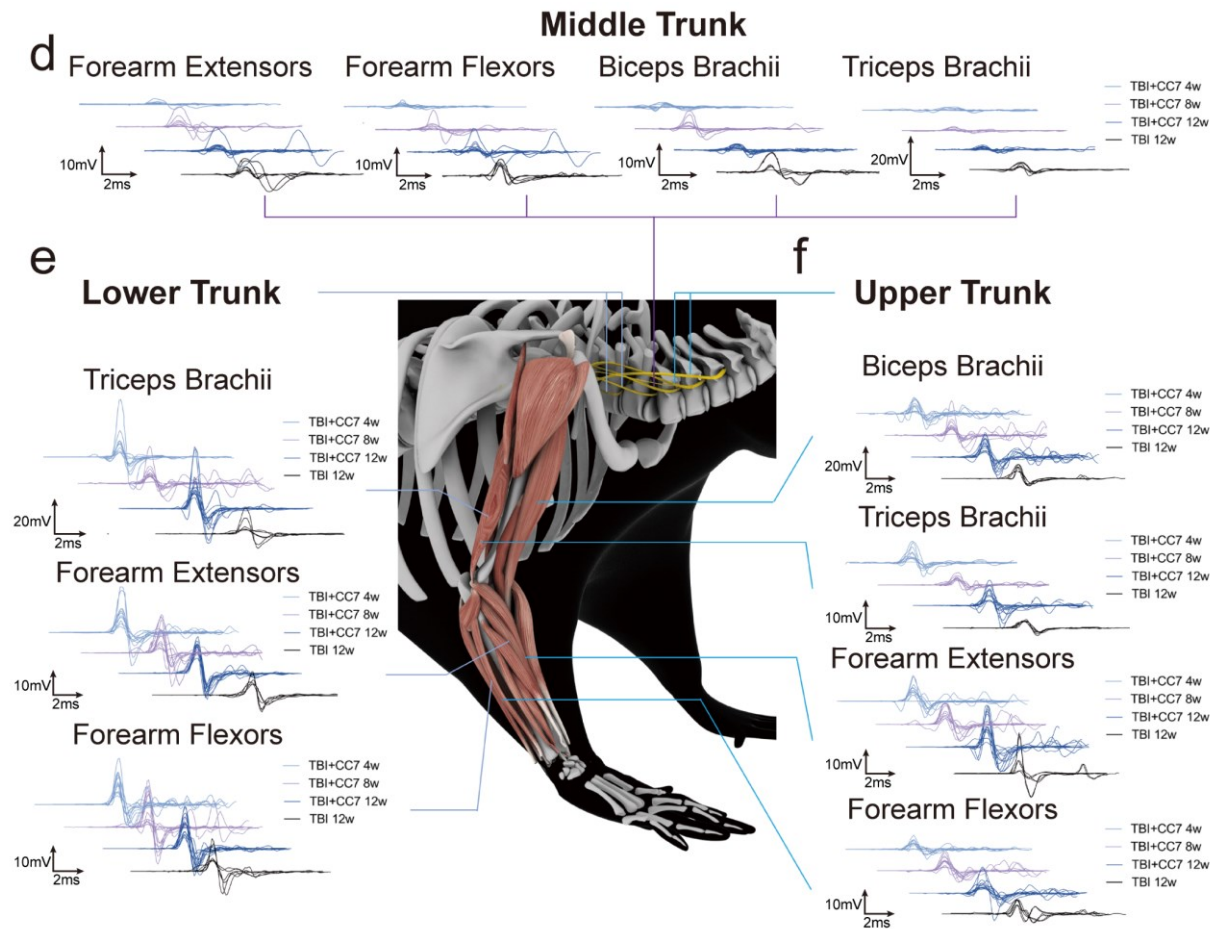


Figure.6 in revised manuscript. Electrophysiology change pattern of brachial plexus after CC7 transfer evaluated by multisite optogenetic stimulation method. d,e,f CMAP curve of forearm extensors, forearm flexors, biceps brachii and triceps brachii induced by spatially selective optogenetic stimulation of regenerating middle trunks (d), lower trunks (e) and upper trunks (f). CMAP curves from each mouse in TBI+CC7 groups (mice undergoing TBI and CC7 surgery) at 4,8 and 12weeks after surgery and TBI groups at 12weeks after surgery were presented.

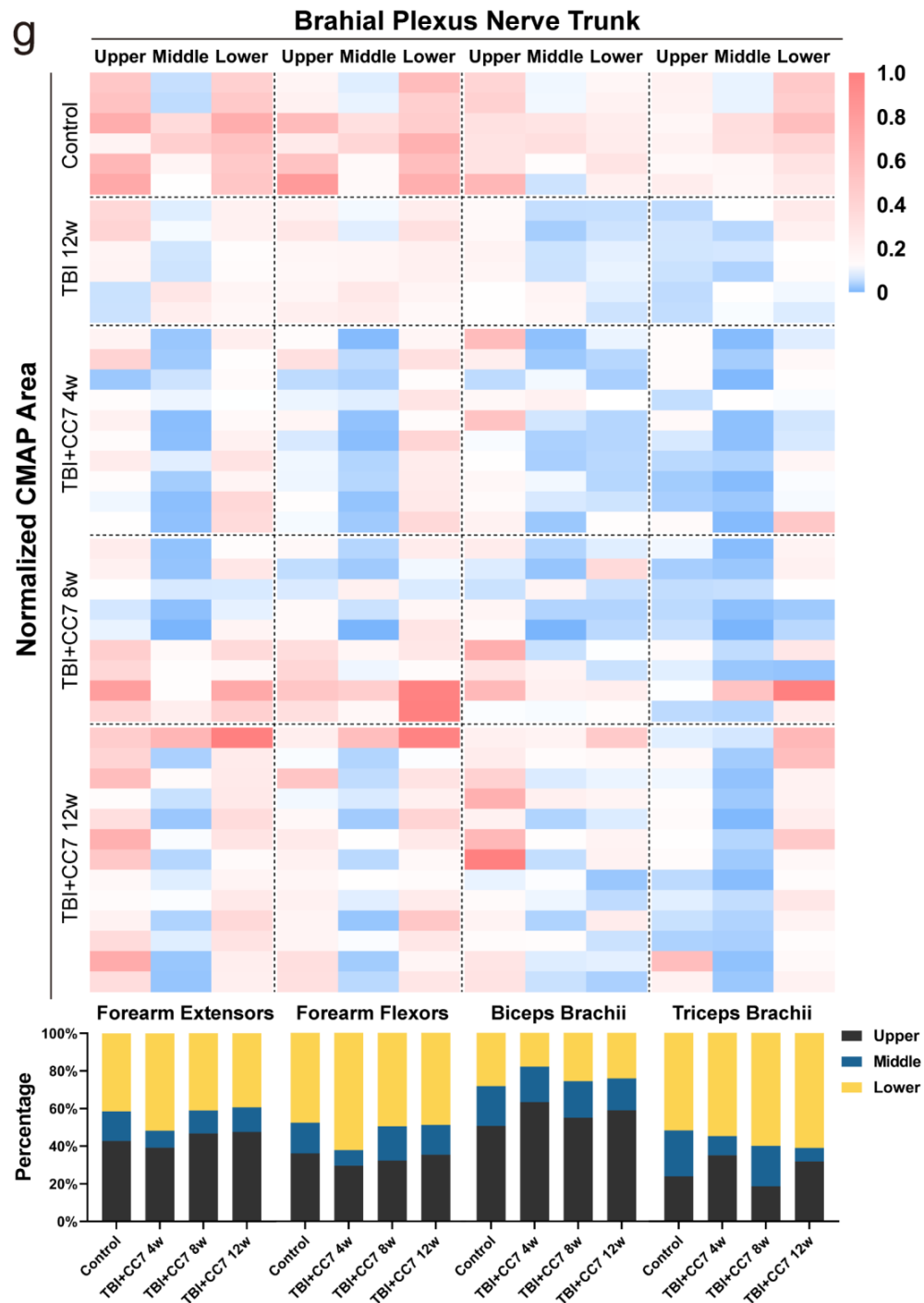


Figure.6 in revised manuscript. Electrophysiology change pattern of brachial plexus after CC7 transfer evaluated by multisite optogenetic stimulation method.
g The heatmap of CMAP area from different mice groups and weighted analysis of brachial plexus innervation to 4 muscle groups on the affected limb.

Methods in revised manuscript, line 1189-1191

Mice from all groups in Figure.5 and Figure.6 were implanted a FIMOSS implant to conduct electrophysiological and induced-behavioral evaluation. However, control group in Figure.4 didn't have an FIMOSS implant.

14. Figure 5g heatmap can benefit from a different color scale, and perhaps normalization to control, to more clearly visualize effect of TBI and CC7. Please also clarify what is meant by “weighted analysis” and the Percentage scale.

Author’s reply:

Thank you for your valuable suggestions.

We have normalized the data using the maximum and minimum values of target muscle groups using the following formula:

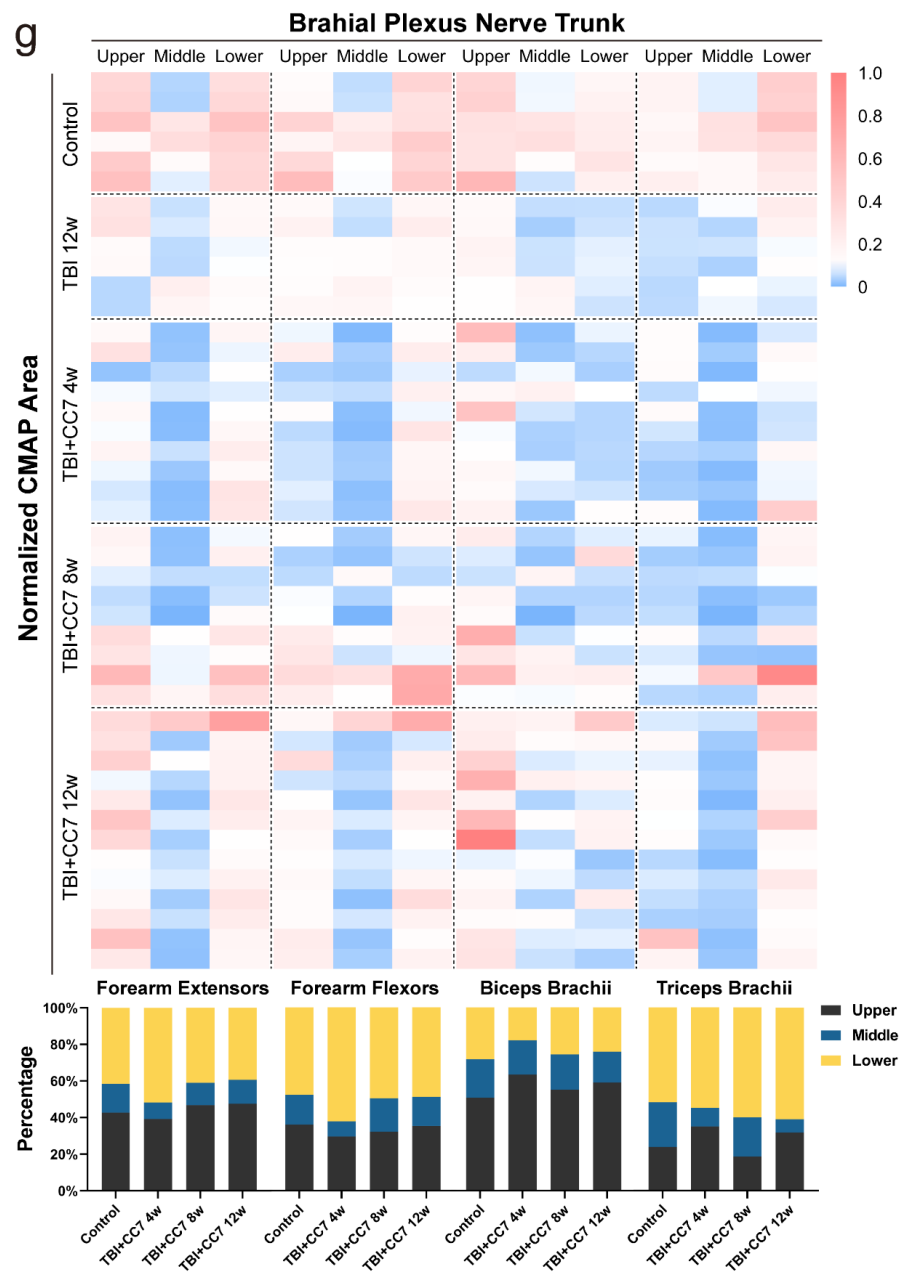
$$\text{Normalized CMAP Area} = \frac{\text{Raw Area} - \text{Min (Muscle)}}{\text{Max (Muscle)} - \text{Min (Muscle)}} \quad (\text{Equation 5})$$

We also changed the color scale of the heatmap to further accentuate the differences between distinct data **(Figure.6g in revised manuscript)**.

