DISPENSE & IMAGING ASSAYS

moving towards a more balanced use of both fluorescence and flash luminescent readouts

Progress towards the uptake and adoption of flash luminescence readouts in screening were the subject of HTStec's recent Dispense & Imaging (D&I) Trends 2007 report. The report suggests that the transition process may be less of a displacement of fluorescence with flash luminescence, but rather a wider more balanced implementation of both readouts. This trend is facilitated by the recent availability of several highly flexible dual mode D&I instrument platforms, improvements to existing and new fluorescence assay kits, complemented by increases in the flexibility and sensitivity of photoproteins and a transformation in the assay protocol (wider use of 1536, suspension and primary cells, frozen cell aliquots, etc). Overall, we can expect D&I assays using both fluorescence and flash luminescence readouts to continue to perform a leading pivotal role in the primary screening of GPCR and ion channel targets for many years to come.

By Dr John Comley

We previously have reported in *DDW* on Dispense and Imaging (D&I) instruments (new flash imagers)[1], and on the changing dynamics of D&I assays[2]. HTStec recently revisited this subject with the publication of its second Dispense & Imaging Trends 2007 Report (November 2007)[3], to learn how the use of these technologies has progressed. This article is based around feedback obtained in that report. The objectives of the new market study were to comprehensively document the current status, future need and user requirements for D&I instruments, readouts and assays. In particular the aim was to provide supporting evidence on the extent of the apparent shift in D&I assays away from fluorescence towards the wider use of flash luminescence, what is motivating that shift, and what is limiting the transition. The report also looked at whether recent vendor support in this area (eg new dual instrument developments, enhanced 1536 assay capabilities, improved no-wash assays, brighter cell lines, frozen assay ready cell aliquots, new fluorescence reagent kits, etc) will facilitate the mixed use of a broader range of D&I applications (ie based on both fluorescence and flash luminescence readouts) in the future.

Figure 1: Distribution of all D&I data points generated between readouts

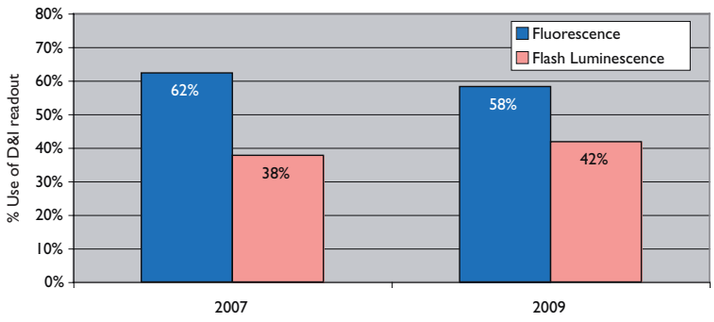


Figure 2: Use of 1536 plate format in D&I assays

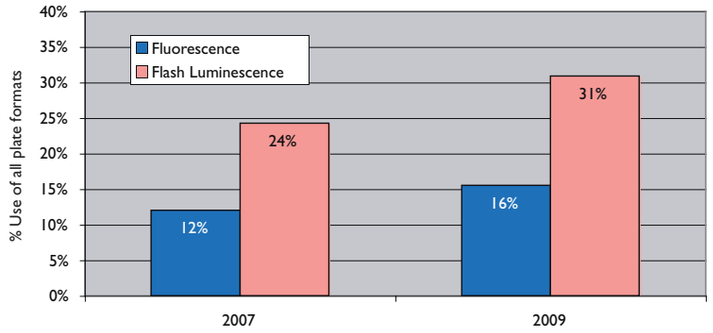
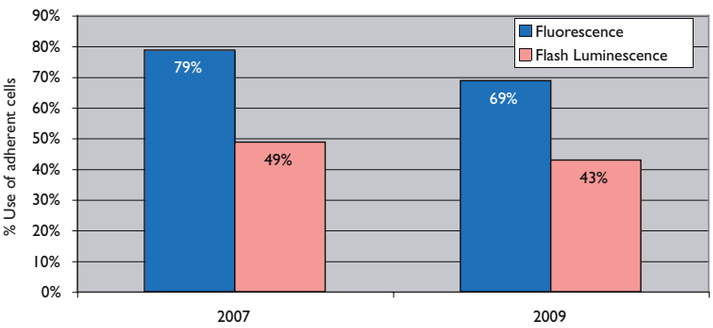


Figure 3: Use of adherent cells in D&I assays



Readouts flexibility still a high priority

The survey showed that 62% of all D&I data points generated today (2007) were based on fluorescence, the remaining 38% were done using flash luminescence. By 2009 the proportion of flash luminescence data points is expected to increase slightly (to 42%), but overall mixed use of both readouts will remain significant and readout flexibility appears is of major importance to end users (**Figure 1**).

Greater use of 1536 well-plates anticipated

The 1536-well plate format is currently (2007) used in only 12% of D&I fluorescence and 24% of flash luminescence assays. For both readouts the remaining percentages are made up of a mix of all the other formats, predominantly 384-well plates. With vendors significantly upgrading 1536 capability on their D&I instruments, it is not surprising that in the future (2009) increases in the use of 1536-well plate format are expected in both fluorescence (up to 16%) and flash luminescence assays (up to 31%) (**Figure 2**).

Use of suspension and primary cells increasing

Adherent cells are currently (2007) used in 79% of D&I fluorescence and 49% of flash luminescence assays. In each case the remaining percentage are assumed to be made using suspension cells or suspensions of adherent cells. In the future (2009) reductions in the use of adherent cells are expected in both fluorescence (down to 69%) and flash luminescence assays (down to 43%) (**Figure 3**).

Recombinant cells are the main cell type used in 78% of all D&I assays today (2007), followed by transiently transfected cells (12% use) and then primary cells (8% use). In future the use of recombinant cells in D&I assays is expected to decline (down to 64%), with greater use made of transiently transfected cells (up to 18% use) and the use of primary cells expected to nearly double (up to 14% use) (**Figure 4**).

