New options for CELL-BASED ASSAY AUTOMATION

The findings of a recent survey on cell-based assay automation revealed that no single factor stands out as the predominant reason productivity is limited in current cell-based automation. However, improving the quality of assay results and enhancing throughput were seen as the most important objectives of those groups applying cell-based assay automation today. Interest in acquiring dedicated systems offering full automation of the entire process from cell culture to the assay data on a single system has declined. The market for new automated systems has expanded beyond the high throughput primary screening segment and is now focused towards more compact scalable automated low/medium throughput systems that can be flexibly adapted to meet changing needs. Some of the latest vendor offerings and new technology developments that meet these criteria are reviewed in this article.

By Dr John Comley

In Spring 2005 HTStec undertook a survey of Pharma and Biotech labs on current practices, requirements and future trends in the automation of cell growth and assays. The findings were subsequently published in HTStec's Cell-Based Assay Automation Trends 2005 Report. Of the various factors that could limit the productivity of current cell-based automation, the survey found that scheduling the effects of transfection and cell manipulation with respect to throughput and staffing levels were ranked as the most limiting. These factors were closely followed by a large number of other factors, all considered equally important in limiting productivity by respondents. These included the automation of the actual cell-based assay protocols; the maintenance and sub-cloning of cells and the time required to screen cell-based assays etc (**Figure 1**). Clearly, based on this result no single factor stands out as the predominant reason productivity is limited in current cell-based automation and enhancing productivity may require the implementation of multiple fixes in parallel.

Quality and throughput drive automation

It was interesting to understand what motivates groups to invest in automation. Although there are many reasons why the automation of cell-based assay processes would be desirable, improving the quality of assay results and enhancing throughput clearly stood out as the most important objectives of survey respondents (**Figure 2**).

Less interest in full automation

Respondents were asked about the degree of automation they required for their cell-based assay processes (**Figure 3**). From these questions it was

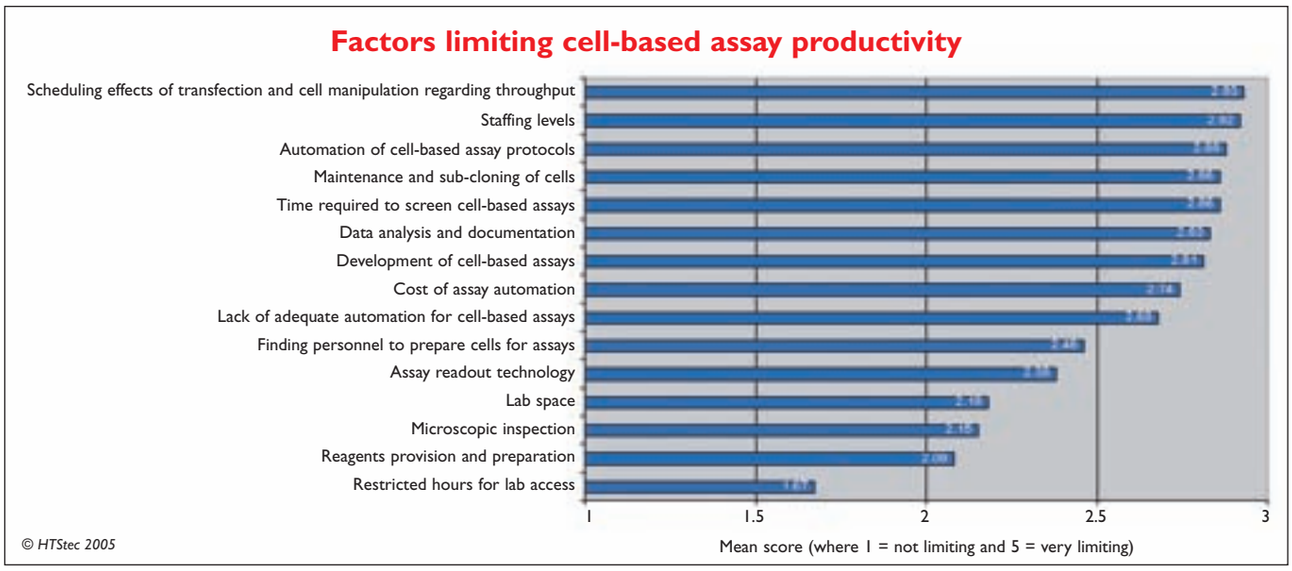


Figure 1

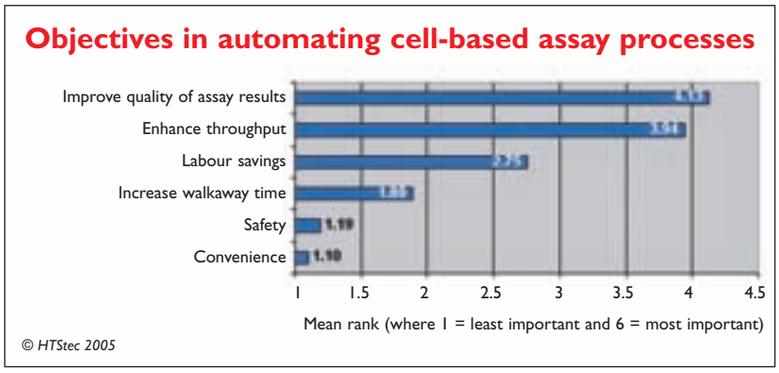


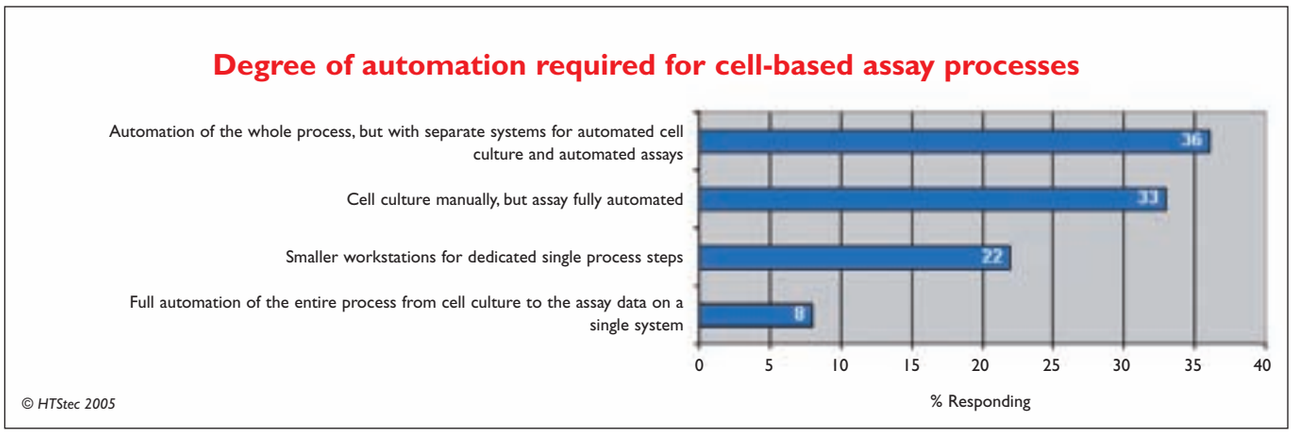
Figure 2

clear that only a small minority (8%) of persons surveyed actually desired full automation of the entire process from cell culture to the assay data on a single system. The majority of respondents wanted some degree of automation ranging from automation of the whole process but with separate systems for automated cell culture and automated assays (36%); through to cell culture manually, but assay fully automated (33%); to smaller workstations for dedicated single process steps (22%).

Dedicated systems lose favour

There was little doubt about respondents' lack of interest in dedicated automated systems (eg suitable for one type or a few similar types of cell-based assays or for growing up larger numbers of cells of a smaller number of cell lines) and their strong preference for flexible automated systems (eg suitable for a diverse range of cell-based assays or for maintaining a diverse number of cell lines but a smaller number of cells per line). This trend was evident for both their cell culture and cell

Figure 3



assay needs, but more marked in the case of the latter (**Figure 4**).