In the weighted analysis, we calculated the innervation percentage of target nerve trunk to target muscle groups from different groups using the following formula:

The definition of percentage scale is as follows:

$$Percentage_{nerve} = \frac{\text{Mean}(Nerve)}{\text{Mean (Upper)}+\text{Mean (Middle)}+\text{Mean(Lower)}} \times 100\% \text{ (Equation 6)}$$



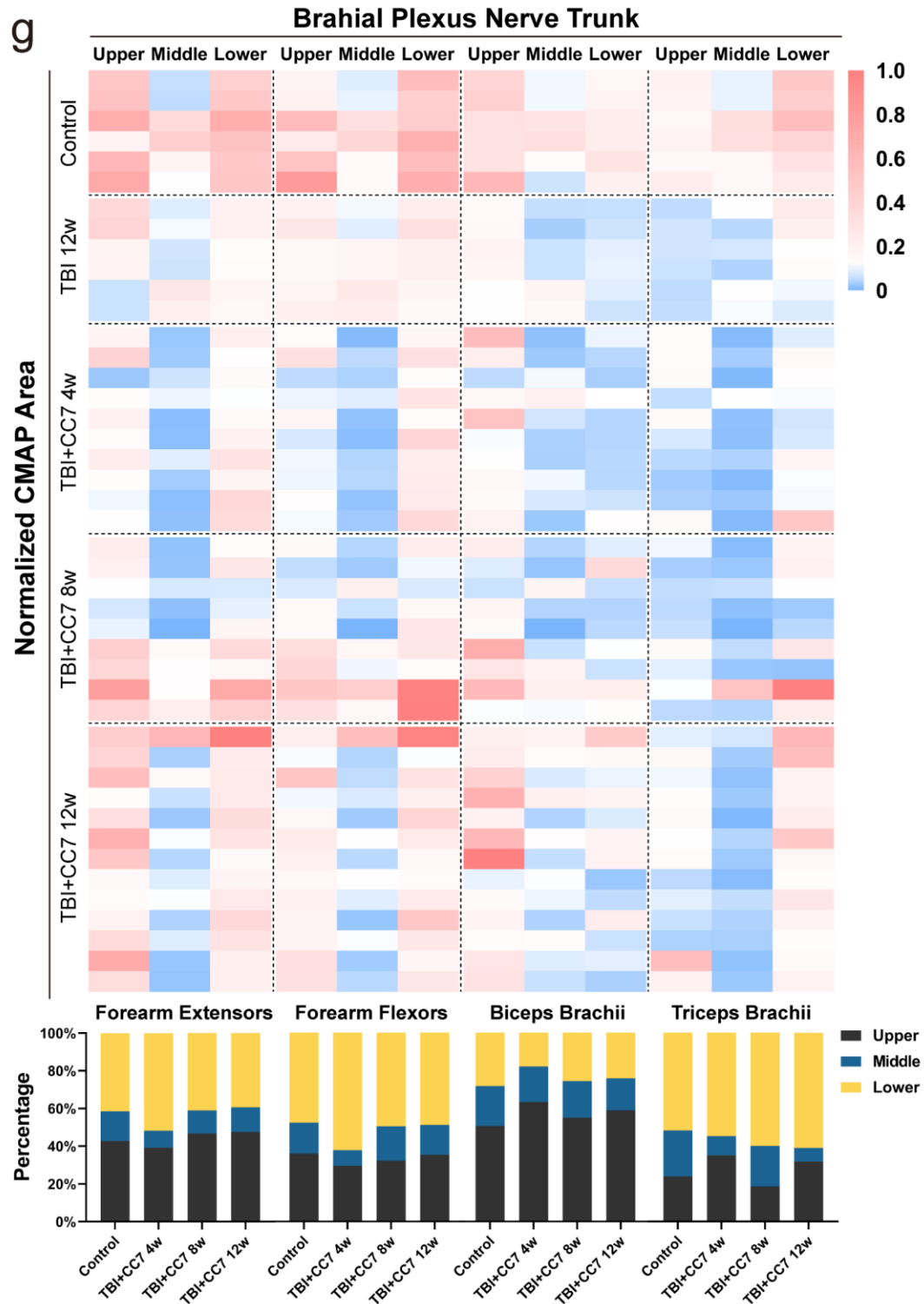


Figure.6 in revised manuscript. Electrophysiology change pattern of brachial plexus after CC7 transfer evaluated by multisite optogenetic stimulation method.
g The heatmap of CMAP area from different mice groups and weighted analysis of brachial plexus innervation to 4 muscle groups on the affected limb.

Results in revised manuscript, line 692-696

The heatmap showed the comparison between CMAP area magnitude of different brachial plexus trunks recorded from 4 muscle groups in forelimb. (Figure.6g)

top) And weighted analysis of electrophysiological results of brachial plexus nerve trunks to different forelimb muscle groups showed the functional compensation of upper and lower trunk to 4 muscle groups in forelimb in the time course of 12 weeks. (Figure.6g bottom)

Methods in revised manuscript, line 1220-1226

Then in the heatmap Figure.5g top, we normalized the CMAP areas using the maximum and minimum values of target muscle groups using the following formula:

$$\text{Normalized CMAP Area} = \frac{\text{Raw Area} - \text{Min (Muscle)}}{\text{Max (Muscle)} - \text{Min (Muscle)}} \quad (\text{Equation 5})$$

In the weighted analysis of brachial plexus in Figure.5g bottom, we calculated the innervation percentage of target nerve trunk to target muscle groups from different mice groups using the following formula:

$$\text{Percentage}_{\text{nerve}} = \frac{\text{Mean(Nerve)}}{\text{Mean (Upper)} + \text{Mean (Middle)} + \text{Mean(Lower)}} \times 100\% \quad (\text{Equation 6})$$

15. Define OS acronym (I'm guessing optogenetic stimulation).

Author's reply:

Thank you very much for pointing this out. We apologize for the typo.

We have deleted OS in the revised manuscript.

Discussion in revised manuscript, line 870-871

However, a degradable and flexible MOSD II is still a more suitable solution for **optogenetic stimulation** of peripheral nerve in distal forelimbs and clinical translation.

Methods in revised manuscript, line 1217-1220

CMAP responses were defined as a repeatable and typical two-phase curve along with visible target muscle contraction, which was induced by **optogenetic stimulation** of FIMOSS and recorded by Medtronic KeyPoint Portable EMG device afterwards.

16. I do not follow the summary for Figure 5 in the text, as the Figure does not measure wrist/hand/elbow function:

“The electrophysiology changes of brachial plexus after CC7 transfer implied that although transferred C7 nerve participated in the innervation of various forelimb

muscle groups, it played a more important role in the reinnervation of the wrist and hand muscles on the affected side, while upper and lower trunk compensate for the motor function of transferred C7 in elbow joint and showed a tendency to compensate for wrist and hand function simultaneously”

Author’s reply:

Thank you very much for pointing this out. We apologize for the inaccurate description of the results in Figure 5.

We have changed “wrist/hand/elbow function” into the function of the corresponding muscle groups in the revised manuscript as the reviewer suggested.

Results in revised manuscript, line 698-707

The electrophysiology changes of brachial plexus after CC7 transfer implied that although transferred C7 nerve participated in the innervation of various forelimb muscle groups, it played a more important role in the reinnervation of the wrist and hand muscles on the affected side. Meanwhile, the upper trunk showed a tendency to innervate the triceps brachii with a higher proportion, which was mainly controlled by C7 nerve and lower trunk in control group. Both the upper and lower trunk were compensating for the innervation of forearm extensors and flexors which is responsible for the motor control of wrist and hand. As a consequence of the motor function reorganization in brachial plexus, TBI+CC7 group demonstrated significantly better CMAP results compared with TBI group after 12 weeks.

17. *Why does elbow extension seem much larger in Fig 6b than in Fig 4?*

Author’s reply:

Thank you very much for your observation and comment.

In brief, the difference in the elbow extension in **Figure.7b and Figure.5 in revised manuscript** is mainly due to the anatomical variation among different animals. As seen in Figure. 7j, the angle change varies from 7.77° (as seen in **Figure. 5 in the revised manuscript**) to 38.89° (as seen in **Figure. 7b in the revised manuscript**). Such large variation may be due to the fact that elbow extension is mainly induced by the contraction of triceps brachii muscle, which is innervated by the radial nerve in the brachial plexus of mice. The radial nerve contains nerve fibers from the upper,

middle, and lower trunks, among which middle and lower trunks play an important role in innervating triceps brachii muscle. However, the proportion of the triceps brachii muscle innervated by the middle and lower trunks varies among different mice.

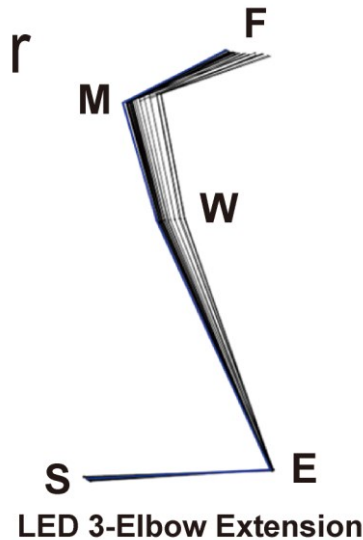


Figure.5 in revised manuscript. The application of FIMOSS on brachial plexus to induce distinct movements of forelimb in mice. r Representative kinematic trajectory of mice forelimb elicited by FIMOSS implant. S, shoulder. E, elbow. W, wrist. M, metacarpophalangeal joint. F, fingertips. Lower trunk is mainly responsible for wrist extension with finger abduction (q), elbow extension (r) and wrist flexion (s).

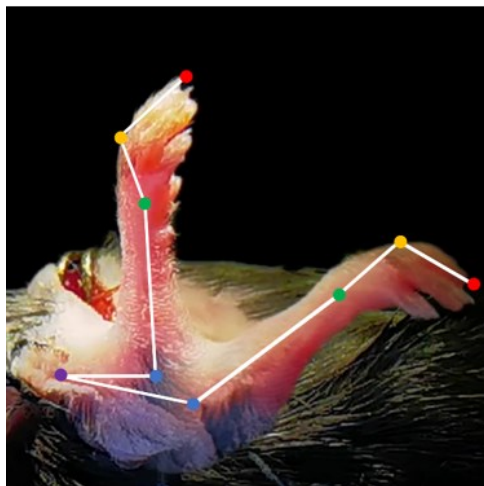


Figure.7 in revised manuscript. Induced-behavioral changes of impaired forelimb after CC7 transfer. b Representative image of induced movements including elbow extension. (20ms pulse width, default wireless condition)

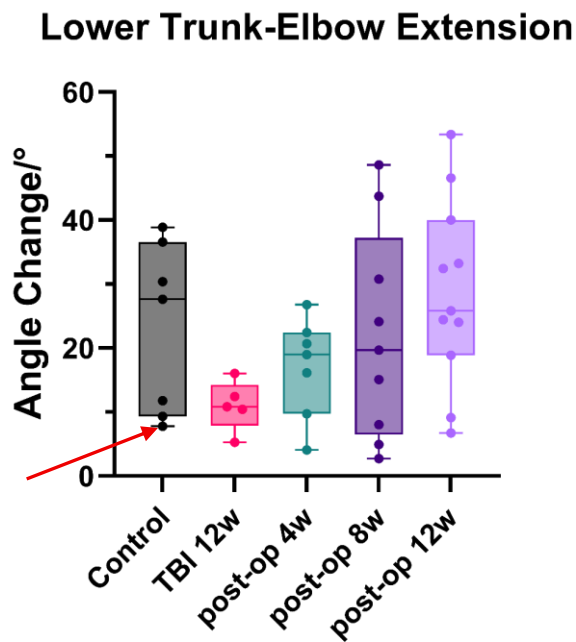


Figure.7 in revised manuscript. Induced-behavioral changes of impaired forelimb after CC7 transfer. j The maximum angle changes of induced movements when different μ LEDs illuminated lower trunk. The plot indicated by the red arrow is the data point corresponding to Figure.5r.

