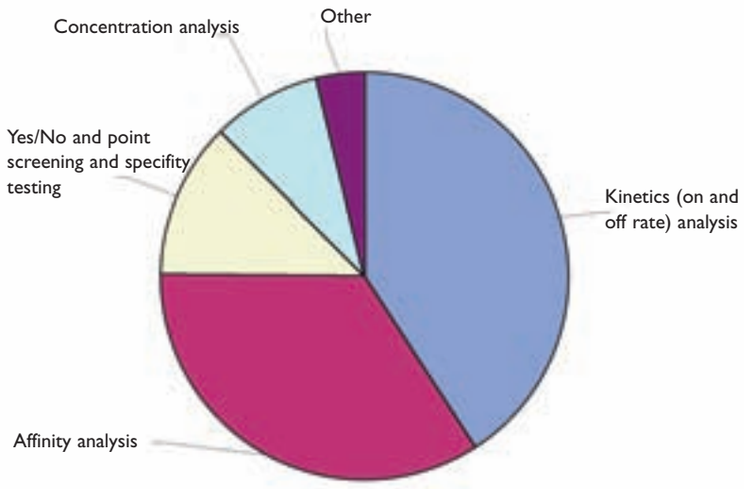
Current biosensor technologies in drug discovery

By Dr Matthew A. Cooper

Over the past two decades the benefits of biosensor analysis have begun to make an impact in the market, and systems are beginning to be used as mainstream research tools in many laboratories[1,2]. Biosensors are devices that use biological or chemical receptors to detect analytes in a sample. They give detailed information on the binding affinity, and in many cases also the binding kinetics of an interaction. Typically, the receptor molecule must be connected in some way to a transducer that produces an electrical signal in real-time. Label-free biosensors do not require the use of reporter elements (fluorescent, luminescent, radiometric, or colorimetric) to facilitate measurements. Detailed information on an interaction can be obtained during analysis while minimising sample processing requirements and assay run times[3]. Unlike label- and reporter-based technologies that simply confirm the presence of the detector molecule, label-free techniques can provide direct information on analyte binding to target molecules typically in the form of mass addition or depletion from the surface of the sensor substrate, or measuring changes in the heat capacity of a sample[4]. However, these technologies have failed to gain widespread acceptance due to technical constraints, low throughput, high user expertise requirements, and cost. While they can be powerful tools in the hands of a skilled user evaluating purified samples, they are not readily adapted to every day lab use where simple to understand results on high numbers of samples are the norm. This article seeks to address some of the issues surrounding the un-met needs in the market place, and the difficulties faced by technology developers in meeting these needs with innovative products. It also reviews recent entries from newer technology developers who are in the race to release products for primary and secondary drug screening, mode of action studies, and screening of pharmacokinetic properties.

The past five years have seen the emergence of a number of new players in the label-free development arena that are bringing new products to the market, and that will stimulate the development of new products from existing players. These companies are detailed in **Table 1**, and selected technologies and accompanying products are briefly reviewed in later sections. A recent report from HTStec[5] summarises the opinions of researchers and managers in 37 different big pharma labs and 15 small pharma/biotech labs towards label-free technologies. The majority of respondents surveyed used label-free technology for kinetic (on and off rate) analysis, followed by affinity analysis (**Figure 1**); a view most likely predicated by the capabilities of the dominant player in the market today: Biacore. Label-free was predicted to displace radiometric assays (a well noted general trend in the industry) as well as ELISA assays, however a sizable minority of respondents did not see label-free replacing any assay formats (**Figure 2**).

Figure 1: Most common use of label-free detection today



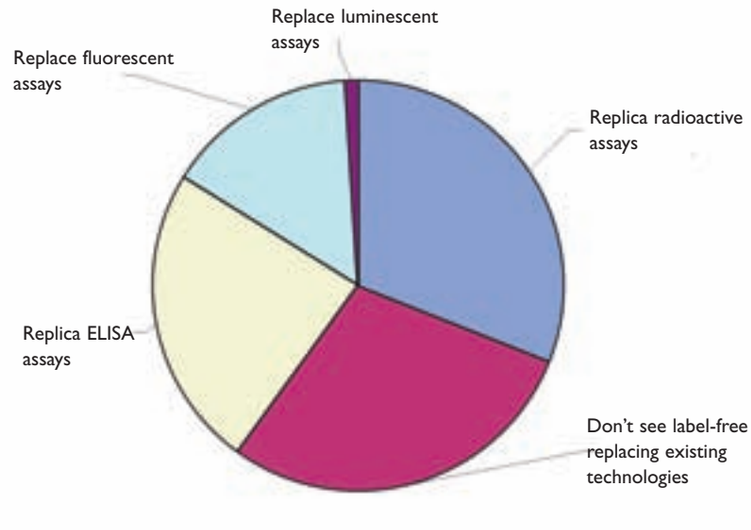
Is label-free difficult to use?

In theory, a label-free assay should be much easier to develop and validate than a labelled assay. Most survey respondents found label-free assays easier, or similar to labelled assays to develop (**Figure 3**). With flow-based (microfluidic) biosensors, it is possible to monitor each step in assay development as it happens, rather than monitoring an endpoint after multiple steps or additions.

