CAMBRIDGE NeuroTech

Advanced Electrophysiology Systems

Silicon probe implantation protocol

Photographs courtesy of:

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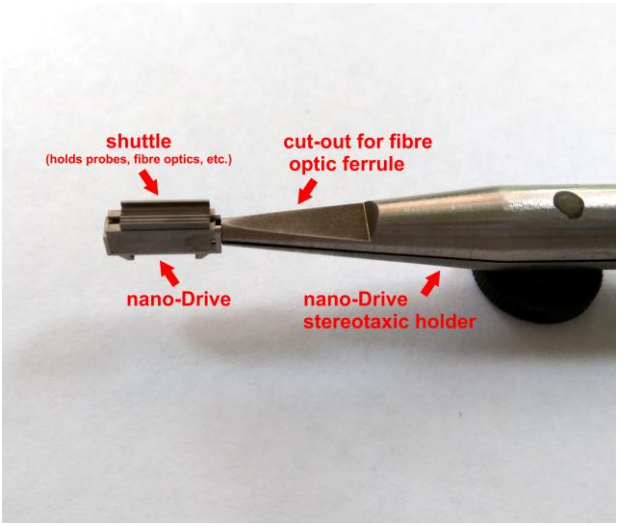
PART 1: Preparation of the Silicon Probe and Nano-Drive mounting

The following steps describe the procedure for mounting a silicon probe on a Nano-Drive. If you do not intend to use a drive start at step 1.6. For non-moving probes, a stereotaxic vacuum-chuck is available for holding and implanting the probes. Most of the surgical steps in Part 2 remain relevant for non-moving probes.

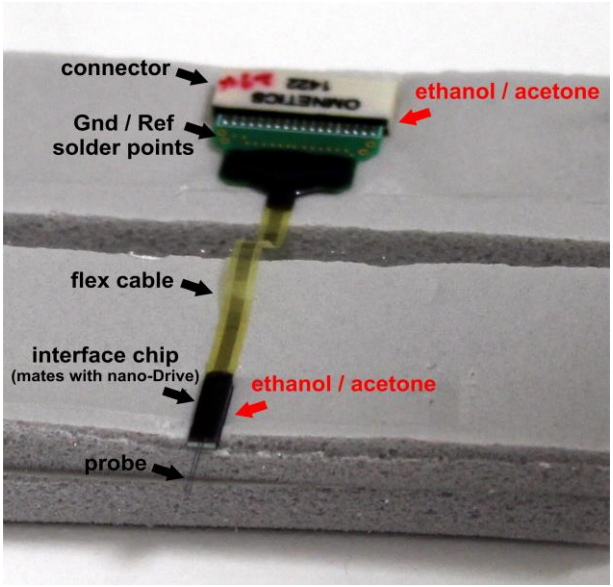
You will need:

Nano-Drive stereotaxic holder tool
Nano-Drive
micro-spatula
fine forceps
Acetone
70% ethanol
cotton-buds / swabs
vaseline
superglue gel
blu-tak / adhesive tape
soldering iron with fine tip
multi-stranded stainless steel wire (teflon insulated) or similar
solder flux (phosphoric acid - of using stainless steel wire)
Low-temperature cautery (D905 Disposable cautery with fine tip - Low temp - Williams Medical)

1.1 Insert the screw-head of the Nano-Drive into the holder tool and clamp by gently tightening the thumb-screw.
Consider your desired travel in the brain - for maximal travel, ensure the Nano-Drive shuttle is at its upper limit by spinning the drive in the holder to turn the lead screw. Full-travel is ~5 mm - the first 2.5 mm will place the shuttle end level with bottom of the Nano-Drive housing, although the shuttle can travel an additional ~2.5 mm below the drive housing. For full-travel note that the drive housing will need to be offset from the skull by ~2.5 mm - see later. Arrange the Nano-Drive in the holder so that that the thumb-screw rests on the bench, allowing the Nano-Drive shuttle to face upwards.

