A Pump Based Microfluidic Image Processing System for Droplet Detection and Counting

Faisal Mehmood\textsuperscript{1}, Zeeshan Haider\textsuperscript{2} and Baoqun Yin\textsuperscript{1}\textsuperscript{*}

\textsuperscript{1}Department of Automation, University of Science and Technology of China, Hefei, Anhui, China
\textsuperscript{2}Department of Biomedical Engineering, School of Information Science, USTC, Hefei, Anhui, China
E-mail: faisalmehmood7@yahoo.com, zeeshanh1234@gmail.com, bqyin@ustc.edu.cn.

Abstract. Droplet Microfluidics has attracted great attention in recent years because of its interdisciplinary nature and had a wide application in various fields especially biomedical applications. Till now researcher focus on controlling the size of droplet through flow rates but none of them focus on droplet detection and counting. This is the first research in which we have presented an automatic system based on Image processing algorithm for detection and counting of the droplets. The image processing algorithm is based on temporal differencing-based motion detection algorithm for continuous flowing droplet detection and counting. Moreover, the overall system was tested to generate droplet of phosphate-buffered Saline and 8\% polyethylene glycol diacrylate pre-polymer droplet. The results depicted that our algorithm is capable of detecting the droplets as well as counting them with lowest statistical errors.

1. Introduction
Microfluidics emerged in the early 1980s as an appealing interdisciplinary technology and has since gained substantial consideration. It is basically the technology that permits the manipulation of small volumes of fluids inside microchannels and has uncovered applications in numerous fields, such as biology, physics [1, 2], biotechnology and pharmacy. It offers the potential to utilize tiny quantities of samples as well as reagents to detect, separate and synthesize materials with high sensitivity and resolution, or to make use of the flow patterns that occur inside microchannels.

Droplet microfluidics revolutionizes the detection of molecules to replace complicated experiments in chemical laboratory by employing integrated and miniaturized systems [3], explaining the benefits of appropriate liquid handling for chemical assays, minimized reagent consumption, as well as optimized results for higher-throughput configurations [4]. Further, droplet-based microfluidics enables significant number of experiments to be conducted and repeated inside a single device, substantially enhancing the statistics of results. After the generation of droplets, they move in microchannels in a steady manner. To meet specific objectives and functions, actuation technologies are demanded for handling of these droplets in accordance to their contents. Droplet microfluidic systems have already been practiced in analytical detection by applying various techniques for high quality content analysis in droplets. These analytical detection methods involve image-based analysis, electrochemistry, laser-based molecular spectroscopy, mass spectrometry, capillary electrophoresis, nuclear magnetic oscillation spectroscopy, chemiluminescence detection [5]. A couple of most used droplet detection techniques can include surface-enhanced Raman scattering (SERS), fluorescence, capacitive, electrochemistry, and mass spectrometry. As an example, fluorescence droplet detection is one of the methods that are used most
commonly for biological applications. It is carried out by illuminating the droplet of great interest and detecting photons emission, e.g., a fluorescence microscope combined with a delicate CCD. Unfortunately, the sensitive and real-time individual droplet detection is limited because of the slow feedback of conventional CCDs. To deal with this concern, expensive high-speed cameras, traditional photomultiplier tubes or photodiode detectors are usually preferred to obtain information regarding the fast-moving droplets. Moreover, Electrochemistry is yet another detection technique that entails the transfer of electrons between sensing electrodes and aqueous droplet content. Making use of imaging analysis is increasing globally and guaranteeing optimal research and medical diagnosis. In imaging techniques, to enhance the quality and clarity of an image by using contrast agents is very important. Image processing is implemented in Droplet microfluidics for size-controlled, synthesizing monodisperses, and higher-quality microparticles [6, 7]. Regardless of the noticeable success of imaging microfluidic systems towards a wide range of applications, droplet examination is mostly restricted towards simplified morphological information.

Presently, papers are lacking the ability to track and observe droplets. Our paper is focusing on the detection and counting of microfluid droplets by means of temporal difference image processing algorithm. The rest of the paper is organized as follows: Section 2 explains the materials and methods used in the system. Section 3 represents the image processing algorithm for detection and counting of droplet. Section 4 shows the experimental setup and result and discussion. Section 5 depicts overall conclusion of Paper.

2. Materials and methods

2.1 Device design and fabrication

We have designed a simple microfluidic device consists of flow focusing region, as shown in fig.1. The device has one outlet (O1) with a diameter of 2 mm and two inlets (I1 and I2). The main channel has height and width of 100 m and 200 m respectively, fig. 1. For the fabrication of the microfluid channel we applied a standardized soft-lithography protocol [8].

