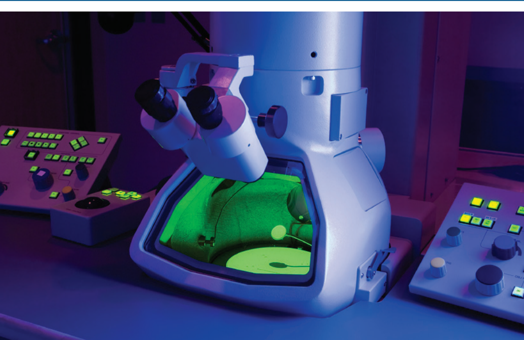
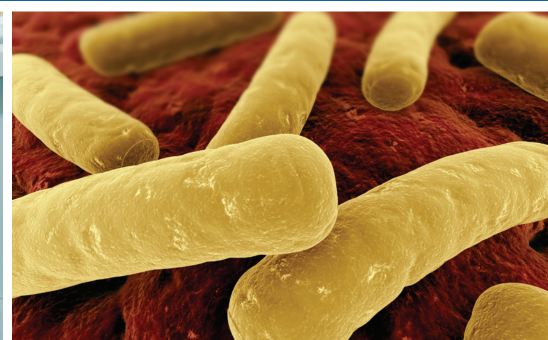
Advances in Analytical Chemistry: Processes, Techniques, and Instrumentation





An ACS-e! Discovery Report that examines the latest developments in analytical chemistry

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Advances in Analytical Chemistry: Processes, Techniques, and Instrumentation

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I. INTRODUCTION

Analytical chemistry laboratories — in academia, government, or industry — run on instrumentation. Whether they are establishing the identity of a newly synthesized material, or probing the properties of some bioactive compound, researchers have a broad array of hardware that can help. Indeed, today's analytical chemists have at their disposal a veritable alphabet soup of options, from GC-MS and NMR to ICP and MALDESI. And that toolbox is growing, with new developments appearing in the literature seemingly every week.

Many of these tools are used not only in the chemistry lab, but increasingly, in the biological sciences and other non-traditional realms as well. Inductively coupled plasma mass spectrometry (ICP-MS), for instance, is growing in popularity as a method to multiplex protein detection and quantitation at the cellular level, a method called "mass cytometry." Raman and infrared spectroscopy are being coupled with microscopy to create tools capable of detecting molecular signatures in biological tissue. And of course, researchers are constantly discovering ever more creative ways to apply nuclear magnetic resonance (NMR) and mass spectrometry (MS) to protein complexes.

All these different tools and techniques fall under the broad description of "analytical chemistry." Yet just what, exactly, does that term even mean? It depends on whom you ask. J. Michael Ramsey, the Goldby Distinguished Professor of Chemistry at the University of North Carolina (UNC) at Chapel Hill, relates that one colleague used to define analytical chemistry cheekily as "what analytical chemists do." Ramsey's definition was a bit more expansive: "Analytical chemists tend to focus on chemical and biochemical measurements and the way that they can best be done." (J. Michael Ramsey, personal communication, 2016)

Whatever you call it, analytical instrumentation certainly isn't new, per se. As *Chemical & Engineering News (C&EN)* reported in June 2016, one of the stalwarts of analytical chemistry laboratories — high-performance liquid chromatography — just celebrated its 50th anniversary in June.[1] Raman spectroscopy dates back to the 1920s, while mass spectrometry is well into its second century. But that doesn't mean researchers aren't discovering new ways to ply their trade.

Many researchers have been working to downsize the traditional tools of analytical chemistry. Among other examples, researchers such as Ramsey and R. Graham Cooks at Purdue University have devised ways to shrink mass spectrometers down into

portable, field-usable instruments. NMR spectrometers, too, are getting smaller, at least for some applications, with benchtop devices proving increasingly popular.[2]

Ramsey and others are also miniaturizing and simplifying analytical assays themselves, through the use of microfluidics. In one recent study reported in *Analytical Chemistry*, Ramsey's team used a custom capillary electrophoresis-electrospray emitter "chip" to characterize the drug loading on antibody-drug conjugates.[3] Similarly, Aaron Wheeler, Professor of Chemistry at the University of Toronto, is developing point-of-care miniaturized diagnostics based on a concept he calls "digital microfluidics" — the use of an electric field to manipulate fluid droplets on an array of electrodes.[4]

Other researchers and technology firms are pushing the envelope in terms of what such traditional analytical methods can achieve. At Northwestern University, Neil Kelleher and his team recently demonstrated their ability to apply MS methods to characterize mixtures of intact protein complexes in king cobra venom, a strategy Kelleher and others call "native top-down proteomics."[5] And on the NMR front, Chad Rienstra, Professor of Chemistry at the University of Illinois at Urbana-Champaign, and colleagues used magic-angle-spinning solid-state instruments as powerful as 750 MHz to solve the structure of alpha-synuclein fibrils, a previously recalcitrant protein aggregate associated with Parkinson's disease.[6] The protein, they found, adopts the peculiar folding of a "Greek key," with those keys stacking atop one another to form Parkinsonian fibers.

Meanwhile, researchers such as James Jorgenson, the W. R. Kenan, Jr. Professor of Chemistry at UNC Chapel Hill, are advancing the seemingly staid field of liquid chromatography (LC), with ultra-high-pressure LC systems capable of some 500,000 theoretical plates of separation — a five-fold improvement in resolution, he says. (James Jorgenson, personal communication, 2016).

According to Jonathan Sweedler, Editor-in-Chief of *Analytical Chemistry*, analytical chemistry is increasingly moving into non-traditional applications like transcriptomics, single-cell biology, and MS imaging, areas that highlight the cross-disciplinary nature of modern analytical chemistry. "One of the areas that's having a big impact in terms of measurement science is combining approaches that give a higher dimensionality," he says. (Jonathan Sweedler, personal communication, 2016)

That's not to say any area of analytical chemistry is stagnant — most are experiencing improvements. But four areas have seen dramatic advances, according to Sweedler – microfluidics, mass spectrometry, transcriptomics, and vibrational (Raman and infrared) spectroscopy.

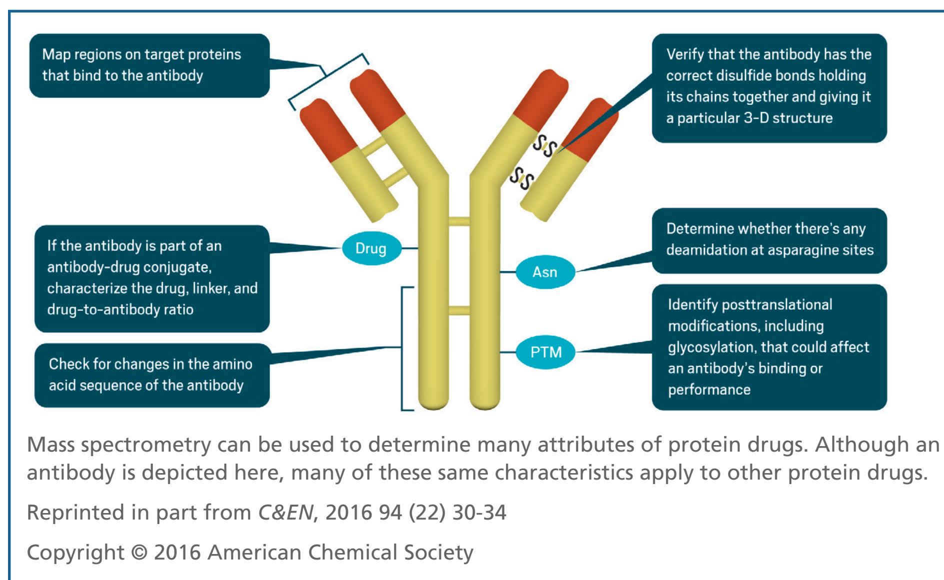
These techniques are exerting their influence both within chemistry laboratories and in the greater research community. And that trend shows no sign of slacking. As the confluence of chemistry, biology, and biopharma continues to grow, analytically inclined researchers can take comfort: With their field continually refreshing and reinventing itself, analytical chemists can expect to be in demand for quite some time to come.

II. MASS SPECTROMETRY

From its origins in the late 19th century, mass spectrometry has emerged to become one of the most versatile tools in the analytical chemistry toolbox. Today, it is used for everything from discovering metabolite biomarkers and monitoring organic syntheses to pharmaceutical process control and screening for toxins, pesticides, and explosives.

MS has also become the go-to tool for proteomics research. As described in a May cover feature in *C&EN*, the technique increasingly is being applied in biopharmaceuticals development, to help quality-control protein production and characterize protein-drug interactions:

> *Of the many analytical techniques available to the industry, mass spectrometry is one of the most versatile. It allows companies to identify proteins on the basis of their amino acid sequences and gives them information about those proteins' higher-order structures. It allows them to assess a protein's purity. And in the right circumstances, it can tell them many of these attributes simultaneously.*[7]



Mass spectrometry can be used to determine many attributes of protein drugs. Although an antibody is depicted here, many of these same characteristics apply to other protein drugs.

