The Next Big Thing In Chromatography?

Find out how microscale chromatography is making a big splash in analytical science

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Technology with a Real Edge

Since scooping a TASIA in 2017 with their µPAC™ technology, PharmaFluidics has made a real splash. We caught up with CEO Johan Devenyns to find out what's new at "the micro-chip chromatography company."

For any readers unfamiliar with PharmaFluidics, could you give a little background?

PharmaFluidics was formed as a spin-out at Vrije Universiteit Brussel in late 2010 and set up shop as a microfluidics consulting boutique. Since 2014, we've focused exclusively on microfluidic devices for chromatography. In late 2017, we launched our flagship product – the µPAC™ nano-LC column – onto the market, which immediately attracted a lot of interest, including an innovation award from The Analytical Scientist! In brief, the µPAC™ is a microfluidic chromatography column with its pillar array stationary phase backbone etched out of silicon wafer; this technology provides exceptional resolution, sensitivity, robustness, reproducibility and retention time stability. Following feedback from our users, we added the µPAC™ Trapping column to our portfolio in 2019.

µPAC™ at the Edge

As uptake of µPAC™ grows, the technology is contributing to exciting advances in biology and beyond. Here are just three projects that hit the headlines in 2019...

Tree of Life

At the EMBL Wellcome Genome Campus Conference in March 2019, the Matthias Mann Group (Max Planck Institute, Munich, Germany) presented the quantitative proteome atlas of 100 organisms across all three kingdoms, fingerprinted thanks to the high retention time stability and reproducibility of the µPAC™. The Tree of Life is the largest open access proteome data set ever reported, with more than 250,000 proteins, and growing.

Labs around the world can use the open access database together with µPAC™ and machine learning to predict a retention time fingerprint for each individual protein in the Tree of Life – the potential for hyper-resolved target data deconvolution is immense.

Doubling Up on Single Cells

Single-cell proteomics is poised to revolutionize many fields of biological research, with important implications for therapeutics, discovery, genomics and translational research. In a presentation titled "Double protein IDs in Single Cell protocols", Karl Mechtler (Institute of Molecular Pathology, Vienna) explained how his group have identified 3,500 proteins in a 10 ng HeLa cell sample using the µPAC™ LUMOS workflow at EUPA Potsdam in March 2019.

Deep-dive DIA

Also at EUPA Potsdam, Lukas Reiter (BIOGNOSYS, Schlieren CH) presented results from 9,000 quantified HeLa proteins, covering five orders of magnitude in a µPAC™–HFX–DIA workflow. Single-shot quantitative deep-dive µPAC™-DIA proteomics has great potential for pathway elucidation and endpoint design during clinical trials, and later in body fluid multiplex/panel proteome diagnostics.

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Proteomics researchers have been keen early adopters of the technology – why?

Having decrypted the genetic heritage of numerous species (including humans), biologists now want to characterize the totality of the proteins in an organism or even a single cell. Technically, this is extremely difficult because of the proteome's complexity and highly dynamic concentration range – and the absence of amplification techniques comparable to PCR; it is not surprising that researchers in this field welcome new technology that can make the task a little easier. The uniform pillar array backbone of the µPAC™ minimize peak dispersion, and hence allows for the longer gradient separations needed for complex biological samples, for example by eliminating the need for prefractionation (read more about proteomic analyses with µPAC™)

What's new for PharmaFluidics?

The company is steadily expanding as µPAC™ moves into more labs. It's been great to hear from our customers about the difference it is making to their research and to feel that we have played a part in some truly groundbreaking advances (see "µPAC™ at the Edge").

We have expanded our operations, and we will be commissioning, by fall 2019, a second dedicated assembly and QC line at our Ghent facility.

A special highlight of 2019 so far has been receiving the European Proteomics Association (EUPA) Award. We are very grateful to EUPA and the proteomics community for recognizing the contribution of µPAC™ to improving and standardizing nanoLC/MS proteomics workflows. It is an achievement that belongs primarily to our pioneer users and product champions, who are setting new standards, breaking records and advancing the field of proteomics.

What's next?

Looking to the future, we are focused on two main growth areas: deepening our reach in the proteomics space and broadening access to our technology in other chromatography segments by accommodating higher-flow-rate regimes.

The company has become well known as a solution provider for deep-dive proteomics, where sensitivity is paramount. As proteomics moves closer to the clinic, other benefits of µPAC™ – namely, retention time stability and reproducibility – have started to attract more attention. For longitudinal, large-cohort or multiple-site studies, the robustness and reproducibility of all steps in the HPLC-MS-proteomics workflow are of paramount importance, as this is critical to build large data pools with maximum statistical power and minimal noise and bias. Many of the greatest workflow challenges for large-cohort (clinical, diagnostic) proteomics relate to lab-to-lab reproducibility, method standardization, and scalability – all areas where µPAC™ has a real edge.

Currently, µPAC™ is mostly used in nanoflow regimes, so the second focus is to move into areas that are using higher-flow-rate regimes, such as routine biopharma applications. Building on the design and engineering of the popular µPAC™ Trapping columns we launched earlier this year, we introduced our capLC µPAC™ column at ASMS 2019, and start taking pre-orders for deliveries in September.

Has the company faced any "growing pains"?

Early on, it was clear that high-end proteomics was the most suitable entry point into the analytical HPLC market, compatible with PharmaFluidics' capabilities and limitations. Having a clear focus on one high-end, niche market proved to be the right thing to do, allowing PharmaFluidics to get a place on the scene and to grow at a manageable rate. Now that we have further developed our manufacturing and supply chain resources, we are ready to take on broader market segments.

Our customers are now spread over a much wider area, which makes our operations more complex. Although it makes for long days, it's a nice problem to have!

µPAC™ 101: What's so different?

Paul Jacobs, PharmaFluidics COO, explains.

Conventional columns are fabricated by stacking (packed beds) or depositing (monoliths) material into a capillary – the resulting random structure of the solid phase results in an irregular flow path through the column, which causes peak dispersion. Furthermore, back-pressures in packed bed columns rise quickly as column length increases (the touching surfaces of the beads block the flow of the mobile phase). These physical limits hamper the analysis of small, complex biological samples, which require long gradient separations. Such samples are the standard in the growing fields of proteomics, metabolomics and lipidomics, so, historically, scientists have resorted to prefractionation to extract sufficient data, which is labor intensive and wasteful of the sample.

