A Surface Acoustic Wave Pumped Lensless Microfluidic Imaging System for Flowing Cell Detection and Counting

Xiwei Huang*, Member, IEEE, Umar Farooq#, Jin Chen, Yakun Ge, Haijun Gao, Member, IEEE, Jiangtao Su, Xiang Wang, Shurong Dong, and Ji-Kui Luo

Abstract—The future point-of-care diagnostics requires miniaturizing the existing bulky and expensive bio-analysis instruments, where Lab-on-CMOS-Chip based technology can provide a promising solution. In this article, we presented a surface acoustic wave (SAW) pumped lensless microfluidic imaging system for flowing cell detection and counting. Different from the previous lensless systems which employ external bulky syringe pump for cell driven, the developed system directly integrate SAW pump on the CMOS image sensor chip to drive the cell-containing microfluid. Moreover, an efficient temporal-differencing based motion detection algorithm is proposed for continuous flowing cell detection and counting. Experimental results show that the SAW pump can drive the cells to flow at different driven powers, also can keep the channel temperature below 40 °C so as not to harm the cells. The human bone marrow stromal cells flowing in the microfluidic channel can be automatically detected and counted with a low statistical error rate of -6.53%. The developed system thereby is competitive for point-of-care cell detection and counting application.

Index Terms—Lab-on-CMOS, lensless microfluidic imaging, surface acoustic wave, temporal-differencing, flowing cell, detection and counting

I. INTRODUCTION

The world population is rapidly aging with the proportion of people aged 60-year-old and over growing faster than any other age groups [1]. Along with the aging society also come special biomedical diagnostic needs and associated challenges. Conventional biomedical diagnostic instruments such as microscopy and flow cytometer for cell detection and counting have improved the early diagnosis and accurate monitoring of existing diseases through blood cell testing [2]. However, these diagnosis systems are usually bulky and sophisticated, hence are expensive, only available in established hospitals or clinics, and require professional personnel to operate. As a result, portable and affordable biomedical devices that miniaturize the traditional bulky and expensive bio-analysis instruments are imperative for the future point-of-care (POC) diagnosis [3-4]. And various meaningful POC diagnostic systems have been developed recently [5-10], such as a smartphone-based electrochemical analyzer and a tumor cell cytometer demonstrated by Guo et al. for biomedical diagnostics.

For bio-analysis instrument miniaturization, one effective solution comes from the development of Lab-on-CMOS-Chip technology, which integrates the mass-manufacturable and low-cost CMOS sensor chip with microfluidic device to form a miniaturized microsystem [11-12]. The advantages of CMOS sensors are that they can be designed to incorporate large numbers of sensing pixels to improve the sensing throughput, and to incorporate multiple biomedical sensing capabilities such as optical or electrochemical sensing [13-16]. Microfluidics, which deals with the manipulation of fluidic samples at microscale volume, are just suitable for preparation and delivery of biomedical samples such as cells or bacterium in an aqueous environment to CMOS sensing sites [17-19]. In addition, microfluidic package can encapsulate and protect other electrical part of the CMOS sensor chip. Therefore, microfluidics-based Lab-on-CMOS-Chip is promising to tackle the bio-instrument miniaturization challenges.
One recent actively investigated Lab-on-CMOS-Chip example is the lensless microfluidic imaging system, which attaches one poly-dimethylsiloxane (PDMS) microfluidic channel on top of a CMOS image sensor (CIS) [20-22], as shown in Fig. 1(a). When a light source illuminates above the channel, the projected shadow images of flowing samples in the channel are recorded by the CMOS sensor underneath without magnification due to any lens elements. For lensless microfluidic imaging, the light intensity and contrast of one cell’s shadow image are determined by the distance $D_{obj}$ from the object to the pixel array plane, as shown in Fig. 1(c). Shorter object distance provides better contrast and resolution due to less diffraction effect [23]. Therefore, to achieve better imaging quality, the distance between cell samples and the image sensor pixels should be reduced in a microfluidic imaging system.