Strong interest in adopting flash luminescence

The majority (42%) of survey respondents rate their interest/status in adopting flash luminescence D&I assays (like aequorin or Photina®) as "considering the practicalities of using flash luminescence D&I assays". To date, around 26% of respondents answered they had "already implemented and are using flash luminescence D&I assays", with a further 16% answering they were

"in the process of implementing flash luminescence D&I assays" (**Figure 5**).

Progress made in using flash luminescence

The majority (35%) of survey respondents answered that the progress they had made using flash luminescence D&I assays (such as aequorin or photina) in screening was "none – still at the planning stage". However, 16% answered they had made "extensive progress – operational screening, with several fully automated screens completed", with a further 13% answering they had made "high progress – operational screening, but with limited or no automation applied" (**Figure 6**).

What's driving and limiting the use of flash luminescence?

Improved assay sensitivity (better quality hits) was rated as the highest motivation considered when evaluating new technologies (such as flash luminescence using aequorin and Photina®). This was followed by screening cycle time improvements. Least motivation was derived from aspects related to cell provision, using less cells and/or frozen cells aliquots (**Figure 7**).

The lack of a budget to purchase new detection instruments as rated the most limiting challenge encountered in the transition process to flash luminescence assays. This was closely followed by ability to convert existing fluorescence cell lines to flash luminescence and then sourcing cell lines. Least limiting in the transition process to flash luminescence assays was scaling up production of cells or frozen cell aliquots, applying required automation to detection instruments and setting up necessary lab infrastructure (**Figure 8**).

Most interest in dual mode instruments

When survey respondents were asked to rank their future interest in purchasing different types of D&I instrument platforms, a dual mode D&I instrument based on a single camera with readout selected through software was ranked most interesting. A system with this configuration is obtained when the aequorin camera option is purchased on the MDS Analytical Technologies FLIPR^{TETRA}® system. The next most highly ranked platform was also a dual mode D&I instrument with two different cameras permanently installed. Systems with this configuration included both the Hamamatsu FDSS 6000 and its newly launched FDSS 7000. Lagging considerably behind in respondent's purchasing

Figure 4: Use of different cell types in D&I assays

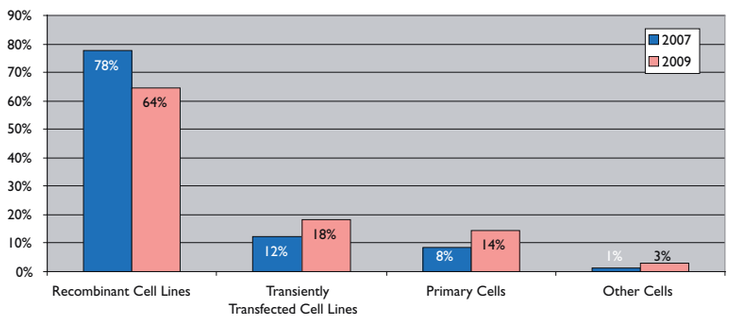


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Figure 5: Interest/status in adopting flash luminescence D&I assays

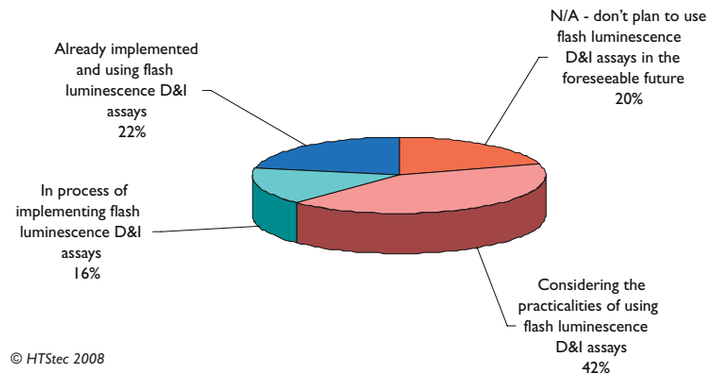

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Figure 6: Progress made using flash luminescence D&I assays in screening

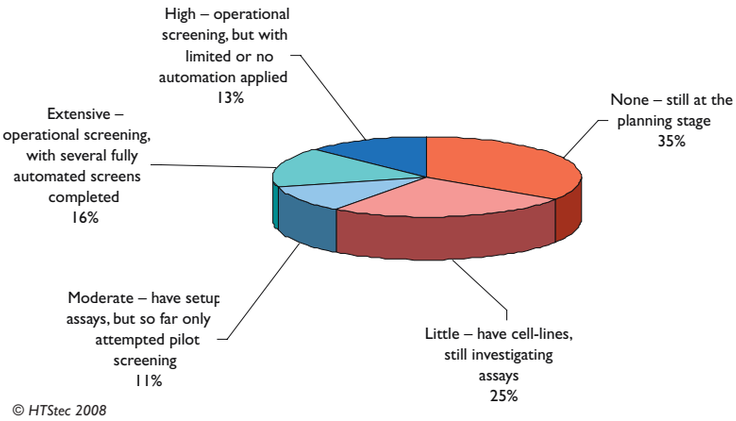

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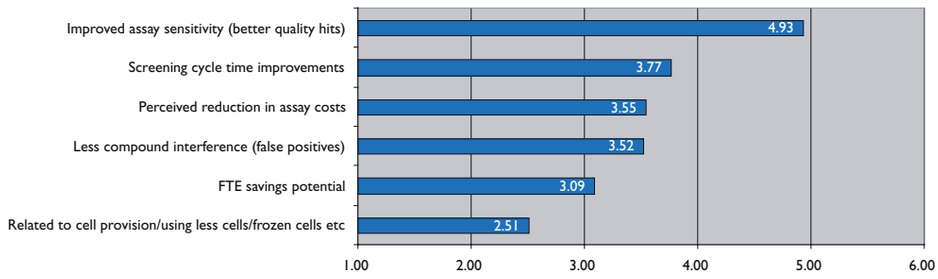
Figure 7: Motivation for evaluating flash luminescence using Aequorin or Photina®



interest was a dual mode D&I instrument with one camera only installed, where the user swaps out the camera to change the readout. A system with this configuration is obtained when the fluorescence only camera option is purchased on the MDS Analytical Technologies FLIPR^{TETRA}® system, where upgrade to the aequorin camera option then gives enhanced flash luminescence sensitivity combined with fluorescence. Least overall interest expressed in purchasing was single mode luminescence or fluorescence D&I instruments, such as PerkinElmer's LumiLux or CellLux imagers. It should be noted that this trend is among all survey respondents who come from a variety of organisations and diverse lab types and reflects the overall general desire for maximum instrument flexibility. However, some labs which are running the same assays type routinely require less flexibility, in these labs a dedicated single mode instrument, with optimum sensitivity, may be a more attractive option (**Figure 9**).

