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Parallel Analysis with Optically Gated Sample Introduction on a Multichannel Microchip

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As an alternative to the T-type injection on microchips, optically gated sample introduction previously has been demonstrated to provide fast, serial, and reproducible injections on a single-channel microchip. Here, the ability to perform high throughput, multichannel analysis with optically gated sample introduction is described using a voice coil actuator. The microchip is fixed on a stage, which moves back and forth via the voice coil actuator, scanning two laser beams across the channels on the microchip. For parallel analysis on a multichannel microchip, both the gating beam and the probe beam are scanned at 10 Hz to perform multiple injections and parallel detection. Simultaneous, fast separations of 4-choloro-7-nitrobenzofurazan (NBD)-labeled amino acids are demonstrated in multiple channels on a microchip. Serial separations of different samples in multiple channels are also reported. Optically gated sample introduction on multiple, parallel channels shows the potential for high-speed, high-throughput separations that are easily automated by using a single electronic shutter.

Within the past decade, microfabricated chips have been applied in almost every area of separation science.[1−8] Microchips have many attractive advantages, including rapid analysis time, minimal sample consumption, and eventually reduced cost. Another important advantage is the ability to multiplex microstructures on a small substrate to increase throughput. High-throughput analysis is desirable for many biological analyses, especially for genetic and proteomic analysis and for drug discovery in the pharmaceutical field. Several developments have been explored to take advantage of high throughput separations on microchips.[9−11] Recently, multiple detection schemes on one microchip were used to provide orthogonal information to increase or improve throughput.[12,13] Meanwhile, much work has been focused on increasing the density of separation lanes. Mathies' group has developed devices with as many as 384 channels for use in genetic analysis.[11,14−16] Simultaneous immunoassay studies in six channels have also been accomplished on a microchip.[9] Two-dimensional capillary electrophoresis system has been fabricated in poly(dimethylsiloxane) to perform 2D gel electrophoresis for protein separations.[17] Sub-millisecond separations have also been accomplished on a microchip.[18] Combining parallel separations in multiple channels with ultrafast serial separations will obviously provide higher throughput than using a single channel.

The most commonly used method to introduce samples to chips for multiple channel separations is T-type injection.[10,11] Typical T-type injections need three or four reservoirs per channel on the microchip. There are usually one or two sidearms perpendicular to the separation channel. A potential is applied to electrokinetically move the sample from the sample reservoir to the separation channel. Then the potential is switched to the separation channel to achieve the separations. To improve performance with this injection method by minimizing leakage from the sidearms, many variations on the T-type injection have been explored.[19−21] These variations involve controlling the potential applied to each reservoir. Thus, as the number of separation lanes increases, the electronics become more complicated, and it is more difficult to accomplish fast, serial separations using this injection in multiplexed channels. In addition, reservoirs take up a large amount of chip space. Typically, each reservoir on the microchip is ∼2−3 mm in diameter. With fewer reservoirs on the microchip, space can be saved for more lanes.

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Monnig and Jorgenson developed optically gated sample introduction in the 1990s to perform ultrafast separations in a capillary.[22,23] With optically gated sample introduction, fluorescently labeled sample is continuously electrophoresed through a capillary. A laser beam is split into two beams and focused onto two points of the capillary. One beam (the probe beam) has less laser power and is used for laser-induced fluorescence (LIF) detection. The other beam (the gating beam) has high laser power and is used to perform sample introduction by time-discriminated photobleaching of the sample. The gating beam continuously photobleaches the fluorescently labeled analytes as they pass through the channel. An injection is accomplished by blocking the gating beam for a specific time. Thus, a plug of unphotobleached sample moves past the injection window, is separated, and then is detected by the probe beam. Since optically gated sample introduction allows fast, serial, and reproducible injections while maintaining constant potential, this technique has been applied for rapid analysis in multidimensional separations and dynamic chemical monitoring in capillaries.[24,25] Recent work has applied optically gated sample introduction on a single-channel microchip.[26] Fast, serial separations have been accomplished using this injection technique.

In this paper, parallel separations using optically gated injections are demonstrated on a multichannel chip. The effect of switching the gating laser beam between channels is studied by chopping the beam focused on a single channel. To perform multichannel analysis, a voice coil actuator is used to scan both the gating beam and probe beam across the channels on a chip. Simultaneous separations of different analytes in multiple channels are presented.