![Figure 1. The schematic and fabrication of the device (A) Image of the real PDMS device (B) Step-by-step process of device fabrication.](image-url)
Some of the core steps are shown in Fig. 1 (B). Concisely, a positive photo resistor (AZ4620, Microchem) was spun-coated on a 4-inch silicon wafer at 4000 RPM for 120 s to achieve 6m thickness. Subsequently the silicon wafer was prebaked for 5 minutes at 100 °C, revealed under UV light for 35 s, developed in AZ 300 MIF developer (Microchem) for 6 min, as well as post-baked at 110 °C for 5 min. To perform etching process a deep silicon etching device (Plasma Pro System100 ICP380, Oxford) is used to acquire 100m etching. After that the silicon wafer was immersed into acetone for the removal of residual photo resistor by washing using deionized water and drying out with nitrogen gas. The Dow Corning pre-polymer, polydimethylsiloxane (PDMS; Sylgard184, along with curing agent were mixed-up at a ratio of 10:1 (w/w), de-gassed to remove bubbles, poured onto the silicon wafer, and cured at 78 °C for 3h. Next the PDMS slab with a microchannel was demolded, bonded and punched to a glass slide by using plasma cleaner (PDC–32 G, Harrick) to use further.

### 2.2 Material preparation for droplet generation

Two different aqueous phases are prepared in this research, including phosphate buffered saline (PBS, Life Technologies, Carlsbad, CA, USA) and 8% w/v polyethylene (glycol) diacrylate (PEGDA, with molecular weight: 700Da, Sigma-Aldrich, St. Louis, MO, USA) hydrogel prepolymer solution with 0.5% w/v Irgacure 2959 (Ciba Chemicals, Basel, Switzerland) in PBS. The oil phase has been prepared making use of the white high purity mineral oil (VWR International, Radnor, PA, USA) having 20% v/v Span 80 surfactant (Sigma-Aldrich, St. Louis, MO, USA).

| Table 1. Represents the symbolic representation of Temporal Difference image processing algorithm |
| --- |
| Symbol | Parameter | Symbol | Parameter |
| $R$ | Pump flow rate | $V$ | Microfluidic flow speed |
| $W$ | Microfluidic channel width | $H$ | Microfluidic channel height |
| $L$ | Microfluidic channel length | $f_i$ | Current frame |
| $N$ | Reference frame | $\Delta t$ | Temporal difference |
| $f_{fr}$ | Sensor frame rate | $m$ | Total horizontal pixel number |
| $n$ | Total vertical pixel number | $I$ | Droplet shadow image intensity |
| $\Delta x$ | Total vertical pixel | $x$ | Horizontal pixel coordinate |
| $y$ | Background intensity variation | $d_t$ | Temporal-diffusing image |
| $D_t$ | Temporal intensity difference | $\ell$ | Negative intensity difference |
| $B_t$ | Binarized difference image | $T_i$ | Droplet image intensity threshold |
| $T_r$ | Droplet pixel removal threshold | $N_r$ | Frame droplet count |
| $C$ | Droplet centroid coordinate | $k$ | $k_{th}$ droplet in frame $i$ |
| $N$ | Total number of droplets | $M$ | Total number of images in a test |
| $P$ | System droplet throughput | $p$ | Droplet concentration in the test agent |

It is observed that by adding additional surfactant can improve the viscosity of oil phase and therefore help the process of droplet generation, particularly when it comes to relatively viscous hydrogel prepolymer solution. Thus, 20% surfactant is widely-used for generating hydrogel droplets.

The viscosity of 8% PEGDA solution was measured by a viscometer (Cannon Instrument, State College, PA, USA).
3. Image processing algorithm

One of the challenges towards continuous flowing droplet detection and counting certainly is the motion detection by detecting the intensity difference of droplets contrary to the channel background [9]. While in most cases droplets have particular transparency, their particular images do not offer high intensity difference, i.e., contrast, contrary to the background. The variation in the background brightness and darkness directly affects the contrast. In our work, an efficient motion detection technique based on temporal difference is developed for the flowing droplet counting and detection in a series of frames [9, 10]. Taking into account the intensity characteristics of droplet images, there are actually two steps required. First of all, motion detection is carried out for all the droplets in every single frame. After that, complete droplet counting is performed based upon the detected moving droplet counts in each frame. A comprehensive discussion is presented underneath for each step. All the terms and symbols used for the analysis of given system are summarized in Table I.

