2.2 Experimental Microfluidic System

FLIGHT DATES:
June 28 - July 2, 2004

PRINCIPAL INVESTIGATORS:
Christopher Culbertson, Kansas State University
Steve Gonda, NASA/Johnson Space Center
John Michael Ramsey, University of North Carolina

GOAL:

The ultimate goal of this project is to integrate microfluidic devices with NASA's space bioreactor systems. In such a system, the microfluidic device would provide realtime feedback control of the bioreactor by monitoring pH, glucose, and lactate levels in the cell media; and would provide an analytical capability to the bioreactor in exterrestrial environments for monitoring bioengineered cell products and health changes in cells due to environmental stressors. Such integrated systems could be used as biosentinels both in space and on planet surfaces.

OBJECTIVE:

To demonstrate the ability of microfabricated devices to repeatedly and reproducibly perform bead cytometry experiments in μ, lunar, martian, and hypergravity (1.8g).

BACKGROUND:

Microfluidic or Lab-on-a-Chip devices are small platforms upon which a complete chemical analysis can be performed. These devices consist of a series of small interconnecting channels (10µm deep and 40 to 200mm wide) etched in glass, or molded in polymers, through which fluids can be moved. The fluids can either be controlled with electric potentials generating electric fields within the channels that move fluid electrokinetically, or by generating pressure differentials using a syringe pump or peristaltic pump that moves the fluid hydrodynamically. Using these two methods of controlling fluid flow it is possible to generate devices that have multiple uses. For example it is possible to use hydrodynamic flow to rapidly move large numbers of cells or large particles, such as beads, through a focusing intersection much faster than if one were to use electrokinetic flow. On the other hand electric fields allow for the separation of differentially charged analytes using capillary electrophoresis, something hydrodynamic flow alone can not accomplish. These devices provide many advantages over conventional bench top scale instrumentation as a result of their ability to integrate sample handling and sample processing operations with analyte detection on a single, monolithic substrate. Such integration allows for the efficient automation of chemical analyses. In addition to automation and integration, microchips have several other inherent advantages over conventional chemical analysis instrumentation. These advantages include (1) the ability to perform faster separations with no loss in separation efficiency, (2) lower reagent and sample consumption (< 1mL/year), (3) less waste production, and (4) the ability to fabricate many parallel systems on the same device. Thus far, their performance has been either equivalent to or better than conventional laboratory devices in all cases investigated. They appear to offer the rare combination of better-faster-cheaper simultaneously, and their ability to manipulate reagents and reaction products "on-chip" suggests the potential to perform virtually any type of "wet-chemical" bench procedure on a microfabricated device.

The advantages described above make these devices especially interesting for use in extraterrestrial environments where small, portable, rugged, and reliable devices capable of sustained remote automated operation will be required.

METHODS AND MATERIALS:

Microfluidic Experiment Description

The portable microfluidic device developed for these tests contained in a Bud box enclosure (NBA10148) which had exterior dimension of approximately 30 cm wide x 18 cm deep x 40 cm high. The microchips, in their custom machined 2-part PMMA holder, were attached to an x-y positioning plate (ST1XY-S; Thor Labs Inc.; Newton, NJ) and positioned above a microscope objective (CD-240-M40X; creative devices, Neshanic Station, NJ). This objective was used to focus the excitation light of a green laser pointer (The Laser Guy, Seabrook, TX). The laser was modified to have a remote switch and power supply (2 D cell batteries). The laser module consisted of an 808 nm laser diode which was shifted to 1064 nm and then frequency doubled to provide a continuous output of 5 mW at 532 nm. The laser beam was reflected off a dichroic mirror (560 DRLP: Omega Optical, Brattleboro, VT) prior to being focused into the microchip channel by the microscope objective. The fluorescence from the labeled amino acids was collected by the same microscope objective, passed through the dichroic mirror, a 1.0 mm pinhole, and a 565 nm longpass filter (565ALP, Omega Optical) prior to being detected at a channel photomultiplier tube (MD972; Perkin Elmer; Fremont, CA). The PMT was powered by a 5 volt power supply. The gain was manually controlled by a potentiometer which had a locking mechanism to prevent accidental change.