A variety of works have investigated such lensless microfluidic imaging system for different applications such as bacteria imaging and blood and tumor cell cytometry [24-26]. However, most of the previous works only focused on the design and optimization of the core components, i.e., CIS and microfluidic chip. While the fluidic delivery and control part, albeit indispensable, still employs external bulky syringe pump as shown in Fig. 1(b). This leads to problems such as large dead volume and excessive waste of bio-samples and reagents which are usually precious in biological analysis. The syringe pump cannot be miniaturized and integrated with the microsystem, causing the whole system not actually fit for POC.

The most widely used micropumps in microfluidic systems are mechanical and electrical types. But mechanical pumps usually need high power and are easy to fail due to fatigue issues. Recently, one electric type of micropumps are rapidly developing which employs surface acoustic wave (SAW) generated on a piezoelectric substrate as the actuation force [27-31]. When an alternating (AC) electric field is applied to a piezoelectric material, mechanical waves are generated that propagate in the substrate or on the surface of the materials as shown in Fig. 1(c). When acoustic wave generated by the IDT meets a liquid droplet on its wave path, the momentum and energy of the acoustic wave are coupled to the droplet. If the substrate surface is hydrophilic and the acoustic pressure is sufficiently large, it will push the liquid forward. In a continuously flowing system, if the channel width is smaller than the vortices of the acoustic streaming to suppress the reverse flow, then the acoustic pressure will pump the liquid to form a continuous flow. With the SAW based micropump integrated, a miniaturized and more compact bio-instrument can be developed.

In this article, we introduce a surface acoustic wave pumped lensless microfluidic imaging system targeted for automated and high-throughput cell detection and counting in continuous microfluidic flow. A SAW-based micropump is developed and integrated beside the CMOS image sensor to deliver microfluidic sample into the sensing pixel array area with steady flow. As the cell motions in consecutive frames are affected by both the microfluidic flow rate and sensor frame rate, one rapid temporal-differencing based background subtraction technique is deployed for cell motion detection. The lensless microfluidic imaging system is fabricated with successful detection and counting for the flowing human bone marrow stromal cells. During SAW pumping, the channel fluidic temperature is kept below 40 °C so as not to harm the cells. The system is verified by a number of experiments with an error rate of -6.53%. This is the first lensless microfluidic imaging system integrated with SAW based micropump for flowing cell detection and counting, demonstrating the potential for portable POC biomedical diagnosis application.

The remaining part of this paper is organized as follows. Section II overviews the SAW pumped lensless microfluidic imaging system. Section III describes the SAW device. Section IV presents the cell detection and counting processing. Section V shows the system setup and experimental results with discussions. Finally, Section VI presents the conclusions.

II. SAW PUMPED LENSSLESS MICROFLUIDIC IMAGING SYSTEM

The SAW pumped lensless microfluidic imaging system can be realized in a miniaturized and portable fashion as shown in Fig. 2. It is composed of one PDMS microfluidic chip integrated on top of a lithium niobate (LiNbO3, LN) substrate. An IDT for SAW generation is fabricated on the same LiNbO3 substrate beside the microfluidic chip. The substrate is further attached on one CIS chip, where the microfluidic channel is aligned over the sensor pixel array so that the shadow images of flow-through cells can be projected to the sensor. The CIS and SAW device are mounted on a printed circuit board (PCB) which provides power supply and timing controls to the CIS and SAW device. Hence, the CIS can continuously capture the projected shadow images with SAW based fluidic pumping. No more external bulky syringe pump or long tubing is used. These captured cell images are then rapidly processed by motion detection algorithms for detection and counting.

![Fig. 2. (a) Cross-sectional diagram of the lensless microfluidic imaging system with a SAW based fluidic pumping, (b) microphoto of the developed SAW pumped lensless imaging system built on a custom-designed PCB.](image-url)
III. SAW DEVICE

A. Theoretical Analysis

The SAW-based micropump device was designed to generate the Rayleigh wave propagation on LiNbO$_3$ substrate surface. Rayleigh wave can transport the fluid or mass with their propagation along the surface due to its longitudinal displacement [31-35]. Rayleigh wave velocity $\zeta$ can be calculated by Rayleigh wave velocity equation

$$\zeta = \frac{C_T}{C_L} = \frac{1-2\nu}{\sqrt{2(1-\nu)}}$$

where $C_T$ and $C_L$ are the speed of longitudinal and shear waves, respectively, $\nu$ defines the Poisson’s ratio.