18. The order of the last Figure 7 seems out of place to me, perhaps it is best to combine it with earlier Figures that characterize device durability.

Author's reply:

Thank you for your valuable suggestions. We repositioned Figure.7 to Figure. 4 in the revised manuscript, as biocompatibility of the device is undeniably critical and foundational for subsequent long-term implantation experiments.

19. Fig 7c: please clarify in caption whether the shown trajectory is for animals with or without FIMOSS implantation. The trajectory both with and without should be shown. Also please state clearly in caption what is meant by "control" condition in 7d-n. Is this without implant and no surgery?

Author's reply:

Thank you for your suggestions. The Figure.7c in the original manuscript depicted the forelimb movement trajectory of a mouse with an FIMOSS implant. In the revised Figure.7, we have included the movement trajectory of a mouse without FIMOSS

implant (control group), along with detailed explanations in the caption. We have added corresponding descriptions in the revised manuscript.

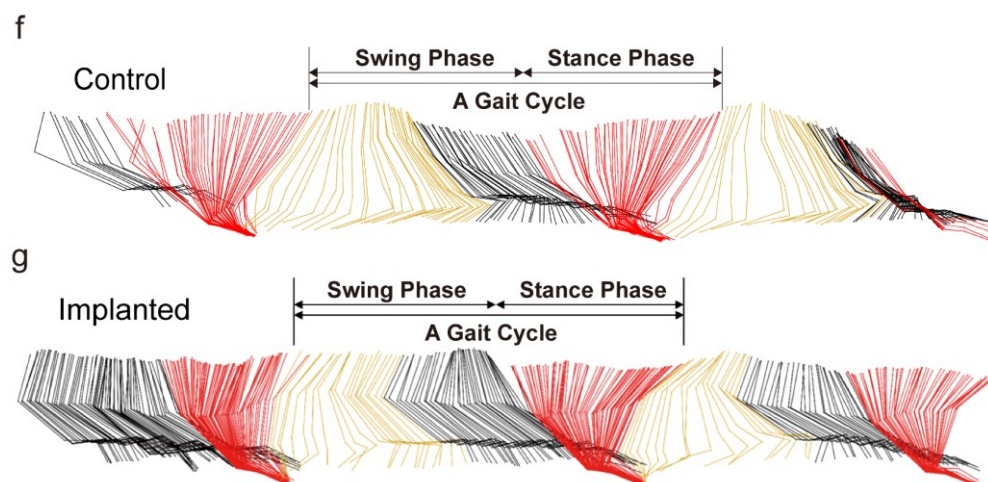


Figure.7 in revised manuscript. Biocompatibility of long-term FIMOSS implantation in brachial plexus after 12 weeks. f~g Representative trajectories of forelimb movements in mice from control group without FIMOSS implant (f) and middle trunk implantation group with an FIMOSS implant 12 weeks after implantation (g). Several gait cycles were showed in the image. Golden and black lines represented the forelimb trajectory of lifting and landing process (swing phase). Red lines represent the stance phase.

Results in revised manuscript, line 473-477

Figure.4f,g showed representative movement trajectories of the forelimb from mice without FIMOSS implant (control group) and the implanted forelimb from mice with FIMOSS implant (implanted group), respectively. As a result, no significant difference was observed in range of motion of shoulder, elbow, wrist and MP joints in forelimb between groups (Figure.4h~k).

20. The authors mention that the device can accommodate more than 4 LEDs. For which applications would this be useful – e.g. which nerves would have more than 4 fascicles that would be interesting to stimulate? How difficult would it be to modify the device (e.g. power requirements, size). Please add this to the Discussion section.

Author's reply:

Thank you for your constructive suggestions.

As the reviewer has kindly implicated, multiple nerves have more than 4 fascicles that would be suitable for FIMOSS applications with more than 4 LEDs, including radial nerve (≈ 11 motor fascicles)^{21,22}, median nerve (≈ 9 motor fascicles)²³⁻²⁵ and ulnar nerve (≈ 14 motor fascicles)^{26,27}.

It would not be too difficult to modify the device to accommodate more than 4 LEDs. In terms of power, as we designed the software so that only one LED would source power at a given instance, thus having more than 4 LEDs does not add difficulties. In terms of size, the current microchip on the FIMOSS implant can already support individual controls of up to 8 LED channels (**Figure.2d in revised manuscript, highlighting that the driver already has 8 current sources**), therefore the size of the driver can remain unchanged. Adding more LEDs on the existing branch of MOSD/II is possible but would make the cuff more rigid, thus adding difficulties to implant the cuff around the nerve. It would be less difficult to add a second branch of MOSD/II with 4 LEDs, where the two branches of MOSD/II can target two separate nerves to elicit a wider range of movements with one implant.

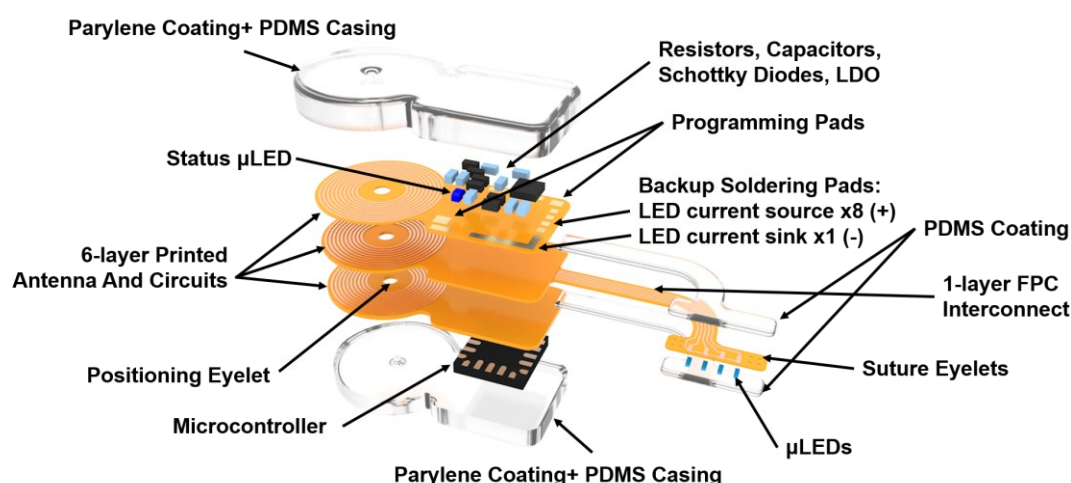


Figure.2 in revised manuscript. Design and structure of FIMOSS implant for long-term multisite optogenetic stimulation. d A layered schematic illustration of FIMOSS implant.

Detailed anatomy and functions of radial, median and ulnar nerves are as follows:

Radial nerve: The radial nerve contains primary nerve branches to muscle groups including triceps brachii, anconeus, brachioradialis, supinator, extensor carpi radialis longus, extensor carpi radialis brevis, extensor digitorum, extensor carpi ulnaris.

These branches are primarily responsible for elbow extension, forearm supination, wrist extension, and finger extension^{21,22}.

Median nerve: The median nerve contains primary nerve branches to muscle groups including pronator teres, flexor carpi radialis, palmaris longus, radial part of flexor digitorum muscles, pronator quadratus, opponens pollicis, flexor pollicis longus, 1st & 2nd lumbricals and abductor pollicis brevis.

These branches mainly controls forearm pronation as well as flexion of the wrist, thumb, and other fingers^{23–25}.

Ulnar nerve: The ulnar nerve contains primary nerve branches to muscle groups including flexor carpi ulnaris, ulnar part of flexor digitorum muscles and hand intrinsic muscles including abductor digiti minimi, flexor digiti minimi brevis, opponens digiti minimi, interossei muscles, 3rd & 4th lumbricals and adductor pollicis.

These branches mainly governs wrist flexion, finger flexion and fine motor skills like grasping and pinching^{26,27}.

Reference:

21. Adcock, K. S. *et al.* Radial nerve injury causes long-lasting forelimb sensory impairment and motor dysfunction in rats. *Pain Rep* **6**, e957 (2021).
22. Ljungquist, K. L., Martineau, P. & Allan, C. Radial nerve injuries. *J Hand Surg Am* **40**, 166–172 (2015).
23. Planitzer, U. *et al.* Median nerve fascicular anatomy as a basis for distal neural prostheses. *Ann Anat* **196**, 144–149 (2014).
24. Zhao, X. *et al.* Selective neurotization of the median nerve in the arm to treat brachial plexus palsy. Surgical technique. *J Bone Joint Surg Am* **87 Suppl 1**, 122–135 (2005).
25. Zhao, X. *et al.* Selective neurotization of the median nerve in the arm to treat brachial plexus palsy. An anatomic study and case report. *J Bone Joint Surg Am* **86**, 736–742 (2004).
26. Chambers, S. B. *et al.* Interfascicular Anatomy of the Motor Branch of the Ulnar Nerve: A Cadaveric Study. *The Journal of Hand Surgery* **48**, 309.e1-309.e6 (2023).
27. Pautler, B., Marchese, C., Swancutt, M. & Beutel, B. G. Anatomical Characterization and Topographic Mapping of the Distal Ulnar Nerve and Its Peripheral Branches: A Cadaveric Analysis. *Hand (N Y)* 15589447241306151 (2024) doi:10.1177/15589447241306151.

Discussion in revised manuscript, line 839-843

Despite brachial plexus trunks mentioned in our study, radial nerve^{62,63}, median nerve^{11,64,65}, and ulnar nerve^{66,67} in the forelimb also serve as ideal target nerves due to their composition of more than 4 nerve fascicles. They control the extension of elbow, the rotation of the forearm, the flexion/extension/rotation of wrist as well as flexion/extension/adduction/abduction of each finger intricately and precisely.

21. *There are many small grammatical errors in the text that sometimes make the meaning difficult to understand. Also a few typos, e.g. “which is is”, Figure 3e “Tememprature”. For clarity, some detailed descriptions can also be moved to supplementary, for example the current-limiting resistor choice.*

Author’s reply:

Thank you for the constructive comment.

We have gone through the entire manuscript and fixed the grammatical errors as much as we can.

We have also moved detailed but less relevant descriptions, including the choice for current-limiting resistor, into supplementary.

Reviewer #3 (Remarks to the Author):

Zhou et al. present a fully implantable, wireless optogenetic stimulation system designed for long-term monitoring of upper limb function with sub-nerve resolution in vivo. The manuscript details the development of a miniaturized, battery-free device to drive four blue LEDs positioned on a nerve cuff. Power and control signals are delivered via magnetic induction, enabling battery-free operation and illumination parameter reconfiguration. The authors demonstrate the device's utility in acute upper limb optogenetic stimulation in Thy1-ChR2-EYFP mice and, importantly, its application in monitoring nerve function following traumatic brain injury and CC7 nerve transfer surgery. Validation is supported by physiological characterization of upper limb motion based on this device stimulation and other clinical stimulation standards.

The work is conceptually sound, and the presented biological results are compelling. The manuscript presents an interesting and potentially valuable contribution to the field of optogenetic stimulation for nerve injury/surgery evaluation. However, the manuscript lacks technical details regarding the device's engineering, raising concerns about the reproducibility and generalizability of the described system. Therefore, authors are strongly encouraged to address the comments outlined below, particularly regarding the engineering aspects of the device.

Major concerns:

1. An important design consideration for optogenetic devices is the irradiance at the region of interest. In this case, the LEDs illuminate the nerve tissue and, due to scattering and absorption, the light irradiance decreases at the site of need (the nerve fibers). Authors report optical power levels produced by the LEDs, but how much is the expected light irradiance at the nerve fiber of interest and how much is required to elicit functional optogenetic stimulation?

Author's reply:

Thank you for your constructive comment. We agree with the reviewer that the irradiance at the nerve fibers are important determining factors for effective areas of optogenetic stimulation.

In the revised manuscript, we added 3D finite element analysis of the light irradiance propagation from the light source (μ LEDs) across the target nerve (**Figure.3k in revised manuscript**). The μ LED was measured to emit at an optical power of 2.5mW (42mW/mm^2 irradiance at the LED's surface), which was used as the source term of the finite element analysis. A 2D representative slice out of the 3D analysis was used to visualize the irradiance propagation in the cross-section of the nerve fiber (**Figure.3l,m in revised manuscript**), where contour lines of 13mW/mm^2 (at which 100% of maximum force is expected to be induced by optogenetic stimulation, **Response Figure.2**)²⁰ and 5mW/mm^2 (50% of maximum force, **Response Figure.2**)²⁰ irradiance were illustrated.