The high voltages used for making injections and performing the electrophoretic separations on the microchip were provided by two independent high voltage power supplies cabable of 125 ma outputs at up to 8 kv (C80; Emco High Voltage Corp.). Each high voltage power supply was powered by a 15 VDC source. The HV output was determined by a 0-5 VDC control signal provided by a National Instruments AO card (DAQCard AO-2DC). These power supplies each occupied only 19 cm^3 and weighed 51 g, making them very suitable for portable applications.

A syringe with a locking stop was used to provide pressure to drive the hydrodynamic flow for the bead and cell focusing experiments.

The entire instrument was controlled and data were acquired using in-house written LabVIEW software run off a laptop computer.

Protocol

Orange fluorescent polystyrene microspheres that have a 10µm diameter were diluted into the running buffer to make a bead concentration of $6.5x10^6$ beads/ ml (Molecular Probes, Eugene, OR). The run buffer was composed of 25 mm NaBorate and 0.01% (v/v) Silwet. The Silwet helped prevent aggregation of the particles and particles adsorption to the capillary sidewalls. Sonication of this solution was performed prior to focusing experiments to further reduce bead aggregation.

To perform the bead focusing experiments a 25 mm NaBorate and 0.01% Silwet solution were placed in channels B and C (Figure 1), while the bead solution was placed in channel A. A low pressure was applied to channel D using a syringe pump. This caused fluids from A, B, and C to converge at the cross intersection. The beads were consequently focused and detected using the LIF system described above.
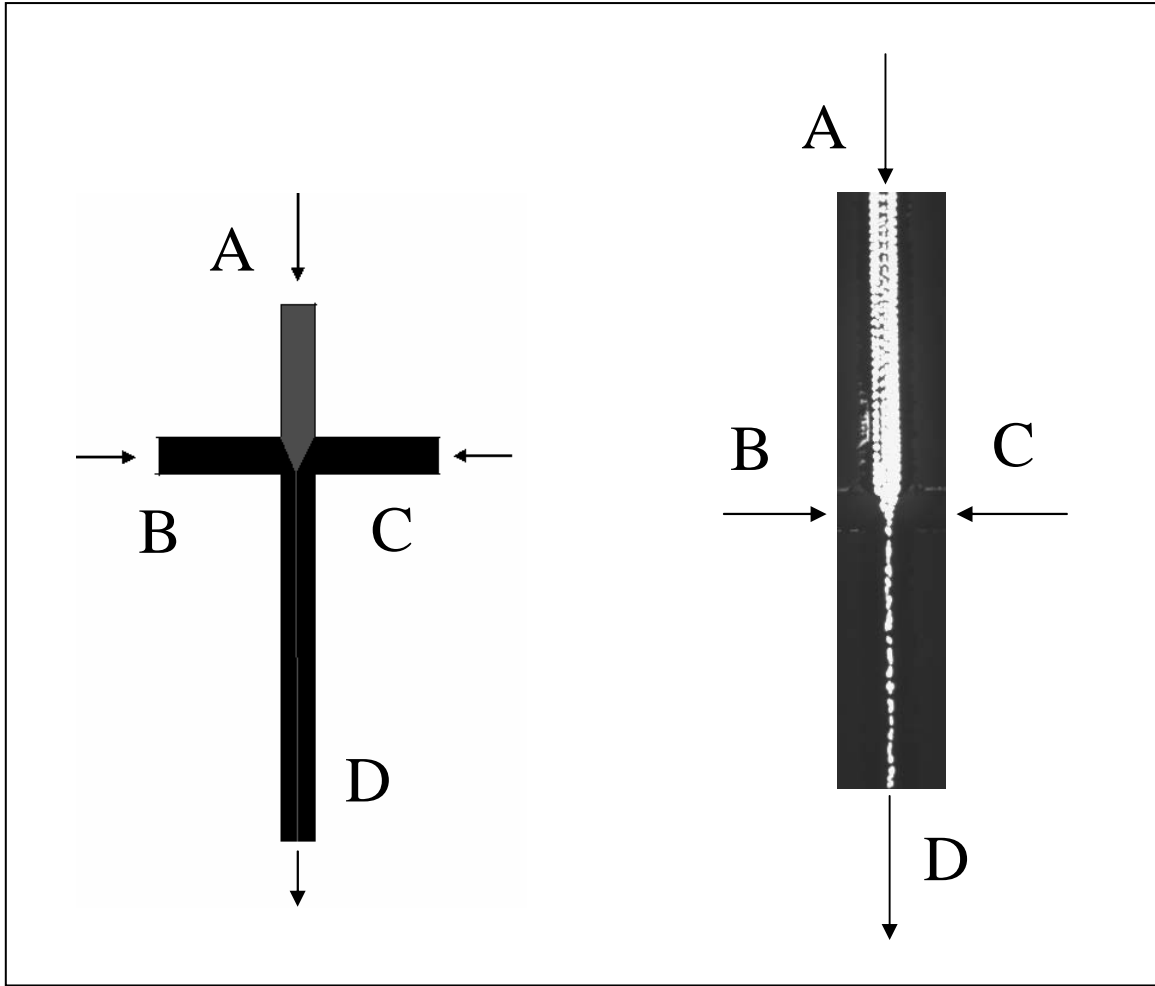
Figure 1: Schematic of bead cytometry experiment, and CCD imaging of 10 micron spheres being focused at the cross intersection.

