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# A Micro-Advancer Device for Vitreal Injection and Retinal Recording and Stimulation

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## Abstract

A micro-advancer device that positions a narrow-gauge needle within the vitreous humor of the rat eye is described. The device is compact, simple and inexpensive to manufacture. It consists of an outer guard needle and an inner injection needle that is advanced through the guard needle. With the rat held in a stereotaxic holder and the globe fixed to a stabilizing ring, the outer 25-gauge guard needle is advanced through the sclera using a standard micromanipulator. The inner 31-gauge injection needle is then advanced through the guard needle with a manually controlled leadscrew and carriage mechanism. The inner injection needle is attached to a Hamilton syringe and can be positioned to within microns of the retinal surface under visual observation through a microscope. The injection needle is fixed to the device by a quick-release clamp on the carriage and can be rapidly exchanged while the guard needle remains in place in the vitreous. This permits different solutions to be injected sequentially into the vitreous humor. Recording electrodes, stimulating electrodes, and optical fibers can also be advanced through the guard needle and positioned accurately near the retinal surface or within the retina.

### Keywords

Micro-advancer device; vitreous humor; retina; intraocular injection; multiple injections; recording electrode; stimulating electrode; inexpensive

## 1. Introduction

Vitreal injections are commonly used in the clinic and in research laboratories to deliver pharmacological agents to the retina. Injections can be made by manually inserting a hypodermic needle through the sclera and into the vitreous. More complex injection protocols are used when accurate placement of the injection needle is required. Needles can be advanced through the sclera and into the vitreous using a micromanipulator. Sophisticated devices have also been designed to advance recording microelectrodes through the vitreous and into the retina of experimental animals (Steinberg et al., 1968;



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Enroth-Cugell et al., 1983; Brown and Wiesel, 1959). These devices allow accurate placement of the electrode in the retina and sometimes utilize an outer guard needle to protect the electrode. In these cases, a recording electrode is advanced through the guard needle and is positioned near or within the retina under visual observation. However, previously designed devices are bulky, complex, expensive to manufacture, and are not suitable for use on small rodents.

We describe here an intravitreal injection device that is compact, simple, and inexpensive to manufacture. It can be used to position precisely an injection needle within the rodent eye. The device consists of an outer guard needle that is positioned with a standard micromanipulator and an inner injection needle that is advanced through the guard needle by a manually controlled leadscrew. An important benefit of the device is that the injection needle is attached by an easily adjusted clamp. This permits different injection needles to be exchanged rapidly while the outer guard needle remains in place in the eye, allowing different solutions to be injected sequentially into the eye. We describe the design of the device and its use in making intravitreal injections in the rat eye.

## 2. Materials and supplies

The design and use of the micro-advancer is described below in the Detailed Methods section.

A 10 μl Hamilton syringe (no. 701, Hamilton Company, Reno, NV, USA) is used to make intravitreal injections. The syringe is attached to the injection needle of the micro-advancer by flexible PE-10 tubing (polyethylene tubing; 0.61 mm OD × 0.28 mm ID).

The rat is held in a modified stereotaxic holder, which has been described previously (Srienc et al., 2011). The stereotaxic holder is mounted on a moveable microscope stage, with the right eye of the rat, sutured to a metal ring, facing upwards into the objective lens of a microscope.

The micro-advancer is clamped onto and positioned by a 3-axis micromanipulator. Any high quality, motorized or manually-driven manipulator can be used. We use a motorized Sutter manipulator (MP-285, Sutter Instrument Company, Novato, CA, USA) to position the micro-advancer. The micromanipulator is fixed to the movable microscope stage.

A contact lens (5.4 mm fundus laser lens, no. OFA5.4, Ocular Instruments, Bellevue, Washington, USA) is placed over the cornea and the retina is viewed through a 4X objective lens of an upright microscope. We use an Olympus FV1000 confocal microscope to image the retina, but any compound microscope or high quality dissecting microscope with epi-illumination can be used.

The guard needle is made from a standard 1 1/2 inch 25-gauge needle (0.514 mm OD × 0.260 mm ID). The injection needle is made from 31 gauge stainless stock (0.260 mm OD × 0.133 mm ID; Small Parts, Miami Lakes, FL, USA)

## 3. Detailed methods

### Micro-advancer design

Except where otherwise noted, all components are made of stainless steel. Computer-aided design drawings of the device are provided in DWG, IGES and STEP formats in the Supplemental Material.

