JUNE 2014 # 17

the Analytical Scientist

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Online this Month

Speak Up!

(tas.txp.to/0614/respect)

I support what this author has said [about equality for women]; disappointingly, not much has changed in the past 30 years in science. If you notice subtle discrimination against anyone, simply speak up and let the offender know that it's not OK. That's how we can change this. – *Margaret Coe, Technician/Engineer, New Zealand.*

Hear, hear!

(tas.txp.to/0614/toolbox)

Good comment. I can't count the number of times I'm asked to move an analytical method from the tried-an-true LC-UVD to UPLC-MS/MS. Why? To make it faster and more sensitive (and more difficult!). There has to be a good reason to change the test procedure and go through all the new validations, accreditations and expenses. Until I hear those reasons, I'm not interested. Multi-analyte, ppb levels, large sample numbers ... then I'll listen. A single high-dose pharmaceutical - give me a break! – *David Woollard, Senior Chemist/Scientist New Zealand.*

Sign up online for free to have your say: *theanalyticalscientist.com/subscribe*

Tea With Rich

Seriously, what's better than a nice cup of tea and a chat? Whilst at Riva 2014 (the 38th ISCC and 11th GCxGC Symposium), Rich Whitworth, editor of The Analytical Scientist, invited three participants of the conference to take part in an exciting new video project: "Tea With Rich", an ongoing series of informal interviews with key analytical scientists in glorious settings around the globe.

And who would be a more fabulous first interviewee than the Chair of Riva 2014 himself, Luigi Mondello? Set in the gardens of a beautiful old villa in Riva del Garda, Italy, our first video sees Luigi thanking Pat Sandra for the wonderful toy (Riva) and introducing his latest grand research project. Not to mention camera drone footage over the lake…

Watch the video now: *tas.txp.to/0614/teawithluigi*

Contributed Content

Given our fast-growing online community of readers, we have now introduced a new style of content, which is brought to you courtesy of our "Manufacturer Channels".

Malvern is the first to take advantage of the new platform and offers up a quartet of articles that cover everything you need to know about the use of light scattering detectors in gel permeation chromatography (GPC) and size exclusion chromatography (SEC).

You can read the first article "When only a MALS detector will do...", here: *tas.txp.to/0514/cc001w*

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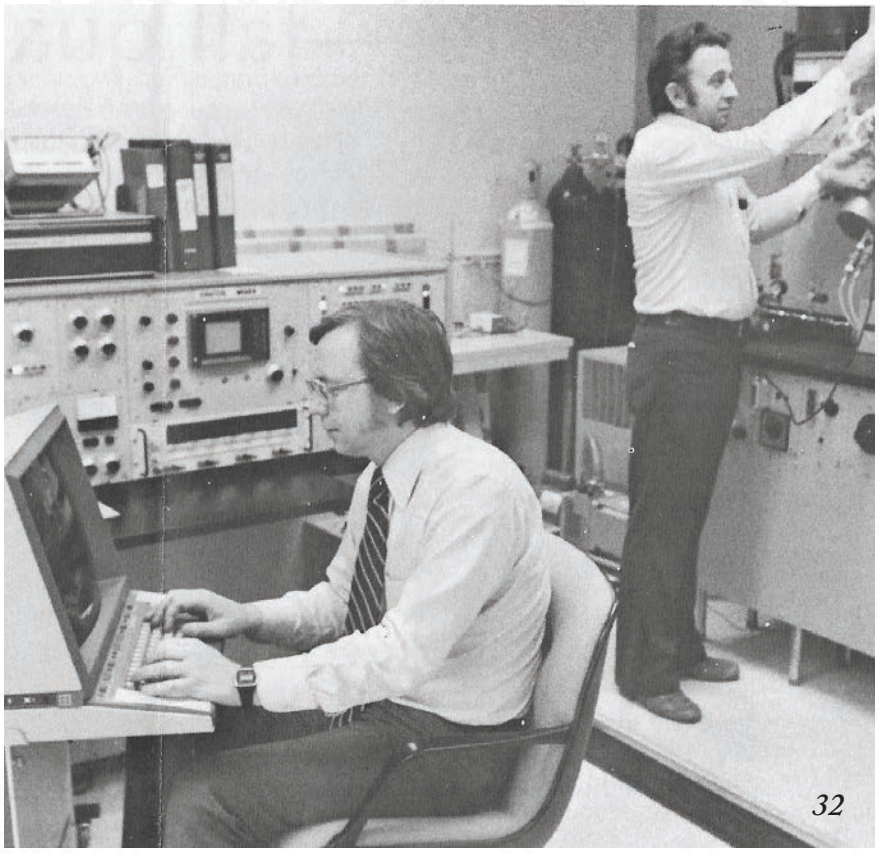
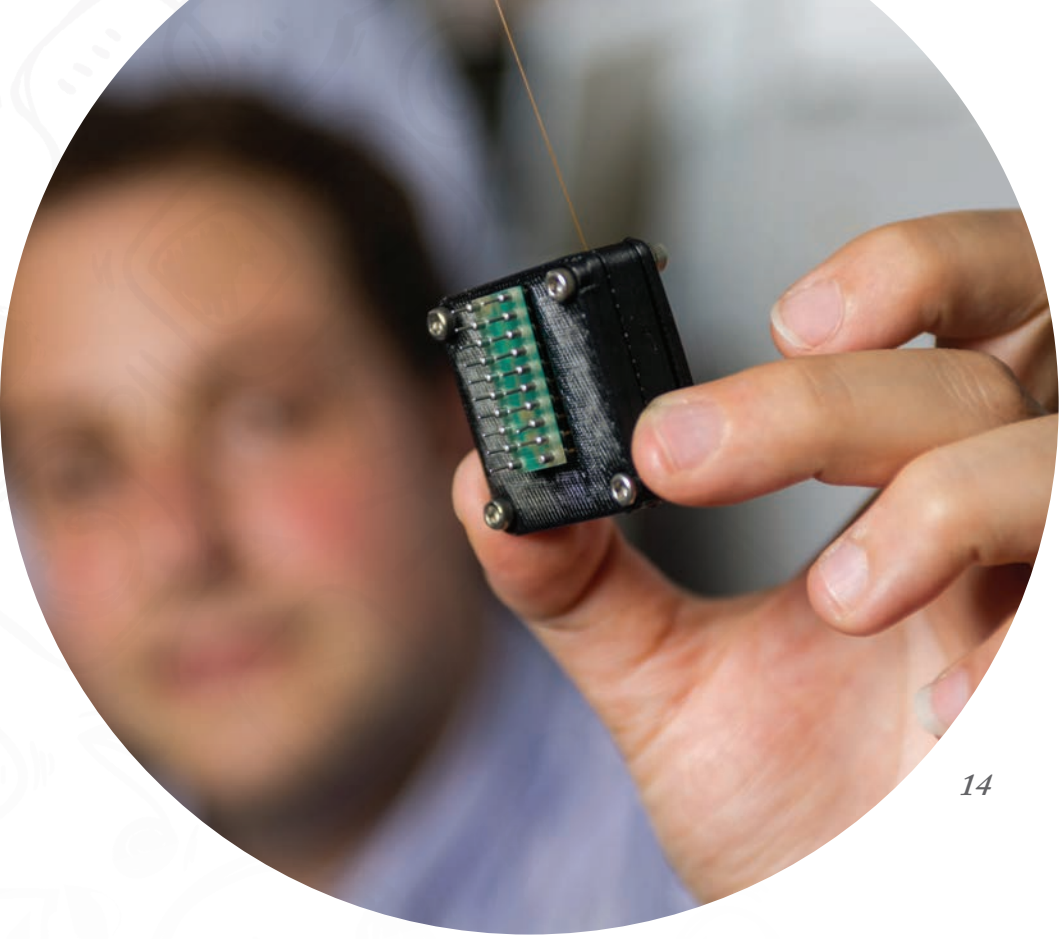


On The Cover

Students raise their hands in favor of the "Flipped Classroom" – only made possible by modern technology.

Upfront

In My View



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the Analytical Scientist

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Lagom är bäst in the Lab

When and where should we apply the concept of "just enough is best"?

I use an iPod Classic that's about 10 years old. On a recent flight out of San Francisco, it was passed around the cabin by astonished fellow passengers who treated it like a relic – and me like a time traveler.

This incident caused me to do some soul-searching, from which I concluded that while I am not a Luddite – I don't oppose technological development – I am definitely not a technophile either. Products that I consider to be needlessly over-specced mildly irritate me (for example, I don't want to watch movies on my phone).

My mindset can be best described by the Swedish phrase "lagom är bäst," which essentially means "just enough is best." If you have ever visited Sweden, you will know that it is (generally) relaxed, low-key and with an emphasis on harmony. It is a little at odds with my adopted country's general desire (for the most part) for more of everything.

One alluring thread that has run through this magazine's content in recent months is the emergence of a "lagom är bäst" approach to the development of products in the analytical sciences. For example, a couple of issues ago, George Whitesides stated that "while complexity can be beautiful, simplicity works better". Further support for that view can be found in a pair of stories on fiber-based diagnostic devices on page 12.

Last month, Hans-Gerd Janssen argued against the need for new methods that focus only on increased sensitivity and resolution. "Performance should be fit-for-purpose and not necessarily a World Record attempt," he said.

Ben Potenza's article on recycling equipment (page 19) is a variation on the same point. Why get the newest, more advanced version of a piece of kit, if the previous generation does the job you need at a much lower price – and in a more sustainable fashion?

Yes, lagom är bäst in the lab works well… so long as you know what you are looking for. Two articles in this issue illustrate where it breaks down. One is John McLean's vision of data-driven discovery, where the more data we acquire, the more knowledge we can gain. The other is Ian Jardine's feature on the development of mass spectroscopy tools over a quarter century. By definition, the development of fantastic new technologies requires innovation that lies outside the comfort zone of what we know.

My point is that, where it makes sense, the concept of "just enough is best" should be applied. But that's not everywhere.

Also, "just enough" is a moving target. I can't watch "Tea with Rich" on my iPod, but I've just remembered that I can on my phone.

Richard Gallagher
Editorial Director

Contributors:

John A. McLean

"Throughout my childhood I was curious about the nature of things, but more in areas like economics and political science. When I was in my 20s, a persuasive series of teachers and mentors lit my passion for chemistry." McLean began his research career in plasma spectrochemistry and later moved into biological mass spectrometry where he and his group colleagues perform research in instrumentation construction for application areas in biology and medicine. "There are few more exciting things in life than working with enthusiastic student colleagues and aggressively asking questions that can change how we think about the world around us."

John emphasizes the need for data-driven discovery on page 17.

Janice Manzi Sabatine

From her days as a PhD student in biochemistry to her current role as an executive coach, Janice Manzi Sabatine has been intrigued by how important interpersonal skills are to success. "I saw my colleagues in non-technical fields receive management training and leadership development, and resented that those of us in the sciences and medicine, particularly in academia, did not receive those same benefits." To address that inequity, she became a certified executive coach, founded Avanti Strategies, and now provides this much-appreciated service to her technical colleagues.

Janice helps us tackles our "immunity" to change on page 40.

Bill Anderson

Bill Anderson couldn't decide between analytical chemistry and biochemistry for his PhD at the University of Cincinnati, so he did both. That (in)decision led him to an academic career at Hampden-Sydney College in Virginia (preceded by a decade at Duke University, the University of the Virgin Islands, and Liaoning Normal University). "My research interests have been in the electrochemistry of biological systems ever since realizing that the human body runs on almost 100 amps, just based on the oxygen reduction we do."

Bill explains why he is wearing an EEG headset on page 16.

Ian Jardine

"For the past 25 years, one person has stood out as Thermo Fisher's greatest champion of innovation," according to CEO Mark Caspar. That person is Ian Jardine. In presenting Thermo Fisher Scientific's 2014 Lifetime in Innovation Achievement Award to Jardine, Casper described his "infectious passion for science," and his "uncanny ability to predict what customers would need long before they knew themselves." This publication also benefited from that insight: Ian served on the Editorial Advisory Board from pre-launch days up until this issue.

Marking his retirement from Thermo and from The Analytical Scientist, Ian Jardine discusses his life and times in the development of mass spectrometry on page 32.

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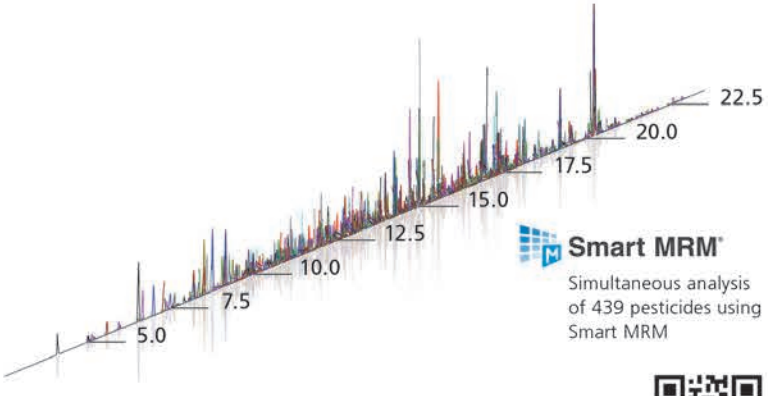

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Upfront

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Mapping the Human Proteome

Team one: ProteomicsDB catalogues more than 18,000 human proteins – or 92 percent of the human proteome

In February 2001, scientists celebrated the initial sequencing of the human genome by two competing groups. Now, not one but two draft human proteomes form the next logical link in a chain. Bernhard Kuster, who led the Technische Universitat Munchen (TUM) research team's effort (1), says that the initial spark of inspiration for the work was a desire to generate a database that could potentially have a big impact on proteomics. "A common problem in science is the sheer amount of data produced. Unfortunately, we do not always have the capabilities or the right tools to mine this information," explains Kuster. "This project was the result of a joint venture between TUM and the software company SAP – technology was key to developing an intellectually clear, useable database."

Kuster and colleagues compiled raw mass spectrometry data from various public databases, as well as generating their own data from human tissue, bodily fluids and cancer cell lines to help fill the gaps. Overall, the team catalogued more than 18,000 human proteins, which is about 92 percent of the human proteome – the work clearly isn't finished yet. The group intends to continue profiling human tissue and will also be using the data to examine how cancer drugs interact with proteins and signaling pathways.

The data has been made publicly available in ProteomicsDB, a "high-performance, in-memory database for real-time analysis of terabytes of big data," which includes information on the types, distribution and abundance of proteins in various cells, tissues and bodily fluids.

"Proteomics and genomics aren't science fiction anymore," says Kuster, "We have shown that it can be done. Many advances were made after it was demonstrated that the human genome could be mapped, so we hope our work will spur other researchers into action, benefiting the field of proteomics." *SS*

Parallel Proteomes (below) explores the efforts of the second team of researchers at Johns Hopkins University in Baltimore, US, and the Institute of Bioinformatics in Bangalore, India.

Reference

1. *Mathias Wilhelm et al., "Mass-spectrometry-based Draft of the Human Proteome", Nature 509, 582–587 (2014).*

Parallel Proteomes

Team two: MS data offers 84 percent coverage of the predicted human proteome and presents 193 new proteins not predicted by genomic data

While Bernard Kuster and his colleagues were compiling ProteomicsDB, a separate team of researchers at Johns Hopkins University, Baltimore, USA, and the Institute of Bioinformatics, Bangalore, India, tackled the challenge in a different way, using a single mass spectrometry platform and data analysis pipeline to generate data (1). Their study identified proteins encoded by about 84 percent of all the genes in the human

genome predicted to encode proteins, plus 193 proteins that geneticists had not predicted. Here, two of the authors of the study – Akhilesh Pandey, Johns Hopkins professor and founder and director of the Institute of Bioinformatics, and Harsha Gowda, a scientist at the same institute – discuss the project.