On the other hand, µPAC™ separation beds are formed by carefully etching away interstitial volumes out of a silicon wafer support, leaving an array of pillars. This is done by using standard photo-mask and lithographic techniques very similar to those used for the production of the micro-electronic chips in computers, telephones, and so on. The resulting stationary phase support structure, which we call the "backbone," is organized in a reproducible, highly-ordered pattern, eliminating heterogeneous flow paths found in conventional columns. As a result, peaks are sharper with higher resolution as a consequence, and sensitivity is also increased.

The micromachined backbone of the separation bed forms a rigid structure that is not influenced by pressure. There are no obstructions caused by touching surfaces, and there is no risk of perturbations by pressure fluctuations. And because we're not limited by backpressure, we can create columns of unprecedented length (up to 2 m right now) in a tiny footprint, by interconnecting several separation lanes using carefully engineered (and proprietary!) turn structures that do not contribute to peak dispersion.

The current generation of µPAC™ technology is designed to offer more information from long gradient separations; no prefractionations are needed, as higher numbers of sharper peaks can be identified with higher sensitivity in a single run, saving labor costs and reducing small sample consumption. These unique advantages led us to focus on systems biology for the initial applications of µPAC™ columns. For example, highly detailed characterization of protein expression in cell populations or the concentration of certain metabolites.

We believe µPAC™ technology will also have an impact on the development of biological drugs. For peptides or antibodies, small modifications (such as glycosylations or deamidations) can have a tremendous influence on the therapeutic efficacy of a given compound. The ability to characterize a biological drug more thoroughly is of substantial value for biologic originators, for strain selection, process development, and ultimately also for tracking GMP production. For development and regulatory filing of biosimilars, precise analytical data can be used as a substitute for extensive additional clinical studies.

In forensic investigations, µPAC™ technology can also prove its worth by extracting more information from the minute sample volumes that typically constrain analyses in this field.

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Perfecting Proteomic Separations

μPAC™ promises high-resolution separations of complex biological samples. Kris Gevaert and Francis Impens, from Ghent University and the VIB Proteomics Core, embedded in the VIB-UGent Center for Medical Biotechnology, have been trialing the new column. We caught up with them to find out more about their work, and how μPAC™ can expedite routine analysis.

How did you get involved in proteomics research?

Kris: I did my Master's thesis in a proteomics lab in the mid-1990s, and have been in the field ever since. I became a professor at Ghent University in 2004 and a group leader at VIB in 2005. Proteomic technologies developed in our lab are made available to other researchers via our VIB Proteomics Core.

Francis: I'm a biomedical scientist by training. It was Kris who introduced me to proteomics – I did my PhD thesis in his lab. After a stint at the Pasteur Institute in Paris, working on bacterial pathogens, I returned to Ghent University and VIB in 2015. I manage the proteomics core facility – a spin-out from Kris' lab. I also have a small research group "on the side," which focuses on host–bacteria interactions using proteomics technologies.

What motivates you?

Francis: For me, it's the strong belief that this technology is enabling real discoveries, as we witness on a weekly basis. There's still so much to discover – novel interaction partners, novel protein modifications, novel biology!

Kris: I also enjoy the opportunity to work with scientists in a variety of disciplines, from plant biologists to clinical scientists.

What recent trends have you noticed in the daily routine of the proteomics center?

Francis: Proteomics technology as a whole has matured, with a very rapid evolution of mass spectrometry instruments, separation instruments, and supporting technologies. It's a very different landscape compared with just a decade ago. As a consequence, some proteomic analyses (such as shotgun analysis for biological samples and mapping protein interaction after affinity purification) have become routine. Of course, there are still many novel kinds of analyses, which are still very much dependent on the expertise of single labs or even single people.

Kris: There are certainly some exciting new techniques that allow us to focus on aspects of a protein that we could not study some years ago. In my lab, we invent or trial such techniques to master them and then transfer them back to our Proteomics Core as a new tool for the wider user community.

What are the biggest limitations in proteomics?

Francis: With shotgun analysis, we can only routinely measure about half of the proteins expressed in the average mammalian cell. And that is not taking into account the millions of different proteoforms that could also exist. The question is: do they all have meaning? To me though, it's not a limitation – it's a challenge.

Will the resolving power of mass spectrometers eventually eliminate the need for separation technology?

Kris: The short and simple answer is no. We will always need resolving power, be it LC or other types of chromatography. It is simple mathematics: there could be hundreds of thousands of different peptides in a sample – and it's impossible to identify that number of molecules without separations.

Can improvements in separation technology help overcome the challenges facing proteomics?

Francis: In my view, improving separations is key. Mass spectrometry has seen huge advances over the past 10 years, for example, Orbitrap technology and hybrid machines. To help mass spectrometry reach its full potential, we need to see advances in fractionation too – smaller peaks, more resolution, and higher peak capacities can only increase the overall performance of the analysis. To that end, we have been excited to trial the new micro Pillar Array Column from PharmaFluidics.

How can µPAC™ technology improve proteomic analyses?

Francis: Personally, I think it has the potential to revolutionize the field of mass spectrometry-based proteomics. It is the type of technology that speaks for itself – you can look inside the column, see the perfectly ordered structure, and understand exactly how it increases separation power.

Kris: In comparison with traditional columns, µPAC™ columns result in more sensitive analysis, so you need less material to identify the same number of proteins. Another positive of the µPAC™ columns is the ease of use – it's very quick and efficient to put a µPAC™ column in front of your mass spectrometer. The back pressure is very low, meaning that you can work with long columns that allow greater sensitivity.

The µPAC™ columns have been designed by scientists who have been in the field of chromatography for decades, so we felt confident in agreeing to collaborate with them to implement the technology.

How have you applied µPAC™ technology?

Kris: So far, we have been evaluating them for routine shotgun analysis, where we try to gain a total protein profile. Compared with traditional columns, we see an overall increase in identifications, both on the peptide and the protein level, which results from the improved LC parameters that you can readily see just by looking at the chromatograms.

