

Automated Electrophysiology: High Throughput of Art

Xiaobo Wang¹ and Min Li²

Abstract: Electrophysiological measurements, in particular, patch clamping, have long been regarded as the “gold standard” for assaying ion channels. Despite its high information content, the technique suffers from laborious, manual processing by highly skilled workers and extremely low throughput. Recently, a number of researchers have started to automate patch clamping by either automating conventional micropipette-based patch clamping or developing planar microelectrode arrays. This article reviews the brief history of these emerging technologies and discusses the technical details, advantages, and disadvantages of each approach and technique. As will be evident from the discussion, two types of automated patch-clamping technologies are emerging. The first places emphasis on data quality, comparable to conventional patch clamping, and the second has much higher throughput. Future developments will include sophisticated patch-clamping devices with both high-quality data and high throughput capabilities and further integration of patch clamping with other cell-based assays.

Ion Channels and Ionic Current Flow

ION CHANNELS ARE PROTEINS LOCATED in cell membranes that permit and regulate movement and conduction of ions down their electrochemical gradients at a rate of $>10^6$ ions/s across a normally ion-impermeable lipid bilayer. These proteins play important roles in affecting the physiological state of cells. Ion channels exist in all excitable and nonexcitable cells. In excitable cells such as neurons and muscle cells, ion channels produce (or mediate) electrical signals leading to action potential generation that controls a number of key processes, including heart beat, brain function, sensory transduction, and muscle contraction. In nonexcitable cells, ion channels are involved in cell cycle, hormone secretion, ion distribution, cell membrane potential, and more.

A typical mammalian cell has on the order of hundreds to thousands of ion channels of different kinds. Each ion channel exhibits high selectivity for one or a few ion

species. Common ion channels include Cl^- channels, Ca^{2+} channels, K^+ channels, and Na^+ channels, and nonselective cation channels. Functionally, the opening or closing of ion channels can be controlled or gated by the binding of signaling molecules (ligand-gated channels), by a change in the membrane potential (voltage-gated channels), or by mechanical stimulation (mechanosensitive channels) that results in conformational changes within ion channel structures. For example, a voltage-gated Na^+ channel is closed at a resting membrane potential below -60 mV, and it opens upon depolarization of the membrane (*i.e.*, a shift in the membrane potential to a less negative value).

More than 100 ion channels have been cloned and functionally investigated, and with the Human Genome Project predicting >300 ion channel genes,¹ new ion channels are still being discovered. Identifying new ion channels, testing their functions, and validating them as drug targets are the efforts of many biotech companies and academic researchers.

¹ACEA Biosciences, Inc., San Diego, CA.

²Department of Neuroscience and High Throughput Biology Center, Johns Hopkins University School of Medicine, Baltimore, MD.

ABBREVIATIONS: hERG, human ether-a-go-go-related gene; HTEP, high-throughput electrophysiology; PDMS, poly(dimethylsiloxane); POETs, Parallel Oocyte Electrophysiology Test station; TEVC, two-electrode voltage clamp.

One of the equations governing the flow of ions down the electrochemical gradient through opened ion channels located in cell membranes is the Nernst equation:

$$V_{\text{eq}} = \frac{RT}{zF} \ln \frac{C_o}{C_i} \quad (1)$$

where V_{eq} is the equilibrium potential for the ion (*i.e.*, the membrane potential at which the electrochemical gradient for the ion is zero and there is no net flow of the ion through the channel), C_o and C_i are outside and inside concentrations of the ion, respectively, R is the gas constant, T is the absolute temperature, F is Faraday's constant, and z is the charge of the ion of the interest.

Equation 1 has several important implications:

- Different ions have different equilibrium potentials (V_{eq}), depending on their inside and outside concentrations.
- When an ion channel opens, the ionic current (I_{ion}) through the channel is a function of the channel conductance (g_{ion}), as well as the difference between the actual membrane potential (V_{mem}) and the equilibrium potential (V_{eq}) of the ion:

$$I = g_{\text{ion}}(V_{\text{mem}} - V_{\text{eq}}) \quad (2)$$

- The resting membrane potential, defined as the membrane potential at which there is no net flow of ionic current across the membrane, is determined by a set of ionic current equations for all the ion channels in the membrane. Those ion channels that open at the resting potential dominate the value of the resting potential. For animal cells, the resting membrane potential is generally maintained by "so-called K^+ leaky channels," which are permeable to K^+ and do not seem to require specific membrane perturbation to open.

Ion Channels in Drug Discovery

Ion channels are of particular importance in the pharmaceutical industry in two areas: ion channels as drug targets and ion channel safety pharmacology. It is estimated that existing drugs that modulate ion channels already account for more than \$6 billion in sales per year. Although abnormal ion channel functions or reduced ion channel expressions have been linked^{2,3} to a number of therapeutic areas, including cardiac arrhythmia, hypertension, anxiety, epilepsy, pain, neuroprotection, and diabetes, novel ion channel modulator drugs for these areas have yet to be developed. Thus, essentially all major pharmaceutical companies have ongoing, active ion channel drug development projects or programs. Additionally, a number of biotech or biopharmaceutical companies focus exclusively on ion channel drug development.

With hERG as an obvious example for compound safety screening, there is a demand that drug molecules that have undesired ion channel interactions should be eliminated earlier in the drug discovery process. Thus, for both drug development and safety assessment, there is a growing demand for high throughput ion-channel assays.

Patch clamping as the gold standard for ion-channel assays

Due to the unique properties of ion channels in conducting electrical current through cell membranes, electrophysiology methods based on modern microelectronic measurements have long been applied to the study of ion channels. Over a half century ago, Hodgkin and Huxley conducted a series of experiments to study the propagated action potentials and membrane electrical conduction on squid giant axons.⁴⁻⁷ They realized that the membrane potential is the governing factor in membrane conduction and developed a voltage-clamp method that allowed for the electronic control of the membrane potential at any chosen command voltage. This enabled the simultaneous measurement of the electrical current of ions that flow through the ion channels. Two intracellular electrodes in the form of electrical wires inserted along the length of squid giant axons were used, one for monitoring and controlling membrane potential with respect to an external electrode and the other for passing electric current into the cell. With this voltage-clamp technique, they were able to explore the voltage dependence of the conduction properties of ion channels in the cell membranes.