3.1 Droplet detection

All the flowing droplets in each frame should be detected in the first step before droplet counting which demands a motion detection achieved by making use of temporal-differencing based on background subtraction. By using pixel-by-pixel intensity difference of each two consecutive frames, it detects moving droplet regions where one is a reference frame and other one is the current frame. Such differences are triggered because of the droplets motion in the channel, or perhaps due to the addition and reduction of droplets from the sensor field-of-view (FOV). The process for droplet detection is presented in fig. 2. Firstly, the droplet images are loaded for detection. As shown in fig. 2, one microfluidic channel having droplet flowing inside channel. It is to be noted that one complete frame includes m (H) × n (V) pixels corresponding to the size of sensor array. However, fig. 2 demonstrates...
only part of the frame with one droplet in every channel so as to make the small droplet clearer to observe. The movement of one droplet flowing from left to the right is captured at two different points in two consecutive frames, $f_t$ and $f_{t-D}$. In this, $f_t$ is the current frame and $f_{t-D}$ is the reference frame (or background frame) as shown in fig. 2(a) and (b).

The temporary difference between two frames $f_{t-D}$ and $f_t$ is $\Delta t$, which can be determined by the CMOS image sensor frame rate $F_{ps}$, i.e., $\Delta t = \frac{1}{F_{ps}}$. By assuming the system is operating under a specified white light illumination, we can define the current frame as,

$$f_t = I_t(x,y) + \Delta I_b(x,y) \mid x \in m, y \in n \quad (1)$$

Where $I_t(x,y)$ stands for the part of pixel intensity as a result of droplet shadows for the current frame $f_t$ at pixel coordinate $I_t(x,y)$ furthermore $\Delta I_b(x,y)$ stands for the background intensity variations that are ascertained by illumination along with the possible stains from the sensor or channel surface. Because of droplet transparency, the images of droplet captured by lensless microfluidic imaging system shows brightness characterization at the center and darkness at droplet boundary as found in fig. 2(h). This indicates that the pixels in center possess higher intensity levels as compared to those in background, and the ones at the boundary possess lower intensity levels compared to the background. By carrying out background subtraction, we can find out a temporal-differencing image $d_t = f_t - f_{t-D}$ the corresponding intensity matrix is;

$$D_t(x,y) = [I_t(x,y) + \Delta I_b(x,y)] - [I_{t-D}(x,y) + \Delta I_b(x,y)] = I_t(x,y) - I_{t-D}(x,y) \quad (2)$$

Where $D_t(x,y)$ is the difference of intensity between two consecutive frames at the same location $(x,y)$. Now in $d_t$, there are only two regions where droplet is present in $f_t$ and $f_{t-D}$ which is obvious in fig. 2(c). However, the other regions in the background are all subtracted to zero. Consequently, the background variation impact is reduced. The difference amongst these two droplet regions can be defined as the region affiliated to the droplet in current frame $f_t$, yet displays brightness in the center and darkness on the boundary as shown in fig. 2(i). Whereas the region created by the droplet in preceding frame $f_{t-D}$ is reversed, in which darkness is in the center and brightness is at the boundary as shown in fig. 2(j). This particular droplet shadow in fig. 2(j) turns out to be an artificial ‘tail’ following the previous droplet as provided in fig. 2(g). For the detection of the actual droplet but not the ‘tail’ droplet in existing frame, the ‘tail’ must be removed, and this can be simply attained by setting the regions of $D_t(x,y)$ to zero in which they were larger than zero, i-e,

$$D'_t(x,y) = \{D_t(x,y), \text{if} D_t(x,y) > 0$$

$$= 0, \text{if} D_t(x,y) \leq 0 \quad (3)$$

Where $D'_t(x,y)$ is up to date from $D_t(x,y)$ on the basis of the above elimination using corresponding image found in fig. 2(d). One can witness that $D'_t(x,y)$ contains only zero and negative intensity values in contrast to $D_t(x,y)$. Afterwards, by comparing further with a droplet image intensity threshold $T_t$, the image binarization can be understood by,
\[ B_i(x, y) = 1, \text{ if } D_i(x, y) < T_i, = 0 \] (4)

