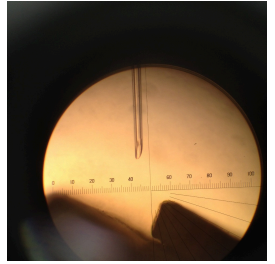


Feb 25, 2020

Virus injection

DOI

<https://dx.doi.org/10.17504/protocols.io.bctxiwpm>



Liu Liu¹, Susu Chen¹, Nuo Li¹, Karel Svoboda¹

¹Janelia Research Campus

HHMI Janelia Research ...



Liu Liu

Janelia Research Campus

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**Manuscript citation:**

This procedure was originally developed in: Petreanu, L., T. Mao, S. M. Sternson, and K. Svoboda. "The Subcellular Organization of Neocortical Excitatory Connections." *Nature* 457, no. 7233 (January 18, 2009): 1142–45.

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Protocol status: Working

We use this protocol and it's working

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Last Modified: February 25, 2020

Protocol Integer ID: 33367

Keywords: Mouse, virus, injection, GCaMP, ChR2, GFP, AAV, Stereotactic, virus injection this protocol, virus injection, headbar implantation protocol for more detail, headbar implantation protocol, conjunction with the headbar implantation protocol, viral vector, pseudorabies virus, adenovirus, modified herpes simplex virus, lentivirus, protocol, aav, proteins such as ion channel, expressing protein

Abstract

This protocol is used to inject viral vectors expressing proteins such as ion channels, transcription factors, enzymes, receptors, fluorescent proteins or other non-toxic, non-hazardous payloads. The viral vectors include AAV, Adenovirus, modified RV, Lentivirus, Modified Herpes Simplex Virus, and Pseudorabies Virus.

The protocol is often performed in conjunction with the headbar implantation protocol ([dx.doi.org/10.17504/protocols.io.bcrsiv6e](https://doi.org/10.17504/protocols.io.bcrsiv6e)). Please refer to the headbar implantation protocol for more details.

Guidelines

Standard sterile procedures for surgeries apply.

Materials

MATERIALS

-  Petri Dish **P212121 Catalog #LI-PD01100**
-  Microcentrifuge
-  Micropipets (2-20 ul, 50-250ul, 100-1000ul)
-  1 ml syringes (or U-100 Insulin Syringe) **Becton Dickinson (BD) Catalog #329461**
-  Anesthetic (Isoflurane) **Fisher Scientific Catalog #NC9259743**
-  ethanol
-  Betadine solution **VWR International (Avantor) Catalog #AJ159701**
-  Stereomicroscope **Microscope Depot Catalog #GC01650**
-  Gloves **Ansell Catalog #PK20782**
-  Heating Pad **Gaymar Catalog #TP22B**
-  Clippers Oster MiniMax trimmer **Patterson Veterinary Catalog #07-842-4245**
-  Ketoprofen **Patterson Veterinary Catalog #07-803-7389**
-  Buprenorphine (Buprenex) **Midwest Veterinary Supply Catalog # 191.26890.3**
-  Ice Bucket **Catalog #M16807-1104**
-  Applicators, Applicator stick; Length: 6 in.; Cotton-tipped **Thermo Fisher Catalog #23400101**
-  Shandon™ Disposable Scalpel No. 10, Sterile, Individually Wrapped, 5.75 (14.6cm) **Thermo Fisher Catalog #3120032**
-  Shandon™ Iris Scissors, Probe/Point, Angular, Premium, 4.5 in. (11.4cm) **Thermo Fisher Catalog #71906**
-  Dumont Forceps (Cover Slip Forceps) **Fine Science Tools Catalog #11251-33**
-  Glass Pipettes **Drummond Scientific**
-  Virus
-  Light Source
-  Stereotax
-  Drill
-  Eye lubricant
-  Dental Cement
-  Cortex buffer
-  Marcaine



