Feature Article

Development of Blood Cell Counter for Point of Care Testing (POCT)

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Palmtop-type blood cell counter with impedance method is our primary design target. The focus is on the development of disposable cartridge-type sensor and sampling part by Micro Electro Mechanical Systems (MEMS) technology. The disposable cartridge needs no cleaning and eliminates the fear of contamination by other samples. The sensor part is structured by silicon and glass which are bonded together. On the silicon side, we fabricate flow channel, filter, aperture and electrodes using MEMS technology. Since it is a palmtop-type device, the sensor structure is closed, which can cause a serious problem that miscount of blood cells occurs due to the bubbles generated by electrolysis at electrodes. We solved the problem by changing the positions of flow channel and electrodes, and achieved a progress in developing the palmtop-type blood cell counter.

Introduction

The demand of immediate test in diagnosis (POCT: Point of Care Testing)*1 has been increasing. To meet this demand, we are developing the palmtop-type model of the main medical product, blood cell counter. For the development, it is essential to downsize the sensor for blood cell counting and preprocessing part for dilution. We used HORIBA's MEMS (Micro Electro Mechanical Systems) technology and succeeded in a chip-type sensor including a cartridge-type preprocessing part. After the experimental stage, we are approaching the first stage of manufacturing.

*1 POCT is a diagnostic test performed at or near the site of patient whenever necessary. The advantage includes a quick test result as well as its visibility to the patient himself/herself. With these features, the test contributes to improve the quality of medical treatment, welfare, and QOL(Quality of life), allowing quick and appropriate diagnosis, nursing and health care[1].

Downsizing of Blood Cell Counter

The dimensions of the current blood cell counter are approximately: W: 30 cm, D: 40 cm, H: 40 cm with the weight of approximately 18 kg [Figure 1 (a)]. It is compact enough for a desktop-use with high performance, but still requires downsizing as a portable system. The downsizing is especially required to the preprocessing system and the sensing system. In the current preprocessing system, multiple syringe pumps are used. This structure obstructs downsizing of the system and requires an open system (system open to air) where the dilution liquid should be injected in a cell container, etc. Obviously, the open system is not suitable to a palmtop type since the palmtop device is highly supposed to be turned upside down or dropped. This is the reason why the measurement with closed system is required. In the meantime, the palmtop-type device always aims for quick and simple in-situ measurement, so the convenience of the device and reduction of measurement time are inevitable.

Our development focused on the cartridge-type preprocessing part and the chip-type sensor, which enable preprocessing and sensing without syringe pumps in a

closed space [Figure 1 (b)]. Furthermore the cartridge is designed to be disposable to save the need of cleaning (reducing measurement time) and to eliminate the possibility of contamination by other samples.

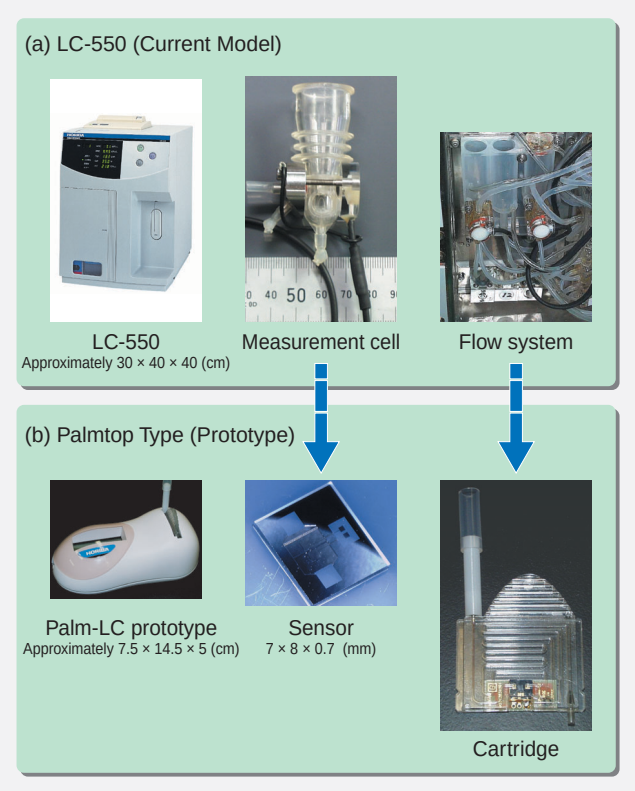

Figure 1 Comparison between the Current Model LC-550 and the Palmtop Type

Blood Cell Counter with Impedance Method using MEMS Technology

Joint Study with Prof. Ishida's Group (Toyohashi University of Technology)

