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Automated microchip platform for immunoassay analysis

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Abstract

An instrument comprising the components and subsystems of an automated microfluidic chip-based platform for bioanalysis by immunoassay is described. The device uses micromachined glass plates for the fabrication of channel networks and combines electroosmotic pumping and capillary electrophoresis for fluid transport and separation with a miniature peristaltic pump for sample introduction. The complete system enables the chips to perform the key elements in analytical processing. sampling, injection, mixing, separation, detection, and elim nation within about 3–5 min We describe the design and subsystem configurations required to engineer a compact instrument box with an overall size of 30 × 35 × 50 cm. The instrument provides computer-based control of three peripheral subsystems incorporated into the box. These subsystems include high-voltage power supplies and relays for on-chip fluid control; a fluidic interface for transporting liquids to and from the chip; and a confocal, epiluminescent optical assembly for detection by laser-induced fluorescence using a solid-state diode laser source. The unit has a detection limit of 0 21 nM for cyanine 5 dye with a peak height variation of <±3%. Short-term migration time variations of ±1%, variations of <±3% over 14 days, and device-to-device variations of <±3% were obtained Linear calibration curves (R^2 = 0.9986) were obtained for the direct immunoassay of 10–100 µg/mL ovalbumin with Cy5-labeled antiovalbumin.

Microchip-based fluidic devices can be fabricated on a variety of substrates such as glasses,[1–5] plastics,[6] silicon,[7] and polymers,[8] and have been demonstrated for bioanalyses such as immunoassays,[9–12] DNA assays,[13–16] serum protein screening,[17] and cell responses.[18,19] Recent work has shown that a wide range of chemical processing can be built into microchip platforms.[20,21] Technological advancements are being directed toward developing complex and automated processing on a single chip. However, to utilize microfluidic technology for analytical instruments, there are requirements for system integration on two levels One level is the integration of chemical function on the chip Since an analytical process is a set of defined steps that must be carried out in a fixed order, each step must lead into the next with respect to position on the chip and sequence of events. The other level is a combination of peripheral system components for control and operation of the chip. This requires having the peripherals (such as high-voltage supplies, optical detection unit, and external pump) under computer control, ready to respond to a predetermined set of software-based commands In this way, the analysis can be initiated by the operator. The peripheral components will respond by making the chip perform the analysis, collecting the data from the analysis, and resetting the system for the next run.

This paper describes the development of an automated, microchip-based platform for immunoassay analysis. For a demonstration, we have developed an immunoassay for the protein ovalbumin, which is used in field-trial testing and evaluation of aerosol collectors and environmental monitoring systems. Capillary electrophoresis has emerged as a useful tool for immunoas-

Indexing terms

Microfluidics, microchip, immunoassay, capillary electrophoresis, fluorescence

Abbreviations

LIF, laser-induced fluorescence; SIC, sample introduction channel; PMT, photomultiplier tube; PTFE, polytetrafluoroethylene

says[22–25] and is well suited for microchip applications. In our assay, fluorescently labeled monoclonal antibodies were reacted with ovalbumin and the product was detected by laser-induced fluorescence (LIF). The channel networks in the device were fabricated on micromachined glass plates. Electroosmotic pumping was used for quantitative injection of reagents and for liquid transport on the chip at velocities up to 1 cm/sec. Thus, by voltage control alone, chemical processing can be carried out within a single manifold of channels on-chip through electroosmosis and CE.

The system we describe enables the on-chip operation of the key elements in analysis: injection, mixing, separation, detection, quantitation, and elimination plus direct-time data display. It was designed to connect to a large-volume aerosol sample collector for environmental monitoring, although any low-pressure fluid delivery apparatus would be suitable for use with the microchip system. Compact peripheral subsystems were developed, so that all of the components could be engineered into a single 30 × 35 × 50 cm box. The unit consists of a programmable high-voltage subsystem for electroosmotic pumping and CE, coupled to a rapid wafer exchange, fluidic, and electrical interface to the chip The fluid interface was designed to allow the use of a miniperistaltic pump to deliver analyte solutions from an external reservoir to the chip Detection was accomplished with a built-in, compact, confocal epiluminescence LIF microscope utilizing a 635-nm diode laser Operational control of the system was carried out by an on-board microprocessor under the command of Microsoft® Windows™ (Redmond, WA, U.S.A.)-based software on a notepad computer

Experimental

Materials and reagents

Ovalbumin and monoclonal antichicken egg albumin (ascites fluid) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A). Water-soluble bisfunctional N-hydroxysuccinimide (NHS) ester of Cy5 dye (Amersham Pharmacia Biotech, Piscataway, NJ, U.S A.) was used for antibody labeling. Other reagents were of analytical grade and were purchased from Sigma. The antibody (ascites fluid) was purified over Protein G beads (Pierce, Rockford, IL, U.S.A.) and then labeled with Cy5 to yield a dye/protein ratio of 1.3. The recovery was about 10–15% Nonreactive Cy5 dye standard was from Beckman Instruments (Fullerton, CA, U.S.A.). Aqueous solutions were made from Milli-Q purified water (Millipore, Bedford, MA, U.S.A).

