A feedback control system for high-fidelity digital microfluidics†

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Digital microfluidics (DMF) is a technique in which discrete droplets are manipulated by applying electrical fields to an array of electrodes. In an ideal DMF system, each application of driving potential would cause a targeted droplet to move onto an energized electrode (*i.e.*, perfect fidelity between driving voltage and actuation); however, in real systems, droplets are sometimes observed to resist movement onto particular electrodes. Here, we implement a sensing and feedback control system in which all droplet movements are monitored, such that when a movement failure is observed, additional driving voltages can be applied until the droplet completes the desired operation. The new system was evaluated for a series of liquids including water, methanol, and cell culture medium containing fetal bovine serum, and feedback control was observed to result in dramatic improvements in droplet actuation fidelity and velocity. The utility of the new system was validated by implementing an enzyme kinetics assay with continuous mixing. The new platform for digital microfluidics is simple and inexpensive and thus should be useful for scientists and engineers who are developing automated analysis platforms.

Introduction

Digital microfluidics (DMF) is a technique in which discrete droplets are manipulated by applying electrical fields to an array of electrodes.[1] An advantage of DMF is that droplets serve as discrete microvessels in which reactions can be carried out without cross-talk between samples or reagents. In contrast to the more conventional geometry of enclosed microchannels, each sample on a DMF device can be addressed individually,[2] and reagents can be dispensed from reservoirs, moved, merged and split.[3]

There is much enthusiasm for using DMF for applications such as cell culture and assays,[4–8] DNA sample processing and analysis,[9–12] protein sample processing and analysis,[13–19] enzyme assays,[20–22] immunoassays,[23,24] and clinical sample processing.[25] Despite this enthusiasm, the technology is still new, and there are significant challenges that prevent its widespread adoption in the lab-on-a-chip community. One such challenge is imperfect fidelity between driving voltage and actuation. In an ideal DMF system each application of driving potential would cause a targeted droplet to move onto an energized electrode; however, in real systems (particularly in those not submerged in oil), droplets are sometimes observed to resist movement onto particular electrodes. A likely cause for this phenomenon is droplet stiction on surface heterogeneities, such as scratches, dust, or reagents that have adsorbed onto the surface from other droplets. The latter (unwanted adsorption onto surfaces) is of particular concern, and we[26,27] and others[28] have developed strategies to limit its effects; however, actuation fidelity continues to be problematic for many applications.

A potential solution to this problem is to implement a sensing and feedback control system. In such a scheme, all droplet movements are monitored such that when a movement failure is observed, additional driving voltages can be applied until the droplet completes the desired operation. We are aware of two such methods reported in previous studies. In the first, Ren *et al.*[29] used a ring oscillator circuit to facilitate high-precision droplet dispensing. In the second study, Gong and Kim[30] used a similar circuit for sensing, and in addition, applied a proportional integral derivative control algorithm to facilitate even greater precision in droplet dispensing. Both of these methods used sophisticated high-frequency electronic circuits that require significant user expertise to implement. In addition, these schemes required that the droplet driving potentials be DC rather than the more common AC droplet driving signals; DC driving potentials for digital microfluidics are often avoided because devices suffer greater risk of unwanted dielectric breakdown and electrolysis.[31] Perhaps most importantly, the performance of these initial techniques[29,30] was not reported for liquids containing proteins and other sticky constituents (a necessity for many applications).

In response to these limitations, we developed a new sensing and feedback control system compatible with AC driving potentials, driven by an inexpensive, passive circuit comprising only a few resistors and a capacitor. We applied this system to actuating a range of fluids, including water, methanol, and cell culture media containing fetal bovine serum (a mixture of sticky proteins), with nearly perfect droplet actuation fidelity. The system was validated by application to an enzyme kinetics assay with continuous mixing. Here, we present the assembly and operation details for the new system, and speculate that it may be useful for scientists and

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† Electronic supplementary information (ESI) available: (1) a text file describing reagents and materials, device fabrication, and device assembly and operation; and (2) a LabView (National Instruments, Austin, TX) program written to implement the new feedback and control system. See DOI: 10.1039/c0lc00223b.

engineers who are developing automated digital microfluidic analysis platforms for a wide range of applications, including those that involve sticky proteins and other biomolecules.

