Validating and optimizing single cell sorting of FACS using Celigo image cytometry

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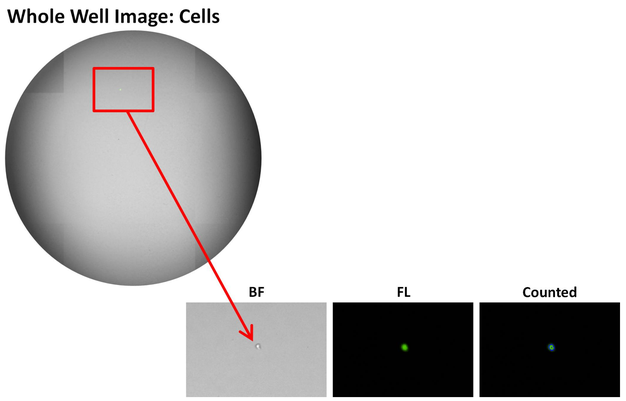
1. ABSTRACT

One of the major applications that is commonly performed on a fluorescence activated cell sorting (FACS) instrument is single cell sorting. Single cell sorting application is mainly used for research such as cell line development to ensure monoclonality for protein production. In addition, regenerative medicine often uses single cell sorting to study stem cell proliferation from single cell to single colony. Currently, single cell sorting is validated via light microscopy several days after initial sorting, where the cells have grown into an observable colony. However, manual observation using microscopy is highly tedious and time-consuming. Therefore, there is a need for a high-throughput, practical, and accurate detection to validate and optimize single cell sorting of FACS. In this work, we demonstrate a novel high-throughput detection method to validate and optimize single cell sorting using the Celigo Image Cytometry (Nexcelom Bioscience, Lawrence, MA). The instrument was used to image the entire well of all 96 wells on a microplate to detect a single object sorted into the well in less than 4 min. Initially, the FACS (MoFlo Astrios EQ) was used to sort single green fluorescent bead into multiple 96-well microplates in two separate experiments. The microplates were used without any buffer, thus the number and location of beads can be accurately detected. Next, the results from the two experiments showing a sort efficiency of 82 and 90% were used to optimize the FACS to increase the efficiency closer to 100%. Finally, a practical experiment was performed where GFP expressing cells were sorted into multiple 96-well plates to determine the final efficiency of sorting actual cells. After imaging and analyzing the plate data, more cells can be sorted into wells without single cell. If more than one cell exist, then this allows the users to quickly disqualify those wells and minimize the amount of reagents or time needed. The ability to rapidly detect single cell in multi-well microplates is highly important to both flow core laboratories to optimize their sorting instruments as well as to the users, who would like to confirm single cell in each well. The proposed method can highly improve the efficiency of work flow for both flow core managers and users.

2. CELIGO IMAGING CYTOMETRY FOR HIGH-THROUGHPUT SINGLE CELL DETECTION



1. Celigo Imaging Cytometer is a plate-based cytometer that can scan the entire well of standard microplates and capture bright-field and fluorescent images
2. The captured images are analyzed with the Celigo software to measure size, morphology, cell count, confluence, and fluorescent intensity
3. The measured parameters are used to generate cell proliferation kinetic data, GFP/RFP expression, tumor spheroid size change, DNA cell cycle analysis, apoptosis, and ADCC cytotoxicity results

3. SINGLE CELL SORTING AND DETECTION PROTOCOL

Prepare Green Fluorescent Beads or Jurkat cells stained with calcein AM

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Prepare a half-area 96-well Greiner plate (675090) with 20 µL of PBS

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Sort single cells into 96-well plate using Beckman Coulter MoFlo Astrios EQ

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After sorting, allow cells to settle for 15 min