However, while one of the key unique selling points claimed by the technology developers (the ease of assay development that arises from the real-time nature of biosensor assays) has been accepted by the early adopters and technical user experts, it has yet to convince the market as a whole. The fact that a sizeable minority did not find label-free assays easier to develop confirms that a significant requirement for detailed technical knowledge of the relevant technology still exists. Comments Ian Campbell, Business Development Director, Akubio: "We have concentrated our efforts on generating a simple to use platform that reproducibly generates high quality data with the minimal number of steps and interventions by the user." Stefan Löfås, CSO at Biacore, has a more sanguine view regarding 'ease of use' which "depends upon the specific technology being used, but in general people see assay development as difficult whatever the technology."

Ronan O'Brien from MicroCal comments further: "[Calorimetry] requires virtually no assay design, is a universal application with no molecular weight limitations and no immobilisation on surfaces required. The drawbacks are mainly the

Figure 2: Choice of detection technologies to replace with label-free



Assays

Table 1: Selected label-free platform developers

PROVIDER	TECHNOLOGY	PRODUCT	WEBSITE
Acea Biosciences	Cell Electronic Sensing	RT-CES™	www.aceabio.com
Akubio	Resonant Acoustic Profiling	RAP◆id™	www.akubio.com
Applied BioPhysics	Electric Cell-substrate Impedance Sensing	ECIS™	www.biophysics.com
Axela	Diffractive Optics Technology	DOT™	www.axelabiosensors.com
Bioanalytic Jena	Surface Plasmon Resonance	BIAffinity®	www.analytik-jena.de
Biacore	Surface Plasmon Resonance	A100, T100, S51, FLEXchip™	www.biacore.com
Calorimetry Sciences	Differential Scanning Calorimetry Isothermal Titration Calorimetry	N-ITC III, N-DSC III MC-DSC, IMC, INC	www.calscorp.com
CSEM	Waveguide Grating Evanescence	WIOS	www.csem.ch
Corning	Resonant Waveguide Grating	Epic™	www.corning.com
EcoChemie	Surface Plasmon Resonance	AutoLab Espirit	www.ecochemie.nl
ForteBio	Biolayer Interferometry	Octet	www.fortebio.com
GWC Technologies	Surface Plasmon Resonance	SPRimager®II, FT-SPRi200	www.gwctechnologies.com
IBIS	Surface Plasmon Resonance	IBIS-1, IBIS-2, IBIS-iSPR	www.ibis-spr.nl
Johnson & Johnson	ThermoFluor®	ThermoFluor®	www.jnjpharmarnd.com
MDS Sciex	Cellular Dielectric Spectroscopy	CellKey™ System	www.mdssciex.com
MicroCal	Differential Scanning Calorimetry Isothermal Titration Calorimetry	VP-ITC, VP-DSC VP-Capillary DSC	www.microcalorimetry.com
Q-Sense	Quartz Crystal Microbalance	E4, D300	www.q-sense.com
Solus Biosystems	Isoelectric Focusing/IR	Solus100™	www.solusbiosystems.com
SRU Biosystems	Colorimetric Resonant Reflection	BIND™	www.srubiosystems.com
TechElan	Thermal IR	_	www.techelan.com
Thermometric	Isothermal Titration Calorimetry	_	www.thermometric.com
Vivactis	Microplate Differential Calorimetry	MiDiCal™	www.vivactis.com
Xerical	Nanocalorimetry	_	www.xerical.com

high sample amounts required. I would guess that biochemists think that ELISA and radiometric assays are easy to develop because they are brought up on it. They do have a perception that anything biophysical is hard. The value of developing quantitative assays is not obvious to ELISA users for whom the semi-quantitative information it provides is often sufficient." Nevertheless there is little argument that the precision and accuracy of biosensor assays are superior to ELISA-based assays. In addition, it is not necessary to develop, or purchase an additional secondary binding partner (antibody) as is the case with ELISA. There is also a significant trend in industry (particular for those companies involved in the development of protein-based biopharmaceuticals) away from ELISA-based screens towards biosensor-based screens.

David Myszka, Director of the Centre for Biomolecular Interaction Analysis, University of Utah, adds: "SPR is very useful for protein-protein interactions, and also for small molecule work. Companies that claim SPR does not work with small molecules probably do not have the expertise to run the system properly. Key is to ensure one has enough active protein on the chip. This is one of the drawbacks of SPR – the majority of proteins on the surface have to be active." This view is reenforced by Geoff Holdgate at AstraZeneca: "[Biosensors] do work with small molecules. More time is required to optimise the conditions, and without this investment, meaningful rate constants and K_D values will not be obtained."

Format of assay – plate based or microfluidics?

The majority of commercially available systems in use over the past 15 years use either cuvettes or microfluidic sensor chips. In order to achieve accurate measurement of interaction kinetics, microfluidic delivery and removal of analyte is essential. However, if an end-point equilibrium binding assay to determine affinities or to rank order compound binding is all that is required, then label-free technologies can be configured to standard plate-based formats. Users from pharma showed a clear preference for 384 well or higher density plate formats that would enable confirmatory/secondary screening, or even primary (HTS) screens (**Figure 4**).

