BIO WORLD EUROPE

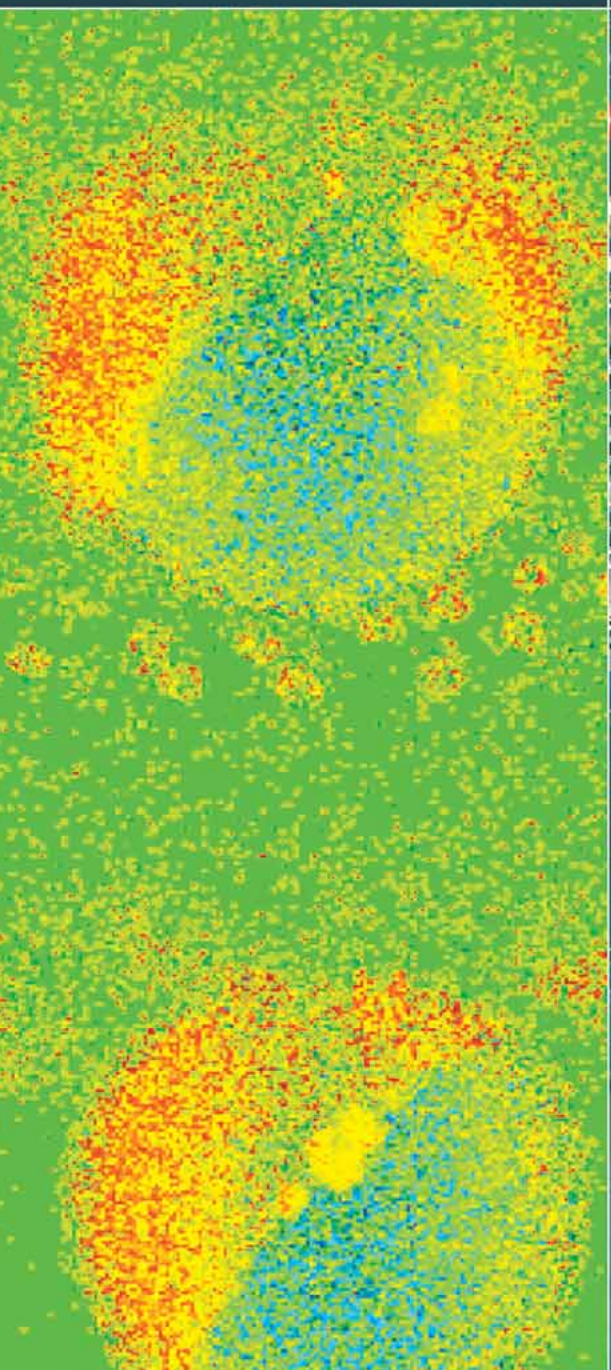
THE MAGAZINE FOR THE LIFE SCIENCE INDUSTRY IN EUROPE

01 | 2008 | March

www.bioworld-europe.com

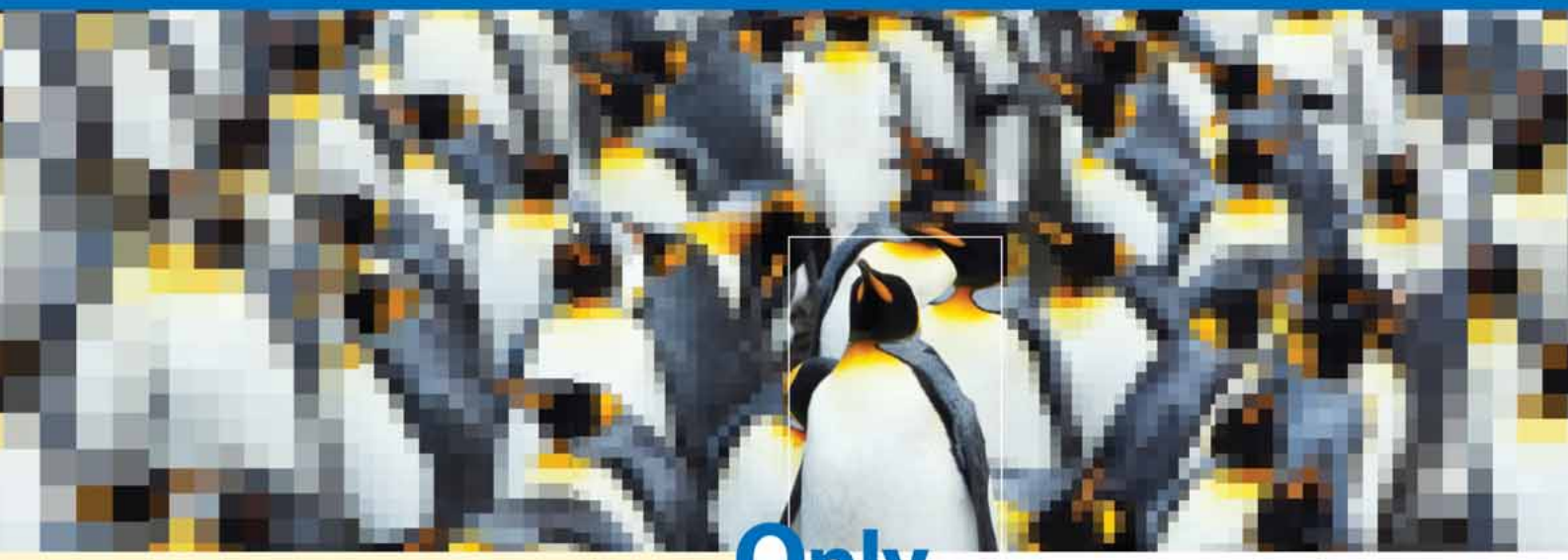
OPTICAL DETECTION TECHNOLOGIES
RESEARCH & DEVELOPMENT
MAGAZINE
SOLUTIONS
SERVICE

Label Free Optical Detection | Cholesterol Changes | Optical Biosensors
Grünenthal | R&D Policies in Canada, USA, and Europe
Trade Fairs in China | Downstream Issues | Analytica
Applied Biosystems | Tecan | Roche Diagnostics | Millipore
Events



Roche

We are High-Definition Genomics℠



Only NimbleGen Microarrays

High-Definition Genomics means high-resolution, higher-quality genome-wide analysis, providing a higher standard in genomic and epigenomic research. Only NimbleGen offers both ultra-high density and long oligo probes, allowing you to obtain both high volume and quality data in each experiment.

NimbleGen cutting-edge applications:

Array CGH
DNA copy number with sub-Kb resolution

ChIP-chip
genome-wide transcription factor mapping

DNA Methylation
entire genomes at high resolution

Gene Expression
cost-effective multiplex options

Sequence Capture
enrichment for targeted DNA sequencing

Long oligonucleotide probes
Ensure highest sensitivity and better signal-to-noise ratios compared with short probes.

Ultra-high density arrays
Capture more information than other platforms with up to 2 million data points per experiment.

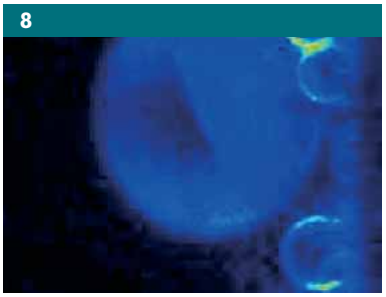
Whole genome or targeted region analysis

■ offer both ultra-high density and long oligo probes, allowing you to obtain both high volume and quality data in each experiment ■ Until you've compared our data with your current approaches, you won't realize just how much you're missing.

NimbleGen®

Start your microarray project with NimbleGen today, and experience High-Definition Genomics.

■ www.nimblegen.com ■ ngsales@nimblegen.com
■ (877) NimbleGen



Optical Detection Technologies

Optical detection technologies are indispensible in bio-analytics and bio-imaging. Biotechnologists interested in putting this technologies to work may not wait for ready-to use kits and instruments. A lot can be picked up from fields like electronics, computer science, aerospace, defense industries, or telecommunications.

TEXT | MICHAEL STEINWAND, GUEST EDITOR

When Kirchhoff and Bunsen in the middle of the 19th century used the absorption phenomena of atomized atoms (discovered by Fraunhofer around 50 years earlier) for qualitative and quantitative element analysis, they took advantage of the availability of some industrial competencies like the production of glass in optical qualities and the capability for the treatment of glass mostly by mechanical means such as grinding and polishing with high and reproducible precision. These competencies were key for the foundation of the analytical industry which to a large degree is based on optical detection. Similarly, in the fifties of the last century lasers became available and e.g. the quantitative protein analysis by means of nephelometry in clinical urine samples was a very early analytical application of this industrial development. Or, when vidicon tubes, diode arrays and charge coupled devices have been developed in the 60s and 70s in the Bell Laboratories, among other places, they originally were intended to be used for picture telephones, not for analytics. However, analytical chemistry took advantage from this industrial development and created detectors using it for registering complete UV and UV/VIS spectra on a time axis, particularly useful in HPLC.

These examples demonstrate that the analytical sciences always have benefitted from industrial developments in other fields. Today it is interesting to see optical detection methods coming along which are based on glass fiber components developed for the telecommunications industry or to observe that the interest in terahertz spectroscopy, induced through its applicability in security applications, suddenly receives attention and opens the oportunity that necessary components will be manufactured on an industrial basis which means at costs affordable for the use in analytics.

These examples also provide a flavor of the complexity of analytical instrumentation and, conclusively, the analytical industry itself. The articles in this issue are vivid examples of it: Dynamic measurement of cholesterol efflux from and to bilayers by two-photon excited fluorescence imaging, a robust optical method for the label-free detection of molecular interactions, complemented by an article which gives readers a glimpse to a rather new technology for the same field which is magneto-optical detection. How the application of existing detection principles can be extended in to new spaces, such as the space of conformational analysis of proteins, is discussed along with a critical review of SPR. Finally, in a reflection on chromatographic applications in large-scale manufacturing processes readers may have a look beyond chromatography: What are the drivers? Is it technology itself, or «the» market? What role do organizational processes play for the adoption of new technologies?

Most important however, from all the contributions highlighting optical detection in this issue it becomes very clear that biological questions are the key motivation for analytical scientists in their interdisciplinary efforts to strive for quantum leaps in bioanalytics. Cross-linking scientific questioning, industrial development and – sometimes genius – inventorship are the bedrock for progress in this field.

Highlights of Optical Detection Modes in Bioanalytics

One key feature of optical detection is that the principle of using electromagnetic waves as means to interact with the sample resp. the analyte contained therein allows non-invasive interrogation. Analytical instrument and sample are kept separate. Conversely, e.g. in electrochemical detection, hardware components like electrodes have to be brought into

INFO

Michael Steinwand (1952) studied chemistry at Universität Tübingen (DE) where he obtained his PhD in 1981 and participated later in courses on business administration at Babson College (USA). In 1982 he joined Perkin-Elmer and in 1993 Applied Biosystems where he stayed until 2007. His latest function was Director of the Science Center Europe reporting directly to the CSO of Applied Biosystems. Today he is the owner and managing director of Innovendia Consulting Services located in the international geography of Lake Constance. He is heading up a working group for Chemo- and Biosensors within the German Chemical Society, acts as a reviewer for several journals and as an invited member of appointment committees at two Universities.

direct contact with the sample. This may not be a problem per se, but if the sample container is supposed to be a biological cell, the non-invasive character of optical detection becomes a key advantage. Moreover, optical detection usually is very fast and in most cases the sample remains unchanged. This is not only important for reasons of recovery of the sample for further processing, but it enables multiple interrogations of the sample allowing process analysis in real time or in the spatial dimension.

In many situations, however, there are no strong enough interactions between an electromagnetic beam and a sample to receive signals which are highly sensitive and sufficient selective for the intended analysis. Widely used procedures to make such samples accessible to optical detection are chemical or enzymatic derivatisations of the sample introducing a label, mostly but not exclusively a dye label. Such procedures surely change the chemical character of the species and ultimately destroy its original entity, however, the signal induced by the label typically even increases the specificity for the analyte of interest. Considering again the biological cell as an eminent sample container, one should mention the possibility to let the cell itself generate such labels in the form of e.g. expressed fluorescent proteins or other so-called genetically encoded labels which came to the market just in the last couple of years. Thus, it is the capability of genetic engineering which in this case helped to progress the same science in terms of new analytical tools. It also should be mentioned that providing such labeling reagents, classical ones as well as the genetically encoded ones, created a significant business on which many firms, particularly in the diagnostics sector, are based upon.

Biology always has been a driver for the analytical sciences. A major challenge in the past twenty years has been genetic analysis. When polymerase chain reaction (PCR) became commercially available – by an industrial instrument supplier, not as one would have expected by a specialized supplier of biochemical reagents! – PCR was a very unique way for the enrichment of a very interesting analyte, namely DNA from biological samples. Originally, PCR has been used to amplify DNA fragments to amounts allowing further manipulation and analysis. Very quickly, though, the process itself has been turned into a powerful, self-consistent analytical technology. In various ways scientists managed to incorporate fluorescent probes into the emerging DNA material allowing to optically monitor the progress of the amplification. Based on the characteristics of the amplification process, it became even possible to deduct the quantity of starting material of DNA from the detection curve. This development opened the way into very sensitive, very specific and quantitative gene expression analysis. Today, these methods generate a research market in the billion dollar range and a diagnostic and clinical study market in the multi-billion dollar range.

PCR stimulated researchers and commercial developers analyzing the human and other genomes as well as exploiting these results. Today, the challenge is to analyze an entire genome in a manageable time, eg 1 day, at reasonable cost, say 1000 dollars. Companies like deCode in Reykjavik (IS) and Seattle (US) or LifeCode in Konstanz (DE) are about to demonstrate that individuals can directly benefit from sequencing personal genomes. Other international initiatives like iBOL (www.dnabarcoding.org), aim at creating a DNA-based taxonomy from some 500.000 eucaryontic species within the next five years, revolutionizing existing classification systems.

Despite alternative methods using electrochemical detection, fluorescence continues to play a major role for the development of the third generation sequencing technologies. For example, so-called «zero-mode» wave guides are being used to follow the enzymatically controlled synthesis of a DNA strand from fluorescence labeled nucleotide building blocks. The signal is being monitored on a single molecule level in a continuous and multiplexed read-out process which is under development at academic institutes as well as in industrial R&D programs. If this new analytical technology can successfully be introduced, it will add a completely new dimension to DNA sequencing and consequently will create completely new markets. Optical detection of single molecules being the key again.

Developing Bioimaging Further

Another area requiring pluri-disciplinarity is the scientists' dream to break Abbe's law. This principle postulates that no optical distinction can be made for objects smaller then half a wavelength of the light being used to illuminate the objects (diffraction resolution limit). However, for example two major inventions are challenging this principle: 4Pi-microscopy and Stimulated Emission Depletion Microscopy (STED-Microscopy). While neither of these methods does break the diffraction resolution limit, they allow to image objects much smaller then the wavelengths of light. Innovative optical designs in combination with photochemical effects render it possible. This development quickly caught the attention of biologists, particularly those in cell biology, who wanted to read events within a biological cell, *in-vivo* and on a molecular level. Again, demand beyond existing markets is emerging, and this in turn will spur biological research and generate new business in bioanalytics.

Discovering Solutions in Other Worlds of Science

An important complement to optical detection are applications of computer-based principles. Algorithms, e.g. chemometric resp. informatic approaches are of great help to turn analytical measurements into knowl-

edge. Particularly high-throughput techniques, imaging methods and – above all – the next generation DNA sequencing methods create huge amounts of data whose value cannot be exploited without sophisticated computational methods. Or, to make another example, high-throughput screening experiments often are restricted to establishing the existence or the non-existence of an intermolecular interaction. A further qualification by elucidating e.g. kinetic or thermodynamic characteristics of this interaction is possible using analytical methods, some of them described in this issue. A lack of appropriate computational procedures impedes their use and high-throughput screening does not evolve into high-content screening.

Hence, optical detection technologies are indispensable in bioanalytics and bioimaging. As a memeber of a cross-cutting discipline such as biotechnology, you may pay lip service to interdisciplinarity, if you don't interact with colleagues from electronics, computer science, aerospace or defense industries, or telecommunications, you might not reach beyond bricolage or follow belated adoption of very useful techniques. □

Correspondence:

Dr. Michael Steinwand
Innovendia Consulting Services
Bartholomäus-Moser-Weg 4
DE – 88696 Owingen
Tel: +49 7551 83 8411
msteinwand@innovendia.de

Label-Free Optical Detection

TEXT | **GÜNTHER PROLL, GÜNTER GAUGLITZ**

The monitoring of biomolecular interaction is a typical modern approach in various types of bioanalytics in biotechnology, diagnostics, fermentation control, and even analysis of drinking water and food. Many of these applications are based on immunoreactions, i.e. interaction between antibodies and antigenes. At present, these interaction processes are either monitored using ELISA techniques (Enzyme-Linked Immuno-Sorbent Assay), or fluorescence-based immunoassays. Although techniques have advanced considerably in recent years, upcoming analytical problems in genomics and proteomics have led to increased interest in methods which do not rely on labeled reactants since labeling may influence these biointeraction processes. For this reason, direct optical techniques for monitoring these processes are gaining in importance, especially since protein/protein interaction is becoming increasingly interesting. In addition, it showed that these techniques can also be applied to many of the above mentioned analytical issues, reducing cost and expenditures, and in many cases reducing the number of required assays. Furthermore, time-resolved measurements of biomolecular interaction processes provide additional information about kinetics and thermodynamic constants (see Fig. 1). These properties of biomolecular reactions are the key to understanding biological regulation processes or developing drugs with designed activities.

Direct optical techniques

Looking just at direct optical techniques, two principles can be distinguished: micro-refractometry and micro-reflectometry [1]. The former method is widely used since it has first been commercialized by introducing surface plasmon resonance into the market. Both use the dependency on the thickness of the layer and/or the refractive index, which influences the phase and/or

TABLE 1 Characteristics of direct optical detection principles

	parallelisation	transducer	LOD	measurand / compensation of changes in temperature or thermostatisation necessary
micro-refractometry				
SPR	MAI	gold, silver	+	n / yes
grating couplers	MTP	glass, plastics	+	n / yes
resonant mirror		glass	+	n / yes
Mach-Zehnder		glass, silicium nitrite	++	n / yes
micro-reflectometry				
RIfS	MAI, MTP	all transparent materials	+	n*d / no
ellipsometry	MAI	silicium	+	n and d / no

amplitude of the electromagnetic radiation penetrating this layer or being reflected. The principle of measuring changes in the reflective index upon interaction in a planar transducer (in this case a modified gold layer with an immobilized bio-shielding polymer film and biomolecular receptors) can be called an evanescent field technique.

Methods based on micro-refractometry

Currently, there are many different SPR-based devices for a variety of applications on the market. A reason why up to now market-penetration has not yet reached the level one would expect is the need of gold layers as part of the disposable sensor chips. This surface is unusual in biological assay technologies, and prevents the implementation of this technology in standard analytical process streams. As soon as these problems can be overcome, label-free technologies will have their breakthrough because of the added value generated by the information about kinetics and thermodynamic constants. The present situation is the driving force for developing alternative devices based on different transduction principles.

Grating couplers, resonant mirrors, and Mach-Zehnder interferometers as well as Young interferometers are based on the same principle of reading out in various ways changes in the refractive index close to the transducer element. The two latter techniques (Mach-Zehnder and Young) also take advantage of interferometric principles which allow the most sensitive detection of phase shifts in the electromagnetic radiation caused by phase shifts in the refractive index. In theory, especially Mach-Zehnder interferometers provide the most sensitive label-free transduction method. But due to noise, drift and missing robustness, these evanescence field based systems have achieved only little commercial success. Compared to SPR, however, these systems offer the possibility to work with the well established glass-surface chemistry.

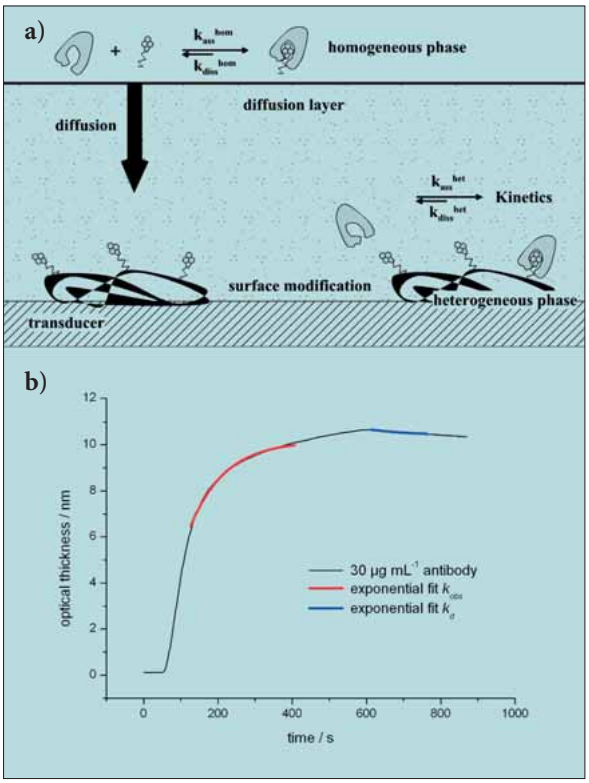


Figure 1: a) Assays using interaction between biomolecules in homogeneous phase and/or at heterogeneous interfaces. In both cases, thermodynamics (equilibrium constant) and kinetics (association and dissociation rate constants) determine the interaction. Direct assays immobilize the receptor at the surface to measure the analyte. Here a binding inhibition assay is demonstrated where derivatives of the analyte or ligand to detect are immobilized. In the pre-incubation phase receptor and ligand are mixed in the homogeneous phase, concentration of non-blocked receptor molecules is detected via the heterogeneous phase. High numbers of interaction sites at the transducer make this process diffusion controlled, at low loads, the kinetics at the heterogeneous phase can be measured. b) This typical label-free measurement of an antibody/antigen interaction shows in the beginning of the association phase a linear behavior because of mass transport limited conditions followed by a kinetically controlled phase (kobs; indicated by the red exponential fit) and a biexponential phase. The dissociation phase is indicated by the blue exponential fit (kd).

Methods based on micro-reflectometry

The second principle, micro-reflectometry, is based on interferometry as well. Since usually at the interface

INFO | **AUTHORS**

Dr. Günther Proll (1974) studied technical biology at Universität Stuttgart and obtained his PhD from Institute of Physical and Theoretical Chemistry, Universität Tübingen (DE) in 2005, where he is working as post-doctoral researcher. In 2007 he co-founded Biametrics Marken und Rechte GmbH a company developing and licensing IP in chemo and biosensors. He is CEO of Biametrics.

Prof. Dr. Günter Gauglitz (1944) obtained his M.S. at Iowa State University, Ames (US) in 1965 and his Ph.D. at Universität Tübingen (DE) in 1972. Thereafter we worked as post-doctoral researcher in Philadelphia on rapid detection systems in pharmacology. In 1979 he received his habilitation in physical chemistry. He is Full Professor for Analytical Chemistry at Universität Tübingen since 1987. He is editor of the Bioanalytical Chemistry Journal and has authored many books on spectroscopy and optical sensing. Günter Gauglitz holds 15 patents in the area and is among other secretary member of IUPAC.

of a thin layer one part of the radiation is reflected, whereas the other penetrates the layer and is there reflected at the other interface, these two partial reflected beams can superimpose and form an interference pattern, resulting in constructive or destructive interference depending on the angle of incidence, wave length, and optical density of this layer which is given by the product of refractive index and physical thickness of the layer. This method called reflectometric interference spectroscopy (RIfS) provides a simple and robust technique in biosensing. It is far less dependent on temperature than the other methods mentioned above since the refractive index and physical thickness show opposite temperature effects and therefore cancel each other out to a large extent. Thus, changes in optical thickness either caused by sorption of molecules in the layer or adsorption to the layer, or even affinity reactions can be measured down to the picometer range without requiring thermostatisation. With this limit of detection RIfS is fully comparable to SPR, but offers the advantage of glass and polymer surfaces. In addition, the simplicity of this transduction method allows for very robust and cost effective sensor systems and consumables.