EXPERIMENT SECTION

Reagents. All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise noted. Stock solutions of amino acids (10 mM) were prepared in 10 mM borate buffer (pH = 9.2, filtered, degassed) and 10 mM NBD (Molecular Probes, Eugene, OR) was made in acetone (Burdick & Jackson, Muskegon, MI). NBD solution and amino acids were mixed to produce a 10-fold molar excess of NBD, reacted for 10 min, and then diluted to the appropriate concentration with borate buffer.

Microchip Fabrication. Two chip designs have been used in these experiments (Figure 1). Microchips were fabricated in the EMPRL Nanofabrication Facility (Pennsylvania State University, University Park, PA) using traditional photolithographic and wet etching techniques, which have been described previously.[26] Chip A consisted of a single lane, which used a chopped gating beam to simulate a scanned laser. Chip B had five channels for multichannel experiments, as shown in Figure 1. All channels in chip B were designed to have the same length, 8 cm. In addition, both the gating beam and the detection beam were focused in the straight area of the channels during experiments, and thus, the separation distances were the same for each channel.

Instrument Design. The system design is depicted in Figure 2. For chip A, a stationary stage was used with a chopper placed

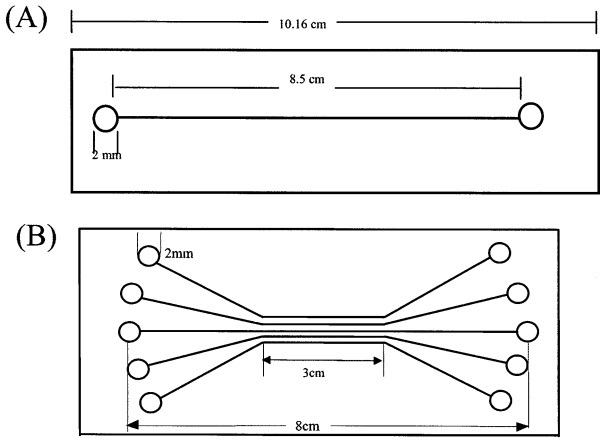

Figure 1. Schemes of microchips: (A) single channel chip; channel depth is 17 μm, and the width is 44 μm. (B) multichannel microchip; all channels on the same chip have the same length and depth; channel depth is from 10 to 42 μm, and the width is from 30 to 100 μm. The straight area in the middle is for gating and probe. The center-to-center distance between channels in this region is 200 μm.

in the pathway of the gating beam. For multichannel experiments, the chopper and the stationary stage were removed, and the voice coil actuator (BEI, San Marcos, CA) was used to move the stage. The system to perform optically gated sample introduction on a microchip is similar to the one that has been described previously[26] with three exceptions: First, the inverted microscope was not used to make the adjustment of optics easier. Second, for most experiments, the collected signal was amplified using a current amplifier (Keithley Instruments, Cleveland, OH) and sent directly to a data acquisition board (National Instruments, Austin, TX) in a computer (Micron Electronics Inc., Nampa, ID). For some experiments (noted in figure legends), data were collected by a computer using an A/D converter (Hewlett-Packard, Wilmington, DE) and analyzed by HPChemstation software (Hewlett-Packard, Wilmington, DE).[26] Third, the chip was rotated so that the gating beam (~50-μm spot size) and probe beam were focused on different channels at any given time. The offset distance was 400 μm.

A voice coil actuator was used as a direct drive linear actuator.[27] Generally, this device can move accurately over a small range from micrometers to centimeters, and it is ideal for applications that require positioning over short distance. The voice coil actuator was composed of two parts, the moving part (a group of coiled wires) and the fixed part (a permanent magnet and a ferromagnetic magnet). An electromagnetic field provides the force to translate the moving part and create linear motion when a voltage is applied. The force is rated to the voltage applied and the loaded weight. Thus, accurate control of movement can be achieved by controlling voltage using the computer program via the motor controller (Galil Motion Control Inc., Mountain View, CA). During multichannel experiments, the scanning frequencies of the actuator were measured from actual movements and were for a complete cycle. The position of the motor was obtained from the motor controller.

