Single nanopore transport of synthetic and biological polyelectrolytes in three-dimensional hybrid microfluidic/nanofluidic devices

Travis L. King,^{1,2} Enid N. Gatimu,² and Paul W. Bohn^{2,3,a)}

¹*Department of Chemistry, University of Illinois at Urbana-Champaign, 600 South Mathews Avenue, Urbana, Illinois 61801, USA*

²*Department of Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, Indiana 46556, USA*

³*Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556, USA*

(Received 1 December 2008; accepted 8 December 2008; published online 2 January 2009)

This paper presents a study of electrokinetic transport in single nanopores integrated into vertically stacked three-dimensional hybrid microfluidic/nanofluidic structures. In these devices, single nanopores, created by focused ion beam (FIB) milling in thin polymer films, provide fluidic connection between two vertically separated, perpendicular microfluidic channels. Experiments address both systems in which the nanoporous membrane is composed of the same (homojunction) or different (heterojunction) polymer as the microfluidic channels. These devices are then used to study the electrokinetic transport properties of synthetic (i.e., polystyrene sulfonate and polyallylamine) and biological (i.e., DNA) polyelectrolytes across these nanopores using both electrical current measurements and confocal microscopy. Both optical and electrical measurements indicate that electro-osmotic transport is predominant over electrophoresis in single nanopores with $d > 180$ nm, consistent with results obtained under similar conditions for nanocapillary array membranes. © 2009 American Institute of Physics.

[DOI: [10.1063/1.3059546](https://doi.org/10.1063/1.3059546)]

I. INTRODUCTION

The use of tailored nanofluidic elements to couple vertically separated microfluidic channels adds considerable power and flexibility to micrototal analytical systems (μ -TAS), by freeing them from the design constraints imposed by planarity.¹ The resulting three-dimensional hybrid microfluidic/nanofluidic devices allow for a wide variety of analytical unit operations to be implemented in these devices. Gated analyte injection,² fraction collection after electrophoretic separation,³ reactive mixing,^{4,5} and preconcentration⁶ have all been demonstrated using polycarbonate track etched nanocapillary array membranes (NCAMs) as the nanofluidic interconnects. NCAM-based structures have also been used to maintain discrete chemical environments in different layers of a chip while simultaneously allowing analyte molecules to be transferred between layers.⁷ Thus, independent microfluidic channels connected by independently addressable nanofluidic elements are of considerable interest.

Clearly, effective implementation of these chemically modulated fluidic switching functions relies on a thorough understanding of the special properties of the nanofluidic interconnects. To this end, significant experimental and computational effort has been expended to understand the detailed transport properties of nanofluidic interconnects, for example, elucidating ion enrichment and depletion effects due to differential transport rates for cations and anions in permselective

^{a)} Author to whom correspondence should be addressed. Electronic address: pbohn@nd.edu.

NCAM-based systems.⁸ However, gaining a more complete understanding of transport processes in these pores is complicated by the innate dispersion of nanopore characteristics (e.g., length, diameter, angle) averaged over the thousands of nanopores in an NCAM. Thus, study of a single nanopore providing connection between two microfluidic channels is highly desirable; any dispersion in pore characteristics is eliminated and, with proper fabrication and characterization, the experimental system, i.e., pore dimensions, materials, chemistry,⁹ etc., can be defined at will. The ability to fabricate high aspect ratio nanopores of arbitrary size, spacing, and location in polymer films has been demonstrated previously.¹⁰ In this paper, we describe the fabrication methods to integrate these nanopores into hybrid microfluidic/nanofluidic devices and subsequent electrical and optical characterization of molecular translocation across the single nanopore.

Studying single nanopores in microfluidic devices presents an array of analytical challenges due to the small amounts of material involved in single pore transport events. Synthetic [polystyrene sulfonate (PSS[−]) or polyallylamine (PAA⁺⁺)] and biological (DNA) polyelectrolytes are ideally suited as probes of nanofluidic behavior, because their size is commensurate with the nanostructures of interest. Thus, substantial theoretical attention has been directed toward the transport of polyelectrolytes in nanopores^{11,12} and nanoslits.¹³ Simulations of the behavior of polyelectrolytes in confined environments¹⁴ such as viral capsids¹⁵ have also been carried out. However, due to the technical challenges of obtaining information on transport in single nanopores, experimental work has lagged behind.