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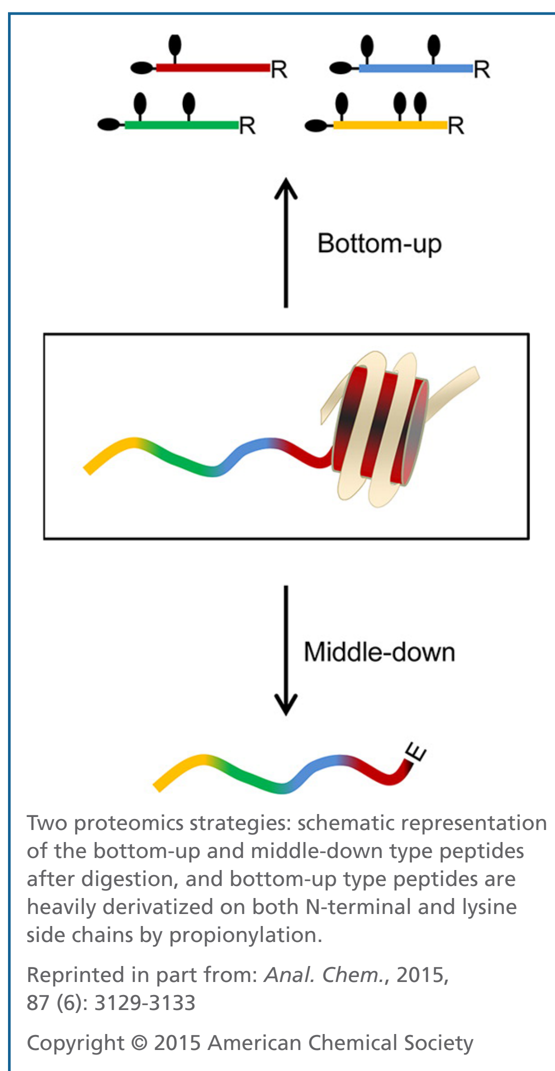
Whatever their goals, such studies commonly take one of three forms.[8] In "bottom-up" proteomics, protein mixtures are digested with proteinases into peptides, which are then separated by LC and sequenced in the mass spectrometer. This approach is straightforward, but it sacrifices any available information regarding which post-translational modifications and peptides are physically connected to which — that is, of the so-called "proteoform."[9] "Top-down" proteomics, a more challenging strategy, analyzes proteins intact in order to retain those compositional details. A final strategy, called "middle-down," splits the difference, applying top-down concepts to large protein fragments (as opposed to entire proteins).

In one recent demonstration, Benjamin Garcia, of the University of Pennsylvania Perelman School of Medicine, and colleagues applied both middle-down and bottom-up proteomics to the N-terminal tails of histone proteins.[10]



Two proteomics strategies: schematic representation of the bottom-up and middle-down type peptides after digestion, and bottom-up type peptides are heavily derivatized on both N-terminal and lysine side chains by propionylation.

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Both approaches yielded "comparable" abundance values of post-translational modifications (PTMs), they found. Even though middle-down is more complicated, it can provide information that bottom-up strategies cannot, they said, especially the co-occurrence of different modifications on the same protein molecule. "Proving the reliability of the middle-down workflow is highly relevant," the authors wrote, "as this is currently the only quantitative technique to estimate the coexistence frequency of histone PTM all along the N-terminal tail."[10]

Recently, a fourth proteomics strategy has emerged, applying the top-down concept to entire protein complexes.[11] In the method adopted by Northwestern University's Neil Kelleher, protein mixtures containing intact complexes are separated using a novel tube-gel-based strategy called "native GELFrEE" (gel-eluted liquid fraction entrapment electrophoresis), and then subjected to native MS on a high-end Orbitrap mass spec.[12] In essence,

the protein complexes are selected, weighed, and controllably broken apart, with the individual subunits analyzed by top-down MS. Finally, the resulting spectra are decoded using bespoke computational approaches.[13]

Working with researchers in Brazil, Kelleher's team applied this approach to the "venome" of the king cobra (*Ophiophagus hannah*).[5] Their analysis (which also collected denaturing top-down and bottom-up datasets) identified four multiprotein complexes and their glycan modifications, as well as some 184 individual proteoforms, to produce what the authors term "a superlative catalog of venom protein content."

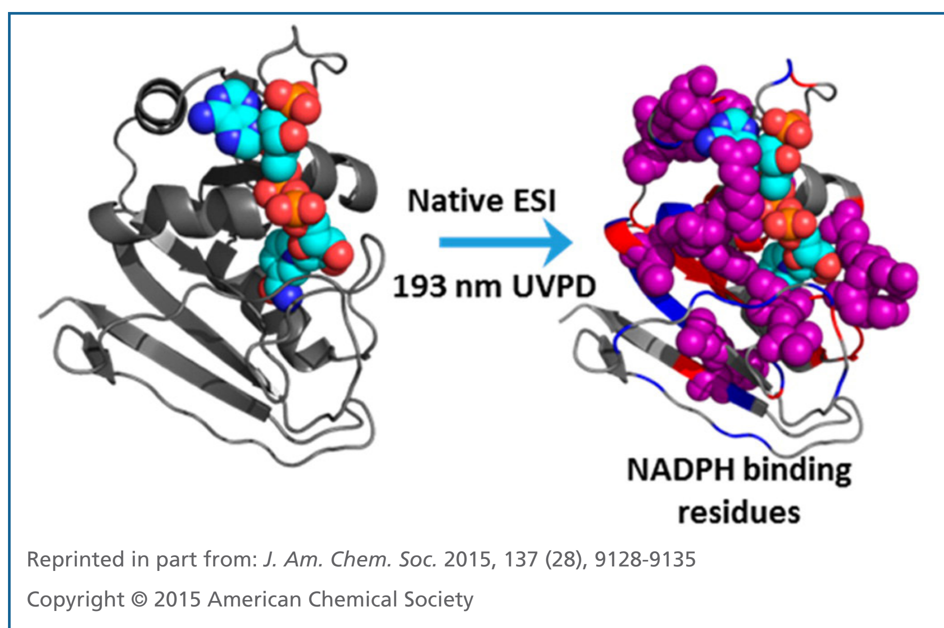
New LC-MS Fragmentation Methods

Tandem mass spectrometry (i.e., MS/MS, MS3, and so on) requires the ability to select an ion of interest, fragment it, and then select and analyze the resulting pieces. In liquid chromatography-coupled mass spec (LC-MS), the most common fragmentation strategies are collisional. In collision-induced dissociation (CID), ions are fragmented by collision with an inert gas. But CID isn't always ideal: it displays sequence biases, and breaks certain protein-post-translational modification linkages, thereby blocking the acquisition of such data. In recent years, researchers have devised a number of alternative, and very promising, strategies.

One popular alternative is electron transfer dissociation (ETD), first described in 2004 by Donald Hunt's laboratory at the University of Virginia.[14] Based on an earlier method called electron capture dissociation (ECD), which was restricted to use in high-end Fourier transform ion cyclotron resonance mass spectrometers, ETD introduces a negatively charged anthracene ion into the mass spec. This induces peptide backbone cleavage while retaining post-translational chemical modifications, effectively by carrying out a chemical reaction in the instrument. Importantly, the technique works on less-exclusive instrumentation, such as the quadrupole linear ion trap used in Hunt's original study.

ETD is a positive ion mode fragmentation strategy; it requires cationic peptide ions. More recently, researchers such as Joshua Coon at the University of Wisconsin, Madison (coauthor of the 2004 paper with Donald Hunt) have developed anionic (negative ion) ETD variants to probe more acidic proteins.[15] In 2015, Coon and his team described a method called "activated ion negative electron transfer dissociation" (AI-NETD), with which they identified 3,730 proteins from the *Saccharomyces cerevisiae* proteome.[16]

Another burgeoning fragmentation strategy is ultraviolet photodissociation (UVPD). Developed by several research teams in the US and Europe, including that of Jennifer Brodbelt at the University of Texas at Austin, UVPD uses ultraviolet laser light to induce peptide cleavage. Like ETD, it tends not to cleave protein-PTM linkages, and it seems to cleave bonds with equal efficiency all along the peptide chain (Jennifer Brodbelt, personal communication, 2016). However, practical considerations, including multiple fragmentation pathways and mass resolving power tend to cap efficient UVPD analysis at about 35 kDa, she says. In one recent application, Brodbelt and her team applied UVPD (using a 193-nm laser and a customized Thermo Fisher Scientific Orbitrap Elite mass spec) to study the interaction of native dihydrofolate reductase (DHFR), a 19-kDa protein, with its cofactor NADPH, and an inhibitor, methotrexate.[17] The spectral data provided sufficient information for the team to map the NADPH-binding sites within the protein.