Obstacles in the future use of D&I assays

Survey respondents rated the need to have good stable cell lines as the biggest obstacle (major limi-

Figure 8: Challenges encountered in the transition from fluorescence to flash luminescence

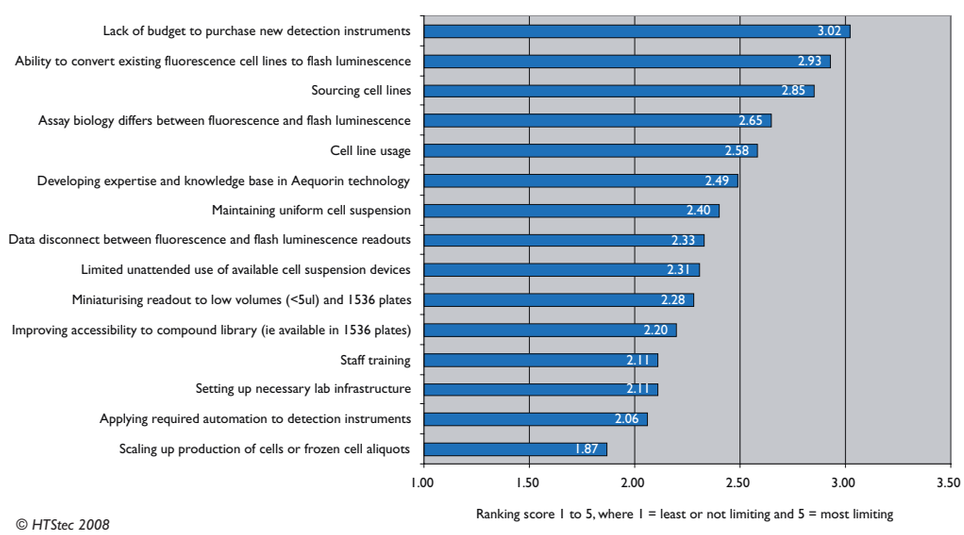
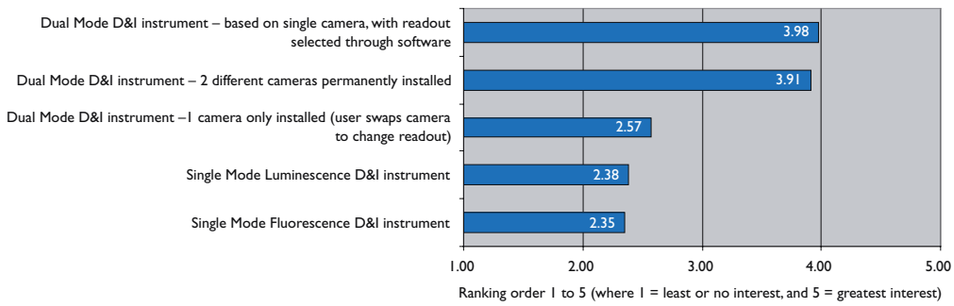


Figure 9: Interest in purchasing different D&I instrument platforms



tation) to their future use of D&I assays. This was closely followed by validating cell lines to have the correct biology of interest, then cell line availability and pricing, and then cost/availability of equipment. Transferring cell passage assays to frozen aliquot platforms was the obstacle rated least limiting (**Figure 10**).

In **Table 1** we summarise the commercial sources for D&I assay technologies that are reviewed in this article.

D&I instrument vendor updates

The **Hamamatsu** (www.hamamatsu.com) FDSS 7000, which will be launched at the SBS conference in April 2008, is the newest addition to the expanding FDSS product line for cell-based assays using fluorescence or luminescence probes. Kinetic assays using fluorescence calcium dyes such as Fluo-4 or flash luminescence like Aequorin are commonly used in compound screening for GPCRs and ion channel targets. However, reliable miniaturisation to 1536-well plate formats remains a challenge and the FDSS 7000 hopes to address this issue. The previous FDSS 6000 has been well accepted since its introduction six years ago. However, its circular rotation platform, with many advantages, prevents it from supporting the 1536 well plate format. By contrast the FDSS 7000 uses 'X-Y' movement for precise alignment of 1536 tips into wells. However, the FDSS 7000 preserves

Figure 10: Potential obstacles proving limiting in future use of D&I Assays

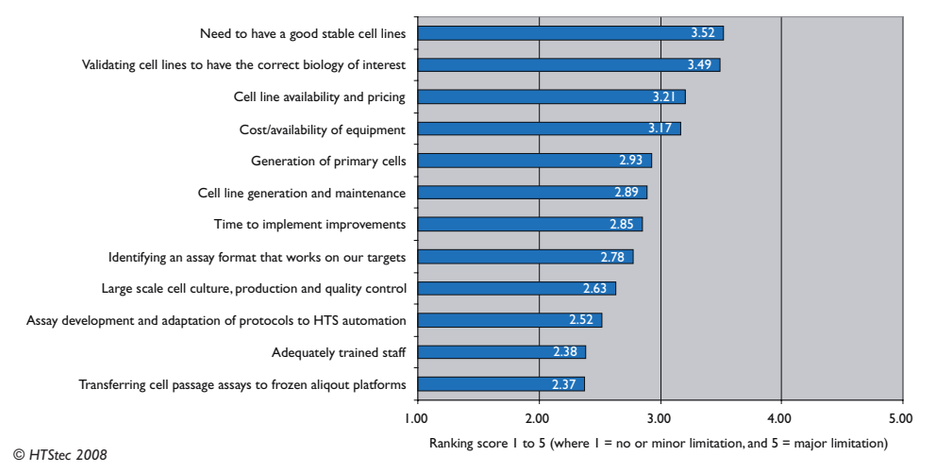


Figure 11
Right: The new Hamamatsu FDSS7000 system

Below: Inside the new Hamamatsu FDSS7000 system with dual modes (fluorescence and luminescence), simultaneous 1536 dispense capability, dual dispensers, automatic tip loading, and a multi-washing unit equipped with ultrasound for maximal washing efficiency

