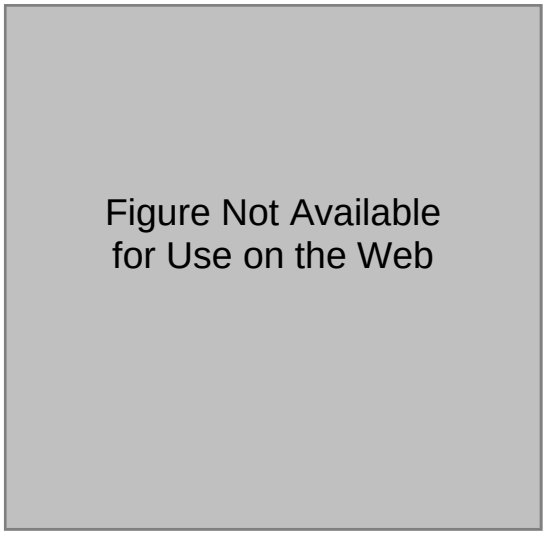
ANALYTICAL CURRENTS

Microfluidics for chemotaxis

In microscale devices, laminar flow often challenges researchers because liquids only mix by diffusion. However, Paul Cremer, Hanbin Mao, and Michael Manson at Texas A&M University use laminar flow to their advantage in a new device to test bacterial chemotaxis.

Chemotaxis is the propensity of an organism to move in relation to a chemical. For example, bacteria move toward a higher concentration of an attractant or away from a repellent. The assay takes advantage of laminar flow to create a concentration gradient across a microchannel by injecting two liquids, a buffer and a chemical attractant or repellent. The bacteria are added just as the two liquids come together and are carried down the channel (by the continuous addition of liquid by syringe pumps) while the bacteria move themselves within the concentration gradient. Numerous channels fan out from the end of the main channel, allowing the bacteria to be separated by their position in the gradient.

The researchers says this method's detection limit is 3 orders of magnitude lower than the standard capillary assay for chemotaxis. Another advantage is that every cell is counted whether it responds to the gradient or not. This is an improvement over capillary systems that only detect bacteria if they respond to a gradient by entering the capillary. In addition, video recording of a bacterium's run can determine how fast it responded to a gradient. (*Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 5449–5454)

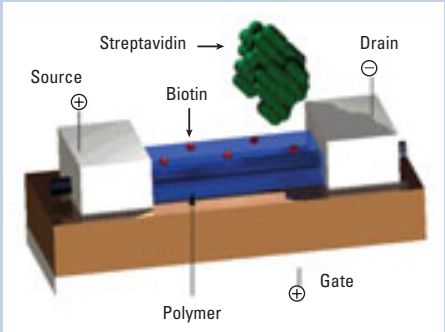


Nanotubes detect protein binding

Carbon nanotubes aren't just for synthetic chemists anymore. Recent papers have shown that nanotubes can be used as gas and flow sensors. Now, Alexander Star and colleagues with Nanomix, Inc., build field effect transistors (FETs) with nanotubes and show how they can detect protein binding—in this case, classic biotin–streptavidin coupling. Such a system could be useful as detectors in labs-on-a-chip and other miniaturized devices.

To create the FET, the researchers deposited the nanotubes on silicon dioxide and doped silicon. Electrical leads composed of titanium films and capped with gold were patterned on top of the nanotubes and formed the transistor's source and drain. Because multiple nanotubes, with properties varying from semiconducting to metallic, spanned the electrical connections, the FET could be modulated over a gate voltage range of –10 to +10. The nanotubes were coated with a mixture of poly(ethylene imine) (PEI) and poly(ethylene glycol), and the protein biotin was attached to PEI amines. The final FET showed n-type characteristics, probably due to the electron-donating properties of the PEI amines.

Binding of streptavidin was accompanied by a huge loss of current at negative gate voltages. Control experiments established that the device was not subject to false positives. Based on data from additional experiments, the authors estimate a current detection limit of 10 streptavidin molecules. (*Nano Lett.* **2003**, *3*, 459–463)



A polymer- and biotin-coated nanotube FET waits to grab a streptavidin molecule.

ANALYTICAL CURRENTS

Virus particles take the gold

Bogdan Dragnea and co-workers at Indiana University report the first application of Rayleigh resonance (RR) spectroscopy for the detection of gold nanoparticles within single virus particles. The method tracks individual viruses in vivo, which ultimately may help answer the question of how viruses infect organisms.

Virus particles, or virions, are essentially protein-wrapped packets of RNA. To get gold nanoparticles into brome mosaic virus (BMV) particles, Dragnea's team disassembled the protein wrapper, or capsid; added negatively charged gold nanoparticles (2.5–4.5 nm diam); and reassembled the capsids. Using transmission electron microscopy, the researchers determined that ~2% of the virions had reassembled around either one or two nanoparticles.

