Biological Sample Preparation Infrastructure

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Droplet-based microfluidic systems allow the ability to manipulate independent microdroplets as individual reactors, enabling reliable and quantitative sample processing and detection. Aqueous droplet formation in an oil crossflow at low Reynolds numbers is highly reproducible using extremely simple microfluidic devices.

We are now using an LLNL-created chip (to generate and stop microdroplets) to perform polymerase chain reaction (PCR) on viral nucleic acid samples. PCR is an enzymatic process that amplifies a specific DNA target sequence in response to temperature cycling that leads to denaturation, annealing, and extension. Each microdroplet contains the necessary biochemical constituents for selectively amplifying and fluorescently detecting a specified portion of a sample DNA via PCR.

Isolating the PCR reaction in such small volumes provides an order of magnitude reduction in overall detection time by significantly reducing the total number of cycles required. Dilution of the fluorescently generated signal is largely eliminated in such a small volume, allowing much earlier detection, since the brightness of the small droplet, for a fixed quantity of product, is much greater than the brightness of a larger sample with the same quantity of product. This capability will enable the next generation of biothreat detectors to operate faster with lower reagent consumption, and provide the ability to amplify individual viral or bacterial genomes without interference.

Figure 1. Integrated and Labview-automated microfluidic workstation used to optimize on-chip PCR devices. Shown are the computer, programmed pumps, imaging electronics, and thermal control system hardware.

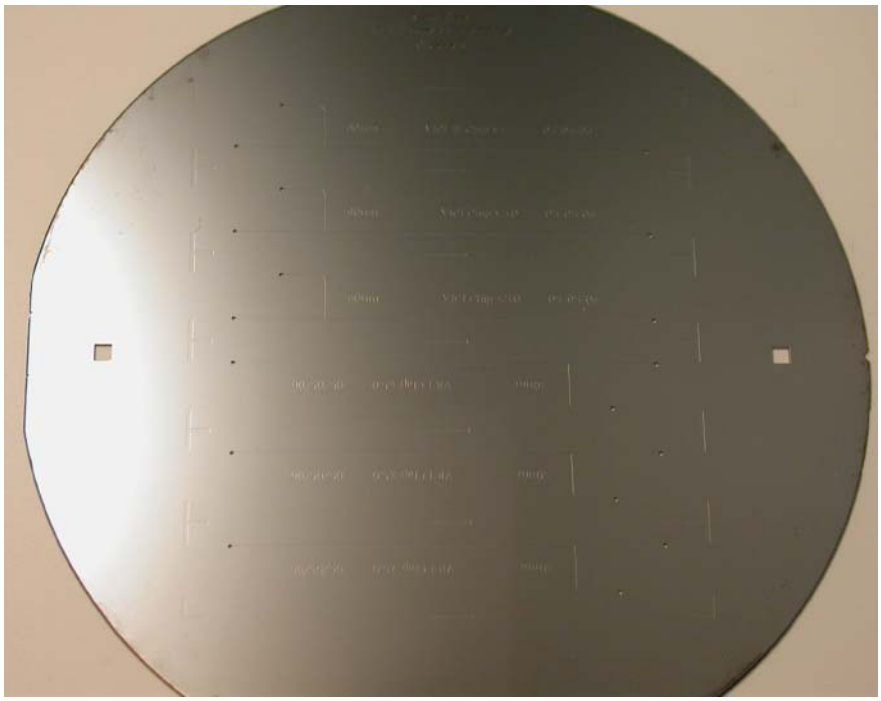

Figure 2. Silicon wafer fabrication process after DRIE, performed at LLNL. The wafer shown awaits anodic bonding to Pyrex, dicing into individual devices, and hydrophobic surface coating.

Project Goals

The result of this work will be the capability to fabricate the microfluidic devices, including an optimized molecular vapor deposition (MVD) coating process for the microfluidic channel surfaces, as well as the implementation of a Labview-automated instrumentation system to operate the microfluidic experiments. Deliverables include:

1. complete benchtop system integration and optimization;
2. automate data acquisition and control system;
3. fabricate microfluidic channels;
4. optimize microfluidic model; and
5. optimize fabrication processes.

Relevance to LLNL Mission

Optimized microfluidic devices and instrumentation systems enable greatly improved chemical and biological detectors for countering emergent chemical and biological threats. The optimization of these technologies advances the core microfabrication competencies at LLNL, while supporting a vital counterterrorism program.

FY2007 Accomplishments and Results

As a result of this work, LLNL now has an integrated and automated benchtop system for conducting microfluidic testing, including on-chip PCR amplification and detection (Fig. 1). This system integrated Watlow PID controllers and power supplies for Peltier Thermoelectric Cooling (TEC), a Coolsnap HQ2 CCD camera for fluorescence imaging (data acquisition), a Redlake high-speed CMOS camera for droplet production dynamics analysis, two programmable KD Scientific infusion syringe pumps, an Excite metal-halide arc-lamp light source, a Nikon TE-2000U fluorescence microscope, a Uniblitz shutter and controller, a cooling fan, and three Valco Instruments multiport valves. Integration and automation allowed repeatable and efficient experiment processing, resulting in more data and faster iteration cycles.

This work also provided an optimized fabrication and device surface coating process for the silicon substrate (Fig. 2). The optimized device used a Deep Reactive Ion Etched (DRIE) 0.5-mm-thick silicon wafer with topside etching for the microchannels and backside etching for the fluidic vias. The device was then anodically bonded to a 0.5-mm Pyrex wafer and diced into individual devices.

Furthermore, device coating optimization was performed, with the results that liquid treatment with SigmaCote was the most cost-efficient method, but molecular vapor deposition (MVD) with FDTS was the most resilient and effective method.

The results of the device optimization are visible in Fig. 3, where a stream of monodisperse droplets with diameters of only 27 µm are visible after droplet generation and stopping on-chip. The droplets are now ready to undergo the PCR amplification and real-time detection process. Due to the optimized channel surface coating, the droplets do not wet the channel walls.

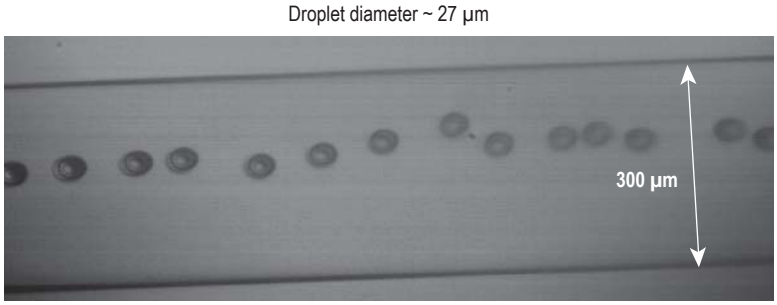

Figure 3. Image of the device in operation showing the generation and trapping of a monodisperse stream of 27-µm droplets. After droplet-stopping the device is thermally cycled to power the PCR amplification process.