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Raymond Mariella, Jr

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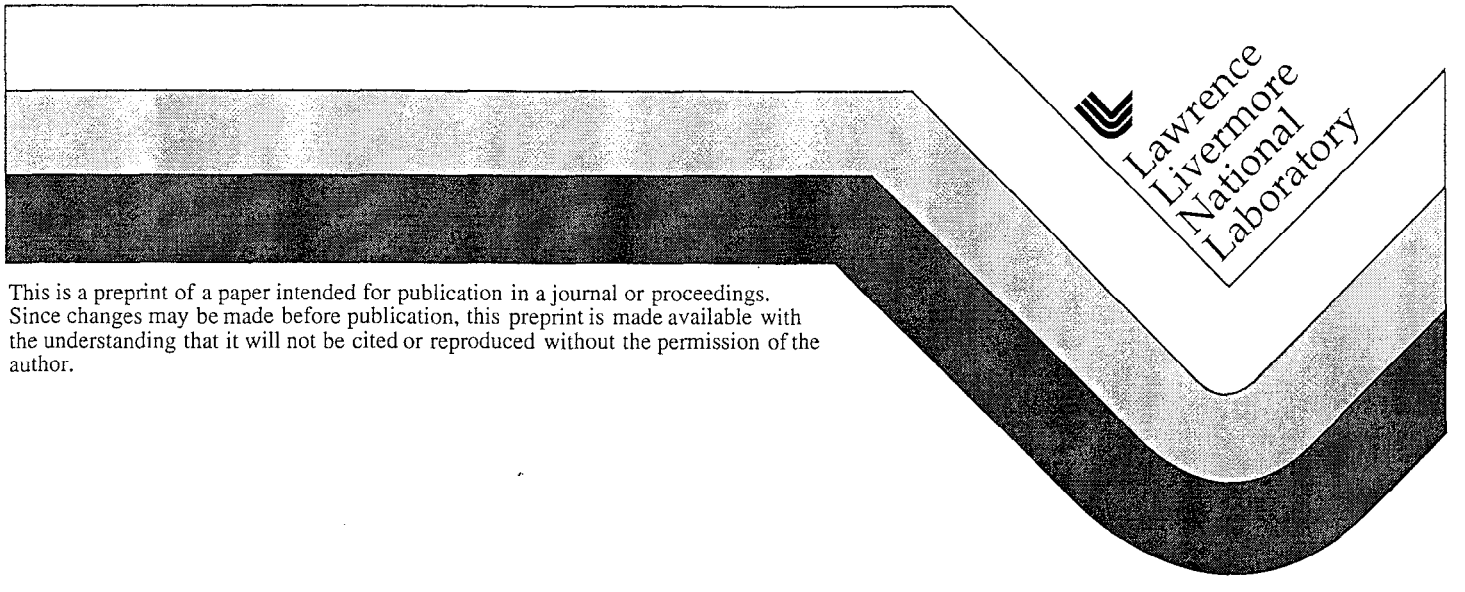

InstrumentationforBiomedicalandEnvironmental Applications based on Microtechnology - Lessons Learned

Raymond Mariella Jr.
Lawrence Livermore National Laboratory, PO Box 808, Mail Stop L-222
Livermore, CA 94551

ABSTRACT

Over the last ten years, LLNL has been developing Microtechnology for instrumentation with applications in the biosciences and environment. In order to build and field high-performance instruments, we have often had to alter our original premises and assumptions, significantly. This meant that we were forced to abandon materials and dimensions that were appealing to us when we began the R&D. Examples include our work on silicon-based electrophoresis systems, etched-fluidics for sample/sheath flow nozzles in flow cytometers, and polymerase-chain-reaction thermal-cycling chambers based on silicon-nitride. This presentation will discuss these and our work on other devices and instruments.

