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Laboratory Experiment

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A Comprehensive Microfluidics Device Construction and Characterization Module for the Advanced Undergraduate Analytical Chemistry Laboratory

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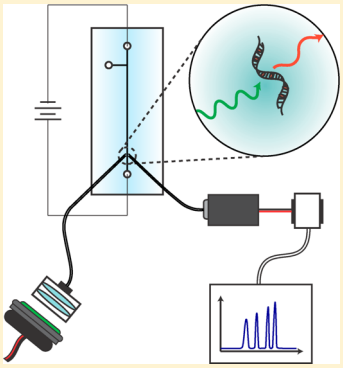
Supporting Information

ABSTRACT: An advanced analytical chemistry undergraduate laboratory module on microfluidics that spans 4 weeks (4 h per week) is presented. The laboratory module focuses on comprehensive experiential learning of microfluidic device fabrication and the core characteristics of microfluidic devices as they pertain to fluid flow and the manipulation of samples. Experiments include the cleanroom-free fabrication of glass-polydimethylsiloxane (PDMS) microfluidic devices by use of thermoplastic molds and soft lithography, determination of the zeta potential at the microchannel walls, investigations of electroosmotic flow, determination of Péclet numbers, and diffusion coefficients based on diffusion-limited reagent mixing. The module concludes with the development of a microchannel electrophoresis lab-on-a-chip device with in-line fluorimetric detection for the separation of a DNA ladder. This laboratory module has been offered for two consecutive years and has received favorable student feedback.



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Over the past two decades, miniaturization of analytical instrumentation has become a dominant trend. The development of microfluidic devices is clearly one of the preeminent themes in analytical chemistry research as indicated by the growth of dedicated journals, conferences, and symposia. Although undergraduate students are exposed to concepts of microfluidic design and operation in lectures, relatively few laboratory experiments have been reported[1−10] that provide students with rigorous hands-on exposure to the core concepts of how fluid manipulation in microchannels differs from that in the macroscale[3,5,6] and highlight lab-on-a-chip type applications.[1,2,5,9] Until very recently, few experiments addressed both of these areas.[5]

A novel experiment in an advanced undergraduate analytical chemistry laboratory is described that provides students with practical experience in techniques related to current trends in analytical chemistry research and teaches students the core concepts of microfluidics through experiential learning. The experiment has been implemented in a 4-week laboratory module (4 h per week) in which students work in pairs. In the first week, students investigate design and fabricate microfluidic devices. In the second week, students investigate diffusion-based mixing, followed by investigation of the concepts of zeta potential and electroosmotic flow in the third week. In the fourth week, students build and use a lab-on-a-chip device for microchannel electrophoretic separation of DNA ladder fragments. This laboratory module on channel microfluidics has been offered for two years and has received favorable student feedback.

MATERIALS AND EQUIPMENT

Polydimethylsiloxane (PDMS) pre-polymer solution and curing agent (Dow Corning Sylgard 184 Silicone Encapsulant) were purchased from Ellsworth Adhesives (Burlington, Ontario, Canada). Polystyrene thermoplastic sheets were from K & B Innovations, Inc. (North Lake, WI) onto which microchannel patterns were printed using a Brother HL-2140 Laser Printer (Brother Canada, Dollard des Ormeaux, Québec, Canada). Plasma oxidation of PDMS castings and glass slides was done using a Harrick PDC-32G plasma cleaner (Harrick Scientific Corporation, Ossington, NY) at an internal chamber pressure of 5 Torr and an applied power of 10.5 W. Pressure driven dispensing of solutions for investigations of diffusion-based mixing was done using a syringe pump (KDS20, KD Scientific, Holliston, MA) fitted with two 1 cc syringes. The syringes in turn were fitted with 16 gauge needles with blunted tips. Silicone tubing (0.030 × 0.065 in., VWR International, Mississauga, Ontario, Canada) connected the syringes to the fluid connection posts (also made from 16 gauge needle

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tubing) affixed to the Y-channel chip. Imaging of fluid flow and reagent mixing in the microfluidic chips was done by use of an inexpensive (<$40) 10× to 200× variable zoom 1.3 megapixel USB digital microscope (Deal Extreme, dx.com).