The basic voltage-clamp technique has been adapted and refined, and it now is practiced as the modern form of TEVC⁸ of *Xenopus* oocytes and the patch clamping of small cells with a single microelectrode made of a glass pipette.⁹ A number of configurations can be used for patch clamping, including whole-cell configuration and cell-attached, inside-out, and/or outside-outside configurations in macro-patch or single-channel mode. These modern electrophysiology techniques have been widely used for studying ion channel structure, function, and pharmacology, and for screening lead compounds for ion channel targets.

In addition to electrophysiological methods, a number of other technologies, including fluorescence-detection methods, ion flux assays, and receptor binding assays, have also been developed and applied in recent years for evaluating and screening physiological and pharmacological properties of ion channels because of their higher throughput capabilities. These technologies, together with electrophysiological techniques, have been extensively discussed and reviewed in several articles¹⁰⁻¹³ for their advantages and disadvantages for ion channel assays in different application settings, and the electrophysiology method is indisputably regarded as the gold standard for

ion-channel assays. The important features of electrophysiological methods are summarized in the following:

- They provide the most direct and sensitive (down to picoampere range) measurement of ions flowing through ion channels in individual cells. The use of modern, low-noise electronic signal acquisition and processing permits the recording of current passing through a single channel protein. Other nonelectrophysiology-based methods cannot resolve ion channel functions at single cell and/or single channel levels.
- They allow for the membrane potential to be clamped at specific voltages with precise temporal control during the ion-channel assay (Eq. 2). Thus, ion channel currents are measured under well defined conditions, providing more specific and relevant information. This is a critical advantage over other nonelectrophysiology-based ion-channel assays where membrane potentials are not controlled.
- Kinetic responses of ion channels in the time resolution of tens of microseconds can be accurately determined, allowing for the measurement of the intrinsic properties of ion channels, such as the time constants for channel opening, closing, and inactivation. No other technologies permit such detailed studies of ion channel kinetic behaviors.

The conventional patch-clamp method

Typically, patch-clamping experiments are conducted under a microscope, with a glass pipette microelectrode, by highly trained personnel following a series of well defined steps (Fig. 1):

- *Positioning the glass pipette (Fig. 1A)*. A micromanipulator is commonly used to hold and manipulate the glass pipette so that the pipette tip is moved toward a selected cell on a culture dish and pressed gently against the cell membrane.
- *Forming gigaohm seal (Fig. 1B)*. A small negative pressure is then applied to the solution inside of the pipette to pull a patch of the membrane into the opening of the pipette tip. Applying a series of seal test voltage pulses between the extracellular solution and the pipette solution continuously monitors the seal resistance, which results from the contact and interaction between the cell membrane and the surface of the pipette tip. A gigaohm range of seal resistance, known as a gigaseal, is required for a low background noise recording and for mechanical stability of the cell–pipette contact.
- *Accessing the whole cell (Fig. 1C)*. After forming a gigaseal, the membrane patch under the pipette tip is ruptured by suction and/or voltage pulses, providing a low resistance (known as “access resistance” or series resistance) access to the cytoplasm via the glass pipette.

- *Whole-cell recording (Fig. 1D)*. A current across the whole cell membrane is measured under a clamped membrane potential by controlling voltage applied between the glass pipette and electrodes in the extracellular solution. Molecules to be tested for modulation of ion channel functions may be applied either to the extracellular solution or to the glass pipette.

Electrical analysis of whole-cell recording

An equivalent circuit for a whole-cell configuration shown in Fig. 2 indicates that the total current I_t measured at the amplifier has several components:

$$I_t = I_{IC} + I_{L1} + I_{L2} \quad (3)$$

where I_{IC} is the current due to the opening and closing of all the ion channels located in the membrane, given by:

$$I_{IC} = \text{Function}(V_{\text{mem}}) \quad (4)$$

I_{L1} and I_{L2} are the leakage currents across the pipette/membrane interface and through the cell membrane, respectively:

$$I_{L1} + I_{L2} = \frac{V_{\text{cmd}} - I_t * R_a}{R_{\text{seal}}} + \frac{V_{\text{cmd}} - I_t * R_a - (I_t - I_{L1}) * R_{\text{cell}}}{R_{\text{mem}} // C_{\text{mem_impedance}}} \quad (5)$$

Equations 3–5 highlight the importance of gigaseal:

- A small seal resistance R_{seal} would cause a large leakage current, leading to a large error in the whole cell current as measured from the amplifier. As an example, for a command voltage V_{cmd} of 100 mV, a seal of $10^9 \Omega$ (1 G Ω) and $10^8 \Omega$ would result in an error in the whole-cell current of ~ 0.1 and ~ 1 nA, respectively.
- A large leakage current resulting from a small seal resistance R_{seal} would also cause large error in the clamped voltage (Eq. 6, below) and increase the noise of the total current measured at the amplifier.
- From an electronics point of view, if the seal resistance R_{seal} is so small to be on the order of magnitude of the access resistance R_a and cell resistance R_{cell} , accurate determination and thus successful compensation of the access resistance R_a will be difficult by electronic means. The uncompensated access resistance will limit the temporal resolution of the whole-cell current measurement. Different ion channels may demand different kinetic resolution. Therefore, the ability to resolve fast kinetic change in whole-cell current is an important aspect of whole-cell recording.
- Small seal resistance sometimes leads to the deterioration of the seal with time and causes instability for the whole-cell access.

Thus, forming a gigaseal is a basic requirement of a successful patch clamping.

Typically, R_{cell} is small so that the voltage drop across the cell may be ignored: $(I_t - I_{L1}) * R_{\text{cell}} \approx 0$. The seal resistance R_{seal} and membrane resistance R_{mem} are effectively in parallel with each other, and it is impossible for the electronic circuits to separate their contributions. A number of instruments typically term their combined effect as R_{mem} . For a successful recording, large values of R_{seal} and R_{mem} are desirable to ensure the accuracy of patch clamping.

The error in the membrane voltage V_{mem} is given by:

$$V_{\text{mem}} - V_{\text{cmd}} = -[I_t * R_a + (I_{L2} + I_{IC}) * R_{\text{cell}}] \quad (6)$$

The voltage escape for the membrane potential is related to the access resistance R_a , the cell resistance R_{cell} , as well as the total current I_t and the current across the cell membrane. For accurate clamping of the membrane potential, the access resistance R_a should be as small as possible. R_a is the sum of the electrode resistance and the resistance due to a number of environmental factors around the pipette tip, such as cell debris.

