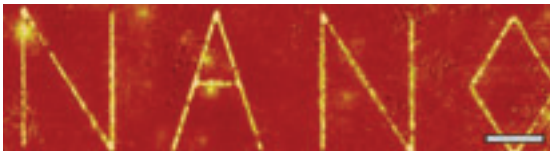
ANALYTICAL CURRENTS

Superlens overcomes diffraction limit



An AFM image of features etched onto a photoresist by evanescent waves focused by a silver superlens. Scale bar = 2 μm. (Adapted with permission. Copyright 2005 American Association for the Advancement of Science.)

Five years ago, John Pendry at Imperial College, London (U.K.) postulated that the diffraction limit, which restricts the resolution of current optical lenses to ~400 nm, could be overcome with a superlens (*Phys. Rev. Lett.* **2000**, *85*, 3966–3969). Now, Xiang Zhang and colleagues at the University of California, Berkeley, have confirmed Pendry's prediction by creating a superlens with a resolution of ~60 nm.

Pendry's superlens theory stemmed from an idea proposed by Victor Veselago in 1968 about materials with a negative index of refraction. The electromagnetic field of an object has both propagating waves and near-field evanescent waves. The evanescent waves, which contain fine details about the object, decay exponentially as the distance from the object increases. Unfortunately, conventional lenses with a positive refractive index can't capture these waves. Pendry suggested that a planar layer of a material with a negative refractive index should be able to refocus the evanescent waves and produce an image with sub-diffraction-limit resolution.

Zhang and colleagues experimentally demonstrated Veselago and Pendry's predictions with a 35-nm planar layer of silver that had a negative refractive index. Light of 365-nm wavelength illuminated a 50-nm chromium mask. The mask was etched with features that had dimensions <365 nm.

The silver focused evanescent waves emanating from the mask's features onto a layer of photoresist. The etched features were then traced with an atomic force microscope (AFM) to produce an image.

The investigators patterned the word "NANO" into a chromium mask. Without the layer of silver in place, the resulting lines in the photoresist were >300 nm in width. When the superlens was present, the line widths in the image were <90 nm. (*Science* **2005**, *308*, 534–537)

Enzyme assay in droplets

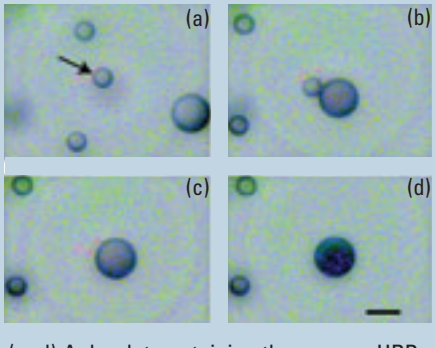
Gregory Faris and colleagues at SRI International have developed a simple protein assay in microdrops. The approach, which relies on optical microfluidics, can be applied to small-volume analyses, high-throughput screening, and combinatorial techniques.

In the new assay, a thermal gradient is optically induced across microliter to picoliter-volume droplets. The thermal gradient in turn induces a surface-tension gradient on the droplet surface, which causes the droplets to move.

The investigators used the assay to monitor the activity of an enzyme. One droplet contained horseradish peroxidase (HRP). Another droplet contained hydrogen peroxide and a chromogenic substance, 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS). When ABTS is oxidized, it turns a dark green color.

Faris and colleagues fused the two droplets together by moving one droplet into the other, and they monitored the activity of HRP by the color change in ABTS. They calculated that their approach can reach zeptomole detection limits.

Evaporation from the droplets was prevented by surrounding the aqueous droplets in decanol, an immiscible, nonvolatile liquid. The investigators point out that the thermal gradient causes the temperature in the droplet to increase by ~10 °C. However, because the temperature rise occurs only during droplet movement and not during the chemical reaction, they argue that the temperature change does not have a deleterious effect on the enzyme or the substrates. (*J. Am. Chem. Soc.* **2005**, *127*, 5736–5737)



(a–d) A droplet containing the enzyme HRP (shown by arrow) is optically moved and fused to a droplet containing HRP's substrates. The chemical reaction is monitored by a color change. Scale bar = 250 μm.

