

# Dynamic Solid Phase DNA Extraction and PCR Amplification in Polyester-Toner Based Microchip

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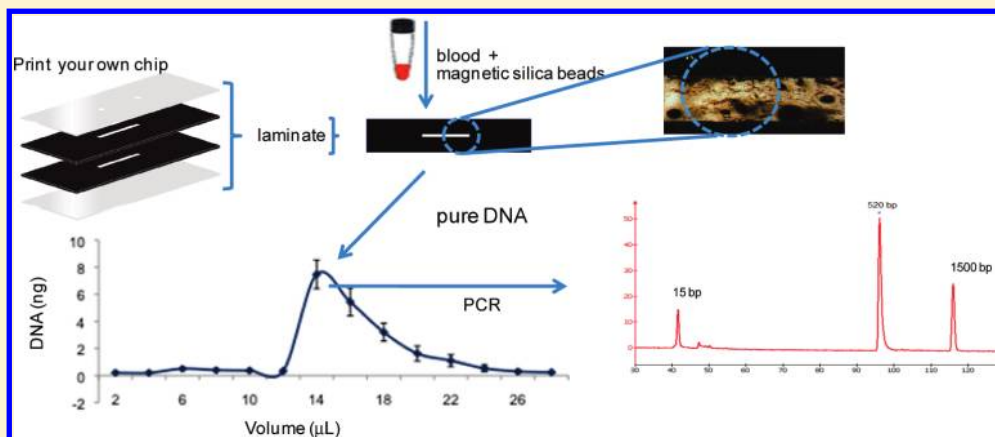
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**S** Supporting Information

## ABSTRACT:



A variety of substrates have been used for fabrication of microchips for DNA extraction, PCR amplification, and DNA fragment separation, including the more conventional glass and silicon as well as alternative polymer-based materials. Polyester represents one such polymer, and the laser-printing of toner onto polyester films has been shown to be effective for generating polyester-toner (PeT) microfluidic devices with channel depths on the order of tens of micrometers. Here, we describe a novel and simple process that allows for the production of multilayer, high aspect-ratio PeT microdevices with substantially larger channel depths. This innovative process utilizes a CO<sub>2</sub> laser to create the microchannel in polyester sheets containing a uniform layer of printed toner, and multilayer devices can easily be constructed by sandwiching the channel layer between uncoated cover sheets of polyester containing precut access holes. The process allows the fabrication of deep channels, with  $\sim 270 \mu\text{m}$ , and we demonstrate the effectiveness of multilayer PeT microchips for dynamic solid phase extraction (dSPE) and PCR amplification. With the former, we found that (i) more than 65% of DNA from  $0.6 \mu\text{L}$  of blood was recovered, (ii) the resultant DNA was concentrated to greater than  $3 \text{ ng}/\mu\text{L}$  (which was better than other chip-based extraction methods), and (iii) the DNA recovered was compatible with downstream microchip-based PCR amplification. Illustrative of the compatibility of PeT microchips with the PCR process, the successful amplification of a 520 bp fragment of  $\lambda$ -phage DNA in a conventional thermocycler is shown. The ability to handle the diverse chemistries associated with DNA purification and extraction is a testimony to the potential utility of PeT microchips beyond separations and presents a promising new disposable platform for genetic analysis that is low cost and easy to fabricate.

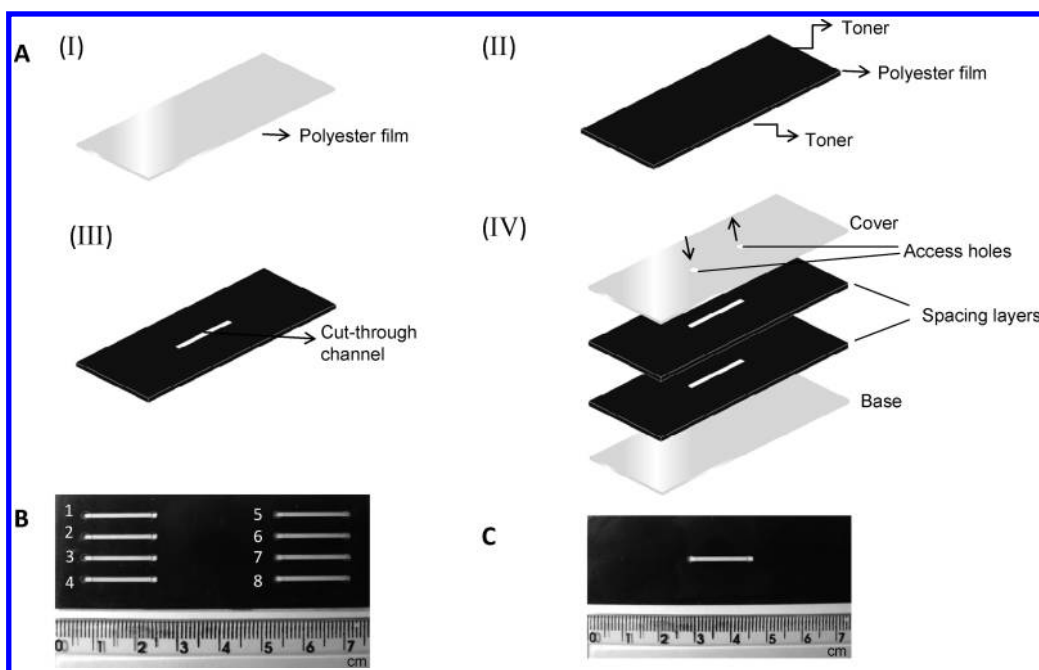
Efforts to develop a microfluidic-based total analysis system ( $\mu\text{TAS}$ )<sup>1</sup> have been intense in the scientific community. The goal of achieving a system comprising DNA extraction, amplification, and detection in one device, characteristics of a true “lab on a chip”, is driven by the substantial advantages associated with such integration. These advantages include (1) reduced cost as a

result of both decreased sample and reagent use and shorter analytical time, (2) fewer manipulation steps and, consequently,

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**Figure 1.** (A) Representation of the steps for the microfabrication process, (I) polyester film, (II) polyester film coated with toner on both sides, (III) microfluidic channel cut by laser cutter, (IV) alignment and lamination of four layers (bottom and top enclosing two middle layers with channel); arrows indicate inlet and outlet access, (B) photograph of an eight-channel microdevice used for parallel DNA extraction, and (C) photograph of PeT microdevice used for PCR amplification.

less chance for sample contamination, and (3) the potential to revolutionize sample analysis via on-site testing and increase access in remote locations. Toward that goal, intense research into the materials and design of microfluidic devices has been undertaken.<sup>2–4</sup>

As microfluidic devices become more widespread as powerful tools for DNA analysis, the focus has shifted from the glass and quartz devices initially developed<sup>5–7</sup> to new polymeric materials. These new materials, ideally, would offer substantial benefits over the glass pioneers. Such polymeric materials allow for a fast and simple fabrication, yielding low cost production of disposable and single-use devices. These materials allow production of many replicas from a single template with high fidelity—high similarity between templates and copies.

