Published in final edited form as:

Electrophoresis. 2010 September ; 31(18): 3074–3082. doi:10.1002/elps.201000209.

Polymer-Based Dense Fluidic Networks for High Throughput Screening with Ultrasensitive Fluorescence Detection

Paul I. Okagbare^{1,3} and Steven A. Soper^{1,2,3,4}

¹ Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803

² Department of Mechanical Engineering, Louisiana State University, Baton Rouge, LA 70803

³ Center for BioModular Multi-Scale Systems, Louisiana State University, Baton Rouge, LA 70803

⁴ Nano-Biotechnology and Chemical Engineering, Ulsan National Institutes of Science and Technology, Ulsan, South Korea

Abstract

Microfluidics represents a viable platform for performing High Throughput Screening (HTS) due to its ability to automate fluid handling and generate fluidic networks with high number densities over small footprints appropriate for the simultaneous optical interrogation of many screening assays. While most HTS campaigns depend on fluorescence, readers typically use point detection and serially address the assay results significantly lowering throughput or detection sensitivity due to a low duty cycle. To address this challenge, we present here the fabrication of a high density microfluidic network packed into the imaging area of a large field-of-view (FoV) ultrasensitive fluorescence detection system. The fluidic channels were 1, 5 or 10 μm (width), 1 μm (depth) with a pitch of 1–10 μm and each fluidic processor was individually addressable. The fluidic chip was produced from a molding tool using hot embossing and thermal fusion bonding to enclose the fluidic channels. A 40X microscope objective (numerical aperture = 0.75) created a FoV of 200 μm , providing the ability to interrogate ~25 channels using the current fluidic configuration. An ultrasensitive fluorescence detection system with a large FoV was used to transduce fluorescence signals simultaneously from each fluidic processor onto the active area of an electron multiplying charge-coupled device (EMCCD). The utility of these multichannel networks for HTS was demonstrated by carrying out the high throughput monitoring of the activity of an enzyme, APE1, used as a model screening assay.

Keywords

High Throughput Screening; Microfluidics; Ultra-sensitive Fluorescence; Enzyme Activity

1. Introduction

High Throughput Screening (HTS) is the process of selecting a potential drug candidate from a pool of diverse chemical elements and represents an integral part of the drug discovery pipeline, which is continuously demanding higher sample processing throughput to reduce drug development cost [1–5]. The number of potential therapeutic targets is rapidly increasing due to a better understanding of the genetic basis of diseases and as combinatorial chemical synthesis has now generated large compound libraries for screening, high demands have been placed on drug discovery screening technologies to provide the necessary throughput. Such HTS systems must be able to sustain extremely high processing rates without sacrificing data quality.

The primary approach toward providing extremely high throughput has been the adoption of high-density titer plate systems capable of screening ~100,000 assays per day [3,6–8]. The consequence of using these high density plates is that sample well volume is significantly reduced compared to low-density plates. To provide the necessary limit-of-detection for ultra-small volume wells, fluorescence-based detection techniques have become increasingly popular due to the inherent sensitivity advantage afforded by fluorescence measurements. For example, modern HTS technology, such as that offered by the Evotec robotic micro-plate system (EVOscreen Mark-II and EVOscreen Mark-III) [9], employs a confocal fluorescence reader that serially addresses assay results [9–11]. Unfortunately, the serial strategy associated with the confocal fluorescence scanner places a severe limitation on the capability of the system to further enhance throughput without sacrificing detection sensitivity. Besides detection, implementation of higher density micro-plates with reduced well volumes is faced with a number of other challenges such as evaporation resulting from the high surface-to-volume ratio of these wells and the difficulty in manipulating small volumes (<1 μ L).

Advances in microfabrication technology has created opportunities for exploring the potential of microfluidics for HTS [12]. Microfabricated devices are capable of manipulating and processing ultra-small volumes (10^{-9} to 10^{-18} L) in microchannels with dimensions of a few micrometers. In addition, several processing units can be configured in a small footprint suitable for high sensitivity optical imaging, which provides the opportunity for massively parallel sample processing. Furthermore, microfluidic devices are characterized by their low variation in structure sizes resulting from the highly parallel nature of the fabrication process and coupled with the ability to employ either hydrodynamic or electrokinetic pumping for fluid transport [13–17], can simplify fluid handling and reduce inter-assay variability. These unique features of microfluidics make them viable platforms for HTS [18,19]. Employing microfluidics for HTS provides several other advantages as well including, reduction in solvent evaporation [20], low consumption of samples and reagents, shorter analysis times and the ability to automate fluid handling [21–24]. Another key feature of microfluidics is the ability to integrate several processing steps into a single system, which simplifies routine operation and may reduce sample cross-contamination [25–27]. Consequently, microfluidics can provide massive parallelization brought about by configuring multiple fluidic processors in a single device [28].