There is a contact angle when the fluid is in contact with the substrate surface. That angle depends on the surface hydrophilic properties. LiNbO$_3$ is hydrophilic with a contact angle smaller than 90º with water, depending on the surface treatment. When the IDT generates surface acoustic waves on the surface, the energy and momentum are transferred at the Rayleigh angle. According to Snell’s law of refraction, the Rayleigh angle can be calculated by

$$\theta = \sin^{-1}\left(\frac{v_l}{v_s}\right)$$

where $v_l$ and $v_s$ are the velocity in liquid and solid medium.

Acoustic pressure gradient $P_{saw}$ can drive the liquid or mass in the direction of surface acoustic waves as

$$P_{saw} = \rho_m V_s^2 \left(\frac{\Delta \rho}{\rho_m}\right)$$

where the $\rho_m$ is the density of the liquid on surface and $\Delta \rho$ the density change under acoustic pressure.

The fluid in the reservoir of the micropump can “feel” the acoustic pressure in the SAW propagation direction, pushing the fluid forward continuously, thus continuous flow is generated in fluid, as schematically shown in Fig. 3. In this figure, the x-component shows the displacement of fluid due to acoustic pressure in a specific acoustic direction along with Rayleigh waves and also the fluid bear acoustic streaming force on the substrate surface.

B. SAW Device Design

Based on the above analysis, the simulation and design of one-port SAW devices were conducted using the COMSOL Multiphysics software. For the model, aluminum (Al) was used as IDT electrode, one two-side polished Y-Cut LiNbO$_3$ was used as the piezoelectric substrate. The thickness of the LiNbO$_3$ substrate and Al electrode are 500 µm and 100 nm, respectively, while the wavelength, $\lambda$, of the SAW pump is 200 µm. The displacement of the LiNbO$_3$ surface along the x-component and the impedance spectrum (Y11) obtained from the simulation are shown in Fig. 4 and Fig. 5, respectively. The resonant frequency can be calculated as:

$$F_r = \frac{V_s}{\lambda}$$

where $V_s$ is the sound velocity in the substrate. $F_r$ is 19.43 MHz, in agreement with that of the fabricated SAW devices.

A 2D COMSOL Multiphysics model that consists of a one port SAW resonator was built to study changes to the wave propagation characteristics resulting from alterations to mechanical properties inside the microchannel of micropump. A simplified 2D cell model of LiNbO$_3$ SAW IDT was built to obtain the resonance frequency shifts by the eigen frequency module of the COMSOL software. The 2D cell was set to be periodic to simulate the entire SAW device with semi-infinite boundary. Fig. 6 shows the one wavelength cell of the simulation model with one pair of IDT finger. The IDT of the SAW device has 50 pairs of fingers and 10 pairs of reflector fingers, both with $\lambda = 200$ µm. Two IDT fingers are illustrated where one of them was grounded. From this simulation, we obtained the resonance frequency of the Rayleigh wave and the
potential stress along the substrate surface. Fig. 7 shows the stress at the LiNbO$_3$ surface due to the wave propagation, and the change of potential across the IDT can increase the stress and control the flow pressure.

Fig. 6. Total displacement of wave propagation at the surface of LiNbO$_3$ excited by an AC electric field through the IDT on the surface.

Fig. 7. Stress on LiNbO$_3$ piezoelectric surface due to wave generation induced by an AC electric field through the IDT.

IV. TEMPORAL-DIFFERENCING BASED CELL DETECTION AND COUNTING

One challenge for continuous flowing cell detection and counting is the motion detection by detecting the intensity difference of cells in contrast to channel background [36]. As cells are usually with certain transparency, their images cannot show high intensity difference, i.e., contrast, against the background. The contrast is affected by background brightness and darkness variation. In this work, an efficient temporal-differencing based motion detection technique is developed for the flowing cell detection and counting in a series of frames [36-37]. Considering the intensity characteristics of cell images, there are two steps involved. Firstly, motion detection is performed for all the cells in each frame. Next, the total cell counting is performed based on the detected moving cell counts in each frame. More detailed discussion is presented below for each step. Note that all the symbols and terms used for the system analysis are summarized in Table I.

