HIGH CONTENT SCREENING

emerging importance of novel reagents/probes and pathway analysis

The number of high content screens will increase by 50% over the coming year; signal pathway analysis was seen as the most relevant high content screening (HCS) application; with greatest interest in applying HCS coming from oncology groups. Novel reagent/probes and pathway analysis developments were considered as the tools and technology developments which will impact most on HCS over the next few years. These were the main findings of the recent market survey reviewed in this article and are used as a setting to discuss some of the latest technologies now being applied to HCS.

By Dr John Comley

Over the past 18 months HTStec has extensively surveyed the assay technology scene in drug discovery. None of these investigations specifically focused on High Content Screening (HCS), although it was very clear from the input we have received in more general surveys that HCS was an area of much interest to respondents and one where significant resources were now being invested, particularly with respect to new detection instrumentation. This article attempts to summarise some of the findings of a survey HTStec undertook in February 2005, specifically addressing HCS. The survey looked at the extent to which HCS instruments have been deployed; interest to acquire new HCS systems; which research groups are planning to use HCS; where and for which applications HCS is currently being applied in drug discovery; instrumentation preferences for HCS; live-cell imaging requirements; HCS informatics; future trends, limitations and unmet needs in HCS.

Current Application of HCS

The survey predicted a 50% annual growth in the number of high content screens. The mean number of high content screens run in large pharma was estimated to be 6.8 screens per year in 2005, this is expect to grow 10.4 screens in 2006. In contrast, small-medium pharma and biotech were estimated to be 5.3 screens in 2005, this is expect to grow to 7.8 screens in 2006 (**Figure 1**). Bear in mind that these are different high content assays, but may not necessarily be high throughput screens. It was clear that a large range of different research groups are applying HCS today and have expertise in the technology and tools. Overall the survey suggested that greatest interest in applying HCS today was among oncology groups (67% responding), although there also was significant interest from neurology, *in vitro* toxicology and immunology groups (**Figure 2**). Those areas of the drug discovery process most interested in applying HCS are summarised in **Figure 3**. The results showed greatest

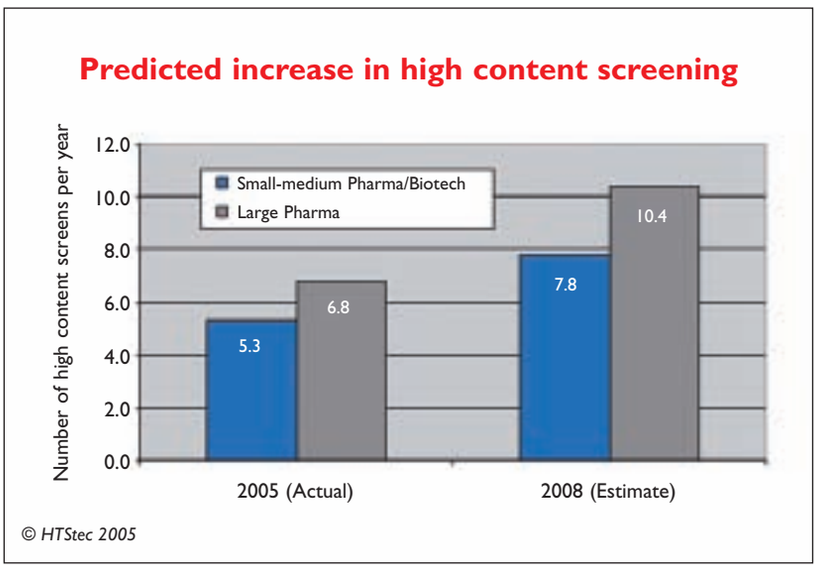


Figure 1

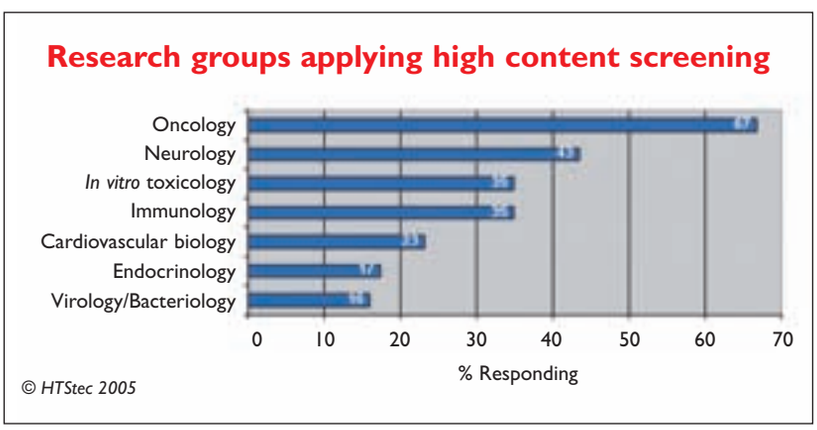
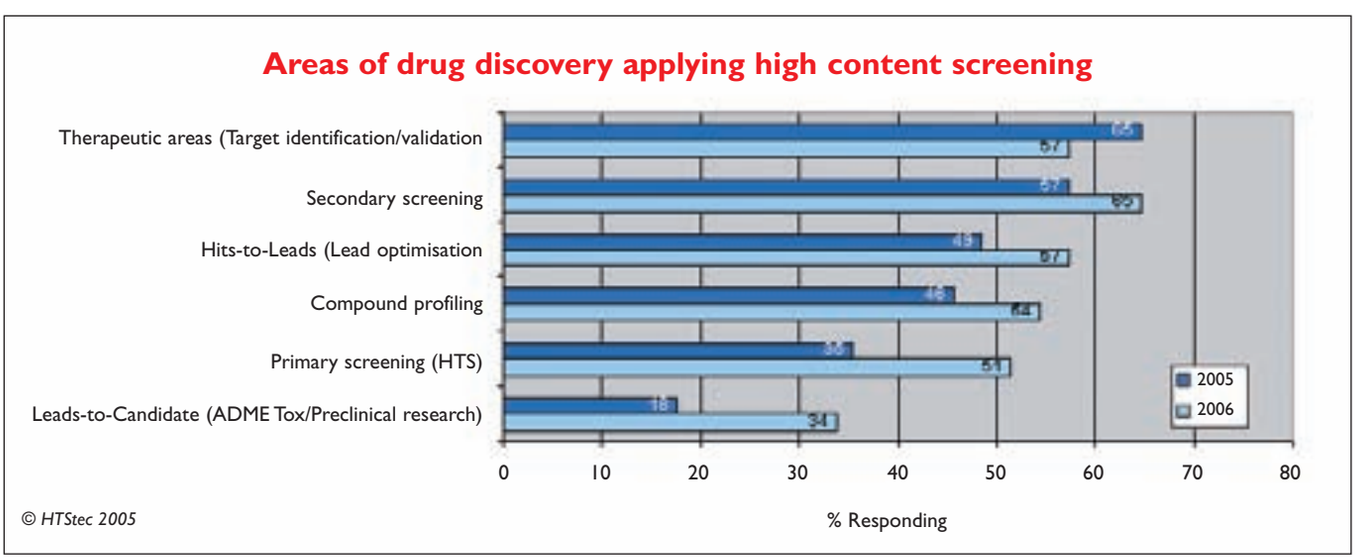


Figure 2

Figure 3

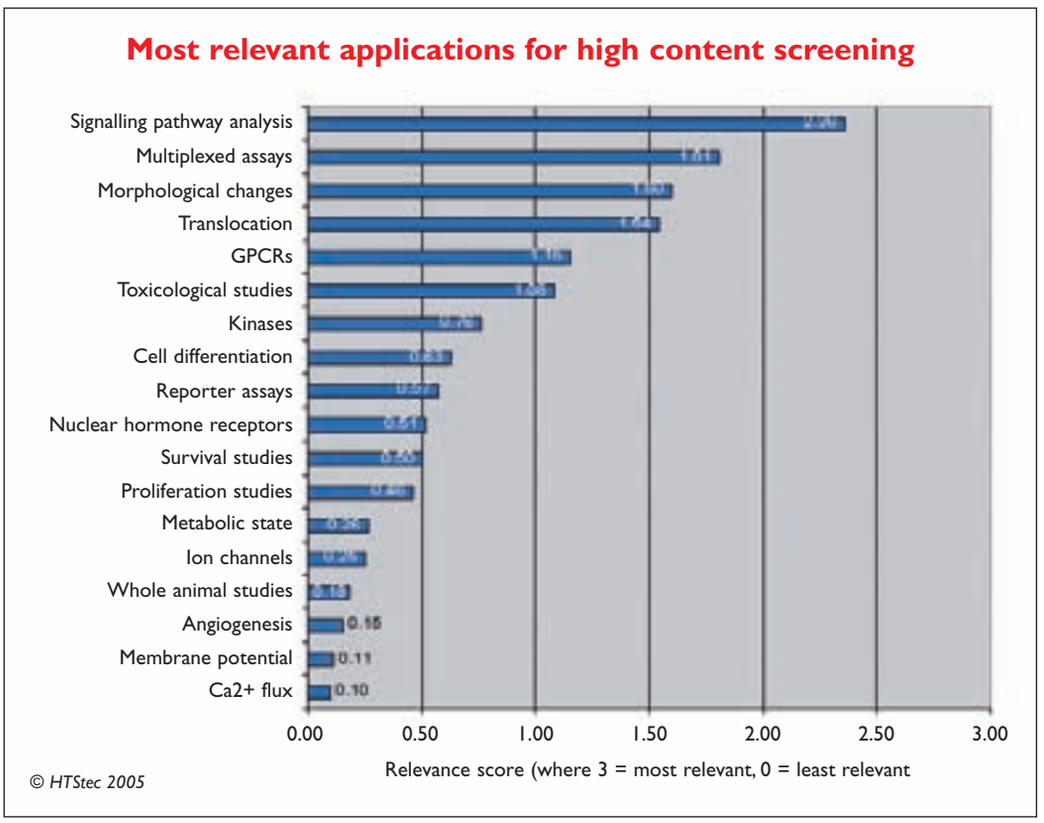


interest today (2005) was in therapeutic areas (target identification/validation) followed by secondary screening, hits-to-leads (lead optimisation) and compound profiling. Less interest today was evident in primary screening (HTS) and least interest from leads-to-candidate areas (ADMET and preclinical research). In 2006 all areas, except therapeutic areas, are expected to show increased application of HCS relative to 2005, this predicted increase in application was most evident for primary screening and leads-to-candidate groups. The relative rankings by survey respondents of those applications seen as most relevant for high content screening are presented in **Figure 4**. This clearly shows that signal pathway analysis was seen as the most relevant application by all respondents, this was followed by multiplexed assays, morphological changes and translocation. Other possible HCS applications eg toxicological studies and cell proliferation, were ranked of lesser importance.

