ANALYTICAL CURRENTS

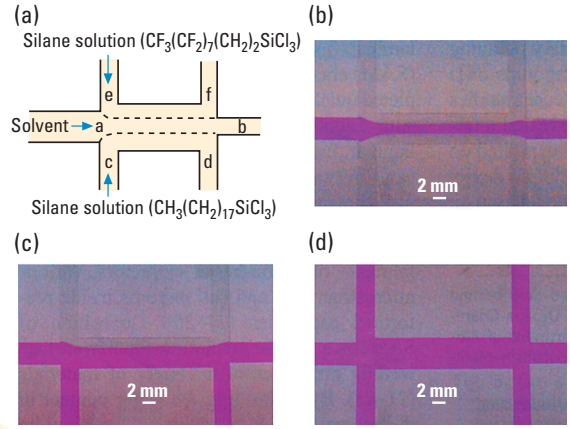
"Virtual" walls and valves for microchannels

For those who feel claustrophobic in the microscale realm, Bin Zhao, Jeffrey Moore, and David Beebe at the University of Illinois at Urbana–Champaign and the University of Wisconsin–Madison have replaced microchannel side walls with "virtual" walls to guide fluids. The "walls" are made by depositing hydrophobic and hydrophilic self-assembled monolayers (SAMs) inside the microchannels—an approach that is designed to make fabrication faster and simpler.

Under normal conditions, the fluids flow only along the hydrophilic pathways. If two virtual channels are fabricated side by side, volatile compounds can be exchanged between two fluid streams separated by a gas membrane.

Fluid may breach the virtual wall if the pressure is above a critical value determined by the surface free energy of the liquid, the advancing contact angle of the liquid on the hydrophobic regions, and the channel depth. Although this may seem like a drawback, the researchers used it to their advantage by combining monolayers that yield various pressure thresholds to create pressure-sensitive valves for regulating fluid flow.

In early experiments, the SAMs were deposited inside preformed channel networks. In later work, the researchers used a photolithographic method to pattern the surface free energies inside microchannels. (*Science* **2001,** *291,* 1023–1026)

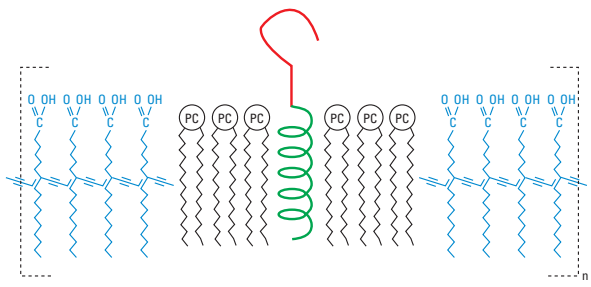


(a) Diagram of fluid flow in a pressure-sensitive valve. (b) At a pressure of 10-mm H_2O, fluid only flows straight through the channel. At (c) 26-mm H_2O and (d) 39-mm H_2O, fluid enters the side channels. (Adapted with permission. Copyright 2001 American Association for the Advancement of Science.)

Colors of recognition

Peptide–antibody interactions are important in biochemical and diagnostic processes, but detection methods are often slow and require several preparation and processing steps. So Raz Jelinek and colleagues at Ben Gurion University of the Negev (Israel) introduce a colorimetric method that responds within seconds or minutes after a single mixing step.

The method employs a biomimetic membrane, made from dimyristoylphosphatidylcholine surrounded by polydiacetylene, which displays an epitope at the N-terminus of a helical sequence of hydrophobic amino acids. The membrane's color changes from blue to red-purple when it mixes with an epitope-specific antibody. Transmission electron microscopy (TEM) indicates that cracks and grooves appear in the surface of the membrane under these conditions. On the other hand, when nonspecific antibodies are added, there is no color change, and TEM shows little change in the smooth surface and shape of the membrane particles.



Schematic diagram of the colorimetric assembly interface, including (blue) conjugated polydiacetylene, (black) dimyristoylphosphatidylcholine, and (red) epitope displayed at the N-terminus of a (green) helical membrane-associated peptide. Molecular components are not to scale.

The authors used this method to detect antibody concentrations in the 100- to 700-μg/mL range. They found that 2 μM of immobilized epitope was optimal. Although a higher concentration of the epitope resulted in a greater color change, it induced aggregation and impeded the assay. Enzyme-linked immunosorbant assays confirmed the general applicability of the colorimetric system and verified that significant color changes occurred only when the displayed epitopes were recognized by their specific antibodies. (*J. Am. Chem. Soc.* **2001**, *123*, 417–422)

ANALYTICAL CURRENTS

Rapid cocktail test for enzymes

New drug candidates are being developed at an ever-faster rate, so faster testing methods are needed. More rapid techniques to test for the inhibition of human drug-metabolizing enzymes are in particular demand because inhibitors can cause possible adverse drug interactions.

Simon Ball and colleagues at Wyeth-Ayerst Research have developed a method for simultaneously evaluating the in vitro metabolic activity of seven major cytochrome P450 (CYP) enzymes. The researchers incubated a cocktail of substrates with well-characterized specific inhibitors and 10-µM enzymes from human liver microsomes. The assay determined the percentage of activity remaining for each enzyme relative to a control sample containing no inhibitor. Incubation of individual substrates under the same conditions confirmed the results of the cocktail substrate method.

A similar experiment with varying inhibitor concentrations established the IC_{50} values for each inhibitor–CYP enzyme combination; values <10 µM indicated potent CYP inhibition. Further assays, performed with enzymes expressed in *Escherichia coli*, established that the substrates were metabolized almost exclusively by specific enzymes.

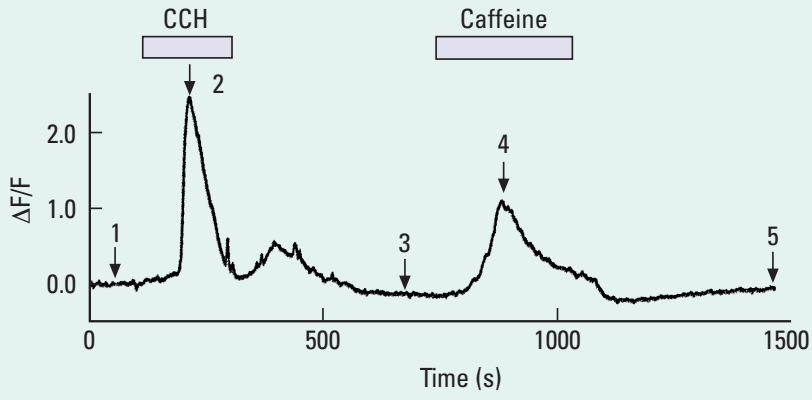
Fast-gradient LC/MS/MS analyzed the metabolic products in a four-min sample run time. A further time-saver is that samples are stable for at least four days at 4 °C, so many samples can be prepared at once. (*Drug Metab. Dispos.* **2001**, *29*, 23–29)

Green Ca^{2+} probe

Although there are already several sensors for measuring intracellular calcium—an important cation involved in activities ranging from synaptic transmission to the development of new cells—each has limitations. Now, Junichi Nakai and co-workers at the National Institute for Physiological Sciences (Japan) describe a new green fluorescent protein (GFP)-based probe for calcium, which features a high S/N and a fast response, making the probe ideal for "excitable cells".

