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TITLE:

Open-source Toolkit: Benchtop Carbon Fiber Microelectrode Array for Nerve Recording

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SUMMARY:

Here, we describe fabrication methodology for customizable carbon fiber electrode arrays for recording *in vivo* in nerve and brain.

ABSTRACT:

Conventional peripheral nerve probes are primarily fabricated in a cleanroom, requiring the use of multiple expensive and highly specialized tools. This paper presents a cleanroom “light” fabrication process of carbon fiber neural electrode arrays that can be learned quickly by an inexperienced cleanroom user. This carbon fiber electrode array fabrication process requires just one cleanroom tool, a Parylene C deposition machine, that can be learned quickly or outsourced to a commercial processing facility at marginal cost. This fabrication process also includes hand-populating printed circuit boards, insulation, and tip optimization.

The three different tip optimizations explored here (Nd:YAG laser, blowtorch, and UV laser) result

in a range of tip geometries and 1 kHz impedances, with blowtorched fibers resulting in the lowest impedance. While previous experiments have proven laser and blowtorch electrode efficacy, this paper also shows that UV laser-cut fibers can record neural signals *in vivo*. Existing carbon fiber arrays either do not have individuated electrodes in favor of bundles or require cleanroom fabricated guides for population and insulation. The proposed arrays use only tools that can be used at a benchtop for fiber population. This carbon fiber electrode array fabrication process allows for quick customization of bulk array fabrication at a reduced price compared to commercially available probes.

INTRODUCTION:

Much of neuroscience research relies upon recording neural signals using electrophysiology (ePhys). These neural signals are crucial to understanding the functions of neural networks and novel medical treatments such as brain machine and peripheral nerve interfaces[1–6]. Research surrounding peripheral nerves requires custom-made or commercially available neural recording electrodes. Neural recording electrodes—unique tools with micron-scale dimensions and fragile materials—require a specialized set of skills and equipment to fabricate. A variety of specialized probes have been developed for specific end uses; however, this implies that experiments must be designed around currently available commercial probes, or a laboratory must invest in the development of a specialized probe, which is a lengthy process. Due to the wide variety of neural research in peripheral nerve, there is high demand for a versatile ePhys probe[4,7,8]. An ideal ePhys probe would feature a small recording site, low impedance[9], and a financially realistic price point for implementation in a system[3].

Current commercial electrodes tend to either be extraneural or cuff electrodes (Neural Cuff[10], MicroProbes Nerve Cuff Electrode[11]), which sit outside the nerve, or intrafascicular, which penetrate the nerve and sit within the fascicle of interest. However, as cuff electrodes sit further away from the fibers, they pick up more noise from nearby muscles and other fascicles that may not be the target. These probes also tend to constrict the nerve, which can lead to biofouling—a build-up of glial cells and scar tissue—at the electrode interface while the tissue heals. Intrafascicular electrodes (such as LIFE[12], TIME[13], and Utah Arrays[14]) add the benefit of fascicle selectivity and have good signal-to-noise ratios, which is important in discriminating signals for machine interfacing. However, these probes do have issues with biocompatibility, with nerves becoming deformed over time[3,15,16]. When bought commercially, both these probes have static designs with no option for experiment-specific customization and are costly for newer laboratories.

In response to the high cost and biocompatibility issues presented by other probes, carbon fiber electrodes may offer an avenue for neuroscience laboratories to build their own probes without the need for specialized equipment. Carbon fibers are an alternative recording material with a small form factor that allows for low damage insertion. Carbon fibers provide better biocompatibility and considerably lower scar response than silicon[17–19] without the intensive cleanroom processing[5,13,14]. Carbon fibers are flexible, durable, easily integrated with other biomaterials[19], and can penetrate and record from nerve[7,20]. Despite the many advantages of carbon fibers, many laboratories find the manual fabrication of these arrays arduous. Some

groups[21] combine carbon fibers into bundles that collectively result in a larger (~200 µm) diameter; however, to our knowledge, these bundles have not been verified in nerve. Others have fabricated individuated carbon fiber electrode arrays, although their methods require cleanroom-fabricated carbon fiber guides[22–24] and equipment to populate their arrays[17,23,24]. To address this, we propose a method of fabricating a carbon fiber array that can be performed at the laboratory benchtop that allows for impromptu modifications. The resulting array maintains individuated electrode tips without specialized fiber populating tools. Additionally, multiple geometries are presented to match the needs of the research experiment. Building from previous work[8,17,22,25], this paper provides detailed methodologies to build and modify several styles of arrays manually with minimal cleanroom training time needed.

PROTOCOL:

All animal procedures were approved by the University of Michigan Institutional Animal Care and Use Committee.

1. Choosing a carbon fiber array

1.1. Choose a printed circuit board (PCB) from one of the three designs shown in **Figure 1**.

NOTE: For this protocol, Flex Arrays will be the focus.

1.1.1. Refer to PCB designs on the Chestek Lab website (https://chestekresearch.engin.umich.edu), free of charge and ready to be sent to and ordered for printing through a PCB printing house.

1.1.2. See **Table 1** for a summary of connectors for each board and their specifications to help choose the connector that will work for the specific experimental setup.

2. Soldering the connector to the circuit board

2.1. Set a soldering iron to 315 °C (600 °F).

2.2. Apply flux to all soldering pads on the PCB.

NOTE: Flux within a tube can be squeezed across the pads, while flux in a pot can be applied with the wooden end of a cotton-tipped applicator by smearing the flux across all pads liberally.

2.3. Form small mounds of solder on the back pads of the Flex Array (**Figure 2A**).

2.4. Solder the bottom row of connector pins to the back row of solder pads (**Figure 2B**).

NOTE: All board designs provided by the Chestek lab were designed so that the connectors would pair precisely with their designated board.

2.4.1. To do this, solder the pins on either side of the connector with easy access to the solder mounds. Once secure, gently push the soldering iron tip between the front pins to solder the remaining connections in the back.

NOTE: Once the back row of pins is secure, the rest of the connector will align with each pin above its assigned solder pad.

2.5. Solder the front row of pins to the board by applying a small amount of solder to each pin. Apply an additional layer of flux if soldering is not happening quickly.

2.5.1. Clean excess flux away with 100% isopropyl alcohol (IPA) and a short bristle brush.

2.6. Encapsulate the soldered connections in delayed set epoxy (**Figure 2 C,D**) using a 23 G needle and 1 mL syringe placed bevel side down on the pins. Push epoxy through the syringe slowly so that it flows into and along the connections.

2.6.1. Leave the board overnight so that the delayed set epoxy can cure.

NOTE: While the product insert for the delayed set epoxy states that it cures in 30 min, leaving it overnight allows a more stable connection to form.

2.7. Secure the backside of the board to the sides of the connector by laying a small line of delayed set epoxy across the back side of the board and pulling that onto the edges of the connector.

2.7.1. Leave the board to cure overnight again.

NOTE: At this point, either store the arrays or continue the build. If pausing in the build, store the arrays in a clean, dry box at room temperature.

3. Fiber population

3.1. Cut a pulled glass capillary so that its tip fits between the traces of the array (**Figure 3A**).

3.1.1. Using a glass puller and filament, make capillaries using the following settings: Heat = 900, Pull = 70, Velocity = 35, Time = 200, Pressure = 900.

NOTE: Numbers are unitless and specific to this device (see the **Table of Materials**).

3.2. Use the wooden ends of two cotton-tipped applicators (one per each part of silver epoxy) to scoop a small, ~1:1 ratio of silver epoxy in a plastic dish and mix using the same sticks used to scoop. Discard the applicators after mixing.

3.3. Cut 2–4 mm off the end of the carbon fiber bundle onto a piece of printer paper using a

razor blade. To easily separate the fibers in the bundle, which are difficult to tease apart, pull a laminated piece of paper gently over the top of the bundle.

NOTE: The laminated piece of paper transfers static into the fibers, which will separate by themselves.

3.4. Apply silver epoxy between every other pair of traces on one side of the board with the glass capillary (**Figure 3B**).

3.4.1. Take a small drop of epoxy onto the end of a pulled capillary. Gently apply between every other trace on the end of the board, filling the gap.

NOTE: The gap should be filled to the top of the two traces without overflowing to touch neighboring traces. Each trace is connected to one channel. This method of epoxy population means that each fiber will have two channels connected to it. This is because two traces allow for better fiber alignment, and redundancy in channel helps ensure electrical connection.

3.5. Use Teflon-coated tweezers to place one carbon fiber in each epoxy trace (**Figure 3C**).

3.6. Use a clean pulled capillary to adjust the carbon fibers, so they are perpendicular to the end of the Flex Array board and buried beneath the epoxy (**Figure 3D**).

3.7. Place the arrays on a wooden block with fibered ends overhanging the edge of the block.

NOTE: The weight of the back end will keep the array on the block.

3.8. Bake the wooden block and arrays at 140 °C for 20 min to cure the silver epoxy and lock the fibers into place.

3.9. Repeat steps 3.4–3.8 for the other side of the board.

NOTE: Arrays can be stored after any baking step; however, static from the storage boxes may cause the fibers to pull away from the board if too little silver epoxy was applied when populating the board.

3.9.1. Create a raised adhesive platform within a box so that the bulk of the board can be stuck to the adhesive allowing the fibered ends of the board to be suspended within the box to prevent fiber breakage. Store at room temperature.

NOTE: If fibers pull away from the board during storage, scrape the epoxy out of the traces with a clean pulled glass capillary and repeat steps 3.1–3.8 to replace the fibers. From this point on, arrays must be stored with the fibers suspended in this manner to prevent fiber breakage.

4. Applying ultra-violet (UV) epoxy to insulate the carbon fibers

4.1. Use a clean capillary and apply a small droplet (~0.5 mm in diameter of UV epoxy on the exposed traces on one side of the board (**Figure 4A**). Continue to add UV epoxy droplets until the traces are completely covered.

NOTE: Do not allow the UV epoxy to get on the carbon fibers past the end of the PCB to ensure a smooth insertion later.

4.2. Cure the UV epoxy under a UV pen light for 2 min (**Figure 4B**).

4.3. Repeat steps 4.1–4.2 for the other side of the board.

4.4. Cut the fibers to 1 mm using a stereoscope reticle and surgical scissors.

NOTE: Arrays can be stored at this point until ready to proceed to the next steps. They should be stored in a box that will elevate the carbon fibers away from the box itself. Arrays can be stored at room temperature indefinitely.

5. **Checking electrical connections with 1 kHz impedance scans** (**Figure 5**)

5.1. Submerge carbon fibers 1 mm into 1x phosphate-buffered saline (PBS).

5.2. To complete the circuit, use a silver-silver chloride (Ag|AgCl) reference electrode and a stainless steel rod (counter electrode).

5.2.1. Using a beaker clamp, suspend the Ag|AgCl electrode in the 1x PBS and connect it to the reference of the impedance system being used.

5.2.2. Using a beaker clamp, suspend the stainless steel rod in the 1x PBS and connect to the counter electrode input of the impedance system being used.

5.3. Run a 1 kHz impedance scan for each fiber using a potentiostat set to a 1 kHz scan frequency at 0.01 V_{rms} in a single sine waveform. Set the potentiostat to 0 V at the beginning of each scan for 5 s to stabilize the recorded signal. Record the measurements via the potentiostat-associated software.

NOTE: Measurements can be taken at any point in the build; however, they are only necessary before insulation and during tip preparation. **Table 2** lists typical ranges of impedances after each build step at 1 kHz for the user's reference.

5.4. Rinse the fibers in deionized (DI) water by dipping them into a small beaker three times and leave them to dry at room temperature.

NOTE: Arrays can be left in storage until the user can continue onto the next step.

6. Parylene C Insulation

NOTE: Parylene C was chosen as the insulation material for the carbon fibers as it can be deposited at room temperature over batches of arrays and provides a highly conformal coating.

6.1. Mask the Flex Array connector using the mating connector.

6.2. Place a batch of 8–12 arrays into a storage box with a raised adhesive platform so that they can be insulated in one run. Place the arrays so that the connector end of the array is on the adhesive platform with the fibered end of the array overhanging (**Figure 6**) to prevent the fibers from sticking to the adhesive and pulling off and to ensure a uniform Parylene coating on the fibers.

6.3. Coat the arrays in a Parylene C deposition system to a thickness of 800 nm in a cleanroom, wearing appropriate personal protection equipment (PPE) as defined by the individual cleanroom being used.

NOTE: Here, PPE was defined as cleanroom shoes, suit, head covering, goggles, mask, and latex gloves. It should be noted that this is standard PPE for entering a cleanroom. This step can be outsourced to a Parylene coating company for a fee; however, a commercial service may be able to coat more arrays at one time. Each Parylene C deposition system may have different safety precautions. Contact the technician before use to ensure user safety.

6.4. Remove the mating connector used as a mask from the Flex Array.

6.5. Place the arrays into a new box for storage until ready to use.

7. Tip preparation methods

NOTE: Two tip preparations in this section use lasers to cut fibers. Proper PPE, such as goggles resistant to the wavelengths used, should always be worn when using the laser, and other lab users in the vicinity of the laser should also be in PPE. Although fiber lengths listed in these steps are recommended lengths, users may try any length that suits their needs. The user must choose one of the following tip preparation methods as scissor cutting alone will not suffice to re-expose the electrode[25].

7.1. Neodymium-doped yttrium aluminum garnet (Nd:YAG) laser cut

7.1.1. Cut the fibers to 550 µm with surgical scissors.

7.1.2. Use a 532nm Nd:YAG pulsed laser (5 mJ/pulse, 5 ns duration, 900 mW) to cut 50 µm off the tip of the fibers to re-expose the carbon underneath the Parylene C (usually takes 2–3 pulses).

7.1.2.1. Align the fiber tips using the built-in stereoscope that comes with this laser system.

NOTE: This system allows the user to align a window (here, 50 µm x 20 µm (height x width)) was used to encompass the end of the fiber.

7.1.2.2. Focus the stereoscope on the end of the fiber at 500x magnification for an accurate and precise cut.

NOTE: Parylene C will ablate slightly (<10 µm) from the tip leaving a blunt, cylindrical tip.

7.2. Blowtorch Sharpening[25–27]

7.2.1. Cut the fibers to 300 µm with surgical scissors.

7.2.2. Submerge the array in a dish of deionized water, connector side down, and secured to the bottom of the dish with a small amount of putty.

7.2.3. Use a pen camera to align the fibers with the surface of the water so that the fibers are just barely touching the surface of the water.

7.2.4. Adjust a butane blowtorch flame to 3–5 mm and run it over the top of the fibers in a back-and-forth motion to sharpen fibers.

NOTE: Fiber tips will glow orange when the flame passes over them.

7.2.5 Remove the array from the putty and inspect it under a stereoscope for pointed tips under 50x magnification.

NOTE: If pointed tips are observed, then no further blowtorching is necessary. If tips appear blunt, repeat steps 7.2.2–7.2.5.

7.3. UV laser cut[28]

NOTE: UV Laser can only be used on zero insertion force (ZIF) and Wide Board designs at present due to the large focal point of the UV Laser used being larger than the pitch of the Flex Array carbon fibers.

7.3.1. Cut the carbon fibers to 1 mm with surgical scissors.

7.3.2. Affix a UV laser to three orthogonally configured motorized stages.

NOTE: The UV laser is a multimode Indium Gallium Nitride (InGaN) semiconductor with 1.5 W output power and 405 nm wavelength.

7.3.2.1. Ensure that the laser has a continuous beam for fast and effective alignment and cutting.

7.3.3. Secure the array in place to keep a still, level plane of electrodes for the laser to pass over. Ensure that array is held at an appropriate distance from the laser so that the fibers will be in light with the laser's focal point. To do this, provide a lower power to the laser and adjust the distance to best focus on the fiber[28].

7.3.4. Move the UV laser focal point across the fiber plane at a speed of 25 µm/s to cut the fibers to the desired length (here, all fibers are cut to 500 µm).

NOTE: Fibers will emit a bright light before being cut. Store the fibers after treatment until they are ready to be coated with a conductive polymer.

8. Poly(3,4-ethylenedioxythiophene):p-toluenesulfonate (PEDOT:pTS) conductive coating for lowered impedance

8.1. Mix solutions of 0.01 M 3,4-ethylenedioxythiophene and 0.1 M sodium p-toluenesulfonate in 50 mL of DI water and stir overnight on a stir plate (~450 rpm) or until no particulates can be observed in the solution.

NOTE: Store the solution in a light-resistant container. Refrigerate the solution after mixing to keep the solution useable for up to 30 days.

8.2. Run a 1 kHz impedance scan using the same parameters as before (steps 5.2–5.3) in 1x PBS. Note which fibers have a good connection (<1 MΩ, typically 14–16 of 16 fibers).

8.3. Electroplate with PEDOT:pTS to lower the impedance of the electrodes.

8.3.1. Submerge the fiber tips in PEDOT:pTS solution.

8.3.2. Follow the steps outlaid in step 5.2, switching the 1x PBS solution out for PEDOT:pTS and short all connections to the board to the applied current channel.

8.3.3. Apply 600 pA per good fiber for 600 s using a potentiostat.

8.3.4. Turn the cell off and allow it to rest for 5 s at the end of the run.

8.4. Remove the fibers from the solution and rinse them in DI water.

8.5. Retake 1 kHz impedances to check that the fibers were successfully coated (use the same parameters listed in steps 5.2–5.3).

NOTE: Good fibers are designated as any fiber having an impedance of less than 110 kΩ.

9. Connecting ground and reference wires

9.1. Gently scrape away Parylene C from the ground and reference vias on the board using tweezers. Short the ground and reference vias together in pairs on this board design.

NOTE: Ground and reference vias can be found near the connector on the Flex array and are the four small gold circles near the connectors. Users will only need to remove Parylene C from the vias closest to the carbon fibers for measurements.

9.2. Cut two 5 cm lengths of insulated silver wire with a razor blade. Deinsulate the ends of the wires 2–3 mm from one end to be attached to the Flex Array and ~10 mm from the opposite ends to allow for easier grounding and referencing during surgery.

9.3. Heat the soldering iron back to 600 °F. Apply a small amount of flux to the vias.

9.4. Insert one wire (2–3 mm exposed end) into each of the ePhys vias on the board. Apply solder to the top of the vias (**Figure 7A**). Allow the probe to cool, then flip it over to apply a small amount of solder to the backside of the via (**Figure 7A**).

