A Novel Pressure Regulating Brain Imaging Implant For Ultra-Large Field-of-View Microscopic Imaging in NHPs

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Problem Definition & Need

Problem Definition

Several problems challenge mesoscopic imaging in the brain: 1) Difficulty with positioning high-NA objectives near the brain; 2) Creating a flat imaging window against the surface of the brain; 3) Adjusting the imaging window to changes in swelling and pressure in the brain, such as those that may occur due to hydration changes and other physiological factors; 4) Preventing growth of dura and biofilms that cloud the imaging window; 5) Follow-on MRI imaging of the animal post-implantation.

Need Statement

Need a thin, strong, radiolucent, and pressure regulating implant for ultra-large field-of-view microscopic imaging in primates.

Design Inputs

Functional Requirement

Maintain a flat, clear, imaging window against the surface of the brain.

Constraints

- Radiolucent
- Thin enough to allow for an objective with a working distance of 2mm to image the brain.
- Allow for water immersion objective.
- Capable of holding attachments & bearing weight (at least 5 lbs).
- Imaging window must be at least 20 mm in diameter.
- Coverslip must move parallel to chamber to allow for manual control of the imaging window.
- Must be durable, last at least six months and be usable on awake monkeys.
- Must be hermetically sealed to prevent infection.

Failures & Modifications

Original Idea for pressure regulation: Sodium Alginate based hydrogel → (Hydrogel breaks / Hydrophilic & Bioactive) → Aseptic inflammatory reaction → (Replace with silicone / Hydrophobic & Inert) → Reduced inflammation and environment remained sterile

Modification: Redesigned the silicone gasket to include two O-rings. Previous design was a flat disk

Original method for adhering silicone cylinder to cup & rotating ring: Silicone based glue → (Leakage occurs after 1 day / Sterile environment compromised) → Inflammatory reaction → (Test adhesives: • Cyanoacrylates • LocTite • Lorde / Sterilizable, hermetic seal & long lasting) → Reduced inflammation and environment remained isolated

Modification: Implemented use of Lorde medical grade adhesive. 3D printed structures to accommodate improved adhesion.

Original method for head stabilization: Single titanium arm on air table → (Difficult to quickly stabilize / Arm requires multiple points of tightening) → Allows for +- 50 um of movement → (Developed aluminum block for head stabilization / Easy to assemble with commercially available parts) → Reduced movement to +- 25 um and experimental set up time

Modification: Switched from multi-axis titanium arm to a limited, bulkier, aluminum block.

Original method for cortical stabilization: Stabilizing screws in imaging cylinder → (Damages silicone if inserted at wrong angle / Compromises sealed environment) → Allows for +- 25 um of movement → (Developed suction system for cortical stabilization / Maintains closed environment and doesn't damage cylinder) → Achieved low micron movement in ideal conditions

Modification: Redesigned cylinder to have permanent , metal, 21G needle guides sealed with silicone adhesive to allow for penetration and subsequent suction. Works in conjunction with head stabilization to reduce motion.