Otherwise wherein \( B_i(x, y) \) is the binary difference image in fig. 2(e) according to \( D_i(x, y) \). The option of \( T_i \) is dependent upon the comparison amongst the intensity levels of droplet boundary contrary to the background level after subtraction. The binary image that indicates a larger region of white circle signifies the actual droplet in current frame \( f_i(x, y) \), fig. 2(l), as well as a small region concerning white brick showing ‘tail’ droplet in the previous frame \( f_{i-1}(x, y) \) fig. 2(k). Each one of these regions is marked and therefore their pixel counts can be obtained. The pixel counts present in white regions are compared with a pre-defined removal threshold \( T_r \). In case the pixel counts are smaller compared to \( T_r \), the white region can be determined as the ‘tail’ droplet region and therefore it will be removed fig. 2(f). Likewise, considering our experiment setup, in this paper \( T_r = 8 \) is obtained. Here, a valid and efficient flowing droplet detection method can be noticed for the developed pumped based lensless microfluidic imaging system.

### 3.2 Droplet counting

Once finishing the droplet detection of each frame, the count \( N_i \) of droplets and their centroid coordinates \( C_{i,k}(x, y), 0 \leq k \leq N_i \), are obtained, wherein \( k \) represents the \( k \)th droplet in the frame \( f_i \). For the counting of complete number of droplets flowing through the microfluidic channel obtained by a series of images, \( f_1 = 1 \) to \( f_M = M \). Here \( M \) defined as the complete number of images for one test with duration \( M \Delta t \), a sum of positive temporal difference concerning all the frames is conducted. In the same way, two frames \( f_i \) and \( f_{i-1} = 1 \) in one series of images are to be processed, and also the amounts of droplets in these two frames, i.e., \( N_{i-1} \) and \( N_i \), is already acquired right after the droplet detection step.

For getting the complete number of flowing droplets, undertake the temporal subtraction \( (N_i - N_{i-1}) \) to determine the new droplets approaching microfluidic channel in the latest frame \( f_i \). As opposed to previous frame \( f_{i-1} \), droplets in the current frame \( f_i \) can have three situations to investigate:

1) The new droplets flowing towards the sensor FOV for increasing the droplet count over \( N_{i-1} \);

2) Current droplets flowing out from the sensor FOV to reduce \( N_{i-1} \);

3) Absolutely no new droplets flowing towards or none existing droplets are flowing out.

For that reason, just the positive temporal droplet count difference is substantial to be added to actual total droplet number \( N \). Once process each new frame in one test, the finalized total droplet count \( N \) can be obtained by summing up the differences of all these positive droplet count in the consecutive frames,\n
\[ N = \sum_{i=2}^{M} (N_i - N_{i-1}) \text{ If } N_i - N_{i-1} > 0 \] (5)

As a result, here obtain the number \( N \) for overall droplets that are flowing through the microfluidic channel captured by series of images in one test.
4. Results and discussion

4.1 Experimental procedure and system setup
Two syringe pumps (Kent Scientific, Torrington, CT, USA) are used to infuse oil and aqueous phase flows. One grayscale CMOS image sensor (Aptina MT9V032, San Jose, CA) with a pixel size of 6 μm × 6 μm is used for imaging. Its pixel array size is 752(H) × 480(V) and active sensing area is 4.5 mm (H) × 2.9 mm (V). The sensor frame rate is 60 frames/second (fps) at its full resolution. This CMOS camera was placed under the PDMS Chip. The schematic demonstrating complete setup is shown in fig. 3. The system was connected with one laptop (3.3-GHz Intel Core i5) through a USB interface for testing.

One MATLAB-based Graphical User Interface was developed to set the sensor working mode and control the image processing. Finally, droplet images and counted droplet numbers in the sample can be displayed. To analyze the accuracy of the system compared to the manual measurement with that from the image processing. Every single group of experiments captures 64 lensless images in 4 seconds. By calculating the flowing distance of the same droplet in two consecutive frames, the flow rate of 0.3 μl/min and 1.3 μl/min were achieved. The raw lensless images of droplet that were captured before doing background subtraction are presented in Fig. 4, which provides a similar annulus intensity pattern of high intensity at the center and low intensity at the boundary as the microscope droplet image in Fig. 2(g).
Figure 4. Captured lensless image of droplet (a) Slow flow rate (b) Moderate flow rate (c) High flow rate

