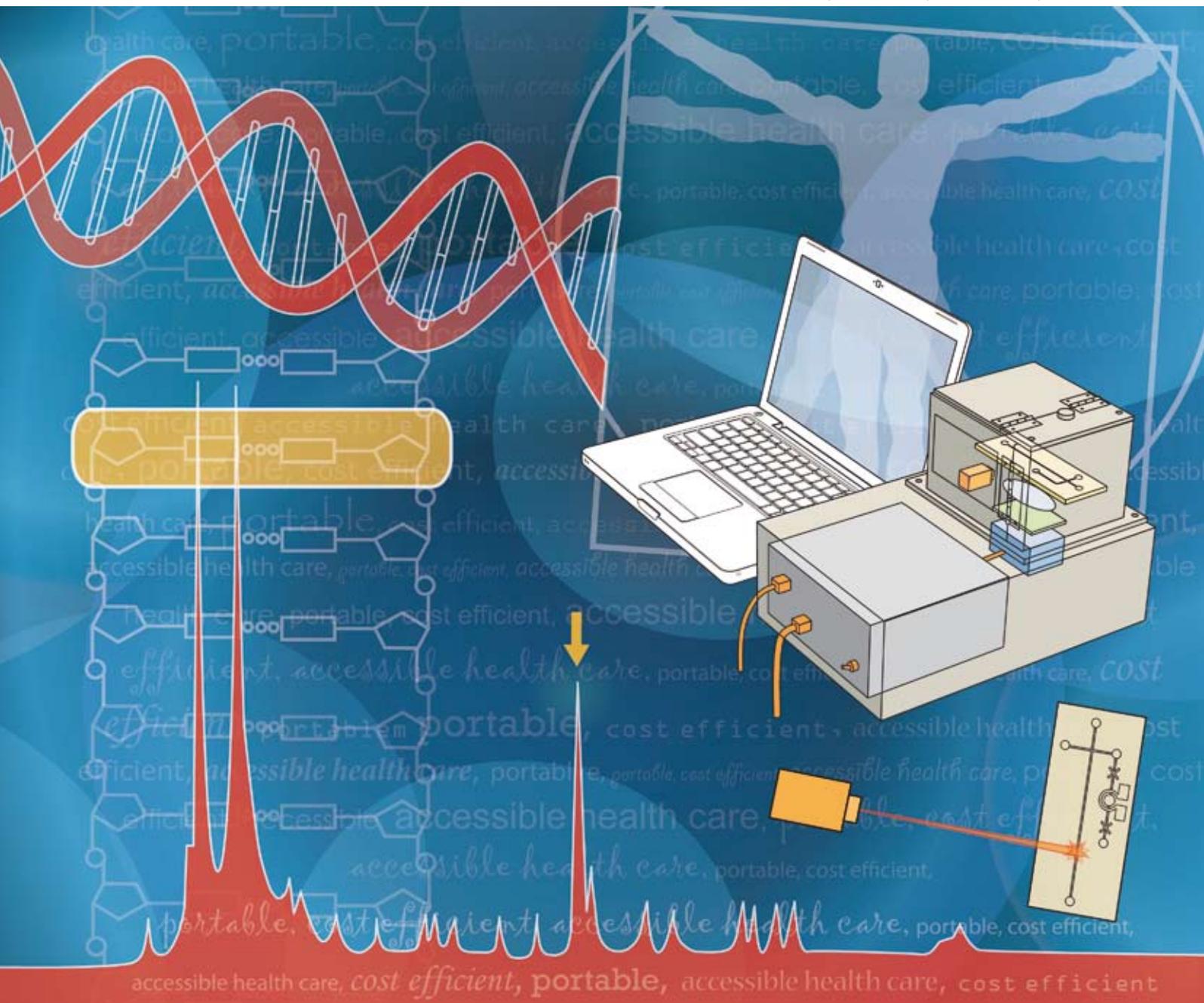


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PAPER

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An inexpensive and portable microchip-based platform for integrated RT-PCR and capillary electrophoresis

An inexpensive and portable microchip-based platform for integrated RT-PCR and capillary electrophoresis†

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We present an inexpensive, portable and integrated microfluidic instrument that is optimized to perform genetic amplification and analysis on a single sample. Biochemical reactions and analytical separations for genetic analysis are performed within tri-layered glass-PDMS microchips. The microchip itself consists of integrated pneumatically-actuated valves and pumps for fluid handling, a thin-film resistive element that acts simultaneously as a heater and a temperature sensor, and channels for capillary electrophoresis (CE). The platform is comprised of high voltage circuitry, an optical assembly consisting of a laser diode and a charged coupled device (CCD) camera, circuitry for thermal control, and mini-pumps to generate vacuum/pressure to operate the on-chip diaphragm-based pumps and valves. Using this microchip and instrument, we demonstrate an integration of reverse transcription (RT), polymerase chain reaction (PCR), and capillary electrophoresis (CE). The novelty of this system lies in the cost-effective integration of microfluidics, optics, and electronics to realize a fully portable and inexpensive system (on the order of \$1000 in component costs) for performing both genetic amplification and analysis – the basis of many medical diagnostics. We believe that this combination of portability, cost-effectiveness and performance will enable more accessible healthcare.

Introduction

Microfluidic technologies are expected to have a strong impact on the future of healthcare by providing a rapid and cost-effective platform for the implementation of molecular diagnostic techniques. These molecular diagnostic techniques are known to be highly sensitive and selective, but their application is greatly constrained by high reagent, equipment and labour costs. There is a significant impetus towards moving molecular diagnostics from the laboratory bench to the bedside,¹ with a compelling need in public health monitoring.² For many such applications, technology is required to cost-effectively test individual patient samples to avoid delays associated with accumulating a large number of samples (sample batching or pooling is a common cost-containment strategy). There is therefore an important niche for microfluidic systems that are able to perform entire molecular biology protocols on a single sample while remaining inexpensive and portable – we present such a system.

In this demonstration, we have developed individual modular components that have been integrated into microchips that couple reverse transcription (RT), polymerase chain reaction (PCR) and capillary electrophoresis (CE) along with components such

as integrated valves, pumps and heating/sensing elements. For these integrations, we adapted conventional molecular biology assays to the microchips, and the microchip to the assays. Although there have been major advances in microfluidic chip-based technologies in the past several years, a factor that often limits the applications of these technologies is the need for considerable supporting equipment, whether for temperature control (*e.g.* Peltier cells or infrared lasers), high voltage power, electronics interfacing, valve control or analyte detection. The platform developed here consists of a fluid handling capability, drive electronics for the on-chip heaters, a high voltage system for CE, and an optical system for the detection of fluorescently-labelled analytes. We believe that the platform and microfluidic chip presented here are a significant step towards the practical realization of genetic testing within a clinic or for point-of-care applications. The system is designed to be portable and inexpensive, with component costs of about \$1000.

