1536 progress

done deal but difficult transition?

After nearly 15 years of challenging maturity 1536 adoption, particularly in primary screening, finally appears to have taken hold. There is now available a broad range of 1536 plate types; an ever expanding variety of tools that make most assays types and profiling approaches doable in 1536; and a diversity of validated reagents and technologies optimised to 1536 plates. Dissatisfaction with some aspects of 1536 liquid handling still persists, but new dispensing options, aimed at addressing concerns, are on the horizon. Despite some reported loss in assay quality (Z' factor) in 1536 relative to standard volume 384, the overall performance of most assays has not been compromised on miniaturisation. Furthermore, 1536 availability has enabled incremental changes in the high throughput potential of some assay technologies to be realised. Overall there probably has never been a better time to reconsider 1536 adoption.

By Dr John Comley

The first 1536-well microplates were manufactured in the mid 1990s, although arguably no significant screening (in terms of numbers of plates used) was attempted in this plate format until the early 2000s. The initial optimism in terms of the payback from assays miniaturisation were largely checked by the realities of problematic liquid handling, which prevented all but the determined from achieving a significant return on their 1536 infrastructure investment. Clearly, although there are organisations who believed it was a done deal some time ago (ie everything in 1536 is doable and all significant problems have been adequately addressed), there are many more labs which have struggled over the past decade to reap the benefits of 1536 and have regarded the implementation process as a difficult transition. This review highlights some of the findings of a recent market survey and report[1], the purpose of which was to dispel the myths that surround 1536, to find out how widely it has been adopted, what success has been achieved and which aspects are still problematic and restrict its wider implementation.

Current status

Feedback from a sample of persons in drug discovery labs worldwide established that implementation of 1536 assays was still primarily confined to Large Pharma, with these facilities representing about 70% of all current use. 94% of survey respondents using 1536 were applying it to primary screening today, with 60% of respondent's total primary screening effort currently done entirely in 1536 plates. 1536 would seem to have progressed from being a niche microplate format

Figure 1: Main motivators/drivers when considering or enabling 1536

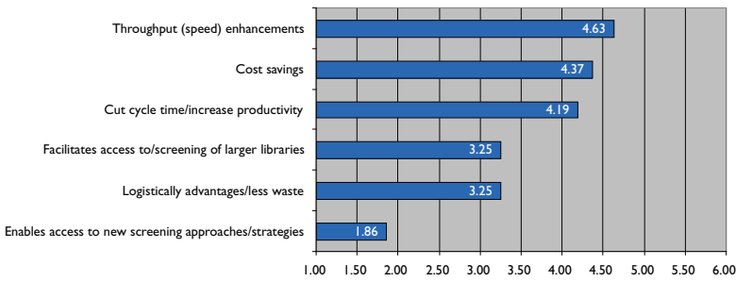
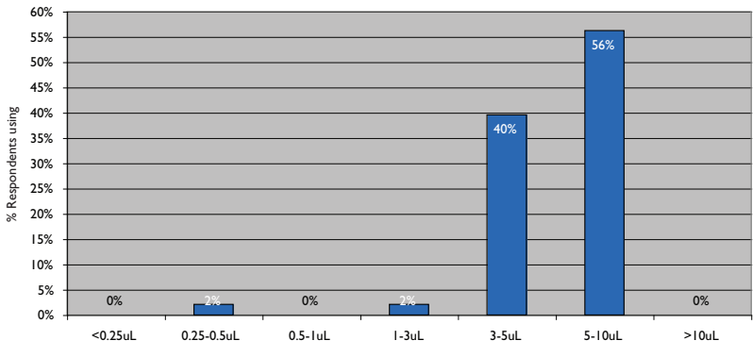


Figure 2: Total assay volume used in 1536 assays



to the mainstay of several companies' screening and profiling operations. Some significant numbers of 1536 plates are finally being purchased and the expected future change in the number of 1536 plates to be used over the coming few years shows a minor increase (0-25% rise). Overall the current rate of adoption for 1536 is expected to be around 9% growth per year.

Main motivation for enabling 1536

Survey respondents ranked throughput (speed) enhancements as the main motivator/driver when considering or enabling 1536. This was closely followed by cost savings and then cut cycle time/increase productivity. Of least motivation was enabling access to new screening approaches/strategies (**Figure 1**).

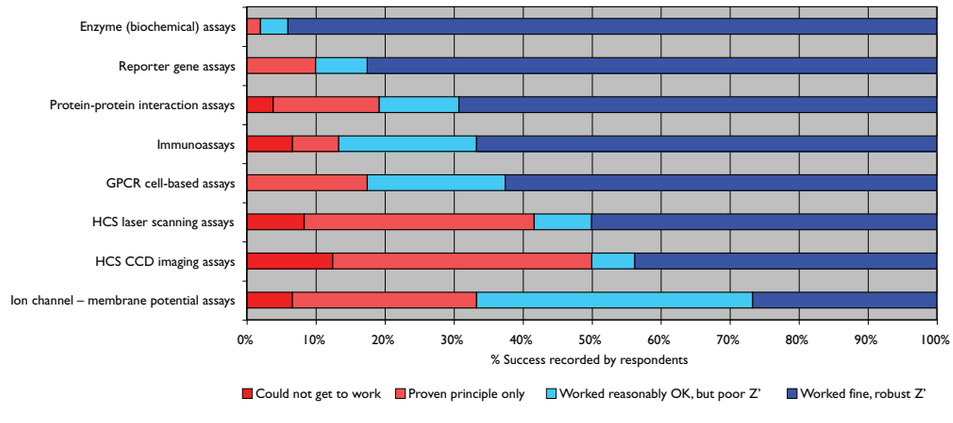
Assay miniaturisation and cost savings

The majority (56%) of survey respondents used a total assay volume of 5-10µL for their 1536 assays. A further 40% of respondents used 3-5µL, with the remaining 4% only using assays volumes below 3µL. The average cost savings reportedly gained in 1536 relative to assays in a 384 standard well plates was 2x to 3x (**Figure 2**).

Assays where greatest 1536 success was achieved

Survey respondents reported greatest success (worked fine, with robust Z') when assaying enzyme (biochemical) assays in 1536, this was followed by reporter gene assays. The target type or assay class where survey respondents reported the highest percentage worked reasonably okay in 1536, but had poor Z', was ion channel membrane

Figure 3: Success in assaying target type or assay class in 1536 plates



potential assays. The target type or assay class where survey respondents reported the highest percentage had proven the principle only in 1536, was HCS CCD imaging assays. The target type or assay class where survey respondents reported the highest percentage could not get to work in 1536, was HCS CCD imaging assays (**Figure 3**).