Electroosmotic flow and electrophoresis experiments were done within an in-house constructed poly(methyl methacrylate) (PMMA) chamber, shown in Figure 1, which was

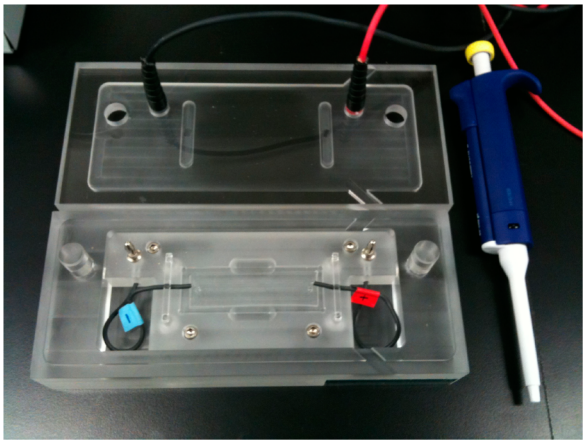

Figure 1. In-house constructed PMMA chamber for investigations of electroosmotic flow and microchannel electrophoresis using microfluidic chips (linear channel chip shown) based on a standard glass microscope slide and PDMS cover. Electrical leads terminated with female connectors secured within the lid of the chamber (top) permitted electrical contact to the leads contacting the microfluidic chip placed in the chamber base (bottom) only when the lid was secured to the base. The shallow grooves in the lid and base of the chamber (right-side periphery of the chamber) permitted introduction of optical fibers for on-chip fluorimetric detection.

prepared for routine student use. The chamber was designed to accommodate microfluidic devices prepared on standard 25.4 mm × 76.2 mm glass microscope slides and permitted electrical connection to the chip only when the lid was secured onto the chamber base, similar to that done for gel electrophoresis tanks, thereby minimizing the risk of electrical shock. Voltage was provided to the microfluidic chips by use of a surplus Gelman Model 38206 electrophoresis power supply (Gelman Instrument Company, Ann Arbor, MI). Monitoring of current and voltage applied to the chip was achieved by use of a current to voltage converter and voltage follower circuit developed in-house by our microelectronics shop, as detailed in the Supporting Information section. The device provided an output voltage over the range of 0−10 V dc in one channel that correlated linearly with current passing through the microfluidic chamber over the range of 0−100 μA. A second output channel provided a low voltage output over the range from 0−10 V dc that correlated linearly with potentials over the range of 0−500 V dc applied to the microfluidic chip. The low voltage outputs were connected to a NI 6008 USB data acquisition module (National Instruments Corporation, Austin, TX), and software developed in-house using LabVIEW (National Instruments) recorded voltage and current data with respect to time. This software was developed by the instructor and provided to the students for use during their experiments. Platinum wire was affixed to the ends of the insulated conductors present in the base of the PMMA chamber so that only the inert platinum tip contacted the fluid reservoirs of the chips.

Fluorimetric detection of DNA fragments separated by microchannel electrophoresis was done using an optical fiber coupled excitation and detection system, as illustrated in Figure 2. The output from a green (520 nm max) light emitting diode

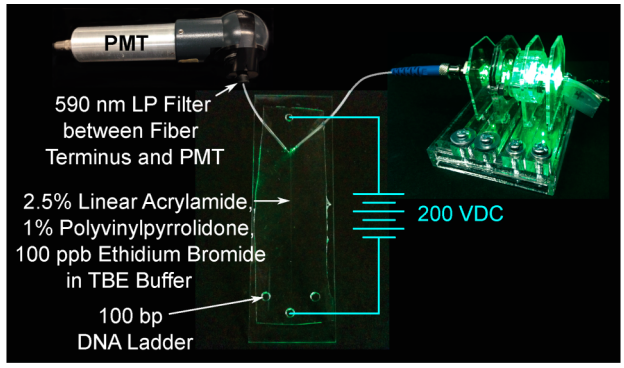

Figure 2. Pictorial representation of the components used in the microfluidic electrophoresis experiment for the separation of a 100 base pair (bp) DNA ladder.

(LED) was passed through a 530/55 nm optical bandpass filter (Newport Corporation, Irvine, CA) and coupled into an optical fiber (3M Powercore FT-400-URT, distributed by Thorlabs, Inc., Newton, NJ) by use of two plano-convex coupling lenses (12.7 mm diameter, 19.0 mm EFL, Newport Corporation), which served as the excitation source. For the detection arm, a second optical fiber was used to capture and deliver a portion of the fluorescence emission from the microchannel to a 590 nm colored glass long pass filter (Oriel Instruments, Stratford, CT) and a PMT module (recovered from an obsoleted Carl Zeiss IM fluorescence microscope (Carl Zeiss Canada, Toronto, Ontario, Canada). The optical fibers were introduced to ports cast on either side of the separation channel of the microfluidic electrophoresis device, as shown in Figure 2. The output of the PMT module was connected to the NI 6008 USB data acquisition module (National Instruments), and software developed in-house was used to record fluorescence intensity data with respect to time. The fiber-optic fluorescence detection system and data logging software were prepared ahead of time by the instructor for routine student use.