Introducing polarized light, the information content is even larger since ellipsometry allows a separation of the refractive index and the physical thickness when using many wave lengths. Ellipsometry allows to characterize sensitive layers and is used not only to characterise simple polymer films, but also biopolymers. Table 1 summarizes all the above mentioned direct optical techniques.

Conclusions and outlook

Direct optical detection has proven its feasibility in many applications. To compete with fluorescence-based methods, the limit of detection has to be lowered and non-specific binding as an interfering process has to be reduced. Therefore, future development will have to concentrate on further improvement of technology and, as the most urgent requirement, on the improvement of surface chemistry to increase the loading which in turn will increase the number of recognition sites and prevent non-specific interaction. Moreover, there is a trend in direct optical detection development towards providing additional information about structure and conformational changes. The most promising technologies are Raman and Terahertz spectroscopy [2]. In addition, hyphenated techniques open new fields of application and widen the area of bioanalytics [3]. Recently, high throughput screening approaches have been added to high-content screening methods. Direct optical detection methods provide new aspects for examining multiplexed, functional cell-based screening technologies. The prospects for direct optical detection methods are therefore promising. □

References

[1] Gauglitz G (2005): Direct optical sensors: principles and selected applications. Anal Bioanal Chem, 381(1), 141-155.

[2] Ulber R, Frerichs JG, Beutel S (2003), Optical sensor systems for bioprocess monitoring. Anal Bioanal Chem, 376(3), 342-348.

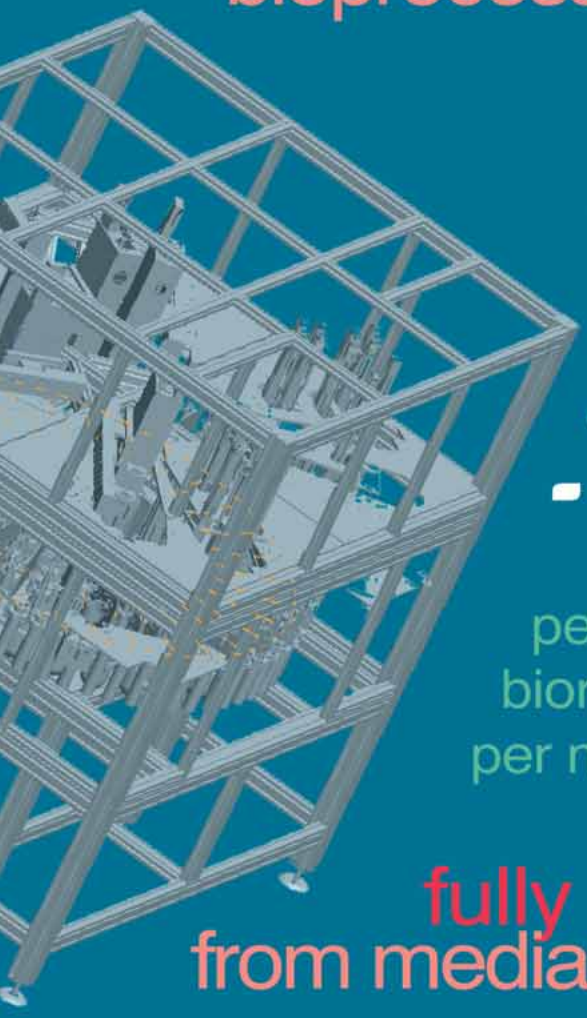
[3] Proll G, Steinle L, Pröll F, Kumpf M, Möhrle B, Mehlmann M, Gauglitz G, (2007): Potential of label-free detection in high-content-screening applications. Journal of Chromatography, A, 1161 (1-2).

Correspondence:

Dr. Günther Proll
Eberhard Karls University Tübingen
Institut für Physikalische und Theoretische Chemie
Auf der Morgenstelle 8
DE – 72076 Tübingen
Tel: +49 7071 297 3048
guenther.proll@ipc.uni-tuebingen.de

Prof. Dr. Günter Gauglitz
Tel: +49 7071 297 6927
guenter.gauglitz@ipc.uni-tuebingen.de

new generation
bioprocess development



clone screener

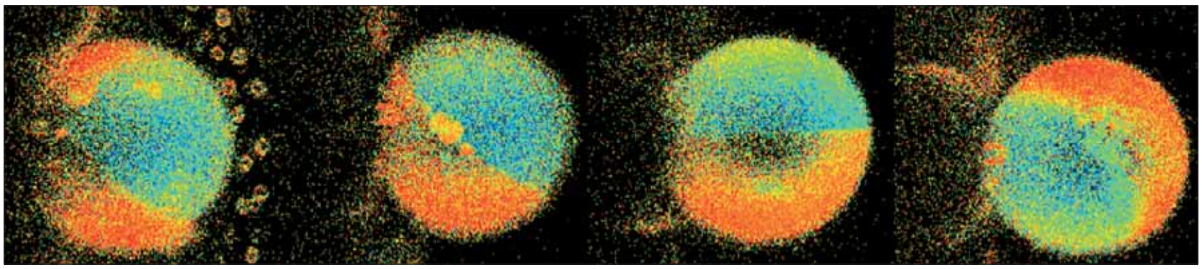
- clone selection
- media optimisation
- process parameter optimisation

performance of up to **256**
bioreactor-quality fermentations
per month by only **1** operator

fully automated
from media mixing to report generation

biospectra
faster from clone to product

Biospectra AG - Switzerland - +41 (0)44 755 67 00 - www.biospectra.ch



Laurdan GP images of a giant unilamellar vesicles (GUVs) made of DOPC:DPPC:CHOL 1:1:1 at 25°C. At this temperature cholesterol segregates between two phases (liquid-ordered and liquid-disordered). These images were used to determine the specificity of different acceptors to remove cholesterol.

Detecting Cholesterol Changes in Lipid Bilayers

Developing controlled methods for cholesterol manipulation in biological and artificial systems is an exciting goal and attracts the attention of bio-scientists. A technique is needed sensitive to cholesterol content and with good spatial resolution to look for changes in membrane cholesterol content in intact cells. This article describes the detection of cholesterol changes in lipid bilayers by laurdan generalized polarization and two-photon excitation fluorescence microscopy.

TEXT | SUSANA A. SÁNCHEZ, MARÍA ALEJANDRA TRICERRI, ENRICO GRATTON

Changes in cholesterol content in the plasma membrane are believed to be responsible for the regulation and fine tuning of many physiological processes. There are several biochemical techniques designed to measure the amount of cholesterol at the membrane and usually they imply the isolation of the plasma membrane with the resulting destruction of the system. We need a technique sensitive to cholesterol content and with good spatial resolution to look for changes in membrane cholesterol content in intact cells. One technique appropriated for this set up is the measurement of Laurdan Generalized Polarization.

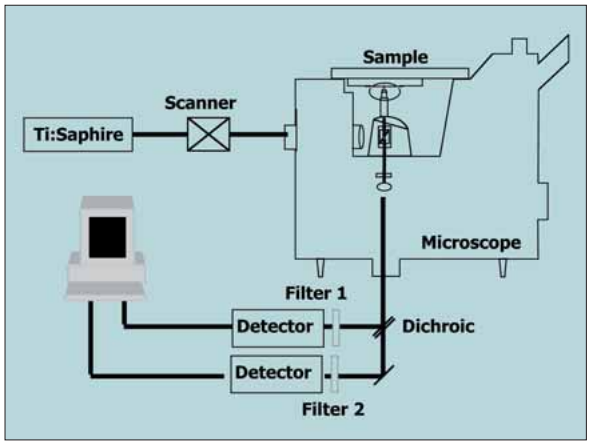


Figure 1: GP measurements in the microscope. The microscope set up for GP measurements includes the two-photon excitation source (Ti:Sapphire laser pumped by a Argon ion laser), mirror scanner and two detectors on the emission to register the filtered signal.

Laurdan Generalized Polarization (GP)

Laurdan (6-lauroyl, 1,2-dimethylamino naphthalene) is a fluorescent probe that detects changes in membrane phase properties due to its sensitivity to the polarity of the environment. Polarity changes are detected by shifts in the Laurdan emission spectrum, which is quantified by the generalized polarization (GP), as:

$$GP = \frac{I_{440} - I_{490}}{I_{440} - I_{490}} \quad (1)$$

where I440 and I490 are the emission intensities at 440 and 490 nm respectively.

Cuvette measurements of Laurdan GP are done in a conventional fluorometer using excitation light at 340-360 nm and by simply registering the two mentioned emission intensities.

Laurdan GP measurements in the microscope are done using two-photon excitation. Two-photon excitation minimizes the severe photo bleaching produced by one-photon excitation due to the confined excitation volume that leaves the areas above and below the focal plane untouched. An additional advantage of the small excitation volume is the intrinsic sectioning (confocal) effect that allows the researcher to collect images from different focal planes.

Several reviews have been written about the use of this technique (Sánchez et al. 2007a, Bagatolli et al. 2003). Here we summarize the basic fundaments in Figure 1 and 2. Figure 1 shows the scheme of the 2-photon microscope, and Figure 2 shows in

more details the filters used in the emission path in order to separate the 440 and 490 nm region of the emission spectrum.

Detection of Cholesterol changes

A well-known effect of cholesterol is to regulate lipid bilayer fluidity. When solubilized in fluid domains cholesterol enhances lipid order, while in solid domains it induces a disorganizing effect. GP values are sensitive to cholesterol content and increase in a linear trend as cholesterol molar ratio increases in Giant Unilamellar Vesicles made of POPC (palmitoyl-oleoyl phosphatidylcholine) (Figure 3). In this case (a single lipid phase containing cholesterol) the images are homogeneous in terms of GP value and the color scale usually used shows images like the ones presented in Figure 3. For this homogeneous case, the analysis is done taking an average GP value obtained from the histogram of all the pixels in the image (Figure 4A)

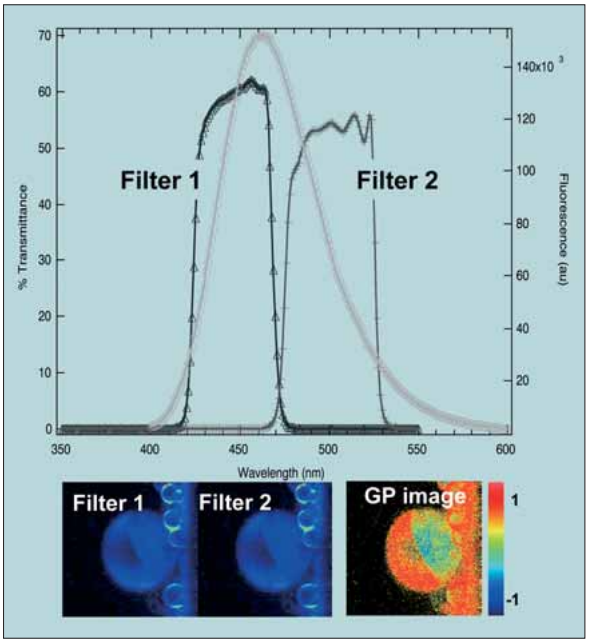


Figure 2: GP measurements in the microscope. Transmittance for Filters 1 and 2 are centered at 440 (triangles) and 490 nm (dashed) respectively, overlapped with the emission spectra of Laurdan (in grey). The GP image is generated from the images acquired simultaneously in the two channels and using equation 1 pixel by pixel using the appropriate software (SimFCS, Laboratory for Fluorescence Dynamics, UCI, USA)

Instead, when cholesterol is distributed into two phases, the GP images and the color representation clearly show the two coexisting phases. In this case one can separate the two phases and calculate the average GP value and the size of each domain (i.e number of pixels) (Figure 4B). For both situations, the GP values and size of the domains can be followed in real time when cholesterol changes due to transport to or from the bilayer occurs (Sánchez et al. 2007b).

Controlled cholesterol manipulation

Developing controlled methods for cholesterol manipulation in biological and artificial systems is an exciting goal and attracts the attention of bio-scientists since several physiological activities are related to the existence of membrane cholesterol-rich domains called "rafts". Cholesterol content is normally manipulated using molecules such as methyl-beta-cyclodextrins (MBCD). These molecules can remove, depending on the system, up to 90% of the cholesterol content of the membrane and they are usually used for short incubation times and in concentration ranges from 1 to 10 mM; higher concentrations might result in cellular damage. Alternative cholesterol acceptor with the potentiality of being less toxic are reconstituted HDL (rHDL).

INFO | AUTHORS

Dr Susana A. Sánchez (1963) received a degree in biochemistry from Universidad Católica de Valparaíso (CL) and her PhD in Chemistry in 1997 from the same University. She went to the Laboratory for Fluorescence Dynamics (LFD) at the University of Illinois at Urbana Champaign in 1997 as Post doctoral fellow and become part of the LFD staff in the year 2000 as a User Coordinator of the NIH facility. She moved with the LFD in 2006 to the University of California at Irvine, Biomedical Engineering Department (US) where she currently is the LFD User Coordinator and Senior Research Scientist applying fluorescence microscopy techniques to the study of protein-protein and protein-lipid interactions in vitro and *in vivo*.

Dr María Alejandra Tricerri (1962) is Professor of Clinical Biochemistry at the Faculty of Medicine, Universidad Nacional de La Plata (AR), UNLP and Associate Researcher with the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) in Argentina. She studied Biochemistry and obtained her PhD from UNLP in 1998. Thereafter she did post-graduate research at the University of Illinois at Urbana-Champaign (US). During 2003 and 2004 she was a visiting researcher at Universidad Federal do Rio de Janeiro, supported by the Conshelo Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq) and the Fundação Carlos Chagas Filho de Amparo a Pesquisa do estado do Rio de Janeiro (FAPERJ), Brazil.

Dr Enrico Gratton (1946) is Professor in the Department of Biomedical Engineering at the University of California, Irvine,(US) since 2006. He obtained his doctorate in Physics from the University of Rome (IT) in 1969. Thereafter he worked as a researcher with Snam Progetti SpA (IT) from where he moved on to University of Illinois (UIUC) in 1976. He holds numerous patents in his field and is among others on the Scientific Advisory Board of Max-Planck-Institut für Biophysik, Göttingen (DE) and Istituto di Biofisica, Genova (IT).

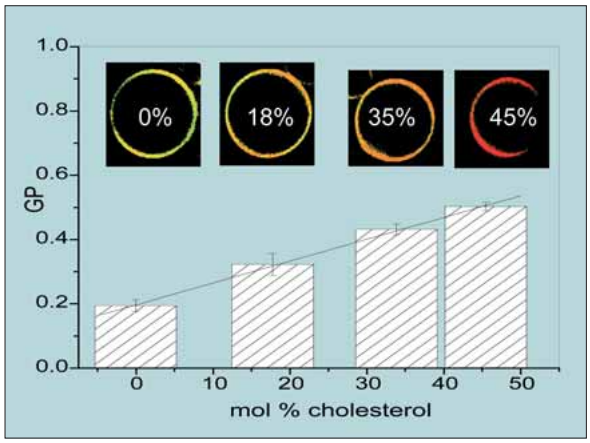


Figure 3: GP changes depending on cholesterol content. GUVs of POPC containing different percentages of cholesterol were made at 37°C. Independent chambers were prepared for each cholesterol concentration (numbers inside the vesicles) and ten GUVs were analyzed from each chamber (standard deviation is shown). Line corresponds to the linear fit of the experimental data.

Cholesterol manipulation by rHDL particles

In the physiological process called reverse cholesterol transport, cholesterol is removed from the peripheral cells and transported to the liver for clearance. The key players in this process are the human high density lipoproteins (HDL). HDL particles consist of a heterogeneous population of particles of different sizes and composition, carrying essentially apolipoproteins (mainly apolipoprotein A-I (apoA-I)) and lipids. As a model system, reconstituted discoidal HDL particles were designed in 1982 (Matz et al.1982). These particles can be made in a reproducible manner and their composition (lipid and apoA-I content) can be precisely controlled. Homogeneous rHDL containing 2 apoA-I and different amount of lipids can be isolated (Figure 5), showing specific biochemical properties as well as binding affinities (Tricerri et al. 2002).

rHDL have been used extensively as a model system to study the role of the structural integrity of the apoA-I in the process of cholesterol removal. In addition, rHDLs by themself are powerful cholesterol acceptors and can be used as a tool for biological studies as an alternative of MBCD. Initial characterization of the efficiency of this particles for extracting cholesterol compared with the MBCD have been recently published (Sánchez et al. 2007b). Particles interact with membranes individually and increase in size as the transfer of cholesterol and phospholipids from the membrane occurs. They have been shown to be ~ 1000 times more efficient in removing cholesterol than MBCD from giant unilamellar vesicles of POPC plus cholesterol. When incubated with GUVs showing lipid phase separation, GP imaging studies demonstrated that rHDL selectively remove cholesterol from liquid disordered phases (Figure 6) (Sánchez et al. 2007b). This last result is particularly important in experiments involving lipid domains, where usually the question to be answered is whether cholesterol is being extracted from the rafts (conceived as liquid-ordered phase) or from the areas surrounding them (conceived as liquid-disordered).

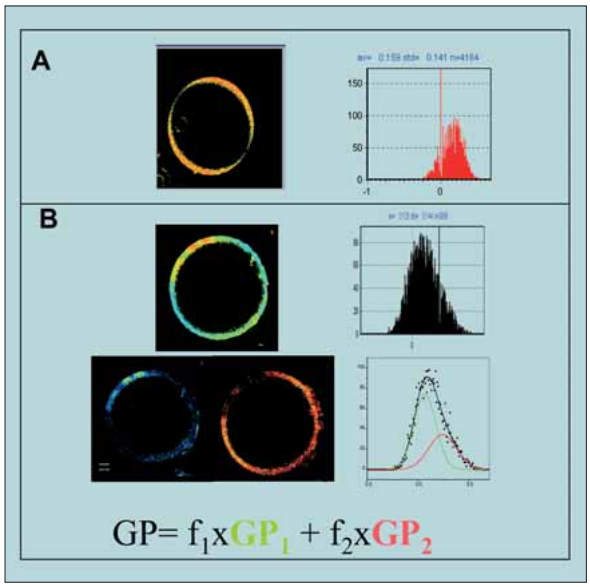


Figure 4: Analysis of the GP images: [A] For homogeneous GP images the analysis of the histogram of number of pixels with a given GP value, will give an average GP. [B] For images showing areas with different GP values both, the image and the histogram can be deconvoluted and the average GP with their corresponding fractions can be obtained by using the equation GP= f1xGP1 + f2xGP2, where GP1 and GP2 are the GP values and f1 and f2 are the fraction of pixels corresponding to the lower and the higher-GP domains, respectively.

A technique advancing biomedical and industrial research

Cardiovascular disease (CVD) is the leading cause of death in Europe, accounting for over 4 million deaths each year, and it represents about 1/3 of all deaths worldwide. The correlation between CVD and high circulating cholesterol levels is supported by a strong body of statistical and biomedical research, which is focused in the study of interactions of cholesterol acceptors with the plasma

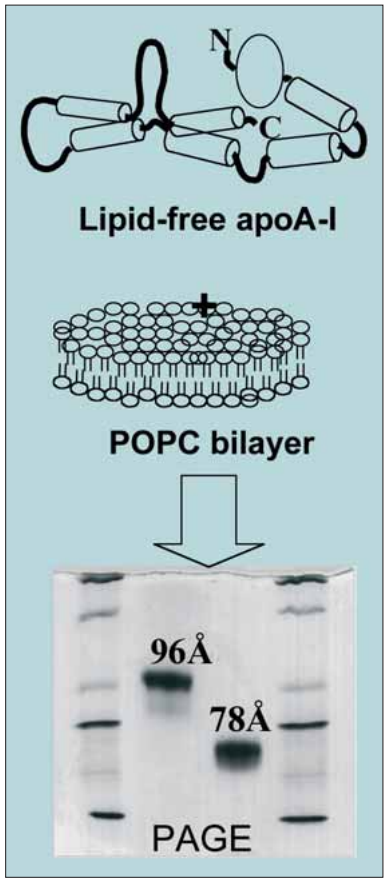


Figure 5: Reconstitution of HDL particles (rHDL). Lipid-free apoA-I and phospholipids (with or without cholesterol), are combined in right amounts in the presence of sodium cholate, followed by extensive dialysis to remove the detergent. Complexes can be further purified by size exclusion chromatography, and characterized by polyacrylamide gel electrophoresis (PAGE) under native conditions. Stokes diameters of the rHDL, 96 and 78 Å, are determined as compared with high molecular weight standards (lines 1 and 4 in the PAGE).

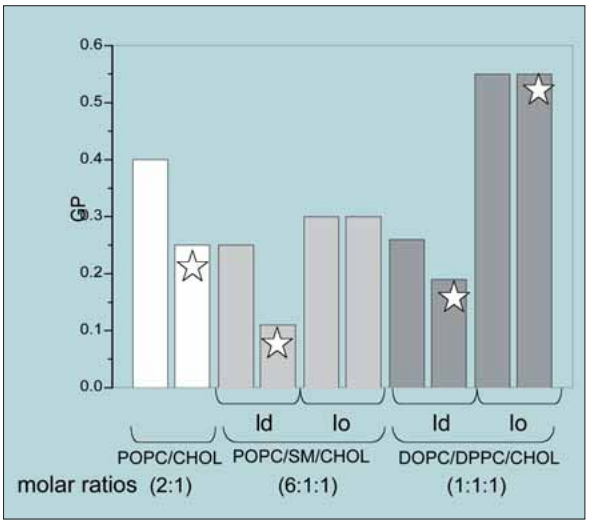


Figure 6: GP values before (first bar) and after (second bar) incubation for 1h with 10µg/ml rHDL to GUVs made of POPC/CHOL (white), POPC/SM/CHOL (clear gray) or DOPC/DPPC/CHOL (dark gray). The last two mixtures show lipid domains coexistence, and then liquid ordered (lo) and liquid disordered (ld) phases are analyzed. Stars inside the bars represent significant changes observed after cholesterol removal.

membrane. Here we describe a non-invasive approach to evaluate with high spatial resolution, and in the real time, cholesterol efflux from or to bilayers. The appeal of this technique resides in its capability to follow kinetics of lipid transport in natural or artificial membranes and the locations in the cell affected by the changes in lipid content. This unique detailed spatial information can be very attractive for the understanding of the action of drugs in regulating lipid transport. In addition, organization of cholesterol in specific lipid domains at the plasma membrane can regulate the activity of proteins involved in cellular signaling processes. In fact, deregulation of such cellular events is correlated with severe pathologies, such as cancer, Alzheimer disease, and atherosclerosis among many others. Thus, our experimental approach will also help the pharmacological industry for the rational design of raft-modulating agents as therapeutic strategies such as anti-cancer. □

This work is supported by a grant from the National Institute of Health (RR03155).

References

- Sánchez, SA,Tricerri, MA, Gunther, G, Gratton, E (2007a): Laurdan Generalized Polarization: from cuvette to microscope. pp. 1007-1014 in: A. Méndez-Vilas A, Díaz, (Eds.): Modern Research and Educational Topics in Microscopy – Applications in Physical/Chemical Sciences. Badajoz: Formatex.
- Bagatolli, LA, Sánchez, SA, Hazlett,T, Gratton,E (2003): Giant Vesicles, Laurdan, and Two-photon Fluorescence Microscopy: Evidence of Lipid Lateral Separation in Bilayers. Methods in Enzymology. 360: 481-500.
- Matz, CE, Jonas, A (1982): Micellar complexes of human apolipoprotein A-I with phosphatidylcholines and cholesterol prepared from cholate-lipid dispersions. J Biol Chem. 257: 4535-4540.
- Tricerri, MA, Sánchez, SA, Arnulphi, C, Durbin, DM, Gratton, E, Jonas, A (2002): Interaction of Apolipoprotein A-I in Three Different Conformations with Palmitoyl Oleoyl Phosphatidylcholine Vesicles. J. Lipid Res. 43: 187-197.
- Sánchez, SA, Tricerri, MA, Gratton, E (2007b): Interaction of High Density Lipoprotein particles with membranes containing cholesterol. J Lipid Res 48: 1689-1700.