Beyond routine analyses, we are excited to try out µPAC™ technology as part of a wide range of proteomic methods. These columns have already made it possible to do incredibly long runs of eight or ten hours – something that was previous unheard of. We're looking forward to finding out what else we can do that we haven't been able to do before! In the beginning of 2019 we decided to equip all high-end MS instruments in our facility routinely with µPAC™ columns.

In the longer term, where do you expect µPAC™ technology to have the greatest impact?

Francis: One of the biggest gains the company reports is in analyzing minute amounts of sample – µPAC™ boosts the performance of the mass spectrometer by keeping peptide concentrations undiluted and high prior to ionization. This particular aspect could be a game changer for analysis of small samples; for example, in biomarker discovery.

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Chromatography Peaks in Silicon Valley

When is a column not a column? We tell the origin story behind PharmaFluidics.

By Rich Whitworth

"Quietly confident." That's how I would describe the team at PharmaFluidics and, in particular, its Managing Director, Johan Devenyns. Despite all the signs that a disruptive – but minimally dispersed – wave is about to break on a very "particula" (Latin pun, folks) liquid chromatography community, Johan is keen to attract the right kind of attention, by allowing the technology to speak for itself rather than shouting about a "microchip revolution" from the rooftops.

I admire his restraint. After all, at HPLC2017 in Prague, I had a number of conversations about a young spin-off company that was "shaking things up" – with some people expressing open concern about the future of "regular" columns.

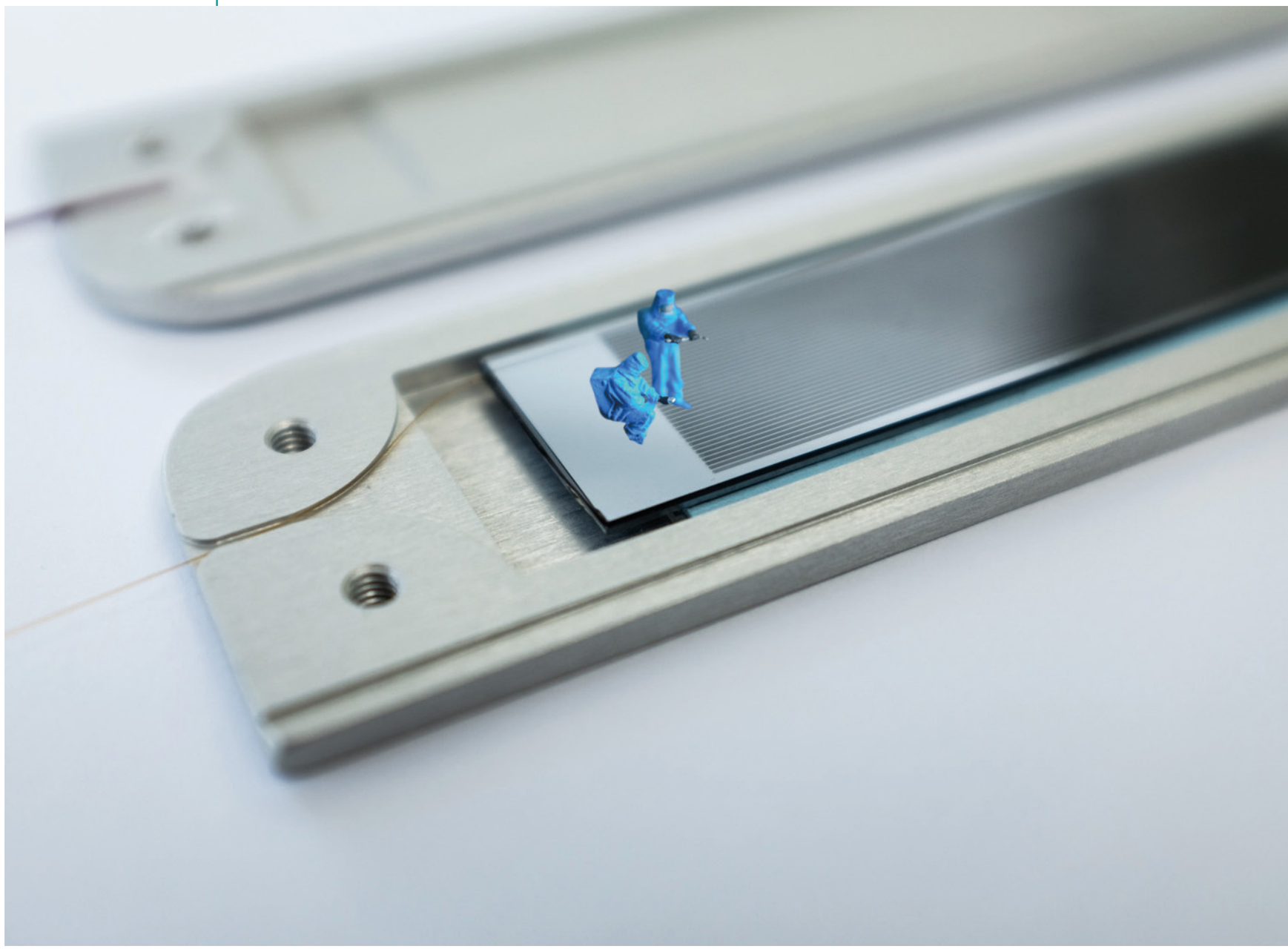
Meanwhile, Gert Desmet (one of the co-founders of PharmaFluidics) makes regular appearances in the upper echelons of The Analytical Scientist's annual Power List, and it feels like momentum is building for change.

I sat down with Johan to learn more about the technology, the company, and its plans for the future.

A brief history of μPAC™

Always wanting to work at the very limit, it was Desmet's exhaustive computational fluid dynamic simulations – probing the effect of column order (or disorder) on chromatographic performance – that laid the foundations for PharmaFluidics' micro Pillar Array Column (μPAC™) technology. In fact, Desmet's theoretical research in this space is ongoing – and he is now using even more complex three-dimensional models in his pursuit of "the perfectly ordered column".

