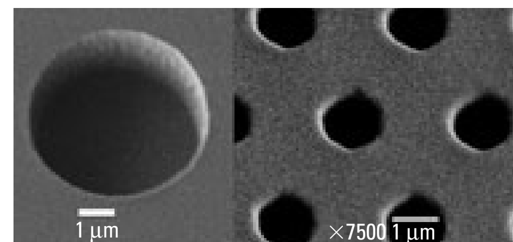
ANALYTICAL CURRENTS

Single-molecule assays in femtoliter chambers

Hiroyuki Noji and colleagues at the Laboratory for Integrated Micro-Mechatronic Systems, the University of Tokyo, and the Japan Association for the Advancement of Medical Equipment (all in Japan) have developed a device with micrometer-sized chambers for performing ultrasenstive bioassays at the single-molecule level. The device, which is made out of PDMS, consists of uniform cylindrical chambers regularly spaced in an array.

To distribute femtoliter volumes of solution into the chambers, Noji and colleagues sandwiched a droplet between a microscope slide and the PDMS device. Capillary forces evenly and rapidly distributed the solution into the chambers. Because PDMS is impermeable to water-soluble compounds, no solution leaked from the chambers. The investigators also found that biomolecules, such as DNA, were not denatured in the confined spaces of the chambers.

Noji and colleagues used the device to measure the activity of single molecules of horseradish peroxidase and β-galactosidase. The enzymes didn't bind to the surfaces of the chambers and remained active in solution. The researchers suggest that the device could be applied in population studies of a given enzyme to analyze differences in activity as a result of posttranslational modifications. (*Nat. Biotechnol.* **2005**, *23*, 361–365)



Scanning electron micrographs of micrometer-scale chambers in a PDMS device that can hold femtoliter volumes. (Adapted with permission. Copyright 2005 Nature Publishing Group.)

Lipid tubule arrays

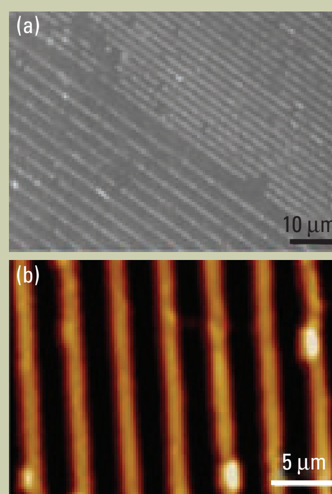
Jiyu Fang and Nidhi Mahajan at the University of Central Florida have developed a new method that combines microfluidic networks and dewetting to produce ordered arrays of lipid tubules on glass surfaces. Ordered tubules on surfaces could serve as substrates for protein crystallization or as templates for the formation of inorganic materials.

The researchers created lipid tubule arrays by placing a PDMS stamp with parallel rectangular channels upside down on a glass surface. A droplet containing lipid tubules was pulled into the channels by capillary action, and the tubule solution was dried at room temperature. Once the PDMS stamp was removed, lipid tubules remained on the glass surface in a parallel array.

Fang and Mahajan used atomic force microscopy (AFM) to determine the height of the tubules. The first set of arrayed tubules were ~470 nm in height, which is the expected diameter of a single lipid microtubule. After a week of drying at room temperature, the lipid microtubules flattened against the glass surface. The flattened tubules resumed their original shapes, however, once they were placed in water. Microtubules left to dry for one month cracked along their lengths and collapsed in the middle. When the researchers kept the tubules in a humid chamber for one month, the tubules did not collapse.

The use of a different set of lipids resulted in ordered nanotubules. Although other groups have reported that lipid nanotubules undergo phase transitions when the solution temperature rises, Fang and Mahajan observed that their nanotubules were stable, possibly because of their rapid drying protocol.

The researchers used the aligned tubules as templates for silica films. Glass surfaces coated with lipid tubules were placed in a silica colloid for six days without agitation. The tubules were rinsed, dried, and then imaged by AFM. Silica films formed on the exposed surfaces of the tubules; this allows the researchers to synthesize films with specific morphologies and patterns. (*Langmuir* **2005**, *21*, 3153–3157)



(a) Optical and (b) AFM images of aligned lipid microtubules.

