Tips on In Vivo Imaging & Behavior

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Experimental timeline

Plan your experiment's schedule accordingly. Here is a typical timeline for getting mice ready for experiments in freely moving mice.

Week 1: virus injection + GRIN lens implant surgery*

Week 2-3: recovery

Week 4: base plate surgery + acclimation to experimenters/handling

Week 5: habituation to wearing Miniscopes & setting imaging focal plane

Week 6: begin experiment!

*We have been combining the GCaMP injection and GRIN lens implant surgeries with no noticeable disadvantages. We recommend drilling a small burr hole for the GCaMP injection, then waiting 5 min after injection before removing the injection pipette and doing the larger craniotomy for the lens implant.

Virus injection + GRIN lens implant surgery

- In lieu of the vacuum method for lens implants, our lab has had success using a cannula holder to grip the lens to lower it during surgery. Since this cannula holder only holds objects with a minimum diameter of 3.4 mm, we fashioned "sleeves" that fit around the GRIN lenses that allow them to be gripped. More details on this procedure can be found here. Other users have reported using this device that can grip the lens directly.
- Dry and score the skull to increase the surface area that the glue will adhere to.
- Blunt and bend the aspiration needle into a 45 degree angle for more comfortable positioning.
- Decrease the needle size (increase the gauge) once you see white matter (27g → 30g).
- Clean the top and bottom of the lens prior to implant.
- Use Surgifoam to soak up blood right before the implant. Follow these steps to cover most if not all of the craniotomy. First, soak a tiny piece in saline or cortex buffer. Insert it into craniotomy. Press a cotton tip applicator to the Surgifoam to dry it out slightly. The Surgifoam will shrink and fit nicely into the craniotomy. Apply a little bit more cortex buffer to the Surgifoam to have it swell up snugly into the whole craniotomy. After a few minutes, make sure the Surgifoam is wet before carefully removing it.
- We tend to have clearer images when draining all cortex buffer from the brain surface prior to lowering the lens. If necessary, you can use a very small piece of slightly moistened Surgifoam to do this.
- Use Kwik-Sil to seal the craniotomy and lens, then apply glue over the seal and around the side of the lens.
- Cover the top of the lens with Kwik-Sil, then apply a layer of dental cement/acrylic to protect the lens until base plating. Don't skimp on the cement/acrylic. Not applying enough could result in all of it scraping off and exposing the Kwik-Sil and eventually the lens. For some reason, this is common in group-housed cages, and rare in single-housed cages.
- For deeper brain regions, we typically do not aspire out brain tissue. Instead, we take a beveled needle about the same gauge as the lens we're implanting (typically 0.5 or 0.6mm diameter, so ~26 gauge needle), and attach it to the stereotax. We slowly lower this into the craniotomy until

just above the intended brain region; this is intended to make a track into which the lens can fit. Then, the needle is slowly removed and the lens is slowly inserted, as described above.

Base plate surgery

- Using the acquisition software, connect your Miniscope and set the electrowetting lens (EWL) slider to 0. This will ensure that if your base plate somehow shifts majorly, you still have some dynamic range to find the optimal focus.
- Before attaching the base plate, this is your last good chance to clean the top of the GRIN lens while the animal is anesthetized.
- Screw in the base plate to the Miniscope as tightly as you would during an experiment. Screwing in loosely could result in an image that is slightly off the next time you screw the Miniscope tightly.
- Make sure that as much of the field of view (FOV) is in focus as possible. Oftentimes, when lowering the Miniscope to the lens, the lens will not be parallel to the Miniscope, and thus, a portion of the FOV will be out of focus (e.g. lateral side out of focus but medial side in focus). You can either adjust the angle of the Miniscope or the lens to align the FOV. Glue baseplate on when FOV is clear for most of the lens.
- We typically use super glue (Loctite Gel Control Super Glue) to make bridges between the cement on the mouse's head and the baseplate. This glue is very viscous and does not fill crevices like a liquid glue does. This makes it easy to mold the glue as you wish.
- Before applying dental cement, make sure there are no gaps in the hardened glue that could allow cement to seep in and harden. If this happens, the Miniscope may become obstructed by that cement and not seat properly.