■ HAZARDS

Students should use great care when handling solutions containing ethidium bromide and acrylamide. Ethidium bromide is a toxin, and potential carcinogen and mutagen (though not formally classified as such by the International Agency for Research on Cancer or the U.S. National Toxicology Program). Acrylamide, used in the preparation of the sieving matrix for the microchannel electrophoresis experiment, is a neurotoxin and carcinogen. Lab coats, safety glasses, and gloves should be worn at all times during the course of these experiments.

■ RESULTS AND DISCUSSION

Fabrication of Microfluidic Devices, Characterization by Scanning Electron Microscopy, and Device Assembly

Microfluidic master molds were prepared by laser printing patterns onto biaxially oriented polystyrene thermoplastic sheets as per the method of Grimes et al.[11] These thermoplastic sheets shrink to approximately 40% of their original size when heated to 125 °C for 30 min, as shown in Figure 3A, causing

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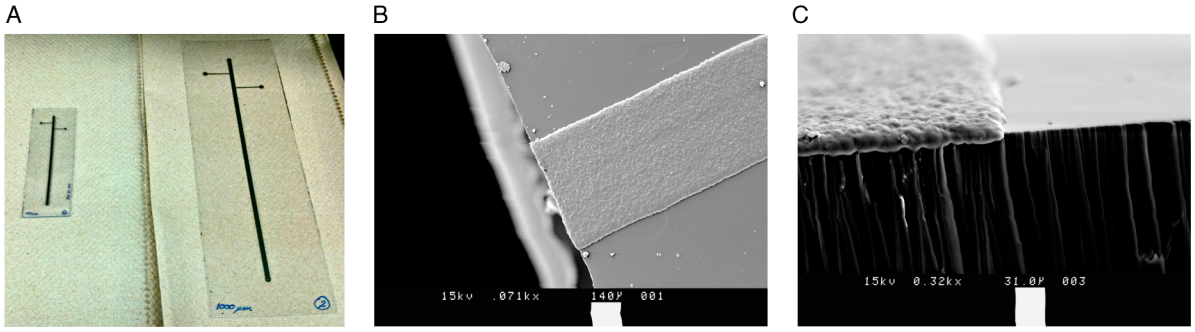

Figure 3. Thermoplastic microfluidic molds: (A) thermoplastic substrate before (right) and after (left) heat treatment (125 °C for 30 min). (B) Scanning electron microscope image of a 550 μm wide toner channel feature on a shrunken thermoplastic substrate. (C) Substrate shown in (B) oriented at 75° incidence with respect to the electron beam, revealing a ca. 20 μm microchannel height.