Device fabrication

The chip layout is shown in *Figure 1* The thin

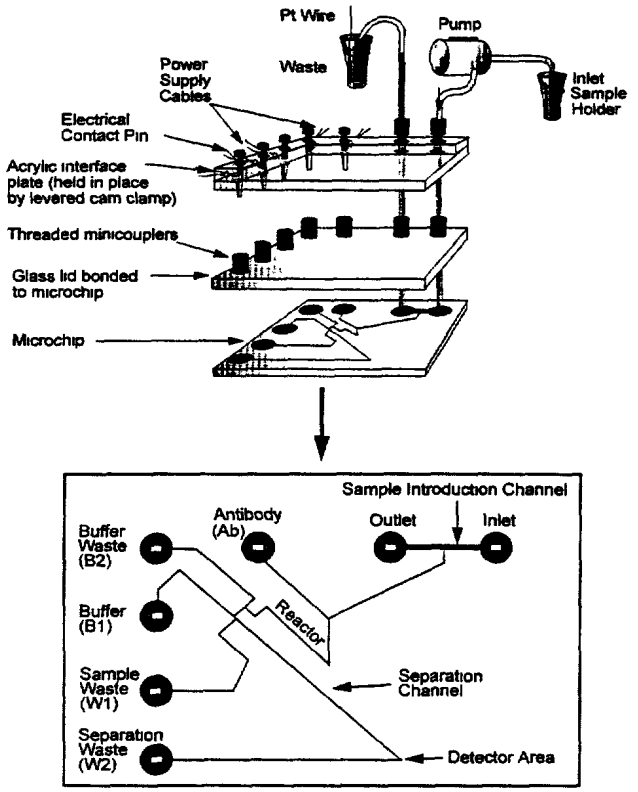

FIGURE 1 *Microchip layout and interconnection assembly for automated immunoassay platform*

lines depict the 13 × 65 µm channels; the heavy line depicts the sample introduction channel (SIC). Devices were fabricated in 4 × 4 in. 0211 glass using a 2-mask microlithographic pattern and an HF/HNO_3 etchant [2,10] Devices consisted of two pieces of 0 6-mm-thick glass. One mask pattern was used to produce a channel network etched to a depth of 13 µm and a trapezoidal cross-section with a top width of 65 µm, and a bottom width of 26 µm The other pattern resulted in a large sample introduction channel etched 0.3 mm deep, 1 mm wide, and 1.8 cm long. Access holes (1.6 mm diam) were drilled in the top plate. Both plates were cleaned and bonded, as described previously [11] The region at the top of the separation channel formed a double-T injector [5,26] The center-to-center distance between the two arms was 491 µm, corresponding to an injection volume of about 290 pL.

High-voltage power supply

A pair of power supplies (UltraVolt, Inc., Ronkonkoma, NY, U S.A.) was equipped with ripple strippers, mu-metal shielding, and aluminum cases to minimize the supply noise The power supplies had a range of

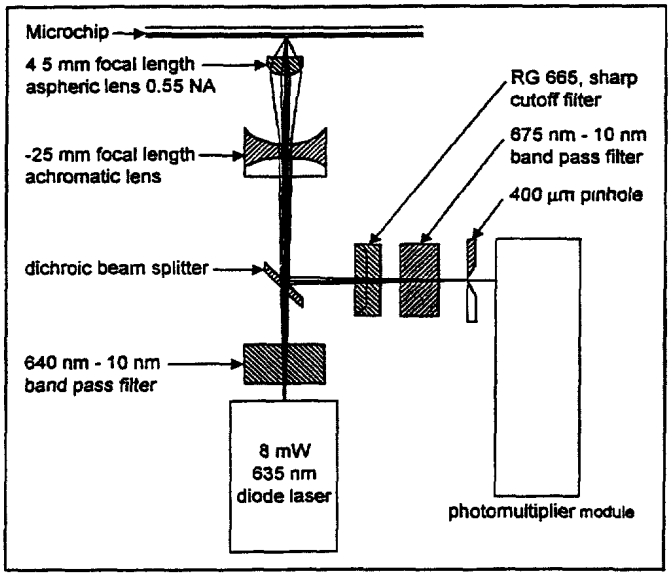

FIGURE 2 *Layout of epiluminescent, confocal microscope for diode laser-induced fluorescence detection of the red absorbing dye, Cy5*

0–3 and 0–10 kV Reed relay switches (Kilovac, Santa Barbara, CA, U.S.A.) were used to deliver voltage to gold-plated electrodes for controlling the fluidic processing on the microchip. The high-voltage supply was always turned off prior to switching the relay to eliminate hot switching. A bleeder resister was used to terminate the high-voltage power supplies The voltage decayed to under 100 V within 300 msec. This procedure prevented arcing at the relay contacts and protected the electronic components in the system. It also improved the formation of sample plugs at the separation channel. The rapid decay of voltage promptly stopped the electroosmotic flow and provided better fluidic control on the chip.