 Gelfoam

 Krazy glue

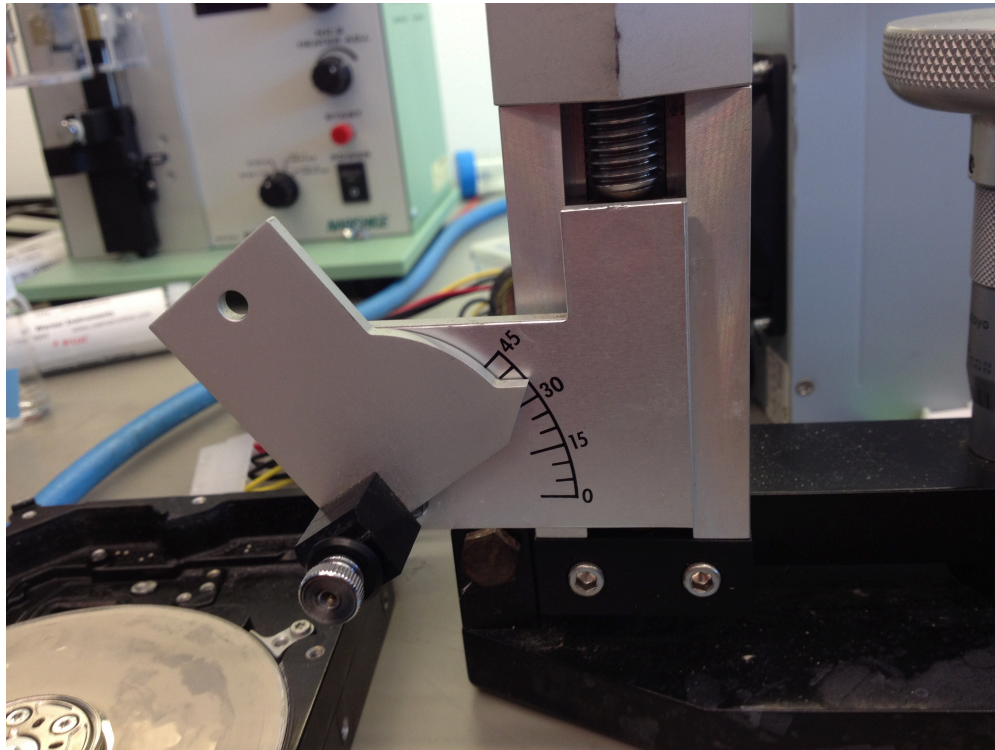
 Volumetric microinjection system

Before start

Please refer to the headbar implantation protocol for more details on the surgical setup (dx.doi.org/10.17504/protocols.io.bcrsiv6e).

Pipette preparation

- 1 Using thin-walled Drummond glass, pull symmetrically on the P-2000 (Sutter) or equivalent.
- 2 Under visual inspection (e.g. microforge or microscope) inspect the tip and carefully break the tip by hitting it against a solid surface. Aim for approximately 20µm tip diameter.
- 3 Mount pipette on a beveler at a 35° angle or less to horizontal, with the broken end roughly flush with the platter. Wet a kimwipe with 70% EtOH. Start the hard drive. Maintain a wet drive surface by holding the wet wipe softly above the platter. The EtOH prevents glass dust from kicking up into the pipette. Lower the pipette and very briefly touch the spinning surface and return.



Beveler on top of a haddrive.

