LABEL-FREE DETECTION

New biosensors facilitate broader range of drug discovery applications

This article highlights some of the findings of a recent market report on label-free detection trends. In particular, it looks at the current perception, main use and biggest benefit of label-free detection today. It considers the potential of label-free to displace other detection technologies and the impact that label-free might have on enabling access to difficult target classes. It reviews the latest and emerging developments in label-free detection systems, describes their underlying technology principles and discusses their prospects in enabling higher throughput processing and getting out more information about an interaction from a single experiment. The next generation of label-free tools will predominantly be microplate-based sensors which for the first time may facilitate wider interest in label-free detection, particularly from those involved in primary screening. Applications enabled by these new label-free tools include cell-based receptor/ligand interactions particularly of orphan receptors; non-invasive cell proliferation and *in vitro* cytotoxicity testing; and antibody and small molecule affinity analysis. The prospects for label-free detection expanding beyond the market focus and customer niche created by surface plasmon resonance have never looked brighter.

By Dr John Comley

According to a recent pharma market report on label-free (HTStec's label-free Detection Trends 2004) the majority of respondents surveyed perceive label-free 'as a promising analytical tool that needs to mature' (**Figure 1**). This maturation process looks set to escalate as several new label-free detection systems are launched over the coming year, facilitating wider exploitation of the technology across the drug discovery process, including, for the first time, those areas that require higher throughput (eg primary screening). Currently the main use of label-free detection is kinetics (on and off-rate) analysis closely followed by affinity analysis. Most of these analyses are done by secondary screening (Hits-to-Leads) and lead optimisation (Leads-to-Candidate) groups. Use of label-free today in Yes/No end point screening and specificity testing

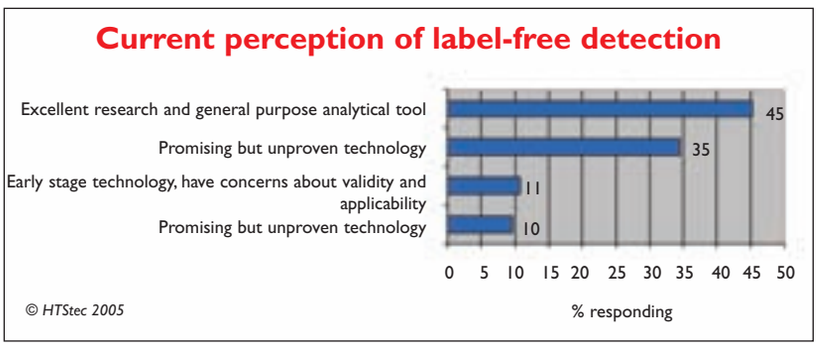


Figure 1

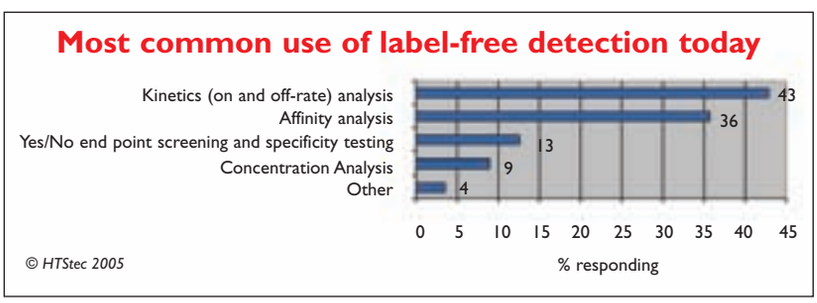


Figure 2

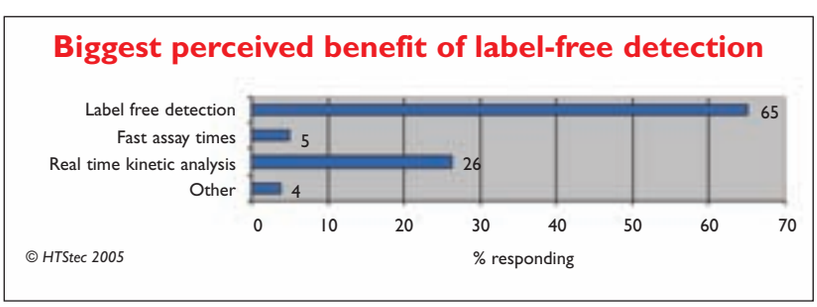


Figure 3

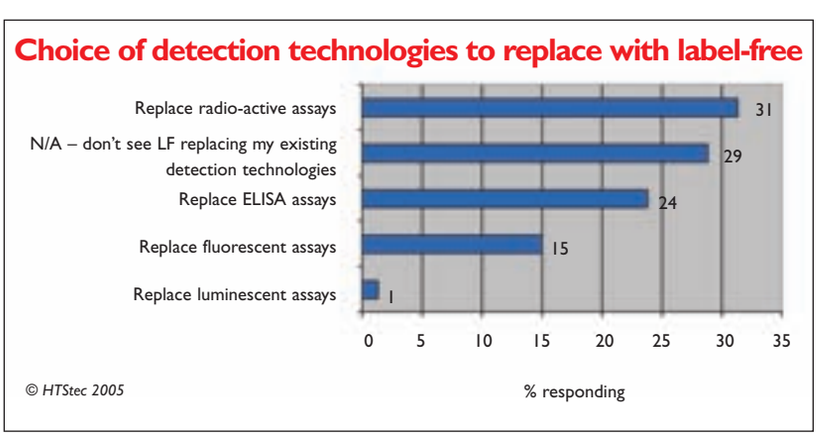


Figure 4

is minimal in comparison (**Figure 2**), although this could all change if label-free finally impacts primary screening. Two-thirds of survey respondents think that being 'label-free' was the biggest benefit of label-free detection (**Figure 3**).The other main advantages of label-free detection are summarised in **Table 1**.

Label-free may help address difficult targets

Of the main detection technologies in use today greatest interest was shown by respondents in replacing radioactive and ELISA assays with label-free (**Figure 4**). However, a significant proportion of respondents do not see label-free replacing any of their existing detection technologies. They see label-free as one of several tools that can aid in the characterisation of drug-target interactions and complementary to their existing range of preferred detection techniques. These respondents would even like to see label-free as an additional readout on a multimode reader. If an enabling label-free system were to become available (ie one that matched users' criteria of sensitivity, cost/well and throughput) survey respondents could envisage a situation where up to a third of their existing primary screens might be replaced by label-free. HTStec's report also documents feedback on the impact that label-free detection might have on enabling access to difficult target classes. The findings suggests that on average around one in four targets do not make it to primary screening. The main reasons these 'difficult targets' do not get screened is the lack of quality (robust) assay methods available to screen that target or the target in question is an orphan or unvalidated and is given lower priority relative to other targets. Respondents were split 50:50 as to whether label-free will help them address these difficult targets. Of those who thought label-free might help, an enabling label-free system could potentially address up to one-third of their difficult targets. Protein-protein interactions were seen as one of the main difficult target classes for which existing assay technologies are currently inadequate. Although, receptor-ligand assays were the most investigated molecular interaction, and of greatest interest to survey respondents.