A single virion is a very small sample, so Dragnea and co-workers used RR spectroscopy, which can provide information at the single-particle level. The researchers found that the RR peak for gold nanoparticles encapsulated by capsids (518 nm) was blue-shifted and broadened when compared to the RR peak for bare gold nanoparticles (524 nm). Typical explanations for differences in the spectra, such as an increase in the index of refraction caused by the protein capsid, would predict a red shift instead. They speculate that the unexpected blue shift may be due to strong interactions between the capsid proteins and the gold nanoparticle surface.

Purified gold-containing capsids appeared red by confocal dark-field microspectroscopy. Two populations were observed, one half as bright as the other, indicating again that some capsids contained one gold particle but others contained two. The population of BMVs containing a pair of nanoparticles was heterogeneous, yielding different spectra depending on the distance between the gold particles within a single capsid. The researchers explain that nanoparticles are close together and coupled when the spectra have two peaks, but the gold particles are too far from each other to couple when a single peak is seen. (*J. Am. Chem. Soc.* **2003**, *125*, 6374–6375)

SERS technique spins away degradation troubles

M. J. Sepaniak and colleagues at the University of Tennessee–Knoxville rapidly spin their samples to avoid, as much as possible, substrate damage and thermal and photolytic decomposition of the sample under surface-enhanced Raman spectroscopy (SERS). Their sample translation technique (STT) decreases the effective residence time of the analytes and substrate within the irradiated zone without reducing spectral acquisition time or analyte density.

Continuous irradiation of samples under traditional SERS conditions can cause permanent damage to the substrate surface—even at powers as low as ~2 mW—and significantly broaden and reduce the intensity of observed spectral bands. Researchers can

sometimes solve these problems by rastering the laser beam with a rotating mirror, which defocuses the laser beam. But sensitivity is compromised using such methods, and Raman microscopes can't reproduce matching defocusing conditions.

Sepaniak's group reports that STT continuously irradiates the sample with laser powers up to ~9 mW with minimal damage to the substrate surface. In most cases, STT improved spectral resolution by >20%, and signal intensity increased by at least a factor of 2. The group says the rapid spinning of the sample improves the precision of the analytical results because it compensates for microscopic aberrations of the substrate by generating a solid revolution that's viewed by the detector as a uniform surface. STT also enhances vibrational modes weakly enhanced or even absent under stationary conditions.

The researchers report that STT significantly decreased SERS bandwidths with solutions of Naproxen, riboflavin, rhodamine 6G, 4-aminothiophenol, and folic acid. Results for Naproxen, a drug susceptible to thermal and photolytic decomposition, were particularly favorable. Sepaniak's group observed a 37% RSD of the Naproxen bandwidths under stationary conditions compared with 7% RSD change under spinning conditions. (*Appl. Spectrosc.* **2003**, *57*, 428–438)

Using KOD DNA polymerase in PCR-MS studies

The DNA polymerases from *Thermus aquaticus* (Taq) and *Pyrococcus furiosis* (Pfu) are commonly used in PCR to copy DNA strands. Although amplicons generated by Taq and Pfu work well for many applications, David Muddiman and co-workers at the Mayo Clinic and Foundation and Virginia Commonwealth University report that *Thermococcus kodakaraensis* (KOD) DNA polymerase generates products that are better suited for MS analysis. They find that KOD yields more product than either Taq or Pfu polymerases, and the resulting fragments are all blunt-ended, which facilitates MS analysis.

Using human genomic DNA as a template, Muddiman and co-workers set up three PCR reactions to compare the amount of product obtained using each polymerase. KOD generated 2–3 times more product than Taq or Pfu as quantitated by densitometry and UV absorbance. The researchers found they could detect product from as little as 5 ng of genomic DNA. Thus, KOD polymerase is particularly useful when template material is hard to come by, such as in forensic investigations.

Taq, Pfu, and KOD PCR products were run separately on an electrospray ionization (ESI) FT ion cyclotron resonance (ICR) mass spectrometer. Pfu and KOD generated blunt-ended species only, but Taq produced three species of fragments (blunt-ended, mono-adenylated, and di-adenylated), which complicated the resulting mass spectrum. Because there was only one species obtained in the reaction with KOD, the researchers achieved unit mass resolution of a 116-base-pair product (~72 kDa) using ESI-FTICR MS at 9.4 T. (*J. Am. Soc. Mass Spectrom.* **2003**, *14*, 601–604)

Figure Not Available for Use on the Web

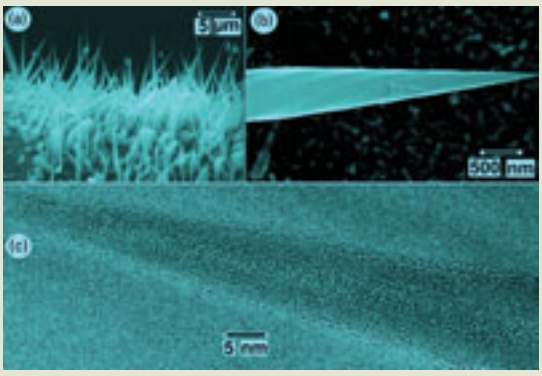
PCR products generated with either (a) Taq, (b) Pfu, or (c) KOD DNA polymerases were analyzed by FTICR MS.