How relevant is label-free to drug discovery and development?

This is a multifaceted question that elicited a varied response from users developers, however there

Figure 3: How easy is assay development with label-free?

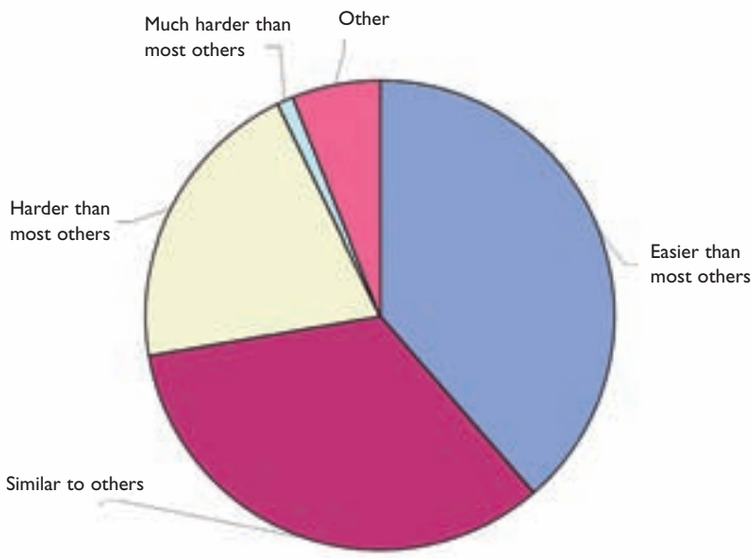


Figure 4: Preferred label-free carrier format

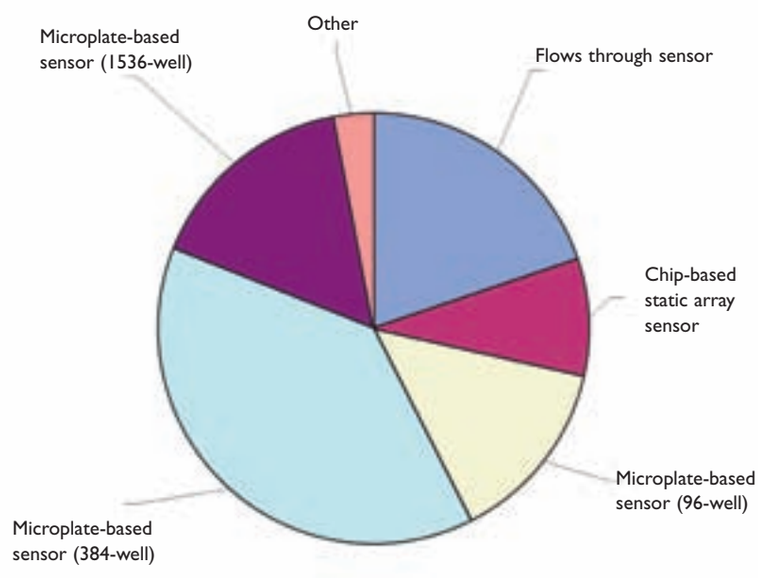


Table 2: Key application areas and benefits of biosensors in drug discovery

APPLICATION	BENEFIT
Target identification and validation	Receptor de-orphaning. Binding-based confirmation of pathway based-screen hit
Binding	Hit validation/secondary screening
Lead characterisation	Optimisation (affinity QSAR and kinetic QSAR). Mode of action
Assay Design	QA/QC of assay components used in HTS
Rank order, affinity, kinetics, and specificity	High information content – triaging of real positives
Early ADME	Predictive bioavailability data
Concentration measurement	Improved accuracy and precision in manufacturing and QA/QC for HTS assays

were several recurrent themes regarding the immediate utility of label-free screening (**Table 2**).

Biosensors are now often used to confirm 'hits' from functional-based assays via use of a receptor-binding assay. Developers of label-free technologies view their data as having high information content, which can enable researchers in pharma to make better decisions during lead optimisation. This information can have additional impact when coupled to quantifiably structural information (eg affinity QSAR, thermodynamic QSAR and kinetic QSAR). Vivactis' Katarina Verhaegen says: "[Users want] any type of high-content assay for any given library in a miniaturised, reliable, cost- and time effective manner. Calorimetry methods provide sophisticated information and direct thermodynamic measurements for almost all possible interactions. The most prominent advantages are its label- and immobilisation-free character, and its universal applicability. Calorimetry will enter drug discovery and development as a high-content discovery tool, but also as a tool to assay 'tough targets' for which no assays are available. A few years ago, people were sceptical of electrophysiology at high-throughput rates and Vivactis is now successfully overcoming the same scepticism concerning calorimetry."

What are the intrinsic advantages with label-free assays and their configuration?