Keywords: Microtechnology, Instrumentation

1. INTRODUCTION

Over the last ten years, LLNL has been developing Microtechnology for instrumentation with applications in the biology, medicine, and the environment. These instruments typically perform analytical chemistry or biomedical assays, where the effortat LLNL has been to improve the performancebeyond the existing state of the art for one or more of: portability, sensitivity, reliability, throughput, etc. Oftentimes, but not always, the improved performancehas been enabled by components or subsystems that were based on inventions in the field of microtechnology. This has, then, required the inventors, typically engineers, to work with the operators of the existing state-of-the-artinstrumentation in order to test and refine the new instrument. It is at this juncture that recently-invented components or subsystems can be forced to undergo major changes, including the abandonment of materials and dimensions that were originally appealing from an engineering viewpoint.

2. EXAMPLES OF INSTRUMENTATION

2.1. Etched-microchannel Electrophoresis

In 1991, Microtechnology researchers at LLNL began investigating the use of etched microchannels for use as a form of capillary in which to separate DNA fragments using electrophoresis. The first substrate that we chose was silicon, since LLNL had an active program ongoing in the microfabrication of structures using silicon wafers as substrates. Initial results were encouraging, but it became clear that silicon was not the correct choice for an electrophoresis plate because the typical oxide that we could produce inside the channel was insufficient to hold offthe thousands of volts that were preferred for use in electrophoresis. Therefore, we abandoned silicon in favor of 10-cm-long Borofloat® glass plates. The use of glass allowed our use of the necessary voltages, but we desired to fabricate longer channels in which to perform the electrophoretic separations. Because it is much easier to microfabricatechannels on 4-inch glass plates rather than on larger plates, we first attempted to fabricate looped or serpentine microchannels in order to achieve the increased channel lengths. A glass plate that we fabricated[1] with several such channels is shown in Figure 1. Although this was conceptually satisfying, since microlithography permitted us to design and fabricate longer capillaries, we determined

experimentally that the curves in the microchannels degraded the performanceof the electrophoretic separations, and we were forced to use longer plates. A photograph of our instrument, running with a 50-cm long glass plate, is shown in Figure 2. Therefore, in the process of making the microchannel electrophoresis system work, today with 384 channels per plate[2], we had to abandon both our favorite substrate material and size.

