MOLECULAR DEVICES

# The Patch-Clamp Rig

Developed by Erwin Neher and Bert Sakmann in the 1970s, patch-clamping is an important electrophysiology technique with a broad range of applications in both basic research and drug discovery and development. However, the complexities of the method can seem intimidating to beginners. To help researchers get up-to-speed on patch-clamping, Molecular Devices has created this brief introduction to the experimental setup.

## 1 AMPLIFIER(S)

**What is it?** An instrument that contains the circuitry required to measure electrical currents passing through ion channels or changes in cell membrane potential.

**Why use it?** To measure changes in current or voltage.

The amplifier contains the circuitry necessary to measure current passing through the cell membrane both in magnitude and direction. The amplifier can also measure the cell membrane potential in response to the movement of current. To initiate current movement, the experimenter can deliver a voltage command to the cell, and the cell will respond by passing the current necessary to maintain that voltage command. Conversely, the experimenter may also inject current and then measure the change in membrane potential resulting from that change in current. Choosing where to amplify and filter the signal of interest has implications on signal fidelity. The ideal place to amplify the signal is inside the recording instrument. All models of Axon™ amplifiers use this strategy with variable gain control on the output to provide low-noise amplification of the pipette current or membrane potential. Placing the amplification inside the recording instrument minimizes the amount of circuitry between the low-level signal and amplifying circuitry reducing extraneous noise sources.

Available amplifiers: **Axopatch™ 200B, MultiClamp™ 700B, Axoclamp™ 900A**

## 2 DIGITIZER(S)

**What is it?** A data acquisition instrument that converts analog signals into digital signals.

**Why use it?** To capture data for analysis.

The current acquired by the amplifier is an analog signal, but in order to perform data analysis needed for high resolution patch-clamp measurements, the analog signal must be converted into a digital one. Positioned between the amplifier and the computer, the digitizer accomplishes this important task. The signal quality the computer receives is extraordinarily important, and this is determined by the sampling frequency, or sampling rate. Actual sampling frequency is user-definable, but in general the Nyquist Sampling Theorem states that at a bare minimum, the sampling rate should be twice the signal bandwidth. However, higher sampling rates are normally used in practice in order to maximize data fidelity with a rate of five or more times the bandwidth frequency often chosen. The latest generation of Digidata® digitizers has the capability of sampling at 500 kHz and is equipped with the HumSilencer™ feature, which can eliminate 50/60 Hz line-frequency noise. The chosen sampling rate may vary by application requirement, but a frequency should be chosen that optimizes balance between recording fidelity and data storage requirements.

Available digitizers: **Digidata 1550B Low Noise Data Acquisition System plus HumSilencer**

