PATCHERS V SCREENERS

divergent opinion on high throughput electro-physiology

The prospect of enabling higher throughput electro-physiology (patch-clamping) has created much expectation from scientists within the ion channel community. Yet there are opposing views among traditional electro-physiologists and screeners as to the needed specification and the preferred mode of operation for such devices. More importantly there appears to be significant disconnect between most technology developers and their target end-users. Throughput restrictions and unrealistic pricing strategies for the consumable component of emerging devices seem destined to limit the deployment of the technology to secondary screening or hit retest segment and the much-hyped impact on primary screening and safety testing remains elusive.

By Dr John Comley

Few would argue that indirect fluorescent-based measurements of ion channel activity enabled on such devices as the FLIPR are ideal from a pharmacological perspective or acceptable in terms of their high rates of false hits[1]. Yet in the absence of higher throughput alternatives these assays, which are relatively cheap to perform, remain the principle method used today for the primary screening of ion channels. Patch clamping is widely recognised as the definitive 'Gold Standard' method for studying ion channels, since this electrophysiological technique enables analysis of ion channel function through direct measurement of ion current flowing through one or more ion channels, yielding information about voltage, rate- and use-dependence of compound binding[2]. The advantages and disadvantages of patch-clamping are summarised in **Table 1**. However, the limited throughput of traditional patch clamping has restricted its use to all but late

stage hits-to-leads, safety testing and basic research. Recent technology developments, particularly the planar patch clamp, have created considerable excitement and expectation that high throughput electrophysiology will finally become a reality and impact on drug discovery by significantly shortening development times[3]. At least two high throughput electro-physiology developers (Molecular Devices and Axon Instruments) now offer full production units for sale and four further systems (Cytocentrics, Flyion, Nanion and Sophion) are in advanced stages of development. See **Table 2** for a comparison of the products available and in development for high throughput electrophysiology technology. Flyion FlyScreen® 8500 relies on 'flipping the tip', ie filling the inside of a glass micropipette with a cell suspension and flushing cells toward the pipette tip, where a single cell forms an extremely stable seal[4]. All of the other systems (apart from Flyion's) are based on a planar patch clamp seal. The planar patch clamp process reverses the traditional operating sequence and moves the cell to the patch pipette (see **Figure 1**). In most cases this is achieved by having a fine pore (typically <10μm diameter) in a flat substrate (eg glass, silicon, quartz or plastic, usually with specific customised surface coatings) and using suction to attract, position and hold the cell over the pore. Many of the devices under development are hybrid structures in which the patch substrate is embedded or injection moulded into other supporting materials that provide the microfluidic interface/network to the patched cell. Some planar chips, like Cytocentrics, have two openings, to separate suction from seal formation[5], but the value of this design has still to be proven. Irrespective of the design it is the ability to micro-fabricate multiple planar patch structures in a substrate and to address those patch sites with multiple independent amplifiers that is of prime importance as this offers the potential for parallel simultaneous recordings and opens up the possibility for fully automated cell and test compound additions. However, herein lies several issues that currently threaten to divide electro-physiologists and ion channel scientists into two polarised groups; traditional 'patchers' and their more results-focused colleagues, the 'screeners'.

Giga Ohm (GΩ) Seals

The classical patch clamp process has its origins in single channel recordings[6,7] and involves locating a cell, breaking the membrane with a drawn out tip of a glass pipette, forming a high-resistance seal at the interface of the membrane and the glass electrode and gaining intracellular access to the cell (going 'whole cell'). **Figure 1** illustrates the difference between a classical, a Flyion 'flip-the-tip' and a planar patch clamp. It is a widely held view that this tight seal must be of a GOhm (GΩ) resistance

Table 1
The pros and cons of traditional patch clamping

ADVANTAGES

- High information content
  - controls (clamps) the voltage across a membrane
  - single cell recordings
  - very fast temporal (μsec to msec) resolution
- High sensitivity
  - pA resolution, ability to detect single molecule and channel activity
- Flexibility
  - ability to manipulate the solution composition on either side of the cell membrane
- Wide applicability
  - assays all channel types (K^+, Na^+, Ca^{2+} etc)
- Less false hits than fluorescence-based or flux assays

DISADVANTAGES

- Requires a trained, skilled biologist
- Low throughput (typically <10 individual cells/day)
- Very labour intensive
- Impractical for primary screening
- Full automation is technically very difficult
- Not suited to parallel processing
- Current practise involves multi-compound additions (with washout) to the same cell

