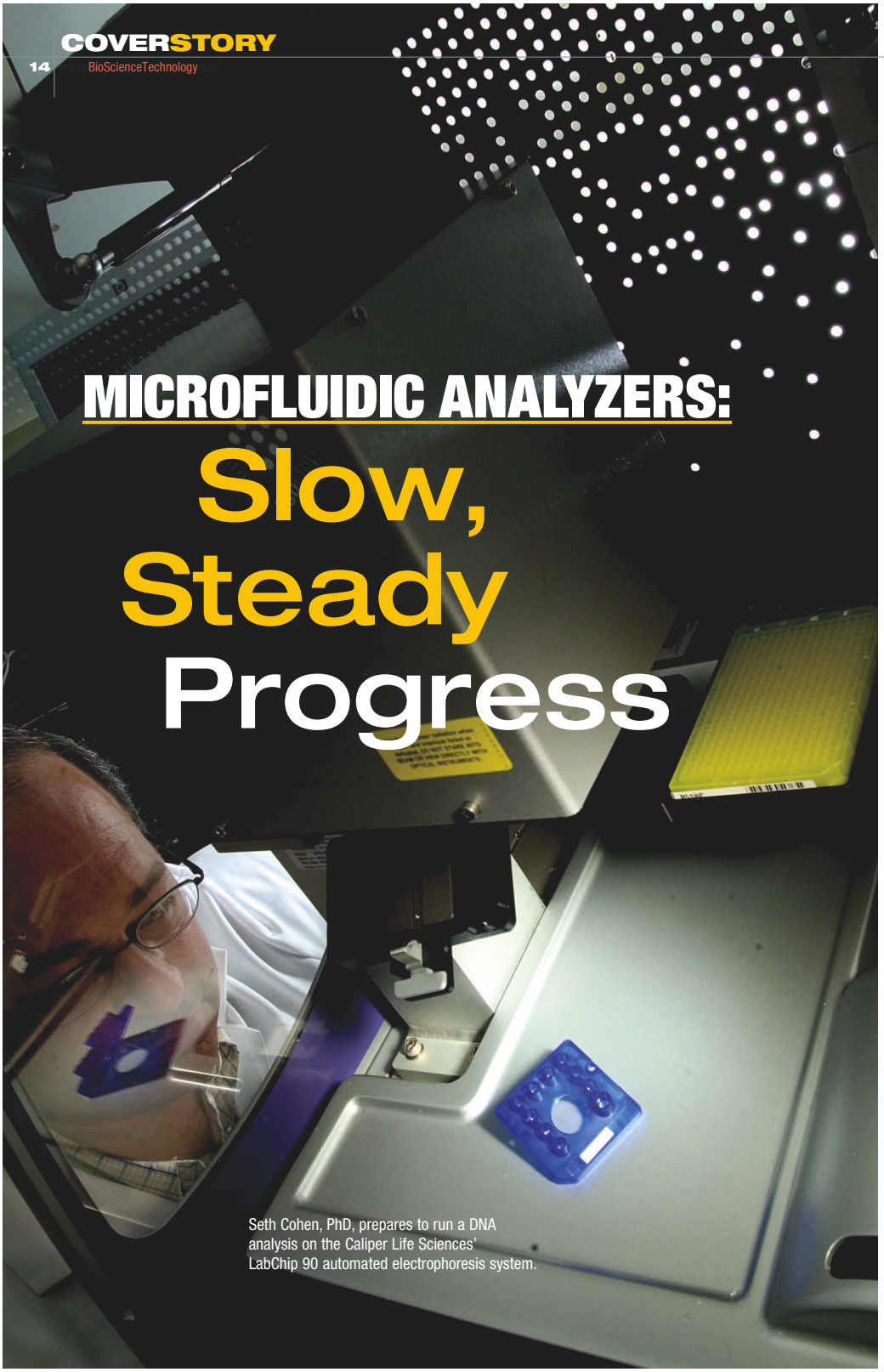

MICROFLUIDIC ANALYZERS: Slow, Steady Progress

Seth Cohen, PhD, prepares to run a DNA analysis on the Caliper Life Sciences' LabChip 90 automated electrophoresis system.

By Angelo DePalma

Once a sidebar of the nanotechnology revolution, microfluidics-based analysis chips have come of age through periods of hype, apathy and finally, commercialization. Originally called "laboratory-on-a-chip" in the United States and "micrototal analytical systems" in Europe, microfluidic chips have progressed rapidly through breakthroughs in materials science, micro/nanofabrication, and microelectronics, but nowhere nearly as rapidly as semiconductors – no Moore's law here.