Acclimation to experimenters/Handling

We typically wait at least a day after base plate surgery to begin handling. We recommend that mice become well-acclimated to the experimenters prior to mounting Miniscopes on awake moving mice. It is important that the mice feel comfortable with the experimenters as stress during the mounting of the miniscope during the experiment can cause adverse effects for the mice and thus, experiment. After gently handling the animals a few times, acclimate the mice to getting something placed on and off their heads by gently taking the protective caps on and off their baseplate (that is cemented on the mice's heads). The experimenters should be able to quickly remove the cap without much struggle from the animal before continuing onto the habituation phase.

Habituation to wearing Miniscopes

Once the mice (and experimenters) are comfortable with the cap removal, habituate the mice to wearing the Miniscopes while freely moving. To attach the Miniscope, lightly grip the base plate cap with one hand, and unscrew it with the other. Remove the cap, and while holding the screwdriver in place, insert the Miniscope. Make sure the Miniscope is seated as deep as possible, then screw it down. With the V4 Miniscope, there is a hole in the objective piece of the Miniscope to which the baseplate screw will press up against; this ensures that the Miniscope is in the exact same position as it was during the previous recording. Make sure to screw the screw in until the Miniscope is stable but not too tightly beyond this. This can start to thread into the hole of the Miniscope and gradually change the position of the Miniscope in the baseplate, which will affect your field of view.

Especially the first time, the animal may struggle. If the animal is struggling too much, let go and calm the animal. If the animal resists and struggles TOO much while you are holding onto the base plate, it can lead to the dental cement holding the base plate to come off the skull. Once the Miniscope is secured, let the animal explore your habituation environment of choice. We typically just let them move

freely around in their home cage (even if there are other mice in the cage). Depending on your experimental demands, you will need to habituate to different levels of movement. If the experiment only requires mice to walk around and explore, we recommend habituating for at least 3 days for 10 minutes each day. If the experiment requires the mice to run swiftly (e.g. down a linear track), then we recommend habituating for at least 5 days for 10-30 minutes each day.

Setting imaging focal plane, LED intensity, and imaging configuration

We recommend setting the focal plane of imaging and LED intensity prior to the experimental day. This can be done during the habituation sessions. With the new V4 scope, this has become as simple as moving the electrowetting lens (EWL) slider to the desired value in the acquisition software.

- How do you know what a good focal plane is? Minimize the size of the cells in the center of the field of view (FOV). Where they are smallest is where they are most in focus. Because of aberration in the GRIN lens, the cells in the outer periphery of the field of view will appear slightly out of focus and this is normal.
- In some cases, blood may be obstructing a large portion of the field of view so that you may only see cells in parts of it. In such cases, pick a focal plane where the highest number of cells are visible and in focus.

During this session, you may also want to find the optimal level of LED intensity and gain for imaging. Make sure that there is enough blue light to see both the firing of the cells as well as some blood vessels to use as landmarks to align frames. In the new acquisition software, pixels will appear red when they saturate. Pick an LED intensity and gain where cells are visible, but do not saturate. If enough pixels saturate, you may have difficulties differentiating between adjacent cells. For CA1 recordings, we typically use a gain of “Low” or “Medium” and an LED intensity of 1-20, but this can vary depending on the quality of the implant. We recommend taking a video during the habituation session with your settings and analyzing the video to make sure the settings are optimal prior to the start of the experiment. We also recommend creating configuration files for each mouse prior to starting the experiment. These will contain mouse-specific information such as the EWL focus and excitation light settings as well as metadata on the mouse name, experiment type, etc. For more information on configuration files, see here.

Take a snapshot of the brain that you will use to reference for all future recording sessions. If the Miniscope is not properly attached for any given recording session, you can use these previous recordings to ensure that the same FOV is maintained. You can use landmarks such as the presence of blood vessels to ensure that the FOV is the same as before. Ensuring that the FOV is the same throughout the experiment is critical for ensuring that you are able to track the same cells accurately across sessions.

Miscellaneous experimental tips

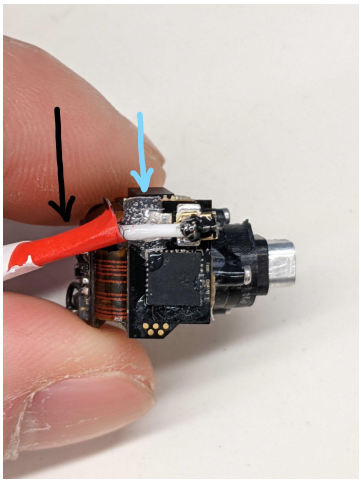
- Check if there is enough space on the hard drive for the data files that will be collected throughout the experiment. If you are using a behavioral camera, don't forget to include that as well.