Flexibility is essential

For automated cell-based assay systems high flexibility (eg complex automated solution that requires dedicated highly trained personnel to operate) closely followed by moderate flexibility (eg automated solution requiring moderate training to operate) were the preferred automation strategies. For automated cell culture systems moderate flexibility (eg automated solution requiring moderate training to operate) was the clear preference (**Figure 5**).

Instrument budgets increase especially for small and medium-sized automation

The mean cell automation budget per lab was estimated to be $0.90 million in 2005, this is expected to grow by 15% in 2006. The biggest part (37%) of this cell-based assay automation budget was spent on instruments. The largest proportion (42%) of the cell-based assay automation instrumentation budget was spent on stand alone instruments (mainly detection devices and platforms which may include integrated stacking). For the remainder of the assay automation instrumentation budget small workstations combined with medium-sized automation systems proportionally represent about double the expenditure on large fully automated robotic systems (**Figure 6**).

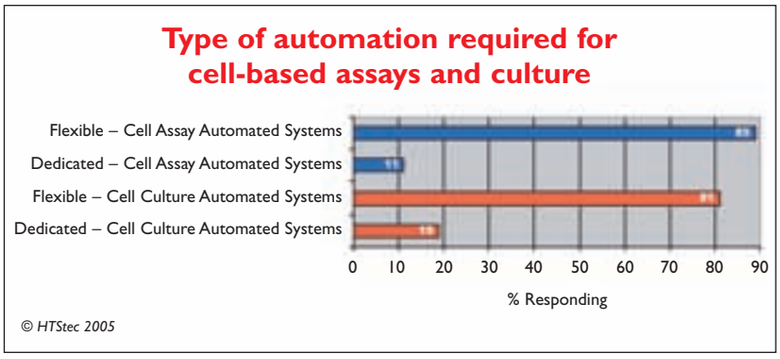


Figure 4

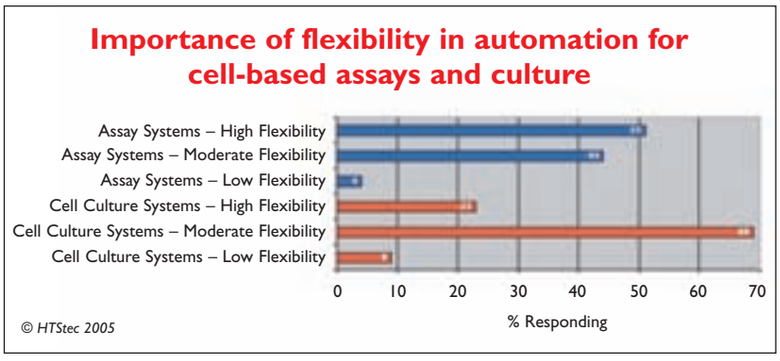


Figure 5

Primary screening still leads in applying automation

It comes as no surprise to learn that primary screening (HTS) and secondary screening were the areas of cell-based drug discovery that have

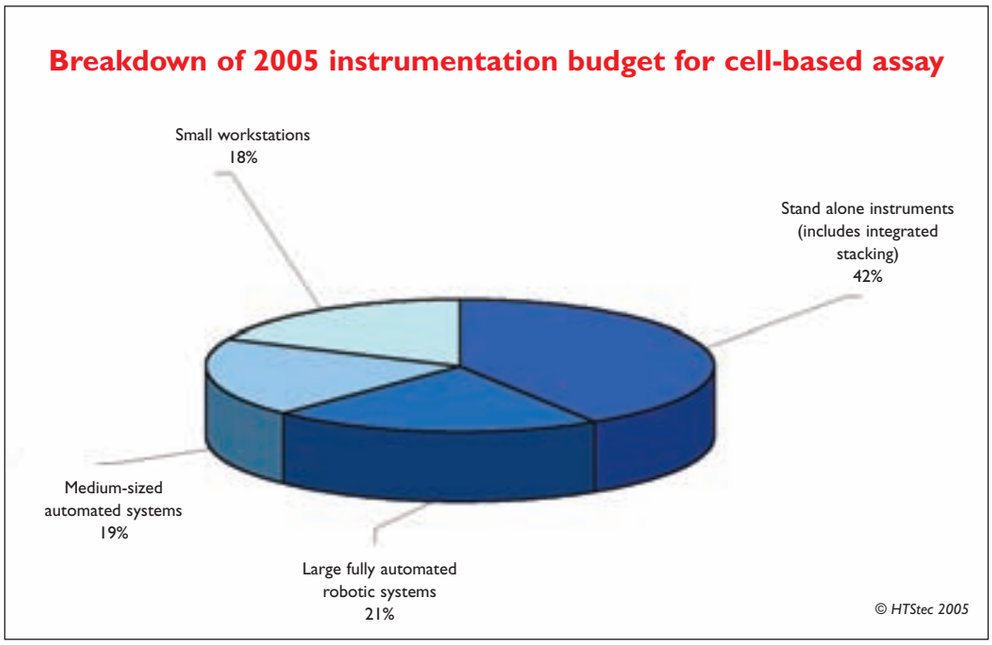
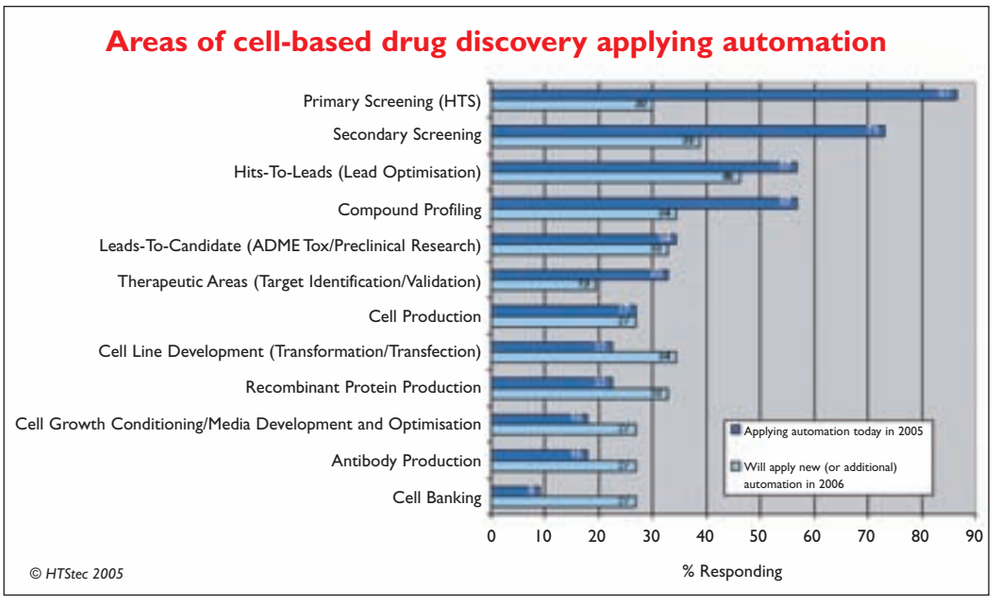


Figure 6

Figure 7



applied automation to the greatest extent today (2005). But new installs of cell-based assay automation in HTS may have peaked as hits-to-leads (lead optimisation) followed by secondary screening were identified as the areas where most new (or additional) automation will be applied in the future (2006). Several areas of drug discovery are predicting greater application of automation in 2006 than in 2005, these include cell line development (transformation/transfection); recombinant protein production; cell growth conditioning/media development and optimisation; antibody production; and cell banking. Of these cell banking was the area where the biggest increase over current automation is expected in the future (2006) (**Figure 7**).

