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Demonstration of Automated Analysis of Multiple Analytes on an Integrated Digital Microfluidic Platform

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Featured Article: Srinivasan V, Pamula V, Fair R. An integrated digital microfluidic lab-on-a-chip for clinical diagnostics on human physiological fluids, Lab Chip 2004;4:310–15.[2]

Our 2004 article highlighted here was the first report of a droplet-based microfluidic device that integrated on a monolithic platform all the functions for analyzing human physiological fluids, including sample injection, on-chip reservoirs, droplet formation structures, fluidic transport, mixing of reagent and sample droplets, and optical detection. The device could also be run by a computer. Thus, the device proved the potential for automated analysis of multiple analytes for clinical diagnostics using a programmable lab-on-a-chip.

The use of microfluidic lab-on-a-chip technology for clinical applications in the early 2000 timeframe was almost exclusively based on continuous fluid flow in microchannels. Fluid pumping based on electrokinetic phenomena (electrophoretic separation and electroosmotics), external pressure sources, centrifugal effects, and passive capillary flow were being investigated. However, continuous-flow based devices offered very little flexibility in terms of scalability, reconfigurability, and suitability for use with a variety of liquids. For instance, physiological liquids with high ionic strength, such as blood and urine, could not be pumped using electrokinetic effects due to excessive Joule heating. In addition, continuous flow systems relied on fixed channels through which liquids would flow in a single direction unless routed into intersecting flow channels using valves. However, in 2000 valves were big structures that consumed watts of power and were leaky. Both fixed channels and valves easily became fouled with physiological liquids, and thus needed to be cleaned or disposed of after use. And each application required a specifically designed device. As a result, most commercial microfluidic devices used in clinical diagnostics were mostly simple, valve-less, disposable, and had at most one or two fixed channels. In addition, the disposable devices were inserted into an expensive cabinet with regent bottles, tubes, and control electronics.

Other than ink jet cartridges and diagnostic arrays, a surprisingly small number of practical commercial lab-on-a-chip microfluidic devices had been successfully introduced. We began investigating microfluidic chips that could be designed with high integration density and that were reusable, trying to mimic the success of microelectronic chips. Based upon available technology we were completely stymied. As we looked for similarities between electronic chips and microfluidic chips, we came to the conclusion that we needed a "digital" microfluidics technology, one where we could manipulate discrete boluses of liquid rather than continuous streams. However, the liquid bolus approach flew in the face of conventional wisdom at the time, which believed that the traditional bolus approach was too complicated, inefficient, laborious, and didn't scale. Rather, it was believed that biochemical processing should be synchronized by a continuous-flow approach in which the biomedical fluids continuously flow through channels, reaction sites, processing sites, and measurement sites *(1)*.

We started down the continuous flow path and burned a year on it. After the first student dropped out and after suffering from chip architectural ideas that couldn't be made to work, we determined that the maligned bolus approach was actually a better basis for programmable microfluidics. We simply lacked a way to implement it!

Thus, the research problem was framed. We looked at how we could mimic the bolus approach used in a chemistry laboratory, where all resources (e.g., test tubes, centrifuges) could be used in any order, cleaned, and reused in multiple applications. Since people and test tubes don't scale to the chip level, you need to be able to actuate discrete volumes of liquids (boluses) and route them through shared resources in a reconfigurable architecture. In computer chips, discrete packets of charge are switched and routed with transistors. But in 2000 there was no microfluidic equivalent to a transistor that could switch and route discrete volumes of liquids.

The idea for a microfluidic transistor came from a Russian instrument maker who had been a post doc in Cell Biology at Duke. He had a notion of how to move droplets (boluses) on a hydrophobic surface under voltage control. A colleague in Biology at Duke referred Alex Shenderov to me as someone who knew about liquid

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[2] This paper has been cited 546 times since publication.
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actuation using an electrowetting-on-dielectric method and who had filed a patent application on the topic. We had never heard of electrowetting.

The most knowledgeable treatises at the time described electrowetting on dielectric as "troublesome," since subtle uncontrolled changes in the liquid/surface interface made this actuation principle difficult to control and too dependent on the liquid's properties. Also, most of the work in the field was 10–20 years old. Optimistic with our knowledge of modern microfabrication and how to control surfaces, we embarked on building electrowetting-based chips. I hired Alex as a consultant in 1999, and together he and my student, Michael Pollack, built the first working electrowetting-on-dielectric microfluidic devices in early 2000 *(2)*. The videos of water droplets moving under voltage control on a hydrophobic surface were captivating. Defense Advanced Research Projects Agency (DARPA) called them the "dancing droplets," which even got the attention of DARPA's Director. In early 2000 we put up a web site showing the dancing droplets and had tens of thousands of hits in the first days *(3)*. Digital microfluidics had been demonstrated. Within a year, over 35 laboratories worldwide were also working on electrowetting microfluidics.

Today, electrowetting-on-dielectric has reached the commercial stage with Illumina's NeoPrep library prep system *(4)*, Baebies newborn screening and pediatric testing products *(5)*, and GenMark's ePlex System for nucleic acid extraction and detection *(6)*. My coauthors of the featured article cited at the outset, who are former students in my laboratory at Duke, have greatly contributed to the technological development of these products.

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