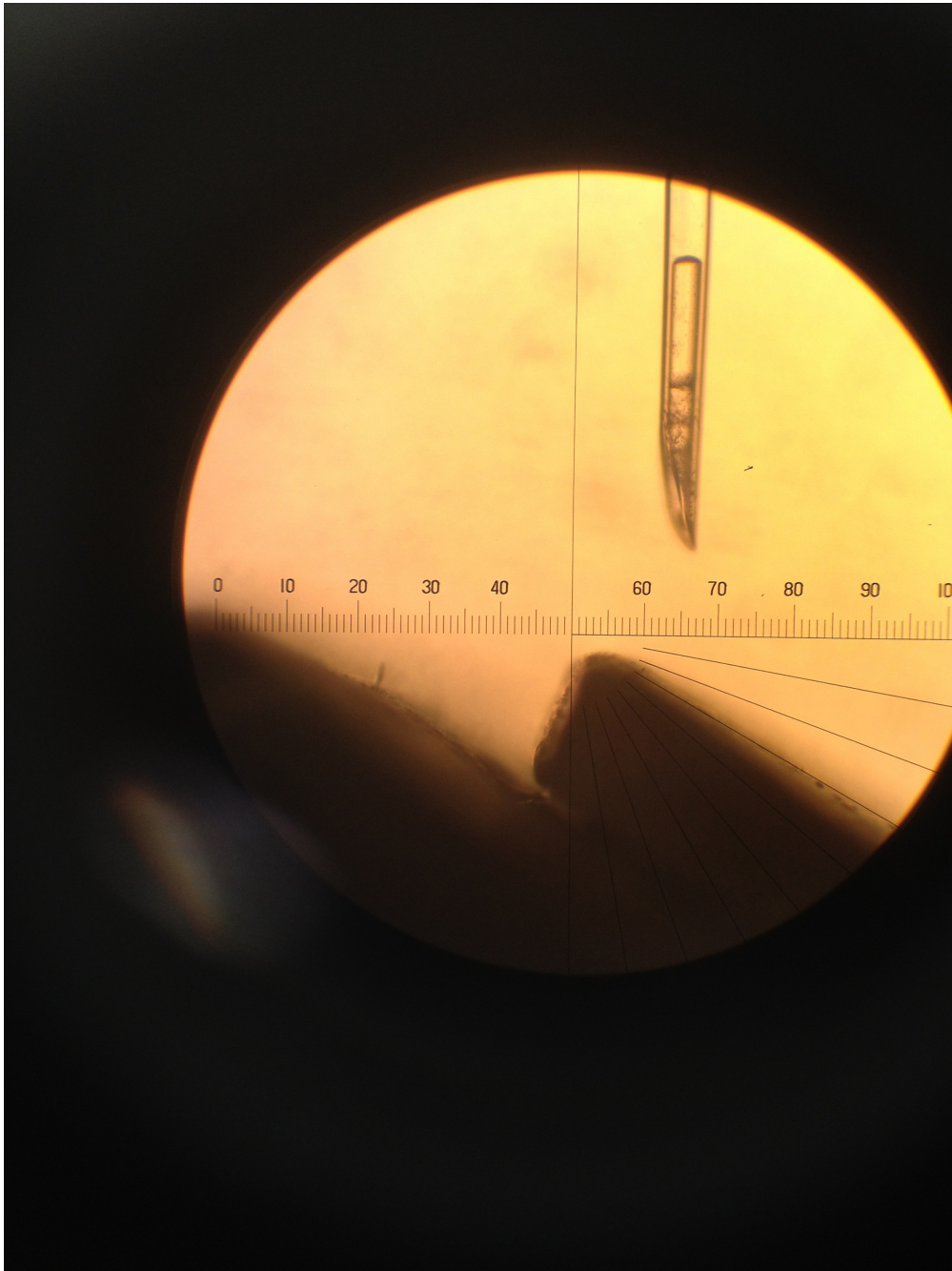
Note

EtOH going inside through the tip of the pipette during sharpening usually indicates a successfully sharpened tip.

- 4 Inspect the pipette. Try to blow dust out of the inside and off the tip with a canned air.

