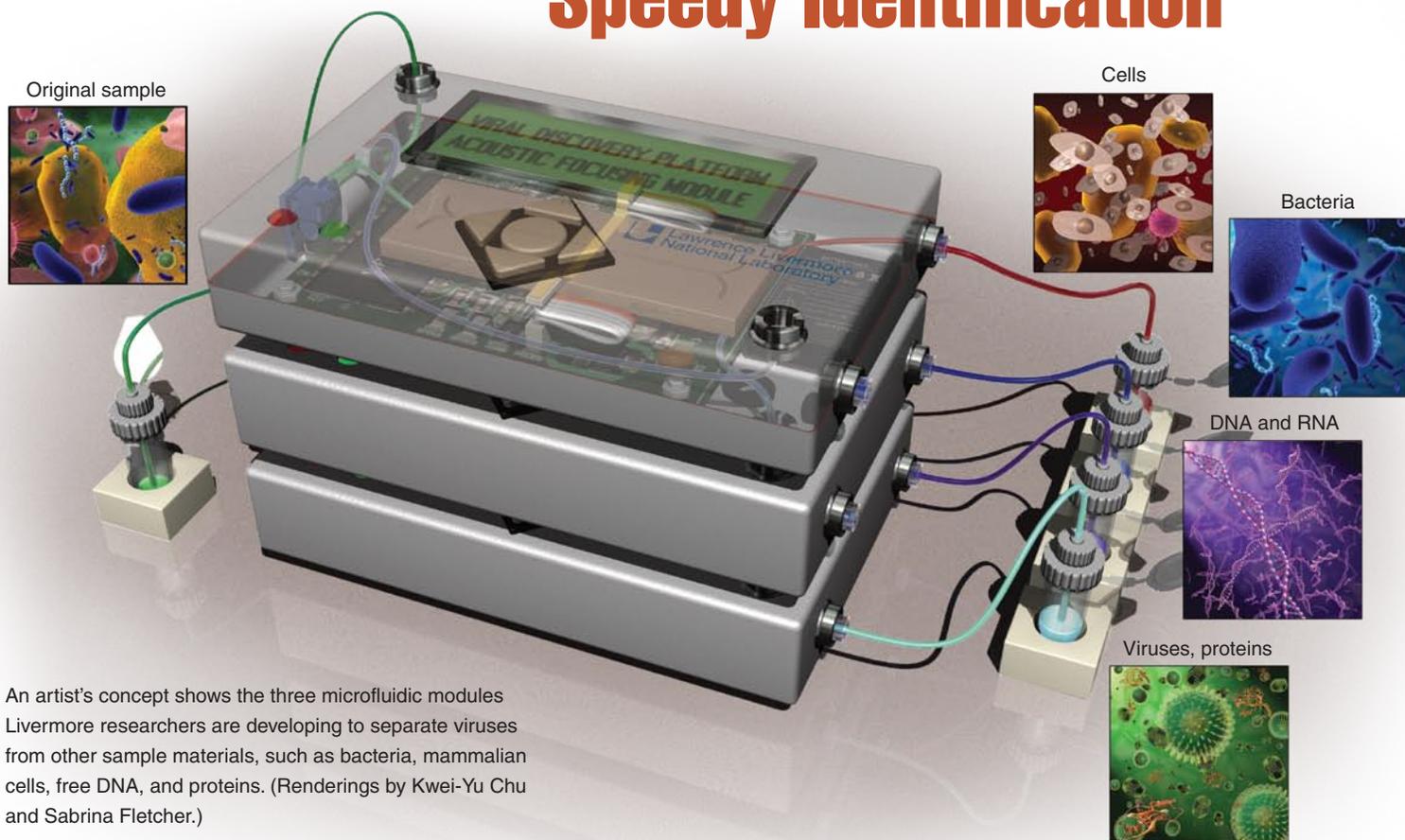


Isolating Pathogens for Speedy Identification



An artist's concept shows the three microfluidic modules Livermore researchers are developing to separate viruses from other sample materials, such as bacteria, mammalian cells, free DNA, and proteins. (Renderings by Kwei-Yu Chu and Sabrina Fletcher.)