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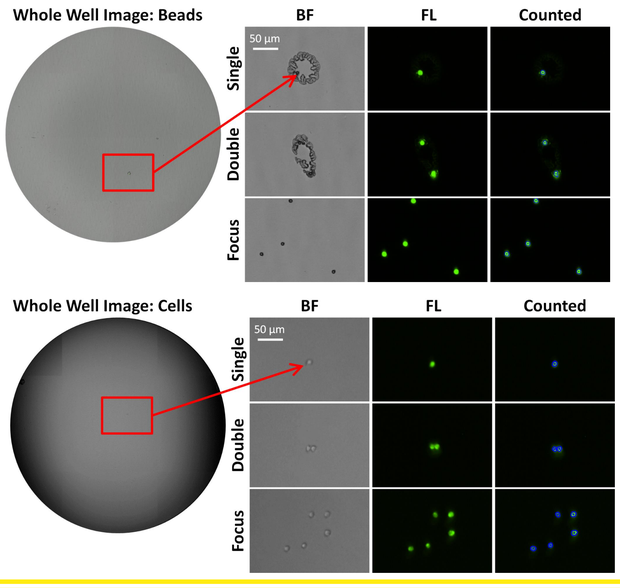
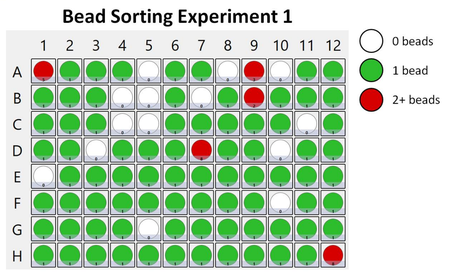
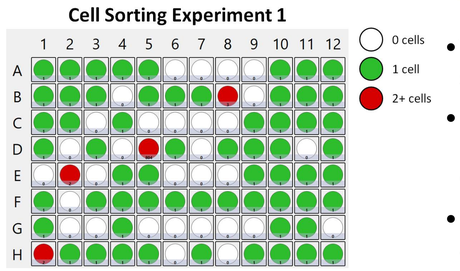
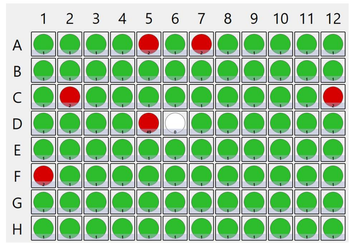
Use the Celigo to scan and count single cell in each well

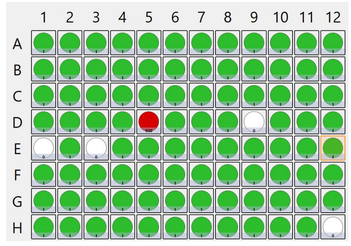


4. SINGLE CELL SORTING EFFICIENCY MEASUREMENT USING CELIGO IMAGE CYTOMETER

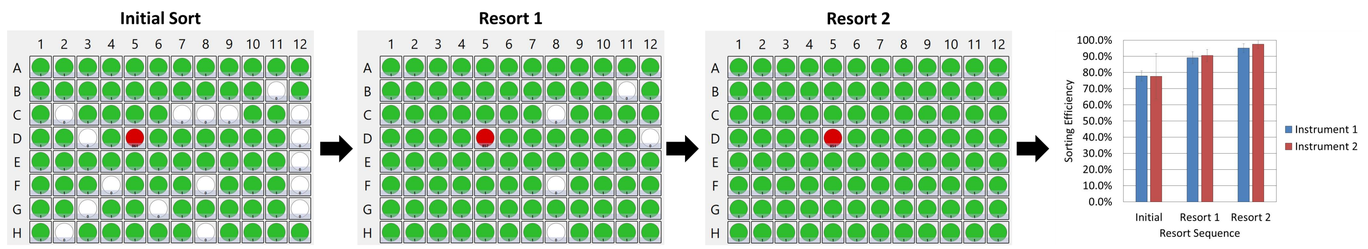








- The initial experiment was performed with sorting single bead into each well
- The singles beads were easily sorted on the MoFlo Astrios EQ, and easily detected on the Celigo Image Cytometer
- The bead sorting efficiencies ranged from 85% to 95%
- When we replaced beads with cells, the initial sorting efficiency significantly reduced to 60%
- We followed a set of rigorous procedures to optimize the sorter to increase the efficiency to above 90%



- One of the advantages of the ability to quickly determine single cell sorting efficiency is to disregard any wells that have more than 1 cell, or resort into any empty wells
- This improves the efficiency of the downstream assays such as single cell sequencing or colony outgrowth
- The results showed that we can step-wise increase the efficiency after each resort

5. SUMMARY AND CONCLUSION

- The Celigo was able to rapidly image and count single cell in the wells of 96-well plates
- Fluorescent beads are much easier to sort with the MoFlo Astrios EQ sorter in comparison to cells
- A rigorous procedure was developed for the MoFlo Astrios EQ sorter to improve the sorting efficiency
- Using Celigo Image Cytometer to quickly determine empty wells, allow quick resort to improve efficiency