

## Planar Array Electrophysiology: Methods and Applications

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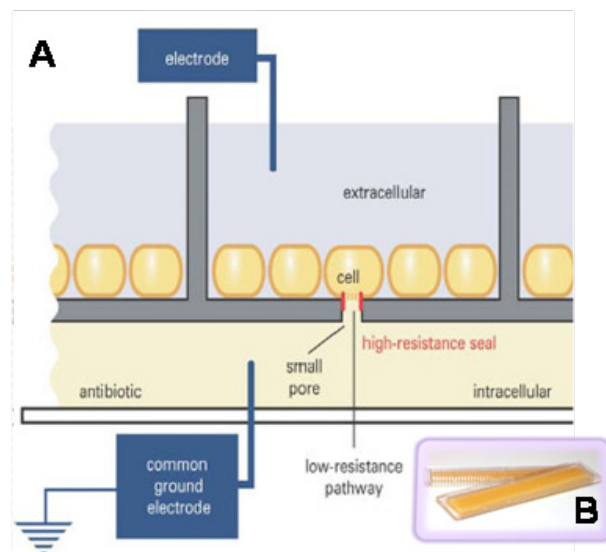
### Discovery Application Note

#### Introduction

Measurements of bioelectric signals from cells and tissues have been critical to understanding physiology and pathology. One particular technique – patch clamp electrophysiology – transformed our awareness of the ionic events that occur at the cell membrane, and provided definitive evidence for the existence of discrete conductance pathways, or ion ‘channels’. The method involves positioning a glass microelectrode on the surface of a single cell and using low noise amplifier circuitry to measure ionic currents with exquisite sensitivity and temporal resolution. Whilst extremely powerful, the conventional patch clamp method is technically demanding and slow and this has hindered the application of this method for drug screening and other types of experiments.

In 2002, Essen Instruments provided a quantal increase in the throughput of patch clamp electrophysiology, with the introduction of the IonWorks platform (Schroeder *et al.*, 2003). This system uses machined apertures (1-2 $\mu$ m) in a planar, kapton substrate as the recording sites and is formatted as a 384-well microtitre plate. This paradigm shifting technology increased the number of possible recordings from 5-10 to in excess of 3000 per day. In a later advance, measurements from populations of cells (up to 64) via multiple apertures were introduced – the ‘population patch clamp’ or PPC method (Finkel *et al.*, 2006). Together, the increased speed and technical ease of electrophysiology has enabled new approaches to ion channel science, notably in the industrial setting.

Most obviously, planar array electrophysiology has been applied to small molecule drug screening with recombinant cell lines. Voltage-gated ion channels, hitherto inaccessible due to the absolute requirements for close voltage-control and millisecond signal resolution, have been extensively exploited. Whilst not true ‘high-throughput’ screening, compound sets up to 50-100K in size can be screened.

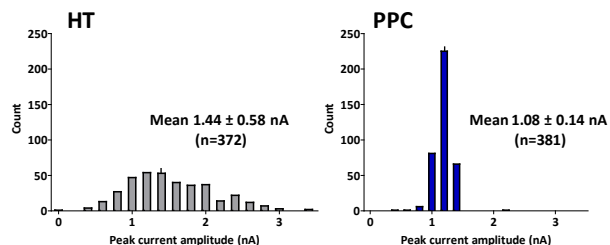


**Figure 1. Schematic diagram of IonWorks planar array electrophysiology:** (A) a section through a single well of the 384-well microtitre-plate consumable (B). In HT mode a single 1-2 $\mu$ m diameter aperture is machined in each well – in PPC mode there are up to 64 apertures per well. Cells are added in suspension via a fluidics head and are positioned over the recording site(s) via suction applied from beneath the plate. Once a high resistance seal has been achieved between cell and substrate, an intracellular solution containing the permeabilising antibiotic amphotericin is circulated to provide ‘perforated patch’ electrical access to the cell interior. Voltage-clamp recordings are made via a pair of Ag/AgCl electrodes. Test compounds can be applied to each well on line via the fluidics head.

This provides access to targeted libraries and some chemical diversity. Repeated gating protocols can be employed to screen for use-dependent blockers, and mechanistic profiles such as open- and closed- channel block can be readily determined. Mechanistic assays looking at shifts in Boltzmann gating parameters are also possible. Such screens require high quality cell lines and the ability to screen more clones afforded by the IonWorks system has improved and accelerated this process. This is most evident for multi-subunit ion channels where the requirements for trialing different transfection protocols and expression constructs are most demanding (Clare *et al.*, 2009). Both safety (e.g. hERG, Na<sub>v</sub>1.5 etc.) and discovery (e.g. Na<sub>v</sub>1.7, Ca<sub>v</sub>1.2) voltage-gated ion channel assays are well described.