Correspondence:

Dr Susana A. Sánchez
University of California, Irvine
Department of Biomedical Engineering
3120 Natural Sciences II
US – Irvine, CA 92967-2715
Tel: +1 949 824 4471
s.sanchez.donoso@gmail.com

Magneto-optic Sensitivity Enhancement in Plasmonic Sensors

Most of today's tests for diseases detection are based on time-consuming, expensive and sophisticated techniques handled only by specialised technicians in laboratory clinical environments. The optical sensing approach is one of the most promising ones to meet expectations of novel biosensors overcoming present limitations of diagnostics.

TEXT | B. SEPÚLVEDA, ANA CALLE, L. M. LECHUGA, G. ARMELLES

Nowadays most of the tests for diseases detection are based on time-consuming, expensive and sophisticated techniques which can only be done by specialised technicians in laboratory clinical environments. Usually those tests require sampling and labelling with fluorescent or radioactive labels. There is an urgent necessity to develop biosensors which can allow the identification of any disease at the earliest stage possible in a fast, simple and cost-effective way.

Among the different biosensing technologies, the optical sensing approach is one of the most promising due to its immunity to electromagnetic interferences, high sensitivity and possibility of miniaturization. In addition, optical transducers have a potential for parallel detection making possible array or imaging detection. In addition, their high sensitivity allows the direct detection of biomolecular interactions without the use of labels and in real time, thus facilitating kinetic analysis.

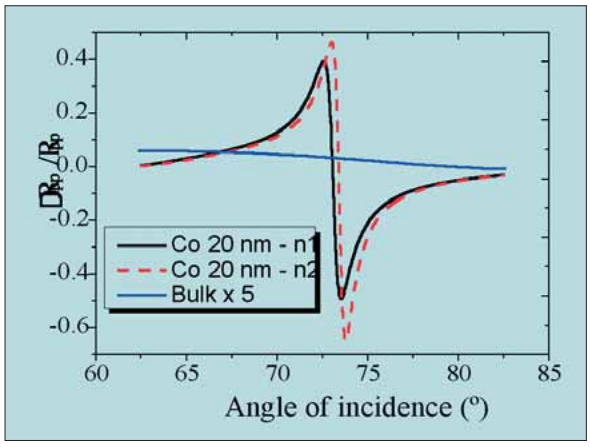


Fig. 1. Comparison of the MO Kerr effects as a function of the angle of incidence for a Co layer of 20 nm and a semi-infinite Co medium (bulk). In the case of the semi-infinite medium, the results are multiplied by 5 for the representation. The change of the MO effects under a $5 \cdot 10^{-3}$ variation in the refractive index of the dielectric is represented as dashed curves. We have assumed that the light source is a He-Ne laser with a wavelength of 632 nm.

Overcoming Limitations of Surface Plasmon Resonance

One of the most successful label-free and commercially accepted optical biosensor is the Surface Plasmon Resonance (SPR) device. The conventional SPR biosensor relies on the excitation of the surface plasmon at the interface between a thin gold film and a dielectric medium. The surface plasmons are charge density oscillations that propagate at the metal/dielectric interface, where they generate highly confined electromagnetic fields. The excitation condition of the surface plasmons strongly depends on the refractive index of the dielectric medium, providing the principle of detection of the SPR biosensors. This technique has been applied in biomolecular engineering, drug design, monoclonal antibody characterization, epitope mapping, phage display libraries, virus-protein interaction, hormone and environmental pollutants detection, among other interesting problems.

However, the sensitivity and limit of detection of the SPR sensors are still not enough for the direct detection of very low concentration of low weight molecules, as for example some pathogens or pollutants, the detection of single nucleotide polymorphisms in DNA, or protein detection at femtomolar level. Recently, several SPR configurations have been described to improve these limits of detection as, for example, the phase-sensitive SPR based on a Mach-Zehnder configuration (Yu et al. 2004), the differential ellipsometric SPR (Hooper et al. 2004) or the optical heterodyne SPR (Kuo et al. 2003).

Magneto-optic sensitivity enhancement

As an alternative, we are investigating a new Magnetooptical Surface Plasmon Resonance biosensor (MOSPR) (Sepulveda et al. 2006) which can improve the sensitivity of the conventional Surface Plasmon Resonance (SPR) sensors. This MOSPR sensor arises from the combination of the Surface Plasmon Resonance in thin metallic layers and the magneto-optic (MO) activity of ferromagnetic metallic materials. This combination

can produce a significant enhancement of the magneto-optic (MO) Kerr effects in the reflected p-polarized light when the SPR condition is satisfied. Such MO effects depend on the orientation of the magnetization with respect to the direction of propagation of the light. Thus, when the magnetization is within the magnetic layer and perpendicular to the propagation plane of the light (transversal configuration), the MO effect produces a relative variation of the reflectivity in the p-polarized (R_{pp}) light when the direction of the magnetization (M) is reversed, i.e.:

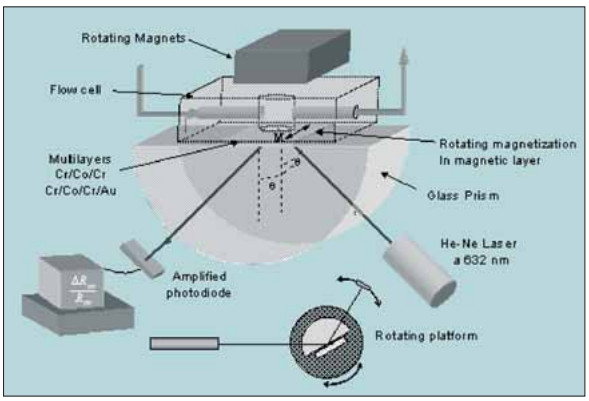


Fig. 2. MOSPR biosensor experimental set-up. We employ rotating magnets to induce the variations of the magnetization. The field intensity of the magnets is around 400 Oe.

$$\frac{\Delta R_{pp}}{R_{pp}} = \frac{R_{pp}(M) - R_{pp}(-M)}{R_{pp}(0)} \qquad (1)$$

The combination of the SPR and the MO activity can be found in ferromagnetic metals, like Fe, Co or Ni. The SPR enhancement of the MO effects can be observed in Fig. 1, where we represent the theoretical MO effects of a 20 nm Co layer in a prism coupling configuration to excite the surface plasmon, compared to the MO effects of a semi-infinite Co medium. As can be noticed, the enhancement of the MO effects is larger than one order of magnitude and this effect is closely localized at the angle of incidence that satisfies the resonant condition of the surface plasmon.

Similarly to the reflectivity measurements in the conventional SPR sensors, the MO curves shift to higher (lower) angles when the refractive index of the dielectric increases (decreases) (see Fig. 1). The sharpness of the MO curves yields that small variations of the refractive index of the dielectric medium will induce large changes of the MO effects. The quantification of such changes constitutes the principle of detection of the MOSPR sensor.

The MOSPR sensor must comprise, at least, the following elements (Fig. 2): one metallic layer in which is possible to excite the SPR, and at least one layer with MO activity. The MOSPR sensor requires a coupling element to excite the surface plasmon like a prism, a TM light source, a light detector and a magnetic actuator to control the orientation of the magnetization in the magnetic layer (see Fig. 2). In thin ferromagnetic films, the magnetization is commonly in-plane and, therefore, we have chosen the transversal configuration of magnetization, measuring $\Delta R_{pp}/R_{pp}$

These measurements show, as an additional advantage, independence from the fluctuations of the light source.

An example of the experimental operation of the MOSPR sensor is presented in Fig. 3 (a). Such plot shows the real-time detection of the changes of refractive index in aqueous solutions with a Cr/Co/Cr multilayer, by measuring the variations of $\Delta R_{pp}/R_{pp}$ at the fixed angle of incidence that maximizes the slope of the MO curve. The Cr layers are intro-

INFO AUTHORS

Borja Sepúlveda Martínez (1976) is Assistant Researcher at Centro de Investigación de Nanociencia y Nanotecnología (CIN2), Bellaterra (ES) since 2007. He received his PhD in physics from Universidad Complutense de Madrid (ES) in 2005 carrying out his research at Instituto de Microelectrónica (CNM-CSIC). Thereafter he joined the Bionanophotonics and Bioimaging Group at Chalmers Tekniska Högskola, Göteborg (SE) and returned to Spain in 2007.

Laura M. Lechuga Gómez (1962) is Head of the Nanobiosensors and Molecular Nanobiophysics Group at Centro de Investigación de Nanociencia y Nanotecnología (CIN2), Bellaterra (ES) since 2008 and Full Professor of Consejo Superior de Investigaciones Científicas. She obtained a PhD in Chemistry by Universidad Complutense de Madrid (ES) in 1992 and went for postdoctoral research to the MESA+ Institute for Nanotechnology at Universiteit Twente (NL). From 1995 to 2007 she was the Head of the Biosensors Group at Instituto de Microelectrónica (CSIC), Madrid (ES).

Ana Calle Martín received her M.S. degree in chemistry from Universidad Complutense, Madrid (ES) in 1983. Since 1988 she is a Member of Technical Staff at IMM-CNM, CSIC where she is involved in fabrication technology of microelectronic devices as laser diodes, LED´s sensors, and biosensors.

Gaspar Armelles Reig is Director of Instituto de Microelectrónica (CNM-CSIC) at Madrid (ES). He received his MA from Universidad Zaragoza (ES) in 1981 and his PhD degree in physics from INSA, Toulouse (FR) and Universidad Valladolid (ES) in 1986. He joined CNM-CSIC in 1987.

duced to improve the adhesion of Co and protect it from oxidation.

Despite Fig. 3(a) shows a high sensitivity of the Co layer to the changes of refractive index, such sensitivity can be improved with the combination of ferromagnetic metal layers with gold layers typically employed in SPR biosensing. The introduction of Au layers enhances both the slope of the MO curves and their angular displacement when refractive index of the dielectric changes, being responsible for the sensitivity increase. In addition, this combination offers other advantages as the compatibility with the well-known gold-based immobilization protocols of biomolecules, and the protection of the ferromagnetic layer from oxidation. In fact, Fig 3 (b) shows that the sensitivity of a Cr/Co/Cr/Au multilayer to the changes of refractive index is six times higher than the Cr/Co/Cr sensitivity.

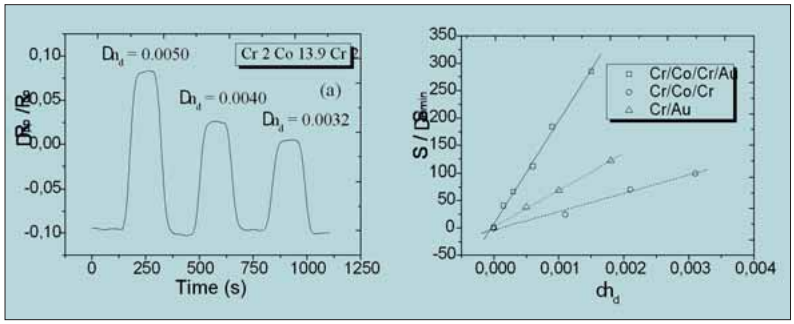


Fig. 3. (a) Real time detection under rotating magnetic fields of the changes of refractive of the solutions measuring $\Delta R_{pp}/ R_{pp}$ at a fixed angle of incidence with the Cr(2nm)/Co(13.9nm)/Cr(2nm) multilayer. (b) Experimental sensitivity to the changes of refractive index of the MOSPR sensor in the Cr/Co/Cr and Cr(2nm)/Co(7.5nm)/Cr(3nm)/Au(23nm) configurations compared to the experimental sensitivity of the conventional SPR.

Due to the different nature of the MO measurements of the MOSPR sensors and the reflectivity measurements of the conventional SPR sensors, the comparison of their sensitivity must be done taking into account the signal-to-noise-ratio of the experimental measurements. For such purpose we define the experimental sensitivity (h_E) as the variation of the acquired signal (S) when the refractive index of the external medium (n_d) changes, normalized by the minimum detectable signal (DS_{Emin}):

$$h_E = \frac{1}{DS_{E\min}} \frac{\partial S}{\partial n_d} \quad (2)$$

where DS_{Emin} is defined as five times the RMS deviation of the experimental signal. In Fig. 3 (b) we compare the experimental sensitivity of the MO measurements with the reflectivity measurements of the typical SPR sensor (2nm of Cr and 45 nm of Au) using the same experimental set-up. As can be observed, the comparison of these measurements shows that the experimental sensitivity of the MOSPR sensor in the Cr/Co/Cr/Au configuration shows a 300% improvement with respect to the conventional SPR. In contrast, the experimental sensitivity of the MOSPR sensor in the Cr/Co/Cr configuration is below the conventional SPR.

Conclusion

The experimental characterization of the MOSPR biosensors has shown sensitivities for the detection of changes of refractive index three times larger than the conventional SPR sensors when the ferromagnetic metal layer is combined with gold layers. The simplicity of the experimental set-up, the compatibility with the immobilization chemistry of the SPR sensors and the immunity to the fluctuations of the light source add more interesting features to the magneto-plasmonic biosensing concept. Moreover, the optimization of the magnetic layers to avoid the use of the highly absorptive Chromium films together with the utilization of coils to generate the magnetic fields to reduce even more the noise level, could lead to an experimental sensitivity enhancement of the MOSPR sensor of one order of magnitude with respect to the conventional SPR. Such improvements can open the way to the direct and ultra-sensitive detection of very low concentrations of small molecules. On the other hand, the introduction of multiple reflection effects in band-gap multilayer structures can amplify the non-reciprocal effects of the magnetic layer, and other magnetic configurations as the longitudinal or polar con provide additional information and increment the dynamic range of the measurements. Finally, the nanostructuration of the MO multilayers can offer as well the possibility to develop promising localized surface plasmon resonance sensing platforms (González-Díaz, et al. 2008). □

References

- Wu, SY, Ho, HP, Law, WC, Liu, C, Kong, SK (2004): Highly sensitive differential phase-sensitive surface plasmon resonance biosensor based on the Mach-Zehnder configuration. Optics Letters 29: 2378-2380.
- Hooper, IR, Sambles, JR (2004): Differential ellipsometric surface plasmon resonance sensors with liquid crystal polarization modulators. Applied Physics Letters 85: 3017-3019.
- Kuo, WC, Chou, C, Wu, HT (2003): Optical heterodyne surface-plasmon resonance biosensor. Optics Letters 28: 1329-1331.
- Sepúlveda, B, Calle, A, Lechuga, LM, Armelles, G (2006): Highly sensitive detection of biomolecules with the magneto-optic surface-plasmon-resonance sensor. Optics Letters 31: 1085-1087. Sepúlveda, B, Armelles, G, Calle, A, Lechuga, LM, Patent application: PCT/EP2005/006273.
- González-Díaz, J,B, García-Martín, A, García-Martín, J,M, Cebollada, A, Armelles, G, Sepúllveda, B, Alaverdyan, Y, and.Käll, M (2008): Plasmonic Au/Co/Au nanosandwiches with enhanced magneto-optical activity. Small 4: 202-205.

Correspondence:

Dr. Borja Sepúlveda
Centro de Investigación de Nanociencia y Nanotecnología (CIN2)
Campus de la UAB - Edificio Q - 2ª Planta
ES – 08193 Bellaterra
Tel: +34 93 581 4969
borja.sepulveda@cin2.es

Process Analytics – Chromatography and Beyond

A conversation among two industry consultants – Andy Lyddiatt and Michael Steinwand.

Michael Steinwand:When working for large analytical instrument companies an issue always is to guide and inform customers. Many might not be aware of what cutting-edge technologies are available and how they could benefit in their daily routines from implementing these methods and devices. With regard to manufacturing what do you see are current technical constraints in chromatography, particularly in process chromatography?

Andy Lyddiatt: What people want is robustness, long operational life, and media of higher capacity with appropriate diffusion characteristics to readily accommodate rapid product transport in adsorption/desorption steps so that process cycles are not extended. Economics are important as well in terms of running costs, operational longevity, and practitioners also want cheaper ligands which are as reliable as the more expensive ones.
However, increasingly a limitation of chromatography crops up that can't be handled just by installing bigger adsorptive contactors. Host cell expression and productive fermentation has improved so much that attainable product titers are rising rapidly. People are now realizing that their existing chromatography media of choice may not have the adsorptive capacities and retained resolution to deal with this. For example, current monoclonal antibody, MAb-titers may commonly exceed 2 g/L and the capacity of a protein A column may be less than 70 g/L. When fermentation batch sizes exceed 10,000 liters there is a significant challenge flowing down the outlet from the fermenter hall toward the capture chromatography step. Running process chromatography steps in parallel may be a pragmatic solution.

Steinwand : Considering such high efficiency processes with increasing yields, how sure can we be to recover all useable product when the process is being monitored by off-line methods? Theoretically, I could see many advantages of on-line optical detection. You can direct your light beam of whatever wave length at your sample and get back any signal there might it be and interpret it terms of in absorption, fluorescence or scattering. You keep the sample unchanged and take measurements either on a time axis or even in a spatial dimension, if you think of following the analyte flowing in a tube. But it seems to me, optical detection is under-exploited in manufacturing.

Lyddiatt: That's right, it's non-destructive and non-invasive and that makes it attractive in process analytics. However, in the manufacturing environment it is mostly used as a monitoring aid. The performance of a particular process step will be defined by more sophisticated biochemical analysis of samples which are lost to the product yield. Such analyses achieved in a separate Quality Control lab, will contribute to the batch records for that particular process. This is not yet generally done in real-time in manufacture, but could greatly contribute to process development,

Steinwand: Instead of online monitoring you undertake a discontinuous analysis of samples taken out of the process from time to time although you know, you won't get results in real time.

Lyddiatt: Retrospective information isn't of much use in terms of intervention, particularly if you're running at high process throughputs. You only got a record of what happened.

INFO

Andy Lyddiatt (1949) received a BSc (First Class Honours) in 1971 and his Ph.D. from Durham University (UK) in 1975. Thereafter he worked as post-graduate researcher at Trinity College, Dublin (IE), and Imperial College, London (UK). In 1980 he moved as Production Biotechnologist to Cambridge Life Sciences and then as Senior Scientific Officer to the Institute of Animal Physiology, Cambridge (UK) until 1983. That year he returned to pursue his academic career at University of Birmingham for nearly two decades. In 2003 he went to Millipore where he was Director R&D of Technology and Predevelopment in the Bioprocessing Division at Consett (UK) until 2006. Last year he founded Lydallan Consultancy Ltd. and is working today as Bioprocess Consultant both to industry and transfer initiatives like BRIC, the British Bioprocessing Research Industry Club. Since 2007 he also is Professor of Practice in Bioprocess Technology at the Business School of Newcastle University (UK).

Dr. Michael Steinwand
see Introduction by Guest Editor

Steinwand: What prospects then do you see for on-line monitoring applications?

Lyddiatt: This may be applied in process development, but not to my knowledge in manufacture. Over twenty years ago, I talked to a company making automated raid chromatography equipment and suggested they arrange for such a machine to analyse the output of, and talk back and control, the operation of a preparative chromatography device. At that time it was actually very difficult to get people to understand why you would want do this. I am not aware that sort of approach is actually widely adopted, but it would seem to me an ideal way of running interactive preparative processes. Such an approach might inform on the robustness of a process in development by virtue of its sensitivity or otherwise to modified operating parameters.

The limitations of optical detection , when you are talking about protein separations, are that products and/or impurities will not generally have characteristic absorption or fluorescent spectra which allow identification of one from the other – particularly where concentrations are widely different. This may not be a problem since you could infer how a separation was going just on the basis of comparing spectral analyses from previous batches with the current one.

Steinwand: This reminds me of chemometric tools: you may not just be able to describe a certain process status by a summary spectrum, but when you know the spectra of each of the constituents you even may be able to achieve a relative quantification of the constituents. Because of the basic limitations in protein separations which you mentioned, I would guess there is not much use of this type of chemometry in process analysis of proteins? However, what if you were looking for a representative surrogate or a set of surrogates in the process mixture which may tell you about the process or about the purification status instead of directly analysing the protein?

Lyddiatt: That could actually be applied to key impurities. In any process you need to know the molecular and physical characteristics of both, product and impurities. If you are applying optical detection, then of course it makes sense to be able to interrogate the impurity if they have a characteristic spectrum to see where they're going relative to what you take to be the products in them. For example, some of those impurities – for example media pigments, low molecular weight species – may be present in a monoclonal antibody feedstock and need to be eliminated or greatly diminished at the protein A capture step. If you're able to run a multi-wave length interrogation of a process stream that's very viable. Similar approaches might apply to product isoforms or conformers.

Steinwand: Talking more about indirect methods. Surface Plasmon Resonance, SPR, is acclaimed providing a method for monitoring bioprocesses, both in bypasses as well as in off-line modes. You introduce specificity into the analysis through a biological interaction between the species of interest in your sample and an affinity molecule bound to the SPR sensors. However you argue, this is rather a discontinuous process requiring time. Is there really so little room for these methods, ultimately optical detection modes?

Lyddiatt: When people purify protein products such as therapeutic antibodies from cell culture medium, the feedstock is actually remarkably enriched in product post cell clarification. The target product may account for far greater than 70% of the total protein in the feedstocks. The remainder comprises trace concentrations of contaminant host cell protein, unwanted product isoforms and spent media components. So actually, a 280 nm absorption signal you can retrieve from feedstock once any low molecular UV absorbers are reduced at capture is a strong indicator of product behaviour. If you have a much more heterogeneous feedstock, where there is a number of proteins or a lot of product isoforms at broadly equivalent concentrations, interrogation with something like the technique you mention is ideal again for process development in pinning down how your separation process can be optimised. It might be a good method for retrospectively monitoring, how a real manufacturing process might have worked. But it is presently unlikely that you can use

the information in any quality description of the process.
Surface plasmon resonance has been demonstrated in process development. A few years ago, work published by the Advanced Centre for Biochemical Engineering at University College London demonstrated such a device in the monitoring of optimal product release in a multi-stage high pressure homogenisation process applied to host recombinant E coli. A balance was necessary between maximal product release without complication of deleterious micronisation of cell debris after multiple treatments. Again, a generic tool to apply in process development.

Steinwand: So far we were talking about the early stages of getting a protein out of its production batch. Are there other analytical challenges if we go more down stream?

Lyddiatt: A real issue here is in the later stages of a purification, where key contaminants are regarded as hazardous in the end product when present at concentrations that cannot be reliably detected by optical methods. For example in MAb manufacture, these might comprise traces of host DNA, residual host cell protein, and even protein A fragments released from an earlier protein A capture step. Off-line proteomic and molecular analysis supported by mass spectrometry seems to be the answer for batch characterization, but not presently for near online process monitoring.

Steinwand: That's right. Besides sensitive analytical instrumentation, you even may need some powerful enrichment or amplification or completely different methods?

Lyddiatt: Physical or biochemical detection methods are needed there, generally with a lot of amplification. Actually the presence of a product at high concentrations is the real issue in running robust analyses of that kind.

Steinwand: Let's come back to the purification process itself: If you were to advise on investments in new plants, what would you say with regard to chromatography?

Lyddiatt: The decisions for the immediate future are already made, and protein manufacturers will stick with some form of chromatography. Subtractive methods may contribute to smaller contactors and reduced operating costs. Simplicity and robustness of operation are the watch words in industrial-scale applications.

Steinwand: How is process development evolving, particularly with regards to separation technologies? Are there any alternatives to chromatographic approaches on the horizon?

