

# Image Processing-based Measurement of Volume for Droplets in the Microfluidic System

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**Abstract** - This paper describes a new approach on the measurement of droplets volume in microfluidic system based on image processing. This approach successfully detects the diameter of a single droplet. Image processing via MATLAB is used to capture droplets volume in the microfluidic system. Compared with other methods, this approach has enormous advantages of real-time, simpleness and wide applications. Because of the demand of constant accurate flow in microfluidic system, the approach to measure volume of microfluid is in urgent need. The accuracy of the volume measurements is limited by the selected parameters of the image processing. During the experiment, the optimum parameters of the image processing are selected through repeated tests. The obtained results are in good agreement with the demand. The results show that microfluid can be controlled in quantitative. That makes an important application field of microfluidic is in pharmacy, microchemistry, biochemistry and bioanalysis.

**Key words** - *Microfluidic system, Image processing, Droplet volume measurement*

## I. INTRODUCTION

Based on microelectromechanical system (MEMS) technologies, microfluidic has a significant progress, such as micro-pump, micro-valves, bioanalysis systems and flow sensors. Precise flows as well as effective mixing have led to the intensive development of the microfluidic mixture in fields of pharmacy, microchemistry, biochemistry and analysis. And the field has undergone rapid development for over 20 years [1]. Compared with traditional devices, integrated microfluidic devices offer many advantages, such as small size, short sample-to-result time, high-efficiency, low cost, small required and sample volumes [2]. Because of those advantages, it is widely used in analysis of DNA, protein, drug or blood screening of rare cell, inspector of Escherichia coli in water [3]-[7]. Droplets are used in many microfluidic systems because of short reaction time, rapid mixing of liquids and precise flow controlling. In order to meet the feature of high accuracy, this paper describes a method to measure volume of droplets in microfluidic system. The approach is particularly true of microfluidic systems, whose designs are used by the field of multi-fluid mixed in precise blending. Real-time volume measurement can provide information about flow of microfluidic that agreement with the feature of accurate flow.

Droplet based microfluidic devices have a bright applicable prospects. Bo Zheng et al. has used demonstrated a

microfluidic system for screening of protein crystallization conditions using nanoliter-size droplets in the flow of immiscible fluids [8]. Hundred of protein were screened in one second and used only 4nL of protein sample in once trail. Michelle R. Bringer et al. has implemented rapidly mix multiple reagents isolated in droplets rely on chaotic advection [9]. The mixing time of reagent is less than 2ms. A. Huebner et al. used droplet microfluidics to detect the expression of protein in single cell by measure size of droplet [10]. Schaerli et al. has designed a circular chip with the channel of different temperature zones can be used to analyse DNA based on the PCR reaction and it could complete 34 cycles within 17 min [11]. Krzysztof Churski et al. has constructed a droplet on demand (DOD) technique for scanning of arbitrary combinations of 3 miscible solutions [12]. The DOD system can be used in 10,000 conditions of chemical and biochemical field. There are many other applications of droplets based microfluidic devices, such as using the micro-droplets to synthetic nanoparticles, cell analysis, self-assembly of nanoparticles and so on.

Droplet based microfluidic devices have been developed for a wide spectrum of applications and have been successfully applied in laboratory scale applications. They have some strong points: 1) Mixing of reagents is very rapid. 2) The consumption of sample is low. 3) The diameter of droplets is uniform. But it is very difficult to keep the diameter of droplets in uniform. To solve the problem, the new approach to measure the volume of the droplet is described in this article.

