Published in final edited form as:

Lab Chip. 2010 May 21; 10(10): 1308–1315. doi:10.1039/b925705e.

Multiphase Bioreaction Microsystem with Automated On-Chip Droplet Operation

Fang Wang^a and Mark A. Burns^{a,b,*}

^a Department of Chemical Engineering, University of Michigan, Ann Arbor, MI 48109

^b Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109

Abstract

A droplet-based bioreaction microsystem has been developed with automated droplet generation and confinement. On-chip electronic sensing is employed to track the position of the droplets by sensing the oil/aqueous interface in real time. The sensing signal is also used to control the pneumatic supply for moving as well as automatically generating four different nanoliter-sized droplets. The actual size of droplets is very close to the designed droplet size with a standard deviation less than 3% of the droplet size. The automated droplet generation can be completed in less than 2 sec, which is 5 times faster than using manual operation that takes at least 10 sec. Droplets can also be automatically confined in the reaction region with feedback pneumatic control and digital or analog sensing. As an example bioreaction, PCR has been successfully performed in the automated generated droplets. Although the amplification yield was slightly reduced with the droplet confinement, especially while using the analog sensing method, adding additional reagents effectively alleviated this inhibition.

Introduction

Droplet-based microfluidics is increasingly being studied for applications in chemical and biological assays. A variety of techniques have been exploited to generate and manipulate droplets on-chip.^{1–19} For example, T-junction^{1–3} and flow focusing^{4–5} are two major mechanical approaches that depend on the channel geometry and flow rates to control droplet generation, while electrowetting on dielectric (EWOD) is an electrohydrodynamic (EHD) approach for generating droplets by electronically changing the interfacial energy.^{6–8} Benefiting from the on-chip controllable droplet size and the isolation of individual droplets, chemical reactions and bioassays such as nanoparticle synthesis,^{14,20–21} cell analysis,^{22–23} protein expression^{24–25} and DNA/RNA amplification,^{26–33} have all been implemented in droplet-based microdevices.

While these droplet-based microsystems have been widely demonstrated to offer robust control and operation, fully automating microsystems could make them more amenable to applications in portable, point-of-care diagnosis. A critical step in this automation is fluid detection that includes not only tracking the location and velocity of the fluid, but also, particularly for the droplet-based microsystems, monitoring the droplet size and generation frequency. One of the most widely used detection methods is optical detection using either charge-coupled device (CCD) camera with microscopy and image analysis or photo diodes for detecting the laser diffraction caused by the presence of droplets.³⁴ However, the optical

* maburns@umich.edu; Fax: 1-734-763-0459; Phone: 1-734-764-1516.

techniques usually rely on cumbersome or expensive accessory instruments and cannot be employed on opaque substrates.

Recently, thermal and electronic sensing have been used as alternative approaches to optical detection. Thermal sensing is based on the convective heat loss to the fluid as it passes over a predefined region.^{35–37} Heating around the detection region is usually required during thermal sensing, and, therefore, this method may not be practical for biological applications since most bioreagents are temperature-sensitive. Electronic sensing is based on the change of electrical resistance, impedance or capacitance as different liquid phases pass over microfabricated electrode sensors.^{37–45} In addition to measuring droplet size and generation frequency, electronic sensing has been used to analyze the composition of the droplets^{42–44} and control the droplet flow according to the droplet size and content.⁴³ Automated droplet generation in electrowetting-based microsystems has also been achieved using electrical capacitance sensing, but most droplet generation using mechanical means are still manually controlled.

In this paper, we present a droplet-based bioreaction microsystem with automated mechanical means of on-chip droplet generation and confinement. The system uses paired electrodes to electronically track the oil/aqueous interface. The sensor reading not only indicates the location of the liquid interface, but also can be used to control the pneumatic system to generate droplets of precise sizes and position single droplets in desired locations. This automated droplet-based microsystem can be used for a variety of applications including biochemical analysis (e.g., polymerase chain reaction (PCR)).

Materials and methods

Device fabrication and assembly

Top side (channel) microfabrication—The detailed procedure for fabricating the fluid network on a glass wafer using photolithography and wet chemical etching have been outlined elsewhere.⁴⁶ Briefly, a thin metal film (500Å Cr/2500Å Au) is deposited and patterned on a glass wafer. The wafer is then etched to the desired depth in hydrofluoric acid (49%) (CMOS grade; J.T. Baker, Philipsburg, NJ). Next, the photoresist and metal layers are removed. The glass wafer is then diced to obtain individual top dies. The dies are coated with a 3µm thick layer of Parylene-C (PDS 1020 Labcoater, Speciality Coating Systems, IN) before bonding it to the bottom dies.