Data point metrics

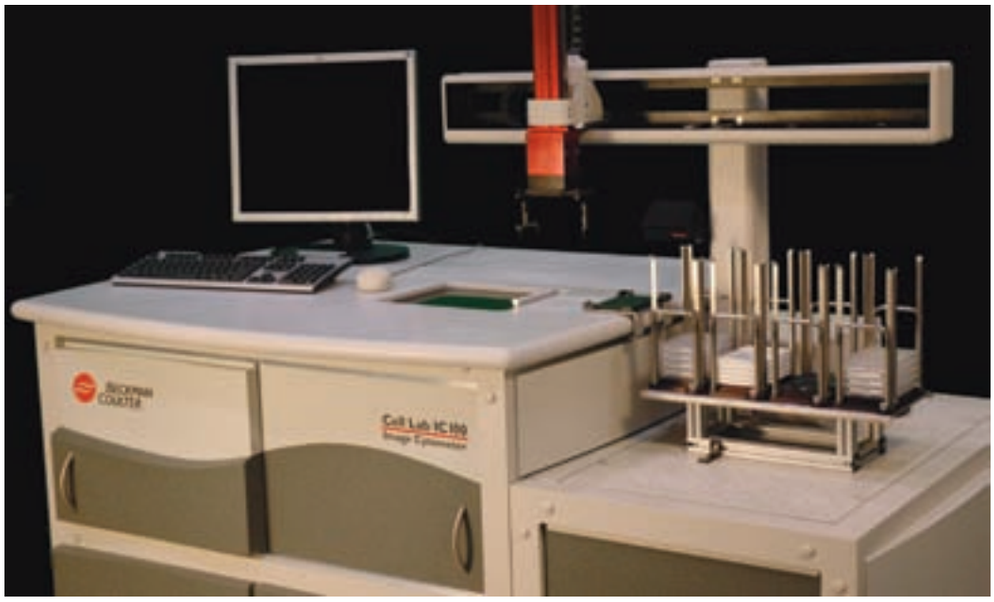
The 2005 data point metrics for automated cell-based assays are summarised in **Table 2**. On average 8,700 cells were used per screening data point in 2005, and this value was expected to decrease by 23% in 2006. It is estimated that 74 billion cells per lab will be required to support cell-based screening in 2005. This translates to 8.55 million cell-based assay wells to be screened per lab in 2005 (**Table 1**).

Latest vendors offerings in cell-based assay automation

Taken as a whole, the findings reported above, particularly those on the degree of automation and flexibility, are consistent with a move away from very large (room-sized) fully-automated robotic

Number of data points per screen (millions)	0.499
Number of targets screened per lab per year	24.2
% of all screens that are cell-based	70.8
Number of cell-based targets screened per year	17.1
Number of cells needed per data point (thousands)	8.7
Total number of cells required to support screening per lab in 2005 (billions)	**74.65**
Total number of cell-based wells to be plated and screened per lab in 2005 (millions)	**8.55**

Table 2: 2005 data point metrics for automated cell-based assays



Beckman Coulter Cell Lab IC 100 Image Cytometer with the BRT Robotic Transport

systems or dedicated high throughput solutions that historically have been mainstay of this market over the past five years. The current trend is towards more compact scalable lower throughput automated systems ranging from stand alone instruments through small workstations to medium-sized automated systems that can be flexibly adapted to meet changing needs. It is these systems that are summarised in **Table 2** and form the majority of the discussion for the remaining part of this review devoted to the latest vendor offerings and new technology developments.

Beckman Coulter (www.beckman.com) offers a range of solutions and automation levels to meet the needs of cellular analysis researchers. It offers a full line of cellular analysis tools based on flow and image cytometry as well as the advanced automated liquid handling and robotics tools of its Biomek® line. The Cell Lab IC 100 Image Cytometer is a flexible, fully automated tool for quantitative cellular analysis, delivering fast and precise high-throughput imaging and analysis of cell populations. The IC 100 can be integrated with Beckman's BRT Robotic Transport to provide an even higher degree of walkaway automation with the ability to transport from four microplate stacks. Further automation with the Biomek FX Automated Liquid Handling Workstation is also possible. The Beckman Coulter FC 500 MPL flow cytometer, with streamlined setup and analysis capabilities, automates tube and microplate-based assays for increased throughput, all in a secure and validated environment. Its multi-platform loader enables cellular and bead-based analysis of samples in tubes or a variety of 24- and 96-well plates. Ideal for biotech and pharmaceutical research applications, the 21 CFR Part 11 module offers password-enforced login and inactivity timeouts, complete audit trails, data archival/retrieval and electronic signatures. Beckman Coulter also offers an extensive line of more than 3,200 cellular analysis research reagents, including human and non-human antibodies, cell signalling reagents, multiplex bead assays and, for the first time, a unique menu of MHC tetramers for T-Cell research. These assays can be automated on any flow cytometer with a 488nm laser, including the Beckman Coulter FC 500 and new Cell Lab Quanta™ benchtop instrument.

Recently, **Caliper Life Science** (www.caliperls.com) has extended the capability of its LabChip 3000 microfluidic platform through the introduction of a cell-stirring capability. This capability expands the type of cell lines compatible with the platform to include adherent cell types such as CHO and HEK293 as well as suspension cells. The microfluidic assay provides a ratiometric measurement of the response of individual cells to agonists and antagonists through coupling of the response to calcium signalling via fluorescent dye detection upon laser excitation of the cells in the microfluidic channels. Small amounts of cells are dye-loaded and placed on the chip into four specially designed

Table 1: Comparison of vendor offerings for cell-based assay and culture automation

Company name	Beckman Coulter	Caliper Life Sciences	CyBio	Evotec Technologies	Genetix	Guava Technologies	Hamilton Li Science Robo
Product name(s)	Cell Lab IC 100 Image Cytometer with BRT Robotic Transport, FC 500 MPL flow cytometer	LabChip 3000	CyBi®-Cellight; Cell Seeding Workstation	EVOscreen®; plate::explorer™	ClonePixFL; CloneSelect	Guava EasyCyte	Cell[host]
Automation focus Cell culture and maintenance Cell assays Both Other	✓	✓	✓ CyBi-Cellight ✓ cell seeding and culture in microplates (Cell Seeding WS)	✓ automated cell screening	✓ CloneSelect ✓ find best clones (ClonePixFL)	✓ mainly cell assays	✓
Main application focus	Basic research, drug discovery – siRNA, protein expression, nuclear translocation, toxicology	Cell-based screening assays including GPCR and receptor signalling assays. Uses advanced microfluidic chip technology to generate the highest cell-based assay data. The platform is capable of detecting specific fluorescent signals from individual cells	CyBi®-Cellight for GPCR, calcium channel and reporter gene assays; full assay automation including preparation of assay plates and measurement; also capable of compound reformatting and cell dispensing; Cell Seeding WS for dispensing of cells into microplates up to 1536 format, including incubator for further culture of the cells in the plates	Screening of cellular assays	ClonePixFL screens, images and picks the highest value clones; CloneSelect for automated high speed confluence calling, cell line maintenance and expansion	Provides absolute cell counts in all assays without using beads	Fully automated system for the culture of prima cells, cell lines an embryonic stem
Degree of automation enabled Large fully automated robotic systems Medium-sized automated systems Smaller workstations for dedicated single process steps Stand alone instrument + integrated stacking Other – please specify	(supports all levels) ✓ ✓ ✓ ✓	✓	✓ CyBi-Cellight ✓ Cell Seeding WS	✓	✓	✓	✓
Flexible vs dedicated automation Flexible automated system – eg suitable for a diverse range of cell-based assays OR for maintaining a diverse number of cell lines but a smaller number of cells per line Dedicated automated system – eg suitable for one type or a few similar types of cell-based assays OR for growing up larger numbers of cells of a smaller number of cell lines	(supports all levels) ✓ ✓	✓	✓ CyBi-Cellight ✓ Cell Seeding WS	✓	✓	✓	✓
Degree of flexibility enabled High flexibility – complex automated solution that requires dedicated highly trained personnel to operate Moderate flexibility – automated solution requiring moderate training to operate Low flexibility – simple automated solution, with dedicated functionality limited by the manufacturer that requires very limited training to operate	(supports all levels) ✓ ✓ ✓	✓	✓ CyBi-Cellight ✓ Cell Seeding WS	✓	✓	✓	✓
Scheduling software (included)	SAMI Scheduling Software	LabChip vers 5.2 software	CyBio Control with parallel execution or CyBio Scheduler	EVOscreen – Bernstein Real Dynamic Scheduling; plate::explorer – plate::works	ExCellerate	None	Cell[task]