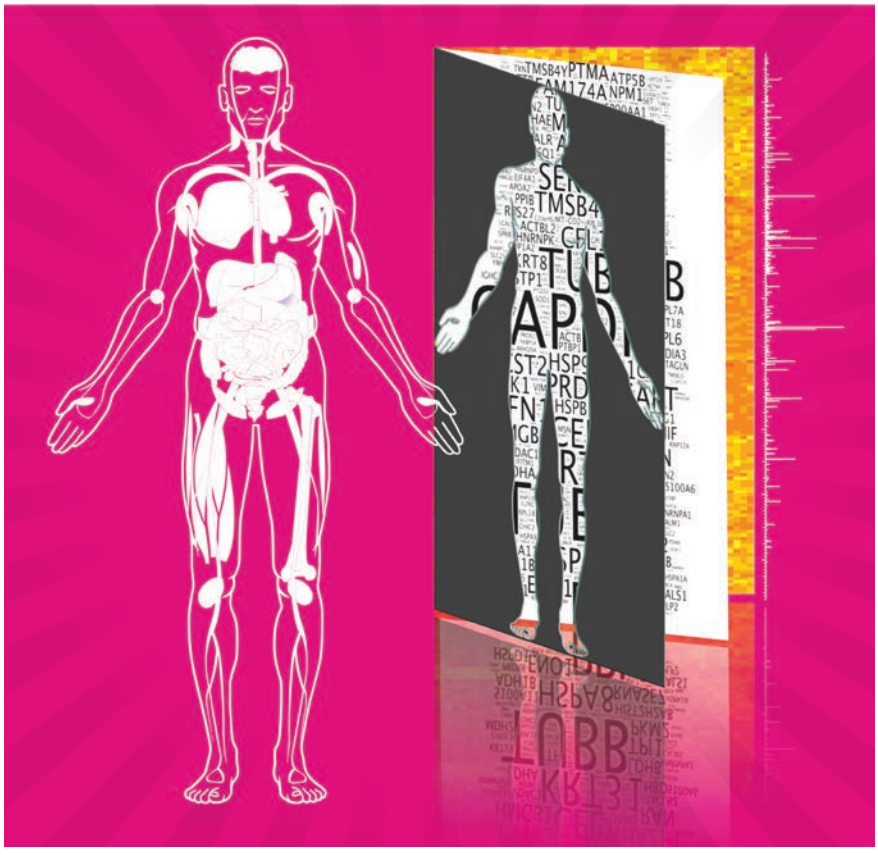
Why take on such a big challenge?

One of the main goals of the Human Genome Project was to discover all the protein-coding genes in humans, but this was not satisfactorily achieved after the completion of the project. Although many biomarkers and therapeutic targets used in clinics are proteins, all the protein estimates and their sequence were largely derived using indirect methods without using any technology or platform that directly monitors proteins. Advances in mass spectrometers and our vast experience and familiarity with proteomic methods and the human proteome prompted us to take up this study.

Another driving factor was that we knew this was important to the proteomics community, but realized that scientists were trying to figure out the 'best' way to accomplish it. We took the most straightforward approach of using mass spectrometry. Finally, although there is a lot of focus on trying to understand disease, we have been frustrated by the lack of understanding of what is normal in the first place, without which we cannot hope to understand what is abnormal.

How does your study compare with the work led by Bernhard Kuster?

Actuallly, we were not aware of a parallel effort by Kuster and colleagues to map the human proteome until three days before the publication in Nature. It was a surprising coincidence! The datasets are complementary and the research community now has the advantage of referring to multiple lines of evidence for each protein from independent laboratories. It will be interesting to compare our datasets to extend our understanding of the human proteome.



H. Habne/TUM

What did you hope to achieve?

One of the main aims was to obtain experimental evidence for most of the annotated human proteins in public databases. In the process, we wanted to generate high quality MS dataset for the human proteome along with a baseline protein expression pattern for histologically normal human tissues. We were also clear from the beginning that we should carry out proteogenomic analysis. This was important for us to demonstrate that the traditional way in which we analyze proteomic data may be missing a lot of information simply because of the limitations of existing protein databases.

What were the big challenges?

The first challenge was simply to get sufficient instrument time. The second challenge was to deal with the large amounts of data generated. Analyzing vast amounts of data is computationally expensive, so we had to figure out efficient ways to achieve our goals with minimum resources; there was a significant manual analysis component. If we had to rely on specific funding for this project, we would probably still be waiting. Instead, we had to be ingenious, using funds that were for technology development.

Next steps?

The identification of several novel proteins that are not part of existing protein databases hints at the possibility of an expanded human proteome that has evaded detection. Further exploration is needed in that area. Post-translational modifications and alternatively spliced forms further add to the complexity – in that sense, there is much more work to be done before we can say we have a truly complete map of the human proteome.

Reference

1. *Min-Sik Kim et al., "A Draft Map of the Human Proteome", Nature 509, 575–581 (2014).*

Crafty Diagnostics

Could a simple flower-shaped paper-based assay be the answer to an undiagnosed hepatitis C pandemic?

Courtesy of Xuan Mu

Xuan Mu, assistant professor at the Institute of Basic Medical Sciences at Peking Union Medical College, China, was shocked to discover the "astonishing circumstance" surrounding global hepatitis C virus (HCV) infections. "The majority of virus carriers are simply unaware of their infections – and millions of people at risk (like baby boomers) are recommended to receive HCV screening tests," says Mu. "We believe that diagnostic advances are crucial to address the 'silence' of the HCV pandemic." With that in mind, Mu and colleagues set out to use advances in paper-based microfluidics and a flower-shaped craft tool to tackle the issue (1).

Those who read George Whitesides' feature in the April issue (tas.txp.to/0614/whitesides) will already realize the potential of simple and cheap diagnostic tools. "Certainly, we were inspired by previous paper-based analytical devices from the Whitesides Group," says Mu, "The unique insights of George Whitesides for patterning paper and stacking it in a 3D manner will undoubtedly launch a new era of paper-based analysis. And we feel fortunate to be involved in this trend."

Not only is paper cheap, it can also be appropriately patterned to create the specific zones required for the lengthy and segmented HCV diagnostic, which uses an enzyme-linked immunosorbant assay (ELISA) for screening and a recombinant immunoblot assay (RIBA) for confirmation. However, patterning the paper also poses a challenge. Most conventional patterning methods use high temperatures and it is therefore unwise to use them with highly flammable paper, such as nitrocellulose – one of the few types of paper that can efficiently bind proteins.

"The idea of using scrapbooking tools or craft punches came to me by chance. My original concept was to combine paper origami and scissors to pattern complicated features on paper," says Mu. "But when I searched online to learn how to easily cut a curved line on folded paper, I chanced upon a craft punch that could do the job far more reproducibly. I soon realized that this scrapbooking tool could be very useful in patterning paper for analytical purposes". The group has called the approach "craft punch patterning" (what else?) and touts it as precise, inexpensive, versatile and convenient for prototype research.

After a few false starts and optimization steps, the team realized they had successfully created a quantitative, multiplex immunoassay that can be performed in minutes rather than hours. What's more, the test can be completed with around 2000 times less serum (around 6 nL per detection zone) than the conventional ELISA and RIBA tests – something that shocked the team. "Being able to perform the test with such extremely low volumes of serum is not only beneficial to patients, but also highlights opportunities for non- or minimally-invasive detection," says Mu.

With an eye on the horizon, Mu believes the flower assay can help change the diagnostic landscape for HCV and hopes to roll out similar devices for other infectious diseases, such as HIV and hepatitis B. Until then, the group plans stepping up hospital collaborations to get its hands on more samples to help fully optimize the test. *RW*

Reference

1. *Xuan Mu et al., "Multiplex Microfluidic Paper-based Immunoassay for the Diagnosis of Hepatitis C Virus Infection", Anal. Chem. 86 (11), 5338-5344 (2014).*

From Diagnostics to BiognostiX

Teams in the UK, Finland, Spain, Switzerland and Italy join forces to tackle microfluidic-based assay development

We spoke with George Hutchinson, director of FFEI Life Science – the company driving the BiognostiX engine forward.

In the length of a Tweet, what is BiognostiX?

Novel microfluidic technology with broad application potential in point-of-use testing for veterinary, agri-food and human diagnostic markets.

How did BiognostiX get started?

The BiognostiX Consortium has been developing the technology under an EU funded FP7 project that aims to change the way diagnostic tests are performed. The consortium is led by FFEI Life Science and also comprises the Institutes of Biotechnology and Manufacturing from the University of Cambridge, Parco Tecnologico Padano SRL, Prionics AG, Proteomika and VTT. Together, we have developed a multiplex microfluidic platform and invented Immuno-Ink to deliver fast, flexible, single-use tests.

What makes BiognostiX unique?

BiognostiX combines the necessary biochemistry, microfluidics and a novel particle technology on a chip composed of a paper-based substrate, which has been mechanically treated to create a microfluidic channel pattern. Reagents are printed using fluid-jet technologies to deliver picoliter quantities of capture complex – the Immuno-Ink – into specific zones in the channels, which enables simultaneous performance of multiple tests per sample.

The simplicity and flexibility of the manufacturing process allows for changes in configuration. For example, the number of microfluidic channels can be varied depending on assay requirements and the residence time of the flowing sample can be adjusted to control interaction time. Once the biochemistry and chip are optimized, they are then fixed for simple, low-cost manufacture. The output of the immunoassays can be quantified using densiometric, colorimetric or fluorometric techniques.

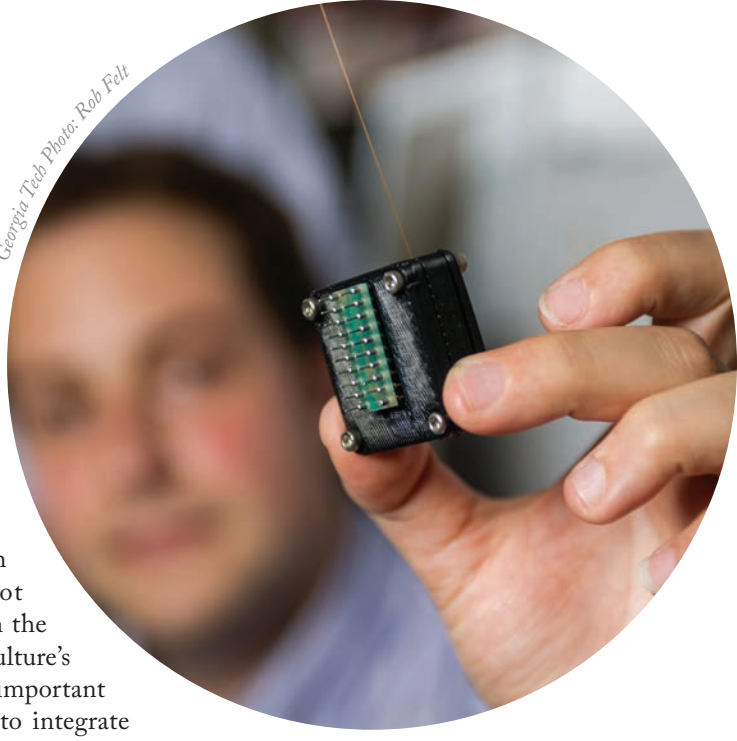
Collaboration seems key in the project…

Well, BiognostiX required several different areas of research, which is why we pulled together partners with various areas of expertise from across Europe. In terms of fiber-substrate engineering, the goal was to develop a cost-effective, disposable fiber-based microfluidic device that enables the controllable movement and processing of fluids while preserving the functionality of printed biomaterials. Our biomaterials engineering research team has conducted pioneering work that has led to the development of specially formulated Immuno-Ink. The fluid-jet manufacturing team has been working to optimize the placement of the biochemical reagents and optical labels without losing biofunctionality. And the BiognostiX Reader has been designed and validated for multiple reaction zones.

What's next?

The next step is to further develop the technology by undertaking collaborative research projects that integrate some or all of our component technologies into diagnostic assays that are in development.

For more information, visit www.biognostix.com.



Georgia Tech Photo: Rob Felt

Micro GC Robots for Farmers

Taking gas chromatography into the field has the potential to detect crop disease sooner

US farmers are estimated to lose around 12 percent of their crops every year to disease. Typically, they look for discolored or wilting leaves as tell-tale signs but, by the time such physical symptoms manifest, the pathogen may have already spread to nearby plants. Now, a micro GC device (roughly the same size as a 9-volt battery) developed by Georgia Tech Research Institute (GTRI) to detect VOCs emitted by plants and pathogens looks set to improve upon sharp eyes. According to Gary McMurray, division chief of GTRI's Food Processing Technology Division, dozens of such micro GCs could be fitted onto a ground robot, which a farmer could then use to take samples from plant to plant, with the aim of detecting disease before it runs riot.

"It is predicted that by the year 2050, we will need 75 percent more food," says McMurray, division chief of GTRI's Food Processing Technology Division, "We felt that eliminating losses on the farm would be a good first step to meeting that need, which is why we set out on this project."

"The science behind early detection of diseases and pests in crops is fairly well understood, but the details are still missing. The ability to link data from the micro-GC to a biological process in the plant is still challenging," says McMurray. Indeed, changes in temperature, humidity, soil moisture and nutrient levels can all affect VOCs emitted by plants, so researchers will need to establish indicative chemical signatures by studying VOCs released under different environmental conditions.

Field tests are planned for the summer and will use a bench top model of the micro GC to test peach trees for Peachtree Root Rot disease, working with the US Department of Agriculture's research service. The next important step beyond that will be to integrate the micro GC into an autonomous robot suitable for crop field sampling and analysis, a task that will involve several other collaborators.

Agriculture isn't the only potential application for the micro GC. "I think it has a very bright future," says McMurray, "People are very interested in this type of device for a variety of applications, including food safety, environmental monitoring, detection of explosive gases, and so on. Our ability to move a technology from the laboratory into the field is always a significant step forward. In time, many of the current applications for a traditional GC could be transferred to this new technology." *SS*

Most scientists regarded the new streamlined peer-review process as "quite an improvement."

Courtesy of www.scienceandink.com

In My View

In this opinion section, experts from across the world share a single strongly-held view or key idea.

Submissions are welcome. Articles should be short, focused, personal and passionate, and may deal with any aspect of analytical science. They can be up to 600 words in length and written in the first person.

Contact the editors at edit@texerepublishing.com

Command the Box

Surely, we've gone beyond thinking outside the box – the time has come to tell the box to respond to our thoughts.

By Bill Anderson, McGavack Professor of Biochemistry at Hampden-Sydney College, VA, USA.

One of the advantages of being old is that one can look back over time and see trends. But often, the time spent looking in the rear-view mirror comes at the expense of looking at what could be ahead in the road. Let's start with a short historical tour of instrumentation. If someone put a random analytical instrument in front of you, you could reasonably tell – at least within a decade – when it was built, whether or not you were familiar with it. But not because of the instrument's resolution or its sample size or even its detection limits. Rather, you would know because of the way you interacted with the system.

A hundred years ago, in order to conduct your analyses, you may have needed to place gratings in the right place, apply samples manually, add weights where appropriate, and so on. Then electronic instruments provided switches and knobs to operate. Likely a dial or meter appeared. Perhaps a light indicated the presence of a feature or condition. Indeed, buttons, knobs and dials were the operational interface for instruments for a while (and covered every available surface in fantasy star ships, such as the USS Enterprise, from the same era). Then came computer control and acquisition, so a keyboard (first defined keys, then "qwerty") was affixed to the core instrument. Joysticks, mice, trackballs, and touch screens followed to provide what most would consider the modern instrument-human interface.

Now, think about every block diagram you have seen in a textbook. Over the last hundred years, the diagrams have become more graphically elegant and frequently more intricate as the instruments (and publishing technology) has evolved. But there is one very important piece of every instrument missing from those block diagrams: the user. Recall how you can tell when an instrument was made based on how you are expected to operate it? Perhaps more attention should be paid to the all important user-instrument interface.

Back to the present and future. What is next for human-instrument interaction? Initial attempts at eye movement, gesturing and voice recognition are already here for some cell phone users. I mean, what comes after that?

> *"If someone put a random analytical instrument in front of you, you could reasonably tell – at least within a decade – when it was built"*

I propose that we begin using our cognitive processes and our ability to observe them as the method of controlling instrumentation. In other words, using our thoughts to control the box.

We have begun doing this in our labs and I see a wide expanse of opportunity ahead. There are several electroencephalography (EEG) headsets available. We have chosen to use the Emotiv EEG headset, which sells for a few hundred dollars – and even includes the software needed to create a pseudo-keyboard interface. You train the software by recording your 16-electrode EEG pattern when thinking a thought or command; when that thought pattern is duplicated, a flag is set that triggers whatever keyboard output you have previously entered. Pipe that keyboard output to the instrument's software and – boom – you're commanding the instrument to do a task simply by thinking the previously coupled thought.

"I have been able to teach students to take backgrounds or sample spectra, label peaks and conduct database searches all under the direction of only their cognitive processes."

I know what you're thinking... But we have been able to make this very concept work on UV-Vis, IR, plasma atomic emission and NMR spectrometers, as well as on our GC-MS system. And we have gone well beyond simply telling an instrument to turn on and off. I have been able to teach students to take backgrounds or sample spectra, label peaks and conduct database searches all under the direction of only their cognitive processes. No muscular movement of any kind was required. Moreover, they all learned to do it within 15 minutes and without much problem (oh, to be a student sponge once again). So, what are you waiting for? Go tell that instrument to read your mind!

Targeting the Untargeted

Our capacity to generate data is unsurpassed, but how do we cope with the data deluge? It's time to embrace data-driven discovery in biology and medicine.

