Because of the confined and optimally oriented current flow, thresholds should be low and quite uniform for all of the fibers of a given caliber. To obtain stable differential thresholds for various populations of nerve fibers in the face of scar tissue formation around the nerve and contacts, it will be necessary to use accurate controlled current stimulation rather than voltage stimulation.

Cleaning and Preparing Cuffs for Re-Use

After removing the cuff from the animal, it should be cleaned and made ready to be used for the next surgical procedure. Sonically clean the cuff in 50% bleach or other tissue solvent for 1 minute or until all tissue has been removed. After tissue removal, sonicate the cuff in distilled water for 2 minutes and then in alcohol for 1 minute.

To reopen the cuff, gently slide the cuff over a blunted and tapered needle such that the slit is open sufficiently to slip the cuff over a nerve. Immerse the needle holding the cuff into boiling distilled water for about 15 to 20 seconds. Store the cuff over the needle in a clean, dry location until the next procedure.This step will allow the Nano Cuff to remain open after removing it from the tapered rod.

Testing

If you have any doubts about the integrity of the electrical leads or their insulation, the best test is a "bubble test" done in vitro in a bowl of saline. Use a low-voltage DC battery (4-6 volts) to apply a negative voltage to each lead in succession while immersing the cuff in a saline bath. The positive side of the battery should go to a large surface area ground (such as the outside of a stainless steel bowl). When contact is made, you should see a stream of bubbles (hydrogen gas) coming from the connected electrode contact within the cuff tube and from no other electrodes or external points along the tube or leads. Bubble formation at multiple contacts within the cuff indicates that a short circuit may be present within the cuff or connector. Bubble formation at any place along the leads suggests insulation damage.

The best test of an implanted nerve cuff is the contact impedance measured by a low-current AC (1 kHz) impedance meter as detailed on **Impedance Testing** section. Measure both the impedance of each contact versus remote ground and the inter-electrode impedances for various contact pairs. The pair-wise impedance will be somewhat less than twice the individual contact impedance and will tend to be somewhat larger for more distant pairs than for adjacent pairs. Individual contacts near the ends of the tube generally have slightly lower impedances vs. ground and vs. each other than do central contacts. A fair amount of contact impedance variability can be expected between contacts and over time, due to changing conditions. Changes of a factor of two in either direction are not significant. This test is most useful for detecting gross loss of continuity or insulation integrity, both of which are most likely to occur at the connection point rather than in the cuff itself.

Consider the mechanical dynamics between nerve and adjacent tissues carefully before selecting a fixation technique. They may cause the very traction that you seek to avoid.

Common Issues

The most common electrical failures observed during the use of **Nano Nerve Cuffs** are poor solder joints or fluid leakage at the connection point to the leads, resulting in open circuits (noisy recordings) or degraded common mode rejection and spurious stimulation. The latter may be difficult to detect because of the normally low contact impedances for larger cuffs. Sudden large impedance decreases can be indicative of this problem.

The most common cuff failure is incomplete closure of the slit, resulting in degraded common mode rejection of external signal sources, such as EMG.
The most common surgical/biological problem is nerve blockage. This can occur immediately during implantation surgery as a result of stretching, in which case it may recover over a day or two, or from ischemia of the nerve, which will generally not show up for many hours and will not recover except by the slow process of distal regeneration.

A more pernicious problem arises from constriction of the nerve by the inevitable pseudomesothelial encapsulation of the silicone coated polyimide. This tends to occur at 7-10 days post-op, and can produce an abrupt complete blockage (particularly in larger diameter fibers) in a nerve that has been quite functional since surgery. **The recommended margin of the cuff inner diameter of 1.4 times the nerve bundle outer diameter is critical.** While excessive over-sizing degrades the recorded signal or stimulation threshold only gradually, only a slight under-sizing can result in a complete block due to this issue.

If a block develops in a tube that is clearly not undersized, the most likely cause is kinking of the nerve at the ends, caused by poor surgical placement, inadequate dissection of connective tissue, or traction from the leads. If cuffs are reused, careftully examine the placement of each contact along the walls of the tubing after it has been removed and cleaned. Occasionally a loop of wire becomes bent away from the wall and will act as a guillotine on any nerve bundle implanted into such a cuff.

Terms and Conditions

Please inspect the package carefully upon arrival and report any damage to us within 7 days of receipt of the package.
Unused items may be exchanged if items and packaging are undamaged and in good condition. Exchange must be made within 30 days of invoice date and with prior permission from our Customer Service Department.
Please call 301-330-9788 or email support@microprobes.com to request a Return Material Authorization (RMA) number. We do not accepted returns after 90 days from invoice date.
Custom design products are non-returnable.