Importance of HCS informatics and data management

A critical aspect of high content screening is the informatics and data management solution that the user needs to implement to process and store the images. Typically multiple images are collected per microplate well at different magnifications and processed with pre-optimised algorithms (these are the software routines that analyse images, recognise patterns and extract measurements relevant to the biological application, enabling the automated quantitative comparison and ranking of compound effects) to derive numerical data on multiple parameters. This allows for the quantification of detailed cellular measurements that underlie the phenotype

Figure 4



observed. From an image analysis perspective the following should not be overlooked when reviewing vendor offerings: the breadth of biology covered; how the software is delivered, does it run quickly, or open a script; is analysis done on-the-fly or offline; have the algorithms been fully validated with biology; the ease of exporting image files to other software packages; and access to new algorithms, is the user dependent on the supplier or is it relatively easy to develop your own or adapt existing algorithms?

Figure 5

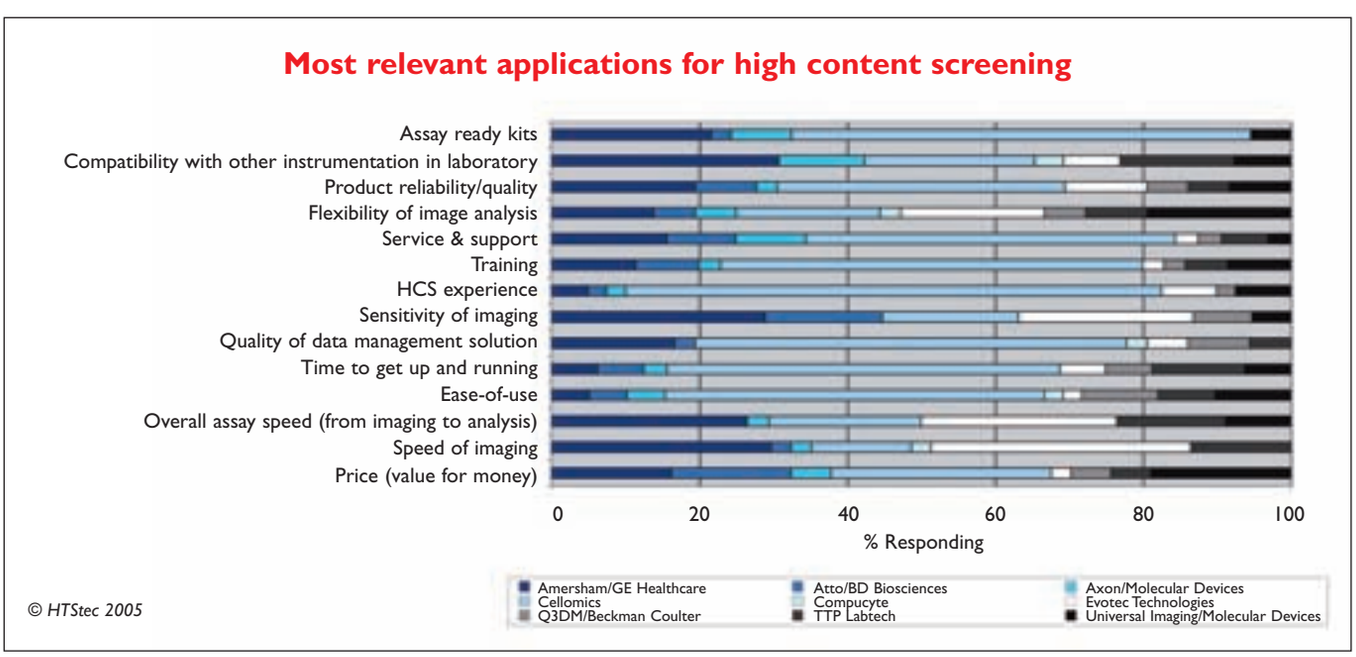


Table 1: Comparison of vendor's informatics and data management solutions for high content screening

VENDOR	INSTRUMENT NAME(S)	SOFTWARE NAME	IS THERE A DATABASE (DB), DOES IT STORE IMAGES AND DERIVED DATA AND ARE THEY LINKED?	CAN THE DATABASE BE SHARED BY MULTIPLE HCS INSTRUMENTS?	CA AND
BD Biosciences	BD Pathway Bioimager	Attovision	Yes, images and derived data are stored in a structured file system where they are linked for navigation via the user interface	Yes, but must be appropriately configured to ensure transaction integrity	Yes
Beckman Coulter	Cell Lab IC 100 Image Cytometer	CytoShop 2.1	Yes	Yes	Yes
Bioimagene (Scimagix)	N/A	CellMine	Yes	Yes	Yes
Cellomics Inc	ArrayScan® HCS Readers, KineticScan® HCS Readers	High Content Informatics (HCi™ Software)	Yes, Cellomics® Store, each data point is linked back to the cell from which it was generated	Yes, Includes licence for up to five licensed HCS Readers (including Cellomics')	Yes, a cor (vHCS™ (HCS Ap provides
CellProfiler	N/A	CellProfiler	N/A	N/A	N/A
Compucyte	iCyte® Automated Imaging Cytometer, iCys™ Research Imaging Cytometer, iColor™ Fluoro-chromatic Imaging Cytometer	iCyte Instrument Software	Yes	No	Yes
Definiens	N/A	Cellenger HCA	Yes	Yes	Yes
Evotec Technologies	Opera™	Opera™ Instrument Software including Acapella™ Analysis System	Images are stored in a separate file space. Derived data and high level experimental descriptions are stored in an XML file tree hierarchy	Yes	Yes
GE Healthcare	IN Cell Analyzer 1000, IN Cell Analyzer 3000	Image Analysis Modules 1000, Image Analysis Modules 3000, IN Cell Developer 1.0 Toolbox	Yes	Yes	Yes
MAIA Scientific	MIAS-2™ microscopy reader	eaZYX™ Control and Imaging Software	No, images and data are stored in the system in an open format to be used by any 3rd party database or other analysis software specified by the user	No, the stored images and data can be shared over the network by 3rd party DB systems resident at the user's location such as Nugenesis, etc	Yes. Data Addition commun CORBA
Molecular Devices	ImageXpressMICRO™, Discovery-1, ImageXpress5000	MetaXpress™ Software and Assay Application Modules; AcuityXpress™ Cellular Informatics Software	Yes, all hardware and applications talk to DB	Yes and offline seats as well	Yes
TTP LabTech	Acumen Explorer	Acumen Explorer software	No DB for two reasons: 1) no images are generated by the system. 2) the small file sizes of derived data do not justify a standalone database	No DB. Derived data from screens is uploaded into client's screening DB	Data is s database
Vala Sciences	N/A	Thora Image Analysis Platform	Yes	Yes	Yes

CAN MULTIPLE USERS AND SITES ACCESS THE DATA?	ARE THERE LINKS TO POPULAR THIRD PARTY TOOLS?	IS THERE ONLINE AUTOMATED STORAGE (WRITE) OF IMAGES/DATA AS THE INSTRUMENT SCANS PLATE?	WHAT ARE YOUR IMAGE AND DATA FORMATS, IS IT AN OPEN SYSTEM?	DOES YOUR OFFERING SUPPORT DIFFERENT VENDORS' HCS READERS?
es	Yes, via text export of data. Also provides direct export into Microsoft Excel-based BD™ Image Data Explorer	Yes	Images – TIF, BMP Data – tab delimited text export Open System	No
es	Yes, but may require site-specific configuration	Yes	Open system, DBF, XML, bitmap	Yes, but may require site-specific configuration
es	No	N/A	Image format varies depending on which instrument they are coming from. However, Cellmine can convert them to common format and bring them into a common platform	Yes
es, a combination of desktop PC software HCS™ Discovery ToolBox), middleware HCS Applications Server) and database rovides an enterprise solution	Yes, direct Spotfire link, export to IDBS ActivityBase and Image Pro Plus. Variety of text/XML/image export tools	Yes, data is automatically transferred to the Cellomics Store Database and there is no need for the user to manually copy/find data	Yes, TIFF, DIB, BMP. Data can be exported as text and XML	Yes, via HCS Gateway product
I/A	Data can be exported to Excel, Matlab, mySQL, and other databases	N/A	CellProfiler is open-source software that supports the following image/image stack/movie formats: AVI, BMP, CUR, DIB, FTS, FITS, GIF, HDF, ICO, JPG, JPEG, PBM, PCX, PGM, PNG, PNM, PPM, RAS, STK, TIF, TIFF (including stacks produced by NIH image or metamorph), XWD	Yes
es	Data may be easily pasted into 3rd party applications such as Excel	Yes	Images may be stored as JPEG or FLT files	No
es	Yes, ODBS DB interface, Cellomics Store, Spotfire, Excel, XML, CSV and Flat File	N/A	TIFF, JPEG, GIF, PNG, BMP, platform independent	Yes
es	Yes, Spotfire is linked via A+plus data evaluation software	Images are stored as the plate is scanned. High level and derived data are stored after a plate is finished	Images are stored in TIFF (preferred) and several other formats. All derived and high level data is stored as XML files. Convert to other formats (eg simple TXT, CSV) are included	Images from other open systems can be read and analysed with the Acapella™ Analysis System. Customised algorithms are available upon request
es	Yes, MS Excel, GraphPad Prism, Spotfire DecisionSite	Yes	TIFF, XDCE, DCE, open system	Yes
es. Data is accessible over the network. dditionally standard crossplatform ommunications protocols such as ORBA and (.NET) are supported	Yes. Spotfire, Excel, other third party software. Open data file structure (ASCII-tables)	Yes	TIFF, JPEG, QuickTime movies	No, however the standalone iBOX computer system with eaZYX™ software is able to analyse TIFF and JPEG image files from any other source
es	Open Application Programming Interface for DB	Yes	TIFF; open system	Supports all MDC imagers, software can import from other HCS readers and Excel
ata is shared via client's screening atabase	No. Derived data is exported in CSV format which is readily imported by third party tools	Yes. Data acquisition, analysis and saving is automated during scanning.	No images. Data stored in CSV or FCS format. Open system	No
es	Yes	N/A	All image formats, data open (XML)	Yes, Thora software will analyse images generated by any HCS reader

The Cell Lab IC 100 Image Cytometer from Beckman Coulter with GPCR pit formation data shown in CytoShop 2.1 Analysis Software. Images taken using 40X 0.95 N.A. optics

All systems (except the laser scanners) will require a data storage and management solution. This is particularly important as the majority of survey respondents indicated they wanted to store raw image data, processed image data and the numerical data derived from images for, on average, 6.3, 6.5 and 10.4 years respectively. To understand better what the different instrument vendors and software developers provide in this respect the various offerings are compared in **Table 1**.

HCS instrumentation and software developments

Survey respondents were also asked which of the instrument vendors currently supporting HCS they most associated with a list of HCS-related product attributes (**Figure 5**). Based on the survey sample, Cellomics was the HCS instrument manufacturer most associated with assay ready kits; service and support; training; quality of data management solution; ease-of-use; time to get up and running; HCS experience; product reliability/quality; and price (value for money). Amersham/GE Healthcare was the HCS instrument manufacturer most associated with sensitivity of imaging and compatibility with other instrumentation in laboratory. Evotec Technologies was the instrument manufacturer most associated with speed of imaging. Amersham/GE Healthcare and Evotec Technologies were the HCS instrument manufacturers most associated with overall assay speed (from imaging to analysis). Cellomics, Evotec Technologies and Universal Imaging/Molecular Devices were the HCS instrument manufacturers most associated with flexibility of image analysis.