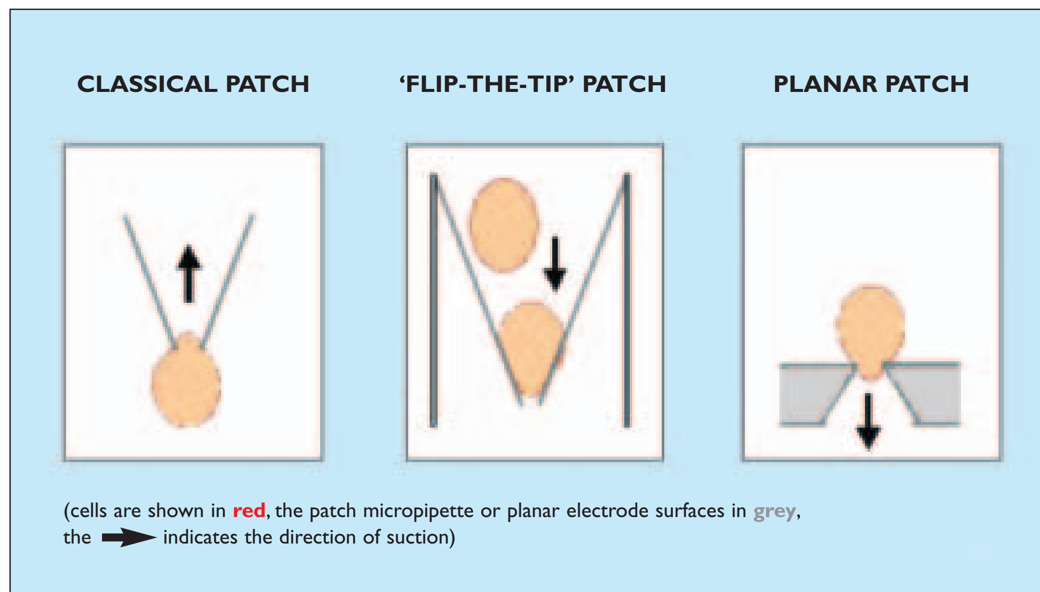


Figure 1
Schematic representation of the patch clamp approaches

if successful patch clamp recordings are to be made. In some planar patch clamping devices (eg Molecular Devices IonWorks™ HT) the cell seals to the substrate with a lower resistance (typically between 100-200 MΩ), which is well below the GΩ seal of conventional patch-clamping. Yet despite this limitation several groups[8,9] have reported the ability to control membrane potential and record adequate ion current directly through different ion channels with ~150 MΩ seals. These individuals (for the most part 'screeners') appear to have adopted a pragmatic approach to the IonWorks™, and are prepared to compromise on the recordings provided the basic pharmacological response is identical to that which can be obtained by traditional patch clamping methods. In contrast, most traditional electro-physiologists ('patchers') remain focused on the quality of the data recordings, have an intrinsic bias towards glass as a substrate and for the most part do not really understand or appreciate the demands of the screening process. Such patchers dislike the way the data is generated (on the IonWorks™ HT), based on a before and after measurement, it is not established patch clamping to them, too many corners have been cut, a lot of the quality is taken out of the reading. They see HTS as mainly being about simple yes/no answers, in the real world of ion channels they argue you need to take into account subtle qualitative differences. The hard sell of convincing such 'patchers' to adopt systems, like the IonWorks™ HT, may never ever be realised until the discovery of new leads can be directly attributed to screening with this system. In the interim, product developers such as Axon, Cytocentrics, Sophion, Nanion and Flyion will try to reap the benefits by stressing the higher quality of their seals relative to the Molecular Devices IonWorks™ HT.

Parallel vs sequential processing

Historically, manual patch clamping has involved making as many recordings from a single cell as possible, a strategy in part adopted to offset the very low success rates achieved. So provided the baseline returns to normal it was not unusual to evaluate different compounds or drug concentrations against the same patched cell. However, the downsides of this are that washouts can be very time-consuming, compounds can be sticky and cells progressively run down after repetitive washouts. Since the duration (time to set up, washout, preread and incubate) of an individual recording is highly variable, there is often little method consistency between these observations. We owe many of today's new technology developments in this area to electro-physiologists turned into instrument developers, but the desire to maintain flexibility and get maximum value out of each patched cell is strongly embedded in their 'patcher' thinking. The effect of this is that some high throughput patch clamp systems in development are highly focused on preserving this flexibility through sequential or parallel asynchronous operation. In direct contrast most screeners, although occasionally experienced with compound pooling, for the most part are interested to derive only one data point (ie one compound addition at one concentration) per cell.