Bottom side (electronics) microfabrication—The electronic components including heaters, resistance temperature detectors (RTDs) and electrodes are fabricated on either a silicon wafer with a 5000Å thick silicon oxide layer or a glass wafer. Firstly, photoresist is patterned on the wafer using photolithography, followed with evaporating 300Å Ti/1000Å Pt for heaters and RTDs. The wafer is then left in acetone (CMOS grade; J.T. Baker, Philipsburg, NJ) to lift off unwanted metal. Next, a second lithography, evaporation and lift-off are conducted to fabricate the electrodes (500Å Cr/2500Å Au). The wafer is then diced to yield individual bottom dies.

Device assembly—A custom designed printed circuit board (PCB, Advanced Circuits, Aurora, CO) serves as the platform for electrical connections. The bottom die is fixed on the PCB using standard quick cure epoxy, and then wire bonded (Kulicke & Soffa 4124 Ball Bonder) using 1.0 mil gold wire. After wire bonding, the top die is visually aligned to the bottom die, and UV curable optical glue is wicked between the top and bottom dies through the edges. The device is then cured in UV light for 1.5 hours.

Experimental setup and operation

Data acquisition—The setup for data acquisition of the automated droplet-based microsystem consists of a function generator (8165A, Hewlett Packard), a data acquisition (DAQ) board (National instruments PCI 6031E, Austin, TX), a digital relay board (National Instruments ER-16, Austin, TX), a computer and LabVIEW program (National instruments, Austin, TX) (See Fig. 1 in ESI). Edge connectors from the PCB connect each pair of on-chip electrodes in series with a 120 k Ω external reference resistor; one of the electrodes in each pair is connected to the function generator *via* the digital relay board while the other is grounded. In most experiments, an AC signal (Amplitude = 1.5V, Frequency = 230Hz) from the function generator is applied across the electrodes. The digital relays can selectively shut off the power input to a pair of electrodes. The change of electrical impedance between two electrodes in each pair is represented by the voltage drop across the reference resistor. This voltage drop is acquired by the LabVIEW VI using the analog input of the DAQ board.

Pneumatic control—The pneumatic control is carried out by adjusting the voltage input to electropneumatic regulators (VSO-EP or VSO-EV; Parker, Cleveland, OH): there is a linear relationship between the input control voltage and the output pressure/vacuum from the regulator. Lab air supply serves as the pneumatic source to the regulator. The pulsing of pressure or vacuum is controlled by a set of three-way solenoid valves (Numatech, Wixom, MI). Opening and closing of the solenoid valves are operated through a combination of a DC power supply (Electro Industries, Model Digi 35A) and a digital relay board (ER-16). Functioning of the relay board and the electropneumatic regulator are both feedback controlled by the voltage signal from the reference resistor through the LabVIEW program using a digital I/O card (National Instrument PCI-DIO-96, Austin, TX) and a DAQ board (National instruments PCI-6704, Austin, TX), respectively.

On-chip temperature control—The on-chip temperature control is also conducted through the LabVIEW program. The RTDs are calibrated by heating the device in a convection oven and recording the temperature-resistance data *via* the PCI 6031E board. The slope and intercept from a linear fit of the temperature and resistance data are read into the control algorithms that use a proportional-integral (PI) module to control temperature *via* the PCI 6704 board. The heaters are connected to a DC power supply (B+K Precision Model 1760, Yorba Linda, CA) through a signal conditioning circuit that boosts the supply voltage from the computer with an op-amp gain of 3. The precision of temperature control is $\pm 0.2^{\circ}\text{C}$.

