Comparing Micromanipulators for Electrophysiology

Several different technologies offer a choice of performance for patch clamp studies, intracellular recording and other demanding experiments in electrophysiology within the confines of an optical microscope.

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Introduction

Electrophysiology encompasses the measurement and analysis of transient ion flows and resultant voltage changes across cellular membranes, e.g., sodium ion channels that enable nerve cell signal transmission. A key, but often unheralded, component of these experiments is the micromanipulation system used by the researcher to maneuver the micropipettes (or other electrodes). Several quite different micromanipulator technologies are currently available, and each offers a unique set of advantages. This article compares these three technologies and examines how each best meets the needs of specific applications.

Voltage and Patch Clamps

The simplest way to study ion flow and resultant electrical potential changes across a membrane is to place an electrode on either side of that membrane. The oldest and most popular method is to insert a micropipette inside the cell, neuron or other study target; electrical measurements are then made relative to a reference electrode located in the external buffer in which the sample is bathed.

Depending on the type of cell, the current flow can be extremely small (picoamp to nanoamp regime). To overcome noise and quantitatively measure the current, specialized circuitry is used to maintain a set potential difference between the two electrodes, i.e., to clamp the voltage difference. This is often chosen to be a zero difference, that is, to temporarily depolarize the membrane. The transient currents required to maintain this difference are recorded as a function

of time as the ion channels in the membrane respond to this externally induced perturbation in the potential across it.

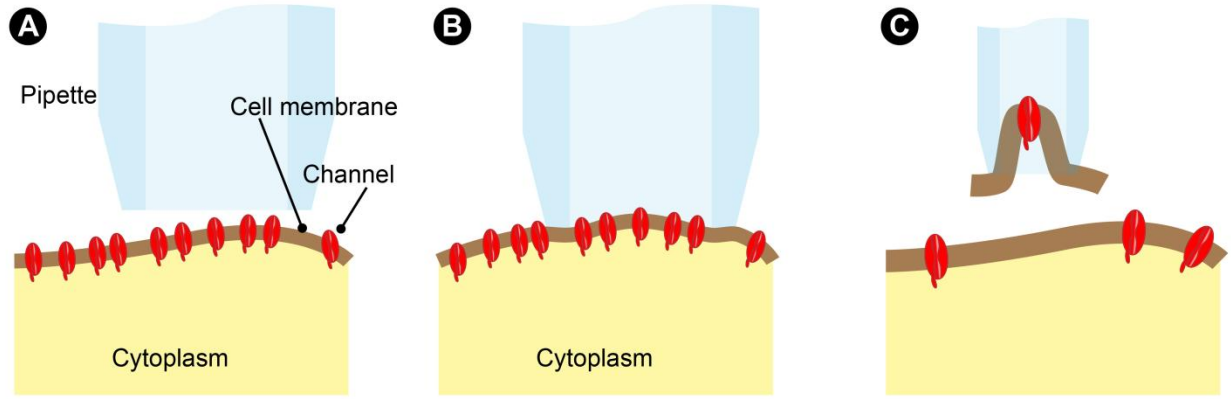

Figure 1. A) The pipette is brought into proximity of the cell wall. B) In simple "cell attached" patch clamp studies it is placed over a small area of membrane and suction is applied to make an ion-impervious seal. C) In some "inside-out" studies, the pipette is retracted to separate some of the membrane in the " inside out " configuration. The number of channels in the sampled patch can vary from a single channel to hundreds of channels.

The term "patch clamp" refers to the most common experimental configurations where the aperture of a micropipette is used to define a test area or membrane patch as shown schematically in figure 1. A low level of suction draws the membrane into the aperture and makes an ion-impervious seal. By using a very small micropipette aperture a patch or membrane can be sampled containing anywhere from just a single ion channel protein (typ. pipette diameter < 0.3 microns) up to 100's of ion channels. "Cell-attached" measurements can then be made by depolarizing and polarizing the patch and observing the transient current flows that characterize the ion channels under study. Alternatively, the suction can be increased to break the membrane and physically remove the patch for isolated study. Clever tricks allow it to be studied from either side in so-called "inside-out" or "outside-in" configurations. Or, the patch can be more abruptly torn by suction so that the cell contents and pipette interior are at least temporarily contiguous. This allows "whole-cell" recording in an alternative configuration to piercing the membrane with a pipette/electrode pushed into the cell.

There are many variants of these basic methods that allow researchers to target intracellular membranes or make measurements on specific intracellular volumes. All are dependent on

precise *xyz* positioning of one or more pipette electrodes. Specifically, they require the ability to move the pipette along its axis direction with precision and control on the micron scale, and with minimal lateral uncertainty or errors.