9.5. Using surgical scissors, snip off any exposed wire sticking out of the back solder mound as this helps reduce noise seen in recording (**Figure 7B**).

9.6. Place the arrays back into the storage box, bending the wires back and away from the fiber. Secure the wires on the adhesive tape to prevent potential fiber-wire interactions (**Figure 7C**).

10. Surgical procedure

NOTE: Rat cortex was used to test the efficacy of the UV Laser-prepared fibers as this has been described previously[7,20]. These probes will work in nerve due to their similar geometry and impedance levels to blowtorch prepared fibers. This surgery was performed with an abundance of caution to validate that the UV laser did not change the response of the electrodes.

10.1. Anesthetize an adult male Long Evans rat using a combination of ketamine (90 mg/kg) and xylazine (10 mg/kg). Confirm anesthesia with a toe pinch test. Apply ointment to the eyes to prevent the rat's eyes from drying out during the surgery.

10.2. Create a 2 mm x 2 mm craniotomy above the right hemisphere's motor cortex. Identify the lower left corner of the craniotomy by measuring 1 mm anterior of bregma and 1 mm lateral of midline.

10.3. Mount the array into a stereotaxic instrument, and zero the stereotaxic instrument at the

dura by gently lowering the fibers until they touch the dura's surface. Raise the array away from the surgical site and move it to the side until it is ready for insertion.

10.4. Resect the dura by gently pulling a needle with a barbed end over the surface of the tissue. Once a portion of the dura opens to the brain, use a pair of fine forceps to further assist in pulling away the dura.

10.5. Insert the fibers into the craniotomy and 1.2 mm into the brain using a stereotaxic instrument, lowering slowly by hand.

10.6. Record ePhys data for 10 min with an ePhys-specific headstage and preamplifier.

10.6.1. Set the preamplifier high-pass filter to process the signal at 2.2 Hz, antialias at 7.5 kHz, and sample at 25 kHz.

NOTE: For these measurements, only spontaneous activity is recorded. No stimulus is applied.

10.7. Euthanasia

10.7.1. Place the rat under isoflurane at 5% under 1 L/min of oxygen until signs of life have ceased (20–30 min). Confirm euthanasia with decapitation.

11. Spike sorting

11.1. Use spike-sorting software to sort and analyze the data using previously reported methods[8].

11.2. Use a high-pass filter on all channels (250 Hz corner, 4^{th} order Butterworth), and set the waveform detection level to -3.5 × RMS threshold.

11.2.1. Use a Gaussian model to cluster and spikes with similar characteristics. Combine and average clusters of at least 10 waveforms to include in further analysis.

11.2.2. Eliminate or delete all waveforms that are not spikes from the data set.

11.3. Export data once all channels have been sorted and use analysis software to plot and further analyze the waveforms.

12. Scanning electron microscopic (SEM) imaging

NOTE: This step will render arrays unusable and should be used only to inspect tip treatment results to check that the arrays are being properly processed. This step does not need to be done to build a successful array. Summarized below is a general outline of the SEM process; however, users who have not previously used SEM should receive help from a trained user.

12.1. Snip off the fibered end of the PCB and mount it on a carbon tape-masked SEM stub. Place the arrays on a small platform of stacked carbon tape (4–5 layers) to prevent the carbon fibers from sticking to the SEM stub.

12.2. Sputter-coat the arrays with gold (100–300 Å) following procedures outlined by the manufacturer of the gold sputter coater.

12.3. To inspect the tip treatment effects, image the arrays in an SEM at a working distance of 15 mm and 20 kV beam strength.

NOTE: Arrays can be imaged without sputter-coating under a low vacuum, as shown in **Figure 8D** for UV laser-cut fibers. For this setup, it is recommended to have a working distance of 11–12 mm and a 4 kV beam strength.

REPRESENTATIVE RESULTS:

Tip validation: SEM images

Previous work[20] showed that scissor cutting resulted in unreliable impedances as Parylene C folded across the recording site. Scissor cutting is used here only to cut fibers to the desired length before processing with an additional finish cutting method. SEM images of the tips were used to determine the exposed carbon length and tip geometry (**Figure 8**).

Scissor and Nd:YAG laser-cut fibers were previously reviewed[17,20]. Scissor-cut fibers (**Figure 8A**) have inconsistent tip geometries, with Parylene C folding over the end when cut[20]. The Nd:YAG laser-cut fibers remain consistent in the recording site area, shape, and impedance (**Figure 8B**). Blowtorched fibers[20] lead to the largest electrode size and shape variability and a sharpened tip, allowing for insertion into tough tissue. On average, 140 µm of carbon was re-exposed, with a smooth transition area between the carbon and Parylene C insulation (**Figure 8C**). UV laser-cut fibers were similar to blowtorched fibers, showing 120 µm of carbon exposed from the tip (**Figure 8D**). Impedances indicated that either the UV laser or blowtorch tip cutting methods are suitable for ePhys and are viable solutions for laboratories without access to an Nd:YAG laser.

Tip validation: electrical recording

Figure 9 shows the resulting impedances from each preparation method using Flex Arrays. The resultant values are within an appropriate range for ePhys recording. Nd:YAG laser-cut fibers resulted in the smallest surface area but the highest impedances, even with the PEDOT:pTS coating (bare carbon: 4138 ± 110 kΩ; with PEDOT:pTS: 27 ± 1.15 kΩ; n = 262). This is followed by the inverse relationship in blowtorched (bare carbon: 308 ± 7 kΩ; with PEDOT:pTS: 16 ± 0.81 kΩ; n = 262) and UV laser-cut (bare carbon: 468 ± 85.7 kΩ; with PEDOT:pTS: 27 ± 2.83 kΩ; n = 7) fibers that have a large surface area and low impedances. However, in all cases, the PEDOT:pTS-coated fibers fall under the 110 kΩ threshold set previously to indicate a good, low impedance electrode.

Acute ePhys recordings were taken from a Long Evans rat acutely implanted with a ZIF array with UV laser-cut and PEDOT:pTS-treated fibers to demonstrate the viability of this method. ePhys has

previously been tested and proven with scissor-cut[20] and Nd:YAG-[17] and blowtorch-treated fibers[7,8] and so was not revalidated in this text. Acute recordings from four UV laser treatment fibers (2 mm in length) that were simultaneously implanted in rat motor cortex (n = 1) are presented in **Figure 10**. Three units were found across all fibers, suggesting that the treatment of the fibers with the inexpensive UV laser is similar to other cutting methods that enable the carbon fiber to record neural units, as would be expected by the SEMs and impedances. While carbon fiber arrays are easily built and modified to suit the user's needs, it should be noted that additional validation is necessary for some builds (**Table 3**), while others are less suitable for certain end tasks.

Commercial Parylene C

Commercially coated arrays were determined to have a Parylene C thickness of 710 nm by the vendor, well within the target range of insulation. The arrays were prepared for ePhys recordings using the blowtorch tip preparation. Impedances were taken after the preparation of the tips and compared to existing data. A blowtorched and PEDOT:pTS-coated probe had an average of 14.5 ± 1.3 kΩ impedance across 16 fibers. SEM images were taken of the tip and shank to compare Parylene C deposition (**Figure 11 A,B**, respectively). These results show that the use of a commercial vendor did not change the expected impedance values, suggesting that this will be an equally viable substitution to deposition in the university cleanroom.

Device cost analysis

Provided all tools and bulk materials (e.g., epoxies, solder) are accessible to the researcher, a Parylene C user fee of $41, and a batch of 8 probes, the total materials cost is $1168 ($146 per probe). Personnel effort (**Table 4**) is ~25 h for the batch. If using a substituted fabrication step, the cost of the probes will vary based on commercial Parylene C coating cost ($500–800 quoted). The time for the build steps (**Table 4**) is grouped for all instances of a repeated task for simplicity. Build times for designs with a larger pitch (Wide Board and ZIF) are dramatically reduced as the manually intensive steps (e.g., carbon fiber placement) are easier and faster to complete.

FIGURE AND TABLE LEGENDS:

Figure 1: Connectors and associated printed circuit boards. (**A**) Wide Board with one of sixteen necessary connectors in inset (inset scale bar = 5 mm). (**B**) ZIF and one of two connectors and one shroud. (**C**) Flex Array with a 36-pin connector; scale bar = 1 cm.

Figure 2: Soldering and insulation steps for the Flex Array. (**A**) Laying the solder for the bottom connector pins. (**B**) Back pins secured in place with the front pins ready for soldering. (**C**) Delayed set epoxy insulated Flex Array; note that the delayed-set epoxy does not cover the reference and ground vias on either side. (**D**) Backside of the Flex Array with a band of delayed set epoxy across the pad vias (not the ground and reference vias) and wrapped around the side of the board toward the edge of the connector. Scale bar = 0.5 cm (**B**) and 1 cm (**A, C, D**).

Figure 3: Applying silver epoxy and aligning carbon fibers between the traces of the Flex Array. Capillaries have been highlighted with a white overlay. (**A**) The end of the capillary fits between

the traces to get (**B**) clean silver epoxy (denoted with arrows at the end of the capillary and within the traces) deposition without spillover outside of the trace pairs. (**C**) Carbon fibers are placed into the epoxy and then (**D**) straightened with a clean capillary. Scale bars = 500 µm.

Figure 4: Insulation with UV Epoxy Application (**A**) UV epoxy is applied using a clean capillary and two drops of UV epoxy (marked with white overlays). UV epoxy is applied in droplets of 0.25–0.75 mm diameters until the UV epoxy forms a smooth bubble over the top of the traces. (**B**) UV epoxy is cured under UV light. The Flex Array is placed in putty on a wooden block for ease of movement and alignment underneath the UV light. The UV light is held with a holder ~1 cm above the end of the Flex Array. Inset (**B**) shows the side profile of a properly UV epoxy-insulated Flex Array. The UV epoxy bubble on either side of the board is roughly 50 µm in height. Scale bars = 500 µm (**A** and inset **B**).

Figure 5: Setup for impedance measurements. All parts are labeled, and system connectors and adapters are system-dependent. PBS is starred as the solution is swapped for PEDOT:pTS later on in the build; however, the setup is identical otherwise. Abbreviations: PBS = phosphate-buffered saline; PEDOT:pTS = poly(3,4-ethylenedioxythiophene):p-toluenesulfonate.

Figure 6: Flex Array prepared for Parylene C coating. The Flex Array is secured to a raised foam platform with tape, adhesive side up during the coating process. Scale bar =10 mm.

Figure 7: Ground and reference wires attached to the finalized Flex Array. Solder was applied to each side of the via on either side of the board (**A**) to create a secure bond. ePhys vias are labeled on the board as **GND** and **Ref** and paired on opposite sides of the board from one another. There are two additional vias also labeled **GND** and **Ref2**. Both **GND** vias are shorted together. **Ref2** is meant to be used in electrochemical experiments. Excess wire in (**A**) is denoted with a red box and is removed (**B**) from the backside of the probe (red box shows where wire used to be) to help with noise reduction and handling the probe. (**C**) Final Flex Array stored for future use. Note that the paired **GND** and **Ref** vias on this board make it designated for ePhys recordings. Scale bars = 200 µm (**A, B**). Abbreviations: ePhys = electrophysiology; GND = ground; Ref = reference.

Figure 8: SEM images of fibers with different tip-cutting techniques. (**A**) Scissor-cut fiber with very little exposed carbon. (**B**) Nd:YAG laser cut. (**C**) Blowtorched fiber with ~140 mm of carbon exposed from the tip. (**D**) UV laser-cut fibers with ~120 mm of carbon exposed from the tip. Red arrows indicate the transition area between Parylene C and bare carbon fiber. Scale bars = 5 µm (**A**), 10 µm (**B**), 50 µm (**C, D**). Abbreviations: SEM = scanning electron microscopic; Nd:YAG = Neodymium-doped yttrium aluminum garnet.

Figure 9: Impedance differences between only applying the treatment (bare carbon exposed) and with the addition of PEDOT:pTS. In all cases, the addition of PEDOT:pTS decreases the impedance by an order of magnitude. Sample size: Nd:YAG = 262, Blowtorch = 262, UV = 7. UV sample size difference is due to the novelty of the preparation method; however, it shows a similar range to blowtorch, as expected. Impedance data are expressed as mean ± standard error. Abbreviations: PEDOT:pTS = poly(3,4-ethylenedioxythiophene):p-toluenesulfonate;

Neodymium-doped yttrium aluminum garnet.

Figure 10: Acute electrophysiological spiking data from four UV laser-cut electrodes.

Figure 11: Commercial Parylene C-coated arrays. (**A**) The sharpened array shows uniform sharpening across all fibers indicating that there are no drawbacks to commercial coating. (**B**) After blowtorching, the transition (red box) between bare carbon fiber and Parylene C shows no discernable difference between arrays coated in a cleanroom facility. Scale bars = 200 µm (**A**) and 10 µm (**B**).

Table 1: Each PCB has a different connector and pitch associated with it. Abbreviation: PCB = printed circuit board.

Table 2: Typical range of impedances after each build stage (n = 272). *n = 16. PEDOT:pTS-treated probes above 110 kΩ may still record signals; however, all treated electrodes typically fall under this value. Abbreviations: PEDOT:pTS = poly(3,4-ethylenedioxythiophene):p-toluenesulfonate; Neodymium-doped yttrium aluminum garnet.

Table 3: Validated uses of each board with the cutting methods described. All cutting methods included electrodeposition of PEDOT:pTS. 'Not Viable' indicates that a form factor of the design prevents this tip treatment from being tested at this time (i.e., fiber pitch). Abbreviations: Neodymium-doped yttrium aluminum garnet; SEM = scanning electron microscopy; ePhys = electrophysiology; ZIF = zero insertion force.

Table 4: Time required for each step of a fabrication process. Soldering of the connector and ground and reference wires have been combined here to simplify the activity list. Abbreviations: PEDOT:pTS = poly(3,4-ethylenedioxythiophene):p-toluenesulfonate; Neodymium-doped yttrium aluminum garnet.

DISCUSSION:

Material substitutions

While all materials used are summarized in the **Table of Materials**, very few of the materials are required to come from specific vendors. The Flex Array board must come from the listed vendor as they are the only company that can print the flexible board. The Flex Array connector must also be ordered from the vendor listed as it is a proprietary connector. Parylene C is highly recommended as the insulation material for the fibers as it provides a conformal coating at room temperature in a reliable manner that can then withstand the *in vivo* environment. The polyimide board and epoxies on the board cannot tolerate the high temperatures required for other insulation techniques. All other materials can be purchased from other vendors or be swapped out for alternatives at the users' discretion. This build is meant to be flexible and customizable to fit the end user's experiment. However, it should be noted that any changes from the materials or vendors listed must be validated by the end user.

Troubleshooting build issues

Silver epoxy deposition tends to fail for several reasons: the width of the capillary is too wide to fit between traces, the width of the capillary is too thin to pick up and deposit epoxy, or an excess of epoxy is on the capillary. The first two problems can be solved by cutting a new capillary of a more appropriate size; the latter by dipping the capillary into the epoxy with a lighter hand or removing a portion of the epoxy blob by gently dabbing the capillary onto a spare nitrile glove.
Deciding how to prepare the electrode is often a difficult decision for many users. However, determining what is needed for the experiment will help illuminate the decision. For acute surgeries, blunt tips can be used if the site size of the electrode is important; however, they will only insert into softer tissue (brain) and only at sub-500 µm target depths.

Going into deeper brain structures is possible using a glass cannula[22]; however, this can cause scarring and associated unreliability in ePhys recordings. Fibers must be less than 300 µm when sharpened to be able to penetrate harder tissues (nerve) as the shorter length provides a stiffer backbone for insertion[7,8]. Sharpened fibers have also recently been observed to penetrate to 1 mm depths in the brain[8].

While the arrays discussed in this paper are an excellent starting point for many labs, newer probes using carbon fibers have also been developed to chronically target deeper areas in brain[21,22,29]. In nerve, electrodes of low invasiveness and high selectivity are an ongoing research topic[5,8,30]. Jiman et al.[7] were able to detect multiunit activity within the nerve with minimal invasiveness and increased selectivity using a carbon fiber silicone array[8], which mirrors the design of the Flex Array presented here.

Parylene C accessibility

Parylene C is a method of conformal coating at room temperature that has been used as a biocompatible insulator in many implanted devices. The technique requires a specialized tool in a cleanroom and takes about an hour to learn. A cursory survey of institutions that have previously requested carbon fiber arrays from our group was conducted to determine Parylene C deposition accessibility. We found that out of 17 institutes, 41% had access to Parylene C-coating systems on their campus. For universities without access to a Parylene C-coating system, commercial coating services are a viable alternative, as demonstrated here. Alternatively, outsourcing to a nearby university cleanroom may also be of interest to laboratories with no direct access to a Parylene C deposition system. To reduce the cost per device, we advise sending out larger batches of arrays as commercial systems can often accommodate larger samples.

Optimizing tip preparations

Additional tip preparations need to be investigated for these fibers as the current tip preparations require the end user to choose between penetrating ability and a small recording site. While the Nd:YAG laser-cut fibers provide a small site size[20], the ability to penetrate stiffer tissue (muscle, nerve) is almost non-existent, and access to a laser setup capable of this cutting technique can be difficult and expensive. While blowtorching allows for a quick and economical way to get sharpened tips that can penetrate many tissues[7], the tip geometry is large and may be inconsistent from fiber to fiber[20]. UV laser cutting also provides low impedances and large surface areas but with the added benefit of more consistent exposure. The UV laser is more

accessible than the Nd:YAG laser; however, laboratories would need to engineer a way to align the laser with fibers and would not be able to use the Flex Array due to the pitch of the fibers being smaller than the laser's focal point diameter. Previous work showed the fabrication of small, sharpened fibers via etching[31,32]. This approach could result in a small, reliable electrode geometry and preserve the sharpened tip necessary for penetrating nerve and muscle.

Our current tip coating, PEDOT:pTS, may also need to be replaced as it tends to degrade over time, which is an undesirable trait for a chronic probe[17,25,33]. A lack of PEDOT:pTS longevity leads to higher impedances and, therefore, lower signal quality, in part due to increased background noise. To increase longevity in these fiber tips, investigation into the feasibility of platinum-iridium coatings is being conducted. Platinum-iridium would allow for a greater surface area[25,34] concentrated on the tip of the electrode, keeping a low impedance[34–36] and allow for longer, chronic stability[34,36]. Other coatings, such as PEDOT/graphene oxide[37] and gold[38], have been utilized to lower carbon fiber electrode impedances, although these coatings are typically used for chemical-sensing probes rather than for ePhys recordings. Due to the inherent properties of carbon fibers[39], the carbon fiber array presented here can be converted from a probe optimized for ePhys to a chemical-sensing device with a simple change of tip preparation[22,40].