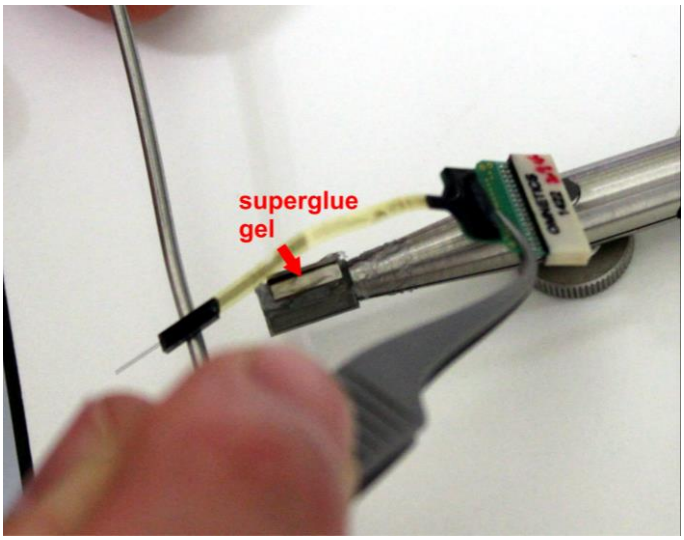


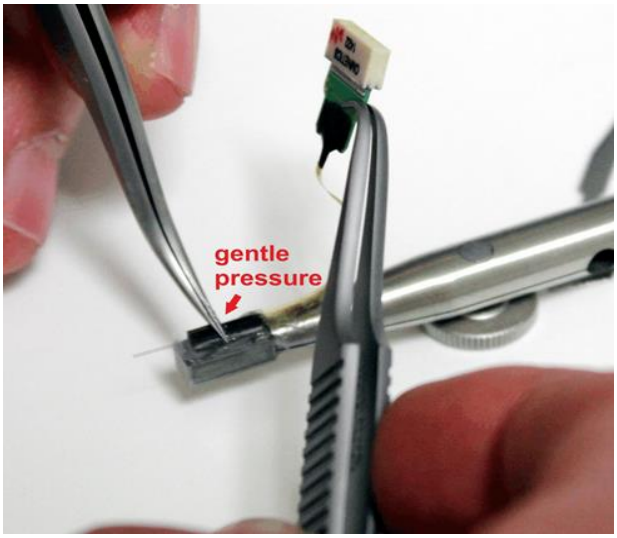
1.2 Carefully open the shipping tray containing the probe, ensuring you correctly orient the tray upwards before opening. Gently prize the connector free from its secure housing and carefully remove protective adhesive tape covering the interface chip. Hold the probe by the connector and flip the tray upside down to allow the probe to fall free from the tray.