Tubular cones offer new scanning dimensions

Enge Wang, Xin Jiang, and Guangyu Zhang at the Fraunhofer Institute for Surface Engineering and Thin Films (Germany) and the Chinese Academy of Sciences use a chemical vapor deposition method to synthesize tubular graphite cones (TGCs) that are stronger and easier to mount than currently used carbon nanotubes (CNTs). The researchers say that these hollow nanometer-sized cones, with interior diameters ranging from ~2 nm to several tens of nanometers, could be used for scanning probe microscopy (SPM).

The radial flexibility makes CNTs susceptible to lateral bending, which can pose problems during some SPM and field emitter applications. For example, mechanical or thermal vibrations can create poor signals or noise, and the shape of CNTs can make them difficult to mount and handle. The researchers say that the design of their TGCs, which have faceted, mostly octahedral surfaces with a spiral direction that is clockwise or counterclockwise, overcomes such problems.

The researchers grew their TGCs using iron needles as substrates and a microwave plasma-assisted chemical vapor deposition system. The average length of the TGC tips is ~12 μm, and the average apex is 6–7°. TGCs are always similar in shape regardless of their size. (*Science* **2003**, *300*, 472–474)



Microscopy images showing (a) aligned TGCs grown on an iron needle surface, (b) the faceted and helical appearance of a TGC, and (c) a TGC tip. (Adapted with permission. Copyright 2003 American Association for the Advancement of Science.)

ANALYTICAL CURRENTS

Doing FTIR imaging one step scan at a time

Ira Levin and Rohit Bhargava at the National Institutes of Health introduce a time-resolved (TR) FTIR imaging technique to examine repetitive dynamic processes that occur on fast time scales. They report that their step-scan FTIR imaging system provides simultaneous spectral, microscopic spatial, and millisecond temporal characterizations of the molecular dynamics in polymer-dispersed liquid crystals (PDLCs). They say they know of no other method that provides such chemically and spatially sensitive information.

Because current large-format focal-plane array detectors for IR imaging require long data acquisition times, many biological and materials processes that happen in less than a second go unexplored, say Levin and Bhargava. Although IR and TR spectroscopic methods have helped to determine molecular responses to external stimuli, spatial specificity tends to be low or nonexistent.

To test their technique, the researchers examined the molecular responses to external electric-field perturbations of a PDLC composite in which most LC molecules occur in droplets containing a small amount of dissolved polymer. Few TR spectroscopic studies exist of low-molecular-weight nematic LCs in confined geometries, including the complications of interfacial and dissolved species interactions found in those composites, add Levin and Bhargava.

By examining the absorbance distributions of the LC-specific peaks as a function of time over the entire field of view, Levin and Bhargava were able to detect spatially different molecular relaxation phenomena, observe local structural defects, and monitor molecular interactions. They were also able to determine that LC–polymer interactions are not restricted to the interface, as previously thought, but occur within the droplet bulk, which they say is critical in understanding LC dynamics in confined geometries. (*Appl. Spectrosc.* **2003**, *57*, 357–366)

A new lead biosensor using DNAzymes

Colorimetric methods are a potentially cheaper and easier way to detect lead in paint than the currently used fluorescence detection. Now, Yi Lu and Juewen Liu at the University of Illinois at Urbana–Champaign have developed a colorimetric test for Pb(II) ions using gold nanoparticles and a tunable DNAzyme system. The researchers say the method could be adapted to detect other metal ions by substituting DNAzymes with different sensitivities.

Lu and Liu used a DNAzyme—a DNA molecule with enzymatic activity—composed of an enzyme strand called 17E and a substrate strand called 17DS. The researchers extended 17DS on both ends to give DNA-coated gold nanoparticles a place to dock. When no Pb(II) is present, the DNAzyme–gold nanoparticle units aggregate, resulting in a blue color. In the presence of Pb(II), 17E cleaves 17DS, which prevents aggregation and turns the solution red.

The system can detect 100 nM–4 μM Pb(II), which is within the range of environmentally relevant levels. Varying the ratio of normal DNAzyme to mutant, inactive DNAzyme (17Ec) tuned the sensor without creating a whole new one. When the ratio 17E:17Ec was 1:20, for instance, the detection range was 10–200 μM.

Spotting the resulting liquid onto a TLC plate allowed the researchers to make qualitative and semiquantitative determinations. The solution turned purple, then red, with increasing Pb(II) concentration through 5 μM, but remained blue in the presence of other divalent metal ions. They also tested the sensor with lead paint samples of varying Pb(II) compositions and found that most samples turned red, including the one containing 0.5% Pb(II), which is the U.S. EPA threshold for leaded paint. (*J. Am. Chem. Soc*, **2003**, *125*, 6642–6643)