By John A. McLean, Stevenson Professor of Chemistry, Department of Chemistry, Vanderbilt University, Nashville, TN, USA.

The rising areas of systems, synthetic, and chemical biology offer an exciting prospect. With allied advances in molecular biology, such as rapid genome editing, the questions posed of biology have increased in their breadth. Our potential to understand the answers to those questions may lie directly in our ability to observe and translate complex biological responses as objectively as possible. But purely compartmentalized, hypothesis-driven research tends to suffer from a subjective bias towards what is being asked and how we are listening for the answers. Such targeted analysis is like a Rosetta stone that may – or may not – hold all the key characters. In contrast, big data generation and interrogation strategies promote the concept of measuring all that we can and allowing the data to drive discovery. There is, of course, a continuum from specific hypotheses to data-driven discovery.

"Hypothesis-driven research tends to suffer from a subjective bias towards what is being asked and how we are listening for the answers."

Four elements make untargeted analyses suitable for driving new discovery in biology and medicine: (i) the increased prevalence of instrumentation and hyphenated techniques that are capable of

generating high dimensional datasets, (ii) the opportunities for interdisciplinary advances in big data strategies that can be imported from fields such as astronomy, business, and systems theory, (iii) the abstraction of salient biological information from complex biological "noise," and (iv) the iterative refinement of coarse-grained untargeted analyses to develop fine-grained understanding of specific hypotheses.

Research over the past several decades to interface distinct approaches (often with disparate operating characteristics, such as flow rates and pressures) has resulted in many contemporary studies that integrate techniques much like individual building blocks. We can now pair the most selective separation mechanism with the most sensitive detector even for complex samples. In other words, the rise of hyphenated strategies provides a means to tailor the analytical approach to the experiment at hand, rather than the other way around. Guided by lab-on-a-chip and microfluidic platforms, we can also scale the analytics appropriately to many questions asked in biology and medicine, ranging from measurements on tissue biopsies to single cells and cell cultures, to replicating human physiology in "organs-on-chip" and "human-on-chip" efforts. In all of these cases, the sample sizes are vanishingly small and yet the samples are exceedingly complex.

"Direct analogies can be drawn from the data-mining of Internet usage for advertising and commerce."

A variety of strategies, including chip-based genomics and mass spectrometry detection, provide data rates on the order of 10^4 to 10^5 detected hits or peaks within minutes or greater than 10^6 to 10^7 molecular features per hour. Generating data density at this rate vastly surpasses our ability to interrogate, identify, and validate each and every signal that is recorded. Indeed, the double-edged sword of untargeted analyses is that in the deliberate attempt not to miss hitherto unknown biology by measuring all that we possibly can, a tremendous amount of "noise" is generated in the measurement. In this context, noise can be considered anything that does not pertain to the question being asked and can arise from a variety of sources, including the biology itself and the superposition of biological function – how does one parse inflammatory response signals from those at the root cause of the inflammation?

Clearly, we must in order to translate the sea of data into signals that contain pertinent information – a task that is not dissimilar to contemporary research directions in areas such as astronomy or even Internet marketing. In fact, direct analogies can be drawn from the data-mining of Internet usage for advertising and commerce; the best way to make accurate, individualized purchasing recommendations is to compare enormous datasets of page views, searches and purchasing patterns for large numbers of customers and to recommend the last action of one individual to the individual with the most closely related pattern. Increasingly, these tasks are performed by strategies that use the self-organization of data to sort salient features from the noisy data. Many of these strategies are beginning to find application in biology and medical research – a trend that is likely to continue in the foreseeable future.

"The rise of hyphenated strategies provides a means to tailor the analytical approach to the experiment at hand, rather than the other way around."

One of the well-acknowledged challenges of big data strategies is that while self-organization of data can reveal otherwise unknown trends and relationships, it is tantamount to observing correlation rather than implying causation. Therefore, this coarse-grained view of the massive dataset should be used to focus on a smaller subset of signals that likely contain the answers that are sought. Iterative interrogation, identification, and validation of those subset signals is then critical to gain insight into the system and to refine hypotheses.

Many exciting avenues are being opened up by data-driven discovery. And we are only just at the beginning; new paradigms for parsing high-dimensional data in near-real time may be necessary as studies increasingly weave spatial and dynamics information from complex biological or ecological interactions into the broad tapestry of questions we are now wanting to ask.

Analytical Asset Management

The promise of cash generated from idle and surplus equipment sounds great. But how can you turn theoretical gains into practical benefits?

By Ben Potenza, vice president of marketing at EquipNet Inc., MA, USA.

Much has been written about the benefits that a proactive asset management strategy can deliver to a company's bottom line. But despite significant drivers, most managers have to focus their day job, lacking the time or resources necessary to establish a successful program. Here, I'll show what an optimized approach can deliver in practice, using the clearance of R&D equipment from a pharmaceutical site as an example.

To achieve a decent level of return, a holistic approach should be considered. Sophisticated tools now exist that allow the creation of a comprehensive inventory of surplus and idle assets, supporting their redeployment and utilization across multiple sites. In my experience, the redeployment of equipment within a business always delivers the highest value. After that, selling items that are no longer required gives the next best return on investment. In other cases, especially with older equipment, scrap provides optimum value. In most projects, a number of strategies – redeployment initiatives, online marketplaces, competitive auction events, and clearance programs – must be used together to reap the highest possible returns. We use a "value control" approach to manage this process effectively.

Let me provide a real-world example. The relocation of a major pharmaceutical company's R&D activity meant that an entire site needed to be cleared of all company assets. Already an active and established EquipNet client, the company had earmarked in excess of 500 individual pieces of equipment for disposal using our Asset Redeployment Management System (ARMS). Starting with this inventory master list, each item was expertly valued, which served not only to provide reliable figures for equipment being transferred to partner companies or subsidiaries, but also allowed us to agree financial targets for the project.

We adopted a 'divide and conquer' policy, splitting the disposal process into a series of phases. Equipment, ranging from tablet presses to liquid chromatography-mass spectrometry systems to stirrers, was grouped together on the basis of a number of criteria, functionality and disposal date being two of the most important. Each phase began when equipment use was at an end and when individual labs or buildings were ready for clearing. Certainly, such nimble project management underpins success and timely progress, but phasing disposal in this way has further rewards, for example, by preventing a flood of similar instrument types into the marketplace, which helps keep interest and returns high.

Even before any equipment left the site our value control approach began, with items initially being posted for sale on our marketplace. At this stage, kit carries a specific asking price and an undisclosed minimum price tag. This is where high value assets are identified; every agreed sale comes with direct client approval. Our sales team works hard, using its network of contacts, to achieve or exceed valuations.

"Sophisticated tools now exist that allow the creation of a comprehensive inventory of surplus and idle assets."

Equipment that remains unsold after being showcased in the marketplace is moved into an auction event. Price expectations are lower here, so the reserve price is typically reduced. If the auction succeeds in generating a bid above the minimum then the sale goes through automatically. However, if the minimum isn't met then the seller has the choice of taking the highest bid or retaining the piece for a later auction. Once all avenues of profitable disposal are exhausted the equipment will be assessed for donation – or scrapped.

In this particular example, all equipment has been extracted from site and the selling process continues – a notable success in the marketplace was the sale of a tablet press for $300,000.

There is no doubt that undertaking a project on this scale can be a logistical nightmare but, by applying the right processes, industry experience and project management know-how, it is already delivering value, turning what could have been a problem into a significant bottom line boost. Working alongside a specialist not only reduces workload but also allows companies to find the best market for their assets. So, the big question is, what have you got gathering dust in the corner of your lab?

FLIPPING THE ANALYTICAL CHEMISTRY CLASSROOM

With so many modern teaching tools at our fingertips, shouldn't we be making more of the time we spend with our students? I decided to "flip" my classroom in an attempt to answer that question. Here's how it went.

By Christopher Harrison

For the spring semester of my analytical chemistry class this year, I chose to implement the "flipped classroom". In it, the conventional approach to teaching is turned on its head: instead of using class time to learn material in a lecture format, students use it exclusively to answer questions and work on problems. The time that the students would normally spend doing problems or homework for a traditional lecture is instead used to watch pre-recorded lectures in preparation for the work to be done in the class. Simple!

"Given your level of education and expertise in the subject, your time could inevitably be better spent doing something other than what amounts to reading. Right?"

The flipped classroom is in no way my creation – I learned of it from a seminar on teaching held at San Diego State University. For me, it's the latest installment in my quest to find the best way to engage a large group of students in the beauty of analytical chemistry.

The back story

I have been teaching analytical chemistry at San Diego State University (SDSU) since the fall semester of 2007. The course is the traditional introductory analytical chemistry course, often called quantitative analysis or just "quant" for short. Its focus is to get students to think analytically about chemistry: to begin to consider the complexities of equilibria, to understand the statistical significance of the numbers that they see and report, and to gain a foundation in how accurate measurements can be made in chemistry, both in the classroom and in the laboratory. To a large extent, it requires the application of a lot of math to chemical systems to understand what is happening within them. As with any university level course, some students love it, most work their way through it, and a few dislike it.

My early teachings followed the traditional lecture format. I would spend most of the class time explaining the concepts, theories, or equations pertinent to that section of the class, including some sample problems that I would solve for the students by walking through each step. The problem with this approach is that it doesn't provide a significant amount of experience to the students. Sure, they get to listen to me – and hopefully learn something from that. And they get to see me solve a few problems along the way. But it does seem like a very odd way to learn how to do something. Would the traditional method work if you were teaching someone to do something complex, such as fly an airplane or perform surgery? Absolutely not! Practical experience is crucial, which is why we have practice problems and homework. But when do students do this work? At home in the evenings? The night before a deadline? Ever? From my experience, I would say one or two days before the deadline is when most students try to complete the problems. I can quite accurately gauge this by counting the number of students that drop by my office with questions. In a week without any homework deadlines, I have nobody coming by. But on the week of a deadline, my office is packed, and I am usually answering the same questions over and over…

It is likely that many lecturers reading this will have had a similar experiences. I would even go so far as to venture that, much like myself, you have probably come to feel that you are better able to teach your students during your office hours than you can during your lectures. After all, it is in these office hours where you can determine what their individual difficulties are and how best to aid them. If only all interactions could be like that. Instead, most contact hours with your students are spent presenting a repackaged version of the textbook or other written course material. Given your level of education and expertise in the subject, your time could inevitably be better spent doing something other than what amounts to reading. Right?

Student engagement

It was during Pittcon 2011, in the middle of our spring semester, that I was inspired to make some serious changes to how I would teach the analytical chemistry course. The inspiration came from a talk by Steven Weber from the University of Pittsburgh, who described how he got his students to calculate the pH values for the titration curves of various amino acids; each student was assigned an amino acid. Steven would introduce the material and then have the students dive into the work during class, so that he could supervise and answer questions. The approach struck a chord with me and I realized it could be nicely adapted for use with my material.

With 40 students in the class there weren't enough amino acids for everyone, so I asked the students to work in groups. Each group was given one of five amino acids whose pH they needed to calculate at various points along a titration curve, which allowed me to have numerical answers that I could share and compare with the students. I used the first half of the class to cover some basics related to polyprotic titrations and then commenced the group work.

The hard part was just sitting back and letting them do the work. Until you have actually tried to leave a lecture to its own devices, it is hard to describe how uncomfortable it feels. That doesn't mean that it didn't work out – but not everything went perfectly. A couple of students adamantly refused to work in groups and elected to leave the class. Most students did get into groups and, after some chatting, began the work. As I circulated around the classroom, I answered questions that arose in each group. I also noticed that groups were benefitting from peer mentoring, with more advanced students taking the lead.

The experiment offered two positive insights into how effective active learning can be. The first was that many of the students wanted to know the correct answers to the calculations at the end of the lecture. It was great to realize how engaged the students were with the problem. The second discovery was actually quite unexpected. All instructors can easily tell when there are less than five minutes left in any class; students start to pack up their books and stop listening entirely. But in my experimental class, this didn't happen. In fact, students were still working on the problems at the end of the class when the next lecture group started to enter the room.

Given my success, I continued with the approach for the next few years. Though it was somewhat effective in getting a large number of students to do problems during class, the amount of time for doing problems was rather limited. After introducing the materials and possibly solving a sample problem, there was little time left for group work. Additionally, some lecture topics were not as conducive to such an approach or simply needed more explaining. And there was the inevitable decrease in attendance for the class, with only a third to a half of the class attending any given lecture, which pretty much reflected declines I'd seen when using traditional lectures. Yes, the approach was working pretty well, but it could certainly be improved; I was still spending at least half of my class time lecturing, rather than letting students work on problems or ask me questions. At this point, the concept of the flipped classroom began to make much more sense to me (see Flipping the Classroom on page 24).

Learning to flip

I should note that the implementation or rigidity of a flipped classroom is entirely up to the instructor. I opted for a more open structure, without any imposed deadlines on the watching of lecture videos or the submission of questions. Rather I had prepared problem sets (those used in the previous years of the course) that I wanted the students to work on in groups. For the first couple of lectures, I had created video reviews of the labs that they would be doing during the course of the semester. In keeping with that theme, I provided the students with a summary of a lab, along with the "data" collected from the analysis – a silver chloride precipitation titration experiment. The objective was to get the students to do the calculations for the standardization of the titrant, during which they would need to deal with statistical issues, such as the exclusion of outlier data points. It was a glorious teaching plan in my mind – the students would complete so much in just 50 minutes. It was an utter failure…

In reality, after introducing the class and answering a few questions, there was little more than 30 minutes left,

Flipping the Classroom

What is "Flipping the Classroom"?

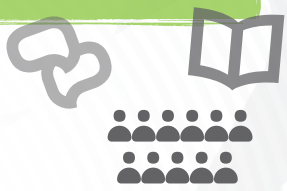
In the Flipped Class format, the locations of instruction and homework are reversed. Instruction takes place outside of the class, through online materials, while 'homework' is done in class. The liberated classroom becomes a more active learning environment.

The Flip

Instruction that was previously given in class time, is now done online.

The Flipped Class.

Class time is used for active work: Q&As, group worksheets, projects. The result: engaged learning.



How does it work

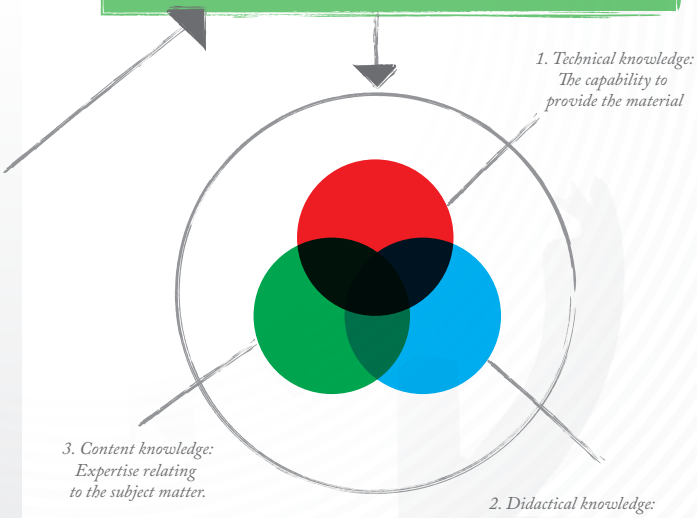
Results – together
Presentations; discussions; questions; applications; problems

Activities – together
Involvement by doing: playing games, experimenting.

Digesting: individual
Give meaning: preparing videos and podcasts, doing tests.

Contents: individual
Deepening: watching video lessons, listening to podcast, surfing websites.

Teachers need to master three things to integrate the Flipped Classroom:



Based on content from www.kennisnet.nl

"I had been toying with the idea of doing a flipped classroom for some time, but never found the time to record the lectures, so I found myself scrabbling."

which was nowhere near enough for the students to grasp the complexities of the problem. Instead of students asking questions about the validity of data and how to interpret results, questions revolved around how to get started.

Unfortunately, this scenario repeated itself in the next period, as there had been no time to alter the plan. Once again, my students were confused with material presented to them. Clearly, I was not preparing them well enough and had overestimated their capabilities. Fortunately, I was able to adapt my plans for subsequent classes; I resorted to using problems from the more traditional format of the course, which made classes much smoother.

I have to say that I really did not account for how much time the lecture recordings would take out of my schedule. I had been toying with the idea of doing a flipped classroom for some time, but never found the time to record the lectures prior to the semester when I decided to implement the process. As such, I found myself scrambling to prepare videos each week for the topics to be covered. Though I had lecture materials from previous iterations of the course, significant modification of those materials was necessary to make them amenable to a lecture video. Add to that the time to record the lecture, edit the final product, and upload it, and the workload starts getting heavy. Sometimes I was only able to get the lectures uploaded the day before the class period – clearly not ideal; however, it did not prove to be such a big problem as most students were a few lectures behind after the first few weeks of the course.