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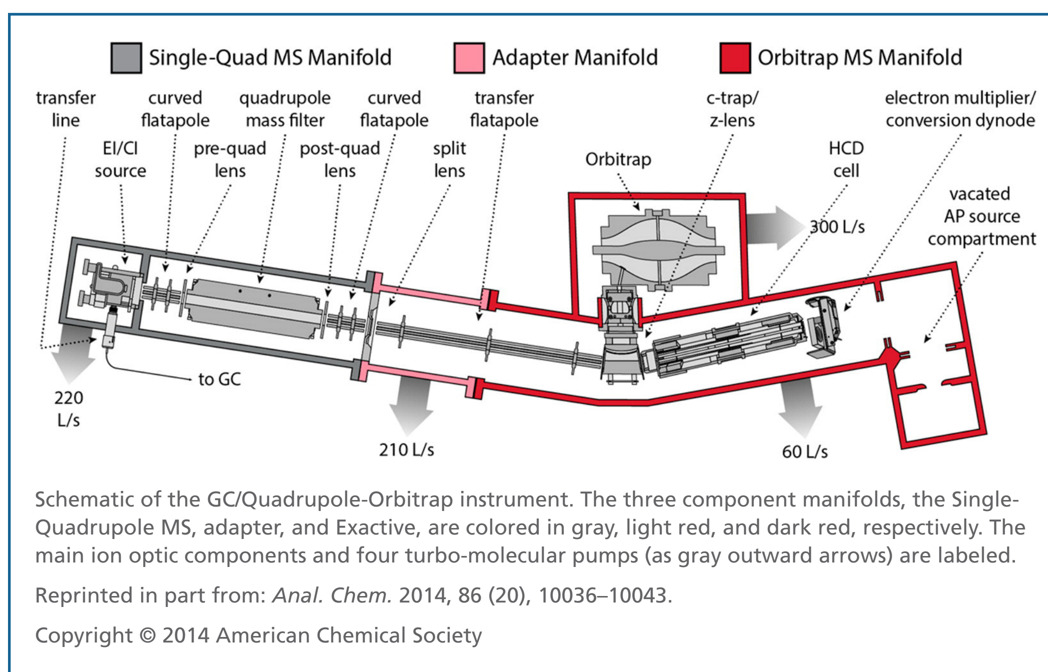
At Ohio State University in Columbus, Vicki Wysocki has been pursuing yet another fragmentation approach. As described in one recent review, surface-induced dissociation (SID) is an approach for native protein complex analysis that "slams protein ions of interest into a nonreactive surface inside the instrument — essentially, a wall — causing complexes to fracture into subcomplexes that reveal the assembly's inner architecture."[11] Wysocki's team has used SID, in addition to ion mobility separation and "solution disruption" strategies, to deconstruct the heterohexameric protein complex, toyocamycin nitrile hydratase (TNH), revealing key details about its organization.[18] "The results," the team wrote, "show that TNH is a dimer of $\alpha\beta\gamma$ trimers, that within the trimer the α and β subunits bind most strongly, and that the primary contact between the two trimers is through a γ–γ interface."

Ion mobility separation, available commercially from both Waters Corp. and Agilent Technologies, has emerged as a key tool for proteomics mass spectrometry. "This is definitely something that's swept the world," Brodbelt says (J. Brodbelt, personal communication, 2016). The technique adds an additional layer of separation between the upstream liquid chromatography system and downstream mass analyzers, fractionating otherwise isobaric molecules by their "collisional cross-section." Thus, by coupling ion mobility and LC-MS, researchers can effectively generate two-dimensional chromatographic separations.

Suppose, Brodbelt explains, a researcher digests a complex protein mixture with trypsin, then passes the resulting mixture, which may contain hundreds of thousands of peptides, over a LC column. Even the most efficient separation will yield multiple co-elating peptides, many of which may be overlooked by typical data-dependent MS/MS acquisition strategies (which fragment ions by order of abundance). By better resolving the samples that emerge from the LC column, Brodbelt says, ion mobility makes it possible to dig deeper into their composition, and thus to extract more information than LC alone, especially for low-abundance species (J. Brodbelt, personal communication, 2016).

Gas Chromatography Gets an Orbitrap

Gas chromatography-coupled mass spectrometry (GC-MS) also has seen some new developments over the past few years. Particularly exciting, says David Muddiman, the Jacob and Betty Belin Distinguished Professor of Chemistry at North Carolina State University, is the recent launch by Thermo Fisher Scientific of a GC-coupled Orbitrap system.[19]



Schematic of the GC/Quadrupole-Orbitrap instrument. The three component manifolds, the Single-Quadrupole MS, adapter, and Exactive, are colored in gray, light red, and dark red, respectively. The main ion optic components and four turbo-molecular pumps (as gray outward arrows) are labeled.

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GC-MS, used for the analysis of volatile compounds, produces highly reproducible spectra. Thus, compound identification by GC-MS is relatively straightforward, as researchers have been able to compile detailed spectral libraries. But they are not, of course, comprehensive. To identify unknown molecules, researchers traditionally rely upon exact mass to determine elemental composition, as well as fragmentation spectra. But for most of its history, Muddiman explains, GC-MS has been coupled to relatively simple, nominal mass analyzers, such as linear quadrupoles, limiting researchers' confidence in their molecular assignments. But with its high resolution and mass accuracy, Thermo Fisher Scientific's Orbitrap analyzer suffers no such limitations.

Researchers at Thermo, in collaboration with Joshua Coon, described the new system in a pair of reports in *Analytical Chemistry* in 2014. In the first paper, they document mass resolving power of 100,000 at a scan rate of 4 Hz and mass accuracy of around 1 ppm.[19] In the accompanying article, the teams use that system, in conjunction with stable isotope labeling and an "intelligent" data-dependent acquisition strategy that focuses on intact mass ions, to determine "plausible identifications for over 80 compounds" from *Arabidopsis thaliana* extracts, with a mean mass error of 2.4 ppm.[20]

Imaging at Atmospheric Pressure

Another ongoing development in the mass spectrometry sphere is mass spec imaging (MSI). The technology allows researchers to create spatial maps of the abundance of proteins and metabolites in tissues and cells – data that can be overlaid on more traditional images to correlate chemical makeup with cellular morphology.[21]

As a general rule, MSI methods can take one of two forms.[22] "Microprobe mode" strategies couple a mass analyzer with a movable sample stage, thus mapping chemical composition to spatial position point by point. In "microscope mode" strategies, ions from a relatively large sample region are desorbed and analyzed en masse and while retaining their relative position, providing a wider field of view.

Multiple MSI methods have been described in the literature, but according to a recent review in *Analytical Chemistry*, the most popular between 2012 and 2014 (based on citation counts) all employed microprobe-based approaches: matrix-assisted laser-desorption ionization (MALDI), desorption electrospray ionization (DESI), laser ablation electrospray ionization (LAESI), and secondary ion mass spectrometry (SIMS).[23]

Yet researchers continue to develop new variants and flavors. For example, since 2006, Muddiman has been developing and optimizing infrared matrix-assisted laser-desorption electrospray ionization (IR-MALDESI), a method that effectively blends MALDI and electrospray ionization to create a cloud of volatile “neutrals” over an ice-encrusted sample, which are then analyzed in an Orbitrap mass spectrometer. (The ice in this case serves as a matrix to absorb the IR laser.) As described in a recent review:

> *The desorbed tissue volume is ejected normal to the surface where the ablation plume overlaps with an orthogonal electrospray plume…. Analytes from the desorbed tissue material partition into the electrospray droplets where they undergo desolvation and charge transfer in a manner similar to ESI. The ions are then sampled by a high resolving power mass analyser.*[24]

According to Muddiman, the technique (which earned his research team the 2015 ACS Award for Chemical Instrumentation) has been used to image everything from tissues and plants to fabrics and forensic samples. In one recent example, Muddiman’s team used the approach to quantify antiretroviral drug concentration in human hair without analyte extraction, with detection limits as low as 1.6 ng/mg hair.[25]

Also pursuing ambient pressure mass spectrometric imaging strategies is R. Graham Cooks at Purdue University, who pioneered DESI. Unlike MALDESI, DESI does not use a laser for ionization; rather, as described in one recent review, “a stream of solvent is sprayed at an untreated tissue surface, where it pools and extracts surface molecules. Additional droplets splash that extracted material into the mass spectrometer, where it is ionized and analyzed.”[21] Cooks, with Nathalie Agar at Harvard Medical School, has been working to integrate DESI-based mass spectrometric imaging into the clinical setting — specifically, to aid surgeons in defining tumor margins in real time based on metabolite concentrations in vivo (as opposed to post-operative biopsy analysis), and thus, to improve patient survival. In 2014, the team successfully demonstrated that capability on a pair of patients with glioma brain tumors, detecting the cancer-associated metabolite, 2-hydroxyglutarate (2-HG), in the operating room as the patients were still in surgery.[26]

At Imperial College London, Zoltán Takáts is also pursuing ambient mass spectrometric analysis in the clinic. Takáts has developed a strategy called rapid evaporative ionization mass spectrometry (REIMS), which samples the “smoke” produced during electrosurgery for specific lipid biomarker signatures of disease. In 2013, Takáts and his colleagues applied the technique to 81 patients, accurately matching post-operative histopathology assessments in all cases.[27]

Single-Cell Proteomic Analysis With Mass Cytometry

Another recent analytic advance has been the fusion of inductively coupled plasma (ICP) mass spectrometry with the single-cell analysis technique called flow cytometry to produce "mass cytometry." In flow cytometry, cells are stained with fluorescently labeled antibodies to specific markers (or fluorescent proteins conjugated to those protein markers), then interrogated one by one as they pass a bank of lasers and detectors. The results provide a single cell-level profile of the cellular population. But there's a limit to how comprehensive such analyses can be. Though cells contain tens of thousands of different proteins, few researchers examine more than six or perhaps a dozen markers at a time, owing to issues such as spectral overlap or antibody cross-reactivity, for example. In 2009, Scott Tanner of the University of Toronto described a strategy to circumvent this problem.[28] His method, "mass cytometry," uses rare earth elements rather than fluorophores to tag antibodies, which are then quantified cell by cell as they pass into an ICP MS. In his original 2009 paper, Tanner quantified some 20 protein antigens in leukemic cell lines; today, panels comprising more than 40 separate antibodies are possible.[29]

More recently, Stanford University researcher Garry Nolan and Bernd Bodenmiller of the University of Zürich independently demonstrated that they could couple mass cytometry to imaging mass spectrometry, thereby allowing for a cell-by-cell assessment of protein abundance in a tissue-wide context.[30,31]

III. MINIATURIZATION

Another popular trend in the world of analytical chemistry involves miniaturization. Thanks to advances in instrument design, microfluidics, microfabrication, and more, researchers are downsizing both instruments and assays. Among other things, miniaturization can cut costs by decreasing reagent and sample requirements, increase ease-of-use for non-experts, and enable deployment beyond chemistry labs and into physicians' offices, first responders, or in the field.