Label-free detection system requirements

Sensitivity and throughput were cited as the biggest limitations to the current widespread use of label-free detection. In terms of required sensitivity, a label-free system needs to be able to detect the

binding of small molecular weight ligand (typically 200-300 Daltons) at a surface mass detection limit of <5pg/mm^2 and at 10^{-9}M concentration in the assay buffer. The throughput required in a label-free system varies with different parts of the drug discovery process, but 5,000 data points/hour (ie about 13 x 384 plates/hour) are needed for primary screening while 200 data points/hour would be acceptable for lead optimisation. The lack of availability of microplate-based label-free systems and Cap-Ex costs were also rated very highly as limiting factors in the deployment of label-free. The preferred label-free sample carrier format was a microplate-based sensor (384-well) (**Figure 5**) and the maximum acceptable price for an instrument able to read such plates was no more than $150,000. The median price per data point at which all respondents would consider implementing label-free primary screening was <$0.2 per data point.

Most survey respondents were aware of surface plasmon resonance (SPR) and its use in label-free detection, many were experienced Biacore users. However, awareness of other types of label-free detection, including emerging technologies, was much less evident. **Table 2** summarises and compares some of latest developments in label-free detection, with a particular focus on those systems that will utilise microplate-based sensors. A more detailed description of these new developments, grouped according to their underlying technology principles, is given below.

Surface plasmon resonance

Biacore (www.biacore.com) was the pioneer and is the world leader in monitoring protein interactions using surface plasmon resonance (SPR) technology. Most people associate label-free detection with SPR and Biacore instruments. SPR is a phenomenon that occurs in a thin metal film at an optical interface under conditions of total internal reflection and is observed as a decrease or dip in reflected light intensity at a specific angle. The system uses polarised light and can detect subtle changes in optical resonance that occur when molecules bind to or dissociate from an immobilised target biomolecule. Essentially, SPR detects changes in mass of dissolved material in the aqueous layer close to the sensor surface by measuring changes in refractive index (SPR angle). Over the years Biacore has offered a range of systems designed to study the interaction of a protein with other molecules, typically another protein or a small molecule drug. Systems have been configured to meet the demands of performance and regulatory support,

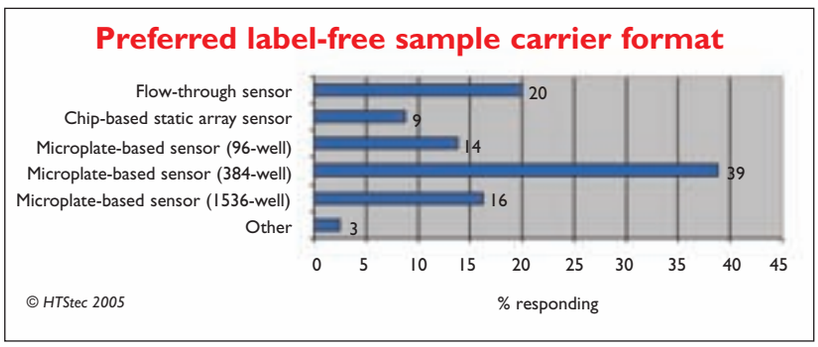
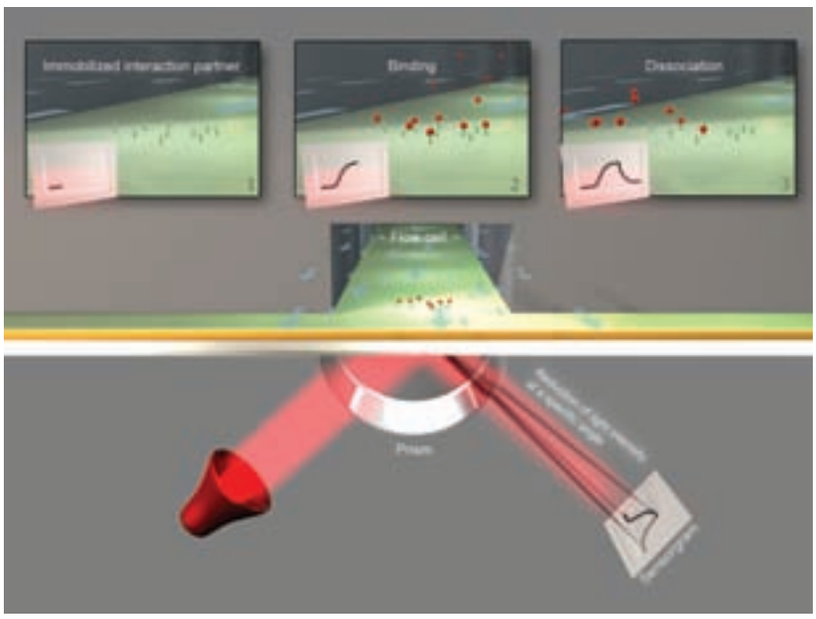


Figure 5

Table 1
Advantages of label-free detection

- **LABEL-FREE**
  - study molecular interactions without modifying molecules of interest
  - no influence of label on physicochemical or binding properties
  - closer resemblance to wild type, enables use of natural ligands & substrates
  - reduced interference from compound fluorescence and colour quenching
  - hopefully less potential for false positive/negative
  - potential for assays to be cheaper (no labelling/reagent cost)
- **GENERIC**
  - systematic/universal approach to assay development and screening
  - prospect of faster cycle times
- **DIRECT MONITORING**
  - measures interactions using species of interest (rather than altering them)
  - measures on and off kinetics of compound binding without resort to complex competition/displacement assays
- **NON-RADIOACTIVE**
  - avoidance of radioactive labels
  - hazard free with less lab safety and waste disposal issues/costs
- **FAST**
  - real-time monitoring of binding interactions
  - eliminates problems with secondary detection or auxiliary reagents
  - increased sensitivity and specificity
  - shorter assay development times



Biacore Figure 1 : Principle of surface plasmon resonance (SPR) on the Biacore. A flow cell is formed when a sensor surface encloses an open flow channel through which a solution containing analyte is injected. As the analyte passes over molecules immobilised on the sensor surface, the association and dissociation phases of the interaction are recorded in real time on a plot called a sensorgram

Biacore Figure 2: The new Biacore T100™ protein analysis system

as these vary through the drug discovery process: from early research to lead characterisation, through to clinical immunogenicity studies and in-process quality control of biopharmaceuticals.

