New Products

Damage-less Sorting System for Live Cells

1. INTRODUCTION

Research on live cells, especially on human embryonic stem (hES) cells and stem cells, is crucially important for regenerative medical treatment, clarification of oncogenesis mechanism and tailor-made medical treatment. A system, which can acquire such kinds of living cells in good condition, will be a very useful tool for medical researchers worldwide. Taking advantage of its proprietary technologies in fiber optics and mechatronics, Furukawa Electric has developed and commoditized a flow cytometer (cell sorter) that features damage-less sorting for the first time in the world. Here we will describe the features and performance of our new product.

2. FEATURES

2.1 Fiber-Optic Flow Cytometer

A flow cytometer is an analytical apparatus, which is employed to identify in a short time various kinds of cells existing in a cell population, whereby single cells which flow in a flow cell are illuminated by an exciting light beam to emit the scattered and fluorescent light signals, and these light signals are analyzed to achieve cell analysis. We have developed an entirely new fiber optic flow cytometer that directly connects optical fibers to the flow cell with the aim of both laser light illumination and fluorescent and scattered light reception. Figure 1 shows the structure of the optics system. Figure 2 shows the measurement results of 8 kinds of fluorescent beads with different fluorescent intensity spectra, demonstrating that the fluorescence sensitivity of our system is below 200 MESF (molecules of equivalent soluble flourochrome).

Moreover this optics structure allows for obtaining new information from the transmission light (which corresponds to the projection area of cells) instead of the forward scatter light conventionally used, taking advantage of the fact that the cells to be measured and the endface of the optical fiber for reception are in close proximity with

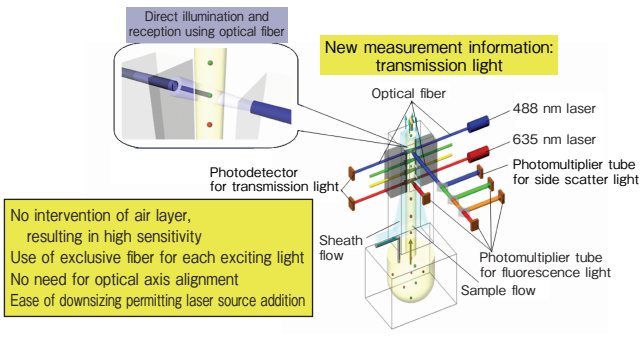


Figure 1 Structure of flow cytometer using optical fiber.

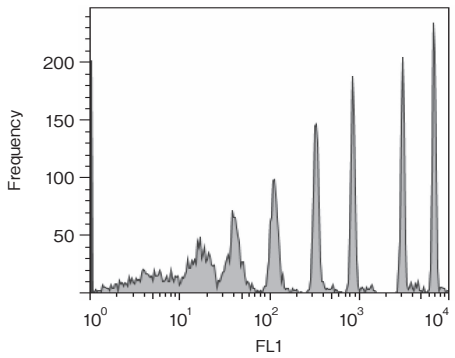
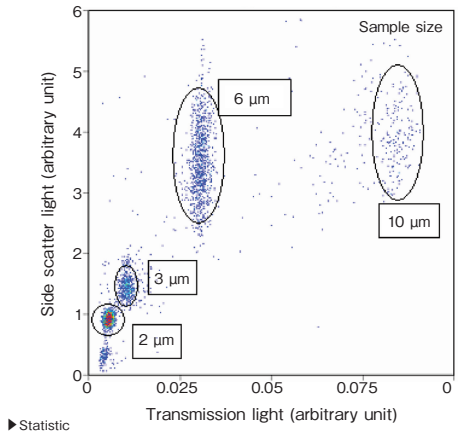


Figure 2 Measurement results of fluorescence sensitivity.



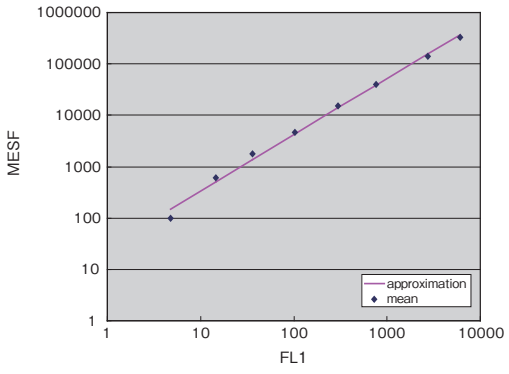


Figure 3 Evaluation results of transmission light information.

each other (i.e., several hundreds μms). Figure 3 shows the measurement results of the beads with different sizes. A strong correlation between the transmission light and the beads size can be seen, demonstrating a significant improvement in the accuracy of sample size recognition. It is expected that this transmission light information offer new valuable information on cell analysis in the future, so that we are promoting development of new application software to this end.

