Moving forward with label-free technology

Label-free technology is gaining acceptance and opening doors for new drug discovery. Relatively new in the well-established world of high throughput screening (HTS), label-free technology provides opportunities for probing biomolecular interactions without spatial-interference, autofluorescent or quenching effects of labels. Endogenous targets and multi-component pathways can be explored in a cellular background that more closely reflect their natural environment. According to the recent report *High Throughput Screening 2010: Effective Strategies, Innovative Technologies, and Use of Better Assays*, based on interviews with 52 HTS directors at pharmaceutical companies and government-sponsored institutes, label-free technology adoption is reported to be one of the three most important trends that will impact HTS in the future.

By Dr Jennifer Hartigan, Cindy Liu and William Downey

Interest in and use of label-free technology has increased in the past several years in drug discovery HTS labs. As compared to previously reported in our 2007 study, 42% versus 31% of the respondents are considering adopting the technology now than in 2007 (**Figure 1**).

According to the HTS report findings, cost of instruments and plates and low throughput are the most significant barriers to increased use of label-free technology (**Table 1**). These factors make it challenging to use label-free as a main screening technology for primary screening. Many directors who have label-free instruments do not use the technology for primary screening of large-scale compound libraries, but instead for smaller screens, for example, fragment libraries with smaller numbers of compounds or hit-to-lead optimisation. In addition to cost and throughput, these directors indicate there is limited understanding of the phenotypic changes observed in cells using label-free technology and the sensitivity and the limits of detection for biomolecular interactions need improvement.

Selected comments from respondents discussing their use and the limitations of label-free technology are shown below.

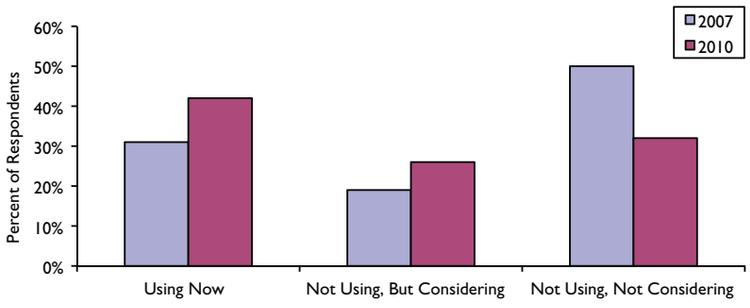
"We want to get away from fluorescence-based assays and some of the artifacts of these assays as well as the cost of labelling. The limitations of label-free technologies are that you see an affect in the phenotypic assay and then you need to try to correlate that to a biologic process. It works for GPCRs but it's not always generalisable."

A non-commercial screening lab

"Yes, we are using label-free technologies but not for primary screening. We use them for hit verification and hit to lead optimisation, mainly because of the cost and throughput."

A High Throughput Screening lab

Figure 1: Trending of label-free instrument usage



"Presently, we are using label-free technologies in collaboration with another company. We're looking at and thinking about purchasing a surface plasmon resonance instrument, however, the limitation is the throughput. We've also looked at a number of the impedance-based technologies. The cost and applicability is more for secondary screening not primary."

A medium throughput screening lab

"For binding assays the current format limits the size of the protein, above ~65,000 you won't detect the change in molecular weight. On the cell-based side, I am seeing label-free technologies as pretty promising. It is kind of a black box, but as long as we characterise the hits and the pharmacology lines up, I think this is useful."

A High Throughput Screening lab

Table 1: Limitations of label-free technology

LIMITATIONS	NUMBER OF MENTIONS
Cost of instruments and plates	16
Low throughput	11
Less useful for primary screening, used more in other areas	10
Unclear biological result, no functional meaning, black box	8
Low sensitivity or limited detection	5
Not applicable for all assays	1
Technology needs specialised plates and linkers	1

Advances in label-free technology

Both biomolecular interactions and whole cells can be assayed using a variety of label-free instruments available today. Recent advances in label-free instruments offer improved cost, throughput, increased sensitivity and more sophisticated data analysis. Some examples of these advances made by label-free instrument suppliers are described below.