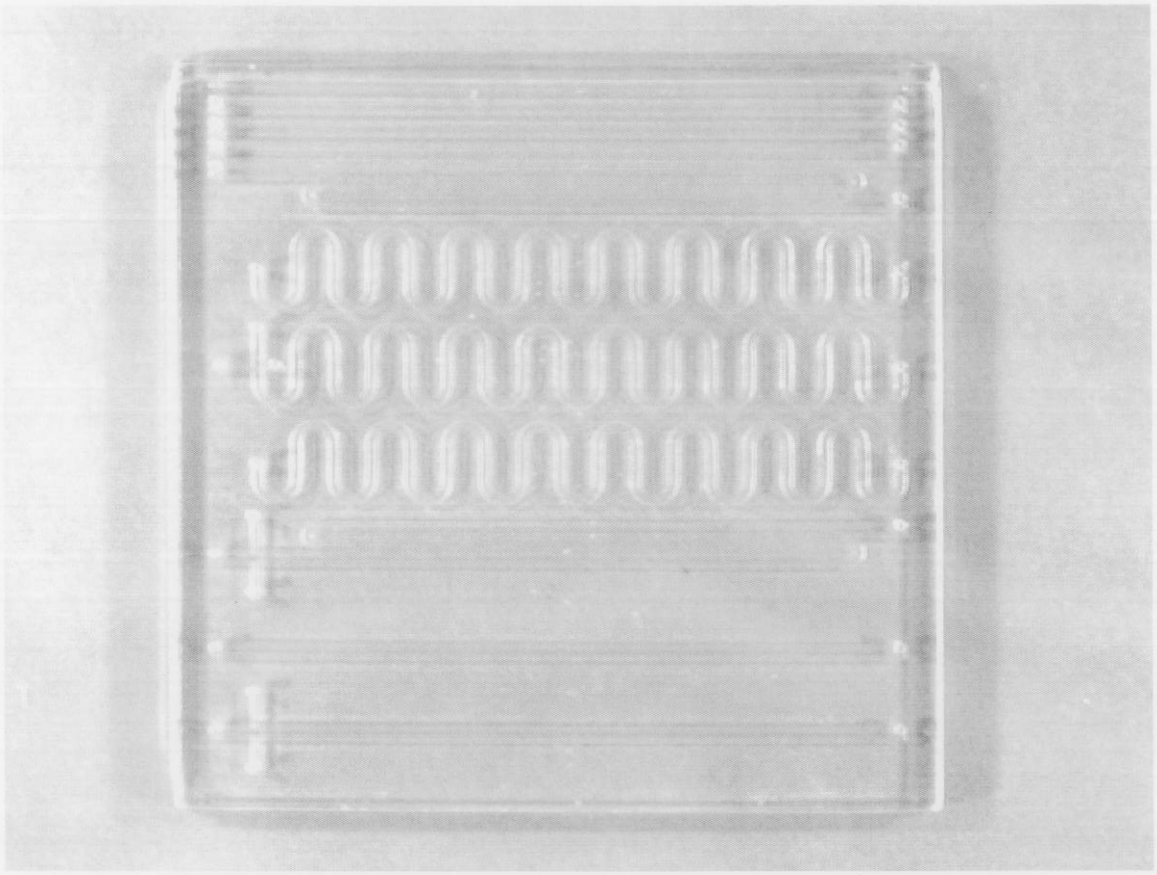

Figure 1. Photograph of bonded 10-cm glass plates with one plate having been microfabricated with electrophoresis channels.

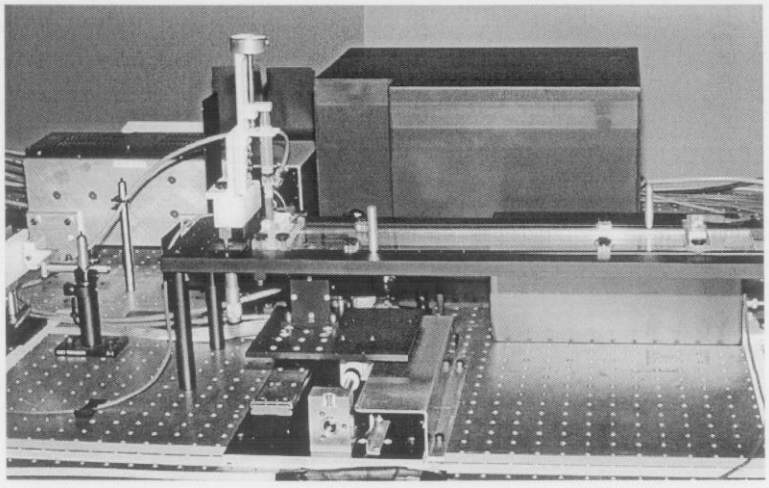

Figure 2. Photograph of the LLNL DNA electrophoresis system with 50-cm-long bonded glass plates.

2.2. Flow cytometry

In 1993, researchers at LLNL invented a new method to collect the scattered light in flow cytometry using the flow stream itself as an optical waveguide[3]. Using simultaneous measurements, we observed that the coefficient of variation from this technique was superior to that from the traditional system with an external lens[4]. The data shown in Figure 3 are these measurements of perpendicularly-scattered light ("side scatter") for a solution containing a mixture of three sizes of polystyrene spherical beads. The vertical and horizontal axes are the same linear scale. The height and width of each bead grouping is indicative of the coefficient of variation for light collection via the external lens and via the flow-stream waveguide ("FSW"), respectively. The origin of this improvement is that an external imaging lens has a light-collection efficiency that depends strongly upon the exact 3-dimensional position of the latex bead, while the FSW has no comparable depth-of-focus issues.

