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Red Blood Cell Concentration Measurement Method Using Two Cell Counters

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Here, we present a novel cell concentration measurement method that can be used to count cells in a fixed control volume. Previous cell concentration measurement methods (e.g., those using a Coulter counter and a flow cytometer) can be used to count cells in a given fluid volume or at a known flow rate. Thus, the accuracy of the cell concentration measurement depends on the performance of external facilities, such as accurate fluid volume and flow rate controllers. However, the proposed method based on the measurement of the number of cells in a fixed control volume can measure cell concentration without requiring accurate fluid volume measurements or precision flow rate control. Using the fabricated devices, we realized two different measurement methods: 1) a cell concentration measurement method using a single cell counter and a fixed flow rate (conventional method), and 2) a cell concentration measurement method using two cell counters and a fixed control volume (proposed method). Compared with the conventional method, which showed cell concentrations ranging from 1.18×10^5 to 3.28×10^5 cells/ml under various flow rate conditions, the proposed method shows a maximum error of 5.32%, which is within a hemacytometer's standard deviation. Finally, we have not only enhanced its simplicity but also reduced its size and cost, because our device requires no expansive flow sensors or accurate pumps.

1. Introduction

Cell concentration measurement is a basic process for medical diagnosis and cell research.[1–4] In diagnosis,[1,2] the concentrations of red blood cells (RBCs) and white blood cells (WBCs) are two of the most important indices. A low concentration of RBCs indicates anemia, and a high concentration of WBCs indicates leukemia. In a general biological experiment, controlling cell concentration is an important process for the quantitative analysis of biomolecules.[1,3,4] For these needs, many cell concentration

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measurement devices have been developed at the commercial level. These devices can be divided broadly into two categories: manual and automatic devices. The first device, the hemacytometer,[5] is a special glass slide that has a grid for counting cells in a fixed volume. While it is portable and cheap, its process of cell counting is manual and requires an optical microscope. Because medical diagnosis (hematology) and some biological experiments that use many samples require an automatic process that takes only a short time for cell concentration measurement, an automatic cell concentration measurement method is also necessary. Automatic cell counters, such as Coulter counters[6,7] and flow cytometers,[8–11] count cells passing through an orifice. They measure cell concentration on the basis of two factors: 1) the number of cells passing through an orifice per unit of time, and 2) the total volume of the sample passing through a microchannel or the flow rate. Since previous works measured cell concentration on the basis of flow rates or the fluidic volume passing through a microchannel, the accuracy of the measurement depends on the performance of external facilities such as delicate pumps and flow sensors. Thus, these methods require accurate fluidic control systems, which are expensive and require a large amount of space.

For a novel method of measuring cell concentration, we propose the use of two cell counters at the inlet and outlet of the control volume (see Fig. 1). Our method is independent of flow rate and fluid control because it calculates cell concentration by counting cells in the control volume. The number of cells in the control volume is the numerical difference between the number of cells that flow in and the number of cells that flow out of the control volume. To count the cells that flow in and out of the control volume, we use electrical cell counters at the inlet and outlet of the control volume. Because the control volume is initially zero, as shown Fig. 2, the cell number ($N_{cv} = N_{in} - N_{out}$) in the fixed control volume increases but converges to a value determined by the cell concentration of the sample. The converged (or mean) value is proportional to the cell concentration of the sample. Therefore, regardless of the flow rate, we can easily calculate the cell concentration by dividing the converged value by the known control volume.

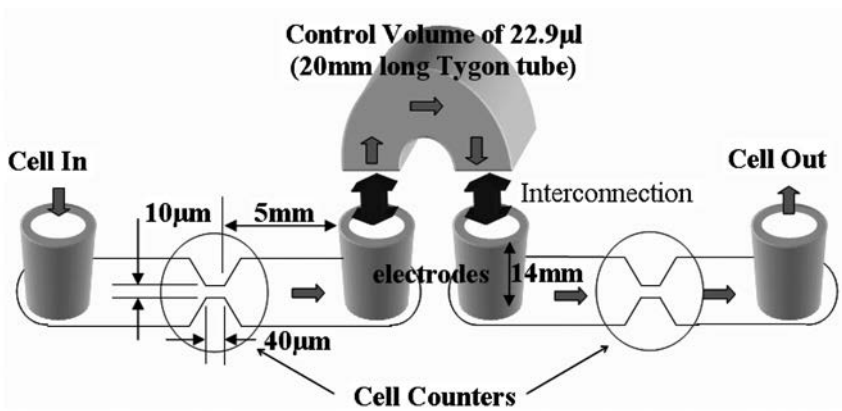


Fig. 1. Schematic of present cell concentration measurement device.