Liquid handling, still the biggest problem

Only 47% of respondents surveyed were generally satisfied with existing commercially available liquid handling that supports 1536. Tip clogging, particularly on small orifice devices was rated as the liquid handling problem that was the most persistent issue when working in 1536 plates. This was followed by unsatisfactory retrieval of unused amounts of reagents (eg dead volume too high). The need to continuously QC and recalibrate dispenser; uniformity of bead and cell dispensing; progressive valve/tip/probe/pin deterioration; and then instrument robustness, lack of true industrial reliability were the next most important liquid handling issues. All other issues were rated less than 2.50, ie were perceived as only moderately limiting as problems in 1536 plates, this included routine fluidics path clean-up and wash-out (eg takes too long, uses too much fluid) and inadequate percentage CV routinely achieved (**Figure 4**).

Fixes adopted to make 1536 doable

The fix or solution most adopted by survey respondents to make 1536 assays doable in their labs was use of specially designed plate lids (63% using). This was followed by use of plate seals (56% using); use of humidified chambers (51% using); restrict duration of assay incubation (49% using); and then use of plate centrifugation between liquid additions (47% using). Least use was made of removable or reduced concentration of detergents/proteins (7% using); and adding an oil layer to prevent evaporation (only 2% using) (**Figure 5**).

How is mixing achieved in 1536?

The majority (31%) of survey respondents felt that mixing in their 1536 assays was adequately addressed by using liquid handler pipetting or the force of droplet ejection. This was followed by centrifugation (27% using), and then a large proportion that do not use any active mixing (ie rely on diffusion) (24% using). Currently, least use is made of acoustics methods for 1536 mixing, although several acoustics systems have recently emerged to address mixing (**Figure 6**).

Figure 4: Liquid handling problems that are persistent issues in 1536

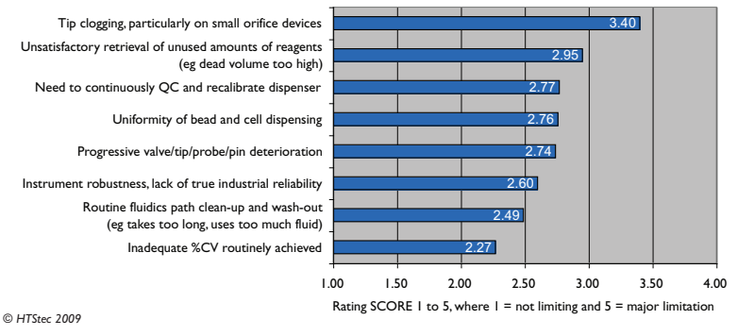


Figure 5: Fixes or solutions adopted to make 1536 doable

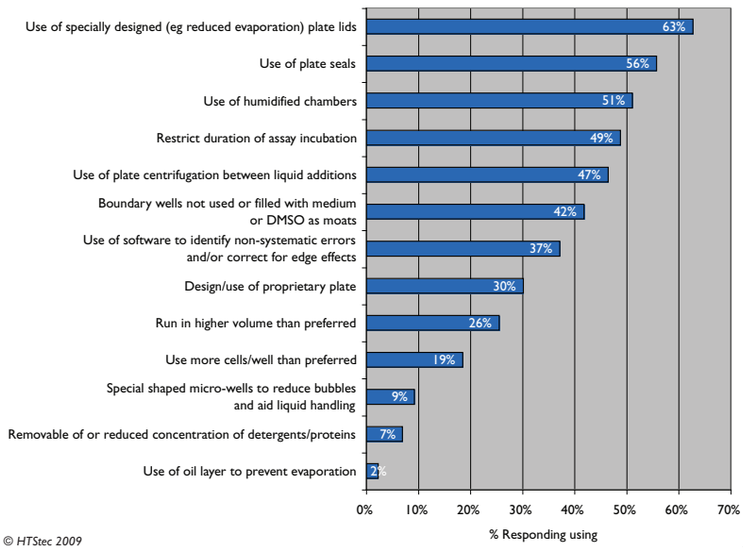


Figure 6: How respondents have addressed mixing in their 1536 assays

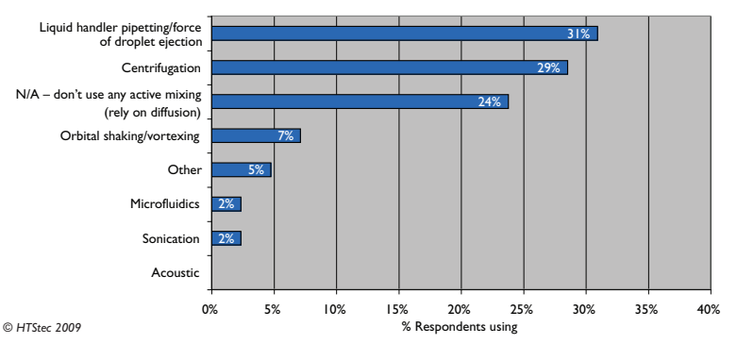


Figure 7: Factors limiting ability to implement 1536 assays

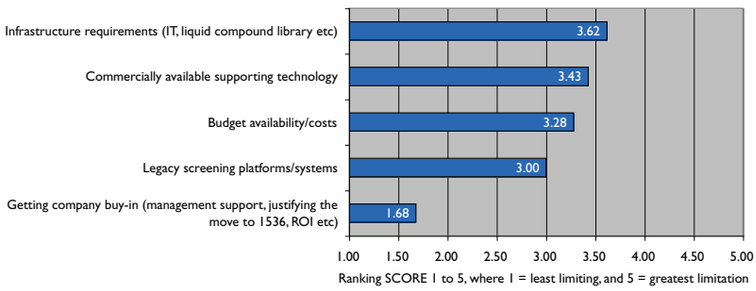
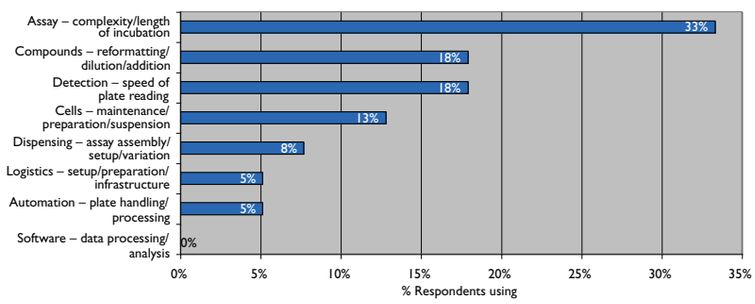


Figure 8: What most limits screening throughput in 1536 plates



What most limited 1536 implementation?

