AUTOMATED SORTING OF ADULT STEM CELLS AND MOUSE EMBRYONIC BODIES

ABSTRACT

In this report, we discuss and demonstrate use of the BioSorter instrument for the analysis and sorting of individual and clusters of adult stem cells and embryoid bodies, based on both size and fluorescence intensity properties. Specifically, based on fluorescence intensity of either antibody conjugated or internally expressed fluorophores, we use the instrument to track the differentiated status of stem cells or expression profiles of embryoid bodies, and it is our assertion that the BioSorter can be a versatile tool for stem cell research labs. *Due to confidentiality agreements, specific sample name and source information cannot be disclosed.

INTRODUCTION

Flow cytometry is a useful technique for the analysis and sorting of individual stem cells. Now, high throughput functional studies and sorting of stem cell clusters are possible using with large particle flow cytometry. This may provide researchers with additional information regarding cell function in the context of a cell cluster, offering greater cell survival efficiency, as well as exploring other preparative and culture-growth conditions. In this document I describe tests using the BioSorter™ 250 flow cytometer from Union Biometrica, Inc. for the in-flow analysis and sorting/dispensing of specific cell types derived from adult stem cells and embryoid bodies derived from mouse embryonic stem cells. The large, 250 micron flow cell of this instrument can accommodate objects up to approximately 125 microns in diameter, which is suitable for the analysis of both single cells and cell clusters.

Based on the principles of flow cytometry, BioSorter® instruments, featuring our proven COPAS™ – Complex Object Parametric Analyzer and Sorter technology, analyze large (20 - 1,600 micron) particles in a continuously flowing stream at a rate of up to 100 objects/second. Using object size, optical density, and intensity of fluorescent markers as analytical criteria, particles can be sorted and dispensed into Petri-dishes or multi-well microtiter plates for further analysis. A gentle pneumatic sorting mechanism located after the flow cell does not harm or alter sensitive objects, thereby making the instrument suitable for live biological materials or sensitive chemistries. (Figure 1)

For these experiments, we used a standard instrument featuring a red diode laser (670 nm) which is used to measure the axial length and the optical density of the object, and a multi-line argon laser (488/514 nm) which is used to excite multiple fluorophores with different excitation wavelengths.

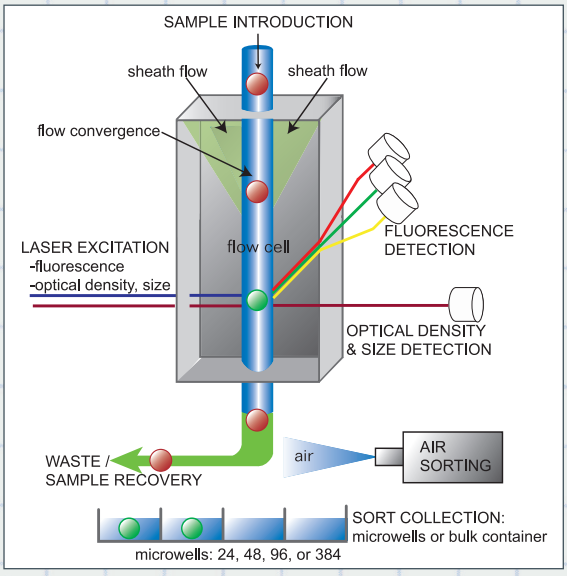

Figure 1. Analysis of objects inside the flow cell. *Objects are carried through the flow cell by a liquid stream while their physical properties are being measured. Convergence of the sheath and sample fluid allows "hydrodynamic focusing" of the objects, forcing them to go through the center of the flow cell along their longitudinal axis. Inside the flow cell objects are illuminated by two lasers and the object's optical properties (of: size, optical density, and fluorescence intensity) are measured. Those objects meeting sort criteria are permitted to drop into a collection vessel, while those that do not are diverted to waste recovery using a pneumatic sorting device.*

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Adult stem cells and mouse embryonic bodies were obtained from an external confidential lab.