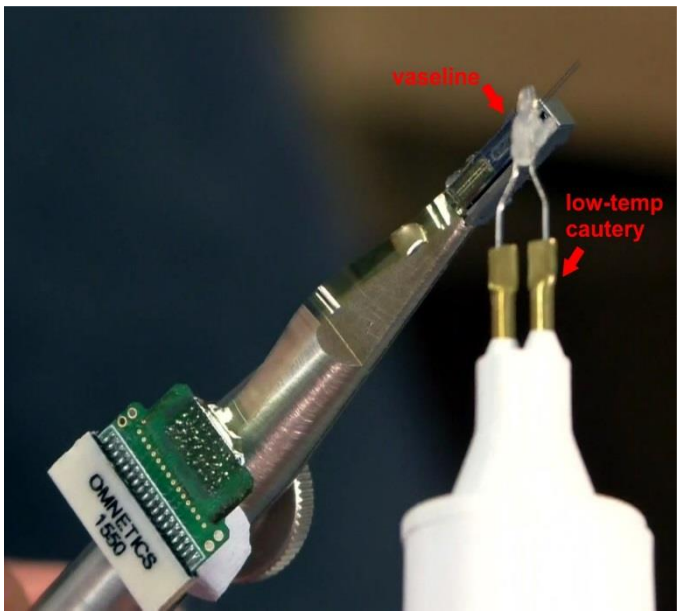


1.3 Apply three small blobs of superglue gel to the nano-Drive shuttle surface Carefully drag the interface chip of the probe in to position on the shuttle and gently nudge the interface chip in to alignment with the shuttle. Consider the working length of the probe with respect to the Nano-Drive shuttle travel limit (see 1.1) and when satisfied apply gentle downward pressure to the probe-body to initiate superglue bond. IMPORTANT: The length of the probe sticking out from the drive should be adjusted depending on how deep you want to lower your probe into cortex (e.g. 1 mm (skull thickness) + 1mm (layer 4/5 rat cortex) + 1 mm (working distance) = 3mm).

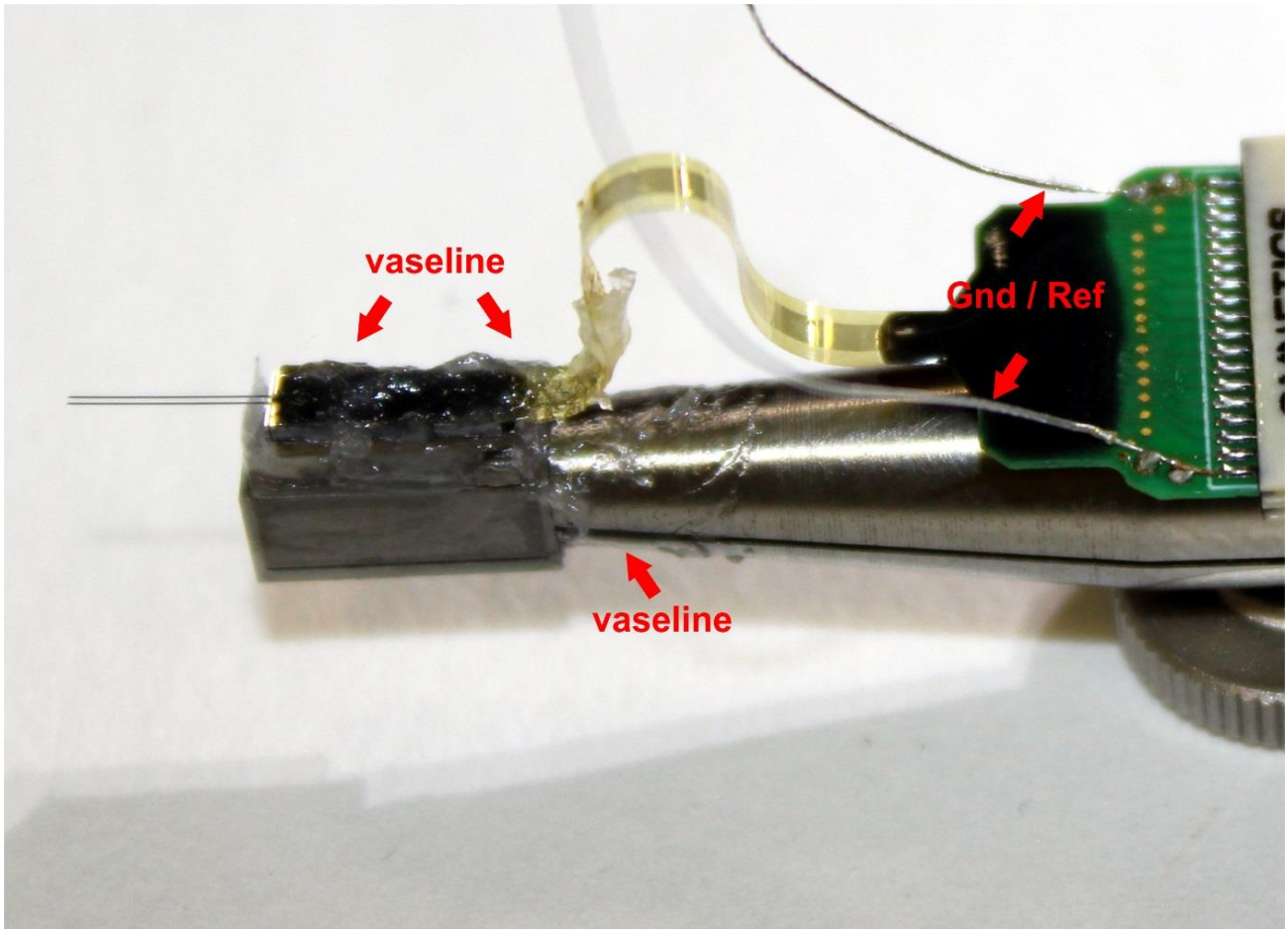
1.4 Carefully pick up the Omnetics connector and with the aid of blu-tack / adhesive tape fix the connector in place to the stereotaxic holder.