- Connect the Miniscope cable with the DAQ, connect the USB 3.0 cable with the DAQ and computer, and open the Miniscope Control application on the computer. Use a high quality USB cable. Low quality ones may result in poor data transfer.

- Your experiment may require you to trigger Miniscope data acquisition from an external source. The new Miniscope DAQ boxes (v3.3) are now tolerant of 5V, which is the voltage that Arduinos output, so the DAQ can be connected directly to an Arduino to externally trigger a recording. For

older generations DAQ boxes, you can use a potentiometer to modulate the voltage. The Miniscope acquisition software has a toggle button to enable externally triggered recordings, labeled “Triggerable”. Importantly, if the recording will be triggered by an external trigger, it is important to change the recording length in the user configuration file to 0; otherwise, the recording will shut off when that recording length has been reached.

- Make sure the 3 lights on the DAQ turn on and the red light on the Miniscope turns on.
- Make sure that the Miniscope is flush against the base plate or as much as possible and the Miniscope is not tilted.
- If your Miniscope wire twists at the U.FL connector, try securing the base of it as shown on the right. Wrap a small piece of tape around the wire right above where it connects to the Miniscope (black arrow). This will prevent the wire from twisting there. You can also try securing the cable in place with a small dab of Kwik-Sil to join the cable to the PCB (blue arrow). This can always be carefully removed later.



- Compare the live image you see with the snapshot of the same region you took during habituation. For every session, pull up this snapshot and compare the current field of view to it. You should be looking for signs that the blood vessels, contrast, and brightness look almost exactly the same. It is very important that you visually check this each time you connect the Miniscope. Doing so from memory is unreliable. What do you do if the image looks slightly out of focus? Chances are you screwed the Miniscope too loosely or too tightly, or the attachment was slightly off. Detaching the Miniscope from the base plate and reattaching it usually fixes this problem. In some cases, there may be an obstruction that can be cleaned off before reattachment. Also, you can change the EWL focus as a last resort. If you have tried all these and the image still looks different, the implant may have shifted and recovery may be impossible.
- Gently tighten the set screw to secure the Miniscope onto the base plate. To check if the miniscope is secure, GENTLY wiggle and tug on the Miniscope when attached to the base plate while watching the imaging of the brain. Moving the Miniscope should not cause movement of the image on the video.
- Make sure to have enough slack on the cable so the mice can move freely but not so much that the mice can chew on the cable. If you're not using a commutator, attend to the possible twisting of the cables while the animals are moving. Too much twisting of the coaxial cable can compromise the connection between the Miniscope and the DAQ, which can lead to dropped frames and/or disconnections.
- If your animal will be moving around a lot and for a long period of time (>10 min), a commutator is recommended to prevent wires from bunching and kinking. For commutator options, Miniscope users have historically used DragonFly (ask jamesgroer@mindspring.com for the #FL-4-UCLA-C-MICRO). Our lab has also had success with commutators from Neurotek. Though it is not listed on their website, you can email Hendrik Steenland (wsneurotek@gmail.com) and ask about their Miniscope commutator. For cheaper, DIY options, ONE Core at U Colorado have designed their own commutator. Another DIY option is described by a Miniscope user here.

- Check to see that the write speed of the computer exceeds the FPS collected.
- After the session, visually inspect that data folder to make sure the data was correctly collected.
- Back up your data!!!
- To sync your behavior and imaging, in the timeStamp.csv files, find the closest timestamps and match the frames. If your sampling rates are different, you will have duplicate behavior frames for each Miniscope frame. Basic code to do this can be found here: https://github.com/wmau/CaImaging/blob/master/CaImaging/util.py#L647
- You may find that when you attach the Miniscope and boot up the software, there is dirt on the field of view that obscures the cells. It is important to remove this dirt. This dirt can move during the recording and can disrupt motion correction and cell registration during data processing. To do this, we remove the Miniscope and rub the surface of the lens with a cotton tip applicator that's damp with lens cleaning solution. Make sure the surface is dry before reattaching the Miniscope.