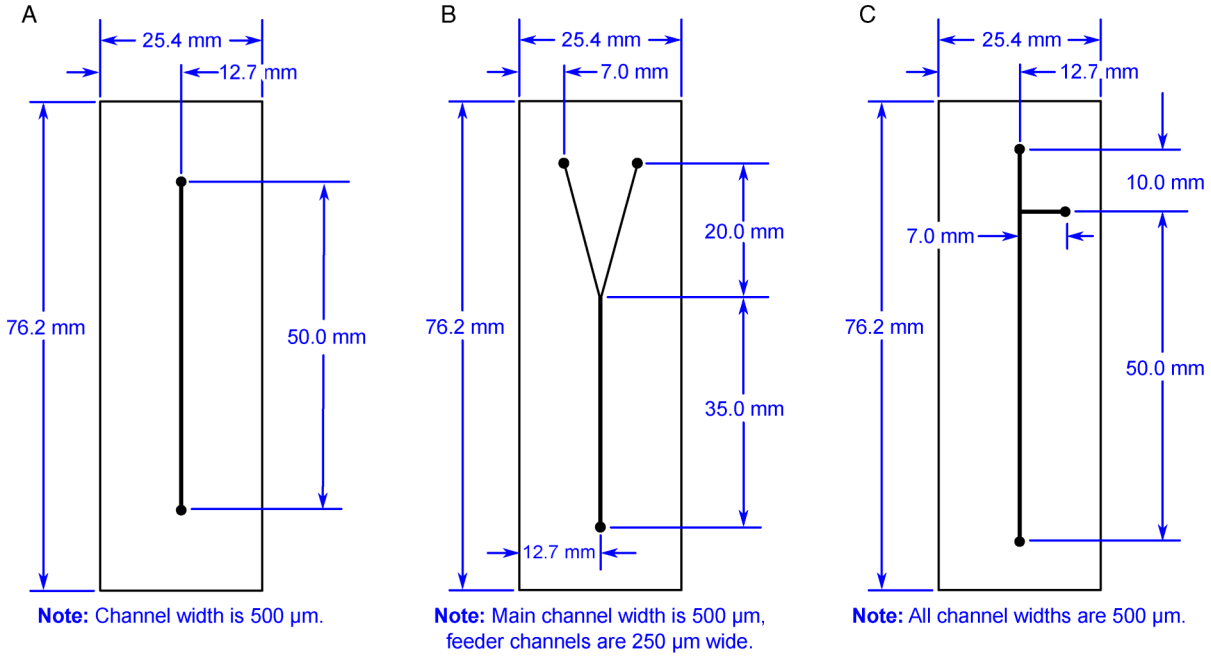

Figure 4. (A) Linear channel (for channel characterization by SEM, electroosmotic flow investigations, and zeta potential determinations). (B) Y-Channel (for Péclet number determination). (C) Linear separation channel with L-shaped injector for microchannel DNA electrophoresis.

toner features printed onto the sheets to become more pronounced and stand, for the laser printer model we used, ~20 μm above the substrate material. Typical desktop laser printers provide 600 dots per inch (dpi) resolution, which translates to a minimum line width of 42 μm. This further translates to 17 μm minimum feature widths for lines printed onto polystyrene thermoplastic substrates after shrinkage. In terms of cost and practicality, this approach to cleanroom-free template fabrication proved to be the least expensive (~$0.25 per template), provided the best quality templates (see Figure 3B,C) and greatest ease of fabrication, and required the least preparation time (30 min) of a number of methods surveyed, which included photolithography and toner transfer done on both copper sheets and on printed circuit board substrates.[12−14]

Using Inkscape,[15] an open-source vector-based graphics program, students learned to create accurate vector-based microchannel design drawings. The drawings were prepared at 2.5× scale so that the templates created would be of the dimensions shown in Figure 4 after shrinking. Students provide a sample of a shrunken thermoplastic mold (as per the design shown in Figure 4A) to the laboratory technician for imaging by scanning electron microscopy (as shown in Figure 3B,C). This permits the students to accurately determine microchannel dimensions, as required for calculations of zeta potential, electroosmotic flow velocity, and Péclet numbers.

PDMS castings are created by application and curing of a PDMS pre-polymer and curing agent solution onto the thermoplastic template, as detailed in the student manual (see the Supporting Information). A photograph of a microfluidic device created from the design illustrated in Figure 4B is shown in Figure 5. Templates and castings may be prepared and microfluidic devices assembled by the students in less than an hour at a total cost of ~ $1 per device. All of the templates and PDMS castings are prepared in the first week of the laboratory module, as well as most of the microfluidic chips. This exposes students to the key concepts underlying microfabrication and provides them with the opportunity to develop their dexterity in a laboratory environment, a pivotal step in the transition from chemistry laboratory exercises to analytical chemistry research.

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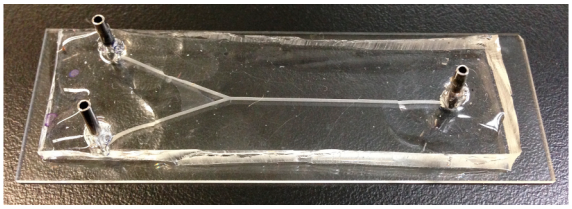

Figure 5. Photograph of a Y-channel microfluidic device with stainless steel tubing connection ports. The microfluidic chip was prepared using the template design shown in Figure 4B.

Mixing in Microchannels: Péclet Number and Diffusion Coefficient Determination

One of the most interesting features of microfluidic devices is the behavior of liquids flowing in microchannels and how it differs from the macroscale. Experiments were devised to demonstrate the concepts of laminar flow and diffusion-based mass transport. The metric most commonly used to report requirements for diffusion-based mixing in systems undergoing laminar flow is the dimensionless Péclet number (*Pe*), which may be calculated as

$$Pe = \frac{L_{LD} \cdot v_{avg}}{D} \quad (1)$$

where L_{LD} is the length scale for lateral diffusion, v_{avg} is the average linear flow velocity, and D is the diffusion coefficient of the solute within the fluid system.[16] In practice, the *Pe* represents the ratio of the distance traveled by a species along a microchannel to the lateral diffusion distance.

