

Headmount Attachment Surgery

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Scientific Support Webinar Series

April 2023

Webinar Series

- Deep dive of relevant topics for success with Mightex products such as OASIS Implant, Macro, Polygon 1000
- Current and soon-to-be users, distributors, core facility management teams, etc.
- Offer opportunity to meet with Mightex scientific team members and ask questions/ learn new tips and tricks to optimize data collection and experiment success
- Meet other Mightex product users (e.g., OASIS Implant, OASIS Macro, Polygon, etc.)

Webinar Topics

~~☒ OASIS Implant GRIN lens implantation surgery~~

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~~☒ Virus selection and infusion for calcium imaging~~

☒ **Headmount surgery and preparation for data collection – Today!**

☒ Optimizing data collection with the OASIS Implant + PolyScan3 Workflow

☒ Pattern generation methods for the Polygon 1000

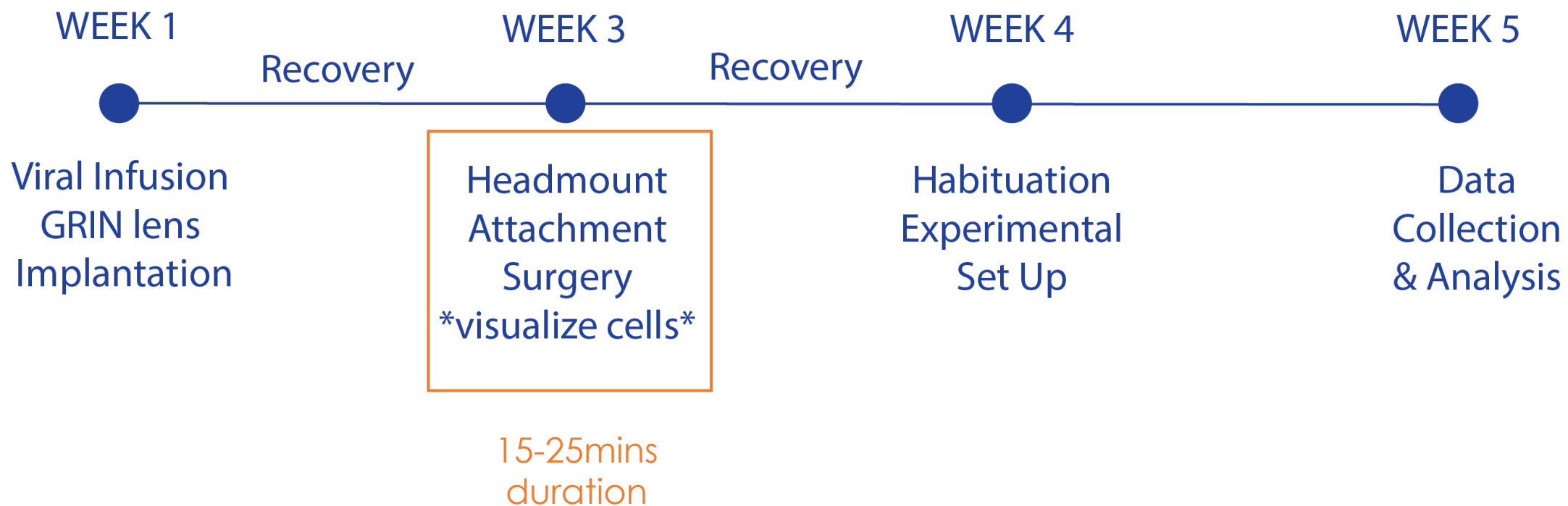
☒ Closed loop control of the Polygon 1000

Design & Purpose of Headmount



- Allows interface and positioning of imaging fiber relative to GRIN secured within the brain
- Allows plane of focus to be determined and FOV set
- Preview cellular activity visualization
- **OASIS Implant works with standard and compact headmounts**