QUICKLY identifying pathogens, especially viruses, can be crucial at the start of a disease outbreak such as H1N1 influenza, severe acute respiratory syndrome (SARS), or any of the many viruses that can initiate an epidemic. Rapid characterization could also speed the response following the deliberate release of a viral biological warfare agent.

However, distinguishing the components in a sample of blood, urine, or nasal mucus is a tedious and time-consuming process. The different materials—viruses, bacteria, mammalian cells, free DNA, and proteins—must be separated before they can be characterized, and the separation process requires the services of highly trained researchers or health-care workers.

A team of Livermore engineers and bioscientists is responding to the nation's critical need for a technology that quickly isolates viruses and other biological particles. The team's goal is to deliver a fully automated modular platform capable of accepting a diverse

range of clinical and environmental samples, separating and "binning" the bioparticles, and removing background signals and contaminants that could distort the results.

The research project, led by mechanical engineer Klint Rose, is part of a Laboratory Directed Research and Development initiative led by Livermore scientist Chris Bailey to detect emerging viruses. "We want to identify existing pathogens and discover emerging ones quickly enough for public health officials to implement countermeasures such as quarantines and antibiotic prophylactic administration," says Rose, who works in the Laboratory's Science and Technology Principal Directorate.

The development effort focuses on introducing up to 1 milliliter of a raw sample into a device about the size of a hardcover book. Modules then separate the sample into components and concentrate the materials targeted for identification—all within 60 minutes. The Livermore separation

method uses extremely small amounts of reagents such as saline and deionized water. Traditional methods require more expensive reagents and antibodies, and these technologies reduce the amount of virus available for analysis.

“Other techniques for separating bioparticles use beads that bind to specific types of cells, bacteria, and viruses,” says Rose. “The beads must be extracted, and then the bacteria removed. We wanted a more generic approach that would separate all of the bioparticles in a sample, including the particles we have yet to identify. The most generic attribute we found was size.” A typical sample contains a wide range of bioparticle sizes, from mammalian cells (which measure 2 micrometers in diameter and larger) to bacteria (0.5 to 2 micrometers in diameter), viruses (20 to 200 nanometers in diameter), and proteins (2 to 20 nanometers in diameter).

The Livermore instrument will use compact modules designed to accept various types of clinical samples, such as blood, urine, nasal washes, and dry and wet material trapped by environmental collectors. The team is focusing on nasal samples because a pandemic would likely start with a respiratory virus, and some virus particles would likely lodge in a patient’s nostrils. Mucus is collected by flushing the nasal cavity with a wash, usually sterile saline. Samples contain cells, bacteria, viruses, loose genetic material, and proteins, representing a vast range of particle sizes.

The sample preparation effort complements the work of two Livermore groups that are developing similar advanced modules for identifying purified virus fractions, or aliquots, using microarrays and a process called polymerase chain reaction (PCR). “Our modules will ‘hand off’ the cleaned-up sample to the analysis modules,” says Rose. “We’re in frequent contact with the other researchers to ensure that the different devices work well together.” He notes that because PCR is based on amplifying DNA or RNA,