Duffy et al. was the first to describe a procedure for fabricating closed microfluidic systems using poly(dimethylsiloxane) (PDMS).<sup>8</sup> Their technique allowed them to design and fabricate microdevices in less than 24 h, a significant improvement over time required for glass chip fabrication.<sup>5</sup> UV laser ablation has been used to create microdevices from polycarbonate, polystyrene, cellulose acetate, and poly(ethylene terephthalate) (PET),<sup>9–11</sup> while several groups have used hot embossing and wire-imprinting to create channels by pressing heated metallic templates into polymeric materials.<sup>12–17</sup> Microfluidic devices made from a low-cost process involving lamination of individual thin layers of plastic to create structures having mixer, separator, and detector functions have been described and used with complex biological samples.<sup>18</sup>

Tan et al. were the first to use toner, deposited by a photocopier, to define a channel in a PDMS replica.<sup>19</sup> They photocopied a printed channel design onto a transparency sheet and used that as a mold to create a PDMS replica. This replica was then sealed to a glass plate to create the microfluidic architecture with a 12  $\mu\text{m}$  depth. The use of toner to create microfluidic

devices was refined and simplified by Lago et al. in 2003 when they printed toner onto polyester sheets and then laminated the sheets together, creating a closed channel.<sup>20</sup> In this case, the toner serves as an adhesive to bind the two sheets of polyester and the channel is defined by the lack of toner.<sup>20</sup> The depth of the channel created by lamination of two polyester sheets is determined by the depth of toner, 6  $\mu\text{m}$  of toner per sheet (single toner layer (STL), 12  $\mu\text{m}$  for two sheets bonded together (double toner layer (DTL), 12  $\mu\text{m}$  channel depth). In both cases, the channel surfaces are polyester at the top and bottom while the walls are composed of toner. This direct printing technique has been used to fabricate electrophoresis microchips with end-channel amperometric detection<sup>21</sup> and other electrochemical detection methods.<sup>22</sup> Toner-based techniques have been used as a fabrication step for other microfluidic devices,<sup>23–27</sup> but to date, there is no report describing the use of PeT microchip in genetic analysis. For the success of using PeT microchip for integrated DNA analysis, it is crucial to perform all steps, extraction, amplification, separation, and detection.

Solid phase DNA extraction in microfluidic devices has been reported in both glass and polymer-based devices.<sup>28,29</sup> We recently described a technique for DNA extraction, dynamic solid phase extraction (dsPE), in which magnetic silica beads are manipulated in the channel of a glass microchip. In this technique, the movement of the beads during the extraction process ensures thorough mixing of the sample with the magnetic silica beads to optimize binding to and elution from the magnetic silica beads. This contrasts DNA in a flowing stream interacting with a static (packed) bed of beads where the flow is critical to efficiency;<sup>30</sup> here the solution remains static and elution of bound DNA is dependent on bead movement.<sup>31</sup>

In the current report, we utilize a DNA extraction technique developed for glass microchips but demonstrate, for the first time

that (i) the dynamic solid phase extraction can be carried out in a unique PeT microchip containing deep microchannels, (ii) this system allows parallel extractions, and (iii) that the fabricated PeT microchip is compatible with DNA amplification via the polymerase chain reaction (PCR). This work presents the first effort for generating a mass-producible, cost-effective (pennies per chip), disposable polymeric microdevice for integrated genetic analysis.

## EXPERIMENTAL SECTION

**Reagents.** PCR reagents were obtained from Fisher Scientific (Fairlawn, NJ). Primers for amplification of a 380 bp fragment of the  $\beta$ -globin gene and for amplification of 520 bp fragment of  $\lambda$ -phage were synthesized by MWG Biotech (High Point, NC). PicoGreen intercalating dye was obtained from Invitrogen (Carlsbad, CA). MagneSil beads from Promega (Madison, WI) were diluted 3.3-fold with 8 M guanidine HCl (GuHCl) pH 7.6. *HinD* III digested  $\lambda$ -phage DNA was obtained from Sigma-Aldrich (St. Louis, MO). Human blood with a known white blood cell count was obtained from the blood bank of the University of Virginia Hospital.

**Microdevice Fabrication.** Fabrication of PeT microdevices was modified from previously reported methods.<sup>20</sup> Two important changes were made (1) four polyester layers were used instead of the original two layers, (2) a CO<sub>2</sub> laser cutter was used to define the channels. The main steps of the fabrication process are shown schematically in Figure 1A. Polyester sheets are first covered uniformly by toner on both sides using a laser printer (HP LaserJet 4000). The design for the device channel was drawn using CorelDraw 11.0 software, and the microchannel was created by cutting with a laser cutter with a 50 W CO<sub>2</sub> laser (VersaLaser 350, Universal Laser Systems). Four layer devices were created by sandwiching two polyester layers with precut channels and coated with toner between uncoated sheets of polyester with precut access holes in the top sheet. The four layers were aligned and laminated using a standard office laminator at 120 °C. This lamination step seals all PeT sheets as a result of the interaction between the toner layers and polyester sheets.

The microchips for dynamic solid phase extraction experiments were designed to have a 14 mm length by 1.2 mm width by 272  $\mu$ m depth (total volume of 4.0  $\mu$ L). The microchips for parallel dynamic SPE experiments with 8 channels (Figure 1B) were designed to have 8 identical channels of 14 mm length  $\times$  1.2 mm width  $\times$  272  $\mu$ m depth. Microchips for PCR amplification experiments (Figure 1C) were designed to have a 22 mm length by 2.4 mm width by 272  $\mu$ m depth (total volume of 10  $\mu$ L).