Stefan Löfås (Biacore) states: "When looking at a protein interacting with other proteins or small molecules, [label-free] can provide such comprehensive high quality information in real time and in one instrument." Ian Campbell (Akubio) adds: "[Label-free] provides a mechanism to look directly at the native receptor, without potential reporter interference, in a format where the analyte (drug candidate) is introduced in solution in real time to generate high levels of novel and confirmatory data on interaction specificity, affinity, rank order binding and kinetics. This can be achieved with minimal assay development times and empowers the users to make better decisions on these key biophysical parameters using a very simple assay."

Katarina Verhaegen (Vivactis) sees additional advantages for calorimetry over optical biosensor systems in the accurate determination of true binding characteristics and mechanism of action (SAR), real-time monitoring of enzyme and cellular kinetics. These include the lack of a receptor immobilisation requirement, compatibility with DMSO, applicability to both biochemical and cellular assays and minimal sample usage. Commenting on the main disadvantages for calorimetry, Katarina adds: "The limit of the [Vivactis] screening technology today is the requirement for approximately 3 to 15µM [ligand]. With our longer-term research programs, we will steadily evolve towards lower concentrations. However, calorimetry must not be seen as the uttermost sensitive technology, but as a high-content, all-target, label- and immobilisation-free screening technology."

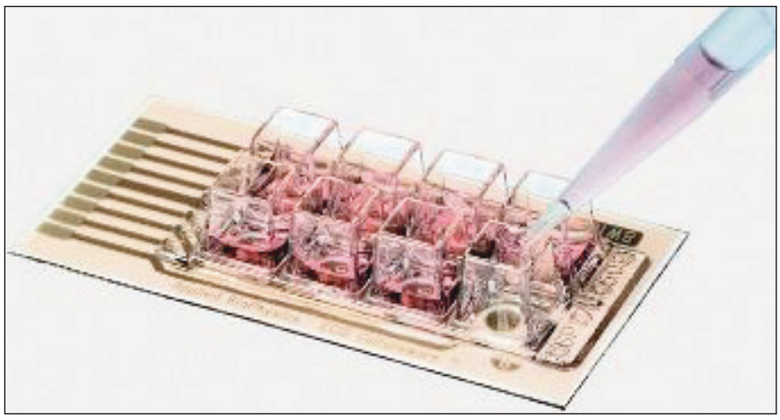


Figure 5: An 8-well electrode array from Applied BioPhysics

Impedance assay systems for cell-based screening

The basic principle of Electric Cell-substrate Impedance Sensing was first reported by Giaever and Keese, then at the General Electric Corporate Research and Development Center[6]. In contrast to other methods of monitoring cellular signal transduction, impedance measurement of cellular responses can provide high information content in a simplified, label-free and non-invasive fashion. Three companies have commercialised this technology and are summarised briefly below.

ACEA Biosciences

ACEA Biosciences (San Diego, CA) has released the RT-CES™ (Real-Time Cellular Electronic Sensing) based on a microelectronic cell sensor array integrated into the bottom of standard SBS format microtitre plates. RT-CES works by measuring electrical impedance across the sensors to detect the presence, absence, or change in condition of cells. For cell-based assays, cells are grown in the individual, sensor-containing wells of the microtitre plate and placed in a standard incubator. The system can be programmed to collect data as frequently as every minute by sending nominal current through the sensors at the user-defined intervals. The electronic sensors provide information on impedance values, which is then converted to a measure known as Cell Index (the impedance of the cells normalised for the impedance of the media alone). Major application areas include: cancer biology, cell adhesion and spreading, receptor-ligand binding and signal transduction analysis, cell proliferation, cytotoxicity, cellular differentiation, and infectious disease and environmental toxicology. ACEA foresee key applications in the drug discovery field to be centered on cell proliferation assays, and on the elucidation of compound toxicity (eg for the prediction of *in vivo* toxic effects and for lead optimisation in secondary screening and the early phases of drug development). Further key applications involve the analysis of receptor-ligand binding, in particular for agonists, partial agonists, and antagonists of GPCRs.

Applied BioPhysics

Electric Cell-substrate Impedance Sensing (ECIS) from Applied BioPhysics (Troy, NY) is also based on a technique of measuring the change in impedance of a small electrode to AC current flow. The heart of the measurement is a specialised slide that has eight individual wells for cell culturing. The base of the device has an array of gold film electrodes that connect to the ECIS electronics to each of the eight wells (**Figure 5**). Cell densities ranging from a heavy confluent layer to very sparse layers can be measured with this approach. The size of the electrodes restricts the maximum number of anchored cells that can be observed from 100 to 1,000 cells (dependent upon the type of electrode array used in the instrument), but even a single isolated cell results in impedance changes that can be monitored. Key applications exemplified by Applied BioPhysics include the monitoring of cellular behaviour properties such as growth, proliferation, attachment, spreading, migration, motility and behaviour under flow. The technology has also been refined to enable profiling of signal transduction, metastatic potential and *in vitro* toxicity.