Another surprising lesson that I learned during the semester (which probably shouldn't have been surprising at all) is that, if classes are unstructured and optional, many students will not attend. I realize this response is not unprecedented. Certainly, in the past when I had taught this course as a traditional lecture, I would consistently see below 50 percent attendance in the latter third of the semester. Some students had just given up on the course, others made use of the course materials that I provided (audio recordings, sample problems, lecture slides) rather than coming to lectures directly. However, using the flipped classroom, the decline in attendance started sooner and went to a much lower level, with as few as a quarter of my 80 students attending lectures regularly.

Assessing the flipped classroom

I would be lying if I said that I didn't want all my students to come to every class, but the reality is that, though working on problems in groups is a great way for most students to learn, it may not holds true in every case. Moreover, because of the lack of strict deadlines, the course effectively became a self-paced program; students were less likely to be at the same point and thus less likely to be able to work in groups. The big question is whether or not low attendance is a problem.

In a traditional course it would clearly be problematic – students would be missing out on the basic instruction for the course. However, with a flipped classroom, that's not necessarily the case. The lecture materials (including videos, problem sets, solutions, and online homework) are fully available, so presence in the class is not a direct indication of their efforts to learn the material. In fact, if the dropout rate for the class (meaning those who did not withdraw from the course but elected not to write the final exam), is compared to the historical average, the change is stunning. Under the flipped classroom approach, the only student who did not

"It was easy enough to accomplish with a whiteboard, iPhone, and a camera tripod, and it added a more dynamic feel to the narration of slides."

complete the class withdrew in the first few weeks of the course. Historically, about 10 percent will not write the final exam, having given up before the course finishes.

I have to say, it is not fully clear if the flipped classroom approach was the principle factor in the improved retention of the students. Other changes, including a revision to the structure and style of exam questions may have also played a role. However, comments from some of the students, including one who had failed to complete the course in the more traditional lecture format, shed some light. In the traditional lecture format, if a student does not grasp the material being taught, it's rarely possible to get a second chance. Of course, with lecture videos, the student can replay sections over and over, so if motivated, would have no reason to fall behind in their understanding.

An inability to ask questions of the lecturer while watching videos was inconvenient for some. But there are two solutions to this problem: (i) email the instructor the question or (ii), as I suggested to my students, they could actually opt to watch the lectures during class, where I would be available to answer questions on the spot. Admittedly, this takes us almost full circle, but because the recorded lectures are much shorter than the class time allocated, it still presented a better alternative, since I was available to answer as many questions as required.

Over the course of the semester I elicited feedback from my students about their feelings on the flipped classroom. The responses varied widely – some loved the new approach and others hated it. Complaints fell into two main categories, students either preferred the live lectures over the videos or wanted to see sample problems solved rather than stepwise calculated solutions.

The craving for traditional lectures may stem from comfort and familiarity – at least one student admitted as much in the feedback. And yet, given the rate at which I normally see students stop attending classes – and their total lack of participation – I am struggling to understand what benefit they derive from a formal lecture period. The inclusion of videos illustrating solutions to sample problems was something that I did change. It was easy enough to accomplish with a whiteboard, iPhone, and a camera tripod, and added a more dynamic feel to the narration of slides.

Am I a flipping convert? Absolutely! Overall, I feel that the flipped classroom was very successful. The students completed the course, and did so with far better grades than my previous traditionally taught classes. Despite the success, I do know that I can make the flipped classroom an even better experience for my students. Many students lamented the low numbers attending the class times, echoing my feelings. And while I recognize that students can be (and often are) successful without coming to class, I would like to be able to push them further.

I will be redoing my videos before the start of the semester to better integrate examples of problem solving. I will also significantly shorten the videos, likely making more in the process. After all, the traditional lecture habit of repetition is not really necessary when students have access to a rewind button!

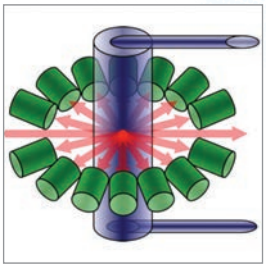
I see the flipped classroom as the inevitable evolution of much of our teaching, if only for the reason that as educators we can be far more effective when we directly engage our students and help them solve their specific problems. Given the success that I saw with my first – and admittedly clumsy – attempt at flipping the classroom, I see no reason to go back to the traditional lecture format.

Christopher Harrison is Associate Professor, Analytical and Bioanalytical Chemistry at San Diego State University in San Diego, CA, USA.

Malvern

Manufacturer Channels

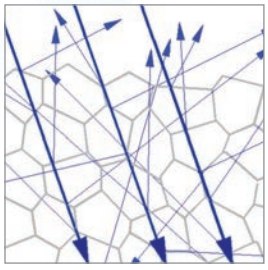
When only a MALS detector will do…



Light scattering detectors play an important role in gel permeation chromatography and size exclusion chromatography (GPC/SEC) analysis because of their ability to directly measure molecular weight distribution. Multi angle light scattering (MALS) detectors are often chosen, sometimes because they provide the most accurate data for the radius of gyration, and in other cases because they have become an accepted industry standard. The launch of Malvern Instruments' new Viscotek SEC-MALS 20 detector extends commercial choice in this area and draws the technology into the spotlight.

http://tas.txp.to/0514/cc001

Static Light Scattering for GPC/SEC Explained



Static light scattering is a technique to measure the molecular weight using the relationship between the intensity of light scattered by a molecule and its molecular weight and size.

The aim of this introductory article is to provide the reader with a clear understanding of the different technological approaches used to measure molecular weight by static light scattering in a GPC/SEC experiment, including those used in SLS, MALS, RALS, and LALS.

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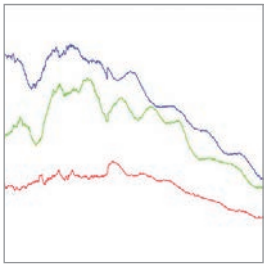
Characterization of Branded Co-Polymers by Triple Detection GPC



A linear polymer is composed of a single main chain of repeating units, linked in a regular end to tail fashion. Branched polymers on the other hand, come in various different forms. Comb polymers have branches of similar structure emanating from the main polymer backbone. Random branched polymers have many different branching points, with variable branch lengths, attached not only to the backbone, but to the branches as well. Star branched polymers, and dendrimers, begin at a central point, with symmetrical branches radiating outward from the core.

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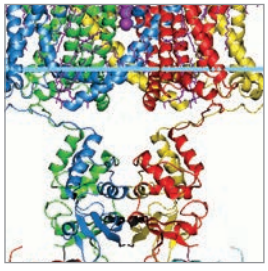
Analysis of Membrane Protein by Multi-Detector SEC



SEC is commonly used in the biological sciences to measure the molecular weight of an unknown sample by comparing its elution time with standards of known molecular weight, using a single concentration detector, such as UV or refractive index (RI). However, to make the measurement accurate, additional detectors must be used. Now, advanced multi-detector SEC systems include up to four detectors, namely UV, RI, light scattering and intrinsic viscosity for more advanced analysis.

http://tas.txp.to/0514/cc002

Harnessing 2D-LC for Big Pharma

Over 10 years ago, the potential of two-dimensional liquid chromatography seemed obvious to me – so I built a system and never looked back. Now, a more mature 2D-LC is ready for a bigger role in pharmaceutical analysis.

By Cadapakam J. Venkatramani

At graduate school, I became fascinated by the research of the late professor John B. Phillips, who invented a comprehensive two-dimensional gas chromatographic (GC) system in early 1990. My two-dimensional journey began with me joining his group; I was extremely excited about the prospect of sampling entire primary column eluent into the secondary column for further separation using complementary phases. I devoted much of my graduate research to 2D-GC – in particular, separations of petroleum samples. A highlight was the resolution of over 4,000 sample components in a petroleum sample using comprehensive 2D-GC, which fully demonstrated the potential of the technique (1). In the late 1990s, I joined a pharmaceutical company and figured it would be the perfect place to extend 2D-GC concepts to liquid chromatography (LC). However, it wasn't until the early 2000s that an opportunity came my way and I actually got to pursue my 2D-LC goals; a case of right place, right time.

Homemade 2D-LC

Two-dimensional GC worked, so I knew that 2D-LC should too. The big question was, "how do I build a system?" There were no commercially available instruments, but luckily we had multiple HPLC systems sitting side by side, so it really was a matter of configuring them and designing a 2D-LC interface to transfer primary column eluent to the secondary column. To cut a long story very short, I put together a system using a 12 port dual position valve and published the work back in 2003 (2). The paper highlighted several different 2D-LC setups, including single and dual columns in parallel in the second dimension, multiple detectors – amongst other things. In fact, what we were doing back then is very similar to current research in multidimensional LC. I guess we were ahead of our time!

Of course, building your own system presents challenges. One of the problems was the noise generated by back and forth switching of the valve, which made it difficult to discern co-eluting impurities from noise spikes. I needed to fully synchronize the valve timing, which was no small feat given system constraints. I had to integrate a high-speed electronic timer that, once triggered, would automatically and reproducibly start the switching sequence every 30, 60, or 90 seconds as per project needs. To summarize, I had to take into account three main considerations: (i) how do I configure the two HPLC systems so that they can communicate, (ii) how do I successfully take a fraction from the primary column and focus it at the head of the secondary column for further separation, and (iii) how do I reduce the baseline noise created by valve switching. And that's before we even got any data.

Retrieving the two-dimensional data from the 2D-LC system was equally challenging. The HPLC systems gave a series of detector responses as a function of primary column retention time. The second dimension's retention time was a real missing link. This had to be manually recreated in Excel taking into consideration the data acquisition frequency and the switching frequency of the valve (3, 4). A 2D contour plot of a sample mixture made of acidic, basic, and neutral compounds resolved on mixed mode stationary phases in two-dimensions, acidic in primary and basic in secondary, is shown in Figure 1 (4). The sample components are separated into acidic and basic zones with the neutrals along the diagonal. The location of sample components in the two-dimensional plane reflects its chemical nature.

So, despite the challenges, we still got great data to demonstrate the concept, which made the extra efforts worthwhile. Funnily enough, we were using sub-2 µm columns even back then for some of our proprietary, unpublished work without realizing one day it would emerge into what is commonly known as sub-2 µm chromatography. We just knew we needed to use columns with small particle sizes (1.8 µm) for high-efficiency, high-speed separations.

In short, 2D-LC required significant creativity and hard work on our part in those early years but, in return, provided excellent rewards.

Taking 2D-LC to the next level

I was using my homebuilt system until about two years ago when Agilent introduced its own 2D-LC-MS model.

Now, researchers like myself don't need to worry about many of the problems we faced. 2D-LC has become a very seamless and intuitive process; it's no longer a research tool in the hands of few researchers but a commercial tool with repetitive gradient capability. Each gradient starts at progressively higher organic strength than the previous gradient, improving efficiency. Previously, I was forced to use shallow gradients in both dimensions, but in modern systems, repetitive gradient programming is

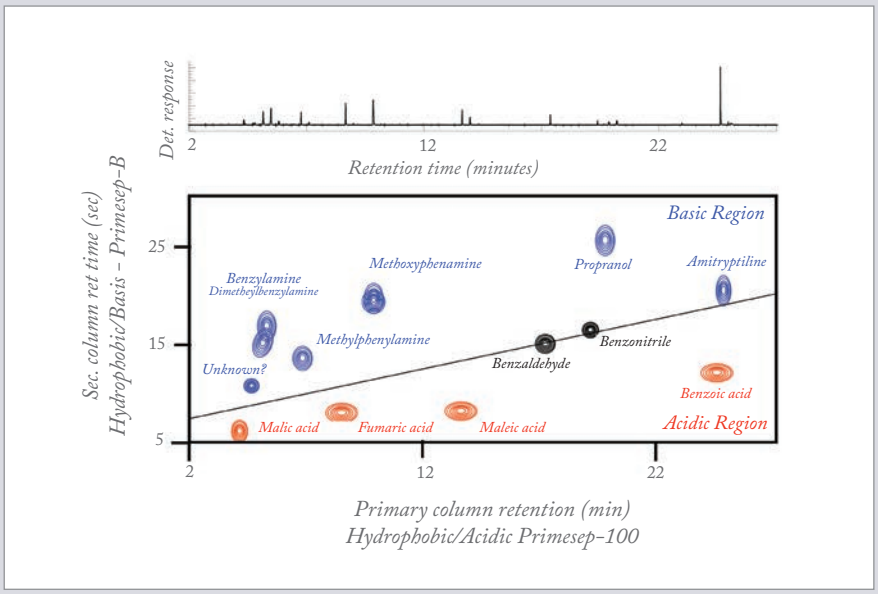


Figure 1. Complementary 2D-LC separation of a test mixture on a Primesep-100 column in the primary dimension and Primesep-B column in the secondary dimension. The one-dimensional chromatogram (top) was used in the generation of the two-dimensional contour plot. The primary column flow rate was 0.5 mL/ min and the secondary column flow rate was 3.25 mL/min. The UV detection was at 215 nm.

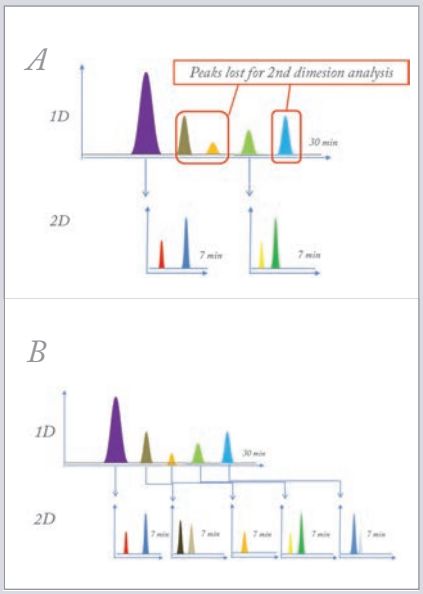


Figure 2. A. Standard heart-cutting 2D-LC. B. 2D-LC with peak parking solution for multiple heart-cutting, which allows the collection and storage of multiple fractions in 12 sample loops.

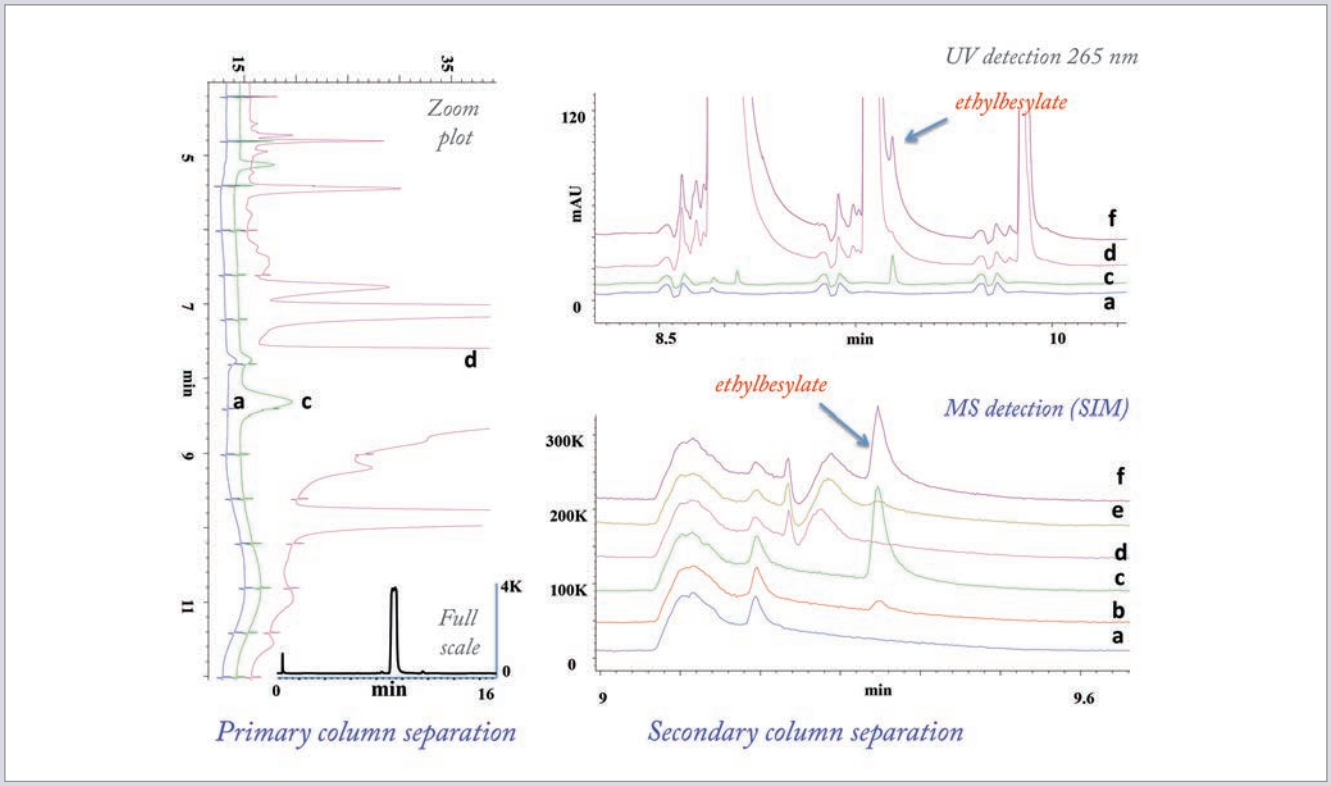


Figure 3. 2D-LC-MS separation of ethylbesylate, a genotoxic impurity, spiked into an active pharmaceutical ingredient (API). The plot to the left is the primary column separation on a C18 column (5 cm x 2.1 mm x 1.8 µm). The full-scale plot of the primary column separation is inset. The plots to the right are the secondary column separations of transferred fractions monitored using UV detection at 265 nm (top) and MS-SIM of ions characteristic ethylbesylate (M+1 ion, bottom). A phenylhexyl column (5 cm x 2.1 mm x 1.8 µm) was used in the secondary dimension.
Ethylbesylate is partially resolved in the secondary column with UV detection. The MS trace of unspiked and spiked samples at the bottom demonstrates the power of 2D-LC with MS detection. a: diluent blank, b: 0.5 ppm standard of ethylbesylate, c: 5 ppm of ethylbesylate standard, d: unspiked API, e: 0.5 ppm ethylbesylate spiked into API (10 mg/ml), f: 5 ppm ethylbesylate spiked into API (10 mg/ml).