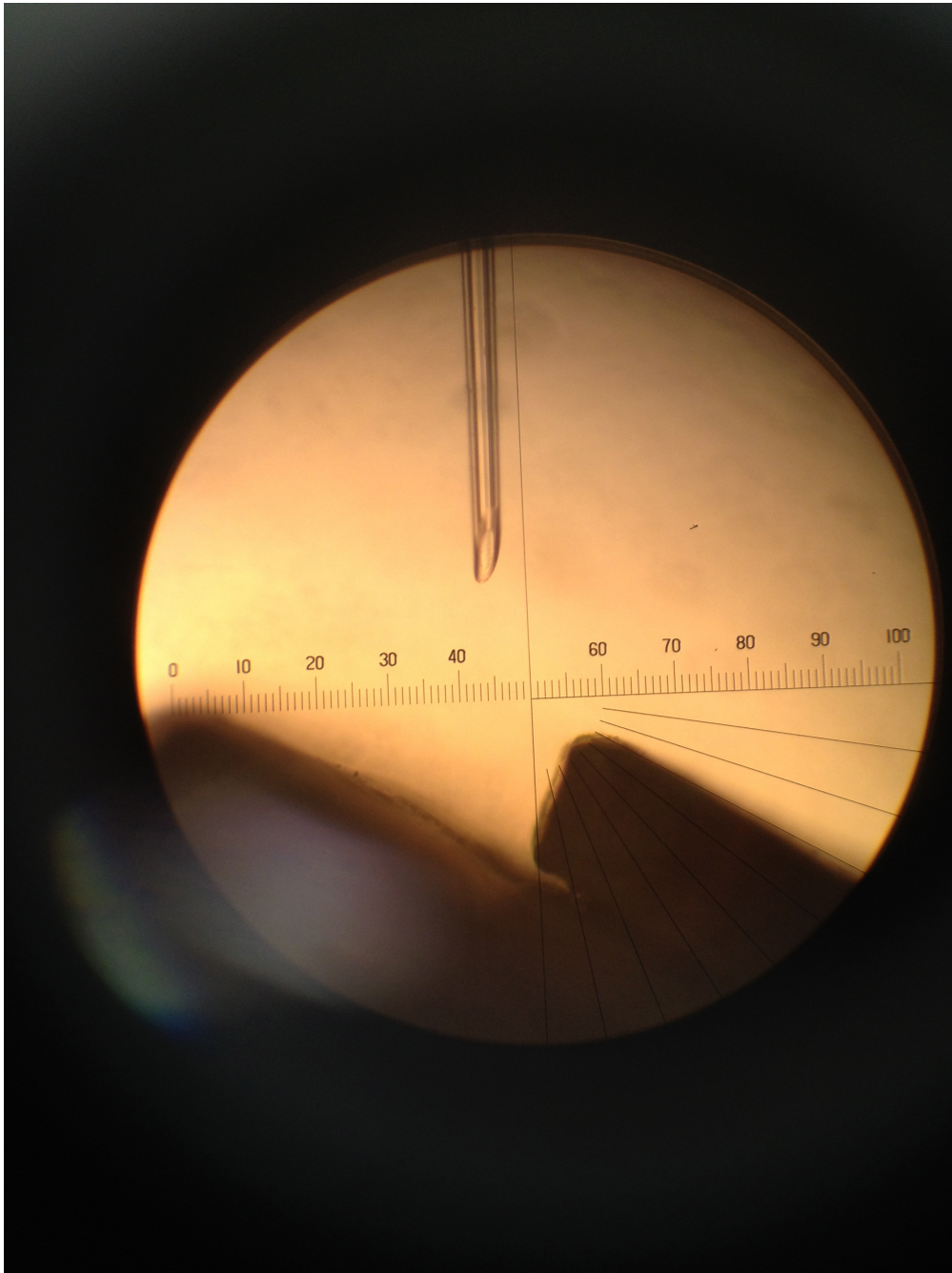
Note

If you did a bad job it looks dusty like this.



Pipette with dust.

If you did a good job it looks clean and sharp like this.

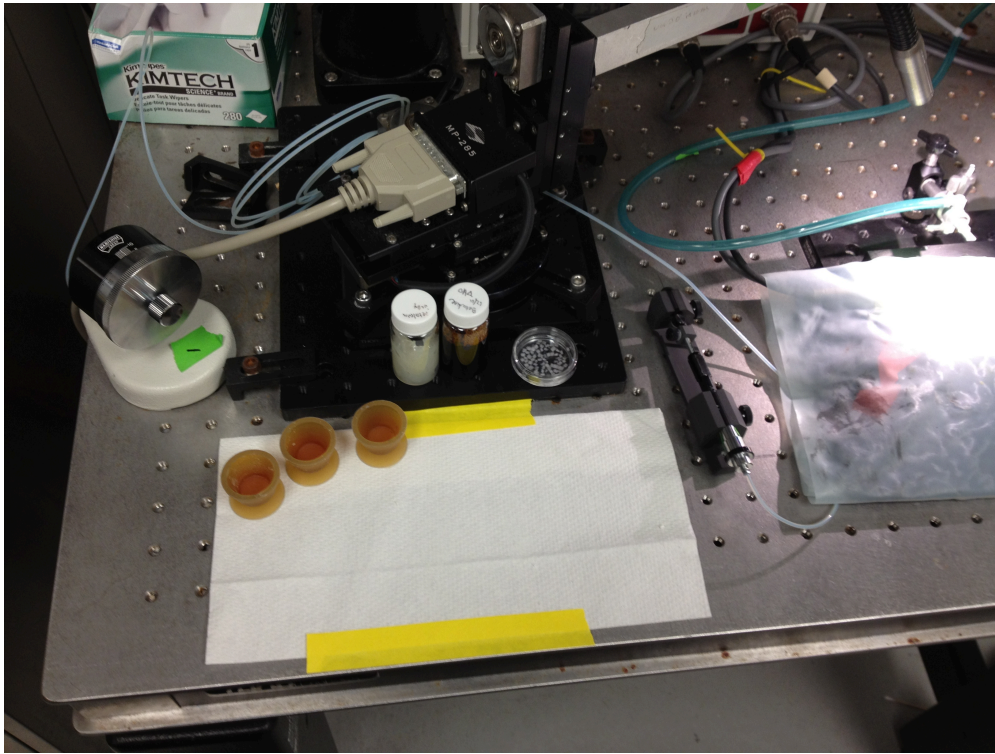


Clean and sharpened pipette.