1.5 Apply vaseline to all moving surfaces of the Nano-Drive - this is easily achieved using the cautery; a small amount of heat will melt the vaseline and enable it to run freely - moreover, the surface tension of the vaseline will take it from the cautery tip on to the Nano-Drive. Apply vaseline around the tip of the drive-holder - this will prevent cement sticking to the holder during surgery and allow the Nano-Drive screw to end up counter-sunk into the cement. Once cooled, remove any excess or undesired vaseline.



1.6 Prior to implantation, wash and sterilize the probe with 70% ethanol and allow to air-dry.

PART 2: Surgery

The following instructions assume the animal is placed in a stereotaxic frame under general anaesthesia (Isoflurane / Oxygen recommended) and that the skin, fascia and periosteum overlying the skull have been appropriately removed with the skull landmarks bregma and lambda properly accounted for. We strongly recommend the use of the NeuroStar stereotaxic atlas integrated surgery robot for precise and reliable placement of your probes. This system automatically corrects many common errors during stereotaxy and offers combined precision drilling along with live-update atlas driven micro-injections and probe placement. Please contact us for more details.

You will need:

standard surgical tools and consumables
operating microscope,
skull screw / anchor bolts for Ground / Reference electrodes
probe-mounted Nano-Drive in holder
micro-spatula
fine forceps - curved tip and serrated tip
vaseline
cotton-buds / swabs
low temperature, fine tip cautery
Cambridge NeuroTech Dura-Gel
dental cement: RelyX Unicem 2 Automix Translucent Value Pack 1113427 (Henry Schein)
BA Optima-10 LED Curing Light Blue 1117552 (Henry Schein)

Skull screws / anchor bolts

2.1 Wash the skull screw (s) / bolt (s) with 100% alcohol and flame / air-dry them, allowing them to cool before use.

2.2 When planning your skull screw positions be sure to allow for the foot-print of the Nano-Drive (2 x 4 mm) - note that the Nano-Drive can sit directly atop a screw given the ability of the Nano-Drive shuttle to extend ~2.5 mm beyond the bottom of the Nano-Drive housing (see step 1.2).

TIP – the recommend RelyX Unicem 2 is a direct to bone adhesive – therefore, you do NOT need classic bone screws for mechanical anchoring of your headpiece – this reduces surgical time and minimizes soft-tissue / brain surface trauma for your animal.

Probe Placement

2.3 Drill a small craniotomy at your entry point of your interest; be sure to remove all bone fragments. Carefully clean the remainder of the skull surface and lightly score / scratch with a scalpel to provide a key for the RelyX cement.

TIP – run a small bead of cement around the edge of the craniotomy to form a small dam – this will help to catch any stray molten vaseline that arises during the later steps of this protocol. Consider carefully the height of this cement dam versus your intended clearance of the nano-Drive housing above the skull surface so that you do not create an obstacle for yourself!