Quick Tips for Getting into 2D-LC

1. *Know your sample.*
2. *Ask yourself, "what am I trying to achieve?" and "will 2D-LC help solve my problem?"*
3. *Review the literature. Find out what has been done before; do not reinvent the wheel.*
4. *Find out what instrumentation is commercially available; make things easy on yourself.*
5. *Experiment and have some fun playing around.*

almost unlimited, which opens up a whole new range of applications.

In pharmaceutical analysis, we are particularly interested in resolving and identifying any trace co-eluting impurities in the midst of the main active pharmaceutical ingredient. But because chemical components that elute in the proximity of the main peak are often similar in structure (or isomeric), developing a specific and sensitive HPLC method can be difficult and conventional detection techniques like the diode array detectors (DAD) and MS have their limitations. Furthermore, the concentration levels of these impurities are often a few orders of magnitude lower. It's like trying to look at a main component peak the size of the tallest structure in the world (the Burj Khalifa in Dubai) whilst not wanting to miss an impurity the size of a pedestrian on the street beneath it. Using 2D-LC, we get a second chance to find any co-eluting components by using another mechanism of separation in the second dimension. That's a major advantage for the pharmaceutical industry.

In small molecule pharmaceutical science, impurity analysis confined to the proximity of main component does not warrant a fully comprehensive 2D-LC run. We tend to adopt a selective or pseudo comprehensive method (which is somewhat like extended heart-cutting). Currently, as we can only generate secondary chromatograms every 30 or 60 seconds, we slow the primary column flow rate (for example, from 1 ml/min to 0.05 ml/min) over the course of the main peak, which allows us to send more fractions to the secondary column. A recent innovation introduced at HPLC 2014 by Agilent is the peak parking solution for multiple heart-cutting, which allows the collection and storage of multiple fractions in 12 sample loops that can then be analyzed sequentially (see Figure 2). Using it, we should no longer need to slow the flow in the first dimension. I believe it will be of great value in the pharmaceutical industry. We are awaiting our demo version.

Another example of 2D-LC's potential in pharma is assessing MS incompatible methods for potential co-elution. You can take a small portion of the non-MS compatible mobile phase from the primary column and introduce it onto the secondary column for further separation without impacting the MS. I also see potential application of 2D-LC in the analysis of genotoxic impurities, which have significant bearing on patient health and must be quantified at very low levels. Application of 2D-LC-MS in the analysis of co-eluting genotoxic impurities is shown in Figure 3. Ethylbesylate, a genotoxic impurity co-eluting in the midst of API peak in the primary column, is partially resolved in the second dimension using UV detection. Use of specific, sensitive detector like MS enables the detection of the co-eluting impurity, which differs in concentration by more than four orders of magnitude, demonstrating the power of 2D-LC.

Achiral/chiral analysis is another area where 2D-LC could play a pivotal role, especially with increased numbers of chiral centers. We demonstrated the proof of concept in our earlier work on simultaneous, achiral/chiral analysis using 2D-LC (5).

In the future, I am positive that 2D-LC will be used to assess stability indicating methods for co-elution and as a feedback mechanism to optimize or improve routine methods. By embracing 2D-LC as a research tool earlier in development, I believe we can produce more robust stability indicating methods.

Given the advantages, I would not be surprised if 2D-LC starts to take center stage in the next 5-7 years – especially, if regulatory agencies take a firmer stance and scrutinize stability-indicating analytical methods for potential co-elusion.

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References

1. *C. J. Venkatramani and J. B. Phillips, "Comprehensive two-dimensional Gas Chromatography Applied to the Analysis of Complex Mixtures", J. Microcolumn Sep. 5, 511-519 (1993).*
2. *C. J. Venkatramani and Y. Zelechonok, "An Automated Orthogonal Two-dimensional Liquid Chromatograph", Anal. Chem. 75, 3484–3494 (2003).*
3. *C. J. Venkatramani and A. Patel, "Towards a Comprehensive 2-D-LC-MS Separation", J. Sep. Sci. 29, 510-518 (2006).*
4. *C. J. Venkatramani and Y. Zelechonok, "Two-dimensional Liquid Chromatography with Mixed Mode Stationary Phases", J. Chromatogr. A 1066, 47-53 (2005).*
5. *C. J. Venkatramani et al., "Simultaneous, Sequential Quantitative Achiral-Chiral Analysis by Two-dimensional Liquid Chromatography", J. Sep. Sci. 35, 1748-1754 (2012).*

Webinars

Virtual Events

Need to Boost GC Speed without Sacrificing Resolution?

Increase GC Speed without Sacrificing Resolution: The Principles of Fast GC

Event Overview:
Providing a background to the basic theory behind Fast GC, this webinar will highlight the practical aspects of actually making it work. Popular applications, such as BTEX, PAHs, FAMEs, volatiles, and semivolatiles, will be shared as real world examples.
Speaker Michael D. Buchanan *Product Manager, Gas Separations*

Date: June 25th – 11am EDT (UTC -4)
Register Free at: http://tas.txp.to/0514/supelco/webreg

SUPELCO Solutions within.™

Advances in Fracking Contamination Analysis

Measuring Fracking Contamination: Rapid and Reliable Determination of Methane and Other Dissolved Gases in Water

Event Overview:
This webinar will look at new high throughput test methods that use robust and automated headspace and GC instrumentation for the quantitative determination of dissolved gases in ground, waste, and drinking waters.
Speakers Massimo Santoro, *GC and Single Quadrupole MS Marketing Manager* and Andrea Caruso, *GC Application Chemist*

Date: June 26th – 8am PDT / 11am EDT (UTC -4)
Register Free at: http://tas.txp.to/0514/thermo/webreg

Thermo SCIENTIFIC

Fast and Furious: Reducing Analysis Time in GC

Increased sample numbers and a drive to improve cost effectiveness is the motor behind changes in GC methodology.

Event Overview:
This webinar sets out the possibilities and limitations of some Fast GC options to improve productivity – from simple changes in carrier gas or column dimensions to backflushing techniques and ultra-fast column heating/cooling modules.
Speakers Johan Kuipers, *GC Specialist, Training & Development*

Date: July 17th – 11am BST (UTC +1)
Register Free at: http://tas.txp.to/agilent-webreg/17/7/14

Agilent Technologies

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Photos, clockwise

1. The LCQ ion-trap. Customers love to see the 'heart' of these instruments, but are always confused why such a small device should cost so much. Of course, vacuum systems, electronics, software, and so on, have to be added… as well as a profit incentive!
2. Me chatting with two young mass spectroscopists – John Yates (center) and Pat Griffin – in the early 1990s. Our interaction with a number of key consultants and collaborators in academia has always been a major driver of our success. Not only do we receive valuable input, but they often are the early adopters of new systems, their students usually go on to buy our systems, and perhaps most critically, we often hire their students.
3. From ASMS 2012 in Vancouver: the "Giant Orbitrap". ASMS 2014 in Baltimore marks my 40th consecutive attendance. It is such a wonderful scientific meeting: well organized, great science and scientists, great opportunities for companies to interact with customers.
4. From Scotland to Scotland (mid 1970s). I became a US citizen many years ago.
5. My three early MS "heroes" – Klaus Biemann (center), John Beynon (to Biemann's left), and Fred McLafferty (to Biemann's right) – perform "kagami biraki" (breaking open the Japanese sake barrel) at the 1992 international Biomedical MS meeting in Kyoto.

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Building Mass Spectrometry from the Inside

The development of mass spectrometry is one of science's great technology stories. I've been fortunate to spend my career in applying mass spec to the life sciences, both in academic research and in building new technologies within industry. From the edge of retirement, and looking back over 25+ years, here's my version of how it unfolded.

By Ian Jardine

The year 1988 was a big one for my career. In terms of technology, it saw the introduction of electrospray ionization. This was a real game-changer: it not only allowed us to ionize large and polar molecules, but also let us use liquid chromatography (LC), which was particularly important for biological samples. It also meant that we could fragment peptides directly to gain sequence information.

That same year, matrix-assisted laser desorption/ionization (MALDI) was also invented. And, on a personal note, I moved from a professorship at the Mayo Clinic – arguably one of the best jobs in mass spectrometry in US academia – to industry, joining Finnigan Corporation. My role in the company, you won't be surprised to hear, was to bring MALDI and electrospray into the portfolio.

The intervening 25 years have been a scientific adventure that I feel incredibly privileged to have experienced. I still marvel at the fact that when I read the newspaper on any given day there is always at least one article, be it in genomics, biochemistry, pharmaceuticals, earth sciences or whatever, that my company is significantly involved in.

Here, I want to give a sense of the excitement and intellectual stimulation that I enjoyed right up to my retirement earlier this year. My passion for new technology is really what has driven me.

Academic beginnings

One thread that has run through my career is an involvement in the use of mass spectrometry to advance the field of proteomics. I started working on the topic in the 1980s at the Mayo Clinic and in that pre-electrospray era it was extremely difficult to look at proteins using mass spectrometry. Generally, we had to cut proteins up with trypsin, digest each peptide with acid to dipeptides, and derivitize those ahead of the gas chromatography (GC)-MS setup. This was extremely laborious and tedious – and, to be honest, not even that useful. The mainstream approach at the time was Edman chemical degradation sequencing which, while also very tedious, was at least effective. Being a mass spec guy with a passion for the latest instrumentation and a thirst for tough challenges, I went my own way.

It's easy to trace out how I got to that point. I started as a

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"The buzz at conferences surrounding electospray and MALDI that year only added to the excitement."

chemistry undergraduate at Glasgow University in Scotland, and followed it with a graduate degree in mass spectrometry with one of the leading mass spectroscopists of the time – Ivor Reed, who was also at Glasgow. Ivor was an excellent scientist and, while working with him, I actually built a mass spectrometer. It used photoionization and simultaneously detected the electron and ionized molecule: it was a coincidence time-of-flight (TOF) MS. And it kind-of worked – at least, well enough to get my PhD. After that, Ivor arranged a post-doc for me at Johns Hopkins medical school in Baltimore working with Catherine Fenselau – another highly-respected mass spectroscopist. There, I focused on pharmacology and drug metabolism, and found that it was a wonderful experience to use instrumentation in a very applied sense. My next stop was Purdue University, where once again I worked mainly on drug metabolism. Whilst there, I tried to tackle the kinds of molecules that were extremely difficult for other techniques; for example, complex anti-cancer alkaloids or the immunosuppressive drug cyclosporin, which is a complex cyclic peptide. When I moved to the Mayo Clinic in Minnesota I got very interested, to the point of distraction, by the problem of protein structural analysis and quantitation. It seemed like an extremely important field to me and over a 10-year period I worked with a number of collaborators at Mayo on using MS in an attempt to sequence proteins.

While some of the target molecules changed, the main thread throughout my time in academia was a desire to use the most sophisticated MS instrumentation available to tackle the most complex challenges in terms of structural analysis. I ended up as a full professor and, to be quite honest, I thought I would be at Mayo for my entire career – why wouldn't I?

During this extremely satisfying point of my life, the folks at Finnigan Corporation approached me to ask if I would join them. My initial response was, "You must be kidding!" I went back home and must have mentioned it to my wife (who is from Baltimore). She said, "Where are they based?" "The San Francisco Bay Area," I told her. "Well, I'm packed," she said, "let's go!" We took on the move as an adventure, both quitting good jobs to move to the sunshine. Colleagues were surprised, but could see our reasoning; no one in the Midwest tends to argue with a move to California.

Finnigan was clearly a very forward-thinking company at the time. Its main business was in environmental analysis using GC-MS, but the leaders realised that in order to grow, mass spectrometry had to move into other areas, particularly life sciences. They didn't quite know how that should be done, which is why they recruited me. They wanted to use my applications expertise to help them figure out how to make MS systems that could solve problems in the life sciences. The buzz at conferences surrounding electospray and MALDI that year only added to the excitement.

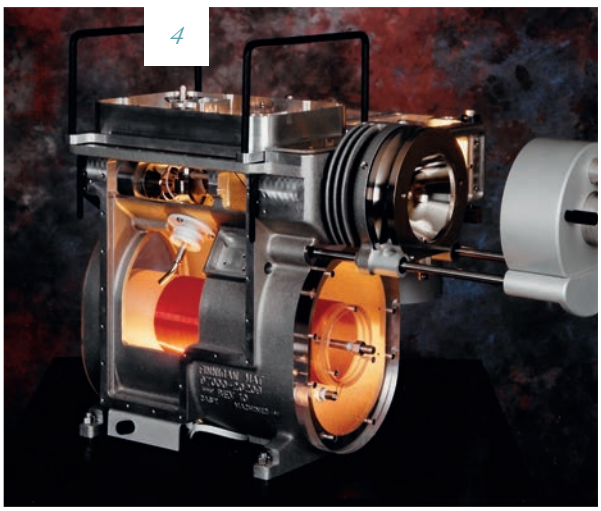
Peptide pioneers

Since 1988, there has been continual – and sometimes step-function – improvement in how well we can measure proteins, but one of the biggest advancements has been in data analysis. Most researchers, especially Don Hunt at the University of Virginia, were performing fragmentation of peptides and then manually interpreting – as best as they could – the sequence of those peptides. The breakthrough that was clearly needed was the ability to automate interpretation of fragmentation data – and that came from John Yates in the late 1990s.

John had recognized the real benefit of all the protein sequence data that had been generated from genome sequencing. Why not take all that sequence information, look at the tryptic peptides that would result, then calculate what the fragments would be?

Photos, clockwise

1. LCQ Ion trap marketing, development and manufacturing team from San Jose. I was then mass spec marketing manager (bottom row, third from left). George Stafford (R&D program manager) is on the far right. Jae Schwartz (principal scientist) is behind LCQ logo on instrument. The LCQ system shipped in 1996.
2. From the 40th class reunion at Glasgow University in 2010. Me with two other chemists who made a great living in analytical science with a major MS focus. Left, Tom Baillie (Dean of School of Pharmacy at University of Washington, previously head of drug metabolism at Merck), center, John Halket, Professor at King's College, London.
3. In my student days.
4. Marketing literature, featuring the internal workings of an LCQ. The actual models were used to attract customers at trade shows and other events.



