

Towards Automating Micro Cellular Detection Process Using Micro Vortex Pump Arrays

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Abstract—This paper reports a polymer based microfluidic analysis system integrated with a surface plasmon resonance (SPR) biosensor for detecting the specific binding of biomolecules and qualitatively monitoring of cell adhesion on the sensor surface. Micropumps, microchannels, and SPR biosensor were integrated into a single polymer (PMMA) based microfluidic system. The integrated system has been studied in its potential application in bio-molecules detection and drug discovery. Two experiments, 1) monitoring the reaction between BSA-BSA antibody, and 2) monitoring the activities of living cells in the presence or absence of trypsin in RPMI-1640 medium, were conducted to show the feasibility of real-time cellular and molecular detection. Based on these successful experimental results we have also developed a computer-controllable vortex micropump system that will eventually automate many bio-molecular detection and drug discovery processes.

I. INTRODUCTION

The research on microfluidics involves the development of miniaturized devices, miniaturized systems, and applications related to the handling of fluids. Over the past ten years, microfluidic lab-on-chip system has been rapidly developed from early single channel devices [1] to current complex analysis systems [2]. The rapid growth of this field is partly due to the rapid developments in MEMS devices, such as micropumps, micromixers, and biosensors, as well as the need for high throughput biomedical analysis and drug delivery system. New generations of automated microfluidic devices have made it possible to achieve biomedical instruments with new levels of performance and capability. Moreover, in the area of biosensors, surface plasmon resonance (SPR) has become a leading technology in biological and chemical sensing because of its real-time and label-free detection capabilities for bio-molecules [3]. Recently, we have proposed a new SPR biosensor design based on measuring the differential optical phase between the

s and p polarizations [4]. The new technique offers a better sensitivity because of its effectiveness in minimizing common-mode noise through the use of differential measurement.

In this paper, a fully automated microfluidic system integrated with a phase-sensitive SPR biosensor is presented. The system consists of three vortex micropumps [5] and an SPR biosensor head [4]. The entire microfluidic system can be fabricated using a low-cost micro-molding replication technique. The working principal of the vortex micropump is based on the vortex flow generation inside the pump chamber by an SU-8 fabricated micro impeller. From our pervious experiments, the vortex micropump can generate a flow rate from 0.11 to 9.5ml/min at an applied voltage from 0.6 to 2.5V with linear relationship. Integrating three vortex micropumps and an SPR biosensor into a single chip, an automated fluid manipulation and bio-detection system can be achieved. The micropumps are software-controlled to pump different solutions sequentially into the SPR biosensor head. For the SPR sensor, results from our pervious work [4] using glycerin-water mixtures indicate that the sensitivity limit of our design can be as high as 5.5×10^{-8} refractive-index units. Such an improvement in the sensitivity limit should put SPR biosensors as a possible replacement of conventional biosensing techniques that are based on fluorescence. We have used our experimental setup, which has real-time phase extraction and software control capabilities, to perform experiments including monitoring of the bovine serum albumin (BSA) binding reaction with BSA antibodies and cell adhesion properties under the influence of trypsin.

II. EXPERIMENTAL SETUP

A. Surface Plasmon Resonance

A prism-coupled Kretschmann scheme depicted in Figure 1 is often used in SPR sensor system. The surface plasmon wave (SPW) vector (k_{sp}) between the metal and dielectric medium can be expressed as:

$$k_{sp} = k_o \sqrt{\frac{\epsilon_{metal} \epsilon_{sample}}{\epsilon_{metal} + \epsilon_{sample}}} \quad (1)$$

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where k_o is the free space wave vector of the optical wave, ϵ_{metal} and ϵ_{sample} are the complex dielectric constants of the metal and the sample medium respectively.

The enhanced wave vector of incident light (k_x) is given by:

$$k_x = k_o n_{\text{glass}} \sin \theta_{\text{inc}} \quad (2)$$

where n_{glass} is the refractive index of the prism, θ_{inc} the angle of incidence.