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DISCLOSURES:

The authors declare that they have no competing financial interests.

REFERENCES:

1. Szostak, K. M., Grand, L., Constandinou, T. G. Neural interfaces for intracortical recording: Requirements, fabrication methods, and characteristics. *Frontiers in Neuroscience*. **11**, 665 (2017).
2. Cunningham, J. P. et al. A closed-loop human simulator for investigating the role of feedback control in brain-machine interfaces. *Journal of Neurophysiology*. **105** (4), 1932–1949 (2011).
3. Yoshida, K., Bertram, M. J., Hunter Cox, T. G., Riso, R. R. Peripheral nerve recording electrodes and techniques. in *Neuroprosthetics: Theory and Practice*. Horch, K., Kipke, D. (Eds), World Scientific, 377–466 (2017).
4. Dweiri, Y. M., Stone, M. A., Tyler, D. J., McCallum, G. A., Durand, D. M. Fabrication of high contact-density, flat-interface nerve electrodes for recording and stimulation applications. *Journal of Visualized Experiments: JoVE.* (116), 54388 (2016).

5. Kim, H. et al. Cuff and sieve electrode (CASE): The combination of neural electrodes for bi-directional peripheral nerve interfacing. *Journal of Neuroscience Methods*. **336**, 108602 (2020).
6. Ciancio, A. L. et al. Control of prosthetic hands via the peripheral nervous system. *Frontiers in Neuroscience.* **10**, 116 (2016).
7. Jiman, A. A. et al. Multi-channel intraneural vagus nerve recordings with a novel high-density carbon fiber microelectrode array. *Scientific Reports*. **10** (1), 15501 (2020).
8. Welle, E. J. et al. Sharpened and mechanically robust carbon fiber electrode arrays for neural interfacing. *IEEE Transactions on Neural Systems and Rehabilitation Engineering*. **29**, 993–1003 (2021).
9. Moffitt, M. A., McIntyre, C. C. Model-based analysis of cortical recording with silicon microelectrodes. *Clinical Neurophysiology*. **116** (9), 2240–2250 (2005).
10. Ardiem Medical. Neural cuff. http://www.ardiemmedical.com/neural-cuff/ (2021).
11. Micro-Leads Neuro. Nerve-cuff electrodes. https://www.microleadsneuro.com/research-products/?jumpto=nerve-cuff (2021).
12. Mortimer, J. T. et al. Perspectives on new electrode technology for stimulating peripheral nerves with implantable motor prostheses. *IEEE Transactions on Rehabilitation Engineering*. **3** (2), 145–154 (1995).
13. Boretius, T. et al. A transverse intrafascicular multichannel electrode (TIME) to interface with the peripheral nerve. *Biosensors & Bioelectronics.* **26** (1), 62–69 (2010).
14. Grill, W. M., Norman, S. E., Bellamkonda, R. V. Implanted neural interfaces : biochallenges and engineered solutions. *Annual Review of Biomedical Engineering.* **11**, 1–24 (2009).
15. Larson, C. E., Meng, E. A review for the peripheral nerve interface designer. *Journal of Neuroscience Methods*. **332**, 108523 (2020).
16. Christensen, M. B. et al. The foreign body response to the Utah Slant Electrode Array in the cat sciatic nerve. *Acta Biomaterialia*. **10** (11), 4650–4660 (2014).
17. Patel, P. R. et al. Chronic in vivo stability assessment of carbon fiber microelectrode arrays. *Journal of Neural Engineering.* **13** (6), 066002 (2016).
18. Yoshida Kozai, T. D. et al. Ultrasmall implantable composite microelectrodes with bioactive surfaces for chronic neural interfaces. *Nature Materials*. **11** (12), 1065–1073 (2012).
19. Saito, N. et al. Application of carbon fibers to biomaterials: A new era of nano-level control of carbon fibers after 30-years of development. *Chemical Society Reviews.* **40** (7), 3824–3834 (2011).
20. Welle, E. J. et al. Fabrication and characterization of a carbon fiber peripheral nerve electrode appropriate for chronic recording. *FASEB Journal*. **34** (S1), 1–1 (2020).
21. Guitchounts, G., Cox, D. 64-Channel carbon fiber electrode arrays for chronic electrophysiology. *Scientific Reports*. **10** (1), 3830 (2020).
22. Patel, P. R. et al. High density carbon fiber arrays for chronic electrophysiology, fast scan cyclic voltammetry, and correlative anatomy. *Journal of Neural Engineering*. **17** (5), 056029 (2020).
23. Massey, T. L. et al. Open-source automated system for assembling a high-density microwire neural recording array. *2016 International Conference on Manipulation, Automation and Robotics at Small Scales (MARSS).* 1–7 (2016).
24. Schwerdt, H. N. et al. Subcellular probes for neurochemical recording from multiple brain sites. *Lab Chip*. 17, 1104–1115 (2017).

25. Welle, E. J. et al. Ultra-small carbon fiber electrode recording site optimization and improved in vivo chronic recording yield. *Journal of Neural Engineering*. **17** (2), 026037 (2020).
26. Guitchounts, G., Markowitz, J. E., Liberti, W. A., Gardner, T. J. A carbon-fiber electrode array for long-term neural recording. *Journal of Neural Engineering*. **10** (4), 046016 (2013).
27. Gillis, W. F. et al. Carbon fiber on polyimide ultra-microelectrodes. *Journal of Neural Engineering*. **15** (1), 016010 (2018).
28. Dong, T., Chen, L., Shih, A. Laser sharpening of carbon fiber microelectrode arrays for brain recording. *Journal of Micro and Nano-Manufacturing*. **8** (4), 041013 (2020).
29. Massey, T. L. et al. A high-density carbon fiber neural recording array technology. *Journal of Neural Engineering.* **16** (1), 016024 (2019).
30. Romeni, S., Valle, G., Mazzoni, A., Micera, S. Tutorial: a computational framework for the design and optimization of peripheral neural interfaces. *Nature Protocols*. **15** (10), 3129–3153 (2020).
31. Khani, H., Wipf, D. O. Fabrication of tip-protected polymer-coated carbon-fiber ultramicroelectrodes and pH ultramicroelectrodes. *Journal of The Electrochemical Society*. **166** (8), B673–B679 (2019).
32. El-Giar, E. E. D. M., Wipf, D .O. Preparation of tip-protected poly(oxyphenylene) coated carbon-fiber ultramicroelectrodes. *Electroanalysis*. **18** (23), 2281–2289 (2006).
33. Venkatraman, S. et al. In vitro and in vivo evaluation of PEDOT microelectrodes for neural stimulation and recording. *IEEE Transactions on Neural Systems and Rehabilitation Engineering*. **19** (3), 307–316 (2011).
34. Petrossians, A. et al. Electrodeposition and Characterization of Thin-Film Platinum-Iridium Alloys for Biological Interfaces. *Journal of the Electrochemical Society.* **158** (6), D269–D276 (2011).
35. Lee, C. D., Hudak, E. M., Whalen, J. J., Petrossians, A., Weiland, J. D. Low-impedance, high surface area Pt-Ir electrodeposited on cochlear implant electrodes. *Journal of The Electrochemical Society*. **165** (12), G3015–G3017 (2018).
36. Cassar, I. R. et al. Electrodeposited platinum-iridium coating improves in vivo recording performance of chronically implanted microelectrode arrays. *Biomaterials*. **205**, 120–132 (2019).
37. Taylor, I. M. et al. Enhanced dopamine detection sensitivity by PEDOT/graphene oxide coating on in vivo carbon fiber electrodes. *Biosensors and Bioelectronics*. **89** (Pt 1), 400–410 (2017).
38. Mohanaraj, S. et al. Gold nanoparticle modified carbon fiber microelectrodes for enhanced neurochemical detection. *Journal of Visualized Experiments: JoVE*. **2019** (147), 59552 (2019).
39. Pusch, J., Wohlmann, B. Chapter 2 - Carbon fibers. in *Inorganic and composite fibers*. *Production, properties, and applications.* Woodhead Publishing, 31–51 (2019).
40. Budai, D., Hernádi, I., Mészáros, B., Bali, Z.K., Gulya, K. Electrochemical responses of carbon fiber microelectrodes to dopamine in vitro and in vivo. *Acta Biologica Szegediensis*. **54** (2), 155–160 (2010).

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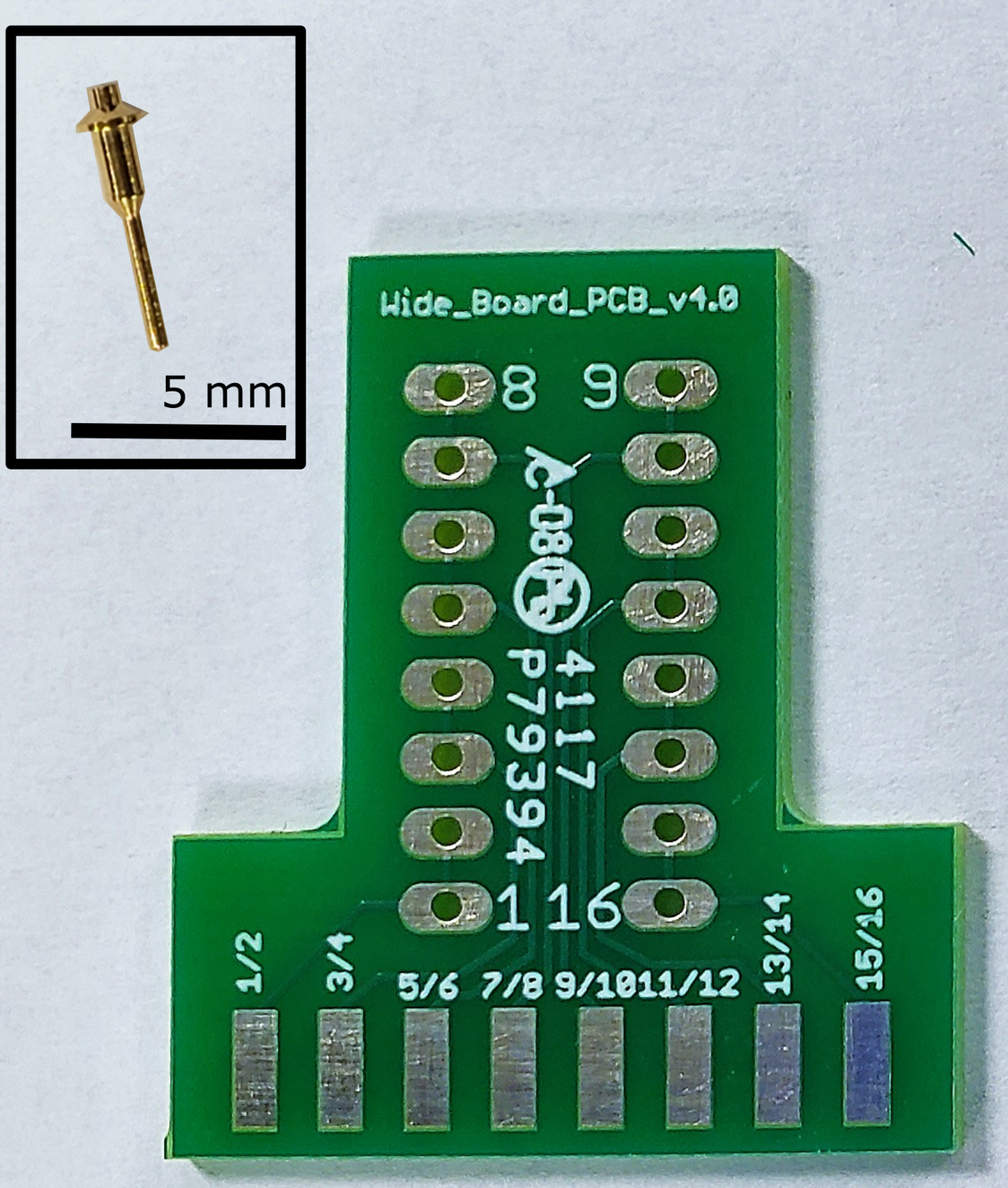

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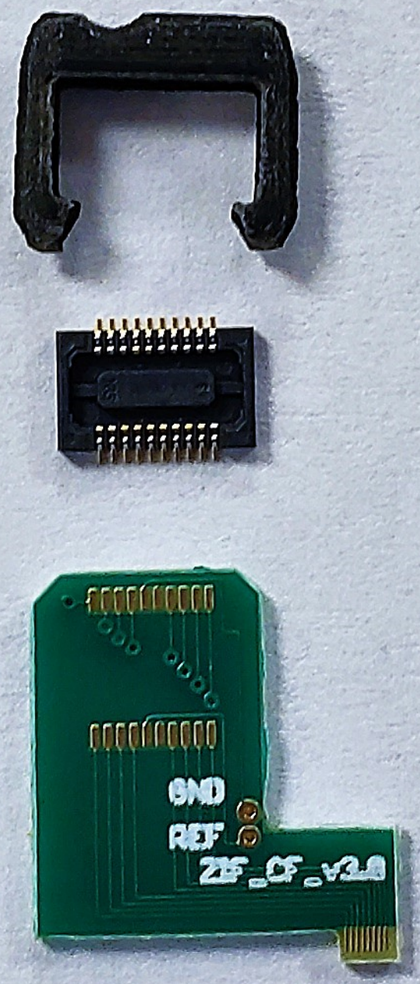

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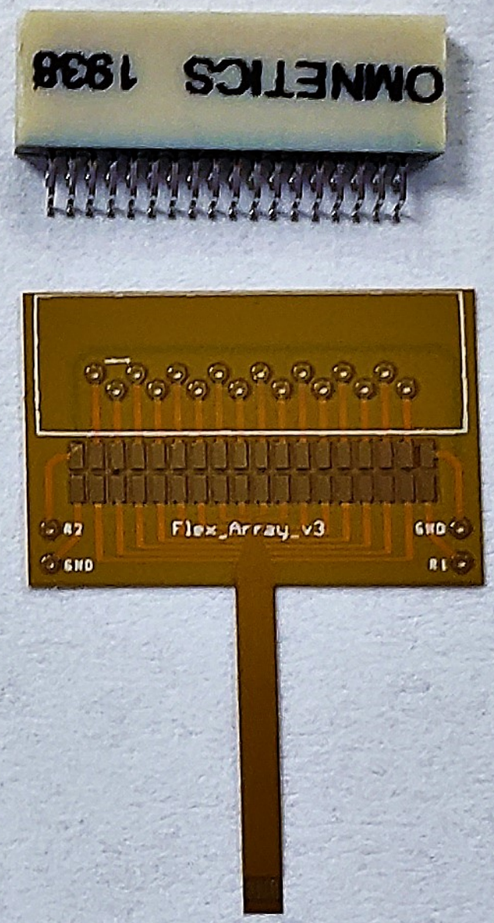