## II. EXPERIMENTAL TECHNIQUE

### A. The microfluidic system

The system of the study is shown in Fig. 1. It is combined with digital microscope system and microfluidic system. The digital microscope system consists of a computer (monitor of microscope system), a microscope and CCD camera. The microfluidic system contains an air pump, two micropumps, a micro-chip, a droplet collection device. The monitor of microscope system is used to show the situation of microfluid in real-time, obtain images, save images, process images and so on. The microscope obtains illumination provided by halogen lamp used to observe the shape of droplets within micro-chip. The microscope contains six objective lenses which are 20X, 30X, 50X, 100X, 150X and 200X. The particle images are recorded using CCD camera. Combination

of CCD with microscope changes the former type of optical microscope. The camera in digital microscope regulates the effect of images and photographs the best condition. It can be preserved, processed and enabling the result in reality and real-time. Images are shown on the screen and save. Then it can be used in the next experimental step. With relevant software, it is available to implement dynamic real-time preview, dynamic and static capture, video, photograph, three-dimensional composite. In microfluidic system, the air pump provides air pressure to the micropump which controls the condition of the fluid, sets the pressure according to the velocity of the microliquid and the maximum pressure is 10bar. Microchip is the channel for microfluid. Its length and width are set based on the mixture time and portfolio. In this experiment, the width of the Y-shaped channel is  $190\mu\text{m}$  (Fig. 2). Droplet collecting device is used for droplet preparing. If further step is required, microfluid will be injected into next device through microchannels.

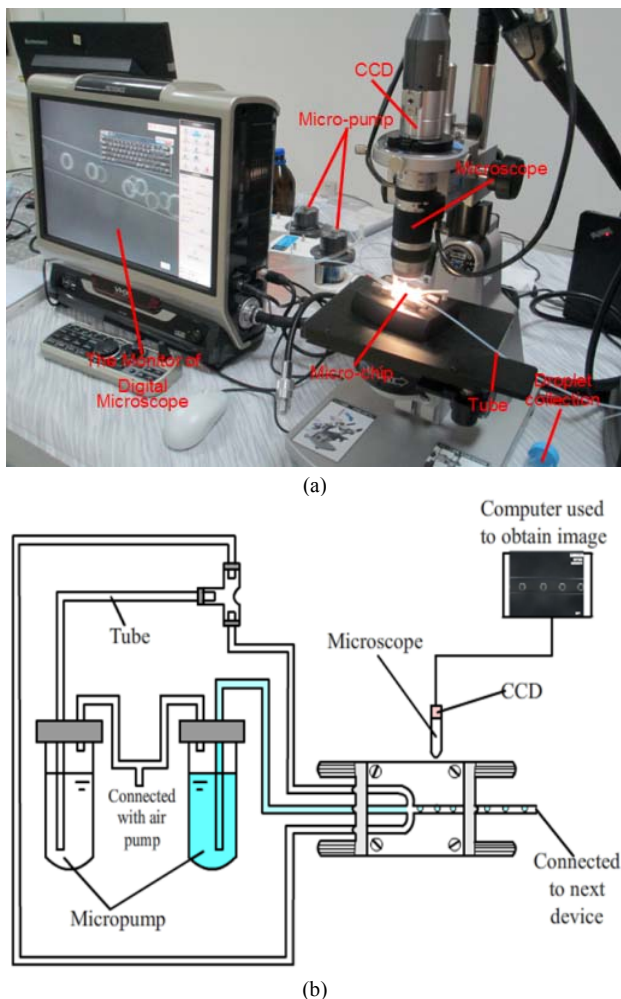


Fig. 1 A picture of the microfluidic system: (a) an actual photograph and (b) a schematic

The integrated microchannel is embedded in chip-groove. Three inlets of microchannel are connected to the pressure pump used as the flow driving pumps by means of

flexible Teflon tubes. The illuminated beam provided by halogen lamp is adjusted by a set of lenses and delivered into microscope.