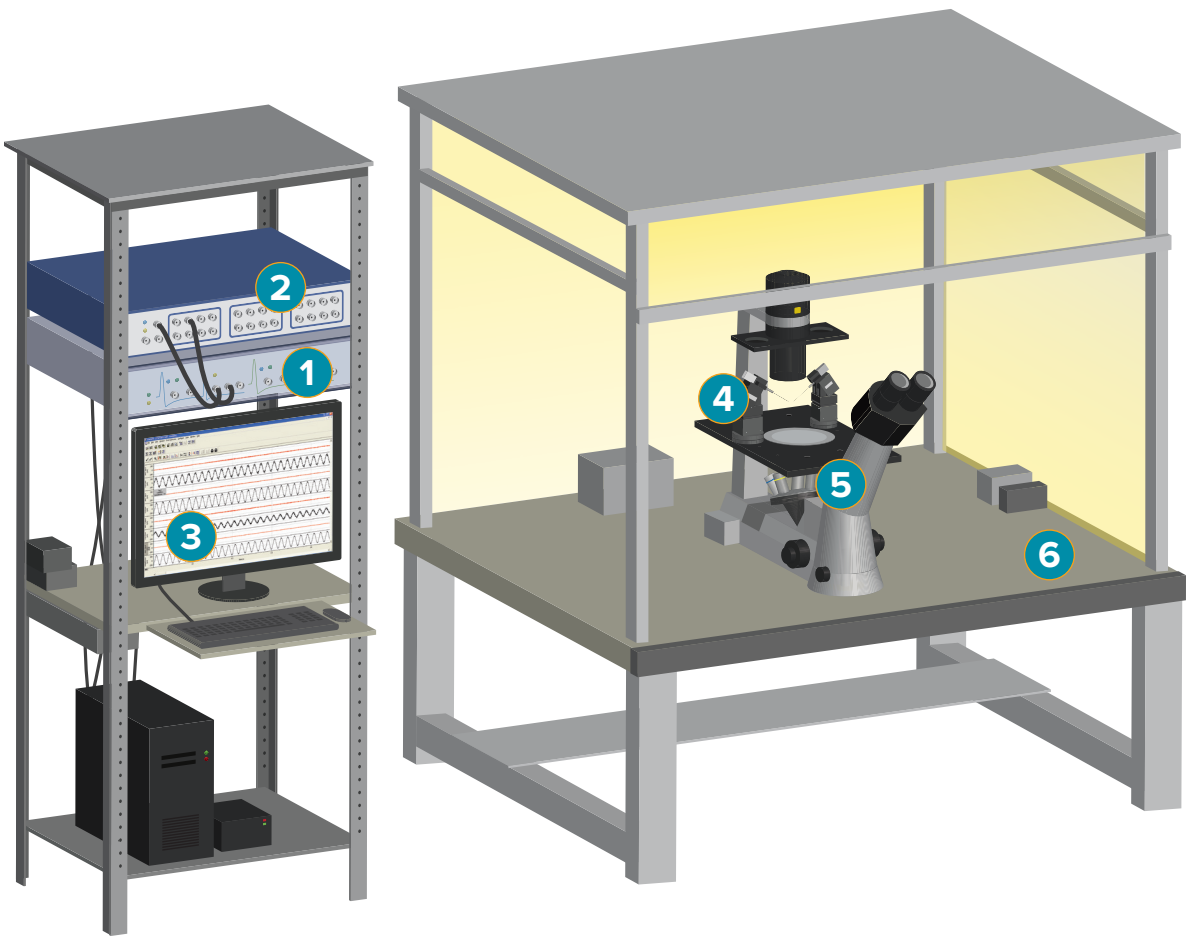
## 3 SOFTWARE

**What is it?** Your interface with the amplifier, digitizer, and any other patch-clamp electronics.

**Why use it?** To perform data acquisition and data analysis, as well as to control the digitizer and amplifier.

While the amplifier and digitizer together hold the key circuitry that implements a patch-clamp experiment, the software controls these instruments so they deliver the desired potential(s) and measure the resulting current or voltage. In addition, the software processes the acquired signal with user-defined settings, which can include filtering, normalization, noise removal, curve fitting, and parameter determination.

Available software: **pCLAMP™ 10 Software**




## 4 HEADSTAGE

**What is it?** A device that holds the micropipettes with built-in circuitry to transmit electrical signals from the micropipettes onto the amplifier.

**Why use it?** The electrical signal acquired by the micropipette needs to be transmitted to amplifier systems for signal processing.

Each headstage is specifically tuned for the amplifier. All headstages contain critical electric circuitry that reduce noise. The headstage is also mechanically controlled by the micromanipulator.

Available headstages: **Axon headstages**

## 5 MICROSCOPE WITH MICROMANIPULATORS

**What is it?** The microscope is an optical magnification tool. The micromanipulator is a device that mechanically maneuvers the micropipette with nanometer precision, typically allowing 3-dimensional movements.

**Why use it?** To precisely and stably position the micropipette to the area of cell membrane, which is critical for successful recording.

Accurately placing a patch electrode onto a 10-20 µm cell requires an optical system that can magnify up to 300- or 400-fold with contrast enhancement (e.g. Nomarski/DIC, Phase, or Hoffman) and a micromanipulator that stably positions the electrode in 3D space. An inverted microscope is preferable because it allows easier access for electrodes from above the preparation and also provides a larger, more solid platform to bolt the micromanipulator. A micromanipulator has the ability to move the electrode in very minute distances along the X, Y, and Z axes. The micromanipulator can then hold that position indefinitely.

## 6 FARADAY CAGE AND AIR/ANTI-VIBRATION TABLE

**What is it?** A table and cage around your patch-clamp setup to isolate sources of interference.

**Why use it?** To shield your setup from external interference.

Electrical currents measured during patch-clamp experiments can be extremely small (in the pico-amp range), and any small sources of interference, such as radio waves, can distort or obscure these signals. A Faraday cage is a wire mesh enclosure around your microscope and recording chamber; it is useful in preventing the electrodes from picking up extraneous noise sources. Additionally, small sources of vibration on the order of pico-meter magnitude can disrupt your recording. Hence, all components must be perfectly positioned throughout the time-course of your experiment, and the air or anti-vibration tables are used to isolate your setup from external sources of vibration that may disrupt this alignment.

**If you want even more in-depth information, be sure to download the Axon guide at moleculardevices.com/axon-guide.**

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