**Sample Preparation for dSPE Extraction.** Blood samples were prepared by mixing 6  $\mu$ L of whole blood with 5  $\mu$ L of proteinase K (20 mg/mL, Qiagen, Valencia, CA) and 9  $\mu$ L of 8 M GuHCl pH 7.6/1% Triton X-100 and incubating the mixture for 10 min at 56 °C. A 2  $\mu$ L aliquot, equivalent to 0.6  $\mu$ L of blood, was used in each extraction. The amount of DNA loaded was calculated using the known white blood cell count for each sample and an assumption of 6.25 pg of DNA/cell.

**Dynamic Solid Phase Extraction Procedures.** The channel was filled by first flowing 4  $\mu$ L of 8 M GuHCl pH 7.6 into the channel, followed by 5  $\mu$ m magnetic silica particles. Bead loading was aided by use of an external magnet, and the excess of solution above the chip capacity was withdrawn from the outlet hole. With the external magnet holding the beads in place, 2  $\mu$ L of sample

was loaded into the channel and 2  $\mu$ L of 8 M GuHCl was withdrawn from the outlet. The microchip was placed in the rotating magnetic field, and the beads were mixed by this motion in conjunction with an additional external magnet held above the chip for up to 5 min (see Figure 1S from the Supporting Information for an image of the apparatus for generating a rotating magnetic field). The beads were then washed by flowing 6  $\mu$ L of 80% isopropyl alcohol (IPA) through the chip, with magnetic bead manipulation. After washing the beads with IPA, the beads were washed with 8  $\mu$ L of 0.1 $\times$  Tris-EDTA (TE) without magnetic bead agitation. Finally, DNA was eluted from the beads as fractions with 0.1 $\times$  TE using the same magnetic manipulation for 2 min for each 2  $\mu$ L fraction. Each fraction was analyzed for extraction efficiency or used for PCR amplification. For quantification of DNA extracted in each fraction, the aliquots were fluorescently labeled with PicoGreen (PicoGreen assay, Invitrogen—Molecular Probes, Eugene, OR, according to the manufacturer's instructions) and quantified on a NanoDrop 3300 fluorospectrometer (NanoDrop, Wilmington, DC). For multiple, simultaneous DNA extraction, the protocol was the same as that for a single extraction. In all extractions, the PeT microchip was used only once and discarded at the end of extraction, even though the PeT microchip could be used more than once if a postextraction wash step was added.

**Sample Amplification by Conventional Methods.** PCR amplification of DNA extracted from blood by dSPE was performed in a tube in a Bio-Rad MyCycler (Hercules, CA) using primers to amplify a 380 bp fragment of the  $\beta$ -globin gene using the following cycling protocol: 95 °C for 1 min (initial denaturation), 30 cycles of denaturing at 95 °C for 30 s/annealing at 60 °C for 30 s/extension at 72 °C for 30 s, followed by 72 °C for 2 min (final extension). All PCR products were analyzed on a Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA) according to the manufacturer's instructions.

**PCR Amplification in PeT Microchip.** The compatibility of PeT microchips with PCR DNA amplification was tested using  $\lambda$ -phage DNA and primers to amplify a specific 520 bp DNA sequence. A sufficient reaction master mixture was prepared for both the microchip and conventional tube PCR: 10 mM Tris-HCl buffer, pH 8.3, 50 mM KCl, 2.4 mM MgCl<sub>2</sub>, 0.4 mM of the each primer, 0.2 mM of each dNTP, 1 ng/ $\mu$ L  $\lambda$ -phage DNA, 0.24 mg/mL of BSA, 0.1 units/ $\mu$ L Taq polymerase. The stock solution was divided and used for conventional amplification in a tube (positive control) and microchip amplification. The PCR chamber of the PeT microchip was filled with approximately 10  $\mu$ L of the stock solution, and mineral oil was overlaid on the reservoirs to prevent evaporation of solution. Both the PeT microchip and tube controls were placed in a conventional thermocycler (GeneAmp 2400 Perkin-Elmer) with thermocycling conditions as follows: 120 s at 95 °C for initial denaturation of DNA, 30 cycles of 30 s each at 95 °C (denaturation) and 68 °C (annealing/extension), followed by 120 s at 72 °C for a final extension. PCR products were removed from the microchip and tube controls and were analyzed on a Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA) according to the manufacturer's instructions. Negative controls lacking  $\lambda$ -phage DNA were included among the tube controls.

## RESULTS AND DISCUSSION

Traditionally, DNA extraction and PCR amplification are carried out in polypropylene tubes, the former using silica

Table 1. Comparison of PeT Microchip with Glass Chip and Others Polymeric Microchips

	PeT chip	glass chip	PDMS chip	PMMA chip
cost per chip	~\$0.15	~\$40	~\$2–5	~\$5–10
time to fabricate	less than 10 min	~24 h	~3–4 h	~4–5 h
reproducibility	moderate	excellent	excellent	excellent
durability	single use	reusable	reusable few times	reusable
solvent compatibility	compatible with SPE and PCR reagents	excellent	poor	poor