Procedures. General procedures for single channel and multichannel chip separations were similar. A vacuum pump

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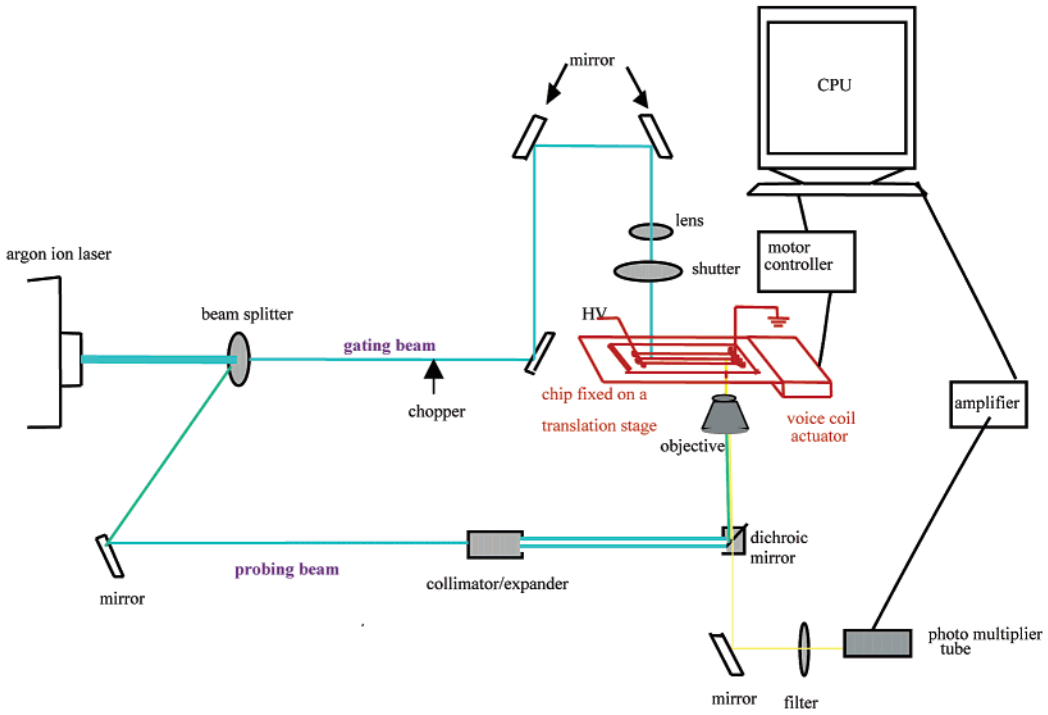

Figure 2. Multichannel system setup with optically gated sample introduction. The arrow points to where the chopper is placed during simulation studies. A stationary stage is used for simulation studies instead of moving the stage attached to the voice coil actuator.