The new probe consists of three sections: a circularly permutated enhanced GFP (chosen because of its strong fluorescence), the calcium-binding protein calmodulin, and the M13 fragment of myosin light chain kinase, which is a target sequence of calmodulin. When calcium binds to calmodulin, the probe undergoes conformational changes that result in fluorescence changes. For example, in in vitro studies, the probe fluorescence increased ~4.5-fold following calcium addition.

At high Ca^{2+} concentrations, the probe binds the cation quickly, taking <10 ms at a [Ca^{2+}] of >500 nM. Dissociation is much slower (~200 ms) and is independent of calcium concentration. Thus, this probe is well suited for determining Ca^{2+} levels following the application of drugs or electrical stimulation. However, other probes are better at measuring micromolar Ca^{2+} levels. In addition, the probe is pH sensitive and unfolds at temperatures of 37 °C or higher, but the researchers do not see these problems as significant drawbacks. (*Nat. Biotechnol.* **2001,** *19,* 137–141)



A calcium jolt. Mouse myotubes expressing the calcium-detecting GFP respond to changes in calcium levels following the introduction of carbachol and then caffeine. (Adapted with permission. Copyright 2001 Nature America.)

A guide to thin-layer electrochemistry

A simple way to study electron-transfer properties across the interface between aqueous solutions and immiscible organic thin layers is to coat a graphite electrode with the organic layer and measure the steady-state currents. However, some of the electron-transfer rate constants that have been determined from this approach do not match values obtained by other methods, leading some to question its value. Fred Anson and Chunnian Shi of the California Institute

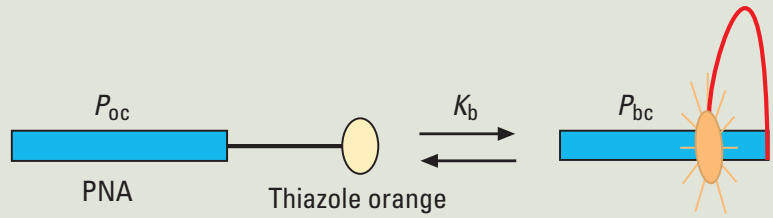
of Technology came to the rescue, deriving and testing an equation that guides researchers toward the appropriate conditions for successful thin-layer electrochemistry.

The derived equation is in the form of an inequality, which sets upper and lower limits on the molar concentration of the reactant in the aqueous phase. The equation was tested by studying the electron transfer between $IrCl_6^{2-}$ in the aqueous phase and a modified ferrocene compound trapped in a nitrobenzene thin layer. The results verify that adhering to the constraints of the inequality produce essentially constant steady-state currents, which yield reliable rate constants. (*J. Phys. Chem. B* **2001,** *105,* 1047–1049)

New light on PNA probes

Light-up probes, in which fluorescent dyes are attached to short peptide nucleic acids (PNAs), are named for the large signal increase generated when they hybridize to their targets. However, the fluorescence enhancement of these probes depends on the PNA sequence, mainly because of variations in the free-probe fluorescence. Now, Mikael Kubista and colleagues at the Chalmers University of Technology (Sweden) explain why.

The researchers characterized three light-up probes made from thiazole orange conjugated to various PNAs. They found that the dye sometimes "folds back" and interacts with the PNA, even though it is not attracted to the PNA electrostatically. The resulting variation in free-probe fluorescence is, for the most part, caused by the fluorescence quantum yield of the probe in the back-bound conformation. The probe's tendency to adopt the back-bound conformation contributes to a much lesser extent. (*J. Am. Chem. Soc.* **2001,** *123,* 803–809)



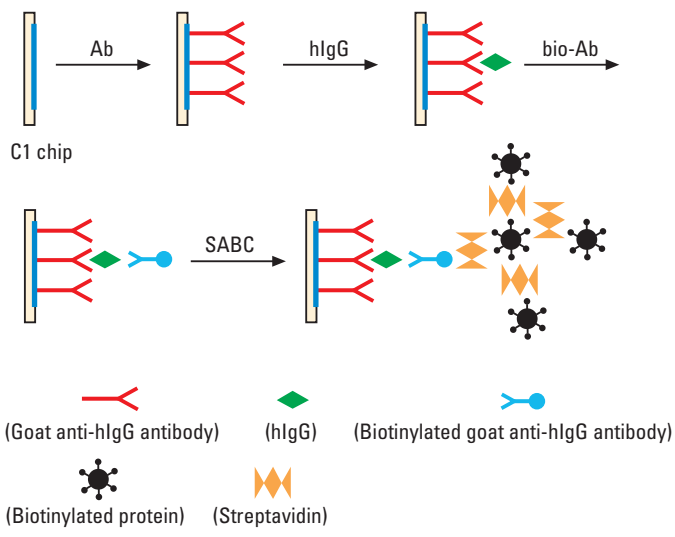
Schematic of a light-up probe in an open and a back-bound configuration.

Enhancing SPR sensitivity

Surface plasmon resonance (SPR) sensing is convenient because it is label free, rapid, and performed in real time. Unfortunately, its sensitivity also tends to be limited. To enhance sensitivity, researchers have coated SPR chips with dextran to increase the loading of biomolecules or employed latex particles, colloidal Au, or liposomes. Now, Renjun Pei, Xiurong Yang, and Erkang Wang at the Chinese Academy of Sciences describe a new technique that takes advantage of the high-affinity binding of streptavidin and biotin.

In the new method, a capture antibody is immobilized on the SPR chip. When the analyte (an antigen) and a biotinylated detection antibody are added, an analyte "sandwich" is formed. At this point, the amount of bound detection antibody is related to the amount of analyte. Then, a complex of streptavidin and a biotinylated protein, each of which provides multiple binding sites, is added to amplify the signal. The researchers note that the quality of amplification depends on the ratio of biotin-labeled protein to streptavidin within the complex.