Experimental timeline



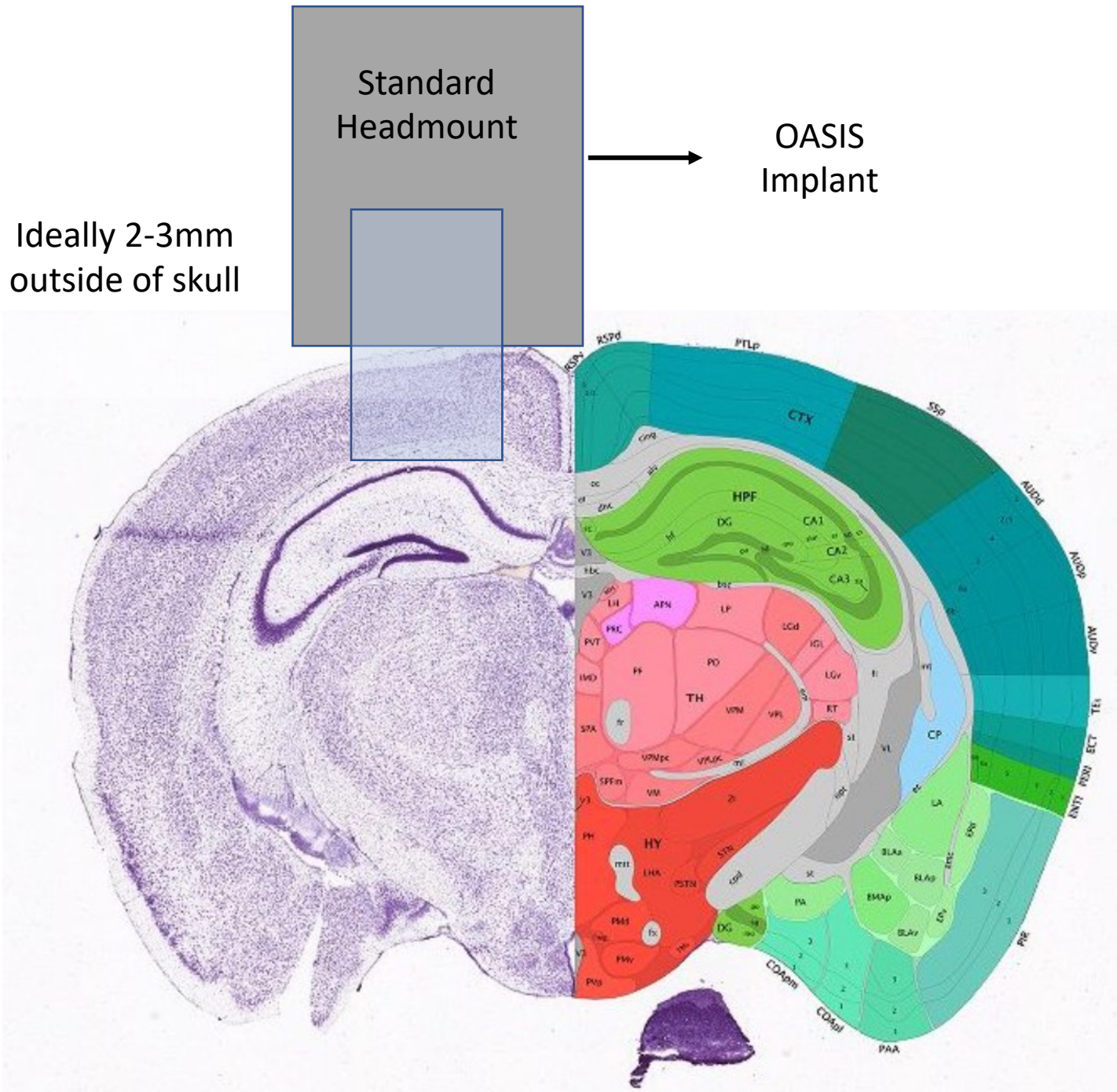


Image courtesy of Allen Brain Atlas

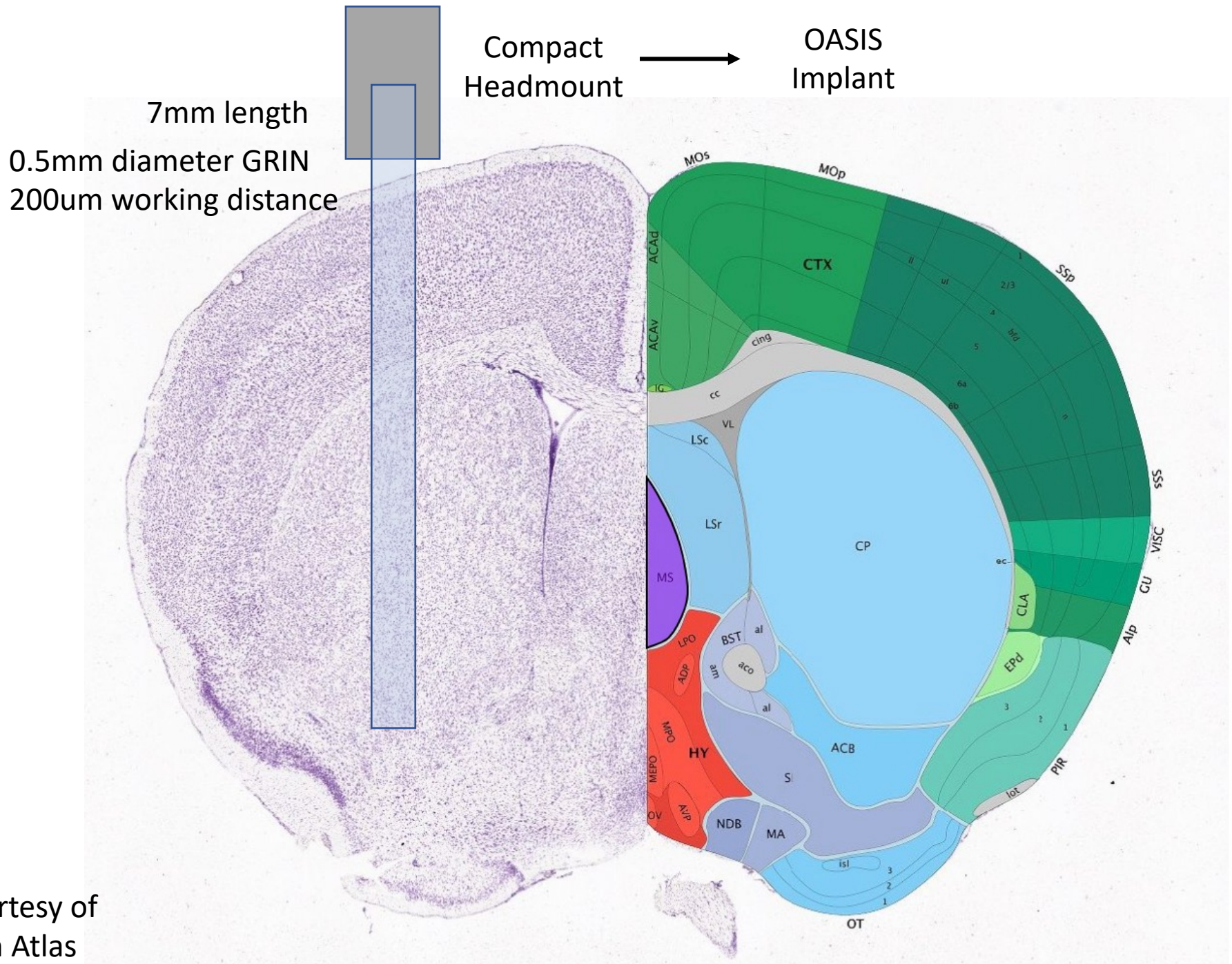
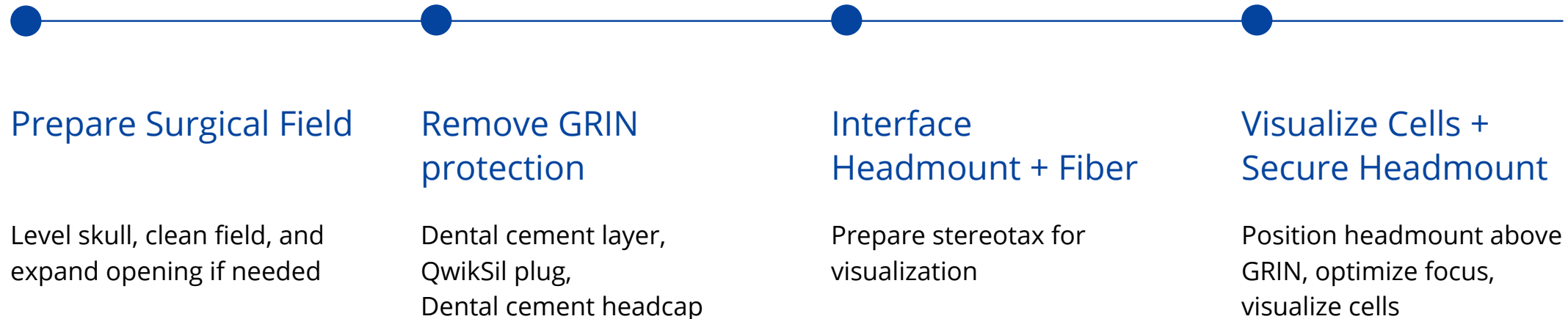


Image courtesy of Allen Brain Atlas

Overview of Headmount surgery



Overview of Headmount surgery

- Everyone does surgery slightly differently
 - Equipment and surgical set up
 - Brain region of interest
 - Additional hardware or techniques
- Best way to optimize your surgery is to **practice, practice, practice!**
- Important Notes:
 - Practice interfacing the imaging fiber and the headmount on your stereotax **before** starting surgery (refer to imaging fiber handling guide)
 - Securing your headcap appropriately will ensure longevity of recording possibilities
 - Always follow AUP and surgical policies for your own institution/ lab; steps outlined here are for educational purposes and may not match your own AUP requirements

Imaging Fiber handling guide

- Whenever fiber is NOT in use, store in protective tubing
- Always hold the imaging fiber by the stainless steel ferrule, be conscious of the location and delicacy of the tip
- Steps for attaching the headmount to the stereotaxic arm:
 - Connect FIXT-000 to the stereotaxic arm
 - Connect the headmount with the front face (where the removable plate is located) facing outwards
 - Tighten screw on the fork with 3mm Allen key
- Steps for attaching the imaging fiber to the headmount:
 - Holding the ferrule, push the imaging fiber into the rubber padded top fork
 - Loosen side screw of the headmount
 - Carefully lower the fiber tip into the headmount; observe location from front plane
 - Tighten upper fork with 3mm Allen key