Infrastructure requirements (eg IT, liquid compound library, etc) were ranked as the factor most limiting their ability to implement 1536 assays. This was closely followed by commercially available supporting technology and then budget availability/costs. Least limiting was getting company buy-in (eg management support, justifying the move to 1536, return on investment, etc) (Figure 7).

What currently limits 1536 screening throughput?

The majority (36%) of survey respondents reported it was the assay itself (ie complexity/length of incubation) that most limited the 1536 screening throughput they achieved. This was followed by compounds (ie reformatting/dilution/addition) and detection (ie speed of plate reading), both with 18% reporting. Software (ie data processing/analysis) was reported to have no impact on the screening throughput (**Figure 8**).

Latest vendor offerings

The following snapshots provide details of some of the latest progress vendors have made to further the implementation of 1536 assays. The main criteria in accepting these updates was that the products/development reported on should either be new or have been launched in the past 12 months.

Aurora Biotechnologies' (www.aurorabiotechnologies.com) most recent product developments in 1536-well plate densities have been aimed at broadening the flexibility of well shapes, and plate layouts. Aurora now offers a complete line of round-well and square-well 1536 plates, in low-base and high-base configurations. All Aurora plates are available as Black, White and Clear Plate 'bodies' with three well-bottom choices: solid, 100 micron film and 188 micron film. All Aurora plates are made from 100% Cyclo-Olefin Polymer (COP), and are available Tissue-Culture Treated, Coated, Sterile and Bar-Coded. Aurora 1536-well plates are available with extra evaporation-barrier-wells which provide significant reduction in edge-effects resultant from differential evaporation in low-volume assay plates. A recent addition to Aurora's 1536-well offering is the Multi-Coat Plate which has up to four different coating materials regionally located in different sectors of the plate. Multi-Coat Plates are used for assay-optimisation when working with new or difficult cell-lines as a determinant for selection of best cell environments. Researchers can review the use of Poly-D-Lysine, Collagen, Laminin and Fibronectin on their cell performance in one plate, with one dispensation of cells. All Aurora plates are available with lids, and all plates are easy to seal with adhesive or heat-sealing techniques. Aurora plates are used extensively in fluorescence/luminescence assays, imaging applications and in acoustic-dispensing applications as both source and destination articles (**Figure 9**).

Figure 9: Aurora Biotechnologies' MaKO™-1536 well microplate

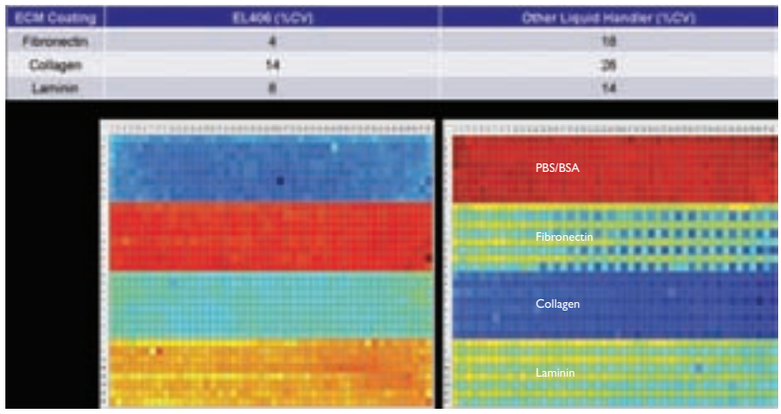


Figure 11: Cell-based assays based on label-free detection with SRU's BIND® platform were prepared in 1536-well microplates using the BioTek EL406™ for the sequential dispensing of microplate coatings (fibronectin, collagen or laminin) and cells expressing the target of interest. The figure shows a heat map comparison demonstrating the reproducibility of protein extracellular mix deposition across a 1536 biosensor microplate for both the EL406™ and another liquid handling device

Figure 10: BioTek's EL406™ 1536-well Microplate Washer Dispenser

BioTek Instruments (www.biotek.com), the global leader in microplate washing technologies, has perfected 1536-well microplate washing and dispensing. The new EL406™ 1536-well Microplate Washer Dispenser provides fast and efficient microplate washing and reagent dispensing in 1536-well microplates. It also can be used with 384-well and 96-well formats for increased versatility. By combining microplate washing and up to three reagent dispensers, researchers no longer need to purchase and maintain separate instruments for each assay wash and reagent dispense step. The EL406 incorporates many of the washing features as the market-leading ELx405™ Microplate Washer. BioTek's patented Dual-Action™ manifold provides fully optimised applications from gentle washing of loosely adherent cell monolayers to complex washing routines. In addition, the EL406 is self-maintained, using integrated and patent pending Ultrasonic Advantage™ to reduce or eliminate clogged manifold tubes – the largest contributor to assay failure. A valve switching module automates reagent switching of up to four different wash buffers. Additionally, researchers no longer need to limit themselves to one reagent dispenser technology. Accurate and precise reagent dispensing is available in both peristaltic and microprocessor-controlled syringe drive reagent dispensing technologies; each with its unique advantages for specific assay requirements. All functions are controlled via Liquid Handling Control™ (LHC) PC software or the instrument's onboard keypad, including multiple custom protocols, instant recall via QuickWash and QuickDispense, and downloaded protocols from LHC software. All EL406 models are BioStack™ Microplate Stacker compatible to automate long and tedious assay processes of up to 50 standard height microplates (**Figures 10 & 11**).