Methods

Reagents and materials, device fabrication, and device assembly and operation are in the ESI†.

Impedance measurement circuit

The control system and the electrical model including the measurement circuit is shown in Fig. 1(a) and (b) respectively. Briefly, the measurement circuit is attached to the device top plate (*i.e.* the ITO-coated slide) and it consists of two parts: (1) a voltage divider (a 1 MΩ resistor in series with a 1 MΩ resistor and a 1 pF capacitor in parallel) and (2) a parallel resistor, R_{adj} (a potentiometer). The output of the circuit, V_{feed}, is measured across the parallel combination of the 1 MΩ resistor and 1 pF capacitor to ground as shown in Fig. 1(b). The circuit was designed such that ~90% of the applied voltage dropped across the insulating (parylene) layer of the bottom plate of the device, leaving ~10% of the voltage to drop across the measurement circuit. This was achieved empirically for each device design and fluid by tuning the resistance of the potentiometer, R_{adj}. Typical values for R_{adj} in the devices and liquids used here were ~1 to 20 kΩ.

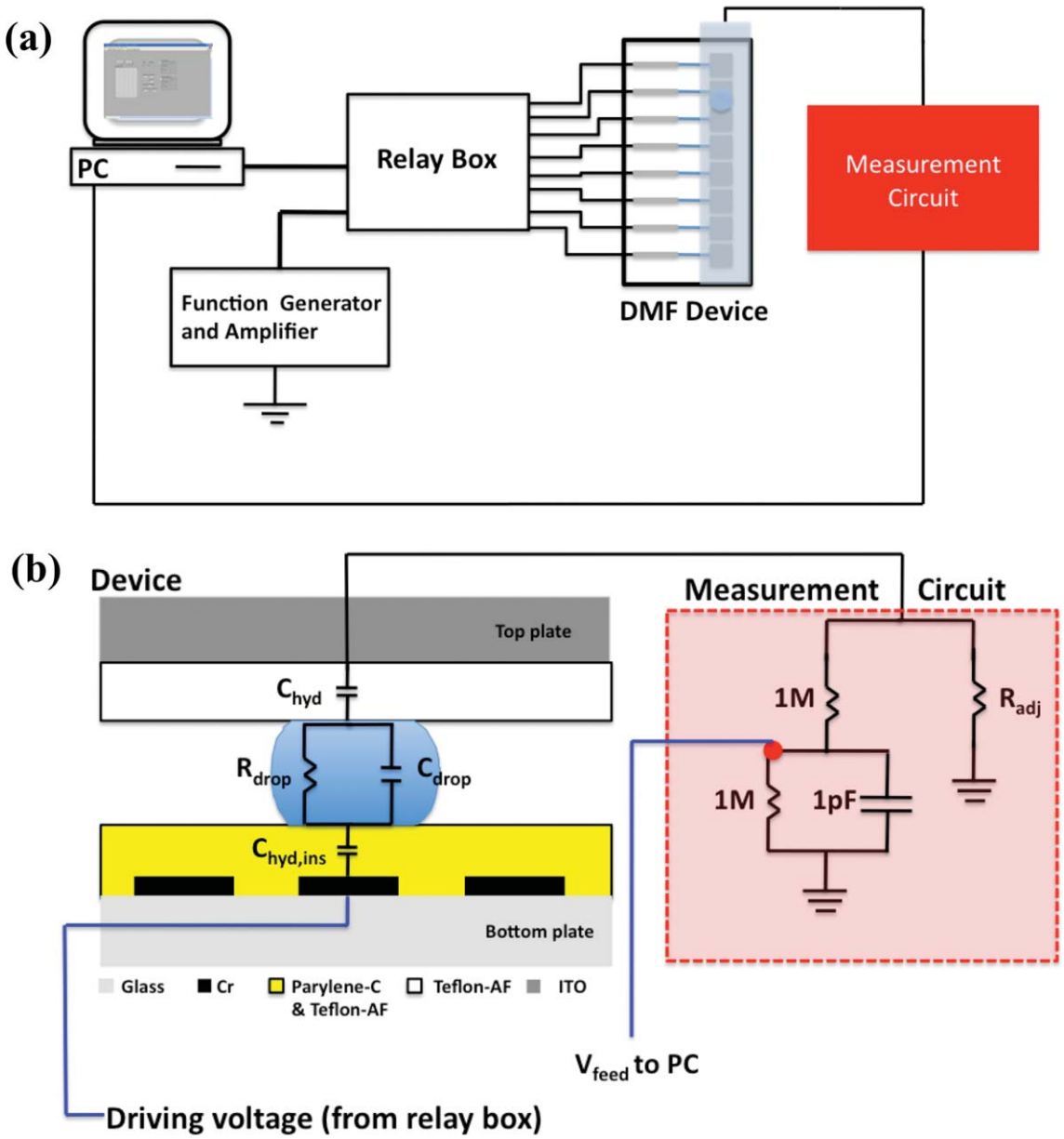

Fig. 1 Schematics of the droplet control system. (a) Overview schematic of the droplet control system, showing the relationships between the PC, the function generator and amplifier, the relay box, the DMF device, and the measurement circuit. (b) Detailed schematic and circuit model of the DMF device and the measurement circuit.

Feedback control system

A simple LabView (National Instruments, Austin, TX) program was written to implement the new feedback control system; this program is available in the ESI†. In the new feedback system, droplet position is sensed as a function of the measured potential, V_{feed}. When V_{feed} is measured across an electrode not bearing a droplet (*i.e.*, with air between the top and bottom plates), V_{feed} is ~0 V because air has very high impedance (*i.e.*, almost infinite impedance). In contrast, when V_{feed} is measured across an electrode bearing a liquid droplet, the finite impedance of the liquid (~MΩ) causes V_{feed} to be greater than 0 V. The value of V_{feed} depends on the magnitude and frequency of the applied voltage, the size of the electrode, the thickness of the dielectric layer, and the conductivity of liquid. Before operating with feedback, a threshold value, V_{thresh}, is determined empirically by actuating an electrode bearing a droplet and storing the measured V_{feed} as V_{thresh}. Typical values of V_{thresh} in the devices and liquids used here were ~3 to 20 V.