Table 2: Key players in the development of high throughput electrophysiology*

MANUFACTURER/ WEBSITE	PRODUCTS	SUBSTRATE/ SEAL	DESCRIPTION/ THROUGHPUT	AI
Axon Instruments Inc Union City, CA, USA www.axon.com	PatchXpress™ 7000A	planar patch glass chip/ GOhm seal	16 channel SealChip, 16 channel washout head, 1 channel dispenser 2,000 dp/day	16 ch Axon
Cytocentrics CCS GmbH Reutlingen, Germany www.cytocentrics.com	CytoPatch™ Automat	planar patch quartz chip/ GOhm seal	modular instrument, with up to 20 patch clamp sites 2,000 dp/site/day (if fast acting compounds) 40,000 dp/day with 20 sites/unit	up to switc parall npi el Multi Syste
Flyion GmbH Tubingen, Germany www.flyion.com	FlyScreen® 8500	glass micropipette embedded in plastic jacket (FlipTip)/ GOhm seal	single channel FlipTips® dispenser with 2 channels (functions) 3 – 6 FlipTip recordings positions (scalable) 300-1,000 dp/day	up to chann
Molecular Devices Corp Sunnyvale, CA, USA www.moleculardevices.com	IonWorks™ HT	planar patch plastic chip/ Avg. ~150MOhm seal	384 PatchPlate at 1536 spacing (~14µL/well), 12 channel dispenser 3,000 dp/day	48 ch propr devel
Nanion Technologies GmbH Munich, Germany www.nanion.de	NPC©1 (Port-a-Patch), NPC©16s (sequential) NPC©16p (parallel)	planar patch glass chip perfusion cartridge/ GOhm seal	1, or 16 channel chips (scalable) 1 channel dispenser NPC©1 – 50 dp/day NPC©16s – 200 dp/day NPC©16p – 2,000 dp/day	1char HEK/ 16 ch (asyn Daga
Sophion Bioscience A/S Ballerup, Denmark www.sophion.dk	QPatch™ 16 QPatch™ 96	planar patch silicon chip/ GOhm seal	QPatch™ 16 – 16 channel – 250-1,200dp/day QPatch™ 96 – 96 channel - 1,500-7,000 dp/day 4/8 channel dispenser	16 ar parall (asyn propr devel

* in addition Xention Discovery (www.xention.com) and Affymax (www.affymax.com) also have internal high throughput patch clamp developments which currently are not commercially available.
** cost per data point calculated assuming a 50% patching success rate, with an average of 5 data points obtained per successful patch in sequential processing.
*** for the IonWorks™ HT we have assumed that each 384 PatchPlate yields data on 96 compounds, in reality the number of successfully patched cells maybe significantly greater than 96, so price per patch (dp) could theoretically be cheaper.

MPLIFIERS/ SOURCE	COSTS PER CHIP PER DATA POINT**	STATUS/ PARTNERS
annel parallel/ n	range $140-180 <$3.8/dp	available/ Aviva BioSciences
20 channels, hed mode el operation/ lectronic and Channel ms	$8 <$3.2/dp	ß-test in Q3 03/ BionChip, Multi Channel Systems, NMI, npi electronic, no sales/ commercialisation partner
6 independent nels/HEKA	Fliptips® A – $3 Fliptips® L – $4 <$1.6/dp	available in Europe/ HEKA, Tecan, Manz Automation no sales/ commercialisation partner
annel parallel/ rietary opment	range $145-200 per patch plate, <$2.0/dp ***	available/ lower throughput Ionworks™APC target launch Q1 04
nnel sequential/ A annel parallel chronous)/ n	NPC©1 -$10 NPC©16 – $100 <$2.5/dp for NPC©16	NPC© in ß-test, target launch Q4 03/ HEKA, Dagan, Tecan, Bruxton, no sales/ commercialisation partner
d 96 channel el chronous)/ rietary opment	QPatch™ 16 – $100 <$2.5/dp	QPatch™ 16 in ß-test target launch Q3 03/ CRL, no sales/ commercialisation partner

Although most don't see any downsides to making repeated additions of the same compound to one cell in the generation of a dose response curve (IC50), none would be willing to accept different compounds additions to the same cell. Screeners generally want to acquire data as fast as possible, to make parallel additions and simultaneous parallel recordings, and to enable the direct 1:1 mapping of the compound addition from the compound library plate to the planar patch device. They want to avoid handling compound plates containing unscreened compounds. The logistics of planning and scheduling how long any experiment will take, the need to produce enough cells at the same stage of development under identical conditions of batch processing and the desire to test all drugs in the same manner mitigate against a more variable sequential approach. In addition, many screeners simply do not believe that a parallel asynchronous mode of operation, where patched cells are processed differentially, at different states of recording, after different number of compound additions, after losing current at very different rates, with compound specific preincubation and washout times etc can be adequately scheduled for really high throughput operation. Many believe that the good stability of the IonWorks™ HT signal is partly the result of not trying to attempt multiple recordings and washouts, ie having taken the variable decision-making steps out of the process.

