

Rapid Defense Against the Next-Generation Biothreat



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Bioengineered and emerging pathogens represent a significant threat to human health. The best defense against a rapidly expanding pandemic is to isolate the pathogen quickly from biological samples so that it may be identified, characterized, and have treatments developed against it. The one persistent technology gap in the process of identifying and quantifying the presence of pathogenic agents has been sample handling and preparation that must precede any assay. Also, we need higher-performance, multiplex assays for families of rapidly-mutating RNA viruses.

Project Goals

The objective of this project is to replace burdensome manual techniques for sample handling and preparation with new automated technologies. We will use microfluidics with ultrasonics, and electrokinetics to separate and purify viruses from biological and environmental samples. We will also create less costly, but more general multiplex assays for viruses, using multiplex,

ligation-dependent probe amplification (MLPA).

Relevance to LLNL Mission

Sample preparation is a critical requirement for biological assays and is a major bottleneck in the process of detecting and identifying biological agents, particularly unknown/engineered/emerging pathogens. Viruses are an important category of pathogens because some of its members, such as influenza and smallpox, are extremely infectious and very virulent forms could result in sudden, massive pandemics. Viruses are often difficult to isolate due to their small size (diameter < 200 nm), compared with the bulk of the particles in a sample. Multiplex assays are also a key part of this work. This project strongly aligns with LLNL's missions in national security.

FY2007 Accomplishments and Results

We have created new simulation capabilities, along with experimental validation, including full 3-D models

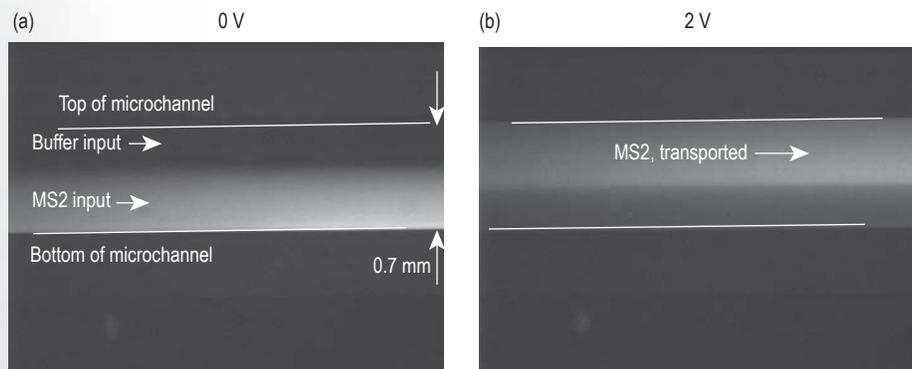


Figure 1. Monochrome photographs of a low-conductivity solution of fluorescently-labeled virus MS2, flowing in a microchannel with top and bottom electrodes (not shown). Two solutions are being introduced from the left of the channel: the upper solution is a clean buffer and the lower solution contains the labeled MS2. In (a) there is no voltage applied and the laminar flow and relatively low diffusivity of the MS2 confine the MS2 to the bottom half of the flow. In (b) with 2 V applied perpendicular to the flow, the MS2 has been almost entirely transported up into the buffer solution, as desired.