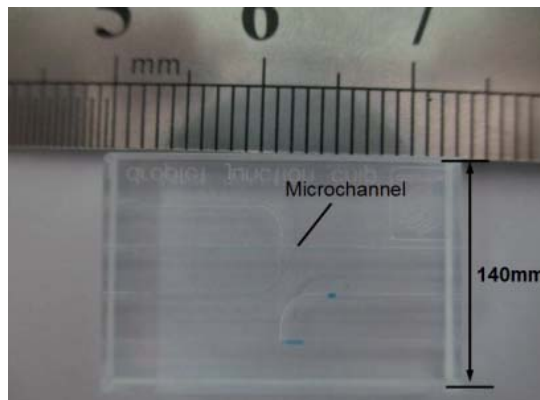


Fig. 2 An actual photograph of micro-chip in the experiment

### B. Image acquisition

Image processing begins with a digital capturing of the image as data. An image comes from an image collection module. The digital microscope system has a light source placed in the bottom of the computer. The computer is connected with the optical microscope by cable. Adjustment to the intensity of the light source is necessary to acquire high quality images. The image acquisition module is a CCD (Charge Coupled Device) image sensor. The sensor photo sensitive surfaced by the spatial distribution of light intensity incident on the information, is converted to an electrical signal timing of the serial output. Under control of the synchronization signal, the CCD camera can read the pre-transfer signal to complete image acquisition.

The size of microchannel is  $190\mu\text{m}$ . The microscope provides different object lens to view microfluid in the microchannel. Firstly, adjust to the focal length of microscope and make the microfluid in microchip clearly show on the monitor of digital microscope. In this module, the computer waits to acquire the image which contains the information of microfluid. The microscope system allows the entire process to be viewed in real time. The image can be acquired when the microfluid is stable and the droplets are uniform. Within a few time after exposure, the image field is transferred to storage pixels on the CCD camera, so that a second image can be recorded. Both images are read out of the CCD camera and download to a computer for processing.

### C. Image processing

The basic of image processing for MATLAB: original image reading—Wiener filtering—erosion—edge detection—diameter and distance extract—using the formula to calculate the required characteristic parameter.

1) *Wiener filtering*: Wiener filtering, which is one of the simple noise reduction methods, was proposed by Rorbert Wiener in 1942. It is an optimal linear least squares filter according to the statistical properties of the useful signal and interference signal and an optimal filter based on the linear minimum mean square deviation estimation principle.

For the acquisition images have noise, signal model is assumed as:

$$a(x, y) = S(x, y) + N(x, y) \quad (1)$$

where  $S(x, y)$  is the true signal,  $a(x, y)$  is the signal recorded with noise,  $N(x, y)$  is Gaussian white noise with mean value of zero, variance is constant  $\sigma_N^2$ . Filter  $\lambda$  that satisfies the mean variance is required.

The mean square error of the signal can be expressed as follows:

$$MSE = E[S(x, y) - \lambda a(x, y)]^2 \quad (2)$$

MSE is required to minimum. Equation (2) can be re-written as follows:

$$\begin{aligned} MSE &= E[S(x, y)^2 - 2\lambda S(x, y)a(x, y) + \lambda^2 a(x, y)^2] \\ &= ES(x, y)^2 - 2\lambda E[S(x, y)a(x, y)] + \lambda^2 Ea(x, y)^2 \end{aligned} \quad (3)$$

Derivation of (3), the result is equal to zero:

$$\lambda = \frac{E[S(x, y)a(x, y)]}{Ea(x, y)^2} = \frac{E[S(x, y)(S(x, y) + N(x, y))]}{Ea(x, y)^2} \quad (4)$$

Since  $S(x, y)$  is independent from  $N(x, y)$  and  $EN(x, y)$  equals zero:

$$\lambda = \frac{E[S(x, y)a(x, y)]}{Ea(x, y)^2} = \frac{ES(x, y)^2}{Ea(x, y)^2} \quad (5)$$

And because:

$$Ea(x, y)^2 = E(S(x, y) + N(x, y))^2 = ES(x, y)^2 + \sigma_N^2 \quad (6)$$

Thus the filter  $\lambda$  is required in (5) can be expressed by:

$$\lambda = \frac{ES(x, y)^2 - \sigma_N^2}{Ea(x, y)^2} \quad (7)$$

The estimated signal is obtained and can be described as follows:

$$b(x, y) = \mu + \lambda(a(x, y) - \mu) \quad (8)$$

where  $\mu$  is the local mean and variance around each pixel.

2) *Image erosion*: The image erosion operation is one of the basic form operations. It means by using some structural elements to detect the image in order to identify the area within it where structural elements can be put down. The structural elements are the basic operators of morphology. The optimal structure elements is selected can affect effectiveness and quality of the image processing in directly.