unique features that customers found useful in FDSS 6000, most noticeably the design of dual dispensers and integrated stackers. Dual dispensers isolate sticky agonists from compounds thus eliminating carryover artifacts. Further, the FDSS 7000 has three wash stations with a sonicator plus a tip wipe stage to catch drips. The most important feature on FDSS 7000 is the simultaneous 1536 dispense capability. Disposable 1536 tips (1-10uL) are used on a dispenser head. In addition, a 1536-well pintool head can be accommodated with either short or long pins ranging in dispense volume from

VENDOR	WEBSITE	DISPENSE & IMAGING OFFERING			
		Detection instruments	Fluorescent kits	Photoproteins	Assay services
Hamamatsu	www.hamamatsu.com	✔			
MDS Analytical Technologies	www.moldev.com	✔	✔		
PerkinElmer	www.perkinelmer.com	✔		✔	✔
Axxam	www.axxam.com			✔	✔
BD Biosciences	www.bdbiosciences.com		✔		
DiscoveRx	www.discoverx.com		✔		
Invitrogen	www.invitrogen.com		✔		✔
Millipore	www.millipore.com			✔	✔

Table 1: Commercial sources for Dispense & Imaging assay technologies

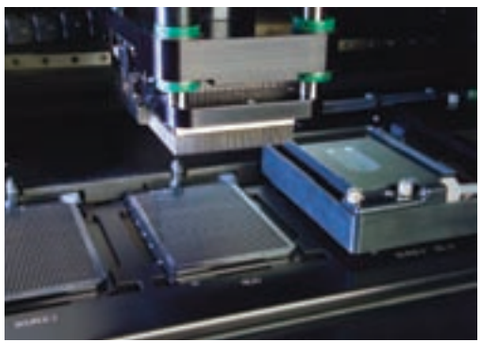


Figure 12
Above: The MDS Analytical Technologies FLIPR^{TETRA} system is a slim, modular platform that addresses both fluorescent and luminescent kinetic cell-based assays during drug discovery

Right: A 1536 pipetting head at the read position in a FLIPR^{TETRA} fitted with the Aequorin Option

10nL to 50nL. Both pintool and pipette heads are interchangeable in the same protocol. Like the FDSS 6000, the FDSS 7000 has two modes of detection: fluorescence and flash luminescence. However, the FDSS 7000 now has a more sensitive luminescence sensor with better resolution. This is particularly important for Aequorin assays in 1536 well format because of low signal intensities. The improved sensitivity even allows for detection of most blue SPA (radiometric) assays (**Figure 11**).

The **MDS Analytical Technologies** (www.moldev.com) FLIPR^{TETRA}® system is a modular platform that can be configured based on detection mode, screening format and target, making the transition from assay development to lead optimisation a seamless process. Two camera options: 1) standard for fluorescence only, or 2) aequorin for both fluorescence and flash luminescence; allow the system setup to be tailored to an individual screen. Both cameras are compatible with the standard FLIPR^{TETRA} system optics to monitor a variety of traditional fluorescence assays, including calcium mobilisation and membrane potential. The aequorin camera can be adjusted to the appropriate window to detect signals from bright fluorescent or dim photoprotein luminescent assays. This optimisation helps to avoid camera saturation associated with some brighter cell lines or densities, while accommodating day-to-day variations in signal intensity. In addition, it enables users to choose the detection mode and cell line that is most appropriate for their target. User-exchangeable pipettor heads, available in 96-, 384- and 1536-well formats, allow for the adjustment of screening rates based on throughput, material consumption and assay requirements. When combined with the cell suspension option, cells can be delivered in uniform suspension, providing consistent results with low noise over extended runs. Different cell lines can be accommodated by fine-tuning the protocol parameters, including the cell mixing and pipetting. Recirculating the cells to a spinner flask, available in multiple sizes, lowers the volume used, minimising the cell culture requirements. User-defined cleaning protocols allow the use of up to four wash solvents to facilitate automated cleaning of the instrument and components, preventing cross-contamination. To adjust with varying throughput, the system can easily be configured as a standalone workstation or full-integrated system. Using the ScreenWorks™ system software, the transition from standalone to full-scale automation is simplified with a single protocol setting. ScreenWorks software further enhances the user-experience with its 'drag-n-drop' interface, which supports multiple users with basic to complex protocol requirements during multiples stages of drug discovery (**Figure 12**).