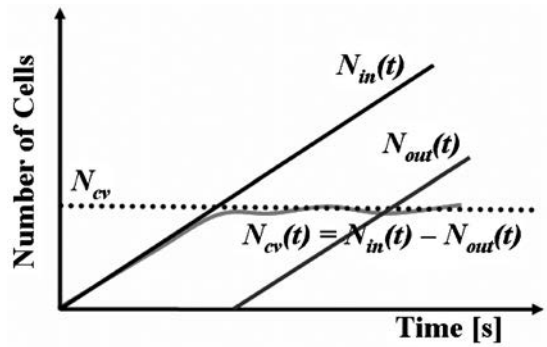


Fig. 2. Cell concentration measurement principle based on total number of cells in the fixed control volume.

2. Theoretical Principle and Design

As shown in Fig. 1, our device for measuring cell concentration has a control volume compartment between two electrical cell counters. We designed the two electrical cell counters, which are made from microchannels including an orifice, and the control volume compartment, which is made from a tygon tube. The design of the cell counters is based on an electrical cell counting method, as well as on considerations of the sensitivity of the cell counting and the coincident effect (where two or more cells are counted as one). As for the control volume compartment, we designed its size in relation to the range in which the cell concentration could be measured.

2.1 *Electrical cell counter*

The cell counting principle relies on changes in resistance caused by a particle passing through the orifice.[12,13] Because the polarization effect at the particle-electrolyte interface prevents any current from flowing through the particle itself, the electrical resistance (R_{sensing}) of the orifice changes and, as shown in Fig. 3, the change in the resistance of the orifice ($\Delta R_{\text{sensing}}$) depends on the integral of the particle cross section. We can determine any change in the element resistance $\delta(\Delta R_{\text{sensing}})$ over a finite distance, Δx, as

$$\frac{\delta\left(\Delta R_{\text{sensing}}\right)}{R_{\text{sensing}}} = \left(\frac{a \cdot \Delta x}{A \cdot L}\right) \Big/ \left(1 - \frac{a}{A'}\right), \tag{1}$$

where A and a are the cross-sectional areas of the orifice and particle, respectively, and L is the length of the orifice. This equation can be integrated as follows to give the resistance change $\Delta R_{\text{sensing}}$ due to the particle:[12,13]

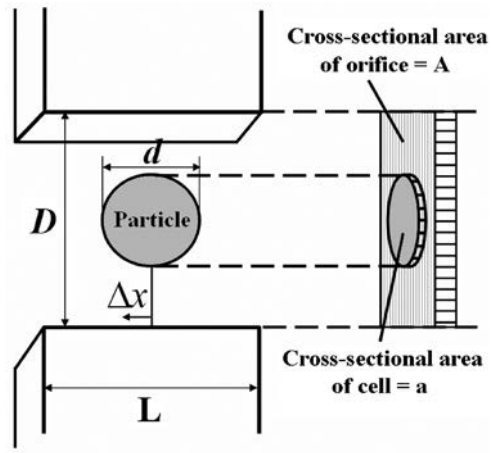


Fig. 3. Schema of principle of electrical cell counter, where *D* is width of orifice, L is length of orifice, and d is diameter of particle. The cross-sectional areas of the orifice and particle are represented by *A* and *a*, respectively.

$$\Delta R_{\text{sensing}} = R_{\text{sensing}} \int_{-\frac{L}{2}}^{\frac{L}{2}} \left(\frac{a}{A \cdot L}\right) dx \Big/ \left(1 - \frac{a}{A}\right)$$

$$= \frac{\rho_f}{A} \int_{-\frac{L}{2}}^{\frac{L}{2}} \frac{a}{A - a} dx, \tag{2}$$

where ρ_f is the electrical resistivity of the fluid.

