

Supplementary information for Protocol for In Vivo Two-Photon FCS to Measure Nanocarrier Number and Flow Velocity in Mouse Cerebral Microvasculature

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Table S1: Acute Cranial Window Preparation Guide

Protocol: _____

Surgery Type: _____

Surgery Classification: Non-Recovery

Date of Log Creation: _____

Principal Investigator: _____

Lab Contact: _____

Mouse Information

- Mouse ID: _____
- Strain: _____
- Sex: _____
- Age: _____
- Weight (g): _____ (Baseline, measured 1 week prior)
- Cage Number: _____

Pre-Surgery Observations (Week Leading to Surgery)

Observations every other day (~5 min/day) for grooming, coat, eyes, tumors, locomotion, responsiveness, and weight fluctuations (>10%).

Date	Time	Grooming (Normal/Abnormal)	Coat (Clean/Dirty)	Eyes (Clear/Cloudy)	Tumors (Y/N)	Locomotion (Normal/Abnormal)	Responsiveness (Normal/Reduced)	Weight (g)

Surgery Details

- Date of Surgery: _____
- Start Time: _____
- End Time: _____
- Duration (min): _____
- Surgeon Name: _____
- Surgical Workstation Disinfected (Y/N): _____
- Sterile Instruments Confirmed (Y/N): _____
- Surgeon PPE (Mask, Sterile Gloves, Clean Gown): _____
- Support Equipment Used: Stereotaxic apparatus, surgical tools, oxygen/isoflurane delivery, heating pad, glass bead sterilizer

Pre-Anesthetic Medications

May be administered at 30 minutes prior to surgery by intraperitoneal (IP) injection or subcutaneous (SQ) injection in scruff or flank.

Medication	Dose (mg/kg)	Time Administered	Site (IP or Scruff/Flank SQ)	Notes
Dexamethasone	2.0			
Meloxicam or Carprofen	5.0			

Operative Anesthesia

- Induction Method: Isoflurane (2-4% in O₂) in an induction chamber
- Maintenance Method: Isoflurane (2-3%) via breathing circuit and nosecone
- Anesthetic Depth Monitoring:
 - Loss of eyeblink reflex: _____ (Y/N, Time)
 - Regular, rapid breathing: _____ (Y/N, Time)

- Loss of toe pinch response: _____(Y/N, Time)

Surgical Preparation

- Shaving Completed (Y/N): _____
- Hair Disposed (Y/N): _____
- Artificial Tear Ointment Applied (Y/N): _____
- Surgical Site Disinfection (3 cycles povidone iodine/chlorhexidine & 70% ethanol): _____(Y/N)
- Sterile Drape Applied (Y/N): _____
- Mouse on Heating Pad (Y/N): _____

Surgical Procedure

- Stereotaxic Apparatus Used (Y/N): _____
- Incision Details:
 - Horizontal cut at base of head: _____(Y/N)
 - Two rostral cuts near eyelids: _____(Y/N)
 - Two oblique cuts converging at midline: _____(Y/N)
- Skull Preparation:
 - Surface scraped for glue adhesion: _____(Y/N)
 - Surface bleached with 3% peroxide (Y/N): _____
 - Topical Lidocaine/Bupivacaine (0.25% + 1:10 epinephrine) applied if bleeding (Y/N): _____
 - 4mm circle drilled: _____(Y/N)
 - Bone window removed: _____(Y/N)
- Cranial Window Installation:
 - 5mm glass cover slip placed on dura mater: _____(Y/N)
 - Cyanoacrylate glue applied to skull: _____(Y/N)
 - Dental acrylic applied to seal cover slip edges: _____(Y/N)

- Wound Closure:
 - Dental acrylic applied to skull: _____(Y/N)
- Second Window Created (Y/N): _____

Mouse may be moved to in-vivo imaging next OR euthanized by cervical dislocation or decapitation while under anesthesia.

2P *In Vivo* Imaging and FCS

Monitor breathing during imaging to ensure proper anesthesia

- Mouse Transferred to Imaging Stage Without Awakening (Y/N): _____
- Toe Pinch Reaction Loss Recorded (Y/N): _____
- Supplemental Heat Provided (Y/N): _____
- Start Time: _____
- End Time: _____
- Dyes Injected:

Dye Used	Time	Volume (mL)	Route	Notes

Euthanasia and Additional Notes

- Mouse Remained Anesthetized During Euthanasia (Y/N): _____
- Cervical Dislocation Performed (Y/N): _____
- Decapitation Performed (Y/N): _____
- Complications Observed: _____
- DLAR Veterinary Staff Contacted (Y/N, Date, Outcome): _____
- Other Observations: _____