Experimental efforts to study polyelectrolyte transport can use either electrical or optical measurement techniques. Measurements of transient current reductions associated with DNA translocation across single protein nanopores have been used to elucidate the effects of voltage¹⁶ and pore asymmetry.¹⁷ Special effort has been directed to the study of DNA transport in nanopores, both because DNA is a model polyelectrolyte and because it presents the possibility of manipulating and sequencing single DNA molecules.¹⁸ Both integrated electrodes¹⁹ and optical tweezers^{20,21} have been used to study and control the dynamics of DNA-nanopore interactions. Fluorescence microscopy has been used to study the effect of ionic strength²² and molecular size,²³ and confocal fluorescence microscopy has been applied to observe the dynamics of single DNA molecules in silica nanoslits^{18,24} and lipid nanotubes.²⁵

In this paper, we report robust fabrication strategies for the incorporation of single focused ion beam (FIB) milled polymer nanopores into three-dimensional (3D) hybrid microfluidic/nanofluidic devices, as well as electrical and optical characterization of transport in these structures. Although facilitating the construction of highly functional vertically integrated microfluidic structures, optical interrogation of transport in hybrid nano/microfluidic 3D structures is complicated, when compared to planar structures, because molecular translocation occurs along the optic axis. Spherical aberration due to refractive index mismatches in these devices reduces the depth discrimination of confocal microscopic images,²⁶ so special strategies have been developed to enable the confocal fluorescence detection of interchannel DNA switching.

II. MATERIALS AND METHODS

A. Reagents and materials

Polystyrene sulfonate sodium salt (PSS), polyallylamine chloride salt (PAA), tris(hydroxymethyl)aminomethane (TRIS), ethylenediaminetetraacetic (EDTA), λ -phage DNA, and acetic acid (Sigma Aldrich Co., St. Louis, MO) and YOYO-1 (Invitrogen, Carlsbad, CA) were used as received. All aqueous solutions were prepared using deionized (DI) water (18.2 M Ω cm, Milli-Q Gradient, Millipore, Bedford, MA). All buffer solutions were filtered through 0.1 μ m membrane filters (Millipore, Bedford, MA) before use. Stock solutions of PAA and PSS were prepared by dissolving 1% [weight/volume (w/v)] PSS or PAA in deionized (DI) water. YOYO-1 dyed λ -phage DNA was prepared by dissolving either 5 or 50 μ g/mL DNA salt in a solution of pH 8.2 40 mM Tris-acetate, 1 mM EDTA (TAE buffer) containing YOYO-1 in a ratio of 1:5 YOYO-1:DNA base pairs and incubated at room temperature for 1 h.

FIG. 1. (a) SEM of a single FIB-milled nanopore in a 10 μm thick PDMS membrane; $r_{\text{pore}}=150$ nm. (b) SEM of a 5 $\times$ 5 nanopore array with fiducial marks around each pore ($r_{\text{outer}}=10$ μm) and at the array corners ($r=25$ μm) to facilitate alignment in completed devices.

Standard rapid prototyping protocols were used to create poly(dimethylsiloxane) (PDMS) layers with embedded microchannels and alignment marks. All positive relief molds were fabricated on piranha-cleaned silicon wafers (Silicon Quest International, Santa Clara, CA) using SU-8 2010 or SU-8 2050 photoresist (Microchem Corp., Newton, MA). These molds were then exposed to tridecafluoro-1,1,2,2-tetrahydrooctyltrichlorosilane (Gelest, Morrisville, PA) vapor overnight to aid in the release of cured PDMS from the underlying mold. Negative-tone photolithography masks were created via high-resolution transparency printing (5080 dpi). PDMS (Sylgard 184, Dow Corning, Midland, MI) was thoroughly mixed in a 10:1 ratio, poured or spin-cast (for confocal microscopy studies, *vide infra*) on the positive relief molds, and cured at 85 $^{\circ}\text{C}$ to form all microfluidic channels.