For instance, multiple researchers and technology firms have worked to shrink mass spectrometers from benchtop to portable instruments.[32] UNC Chapel Hill's Ramsey, with funding from the U.S. Department of Defense, has shrunk ion trap mass spectrometers from benchtop-sized instruments down to about 2 kg, including a battery, for use in defense and first-responder applications. Recently, Ramsey and his team developed a so-called "high-pressure mass spectrometer" (HPMS), which is a portable ion trap mass spectrometer capable of operating at pressures greater

than 1 Torr. The device has been commercialized by 908 Devices Inc. (cofounded by Ramsey) as the M908 handheld mass spectrometer.[33] Normally, Ramsey explains, mass spectrometry requires pressures of between 10e-6 T and 10e-10 T; ion traps typically operate near the 1 milliTorr range. "So we're at more than a thousand-times higher pressure than normal mass spectrometers," he says. "That has allowed us to reduce the complexity of the vacuum system, and the development of the world's first handheld mass spectrometer." (J. Michael Ramsey, personal communication, 2016). In one example, Ramsey's team detected 2-chloroethyl ethyl sulfide, a volatile chemical warfare agent, at 1.2 T, albeit with some peak broadening.

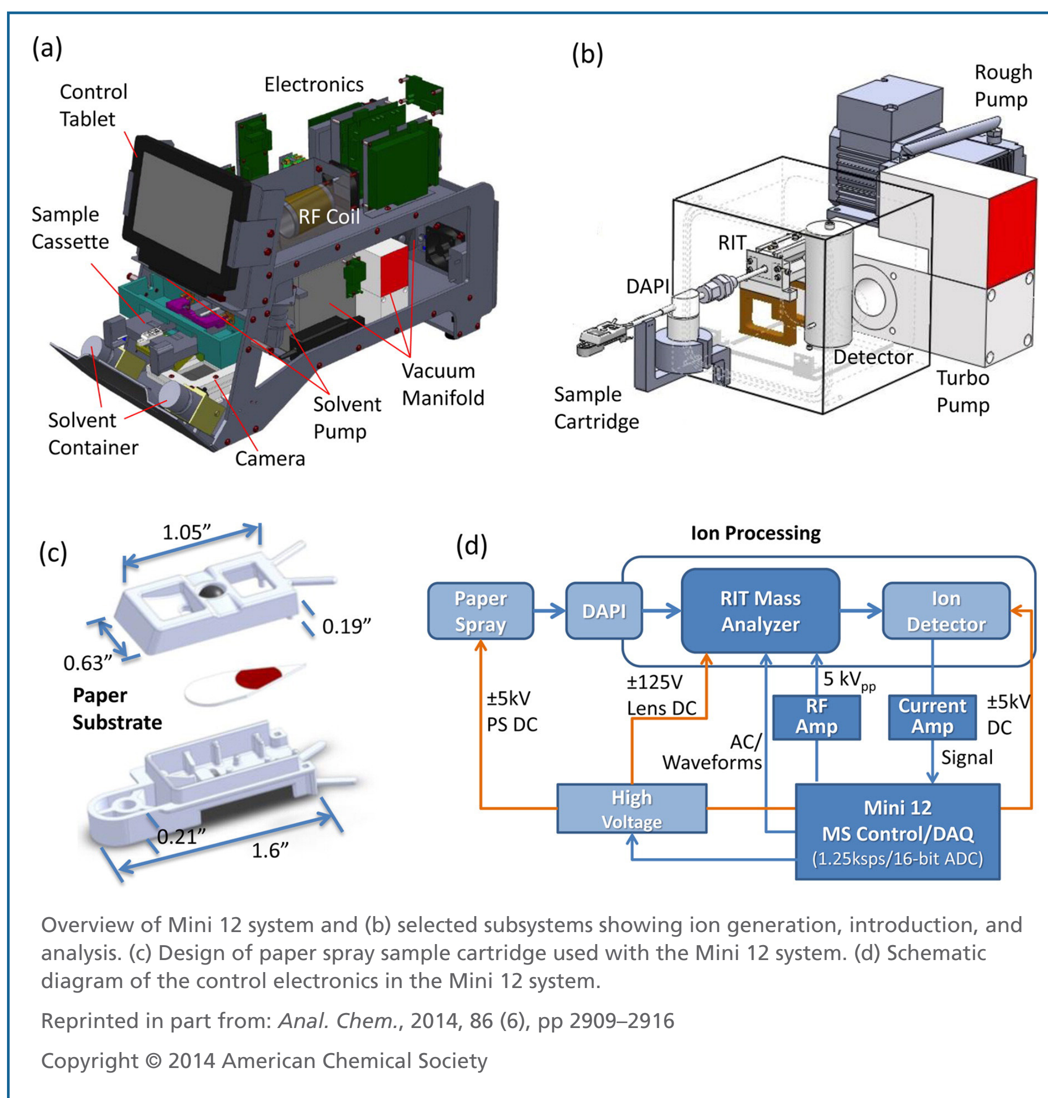
Ramsey has also designed microfluidic circuitry to accelerate and reduce the cost of MS analyses for biological and biopharmaceutical applications. His team has integrated a capillary electrophoresis-based separation system and electrospray nozzle into a single microfluidic chip, called a CE-ESI device. By coupling that chip to a time-of-flight mass spec, Ramsey and his team have been able to characterize differently charged variants of therapeutic monoclonal antibodies,[34] the drug loading of antibody-drug conjugates,[3] and the glycation state of hemoglobin.[35] According to Ramsey, the key to CE-ESI is the chemical vapor deposition process used to coat the CE channels. Traditionally, CE columns are treated by pumping reagent-filled solutions through the column in order to passivate the surface (with aminopropylsilane, for example), thus preventing proteins from sticking as they pass through. But, as that process proved unsatisfactory, he says, his team switched to vapor deposition instead, yielding approximately three-fold better performance (J. Michael Ramsey, personal communication, 2016).

Paper-Based Analytics

R. Graham Cooks at Purdue University is also pursuing portable mass spectrometers, and has been doing so for years. Over the past two-plus decades, he and his colleagues have rolled out a series of mini mass specs, the smallest of which weighed about 5 kg.[36] The most recent iteration is the Mini 12, a benchtop-sized (25-kg) "rectilinear ion trap" tandem mass spectrometer intended for use as a point-of-care analyzer in doctors' offices.[37]

Among other things, the Mini 12 employs "paper spray ionization" to introduce ions into the mass spectrometer.[38] By wetting a triangular piece of filter paper dotted with sample material and applying a high voltage, samples can be ionized and volatilized in a manner akin to electrospray ionization.

And that's just one of the emerging applications of paper, which has proven itself to be a surprisingly useful and robust material for downsizing and simplifying analytical



Overview of Mini 12 system and (b) selected subsystems showing ion generation, introduction, and analysis. (c) Design of paper spray sample cartridge used with the Mini 12 system. (d) Schematic diagram of the control electronics in the Mini 12 system.

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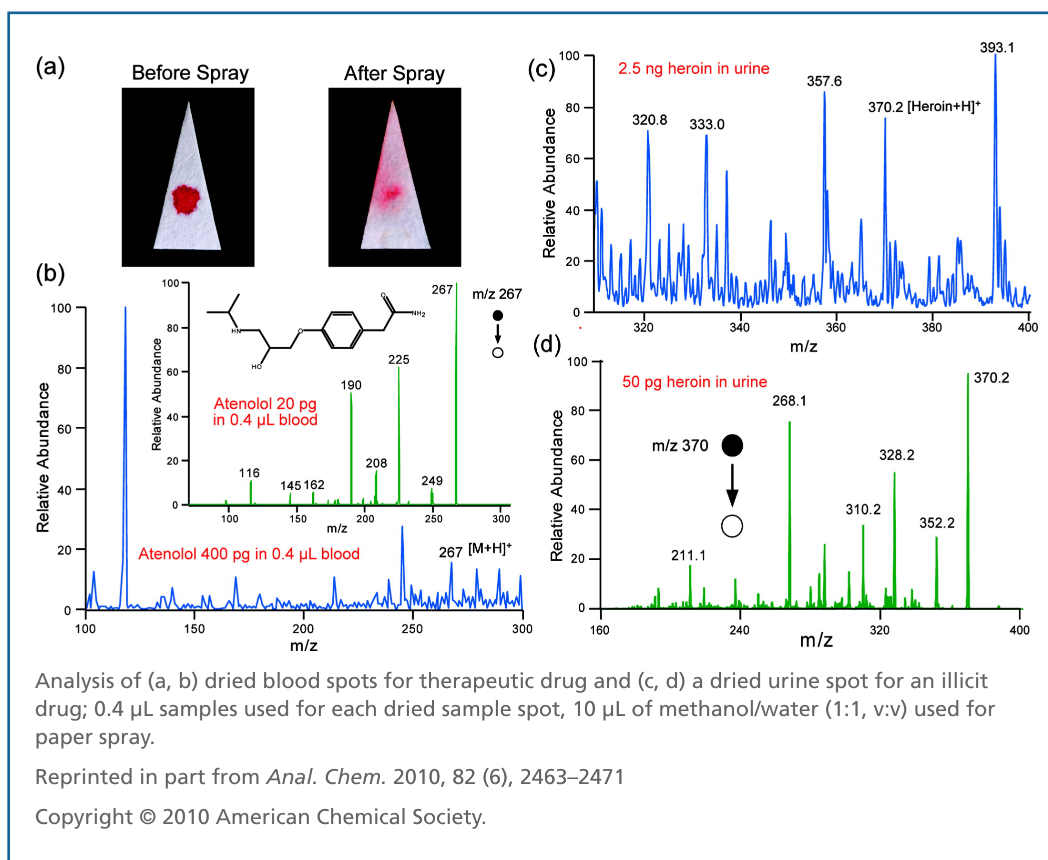
chemistry. George Whitesides of Harvard University, has developed several point-of-care microfluidic devices built of filter paper.[39] As described in one 2012 example:

> *Unlike traditional POC [point-of-care] paper-based devices (for example, lateral flow tests), which typically run one or two tests in series (requiring that each assay on the device be compatible with the others in terms of buffers and other reagents and that assays not cross-react), paper-based microfluidic devices, with their ability to modify and direct flow within microfluidic channels [and in three dimensions (3D)], have the capacity to split a single, low-volume (<40 ml) sample into multiple separate portions, which can each be assayed in parallel. This allows for high-level multiplexing of independently optimized assays with discrete assay conditions and eliminates concern about cross-reactivity between assays. Like lateral flow tests, paper-based microfluidic devices require no external pumps, instrumentation, or power and are both portable and disposable.*[40]



Analysis of (a, b) dried blood spots for therapeutic drug and (c, d) a dried urine spot for an illicit drug; 0.4 μL samples used for each dried sample spot, 10 μL of methanol/water (1:1, v:v) used for paper spray.

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That paper from 2012 detailed a multiplexed paper-based assay for two different liver enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in blood. A droplet of blood is spotted onto a multilayered stack of filter paper onto which fluidic channels and detection zones have been patterned with wax. Detection is based on a simple colorimetric assay. That technology was subsequently licensed to Massachusetts-based Diagnostics For All, which in 2013 published the results of a field trial using a simplified assay (assaying only ALT) for 600 patients on HIV antiviral medication in Vietnam.[41] Whitesides' team has since developed a handheld paper-based microfluidic nucleic acid amplification test, which they used to detect as little as a single copy of bacterial DNA in human plasma.[42]

Also developing paper-based devices is Aaron Wheeler of the University of Toronto. Wheeler has pioneered an approach he calls digital microfluidics. Digital microfluidics, he says, refers to "the subset of microfluidic techniques that allow for the manipulation of droplets on an open surface, without microchannels." (Aaron Wheeler, personal communication, 2016). Basically, droplets are sandwiched between two plates, one containing an array of electrodes, the other a counter electrode. By applying a potential between the counter electrode and one of the arrayed electrodes, the droplet can be forced to move from station to station on a surface, like iron filings controlled by a magnet. The advantage of this approach,

Wheeler says, is that it is highly flexible, unlike traditional microfluidic designs, in which each step is precisely engineered. For instance, users can change running parameters to allow for sample concentrations outside idealized ranges.

In 2015, Wheeler's team used this strategy to design an assay for rubella (German measles) immunity and infection.[4] The assay captures anti-rubella virus antibodies in blood on magnetic beads. These beads are then washed, incubated with enzyme-conjugated secondary antibodies, then mixed with detection reagents. Finally, the assay is read via chemiluminescent detection. And all these steps are accomplished by moving reagents across the chip surface like pieces on a chessboard. Wheeler originally developed these electrode arrays on glass or silicon. But those are expensive technologies, he says, and difficult to fabricate. So more recently, his team has developed methods to build these arrays on paper, producing hundreds of devices per day at about $1 each, he says.[43] Using that technology, Wheeler and his team were able to fabricate several hundred assay plates, which he used to field-test his rubella assay in a refugee camp in Kenya, "I don't think I could have afforded to make 600 devices to send to Kenya if we hadn't come up with new ways of making the devices," he says. Those findings from the Kenyan rubella assay have yet to be published (A. Wheeler, personal communication, 2016).

Single Cell Assays

Some researchers are miniaturizing assays in another way, by taking them to the single-cell level — particularly for the assessment of protein or RNA content.[44] As it turns out, supposedly homogeneous cell populations — a plate full of cultured immortalized cells, for example — are anything but truly homogeneous. Different cells in the population may be at different places in the cell cycle, or may experience slightly different microenvironments. As a result, gene expression patterns may vary from cell to cell, a level of heterogeneity that is lost when doing bulk gene expression analyses. Over the past few years, researchers have improved at probing that heterogeneity by examining transcription at a cellular level.

Traditionally, single-cell RNA sequencing strategies are accomplished by isolating and analyzing individual cells. In one recent study, Corbin Jones of UNC Chapel Hill and colleagues used a "microraft array" (designed in the lab of Nancy Allbritton at UNC Chapel Hill) to effect single-cell isolation, thereby demonstrating the array's utility for single-cell RNA-seq applications.[45] But it's also possible to tag cells in such a way that their contents can be differentiated even in bulk analyses. James Eberwine of the University of Pennsylvania recently described a strategy called TIVA (transcriptome in vivo analysis) for capturing the mRNA of specific cells in a large population in situ.[46] Instead of isolating cells prior to RNA capture,

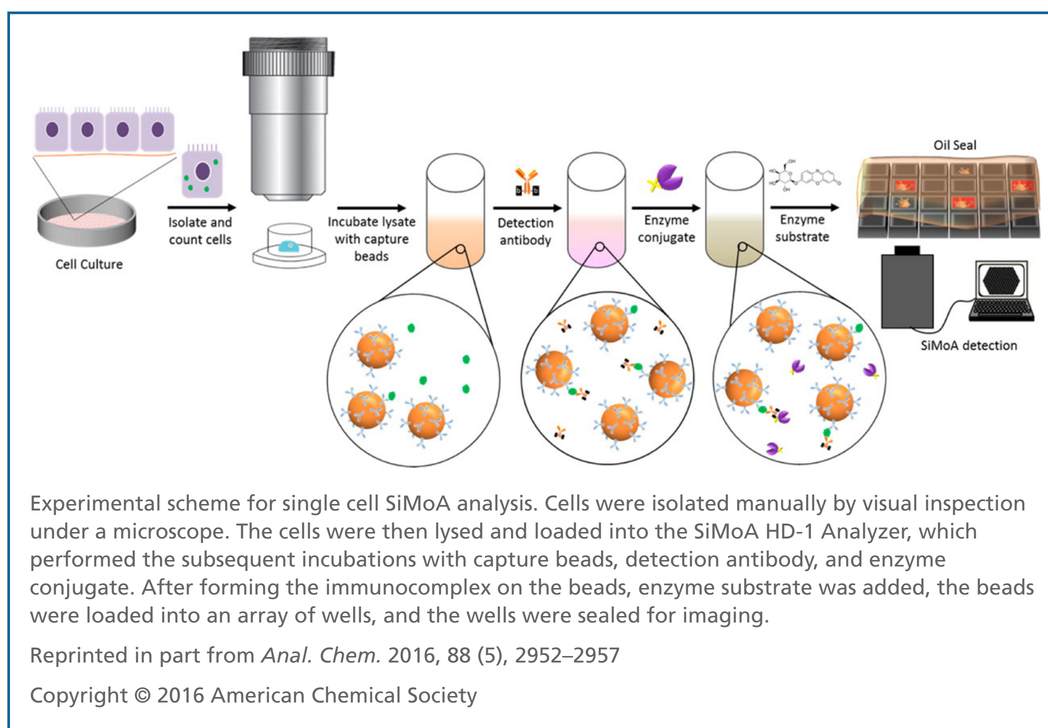
TIVA allows researchers to work with cells in intact tissue sections. The method uses a photoactivatable mRNA capture reagent that can freely enter cells but only becomes active when hit with light, and Eberwine and his team used it to study the heterogeneity of neuronal transcription in both mouse and human brain tissues. Other researchers are probing the proteome content of individual cells. In the previous section we discussed the research of Garry Nolan and Bernd Bodesmiller, both of whom have modified a hybrid ICP-MS/flow cytometry system, called a mass cytometer, to retain spatial information akin to imaging mass spectrometry.

Different strategies have been adopted by other researchers. Jonathan Sweedler, Editor-in-Chief of *Analytical Chemistry* and a Professor of Chemistry at the University of Illinois at Urbana-Champaign, has developed a method for probing the neuropeptide content of tens of thousands of cells dispersed on a microscope slide using mass spectrometry — information that can be used to identify cells based on their specific peptide signatures.[47] Rather than raster scanning across the slide as in traditional imaging mass spectrometry — a process that would require "several hundred hours" of data collection — this method stains each cell nucleus with a fluorescent dye. By first imaging the slide under a mercury arc lamp to excite fluorescence and then feeding the resulting coordinates to the mass spectrometer, thousands of cells can be assayed "in about an hour."

David Walt, a Howard Hughes Medical Institute Professor at Tufts University, has pioneered yet another approach. Inspired by the lack of anything akin to PCR for proteins, Walt's single molecule array assay (SiMoA, commercialized by Quanterix, a company Walt cofounded) allows researchers to precisely count the number of specific protein molecules per cell by distributing the molecules across many wells, in a manner akin to digital PCR.

The assay involves capturing and lysing individual cells in microfuge tubes, incubating the lysates with capture beads and secondary antibodies for the protein of interest, and then depositing those beads into wells that are sized to hold precisely one bead each. A fluorogenic substrate is added to the wells, which are then sealed, making it possible to concentrate and thus detect the small fluorescent signal that results.