There are several commercial bench-top, microchip-based CE analysis systems such as the Agilent 2100, BioRad's BioFocus®/Experion™, Micralyne's Microfluidic Tool Kit™ and Caliper's LabChip® 90 System. However, these have only the CE functionality, are directed towards bench-top laboratory usage, and are not meant to be portable diagnostic platforms. Sandia Laboratories recently demonstrated³ one of the first portable CE-based systems, but it does not include functionality such as genetic amplification, which, when integrated effectively with CE, provides the basis for many medical diagnostics.

From the perspective of medical diagnostic applications, several chip-based diagnostics have been realized for: hepatitis C (HCV),⁴ influenza,⁵ *Escherichia coli*,⁶ amino acid biomarker

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†The HTML version of this article has been enhanced with colour images.

detection,⁷ Duchenne muscular dystrophy,⁸ human papilloma virus (HPV),⁹ malaria,¹⁰ and *Streptococcus pneumoniae* bacterial detection.¹¹ Many of these demonstrations are based on microchip PCR techniques. Microchip PCR itself has recently been well-reviewed by Landers and co-workers¹² and by Verpoorte *et al.*¹³ Though several independent and partially-coupled technologies have been demonstrated on microchips,¹⁴ fully integrated, inexpensive microchips that are compatible with portable platforms are yet to be realized. Microchip-based and integrated RT, PCR and CE are critically important functionalities for a broad range of diagnostic applications.

Several integrations of RT and PCR functionalities have been demonstrated on a single microchip using a two-step reaction approach (performing RT and then moving the mix to another chamber to perform PCR).^{15,16} In ref. 15, RT–PCR for the detection of dengue virus type-2 and enteroviruses 71 was performed in separate chambers for RT and PCR followed by off-chip detection. Single-step RT–PCR approaches similar to the approach demonstrated here (wherein both RT reaction and PCR are performed in the same reaction chamber with no human intervention between these steps) have been reported only recently (see refs 17, 18 and 25). Quake and co-workers¹⁷ were one of the first groups to demonstrate microchip RT–PCR in a single chamber. Although highly effective in a non-portable, high density array format, owing to water-loss issues this chip architecture requires guard channels (to replenish water) and is not amenable to a lower density RT–PCR–CE approach. Toriello *et al.*²⁵ present an impressive multi-channel-based sensitive RT–PCR–CE system; however, this system is not portable in its current form. Similarly, the single-step, continuous flow-based RT–PCR of Obeid and Christopoulos¹⁸ demonstrated chip-based integration of CE. While the work of both refs 16 and 18 demonstrates the feasibility of a continuous flow reaction system, we believe that a stationary PCR approach is more suitable for a versatile, portable system. Although there have been relatively few demonstrations of integrated RT–PCR–CE microchips, numerous PCR–CE demonstrations have been reported in the literature to date: SARS-coronavirus detection within a glass microchip,¹⁹ λ DNA in a glass/PDMS chip with volumes of up to 50 μ L,²⁰ and viral load assessment (BKV) using a glass/PDMS microchip.²⁸ Landers and co-workers make use of valves, as in ref. 23, within an integrated PCR–CE chip to detect *Salmonella typhimurium* DNA.²⁴ In ref. 25, Mathies and co-workers further integrated the RT step onto the PCR–CE platform. Both groups (of Mathies^{21,22} and of Landers²⁶) make use of a four-layer chip architecture, based on a multi-channel approach directed towards high throughput applications. Recently, an interesting demonstration¹¹ integrated RT–PCR–CE in a way that combines a polymer chip and a glass chip that is fibre-coupled to an off-chip, PMT-based optical system for intercalator-based fluorescence detection. Although this system represents significant progress towards lower-cost systems through its level of on-chip integration, it still relies on a relatively complex hybrid chip approach and considerable off-chip infrastructure (not described in detail). The use of intercalators complicates the CE implementation for an integrated platform and typically lowers resolution.

To date, demonstrations of PCR–CE (*e.g.* refs 5 and 26) have shown systems of great functionality, but these typically

require considerable external infrastructure for operation and hence are not readily portable. However, two demonstrations from the Mathies group,³¹ and in more recent work,²⁷ are portable systems largely due to the use of mini-pumps similar to those used here. While we acknowledge that refs 27 and 31 give impressive demonstrations of portable genetic analysis platforms, the confocal optics used preclude these systems from being inexpensive. In the present work, we have instead sought a portable and inexpensive lab-on-chip approach suitable for routine and widespread use for point-of-care applications. We believe that resistive heating is eminently suitable for portable operation, particularly since we use a single resistive element approach to perform both heating and temperature sensing (as opposed to two separate elements for heating and sensing as in ref. 22). This heating approach simplifies the electronic interface and improves the real-estate utilization on the microchip. We also employ a three-layer chip architecture based on standard lab-on-a-chip (LOC) technologies for microfabrication, as opposed to four layers as in refs 26 and 27. This three-layer microchip has been optimized for operation using low-power mini-pumps that generate pressure and vacuum for actuating the microvalves, thus eliminating the need for external infrastructure. We employ a relatively large reaction volume (600 nL) that is ideally suited for clinical samples having a low concentration of target DNA. We earlier demonstrated that optimized reaction volumes are necessary for the detection of viral agents (BK virus) from urine.²⁸ That application dictated this reaction volume, and the heater geometry was optimized to maintain a uniform temperature (less than 1 °C variations) in the reaction chamber. The conventional approach for detection is to apply a more expensive confocal optical system. However, the use of a simple optical assembly based on a CCD for detection renders our system amenable to multiple-channel detection while remaining inexpensive. The present work combines ease of manufacture with low cost in a portable system suitable for single patient testing.