For SPR to take place, which leads to the strongest SPW, the two vectors should be matched. i.e.

$$k_x = k_{\text{sp}} \quad (3)$$

The simplest way to express the phase property of SPR is to use Fresnel equation, the reflection coefficients of the p- and s-polarized light (r_p and r_s), which are given by [7]:

$$r_p = |r_p| e^{i\phi_p} \quad \text{and} \quad r_s = |r_s| e^{i\phi_s} \quad (4)$$

Across the resonant peak, the phase angle will exhibit a steep change, which means that a small variation of ϵ_{sample} or refractive index of sample will lead to a large phase change in the reflected light. Due to the fact that SPR effect will only affect p-polarized light, the value of phase different ($\Delta\phi$) between p-polarization and s-polarization ($\phi_p - \phi_s$) can be used as a refractive index probe on the sensor surface (for detailed explanation, see [4]).

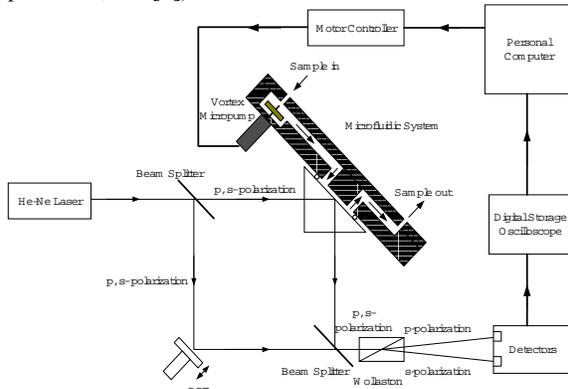


Figure 1. Experimental setup of the microfluidic system integrated with the SPR biosensor.

B. Microfluidic pump

In our automated microfluidic system, fluid flow is driven by vortex micropumps. Detailed fabrication and modeling results developed by our group have been presented in [5] and [6]. The vortex micropump uses kinetic energy to move fluid through the use of an impeller and a circular pump chamber. The basic design concept is illustrated in Figure 2. As fluid enters the pump near the center of the impeller, it is moved towards the outer diameter of the pump chamber by the rotating motion of the impeller. The confinement provided by the pump chamber forces the fluid to enter the microchannel and a pumping flow is thus created. Since the generation of pumping flow is due to the rotating motion of the impeller, the pumping flow rate can be controlled smoothly by changing the rotational speed of the impeller. This allows

digitally controlled fluidic manipulation through straightforward control software.

As mentioned earlier, microfluidic systems are required to be optically transparent and bio-compatible for bio-optical detection and chemical applications. For our vortex micropump, we choose polymethyl methacrylate (PMMA) to be the structural material. The SU-8 micro impeller is placed inside the pump chamber. When the fluid enters the micropump from the center of impeller, the rotational motion of impeller, driven by a DC motor, can induce a fluid pressure gradient and thus create a continuous flow. In our vortex pump design, two structural layers are needed. The lower layer includes pump chamber and microchannel, while the upper layer is a cover layer providing fluidic connection. A completed vortex micropump with microchannel is shown in Figure 3. The diameter of pump chamber is 5mm. The fluid is pumped through an output microchannel of 300 μm in width and 200 μm in depth.

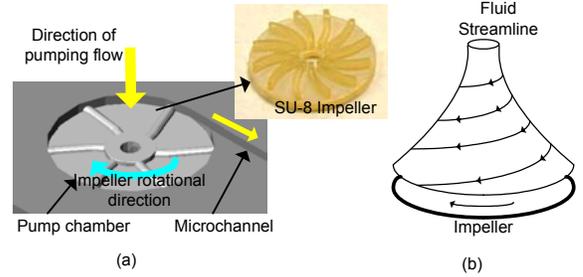


Figure 2. (a) Illustration of the vortex micropump working principle. (b) Fluid streamline inside the pump chamber.