RESULTS:

Bead cytometry was performed on two of the three flights, June 30th and July 1st. Figure 2 below shows an example of the bead cytometry experiment at 0.026 g. There was no significant difference between the cytometry experiment at 0.026 g, and 1.768 g. The particle velocity was 2.3 meters per second and the volumetric flow rate was 0.036ml/s. To properly sample the rapidly flowing beads, the data acquisition rate used was 20 kHz. The maximum particle count rate at this data acquisition rate is about 3,000/s. Our experiment had a count rate of 200/s with an average intensity of 0.95±0.4. The velocity of the beads, maximum count rate, average and standard deviation of the intensity and flow rate can be seen in Table 1.
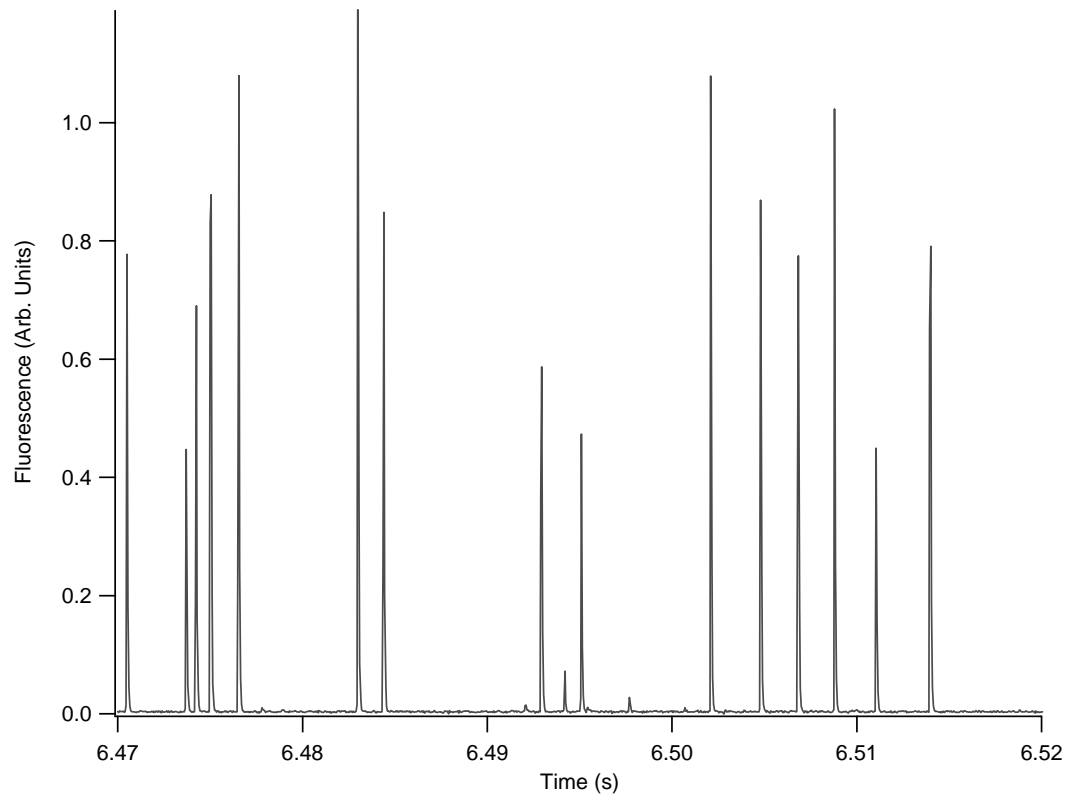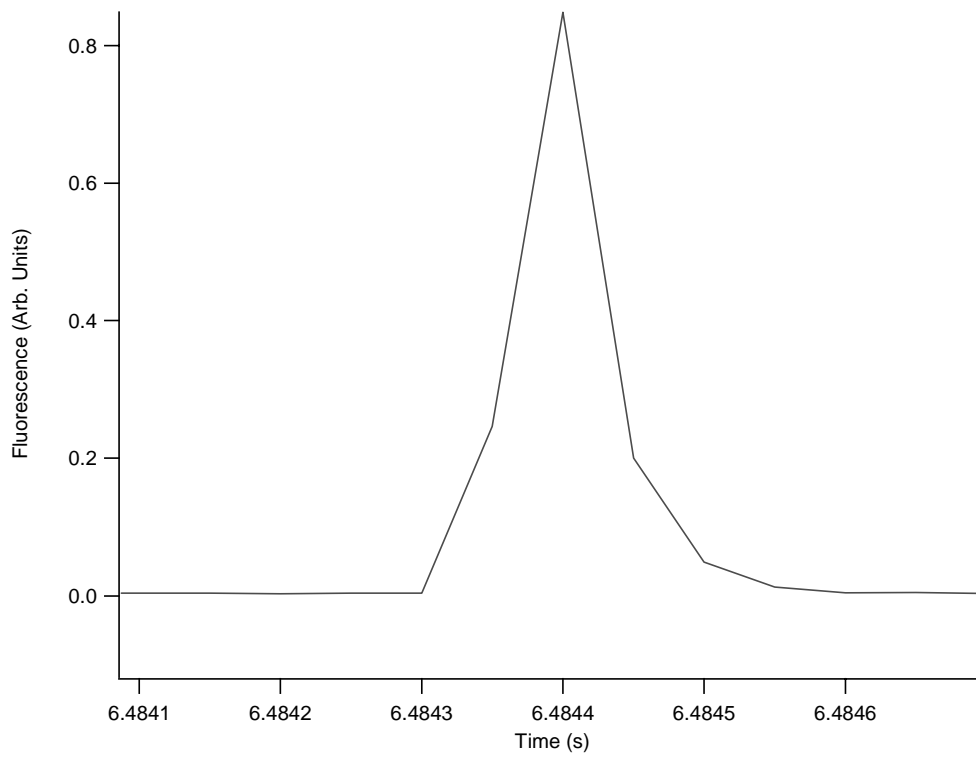
Figure 2: 10 seconds of bead cytometry at 0.026 g