The utility of microfluidics for throughput enhancement in different types of assays has been demonstrated [29–34]. The potential of microfluidics for large-scale biochemical screening was demonstrated through the use of microfabricated microchannel array systems with automated sample loading from microtiter plates to screen 96 samples in less than 90 s [35]. Other examples includes on-chip immunoassays for screening fluorescently-labeled antibodies [36], continuous-flow sandwich hybridization assay for pathogen detection [37], bead-based assays for detection of gene expression [38], detection of low-affinity transient binding events in biomolecular interactions [39], parallel screening of in-situ click chemistry [40], kinase assays based on the separation of reaction products from substrates and cell-based assays [3,41–43]. Therefore, the application of microfluidics in HTS can provide a way to resolve current challenges in drug screening and significantly increase throughput [28].

While microfluidics can provide a suitable platform for HTS by generating high numbers of sample processing units over a small footprint, a suitable detection system is required for the parallel interrogation of the fluidic processors such that the resulting fluorescence from each assay is imaged onto an array detector possessing the prerequisite sensitivity for parallel readout. An array detector that can be employed for ultra-sensitive fluorescence detection is the electron multiplying charge-coupled device (EMCCD) [44–46]. Because throughput is

directly related to the number of fluidic processors that can be imaged onto the detector, the configuration of fluidic processors in a high density format can provide the necessary throughput to realize the full potential of microfluidics in HTS [47].

In this paper, we present a high density fluidic device with a series of individual fluidic sample processors configured in a small footprint suitable for high sensitivity optical imaging. The fluidic device was fabricated using UV-LiGA to first produce the prerequisite structures onto a metal molding tool (Ni), which was transferred onto a polymer substrate during replication using hot embossing. The polymer microchip possessed multiple fluidic processors with an input reservoir for each fluidic processor. An ultrasensitive fluorescence detection system with a large field-of-view (FoV) was used to transduce fluorescence signals simultaneously from each fluidic processor onto the active area of an EMCCD. The utility of the multichannel network for HTS was demonstrated by parallel monitoring of fluorescence signals generated from AlexaFluor 660 labeled oligonucleotides that were hydrodynamically pumped through the individual fluidic channels. This capability was further demonstrated by high throughput monitoring of the activity of AP Endonuclease 1 (APE1) enzyme on a DNA duplex target that was dual labeled with a fluorophore (AlexaFluor 660) and a quencher (BHQ-3). APE1 is known to recognize abasic (Apurinic, AP) sites in genomic DNA, where it cleaves the phosphodiester backbone on the 5' end of the AP site leaving a 3'-hydroxyl nucleotide and deoxyribose 5'-phosphate at the termini [48]. Although this is an essential step in DNA base excision repair (BER), over expression of APE1 has been linked to radio- and chemo-resistance in human tumors.

2. Materials and Methods

2.1 Fabrication and layout of the fluidic network

Fluidic structures were fabricated using UV-LiGA to produce Ni electroforms (molding tools) according to standard lithography procedures already reported [49,50]. To achieve a precise depth of 1 μm for the fluidic channels, an SU-8 photoresist was spin coated (3500 rpm; 30 s) onto a Si disc to a thickness of 1 μm and baked for 1 min at 95°C on a hot plate. A UV mask containing the fluidic patterns was designed (see Figure 1) and used for the UV exposure of the resist prior to development and electroplating steps to make the appropriate metal molding tool. The UV mask (see Figure 1) consisted of three different device designs: (1) 10 μm width (pitch = 10 μm); (2) 5 μm width (pitch = 2 μm); and (3) 1 μm width (pitch = 1 μm) channels that could be used interchangeably to make any of the three different structure [49]. The Ni molding tool was subsequently used to hot emboss to transfer the fluidic pattern into poly(methylmethacrylate), PMMA, substrates by employing a JenOptik HEX02 high-precision hot embossing system.

The device consisted of 90, 25 or 18 fluidic processors configured in a footprint of ~180 μm in the high density region for fluorescence detection. A PMMA cover plate (thickness = 125 μm) was positioned onto the device and subsequently fusion bonded to the substrate in a GC oven at 107°C (slightly above the T_g of PMMA) after being clamped between two glass plates to render uniform pressure. Each fluidic processor possessed an individual sample input reservoir (see Figure 1) for introduction of reactants (enzyme and substrate) and also a reaction zone equipped with a strip heater for incubation of the enzyme and substrate. The fluidic channels gradually fanned-out from the high density region to allow connection to individual sample input reservoirs. The width of the channel was 22 μm at each individual reservoir and tapered to 14 μm at the reaction zone and then to 1, 5 or 10 μm at the high density (detection) region. All fluidic processors had equal lengths (3 cm) and equal depths (1 μm) from the sample input reservoir to the common reservoir. This was important to maintain uniform hydraulic pressure in all of the fluidic processors. The volume capacity of each individual fluidic processor was ~0.15 nL.