Today, there are numerous different technological approaches (mechanical, electrical and hydraulic) to this challenge, as well as supplier-specific details of implementation. The ideal choice for a given application is determined by matching the performance specifications to the requirements of the experiments, as well as a few practical factors and cost considerations. Typically, the complete solutions are modular so as to optimally combine one or more axes of lower-cost coarse positioning/adjustment with the requisite amount of fine positioning. (Performing experiments with all-fine adjustments over the necessary range of travel would be unnecessarily and prohibitively costly.)

Huxley-Style Flexures – Tried and True

The original experiments of electrophysiology co-pioneer Andrew Huxley were performed using all-mechanical micromanipulation systems. These were based on flexures where the position is controlled by driving the pipette holder – usually mounted on a so-called head stage –through manually turning a fine-pitched lead screw against resistance provided by a metal flexure. This type of simple, time-proven mechanical flexure combines modest resolution with low cost. A commercial example of this is the MX300 Series from Siskiyou which utilize manually driven, 100 threads per inch (TPI) screws and ball bearing translation stages to deliver a resolution of <1 µm.

Figure 2. Traditional Huxley-style mechanical micromanipulator are based on flexures and usually incorporate dial-like knobs for manual adjustment. This modular MX310L system from Siskiyou, coarse adjustment is provided by incorporating lower resolution stages at the top of the assembly, and simple pipette replacement is supported by a rotary stage at the base of the system with a hard mechanical reference stop.

Experiments are typically performed under an optical microscope, often with less than 2 mm of working distance under the objective. As a result, the probe has to be brought into the sample within a very limited angular range. To enable simple probe/pipette replacement under these constraints, the complete assembly incorporates a low-resolution rotation stage equipped with a hard (settable) mechanical stop. This allows the headstage to be swung out of the experimental position and then returned with kinematic fidelity. Coarse adjustment is optionally provided by mounting a 5-axis stage module on top of the fine flexure motion system. These axes are also driven manually, but with 20 TPI screws. Along the critical pipette axis, the end result is 20 mm of coarse motion and 2 mm of fine motion.

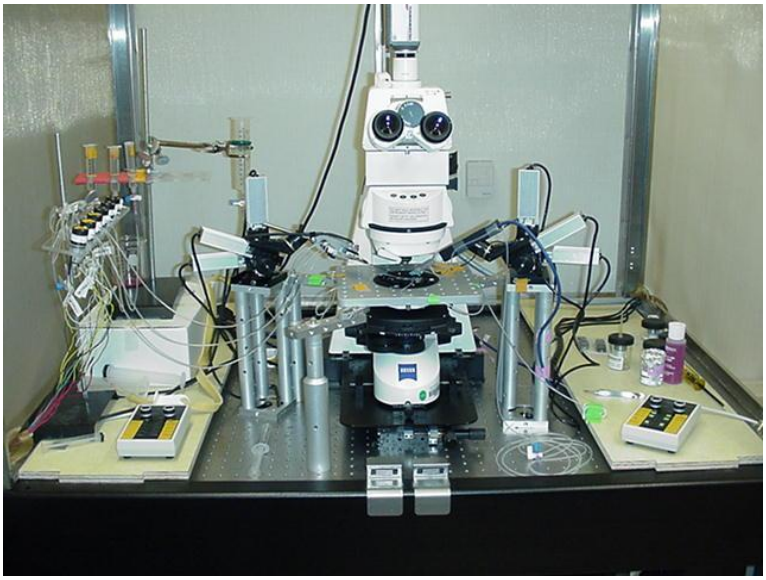

Figure 3. In electrophysiology studies, the confined experimental space under a microscope objective means that the micropipette electrode(s) can only be introduced in a very narrow range of angles.

These Huxley-style flexures are optimized primarily for teaching laboratory applications, where typical experiments require lower resolution and are targeted at larger cells (50 µm to 1 mm in diameter). Compared to other micromanipulator systems, they deliver the robust durability and low cost needed for a teaching lab, albeit in a somewhat bulky format.

As with all micromanipulators for electrophysiology applications, it is very important that the rotational axis of the motion is perfectly aligned with the electrode/pipette on its accompanying headstage. At the same time, it is advisable to electrically isolate the pipette and headstage from the micromanipulator assembly. In principle, both plastics and ceramics could be used for this isolation. However, Siskiyou has found that using a solid cylindrical ceramic rod for this mounting connection minimizes thermal drift and provides maximum rigidity.