2.4 Make a small durotomy to allow the probe to enter the brain. There are two ways to make a durotomy:

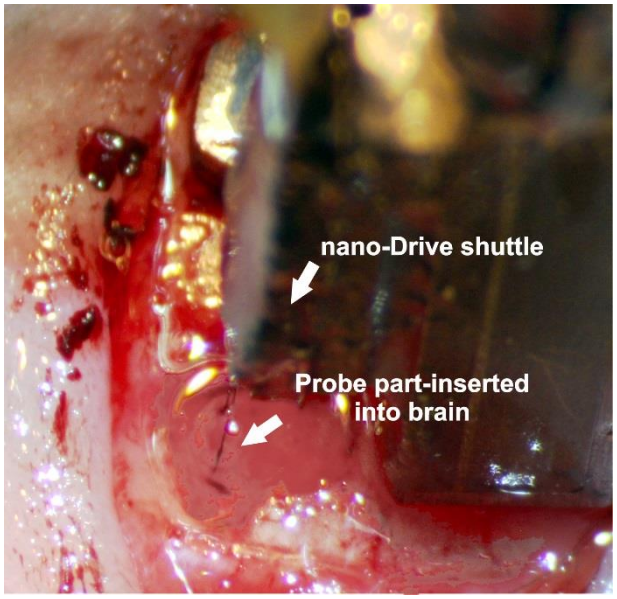
Chemical, via the use of collagenase enzyme to dissolve the connective tissue in the dura (please contact us for an approved protocol, if desired), or

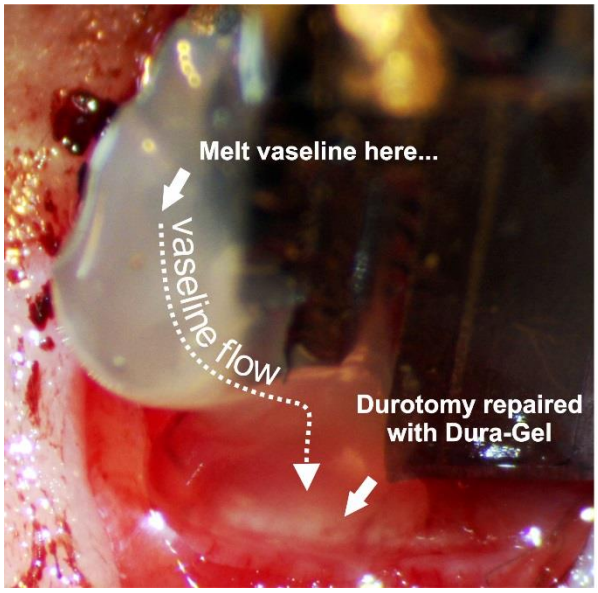
Mechanical, via the use of sharp hook to make a small cut in the dura. A dura hook can be fashioned using a 1ml syringe and 25G needle - bend the needle tip perpendicular to the tip-bevel (either inwards or outwards, depending on personal preference) the to form a sharp hook.

TIP – Our sharpened-tip probes are capable of penetrating dura in mice and most of the rat dura, thereby removing the need for mechanical / chemical durotomy, in turn reducing the likelihood of brain surface trauma and saving surgical time.

2.4 Carefully lower the probe tips to touch the brain surface. Observe each probe shank while advancing to ensure a smooth advance without significant shank bending or deviation. If probe bending is observed, check for the necessity of minor adjustments to the entry point to avoid surface vasculature. If probe bending is still occurring then repeated 'tapping' of the brain surface will often persuade the probe to penetrate; do this by raising the probe clear of the brain surface and then lowering down just until the probe starts to bend - you will need to repeat this for a few minutes as the sharp tips of the probe gradually disrupt the connective tissue that is impeding your path. As a final resort, with the probe pressed against the brain surface with an apparent bend, apply gentle horizontal pressure by means of fine forceps (or similar) to the maximally convex point of curvature in order to generate additional penetrating force (note that you might find the probe 'flips' bend when you do this, if so, retract and start-over).

2.5 When setting the initial implanted position, we recommend that you implant a few hundred microns distance above your target and then spend a couple of days slowly descending to target – this approach is intended to reduce the chances of local tissue damage / micro-bleeding arising from the relatively fast insertion speeds employed during surgery compared to 100-200 microns per day descent thereafter. Recall that the Nano-Drive may either be lowered to the skull surface (half-travel) or remain offset above the skull (full-travel) - see step 1.1. It is at this stage that the atlas driven live-update feature of the NeuroStar stereotaxic surgery system is especially helpful.



