SET-UP and Instruction Manual for Xenoplace™ Workstation

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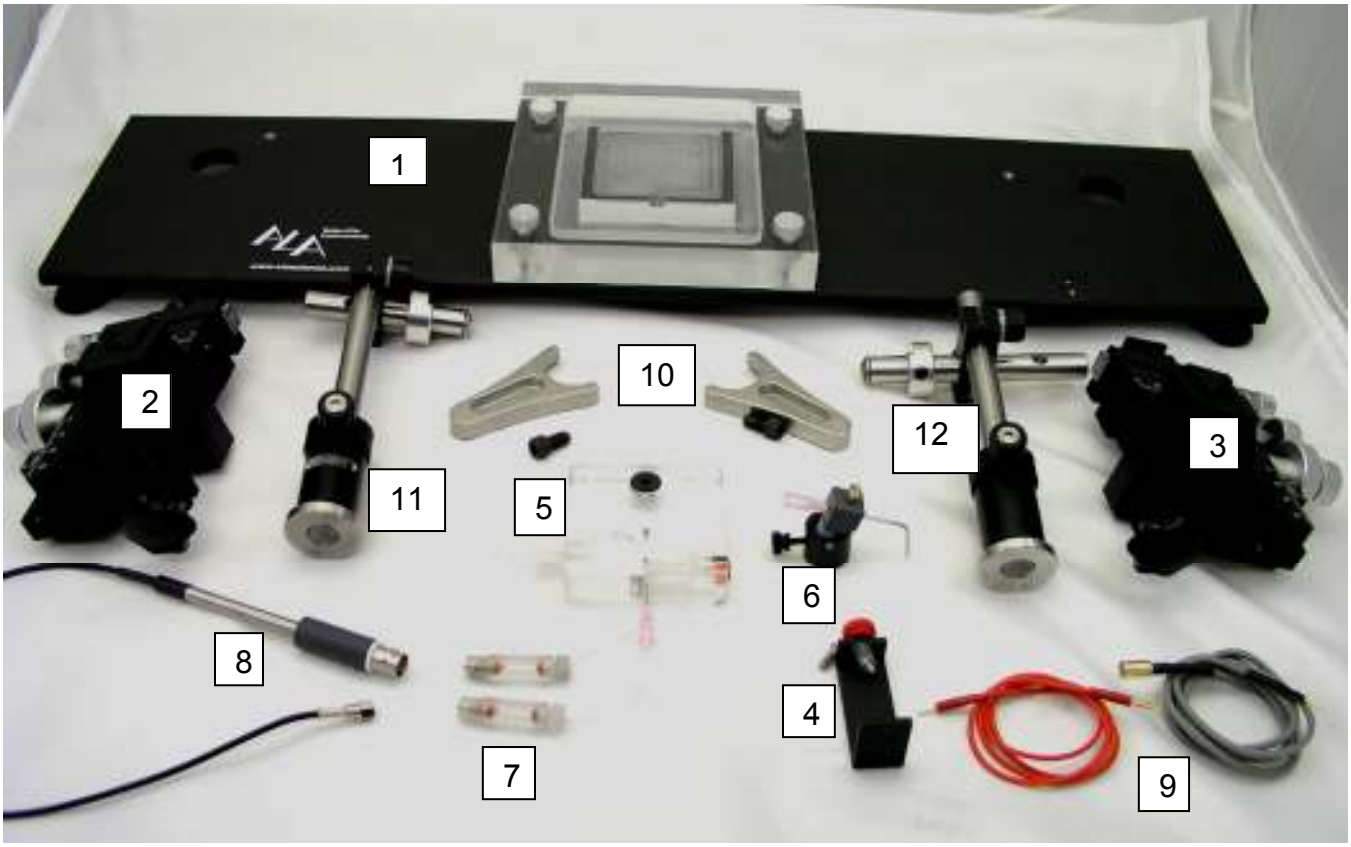
Components



1. Base plate and chamber holder.
2. Right MM-33 Manipulator
3. Left MM-33 Manipulator
4. Current Head Stage Bracket
5. Chamber
6. Adjustable suction port
7. Pipette holders (2) (Optional)
8. Current Injection pipette holder extension
9. Reference electrode wires (2)
10. Securing Forks (2)
11. Left Tilt & Swivel Post
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Introduction