MDS Sciex

MDS Sciex (Concord, Ontario) has recently released the CellKey™ System (**Figure 6**), which uses cellular dielectric spectroscopy (CDS) to quantitatively and kinetically measure endogenous cell surface receptor responses to ligands in live cells. Using this technology, a series of receptor-specific, frequency-dependent impedance patterns (called CDS response profiles) resulting from changes in cellular bio impedance are collected every two seconds as spectrum of frequencies (1KHz to 10MHz). The characteristics of the CDS response profiles are used to determine the identity of the signalling pathway being activated by the receptor-ligand interaction and provide easy to access information on compound selectivity. In addition, these profiles allow quantitative pharmacological analyses such as potency and Schild analysis. Recently published work demonstrates the effectiveness of the system in profiling many endogenous ligand-induced cellular responses mediated by the three major classes of G-protein-coupled receptors, G_s, G_i,

and G_q, as well as a number of protein tyrosine kinase receptors in many different cell types including primary cells[7].

Optical biosensors

Biacore

Over the past 3-5 years Biacore's S51 and 3000 instruments have demonstrated the sensitivity required for typical drug candidates and lower molecular weight (~150Da) drug fragments or 'needles'. The sensitivity for drug 'needles' is poorer than for larger molecular weight drugs as SPR measures changes in refractive index that are directly related to the molecular weight of the binding molecule. After more than 20 years of research and development, SPR sensitivity may be approaching theoretical limits in terms of the detection interface sensitivity, however there is still significant scope for improving usability, increasing throughput and integrating better with existing liquid handling capabilities in HTS, and with industry standard data archiving and data mining software. Relevant commercially available Biacore systems (**Figure 7**) are reviewed briefly below.

The Biacore S51 system was the company's first product designed for the pharmaceutical industry based on a three-spot flow cell system with automated delivery of drug candidates, analysis of binding profiles and output of key kinetic and affinity parameters. The system exploits hydrodynamic focusing of fluid flow to selectively pass analyte across a control spot and either one, or both of two active receptor spots. The Biacore T100 system is based on the S51, with the addition of automated temperature control for vant Hoff determination of steady state parameters (ΔG°, ΔH°, ΔS°) and transition state parameters (ΔG°‡, ΔH°‡, ΔS°‡), which enable the activational energies of an interaction to be probed. Up to 384 samples can be run automatically and, as with the S51, software wizards are provided for fast assay development, analysis and evaluation of interaction parameters. The system also possesses integrated sample cooling for temperature-sensitive samples and is 21 CFR Part 11 compliant for use in GxP environments. David Myszka, Director of the Centre for Biomolecular Interaction Analysis at the University of Utah and a senior Biacore consultant, comments: "We find Biacore T100 to be exceptionally easy to run, with intuitive operating and analysis software. The ability to collect high quality data at elevated temperatures and to automatically switch between multiple buffer systems expands the application of Biacore as a biophysical tool for characterising protein interactions."

Figure 6
The CellKey™ System from MDS Sciex

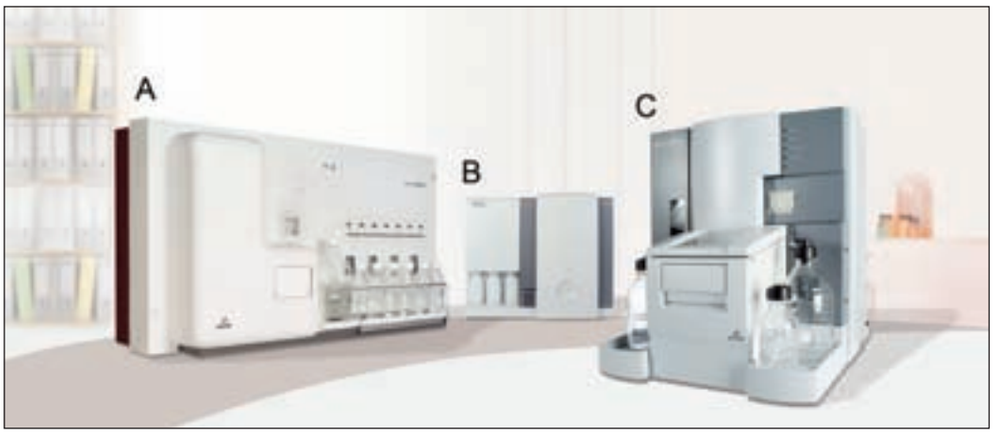


Figure 7
Commercially available systems described in this article from Biacore. A) A100, B) FLEXCHIP and C) T100

The Biacore A100 system was released in 2005 to enable higher throughput processing of protein therapeutic and small molecular weight drug candidates. Again the system uses the S51 hydrodynamic focusing fluidics, except that six sensor chips with five spots per chip are employed. This enables up to 3,800 interactions to be screened per day.