2.2 Damage-less Sorting

A flow cytometer has a function to sort target cells, besides cell analysis function. In the conventional sorting method, cell-containing droplets are formed using a high hydraulic pressure and ultrasonic waves, and subsequently a high-intensity electric charge is applied to the droplets before sorting. Thus there is a danger that live cells may be damaged or stressed through this process, rendering in some cases this process unsuitable to be used for sorting the cells with multi differentiation potency, such as ES cells and stem cells, as well as other tender cells.

We have introduced a new sorting method into our flow cytometer, which has completely eliminated ultrasonic-based droplet formation and high-voltage droplet charging conventionally used. In our flow cell, multiple optical fibers are accurately arrayed along the fluid channel, allowing for precise measurement of the flow velocity of each sample. As illustrated in Figure 4, the time when a target sample reaches the front end of the sorting nozzle is calculated based on this flow velocity information, and in synchronization with the arrival time, the nozzle front end is moved from the drain tank to the specified well on the culture plate, thereby achieving sorting of the target cell. This method has completely eliminated the use of ultrasonic waves and high-intensity electric charges that constitute the potential factors of damage and stress to the cells, thus realizing a damage-free sorting method that is boundlessly friendly to the cells. Moreover, since this method is able to sort a single cell to an arbitrary well, the method is expected to make a significant contribution to the study of ES cells and stem cells in particular that are required for regenerative medical treatment.

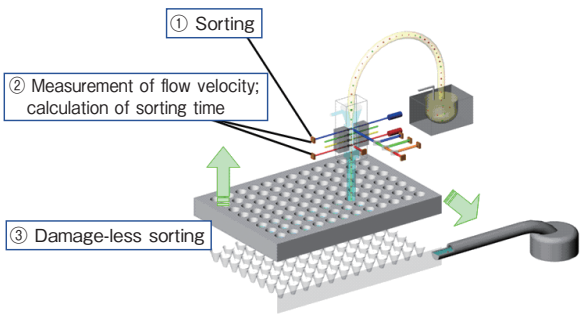


Figure 4 Schematic of damage-less sorting system.

3. EVALUATION EXAMPLES

Figure 5 shows the result of lymphocyte subset measurement for human peripheral blood mononuclear cells, using the FITC-conjugated anti-CD4 and PE-conjugated anti-CD8 antibodies. Erythrocytes and granulocytes were removed in advance from the sample by centrifugal separation in conjunction with a Ficoll-Paque fluid. Subsets are identified through fluorescent signals generated by each antibody specifically bound to its special cluster of differentiation (CD).

Figure 6 shows the sorting results for megakaryocite (i.e., a kind of cell constituting blood platelet, approximately 150 μm in diameter) which is, known as one of the

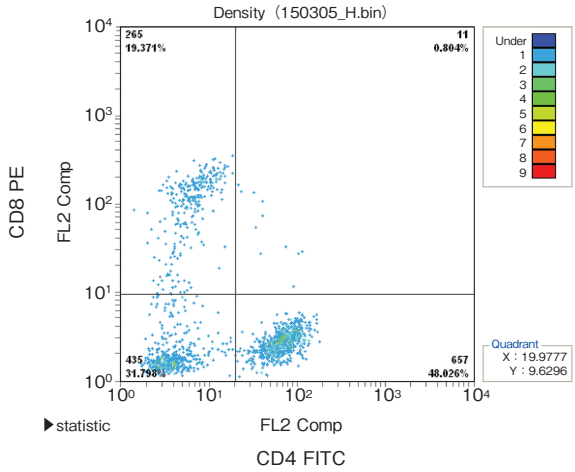
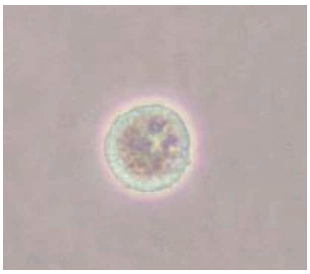
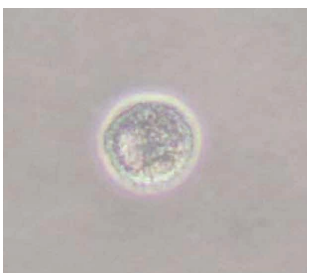


Figure 5 Measurement result of subset of human peripheral blood mononuclear cells.