Cost per data point

What end-users are willing to pay per data point for high throughput electrophysiology is directly related to where the technology will be deployed. Most do not need to be convinced about its value in enabling access to ion channel assays and theoretically it could be implemented widely across drug discovery. In addition, few believe that the technology will result in major savings in FTEs, as skilled electro-physiologists will still be required to develop the assays and interpret the results, but they do expect the availability of the technology to yield significant improvements in productivity. Instrument developers have, however, been slow to realise that consumable budgets are finite and it is a myth that Pharma will pay any cost to access these assays. In primary screening most big Pharma have a reagent cap per screen of around $100,000 to $200,000, and expect to get 1 million data points for this price. Although there is always a degree of flexibility to accommodate a unique or particularly difficult target using more expensive reagents, it is doubtful whether any would pay >$1/data point for a full diversity HTS. This would

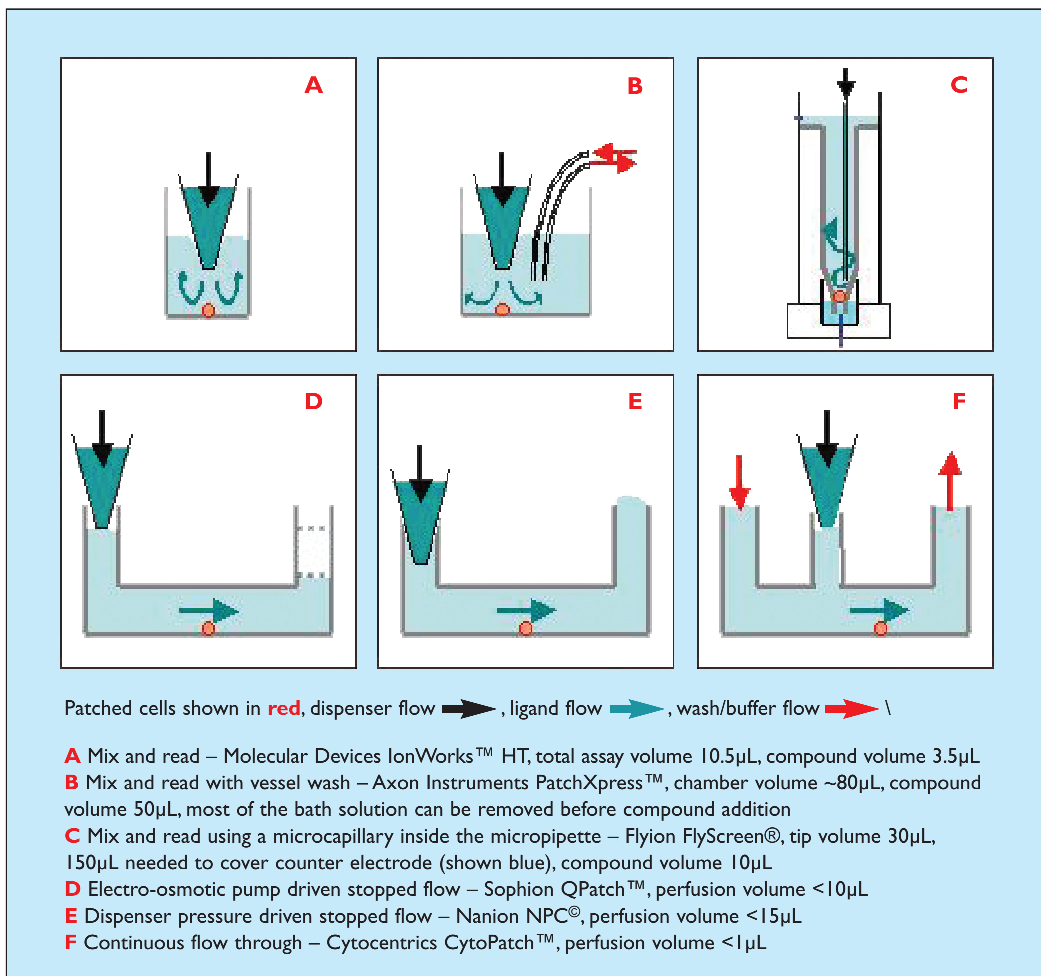


Figure 2
Schematic of ligand perfusion enabled in the patch clamp devices

only be considered with a focused (targeted) library of much fewer compounds. On average screeners are paying somewhere up to 20¢/well (per data point) for reagents today, but this is the upper limit. Even if the throughput were adequate for patch clamping, there is NO way it will ever be implemented in routine primary screening with the current projected cost for these disposable patch device (ranging from approximately $1.6-$3.8/data point), particularly as screeners do not envisage lowering their costs per data point by making multiple compound additions. In secondary screening and safety testing these consumable costs will probably be accepted as a starting point for a new technology, but most expect and will demand that this price goes down once there is more competition. Further expansion into the secondary screening market will also be ultimately limited at this cost. Most of alternative offerings to the IonWorks™ HT are focused on trying to get multiple data points per patched cell, to minimise the even higher projected costs of their consumable patch devices. This flexible approach may work OK in low throughput secondary screening, where the numbers screened as dose response curves are low enough to be afforded, but the consumable costs and the variable processing is unlikely to ever be accepted into primary screening (HTS).