lton Life e Robotics	Hamamatsu	Molecular Devices	Panasonic Factory Solutions	PerkinElmer LAS	RTS Life Science	The Automation Partnership	Tecan	Velocity11
	FDSS6000	FLIPR^{TETRA}™ IonWorks Quattro™ ImageXpress family	HTS-10HD; HTS-100	JANUS™ Automated Workstation; LumiLux™ Cellular Screening Platform	Assay Platform	CompacT SelecT	Cellerity	BioCel 1200; Biocell 1600
	✓	✓	✓ HTS-100 ✓ HTS-10HD	✓	✓	✓ plus delivery into plate ready for assay	✓	✓
mated r the primary lines and c stem cells	Image-based plate reader for cell based assay, mainly focus on fast kinetic readout, such as calcium immobilisation, ion channel FRET assay, Aequorin assay etc. Also good for suspension cell lines	FLIPR® is a widely used tool for GPCR and ion channel screening. IonWorks measures direct ion channel current. The ImageXpress family for automated high content imaging provides both fluorescent and bright field microscopy options to cell biology, signalling and physiology research	HTS-10HD is an all-in-one high throughput screening platform tailored towards drug discovery; HTS-100 is a high throughput liquid handling system which contains three parallel belt-conveyor stacker to stacker lines to enable a true high throughput automated liquid handling processing	JANUS provides complete walk-away assay automation from sample prep to results, including vacuum filtration, nucleic acid clean-up, solid phase extraction and ADME. LumiLux™ Cellular Screening Platform enables uHTS calcium assays by combining luminescence technology and suspension cells in 1536-well format	Automation of simple and complex screening protocols in HTS, assay development, secondary screening and eADMETox	Simultaneous culture of multiple cell lines in T175 flasks for cell line maintenance and the production of assay ready 96/384 well plates for cell based assays on demand.Proven aseptic processing, no cross contamination and full audit trail	Automated maintenance and production of cells and proteins	Variety of high to medium throughput assays, including cell based assays, enzymatic assays, ADME/Tox assays, molecular biology applications and compound management applications
	✓	✓	✓	✓	✓	✓	✓	✓
	✓	✓	✓ HTS-10HD ✓ HTS-100	✓ JANUS ✓ LumiLux	✓	✓	✓	✓
	✓	✓	✓ HTS-10HD ✓ HTS-100	✓ JANUS – high flexibility but easy to operate ✓ LumiLux	✓	✓	✓	✓
	None	None	HTS-10HD only – Biopro	WinPREP® applications software	SPRINT	SelecT Operating Software	EVOware and CellGEM	VWorks

The Caliper Life Sciences LabChip 3000 platform uses advanced sipper chip technology and precision chip cassettes with cell stirring and heat capability to enable the effective use of both suspension and adherent cells

Caliper Life Sciences' LabChip 3000 HTS Workstation

wells, containing the stirring mechanism. Typically one to five million cells is sufficient for an entire screening run of 4-8 hours. Using a precise vacuum pressure, cells are drawn from the cell wells into the microfluidic channel towards the detection window. Compounds are introduced from 96 or 384 well plates on to the LabChip through four capillary 'sippers'. For antagonists screening, the agonist addition occurs as the cells flow through the microfluidic channel from additional agonists wells on the chip. For agonist screening or deorphaning assays, the putative agonists are introduced via the sippers and combine with the cells upstream of the detection window. The greatly reduced cell consumption enables the use of difficult to obtain cell types such as primary cells and reduces the need for large-scale cell culture automation facilities. Similarly, the reduction in agonist consumption also enables screening where the agonist is in short supply or prohibitively expensive. Since each cell is analysed individually, mixed cell populations and transient transfectants, in which only a fraction of the cells receive or express the receptor can be easily studied.

Below left: CyBio CyBi®-Cellight System
Below right: CyBio Cell Seeding Workstation for uniform dispensing of cells into microplates up to 1536-well format

Unintentional loss of receptor expression can also be quickly identified. The microfluidic cell-based assays have throughputs of approximately 4,000 datapoints in an eight-hour run.

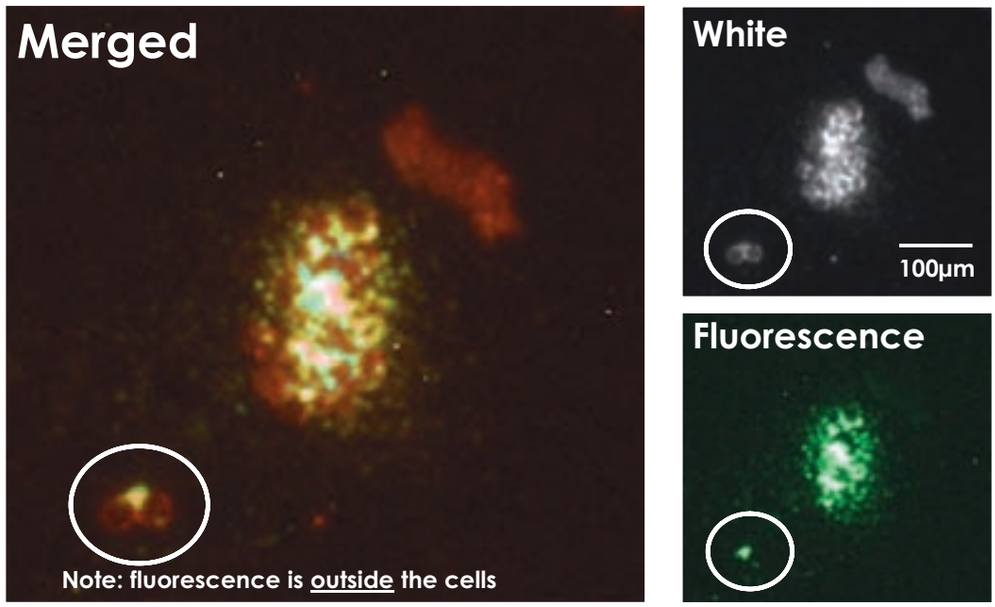
CyBio (www.cybio-ag.com) has recently launched a flexible system called CyBi®-Cellight for the complete automation of all kinds of cell-based luminescence assays such as GPCR, calcium channel, reporter gene and cell viability/cytotoxicity assays. This system is scalable for various needs from assay development through small focused screening sets to high throughput screening. With excellent reliability, full 1536-well and high throughput capability of all components, the CyBi®-Cellight is the ideal solution for miniaturised, efficient and fast automation of cell-based assays. A key component contributing to the superior data quality and productivity is the new flash luminescence reader CyBi®-Lumax flash HT. Due to its superior sensitivity and cell dispensing capability, flash luminescence assays can be performed in 1536-well plates and with less than 1,000 cells per well. The CyBi®-Lumax flash HT has two integrated dispensers and a unique recirculation function to eliminate cell settling effect. Special attention has been given to the optimal handling of cells resulting in high cell viability, low cell stress and producing levels of uniformity in cell dispensing previously not achievable. With its capability to dispense cells into microplates containing the compounds to be screened, the CyBi®-Lumax flash HT provides several important advantages. It reduces costs for material and cell culture significantly and provides better consistency of results, because the cells do not need to be grown in microplates. Since it eliminates the need for tip washing between plates, non-contact dispensing of cells increases the throughput and prevents cross-contamination. Furthermore, it streamlines the assay process and enables fast collection of agonist and antagonist data from one compound plate. For extremely difficult targets and assays CyBio launched at the SBS Meeting (September 2005) the CyBi®-Lumax flash HS, which combines perfect cell dispensing capability with ultimate sensitivity. The unique cell recirculation function is also offered on the stand alone CyBi®-Drop dispenser and will be integrated into CyBio's new cell seeding workstation composed of the cell dispenser, an incubator and automated lidding/delidding of microplates.