With Desmet's concept in hand, Wim De Malsche took on the not insignificant challenge of transposing theoretical models into a practical material substrate in the mid 2000s. By using lithographic micromachining processes to etch out interstitial volumes in silicon



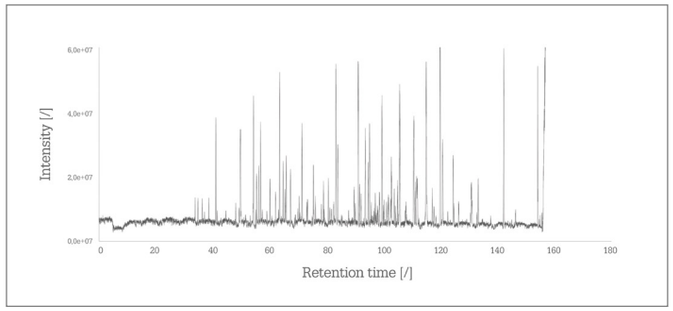


Figure 1. Basepeak chromatogram acquired on a PharmaFluidics *µ*PAC™ column coupled to a Thermo Fisher Scientific Orbitrap Fusion Lumus tribrid mass spectrometer. Only 10 ng of tryptic Hela cell digest sample was injected, which corresponds roughly to the protein content of 50 cells. Over 3,000 unique protein groups could still be identified in a single 2h gradient run.

wafers to create an ordered and reproducible array of pillars, De Malsche was able to give form to Gert's vision – but there was more work to do. And so PharmaFluidics was co-founded by De Malsche, Desmet and Paul Jacobs in late 2010 as a spin-off company from Vrije Universiteit Brussel (VUB) in Belgium, to bring a radically different column to the market.

The Pittcon promise

After a relatively quiet first few years, PharmaFluidics became much more visible in 2017, with an enthusiastic team attending more than a dozen events. "I think three landmarks stand out," says Devenyns. "The first, of course, was our 'premiere' at Pittcon, when we launched the first generation of *µ*PAC™ technology."

All commercial launches aim to make some sort of a "splash", but Devenyns recalls how overwhelming it was to capture so much attention as a small company introducing its first product. "The first day, we attracted a crowd of R&D experts; the second day, R&D managers came to see us," he says. "And by the end of the second day, we were being visited by decision makers and top executives." An excellent start – but also daunting, says Devenyns. "The extremely positive response to the launch really highlighted the need for us to follow through on the promises we made in terms of getting *µ*PAC™ technology out into the market..."

The second "landmark" was the pricing of the *µ*PAC™ column, which made it a tradable item and coincided with ASMS and HPLC in June. And in September 2017, PharmaFluidics held true to its Pittcon promise with a third landmark that coincided with HUPO in Dublin: the first commercial shipments of products. The most striking feedback has been about the robustness of the device, with some people reporting that *µ*PAC™ columns are being used for many more cycles than was expected – perhaps to the slight consternation of a consumables company!

A band of pioneers

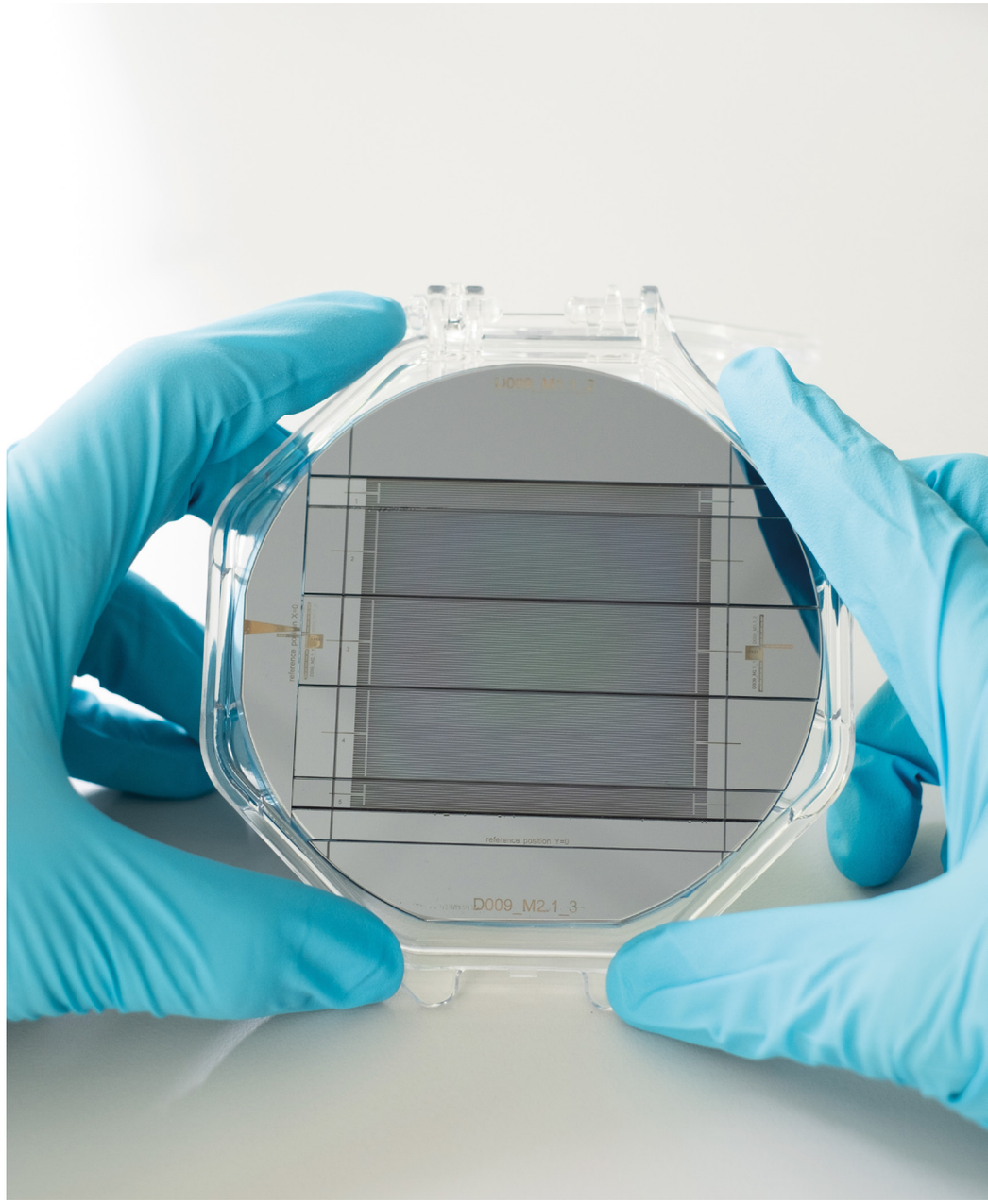
Bringing together a strong network is essential for the wellbeing and further development of any company – especially for "David" facing several "Goliaths," as is the case with any new chromatography column manufacturer trying to break into a busy market.