B. Single nanopore fabrication

PMMA (5:1:1 350 kDa PMMA:methylmethacrylate:anisole) or PDMS prepolymer (5:1 base:curing agent) films were spin cast on 5 mm $\times$ 5 mm (PMMA) or 3 in. (PDMS) sacrificial Si carrier wafers at 6000 rpm and cured at 195 $^{\circ}\text{C}$ (PMMA) or 100 $^{\circ}\text{C}$ (PDMS) for 1 h. A small amount of poly(ethyleneoxide)-poly(dimethylsiloxane) copolymer (Gelest, Morrisville, PA) was added to PMMA solutions before spin casting to aid release of the cured film from the underlying substrate after fabrication. All films were coated with Au to reduce charging during FIB milling and scanning electron microscopy (SEM) imaging. Nanopores were milled via a 30 kV focused ion beam (FIB) (Strata Dual Beam 235, FEI, Hillsboro, OR) using beam currents in the range 1 $< i < 30$ pA to yield cylindrical pores $80 < d < 400$ nm in either polymer [Fig. 1(a)]. Two shallow concentric circular fiducial marks ($r_{\text{outer}}=10$ μm) were milled around each pore to allow location of a single pore during confocal microscopy experiments. Pores were fabricated in 5 $\times$ 5 or 10 $\times$ 10 arrays, with individual pores separated by the width of the microchannels in the finished device to ensure that a single nanopore would be aligned to the two microchannels (see below). Additionally, another shallow circular fiducial mark ($r=25$ μm) was milled at each corner of the array to aid in visualization during alignment [Fig. 1(b)].

C. Homojunction device fabrication

The device design used in these experiments is shown schematically in Fig. 2. Two PDMS microfluidic channels, a “source” microchannel and a “receiving” microchannel (50 μm wide $\times$ 10 μm deep $\times$ 10 mm long), are fluidically connected by a single PDMS nanopore. To construct this homojunction device, O_2 plasma (1 Torr) was used to bond coverglass to an ~ 20 μm

FIG. 2. Schematic diagram showing the relative dimensions of a three-dimensional hybrid microfluidic/nanofluidic device with a single nanopore providing fluidic connection between the microfluidic elements. In the functioning devices studied here, the source and receiving microchannels are orthogonal.

thick PDMS film formed by spin casting directly onto a positive-relief mold of the microchannel features. This covalently bonded entity was then carefully removed from the underlying Si mold to yield the bottom half of the device, a piece of microscope coverglass with an upward-facing PDMS microchannel. The top microchannel was then fabricated in a relatively thick (~ 3 mm) PDMS layer. Fluidic vias were cut to allow access to the two microchannels, and the top monolith was covalently bonded to the PDMS nanofluidic membrane via O_2 plasma exposure. This moiety was then lifted from its underlying Si carrier, and the portions of the nanofluidic membrane occluding the fluidic vias were removed. Alignment of a single nanopore to the top microchannel was achieved by aligning the microchannel between the FIB-milled fiducial marks on the nanofluidic membrane prior to placing the two plasma-treated PDMS layers into contact. The upper (top microchannel and nanopore membrane) and lower (coverglass and bottom microchannel) constructs were then O_2 plasma bonded to complete the device; alignment of the nanopore to the bottom microchannel was achieved as for the top microchannel.

D. Heterojunction device fabrication

A schematic of the device design used is shown in Fig. 2. In this device, two PDMS microchannels, a source microchannel and a receiving microchannel ($50\ \mu\text{m}$ wide $\times$ $50\ \mu\text{m}$ high $\times$ $10\ \text{mm}$ long), are fluidically connected by a single PMMA nanopore. Both microchannels are formed by pouring PDMS prepolymer into a relatively thick (~ 3 mm) layer on the Si mold. The bottom microchannel is then bonded to a glass carrier with the microchannel facing up. To transfer the PMMA membrane to this bottom construct, the fiducial marks on the PMMA membrane are carefully aligned to the microfluidic channel, and the two surfaces are gently pressed into conformal contact. A piece of adhesive tape is pressed onto the sacrificial Si carrier holding the nanofluidic membrane, and the carrier is rapidly pulled away, leaving the free PMMA layer behind. The top microchannel is fabricated in the same fashion as the bottom with the addition of two sets of vias: large vias to provide fluidic access to the microchannels and small ($\sim 1\ \text{mm}$) vias to allow sealing at the PMMA-PDMS interface. The top microchannel is then O_2 plasma sealed to the bottom construct with careful alignment of the large vias to the microchannels and the small vias to the corners of the PMMA membrane. To seal the corners of the PMMA membrane, the entire chip is heated to $90\ ^\circ\text{C}$, and PDMS prepolymer is inserted into each small via and cured for $\sim 1\ \text{h}$.

FIG. 3. Schematic of the confocal laser-induced fluorescence microscope constructed to study molecular translocation across single nanopores. L is the excitation laser; L1-2 is the beam expanding telescope; BS is the dichroic beamsplitter; O is the microscope objective; M1 is the turning mirror; TL is the tube lens; PH is the pinhole; L3 is the focusing lens; EF is the emission filter; APD is the avalanche photodiode; PC/MCS is the multichannel scaler/computer.