A cell counter should be operated at a sufficiently low concentration to prevent two or more cells from passing though the cell counter at one time (coincident effect).[12,13] We can theoretically determine the coincident effect by assuming a Poisson probability for the concurrent detection of two particles in the cell counter. This probability yields the following relationship between the true cell number, *N*, and the observed cell number, *n*:

$$N = n + pn^2$$

where

$$p = 2.5\left(\frac{D}{1000}\right)^3 \left(\frac{500}{v}\right) \times 10^{-6}, \tag{3}$$

D is the diameter of the orifice (in micrometers) and v is the sample volume (in microliters).[12,13]

Because we used RBCs with a volume ranging from 60 fl to 120 fl,[14] we designed the orifice of each electrical cell counter with the following dimensions, as shown in Fig. 4: a

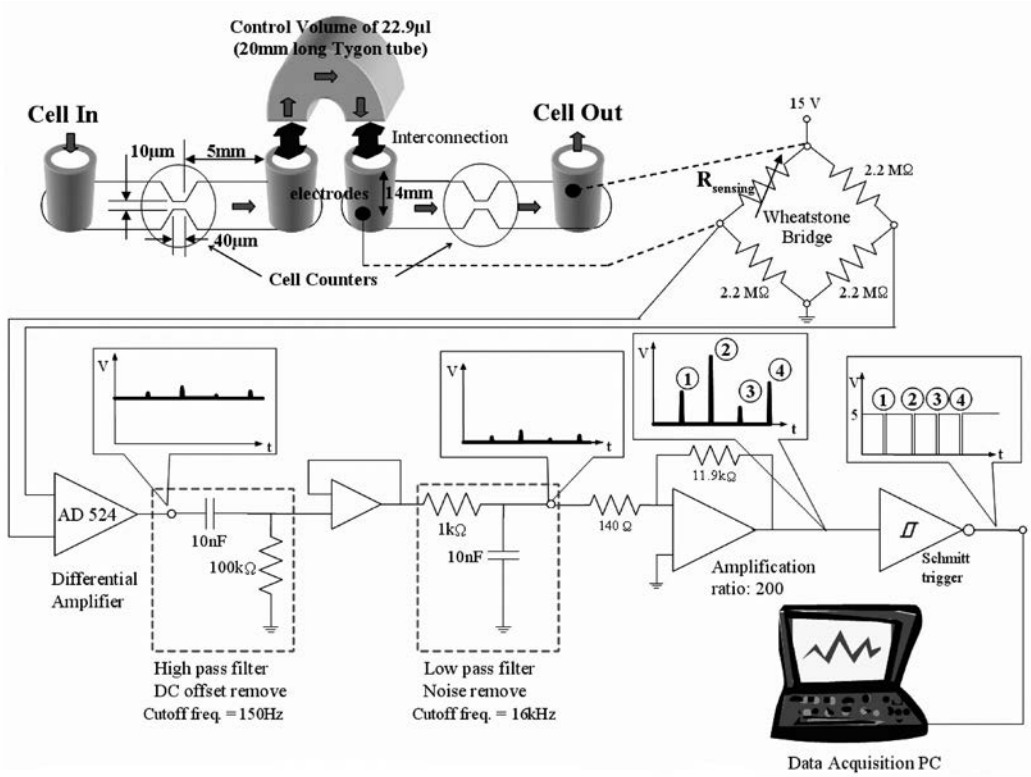


Fig. 4. One of the two electrical cell counters and its measurement circuit.

height of 10 μm, a width of 10 μm, and a length of 40 μm. In this dimension, the cross-sectional area of the orifice is about 4 times wider than that of RBC to prevent an RBC clogging problem at the orifice. With this cell counter and a buffer solution of phosphate-buffered saline (PBS: with a conductivity of 1.6 S/m), we used MATLAB 7.0 to estimate the following values: a sensitivity level ($\Delta R_{sensing}/R_{sensing}$) ranging from 0.19% (for an RBC volume of 60 fl) to 0.42% (for an RBC volume of 120 fl); an orifice resistance ($R_{sensing}$) of 2.35 MΩ; and a change in resistance ($\Delta R_{sensing}$) ranging from 4.4 kΩ (for an RBC volume of 60 fl) to 9.86 kΩ (for an RBC volume of 120 fl). As shown in Fig. 4, the voltage theoretically changes from 2.8 V (for an RBC volume of 60 fl) to 6 V (for an RBC volume of 120 fl) in relation to the volume of passing RBCs at a bias voltage (±15 V) and a voltage amplification of 200. A Schmitt trigger (74LS14) converts analog signals above a threshold voltage of 1.7 V to digital signals. We counted the number of digital signals, which indicate the number of RBCs. Using eq. (3), a cell concentration of 1×10^7 cells/ml, and a sample volume of 22.25 μl, which is the control volume, we can also calculate that the coincident effect causes a maximum cell concentration error of 1.25%.