Lyddiatt: In many current manufacturing operations applied to protein products, so much successful effort has been invested in optimizing cell culture that the starting purity of the product is relatively high at the outset. It is ironic that advancing titers may be reversing this trend since these are often obtained at the expense of lower cell viabilities and concomitant cell lysis. This invites a practitioner to say: 'Let's try to take out the impurities in an adsorptive way and leave the product in solution until a later stage of concentration'. I think there is a lot of sense and mileage in that approach.
As an academic researcher I had lots of interest in solvent-based processes, particularly aqueous two-phase partition processes which have never caught on. In my view, this has been in part because there is no vendor or supplier associated with the technique or applications. Just think about how most current practitioners learn chromatography technologies. Not so much in the lecture theatre but more from the technical literature of the various vendors. So people don't have much practical understanding of how solvent partition might be working or be usefully deployed. But it's clear that volumetric capacity is much higher for such solvent systems than most porous chromatography matrices. There are also practical possibilities for selective crystallization or affinity precipitation. New technologies might more quickly be applied in new product niches where chromatography is not readily suited at all. For example in the capture of very high protein titers, or the capture of nano-scale or micro-scale products, for example whole viruses and cells. However in a very conservative business, somebody needs to break the ranks and say: This is the way we're going to do – and for these reasons. Others will generally follow compelling case history.

Steinwand: Quite understandable for operating in validated conditions. You have to be conservative somehow.

Lyddiatt: Absolutely. The issue then is to find solutions to the challenges we 've discussed of increased titers and process analyses, and perhaps to increase the sophistication of interrogation, let's say in advanced optical methods. Change may come suddenly, if you develop new processes to bring about a new family of products. In this context selective recovery of nano-scale products – viruses, plasmids etc. – is quite an interesting challenge. It seems the dogma is not established there. There is scope for new technologies and new ways of measuring that can develop in tandem with a new product area. □

Correspondence:

Dr Andrew Lyddiatt
Lyddallan Consultancy Ltd.
Wood Manor, Wood Street
UK – Shotley Bridge, DH8 0JE
Tel: +44 1207 58 3615
al@lyddallan.co.uk

Plasmon Resonance Revisited

Today technologies are emerging that add value to rather conventional optical bioanalytics and allow access to new dimensions in proteomics studies and bioanalytics in general. Very interesting technologies are available from telecommunications, and in defence and electronics industries.

TEXT | MALCOLM BUCKLE

Proteomics studies requiring the identification and characterisation of macromolecular interactions as well as being able to follow small molecule binding to target molecules can be greatly improved by developing optical detection technologies which provide access to completely new dimensions such as conformational or kinetic space.

INFO

Dr Malcolm Buckle (1954) is a CNRS Research Director of the Group of Enzymology and Structural Kinetics at LBPA, ENS since 2002. He studied Biochemistry at University College N. Wales and obtained his PhD in Chemistry at The Polytechnic Wolverhampton (UK) in 1980. Until 1982 he worked as a post-doctoral fellow at McMasters University, Hamilton (CA) and moved on as Assistant Professor at Istituto di Chimica Biologica, Università di Bari (IT) from where he joined Institut Pasteur in 1986 and Institut de cancérologie Gustave Roussy at Villejuif (FR) in 2000. He is the inventor of a novel method for the production of peptide banks and has worked with companies like Amersham Biosciences (GE healthcare), Biacore, Ciphergen, and GenOptics SA.

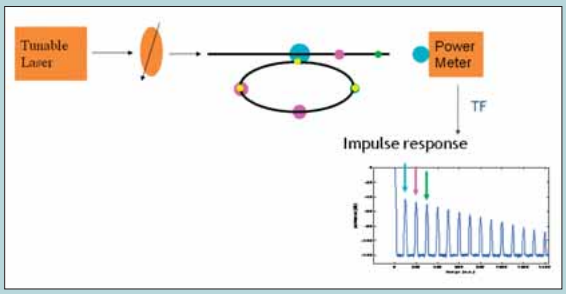
However, even here old problems persist such as having to work with heterogeneous surfaces and the major difficulty of directing molecules within the experimental space. Moreover, it is still a challenge to relate measurements in changes in a property such as refractive index which is not easily perceived by biologists as a useful parameter to an actual local change in mass which is what the biologist really wants to measure. Furthermore, to mention another roadblock in bioanalytical studies of binding of molecules, the dynamic range offered by current methods and instruments is far too narrow. For example in drug discovery, you have to immobilize your target molecule, and introduce a smaller molecule, let's say a drug, that is going to bind; if the detection limit of the measuring device is limited to 10% of the mass of the material that is immobilised and yet the drug is a mere 1% of the target molecule, then you will see no signal (as for example a 20kDa protein target binding a 200 Da drug); even worse if the drug interacts with the surface and you do see this interaction then you will interpret this as a specific event, with perhaps expensive and embarrassing consequences. Finally, all this assumes that the immobilised target is completely active and is not denatured by its interaction with the surface, and unfortunately often you quite simply do not know that this is so!

The road to solve these problems is fortunately rather clear: Techniques are needed which chemically optimise the functionality of surfaces, and that require a targeted interface between molecules at the surface and the detection device. Methods are in demand to measure interaction on the surface and conformational changes of micro-molecules on the surface.

Overcoming limits of routine surface plasmon resonance

Today technologies are emerging that add value to rather conventional optical bioanalytics and allow access to new dimensions. This can be seen with surface plasmon resonance (SPR), which many people consider a 'label-free' detection technique. It is not. In fact, in SPR you are labelling the molecules with the surface, because you stick a surface on them! If you use most commercially available SPR instruments or any surface measurement technique, then the dynamic range you can look at is quite restricted. For example, as stated above if you have a large molecule and a small ligand, it is very difficult to see the binding of a small molecule to a complex one. In SPR one is obliged to use a metal surface (generally silver or gold) at the interface between the optical detection system (a glass prism or diffraction grating) and the biological molecules. The issue is not just how the biological molecule binds to a metal surface. The nature of the interface between the metal and the prism is also crucial since this dictates the form of the plasmon and this in turn impacts on the sensitivity and dynamic range of the measurement thus dictating you can then see small molecules binding to big molecules. Different configurations may dramatically change the nature of the curve measured. By various solutions you can decide whether to look at sensitivity or fewer molecules binding, or whether you want to locate a bigger dynamic range that is a small molecule bind-

INFO MICRO-RESONATORS



Micro-resonators are composants having a resonance cavity which may be coupled with an incoming and outgoing wave guide. Interrogation of evanescent waves extending beyond the wave guide produces a series of resonance peaks that may provide important information about the nature of the surface of the resonator cavity. In simple words, in the diagram shown below, any changes at a point on the resonator cavity polymer will be reported by changes in the impulse response. In practice such resonator cavities may have radii of the order of ~10 µm and thus coupling of a microfluidic delivery system to a small part of these polymer rings will allow the delivery of small volumes of macromolecules to interact with immobilised targets at the surface. Subsequent changes in refractive index will be picked up by changes in phase measured by analysis of the resonance peaks.

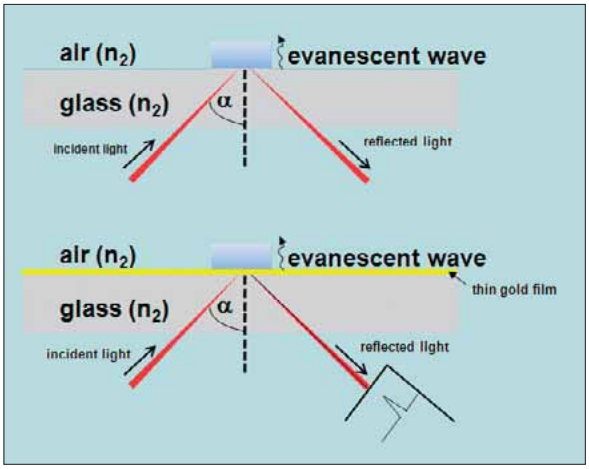
ing to the surface. And that requires a model to change this. We (Nogues et al. 2008) solved this by modifying how the gold is maintained at the prism surface. This newly developed technique allows for flexibility and easy visualisation of a protein, eg one with 45.000 Da binding to a drug with about 400 Da. The procedure is opposite to the conventional one: in this case the protein is bound to the surface and thereafter the small molecule is added, and lends itself to the production of protein microarrays where peptide libraries may be applied to arrays of immobilised protein targets for high throughput analysis.

Another aspect of bio-detection is kinetics-based. In general biological assays report the "affinity" between two or more molecules based on a steady state (often confused with equilibrium) measurement of how much complex is present compared to how much individual components are present. It is very informative to measure the on and off rates associated with the formation of a macromolecular complex. Quantification of the reaction studied informs you about the nature of the molecules that are interacting. Very often this information is neglected in proteomics studies. Standardising accumulated data would aid identification of unknown components. If you see an interaction you would get the on- and -off rates, you would be able to calculate an apparent affinity constant and these three parameters, measured under the conditions used, are also specific for the interaction and thus would aid in identification if one of the partners were an unknown.

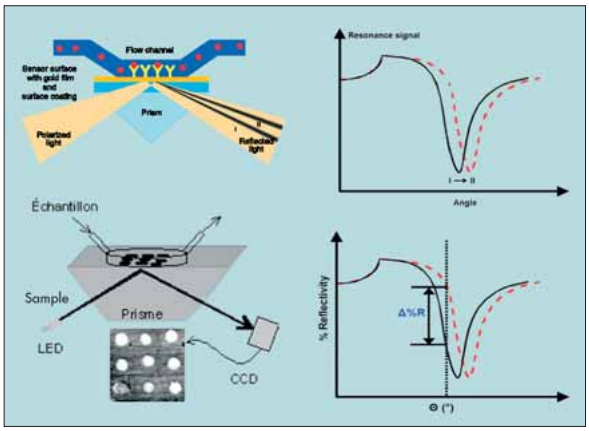
In this context the stochiometry of the interaction is gaining importance (Bayley/Cremer 2001) and incidentally, this is one of the most under-used aspects of SPR techniques today. Most people analysing data are happy with some equilibrium affinity number, say a Kd of 10 nM for subsequent comparison but it is meaningless unless you really understand the model behind this data. Stochiometry allows you to build a simple model that guides your interpretation of data – a further caveat however is that none of these numbers have any meaning in an absolute, thermodynamically valid sense! They are useful as comparisons, much as the concept of weight is useless outside of a gravitational field.

Coupling upstream to downstream techniques

Why bother coupling upstream detection and downstream identification techniques? A main advantage is being able to monitor very carefully your ongoing analytical process, getting immediate feedback, assessing if it is working and control-



Surface Plasmon Resonance stems from one of the basic principles of optics, that of total internal reflectance, (TIR). This occurs at the interface between materials with differing refractive indices, n1 and n2. Total reflection occurs above a critical angle, when n1 > n2. At the same time, an evanescent electromagnetic wave propagates away from the interface. SPR occurs when a thin conducting film is placed at the interface between the two optical media. At a specific incident angle, greater than the TIR angle, the surface plasmons (oscillating electrons at the edges of the metal) in the conducting film resonantly couple with the light because their frequencies match. Since energy is absorbed in this resonance, the reflected intensity, I, shows a drop at the angle where SPR is occuring



Principle of SPRi (surface plasmon resonance) and (SPRi) surface plasmon imagery

ling the experiment, actually seeing if something is retained on the surface in a meaningful fashion. And you can do that before investing time in expensive and time consuming downstream analyses which are more often than not nowadays based on mass spectrometry. Furthermore if you carry out routine mass spectrometry (LC-MS or MALDI) or as we do, SELDI which is just a MALDI coupled with retentate chromatography, you are doing mass spectrometry, but you don't see your result until the experiment is over; having an SPR technique upstream allows you to be sure that something is binding to the target surface.

The foremost requirement in upstream techniques is very specific surfaces. A key feature is functionalisation of the surface and the corresponding chemistry that allows you to fish out what is unknown. The analytical question is: Can we pass a complex mixture across a surface that contains a bait molecule – an antibody, a piece of DNA –-, see an interaction, and probe what was involved in that interaction? You have to have a surface that contains only your target molecule. The answer requires that the bait molecule is functional in contrast to the surface which has to be non-functional. In other words, nothing is binding to that surface that shouldn't be binding. The challenge is developing inert surfaces with an almost negligible level of non-specific binding.

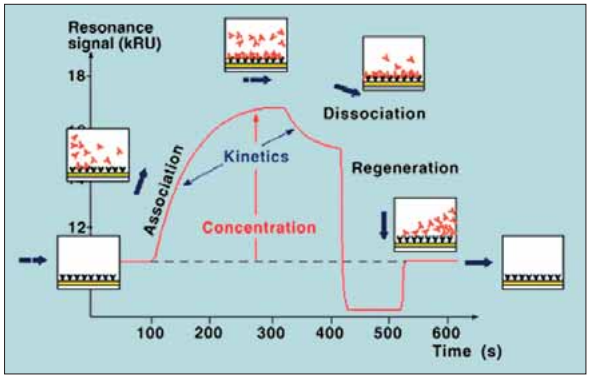
Moreover, at issue is understanding the hierarchy of interactions. If you are able to observe the interaction in real-time, you can also remove the material by taking the SPR surface for example recovering the material onto a SELDI support for analysis of retained material. (Bouffartigues et al 2007).

A problem in looking at complex mixtures with MALDI is that this technique is terribly non-quantitative, and the signature you get from molecules flying in MALDI is not necessarily typical of the number of molecules present. The measurement just tells us which molecules fly the best. One way around this is the use of labelling techniques using selective mass tags (ITRAQ), or stable isotopes (SILAC), whatever the choice, it provides a neat combination with the upstream quantitative analysis.

Using available techniques from telecommunications, defence and electronics industries

Now, in order to methodically tackle these problems, stepping outside of biology and biochemistry offers great opportunities. Often, you find that physicists have smart techniques at hand that can be put to use to solve bioanalytical problems. Such is the case with exploiting micro-resonance in cavities which is well known in telecommunication, defence and electronics industries. However, its use still has not entered into the biological domain.

There is strong evidence in physics that were we to orient molecules on the surface of a micro-resonated cavity, then we will be able to characterise attomol quantities of small or larger molecules – we are talking about nm dimensions – by the phase change and, more importantly, measure the conformational space change as other molecules brought in will change that conformation. For example you can reduce a conformational change by a change in pH or temperature and already receive a signal. Of course, the advantage is that this will be a time-resolved analysis, and maybe we get down to microseconds. The interface with biology still needs to be worked out, and that is the part of basic research whereas in terms of the actual physics this is rather straightforward. Thinking about putting phenomena like micro-resonance to use in bioanalytics allows integrating available devices like micro-resonators*. When these components are built into fiber optical sensors you can exploit rather microscopic optical cavities and measure minimal



Principle of kinetic analysis by SPR
The change in relative resonance signal is interpreted as a change in mass bound to the surface as a function of time, assuming that material binds only to the immobilised target molecule then these changes can be used to calculate apparent off (k_d) and on (k_a) rates that in a simple Langmuir process (A + B $\rightleftharpoons$ AB) allows calculation of an apparent equilibrium dissociation constant (K_d).

changes in the properties of the surface, which you then relate to changes in phase and get a very accurate analysis of the number of molecules present on the surface. Again, this provides you with valuable information about conformational changes in those molecules on the surface.

And, at the end of the day, you can happily return to biochemistry with added tools from physics. Such is the case, when applying nanoparticles where one uses resonance effects or whispering gallery modes ** to create plasmon particles which change colour whenever a molecule interacts with it. This allows you to measure multiple interactions, conformational changes in the sub nanometre range and discriminate individual interactions even in complex physiological fluids.

Sensing futures

Optical detection techniques are not sure-fire solutions to major bioanalytical problems. They have to prove themselves against breath-taking electrical bioanalytical technologies. This can be seen in research by Hagan Bayley at Oxford University (UK). His group develops protein channel sensor chips, where a Teflon film with an inner orifice 100 [mu]M diameter is sandwiched between two polymer films both having an outer orifice of 200 [mu]M diameter. The lipid membrane (eg a bilayer film made of glycolipids) has a single channel protein surrounded with and protected by a gel. This can be used then to measure electrical properties of a solution and correlating their changes to the analyte which in the end allows detection almost of interactions between single molecules.

Basic research in photonics will provide further surprises in the field. The future is already here while remaining uncertain. Take terahertz imaging***. Some decades ago these waves were applied in studies of plant leaves, certainly not a field offering much return on investment to a costly technology. Then, T-rays have been hailed in defence and space industries, but companies and inventors trying to commercialise the technologies went into insolvency or refolded into the academic domain. Since the turn of the century terahertz imaging is slowly making its way back into biology (cp. Baras et al. 2002; Nishizawa 2005). However, its success is open-ended. Many in this field are physicists, not particularly interested in bioanalytics. Procurement of suitable instruments requires changes in existing infrastructures and additional investments. At present, demand from biology is strong enough to motivate manufacturers. This may change with technology adaptation in high-margin sectors like breast cancer screening. A non-negligible advantage against existing devices are less cost and less side-effects. THz radiation is non-ionizing and lead shields can be dispensed with. However, in bioanalytics, cost is still too high and, most important there are still technical hurdles to achieve the level of precision required in bioanalytics. For example, by using conventional lenses and mirrors THz radiation cannot be exactly focused onto objects as small as cancer cells. This implies that despite breath-taking developments in electrochemical detection, optical detection methods still hold great potential, especially with regard to plasmon resonance techniques and ellipsometry. □

This article originated from a lengthy and fruitful conversation with Michael Steinwand and Wolf Kroner when our professional experience meshed gears and provided a taste for what I hope this final version for which I am solely responsible will spur within a wider network of colleagues.

Comments

* Micro-resonators are part of a fiber optic sensor which measure the changes caused by the target parameter in the natural frequency of the micro-resonator. Resonance is occurring in micro-cavities on the surface, if molecules bind to it. Coupling induces a phase change which a laser measures when the beam interferes.

** For a given particle size light is internally reflected at particular resonating wavelengths at the surface of the particle. This internal reflection lasts only nanoseconds and the time interval then can be used for measurements.

*** Depending on frequency (range 100 GigaHz to 10 THz) electromagnetic terahertz waves operate from millimetre- to submillimeter ranges and then have properties of either radio waves, which pass solid materials, or optical emissions.

References

- Bayley H, Cremer PS (2001): Stochastic sensors inspired by biology. Nature 413: 226-230.
- Baras T, Kleine-Ostmann T, Koch M (2002): Design considerations for on-chip THz analysis of biomolecules. Presentation 10th International Conference on Terahertz Electronics 2002, Cambridge (UK), September.
- Bouffartigues E, Leh H, Anger-Leroy M, Rimsky S, Buckle M (2007): Rapid coupling of Surface Plasmon Resonance (SPR and SPRi) and ProteinChip™ based mass spectrometry for the identification of proteins in nucleoprotein interactions. Nucleic Acids Research. 35 (6):e39. Epub 2007 Feb 7.
- Nishizawa J, Sasaki T, Suto K, Yamada T, Tanabe T, Tanno T, Sawai T, Miura Y (2005): THz imaging of nucleobases and cancerous tissue using a GaP THz-wave generator. Optics Communications. 244: 469-474.
- Nogues C, Leh, H, Lautrou J, Buckle M. (2008): Optimisation of SPRi leading to enhanced sensitivity and dynamic range. manuscript in preparation.

Correspondence:

Dr Malcolm Buckle
École Normale Supérieure de Cachan
Laboratoire de biotechnologie et de pharmacologie génétique appliqués
61, avenue du Président Wilson
FR – 94235 CACHAN
Tel: +33 1 4740 7673
buckle@lbpa.ens-cachan.fr

Grünenthal opts for Pragmatic Innovation

Eric-Paul Pâques, Member of the Executive Board of Grünenthal for Research, Development and Global Alliances describes how his company will become Number One in centrally acting analgesic drugs and the strategy behind the pipeline.

IINTERVIEW | **WOLF G. KRONER**

«Divinum est sedare dolorem» said Galen of Pergamon. What divine developments are in Grünenthal's pain pipeline?

As you know still a significant part of the population experiencing pain is therapeutically underserved. While many pain killers are on the market there is still a great need for drugs with a better profile than those presently available. In order to address these questions we build up a pipeline based on new chemical entities, NCEs, as well as based on known molecules we re-formulate and/or develop for new indications. This approach seems to pay off since Grünenthal has been able to launch three products based on "re-designed" drugs i.e Zaldiar which is a combination of Tramadol and Paracetamol, Transtec which is a Buprenorphine patch, as well as the recently approved Lidocaine patch, Versatis, for the treatment of post herpetic neuropathy.

wk

In terms of NCE's we have at present several compounds in advanced clinical trials. All belong to new classes of compounds. Recently Grünenthal together with its partner for the US J&J completed the Phase III program in acute moderate to severe pain with Tapentadol which is Grünenthal's most advanced compound. The data obtained are extremely convincing and reveal a compound having an efficacy similar to that of morphin and oxycodon – two golden standards – while having a side effect profile which is impressively improved. Such a compound really addresses the needs of the patients. The application for marketing authorization has been recently filed at the FDA. All the other compounds Grünenthal has under clinical development also displaced profiles which let us hope that we are on the right way to provide the patients with drugs which better address their needs. In total we can say that as much as 80 % of our R&D effort is dedicated to pain, while the remaining 20% are focused on contraceptives.

Belara has been a cash cow for long. What's coming up in the gynaecology business line?

Indeed Belara has been a success in Germany, and we are in the process of introducing this contraceptive in several European countries. As our competitor Bayer-Schering, we have also developed a new contraceptive based on the same gestagen as the one used in Belara, Chlormadinone acetate, but with a lower estrogen dose. This product has been filed for registration. Nonetheless if we want to stay in this field we need to keep developing new products. This is indeed not very simple since we do not invest in basic research activities in this field and the in-licensing opportunities for compounds and products are extremely limited. It is probably not without reason that several of the already limited number of companies active in this area are getting out of the business. Therefore, we have been pleased to make a deal with Sanofi-Aventis, which enables Grünenthal to get access to a new gestagen. We have already started the clinical development of a new oral contraceptive based on this new gestagen. It looks as we would have the opportunity to build a new family of contraceptives.

What about other product lines?

We come from anti-infectives, but we decided to stop all our R&D activities in antibiotics respectively anti-infectives, because – that's my personal conviction – the size we have, we do need to focus in order to compete. Take for example anti-viral drugs: to have a reasonable chance to find a drug to combat the one and same virus you need several R&D approaches and that's what big pharma is doing. They follow many routes in parallel. Therefore we

TABLE 1 Grünenthal at a Glance

m €	2005	D 05/04 %	2004	D 04/03 %	2003	D 03/02 %	2002	
GRT Group turnover	780	7	726	2	713	7	666	
GRT GmbH balance sheet total	491	12	439	0.2	437	-1	444	
turnover	374	10	340	2	327	2	312	
cash flow	175	32	132	-2	144	-4	167	
equity capital	316	11	286	0	286	1	278	
operating profit	-7	71	-22	-200	-7	600	-47	
GRT GmbH expenses		% of turnover		% of turnover		% of turnover		%
expenses marketing & sales	115	31	106	31	113	34	118	38
expenses R&D	125	33	101	30	91	28	86	28
expenses PGA	38	10	40	12	35	11	37	12
GRT Group employees							4.961	
GRT GmbH employees	1.784		1.784		1.784		1.841	
R&D	481		437		422		405	
Production	463		407		425		452	
Marketing & Sales	570		635		616		659	
Administration	388		319		321		325	

Group Turnover 2006/2007	€ 813m / € 830m (exp.)
R&D investment 2007	€ 140m
Employees 2006 ww / DE	4.800 / 1.900
Founded	1946
Company Shareholders	100 % Wirtz family
Production Sites	6 (DE, EC, IT, MX, CH, ES)
key investements	€ 6m production & distribution EC; € 25m central packaging DE

Sources: Grünenthal 2/2008; BioWorld Europe

decided to concentrate on pain but here having the ambition not to be at the level of big pharma but to be the innovation leader.

How are you going to bring that about?