By counting the number of positive wells and applying a Poisson correction, the assay could detect as few as 12,000 PSA molecules in one demonstration.[48] Walt's SiMoA can also be multiplexed with fluorescently tagged beads (in a manner similar to Luminex Corp.'s xMAP technology) to quantify up to 10 different analytes, including both nucleic acids and proteins simultaneously, he adds (David Walt, personal communication, 2016).



Experimental scheme for single cell SiMoA analysis. Cells were isolated manually by visual inspection under a microscope. The cells were then lysed and loaded into the SiMoA HD-1 Analyzer, which performed the subsequent incubations with capture beads, detection antibody, and enzyme conjugate. After forming the immunocomplex on the beads, enzyme substrate was added, the beads were loaded into an array of wells, and the wells were sealed for imaging.

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Meanwhile, at the University of California, Berkeley, Amy Herr has developed microfluid methods to perform Western blotting on thousands of single cells simultaneously, detecting up to 9 proteins per cell.[49] The scWestern method employs an array of 6,720 cell-sized microwells arranged in 16 blocks in a photoactive acylamide layer atop a glass microscope slide. Cells settle into the wells by gravity and are lysed to release their proteins. Those proteins are then electrophoretically separated over a distance of 500 mm, fixed with UV light, probed with fluorescent antibodies, and imaged. Because these proteins are physically separated prior to detection, the technique, the authors note, provides a level of molecular specificity absent from such approaches as immunocytochemistry and flow cytometry, as these methods cannot distinguish on- and off-target binding events. Herr's team used the approach to probe the cell-to-cell variability in growth factor signaling responses and differentiation of cultured neural stem cells, detecting a "rich, graded heterogeneity in stem cell signaling trajectories."[49]

IV. SEPARATION TECHNOLOGIES

Separation technologies are also seeing substantial improvements in the analytical realm, including liquid chromatography. High-performance liquid chromatography (HPLC) just celebrated its 50th anniversary, according to a cover story from a June 2016 issue of *C&EN* — though the precise birthdate of the technology remains up in the air, and some say the technology originated two years earlier, in 1964.[1] Whatever its true origin story, there's no denying the impact HPLC has had. As described in an accompanying article in the previously mentioned issue of *C&EN*:

> *High-performance liquid chromatography (HPLC) gives chemists access to a world of knowledge about molecules both large and small. When combined with high-resolution detection methods such as mass spectrometry, the historic separation technique can not only help reveal hitherto unseen paths leading to new treatments for diseases, but it can also shed light on the complex array of contaminants in the water we drink or reveal banned pesticides in the food we might place on our tables.*[50]

And the technology continues to evolve. In June, liquid chromatography experts gathered in San Francisco for HPLC 2016, the "44th International Symposium on High Performance Liquid Phase Separations and Related Techniques." Robert Kennedy, Chair of the Department of Chemistry at the University of Michigan in Ann Arbor, who chaired the meeting, said that about 1,000 researchers attended the conference, which focused on column development, biopharmaceutical and protein separation, and the interface to mass spectrometry (Robert Kennedy, personal communication, 2016). Although liquid chromatography technology may be considered mature, Kennedy says, it is also evolving as researchers confront new technical challenges. Proteomics, for instance, "is a very hot topic right now," Kennedy says, with researchers constantly looking for new ways to sift through complex protein mixtures. Also hot, he says, are separation strategies for biopharmaceutical development quality control — ensuring, for example, that protein therapeutics are properly modified and free of undesirable side products.

At Purdue University, Mary Wirth has been pushing the limits of liquid chromatography with ever-smaller column packing materials. Traditional LC columns use particles between about 1.7 mm and 2 mm, says James Jorgenson at UNC Chapel Hill. As particle size drops, separation power increases and separation time decreases — but so too does the pressure required to run the column (James Jorgenson, personal communication, 2016). "Going from 1.7 [mm] down to even half that, 700 nm, that means basically eight times the problems, because everything gets harder to pack,

harder to drive pressure through, all that sort of thing," Kennedy says. Nevertheless, in 2014 Wirth and her team demonstrated a nano-flow-scale 4-cm reversed-phase C18 capillary column with 470-nm particles, which they used to separate both a mixture of intact model proteins and a crude bacterial lysate prior to mass spectrometry.[51]

Key to that separation is the phenomenon of "slip flow." As described in Wirth's 2014 study, "Slip flow enhancement is a nanoscale phenomenon that owes to the attractive interactions between mobile and stationary phases being weak, resulting in a nonzero velocity for the fluid at the wall and consequently an enhancement in flow rate." In this case, the researchers observed a "10-fold enhancement," with a back pressure at 200 nL/min flow rate of just 600 bar, rather than the predicted 7,000 bar.[51]

Going to Ultra-High Pressures

According to Jorgenson, a significant part of the history of liquid chromatography has been a battle pitting ever-smaller particles, which effect better separations, against ever-increasing pressures. Most HPLC systems run at about 6,000 psi, and commercial ultra-high-pressure systems operate at pressures of about 15,000 to 20,000 psi. But historically, as particles shrank, instrument manufacturers tended to pack them into shorter and shorter columns to keep the pressures manageable. As a result, separations got faster but not better, he says (James Jorgenson, personal communication, 2016). Jorgenson's team has been working since the mid-1990s to reverse that trend, developing systems that can handle pressures as high as 100,000 psi, with the goal of taking advantage of the separation power that smaller particles should be able to afford. Typical HPLC columns, he says, are about 15 cm long. His lab has produced columns of 2 mm particles measuring 1 m in length, producing some 500,000 theoretical plates at 30,000 psi.[52] That, he says, should produce separations about five times better than traditional HPLC columns, which have about 20,000 theoretical plates. "If you had two peaks that were right next to each other, they'd now have room for five peaks to be put in between them, which is a mind-blowing difference," he says (James Jorgenson, personal communication, 2016).

Shrinking Columns

Another option for improving separations involves downsizing the columns into the realm of nano-LC or microfluidics. As mentioned earlier, Ramsey of UNC Chapel Hill has built monolithic microfluidic capillary electrophoresis systems with integrated nano-electrospray emitters, which he has used to resolve therapeutic antibodies,[34]

antibody-drug conjugates,[3] and variants of hemoglobin.[35] According to Ramsey, that chip separates based on charge-to-size ratio — a characteristic that, among other things, can reflect the post-translational modification of protein molecules. These molecules are then evaluated on a simple time-of-flight mass spec (or quadrupole-time-of-flight, in the case of the hemoglobin study). "We don't need a super-high-performance mass spectrometer, because we're decluttering the sample by sorting out these charge variants using the CE," he explains. Ramsey has commercialized his CE-ESI chip designs through the company 908 Devices, which sells the technology under the ZipChip brand.

Other companies have also commercialized micro-scale chip-based LC systems, including Waters Corp., with its ionKey/MS system, and Eksigent (now part of SCIEX). David Muddiman at North Carolina State University, says he has obtained impressive separations with the Eksigent system, though his own team members are no slouches when it comes to column packing. "We could not pack a better column; we got the best chromatography we've ever seen." (David Muddiman, personal communication, 2016). According to Muddiman, the advantage of such columns is neither price nor speed, but reproducibility. "Most people who do nanoflow chromatography pack their own columns," he explains. "And [when] you pack your own columns, you don't pack a 15-cm column every time; sometimes it's 14.5, sometimes it's 16. And the density of the particles is different each and every time." With a microfluidic chip, however, the columns are more or less identical from column to column and batch to batch — so, therefore, are the resulting analyte retention times. "We really like that about the chip technology," he says (David Muddiman, personal communication, 2016).

V. NUCLEAR MAGNETIC RESONANCE: FIELD SIZE GOES UP & DOWN

Nuclear magnetic resonance is also keeping pace with the times, including improvements in both instrumentation and applications. On the hardware front, NMR magnets continue to grow, a parameter that improves spectral resolution. Today's current top-of-the-line superconducting magnets weigh in at 1 GHz, but Bruker has announced plans for "ultra-high-field" 1.2 GHz instruments to begin shipping in "late 2017 or 2018."[53] NMR developer JEOL has also cracked the 1 GHz barrier and, according to *C&EN*, has a 1.3 GHz instrument in development.[2] At the other end of the spectrum, sub-100-MHz benchtop systems powered by rare earth magnets are also growing in popularity, with applications in education, quality control, and reaction monitoring.[2]

Decoding Protein Structure with Solid-State NMR

Though solution-state NMR traditionally has held the edge in biological NMR, solid-state NMR is now able to rival the technology, at least when it comes to complicated protein structures.

As Christopher Jaroniec, Professor of Chemistry and Biochemistry at Ohio State University, explains, there's an upper limit to the structures that can be solved practically using solution-state NMR, because spectral lines broaden with increasing molecular size and decreasing molecular tumbling rate. In solid-state NMR, molecules don't tumble either. But researchers can overcome this problem by spinning the sample inside the magnet at tens of thousands of revolutions per second at the so-called "magic angle" of 54.7 degrees, equivalent to the body angle of a cube. "This magic-angle spinning trick essentially allows the interactions that depend on molecular orientation to be averaged to zero. As a result one is able to obtain solution-like quality NMR spectra, but in the solid state." (Christopher Jaroniec, personal communication, 2016). This makes solid-state NMR the method of choice for the analysis of very large protein molecules and complexes, he says.