Thus, quality parameters for whole-cell recording include: (a) large seal resistance (e.g., $R_{\text{seal}} > 1 \text{ G}\Omega$); (b) large membrane resistance (e.g., $R_{\text{mem}} > 200 \text{ M}\Omega$); (c) small access resistance (e.g., $R_a < 10 \text{ M}\Omega$); and (d) stable whole-cell access for an extended time period (e.g., $> 10 \text{ min}$).

Toward automated, high throughput electrophysiology

Despite being viewed as the gold standard for ion-channel assays, patch clamping, as exemplified in the recording procedure described above, remains one of the most labor-intensive and skill-demanding assays in cell biology. Even for the experienced electrophysiologist, the assay throughput is, at best, 10–20 cells per day. Such a low throughput has greatly limited the use of the electrophysiology method for ion channel screening. Since as early as 1994,¹⁴ efforts have been undertaken to automate electrophysiology and to improve throughput of the electrophysiology method.

Several major categories of automated electrophysiology technologies have been and are being developed: (a) automated glass pipette-based patch clamping (Table 1); (b) microfabricated planar electrode-based patch clamping (Table 2); and (c) automated, TEVC on *Xenopus* oocytes.

Automated glass pipette-based patch clamping

Bristol-Myers Squibb Company (Princeton, NJ, U.S.A.). Bullen and Weaver of the Bristol-Meyers Squibb Company have described an automated patch clamp recording method,¹⁵ which involves suspending cells into one

or more cell layers in a density-gradient solution and inserting a glass pipette into the cell layer(s) (Fig. 3A). After positioning of the tip of the pipette to a cell surface, gigaohm seal formation and whole-cell access or other patch-clamp configuration may be achieved in the same fashion as the conventional patch clamping. The performance details of this method are undisclosed.

CeNeS (Cambridge, U.K.). AutoPatch (AP) systems developed by CeNeS used a technique called interface patch clamping,^{13,16} where cells are suspended a liquid medium at a liquid/air interface (Fig. 3B) and are accessible to patch-clamp recording electrodes (such as a pipette tip). The advantage of this approach over the conventional patch clamping is that no optics are required to move the pipette tip to a cell surface and the whole-cell patch clamping can be automated. A whole-cell recording rate of ~50–60% has been reported with an AutoPatch system.

Cytocentrics (Reutlingen, Germany). An automated patch-clamping system being developed by CYTOCENTRICS is based on their “Cytocentering” technique.¹⁷ In the proof-of-principle studies of the technique, a conventional glass pipette was vertically positioned with the tip placed in the center of an opening in a polyimide sheet (Fig. 3C). In operation, a suspended cell was attracted to the pipette tip by application of suction to the opening in the polyimide sheet and surrounding the pipette tip. After positioning of the cell, the patch contact was used for patch clamping in the conventional way. Success rates of 97% for positioning cells to the glass pipette and 68% of gigaseals were achieved with the Cytocentering technique. The company is further developing a CytoPatch™ chip technology¹⁷ based on the Cytocentering approach, with a goal of full automation and parallel and asynchronous whole-cell recording.

Flyion (Tubingen, Germany). In developing automated patch-clamp systems, researchers in Flyion GmbH noted that glass is a low-cost, proven gigaseal substrate, and pulling glass capillaries to small tips is a robust and simple method to produce ultraclean, smooth surfaces suitable for gigaohm sealing. Based on this, they developed a technology¹⁸ to produce gigaseals and whole-cell preparations inside a glass micropipette by simply filling the pipette with cell suspension and flushing cells toward the pipette tip (Fig. 3D).

The success rate for obtaining a gigaseal was ~80%, with average seal resistance above 9 GΩ. The gigaseal was formed within 10–90 s. Whole-cell access was achieved by simply applying suction pulses to the cell sealed inside the pipette. Over 80% of whole-cell preparation had a seal intactness and whole-cell access stability for $> 15 \text{ min}$. Where pipette tips had electrode resis-

