Automated analysis and sorting of human induced pluripotent stem cell (hiPS) clusters using large particle flow cytometry.

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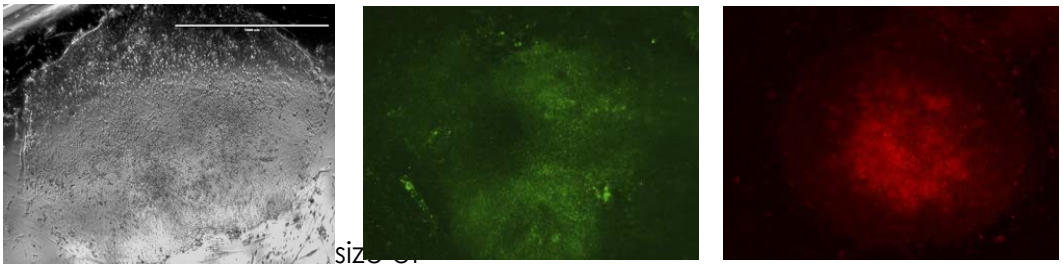
Objective

The purpose of this experiment was to test the feasibility of using the COPAS PLUS HTS instrument (Union Biometrica, Inc.) to analyze and sort intact human induced pluripotent stem cell (hiPS) clusters based on their size, optical density and fluorescent properties.

Introduction

The reprogramming of somatic cells into human induced pluripotent stem cells (iPS cells) has opened unique perspectives for producing disease and patient specific human cell products. To take full advantage of this technology there is a need to produce a high number of iPS cell lines using high throughput techniques, to standardize the respective protocols and to deliver fully characterized cells. A high throughput technique, which is fully automatable and capable of selecting and sorting cell clusters, is highly desirable. Union Biometrica's large particle flow cytometry technology (COPAS and BioSorter) allows the analysis and sorting of intact hiPS cell clusters based on size, optical density and fluorescent parameters. Figure 1 depicts a typical cell colony of interest as dispensed by the COPAS flow cytometer (approximately 300 µm diameter). The micrographs show a phase contrast image of a colony and additionally colonies stained with cell tracking dyes CFSE- (middle) and PKH26 (right). The colonies remain intact throughout the procedure.



size of

Figure1. Intact hiPS colony before processing by COPAS PLUS HTS showing (left). HiPS colonies (middle) stained with CFSE (middle) and PKH26 (right)

Materials and Methods

The COPAS PLUS HTS instrument (Union Biometrica, Inc) is a large particle flow cytometer which is able to analyze and sort large objects (~20-700 µm diameter) at a high rate (up to 50 events per second) on the basis of the physical characteristics of size, optical density and fluorescence signals. A 488 nm solid state laser is used to measure both the size (TOF) and optical density (EXT) of the cell colonies. Additionally, if the sample contains certain fluorophores that can be excited by the 488 nm light, their emission levels can be detected for each of the objects in the sample. The system used during the tests described here was also configured with a 561nm solid state laser in order to excite the red dye PKH26.
The COPAS PLUS HTS instrument is also capable of sorting. A gentle pneumatic sorting mechanism provides a means for dispensing sensitive objects like cell clusters without disrupting the clusters. Consequently this instrument can identify objects with similar features and dispense these to various formats (Petri dishes or multiwell plates) for further use and analysis.

HiPSC were cultured on MEF and Matrigel in 6-well plates. A collagenase treatment was used to detach the colonies. Cells were physically disrupted into smaller clusters by pipetting. After a washing step the cells clusters were stained with the CFSE (FITC) or PKH26. CFSE has an emission max of 494 nm and is a reactive dye which binds covalently to cytosolic and membrane proteins. PKH26 has on emission max of 567 nm and is a lipophilic dye which partitions non-covalently into cell membranes. Both dyes are used for tracking of cells. After staining the cell clusters were diluted into 50 ml conical tubes (Figure 2). These conical tubes can directly be supplied to the system for analysis. To experimentally determine whether the COPAS PLUS HTS is suitable to preserve clonality of the cell clusters, the PKH26 and CFSE batches were mixed and subseqently sorted under sterile conditions.