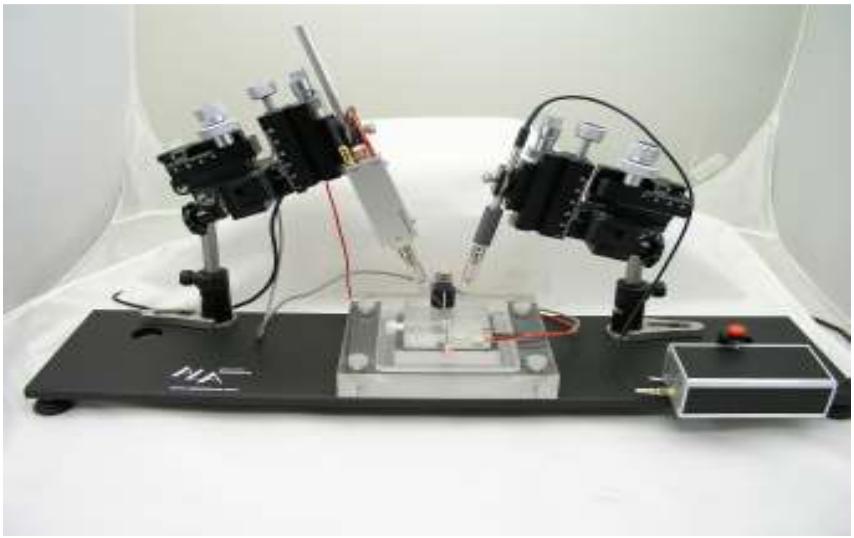
The Xenoplace™ workstation is designed to be a simple yet comprehensive workstation for investigating the electrical properties of Xenoplace Oocytes, the egg of the African leopard frog. Oocytes are useful in electrophysiology and pharmacology research because the pre-emergent egg is basically a factory ready to make a frog, and therefore able to process a wide variety of RNA. As such it is very capable of processing the RNA that codes for membrane ion channels and can easily express those channels in the membrane of the egg. Since a typical egg is about 1mm across, millions to billions of channels can be expressed giving rise to large currents that can be more easily studied than in a neuron or other excitable tissue. The large size of the egg can give rise to other electrical problems that must be dealt with such as large capacitance and high current. A good amplifier such as the TEC 03 from npi makes the perfect system to use with the Xenoplace™ since is contains many of the features necessary to discriminate membrane currents and give accurate recordings.

The typical Xenoplace set-up consists of the TEC amplifier from npi and two MM-33 manipulators mounted on a base plate. The two manipulators are positioned so that electrodes can descend into a chamber to pierce an oocyte which can also be perfused, very easily with drugs, or other substances. The chamber, specifically, and the design layout were perfected by Lonnie Wollmuth in his lab at SUNY Stony Brook in Long Island, New York. The system provides two manipulators, one for the measuring electrode and one for the current injecting electrode. There is a unique channel with a dimple to hold the oocyte, and two reference electrode (AgCl pellets) are positioned adjacent to the dimple. Convenient plug-in connections allow the user to connect the reference electrodes to the head stage. Perfusion liquid entering the chamber is channeled directly at the oocyte. Fluid is removed via a suction port from just behind the oocyte, or from a Luer connection on the side of the chamber block. There is a pedestal catch basin that supports the chamber and serves to catch the liquid if the removal system should fail. A clip holds the current injecting head stage for convenience. All the components are tied to a common base plate for convenience and stability.

Setting up your Xenoplace

The workstation is very easy to assemble. First determine where you want to put it. A firm table or an anti-vibration table is preferred. You will also need a dissecting microscope. Make sure it has a long enough reach to be positioned over the chamber to provide a good view for impaling the oocyte. Start with the base plate. The base plate comes with the chamber base and four adjustable legs already mounted.

First, mount the manipulators When finished they should appear like this. The tilt and swivel mechanisms will return the current injecting and the recording electrode to the correct location for easy impalement.



