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Pipette Drift Sources and Solutions for Patch Clamp Recording Experiments

Negligible pipette position drift is a critical requirement of most patch clamp recording experiments. In a typical experiment this is less than one micrometer (10^{-6} meters) per hour. When mechanical systems are used on the micrometer scale, physical phenomena that normally can be ignored at other resolutions become very significant. These phenomena include thermal expansion, mechanical stiffness, dynamic forces and deflection.

Application Note
Burleigh December 2010

Introduction

As a world leader in ultra-precise mechanical systems, Lumen Dynamics' Burleigh Products Group (formerly Burleigh Instruments) has pushed the limits of stability with its solid-state piezoelectric micromanipulator systems. Regardless, the entire mechanical system, not just the micromanipulator, must be taken into consideration when evaluating drift in patch clamp recording experiments. In recent tests we analyzed some of the factors that cause drift including pipette/pipette holder materials, environmental changes and cable forces.

Defining Pipette Drift

Position 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. This ensures that the drift being measured is not coming from the optics or camera. Any potential movement of the camera and microscope optics usually is not considered drift. Pipette drift can come from numerous sources that can be listed 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.

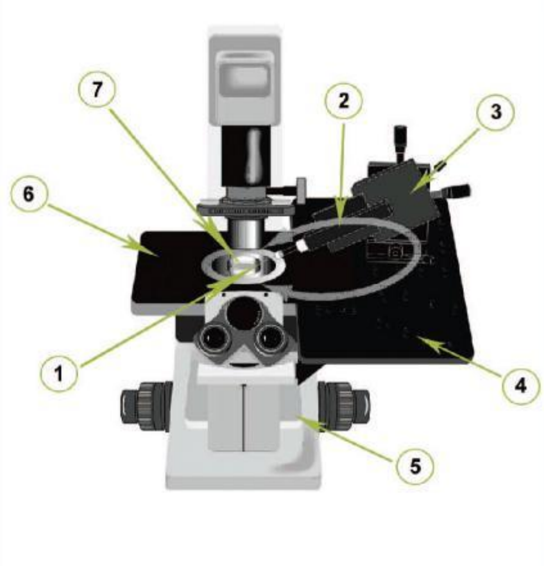

Figure 1: Mechanical loop of pipette drift source

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

General Guidelines

Pipette drift can be caused by any of the mechanical components listed above. To avoid pipette drift follow these simple guidelines:

- Avoid temperature-sensitive materials such as polymers. Table 1 shows the thermal expansion coefficient of materials typically used in patch clamp workstations and illustrates the relative thermal sensitivities. The larger the number, the greater the thermal sensitivity.

- Minimize temperature gradients and use the same structural materials throughout the mechanical loop. Aluminum is a good material because it has high thermal conductivity and quickly dissipates thermal transients. Generally when the same material is used the structure is insensitive to the slow changes in temperature that typically occur in a laboratory over a 24-hour period or as the seasons change.
- Build a solid, stiff structure.
- Minimize force changes on the system.
- Prevent temperature variations. Air conditioning vents, open windows, sunshine, and even body heat can create significant thermal variations.

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.

Table 1 - Thermal Expansion Coefficient of Selected Materials (units are x $10^{-6}/°$ C):

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

Localizing Drift Sources

If you experience pipette drift we recommend that you check these items in this order of priority:

1. Remove the headstage, clamp the pipette directly to the aluminum mounting plate and check for pipette drift. This test helps to determine if the headstage and pipette holder contribute to 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 (see F. Sachs reference above).
2. Keep the microscope away from air conditioning vents, sunlight or any other sources of heating or cooling. In the most sensitive cases, isolate the workstation from body heat using clear plastic. If the equipment is exposed to temperature changes due to installation, handling or environmental changes, let it stabilize for at least 30 minutes. More time may be needed if the temperature disturbance is severe.
3. Ensure the piezoelectric movement is smooth and achieves full travel. This confirms that the actuators are working properly and the axis movement is not obstructed. Make sure all cables are free and not applying forces to the manipulator (see discussion below on cable forces).
4. The microscope mounting system should be stiff and all mechanical connections should be tight. Hold the headstage and gently try to move it back and forth rapidly in all directions. Any movement should be small and quiet. If your fingertips feel a small "click," there is a loose connection somewhere in the system. Another test is to tap the microscope and observe the pipette. If the pipette vibrates excessively, this could indicate problems with the mounting structure. Loose or unstable connections can produce drift.