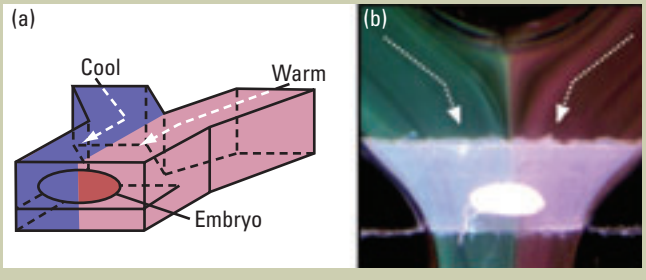
ANALYTICAL CURRENTS

Microfluidic investigation of fruit fly development

Rustem Ismagilov at the University of Chicago, Nipam Patel at the University of California, Berkeley, and colleagues have developed a microfluidic method to investigate the development of the *Drosophila melanogaster* embryo. The technique can be used to determine how embryonic biochemical networks respond to spatiotemporal changes in the environment.

The investigators created a microfluidic device that has two converging aqueous streams and can be built around a live embryo in 1 min. The device was made out of PDMS because of the material's low thermal conductivity.

To investigate the effect of temperature on the embryonic fruit fly, the investigators created a temperature step by heating one laminar flow stream and cooling the other. They exposed half of the embryo to the cool stream and the other half to the warm one. Embryos were exposed to the temperature step (17 °C/27 °C or 20 °C/27 °C) for 150 min and then were returned to room temperature.



(a) A schematic of a fruit fly embryo developing inside a PDMS microfluidic device. (b) Thermochromic liquid crystals show a temperature step inside a microfluidic device—the green stream is 21 °C, and the red stream is 24 °C. (Adapted with permission. Copyright 2005 Nature Publishing Group.)

Ismagilov, Patel, and colleagues had originally assumed that the perturbation in temperature would disrupt normal embryonic development. Although the halves of the embryos that were exposed to warmer temperatures developed faster than the halves exposed to cooler temperatures, the larvae that developed from the embryos were normal.

The investigators concluded that compensatory networks in embryos counteracted the effects of the environmental perturbations. By applying time-specific reversals of the temperature step, they found that the compensatory networks were activated within 65–100 min after the start of embryonic development. (*Nature* **2005**, *434*, 1134–1138)

Identifying respiratory pathogens

Current techniques for identifying pathogens responsible for epidemic respiratory infections are time-consuming and targeted at certain suspected agents. To overcome these limitations, David Ecker and colleagues at Isis Pharmaceuticals, the Naval Health Research Center, and Science Applications International Corp. developed a new method that rapidly identifies multiple species of bacteria from complex mixtures in respiratory samples.

In the new method, samples can be analyzed by PCR either directly or after a culturing step. Once the microbial DNA is amplified, the researchers determine the base compositions of the PCR amplicons by ESI-MS. Amplicons generated by surveillance primers allow the classification of bacteria into broad subdivisions, whereas amplicons from genotyping primers allow the identification of the specific bacterial species.

The researchers tested the method on samples taken from recruits at a military training center in 2002 during a pneumonia outbreak. Most of the samples contained a highly virulent strain of *Streptococcus pyogenes*, and the results were consistent with those obtained by two conventional methods. In a second experiment, duplicate swabs were analyzed from 15 recruits. One set of samples was cultured before PCR and MS analysis. All the samples tested positive on selective media for *S. pyogenes*, and the new method verified the finding. The other set of samples was analyzed directly by PCR, followed by MS. Many different types of bacteria were identified in the samples, and 12 tested positive for *S. pyogenes*.

Later in the winter, samples were taken from recruits at other facilities. For eight of the samples, duplicates were tested. One set was directly analyzed by the new PCR method, and the other set was cultured before amplification. For both sets, five tested positive for *S. pyogenes* and three were negative.

The detection limits and dynamic ranges for bacteria were dependent on the concentration of other microbes in the sample. According to the researchers, the new method can be generalized to other types of microbes, such as fungi, viruses, and protozoa. (*Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 8012–8017)

Nonintrusive droplet analysis

By combining evanescent wave sensing and thermocapillary actuation, Sandra Troian and colleagues at Princeton University have developed a nonintrusive optical method for analyzing discrete droplets in microfluidic devices. The system can monitor droplet position, sample concentration, and the dependence of temperature on kinetic rate constants. In addition, it can be readily adapted to any fluidic device that uses capillary forces to manipulate droplets.