The binary image is corroded by structural element can be expressed as follows:

$$E = I \ominus S = \{x, y | S_{xy} \subseteq X\} \quad (9)$$

where  $I$  is a binary image,  $X$  is connected region,  $S$  is the structural element,  $S_{xy}$  is  $S$  move to  $(x, y)$  from the origin point. The equation means if  $S$  is completely contained in  $X$ , the point in the image after etching is 1, and 0

otherwise when  $S$  move to  $(x, y)$  from the origin point. E is the results after erosion.

3) *Edge detection*: Canny operator [13] is an edge detection operator with excellent performance and it has been widely used in many image processing areas.

The basic idea is: Firstly, Canny operator chooses certain Gaussian filter to smooth processed image, and then use a technology called "non-extremum suppression". The final edge image is available after processing the certain image.

The finite difference of one-order partial derivative is used on each pixel of the filtered image to calculate the size  $M$  and orientation  $\theta$  of the gradient. That can be expressed as:

$$\phi(x, y) = \sqrt{[\sigma(x, y) * P(x, y)]^2 + [\sigma(x, y) * Q(x, y)]^2} \quad (10)$$

$$\theta(x, y) = \arctan \frac{\phi_2(x, y)}{\phi_1(x, y)} \quad (11)$$

where  $\sigma(x, y)$  is the parameter of smoothing degree,  $P(x, y)$  and  $Q(x, y)$  are the  $2 * 2$  template

The result of  $\phi(x, y)$  is not sufficient to determine the edge. It must be refined to determine the edge amplitude of the gradient, as this will generate refining edges. The refinement of the method is to use the direction of the gradient to fulfill "non-maxima suppression".

The refinement gradient amplitude is:

$$N(x, y) = NMS(M(x, y), N(x, y)) \quad (12)$$

The result is an edge of the image array. There may be false edge array through non-maxima suppression amplitude as threshold. A typical method to reduce the number of false edge section is to use a threshold value on  $N(x, y)$ . Let  $N(x, y) = 0$ , when the true value is lower than the threshold.

4) *Measurement*: The volume of one droplet can be described as follows:

$$V = \frac{4}{3} \pi \left(\frac{d}{2}\right)^3 = \frac{1}{6} \pi d^3 \quad (13)$$

where  $d$  is the diameter of droplets.

### III. RESULTS AND DISCUSSIONS

#### A. The image processing and comparison

A series of benchmark tests are conducted using different method to de-noise. These methods include median filter, average filter, wiener filter, two-dimensional statistical sequential filter and so on. The results are detected by four parameters. There are NMSE (Normalized Mean Square Error), MSE (Mean Square Error), SNR (Signal Ratio) and PSNR (Peak Signal Noise Ratio). They are expressed as:

$$NMSE = 10 \lg \frac{\sum_{x=1}^M \sum_{y=1}^N |a(x, y) - \hat{a}(x, y)|^2}{\sum_{x=1}^M \sum_{y=1}^N |a(x, y)|^2} \quad (14)$$

$$MSE = \frac{1}{M \times N} * \sum_{x=1}^M \sum_{y=1}^N (a(x, y) - \hat{a}(x, y))^2 \quad (15)$$

$$SNR = 10 \lg \frac{\sum_{x=1}^M \sum_{y=1}^N |S(x, y)|^2}{\sum_{x=1}^M \sum_{y=1}^N |N(x, y)|^2} \quad (16)$$

$$SPNR = 10 \lg \frac{(L-1)^2}{MSE} \quad (17)$$

where  $a(x, y)$  is the pixel of original image.  $\hat{a}(x, y)$  is the pixel of the image after de-noising, L is the gray level of image. In general, the value is 0-255. The size of image is  $M \times N$ .

A smaller value of MSE and NMSE means better quality of the processed image. The processed image has smaller noise signals when SNR is bigger. PSNR is greater as the image distortion is smaller. Table I shows the results of four kinds of filters are used.

