

OASIS Implant Surgical Guide

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Introduction

This guide outlines the necessary surgical procedures required to use the OASIS Implant for in vivo imaging. This protocol will focus on describing Graded-Index (GRIN) lens implantation and attachment of the OASIS Implant headmount (Figure 1). Please use this protocol as a guideline that can be adapted for your standard surgical setup.



Step 1: Imaging Indicator Expression

Fluorescent imaging indicators (e.g. GCaMP6) allow researchers to image in vivo brain activity in freely-behaving rodents. Imaging indicators can be genetically expressed (e.g. transgenic mouse lines) or virally introduced into the brain of a rodent, depending on the preferred methodology.

Using the viral strategy, a virus is infused into the brain region of interest to express the imaging indicator (see Figure 2). Sufficient viral expression is commonly achieved ~2-4 weeks following infusion (depending upon virus concentration)¹. Prior to GRIN Lens implantation, optimal fluorescent imaging indicator expression should be tested and verified by sacrificing an animal and examining viral expression.

¹ The GRIN lens can be implanted directly following viral transfection. However, it cannot be guaranteed that the lens will be implanted in an area expressing the fluorescent imaging indicator.



Figure 1. OASIS Implant headmount.

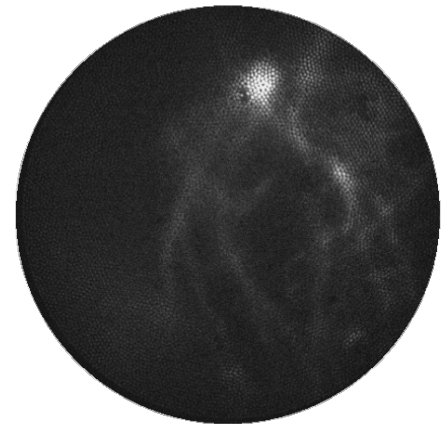


Figure 2. GCaMP6 image taken from the striatum of a mouse with Mightex's OASIS Implant.

Step 2: GRIN Lens Implantation

The implantation of a GRIN lens allows researchers to image fluorescent imaging indicators in deep-brain regions. The diameter (field of view) and length (penetration depth) of the GRIN lens can vary depending on the application or brain region of interest. For deep-brain regions, a GRIN lens with 0.5mm diameter is commonly used to minimize tissue damage. Select the appropriate GRIN lens size depending on the brain region of interest. Please follow these steps for proper implantation of the GRIN lens for in vivo imaging.

NOTE: The working distance of the GRIN lens supplied by Mightex is $\sim 200\mu\text{m}$. Adjust your dorsal/ventral coordinates accordingly to image the region of interest.

1. Insert 2-3 skull screws surrounding the area of the implant. These will be used to adhere/secure the dental cement to the skull².
2. Align the GRIN lens with Bregma (without touching the skull) and zero the coordinates.
3. Move the GRIN lens to the appropriate coordinates (region of interest) relative to Bregma and mark this region. Raise the GRIN Lens from the region of interest.
4. Perform a craniotomy in the region of interest to accommodate the implantation of the GRIN lens. Remove any dura using a bent needle.
5. Prior to GRIN lens implantation, confirm there is no bleeding in the craniotomy area.
6. Slowly lower the GRIN lens into the brain region of interest ($\sim 100 \mu\text{m}/\text{min}$) to reduce tissue damage. To further prevent tissue damage, stop lowering the GRIN lens periodically and raise it to let the tissue settle.

Step 2: GRIN Lens Implantation

7. Following implantation, the OASIS Implant can be used to image through the GRIN lens to confirm fluorescence and correct placement of the GRIN lens.
8. Once the GRIN lens has been lowered to the correct coordinates, apply an adhesive cement (e.g. Metabond®) around the GRIN lens to secure it to the skull³.
9. Once the adhesive cement has completely dried, apply a layer of dental cement surrounding the lens and skull screws.
10. Once this layer of dental cement is dry, carefully remove the stereotaxic arm from the GRIN lens. Apply another layer of dental cement on the skull around the GRIN lens and skull screws to build a surface for attaching the headmount at a later time (CAUTION: Careful not to get dental cement on the top of the GRIN lens)⁴.
11. Let the dental cement dry completely.
12. Apply a silicone covering (e.g. Quickcast®) over the GRIN lens to protect it from any potential damage during the healing process.
13. Let the mouse heal for ~4 weeks following GRIN lens implantation to obtain high quality imaging.

