

# PIPETTE DRIFT

LOCALIZING AND ELIMINATING  
PIPETTE DRIFT FOR SUCCESSFUL  
PATCH CLAMPING

## APPLICATION NOTE

The following frequently asked questions were assembled as a guide to understanding, isolating and eliminating the most common causes of pipette drift. We hope this guide will serve as a useful reference guide for your entire lab. Before delving into the questions and answers on pipette drift, let's start with a definition of pipette drift.

Pipette drift is defined as the relative movement of the pipette tip with respect to the cell and usually is observed using a high-powered microscope objective and CCD camera. Because it is important to understand magnitude when analyzing sources of drift, pipette tip movement should be measured relative to a cell or other feature on the slide.

### Q. IF I OBSERVE PIPETTE DRIFT, WHAT ARE THE FIRST THINGS I SHOULD CHECK?

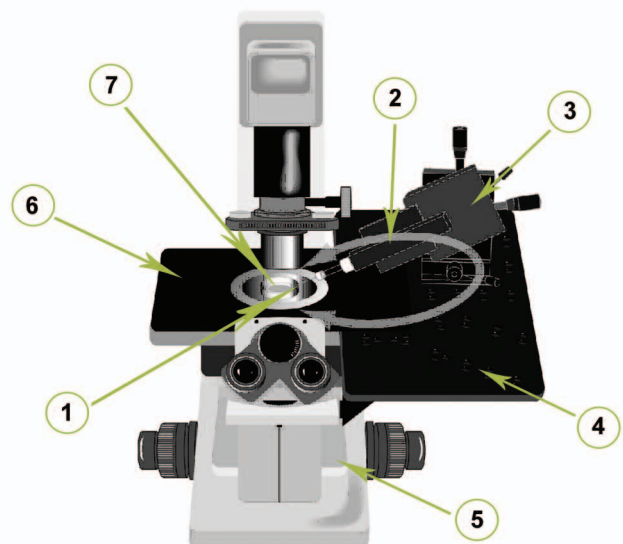
A. There are a number of things that should be checked. However, temperature changes in the room should be considered first. For example, has the air conditioning just turned on or have other lab members been walking in and out of the room and leaving the door open? If you're reasonably confident that there are no obvious signs of temperature variation, the second check should be that everything is securely tightened. Ensure the pipette is held securely, the rotary stages are in a locked position, etc. In general, a quick check of the environment, then securing pipettes, rotary stages and any fixtures or mounts should be the first things undertaken to ensure that the basics have been covered. Quite often, these initial checks may be all that is required to eliminate the pipette drift.

### Q. WHAT ARE THE MOST COMMON CAUSES OF DRIFT?

A. Pipette drift can come from numerous sources that can be identified by visualizing the mechanical loop connecting the cell and the pipette tip. Starting with the pipette tip, the potential drift sources shown in Figure 1 should be considered.

Figure 1: Mechanical loop of pipette drift source

1. Pipette
2. Headstage and pipette holder
3. Manipulator
4. Mount
5. Microscope body
6. Microscope stage
7. Cell preparation



## Q. HOW CAN I TELL IF THE DRIFT IS COMING FROM THE HEADSTAGE OR THE MANIPULATOR?

A. To determine if the headstage and pipette holder contribute to drift, remove the headstage, clamp the pipette directly to the aluminum mounting plate and check for pipette drift. If direct connection to the mounting plate eliminates the drift, refer to the discussion about headstage drift below. Our experience indicates that most drift results from the headstage and pipette holder. A very good system analysis of pipette drift is found in "A Low Drift Micromanipulator Holder" by F. Sachs, European Journal of Physiology 1995, 429:434-435.

## Q. IF I HAVE HEADSTAGE DRIFT, WHAT ARE THE RECOMMENDATIONS FOR ELIMINATING IT?

A. Pay particular attention to the connection of the pipette to the headstage. Improper connections are a potential and damaging source of drift. Below are some general comments followed by guidelines from Axon Instruments and HEKA.