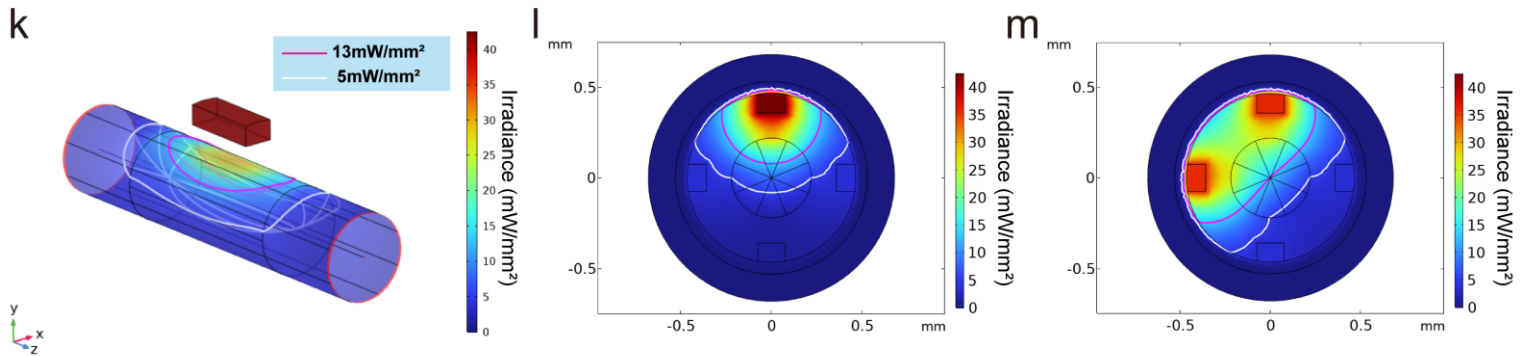
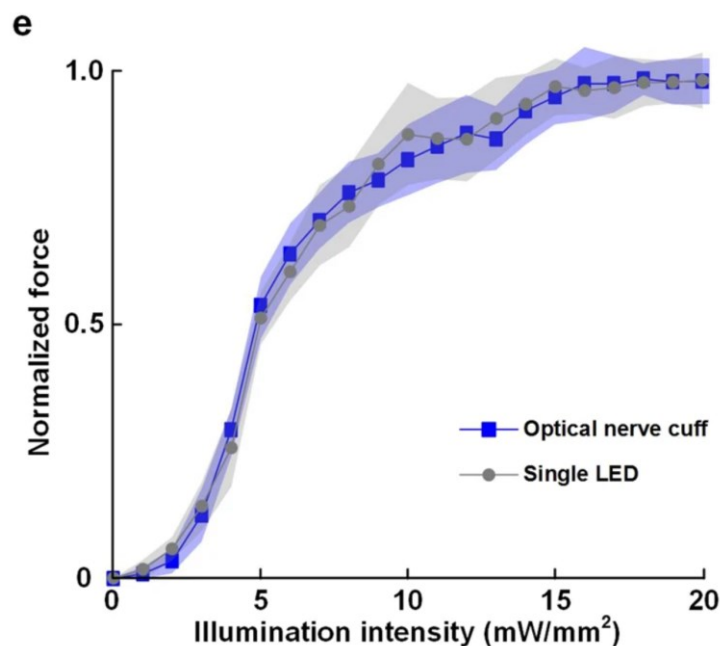


Figure.3 in revised manuscript. Characterization of FIMOSS implant. k Heat map of the light irradiance distribution in 3D optical simulation, when one μ LED was illuminated at 2.5mW of optical power. The color legend is the same as in Figure.3i,m. Domains except for the activated μ LED and the target were hidden. The plot was set at 40% transparency to allow visualization of the contour lines of 13mW/mm^2 and 5mW/mm^2 depicted in magenta and white. **l,m** Heat map of light irradiance in a representative 2D slice out of the 3D optical simulation result. The 2D slice was defined as the XY-plane slicing down the middle (Z-direction) of the μ LED in Figure.3k. One μ LED (l) was illuminated at the standard optical power of 2.5mW.

Two neighboring μ LEDs (m) were illuminated at a reduced optical power of 2.0mW. The color bar represents light irradiance in mW/mm^2 .



Response Figure.2 Muscle force measurements during the optogenetic stimulation using the optical nerve cuff electrode and using a single LED. From Kang-II Song et. al. 2018, Figure. 2e.

Reference:

20. Song, K.-I. *et al.* Compact Optical Nerve Cuff Electrode for Simultaneous Neural Activity Monitoring and Optogenetic Stimulation of Peripheral Nerves. *Sci Rep* **8**, 15630 (2018).

Results in revised manuscript, line 403-411

A set of 3D finite element analysis (FEA) studies were performed to simulate the propagation of light irradiance from the μ LED (2.5mW optical power, $42\text{mW}/\text{mm}^2$ irradiance at μ LED's surface) in the implanted environment (Figure.3i,j,k). A 2D representative slice out of the 3D result was used to visualize the irradiance distribution in the cross-section of the nerve fiber, where contour lines of $13\text{mW}/\text{mm}^2$ and $5\text{mW}/\text{mm}^2$ irradiance were illustrated (Figure.3l,m). These two irradiance thresholds were chosen because the transgenic mice line Thy1-ChR2-EYFP has been previously characterized that the muscle force elicited by optogenetic stimulation of the peripheral nerve reaches 50% of the maximum force when light irradiance is $5\text{mW}/\text{mm}^2$, and reaches the maximum force at $13\text{mW}/\text{mm}^2$ or higher³⁰.

2. Following on the previous comment. The authors present a strategy to identify the optimal electronic configuration to set the proper optical power, thermal

performance, and device operation for UART Rx based on the choice of a resistor. The trial-and-error approach undertaken in this manuscript is too empirical. I would expect the authors to understand the total power budget of the device and the available power harvested by the device and based on this to propose design parameters that would be demonstrated experimentally. This is important since the loading effect of relatively high current demands lowers the power performance of the antenna. Thus, the characterization on a wired system (with external power supply with sufficient power) is not directly transferable to the wireless system where only a maximum electrical power is available. It is easy to establish LED operation parameters based on desired optical power which requires LIV LED characterization, but none of these are presented in this manuscript.

Author's reply:

Thank you for the detailed and constructive comment. We agree that a more thorough engineering characterization of the FIMOSS system is needed to uncover the design constraints to assure proper μ LED optical power output in the wireless system. Hence, we conducted the suggested experiments, and report on the following:

First, we measured the power consumption of the FIMOSS implant across a typical stimulation event. When powered at the standard operating voltage of 3V, the current consumption of the device is 0.6mA (~ 1.8 mW) at minimum when idle, and 2.6mA (~ 7.8 mW) at maximum when one stimulation LED is turned on (**Supplementary Figure.6a in revised manuscript**). Then, we measured the voltage and current through a variable load when it is sourcing power from the FIMOSS implant being wirelessly powered under default wireless condition (defined in revised manuscript) to characterize the available power harvested by the device (**Figure.3d in revised manuscript**). The load sweep showed that the device was able to maintain a stable voltage output of 3V when the load was sourcing 4mA or less (**Figure.3d in revised manuscript**). Therefore, we were able to confirm that the power budget of the device is within the limit of its wireless power transfer capability under the default wireless condition. Lastly, we also included the LIV characterization of the μ LED to guide the selection of the μ LED's current-limiting resistor (**Supplementary Figure.6b in revised manuscript**).

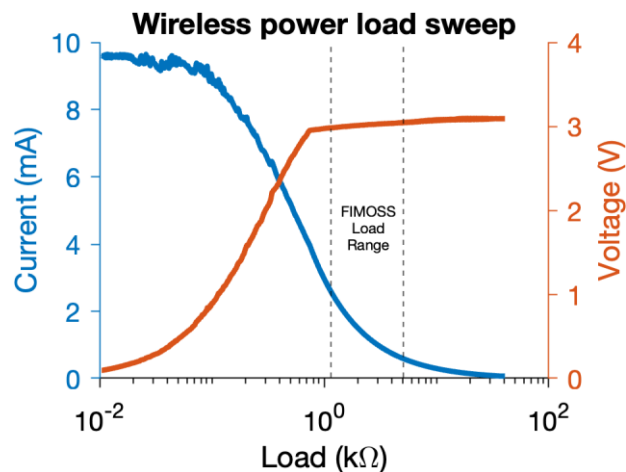
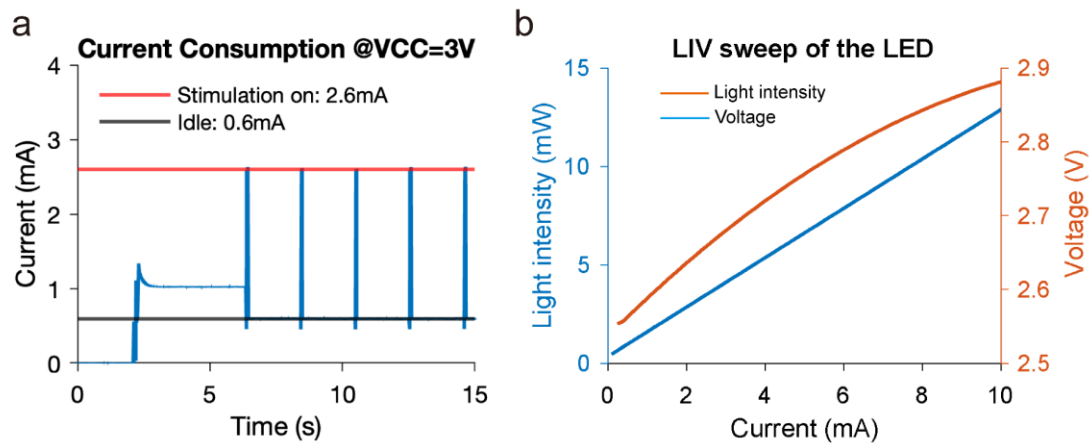


Figure.3 in revised manuscript. Characterization of FIMOSS implant. d Current and voltage across a variable load resistor while it sourced power from the FIMOSS implant that was wirelessly powered under the default wireless condition. The two vertical lines corresponds to the minimum (0.6mA) and maximum (2.6mA) current consumption of the FIMOSS implant at 3V.



Supplementary Figure.6 in revised manuscript. Power consumption of FIMOSS implant and LIV characterization of LED. a Power consumption of the FIMOSS implant in a typical stimulation event. **b** Luminous intensity, current and voltage across the LED were measured simultaneously to obtain the LED' s LIV characterization.

Results in revised manuscript, line 302-310

The wireless power transfer (WPT) capability of the FIMOSS implant was evaluated by a load sweep sourcing power from the implant while it was wirelessly powered by the external device under the default wireless condition (external device set at 3.3V, 60~80mA; the emitting antenna and receiver antenna were concentrically aligned, in parallel and 1mm apart). The load sweep showed that the implant was able to maintain the target voltage output of 3V when the load is sourcing 4mA or less (Figure.3d). The current consumption of the implant (when operating at 3V) was characterized to be between 0.6mA when idle to 2.6mA when stimulation was on

(Supplementary Figure.6a). Thus, we confirmed that the default wireless condition was sufficient to support the power budget of the implant.

Results in revised manuscript, line 399-402

Light-current-voltage (LIV) sweeps were conducted to characterize the μ LED's optical power output as its current increased from 0mA to 10mA. FIMOSS implant used an 160 Ω current-limiting resistor in series with the μ LEDs, which limited the current through μ LED to 2.0mA resulting in an optical power output of 2.5mW (Supplementary Figure.6b).

Results in revised manuscript, line 391-395

The realistic operating condition of the μ LEDs used in our animal experiments was far less demanding, where the μ LED was activated using 2.0mA of current (Supplementary Figure.6a), and with a low duty cycle (~1%) in the typical stimulation parameter of 20ms pulse width and 2000ms inter-pulse interval.

3. In addition, the authors claim sub-nerve stimulating resolution, which is obviously demonstrated in the biology characterization in the second half of the manuscript. As a device engineering paper, I would have expected the authors to support this claim at least with more quantitative analysis, for example, via optical simulations. Authors do present optical simulation (Fig 3j-m), however, what quantitative insights do we gain from these results? How much is the irradiance thresholds used to establish the sector photon concentrations? What do the color bar even mean?