Patching success

Unlike other screening methodologies, high throughput electrophysiology is based on single cell recordings and a significant proportion of cells screened will not yield data. Many factors contribute to the lack of ability to establish a stable physiological recording in mammalian cells (typically between 40-80%). These include: the controlled and stable positioning of cells over the pore in the patch device; lack of adequate suction control in some systems; unacceptable seal resistance, inability to achieve electrical access and non-expression of a channel in the given cell[7]. This problem led Molecular Devices to design in a redundancy factor into its IonWorks™ HT platform, to guarantee get-

ting at least one data point per patched cell the system makes four replicates patches with each compound. Other manufacturers do not have redundancy strategies. Although they face similar patching success rates and the effect this creates will become more evident if they make multiple recordings per patched cell. In a parallel recording 16-channel device such as Axon's PatchXpress™ or Sophion's QPatch™16 not all wells will have functionally active patched cells, and only a proportion of these will give repetitive stable recordings. Systems based on a scalable format (eg Cytocentrics, Flyion and Nanion) could process multiple single patch sites simultaneously offering the potential to discard cells (patch sites) that do not give an adequate response within a defined timeframe, so that new patches can be rapidly initiated. However, these systems have a degree of operational complexity which to date has been largely unproven in anything more than very low throughput mode.

Throughput

The daily throughput desired of a high throughput electrophysiology instrument is related to the area of application. Many Pharma currently run ion channels primary screens of between 500,000-1,000,000 compounds/campaign and plan to continue doing so over the next few years. HTS really only begins at 10,000 data points/day (100x96 well plates), to impact on this segment today an instrument ideally needs to be able to compete with the FLIPR3, ie to screen around 100x384 plates/day (40,000 data points). All these data points are single drug concentration per cell. Typical big Pharma secondary screening operations are looking to replace oocyte screening systems based on two electrode voltage clamp (TEVC) and desire a throughput of at least 1,000 data points/day, and the majority of these are IC50s (dose response curves), based on multiple drug additions. Ascending drug concentrations added to the same cell would be acceptable here. Many Pharma are thinking of implementing non-GLP compliant safety testing (hERG and other ion channel liabilities) at a much earlier stage in the process. What is clear is only one of the proposed systems discussed in **Table 2** (Cytocentrics), claims to be able to deliver a throughput close to expectations of some HTS today and that capability has not yet been demonstrated. Even if this throughput were achieved, pricing at approximately $3.2/data point would make its operation in HTS prohibitive. The maximum estimated throughputs of the other developments are around 3,000 data points/day (12,000 patched cells/day allowing for the four replicate redundancy built into the IonWorks™ HT). We have attempted below to calculate what type of device might be required to realistically achieve primary screening throughputs. Almost certainly this will necessitate the abandoning sequential and asynchronous approaches in favour of a highly miniaturised and parallelised fixed-process planar device, so as to minimise the time wasted in compound washouts, etc. Assuming planar patching success rates remain low (<50%) and anywhere up to 4x the number of actual patched wells are required per data points the following scenarios might be possible:

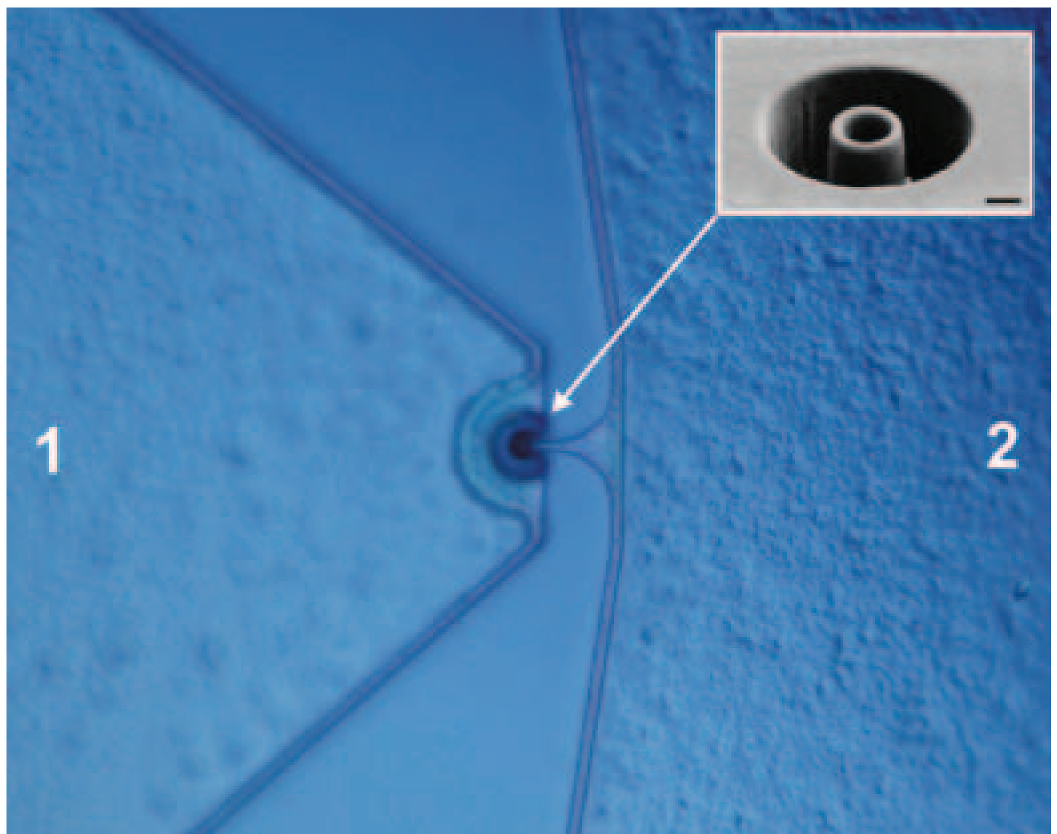
Throughput Needed/8h. Day	100x96	100x96	100x96	100x384
Required Data Points	10,000	10,000	10,000	40,000
Actual Patched Wells	40,000	40,000	40,000	160,000
Amplifier Channels	96	384	1536	1536
Dispenser Channels	24	96	384	384
No. Patch Cycles/8h. Day	416	104	26	104
Maximum Patch Cycle Time	1.1min	4.6min	18.5min	4.6min

Based on these assumptions it is clear that for high throughput electrophysiology to significantly impact on HTS a 1536 patch-plate would be needed, with a 384 dispense head and a highly parallel amplifier. The exact number of amplifier channels needed is debatable particularly if the readout can be multiplexed. If Pharma were willing to pay up to 20¢/data point (ie 5¢/patched well plus the additional cost of the four 384 plates they would ordinarily use for a FLIPR or alternative assay) this would mean that a 1536 device might command a customer price of around $100. Clearly to make such a patch plate with sufficient margin the device needs to be fabricated in a technology that can be mass produced very cheaply. It is possible to imagine that a 1536 IonWorks™ HT plastic patch-plate could be relatively easily manufactured as the current design is based on a quadrant of a 1536 array, although proportionally it would need to be priced at approximately 10-15% of the current cost, which seems highly unlikely, if the current margins are industry standard. Possibly a 1536 well glass planar substrate integrated into a standard 1536 top plate might be manufactured more cheaply, although the application of such a device may be limited to voltage-gated channels.

Axon Instruments PatchXpress™7000A. View of dispensing into the 16-channel SealChip on the bed of the PatchXpress™7000A (right)

Voltage vs ligand-gated ion channels

There exists a requirement for high throughput electrophysiology devices to be able to assay both voltage and ligand-gated ion channels. Overall, voltage-gated channels are more important to most Pharma and represent the majority of channels under investigation today, although there is wide variation between companies. Some companies believe that they can fill this gap adequately by using FLIPR assay strategies (eg with Ca^{2+} per-



Top view on the inner area of the Cytocentrics CytoPatch™ Chip, with the cytocentring channel (1) and the CytoPatch™ channel (2). The SEM image insert (scale bar 1µm) shows the centre of the CytoPatch™ Chip with its two concentric openings in a quartz substrate

meable dyes and some of the newer Cl^- sensitive dyes), others predict the need to acquire additional high throughput electrophysiology systems just to screen these ligand-gated channels and might even be prepared to pay more for this capability, but would only need about 1/10th of the data points they currently require for voltage-gated channels. Typically, desensitising ligand channels requires fast solution exchange in the 20 milli-sec timeframe. To address the fastest channels a device ideally needs to create a laminar front of ligand to hit the cell and rapidly move across it, avoiding turbulence, blurring of the ligand front and mechanical disturbance of the patched cell, otherwise the recording can be distorted. **Figure 2** is a schematic depicting the ligand flow capabilities of the systems discussed in **Table 2**. On the IonWorks™ HT the liquid handling head must be withdrawn before the electronics head can be inserted to record, this means it can currently only assay the slowest of ligand-gated channels. The Axon system can record simultaneous with ligand addition, but simply increasing the dispense speed from a standard pipette tip positioned over a patched cell may not give fast compound exchange. In this respect those approaches that use laminar flow (Sophion, Nanion and and Cytocentrics) over the patched cell could be advantageous in enabling thorough wash out relative to Axon's open well system or the highly confined space within Flyion's micropipettes. Sophion's system uses an electro-osmotic pump with a soft capillary stop, the benefit of this system is that no pressure needs to be applied at the QPatch interface. In contrast, Nanion's flow cell, relies on the pipette tip forming a tight seal at the device interface with direct pressure from the dispenser syringe driving ligand flow over the cell. Only Cytocentrics' approach allows for continuous cell perfusion and minimal dead volume by using separate channels for the ligand and buffer/wash additions, potentially enabling fast wash in and wash out; a prerequisite to speeding up assays based on sequential operation. However, the extent to which any of these systems can achieve very rapid and robust solution exchange is largely unproven and the limitations on assaying ligand-gated channels will only become apparent when customers start investigating their own targets.