The latest system, Biacore T100™, will be introduced in Q1 2005. This instrument represents the next generation of protein interaction analysis systems from Biacore. The company has seen an increasing use of their technology throughout the drug discovery process. The ability to gain unique data about the interaction of proteins, with other proteins or with low molecular weight molecules, has enabled faster, better decisions which in turn has improved productivity, for example, by earlier elimination of sub-optimal compounds or by facilitating selection of the most likely potential biotherapeutics.

To significantly improve on ease of use compared to earlier systems, the Biacore T100™ uses software wizards, built upon the years of experience within Biacore, to assist at every step of an analysis from method development through to data evaluation. This ensures that interactions are performed and interpreted in the best possible way. An additional feature which further enhances data quality is the stability of this system at higher temperatures. Studying interactions at 37°C rather than room temperature will give far greater insight into the potential behaviour of a molecule *in vivo*, again shortening the decision process for selection of potential biotherapeutics. To ensure compliance with regulatory demands both the hardware and software of the Biacore T100™ have been designed for 21 CFR Part 11 compliance. A GLP/GMP support package is available to save time during the otherwise time-consuming validation process.

Biacore has never positioned itself to address the primary screening segment of drug discovery. The launch of Biacore T100™ reaffirms the company's focus on higher content and data quality ie getting out more information about an interaction from a single experiment, like kinetics, affinity, specificity, concentration and even thermodynamic information, in order to make better, faster decisions.

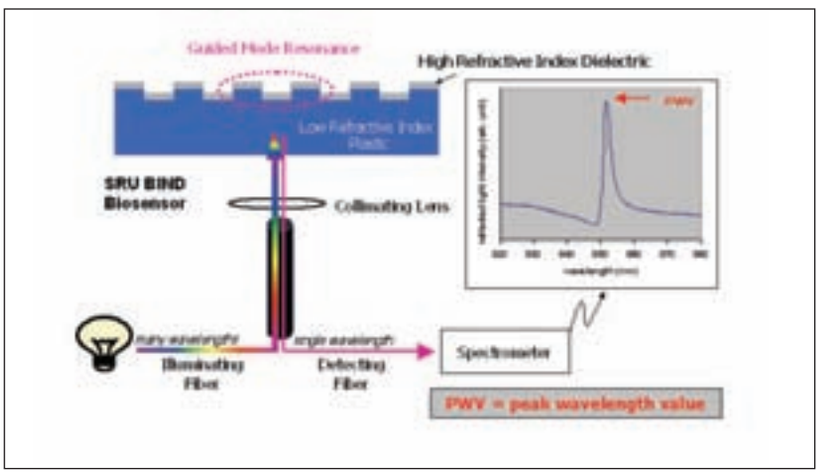
Guided mode resonance

SRU BIND™ (Biomolecular Interaction Detection) from **SRU Biosystems** (www.srubiosystems.com) is a novel optical biosensor that is ready for use in drug discovery applications. SRU BIND™ is based upon the optical principle known as guided mode resonance. The biosensor incorporates a proprietary nanostructure replicated on to a plastic substrate, enabling the high-volume manufacture of optical biosensors in a microplate format. BIND

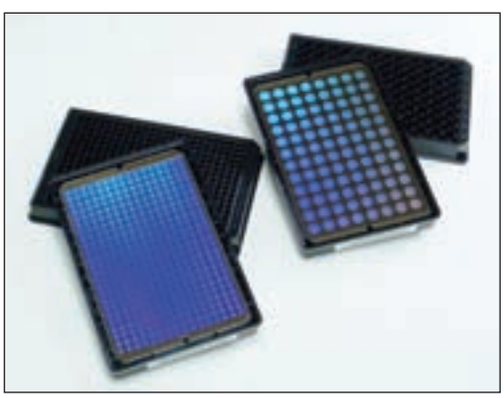
sensors are designed so that when a collimated linearly-polarised beam of broad-spectrum light is incident on the sensor, a narrow wavelength light beam is reflected directly back to the BIND Reader. Binding interactions on the biosensor are detected as a shift (increase) in the wavelength of this directly reflected beam. Due to the subwavelength structures that are part of the biosensor, rainbow colours are observed on the biosensor in regular laboratory light, even though the resonance reflection is in the infrared.

The SRU BIND™ system consists of single-use biosensor plates in industry-standard formats (96-well or 384-well configuration) and the BIND Plate Reader. A 384-well plate can be read in approximately one minute, while a 96-well plate requires as little as 15 seconds. The BIND Reader has been designed to interface with robotics for automated plate-handlers or liquid-dispensing robots. The wide dynamic range of the BIND sensor allows quantitative analysis of cells, macromolecules, DNA and small molecules. BIND will replace existing label-free systems that suffer from a lack of throughput when performing affinity analysis. In addition, SRU BIND will be a powerful tool in combination with existing labelled methods, providing multi-mode readouts and biological system analysis.

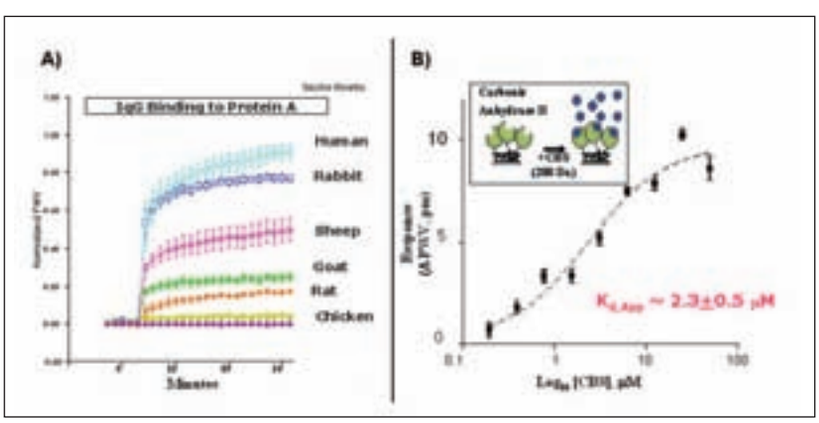
Over the past 12 months, SRU Biosystems has worked with multiple beta site users. A primary application focus of the BIND system has been quantitative analysis or affinity ranking when hundreds to thousands of samples need to be processed. For example, the BIND system can be used to perform direct binding assays for large or small molecules in secondary screening and lead optimisation. Sensitivity and throughput of the system meet the needs for the next generation of label-free analysis. The system was commercially available from Q4 2004. Instruments will be available at a US list price of $150,000. BIND microplates are expected to cost less than $100 each. Plates come pre-packaged and derivatised to offer the user a variety of attachment methods of the target to the biosensor.