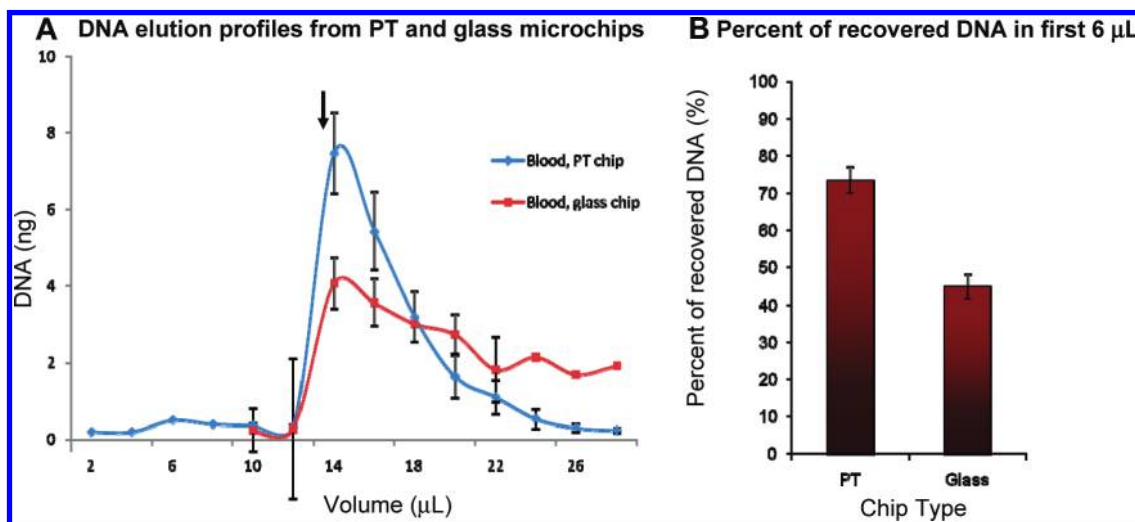


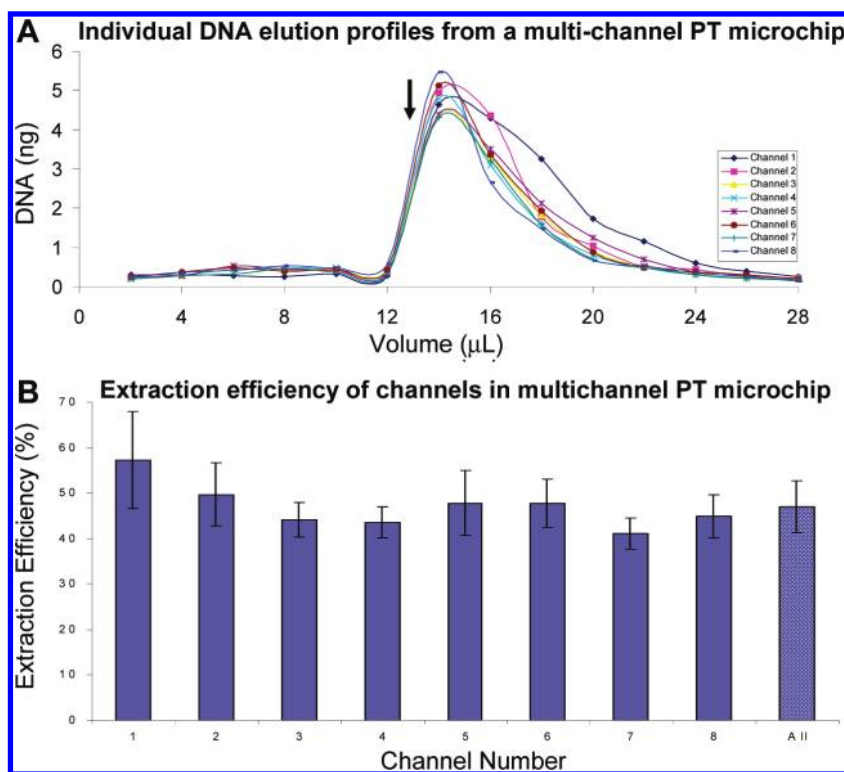
Figure 2. (A) Elution profiles showing that DNA extraction from blood in a PeT microchip is more efficient than extraction in a glass microchips using dSPE ( $n = 3$ ). Arrow indicates the initiation of bead movement after the 12  $\mu\text{L}$  fraction. (B) The amount of DNA recovered in the first 6  $\mu\text{L}$  (three fractions) is much higher for the PeT microchip versus the glass microchip.

particles for DNA purification in a centrifuged format, the latter using a conventional thermocycler. Microfluidic devices present the opportunity for significantly reduced volumes in PCR reactions, e.g., Easley et al.<sup>32</sup> showed the successful amplification of a portion of the *B. anthracis* genome from 500 nL of whole blood, which is 400-fold less than the typical volumes used for macroscale DNA extraction. The ramifications of reduced PCR volume are that it allows for shorter reaction times and easier integration of PCR with upstream extraction and downstream separation and analysis. It is for these reasons that efforts have been focused on performing PCR in microfluidic devices.<sup>33–35</sup>

Materials used for microchip-based PCR amplification have included a wide range of substrates, including glass,<sup>36</sup> silicon,<sup>37</sup> and polymers.<sup>38</sup> Although glass is the most popular material used in microfabrication, it is not an ideal substrate because, in addition to costly and time-consuming fabrication, the surface must be passivated to minimize *Taq* adsorption to the surface. Because of this, passivation of the glass surface is necessary for successful PCR amplification. The limitations of glass have led to investigation of alternative materials for microchip fabrication. On the other hand, PeT microchips represent a rapid, simple, and inexpensive fabrication method<sup>39,40</sup> for the development of microchip-based integrated DNA extraction and PCR. We show here that PeT chips are compatible with the PCR reaction without passivation of the surface and that both DNA extraction and PCR can be accomplished on a single disposable device, making this an excellent option for genetic analysis.

**Microfabrication Processes.** The conventional process for fabricating PeT microchips has been described to create electrophoresis microchips and mixers.<sup>21,41</sup> In all cases, the PeT microchips were made with two polyester films and the channels were defined by voids in toner in a direct-printing process using a laser printer to selectively deposit a toner layer on a polyester film. The maximum depth of the channels described in these earlier reports was 12  $\mu\text{m}$ , achieved by the deposition of a 6  $\mu\text{m}$  toner layer on both films. While adequate for electrophoretic separation, this design cannot be used for solid phase extraction because the shallow depth (or height) of the channels does not accommodate the solid phase (beads) or allow bead movement in the channel. In response to this, a novel method for fabrication of the PeT microchip was developed. In order to create a channel approximately 270  $\mu\text{m}$  deep, four polyester films, instead of two, were used. DNA extractions using microchips with three polyester films (channel depth of approximately 135  $\mu\text{m}$ ) were also tested, but the efficiency of extraction was higher when four layer microchips were used (data not shown). The basis for the higher extraction efficiency with the four layer devices is thought to lie in the dependence of elution on bead movement, which is more pronounced in the four layer microdevice (see Dynamic Solid Phase DNA Extraction from Human Blood).

The fabrication process was modified from that previously described by Lago et al.<sup>20</sup> by using a CO<sub>2</sub> laser cutter to define the channel. The main steps of the fabrication process are shown schematically in Figure 1A. The materials required for the PeT fabrication are simply transparency film and toner, and the



**Figure 3.** (A) Elution profiles from eight extractions in a multichannel PeT SPE microchip. Arrow indicates the initiation of bead movement after collection of the 12  $\mu\text{L}$  fraction. (B) Extraction efficiency in each channel and the average from all extractions ( $n = 3$ ).