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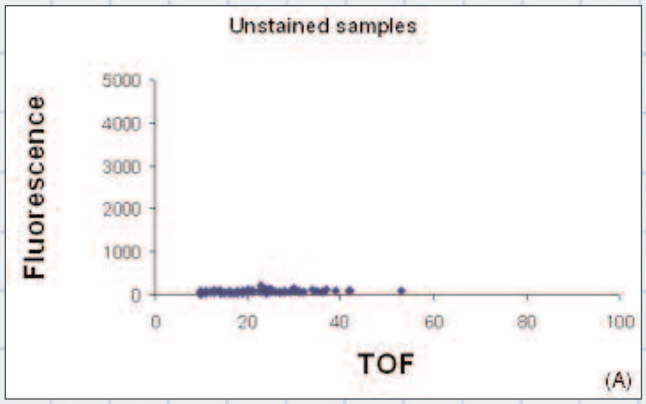
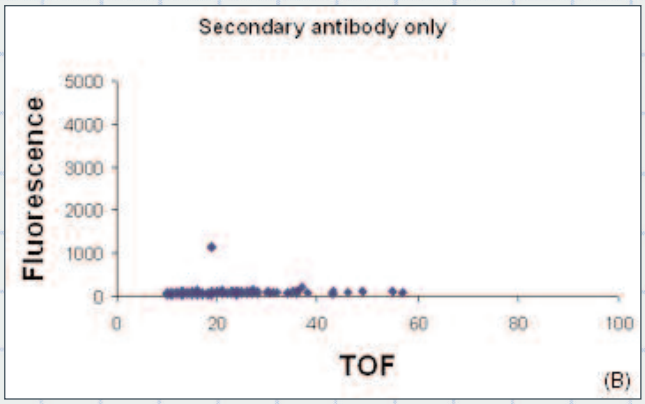
RESULTS

The BioSorter instrument measures five parameters of an object: TOF (Time of Flight, equivalent to length or diameter along the longitudinal axis), EXT (EXTinction, equivalent to the optical density), and three fluorescence intensities (Green, Yellow and Red). The COPAS software displays data in real time in two possible ways: a single parameter representation in histogram mode, and a dual parameter representation in dot-plot mode. In either mode, a subset of data can be defined through creating a "gate" which is a numerical or graphical boundary that can be used to define physical characteristics of objects to include for further analysis and/or sorting. (Figure 3 shows a gate drawn in dot-plot mode.)

Detection of specific cell types derived from adult stem cells

Specific cell types derived from the adult stem cells can be identified by staining with fluorophore conjugated antibodies against the corresponding cell markers. Figure 2 shows the comparison of the measured fluorescence intensity between an antibody-stained adult stem cell sample and its negative controls. As expected, fluorescence was only detected in the stained sample.





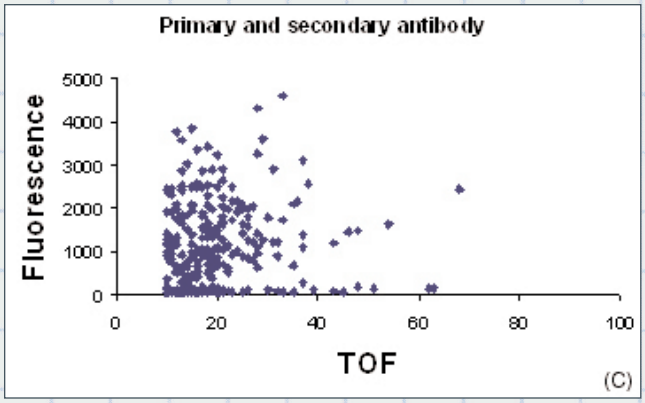


Figure 2. Detection of specific cell types derived from adult stem cells based on antibody staining. *TOF: Time of Flight, equivalent to length; Fluorescence: Green fluorescence intensity. Fluorophore was conjugated to the secondary antibody. Analysis of raw COPAS data exported into Microsoft® Excel®.*

Because of the ability to differentiate into multiple cell types, stem cells are considered to bear great potential for research and medical uses. Therefore, it is important to establish conditions where stem cells can be coaxed into adopting specific cell types. As shown in Figure 3, by staining with two different primary antibodies, we were able to compare the percentage of adult stem cells adopting two different cell types. The result shows that the experimental condition facilitates the stem cells to differentiate into cell type II, but not cell type I.