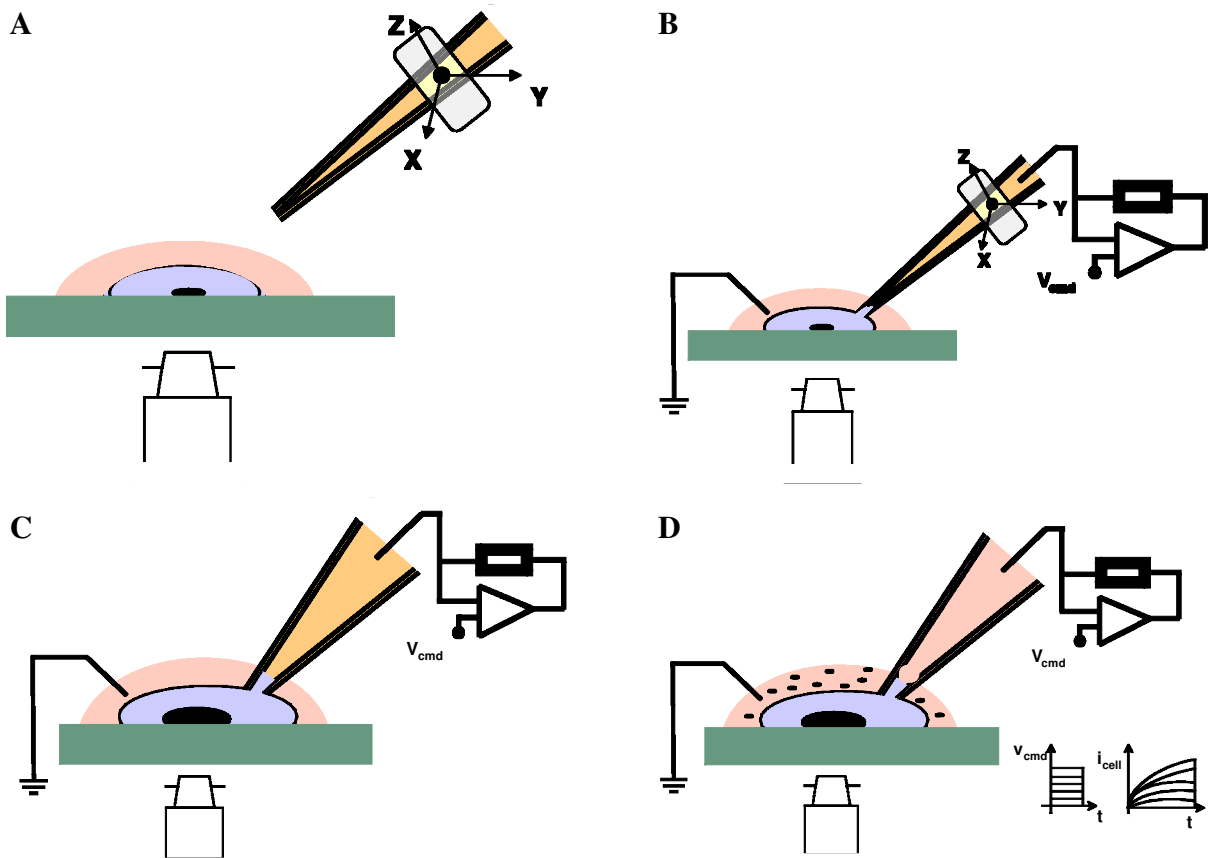


FIG. 1. The sequence of whole-cell patch clamping with a glass pipette electrode: (A) positioning the pipette to a cell; (B) forming gigaohm seal; (C) accessing the whole cell; (D) whole-cell recording. Black spots in D represent ion channel modulator molecules added to the extracellular solution during the patch clamping.

tance of $0.9 \text{ M}\Omega$, the access resistance was $<3 \text{ M}\Omega$ and remained stable for $>15 \text{ min}$.

Compared with conventional patch clamping, we might speculate that patch clamping inside a glass pipette in Flyion technology may result in a larger contact area between the cell membrane and the glass pipette, and may lead to a higher rate and a higher quality for gigaseals and more stable whole-cell access. Furthermore, small

access resistances ($<3 \text{ M}\Omega$) may be a direct result of cells sealed inside and located very close to the pipette tips, having a very small distance between the pipette tip and the cell interior.

The company is developing a fully automated patch-clamp system based on robotic tip arrays with expected throughput of 300–1,000 independent whole-cell screens per day.

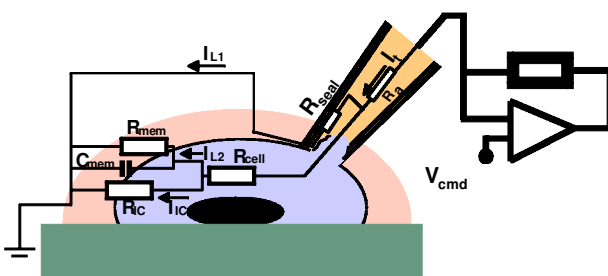


FIG. 2. Schematic representation of an equivalent circuit for a cell under whole-cell patch-clamp recording configuration. The definitions of the symbols are as follows: V_{cmd} , the command voltage applied at the amplifier; I_t , the total current measured at the amplifier; I_{IC} , the current due to all the ion channels located in the cell membrane; I_{L1} the leakage current across the pipette/membrane interface; I_{L2} , the leakage current through the cell membrane; R_a , the access resistance from the amplifier to the cell interior; R_{seal} , the seal resistance between the pipette and the cell membrane; R_{cell} , the resistance of cell interior; R_{mem} , the membrane resistance; C_{mem} , the membrane capacitance; R_{IC} , the equivalent resistance of all the ion channels located in the cell membrane. Values of R_{IC} depend on whether the ion channels are open or closed and how many ion channels and what types of ion channels are open. For the membrane containing the voltage-gated ion channels, the R_{IC} value depends on, among other things, the voltage applied to the membrane.

TABLE 1. MAIN PLAYERS IN THE DEVELOPMENT OF AUTOMATED, PIPETTE-BASED PATCH-CLAMP TECHNOLOGY

<i>Company</i>	<i>Cell/pipette positioning</i>	<i>Gigaseal rate</i>	<i>Whole-cell access</i>	<i>Whole-cell recording rate</i>	<i>Status</i>
Bristol-Myers Squibb Co.	Cells suspended in solution having a density gradient into cell layers; a pipette tip manipulated to a cell ^{1,5}	N/A ^a	N/A	N/A	N/A
CeNeS (AutoPatch)	Cells suspended at an air/water interface; a pipette tip is moved to a cell ^{13,16}	N/A	Suction	50–60%	AP1 installed and AP2 under development
Cytocentrics CCS	Cytocentering technique ¹⁷ >95%	68%	Suction	77%	CytoPatch chips under development
Flyion	Flushing cells into pipette tips from the inside pipette ¹⁸	75–82% 10–90 s to seal	Suction (loss <10%)	80%, > 15 min, $R_a \approx 2.7 \text{ M}\Omega$ Perforated patch possible, $R_a = 5\text{--}12 \text{ M}\Omega$	Robotic tip array system under development
Sophion Biosciences (Apatchi-1™)	Imaging-based, pipette positioning ¹⁹	N/A	Suction	N/A	Commercially available

^aN/A, not available.

TABLE 2. MAIN PLAYERS IN THE DEVELOPMENT OF AUTOMATED, PLANAR ELECTRODE-BASED PATCH-CLAMP TECHNOLOGY

<i>Institution</i>	<i>Chips</i>	<i>Cell positioning</i>	<i>Gigaseal rate</i>	<i>Whole-cell access</i>	<i>Whole-cell recording rate</i>	<i>Status</i>
Affymax AVIVA Biosciences, Axon Instruments	Glass, laser drilling ²⁴ Glass ^{31–34} with modified surface	Suction Suction dielectrophoresis	N/A ^a >90%, >1 G Ω	N/A Suction (loss < 1%)	N/A >75%, 15 min, R_a < 15 M Ω , R_m > 200 M Ω ~50%	N/A PatchXpress™ and SealChip™ available
Axon Instruments CeNeS	PDMS, molding ⁴⁷ Various substrates, laser drilling, and other, methods ³⁰	Suction Suction	~50% N/A	Suction N/A	~50%	N/A N/A
Cytocentrics CCS Cytion	Quartz glass ¹⁷ Si ₃ N ₄ membrane ³⁷ supported by a Si chip	Suction Electrophoresis	N/A N/A	N/A N/A	N/A N/A	Under development Under development
Essen Instruments, Molecular Devices	Plastic chip, laser drilling, with a glass coating ^{28,29}	Suction	20–250 M Ω	Perforated patch	60–80%, 6 min, R_a = 10–15 M Ω , R_m = 10 M Ω 30–50%, 30 min	IonWorks™ HT and PatchPlate™ available NPC [®] 16 under development
Nanon Technologies	Glass chip, ^{42–45} gold ion-etched track followed by wet etching	Suction	30–50%	Suction		
Sophion Biosciences	Silicon chip, ¹⁹ etching with biocompatible coating	Fluid channel, suction	N/A	Suction	N/A	QPatch 16™ and 96 under development
Yale University	PDMS, molding ⁴⁶	Suction	<i>Xenopus</i> oocyte 13%, gigaseal	N/A	N/A	N/A

^aN/A, not available.