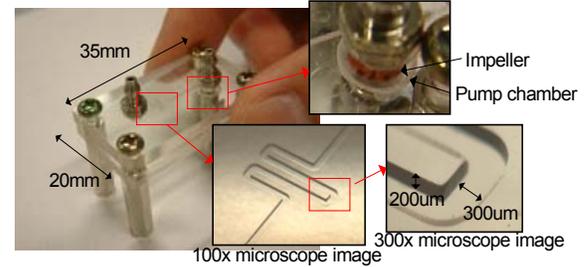


Figure 3. Photo of a vortex micropump with microchannel. The chip size is 20mm \times 35mm. The diameter of pump chamber is 5mm. The output microchannel is 300 μm in width, 200 μm in depth.

Experimental and simulation results on the flow rate and pump pressure as functions of rotational speed of the impeller are shown in Figure 4. The pump performance of two different sizes of the pump chamber, which are 3mm and 5mm in diameter, feeding identical output microchannels are also compared. Our results confirm that the fluid flow rate and pump pressure are proportional to the impeller rotational speed. This also means that the flow rate and pump pressure increase linearly with the DC voltage applied to the motor. From the comparison between two different pump chamber sizes, we found that the larger pump chamber can produce higher fluid flow rate and pressure level. Our experimental

data shows that the minimum pump rate and pump pressure are 0.11ml/min and 166Pa at the startup voltage (0.75V) of the DC motor, respectively. The maximum flow rate and pump pressure are 9.5ml/min and 8000Pa at the applied voltage of 2.5V. We have derived an analytical model to approximate the vortex pump performance using the Hagen-Poiseuille equations [6]. The simulation results are compared to experimental results in [4].

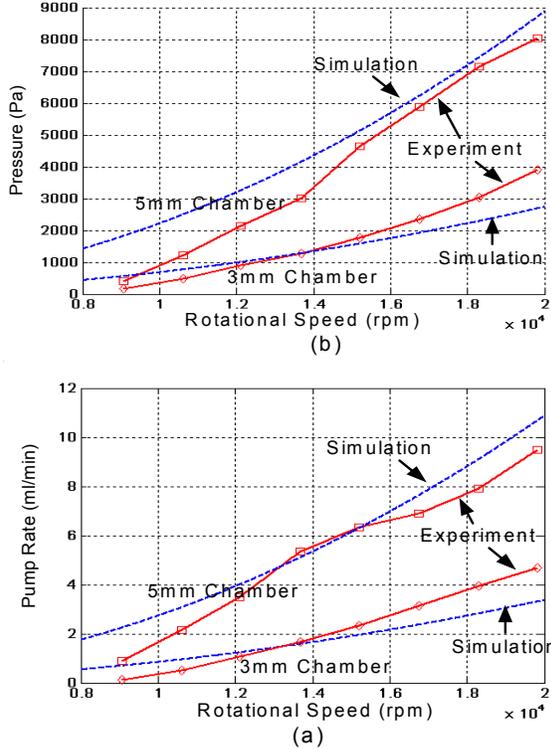


Figure 4. Comparison of the experimental (solid line) and simulation (dotted line) results. (a) Comparison of pump rate (water as pump medium) as a function of rotational speed of impeller at zero back pressure. (b) Comparison of pumping pressure (water as pump medium) as a function of rotational speed of impeller at zero flow rate.

C. Integrated microfluidic system

To test the concept of driving fluids using multiple vortex pumps, we have fabricated 3 vortex pumps and integrated them with an SPR biosensor. The pumps were driven by individual voltage supplies in the experiments described below. The experimental setup of this integrated microfluidic system is illustrated in Figure 5. Three vortex micropumps and an SPR biosensor were integrated into a polymer-based chip to provide a controlled flow sequence and real-time SPR signal detection. In this setup, three different solutions were pumped into the SPR biosensor head independently. A Mach-Zehnder interferometer with a 10mW polarized He-Ne laser operating at 632.8nm was used as the light source. The polarization direction of the output beam was tuned at 30° from p -polarization so that a larger amount of light intensity could be contributed to p -polarization to enhance the signal-to-noise ratio of the probe beam. For the sensor head, we used a nominally 45nm gold-coated glass plate attached to