The following is an update of the some of the latest developments in instrumentation and software now available to support HCS.

The Cell Lab IC 100 from **Beckman Coulter** (www.beckman.com), built upon the technology it acquired from Q3DM, expands Beckman's broad offering of cellular analysis tools that help researchers obtain a deeper and contextual picture of processes on the cell surface and within the cell itself and is indicative of Beckman's strong commitment to imaging technologies.

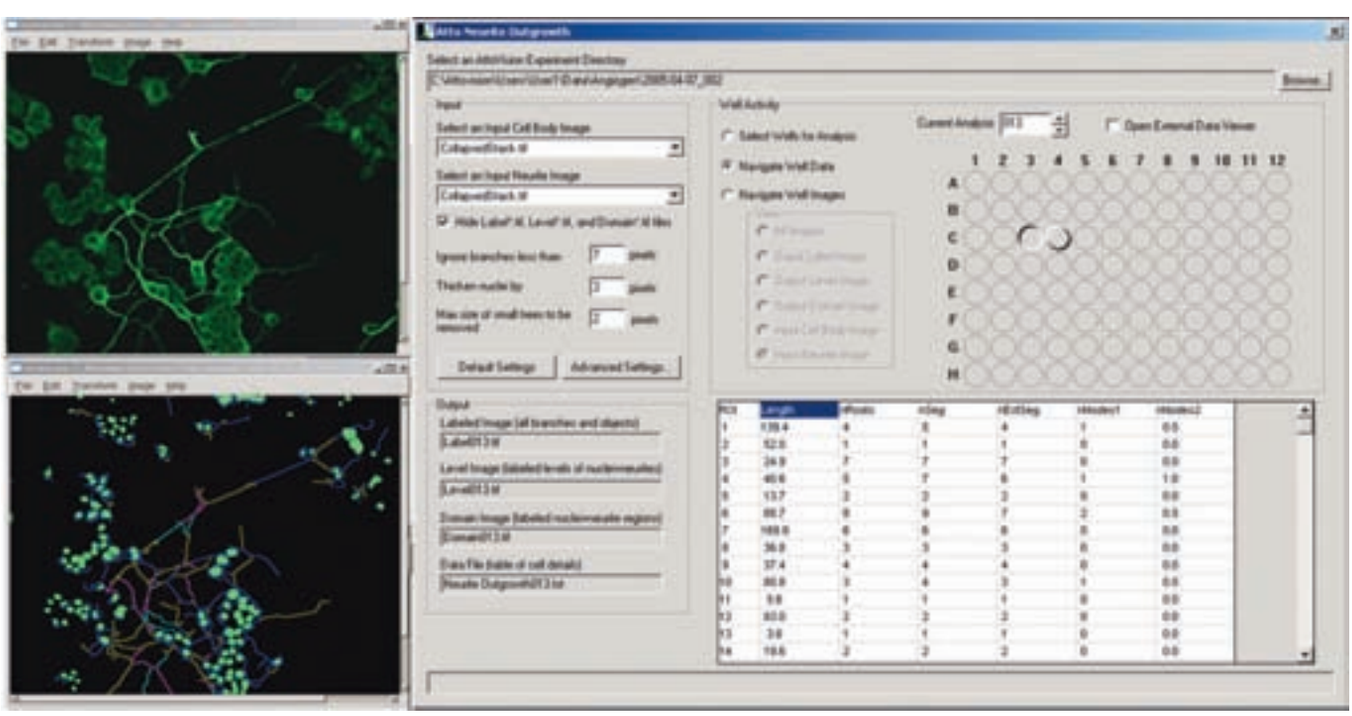
The Cell Lab IC 100 is not only a highly effective high content analysis tool for drug discovery applications like siRNA, nuclear translocation, foci formation, mitotic indices etc, but the system has also been accepted and proven especially valuable in academic settings. In large part, this is a direct consequence of the open, standards-based philosophy applied throughout the development process. In conjunction with the control and analysis software CytoShop 2.1, the IC 100 is built from the ground up to be an open, accessible, extendable cell analysis tool, eg every hardware, software, system or protocol setting is always available via well accepted standard XML, database, text and image formats. Extensibility is shown through easy to use

cell gating tools and core integration of a plug-in architecture for new imaging processing and measurement algorithms. Importantly, CytoShop treats the imaging data as a population of cell-by-cell data. Though simple in concept, the extra architectural step to separate and organise the data as populations of cells is very powerful and better approximates to the biology being observed. This enables native cytometry gating tools and makes writing plug-ins much more approachable.

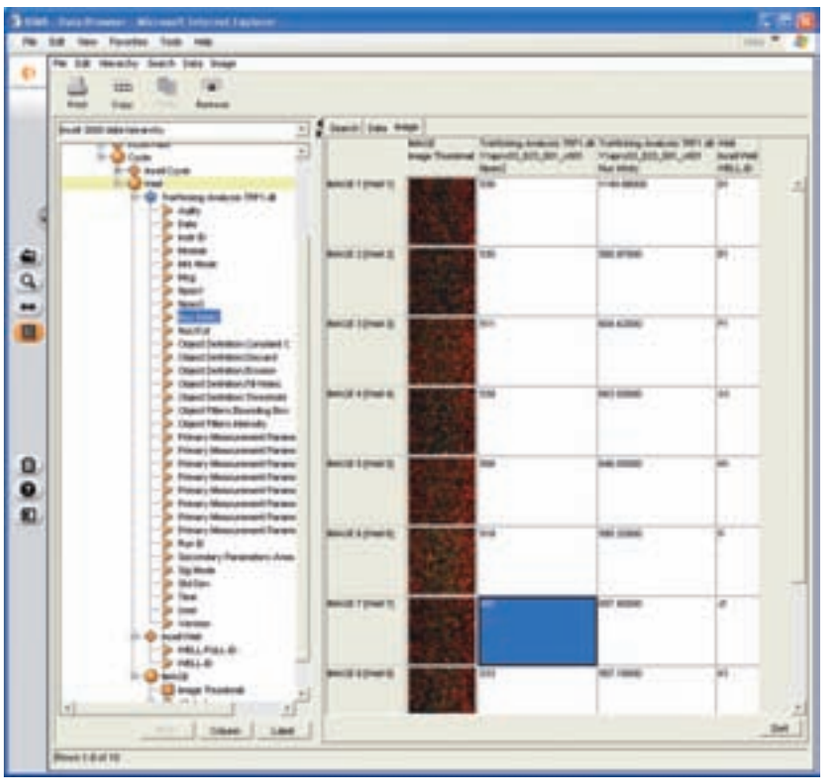
The recent addition of z-stacks (serial axial image acquisition), time series and expanded automation support has truly expanded the available applications. Z-stacking is especially interesting because it is an excellent complement to the fast highly accurate focusing system available in earlier versions of the system. Fast high resolution 3-D imaging of entire plates and slides is now in routine use on the Cell Lab IC 100 in many academic centres.

The BD Pathway from **BD Biosciences** (www.bdbiosciences.com) is unique among automated CCD imagers owing to its full-spectrum confocal imaging and endpoint and kinetic capabilities. The system utilises a spinning disk confocal device using dual white-light sources for illumination. This provides the advantages of true confocal imaging, including three-dimensional modelling, without the spectral limitations of laser-based systems. Since the confocal device can be removed from the light path with the push of a button, the system also allows automated widefield imaging.

The BD pathway is designed to permit both kinetic and endpoint experimentation. The system can acquire several images per second and can acquire images during the addition of a cellular stimulus through an on-stage liquid handling system. The high image capture rate coupled with the environmentally controlled imaging chamber allows for both rapid kinetics and extended time-lapse observations of living cells. The provided AttoVision software controls all operating features of the platform, and allows a wide range of customisation for developing novel assays. The system permits evaluation of several translocation features including molecular translocations and trafficking, morphometric features including neurite outgrowth, and fluorescence intensity fluxes within cells – all in fully-automated or semi-automated modes. New application workflows provide the user with a broad range of applications in an intuitive user interface, making the system easy to operate for those new to imaging. For experienced users, the system still provides full control of all operational features of the instrument to develop novel cell-based applications. The versatility of the BD Pathway Bioimager allows the researcher to explore biological events both spatially and kinetically, opening up new possibilities in cell analysis.



Determination of neurite outgrowth in PC-12 cells stimulated with NGF. Cells were treated with varying concentrations of NGF and analysed using the BD Biosciences AttoVision Neurite Outgrowth algorithm that reports data regarding number of new neurites, length of neurites, as well as number of roots and branches per neurite



BioImagene Scimagix Cellmine Data Browser List View

BioImagene (www.bioimagene.com) now offers Scimagix's CellMine™ HCS, an instrument and application data repository for storing and mining HCS cell-based assay data. It is a major step forward in BioImagene's delivery of cutting edge solutions for faster lead identification and prioritisation studies in drug development and further evidence of the utility of image informatics.

Using CellMine™, distinct sets of information can be organised in a single interface and combined with complementary data such as biochemical assay, compound and toxicology data to better understand a compound's effects. HCS data can also be made available to chosen applications and researchers when and where needed to optimally support established workflows. It helps in streamlining the drug discovery process by integrating data navigation, changing workflows, mining and high content screening. It provides rapid mining and retrieval of the precise data that best informs the lead selection and optimisation decision-making, enhances the operational efficiency and saves substantially in terms of time and money allowing our customers to recoup their upfront CellMine HCS investment.

Apart from facilitating workflow automation, it also streamlines the key components of the HSC workflow such as image quality verification and validating analysis algorithms, review of control data, 'hit' identification, data reanalysis and compound report generation. It supports development and execution of single parameter or multi-parameter searches which can be saved for future use. It also provides a feature to accommodate changes in HCS operations such as a change of instrument, plate dimensions, assay, method of analysis and/or research focus. Its multi-tiered Internet-based architecture ensures no loss of data processing time and throughput takes place each time HCS infrastructure functional capabilities are upgraded or a new instrument and/or application and/or database is interfaced or IT infrastructure is scaled-up.