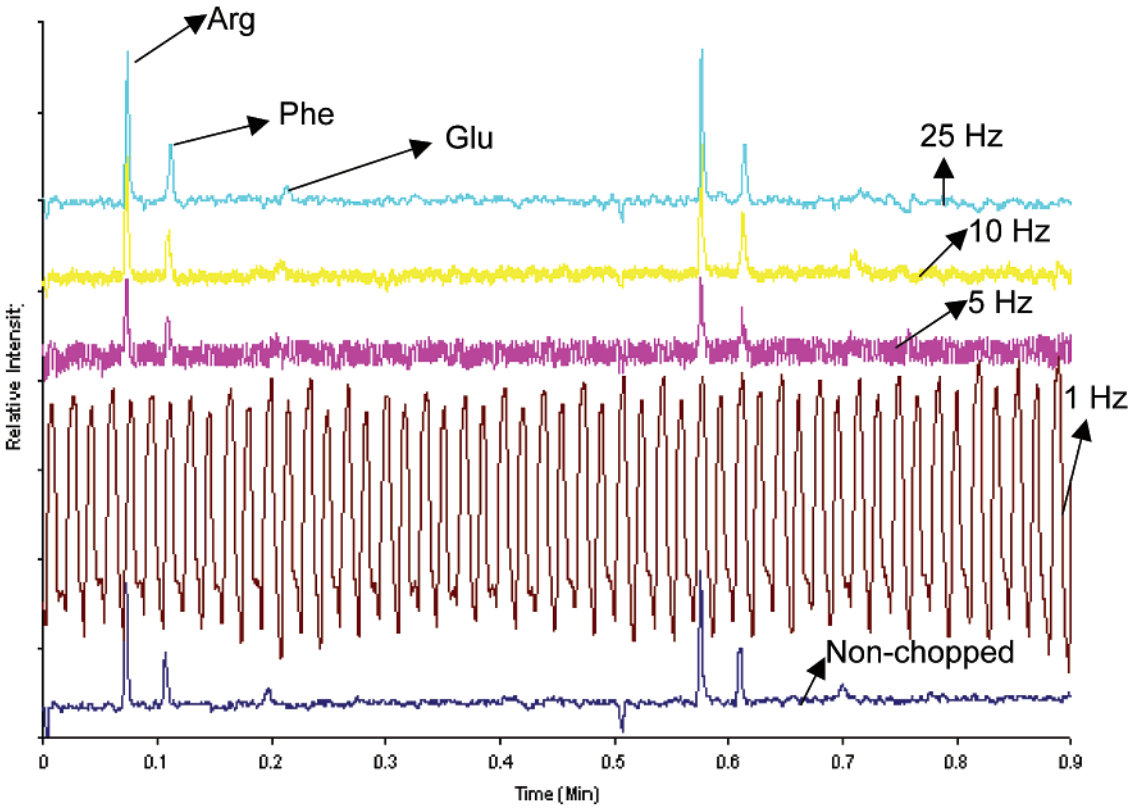

Figure 3. Two serial separations of three NBD-labeled amino acids in a single channel microchip at different frequencies of a chopped gating beam. The separations were performed using a 600 V/cm separation field and 250 mW laser power on a 17-μm-deep channel. Injections (170 ms) were carried out every 30 s. The concentrations of analytes were follows: 800 μM arginine, 400 μM phenylalanine, and 1.1 mM glutamic acid. (Data were collected using HPChemstation.)

(Welch 8890, Thomas Industry, Skokie, IL) was used to fill the separation channels before use. For single channel analysis, one electrode was placed into each reservoir. For multichannel analysis, the microchip was fixed on the stage attached to the voice coil actuator. For these experiments, two sets of five platinum electrodes were connected together, fixed to the stage, and used

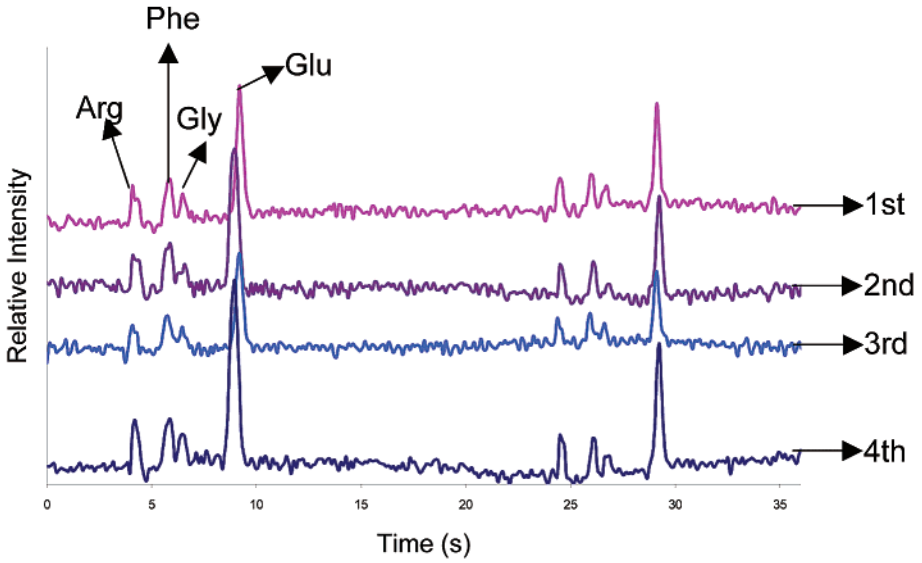

Figure 4. Simultaneous separations of four NBD-labeled amino acids in four channels with two serial injections. The four electropherograms were offset. A 438 V/cm separation field and 240 mW laser power were used in the separations. The first injection time was 500 ms, and the second one was 300 ms with a 20-s delay between them. Separations were performed on 35-μm-deep channels with analyte concentrations of 1 mM, 500 μM, 500 μM, and 3.3 mM for arginine, phenylalanine, glycine, and glutamic acid, respectively.

to apply uniform potential (Bertan, Hicksville, NY) and ground to the five inlet and outlet reservoirs, respectively.