SRU Figure 1 (above): Schematic diagram illustrating the principle of operation of the SRU BIND™ sensor



SRU Figure 2 (left): SRU BIND™ 96 and 384 well plates



SRU Figure 3: A) Protein A was adsorbed on to the BIND sensor in a 96-well format and IgG was added at the same concentration from different species. The sensor is fully capable of ranking antibody affinity within 1-2 minutes of addition to the well. B) Carbonic Anhydrase II was covalently attached to the sensor in 96-well format and varying concentrations of the carboxybenzene sulfonamide drug CBS (200Da) were added to individual wells

Resonant waveguide grating sensors

Corning Life Sciences (www.corning.com) is developing a label-free detection platform for drug screening called the Epic™ System. The Epic™ System allows researchers to explore biomolecular interactions in a direct, label-free, high-throughput manner. The system consists of the following: 1) a disposable 384-well microplate (SBS standard) with optical sensors and attachment surface chemistry inside each well; 2) an HTS-compatible microplate reader that is capable of reading 40,000 wells per eight hours; and 3) a set of label-free, direct binding assay protocols.

The optical sensors (the square region in the picture) in each well of Corning's microplate are resonant waveguide grating sensors. When illuminated

Table 2: Comparison of newly emerging label-free detection systems

COMPANY	WEBSITE	PRODUCT NAME	TECHNOLOGY BASIS	FORM
ACEA Biosciences	www.aceabio.com	RT-CES™ (Real-Time Cell Electronic Sensing) System	Real-time monitoring of cell status – morphology, adhesion and number – with electrical impedance	16 and 96-well
Akubio	www.akubio.com	RAP™ Real time, Label-Free Analysis System	Resonant Acoustic Profiling	8 and 96-well
Biacore	www.biacore.com	Biacore T100™	Detection of protein interactions using Surface Plasmon Resonance (SPR)	Samples loaded in 384-well plat analysed in chip sensor
Corning	www.corning.com	Epic™ Label-Free Detection System	Resonant waveguide grating sensor	384-well
CSEM	www.csem.ch	WIOS (Wavelength-Interrogated Optical Sensor)	Evanescent wave technology	8 detection cha parallel, various array and micro configurations
MDS Sciex	www.mdssciex.com	Under review	Cellular Dielectric Spectroscopy (CDS)	96-well, with 96 dispense & reac capability
SRU Biosystems	www.srubiosystems.com	SRU BIND™ (Biomolecular Interaction Detection)	Guided Mode Resonance	96 and 384-wel

with broadband light, these sensors reflect only a specific wavelength that is a sensitive function of the index of refraction close to the sensor surface. The sensors are coated with a surface chemistry layer that enables covalent attachment of protein targets via a primary amine group. After a target is immobilised, a baseline reading is established (**Figure 1**). When the binding assay is performed and analyte molecules bind to the immobilised target, a change in the local index of refraction is induced, and results in a shift in the wavelength of light that is reflected from the sensor (**Figure 2**). The magnitude of this wavelength shift is proportional to the amount of analyte that binds to the immobilised target. The entire process is label-free and requires only the binding partners of interest.

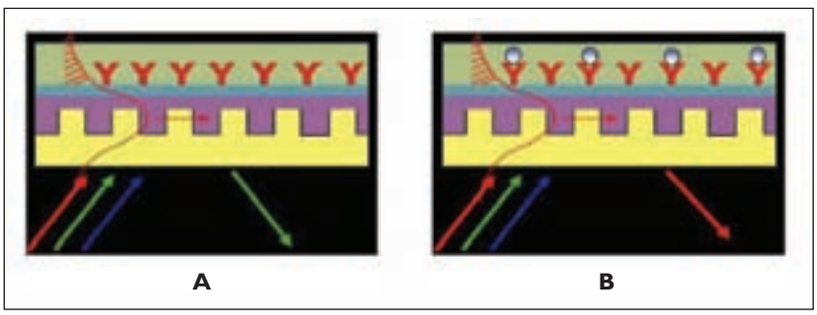
With its 384-well microplate format the Epic™ System will enable researchers to screen compounds in a high-throughput manner that is compatible with existing automation in HTS labs. Because the binding assay involves only the target protein and drug candidate, assay development is simple and straightforward. In its most basic format, Epic™ assays consist of the immobilisation of

ORMAT	THROUGHPUT (PER 8 HOUR DAY)	SENSITIVITY	KEY APPLICATIONS	PRODUCT LAUNCH
-well	46,080 data points	10 – 100 cells	Cytotoxicity, compound profiling, siRNA detection, receptor/ligand interactions	Q1 2004
vell	3,000 wells	10pg/ml conc., uM to pM Affinities, 200Da compounds to whole cells	Affinity and Kinetics, Concentration, Secondary Screening	Q1 2006
aded manually l plate, chip-based	Typically 100 interactions fully analysed within 24 hours	Detects binding of 100 Da compounds to a target	Antibody characterisation during biotherapeutic or vaccine development	Q1 2005
	40,000 wells	5pg/mm^2 (ie detects the binding of a 300Da compound to a 70KDa immobilised target)	Small-molecule/protein, orphan receptors, protein/DNA, antibody/antibody, kinase direct bind and functional assays, and cytokine/cytokine receptor, receptor/ligand, antibody/antigen	Beta Q2 2005
n channels in rious chip microplate ons	Depends on final product configuration	<1pg/mm^2 (more than 10x above detection limit for 244Da biotin binding to neutravidin)	Small-molecule binding/ receptor binding/affinity and kinetics/antibody-antigen affinity	Searching for manufacturing and distribution partners
th 96 channel read	5,000 wells, update rate 1sec	Capable of consistently and robustly measuring endogenous receptor activation	Universal assay for endogenous orphan and non-orphan GPCRs, TK receptors; deconvolution of signalling pathways	Q3 2005
4-well	50,000 wells	1pg/mm^2	Antibody and small molecule affinity analysis	Q4 2004

a target protein on the sensor surface followed by wash-free binding assays using potential drug candidates. Some examples of application for this universal platform include small-molecule/protein assays, protein/DNA interactions, antibody/antigen interactions, kinase direct bind and functional assays, and cytokine/cytokine receptor assays. A major advantage of these direct bind assays performed on the Epic™ System is the ability to screen orphan receptors (receptors in which the natural ligand is not known). In other words, it will be capable of detecting the binding of a 300 Dalton compound to a 70K Dalton immobilised target with CVs of 10% or less (depending on assay type). In addition, if the immobilised target is smaller (eg 25K Dalton) it is possible to detect the binding of smaller compounds (eg 150 Dalton). The Epic™ System is currently in an Alpha evaluation phase. Corning will initiate a Beta evaluation phase in 2005, followed by a commercial launch.