PerkinElmer (www.perkinelmer.com) continues to develop its cell-based assay reagent technologies that can be applied to two major classes of drug discovery targets – G protein coupled receptors (GPCRs) and ligand gated ion channels. Both of these target classes, when activated, can increase

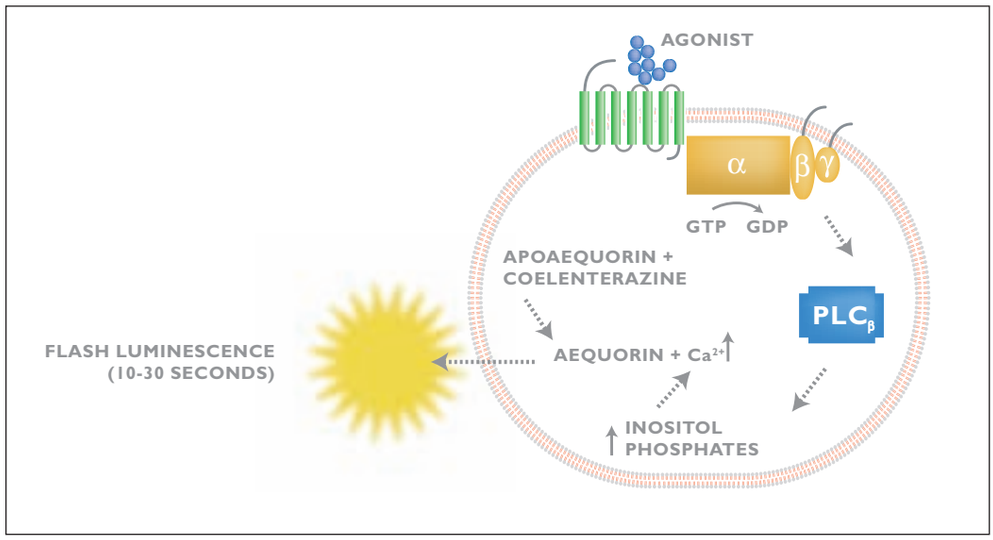


Figure 13
AequoScreen Assay Principle: the apo-enzyme (apoaequorine) is a 21kDa protein and needs a hydrophobic prosthetic group, coelenterazine, to be converted to the active enzyme, aequorin. Upon calcium binding, aequorin oxidises coelenterazine into coelenteramide, producing CO_2 and emitting light. The light emission is measured as luminescence

intracellular calcium levels, the changes of which can be detected using the photoproteins, aequorin and Photina®. These proteins in the presence of calcium yield a flash luminescent response of high intensity (**Figure 13**). Due to the low inherent background, and the large signal generated, extremely large signal:background ratios can be detected from intact cells. Consequently, the approach is ideal in terms of providing assay where high sensitivity is required. Specifically, this photoprotein technology can be used to measure changes in calcium from ion channels or receptors that are either expressed at low levels or that couple poorly to calcium signalling (as occurs in transiently transfected cell lines). In addition, the technology can be used in protocols that use very small fluid volumes, such as 1536-well microtiter plate assays. PerkinElmer now has available a growing series of AequoScreen® and PhotoScreen™ cell lines engineered with aequorin or Photina®, respectively, to facilitate cell-based assays for GPCRs or ion channels. These reagent platforms have been optimised for PerkinElmer's LumiLux® and LumiLux CS dispense and imaging systems. These highly sensitive, high throughput cellular imaging platforms have the ability to image flash and glow cellular assays (suspension or adherent), and biochemical luminescence assays with advanced liquid handling functionality. Recently introduced at LabAutomation 2008, the LumiLux CS Cellular Luminescence Platform features a compact design for standalone assay development or SAR labs or as an integrated imaging system within existing HTS robotic platforms (**Figure 14**). Its modular design can also be upgraded with a robotic arm for use with external stackers or incubators. Both LumiLux instrument platforms measure suspension or adherent AequoScreen® and PhotoScreen™ technologies, offer 384-channel and 1536-channel dispense heads, and utilise a unique cell stirrer to facilitate assay development and high-throughput screening.

Figure 14: PerkinElmer's LumiLux CS Cellular Luminescence Platform features a compact design for standalone assay development or SAR labs or as an integrated imaging system within existing HTS robotic platforms

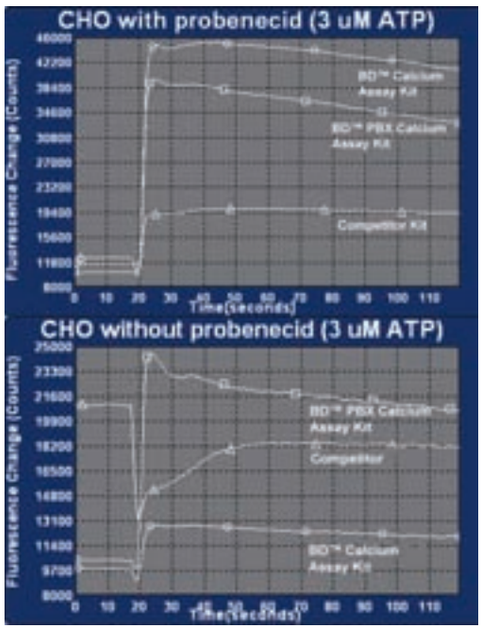


Figure 15
Comparison of BD Calcium Assay kits with a competitive product in CHO cells in the presence or absence of Probenecid
Top: With probenecid, both BD kits outperform a competitive product, providing significantly higher signal over background
Bottom: In the absence of probenecid, not only does the BD PBX Calcium Assay kit provide a better signal over background, but there is a noted absence of the 'addition artifact' seen using the competitive product

D&I reagent/biology vendor updates

The use of photoproteins for cellular Ca2+ mobilisation assays has increased over the past few years. There are several factors contributing to this phenomenon: the availability of more sensitive instrumentation for flash luminescence detection in HTS as well as the wider range of available reporter cell lines expressing photoproteins. Among these, Photina® is a particularly bright Ca2+ activated photoprotein developed by **Axxam** (www.axxam.com) and currently distributed by PerkinElmer. Photina® has been tested and validated for use on several different instruments designed for flash luminescence detection, such as LumiLux (PerkinElmer), FLIPR^{TETRA}® system (MDC) and the CyBi-Lumax (CyBio). CHO and HEK293 cells stably expressing Photina®, together with GPCRs or calcium permeable ion channels of interest, were tested on all of these instruments. The aim of these tests was to assess the suitability of these cell lines for use in HTS, therefore the fulfilment of several important parameters was considered as criteria for Photina®-based assays evaluation. **Table 2** summarises the results obtained testing CHO-Photina®/Adenosine 3 cell line on different instruments using both fluorescence and flash luminescence detection. The results obtained show that the readout system does not change the observed **in vitro** pharmacology. The absence of background luminescence allows for a higher S/B ratio than for fluorescence and therefore a larger range for identifying putative active compounds in a screening campaign. Photina®-based cellular assays can be loaded with the coelenterazine substrate for several hours up to 24 hours longer than the usual suggested time without affecting cell viability or assay performance. This allows a greater flexibility in scheduling HTS operations compared to fluorescence-based assays, where dye toxicity strictly limits the cell loading time. In conclusion, Photina® is a valuable and flexible method for cellular calcium mobilisation assays on most of the instrumentation platforms currently available on the market.