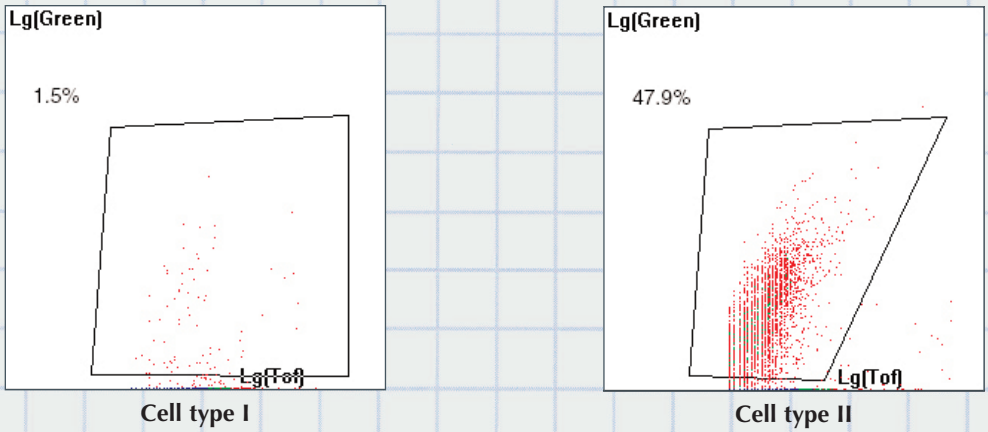


Figure 3. By staining with antibodies against different specific cell markers, different cell types that are derived from the same batch of adult stem cells can be identified using the BioSorter instrument. As shown in the dot-plots, the cells differentiated predominantly into cell type II.

Size analysis

Figure 4 shows the comparison of standard 42 µm beads and the adult stem cells on the TOF-EXT dot plot. The 42 µm beads form a tight cluster all within a narrow size distribution. The cells form a more broad distribution, primarily smaller than the 42 µm beads on the TOF axis. While not performed for this report, users may also determine the actual cell sizes by using a series of standard beads with known sizes as reference points.

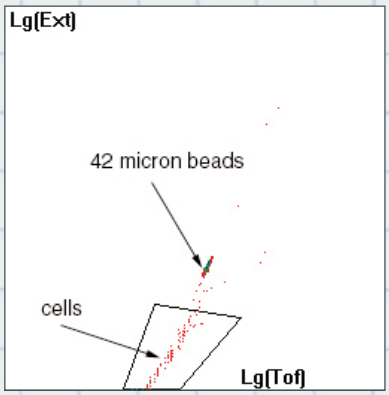


Figure 4. Comparison between standard 42 µm beads and stem cells. *Lg[TOF]: Log scale of Time of Flight. Lg[EXT]: Log scale of Extinction.*

Sorting based on size and fluorescence

As discussed above, the user can define "gate" regions within a dot-plot to define physical characteristics of objects to include for sorting. In this example, size and fluorescence intensity properties were used. Figure 5 shows the sorting regions for collecting fluorescent objects with different sizes from the stained adult stem cell sample. Figure 6 shows the microscopic images of the sorted objects. By using the log scaling feature within the Advanced Acquisition Package, even small objects are detected with the increased detection sensitivity.