Molecular Devices

In late 2008, Molecular Devices (www.moleculardevices.com) launched the CellKey® 384 System; the only 384-well impedance-based label-free system for cell-based assays. With fully integrated fluidics and thermal control, the system delivers robust and sensitive universal assays for measuring a wide range of targets important to the drug discovery community. The system has well proven abilities to measure G-protein coupled receptor (GPCR) and Tyrosine Kinase Receptor (TKR) activation and also demonstrated capabilities in the measurement of ligand-gated ion channels and adhesion molecules, among other targets.

Debra Gallant, Product Manager at Molecular Devices, comments: "Two growing trends in receptor biology can be hugely benefitted by the adoption of label-free cell-based assays. The first is the study of more biorelevant systems. The nature of the non-invasive label-free measurement provides the sensitivity to measure endogenous receptor targets in native cell types, including primary cells. Therefore, one is able to study receptors in their natural environment, not the artificial environment that is imposed when using a recombinant expression system or artificial dyes and tags which may disrupt native signalling cascades. The second trend is an ever growing understanding that signal transduction pathways, particularly those of 7 transmembrane receptors, are far more complex than previously understood and that current technologies don't do enough to address this complexity. The lack of high quality assay data frequently leads drug discovery researchers down false paths during hit identification and characterisation. The ability to study multiple signalling cascades and deliver high information kinetic data makes the CellKey® System an ideal tool for addressing this need."

GE Healthcare Life Sciences

GE Healthcare Life Sciences (www.gelifesciences.com) has launched two new systems for label-free interaction analysis in 2010, according

to Christina Burtsoff Asp, Marketing Manager, GE Healthcare Life Sciences, Sweden – Biacore™ 4000 for candidate selection and Biacore™ T200 for characterisation of biomolecular interactions.

Biacore™ 4000 is designed for confident candidate selection in biotherapeutic and small molecule drug discovery. It is developed for large-scale parallel interaction analyses, with the capability to analyse up to 4,800 interactions in 24 hours. The system provides high quality binding, kinetic, affinity, concentration and specificity data in both screening assays and detailed characterisation studies.

Biacore™ T200 is a versatile, label-free interaction analysis system with outstanding sensitivity for comprehensive characterisation of biomolecular interactions. The system pushes the detection limits in label-free analysis by enabling new applications such as GPCRs, offering increased assay flexibility and providing more confidence in data from early research to drug discovery and development, and on to QC.

"Label-free technologies including surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC) are increasingly being used in drug discovery and development, providing key insights into biological process and molecular binding mechanisms," says Burtsoff Asp. "The use of biophysical methods is becoming more and more important in fragment-based drug discovery and vaccine development, demanding increased sensitivity, reproducibility and improved data processing. GE Healthcare Life Sciences' newly launched products within label-free interaction analysis address these needs enabling better-informed decisions earlier. Similarly, label-free technologies for small organism imaging and analysis will allow phenotypic screening at the organ level, complementing the molecular-level insights allowed by SPR and ITC."

Roche Diagnostics

Roche's (www.roche.com) flagship label-free technology is the xCELLigence™ system, according to Steven Hurwitz, Manager of Marketing, Cellular Systems at Roche Diagnostics Corporation.

New for 2011 is the xCELLigence™ RTCA HT instrument that offers a modular 384-well format where up to four plate stations can be run on a

PerkinElmer is launching the first bench-top multimode reader that incorporates the established Corning Epic® optical label-free technology in early 2011

robotic platform. This system is well suited for secondary screening in drug discovery due to its ability to perform short-term GPCR assays as well as longer-term cytotoxicity applications in addition to utilising primary cells and assessing endogenous receptors for more physiologically relevant data.

Also new for 2011 is the xCELLigence™ RTCA Cardio instrument for pre-clinical safety testing of lead compounds in drug discovery. The ultra-high data capture rate of this system resolves the actual beating patterns of culture cardiomyoctyes, thus allowing for the detection of compound induced arrhythmias in real-time. "Utilising human stem cell-derived cardiomyocytes or induced pluripotent stem cell cardiomyocytes provides an earlier predictive link to possible live human drug reaction and should lead to the possibility of reduced animal testing and earlier identification of failed hits in the screening process," says Hurwitz.