Bioreaction and fluorescence detection

PCR amplification—The PCR mixture consists of λ DNA template (Invitrogen, Carlsbad, CA) with the concentration of 3.5ng/ μL , dNTPs (0.2mM each dATP, dGTP, dCTP and 0.4mM dUTP), 10mM Tris-HCl (pH 8.3), 50mM KCl, 0.01mM EDTA, 3.5mM MgCl₂, 0.9 μM each primer, 0.25 μM probe, 0.01units/ μL AmpErase UNG, and 0.175units/ μL AmpliTaq Gold[®] DNA polymerase (Applied Biosystems, Foster City, CA). The sequences of forward and reverse primers are 5'-CATCAAAGCCATGAACAAAGCA-3' and 5'-TCAGCAACCCGGTATCAG-3', respectively. The sequence of probe is 5'6FAM-CCGCGCTGGATGA-3'MGBNFQA. This size of the target product is 56bp. The thermocycling protocol used in most of the on-chip droplet-based PCR consists of 50 $^{\circ}\text{C}$ for 2min, 95 $^{\circ}\text{C}$ for 9min, followed by 30 cycles of 95 $^{\circ}\text{C}$ for 9s and 60 $^{\circ}\text{C}$ for 30s. PCR grade mineral oil (Sigma Aldrich, St. Louis, MO) is used as the continuous oil phase.

Fluorescence detection and data analysis—An inverted fluorescence microscope (Nikon Eclipse TE2000-U) with a 2x objective (Nikon) is used to monitor fluorescence of the reaction droplet. An X-cite Series 120 lamp (EXFO Life Science Divisions, Ontario,

Canada) with FITC filter is used as the excitation source. The fluorescence images are captured using a digital CCD camera (Photometrics Cascade 512F; Roper Scientific, Tucson, AZ) with a 500ms exposure time. The relative fluorescence intensity is used to quantify the amplification yield in the droplet, which is calculated by scaling the fluorescence intensity of the droplet by that of the reference area on the device. Data analysis is done by MetaVue Software.

Results and discussion

Electronic sensing of the oil/aqueous interface

The approach for tracking the oil/aqueous interface in our device is based on the fact that the electrical impedance across two electrodes is dependent on the electrical properties of the fluid above those electrodes. Thus, using a simple electrical circuit (See Fig. 1 in ESI), the liquid interface can be tracked by measuring the impedance change across a pair of electrodes (each 100 μ m wide, 50 μ m apart) microfabricated at predetermined positions in a fluid channel. The arrangement of those electrodes will affect the way in which the interface location can be measured: electrodes orthogonal to the fluid path produce a “yes/no” signal and electrodes parallel to the fluid path can produce a more complex location signal.

Fig. 1 shows the arrangement and responses of four pairs of electrodes placed orthogonal to the fluid channel. The voltage responses from these sensors when DI water displaces mineral oil in the channel produce characteristic digital (“yes/no”) signals. An increase in the output voltage (*i.e.*, the voltage across the reference resistor) is sequentially observed for the four digital sensors as the moving oil/aqueous interface crosses each one. In this experiment, pressure (P_{H_2O}) is first applied through the aqueous phase to push it into the channel (*i.e.*, passing over Sensor 1). Once the head of the aqueous phase reaches the junction, which can be detected using Sensor 2, the aqueous phase can flow to both the left and right of the junction. Then pressure (P_{Oil}) is also applied through the oil phase to guide the aqueous phase to flow to the right of the junction without overflowing too much to the left. Therefore, the oil/aqueous interface continues to reach Sensor 3 and 4. Note that the input signal from the function generator will be shut off once the voltage response is above a threshold value to prevent possible damages to the reaction solution.

In this digital sensing method, the digital signal can serve as a switch for the pneumatic control to drive the liquid flow in the device provided a proper voltage threshold is used. The setting of the voltage threshold is determined by the voltage response from the sensor and is affected by the input voltage signal. The output voltage from a digital sensor with different input frequencies (150~7500Hz) has been measured (See Fig. 2 in ESI). The input signals used in the experiment have the same amplitude of 1.5V. With increasing input frequency, the output voltage signal rapidly increases and then gradually decreases when the input frequency is above ~2600 Hz while the base signal (*i.e.*, with only the oil phase) monotonically increases within the tested frequency range. This result indicates that input signals with lower frequencies (< 1kHz) may be more amenable to be used for control since the background (*i.e.*, base signal) is low. In addition, the total cost of the automated microsystem could also be reduced by not requiring high input power and frequency.

The voltage response from the digital sensor may be also affected by the properties of the fluids especially the aqueous phase. Using buffer solution (10mM Tris-HCl) with different pH values (pH=7.0, 7.5, 8.0, 9.0), the voltage signal increases with the decreasing pH values at a fixed input frequency (Fig. 2 in ESI). This trend can be explained by the fact that the ion concentration of the buffer solution also increases with the decreasing pH values. Higher ion concentration results in lower electrical impedance across the electrodes and thus higher voltage drop across the reference resistor. This result also indicates a detection sensitivity of

a pH difference of 0.5 or an ion concentration difference less than 1.5mM. Moreover, since the buffer solution is one of the most common reagents involved in bioreactions and its pH value varies according to the applications, this system can be used to track and sort droplets containing different solutions or reagents.