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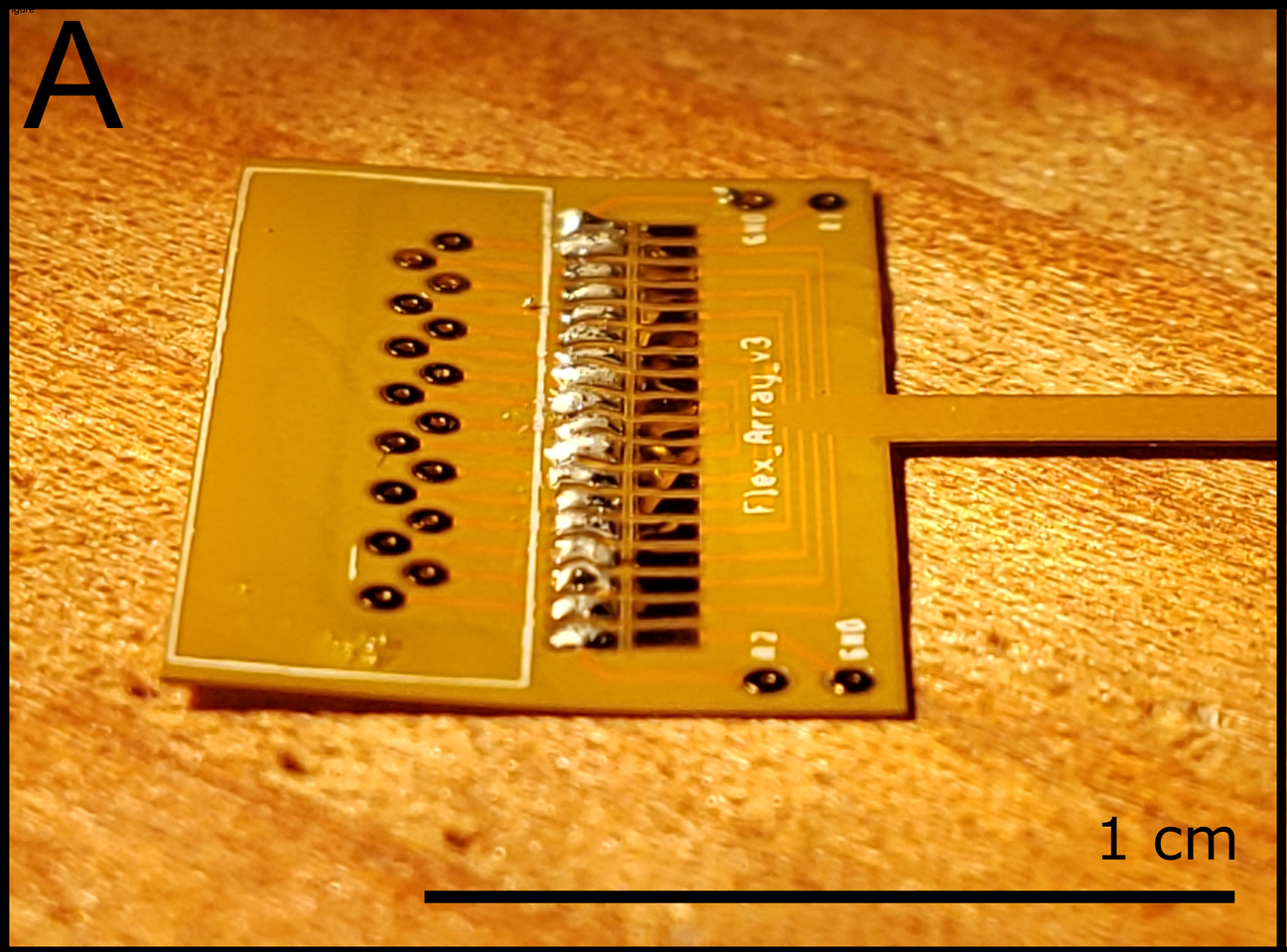
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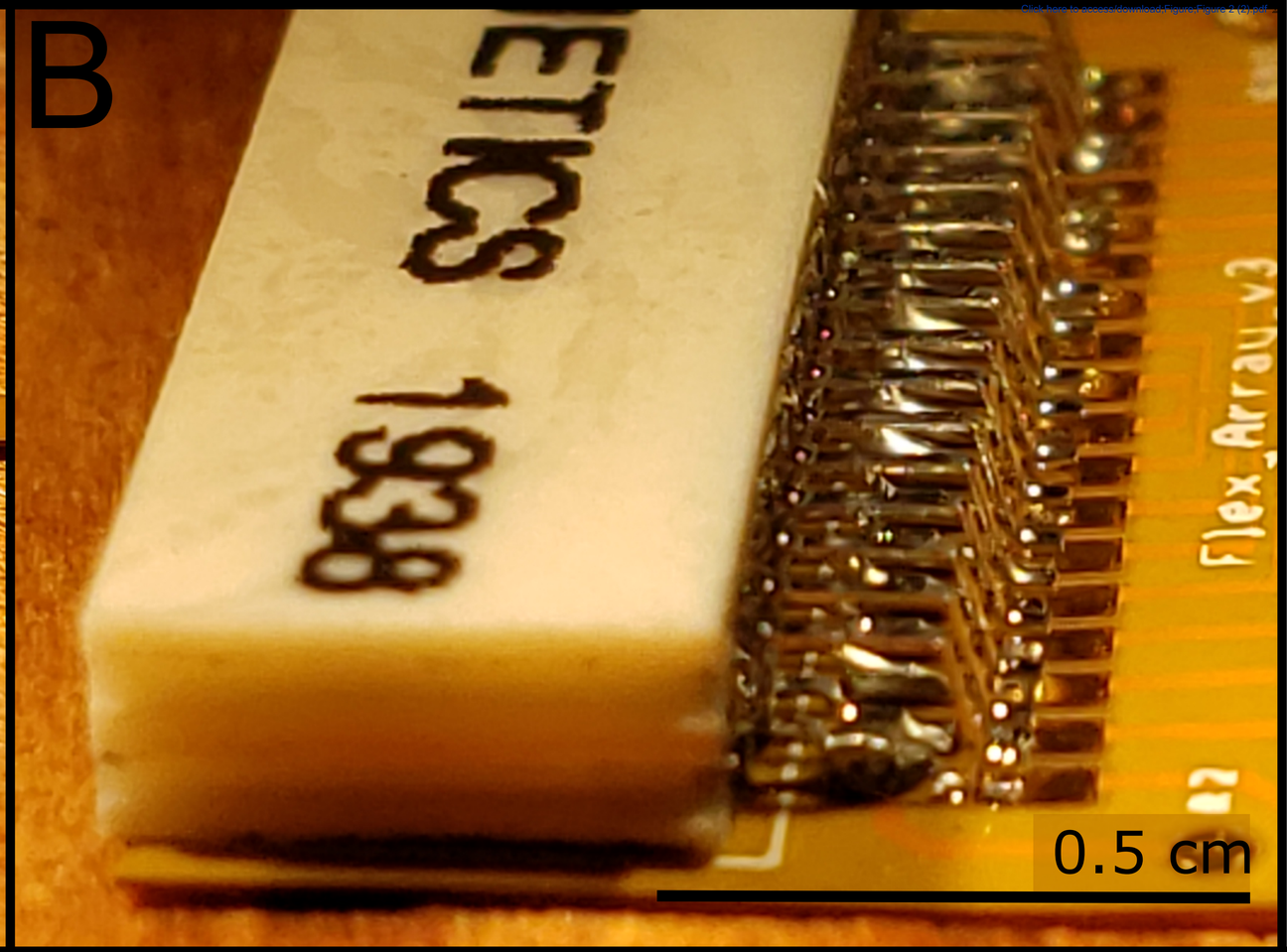
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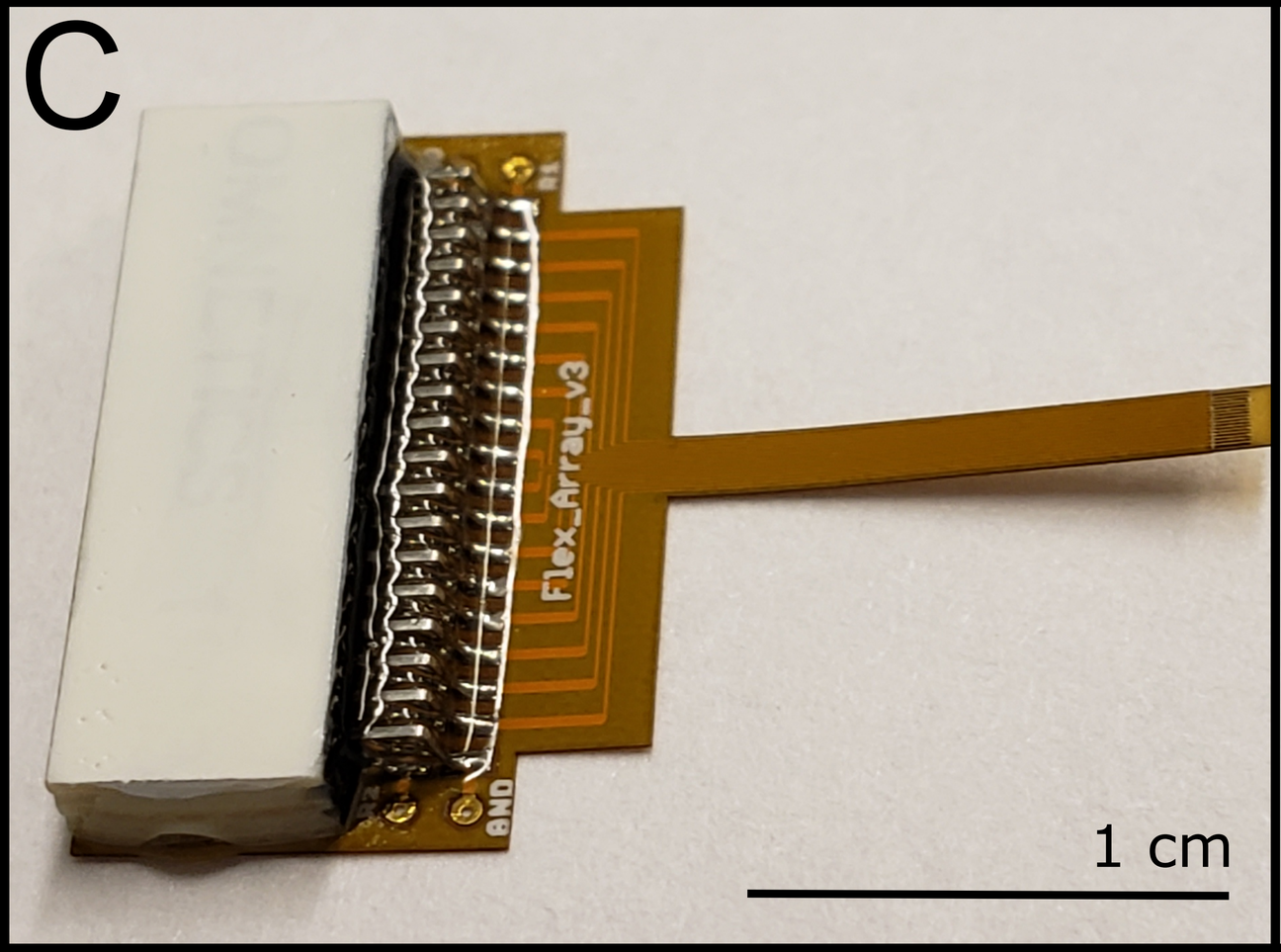
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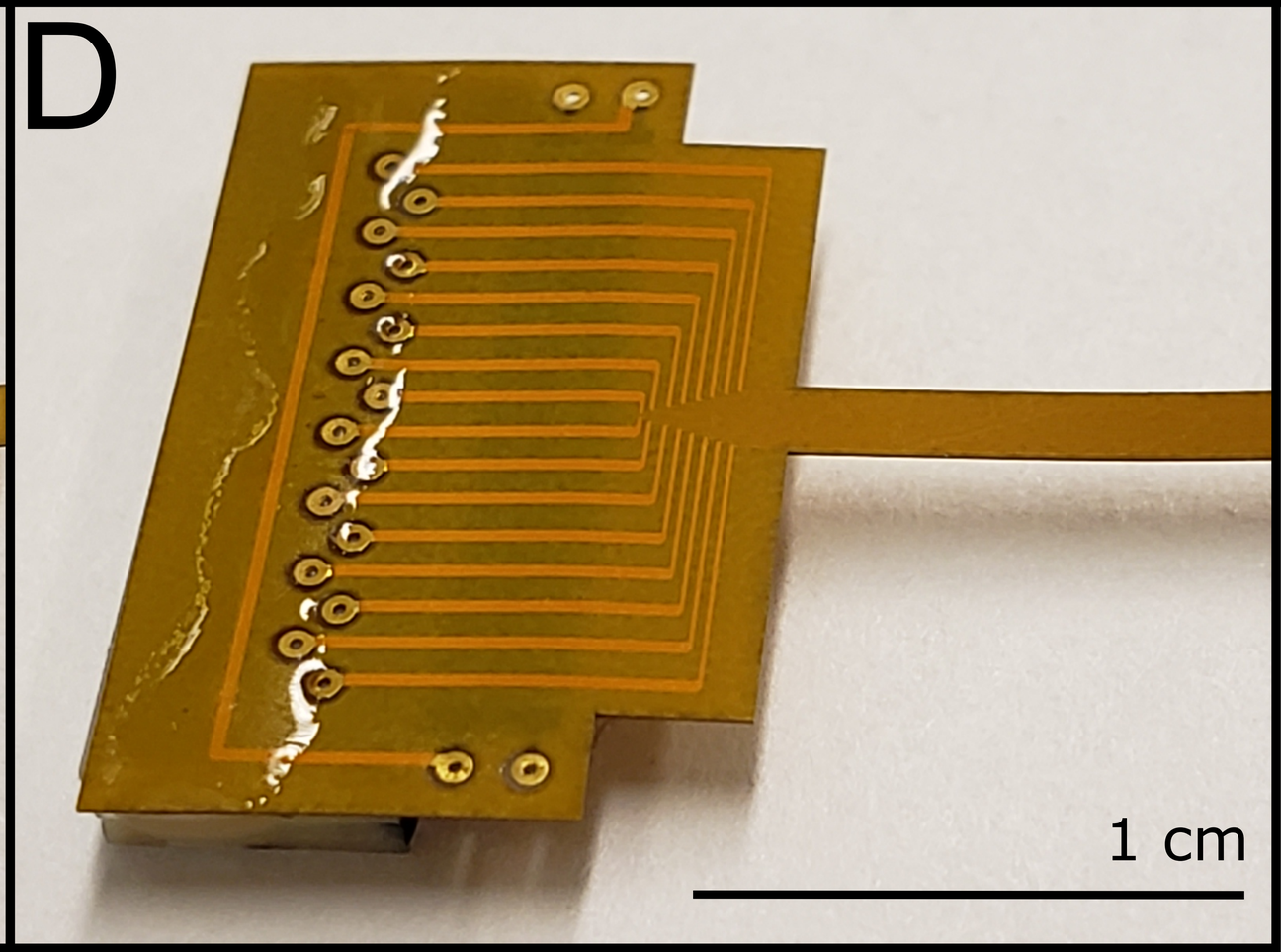
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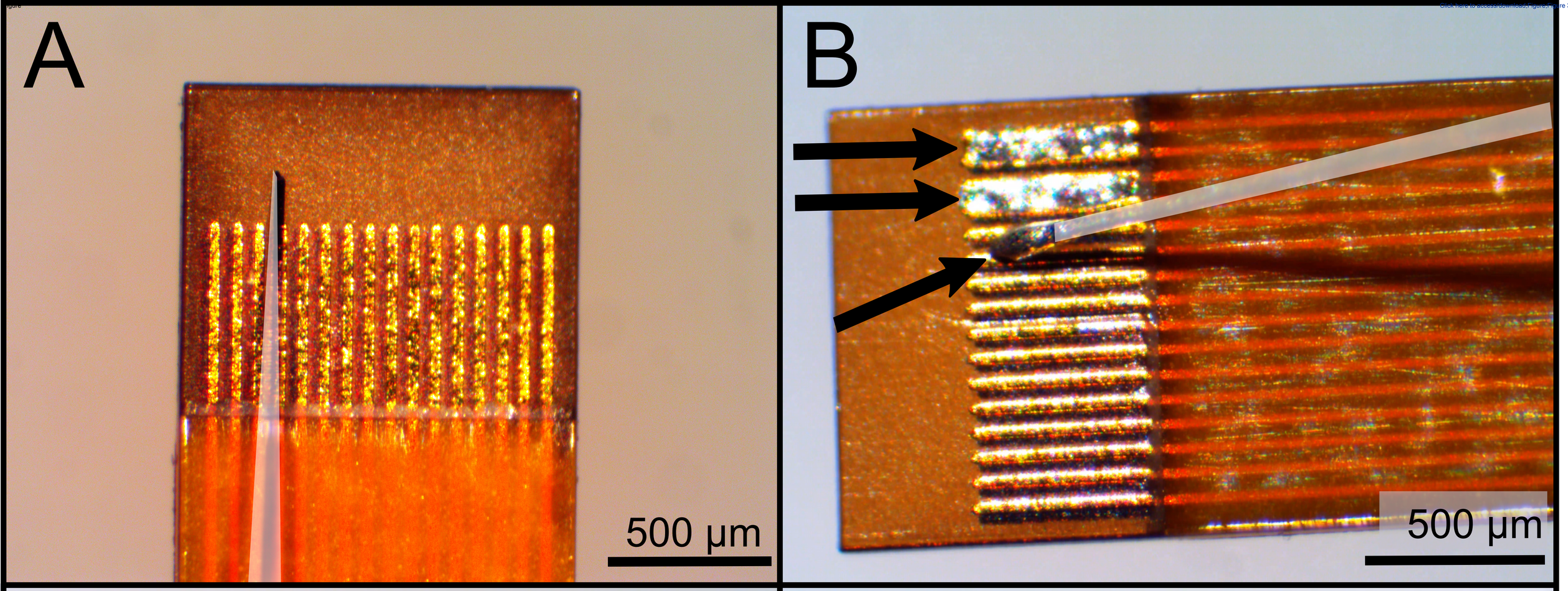

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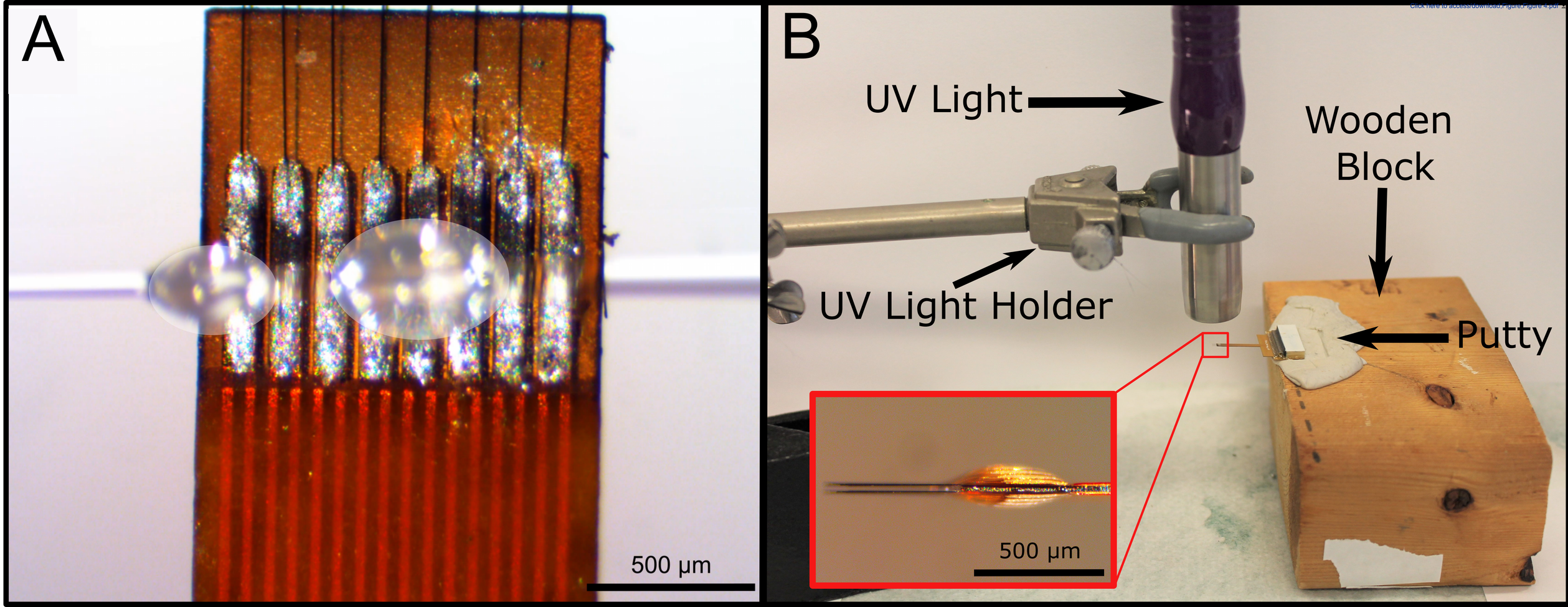