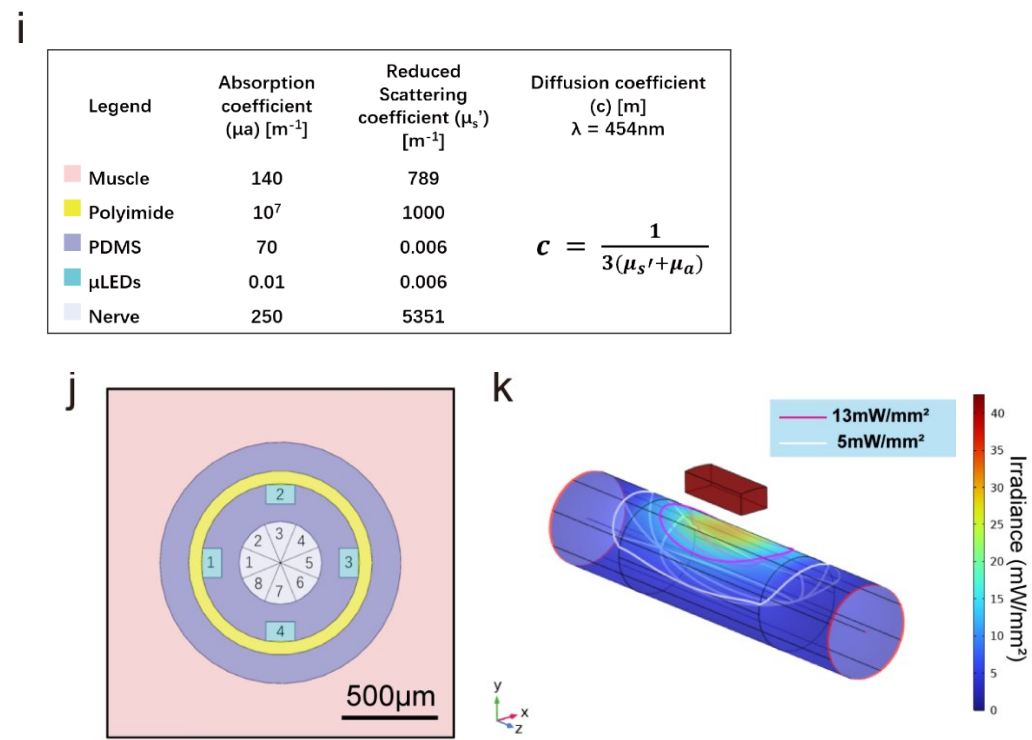
Author's reply:

Thank you for the constructive comment. We apologize for missing out the label for the color bar.

As also seen in **reply to Reviewer 3 Major Concerns 1**, we conducted new optical simulation and showed that 22.3% of the nerve's cross-sectional area is illuminated above 13mW/mm² irradiance, and 68.8% of the area is illuminated above 5mW/mm² when one μ LED was turned on at 2.5 mW (**Figure.3l in revised manuscript**). These two irradiance thresholds correspond to achieving 100% and 50% of the maximum muscle force by optical stimulation of the peripheral nerve²⁰. We also added quantitative analysis that when one μ LED was turned on at 2.5mW, sector 3 of the nerve (**labeled in Figure.3j in revised manuscript**) that directly faced the μ LED

received an average irradiance of 21.9 mW/mm², where sector 7 of the nerve that was the furthest away from sector 3 received an average irradiance of 2.5 mW/mm² (**Figure.3n in revised manuscript**). This demonstrated that irradiance gradient allows for selective activation of a sub-section of the target nerve, and was further demonstrated in *in vivo* animal experiments (**Figure.5h.i,j in revised manuscript**).

We have added color bar for the rainbow map in **Figure. 3 in revised manuscript**.



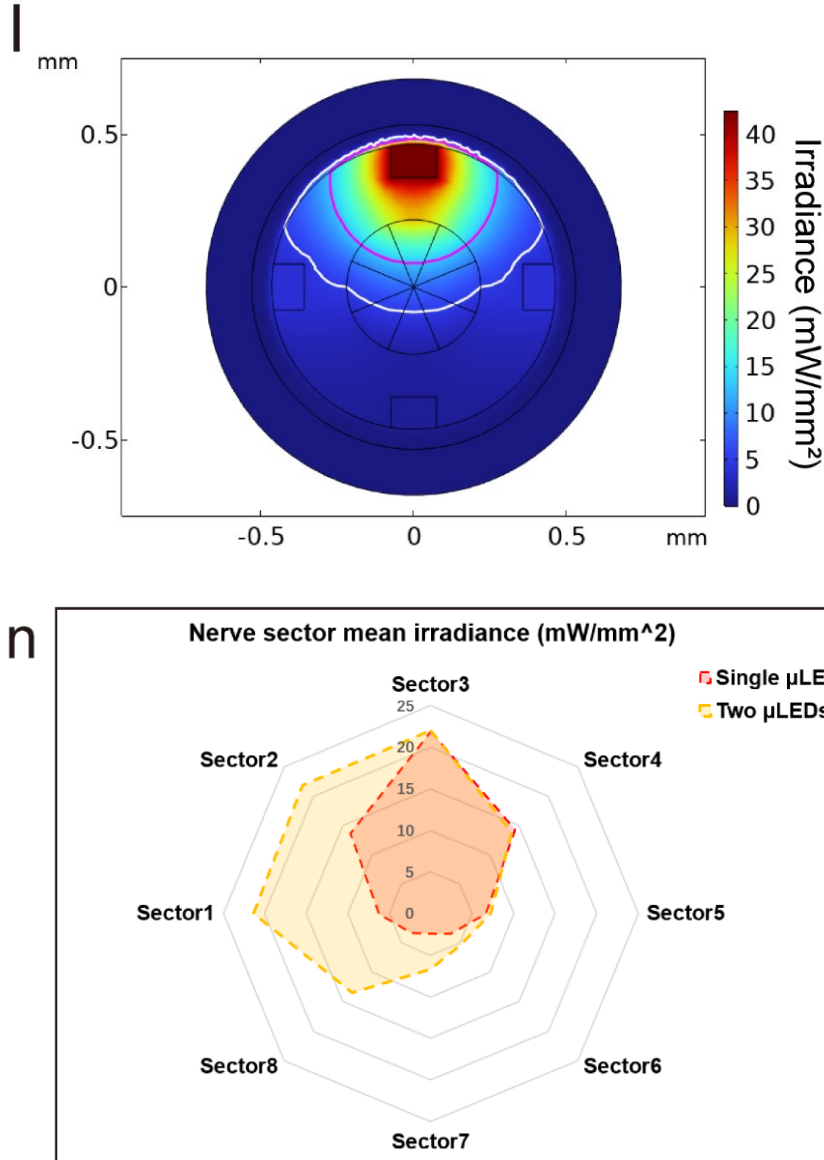


Figure.3 in revised manuscript. Characterization of FIMOSS implant. **i** The absorption coefficients and reduced scattering coefficients used for the optical simulation. **j** Cross-sectional view of the 3D model of a MOSD II cuffing on a typical target nerve (440 μ m diameter, scale bar=500 μ m). This 3D model was used in the optical simulation, and each domain was color-coded corresponding to the color legend in Figure.3i. The four μ LEDs were numbered, and the nerve was equally divided to eight sectors and numbered as references for the quantitative analysis result in Figure.3n. **k** Heat map of the light irradiance distribution in 3D optical simulation, when one μ LED was illuminated at 2.5mW of optical power. The color legend is the same as in Figure.3i,m. Domains except for the activated μ LED and the target were hidden. The plot was set at 40% transparency to allow visualization of the contour lines of 13mW/mm² and 5mW/mm² depicted in magenta and white. **l** Heat map of light irradiance in a representative 2D slice out of the 3D optical simulation result. The 2D slice was defined as the XY-plane slicing down the middle (Z-direction) of the μ LED in Figure.3k. One μ LED (l) was illuminated at the standard optical power of 2.5mW. **n** The average irradiance in each sector of the nerve computed by optical

simulation of one- μ LED (orange) and two- μ LED (yellow) illumination in Figure.3l,m.

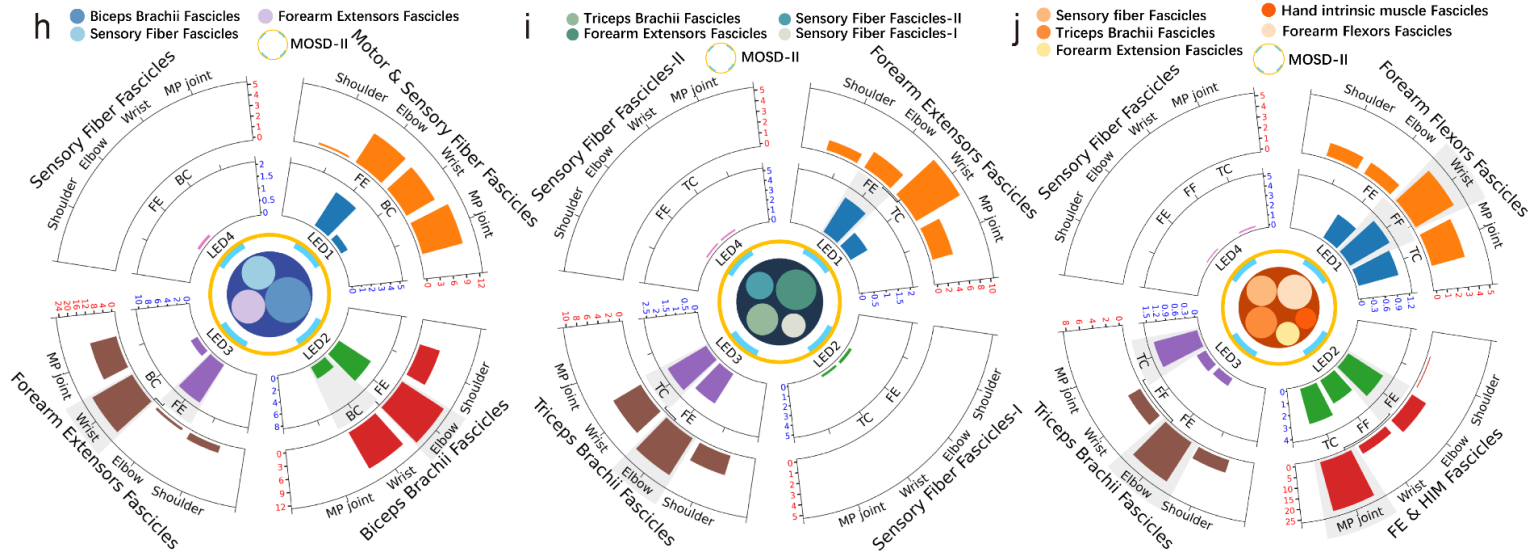


Figure.5 in revised manuscript. The application of FIMOSS on brachial plexus to induce distinct movements of forelimb in mice. h~j Circular statistical graph of representative CMAP area/mV*ms (blue axis) from target muscles and angle changes/° (red axis) of distinct forelimb movements induced by different μ LEDs (20ms pulse width, 2000ms inter-pulse interval, default wireless condition) during multisite optogenetic stimulation of upper trunk (h), middle trunk (i) and lower trunk (j). BC, biceps brachii. TC, triceps brachii. FE, forearm extensors. FF, forearm flexors. HIM, hand intrinsic muscles. MP joint, metacarpophalangeal joint.

Reference:

20. Song, K.-I. *et al.* Compact Optical Nerve Cuff Electrode for Simultaneous Neural Activity Monitoring and Optogenetic Stimulation of Peripheral Nerves. *Sci Rep* **8**, 15630 (2018).

Results in revised manuscript, line 403-421

A set of 3D finite element analysis (FEA) studies were performed to simulate the propagation of light irradiance from the μ LED (2.5mW optical power, 42mW/mm² irradiance at μ LED's surface) in the implanted environment (Figure.3i,j,k). A 2D representative slice out of the 3D result was used to visualize the irradiance distribution in the cross-section of the nerve fiber, where contour lines of 13mW/mm² and 5mW/mm² irradiance were illustrated (Figure.3l,m). These two irradiance thresholds were chosen because the transgenic mice line Thy1-ChR2-EYFP has been previously characterized that the muscle force elicited by optogenetic stimulation of the peripheral nerve reaches 50% of the maximum force when light irradiance is 5mW/mm², and reaches the maximum force at 13mW/mm² or higher³⁰.