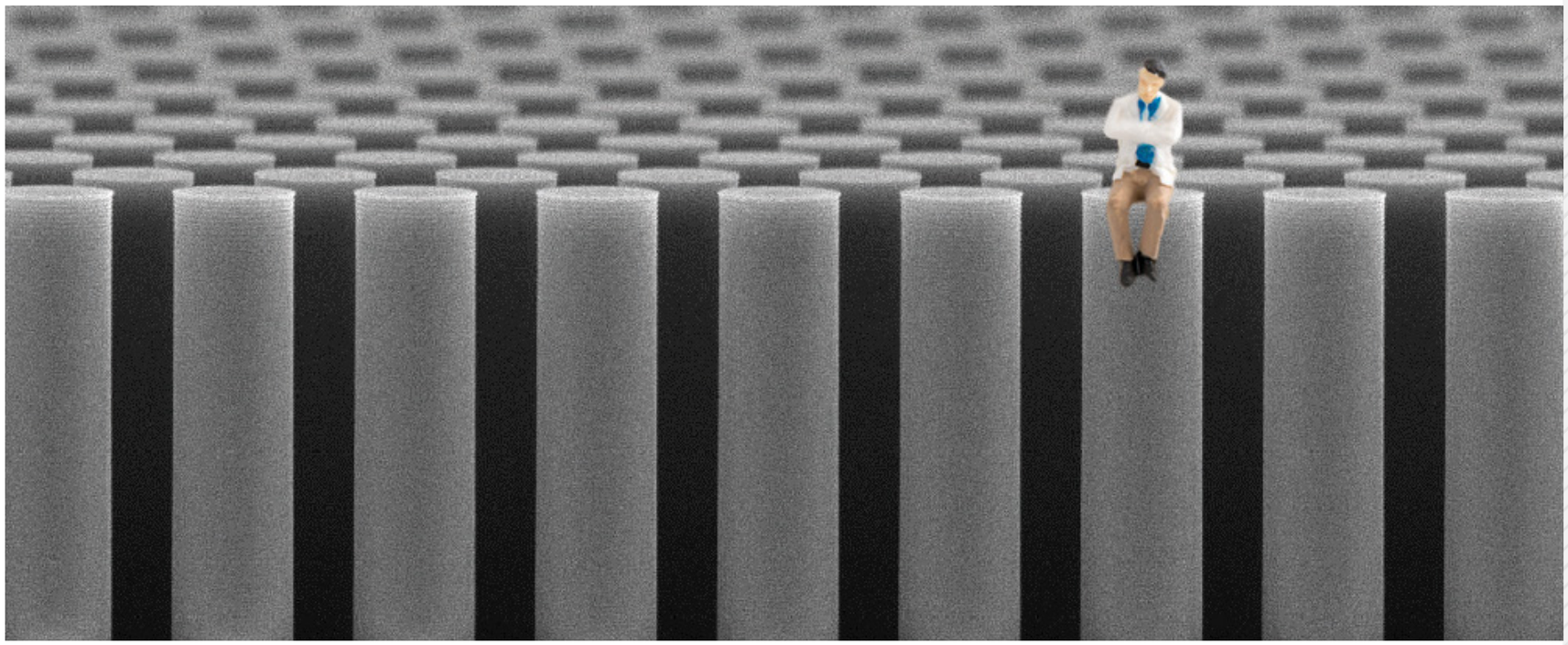
Most analytical scientists – and all chromatographers – will be familiar with the gurus joining Desmet on PharmaFluidics' strategic advisory board (SAB). "Pat Sandra is a veteran of the industry and brings with him great technical and scientific skill, but he is also a very keen businessman," says Devenyns. "The same can be said of Jean-Pierre Chervet, who is very sharp from a scientific point of view, but also knows the industry inside-out, having built, sold and re-built several companies." Gerard Rozing is another very well-connected individual with an impressive and long career in chromatography. In October 2019, PharmaFluidics announced that Matthias Mann had joined the Advisory Board. Mann is a pioneer, eminent researcher and global authority in the field of proteomics.

"The SAB is just one visible part of our network, but provides us with clear guidance on when, where and how to move forward, both in scientific but also business terms," Devenyns says.

PharmaFluidics is also supported by a board of directors, made up of co-founders and investor representatives. Another familiar face joined in 2017: Fasha Mahjoor, who made big news in 2016 by securing Phenomenex's acquisition by Danaher Corporation. "Fasha always offers plenty of words of wisdom, in addition to his famous sense of humor," says Devenyns. "Beyond his strong business acumen and knowledge of the market, Fasha is a colorful character with an enchanting personality. I think it's fair to say that he brings a further innovative twist to the profile of the company!"

According to Devenyns, the strength of the team extends well beyond the more visible members of the boards; only the talented and passionate survive when gunning for success as a small but fast-growing enterprise. "Everyone in the company has been hand picked – and I think we all feel privileged about the core team that now stands together."





Starting small (with big plans)

PharmaFluidics' initial focus has been on nano LC, which has seen the technology being first embraced by the omics and biopharmaceutical communities, to aid in the extraction of sufficient information from complex samples. "It is a natural entry point for a technology that pushes the limits," says Devenyns. "A rather wonderful match exists between the relatively small but 'high-end' world of omics – a community that welcomes enabling technology – and the intrinsic status of µPAC™ technology at launch, as well as the size of our company as we scale up production."

The company will continue serving the needs of the omics community by working on the current generation of chips and on ancillary products that will support the µPAC™ itself for example, the company released a trapping column in 2019.

However, Devenyns sees opportunities in all segments of chromatography – and the team is working very hard behind the scenes on the development of a next generation of products that will target more routine analytical HPLC and UHPLC applications – in pharma and beyond.

"Right now, we're targeting applications that require ultra-high resolution, so the current generation µPAC™ product specifications aim for half a million plates to reflect that need," says Devenyns. "As we move into more routine applications, we will leverage the same technology – or rather the same performance – on a plates-per-minute level, which will position µPAC™ technology very competitively in labs that are time constrained." In other words, PharmaFluidics is able to tailor chips to meet performance expectations, whether they be resolution, sensitivity or speed oriented.