In the analog sensing method, the voltage response from electrodes parallel to the fluid channel is proportional to the coverage of aqueous phase over the two electrodes (Fig. 2). The oil and aqueous phases between the two electrodes can be treated as two resistors (R_c and R_d , respectively) connected in parallel (See Fig. 3 in ESI). We also assume no phase shift between the voltage and current, and thus the impedance (Z) equals the resistance (R). Since the resistivity of mineral oil ($\sim 10^{11} \Omega \cdot m$) is much higher than that of water ($1.82 \times 10^5 \Omega \cdot m$), the current through R_c can be neglected. Moreover, the resistance of the reference resistor (R_f) is 120 k Ω , much smaller than that of the aqueous phase between the two electrodes (R_d) (on the order of at least 10^5 k Ω).

Therefore, the output signal (V_{out}) can be approximated using equation (1):

$$V_{out} = V_{in} R_f / R_d. \quad (1)$$

Here, V_{in} is the voltage signal input to the two electrodes and

$$R_d = \rho \frac{w}{Lh} \quad (2)$$

where ρ is the electrical resistivity of water, w is the gap between the two electrodes, L is the length of water coverage along the electrodes, and h is the depth of the channel. Substituting equation (2) into equation (1) produces

$$V_{out} = \frac{R_f V_{in} Lh}{\rho w}. \quad (3)$$

Thus, V_{out} is linearly proportional to L or the fractional coverage L/L_e (L_e is the length of the electrode). This electronic signal accurately corresponds to the location of the oil/aqueous interface and can be used to precisely control the position of droplets. However, calibration may be required for different aqueous phases based on their electrical conductivity for use in such control.

Automated droplet generation

This electronic sensing can be used to automate droplet generation in conjunction with computer-controlled pneumatic system (Fig. 3). First, pneumatic control (P_{H_2O}) is switched on to inject aqueous solution (DI water) into the oil-filled channel after desired droplet size is chosen (Fig. 3(a)). Once the oil/aqueous interface passes over the appropriate sensor (Sensor 11), pressure (P_{Oil}) is also applied to facilitate the aqueous solution passing the neck region and prevent the aqueous solution from entering the oil reservoir (Fig. 3(b)). When the target sensor (Sensor 9) is tripped, P_{H_2O} is switched off and P_{Oil} is increased to quickly break the aqueous stream (Fig. 3(c) and (d)). Once the aqueous phase is sensed back to the reservoir (*i.e.*, Sensor 3 becomes untripped), vacuum is applied through the outlet to pull the aqueous slug into the chamber (Fig. 3(e)). After the entire droplet has entered the chamber

(i.e., Sensor 7 becomes tripped and then untripped), both the pneumatic supply and digital sensing are turned off and the droplet generation is complete (Fig. 3(f)).

Our device is designed to automatically generate four different sized droplets corresponding to four digital sensors (Sensor 7–10) in the main channel (See Movie 1–4 in ESI). As shown in Fig. 4, the size of automatically generated droplets is quite close to the designed droplet size (a standard deviation less than 3% of the droplet size). Moreover, the pneumatic control is triggered by computer signals and is significantly faster than manual operations. This automated droplet generation can be completed in less than 2 seconds, and is much faster than the manual generation (at least 10 seconds). In addition, since the only manual part of the operation is choosing droplet size in the program, even a user without any professional training should be able to generate droplets using this system.

The effect of pneumatic parameters on the droplet formation time and droplet size has also been studied. In this experiment, designed droplet sizes of 98nL and 197nL were used, and the ratio of pressure through both phases (P_{Oil} and P_{H_2O}) was chosen as 1 to simplify system operation. The formation time for both sized droplets decreases with increasing pressures (See Fig. 4(a) and (b) in ESI). At high pressures, the actual droplet size varies from the original designed size with the larger droplets (197nL) being below their designed size and the smaller droplets (98nL) being above (See Fig. 4(c) in ESI). However, this size variation is not obvious within a pressure range of 0.6–0.75 psig, indicating that the pneumatic control responds rapidly enough that the applied pressure does not limit the performance of our system. In contrast, most droplet-based microsystems adjust the pressure to control droplet size. That technique then requires a calibration to relate the applied pressure to droplet size and produces non-uniform sized droplet during startup.