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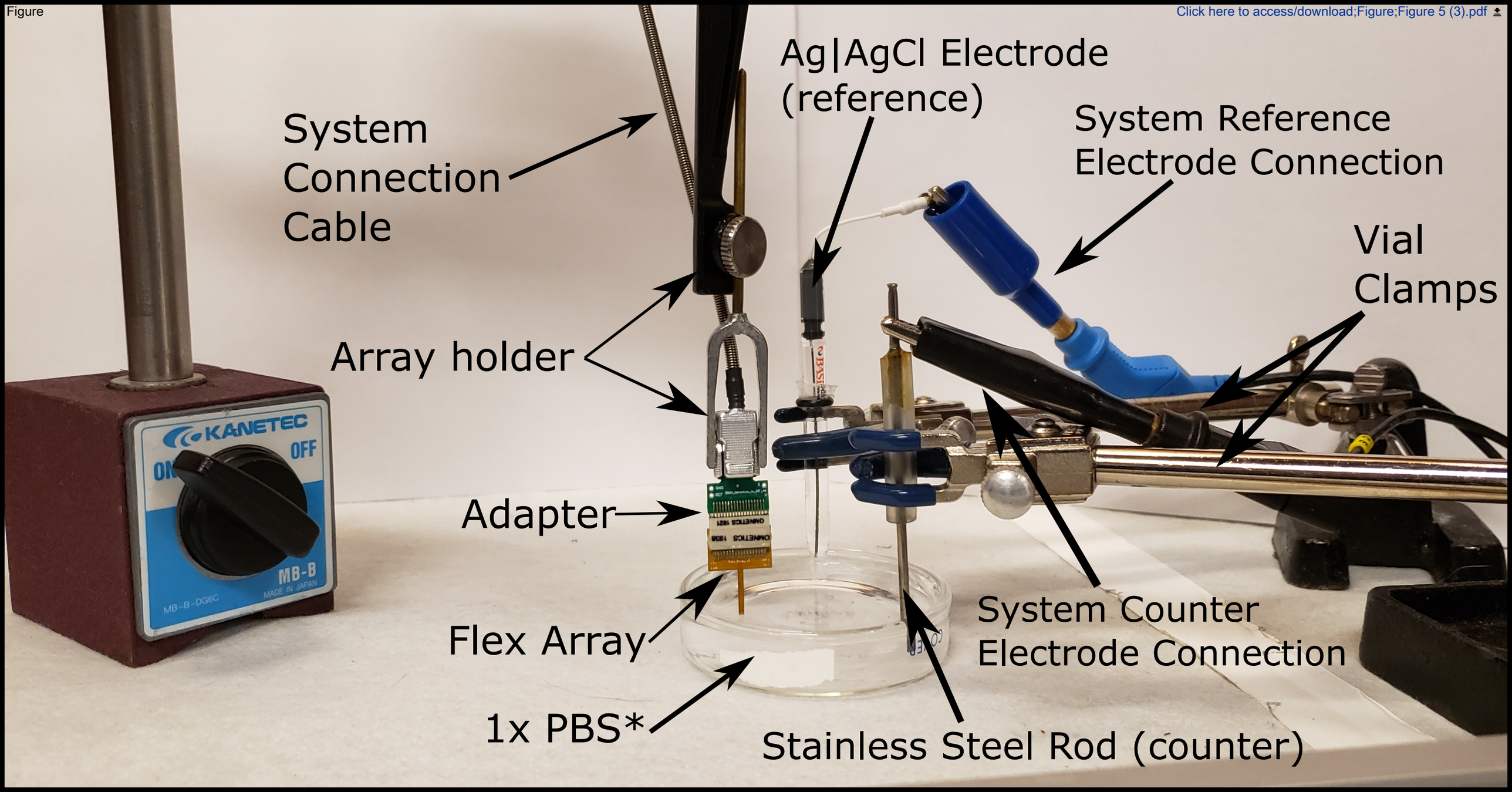

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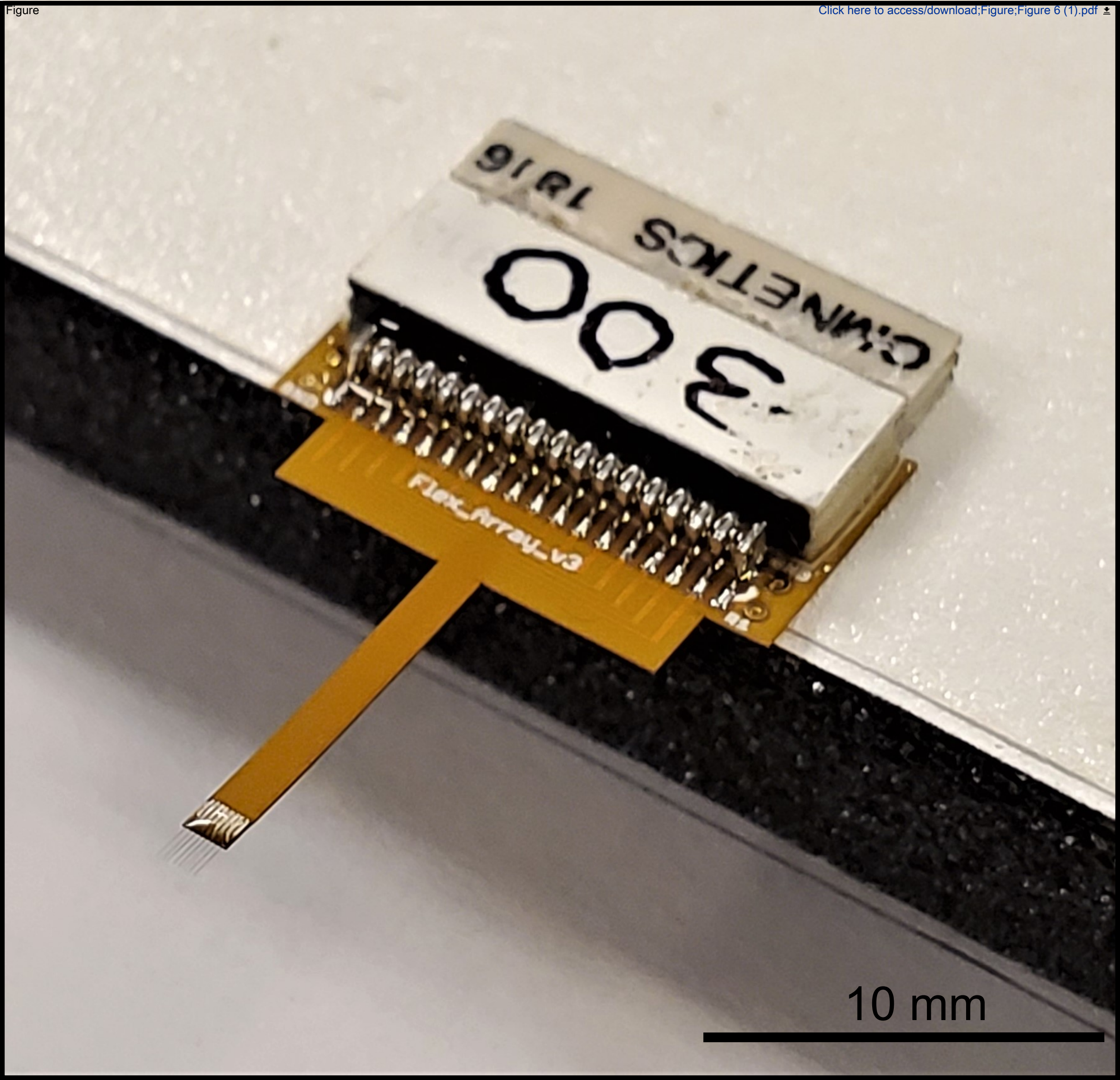

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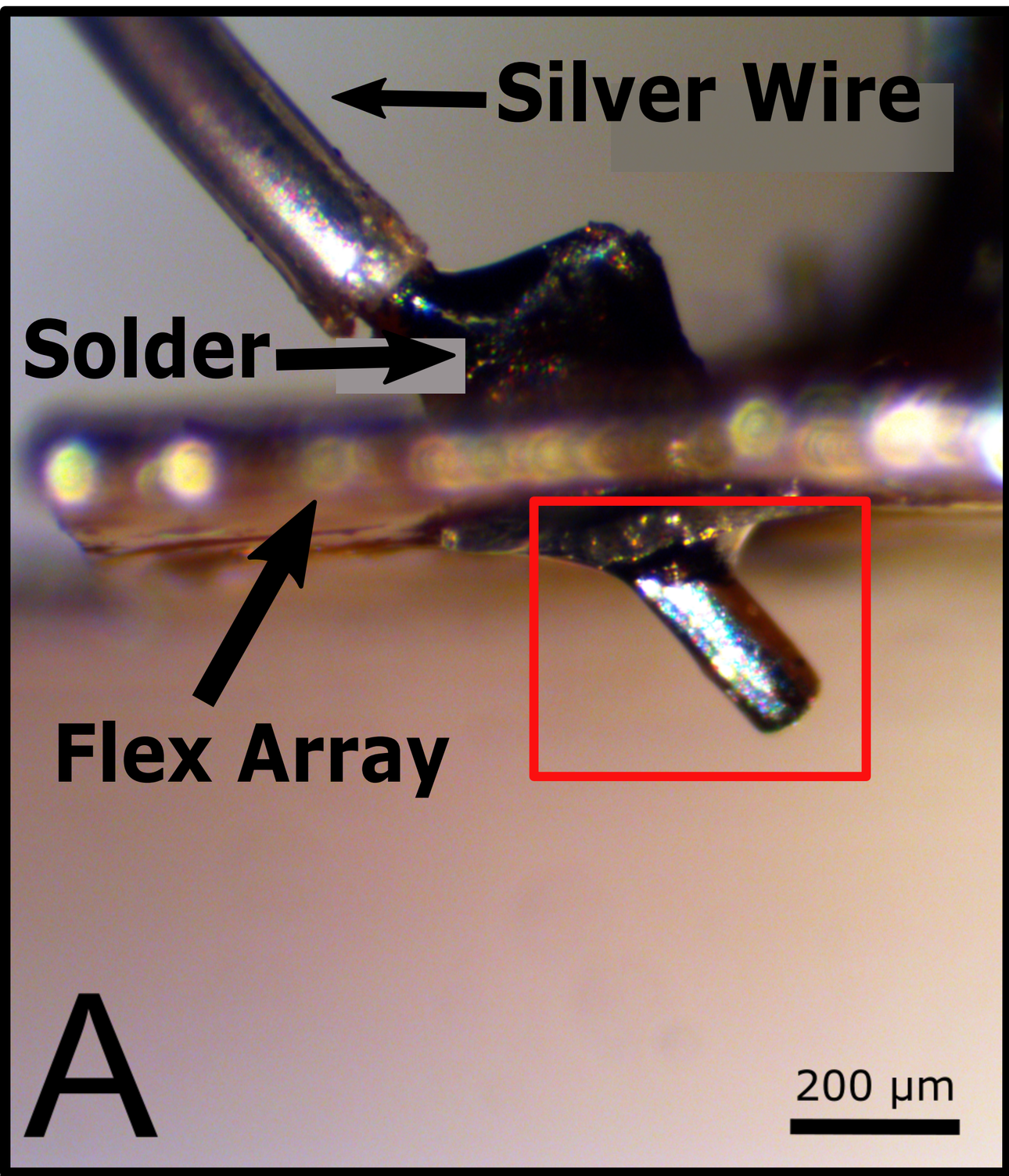