Each channel on the microchip was rinsed using sodium hydroxide (0.1 M) electrokinetically for 5 min before use each day. Then the separation channels were rinsed with buffer solution for 5 min every hour. For single-channel chip separations, the gating beam and probe beam were focused onto the channel 1 cm apart. Because of the scanning nature of the multichannel microchip separations, it was not necessary to precisely focus the two beams on the channels. In this case, the distance between the two beams for every channel was 0.9 cm. During the multiple separation experiments, the actuator was moved back and forth so that the gating and probe beams were scanned across all of the channels.

Data Processing. The computer used an in-house Labview (National Instrument, Austin, TX) program to simultaneously control the actuator, initiate the data acquisition, and begin the injection. A 1000-Hz scan rate was used to collect data through the data acquisition board. The rise time on the signal amplifier for the single-channel chip was 300 ms. To obtain more data points for each channel on the multichannel chip, the rise time was decreased to 3 ms. A photomultiplier tube (Hamamatsu, Bridgewater, NJ) collected data continuously during scanning in both the channel regions and interchannel regions. Data were sent serially to the computer. Owing to scattering, the signal from the middle of each channel is higher than the signal from other areas of the chip. Thus, a peak is observed when the probe beam is scanned over one channel, even when no fluorophore is present. An in-house written program in Visual Basic was used to select and sort the data collected into groups to represent each individual channel on the basis of this scattering. In this program, the data point at each peak maximum in the original data set was selected to represent data from that channel. Following this, data was discriminated from each channel on the basis of motor position. Data points representing the same channel were collected together to compose the electropherogram for the channel.

RESULTS AND DISCUSSIONS

Characterization of Intermittent Optical Gating. In multichannel separation systems,[9,15,16] a single beam has been commonly used to detect the signal with simpler design and ease of optics alignment. There are two ways to collect signal from multiple channels using a single excitation beam, either moving the channels[14,15] or moving the laser beam.[9−11,16] Since optically gated sample introduction has two beams to scan over all the channels, moving the microchip instead of both beams is much simpler. All previous work with optically gated sample introduction has employed single-channel or capillary analysis. To accomplish multichannel separations with optically gated sample introduction using a scanning-beam method, the laser beams for both sample introduction and detection are on each channel for only a short period of time.

A chopped laser beam on a single channel chip was used to simulate a moving chip in and out of the gating beam to characterize the effects of this process. Chopping the beam simulates the effect of moving the beams from channel to channel with the result of intermittent exposures but allows evaluation of a single channel without physically moving the chip or the beam alignment. The channel was exposed to the gating beam for a portion of the total analysis time with varied frequencies.

The separations of three different NBD-labeled amino acids at five different chopping frequencies, 1, 5, 10, and 25 Hz, and no chopping, are shown in Figure 3. The S/N for arginine increases from 4 (5 Hz) to 25 (25 Hz) as frequency increases. Apparently, a higher chopping frequency allows adequate optically gated sample introduction. Interestingly, the 25 Hz chopped electropherogram (S/N = 25) has a better S/N than the nonchopped electropherogram (S/N = 14). This may be a result of less heating of the sample induced by the chopped beam when compared to