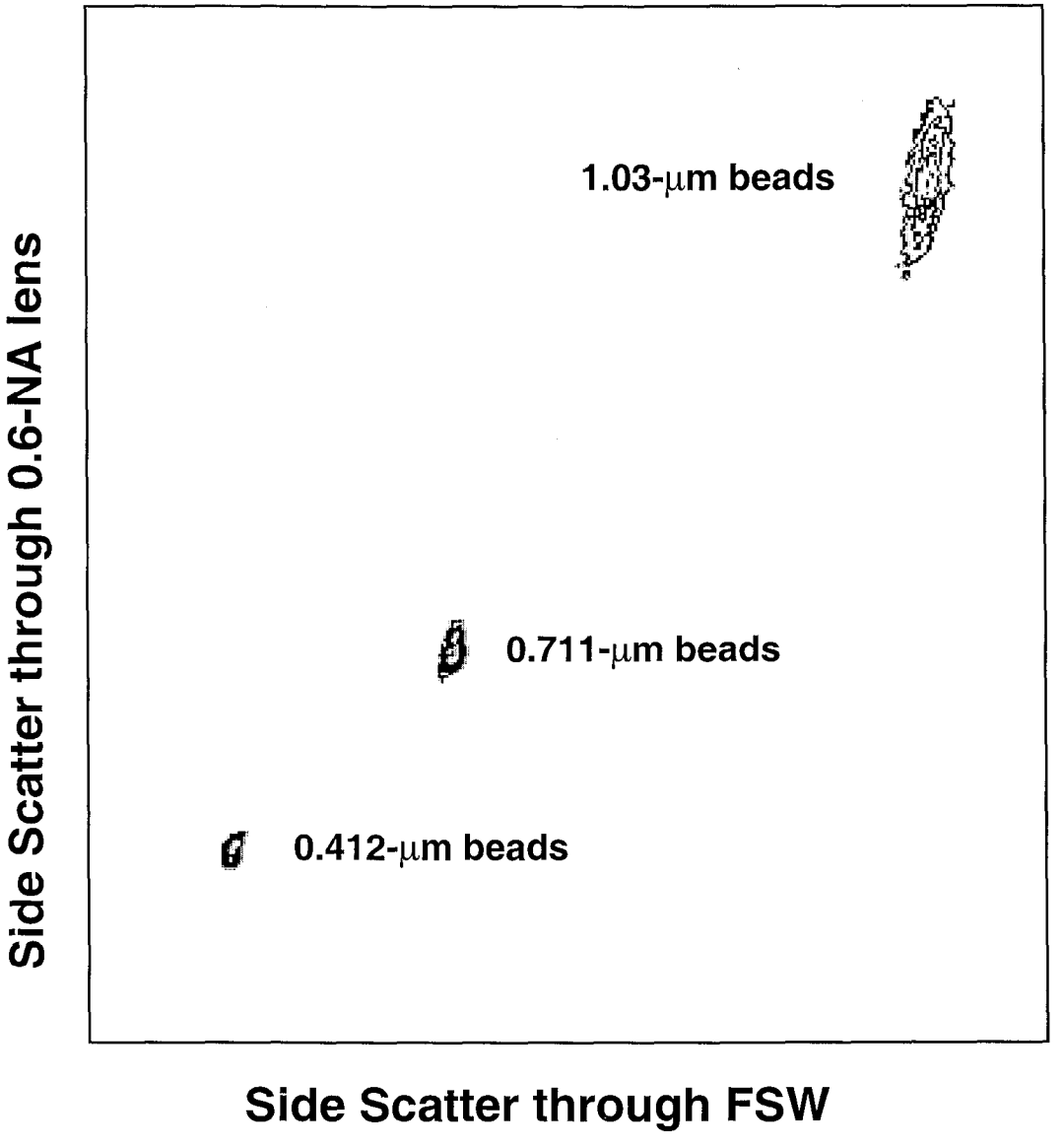

Figure 3. Cross-correlating histograms of simple side scatter from a solution containing three sizes of polystyrene beads, measured using both a conventional external high-numerical-aperture lens and the flow-stream waveguide.

Encouraged by these results, we built a luggable flow cytometer[5], pictured in Figure 4, that incorporated the FSW technique for light collection, and this instrument has performed well in the field[6] and has recently demonstrated the capability to resolve the side scattering from 0.093-μm diameter polystyrene beads[7].