Through the joint study with Prof. Ishida's group (Toyohashi University of Technology) since 1999, we have succeeded in manufacturing the world's first sensor chip for blood cell counter (Figure 2) using MEMS technology[2]. MEMS technology enables fabricating detailed patterns on silicon substrate and allows mass production of the sensors. With this blood cell counter, the output signal pulses of WBC (white blood cell) and RBC (red blood cell) of actual samples were detected (Figure 3). In this article, we describe HORIBA's development for further improvement of detection sensitivity and reproducibility, which is now in progress.

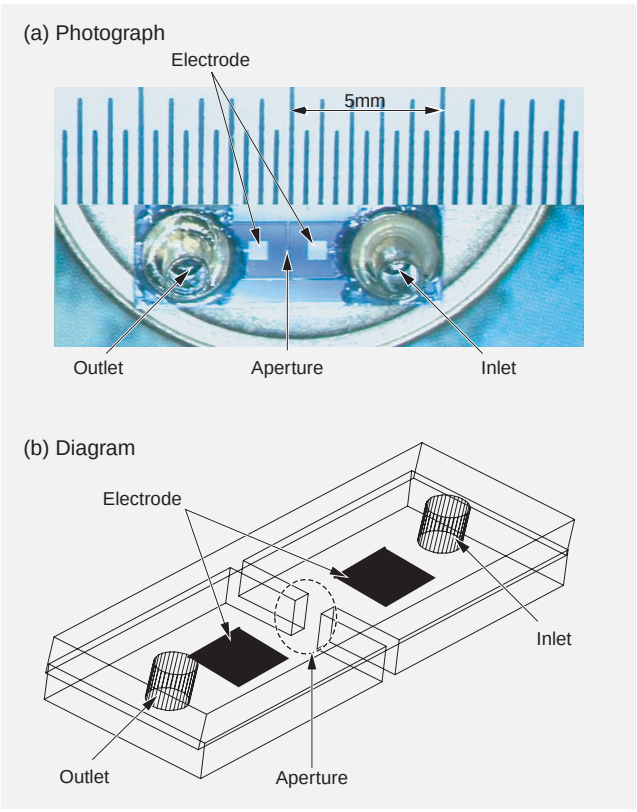

Figure 2 The Proposed Sensor Device for Blood Cell Counting

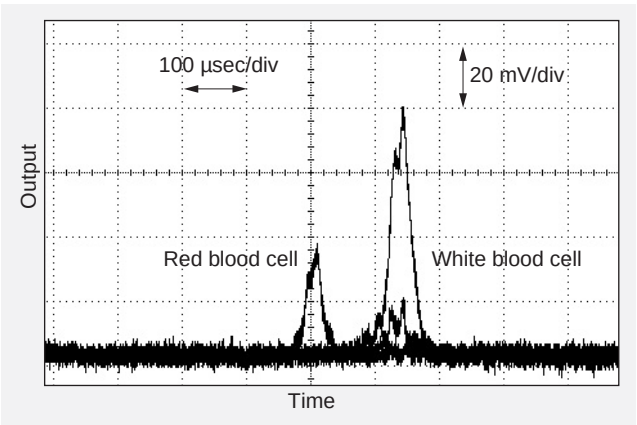

Figure 3 Signal Pulses from Blood Cell

Sensor Structure and Fluid Flow

The blood cell counter sensor is basically structured by silicon substrate and glass substrate which are bonded together. The flow channel is fabricated by creating microscopic grooves and an aperture on the silicon, patterning electrodes on both sides of the aperture, then bonding the glass on which a fluid contact hole is created. Sample enters the contact hole, and passes through the flow channel on the silicon groove and through the aperture. At this time, pulse signals are output due to change of impedance between the electrodes (Figure 4). Then the sample is discharged from the other end of the contact hole.