The ForteBio Octet® RED384 and Octet® QK384

PerkinElmer

PerkinElmer (www.perkinelmer.com) will be launching the first bench-top multimode reader that incorporates the established Corning Epic® optical label-free technology in early 2011, according to Achim von Leoprechting, Vice-President and General Manager of Imaging and Detection Technologies, PerkinElmer, Inc. The new system is a lower cost, compact platform and, along with the label-free detection, can include classical labelled technologies such as AlphaScreen, absorbance, fluorescence and luminescence readout modalities.

"The use of optical label-free detection allows the researcher to measure biochemical and cellular events without introduction of labels and therefore minimises perturbations in the activity to be measured," says von Leoprechting. "This has particular relevance when looking at cellular activity, either in basic research or in drug discovery." The use of label-free detection in a multimodal reader offers the researcher complete flexibility with biochemical and cellular analysis. The effects of drugs as measured in classical HTS formats can be directly compared in the same instrument and on the same cells, thereby allowing for the first time direct comparisons to be made of drug pharmacology.

The system is "specifically powerful in orthogonal testing as part of the hit confirmation process for structure activity relationship (SAR) studies due to its exquisite sensitivity and for biophysical tests as a higher throughput front end screen, for example, for the determination of binding strength," says von Leoprechting. "This new system would also be used as an assay development tool in disease research labs in the drug discovery process to run label-free assays on a cost-effective, flexible and compact platform. This is made possible with the unique offering of multimodal functionality and robust proven label-free technology, at an affordable price."

ForteBio

ForteBio, Inc (www.fortebio.com) has launched two next-generation instruments during the last two years, according to Christopher Silva, ForteBio's Vice-President of Marketing – the Octet® RED384 for protein, peptide, small molecule and fragment screening and the Octet® QK384 for protein and antibody assays. The Octet® RED384 and the Octet® QK384 will enable 384-well detection, 16-channel simultaneous readout, biosensor regeneration and reracking, and automation capabilities for biotherapeutic and pharmaceutical drug discovery assays.

Recently, the company also launched two new

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assay modules to support development of biotherapeutic and pharmaceutical products for use on the company's Octet® instrumentation platform, for high-sensitivity protein quantitation and small molecule and fragment screening, respectively. The new modules are available via software upgrades and will enable, for the first time, life science researchers who require these capabilities to access the high-throughput, ease-of-use and cost-effectiveness of ForteBio's leading label-free technology.

"The Octet® RED384 and Octet® QK384 will deliver revolutionary efficiency improvements for life science research applications that have traditionally been conducted using ELISA or SPR-based methods," says Silva. "For example, our new label-free instruments enable researchers to perform protein kinetic screening and characterisation on a 384-well plate in about two hours."

The new high-sensitivity assay module can detect subnanogram per milliliter levels of analytes in quantitation mode. "For protein quantitation, life science researchers have traditionally run fluorescence and luminescence ELISA methods to achieve greater sensitivity in their assays. Now, using ForteBio's new modules, they can obtain highly precise results, enabling follow-up research to be conducted the same day," says Silva. "Additionally, labs that have transitioned from ELISA techniques to the Octet® platform have improved their productivity by 50-70%, with no additional cost per well."

Benefits of label-free technology

"The trend towards biologically relevant assays is a large opportunity for label-free technologies," says Gallant. "Scientists are able to study not only endogenous receptors in cell lines, but also primary cell types – both of which deliver access to receptors in more natural states. We have found that as scientists become more comfortable with technologies that measure and interpret whole cell responses, they discover the greater power of the data generated, especially when compared to single point/single pathway assays. Though the data output may seem initially more complex, it is also

more relevant, and a more accurate reflection of the target biology."

von Leoprechting agrees: "Label-free detection greatly expands the types of cells that can be used, including human primary cells and stem cells, as well as the types of targets that can be studied. Since the cells are not genetically manipulated to introduce a label, label-free methods allow the researcher to measure the response in a manner as close to the authentic cell physiology activity as possible."