BD Biosciences (www.bdbiosciences.com) offers two calcium assay kits for detection of calcium flux in living cells. The kits provide increased signal to background ratios, in an easy to use no-wash assay format, with no known interference to ligands (small molecules, peptide/proteins and lipids). Also, the 'addition artifact' seen with some kits is significantly reduced with these new dye formulations. The kits have been successfully used with a variety of cell lines including CHO, HEK293,

INSTRUMENT	LUMILUX®	CYBI®-LUMAX	FLIPR^{TETRA}®	FLIPR^{TETRA}®
Readout Mode	Luminescence	Luminescence	Luminescence	Fluorescence
Cell Type	Suspension	Adherent	Adherent	Adherent
Cells/Well	5,000	5,000	5,000	10,000
IB-MECA EC50	1.49nM	5.30nM	6.02nM	3.87nM
S/B (RLU max/RLU min)	46.4	17.3	37.5	3.84
Z'	0.82	0.75	0.71	0.67

Table 2: Results obtained testing CHO-Photina®/Adenosine 3 cell line on different instruments using both fluorescence and flash luminescence detection

Jurkat, Hela and U2OS, but also with primary cells. The kits are compatible with all D&I devices used in compound screening (eg FLIPR^{TETRA}™, CellLux™ and FDSS 6000). Many dye kits require addition of probenicid, a transport inhibitor, to avoid leaking of the dyes after loading to cells. Probenicid is toxic to some cell types and can strongly interfere with the calcium flux. In this situation the PBX-format of the BD kit is the assay of choice. In particular, the PBX-format is recommended for the BD ACT*One*™ GPCR screening technology. Evaluating both kits is recommended to select the best dye kit for each screening campaign. The kits are available in two sizes providing sufficient material for either 10 plates, or 100 plates (96, 384, or 1536 well). Additionally, for higher volume applications, custom packaged materials are available on request. Optimisation of screening assay for GPCR targets is possible by combining the BD ACT*One* technology with BDTM Calcium Assay Kits and BD Falcon™ or BD BioCoat™ microplates for HTS applications. For adherent cells, BD BioCoat Poly-D-Lysine coated microplates are strongly recommended (**Figure 15**).

DiscoveRx's (www.discoverx.com) D&I offering includes fluorescent calcium no wash technology and a coelenterazine reagent assay kit to evaluate the mobilisation of calcium within cells. DiscoveRx Ca NW plus was launched in the middle of 2007. The new assay formulation is based on a homogenous technique for the detection of calcium mobilisation, but has an improved signal to background of 50% over the previous formulation (**Figure 16**). Ca NW plus contains proprietary enhancer reagent, which out-competed competitive no-wash systems in multiple big pharma evaluations (**Figure 17**). In providing a complete GPCR solution portfolio, DiscoveRx also offers one of the purest and most sensitive forms of coelenterazine h on the market. Coelenterazine h is a derivative of native coelenterazine that has greater sensitivity to calcium, making it the ideal choice for luminescent calcium HTS.

For assay of GPCRs or Ca2+-permeable ion channels, **Invitrogen**'s (www.invitrogen.com) Fluo-4 NW Assay has a unique formulation that eliminates the need for either a quencher dye or a wash step, while delivering a large increase in fluorescence intensity. This new formulation results in a more reliable Ca2+ assay designed to meet the requirements of automated applications. The Premo™ Cameleon Calcium Sensor is a genetically

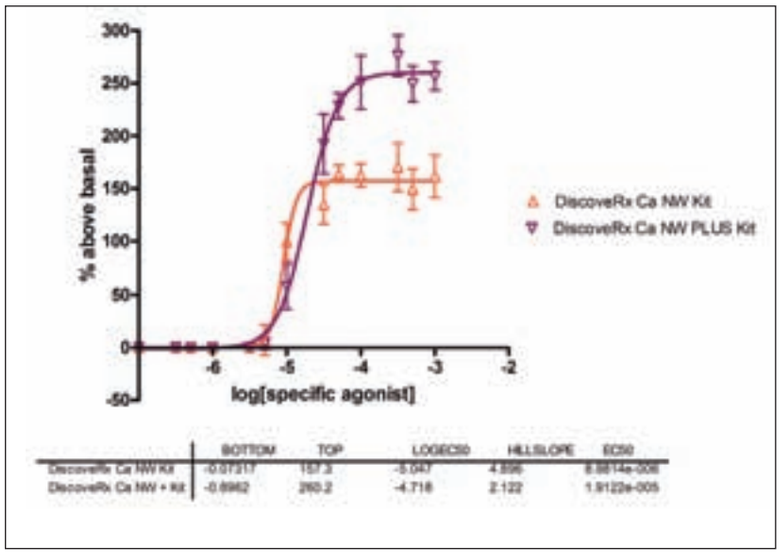


Figure 16: DiscoveRx Ca NW plus reagent was tested against DiscoveRx's previous Ca NW in ligand-gated ion channels in astrocytomas

encoded ion indicator delivered by BacMam technology that readily and accurately detects intracellular calcium flux. The ratiometric readout significantly reduces assay variations, and excellent Z' values in complete medium define the true no-wash nature of the assay. Invitrogen's FluxOR™ Thallium Detection Kit is a fluorescence-based, homogenous HTS assay for potassium ion channel