When one μ LED (μ LED2 in this simulation) was activated at the standard optical power of 2.5mW, sector 3 that directly faced it received an average irradiance of 21.9mW/mm², where sector 7 that was further away from it only received an average irradiance of 2.5mW/mm² (Figure.3l,n). Despite the two sectors are only ~300 μ m (centroid-to-centroid distance) apart, a fascicle in sector 7 would elicit 90% less force than a fascicle in sector 3 due to the irradiance differences³⁰. Therefore, we estimate the spatial resolution of FIMOSS's optogenetic stimulation to be around 300 μ m. This demonstrated irradiance gradient was expected to allow for selective activation of a sub-section of the target nerve. By illuminating different μ LEDs, fascicles across different sections of the nerve can be targeted, thus triggering distinct downstream muscle movements.

Minor concerns:

1. Confusing terminology: 1) The title delivers the message that the device will monitor upper limb function over long-term, which is not the case, it is just an optogenetic stimulator. Author should update the title accordingly. 2) The terminology used for describing optogenetic stimulation parameters is not standard. e.g. "on-Time (range 0.1~200ms, and special case of 2000ms), off-Time (range 0~199ms, and special case 2s)". Authors must reframe this terminology such as pulse widths (pw), frequency (f) and/or periods.

Author's reply:

Thank you for the constructive comment. We apologize for the confusing terminology and made revisions accordingly:

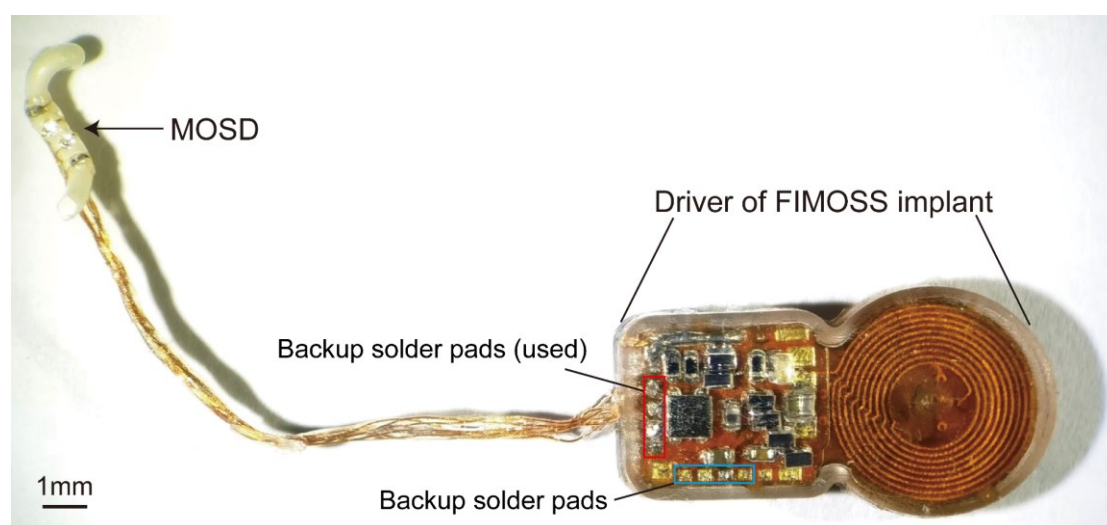
- 1) We agree that monitoring upper limb function was not an accurate expression. We have changed the title to "A wireless and fully implantable optogenetic stimulation system for long-term function evaluation of mice forelimb with sub-nerve resolution". We used the term "function evaluation of mice forelimb" because we used the FIMOSS device to evaluate the recovery of nerve function to control its forelimb after the nerve transfer surgery in Figure.5 and Figure.6 in the revised manuscript.
- 2) We have revised the manuscript to use standard terminology such as pulse width and inter-pulse interval (in the case of multiple μ LEDs are activated in sequence) for describing optogenetic stimulation parameters.

2. There is a reference for eight channel operation as an option for future development, but this is not demonstrated in the manuscript. Therefore, this should be removed from the manuscript or include validation experiments that demonstrates it.

Author's reply:

Thank you for the constructive comment. We have removed the reference for eight channel operation reported by Zheng et. al.¹⁷.

In the revised manuscript, we mentioned that FIMOSS implant contained backup solder pads to provide compatible extension for 4-channel MOSD (**Supplementary Figure.2 in revised manuscript**).



Supplementary Figure.2 in revised manuscript. A photo of the FIMOSS driver soldered with a 4-channel MOSD and with complete encapsulation. (scale bar=1mm)

Reference:

17. Zheng, H. *et al.* A shape-memory and spiral light-emitting device for precise multisite stimulation of nerve bundles. *Nat Commun* **10**, 2790 (2019).

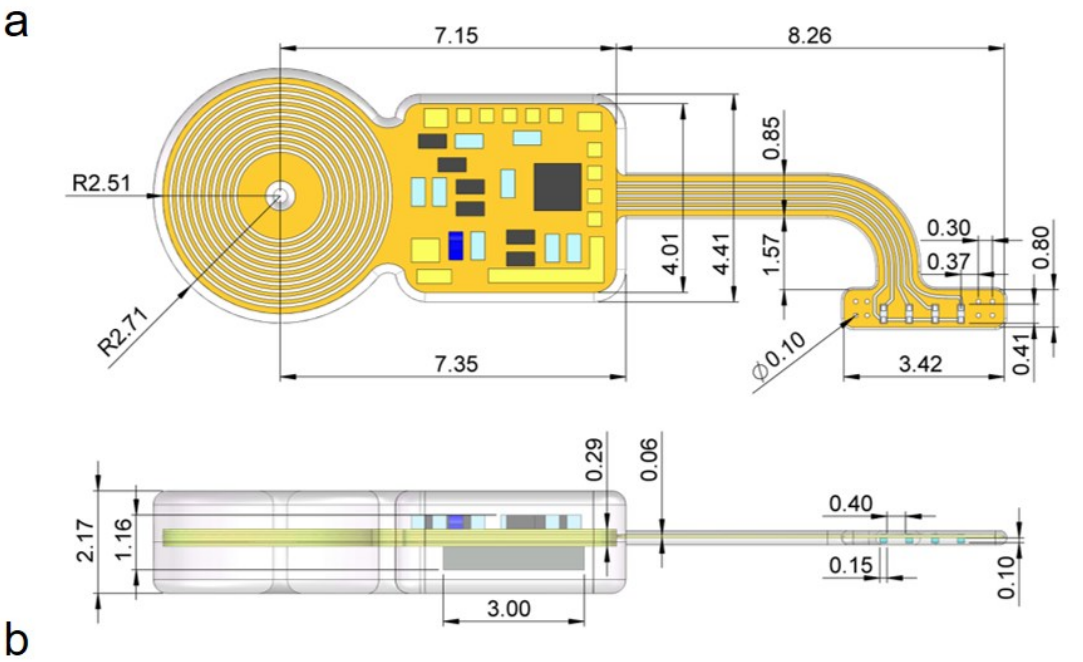
Results in revised manuscript, line 151-155

The electronic hardware of the driver region was a 6-layer Flexible Printed Circuit (FPC) Board containing the printed antenna, and the driver circuitry mounted with a micro-controller-unit (MCU), supporting electrical components, and backup solder pads to provide compatible extensions for MOSD²² reported by Zheng et al. previously (Supplementary Figure.2).

3. It is surprising the device is 1.2 mm thick. Even when the polyamide substrate is somehow flexible, this thickness is quite unusual. What is the justification for such a 6-layer flexible PCB?

Author’s reply:

Thank you for the comment. We apologize for the confusion. We have added further clarification in the manuscript that, although the driver region of the device is a 6-layer flexible PCB (0.29 mm thick without components, 1.2mm thick with components), the interconnect and the cuff region is just a 1-layer flexible PCB with 0.065mm thickness (**Supplementary Figure.1 in revised manuscript**). We designed the device so that the driver part can remain rigid to facilitate stable solder joints with the main electronics, while the interconnect and the cuff be flexible to navigate through the surgical route and encircle the target nerve fiber.



	PI coverlay	Solder Mask	12.5	μm
Top	Adhesive	Base Copper+plated	15	μm
		Polyimide	12	μm
Layer2		Base Copper	25	μm
		Adhesive	12	μm
		PI coverlay	15	μm
		Adhesive	12.5	μm
Layer3		Base Copper	15	μm
		Polyimide	12	μm
Layer4		Base Copper	25	25 μm
		Adhesive	12	μm
		PI coverlay	15	15 μm
		Adhesive	12.5	12.5 μm
Layer5		Base Copper	15	μm
		Polyimide	12	μm
Bottom		Base Copper+plated	25	μm
	Adhesive		12	μm
	PI coverlay	Solder Mask	15	μm
			12.5	μm
Total	Driver part=287±30μm		Interconnect part=64.5±30μm	

Supplementary Figure.1 in revised manuscript. FIMOSS implant engineering drawing, and FPC layer management. a Engineering drawing of the FIMOSS implant with key dimensions annotated. Units are in millimeter(mm). **b** Layer stacking of the FIMOSS implant's printed circuit board

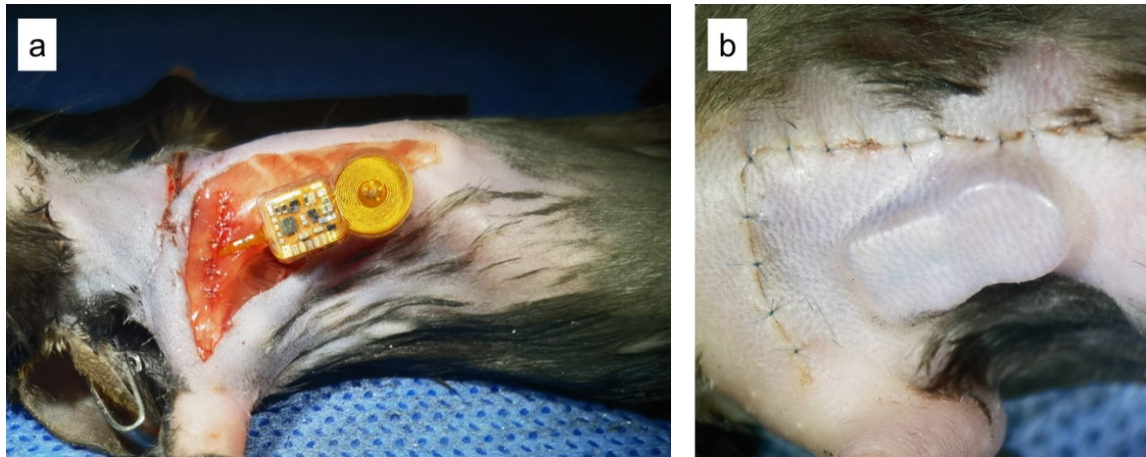
Results in revised manuscript, line 146-150

The circuit board thickness of the driver region was 0.29mm (1.2mm with components), and the total device thickness increased to 2.2mm after encapsulation, as 500µm of encapsulation was added to each side. The interconnect and the nerve cuff extended from the driver were 0.065mm thick, and increased to 0.365mm after PDMS encapsulation (Supplementary Figure.1a,b).

4. Although it is true that the efficiency of WPT scales with the area of the planar coil and the number of turns, it is not validated how, for example the 6-layer coil system compares with the standard 2-layer counterpart. The resonance quality factor benefits for a larger turn count though. Is this the benefit the authors were looking for? If so, how does it change based on loading conditions?

Author's reply:

Thank you for the insightful comment. One key design for the long-term *in vivo* application of our FIMOSS is its miniaturization in size to be placed onto the pectoralis major of the mice, so that we can leverage its flat and stable anatomic structure (**Response Figure.3a in revised manuscript**). We have previously attempted to implant a device with a slightly larger coil (8mm in diameter compared to the current 5mm diameter coil). The skin cannot be closed by suture due to the bulky size of the implant. Therefore, the size of the coil was limited to 5mm diameter for the implant to fit within the pectoralis major region and for successful skin closures (**Response Figure.3b in revised manuscript**).



Response Figure.3 **a** A photo of the implantation procedure of FIMOSS on pectoralis major of the mouse before skin closure. **b** A photo of the successful skin closure after the implantation of FIMOSS.