In conventional operation (without feedback), each droplet movement was initiated by application of a voltage pulse to a specific electrode; this step was then iteratively applied to adjacent electrodes to cause the droplet to move across the device. The droplet velocity (V) is the ratio of the length of an electrode, L, to the duration of the voltage pulse (D_P), $V = L/D_P$. In feedback control mode, each voltage was applied for a set duration during which an impedance sensing measurement was made. This period was followed by a comparison step (with duration D_C), in which the software compared V_{feed} to V_{thresh}. If V_{feed} was less than V_{thresh}, the driving potential was applied (again) to the same electrode. If V_{feed} was greater than or equal to V_{thresh}, the program continued to the next electrode. At the conclusion of each assay, the total number of voltage pulses (N_P) was recorded by the software, and velocity was calculated as $V = L \times N_M/[N_P \times (D_P + D_C)]$, where N_M was the number of programmed electrode movements.

Droplet actuation reliability

To evaluate digital microfluidic actuation fidelity, a simple assay was devised in which a droplet was programmed to move back and forth across the eight electrodes on device 1 (see Table S1† in the ESI) twenty times, a total of 140 movements. In conventional mode without feedback, experiments were performed with different actuation pulse durations (D_P = 200, 400, 800, 1600, 3200, 6400, 12 800 ms) per electrode. In feedback mode, a voltage pulse duration of D_P = 200 ms was used, with a comparison step between each voltage pulse of D_C = 15 ms. A "% completion" parameter was defined as the number of successful droplet movements divided by the total number of programmed steps (*i.e.*, 140). In all experiments, five trials on five separate devices were performed. A *t*-test was used to evaluate significance between the results. Droplets of several different liquids were tested including methanol, deionized water, phosphate buffered saline, a 50 : 50 mixture of deionized water and glycerol, cell culture medium containing 10% v/v fetal bovine serum and 0.05% w/v Pluronic F-68 additive, and phosphate buffered saline containing 1% w/v bovine serum albumin and 0.05% w/v Pluronic F-68 additive. Viscosities were measured using a U-tube viscometer (Fisher Scientific, Ottawa, ON).