TABLE I

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Parameter</th>
<th>Symbol</th>
<th>Parameter</th>
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<tbody>
<tr>
<td>$R$</td>
<td>Pump flow rate</td>
<td>$V$</td>
<td>Microfluidic flow speed</td>
</tr>
<tr>
<td>$W$</td>
<td>Microfluidic channel width</td>
<td>$H$</td>
<td>Microfluidic channel height</td>
</tr>
<tr>
<td>$L$</td>
<td>Microfluidic channel length</td>
<td>$f_i$</td>
<td>Current frame</td>
</tr>
<tr>
<td>$f_{i,\text{ref}}$</td>
<td>Reference frame</td>
<td>$\Delta t$</td>
<td>Temporal difference</td>
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<td>$F_m$</td>
<td>Sensor frame rate</td>
<td>$m$</td>
<td>Total horizontal pixel number</td>
</tr>
<tr>
<td>$n$</td>
<td>Total vertical pixel number</td>
<td>$I$</td>
<td>Cell shadow image intensity</td>
</tr>
<tr>
<td>$\Delta I_B$</td>
<td>Background intensity variation</td>
<td>$x$</td>
<td>Horizontal pixel coordinate</td>
</tr>
<tr>
<td>$y$</td>
<td>Vertical pixel coordinate</td>
<td>$D_i$</td>
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</tr>
<tr>
<td>$D_t$</td>
<td>Temporal intensity difference</td>
<td>$D_i'$</td>
<td>Negative intensity difference</td>
</tr>
<tr>
<td>$B_r$</td>
<td>Binarized difference image</td>
<td>$T_r$</td>
<td>Cell image intensity threshold</td>
</tr>
<tr>
<td>$T_r$</td>
<td>Cell pixel removal threshold</td>
<td>$N_i$</td>
<td>Frame cell count</td>
</tr>
<tr>
<td>$C$</td>
<td>Cell centroid coordinate</td>
<td>$k$</td>
<td>$k^{th}$ cell in frame $f_t$</td>
</tr>
<tr>
<td>$N$</td>
<td>Total number of cells</td>
<td>$M$</td>
<td>Total number of images in a test</td>
</tr>
<tr>
<td>$P$</td>
<td>System cell throughput</td>
<td>$\rho$</td>
<td>Cell concentration in the test reagent</td>
</tr>
</tbody>
</table>

A. Cell Detection

Before cell counting, all the flowing cells in each single frame should be detected in the first step. This requires a motion detection realized through temporal-differencing based background subtraction. It detects moving cell regions using the pixel-by-pixel intensity difference of each two consecutive frames in which one as a reference frame and one as the current frame. These differences can be caused by the motion of cells in the channel, or the addition and removal of cells from the sensor field-of-view (FOV). The procedure for cell detection is shown in Fig. 8.

At first, the cell images are loaded for detection. Fig. 8 shows one microfluidic channel with cell flowing inside driven by SAW. Note that one full frame includes $m(H) \times n(V)$ pixels corresponding to the sensor array size. But Fig. 8 shows only part of the frame with one cell in each channel in order to make the small cell clear to see.

One cell flowing from the left to the right is captured at two different places in two consecutive frames, $f_{i,\text{ref}}$ and $f_i$. Here, $f_{i,\text{ref}}$ is the reference frame (or background frame) and $f_i$ is the current frame as shown in Fig. 8 (a) and (b), respectively. The temporal difference between the two frames $f_{i,\text{ref}}$ and $f_i$ is $\Delta t$, which is determined by the CMOS image sensor frame rate $F_m$, i.e., $\Delta t = 1/F_m$. Assume the system is working under a certain white light illumination, the current frame can be defined as

$$f_i = I(x, y) + \Delta I_B(x, y) \mid x \in m, y \in n$$  \hspace{1cm} (5)

where $I(x, y)$ represents the part of pixel intensity caused by cell shadows for the current frame $f_i$ at pixel coordinate $(x, y)$, and $\Delta I_B(x, y)$ represents the background intensity variations that is determined by the illumination as well as the possible stains from the channel or sensor surface.