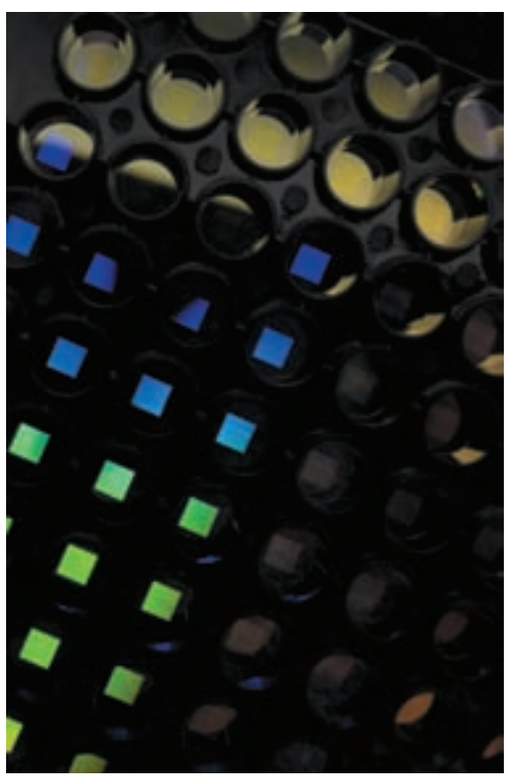
Evanescent wave sensing

Evanescent wave sensing utilises the evanescent part of an electromagnetic wave to sense a reaction



Corning Figure 1: Principle of Corning's Epic™ System. A) Biosensor without added analyte has given resonant wavelength; B) Binding analyte to target molecule gives resonant wavelength shift

Corning Figure 2 (right): A close-up view of Corning's Epic™ microplate. The optical sensor surfaces are the square regions in the centre of each well



Corning Figure 3 (below): A) Plot showing the binding of 25uM warfarin (308 Da) to immobilised human serum albumin (HSA); an average response of 8.6pm ± 1.8pm was observed with a baseline signal-to-noise ratio of 45. Wells with no immobilised HSA were used as negative controls and to correct for bulk index of refraction effects. B) Competitive Plot showing the results of binding and competitive inhibition assays for the binding of the antibiotic vancomycin to immobilised Lys-d-Ala-d-Ala (DADA); wells with no immobilised DADA groups were used as negative controls. The inhibitor (free DADA) concentration was 500uM

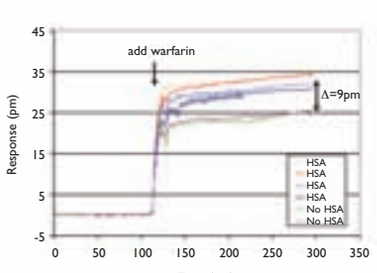


Figure A: Drug binding assay. Plot showing the binding of 25uM warfarin (308Da) to immobilised human serum albumin (HSA) on the Epic™ System. An average response of 8.6pm ± 1.8pm was observed with a baseline signal-to-noise ratio of 45. Wells with no immobilised HSA were used as negative controls and to correct for bulk index of refraction effects.

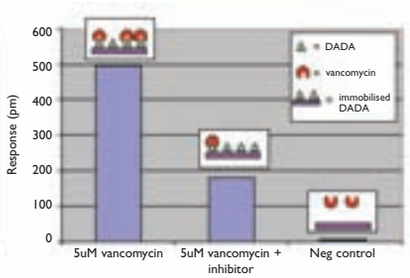


Figure B: Competitive inhibition assay. Plot showing the results of binding and competitive inhibition assays for the binding of the antibiotic vancomycin to immobilised Lys-d-Ala-d-Ala (DADA) on the Epic™ System. Wells with no immobilised DADA groups were used as negative controls. The inhibitor (free DADA) concentration was 500uM.

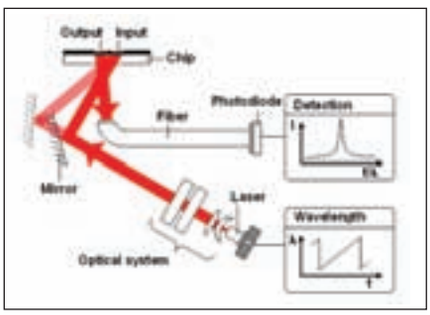
which takes place at the interface where the wave is generated. Evanescent waves can be found in different optical configurations (total internal reflection, surface plasmon mode, waveguide mode, etc). The advantage of this sensing approach is that a part of the propagating guided light, (the evanescent wave), penetrates into the sensing area (where the target and analyte interact) and is affected by optical changes occurring only in this region. The sensors are based on a resonant structure composed of a substrate (eg plastics, glass, silica); a high refractive index waveguiding layer (eg Ta2O5, TiO2, Si3N4) with grating structures; an immobilised target layer (eg antibodies, DNA, enzyme, proteins etc); and the analyte whose binding is being studied in the medium.

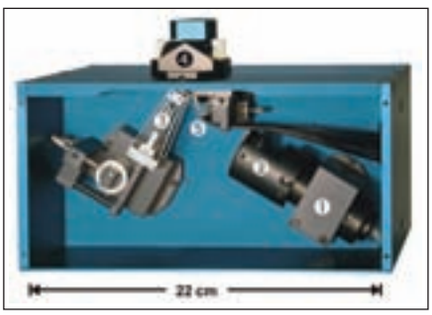
CSEM has developed (www.csem.ch) WIOS (wavelength-interrogated optical sensing) label-free detection technology, which is based on a waveguide grating coupling with wavelength modulation. The wavelength at which the resonant coupling occurs depends on the mass of the target adsorbed to the sensing site. The waveguiding layer is in contact with the target being examined and the interaction takes place through the evanescent part of the waveguide mode. Changes occurring in the evanescent wave as a result of analyte binding to the immobilised target result in a change in the resonance wavelength. By monitoring the resonance wavelength with time, extremely small optical changes at the sample waveguide interface can be observed with high sensitivity. In order to take into account variation which might be introduced by unwanted effects like temperature changes, laser wavelength perturbation and non-specific binding on the sensing sites, one or several sensing sites of the chip can be used as a reference site.