In 2005 Biacore acquired the FLEXchip™ system and patents from HTS Biosystems/Applied Biosystems that were developed to enable parallel screening of up to 400 micro-arrayed receptors against multiple potential protein binders. The system is based on micro-arrayed proteins, nucleic acids or thiolated peptides. These receptors are probed with a movable light source applied for plasmon coupling mediated via a proprietary diffraction grating. In contrast to other Biacore systems, in which receptors are deposited 'on-line' in liquid phase, the FLEXchip™ system requires a separate dedicated protein micro-arrayer to deposit receptors 'off-line'. Applications have been developed for receptors that can be readily micro-arrayed, such as Protein A/G for immunoglobulin binding, consensus oligonucleotides for DNA-binding proteins and biotinylated SH2-, 14-3-3-, WW domain- and kinase-binding peptides. The system is not suited for routine pharmaceutical screening applications due to lower detection sensitivity (~10µg/ml) and higher analyte volume requirements (~1.8ml) than is the case with other Biacore and competitor systems.

SRU Biosystems

The BIND™ instrument from SRU Biosystems is comprised of the BIND Reader™ and 96-, 384-, and 1536-well microplate consumables. The BIND™ system takes advantage of a novel optical effect to provide very sensitive measurement of binding on the biosensor surface. The biosensor incorporates a proprietary nano-structured optical device that forms the base of micro-well plates in industry standard formats. A photonic crystal optical filter produces a specific single wavelength of reflected light, and assays are performed on the structure to test for binding. The sensors can detect a shift in wavelength down to half a picometer (one picometer is one-trillionth of a metre). Binding interactions can be quantified with proteins, cells, and small molecules with between 2-200uL total sample per well. Sensitivity is quoted in the 0.05µg/ml to 1mg/ml range with molecular weights demonstrated <200Da. A high resolution imaging detection instrument has also been developed that is capable of assay multiplexing with multiple spots per well, self-referencing of assay artefacts such as bulk refractive index effects and non-specific binding, and imaging detection of cell attachment with single-cell resolution.

Corning

Corning has also developed a label-free detection platform that utilises resonant waveguide grating sensors. The Corning® Epic™ System consists of 384-well microplates (**Figure 8**) with an optical interface and surface attachment chemistry within each well, and an HTS reader capable of analysing up to 40,000 wells in an eight-hour period. When illuminated with broadband light, the optical sensors inside each well of the Epic™ micro-plate reflect only a specific wavelength that is a sensitive function of the index of refraction close to the sensor surface. The platform can be used for both biochemical and cell-based assays.

This plate-based approach not only facilitates true high-throughput label-free detection, but also affords the user the benefit of being able to integrate an already installed HTS capital base. The platform has a sensitivity of 5pg/mm^2, which enables the detection of the binding of a 300Da compound to a 70kDa immobilised target with CVs of 10% or less (depending on assay type). If the immobilised target is smaller (eg 25kDa) it is possible to detect the binding of a smaller compound (eg 150Da). For biochemical assays, the sensors are coated with a surface chemistry layer that enables covalent attachment of protein targets via a primary amine group. The surface chemistry provides a high binding capacity surface, with low levels of non-specific binding. Users may choose to immobilise proteins, peptides, small molecules and DNA (containing primary amine groups). After a target is immobilised, a baseline reading is established. 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 this results in a shift in the wavelength of light that is reflected from the sensor. The magnitude of this wavelength shift is proportional to the amount of analyte that binds to the immobilised target.

Corning Life Sciences' Dana Moss elaborates: "The Corning© Epic™ System is a universal platform that can perform a range of assays from label-free, direct binding assays to label-free, functional assays. The label-free, direct binding features enable the screening of 'intractable' targets and pathway interactions that cannot be screened today because of labels, licence fees, or a lack or adequate methods. The universal applicability of the Corning® Epic™ System across biochemical assay types enables the discovery of new chemical entities (small molecule) as well as new biological entities

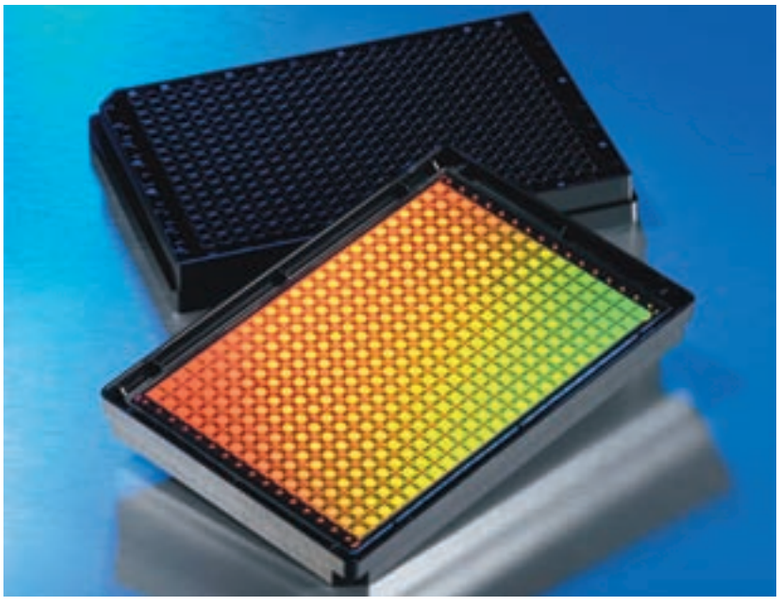


Figure 8
The Epic™ System 384-well plate from Corning®

(large molecule). Some examples of label-free, biochemical, direct bind and functional assay types for this universal platform include enzyme/small-molecule drug, enzyme/natural substrate, protein/DNA, antibody/antigen, protein/protein, antibody profiling, label-free safety panels, K_D estimations, and cytokine/cytokine receptor assays, to name a few."