Enzyme assay

A digital microfluidic protease activity assay was implemented in a manner similar to our previous study.[27] Briefly, solutions of 10 μg mL^{-1} trypsin and 2.5 μg mL^{-1} labeled, quenched bodipy-casein (each containing 0.08% w/v Pluronic F127 additive) were prepared using an E6638 EnzChek Protease Assay Kit (Life Technologies, Burlington, ON). ~0.3 μL droplets of each solution were dispensed from separate reservoirs on device 2 (Table S1†) onto the electrode array and then merged and mixed by moving the coalesced droplet around a loop of six actuation electrodes. After mixing, the device was positioned on the top of a microtiter plate and inserted into a PheraStar multiwell plate reader for fluorescence detection (λ_{ex} = 485 nm, λ_{em} = 520 nm, focal height 15.0 mm) for a total analysis time of 1 min, after which the device was removed and droplet movement resumed. The fluorescence from the merged droplet was measured every 3 min (not including analysis time), and three replicate trials using three different devices were conducted in conventional mode and with feedback control. As a comparison, macroscale experiments were conducted using 250 μL total volume in a 96 well-plate with continuous shaking at 700 rpm. A fluorescence intensity measurement was collected every minute using the same reader and conditions as above. The fluorescence data for all assays were normalized and plotted as a function of time and fitted with exponential functions to extract apparent first-order rate constants.

Results and discussion

High-fidelity actuation of simple liquids

As a first step towards implementing a high-fidelity digital microfluidic control system, we evaluated the actuation fidelity for droplet movement programmed in conventional mode (*i.e.*, without feedback control). Device 1 (Table S1†) was used to evaluate a series of simple fluids (*i.e.*, those that do not contain molecular constituents that are likely to stick to surfaces) including methanol, deionized water (diH_2O), phosphate buffered saline (PBS) and a 50 : 50 mixture of diH_2O : glycerol. A "% completion" parameter was defined as the number of successful movements divided by the total number of electrode steps. This parameter represents the probability that a given droplet will complete the programmed sequence of movements without failure. As shown in Fig. 2, when droplets were moved at low velocities, the % completion was high, but when droplets were moved at high velocities, the % completion was low. Interestingly, this velocity-trend is a function of viscosity—the actuation fidelities at higher velocities are best for liquids with low viscosities—*i.e.*, methanol (0.54 cP) > diH_2O (0.89 cP) > PBS (1.05 cP) > 50 : 50 diH_2O : glycerol (14.6 cP). We attribute this viscosity/velocity trend to the higher friction shear forces associated with high-viscosity liquids.[32–35]

For some applications, slow droplet velocities are fine, but for others, it would be useful to move droplets as rapidly as possible. We hypothesized that the new feedback control system would be useful for moving droplets at rapid velocities with high

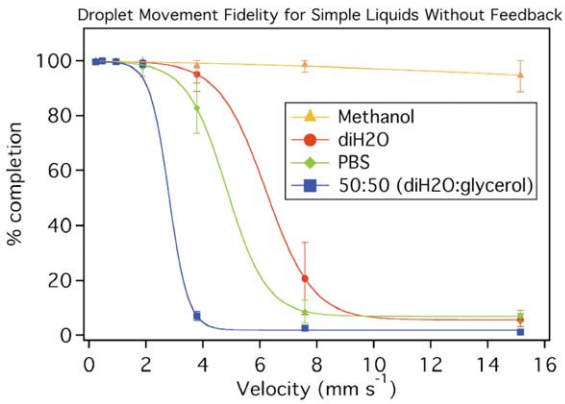

Fig. 2 Droplet movement fidelity (measured as "% completion") for digital microfluidic actuation of droplets of simple liquids (not containing protein) without feedback. % completion is defined as the number of droplet movements divided by the total number of scheduled movements (in this case, 140). Each experiment was repeated five times on separate devices, and error bars are ±1 SD.