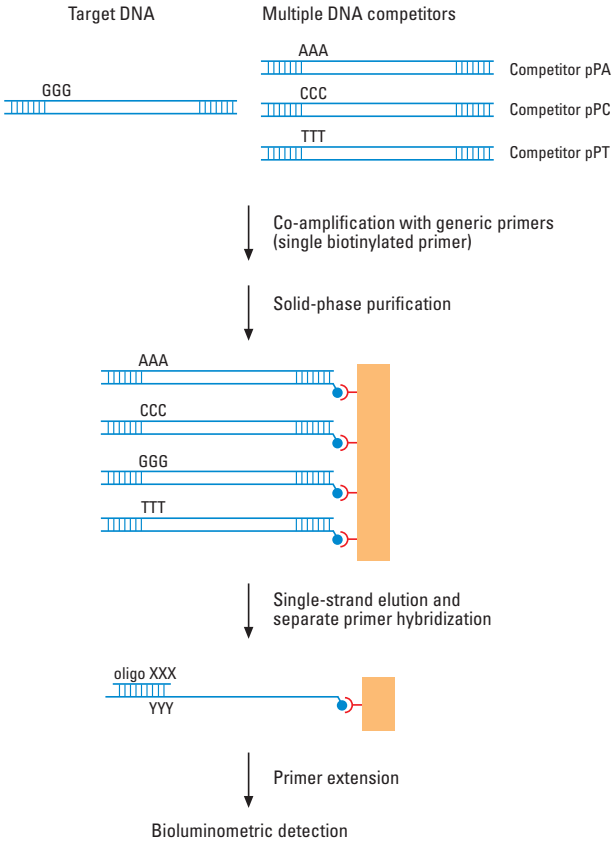
In experiments conducted with human immunoglobulin G (hIgG) and goat anti-hIgG antibodies, a 200-fold signal enhancement was observed when the streptavidin–biotinylated protein complex was added, allowing detection in the range of 0.005–10 μg/mL. (*Analyst* **2001,** *126,* 4–6)



Schematic of a human immunoglobulin G assay enhanced with a streptavidin–biotinylated protein complex. (Adapted with permission. Copyright 2001 Royal Society of Chemistry.)

ANALYTICAL CURRENTS

Quantifying HIV infection levels



Schematic illustrating the bioluminometric detection technique for quantification of PCR products. (Adapted with permission. Copyright 2001 Academic Press.)

Determining the degree of human immunodeficiency virus (HIV) infection, also known as the viral load, has been a long-standing priority in health care. Now, Malin Nygren, Joakim Lundeberg, and colleagues at the Royal Institute of Technology and the Karolinska Institute (both in Sweden) describe a PCR-based assay that uses multiple quantitative standards and replaces gel electrophoresis with rapid bioluminometric detection.

The new method is a form of competitive PCR, in which one or more control templates, which are very similar to the test template, are amplified. The final amounts of all PCR products are compared, usually by running them on an electrophoretic gel and comparing band intensities. Because the initial amount of control template is known, the initial amount of test template can be determined.

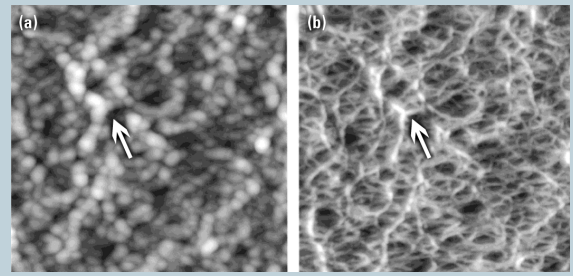
One drawback to competitive PCR methods has been the need to perform many parallel amplification reactions with various dilutions of each control template to generate a standard curve. The new method reduces the number of parallel PCR reactions by employing three control templates, which differ from the test template at only three or four nucleotides and produce PCR products of the same length, in the reaction with the test template. The four PCR products are immobilized onto beads, denatured to produce single-stranded DNA, and divided into four aliquots.

Each aliquot is then subjected to another reaction in which a modified DNA polymerase extends a primer if the primer and target match. Successful extension releases inorganic pyrophosphate, which is converted into ATP. A luciferase enzyme then takes up the ATP and converts it to a proportional amount of visible light. (*Anal. Biochem.* **2001,** *288,* 28–38)

Check those AFM tips

Here is a simple and easy method to ensure that your atomic force microscopy (AFM) tip is free of contaminants and is undamaged. H.-Y. Nie and N. S. McIntyre of the University of Western Ontario (Canada) propose testing the tip's resolution with biaxially oriented polypropylene (BOPP), a polymer film with nanometer-sized fiber structures.

AFM tips contaminated with impurities the size of the features being studied can yield images that mix surface features with contaminant structures. BOPP films are a good way to test the tip before a measurement. Not only does the polymer help check resolution, but the material's high hydrophobicity and low surface energy keep BOPP's own surface contaminant-free, thereby preventing new tip contamination. BOPP is also soft, and in this paper, that property was used to remove tip contaminants by pressing the tip repeatedly into the polymer without damaging the AFM probe. (*Langmuir* **2001,** *17,* 432–436)



Before and after. An AFM image of BOPP film with (a) a dirty tip and (b) a cleaned tip.

RESEARCH PROFILES

Single molecule imaging sheds light on chromatography

In chromatography, a single step can dominate the entire process, so looking at several steps simultaneously may not provide the correct interpretation of molecular behavior. But in the March 15 issue of *Analytical Chemistry* (pp 1091–1099), it's single molecule imaging to the rescue. Edward S. Yeung and co-workers at Iowa State University–U.S. Department of Energy's Ames Laboratory describe the use of single molecule imaging to study the adsorption and desorption of single molecules of DNA at chromatographic surfaces. "This is a totally different way to look at chromatography," says Yeung. "We can get information that is much more detailed."

The researchers labeled λ-DNA molecules with the fluorescent dye YOYO-1 and excited them in an evanescent field using a total internal reflection optical geometry and an argon ion laser. An intensified charge-coupled device camera acquired images and even movies of individual DNA molecules being adsorbed and desorbed on fused-silica and C_{18} surfaces at flow rates similar to those used in chromatography.

"We can use the DNA molecule as a probe of what is happening on a chromatographic surface," explains Yeung. DNA is well suited to this task because most of the molecule has ionic properties, whereas the ends are hydrophobic. "We can see the interplay between ionic and hydrophobic interactions and which is more important under different experimental conditions," he says.

"For DNA, we found that hydrophobic interactions are the only important interaction in chromatographic events," Yeung says. The extent of the hydrophobic effect can be altered by conditions such as pH, type of surface, and the water/organic composition of the buffer. Electrostatic interactions had no role in adsorption, he adds, but are important for keeping DNA away from the capillary walls.

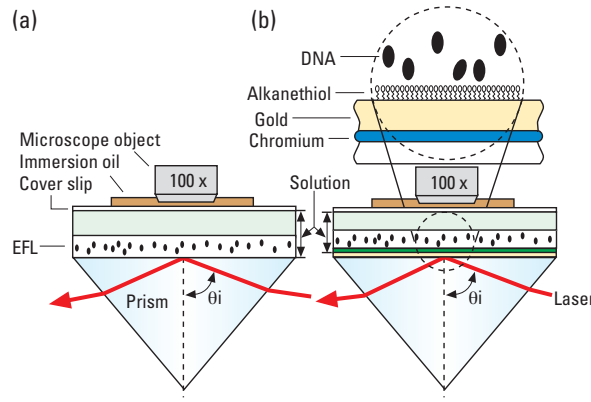
The researchers found that DNA began adsorbing onto the fused-silica surface as the pH decreased, beginning at around pH 5.5 and continuing to around pH 3.5. Adsorption began at the hydrophobic ends of the molecule, showing that hydrophobic interactions are the driving force for adsorption. "You can see the ends of the molecules being adsorbed, [whereas] the center [is] not adsorbed," says Yeung.