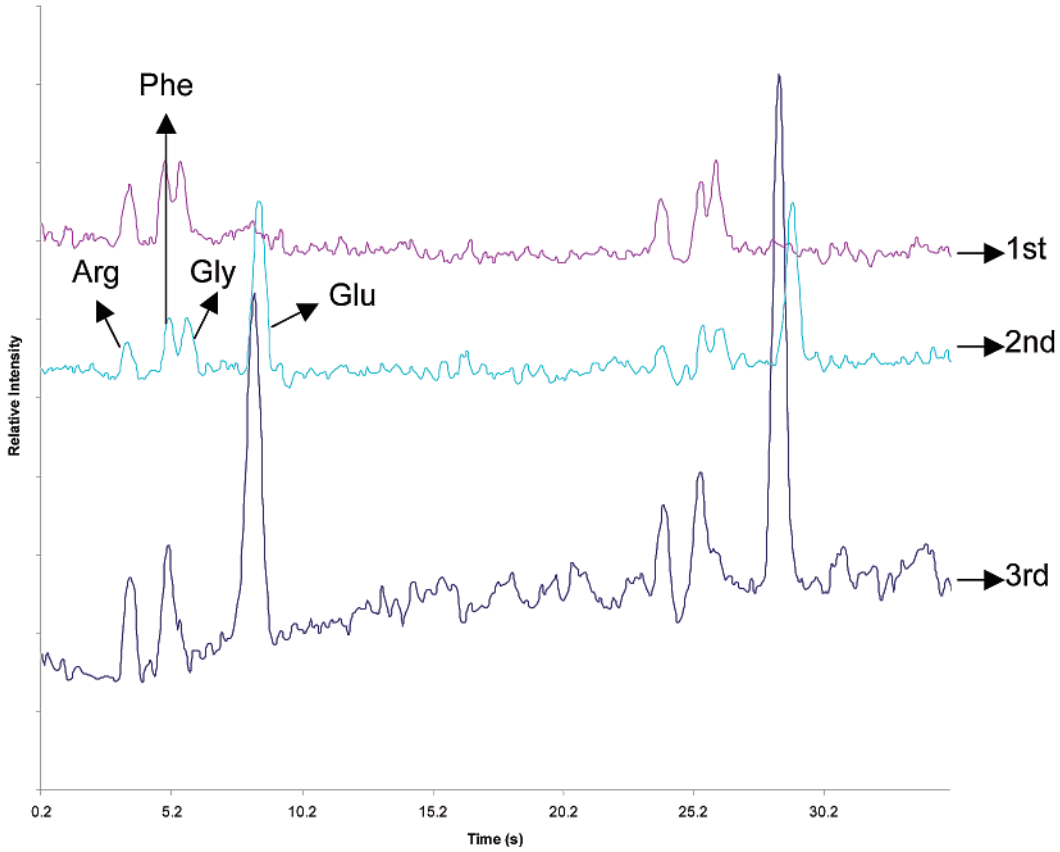

Figure 5. Simultaneous separation of NBD-labeled analytes in three parallel channels. Channel 1 had arginine, phenylalanine, and glycine. Channel 2 had arginine, phenylalanine, glycine, and glutamic acid inside. Channel 3 had arginine, phenylalanine, and glutamic acid. Separations were performed with a 375 V/cm separation field and 300 mW laser power on 42-μm-deep channels. Injections (500 ms) were carried out every 30 s with analyte concentrations of 240 μM arginine, 130 μM phenylalanine, 130 μM glycine, and 600 μM glutamic acid.

the constant beam. However, the voice coil actuator, which is used to implement multichannel scanning, moves unstably, and vibration of the system occurs when scanning frequency is higher than 10 Hz. This is very important, since vibrations can move the small channels into and out of the beam focus and result in increased background noise. Thus, a frequency of 10 Hz was chosen for the chip-scanning experiments. With the current selection of parameters for collecting data, the signals from each channel are well-separated from each other during multichannel separations.

Multiple Fast Separations. Parallel, serial separations of four NBD-labeled amino acids in four different channels are demonstrated in Figure 4. Two serial injections have been performed in each channel using 500-ms and 300-ms injection times, respectively. There is slight variation in the migration times of the analytes in different channels as a result of the differences in channel geometry and design. Nonuniform surface conditions and nonuniform etching might also account for some of this difference. In addition, adsorption of analytes on the wall of the channel might affect electroosmotic flow and, therefore, the migration time. Rinsing with buffer or NaOH reduces the variation in migration time, as an additional indication of this adsorption. Different concentrations of NBD-labeled amino acids (arginine, phenylalanine, glycine, and glutamic acid), from 3.3 mM to 30 μM (data not shown here) have been separated. Using this procedure, the differences in mobilities between different channels are found to be $<1\%$.

One advantage of using optically gated sample introduction in multiple channels is the ability to perform fast separations of different samples. In Figure 4, each separation per channel takes less than 6 s without any optimization. By decreasing the separation distance between the gating and probe beams, separation time can be further decreased. However, if the two beams are too close to each other, noise becomes a problem as scattered light from the gating beam reaches the detector. Thus, there is a tradeoff between short separation time and high S/N when varying separation distance. Another way to increase throughput is to reduce the dead time between injections. Currently, the delay between two injections is 20 s, which can be decreased, since a single separation requires only 6 s, allowing more separations to be carried out in a given time period. Faster separation can also be performed if higher separation voltage is used.