Table 2. Counting results of the pumped lensless imaging system

| Flow Rate | Group # | Manual Count | Auto Count | Error Rate |
|-----------|---------|--------------|------------|------------|
| 0.3 μl/min | 1       | 16           | 16         | 0.00%      |
|           | 2       | 33           | 31         | −6.06%     |
|           | 3       | 26           | 26         | 0.00%      |
|           | 4       | 30           | 30         | 0.00%      |
|           | 5       | 20           | 20         | 0.00%      |
| Average1  |         | 25           | 24.6       | −1.60%     |
| 1.3 μl/min | 6       | 56           | 46         | −17.86%    |
|           | 7       | 57           | 46         | −19.30%    |
|           | 8       | 50           | 47         | −6.00%     |
|           | 9       | 45           | 45         | 0.00%      |
|           | 10      | 50           | 51         | 2.00%      |
| Average2  |         | 51.6         | 47         | −8.91%     |
| Total Average |       | 76.6         | 71.6       | −6.53%     |

The automated counting results were given in Table II. By manually analyzing all the droplets flowing through the channel in every group of 64 frames, the counting results are achieved for comparison, which can be assumed as valid. For automated counting, the average droplet throughput at a low flow rate of 0.3 μl/min is 6 droplets/second and the error rate is −1.6%; at a high flow rate of 1.3 μl/min, the average droplet throughput is 12 droplets/second and the error rate is −8.9%. Both accuracies are within the 10% error margin, demonstrating the effectiveness of the developed lensless microfluidic imaging system for the flowing droplet detection and counting. The error rate increased with flow rate, because of the misdetection of some droplets with low contractor noise. It can be improved by an improved system integration to shorten the distance between sensor and droplet. This is a prototype detection system with many to improve. The throughput of the system is rather low compared to others, and this can be improved by integrating multi-channels in parallel. The images resolution is low at the moment; this can be improved by image processing algorithms such as super-resolution.

5. Conclusion
In this paper for the first time we have developed the automatic system for detection and counting of droplet. The CMOS camera was placed under the PDMS Chip for getting the images of the droplets. In addition to that an efficient temporal difference image processing algorithm has been designed for detection and counting of droplets. Moreover, extensive experiments have been performed to demonstrate the effectiveness of the proposed system. The results indicate that the developed system is capable of detection and counting of droplet at different flow rates. This kind of system can be utilized
for further investigation of droplet applications like droplet encapsulation with single droplet and droplet analysis within single droplet.

6. References

[1] J. C. Baret, O. J. Miller, V. Taly, M. Ryckelynck, A. El- Harrak, L. Frenz, C. Rick, M. L. Samuels, J. B. Hutchison, J. J. Agresti, D. R. Link, D. A. Weitz and A. D. Griffiths, Fluorescence-activated droplet sorting (FADS): efficient microfluidic cell sorting based on enzymatic activity Lab Chip. 9 (2009) 1850.

[2] D. Lombardi and P. S. Dittrich, Anal. Bioanal. Chem., Droplet microfluidics with magnetic beads: a new tool to investigate drug–protein interactions 399 (2011) 347

[3] Pan, I.; Mukherjee, R.; Rahaman, H.; Samanta, T.; Dasgupta, P. Optimization algorithms for the design of digital microfluidic biochips: A survey. Comput. Electr. Eng. 39 (2013) 112–121.

[4] Zhao, Y.; Chen, D.; Yue, H.; French, J.B.; Rufo, J.; Benkovic, S.J.; Huang, T.J. Lab-on-a-chip technologies for single-molecule studies. Lab Chip. 13 (2013) 2183–2198.

[5] Zhu, Y.; Fang, Q. Analytical detection techniques for droplet microfluidics—A review. Anal. Chim. Acta. 787 (2013) 24–35.

[6] Rademeyer, P.; Carugo, D.; Lee, J.Y.; Stride, E. Microfluidic system for high throughput characterization of echogenic particles. Lab Chip. 15 (2015) 417–428.

[7] Duncanson, W.J.; Arriaga, L.R.; Ung, W.L.; Kopechek, J.A.; Porter, T.M.; Weitz, D.A. Microfluidic fabrication of perfluorohexane-shelled double emulsions for controlled loading and acoustic-triggered release of hydrophilic agents. Langmuir 30 (2014) 13765–13770.

[8] M.A. Unger, H.-P. Chou, T. Thorsen, A. Scherer, S.R. Quake, Monolithic microfabricated valves and pumps by multilayer soft lithography. Science 288 (2000) 113–116.

[9] J. Lipton, H. Fujiyoshi, and R. S. Patil, “Moving target classification and tracking from real-time video,” in Proc. 4th IEEE Workshop Appl.Comput. Vision. (1998) pp. 8–14.

[10] M. Piccardi, “Background subtraction techniques: A review,” in Proc.IEEE Int. Conf. Syst., Man Cybern. (2004) 3099–3104.