Engineering Design Solution

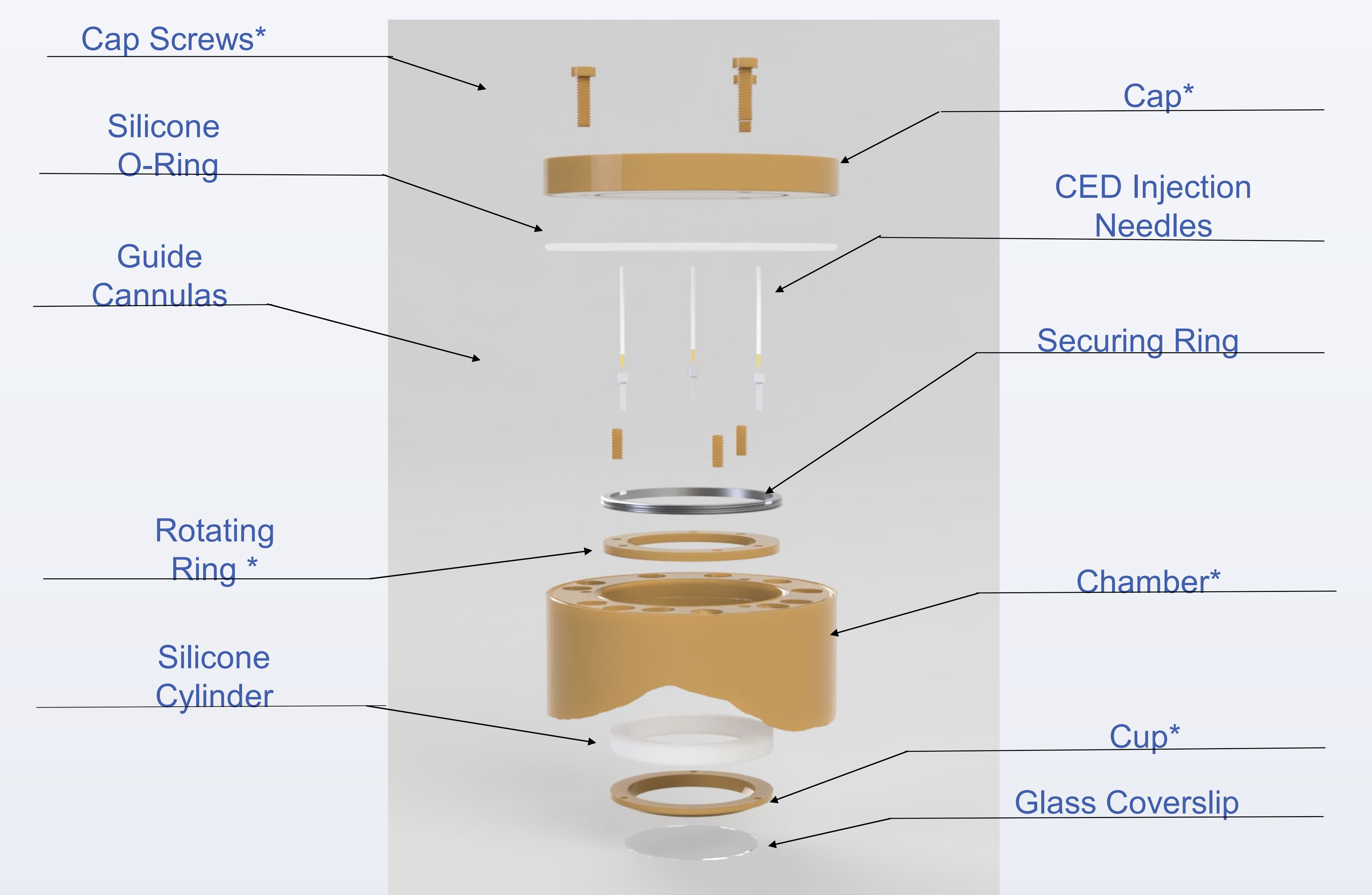


Figure 1: Exploded view of annotated imaging implant prototype. *Indicates part is made of PEEK plastic, chosen for its radiolucent properties, strength, and ability to be sterilized.

Component Listing with Description

Cap & Cap Screws- Removable cap for imaging and cleaning.

Silicone O-Ring - Approximately .4 mm thick, prevents bacterial movement between chamber and cap. There are two O-rings, one on the outer and one on the inner diameter of the chamber. The cap and chamber have indents to accommodate the rings and squeeze them when the cap is completely secured.

Thin Securing Ring - Secures rotating ring against the bottom shelf of the chamber and prevents it from moving up.

Guide Cannulas - Cannulas are threaded into the rotating ring at 3 different locations and sealed with silicone glue. These cannulas allow for 21G CED needles to penetrate the cylinder.

CED Injection Needles – Convection enhanced delivery needles. Designed for cortical injections but can also be used to deliver drugs or imaging contrast agents into the soft tissue.

Rotating ring – Multiple threaded holes for the height adjusting screws and can rotate to adjust the positions of the screws. Sits between shelf in chamber and thin securing ring.

Chamber - Has holes for bone screws that are perpendicular to the surface of the bone to increase the strength of the bond between the chamber and bone[2]. It also includes three threaded holes on the top to allow for attachments and to secure the cap.

Silicone – Chosen for its ease in manufacturing, control of mechanical properties, and ability to be sterilized. Serves as a spring to adjust for pressure changes caused by the variations in swelling in the brain. Although not indicated in the drawing, the silicone connects to the lip of the imaging cup and rotating ring to create a sealed environment.