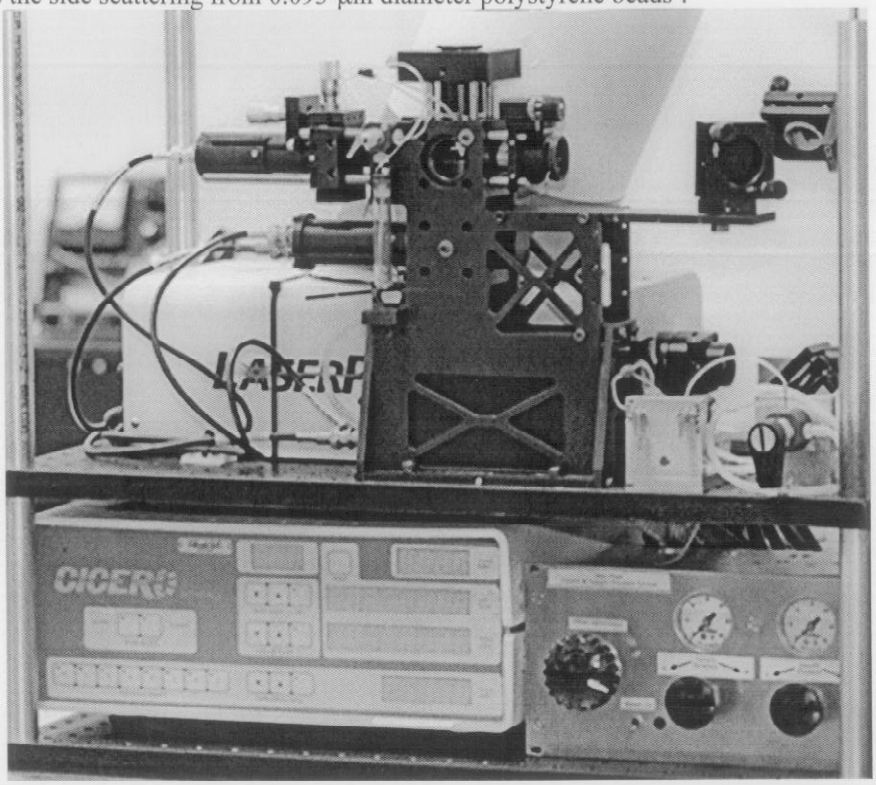

Figure 4. Photograph of "miniFlo", a luggable flow cytometer based upon the flow-stream-waveguide technique to capture side scatter and fluorescence.

Because the data shown in Figure 3, we fabricated a sheath-flow/sample injector from three etched and bonded silicon wafers, as shown in Figure 5, along with its plastic housing for fluidic connections[8]. Such a design produced a 100-μm diameter flow stream that appeared smooth and stable to the eye, but internally it lacked true 3-dimensional hydrodynamic focusing, and possessed internal surfaces that did not produce a single, well-defined path for sample particles to traverse. As a result, the sample particles traveled on a variety of widely-dispersed paths within the silicon component and, upon exiting, passed through correspondingly widely-dispersed points in the interrogating laser light beam. The 2-dimensional histogram "dot plot" shown in Figure 6 would, ideally, have been a single spot on the graph. The large blob near the origin indicates that a large fraction of the beads nearly missed the 25-μm-diameter laser beam, and the multiple series of arms extending from the origin show other unsatisfactory performance of this first unit, too. Thus, although the miniFlo does use a tiny, conically-polished fiber optic to convey the light, captured within the FSW, to the optical detectors, the traditional silicon-based microfabrication technologies did not contribute to its performance. We understand that Micronics, Inc., of Redmond, WA, has solved the problem of producing true 3-D hydrodynamic focusing by using multiple thin layers of bonded plastics to fabricate a sheath-flow/sample injector for analysis by flow cytometry[9].