"Kinetic profiles can be obtained that are quantifiable and lead to information suitable for applications such as cell culture QC, proliferation, cytotoxicity, receptor studies and cell invasion/migration to name a few," adds Hurwitz.

Biochemical interactions also benefit from label-free analysis "showing the interaction as it happens in contradiction to an end-point assay", says Burtsoff Asp. "The label-free interaction data provides not only information such as kinetics, affinity, thermodynamics, specificity, concentration and enzyme kinetics, but can also be used for data quality control or trouble shooting supporting confident decisions in research."

"Fragment-based lead generation is becoming an increasingly important component of drug discovery," says Silva. "The Octet® platform's sensitivity and ability to assay precipitating compounds using a 'dip and read' format, its greater dynamic range and our new analysis software capabilities, enable rapid screening of fragment libraries in a microplate format."

"Label-free assays also provide great opportunity in delivering simplified methods for studying the complexity of biological pathways," says Gallant. "The universal assay format allows one to study multiple pathways at once, making assay development less tedious and data more easily comparable."

We expect to see "greater adoption of label-free in non pharma/biotech areas through greater accessibility due to decreased cost, decreased instrument size and higher value for money capital", says von Leoprechting. "This may also assist the pharmaceutical industry in widening the outsourcing and collaboration potential with academic partners and CROs."

Continuing challenges

"Label-free technology is still early in its growth phase, especially for cell-based assays. How it can be used and integrated into standard procedures offers the greatest possibilities. Deconvolution of the high content data has been one featured challenge and is one that both industry and customer have been working together to improve," says Hurwitz. "There is continuous work going on to further develop system performance and also to improve support for data handling and data interpretation," adds Burtsoff Asp.

One of the challenges limiting the use of label-free technology is that the experimental results are perceived to be a black box. The technology is perceived to give an observable result that is only circumstantially connected to a biological event. While this perception is more relevant to cell-based experiments, it colours researchers' interest in label-free technology in general.

Gallant comments: "Moving to label-free assays from labelled assays requires adopting a new approach – a change in the mindset of how drug discovery gets done. Scientists are accustomed to the use of labelled assays where the labelled reagent or reaction occurs due to one specific effect in one specific pathway. The change to label-free assays requires moving away from low information HT assays to an assay type of moderate throughput with more informative, yet more complex, data output than that of binary assay data of typical HTS assays.

"Our main strategy to overcome the perception that label-free assays are 'black box' has been to focus on education about the approach and its benefits," says Gallant. "We sponsor talks at shows, perform tutorials and webinars to educate people about the power of label-free, and we have generated a strong set of data using modulators of different parts of the signalling pathways, showing how pathways are specifically blocked or upregulated; proving the connection of the receptor activation to the cytoskeletal rearrangement leading ultimately to the change in impedance."

In addition, Hurwitz says: "Application notes, sponsored tutorials, and webinars driven by user success stories provide the best examples of how the perceived 'black box' data is translated into meaningful results. An increase in peer reviewed publications also supports the technology's true value and discoveries that previously were unattainable."

von Leoprechting comments: "We work with leading researchers who are successfully using label-free technologies such as the EPIC system in their mix of tools and workflows in modern drug discovery, and enable a wider range of researchers to use and validate label-free alongside other labelled technologies on a cost-effective multi-modality plate reader platform." There are clear examples of the complementary and correlated nature of label-free approaches to single target measurements. We are able to demonstrate that label-free traces "give clear information on more

complex pathway regulation and show further differences between compounds which appear the same in Ca2+ assays, therefore distinguishing a partial agonist vs a full agonist, for example", adds von Leoprechting.

"Scientists working in research, drug discovery, toxicology, immunogenicity testing or vaccine development today are more aware of how to interpret the data from these techniques. The increased understanding will drive the technology and open up for more applications in the future," concludes Burtsoff Asp.

Conclusion

Label-free technology, a newcomer to high throughput screening, is being used increasingly as costs decrease and throughput and detection sensitivity improve. A valuable tool for drug discovery, label-free technology provides simple platforms for studying biomolecular interactions and complex signalling pathways without interference. DDW

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