Materials and methods

Microchip architecture

A critical element for an integrated microfluidic chip is the development of an appropriate microvalve technology (both on-chip components and off-chip drive systems, be they electronic or mechanical) that is easy to implement. The Mathies-style valves²³ and the Quake-style²⁹ valves are both widely-used valve architectures consisting of multi-layers and external pressurized air and vacuum connections. Here, we implement a modified version of the Mathies-style valves since this architecture mitigates vapor loss through PDMS, thereby avoiding the need for using guard channels (fluidic channels to hydrate the PDMS) or techniques as in ref. 30. The microchip used here (Fig. 1) applies a simpler tri-layer architecture that includes a Mathies-style valve.²³ In this microchip architecture, for fluid handling, the top glass layer (flow layer) was etched (40 μ m deep) to form discontinuous fluidic channels while the PCR chamber in this same layer was etched to a depth of 90 μ m. The bottom glass layer (control layer) was etched (70 μ m deep) to complete the valves at the discontinuities of the fluid layer. Both the pressure

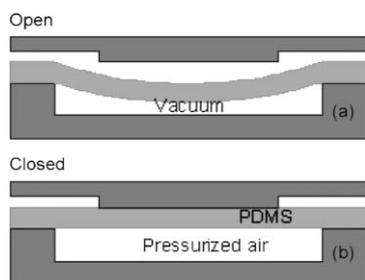


Fig. 1 Side-view of the tri-layer microchip that depicts the two states of the microvalve. The chip comprises a top etched glass layer (flow layer), a bottom etched glass layer (control layer), and a PDMS membrane between these two glass layers, which is actuated by pressurized air and vacuum. The valves are normally closed when no external vacuum is applied. When external vacuum is coupled to a valve, then as in (a), the valve opens, providing continuity in the channel within the flow layer for fluid flow (open state). When pressurized air is coupled to the valve, then as in (b), the valve is sealed shut (closed state).

and vacuum required to actuate the valves are generated by low-power mini-pumps (Virtual Industries, Inc., Colorado Springs, CO, USA) within the developed system and are controlled by a custom-built microcontroller-driven circuitry to actuate the PDMS membrane.

Three such valves, when placed in series and actuated in sequence, act as a pump which can direct the flow of fluid. Geometries in the fluidic layer were designed such that bubble-free loading of the fluid is achieved by avoiding sharp corners along the fluid flow path. For thermal cycling, the microchips are patterned with a platinum (Pt) thin-film resistive element (200 nm thick Pt, with a 20 nm thick layer of titanium that serves as an adhesion layer between glass and Pt).

Microchip fabrication

The microchip designs were drawn in L-Edit v3.0 (MEMS Pro 8, MEMS CAP, CA, USA) and transferred to a mask wafer using a pattern generator (DWL 200, Heidelberg Instruments, Torrance, CA, USA). The 4" × 4" Borofloat® glass substrate (Paragon Optical Company, Reading, PA, USA) is cleaned in hot Piranha (3 : 1 of H₂SO₄–H₂O₂) and sputter-coated with 30 μm of Cr and 180 μm of Au. HPR 504 photoresist (Fujifilm USA Inc., Valhalla, NY, USA) was spin coated with a spin speed of 500 rpm for 10 s and a spread speed of 4000 rpm for 40 s. The photoresist-coated substrate was then baked in an oven at 115 °C for 30 min. UV exposure (4 s, 356 nm and with an intensity of 19.2 mW/cm²) of the spin-coated substrate was performed through the chrome mask using a mask aligner (ABM Inc., San Jose, CA, USA). The substrate was then chemically developed with Microposit 354 developer (Shipley Company Inc., Marlborough, MA, USA) for *ca.* 25 s. Glass was etched at *ca.* 1.1 μm min⁻¹ using hydrofluoric acid [20 : 14 : 66 HF(49%)–HNO₃(70%)–H₂O]. The control layer was etched to a 70 μm depth, and the flow layer had the CE channels etched to 40 μm, while the PCR chamber was etched 90 μm deep to realize the required reaction volume. Subsequently, Au etch (0.0985 M I₂ + 0.6024 M KI) and Cr etch (Arch Chemicals Inc., Norwalk, CT, USA) were used to strip the metal with etch times of *ca.* 45 s for Au and *ca.* 30 s for Cr. Holes in the flow layer for accessing

both the flow and the control layers of the assembled chip were drilled using a Waterjet system (Bengal, Flow International Corp., Kent, WA, USA). Pt was patterned on the control layer *via* a lift-off technique. The metal-stripped etched glass (control layer) was cleaned in freshly-prepared Piranha, and 20 μm of Cr was then sputter deposited on the glass substrate. Next, AZ 4620 photoresist (AZ Electronic Materials Corp, Branchburg, NJ, USA) was spin coated for 10 s at a spread speed of 500 rpm and spin speed of 2000 rpm for 25 s, the substrate was soft-baked on a hot-plate for 90 s, and then hydrated for 2 h. The photoresist was then UV exposed for 30 s and developed using AZ 400 K (AZ Electronic Materials USA Corp., Branchburg, NJ, USA) developer for *ca.* 120 s, after which the Cr was etched. Then, 20 nm of Ti and 220 nm of Pt were sputter deposited, and using lift-off, the Pt/Ti electrodes were defined on the control layer. Post-fabrication, the heaters were annealed (*ca.* 200 °C for 2 h) to ensure highly repeatable performance at elevated temperatures. As the final step in producing the tri-layer microchip, the PDMS membrane was irreversibly bonded³² to the etched faces of both the flow and the control layers using a custom-built UV-Ozone cleaner. For efficiency we re-use the microchips after a process that, although rapid, exposes the microchip components to harsh processing environments similar to those initially used to fabricate the device. These harsh conditions are sufficient to destroy any residual DNA – there is no difference in behaviour between reprocessed chips and new chips and they can be re-used indefinitely. To re-use a chip (*e.g.* after each PCR–CE run), the PDMS is dissolved in Dynasolve 210 (Dynamol, Indianapolis, IN, USA), and the flow layer is Piranha-cleaned (cold followed by hot, *i.e.* *ca.* 110 °C) and subsequently annealed at 400 °C for *ca.* 4 h. The flow and control layers are then prepared using the procedure as described in ref. 24, including a surface treatment of Sigmacote (Sigma-Aldrich Co., St Louis, MO, USA). Subsequently, the treated glass plates are reassembled with a fresh PDMS layer. Thus, without the need to re-fabricate the glass layers (the most time-consuming fabrication steps), the chips are reassembled and it has been established that no cross-contamination between runs exists.