Sophion Biosciences (Ballerup, Denmark). The Apatchi-1™ system¹⁹ developed by Sophion Biosciences is a machine-vision-based, automated conventional patch-clamp system. Cells to be patch-clamped are identified and selected by an imaging software, and a micromanipulator in the system automatically grabs a pipette from a pipette rack and positions the pipette tip to the membrane of the selected cell. Gigaseal and whole-cell access is accomplished by the application of appropriate pressure protocols. This imaging-based system performs patch clamping one-at-a-time, and its throughput is two to five times higher than the conventional patch clamping.

Planar electrode-based patch clamping

In recent years, microfabrication and micromachining technologies have been used to develop a variety of biochip devices and systems for significant improvement of biological assay throughput. Planar DNA microarrays are being widely used for DNA sequencing, mutation detection, and gene expression studies. By immobilizing a large number of different DNA probe molecules onto different locations, arranged typically in a row by column format on a single substrate, massively paralleled experiments for DNA hybridization are conducted.^{20,21} Planar microfabricated chips have also been developed for electrophoresis separation of molecules and for dielectrophoretic and other physical force-based cell manipulation and processing.²² Furthermore, there is a growing interest in developing functionally integrated lab-on-a-chip devices and systems. A natural extension of these biochips into the ion-channel assay field is a planar patch-clamping chip with an array of recording apertures microfabricated on a substrate. Figure 4 shows a sequence of patch clamping with such a planar recording structure. The potential benefits of such an approach are obvious and include:

- Parallel patch clamping by incorporating many recording apertures on a single substrate;
- Precise and consistent recording aperture fabrication using matured engineering methods;
- Improved stability and longevity of the seal and the whole-cell recording because of cells being supported by a planar surface;
- Reduced electronic noise interference from the environment because of smaller, compact size of the recording chip;
- Use of less reagents because of the small volume of the recording chambers;
- A microfluidic approach facilitating compound solution applications;
- A wide range of dielectric materials available as the substrates.

On the other hand, there are a number of new issues arising from the use of planar patch-clamping microelectrodes:

- Is gigaseal possible for recording apertures on substrate materials other than commonly used glass and quartz?
- How would the geometry, surface smoothness, and other properties of recording apertures affect gigaseal?
- How are individual recording sites controlled, from gigaseal formation to whole-cell recording, to maximize successful recording rates?
- How are the system and recording process fully automated?
- How is the potential for electronic cross talk and interference between neighboring recording sites minimized?
- How is the vast amount of data processed?
- How expensive is the system and the patch-clamp recording chip on a cost-per-test basis?

Many of these issues and challenges remain unanswered today, and are being addressed by a number of companies, as well as academic institutions that have endeavored to develop planar patch-clamping technology. In the following, each of their approaches will be discussed in the context of the questions and issues raised above. It is worthwhile to point out that the use of planar structures for measuring whole-cell current of neurons under voltage-clamping conditions has long been described by Kostyuk *et al.*²³ In the experiment, a neuron cell was positioned by suction into a conical aperture on a polyethylene membrane sandwiched between an upper and a lower fluidic compartment. The aperture wall was coated with adhesive material to improve “seal” between the cell membrane and the aperture wall. Whole-cell break-in was achieved by the perfusion of the lower chamber with a potassium solution. Electrical current through the cell membrane was then measured under voltage-clamping conditions. The basic device configuration and voltage-clamping methods described by Kostyuk *et al.*²³ are very similar to those used in the modern planar patch-clamping technologies to be discussed below (Table 2), except that their aperture was somewhat larger.

Affymax (Palo Alto, CA, U.S.A.). In their patent application, Kelly *et al.* described their planar patch-clamping devices²⁴ made of a variety of substrate materials, including glass, plastics, or silicon. The recording apertures, such as conical holes having a diameter of 1–5 μm , are microfabricated in substrate plates using laser drilling. In the patent application, an emphasis was placed on various methods for modifying recording apertures to achieve gigaseals, including heat treatment, adhering glue-like substances, or covalently bonding lipid mole-

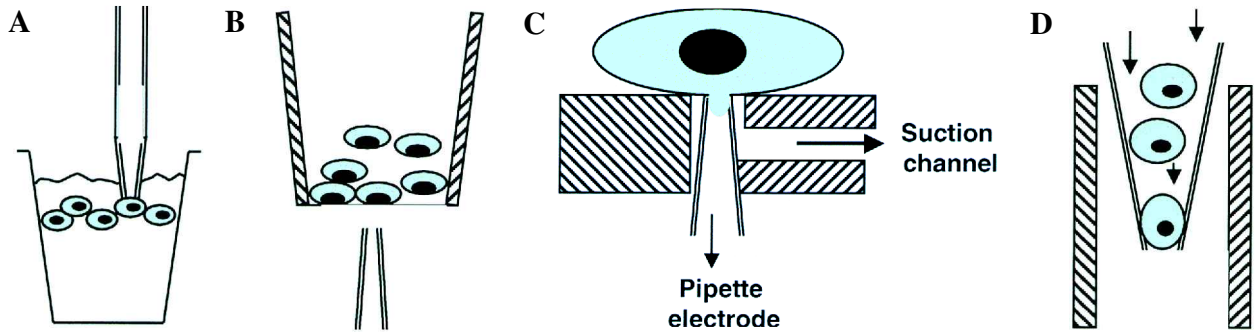


FIG. 3. Schematic representation of several automated, pipette-based patch-clamp technologies. (A and B) A pipette electrode is moved to contact the surface of a randomly chosen cell that is suspended in a layer within a density gradient (A) or at the air/liquid interface (B). (C) A cell is positioned to the recording pipette electrode under negative pressure at the suction channel. (D) Cells are filled into a pipette and are flushed into the inside tip of the pipette.

cules. Nevertheless, little is known regarding the details of Affymax's planar patch-clamping technology.