2.2 *Control volume*

During the design of the control volume compartment, we determined its size. The size of the control volume compartment is related to the cell concentration measurement range and time. Because the number of cells in the control volume compartment at a low cell concentration is too small to converge to a number determined by the cell concentration, it fluctuates owing to a heterogeneous cell distribution in the sample; thus, cell concentration cannot be measured. In this case, it is necessary to increase the size of the control volume to obtain a sufficient number of cells in the control volume. A large control volume, however, needs a long measurement time owing to the longer filling time. Therefore, for a short-time cell concentration measurement, we optimized the size of the control volume compartment according to the target measurement range of cell concentration. Before designing the control volume compartment, we chose a target measurement ranging from 1×10^5 to 5×10^5 cells/ml, which is the general measurement range of a hemacytometer. As shown in Fig. 1, the control volume compartment was made from a Tygon tube and two electrode pins. We used electrode pins to connect the Tygon tube and each cell counter, as well as for measuring the resistance at each cell counter. The inside diameters of the Tygon tube and electrode pins were 800 and 700 μm, respectively, which are large enough to prevent an RBC clogging problem in the Tygon tube and electrode pins. The lengths of the Tygon tube and the electrode pins were 20 and 14 mm, respectively, to make a control volume of 20.8 μl. For a cell concentration of 1×10^5 cells/ml, the control volume compartment contains approximately 2080 cells, which is a large enough number to make the number of cells in the control volume converge to a number determined by cell concentration.

3. Experimental Study

To measure cell concentration, we measured the exact control volume and determined the RBC counting condition (i.e., the threshold voltage for counting RBCs). We then measured the cell concentration using our proposed method and a conventional method. After comparing the results of the two methods, we characterized the performance of the proposed method. Finally, using various flow rate conditions, we demonstrated that the proposed method is independent of flow rate.

3.1 *Fabrication process*

As shown in Fig. 5, to make the electric cell counters, we used a polydimethylsiloxane (PDMS) microchannel (with a 10:1 mixing ratio of the monomer to the curing agent), which was bonded with Pyrex glass, and the present device was then made by interconnecting the cell counters with the control volume compartment made from the Tygon tube. To fabricate the PDMS microchannel for the cell counters, we first made a 10-μm-thick mold of positive photoresistor, AZ9260. We then poured PDMS into the mold and cured it on a hot plate at 85°C for 2 h. Next, we used a punch for the inlet and outlet ports at each microchannel, and made the cell counters by bonding the PDMS microchannel and the Pyrex glass using plasma treatment with a BD-10AS high-frequency generator (Electro-Technic Products, Inc.). Finally, we interconnected two fabricated cell counters with the control volume compartment using electrode pins. Figure 6 shows the assembled cell concentration measurement device and an enlarged view of a cell counter.

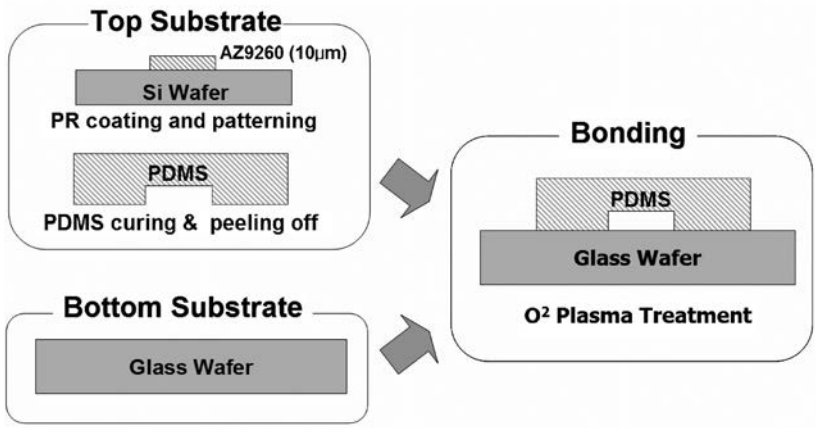
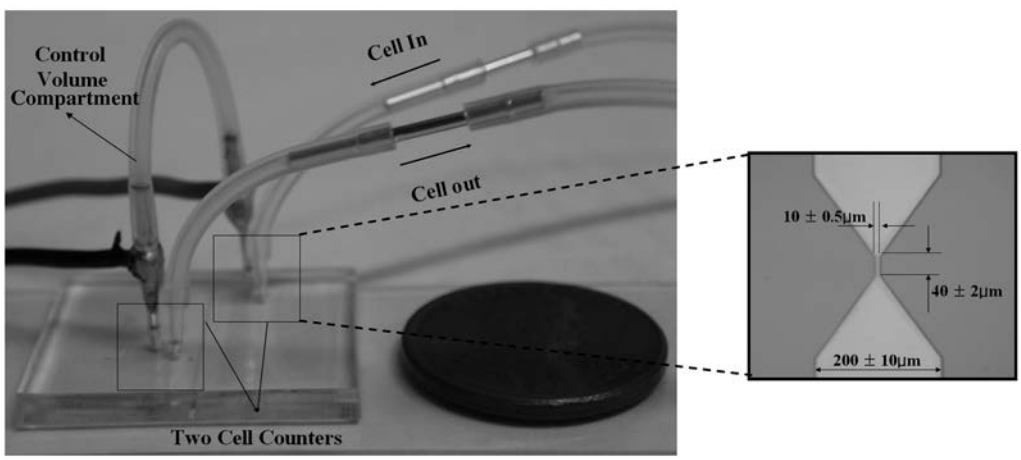