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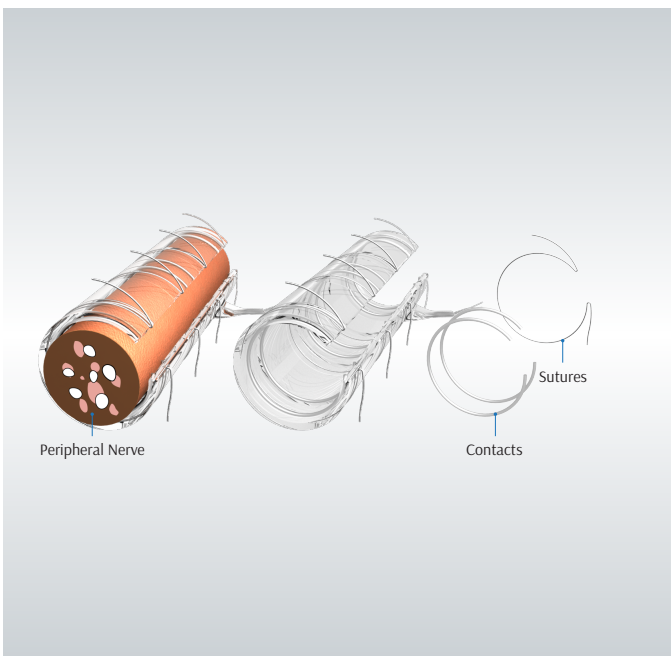
NANO NERVE CUFF

Handling and Implantation Manual



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Introduction

Unlike Standard and Micro Cuffs which can employ sutures to close and open the cuff, **Nano Nerve Cuffs** are too small to accommodate even very fine sutures. Please carefully read the implantation and extraction protocols below, especially if the cuffs are to be used acutely for more than one procedure. While Standard and Micro Cuffs have silicone as the base substrate, **Nano Nerve Cuffs** have polyimide with an overcoat of silicone elastomer.

Because silicone and polyimide are hydrophobic, additional steps may be required to insure that the cuffs are adequately wet prior to recording or stimulation. If the cuffs are not sufficiently wet, the small metal contacts within the cuff may not maintain proper connection to the nerve (or saline during in vitro testing). It is highly recommended that an impedance meter be used to measure the impedance of the contacts in saline prior to implantation, and then again after the cuff has been implanted.

The impedance after implantation should be within at least 150% of the pre-implantation impedance values.

Sterilization and Handling

The **Nano Nerve Cuff** is packaged and shipped unsterilized within a sterilizer-compatible envelope. The cuff can be sterilized using any one of many sterilization protocols including **ETO, STERRAD®**, or **autoclave**.

Nano Nerve Cuffs are very fragile and care must be used when removing them from the packaging. The Teflon-insulated stainless steel lead wires are wrapped around a thin cardboard holder, while the cuff and fine Pt/Ir lead wires are left "floating" within the envelope.

When removing the cuff from the envelope packaging, it is recommended that you grab only the cardboard holder while taking care to not snag the cuff on any portion of the envelope. When re-loading the cuff back into the envelope first wrap the stainless steel lead wires around the cardboard holder and use the same procedure in reverse.

Impedance Testing

It is strongly recommended that the impedance of the **Nano Nerve Cuff** contacts be measured in a saline bath prior to implantation. The in vitro values that are measured serve to validate the proper function of the cuff, and may be used as benchmark values for comparison against in vivo impedance values measured after implantation.

Each **Nano Nerve Cuff** is visually inspected and impedance tested prior to shipping. Shipped impedance values are provided to the investigator on the quality control form provided with each cuff. MLS measures impedances using an impedance meter (Model IMP-2A, available for purchase from MLS), using a 1 KHz sine wave. Measurement is performed in a two-point configuration against a platinum counter electrode.

Different measurement configurations and conditions can result in variations in measured impedance. Impedances measured by the investigator may be different than those provided by MLS.

Some commercially-available impedance meters use a measurement frequency other than 1 kHz, which will result in very different measured values.

Due to the hydrophobic character of the polyimide surface, special considerations need to be made to insure that the electrode contacts within the cuff maintain continuity with the saline during in vitro measurement. Even though each cuff is delivered spread open for easy implantation, saline may not properly fill the cuff even after being fully submerged. MLS has found that by blowing can air onto the cuff as it is submerged in saline less than 1 cm will in most cases fill the cuff cavity and allow the saline to make complete contact with the electrodes.

For in vivo measurements, a steady stream of saline or ringers solution should be squirted onto the nerve using a syringe with a 23 gauge needle at the point where the nerve enters the nerve cuff.

Surgical Handling and Implantation

Cuffs should be handled while being visually observed under a binocular microscope, due to their very small size.

Avoid direct contact with the cuff while handling. Instead, manipulate the cuff using the stainless steel leads whenever possible.

- After the nerve has been exposed and prepared for implantation, the cuff should be initially positioned close to the nerve by holding the steel leads and maneuvering the cuff over the nerve.
- Once the cuff has been positioned close to the nerve, the steel leads should be held in place and stabilized with sufficient compliance that when the cuff has been implanted any movement of the leads will not pull on the implanted cuff.
- The nerve should be completely freed from surrounding tissue for as long a length as possible. Avoid cutting nerve branches, as these usually contain essential feeding blood vessels; include them in the tube if necessary. For acute recording or stimulation this may not be necessary.