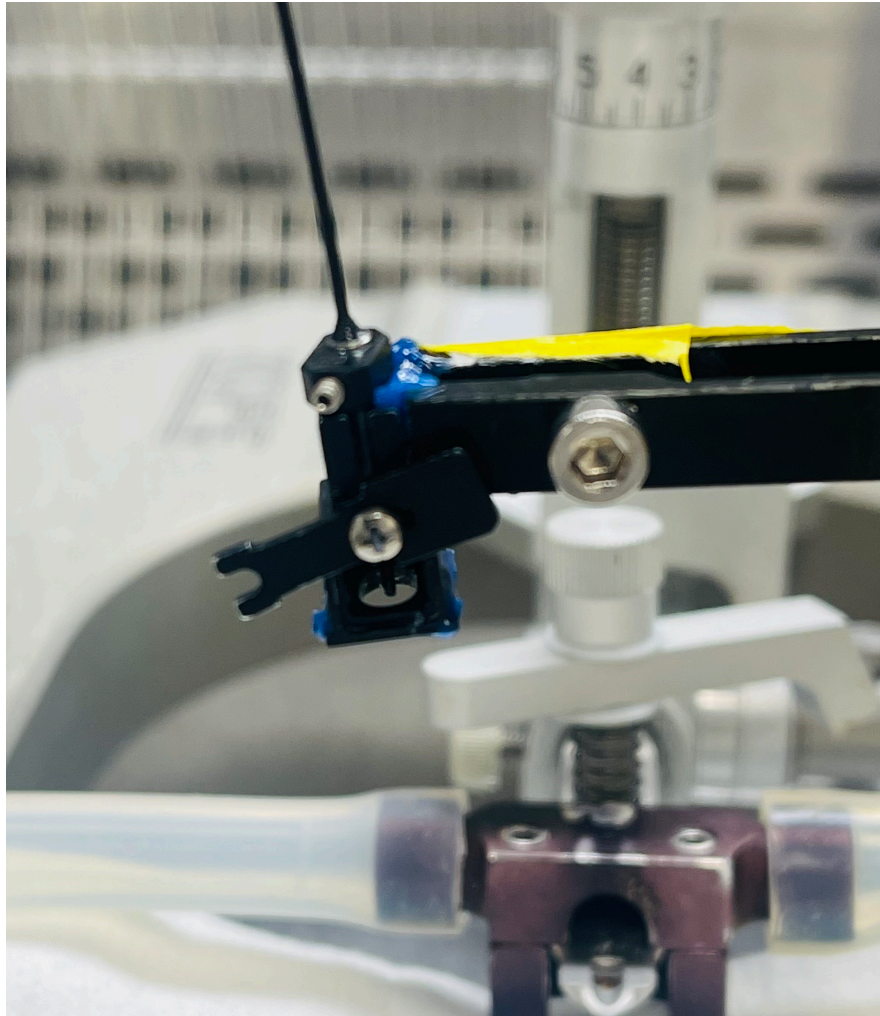
More details and images can be found in the imaging fiber handling guide on MyPage

GRIN lens surgery summary



- GRIN lens surgery involves:
 1. Skull prep and craniotomy (viral infusion optional)
 1. Lens insertion
 2. Lens securing and headcap
 3. Recovery (1 – 3 weeks)
- GRIN is typically covered with silicone plug and dental cement

Preparing the headmount + imaging fiber



- Turn on OASIS Implant LEDs and PolyScan3 software
- Ensure LEDs are working (i.e. you can see light from the distal tip of fiber); Reduce to 0%
- Remove front plate (if using standard headmount)
- Clean headmount with alcohol
- Attach to stereotaxic fixture arm
- Clean tip of imaging fiber by dabbing with Kim wipe and alcohol
- Insert imaging fiber into headmount; swing arm away from location of animal

****PolyScan3 workflow webinar
upcoming****

Headmount Preparation



Figure 3. OASIS implant headmount with fiber inserted. Side screw locks the fiber in place. Front screw locks fiber focus.

