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Applications of MEMS-Based Biochemical Analytical Instrumentation

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The MicroTechnology Center at Lawrence Livermore National Laboratory is developing a variety of MEMS-Based analytical instrumentation systems in support of programmatic needs, along with numerous external customers. Several of the applications of interest are in the area of biochemical identification and analysis. These applications range from DNA fragment analysis and collection in support of the Human Genome Project, to detection of viruses or biological warfare agents. Each of the applications of interest has focused in micro-machined MEMS technology for reduced cost, higher throughput, and faster results. Development of these analytical instrumentation systems will have long term benefits for the medical community as well. The following describes the technologies several specific applications.

Capillary Electrophoresis for Large DNA Fragment Analysis and Collection

Sequencing of DNA by gel electrophoresis is typically performed in slab gel systems. The advantages of micromachining and MEMS technology for this application are that microchannel arrays can be fabricated enabling multiple analyses to be completed in a single run. Double-stranded (undenatured) DNA fragments in the range of about 100 to 20,000 base pairs (bp) are best fractionated by embedding them in an agarose gel sieving matrix, then applying a constant voltage across the gel. Such electrophoretic gel separations usually take 2-12 hours. Resolution resulting in accurate base calling to greater than 500 DNA base per channel has been obtained (0.2 mm deep by 1 mm wide by 25 cm long microchannels filled with 6% polyacrylamide gel composition). In order to meet the needs of the genomic research community, high throughput separation is required, hence the emphasis to conduct parallel analysis. Present systems analyze 24 channels in parallel, with a 2-4 fold increase in channel density required for next generation systems. Further increases in channel density depend upon novel methods of sample introduction, since the limits of manual techniques, such as pipetting, are presently being approached. Further advances require microfabricated sample injectors capable of delivering nanoliter volumes. Ultimately, systems will include complete sample preparation, injection, separation, and collection capabilities. In order to meet these needs, development in the areas of micropumps, microvalves, reaction chambers, and sample injectors is continuing. Address inquiries to Joe Balch or Courtney Davidson.

Microreactors for PCR

Microfabricated polymerase chain reaction (PCR) systems are being developed using silicon micromachining technology. PCR is a biochemical reaction used to selectively reproduce DNA to allow detection and identification of targeted DNA molecules. The development of enhanced DNA amplification and analysis devices and methods having improved speed and reduced reagent volumes will be critical for the completion of the Human Genome Project and for subsequent utilization of this sequence information. PCR
advanced DNA amplification dramatically, but currently requires long cycling times because of the large thermal mass of typical systems with concomitant slow heating and cooling rates. These problems have been previously addressed by placing samples in small glass capillaries and using heated air to drive the cycling. This approach is limited since the introduction and removal of small sample volumes and the handling of capillaries can be problematic. Microfabricated silicon PCR reactors have the advantage of rapid thermal cycling capabilities (10°C/second heating, 2.5°C/second cooling). This allows for the fabrication of 20-50 µL silicon chambers with integrated heaters. Chambers with integrated heaters are advantageous because they match the size of the thermal cycler to the sample volume, thereby reducing the time for amplification to as low as 30 seconds/cycle. Thus, a typical PCR amplification takes approximately 15 minutes.

In order to realize a single microdevice analytical instrument, a silicon PCR chamber has been directly integrated with a microfabricated capillary electrophoresis (CE) chip fabricated on glass substrates. This instrument will subsequently perform very rapid (<120 seconds) separations of DNA restriction fragment digests and PCR products. This instrument has demonstrated that DNA sequencing fragments can be separated on CE chips with single base resolution. The integration of the amplification and analysis of DNA on-chip will require the development of methods for reliably fabricating multiple components on a single microdevice and controlling the transfer of DNA between systems. For example, electroosmotic flow, electrophoresis and thermocapillary pumping have been used to move and mix solutions on chips. Although many of these individual components have been developed, it is important to demonstrate that two fundamentally different device functions such as PCR and CE can be functionally integrated onto a single device. In this case, the PCR chamber and CE chip were directly linked through a photolithographically fabricated channel filled with hydroxyethylcellululose (HEC) sieving matrix. Electrophoretic injection directly from the PCR chamber through the cross injection channel was used as an "electrophoretic valve" to couple the PCR and CE devices on-chip. The viscous HEC sieving matrix in the interconnecting channels on the CE chip is used to prevent the flow of PCR reagents into the separation channel during thermal cycling, while still enabling the DNA to be electrophoretically driven out of the PCR chamber for CE analysis. These integrated devices offer the unique capability of performing real-time monitoring of PCR amplification. To demonstrate the functionality of this system, a 15 minute PCR amplification of a B-globin target cloned in M13 was immediately followed by high speed CE separation in under 120 seconds, providing a rapid PCR-CE analysis in under 20 minutes. A rapid assay of genomic Salmonella DNA was performed in under 45 minutes, demonstrating that challenging amplifications of diagnostically interesting targets can also be performed.