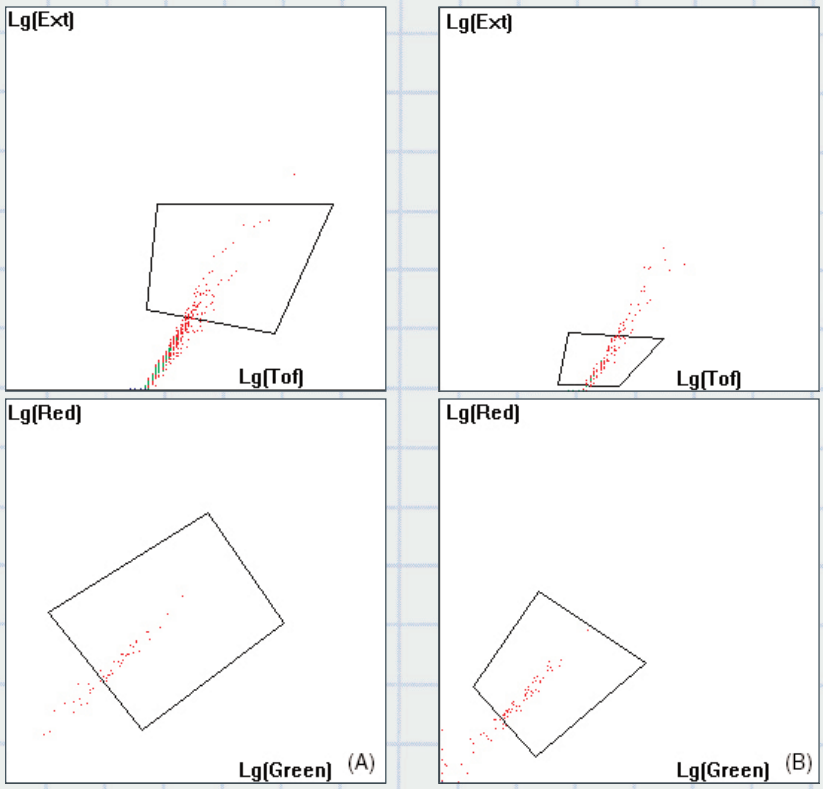
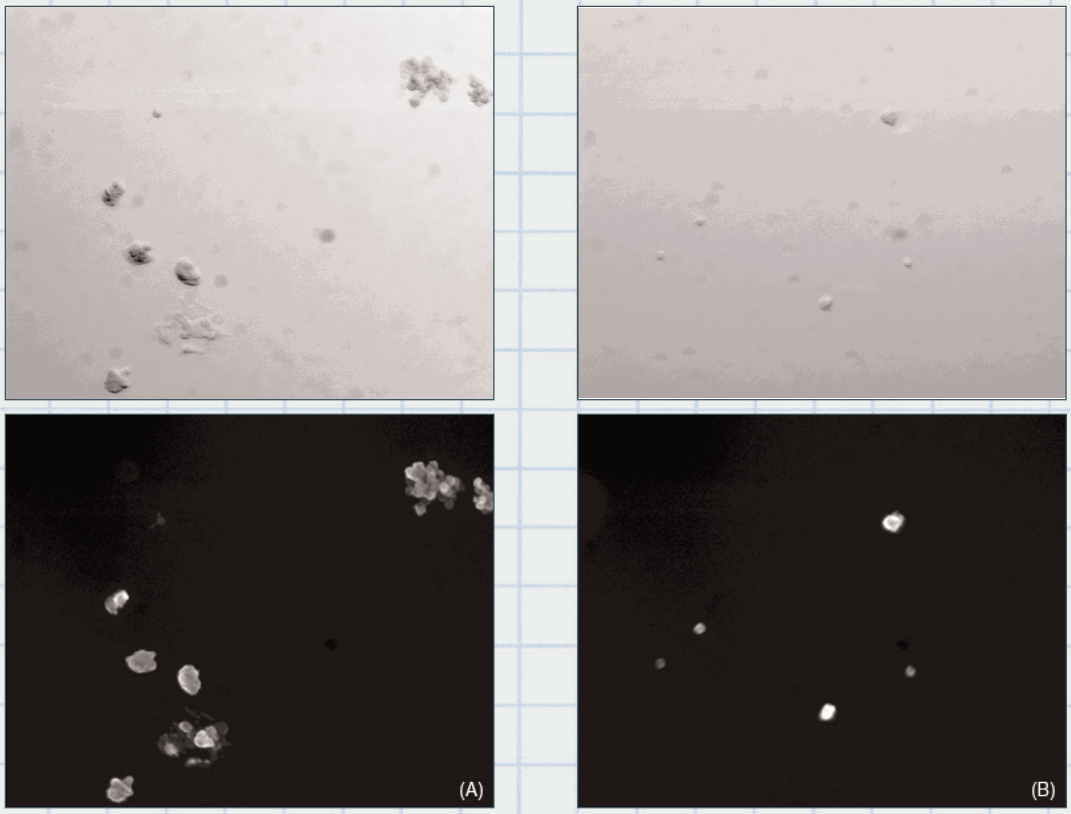