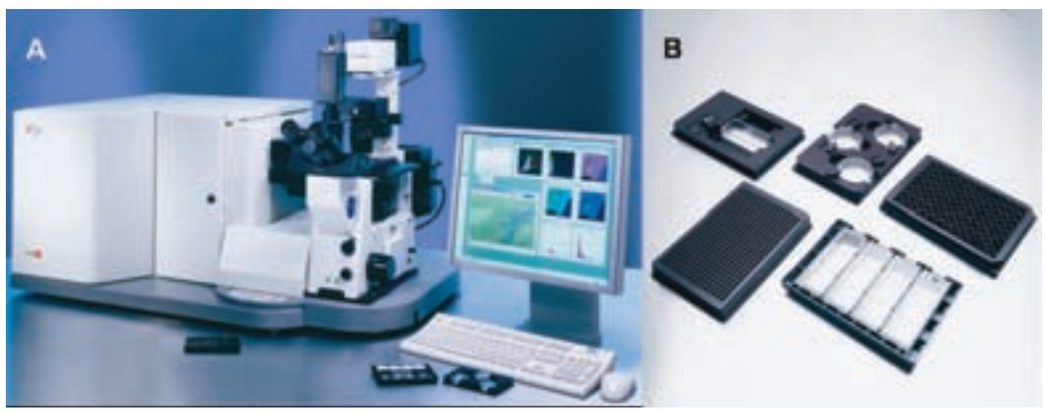
Maximum productivity in HCS is provided by Cellomics' total HCS solution, powered by informatics, which seamlessly integrates instrumentation, quantitative analyses, visualisation and data management to get from image to decision in the fastest and most efficient manner

Cellomics (www.cellomics.com) is automating drug discovery through a unique, cell-based assay platform that addresses the needs of Drug Discovery and Systems Biology groups by offering complete systems HCS. The platform includes HCS instrumentation, informatics, cellular image analysis software (BioApplications), fluorescent reagents, kits, cell lines and multiparametric assays. Cellomics is actively developing new products which will carry its large installed base of scientists using HCS tools to the next level of HCS analysis and assay development. When developing new products, Cellomics makes it a policy to implement an upgrade path for scientists who own their older model systems. This insures that all Cellomics HCS customers can take advantage of the latest innovations in HCS technology. In the past six months, Cellomics has launched six BioApplications software updates, an upgrade for its v. 3.1/4.0 instrument systems as well as new software for its latest model instrument, the ArrayScan® VTI HCS Reader. In addition, it is in the process of launching an annual maintenance and support update called HCi2005

which will go out to all customers with an active warranty or maintenance and support contract. This annual update insures that its customers have the latest software and informatics improvements to keep their productivity levels high. By utilising cutting edge market research and by implementing feedback from its HCS customers, Cellomics is able to make sure it is focusing on addressing the needs of HCS scientists everywhere through product updates, enhancements and innovations. When applied to early drug discovery, the Cellomics platform is proving to reduce the 'idea-to-discovery' cycle time in drug discovery, while increasing the probability of the therapeutic success of leads as well as enhancing throughput in systems biology and basic research. Cellomics' proprietary platforms, including the ArrayScan® and KineticScan® HCS Readers, along with BioApplications software and High Content Informatics (HCi™) suite, are in use at multiple sites within all of the top 15 pharmaceutical companies, as well as leading pharmaceutical and biotechnology companies and academic centres globally.

CompuCyte's (www.compucyte.com) unique laser scanning cytometry (LSC) technology performs high-precision, high-accuracy quantitative cellular or cell-based analysis on a par with flow cytometry, while delivering high-quality images and image processing of the specimens under analysis. The iCyte® and iCys™ Imaging Cytometers, based on LSC technology, allow analysis of cellular and tissue specimens on a variety of labware, including microscope slides, microtiter plates, chamber slides, Petri dishes or additional user-defined carriers. Although the high-quality digital imaging produced by the technology eliminates the need for an optical microscope, one can be provided to allow post-scan visualisation of any location of interest. Because of the technology's broad depth of focus compared to confocal and non-confocal microscopy systems, precision of <2.5% FWHM (full-width, half-maximum) and sensitivity below 1000 MEF (molecules of equivalent fluorescence) are possible.

Recently developed multicolour laser absorption

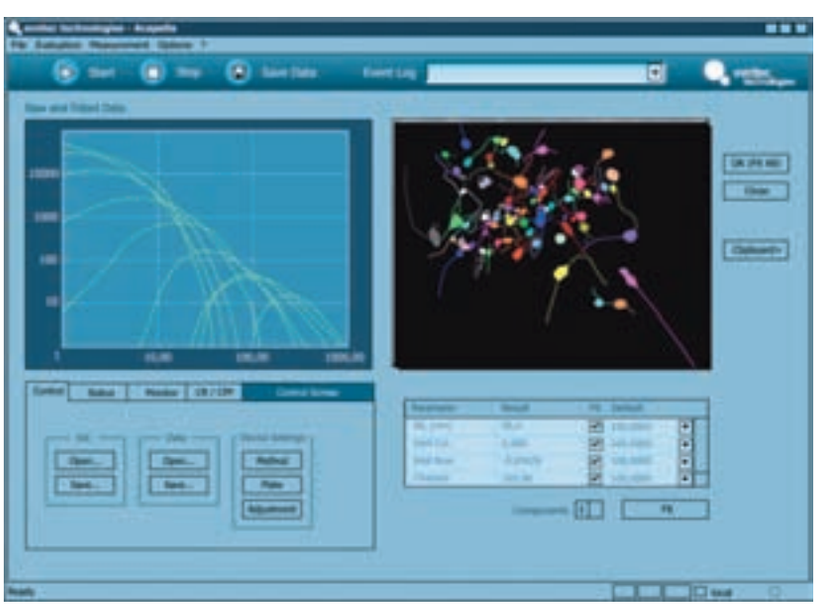


The Compucyte iCys™ Imaging Cytometer is shown in panel **A**. Panel **B** shows a sampling of the carrier types that can be analysed on the instrument

allows precise quantitative measurement of chromophore staining and specific contributions of multiple dyes. The technology is applicable to tissue, tissue microarrays (TMAs), and adherent cellular specimens. Combined fluorescent and chromatic analysis brings new capabilities to quantitative image analysis, including correction for the effects of auto-fluorescence. CompuCyte has also developed automated quantitative sampling methods for tissue sections and TMAs, and has shown correlation between the results of manual sampling and this automated method. The iColor™ Fluorochromatic Imaging Cytometer, scheduled for full release in summer 2005, offers near-true colour imaging by combining the absorption signals from blue, green and red lasers.

Variable resolution scanning (VRS) is available on all instruments and is proving to be the most effective method for adjusting assay throughput while maintaining instrument sensitivity. Increasing the spacing between laser scans increases the scan speed by up to 40 times; using high-power objective lenses maintains instrument sensitivity. VRS is applicable to both tissue and cellular analysis, and allows the instrument to be tuned to a given assay's requirements.

The multi-faceted flexibility of CompuCyte LSC technology, obtaining quantitative and imaging data for both fluorescent expression and chromatic staining, on sample types ranging from adherent cells to tissue sections and tissue microarrays, allows the use of a single LSC platform, where previously multiple instruments were required.



Evotec Technologies' Opera software Acapella™ for neurite outgrowth evaluation

Cellenger for High Content Analysis from **Definiens** (www.definiens.com) has been designed to meet demand in the pharmaceutical and biotechnology sectors for a flexible and easy to use solution for automated image analysis in high content applications. Cellenger supports the user with unmatched accuracy in extracting cells and subcellular structures. It offers several levels of customisation complexity. The vast majority of current and future applications can be configured by the research scientist during the assay development process using the Cellenger Library. This is a set of modules which can be mixed-and-matched to create exactly the desired image analysis procedure in seconds with no programming or scripting required. If none of the modules exactly meet your requirements then these modules can be modified, or entirely new modules created using Cellenger Developer Studio. Once you are happy with your application then it can be transferred to a fully automated production environment which supports a scalable processing architecture to meet your throughput requirements.

Cellenger provides access to many powerful predefined modules from the Cellenger Library.

Throughout the development process support is given by workflow templates which guide the user through experimental design and metadata import and set-up, module selection and configuration, assay development runs, data analysis and application validation. During the assay development process it is important to be able to characterise the performance of a newly developed application for validation purposes. Cellenger provides you with the tools to perform bottom-up or top-down interrogation of your data on an experiment-plate-well-image-cell or sub-cellular basis. Each data representation is linked with all the other relevant representations for all objects down to the sub-cellular level allowing you to rapidly drill down through the data without having to go to external tools such as MS Excel. Because Cellenger is platform independent, applications can be run on data from any instrumentation. This means assays can be developed on one type of instrument and transferred to a different type of instrument without needing to develop a new image analysis application.

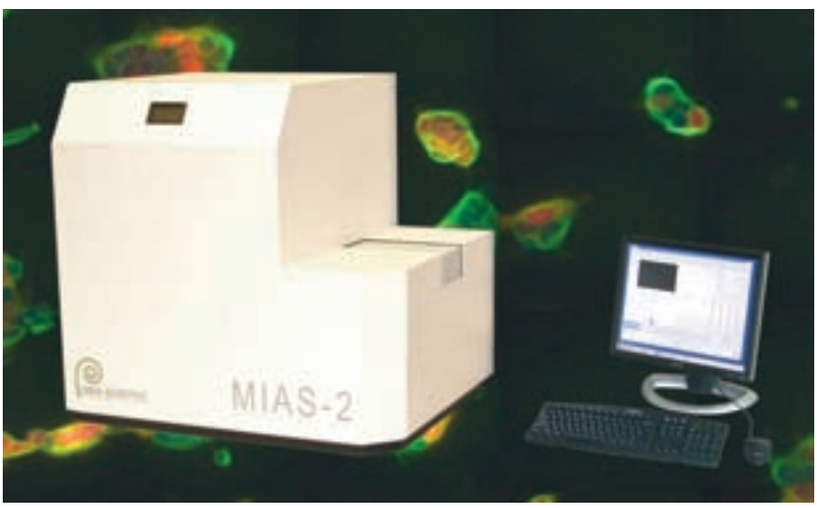
The Opera™ is **Evotec Technologies**' (www.evotec-technologies.com) high speed, confocal HCS imaging reader. It now has four lasers (405nm, 488nm, 532nm, 635nm) plus a high pressure Xenon lamp available for fluorescence excitation in the range from 360nm to 635nm. Images can be acquired by up to four CCD cameras in parallel, enabling high speed multicolour image acquisition and analysis. The Opera™ is equipped with the script-based runtime system Acapella™ which performs a fully automated online analysis of images during acquisition in high throughput mode.

Acapella™ is composed of a modular library of routines for script development. For many standard applications and analysis classes a variety of basic algorithms are already available. These algorithms include the detection/separation of cells, detection of cell membrane, cytoplasm, nuclei as well as small spots such as endosomes, vesicles and clathrin-coated pits. Assays enabled by these algorithms include cytotoxicity, endocytosis, translocation of signalling molecules and many more. Acapella™ is an open software concept offering pre-made solutions as well as interfaces to integrate home-made applications and third party data-sets. Applications include today's state-of-the art high content cellular assays, tissue image processing, kinetic data streaming as well as single molecule detection and analysis, thus covering extensively the life science research field and the drug discovery process.

A new data analysis package for the evaluation of Neurite Outgrowth is expanding the list of Acapella's pre-made data analysis tools. The package is based on CSIRO's neurite outgrowth evaluation code and allows versatile image analysis routines on neurons and neuron-like cells. Neurite outgrowth has conventionally been regarded as being rather difficult, subjective and labour-intensive due to manual counting of cells and manual allocation of neurites to the individual cells using conventional fluorescence microscopy. Automated imaging of neurons and quantification of neurite formation is now available as an integral bundle for the Acapella™ framework allowing not only counting of neurons and neurites but also individual cell labelling and neurite allocation as well as branch point analysis.