E. Electrical characterization of polyelectrolyte transport

Electrokinetic polyelectrolyte transport through single nanopores was monitored by measuring the *trans*-nanopore current when the pore was electrically biased. Pt-wire electrodes inserted into each reservoir provided electrical connection to an external eight-relay switching system, voltage source (SourceMeter 2400, Keithley Instruments, Cleveland, OH), and nanovoltmeter (Model 2182, Keithley Instruments, Cleveland, OH). Current was recorded by measuring the voltage across a 10 k Ω resistor in series between the voltage source and nanopore. In each experiment, both microchannels were filled with the polyelectrolyte to be studied, and electrical potentials were applied between the two microchannels; $\Delta V = V_{\text{receiving}} - V_{\text{source}}$.

F. Optical characterization of polyelectrolyte transport

1. Confocal microscope

All confocal microscopy experiments were conducted on a specialized system constructed in-house (Fig. 3). The output of a 488 nm solid-state laser (Sapphire488-20, Coherent, Santa Clara, CA) was expanded to overfill the back aperture of a 40 $\times$, 1.3 NA objective lens (Fluar 40 $\times$, Carl Zeiss) which focused the beam to a diffraction-limited spot in the sample plane. A long-pass dichroic mirror (Q505LP, Chroma, Rockingham, VT) reflected excitation light into the objective lens while allowing the collected fluorescence to pass through. Collected fluorescence radiation was focused by a 200 mm focal length tube lens onto a confocal pinhole (25 or 50 μm) (Newport Corp., Irvine, CA). Light passing the confocal pinhole was passed through a 535 ± 50 nm bandpass filter (HQ535/50, Chroma, Rockingham, VT) to exclude any stray or scattered light and focused onto an avalanche photodiode (APD) (SPCM-AQR-16, Perkin-Elmer Optoelectronics, Fremont, CA). Output pulses from the APD were counted into discrete time bins by a PC-based multichannel scalar (MCS-PCI, Ortec, Oak Ridge, TN). Coarse objective positioning along the optical axis was accomplished by a closed-loop computer controlled actuator (CMA-25CCCL, Newport Corp., Irvine, CA), while fine positioning along the same axis was controlled by a piezoelectric actuator (P-721, Physik Instrumente, Auburn, MA). Sample positioning in the two dimensions orthogonal to the optical axis was accomplished by a piezoelectric stage (P-517.2CL, Physik Instrumente, Auburn, MA) fitted with a custom sample holder. To position the sample prior to data collection, the sample was illuminated with white light from above, and a removable mirror was placed before the confocal pinhole to direct the transmitted light to a video-rate charge-coupled device (CCD) camera.

2. Optical characterization of electrokinetic DNA transport

Trans-nanopore DNA transport was monitored by measuring laser-induced fluorescence from the YOYO-labeled DNA via confocal microscopy in the receiving channel directly below the nanopore. The source channel was filled with 5 $\mu\text{g/mL}$ YOYO-1 labeled λ -phage DNA in 40 mM TAE buffer, and the receiving channel was filled with 40 mM TAE buffer only. Pt-wire electrodes were inserted into each reservoir of the device to provide electrical contact to a custom eight-relay switching system and a voltage source (Sourcemeter 2400, Keithley Instruments, Cleveland, OH), both controlled by Labview software (National Instruments). Fluorescence intensity was recorded at a rate of 100 Hz with 1 mW excitation power, while electrical potentials were applied between the source and receiving channels for 3 s followed by grounding both channels for 20 s.

III. RESULTS AND DISCUSSION

A. Homojunction and heterojunction device fabrication

Developing the protocol to study molecular translocation across single vertical nanopores connecting orthogonal microfluidic channels begins with a strategy to fabricate a single nanopore and position it between the cross microchannels. The approach taken here utilizes a periodic square array of identical nanopores with each nanopore prepared by FIB milling and separated from its nearest neighbor by the width of the microfluidic channels. Figure 1(a) shows a single nanopore prepared by FIB milling a 10 μm thick PDMS membrane for 10 s with $i_{\text{milling}} = 30$ pA. Two features are readily apparent in the SEM micrographs: a small depression around the pore mouth and minor cracking along the margins of this depression. It is likely that beam steering due to charging of the insulating sample during the milling process is the cause of the depression. SEM images (not shown), acquired in backscattering geometry to provide atomic number contrast, confirm that the cracking is only in the Au coating of the membranes.