2.2 Optical set-up and multichannel illumination

A wide field-of-view (FoV) fluorescence detection system described previously was used to monitor fluorescent signals from the fluidic vias using a highly sensitive EMCCD operated in a frame transfer mode [46]. Briefly, the optical system was arranged in an epi-illumination format with an HL6535MG laser diode (Thorlabs, Newton, NJ, USA) providing the excitation (660 nm) with a peak power of 30 mW. The laser output was collimated using an aspheric lens (C230TM-B, Thorlabs) and passed through a 660 nm laser line filter (660DF10, Omega Optical, Brattleboro, VT, USA) that was placed in the optical path of the excitation source. A dichroic mirror was used to direct the laser into a microscope objective (40x, or 20x, Zeiss, Thornwood, NY, USA) and then, onto the sample after being shaped using a planoconvex lens (LA1509, Focal length = 100 mm, Thorlabs). The shaping lens was used to generate a collimated light output from the objective with a diameter determined by the aperture of the objective; the diameter of the collimated light output determined the FoV of the imaging system. For example, a 0.2 mm FoV was generated for a 40x microscope objective with NA = 0.75. The system could accommodate other microscope objectives as well with the appropriate NA and magnification selected to expand the FoV. For example, a 20x microscope objective with a similar NA (0.75) generated a FoV of 0.4 mm. The fluorescence emission from the sample was collected with the same objective and directed through the dichroic filter, then a band pass filter (719AF40, Omega Optical), and finally a long pass filter (690ALP, Omega Optical) to spectrally isolate the emission from scattered radiation. The isolated emission was then directed onto a reflecting surface of a mirror.

The EMCCD camera (Cascade 1K, Photometrics, Tuscon, AZ, USA) was a frame transfer EMCCD equipped with signal enhancement via on-chip gain and possessed $8\text{ }\mu\text{m} \times 8\text{ }\mu\text{m}$ pixels configured in a 1004×1002 front illumination format. The camera had a 10 MHz digital converter and a 16-bit digitization range. The EMCCD camera was thermoelectrically cooled to -30°C .

2.3 Chemicals and Reagents

TRIS-Taps-EDTA (TTE) buffer (10x) was obtained from Amresco (Amresco, Ohio, USA) and was diluted to obtain a 1x TTE buffer solution (pH = 8.7) using nanopure water obtained from a Barnstead NANOpure System (Model D8991, Dubuque, IA). Oligonucleotides (50 bp) were custom synthesized by Midland Certified Reagent (Midland Certified Reagent Company, Inc., Midland, TX, USA). Two were used in these measurements with both being complementary to each other. One oligonucleotide contained a tetrahydrofuran (THF), an abasic (Apurinic, AP) site analogue, modification at position 8 from the 5'-end of this strand (see Figure 4a). This oligonucleotide strand was also labeled with AlexaFluor 660 at its 5'-end (designated as the sense strand). The complementary strand (anti-sense strand) was labeled with the Black Hole Quencher-3 (BHQ-3) at its 3'-end. A duplex of the sense and anti-sense strands was made through hybridization and stock solutions ($0.5\text{ }\mu\text{M}$) of the duplex were prepared in TRIS/HCl buffer containing 20 mM TRIS-HCl, 10 mM MgCl_2 10 mM KCl and 2 mM dithiothreitol (DTT) at pH 7.5. Dilutions from this stock solution to the desired concentrations were accomplished with the same buffer. AP Endonuclease (APE1) was purified and supplied by Professor Deininger at Tulane Medical School, New Orleans. In all cases, buffer solutions were filtered through a $0.2\text{ }\mu\text{m}$ filter prior to insertion into the fluidic chip.

3. Results and discussion

Several design constraints were imposed on this chip to not only provide high throughput processing capabilities, but also high detection sensitivity, high operational stability and

favorable performance of enzymatic reactions typically required for HTS. For example, the chip material selected for this application was PMMA due to its superior optical properties compared to many other thermoplastic materials in terms of minimal amounts of autofluorescence [51]. In addition, PMMA tends to show surface properties that produce minimal amounts of non-specific adsorption and thus, biological enzymes can remain in their active form [51]. PMMA also possesses a rather low sessile water contact angle (73°) and therefore is easily wettable with aqueous solvents and therefore, channels with small dimensions ($1\ \mu\text{m} \times 1\ \mu\text{m}$ channels) can be easily primed. We selected as the channel depth in all designs $1\ \mu\text{m}$ to keep the sample within the depth-of-field of the microscope objective to assist in enhancing the limit-of-detection and image quality. For a 40x microscope objective with an NA = 0.75, a depth-of-field of $\sim 1.2\ \mu\text{m}$ can be calculated. Therefore, the $1\ \mu\text{m}$ channel depth selected was smaller than the depth-of-field of the imaging microscope objective and produced a sharp image on the EMCCD as well as provided high photon processing efficiency.