As the reviewer pointed out, the efficiency of WPT scales up with the area of the planar coil and the number of turns. Due to surgical consideration mentioned above, we can only increase the turn count by stacking more layers to maximize the WPT throughput to suffice the power budget of the device. The 6-layer coil design has 60 turns in total (10 turns per layer), where the standard 2-layer counterpart would have 20 turns. If all other WPT conditions remain unchanged (for example, the planar area of the receiving coil, the physical alignment between the emitting and receiving coil, the power emitted by the emitting coil, resonance tuning), the 2-layer counterpart can support one third of the load that the 6-layer coil system can power (Equation 1 in revised manuscript, 2-layer coil's total turn count N_2 is one third of that in 6-layer coil). As the 6-layer coil system was able to provide up to 4 mA of current ($V_{CC}=3V$) to the load, the 2-layer coil system could in theory only provide up to 1.3mA of current, which is not sufficient for the device's current requirement of 2.6mA.

The resonance quality factor (Q) does benefit from a larger turn count (N), as the increase of N increases Q value quadratically. The Q value of the 6-layer coil (10uH) is measured to be 14.8 at 2MHz. A 2-layer counterpart with the same number of turns on each layer (predicted to be 1.3uH by TI Coil Designer) result in a lower Q value as its inductance L is much smaller (Equation 9). Lastly, we do not expect the Q value to change significantly within the loading condition of our device (current consumption 0.6~2.6mA, at $V_{CC}=3V$), because the factors that influences Q value, namely operating frequency f , inductance of the coil L , and the resistance of the coil R , do not change during the device's operation.

$$M_{21} = \frac{N_2 \Phi_{21}}{i_1}, \text{ where } \Phi_{21} = B_{21} A_2 \cos \theta_{21} \text{ (Equation 1)}$$

$$Q = \frac{2\pi f L}{R}, \text{ where } L = \frac{K_1 \mu_0 N^2 d_{avg}}{1 + K_2 \rho} \text{ (Equation 9}^{28}\text{)}$$

Reference:

28. Mohan, S. S., Del Mar Hershenson, M., Boyd, S. P. & Lee, T. H. Simple accurate expressions for planar spiral inductances. *IEEE J. Solid-State Circuits* **34**, 1419–1424 (1999).

Discussion in revised manuscript, line 790-796

In order for FIMOSS implant to operate in larger arena sizes, the energy harvesting capability of the receiving (secondary) coil needs be improved by increasing its mutual inductance with the emitting (primary) coil (M_{21}). The mutual inductance scales positively with the number of turns of the secondary coil (N_2) and the magnetic flux through the secondary coil from primary coil (Φ_{21}) (Equation 1). Therefore, one can increase N_2 or coil area A_2 for a more robust energy harvesting capability.

$$M_{21} = \frac{N_2 \Phi_{21}}{i_1}, \text{ where } \Phi_{21} = B_{21} A_2 \cos \theta_{21} \text{ (Equation 1)}$$

5. Authors describe and characterize thermal levels produced by the device. However, what about the LEDs? LEDs are inefficient, so, the electrical power delivered to the LEDs produce light and heat. Is the expected heat a parameter of concern?

Author's reply:

Thank you for the constructive comment. We have conducted new experiments to characterize the LED's thermal level. A thermistor (GAG22K7MCD419, TE Connectivity Measurement Specialties) that has a size (0.38 mm in diameter) similar to our target nerve (0.44 mm in diameter) was fixated onto the device's LED to track its temperature change across a period of constant illumination with 2.1mA of current injection into the LED. The temperature of the LED rose 1.2 °C, and maintained at a steady state (**Figure.3h in revised manuscript**), which is within the safety limit of 2°C temperature increase for implantable devices.

In the optogenetic experiments conducted in this work, the LEDs were turned on in a pulse mode: 5~50ms pulse width, with a 1000~2000ms period. Hence, the

temperature increase of the LEDs during *in vivo* experiments are lower than 1.2°C and thus within the safety range of operation.

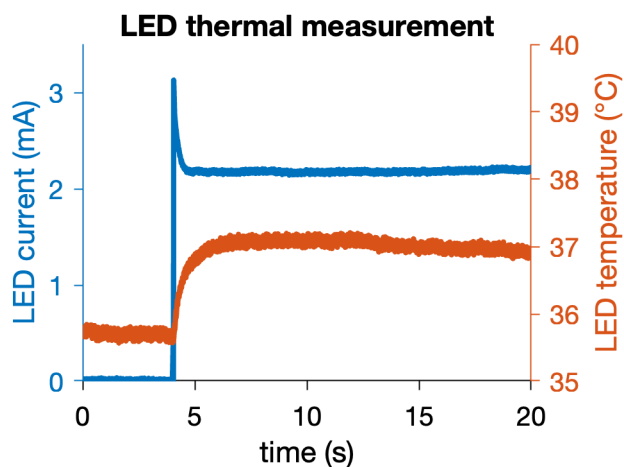


Figure.3 in revised manuscript. Characterization of FIMOSS implant. h
 Temperature change of the μ LED *in vivo* during a 20s test period, where the current through the μ LED was simultaneously recorded. The μ LED was powered by a constant current source that was set to 2.1mA.

Results in revised manuscript, line 388-391

The temperature change within the μ LED nerve cuff was also measured *in vivo* by replacing the nerve with a thermistor of the same size. Under the extreme case of constant illumination at 2.1mA, the μ LED temperature increased from 36°C (body temperature) and stabilized to 37°C during the 20s testing period (Figure.3h).

6. Authors argue that this device is compatible with commercial RF drivers such as those sold by the company Neurolux. However, operating the device introduced here within a large antenna lowers the wireless power transfer efficiency considerably. So, authors are cautioned to claim operation of the device with the commercial RF hardware if the proper validation of illumination characteristics is not validated or confirmed.

Author's reply:

Thank you for the comment. We have removed the claim that the device is compatible with the commercial RF driver sold by NeuroLux in the revised manuscript, as we agree with the reviewer that more thorough validation of the optical characteristics is required for this claim. Instead, in the revised manuscript we conveyed that

compatibility with NeuroLux system is an important future direction for the FIMOSS device.

7. Furthermore, authors introduce here a mechanism for reprogramming in real-time. Since this is not even demonstrated in the manuscript, this should be removed from the claims of the paper or include demonstration of it.

Author's reply:

Thank you for the comment. We have removed the software proposal for reprogramming and triggering stimulation in real-time in the revised manuscript.

We would like to kindly remind the reviewer that the point-to-point reply below uses the following format:

Reviewer's Comments: *Times New Roman (black, italic)*

Author's reply: *Times New Roman (blue)*

Reference: Times New Roman (black)

Quote from the revised manuscript: *Arial (red)*

Reviewer #1 (Remarks to the Author):

The MS improved a lot especially in terms of biological characterisation. There are minor things which have to be corrected in wordings and explanations but I do not think that more and new experiments are needed:

Thank you for your approval of our revision work.

1. Please compare the indirect optogenetic stimulation of skeletal muscles to direct optogenetic stimulation of skeletal muscles (Not only electrical). This is especially important for the muscle specific stimulation or cases in which nerval input is completely lost.

Thank you for this important and constructive comment. Below is our comparative analysis between indirect and direct optogenetic stimulation of skeletal muscles:

1) Differences in the activation patterns of muscles

Indirect stimulation of nerve fibers: Indirect optogenetic stimulation using FIMOSS activates muscles by targeting motor neurons or axons, preserving the physiological order of muscle fibers recruitment (sequential activation from slow to fast fibers). However, its efficacy depends on an intact motor pathway including functional spinal motor neurons, axons, neuromuscular junctions, and muscle fibers, limiting its application in peripheral nerve or spinal cord injuries.

Direct stimulation of skeletal muscles: Direct optogenetic stimulation of skeletal muscles bypasses neural pathways by targeting muscle fibers directly. This method is particularly valuable for denervated or single target muscle. A major drawback is the induction of unnatural, synchronous muscle contraction due to reversed recruitment order, which accelerates fatigue. Uniform optogenetic protein expression across muscle tissues is also a challenge.

2) Differences in the surgical procedure

Indirect stimulation of nerve fibers: The surgical procedures for the implant surgeries in nerve fibers are complicated due to their anatomical location deep in tissues, low Young's modulus and proximity to critical vasculature, such as arterial/venous plexuses.

Direct stimulation of skeletal muscles: The accessibility of skeletal muscles simplifies the procedure of implantation. Sufficient light without interfaces is sometimes enough to activate muscle contraction^{1,2} (**Response Figure 1**).

3) Differences in the stimulation device

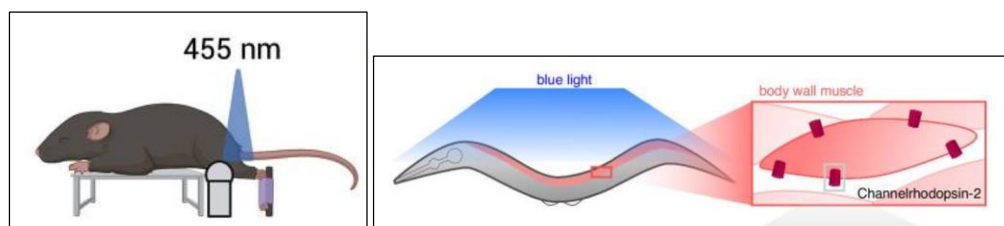
Indirect stimulation of nerve fibers: Indirect optogenetic stimulation such as FIMOSS requires flexible electrodes such as cuff^{3,4}, intrafascicular electrode⁵(**Response Figure 2-3**). In addition, multisite interfaces are usually utilized for selective nerve fascicle activation.

It's noteworthy that since nerve trunks are consisted of multiple nerve fascicles innervating different muscles, this approach enables coordinated multi-muscle activation through fascicle-selective neuromodulation, making it suitable for complex motor tasks such as locomotion or steering behaviors.

Direct stimulation of skeletal muscles: The broad, superficial distribution of skeletal muscles presents key hurdles including the uniform presentation of light stimulus in anatomically complex areas (e.g., diaphragm). Limited light penetration may also lead to insufficient activation in thicker muscle tissues⁶.

In summary, direct muscle stimulation bypasses neural dependency yet incurs unnatural contraction patterns and technical barriers to uniform opsin expression and light delivery. Indirect optogenetic stimulation leverages physiological recruitment order and coordinated multi-muscle control, but is constrained by neural circuit integrity and surgical complexity.

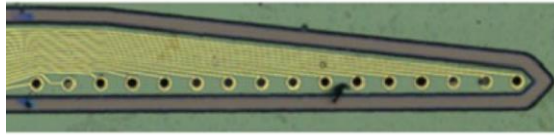
We have included these considerations into the discussion of the manuscript.



Response Figure.1 Optogenetic Stimulation of muscles to induce contraction. From Ganji, E. et al. Sci. Adv. 9, eadf4683 (2023) Figure.1 and Kutzner, C. E. et al. Nat. Commun. 15, 6879 (2024) Figure 1a.



Response Figure.2 Multisite optogenetic stimulation cuff (left) and flexible multichannel cuff electrode(right) for indirect stimulation. From Zheng, H. et al. Nat. Commun. 10, 2790 (2019) Figure.5h and Bong, J. et al. Biosens. Bioelectron. 142, 111493 (2019) Figure 1a.



Response Figure.3 Transverse intrafascicular multichannel electrode. From Viana, D. et al. Nat. Nanotechnol. (2024) Figure 3g (right)

Reference:

1. Zheng, H. et al. A shape-memory and spiral light-emitting device for precise multisite stimulation of nerve bundles. Nat. Commun. 10, 2790 (2019).
2. Bong, J. et al. Flexible, multichannel cuff electrode for selective electrical stimulation of the mouse trigeminal nerve. Biosens. Bioelectron. 142, 111493 (2019).
3. Viana, D. et al. Nanoporous graphene-based thin-film microelectrodes for in vivo high-resolution neural recording and stimulation. Nat. Nanotechnol. (2024) doi:10.1038/s41565-023-01570-5.
4. Ganji, E. et al. Optogenetic-induced muscle loading leads to mechanical adaptation of the Achilles tendon enthesis in mice. Sci. Adv. 9, eadf4683 (2023).
5. Kutzner, C. E. et al. Optogenetic induction of mechanical muscle stress identifies myosin regulatory ubiquitin ligase NHL-1 in C. elegans. Nat. Commun. 15, 6879 (2024).
6. Raman, R. et al. Optogenetic skeletal muscle-powered adaptive biological machines. Proc. Natl. Acad. Sci. 113, 3497–3502 (2016).