Start by securing the two post mounts to the base plate. The posts are held in place by Securing Forks that permit some freedom in choosing the exact location at which to lock down the posts.

Right Side Fork.

Left Side Fork

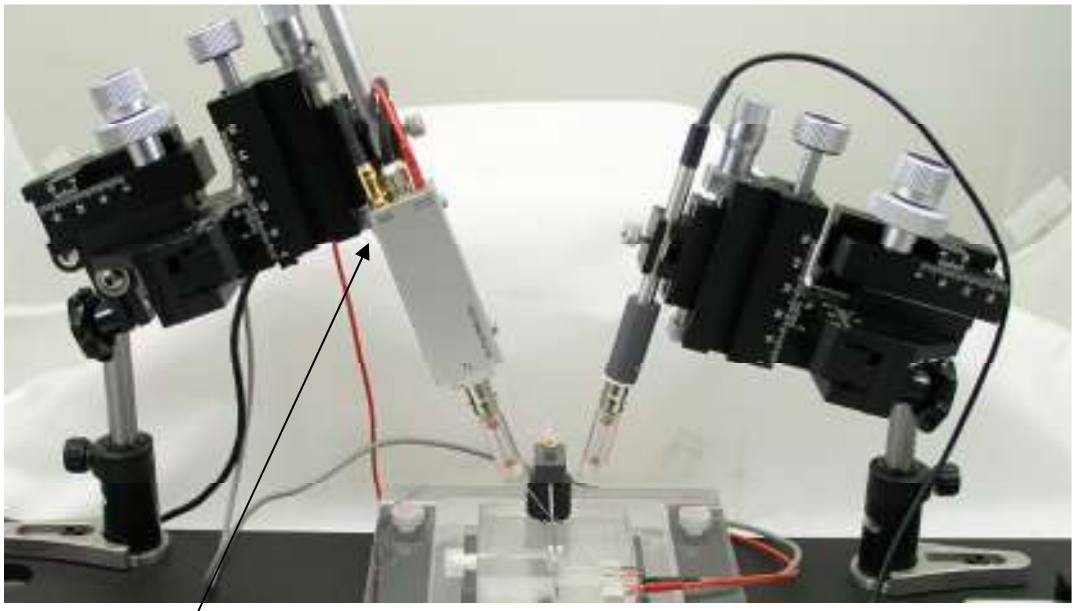
A ½" ¼-20 screw holds each fork in place. The ¼-20 mounting hole on the left and right are in slightly different locations to facilitate the pipettes reaching the Oocyte in the chamber. Lock the posts down temporarily so that the manipulators can be mounted.

Slip each MM-33 manipulator on the shaft. Make sure the stop pin on the manipulator mount goes into the slot on the Tilt Stop. Tighten the flange screw on the end of the shaft to trap the manipulator mount.

Slot on Tilt-Stop, Pin on manipulator mount must orient in slot.

Pin on MM-33 mount.

Once the manipulators are mounted, Put the Pipette Holder Extension in the right MM-33 and put the Current Injecting Head Stage in the Left MM-33. Carefully tilt the manipulators toward the Oocyte chamber. Make sure all fittings are tightened before you release your grip so that they do not fall and damage the chamber. Loosen the Forks and slide the posts around until you are sure the pipettes line up with the Oocyte spot. Tighten the forks securely when you are finished.



NOTE: Current Head Stage bar needs to be all the way back in MM-33 clamp.

Setting the Tilt and Swivel Stops

The tilt and swivel stops are provided so the user can easily tilt the manipulators back, and swivel them out, to make pipette changes and working with the chamber, easy.

The tilt stop is set using the set-screw on the stop ring. Loosen the black clamp at the base of the manipulator, tip it to the desired angle then tighten the black clamp. Rotate the stop ring until the pin rests on the stop. Tighten the set screw. The manipulator will now have a “tilt-in” and “tilt-out” stop.

Black knob on manipulator clamp.

Loosen and tighten the black knob to tilt the manipulator back and forth during experiments.