The micro-advancer is 21 cm long. It is made of a 9.5 mm (3/8 inch) diameter rod with the micro-advancer mechanism attached to the end of the device pointing towards the eye and a holder for the Hamilton syringe (machined from Polyoxymethylene) attached to the other end (Fig. 1A). The micro-advancer is clamped onto a standard 3-axis micromanipulator (Fig. 2A).

A sharp guard needle, with its beveled end facing towards the eye, is clamped to the end of the micro-advancer mechanism by a Philips round-head screw (refer to Fig. 1, where individual parts of the micro-advancer are labeled). The guard needle is a 34 mm long, 25-gauge hypodermic needle. It is made by cutting off the Luer fitting from a standard needle and de-burring the cut end. A second 10 mm length of 25-gauge tubing is also clamped to the device by a screw and serves as a guide tube for the inner injection needle. The guard needle and the guide tube are both seated in grooves that are machined into the device.

A thinner, 31-gauge injection needle is attached to the movable carriage of the device by a clamp made of 0.5 mm thick acrylic sheet tightened onto the needle by a Philips-head screw. The injection needle is seated in a groove machined into the carriage. The injection needle is fashioned from an 8 cm long piece of 31-gauge tubing. The end of the tubing facing towards the eye is beveled at a 55 degree angle. The injection needle is threaded through the device, first through the guide tube, then through the clamp on the movable carriage and finally through the guard needle. The end of the guide tube facing away from the eye is fitted into a metal block with a conical opening machined into its surface that facilitates the threading of the injection needle into the guide tube. When the injection needle is positioned correctly within the guard needle, the carriage clamp is tightened.

Once the injection needle is clamped to the carriage, it can be advanced through the guard needle by rotating a knurled wheel that is attached to a leadscrew. As the leadscrew is rotated, the movable carriage is slowly advanced, pushing the injection needle through the guard needle. The leadscrew is made from a 3 cm long, 2–56 screw. The leadscrew rotates within holes in two vertical extensions of the device and is held in place by three nuts. The movable carriage is tapped with a 2–56 threaded hole and is machined from brass to reduce binding between the carriage and the steel base.

A 10 μl Hamilton syringe is attached to the injection needle by a 9 cm length of PE-10 tubing (Fig. 2Ac). The tubing is attached to the syringe by a short length of 30-guage needle mated to the syringe. The other end of the tubing is stretched in order to reduce its inner diameter so that it fits tightly around the 31-guage injection needle. The connection is sealed with nail polish. The syringe is secured to the micro-advancer by a syringe holder (Fig. 2Ae).

## Rat preparation

The anesthetized rat preparation we use for non-survival experiments has been described previously (Srienc et al., 2010; Srienc et al., 2011). The left femoral vein and artery are cannulated for drug administration and monitoring of blood pressure, respectively, and a tracheotomy performed for artificial ventilation. The animal is placed in a modified stereotaxic holder with a three-point head restraint. The stereotaxic holder is pictured and described in detail in Srienc et al (2011). The pupil is dilated and a metal ring is sutured to the conjunctiva to stabilize the globe. The connective tissue over the nasal aspect of the globe is removed to expose the sclera near the limbus so that the guard needle can be positioned accurately with respect to the limbus. The stereotaxic holder with the rat attached is fixed to a movable microscope stage and the holder rotated so that the right eye is oriented upwards into the microscope objective. A plano concave contact lens whose curvature matches that of the rat cornea is placed over the cornea to neutralize the optics of the eye

and to bring the retina into focus. A film of gonioscopic prism solution (Wilson Ophthalmic, Mustang, OK, USA) between the cornea and contact lens prevents the cornea from drying out.

The rat is anesthetized during the initial surgery with isoflurane (2% in 30% $O_2$/70% $N_2$, 1 L/min) and is maintained on α-chloralose-HBC (550 mg/kg/hour IV) for the duration of an experiment. The rat is artificially ventilated (40–50 breaths/min) with a mixture of $O_2$ and $N_2$ (nominally 30%/70%) and paralyzed with gallamine triethiodide (20 mg/kg bolus, maintained at a rate of 20 mg/kg/hour IV) to minimize eye movements. Blood oxygen saturation level and heart rate (MouseOx, Starr Life Sciences Corp), arterial blood pressure (Pressure Monitor BP-1, World Precision Instruments), and end-tidal $CO_2$ (microCapStar, CWE), are continuously monitored. Blood $pO_2$, $pCO_2$, and pH are sampled periodically (Radiometer ABL 810), and maintained within physiological limits (100–125 mm Hg, 35–45 mm Hg, and 7.35–7.45 respectively). Fluid is continuously infused into the animal IV at a rate of 1.1 ml/kg/hr. The rat is sacrificed at the end of an experiment while still under anesthesia.