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ANALYTICAL CURRENTS

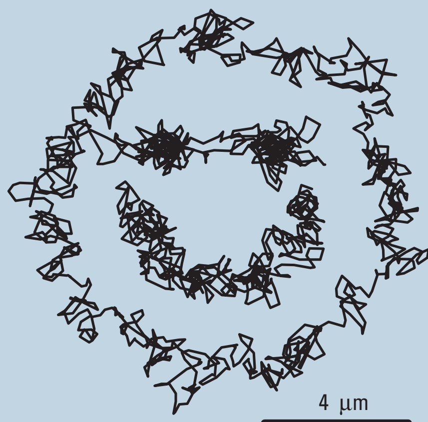
Manipulating nanoscale objects in solution

Adam E. Cohen and W. E. Moerner at Stanford University have designed a device that can manipulate nanoscale objects in solution at ambient temperatures. The device is noninvasive and can trap objects smaller than those trapped by laser tweezers. The instrument could be used for single-molecule spectroscopy and in the identification of biological molecules.

The new device, which is called the anti-Brownian electrophoresis (ABEL) trap, contains four microfabricated electrodes arranged in a diamond configuration on a glass slide. Fluorescence microscopy is used to monitor the Brownian motion of a single particle in the trap. A time-dependent feedback voltage is applied to the solution to exactly cancel out the Brownian motion with electrophoretic drift. The trap works on any object that acquires a charge in solution and can be visualized by optical microscopy.

Cohen and Moerner used the ABEL trap to capture polystyrene particles with diameters down to 20 nm in water. They demonstrated that a 20-nm particle could be confined within 1.7 nm. They also showed that particles could be moved in a controlled manner within the ABEL trap along a defined trajectory.

The researchers point out that the ABEL trap does not have confinement on the *z* axis, although they say it is possible to build a trap with eight electrodes arranged at the corners of a cube. They hope that future versions of the device will be in an array format so that many particles can be simultaneously trapped and manipulated. (*Appl. Phys. Lett.* **2005**, doi 10.1063/1.1872220)

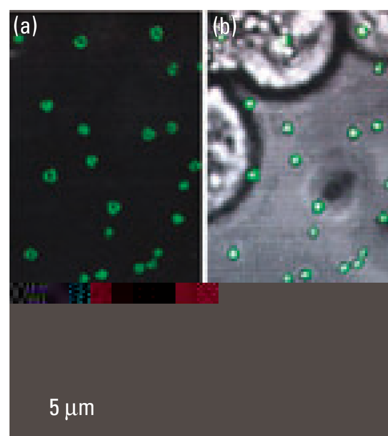


A 200-nm particle followed a trajectory that drew a smiley face in the ABEL trap. (Adapted with permission. Copyright 2005 American Institute of Physics.)

A substitute for cells?

Horst Vogel and colleagues at the Swiss Federal Institute of Technology in Lausanne have developed a method to monitor cell signaling in single attoliter vesicles. The tiny vesicles, which can be stored for weeks, will enable researchers to study many processes, such as cellular responses to drugs. Vesicles could potentially replace live cells for screening in miniaturized, highly parallel formats.

When Vogel and colleagues treated human kidney cells with the drug cytochalasin, tubular extensions formed from the cells. Upon agitation, these extensions broke off into ~50 submicrometer-sized vesicles per cell.



Submicrometer-sized vesicles containing a fluorescent protein are shown (a) by themselves and (b) together with the cells from which they originated.

The orientation of the vesicles is important if they are to be used as substitutes for cells. To ensure that the vesicles did not assume a reversed orientation after being agitated, the researchers used a line of kidney cells that expressed a green-fluorescent-protein-labeled reporter protein in the cytoplasm. Green fluorescence observed inside the vesicles from these cells indicated that they contained cytoplasm.

The researchers also monitored the binding of a particular radioactively labeled ligand to its receptor on the submicrometer vesicles. They observed that both the number of receptors on the vesicles and the dissociation kinetics of the ligand from the receptor were similar to those measured on the initial kidney-cell population. Other cell types that express the same receptor produced vesicles with identical properties.