In addition to biochemical assays, the Corning® Epic™ System also enables cell-based assays. The detection principles for performing whole cell assays are similar to those for biochemical assays: changes in local index of refraction are manifest by a shift in response of the sensor. The sensors in each well detect index of refraction changes that occur within the first 200nm from the sensor surface. This surface sensitivity means that only the bottom portion of whole cells cultured on the sensor is monitored during an assay. When endogenous macromolecules within the cytoplasm of mammalian cells move into or out of the sensing volume, a change in the local index of refraction is induced which leads to a shift in sensor response. Moreover, if in response to a stimulus, the cell changes shape, or the endogenous material within the cell that is in close proximity to the sensor reorganises, a shift in sensor response is also induced. Thus, the Epic™ System is sensitive to both whole cell movement and to mass redistribution within a cell. Examples of label-free cell-based assays for the Epic™ System include signal transduction (EGFRs, GPCRs, cytoskeleton modulators), toxicity screening, lipid signaling and cell proliferation. Corning sees a label-free detection as a key milestone for the Life Sciences division, comments Moss: "The Corning® Epic™ System fuses together Corning's history in biochemistry, microbiology, photonics, surfaces and materials science. The Beta testing evaluations of the Epic™ System are currently being conducted at select pharmaceutical, biotechnology and academic institutions. Full commercial launch of the Epic™ System will commence in the fourth quarter of 2006."

ForteBio

ForteBio (Menlo Park, CA) has this year released the Octet System, based on a proprietary technique called BioLayer Interferometry (BLI). The Octet System utilises disposable sensors with an optical coating layer at the tip of each sensor (**Figure 9**). This optical surface is coated with a biocompatible matrix that can interact with molecules from a surrounding solution. A sample volume of 80µl can be used in low volume microplate wells to make accurate measurements, as volumes less than this can generate measurement artefacts due to internal reflections during measurement. To overcome the effects of diffusion on kinetic measurements, the sample plate is subject to orbital motion relative to the biosensor. Experiments can be performed with static samples (for binding steps), or with motion ranging from 100 to 1,500rpm.

The Octet instrument then shines white light down the biosensor and collects the light reflected back. Interference patterns in the reflected light are captured by a spectrometer as a characteristic profile of wavelength peaks and troughs. When biological molecules bind to the biosensor surface its thickness increases, and the binding can be monitored by analysing changes in the interference pattern at the spectrometer. Unbound molecules and changes to the matrix do not change the interference pattern, which allows the ability to use crude cell lysates or periplasmic samples. Samples can be presented in a variety of buffers or diluents including common cell culture media, Serum-containing media (up to 25%) and DMSO-containing buffers (up to 10%) are commonly used. ForteBio's Octet System can measure molecular interactions and can be used to determine affinity, kinetic analysis or concentration. Joy Concepcion, Product Manager, ForteBio, says: "BLI differs from [SPR] in that the technique does not involve measurement of dielectric constant or refractive index of the solution using an evanescent sensing field, and is hence only minimally perturbed by changes in the medium."

Stephen Oldfield, VP Sales and Marketing, elaborates: "We shine white light down an optical fibre on to a proprietary optical coating with

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biomolecules attached. Most of the light passes through the coating, however ~4% is reflected back through the optical fibre to a spectrometer. Some wavelengths of light are subjected to constructive interference, and others to destructive interference, which give rise to an interference pattern across the visible spectrum. This pattern is sensitive to the thickness of the biological layer at the surface of the coating. As the surface layer thickness increases, the interference pattern is red-shifted, which can be accurately measured in real time. Since the binding measurements are made from the 'clean' side of the fibre, refractive index changes and even particulates in the surrounding solution have minimal effect on the signal."

Acoustic biosensors

Akubio

Resonant Acoustic Profiling (RAP) from Akubio (Cambridge, UK) provides researchers with the ability to perform accurate real-time, label-free characterisations of interactions with reagents in buffered solutions, DMSO solutions and crude matrices such as culture media, hybridoma supernatants, urine and serum. RAP, which is based on piezoelectric quartz crystal technology, measures the build up of molecules on the surface of an oscillating crystal and provides real-time binding information on the binding interactions. In the past, acoustic detection has been used to characterise interactions with peptides[8,9], proteins and immunoassay markers[10-13], oligonucleotides[14,15], bacteriophage[16,17], viruses[18,19], bacteria[20-22] and cells[23-28]. The technology can thus be applied to an extremely wide range of biological and chemical entities. However, any system that utilises a highly sensitive transducer such as piezoelectric quartz to measure molecular interactions must possess a variety of integrated technical controls to facilitate the highest level of sensitivity, accuracy and precision. Previous attempts to exploit this detection method in a commercial format have been limited by poor sample delivery mechanics, inadequate thermal controls and the lack of a multi-sensor analysis platform. The development of proprietary electronics, fitting algorithms and a low-stress mounting system for the sensor has enabled Akubio to integrate microfluidic delivery together with automated liquid handling in the RAP◆id system (**Figure 10**). "Ease of use is further facilitated by a number of simple to use coupling chemistries for attachment of target proteins to the sensor surface, and flow-chart based assay design software," says Ian Campbell, Director of Business Development. "Currently, the RAP technology fits into a diverse range of activities in pharmaceutical drug discovery and development R&D. RAP can be easily integrated into key processes in target selection, clone profiling, tertiary screening (lead optimisation and SAR studies). With our initial product offering, Akubio are focused on serving the proteomics and new biological entity discovery market, with a platform that has high (sub-nanomolar) sensitivity and moderate throughput (>400 samples/day). Users