The size variation in our system with higher applied pressure could be associated with the neck region at the T-junction. This neck is designed to provide higher shear rate to facilitate the breakup of the aqueous stream, but it also increases flow resistance. When the aqueous phase reaches the T-junction, P_{Oil} is switched on to force the aqueous phase to flow through the neck region instead of into the oil phase channel. The left oil/aqueous interface needs to be maintained at the T-junction till the right interface reaches the target sensor in the main channel. However, when generating larger droplets (e.g., 197nL), if P_{Oil} is too high, the aqueous stream may be split at the neck before the right interface reaches the target sensor, producing droplets smaller than the designed size. Also, when forming smaller droplets (e.g., 98nL), if P_{H_2O} is too high, the aqueous stream may overshoot the target sensor, making the actual droplet size larger.

Bioreaction with automated position control

In addition to automated droplet generation, the combination of droplet sensing and controlled pressure sources can be used to maintain droplet position in microfluidic bioreaction systems. Ideally, droplets need to stay in the temperature-controlled reaction region in order for the reagents to reach and maintain the desired temperature. However, temperature variations during liquid heating and cooling can cause droplet motion⁴⁷ and cause the droplet to be displaced from the reaction region. Confinement of the droplet is possible but requires additional fabrication steps in order to include valves or other sealing systems. Using our system, droplet location can be controlled using electronically triggered pneumatic pulses. The sensors indicate the location of the droplets and pressure pulses are used to prevent the droplets from leaving the reaction region.

The droplet has been automatically positioned using digital sensing during temperature cycling (See Movie 5 in ESI). Pressure is applied from left to right when only Sensor 8 (Fig. 5(a) in ESI) reads “True”, and from right to left when only Sensor 5 (Fig. 5(a) in ESI) reads

“True”. At room temperature, the droplet stays between Sensor 5 and 8 (Fig. 5(b) in ESI). During the temperature transition from 95°C to 55°C, the droplet tends to move, turning on the pneumatic control. The droplet then oscillates between Sensor 5 and 8 because one of the two sensors is always tripped (Fig. 5(c) in ESI). When the temperature reaches 55°C, the droplet remains between Sensor 5 and 8, and the pneumatic oscillations cease.

The voltage signals of Sensor 5 and 8 during one thermal cycle with automated confinement are plotted in Fig. 5(a). If both sensors read “T” or “F” at the same time (Fig. 5(b)), which indicates that the droplet is essentially centered in the chamber, the pneumatic control will remain off. If Sensor 5 and 8 read “T” alternatively (Fig. 5(c)), pressure will be applied from right and left correspondingly thus causing the droplet to oscillate between the two sensors. It is difficult to completely prevent the droplet from oscillating while using digital sensing because the pressure is controlled digitally (i.e., either on or off). However, this oscillation can result in favorable mixing of the components inside the droplet.^{48–49}

The droplet can also be positioned using analog sensing signals and feedback pneumatic control. The electrodes in Sensors 5–8 are parallel rather than orthogonal to the channel (the inset in Fig. 6(a)). According to equation (3), the voltage output signals from these sensors correspond to the location of the oil/aqueous interface (i.e., the location of the droplet). A proportional-integral (PI) control module (equation (4)) is used to adjust the applied pressure in real time according to the location of the droplet and offers finer positioning than the digital sensing based approach.

$$V_{r,n} = K_p(V_{out,n} - V_{out,0}) + K_i \sum_n (V_{out,n} - V_{out,0}) \quad (4)$$

In equation (4), $V_{r,n}$ is the voltage input to the electropneumatic regulator; $V_{out,n}$ is the measured signal from the analog sensor, $V_{out,0}$ is the base signal from the analog sensor (i.e., the output signal when the sensor is completely covered by mineral oil), and n denotes different sampling time for the measured or responded voltage signal; K_p and K_i are the proportional and integral coefficients, respectively. According to equation (1)–(3), $V_{out,n} - V_{out,0}$ varies as the resistance of the aqueous phase (i.e., the coverage of the aqueous phase) along the analog sensing electrodes varies. In one feedback control loop, $V_{out,n}$ is acquired from the analog sensors and read into a LabVIEW program where the PI control module (equation (4)) calculates different $V_{r,n}$ based on $V_{out,n} - V_{out,0}$. Then is $V_{r,n}$ output from the computer and input into the pressure regulator. The pressure regulator will respond with different pressure output connected to the device to move the droplet, which then changes the $V_{out,n}$ in the next control loop.