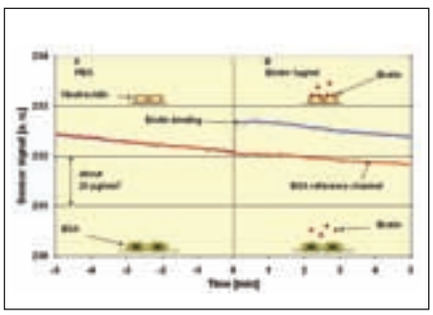
The key features of CSEM's WIOS instrument are: 1) simultaneous measurement of up to eight channels; 2) fast readout for real-time monitoring (10Hz); 3) detection limit <0.5pg/mm^2; and 4) modular optical system which can be adapted either for microplate use or further miniaturised into a chip-based diagnostic system. WIOS is a market-ready detection platform, which is flexible to address drug discovery, as well as the diagnostic and food technology markets. CSEM is currently searching for licensing, manufacturing and distribution partners of the WIOS technology platform.

Impedance-based sensors

MDS Sciex (www.mdssciex.com) is developing a system based on Cellular Dielectric Spectroscopy (CDS), a novel technology that enables comprehensive pharmacological evaluation of endogenous





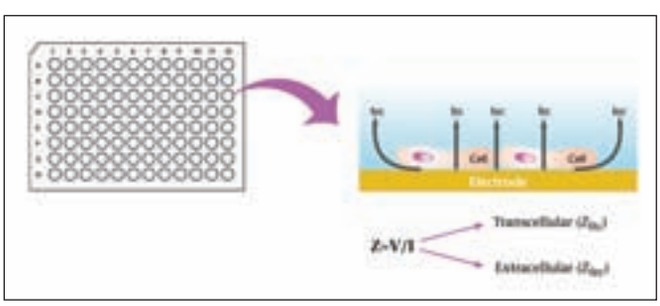


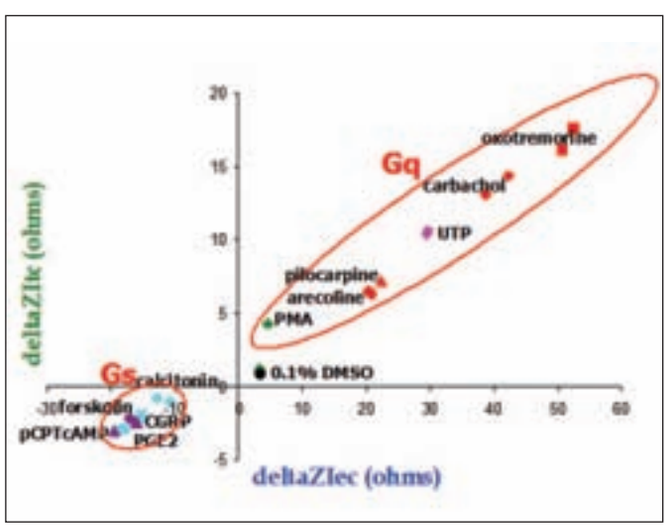
WIOS Figure 1 (left): CSEM's WIOS working principle; **WIOS Figure 2 (middle):** CSEM's WIOS research instrument: laser (1), optics (2), mirror (3), fluidic cell with sensor chip (4), and optical fibres to detector (5); **WIOS Figure 3 (right):** An example of small molecule detection using CSEM's WIOS instrument (raw data). Two channels were used, a reference site (coated with BSA) and the signal site (with immobilised neutravidin). Biotin (244 Da) was simultaneously applied at time 0 to both sites. The BSA-blocked channel shows no reaction (lower curve) with the biotin. Binding was evident on the neutravidin channel (upper curve)

cell surface receptors using a label-free, real-time, kinetic cell-based assay. CDS is a universal assay that allows measurement of multiple types of receptors including G protein-coupled receptors (GPCRs) and tyrosine kinase receptors using the same platform and without the need for any modification of the cell. The unique information generated by CDS allows deconvolution of receptor-mediated signal transduction, which can be used in characterising GPCRs including orphan GPCRs.

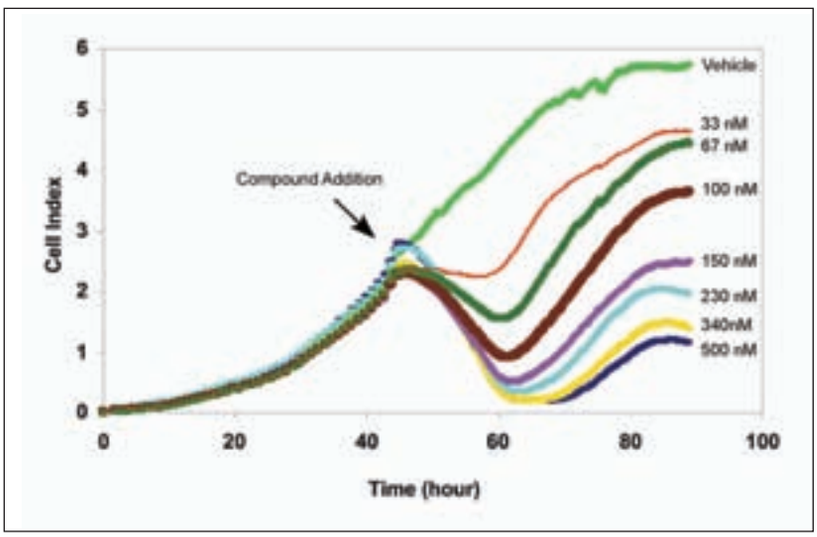
CDS technology is based on the measurement of complex impedance changes (ΔZ). Impedance (Z) is related to the ratio of voltage to current as described by Ohm's law (Z=V/I). Cells are seeded onto a custom 96-well microplate that contains electrodes at the bottom of the wells. The CDS instrument supplies constant voltage producing a current that flows around and between cells (extracellular current or Iec) and through cells (transcellular current, Itc) (**MDS Sciex Figure 1**).