Figure 1(b) shows a 5×5 array of nanopores with all accompanying fiducial marks. The array spacing is chosen such that two crossing microchannels define an area containing one and only one nanopore (see Fig. 2 and below). The fiducial marks at the corners of the array facilitate the alignment of the array to the microchannels. Because the array is 200 μm across, and a typical microchannel is 50 μm wide, alignment is readily accomplished with the naked eye and manual manipulation of the various components. Two rings also surround each nanopore; these markings allow each nanopore to be located via brightfield microscopy so that the confocal microscope focal volume can be precisely positioned under the exit aperture of the single pore that provides fluidic connection between microchannels.

Both homojunction (all-PDMS) and heterojunction (structures with PMMA membranes separating PDMS microchannels) devices were fabricated and studied. Each structure presents characteristic benefits as well as challenges and limitations. Heterojunction devices are hampered by surface energy differences at the PDMS-PMMA interfaces, making interfacial bonding difficult and necessitating the use of PMMA membranes (e.g., 5 mm $\times$ 5 mm) smaller than the surrounding PDMS, to permit a strong, stable PDMS-PDMS seal to be established. However, this creates a height mismatch at the edge of the PMMA membrane, and thus a gap. This is an especially pernicious problem when conducting experiments with single nanopores, since even very small leakage currents circumventing the nanopore can quickly overwhelm the current due to ion transport through the nanopore. To address this problem, the gaps were backfilled with PDMS prepolymer via a series of ~ 1 mm vias positioned at the membrane corners. In contrast, homojunction devices do not suffer from interfacial energy problems, and PDMS can readily be bonded to itself. This allows the nanopore-containing membrane to span the entire area of the device structure, thereby eliminating leakage current around the membrane. On the other hand, PDMS is inherently hydrophobic, and filling PDMS nanopores with aqueous solutions is problematic. Rendering the PDMS surface hydrophilic by O_2 plasma treatment can temporarily alleviate nanoscale surface tension issues, but experience shows that the nanopores ultimately revert to their initial hydropho-

FIG. 4. Current vs voltage behavior of a single PDMS nanopore connecting two vertically separated microfluidic channels. Ohmic response yields $R_{\text{pore}}(-10-0 \text{ V})=49.2 \text{ M}\Omega$.

bic character, and once the device is sealed, further O_2 plasma treatment is precluded. Filling can also be aided with mixtures of organic solvents and/or surfactants with water, but care must be exercised lest the large-area PDMS-PDMS seal be disrupted.

Figure 4 shows the current-voltage behavior for a hybrid microfluidic/nanofluidic device in which a single PDMS nanopore connects the microchannels. The device was filled with 1.0 M KCl, and all potentials were applied between receiving and source channels, $\Delta V = V_{\text{receiving}} - V_{\text{source}}$. Due to the high resistance of the device, the current-voltage plot was constructed in stepwise fashion, applying potentials in 1 V steps and averaging the current over time to construct the curve. A small amount of directional hysteresis due to capacitive effects is evident in the i - V characteristic. This can be explained by ionic reorganization in the source and receiving channels. More specifically, differential transport rates for cations and anions across the nanopore cause the formation of space-charge regions at the nanofluidic-microfluidic interface.²⁷ Linear fitting to portions of the i - V curve yields a resistance of 49.2 M Ω . The mobilities of the constituent K^+ and Cl^- ions in the solution, the 10 μm membrane thickness, and a cylindrical pore assumption yield an effective pore diameter of 132 nm. Note that this is smaller than the $\sim 300 \text{ nm}$ diameter obtained from the entrance aperture of a pore shown in Fig. 1, as would be expected for the expected tapered pore shapes produced by FIB milling in thick (relative to the electron backscatter length) polymer films.

B. Electrical measurement of synthetic polyelectrolyte transport across single nanopores

Figure 5 shows the measured current during episodic electrokinetic transport of (a) 1% PSSⁿ⁻ and (b) 1% PAA^{m+} solutions across a single 180 nm PMMA nanopore. The current associated with transport of the polyelectrolyte species is clearly visible in both cases under negative injection potentials. Because both PDMS microchannels and the PMMA nanochannel in this system have negative ζ -potentials, this observation indicates that transport is dominated by cation-driven electro-osmosis for both probe species. The average current associated with forward bias PSS transport is $\sim 15 \text{ nA}$, while it is 400 nA for PAA, i.e., an $\sim 25\times$ larger current for PAA. Since a large fraction of the monomer units in these polyelectrolyte molecules are charged, the total charge on the polyelectrolyte molecule can dominate the charge interaction with the walls of the nanopore. The 180 nm diam $\times$ 5 μm long nanopore studied here has a volume of $\sim 130 \text{ aL}$, meaning