Capillary electrophoresis instrumentation

The CE functionality of the instrument (dimensions: 8" × 10" × 12") is provided by a high voltage sub-system and an optical assembly for fluorescent detection. Microcontroller-based circuitry communicates with a PC *via* a custom-built graphical user interface (GUI) through which the experiments are controlled. The high voltage output of an EMCO DC-DC converter (C60, Sutter Creek, CA, USA) sources up to 6 kV. High voltage relays (Crydom, San Diego, CA, USA; Model DAT71210) control the state for each electrode, to either apply a known fixed voltage (as set by the user), floating, or ground. During electrophoresis, the current is measured by a transimpedance amplifier (LT1114; Linear Technology Corp., Milpitas, CA, USA). The precision amplifier has a maximum input bias of 250 pA and a 60 μV offset voltage, making it appropriate for use as a micro-ammeter. A laser (M635-5; US Lasers, Hazlehurst, GA, USA) is directed so that it enters perpendicularly through the edge of the microchip to intersect the CE separation channel (Fig. 2). The laser output is 5 mW,

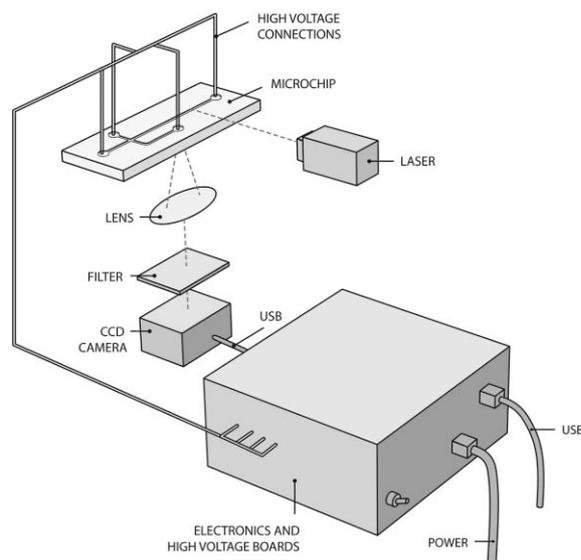


Fig. 2 Optical path (and electrical connections) for fluorescence detection. A laser beam is shone on the edge of the microchip, and a fraction of this light is coupled into the sieving matrix-filled CE channel. Fluorescently tagged DNA electrophoretically migrates through the illuminated region of the channel, and the resulting emitted fluorescent light is focused, filtered and captured by a CCD camera.

with only a small fraction of this power eventually coupled to the CE channels in the microchip.

Automated image processing to capture the fluorescence images

The CCD detector images a substantially larger area than the CE channel itself (typical channel widths are of *ca.* 100 μm), thus allowing multi-channel imaging with change in software. When DNA migrates through the channel along the field of view of the detector, the fluorescent light emitted from the channel is gathered by a lens (15 mm diameter, NT45-879; Edmund Optics Barrington, USA) and then passed through an interference filter (HQ710/50 m; Chroma Technology Corporation, Rockingham, USA) to block excitation light (primarily from scatter). The CCD camera (Deep Sky Imager; Meade Instruments Corp., Irvine, CA, USA) itself required that the operator set the black and white values of the light (version 2.0 of the Autostar Envisage software; Meade Instruments Corp., Irvine, CA, USA), which is used to determine the contrast of the images and define the resolution with which the image intensity is eventually measured. The black value for contrast in the camera is established by observing the refraction of light from the channel of the chip loaded with polymer prior to beginning electrophoresis. The black value is set to remove *ca.* 90% of the baseline in the analysis region. The white value is set such that it is approximately 10% above the maximum peak intensity in order to prevent clipping. The CCD is set to have an exposure time of 170 ms, a value that was empirically found to be suitable for the end-labelled PCR products used in our experiments (sampling time of 1 s). During the DNA separation in the analysis phase, a typical band of DNA generates a fluorescent signal that is caught by the CCD in several successive frames. These are sent to and stored on the controlling PC in real-time *via* the USB port (using the Meade software).

Custom-developed image processing software running on the PC performs the data processing of the CCD images. The average intensity of a particular image section (where the fluorescent spot is visible) is quantified and plotted over time to form an electropherogram. This quantification of the intensity of the signal by the automated software is performed by first establishing a known dark region in the image as a background by using the first image during the CE – this is when the DNA has not yet migrated into the field of view of the camera. By comparing this background against the brighter regions in each image, a box of a pre-defined number of pixel intensities is then averaged to minimize the noise. This same box is then used to extract the average intensity information from all subsequent images captured during the electrophoretic run. Empirically, it was established that reasonable variations in the selected box size did not significantly affect the average or the noise level. For a given CE run, each acquired image is eventually reduced to a single data-point in the electropherogram. The sampling period (*ca.* 1 s) is short enough to ensure that the migration of DNA is captured at typical DNA electrophoresis migration velocities as dictated by the applied separation electric field. Such electropherograms can be interpreted by a qualified clinician and are a common endpoint in diagnostic methods.

Capillary electrophoresis (CE) protocol

To avoid the need for complex electrophoresis protocols (steps such as denaturing the DNA prior to CE either by chemical or thermal means), the CE was performed on double-stranded DNA. A linear polyacrylamide (LPA) separation matrix was optimized to be used with our microchips (similar to that used in ref. 25). We found that 4% LPA resulted in adequate resolution in CE separations (sufficient to allow correct sizing of the DNA fragments/PCR products in relation to the size standards) and optimal viscosity for ease in channel filling. 4% LPA is prepared by mixing 900 μL of water with 100 μL 10 \times Tris TAPS EDTA (TTE) buffer,²⁵ and 400 mg of 10% LPA (Polysciences, Inc, Warrington, PA; cat# 19901, MW 600 000–1 000 000). This mixture is then vortexed and centrifuged for 30 s at 400 rpm. To suppress the surface effects that may potentially interfere with the electrophoretic migration during CE and to improve the separation efficiencies (and repeatability), prior to the first filling of the sieving matrix, the CE channels are filled with Dynamic coating (The Gel Co., San Francisco, CA; cat# DEH-100) as in ref. 22 and the chip is allowed to sit for 45 min before removing the solution. To perform CE, the channel is filled with 4% LPA using a syringe, and 4 μL of 1 \times TTE buffer is then pipetted into each of the wells except the injection well (Fig. 3). Post-PCR, we flush out the contents of the PCR chamber by loading 0.1 \times TTE in the input well and pumping (with the on-chip pumps/valves) through to the CE section. This ensures that the 0.1 \times TTE and the amplified PCR sample are mixed and loaded into the injection well of the CE section of the chip. An injection voltage of 300 V is then applied for 60 s, followed by a separation voltage of 1000 V for 260 s. Optical detection is performed at 24 mm from the CE channel intersection. After the CE run is completed, the polymer is flushed out of the channels and the chip is stored in 10 \times TTE buffer for subsequent re-use.