Jaroniec has used this magic-angle spinning (MAS) approach to study the histone proteins around which DNA wraps, looking for the boundaries between the flexible tails and more rigid protein cores in the context of chromatin.[54] Their data suggest that even in their compact state, histone tails retain remarkable flexibility — a counterintuitive observation, he says. "The naive assumption would be that when chromatin compacts, the tails should become completely rigid… But that's in fact not what you find." (Christopher Jaroniec, personal communication, 2016).

Chad Rienstra, Professor of Chemistry at the University of Illinois at Urbana-Champaign, used MAS solid-state NMR to investigate the structure of the 14.5-kDa alpha-synuclein, a protein that aggregates in the neurons of patients with Parkinson's disease but whose structure has remained elusive. Taking advantage of improvements in sample preparation, a powerful 750-MHz wide-bore NMR instrument, and customized pulse sequences, Rienstra's team solved the structure in May 2016.[6] The structure, they reported, resembles a Greek key, with protein aggregates forming via an in-register beta-sheet stacking.

Both Jaroniec and Rienstra completed their studies with MAS probes spinning at around 10,000 Hz. But the faster the spinning, Jaroniec says, the better the resolution, especially for fully protonated samples. Bruker now offers MAS probes capable of up to 111,000 revolutions per second with a sample volume of 0.5 ml.

Structural Constraints with Paramagnetic Tags

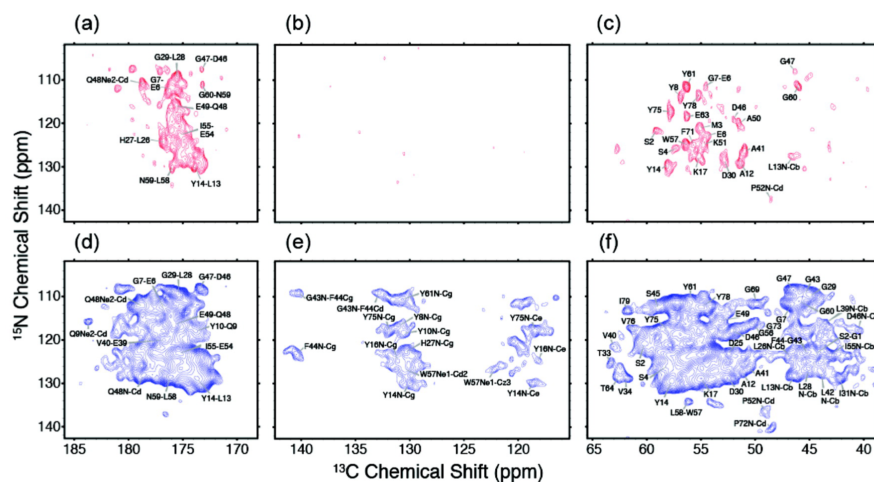
Another new development, Jaroniec says, is the use of paramagnetic tags to enhance the utility of solid-state NMR in structure determination. Normally, he explains, researchers cannot probe distances greater than 5 Å or 10 Å in NMR, because the signals reporting on internuclear interactions become too weak. "To go beyond that is effectively impossible because the nuclear dipolar couplings become very, very small." As described in one recent study:

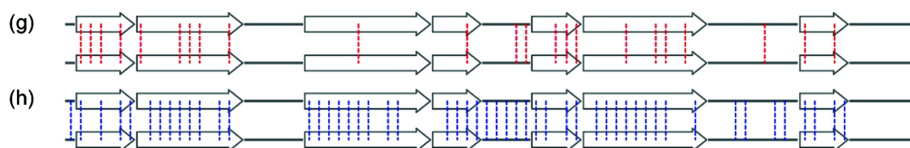
> *The fundamental limitation in most solid-state NMR structure determination endeavours has been the relative scarceness of unambiguous long-range interatomic distance restraints. This is primarily because dipolar couplings between ^{1}H, ^{13}C and ^{15}N spins, which scale with the inverse third power of the internuclear distance, become vanishingly small for distances in the 5–10 Å regime and beyond.*[55]

Using paramagnetic tags, however, researchers can push that limit to 20 Å or even 25 Å, Jaroniec says, providing unique long-range structural constraints that simplify structure elucidation. In 2012, his team demonstrated that possibility by using copper-tagged mutants and nuclear paramagnetic relaxation enhancements (PREs) to calculate the de novo structure of protein G, a 56-amino acid immunoglobulin-binding protein.[55]

At the Massachusetts Institute of Technology, Robert Griffin has developed another strategy that also leverages paramagnetic centers to enhance structural studies. Dynamic nuclear polarization (DNP) is a solid-phase NMR strategy that increases spectral sensitivity from 100- to 1000-fold by effectively blending electron paramagnetic resonance with NMR. As explained in a 2013 review in *Accounts of Chemical Research*, "In DNP, the sample is doped with a stable paramagnetic polarizing agent and irradiated with microwaves to transfer the high polarization in the electron spin reservoir to the nuclei of interest."[56] As Jaroniec puts it, "One can dramatically enhance the sensitivity of conventional solid-state NMR spectra essentially by transferring the very large electron spin polarization to the nuclear spins" (Christopher Jaroniec, personal communication, 2016).

In one example, Griffin's team scanned a sample comprising amyloid protein fibers in a 750-MHz instrument for 16 days at 300 K, or on a 400-MHz instrument with DNP for 32 hours at 100 K.[57] "The number of intermolecular ^{15}N-^{13}C constraints detected was more than doubled due to the DNP," the authors wrote, adding, "The additional constraints obtained from DNP permitted us to establish that the PI3-SH3 protein strands are aligned in a parallel and in-register β-sheet arrangement."





Comparison between room-temperature and DNP-enhanced, low-temperature intermolecular correlation spectra. (a–c) 750 MHz intermolecular ^{15}N–^{13}C correlations in PI3-SH3 fibrils recorded at 300 K with 16 days of acquisition. The three panels correspond to the ^{15}N–^{13}C–O, ^{15}N–^{13}C (aromatic), and ^{15}N–$^{13}C\alpha$ regions of the spectra. (d–f) The identical spectral regions recorded at 100 K and 400 MHz with DNP enhancement in 32 h of signal averaging. The spectra were obtained with ZF-TEDOR recoupling (τ_{mix} = 16 ms) from mixed PI3-SH3, a sample prepared from a mixture of [15N] monomers and [2-^{13}C] monomers. (g) Illustration of the 23 interstrand contacts established from ^{13}C–^{15}N cross-peaks in the 750 MHz spectra acquired at 300 K in panels a–c; (h) the 52 interstrand contacts established from the 400 MHz DNP enhanced spectra recorded at 100 K shown in panels d–f.

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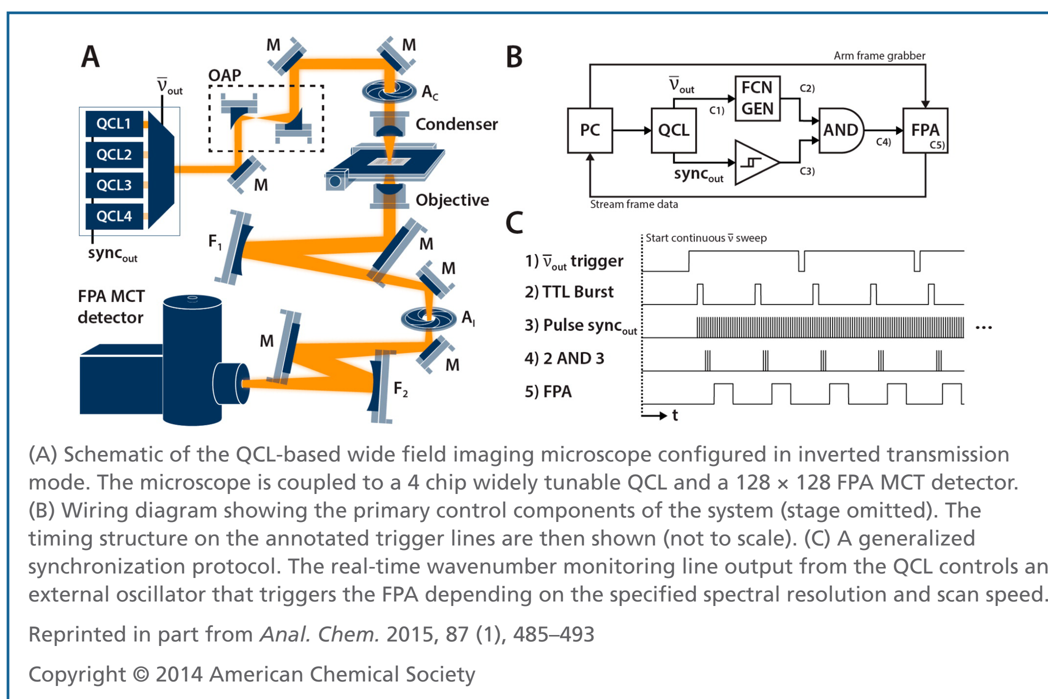
Another team, led by Lyndon Emsley of the University of Lyon, France, has used DNP signal enhancement to characterize surface chemistry, a technique Emsley calls DNP surface enhanced NMR spectroscopy (DNP SENS).[58] In one example, Emsley's team studied a phenol-functionalized silica matrix, achieving a 25-fold enhancement of the proton signal, and 56-fold for carbon-13.[59]