FIG. 5. Current vs time behavior during periodic injections of (a) 1% (w:v) PSS and (b) 1% (w:v) PAA across a single $5\ \mu\text{m}$ long by $180\ \text{nm}$ diameter PMMA nanopore. Forward bias injection potentials ($V_{\text{receiving}} - V_{\text{source}} < 0$) were applied for 5 s, during the indicated time intervals, after which the potential program was switched back to support microchannel flow for 50 s.

that a well distributed 1% (w:v) solution of a 70 kDa polymer would constitute $\sim 1.1 \times 10^4$ molecules pore^{-1} . A 70 kDa PSS molecule is comprised of $n \sim 180$ repeat units, so even if only 10% of the sulfonic acid residues were ionized, the polyions in the pore would carry 2×10^5 elementary charge units. This must be considered in the context of the surface charge density, which dictates the electrical properties of the nanopore. The pore studied here has a surface area of $2.8 \times 10^{-8}\ \text{cm}^2$, and a moderate surface charge density, such as the $0.35\ \text{mC m}^{-2}$ used in Ref. 27 representing ~ 6100 negative surface charges, would make the PMMA nanopore permselective for cation transport. Thus, the strong bias in favor of PAA^{m+} transport over PSS^{n-} transport is entirely consistent with the permselective character of a negative ζ potential nanopore. Finally, we note that the actual behavior likely reflects multiple effects, some of which are far more subtle than the above arguments based on charge balance. For example, because the radius of gyration of these polyion probes is of the same order of magnitude as the pore diameter, they experience many more interactions with the pore walls than a small molecule probe, thereby accentuating the ionic exclusion effects in the case of the negatively charged PSS, but not the positively charged PAA.

C. Confocal microscopy in 3D fluidic devices

Figure 3 shows the custom confocal microscope that was constructed specifically for the study of these vertically integrated microfluidic/nanofluidic systems. The sample scanning design was chosen so that the focal volume of the microscope could be positioned at a single point in space (i.e., the exit aperture of a single nanopore) and data could be recorded from that position for extended periods. The performance of the microscope was characterized by measuring the axial point spread function (PSF). Figure 6(a) shows a Gaussian fit to the axial PSF, measured as the excitation volume was scanned through a planar, mirrored interface, yielding a half-width $\sigma = 240 \pm 10\ \text{nm}$, in close agreement with theoretical predictions of ideal confocal microscope performance. Figure 6(b) shows that the axial PSF is severely degraded by scanning through the interface between PDMS and fluorescein solution in the bottom microchannel of the integrated microfluidic device. The width of the Gaussian fit measures $\sigma = 8.3 \pm 0.2\ \mu\text{m}$, an increase of a factor of nearly 35. This is in good agreement with the predictions of Hell and co-workers for confocal imaging through water.²⁶ This is especially problematic because the working distance of a 1.3 NA objective lens is $160\ \mu\text{m}$ beyond the coverglass, with throughput of fluorescence photons decreasing greatly as the thickness of nondesign index material (i.e., water or PDMS) between the objective and the collection region increases. Worse yet, the axial PSF in these devices is on the order of the individual layer thicknesses. Thus, attempts to monitor fluorescence in the source channel can be obfuscated by fluorescence excited in and collected from the source channel, since source channel concentrations are typically orders of magnitude greater than concentrations in the receiving channel. This problem can be effectively addressed by sputtering $\sim 100\ \text{nm}$

FIG. 6. Axial point spread functions measured from the confocal microscope in Fig. 3 by (a) scanning the excitation volume through a mirrored interface, and (b) scanning the excitation volume through the PDMS/water interface inside a fluidic device. Individual points (red) are measurements, and solid curve (blue) is a Gaussian fit. Note the difference in the x -axis scale.

of Au (greater than the optical skin depth) onto the receiving side of the nanofluidic membrane, blocking any fluorescence excited in the source channel from bleeding through into the receiving channel. Although this is certainly effective in isolating the optical signal from the receiving channel (data not shown), the presence of Au places limits on the electrokinetic transport that must be taken into account in characterization experiments.⁸