As a final note, while the use of the $1\ \mu\text{m}$ wide channels with a pitch of $1\ \mu\text{m}$ would provide the highest throughput capability (90 channels) due to the higher packing density of the fluidic network, the replication fidelity was poor using these structure sizes and the embossing conditions selected herein. In many cases, the walls between these channels were removed from the substrate during demolding. In addition, the $1\ \mu\text{m}$ channels were found to be highly deformed following thermal fusion bonding. None of these artifacts were seen in the case of the $10\ \mu\text{m}$ or the $5\ \mu\text{m}$ channels. Therefore, all experiments reported herein used the $5\ \mu\text{m}$ or $10\ \mu\text{m}$ channel designs. However, work in our laboratories is currently underway to optimize the embossing conditions to improve replication fidelity for these small features.

3.1 Structural integrity following thermal fusion bonding

The utility of the fluidic device for continuous-flow assays could be affected by slight variations in the structure's dimensions resulting from thermal fusion bonding the polymer substrate to its cover plate. Therefore, we investigated possible variations in channel dimensions by measuring the width and depth of the fluidic structures before and after thermal fusion bonding to the polymer cover plate. The measurements were carried out using a Nikon measurescope (Nikon SC – 112, MM – 22, Japan). For the $5\ \mu\text{m}$ channels, we found that the channel width changed by approximately 5.5% (average), but the channel depth changed by $<1\%$ of the designed depth when using the thermal fusion bonding process adopted here. It would appear that minimal changes in the $1\ \mu\text{m}$ designed depth were observed, but we should note that slight variations in channel depth $<200\ \text{nm}$ were not registered by the microscope due to the high depth-of-field of the microscope objective used in this case. For the $10\ \mu\text{m}$ channels, no visible changes in the structure width were noticed. However, as noted above, for the $1\ \mu\text{m}$ channels, significant deformation of the channels was observed with many channels collapsed following thermal fusion bonding.

3.2 Parallel fluorescence measurements using the multichannel device

Hydrodynamic pumping was used to drive an aqueous fluid seeded with fluorescently labeled oligonucleotides through the channels of the device. This was achieved by connecting a syringe pump to the common reservoir while each fluidic channel was fed with a prepared fluorescent sample at its appropriate reservoir. The syringe pump was operated in the refilling mode, which transported the liquid sample from its input reservoir through each fluidic channel at a constant flow rate (linear velocity = $0.03\ \text{cm/s}$). Using this set-up, a $2.0\ \text{nM}$ solution of AlexaFluor 660 labeled oligonucleotides were hydrodynamically pumped through each fluidic channel and a fluorescent signal was acquired simultaneously from all of the fluidic processors. Figure 2a is a fluorescence image obtained with the EMCCD

showing fluorescent signal from 25 microchannels that were 5 μm in width with a 2 μm pitch, while in Figure 2b is shown 10 μm wide channels with a 10 μm pitch.

In Figure 2c, an intensity line scan was taken across the image shown in Figure 2a (see white dashed line) and the intensity was plotted for each channel across the array. The fluorescence intensity profile across the FoV gave an average intensity of 38,338 cps for the intensity values secured at the x-coordinate corresponding to the middle of each fluidic channel (not background corrected). The standard deviation across the fluidic channels within the microscope's FoV was calculated to be 10,165 cps (RSD = 26%). As can be seen from this line scan, the major intensity deviations occurred at the edges of the FoV. This resulted from the Gaussian intensity profile of the excitation beam and the non-flat field response of the optical system used herein. These can be rectified by using a top-hat shaped input laser beam instead of a Gaussian shaped beam to give a more uniform intensity profile across the FoV that would provide a more uniform excitation intensity for every channel of the array.

Using the data shown in Figure 2a, the signal-to-noise ratio (SNR) calculated from the background corrected signal divided by the square root of the average background level was determined to be 285 for the 2.0 nM dye/oligonucleotide solution. Therefore, the calculated limit-of-detection at a SNR = 2 was determined to be 14 pM. This detection limit could be improved by using a higher power laser due to the fact that the current system is operating below optical saturation, where the optimal SNR typically results for ultra-sensitive fluorescence measurements [52]. For a 30 mW laser power spread over an area of $3.14 \times 10^{-4} \text{ cm}^2$ ($r = 0.01 \text{ cm}$), the photon density (laser fluence) was $3.17 \times 10^{23} \text{ photons cm}^{-2} \text{ s}^{-1}$. The absorption rate (k_a) for the labeling dye used herein could be determined from the product of the laser fluence and the absorption cross section (AlexaFluor 660, $\sigma = 5.02 \times 10^{-16} \text{ cm}^2$ at 660 nm) and was found to be $1.59 \times 10^8 \text{ s}^{-1}$. Optical saturation can be estimated from $1/\tau_f$, where τ_f is the fluorescence lifetime. For AlexaFluor 660, $1/\tau_f$ was calculated to be 8.3×10^8 . Therefore, we are approximately 10-fold below optical saturation and thus, the use of a higher power laser will improve the detection limit from that observed here.