With the analog sensing based feedback control, the droplet can be centered in the chamber without oscillation (See Movie 6 in ESI). As shown in Fig. 6(a), the output signals from Sensor 6 and 7 reach the same value after ~1.5sec, indicating the droplet volumes covering Sensor 6 and 7 are equal, and thus the droplet is symmetrically located relative to the microheaters. The voltage input to the electropneumatic regulator shows a curve similar to the output signal of related sensors (Fig. 6(b)). The difference between the two input voltages even after 1.5sec is because different proportional coefficients are used for the two sensors, taking account of the different flow resistance in the left and right sides of the chamber. Another interesting result is that, during thermal cycling (see Movie 7 in ESI), both the sensor reading and regulator input voltage show a cycled profile similar to the temperature, indicating our system was able to capture and respond to the change of droplet volume caused by temperature changes (See Fig. 6 in ESI).

With the droplet generation and positioning automated by electronic sensing, bioreactions (*e.g.*, PCR) can be performed in the droplet-based system. Automatically generated droplets (98nL and 197nL) containing PCR reaction mixture show significant increase of fluorescence intensity after 30 temperature cycles (See Fig. 7(a)–(c) in ESI). This increase is approximately the same as that in manually generated droplets (data not shown), which suggests that the input voltage signal during droplet generation did not interfere with the reaction. During the droplet confinement, the amplification was inhibited, but the yield reduction was not significant (See Fig. 7(d) in ESI). On the other hand, the amplification yield was much lower in the analog sensing device, although no difference has been observed between with and without input electrical signals. A similar inhibition has been observed when using platinum electrodes.⁵⁰ One possible explanation to this yield drop is that the droplet is exposed to a much larger surface area of gold electrode in the analog sensing device, and the enzyme loss becomes more severe since the gold surface binds some functional groups of amino acids (*e.g.*, thiol (–SH)) with quite high affinity.⁵¹ Indeed, the fluorescence intensity can be increased to approximately the same as in the digital sensing device by increasing the enzyme concentration (Fig. 7(d) in ESI). Surface passivation or changing the electrode size could also reduce enzyme loss.

Conclusion

We have developed a droplet-based microsystem with automated mechanical means of droplet generation and manipulation. On-chip electronic sensing is employed to track the liquid-liquid interface and trigger the pneumatic control. PCR reaction in the automated generated droplet shows no inhibition caused by the electronic sensing during the droplet generation. Although the reaction was interfered adversely during the droplet confinement especially while using the analog sensing method, adding additional reagents can effectively alleviate the inhibition.

Our system demonstrates a successful integration of the automated droplet operation and PCR reaction, and can certainly be applied to other RNA/DNA analyses, for example, reverse transcription PCR (RT-PCR) and ligation detection reaction (LDR), both of which require thermal cycled bioreactions similar to PCR and play very important roles in clinical diagnosis. Our system is also ideal for other biochemical analyses such as cell-based assays and biological fluid assays, which could take advantages of the droplet environment and are not sensitive to the input electrical signals during the automation.

Our automated system can be operated much more efficiently than using manual controls. With the electronic sensing, it is highly feasible to eliminate the visualization instruments in the system setup, and image analysis is also not required to determine the droplet generation (*e.g.*, droplet size and generation frequency) and even the droplet content (*e.g.*, reagent concentration in the droplet). In the future work, photodiodes could also be integrated onto the microdevice to perform light intensity detection on the reaction droplets, and the electronic signals from the photodiodes could be used to automatically monitor the reaction progress. With these automations, the microsystem could be constructed in a more compact size, and could provide portable, point-of-care biochemical analyses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to gratefully acknowledge the funding of this work through the grants (5-R01-AI049541-06 and 1-R01-EB006789-01A2) from the National Institutes of Health. The authors would like to thank Brian N.

Johnson for his help with LabVIEW and related electronics. The authors would also like to thank the staff and members of the Lurie Nanofabrication Facility at University of Michigan for their assistance in device fabrication.