Immediately after sorting



After three days cultivation

Figure 6 Sorted megakaryocite.

most fragile live cells, absolutely impossible to be treated using conventional cell sorters. It has been confirmed that the cells are alive for three days after sorting.

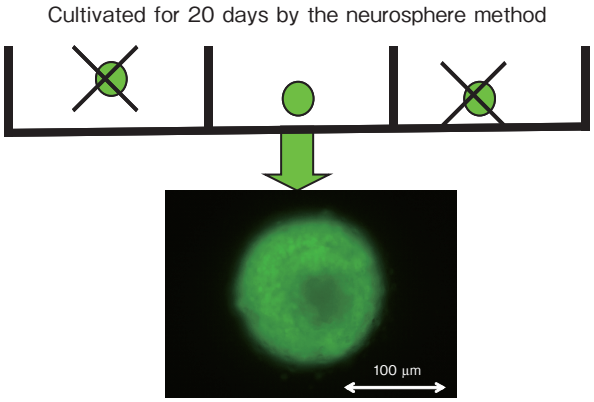


Figure 7 Colony formed from sorted single neural crest stem cell.

Figure 7 shows the result of an experiment carried out to find neural crest stem cells. In this experiment, the green fluorescent protein (GFP) gene was injected into a fertilized egg of mouse to obtain the mouse embryo expressing GFP. Brain tissue was taken out 9.5 days after fertilization, and resultant single cells were sorted into each well on a 96-well culture plate. As shown in Figure 7, a cell colony has been formed after about 20 days of cultivation. This result confirms that sorting has been successfully achieved with less damage even for the fragile brain tissue.

4. PRODUCT SPECIFICATION

Figure 8 shows the appearance of two models of our products, and Table 1 main product specifications. The PERFLOW Ana is the model for analysis only, capable of measuring four fluorescence lights, as well as the scatter and side scatter lights, using its two lasers with different wavelengths. The PERFLOW Sort is equivalent to the

PERFLOW Ana. PERFLOW Sort.

Figure 8 Appearance of PERFLOW Ana and PERFLOW Sort.

Table 1 Specifications of PERFLOW Ana and PERFLOW Sort.

Model	PERFLOW Ana	PERFLOW Sort
Light source	Blue (488 nm) semiconductor laser Red (635 nm) semiconductor laser	
Measurement parameters	Transmission light (corresponding to forward scatter light), Side scatter light, Four fluorescence lights (FITC, PI (PE), PeCy5, APC)	
Detection sensitivity	FITC < 200 MESF (when Spherotech Rainbow Beads is used)	
Fluorescence light detection resolution	CV value (variation coefficient): 4 % or less (when BD QC Particles is used)	
Side scatter light detection sensitivity	Capable of detecting ϕ2-μm beads	
Sample analysis speed	20,000 event/sec	
Optical axis alignment	Unnecessary	
Sorting method	—	Mechanical flow switching
Sample acquisition speed	—	1 sample/sec
Sample acquisition function	—	6~384 multiple-well plate supported
Software	Exclusive control and analysis software is installed in standard specification (FCS compatible)	
Power supply	100 V AC, 50/60 Hz, 4 A	100 V AC, 50/60 Hz, 9 A
Dimensions	400 x 550 x 450 mm (WDH)	550 x 600 x 600 mm (WDH)
Mass	Approx. 40 kg	Approx. 90 kg

PERFLOW Ana in terms of the analysis function, and is provided with a damage-less sorting function which allows for sorting of a single cell to an arbitrary well. Both models have achieved considerable downsizing taking advantage of their proprietary optical fiber-based optics system. Moreover, since the number of the optical fiber array can be expanded to eight at the maximum, it is possible to add UV lasers and the like as options.

In an effort to achieve cost-effectiveness of this apparatus so that every laboratory can afford it, as well as to break down the traditional knowledge such that a flow cytometer is an expensive apparatus, we have employed the brand name "PERFLOW" to carry a meaning of "Personal Flow Cytometer". It is expected that the PERFLOW featuring transmission light exploitation and damage-less sorting will contribute to the leading-edge life-science study involving ES cells and stem cells, as well as to the development of regenerative medical treatment.

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