The EVOscreen® and the new plate::explorer™ are the two major platforms of **Evotec Technologies** (www.evotec-technologies.com) for automated cell screening. Both platforms are modular and can be configured to meet customer requirements. EVOscreen® is focusing on providing high-throughput, high-content screening solutions while the plate::explorer™ system is a flexible platform aiming to automate routine applications and which provides highly customisable solutions which grows with growing demands. One core part of cellular screening is the readout. For image-based readouts the Opera™ reader provides both high throughput and high resolution imaging. It can be fitted with up to four lasers (405nm, 488nm, 532nm, 635nm) and also a high pressure Xenon lamp to excite fluorophores in the 360nm to 635nm range. Up to 100,000 images can be acquired in 24 hours by up to four CCD cameras working in parallel, enabling high speed multicolour image acquisition and analysis. The Opera™ is equipped with the script-based image analysis package named

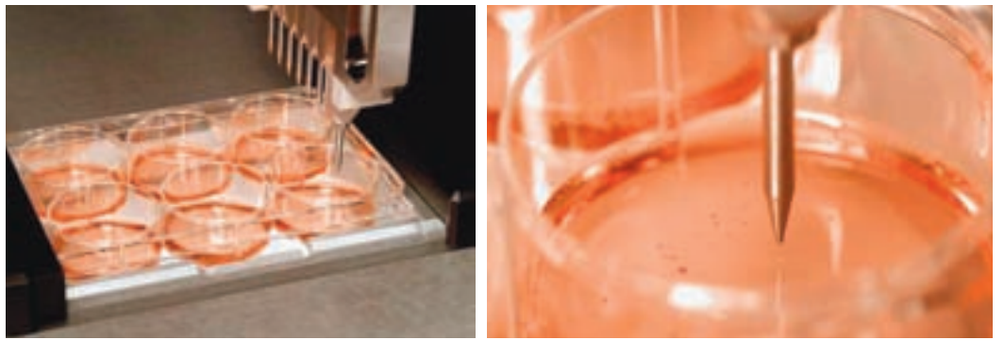
Above: Evotec Technologies' EVOscreen®

Left: Evotec Technologies' plate::explorer™

Images from a Genetix ClonePixFL of IgG-secreting myeloma cells, grown four days in semi-solid medium containing fluorescein-conjugated anti-IgG antibody, ~20 cells/colony



Genetix ClonePixFL picking in a 6-well plate



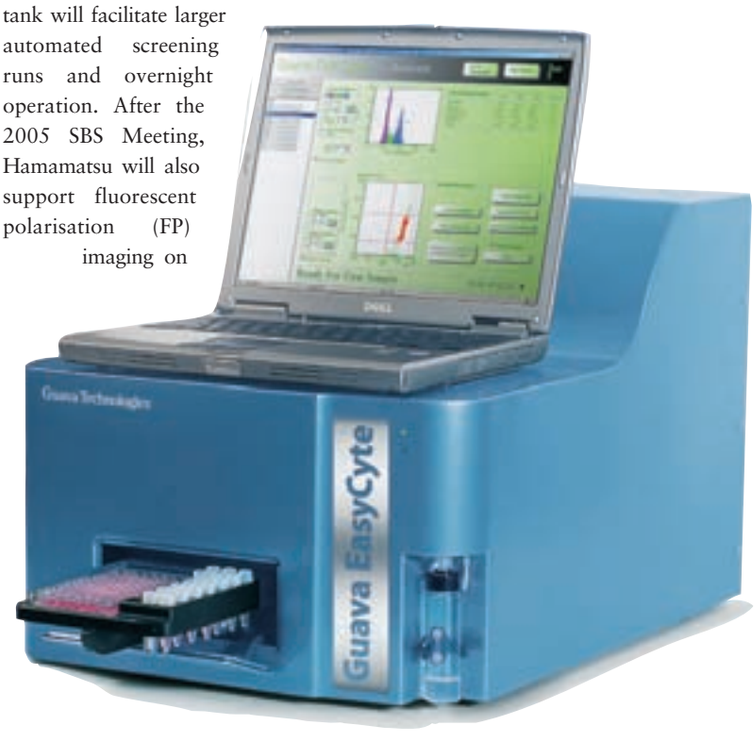
Acapella™ which performs a fully automated online analysis of images in high throughput mode. For whole cell fluorescence or luminescence readouts both EVOscreen and plate::explorer can be fitted with high-throughput multimode readers like the plate::vision™. Both screening platforms can be equipped with online cell dispensing units, plate washers, pipettors for fast online compound addition and various incubators. The EVOscreen system can be used to screen in 384 and 1536-well plates and features intelligent reagent dispensers which constantly monitor the amounts of liquid dispensed. Another unique EVOscreen feature is the truly dynamic scheduling software Bernstein which gives the user control over the entire screening process and which guarantees that all plates are handled equally. The plate::explorer™ can handle 96, 384 and 1536 plates and can be equipped which a range of reliable state-of-the art liquid handling technologies, eg CyBi-Well pipettors, CyBio Nanojet dispensers or Thermo Multidrop. The screening process is controlled by the easy-to-use (click and place) plate::works™ software.

With the ability to automatically screen, select and then pick transfected cell colonies, the ClonePixFL from **Genetix** (www.genetix.com) revolutionises the entire cell line generation process by completely removing bottlenecks and enabling screening of larger populations with significantly earlier selection of cells. This unique system identifies single adherent colonies in 6-well plates using white light multiplexed with selective fluorescence at up to five wavelength combinations to enable selection on the basis of parameters such as size, roundness, proximity to neighbours and expression of fluorescent proteins associated with a co-transfection experiment or a fluorescence reporter gene. Clonal colonies are ranked on the basis of fluorescence

intensity and then immediately picked into 96 well well plates using purpose designed tools mounted on a high precision motion system. Not only does this approach diminish the need for sub-cloning, it also eliminates many of the issues associated with manual picking. Furthermore, process efficiency is greatly improved as larger cell populations can be screened and viability maintained as only the most desirable colonies are picked. These improvements present a new challenge to downstream tissue culture; the number of plates that need handling has significantly decreased but the potential value of each picked plate has dramatically increased. Consequently, potential errors associated with manual management become very expensive and a large scale automation strategy is not practical. To meet this new challenge Genetix has launched the Clone^{Select} family, a series of modules for automated confluence determination, expansion, stratification and cherry-picking. The Clone^{Select} Imager module is a bench top system that fulfils the previously unmet need for fast, automated confluence scoring for 96, 24, 6 and single well microplates.