GE Healthcare's (www.amershambiosciences.com) IN Cell Analyzer 1000 is an automated cellular and sub-cellular imaging system for fast, automated multi-wavelength imaging and analysis in fixed and live cells. The basic end-point system can be upgraded to enable kinetic assays, transmitted light imaging and microscope slide handling. The IN Cell Analyzer 1000 uses pre-packaged, fully validated software, accessed via cost-effective seat licensing, to analyse a broad range of cellular and subcellular applications. The instrument can be integrated with laboratory automation and with proven data integration tools such as discoveryHub™ and leading commercially available management, analysis and visualisation tools. IN Cell Developer Toolbox is new image analysis

GE Healthcare's IN Cell Analyzer 1000



MAIA Scientific's MIAS-2™ microscopy reader

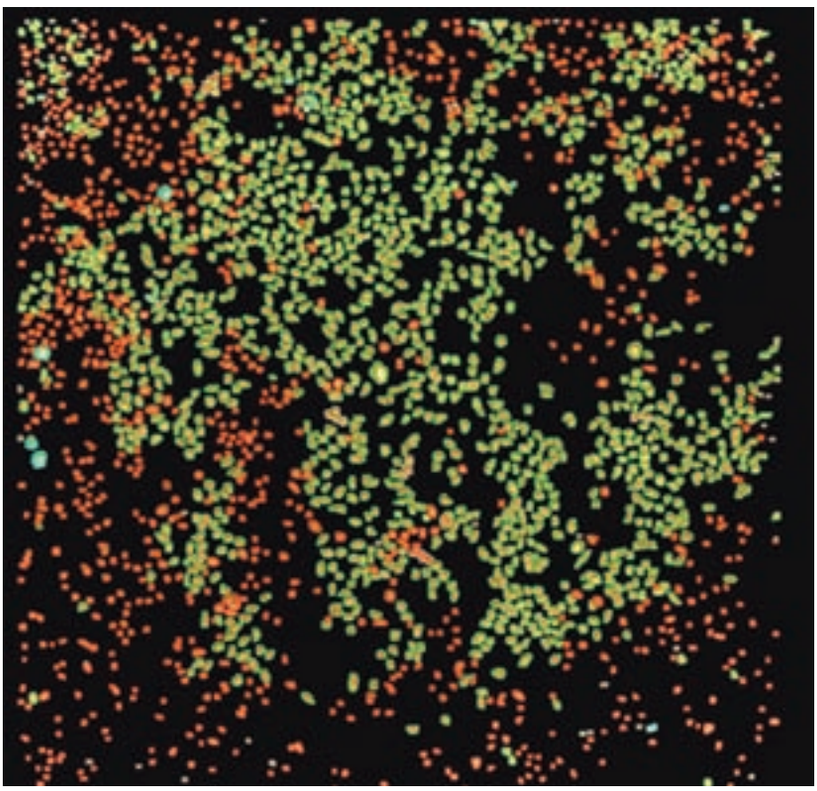
software designed for specialised information-rich analysis applications where pre-developed image routines are not suitable. This analysis software gives users the flexibility to develop new cellular assay routines in an automated software environment. Renewable seat licensing gives cost-effective access to IN Cell Developer Toolbox software.

MAIA Scientific (www.maia-scientific.com) is a wholly-owned subsidiary of Harvard Bioscience which develops, manufactures and markets novel instrumentation and applications for high content screening. MAIA Scientific was founded in January 2002 as the European branch of Union Biometrica. Four scientists, who previously held key positions in technology development for high throughput, high content and model organism screening at Johnson & Johnson PRD, Beerse, Belgium, in-licensed key technology from Johnson & Johnson and developed novel instrumentation for high content screening.

The MIAS-2™ microscopy readers, having both brightfield and fluorescence parameters, and the eaZYX™ imaging software, utilising the concept of 'Scale Space' object oriented analysis algorithms, offer unprecedented sensitivity, speed and flexibility across the drug discovery and development value chain. The MIAS-2™ and eaZYX™ platforms allow genomics, target validation, assay development, drug discovery and ADMET R&D programs to be conducted on the same platform. In addition, MAIA Scientific provides services for compound and genetic screening and toxicology assay development with cells, tissues, tissue sections and small animal model organisms. MAIA Scientific also builds partnerships to integrate biological imaging, preclinical and clinical R&D into customer-oriented platform technology.

Molecular Devices' new automated imaging system – ImageXpress$^{MICRO™}$

By integrating and leveraging the imaging expertise of both Universal Imaging and Axon Instruments, **Molecular Devices** (www.moldev.com) is launching a new suite of tools for high content screening. The key components of this suite are the commercial releases of ImageXpress$^{MICRO™}$, a new automated imaging system; MetaXpress™, an expanded software package for image acquisition and analysis; and AcuityXpress™, an integrated cellular informatics platform. ImageXpress$^{MICRO™}$ is a cost-effective, high resolution CCD imager with expandability and modularity. The system's utility can be expanded with upgrades such as laser autofocus for rapid plate scanning, and bright field illumination optics. Built on MetaMorph™, MetaXpress™ now controls all of MDC's existing and new high content imaging systems, providing a simplified but fully flexible interface for configuring image acquisition. MetaXpress™ also optionally includes a suite of more than 10 fully validated assay-specific application modules, enabling turnkey configuration and operation of image segmentation and analysis for high content assays. The script writing capability provided in MetaMorph™ is also available for developing novel assays. AcuityXpress™, a new cellular informatics platform, provides powerful statistical and data visualisation tools for high content screening. AcuityXpress™ provides automatic calculation of IC50s, EC50s and Z' values, as well as the ability to drill down from analysis data to the original images and segmentation overlays. Both



Virtual Well View in TTP Labtech's Acumen Explorer software showing variable protein kinase activation. Green cells are active, red are inactive

MetaXpress™ and AcuityXpress™ are seamlessly integrated with either MDC's enterprise-level database, MDCStore or customer's internal database systems, for streamlined image and data management.

For cell-based primary screening, **TTP LabTech**'s (www.ttplabtech.com) Acumen Explorer microplate cytometer combines the object-recognition capabilities of microscope-based systems with the fast read speeds of bulk fluorescence readers. Unlike microscope-based high content instruments, the Acumen Explorer uses scanning laser excitation with photomultiplier tube detection to resolve fluorescent objects. Four colours can be monitored simultaneously, allowing true multiplexing within experiments. Signal thresholding algorithms identify fluorescence above the solution background from which virtual three-dimensional models of fluorescent objects are created for calculation of morphological and fluorescent parameters (**Figure 1**). This method of analysis results in a 99% reduction of data when running in HTS mode and file sizes of around 50 KB. This is in contrast to the requirement for terabyte data servers for many other systems.

A key design feature of the Acumen Explorer is the large field of view afforded by the precision optics. At 400mm^2 (20 x 20mm), the field of view is far greater than that offered by the objectives in microscope-based systems (about 1mm^2 for x10 objective). The application of laser scanning over such a large area means that analysis is performed by area and not well, resulting in rapid scanning and analysis of any SBS standard microplate – including high density 1536 well plates – at times which average only 10 minutes a plate. On the Acumen Explorer, reconfiguration of assays into higher density plate formats thus results in a concomitant increase in throughput. In addition, the design enables analysis of the entire well permitting normalisation to total cell number, and overcoming problems of variable stimulation and random cell distribution often observed in screening plates. Where cell culture and assay plate preparation is highly automated, and mistakes can occur, this capability can greatly improve the quality of primary screens.

Recently, the Acumen Explorer has been enhanced by the introduction of 405nm laser excitation. The Acumen Explorer 405 model can read the ß-lactamase reporter gene assay which, until now, has routinely been measured by bulk fluorescence readers. By fully exploiting the ratiometric detection of fluorescent enzyme substrates on a cell-by-cell basis, the instrument provides greater assay sensitivity and up to 100-fold reduction in cell requirement.

Scientists at the **Whitehead Institute for Biomedical Research** and **Massachussetts Institute of Technology** have collaborated to develop CellProfiler cell image analysis software (www.cellprofiler.org). Unlike bundled image acquisition/image analysis/data storage systems, CellProfiler handles image analysis only and as a result, it works with any image acquisition instrument that exports images. CellProfiler uses algorithms culled from the academic literature to quantitatively measure complex cellular phenotypes from thousands of images in a high throughput manner. It accurately identifies cells from even 'difficult' cell types and is more robust to assay conditions such as illumination variation and cell clumping than many commercial packages. Measurements of size, shape, intensity and texture are made for every cell and the data are exportable to any database or spreadsheet program. As a free software package, this software fills an entry-level niche for labs exploring high content screening – its user-friendly interface is usable by biologists without training in computer vision or programming. For high-end users, it offers greater flexibility for assay developers and computer vision researchers

than is available in commercial software packages, because it is an open-source academic project where all the code is available and adaptable by the user. The modularity of the software allows programmers to write custom modules for any visual phenotype. It is anticipated that CellProfiler will become a useful infrastructure for the exchange and development of new algorithms to drive the image analysis side of high content screening.

HCS Reagent Technologies

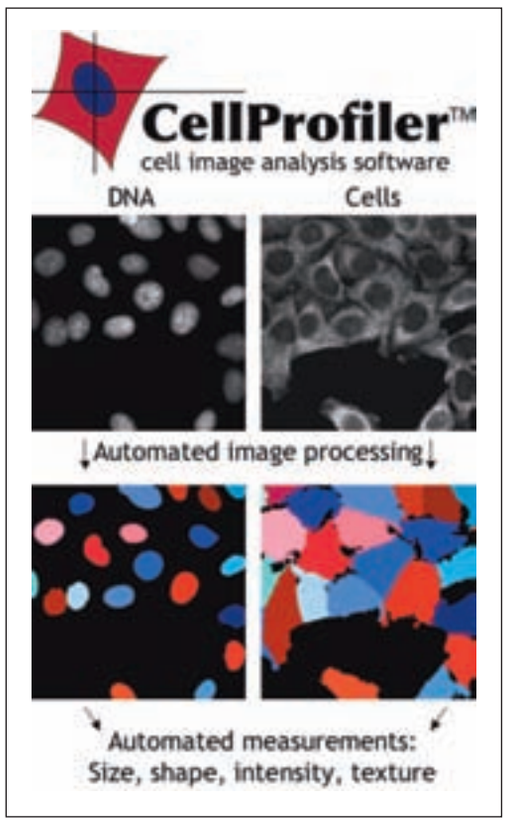
In terms of the tools and technology developments which may impact on HCS over the next few years all alternatives presented were viewed as important (**Figure 6**). However, novel reagent and probes and pathway analysis developments were viewed as the most important in terms of their potential to impact on HCS. New fluorescent reagents make possible improvements in the quality of data generated, the capacity for assaying cell by cell rather than integrated responses and the ability to query multiple analytes per well.

