

## Handling Instructions for Imaging Fibers and Head Mounts

Imaging fibers are very delicate and can be damaged when handled improperly. In particular, the tip at the end of the imaging fiber is glass and can be scratched or chipped easily. This document contains several instructions on how to handle the imaging fiber in a safe way that minimizes the risk of damaging it.

### General Instructions

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1. Whenever the imaging fiber is not in use, keep the protective tube covering the end of the imaging fiber securely attached.
2. Once the protection tube is removed, always hold the imaging fiber by the stainless steel ferrule. This minimizes the possibility of the tip accidentally contacting another object.



Step 1.



Step 2.



To perform the following steps you will need:

- 1.5mm Allen Key
- 3mm Allen Key
- Small Phillips screwdriver

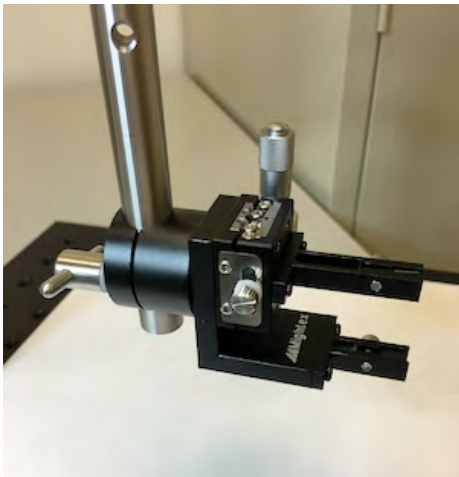
## Interfacing the Imaging Fiber & Headmount - Part I

When attaching the headmount to the animal for the first time, the distal end of the imaging fiber (connected to the OASIS Implant) needs to be inserted into the headmount for live visualization of fluorescent signal.

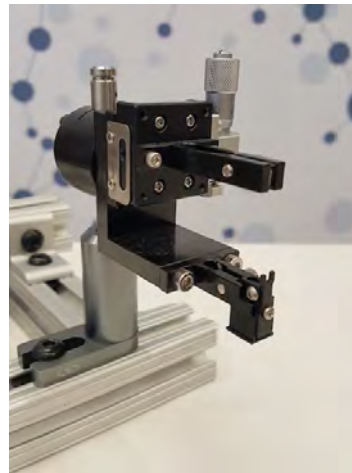
By using the imaging readout from the camera on the OASIS Implant, the user will be able to verify that they are mounting the headmount in the right position.

The next few pages provide description of how to interface the imaging fiber and head mount

1. Connect the focusing fixture [FIXT-000] to the post of a stereotaxic apparatus.
2. Connect the headmount (front plate facing outward) to the lower fork of the focusing fixture. Tighten the screw on the fork with the 3mm Allen key to secure the head mount in place. The front plate of the head mount should be removed by loosening the front screw with the Phillips screwdriver.



Step 1



Step 2 (front view)



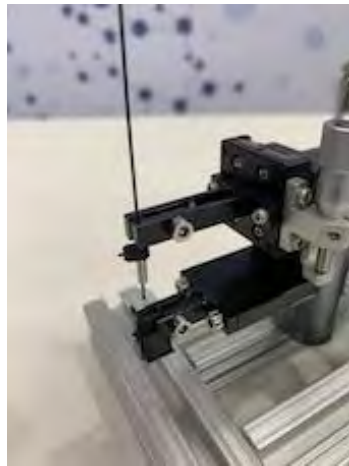
Step 2 (side view)

3. Using the stereotaxic instrument, lower the focusing fixture with the head-mount in place until the head-mount is positioned on top of the skull. We recommend that the GRIN lens protrudes less than 3mm above the skull. Please build up the dental cement surrounding the GRIN lens if the GRIN lens protrudes by more than 3mm. For example, if the GRIN lens protrudes by 4mm, please build at least another 1 mm of dental cement around the GRIN.

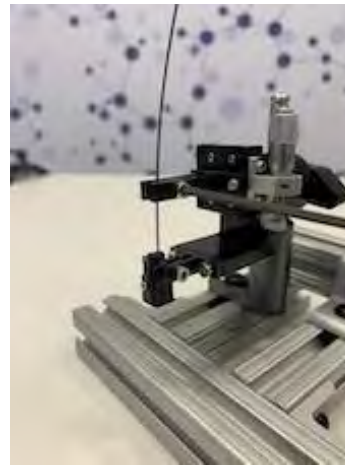
## Interfacing the Imaging Fiber & Headmount - Part II

4. Push the imaging fiber into the rubber-padded top fork first before carefully lowering the fiber tip into the headmount. The side screw on the headmount should be loosened by using the 1.5mm Allen key so that the stainless steel ferrule can slide into the headmount easily. The fork helps to stabilize the fiber to minimize the risk of hitting the fiber tip with any part of the headmount.