a 60° equilateral prism made from BASF10 glass using matching oil. The first 50:50 beam splitter divided the laser into two halves. One beam (probe beam) went to the sensor head while the beam (reference beam) went to the plane mirror. A piezoelectric transducer (PZT) was attached to the back of the mirror and a saw-tooth wave oscillating at 120Hz was used to provide the phase modulation signal required by the phase measurement software. Constructive and destructive interferences occurred periodically as a time-varying path difference were introduced by the back-and-forth movement of the mirror. The probe beam and reference beam were combined again at the second beam splitter. In the output beam p - and s -polarization light were separated by the use of Wollaston prism. Two detectors together with a digital oscilloscope were used to capture the signal and finally the differential phase quantity was extracted.

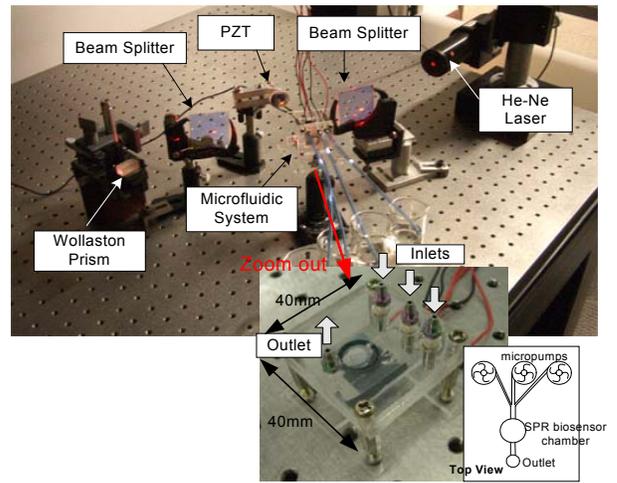


Figure 5. Experimental setup of the automated microfluidic system integrated with 3 pumps and an SPR biosensor for micro cellular detection. (zoom out) A photo of the polymer based microfluidic system integrated with SPR biosensor. The size of the whole chip is 40mm x 40mm. There are three independent inlets for three different solutions.

III. MICRO CELLULAR DETECTION

A. Material

The cells that we used in our experiment were mouse L929 cell (4×10^6 cells/ml) obtained from ATCC (American Type Culture Collection). Prior to the experiment, the mouse cells were put in a rich nutrition buffer, RPMI Medium 1640 from Invitrogen Corporation. RPMI (Roswell Park Memorial Institute) Medium 1640 are liquid medium with enriched formulations for culturing living mammalian cells [8]. With the aid of RPMI, living L929 cells will naturally attach to the gold surface. Trypsin-EDTA (0.25% Trypsin) obtained from Invitrogen Corporation is a common reagent used to remove and detach cells from culture substratum. Trypsin was thus used in our experiment to detach the cells from the gold surface by breaking the adhesion proteins between the cell and the gold surface.

B. Results

The process sequence is illustrated in Figure 6. Mouse L929 cells (4×10^6 cells/ml) were first cultured on a 45nm gold-coated glass plate at 37°C for two hours. Then the gold surface was observed under an optical microscope to ensure cells were well adhered to the glass plate. As shown in the in-set of Figure 7, cells were adhered on the gold surface as a monolayer. This glass plate was then attached to a prism using matching oil for phase-sensitive SPR bio-sensing studies. RPMI Medium 1640 was first flowed into the chamber to measure differential phase as a baseline. Trypsin-EDTA (0.25% Trypsin) was then injected into the sensor head. As shown in Figure 7, an exponential decay curve was observed indicating that the cells were detached from the gold surface. Finally, RPMI was circulated to ensure all the cells were suspended. Another set of control experiment was carried out by repeating the experiment with bare gold glass plate. A small phase angle changed was observed when trypsin is added. Thus, we conclude that the large phase change observed in Figure 7 must be largely caused by cells detachment. At the end of the experiment, the glass plate was taken out from the prism and observed again under an optical microscope. Visual inspection confirmed that almost all the cells were removed from the surface. Our results show that one can monitor cell detachment affinities using SPR. This is a very useful platform for a range of biomedical applications e.g. drug discovery by simply replacing trypsin with newly invented drugs.