fidelity—specifically, the new system should move droplets rapidly over most electrodes, but should allow more time for droplets to move slowly over "sticky" electrodes (*i.e.*, those that have a flaw or heterogeneity that may cause the droplet to resist movement). The data validated our hypothesis—for example, in five trials of 140 droplet movements (representing 700 total movements), diH_2O droplets were observed to require only one or two 200 ms voltage pulses for the majority of the movements (693 of 700), and required three or more 200 ms voltage pulses for the remainder (7 of 700). This translated into higher average droplet velocities—as listed in Table 1, the maximum velocities achieved with high fidelity (defined as % completion ≥ 99.5%) were greater for droplets actuated with feedback than without feedback for all of the fluids tested. As shown, the observed gains in velocity are modest, ranging from 2.5 to 5.5× higher for feedback mode compared to conventional mode, but they are statistically significant ($p < 0.01$).

High-fidelity actuation of complex liquids

Actuation by digital microfluidics is problematic for complex liquids containing proteins that are likely to stick to hydrophobic device surfaces.[26–28] To test this phenomenon in our new system, we evaluated two fluids containing proteins that are known to be extremely sticky in microfluidic systems: fetal bovine serum (as a mixture of 10% FBS in RPMI 1640 cell culture media—this combination is routinely used to culture cells in microfluidic devices[4]), and bovine serum albumin (as a 1% solution in PBS, this solution is commonly used in microfluidic systems because it adsorbs strongly to hydrophobic device surfaces[36]). As shown in Fig. 3, the behavior of these two complex fluids when moved without feedback was quite different when compared to that of simple fluids (Fig. 2). When droplets containing FBS and BSA are moved at high velocity, movement fidelity is poor, and as the velocity is reduced to moderate levels, movement fidelity improves. However, in contrast to simple fluids, as the velocities are reduced to very low levels, the movement fidelity of complex fluids decreases dramatically. Thus, droplets moved without feedback in such systems never achieve high movement fidelity under any conditions. We note that this poor performance is observed despite the fact that these samples were prepared to contain Pluronic additives to reduce protein sticking.[27] Thus, this represents a significant problem for digital microfluidics.

In an ideal system, a balance would be struck such that droplet velocities would be slow enough to account for viscous drag and device surface heterogeneities, but fast enough to limit the extent of protein adsorption. We hypothesized that the new feedback system would be useful for moving droplets of complex fluids with high fidelity. As listed in Table 2, the results confirm this hypothesis—droplet movement fidelity is dramatically improved for liquids containing proteins (*e.g.* 56.1% completion without feedback and 98.1% completion with feedback for cell culture media containing FBS). In addition, droplets manipulated with feedback are observed to have higher average velocities. Clearly, the feedback control system represents significant improvement for digital microfluidics for applications involving proteins.

Table 1 Transport of simple liquids with and without feedback control

	Velocity with ≥99.5% completion/(mm s^{-1})	
Reagents	Without feedback	With feedback
Methanol	1.87	7.58
diH_2O	1.51	5.05
PBS	0.62	3.03
50 : 50 diH_2O : glycerol	0.55	3.03

An application for high-fidelity DMF actuation

To illustrate the utility of feedback control for DMF, we implemented an enzyme-kinetics assay requiring the mixing of two different proteins. In such assays, it is critical that the reagents be mixed as rapidly as possible to be able to measure kinetics with good fidelity. It has been shown previously[37–39] that in DMF systems, reagents in a droplet mix up to