This work demonstrates the feasibility of performing high speed DNA analyses in microfabricated integrated fluidic systems. Additional applications for this instrument are detection and identification of biological warfare agents, rapid analysis of blood samples, forensics, and food processing.

Address inquiries to Ray Mariella or M. Allen Northrup.

Flow Cytometry

Flow cytometry is a powerful diagnostic tool for monitoring microscopic particles in solution. An important application for such analytical instruments is for the detection of viral pathogens in blood-bank samples, and detection of biological warfare agents extracted from air samples. A microfabricated sheath flow/sample injector in a miniature flow cytometer provides larger signals, easier assembly, reduced noise levels, increased experimental accuracy, and reduced costs. A new physical configuration has been
developed for the detection of right-angle-scattered (RAS) light using a free aqueous stream in flow cytometry which increases the signal-to-noise ratio, narrows the coefficient of variation for uniformly sized latex spheres, and greatly eases alignment requirements. The new flow-stream waveguide (FSW) technique views the scattered light which is trapped within the optical waveguide of the flow stream in air.

In previous flow cytometers, the RAS light has been viewed perpendicularly to the liquid flow, typically using a high-numerical-aperture (NA) microscope objective lens or fiber optic. Some of the difficulties associated with this approach are the very limited depth of focus of high-NA optics, and the necessity to align precisely the exact focal point of the lens with the point where the excitation light source intersects the sample flow stream. This approach makes use of the unconfined aqueous flow stream itself as an optical waveguide. There is no "focal point" for this configuration. Alignment simply requires centering the light source on the flow stream; the liquid optical waveguide is then automatically "aligned". This approach provides robust, stable light collection. For the collection of elastically-scattered light, another advantage accrues; no light is reflected towards the detector, so no obscuration bar is needed for the collection of right-angle-scattered light. Experimental results show the FSW-based scatter detector collects the RAS light from 7 to 10 times more effectively than the conventional high-NA microscope-lens system while providing easier alignment and a smaller coefficient of variation for uniformly-sized spheres. Address inquiries to Ray Mariella.

MicroTools for Intravascular Surgery
LLNL is developing microfabricated structures for use in catheter-based surgery. One example is a coil release mechanism needed for repair of brain aneurysms. Utilizing silicon and other materials, we are building sub-millimeter-sized actuators and actuator-feedback systems for intravascular therapies. We expect to be able to significantly augment the level of sophistication of intravascular tools such as catheters in order to improve minimally-invasive medical procedures, and provide increased cost-effective healthcare in the U.S. Address inquiries to M. Allen Northrup.

Conductive Polymer Microactuators
Thin film conductive polymer/polyimide bimorphic microcantilevers have been actuated 100 μm vertically (out-of-plane) upon the volumetric changes induced by electrochemical doping/undoping of the polymer. The microcantilevers are 200-500 μm in length, 50-100 μm in width, and can be extended from a circularly-curled geometry, and thus generate more than 100 μm displacement. The power required to drive these actuators was typically less than 2 mW with voltages as low as 2-3 volts. Dynamically the microcantilevers have been driven as fast as 1.2 Hz. The polymer was stable for over a week stored in air and light. Residual stress in the polymer film is estimated to be as high as 254 MPa, and actuation stresses are as high as 50 Mpa. Address inquiries to Abe Lee.

Microgripper
A silicon-based microgripper with a large gripping force, a relatively rigid structural body, and flexibility in functional design was developed. The normally-closed actuation is generated by Ni-Ti-Cu shape memory alloy (SMA) films. Heating to the relatively low actuation temperature (70°C) induces stress which deflects each side of the microgripper by up to 55 μm for a total gripping motion of 110 μm. When fully open, the force exerted by the film corresponds to a 13 mN gripping force on the tip of the gripper. Fine alignment, Au-Si eutectic bonding, precision sawing, Ni-Ti-Cu SMA deposition, as well as bulk silicon micromachining are the core of the process. The processes, although not standard, do not involve exotic fabrication equipment. All the individual processes could be streamlined for batch processing and therefore reduce fabrication costs.
For future developments, the outer surfaces of the gripper are being integrated with heaters and strain sensors for remote active heating and feedback control. Another advantage is the possibility to apply alternative actuation mechanisms on the microgripper structure, either hydraulic or thermal bimorphic. Many creative designs of practical microgrippers can be conceived using this basic process. Address inquiries to Abe Lee.

Ni-Ti-Cu Shape Memory Films

Mixed-sputter deposition was proven to be a viable method for producing shape memory Ni-Ti-Cu films, providing the compositional control necessary for optimization of SMA properties, and substrate curvature was shown to be an effective technique for characterizing SMA films. The Ni-Ti-Cu film properties were found to be comparable to the properties of bulk alloys, with transformation temperatures from 20-620°C, 10-130°C hysteresis, and up to 330 MPa recoverable stress. The addition of 7 at.% Cu was shown to make the transformation temperatures less sensitive to titanium content. Films with 51 at.% Ti have transform near body temperature, making them ideally suited for medical applications. Address inquiries to Peter Krulevitch.

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