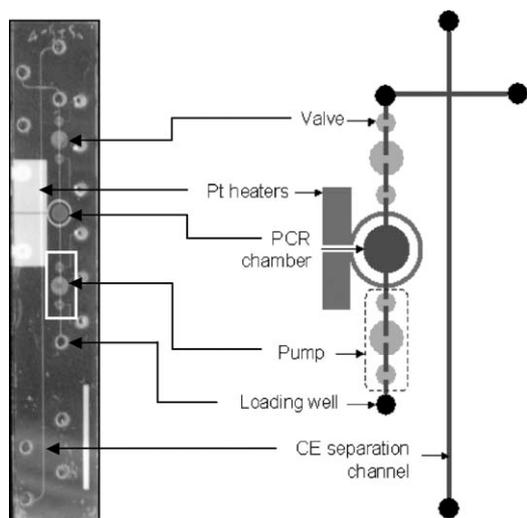


Fig. 3 Photograph (left) and schematic (right) of the integrated RT-PCR-CE microchip. The PCR/RT-PCR biochemical mixture is externally prepared and placed in the loading well of the chip. With the appropriate sequence of actuation for the valves comprising the pump, the RT-PCR mixture is loaded into the reaction chamber for thermal cycling. After the genetic amplification is completed, the fluid is pumped into the injection well of the CE section of the microchip. Subsequently, CE is performed to detect and size the PCR product with a DNA ladder. Chip dimensions: $95 \times 18 \times 2.5$ mm.

Microchip RT-PCR

All RT-PCR mixtures included $25 \mu\text{L}$ of $2\times$ reaction mixture (a buffer containing 0.4 mM of each dNTP, 2.4 mM MgSO_4), $1 \mu\text{L}$ of the enzyme mixture comprising SuperScript III RT and high-fidelity Platinum Taq polymerase (Invitrogen Life Technology, Burlington, ON, Canada), $15\text{--}20 \mu\text{L}$ 5 mM MgSO_4 , $1 \mu\text{L}$ of each forward and reverse primer ($10 \mu\text{M}$) (primer set-I: forward: $5\text{-CCA GCA GAG AAT GGA AAG TC-3'}$, and reverse: $5\text{-ACT TAA CTA TCT TGG GCT GTG AC-3'}$), $1 \mu\text{g}$ of RNA template, and double distilled water to reach a $50 \mu\text{L}$ volume. The primers were labelled with VIC dye and synthesized by ABI (Applied Biosystems, Foster City, CA). The expected product size is 243 bp . Thermal cycling conditions using the microchip were 45°C for 30 min , 94°C for 2 min , 35 cycles of 94°C for 15 s , 60°C for 30 s , 68°C for 30 s , and a final extension time of 7 min at 68°C . For additional control experiments on the reagents, a second primer (set-II) set was used: forward: $5\text{-CCA GCA GAG AAT GGA AAG TC-3'}$, and reverse: $5\text{-GAT GCT TAC ATG TCT CG-3'}$, designed to amplify a 268 bp fragment from RNA (cDNA) or a 884 bp fragment from genomic DNA.

Thermal management

Platinum exhibits a highly linear dependence of resistivity on temperature³¹ and this feature is exploited here for using the resistive element simultaneously as a heater and the temperature sensor. Such a heater/sensor design eliminates the need for two resistive elements, simplifying the microchip electrical interfacing while also reducing the real-estate usage on the microchip, both of which are critical to realizing a portable system. Heater geometry optimization coupled with adequately spaced component placement on the microchip ensures adequate

spreading of the heat flux, thus ensuring temperature uniformity within the reaction chamber during the PCR operation. As we will report elsewhere, we have characterized the thermal performance of the system *via* simulation and the use of custom-synthesized thermochromatic liquid crystals (Hallcrest Inc., Glenview, IL, USA). That work indicates that temperatures within the chamber are kept uniform to within 1°C (during steady state) along the resistive element and throughout the reaction chamber.

Instrumentation for thermal cycling and fluidic control

Custom-built circuitry was used to control the valves and pumps as well as for controlling the thin-film elements to perform localized heating and temperature sensing. The pumps and valves were actuated by connecting the on-chip control layers to either a pressure of -7.4 psi (to open) or a pressure of 20 psi (to close) generated by the mini-pumps (one for pressure and one for vacuum) included in the platform (run at 12 V and 150 mA). The pressure and vacuum generated are higher than the manufacturer's specification since these pumps are being coupled to dead-end reservoirs/channels. This control was performed by off-chip valves (LHDA1233115H; The Lee Company, Westbrook, USA) under computer control. For precise thermal cycling, within the microcontroller software, a Proportional Integral Derivative (PID) controller is employed to regulate the current applied to the resistive element. The proportional-derivative (PD) component is used to ensure rapid transients between the different temperature stages during PCR, while a proportional-integrative (PI) component maintains constant temperature during the dwell times of the three stages of PCR. The PID controller ensures that transitions between the PD and the PI controller are enforced with minimum overshoots and undershoots in temperature. A DC current is applied to the resistive element, and the temperature of the element is subsequently computed from its resistance with a pre-determined resistance *vs.* temperature function. With this temperature as the feedback, the applied current was controlled and adjusted to the desired temperature for thermal cycling. The resistive element heats almost instantaneously when the desired current is applied *via* the control circuitry, and therefore, the temperature cycling rate of the PCR chamber was limited primarily by the thermal conductivity between the resistive thin-film and the chamber (with heating rates of up to 10°C s^{-1} within the reaction chamber) and also by the cooling rate of the system. Cooling of the reaction chamber (and the microchip) was achieved *via* passive natural convection and conduction to cooler areas of the microchip and through the base of the stage on which the microchip is placed in the platform.

Results and discussion

As a prototype for work with total RNA, we demonstrated the use of the microchip platform to amplify and detect transcripts encoding $\beta 2$ microglobulin ($\beta 2\text{M}$), a housekeeping gene expressed in human cells.³³ Such transcripts are commonly used as positive controls. For this microchip validation, RNA was isolated from KMS-34, a multiple myeloma (MM, cancer) cell line. The primers (set-I) were designed to amplify a 243 bp fragment