The researchers then modified the experimental conditions to change the proportion of hydrophobic versus ionic interactions. Changing to C_{18}, a more hydrophobic surface, was expected to enhance hydrophobic interactions, and it did: DNA was more strongly adsorbed on C_{18}. Adsorption on C_{18} occurred over the pH range 4–6, with a high fraction of molecules permanently adsorbed and more molecules breaking below pH 6 than with the fused-silica surface. On the other hand, adding methanol to the aqueous buffer decreased hydrophobic interactions between DNA and the C_{18} surface, as was expected. "Methanol is hydrophobic compared to water," Yeung explains, "and it disrupts the interaction between DNA and the surface."

The duration of adsorption on the fused-silica surface ranged from 33 to 267 ms, peaking at 67 ms. A histogram of the adsorption duration shows an asymmetric plot skewed toward the shorter adsorption times. "Molecules would get stuck," says Yeung, "go back and swim around, then get stuck again." At low pH, the molecules became permanently adsorbed.

To show that the imaging results carry over to chromatography, the researchers used the same conditions for CE and capillary LC as in the imaging experiments. Chromatographic bands broadened and showed tailing as pH decreased; these effects were similar to the pH-dependent adsorption effects in the imaging experiments.

In chromatography, explains Yeung, both retention and band broadening effects have been assumed to be related to adsorption. "We now see that this is true in the single molecule case," he says. There was a 1:1 correlation between band broadening in chromatography and increases in adsorption time in imaging experiments, he says, and the longer the adsorption time, the more the progress of the molecule was delayed. The asymmetric plot of adsorption duration in imaging experiments has the same shape seen in CE, he says, showing that as an interaction becomes important, peaks start to show broadening and tailing.



Experimental setup for monitoring single molecules of DNA at (a) fused-silica and (b) C_{18} surfaces within the evanescent field layer.

Yeung plans to continue the imaging experiments using proteins. "Proteins are more complicated than DNA," he says. In proteins, hydrophobic and ionic regions are all mixed up in different parts of the molecule. "We want to see how these same effects compete with each other in protein separations," he says. ◥

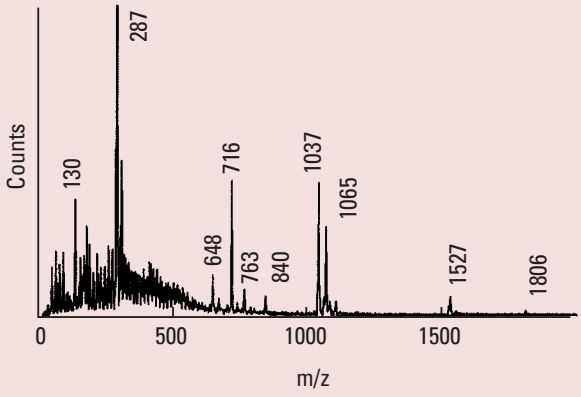
–Teresa Calafut

RESEARCH PROFILES

"Matchsticks" for MALDI

The future of MALDI MS may be matchsticks. That best describes the novel silicon surface for housing analytes for MALDI, which is described by Joseph Cuiffi, Stephen Fonash, and their colleagues at Pennsylvania State University in the March 15 issue of *Analytical Chemistry* (pp 1292–1295).

MALDI experiments are typically performed using organic matrixes that provide a shield from the harshness of ionization conditions, allowing good detection of large, delicate analytes. The trouble is that the matrix itself breaks down under ionization, and any analyte that shows up in the mass spectrum at <500 *m/z* gets lost in the signals originating from the matrix components. The randomness of analyte deposition in the matrix also leads to clumping and uneven distribution, and therefore inconsistent spectra. As a result, says Cuiffi, "It's tough to get the same results every time."



Mass spectrum obtained from a column-desalted (1-mM predigest) tryptic digest of ubiquitin with the addition of ammonium citrate (250-μM ammonium citrate, 1:1 mixture).

Those problems led other research teams to develop matrix-less methods using electrochemically etched porous surfaces in silicon, but they have proven to be difficult to manufacture. The silicon surfaces also still suffer from nonuniformity, and contaminants can get trapped during the etching process—effects that interfere with ionization. That's a problem, notes Cuiffi, especially if you are developing automated systems. Low ionization leads to weak signals. "In high-throughput systems, computer programs are looking for a peak, but if their criteria are not stringent enough, they won't be able to detect it," says Cuiffi.

To get around this problem, Cuiffi and his collaborators used a plasma-enhanced chemical vapor deposition technique to produce a thin film of rodlike silicon columns, which rise from a glass or plastic substrate. The columns are ~10 nm in diameter, and they form in clusters ~100 nm in diameter. "It's like bundling match sticks together. If you bundle them together—not necessarily tightly—that's what our film is," says Cuiffi.

Under specific conditions, the clusters are uniform, and that should lead to consistent ionization yields, more reproducible spectra, and an automation-friendly approach, says Cuiffi. Even better, the films are easily manufactured, he says, and their properties are easy to manipulate. "It's done in a plasma deposition system," he notes. "These machines are used to coat [many] things, from screens for laptops to the aluminum they use to coat every compact disc. They can be high-throughput."

The conditions can also be varied to yield different surface characteristics. The nucleation density changes with variations in the pressure and power of the plasma source, explains Cuiffi. The researchers have a range of cluster and column sizes and crystallinity that they can tailor, he says. They also can tailor the reflectivity of film and its porosity to enhance ionization. "You can imagine changing the porosity, and having various surface areas and pocket sizes that molecules can stick on or in," he says. "You could get a lot of molecules in a small area to get a higher ion count," and thus get more samples on to a slide.

In addition, it turns out that ionization and analyte fragmentation are comparable with those obtained with a standard MALDI matrix, making the films suitable for delicate analytes. "I think it has to do with the fact that [the clusters] absorb UV light very well," says Cuiffi. "You get a lot of incident light in a small space, and there is excellent heat transfer."

Like silicon used in microelectronic fabrication, the new surface is versatile. It can be patterned, using photolithography, chemical etching, and other techniques. It's also easy to chemically modify the surface.

"You can attach molecules to the surface of the film," Cuiffi explains. "That changes your surface chemistry without having it be a free-floating matrix. We used molecules to make the surface hydrophobic or hydrophilic. When you drop the peptides on the surface, it still works [for laser desorption/ionization]." It's also possible to tailor the surface to absorb different contaminants or proteins out of the solution, he adds.