Increasing demands for high-throughput screening has led to the development of the high density 1536-well microplate format. Although numerous laboratories in the pharmaceutical industry now use this format, it has not become universal. One of the key reasons that 1536-well format has not received wide acceptance is that many microplate readers cannot read 1536-well plates in all detection modes. To meet all of the current screening demands, multi-detection capability and plate format flexibility up to 1536 is a prerequisite for selecting a microplate reader. **BMG Labtech** (www.bmglabtech.com) has launched its new HTS multi-detection microplate reader, the PHERAstar FS, which is capable of reading 1536-well format in all detection modes. The PHERAstar FS incorporates BMG Labtech's unique Tandem Technology, a combination of two technological advances – an ultra-fast UV/Vis absorbance spectrometer, and high performance filter-based assay specific modules for all other detection modes. This unprecedented technology allows for experimentation which was previously unimaginable. The PHERAstar FS is able to read full absorbance spectra from 220-1,000nm in 1536 plate format in less than 1s/well. In addition, the PHERAstar FS reads 1536-well microplates in all of the following detection modes: fluorescence polarisation,

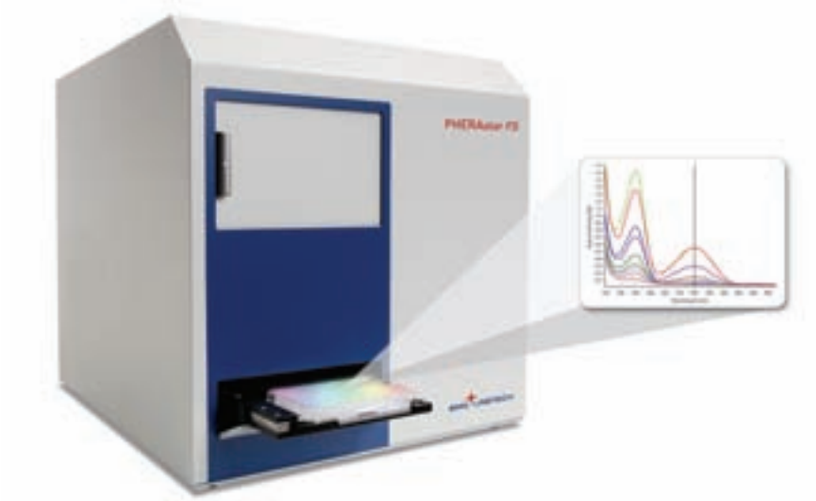


Figure 12: PHERAstar FS – the next-generation HTS microplate reader from BMG Labtech

fluorescence intensity (including FRET), time-resolved fluorescence (including TR-FRET, eg HTRF®), luminescence and AlphaScreen®. Coupled with unmatched bottom reading, on-board reagent injectors, and three excitation sources – high energy xenon flash lamp, nitrogen laser for TR-FRET, and solid state laser for AlphaScreen® – the PHERAstar FS meets all HTS needs (**Figure 12**).

Unlike emissions such as radioactivity or luminescence which are quantity dependent, fluorescence intensity is only related to concentration. This property has allowed homogeneous fluorescence technologies, and **Cisbio**'s HTRF® in particular (www.htrf.com), to be optimised for high density formats. In addition, because all assay components are solubilised and non-bead based, HTRF biochemical assays progressively reaching uHTS 1536-well format have been successfully developed to address a broad number of targets such as kinases, proteases or other metabolic disease-related-enzymes. In most instances, miniaturisation conditions are extrapolated from lower density format by a proportional downsizing of all assay component volumes. In a recent study, Shelton et al[2] exemplified this process with the development of a gamma-secretase assay miniaturised down to a 10µL final volume. The assay displayed a Z' factor over 0.7, shown to be sensitive enough to identify reference modulators such as pepstatin A with micromolar activity. The emergence of cell-based assays has, however, added new challenges requiring innovations in both 1536 liquid handling and microplate design to enable them to be overcome. GPCR screening has naturally been an important investigation area where cells have progressively replaced solubilised membranes. As a consequence, quantification procedures of second messengers like cAMP have been redeveloped for 1536-well plate format. More recently, Bergsdorf et al[3] and Lui et al[4] have described the optimisation of Cisbio's IP-One assay in 1536-well plates. In both cases, the miniaturised assay was compatible with the use of various cell materials, eg adherent, suspended or frozen cells, and easily met the specification for uHTS with Z' factors ranging from 0.62 to 0.78 (**Figure 13**).

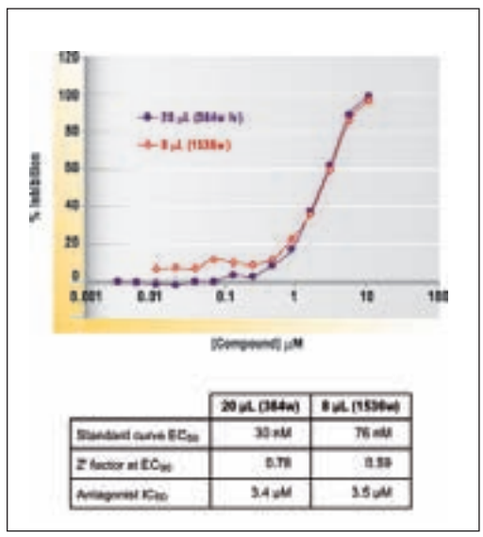


Figure 13: Miniaturisation of the Cisbio IP-One cell-based assay to 1536. (From Tozawa-Takahashi et al. Poster at SBS 11th Annual Conference)

Corning® (www.corning.com) is a global supplier of innovative tools for research, drug screening, cell culture and compound storage. To support recent improvements in automation for ultra high throughput (uHTS) applications and reducing per-well assay costs, Corning has developed a 1536-well microplate optimised for compatibility with low base readers such as FLIPR™. Most recently, Corning has developed 1536-well microplates compatible with uHTS consolidated systems such as Kalypsys and GNF, which require specific and unique microplate attributes. The advances in microplate handling, liquid handling, reader throughput and microplate attributes have culminated in truly uHTS primary screening, and Corning is a leader in the development of microplates optimised for the instrumentation designed to use them. Additionally, Corning is expanding its line of label-free microplates by

introducing the Epic® 1536-Well Cell Assay Microplate designed for uHTS. Like its 384-well counterpart, the Epic 1536-well microplate has an optical biosensor integrated in the bottom of each well. These biosensors monitor changes in light's index of refraction caused by the dynamic mass redistribution (DMR) of intracellular proteins within ~150nm of the sensor's surface. This technology enables a wide variety of cell assays including evaluation of GPCRs, ion channels, chemotaxis, toll-like receptors and viral detection. This novel approach removes the need for fluorescent, luminescent, and/or radioactive reagents and enables researchers to obtain more physiologically relevant data by screening native cells, recombinant/engineered cell lines, and primary cells. The Epic® 1536-Well Cell Assay will be available in late 2009 and will be compatible with the existing Epic® reader (**Figure 14**)