Epiluminescence confocal microscope and fluorescence detection

A compact, custom-built epiluminescence confocal microscope was designed (see *Figure 2*). A solid-state laser (Power Technologies, Mabelvale, AR, U.S.A.) having a circular Gaussian beam profile and a nominal power output of 8 mW at 635 nm was used as the excitation source. The actual wavelength of the diode laser was determined to be 642 nm. The diode laser generated a significant amount of out-of-band radiation, a phenomenon similar to discharge glow in gas lasers, which was eliminated with a 640 (10)-nm bandpass filter (Melles Griot, Ottawa, Ontario, Canada). The laser beam was focused on the capillary channel perpendicular to the plane of the microchip with a 40× aspheric lens, 0.55 numerical aperture, 4.5-mm focal length (New Focus Optics, Santa Clara, CA, U.S.A.) that had a working distance of 2.7 mm. The emission was collected with the same aspheric lens, projected through an achromatic negative focal length lens (–25 mm f.l., Edmond Scientific, Barrington, NJ, U S.A.) onto a heat-reflecting filter (Roland Optics, Corvena, CA, U.S.A.) that was used as a low-cost beam splitter. The split beam then passed through a filter set consisting of a 665-nm short-wave cutoff filter (Roland Optics), a three-cavity 675 (10)-nm bandpass filter (Melles Griot), and a 400-µm pinhole (Melles Griot) to the photomultiplier tube (PMT) (HC 577301, Hamamatsu Corp , Bridgewater, NJ, U.S.A.). The PMT had a multialkali photocathode, which enhanced the sensitivity in the red and near-IR portion of the spectrum The narrow spread of the light, less than 40 nm, allowed the use of a chromatic optical design, that is, a single lens in the microscope objective. Thus, with the single-lens objective and the negative focal length element, a compact (4 × 8 × 13 cm), low-cost optical unit was achieved.

Electrical and fluid interface plates

A fixed, reusable interface plate for electrical and fluidic connections was fabricated from sheet acrylic (Cadillac Plastics, Edmonton, Alberta, Canada) The electrical leads from the high-voltage power supply were connected to gold-plated spring pin electrodes (Interconnect Devices Inc., Kansas City, KS, U.S.A.) through the sides of the interface plate and were held in place by threaded-screw press fittings. Electrical contact with the fluid reservoirs was made through the gold-plated electrodes.

A disposable fluid interface was molded onto the chip. Threaded flange-free liquid chromatography minicouplers (for 1/16-in.-o d. tubing) (Valco Instruments, Houston, TX, U S.A.) were used as fluid reservoirs, as suggested in Figure 1. The reservoir vials were bonded to the chip over the access holes using double-sided tape and then reinforced at the base with 5-min epoxy glue. After bonding, a mold was placed over the top of the wafer, and epoxy encapsulating resin (Sealtronics #221AC-7V, Industrial Formulators of Canada, Burnaby, BC,Canada) was poured to a depth of 5 mm and allowed to cure for 24 hr. A small amount of lampblack (0.25% wt/wt, Industrial Formulators) was added to make the resin opaque. The minicouplers were used for both fluid reservoirs and pressure fittings to the SIC. This design provided a leak-free interconnection to the chip. The chip assembly was mounted on an *XYZ* translation stage (Newport Instruments, Irvine, CA, U.S A.) so that it could be positioned and aligned with respect

to the optical system. Analyte sample was delivered to the chip by means of a miniperistaltic pump (model P-625/900, Instech Laboratories, Plymouth Meeting, PA, U.S.A.) The fluid lines for delivering the sample were 0.020-in.-i.d. silicone tubing (Instech) connected to the interface chip by the flangeless minicouplers (Valco), as shown in Figure 1. The fixed interface plate was held on a levered cam and was lowered into place on the disposable interface plate by the operator. When lowered, the gold electrodes rested in the fluid reservoirs to make the electrical connections. The high-voltage connection for the SIC was made off-chip in the sample tube. The sample fluid in the silicone tubing completed the circuit.

System description

The analysis platform instrument used an 8-bit PIC electronic microprocessor (chip no. 16C77, Microchip Technologies Inc., Chandler, AZ, U.S.A.) (14 kbytes of on-chip read-only memory and 368 bytes of random access memory) that combined serial communication and I/O ports in a single device and required a host as the controller. The host computer sent instructions, such as the switch-matrix and timing information for the injection, mixing, and separation events, to the on-board PIC processor to operate the system. The on-board PIC executed the instruction sequence and controlled the on/off switching of the high-voltage power supplies and the switching of the relays that directed the voltages to the electrodes at the fluid reservoirs on the (fluidic) microchip The program for controlling the voltage sequence in the fluidic microchip, the data acquisition, and the communication link was custom-written in C and stored on the PIC processor. The instructions for the control program were passed from the host to the PIC. An isolated RS232 communications port on the analysis platform connected the instrument to the host computer in order to further isolate the host computer from possible arcing at the electrodes. As a safety feature, a set of interlocks was installed that terminated the high voltage when the cover was removed from the instrument.