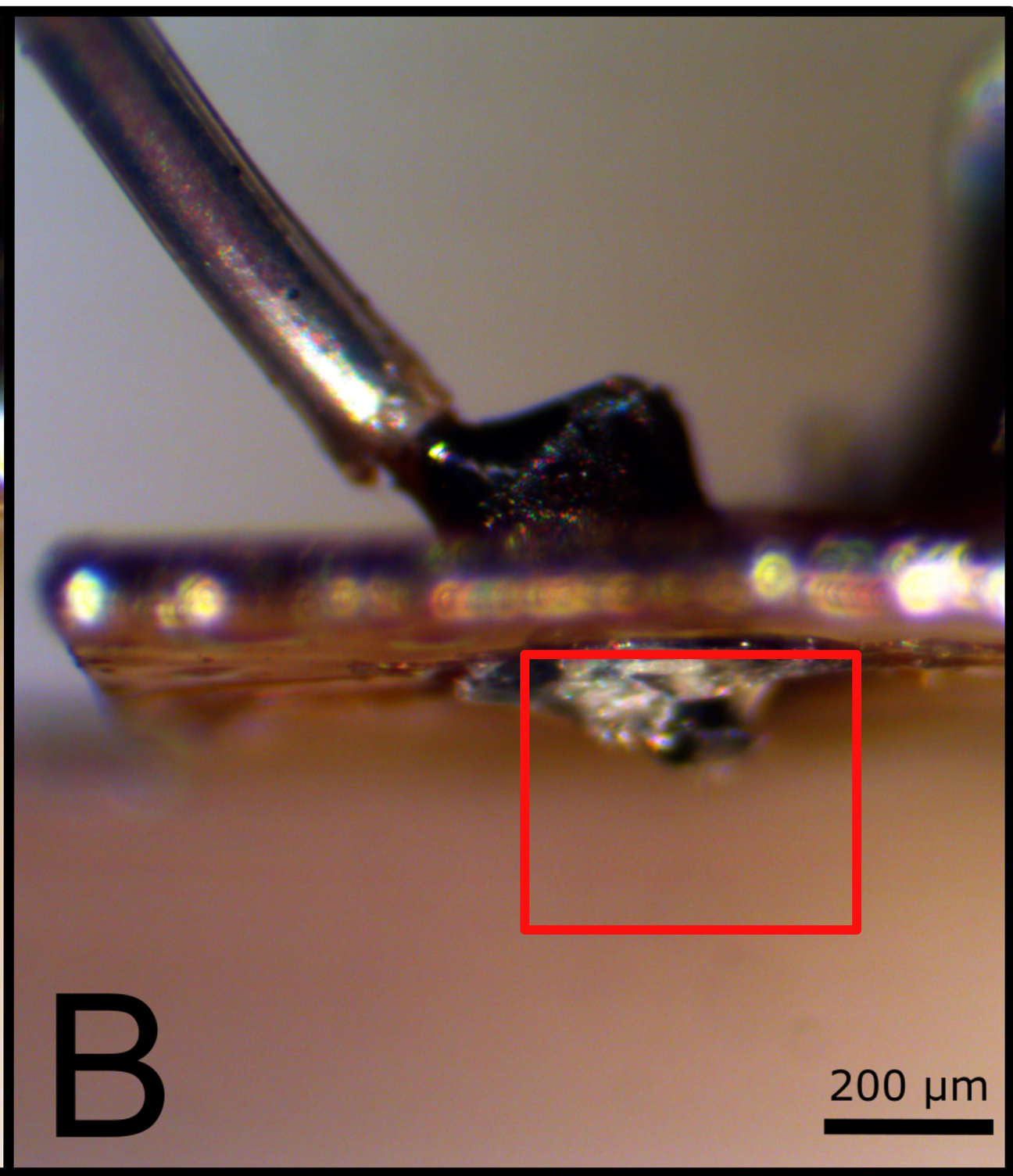

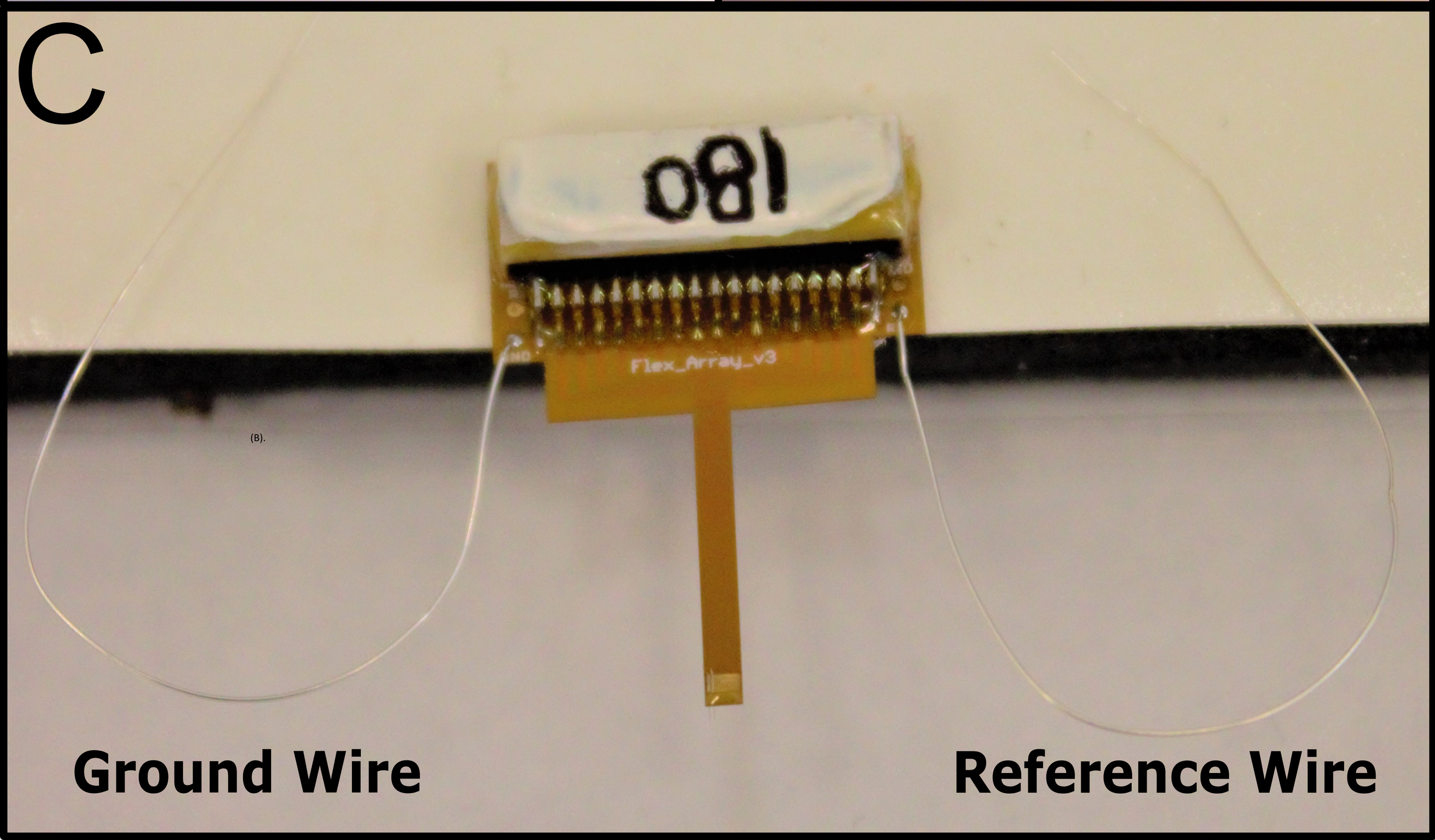

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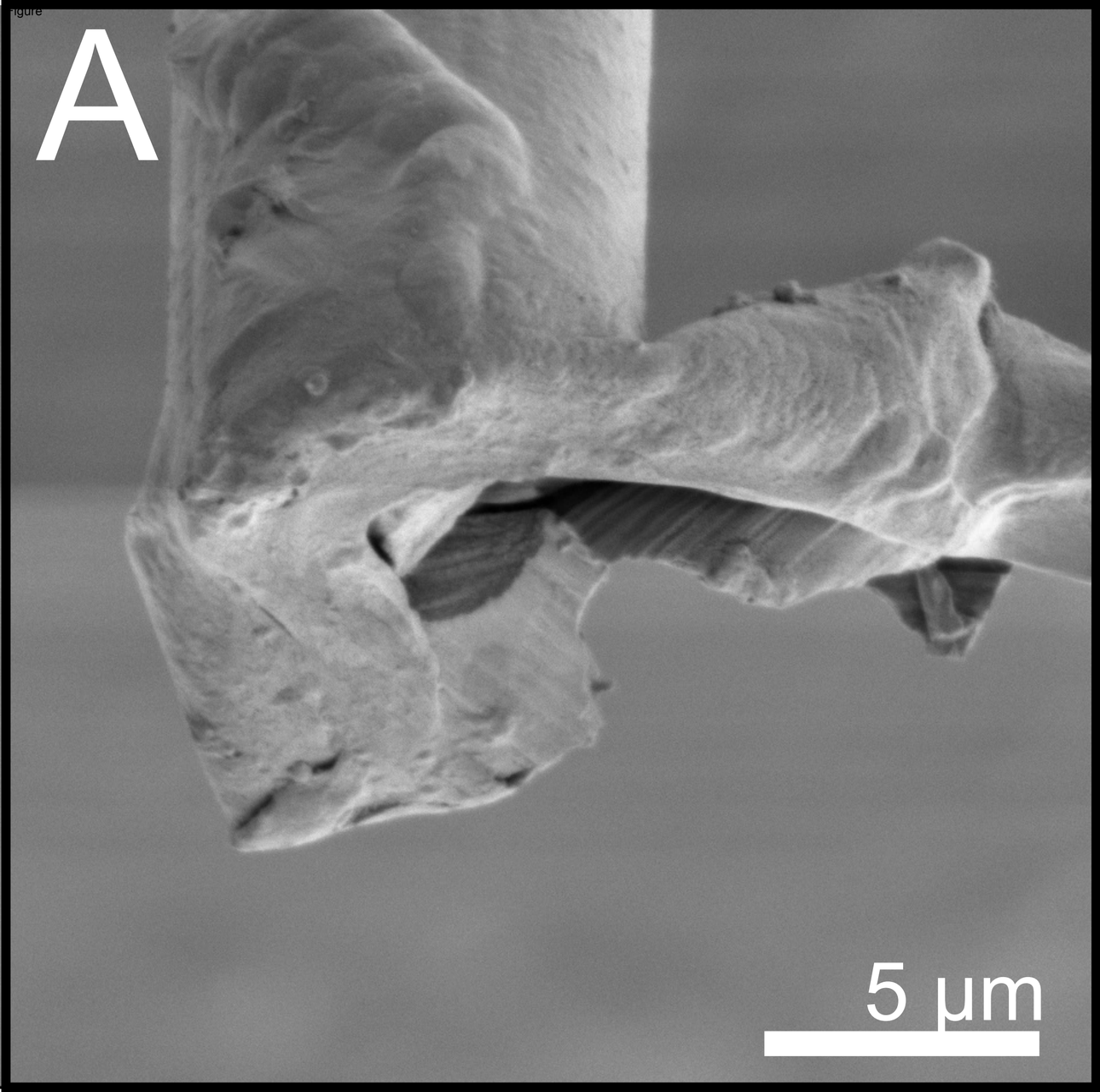

