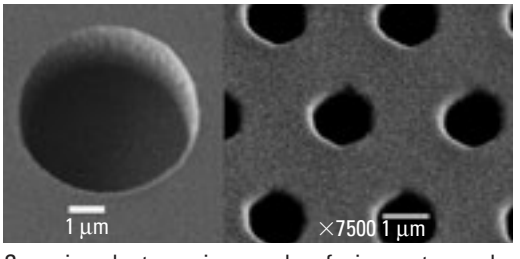
ANALYTICAL CURRENTS

Single-molecule assays in femtoliter chambers



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

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.

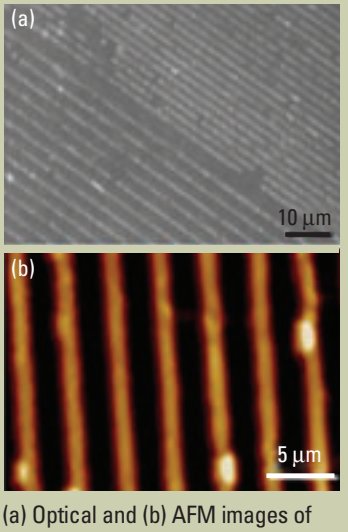
Noji and colleagues used the device to measure the activity of single molecules of horseradish peroxidase and β-galactosidase. The enzymes didn't bind to the surfaces of the chambers and remained active in solution. The researchers suggest that the device could be applied in population studies of a given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

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.



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

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)

ANALYTICAL CURRENTS

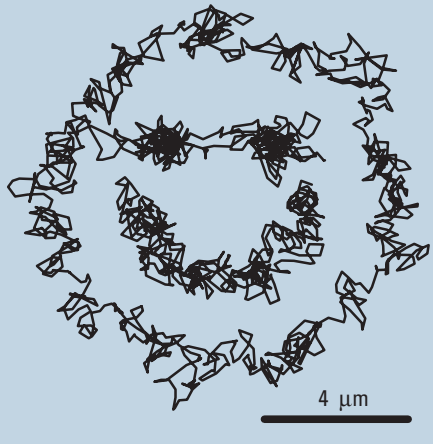
Manipulating nanoscale objects in solution

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)

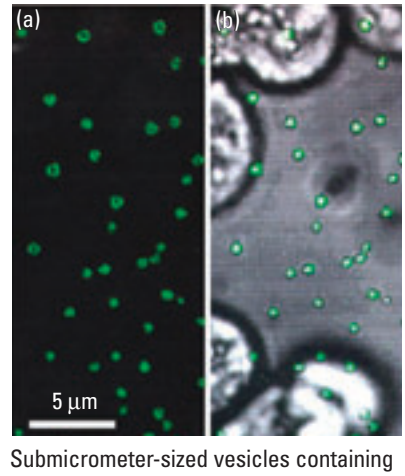


A 200-nm particle followed a trajectory that drew a smiley face in the ABEL trap. (Adapted with permission. Copyright 2005 American Institute of Physics.)

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.

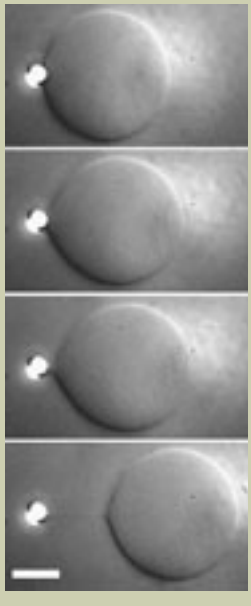
Finally, they assayed transmembrane signaling. When a particular ligand binds to its receptor, intracellular calcium levels increase transiently. Vogel and colleagues, therefore, measured the calcium concentration within immobilized vesicles when the ligand was present. Calcium levels rose briefly and 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.

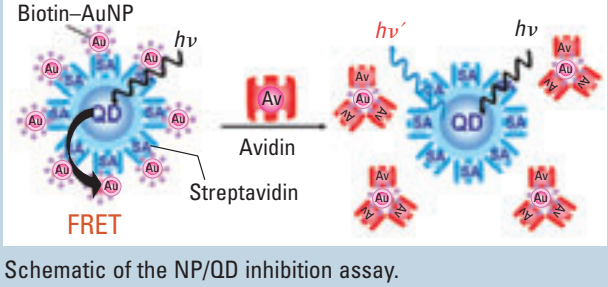
As a first step toward finding a reliable measurement method for ultralow-level particle emissions, Martin Mohr and colleagues at the Swiss Federal Laboratories for Materials Testing and Research investigated 16 different instruments comprising both mass- and nonmass-related methods. The instruments 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.