The average breakdown of survey respondents operating budgets (non-instrument purchases) are summarised in **Figure 7**. This shows that approximately 50% of the operating budget (non capital purchases) was spent on bulk reagents (eg antibodies, fluorescent probes, etc) (31%) and consumables (eg special microplates) (21%). In contrast, the other components of respondents' operating budgets were costed at: in house support (eg for expression of target, software etc) for HCS – 14%; off-the-shelf HCS assay kits – 14%; new software purchases (eg HCS algorithms) – 11%; technology license fees for fluorescent proteins etc – 5%; and outsourced (ie third party) HCS assay development or screening – 4% of the operating budget. It seems that is no longer possible to effectively investigate HCS without licensing fluorescent proteins, probes and/or alternative technologies, and up to 73% of Large Pharma and 30% of Small/Medium Pharma & Biotech now have such licences. The main licences cited are green fluorescent protein (GFP) – GE Healthcare, Bioimage: Living Colors – BD/Clontech; Redistribution – Bioimage; and Transfluor – Xsira (now available from Molecular Devices).

The following is an update of some of the latest developments in reagent technologies now available to support HCS:

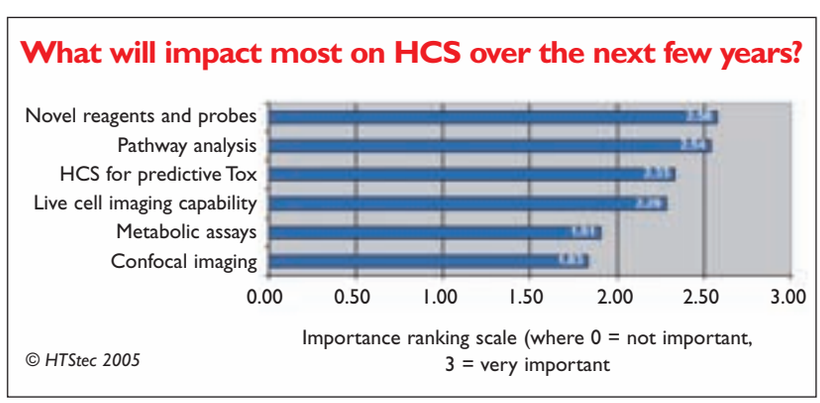
Fluorescent proteins are ideal for use as fusion tags when visualising and tracking proteins of interest in living cells. With distinctly separate excitation and emission spectra, DsRed-monomer (a red fluorescent protein derived from Discosoma sp. reef coral) and AcGFP1 (a green fluorescent protein derived from jellyfish Aequorea coerulescens), are monomeric fluorescent proteins ideal for use in multicolour applications to simultaneously visualise subcellular localisation events of two proteins (**Figure 1**). The BD Living Colors™ suite of novel fluorescent proteins are available to non-profit institutions through the **BD Clontech™** (www.clontech.com/clontech/gfp/index.shtml) product catalog. For-profit institutions should contact BD Clontech for licensing details.

Fluorescent RNAi-Ready pSIREN vectors from BD Clontech generate effective, tagged shRNA



CellProfiler is a free, flexible, academic, open-source software package to be released later this year which allows sophisticated identification and analysis of cells in images

Figure 6



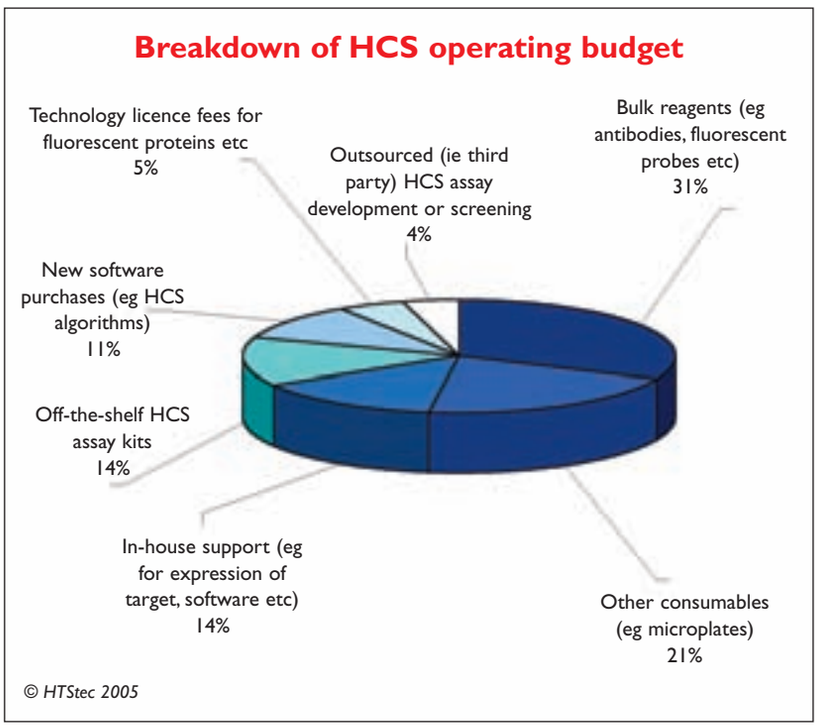
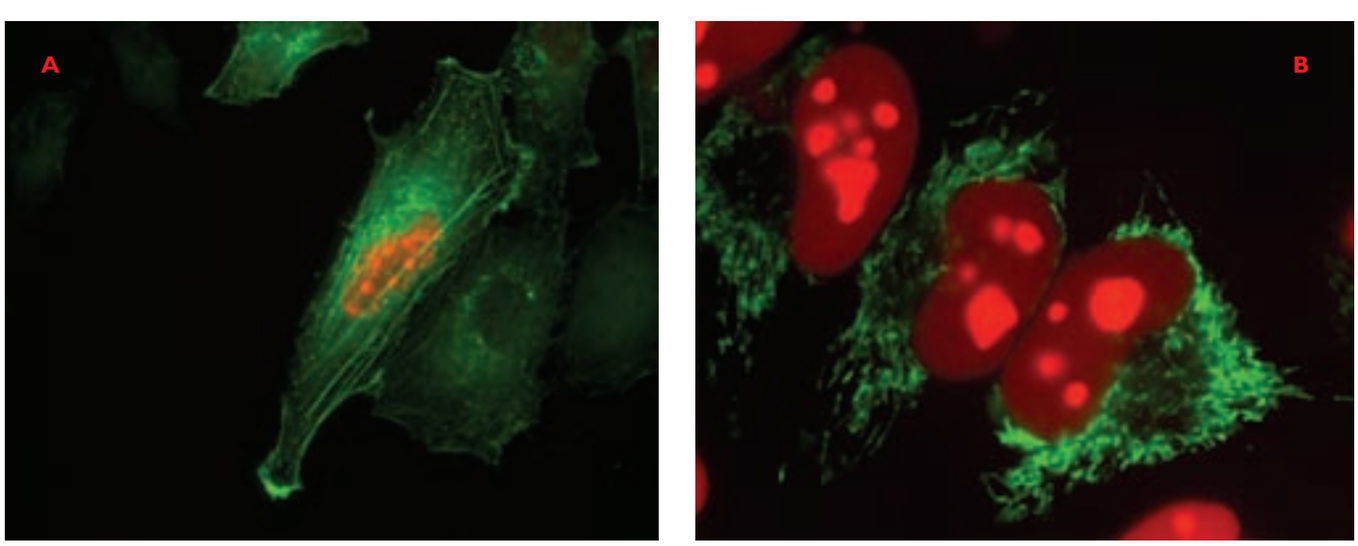


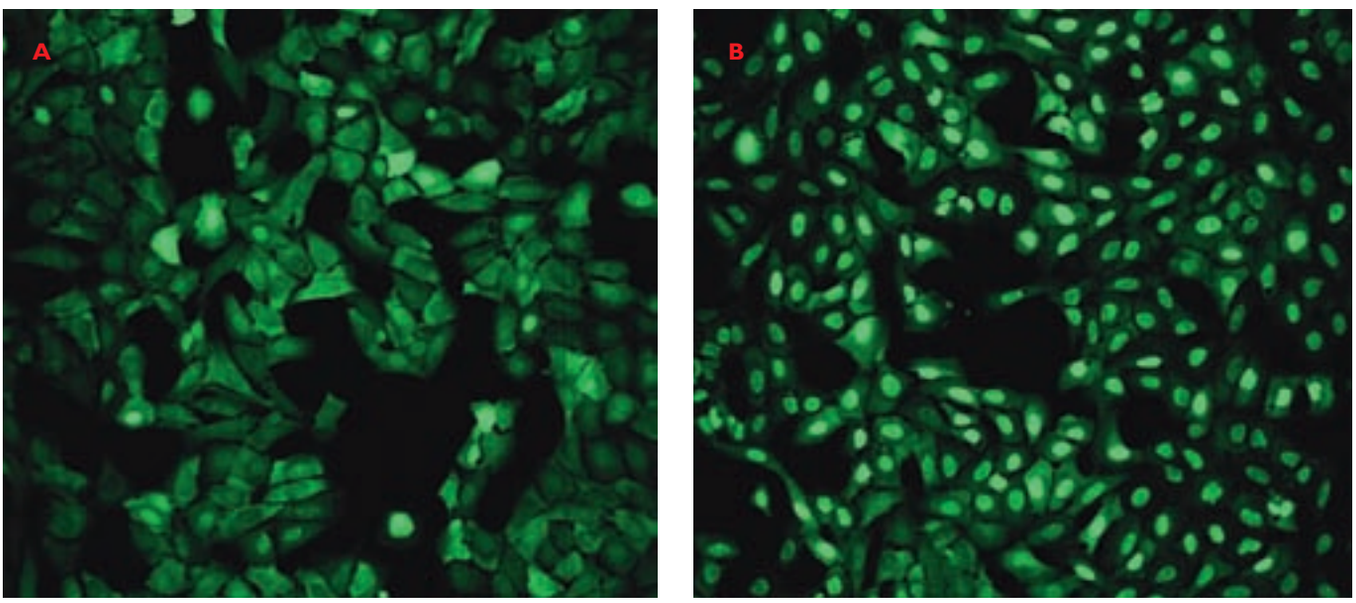
Figure 7

expression cassettes (SECs) which allow direct determination of delivery efficiency, enrichment for cells expressing a silencing construct, and provide a ligation-ready vector for plasmid or viral delivery. Retroviral delivery permits high-efficiency integration of your shRNA construct into the genome of virtually any mitotically active cell, making it an excellent option for consistent and stable suppression of your target gene. To generate stable HeLa cell lines differentially lacking the STAT1 protein, BD Biosciences used its retroviral vector system to encode an shRNA targeted to the STAT1 protein. Western blot, BD Cytometric Bead Assay, and the BD TransFactor Profiling Kit all confirmed a minimum of 90% knockdown of the endogenous STAT1 protein. These compelling results were obtained after 153 days of continuous culture, showing that retroviral-based BD™ Knockout RNAi technology reliably generates persistent, stable gene knockdown in mammalian cell lines. This technology is an excellent means of identifying and profiling stable lines for functional RNAi experiments and long-term disease pathway studies.