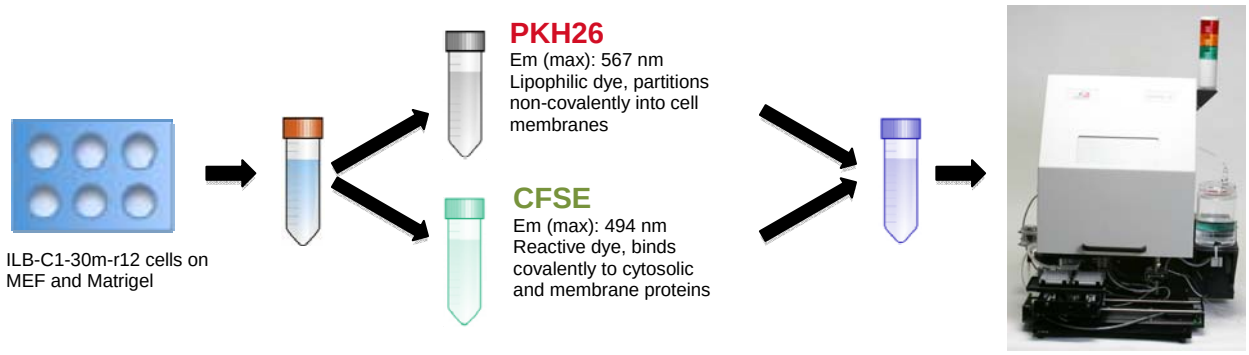


Figure2. Experimental outline for COPAS PLUS HTS clonality experiment

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Results

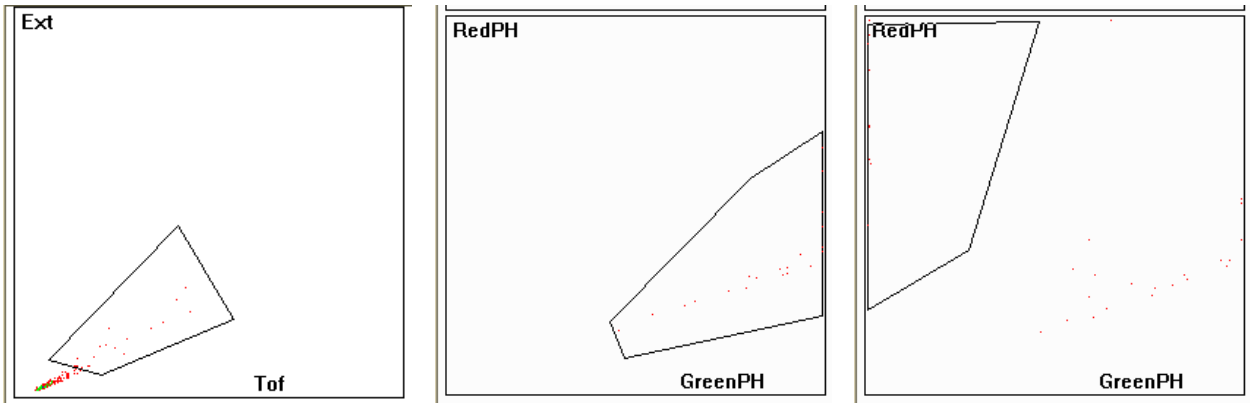

Figure3. Dot plots from analysis of the hiPS clusters on the COPAS PLUS HTS. The left panel shows a dot plot of Tof (time of flight, size) vs. Ext (extinction, optical density). The region (polygon) was set around cell clusters of approximately 300 micron diameter to eliminate small debris and very large clusters. Sorting regions were drawn around clusters stained with CFSE (green label, middle panel) and clusters stained with PKH26 (red label, right panel). Data display for peak height is a feature of the Profiler function and shows the highest intensity level within a cluster. The dot plot shows Green fluorescence Peak Height (GreenPH) vs. Red fluorescence Peak Height (RedPH).