We have two dreams. The first is Grünenthal being the Number One innovator in the field of centrally-acting analgesics. The second is Grünenthal being the market leader in Europe in this field. First of all we need talented people with a high level of commitment with whom we can create a rich pipeline of attractive compounds. Today I believe that we have reached that stage. So for illustration the first wave is combining in one drug two or three different well established mechanisms of action. This is indeed a conservative but highly rewarding approach. An example of this approach is the combination of μ-agonist with serotonin-noradrenaline re-uptake inhibitor. Such a combination makes possible to design drugs having at least the same efficacy as strong opioids while as we could demonstrate with Tapentadol having a significant reduction of the side-effects. Thus, playing with the combination of different mechanisms and/or with the relative contribution of the mechanisms involved in a single molecule as well as for example with the pharmacokinetic then it is possible to generate a variety of compounds with very different profiles that can be developed into very different drugs. Beside Tapentadol we have presently another powerful analgesic compound which displays a strong antidepressive activity, This compound is just entering Phase ll b. A further compound just passed the Phase ll b status. Interestingly all of these compounds have very different profiles and very different positionings in terms of medical specialties. To me, that's the beauty of such a concept.

But we are also working at further waves of compounds. Let us dream to combine a mechanism leading to pain relief, and another one – for instance some known receptor cross-talking to other receptors – which by itself intervenes in the path leading to these undesired side-effects.

wk

Is that still in discovery?

In preclinical development stage. We are on a very interesting path. But the approach is very different: not combining efficacy and diluting side effects. It seems that we could be close to what I just described as a dream.

INFO

Perry Blocher

Prof. Eric-Paul Pâques (1954) studied chemistry chemistry at Université Catholique de Louvain (BE) and obtained his Ph.D. at the age of 25, supervised by Prof. Robert Huber (Max Planck Institute for Biochemistry, München) and Prof. Robert Crichton (Louvain). In 1980 he started his professional career in Behringwerke AG, Marburg (DE) as Head of Research until 1993, when he entered Grünenthal GmbH, Aachen (DE). In 1998 he was appointed Member of the Executive Board for Research & Development and Alliances. He also teaches part-time at Université Louvain and Universität Aachen.

You also co-operate with biotech companies to generate your NCE-portfolio.

Yes, for example we have a very promising co-operation with Digital Biotech from Korea. This approach is part of the third wave where we try to find new mechanisms which allow developing centrally-acting compounds with the same efficacy as the strongest opioids but without their side effects. There, we have a portfolio of projects in this field. But, as we all know, new approaches also generate new problems.

Which ones are you thinking of?

As several others we can demonstrate an antinociceptive activity while at the same time we observe a rise of the body temperature.

What is the role of genetics?

Of course the role of genetics is important. We continuously use genetic tools these days. A company of our size however cannot and probably should not establish all these methodologies in-house; that's why we work with third parties. The same applies to the discovery of new molecular targets. We are not the 'avanguardistes'. We leave it to big companies, biotech as well as academia running five years ahead and finding new receptors.

Don't you think that genetic testing or biomarkers can discover early undesired side-effects?

For sure a battery of powerful in-vitro tools helps the researchers to identify at an early stage existing problems or potential risks. But let us consider the broader perspective of applying 'new technologies'. There are numerous highly sophisticated, impressive and exiting technologies. I am extremely careful with all these fashions claiming some methodology is indispensable or is going to bring the answers to our problems. Ten years ago everybody would call for combinatorial chemistry, or high throughput screening. Twenty years ago everybody was praising computer-assisted design. All of these are tools, and each of them is only one in a box of tools. As human being we have the propensity to reductionism and we have a tendency to believe, there is only one single way to solve a problem, and we own the one and only solution. Beside all these new fascinating *in-vitro* techniques we should not lose one of the core competencies which is the *in-vivo* pharmacology. In pharmaceutical development you cannot solve all your problems with «biotech». We have to avoid this binary thinking which is 'There is nothing but biotech'.

How do manufacturing concerns influence R&D?

We need to be extremely cautious to give a lot of attention to API cost. From the very beginning we have to have the API-costs in mind and to extrapolate from the lab scale in the production scale. Having this said, we will not kill a project too early for reason of cost of goods. In our field, you get a good appreciation of the price you can reach on the market after completion of Phase II b. At that stage you have a really good understanding of the features of the compound.

Is prevent copying an issue in your R&D?

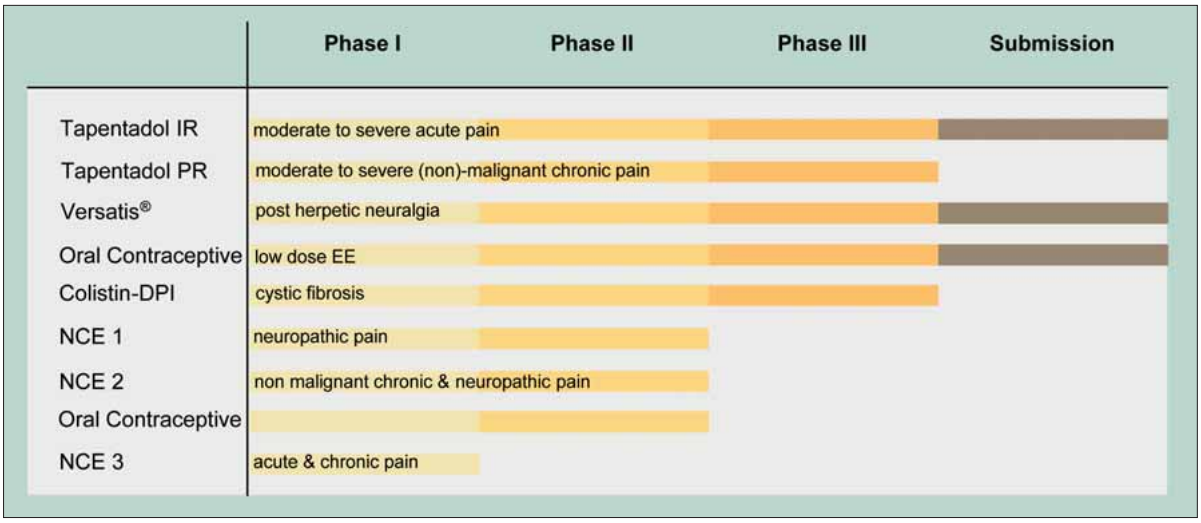
We invest a lot in in R&D, but we also invest a lot in IP-protection.

Grünenthal currently has some 4.100 patents –

That's right, but it's not only a question of getting a patent and of having freedom to operate. A huge topic the research based pharmaceutical industry has to deal with these days is litigation in the US. Today, it is also to make sure that you protect yourself from litigation, and it is

wk

Biology and chemistry side by side.



Grünenthal Pipeline; (NCE = New Chemical Entity)

very, very important for us.

For a mid-size pharma company what do you consider the right ratio of R&D budget to turnover?

Just to give you a number, I would say, a company like Grünenthal needs 17, 18 percent of the turnover. Maybe the percentage is less important as the actual amount. I would say there is a magic number around 200 million Euros per year. But all depend of course of your ambitions and of the level of risk a company is ready to take. At Grünenthal we set a goal of delivering a NCE every 7 to 10 years and 1 re-formulated / re-developed compound as our Transtec, Zaldiar, Versatis etc. every 3 years. Another important aspect beside the numbers is the partnering strategy. We follow the strategy to share the North-American rights to our compounds with partners after completion of Phase II b. The compound is then further co-developed with the partner.

If you had to reduce cost, where would you axe the R&D budget?

I would definitively concentrate on the more advanced projects and I would probably get rid of some research activities. Unfortunately

It strikes me that Grünenthal still follows the model of «Zentralforschung», centralised R&D. What are the benefits compared to a decentralised research organisation or spinning off projects into separate profit centres.

When I came to Grünenthal we had beside our activities in Germany a little bit of research in Spain, a little bit in Italy. It was piecemeal R&D. Since we have around 600 people in R&D we are certainly more efficient having our activities concentrated in one site: You have quick access to people, quick response, and a quick integration of newcomers. On the other side it bears the risk of limiting ourselves, that is why we try to compensate that potential deficit with co-operation.

Why is Grünenthal so mysterious about its partnerships?

We are not very public on that, indeed. We don't have pressure from stock markets and do not need to make announcements. The only pressure we do have is to deliver molecules, to deliver products not newspaper articles. We have research co-operations all over the world with academia and with small biotech which allows us to access different knowledge and to maintain flexibility. We are working with companies in the Netherlands, Korea or the US, with different institutes in Germany, Switzerland, Spain, and France. We have a research level network in Japan and India.

How many?

I would say about 15 to 20 doing very different jobs for us, from identifying new targets and creating new molecules to services up to developing technologies.

What lesson did you take working as post-doctoral researcher with nobel prize winner Robert Huber?

At the time I was very impressed about Robert's modesty. He is a brilliant intellectual, a very analytical person and hard worker – definitely, I learnt that. There is nothing easier than to jump from one nice idea to the next, Robert had perseverance in the many ideas behind what he did. In that respect he is what I would call somehow conservative but in the very best sense of the word. □

R&D at the Interface of Politics and Industry

Alastair Glass, Chairman of Irish Tyndall Institute and former Executive with Alcatel-Lucent's Photonics Research and Deputy Minister of Research and Innovation, shares his thoughts on R&D policies in Canada, Ireland, the US and Europe.

IINTERVIEW | **WOLF G. KRONER**

As a US citizen you were appointed the first Deputy Minister of Research and Innovation in February 2006. Is there something special about Canada to nominate foreigners the top of public institutions?

Hiring the right person rather than who you know is a good thing to do, but it also is a brave thing to do, because you are hiring somebody that doesn't actually know the culture. In my case Ontario went for my background and experience in the innovation space. Ireland also hired foreigners when they established the Science Foundation Ireland.

Photo Montage: MaRS

Scheduled to open in 2009, Phase II expansion will bring Toronto's MaRS centre to 457.200 sqm. MaRS is the kernel of Ontario's life science cluster, situated in the heart of Canada's largest urban centre.

You were educated in the UK, then in the British Columbia academic systems. You started your career in the US industry, lived in Germany, founded a public research institute in Ireland and then worked in the Ontario civil service. How did this enrich your experience?

I would say that science, technology, and innovation are completely international. People around the world are trying to do very similar things: build prosperity and the economy – albeit with a different perspective depending on their state of development. Now in the United States, research and innovation has had a very strong foothold for many years and is an integral part of the culture. Ireland has been building theirs over the last several years. Until 2000 Ireland did not have a strong record of investing in research and innovation - before that most of the research grants came from the European Union. Now, the Irish are eager to grow, and are making considerable investments in research and innovation. Canada has been a wealthy country for many years and has a very strong research base, but now they are putting more focus on innovation – that is driving ideas from the research laboratory to the market place quickly. Speed is the new global reality. In Ontario there is a very significant move to support innovation wherever it is, in academia, industry or society, and to focus on areas of strength. In Europe, state aid rules limit how much a government can support development in industry.

A major reason is to curb regional protectionism and foster competition …

In fact, the EU philosophy is to build partnership between the Member States and building Europe as an entity. They've done remarkably well in bringing countries together in the science space. In the case of Canada, they need to do more in building inter-provincial partnerships. Canada has a fairly small population on a global scale and by combining the strengths of the different provinces it could build a stronger science and technology base.

Could that be a model for transactions between the Republic of Ireland and the Northern UK province?

Absolutely. The Republic of Ireland is building partnerships with Northern Ireland. At Tyndall we do that. The Northern universities can use the very good facilities at Tyndall through an access program established by the Science Foundation Ireland. There is a strong desire in Ireland to build these partnerships between the North and the South, and even the United States is involved in helping put these partnerships together.

How's that?

Remember Ireland's R&D policy is new. The Science Foundation of Ireland was created in 2000. Its first director general was Bill Harris, formerly director of the National Science Foundation in the United States. Bill Harris hired me from Bell Labs to be the first Director of information and Communication. The third leader was John Atkins – director of biotechnology programs. He was Irish born, but came from the University of Utah. So of all of the Science Foundation Irelands leadership for ICT and biotechnology came

from the United States. That was the beginning of major Irish investment in research – some 650 million Euros. Such a big public investment in R&D was a huge change to the very small funding base before, and you can't imagine, how exciting this was for the Irish R+D community.

What do you see as typical mistakes by politicians in supporting innovation?

In my view, science and innovation is best led by the technical community based on excellence and on strategic importance. Now, if governments start making the investment, then politics comes in as well. There is nothing wrong with political support, however for clarity it is best to keep the political systems and the science funding systems at arms length.

A Minister of R&D is typically expected to empty his horn of plenty. With limited public resources, what did you do to pull in your horns in Ontario?

You are right; the system always wants more money. But innovation effectiveness is less about the amount of money than changing the way money is spent. It is important that researchers understand why they are doing research. In the old days, they didn't have to ask that question. Researchers just did exploration. Nowadays, they need to understand who the customer is, and need to develop the relationship with the customer. Clearly, the marketplace and industry inspires research as much as research delivers new ideas and products to industry. So that partnership is very important. I don't mean that on a short-term basis. Universities are good at the longer-term objectives. The big issue is to engender a cultural shift that brings the various players in the innovation ecosystem closer together.

Politicians have to heed elections. I wonder if they can maintain the long-term view needed for an innovation policy?

That of course depends on the politician. I am not a politician. As deputy minister I was close to the political system, but I was a civil servant and our view was long-term. Ontario Premier Dalton McGuinty was my Minister for the first year and a half, and he cared very much about the long term. His view was, if the public can see the benefit of the government's long-term investments, then that's good politics. Indeed he was re-elected last year and so he is able to get a second go at delivery on the innovation agenda. Think of others like John F. Kennedy. He set the target for putting a man on the moon and that was not a short-term agenda. You need leaders with vision, and these politicians will actually do better than those who think merely about re-election.

And the scientists?

From a scientist's standpoint we must always bear in mind that the system is not as patient as it was. Things are moving faster. The public want to see results on their investments in research and innovation. This is also true in industry. When I was vice president of photonics research at Bell Labs, the partnership with the business units helped enormously. When we got together with them and with customers, we were able to understand what the customers wanted and what the business needed in the long term. Then we could feed that back to the research people and the efficiency of research, in terms of return on investment, greatly increased.

INFO

Perry Blocher

Alastair M. Glass (born 1940) serves today as consultant to government agencies on R&D policy. Born in the US he graduated from University College London (UK) in 1961 (B.Sc. Physics) and obtained his Ph.D. in physics from University of British Columbia at Vancouver (CA) in 1964. He began his professional career as a research scientist with Bell Laboratories at Murray Hill, NJ (US) where he moved up to vice president of the Bell Labs Photonics Research and CTO of Lucent Technologies' Optical Networking business. In 2001, he went to the newly established Science Foundation Ireland as the first Director of Information and Communications Technology and was called to become founding Chairman and acting Chief Executive Officer of Tyndall National Institute in Cork (IE) in 2004. In 2006 he went to Ontario to join the newly established Ministry of Research and Innovation (MRI) as its first Deputy Minister, from which he retired early this year.

In the innovation arena a rather large tier of intermediaries has evolved between policy makers and those thought to innovate, primarily researchers or businesses. Today you have innovation consultants, many more tech transfer officers, and so on. How can policy makers ensure that this intermediate tier is not just perpetuating itself?

There is a compromise there between creating new organizations and building an effectively linked community. In Ontario we created regional innovation networks to

Alcatel-Lucent

Global headquarter of Bell Labs, Alcatel-Lucent, at Murray Hill NJ (US). Around 2.300 people work on some 500.000 m2 innovating electronic switching, data communications, and optical transmission applications. Bell labs is the technology forge of transistor, Unix, and the laser.

reduce fragmentation. The objective was to bring the players together, to be like a marriage broker for the various regional players to network together, and help build the local economies. We established MaRS, which really

Tyndall Institute

357 researchers from 33 nations work at the Tyndall National Institute Cork (IE) on an area covering 10,000m² at present. Since 2001 the Irish government have invested more than €115m into the Institute.

embodies this concept for Ontario by bringing researchers, entrepreneurs, investors and business support together under one roof. MaRS provides services to the regional networks and the broader provincial community.

But you are absolutely right, once you have created an organisation like that, these organisations have to sustain themselves and then they have to come back for more money. In the long run it is important that they can demonstrate their value and become self-sufficient. To government, intermediaries are important. As I said before, it's very important to separate out political decisions from the technical decisions. That's why we worked with external expert groups like the Ontario Research Fund Advisory Board, Ontario Genomics Institute, or Ontario Institute for Cancer Research to help make the technical decisions.

Coming back to bringing the players together, biotechnologists are still rather slow in taking up technologies developed in other fields like telecom, electronics or space industries, – optical detection technologies are just one example. It appears that trumpet calls for inter-disciplinary cooperation or joining forces may not be that effective –

You can do that in a variety of different ways. In the case of Tyndall, in Ireland, they have a very strong optical device activity and strong telecom people. In fact, we brought a research group from British Telecom – real leaders in the optics space – over from the UK to Ireland. Besides the photonics, electronics and silicon fabrication activities we also have the biological science interface, which does work in microfluidics and optical detection of biological molecules and reactions. That's all in the same institute and very specifically arranged to bring the people together. We've done similar things in Ontario. Many funding agencies look for interdisciplinary activities to bring the various players together.

What could Ireland learn from Canada to support R&D policy?

It's actually learning from each other. Ireland is still in the growth mode. They are building their capabilities well and have some internationally known research teams, but it's still going to take a few years before they are on a par with the major innovative countries. The Irish initial focus was on creating new jobs. Today they are in a state of full employment and the issue is high-value jobs. Conversely, Ontario and Canada has a wonderful research base, extremely strong. Now, their focus is getting more economic value out of their research. So focus is important, understanding where you want to be strong. In Ireland they don't invest in all areas of science, primarily in those areas that are important to the economy, information technology and biotechnology or biopharma.

So, no further learning required ….

One thing we did in Ontario that might be a good thing to do in Ireland, is to invest in a venture capital fund. For example last November the McGuinty government invested C$90m* and the private sector added C$75m** to build an investment fund that is focused on return on investment. This will help grow early stage companies. It is important that all the links of the innovation chain are in place: a strong education system, research, development, start-up companies, growth companies, and investment, especially for early-stage funding.

What could Canada learn from Ireland?

The Irish remember what it was like to be a poor country with high unemployment and they understand the importance of innovation and inward investment to building the jobs of the future. They have seen the effectiveness of coordinated and consistent government policy over many years. It is important for the Canadian public to recognize that investments in innovation now are essential for the economic health and prosperity of the future. It is also important for the various government agencies to work closely together and to have close federal/provincial partnerships to deliver consistent policies toward this goal.

In terms of geography what should be the scope of an R&D policy?

International partnering is an important building block of an innovation policy – you can't brand yourself as an innovation centre unless you are globally connected, and respected by the rest of the world. From a wider perspective I would say the United States is still seen as the country that's probably got most right in getting ideas out of the research lab into the market place effectively and quickly. Other countries including Europe are trying to replicate that. □

Comment

* C$90m = €60m; ** C$75m = €50m

Accessing Chinese Pharma, Agro, and Food Markets

With over 600 sector shows per year you are spoilt for choice in China. The report assesses major sector trade fairs, describes some opportunities, relates experiences with product pirates, how foreign firms cope with it, and where they get help.

TEXT | **WOLF G. KRONER**

According to WTO (Oct 2007) China is now second (US$10bn) to the US in terms of purchasing power parity. In 2006, the country ranked third in exports (US$969bn) before Japan, France, Netherlands, UK and Italy. Last year 10 out of the 100 companies worldwide with the highest market capitalization were Chinese ones (US 32, UK 11, FR 8, DE 7). Beginning March Xuewu Gu, Professor of Political Science of East Asia, summarised the economic rank of China for his audience at the Liechtenstein Bankers Association meeting. No doubt, a Far-East engagement promises to Western companies a boost in sales or access to cost-effective inputs like ingredients, components and research resources, but Chinese customers and partners

TABLE 1 China's Pharmaceutical Industry

© 2008 BioWorld *EUROPE*	total Jan-Aug 07 [bn €]	total change vs. period 06 [%]	ChemPharma Jan-Aug 07 [bn €]	ChemPharma change vs. 06 [%]	Pharma ex Chem, incl. bioproducts Jan-Aug 07 [m €]	Pharma ex Chem, incl. bioproducts change vs. 06 [%]
Product sales income	182.42	25	182.26	25	164.0	0.09
Total profit	15.33	49	15.64	51	-314.4	-2.01
Product's sales cost	129.79	25	126.96	23	2.831.3	2.23
Operational cost	1.23	15	1.18	11	50.8	4.32
Delivery value of exports	25.15	19	25.28	20	-121.3	-0.48
Output value of new products			28.45	17		
Management expense			13.35	20		
Av. N total personnel			660.093	3		

Total number of chemical pharmaceutical enterprises (incl. chem preparation) 8/2007: 3.304. Euro figures are based on original data in Renminbi (Yuan). Fixed exchange rate (March 2008): 1 RMB = 0,08999 €. Estimate BioWorld Europe based on CPIA (2007)

Booming Cities: Downtown Shenzhen, today 12 million, 20 years ago 12.000 inhabitants.

become more demanding. A sizable domestic demand can be seen for high-priced technologies from abroad – just think of today's 345.000 Chinese US$-millionaires –, Gu pointed out, but the way of doing business continues to be different. A key reason is the political system in place. There is domestic economic competition. Modern management is applied, said Gu, but this is framed within state control of industries regarded as being of national strategic interest.

Foreign investment plays a significant role in growing the China's economy and for many Chinese companies in the life science industries returns on foreign exchange are crucial for expansion in their home market (PWC 2006). According to a report by CPIA, the China Pharmaceutical Industry Association (2007), the country exported 45 % of its output in 24 major drug categories (total 272.919 tonnes, exports +5 % against previous period) during the first half of 2007. Vitamins (total production 89.000 tonnes) took the largest share of which 62% were exported. «Biofermentation technology develops quickly in our country» says CPIA and prognosticates: «It shows a good market prospect» especially with regard to amino acids with many other uses beyond pharma. Indeed, the market for biotech drugs is still negligible. In the first half of 2007 out of 902 drugs approved by SFDA, the Chinese regulatory authority, merely 15 were new biological drugs and an additional 17 biological drugs were what is called «with established national standards.» By contrast, a sizable demand grows outside clinical uses, i.e. in feed and human nutrition. This trend was clearly reflected by API China at Shenzhen last november. Several Chinese manufacturers said they were eagerly looking for partnerships in the agro and food ingredients sectors abroad. Others like German dairy-processor Meggle seize the opportunity to co-develop nutraceuticals for the Chinese market.

The Role of Trade Fairs

Trade fairs provide an excellent opportunity to access customers and gather market intelligence, but you are spoilt for choice in China. There are some 600 exhibitions and conferences per year in the pharma-medtech sector, says Daniel Chan, Head of International Sales and Marketing Department with Reed Sinopharm, one of the largest exhibition organisers in the country. While quite many of the Western companies eying business in China feel comfortable following the flock, experienced business developers point to opportunities aside from trodden paths. Thorsten Böttcher is Director of Business Development in Asia with German Erweka GmbH, a mid-size manufacturer of testing instruments for the pharmaceutical industry, and since 1992 at Hong Kong. It is true, says Böttcher, Shanghai is a large centre of the pharmaceutical industry. However at Nanjing and attending API China, a trade show rotating between different loca-

API China, Shenzhen, Opening.