3.3 Investigation of cross-talk between fluidic channels

Cross-talk between fluidic channels could result from light propagation into adjacent channels due to refractive index differences between the polymer substrate and the fluid filling the channels. Because the refractive index of the polymer substrate (PMMA, $n = 1.489$) is typically higher than the aqueous medium ($n = 1.33$), this can make the polymer substrate behave as a waveguide for the isotropically emitted fluorescence, which can cause fluorescence from one channel to leak into adjacent channels [53]. In addition, poor sealing between the cover plate and the inter-channel space of the substrate due to inadequate fusion bonding could cause sample leakage between channels, generating false positive signals.

To investigate potential cross talk between the fluidic channels, blank samples (buffer) and dye/oligonucleotide samples were loaded into individual sample reservoirs and high sensitivity fluorescence images were acquired. Figure 3a shows an image of the chip in which every other channel was loaded with dye or buffer. The channels loaded with dye gave bright fluorescent signals while those loaded with buffer (marked 'x' in Figure 3a) produced no observable fluorescent signal. A negative control measurement was also taken under similar conditions as those used in Figure 3a, but in this case, only buffer was placed in all of the fluidic channels. The result from this measurement is shown in Figure 3b. A careful examination of Figure 3b revealed approximately the same level of background signal across all of the fluidic channels as those marked with an "x" in Figure 3a. The average background signal in the middle of the channels marked with an "x" in Figure 3a

was found to be $53,000 \pm 13$ cps, while the corresponding channels (also marked with an “x” in Figure 3b) for the buffer filled device was found to be $52,772 \pm 16$ cps. A student’s t-test indicated no statistical difference between these numbers.

The absence of cross talk could be attributed to two factors: (1) Good sealing of the cover plate to the PMMA wafer that prevented sample cross contamination between adjacent channels. (2) The refractive index difference between PMMA ($n = 1.489$) and water ($n = 1.33$) creates a critical angle ($\sim 64^\circ$) that does not provide sufficient amounts of fluorescent light to guide through the substrate. Consequently, the amount of light propagated into adjacent channels by impinging on the surface above the critical angle is minimal.

3.4 Monitoring APE1 activity

The potential of the fluidic system for HTS was evaluated by monitoring the activity of APE1, an enzyme, which is responsible for a double-strand break in genomic DNA and has been linked to radio-resistance and chemo-resistance (resistance to chemotherapy) in human tumors [54], thus making it an important target in the design of therapeutic agents for cancer therapy. A model assay was developed, which was suitable for screening elements from a combinatorial library against APE1 (see Figure 4a). The assay employed a duplexed oligonucleotide (50 bp), which contained a tetrahydrofuran base (represented as ‘P’ in the sense strand; see Figure 4a) as the abasic (Apurinic, AP) site analog that was recognized by the APE1 enzyme. The oligonucleotide has one strand (sense strand) labeled with AlexaFluor 660 at its 5’ end and the complementary strand (anti-sense strand) was labeled with BHQ-3 at its 3’ end to create sufficient proximity for resonance energy transfer quenching of the fluorescence from AlexaFluor 660 for the intact duplexed DNA (see Figure 4a). Activity of the enzyme could then be monitored by incubating the enzyme with the duplexed DNA at 37°C such that the strand break at the abasic site would generate fluorescence following dissociation (melting) of the 7-mer single stranded DNA (ssDNA) containing the AlexaFluor 660 reporter from the strand containing BHQ-3 [54–56].

For this assay, the duplexed oligonucleotide substrate was prepared in Tris/HCl buffer containing 20 mM Tris-HCl, 10 mM MgCl_2 , 10 mM KCl and 2 mM dithiothreitol (DTT) at pH 7.5. Experiments were first carried out using a PMMA cover sheet that was equipped with a strip heater to ensure that the enzymatically generated 7-mers would completely dissociate from the major strand at the incubation temperature of 37°C . The oligonucleotide sample (2 nM) was spotted (using a micropipette) onto a PMMA sheet and the APE1 enzyme (1.0 nM) was added followed by incubation for 30 mins. Following the incubation period, the sample (Enzyme + oligonucleotide duplex) was immediately transferred using a preheated (37°C) micropipette, to the imaging area and fluorescence was acquired. The same protocol was adopted for an oligonucleotide sample that did not contain the enzyme. Figure 4b gives the fluorescence image obtained, which showed fluorescence signal from the sample with enzyme (left) and the absence of fluorescence from the sample without enzyme (right). This indicated that the 7-mer AlexaFluor 660 containing oligonucleotide completely dissociated from the complementary oligonucleotide when incubated at 37°C . This result was further supported by measuring fluorescence from a bulk sample (100 nM) of the duplex at different incubation times (incubation temperature = 37°C) using the same concentration of the enzyme as noted above. A plot of fluorescence intensity at different incubation times was generated (data not shown) and showed precipitous increases in fluorescence until approximately 25 min at which point the fluorescence remained steady. We therefore surmised from this data that a 25 min incubation time was necessary to provide optimal enzyme activity with the conditions employed here.