The fluorescence signal, i.e., the output of the photomultiplier tube, was amplified by a transimpedance amplifier that had 10^7-ohm feedback resistance, then filtered by an eight-pole, 20-Hz Butterworth (3 dB point) low-pass filter to eliminate any noise present on the analog signal It was then digitized by a 12-bit A/D converter. The digitized signal was stored in the processor memory array and sent to the host computer at the end of the data collection sequence when the separation was finished. A block diagram of the system is shown in *Figure 3*; a photograph of the instrument is shown in *Figure 4*.

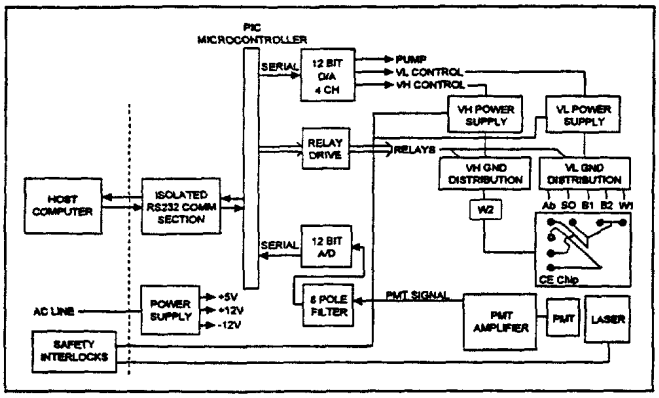

FIGURE 3 *Block diagram of the microchip immunoassay instrument*

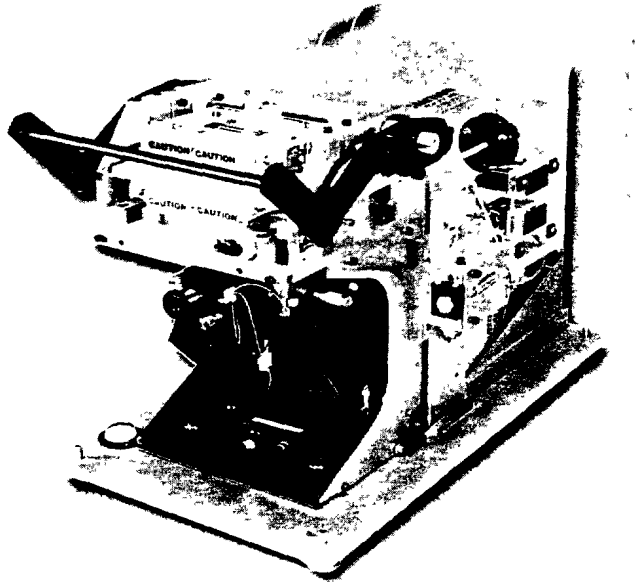

FIGURE 4 *Photograph of the automated immunoassay instrument (overall size 30 × 35 × 50 cm) The microchip device is located behind the lever handle and is mounted on an XYZ translation stage The epiluminescence confocal optical subsystem (4 × 8 × 13 cm) is below the chip; the high-voltage power supplies are at the rear of the instrument*

Chip conditioning and ovalbumin immunoassay

Dry chips were filled by adding buffer solution to reservoir B1, then allowing capillary action to distribute the solution throughout the channel network. After the channels were filled, the other reservoirs were loaded with 10 µL of the appropriate solutions using a syringe Care taken to avoid trapping air in the reservoirs during filling eliminated any problems with bubbles in the channels later. Washing of the devices with 0 1 *M* NaOH for 1 hr, followed by 20 min in 0 1 *M* HCl before their first use, produced devices with similar electroosmotic

pumping rates. At the end of each day, the channels and reservoirs were flushed with distilled water for 15 min using house vacuum, and were then stored filled with distilled water. The water was removed by vacuum prior to introducing buffer and sample solutions. When required, the channels could be cleaned and conditioned by flushing under house vacuum with 0.1 *M* NaOH for 5 min, distilled water for 10 min, and then with buffer for 5–30 min.

The buffer used for the immunoassay was composed of 50 m*M* tricine pH 8.0, 26 m*M* NaCl, and 0.01% Tween 20 (wt/vol). The antibody solution consisted of Cy5-labeled antiovalbumin diluted to 38 µg/mL in buffer. Ovalbumin was dissolved in buffer solution at 10–100 µg/mL. Approximately 300–500 µL of the sample solution was added to the off-chip sample tube and pumped through the SIC at 66 µL/min. Sample solution was pumped for an initial 2–3 min to flush the sample from the previous run.