## Injection procedure

The 10 μl Hamilton syringe, attached to the injection needle by PE-10 tubing, is filled with the injection solution. The syringe is first flushed with saline by removing the plunger and injecting saline into the back end of the syringe using a 1 cc syringe and a blunt 22-gauge needle. The plunger is then reinserted into the Hamilton syringe, the flushing solution pushed out, and the injection solution drawn up into the injection needle.

A guard needle is attached to the device. A new needle is used for each experiment. The injection needle (with the Hamilton syringe attached) is then threaded through the guide tube, movable carriage, and guard needle and clamped to the carriage. The carriage should be positioned near the top end of its travel to allow maximum travel distance and the injection needle clamped so that its tip lies inside the guard needle, just before the bevel of the guard needle begins. The Hamilton syringe is secured to the holder at the end of the micro-advancer farthest from the eye and the device is clamped to the micromanipulator. The micromanipulator is adjusted so that its z-axis is angled down at a 35-degree angle from the horizontal. The injection needle should be clamped to the carriage so that the opening of its beveled tip is facing downward. In this orientation, the bevel of the needle will be parallel to the horizontal.

The micromanipulator is advanced along its z-axis, moving the micro-advancer forward along its long axis. The X- and Y-axes of the micromanipulator are adjusted so that the tip of the guard needle penetrates the sclera approximately 1.5 mm below the limbus of the globe. When the sclera is penetrated at this position, the needle passes behind the crystalline lens. A drop of artificial tears ointment (Phoenix Pharmaceutical, Inc.) is applied at the penetration point to prevent the sclera from drying. Once the sclera is penetrated, the position of the guard needle within the vitreous humor can be monitored through the microscope. The needle and the surface of the retina can be imaged clearly with the confocal microscope operating in a reflected light mode (Fig. 2B); that is, the light reflected back from the eye is detected at the same wavelength as the excitation light. The guard needle is advanced towards the retinal surface until it is approximately 1 mm above the surface. The needle can be moved laterally or up and down (along the X- and Y-axes of the micromanipulator) up to 1 mm to place the guard needle at a desired retinal location. Greater displacement should be avoided as it could distort the globe. The injection needle is then advanced through the guard needle by rotating the knurled wheel on the micro-advancer. The injection needle can be advanced with approximately 5 μm accuracy. It is sometimes desirable to inject solutions close to the retinal surface. In these cases, the injection needle is

advanced until it is near to or touching the surface. Because the opening of the beveled tip faces downwards, the bevel of the injection needle lies parallel to the retinal surface. The plunger of the Hamilton syringe is depressed manually to inject the solution.

The micro-advancer can be used to inject the eye sequentially with different solutions. After the first injection is made, the injection needle is removed while keeping the guard needle in place in the vitreous humor. The injection needle is first withdrawn through the guard needle by rotating the leadscrew in the reverse direction, until the micro-advancer carriage is near the top of its travel. The carriage clamp is then loosened and the injection needle is withdrawn completely from the device. The injection needle is filled with a second solution and is reinserted into the micro-advancer. A reference mark should be made on the injection needle so that it can be advanced into the guard needle the appropriate distance before being clamped to the carriage. The leadscrew is then rotated in a forward direction to advance the injection needle towards the retina.

### Other uses of the micro-advancer

The micro-advancer can be used for other purposes in addition to vitreal injections. Recording electrodes can be advanced through the guard needle and into the retina. Electrodes should have an outer diameter equal to that of the injection needle (0.260 mm) and be insulated except at the tip. Similar electrodes can be used to stimulate the retina electrically. The micro-advancer can be modified by using a finer-pitch leadscrew to achieve greater accuracy in advancing and positioning electrodes within the retina.

The micro-advancer can also be used to position an optical fiber within the vitreous humor. A glass optical fiber the same diameter as the injection needle is threaded through the guard needle and clamped to the carriage. We have used intravitreal optical fibers for two purposes. First, a coherent infrared light (808 nm) is focused into one end of an optical fiber. The other end of the fiber is inserted into the vitreous and used to illuminate the retinal surface. This arrangement is used when making laser speckle flowmetry measurements of retinal blood velocity (Srienc et al., 2010). Such intravitreal illumination yields better laser speckle flowmetry results than does trans-scleral illumination. Second, an optical fiber positioned close to the retinal surface can be used to illuminate a small retinal region with UV light. The UV spot is used to photo-activate caged compounds previously introduced into retinal cells. This method of introducing UV photolysis light is necessary because UV light cannot be focused onto the retina through the cornea and crystalline lens as the lens absorbs at UV wavelengths.