D. Optical characterization of DNA transport across single nanopores

Figure 7 shows the electrokinetic transport of $5\mu\text{g/mL}$ YOYO-1 labeled λ -phage DNA in 40 mM TAE buffer across a single PMMA nanopore (300 nm diam $\times$ 10 μm long) sandwiched between two 50 μm wide $\times$ 10 μm high microchannels. As illustrated in the figure, application of negative injection potentials $V_{\text{receiving}} - V_{\text{source}} < 0$ results in transport of DNA across the pore, as evidenced by an increase in fluorescence intensity, synchronous with the negative bias application. Negatively charged DNA is transported under negative bias conditions, indicating that transport is dominated by electro-osmosis, just as for the synthetic polyelectrolytes. Analysis of the charge balance is, however, very different than for PSSⁿ⁻. A concentration of $5\mu\text{g/mL}$ of λ -phage DNA is equal to a molar concentration of 1.6×10^{-10} M or 9.6×10^{13} molecules per liter, giving an average pore occupancy (assuming free solution concentrations) of 0.07 molecules. Poisson sta-

FIG. 7. Fluorescence intensity and applied electrical potential vs time during electrokinetic injection of $5\mu\text{g/mL}$ YOYO-1 labeled λ -phage DNA in 40 mM TAE buffer across a single 300 nm diam $\times$ 10 μm long PDMS nanopore.

tistics based on this average occupancy would predict the pore contains 0(1) DNA molecules with probability 0.932(0.065), so most of the time the pore does not contain a DNA molecule. Thus, it is quite reasonable to posit the dominance of electro-osmotic transport under these conditions. Injection experiments were conducted with both polarities. Negative injection potentials support transport of DNA from the source to the receiving channel, while positive injection potentials do not, an observation consistent with the dominance of electro-osmotic flow over electrophoresis. Furthermore, under nominal flow conditions at $\Delta V = -100$ V approximately two pore volumes are transferred in each detection interval (10 ms in Fig. 7), but the confocal probe volume is ~ 10 pore volumes. Furthermore, the persistence length of λ -phage DNA is $\sim 17 \mu\text{m}$,²⁵ significantly longer than the length of the nanopore or the depth of the microchannels. Thus, it is highly likely that the signal observed in Fig. 7 results from a small part of a single YOYO-labeled DNA molecule as it transits the probe volume. Finally, it is interesting to compare hydrodynamic scaling lengths with nanostructure dimensions. Although the radius of gyration, R_g , of λ -phage DNA is 532 nm, larger than the pore radius, transport appears to be facile, likely due to the large driving force exerted by the -100 V injection potential, a large portion of which is dropped across the $10 \mu\text{m}$ thick membrane, creating a strong electric field across the pore.

IV. CONCLUSIONS

The work described here presents the first optical/electrical study of polyelectrolyte translocation across a single nanopore integrated into a vertically stacked structure. These three-dimensional hybrid microfluidic/nanofluidic devices, where single nanopores provide fluidic connection between vertically separated microchannels, provide an excellent test bed for the study of fundamental transport behavior in nanofluidic systems. We have demonstrated the construction and characterization of these hybrid devices in both homojunction and heterojunction configurations and with nanopores of arbitrary size, number, and location, employing both electrical and optical detection strategies. Comparison of episodic electrokinetic transport of PSS^{n-} and PAA^{m+} solutions across a single 180 nm PMMA nanopore shows that transport of both polyelectrolyte species is obtained under negative injection potentials, with the current $\sim 25\times$ higher for polycation transport. This suggests that electro-osmosis is the dominant electrokinetic transport mechanism and that permselectivity effects are strong in this single nanopore of negative- ζ potential, as expected from an analysis of the charge characteristics of the pore and of the probe polyelectrolytes. Confocal laser induced fluorescence microscopy studies of the translocation of YOYO-1 labeled λ -phage DNA also exhibit behavior dominated by electro-osmosis, although DNA samples display far different physical characteristics than their synthetic polymer counterparts, i.e., PSS. Finally, the dominance of electro-osmotic transport in these single nanopore systems is consistent with previous results obtained in NCAM-coupled structures at similar values of the dimensionless group, κd , where κ =inverse Debye length and d =pore diameter.²

The interaction of nanofluidic structures with synthetic and biological macromolecules of comparable size is especially fertile ground for future research. Many opportunities exist to apply these detection modalities for the study of the effects of pore size, surface charge density, analyte size and charge, as well as pore chemistry (e.g., molecular recognition) on diffusive, electrokinetic, and pressure-driven transport in these systems.

ACKNOWLEDGMENTS

This work was supported by the Department of Energy through Grant No. DE FG02 07ER15851 and by the National Science Foundation Center for Advanced Materials for Purification of Water with Systems, the Water CAMPWS, through cooperative agreement No. CTS-0120978.

¹ B. R. Flachsbarth, K. Wong, J. M. Iannaccone, E. N. Abante, R. L. Vlach, P. A. Rauchfuss, P. W. Bohn, J. V. Sweedler, and M. A. Shannon, *Lab Chip* **6**, 667 (2006).