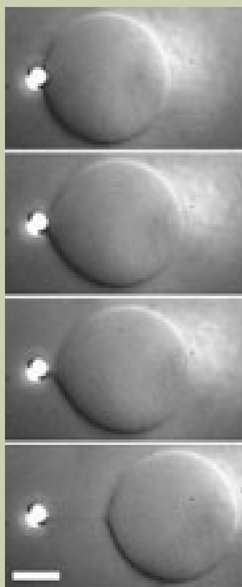
Finally, they assayed transmembrane signaling. When a particular ligand binds to its receptor, intracellular calcium levels increase transiently. Vogel and colleagues, therefore, measured the calcium concentration within immobilized vesicles when the ligand was present. Calcium levels rose briefly and then decreased. The signaling process within vesicles was identical to that in whole cells; this indicates that the attoliter vesicles may be a suitable analytical substitute for cells. (*J. Am. Chem. Soc.* **2005**, *127*, 2908–2912)

Forces in membrane tube formation

Marileen Dogterom and colleagues at the FOM Institute for Atomic and Molecular Physics (The Netherlands) and Eötvös University (Hungary) have measured the forces during the formation of a membrane tube from a giant vesicle. The results may help researchers understand how membrane tubes are formed inside biological cells by motor proteins and the cytoskeleton. The data may also have implications for the creation of microfluidic devices out of vesicles and membrane nanotubes.

Dogterom and colleagues used optical tweezers to measure the force–extension curve in tube formation. A biotinylated giant vesicle was immobilized on a cover slip with a polystyrene bead coated with streptavidin. The optical tweezers were used to bring a second bead into contact with the vesicle. A piezoelectric stage pulled the vesicle at a constant speed away from the tweezers, causing a tube to be formed.

The investigators found that a significant force barrier exists in membrane tube formation. The force barrier increased linearly as the radius of the contact region on the vesicle increased. (*Phys. Rev. Lett.* **2005**, *94*, doi 10.1103/PhysRevLett.94.068101)



Differential interference contrast microscopy images demonstrate the use of optical tweezers to form a membrane tube from a giant vesicle. Scale bar = 10 µm (Adapted with permission. Copyright 2005 American Physical Society.)

Comparing particle emissions methods

Particle emissions from automotive sources are typically measured by collecting the particles on a filter and weighing them with a microbalance. Regulatory restrictions based on this gravimetric filter method have led to a decrease in emissions, but the method's limit is quickly being approached. To reduce particle emissions even further, more sensitive measurement methods are needed.

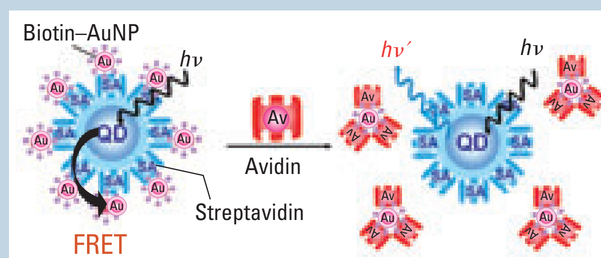
As a first step toward finding a reliable measurement method for ultralow-level particle emissions, Martin Mohr and colleagues at the Swiss Federal Laboratories for Materials Testing and Research investigated 16 different instruments comprising both mass- and nonmass-related methods. The instruments were compared on the basis of reproducibility, detection limit, sensitivity, time resolution, and correlations with the gravimetric filter method and the elemental carbon fraction of the particles. Although opacimeters and light-scattering methods were found to be unsatisfactory at low levels, several time-resolved methods showed good performance. Nonmass-based instruments provided a significant improvement in sensitivity.

The results reveal that more sensitive measurement methods are available. Replacing the gravimetric filter method, however, will make it difficult to compare past and future vehicle emissions, say the researchers. (*Environ. Sci. Technol.* **2005**, doi 10.1021/es049550d)

NP/QD inhibition assay

Inorganic nanoparticles (NPs) and quantum dots (QDs) are commonly used as optical labels for biomolecules. Hak-Sung Kim and colleagues at Ajou University and the Korea Advanced Institute of Science and Technology (both in Korea) have now developed an inhibition assay that uses both NPs and QDs. In the new assay, the researchers assess changes in fluorescence resonance energy transfer (FRET) between molecules tagged with gold NPs (AuNPs) and QDs in the presence of inhibitors that disrupt the interactions between the labeled molecules.