One particular point worthy of discussion is the laser drilling technology used for fabricating recording apertures by Kelly *et al.*²⁴ Laser ablation has long been demonstrated for drilling holes of micrometer sizes in a variety of substrate materials, such as plastics, glass, ceramics, and even metals.^{25–27} The technology relies on the interaction of a series of focused laser pulses with materials, causing vaporization and ablation of a thin

layer of materials. Repeated laser pulses focused on the same spot of a substrate material will result in a through-hole being created. Laser drilling is a robust and accurate process for producing holes in substrate materials and has been widely used in the drilling of nozzles for ink-jet printers. It is noteworthy that laser drilling for producing apertures suitable for electrophysiological measurements is described not only by Kelly *et al.*²⁴ of Affymax, but also by Schroeder *et al.*^{28,29} of Essen Instruments, Inc., and Owen and Nicholas³⁰ of CeNeS.

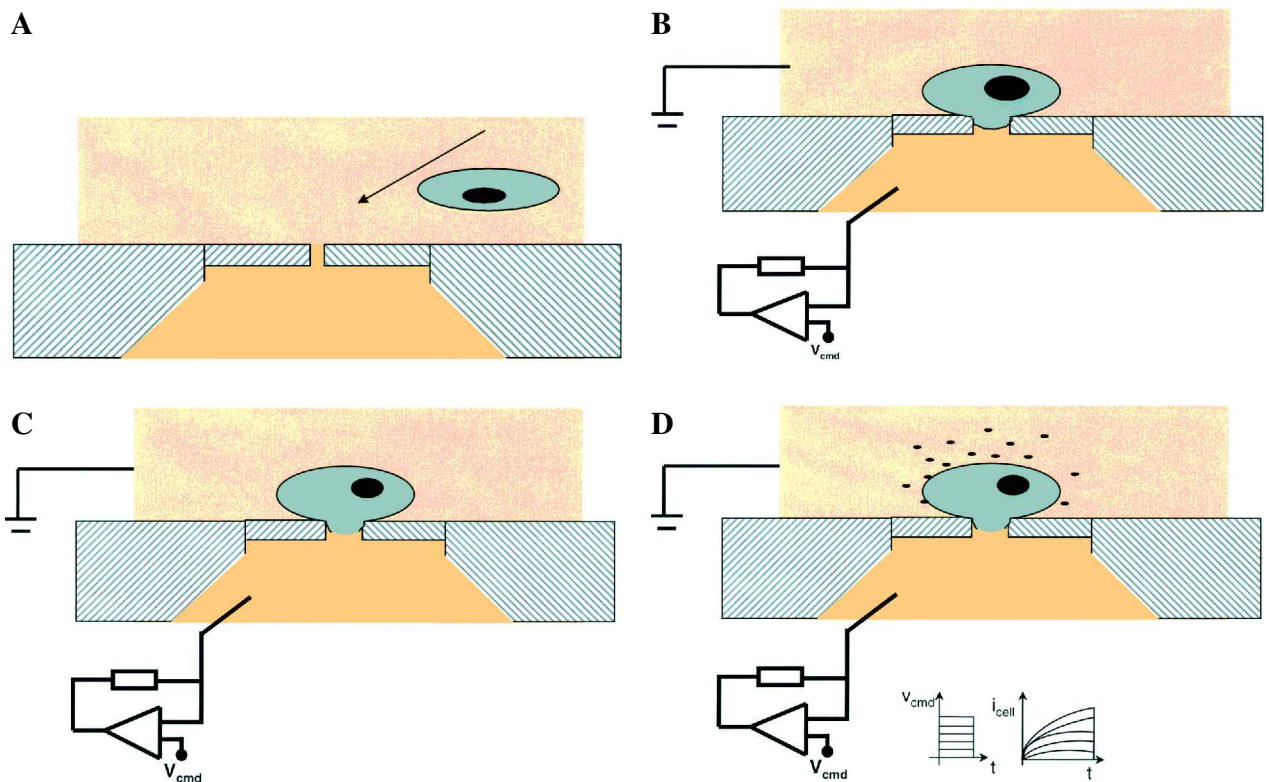


FIG. 4. The sequence of whole-cell patch clamping with a planar patch-clamp electrode: (A) positioning of a cell to the recording aperture; (B) forming gigaohm seal; (C) accessing the whole cell; (D) whole-cell recording.

AVIVA Biosciences Corp. (San Diego, CA, U.S.A.)/Axon Instruments, Inc. (Union City, CA, U.S.A.). SealChip™ planar patch-clamp technology developed by AVIVA Biosciences uses glass as substrate material. Although the methods for producing recording apertures and for surface modification to enhance gigaseal are not disclosed, SealChip™ technology was demonstrated^{31–34} to have a 90% gigaseal success rate and >75% overall whole-cell recording rate at achieving gigaseals, followed by whole-cell access lasting >15 min, with access resistance below 15 MΩ resistance and membrane resistance over 200 MΩ.

SealChip technology has now been commercialized with the launch of a 16-channel automated patch-clamp recording system—PatchXpress™³⁵ by Axon Instruments, Inc. The device consists of 16 planar patch-clamp recording apertures of ~1 μm diameter, aligned on a single strip 4.5 mm apart. Each recording aperture acts independently to record currents from mammalian cells. Noted features in the PatchXpress™ system^{35,36} and SealChip devices^{31–34} include:

- High whole-cell recording success rate (gigaseal, 15-min duration with $R_a < 15 \text{ M}\Omega$ (uncorrected) and $R_m > 200 \text{ M}\Omega$);
- 16 independent patch-clamp amplifiers for automated parallel patch-clamp recording of multiple cells simultaneously;
- 16 independent pressure controllers, ensuring high success rates of gigaseal and whole-cell access and ensuring low access resistance;
- Integrated fluidics for drug application during recordings with rapid solution exchange and test compounds of ~50 μl added only to cells with successful recordings;
- Parallel patch clamping of 16 cells per chip with throughput of testing >2,000 compounds in one 8-h day;
- Cumulative dose-response experiments are supported.

CeNeS (Cambridge, U.K.). In their patent application, Owen and Nicholas of CeNeS described a high throughput electrophysiology method³⁰ where a contiguous layer of cells is adhered to a perforated substrate with high-resistance seals. Electrophysiological measurements are performed on the adhered cells to monitor their ion channel activities and properties using transmembrane impedance measurement and voltage-clamping methods. In one approach, a movable recording head is used to perform electrophysiology measurement on cells located in each chamber. Nevertheless, no detailed information or experimental data of using such technologies are available.