We next evaluated the microfluidic device using this protocol as a model screening assay for APE1 enzyme. The microfluidic device was equipped with a kapton strip heater within the

reaction zone (see Figure 1) that was set to 37°C. The duplexed DNA sample (2 nM) was introduced into all the fluidic processors followed by the addition of APE1 enzyme (1.0 nM) to selected fluidic processors. The reaction mix (Enzyme + oligonucleotide) was hydrodynamically driven through the fluidic processors and incubated at 37°C for the optimal enzyme performance time (25 min, see above). Hydrodynamic pressure was applied to maintain a linear velocity of the sample through the fluidic processor of 0.3 cm/s. Fluorescence images were then acquired from all the fluidic processors at the detection zone of the device. Figure 4c shows a fluorescence image acquired for the duplexed DNA substrate incubated with APE1. There was no fluorescence signal registered from fluidic processors in which the APE1 enzyme was not added (these fluidic processor are labeled 'N' in Figure 4c). Conversely, visible fluorescent signals were observed in fluidic processors that contained APE1 enzyme (see Figure 4c). The absence of fluorescence from the fluidic processors that did not contain the APE1 enzyme indicated that the fluorescence from the fluorophore was sufficiently quenched by BHQ-3 because the duplexed DNA was assumed to remain intact in these channels, which is in agreement with previous studies using this fluorophore-quencher pair with a reported 96% quenching efficiency [57].

4. Concluding remarks

A multi-channel microfluidic system was fabricated using replication technology into a polymer substrate and found to be suitable for HTS. The fluidic channels possessed individually addressable input reservoirs and were configured into a high density format so that all of the fluidic vias could be imaged within the FoV of a high resolution microscope to provide adequate detection limits. The multichannel capability was further demonstrated by carrying out monitoring of the activity of APE1 enzyme in a model screening assay in which resonance energy transfer was used to indicate APE1 activity by excising an apurinic base, releasing a 7-mer carrying a fluorescent dye that was quenched when attached to the intact DNA substrate. This microfluidic system discussed herein will provide several potential advantages over conventional titer plate systems for HTS such as reagent economy, reduction in solvent evaporation, sample handling automation and simultaneous detection from all fluidic channels (parallel readout) using an imaging detection system. Furthermore, the system can integrate multiple steps of the sample processing pipeline into a single fluidic chip to minimize sample cross-contamination while at the same time provide automation that will not only improve data throughput, but also data quality.

Whilst the present fluidic design produced the ability to simultaneously image 25 fluidic channels using the 5 μm channels with 2 μm pitch, the throughput could be significantly increased by reducing the channel width. For example, with a channel width of 1 μm (pitch = 1 μm), 100 fluidic processors could be imaged in a ~200 μm field-of-view. While we did design channels with these dimensions, replication fidelity and structural integrity following thermal fusion bonding the cover plate to the device was poor using these dimensions. Work is currently underway in our laboratories to optimize molding/demolding conditions as well as cover plate assembly conditions to accommodate the use of these channels and pitch dimensions. If this can be accomplished, further reductions in channel width to 500 nm with a pitch of 500 nm would allow the ability to image a total of 200 fluidic processors simultaneously using a 40x microscope objective (NA = 0.75). The fluidic device will be further evaluated by screening a small library of compounds against a specific target. For example, L1 endonuclease (L1-EN) is an enzyme responsible for double strand breaks in genomic DNA and is associated with 45 different diseases, including aging and cancer [58]. A small library of potential therapeutic agents for this important target will be screened to register drug candidates for clinical trials using the model assay presented in Figure 4a.

Finally, the system reported herein is not restricted to only monitoring the output from homogeneous-based fluorescence-based assays. It can also be used to monitor other assays formats as well. For example in terms of drug discovery, mobility shift assays requiring electrophoresis to monitor the association of certain drug candidates to the appropriate target can also be evaluated using this platform and done so with high throughput capabilities [59].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors acknowledge support of this work through the National Institutes of Health (EB-006639), the National Science Foundation (EPS-0346411) and the Louisiana Board of Regents. The authors also thank partial financial support of this work through the World Class University (WCU) program in Korea. The authors also would like to acknowledge Dr. Daniel Park of Louisiana State University for his assistance with the SU-8 processing performed during the fabrication of the molding tools.

