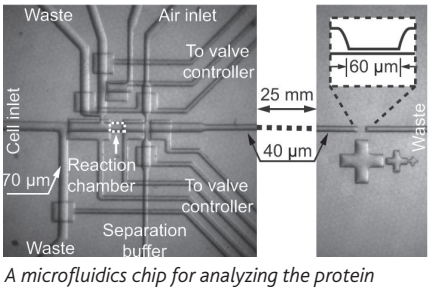
Microfluidics begins to realize its potential

MICRO/NANOFLUIDICS

The control and manipulation of small volumes in microscale fluidic channels is being used to carry out evermore complex biomedical assays. Three recent studies illustrate the great power of microfluidic systems to perform sequential operations and parallel reactions, giving fast results difficult to achieve by other means.

Richard N. Zare and colleagues at Stanford University and the Carnegie Institution in California have used a microfluidics device to count the number of specific proteins in a single cell [Huang *et al.*, *Science* (2007) **315**, 81]. Protein levels can vary markedly between different cells, and some proteins that are present in very low numbers can play very important roles in cell functions. The group's microfluidics platform (shown below) can study these effects as it has the sensitivity to detect single protein molecules from single cells and the ability to quantify multiple protein targets at once.

"This feat is accomplished using a microfluidics system in which a single cell is captured and lysed, followed by fluorescent tagging of the chemical contents," explains Zare. The proteins are then separated electrophoretically and the molecules counted one by one as fluorescent bursts flowing past an observation region. "It is the first time that the chemical contents of a cell have been measured by counting individual molecules," he says. Zare suggests that their method should have many applications in trying to understand the functioning of cells.



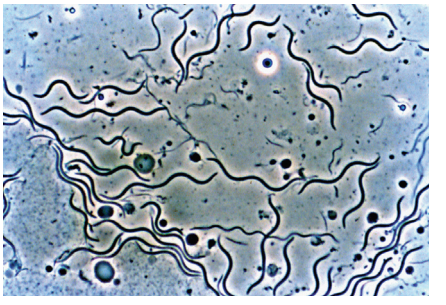
A microfluidics chip for analyzing the protein contents of single cells. (© 2007 Science.)

An interdisciplinary group from California Institute of Technology (Caltech), Auburn University, and Stanford have also recently reported the use of microfluidics to separate out single cells for analysis. Rather than look at proteins, however, they carried out a polymerase chain reaction (PCR) to amplify DNA from single bacterial cells and analyze multiple genes at once [Ottesen *et al.*, *Science* (2006) **314**, 1464].

One of the potential applications for genetic analyses like this is in environmental science. Microbial communities in nature are often complex, rich, and diverse ecosystems that contain many different bacteria. Sometimes potentially useful catalytic activities are found, but identifying the microbial species responsible is a challenge.

The researchers analyzed bacteria from the gut of termites (shown above). These bacteria break down wood into a material that serves as a source of food and energy for the termites, using enzymes of interest to biotechnologists.

The group used a microfluidics device to rapidly separate and partition single bacteria from the complex initial sample. Subsequent PCR reactions were designed to probe for the presence of an enzyme of interest, as well as a standard gene that could be used to identify the particular bacterial species involved. After sequencing of the PCR products, a specific *Treponema* species was found to encode the key enzyme.



Bacteria from the gut of termites. (Courtesy of Jared R. Leadbetter.)

"We're revealing an approach that can lead to a better understanding of the many microbial processes that underlie the environments in which we all live," says Jared R. Leadbetter of Caltech.

Screening followed by optimization experiments are used in areas from the life sciences to chemistry and materials science, and University of Chicago researchers have shown how a microfluidics approach can combine the two processes to minimize the amount of sample needed [Li *et al.*, *Proc. Natl. Acad. Sci. USA* (2006) **103**, 19243]. The group demonstrated the value of their method by crystallizing two model membrane proteins. Membrane proteins tend to be difficult to work with and obtain crystals, as they can be difficult to solubilize. From just 10 μl of solution, ~1300 crystallization trials were set up in under 20 mins. The crystals obtained allowed the proteins' crystal structures to be solved. The method is used in the Chicago lab for crystallization of new membrane proteins, says Liang Li.

Jonathan Wood

Cells mix and match on the same surface

MICRO/NANOFLUIDICS

Scientists at the National Center for NanoScience and Technology, Peking University and The Institute of Medical Equipment in China have developed a simple method that uses microfluidic channels within an elastomeric stamp to pattern multiple types of cells on the same substrate [Li *et al.*, *Angew. Chem. Int. Ed.*, doi: 10.1002/anie.200603844].

"Most laboratory studies of cultured cells use only one or two types of cells without controlling their spatial locations," explains Xingyu Jiang, who led the work. "Studies of the development of normal and pathological tissues and many disease models, however, require the precise spatial ordering of several types of cells."

A Au substrate is covered in a self-assembled monolayer of a thiol molecule that resists protein adsorption and cell attachment. A poly(dimethylsiloxane) stamp with embedded fluidic channels is then placed on the substrate. The individually addressable channels are used to first desorb the thiol from the Au surface, then deliver a particular cell solution. The cells only adhere in the activated areas of the substrate exposed to the microfluidic channels and remain confined after removal of the stamp. A second desorption step can remove the remaining thiol molecules and allow cells to move over the Au surface, interacting with each other. The researchers were able to pattern HeLa and NIH 3T3 cells in separate regions of the Au substrate. The researchers believe their technique should be particularly useful in creating models to study neuronal development and tumor growth.

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