Due to cell transparency, cell images captured by the lensless microfluidic imaging system show a characteristic of brightness at the center and darkness at the cell boundary as
shown in Fig. 3(h). This means that the pixels in the center have higher intensity levels than the background, and those at the boundary have lower intensity levels than the background. By performing background subtraction, one can obtain a temporal-differencing image

\[ d_t = f_t - f_{t-\Delta t} \]

and its corresponding intensity matrix is

\[ D_t(x, y) = \left[ I_t(x, y) + \Delta I_t(x, y) \right] - \left[ I_{t-\Delta t}(x, y) + \Delta I_t(x, y) \right] \]

\[ = I_t(x, y) - I_{t-\Delta t}(x, y), \]  

(6)

where \( D_t(x, y) \) is the intensity difference between the two consecutive frames at the same location \((x, y)\). Now in \( d_t \), only the two regions where cell existed in \( f_t-\Delta t \) and \( f_t \) are clearly shown (Fig. 8(c)), and the other background regions are all subtracted to zero. Hence the background variation effect is reduced. The difference between these two cell regions is that the region corresponding to the cell in current frame \( f_t \) still shows brightness in the center and darkness at the boundary as shown in Fig. 8(i); while the region caused by the cell in previous frame \( f_t-\Delta t \) is reversed, where brightness is at the boundary and darkness is in the center as shown in Fig. 8(j). This cell shadow in Fig. 8(j) becomes an artificial ‘tail’ following the flowing cell as shown in Fig. 8(g).

To detect the actual cell but not the ‘tail’ cell in current frame, the ‘tail’ needs to be removed, which can be simply achieved by setting the regions of \( D_t(x, y) \) to zero where they were larger than zero, i.e.,

\[ D'_t(x, y) = \begin{cases} 
D_t(x, y), & \text{if } D_t(x, y) > 0 \\
0, & \text{if } D_t(x, y) \leq 0 
\end{cases} \]  

(7)

Here \( D'_t(x, y) \) is updated from \( D_t(x, y) \) based on the above removal with the corresponding image shown in Fig. 8(d). One can observe that \( D'_t(x, y) \) has only zero and negative intensity values compared to \( D_t(x, y) \).

Next, by further comparing with a cell image intensity threshold \( T_i \), image binarization can be realized by

\[ B_t(x, y) = \begin{cases} 
1, & \text{if } D'_t(x, y) < T_i \\
0, & \text{otherwise} 
\end{cases} \]  

(8)

where \( B_t(x, y) \) is the binary difference image (Fig. 8(e)) corresponding to \( D'_t(x, y) \). The choice of \( T_i \) depends on the comparison between the cell boundary intensity levels against the background level after subtraction. Fig. 9 shows the intensity distribution for the two cell regions in \( D'_t(x, y) \). Fig. 9(a) corresponds to the cell in current frame \( f_t \) that needs to be counted, and Fig. 9(b) corresponds to the fake ‘tail’ cell that needs to be removed. By comparing the intensity distribution of a number of cells in different frames, \( T_i = -15 \) is chosen in this paper that can allow more pixels of actual cell images but fewer pixels of ‘tail’ cell images to be binarized to ‘1’ (white).
white circle represents the actual cell in current frame \( f_l(x, y) \) (Fig. 8(l)), and a small region of white brick represents the 'tail' cell in previous frame \( f_{l-1}(x, y) \) (Fig. 8(k)). All these regions are labeled and their pixel counts can be thereby obtained. The pixel counts presenting the area of white regions are compared with a predefined removal threshold \( T_r \). If the pixel counts are smaller than \( T_r \), the corresponding white region can be concluded as the 'tail' cell region, and thus it will be removed (Fig. 8(f)). Similarly, based on our experiment setup, \( T_r = 8 \) is obtained in this paper. As such, an accurate and efficient flowing cell detection procedure can be realized for the developed SAW pumped lensless microfluidic imaging system.

### B. Cell Counting

After cell detection of each frame is done, the count \( N_t \) of cells and their centroid coordinates \( C_t(k, x, y) \), \( 0 \leq k \leq N_t \), can be obtained, where \( k \) represents the \( k \)-th cell in the frame \( f_l \). To count the total number of cells flowing through the microfluidic channel taken by a series of images, \( N_{f_{l-1}} \to f_{f_{l+M}} \), where \( M \) is the total number of images for one test with \( M/\Delta t \) duration, a summation of positive temporal difference for all the frames is conducted.