Recent Test Results and Experience

We have performed numerous drift tests of our micromanipulators on commercially available microscopes. Typical drift is less than one micrometer per hour, which is consistent with the changes in room temperature measured. Follow-up visits and communications with laboratories in the US, UK and France have identified several potential drift sources that should be considered by any researcher struggling with pipette drift. Potential sources include headstage drift, cable forces and warm perfusion baths.

Headstage Drift

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 cables 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.

Low-Drift Pipette Holder

- A novel, low-drift pipette holder solution is available from G23 Instruments. Called the ISO range, 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 and HEKA.

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 no more than 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 cap 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. (Axon notes that 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.)

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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 precise positioning guide for two O-rings that eliminate any pipette movements within the holder. A third Oring 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

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Cable Forces That Deflect the Manipulator

When using the rotary and linear stage on Lumen Dynamics PCS-5000 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.

To reduce 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.

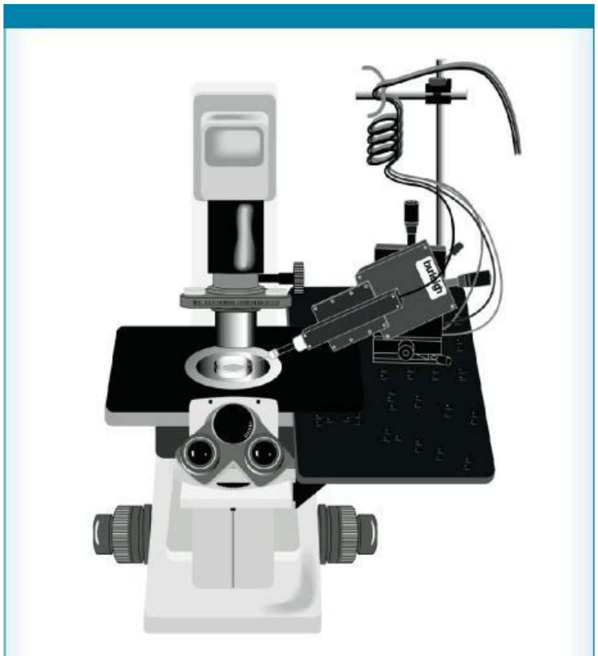

Figure 2: Suggested method of supporting cables exiting the

3. All current-model Axon headstages are designed to provide maximum flexibility without compromising shielding requirements. Very early versions of the CV 203BU headstage had relatively stiff cables, so an engineering change was implemented to improve flexibility in all future units. If you own a CV 203BU manufactured prior to 1997, you may want to contact Axon Instruments' Technical Support to discuss cable replacement options for this headstage.

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.

Thermal Analysis of Warm Perfusion Baths

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 x 10^{-6}/°C) x 0.01 m x 9 °C = 0.29 x 10^{-6} meters.

The above result suggests that a warm perfusion 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.

Conclusion

To minimize pipette drift for patch clamp recording it is necessary to understand the specific sources of mechanical drift and subsequent corrective action that needs to be taken. Generally, the first place to start is ensuring that the area for the microscope and manipulators is free from vibration and abrupt changes in temperature.

The second area to investigate is that all mountings and fixtures are appropriately tightened according to manufacturer's specifications (generally finger tight).

If pipette drift is of concern then the first diagnostic test is to determine whether it originates from the head stage or the micromanipulator. After the source of the drift (head stage or micromanipulator) is identified, pay particular attention to the cables and tubing to ensure that minimal stresses are applied.

On a final note, it often is assumed that higher temperature of the incubating solution compared to the room temperature is responsible for drift. Our calculations show this not the case.

For more information on Burleigh® products, please visit http://www.LDGI-Burleigh.com. If you would like to share your research involving the use of a Burleigh® system in a publication of this type, please contact Dr. Kavita Aswani at Kavita.Aswani@LDGI.com.

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