TABLE 1 Trade Shows in China 2006/2007

	Focus	Date	Location	Organiser	net space m²	Countries Exhib.	Exhibitors (International)	Visitors (1) (International)	prices (2) starting m²
CHTF China Hi-Tech Fair	Biotechnology, Laboratory, Optics	Oct 12, 07	Shenzhen	China Hi-Tech Transfer Center Exhibition Dept.	105.000	5	3.527 (10)	n.a.	n.a.
CMEF Autumn	MedTech, Pharma, Healthcare	Oct 24, 07	Chengdu	Reed Sinopharm Exhibitions Co. Ltd.	40.914	16	2.000 (66)	50.000 (1.428)	2.200 RMB
CMEF Spring	MedTech, Pharma, Healthcare	Apr 10, 07	Dalian	Reed Sinopharm Exhibitions Co. Ltd.	40.905	8	1.925 (126)	50.000 (1.197)	2.200 RMB
API China / Interphex China	API, Biomedicine, Pharma Packaging, Process Technology	Nov 13, 07	Shenzhen	Reed Sinopharm Exhibitions Co. Ltd.	16.389	3	1.119 (18)	30.000 (657)	220 USD
API China / Interphex China	API, Biomedicine, Pharma Packaging, Process Technology	May 15, 07	Nanjing	Reed Sinopharm Exhibitions Co. Ltd.	17.118	2	1.203 (6)	30.000 (821)	220 USD
CISILE - China Int. Scientific Instrument & Laboratory Equipment Exhibition	Biotechnology, Lab., Optics	May 23, 07	Beijing	China Instrument Manufacturer's Assoc. / Beijing Lamp Exhibition Co.,Ltd.	20.000	n.a. (20)	432 (458)	16.630	240 USD
CHINA MED	MedTech, Pharma, Healthcare	Apr 22, 07	Beijing	Messe Düsseldorf China Ltd. / China World Trade Center Ltd. / Medical Department of general Logistics-Chinese People's Liberation Army	12.000	19	559 (147)	31.568 (2.054)	380 USD
ACHEMASIA - Int. Exhibition Congress on Chemical Engineering and Biotechnology	Chemistry Food, Process Technologies, Biotechnology	May 14, 07	Beijing	Dechema e.V.	8797	26	505 (266)	16.047	
Analytica China	Laboratory Equip., Analytics, Life Sci., Diagnostics	Sept 19, 06	Shanghai	Munich Trade Fairs (Shanghai) Co.,Ltd.	4.211	8	270 (124)	8.557 (1.027)	295 USD
BIOTECH CHINA	Biotech., Pharma, Laboratory, Optics	July 4, 07	Shanghai	Deutsche Messe AG / Shanghai Technology Convention & Exhibition Co. Ltd.	1.800	17	134 (16)	4.853 (1.310)	196 EUR
Hong Kong Int. Medical & Health Care Fair	MedTech, Pharma, Healthcare	Aug 14, 07	Hong Kong	Hong Kong Trade Development Council / China Promotion Ltd.	1.392	39	113 (43)	6.707 (1.630)	370 USD

(1): Admission to Trade Fairs in China is regularly free of charge on registration which accounts for the high number of visitors; The indicated number of professional visitors is a subset. (2): Prices in USD/(EUR are typically for foreign exhibitors and often higher than for domestic ones.
With the exception of exhibitions by Reed Sinopharm, trade fairs listed are held at the same locations. ***Sources: Trade Fair Organisers (Dec 07/Jan 08)***

tions, he made an excellent business. API China is but one of a series of complementary exhibitions – pharma, medtech, healthcare, laboratory sector – organised by Reed Sinopharm. This joint venture between Sinopharm Group and Reed Exhibitions runs twelve large shows in China per year with exhibitors from many different regions and sub-sectors. Example are bi-annual shows CMEF focused on MedTech, and API China linked with Interphex, an analytics instrument show. Both fairs attracted 2.000 resp. 1.100 exhibitors in autumn 2007. This was only surpassed by CHTF China Hi-Tech Fair with some 3.500 exhibitors and held a month before at Shenzhen, where API China was held later. Competition is heating up with international organisers, many being joint ventures with domestic ones. They are pushing on the domestic market with an added value in bringing exhibitors at home to China. Competition among trade fairs is of great benefit both to foreign as well as to export-minded domestic exhibitors or visitors. This can be seen in top trade fair organisers (see chart) providing much more transparent data and better services to their customers. Imagine, you are about to decide which show in the sector would be best to attend. The usual expo prose, figures on gross space of the exhibition grounds, or total number of visitors is rather meaningless for planning. Gross space typically includes recreation areas, restrooms and unrented space. Typically, the real exhibition is at less than half of the gross space figure given. Or take the exorbitant number of visitors to a show usually given to confirm the stereotype of a big, big market to wait for the exhibitor. In reality, the number of professional visitors (buyers, distributors, development partners) is regularly of a much lower order. You should know, that acess to a trade show is typically free upon registration (which is how organisers know the number of professional visitors).

Copy the Copy Cats

«With a legal approach, you won't have any chance. It's a waste of time and nothing comes out of it,» comments a marketing manager of a UK instrument supplier engaged in China. The typical reaction of afflicted companies then is, not to litigate in court. Erweka's Böttcher simply recommends: «Be faster than the copy cat!» At API China Nanjing he spotted an illegal copy of his company's combination tester for tablets'

hardness, diameter, thickness and weight. Half a year later, Erweka presented at API China Shenzhen this instrument with newly developed technical features and a re-designed body. «In most cases copied instruments do not deliver the same precision measurements as the original ones, because the technology is rather complicated copying is time-consuming» says Böttcher and points to the competitive advantage of originators: knowing their product better they can outwit product pirates. But this implies to learn from the copy cats' approach in identifying the demand that fits to in-house capabilities, quickly taking up innovations, and organise in-house development along these lines. After all a 100% fake is an art in itself, as successful counterfeit paintings vividly demonstrate.

In addition, further means are available to put pressure on product pirates in the pharmaceutical industry and lobby for IP protection. You might visit those places where you can get in touch with industry representatives. For example, CPIA regularly convenes at the API China trade show. There, odds are quite high to meet a senior executive of the top 140 Chinese pharma manufacturers to bring your case to his attention … provided you are on site the first day.

wk

Product pirates need time. Erweka countered illegal copying of its combination tester (l) in China by developing a newly designed and technically upgraded version (r) within half a year after discovering counterfeiting.

Beyond helping yourself

In principle, the Chinese government recognizes that illegal copying is not a trifling offence, and seriously harms external trade as much-sought technology transfer and expertise does not enter the country to the extent hoped for by Chinese partners. The country is making progress in cracking down on product pirates. Foreign companies can indeed protect themselves as Robert Harrison (2007) has shown. However, interested companies should mind differences betwen legal IP systems, says Harrison, who is Senior Manager with 24IP Law Group. A case in point are different interpretations of 'prior art' in patent litigation. In Chinese law 'novelty' is judged on the domestic use of an invention, i.e. preceding use outside of China are not relevant in the decision to grant a patent. However on Chinese operating levels a striking lack persists to strongly engage against counterfeiting. Major reasons may be that the Chinese judicial systems is still in transition. According to Peter Schulz, whose law firm is licenced in Shanghai since 1995, authorities still have to come to grips with recent reforms with a stress on individual rights (counterfeiting is part of civil law) and the massive lack of jurists. In order to cope with increasing litigation in the wake of these new regulations government hired people who are known 'to decide', says Schulz. No wonder then, many judges are former military officers. Talking as a journalist to Chinese entrepreneurs about imitating products it appears that such instances are more seen as being clever at 'competition'. There might not be many copy cats, but a popular Chinese sentiment that product piracy is a 'problem of the West' is to the detriment of the overwhelming majority of honest Chinese entrepreneurs. Foreign companies reluctant to notify such cases to the authorities and trade fair organisers reinforce a system where odds are high that counterfeiters get away scot-free.

More and more trade fairs offer help

wk

Supply chain for manufacturing: natural ingredients, chemicals, instruments, packaging.

to complaining customers as in fact, many illegal copies are discovered during exhibitions by other exhibitors or professional visitors. Hartwig von Saß, Communication Officer with Deutsche Messe, Hannover (DMH) which operates pharma and biotech trade shows at Shanghai explains their procedure: During each exhibition DHM has designated a person complainants can turn to. DMH refers then to specialized lawyers who come by and decide on site, if the complaints warrants legal

wk

Kunping Hu: «You cannot encounter so many CEO's and Chairman from the top pharmaceutical industry in other occasion or on other platforms than API China.»

prosecution. For urgent cases including weekends, DHM is able to contact by cell phone the President of the Chamber hearing the case. On the trade ground a special room is prearranged which serves as a court room. If the complaint is legally valid, the trade fair then closes down the infringing parties booth. Other international trade fairs have similar measures in place which can be pulled up to national industry associations (in some cases operating special databases) and governments. This is increasingly, effective in the West, and not just against Chinese counterfeitors. For example, during this year's CEBIT, the world's largest IT fair, custom police raided 51 booths and confiscated some 500 products infringing IP right. While the majority of the counterfeitors were from Asia (24 China and Hong Kong, 12 Taiwan), nine of them were from Germany. In fact, when the German Engineering Federation surveyed cases of product piracy among its members in 2005, worldwide second to Chinese companies were Italian firms. Within China foreign trade fair organisers are still reluctant supporting complainants as its a very sensitive political topic. «However,» cautions Hartwig von Saß, «product piracy» has many meanings. It also happens that a supplier produces commissioned products in excess and then sells the original ones into the black market.

Kunping Hu, VP of Reed Sinopharm, explains that unlike many other trade fair organisations in China her company is going to great lengths to educate exhibitors. An IP Bureau has been and a report system to local authorities has been established. Before each show by Reed Sinopharm exhibitors are informed on sensitive issues and warned. The IP office handles complaints on site. Asked if her company were ready to remove an illegal product copy form a show Ms. Hu confirmed: «If this happens we definitely will it remove, but I am very glad so far, at all shows of our company no such incident has been reported.»

Getting to Know Your Lot

Product piracy by Chinese companies is of persisting concern to international Western pharma firms and life science instrument suppliers – even if, «it's not easy to copy that» as Leo Xie, Pall's Senior Regional Manager East China Biopharmaceuticals claims pointing to plastic products Pall imports in China. "There is a lot of technical knowledge included in highly specialized plastics products" Xie explains. The presence in the regional market is crucial in educating customers what 'quality' really means and what is at stake in export, if not complying with tough regulatory standards. Originators and imitators alike have to pass the market test, and trade fairs are a major distribution channel.

Meanwhile Western firms pro-actively structure their Chinese engagements to minimize risks of technology transfer. Arno Krotzky, CEO of BASF-subsidiary Metanomics, which specializes in pharma and nutrition analytics, describes a prudent approach: «First, you have to decide which parts of your technology are you able to develop together with partners, namely for the Chinese domestic market. You should ask yourself: Do I rather have established technologies which you can develop further with partners? But, also question yourself: What can I learn from partners in China.» □

References

- China Pharmaceutical Industry Association CPIA (2007): Market Analysis on Chemical Pharmaceutical Industry: January–August 2007. Shenzhen, November.
- Harrison R (2007): The Great Rip-Off? BioWorld Europe 4:8–12.
- Schulz, Peter (2005): The Second Revolution. Frankfurter Allgemeine Zeitung. December 28 (in German).
- PriceWaterHouse Coopers (2006): Investing in China's Pharmaceutical Industry. March. www.pwc.com/Extweb/pwcpublications.nsf/docid/4D287C2BE1191F4A80257131004D6464/$File/china-pharma-paper-mar2006.pdf

Faster, Smaller, More Accurate, and Less Expensive

In an interview with BioWorld *EUROPE*, Hans-Joachim Heusler, Managing Director at Munich International Trade Fairs, explains how the trade fair analytica helps companies use the dynamic nature of the global market profitably.

WOLF G. KRONER | IINTERVIEW

For more than three decades, analytica has been a trade fair for the laboratory and life-science industries. From a global perspective, what market dynamics keep this trade fair going?

analytica is an exhibition that covers everything related to the laboratory, which includes the sectors for chemistry and biotechnology as well as electronics, IT and related services. It is dynamic in a number of ways. It focuses on technological developments in the micro and nano range, in biosensors and bioinformatics and in automation and material analysis. These developments can positively influence all laboratory applications, be it in industry, the research and development of new products, in-process quality assurance, public safety, environmental protection, healthcare and basic research. The latest buzzwords include point-of-care diagnostics in medicine, drug analysis, food and environmental analysis and the paperless lab. Technology follows economic imperatives, even in the public sector. Technology must contribute to process optimization, increased efficiency and higher quality. Quality at affordable prices is an important criterion for companies that hire laboratories, and laboratories must adapt accordingly. Intelligent financing solutions, selective recruiting and market knowledge are important both to laboratories and to manufacturers of laboratory solutions, which is why they are offered at the fair in the forum of seminars.

In addition to technological development and the pressure to increase efficiency, there is a third factor of market dynamics, which many people like to describe as «globalization». Right now there are fewer trade barriers in the world. Better transportation infrastructures and means of transportation, more efficient data networks and enormous progress in merchandize logistics are bringing people closer together and promoting the flow of goods and opportunities to collaborate with companies in other countries. That is why it is very important to have as many foreign exhibitors at *analytica* as possible, so that attending the fair is worthwhile for customers from around the world. Manufacturers understand the significance of domestic markets: after all, laboratories exist around the world, and their application sectors are also comparable. For example, take medical diagnostics or demand on the part of specific government agencies – a regional epidemic of bird flu must be dealt with, and doping or drug tests must comply with the laws of the country in which they are conducted. But now you can select the necessary technologies and development and production partners for corresponding products and services around the world. And the fact that exhibitors at *analytica* are so international reflects that fact.

INFO


sbA

Hans-Joachim Heusler (1953) is Managing Director at Messe München GmbH since 2005. After obtaining a degree in law (1983) he joined the Bavarian Ministry of Economic Affairs, where his last function was Senior Ministerial Councillor for strategic industry policies in IT, telecommunications, electronics, the media, electrical engineering and life sciences.

How do you turn this internationalization into trade-fair operations?

We do everything we can to approach companies in other countries. If you look at the number of exhibitors currently registered for this year's *analytica*, there are more exhibitors than we had in 2006, when 36% came from countries other than Germany. Of course, it is still too soon to say anything about the number of visitors. To achieve this increase at *analytica*'s central venue here in Munich, we hold press conferences and attend partner exhibitions in other countries. At the same time, we are establishing a network of our own foreign exhibitions. *analytica* in India has grown so much that we had to change locations. We have been in Hyderabad since 2007. *analytica* China is also growing, although the trade-fair centre in Shanghai is still large enough to accommodate expansion. We also enter into partnerships that support this growth trend. We work with the Indian *Analytica*l Instruments Association (IAIA) in India, and our partner in China is the Beijing Conference and Exhibition on Instrument Analysis (BCEIA). Our exhibitions, including those abroad, are not just oriented

to demand on local markets. They also specifically address future demand. Environmental protection is a good example. Right now this is a hot topic in India and especially in China, but many of the analysis and laboratory-technology solutions that are available are not very well known there. This is a great opportunity, even for smaller companies in Europe.

wk

You are not alone in China. There are currently some 600 trade fairs and exhibition conferences in the laboratory and life-science sectors. Moreover, some very modern exhibition centers have been constructed outside Shanghai recently. Shenzhen near Hong Kong is just one of them. Are you nailed down at the Shanghai fairgrounds?

We basically see two locations in China. One is Beijing, where government agencies and universities play a key role. The other is southern China, where Shanghai is undeniably the economic center. That is why we think that our partnership with the BCEIA, which we alternate with every other year – the BCEIA exhibition is held in Beijing one year, and *analytica* China is held in Shanghai the next – is the right way to go. In Shanghai, we also have a chance to create synergy with our own exhibitions. For example, the next *analytica*China is being held at the same time as our environmental exhibition IFAT China in Shanghai, the International Trade Fair for Water, Sewage, Refuse and Recycling. We think this will give both *analytica* China and IFAT China another strong boost.

wk

What is the relationship between the satellite analyticas and the mother exhibition in Munich?

Most of our exhibitors want to sell their products on international markets. So many of the large corporations that exhibit at *analytica* in Munich are also represented at our exhibitions in India or China. However, there are also several smaller manufacturers that have specialized in their domestic markets. If we want to serve these companies, we must have fairs at locations where there is demand for their products. We have a similar situation when it comes to visitors. There are those who can afford to travel to major international exhibitions like *analytica* in Munich to see the market in its entirety and the complete range of products that it has to offer. The exhibitions in China and India are still much smaller, but for many visitors, investigating those events is sufficient.

Are you considering more satellite exhibitions?

We don't limit our vision to China or India. We are always checking to see if other markets are developing at the same dynamic pace and if further expanding our international network to meet our customers' needs is really worthwhile for our exhibitors, our visitors and, of course, for us.

Why don't you expand into the US?

We generally stick to markets that do not already have their own exhibitions. There are plenty of exhibitions in the US where our exhibitors are represented. If we want to support our customers, then the best place to do so is wherever they do not yet have a stronghold.

Compared with other industry trade fairs in Europe like EuroBIO or Forum Labo in France, ScanLab in Sweden, RichMAC in Italy or Biotechnica in Germany – what makes analytica unique?

analytica clearly focuses on «everything related to the laboratory». That also applies to the use of laboratories by and for the biotechnology sector. That is where *analytica* is unique. Take the number of exhibitors and the amount of exhibition space. This year there will be some 1,000 exhibitors on 50,000 m^2 of exhibition space. We expect some 30,000 trade visitors. Then there are the specific visitor target groups that we address, i.e. the industrial sector, the public sector and basic research. Aside from the names given to other events, which do not necessarily reflect the actual exhibits, you won't find this kind of focus anywhere else.

What are the highlights of analytica 2008?

Once again, all the key players in the international laboratory-technology, analysis and biotechnology sectors are exhibiting at this year's fair. But *analytica* is more than just

an exhibition. There is also the scientifically oriented *analytica* Conference, which is being organized by the German Chemical Society (GDCh), the Association for Biochemistry and Molecular Biology (GBM) and the German Association for Clinical Chemistry and Laboratory Medicine (DGKL) and will feature a first-rate program of lectures and presentations with international speakers. There are also two new forums, which serve as a link between the exhibition and the scientific conference. The Business and Markets Forum examines the latest business and market developments: What opportunities and hurdles are there when marketing products? How do the markets in Russia and China work? Speaking of which: I am proud to announce that, for the first time ever, this year's fair will feature a large China Pavilion. The other forum is the Innovations and Technologies Forum, which focuses on technology platforms and solutions instead of specific companies or products. This forum will be located in the new Innovation Area, which is where we give new companies a chance to participate in a world-class exhibition at affordable rates, i.e. companies that could not afford to participate otherwise. Besides German companies, participants include new high-tech companies from the US, the UK, Austria, the Netherlands and even Greece, which truly shows that these start-ups are serious about going commercial and that they think and act in a businesslike fashion.

You are expecting an 11% increase in the number of exhibitors and a 10% increase in trade visitors at analytica 2008. Other trade fairs say attendance will remain more or less the same compared to their previous exhibitions. Why are you so optimistic?

If you look at the exhibitor applications that we have received, we have already met that objective. As far as the visitors are concerned, it is still too soon to say, but we fully expect the market to pick up again and that it will be noticeable at *analytica*. The biotechnology sector is still relatively new and is rebounding from the slump that it experienced over the past few years. We are just beginning to explore the potential that this sector has to offer. However, in other sectors such as public safety, healthcare, consumer protection and the environmental sector, demand for laboratory services should actually be increasing. I already mentioned the challenges that laboratories face when it comes to efficiency. I also think that increased awareness among the general population about issues that concern them will require more and more new laboratory services and that they will want to come to *analytica*, not just to gather information about new equipment and solutions, but to exchange thoughts and ideas about how markets are developing. We are certainly doing everything we can to give the visitors what they need.

In 2006, 79 exhibitors were from the US and Canada, which is 9% of all exhibitors, not a small number from overseas. What attracts these companies?

In actuality, the number of North American companies represented at *analytica* is much higher. That also applies to other countries like Japan, where the number of exhibitors from Japan that have registered for the fair is up considerably over 2006. When we mention a country of origin, we always refer to the country that a registered company actually comes from. In the case of companies that have a subsidiary in Germany, that is not their headquarters in some other country, but Germany. So companies like Thermo Fisher Scientific, Applied Biosystems, Agilent or Shimadzu don't register from the US or Japan: they are represented by their German subsidiaries and are basically considered German companies. As a result, the share of international companies is actually much higher. Incidentally, our fair-related statistics are verified by an independent auditor. Based on our surveys, we know that a large number of exhibitors look forward to returning to the fair – as do 92% of our visitors. And they recommend us to others. The thing that attracts American exhibitors to *analytica* is the circle of visitors that they expect to see. They are particularly interested in visitors from Europe and neighboring countries to the east. When it comes to the Asian market, we see the same American companies at our *analyticas* in Shanghai and Hyderabad.

wk

Two years ago, 59 companies or 7% of all exhibitors came from the UK and Ireland.

The same thing applies there as to North America. *analytica* is quite simply THE exhibition when it comes to the laboratory. In the case of the UK and Ireland, you have to add the fact that they do not have their own exhibition that is as broad-based, as large or as international as *analytica*. Attending *analytica* is worthwhile for companies based in the UK and Ireland because it is the place to meet customers from their own countries. People come to Munich because *analytica* is THE leading exhibition for the laboratory sector. They know they won't find themselves in a department store-like atmosphere. They know exactly what to expect when they come here. In other words, the cost-benefit analysis for participating in or attending the fair is very favorable. □

Downstream Production Impacts Overall Capacity

As upstream bioprocessing yields continue to improve, the capacity bottleneck is stubbornly being pushed downstream. This, in turn, has put the squeeze on biomanufacturers' overall purification and downstream processing. Now it appears that European biotherapeutic developers and contract manufacturers have been pinched harder than many of their US counterparts.

TEXT | ERIC S. LANGER

INFO

Eric S. Langer (1958) is president and managing partner at BioPlan Associates founded in 1989 and incorporated in 2001. He obtained a degree in chemistry from University of Maryland and thereafter a MSB from American University. Today, he also teaches at Johns Hopkins University Marketing in a Regulated Environment, Biotechnology Marketing, Bioscience Communication, and other courses.

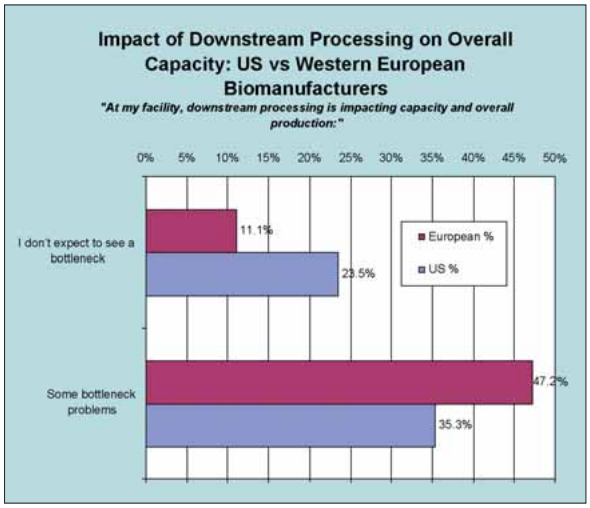
As upstream bioprocessing yields continue to improve, the capacity bottleneck is stubbornly being pushed downstream. This, in turn, has put the squeeze on biomanufacturers' overall purification and downstream processing. Now it appears that European biotherapeutic developers and contract manufacturers have been pinched harder than many of their US counterparts. According to the just-released *5th Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production**, a global survey of 434 biotherapeutic developers and CMOs, only 11% of Europeans said they did not expect to see bottleneck resulting from downstream processing at their facility. This compares with 23.5% of US biomanufacturers who expect no downstream bottlenecks.

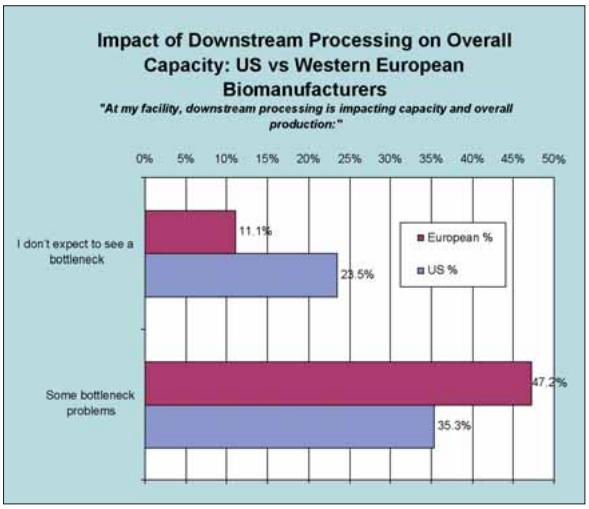
Although only about 5% of biomanufacturers on either side of the Atlantic today are experiencing serious bottlenecks from downstream purification, over 47% of Europeans are seeing «some» bottleneck problems. This compares with 35% of US biomanufacturers who are experiencing more than just a minor squeeze (See Fig 1).