Factors Affecting Separation Efficiency and Detection Limit. The effect of injection time on the separation efficiency has been examined. The peak efficiencies of three analytes, NBD-labeled arginine, phenylalanine, and glycine, have been examined, with the best efficiency for arginine being a 0.5-μm theoretical plate height for a 200-ms injection time. This is similar to the 0.8-μm plate height obtained previously using optically gated sample introduction in a single channel microchip.[26] As expected, the efficiency in theoretical plate numbers decreases as the injection time increases. Theoretically, efficiency is directly proportional to the potential field of the separation. However, to maintain the

high separation efficiency in microchips, small injection plugs are necessary. This result is in agreement with the work with optically gated sample introduction in single-channel microchips.[26] Since the sample is electrokinetically moved, a higher mobility analyte (arginine) spends less time in the separation channel and yields a higher separation efficiency than a low mobility analyte (phenylalanine, glycine).

Several possible solutions can be employed to improve the detection limit. The scattered laser light is usually the main source of background noise when LIF detection is used. When scanning two laser beams simultaneously, this noise becomes worse. Thus, the gating and probe beams have been offset so that they are not focused on the same channel at the same time. This reduces the scattering along the channel from the gating beam to the detector. A better filtering system, including adding another optical filter, also helps to minimize the effects of scattered light. The detection limits for arginine, phenylalanine, glycine, and glutamic acid shown in Figure 4 are 1 mM, 500 μM, 500 μM, 3.3 mM, respectively. Currently, the best detection limit obtained using individual injection, a larger channel (75 μm in depth), and a longer injection time (700 ms) is 17 μM for NBD-labeled arginine, phenylalanine, and glycine and is 120 μM for glutamic acid. Strategies involving reagents that are more efficiently photobleached may improve these detection limits dramatically.

Multiplexed Samples Separations. The general goal of multiplexing separations is to analyze large quantities of samples simultaneously. To show the ability of multiplexed optically gated sample introduction for high throughput analysis, three different samples in multiple channels have been separated, as shown in Figure 5. Two serial injections have been performed in each channel simultaneously. The first channel contains NBD-arginine, NBD-phenylalanine, and NBD-glycine. The second channel has these three amino acids and also NBD-glutamic acid. NBD-arginine, NBD-phenylalanine, and NBD-glutamic acid are used in the third channel. The separations of these unique samples have been successfully accomplished. From these separations in three channels, the technique shows the potential of multiplexed sample analyses in different channels simultaneously. As mentioned previously, the microchip design using optically gated sample introduction is simpler, as compared to T-type injection, and more space is conserved. Thus, more channels can be easily added to the microchip. For multiplexed channels, this is especially important, since more samples can be analyzed simultaneously, with more separation channels fitting onto a limited microchip area. Scaling up the voice coil actuator system with a scan rate of 10 Hz, 355 channels could theoretically be used simultaneously. This extrapolation demonstrates that this technique could be competitive with other techniques using multiplexed channels. Combining this ability of unique sample separations with optically gated sample introduction, this multiplexed separation technique is capable of high-speed separations with high throughput. Separations with optically gated sample introduction can be performed serially in each channel, whereas most multiplexed systems target single separations in many parallel channels. This dynamic nature of the technique also makes it possible to consider its use as a multichannel separations-based sensor.

CONCLUSION

In summary, optically gated sample introduction with a voice coil actuator is easy to automate for parallel channels and allows fast, serial separations, as compared to traditional injection methods used in other multiplexed separation techniques. Theoretically, injections can be as rapid as the time to control an electronic shutter. In addition, the overall microchip design is simple, since only two reservoirs or less, if a common outlet reservoir is used, are needed for each channel, and more space is conserved. This simple design of these multiplexed chips has the potential for a high density of channels in a limited area. Combining this high-density parallel separations channel with rapid analysis time should make it possible to achieve high-throughput separations with this technique. Recently, the multiplexing of other sample preparation and chemical analysis components, including mixing, reaction chambers and multiple detection schemes, onto one microchip has been considered as the next necessary step to achieve the goal of a micro total analysis system with high throughput.[9,13,28] The simple chip layout with optically gated sample introduction in multiple channels will help put more microstructures into this system. The serial injections accomplished using optically gated sample introduction also makes it possible to combine dynamic monitoring with high throughput.

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