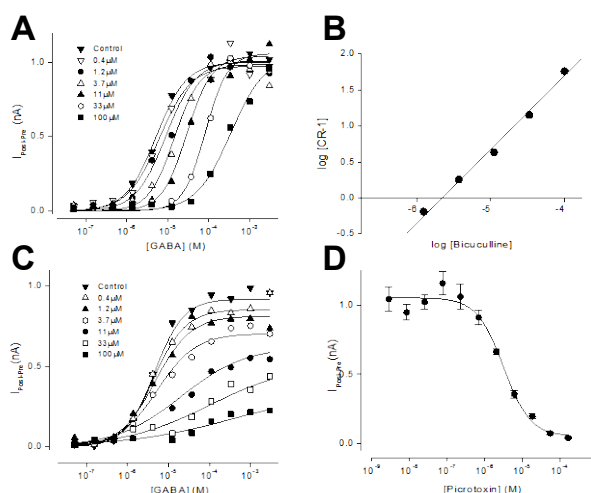
The PPC protocol provides two major advantages over the single-hole measurements. First, the need for well ‘redundancy’ to accommodate for single cells that may not seal or express very well is abrogated – this allows true 384-well screening compared to (only) 96-well in the single hole paradigm, and hence a 4-fold increase in throughput. Secondly, the well to well variability in (pre-

compound) current amplitudes is considerably lower in PPC, allowing direct cross well comparisons. These



**Figure 2.** Distribution of current amplitudes for  $K_v1.5$  expressing CHL cells measured in single hole (HT) and population (PPC) modes. Values shown are mean  $\pm$  SD ( $n$  = cell/well count). Note the much tighter distribution of data in the population patch clamp mode.

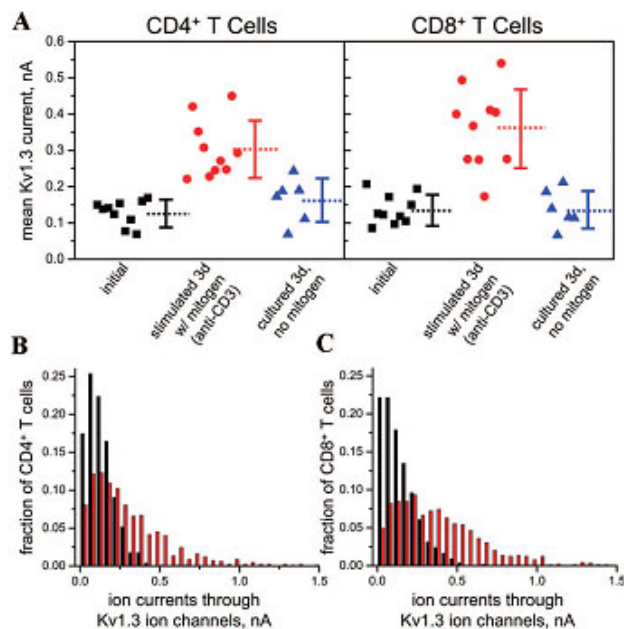
features have not only increased the quality of screens for voltage-gated channel blockers but have also expanded the utility of the IonWorks technology. For example, assays for ion channel openers are now more tractable. In single hole experiments it is hard to differentiate ‘inactive’ compounds from cells that simply do not express much functional channel – this limitation is overcome in PPC (e.g. John *et al.*, 2007). Slow-ligand-gated channels are also more accessible for the same reason, and despite the inability to rapidly apply compounds, robust assays for channels such as  $GABA_A$  and TRPs can be assembled (e.g. Hollands *et al.*, 2009).



**Figure 3.** Example pharmacology of  $GABA_A$   $\alpha1\beta3\gamma2$  channels determined using PPC planar array electrophysiology. Concentration response curves to GABA in the absence (control) and presence of increasing concentrations of bicuculline (A) and picrotoxin (C). (B) Schild analysis for bicuculline showing competitive antagonism. (D) Concentration-response curve for inhibition by picrotoxin. Reproduced from Hollands *et al.*, 2009 with permission.

The greater number of cells that can be sampled with planar array techniques also enables the application of ‘population statistics’ to ion channel questions. For example, the up-regulation of  $K_v1.3$  currents in human T-

lymphocytes following mitogen activation could be measured from whole blood isolates by comparing the expression of different cell populations. Similarly, pharmacological studies that require long incubation times are possible, that would otherwise be prohibitive in classical ‘measure-add drug-remeasure’ electrophysiology protocols. This can be advantageous for studies on biologics such as siRNAs, shRNAs and antibodies where overnight incubations with cells are sometimes required.



**Figure 4.** Functional increase of  $K_v1.3$  ion channel activity in stimulated T cells from 10 independent blood draws. (A)  $K_v1.3$  activity was quantified from freshly drawn blood (labeled “initial”), after 72 h culture in the presence of  $150 \text{ ng mL}^{-1}$  anti-CD3 antibody and after 72 h culture without added antibody (no stimulation). (B) Histograms of  $K_v1.3$  currents from 797  $CD4^+$  T cells before (black bars) and from 823  $CD4^+$  T cells after 72 h culture with anti-CD3 antibody (red bars). (C) Histograms of  $K_v1.3$  currents from 541  $CD8^+$  T cells before (black bars) and from 888  $CD8^+$  T cells after stimulation (red bars). From: Estes *et al.*, 2008.

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