TABLE I  
DETECTION OF FOUR FILTERS

Method	Median Filter	Average Filter	Two-dimensional Statistical Sequential Filter	Wiener Filter
MSE	29.5037	11.9649	36.5509	3.4276
NMSE	0.0080	0.0033	0.0100	$9.3448 \times 10^{-4}$
SNR	15.0948	19.0144	14.1645	24.4437
PSNR	33.4320	37.2889	32.5018	42.7810

Table I clearly shows that the wiener filter can be modeled accurately by the image, as excellent agreement with the requirement of experiment.

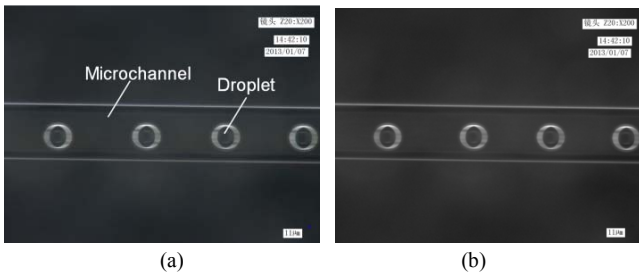


Fig.3 Image recorded by CCD (a) and Image processed by Wiener filter (b)

Fig.3 shows the results at the end of experiment. The results are shown in Fig. 3 b) where it is seen that excellent agreement between the measurements.

### B. The results of image processing

It can only remove the noise which is generated in the process of taking the image by preprocessing image. However, there is a lot of information on the image that does not require during the test. So the image should be processed at a deeper level.

CCD camera of the digital microscope system is not a high-speed acquisition device. There are some smears on the images. Image erosion is used to remove the smear of the image, to display the full round of the droplets, to contract the

target graphics and to eliminate small and medium image meaningless goal. In this experiment, the processed image is circular, so the structural element is spherical. The size of structural element is selected by trial. The optimum size is obtained through comparing with images that are processed by different sizes. Fig. 4 shows the result of erosion.

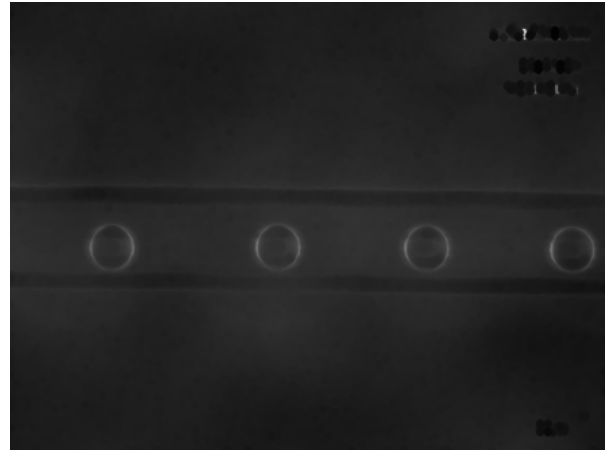


Fig. 4 Image of erosion

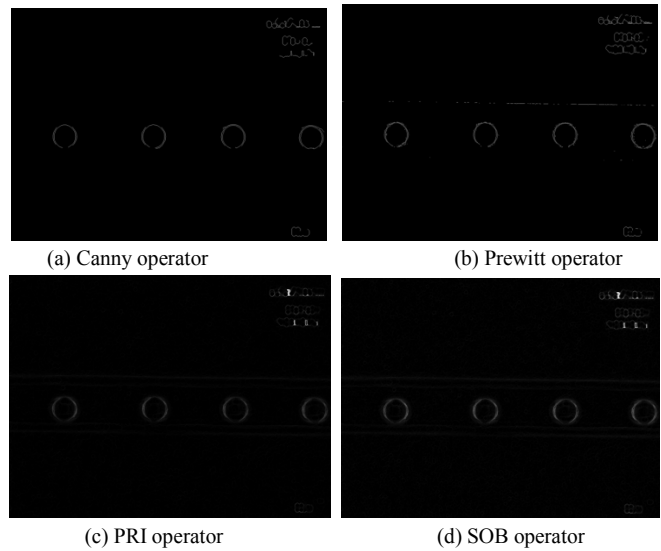


Fig. 5 Image of edge detection by different operator

During the experiment, Canny operator, Prewitt operator, PRI operator and SOB operator are used. The Fig. 5 illustrates the result of edge detection. The result shows Canny operator is more suitable for the image.