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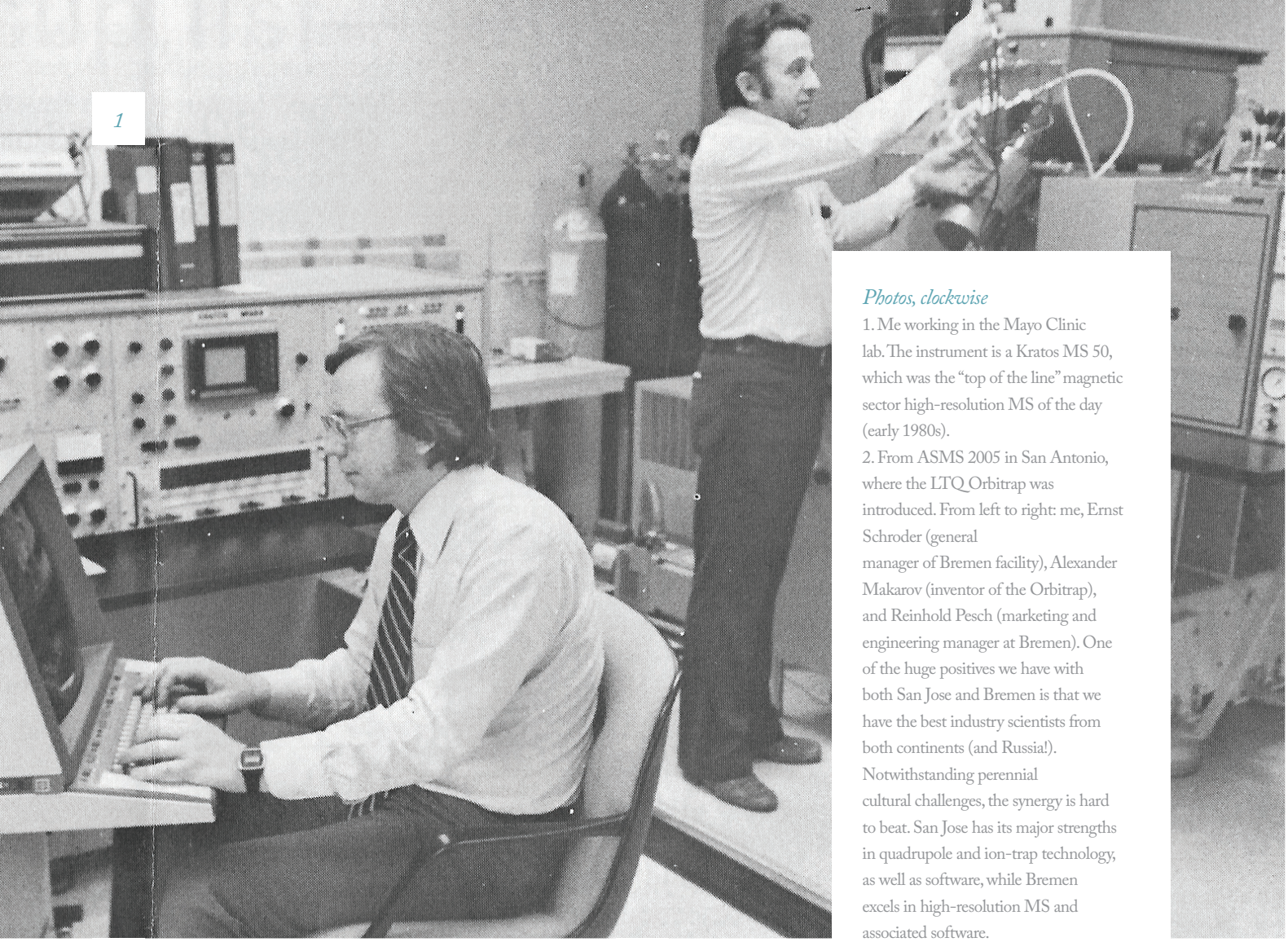

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Photos, clockwise

1. Me working in the Mayo Clinic lab. The instrument is a Kratos MS 50, which was the "top of the line" magnetic sector high-resolution MS of the day (early 1980s).
2. From ASMS 2005 in San Antonio, where the LTQ Orbitrap was introduced. From left to right: me, Ernst Schroder (general manager of Bremen facility), Alexander Makarov (inventor of the Orbitrap), and Reinhold Pesch (marketing and engineering manager at Bremen). One of the huge positives we have with both San Jose and Bremen is that we have the best industry scientists from both continents (and Russia!). Notwithstanding perennial cultural challenges, the synergy is hard to beat. San Jose has its major strengths in quadrupole and ion-trap technology, as well as software, while Bremen excels in high-resolution MS and associated software.
3. A man of leisure.

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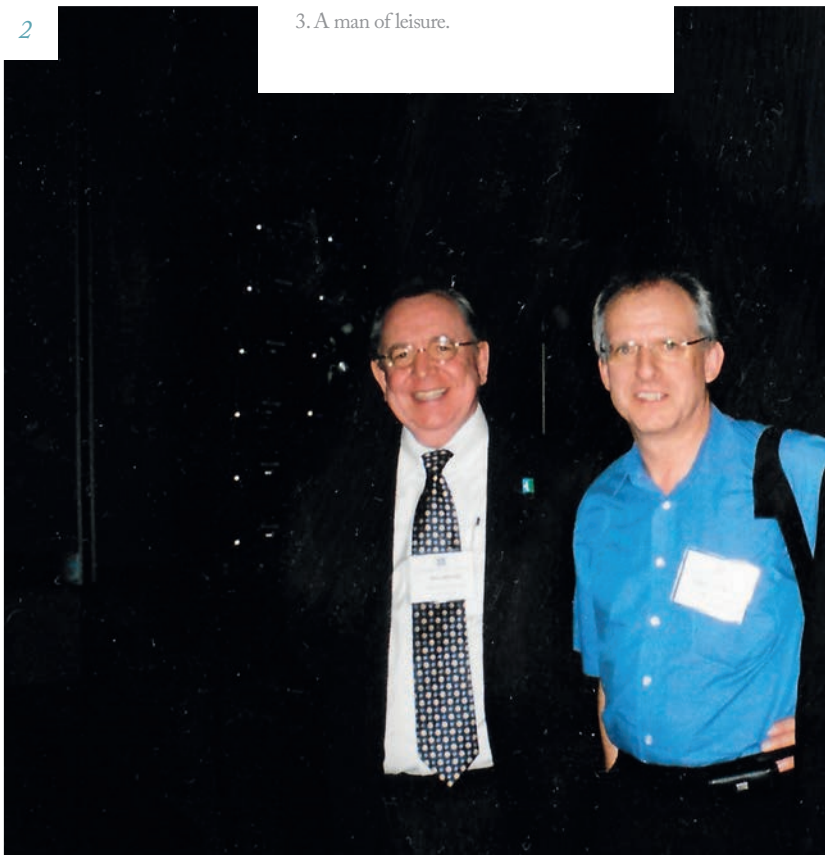

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That way, you could compare your own experimental peptide fragmentation data with the in silica-generated database. The program was called SEQUEST. I was already working with John, who had just moved from Caltech to the University of Washington, when he told me about his work on SEQUEST. He had just filed for patents and I immediately made sure that we licensed the protocol exclusively because the absolute importance of John's work was clear to me at this very early stage. In that regard, I feel that I can take some small credit for the advancements that followed. The best technology that we had back then for fragmenting peptides was the ion trap, because of its scan speed, sensitivity and richness of fragment information. When used in conjunction with John's SEQUEST algorithm, it became a very powerful tool for rapid protein sequencing. There was a sense at the time that Q-TOFs were a better instrument to perform the analysis, but they were actually quite slow.

I remember asking John if he would be willing to talk to The Protein Society at a lunchtime seminar about the SEQUEST algorithm. He dutifully agreed but as I looked around the room, I could sense the sheer lack of understanding. Afterwards, John asked me how it went. "It was fabulous," I said. "No one had a clue what you were talking about, but don't worry, they will." That was not a criticism of the audience who weren't all that familiar with mass spectrometry, let alone advanced algorithms for data analysis.

I've sat in rooms filled with dumbstruck experts more frequently than you might expect. I guess one of the things that I brought to Finnigan and to Thermo Fisher Scientific was an ability to anticipate quite accurately what would be important and useful for researchers in the future. Being in academia for an extended period, trying to solve some of the problems researchers face, certainly helped me get a feeling for the next big thing.

One of the factors that encouraged me to go into industry is the sometimes frustrating nature of the academic world. You might get an inspired idea about how best to use the latest-generation of mass spectrometer one day and apply for a grant; a year or two later, you might actually get the funding, and, by the time you had the instrument you were initially excited about, it would be two or three years too late. That said, it is that very same academic background that has allowed me to remain connected with the research world right the way through my career. Have I been able to apply greater influence on science doing my job within the company than if I had remained an academic? I believe I have, and that makes me feel even more fortunate to have been so involved in the development of the exciting technology around me. I like to believe that I would have made some contributions in academia if I'd stayed but I travelled the more satisfying route for me, especially in terms of being able to make an impact.

Mass spec impact

To give an example of an actual impact (see "The Big Decisions" on page 28), I go back to the mid-1990s and the development of a product that combined the ion trap with electospray (the original instrument was called the LCQ). I initiated the project with Jae Schwartz, one of our key instrument developers. Once the basic principles had been worked out, I moved my office downstairs to be next to the head of R&D. This way, I insisted, we could more easily engage with each other on questions such as, "Why are we doing this again?!" (from him) and "Is it possible to increase the speed and resolution even more?" (from me) – my job title at the time was head of marketing. It was a wonderful working relationship that really linked application knowledge with engineering. When the LCQ finally came out, it was an incredible hit and changed the ability of the whole field to do rapid protein sequencing.

Subsequent experimentation essentially aimed to increase the speed, efficiency, and sensitivity of protein analysis and is really what has driven the advances in mass spectrometry that we've seen over the past 20 years, in my opinion. After the original ion trap, we came out with the linear ion trap, which was much faster with much higher sensitivity. We then coupled the linear ion trap to a high resolution super-conducting Fourier transform MS (FTMS) in 2003, which was yet another breakthrough in

The Big Decisions

Remembering that building successful commercial analytical instruments is very much a team effort, here are the five major advances to which I made key contributions over the last 25 years. These had a significant impact on Thermo Fisher Scientific and on the development of mass spectrometry in the life sciences. They were all very controversial inside the company, but turned out to be correct. Fundamentally, all decisions were based on the philosophy that we had to go down compellingly differentiated paths, relative to all competitors, because they all had clear unique strengths.

1. Coupling ESI to 3D ion trap technology in parallel to ESI quadrupole in the early 1990s, followed by development of a new commercial LCMS ion-trap instrument (LCQ - 1996) before a new LC triple quad (Quantum - 2001). We could only afford to do one development at a time because of business financial realities. The key LCQ developers were Jae Schwartz and George Stafford. My reasoning was that although Finnigan was the first company to introduce commercial triple quad instruments, it was going to take a long time to catch up with the dominant position that Sciex had created with ESI triple quads by the mid-1990s.

2. Championing the potential of 2D linear ion-trap technology – and getting the funding to develop it in the late 1990s – early 2000s. After listening carefully to key scientists (John Syka, Jae Schwartz and Alan Schoen) regarding the potential gains of this technology over 3D ion-traps, especially with respect to sensitivity, dynamic range, and scan speed, I obtained the funding to create the research and then LTQ (2003) instrument program. Again, major pushback came from triple quad and even time-of-flight advocates, but the power of ion-traps to generate the best peptide sequencing data was compelling.

3. John Syka, working in Don Hunt's lab at the University of Virginia, demonstrated the incredibly effective coupling of the linear ion-trap to superconducting FTMS systems. It was clear then that we should build such a commercial system to enter the world of high resolution/accurate mass MS for life science applications, and especially for peptides and proteins. Around 2001, I would not fund the construction of a commercial hybrid LTQ FTMS without a commitment from our brilliant Bremen engineers (under the leadership of Stefan Horning) that it would be an easy to use, automatable system, which was fast enough (high-resolution scan speed of 100,000 resolution in one second) to be essentially a dedicated HPLC detector. With the help of John Syka and Mike Senko in San Jose, they delivered just such a dream system in 2003.

4. Because Q-TOF technology had become very successful, we undertook serious development of such a system in the early 2000s in San Jose. We had very nice TOF systems up and running just as the Orbitrap was coming up to speed in Bremen, but the latter was still very new with many uncertainties. We could not afford to build both systems for commercial introduction (again, business reality), so we cancelled the Q-TOF program and went with the clearly differentiated Orbitrap. It was obvious that every one of our major competitors would have a Q-TOF, and since at that time we were not a major HPLC player, we would certainly lose in the marketplace.

5. After the introduction of the quadrupole Orbitrap configuration (Q Exactive) in 2011, and its subsequent major success, especially against Q-TOF systems, and the overall success of our highly competitive portfolio (thanks in particular to the leadership of Iain Mychreest), it became clear that it was time for us to expand our efforts. We needed competitive LC-MS/MS systems (ion-traps, quadrupoles, Orbitraps and combinations thereof) outside of proteomics, in applications areas where we had not been confident enough to compete intensively, such as drug metabolism, metabolomics and clinical chemistry. Thermo Fisher Scientific's LC-MS portfolio now competes in these areas today. And the programs and projects that I have championed will come to fruition in the next few years, which will ensure that mass spectrometry, and Thermo Fisher Scientific in particular, will revolutionize many areas of clinical diagnostics, which frankly, desperately needs much more accurate and precise analytical systems.

protein analysis. And in 2005, we replaced the super-conducting FTMS with the Orbitrap technology, which was another step-function change. I could go on, up to last year's release of the Fusion instrument, which combines quadrupole, linear ion-trap, and ultra high resolution Orbitrap. Suffice to say that it is hugely gratifying to have witnessed such technological advancement in a relatively short time.

In fact, the Moore's Law-esque advancement in our ability to identify and quantify proteins over the last 20 years is probably one of the few areas where end-users in a field have fully acknowledged the fact that industry has driven much of the progress. And I have to say that organizations, such as the American Society for Mass Spectrometry, have been very clear that they really appreciate not only what industry has achieved, but also the support it has provided. In a field like mass spectrometry, it's not surprising that industry has led the way – you need something akin to a small army to put these extremely complex instruments together. In a bygone era, a research lab could put together hardware – magnets and pumps – and use chart recorders, but as electronics and software have evolved so fast, the breadth of scale required is outside of a typical lab's capability. In fact, it's becoming a problem more generally, even for instrument companies: it is very difficult for us to recruit instrument developers from academia, because there are so few labs that are working in the area. We mainly now bring in raw talent and then train them.

Learning the hard way

Where the ion trap/electrospray project was a clear success, the road hasn't entirely been paved with gold. Though we were actually the first to offer commercial MALDI-TOF systems, we went astray. The business plan stated that we should sell a new benchtop MALDI-TOF for the same price as an Edman sequencer, which made complete sense: competing/complementary technology at a competitive price. However, some colleagues argued that it was such a beautiful machine that it was worth much more, which was quite possibly true. Indeed, researchers came in droves to test out the instrument and told us how wonderful it was, after which we would tell them the price, and we'd never see them again. A year later, competitors released lower-priced instrumentation and we were simply edged out of the MALDI-TOF market. We misjudged the customer and value proposition at hand. However, I can assure you, we didn't make that same mistake twice.

The silver lining is that it meant we focused everything we had on LC-MS, which turned out rather nicely…

The size of the opportunity that arose from coupling the linear ion trap to the FTMS was a huge surprise to us all. The business grew much faster than we could have possibly imagined, up to annual sales of well over $100 million. Despite the success, we realized that the costs and maintenance associated with super-conducting magnets could be a problem for labs. And given its high price, it was unlikely that customers would buy more than one. The brand new Orbitrap technology was ready and waiting, so we decided to replace the FTMS side of the system, which was a huge gamble in many ways. It was new technology, and while we thought it would be great, you never know. Certainly, some of the senior managers in the company were puzzled and extremely nervous as to why we would cannibalize a very successful product with something that was essentially unproven. But, as you may know from Alexander Makarov's Orbitrap Story (see tas.txp.to/issues/0614-Orbitrap), things worked out beautifully. It replaced the FTMS system completely and enabled labs to make multiple purchases and ramp up their research efforts.

What's amazing is the fact that each time we launch one of these new high-end instruments, there is great demand despite flailing economies or the sequestration problem in the US, for example. I guess that when new technology is too good, too exciting and too useful to miss, people somehow find the money. And I am absolutely confident that this will continue to be the case. There is always room (or money) for breakthrough innovation that can be successfully applied.

End game

I've had a great deal of fun and satisfaction over my career. In some ways, there are still many things that I would like to be involved in, such as the rapid move of mass spectrometry into clinical diagnostics, multiplexed protein quantitation that provides astonishing accuracy and precision in biological systems, and "top-down" proteomics. However, there is no getting away from the fact that working in industry is hard; there are deadlines, business pressures, and a huge amount of travel. It feels like the right time to hang up my hat and retire. The acquisition of Life Technologies and an excellent year for the mass spectrometry division have left the business very much in an upward trajectory and it's clearly much better to leave on an up than on a down.

Perhaps more importantly, I'm in the very fortunate situation that my wife Gail actually wants to spend more time with me!