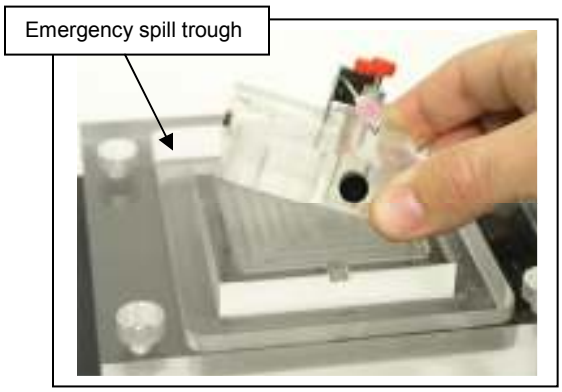
To set the swivel stop, loosen the small black knob on the side of the swivel post base. Loosen the securing fork just enough to be able to rotate the post base in-place. Rotate the post base so that locking knob is on the left side for the left side manipulator, and on the right side for the right side manipulator, as seen in the photo on page 6 above. Tighten the securing fork to fix the post base in place. Swivel the manipulator until the post stops turning at the "in position" stop. Loosen the vertical post lock of the CA-1 X block. (Use caution as this may cause the manipulator to slide down the post.) This will allow the manipulator to swivel freely on the post. Set the manipulator in the desired position at the "in position", and then lock the CA-1 in place. The "in position", stop will be set. The "out position" stop will be about 90° toward the user.

CA-1 X Block vertical post lock.

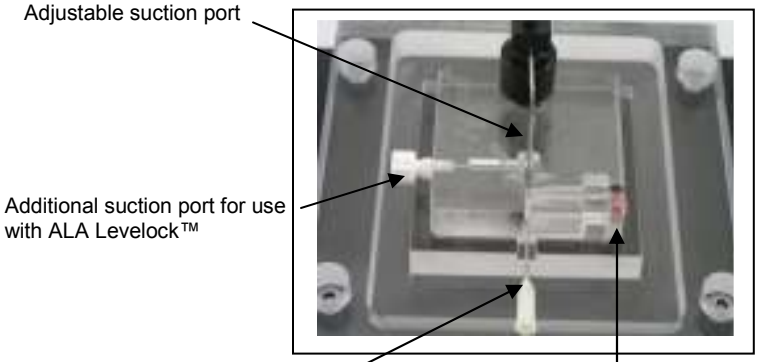
Left side post, adjusting the swivel. Note the locking knob is on the left.

Installing the Chamber

The chamber simply drops in the base. The infusion port, (small steel canula) should be away from you, the suction port, the adjustable one, should be toward the user.



Chamber base with spill trough

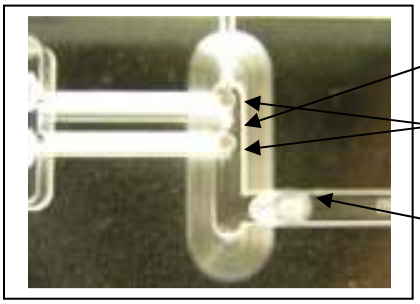


The next step is to orient the electrodes into the chamber so that you can get the manipulators into their general positions. They will remain in the general position most of the time, requiring only small adjustments of the control knobs to change electrodes and penetrate the oocyte. The initial positioning will require you to slide the manipulators around on the spacers. The large holes in the base plate and the anchor system permit you to lock the manipulators in the best possible location. Leave them slightly loose for sliding then turn the knobs tight to lock in.

Mount the Potential head stage on the left side, and the Current head stage on the right.

Install the pipette holders on both sides.

With the pipette holders in place, get the manipulators as close to aligned as possible. Then put electrodes into the pipette holders, and with the aid of a dissecting microscope, orient the tips of the electrodes toward the dimple where the oocyte will be:



Put some water in the chamber and place an oocyte on the dimple. Get the electrodes to line up and pierce the oocyte.

Adjust manipulators as necessary to align the electrodes.

The Potential Head Stage:

The potential head stage has a mounting rod attached to it. The rod is not on the central axis of the electrode. Therefore, one of the degrees of freedom for this electrode is to rotate the head stage about the shaft as it passes through the mounting clamp. In addition to adjusting X-Y-Z on the manipulator, turn the shaft on its axis through the mount to change the position of the electrode. Tighten the camp down when you have found the best orientation.