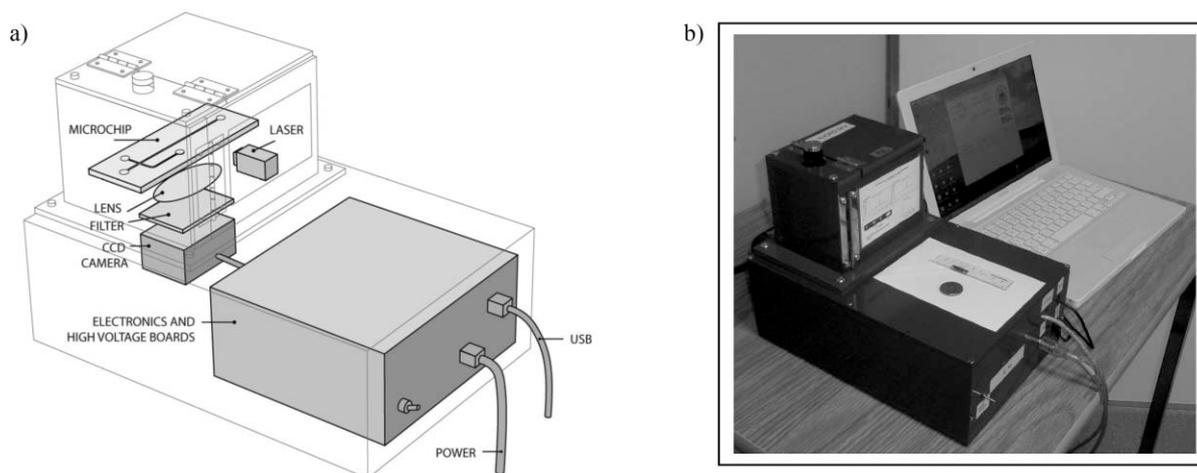


Fig. 4 (a) Schematic depicting major functional blocks of the 'shoe-box'-sized portable platform that can perform high voltage generation (variable from 0 to 6 kV) and control (switching). In addition, this platform is capable of fluorescence detection (CCD-based and hence large area imaging suitable for multi-channel detection) while also providing thermal and fluidic control. (b) Photograph of the PCR-CE system.

from RNA or from genomic DNA. Using the instrument (Fig. 4) and microchips (Fig. 3), the reaction chamber was filled with the RT-PCR mixture, the valves were subsequently sealed, thermal cycling was performed, and then the amplicons were pumped into the loading well where CE is subsequently performed (*i.e.* an on-chip positive control experiment). All of these actions were coordinated by the custom-built microcontroller-based circuitry on the system, requiring no human intervention, with user control *via* the PC. The on-chip RT-PCR amplified a fragment of the appropriate size, indicating successful RT-PCR (Fig. 5a).

For comparison, an identical run of β 2M RT-PCR and CE was performed (Fig. 5b) using a conventional thermal cycler and a commercial microchip electrophoresis system, referred to as a Microfluidic Tool Kit™ or μ TK (Micalyne, Edmonton, AB, Canada). Although the present microchips are relatively easily fabricated, their separation performance is significantly lower than that of glass chips in terms of resolution and sensitivity. The separation resolution was comparable in the commercial and the miniaturized system, with the average resolution in the vicinity of 200 and 300 bps being *ca.* 16.5 bp. Although single base resolution can be obtained with longer chips and different sieving matrices, this resolution in CE separation has been demonstrated to be adequate in our earlier diagnostic applications.^{28,34} The signal to noise ratio (SNR) for the custom-built system presented here was calculated by determining the noise level in the 50 s of signal prior to the arrival of the first peaks. This estimate of the noise level is likely to be too high due to slight variations in the baseline during the 50 s period; however, given the slow sampling rate of the present system this long sampling time appears to be unavoidable. For the glass/PDMS chip the SNR was 23 while that using the same microchip in the μ TK was 32.3. The relatively low SNR in both systems, demonstrated in Fig. 5 (using the microchip as in Fig. 3), is attributed to the present hybrid chip design with its multiple air/glass/PDMS interfaces, each of which scatter significantly. This can readily be fixed by moving to a glass chip as in ref. 27 and as in our earlier work.³⁴ With such glass chips the SNR is

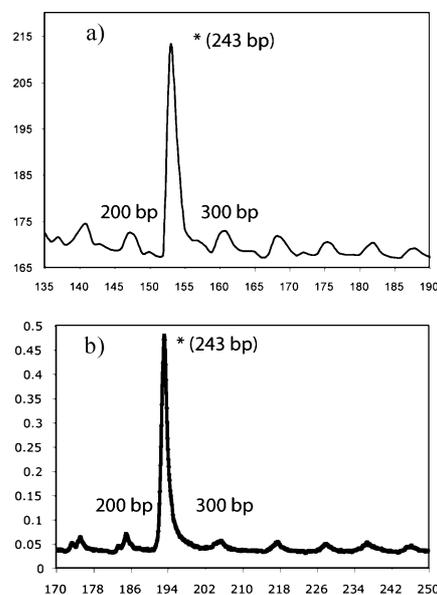


Fig. 5 Fluorescence (arbitrary units) *versus* time (s) for the electrophoresis stage of a single-step RT-PCR-CE performed (using the chip and the protocol described in the Materials and methods section) within the portable platform to detect the β 2M gene (small peaks are from DNA ladder, ALFexpress™ size 50–500; Amersham Biosciences, Piscataway, USA). (a) Portable PCR-CE platform and (b) commercial microchip capillary electrophoresis system for CE analysis (μ TK). Single-step microchip RT-PCR integration was performed to demonstrate the capability of the developed platform to detect transcripts. This test was developed on a β 2M gene with primers designed to amplify a 243 bp fragment from total RNA from a positive multiple myeloma KMS-34 cell line. On-chip PCR product was verified (data not shown) using acrylamide gels, and sequenced (using an ABI3100).

10–20 times higher. Nevertheless, the present SNR is adequate for clinical interpretation. For comparison, recent work by Bliss *et al.*³⁵ is representative of the conventional confocal systems with a SNR of *ca.* 330 (with a glass chip).

Confocal systems such as that in the μ TK typically employ expensive optics, designed to minimize the collection of out-of-focus light (*i.e.* they minimize interference from light emanating from above and below the imaged plane) or scattered light. These systems typically rely on a PMT for detection. By design, the confocal LIF system samples the fluorescence from a relatively constant volume within the microchannel. Thus, as expected, with the confocal system we have experimentally established that the SNR increases weakly with increasing depth of the channel. On the other hand, the signal seen by the CCD is approximately proportional to the volume of the microchannel that is imaged. As a result, an experimental determination found that for glass microchips, the CCD-based imaging system gave a SNR that increases strongly with increasing depth of the channel. We estimate that for channels that are approximately 100 μm in depth, the SNR values for the CCD and confocal systems will be equivalent (data not shown). We will report on this work elsewhere. For applications demonstrated here, it was convenient (and sufficient) to use microchips with channel depths of 40 μm .

We frequently performed on-chip negative controls (*i.e.* the RT-PCR mixture according to the procedure as outlined in the Materials and methods section, but with no template RNA) both on newly fabricated chips and on re-treated chips (procedure in the Materials and methods section) and found that they consistently showed that the system *does not generate false positives* from either RNA or DNA contamination (data not shown). The β 2M RNA is used here as a positive control. Since we reliably detect a product when the sample contained the β 2M RNA it is clear that the system *does not generate false negatives*. Because of the single-use nature of our microchips, on-chip positive (with RNA) and negative (without RNA) runs cannot be performed in parallel (though we plan to design microchips capable of performing parallel runs in the near future).