  Make sure that there will be some slack in the nerve at either end, and that it will neither be kinked by an abrupt change of direction near the cuff end, nor stretched by attached connective tissue.
- The cuffs are delivered spread open sufficiently to allow the nerve to be slipped into the cuff. In some tight access situations the nerve can be started into one end and the cuff gently forced over the nerve using a pair of forceps. The compression on the nerve from the edges of the cuff is usually not severe enough to cause direct fiber damage, but may temporarily occlude blood flow, so be ready to act quickly.

Surgical Fixation

The impedance of the contacts should be measured prior to sealing off the cuff from the surrounding tissue to insure that the electrodes are electrically connected to the nerve.

- For **Nano Nerve Cuffs larger than 160 microns**, two or more size 7 or 8 sutures maybe slipped under and over the cuff and gently tied around the cuff to close it. For chronic and possibly acute implantation it is advised that a very thin strip of "Kwik-Sil", a quick setting surgical silicone elastomer (World Precision Instruments, Sarasota FL), be applied over the cuff slit to completely seal and isolate the nerve from the surrounding tissue. For acute use, the cuff's slit can be re-opened by slipping the cuff over a blunted and tapered needle (supplied by MLS), then using a #11 scalpel blade slice through the Kwik-Sil along the slit such that it is completely open again. For this reason it is extremely important to use as thin a strip of the Kwik-Sil as is necessary for sealing the cuff.
  An alternative procedure to applying and removing the Kwik-Sil for multiple acute experiments is to apply a very thin coat of mineral oil over the cuff and then apply the Kwik-Sil. After extracting the nano cuff the Kwik-Sil removes quite easily from cuff. This procedure has been used and was shared with MLS by Dr. Aswini Kanneganti from Dr. Mario Romero's lab at the University of Texas at Dallas.

- For **Nano Nerve Cuffs** having inner diameters of **160 micron** or less, other techniques to completely close the cuff need to be employed. One method is to pinch the cuff closed using small but smoothly rounded forceps. Another technique is to use specially modified miniature hemostats sold by MLS, which have a tip that has been drilled out to a diameter slightly smaller than the outer diameter of the cuff. Carefully pinch the cuff closed under a microscope. Several pinching iterations may be necessary to completely close the cuff. Squirt saline or ringers along the nerve near the cuff, measure the impedance values of the electrode contacts and apply the Kwik-Sil silicone.

  Consider the mechanical dynamics between nerve and adjacent tissues carefully before selecting a fixation technique. They may cause the very traction that you seek to avoid.

Connection of Leads

The stranded stainless steel wire employed in the cuff contacts and leads was selected for its strength and biocompatibility, but can be somewhat challenging to make good mechanical and electrical connections. The best technique is soldering with the use of special stainless steel fluxing liquid:

- Carefully strip the Teflon® insulation from the end of the leads by nicking and pulling it, taking care not to damage the wire strands.
- Dip the wire end in the acid flux or paint it on generously using a cotton swab. Allow a few seconds for it to take effect.
- Using a freshly tinned, clean soldering iron (regular 60/40 rosin core solder), quickly dip the fluxed wire into the solder ball. You should hear a hiss as the flux evaporates, and the wire strands should be immediately drawn together by a solder bead, which will be firmly fixed to the wire after it cools.
- Cut off any stray ends that have not been well tinned. If you have any doubt about the adhesion of the solder, cut off at least 5 mm past the exposed portion and start again. Do not attempt to re-flux or re-solder the first site, as the rosin will coat the strands and make this ineffectual.
  Once the end is tinned, it can be soldered in the conventional manner to any pin or other termination. Be careful to protect any connection points from fluid leakage.

Configuration of Leads

The contacts are numbered sequentially from distal to proximal according to the handedness of the cuff. The right-hand (standard configuration) can be visualized by holding your right hand palm upward and imagining your thumb to represent the exit of the leads. The cuff then lies with the slit facing up (parallel to fingers) and the first lead comes from the contact closest to your fingertips (distal). This lead is identified by labeled paper tabs. For left-handed cuffs, the conventions are the same but the orientation is for the left hand held palm upward.

For tri-polar recording, a triad of contacts evenly spaced with respect to each other is selected. No contact should be closer to an end than one diameter of the tubing. The two outer contacts are connected to the negative (inverting) input of a differential amplifier and the central contact is connected to the positive (non-inverting) input. The ground or reference of the amplifier must be connected to some other point in the preparation (a fourth cuff contact can be used, preferably at or near an end).

Stimulation

For stimulating, it is common to select a bipolar pair of adjacent cuff contacts and use an isolated source of charge-balanced, biphasic stimulation, with the cathodal-first stimulus applied to the contact closest to the direction in which propagation is desired. If you wish to record the stimulated volley from another set of electrodes in the same cuff, stimulus isolation, an intervening ground contact, and a very fast recovery amplifier will probably be necessary to escape the stimulus artifact.

Always use a series-blocking capacitor even when the stimulus is biphasic in shape to prevent any net DC current. Use stimulus waveforms as short in duration as possible to minimize electrode polarizations.