## 4. Potential pitfalls and trouble shooting

### Surgery

The surgical procedures described above are for non-survival surgery. Animals are sacrificed at the end of experiments, while still under anesthesia. However, surgical procedures could easily be modified so that animals survive following intravitreal injection. Injections would be made while animals are under isoflurane anesthesia. All surgical procedures (femoral vein and artery cannulation, tracheotomy, suturing the globe to a stabilizing ring, removal of connective tissue over the globe) would be unnecessary. A local anesthetic would be applied to the needle insertion point on the globe prior to and following intravitreal injection.

### Bubbles in injection needle

Care must be taken when flushing saline solution through the Hamilton syringe, PE-10 tubing and injection needle to ensure that there are no air bubbles in the system. An air

bubble in the injection needle will be expelled into the vitreous when an injection is made and will interfere with imaging of the retina.

### Leakage of vitreous humor

When making sequential injections, vitreous humor may leak from the end of the guard needle when the injection needle is removed. If leakage occurs, a short length of sold rod, the same outer diameter as the injection needle, should be inserted into the guard needle to plug it up. This will stop leakage of vitreous. If the guard needle is withdrawn from the globe, leakage of vitreous humor may also occur. In our experience, however, leakage is minimal as the hole in sclera seals rapidly.

### Precision advance of injection needle

A finer-pitch leadscrew can be used to advance an injection needle or electrode into the retina with high precision. The micro-advancer could also be modified by adding motorized rotation of the leadscrew.

### Precision of fluid injection

Injections are made by manually depressing the plunger of the Hamilton syringe. Too rapid a rate of injection may cause damage to the retina, particularly if the tip of the injection needle lies against or is within the retina. The micro-advancer could be modified so that the syringe plunger is depressed slowly by a motorized syringe pump. The PE-10 tubing linking the Hamilton syringe and the injection needle would be lengthened so that the syringe could be placed in a syringe pump located near to the micro-advancer.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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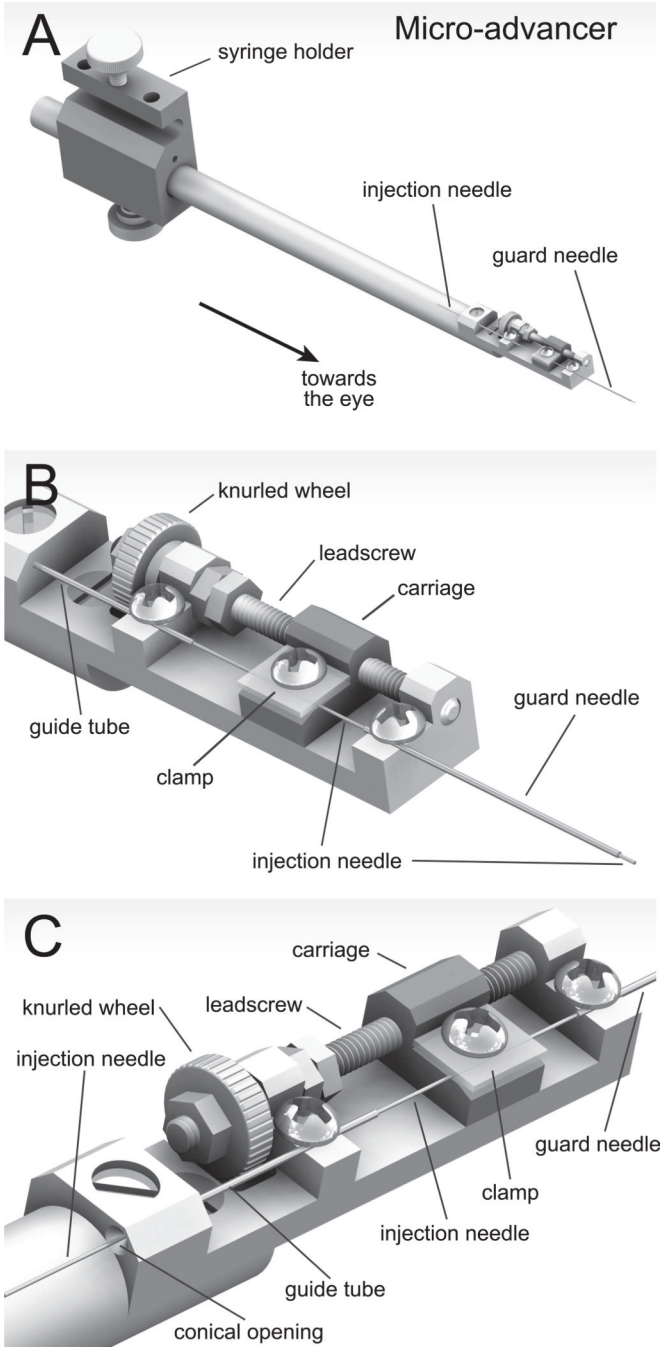