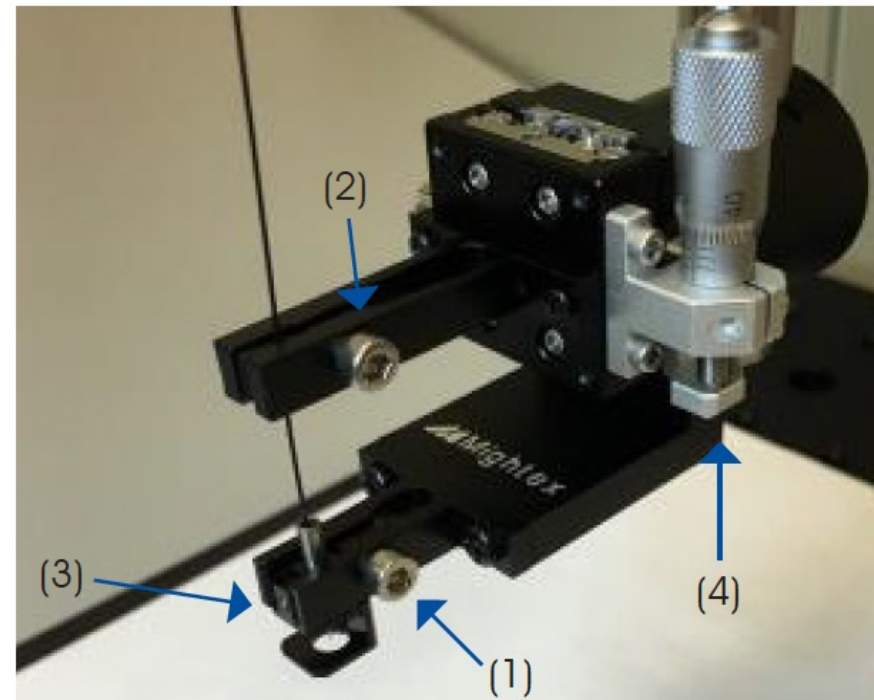


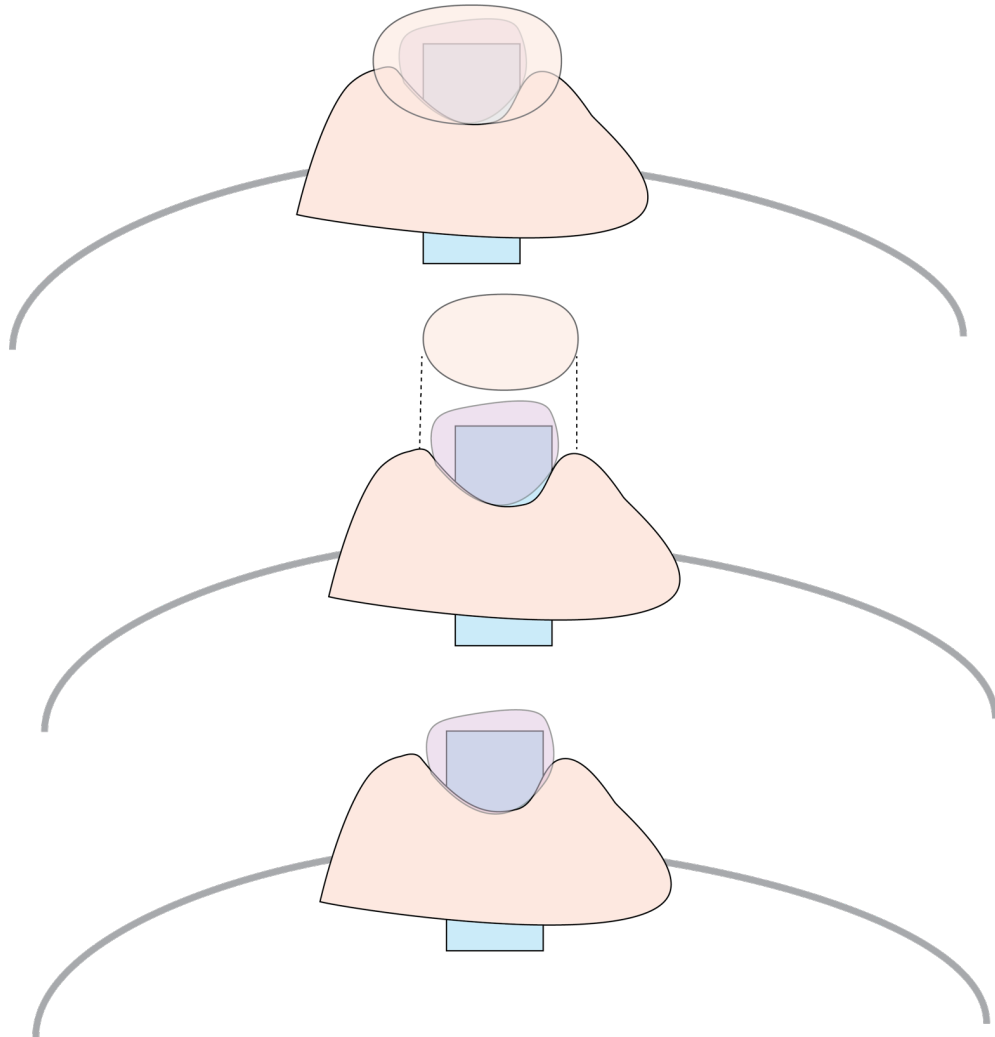
Figure 4. Mightex's focusing fixture. (1) Grip the headmount by tightening this screw. (2) Lightly clamp the fiber by tightening this screw. (3) Loosen the headmount side screw so the ferrule can move freely inside the headmount. (4) Turn the micrometer to move the fiber.

Cleaning and preparing the skull



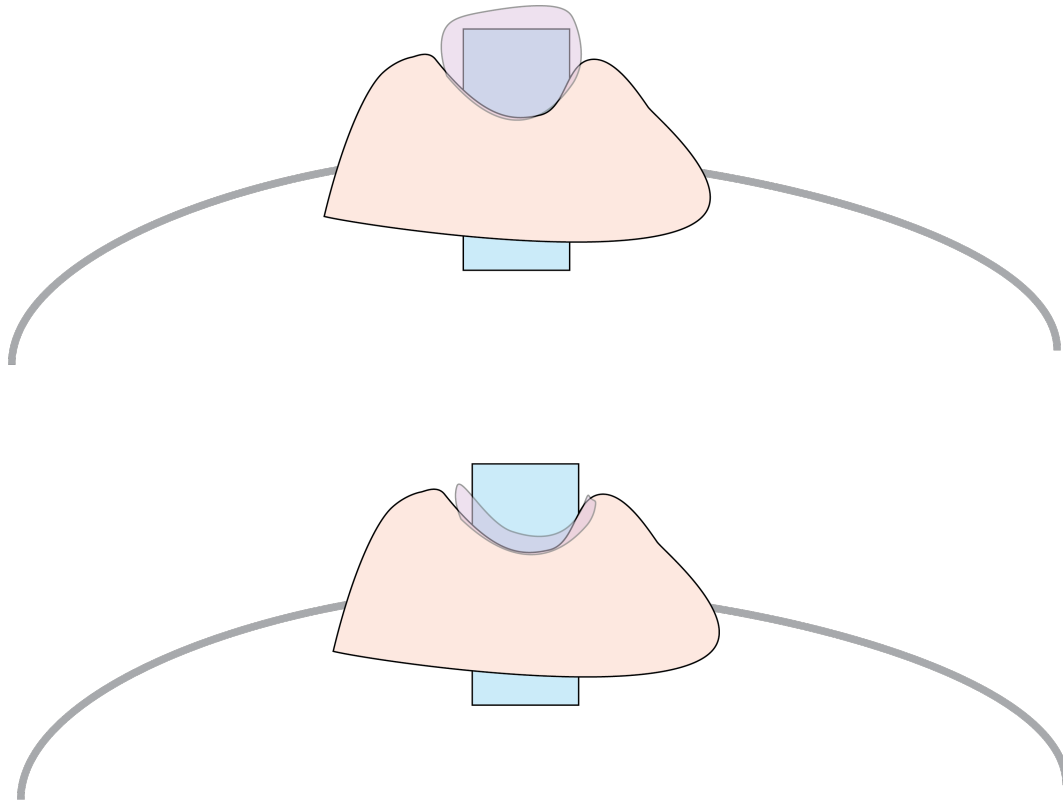
- Anaesthetize animal (isofluorane ideally)
- Head fix and level skull
- Administer IP saline and preoperative analgesic
- Alcohol swab to remove debris and clean headcap
- Use surgical scissors and Q tips to push back/remove any overgrown skin and fascia around GRIN and headmount
- Expand surgical field (remove additional skin/fascia) if you think it is necessary
 - **NB** you can always add a tight suture at the back of the exposed area at the end of surgery to tighten up any areas that are left exposed