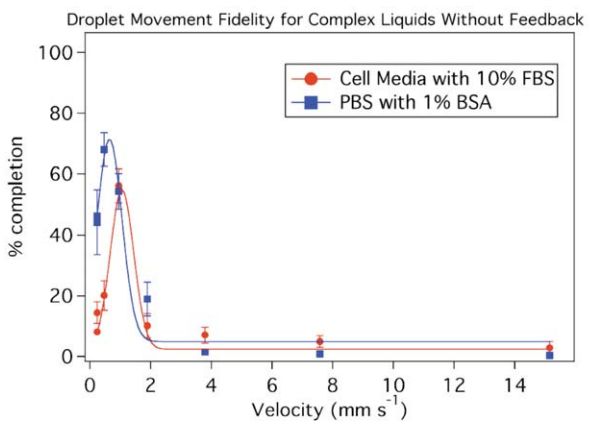

Fig. 3 Droplet movement fidelity (measured as "% completion") for digital microfluidic actuation of droplets of complex liquids (containing protein) without feedback. Each experiment was repeated five times on separate devices, and error bars are ±1 SD.

Table 2 Transport of complex liquids with and without feedback control

	Maximum % completion		Velocity (mm s^{-1}) at which max % completion is achieved	
Reagent	No feedback	Feedback	No feedback	Feedback
Cell media with 10% FBS	56.1	98.1	0.95	1.89
1% BSA in PBS	68.6	99.1	0.47	2.53