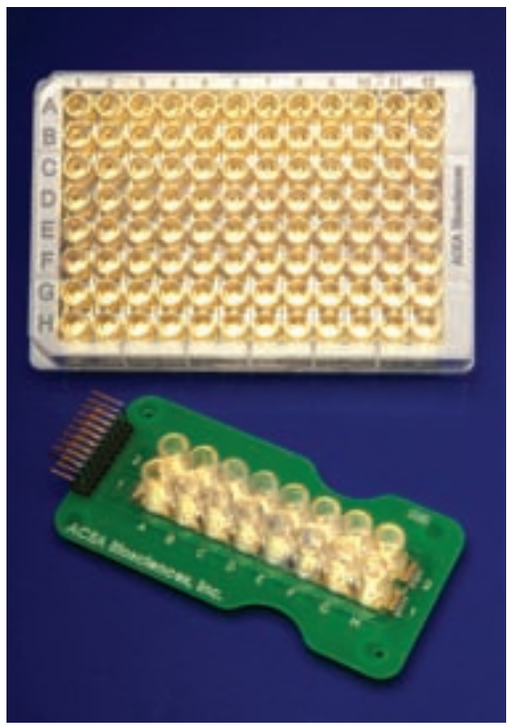
CDS measures changes in impedance upon stimulation of different cell surface receptors. Cell stimulation on addition of an agonist or antagonist is achieved using a 96-channel dispense head with adjustable dispense speed, adjustable pipette height and mixing capability. Impedance measurements are made simultaneous with fluid addition, with an update rate of 1sec. Contributors to the impedance measurements are changes in cell adherence to their substrate, changes in cell shape and volume, and changes in cell-cell interactions. These will affect the flow of extracellular and transcellular current and hence the magnitude and characteristics of the signal measured. Each of these physiological changes can be linked to receptor stimulation through classical signalling pathways that result, for example, in changes in cytoskeletal organisation. MDS Sciex plans to launch the commercial CDS system in late 2005.





MDS Sciex Figure 1 (above): A simplified diagram of the custom 96-well microplate used for MDS Sciex CDS measurements; **MDS Sciex Figure 2 (right):** Example of MDS Sciex CDS clustering data. The unique profiles of the CDS responses for different classes of endogenous and transfected GPCRs cluster based on their signaling pathway. The extracellular (ΔZIec) and transcellular (ΔZItc) components of changes in impedance due to receptor stimulation are measured over a range of frequencies and time





ACEA Figure 1 (right): ACEA Biosciences 16 and 96-well RT-CES™ devices. You can clearly see the gold at the bottom of the wells, which are the sensor arrays; **ACEA Figure 2 (above):** Data from ACEA Biosciences RT-CES™ system. Readings were taken every 15 minutes for a period of 90 hours. A reduction in the Cell Index indicates a loss of viability, and conversely an increase in the Cell Index indicates proliferation. The initial cytotoxic effect (hrs 40-60) and subsequent recovery (hrs 60-90) of the target cells upon treatment with the compound would have been undetected using traditional end-point assays such as MTT

ACEA Biosciences (www.aceabio.com) is developing a broad biosensor platform for label-free, real-time analysis of cell-based assays. The RT-CES™ (Real-Time Cell Electronic Sensing) System is ACEA's first product based on this patented technology platform. The core of the system is the microelectronic cell sensor arrays integrated into the bottom of microtiter plates. RT-CES™ uses a non-invasive impedance-based measurement to detect the presence, absence or change of properties of cells or molecules affecting the electronic properties and ionic passage on the sensor surfaces. For cell-based assays, cells are grown in the individual, sensor-containing wells of the microtiter plate and placed in a standard incubator. With the user-friendly integrated software, the system can be programmed to collect data as frequently as every minute over virtually any time period. The electronic sensors therefore provide hands-free, continuous, quantitative information concerning the biological status of the cells present in the well. Changes to the biological status of the cells are measured automatically in real-time by the RT-CES™ system. The entire process is non-invasive and harmless to the cells.

ACEA's RT-CES™ system is ideally suited for the time-dependent, dynamic evaluation of inhibition of cancer cell proliferation and to *in vitro* cytotoxicity testing in both lead identification and lead optimisation phases of drug development. The ACEA Biosciences RT-CES™ system can also be used to study receptor activation for almost any GPCR target, overcoming traditional assay limitations through its sensitivity to subtle cellular changes, and ability to provide real-time monitoring and kinetic data. The ACEA RT-CES™ system is currently available in 16 and 96-well formats for research applications and assay development. RT-CES™ technology is scalable to 384 or higher, and can be readily adapted to robotic workstations.

Resonant acoustic profiling

Akubio (www.akubio.com) is developing a highly sensitive, real time, label-free molecular interaction analysis system based on piezoelectric technology. Called Resonant Acoustic Profiling (RAP™), the technique measures the changes in oscillation of quartz crystal resonators to give information about the specificity, affinity, kinetics and concentration of molecular binding. Based on the same technology the electronics industry uses for timing devices in everything from cell phones to microwaves, the technology is cost-effective, robust, and very scalable.

During RAP analysis target molecules are attached to the sensor surface through direct linkage or capture. Samples containing potential binding partners are then applied to the sensor surface using either flow-based or static delivery techniques. Frequency and resistance parameters of the

crystal oscillation are then measured over time to characterise binding of sample molecules to the surface bound target.

Measurement of the changes in frequency of crystal oscillation provides information about changes in mass at the sensor surface. The RAP technique has already been employed to characterise the binding of compounds as small as a few hundred Daltons and as large as whole cells.