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The final flight on July 2nd was used to attempt to complete an experiment integrating a bioreactor with a microfluidic device to perform an on-line separation of amino acids within the bioreactor. The separation was working on the ground prior to the flight, but unfortunately an electrical failure with the laser catastrophically caused the experiment to fail. This problem was solved by integrating a new 30 mW laser that has a longer lifetime for future flights.

DISCUSSION:

This data shows that it is possible to perform bead cytometry in reduced gravity environments. The throughput of the device is absolutely outstanding with respect to the number of beads it can count per second, although the standard deviation of the mean amplitude of the fluorescent signal is relatively high. This suggests that the focusing at the intersection needs to be tighter to assure that the particles pass through the interrogation beam single file.

Table 1: Summary of Results for Bead Cytometry Experiments

Bead Velocity	2.3 m/s
Max. Count Rate	3,000 bead/s
Fluidic Flow Rate	0.036 mL/s
Average Peak Amplitude	0.9+/- 0.4

PHOTOGRAPHS:

JSC2004E28082 to JSC2004E28085
JSC2004E28254
JSC2004E28286
JSC2004E28399 to JSC2004E28401

VIDEO:

- Zero g June 29 – July 2, 2004, Reference Master: 718394

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

CONTACT INFORMATION:

Christopher Culbertson, Ph.D.
Kansas State University
Phone: 785-532-6685
Fax: 785-532-6666
culbert@ksu.edu