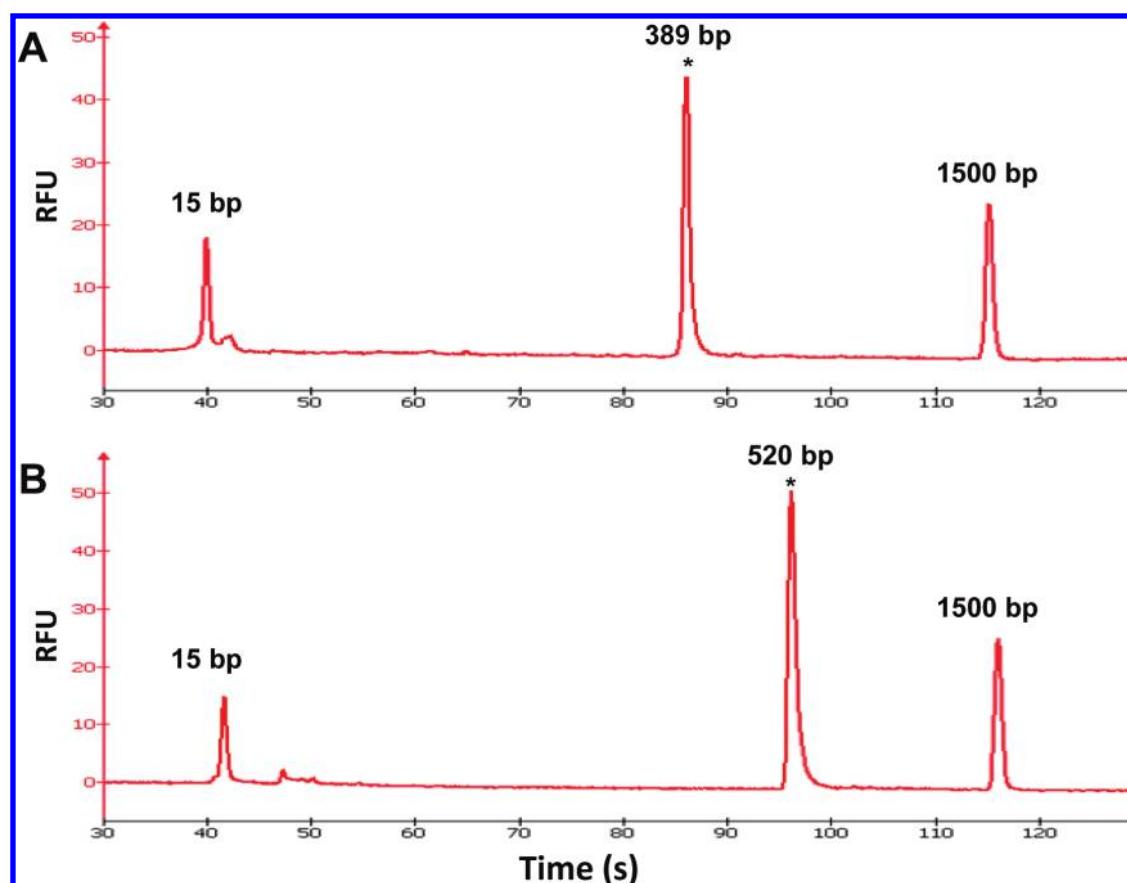
equipment used are a laser printer, a laser cutter, and a laminator. The laser printer and laminator, commonly used and readily available, cost less than U.S. \$300 each while the laser cutter used costs approximately U.S. \$10 000. It may be possible, however, to use less expensive alternatives, such as knife plotters that cost less than U.S. \$5 000<sup>42</sup> or desktop digital craft cutters that cost between U.S. \$150–300.<sup>43</sup> Arguably, the most important advantages of this type of microchip fabrication is the minimal time required (less than 10 min) and negligible cost of consumable materials (transparency film and toner).

The PeT microchip is a promising new platform for genetic analysis for more reasons than just the low cost and easy fabrication. Although it is not comprehensive, Table 1 shows a direct comparison of the costs and time associated with the fabrication of PeT versus glass, PDMS, and PMMA microchips. Table 1 also shows comparison of the reproducibility, durability, and solvent compatibility of them. This comparison makes clear the advantages of PeT microchips, even when compared with other polymer microchips. While PeT solvent compatibility has not been exhaustively studied, the compatibility of PeT microchips with dSPE and PCR is presented in this study.

#### Dynamic Solid Phase DNA Extraction from Human Blood.

Our earlier work has shown that dSPE in a glass microchip is capable of efficiently extracting DNA (more than 60%) from small volume blood samples (0.6  $\mu\text{L}$ ) with recovered DNA at a concentration suitable for PCR amplification.<sup>31</sup> The advantages of an alternative approach using a dynamic (magnetic) silica phase (rather than a packed bed of beads) include the simplification of the setup, which includes eliminating the syringe pump and removing the variability associated with flow through a packed bed of solid phase.

Figure 2A shows the elution profile of a single DNA extraction from 0.6  $\mu\text{L}$  of whole blood by dSPE in a four layer PeT microdevice. Bead movement, and thus elution, was initiated at 14  $\mu\text{L}$  (indicated by an arrow). The first two fractions collected are predominately composed of the 80% IPA wash solution and are not expected to contain DNA. Four washes of  $0.1 \times \text{TE}$  (representing volumes 6–12  $\mu\text{L}$ ) while the beads were immobilized were used to ensure all residual IPA was removed before DNA elution. DNA elution was dependent on bead movement, which was also observed with dSPE in glass microchips (Figure 2A).<sup>31</sup> The first fraction associated with bead agitation in elution buffer is at 14  $\mu\text{L}$ , which contained the majority of the eluted DNA (7.5 ng;  $\sim 34.2\%$ ). Thus, the microchip-based dSPE method described for glass microchips proved to be compatible with the PeT microchip device, providing both high extraction efficiency ( $69.7 \pm 5.7\%$ ) and highly concentrated ( $3.2 \pm 0.08 \text{ ng}/\mu\text{L}$  in the first two fractions) eluted DNA necessary for downstream applications. In fact, dSPE in the PeT microchip was superior to glass due to the high concentration of recovered DNA. Although the total recovery of DNA from whole blood was high in the glass chip, the elution profile did not present a bell shaped profile. Rather than eluting the DNA immediately in a small volume as in the PeT chip, dSPE in a glass microchip resulted in DNA elution steadily over several fractions. The elution profiles from whole blood in both PeT and glass microchips are shown in Figure 2A. Although the extraction efficiency for blood is comparable in both types of chip ( $69.7 \pm 5.7\%$  for PeT chip and  $63.9 \pm 6.0\%$  for glass chip), the amount of DNA recovered in the first 6  $\mu\text{L}$  ( $3 \times 2 \mu\text{L}$  fractions) is much higher for the toner chip (73.5%) than for the glass chip (34%) (Figure 2B) when comparing a single extraction channel.