It is that flexibility and ease of manufacture that Cuiffi believes will make the technique a boon to automated detection, particularly in the field of proteomics. "It doesn't replace MALDI; it's a new super-enabling technology," he says. "There are so many options. Compared to electrochemically etched surfaces, [this method] is very manufacturable, very reproducible, and it has long-term stability." ◥

–Jim Kling

Gradients from a microfluidic "Christmas tree"

It began, as it usually does, with a problem. But instead of setting out to solve the problem, the researchers turned the problem to their advantage. In the March 15 issue of *Analytical Chemistry* (pp 1240–1246), Stephan Dertinger, Daniel Chiu, Noo Li Jeon, and George Whitesides at Harvard University present a method for creating concentration gradients in capillary solutions.

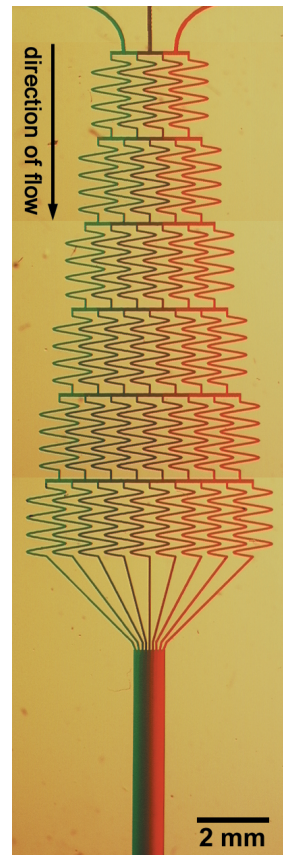
Unlike previous methods, this creates complex and multicomponent gradient shapes that are maintained over long distances (hundreds of micrometers) and long periods of time (hours or days), says Dertinger. Gradients are extremely important in biological and neurobiological systems, and this method may make it possible to study how cells respond to competing gradients of different chemically active substances.

What first caught Dertinger's attention was a figure in an earlier study of laminar flowing solutions, which showed the deposit of a silver wire that begins to broaden. Talking with one of the researchers revealed that diffusion between laminar layers was a problem during microfabrication inside capillaries. But with his background in the biophysics of neural networks, Dertinger says that he "thought it a good starting point" to use this diffusive mixing "problem" to create gradient systems.

The Harvard researchers used soft lithography to mold poly(dimethylsiloxane) into a microfluidic device with a branched system of n vertical serpentine channels feeding into a horizontal channel. This horizontal channel is the starting point for the next branched system, which has one more serpentine channel than the previous system. This pattern is repeated several times. The final branched system, which represents the m output branch, is different because it combines all vertical flows into a single broad channel, where the resulting concentration gradients and shapes are measured. Parallel streams are mixed by nonturbulent diffusion. The researchers describe this system as an l-input/m-output network or, less formally, as a "Christmas tree" structure.

The concentration gradient perpendicular to the flowing stream remains constant because the flow rate is maintained and the rate of diffusion in the broad channel is slow relative to the flow rate through the channels. Branched systems, in which diffusive mixing from the horizontal channel flow is 99%, increase the number of different concentration streams that flow into the broad channel and create complex gradients. From a study of red and green dyes in a 3-input/9-output network, the researchers derived mathematical formulas to describe splitting ratios of the streams at the branch points and concentrations at the ends of the serpentine channels.

In another 3-input/9-output network system, a fluorescence intensity profile graphs the concentration of fluorescein versus the position of the stream across a 900-μm broad channel, which is 500 μm downstream from the junction with the last serpentine system. Depending on the concentration of the solution introduced and the initial inlet position, this system shows either parabolic or linear gradient shapes. The gradient shape is also influenced by branch points farther from the vertical axis of symmetry and at higher orders of the branching system, which cause more solution to channel toward outer branches of the network.



A microfluidic 3-input/9-output device with streams combining into a single wide channel at the bottom, where a gradient forms perpendicular to the direction of flow.

Empirically, the overall shape of the gradients is described by an (n–1) order polynomial curve, where n is the number of inlets. Dertinger says that he wanted a mathematical proof of the polynomial curve fit as well, and its derivation should parallel that of the binomial distribution that has 50:50 splitting ratios. But with microfluidic splitting ratios that change in a very defined way to give polynomial curves, he says the math generated "nasty equations", and so far, he cannot get a mathematician interested.

After exploring the network gradients, the researchers' next step was to combine network systems. This created periodic gradients from a few inlets compared with the large number that would be necessary with other methods. With three networks of 2-inlet/8-outlet systems, each creating a linear concentration profile, different sawtooth gradients formed in the broad combined channel.

The overall sawtooth pattern varied with the concentration of the solution and the position of the initial inlet for the solution. In one case, the linear components showed a regular decrease in the peak maximum and slope; another case showed positively and negatively sloped linear gradient components. The concentration profile at the beginning of the channel showed smaller individual steps within the linear components, which indicates incomplete diffusive mixing of the individual streams, as opposed to the smooth, linear compo-

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nents of complete mixing downstream.

Other variations reported are complex gradients with combined parabolic parts or with parabolic and linear parts; these resulted from three 3-input/8-output parallel networks. The researchers also generated two overlapping sawtooth gradients of green fluorescein and red tetramethylrhodamine ethyl ester dyes in ethanol by injecting them at different inlets. Calculated and experimental gradients were in good agreement throughout these experiments.

What comes next? With such an easy-to-use, robust system, Dertinger says he wants to investigate how cells respond to different gradients and substrates in gradient solutions, finding the distance needed for interaction and determining the effects of different gradient shapes on cell migration, differentiation, and growth.

For these researchers, this "Christmas tree's" gift was a means of finding out, "Just how do those cells function anyway?" ◥

MEETINGS

Elizabeth Zubritsky reports from the **14th International Symposium on Microscale Separations and Analysis (HPCE 2001),** *in Boston, MA.*

HPCE continues to reinvent itself

As announced last year, HPCE is expanding beyond CE and other separation techniques to microscale methods, especially those that pertain to pharmaceuticals and the life sciences (*Anal. Chem.* **2000,** *72,* 321 A–322 A). This change is reflected in the meeting's new name: Last year it was called the 13th International Symposium on High-Performance Capillary Electrophoresis and Related Microscale Techniques. The shift also was apparent in this year's program. Of the 18 nonplenary sessions at the conference, 4 were devoted to genomics, 2 to microscale devices, and 4 to proteomics.