Each microchip run was accompanied with reagent positive and negative controls (RT-PCR mixture with and without template) using conventional equipment (a thermal cycler and polyacrylamide gel electrophoresis) to ensure that no contamination in the reagent mixture occurs. Since we have found that it is difficult to completely empty and clean out the reaction chamber of our microchip, to avoid contamination issues all microchips are used only once, after which the chips undergo the extensive treatment and reassembly procedure described in the Materials and methods section.

Additional control experiments on the reagents were also performed to ensure that only RNA (cDNA) is amplified. This was tested by making use of primer set-II (data not shown) which yields different primer sizes for genomic DNA and RNA (cDNA). As this is a test of the primer design, it was performed using a conventional thermal cycler and run on a polyacrylamide gel. Our RT-PCR conditions consistently amplify only the 268 bp product from cDNA, with no detection of the 884 bp product that would be present if genomic DNA had been amplified (*e.g.* by contamination of the template RNA by DNA).

A primary focus in this work was to show that we have sufficient sensitivity in optical detection despite a dramatic reduction in complexity and cost. In terms of the electrophoretic performance of our system, and as described above, we have found that our SNR is comparable to that of a confocal PMT-

based system when deep-channel (100 μm) glass chips are used. We have been able to reliably detect as little as 0.1 ng μL^{-1} of end-labelled PCR product (tagged with Cy5, data not shown) placed in the injection well of the CE section using a protocol much as outlined in ref. 34 (glass/glass microchip and POP6 polymer). We are in the process of fully assessing the performance of the genetic amplification component of this system. Although we used a high concentration of RNA in this work (40 ng μL^{-1}), in past work²⁸ with a similar PCR-CE microchip we were able to reliably detect 3–4 copies of viral DNA from clinical samples. The specificity of the amplification is established by electrophoretic sizing of the product on the integrated microchip. In addition, the sequence of product amplified conventionally using the same primers was verified on an ABI3100.

Concluding remarks and future directions

In this work the electronics and optics required for performing microchip-based RT, PCR and CE were integrated within an inexpensive, compact and portable platform. We believe that the RT-PCR-CE implementation described here holds great potential for point-of-care testing. The entire system in its current form is 'shoebox-sized' with component costs of less than \$1000 while still demonstrating performance comparable to commercial systems that are several orders of magnitude more expensive.

Further miniaturization of the PCR (RT-PCR) volumes and of the microchip itself is also readily feasible. However, we have found that our present reaction volume (600 nL) is optimal for detecting low concentrations of transcripts and viral or bacterial templates in unconcentrated clinical samples. Additionally, further miniaturization of PCR volume for clinically important applications is likely to be of marginal benefit since the reagent costs are already less than about \$1. In related work, we have recently reported a microchip-based PDMS/glass PCR-CE approach for analyzing the viral load for a DNA virus (BKV) from unprocessed urine,²⁸ the use of a chromosomal translocation method to assist in cancer diagnosis and treatment,³⁶ and pharmacogenetic tests to avert adverse drug reactions.³⁷ These clinically relevant tests can readily be ported to the system demonstrated here.

The present system is a highly portable and practical genetic analysis system that is both versatile and sensitive. With the integration of sample preparation techniques to extract and concentrate nucleic acids from raw clinical samples (*e.g.* whole blood, buccal cells, *etc.*) coupled with strategies to improve the rapidity of the tests, this system should be well-suited for a wider variety of real-time diagnostics in the clinic or in the field. The testing of individual samples at the time they are taken enables more effective clinical decision making. This ability to cost-effectively test individuals one at a time may be crucial to enabling the use of microfluidics in applications such as clinical medicine and public health surveillance.