Kim and colleagues used streptavidin–biotin binding as a model system. Streptavidin was labeled with a QD, and biotin was labeled with an AuNP. Because AuNPs quench the photoluminescence of QDs, the absence of a QD signal indicated that streptavidin and biotin were closely associated with each other. Electron microscopy images confirmed that streptavidin and biotin formed clusters when they were in solution together. When the researchers added avidin, which also binds to biotin, photoluminescence was restored in a concentration-dependent manner. The assay can be adapted to study other biomolecules. (*J. Am. Chem. Soc.* **2005**, *127*, 3270–3271)



Schematic of the NP/QD inhibition assay.

RESEARCH PROFILES

Measuring drugs in individual organelles

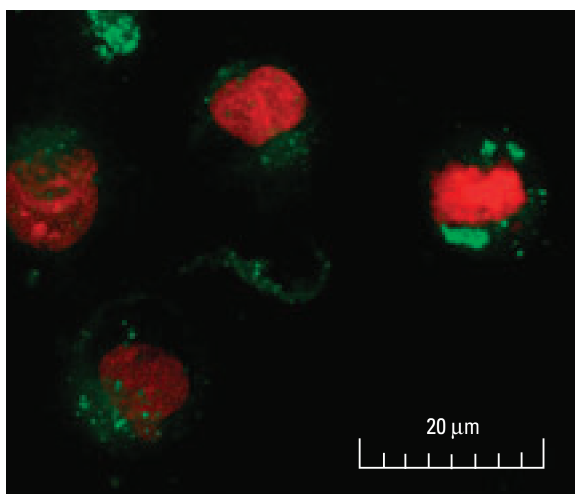
The effects of a drug depend on where it goes in a cell. To get a better idea of how much of a drug accumulates in particular subcellular compartments, Edgar Arriaga and colleagues at the University of Minnesota have developed a CE laser-induced fluorescence (LIF) method with dual-channel detection. The technique can measure the properties of a particular organelle even in the presence of other types of organelles.

In the April 15 issue of *Analytical Chemistry* (pp 2281–2287), the researchers used the CE-LIF approach to detect the amount of the anticancer drug doxorubicin (DOX) in individual acidic organelles. The results could shed light on how acidic organelles mature and their role in multidrug resistance.

DOX is a drug commonly used for treating leukemia and other types of cancer. Some tumors, however, have developed resistance to DOX. Researchers have speculated that acidic organelles sequester DOX, making it less available to the cell. Until now, however, no one could measure exactly how much DOX accumulates in these individual subcellular compartments.

Drugs that are sequestered by acidic organelles are usually weak bases, says Arriaga. "When these drugs are in the cytoplasm, they are neutral and somewhat hydrophobic, so they can cross the acidic organelle membrane. But once they hit the acidic organelle, they are exposed to low pH and become protonated," he says. When the drugs become protonated, they cannot escape the acidic organelles and therefore are not as effective, he adds.

To determine whether the sequestration of DOX by acidic organelles is relevant to drug resistance, Arriaga and colleagues measured the DOX content in individual acidic organelles from two human leukemia cell lines. The acidic organelles were first labeled with commercially available fluorescent nanospheres. The nanospheres were specific for acidic organelles and therefore did not label other organelles, such as mitochondria.



Composite confocal image of human leukemia cells (CEM/C2) incubated with 50-nm-diam nanospheres (green) and 25-µM DOX (red).

All of the acidic organelles successfully encapsulated the fluorescent nanospheres by endocytosis after a 48-h incubation period. The researchers then exposed the cells to DOX for 15 min. Because nanospheres emit at a shorter wavelength (green) than DOX (red), Arriaga and colleagues could easily distinguish the acidic organelles with DOX from those without DOX by monitoring two different spectral ranges. Dual-channel detection also allowed them to determine how much DOX was taken up by other organelles, such as mitochondria, which had no detectable nanospheres.

Most of the acidic organelles quickly took up and concentrated DOX in the nuclear region; however, a small fraction of them did not take up DOX during the 15-min treatment. Arriaga believes that one reason has to do with the size of the acidic organelles. "When an acidic organelle is formed, it is usually very tiny," he says. Over time, however, acidic organelles tend to fuse together, forming larger and larger acidic organelles. To detect DOX, the acidic organelle may need to be really large so that it contains a significant amount of the drug, speculates Arriaga. Another possibility is that some of the acidic organelles will lose their pH gradient with time. "If they lose their pH gradient, the drug is going to escape," he says.