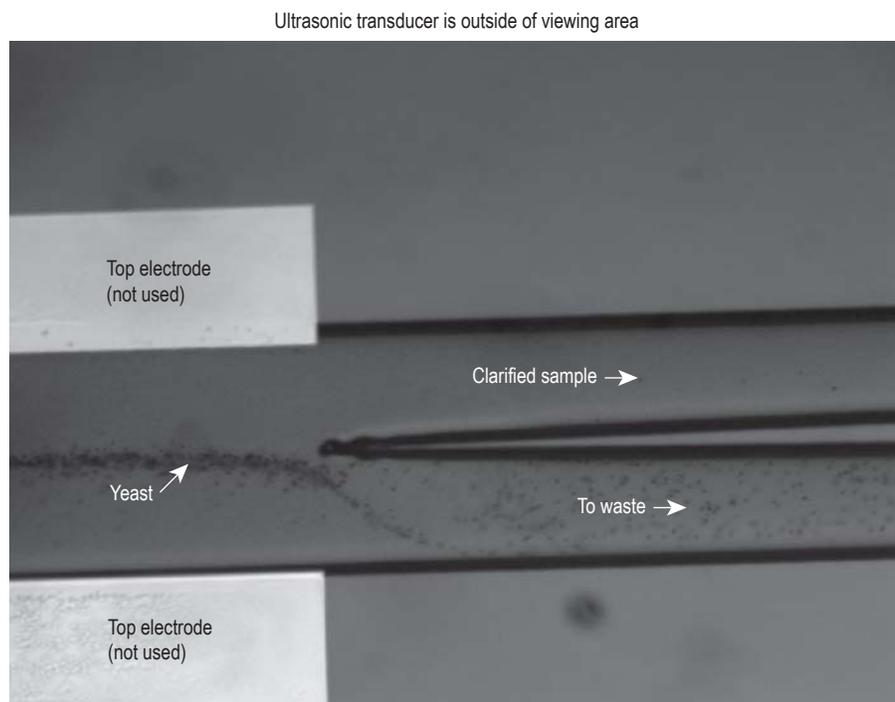


Figure 2. Photograph of yeast flowing in a 0.5-mm microchannel that has an ultrasonic standing wave, perpendicular to the image plane and running along the center of the flow channel. Ultrasonic transducer is outside of viewing area.

with Monte Carlo models for transport of biological particles. In our modeling and experimental research, we have investigated acoustics, electrokinetics (including effects of electroosmosis), electrochemistry, including local pH changes due to wall/electrode processes in our microfluidic systems.

We have established quantitative assays for the titers of MS2, *E. coli*, *S. cerevisiae*, murine herpesvirus, torque teno virus (TTV), Epstein-Barr Virus (EBV) and Adeno virus subgroup C (types 1 and 5). Prior to using human nasopharyngeal samples (NPS), we have used prepared mixtures of viruses both RNA and DNA with bacteria and eukaryotic cells in our research on microfluidic separation techniques. Some examples of these mixtures are the bacteriophage MS2, a RNA virus, with its prokaryotic host bacteria, *E. coli*; and BSL-1, Risk-Group-1 virus such as the murine herpes virus, a DNA virus; and eukaryotic *S. cerevisiae* (Baker's yeast). NPS collection from volunteers has commenced and the samples have been analyzed with quantitative assays

for EBV, Adeno C and TTV as well as spikes of EBV, AdenoC and murine herpes virus.

We designed, fabricated, and tested numerous configurations of microfluidics that demonstrated that we could use ultrasonic standing waves with high-Q at 10- μ l/min flow rates to drive yeast (model organism for human cells) to the energy/pressure-wave nodes and that we could use electrophoresis at 1.4 V or slightly less to transport virus-sized (40-nm-diameter) latex beads. We have conceived of new separation configurations that use continuous flow with dielectrophoretic forces (negative dielectrophoresis). We have established a quantitative flow-cytometer-based assay for the yeast and bacteria with diameters ranging from 0.5 μ m to 10 μ m. We designed a fluidic system that is capable of excluding the host DNA/RNA from well-characterized biological samples consisting of cells, bacteria, and viruses using Isotachophoresis.

Our general research effort has been coordinated with the Viral Identification and Characterization Initiative.

We have initiated an extensive effort to collect published data on the electrophoretic mobilities of DNA, proteins, viruses, bacteria, and other biological particles that we will need to manipulate. These data, coupled with our modeling of transport versus pH and solution conductivity, have driven our designs of microfluidics-based sample processing systems.

We used cyclic voltammetry to characterize the electrode processes, so that we could avoid the extensive electrolysis of water, with its collateral generation of bubbles. We still are addressing the effects of pH changes, when we operate at or slightly above the overpotential.

Non-specific binding of non-ligated, PCR-amplified probes to bead oligos has been a significant and on-going problem for the MLPA assay. This problem has been addressed by optimization of the probe design and MLPA assay parameters (probe concentration, nucleic acid concentration, ligation and hybridization buffer properties, and ligation parameters). None of these modifications resulted in adequate reduction of background signal and therefore the assay probes were redesigned into a Molecular Inversion Probe (MIP) format to allow digestion of unligated probes that may cause a non-specific signal.

A polymerase step was added to the protocol to adjust for the 5-ft truncation of probes that commonly occurs during probe synthesis. A DNA polymerase has been identified for the PCR amplification step that greatly increases the signal-to-noise ratio. The probe hybridization and ligation protocols have been optimized so that the hybridization-ligation reaction time is decreased from 4 h to 90 min. This modified protocol yields a very high signal-to-noise ratio in a 10-plex assay format and is capable of detecting Adenovirus in a clinical nasopharyngeal sample. This is the first time the MIP technology has been modified for use on a bead-based microarray.

Figures 1 and 2 illustrate our accomplishments.