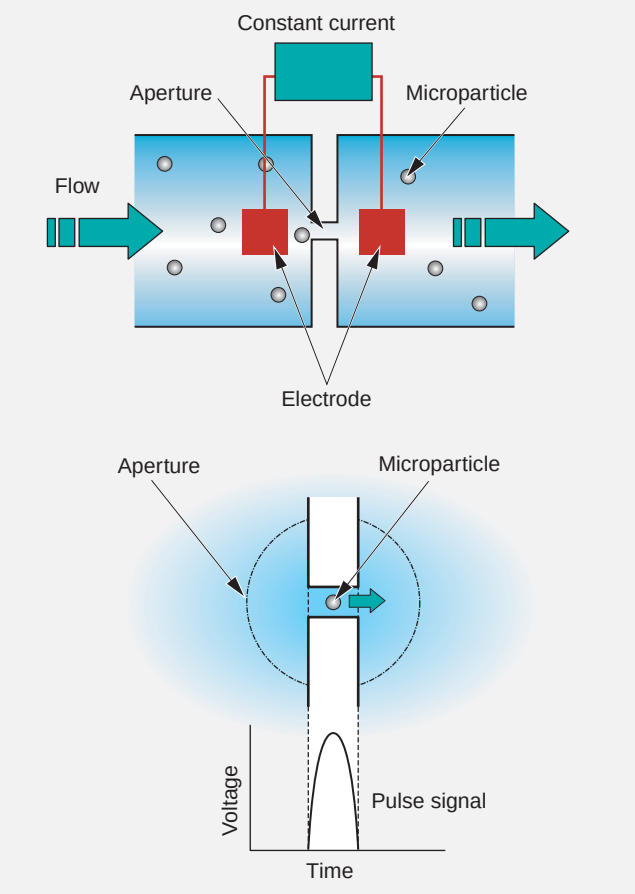

Figure 4 Impedance Method Principle

Improvement of Sensor

The following three are the problems that the sensor structure holds.

- Difficulty in manufacturing electrodes
- Aperture clogged with dust
- Noise by bubbles

These problems and solutions are shown in Table 1 and the details are described below.

Conventionally electrodes were fabricated on a flow channel after it was created on the silicon. However, the flow channel has approximately 50 μm step, which seriously obstructs the effective photolithography, only resulting in less precise processing. We changed the location of electrodes from silicon side to glass side as shown in Figure 5. As the glass side was flat, the precision of processing has been improved. However, the process of bonding glass and silicon requires even higher-precision positioning. We solved this problem with two approaches: a) change the fluid contact hole from glass side to silicon side to eliminate the positioning work to fit to the hole at bonding, and b) create the high-precision adjusting mechanism of electrode positions. Moreover, we changed the processing method of contact hole from sandblast to silicon anisotropic etching to improve the precision of processing.

To solve the problem of clogged aperture, we fabricated a filter with aperture by creating equally-spaced silicon pillars on the inlet flow channel. The improved sensor withstands a few hours of flow in certain conditions while the former sensor was easily clogged with dusts.

Table 1 Problems and Solutions

Problem	Solution
Difficulty in fabricating electrodes	Fabricating electrodes on glass substrate and developing high-precision positioning mechanism
Clogged aperture	Manufacture of filter at the same time with flow channel formation
Noise by bubbles	Change the positions of flow channel and electrodes