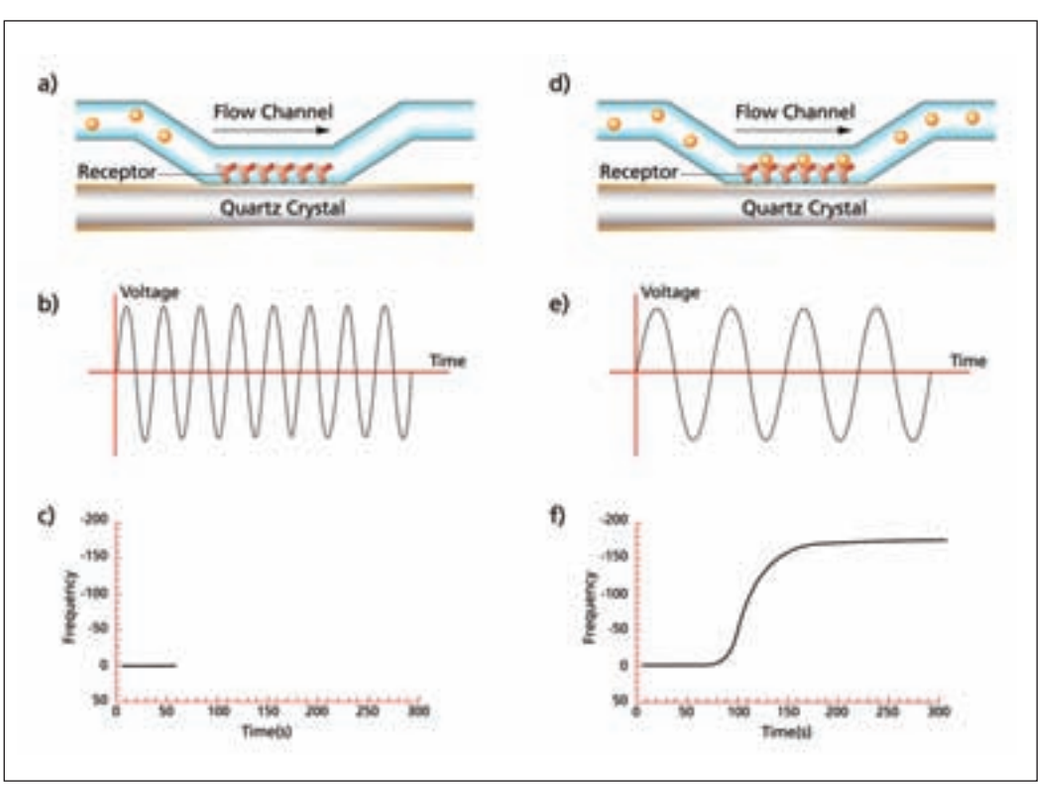
The RAP analysis technique overcomes a number of problems intrinsic to optical based label-free analysis methods. Since RAP measures the change in molecular mass attached to the sensor surface, changes in fluid environment have minimal effect on binding measurements. Analyses of samples in media such as serum and ascities fluid, as well as in organic solvents, cause little if any artefacts in binding measurements. Also due to the simplicity of the detection method RAP technology is very scalable. Both an 8-channel (RAPid) and a 96-well format instrument (RAPArray) are under development. Akubio currently plans the commercial launch of the RAPid instrument in early 2006 and the RAPArray system in early 2007.

Bio-layer interferometry

ForteBio (www.fortebio.com) is developing analytical systems capable of providing rapid real-time analysis of biomolecular interactions (eg protein-protein interactions) in micro-volume sample sizes, providing information on affinity, kinetics and concentration. ForteBio utilises proprietary bio-layer interferometry (BLI) that enables self-calibration and measurement of molecular interactions using simple-to-use and inexpensive instrumentation. The entire analysis can be completed within minutes and does not require labelling of either probe or target. ForteBio's analytical capabilities thus provide greater value in applications where existing methods such as HPLC, ELISA and surface plasmon resonance (SPR) have limitations in throughput, performance and cost.

Conclusions

What is evident from the new product developments described in this article is that the underlying core technologies of some the microplate-based sensors are in fact quite similar. For example MDS Sciex CDS and ACEA Biosciences RT-CES™ are both based on impedance measurements. The ACEA approach essentially allows for cell-based assays to be performed just like doing cell culture but on electronic plate, plus the entire course of the experiment is monitored with the electronic plate inside the incubator. MDS' approach is more like a 'FLIPR' method, where cells are cultured on an electronic plate, and then transferred to their reading instrument which has integrated fluidics for



Akubio Figure: a) A quartz resonator coated with target receptor is integrated with a liquid delivery system for sample delivery; b) While buffer is passed over the sensor surface the crystal is oscillated and its resonant frequency measured; c) The frequency signal is measured vs. time in seconds; d) Sample is then injected across the sensor surface; e) Binding of sample material to the target receptor results in a change in the resonance profile of the resonator; f) The resulting change in frequency signal is measured in real time and is proportional to the amount of sample protein bound

compound addition. MDS is capable of monitoring cellular activation at one-second intervals with a total assay time of less than 10 minutes, while ACEA operates with 60-second updates over many hours. ACEA's initial focus seems to be on cancer biology with an emphasis on cell proliferation and cytotoxicity. MDS is focused on activation of endogenous cellular receptors, aimed at groups performing hit confirmation, potency ranking of ligands and ligand selectivity analysis. In addition, SRU Biosystems' BIND™, Corning's Epic™ and CSEM's WIOS™ also have similarities in that they all utilise a type of optical resonant reflection and have biosensors based on diffraction gratings. It is also these optical systems that in the short term appear to offer the greatest potential, not only in terms of the diversity of label-free applications enabled, but also from a high throughput processing perspective.

The pricing of the disposable microplate-based sensor will also be critical to the future success and impact of label-free. Customers' expectations are high that label-free will bring with it some sort of cost premium (ie savings derived from not having to buy labelled reagents and isotopes) and on average respondents indicated that a 40% cost reduction in current screening costs per well would be required in order to make them switch primary screening to an enabling label-free technology. This means that it will not be enough just to swap current reagents costs for the price of a 384-sensor microplate if technology developers want label-free to be used for more than just difficult or orphan targets.

The market survey concluded that label-free detection is expected to make the biggest impact on secondary screening (Hits-2-Leads), lead optimisation (Leads-2-Candidates) and antibody development over the next one to two years. After three years label-free will impact more on secondary screening, and start to become more widely used in primary screening. However, it is predicted that it will take five years for label-free to become a mainstream primary screening technology. A model was developed to predict the size of the label-free pharma primary screening market. It was estimated that after three years (2007) label-free detection could have captured up to a 4.2% share of the total primary screening market, valued at around $100 million from the sales of instruments and consumables (a label-free sensor plate). It was assumed that only a 384 plate-based label-free sensor will gain sufficient market acceptance to achieve the models predicted sales potential.

The next one to two years will be something of a watershed for label-free detection as the new technology developments described in this article mature and start to be used throughout drug discovery. It will be interesting to see the extent to which these new tools are adopted by end users; the outcome of ongoing IP disputes; the extent to which emerging products may get consolidated within the portfolios of major life science tools providers; and if any of the predictions made in the market survey for label-free hold up. **DDW**

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. Further information on accessing the market report 'Label-free Detection Trends 2004' 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.*