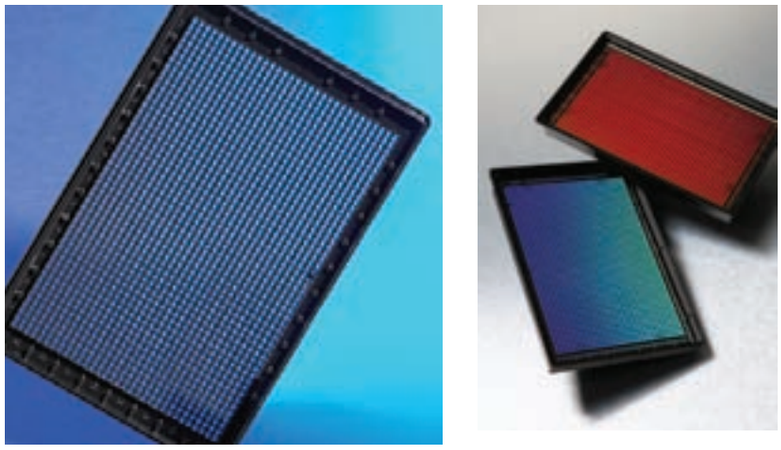


Figure 14: Corning's 1536 low-based FLIPR plate (left) and the 1536 Epic plate (right)

From the very beginning in the late 90's **Greiner Bio-One** (www.gbo.com/bioscience) was the driving force in 1536-well plate formats and still continues to meet the increased interest of 1536 users for specialty plates. Today, the key techniques in common 1536-well applications are biochemical and cell-based assays. These applications present significant challenges in plate performance to address the specific needs for sub-microlitre volume dispensing, low evaporation and confocal imaging in high content screening systems. Recently, plates with features such as recessed wells have been developed by Greiner Bio-One to allow reading on the FLIPR and OPERA systems. In combination with its unique film bottom technology these plates are ideal for confocal imaging and offer the lowest background autofluorescence. Meanwhile, there is a strong demand on more versatile plates which combine features needed for storage, low volume dispensing and imaging applications. One of the first plates that fulfilled these requirements was introduced as a customised plate made out of COC (Cyclic Olefin Copolymer). This resin offers excellent chemical, mechanical and optical properties and is ideal for acoustic dispensing. Two grooves at the plate's perimeter combined with a double baffle lid make this plate ideal for long term applications with extremely low evaporation. In addition, Greiner Bio-One is providing high clarity polypropylene plates for compound storage and acoustic dispensing with superior precision and accuracy on liquid handlers. More plates with major improvements and novel features will be launched by Greiner Bio-One in the summer of 2010 (**Figure 15**).

GNF Systems (www.gnfsystems.com) is a manufacturer of advanced automated systems for genomics and drug discovery. The company's 384 and 1536-well plate compatible uHTS platform has been in industrial use since 2000 and has been well received by a number of academic and pharmaceutical organisations[5]. Best known for its uHTS systems and emphasis on reliability, GNF Systems also manufactures platforms for genome scale cell-based assessment of gene function, genome scale protein production/purification and most recently cell-based compound profiling. Taking advantage of the throughput, compatibility with most types of cell-based assay, 1536-well capabilities and ability to run unattended over nights and weekends, GNF Systems has recently launched a configuration of its uHTS platform optimised for high

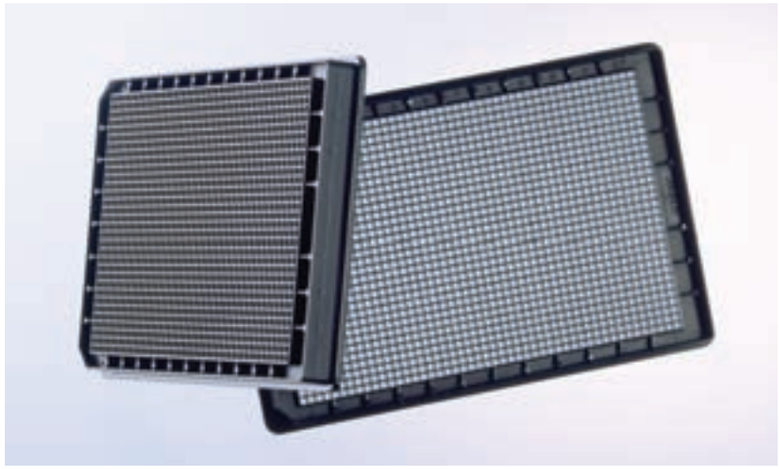


Figure 15: Clear bottom 1536-plates from Greiner Bio-One for high content screening and acoustic dispensing/liquid handling

Figure 16: GNF Systems' compound profiling system; a 384/1536 well compatible high throughput screening system optimised for profiling libraries of compounds against tens to hundreds of cell based assays

throughput profiling of compound sets against collections of cellular assays. Such profiling projects, when compounds are tested in dose-response format, in replicates, against hundreds of unique cellular assays can run into the multiple millions of datapoints per project. The combination of the reliability of the GNF Systems platform and ability to interleave multiple assays simultaneously on the same system significantly enables this approach. Rapid characterisation of collections of molecular entities in a broad array of cellular assays allows researchers to uncover new biological properties and patterns of activity for interesting molecules at an earlier stage in the drug discovery process, thereby enabling positive leads to advance faster and avoiding application of resources to compounds with undesirable biological activity profiles (**Figure 16**).

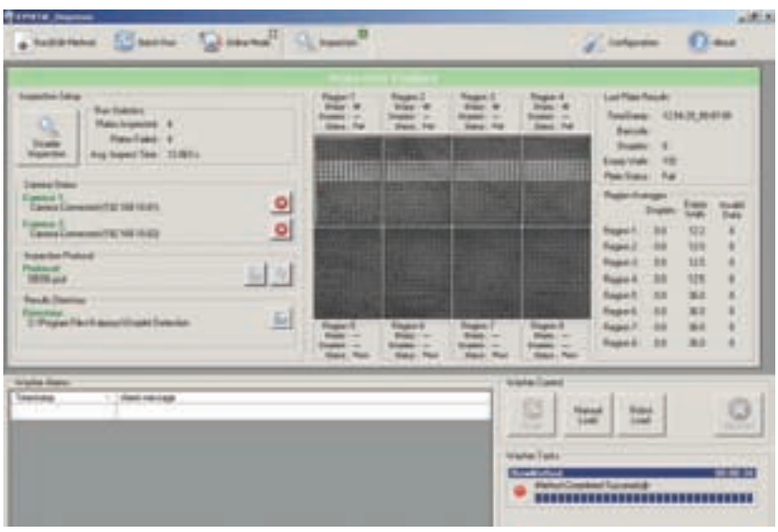


Figure 17: The software interface of Kalypsys Systems' new tip clog detection and recovery system on its 1536 Well Washer/Dispenser