Removing Dental Cement Cover



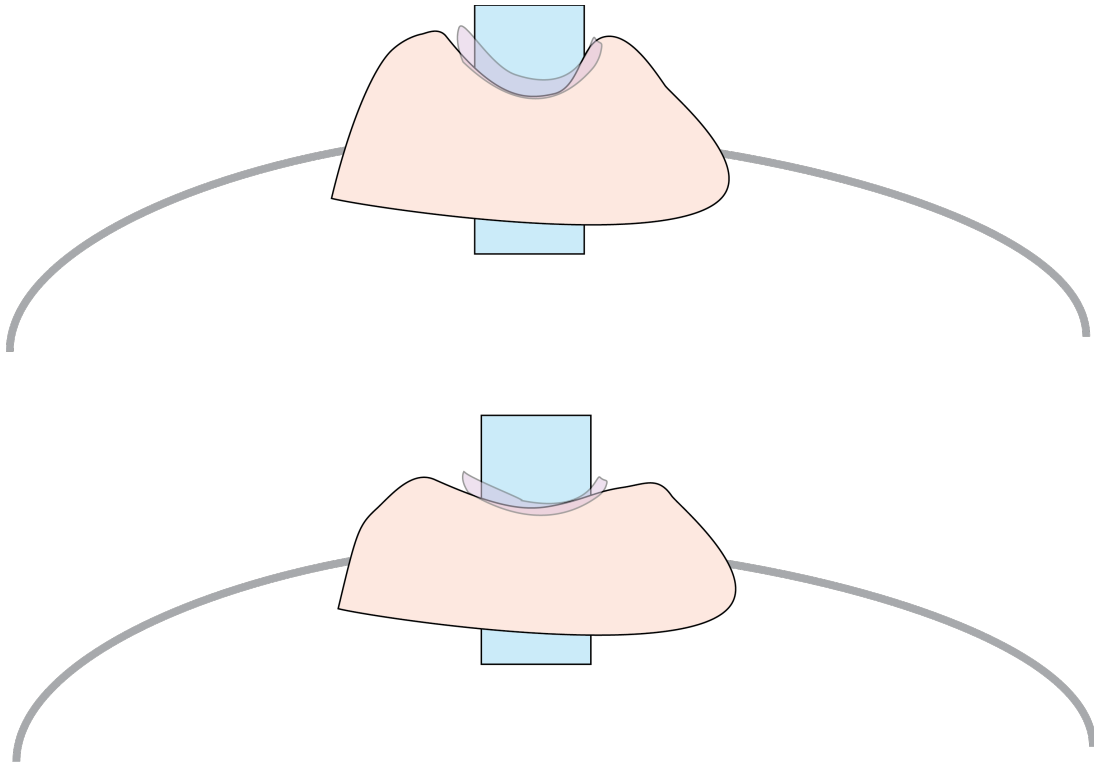
- If you are using a thin layer of cement to cover the GRIN, you can remove this by gently making indentation in a circular pattern around the GRIN (making sure to leave room away from the GRIN itself)
 - Use either a needle tip (bevelled) or a scalpel; use forceps to lift off the loose central circle of dental cement (using alcohol or a drop of acetone if needed)
- Alternatively, use surgical drill to shave off the layer of dental cement
 - Use smallest available drill bit
 - Do not touch center of cement (above GRIN lens)
 - use this approach if you have added a thicker layer of dental cement during your previous surgery
- **Wash surgical field with saline or alcohol as necessary, be sure to remove all cement debris**
- **This requires practice!!**
- **IF your animals are singly housed and you do not wait a long time between surgeries you can try not covering the GRIN at all**

Removing Silicone Cover



- Next remove QwikSil from well surrounding the GRIN.
 - **Goal: clearly visualize the the top of GRIN and make space around sides of GRIN headmount to attach**
 - **NB you can use a fine droplet of alcohol to loosen the QwikSil**
- If there is a small amount of QwikSil left in the well, but the GRIN surface and sides are exposed, you can leave this in place
- If you are finding the QwikSil is difficult to remove, you may need to wait longer for it to cure when first applying (during GRIN surgery)

Preparing headcap surface



- Using your surgical drill, remove the sides of your previously created head cap
- Move in circular motions or zig-zag pattern to reduce the “sides” of the well to a lower level that the top of your GRIN
- Use saline in a hand-held syringe (5ml) to maintain a clear visualization of when your GRIN is located
- Use suction or a Kim wipe to brush away any debris
- **Be careful when conducting this process!**
 - **Do not touch the GRIN; work slowly and precisely using a stereoscope**
- Clean the top surface of your GRIN using a Q tip or Kim wipe with acetone
- **Double check skull is level and adjust to make sure that the GRIN surface is level**

Headmount Preparation



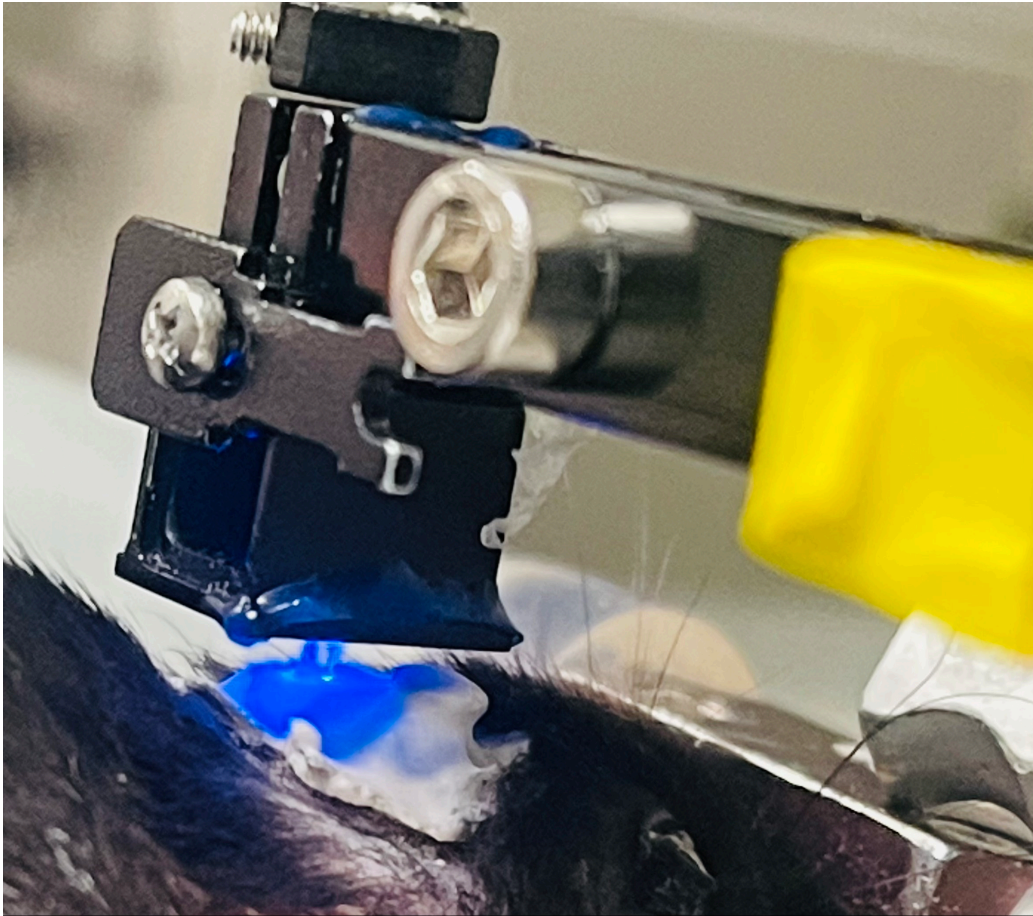
- Confirm that illumination is visible from imaging fiber
- Open camera live stream, position computer monitor to be able to see while working at stereotax/surgical field
- Slowly move stereotax arm around to above surgical area and secure in place
- Use the "window" into the headmount from where the front plate has been removed to see the tip of the imaging fiber