Now that the researchers have the ability to measure exactly how much DOX accumulates in individual acidic organelles, the next step is to compare the behavior of a drug-resistant cell line with that of a cell line that is sensitive to DOX, to determine whether one has the ability to sequester DOX while the other does not. "It may seem simple, but it is complicated because that involves needing to know exactly how many acidic organelles there are in a cell," says Arriaga.

The researchers also hope to eventually look at changes in proteins in acidic organelles as the organelles mature. "We would like to investigate some features that might be responsible for changes in their ability to accumulate doxorubicin," says Arriaga. One area that they hope to investigate is the expression or lack of expression of ATPases, which are responsible for pumping protons in and out of acidic organelles. "It might be that some acidic organelles have the ability to maintain the pH gradient for longer times than others," says Arriaga.

But future work depends on the advancement of proteomics technologies. "That is one of the bottlenecks right now for anybody who is interested in subcellular analysis and working with very small samples. The sensitivity and the limits of detection of present proteomics strategies are still not there," says Arriaga. ◂

—*Britt E. Erickson*

An ion source for all seasonings?

What started as a hobby between two friends has become an innovative new MS ionization source, called Direct Analysis in Real Time (DART), which is described in the April 15 issue of *Analytical Chemistry* (pp 2297–2302). DART can ionize almost any material, including illicit drugs, chemical warfare agents (CWAs), and even seasonings and flavor components, at atmospheric pressure without prior sample preparation.

Robert Cody at JEOL USA and Jim Laramée at EAI Corp., the developers of DART, have known each other since their graduate-school days at Purdue University. They went their separate ways after defending their theses, but ~10 years ago, they reunited at a conference. At one of the sessions, Cody heard Laramée talk about his research on an electron monochromator, which is a type of electron ionization source. "I thought that was a really clever idea, so [JEOL USA] sponsored his research and finally commercialized the product," says Cody.

But Cody and Laramée continued to think about how to build better ionization sources in their spare time. Finally, Laramée called his friend about a "crazy idea" in 2001. He thought that they should develop a source of low-energy electrons. He explains that as an electron slows down, it becomes large, which facilitates electron capture.

After many calculations and phone calls, Cody and Laramée realized that their concept was feasible. Cody's coworkers at JEOL USA put together a prototype source that used an electrical discharge to create a plasma. DART was then coupled to a TOF mass spectrometer, which analyzed the ions. "We found out that the device, which was supposed to work with vapor, actually worked with solids and other materials on surfaces as well," says Cody. "After that, it became a full-fledged project."

The researchers ionized a wide range of molecules, including pharmaceuticals, illicit drugs, metabolites, foods and spices, and fire accelerants. In collaboration with H. Dupont Durst at the U.S. Army Edgewood Chemical Biological Center and under the sponsorship of the Defense Threat Reduction Agency, the researchers also analyzed CWAs, such as G- and V-series nerve agents and HN-series blister agents, and explosives. Cody says he also had a bit of fun when he demonstrated DART at the Pittsburgh Conference in February 2005. DART-TOFMS detected a fungicide on an orange peel, a flame retardant on a wipe that was brushed against the sole of a shoe, and flavor components of candy from the breath of volunteers.

JEOL USA

DART ionizes molecules on the surface of a $20 bill.

For now, JEOL USA sells DART as an option for its TOFMS instrument, but Cody says that DART could be used with other mass spectrometers. He says that TOF systems are ideal, however, for pinpointing the identities of unknown molecules. "The high resolution and exact mass capabilities really help us to figure out what we're looking at," Cody explains. For example, he points out that the m/z-150 peak observed when a dollar bill is placed in front of DART could indicate that either methamphetamines or cosmetics are on the bill. With a TOF instrument, the cosmetics peak at m/z 150.113 is easily distinguished from the drug peak at m/z 150.128.

Although the researchers are still trying to work out the details of the ionization mechanism, they don't think that low-energy electrons are causing ions to form. "That was kind of a red herring," says Laramée. "We started off in one direction, and we found that the real workhorse was the metastable helium neutral, although the use of low-energy electrons for environmental contaminants is still being pursued." Helium gas used in the source is excited in the electrical discharge, and the excited helium neutrals probably react with atmospheric water to produce hydronium ions, which transfer protons to the analyte molecules. Vibronic excited-state species of nitrogen can also ionize molecules.