Results and discussion

Sample introduction channel

In order to deliver analyte fluids to the chip, a novel, low-flow-resistance interface was constructed on-chip. The large sample introduction channel was fabricated 0.3 mm deep, 1 mm wide, and 1 8 cm long, as shown in Figure 1. The other channels on the chip were 13 µm deep and 65 µm wide. Thus, the large difference in channel dimensions provides a large difference in resistance to flow. In this way, sample can be pumped through the low-resistance sample channel without the risk of contamination of the narrower chemical processor portion of the channel network. Successive samples were introduced to the SIC using a miniature peristaltic pump to draw sample from the sample tube through external tubing. The tubing length to the sample tube was not optimized, so that a total of 3 min was used to flush the sample line and SIC at a flow rate of 66 µL/min. No carryover of dye or proteins was observed with this loading protocol.

Sample was drawn into the reaction channel and the double-T injector using electroosmotic pumping. The electrokinetic injections could be precisely and reproducibly controlled, even with the peristaltic pump running continuously. Electropherograms collected for five successive injections of Cy5 showed migration time variations of ±0.21% RSD and peak height variations of ±2.1% RSD. Over a period of 14 days on a single device, the migration times of Cy5 varied by less than 3%.

The interface plate provided a quick and rapid means of exchanging devices within the unit. Opening the lever arm, seen near the top in Figure 4, allowed rapid insertion of a chip within the unit. Electrical contact to the fluids prefilled in the plastic reservoirs on the chip was easily made simply by lowering the arm again. Fluid contact from the peristaltic sample pump line to the SIC was made simultaneously This fluid interface design was very effective Initial difficulties with high-voltage leakage currents in the top interface plate were due to corona discharge and were remedied by sheathing the upper portion of the electrodes with narrow-gauge PTFE tubing.

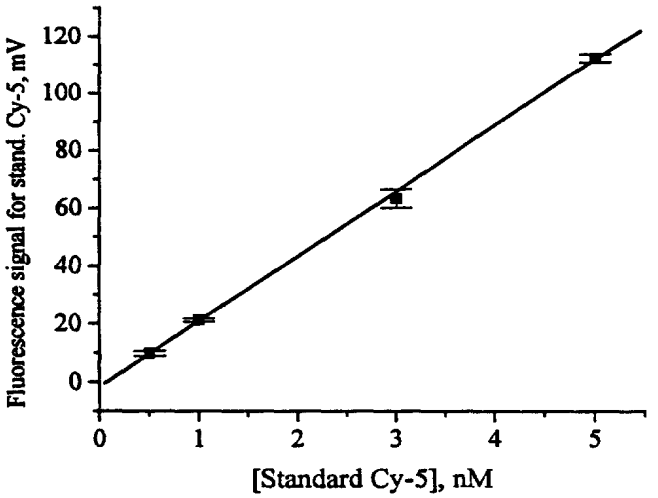

FIGURE 5 *Calibration curve of serially diluted samples of the nonreactive Cy5 dye, introduced via the SIC and injected electrokinetically into the microchip device. The limit of detection of dye was 0 21 nM The data points and bars represent the mean and standard deviation for three consecutive measurements (R^2 = 0 99992).*

Fluorescence detection

Fluorescence detection provides the high degree of sensitivity required for microchip CE, where the sample volumes and hence the total number of detectable molecules are low. The epiluminescence microscope assembly used for LIF was mounted below the chip The diode laser beam was aligned perpendicular to the chip using the translation adjustments on an optical stage. The eyepiece of the assembly could be rotated into place so that alignment could be made visually. Optimal alignment involved focusing the laser beam at the axis of the channel and fine-adjusting the translations to minimize the background.

The performance of the optical unit installed in the platform was measured using nonreactive Cy5 dye. Serially diluted samples of the dye were introduced via the SIC, and 290-pL sample plugs were injected electrokinetically. S/N measurements were made over a range of 0.50–5 n*M* Cy5 dye at pH 9. A plot of fluores-

Table 1
VOLTAGE AND RELAYS SWITCHING MATRIX FOR FLUIDIC CONTROL OF MICROCHIP*

Program A		Reservoirs (voltages in kV)							
Step	Function	B1	B2	W1	W2	Ab	SO	SI	Time (sec)
1	Sample input to SIC	—	—	—	—	—	—	P	180
2	Sample introduction, mixing, loading	—	—	VL –1.2	—	G	G		15
3	Reaction (stop flow)	—	—	—	—	—	—		60
4	Separation	G	—	—	VH –7 0	—	—		80
Program B		**Reservoirs (voltages in kV)**							
Step	**Function**	**B1**	**B2**	**W1**	**W2**	**Ab**	**SO**	**SI**	**Time (sec)**
1	Sample input to SIC	—	—	—	—	—	—	P	180
2	Sample introduction and mixing	—	VL –1.0	—	—	G	G		30–60
3	Reaction (stop flow)	—	—	—	—	—	—		60
4	Injection and plug shaping	G	—	VL –1.2	G	G	G		15
5	Preseparation	G	—	VL –0.5	VH –5 0	—	—		0 6
6	Separation	G	VL –0.8	VL –0 8	VH –7.0	—	—		80