References

1. Aherne GY, McDonald E, Workman P. *Breast Canc Res*. 2002; 4:148–154.
2. Olsen M, Iverson B, Georgiou G. *Curr Opin Biotechnol*. 2000; 11:331–337. [PubMed: 10975452]
3. Sundberg SA. *Curr Opin Biotechnol*. 2000; 11:47–53. [PubMed: 10679349]
4. Sittampalam GS, Kahl SD, Janzen WP. *Curr Opin Chem Biol*. 1997; 1:384–391. [PubMed: 9667878]
5. Zheng W, Spencer RH, Kiss L. *Assay Drug Dev Technol*. 2004; 2:543–552. [PubMed: 15671652]
6. Maffia AM, Kariv I, Oldenburg KR. *J Biomol Screen*. 1999; 4:137–142. [PubMed: 10838422]
7. Oldenburg KR, Zhang JH, Chen TM, Maffia A, et al. *J Biomol Screen*. 1998; 3:55–62.
8. Mere L, Bennett T, Coassin P, England P, et al. *Drug Discov Today*. 1999; 4:363–369. [PubMed: 10431146]
9. www.evotec-technologies.com.
10. Gribbon P, Schaertl S, Wickenden M, Williams G, et al. *Curr Drug Discov Technol*. 2004; 1:27–35. [PubMed: 16472217]
11. Jager S, Garbow N, Kirsch A, Preckel H, et al. *J Biomol Screen*. 2003; 8:648–659. [PubMed: 14711390]
12. Breslauer DN, Lee PJ, Lee LP. *Mol BioSyst*. 2006; 2:97–112. [PubMed: 16880927]
13. Whitesides GM. *Nature*. 2006; 442:368–373. [PubMed: 16871203]
14. Gunther A, Jensen KF. *Lab Chip*. 2006; 6:1487–1503. [PubMed: 17203152]
15. Stone HA, Stroock AD, Ajdari A. *Annu Rev Fluid Mech*. 2004; 36:381–411.
16. Shui LL, Eijkel JCT, van den Berg A. *Sens Actuatur B-Chem*. 2007; 121:263–276.
17. Hong J. *Biochip J*. 2008; 2:12–26.
18. Thorsen T. *BioTechniques*. 2004; 36:197–199. [PubMed: 14989081]
19. Khandurina G, Guttman A. *Curr Opin Chem Biol*. 2002; 6:359–366. [PubMed: 12968633]
20. Litborn E, Roeraade J. *J Chromatogr*. 2000; 745:137–147.
21. Melin J, Quake SR. *Annu Rev Biophys Biomol Struct*. 2007; 36:213–231. [PubMed: 17269901]
22. Dittich PS, Manz A. *Nature Rev Drug Discov*. 2006; 5:210–218. [PubMed: 16518374]
23. Yager P, Edwards T, Fu E, Helton K, et al. *Nature*. 2006; 442:412–418. [PubMed: 16871209]
24. Craighead H. *Nature*. 2006; 442:387–393. [PubMed: 16871206]
25. West J, Becker M, Tombrink S, Manz A. *Anal Chem*. 2008; 80:4403–4419. [PubMed: 18498178]
26. Hong JW, Quake SR. *Nature Biotechnol*. 2003; 21:1179–1183. [PubMed: 14520403]
27. Thorsen T, Maerkl SJ, Quake SR. *Science*. 2002; 298:580–584. [PubMed: 12351675]
28. Brenan CJ, Morrison T, Stone K, Heitner T, et al. *Proc SPIE*. 2002; 4626:560–569.