Headmount Interfacing



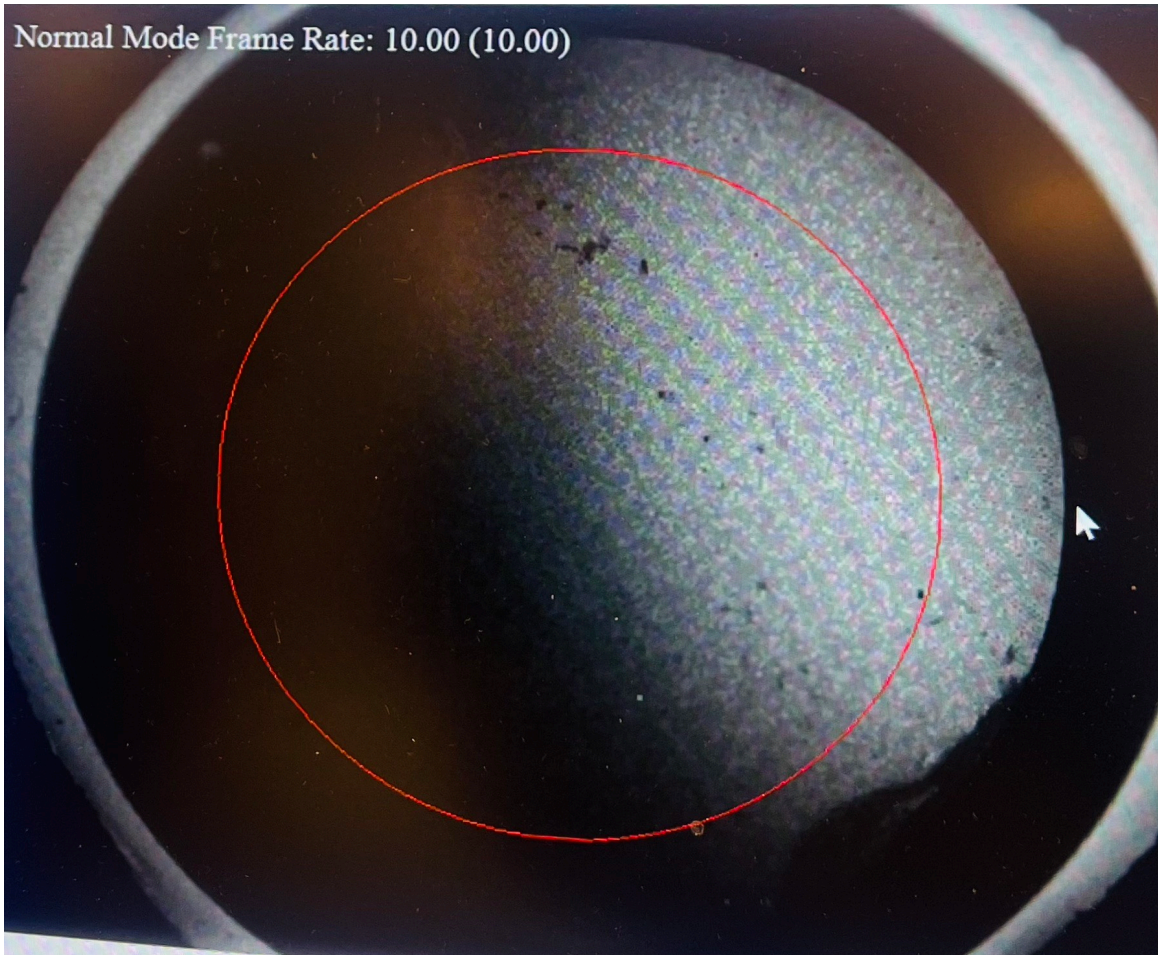
- Position stereoscope so that you are looking at the AP direction of the skull
 - Imaging fiber location within headmount may be visible from the ventral side; you can also approximate this location from where you see the imaging fiber through the headmount opening
 - You may need to adjust your lighting in order to see this (i.e., light from anterior/ medial sides of your surgical field)
- Position headmount centrally above craniotomy
- Turn ON LED and maximize camera live stream on computer screen
- Slowly move your headmount/imaging fiber and position **closely to edge of GRIN**
- Slowly lower until you can see the edge of the GRIN lens in focus on your camera feed

Headmount Interfacing



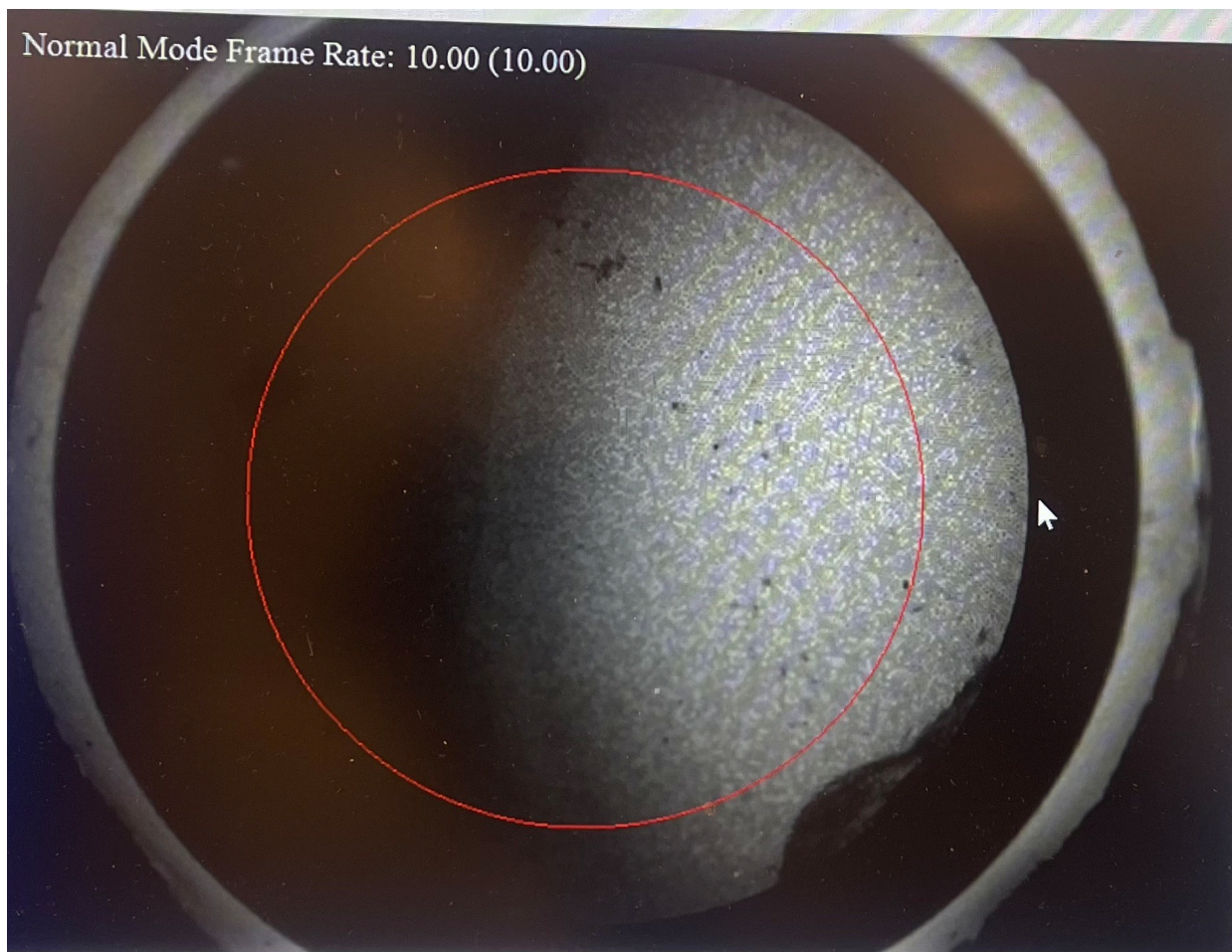
- Position stereoscope so that you are looking at the AP direction of the skull
 - Imaging fiber location within headmount may be visible from the ventral side; you can also approximate this location from where you see the imaging fiber exiting on the dorsal side of the headmount
 - You may need to adjust your lighting in order to see this (i.e., light from anterior/ medial sides of your surgical field)
- Position headmount centrally above craniotomy
- Turn ON LED and maximize camera live stream on computer screen
- Slowly move your headmount/imaging fiber and position **closely to edge of GRIN**
- Slowly lower until you can see the edge of the GRIN lens in focus on your camera feed