Similarly, two frames \( f_{l-1} \) and \( f_l \) in one series of images are being processed, and the numbers of cells in these two frames, i.e., \( N_{f_{l-1}} \) and \( N_f \), have already been obtained after the cell detection step. To obtain the total number of flowing cells, we perform the temporal subtraction \( (N_f - N_{f_{l-1}}) \) to calculate the new cells coming into the microfluidic channel in the current frame \( f_l \). Compared with previous frame \( f_{l-1} \), cells in the current frame \( f_l \) can have three situations to explore: 1) new cells flow into the sensor FOV to increase the cell count over \( N_{f_{l-1}} \); 2) existing cells flow out of the sensor FOV to reduce \( N_{f_{l-1}} \); 3) no new cells flow into or no existing cells flow out. Therefore, only the positive temporal cell count difference is meaningful to be added to actual total cell number \( N \). After processing each new frames in one test, the final total cell count \( N \) is obtained by adding all these positive cell count differences in the consecutive frames, \( \sum_{t=2}^{M} (N_t - N_{t-1}) \), if \( N_t - N_{t-1} > 0 \).

As such, one can obtain the number \( N \) for total cells that flow through the microfluidic channel captured by the series of images in one test.

### V. EXPERIMENTAL RESULTS

#### A. System Setup

The prototype lensless microfluidic imaging system with SAW based pumping is shown in Fig. 2(b). One 128°Y cut two-side polished transparent 5-inch LiNbO₃ wafer for wave propagation in the Y-direction (3800 m/s) was used as the substrate. An IDT with a tilt angle of 10° with reference line perpendicular to the channel was patterned and formed by standard photolithography and lift-off procedure. Once confirmed the SAW device was working, the transparent SAW pump chip was mounted on the CIS chip for lensless detection, and electrical connections were made from wires with IDTs by silicon paste.

One grayscale CMOS image sensor (Aptina MT9V032, San Jose, CA) with a pixel size of 6μm × 6μm is used for imaging. Its pixel array size is 752(H) × 480(V) and active sensing area is 4.5mm(H) × 2.9mm(V). The sensor frame rate is 60 frames/second (fps) at its full resolution.

The PDMS microfluidic channel was fabricated by soft-photolithography [38] and replica molding method [39] using a 5-inch silicon. The channel features were first designed in AutoCAD (Autodesk, San Rafael, CA) and then written to a film mask. Negative photosist SU-8 (SU-8 3050, Microchem, MA) was spin-coated on a glass slide and subsequently through soft baking. After UV light exposure, the developing and silanization were performed with post-baking. Then the SU-8 master was fabricated on a glass slide. Next, a volumetric ratio of 10:1 mixture of PDMS (Sylgard 184, Dow Corning, MI) and curing agent are poured onto the SU-8 master. After degassing and curing, the PDMS replica was peeled off from the master and punched on top for inlet and outlet. To fully use the active pixel area, the channel length was selected as 4.5mm. The channel width was 1mm so that cells can flow in a straight and predictable manner in the channel. The height of the sensor was 30μm as it is just larger than the normal cell diameter. The surfaces of the microfluidic channel and the LiNbO₃ substrate were then cleaned with oxygen plasma, and finally, they were bonded together.

Before integration with the SAW device, the sensor protection glass was removed to reduce the object distance and hence improve the imaging contrast. To make it easy to integrate with SAW device, one 6 cm × 6 cm supporting PCB was designed with only one CIS chip on the top surface, while all the other components were placed on the bottom surface. The system PCB 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, cell images and counted cell numbers in the sample can be displayed.

#### B. Sample Preparation

After the SAW pumped lensless microfluidic imaging system prototype was developed, human bone marrow stromal cells (American Type Culture Collection, MD) were used for the experiments. They were cultured in Minimum Essential Media (MEM) (Gibco, cat# 11095-080) supplemented with 10% fetal bovine serum (FBS) (Gibco, cat# 10270-106), 1 mM sodium pyruvate (Gibco, cat# 11360-070), 0.1 mM MEM non-essential amino acids (Gibco, cat# 11140-050), and grown at 37°C under a 5% CO₂ atmosphere in a T75 flask.