Prospects for the future

Current consumable pricing strategies and throughput limitations look set to impact on the introduction of high throughput electrophysiology and the potential for these 'first generation' instruments to significantly shorten drug development times appears to be rather limited, as they are unlikely to be used in primary screening (HTS). We can expect their use to be restricted predominantly to secondary screening with some application in the direct high quality retest/confirmation of actives from HTS or possibly in the primary screening of smaller focused libraries, provided the number of data points is kept low. Other areas of immediate application will include counter screening/profiling immediately after primary screening, ie to get screening done against hERG, plus an increasing array of other ion channel liabilities, a lot earlier than is currently possible. We should not assume their immediate implementation into safety testing as this environment is GLP constrained and by necessity the data generated must be widely accepted before change is considered. Arguably there is also a significant market for a single channel device giving very high quality data to replace conventional rigs used in basic research and academic labs, if

Flyion FlyScreen® 8500 workstation

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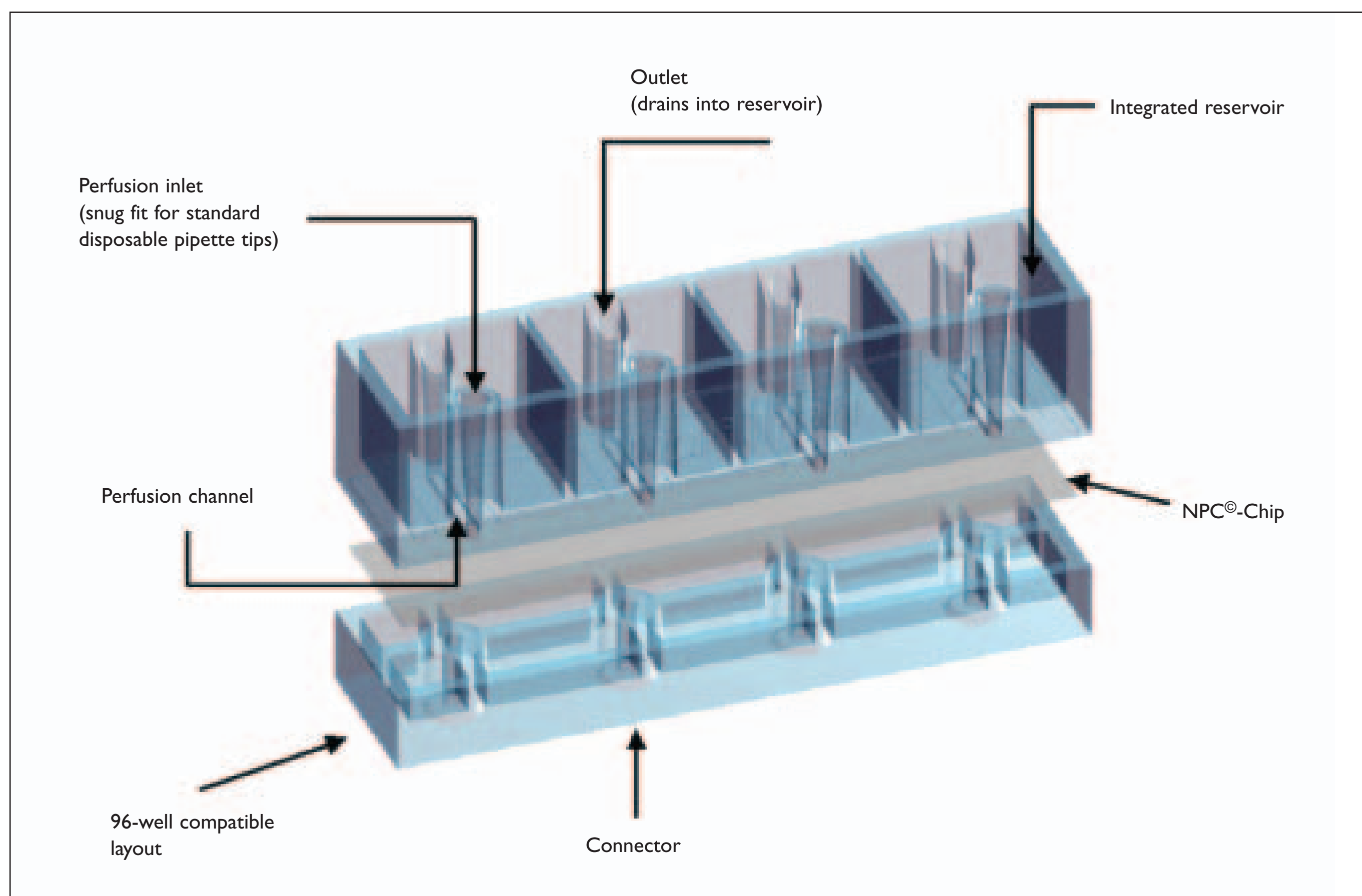
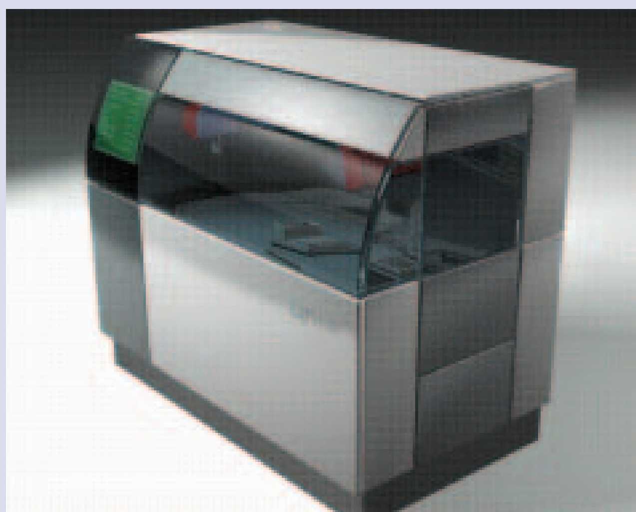
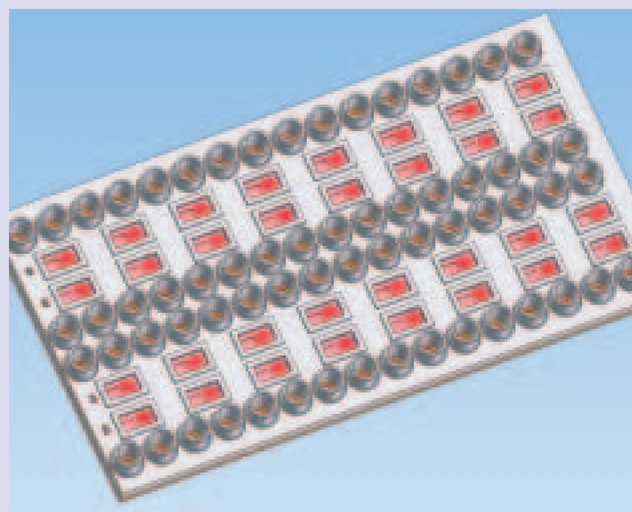
Molecular Devices IonWorks™ HT.

Schematic of Nanion Technologies NPC© 4 channel device







Sophion Bioscience QPatch™ workstation. Schematic representation of Sophion's QPatch™ 16 chip, with 32 separate patch sites (right)

it is competitively priced similar to these systems (around $75,000). The basic Flyion FlyScreen®, a single site Cytocentrics Cytopatch™, Nanion's NPC©1 (Port-a-Patch) or the lower throughput IonWorks™ APC (expected to be launched in early 2004) will all serve this market segment. These customers could probably afford Flyion's FlipTips® at $3-4 each, as most likely they will only use 5-10 tips/day. High throughput electrophysiology not only has the potential to generate extra capacity in secondary screening but its wider availability will promote new thinking and testing in other directions, particularly as most of the new systems are true 'turnkey workstations', offering the inexperienced access to patch clamping. Overall, in addition to realistic consumable pricing, manufacturers need to realise customers are no longer impressed by 'patch-on-a-chip', they need reliable hardware with multi-well parallel readout, with parallel amplifiers and truly robust software to analyse the results and schedule the assays, so as to minimise cycle time between measurements. The ability to generate a high resistance seal is seen as an important factor in convincing the wider electro-physiology community to adopt the technology and looks set to become one of the main differentiators as new product offerings become more widely available. Finally we should not overlook the importance of optimised cell lines for the expression of ion channel targets and the real winners in this technology race are likely to be those developers that can support and enhance their products through the provision of related cell biology services.

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