In the new design, a thin-film waveguide is integrated with a planar microfluidic device. Droplets are transported electronically and positioned over the waveguide surface by thermocapillary actuation. The droplets are in direct contact with only a small part of the waveguide surface. The presence of a droplet in the optical beam path causes detectable intensity modulation.

The researchers are trying to further miniaturize the system by substitution of diffraction grating couplers. In addition, they are investigating the attenuation of each separate mode to improve the device's sensitivity. (*Appl. Phys. Lett.* **2005**, *86*, doi 10.1063/1.1922075)

Characterizing nanoparticle bioconjugates by AU

Analytical ultracentrifugation (AU) has been previously used to obtain detailed quantitative information about the mass and shape of proteins in solution. Now, Vicki Colvin and colleagues at Rice University have demonstrated that AU can provide physical information about nanostructures and their bioconjugates in solution. The technique can evaluate nanoparticle size, dispersity, and protein conjugation in a nondestructive manner, allowing the sample to be recovered for further use. In addition, AU is broadly applicable to any particle type in solution, regardless of the particle composition or solvent.

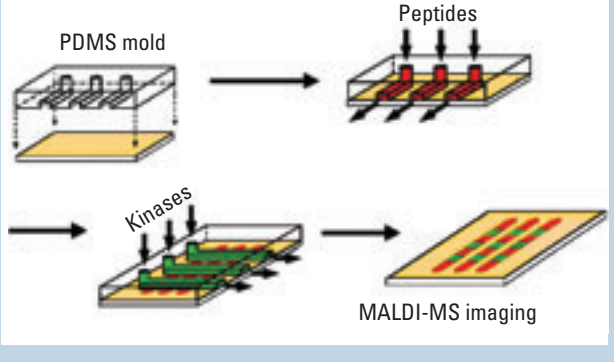
The researchers used AU to detect changes in the sedimentation properties of gold nanocrystals that result when the particles are conjugated to the DNA binding protein LacI. Such data provide information on the extent of conjugation and the number of proteins per nanoparticle in the nanobioconjugates. The distribution in the measured sedimentation coefficients agreed well with the size distribution observed by transmission electron microscopy. AU thus appears to be sensitive to conjugate formation. In the future, the researchers plan to use AU to analyze other parameters that affect conjugation. (*Nano Lett.* **2005**, *5*, 963–967)

A microfluidic peptide array

Milan Mrksich, Rustem Ismagilov, and colleagues at the University of Chicago have developed a multi-enzyme assay that marries microfluidic and biochip technologies. The new microfluidic peptide array can simultaneously measure the activities of several enzymes in small sample volumes.

The researchers used a criss-cross procedure to create the array on a self-assembled monolayer. Six parallel channels were molded into the monolayer, and a different peptide solution was flowed into each channel. After the peptides were immobilized, another set of six parallel channels was molded across the initial set. Solutions containing enzymes and/or small molecules were flowed through the second set of channels. The researchers then used MALDI-TOFMS to analyze the molecules in the cross-over regions.

Kinases were flowed separately and in mixtures through the channels. The kinases phosphorylated the expected peptide substrates. When inhibitors of particular kinases were added to the mixtures, the kinase substrates were not phosphorylated. Cell extracts from human cells were also tested in the presence and absence of small-molecule inhibitors on an array of kinase and phosphatase substrates. Once again, the expected enzyme activities were observed. (*J. Am. Chem. Soc.* **2005**, *127*, 7280–7281)

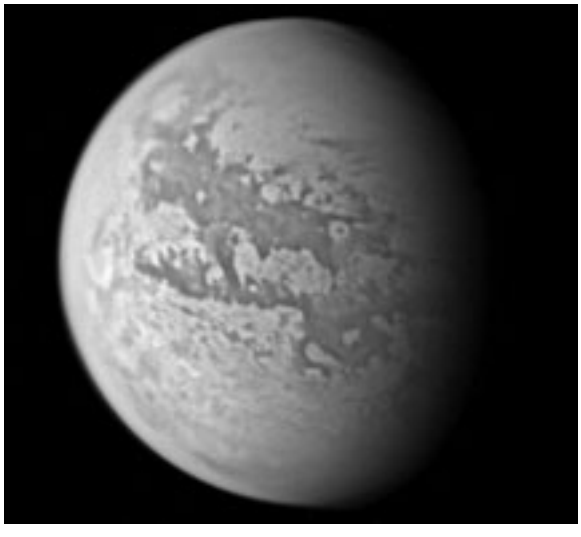


A schematic of a microfluidic peptide array.