VI. VIBRATIONAL IMAGING

Although many analytical techniques are destructive to the sample — requiring, for instance, cellular lysis or staining — vibrational spectroscopy techniques are not. Raman and infrared spectroscopy, says Rohit Bhargava, the Founder Professor of Bioengineering at the University of Illinois at Urbana-Champaign, use light to probe the molecular bonds of a chemical mixture. When applied to complex chemical samples, such as a biological sample, the resulting data cannot reveal specific chemicals, he says, but rather a "spectral signature of the state of the cell," reflecting the relative abundance of proteins, lipids, and nucleic acids.[60] Deviations from the "normal" signature can indicate disease or dysregulation — conditions that researchers can pick up using vibrational microscopy techniques.[61] Like mass spec imaging, vibrational microscopy methods blend chemical analysis with spatial information, resulting in a two-dimensional map of a sample's chemical makeup. By correlating those images with optical images of a sample, researchers can discern the chemical differences between tumor and normal tissues, for example — all without the use of chemical stains.[62]

Both IR and Raman imaging have seen significant technical advances over the past decade, Bhargava says (Rohit Bhargava, personal communication, 2016). On the IR side, for instance, Bhargava and Carol Hirschmugl of the University of Wisconsin, Milwaukee, described in 2011 a diffraction-limited Fourier-transform infrared (FTIR) microscope built using a 3 x 4 array of synchrotron beams and a widefield detector array, which exhibited unprecedented resolution.[63] As the authors demonstrated, the system was able to detect individual white blood cells in prostate cancer tissue sections (which remain unresolved using a standard IR microscope comparator), and even approached traditional hematoxylin and eosin (H&E) staining (the "clinical gold standard for diagnosis") in image quality — all at relatively fast imaging speeds, scanning an area measuring 280 mm x 310 mm in 30 minutes, as compared to 11 days with some older configurations. More recently, Bhargava and his team demonstrated a more compact discrete-frequency infrared (DF-IR) imaging system built using four tunable quantum cascade lasers (QCL), with which they imaged 148 tissue core samples on a tissue microarray in 5 hours.[64]



(A) Schematic of the QCL-based wide field imaging microscope configured in inverted transmission mode. The microscope is coupled to a 4 chip widely tunable QCL and a 128 × 128 FPA MCT detector. (B) Wiring diagram showing the primary control components of the system (stage omitted). The timing structure on the annotated trigger lines are then shown (not to scale). (C) A generalized synchronization protocol. The real-time wavenumber monitoring line output from the QCL controls an external oscillator that triggers the FPA depending on the specified spectral resolution and scan speed.

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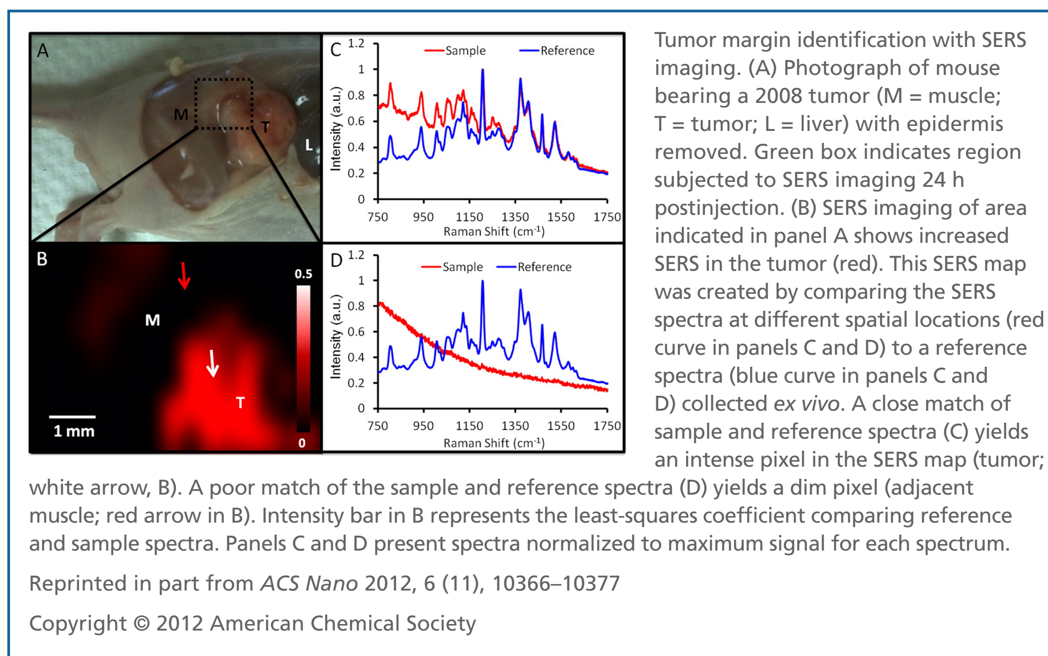
Raman Imaging

Raman imaging has also advanced in recent years, with multiple groups developing methods to use the technique in medical settings.[62] In 2012, Sanjiv Sam Gambhir of the Molecular Imaging Program at Stanford University, and his team described functionalized gold nanorods that could serve as dual-modality photoacoustic imaging/SERS agents.[65] The team demonstrated these agents in mice, using them to identify tumor margins and to guide tumor excision.



Tumor margin identification with SERS imaging. (A) Photograph of mouse bearing a 2008 tumor (M = muscle; T = tumor; L = liver) with epidermis removed. Green box indicates region subjected to SERS imaging 24 h postinjection. (B) SERS imaging of area indicated in panel A shows increased SERS in the tumor (red). This SERS map was created by comparing the SERS spectra at different spatial locations (red curve in panels C and D) to a reference spectra (blue curve in panels C and D) collected *ex vivo*. A close match of sample and reference spectra (C) yields an intense pixel in the SERS map (tumor; white arrow, B). A poor match of the sample and reference spectra (D) yields a dim pixel (adjacent muscle; red arrow in B). Intensity bar in B represents the least-squares coefficient comparing reference and sample spectra. Panels C and D present spectra normalized to maximum signal for each spectrum.

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Gambhir's team has also developed a Raman-based endoscope, which can be used with a series of surface-enhanced Raman scattering (SERS) nanoparticles to detect at least 10 distinct chemical species at a time.[66] As described in the study, the SERS particles "consist of a unique Raman-active layer adsorbed onto a 60-nm gold core and then coated with silica, resulting in an effective diameter of ~120 nm." Multiplexing is achieved by altering the "Raman-active layer," producing a series of particles each bearing a unique spectral signature. In a subsequent study, Gambhir's team updated that design with "a new opto-electro-mechanical device that has the capability to rapidly and systematically scan large tissue surfaces and produce images of structural and multiplexed functional data within the context of the traditional endoscopic-imaging procedure."[67]

Other researchers are also pursuing this line of research. In 2013, X. Sunny Xie of Harvard University described a two-color Raman microscopy system based on the principle of stimulated Raman scattering (SRS). As explained in the study:

> *SRS has several advantages over CARS [coherent anti-Stokes Raman scattering] for biomedical imaging, including a linear relationship between signal intensity and chemical concentration, as well as a non-distorted spectrum almost identical to that of spontaneous Raman, enabling quantitative chemical imaging. Furthermore, by adding spectroscopic information, multi-color SRS microscopy enables the differentiation of normal and tumor-infiltrated tissues based on biochemical and morphological properties.*[68]

Xie's team used their system to differentiate glioblastoma (a brain tumor) from normal brain tissue in mice based on the spectral signatures of lipid and protein. In a subsequent study, they used SRS to image nuclear structures in live cells at interphase at high resolution, relying on "the distinct Raman spectral features of the carbon-hydrogen bonds in DNA."[69]

Raman is also having an impact outside the imaging realm, particularly for the development of analytical assays that leverage the signal boost afforded by SERS. In one recent example, Shuming Nie and Chunhui Xu of Emory Universityin developed antibody-coated SERS nanoparticles for the high-sensitivity detection of pluripotent stem cells.[70] Such an assay, the authors say, could be used in regenerative medicine to ensure that stem cell-derived transplants are free of teratoma-forming pluripotent stem cells, an application that requires sensitivity on the order of one cell in 100,000. Using their custom SERS particles, they could detect as few as one stem cell in a million background cells, the authors report, at least 2,000 times better than they could achieve via flow cytometry.

VII. CONCLUSION

There's no denying that "analytical instrumentation" is a broad topic, encompassing technologies from atomic absorption to x-ray spectroscopy. But the unifying theme, says J. Michael Ramsey of UNC Chapel Hill, is a drive to make chemical or biochemical measurements, and to do them as quickly, accurately, and inexpensively as possible (J. Michael Ramsey, personal communication, 2016).

Over the preceding pages, we have seen technological improvements along those lines across the broad field of analytical methods have been described. And this survey was by no means comprehensive — just about every subdiscipline of analytical chemistry has experienced improvement and advancement as well.

Using scanning electron microscopy as an example, researchers interested in mapping the neural connectivity of the brain can now employ more powerful microscopes, such as focused ion beam and multi-beam SEMs, to collect serial images of ultrathin brain slices.[71] Researchers with an eye toward economy can now build surface plasmon resonance substrates out of silver rather than the more typical gold — a finding reported by a team of China researchers who used that approach (and an SPR microscope) to image and quantify 1,296 binding events in parallel.[72] And those interested in surface properties can now scan those surfaces faster than ever thanks to high-speed atomic force microscopy.[73]

For researchers who would exploit these methods to drive their scientific agenda, it's never been a better time to be an analytical chemist. And for those who aim to develop the next generation of tools, there's plenty of work still to do. Innovation, improvement, and advancement are the operative words to describe the current state of the analytical sciences. So keep checking the pages of your analytical journals. You never know what new innovation you're going to find.

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