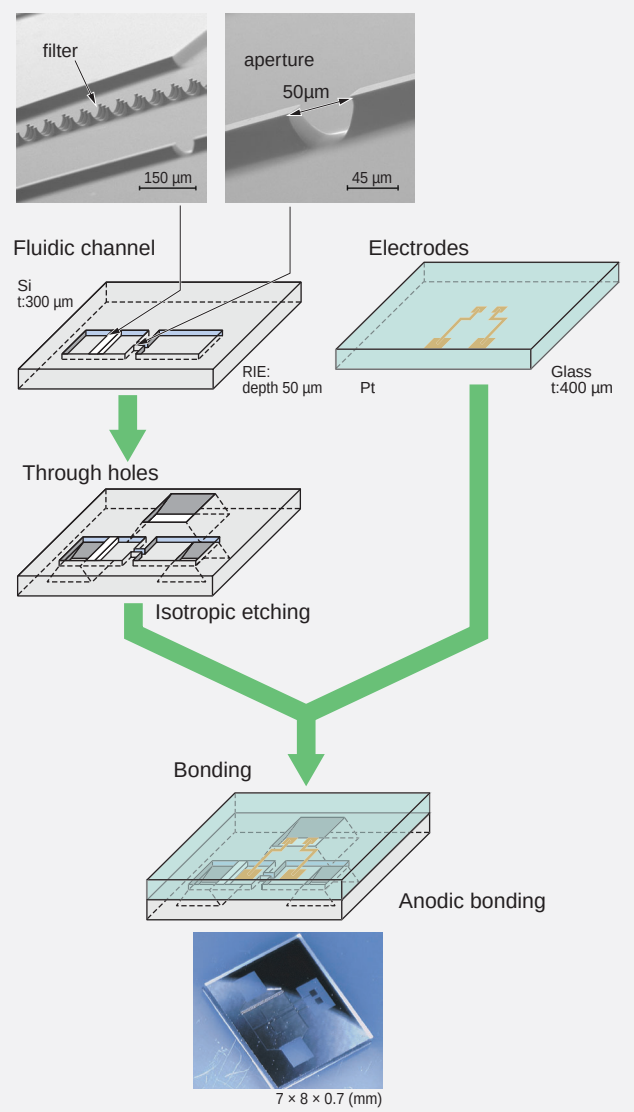

Figure 5 Outline of Sensor Fabrication Process

Of all noises, the noise caused by bubbles is considered to give the most serious effect on blood cell counting. When passing through the aperture, bubbles output pulses equivalent to those of blood cells. A large bubble can be separated into pieces as it flows through the aperture, causing thousands of noises which are miscounted. There are two sources of bubbling: one is inflow from the outside and the other is generation inside. As an example of the former case, a large bubble can be caught before blood sample enters sensor. To avoid the problem, the flow channel should be redesigned not to catch bubbles. The bubbles inside is generated by water electrolysis at electrodes. This is an unavoidable problem as far as impedance method is applied. However, we succeeded in developing an drastic method; As shown in Figure 6 (a), in the conventional sensor, the electrodes were fabricated in up/downstreams of flow channel against the aperture. This structure cannot prevent the bubbles caused at the upstream electrode from passing through the aperture. (Since current LC model has open system, the most bubbles generated at electrodes surface to escape into air.) We divided the flow channel into two at the aperture and fabricated both electrodes in downstream of the aperture as shown in Figure 6 (b). This design allows bubbles generated at electrodes to flow without passing through the aperture. As the electric field is the strongest near the aperture, it is possible to count blood cells at the aperture as conventional procedure. This development has brought a great progress in developing a cartridge-type blood cell counter.

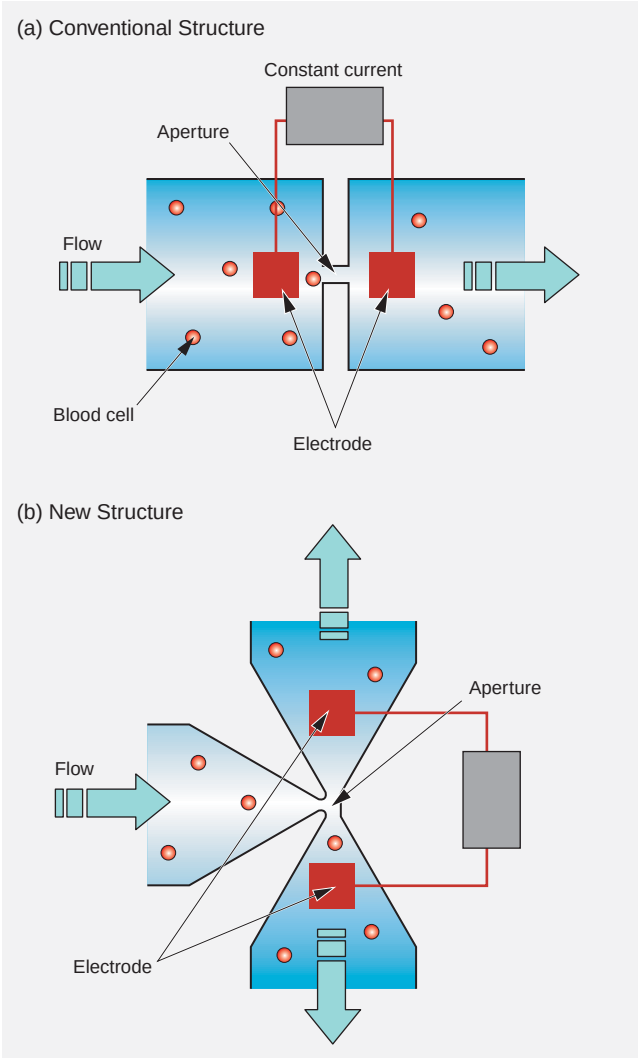

Figure 6 Positions of Aperture, Flow Channel and Electrode

Palmtop Blood Cell Counter Palm-LC (Prototype)