Kalypsys Systems (www.kalypsys.com) understands that the most common challenge associated with most bulk dispensers is the possibility of a clog developing in a dispense tip. Tips on Kalypsys' 1536 Well Washer/Dispenser are separate from the more expensive valves, are easily accessible, and can be changed in seconds. Still clogs do occur, and if left unattended a clogged tip can destroy days of work and waste thousands of dollars in reagents. The Kalypsys Clog Detection System was deployed in 2008 to alert an operator to a fully or partially clogged tip and automatically stop dispensing until an operator corrects the problem. Feedback from customers who have purchased the system has been unanimously positive. Now we have taken the concept one step further and added clog recovery software to our system. When a clog is detected the system can now be set to automatically stop and call an operator or to recover automatically. In automatic recovery mode, the system determines which tip is clogged, disables the clogged tip, and enables one of the remaining 'healthy' tips to compensate by dispensing to the wells previously assigned to the clogged tip. It is finally possible to allow your bulk dispenser to operate completely unattended with no fear of clogged tips and the challenges they could otherwise cause. Kalypsys Systems new Clog Detection and Recovery System will be available in the fall of 2009 as part of Kalypsys Systems' 1536 Well Washer/Dispenser or can be ordered separately to be used on third party systems (**Figure 17**).

HTStec's report[1] found that the number one liquid handling tool capability most needed to advance 1536 dispensing was "compatibility with all types of HTS fluids including cells and beads". **Labcyte** (www.labcyte.com), manufacturer of the Echo acoustic liquid handlers, has taken this to heart and moved beyond the transfer of DMSO. It has calibrated systems for researchers for a wide variety of aqueous solutions. Applications covered by these fluids include cell-based assays, PCR (master mix as well as solutions of oligonucleotides), enzyme assay set-ups of proteins and co-factors, RNAi including transfection reagents and highly viscous solutions that are not easily amenable to transfer by other techniques. Labcyte has also shown the transfer of cells and non-magnetic beads. Acoustic liquid handling eliminates the potential for the number one problem reported by

Figure 18: An acoustically engineered, 1536-well, Echo qualified source plate on the source plate stage of an Labcyte Echo 555 acoustic liquid handler

users of 1536 capabilities, tip clogging, because there is no physical contact with the liquid being transferred. This also eliminates the need for another tool many researchers have requested: an improved capability to wash tips, probes or pin tools. The pre-calibrated Echo systems eliminate the need for researchers to repeatedly have to re-calibrate their systems. Labcyte is able to guarantee excellent precision and accuracy in transfer volume because its Echo qualified plates are acoustically engineered to provide optimum results. With dead volumes as low as 2µL and with the complete elimination of a fluidics path, acoustic transfer reduces waste of samples or reagents. Typical users generate %CV of less than 5% for transfers as low as 2.5nL with these systems that are found in 24/7 use in many pharmaceutical HTS centres. The recently released Echo applications software for 1536 plate reformatting and dose-response set-up have provided researchers with the tools to run as many as 1,400 12-point dose-response curves per hour (**Figure 18**).

Figure 19
MDS Analytical Technologies' new FLIPR® Calcium 5 Assay is a perfect match for 1536-based screens

MDS Analytical Technologies (www.moleculardevices.com) is dedicated to meeting the needs of researchers conducting high-content and high-throughput screens with robust tools that can be scaled for 1536-well plate requirements. The FLIPR® Calcium 5 Assay is a perfect match for 1536-based screens. Its proven quench technology reduces well-to-well variation and a new indicator dye increases signal-to-noise ratio significantly to enable generation of high-quality data even with low cell numbers. No other commercial calcium mobilisation assay gives researchers more confidence in their screening results. The IsoCyte™ Laser Scanning Cytometer can capture an entire 1536 microplate well at micron resolution and supports four-colour imaging, making it ideally suited for cell-based assays, colony morphology studies, and multiplexed analysis of spot and bead arrays. A typical IsoCyte assay is read in 2-5 minutes regardless of format, enabling researchers to transition from 96- to 1536-well plates while achieving up to 16-fold improvement in throughput. From our experience, most high-content screening (HCS) applications appear to transfer well from 96 to 384 to 1536 formats, meaning that it is relatively easy to scale up HCS imaging applications from 96-well based formats to 1536-well based formats as long as the imaging system can accommodate the 1536 plates. MDS Analytical Technologies ImageXpress® Micro or Ultra Imaging Systems respectively offer wide-field or confocal imaging that are fully compatible with 1536 plates, ie they support HCS imaging applications conducted in 1536-well formats, making them an ideal choice for HTS image-based HCS assays. By transferring image-based assays directly to a 1536-well format, researchers reduce costs by one-third compared to 384-well formats, reduce time to scale up, and reduce the amount of consumables and full-time employees (FTEs) required to conduct large screens. Further, with the introduction MetaXpress® Power Core Software, a new high-speed, parallel image analysis option, running HCS assays in 1536-well plates to conduct high throughput imaging for primary library screening is now relatively easy to achieve. In fact, a number of large pharma, including Merck, are now leveraging 1536 HCS exactly for this purpose (**Figure 19**).

Microsonic Systems' (www.microsonics.com) Hendrix SM100 fulfils an unmet need for effective mixing, solubilisation, isothermal thawing and suspension of beads, particles and cells in standard microplate formats, including 1536-well microplates. The company is currently developing several additional applications for the Hendrix SM100. The Hendrix SM100 creates ultrasonic waves in microplate wells with an array of Micro-Electrical-Mechanical Systems (MEMS)-based transducers. The patented Lateral Ultrasonic Thrust™ (LUT) technology based on these MEMS transducers creates strong lateral motion in fluids in addition to the high frequency vibration of the ultrasonic waves. The strong LUT of the Hendrix SM100 can mix 1536-well microplates in seconds ensuring homogeneous testing conditions. Currently, localised reagent concentrations in non-homogenous 1536 wells cause assay data imprecision as seen in high CVs and low Z' values. The ultrasonic waves and improved mixing of the Hendrix SM100 also make possible for the first time, high-speed isothermal compound solubilisation in 1536-well compound storage microplates. Effective solubilisation of compounds in microplates has remained elusive. Compound precipitation in microplates after freeze-thaw cycles causes the effective concentration of the compound in solution to decrease, potentially causing active compounds to be missed during testing. Ultrasonic, isothermal thawing with the Hendrix SM100 now provides a rapid, automated way to transition microplates from frozen storage to automated systems. Isothermal thawing of solids to liquids changes the state of matter without the compound degradation that can be caused by heating. The Hendrix SM100 is able to keep cells, beads and particles in suspension in 1536 wells without damaging the beads or particles or affecting the viability or morphology of cells allowing for accurate dosing during transfers and for improved precision in binding assays (**Figure 20**).