Headmount Interfacing - standard



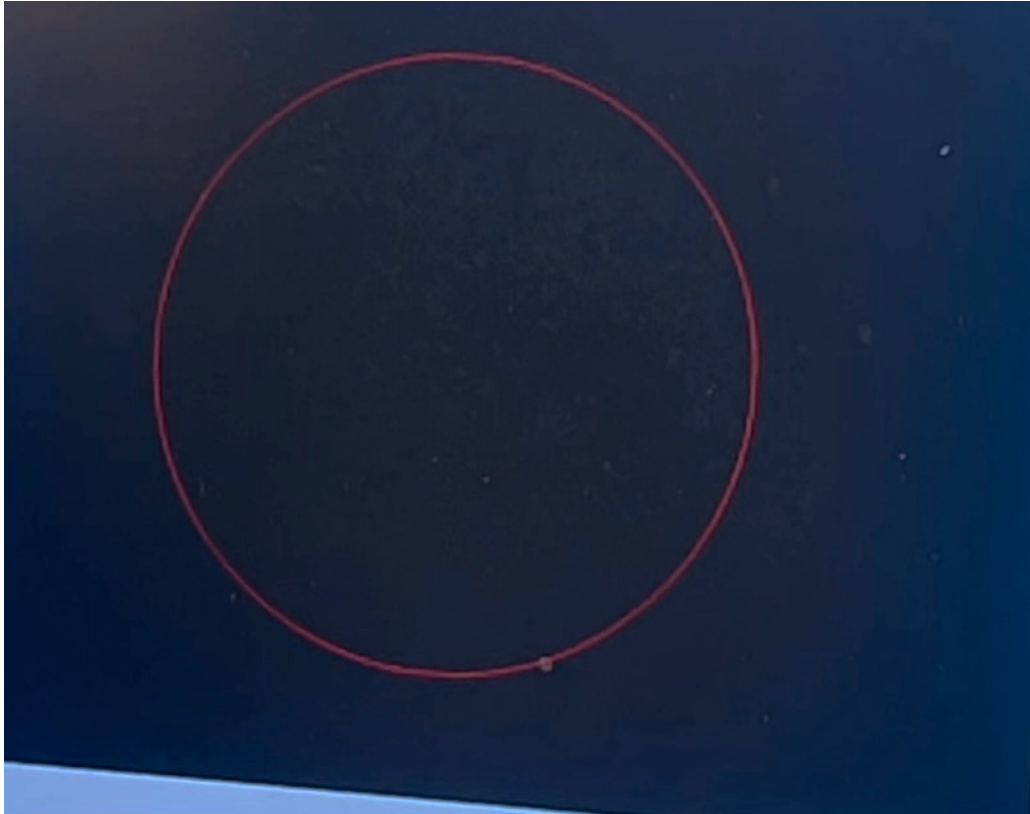
- Next, you can slowly move the GRIN to the center of your camera FOV (it may be slightly out of focus)
- You can then slowly move the imaging fiber relative to the headmount (using the independent micromanipulator) to bring the GRIN into focus
 - Edges of GRIN should be sharply visible and microfibers of the imaging fiber also in focus
- You can then slightly lower the headmount in top of the GRIN and repeat the focusing process
 - You can go back and forth with the position of the headmount and imaging fiber relative to the GRIN
 - You should be able to see landmarks of your brain region as well as vascularizations

Headmount Interfacing - standard



- Next, you can slowly move the GRIN to the center of your camera FOV (it may be slightly out of focus)
- You can then slowly move the imaging fiber relative to the headmount (using the independent micromanipulator) to bring the GRIN into focus
 - Edges of GRIN should be sharply visible and microfibers of the imaging fiber also in focus
- You can then move the the headmount on top of the GRIN and repeat the focusing process if needed
 - You can go back and forth with the position of the headmount and imaging fiber relative to the GRIN
 - You should be able to see landmarks of your brain region as well as vascularizations

Visualizing Cells



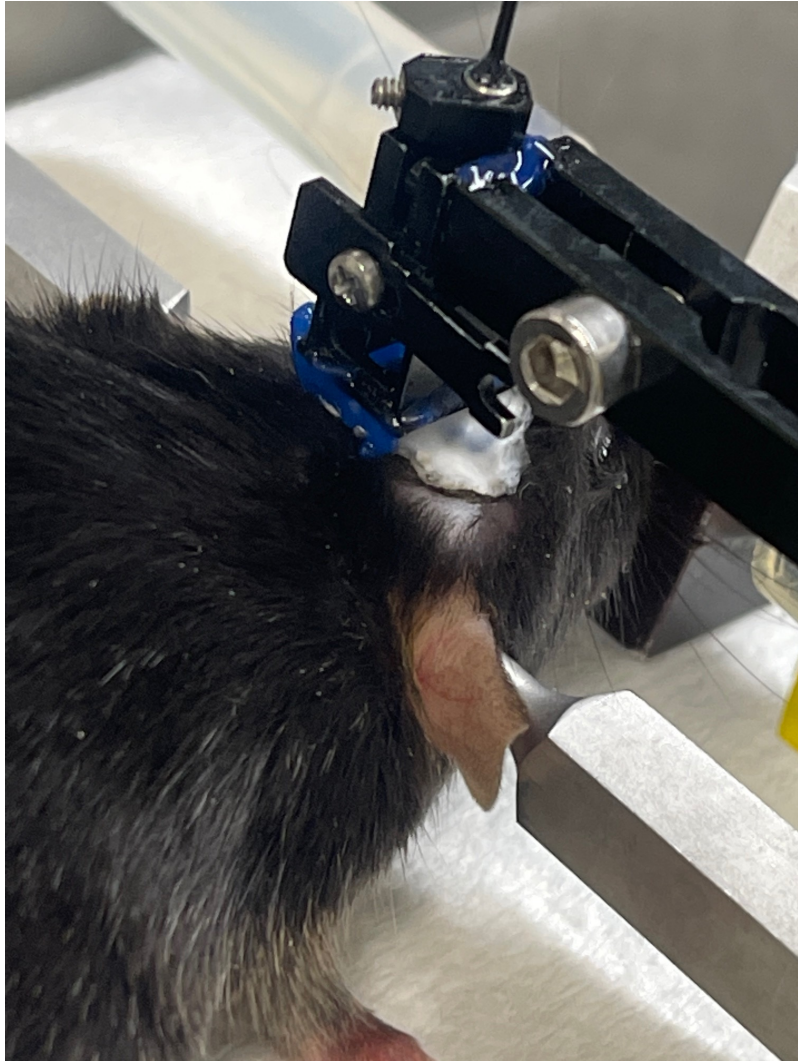
- Once in focus, you can then begin to lessen the anaesthetic of your animal slightly very slowly (or conduct a plantar reflex test and make a noise [e.g., clap/click] near to animal to elicit a response)
 - **Be sure to monitor animal and physiological measures of depth of anaesthesia**
- You can adjust the location of your headmount and imaging fiber within the GRIN diameter (AP; ML) as well as slightly in the DV direction to optimize the cellular population you can see
- Balance factors: depth of anaesthesia, activity of neurons, plane of focus, time taken to 'optimize'
- You can also begin to optimize data visualization by removing background, tweaking camera settings, etc in PolyScan3 to maximize signal to noise and increase likelihood of seeing cells
 - **Covered in next webinar**
 - **Contact our support team for assistance**
 - **See optimization tips in Surgery Primer**