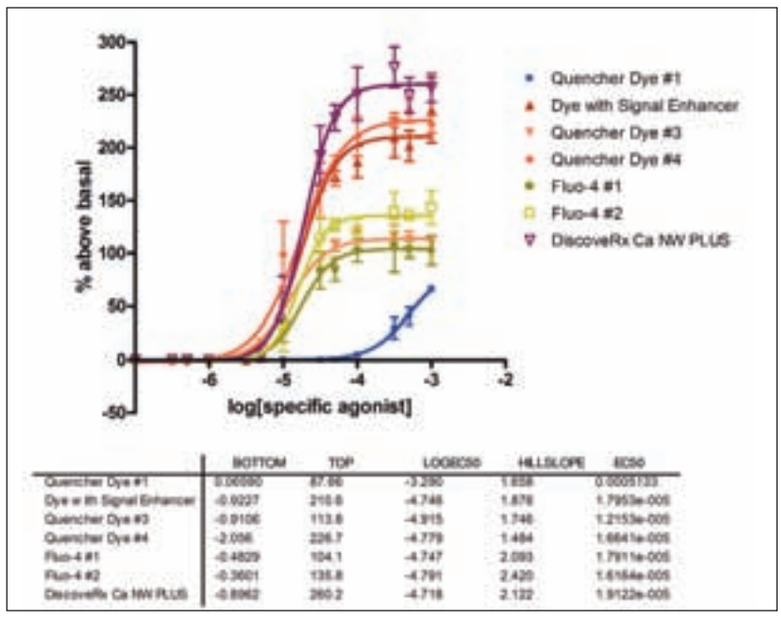


Figure 17: DiscoveRx Ca NW plus reagent tested against various commercially available Calcium No Wash kits. The Ca NW plus assay showed superior performance by comparison

and transporter activities. When potassium channels are opened, thallium influx from the external medium is detected with a highly sensitive indicator dye (**Figure 18**). The FluxOR™ Thallium Detection Kit enables rapid and robust HTS of potassium channel targets reproducibly giving IC_{50} values that are predictive of block or modulation in lower-throughput platforms. Ion channels can also be assayed with Invitrogen's Voltage-Sensor Probe (VSP) technologies. A coumarin-based donor associates with the outer surface of the external plasma membrane; DisBac-based acceptor dyes associate into the lipid phase of the membrane and translocate depending on the charge across the membrane (ie, they move away from the donor during depolarisation, and toward the donor during hyperpolarisation). This allows a FRET readout that is linear with membrane potential from -80 to +40mV (the physiological range for many ion channels) with a sensitivity of approximately 1% change in the fluorescence ratio per mV membrane potential change. Careful assay design and optimisation yields excellent correlation with patch-clamp data for both ligand-gated channels and specific conformational states of voltage-gated channels.

Millipore (www.millipore.com) has developed a portfolio of more than 140 robust ChemiScreen™ Ca2+-optimised pharmacologically validated GPCR cell lines. Chem-1 cells, the prototypical ChemiScreen parental cell, express high levels of endogenous Gα15, facilitating Ca2+ flux readout for most stably expressed GPCRs. This format provides a common readout for a variety of GPCRs, simplifying study of this target class and reducing the complexity of multiple platforms and assay types otherwise required. Cellular Ca2+ flux in conjunction with fluorescent Ca2+ dyes and imaging plate readers have been an industry standard for high throughput screening and ChemiScreen cell lines are ideally suited for this type of application. Millipore's GPCRProfiler™ service utilises these cell lines for selectivity profiling, SAR optimisation and medium throughput screening to advance BioPharma drug discovery efforts. Presently, fluorescence detection of Ca2+ signalling is the preferred primary screening platform for GPCR drug discovery. However, recent advances in other platforms and technologies, such as time-resolved fluorescence, luminescence, high content analysis and label-free detection, offer researchers alternatives to conventional fluorescent-based assays. Millipore is extending its portfolio of GPCR solutions and is validating and expanding the range of products for use with these alternative technologies. Recently, the adoption of flash luminescent detection of Ca2+ flux by photoproteins, such as aequorin, has increased with the availability of new luminescent D&I devices suitable for studying GPCRs. Millipore has developed ChemiScreen Aequorin GPCR cell lines for use with conventional plate readers equipped with injectors or next-generation D&I devices. Two variants of aequorin have been selected for the development of a robust, pharmacologically validated solution for luminescent readouts: the 'flash' variant is a traditional aequorin with a short intense peak; whereas the 'glow' variant has an extended signalling duration that may be amenable to plate readers or even plate imagers with minimal on board liquid handling capabilities. Another common industry trend is the adoption of frozen cell technology to reduce time, cost and scheduling constraints of cell-based projects. Millipore has demonstrated feasibility and reproducibility for manufacturing the ChemiScreen cell lines in a frozen, Ready-to-Assay™ format. Millipore will expand its offering as demand for this new convenient format of cells increases, offering bulk quantities for rapid HTS implementation (**Figure 19**).

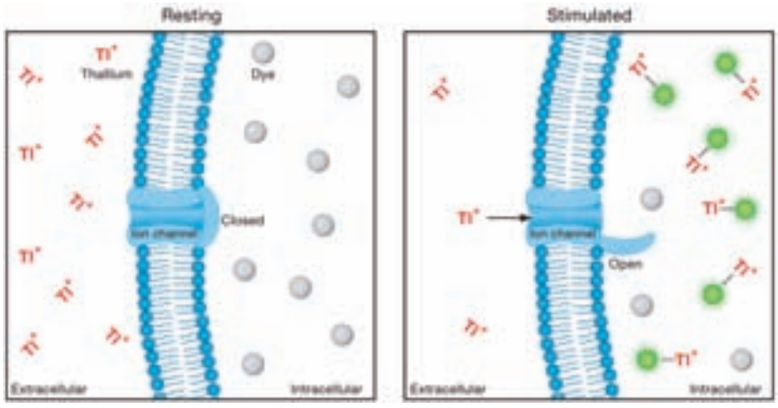


Figure 18
Principle of the FluxOR™ assay. Basal fluorescence from cells loaded with the FluxOR™ dye is low (left) until potassium channels are stimulated. Thallium ions (Tl+) added to the assay with the stimulus flow into the cells, activating the dye (right). The high sensitivity of the FluxOR™ dye gives an excellent signal window for detecting potassium channel activity