**Figure 4.** (A) Electropherogram showing that the DNA purified by dynamic SPE is of high quality: amplification of a portion of the  $\beta$ -globin gene using the first fraction of DNA purified from blood by dSPE on a toner chip. (B) Electropherogram showing the amplification of a 520 bp fragment of  $\lambda$  DNA in a PeT microchip.

**Table 2. Comparison of Dynamic SPE in PeT Microchips with Other SPE Methods: dSPE in Glass Microchips, Packed Silica Bed Extraction, and Qiagen QIAmp Extraction Kit<sup>a</sup>**

	extraction efficiency (%)	concentration of DNA (ng/ $\mu$ L)
dynamic SPE toner	69.7 $\pm$ 5.7	3.23 $\pm$ 0.08
dynamic SPE glass	63.9 $\pm$ 6.0	1.65 $\pm$ 0.39
packed chip	42.5 $\pm$ 5.6	2.26 $\pm$ 0.53
DNA extraction		
Qiagen QIAmp	65.4 $\pm$ 3.0	0.108 $\pm$ 0.011
DNA extraction		

<sup>a</sup>For the concentration comparison, the average from the two most concentrated elution fractions from the dSPE and packed chip extractions is listed.

We hypothesize that the basis for the dramatically different elution profile in PeT microchips is the result of the greater magnetic force experienced by the beads through a thinner polyester film in comparison to the relatively thicker glass. Since effective bead agitation is required for initiation of elution, it stands to reason that more efficient bead agitation will result in more efficient elution. Indeed, we observed that extraction efficiency was lower in PeT microchips made from only three layers of polyester film, which resulted in the movement of the magnetic beads being more restricted. Other factors may also

contribute to achieving the best DNA elution in PeT microchip such as (i) surface smoothness and (ii) the composition of the polyester film. The PeT has a smoother surface than the glass one (after etching with HF). This surface allows the particles to move more freely in PeT than in glass channels (results not shown). The nature of the surface of the channel can influence the extraction efficiency primarily due to irrecoverable loss of DNA. In contrast, without passivation, the PeT surface seems more immune to biofouling (i.e., similar to typical laboratory plasticware, which are known to be of low-binding and of widespread use in DNA analysis).

**Multiple Dynamic Solid Phase DNA Extraction.** The simplicity of the dSPE technique, which obviates the use of syringe pumps, is easily adapted to multisample DNA extraction in a PeT microchip. A PeT microchip with eight channels was designed, fabricated, and assembled as described in Figure 1A. It is noteworthy that fabrication time for this chip was essentially identical to the fabrication of a single-channel SPE device; moreover, the cost and the time per extraction are significantly reduced. The microchips for multiple dSPE experiments (Figure 1B) were designed in a way that all channels were equally subjected to the magnetic field. Figure 3A shows the overlaid elution profiles from the eight DNA extractions, performed simultaneously in a multichannel PeT microchip. Within acceptable experimental variation, the eight parallel experiments yielded the same general profiles and the individual (and average) extraction efficiencies, shown in Figure 3B, are slightly

lower than the extraction efficiency observed with the single channel device (Figure 2A). The differences between both systems are mainly due to the nonhomogeneity of the magnetic field over all the channels. For a single channel, the device is centered in a rotating magnetic field (RMF) and faces a relatively homogeneous magnetic field. For the eight-channel device, the center of the chip is centered in the RMF where all of the channels are not necessarily optimally localized relative to the RMF. This supports other data that indicate that the position of the channels relative to the RMF is critical for the overall efficiency of extraction. A channel lying in the center of the RMF faces a parallel magnetic field to the surface of the chip (see Figure S2 of the Supporting Information for the simulation of the magnetic field). The parallel line fields induce the alignment of the magnetic silica particles along the length of the channel at first (results not shown), then undergo to a DNA-bead interaction as described previously.<sup>31</sup> For the parallel extraction chip, the channels lay over the path of the RMF. Over the magnet, the magnetic line fields are perpendicular to the surface and the magnetic particles and, thus, face smaller amplitude of movement (results not shown).

**PCR Amplification of Target Sequences in DNA Purified by Dynamic SPE in PeT Microchip.** The quality of the DNA extracted by dSPE in a PeT microchip was verified by demonstrating that purified DNA can be amplified by conventional PCR. The ability of PeT chip to isolate pure, PCR-amplifiable DNA from complex samples was first shown by isolating DNA from whole human blood, followed by conventional amplification (tube-based) of a fragment of the  $\beta$ -globin gene (389 bp) in a conventional thermocycler. The first 2  $\mu$ L DNA fraction collected after bead agitation, typically the most concentrated fraction, was used for PCR experiments ( $n = 3$ ). The electropherogram in Figure 4A shows the resultant 389 bp amplicon from a representative experiment, showing that DNA purified by dynamic SPE is of high quality and suitable for downstream applications.

**PCR Amplification of Target Sequences in a PeT Microchip.** Integration of DNA extraction and amplification into a single device is an area of intense study.<sup>32,44</sup> We sought to determine if the PeT chips are compatible with PCR amplification, making PeT devices more powerful in the quest for a  $\mu$ TAS. In order to confirm that PeT microchips are compatible with PCR, a PeT microchip loaded with a solution of PCR master mix and  $\lambda$ -phage DNA was placed in a commercial thermocycler alongside tube controls containing the same reaction mixture. Figure 4B shows the resultant electropherogram from the successful amplification of the 520 bp fragment of the  $\lambda$ -phage genome, demonstrating that compatibility of the materials used in fabrication of the PeT microchip with the biochemistry of PCR is not a concern. The toner is certainly of porous nature and that large surface area could possibly affect the outcome of the polymerase chain reaction. We did not observe phenomena like evaporation of the solution (because we added mineral oils in both reservoirs) or irreversible adsorption, but instead we observed spurious formation of air microbubbles. Nevertheless, such small bubbles do not impair the development of the amplification reaction.