Guava Technologies (www.guavatechnologies.com) has revolutionised the way in which cell culture monitoring and cell-based assays are performed with the Guava EasyCyte, a compact, easy to use, micro-volume cellular analysis system that makes high content, cell-based assays easily accessible at the benchtop. The Guava EasyCyte is a three-colour, microplate-sampling cytometer that now features an optional side scatter detector for flexible, five parameter detection (3 colour, 2 scatter). The side scatter detection allows easy delineation of multiple subpopulations in whole blood samples or identification of live/dead cells without dye stains, leaving the fluorescence channels open for other types of analysis. In addition, the patented Guava microcapillary technology greatly reduces the number of cells per sample over many traditional cell-analysis platforms and allows for absolute cell counting without using reference beads required by traditional flow cytometers. Guava's four new EasyCyte assays round out its current suite of apoptosis assays and create a powerful cell-based, functional analysis platform, particularly for oncology-focused programmes. Two new microplate-based assays for Caspase 3/7 and Caspase 8 provide reproducible, robust and highly sensitive assessments for early and mid-apoptosis to complement the pan-caspase (or multi-caspase) assay Guava already offers. As a mix and read assay for changes in mitochondrial membrane potential, Guava MitoPotential provides rapid, sensitive detection for the earliest stages of apoptosis or cellular responses. In addition, Guava CellGrowth provides reliable detection of proliferating cells out to five generations. All of these assays are optimised to run on the EasyCyte's automated microplate system with integrated data acquisition and analysis software providing highly informative cytometric analysis for both adherent and suspension cells. This makes them ideal for endpoint screening, mechanistic investigation of cellular targets or even, secondary compound screening. For moderate throughput needs, the Guava EasyCyte can also be integrated with robotic systems for continuous assaying.

Hamamatsu (www.hamamatsu.com) offers the FDSS 6000, its imaging-based plate reader for the automation of cell-based assays. New to the FDSS platform later this year will be the Multiple Solvent Washing System, that has the unique ability to switch between three solvents (eg DMSO, ethanol and distilled water) or three different buffers. This washing system is particularly useful in cleaning pin tools, enabling the FDSS to fully support the V&P range of pin tools, including its 1536 pin heads, suitable for precise low volume compound dispensing to cells in 1536 well-plates. Hamamatsu is also working on expanding the volumetric capacity of its Cell Tank. The Cell Tank enables the uniform distribution and dispensing of suspension cells. The new bigger capacity tank will facilitate larger automated screening runs and overnight operation. After the 2005 SBS Meeting, Hamamatsu will also support fluorescent polarisation (FP) imaging on



Guava Technologies' EasyCyte

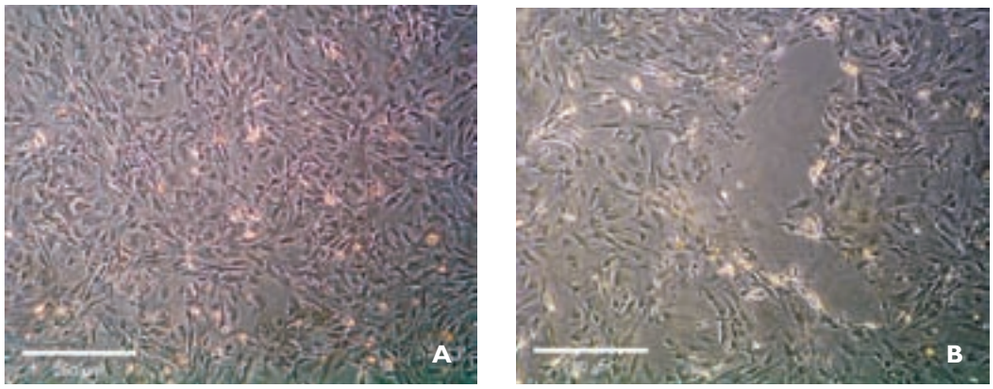
Hamamatsu FDSS 6000

the FDSS, as a minor upgrade to existing instruments. This is in response to customer requests, as there is increasing evidence that FP can aid in discriminating green fluorescent protein from interferring autofluorescence in a microplate assay for cell-based genotoxicity[1]. The FDSS is proving a popular platform of the automated imaging of cell-based fluorescent and luminescent assays with more than 10 units now installed in US Pharma, Biotech and Academic labs.

Hamilton Cell^{host} System with 2 Kendro incubators (37°C incubator for cell cultures; 4°C incubator for media) and MICROLAB® STAR pipetting workstation contained in a sterile housing with laminar downflow

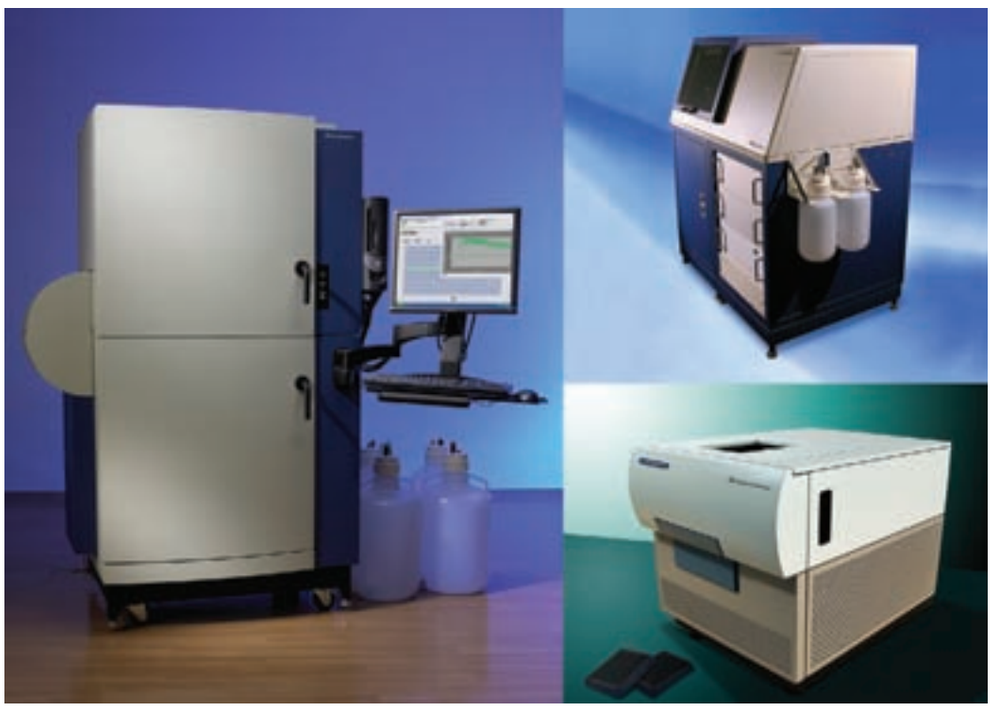
Limitations of the currently used cell culture procedures include the lack of standardisation associated with poor reproducibility and insufficient throughput. Thus, the availability of cells can become a bottleneck in the logistic chain of experiments. **Hamilton** (www.hamiltonrobotics.com) and Life & Brain have joined their expertise in the development of Cell^{host,} a system for the automated culture of primary cells, cell lines and embryonic stem cells which provides high-quality cells in large numbers. The core of the Cell^{host} system is the MICROLAB® STAR pipetting workstation equipped with monitored air displacement pipetting technology. This eliminates use of tubing, pumps or system liquids, thus significantly reducing the risk of contamination by bacterial growth. An internal robotic hand, the iSWAP, handles SBS standard cell culture plates on the workbench. Typical manual processes like plate agitation are perfectly mimicked by analogous robotic movements, The gentle movement of the iSWAP arm enables the Cell^{host} to handle even standard 1-well cell culture plates with ease without the danger of spilling medium. For a complete cell culture automation lifecycle, Cell^{host} can perform cell culture analysis-based decision-making, for example cell splitting based on plate confluency, using selected third party instruments. Any automated system devised for handling large numbers of plates, with each plate undergoing complex operations, requires sophisticated operating and data tracking capability. The Hamilton Cell^{task} software allows easy scheduling of repetitive processes, enabling customer-friendly cell culture automation. The Cell^{track} software monitors and tracks every operation on every single plate and enables data documentation in conformity with CFR 21 Part 11 requirements. For more complex workflow and data management, Cell^{host} uses a dynamic scheduler along with a system wide data management and data security concept. The availability of automated and biologically validated (see microscopic pictures) cell culture systems like Cell^{host} can facilitate a significant reduction in manual workload associated with today's drug discovery.