Limitations in fabrication and application that perplexed early microfluidics researchers still thwart today's efforts. Interfacing chips to anything remotely human-sized is problematic, and efforts to integrate fully-functional sample prep, detection, and microfluidics on one substrate have been largely unsuccessful. And because of the tiny dimensions of microfluidic channels and components, trouble-shooting problems during design (not to mention use) is much more difficult than, say, with macro-scale liquid chromatography.

Microfluidics made great strides between the late 1980s and late 1990s, particularly in the materials science of chip substrates and in techniques for moving fluids through microchannels. With these now routine, today's investigations focus on integration, particularly with biosensors, to create chips with greater function and versatility.

Chia Chang, PhD (Figure 1), based at the University of Notre Dame Notre Dame, has been collaborating with microbiologists and immunoassay experts to increase the speed and sensitivity with which his microfluidic devices detect cells and biomolecules. At the same time, Chang is perfecting alternating current (AC) electrokinetics, which he believes will be the platform of choice for driving fluids through medical and research microfluidic analyzers. (Figure 2). Conventional direct current electro-

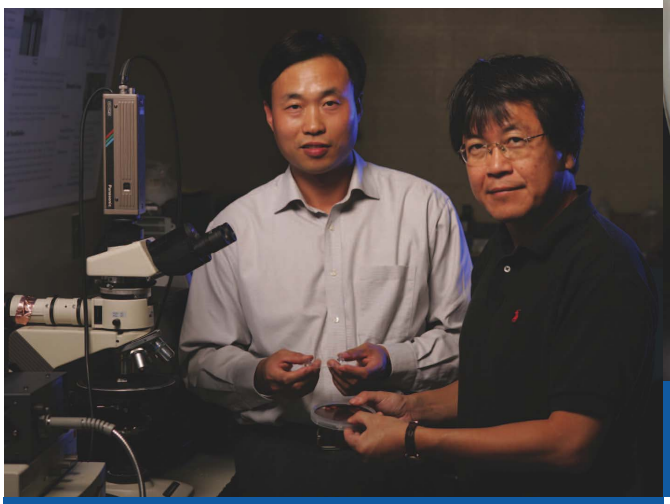

Figure 1. Center for Microfluidics and Medical Diagnostics Director Dr. Chia Chang (right) and Center research professor Dr. Zilin Chen have developed high-pressure, low power pumps for controlled drug delivery through the skin. The patent-pending silica monolith that enables controlled pumping of insulin and other drugs at pressures of up to 4 atmospheres and flow rates as low as 1 nanoliter/minute can also be functionalized for capillary electrochromatographic separations, making it a valuable tool for micro-Total Analysis Systems and mass spectrometers.

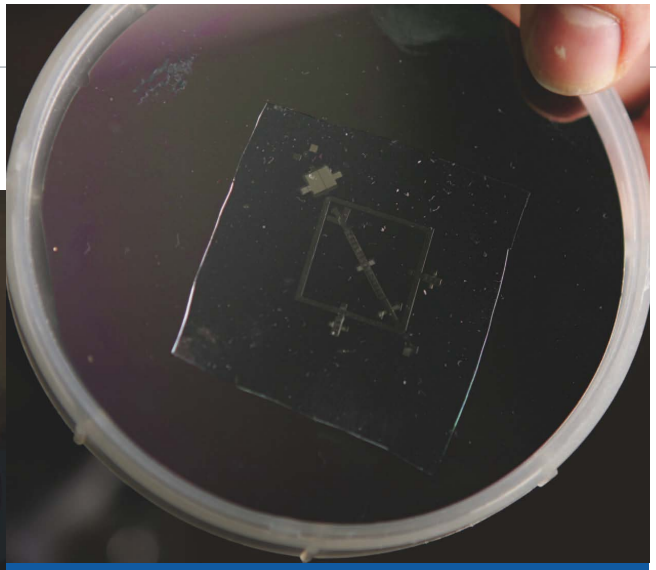

Figure 2. The medical diagnostics of the future, such as this thumbnail sized prototype developed at the University of Notre Dame's Center for Microfluidics and Medical Diagnostics, transform weeks of expensive laboratory testing into seconds of automated analysis.

kinetics, microfluidics' original driving mechanism, generates bubbles and induces chemical reactions at the electrodes, limiting the current that may be applied. Plus DC electrodes must be placed into reservoirs, not directly into channels where they're needed most for precise flow control.