### **General Suggestions:**

- ✓ To eliminate cable forces as a possible source of drift, secure tubing connected to the suction port on the pipette holder. Unsecured tubing connected to the pipette holder suction port may cause a drag on the holder and produce drift. Use the most flexible and lightweight tubing available for this purpose, and secure it to the headstage or suspend it from a hook to reduce the gravitational drag.
- ✓ Headstage drift can be greatly reduced by using a quartz pipette holder as described by F. Sachs. This design eliminates the unstable polymer pipette holder while maintaining excellent electrical isolation.
- ✓ A novel, low-drift pipette holder solution is available from G23 Instruments. Called the DB1 and DB2, the design holds the pipette at two points with O-ring seals to minimize rotation of the pipette. The microelectrode holders are compatible with headstages from Axon Instruments. For more information, contact:

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### **Suggestions for Axon Headstages:**

- ✓ Use the threaded connections on the pipette holder. For example, all current Axon Instruments' headstages use a threaded collar pipette holder (HL-U) to secure the pin cap into the headstage. Non-threaded holders (such as Axon's HL-1 and HL-2) depend on friction fit between a tapered Teflon plug and a Teflon hole. This fit can be unstable after many insertions, because the interference stresses cause the Teflon to creep. The threaded HL-U is a more reliable and stable design. (Note: Axon recommends tightening the threaded collar only finger-tight, because relaxation in the Teflon threads may exacerbate drift.)
- ✓ Very early designs of Axon Instruments' holders used a white (PTFE) threaded cap to clamp the pipette into the holder. This white nut has since been replaced by a more stable clear polycarbonate cap. Contact Axon Technical Support to get a replacement cap.
- ✓ Headstage amplifiers with cooled active elements (for example, Axon Instruments' CV 203BU) dissipate more heat than non-cooled amplifiers. Allow sufficient time for your cooled headstage to warm up and stabilize. (According to Axon, the headstage cooling feature of the Axopatch 200B is most beneficial in the Patch recording mode. If thermal instability seems to be the cause of pipette drift in Whole Cell mode, then headstage cooling can be turned off.)

### **Suggestions for HEKA Headstages:**

- ✓ HEKA uses a high quality BNC plug connector to mount the pipette holder to the headstage. This "all or nothing" type of connector ensures a tight fit and has proven over the years to be very stable mechanically.
- ✓ Several years ago, HEKA improved the design of their pipette holder. The polycarbonate screw cap was lengthened and a polycarbonate cylinder was inserted inside the cap. This cylinder serves as a precise positioning guide for two O-rings that eliminate any pipette movements within the holder. A third O-ring at the level of the gold pin connector prevents movement of the holder within the BNC plug assembly. This design prevents air leakage and there is no pipette movement when suction is applied to the pipette holder suction port. More information about the HEKA pipette holder is available from the HEKA web site, [www.heka.com/physio/equipment/pipette/piphp.html](http://www.heka.com/physio/equipment/pipette/piphp.html)

## **Q. IF I SUSPECT THE MANIPULATOR IS THE CAUSE OF THE DRIFT, HOW DO I ISOLATE THE CAUSE?**

A. If the manipulator is the cause of the drift, ensure that all further tests do not involve the headstage, in order to simplify the tests. The source of micromanipulator drift is likely to result from two areas:

1. Failing to lock one or both of the rotary stages after changing the pipette (an easy fix).
2. Cabling forces that deflect the manipulator.

### **Cable Forces That Deflect the Manipulator**

When using the rotary and linear stage on EXFO PCS-6000, -5000 and -4000 series manipulators, the wires on the manipulator and the headstage are bent and twisted significant distances and angles. Each time a pipette is exchanged, the cables are flexed and the memory in the cable insulation produces residual stresses. As these residual stresses are relieved over time the forces on the manipulator change and can produce apparent drift. If your drift measurements are several micrometers over a few minutes this could be the source. The stiffness of the PCS-5000 series manipulator is approximately 0.13 Newtons/micrometer on each axis. Thus, two micrometers of movement correspond to about 0.26 Newtons (25 grams) change in force.