² T. C. Kuo, D. M. Cannon, M. A. Shannon, P. W. Bohn, and J. V. Sweedler, *Sens. Actuators, A* **102**, 223 (2003).

³ J. J. Tulock, M. A. Shannon, P. W. Bohn, and J. V. Sweedler, *Anal. Chem.* **76**, 6419 (2004).

- ⁴T. C. Kuo, H. K. Kim, D. M. Cannon, M. A. Shannon, J. V. Sweedler, and P. W. Bohn, *Angew. Chem., Int. Ed.* **43**, 1862 (2004).
- ⁵J. S. Kirk, J. V. Sweedler, and P. W. Bohn, *Anal. Chem.* **78**, 2335 (2006).
- ⁶Y. Zhang and A. T. Timperman, *Analyst (Cambridge, U.K.)* **128**, 537 (2003).
- ⁷K. Fa, J. J. Tulock, J. V. Sweedler, and P. W. Bohn, *J. Am. Chem. Soc.* **127**, 13928 (2005).
- ⁸A. Piruska, S. Branagan, D. M. Cropek, J. V. Sweedler, and P. W. Bohn, *Lab Chip* **8**, 1625 (2008).
- ⁹B. Y. Kim, C. B. Swearingen, J. A. A. Ho, E. V. Romanova, P. W. Bohn, and J. V. Sweedler, *J. Am. Chem. Soc.* **129**, 7620 (2007).
- ¹⁰D. M. Cannon, B. R. Flachsbar, M. A. Shannon, J. V. Sweedler, and P. W. Bohn, *Appl. Phys. Lett.* **85**, 1241 (2004).
- ¹¹S. Ghosal, *Phys. Rev. E* **74**, 041901 (2006).
- ¹²S. Ghosal, *Phys. Rev. Lett.* **98**, 238104 (2007).
- ¹³J. Jeon and M.-S. Chun, *J. Chem. Phys.* **126**, 154904 (2007).
- ¹⁴J. E. Butler, O. B. Usta, R. Kekre, and A. J. C. Ladd, *Phys. Fluids* **19**, 113101 (2007).
- ¹⁵D. G. Angelescu, J. Stenhammar, and P. Linse, *J. Phys. Chem. B* **111**, 8477 (2007).
- ¹⁶L. Brun, M. Pastoriza-Gallego, G. Oukhaled, J. Mathe, L. Bacri, L. Auvray, and J. Pelta, *Phys. Rev. Lett.* **100**, 158302 (2008).
- ¹⁷G. Gibrat, M. Pastoriza-Gallego, B. Thiebot, M.-F. Breton, L. Ø. Auvray, and J. Pelta, *J. Phys. Chem. B* **112**, 14687 (2008).
- ¹⁸J. T. Mannion, C. H. Reccius, J. D. Cross, and H. G. Craighead, *Biophys. J.* **90**, 4538 (2006).
- ¹⁹B. C. Gierhart, D. G. Howitt, S. J. Chen, Z. Zhu, D. E. Kotecki, R. L. Smith, and S. D. Collins, *Sens. Actuators B* **132**, 593 (2008).
- ²⁰U. F. Keyser, J. van der Does, C. Dekker, and N. H. Dekker, *Rev. Sci. Instrum.* **77**, 105105 (2006).
- ²¹A. Sischka, C. Kleimann, W. Hachmann, M. M. Schafer, I. Seuffert, K. Tonsing, and D. Anselmetti, *Rev. Sci. Instrum.* **79**, 063702 (2008).
- ²²C.-C. Hsieh, A. Balducci, and P. S. Doyle, *Nano Lett.* **8**, 1683 (2008).
- ²³J. D. Cross, E. A. Strychalski, and H. G. Craighead, *J. Appl. Phys.* **102**, 024701 (2007).
- ²⁴C. H. Reccius, S. M. Stavis, J. T. Mannion, L. P. Walker, and H. G. Craighead, *Biophys. J.* **95**, 273 (2008).
- ²⁵M. Tokarz, B. R. Ökerman, J. Olofsson, J.-F. Joanny, P. Dommersnes, and O. Orwar, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 9127 (2005).
- ²⁶A. Egner, M. Schrader, and S. W. Hell, *Opt. Commun.* **153**, 211 (1998).
- ²⁷X. Jin, S. Joseph, E. N. Gatimu, P. W. Bohn, and N. Aluru, *Langmuir* **23**, 13209 (2007).