Automated cell-based assays are key to drug discovery. **Molecular Devices Corporation** (MDC) (www.moldev.com) provides high-performance bioanalytical systems for improved cell-based



Microscopic picture of a fibroblast cell layer immediately after a media change on the Hamilton Cell^{host} System (**A**). After a less careful manual media change (**B**). The robotic media change leaves the cell layer undamaged

assays. MDC offers three product families that enable cell based assay screening: fluorometric imaging (FLIPR®), automated electrophysiology (IonWorks®) and high content imaging (ImageXpress®). The FLIPR® is a widely used tool for GPCR and ion channel screening. The FLIPR^{TETRA}™, the latest FLIPR entry, is a modular system that converts between 96-, 384- or 1536-well microplates. FLIPR^{TETRA} also offers an expanded set of excitation wavelengths, significantly broadening its use beyond traditional calcium mobilisation and membrane potential assays. The IonWorks family addresses the throughput limitations of direct ion channel current measurement via conventional pipette patch-clamping of individual cells. Utilising this method, many data points are lost due to inconsistencies caused by variable channel expression levels or failed measurements caused by technical problems. The IonWorks Quattro™, with Population Patch Clamp™ (PPC) Technology, measures the average concurrent response of many cells. The PPC Technology dramatically increases the success rate of obtaining a data point to >95% while also increasing data consistency, reproducibility and throughput (384 data points/70 minutes). The ImageXpress family for automated high content imaging provides both fluorescent and bright field microscopy options to cell biology, signalling and physiology research. Automated imaging has two



Left: Molecular Devices' FLIPR^{TETRA}™
Top right: IonWorks Quattro™
Bottom right: ImageXpress^{MICRO}™

categories: fixed 'endpoint' assays (cells stained or contain fluorescent proteins), and live cell assays (changing cell morphology or fluorescent probes tracked over time). The recently released ImageXpress$^{MICRO™}$ provides a low cost solution for endpoint assays but with high throughput. For live cell assays, MDC offers the ImageXpress 5000A, which has options for environmental control and automated reagent addition. Transfluor™ is an endpoint assay, ideally suited for the ImageXpress^{MICRO}, which monitors GPCR desensitisation by the redistribution of GFP-tagged beta-arrestin. With these three product families, Molecular Devices provides automated cell-based solutions for multiple stages of drug discovery.

Panasonic's HTS-10HD System

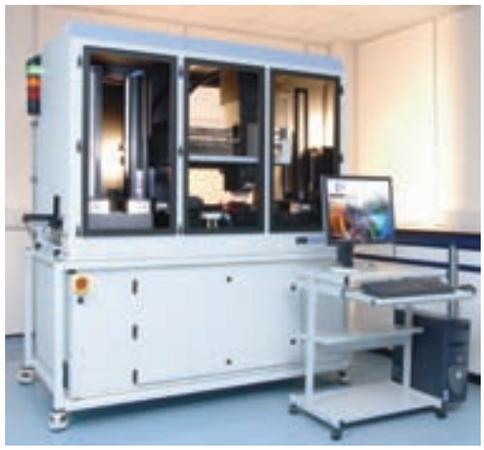
Through the use of its patented electronic and factory automation technology, **Panasonic Factory Solutions** (http://industrial.panasonic.com/ww/products_e/product_cat2/AEAH000_e/AEAH000_e.html) has been able to leverage its strength in electronic components, mounting and fine device bonding to develop reliable and innovative robotic systems for drug discovery and development since 1999. Panasonic's R&D efforts have also focused on the development of proprietary dispensing and image processing technology which has enabled its factory automations division to meet the various application needs of the life science field including cell automation.

Panasonic's HTS-10HD system is an all-in-one custom high throughput screening platform tailored towards drug discovery. Panasonic's diverse library of reliable functional components, including its high precision 96 and 384 channel dispensers, high speed plate shaker, large capacity CO_2 incubator, filtration unit, lid-on/lid-off unit, plate handling transport arm, cooling stage, inclination stage, and plate sealer unit to name a few, offers end users the opportunity to customise a fully integrated system based on their needs and not on availability of components. Panasonic's reliable factory automation technology and engineering staff further enables new end-user features to be developed quickly and with the same high quality offered with all its systems and components.

Panasonic's HTS-100 system is quite unique in its engineered approach at achieving reliable ultra high throughput liquid handling. Mainly, the use of its parallel belt conveyor lines to dispense into, shake and transfer plates at high speed reduces time-consuming plate handling motions typically required by robotic arm systems to accomplish these same tasks. The engineered design of this system as well as the reliability of its components and speed makes it an ideal system for most liquid handling applications.

Panasonic's HTS-100 System

Both systems have been used by extensively by Japanese customers for the automation of cell-based assays for several years and are now available in the US and Europe.

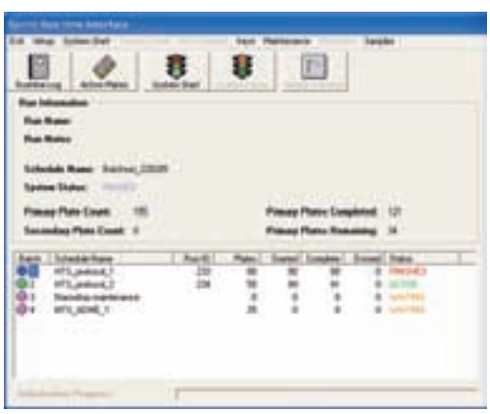


Left: PerkinElmer JANUS™ Automated Workstation with Victor reader
Right: PerkinElmer LumiLux™ Cellular Screening Platform

PerkinElmer's (www.perkinelmer.com) newest advance is the LumiLux™ Cellular Screening Platform which offers, for the first time, a fully automated uHTS luminescence platform for 1536-well, robust high sensitivity calcium assay screens using suspension cells. The LumiLux addresses drug discoverers' needs for high quality data, increased productivity and cost reduction for their screens. The LumiLux's integrated automation and superior sensitivity enable kinetic calcium assays using the flash luminescence aequorin technology, allowing screens to run five days per week. The CellLux™ Fluorescence Cellular Screening Platform is an advanced solution for robust kinetic cellular assays, with particular focus on ion channels and membrane potential assays. Both instruments offer walk-away automation, integrated liquid handling as well as assay development and screening on one platform. The UltraView™ERS Confocal Live Cell Imaging System produces high-resolution real-time multi-dimensional images and movies of the dynamics and structure of cellular and subcellular processes. The system's unique contribution to live cell imaging is its combination of sensitive, high speed imaging with low photobleaching and low phototoxicity. This allows visualisation of both rapid sub-cellular events and studies where cells must remain viable for hours. The powerful confocal optics and multi-line laser excitation produce blur-free optical sections of biological samples in any fluorescent microscopy application, using excitations from the near UV (405nm) to the visible red (640nm). PerkinElmer's new JANUS Automated Workstation employs a modular design that can be configured with a choice of pipetting modules to address throughput requirements and labware movement arms for walk-away capability. JANUS offers the addition and replacement of liquid handling and labware movement technologies as application and throughput demands evolve and is designed to enhance productivity.