Where is the Pinch?

Downstream purification covers a broad range of technologies and systems. When evaluating specifically where these downstream bottlenecks are occurring we found, as expected, that most are associated with chromatography columns. Here, as well, European respondents are feeling the pinch more acutely. Over 23% of European respondents were experiencing «Severe» or «Significant» constraints as a result of chromatography column bottlenecks. This compares with 18.7% of their US counterparts. Similarly, depth filtration steps were causing overall capacity constraints among 16.7% of European manufacturers, compared with 10.4% of US producers. In ultrafiltration steps, the bottlenecks were seen as less acute both in Europe and in the US.

When we asked respondents to comment on their downstream bottlenecks, we found a broad range of reasons. The increasing upstream yields, especially in monoclonals, are driving biomanufacturers to require ever-larger downstream scales. This has resulted in chromatography steps that are slowing overall facility throughput. Many respondents also indicated that poor scalability of individual process steps was also creating major problems, as was the lack of availability of trained staff in these production areas. The problems have been compounded by factors such as costs of protein A.





Downstream Processing vs Other Factors Impacting Capacity

To put downstream processing factors in context, we also evaluated 19 other factors that are creating capacity constraints in biomanufacturing. We then compared US and Western European responses from biotherapeutic developers and CMOs. A few of these are shown in Figure 3. There were significant differences in perception of how future factors may create capacity constraints by 2012. The greatest difference was seen in their perspective on hiring new, experienced technical and production staff. In the US, this factor was indicated by nearly 25.1% of respondents (second only to physical capacity of downstream purification equipment). However, in comparison, 37.1% of European facilities indicated hiring staff would cause capacity problems within 5 years. In the US, lack of financing for production expansion was expected to be causing relatively fewer capacity problems (19.4% in the US vs. 30% in Europe). Although Europeans are expecting physical capacity of downstream purification equipment to be a larger problem (37.1% vs. 28.9% in the US), both regions found this factor to be at the top of their list of factors creating capacity constraints in 2012. Overall, relatively more US facilities indicated that they are unlikely to see capacity constraints in 2012 (24.6% in the US vs 15.7% in Western Europe).

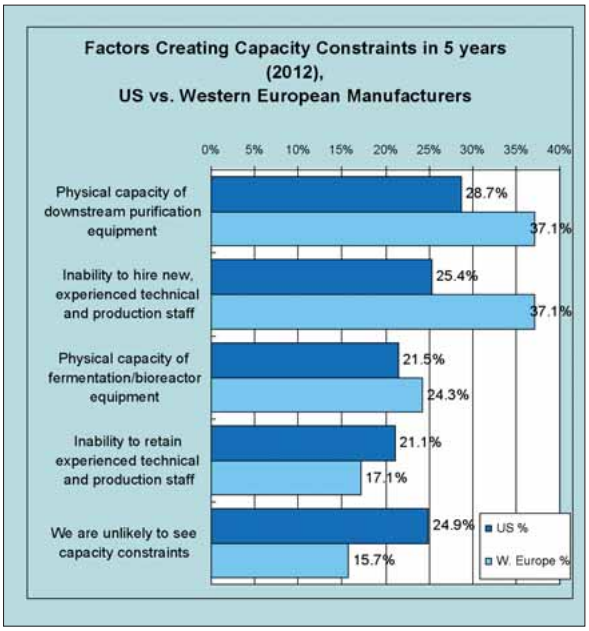


Figure 3: Abbreviated Capacity Constraint Factor List

Annual Trends: Factors Impacting Production Capacity

In 2005 and 2006 the major future production capacity problems were being generated by «Inability to hire new, experienced technical and production staff». Back then, this factor topped the list with over 30% of respondents worldwide indicating it would be a major factor in production capacity. In 2007 this hiring concern dropped in the ratings to second place (with 27.9% of respondents indicating). It is interesting to note that this number has continued to decline from 2003 when over 52% of respondents felt that lack of experienced production staff would be the major capacity bottleneck. And in 2005, 39.6% felt hiring would be the primary source of bottlenecks by 2010.

Today, «Physical capacity of downstream purification equipment» is predicted to be the greatest constraint (indicated by 29.6% of biomanufacturers and CMOs worldwide). In comparison, «Lack of financing for production expansion» rates fourth on the list, with 22.4% today. Back in 2006, «Physical capacity of fermentation/bioreactor equipment,» shared the spotlight with the, «Inability to hire new, experienced technical and production staff», with 21.4% of respondents' votes. This factor is fifth on the list in the current survey.

Downstream Purification Issues Facing the Industry

Today, downstream purification is clearly the area biomanufacturers and the industry recognize must be fixed to avoid future capacity constraints. Indeed, many vendors and biomanufacturers in the industry are evaluating options and developing improved downstream purification systems.

In this year's annual survey we first asked respondents to discuss the current downstream purification systems that are impacting their production. There was agreement that new routes to downstream purification technologies are needed to improve production processes, but the path has not been clearly identified. For example:

- Over 46% are now considering alternatives to protein A to reduce costs in new production projects.
- However, only 19% actually plan to move away from protein A for new production projects over the next 12 months.

Major Areas of Improvements for Downstream Processing

Whilst constraints caused by downstream processing is a hot topic, this concern has yet to be translated into broadly accepted commercialized innovations or products. Whether these innovations will come as stepwise, incremental improvements to current technologies, or be introduced as 'disruptive' technologies continues to be debated. Respondents to the survey were asked to identify areas where they believe major improvements in downstream purification will occur over the next five years (by 2012). Membrane technology was indicated as the area most likely to see major improvements for downstream purification. Following was the development of MAb fragments and the development of synthetic proteins. Interestingly, relatively few biomanufacturers and CMOs had opinions on these new downstream technologies. From this, we infer that the adoption rate for new technologies may be slow, as there may be a long learning curve for the industry. What is clear is that changes are very likely to occur in the way proteins are purified. Downstream innovation will likely drive these changes in an effort to lower costs and increase capacity compared with how current chromatography resins are being used. □

References

– 5th Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production, March 2008, BioPlan Associates, Inc.

Correspondence:

Eric S. Langer
BioPlan Associates, Inc.
15200 Shady Grove Road,
Suite 202
US – Rockville, MD 20850
Tel: +1 301 921 9074
elanger@bioplanassociates.com

IPO of MolMed Supports Promising Clinical Development Program

Beginning March MolMed S.p.A., Milano (IT) has been admitted to Borsa Italiana's MTA (Mercato Telematico Azionario) segment with current investors San Raffaele (28,18%), Airain (28,16%), Fininvest (21,84%), H-Equity (10,91%), and Delfin (10,91%). In order to move forward with its promising R&D programs MolMed needs up to € 40m capital.

Elena Lugagnani

MolMed's Management Team (from left): Holger Neecke, Marina Del Bue, Claudio Bordignon, Enrico Cappelli

Preceding the IPO the company announced in January that it has received approval by Agenzia Italiana del farmaco (AIFA) to start Phase III trial for its therapeutic candidate TK008 in the indication of high risk acute leukaemia. Since 2003 TK008 is designated in the EU as orphan drug (EU/3/03/168) for adjunctive treatment in hematopoietic cell transplantation and targetd at patients with high risk acute leukaemias. TK008 is a Herpes simplex 1 virus-thymidine kinase and truncated low affinity nerve growth factor receptor which is transfected to donor lymphocytes. Claudio Bordignon, CEO of MolMed said that TK008 is developed for those 60% of candidate patients who lack a fully compatible donor (haplo-HSCT).

AIFA clearance is the first one in Italy for a Phase III of a cell therapy in the country and follows approval of the clinical protocol, obtained in December 2007 by the Ethical Committee of the first study site at Milano. AIFA requires the completion of an analytical characterisation of TK components within the treatment of 20% of patients involved in the study. In Italy, a total of four centres are participating in the Phase III trial. Approvals pending another 6 to 11 centres in the rest of Europe will soon join in. A total of 200 patients will be enrolled in the European-wide trial. Holger Neecke, responsible for Business Development, told BioWorld Europe that depending on statistical power preliminary results should be expected within 18 to 24 months from now. The company expects cost of this trial in between € 10 to 30 million Euros.

MolMed announced starting in parallel a Phase I/II clinical trial of TK008 in the US, which will be managed by the MD Anderson Cancer Center in Houston (Texas). This trial studies TK008 for another indication which is hematological malignancies at high risk based on disease progression or presence of negative prognostic factors, who have received a stem cell transplantation from donor HLA mismatched (haploidentical) for 2 or 3 loci. This development line will require in between 1,5 to 6 million Euros. red

www.molmed.com

€ 30m by Novo Nordisk will fuel Affimed's oncology pipeline

Heidelberg and Oslo-based Affimed acquired new funds to fuel its antibody development. Novo Nordisk's corporate VC fund subscribed €30m in equity in Affimed Therapeutics AG, Heidelberg (DE). Rolf Günther, the company's CEO said that funds are needed to move forward with its lead candidates. One is a bi-specific tandem antibody which binds specifically to CD30, a protein which is mainly expressed on Hodgkin tumor cells and to CD16-A surface receptors on Natural Killer (NK)-cells in order to induce cell lysis therefore inducing selective destruction of the Hodgkin's tumor cells through antibody dependent cell mediated cytotoxicity (ADCC). The other TandAb targets CD19 on B-cells, e.g. for the treatment of Non-Hodgkin's Lymphoma (NHL), and a mono-specific one in the autoimmune development program (indication: Psoriasis).

Affimed develops tetravalent antibodies which can be constructed either monospecific or bispecific with two binding sites for an activating receptor on immune effector cells and two binding sites for a target molecule on the surface of tumour cells. According to the target this allows for recruiting either NK-cells (anti-CD16) or T-cells (anti-CD3). The present Series B financing allows Affimed to broaden its pipeline of currently five different programs and add further pre-clinical projects. In particular, this concerns candidates for immunosuppression for which the company has sought licencing partners in the past. Günther told BioWorld Europe that clinical trials are planned to start in spring 2009 with approximately 20 patients for phase I and 40 patients for phase IIa.

Affimed

Rolf Günther, CEO (l) and Melvyn Little, CSO of Affimed Therapeutics

red

Synoptics (UK)

Life Sciences in the Asian State are booming and with it the need to speed up targeted genomics and proteomics research. Synoptics (UK) is one of those companies already well established for its automated imaging systems with more than 70 instruments sold into the region since 2001. The Cambridge manufacturer announced that it installed its first high resolution instrument for analysis of gels and blots at the Institute of Chemical and Engineering Science (ICES), an up and coming research centre on Jurong Island. The imaging system runs with a non-cooled camera thus optimally in genomic research, but can be also used for analysis of thin layer chromatography plates, colony plates or bacterial flasks, commented Paru Oatey, Synoptics Head of R&D.

Newron Pharmaceuticals

Hans-Joachim Lohrisch former CEO of Altana Pharma AG Konstanz (DE) and Chairman of Nycomed GmbH (DE) up to September 2007 joins Newron Pharmaceuticals SpA, Milano (IT). Lohrisch studied at universities Berlin and Bonn where he obtained his PhD in organic chemistry. In 1978 he joined Merck KGaA where his last position was Head of the worldwide ethical pharmaceuticals business until 2000 when he moved to Altana at the Lake of Constance.

Advancell and Protherics

Advanced In Vitro Cell Technologies SL (Advancell) , Barcelona (ES) announced that clinical trials are starting for acadesine, its orphan drug candidate for the treatment of B-cell chronic lymphocytic leukemia (B-CLL). Acadesine is a nucleoside analogue that selectively kills B-Cells, but does not require tumour suppressor protein p53 to kill malignant cells as other treatments do, and it has little effect on T-cells. The present study is designed to elucidate further the action mechanism as well as safety aspects. B-CLL is an orphan disease with 3 patients affected in 100.000 (Europe). Acadesine is marketed by Schering-Plough as treatment of ischaemia reperfusion injury, but Advancell independently discovered the application in the B-CLL indication. Clinical trials will also be in Belgium and France and enroll a total of 30 patients with projected total costs of €50m for all phases. If successful Protherics expects annual turnover of €250m.

Human Genetic Diagnostics

Human genetic testing services are picking up speed and companies offering direct-to-consumer tests recently gained considerable prominence. Today, the battle is on in Europe – not only over quality of service, but also who sets the rules and decide who gets access to the human genetic diagnostics market.

TEXT | WOLF G KRONER

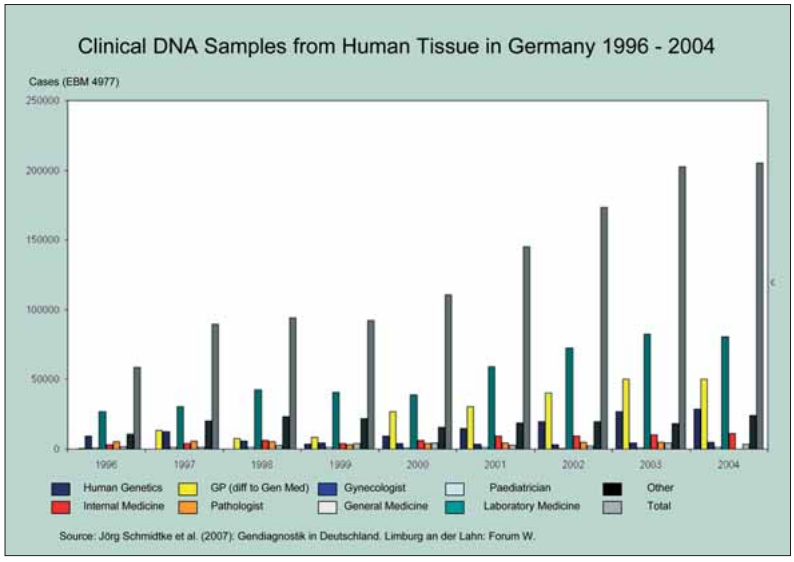
Rapid advances in genomic methods and instrumentation by companies like Applera, Illumina or Roche allow enable ever faster, more accurate and cost-efficient sequencing. Today, instruments deliver much more than SNPs, i.e. methylation studies, microRNA analysis up to chromatin immuno precipitation. The so-called next-generation sequencing technologies enable analyses of individual genetic variants, testing for predisposition of particular diseases and pharmacogenetics. Major demand comes from biomedical research. Beginning march 2008 the GOLD database counted 3.600 genome projects (incl. non-human sequencing). Another continually increasing demand stems from clinical laboratories which are applying these novel technologies in their daily routines. According to Jay Flatley, CEO of Illumina, today that market worldwide totals about $2,25bn (€ 1,5bn) and is expected to rise to $3,7bn (€ 2,5bn) in 2010.

In March 2008 in an activity report on Eurogentest, a EU-funded consortium of human geneticists offering test evaluation and validation services for clinical laboratories and diagnostics companies, co-ordinator Jean-Jacques Cassiman complained about «diverse and heterogeneous quality schemes, lack of reference systems and differing Member States regulations» which he said «have added to the overall disorganization and fragmentation of services».

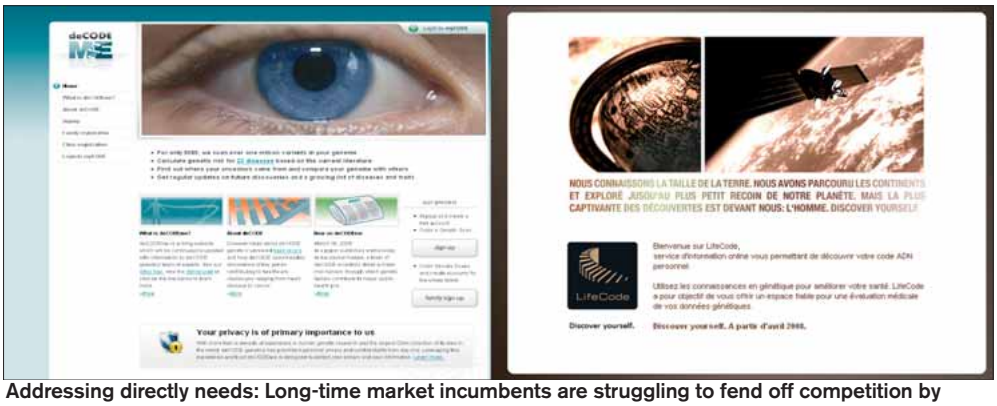
According to this report genetic services in Europe which provide patient diagnostics to the medical community have to be improved as they suffer from technical errors and poor reporting. This echos findings by other auditors like the College of American Pathologists (CAP) also active in Europe. Eurogentest's work is undisputably beneficial to the field of clinical genetic testing services, but up to now the diagnostics industry, and especially biotechnology companies working in the area neither other medical specialties besides human genetics (eg example for Germany) have not been involved in this work to a considerable extent.

Efforts near regulators appear to be justified with companies offering genetic testing direct to consumers (DtC) (Hunter et al. 2008). The Bioethics Committee of the European Council (CDBI 2007) backs up the position: «a genetic test for health purposes may only be performed under individualised medical supervision». DtC-firms offer genetic tests over the counter. They bypass doctors, or in the language of marketing: they do not use the channel of authorized dealers with medical expertise. This provokes indignation as for instance of the German Society

Doctors, Consumers, Researchers – Who is going to Order the Genetic Test



Genetic diagnostics is a sizable business for many years and with many specialties involved. The above services were all reimbursed by health insurers according to the German EMB-system.



Addressing directly needs: Long-time market incumbents are struggling to fend off competition by reputed sequencing companies for genetic testing business

of Human Genetics issuing a position statement (Feb 22, 2008) and railing against «commercial internet-based offerings for analysis of genetic SNP-variants». Indeed, personal genetic tests are ordered by individuals. There is no issue here of 'informed consent', neither question of dual-use of patient data for treatment and research. DtC-companies guarantee confidentiality and process samples only for the purpose of the order. No doubt scientific reservations on DtC-genetic tests have to be taken serious. However fierce critics on the European continent are cautioned by British collegues. Not every genetic test must pass the doctor. Some of them are quite innocuous and regulation of demand should be left to the market (see Stuart Hogarth in an interview on BioWorld Europe Online). But beyond challenging sometimes overblown claims by DtC-companies it is noteworthy that critics make a scarce effort for dialogue with firms concerned and the diagnostics industry in general. They live up to expectations of consumer and patients advocates (eg Hudson 2007) accusing a conspicuous reluctance to translate genomics in tangible public health benefits and giving consumers a larger say how they can directly benefit from publicly funded research. Companies offering genetic testing respectively their individual offerings should be thoroughly scrutinized case by case regarding the analytical validity, the clinical validity, and the clinical utility of their tests. But treating consumers as medical dopes who cannot understand genetic research findings and probability numbers interpreted to them in a responsible manner does not even support the business of clinical laboratories distributing genetic tests via doctors. Today, these testing services esp. in the major healthcare markets of Europe are under pressure to improve both the quality of their genetic tests as well as the ways they are heeding basic privacy rights of patients (see above: double-uses of patient data for research and treatment).

In continental Europe, 23andMe is serving as ('American') scapegoat to obscure the reality of a changing market for genetic diagnostics. You can find similar companies from Iceland to Malta, from Spain to Turkey, several in the large European markets. At present, some 10 companies are offering DtC-diagnostic services according to the BELISDA database. Most of these companies are not exclusively serving consumers, but in parallel doctors, academic researchers, or diagnostics resp. drug developers. They regularly have highly qualified medical experts and laboratory scientists in-house (eg Ice-

wk

deCodeme advert: «my CODE is my past – my CODE is my present – my CODE is my future»

Validity and utility claims are the same, while the distribution and target customers may be different.

landic-US deCODEme) or they work within a network of experienced specialty doctors and test distributors (eg Austrian Genosense Diagnostics). Generally speaking, the quality these companies deliver appears to be comparable at least to non-DtC-Laboratories (eg EN ISO certification). Some start-ups build their business with EU-funding. After all some of the DtC-companies are on the market for several years, and up to now we have not heard of a consumer harmed by directly receiving the results of a personal genetic test. Notwithstanding, with new technologies for genetic analyses and easier distribution of samples and test results there is a more acute need for regulatory and oversight procedures. But this should not be just tailored to individual national health systems or single stakeholders in the market, but encompass the entire sector, as the recent OECD report (2007) underlined with very practical recommendations for regulation. □

References

- Council of Europe, Steering Committee of Bioethics CDBI (2007): Draft Additional Protocol to the Convention on Human Rights and Biomedicine concerning genetic testing for health purposes. Strasbourg, September 27.
- Hudson, K. (2007): Consumer Engagement. Presentation Institute of Medicine Workshop 'Redesigning the Clinical Effectiveness Research Paradigm: Innovation and Practice-Based Approaches. Washington DC. December 13.
- Hunter, DJ, Khoury MJ, Drazen JM (2008): Letting the Genome out of the Bottle – Will We Get Our Wish? New England Journal of Medicine, Jan 10: 105-107.
- OECD (2007): Guidelines for Quality Assurance in Molecular Genetic Testing. www.oecd.org/dataoecd/43/6/38839788.pdf

Roche NimbleGen and Affymetrix Expand Licensing Agreement

Roche NimbleGen Inc., a company of Roche Applied Science, and Affymetrix Inc. announced that they have expanded the terms of the license to a number of Affymetrix patents originally granted in October 2006. The expanded license now provides certain diagnostic rights for array-based DNA copy number analysis and array-based re-sequencing in addition to covering the manufacture, use and sale of nucleic acid microarrays and related products and services in the research field. Financial details of the expanded license were not disclosed.

Roche NimbleGen is a leading innovator, manufacturer and supplier of a proprietary suite of DNA microarrays, consumables, instruments and services. Roche NimbleGen uniquely produces high-density arrays of long oligo probes that provide greater information content and higher data quality necessary for studying the full diversity of genomic and epigenomic variation. The improved performance is made possible by Roche NimbleGen's proprietary Maskless Array Synthesis (MAS) technology, which uses digital light processing and rapid, high-yield photochemistry to synthesize long oligo, high-density DNA microarrays with extreme flexibility.

www.nimblegen.com

Roche Diagnostics GmbH
Phone +49 8856 60 4830

In-process Test for Detecting Microbial Contamination in Biopharmaceutical Applications

The MilliPROBE system is the first solution of its kind for biopharmaceutical manufacturing. It combines Millipore's leading, industry-accepted sample prep methodologies with Gen-Probe's advanced, proven nucleic acid technologies to deliver both speed and sensitivity in one microbial screening tool.

The MilliPROBE system uses Real-Time Transcription-Mediated Amplification (TMA) technology to detect targeted microbial contamination within hours compared to the days or weeks usually required to generate results using traditional culture-based technology. Faster detection allows biopharmaceutical manufacturers to take corrective action earlier in the production process, which reduces downstream processing risks, optimizes product yields, and improves final product quality. These improvements may result in better operational efficiency and lower manufacturing cost for product manufacturers.

Millipore S.A.S.
39 Route Industrielle de la Hardt
FR-67120 Molsheim
Phone: +33 (0)3 90 46 99 86
Fax: +33 (0)3 90 46 91 93
virginie_isner@millipore.com
www.millipore.com

Lowering the Cost of Sequencing Human Genome

Applied Biosystems have announced a significant development in the quest to lower the cost of DNA sequencing. Scientists from the company have sequenced a human genome using its next-generation genetic analysis platform. The sequence data generated by this project reveal numerous previously unknown and potentially medically significant genetic variations. It also provides a high-resolution, whole-genome view of the structural variants in a human genome, making it one of the most in-depth analyses of any human genome sequence. Applied Biosystems is making this information available to the worldwide scientific community through a public database hosted by the National Center for Biotechnology Information (NCBI).

Applied Biosystems was able to analyze the human genome sequence for a cost of less than $60,000, which is the commercial price for all required reagents needed to complete the project. This is a fraction of the cost of any previously released human genome data, including the approximately $300 million spent on the Human Genome Project. The cost of the Applied Biosystems sequencing project is less than the $100,000 milestone set forth by the industry for the new generation of DNA sequencing technologies, which are beginning to gain wider adoption by the scientific community.