These concepts are demonstrated by having students merge solution streams of the colorimetric pH indicator bromophenol blue (aqueous, sat'd, pH 3) and 0.1 M NaOH at various flow rates in a Y-shaped microchannel (as shown in Figure 5) in the second week of this laboratory module. Representative images of the diffusion-based deprotonation of bromophenol blue under various flow conditions are shown Figure 6. The *Pe* of aqueous protons and the dye at various flow rates can be approximated from these images by measuring the distance to which the blue (deprotonated) dye is observed to extend to the right and left of the center line of the channel, respectively, relative to the distance traveled along the channel from the intersection. With *Pe* values approximated, an estimate of the diffusion coefficient of aqueous protons and the dye can be obtained from eq 1.

Fluid Flow in Microchannels: Investigations of Electroosmotic Flow Velocity and Zeta Potential Determination

In the third week of the experiment, students investigate the phenomenon of electroosmotic flow (EOF) and determine the zeta potential at the charged walls of the microchannel devices. This was done by implementation of the current monitoring approach developed by Sze et al.[17] Zeta potential and average EOF velocity directly correlate to the slope of current versus time curves obtained when a low conductivity buffer is replaced by a buffer of greater conductivity in the microchannel by EOF. The slope of the transition in the current versus time curve (Figure 7) is introduced to modified forms of the

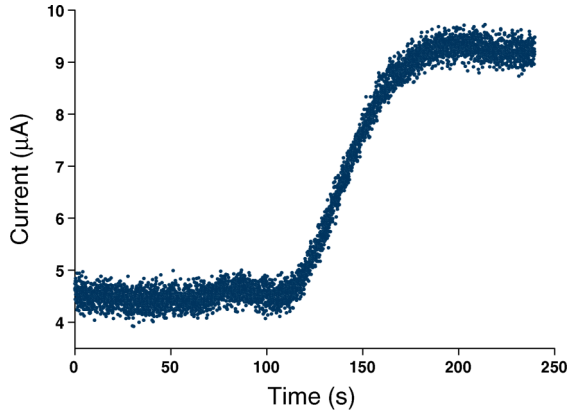

Figure 7. Determination of zeta potential and average electroosmotic flow velocity as per the current monitoring method of Sze et al.[17] EOF was induced in a PDMS-glass microchannel (250 μm × 60 μm × 60 mm, similar shape to Figure 4A) filled with 100 μM KCl by application of 500 V dc potential across the channel length. After ca. 100 s, the solution in the anodic fluid reservoir was exchanged with 1.00 mM KCl. From the slope of the current versus time curve, the zeta potential of the microchannel walls was determined to be −38.5 ± 0.8 mV and the average electroosmotic flow velocity to be 2.00 ± 0.04 mm/s.

Smoluchowski equation[17] to yield the zeta potential (ζ) and average linear EOF velocity ($v_{avg\ EOF}$) in the microchannel, as given by

$$\zeta = \frac{\mu \cdot \text{slope} \cdot L}{\varepsilon_r \varepsilon_0 E_z^2 A_{cross} (\lambda_{b_2} - \lambda_{b_1})} \quad (2)$$