Figure 5. Sorting based on object size and fluorescence. *(A) Sorting regions for fluorescent objects with large sizes (mostly cell clusters); (B) Sorting regions for fluorescent objects with small sizes (mostly single cells).*

Lg[TOF]: Log scale of Time Of Flight.
Lg[EXT]: Log scale of Extinction.
Lg[Green]: Log scale of green fluorescence intensity; Lg[Red]: Log scale of red fluorescence intensity.



Bright field images

Fluorescent images

Figure 6. Images of sorted cells. *(A) Fluorescent objects with large sizes (mostly cell clusters); (B) Fluorescent objects with small sizes (mostly single cells). See Figure 5 for the corresponding sorting regions.*

Sorting embryoid bodies based on fluorescence

The BioSorter instrument is also able to analyze and sort embryoid bodies (EB), as the large flow cell size can accommodate even these large clusters that usually contain hundreds of cells. In this experiment, EBs carrying cell type-specific promoter driven green fluorescent protein (GFP) gene were induced to express GFP under certain experimental conditions. The population of EBs that exhibit this fluorescence property was analyzed and sorted. Figure 7 shows the Lg(TOF)-Lg(Green) dot plot for the sorted mouse EB sample. As shown in the figure, a sorting region was set so that only highly fluorescent EBs were sorted, regardless of size.

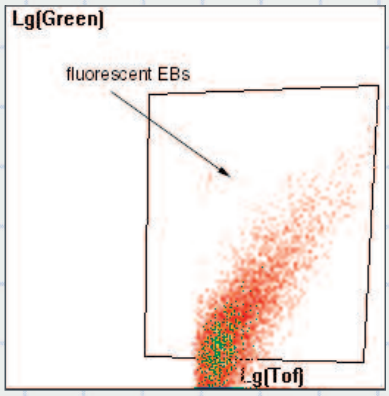


Figure 7. Sorting EBs based on fluorescence. *A sorting region was drawn to collect only fluorescent EBs. Lg[TOF]: Log scale of Time Of Flight. Lg[Green]: Log scale of green fluorescence intensity.*

CONCLUSION

Unlike traditional flow cytometers, the BioSorter instrument features a large flow cell and gentle pneumatic sorting mechanism, making it ideal for analysis and sorting of stem cell derived structures, such as embryoid bodies, neurospheres, cardiospheres, or other cell clusters which are usually too big or too fragile to be sorted on traditional flow cytometers. In this report, we discussed and showed that the BioSorter instrument is able to analyze and sort individual or clusters of adult stem cells and embryoid bodies based on both size and fluorescence intensity properties. Specifically, based on fluorescence intensity of either antibody conjugated or internally expressed fluorophores, we were able to use the instrument to track the differentiated status of stem cells or expression profiles of embryoid bodies,making it a versatile tool for stem cell research labs. With the relatively high analysis and sorting speed (up to 100 objects/second), the BioSorter instrument can greatly enhance the throughput for developmental and functional studies of stem cells, help optimizing culture conditions and allow researchers to do experiments in a multi-cellular context.

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