5. By hand, position the fiber tip within 3mm from the GRIN lens. Once this is done, tighten the screw on the upper fork of the fixture by using a 3mm Allen key. You should be able to see the tip of the fiber from the opening created by removing the front plate.



Step 4



Step 5

6. Align the tip of the imaging fiber with the GRIN lens protrusion in the X-Y plane. To help in this procedure, turn ON the excitation light source (e.g., if imaging GCaMP, blue light should be emitted from the fiber). Using the stereotax, position the fiber tip such that most of the blue light is being collected by the GRIN lens, rather than illuminating surrounding tissues. Now the fiber tip can be lowered down using the micromanipulator on the focusing fixture.

7. Lower the fiber tip down until it is at the working distance of the GRIN lens. For all GRIN lenses supplied by Mightex, the working distance is 200 $\mu$ m, therefore the fiber tip should be positioned 200 $\mu$ m above the GRIN lens. You can also make finer adjustments of the X-Y alignment if needed.

**8. Do not under any circumstance allow the fiber to come into contact with the GRIN lens.**

A simple way to verify if the fiber tip is approximately in focus with the GRIN lens is to position the fiber tip above the edge of the GRIN lens. At the focus plane the edge of the GRIN lens should become clear and in focus. From here, center the fiber tip within the field of view of the GRIN lens and perform minor adjustments to the focus of the fiber until an acceptable image is seen by the system.

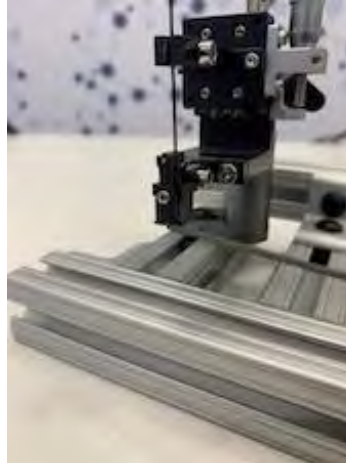
## Interfacing the Imaging Fiber & Headmount - Part III



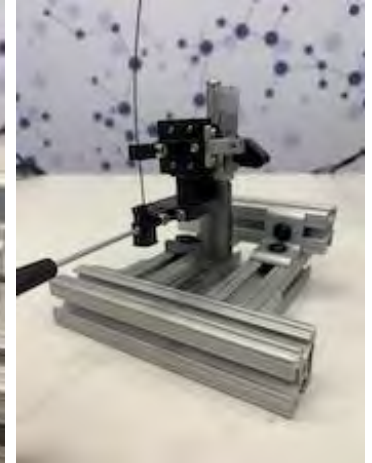
Step 7



Step 8



Step 9a



Step 9b

9a. Once you are happy with the location and image quality, lower the locking ring on the ferrule until it touches the top of the head mount. Lock the locking ring.

9b. Push the front plate up until the pin sets at the bottom of the notch. The fiber should now be in proper focus. Every time the fiber is re-inserted into the head mount it will now be set to the proper focus depth.

10. Once the headmount is cemented in place, you can now remove the fiber from the headmount by performing the instructions above in the reverse order, as below:

- a) Loosen side screw on the headmount.
- b) Use the micromanipulator on the fixture to move the fiber upward until it reaches its maximum point.
- c) Loosen the screw on the upper fork of the fixture.
- d) Carefully, manually move the fiber upward until the fiber slides completely out of the headmount. Use the pads on the upper fork as guides to move the fiber upward.
- e) Slowly pull the fiber outward, disengaging it from the fixture. If needed, loosen the screw on the upper fork even further.
- f) Place the protection tube on the distal tip of the fiber.

11. Use black tape to cover the top of the headmount to prevent any debris from reaching the top surface of the GRIN lens.

12. During subsequent fiber insertions, avoid contact between the fiber tip and any part of the head mount. If necessary, head fix the animal before inserting the fiber.

## Cleaning Instructions

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1. To check for debris or dust on the tip of the imaging fiber, set all the filter positions on the OASIS Implant base station to an empty position and point the fiber tip at a bright light source [ceiling light will work for this]. Observe the image acquired by the camera for any dark spots.
2. Wet a clean lens cloth or lens tissue with a cleaning alcohol.
3. Gently dab the fiber tip with the lens tissue/cloth and then point the fiber at the light source and observe if there are less debris on the image of the fiber acquired by the camera on the OASIS Implant.