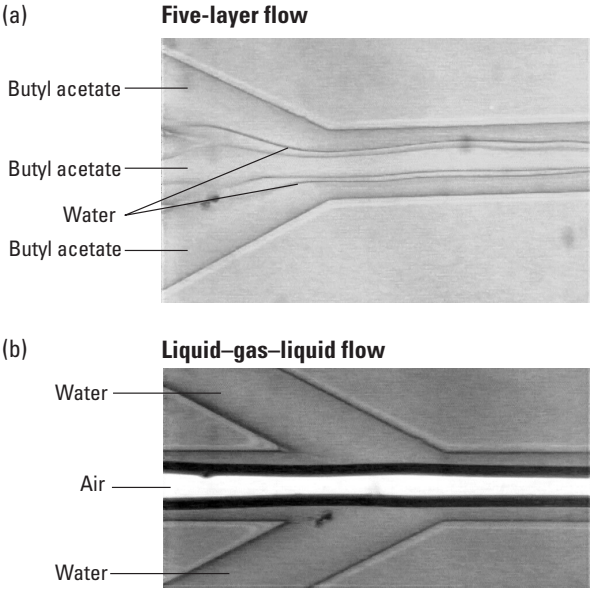
Multiphase flow on chip

For researchers who study the transport of solutes through a membrane, patience is certainly a virtue. Although the models can be simple—an aqueous donor layer and an aqueous acceptor layer are separated by an organic layer that serves as an artificial membrane—achieving equilibrium can take hours or even days. So Takehiko Kitamori, Mariana Surmeian, Hideaki Hisamoto, Akihide Hibara, and Manabu Tokeshi at the University of Tokyo (Japan) decided to speed up the process by moving it onto a microfluidic device that has been optimized for multiphase flow.

The multiphase device has three input streams that quickly merge into one so that the three phases are in contact as they flow in parallel down the length of the chip. Although surface tension tends to preserve the fluid columns, the device has a three-lobed guide structure etched into the bottom of the channel to maintain a stable interface for the entire 30-mm length, says Kitamori. The guide structure is created by fabricating three narrow channels and etching them sideways to form troughs that are semicircular in profile. If etched long enough, the troughs will expand outward to form a single, three-lobed channel, he explains.

The researchers tested the device using 0.1-M HCl with methyl red as a donor solution, *n*-octanol cyclohexane as the membrane layer, and plain 0.1-M HCl as the receptor solution. Viewed from the top, the three-layer column was 30-mm long and 190-μm wide, with the membrane layer occupying the middle 60 μm of the width. For detection, a thermal lens microscope at 514.5 nm was used. The average K_{DA} for the transport of methyl red was 0.426 ± 0.039 s^{-1}.

The researchers have used similar multiphase microfluidic devices—with up to five phases—to perform fast chelations or solvent extractions that require only small volumes of reagents, Kitamori says. For the solute transfer studies, he adds, the next step is to tackle real problems, such as modeling the transport of pharmaceuticals through biological membranes.



(a) Five-layer flow in which three layers of butyl acetate are separated by two layers of water. (b) Three-layer, liquid–gas–liquid flow.

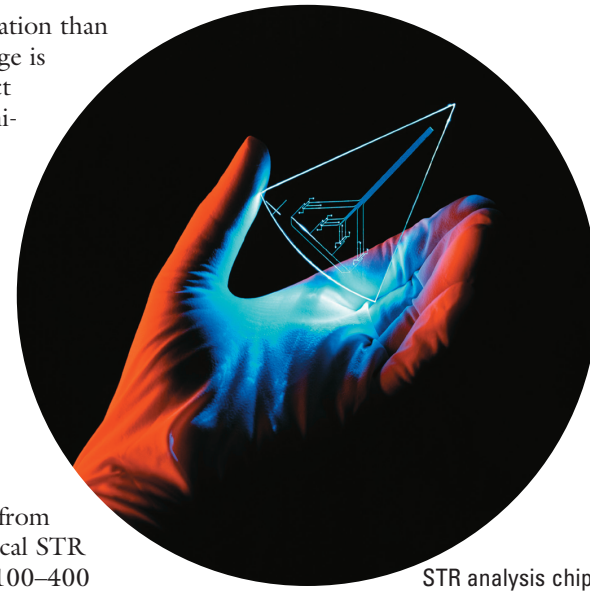
Chips to clear out crime backlog

Despite an expanding arsenal of forensic tools, 1999 statistics show that half of all violent crimes in the United States remain unresolved, says Luba Mitnik of the Whitehead Institute for Biomedical Research. That's one reason that the U.S. Federal Bureau of Investigation developed the Combined DNA Index System (CODIS), which has two nationwide databases: one containing the DNA profiles of convicted felons and another containing profiles from biological samples taken at crime scenes. But before CODIS can fulfill its purpose of cross-referencing crimes and criminals, >600,000 outstanding DNA profiles must be cataloged, Mitnik notes. To clear this backlog, entering profiles must become faster and easier. This is where the Whitehead Institute thinks it can help.

Mitnik and her colleagues have designed two microfluidic devices to perform short tandem repeat (STR) analyses, the latest in a series of forensic DNA profiling techniques. STR analyses—which focus on one- to five-base pair (bp) units that are repeated many times—are often considered more reliable because they can be multiplexed and are less sensitive to DNA degradation than other techniques. The challenge is that STR analyses must detect several kinds of mutations: microvariants, which are 1- or 2-bp shorter or longer than the wild-type sequence; stutters, which often lack one repeat unit; off-ladder variants, in which 4–8 bp may differ from the wild-type sequence; or combinations of the three.

The Whitehead researchers' device simultaneously separates eight STR regions of interest, called loci, from various chromosomes. A typical STR locus is a DNA fragment of 100–400 bp. Each STR "ladder" contains 8–10 alleles, or variations, per locus. Looking at a combination of eight loci leads unavoidably to DNA fragments that overlap in size and, thus, migrate at the same velocity during separation. To avoid this problem, different fluorescent primers are used to amplify these loci during PCR. The result is two allelic ladders that have similar sizes but are labeled with different dyes.

Although the devices are still being optimized, the researchers routinely get 1-bp resolution for DNA fragments as long as ~325 bp using an 11-cm-long separation channel, says Mitnik. The analysis takes only ~20 min, which makes it much faster than conventional gel-based STR analyses, she notes. Another version of the device, which uses a 2-cm-long channel, provides even faster analysis, albeit with lower resolution. Mitnik adds that the device design is flexible enough to accommodate up to 384 channels/chip in the future.



SAM OGDEN

STR analysis chip.

BUSINESS

ICAT proteomics tool commercialized

The isotope-coded affinity tag (ICAT) approach for quantitative proteomics, developed by Ruedi Aebersold and colleagues at the University of Washington–Seattle, has been commercialized by Applied Biosystems, an Applera Corporation group. The announcement was made at the 2001 meeting of the Association of Biomolecular Resource Facilities in late February. The company said then that ICAT reagent kits, which prepare protein samples for MS analysis, would be shipped in March.

The ICAT technique is one alternative to the traditional, yet labor-intensive, approach of separating proteins using two-dimensional electrophoretic gels and then performing MS or MS/MS to obtain sequence information. Aebersold and colleagues have used the ICAT method to compare the relative expression levels of two sets of proteins (*Nat. Biotechnol.* **1999,** *17,* 994–999). In one protein sample, the side chains of cysteinyl residues are derivatized with the isotopically light form of the ICAT reagent. In the other sample, the isotopically heavy form of the reagent is used. The two samples are combined and cleaved to generate peptide fragments, some of which are tagged. The tagged peptides are isolated by affinity chromatography and analyzed by microcapillary LC/MS/MS. No a priori knowledge of the proteins is needed.