BioImage (www.bioimage.com) is a Copenhagen-based biopharmaceutical company which focuses on high information content, cell-based assay technology for early stage drug discovery. BioImage's core technology is Redistribution® which quantifies protein translocation responses in living cells as the primary assay readout. Redistribution® tracks the translocation of relevant protein targets tagged with Green Fluorescent Protein (GFP), and it is used broadly in the discovery process, from target validation through primary and secondary screening, to pathway profiling and lead optimisation. BioImage commercialises Redistribution® through assay development and transfer, licensing, research partnerships and pathway profiling studies. BioImage has an extensive patent portfolio covering Redistribution® technology, and includes Boehringer Ingelheim, Bristol Myers Squibb, Eli Lilly and Merck & Co among its licensees.



BD Clontech Living Colors™ AcGFP1 (green fluorescence) and DsRed (red fluorescence) are ideal for multicolour and fluorescence microscopy applications. **A** DsRed-Monomer-Lamin B (structural nuclear envelope) and AcGFP1-Actin (cytoskeletal microfilaments) in HeLa cells. **B** AcGFP1-Golgi (Golgi apparatus) and DsRed2-Nuc (nucleus) in HEK 293 cells



Bioimage Redistribution assay showing the cytoplasm-to-nucleus translocation response of a GFP-ERF1 fusion construct stably expressed in U2OS cells, with cytoplasmic localisation in vehicle (0.25% DMSO) control wells (**A**), and nuclear localisation in reference compound (10uM UO126) treated wells (**B**) in 384 well plates

A recent example in which Redistribution® provides a unique approach to assaying a Ras/MEKk/erk signalling pathway target is the ERF1 assay. This is a tricky pathway for high content assays since many of the cell lines tend to be stress-sensitive and unstable for screening. ERF is an excellent downstream target and it is very tightly coupled to upstream activity. The ERF1 agonist Redistribution® assay is designed to identify inducers of ERF1 activation by monitoring the translocation of a GFP-ERF1 fusion protein from the cytoplasm to the nucleus in the human ostosarcoma cell line, U2OS. ERF1 nuclear translocation can be promoted by the MEK inhibitor U0126 [1-4] which blocks the Ras/Erk signalling pathway and thereby prevents phosphorylation and cytoplasmic retention of ERF1. In this assay U0126 is used as reference compound with an EC50 value of ~1µM and test compounds are assayed for their ability to induce nuclear accumulation of ERF1. Compounds causing nuclear accumulation of ERF1 could be directly interfering with ERF1 import, acting upstream of ERF1 by interfering with the Ras/Erk pathway, or could be general nuclear import activators/nuclear export inhibitors. The ERF Redistribution® assay is available in both U2OS human osteosarcoma and T24 human bladder cancer cell lines.

Evrogen (www.evrogen.com) offers a wide collection of fluorescent proteins (FPs) for research and commercial use. Ranging in colour from cyan to far-red, Evrogen FPs provide bright fluorescence, demonstrate successful performance in various fusion constructs and have proven availability to create stably transfected cell lines. Besides basic fluorescent proteins intended for cell labelling and protein tagging, Evrogen offers unique photoactivatable FPs allowing direct monitoring of cell, organelle, or protein movement *in vivo*. The Evrogen collection is constantly extended and now includes extra fast maturating green fluorescent protein TurboGFP, allowing it to monitor gene expression at the early stages of biological processes; bright true-yellow fluorescent protein PhiYFP; and red fluorescent protein JRed, specially designed for fusion construction. Evrogen FPs are well distinguished from each other and can be easily used for multicolour labelling. Evrogen photoactivatable proteins include photoswitchable monomeric cyan fluorescent protein, PS-CFP2, capable of irreversible photoconversion from a cyan to a green form in response to 405nm light irradiation, and kindling red fluorescent protein KFP-Red, which reversibly switches from a non-fluorescent to a bright red fluorescent form under the exposure to intense green light irradiation at 530-560 nm. Evrogen provides fluorescent protein expression vectors that can be easily optimised for particular research needs using special Evrogen service. TurboGFP, PhiYFP and JRed related technologies are covered by international

patent applications (in the stage of obtaining national patents) and available for licensing. Evrogen licensing program offers its customers a cost-effective and flexible way to use fluorescent protein technologies for commercial purposes.

GE Healthcare (www.amershambiosciences.com) has now developed a new sensor of the G1/S checkpoint (G1/S CCPM). Together with the G2/M cell cycle phase marker (G2/M CCPM), these cell cycle phase markers can be used to study the effects of cell cycle and cell growth inhibiting drugs in real time in a live cell system. They can be used in screening to uncover novel anti-cancer drugs, toxicology to establish whether a lead compound has adverse effects upon the rate or control of the cell cycle, or in a multiplexed assay to determine the effect of cell cycle position on a separate process. GE Healthcare is also developing other products that can be used in HCS. The Viral Vector Gene Delivery System is a ready to use, high transduction efficiency method for delivering GFP fused genes of interest to a wide range of cell types. Key components of major signal transduction pathways often change their cellular localisation in response to specific stimuli. By enabling these events to be followed in real time in a cell line of choice, more biologically relevant results can be obtained. Viral vectors that deliver reporter genes based on a nitroreductase readout are also being developed.

In addition to its suite of hardware and software HCS solutions, **Molecular Devices** (www.moldev.com) is introducing the Transfluor® GPCR activation assay, now exclusively available from MDC. Specific MetaXpress™ application modules for the Transfluor assay provide validated, robust multi-parametric results. MDC will release general technology licensing and reagents for the Transfluor assay in June 2005. Along with a use licence MDC can provide third party intellectual property for GFP and for the BioImage Redistribution IP for use with Transfluor®. Pricing will be set up for annual by use individual sites. It is the intention to make the use of Transfluor® technology more cost-effective by placing no restriction on the number of receptors screened or number of compound screened. The collection of Transfluor reagents includes cell lines stably expressing ß-arrestin-GFP and plasmids for constructing custom cell lines. Because Transfluor® technology is independent of the particular g-protein coupling, it is very complementary to the use of FLIPR for screening GPCRs. MDC will be now leverage the use of FLIPR and Transfluor® together in developing new assay technology. Taking Transfluor® together with simplified assay development, turnkey application modules, automated imaging instruments, and comprehensive database-driven software for image acquisition, analysis, and informatics MDC will provide a total solution for high-content screening.

Molecular Probes, now part of **Invitrogen** (www.invitrogen.com) is a provider of numerous fluorescent detection reagents suitable for creating HCS assays, including unique non-antibody-based reagents for detecting most organelles and subcellular structures; these reagents bypass the costs and extra steps of antibody-based methods. When antibodies are the only option for detecting a particular

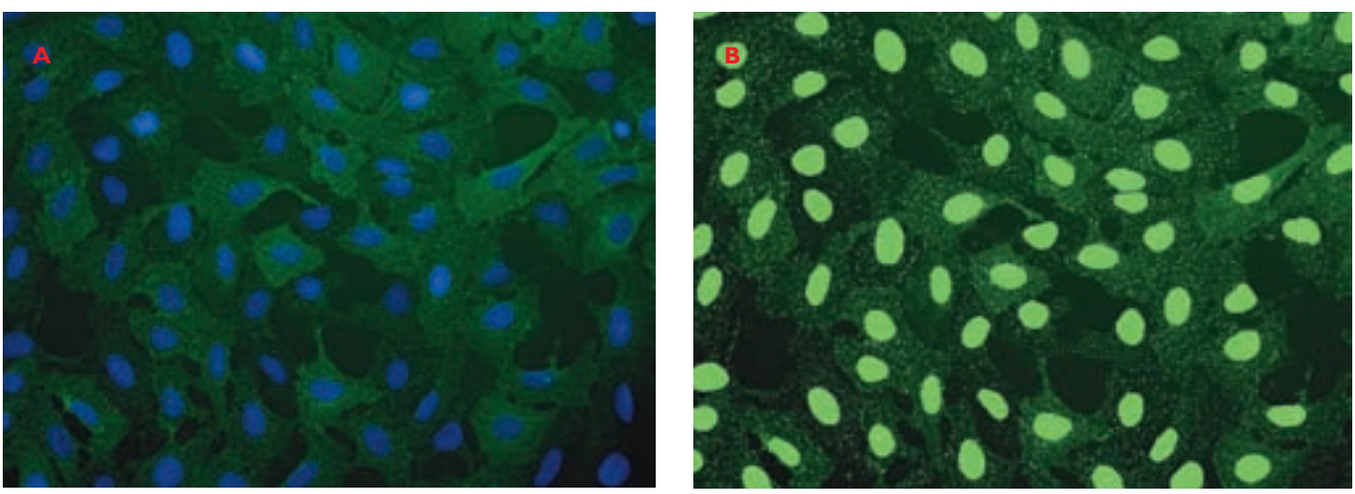
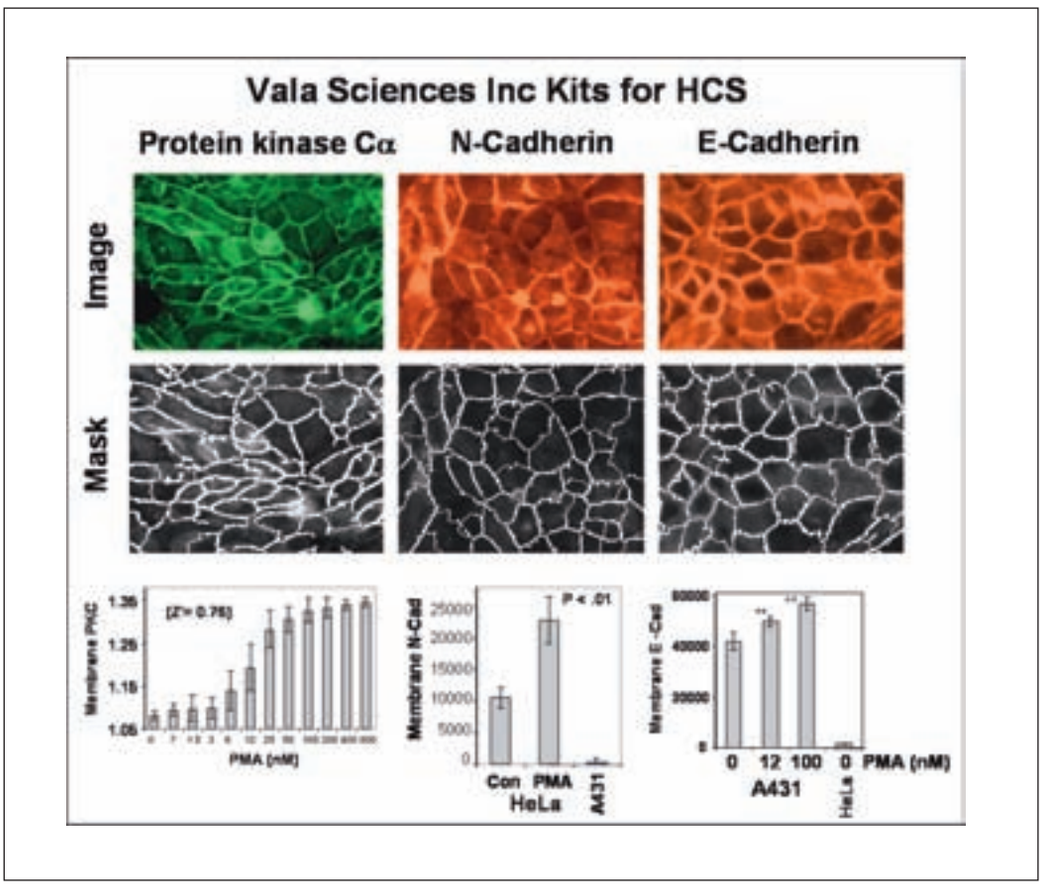