Figure 20
Microsonic Systems' Hendrix SM100 microplate mixer

PerkinElmer (www.perkinelmer.com) continues to develop the high throughput capabilities of its assay portfolio. The company initially reported that its LANCE® *Ultra* Serine/Threonine kinase assays can be automated in miniaturised formats (both 384-well and 1536-well) reducing assay costs while retaining HTS robustness. Current data demonstrates that the LANCE *Ultra* platform, combined with the JANUS® MDT Automated Workstation, is capable of automation and substantial miniaturisation of Ser/Thr kinase assays. In one study[6], two Ser/Thr LANCE *Ultra* kinase assays were initially developed and optimised in 384-well plates (20µL assay) using manual pipettors. Both assays were found to be suitable for HTS purposes as illustrated by Z'-factors of 0.80 (IKKβ) and 0.82 (Akt1). Automation and miniaturisation to the low-volume 384-well (10µL assay) and 1536-well (5µL assay) formats were then conducted by maintaining final concentration of reagents in both the kinase reaction and detection steps. Automated and miniaturised assays showed a satisfactory assay quality for both the low-volume 384 and the 1536-well formats (Z' above 0.7 and 0.6, respectively). Further work will

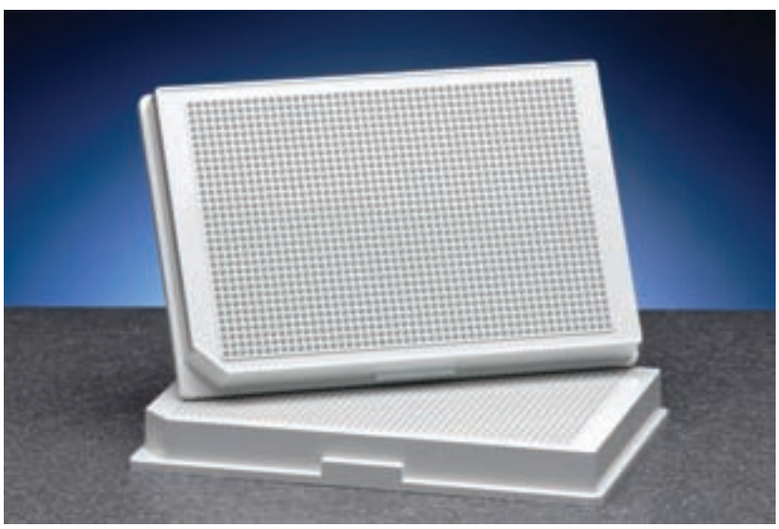


Figure 21: PerkinElmer's new automation-friendly 1536 well AlphaPlate®. Note the plate is engineered to be the same height as a standard 96- or 384-well plate

now include testing of its JANUS MDT NanoHead™ dispense head combined with PerkinElmer's new automation-friendly 1536-well plates (engineered to be the same height as a 96- and 384-well plates, and with extraordinary well flatness) in order to reduce the assay volume to ≤2µL. Another PerkinElmer-assisted advance in 1536-well technologies relates to the company's AlphaScreen® SureFire® "All in One Well" assay. In a recent deployment at the National Institutes of Health's Chemical Genomics Center, researchers were able to miniaturise a whole-cell assay process as part of a qHTS library screen using the SureFire pERK assay, and achieved good signal/background ratios in qHTS using their Kalypsys 1536 automated robotic platform with very few failures (only six failed plates out of 432 plates screened and 662,552 wells) (**Figure 21**).

Redd and Whyte (R&W) (www.reddandwhyte.com) have developed two new products for launch in October 2009 specifically designed for dispensing cells, beads and biochemical reagents into 1536-well plates at low volumes. The R&W Nanoid is an 8-channel 1536 dispenser which uses innovative new electro-magnetic bellows (EMB) pump technology. The EMB pumps were designed by Fluilogic (www.fluilogic.fi) for R&W to produce outstanding dispensing accuracy when working with even the most difficult reagents across a range of 50nL to 3µL. Unlike syringe pumps, the EMB has no moving parts but uses an electromagnetic actuator to provide a pulse which can be modified through the system software to deliver almost infinite control by the operator when pushing or pulling fluid. Protocols can be designed for optimum performance bespoke to the properties of individual reagents. Nanoid has eight EMBs, each fitted with an integral pressure sensor which detects any blockages; the system software then replaces any blocked tips automatically. The second new product from R&W is the Microid 1536 dispenser, which is a pressurised system using an innovative new design of electronic solenoid valve, with simplified fluidic pathways and integral filters to significantly reduce the likelihood of blockages occurring, particularly when working with cells and proteins. Microid is fitted with auto-calibration, which provides outstanding repeatable accuracy. High speed delivery allows a 1536 plate to be filled with 500nL in just seven seconds at a CV of 3%. Microid has a dispensing range of 50nL to 50µL with a dead volume of less than 200µL. R&W have worked with several pharmaceutical companies in the development of both instruments, which have been specifically designed to be used as standalone instruments or fully integrated into automated robotic systems (**Figure 22**).