**Figure 1.**

Three-dimensional drawings of the micro-advancer, with components labeled. A) Overview of the micro-advancer, with the end of the device pointing towards the eye at the right. B) Close-up of the micro-advancer mechanism at the end of the device nearest the eye. C) Close-up of the micro-advancer mechanism, viewed from a different angle. The 3-D drawings were rendered using ViaCAD Pro v7.0 (Encore Software). Computer-aided design drawings of the device are provided in the Supplemental Material.

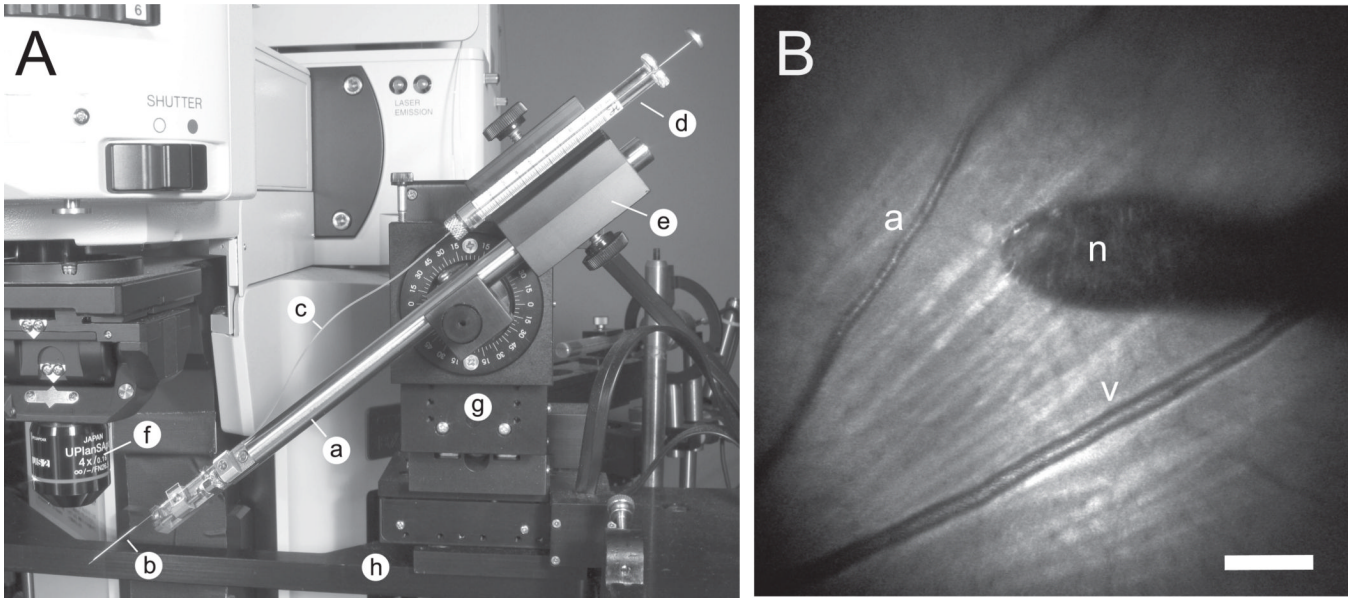


**Figure 2.**

A) Photograph of the micro-advancer. The device is clamped to a micromanipulator, which is attached to a movable microscope stage. A Hamilton syringe is clamped to the syringe holder and is connected to the injection needle by PE-10 tubing. a, micro-advancer; b, guard needle; c, PE-10 tubing; d, Hamilton syringe; e, syringe holder; f, microscope objective lens; g, micromanipulator; h, microscope stage. B) Reflected light confocal image of the retina, showing the tip of the micro-advancer injection needle (n), an arteriole (a) and a venule (v). Scale bar, 250 μm.