The centre coordinate and diameter of circle is measured in the last procedure of image processing. The result is shown in Fig. 6. The volume of droplets is calculated by the captured data of the diameter of circle. Image processing is simple and the result of each step becomes visual with analysis above. Using MATLAB to program throughout the process needs to call MATLAB library a few simple functions which can achieve image processing. This method shows the unique advantages not only to complex image processing but also data calculation. The approach is simple, easy and universal.

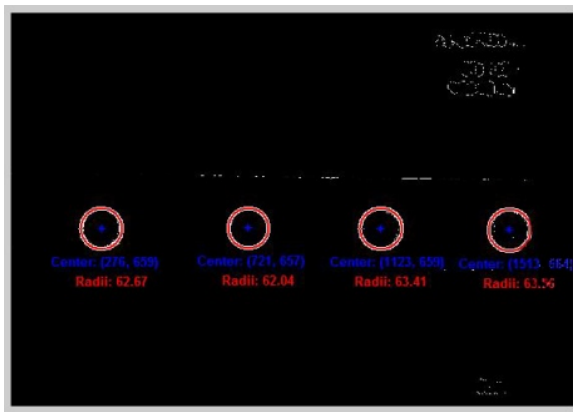


Fig. 6 Measurement of the centre and the diameter of the circle

In the experiment, radii of droplets are measured for 24 times and measurement result data are recorded. Fig. 7 shows the difference of theoretical and measured radius of droplets. The difference between measured and theoretical is very small (Fig. 8). From Fig. 7 and Fig. 8, the data of image processing are close to the true value of droplets and the accuracy can achieve 0.8%. Therefore, the approach can be used to measure the volume of droplets in the microfluidic system.

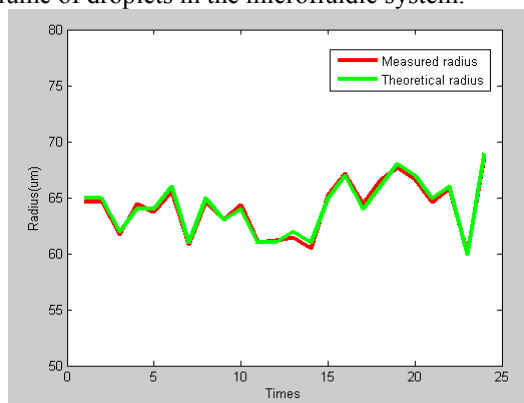


Fig. 7 The measured and theoretical radius of droplets

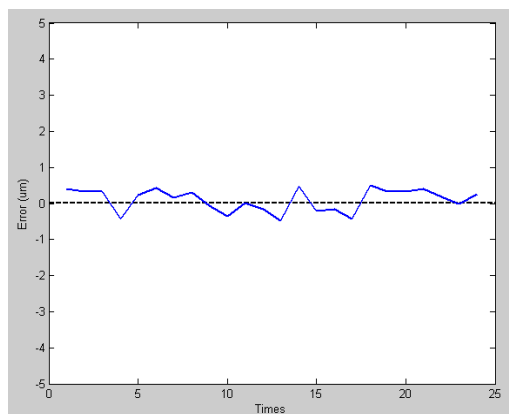


Fig. 8 The difference between measured and theoretical radius of droplets

#### IV. CONCLUSION

The approach described in the statement above can be used to measure the volume of the micro-flow in the microfluidic system. The corresponding numerical simulation

is performed as a comparison. Both experimental and numerical results are in good agreement. The measured results show that the volume of microfluid is easy to be obtained using image processing. In the experiment, the choice of parameters impacts on the effect of image processing directly. Meanwhile, the choice of operator of image processing in each step is important. These two factors result in the enhancement of the image processing in the experiment. The conclusions are helpful to measure the volume of microfluid in new field. The approach can be used in the fields such as pharmacy, biochemistry and analysis.

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