Three ingredients

Success in microfluidics depends on alliances, technology, and applications, ingredients which are related, says Andrea Chow, PhD, VP of Microfluidics R&D at Caliper Life Sciences (Hopkinton, MA). "In forming alliances, we would identify all possible applications for a given level of complexity. By looking long and hard at the low-lying fruit, we find many applications that offer high value but don't require complex integration, for example mechanical valves or MEMS."

The lowest-lying "fruit" for microfluidics has traditionally been electrophoresis for proteins and genes, which can often replace polyacrylamide gels. A bit higher up the food chain are chips that perform enzymatic and cell-based assays, useful in drug discovery. Up a bit higher are products that integrate gene identification with sample prep, for example chip-based polymerase chain reaction. Such automated and semi-automated microfluidic chips have become popular in early-stage pharmaceutical research because of their high reproducibility and low consumption of sample and reagents. (Figure 3)

Caliper's (Hopkinton, MA) business model, which other firms have independently adopted, views the chip as a consumable that interfaces with a much more expensive instrument holding electronics and optics. Chips that perform a day's worth of experiments cost about $5 each; those for high-throughput applications cost hundreds to thousands of dollars but are expected to work reproducibly for hundreds or thousands of cycles. On a per-analysis basis, when time and reagent costs are accounted for, chips are comparable with standard laboratory analysis costs. Moreover, mass-production of specific chip designs drives down their cost substantially.

Caliper's flagship products are the LabChip 3000 drug discovery system whose microfluidic components analyze up to 100,000 samples, and the LabChip 90 electrophoresis system for high-throughput gene and protein analysis. Caliper claims that 75% of the top pharmaceutical and biotech companies have adopted the LabChip 3000 system.

"Systems consisting of consumables bundled with the right instrumentation will provide expert performance to non-experts,"

Agilent's (Palo Alto, CA) leadership in microfluidics predates its formal collaboration with Caliper, which began in 1998 and was terminated about a year ago. Agilent's strength as an instrument company, combined with its experience (through past link to Hewlett Packard) in inkjet cartridges, gives it a unique perspective in a microfluidic marketplace which has not quite shed its nerdy, user-unfriendly reputation. (Inkjet printing is by far the largest microfluidic application in dollar terms about $10 billion per year.)

Agilent has learned from instrumentation's inexorable trend toward greater usability, and applied these lessons to microfluidics. Kevin Killeen, PhD, project manager for microfluidics and biosensors, says Agilent's goal is to take the burden off the end-user. "Systems consisting of consumables bundled with the right instrumentation will provide expert performance to non-experts," he told BioScience Technology. It is time, he says, for microfluidics to deliver usability and reliability, for example nano-electrospray MS without concerns for tips clogging or band broadening. "That's the value of microfluidics to biologists," Killeen adds.

Agilent's three major microfluidic platform products are the Agilent 2100 Bioanalyzer, 5100 Automated Lab-on-a-Chip (which debuted in

November, 2004), and the Agilent HPLC-Chip (March, 2005). The Protein ID HPLC-Chip integrates sample enrichment and separation, while eliminating up to half the fittings typically required in an LC/MS system. According to Agilent this feature reduces leaks and dead volumes. The chip employs a radiofrequency tag for experiment management and compliance.

Drive, she said

Driving and controlling fluids passing through microcapillaries are recurring themes that beg for fresh approaches. At one point researchers believed that micro-actuators might provide the force and control needed for microfluidics, but this idea didn't pan out. Chia Chang at Notre Dame believes that the days of microfluidics employing microelectromechanical systems (MEMS) are behind us. "No MEMS has worked out because these devices break down," he notes.

So for now, at least, non-mechanical fluid drive mechanisms are in. Among the technologies on the horizon: large-scale integration of microfluidic control elements (Stephen Quake's group, Stanford University), and Spinx Technologies' (Geneva, Switzerland) laser-controlled valving. Researchers at Monash University (Melbourne, Australia) are working on plasma polarization methods for pumping, mixing, and concentrating analytes within microchannels. Plasma, which generates "push" without direct contact with working fluids, provides very intense flow, no contamination, and non-sensitivity to electrolyte concentration.

Fluid-driving methods need not be so high-tech. David Juncker, PhD, a post-doc at the Swiss Federal Institute of Technology (Zurich) harnesses simple capillary effects to propel liquids through microchannels. Capillary forces provide "unique advantages," says Juncker: they are self-contained, scalable, free of dead volume, pre-programmable, and allow easy exchange of solutions. Potential applications include immunoassays for drug development or point-of-care diagnostics.