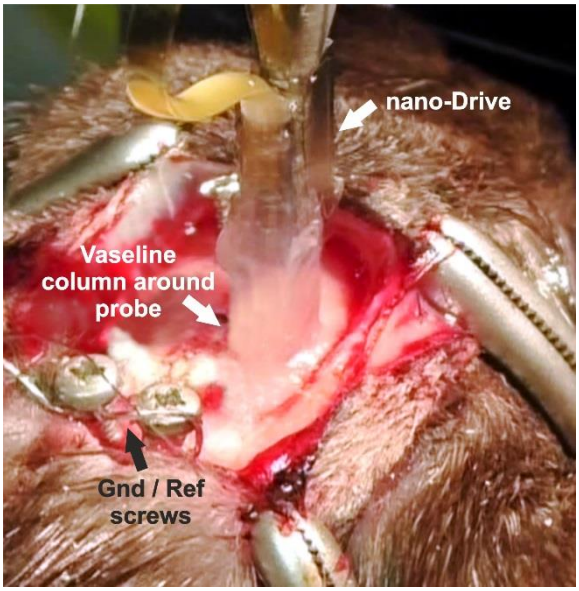


2.6 To repair and reseal the durotomy, mix a few drops of the Dura-Gel compounds (parts A & B, 1:1 ratio) in a mixing crucible and using a spatula, run a droplet of the compound into the craniotomy. Allow 5-10 minutes for the Dura-Gel to part-cure before proceeding with the next step.

For dural repair – see Jackson, N., & Muthuswamy, J. (2008). Artificial dural sealant that allows multiple penetrations of implantable brain probes. Journal of Neuroscience Methods, 171(1), 147–152. http://doi.org/10.1016/j.jneumeth.2008.02.018

TIP – If your target is superficial cortex, we recommend that you implant the probe tip on the brain surface – to achieve this, use your probe to measure brain surface, withdraw the probe clear of the craniotomy and apply the Dura-Gel. Allow 30 mins for full curing of the Dura-gel before returning the probe to your surface reference point, taking care to advance slowly through the Dura-gel.

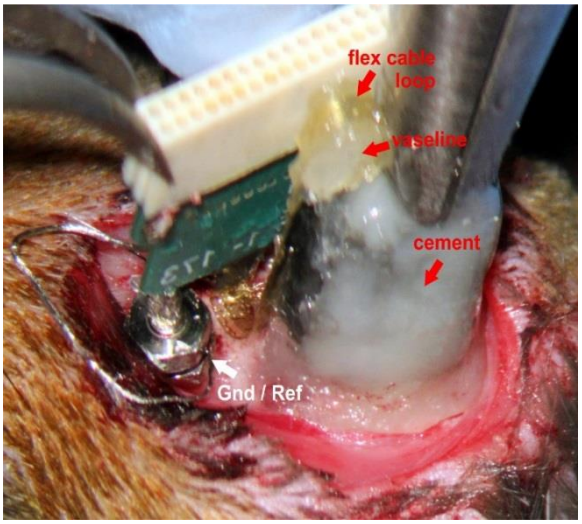
2.7 Using the cautery, carefully apply molten vaseline to the Nano-Drive shuttle. Avoid pushing the drive and similarly avoid direct contact with the probe shanks when applying vaseline. The objective is to form a column of vaseline around the probe and under the Nano-Drive shuttle so as to repel cement from the moving parts. Do this by using the cautery to gently heat the vaseline so it's runs down the Nano-Drive shuttle to create the desired column. Excess vaseline can be easily removed with the spatula or cautery once cooled.



TIP – Run a small bead of cement around the edge of the craniotomy to form a small dam earlier in the protocol – this will help to catch any stray molten vaseline thereby maintaining a clean skull surface for cement-bonding. Consider carefully the height of this cement dam versus your intended clearance of the nano-Drive housing above the skull surface so that you do not create an obstacle for yourself!

2.8 Apply the Relyx cement carefully to the Nano-Drive housing and vaseline column, making sure to run enough cement between the drive and the nearest, clean skull surface - use the curing light to set the cement as you work.