References

1. Thorsen T, Roberts RW, Arnold FH, Quake SR. *Phys Rev Lett* 2001;86:4163–4166. [PubMed: 11328121]
2. Nisisako T, Torii T, Higuchi T. *Lab Chip* 2002;1:24–26. [PubMed: 15100856]
3. Garstecki P, Fuerstman MJ, Stone HA, Whitesides GM. *Lab Chip* 2006;6:437–446. [PubMed: 16511628]
4. Anna SL, Bontoux N, Stone HA. *Appl Phys Lett* 2003;82:364–366.
5. Yobas L, Martens S, Ong WL, Ranganathan N. *Lab Chip* 2006;6:1073–1079. [PubMed: 16874381]
6. Pollack MG, Fair RB, Shenderov AD. *Appl Phys Lett* 2000;77:1725–1726.
7. Pollack MG, Shenderov AD, Fair RB. *Lab Chip* 2002;2:96–101. [PubMed: 15100841]
8. Cho SK, Moon H, Kim CJ. *J Microelectromech Syst* 2003;12:70–80.
9. Jones TB. *J Electrostat* 2001;51–52:290–299.
10. Ahmed R, Jones TB. *J Electrostat* 2006;64:543–549.
11. Link DR, Anna SL, Weitz DA, Stone HA. *Phys Rev Lett* 2004;92:054503-1–054503-4. [PubMed: 14995311]
12. Ting TH, Yap YF, Nguyen NT, Wong TN, Chai JCK, Yobas L. *Appl Phys Lett* 2006;89:234101–234103.
13. Yang H, Park CC, Hu YT, Leal LG. *Phys Fluids* 2001;13:1087–1106.
14. Hung LH, Choi KM, Tseng WY, Tan YC, Shea KJ, Lee AP. *Lab Chip* 2006;6:174–178. [PubMed: 16450024]
15. Sarrazin F, Prat L, Miceli ND, Cristobal G, Link DR, Weitz DA. *Chem Eng Sci* 2007;62:1042–1048.
16. Song H, Bringer MR, Tice JD, Gerdtz CJ. *Appl Phys Lett* 2003;83:4664–4666. [PubMed: 17940580]
17. Bringer MR, Gerdtz CJ, Song H, Tice JD, Ismagilov RF. *Phil Trans R Soc Lond A* 2004;362:1087–1104.
18. Tan YC, Fisher JS, Lee AL, Cristini V, Lee AP. *Lab Chip* 2004;4:292–298. [PubMed: 15269794]
19. Link DR, Grasland-Mongrain E, Duri A, Sarrazin F, Cheng Z, Cristobal G, Marquez M, Weitz DA. *Angew Chem Int Ed* 2006;45:2556–2560.
20. Shestopalov I, Tice JD, Ismagilov RF. *Lab Chip* 2004;4:316–321. [PubMed: 15269797]
21. Chan EM, Alivisatos AP, Mathies RA. *J Am Chem Soc* 2005;127:13854–13861. [PubMed: 16201806]
22. He M, Edgar JS, Jeffries GDM, Lorenz RM, Shelby JP, Chiu DT. *Anal Chem* 2005;77:1539–1544. [PubMed: 15762555]
23. Chabert M, Viovy JL. *Proc Natl Acad Sci* 2008;105:3191–3196. [PubMed: 18316742]
24. Dittrich PS, Jahnz M, Schwille P. *Chem Bio Chem* 2005;6:811–814.
25. Huebner A, Srisa-Art M, Holt D, Abell C, Hollfelder F, deMello AJ, Edel JB. *Chem Commun* 2007:1218–1220.
26. Kumaresan P, Yang CJ, Cronier SA, Blazej RG, Mathies RA. *Anal Chem* 2008;80:3522–3529. [PubMed: 18410131]
27. Srisa-Art M, deMello AJ, Edel JB. *Anal Chem* 2007;79:6682–6689. [PubMed: 17676925]
28. Guttenberg Z, Müller H, Habermüller H, Geisbauer A, Pipper J, Felbel J, Kielpinski M, Scriba J, Wixforth A. *Lab Chip* 2005;5:308–317. [PubMed: 15726207]
29. Lehmann U, Vandevyver C, Parashar VK, Gijs MAM. *Angew Chem Int Ed* 2006;45:3062–3067.
30. Pipper J, Inoue M, Ng LFP, Neuzil P, Zhang Y, Novak L. *Nat Med* 2007;13:1259–1263. [PubMed: 17891145]
31. Beer NR, Hindson BJ, Wheeler EK, Hall SB, Rose KA, Kennedy IM, Colston BW. *Anal Chem* 2007;79:8471–8475. [PubMed: 17929880]