* P = external pressure; — = floating voltage, G = ground, VL– = power supply variable from 0 to –3 kV, VH– = power supply variable from 0 to –10 kV, SO and SI are the outlet and inlet ends, respectively, of the sample introduction channel

cence intensity versus concentration is shown in *Figure 5*. The detection limits (S/N = 3) were 0 21 and 0.24 n*M* for 1000- and 400-μm pinholes, respectively, comparable to the 0.1-n*M* limit we obtained for Cy5 on a commercial P/ACE System 5000 CE instrument (Beckman Instruments) with a 635-nm LIF unit. These values, although poorer than those obtained previously on a chip with fluorescein and a confocal, epiluminescent microscope,[27] were adequate for the immunoassay.

The data in Figure 5 also demonstrate the ability of the SIC to provide sample delivery to the chip without carryover. The correlation between fluorescence signal and concentration of dye indicated the accuracy and reproducibility of the sample exchange.

Fluidic processing and operation of microchip

On-chip mixing and reaction was followed by a capillary electrophoresis separation in a sequence of timed events. Two programmable power supplies and a multistep program were used to control the on-chip processes. *Table 1* illustrates the timing sequences used, indicating the magnitude and location of the potential applied and the length of time it was applied for. The first step was pumping of the sample through the SIC, followed by electrokinetic mixing of sample and antibody within the reactor channel. When the glass devices were well behaved, there was very little leakage during sample loading or separation. In that case, a simple two-stage potential program was used, with potentials applied to the primary flow channels alone, as indicated by program A, Table 1. With an older device, or after several hours of use and before reconditioning the channels, a more complex program was used to minimize leakage at the intersections. Program B in Table 1 illustrates the initial delivery of sample to reservoir B2 during mixing and filling of the 19-nL reactor channel. This was followed by loading of the injector, with plug shaping performed by application of voltages to all channels intersecting at the double-T injector. The fluid flow for loading the injector and plug shaping is shown in *Figure 6a* Using the plug-shaping procedure, reproducible injection volumes were obtained after about 15 sec of injection at 1.2 kV, as shown in *Figure 7* for a 40-n*M* Cy5 sample. A preseparation step was performed to first move sample plug just past the double-T injector Then potentials were applied to the side channels that contained sample to induce a side flow of buffer into the channels to push sample back and prevent leakage during the separation The fluid flow for separation and detection is shown in Figure 6*b*

In order for a set program to run a microfluidic chip repeatedly without human intervention, the migration time must be quite stable. In addition to the immediate reproducibility for replicate injections of ±0.21%, the long-term change in migration time over 14 days on a single device varied by less than 3% for a Cy5 sample. The variation in migration time between devices was about ±2%, when a rigorous cleaning procedure involving both NaOH and HCl was applied. This reproducibility was judged to be adequate for allowing selection of stable operating parameters for routine operation of the devices.

Immunoassay of ovalbumin

Homogeneous immunoassays are solution-phase reactions of antibody and antigen. In this work, the antibody was labeled with the fluorescent dye and thus the antibody–antigen complex was also fluorescent.