2.9 Take the loose end of your ground / reference wire(s) and wrap around your chosen skull-screw(s). Carefully hold the loose end of the wire taut around the screw and apply a small amount of Relyx cement to stabilize the connection. (Do NOT attempt to solder the wires to the skull-screws!)

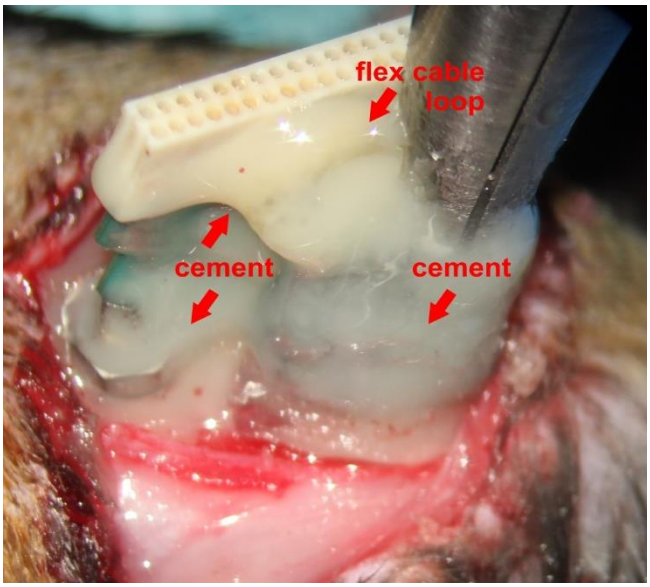


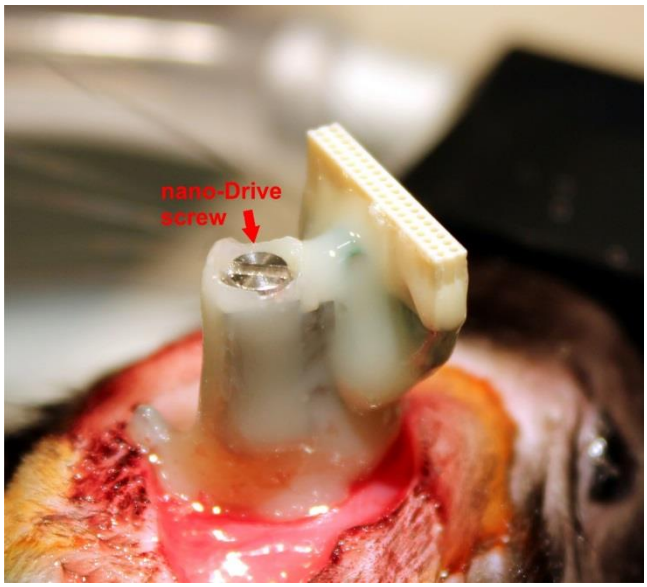
2.10 Carefully loosen the probe connector from the drive-holder and position to your desired location in the implant, allowing sufficient free length of the flex cable to form a loop, which in turn will allow for the descent of the probe on the nano-Drive. Apply a small amount of cement to tack your connector in place. Carefully arrange the flex cable to form a loop, or similar, and protect with molten vaseline on the cable surfaces and within the loop you create.

2.11 Cover the probe flex cable loop with cement and build the

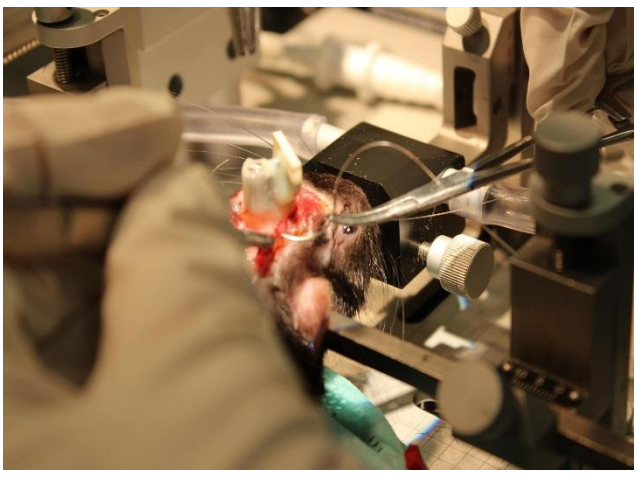
cement up over the loop and around the tip of the drive holder. Note that the vaseline you applied to the drive-holder will prevent cement bonding this to the implant. Carefully tuck the ground / reference wire(s) into the implant and finish cementing all exposed parts of the implant, taking care to avoid any sharp / rough edges near the wound margin.