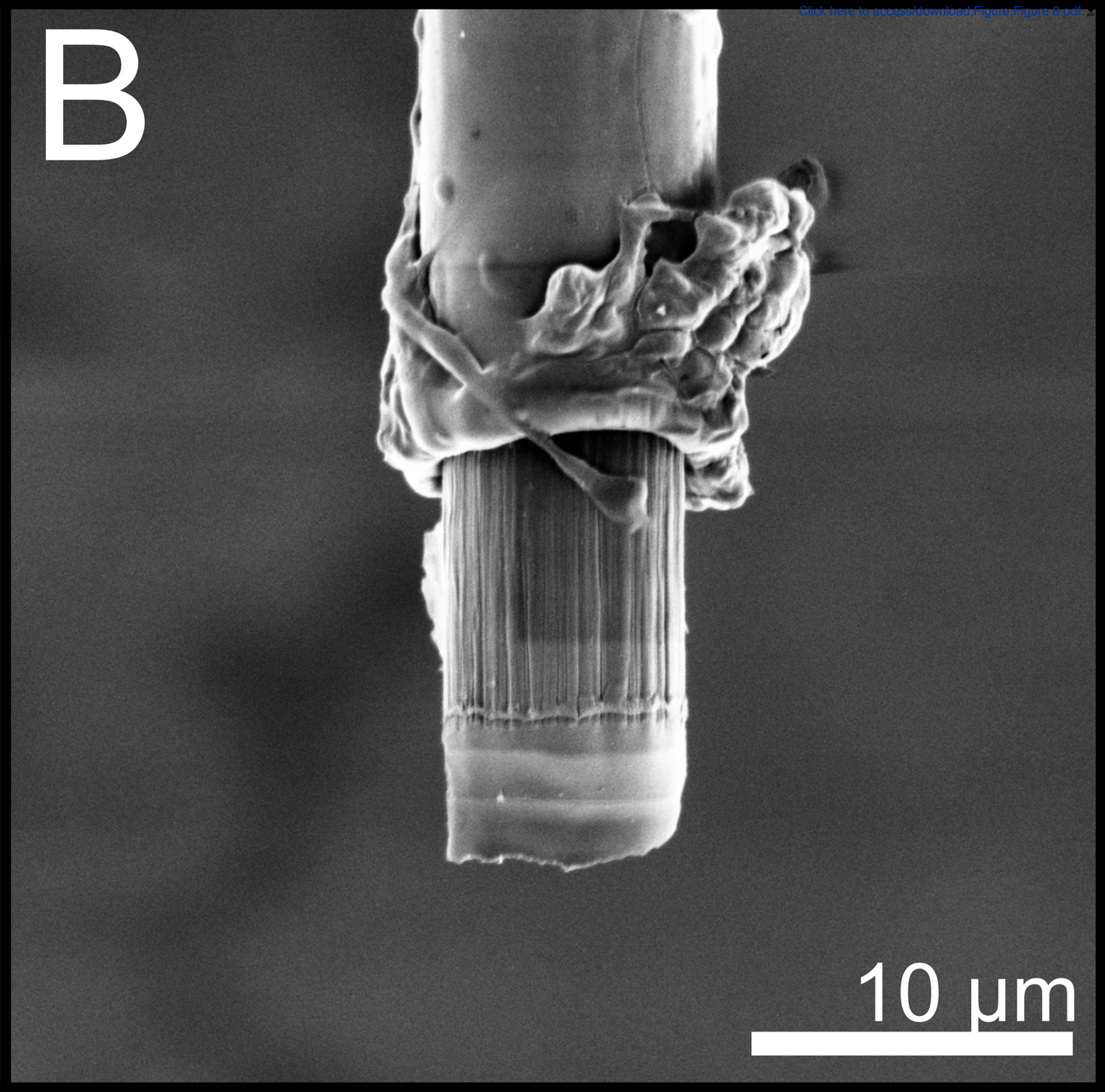

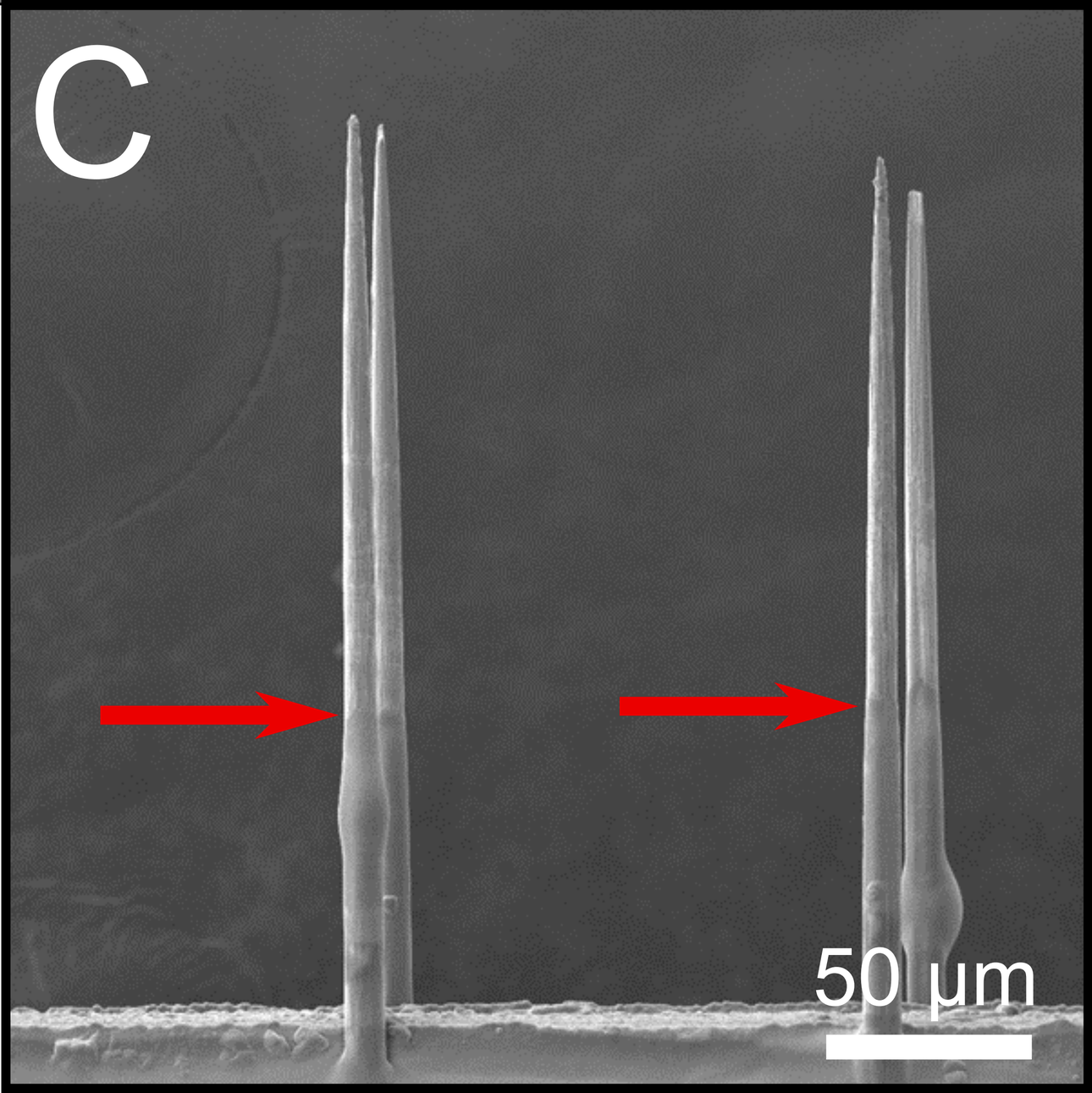

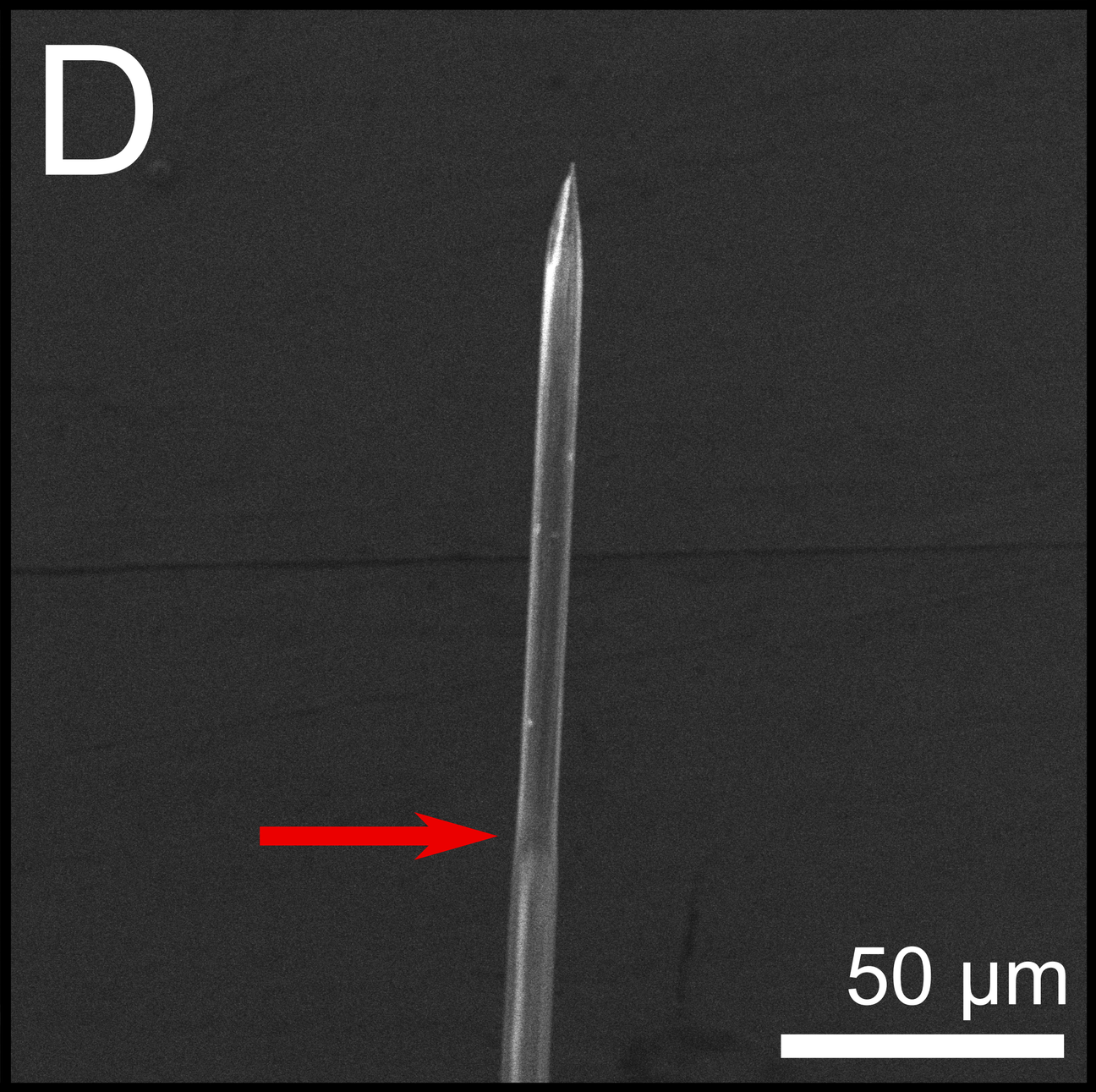

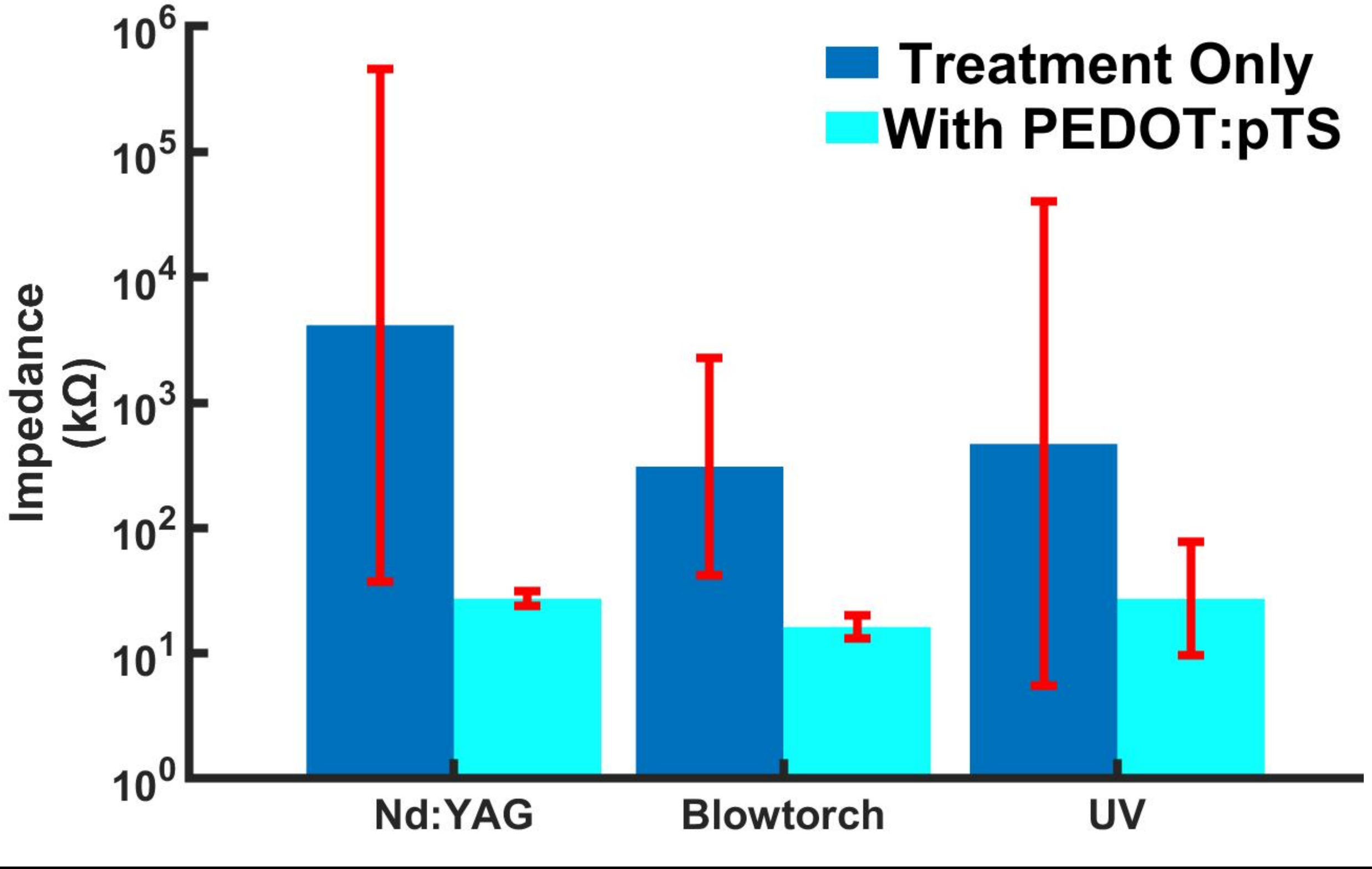

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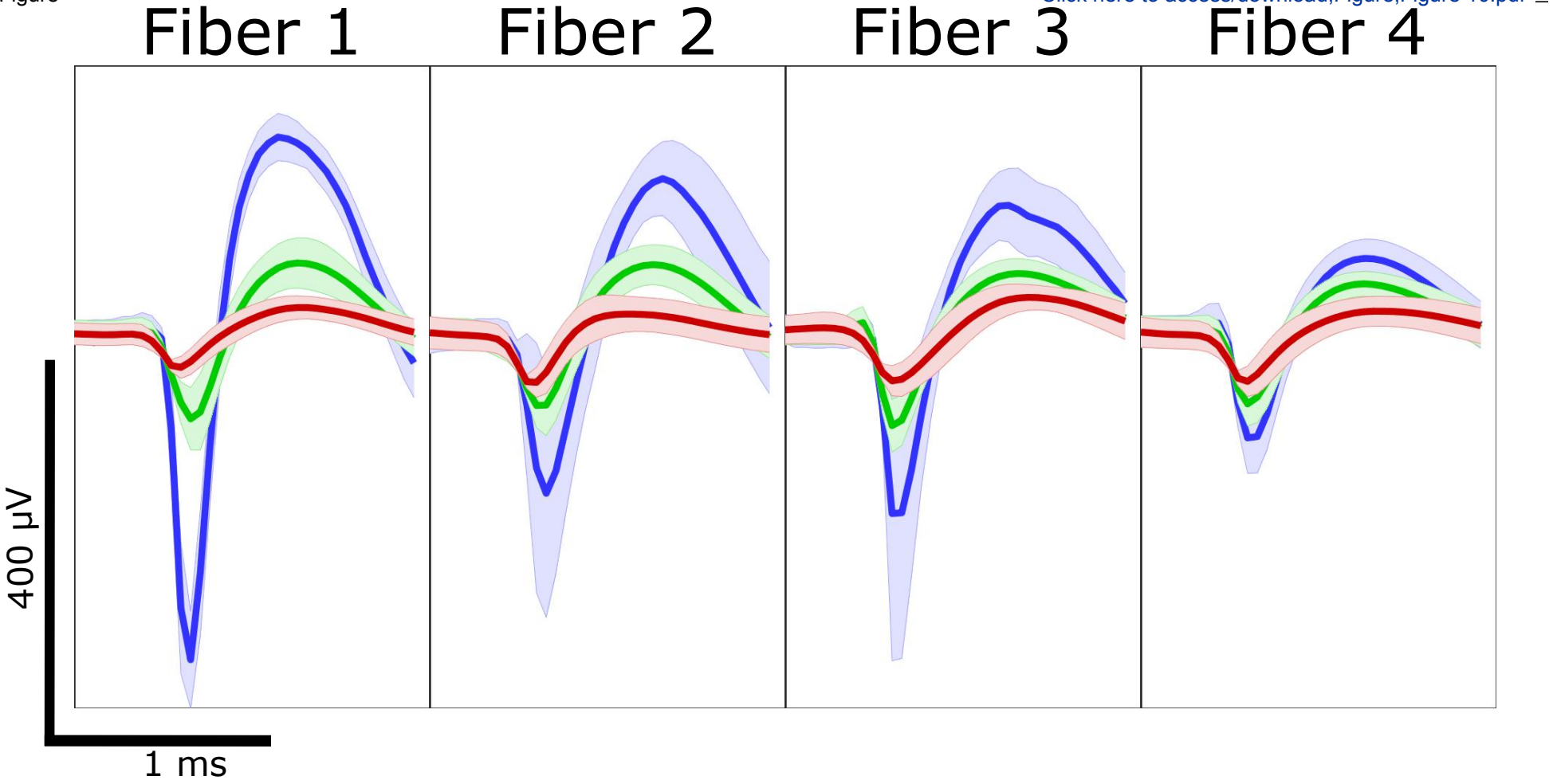

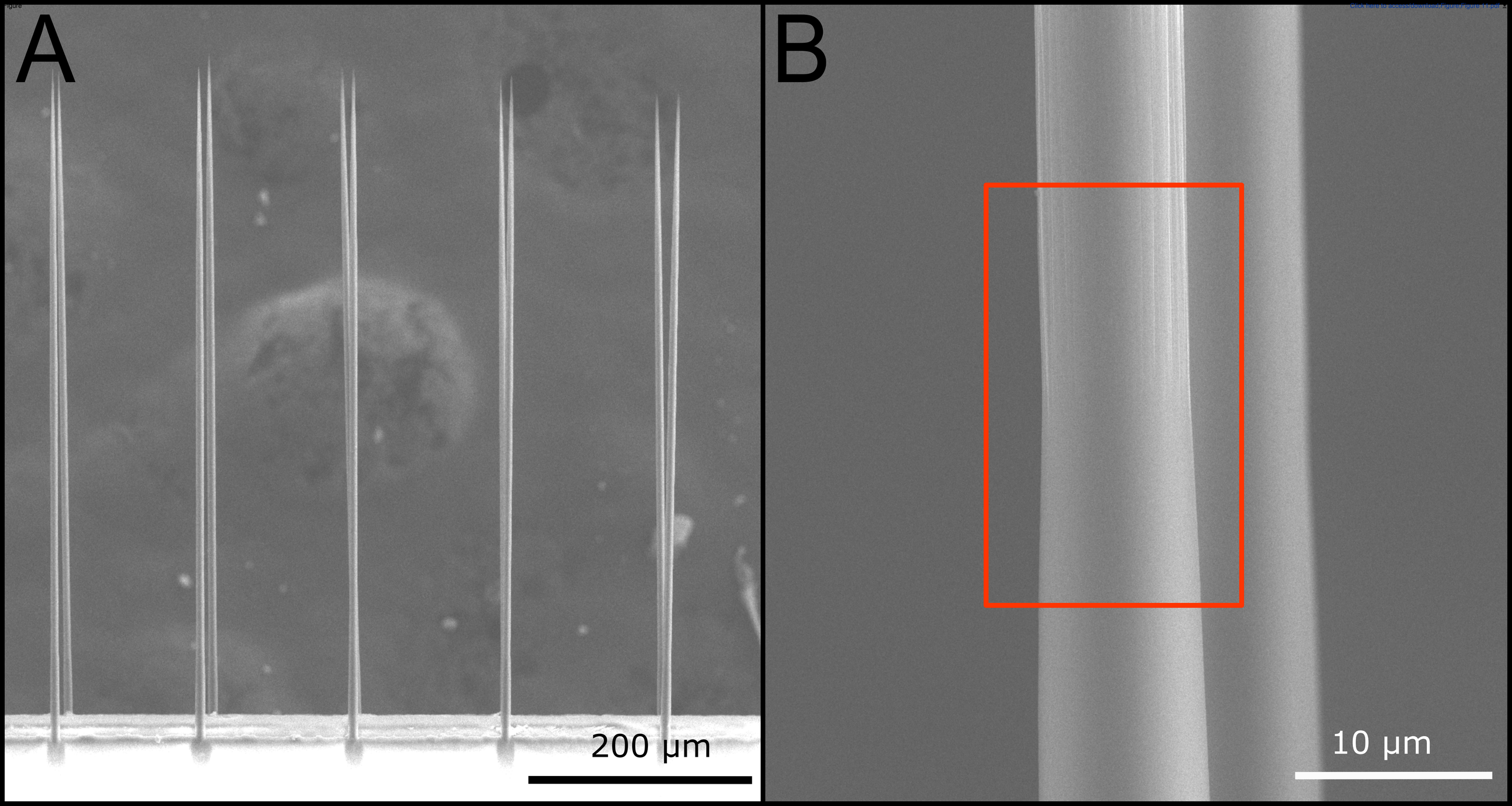

Table 1

PCB Name	Connector	Soldering Pad Size (mm)	Exposed Trace Size (mm)	Trace Pitch (μm)	Channels
Wide Board	Mill-Max 9976-0-00-15-00-00-03-0	3.25 x 1.6	1.5 x 4.0	3000	8
ZIF	Hirose DF30FC-20DS-0.4V,	0.23 x 0.7	0.75 x 0.07	152.4	16
Flex Array	Omnetics A79024-001	0.4 x 0.8	0.6 x 0.033	132	16

Table 2

Build Step	Expected 1 kHz Impedance (kΩ)
Bare Fiber	150-300
Bare Fiber with UV Insulation	400-500
Parylene C Insulated Fibers	>50,000
Nd:YAG Laser Cut	<15,000
Blowtorched	300-400
UV Laser Cut*	300-500
PEDOT:pTS Coated	<110

Preparation Method	Wide Board	ZIF
Nd:YAG	Impedance, SEM, acute ePhys	Impedance, SEM, acute/chronic ePhys
Blowtorch	Impedance, SEM, acute ePhys	Impedance, SEM, acute/chronic ePhys
UV Laser	Not yet validated	Impedance, SEM, acute/chronic ePhys

Flex Array
Impedance, SEM, acute/chronic ePhys
Impedance, SEM, acute/chronic ePhys
Not Viable

Table 4

Activity	Time for 8 Devices (h)
All Soldering	5
Insulating Omnetics	1
Populating Carbon Fibers	10
Insulating Traces with UV Epoxy	0.5
Parylene C Deposition	1.5
Nd:YAG Laser Cutting	1
Blowtorching	1
UV Laser Cutting	1.5
All Impedance Testing	4.5
PEDOT:pTS Deposition	1.5
Recipe Used	Total Hours
Nd:YAG Laser Cut	25
Blowtorch	25
UV Laser Cut	25.5

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Table of Materials

Table of Materials_rev 2.xlsx

Editorial comments:

Changes to be made by the Author(s):

We thank the editor for their comments and have summarized our responses to the concerns below. Please let the authors know if formatting is still an issue, though we have done our best to incorporate the changes.

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please define all abbreviations at first use.

We have gone through the manuscript and corrected all spelling issues and have defined all abbreviations at first use.

2. For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s) before punctuation.

We have updated the references to match the JoVE style and have added some additional reference points for users within the methods sections.

4. Include a space between all numbers and the corresponding unit: 50 mg, 100 mL, 37 °C but NOT 37%.

We have gone through the manuscript and added a space before all units as asked.

5. JoVE cannot publish manuscripts containing commercial language. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials.
For example: Omnetics, Sutter Instrument Co., Novato, CA, New Wave Research, Fremont, CA, MS100, Teslong, Shenzhen, China, WER, Shanghai City, China, Tucker-Davis Technologies OFS, Plexon, Dallas, TX, etc.

We have removed all commercial language from the text and have replaced specific references to tools with generic language (ex: "Autolab" now reads as "potentiostat").

6. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Any text that cannot be written in the imperative tense may be added as a "Note." However, notes should be concise and used sparingly.

We have updated our methods so that they are in the imperative tense. Notes have been used sparingly only to highlight tips for building or to clarify the purpose of a step.

7. Please include an ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution.

We have added a statement summarizing our animal ethics protocol before the procedure steps.

"All animal procedures were approved by the University of Michigan Institutional Animal Care and Use Committee."

This is our standard ethics statement used in other publications, however please let the authors know if this needs to be expounded upon.

8. What happened to the rats after the study? Please specify the following (whatever is relevant to you):
a) Please mention how proper anesthetization is confirmed.

We have added the following line to indicate how we confirm anesthetization:
"10.1.1. Confirm anesthesia with a toe pinch test."

b) Please specify the use of vet ointment on eyes to prevent dryness while under anesthesia.

We have added "10.1.2 Apply ointment to eyes to prevent rat's eyes from drying out during the course of the surgery."

c) For survival strategies, discuss post-surgical treatment of animal, including recovery conditions and treatment for post-surgical pain.
d) Discuss maintenance of sterile conditions during survival surgery.

e) Please specify that the animal is not left unattended until it has regained sufficient consciousness to maintain sternal recumbency.
f) Please specify that the animal that has undergone surgery is not returned to the company of other animals until fully recovered.

For comments 8c – 8f: Surgery was non-survival so we did not include this in our methods.

g) Please specify the euthanasia method. Please do not highlight any steps describing euthanasia.

We have included a statement on euthanasia and have not highlighted this section.
"10.7. Euthanasia: Place rat under isoflurane at 5% under 1L/min of air until signs of life have ceased (20-30 minutes).

10.7.1. Confirm euthanasia with decapitation.

9. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed?
Please specify the array storage conditions.

We have added descriptions of storage conditions within the methods section.

"2.8. Arrays can either be stored at this point or the build can continue

2.8.1. If pausing in the build, store arrays in a clean dry box at room temperature. ”

and

“3.10. Arrays can be stored after any baking step, however static from storage boxes may cause the fibers to pull away from the board if too little silver epoxy was applied when populating the board.

3.10.1. Create a raised, adhesive platform within a box so that the bulk of the board can be stuck to the adhesive allowing the fibered ends of the board to be suspended within the box to prevent fiber breakage.

3.10.2. Store at room temperature.

3.10.3. If fibers do pull away from the board during storage, scrape epoxy out of the traces with a clean pulled glass capillary and repeat steps 3.1-3.8 to replace the fibers.”

Step 2.4, 2.5: What was the distance maintained between each pin? How was this arrangement determined?

The arrangement of the pins is set by the connector manufacturer. All board designs set by the Chestek lab are designed so the pins and solder pads will line up during build. We have highlighted this with the following:

“2.4. Solder the bottom row of connector pins to the back row of solder pads (Figure 2 B)

NOTE: All board designs provided by the Chestek lab were designed so that the connectors would pair precisely with their designated board.

2.4.1. To do this, solder the pins on either side of the connector where there is easy access to the solder mounds. Once secure, gently push the tip of the solder iron between the front pins to solder the remaining connections in the back.

2.4.2. Once the back row of pins is secure, the rest of the connector will align with each pin above its assigned solder pad.

2.5. Solder the front row of pins to the board by applying a small amount of solder to each pin.”

Step 2.6, 3.4: How was the epoxy coating on connections done?

We have further expanded on this with the following:
“2.6. Encapsulated soldered connections in delayed set epoxy (Figure 2 C, D) using a 23-gauge needle and 1mL syringe placed bevel side down on the pins. Push epoxy through syringe slowly so that it flows into and along the connections.”

And

“ 3.4. Apply silver epoxy between every other pair of traces on one side of the board with the glass capillary (Figure 3 B).

3.4.1. Take a small drop of epoxy onto the end of a pulled capillary. Gently apply between every other trace on the end of the board filling the gap completely. The gap should be filled to the top of the two traces without over flowing to touch neighboring”

Step 3.2: What was the epoxy mixed with?

We have included the following to further explain:
“ 3.2. Use the wooden ends of two cotton tipped applicators (one per each part of silver epoxy) to scoop a small, ~1:1 ratio of silver epoxy in a plastic dish and mix using the same sticks used to scoop.

3.2.1. Discard the applicators after mixing.”

Step3.3: How was the carbon-fiber cutting and separation done?

We have added in a more detailed description of using a laminated piece of paper to separate many fibers at once.
“3.3. Cut 2-4 mm off the end of the carbon fiber bundle onto a piece of printer paper using a razor blade.

3.3.1. The carbon fibers will remain in a bundle and will be difficult to tease apart. To easily separate the fibers, pull a laminated piece of paper gently over the top of the bundle.

3.3.1.1. This transfers static into the fibers and they will separate by themselves.”

Step 3.7: How was the array baked?
Step 3.9: How was the oven curing done?

For these two comments, the authors have further explained the process of curing the epoxy on the end of the board in an oven with the following:
“3.7. Place arrays on a wooden block with fibered ends overhanging the edge of the block. The weight of the back end will keep the array on the block.

3.8. Bake the wooden block and arrays at 140 ^{o}C for 20 minutes to cure the silver epoxy and lock the fibers into place.”

Step 5.2: How was the circuit assembled?

The authors have further described the set up and included a diagram showing the set up with annotations to further help the reader.

“5.2. Use a Ag|AgCl reference electrode and a stainless steel rod (counter electrode) to complete the circuit.

5.2.1. Using a beaker clamp, suspend Ag|AgCl electrode in the 1x PBS solution and connect to the reference of the impedance system being used.

5.2.2. Using a beaker clamp, suspend the stainless steel rod in the 1x PBS solution and connect to the counter electrode input of the impedance system being used."

Step 5.3, 8.2, 8.5: How was the impedance applied? How were the measurements taken?

Step 5.3 has been further flushed out and steps 8.2 and 8.5 now reference this step for all impedance measurements.

"5.3. Run a 1 kHz impedance scan for each fiber using a potentiostat.

5.3.1. Potenitostat was set to a 1000 Hz sweep speed at 0.01 Vrms in a single sine waveform.

5.3.2. Potentiostat was set to 0 V at the beginning of each sweep for 5 seconds to stabilize the recorded signal.

5.3.3. Measurements were recorded via the potentiostat's associated software."

Step 6.3: How was Parylene coating done? What were the safety precautions followed?

The authors have further explained the Parylene C coating safety precautions; however, these precautions will vary from lab to lab. Overall, Parylene coating is a very easy and safe process for a new clean room user to learn and use.

"6.3. Coat arrays in a Parylene C deposition system to a thickness of 800 nm in a cleanroom wearing appropriate PPE as defined by the cleanroom.