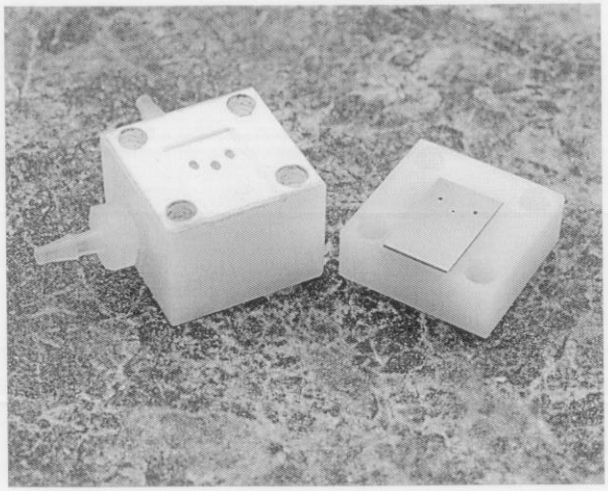

Figure 5. Photograph of a silicon three-bonded-wafer sheath-flow/sample injector with machined plastic block for fluidic connections.

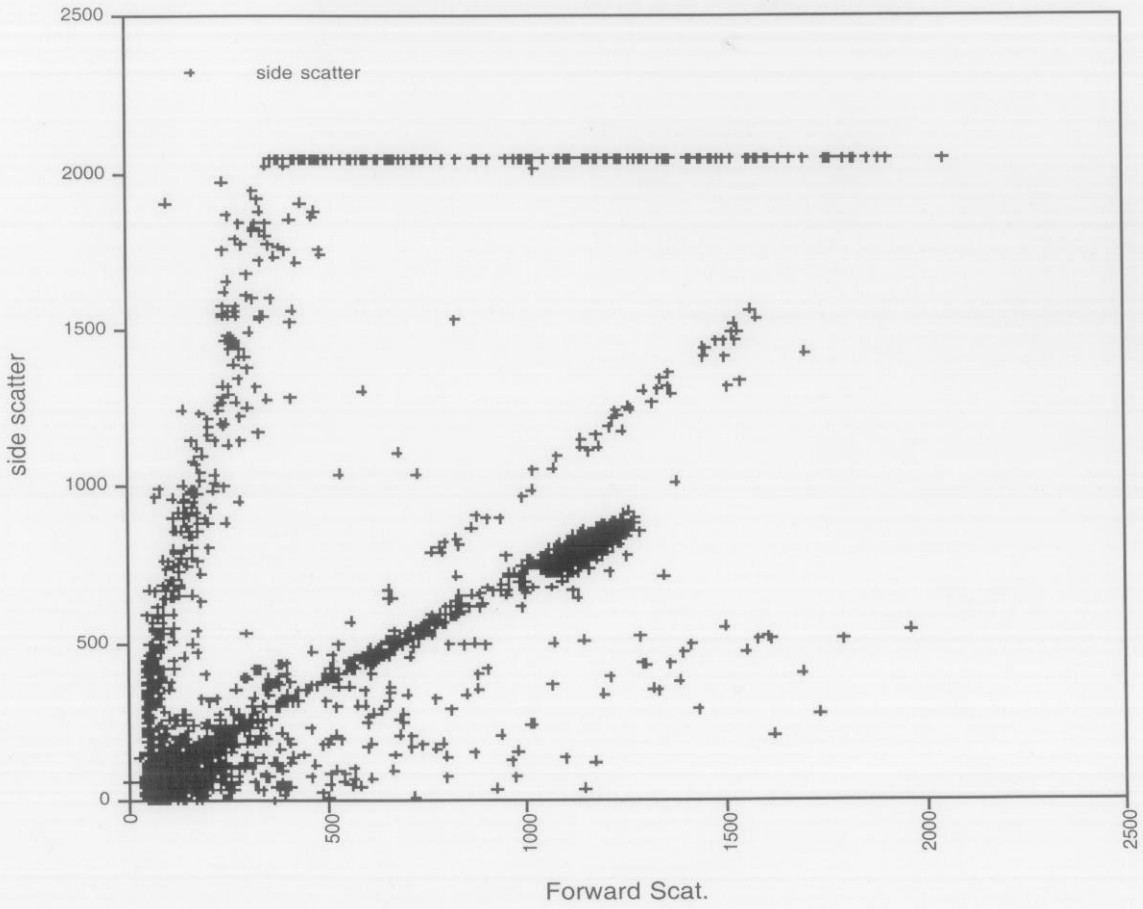

Figure 6. 2-D histogram plotting forward scattering versus side scattering, collected using the sheath-flow/sample injector pictured in Figure 5. Data were collected on a diode-laser-based version of miniFlo (not pictured), using a solution containing 2-μm polystyrene beads.