ANALYTICAL CURRENTS

Sampling the haze around Titan

A wealth of data from the *Cassini* spacecraft, which flew by Saturn's largest moon, Titan, in October and December 2004, was recently reported. The results could help shed light on the composition and structure of Titan's atmosphere as well as the surface beneath it. Titan and Earth are the only bodies in our solar system with nitrogen-rich atmospheres. Although Titan's atmosphere is more reducing than that of Earth and contains high levels of methane, it may offer a glimpse of Earth's early atmosphere before the influence of life.



NASA/JPL/SPACE SCIENCE INSTITUTE

Although scientists still have a lot to learn about Titan's surface and its murky skies, data obtained by the *Cassini* spacecraft are starting to reveal some of the moon's features.

In addition to other instruments, *Cassini* was equipped with a UV imaging spectrometer (UVIS). On the second flyby, the UVIS identified and measured six species: methane, acetylene, ethylene, ethane, diacetylene, and hydrogen cyanide. The measurements were taken at altitudes of 450–1600 km above the surface. Methane was the only detectable carbon-based molecule observed below 600 km; higher-order hydrocarbons and hydrogen cyanide were undetectable at altitudes below 600 km. The results show the presence of a mesopause at 615 km, with a minimum temperature of 114 K. The temperature of the upper atmosphere was estimated to be 151 K.

Cassini was also equipped with an ion-neutral mass spectrometer, which obtained the first in situ neutral composition measurements of molecular nitrogen, methane, molecular hydrogen, argon, and several stable carbon-nitrile compounds in Titan's upper atmosphere (3000–1174 km). The instrument also provided the first direct measurements of nitrogen, carbon, and argon isotopes, which offer important clues about the evolution of the moon's atmosphere. The composition and thermal structure of Titan's upper atmosphere did not appear to have changed significantly since the Voyager 1 flyby in the early 1980s. (*Science* **2005**, *308*, 978–982; 982–986)

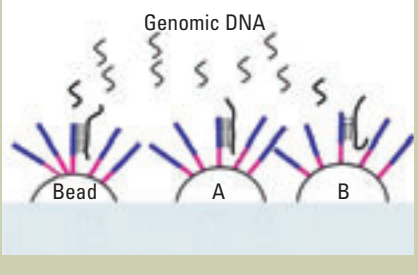
Probing whole genomes

Kevin Gunderson and colleagues at Illumina, Inc., Ambion, Inc., and Prognosys Biosciences, Inc., have developed an array-based whole-genome genotyping (WGG) assay that determines whether mutations are present at particular locations in a genome. The new assay does not require a PCR step and has the potential for unlimited multiplexing.

The researchers first amplify genomic DNA with a method called whole-genome amplification (WGA) to ensure that enough DNA is present for WGG analysis. Unlike PCR-based approaches, WGA allows researchers to amplify genomic DNA with little sequence bias. Therefore, more single nucleotide polymorphisms (SNPs) are available for analysis on genomic DNA with the WGA method.

After amplification, the genomic DNA copies are hybridized to probe DNA on a bead array. Probes that are complementary to one of the two possible forms of a SNP are bound to each of two bead types (A and B). Probes differ in their final base, which is the SNP site. Probe sequences are only extended with labeled nucleotides if the genomic DNA perfectly hybridizes to a probe.

Gunderson and colleagues optimized the assay with a set of 96 sequences. Next, the researchers tested 95 samples of human DNA on a set of 1485 SNPs used by the HapMap Consortium project. The assay had a reproducibility rate of 99.99% and was accurate 99.7% of the time. (*Nat. Genet.* **2005**, *37*, 549–554)



Schematic of a WGG assay. (Adapted with permission. Copyright 2005 Nature Publishing Group.)