"When PharmaFluidics succeeds, chips will no longer be just for computers, you will find them in diagnostics, drug development, and progressively more analytical workflows," says Devenyns. "As Emily Hilder and Robert Shellie predicted (1), the future of separations technology will indeed be 'smaller, faster, smarter.'"

Seven Fast Years at PharmaFluidics

December 2010
PharmaFluidics incorporated as a VUB spin off

Q1 2014 and Q2 2015
Capital increase from Flemish Government, PMV and QBIC

Q4 2016 and Q1 2017
Capital increase from QBIC, PMV, Innovation Fund, Theodorus III Fund and Volksvermorgen

March 2017
µPAC™ nanoLC column launched at Pittcon 2017

June 2017
Product priced

September 2017
First units shipped

December 2017
Capital increase from existing and new shareholders, including FPIM and family offices of Biotech entrepreneurs Rudi Mariën and Annie Vereecken

September 2018
EASY-Spray™ OEM agreementt with Thermo Fisher Scientific Inc

February 2019
µPAC™ Trapping column launched

June 2019
µPAC™ capLC column presented at ASMS

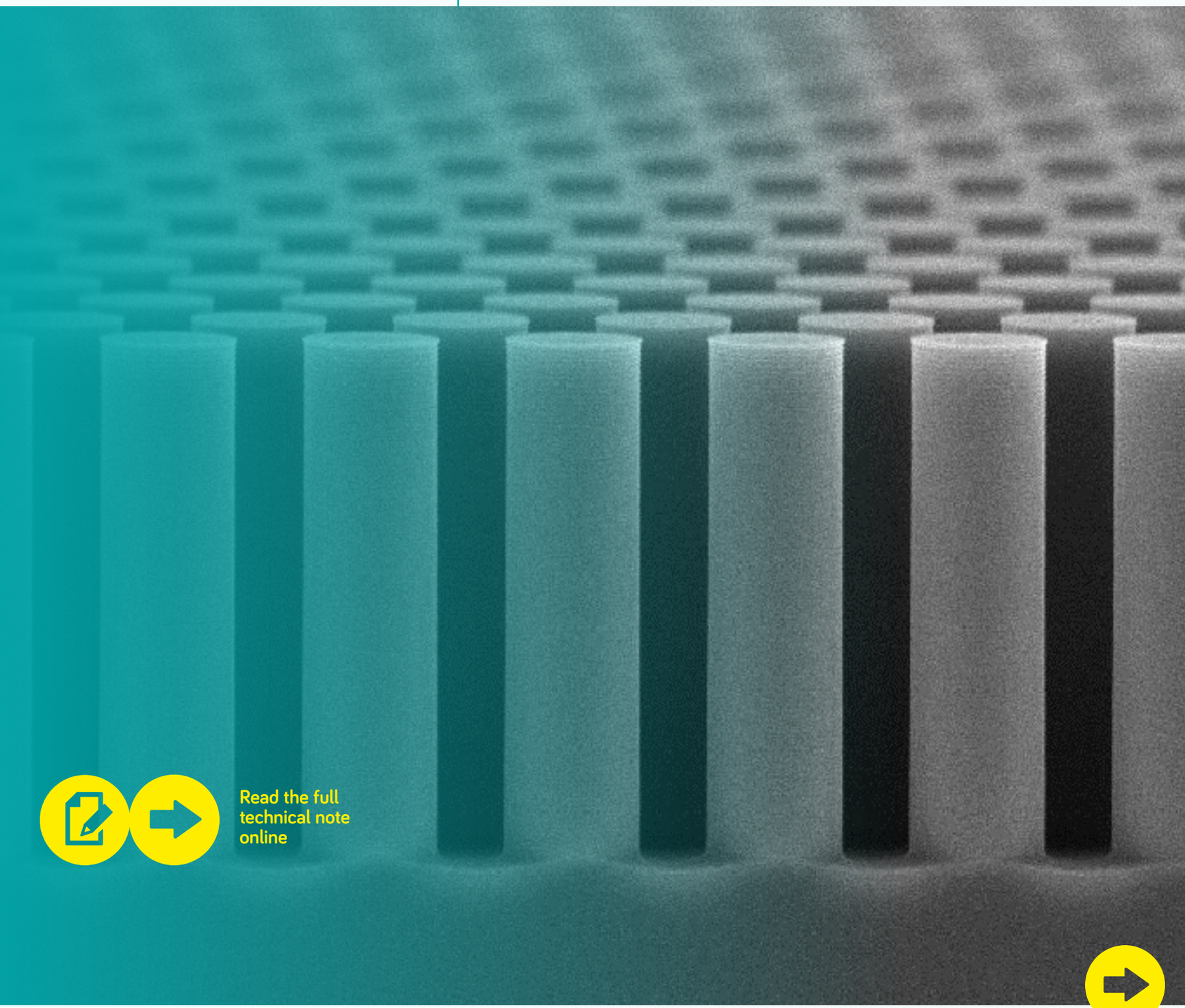
µPAC™ Column Robustness in Bottom-up Proteomics

LC column failure is one of the most frequent causes of LC-MS system downtime - how can nano LC-MS/MS help?

Many factors contribute to the challenge of maintaining LC-MS systems. In addition to technical issues that can occur with the LC-MS system's hard- and software, LC column failure is one of the most frequent causes of LC-MS system downtime. The telltale signs of LC column failure? An increase in column backpressure, deviating peak shapes or gradual shifts in retention time.

As an alternative to classical packed-bed LC columns, PharmaFluidics offers micromachined nano-LC chip columns or micro Pillar Array Columns (µPAC™) that distinguish themselves in a number of ways. The inherent high permeability and low 'on-column' dispersion obtained by the perfect order of the separation bed makes µPAC™-based chromatography unique. The peak dispersion originating from heterogeneous low paths in the separation bed is eliminated (no A-term contributions, meaning components remain much more concentrated (sharp peaks) during separation. The freestanding nature of the pillars also leads to much lower backpressure, allowing the use of very long columns. The result is a high-performance nano-LC column that is very robust and less prone to sample-related column failure.