Fig. 5. Fabrication process of the cell counters.



(a) (b)

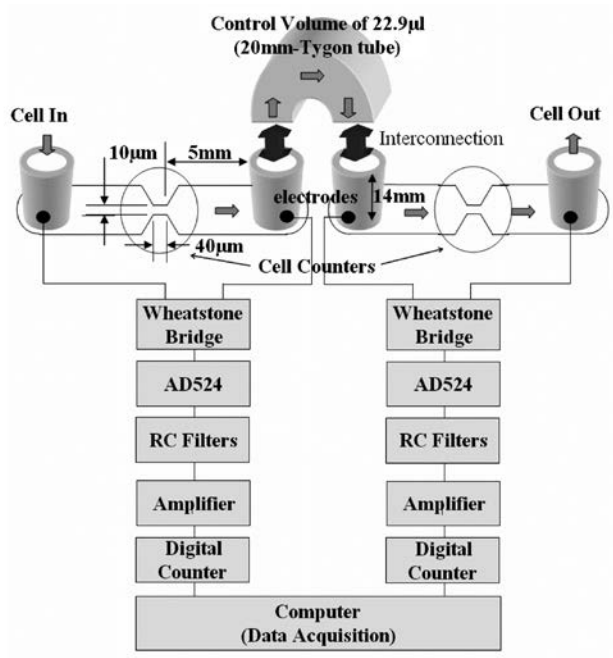
Fig. 6. Photograph: (a) assembled cell concentration measurement device; (b) enlarged view of cell counter.

3.2 *Sample pretreatment*

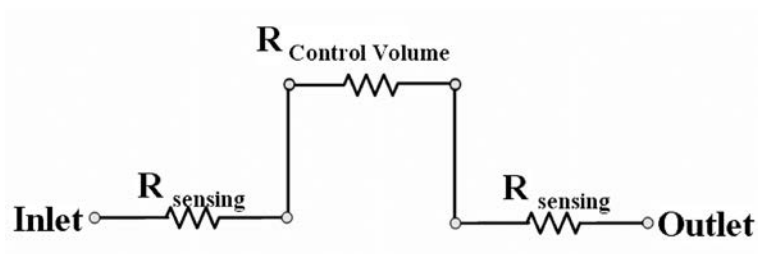
We obtained 1 ml of human blood from a blood donor and centrifuged the sample at 1000 rpm for 5 min to separate the RBCs from the serum. The supernatant was discarded, and we washed the RBCs with a 1×PBS buffer three times by centrifuging until we obtained a clear supernatant. After discarding the supernatant, the RBCs were suspended in a 1×PBS buffer. Finally, we diluted the sample into samples containing 1.18×10^5, 1.79×10^5, or 3.28×10^5 cells/ml.