RTS Life Science Assay Platform™ Automation

RTS Life Science SPRINT™ Batch Schedule



Cell-based high throughput screening (HTS), which is standard on **RTS Life Science**'s (www.rts-group.com) Assay Platform™, now requires higher throughput support from assay development, secondary screening and early

TAP CompacT SelecT

ADMETox in order to optimise the quality of leads produced. RTS has introduced a number of new features to facilitate flexible cell-based assays using SPRINT™ dynamic scheduling software. Increased sample processing flexibility via Batch Scheduling, automated instrument Maintenance Schedules and 'cell nurturing' ensures a broader range of assay types may be successfully run with minimum user intervention. Screening of larger numbers of assays, each at low to medium throughput but collectively requiring HT processing requires a more flexible approach to automation. RTS Batch Scheduling allows multiple different assay schedules to run automatically, in parallel or sequentially, without operator intervention. Each individual schedule retains SPRINT™ functionality for multi-plate assaying and complex dispense/wash/incubation loops. As co-ordination of multiple assays is much more likely to require last minute changes in a screening run; batch schedules are editable once they begin, allowing individual schedules to be added, deleted or the number of plates altered. Instrument maintenance, eg rinsing of reagent or cell handling dispensers may now be conducted automatically. SPRINT™ Maintenance Schedules allow the operator to include instrument maintenance programmes at any point in a batch schedule. A single instrument may be used for multiple schedules, being primed and rinsed automatically during and after completion of the run. Instrument maintenance is optimised and any possible contamination issues minimised. To facilitate automated handling of increasing numbers of sensitive cell lines Assay Platform™ also provides temperature, CO^2 and humidity controlled cell incubation, plate lidding and gentle cell plate transfers. SPRINT™ scheduling dynamically optimises throughput for each assay schedule and also provides a number of operator controlled parameters to ensure process timings and biology are optimised for each assay.

The Automation Partnership (TAP) (www.automationpartnership.com) has launched CompacT SelecT, an automated cell culture and assay ready plating system which delivers all of the benefits of automated cell culture in a smaller system suitable for the medium throughput laboratory and smaller budget. A new generation of more compact robots has allowed TAP to downsize the successful SelecT system and enable the processing of T175 cell culture flasks in a space only slightly larger than a standard class II safety cabinet. CompacT SelecT is only 2.8 metres long and 1m in depth and will fit into a routine cell culture laboratory. CompacT SelecT utilises the proven aseptic processing of SelecT to grow and maintain cells from multiple cell lines in T175 flasks in a controlled environment (negative pressure laminar air flow) eliminating all possibility for cross contamination. It will count and check cell viability before dispensing the cells into microtitre plates ready for cell-based screening and assay development. CompacT SelecT can hold up to 130 T 175 flasks in a temperature and CO_2 controlled incubator enabling the simultaneous culture of multiple cell lines limited only by the pumps for 10 different media. Virtually any attachment-dependent cells can be introduced on to the system without process change and each flask can be processed with its own unique parameters including timings, media and volumes required by each cell type. Output from the system can be either harvested cell suspension or in 96- or 384-well microtitre plates ready for assay any day of the week. CompacT SelecT ensures a full audit trail security via bar code tracking of flasks and plates in the system. CompacT SelecT delivers dramatic improvements in throughput, productivity and quality for cell based assays for drug discovery.

This year **Tecan** (www.tecan.com) formally launched the Cellerity, the latest addition to its line of workstations designed for automated cell culture. The Cellerity is a fully automated system for the production of cells for downstream assays. It automatically performs all the typical cell culture tasks, such as feeding, passaging, harvesting and plating of adherent cell lines utilising novel microplate-sized automation-friendly cell culturing flasks. The system is modular in design enabling different capacities and throughputs depending on the specific users needs. It can handle the processing of several cell lines in parallel. Since the installation of the initial systems, the Cellerity has successfully demonstrated continual operation for more than 30 days with minimal user interaction to replace reagents, the ability to maintain sterility over several months, cultivation of several cell lines in parallel and successful culture of cells in comparable quantity and quality to manual methods. The launch of the Cellerity has also been complemented by a number of additional new or updated instruments. For customers wanting to perform cell-based assays, Tecan has also added a new sensitive cell wash mode to its PW384 microplate washer. Its latest microplate readers, the GENios Pro and Safire^2, can be equipped with different modules for bottom or top reading of fluorescence, absorbance or luminescence and a temperature controlled interior meeting the different needs of both small biotech and large pharmaceutical laboratories. Recent applications have demonstrated the use of these readers for determining assays transfection efficiency, expression level and cell viability and growth. By utilising the multiple readout modes of these readers, it is easy to establish multiplexed assays. The readers can be used manually as standalone instruments, with stacker option for walk away use, or integrated on to Tecan's Freedom EVO line of liquid handling workstations. As an integrated option on the Cellerity, the readers also enable automated detection of mycoplasma contamination. Instruments such as Tecan's GENios Pro and the new Infinity 200, to be introduced later in 2005, have on-board liquid handling for reagent addition by injectors for fast kinetics and flash luminescence measurements.

The BioCel® line of automated screening platforms from **Velocity11** (www.velocity11.com) consists of a variety of solutions accommodating a large range of cell-based applications. The new BioCel 1200 with its compact footprint is ideally suited for less complex applications, where easy set-up, flexibility and affordability are critical. The under-the-deck CO_2 incubator is another important feature keeping the system compact and flexible. The BioCel 1600 can accommodate very complex procedures, including cell culture applications. Cells can be kept viable for longer via efficient enclosure and environmental control of the whole system, allowing sensitive and timely procedures to be run successfully. Both systems are very flexible and easily expandable, meeting the cell-based assay needs of today and tomorrow. All BioCel systems feature VWorks™ as the scheduling software, a dynamic scheduler which is fast becoming the industry standard for lab automation systems. The ease of use makes the transition from the bench to full automation quick and simple. At the same time, it allows such complex procedures as running multiple, different protocols in parallel. The powerful software, combined with the unmatched speed of the Velocity11 robot, opens up possibilities for cell-based assays that were previously difficult to achieve. One typical example where speed is critical is the distribution of cells into plates. By

Tecan Cellerity, with inset showing automation of standard cell culture processing utilising robotic friendly cell culture flasks

Velocity11 BioCel 1200 integrated with a FLIPR$^{TETRA\,TM}$
A Side view. **B** Top view

keeping the stressful time for the cells to a minimum, the success rate of the assay is greatly raised. Multiple protocols are useful when the same assay has to be run on different cell lines, each of them requiring slightly different conditions. Velocity11 offers industry leading support, a critical asset when running cell-based assays in production mode, and is the ideal partner where innovation and new thinking in cell-based assays are required.

Conclusions

Based on this overview, vendors seem to be on the right track by responding to the needs of cell groups not focused on high throughput by offering a range of more medium scale (smaller footprint) cell automation solutions. Low-tech solutions are definitely needed to get some people started. Not all groups want to jump in and go 'full automation' on day one, many want to progress slowly in steps and modular scalable solutions will help here. As much as these new solutions are attractive, affordability cannot be overlooked and the industry will not sustain high cost instrumentation indefinitely with relatively little success from screening or lack of a clear ROI. In addition, desired improvements in instrument robustness, perceived lack of adequate service support, instruments being launched too early with insufficient beta-testing and greater emphasis needed on simple user operation (ie walk-up and press-go automation) seem to be recurrent themes from our survey of end-users opinions. It is not coincidental that those groups that seem to have got the most out of their cell-based assay automation are also those that have adequate engineering support in house to troubleshoot and provide end-users with a quick turnaround on problem solving. Overall the market cell-based assay automation can look forward to a period of moderate growth, particularly within the large pharma segment. DDW

***Dr John Comley** is Managing Director of HTStec Limited, an independent market research consultancy whose focus is on assisting clients delivering novel enabling platform technologies (liquid handling, laboratory automation, detection instrumentation and assay reagent technologies) to drug discovery. Over the past two years HTStec has published 11 market reports on drug discovery technologies and Dr Comley has authored 11 review articles in* **Drug Discovery World**. *Further information on accessing the market report 'Cell-Based Assay Automation Trends 2005' can be obtained by visiting www.htstec.com or e-mail john.comley@htstec.com to receive a free copy of the Report's Executive Summary and Table of Contents.*

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