**Comparison of dSPE in PeT Microchips with Other Available Extraction Methods.** Comparison of dSPE in PeT microchips with other available extraction techniques, in terms of extraction efficiency and DNA concentration for downstream applications, is an important parameter to evaluate the performance of this technique in relation to other techniques. Table 2 shows a comparison of microfluidic dSPE DNA purification in PeT

microchip to a commercial DNA extraction system, (Qiagen QIAamp), silica-based extraction in a packed bed microfluidic device, and dSPE in a glass microchip. The table shows a comparison of both the total extraction efficiency and the concentration of the DNA recovered in the first two fractions ( $\sim 4 \mu$ L), typically the most concentrated fractions obtained from both dynamic SPE and packed chip SPE. The concentration of DNA from the commercial extraction is based on the total extraction efficiency, since only one 200  $\mu$ L fraction is collected (as per the manufacturer's protocol). Dynamic SPE in PeT was found to have comparable extraction efficiency to the dSPE in glass chips and to the commercial method, but it was more efficient than the packed silica microchip. The advantage of dSPE, however, is that the concentration of the purified DNA yielded by this technique was 30-fold greater than that obtained by the commercial method. Thus dSPE in general and in PeT particular offers a distinct advantage over commercial products for microchip based DNA analysis.

## CONCLUSIONS

A novel fabrication process using a multilayer device with a deep microfluidic architecture allowed us to perform dynamic solid phase DNA extraction and PCR in a simple and disposable manner. In this study, we present a different fabrication process using the PeT chips that, unlike earlier reports, produce deep channels (high aspect ratio) by using four stacked layers of polyester film instead of conventional double layer. The key issue for the successful dSPE of DNA is the elevated height of the channel, allowing ample freedom for the magnetic silica particles to move in three dimensions. In PeT chips previously described, the channel height was shallow, typically 12  $\mu$ m, and the use of 5  $\mu$ m particles would have been difficult, if not impossible. The use of cut-through channels in the PeT film allows for the stacking of multiple layers and, thus, provides the necessary space for movement of the particles so that magnetic bead extraction can be carried out. In addition, a base plate thickness (channel floor) of only 100  $\mu$ m, the thickness of one layer, allows close proximity to the magnetic field and, thus, efficient mobilization of the magnetic particles. Increasing intimate contact between the magnetic particles and the DNA allows for a highly efficient extraction. The use of a laser cutter, or even a knife plotter, is an essential step in the fabrication of the microchips, which is basically (i) printing both sides in black, (ii) cutting the channels, and (iii) laminating all layers.

The ease of fabrication of PeT microchips, the simplicity of the dSPE technique, and the low cost (of both fabrication and extraction) make this method even more attractive when eight extractions are carried out in parallel. Considering that a single PeT microchip costs on the order of \$0.15 (as a disposable platform), the cost per extraction on an eight channel chip is less than two cents. The density of channels of this parallel extraction platform can be further increased to, for example, 16 channels, making the cost associated with the platform alone (disregarding the costs of chemicals) negligible. Moreover, with the PeT chips designed for single-use disposability, we avoid the run-to-run contamination experienced with glass chips that are reused.

This work demonstrates the potential of PeT microchips to be used, upon further development, for total genetic analysis, driven by a compatibility of the polyester and toner for processes ranging from DNA extraction to PCR-based amplification. One critical step needed to achieve integration will be the reduction of the chamber dimensions in order to reduce the

PCR reaction volume. A smaller reaction volume will allow for the use of infrared heating<sup>42</sup> to rapidly cycle the temperature in the reaction chamber, thus expediting the amplification process, and the analysis, further enhancing the utility of PeT microchips as  $\mu$ TAS. Improved optics now available for the commercial laser cutter we have utilized may allow for better fabrication with reduced size features.

Even though the ultimate goal in developing PeT microchips is to fully integrate all parts required for genetic analysis (i.e., see ref 32), we have successfully carried out genetic analysis for the first time using simple to use and inexpensive polyester-toner substrate. The PeT devices, with true “sample-in/answer-out” capability, will be of low-cost and have the potential to be extensively used in diagnostic and forensic testing.

## ■ ASSOCIATED CONTENT

**S Supporting Information.** Additional information as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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## ■ REFERENCES