- 5 Bevel at least 3 pipettes and arrange by quality. Use the best one first, with the others as backup (if you break / clog the first).

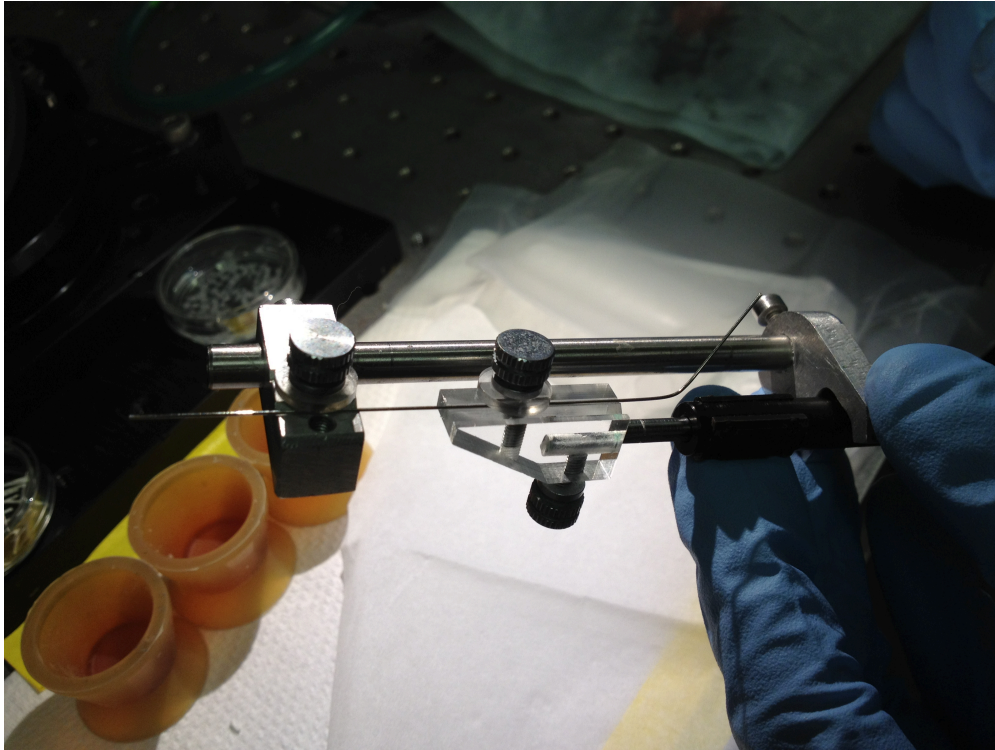
Injection setup

- 6 Thaw virus aliquot (2 μ L) on ice.
- 7 Sterilize the workspace and prepare tools. See headbar protocol for more details.



Workspace setup with the injection system, micromanipulator, and gel foam.

- 8 Cut gel foam into 1mm cubes and soak in cortex buffer
- 9 Adjust the alignment of the pipette holder and the plunger holder. The two attachment channels should be straight and co-linear.



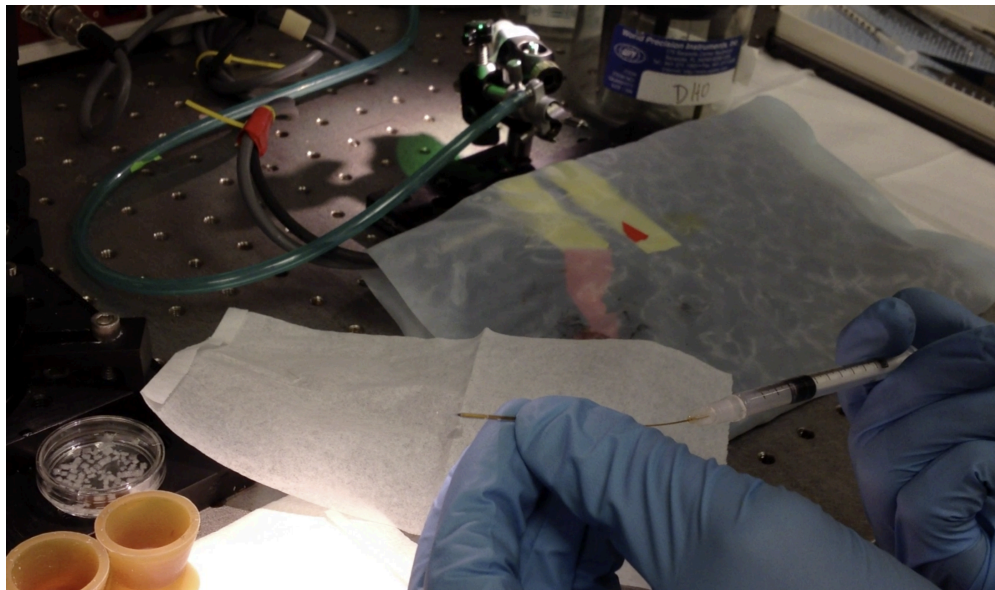
The pipette holder and the plunger in the plunger holder are aligned.