Imaging Cup & Glass Cover Slip – The glass is glued to the cup. Together they create a bowl that can hold liquid for a water immersion objective.

Modifications for 3D printing

Figure 2: Panels (A), (B) & (C) show the progression of replacing damaged threads on the implant using a soldering iron to press new, metal inserts into PEEK plastic at 550 degrees Celsius. Panels (D) & (E) show a modified tab design to replace the threaded ring and it easier to print. Panel (D) is a strength simulation showing >2 e^-4 mm of displacement when 20 N of force is placed on the ring.

Viral Transduction Results

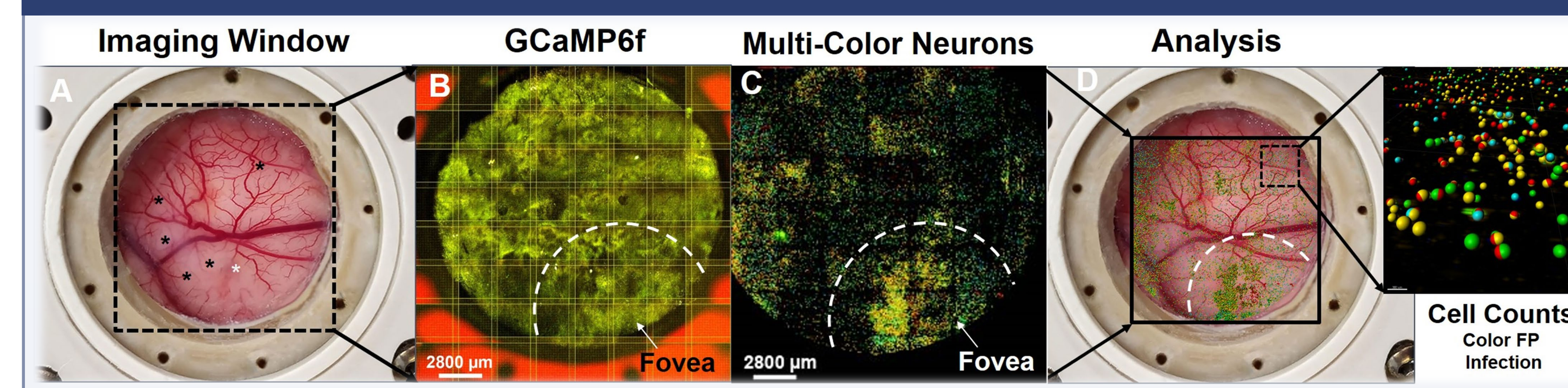


Figure 2: (A) 2cm imaging window (stars indicate previous probe sites). White star indicates the LGN CED injection site. (B) Stitched 2P image (49 tiled 2800 µm stacks) of GCamp6f infected cells.(C) Multicolor scan of multiple fluorescent protein cells. (D) Positions of individual best of multi-color FP cells. E) Hyperspectral brainbow analysis. We were able to successfully transfect the cortical area beneath the implant with five multi-color fluorescent proteins and GCamp6. The implant allowed for impressive imaging using our 2-photon scope and after analysis revealed successful transfection of the virus' injected. The results indicate that the implant was successful in its ability to keep enough dural and pial regrowth at bay to allow for the imaging previously discussed.