3.3 *Experiment setup*

Our experimental apparatus (Fig. 7(a)) was designed to evaluate the fabricated device. Figure 7(b) shows a simplified electrical analogy of the device. The resistance of the control volume ($R_{\text{control volume}}$) and the resistance of the cell counters (R_{sensing}) are connected as an electrical series circuit. When RBCs pass through the orifice, a Wheatstone bridge converts the resistance change of the orificee ($\Delta R_{\text{sensing}}$) into a voltage change. We then use a differential amplifier AD524 (Analog Devices), which is connected to the two electrodes interconnected with the orifice, to measure the voltage change. Next, as shown in Figs. 4 and 7, we use a resistance and capacitance (RC) high-pass filter to remove the DC offset



(a)



(b)

Fig. 7. Experimental apparatus for present cell concentration measurement device with control volume comprtment between two cell counters: (a) experimental setup; (b) electrical analogy circuit of the entire device.

voltage and a RC low-pass filter to reduce the electrical noise. Before counting RBCs, the analog signals are converted to digital signals by a Schmitt trigger (74LS14). Using MATLAB 7.0, we then measure the number of RBCs that flow in and out of the control volume compartment by counting digital signals. Subtracting the number of cells that flow in from the number of cells that flow out, we calculate the number of cells in the control volume compartment over a fixed period. Finally, we measure the cell concentration by dividing the number of cells in the control volume by the known control volume compartment.

3.4 *Control volume measurement*

Although we designed the control volume to be 20.8 ml, there was a possibility of fabrication errors. Hence, before measuring the cell concentration, we measured the control volume in an experiment. First, we calculated the control volume by measuring the filling time under a constant flow rate supplied by an accurate syringe pump (Harvard Apparatus). After measuring the filling time five times, we calculated the control volume to be 22.9 ml ±0.98 ml. Compared with the designed control volume of 20.8 ml, the measured control volume has the error of 10%. For an accurate control volume, a control volume integrated with two cell counters is necessary, because an error in the control volume comes from the dead volume of the interconnection between the counters and the control volume compartment.

3.5 *RBC counting*

In contrast to the theoretically estimated signals, which ranged from 2.8 (for an RBC volume of 60 fl) to 6 V (for an RBC volume of 120 fl), the signals we measured when the RBCs passed through the cell counter ranged from 2 (for an RBC volume of 60 fl) to 4 V (for an RBC volume of 120 fl). The experimental and theoretical analyses revealed a difference due to an effect of the RC filters to remove noise and offset. We observed RBC counting signals of 2×10^5 from all experiments between 2 and 4 V. The variation in the measured signal from 2 to 4 V (Fig. 8) is due to the variable RBC volume distribution.[14] The analog signals above the threshold voltage of 1.7 V are converted to digital signals by a Schmitt trigger (74LS14) as shown in Fig. 8.

3.6 *RBC concentration measurement*

We used RBC samples of the three different concentrations shown in Table 1 and compared the results for the fabricated devices with those for a hemacytometer. First, we measured RBC concentration using a conventional method (i.e., using one cell counter and a fixed flow rate of 10 ml/min) and then using the proposed method (i.e., using two cell counters and a control volume of 22.9±0.98 ml). By measuring the cell concentration with the same cell counting circuit for both the conventional and proposed methods, we were able to exclude other effects in our comparison of the two methods. In the conventional method, we measured cell concentration by counting the cells in one cell counter at a constant flow rate of 10 μl/min. Hence, by determining the total number of cells and the volume of fluid that passed through the cell counter in a 10 min period, we were able to calculate cell concentration. The proposed method measures cell concentration in terms of

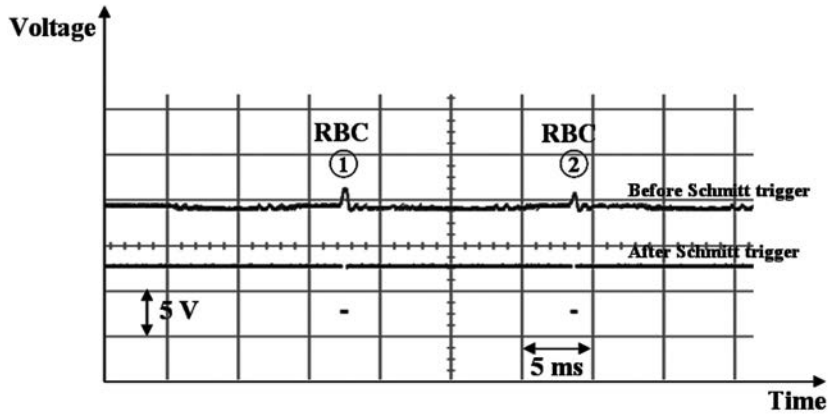


Fig. 8. Measured electrical signals from one of two cell counters.

Table 1
Comparison of experimental results.