- 10 Place a tiny platform adjacent to the mouse head holder and wrap with parafilm for virus loading.



Parafilm for virus loading from the tip

- 11 Fill the pipette with mineral oil, avoiding bubbles.



Back filling the pipette with mineral oil.

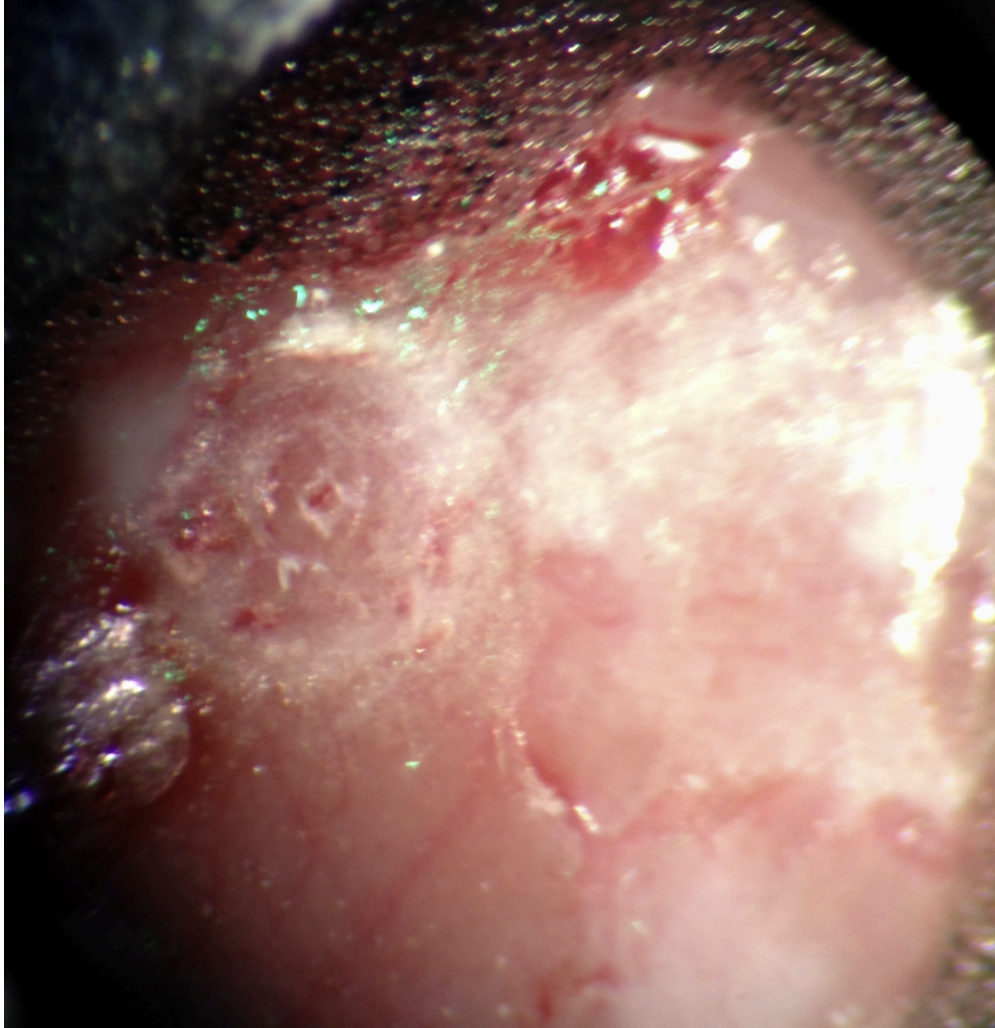
- 12 Mount the pipette in the pipette holder, and insert the plunger into the back end of the pipette. Turn the knob of Narishige injection system clockwise to eject and counter-clockwise to suck.
- 13 Mount the injector, eject any bubbles, then carefully suck up the virus from a drop on the parafilm.



Loading the virus by suction from the tip.