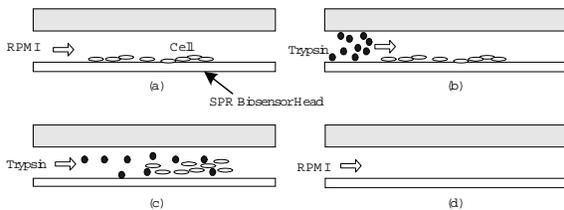


Figure 6. Illustration of detecting of cell detachment. (a) Injection of RPMI. (b) Injection of trypsin. (c) Detaching cells from the sensor head. (d) No cell left on the sensor surface.

IV. INTEGRATED MICROFLUIDIC SYSTEM WITH PARALLEL SAMPLE PREPARATION CAPABILITY

The schematic of the integrated microfluidic system, which has multiple detection chambers that can allow diverse bio-solutions to be transported, is illustrated in Figure 8. Our group has built and tested this system with rudimentary experiments. This section briefly describes the performance of this multi-pump system. The microfluidic chip consists of four microchambers and sixteen inlets. Bio-solutions are pumped to the inlets by vortex micropumps. In each chamber, any combination of the four inlet chemicals can be prepared by adjusting the flow rate of micropumps (A – D). With the designed channel configuration, samples could be delivered to the four target chambers (1 – 4) by micropumps. Each micropump was used to adjust the fluid flow rate from a

particular chemical inlet to a target chamber. The combination and concentration of chemicals were controlled by the pump rate ratios among the micropumps. Integrated with the motor control system discussed in section V, the system enables the parallel sample preparations of the four microchambers, with different pump rates and chemical combinations.

The complete polymer microfluidic chip is shown in Figure 9. The channel patterns on the substrate were fabricated with SU-8 photosensitive polymer. Further machining processes were performed to construct the sixteen inlets. An experiment was also performed to illustrate the flow pattern inside the microchambers. Micropumps A and C were inserted with DI water while micropumps B and D were used to pump red dye. The flow pattern (Figure 10) indicates that all micropumps could successfully deliver inlet fluids into the target microchambers. This result shows that the parallel detection of multiple SPR sensors can be achieved by 1) fabricating an array of detection chambers on a transparent polymer substrate, and 2) using an array of micropumps to transport different bio-fluids into different microchambers. Essentially, the technology that we have developed is scalable, i.e., the number of vortex micropumps and number of reactions chambers can be dictated entirely by the experimental needs.

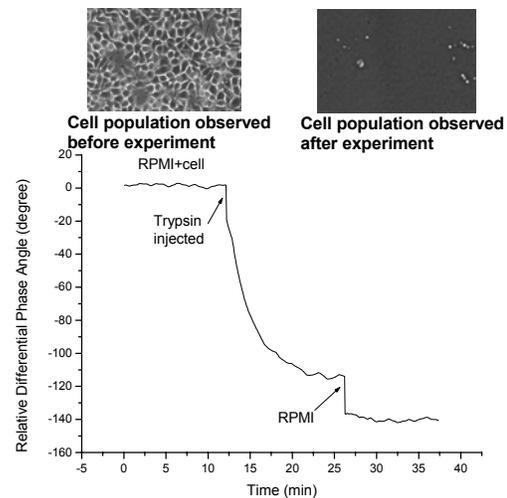


Figure 7. Differential phase response curve due to sequential addition of RPMI containing cells, trypsin, and RPMI into sensor head.