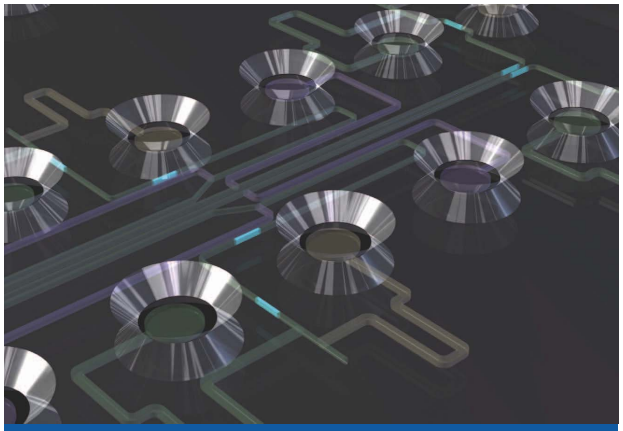

Figure 3. Digital rendition of top surface of a microfluidic chip. Circular wells allow introduction of sample or reagent into the channels of the chip. Pressure or electrokinetic flow inside the channels control movement of fluids and analytes.

Recently, Juncker and co-workers have developed microfluidic probes that enable large protein arrays incorporating gradients and testing of single cells. "This probe combines scanning and microfluidics, thus defining a new experimental space," says Juncker, who envisions applications in cell biology and drug discovery.

Another hurdle that never seems to go away in microfluidics relates to what Agilent's Killeen terms "the point of diminishing returns from smallness." As systems shrink to micro- and nano-scale dimensions, the device-world interface becomes problematic. For microfluidic chips, this means getting material into and out of the channels, and obtaining a reliable signal from nanoliter flows. Some researchers have proposed interfacing microfluidics with "mesofluidics" – add-on devices which are mid-range in miniaturization that concentrate samples for easier detection.

Biologists are also somewhat limited by the geometric constraints of their ubiquitous microplates. Caliper and others are working on systems for loading samples directly from plates to chips, but these manipulations are challenging. "It would be difficult for industry to switch to a completely different platform unless they can demonstrate significant cost savings," observes Po Ki Yuen, Ph.D., Research Manager at Corning (Corning, NY). Yuen's group is involved in MEMS, optics, and microfluidics and is working on tools for label-free detection in drug discovery.

Parallels with chips?

Cali Sartor, Senior Product Manager at Cascade Microtech (Beaverton, OR) sees parallels between microfluidics in the life sciences today and the semiconductor industry of twenty years ago. Computer chip-makers eventually solved problems of integration, design, and increasing complexity, problems microfluidics developers have yet to overcome in any systematic way.

Cascade, whose market was originally test and analysis systems for semiconductor manufacturing, hopes to change that through its L-Series microfluidic characterization and modeling platform. The L-Series consists of a mechanically rigid platform that integrates microscopy, micropositioning, and metrology. Electric fields may be applied anywhere on the chip through a second product, the Microport.

The L-Series addresses a real-life problem faced by every developer of microfluidic devices: Systems that hold the chip and provide "utilities" must be constructed individually, and once the substrate and chip are glued together they are inseparable. "This results in long testing times," says Sartor. "Designers who wish to change a fluid path must start from scratch."

The L-Series test bench simplifies inline testing and "what-if" experimentation since all that is required to test a new design is to swap chips. Current L-Series devices work only in manual mode, one chip at a time, but Cascade is considering a device that handles multiple chips in parallel.

Cascade has two beta customers: Profs. Don DeVoe at the University of Maryland's Maryland Microfluidics Laboratory and Carl Meinhart at the University of California, Santa Barbara.

Another set of tools for microfluidics development come from thinXXS GmbH (Zweibrücken, Germany). The company offers a "construction kit" comprised of a microplate assembly platform and modular slides for mixing, fluid splitting, pumping (including a pump controller and AC/DC converter), connectors and tubing. The modular slides may also be purchased separately.

thinXXS also fabricates custom chips and supplies microfluidic and micro-optic devices, components, and services. Optical detection has been on the microfluidics wish-list for many years. thinXXS is apparently working on this type of integration but could not provide details.

Thomas Stange, PhD, marketing manager at thinXXS, believes that although prototyping is still expensive and risky, microfabrication technology is no longer the principal hurdle to commercializing microfluidic products. For the high-value medical testing and diagnostic markets in which his company operates, regulations and technologic inertia are the primary obstacles.

In June, thinXXS introduced a microchip product, QPlate, which the company claims is the first to incorporate silicon microprocessing, micromolding, and printed circuit board technology. QPlate is part of the QPatch-16 system which measures 16 cellular ion channels in parallel. QPlate was developed with Sophion Bioscience (Ballerup, Denmark).