² Alternatively, Metabond® can be used by itself to secure the dental cement to the skull.

³ Alternatively, super glue can be used to secure the GRIN lens to the skull.

⁴ A head-fixing plate can be attached following GRIN lens implantation. This will be placed on top of the dental cement. Apply as necessary to maintain proper fixation to the skull.

Step 3: Headmount Attachment

The OASIS Implant headmount is designed to couple the imaging fiber and GRIN lens for imaging in the deep-brain of a freely-moving rodent (see Figure 3). This light-weight headmount allows the fiber to be focused relative to the GRIN lens and attached/detached from the rodent. This step should be performed following the GRIN lens healing process (~4 weeks) and once the imaging indicator can be visualized to aid with alignment and focusing. Please follow these steps for proper attachment of the OASIS Implant headmount.

NOTE: To properly align the imaging fiber and GRIN lens, have the OASIS Implant running (including software) to view live images and obtain proper focus.

1. Clamp Mightex's focusing fixture onto the stereotaxic arm.
2. Place the imaging fiber ferrule into the headmount (see Figure 3). Lock the front plate in the middle position to allow for focus adjustments.
3. Attach the headmount and imaging fiber onto the focusing fixture by clamping the headmount using the bottom screw and lightly clamping the fiber using the top screw (see Figure 4). Lightly tighten headmount side screw (see Figure 4) to allow the fiber to be raised or lowered. This will allow proper focus for imaging to be obtained. (CAUTION: If this is too tight, it can damage the fiber).
4. Lower the focusing fixture (see Figure 4) to place the headmount over GRIN lens.
5. Use the focusing fixture to focus the fiber with the GRIN lens by viewing the live image.

Step 3: Headmount Attachment

6. Use the stereotaxic arm to carefully adjust the X and Y position of the fiber (inline with the GRIN lens).
7. Once a proper position is established, cement the headmount to the skull using pink dental (CAUTION: do not cement over the front cover). Cement over the back and sides of the headmount.
8. Loosen the side screw and carefully remove the fiber from the headmount.



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

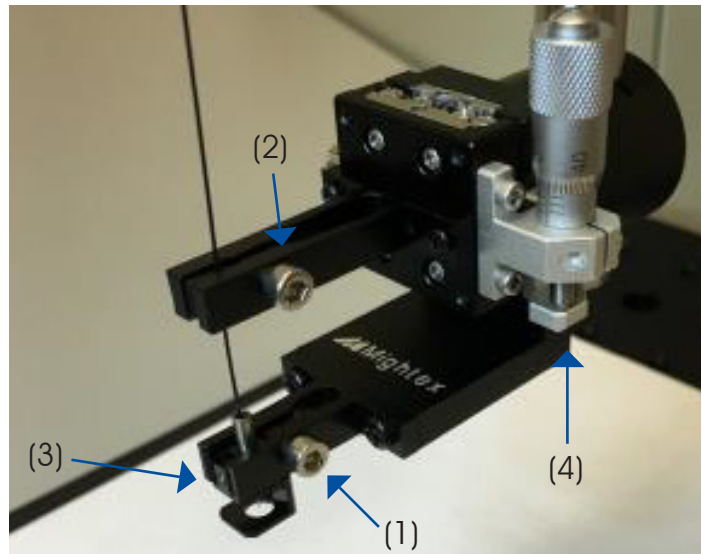


Figure 4. Mightex's focusing fixture. (1) Grip the headmount by tightening this screw. (2) Lightly clamp the fiber by tightening this screw. (3) Loosen the headmount side screw so the ferrule can move freely inside the headmount. (4) Turn the micrometer to move the fiber.

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