2.3. Polymerase Chain Reaction

A team of LLNL researchers has designed and built the world's first battery-powered, real-time PCR instrument[10]. The heart of this instrument is silicon sleeve thermal cycling chamber in which the reaction is carried out[11]. This unit is pictured in use by Dr. Phillip Belgrader at Dugway Proving Grounds in Figure 7. A recent 10-chamber version of this instrument has demonstrated a total time of only 7 minutes to perform automatic detection of a vegetative bacterium[12].

Figure 7. Photograph of the battery-powered, real-time PCR instrument in use at Dugway Proving Grounds in 1997. This instrument has control software that automatically calls a positive if DNA from the target organism is amplified during the thermal cycling. During this exercise, the typical time to detect was 20 minutes or less.

Prior to building the chamber with a silicon sleeve that is used in the instrument pictured in Figure 8, we fabricated our first thermal cycling chambers using thin-film heaters deposited onto thin insulating films of silicon nitride, supported by a frame of silicon. Such a design was conceptually appealing, from the standpoint of the efficient use of power to the heating elements – by isolating the heater from the supporting silicon, no power was wasted. Unfortunately, these chambers did not perform PCR well. The reason was that this enzyme-catalyzed chemical reaction requires highly uniform temperatures and accurate control of time spent at those temperatures in order to achieve a high level of reaction yield. As the photograph of temperature versus position shows in Figure 9, the temperature across the chamber exhibited unacceptably large variations.

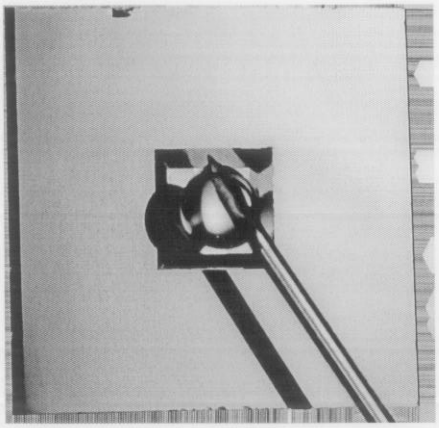

Figure 8. Photograph of an early implementation of a sleeve-type thermal cycling chamber, which used thin film heaters on thin-film insulating windows. The tip of a hypodermic needle dispensing a drop of water is also shown, for scale.

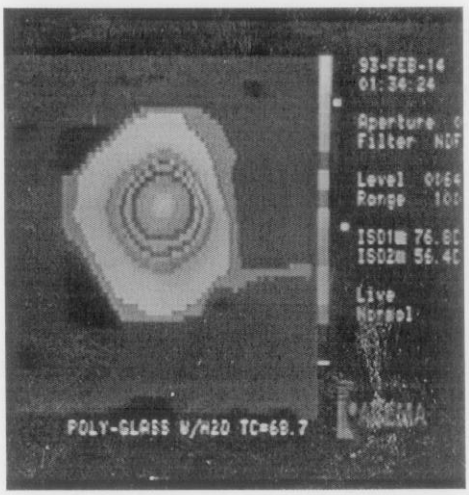

Figure 9. 2-dimensional plot of temperature for a chamber of the type shown in Figure 8. Temperature variations could exceed 15°C across the heated fluid.

3. SUMMARY

In summary, the use of microtechnology has allowed us to increase the performance of instruments, particularly the performance of portable instruments. However, in order to achieve an optimal implementation of microfabricated elements or devices within these instruments, we have typically faced the necessity of abandoning materials and configurations that appealed when we began the efforts, based on the function that the instrument must perform. That is, in order to achieve overall optimization of the full system, the energy efficiency, or size, or convenience of fabrication for an individual element had to be compromised.

ACKNOWLEDGMENTS

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