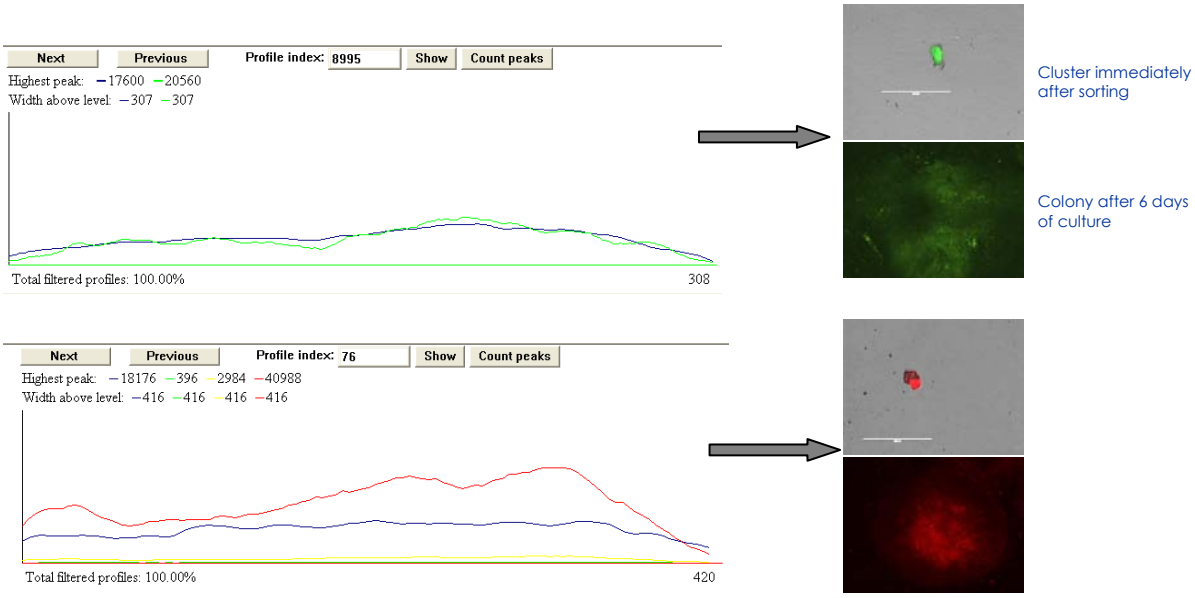

Figure4. Profiler graph shows the distribution of CFSE (green) and PKH26 (red) along the axis of the cell cluster. The blue line represents the optical density profile. The horizontal axis represents the TOF (size) of the cell cluster. The micrographs on the right side show the corresponding cell cluster as seen by a fluorescence microscope.

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The Profiler option (Figure 4) can be used to set additional sorting parameters. For instance, criteria can be set such that only cell clusters will be dispensed that have a homogeneous distribution of fluorescence, thus excluding partially stained cell clumps. All sorted and evaluated cell clusters showed only either red or green fluorescent which indicates that clonality can be preserved during the whole sorting process.

Conclusions

These experiments demonstrate that the COPAS PLUS HTS can be used to analyze and sort primary human induced pluripotent stem cell colonies. The instrument can analyze accurately and sort similar cell clusters from a complex mixture of varying sizes. This automated analysis and sorting process is gentle and does not influence the morphology or viability compared to manually sorted cell clusters. The COPAS PLUS HTS provides a level of automation to the process of handling the stem cell clusters allowing for increased throughput and eliminates any biases that might be introduced by the researcher. Additionally, the Profiler II allows discriminating clusters with full from those with partial fluorescence labelling. Physical properties like size, optical density and fluorescence can be traced back for every dispensed cluster. When combined with a fluorescence microscope, correlation between the profile and the micrograph can be made for every individual cluster. Fluorescence distribution in the cluster allows valuable information about the heterogeneity. All sorted and evaluated cell clusters showed only either red or green fluorescent which indicates that clonality can be preserved during the whole sorting process.

COPAS PLUS HTS instrument brings the method of flow cytometry to the analysis and sorting of stem cell clusters which are otherwise too large and fragile for analysis on conventional single-cell flow cytometers. Cell clusters can be analyzed and dispensed while intact, allowing the automation of conventional and low throughput manual procedures to one of increased throughput. This instrument brings the advantages of flow cytometry – statistically meaningful data, large unbiased data sets, and multi-parametric analysis – to experiments using stem cell clusters.

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