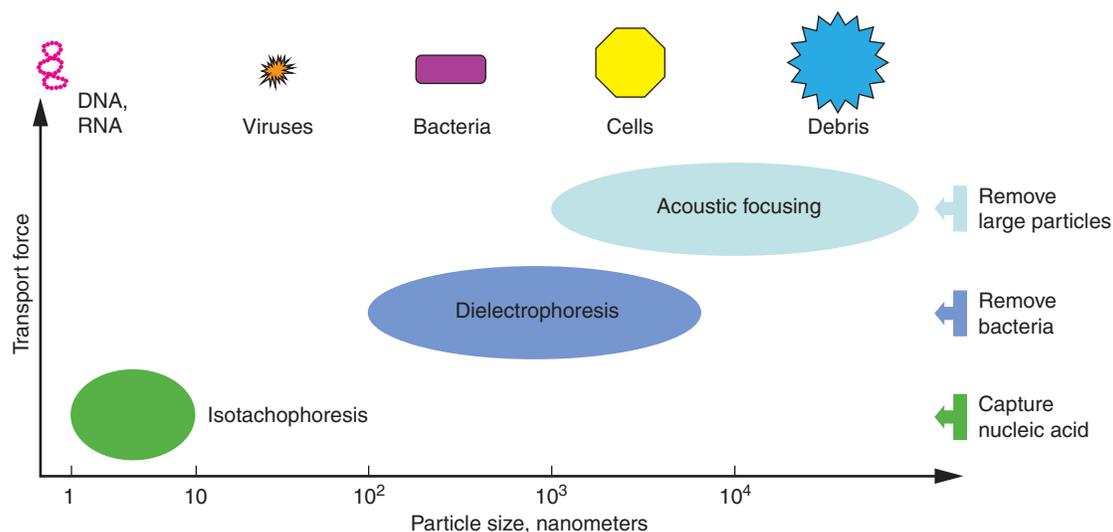
the separation modules must remove all nonviral nucleic acids, including free-floating DNA and RNA, as well as cells and bacteria whose large genomes would complicate analyses.

Microfluidics Tap Subtle Forces

The development team is conducting its research at the Livermore Center for Micro- and Nanotechnology, which brings together researchers from diverse disciplines such as engineering, chemistry, biology, physics, materials science, and computer science. In addition, the team is collaborating with researchers from the University of California at San Francisco and Santa Barbara, San Diego State University, and Stanford University.

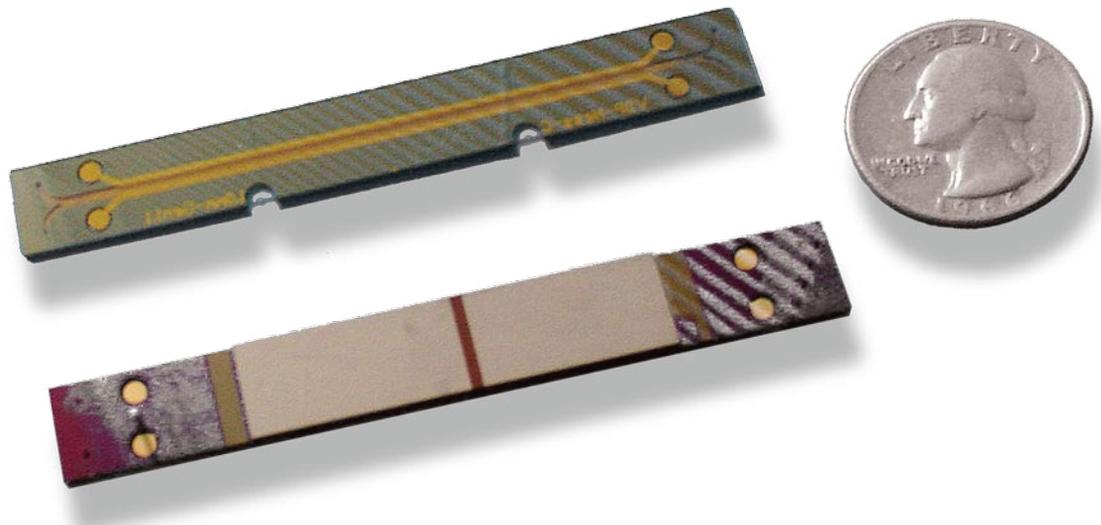
The modular separation technologies are based on microfluidics, a field aimed at manipulating particles suspended in extremely small volumes of liquid within microscopic channels. At the microscale, physical forces such as subtle electrical attractions and repulsions influence particle transport and component separation in ways that are not effective in larger-scale systems. For example, the weak electric forces of dielectrophoresis decay rapidly as a particle drifts more than a few micrometers away. Livermore researchers have been among the leaders in using microfluidic technologies to build experimental devices aimed at reducing costs, sample volumes, and time to complete a diagnostic test.