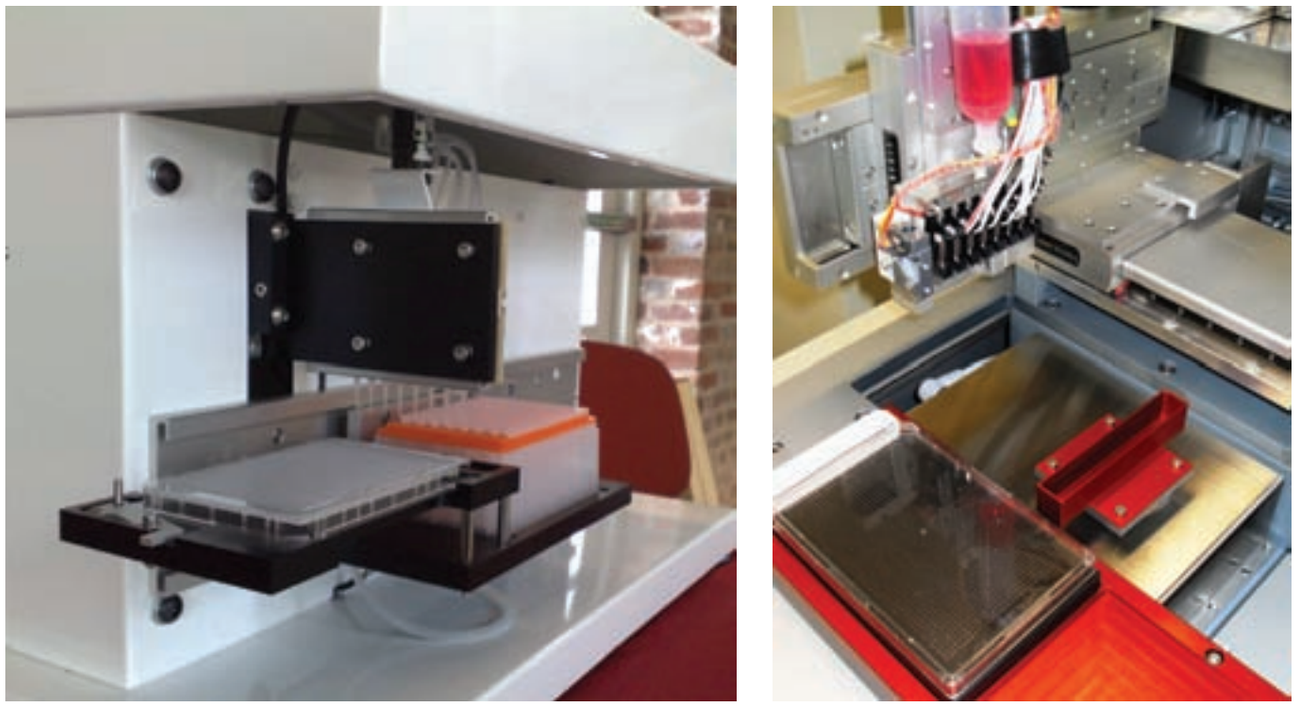


Figure 22: Redd and Whyte's Nanoid with block detection and auto tip replacement (left) and Microid with auto-calibration (right)

Figure 23: Roche Applied Science's LightCycler® 1536 Real-Time PCR System (right) and its 1536-Multiwell PCR Plate shown top and bottom (left)

The LightCycler® 1536 Real-Time PCR System from **Roche Applied Science** (www.lightcycler1536.com) is a novel high-throughput platform, precisely engineered for miniaturisation and parallelisation of high-speed real-time PCR analysis. This powerful system includes instrumentation, software, reagents and a uniquely engineered disposable. This system has been designed for true high-throughput analyses of gene expression and genotyping applications, integrated into an automated workflow environment. The LightCycler® 1536 Instrument is based on the well-established LightCycler® 480 platform, supporting mono- and dual-colour applications (excitation at 465nm and 533nm, and detection at 510nm and 580nm) to detect green intercalating dyes and hydrolysis probes. The innovative LightCycler® 1536 Multiwell Plate (1536-well format) is based on unique Thermaxis® technology and is manufactured under licence from IT-IS International Limited, under Patent Number US 60/970401. The plate enables low-volume PCR reactions (0.5-2.0µL), and facilitates, in combination with the high-performance block cycler technology (Therma-Base), short PCR protocols (<50 minutes). This plate-based format can be easily customised to changing research needs. The LightCycler® 1536 Software is able to generate robust, basic real-time PCR results (eg, Cps, endpoint, and slope values) for gene expression and genotyping analysis, and allocates and manages data in both network and LIMS environments. Together with the next generation of real-time PCR reagents, the RealTime ready chemistry, the LightCycler® 1536 System supports most demanding applications in automated high-throughput low-volume workflows, and offers the industry-first, pipetting error-tracking surveillance capability (**Figure 23**).

Conclusions

It is clear from the market summary and the vendor contributions to this article that 1536 finally seems to have come of age. It has taken nearly 15 years of difficult maturity to reach the point where not only are increasing numbers of end-users reporting 1536 adoption, particularly in primary screening, but many vendors are renewing their interest in the plate format and seizing the opportunity to bring to the market new developments that will support a greater diversity of 1536 assays. There is now available a broad and diverse range of 1536-well plate types, with many recent additions claiming unique plate features and benefits (eg from Aurora Biotechnologies, Corning, Greiner Bio-One, Labcyte, PerkinElmer and Roche Applied Science). An ever expanding variety of tools not only makes most assays types and profiling approaches doable in 1536, but they are now possible with greater precision, ease of automation and unattended run capability (eg from Corning, GNF Systems, Kalypsys and Microsonic Systems). Although many regarded 1536 detection as a done deal nearly a decade ago with the launch of PerkinElmer's ViewLux, only recently has the full range of detection modes and capabilities become available on 1536 readers, with wider support for AlphaScreen (eg Beckman, BioTek, BMG Labtech,

and PerkinElmer), HCS imaging fully supported (eg MDS Analytical Technologies), and even label-free (eg Corning and SRU Biosystems) now available. The next big leap in 1536 detection still awaits the emergence of the second generation of multi-mode whole plate imagers, featuring new camera technology, bench-top footprint and significantly reduced price-tag. Dissatisfaction with various aspects of liquid handling does, however, persist and is the most commonly voiced concern about 1536. Despite significant advances in dispensing over the past decade, particularly in relation to acoustic droplet ejection (eg Labcyte and now EDC Biosystems), the majority of existing liquid handlers cannot be regarded as meeting all user expectations in terms of robustness and reliability. However, light may now be at the end of the tunnel, as several new liquid dispensing developments are revealed for the first time in this article (eg from Kalypsys, Labcyte, PerkinElmer and Redd and Whyte), and 1536-well microplate washing may at long last have been perfected (see BioTek). In addition, it is now common place for assay vendors to validate and optimise their reagents and technologies to work better in 1536 (examples from Cisbio, Corning, PerkinElmer, MDS Analytical Technologies, Roche Applied Science are mentioned in this article). Despite some reported loss in assay quality (Z' factor) in 1536 relative to standard volume 384, the overall performance of most assays has not been compromised on miniaturisation. Furthermore 1536 availability has permitted incremental changes in the high throughput potential of some assay technologies to be realised (eg HCS imaging and real-time PCR) and has enabled screening of large libraries against targets where access to reagents was limited. In addition, some advocates of 1536 have concluded that the cost savings effectively de-risk the higher risk screens and promote an opportunistic approach to exploratory drug discovery. In summary, there probably has never been a better time for the more prudent followers of new technologies, who need cost savings matched with higher throughput and speed, to put aside some their concerns, myths and prejudices about 1536, and give serious consideration to the format's adoption. DDW

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