Surgery and injection

- 14 Wet the skull with cortex buffer to visualize the vasculature.
- 15 Confirm the target, dry the skull and drill a small hole directly above the target. Look carefully for cracking or peeling of the bone, this indicates you are at or very near the dura, and can stop drilling.



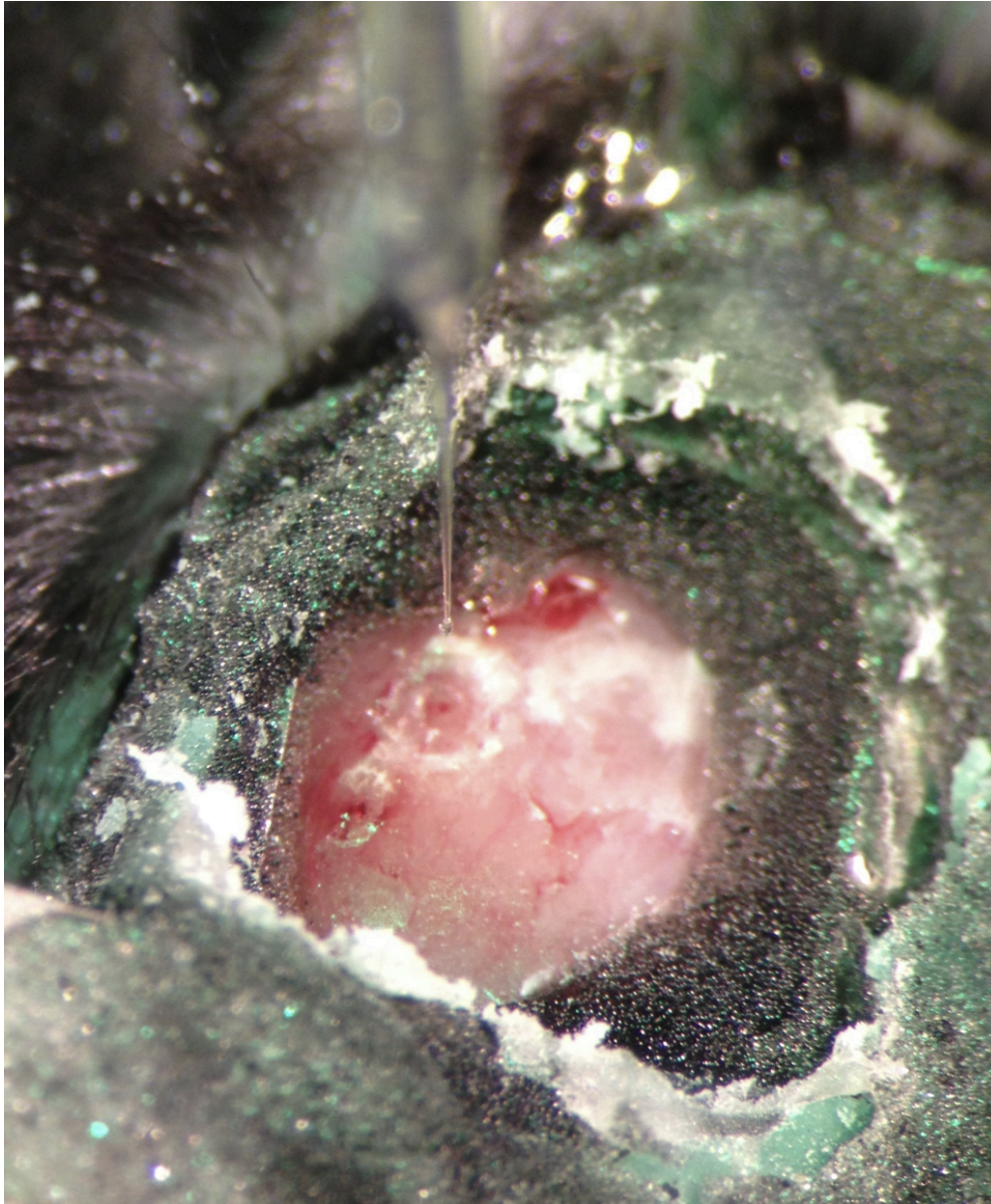
Drill a small hole.

- 16 Re-wet the area and zoom in.
- 17 Position the pipette directly above the craniotomy, Watch out to not hit the hydraulics on the injector with the scope support.