32. Beer NR, Wheeler EK, Lee-Houghton L, Watkins N, Nasarabadi S, Hebert N, Leung P, Arnold DW, Bailey CG, Colston BW. *Anal Chem* 2008;80:1854–1858. [PubMed: 18278951]
33. Wang F, Burns Mark A. *Biomed Microdevices* 2009;11:1071–1080.
34. Nguyen NT, Lassemono S, Chollet FA. *Sens Actuators B* 2006;117:431–436.
35. Ernst H, Jachimovicz A, Urban GA. *Sens Actuators A* 2002;100:54–62.
36. Pagonis DN, Kaltsas G, Nassiopoulou AG. *J Micromech Microeng* 2004;14:793–797.
37. Srivastava N, Burns MA. *Lab Chip* 2006;6:744–751. [PubMed: 16738725]
38. Chen JZ, Darhuber AA, Troain SM, Wagner S. *Lab Chip* 2004;4:473–480. [PubMed: 15472731]
39. Ren H, Fair RB, Pollack MG. *Sens Actuators B* 2004;98:319–327.
40. Collins J, Lee AP. *Lab Chip* 2004;4:7–10. [PubMed: 15007432]
41. Salgado JD, Horiuchi K, Dutta P. *J Micromech Microeng* 2006;16:920–928.
42. Luo C, Yang X, Fu Q, Sun M, Ouyang Q, Chen Y, Ji H. *Electrophoresis* 2006;27:1977–1983. [PubMed: 16596709]
43. Niu X, Zhang M, Peng S, Wen W, Sheng P. *Biomicrofluidics* 2007;1:044101–044112.
44. Liu S, Gu Y, Le Roux RB, Matthews SM, Bratton D, Yunus K, Fisher AC, Huck WTS. *Lab Chip* 2008;8:1937–1942. [PubMed: 18941696]
45. Gong J, Kim CJ. *Lab Chip* 2008;8:898–906. [PubMed: 18497909]
46. Pal R, Yang M, Lin R, Johnson BN, Srivastava N, Razzacki SZ, Chomistek KJ, Heldsinger D, Haque RM, Ugaz VM, Thwar P, Chen Z, Alfano K, Yim M, Krishnan M, Fuller AO, Larson RG, Burke DT, Burns MA. *Lab Chip* 2005;5:1024–1032. [PubMed: 16175256]
47. Sammarco TS, Burns MA. *AIChE Journal* 1999;45:350–366.
48. Handique K, Burns MA. *J Micromech Microeng* 2001;11:548–554.
49. Rhee M, Burns MA. *Langmuir* 2008;24:590–601. [PubMed: 18069861]
50. Fang TH, Ramalingam N, Dong XD, Ngin TS, Zeng X, Kuan ATL, Huat EYP, Gong H-Q. *Biosens Bioelectron* 2009;24:2131–2136. [PubMed: 19162460]
51. Elwing H. *Biomaterials* 1998;19:397–406. [PubMed: 9677153]

Fig. 1.

Output signals from four digital sensors when the oil/water interface passes over. The input signal to the sensor is shut off if the output signal is higher than the threshold value of 40mV. The labels of length and time on top of the signal peaks denote the distance between the adjacent sensors and the time interval for tripping the adjacent sensors. The inset diagram shows the layout of four digital sensors outlined by dashed lines. P_{H_2O} is applied first, and P_{Oil} is applied after Sensor 2 is tripped.

Fig. 2. Output signal of the analog sensor as a function of the droplet coverage across the electrodes. The inset diagram shows the layout of the analog sensor outlined by dashed line.

Fig. 3. Snapshots of the automated droplet generation. The locations of all the sensors are denoted in (a). Sensor 3 is located very close to the aqueous phase inlet, and is underneath the aqueous phase reservoir.

Fig. 4. Comparison between the designed droplet size and the size of automated generated droplet. The straight line denotes that the experimental droplet size equals to the designed droplet size. The error bar represents the standard deviation.

Fig. 5.

(a) The output signals from Sensor 5 and 8 during the droplet confinement with digital sensing. (b) Zoom-in of the left outlined region of the signal curves. (c) Zoom-in of the right outlined region of the signal curves.

Fig. 6.

(a) The output signals from Sensor 6 and 7 during the droplet confinement with analog sensing. The inset shows the image of the reaction region with four analog sensors. (b) The voltage input to the electropneumatic regulators when Sensor 6 and 7 are monitored during the droplet confinement. $K_p=0.0005$ (V_{r_S6}); $K_p=0.0025$ (V_{r_S7}); $K_i=0$.