Securing the headmount



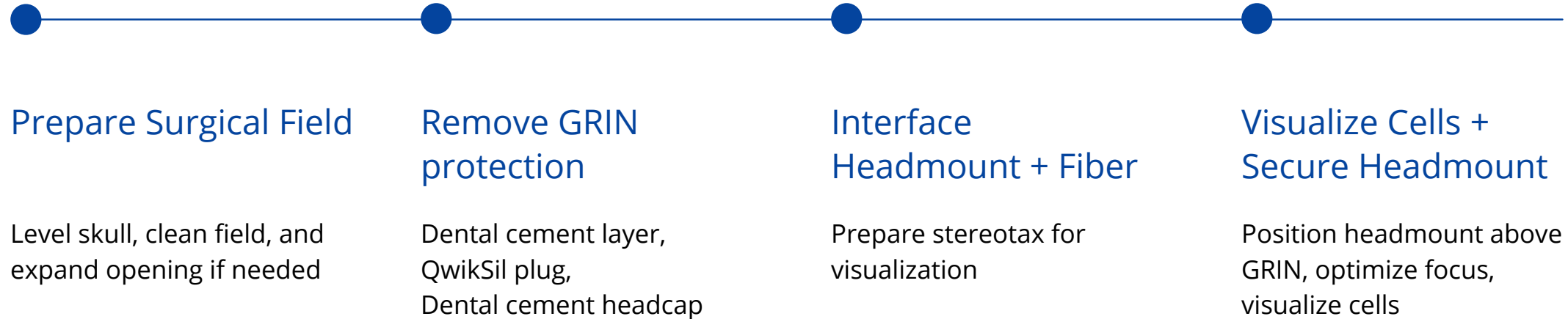
- Once you are happy with the cellular visualization, you can lower the depth of anaesthesia
- Begin securing the headmount by adding a thin layer of metabond (very liquidy consistency) to the exposed surface of the existing headcap and any exposed sides of GRIN lens
- You can also use UV curable epoxy or bonding cement
 - Or LockTite glue can be used along with accelerator → this is more malleable BUT can allow light to pass
- Once almost but not completely dry, begin adding either a second layer of metabond or a first layer of dental cement
 - Black or blue is recommended, pink will also work fine but can allow some light to pass through
 - Apply cement by filling a small syringe (1-3ml) with cement while it is still relatively liquidy, then attach a large gauge bevelled needle tip
 - NB make sure to test viscosity of cement and prime needle tip on paper towel NOT on animal
 - This will let you reach exposed areas underneath the ventral side of the headmount and to fill any remaining empty well area around the GRIN
 - Begin by applying dental cement to the posterior and sides of the headmount; avoid covering the front plate if using the standard headmount

Securing the headmount



- Make sure the first layers of cement are completely dry and hardened before proceeding
- Slowly release the imaging fiber from the headmount (release the screw) and then raise the imaging fiber out of the headmount
- You can then release the headmount from the stereotaxic holder and retract the holder slowly and carefully away from the animal's head
- You should be left with a securely attached headmount on top of the animal's head
- If necessary, you can apply a second coat of dental cement to attach any exposed areas of the GRIN implantation surgery headcap to the headmount cap
- If using the standard headmount you can make sure that the front plate is secured in place but able to be moved when necessary (i.e., the dental cement has not adhered it shut)
- Add a single suture at the back of the surgical area if you have any fascia/ skull exposed

Recap



Post-operative Care

- 3 days of analgesic (e.g., meloxicam i.p.)
- Monitor weight loss/ signs of stress for 7 days
- Hand feed or provide wet food in cage immediately after surgery
- Antibiotics if needed or part of your AUP

- Recovery 7-10 days
- Visualize cells ~2 days after surgery (once gliosis has decreased)

- Habituation and preparation for behavioural experiments/data collection

Important Notes

- It will usually take 2-3weeks after viral infusion and implantation of GRIN to visualize cells; you must time your experiments correctly in order to record/ see cells during the headmount attachment surgery
 - The longer you wait after GRIN implantation, the clearer the field of view (reduced gliosis)
 - This is important for optimizing field of view
- Many neuronal populations are not super active while animal is under anaesthesia
 - Lighten depth of anaesthesia temporarily once you are confident our your imaging fiber and headmount placement to try to see cellular activity
- Make sure that the surface of the GRIN is parallel to the imaging fiber (not on an angle)
- You can scratch lines on the headmount if you are planning long term experiments – this will help the cement keep in place and the headcap attach securely

Important Notes

- Check your animals every day of recovery to check the headcap and headmount
- You may see some photobleaching during the optimization of visualization; this is temporary and you can double check your imaging a few days into the recovery period (during handling or under anaesthesia) to doublecheck the visualization
- Plan out any other surgery or experimental steps (e.g., relearning a behavioural paradigm) around the viral infusion → GRIN → headmount timeline

Preparation for Experiments – Handling & Habituation

- Wait 1-2 days after surgery to begin handling animals daily
- 10-15 minutes of handling by experimenters who will be doing data collection for duration of recovery (ideally 7-10days)
 - Include some resistance holding (e.g., scruff or immobilisation against experimenter's body) during the latter days of habituation → this will be helpful for when attaching imaging fiber
- Once animals are familiar with experimenters, it is worth beginning habituation of attaching the imaging fiber
 - It can be a fiddly process that requires dexterity to properly handle the imaging fiber, insert into the headmount and secure in place
 - It can also be uncomfortable for the animal and requires immobilization
 - Practice attaching and securing (tightening screw) several times until you are confident, and the animal is familiar with the process
- **NB** the animal will struggle when first being held or when imaging fiber is first attached
 - Be gentle but firm, if the animal is struggling too much and you cannot attach the imaging fiber after 2-3 attempts, return the animal to their home cage to calm down and try again after a few minutes
 - Be careful not to hold onto the headmount too tightly or restrict the animal's head movements while interfacing the imaging fiber – this can cause the headmount to become separated from the skull or move the plane of focus and thus ending the animal's viability as a test subject

Summary

- Headmount is used to provide mechanical interface for imaging fiber and GRIN
- Headmount surgery involves:
 1. Preparing headmount and imaging fiber
 2. Skull prep and levelling
 3. Removing dental cap and silicone plug
 4. Positioning headmount and visualizing cells
 5. Optimizing FOV and securing headmount
 6. Recovery and habituation
- Sterile surgical field and clear GRIN lens surface is important
- Individual differences with each experimental design
- Practice, practice, practice!

Available Resources

- Surgery Primer – will be available on MyPage
- Webinar recordings

- Calcium Imaging Guide
- OASIS Implant product focused webinars via website and MyPage
- Future webinars
- Support team – contact us!

Webinar on PolyScan3 workflow and data collection optimization next!

Thank you!

Surgery images courtesy of Jayant Rai – University of Toronto
Webinar and accompanying primer will be available on MyPage
Further questions: Catherine.Thomas@mightex.com