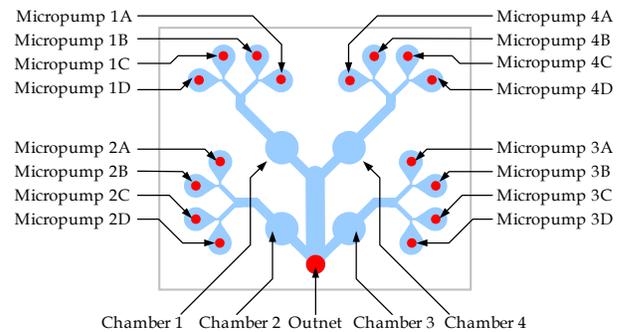


Figure 8. Channel design of the microfluidic system integrated with four microchambers and sixteen inlets connected with micropumps.



Figure 9. Photograph of the SU-8 channels on the PMMA substrate of the microfluidic system with parallel sample preparation capability.

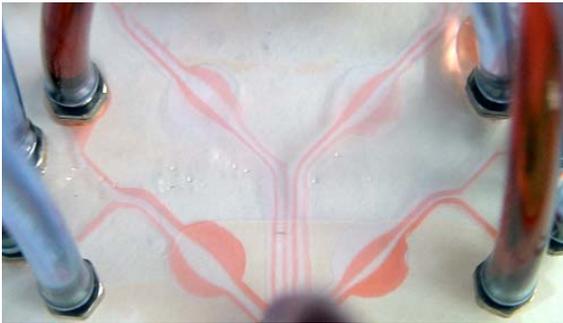


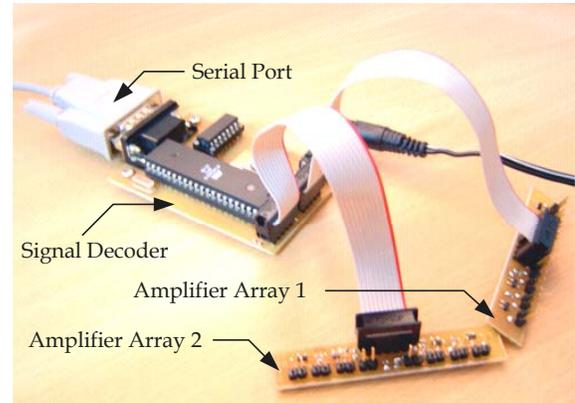
Figure 10. Fluid Pattern of the microchambers pumped with DI water (inlets B and D) and red dye (inlets A and C)

V. MOTOR CONTROL SYSTEM

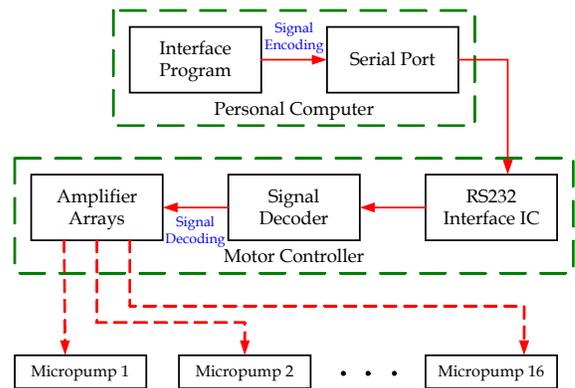
The interface hardware and software were developed to control the vortex micro pumps and eventually automate the entire bio-fluid delivery and transport process. As mentioned earlier, the pump rate of fluid can be controlled by the vortex pump rotation speed. Hence, controlling the DC motor speeds by a computer is needed to automatically control the fluid delivery in a microfluidic system. The motor control system was developed to operate multiple micropumps as shown in Figure 11 (a). The schematic is shown in Figure 11 (b). Users could input commands with an interface program to control the pump rates of micropumps. Transmitting signals via the serial port, the motor controller would collect and decode the received commands. The motor controller is integrated with IC chips AT90S8515, MAX232 and two operational amplifier arrays. It could simultaneously activate up to sixteen micropumps simultaneously. The output signals of the motor controller were programmed in pulse-width modulation (PWM) format. Since the ratio of the equivalent output voltage V_{eq} and the peak voltage V_{peak} is equal to the ratio of its pulse width t_{pulse} and signal period T , the rotational speed of micromotors, and hence the pump rate, could be adjusted by the output pulse width. In our system, V_{peak} is 5V and T is 3.9ms. By regulating t_{pulse} between ~ 0.4 ms and ~ 2 ms,

the desired V_{eq} could be generated based on the characteristic of the micropump as shown in Figure 12.