In the original ICAT paper, Aebersold and colleagues analyzed proteins using an electrospray ionization mass spectrometer. The researchers recently extended the technique to MALDI quadrupole time-of-flight MS (*Anal. Chem.* **2001,** *73,* 978–986).

LABORATORY PROFILES

ISAS regroups

Converting a once-fundamental research center into a forward-looking applied analytical science establishment is no joke. Such transformations do not happen overnight, and the new director of Germany's Institute of Spectrochemistry and Applied Spectroscopy (ISAS) is working hard to build on his predecessors' efforts.

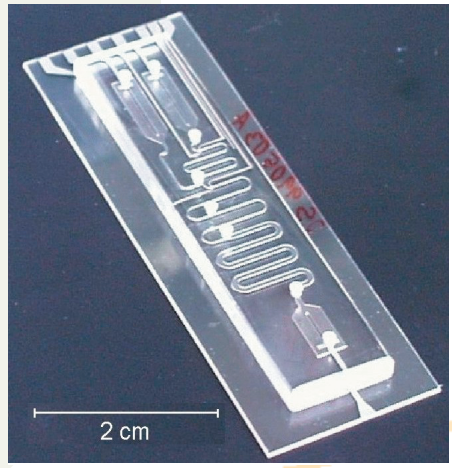
ISAS is based at two centers (called departments), one in Dortmund, founded in 1952, and one in Berlin, which was established in 1992 by integrating a team of optical spectroscopists from the old East German Academy of Sciences after the collapse of communism. ISAS focuses on the development and validation of new analytical methods and instrumentation. With the retirement of long-time director Günther Tölg in 1995 and of his successor, Dieter Klockow, in February 2000, control of ISAS passed to Kay Niemax, a physicist by training, who had been appointed to associate director in 1997.

"ISAS is now concentrating increasingly on problems such as miniaturization of analytical instrumentation and new technologies for improving analytical performance in the life sciences," says ISAS's Antony Davies. The updated focus does not exclude the traditional core competencies in the development of new spectroscopic instrumentation or material sciences analysis, he notes. "Without [these], the more trendy, headline-grabbing advances could not be achieved!"

ISAS formerly grouped projects according to specific techniques, such as IR or Raman spectroscopy. But in 1997, ISAS switched to a problem-oriented structure that combines resources from across the institute. Niemax believes that this interdisciplinary approach is increasingly important for tackling analytical problems, especially as the number of life science projects grows.

Although ISAS is independent, it garners financial support from the German federal government and the states of North Rhein–Westphalia and Berlin. Other backing—which Davies says is critical at a time of reduced centralized funding—comes from the German Research Council, German and international industry, and the European Union.

Whereas once its charge was to carry out fundamental research in spectroscopy, ISAS's role is now influenced by industrial developments and increased public awareness of the need for a strong analytical science base to maintain and improve the quality of life. Thus, the institute works with specialists in other fields who are developing analytical techniques and designing and testing instrumentation and components. The Berlin Department, for instance, has developed the advanced Echelle spectrometer, which has provided the optics for PerkinElmer's new Optima 2000 and for instrumentation from two small, independent companies.



ISAS

A plastic isotachophoresis chip developed by ISAS, the University of Dortmund, and Merck Darmstadt (all in Germany).

ISAS is also heavily involved in miniaturized analytical systems these days. Roland Hergenröder is the coordinator for this research branch and works with scientists such as Michael Heise, who is developing IR optical-fiber sensor systems for the analysis of microsamples. The team's ultimate aim is to develop faster, more economic, and more sensitive chromatography/spectroscopy coupling.

Meanwhile, Jörg Baumbach is striving to make ion-mobility spectrometry (IMS) less of a "black-box" technique. His team is investigating customized IMS spectrometers for use in health and environmental work for process analysis and monitoring. Currently, they are examining how volatile organic hydrocarbons found in contaminated soils, landfills, and groundwater might be analyzed using an ion-mobility sensor (i.e., a miniaturized spectrometer) equipped with a multicapillary column. Field tests on benzene, toluene, xylene, and other selected volatile organic compounds confirm that the instrument has high discriminating power for these compounds, and the researchers believe that the method provides useful information for transfer and risk-assessment models.

Niemax is interested in laser-based methods such as sampling of solids by pulsed laser or analytical laser spectroscopy. He says that he intends to get rid of the large laser installations and convert ISAS scientists to using far less expensive diode lasers. This approach is part of the movement toward finding practical solutions to problems and a new focus on developing flexible, transportable instrumentation. For example, Niemax and his colleagues have developed HPLC/diode laser atomic absorption spectrometry instrumentation as an alternative to HPLC/inductively coupled plasma MS for the speciation of metals, such as Cr(III)/Cr(VI), and the analysis of the controversial fuel additive methylcyclopentadienyl manganese tricarbonyl, commonly known as MMT.

In the future, Niemax hopes to encourage even greater synergy among the specialists in the institute to continue to meet the analytical challenges posed by the recent boom in the life sciences. ◥

–David Bradley

GOVERNMENT AND SOCIETY

Do pacts pay?

Companies large and small are queuing up to join a relatively new consortium in the United Kingdom (U.K.), the Foresight Centre for Process Analytics and Control Technology (CPACT; http://www.cpact.com). Now in its second phase of implementation, CPACT was established with £1.3 million (~$2 million) in funding from the U.K. government's Office of Science and Technology (OST) matched with industrial cash and contributions. The goal is to bring together industrial experts from companies such as GlaxoSmithKline, BP, and Avecia and researchers from the Department of Chemical and Process Engineering and the Department of Engineering Mathematics at the University of Newcastle, the Department of Pure and Applied Chemistry and the Department of Electronic and Electrical Engineering at Strathclyde University, and the Department of Chemistry at the University of Hull (all in the U.K.) in the areas of process spectroscopy and analysis, statistical data interrogation and feature extraction, process modeling, and control engineering.

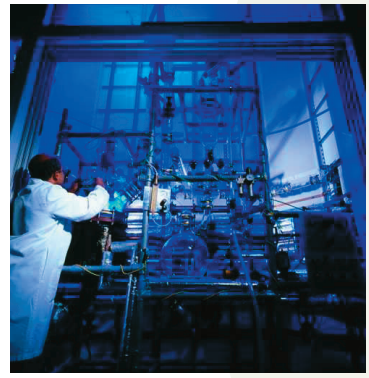
CENTRE FOR PROCESS ANALYTICS AND CONTROL TECHNOLOGY

Individual teams within CPACT focus on finding solutions to generic problems in process measurement, monitoring, control, and optimization. Each project addresses manufacturing challenges identified by industrialists working in the chemical, biochemical, pharmaceutical, food, and materials processing industries. Particular emphasis is placed on new and improved measurement techniques, better signal-processing protocols, online process optimization and model-based control, and the application of multivariate statistical techniques to enhance process performance.