Ian Jardine was Vice President of Global R&D for Thermo Fisher Scientific, and before recent retirement was Chief Technology Officer, Life Sciences Mass Spectrometry at Thermo Fisher Scientific in San Jose, CA, USA.

Profession

Leadership
Talent Development
Career Planning

Overcoming Your Resistance to Change

Is your "psychological immune system" preventing you from moving in positive new directions? By facing the hidden commitments that you harbor and challenging the assumptions that they are based upon, you really can modify your behavior. Here's how.

By Janice Manzi Sabatine

Like it or not, the workplace is constantly changing. Usually discussions about change focus on changing things – processes, structures, formats, and so on. As interesting as those topics are, there is plenty of literature on how to effectively lead, manage, or adapt to such change. Instead, I want to discuss a subject that is rather more personal: how to change ourselves.

As we grow and develop (both personally and professionally), it's natural for us to identify areas that we want to improve – or change. Yet, despite a strong desire to grow and adapt, there are often aspects of personal change that we are unable to make or to sustain. Why is this? According to Robert Kegan and Lisa Lahey, Harvard professors and authors of the book "Immunity to Change" (1), there is a very good reason for our resistance: a psychological protection mechanism.

Here, I will interweave an overview of Kegan and Lahey's approach to overcoming our natural resistance to change with my personal coaching experiences of trying to help clients attain their highest potential through change.

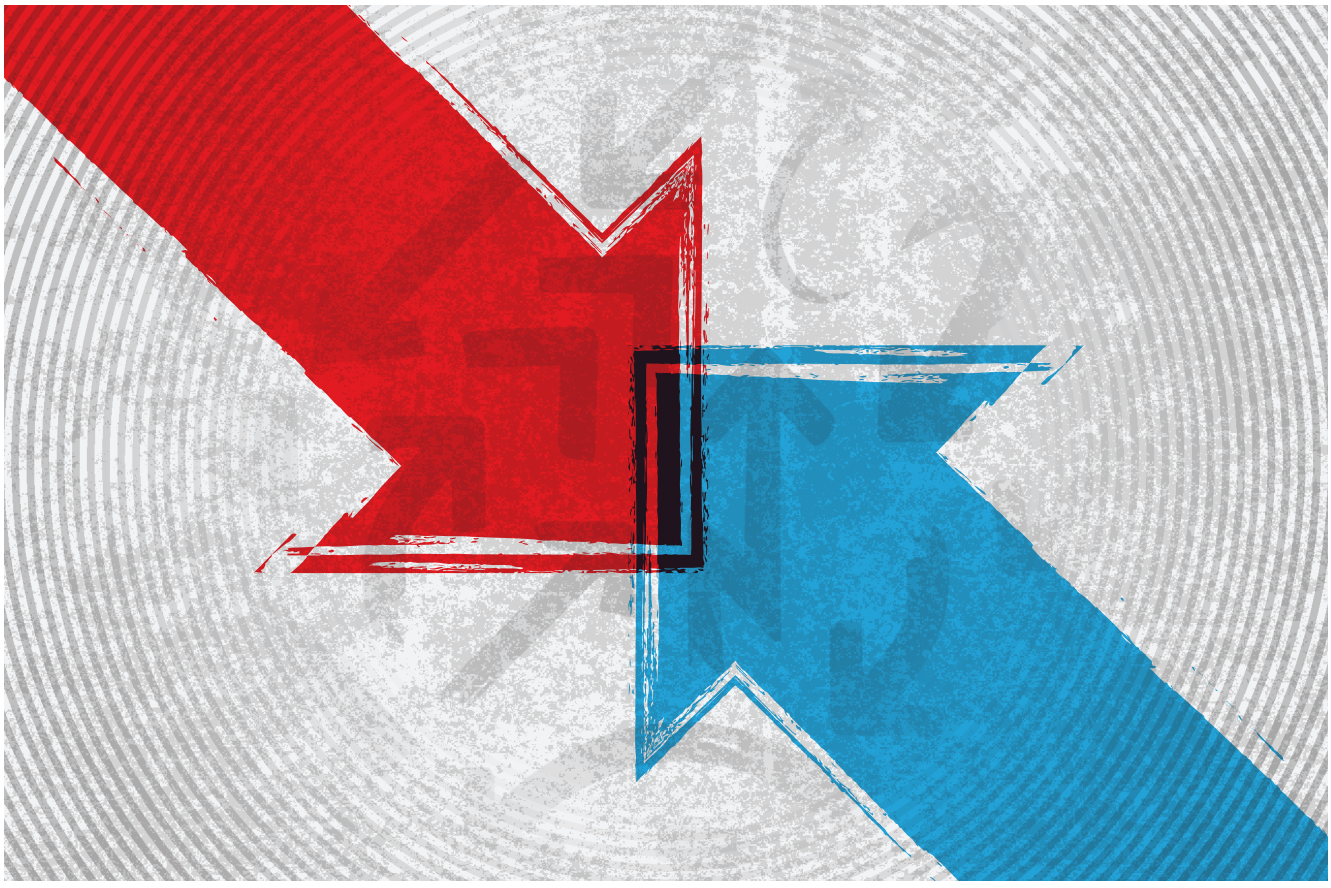
What do you want to change?

Some people don't have to think long to know exactly what they want to change about themselves or what they need to change to be successful. For others, it may take some thought. Constructive feedback about your performance at work can serve as a good starting point and families and partners can also supply 'critical' information on where we could improve. If you don't receive any kind of feedback, I encourage you to seek it out. Identify the peers or colleagues whose perception of you really matters and ask them. Finding out areas where others perceive you positively can be quite validating and, on the other hand, discovering those areas where you are negatively perceived can be a strong motivator for change.

When choosing an aspect of yourself to improve, remember to identify a behavior rather than an outcome. You may want to be more influential, reach or exceed targets, or increase employee engagement – all worthy goals. To achieve these, identify which behaviors you must improve. Perhaps you need to get better at dealing with conflict, or speak up more at meetings, or become a better listener. Notice that these change goals are stated in the positive. If you have a goal to stop doing something or to not behave in a certain way, restate it in the affirmative. For example, if you want to stop interrupting people when they talk, you could say you want to let people finish talking before you interject. The most important thing is that you have to really want to make the change.

Once you have selected behaviors that you truly want to change, write down a list of the things that you currently do or don't do in relation to those behaviors. For example, if you chose to improve how you deal with conflict, you might write down that typically you sugar-coat your words, don't address issues, or avoid certain people. Give this some thought and perhaps observe yourself for a few days. Better yet, tap into those who gave you the initial feedback or enlist the help of a trusted friend or colleague to observe you. Make the list as extensive as you can.

Up to this point, the process may sound somewhat familiar. But this is where things take an unexpected



turn. Typically, improvement plans suggest that you get feedback, identify an improvement goal, get tips on what to do, and start practicing those new behaviors. If you need to stop interrupting people, you are usually advised to take a few minutes before a meeting to remind yourself and to ask a colleague to observe you or give certain signals to warn you when you stray.

When you are merely trying to break a bad habit, this approach might work; however, you may find that you repeatedly slip back to your old ways, which could mean that there is something else lurking in the background. If you have been unsuccessful in changing a behavior by following a new tip sheet with sheer hard work and determination, another tip sheet and more hard work and determination are unlikely to make a difference. Remember that Albert Einstein once said that insanity is doing the same thing over and over again and expecting different results. I challenge you to take this next step.

Hidden commitments

Actually, the next step is a step back. We're not going to tackle new behaviors at this stage; in fact, we're not even going to think about working on the original goal at all. Instead, we will try to uncover a goal or commitment that you didn't even know you had – a so-called "hidden commitment".

Hidden commitments are driven by emotions – our worries, concerns, or fears that cause us to take protective measures. To get at your hidden commitments, go back to your "do/don't do" list. For each of the behaviors you've listed, such as a tendency to sugar-coat your words, imagine doing the opposite and try to write down the emotions that emerge. Don't make this an academic exercise; instead, really imagine yourself in these situations and allow yourself to experience and focus on the most negative, emotionally-charged reactions that could result. For example, you may have listed that "I often interject when other people are talking". Now, imagine the opposite behavior: always allowing people to finish their point before you speak. How

Trapped	*Aware*	*Questioning*	*Free*	
Blind to: *• Worries* *• Hidden Commitments* *• Assumptions*	*Aware of:* *• Worries* *• Hidden Commitments* *• Assumptions*	*Are my assumptions accurate?* *How do I know?*	*New mindset*	*GOAL*
Counterproductive behaviors	*Discovery*	*Observations.* *Repeated testing.* *Refinement of assumptions.*	*New desired behaviors*	

Figure 1. Breaking down the psychological immune system to achieve your behavioral goal.

does it make you feel? It may produce a strong fear of appearing stupid or uninformed, raise concerns that you will have no say in decision-making, make you appear weak or unsuited for promotion, or that you will somehow lose control of the discussion. If you would feel embarrassed to let other people see this list of fears or worries, then you are on the right track!

The next step is to restate these fears as commitments. The key is to retain the fear or worry within the commitment; for the example above it might state "I am committed to not appearing weak" or "I am committed to not losing control". These commitments should read like some form of self-protection rather than the statement of a noble goal.

Once you have written out these clear personal commitments, go back and look at the do/don't do behaviors you have listed. The commitments should now seem like ways of overcoming the fears and worries you've identified. Kegan and Lahey compare these hidden commitments to an immune system. Like the real immune system, the hidden commitments are actually working actively in the background to protect you, and you need to disable them to generate real change.

The key to overcoming this form of immunity is to examine and question its underlying assumptions. Ask yourself what assumptions make those hidden commitments feel so necessary. In the above example, your assumptions may be that you will appear weak or stupid, or that you will lose people's respect, if you listen more than you talk. Each hidden commitment generates numerous assumptions about how you will feel, how others will react, or just about the world in general. You may find that the assumptions don't all make sense from an intellectual point of view, or discover that you believe them to be absolute truth. Either reaction is okay. You may notice that your assumptions put boundaries around you, and that if you cross those boundaries you enter 'unsafe' territory.

Experiment!

The next step is to test and challenge your assumptions. As analytical scientists, this stage may appeal to you. Try to design and execute experiments that can invalidate or refine your assumptions in a way that opens up new, safe territory for you to function in. It is important that these experiments do not actively attempt to directly address your original objective (changing your behavior), rather they should only seek to discover flaws within your own assumptions.

The key is to gather information from our behavioral changes that lead to changes in our mindset. We must believe and think differently to sustain behavioral changes.

Use the SMART acronym to guide you as you design your tests.

S-M: your experiment should be both safe and modest. Attempt doing (or not doing) something small that carries low risk but that will still give you information to assess one of your assumptions.

A: Choose something that is easily actionable in a normal day.

R-T: Take a research stance (not a self-improvement stance) and test your assumption. The point

is to collect data, not try out behaviors necessary to your original goal.

For example, let's say "John" assumes that he will appear stupid or uninformed if he talks less and listens more. John must first ask himself what small changes he can make that would give him valuable information about his assumption. John planned to try a small change in an upcoming discussion with a trusted colleague who is facing a challenging situation and wants to talk it over. First, John planned what he would do and how he would do it. He decided to adopt a position of curiosity, to hold back from interrupting, and to ask questions rather than give advice. He felt that it would be easier to try this unfamiliar approach one-on-one with someone he trusted during a short conversation. He planned to collect data on how he himself felt and to record what his colleague actually said and did. After the conversation, John noted that it was hard to keep quiet at first and then it got easier. He also realized he learned more about the situation by being quiet. He noted that his colleague was quiet at first and then began to talk more and responded thoughtfully to the questions John posed. At the end of the discussion the colleague expressed how helpful John was and that he appreciated John taking the time to help him sort through this challenge.

John's interpretation of this initial experiment was that talking less was actually less taxing and stressful for himself and that he provided value without dominating the conversation. Since this was a trusted colleague, John could further explore his assumption by telling his colleague about his assumption and asking him whether John appeared uninformed by taking this approach. These data could serve to modify John's assumption or to create new ones.

One single test is unlikely to be conclusive or create a major breakthrough. The point is to keep refining your data and expanding your boundaries. John may now decide to try this approach in a more important meeting and ask his colleague to observe and help interpret responses from others on the team. Enlisting supportive friends and colleagues can be invaluable as you continue to modify and test your assumptions. Once the assumptions no longer hold, the self-protective behaviors are no longer necessary. These steps may take some time and practice, but at some point you may find that you are no longer conscious that you are trying to interrupt your main assumptions. Use Figure 1 to help remind you of the process. Once you are unconsciously acting counter to your assumptions, you will be on your way, if not fully successful, with your original goal.

The fact is, that if you have tried repeatedly – and failed repeatedly – to change your behavior, don't be too hard on yourself. You may actually be quite brilliant, having created an incredibly powerful psychological immune system! Examining this system carefully and testing and refining the underlying assumptions that keep you from your goal are the keys to overcoming your resistance to change. Good luck.

Janice Manzi Sabatine is president of Avanti Strategies LLC, which provides executive coaching for physicians and scientists.

Reference

1. *R. Kegan and L. K. Lahey, "Immunity to Change" (Harvard Business Review Press, Boston, MA, 2009).*

Solutions

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The Power of Fully Integrated Informatics

Shell's Pearl GTL brings in the big guns to tackle its vast data-handling requirements with an integrated laboratory information management system.

By Ajith Kumar and Colin Thurston

The Problem

The unprecedented scale of the world's largest gas to liquids (GTL) plant in Qatar – Pearl GTL – creates a massive business and technology challenge: how can quality be maintained and productivity maximized while managing huge volumes of critical data?

Background

As a senior business analyst for Pearl GTL, I was responsible for managing the laboratory informatics components of a project of unprecedented scale, which required billions of investment dollars and created tens of thousands of jobs during peak construction. Data management was a major priority. To maximize production and allow rapid decisions, we needed consolidated, accurate information available at our fingertips – at all times.

Established by Shell and Qatar Petroleum in 2006, and onstream at the beginning of 2011, Pearl GTL is the world's largest GTL plant and cements Qatar's position as the GTL capital of the world. The GTL process converts natural gas to liquid fuels and other products, including gasoil, naphtha, kerosene, normal-paraffin and lubricants. Pearl GTL captures the full value chain, from offshore development and onshore gas processing to the refining of finished products in one project. The project provides the platform for the growth of an entirely new industry with GTL fuels, in particular by opening up opportunities for new markets.

When it comes to illustrating the enormous scale of the project to build the world's largest GTL plant, the statistics paint an impressive picture. Some two million tonnes of freight were shipped into a dedicated berth at Ras Laffan port adjacent to the plant site. More than 750,000 cubic meters of concrete were poured during construction. And enough steel was being used during the peak construction period to erect the equivalent of two and a half Eiffel Towers a month.

Initially, my major challenge was finding a resilient software solution that supported all of our stringent data and integration requirements – something that could help drive success from the very beginning.

The solution

From the start, we knew that we needed a highly sophisticated software solution to manage the interface of a quality control system that receives a constant stream of 34,000 transmitted measurements. These measurements, which include well content, volume, emissions, equipment condition and hundreds of other data points integral to the plant's operation, needed to be mapped onto the data generated by the laboratory operations. In addition to collection and storage, data also needed to be organized, integrated and analyzed constantly to ensure plant safety, product quality, environmental protection and production efficiency. Furthermore, our solution needed to ensure that Pearl GTL's labs remained in compliance with requirements such as ISO 17025, an accreditation that sets an international benchmark for running a testing laboratory. It lays out qualifications for suppliers, training, record-keeping, equipment calibration and much more. In the event of an audit, Pearl GTL would need to quickly retrieve and present data proving compliance.

With so many prerequisites for success, we needed a proven solution: a laboratory information management system (LIMS). The right LIMS should

present accurate, unbiased information necessary for maintaining the highest standards of safety, regulatory compliance and environmental commitment – all without sacrificing financial performance. But clearly, given the operation's complexity, not just any LIMS solution would do. In addition to organizing sample results, Pearl GTL's LIMS would need to be fully integrated with the ability to communicate with a variety of other systems, including operations management, batch tracking and enterprise resource tracking systems. Without such integration, it would be almost impossible to achieve success with a project of this size and scale.

We chose Thermo Scientific's SampleManager LIMS to manage our state-of-the-art testing laboratories, standardizing it across all laboratory equipment and production systems. SampleManager offered unparalleled support for all of Pearl GTL's stringent requirements.

Despite our complex quality, regulatory and interface requirements, we worked with a strong team of people from Thermo Fisher, including Colin Thurston (director of product strategy for informatics). Rolling out the solution across Pearl GTL was seamless, despite some of the LIMS interfaces that were implemented being the first of their kind in any Shell facility.