CytoCentrics CCS (Reutlingen, Germany). The CytoPatch™ chip technology being developed by Cyto-

centrics CCS is based on the “CytoCentering” principle¹⁷ described above. For prototype device development, quartz glasses were used as the chip substrates and recording apertures were produced using a focused ion beam. The CytoPatch™ system is developed as a single cycle device for one-cell-at-a-time recording with fully automated operation and throughput up to 200 cells per day (including overnight operation).

Cytion SA (Lausanne, Switzerland). Cytion SA has been developing silicon-based³⁷ planar patch-clamp chips. Silicon nitrate membranes containing micrometer-sized recording apertures were suspended over holes etched into silicon wafers. The Si₃N₄ membrane was coated with 20 nm SiO₂ and modified to have positively charged surfaces. Electrophoretic positioning of membrane vesicles was demonstrated to the recording apertures from up to 20 μm away. Furthermore, the structures have proven successful for forming gigaseal with vesicles and recording single ion channel activities. However, there has been no report on gigaseal or recording on cells with this approach. Sigworth and Klemic³⁸ have suggested that the narrow sidewalls or sharp corners of the etched recording apertures do not provide sufficient contact area with the cell membrane to allow a seal to be formed.

Essen Instruments, Inc. (Ann Arbor, MI, U.S.A.)/Molecular Devices (Sunnyvale, CA, U.S.A.). Essen Instruments has developed a plate-based electrophysiology measurement platform. The center of this technology is the planar multiwell substrate (a PatchPlate™). The substrate is a plastic sheet (e.g., polyimide or polyethylene terephthalate) into which recording apertures are fabricated with laser drilling. To facilitate a high-resistance seal, the substrate may have been coated²⁹ with silicon dioxide. The PatchPlate™ has been shown^{28,39} to have a seal resistance centered around 100 MΩ with a spread between 20 and 250 MΩ, and to have an overall rate for stable (~6 min) electrophysiology measurement on the order of 60%–80%.

The PatchPlate™ is a planar 384-well substrate arranged in an 8 × 48 well array with the spacing between wells being 2.25 mm and each well holding up to 15 μl. Each well of the PatchPlate contains a centrally located recording aperture and allows the communication between the extracellular and intracellular compartments. All the wells share a common intracellular compartment that is connected to an electrical ground. After cells are placed into the recording apertures and high-resistance seals are formed, a membrane-permeabilizing compound is applied to the common intracellular compartment, resulting in a perforation on the membrane patch and access to the cell interior. Subsequently, the voltage control and current recordings from the cell membrane are

performed. The PatchPlate is commercialized by Molecular Devices as part of a high throughput electrophysiology platform, IonWorks™ HT,^{40,41} consisting of computer-controlled fluid handling, recording electronics, and processing tools capable of voltage-clamp whole-cell recordings from thousands of individual cells per day. The noted features in IonWorks HT^{40,41} include:

- 48 independent voltage-clamp amplifiers for automated recording of multiple cells simultaneously;
- A 12-channel pipettor having <5% CV at 3.5- μ l dispense volume for transferring and dispensing reagents;
- Positioning of cells introduced to extracellular compartments by applying a negative pressure to the common intracellular compartment;
- Electrical access to cell interior by exchanging a perforation compound solution into the intracellular compartment;
- Recording of cells in all 384 wells by moving a 48-channel recording head in eight successive moves/read steps;
- Up to 3,000 recordings per day.

Compared with PatchXpress launched by Axon Instruments, Inc., IonWorks™ HT has a higher throughput. However, IonWorks's "mix and read" mode of fluidics limits its application to simple assay protocols, whereas PatchXpress is capable of sophisticated fluidic procedure, *e.g.*, complete washout. In addition, SealChip is used in PatchXpress, allowing for higher resistance gigaseals and better patch-clamping data quality (*e.g.*, no leak subtraction is needed). It is believed that IonWorks is better suited for high throughput screening where producing several thousand data points per day is required. On the other hand, PatchXpress is well suited for medium throughput screening and for detailed electrophysiology studies on ion channels (*e.g.*, investigating functional interactions of chemical compounds with ion channels).

Nanon Technologies (Munich, Germany). Nanion Technologies was the first to report gigaseals and whole-cell recordings from mammalian cells on a planar glass chip.^{42–45} To produce recording apertures, a 100- μ m-thick glass was first etched to \sim 20 μ m on a local area of the glass defined by a gold mask. Then a single, high-energy gold ion was shot through the 20- μ m glass, leaving behind a narrow etched track. The narrow track was subsequently wet-etched, producing a conical-shaped hole through the glass. Strict process control allowed for reproducibly achieving micrometer-sized apertures. Such a glass chip had an overall success rate for whole-cell recording of 30–50%.⁴⁴

Nanon Technologies is developing NPC[®]-16 chip, which is equipped with 16 recording sites with 16 individual suction controls, allowing for either sequential or

parallel recording and providing a throughput of \sim 150 cells a day. The system is embedded with a microfluidic perfusion system, allowing rapid solution exchange.

Sophion Biosciences (Ballerup, Denmark). In addition to commercializing the pipette-based Apatchi-1™ system, Sophion Biosciences is also developing¹⁹ a silicon chip-based, 16-channel and 96-channel system QPatch 16™ and QPatch 96™. A micrometer-sized hole is etched on the silicon wafer, and the chip is then surface-coated with nonconductive, biocompatible materials that smooth the edges of the hole and facilitate the gigaseal. The success rate of gigaseal and stable whole-cell recordings has not been reported.

Noted features of the QPatch 16 and QPatch 96™ systems include:

- Microfluidic channels and electroosmotic pumps being incorporated into QPlate containing silicon chips and recording electrodes, allowing for washout of the applied compounds and sequential drug application;
- 16 and 96 independent amplifiers in QPatch 16 and QPatch 96 systems, respectively, allowing for parallel recording of multiple cells simultaneously.

Yale University (New Haven, CT, U.S.A.)/Axon Instruments (Union City, CA, U.S.A.). The Sigworth group was the first to report⁴⁶ successful recording of cells using planar patch-clamp electrodes. PDMS was chosen as the substrate material because of its excellent dielectric properties and ease of reliable microfabrication. After recording apertures were micromolded into PDMS substrate, the PDMS substrate was treated with O₂ plasma to convert its hydrophobic surface to the hydrophilic state. This treatment was necessary for forming gigaseals with *Xenopus* oocyte. The low gigaseal rate of 13%⁴⁶ indicates that the surface and/or geometry of the recording aperture is far from optimum, despite O₂ plasma treatment.