To demonstrate this, a single µPAC™ column was operated under standard bottom-up proteomics conditions over a period of 6 months. Sequential injection of a HeLa cell tryptic digest, a blank and a Cytochrome C tryptic digest sample was performed to evaluate column robustness. In addition, several sample sets that are perceived as challenging were injected to demonstrate the column's resilience to sample-related column failure.



Read the full technical note online

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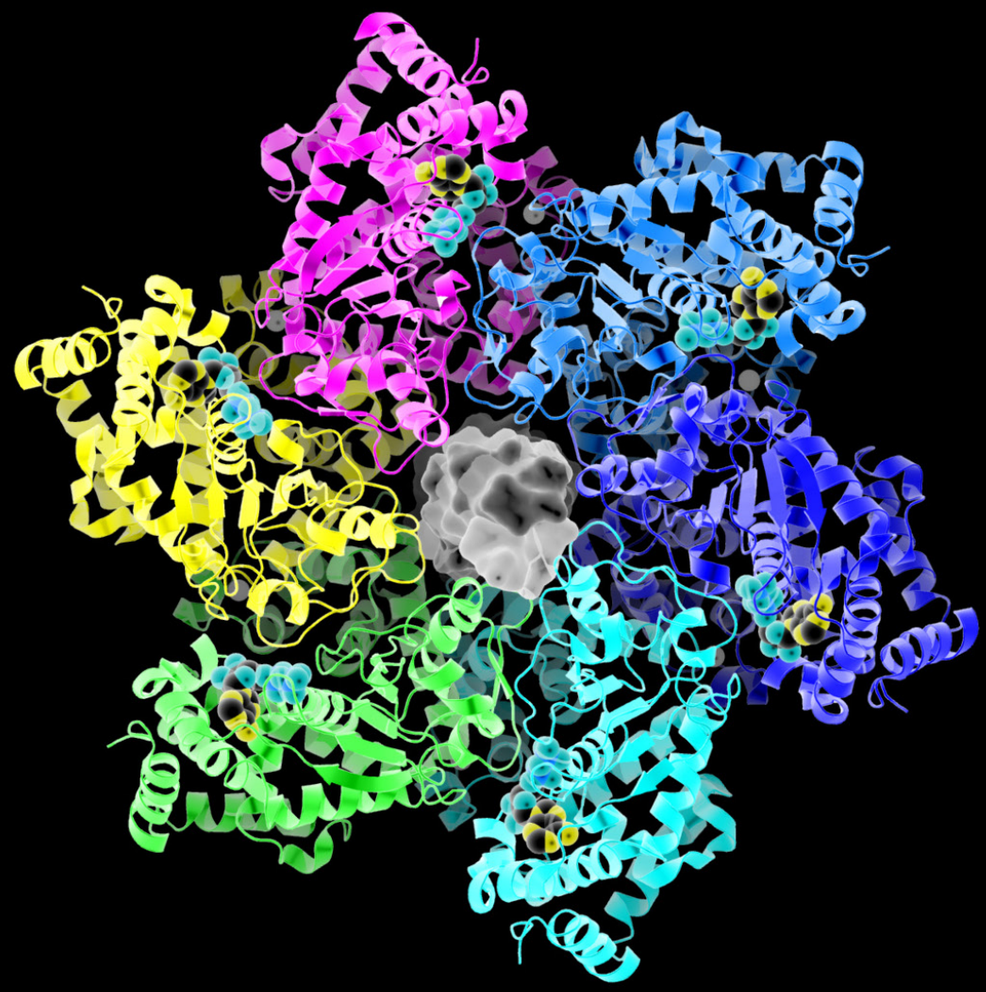
Non-Targeted Host Cell Protein Monitoring in Downstream Process Samples Using μPAC™-MS

Protein biopharmaceuticals have emerged as important therapeutics for the treatment of various diseases including cancer, cardiovascular diseases, diabetes, infection, inflammatory and autoimmune disorders.

They come in many flavors and include monoclonal antibodies (mAbs), antibody-drug conjugates (ADCs), bispecific antibodies, antibody fragments, fusion proteins, hormones, growth factors, cytokines, therapeutic enzymes, blood factors, vaccines and anticoagulants. Given their obvious benefits in terms of safety and effcacy, these molecules have substantially reshaped the pharmaceutical market and today over 350 products have been approved for human use in the United States and the European Union. Protein biopharmaceuticals are commonly produced recombinantly in mammalian, yeast or bacterial expression systems. Next to the therapeutic protein, these cells produce endogenous host cell proteins (HCP) that can contaminate the biopharmaceutical product despite multiple purification steps in a process. Since these process-related impurities can affect product safety and effcacy, they need to be closely monitored. This application note reports on the use of micro pillar array columns (μPAC™) in combination with mass spectrometry (MS) for the characterization of HCPs and their monitoring during downstream processing.



Read the full application note online

Monoclonal Antibody Peptide Mapping Using Micro Pillar Array Columns Combined with Mass Spectrometry (μPAC™-MS)

The use of micro Pillar Array Columns (μPAC™) for monoclonal antibody (mAb) peptide mapping is presented. Herceptin® (trastuzumab) originator and candidate biosimilar tryptic digests were analyzed on a 200 cm μPAC™ C18 column and peaks eluting were detected by ultraviolet (UV) spectroscopy and high-resolution mass spectrometry (MS).

Monoclonal antibodies (mAbs) have emerged as important therapeutics for the treatment of life-threatening diseases like cancer and autoimmune diseases. Compared with small-molecule drugs, mAbs are large (ca. 150 kDa) and heterogeneous (due to the biosynthetic process and subsequent manufacturing and storage) making their analysis very challenging. Despite the fact that only one molecule is cloned, hundreds of variants differing in post-translational modications (N-glycosylation, asparagine deamidation, aspartate isomerization, methionine oxidation, etc.), amino acid sequence, higher order structures, etc. may coexist.

Micro Pillar Array Columns (μPAC™) are perfectly suited to tackle the complexity associated with mAbs.

Read the full application note online

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Routine Proteome Analysis Using 50 cm μPAC™ Columns

The practice of bottom-up proteomics relies on the separation performance that can be achieved with state-of-the art nano LC-MS/MS equipment.