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References

- J. Kling, 'Moving diagnostics from the bench to the bedside', *Nat. Biotechnol.*, 2006, **24**, 891–893.
- P. Yager, T. Edwards, E. Fu, K. Helton, K. Nelson, M. R. Tam and B. H. Weigl, 'Microfluidic diagnostic technologies for global public health', *Nature*, 2006, **442**, 412–418.
- R. F. Renzi, J. Stamps, B. A. Horn, S. Ferko, V. A. VanderNoot, R. Crocker, B. Wiedenman, D. Yee and J. A. Fruetel, 'Hand-held microanalytical instrument for chip-based electrophoretic separations of proteins', *Anal. Chem.*, 2005, **77**, 435–441.
- Y. C. Lin, M. Y. Huang, K. C. Young, T. T. Chang and C. Y. Wu, 'A rapid micro-polymerase chain reaction system for hepatitis C virus amplification', *Sens. Actuators, B*, 2000, **71**, 2–8.
- R. Pal, M. Yang, R. Lin, B. N. Johnson, N. Srivastava, S. Z. Razzacki, K. J. Chomistek, D. C. Heldsinger, R. M. Haque, V. M. Ugaz, P. K. Thwar, Z. Chen, K. Alfrano, M. B. Yim, M. Krishnana, A. O. Fuller, R. G. Larson, D. T. Burke and M. A. Burns, 'An integrated microfluidic device for influenza and other genetic analyses', *Lab Chip*, 2005, **5**, 1024–1032.
- E. T. Lagally, S. Lee and H. T. Soh, 'Integrated microsystem for dielectrophoretic cell concentration and genetic detection', *Lab Chip*, 2005, **5**, 1053–1058.
- A. M. Skelley, J. R. Scherer, A. D. Aubrey, W. H. Grover, R. H. C. Ivester, P. Ehrenfreund, F. J. Grunthaler, J. L. Bada and R. A. Mathies, 'Development and evaluation of a microdevice for amino acid biomarker detection and analysis on Mars', *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 1041–1046.
- J. P. Ferrance, Q. Wu, B. Giordano, C. Hernandez, Y. Kwok, K. Snow, S. Thibodeau and J. P. Landers, 'Developments towards a complete micro-total analysis system for Duchenne muscular dystrophy diagnosis', *Anal. Chim. Acta*, 2003, **500**, 223–236.
- A. Gulliksen, L. A. Solli, K. S. Drese, O. Sorensen, F. Karlens, H. Rogne, E. Hovig and R. Sirevag, 'Parallel nanoliter detection of cancer markers using polymer microchips', *Lab Chip*, 2005, **5**, 416–420.
- P. Gascoyne, J. Satayavivad and M. Ruchirawad, 'Microfluidic approaches to malaria detection', *Acta Trop.*, 2004, **89**, 357–369.
- F. C. Huang, C. S. Liao and G. B. Lee, 'An integrated microfluidic chip for DNA/RNA amplification, electrophoresis separation and on-line optical detection', *Electrophoresis*, 2006, **27**, 3297–3305.
- M. G. Roper, C. J. Easley and J. P. Landers, 'Advances in polymerase chain reaction on microfluidic chips', *Anal. Chem.*, 2005, **77**, 3887–3894.
- E. Verpoorte, 'Microfluidic chips for clinical and forensic analysis', *Electrophoresis*, 2002, **23**, 677–712.
- A. J. deMello, 'Control and detection of chemical reactions in microfluidic systems', *Nature*, 2006, **442**, 394–402.
- C. Liao, G. Lee, H. Liu, T. Hsieh and C. Luo, 'Miniature RT-PCR system for diagnosis of RNA-based viruses', *Nucleic Acids Res.*, 2005, **33**, e156.
- P. J. Obeid, T. K. Christopoulos, H. J. Crabtree and C. J. Backhouse, 'Microfabricated device for DNA and RNA amplification by continuous-flow polymerase chain reaction and reverse transcription-polymerase chain reaction with cycle number selection', *Anal. Chem.*, 2003, **75**, 288–295.
- J. S. Marcus, W. F. Anderson and S. R. Quake, 'Parallel picoliter RT-PCR assays using microfluidics', *Anal. Chem.*, 2006, **78**, 956–958.
- P. J. Obeid and T. K. Christopoulos, 'Continuous-flow DNA and RNA amplification chip combined with laser-induced fluorescence detection', *Anal. Chim. Acta*, 2003, **494**, 1–9.
- Z. M. Zhou, D. Y. Liu, R. T. Zhong, Z. P. Dai, D. P. Wu, H. Wang, Y. G. Du, Z. N. Xia, L. P. Zhang, X. D. Mei and B. C. Lin, 'Determination of SARS-coronavirus by a microfluidic chip system', *Electrophoresis*, 2004, **25**, 3032–3039.
- J. W. Hong, T. Fujii, M. Seki, T. Yamamoto and I. Endo, 'Integration of gene amplification and capillary gel electrophoresis on a polydimethylsiloxane-glass hybrid microchip', *Electrophoresis*, 2001, **22**, 328–333.
- R. G. Blazej, P. Kumaresan and R. A. Mathies, 'Microfabricated bioprocessor for integrated nanoliter-scale Sanger DNA sequencing', *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 7240–7245.
- C. N. Liu, N. M. Toriello and R. A. Mathies, 'Multichannel reverse transcription-polymerase chain reaction microdevices for rapid gene expression and biomarker analysis', *Anal. Chem.*, 2006, **78**, 5474–5479.
- W. H. Grover, A. M. Skelley, C. N. Liu, E. T. Lagally and R. A. Mathies, 'Monolithic membrane valves and diaphragm pumps for practical large-scale integration into glass microfluidic devices', *Sens. Actuators, B*, 2003, **89**, 315–323.
- C. J. Easley, J. M. Karlinsey and J. P. Landers, 'On-chip pressure injection for integration of infrared-mediated DNA amplification with electrophoretic separation', *Lab Chip*, 2006, **6**, 601–610.
- N. M. Toriello, C. N. Liu and R. A. Mathies, 'Multichannel reverse transcription-polymerase chain reaction microdevice for rapid gene expression and biomarker analysis', *Anal. Chem.*, 2006, **78**, 7997–8003.
- C. J. Easley, J. M. Karlinsey, J. M. Bienvenue, L. A. Legendre, M. G. Roper, S. H. Feldman, M. A. Hughes, E. L. Hewlett, T. J. Merkel, J. P. Ferrance and J. P. Landers, 'A fully integrated microfluidic genetic analysis system with sample-in-answer-out capability', *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 19272–19277.
- P. Liu, T. S. Seo, N. Beyor, K. J. Shin, J. R. Scherer and R. A. Mathies, 'Integrated portable polymerase chain reaction-capillary electrophoresis microsystem for rapid forensic short tandem repeat typing', *Anal. Chem.*, 2007, **79**, 1881–1889.
- G. V. Kaigala, R. J. Huskins, J. Preiksaitis, X. L. Pang, L. M. Pilarski and C. J. Backhouse, 'Automated screening using microfluidic chip-based PCR and product detection to assess risk of BK virus-associated nephropathy in renal transplant recipients', *Electrophoresis*, 2006, **27**, 3753–3763.
- M. A. Unger, H.-P. Chou, T. Thorsen, A. Scherer and S. R. Quake, 'Monolithic microfabricated valves and pumps by multilayer soft lithography', *Science*, 2000, **288**, 113–116.
- A. R. Prakash, S. Adamia, V. Sieben, P. Pilarski, L. M. Pilarski and C. J. Backhouse, 'Small volume PCR in PDMS biochips with integrated fluid control and vapour barrier', *Sens. Actuators, B*, 2006, **113**, 398–409.
- E. T. Lagally, J. R. Scherer, R. G. Blazej, N. M. Toriello, B. A. Diep, M. Ramchandani, G. F. Sensabaugh, L. W. Riley and R. A. Mathies, 'Integrated portable genetic analysis microsystem for pathogen/infectious disease detection', *Anal. Chem.*, 2004, **76**, 3162–3170.
- G. V. Kaigala, S. Ho, R. Penterman and C. J. Backhouse, 'Rapid prototyping of microfluidic devices with a wax printer', *Lab Chip*, 2007, **7**, 384–387.
- P. M. Pilarski, S. Adamia and C. J. Backhouse, 'An adaptable microvalving system for on-chip polymerase chain reactions', *J. Immunol. Methods*, 2005, **305**, 48–58.
- C. J. Backhouse, H. J. Crabtree and D. M. Glerum, 'Frontal analysis on a microchip', *Analyst*, 2002, **127**, 1169–1175.
- C. L. Bliss, J. N. McMullin and C. J. Backhouse, 'Rapid fabrication of a microfluidic device with integrated optical waveguides for DNA fragment analysis', *Lab Chip*, 2007, **7**, 1280–1287.
- J. VanDijken, G. V. Kaigala, J. Lauzon, A. Atrazhev, S. Adamia, B. J. Taylor, T. Reiman, A. R. Belch, C. J. Backhouse and L. M. Pilarski, 'Microfluidic chips for detecting the t(4;14) translocation and monitoring disease during treatment using RT-PCR analysis of IgH-MMSET hybrid transcripts', *J. Mol. Diagn.*, 2007, **9**, 358–367.
- J. Chowdhury, G. Kaigala, S. Pushpakom, J. Lauzon, A. Makin, A. Atrazhev, A. Stickel, W. G. Newman, C. J. Backhouse and L. M. Pilarski, 'Microfluidic platform for SNP genotyping of the thiopurine s-methyltransferase gene to evaluate risk for adverse drug events', *J. Mol. Diagn.*, 2007, **9**, 521–529.