Hemacytometer using sample volume of 0.1 μl	Conventional Method*	Proposed Method**
3.28±0.99 [×10^5 cells/ml]	2.97±0.41	3.04±0.42 (2.35%)
1.79±0.13 [×10^5 cells/ml]	1.58±0.15	1.65±0.15 (4.43%)
1.18±0.12 [×10^5 cells/ml]	0.94±0.25	0.99±0.15 (5.32%)

( ): Maximum error of mean value compared with that of conventional method.
*One RBC counter at flow rate of 10 μl/min. **Two RBC counters at control volume of 22.9±0.98 μl.

the number of cells in the control volume, and it calculates this number by subtracting the number of cells that flow in the control volume compartment from the number of cells that flow out.

Figure 9(a) shows that the number of cells in the control volume fluctuates at approximately 7831 at a RBC concentration of $3.28\times10^5\pm0.99\times10^5$ cells/ml. The converged values differ according to RBC concentration, as shown in Fig. 9(b). For example, the converged (or mean) values of 7924 RBCs and 3652 RBCs are $3.28\times10^5\pm0.99\times10^5$ cells/ml and $1.79\times10^5\pm0.13\times10^5$ cells/ml, respectively. The fluctuation was caused by a heterogeneous RBC distribution in the samples. During the experiment, the accumulation of RBCs inside the control volume does not occur, because the number of RBCs in the control volume is converged.

Table 1 compares the RBC concentrations obtained from the proposed method with those obtained from the conventional method for three different RBC samples. Compared with the conventional method, the maximum error of the proposed method is 5.32% for the three samples. The measurement results from both methods are within the hemacytometer's standard deviation of 20% (ten RBC concentration measurements of three samples), which

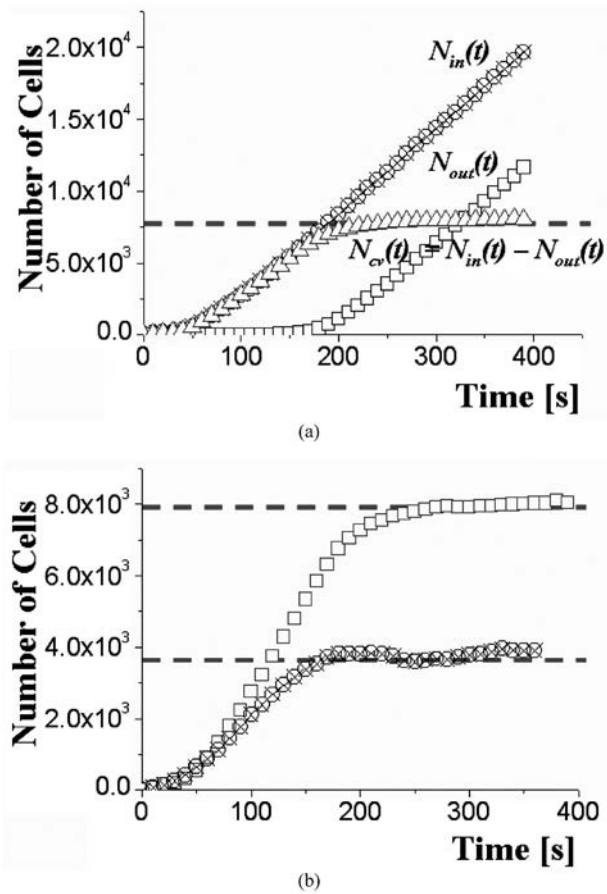


Fig. 9. Number of cells in control volume (22.9±0.98 μl): (a) at $3.28\times10^5\pm0.99\times10^5$ cells/ml; (b) at $3.28\times10^5\pm0.99\times10^5$ and $1.79\times10^5\pm0.13\times10^5$ cells/ml.

causes the heterogeneous RBC distribution in the samples.

To verify that the performance of the proposed device is independent of (or insensitive to) the flow rate, we used the device to measure cell concentration under the following flow rate conditions, as shown in Fig. 10.

- flow rates of 10 and 5 μl/min
- a flow rate that changes (4 μl/min → 2 μl/min → 4 μl/min) during the measurement of cell concentration

By increasing the flow rate, we reduced the measurement time, although, as shown in Fig. 10(a), the converged value of cells in the control volume is constant. In Fig. 10(b), the cell numbers as functions of the time flowing in and out the control volume (slopes of N_{in} and N_{out}) vary in relation to changes in the flow rate, but the number of cells in the control volume (N_{CV}) is constant. In both cases, the converged value of cells is constant, implying