Device Structure

The palmtop blood cell counter Palm-LC consists of three parts: main body, cartridge, and dilution tank. The cartridge includes glass capillary for sampling, sensor for blood cell counting, and fluid level sensor for detecting the end of measurement. The main body basically includes cartridge socket, valve, pump, electronic circuit, and LCD as shown in Figure 7. We are improving cartridge and main body to conform to the newly developed sensor.

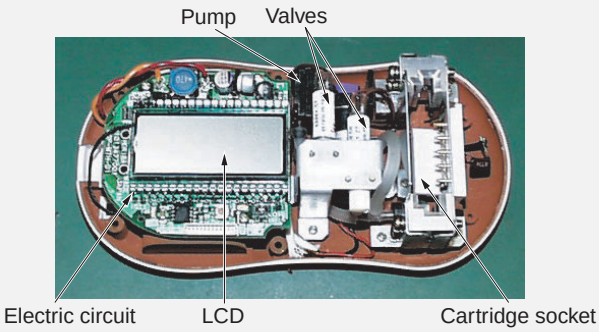

Figure 7 Internal Structure of the Main Body

Measurement Sequence

As shown in Figure 8, prick a fingertip of patient with lancet etc. and collect blood sample with glass sampling capillary inside the cartridge (1 µL of blood sample is collected with glass capillary). Insert a dilution tank into the tip of sampling part. Then insert the cartridge with the tank into the main body. The device power turns on as the cartridge is inserted. The blood sample collected with glass capillary flows into the dilution tank which contains a dose of diluted solution by applying pressure, then the mixing starts (lasts for approximately 20 seconds). After mixing, introduce the sample to the sensor with the pressure reduced and start the measurement. The waste fluid is accumulated in the cartridge gradually up to the fluid level sensor. When it reaches to the fluid level sensor, measurement is completed (measurement time is approximately 1 minute). After calculation, the measurement result is displayed.

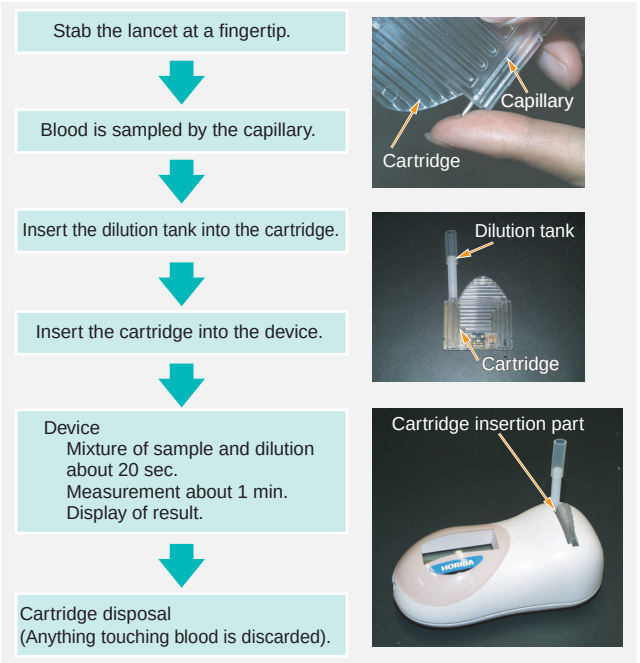

Figure 8 Structure of Palmtop Blood Cell Counter Palm-LC and Measurement Sequence

Conclusion

We are currently developing the palmtop-type blood cell counter which targets WBC as a measurement item. The device to measure multiple blood components including WBC is also planned. There is a growing expectation to MEMS technology for POCT. It is certain that µ-TAS (µ Total Analysis System) will be continuously developed in various applications other than blood cell. We also hope to be a leading company of the world continuously in this field.

Reference

[1] The POC Promotion Committee
http://www1.sphere.ne.jp/jidouka/Q&A.htm

[2] D. Satake, H. Ebi, N. Oku, K. Matsuda, H. Takao, M. Ashiki and M. Ishida, A sensor for blood cell counter using MEMS technology, Sensors and Actuators B 83, 77 - 81 (2002)

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