- (1) Manz, A.; Graber, N.; Widmer, H. M. *Sens. Actuators, B* **1990**, *1*, 244–248.
- (2) Vicckers, J. A.; Caulum, M. M.; Henry, C. S. *Anal. Chem.* **2006**, *35*, 491–499.
- (3) Shadpour, H.; Hupert, M. L.; Patterson, D.; Liu, C.; Galloway, M.; Stryjewski, W.; Goettert, J.; Soper, S. A. *Anal. Chem.* **2007**, *79*, 870–878.
- (4) Hosmane, S.; Yang, I. H.; Ruffin, A.; Thakor, N.; Venkatesan, A. *Lab Chip* **2010**, *10*, 741–747.
- (5) Harrison, D. J.; Fluri, K.; Seiler, K.; Fan, Z.; Effenhauser, C. S.; Manz, A. *Science* **1993**, *261*, 895–897.
- (6) Effenhauser, C. S.; Manz, A.; Widmer, H. M. *Anal. Chem.* **1993**, *65*, 2637–2642.
- (7) Jacobson, S. C.; Hergenroder, R.; Koutny, L. B.; Warmack, R. J.; Ramsey, J. M. *Anal. Chem.* **1994**, *66*, 1114–1118.
- (8) Duffy, D. C.; McDonald, J. C.; Schueller, O. J. A.; Whitesides, G. M. *Anal. Chem.* **1998**, *70*, 4974–4984.
- (9) Roberts, M. A.; Rossier, J. S.; Bercier, P.; Girault, H. *Anal. Chem.* **1997**, *69*, 2035–2042.
- (10) Rossier, J. S.; Schwarz, A.; Reymond, F.; Ferrigno, R.; Bianchi, F.; Girault, H. H. *Electrophoresis* **1999**, *20*, 727–731.
- (11) Rossier, J. S.; Ferrigno, R.; Girault, H. H. *J. Electroanal. Chem.* **2000**, *492*, 15–22.
- (12) Martynova, L.; Locascio, L. E.; Gaitan, M.; Kramer, G. W.; Christensen, R. G.; MacCrehan, W. A. *Anal. Chem.* **1997**, *69*, 4783–4789.
- (13) Locascio, L. E.; Perso, C. E.; Lee, C. S. *J. Chromatogr., A* **1999**, *857*, 275–284.
- (14) Chen, Y.-H.; Wang, W.-C.; Young, K.-C.; Chang, T.-T.; Chen, S.-H. *Clin. Chem.* **1999**, *45*, 1938–1943.
- (15) Chen, D.-C.; Hsu, L.; Zhan, D.-Z.; Chen, C.-H. *Anal. Chem.* **2001**, *73*, 758–762.
- (16) Grass, B.; Siepe, D.; Neyer, A.; Hergenroder, R. *Fresenius J. Anal. Chem.* **2001**, *371*, 228–233.
- (17) Galloway, M.; Soper, S. A. *Electrophoresis* **2002**, *23*, 3760–3768.
- (18) Weigl, B. H.; Bardell, R.; Schulte, T.; Battrell, F.; Hayenga, J. *Biomed. Microdevices* **2001**, *3*, 267–274.
- (19) Tan, A.; Rodgers, K.; Murrhij, J. P.; O’Mathuna, C.; Glennon, J. D. *Lab Chip* **2001**, *1*, 7–9.
- (20) Do Lago, C. L.; Silva, H. D. T.; Neves, C. A.; Brito-Neto, J. G. A.; Silva, J. A. F. *Anal. Chem.* **2003**, *75*, 3853–3858.
- (21) Coltro, W. K. T.; Silva, J. A. F.; Silva, H. D. T.; Richeter, E. M.; Furlan, R.; Angnes, L.; Do Lago, C. L.; Mazo, L. H.; Carrilho, E. *Electrophoresis* **2004**, *25*, 3832–3838.
- (22) He, F. Y.; Liu, A. L.; Yuan, J. H.; Coltro, W. K. T.; Carrilho, E.; Xia, X.-H. *Anal. Bioanal. Chem.* **2005**, *282*, 192–197.
- (23) Daniel, D.; Gutz, I. G. R. *Electrochem. Commun.* **2003**, *5*, 782–790.
- (24) Liu, A. L.; He, F. Y.; Wang, K.; Zhou, T.; Lu, Y.; Xia, X.-h. *Lab Chip* **2005**, *5*, 974–978.
- (25) Do Lago, C. L.; Jesus, D. P.; Silva, H. D. T.; Neves, C. A.; Silva, J. A. F. *Electrophoresis* **2004**, *25*, 3825–3832.
- (26) Jesus, D. P.; Blanes, L.; Do Lago, C. L. *Electrophoresis* **2006**, *27*, 4935–4941.
- (27) Coltro, W. K. T.; Piccin, E.; Silva, J. A. F.; Do Lago, C. L.; Carrilho, E. *Lab Chip* **2007**, *7*, 931–934.
- (28) Wen, J.; Legendre, L. A.; Bienvenue, J. M.; Landers, J. P. *Anal. Chem.* **2008**, *80*, 6472–6479.
- (29) Price, C. W.; Leslie, D. C.; Landers, J. P. *Lab Chip* **2009**, *9*, 2484–2494.
- (30) Breadmore, M. C.; Wolfe, K. A.; Arcibal, I. G.; Leung, W. K.; Dickson, D.; Giordano, B. C.; Power, M. E.; Ferrance, J. P.; Feldman, S. H.; Norris, P. M.; Landers, J. P. *Anal. Chem.* **2003**, *75*, 1880–1886.
- (31) Duarte, G. R. M.; Price, C. W.; Littlewood, J. L.; Haverstick, D. M.; Ferrance, J. P.; Carrilho, E.; Landers, J. P. *Analyst* **2010**, *135*, 501–537.
- (32) Easley, C. J.; Karlinsey, J. M.; Bienvenue, J. M.; Legendre, L. A.; Roper, M. G.; Feldman, S. H.; Hughes, M. A.; Hewlett, E. L.; Merkel, T. J.; Ferrance, J. P.; Landers, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *19*, 19272–19277.
- (33) Niu, Z. Q.; Chen, W. Y.; Shao, S. Y.; Jia, X. Y.; Zhang, W. P. *J. Micromech. Microeng.* **2006**, *16*, 425–433.
- (34) Easley, C. J.; Humphrey, J. A. C.; Landers, J. P. *J. Micromech. Microeng.* **2007**, *17*, 1758–1766.
- (35) Oda, R. P.; Strausbauch, M. A.; Huhmer, A. F. R.; Borson, N.; Jurens, S. R.; Craighead, J.; Wettstein, P. J.; Eckoff, B.; Kline, B.; Landers, J. P. *Anal. Chem.* **1998**, *70*, 4361–4368.
- (36) Roper, M. G.; Easley, C. J.; Legendre, L. A.; Humphrey, J. A. C.; Landers, J. P. *Anal. Chem.* **2007**, *79*, 1294–1300.
- (37) Wilding, P.; Shoffner, M. A.; Kricka, L. J. *Clin. Chem.* **1994**, *40*, 1815–1818.
- (38) Giordano, B. C.; Ferrance, J. P.; Swedberg, S.; Huhmer, A. F. R.; Landers, J. P. *Anal. Biochem.* **2001**, *124*, 124–132.
- (39) Coltro, W. K. T.; Carrilho, E. In *Handbook of Capillary and Microchip Electrophoresis and Associated Microtechniques*, 2nd ed.; Landers, J. P., Ed.; CRC Press: New York, 2007; pp 1169–1184.
- (40) Coltro, W. K. T.; Lunte, S. M.; Carrilho, E. *Electrophoresis* **2008**, *29*, 1–10.
- (41) Liu, A. L.; He, F. Y.; Wang, K.; Zhou, T.; Lu, Y.; Xia, X. H. *Lab Chip* **2005**, *5*, 974–977.
- (42) Fenton, E. M.; Mascarenas, M. R.; López, G. P.; Sibbett, M. M. *Appl. Mater. Interfaces* **2006**, *1*, 124–129.
- (43) Yuen, P. K.; Goral, V. N. *Lab Chip* **2010**, *10*, 384–387.
- (44) Legendre, L. A.; Bienvenue, J. M.; Roper, M. G.; Ferrance, J. P.; Landers, J. P. *Anal. Chem.* **2006**, *78*, 1444–1451.