The availability of this sequence data in the public domain is expected to help scientists gain a greater understanding of human genetic variation and potentially help them to explain differences in individual susceptibility and response to treatment for disease, which is the goal of personalized medicine. Although most human genetic information is the same in all people, researchers are generally more interested in studying the small percentage of genetic material that varies among individuals. They seek to characterize that variation as either single-base changes, or as a series of larger stretches of sequence variation known as structural variants. Structural variants comprise fragments of DNA – which include insertions, deletions, inversions, and translocations of DNA sequences ranging from a few to millions of base pairs that have a higher potential of impacting genes and thus contributing to human disease.

abdirect@
eur.appliedbiosystems. com
http://europe.appliedbiosystems.com

QuadChamber™ is validated for use with 4-Plex Microarrays

Tecan has recently developed the QuadChamber™ for fully automated processing of four different microarrays simultaneously on one slide, using the HS Pro™ automated hybridization station. The QuadChamber was specially developed for use with Agilent's new 4 x 44k 4-Plex Gene Expression as well as CGH Microarrays, which consist of four individual, whole-genome microarrays printed on a single glass slide. This represents the first fully automated system that can independently handle four arrays on one slide with no cross-contamination between the arrays.

The QuadChamber provides a completely sealed environment around each of the four arrays on Agilent's 4-Plex slides, with independent channels for wash buffers, independent agitation mechanisms and drying for each of the four areas. With the new chamber, the HS Pro fully automates the hybridization of the arrays from start to finish while maintaining separate compartments around each array on the slide. The system ensures high quality processing with minimal slide handling, excellent reproducibility and bubble-free hybridization through Tecan's unique active bubble suppression (ABS™) technology.

Agilent's 4-Plex Microarrays concentrate sample hybridization, so provide enhanced sensitivity and allow the detection of even very low abundance transcripts. Having four arrays on each slide allows researchers to generate more genomics data per experiment while considerably reducing the required volumes of samples and reagents.

«Agilent and Tecan have worked closely together to develop a fully-automated solution that provides high quality data and consistently good results,» said Jeff McMillan, Agilent product manager, Genomics Automation. «Now, customers can combine

the economies of four microarrays per slide with the benefits of automated hybridization.»

Tecan Deutschland GmbH
Theodor-Storm-Straße 17
DE-74564 Crailsheim
Tel: +49 7951 94170
Fax: +49 7951 5038
e-mail: info.de@tecan.com
www.tecan.com

FRITSCH Particle Sizing

FRITSCH Particle Sizing Instruments offer state-of-the-art laser technology for an unique price performance ratio, for each particular area of application and utilization. An extra plus: the special, patented FRITSCH measuring method by laser diffraction inside the convergent laser beam. Your advantage: a simple continuous adjustment of the measuring area as well as up to now unknown number of measuring channels.

With the different systems COMPACT, MicroTec, MicroTec XT and NanoTec and the possibility to combine corresponding components, you can configure a measurement system that is precisely adapted to your needs, with reliability and efficiency guaranteed by FRITSCH as a specialist in particle measurement technology.

With the ANALYSETTE 22 COMPACT for example, FRITSCH provides a solution for sample materials in the particle size range from 0.3 to 300 µm who require a particularly easy-to-use bench model instrument with an extremely attractive price-performance ratio.

With its flagship NanoTec for example, FRITSCH offers the option of shape analysis as well as a measurement range from 2000 µm down to 0.01 µm – the entry into the nano range.

Catch up on the state-of-the-art laser technology by FRITSCH.

Please visit us at Analytica, hall B2, booth No. 205/304

FRITSCH GmbH
Manufacturers of Laboratory Instruments
Andrea Köhler
Industriestrasse 8
55743 Idar-Oberstein • Germany
Phone: +49 67 84 70 146
Fax: +49 67 84 70 11
E-Mail: koehler@fritsch.de
www.fritsch.de

Comprehensive Antibody Characterisation

Guava Technologies, Inc., a leading developer of easy-to-use, on-demand, benchtop cell analysis systems,

provides new levels of flexibility and analysis with its Guava RapidQuant™ IgG Assays for quantification of mouse and human antibodies.

Used in combination with the Guava Express®, Guava ViaCount® and other Guava® Assays the new Guava RapidQuant IgG Assays provide unmatched antibody characterisation on a single cellular analysis platform – the Guava EasyCyte™ Plus™ System.

With their simplicity and ease of use, the new microplate-based Guava RapidQuant™ IgG Assays have raised the bar for IgG titering in tissue culture supernatants. Designed to be time-saving, labour-saving and money-saving, RapidQuant Assays eliminate the most tedious portions of antibody titering methods. The no wash RapidQuant Assays are bead-based and rely on sensitive fluorescence detection for the quantitative measurements. Standard curves are automatically produced by the easy to use software interface, removing the need for time consuming calculations performed by the user during analysis. The fluorescent intensity is proportional to the concentrations of antibody captured on the beads. Using fluorescence provides tremendous reliability and precision so you can have confidence in every result. RapidQuant Assays have been tested extensively with human and mouse antibodies of all IgG subtypes and in many types of hybridoma media and have performed well in all cases tested. RapidQuant Kits are extremely specific for IgG and are not hindered by the presence of FBS or IgM in the media. The RapidQuant Assay can be used to determine absolute concentrations of antibody present in cell culture medium or in purified preparations by comparison to a standard curve. Results from this assay can also be combined with results from the Guava Express Assay to determine relative antibody affinities.

The Guava ViaCount® Assay has become a leading standard for viability and cell counting. Used in laboratories characterising antibodies the ViaCount Assay provides a rapid and reliable assessment of the cell concentration and viability of clones for production. Combining results from the RapidQuant and ViaCount Assays delivers the benefit of simultaneous assessment of IgG production and cell health parameters.

The complete antibody characterisation suite from Guava Technologies extends your capabilities for assessing target activity of antibody clones, or identifying cell populations that express a specific protein marker. For example, the Guava Express® Assays provide superior sensitivity and excellent discrimination between cells expressing low and high levels of antigen recognised by the antibody and may also be used to obtain information on specificity of antibodies in the screening steps. The Guava Nexin® and Guava® Cell Toxicity Assays deliver reliable results concerning

the potency of the antibody to mediate apoptotic induction and cell killing, respectively. Using the RapidQuant, Express and other Guava Assays provides a highly specific and quantitative assessment tool for obtaining multiple layers of information about your antibodies.

Optimised for use with Guava® microcapillary flow cytometry systems, all Guava Assays, including RapidQuant, Guava Express and ViaCount, require very small volumes of sample for accurate antibody characterisations enabling multiple analyses of each supernatant to be performed from a single 96-well plate.

Faster and easier than the average enzyme-linked immunosorbent assay (ELISA), the traditional approach for antibody quantification, the Guava RapidQuant IgG Assays efficiently quantify the amount of antibody present. When coupled with the suite of other available microplate-based Guava Assays more comprehensive data on antibodies can be generated enabling users to conveniently and productively select optimal clones from hybridoma screens.

Guava Technologies Inc.
Unit 3, Southview Business Centre
Tinwell Road
Stamford PE9 3UA, UK
www.guavatechnologies.com

Labnet's gradient thermal cyclers and shakers

Appleton Woods Ltd., supplier of a comprehensive range of quality laboratory equipment and consumables for over 40 years, has entered the fifth year of its exclusive agreement with Labnet International, Inc., for the UK distribution of Labnet's gradient thermal cyclers and platform shakers.

Labnet's new Multigene™ Gradient thermal cycler offers affordable and easy-to-use gradient technology in a compact, lightweight unit. The Multigene Gradient is extremely simple to programme and can operate with a uniform temperature across the block, or a gradient with up to 12 temperatures in one run. Appleton Woods also supplies a wide range of PCR plasticware that is perfectly suited for use with the Labnet Multigene Gradient thermal cycler.

The Labnet Orbit™ range of platform shakers provides a variety of options to shake or vortex tubes and plates. Multipurpose versions with interchangeable platforms to accommodate bottles, flasks and tube racks are also available. The VorTemp™ range can simultaneously shake and incubate samples while the Orbit LS low speed shaker is ideal for washing fragile gels. A variety of Labnet instruments are also available for rocking, reciprocal shaking and 3D shaking.

Appleton Woods Ltd.,
Lindon House,
Heeley Road, Selly Oak,
Birmingham,
B29 6EN
www.appletonwoods.co.uk
sales@appletonwoods.co.uk

Miniature, back-thinned FFT-CCD detector spectrometers

For applications requiring high sensitivity, good UV response and wide dynamic range, Ocean Optics has introduced the Maya2000-series high-sensitivity back-thinned 2D FFT-CCD spectrometers. Two new spectrometers, the Maya2000 and Maya2000 Pro, were designed to offer good value and performance for applications not requiring long integration times.

Particularly well suited to low light-level, UV-sensitive applications, such as fluorescence, DNA sequencing and Raman analysis, the Maya2000 and the Maya2000 Pro offer greater than 90% quantum efficiency, high dynamic range and excellent UV response.

Both spectrometers feature a USB 2.0 interface, low-noise electronics, 14 grating options, and a detector collection lens option for enhanced signal collection. Fully programmable strobe signals allow for the selection of single or continuous strobes to suit application needs. The Spectrometers are controlled with Ocean Optics' completely modular, Java-based SpectraSuite software (Windows, Mac OS and Linux compatible).

While the Maya2000 and Maya2000 Pro offer similar performance for most parameters, the Maya2000 has a slightly faster readout time while the Maya2000 Pro provides a better dynamic range and signal-to-noise ratio.

Ocean Optics
Geograaf 24
NL-6921 EW Duiven
Tel: +31 (0)26 319 05 00
Fax: +31 (0)26 319 05 05
Jessica.vanHeck@oceanoptics.eu
www.OceanOptics.eu

Versatile Media Preparation System Fulfils The Needs Of Nematode Research

Using a MEDIAJET vario adapted to work with 35mm petri dishes, supplied by INTEGRA Biosciences AG, the FMI Institute for Biomedical Research in Basel, Switzerland has established an effective technique for automated production of thousands of agar plates on which to cultivate the nematode *C. elegans*.

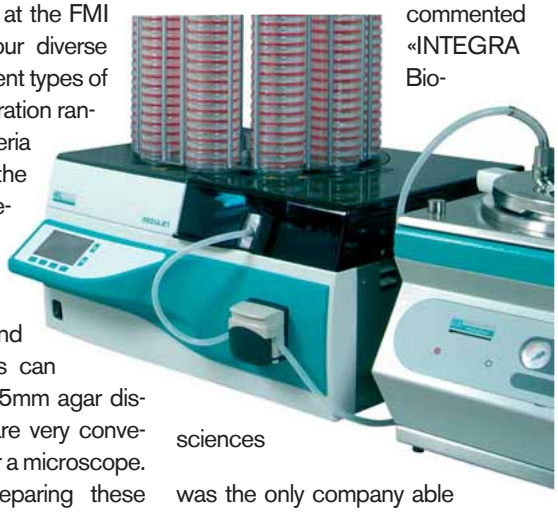
The nematode *C. elegans* has long been a popular geneticist's tool. The simplicity, transparency and speed of its biological functions have made *C. elegans* an ideal model organism for studying genes and their function.

The FMI is internationally recognised as a centre of excellence in biomedical research with a strong record of innovation in the molecular biology of disease. Supported by the Novartis Research Foundation the goal of the FMI is to exploit new technologies to further its understanding of the basic molecular mechanisms of cells and organisms in the fields of epigenetics, growth control and neurobiology.

Mathias Müller, Head of the Media Preparation Laboratory at the FMI commented «at FMI our diverse research requires different types of agar plate media preparation ranging from growing bacteria to the cultivation of the model organism C.elegans». He added «we perform large numbers of screenings with C.elegans worms and therefore our scientists can require thousands of 35mm agar dishes, as these dishes are very convenient to work with under a microscope. However manually preparing these agar dishes has been more than cumbersome and very time consuming».

To address these challenges, INTEGRA Biosciences in collaboration with FMI, adapted a MEDIAJET vario to provide users with the versatility to fill 35, 55/60 and 90/100 mm Petri dishes on the same system. With a compact footprint of only 70 x 70 cm the adapted MEDIAJET vario fits conveniently onto a laboratory bench and is capable of automatically producing 900 filled petri dishes per hour. Converting the system between two different plate types takes only minutes.

Reflecting on the new system, Mathias Müller further commented «INTEGRA Biosciences



was the only company able to adapt their petri dish filler according to our needs. The MEDIAJET vario system uniquely provides us with the versatility we sought as well as saving us time through automating the production of 35mm agar plates for cultivation of *C.elegans* worms».

INTEGRA Biosciences
CHUR, Switzerland
Tel: + 41 81 286 9530
info@integra-biosciences.com
www.integra-biosciences.com

The Only Confocal Microscope that Adapts to the Sample

The pioneering new confocal microscope Leica TCS SP5 X with supercontinuum laser provides freedom and flexibility which were unattainable until now in fluorescence microscopy and allows true optimization of results. The supercontinuum laser is a groundbreaking innovation which now allows the researcher to choose any excitation line within the continuous range of 470 to 670 nm. The Leica TCS SP5 X precisely adapts to any existing or future fluorescent dye. Optimal adjustment of the excitation line to the sample – in 1 nm increments – reduces cross-excitation and minimizes sample damage. The Leica TCS SP5 X allows multi-color excitation with up to eight excitation lines simultaneously.

All broadband capabilities of the Leica TCS SP5 AOBS are included in this pacesetting confocal system. The patented Spectral Detection Technology of Leica Microsystems provides complete freedom in choosing any detection area in up to five truly spectral confocal PMT channels. The acousto-optical beamsplitter (AOBS®), a groundbreaking invention from Leica Microsystems, ensures highest sensitivity and provides a far better transmission than any dichroic mirror. The AOBS® and the supercontinuum laser are a perfect match and open new doors in confocal microscopy applications.

Leica Microsystems CMS GmbH
Am Friedensplatz 3
Fax: +49(0)621/7028-1028
DE – 68165 Mannheim
Tel.: +49(0)621/7028-2801
www.leica-microsystems.com

HPLC for simultaneous measurement of cations and anions

ESA Biosciences, Inc., a Magellan Biosciences company, has announced a simple and reliable HPLC method for simultaneous measurement of cations and anions, using its award-winning Corona CAD universal detector. Since positive ions (cations) and negative ions (anions) are key components of everything from pharmaceuticals to food and beverages to fine chemicals, analysis of these atoms and molecules is a critical step in product development and QA/QC. The new Corona CAD method improves results for users: it is more efficient than traditional techniques, since it measures anions and cations in a single run, and it is also more accurate, reliable, and cost-effective.

«The credit for this novel ion-analysis method actually goes to one of our long-standing Corona CAD users – a scientist in the analytical research and development department of one of the world's leading pharmaceutical companies,» said Dr Jasmine Gruia-Gray, ESA's vice president of life sciences marketing. «After successfully developing, validating, and transferring a number of other Corona CAD methods to facilities around the globe, he decided to turn his attention to a difficult analysis challenge, ion measurements. The separation and quantitation of counterions (cations or anions associated with the drug) in the pharmaceutical industry is an important determination. During drug development, the selection of the correct salt form early in the development process can prevent repeating toxicology, biological, and stability studies down the road. In addition, during pharmaceutical manufacturing, ion measurements of the active pharmaceutical ingredient are a routine part of the QA/QC process to ensure the safety, identity, strength, purity, and quality of the material.»

The traditional method for ion measurement uses dedicated, single-purpose ion chromatography (IC) systems, which must be run by highly skilled operators with specialised reagents under tightly controlled conditions. In IC, conductivity detection is used with a suppressor to reduce the bakkground signal. Separate equipment (exchange columns and suppressors) is required to measure cations and anions. This can be cumbersome and time-consuming. The cost of analysis is high: the use of resources – both human and instrument – is inefficient, and the quality of the data generated can lack the necessary accuracy and precision required.

Dr. Gruia-Gray continued, «Our Corona CAD user came up with an elegant solution combining the CAD's universal detection with hydrophilic interaction chromatography (HILIC).» The beauty of the CAD method is that it is applicable to both the pharmaceutical development laboratory and to the QA/QC process. And easy method validation and transfer is a hallmark of the Corona CAD. The Corona CAD-based method allows simultaneous analysis of anions and cations and exceeds the performance of IC with regard to measurement accuracy and reproducibility. The HILIC chromatography separates both anions and cations from complex samples for easy and reliable detection by the Corona CAD.

«We confirmed the ion-analysis method in our own ESA application laboratories, and have fine-tuned it for use in the pharmaceutical, food and beverage, and fine chemical industries. The Corona CAD method has attracted significant attention by scientists who have been using IC methods reluctantly for lack of an alternative: the Corona CAD method enables them to perform ion analysis quicker, more efficiently, more cost effectively, and with overall better results.»

Equally suited to the methods-development laboratory and the factory floor, ESA's Corona CAD system reliably measures compounds, independent of molecular structure, to low-nanogram levels. Charged Aerosol Detection [CAD] is a robust HPLC detection technology that delivers advanced capabilities to every HPLC-user lab. Besides excellent sensitivity, the Corona CAD shows consistent inter-analyte response independent of chemical structure, enabling quantitation across a range that exceeds four orders of magnitude. This breakthrough detector can effectively analyse a wide diversity of chemical structures and important classes of molecules – from small organic molecules, proteins and peptides, to ions, carbohydrates, lipids, and polymers. The Corona CAD makes methods development, validation, and transfer fast and easy for applications in the pharmaceutical, chemical, polymer, food and beverage and oil/petrochemical industries.

Please visit Analytica
Stand A2:465

ESA Biosciences
Phone: +44-1844-239381
email egoodall@esainc.com

2008 I APRIL

April 1 – 4
Munich (DE)
analytica 2008
www.analytica-world.com

April 2 – 4
Barcelona (ES)
Pharma Forecasting Excellence
www.eyeforpharma.com/forecasting08

April 3 – 4
Florence (IT)
EBSA Conference 2008
www.ebsaweb.eu/EBSA_11

April 7 – 9
Madrid (ES)
BIO-Europe Spring
www.edbgroup.com/bes

April 7 – 10
Barcelona (ES)
6th World Meeting on Pharmaceutical Technology
www.worldmeeting.org

April 12 – 16
Alexandria (EG)
BioVisionAlexandria
www.bibalex.org/bva08

April 16 – 17
Maastricht (NL)
Biomdica – The Life Science Summit
www.biomedica2008.com

April 21 – 24
Vienna (AT)
BioProcess International European Conference & Exhhibition
www.bpi-eu.com

April 22 – 23
Zurich (CH)
European Life Science CEO Forum
www.sachsforum.com

April 27 – 29
Dubai (UAE)
Pharmaceutical & Biotechnology Middle East
www.pabme.com

2008 I MAY

May 13 – 15
London (UK)
European Stem Cells & Regenerative Medicine Congress 2008
www.terrapin.com

May 15 – 17
Lodz (PL)
Bioforum 2008
www.bioforum.pl

May 19 – 21
Philadelphia (USA)
Biomarker World Congress 2008
www.biomarkerworld-congress.com

May 22 – 23
Wädenswil (CH)
BioTech 2008 & 4th Swiss-Czech Symposium
www.biotech2008.ch

May 27 – 29
Jönköping (SE)
World Bioenergy 2008
www.worldbioenergy.se

2008 I JUNE

June 8 - 11
Naples (IT)
IBIC – Industrial Biotechnology International Conference
www.aidic.it/IBIC2008

June 17 – 20
San Diago (USA)
BIO 2008
www.bio2008.org

2008 I JULY

July 2 – 4, Tokyo (JP)
BIO FORUM & BIO EXPO japan
www.bio-expo.jp/english

2008 I SEPTEMBER

Sept 7 – 10, Faro (PT)
7th European Symposium on Biochemical Engineering Science
www.esbes2008.org

Sept 16 – 19
Geneva (CH)
bioLOGIC Europe 2008
www.terrapin.com

Sept 21 – 24
Baden-Baden (DE)
ISPPP 2008
www.dechema.de/isppp2008

Sept 22 – 24
Heiligenstadt (DE)
Technische Systeme für Lebenswissenschaften
www.iba-heiligenstadt.de

Sept 23 – 24
Zurich (CH)
Biotech in Europe Investor Forum
www.sachsforum.com

Sept 23 – 25
Copenhagen (CH)
Biotech Forum / ScanLab
www.biotechforum.org

2008 I OCTOBER

Oct 1 – 2
Milano (IT)
BioForum 2008
www.bioforum.it

Oct 1 – 3
Québec (CA)
BioContactQuébec 2008
www.biocontact.ca

Oct 6 – 9
Lyon (FR)
World Vaccine C ongress 2008
www.terrapin.com

Oct 7 – 9
Paris (FR)
EuroBIO 2008
www.eurobio2008.com

Oct 7 – 9
Hannover (DE)
Biotechnica / European BioPerspectives 2008
www.biotechnica.de / www.bioperspectives.org

Oct 14 – 16
Basel (CH)
MipTec 2008
www.miptec.com

2008 I NOVEMBER

Nov 17 – 19
Mannheim/Heidelberg (DE)
BIO-Europe 2008
www.edbgroup.com/bioeurope

2008 I DECEMBER

Dec 3 – 5
Lyon (FR)
European Antibody Congress 2008
www.terrapin.com

2009 I JAN. - JULY

May 11 – 15
Frankfurt a.M. (DE)
ACHEMA 2008
www.achema.de

IMPRINT

Volume 4, 2008

Publisher
Serge Perriard, BioTalk GmbH

Internet
www.bioworld-europe.com

Editorial Staff
Wolf G. Kroner, Munich, (wk, responsible)
Micheal Lindenmaier (ml)
Gary O. Martini (gm)

Editorial Office
BioTalk GmbH
Kohlrainstrasse 10
CH-8700 Küsnacht
Telefon +41(0)44 912 03 05
Telefax +41(0)44 912 03 17
ISDN +41 (0)44 912 03 21
E-Mail: press@bioworld-europe.com

Advisory Board
Dr. Reinhard Glück, Swiss Biotech Association (SBA)
Prof. Dr. Peter Stadler, Deutsche Industrievereinigung Biotechnologie (DIB)
Dr. Charles Woler, France Biotech
Dr. Stephen C. Taylor, UK bioProcessUK

International Advertising
Beat Nik Hug, BioTalk GmbH
Telefon +41(0)44 912 03 05
Telefax +41(0)44 912 03 17
E-Mail: b.hug@biotalk.biz

Subscription and Events
Beat Nik Hug, BioTalk GmbH
Telefon +41(0)44 912 03 05
Telefax +41(0)44 912 03 17
E-Mail: subscribe@bioworld-europe.com

Circulation
17'500 copies

Subscription Rate

Europe	60.– EURO
Rest-of-World	80.– EURO

ISSN Number
ISSN 1661-741X

Graphic Design BioTalk GmbH

Printed at
Vogt-Schild/Habegger Medien AG, Switzerland

"Never thought that real-time PCR could be so simple!"

Remarkably simple system. Simply remarkable results.

Take your first step into real-time PCR with the affordable 48-well StepOne™ System, and you'll be surprised at how easy high performance real-time PCR can be! Take the second step and upgrade to the 96-well StepOnePlus™ System, whenever you are ready.

StepOne ... a step above the rest!

- High performance real-time PCR
- Remarkably simple to use
- Fast PCR (<40 minutes)
- Full range of applications

You will be even more surprised to hear our competitive prices! info.applied biosystems.com/stepone

StepOne System

- Surprisingly affordable
- 48 wells for lower throughput demands
- 3 colours for basic research applications
- Easily upgradeable to the StepOnePlus System

StepOnePlus System

- 96 wells for higher throughput demands
- 4 colours for greater versatility
- Same easy to use software as the StepOne System
- VeriFlex™ technology – annealing temperature flexibility

It's remarkable how simple high performance real-time PCR can be view our free software demonstration at: info.appliedbiosystems.com/steponeplus

Applied Biosystems