One of our principal reasons for choosing SampleManager was its ability to integrate with other systems. At Pearl GTL, the LIMS is integrated

with an operations management system (known as OTTER), process historian (OSI PI), an oil movement and batch tracking system, laboratory instruments and other production systems. Now, communication between all systems is seamless and bi-directional. And all information necessary to manage complex sample scheduling and stringent safety, quality and regulatory requirements is readily accessible.

The way SampleManager integrates with PI delivers notable efficiencies for Shell and Qatar Petroleum at Pearl GTL. While some other labs manually send test results to operations, technologists and process engineers, the Pearl GTL laboratory results are available to all relevant parties within the PI system as soon as they are authorized in SampleManager. If high accuracy sampling data is important to your job, you can access it through the system in real time.

Other important consumers of lab data within Pearl GTL are users of the oil movement and batch tracking system. When panel operators need to move oil to new tanks in preparation for shipping, for example, they don't need to wait to be notified of test results, and this minimizes demurrage charges for loading delays. As soon as the results are available from the lab, the LIMS notifies operators through the oil movement system.

The LIMS has also enabled Pearl GTL to go paperless, helping us eliminate many human errors common in paper-based laboratories. Human beings can make an average of 3-6 mistakes for every 1,000 lab readings transcribed, so a sampling program the size of Pearl GTL's, could lead to hundreds of errors every day. SampleManager solves this problem by integrating lab instruments that automatically transmit data as soon as final results are produced.

SampleManager aggregates all this data and combines it with information collected from other sampling systems, including technicians in the field, enabling Pearl GTL to collate and present a vast array of data in a logical format for managers to analyze and make fast, effective decisions.

Using the OTTER system, all sample points in the field are marked with radio frequency identification tags. When field operators perform sample rounds, a handheld computer guides them to each sample point and then automatically records the required information, such as sampling time, and whether the sampling task is routine or non-routine. The LIMS also transmits safety related information, (such as the sample container to be used and special instructions for sampling) to the handheld computers to ensure the wellbeing of staff. As the LIMS is fully integrated with OTTER, the data collected is instantly transferred to SampleManager from the field for analysis by managers or technicians back in the lab, which also saves Pearl GTL an estimated 2,400 working hours per year.

It's interesting to note that all the benefits we achieved by implementing SampleManager are completely transferable to other industries, which Colin can explain in more detail.

Beyond the solution

Ajith has outlined nicely the implementation of SampleManager LIMS at Pearl GTL, an enormous accomplishment for both Shell and Thermo Fisher alike. Among the most important benefits of a fully integrated informatics solution is the agility gained in moving assets, reassigning personnel or streamlining production. In essence, the LIMS gives the business the ability to make more timely decisions.

The same principles are echoed in many other industries, for example, downstream of the oil refinery at modern chemical manufacturers. The need for agility is especially true in the production of fine and specialty chemicals, where plants may change over production lines weekly, sometimes even daily. Within these highly flexible environments, laboratories responsible for quality, safety and efficacy of finished products must be equally flexible, which is no simple task.

In the chemical manufacturing lab, LIMS isn't just the first step, it's the most important step towards achieving the agility required to meet more precise customer demands and more stringent regulatory requirements. Once in place, the business isn't simply capturing and collecting data; it's making that data actionable across the enterprise. With the LIMS enabling this type of data mining, management can move more quickly to respond to market trends or new regulations or to recognize and capitalize on cost-saving or margin-growing opportunities.

For businesses to compete in manufacturing, they need to liberate the insights that are too often kept in silos across the site or around the world. Opening up these vast stores of knowledge to the benefit of the enterprise can improve manufacturing technology in new ways. Your laboratories can become real growth drivers for business transformation and enable your business to rapidly capitalize on new growth opportunities and build lasting value, customer loyalty and security for employees.

Ajith Kumar is a senior business analyst at Pearl GTL, Qatar, and Colin Thurston is informatics project director at Thermo Fisher Scientific, Manchester, UK.

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Achiral-Chiral Heart-Cutting 2D-LC Analysis of Chiral Pharmaceutical Substances

Impurity analysis with simultaneous determination of enantiomeric composition using the Agilent 1290 Infinity 2D-LC Solution

By Sonja Krieger, Udo Huber

Introduction

According to ICH guideline Q3A (R2), impurities in new drug substances at levels of 0.05 % or above must be reported, and impurities at 0.1 % or above must be identified. Enantiomers of chiral drugs often show differences in pharmacokinetic behavior and pharmacological activity. One enantiomer might be pharmacologically active, while the other might be inactive, or even toxic. Therefore, the FDA has released guidance on the development of new stereo-isomeric drugs, demanding that the stereo-isomeric composition of a drug with a chiral center is known, and that specifications for the final product include assurance of purity from a stereochemical viewpoint.

Results and Discussion

The analysis of impurities contained in pharmaceutical substances can be accomplished by subjecting a concentrated solution of the substance to liquid chromatographic analysis. Impurities separated from the pharmaceutical substance are detected as small peaks next to a large peak originating from the main compound.

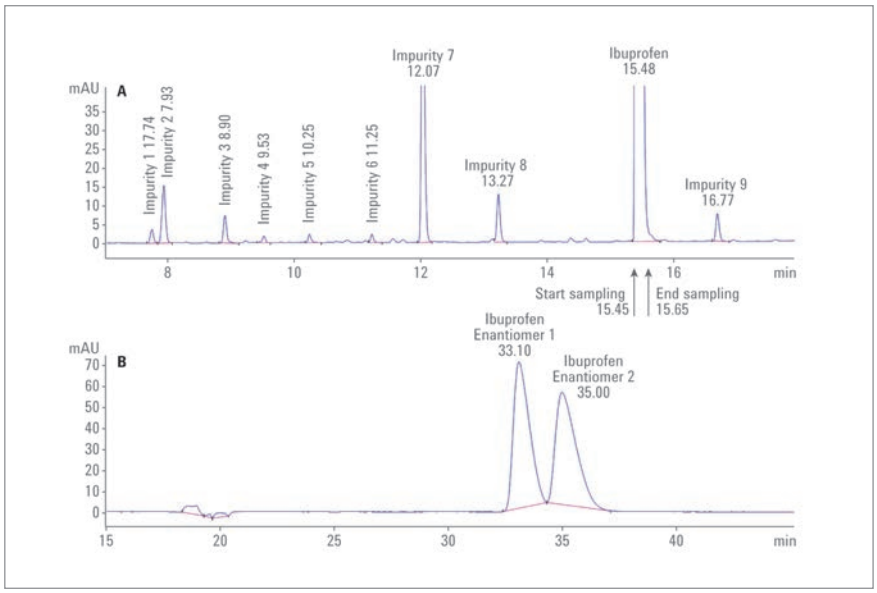

Figure 1. A. Separation of ibuprofen and impurities 1–9 on the first-dimension reversed phase column and B. heart-cutting of the ibuprofen peak and transfer to the second-dimension chiral column for separation of the enantiomers.

Racemic ibuprofen was chosen to prove the principle of the analysis of impurities in chiral pharmaceutical substances with simultaneous determination of the enantiomeric composition of the API. Figure 1A shows the chromatogram resulting from the first dimension reversed phase analysis of ibuprofen. Here, several impurities are separated from the main compound.

The effluent from the first-dimension column was sampled at 15.45 minutes with a loop fill time of 0.20 minutes to transfer the ibuprofen peak to the second-dimension chiral column and enable separation of the enantiomers. Figure 1 shows when the effluent of the first-dimension column was cut and transferred to the second-dimension column (A) and the separation of the ibuprofen enantiomers on the second-dimension chiral column (B). The ibuprofen enantiomers were separated with a resolution of Rs = 1.25 on the second-dimension chiral column.

Conclusion

This article demonstrates that the Agilent 1290 Infinity 2D-LC Solution is ideally suited for the analysis of impurities in chiral pharmaceutical substances and for the simultaneous determination of the enantiomeric composition of the API. In the first dimension, a reversed phase separation was used to separate achiral impurities from the API. A heart-cutting experiment was used to transfer the API to a second-dimension chiral column for determination of the enantiomeric composition.

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Measuring the Opacity of Plastic Tubing with Vis-NIR Transmission Spectroscopy

Plastic tubing is available in a variety of opacities ranging from clear to translucent. The transparency of plastic tubing varies for reasons such as providing contrast for visual monitoring of fluid flow, decreasing exposure to ambient light, and making the tubing more distinct for machine vision technology. Visible-NIR transmission spectroscopy is used to assess the amount of frosting applied to plastic tubing to determine if the tubing meets the required opacity level.

By Yvette Mattley, Ph.D. and Ruud Niesen

Background

Plastic tubing is used everywhere – from the beverage dispenser at your favorite restaurant to the gas and liquid delivery lines in life-saving medical devices.

In many applications that employ plastic tubing, visual contact with the flowing material is required to confirm flow and check for bubbles. Visual monitoring is facilitated by enhancing the contrast between the fluid and plastic tubing. Coatings, frosting and other surface modifications are used to vary the interaction of light with the tubing, making it easier to observe fluid flow.

Modular spectroscopy components can be used to assemble a range of setups to measure the interaction of light with plastic tubing. In the case of frosted tubing, where light transmission must be kept within a narrow range to provide the desired tubing characteristics, Vis-NIR transmission measurements provide a straightforward method to assess frosting level.

Measurement Conditions

Samples of five plastic tubes with varying levels of frosting were used for the analysis (Figure 1). Details on the frosting level for each sample are provided in Table 1.

Transmission measurements were made using an enhanced sensitivity Vis-NIR spectrometer. The tubes were placed between two collimating lenses in a transmission setup and adjusted until the most reproducible orientation was found.

Table 1: Frost Level Analysis of Plastic Tube Samples

Sample	*Status*	*Reason for Rejection*
0	Reject	Excessive frosting
1	Pass	NA
2	Pass	NA
3	Reject	Insufficient frosting
4	Reject	Insufficient frosting
5	Reject	Insufficient frosting

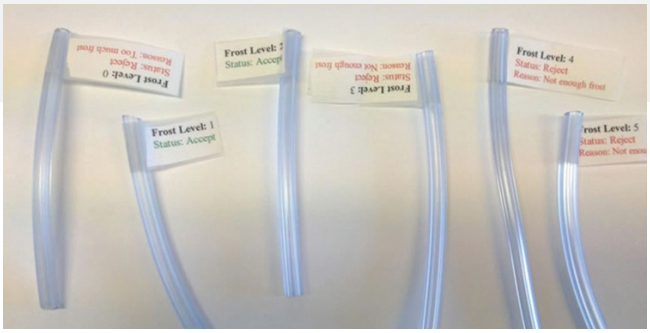

Figure 1: Although differences in the frosting levels of plastic tubing samples are difficult to distinguish visually, spectroscopic methods are highly reliable in characterizing tubing properties.

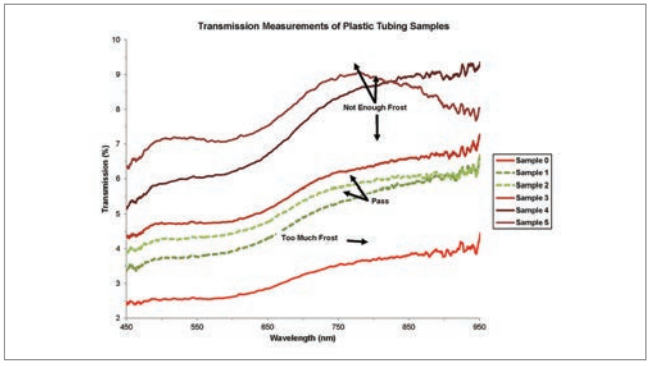

Figure 2: Transmission characteristics of plastic tubing samples varied by the amount of frosting applied to the surface of each sample.

Results

The transmission spectra measured for the frosted plastic tubing samples are shown in Figure 2. The transmission intensity measured for these samples correlates with the frost levels reported for the plastic tubing in Table 1. Note that even though the transmission intensity for Samples 1, 2 and 3 is very similar, the transmission spectra are sufficiently different to reject Sample 3 as having insufficient coating.

Visual observation of the plastic tubing samples showed that the frosting level for each sample was difficult to distinguish (Figure 1). This underscores the value of spectroscopic transmission measurements to discriminate plastic tubing with very similar frosting levels.

Conclusions

The power of Vis-NIR transmission measurements to discriminate tubing samples is demonstrated by the different transmission intensities measured for plastic tubing samples with similar frost levels. The ability to discriminate samples separated by less than 1 % transmission make this technique a good option for use in QA or QC methods to ensure the plastic tubing has the desired light interaction properties and characteristics.

Science and The Great Beauty

Sitting Down With… Salvatore Fanali, Head of the Capillary Electromigration and Chromatographic Methods Unit at the Institute of Chemical Methodologies, National Research Council (CNR), Monterotondo, Italy.

How did you get into capillary chromatography?

Actually, I was a high school chemistry teacher for 13 years before I joined the CNR in 1983. In those early days, I was using paper electrophoresis for chiral separations. I then moved briefly onto capillary isotachophoresis before constructing a very simple capillary electrophoresis (CE) instrument in 1988. I was one of the first to publish separations of chiral drugs using CE – something that is still very memorable for me.

Could you tell us about your research now?

My group focuses on fundamental research in separation science, which includes method development but also the study of new stationary phases, such as core-shell particles, for chiral separations. Equally important is the application of new methodologies to practical problems in areas such as food, environmental or forensics analysis.

At five persons, my group is not very large, but we are totally engaged in optimizing techniques, such as capillary electrochromatography (CEC) and nano-LC. Some say CEC is dead, but I do not believe so. It combines the best features of both HPLC – high selectivity – and electrophoresis – high efficiency. We've applied CEC to chiral separations in a number of currently hot areas. By separating L and D forms of amino acids in fruit juice, for example, we can flag suspect samples that may have been subject to adulteration. And in forensic analysis, the enantiomeric ratio of illicit drugs can provide important clues as to the origin of the compound.

For capillary and nano-LC, we've assembled our own systems and routinely pack our own columns with diverse particle types (for example, HILIC, phenyl-hexyl, and silica derivatized with glycopeptide antibiotics).

Miniaturized techniques tend to be greener than other approaches. Is that important to you ?

I think we should all more deeply consider our ecological impact. In that regard, the miniaturized techniques we focus on do only use a few microliters of mobile phase and nanoliters of sample; the knock-on effect is reduced waste, which is good for the environment – and reduced costs.

What are your plans for the years ahead?

I have a few years to think, but I will probably continue my research even after retirement. I have a great group and they seem to like working with me. But then again, I am based in the wonderful city of Rome and love walking. I've noticed that whenever I wander the streets, I seem to find something new; it would be nice to have more time to rediscover my city – especially having enjoyed the film "La Grande Bellezza" [The Great Beauty] so much, which was, of course, based in Rome. You know, if you walk into a church in Rome, you may be greeted by the paintings of famous artists like Caravaggio for free. Why? Because this art is for the people!

And should science be similarly for the people?

That's a tricky question! It's true that researchers often work with funding from the state and have certain obligations, but I have to say that we, as researchers, must satisfy our own needs and desires first – after all, we often sacrifice time with friends and family to find success – and that can only happen when we are truly happy in what we do. Hopefully, any success ultimately benefits mankind.

Congratulations on receiving the Giorgio Nota Award at Riva 2014!

Thank you. It was a very big surprise. I only learned about the award a few days beforehand. Giorgio Nota was a professor at the University of Naples and was the first to publish on applications of capillary chromatography. The Award recognizes an outstanding contribution to capillary chromatography, so I feel very proud.

Did you ever meet Nota?

Yes, in the 1980s. He had an interest in thin layer chromatography but I had different interests and missed the opportunity to collaborate. I remember him well and know that he was a brilliant scientist and excellent teacher.

You thanked many people in your acceptance lecture…

Yes, it was a long list from all over the world – and included many of the PhDs, post PhDs, and collaborators I have had the pleasure to work with over the years. Many of them are good friends that have gone onto great things – but not always in chemistry. One ex-PhD is head of security at Vienna airport and, whenever I pass through, I always visit him – even after over 20 years. After all, human exchange is one of the most important aspects of science. We should all be able to smell the humanity in what we are trying to construct together.

I lost my wife last year and that's still very fresh in my mind. I dedicated the award to her because she was the one who pushed me to move from teaching and into the position at CNR; she knew that research was what I really wanted to do. I have to say, I've never once looked back. In Italy, we say, "behind every great man is a great woman" – I didn't say that in my speech, because I didn't want to appear boastful – but I did want to stress that I owe any success to her. And if she was still alive today, I am sure she would have been here in Riva to share it with me.

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