The operation of the microfluidic system often requires parallel operation, i.e., multiple micropumps can operate with different pump rates at the same moment. However the information contained by each serial port data package is limited to 8 bits. An appropriate transmission protocol is designed for our application. In the motor control system, the rotational speed values of target micromotors are temporarily stored in the controller buffer. The micromotors would keep their previous rotational speeds until the updated command is sent. This approach can ensure the parallel operation of micropumps. The flow diagrams of implementation are illustrated in Figure 13. In the system, the microcontroller AT90S8515 processes the command data and the output PWM signals with UART and timer interrupt functions, respectively. The microcontroller keeps swapping the output voltages and generated PWM signals by the timer interrupt. When an external command is received from the serial port, the UART interrupt function would instantly transfer the rotational speed values to the buffer storage and check whether the updating of the output voltages is needed. To update the signal outputs, the system would change the pump rates of all micropumps instantaneously.



(a)



(b)

Figure 11. (a) Motor controller developed for micropump operations and (b) schematic design of the motor control system.

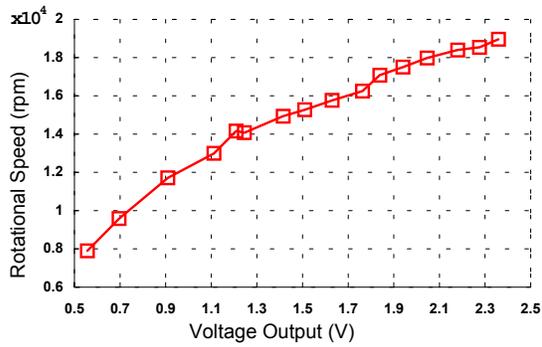


Figure 12. Calibrated rotational speeds of impeller corresponding to different input voltages.

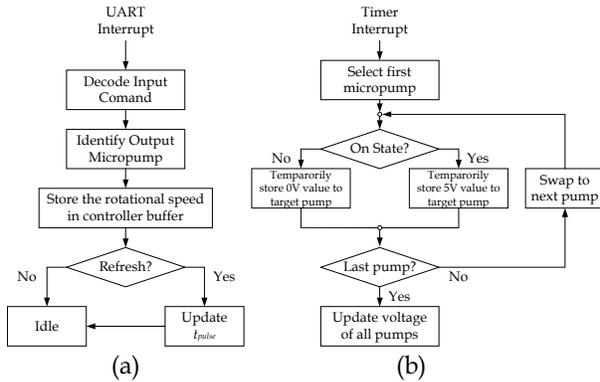


Figure 13. Flow diagrams of (a) UART and (b) timer interrupt functions adopted in the motor control system.

VI. CONCLUSION

Detection of bio-specific protein partners (BSA and BSA-antibody) and monitoring of cell activities have been successfully demonstrated using our new SPR biosensor integrated with automated microfluidic system. This preliminary study on cell activity showed the possibilities of using differential phase SPR bio-sensors in a wide range of biomedical applications. Our integrated system should provide a useful platform for conducting high-throughput diagnostic tests on cells and biomolecules at a very low cost. We have also developed the microfluidic chip integrated with four microchambers and sixteen micropumps for the potential application of the parallel detection with multiple microchambers. Furthermore, arrayed sensing surface with multiple analyses and parallel detection by using a 2-D phase imaging technique will certainly be a very exciting direction for further development of phase-sensitive SPR sensors.

ACKNOWLEDGMENT

The authors wish to thank The Chinese University of Hong Kong for the Direct grant under Project #2050305.

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