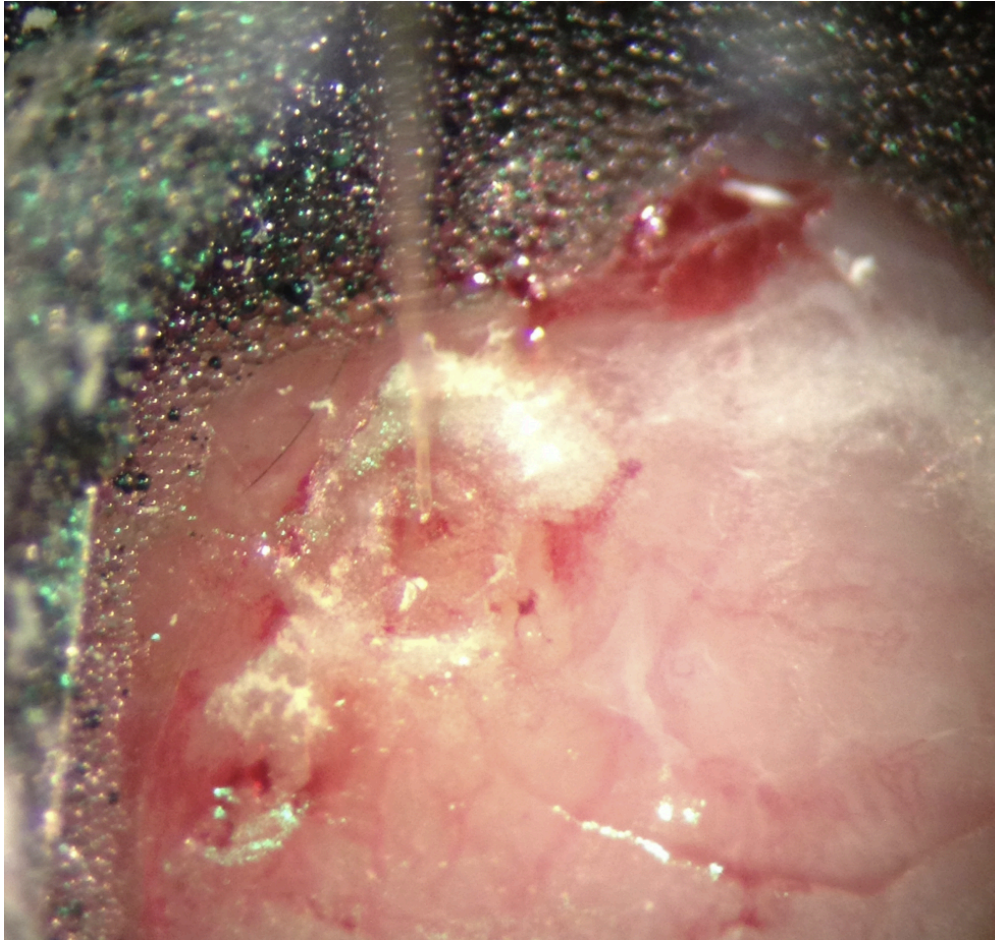
Injection system above the mouse.

- 18 Eject a small volume of virus to remove any bubbles and wick away the drop using a saline soaked piece of gel foam. Keep the lighting at a fair distance from the pipette, at moderate brightness, to reduce volume expansion from heating. Once the lighting is set, do not change it so that the pipette stays in a thermal steady state.



Pipette above the target with a drop of virus suspension at the tip.

- 19 Position the needle just onto the surface of the craniotomy. Zero the manipulator.



Manipulator above the craniotomy.

- 20 Lower the pipette to the desired depth at 2 $\mu\text{m/s}$, and inject the desired amount (typically 10-100 nL) of virus at 0.5 nL/s.
- 21 After each injection, leave the pipette in tissue for  00:20:00 . Maintain a wet surface on the craniotomy to prevent tissue sticking to the pipette.
- 22 Slowly retract the pipette out of the brain at 5-10 $\mu\text{m/s}$. After the pipette is removed from the brain, there should be no bleeding at the injection site.
- 23 Test the pipette for clogs by ejecting a small volume.



Note

If it comes out easily, the injection probably worked. If nothing comes out, don't despair, it might have gotten clogged on the way out. If clogged, then repeat the injection with a new pipette.

- 24 Wipe the pipette with wet gel foam, then eject another tiny drop to seal the end. If you thinned a large area of skull, coat the thinned area with a small volume of Krazy glue. Fill well with Kwik-Cast and cover with dental acrylic.
- 25 Dispose of virus handling parts in a labeled sealed 50mL conical.