Discussion in revised manuscript, line 693-703

~~Some previous studies have also proposed devices for precise motor control through electrical muscle stimulation^{60,61}. Adjusting the stimulation intensity of electrical muscle stimulation rarely affects the untargeted muscles. Consequently, this method can achieve exceptionally precise control over muscle contraction. However, multiple electrodes are required when targeting distinct muscle groups using this method, which may demand a much larger system and cause considerable damage during the implantation process in small animals. Our system offers a reliable and efficient method for spatially selective control of different nerve fascicles in mice.~~

~~Previous studies achieved focused motor control via optogenetic/intramuscular electrical stimulation, minimizing off-target muscle activation^{60,61}. Direct muscle stimulation offers applicability for denervated or single-target muscles^{62–65} with simpler surgical access but incurs unnatural synchronous contraction and limited activation of thick muscles due to inadequate light penetration. In contrast, indirect fascicular stimulation leverages physiological recruitment order for graded force and fatigue resistance while enabling coordinated multi-muscle activation through selective neuromodulation^{22,66}. However, this approach requires an intact motor pathway, necessitates complex neural interfaces with sub-nerve resolution, and involves intricate implantation surgery near critical neurovascular structures. Our system bridges this specificity-invasiveness gap, providing multi-muscle control while preserving physiological recruitment patterns.~~

2. Please specify further: This is a device (Or thats what has been shown) to test engraftment but not to improve engraftment. For the latter you would need optogenetic stimulation during the whole period and not only at one final experiment.

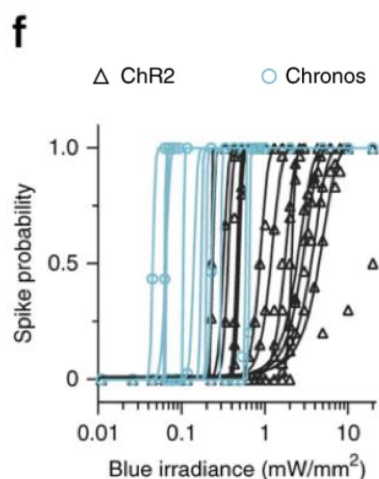
Thank you for highlighting this important distinction. We deleted relevant content about regeneration promotion in the revised manuscript.

Discussion in revised manuscript, line 721-722

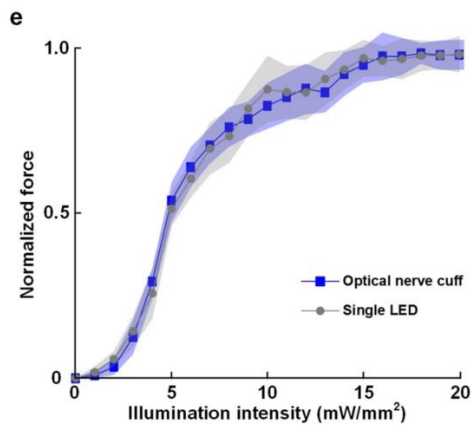
~~Furthermore, optogenetics may also have the potential to promote peripheral nerve regeneration, and inhibitory optrodes with a wavelength of 589nm may be applied in the treatment of neuralgia. Our system provides the possibility of long-term implantation for distinct forelimb movement induction, and further experimental exploration is required to explore other application.~~

3. 2.5 mW/mm² is more than enough and rather supra supra threshold to evoke an action potential in neurons and this is also not matching the light efficiency curve shown in the graph and described as 1:1 match.

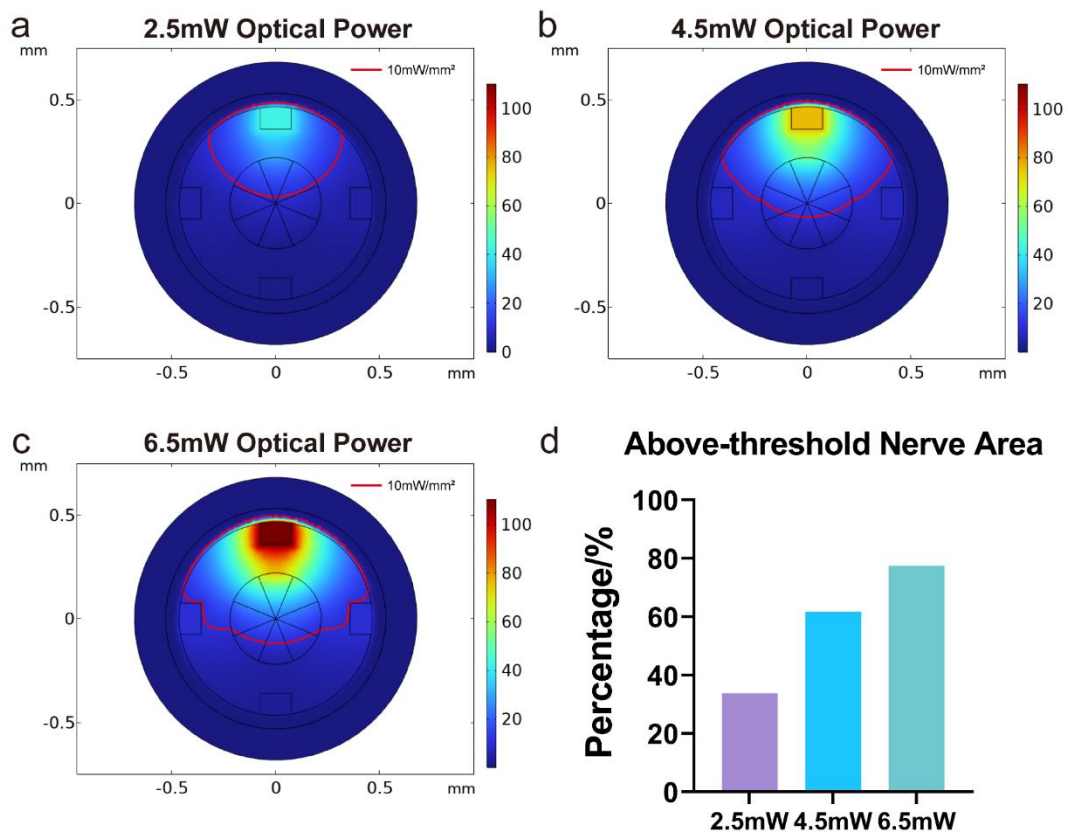
Thank you for the constructive comment. As the reviewer correctly pointed out, 2.5 mW/mm² is enough to evoke action potentials in neurons. Klapoetke et al showed that the spike probability of ChR2-expressing neurons at 2.5mW/mm², however, is less than 100% (**Response Figure 4**)⁷. Song et al further showed that the elicited force at 2.5mW/mm² is rather weak(<0.1 of the maximum force that can be elicited, **Response Figure 5**)⁸. Therefore, when computing the percentage of the nerve's cross-section that can be effectively activated by optogenetic stimulation, we set the threshold to be 10mW/mm² (**Response Figure 6**), under which condition the spike probability of ChR2-expressing neurons is almost 100% (**Response Figure 4**).



Response Figure 4 Spike probability vs. blue light irradiance, plotted for individual Chronos- or ChR2-expressing neurons. From Nathan C. Klapoetke et. al. 2014, Figure 4f.



Response Figure 5 Muscle force measurements during the optogenetic stimulation using the optical nerve cuff electrode and using a single LED. From Kang-II Song et. al. 2018, Figure 2e.



Response Figure 6. Also Supplementary Figure 8 in the revised manuscript. **Optical simulation of light irradiance distribution in the target nerve when illuminated by different optical power of the μ LED.** a,b Heat map of light irradiance in a representative 2D slice. One μ LED was illuminated at optical power of 2.5mW(a), 4.5mW(b) or 6.5mW(c) respectively. The color bar represents light irradiance in mW/mm^2 . The contour lines of $10\text{mW}/\text{mm}^2$ were depicted in red. d Comparison

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Reference:

7. Klapoetke, N. C. et al. Independent optical excitation of distinct neural populations. *Nat. Methods* 11, 338–346 (2014).
8. Song, K.-I. et al. Compact Optical Nerve Cuff Electrode for Simultaneous Neural Activity Monitoring and Optogenetic Stimulation of Peripheral Nerves. *Sci. Rep.* 8, 15630 (2018).

4. The explanation why 20 ms illumination is more efficient than shorter pulses is misleading and I am also not sure whether this is really still a single twitch contraction. Such prolonged illuminations can easily evoke several APs in motor neurons and thus induced rather short tetanic contractions by repetitive AP generation. This has to be discussed as alternative mechanism.

Thank you for raising this point. We agree with the reviewer that the more pronounced forelimb movement can be due to short tetanic contraction induced by repetitive AP generation instead of a single twitch, which is an alternative mechanism that should be mentioned in the manuscript. We have added relative contents in the revised manuscript.

Result in revised manuscript, line 460-462

This can be attributed to several action potentials generated at the targeted nerve fascicles, which induced more obvious angle changes.

Reviewer #2 (Remarks to the Author):

This revised version of the manuscript fully addresses my concerns.

1. Thank you for your response. The added comparison between multisite optogenetic stimulation and electrical stimulation indeed showcases the increased nerve group specificity with the FIMOSS device. The wording changes in the Introduction also help with motivating the scope of the study more clearly.

Thank you for your approval of our revision.

2. Thank you for the clarification. I think this advantage of FIMOSS over MOSD can be emphasized more clearly in the manuscript (i.e. that repeated surgeries are not necessary).

We have added relevant statement in the revised manuscript according to your advice. The details are as follows:

Discussion in revised manuscript, line 651-653

Our FIMOSS system can avoid potential injury to the targeted nerve due to repeated surgeries, which can be an issue in the previous version of FIMOSS, namely the acutely implanted device MOSD²².

3. Thank you for the clarification. I think that adding a statement similar to these lines to the main text of the manuscript will be useful: "So, the implanted MOSD II will not rotate during free moving behaviors in mice. This stability is one important advantage we considered when making decisions on the implant location of MOSD II."

Thank you for your advice. We have added relevant content in the methods. The details are as follows:

Method in revised manuscript, line 872-876

After the procedures above, No.2, No.3 μ LEDs and the interconnect are always located on the ventral side of the mouse (as shown in Figure. 5e,f,g). And the implanted MOSD II will not rotate during free moving behaviors in mice. This stability is one important advantage we considered when making decisions on the implant location of FIMOSS.

4. *Thank you for the clarification.*

Thank you for your approval of our revision work.

5. *Thank you for your response, the updated timeline and immunofluorescence images are important additions to the manuscript.*

Thank you for your approval of our revision work.

6. *The added experiments comparing different pulse width are good additions to the manuscript.*

Thank you for your approval of our revision work.

7-8. *Thank you for the clarifications and changes.*

Thank you for your approval of our revision work.

9-11. *Thank you for the nicely improved characterizations.*

Thank you for your approval of our revision work.

12. *Thank you for adding the important angle change comparison to electrical stimulation, it is encouraging that both optogenetic and electrical stimulation give similar responses.*

Thank you for your approval of our revision work.

13-16. *Thank you for addressing these concerns.*

Thank you for your approval of our revision work.

17. *Thank you for the detailed explanation. Adding this explanation to the Discussion would benefit the manuscript, where you address the potential cause for the large observed anatomical variation.*

Thank you for your approval of our revision work. We have added relevant explanation in the discussion of revised manuscript.

Discussion in revised manuscript, line 653-661

More importantly, since many muscles are jointly controlled by multiple nerve trunks, sometimes the proportion varies significantly among individuals. For example, triceps brachii is innervated by the radial nerve, which receives contributions from the upper,

middle, and lower trunks of the brachial plexus (Figure 5b). Although the middle and lower trunks typically dominate its control, significant individual variations exist between their relative contributions (Figure 7g,j). Our system achieves long-term follow-up and self-control study of both electrophysiology and induced-behavioral experiments in mice, which can detect subtle changes in nerve functions with sub-nerve resolution.

18-21. Thank you for these added explanations and changes to the manuscript.
[Thank you for your approval of our revision work.](#)

Reviewer #3 (Remarks to the Author):

This reviewer acknowledges the comprehensive manuscript revision the authors performed on. They have addressed my concerns and other reviewers' concerns. I do not have further comments or concerns.

[Thank you for your approval of our revision work.](#)