6.3.1. Here PPE was defined as cleanroom shoes, suit, head covering, goggles, mask, and latex gloves. It should be noted that this is standard PPE for entering a cleanroom.

6.3.2. This step can be outsourced to a Parylene coating company for a fee, however a commercial service may be able to coat more arrays at one time.

6.3.3. Each Parylene C deposition system may have different safety precautions. Contact the technician before use to ensure user safety."

Step 7.1, 7.3: Please provide all the steps associated with cutting of fibers in detail including the laser setup, timing, length measurements, etc.

The authors apologize for this confusion. We have included a statement on alignment with the laser system that should help to clarify for the end user.

"7.1. Nd:YAG Laser Cut

7.1.1. Cut fibers to 550 µm with surgical scissors.

7.1.2. Use a 532nm Nd:YAG pulsed laser (5 mJ/pulse, 5 ns duration, 900 mW) cut 50 µm off the tip of the fibers to re-expose the carbon underneath the Parylene C (usually takes 2-3 pulses).

7.1.2.1. Fiber tips were aligned using the built in stereoscope that comes with this laser system. This system allows the user to align a window (here we used 50 µm x 20 µm (height x width) to encompass the end of the fiber.

7.1.2.2. User must focus the stereoscope on the end of the fiber at 500x magnification for an accurate and precise cut."

Step 7.2.5: What were the stereoscope settings and parameters used for inspecting?

We have included the magnification of the stereoscope needed to view sharpened fibers.

"7.2.5.1. Pointed tips can be observed under 50x magnification."

Step 8.1: Was the solution made in water? How was the overnight stirring done?

We have included more details in the procedure to make this clearer to the end reader.
"8.1. Mix a 50 mL solution of 0.01 M 3,4-ethylenedioxythiophene and 0.1 M sodium p-toluenesulfonate in DI water and stir overnight on a stir plate (~320 rpm).

8.1.1. Store solution in a light resistant container."

Step 8.3.2: How was the electroplating done? Please provide all the associated steps.

The authors have further expounded upon this step with the following steps:

"Electroplate with PEDOT:pTS to lower the impedance of the electrodes.

8.4.1. Submerge fiber tips in PEDOT:pTS solution.

8.4.2. Follow the steps outlaid in steps 5.2, but switch the 1x PBS solution out for PEDOT:pTS and short all connections to the board to the applied current channel.

8.4.3. Apply 600 pA per good fiber for 600 s using a potentiostat.

8.4.4. Turn cell off and allow to rest for 5 s at the end of the run.

8.5. Remove fibers from solution and rinse in DI water."

Step 9.1: Please mention how proper anesthetization is confirmed. Please specify the use of vet ointment on eyes to prevent dryness while under anesthesia.

We have added "10.1.2 Apply ointment to eyes to prevent rat's eyes from drying out during the course of the surgery."

Step 9.3: How was dura resection done?

We have added "10.4. Resect dura by gently pulling a needle with a barbed end over the surface of the tissue.

10.4.1. Once a portion of the dura opens to brain, use a pair of fine forceps to assist in pulling away the dura." to the surgery protocol.

Step 9.4: How and at what exact location where the fibers inserted?

Fibers were not precisely aligned to a specific location for validation of probes. However, we have more precisely described the location of the craniotomy for user reference.

"10.2. Create a 2.5 mm x 2.5 mm craniotomy above the right hemisphere's motor cortex.

10.2.1. The lower left corner of the craniotomy was placed 1 mm lateral and 1 mm anterior of bregma and this acted as the reference point for the size of the craniotomy."

Step 9.5: Please provide in detail the steps followed for EPhys measurement including device set up, stimuli given, etc.

We have further expounded upon the recording settings used in ePHys measurements. No stimulus was applied during the validation test.

"10.6. Collect ePhys data for 10 minutes.

10.6.1. For these measurements, only spontaneous activity is recorded. No stimulus is applied.

10.6.2. ePhys data was collected with an ePhys specific headstage and pre-amplifier.

10.6.2.1. The pre-amplifier high-pass filtered the signal at 2.2 Hz, anti-aliased at 7.5 kHz, and sampled at 25 kHz."

Step 11: Please describe in detail how the array was set up, how was the gold spluttering done, how was the SEM measurements taken including all instrument settings and parameters.

The authors have summarized the SEM steps and provided parameters that have worked to image carbon fibers in the past. We have summarized the two key parameters (beam strength and working distance) we use for our images.

"12. SEM Imaging

This step will render arrays unusable and should be used only to inspect tip treatment results as a check that the arrays are being properly processed. This step does not need to be done to build a successful array. Summarized below is a general outline of the SEM process, however, users who have not previously used SEM should receive help from a trained user.

12.1. Snip off the fibered end of the PCB and mount on a carbon tape masked SEM stub.

12.1.1. Place arrays on a small platform of stacked carbon tape (4-5 layers) to prevent carbon fibers from sticking to the SEM stub.

12.2. Sputter coat arrays with gold following procedures outlined by the manufacturer of the gold sputter coater.

12.2.1. Following guidelines posted at the imaging center, coat the arrays with 100-300 Å of gold.

12.3. Image arrays in an SEM to inspect tip treatment effects.

12.3.1. For best results, image at a working distance of 15 mm and 20 kV beam strength. "

10. Please format the manuscript as: paragraph Indentation: 0 for both left and right and special: none, Line spacings: single. Please include a single line space between each step, substep and note in the protocol section. Please use Calibri 12 points and one-inch margins on all the side. Please include a ONE LINE SPACE between each protocol step and then HIGHLIGHT up to 3 pages of protocol text for inclusion in the protocol section of the video.

The authors incorporated these formatting changes, however may not have completely done them correctly. This is the authors' first paper within JoVE and we are still figuring out how to format the paper. If the editor sees any additional changes that need to be made, please let the authors know as we will make any additional changes.

11. Line 265: Please consistently use one abbreviation throughout the manuscript: ePhys or EPhys.

The authors have decided to use the abbreviation "ePhys" and have changed the abbreviation throughout the manuscript for better clarity.

12. Please revise the Discussion to cover the following in detail with citations: Critical steps within the protocol.

We have removed the sections of the discussion focused on "Future Experimental Options" and "Automated Carbon Fiber Placement." However, have kept the remaining sections as these have been noted to be the key sticking points when training new builders and explaining the device build to potential users. We have also added a section "Material Substitutions" as Reviewer 1 pointed out that other labs may want to know if they can substitute in materials from other distributers.

13. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source (ITALICS). Volume (BOLD) (Issue), FirstPage–LastPage (YEAR).] For 6 and more than 6 authors, list only the first author then et al. Please include volume and issue numbers for all references, and do not abbreviate the journal names. Make sure all references have page numbers or if early online publication, include doi.

The authors have downloaded and applied the JoVE formatting file to this paper. This issue appears to be fixed throughout.

14. Please add all items (plastic and glassware, solvents, equipment, software etc) in the Table of Materials so that it serves as a handy reference for users to get everything ready for the protocol. Please sort the Materials Table alphabetically by the name of the material.

We have added all equipment for the build into the table to the best of our ability. We believe this to be a more comprehensive list.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

Overall, this is a clearly presented and written protocol for making carbon fiber arrays. There are no major concerns. Minor concerns are listed below and identified for the purpose of clarifying the protocol as it may be viewed by readers with a wide range of backgrounds.

We would like to thank the reviewer all comments brought up. We have addressed these concerns to the best of our ability, but will happily elaborate more if requested to do so.

Major Concerns:

n/a

Minor Concerns:

Is there a specific soldering iron tip or recommended types that should be used for the PCB designs provided by the Chestek lab? Please see table of materials for source. What about the type of silver epoxy used? Please see table of materials for source. Source for the carbon fibers? Please see table of materials for source. In general, it would have been helpful to know if specific formulations or sources for materials are important to the success of the presented protocol. These should be given as tips to the reader.

The authors have summarized all equipment used in the Table of Materials. JoVE Editorial staff has asked the authors to remove all commercial language from the paper in favor of generic terms. To help address the concern with substitution materials, we have added a section in the description to inform readers that most materials can be substituted out for alternative materials. We have also included that substitution of materials has not been verified by our lab.

"Material Substitutions

While all materials used are summarized in the table of materials, very few of the materials are required to come from specific vendors. The Flex Array board must come from the vendor listed as they are the only company we know of that can print the flexible board. The Flex Array

connector must also be ordered from the vendor listed as it is a proprietary connector. All other materials can be purchased from other vendors or be swapped out for alternatives at the users' discretion. This build is meant to be flexible and customizable to fit the end user's experiment. However, it should be noted that any changes from the materials or vendors listed must be validated by the end user."

A simple diagram of the PEDOT:pTs electroplating setup would be helpful. Likewise, recommendations on the electrochemical impedance settings and setup would also be helpful. Would be helpful to explicitly mention that all impedances are at 1 kHz.

The authors have added a figure showing the set up for impedance measurement (and PEDOT:pTS coating) set up. We have also further expanded upon the impedance parameters to help clear up any confusion a reader may have when running tests.

Parylene was likely selected as the insulation as it can be vapor deposited compared to other polymers which are commonly processed from solution. It might be worthwhile to mention this to readers who might wonder if there are alternative insulation materials that can be used.
The Parylene used is very thin but does coat the entire array and the PCB. Is additional insulation or potting required on the PCB to prevent moisture intrusion?

At the current time, Parylene C is the only insulation option our lab is pursuing. This is due to the ease of application and conformal coating acquired at low temperatures. The polyimide board and epoxies on the board cannot tolerate the higher temperatures needed for a different insulation technique. As these arrays are not implanted chronically, we did not address moisture intrusion. However, we know from previous studies that the Parylene C tends to hold up well without additional insulation in an in vivo environment.

There are minor grammatical issues:
In the abstract, "pro-posed" should be "proposed."
In paragraph 2 of the introduction, the sentence that starts with "As cuff electrodes..." should be revised. The phrase after "and" does not make sense given the prior text.
In paragraph 3 of the introduction, "car-bon" should be "carbon." In the same paragraph, the symbol for "micro" in micron is missing. The symbol is missing in other sections of the paper as well. In some cases, the micro symbol is simply an "m;" this should be checked throughout the document and replacements made as needed.

We thank the reviewer for pointing out these errors. We have gone through and corrected the manuscript.

Reviewer #2:
Manuscript Summary:
The method by Richie et al describes bench-top methods for building carbon fiber arrays using common laboratory equipment. Carbon fiber electrodes have excellent biocompatibility and excellent electrical properties. Methods for building these

electrodes are of broad interest to the physiology community.

We thank the reviewer for the concerns brought up under review. We have done our best to address each concern and would be happy to answer any additional questions.

Major Concerns:
I found 4 things in need of some general attention.

1) The introduction describes electrodes used for recording in peripheral nerves, but the demonstrated recordings are in motor cortex, which is a very different environment. Can the authors please revise the text so we understand why the tests were done in motor cortex and how this might give evidence about the performance of the electrodes in peripheral nerves?

We thank the reviewer for this comment. We have added an additional statement before the surgical protocol to further explain this choice. The fibers made were extremely similar to previous probes (blowtorched) used in nerve, and doing a test in motor cortex provided a more reliable and fast validation for the UV cut fibers.

"9. Note: Rat cortex was used to test the efficacy of the UV Laser prepared fibers as this we know from previous papers 7, 20 these probes will work in nerve due to their similar geometry and impedance levels to blowtorch prepared fibers. This surgery was performed in an abundance of caution to validate that the UV laser did not change the response of the electrodes."

2) I am quite surprised that the electrodes give such good single unit isolation with so much insulation removed. The site of the UV-sharpened tips is over 100 microns in length. Can the authors comment on this?

The authors are also quite surprised by this. However, we have seen this behavior in previous work (Jiman et al 2020, McCallum et. al. 2017) with McCallum probes having a much larger exposed surface area (500 um exposed length and 15-30 um diameters). McIntyre and Moffatt (2005) suggest that the recording of individual units is not impossible, but rather dampened greatly by an increasing electrode surface area. Future designs for this probe will investigate probes with much smaller surface areas and we hope to help solve this mystery with that work.

3) The UV tip treatment is key, but very little detail is provided on the light source or laser source. Is it a coherent source or incoherent source? Pulsed or continuous? How much average power, and/or power per pulse? What wavelength or wavelengths? What is the beam size (and how measured) and how was it focused and aligned with the tips? What was the duration of exposure?

We thank the reviewer for pointing this out. We have added a reference to our collaborator's paper that further discusses the precise set up and laser description. We have also summarized the laser specifications within the manuscript as follows:

"7.3. UV Laser Cut28

NOTE: UV Laser can only be used on ZIF and Wide Board designs at the present time due to the large focal point of the UV Laser used being larger than the pitch of the Flex Array carbon fibers.

7.3.1. Cut carbon fibers to 1 mm with surgical scissors.

7.3.2. Affix a UV laser to three orthogonally configured motorized stages.

7.3.2.1. The UV laser is a multimode Indium gallium nitride (InGaN) semiconductor with 1.5 W output power and 405 nm wavelength.

7.3.2.2. Ensure that the laser has a continuous beam for the faster and effective alignment and cutting.

7.3.3. Secure array in place to keep a still, level plane of electrodes for the laser to pass over

7.3.3.1. Array should be held at an appropriate distance from the laser so that the fibers will be in light with the laser's focal point.

7.3.3.1.1. This can be accomplished by providing a lower power to the laser and adjusting the distance to best focus on the fiber28.

7.3.4. Move the UV laser focal point across the fiber plane at a speed of 25 µm/s to cut the fibers to 500 µm.

7.3.4.1. Fibers will emit a bright light before being cut. Several passes of the laser may be necessary to cut through the fiber."

4) Lasers were used, but I did not see laser safety highlighted. Laser safety precautions need to be indicated.

We have added the following statement to the beginning of our "Tip Preparation Methods" to address safety concerns when using the lasers mentioned in the manuscript.

"Two tip preparations in this section use lasers to cut fibers. Proper PPE, such as goggles resistant to the wavelengths used, should be worn at all times when using the laser and other lab users in the vicinity of the laser should be in PPE as well. Fiber lengths listed in these steps are recommended lengths, but the user may try any length that suits their needs."

Minor Concerns:
31: never -> nerve
62-63: This is difficult to understand; consider changing to "intrafascicular electrodes."
63: revise, perhaps "However, cuff electrodes..."
65: biofouling ? Is that a technical term?
70: , when bought commercially, have . . . no options for experiment-specific customization

100: Need extra words to describe the point of the step. "Attaching the connector to the printed circuit board"
102: How was flux applied? Painting? Describe
107: IPA abbreviation - spell out
108: which epoxy? What is the part number?

The authors have changed references to "two-part epoxy" to "delayed set epoxy" as denoted in the table of materials to better help distinguish between the epoxies used in this build. We unfortunately cannot include commercial language in the manuscript due to restrictions from the journal.

122: What is the part number / item for silver epoxy?

This is addressed in the table of materials. JoVE asks that material part numbers be summarized in the table of materials and not appear in the text.

123: what tool was used to cut the carbon fibers?

The cutting method and separation of the fibers has been more fully expanded upon in the methods section when cutting is mentioned.

132: say "silver epoxy" here instead of just "epoxy", because you use many epoxies in your work

We have clarified the different epoxies throughout the text.

136-137: Why were the electrodes baked? To cure the epoxy or to remove a manufacturer's coating from the carbon fibers (or some other reason)?

The electrodes are baked to cure the silver epoxy and lock fibers into place on the board. We have expounded upon this in the text to better explain the need for the bake step.

141: Again, need more of a description of what the step is. "Mechanical stabilization of the fiber connections to the PCB board by UV-cured epoxy" (or whatever it should be)

We have added a better description for this step.

142: Small droplet of UV-cured epoxy

142: What is the part number / supplier of the UV-curved epoxy?

This is addressed in the table of materials. JoVE asks that material part numbers be summarized in the table of materials and not appear in the text.

144 - 147: recommend replacement of "epoxy" or "UV epoxy" with "UV-cured epoxy" in all instances, for clarity.

The authors have gone through and revised all references to epoxy to better differentiate between the different epoxies.

153: It may be helpful to reference Figure 6 here.

We have added a section (9) to better describe figure 6 and the addition of ground and reference wires to the board. We have also added a better caption to figure 6 to point out the vias and explain their interconnections.

182: How was the laser aligned with each fiber tip? What is the beam size? Was any focusing used or just the naked beam?

Laser was aligned using the laser system which is mounted over a moving stage and a stereoscope through which the laser beam is directed. We have added additional detail to the manuscript to account for this.

"7.1.2. Use a 532nm Nd:YAG pulsed laser (5 mJ/pulse, 5 ns duration, 900 mW) cut 50 µm off the tip of the fibers to re-expose the carbon underneath the Parylene C (usually takes 2-3 pulses).

7.1.2.1. Fiber tips were aligned using the built in stereoscope that comes with this laser system. This system allows the user to align a window (here we used 50 µm x 20 µm (height x width) to encompass the end of the fiber.

7.1.2.2. User must focus the stereoscope on the end of the fiber at 500x magnification for an accurate and precise cut."

204: See major item 3 above and add these details

We have added the additional details and included a reference to a more in depth description of the setup.

260 - 264: It would be helpful to refer to Figures 7C and 7D in these lines as Figures 7A and 7B were referred to earlier in the passage.

References to the figures have been added. Figure 7 is now figure 8.

334-340: Use "UV-cured epoxy" (same suggestion as above) for clarity

We have made this correction.

351-352: It is unclear what is meant by "Solder applied to each side of the via." (Basically, provide enough context so someone who does not design PCBs and does not know the term "via" can figure it out.)

We have added a section (9) and expanded the figure 6 (now figure 7) caption to better explain where vias are located on the boards. We have also explained in section 9 the soldering method to connect these wires to the board.

353: Denote that this is the description for Figure 6C somewhere in this sentence.

We have referenced figure 6 (now figure 7) in another section of the text to better highlight this figure.

362: What is the sample size for each bar in this graph?

We have added sample size to the caption for the figure.

396: nerve tissue -> brain tissue (this is not a peripheral nerve, right?)

We have clarified this sentence to point out that the carbon fibers can be used in various tissues, but require different lengths and geometries to be properly utilized in each. Brain tissue can be penetrated by longer fibers, but nerve and muscle tissues need a shorter, sharper fiber to be penetrated and recorded from.