Summary

In conclusion, progress towards the wider uptake and adoption of flash luminescence readouts has been considerable since our first market D&I report in 2005[3], and looks set to continue. By 2009 it is expected that there will be a significant decline in the number of labs using exclusively fluorescence-based D&I assays. However, the transition appears to be less of a displacement of fluorescence with flash luminescence, but rather a more balanced implementation of both readouts, ie where the detection mode and the cell

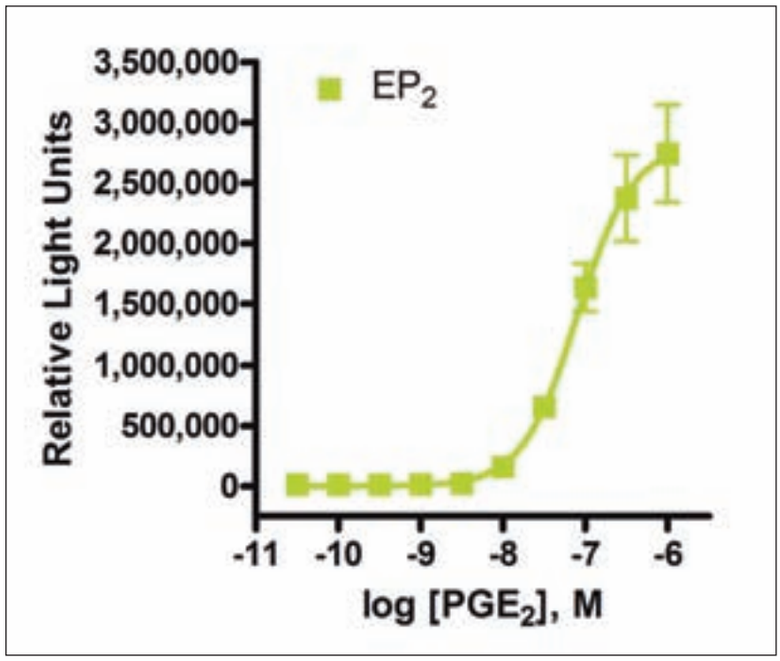


Figure 19: Ca2+ flux readout for ChemiScreen™ Flash Aequorin cells stably expressing EP2 prostanoid receptor. Chem-1 cells stably co-expressing Flash Aequorin and EP2 were loaded with 5uM coelenterazine for 3hrs at room temperature. Luminescence in response to PGE2 was determined in duplicate on a Perkin Elmer Wallac Victor2. Data were collected for area under curve for 20secs

References

1 Comley, J (2004). New flash imagers – facilitating higher throughput functional GPCR assays! Drug Discovery World 5(1): 49-60.

2 Comley, J (2006). Changing dynamics in Dispense and Imaging assays. Drug Discovery World 7(3):35-51.

3 Dispense & Imaging Trends 2007 Report, published by HTStec Limited, Cambridge, UK, November 2007.

4 Dispense & Imaging Trends 2005 Report, published by HTStec Limited, Cambridge, UK, December 2005.

line are preferentially selected to optimise the assay of the target of interest. This trend requires greater readout flexibility in D&I instrument platforms, if multiple purchases of these expensive detection platforms are to be avoided, and vendors have foreseen this requirement by the launch of two new highly versatile dual mode instruments (Hamamatsu FDSS 7000 and MDS Analytical Technologies FLIPR^{TETRA}® system). Responding to a need (also highlighted in the latest report) for more compact D&I instruments with less automation within the box, PerkinElmer has recently introduced the LumiLux CS Cellular Luminescence Platform, with optional external stackers, for standalone assay development or SAR labs or for use as an imaging system integrated within an existing HTS robotic platform. Improvements in instruments for the detection of flash luminescence have been complemented by increases in the flexibility and sensitivity of photoproteins to enable use of these technologies in HTS.

The commercial availability of photoproteins, now unencumbered by licence fees, has been considerably enhanced over the past year with PerkinElmer's growing series of AequoScreen® and PhotoScreen™ cell lines engineered with aequorin or Photina® and will be further improved when Millipore's ChemiScreen Aequorin GPCR cell lines are offered in a frozen, Ready-to-Assay™ format. Recent data generated by Axxam with Photina® demonstrated that the readout system does not change the observed *in vitro* pharmacology. It is noteworthy that a data disconnect between readouts was not rated a major limitation in the transition process from fluorescence to flash luminescence. Interestingly the adoption of flash luminescence seems to be paralleled by the greater utilisation of 1536 plates and reduced use of adherent cells. Fluorescence assays kits have, however, not been overlooked by vendors and several new no-wash calcium assays with improved characteristics are now offered by DiscoveRx, BD Biosciences and Invitrogen. In addition, Invitrogen's FluxOR™ Thallium Detection Kit addresses the demand for new assays for potassium ion channels and transporters. In the future the report predicts wider use of primary and transiently transfected cells, a reduction in assay cycle times (time from start of a screen to its completion, including Hit retests) and a wider use of outsourcing. Overall we can expect D&I assays to perform a leading pivotal role in the primary screening of GPCR and ion channel targets for many years to come. DDW

***Dr John Comley** is Managing Director of HTStec Limited, an independent market research consultancy whose focus is on assisting clients delivering novel enabling platform technologies (liquid handling, laboratory automation, detection instrumentation and assay reagent technologies) to drug discovery. Since it formation in 2003, HTStec has published 30 market reports on drug discovery technologies and Dr Comley has authored 21 review articles in **Drug Discovery World**. Further information on accessing the market report 'Dispense & Imaging Trends 2007' can be obtained by visiting www.htstec.com or by emailing john.comley@htstec.com to receive a free copy of the Report's Executive Summary and Table of Contents.*