The chamber comes with two built-in reference electrodes. They will need to be connected to the potential head stage of the amplifier. There are 1mm pin cables that are provided.

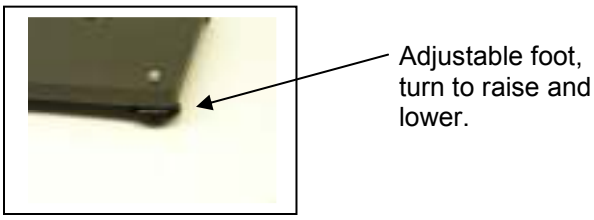
Finally, the **current head stage** has a mounting bracket. It is mounted on the right side of the platform with a single screw. The bracket is screwed in with one screw, and then the head stage is mounted with a hold-down thumb screw to fix it in place. The cable from the electrode holder extension is plugged into this head stage.

Connecting the recording electrode

Screw down the head stage bracket.

Securing the current head stage.

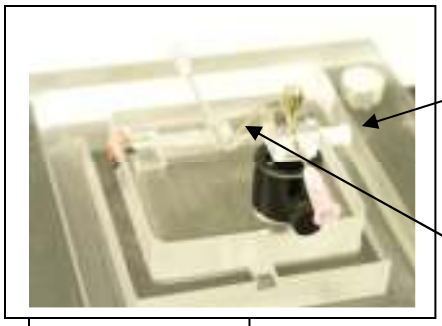
On the bottom of the base plate there are adjustable feet to level the Xenoplace. They should be adjusted as necessary to keep the system level.



Operation

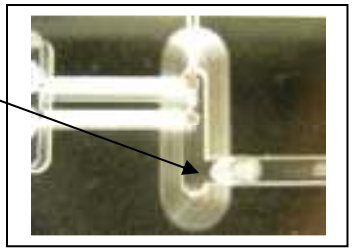
Operation of the amplifier is covered in its manual and the techniques for recording from oocytes are well described in the papers and book chapters in the reference section of the manual.

The Xenoplace™ workstation is very easy to set up each time you will perform a recording. First you will establish the suction line that will drain the chamber. The chamber can be evacuated in two ways, one is with the perfusion canula that is on the tilt device, the other is with the side port.



Side port with Luer-lock fitting. A small piece of tubing acts as a sluice to keep it closed when not in use.

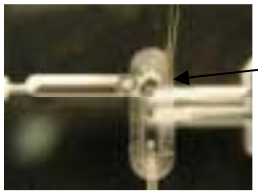
Adjustable perfusion canula



When using the side port, the sluice can be raised to allow fluid to flow underneath, rather than being removed completely. This will help dampen vibrations in the chamber from the fluid removal.

The ALA Tilt-A-Port™ is used as the adjustable suction port for the Xenoplace chamber. It is held in position via magnet.

When using the suction canula, it should be noted that the tip has to touch the side wall of the chamber to improve suction. The canula is designed to be used with house vacuum, so it can make some vibrations, keeping the tip against the wall will help reduce that.

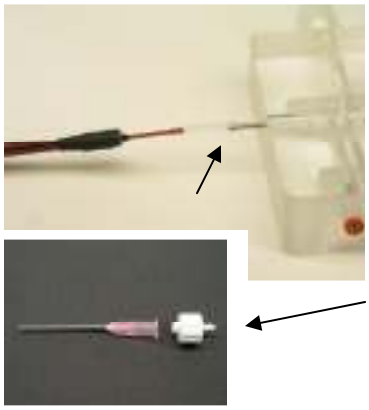


Canula is on the side of chamber, touching the wall.

If you wish to use the side port, we recommend that it be combined with our Levelock™ system that includes our vacuum waste kit. The Levelock™ has a sensor that can be set to the surface of the fluid in the chamber, and it will regulate the fluid height to within .2mm, while the suction comes from the side port, below the surface of the fluid in the chamber, and thus not disturbing the fluid surface. Please contact your representative or ALA directly for information about the Levelock™ and the Vacuum Waste Kit.