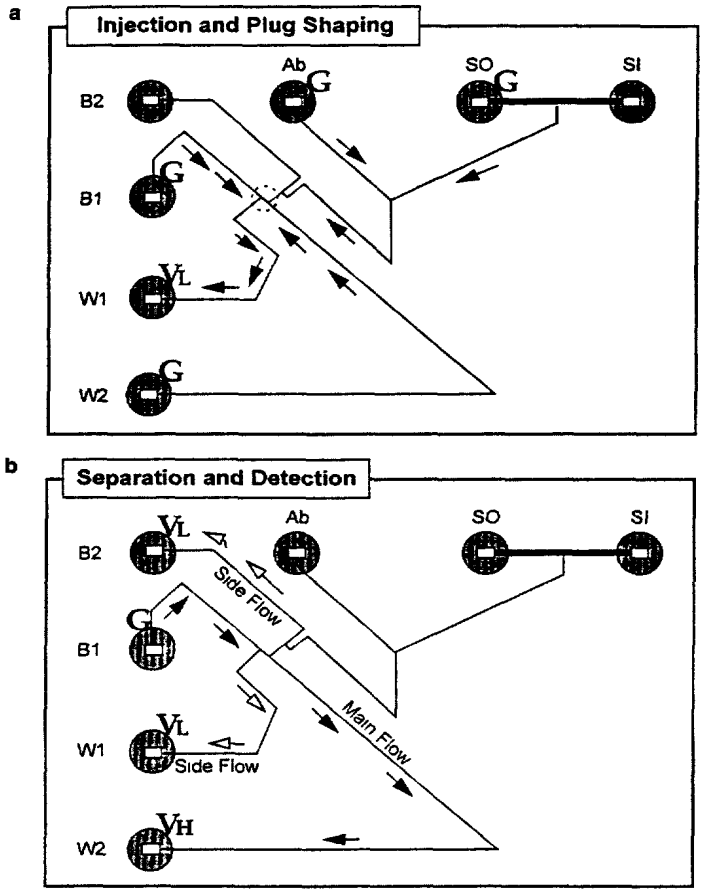

FIGURE 6 *The on-chip processing was a sequence of timed events controlled by high voltages at the electrodes in the fluid reservoirs a) Fluid flow for injection of the double-T and plug shaping, corresponding to step 4 in Table 1, program B The double-T injector is shown in the dashed circle b) Fluid flow for separation and detection, corresponding to step 6 in Table 1, program B*

The reaction of ovalbumin (Ov) and labeled antiovalbumin (Ab*) is given by the following:

$$Ab^* + Ov \rightleftharpoons Ab^*Ov$$

To carry out the immunoassays, aliquots of Ov solution were placed in the sample tube (as depicted in Figure 1) and delivered to the SIC by peristaltic pump From the SIC, Ov was sampled electrokinetically, mixed on-chip with the Ab*, then injected and separated. Electropherograms of Ab* and a mixture of Ov and Ab* are shown in *Figure 8* For Ab* alone, the peak appeared at 23 ± 0 2 sec. The small peak at 33 sec was an impurity, most likely free Cy5 dye For the Ov-Ab* mixture, the complex appeared at 26.5 ± 0.2 sec (n = 5). Peak heights varied by 2–3% (n = 5) The day-to-day variations in migration times were about 6%. The free Ov

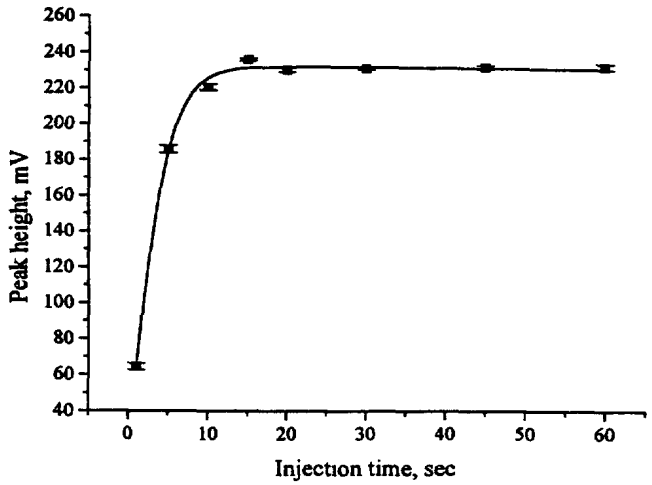

FIGURE 7 *The peak height (representing volume of 40-nM Cy5 dye loaded into the 290-pL double-T injector) as a function of loading time. The injector was loaded using the plug-shaping procedure of step 4 in Table 1, program B The fluid flow during loading is shown in Figure 6a The data points and bars represent the mean and standard deviation for five consecutive measurements*

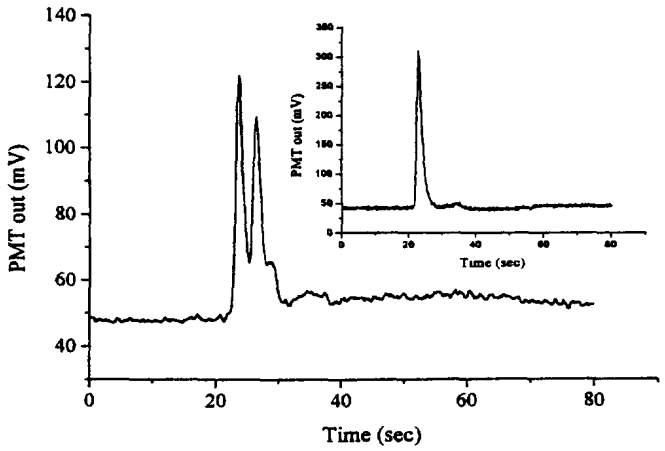

FIGURE 8 *On-chip electropherogram of 38 µg/mL Cy5-labeled antiovalbumin (inset) and a mixture containing 38 µg/mL labeled antiovalbumin and 50 µg/mL ovalbumin The complex appears to the right side of the antibody peak*

peak was not observed, of course; however, Cy5-labeled Ov had a migration time of 35 sec. In this chip assay, complete baseline separation of the free antibody and the complex was not achieved, but deconvolution of the peaks to obtain peak heights was easily done For a series of Ov samples (approximately 10–100 µg/mL), the peak heights varied linearly (R^2 = 0.9986), as illustrated in *Figure 9.*

A difficulty in CE separation of immunoassay products arises from protein adsorption on the capillary walls Through the use of appropriate buffer systems,