According to Laramée and Durst, DART offers several advantages to researchers. Unlike traditional CWA detectors, DART does not use radioactivity, so researchers are freed from a whole host of safety and regulatory issues. Another advantage is that analytes do not have to be placed onto special surfaces or dissolved into certain solvents to be ionized with DART. In fact, no sample preparation is required. Cody says, "I've been an analytical chemist doing MS for a couple of decades now, and it's just the most liberating experience to be able to stick something in front of the mass spectrometer without having to worry about whether you're going to contaminate something or whether it's going to dissolve. It's just been the most fun I've had in years."

—Katie Cottingham

MEETING NEWS

Rajendrani Mukhopadhyay reports from **MicroScale Bioseparations 2005**—*New Orleans, La.*

Cell selection on a chip

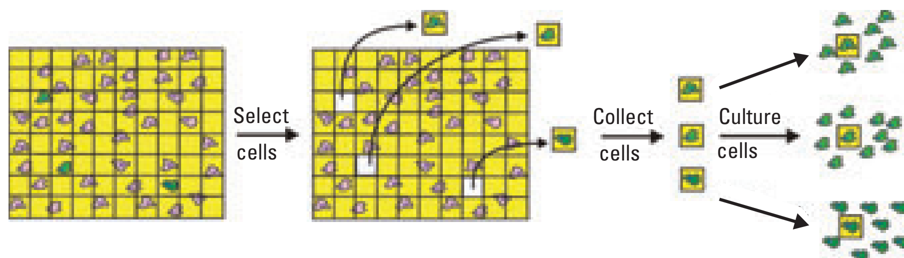
The selection of a single cell from a heterogeneous population can be laborious, time-consuming, and expensive. In addition, hundreds of thousands of cells are needed for the sorting and selection process, of which less than half survive to the end. To overcome these limitations, Nancy Allbritton and colleagues Mark Bachman, G. P. Lee, and Chris Sims at the University of California, Irvine, are developing a chip that could make cell selections easier, faster, and more economical.

The new chip consists of a glass slide with an array of pallets. The pallets are fabricated out of SU-8 by photolithography and can range from 30 to 500 µm in diameter. The gaps between the pallets are usually 5 µm.

The investigators hope to ultimately develop chips that are disposable and cheap. Bachman says that they are currently using SU-8 for research purposes, but the commercial chips they envision for the future will have pallets made out of materials that are more amenable to large-scale manufacturing, such as polyimide or polyester.

The investigators have developed techniques to change the surface chemistry of SU-8 and other polymers so that a variety of biomolecules can bind to it. For cell selection, polylysine and other charged molecules are used so that cells can attach to the pallets. When cells are plated onto the chip at the proper dilution, single cells sit on individual pallets. The pallet coating of the SU-8 is done in such a way as to ensure that the cells prefer the tops of the pallets rather than the intervening spaces on the glass slide. Wells can also be formed on top of the pallets so that the cells comfortably sit on the pallet. The array of cells on the pallets can be visualized by conventional light microscopy.

When a cell of interest is identified on a pallet, Allbritton and colleagues focus a 5-ns pulse from a Nd:YAG laser at the interface of the pallet and the glass slide. Plasma of submicrometer dimensions is produced at the interface, and a shock wave travels out of the plasma at supersonic velocities, causing the pallet to lift off the glass slide. The loosened pallet is either collected by a pipette or moved into a microchannel by fluid flow. Because the plasma formation is a localized phenomenon, the laser pulse only releases a single pallet. The process of releasing pallets can be repeated as many times as needed. Allbritton and Bachman say that preliminary viability studies indicate that the cells are not harmed by the procedure.



Cells are plated on an array of pallets and can be individually selected and released.

Allbritton explains that an advantage of the chip is that a large population of cells is not required for the selection. Small sample sizes are desirable in many biological applications because large quantities of cells can be hard to come by. Conventional cell-selection techniques, such as flow cytometry, require hundreds of thousands of cells, but the chip technology only needs a starting sample of ~10,000 cells. Bachman also points out that with the chip, "you can scan literally half a million sites at a time." This speeds up the selection process.

Applications for the chip are numerous because many areas in biology rely on sorting and selection of cells. Cancer research is one example. Transformation assays involve introducing oncogenic viruses into cells and then separating the transformed cells from the nontransformed ones. Another major application is stem-cell research. Purified stem cells don't often remain in their original state but continue to differentiate, which causes the population to turn into a mix of stem cells and differentiated progeny after several days in culture. This forces biologists to continuously select for pure stem cells.