Bioluminescence Brainbow Spectral Analysis

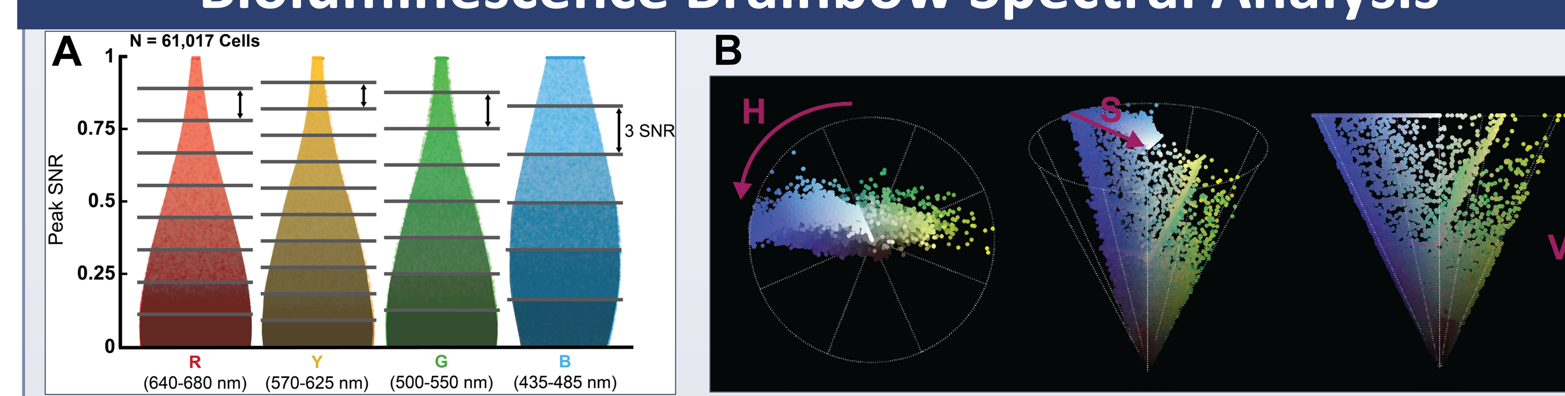


Figure 3: (A) Violin plot shows 4,752 distinguishable colors. (B) indicates the hue, saturation, and values of the cell colors.

Stability Analysis

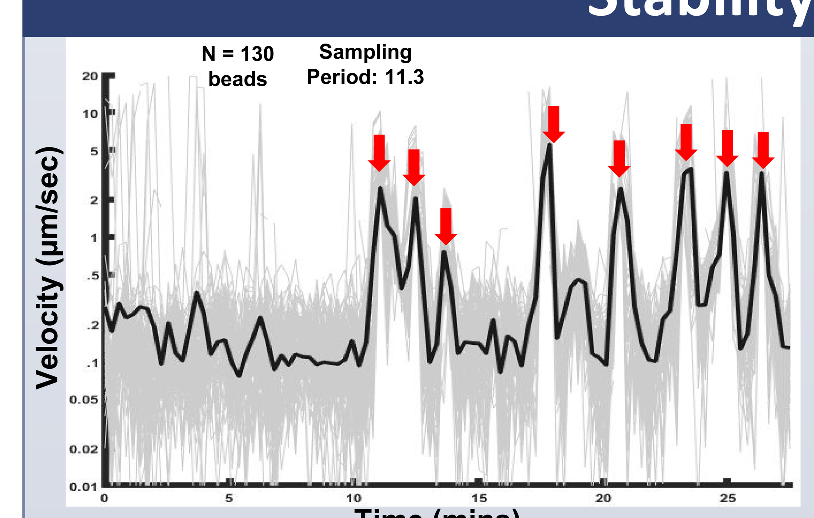
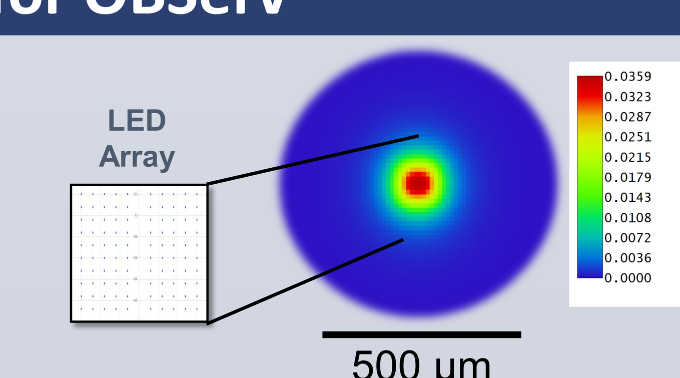


Figure 4: X-Y motion was measured by placing a clear slide of fluorescent od = microbeads on the imaging glass of the chamber. The movement of each bead (gray lines) was recorded & averaged (black line). Large bodily movements of the NHP are indicated with red arrows. As seen in the figure the NHP becomes restless after ~ 30 mins of imaging.

LED Simulation for OBServ

Figure 5: LED light scatter simulation, 1 mm deep into the cortex using Zemax Optic Studio. LED positional array of 100 16 x 16 µm LEDs separated by 8 µm, each LED operates at .012 mW/mm^2 of power.



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