2.12. When the cement is set, gently loosen the thumb-screw on the drive holder and slowly raise the drive holder upwards to release the Nano-Drive.



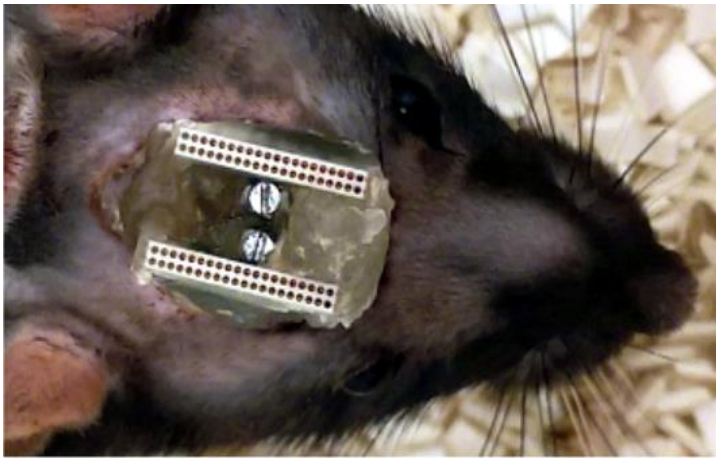


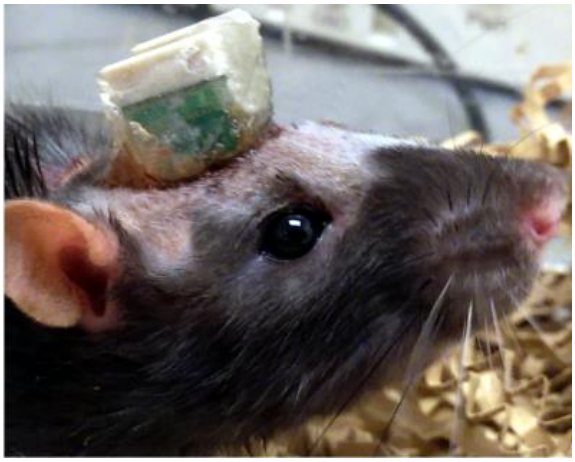
2.13 Thoroughly wash the wound with saline followed by antibiotic (e.g. Baytril). Locally apply Xylocaine around the wood and inject painkiller (e.g. Metacam, sub-cutaneous). Suture the skin using a mattress stitch taking care not to over-tighten the first knot in each suture (over-tightening will cause skin margin death and slower wound recovery).



2.13 Maintain flow of oxygen and homeothermic control while animal recovers from anaesthetic.

2.14 Recordings can be made immediately once the animal is awake and mobile. Most times you will see spikes straight-away, although it may necessary to drive the probe down a short distance over the course of a couple of days following surgery (counter-clockwise is down on the Nano-Drive, 205 um per turn).





For multi-target implants, such as the dual Nano-Drive implant pictured above, proceed part-way through the protocol so that you can partially cement your first Nano-Drive in place, sufficient to release the drive-holder tool, thereby allowing you to approach with the second Nano-Drive. As each multi-target approach may vary according to your desired targets, feel free to adapt the protocol as desired, with the primary concerns being (a) maintaining implant stability and (b) protecting the probes / flex cables from damage whilst partially implanted.

Download the quick-reference surgical protocol

Download the accompanying video of a narrated implant surgery

Additional questions? Need help?

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