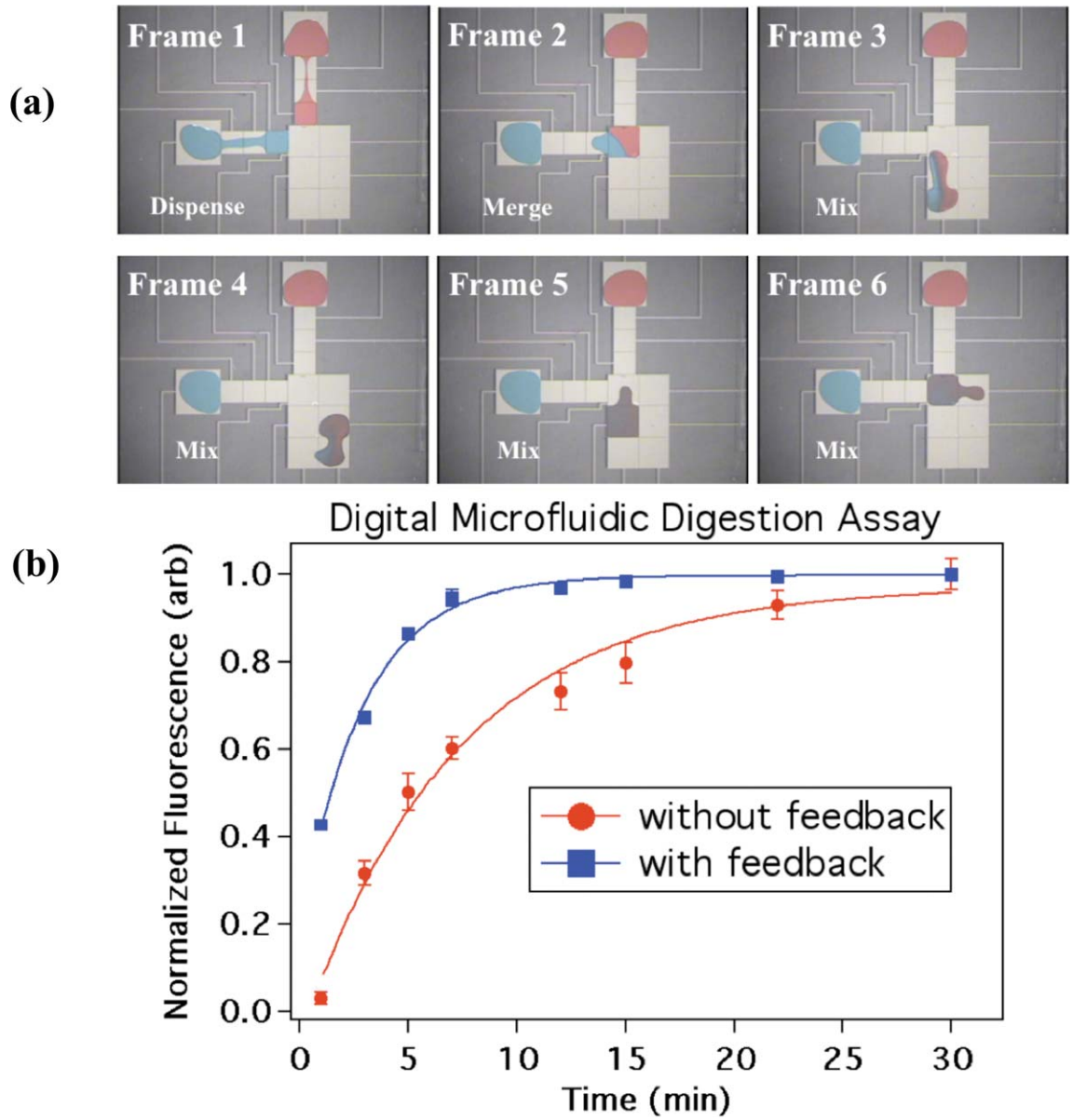

Fig. 4 Enzyme kinetics assay with and without feedback. (a) Series of frames from a movie depicting an enzyme kinetics assay. Droplets containing trypsin and a fluorescent reporter are dispensed (frame 1), merged (frame 2), and then continuously moved around electrodes (frames 3–6) to mix the reagents. Dyes were added to the droplets to assist in visualization. (b) Fluorescence measurements as a function of time collected in three-minute intervals from assays implemented with and without feedback. Droplets moved with feedback had perfect fidelity, while droplets moved without feedback had poor fidelity (and hence, slower mixing). Each experiment was repeated three times on separate devices, and error bars are ±1 SD.

10–50× faster when the droplet is continuously moved across the device relative to cases in which the droplet remains stationary. Unfortunately, if a droplet contains proteins or other sticky reagents, droplet movement may be unreliable (see Fig. 3), which could negate this effect. We hypothesized that application of feedback control to this assay (with reliable, continuous droplet movement) would facilitate faster mixing, leading to a more accurate experiment.

The kinetics assay used here measures the activity of an enzyme (trypsin) by mixing it with a fluorogenic reporter (bodipy-labeled/quenched casein) that has low fluorescence when intact but becomes highly fluorescent when digested. As shown in Fig. 4(a), in each assay, droplets containing the enzyme and the reporter were dispensed, merged and continuously circulated around six electrodes on device 2 (Table S1†). Fluorescent measurements were collected every 3 min, and Fig. 4(b) shows two curves depicting the normalized fluorescent intensity for droplets actuated with feedback and without feedback. In the case without feedback, droplet movement fidelity was poor (~20%) which caused the droplet to remain stationary for much of the time, such that it suffered from poor mixing. In contrast, when the same assay was implemented with feedback control, the combined droplet continuously circulated around the electrodes, resulting in better mixing.

Apparent first order rate constants were extracted from the data in Fig. 4(b) for the system controlled with feedback and without ($k_{\text{feed}} = 0.424\ \text{min}^{-1}$ and $k_{\text{no feed}} = 0.127\ \text{min}^{-1}$, respectively), and were compared to data from an equivalent, well-mixed (with turbulent mixing) macro-scale reaction ($k_{\text{mixed}} = 0.521\ \text{min}^{-1}$). The value generated using the feedback control system was a much better estimate of the actual kinetics than that generated without feedback control. We propose that similar advantages will be apparent for many different kinds of DMF applications that require rapid mixing of proteins or other sticky constituents.

Conclusion

We have developed a sensing and feedback control system for high-fidelity digital microfluidics. We have shown that with the feedback system, droplet movement fidelity is much higher compared to movement operations without feedback, and that this advantage is particularly important for fluids containing proteins. We speculate that this may be useful for scientists and engineers who are developing automated analysis platforms for a wide range of applications.

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