Image **A** of U20S cells where green indicates GFP-ß-arrestin labelled 'pits' formed upon stimulation of GPCR by ligand and blue is DAPI staining of nuclei. Image **B** demonstrates the results of image processing. The image is overlaid with the analysis mask showing that nuclei and 'pits' are correctly identified by Molecular Devices' MetaXpress™ Transfluor® Analysis Module for quantification



Performance of PKCa, N-cadherin, and E-cadherin assays from Vala Sciences. HeLa cells were stained for PKCa (upper left) or N-cadherin (upper middle), and A431 cells were stained for E-cadherin (upper right). The images (acquired with a Beckman IC 100) were analysed by Vala's software to produce membrane or cell junction masks (middle panels). To validate the PKCa assay (lower left), PMA was added for 20min, which increased membrane PKCa with an EC50 of 20nM (each bar, mean a SD for 8 wells). The Z' score of 0.76 signifies that the assay is very robust for screening. For N-cadherin (lower middle), 12nM PMA for 3hr increased membrane protein in HeLa cells by two-fold, while N-cadherin was virtually undetectable in A431 cells (each bar, mean a SD, n=6 wells, Z' = 0.45 for HeLa vs. A431). For E-cadherin (lower right), 12 and 100nM PMA for 3hr induced 18% and 35% increases in membrane protein ($p < .01$), respectively, while E-cadherin was virtually undetectable in HeLa (Z' = 0.86 for A431 vs. HeLa)

analyte, the popular Alexa Fluor® dyes are available as conjugation-ready reactive dyes for labelling primary antibodies, or as labels on secondary antibodies. The Vybrant® kits feature HCS-compatible indicators for viability, proliferation (CyQuant) and apoptosis markers, including the newest mitochondrial transition pore assay for early stage apoptosis. Fluo-4 and other calcium dyes will find extensive use in G-protein–coupled receptor screens, while other ion channel–based screens can be designed using membrane voltage dyes and sodium, chloride and potassium indicators. Assays for lipid degradation, glucose metabolism, general metabolic sensors, nitric oxide and reactive oxygen, including the new MitoSox™ indicator for mitochondrial superoxide, are also among the company's offerings. With a well-conceived combination of gene transfections, RNAi, and fluorescent detection reagents, it is now feasible to study the role of hundreds of genes and lead compounds in automated assays.

Vala Sciences (www.valasciences.com) of La Jolla, CA offers Thora™ image analysis software and reagent kits for the detection and quantification of membrane structure and function imaged by fluorescence microscopes and HCS instruments. Vala Sciences specialises in automated cell image analysis and has just released reagent kits and software for quantification of protein kinase Cα, N-cadherin and E-cadherin for individual digital microscopy users as well as for dedicated HCS instrument assays. Vala's software is compatible with virtually any computer. It has several more cell membrane reagent kits in the pipeline for quantifying membrane, adhesion and junctional proteins, and also provides contract development for custom assays.

High Content Analysis (HCA) versus High Content Screening (HCS)

A prerequisite for HTS of high content assays is their transfer from the development to the screening laboratory. As you can see from the preceding discussion, much progress has been made through the availability of commercial reagent kits and the consolidation of assay protocols between different laboratories and manufacturers, including software algorithms. What is often overlooked, however, is the fact that instrumentation used for high content analysis (HCA) in assay development does

not necessarily meet the different requirements of high content screening (HCS) (**Table 2**). The emphasis of HCA is providing as much information as possible on each cell analysed, and most often for target validation, secondary screening or lead optimisation. The *modus operandi* of HCA commonly involves profiling small numbers of well characterised compounds in assays where the chemistry and biology is well understood. The low sample number allows high resolution analysis of limited numbers of cells (typically about 1,000) with the associated storage of large data files. For these reasons, HCA is predominantly performed using CCD Imagers because they meet all the criteria, especially when performing assays based on immunocytochemistry, whose origins lie in fluorescence microscopy.

In contrast, HCS is geared to profiling compound libraries as fast and economically as possible to generate hits against new therapeutic targets. Detailed information is not required – a simple yes/no readout will suffice – since 99.9% of wells in a typical HTS assay do nothing. To meet these demands, high content assays may require significant modification when transferred from development. They need to be robust (Z' >0.5), ideally fixed endpoint to permit batch processing of large numbers of plates and preferably offer whole well analysis to account for patch effects. In addition, the number of cells required should be low, and data file sizes kept small to cope with the increased throughput and the lack of requirement for reanalysis.

Currently, no single instrument meets the requirements of both HCA and HCS for all biological applications. The CCD Imagers offer multiple strengths for HCA, but for HCS they may be excessively complex, offer limited throughput and create data storage issues. Conversely, PMT-laser scanners offer the high throughput, whole well analysis for HCS, but do not offer the optical resolution or kinetic measurement demanded for certain HCA applications. Many laboratories are choosing to integrate CCD Imagers and PMT-laser scanners in a screening system offering the advantages of both. One approach involves division of assays based on optical resolution: high resolution assays (eg micronuclei analysis, neurite outgrowth) on CCD Imagers; low resolution assays (eg cell cycle, cytotoxicity, apoptosis) on PMT-laser-scanners. Alternatively, where analysis parameters are

Table 2: High Content Analysis (HCA) vs High Content Screening (HCS): are the same tools appropriate?

PARAMETER	HCA – emphasis on content	HCS – emphasis on numbers
Main application area	Therapeutic areas, target validation, assay development, 2° screening, lead optimisation (H2L)	Primary screening, increasingly compound profiling
Number of compounds tested	100s-1,000s	10,000s-100,000s
Throughput needed	Low/medium throughput acceptable	Needs higher throughput
Number of cells required	Algorithms typically report on a 1,000, requires high plating densities	All the cells in a well are processed, requires lower plating densities
Information needed	Want all the info you can get	Only want to know what happened, outcome drilled down to a single number, 99.9% of the wells do nothing
Assay requirements	Need well validated/characterised system with fully understood biology Flexibility to enable live cell or kinetic assay with environmental control	Need robust assay with maximum window, performance criteria to address Z' etc Typically fixed endpoint assays to permit batch processing of large number of plates
Analysis	High resolution analysis of small sample area	Ideally whole well analysis to account for patch effects, auto-fluorescence, cytotoxicity etc
Data storage	Large file size to store all high content information for detailed analysis	Small file size to cope with increased throughput – no reanalysis required

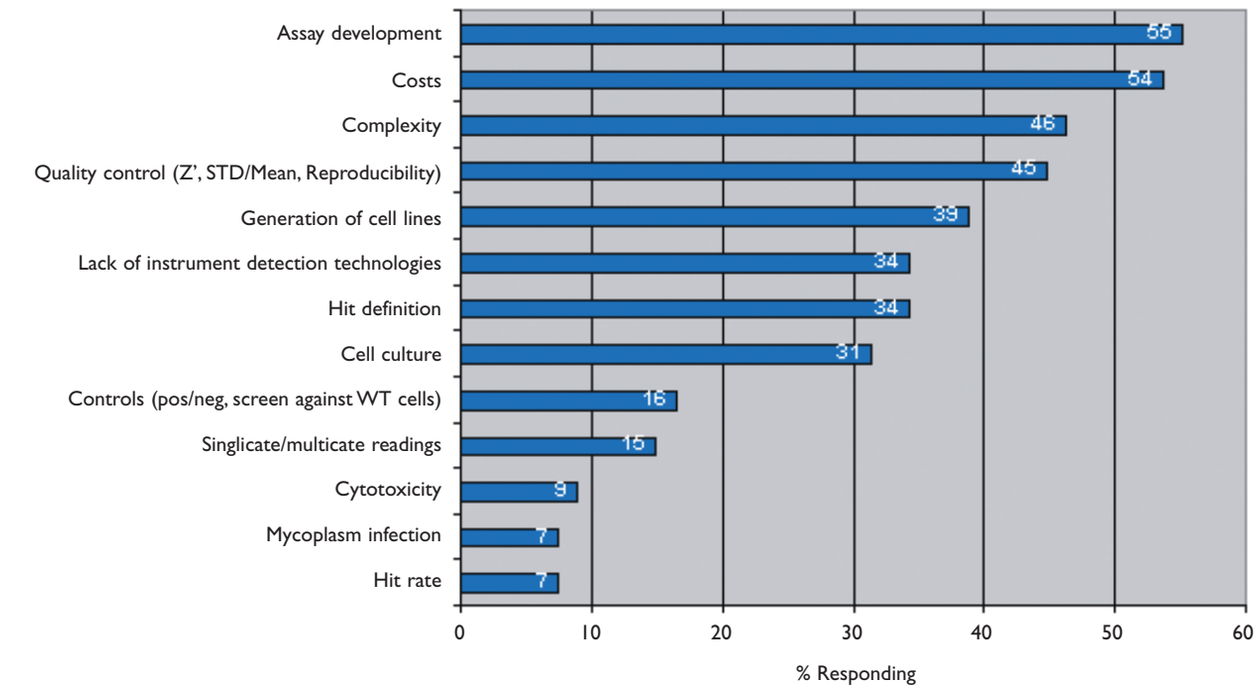


Figure 8

similar on both platforms, assay development, secondary confirmation and lead optimisation is carried out on CCD Imagers, and PMT-laser scanners are reserved for primary screening.

Conclusions

Over a year ago in ***DDW***[1] we concluded that "time will tell if the hype justifies the hope now being invested in HCS!" Clearly HCS has moved on since then and even some of the Pharma which were sceptics regarding the usefulness of high content analysis now have plans to evaluate the technology. Interestingly, assay development and costs were now seen by greater than 50% of survey respondents as the most restrictive limitations in HCS today (**Figure 8**). Complexity, quality control and generation of cell lines were the next most restrictive limitations, affecting at least 40% of respondents (**Figure 8**), all viewed as more limiting than the availability of instrument detection technologies. These findings suggest that HCS has finally moved out of the proving stage into the deployment phase. However, evaluation of high content targets into full diversity primary screening campaigns is not widespread and largely restricted to a few labs. It will be interesting to see if the new tools and technologies reported in this article will enhance the adoption rate by screening (HCS) over the coming years or whether analysis (HCA) will remain primarily a tool used by therapeutic area (target identification/validation), secondary screening, hits-to-leads (lead optimisation) and compound profiling groups.

Acknowledgements

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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 10 market reports on drug discovery technologies and Dr Comley has authored 10 review articles in* **Drug Discovery World.** *Further information on accessing the market report 'High Content Screening Trends 2005' can be obtained by visiting www.htstec.com or e-mail surveys@htstec.com to receive a free copy of the Report's Executive Summary and Table of Contents.*

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