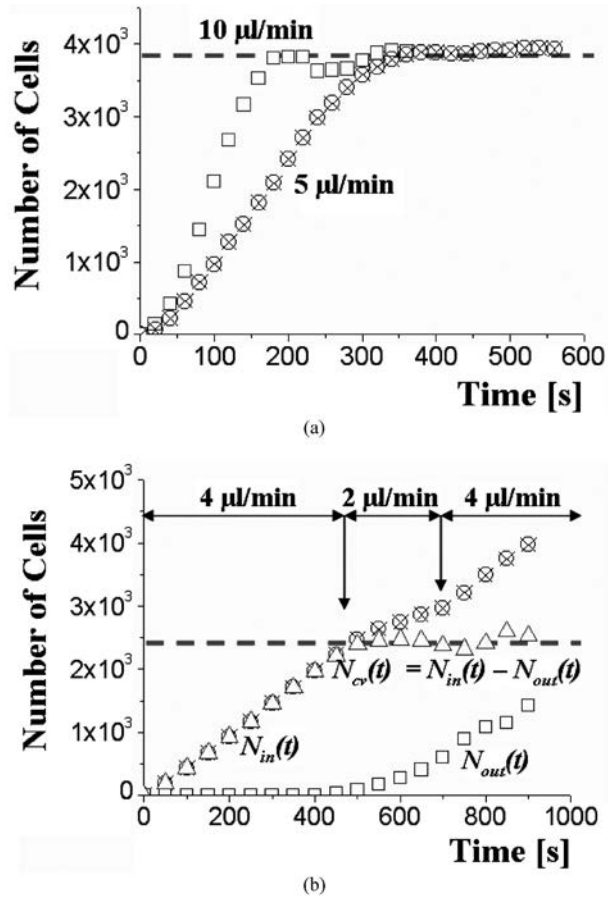


Fig. 10. Number of cells measured by cell concentration measurement device with two cell counters and control volume of 22.9±0.98 μl: (a) under different flow rates of 10 and 5 μl/min for RBC concentration of $3.28\times10^5\pm 0.99\times10^5$ cells/ml; (b) under flow rate that varies (4, 2 and 4 μl/min) during cell concentration measurement for RBC concentration of $1.18\times10^5\pm 0.12\times10^5$ cells/ml.

that the flow rate has no effect on the measurement of cell concentration.

4. Conclusion

By a novel method of measuring cell concentration, we used electrical cell counters across a fixed control volume. Although existing methods require the accurate measurement of fluid volume or the precise control of flow rate, our simple and automated method of measuring cell concentration obviates the need for a delicate pump or flow sensor. When measuring cell concentration, we achieved a normal RBC concentration measure-

ment time of 5 min and an RBC concentration range from 1.18×10^5 to 3.28×10^5 cells/ml. Compared with the conventional method, the proposed method shows a maximum error of 5.32%, which is within a hemacytometer's standard deviation of 20%. The maximum error of 5.32% of the present device was achieved with flow rates of 10 and 5 μl/min and when the flow rate that varied from 4 to 2 μl/min. We have therefore confirmed that our device offers a simple and automated method of measuring cell concentration, without the need to accurately measure fluid volume or precisely control flow rate. Finally, we not only enhanced its simplicity and adaptability but also reduced its size and cost, because the present device requires no flow sensors or accurate pumps.

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From 1982 to 1986 he was a Research Scientist of CAD/CAM Research Laboratory, Korea Institute of Science and Technology (KIST), Seoul, Korea. He worked as a Post-Graduate Researcher (1987-1990) and a Post-Doctoral Research Associate (1991) of the Berkeley Sensor and Actuator Center (BSAC) at the University of California at Berkeley. In August 1991, Dr. Cho moved to KAIST, where he is currently Professor in the Departments of BioSystems & Mechanical Engineering as well as the Director of Digital Nanolocomotion Center.

Dr. Cho's research interests are focused on the nano/micro-electro-mechanical systems (N/MEMS) where bioinspired actuators and detectors are integrated with control circuitry for the high-performance, low-power, low-cost manipulation and processing of nonelectrical nanoinformation carriers or substances. In Korea, he has served as the chair of the MEMS Division in the Korean Society of Mechanical Engineers, the Chair of Steering Committee in Korea National MEMS Programs, and the Committee of National Nanotechnology Planning Board. Dr. Cho has also served for international technical society as the General Co-chair of IEEE MEMS Conference 2003, the Program Committee of IEEE Optical MEMS Conference, the Chief Delegate of the Republic of Korea in World Micromachine Summit. Dr. Cho is a member of IEEE and ASME.