In the Laboratory’s instrument, separation modules serve as “virtual filters” in which three microfluidic techniques—acoustic focusing, dielectrophoresis, and isotachophoresis—replace physical filters. The first two modules separate and concentrate a certain class of constituents according to size. Acoustic focusing extracts cells larger than 2 micrometers. Dielectrophoresis then removes bacteria and other particles small enough to pass through the acoustic separator. In the third module, isotachophoresis pulls out loose DNA and RNA molecules based on their electric charge



The Livermore device uses three microfluidic technologies to separate biomolecules. Acoustic focusing removes large particles, mainly mammalian cells. Dielectrophoresis isolates bacteria. Isotachophoresis then captures nucleic acids and separates them from virus particles.

In the acoustic focusing module, the vibration of sound waves pushes cells to the middle of an H-shaped channel (top), where they can be collected. Smaller components, including viruses, are then transferred to the next module. On the reverse side of the device (bottom), a piezoelectric transducer vibrates the channel walls as fluid flows through.



within the fluid. Once the viruses pass through the third filter, they are collected for analysis.

The microfluidic chips in the three modules are small, measuring about 5 centimeters by 2 centimeters by 1 millimeter thick, with channels 500 micrometers wide and 200 micrometers tall. Manufactured at Livermore from silicon and glass, they are relatively inexpensive and reusable. Together, they form a rugged, automated system.

Each module is built around a single microfluidic chip with an H-shaped channel geometry. With this geometry, samples can be injected at one end, fractionated, and then extracted at the other end. An automated system controls the chips' fluids and electronics. To optimize the module designs, the team ran simulations to determine how the different moving bioparticles respond to the subtle acoustic or electric forces.

Modules are connected so that as each one captures its designated class of bioparticle, it transfers the remaining sample to the next module. The first module uses acoustic waves to remove cells from the complex sample. A piezoelectric chip bonded to the back of the device generates acoustic forces that cause the channel walls to vibrate as fluid moves through. The acoustic waves push cells to the middle of the channel where they can be collected. Smaller constituents are then transferred to the second module, which uses dielectrophoresis.

The second module applies an electric field gradient to polarize particles and isolate them. In effect, the electric field pushes bacteria from the input stream to the recovery outlet, while permitting smaller bioparticles (DNA, RNA, viruses, and proteins) to pass through to the isotachopheresis stage.

In this third module, an electric field applied across specially designed electrolytes creates forces that focus, or pull, bioparticles into different zones depending on their electrophoretic mobility. In this way, DNA and RNA, which have a nearly uniform mobility for

lengths greater than about 200 base pairs, can be separated from the viruses and proteins in the remaining sample.

The three-part separation process produces individual aliquots of cells, bacteria, DNA and RNA, and viruses. Although the team is focusing on viruses, other materials could just as easily be tapped for analysis.

Modular Process Improves Performance

In early tests, the Livermore modules recovered more than 80 percent of input viruses while removing more than 90 percent of the cells from the sample. These results compare favorably to more cumbersome bench-top techniques involving membrane filters or centrifugation, which recover about 50 percent of viruses and remove 99 percent of the cells. In addition, the modules can process samples ranging from 10 microliters to 1 milliliter at a rate of 10 to 100 microliters per minute, resulting in a total processing time of 10 minutes to about an hour. In contrast, current techniques can take several hours.

Over the next year, the team plans to integrate the three modules into a single device. Rose estimates that the Laboratory can transfer this technology to industry in about three years, where it could perform diagnostic tests more cheaply and quickly than is currently possible. The modular device could also be deployed on the front lines of biodefense, where aerosol environmental collectors search for evidence of biological warfare agents.

—Arnie Heller

Key Words: acoustic focusing, bacteria, Center for Micro- and Nanotechnology, dielectrophoresis, DNA, isotachopheresis, microfluidics, polymerase chain reaction (PCR), RNA, virus.

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