#### C. SAW Device Characterization

The one-port SAW devices were characterized by a vector network analyzer E5071C (Agilent) with and without PDMS channel and fluid loaded. For testing, the ethanol alcohol was pass through the micropump PDMS channel for removing air
from microchannel because ethanol viscosity is more than DI water, can remove stain particles and not make bubbles inside the microchannel. The reflection spectra (S11) with off load and on load are shown in Fig. 10. A signal generator (SP2461) combined with an amplifier were used to drive the SAW device.

Furthermore, the SAW device temperature response was measured by infrared Camera (Fluke ti25, USA) at different power of SAW device. Fig. 11 show the low and high power temperature responses in the microfluidic channel obtained from Fluke smartview software connected with Infrared camera. The device is driven by one low power at 26dBm and one high power at 37dBm. The results indicate that even at high power supply, the temperature in the channel can still be kept lower than 40 °C, which will not harm the physiological status of mammals live cell samples.

D. Results of Cell Detection and Counting

To validate the function of the SAW pumped lensless microfluidic imaging system on cell detection and counting, human bone marrow stromal cells were tested. The SAW IDT device was driven at two different power, +26dBm and +37dBm, so the cells were flowing at different flowrates. The CIS is set to work at 16 fps frame rate to continuously capture and output the lensless cell images to external PC for processing. Each experiment group captures 64 lensless images in 4 seconds. By measuring the flowing distance of the same cell in two consecutive frames, the flow rate of 0.3μl/min and 1.3μl/min were obtained. The captured raw lensless images of cells before doing background subtraction are shown in Fig. 12, which presents a similar annulus intensity pattern of high intensity at the center and low intensity at the boundary as the microscope cell image in Fig. 8g.

The automated counting results were shown in Table II. By analyzing manually all the cells flowing through the channel in each group of 64 frames, the counting results are obtained for comparison, which can be assumed as accurate. For automated counting, the average cell throughput at a low flow rate of 0.3μl/min is 6 cells/second and the error rate is -1.6%; at a high flow rate of 1.3μl/min, the average cell throughput is 12 cells/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 cell detection and counting. The error rate increased with flow rate, due to the misdetection of some cells with low contrast or noise. This can be improved by a better system integration to shorten the distance between cell and sensor.

This is a prototype detection system with many to improve. The throughput for the system is rather low compared to others, and this can be improved by integrating multi-channels in parallel. The resolution of the images is low at the moment, this can be improved by image processing algorithms such as super-resolution.

<table>
<thead>
<tr>
<th>Flow Rate</th>
<th>Group</th>
<th>Manual Count</th>
<th>Auto Count</th>
<th>Error Rate*</th>
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<td>0.3μl/min</td>
<td>1</td>
<td>16</td>
<td>16</td>
<td>0.00%</td>
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<tr>
<td></td>
<td>2</td>
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<td>9</td>
<td>45</td>
<td>45</td>
<td>0.00%</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>50</td>
<td>51</td>
<td>2.00%</td>
</tr>
<tr>
<td>Average2</td>
<td>51.6</td>
<td>47</td>
<td></td>
<td>-8.91%</td>
</tr>
<tr>
<td>Total Average</td>
<td>76.6</td>
<td>71.6</td>
<td></td>
<td>-6.53%</td>
</tr>
</tbody>
</table>

*Error Rate = (Auto Count - Manual Count)/Manual Count
VI. CONCLUSIONS

A miniaturized lensless microfluidic imaging system with SAW based pumping has been developed targeting the future point-of-care diagnostics applications. The developed system directly integrates a SAW pump on the CMOS image sensor chip to drive the microfluidic flow so that the previous bulky syringe pump can be removed. In addition, an efficient temporal-differencing based motion detection algorithm is proposed for continuous flowing cell detection and counting. Experimental results show that the SAW pump can drive the cells to flow with different driven powers, meanwhile keep the channel temperature below 40 °C so as not to harm the cells. The human bone marrow stromal cells flowing in the microfluidic channel can be automatically detected and counted with a low statistical error rate of -6.53%. This is the first lensless microfluidic imaging system integrated with a SAW based micropump for flowing cell detection and counting towards portable POC biomedical diagnosis.

REFERENCES