### **Reducing the Effects of Cable Forces**

1. Use large service loops and bend radii on the black manipulator stage cables. The black cables are very flexible when given a few inches of free length. Make sure that all the black cables are loose throughout the range of motion of the linear and rotary stages. The cables should never be compressed, stretched or pinched when moving the stages. In particular, verify that the black wire exiting the vertical stage (axis 2) is not pinched by the bottom stage.
2. Support all manipulator cables vertically above the center of rotation as shown in Figure 2. Minimum stress on the cables is achieved if they are supported vertically above the manipulator centerline. Ideally the cables should be coiled like a telephone cord to minimize stiffness in all directions.
3. Upgrade stiff headstage cables to a more flexible version. For example, the original Axon Instruments' design of the CV 203BU headstage used a relatively stiff cable. (The cable was redesigned to be more flexible in 1997.) If necessary, contact your headstage manufacturer to discuss replacement of stiff cables.
4. The headstages of HEKA's EPC families of patch clamp amplifiers are equipped with a very soft and flexible rubber isolated cable. The special headstage cable design reduces the cable stiffness and, therefore, minimizes mechanical stress applied to the headstage and drag forces to the manipulator.

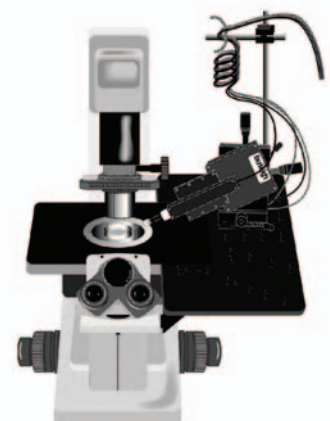


Figure 2: Suggested method of supporting cables exiting the micromanipulator.

## Q. CAN A WARM PERFUSION BATH CREATE A TEMPERATURE GRADIENT THAT WOULD CAUSE PIPETTE DRIFT?

A. Work with mammals sometimes requires the preparation be warmed above room temperature. When working at shallow angles in warm perfusion baths, a significant fraction of the pipette length will be immersed in the warm solution. Can this temperature gradient cause pipette drift of several micrometers in a few minutes?

Analysis (See Table 1)

- ✓ Borosilicate glass with 10 mm of the pipette immersed in the warm solution.
- ✓ The room temperature is 25°C and the bath is at about 34°C.
- ✓ The expected change in length of the pipette for a simple linear expansion is:  $(3.2 \times 10^{-6}/^{\circ}\text{C}) \times 0.01 \text{ m} \times 9 \text{ }^{\circ}\text{C} = 0.29 \times 10^{-6} \text{ meters}$ .

Glass, fused silica (quartz)	0.56
Glass, borosilicate	3.2
Alumina (aluminum oxide)	7.74
Stainless steel	12
Aluminum	22
Polycarbonate	68
Teflon (PTFE)	140

The above results suggest that a warm bath will not cause significant drift unless the pipette is being bent by a temperature gradient. Bending deflections can be an order of magnitude larger than linear deflections. Therefore, we recommend that the pipette be allowed to stabilize in warm solution for a few minutes before establishing a patch.

## Q. HOW DOES EXFO ENSURE THAT THE PRODUCT SHIPPED TO ME DOES NOT DRIFT?

A. We test all of our manipulators to guarantee a drift specification of less than 1 micron per minute. Our tests are performed using three capacitive sensors arranged in orthogonal axes, X, Y and Z. The capacitive sensors can detect movement of as little as 1 nm. This precision allows us to determine the most frequent causes of drift and their remedy.

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