Figure 9: The ForteBio Octect system and associated coupling kits and disposable fibre optic sensor tips

can also work directly with complex samples with no-need for laborious pre-assay purification or post-assay calibration routines."

All optical label-free detection methods ultimately measure changes in dielectric constant or refractive index of a solution in close proximity to the surface of the sensor substrate. While they are powerful techniques under extremely well controlled conditions, the advantages are often minimised when trying to apply these methods in routine analysis procedures. As optical methods rely on proximity-based detection, any analyte that is within the evanescent sensing field (typically 300nm for most SPR devices) is detected as 'bound'. This is the case whether it is physically bound to the receptor or simply in close proximity to the surface of the sensor. In contrast, RAP measures only those materials that are acoustically coupled to the sensor surface; ie binding-based detection rather than proximity-based detection. The process of measuring refractive index changes with optical methods to infer mass changes imparts a number of other intrinsic limitations: in particular the masking of binding events that occurs in sample environments that have variant refractive indices. In cases where the molecules to be tested have been solubilised in organic solvents, or are components of a crude cell lysate, culture medium or a serum sample, optical-based techniques often are incapable of measuring associated binding events without extensive calibration or sample preparation procedures. "One notable advantage of acoustic detection over more established optical label-free detection is the relative insensitivity of acoustics to changes in solvent. When running samples containing DMSO, optical detection systems suffer from large bulk refractive index shifts that arise from the disparate properties of the organic solvent and the running buffer", states Ian Campbell. The dielectric constant of water is 80, whereas DMSO is 40 (a difference of 100%). To normalise for these large bulk effects in an optical system, a calibration routine using known serial dilutions of DMSO in running buffer is normally run at the beginning, middle and end of a screening panel[29]. In contrast, acoustic systems are not effected by dielectric constant/refractive index changes, but are instead sensitive to bulk effects dominated by viscosity and density of the solvent; more specifically the square root of the viscosity:density product[30]. For water, this value is 0.99, whereas for DMSO it is 1.10 (a difference of only 11%).

"Through recent improvements in microelectronics and crystal mounting, RAP technology is beginning to provide researchers with a new generation of analytical tools for producing data on the real time kinetics of molecular interactions," comments Andrew Carr, CEO. "If you need to study the kinetics of molecular interactions in biologically relevant samples, you have a problem today. We believe that a range of new analytical tools based on RAP can now start to address many of the limitations of existing techniques. RAP technology is still at an early stage in its full development but already products such as the Akubio RAP◆id-4 can provide researchers with real benefits".

Figure 10
The RAP◆id-4 system from Akubio

Q-sense

Q-sense offers acoustic label-free sensors based on a novel technique, QCM-D. By collecting both the dissipation and the resonance frequency of a quartz crystal, QCM-D can be used to study the formation of thin films such as proteins, polymers and cells on to surfaces in liquid. By measuring several frequencies and the dissipation, it is possible to judge if the adsorbed film is rigid or soft, and the kinetics of structural changes and mass changes can be obtained. Q-Sense offers two products: Q-Sense E4 and Q-Sense D300. The E4 is the new generation instrument for rapid characterisation of bio-interfaces. Four sensors enable four parallel measurements with an optional electrochemistry cell. The D300 is the original Q-Sense instrument that enables QCM-D studies of a variety of processes taking place on its sensor surface. The company offers sensors pre-coated with gold, stainless steel, silica, titanium and polystyrene for surface science research.

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Conclusion

The past five years have witnessed the emergence of an increasing number of commercially available technology platforms that are driving the development of novel label-free assays. A label-free screening system imparts additional flexibility to the process of assay design with potentially fewer artefacts. Scientists in both academia and industry are using biosensors in areas that encompass almost all stages of the drug discovery process, but the technology remains some way from being accepted as mainstream. However, the wider availability of novel sensor platforms that exploit optical, electrical and acoustic interrogation of a sample should finally begin to break down the resistance to uptake of label-free as a valuable and easy to use tool in drug discovery.

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Disclaimer

The author is a founder of Akubio and a former consultant to Biacore. This article represents the sole opinion of the author; not that of Akubio, nor Biacore, nor any employee, shareholders, consultants, directors or other representative thereof. DDW

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