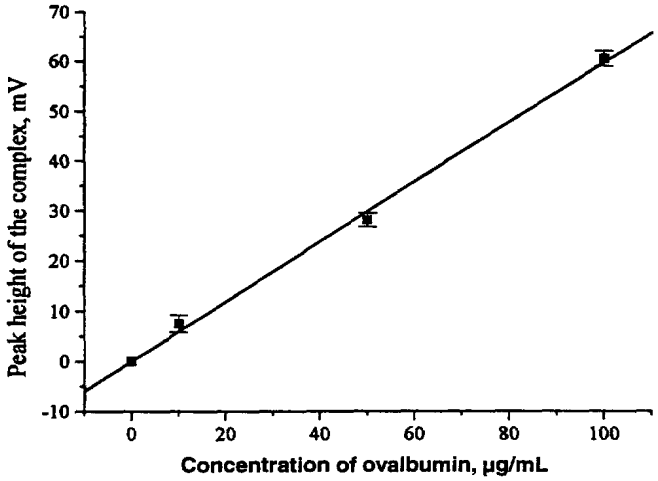

FIGURE 9 *Calibration curve of on-chip ovalbumin assay Samples of ovalbumin in varying concentrations were delivered to the sample introduction channel by peristaltic pump The analysis from that point was completely automated Total assay time was about 3 min The data points and bars represent the mean and standard deviation for three consecutive measurements (R^2 = 0 9986)*

sufficient resolution can be obtained within the microchip for immunoassays[10,11] and clinical serum samples[17] without the need for channel coatings. The use of dynamic coating agents such as surfactants greatly simplifies the fabrication of the devices, and avoids the durability problems associated with coating lifetimes. When protected from particulates and properly cleaned each day with NaOH and water, individual devices had lifetimes of 3 to at least 18 weeks.

Overall instrument evaluation

In order to perform chemical processes within a microfluidic chip, peripheral subsystems such as lasers, detectors, power supplies, and switches are required. Although the microchip devices are small, the use of conventional-sized peripherals can result overall in a large system, occupying a laboratory optical table. One of the goals of this work was to design more compact peripherals, so that the analytical instrument was potentially portable, rugged, and standalone. The automated platform that is shown in Figure 4 has an overall size of 30 × 35 × 50 cm and operates with a standard 120-V ac supply. This was accomplished in part through the use of small, low-current power supplies and high-voltage relays to make an electrical subsystem that was about 20 × 20 × 10 cm in size. The greatest gain in shrinking the size of the instrument was in the design and construction of the epiluminescent confocal microscope for fluorescence detection. This unit contained the laser, detector, optics, and alignment eyepiece and was 4 × 8 × 13 cm in size. We deliberately selected conventional optical subcomponents because the demand for ultra-high-sensitivity detection requires state-of-the-art detection methods, which are difficult to achieve with integrated microoptics or other novel solutions at this point. We obtained high performance of the optical unit at a relatively low cost using standard optical elements.

The system and its subcomponents were subjected to extensive testing over a period of about 10 months. Early problems with computer latch-up due to high-voltage switching were resolved, and the optical detection system was improved to provide the present 0.21-n*M* detection limits for Cy5. The system will run continuously for 8–12 hr with no failures for periods of months at this stage. Assays carried out on the instrument were fast and easy to perform. The fluid interface plate design provides a convenient means of rapidly switching chips. The operator can change a chip within 1–2 min, and be ready to perform the next assay following 15–30 min of chip equilibration time The low-resistance sample introduction channel provided a simple microfabricated solution to interfacing the chip to an external fluid flow. The transition from off-chip peristaltic (mechanical) pumping to on-chip electrokinetic injection was accomplished accurately and without leakage. There was no observed problem with sample carryover in the device for either Cy5 or protein solutions.

Conclusions

This work, to our knowledge, is the first report of a standalone, automated, microfluidic-based instrument platform for immunoassays. In this system, the complete reaction process for immunoassays was performed on-chip. Automation was accomplished by organizing the assay as a set of defined chemical functions that could be executed precisely in a specific electrically controlled and electrokinetically driven sequence. The work reported here represents the first prototype stage in the development of an automated immunoassay instrument. In addition to the microchip-based processing, we were designing compact subsystems for optical detection and high voltage, plus fluidic interface and computer control. The results demonstrate the amount of automation and the rapid analysis times that can be achieved in a microfabricated device. The performance of the system is robust, with fluorescence detection limits of 0.21 n*M*, migration time reproducibility of ±0.21% between runs and ±3% over 14 days, chip durability of many weeks, and stable system peripheral operation. The operator is required to change the sample solution in the sample tube after each run in the present design. However, with appropriate interfacing to an autosampler and less dead volume in the external

fluid lines, such systems could form the basis of automated instruments for on-site environmental monitoring and point-of-care clinical analysis. The instrument also has the potential to be used in the screening of antibodies to determine affinity constants and in hybridoma production to allow selection of suitable antibody producing cells.

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