Hydraulics – Smooth and Fast

Engineers have long known that some of the smoothest continuous motion can be driven using hydraulics. Here liquid is forced into or out of an expandable, sealed enclosure that permits motion in a single axis, e.g., a bellows, or a cylinder and piston. An example is the MX6000 series from Siskiyou which combine a metal bellows for longevity and durability with a crossed-

roller bearing translation stage. Crossed roller bearing stages are widely used in demanding photonic applications because they deliver precise linear motion (eliminating play such as pitch and yaw) with extremely long lifetime. As with the flexure actuators, motion is manually controlled by rotation of a large dial. The fine positioning adjustments on the three axes, probe, Y and Z, use ultrafine 127 TPI adjustment screws to deliver 5 mm of linear motion with 0.5 µm resolution. Also, a coarse positioning system is optionally integrated into the complete modular assembly, as well as an optional rotary stop for accurate re-positioning after pipette exchange.

A key design consideration for any hydraulic system is the choice of fluid. For example, viscosity will directly impact the system responsiveness. Another important factor is thermal expansion; changes in ambient temperature can cause the liquid to expand or contract, potentially resulting in minor positional drifts. We have found that overall, water is the best liquid for actuators for electrophysiology; it delivers the requisite responsiveness but with two to three times lower thermal expansion that typical hydraulic oils. The result is a drift of <2 µm/hour under nominal constant temperature conditions. Water can be problematic when using nylon and related plastics because these organics slowly absorb water. For this reason, Teflon tubing is used to connect the drive and output bellows. Nonetheless, we recommend flushing and refilling every two years to maintain specified performance levels.

The responsiveness of hydraulic systems means that they are well suited to demanding research applications with typical cell diameters around 10 µm or less. They often represent the best solution for intracellular recording, where an abrupt impaling action is needed to pierce the cell with a sharp electrode. And generally, these types of experiments are of less than 20 minutes duration, so minor long-term drift is not an issue. The ability to make rapid piercing movements is the reason hydraulics are often preferred for micro-injection and similar applications. For example, they are widely used by in-vitro fertilization (IVF) laboratories to perform genetic material transfer as well as fertilization.

DC Servo – Highest Stability and Lowest Noise

High-end micromanipulators offer higher resolution as well as automated (i.e., electronic) control, rather than solely manual adjustments. There are several types of electronic actuator widely used to drive stages in all kinds of photonic applications, including stepper motors, DC servos, and piezoelectrics. At Siskiyou, we use only DC servos in our MX7000 series since these best meet the needs of patch clamp studies, which form the majority of the high-resolution segment of the electrophysiology market. And, since these are intended for the highest resolution applications, they are paired with crossed-roller bearing stages. Moreover, these are assembled as four axis micromanipulator modules so that a single DC servo driver moves the pipette smoothly along its axis. Some commercial electronic axis control systems synthesize a " virtual " probe axis by cutting corners; they iterate X and Z motion in predetermined ratios only. In that case, the probe doesn't go straight into the target, but enters the cell by iterating X and Z axes. The system can also include a low-resolution rotary stage to set a micron-accuracy hard stop for simple pipette replacement.

Figure 4. Higher performance micromanipulators provide automated (pushbutton/software) positional control by using incorporating electronic actuators. This MX7600 system from Siskiyou is based on DC servos since these produce zero EMI when stationary, unlike piezos or microsteppers.

Why DC servo? Patch clamp studies are the most electronically demanding of all electrophysiology procedures because they usually involve the smallest signals; a small patch may contain just a single ion channel protein complex. In fact, the signals are so small that no

external electronic noise sources can be allowed to reach the amplifier circuitry. Although the pipette/electrode and amplifier headstage are electrically isolated by a ceramic connector rod, the intimate proximity of the actuators to the experiment means that even minor, free space electro-magnetic interference (EMI) must be avoided. This is a potential problem for steppers (especially when micro-stepping) and piezo systems, where current is continuously drawn in order to maintain position. This ongoing current draw also means these systems create unwanted heat even when stationary, i.e., during the measurements. Thermal drift is always the enemy of precision positioning.

In contrast, a DC servo system only draws current when it is moving. In fact, the power supply can be completely disrupted without causing any shift in position. As a result, DC servos produce no EMI when stationary, and the lowest drift of any electronic actuator.

The term servo refers to the fact that these actuators can be moved directly or in response to feedback from a position sensor. Direct (push-button) control delivers spatial resolution of 0.1 µm or 0.2 µm, depending on the choice of system controller. And, in closed loop operation using integrated optical encoders for servo feedback, these systems deliver even higher resolution. These encoders also support high speed motion – up to 1.7 mm/sec.

Summary

In conclusion, electrophysiology and related studies depend on micromanipulators to move and position a pipette/electrode with micron level precision and stability. However, the spectrum of commercial and research applications has a range of requirements in terms of performance, features, cost, speed and flexibility. In order to optimally meet the needs of each of these diverse uses, equipment suppliers have developed a range of micromanipulator technologies that can be combined in modular ways. This provides the right mix of degrees of freedom, coarse and fine control, and manual and automated adjustment that each user requires.