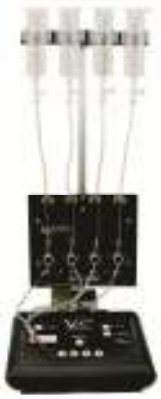
Above: Levelock™, Left: Vacuum Waste Kit.

The fluid input to the chamber comes from either an infusion pump, or a gravity fed reservoir, or a series of reservoirs. The input canula is supplied loose so that it can be adjusted to be either very close to the oocyte or a bit farther away. The closer it is, the more concentrated the output will be. We do not recommend using super glue or very strong epoxy. An epoxy that remains soft or RTV silicone is best since they will form a water tight seal but can be removed. (Vacuum grease is also good).

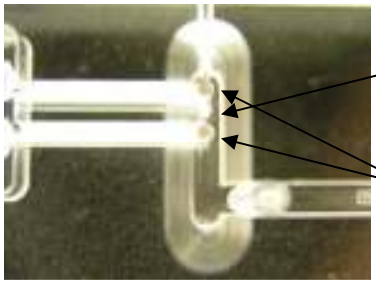


The input canula, shown here connected to an ALA Millimanifold™ via a short piece of silicone tubing. The Millimanifold™ can be connected to a valve controller like the one shown at right. The canula is 18gage and a standard needle with tip ground off can be used. A standard needle also has a Luer for easy connection to barb fittings.

A valve controller such as the ALA VC3-4 is a great way to derive dose response curves.



The input fluid should be able to be controlled, as in started, and stopped as necessary. Test your system by allowing fluid to flow in and then see that your suction tube can remove the fluid before it floods, and maintain a steady level. Once the fluid is established, you can put in an oocyte. Simply place it on the dimple just in front of the input canula. (remember that the chamber is designed to be used with the input canula either facing away from the user, or toward the user.)



Dimple for Oocyte, the tip of the infusion canula can be placed right adjacent to the dimple, over the first reference electrode, or further away, so that the fluid stream spreads out more. Be aware that if the canula is close to the Oocyte, there might be a space behind it that is not flushed out all the time.

Reference electrodes

The oocyte should stick to the dimple, then you can impale it with the electrodes. Check to see that the fluid level always stays above the top of the oocyte.

Maintaining the system

The system does not need maintenance, other than to keep it clean. Since much of the device is made of metal, you will want to wipe up all spills for salt solution as quickly as possible. Once corrosion has had a chance to set it, it can even be a source of electrical noise! The chamber itself should be washed out periodically, and mild detergent can be used for that. If necessary, 70% isopropanol can be used as well. Flush the chamber with distilled water as often as possible.

The reference electrodes will wear out eventually, and need to be replaced. For starters, as they get dirty, you can just gently scrape off the top layer to remove dirt and debris to expose fresh AgCl. After a while you will see a disk appear in the center of the pellet. The pellet will be noticeably shorter by this time, it means it needs to be changed. To change it, you will need to de-solder it, or cut the wire, from the socket. Then pull out the old pellet by removing the silicone adhesive that holds it in place.

Silver wires are soldered to the connectors. Silicone seals in the pellets.

When you put in the new pellet(s)push it up so it is just under the level of the bottom of the chamber, almost even with the bottom. We suggest using a small dab of silicone to get it to stay in place, then when it is dry, add more to complete the seal.

The dimple that holds the oocyte is also prone to fill up with debris after a while. Clean it out carefully with a needle. If you wish to replace it, the dimple is formed by a hole that passes right through the chamber. Just clean out all the RTV silicone with a pin, needle or small drill bit. To replace the silicone, inject it up from the bottom. Watch the top of the hole and when the silicone is about 1mm below the top, stop injecting. Let it cure for a day before using.

For any other questions or concerns, please contact ALA or npi. Please see the list of references for techniques and methods in oocyte research.

References

The Chamber

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Limited Warranty

ALA Scientific Instruments, Inc., warranties this system to be free of defects in workmanship for a period of one year from time of shipment. Remedy shall be limited to repair or replacement of the device or its components as necessary. Repair or replacement is up to the discretion of ALA Scientific Instruments. The user is responsible for return shipment to ALA for any warranty repairs. All returns must receive authorization prior to shipment.

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