CPACT was formed in response to the perception that industrial processing in the U.K. is threatened. The Foresight Industry Report Group has stated that the United Kingdom's process industry will fail unless it pays more attention to customer needs and brings products to market more quickly. Similarly, one performance measure by Harvard University placed the United Kingdom 13th out of 17 industrial nations in terms of its ability to derive commercial benefits from science and technology. According to Keith Pavitt at Sussex University, that report highlighted what U.K. industrialists already know: "British business has not been very good for a long time at production-process engineering."

CPACT's honorary president, Roger Benson of Eutech, has described it as a "one-stop shop" for companies seeking advice and research on process technologies. Comprehensive chemical, biochemical, and polymer-reaction pilot plant facilities have been established at the participating universities, allowing the realistic evaluation of analytical instrumentation and software for process analysis and the testing of calibration and control algorithms.


CPACT


CPACT


CPACT

Process analysis has been identified by both the U.K. government and industry as a strategic research area to ensure educational and industrial development. Within CPACT, novel on-line process analysis techniques are being developed, including low-frequency NMR for the quantitative monitoring of chemical components such as hydrocarbons, fluorocarbons, and catalysts. Additionally, novel hydrocarbon monitoring technologies have been developed for several processes, including food manufacture, wastewater treatment, and the production of drug intermediates.

But such collaborations raise the question, "What, aside from financial resources, is in it for the academics who take part?" Some scientists worry that when research is driven by industrial problems, there is a serious risk that the need for fundamental science will be lost in the company balance sheets. Could academic–industrial partnerships simply turn academia into a service industry for commercial research and development (R&D)?

It's possible but not inevitable, says Pavitt. "CPACT activities in themselves are typical examples of academic engineering research, which [like other applied subjects such as medicine and architecture] have been in universities for a long time," he explains. CPACT projects are not substitutes for academic science, nor for in-house R&D activities at companies, but complements to them, he explains. Nevertheless, ex-

GOVERNMENT AND SOCIETY

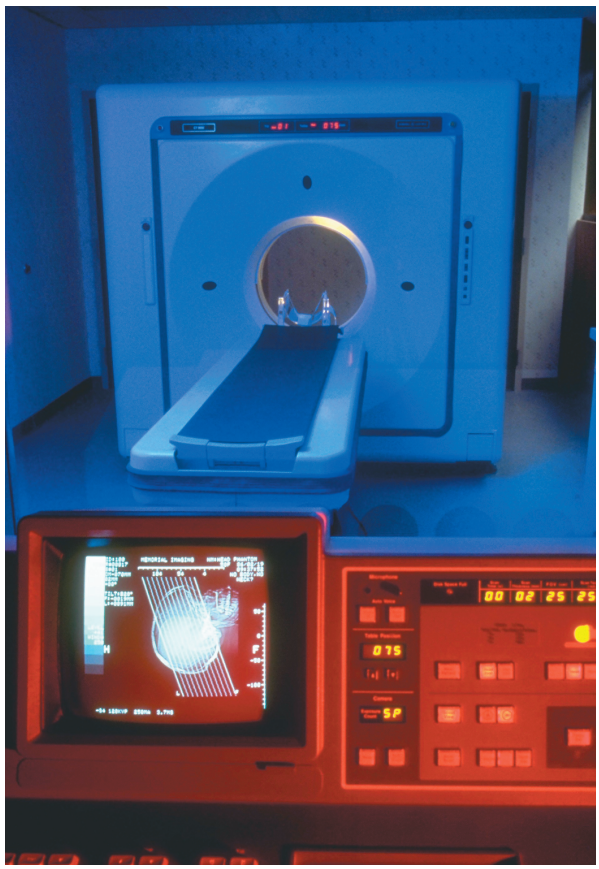
ploratory research seems to be a luxury that few companies believe they can afford, particularly as problems become increasingly multidisciplinary in nature.

According to Jacky Senker of the United Kingdom's Science Policy Research Unit, "In general, these relationships are beneficial, so long as academics are not forced to rely on them because of lack of funds for research." When scientists are free to pursue a project because it is of interest, solving problems faced by industry can lead to fundamental breakthroughs in science, she notes.

Thus, observers see potential benefits for both sides. Industry taps cutting-edge science, and academia accesses industrial challenges with a commercial perspective. Pavitt, however, adds a note of caution: "The great illusion is to think that government-funded academic research and training can replace business-funded R&D." If British firms cannot sustain their in-house research, he adds, "I hope CPACT will attract world-class foreign firms."

New bioengineering institute at the NIH

As one of his last acts, President Clinton signed legislation on December 29 creating the National Institute of Biomedical Imaging and Bioengineering (NIBIB) at the National Institutes of Health (NIH). This institute will conduct and support research training and will disseminate information relevant to biomedical engineering and imaging. Whether the NIBIB will be a boon for analytical chemists seeking funding to develop instrumentation is not yet known. However, Donna Dean, senior advisor to the NIH's acting director, says, "Since this institute's research will not be targeted towards specific diseases, it will reflect what material science and all of chemistry can do."



CORBIS

The new National Institute of Biomedical Imaging and Bioengineering will likely be a boon to researchers in magnetic resonance imaging. Will it do the same for analytical chemists?

Although the institute's formation had broad bipartisan congressional support, it was widely opposed by directors at the NIH and former Secretary of Health Donna Shalala. Directors of the various departments at the NIH were obviously alarmed at the institute's creation, fearing further bureaucracy and seeing the institute as a threat to their own funding interests. However, the American Institute for Medical and Biological Engineering was apparently the primary motivating force behind creation of the NIBIB.

"Most of the bioengineering departments where these people work are funded by the Whitaker Foundation, and the Whitaker Foundation is spending itself out of existence," says Paul Morrison, a physical scientist at the NIH's division of Bioengineering and Physical Sciences. "So these people are looking for ways to stay funded."

Morrison also notes that many scientists conducting biomedical engineering research have real concerns about locating funding, especially if their research does not fit with any of the traditional study sections. "Bioengineering has often been viewed more as support than as cutting-edge research science," he says. "The NIH is heavy into its latest and most successful technology, which is molecular biology, and people have been afraid that this would always take precedence over bioengineering."

But behind-the-scenes maneuvering has focused on forming the institute. "The NIH's position now is that we're beyond [the aforementioned opposition]," says Dean, who heads up the transition team that is writing a mission statement for the NIBIB, requesting funding for the NIBIB's 2002 budget, and searching for an acting director. "This is law and we are moving forward."

Since the institute is evolving quickly, nobody is certain which researchers will be involved directly with the NIBIB. "We're going to make our initial primary focus on extramural grants," says Dean, adding that the eventual permanent director will determine much of the institute's operations. "We'll leave open the possibility of an intramural community, but that will be taken up in the future."

–Paul D. Thacker