Depending on the sample complexity or the instrument time that can be dedicated to a certain sample, different LC columns and corresponding LC-MS/MS methods are often required. When aiming for comprehensive proteome analysis with deep coverage, relatively long columns (lengths up to 75 cm) are typically operated with long and shallow solvent gradients, delivering the highest chromatographic performance. This is a good strategy if very complex samples need to be analyzed and when as much information as possible needs to be retrieved from these samples. However, daily routine proteome analysis often deals with much less complex samples or demands increased sample throughput, making total analysis times above 120 min undesirable or even impossible.

Complementary to its landmark 200 cm column, ideally suited to comprehensive proteome research, PharmaFluidics now also offers a 50 cm μPAC™ column that can be used in a more routine research setting.

With an internal volume of 3 µL, this column is perfectly suited to perform high-throughput analyses with shorter gradient solvent times (30, 60 and 90 minute gradients) and it can be used over a wide range of flow rates, between 100 and 2000 nL/min.

Read the full technical note online

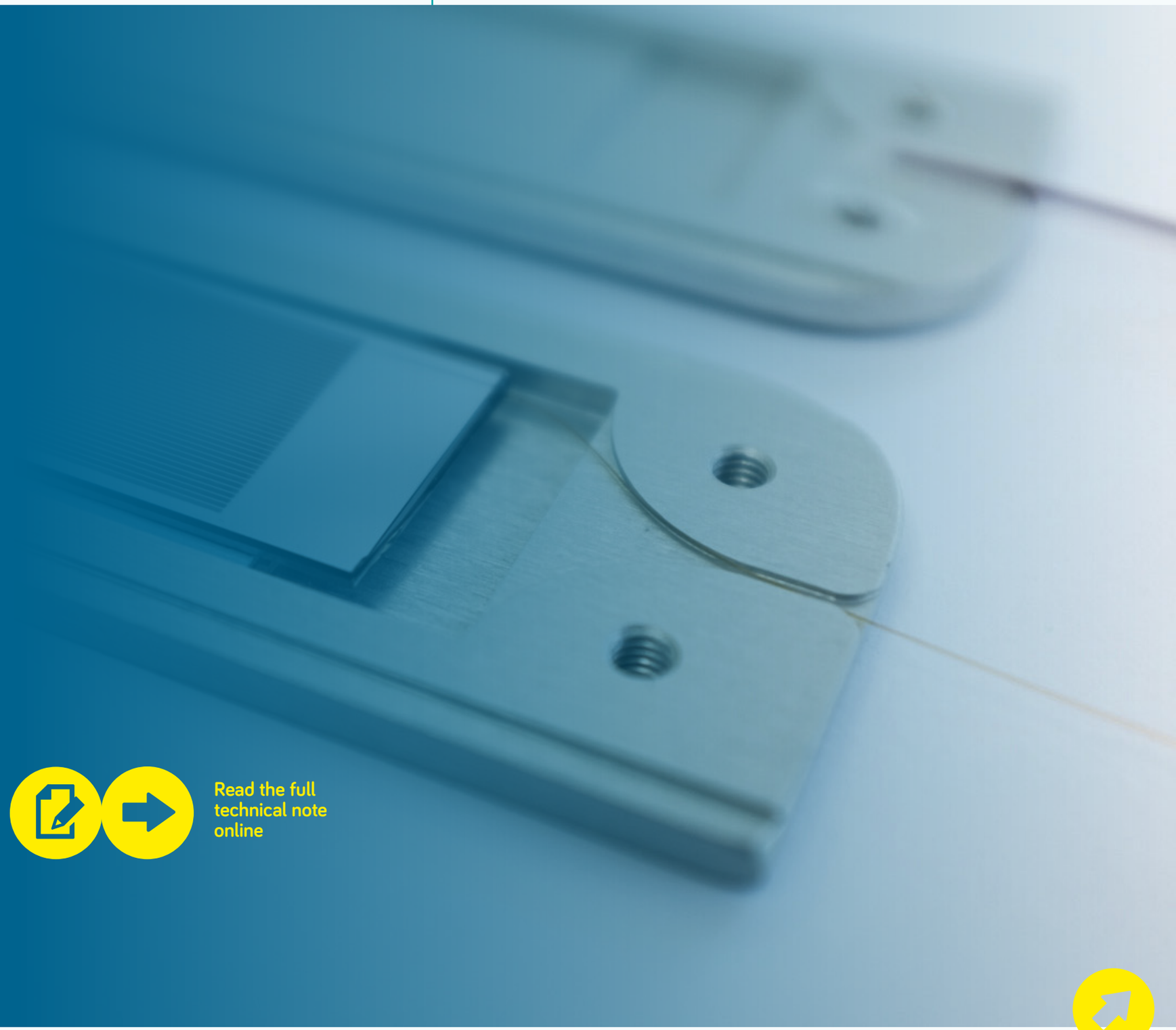
Sponsored by PharmaFluidics | Produced by the Analytical Scientist

µPAC™ Column Performance With Peptide Standards: Thermo Scientific™ Pierce™ Retention Time Calibration Mixture

Peptide standards are critical in mass spectrometry-based proteomics to ensure optimal and consistent system performance before, during, and after sample analysis. They can be used to assess peptide elution, troubleshoot chromatography, predict retention time, and demonstrate that the LC-MS platforms are working properly.

A complex protein digest (HeLa cell lysate) was spiked with 50 fmol/µl of a peptide retention time calibration mixture (Thermo Scientific™ Pierce™ Retention Time Calibration Mixture) and injected onto a 200 cm µPAC™ column using a direct injection method. Short solvent gradients (30 min gradient – 60 min total run time) were used at a flow rate of 750 nl/min to evaluate whether the µPAC™ column was properly installed. These conditions are advised to perform installation qualification, inter-run quality control or blank injections. For triplicate injections, average peak widths of 0.15 min with an average retention time variation below 1.5 percent CV were observed.

Long solvent gradients (240 min gradient – 300 min total run time) were used at a flow rate of 300 nl/min. These conditions are advised when deep proteome coverage is desired. In these experiments, the Thermo Scientific™ Pierce™ Retention Time Calibration Mixture can be used as an internal standard to monitor LC-MS performance or to adjust for variation in injection volumes. For triplicate injections, an average retention time variation below 0.8 percent CV was observed.



Read the full technical note online