And applications for the chip aren't just restricted to cell selection. "You can imagine it as an assay platform," says Allbritton. One example is DNA hybridization. Individual oligonucleotide pieces can be deposited on the pallets by a standard DNA spotter. A library of oligonucleotides modified with fluorophores can be incubated on the chip and the DNA sequences of interest identified. Bachman says, "Now you can actually remove those pallets by the [laser] technique, collect them, and then use PCR on just that piece of DNA. This chip becomes a great screen for purifying DNA."

Britt E. Erickson reports from the **American Chemical Society 229th National Meeting**—*San Diego, Calif.*

Progress toward a flu chip

Although the flu season is now over in the Northern Hemisphere, researchers at the World Health Organization (WHO) and the U.S. Centers for Disease Control and Prevention (CDC) are busy identifying newly emerging strains in the Southern Hemisphere in preparation for next year's influenza vaccine. Developing an annual flu shot typically takes six months.

To speed up the process, Kathy Rowlen and colleagues at the University of Colorado, Boulder, are developing a microarray diagnostic for detecting influenza. This so-called Flu Chip will be able to differentiate between different influenza types (e.g., influenza A and B) as well as subtypes (e.g., the human strains H1N1 and H3N2, and the avian strain H5N1).

"The surveillance problem stems from the fact that few doctors can afford to do any kind of analysis. 'Sentinel' physicians in the United States send isolates from patient samples to the CDC. So most of the detailed strain analysis is conducted in a central location," says Rowlen. As a result, only ~7% of patient isolates actually get analyzed. "A new virus strain could emerge and we could initially miss it because right now we just don't have the resources to analyze a large number of samples," warns Rowlen.

PHOTO ART BY JOHN BIRKS

Researchers are developing a field-portable influenza diagnostic that can fit in the palm of your hand. They envision it looking like the device shown above.

By this summer, Rowlen and colleagues hope to have a working prototype of their Flu Chip for thorough testing in laboratory settings. "We'll have a chip that will have 200–300 sequences on it. But what we won't have worked out is the biology. That is, how to get the viral RNA most efficiently out of patient samples and onto the [chip's] surface," says Rowlen.

The Flu Chip will consist of DNA oligosequences on a surface. The idea is to bind these oligosequences directly to viral RNA extracted from a patient's nasal swab. "The problem is that there is so little viral RNA in patient samples that we are going to have to amplify the signal," says Rowlen. The researchers are exploring several options, including a nonenzymatic on-chip signal amplification technology, surface-enhanced fluorescence, and reverse transcriptase (RT)-PCR. "We'd like to avoid [RT-PCR] because you have to know what sequences you are looking for ahead of time," says Rowlen. In addition, the chemistry is difficult to do, particularly in a field setting.

One of the challenges with influenza RNA is that it tends to self-hybridize and form a significant secondary structure, says Rowlen. "Wherever you have self-hybridization, those regions of the gene are less accessible for hybridization onto the chip," she says. The researchers therefore chemically break up the RNA into small pieces so that it does not self-associate. But if the fragments are too small, they can't be fluorescently labeled. "The optimal length is probably on the order of 100 nucleotides for our system," says Rowlen.

The researchers hope to create an influenza diagnostic that is rapid, affordable (<$5/test), field-portable (~1 lb), and easy to use. Although their primary goal is to get it into the hands of WHO and CDC researchers, they are hoping it will eventually find its way into every doctor's office.

PEOPLE

New HHMI investigators

The Howard Hughes Medical Institute (HHMI) selected 43 biomedical scientists in March as new HHMI investigators. Two of them have contributed to *Analytical Chemistry*. The selection demonstrates a growing trend toward multidisciplinary research and the blurring of the line between chemistry and biology. *Analytical Chemistry* has seen a growing number of biology-related papers in recent years.

Mrksich

Quake

Milan Mrksich, a professor of chemistry at the University of Chicago, was chosen for his work on deciphering the molecular basis of cell adhesion. He has developed mimics of extracellular protein matrix surfaces and created artificial surfaces for biochip arrays that assay specific biological activities.

Stephen R. Quake, a professor of bioengineering at Stanford University, was chosen for his work in microfluidics. He has designed several chip-based systems for analyzing DNA and single cells as well as for growing crystallized proteins.