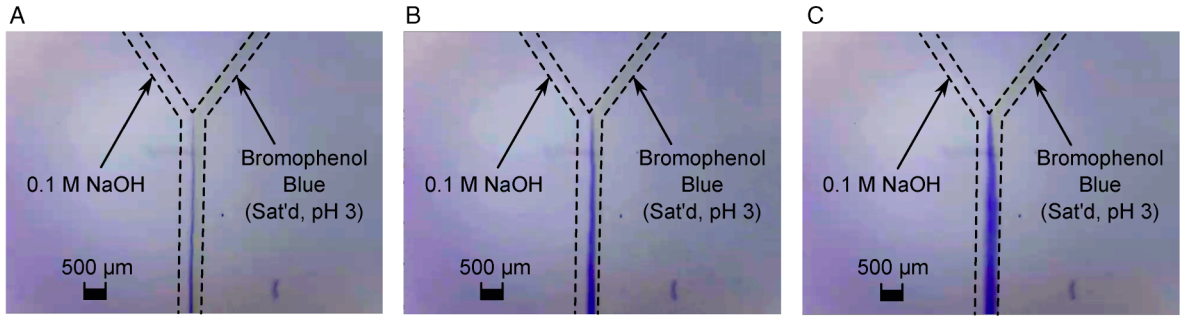

Figure 6. Evaluation of Péclet numbers for the widthwise diffusion of protons and bromophenol blue across the microchannel at flow velocities of (A) 5.8 mm/s, *Pe* = 76, (B) 1.2 mm/s, *Pe* = 33, (C) 0.23 mm/s, *Pe* = 5.7. All images were obtained using an inexpensive USB microscope. Dashed lines have been superimposed onto the images to indicate the location of the microchannel walls.

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$$v_{\text{avg EOF}} = \frac{\text{slope}\cdot L}{E_z A_{\text{cross}}(\lambda_{b_2} - \lambda_{b_1})} \quad (3)$$

where E_z is the applied electric field strength, A_{cross} is the cross-sectional area of the microchannel, μ is the solution viscosity, λ_b is buffer conductivity, L is the length of microchannel, ε_r is the relative dielectric constant of the electrolyte solution, and ε_0 is the electrical permittivity of free space.

Microchannel Electrophoresis with On-Line Fluorimetric Detection for the Separation and Analysis of a DNA Ladder

A simple microchannel electrophoresis device is constructed by the students in the fourth week of the experiment to provide them with the opportunity to create and evaluate a lab-on-a-chip device. The device construct is illustrated in Figure 2 and a representative electropherogram of a 100 bp DNA ladder separated using a student-prepared device is shown in Figure 8.

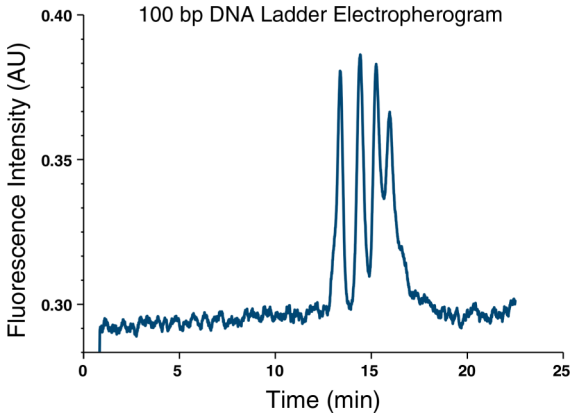

Figure 8. Representative result from a student-built microfluidic electrophoresis device with on-chip fiber-optic fluorescence detection for the separation of DNA fragments of different length.

The left-most peak appearing at ca. 13 min represents the 100 bp fragment, followed by the 200, 300, and 400 bp fragment. Larger fragments were not resolved in this particular electropherogram. In this experiment, students learn about the structure and electrophoretic mobility of DNA and why a sieving matrix is required to achieve separation of DNA fragments as a function of length. Students learn about the binding motifs of small molecules to DNA, in this case, the intercalative binding of ethidium bromide into the base-stacking region of the double-stranded nucleic acid complexes. Students are also exposed to fluorescence theory as they must account for the change in the quantum yield of the ethidium cation on binding to double-stranded DNA and hence the mechanism of signal generation in this experiment. On-chip detection is achieved through the use of a fiber-coupled excitation source and detector, which is provided to the student, and provides for exploration of the basic concepts of how optical fibers transmit light and the design of a fluorescence based detection instrument.

■ CONCLUSIONS

This multi-week laboratory experiment on microfluidics provides an inexpensive and easily implementable route to introduce modern analytical chemistry research techniques (i.e., microfluidic device development) to undergraduate chemistry students. Over four laboratory sessions, students gain hands-on experience with the fabrication of microfluidic devices, learn about fluid flow in microchannels, and build and characterize their own microchannel electrophoresis device. Over the two years in which this laboratory module has been offered, students have participated eagerly and become well engaged in this experiential learning opportunity, as attested to by the students themselves:

"I liked the microfluidics module, as it was cool and exciting to actually perform experiments on a chip."... "to actually perform an experiment that used microfluidic principles was really rewarding. It is a new technique that is growing rapidly, and to actually say that I have experience with performing chemical analyses on microfluidic chips is a good skill to have for the future in terms of the job market." (Student comment provided on an anonymous feedback forum.)

■ ASSOCIATED CONTENT

Supporting Information

Instructor notes, student instructions and software. This material is available via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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