29. Hadd AG, Jacobson SC, Ramsey JM. *Anal Chem.* 1999; 71:5206–5212.
30. Cohen CB, Chin-Dixon E, Jeong S, Nikiforov TT. *Anal Biochem.* 1999; 273:89–97. [PubMed: 10452803]
31. Chiem NH, Harrison DJ. *Clin Chem.* 1998; 44:591–598. [PubMed: 9510867]
32. Hadd AG, Raymond DE, Halliwell JW, Jacobson SC, Ramsey JM. *Anal Chem.* 1997; 69:3407–3412. [PubMed: 9286159]
33. Li PCH, Harrison DJ. *Anal Chem.* 1997; 69:1564–1568. [PubMed: 9109354]
34. Khandurina J, Guttman A. *J Chromatogr A.* 2002; 943:159–183. [PubMed: 11833638]
35. Shi YN, Simpson PC, Scherer JR, Wexler D, et al. *Anal Chem.* 1999; 71:5354–5361. [PubMed: 10596215]
36. Yang TL, Jung SY, Mao HB, Cremer PS. *Anal Chem.* 2001; 73:165–169. [PubMed: 11199961]
37. Erch MB, Locascio LE, Tarlov MJ, Durst RA. *Anal Chem.* 2001; 73:2952–2958. [PubMed: 11467540]
38. Yang L, Tran DK, Wang X. *Genome Res.* 2001; 11:1888–1898. [PubMed: 11691854]
39. Maerkl SJ, Quake SR. *Science.* 2007; 315:233–237. [PubMed: 17218526]
40. Wang J, Sui G, Mocharla VP, Lin RJ, et al. *Angew Chem Int Ed.* 2006; 45:5276–5281.
41. Hung PJ, Lee PJ, Sabounchi P, Lin R, Lee LP. *Biotechnol Bioeng.* 2005; 89:1–8. [PubMed: 15580587]
42. Wolff A, Perch-Nielsen IR, Larsen UD, Friis P, et al. *Lab Chip.* 2003; 3:22–27. [PubMed: 15100801]
43. Ye NN, Qin JH, Shi WW, Liu X, Lin BC. *Lab Chip.* 2007; 7:1696–1704. [PubMed: 18030389]
44. Christenson M. *Single Mol.* 2000; 1:177–179.
45. Hiller WJ, Kowalewski TA, Tatarczyk T. *Proc SPIE.* 1992; 1801:595–601.
46. Okagbare PI, Soper SA. *Analyst.* 2009; 134:97–106. [PubMed: 19082181]
47. Wen Y, Yang ST. *Expert Opin Drug Discov.* 2008; 3:1237–1253.
48. Gorman MA, Morera S, Rothwell DG, Fortelle EdL, et al. *EMBO J.* 1997; 16:6548–6558. [PubMed: 9351835]
49. Qu W, Wenzel C, Jahn A, Zeidler D. *IEEE: Optoelectron Microelectron Mat Devic.* 1999 Dec 14–16.:380–383.
50. Ford SM, Davies J, Kar B, Qi SD, et al. *J Biomech Eng-Transact Asme.* 1999; 121:13–21.
51. Shadpour H, Musyimi H, Chen JF, Soper SA. *J Chromatogr A.* 2006; 1111:238–251. [PubMed: 16569584]
52. Mathies RA, Peck K, Stryer L. *Analytical Chemistry.* 1990; 62:1786–1791.
53. Irawan R, Tjin SC, Yager P, Zhang D. *Biomed Microdev.* 2005; 7:205–211.
54. Madhusudan S, Smart F, Shrimpton P, Parsons JL, et al. *Nucl Acids Res.* 2005; 33:4711–4724. [PubMed: 16113242]
55. Yoshida A, Urasaki Y, Waltham M, Bergman AC, et al. *J Biol Chem.* 2003; 278:37768–37776. [PubMed: 12842873]
56. Pourquier P, Ueng LM, Kohlhaagen G, Mazumder A, et al. *J Biol Chem.* 1997; 272:7792–7796. [PubMed: 9065442]
57. Marras SAE, Kramer FR, Tyagi S. *Nucl Acids Res.* 2002; 30:e122. [PubMed: 12409481]
58. Gasior SL, Wakeman TP, Xu B, Deininger PL. *J Mol Biol.* 2006; 357:1383–1393.
59. Perrin D, Fremaux C, Shutes A. *Current Opinion on Drug Discovery.* 2010; 5:51–63.

Figure 1.

The design of the UV photomask containing the high density fluidic patterns. Three different patterns were poised on this mask (from top to bottom): 10 μm wide (pitch = 10 μm); 5 μm wide (pitch = 2 μm); and 1 μm wide (pitch = 1 μm). The insets show SEMs of the Ni molding tool (1, 2 and 3) or the molded PMMA piece (4). The device had a total of 25 fluidic processors configured in a 180 μm wide footprint, which was within the FoV of the imaging microscope when using channels that were 5 μm wide with a pitch of 2 μm .

Figure 2.

(a) EMCCD image of fluorescent labeled oligonucleotides (AlexaFluor 660) moving through the high density chip (channel dimensions were $5\ \mu\text{m} \times 1\ \mu\text{m}$ (width $\times$ depth), pitch = $2\ \mu\text{m}$). The fluorescence was collected with a 40x/0.75 objective using a 200 ms EMCCD exposure time (25 fluidic channels were imaged). (b) EMCCD image acquired using the same conditions as in (a), but using wider channels (dimension: $10\ \mu\text{m} \times 1\ \mu\text{m}$, pitch = $10\ \mu\text{m}$); 15 fluidic channels were imaged. (c) Fluorescence intensity profile as a function of x coordinate across the fluidic channels. The intensity profile was accumulated using the dashed white line shown in Figure 2a.

Figure 3.

(a) EMCCD image acquired when fluidic channels were filled with buffer or dye labeled oligonucleotides (AlexaFluor 660) samples. The locations marked 'x' are buffer-filled channels. (b) EMCCD image of a buffer only filled chip. Imaging conditions are similar to those noted in Figure 2.

Figure 4a

Figure 4b

Figure 4c

Figure 4.

(a) Fluorescence quenching assay for monitoring the activity of APE1. The oligonucleotide duplex was labeled with AlexaFluor 660 (represented as 'F') on the 5'-end of the sense strand and BHQ-3 (represented as 'Q') on the 3'-end of the anti-sense strand. When hybridized, a 50 bp duplex was formed with the sense strand 'S' and the anti-sense strand 'AS' presented in the rectangular box. The sense strand has a tetrahydrofuran (represented as 'P') as the abasic (Apurinic, AP) site that is excised by APE1. (b) Fluorescence image acquired with the EMCCD when a DNA duplex sample was spotted onto a thin PMMA sheet (250 μm) and incubated with APE1 (left) and without APE1 enzyme (right). (c) EMCCD image acquired when oligonucleotide duplex samples were incubated with and without APE1 enzyme. The locations marked 'N' represent fluidic channels that contain only the oligonucleotide duplex without the APE1 enzyme.