Yale's PDMS-based technology was licensed to Axon Instruments. With improvement in recording aperture fabrication, Mathes *et al.*⁴⁷ demonstrated successful gigaseal and whole-cell recordings of mammalian cells, having an overall success rate of \sim 50%, on PDMS-based planar recording electrodes.

Xenopus oocyte voltage-clamp system

The *Xenopus* oocyte is a popular ion-channel screening system, because of the following:

- Oocytes are large (>1 mm in diameter) and easy for manipulation and recording.
- Oocytes contain few endogenous channels and faithfully express injected RNA and DNA rapidly in 1–2 days.

- Oocytes can be maintained with electrophysiological access for hours, allowing for multiple, serial drug applications.

Several companies have developed TEVC based systems for whole cell recording of *Xenopus* oocytes.

Abbott Labs (Abbott Park, IL, U.S.A.). POETs™⁴⁸ is an automated recording system with six identical TEVC recording stations. It allows integrated drug application from 48, 96, and 384-well plates, can run autonomously for up to 18 h and is capable of automated data acquisition and real-time data analysis.

Axon Instruments, Inc. (Union City, CA, U.S.A.). OpusXpress™ 6000A workstation⁴⁹ is an automated recording system with eight channels (eggs) in parallel. It allows full perfusion of oocytes, integrated drug application from 96-well plates, and rapid solution exchange (95% exchange within 1–5 s), and is capable of automated data acquisition and real-time data analysis, and simultaneous control of eight TEVC amplifiers. It is commercially available.

Hitachi (Japan). An automatic electrophysiology apparatus is being developed,⁵⁰ capable of sequentially performing TEVC recording on *Xenopus* oocytes in a 96-well plate.

Multichannel systems (Reutlingen, Germany). Ro-boocyte⁵¹ is an automated system for performing both cDNA (or mRNA) injection and subsequent TEVC measurement on *Xenopus* oocytes plated in a 96-well microtiter plate.

Scion Pharmaceuticals, Inc. (Boston, MA, U.S.A.). HTEP™⁵² is an in-house automated electrophysiology station with 10-fold throughput improvement over manual electrophysiology methods. Other technical details are not available.

Other Electrophysiology Technologies

Collectricon has recently launched a Dynaflow™ chip technology.^{53,54} The technology integrates a microfluidic chip consisting of multiple channels entering an open volume with conventional patch-clamp equipment. Different compound solutions are loaded into multiple channels and are caused to move into the opening volume, creating a series of no-mixed compound solutions at the outlets of the channels. A patch-clamped cell is scanned across the channel outlets using a motorized stage, and the kinetic response of ion channels to these compound solutions can be measured with the patch-clamp mea-

surement. With this technology, several hundred compounds can be screened and analyzed using the standard patch-clamp method per day.

Conclusions and Perspective

Continued efforts have focused on developing automated electrophysiology technologies for ion-channel assays with the goal of improving assay throughput. For patch clamping, automated technologies can be divided into two categories: pipette-based (Table 1) and planar chip-based (Table 2) approaches. A number of promising technologies are still under development and are expected to be commercialized within the next 12–18 months.

Pipette-based patch-clamp technologies use proven methods for making recording electrodes out of glass pipettes so that the success rates of gigaseal and whole-cell recording are comparable to those of conventional patch clamping. However, parallel recordings for high throughput capability with multiple pipettes operated simultaneously remain to a significant challenge. In addition, methods for positioning pipettes to cell surfaces need to be further improved in order to have a guaranteed success of pipette–cell contact (except for Flyion's method where cells are flushed to the inside tips of the pipettes). For planar chip-based technologies, the advantages are parallel patch clamping with many recording apertures on a single substrate, consistent recording aperture fabrication using matured manufacturing methods, improved stability of recording with cells being supported by a planar surface, and a microfluidic approach facilitating compound solution applications and using less reagents in the recording chambers of small volumes. Potential technical hurdles exist, including gigaseal formation on unproven substrate materials, the automatic control of individual recording sites, and minimization of potential cross talk between neighboring sites. Several companies have overcome these hurdles and launched their planar-chip-based products.

When comparing commercially available automated patch-clamp systems, we note that two general classes of new technologies are emerging. The first is represented by PatchXpress launched by Axon instruments, Inc., with SealChip of AVIVA Biosciences, Inc. as recording electrodes, and said to be capable of high-quality patch-clamp recording, allowing for detailed studies of ion channel function and with a medium assay throughput. The second is represented by IonWorks HT, commercialized by Molecular Devices, Inc., with PatchPlate of Essen Instruments, Inc., and is said to be capable of high throughput (up to several thousand recordings per day) with limitations in performing certain ion-channel assays.

A key technology challenge in developing automated patch-clamp systems lies in the achievement of a reliable gigaseal between the recording electrode and the cell mem-

brane. Despite the popular use of gigaseal-required patch clamping for ion-channel assays for the last 20 years, the physicochemical nature of the seal is not well understood.^{55,56} Nearly 20 years ago, Corey and Stevens⁵⁵ identified four sources of interaction that should contribute to the glass–membrane seal: ionic bonding, hydrogen bonding, divalent ion-mediated salt bridge between glass and membrane, and van der Waals forces. Development of an automated patch-clamp technology with guaranteed gigaseals for all viable cells is every electrophysiologist's dream. To achieve that, understanding the nature of the gigaseal is necessary, and further investigation into the gigaseal formation is warranted.

Looking into the future, we make several predictions regarding ion-channel assay methods and technologies:

- Modern microfabrication and micromachining techniques will produce sophisticated patch-clamp devices capable of producing high data quality with high throughput capability. More elaborate microfluidics will be incorporated, facilitating cell addition and rapid compound application and washout. Active microstructures (e.g., dielectrophoresis-based) may be used for first separating target cells from a heterogeneous cell population and then transporting them to the recording sites.
- With growing emphasis on high information content, electrophysiology measurements will be performed on cells, together with other cell-based assays. Such cell-based assays will likely include conventional end-point detection methods and other emerging information-rich, real-time monitoring of cells.⁵⁷

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Address reprint requests to:

Xiaobo Wang
 ACEA Biosciences, Inc.
 4108 Sorrento Valley Boulevard
 San Diego, CA 92121

E-mail: xbwang@aceabio.com