RaftNote

Applications and protocols for use with CellRaft™ Technology

CELL Microsystems

Cell Seeding Optimization for Low Input Samples on the CellRaft™ Technology

December 2018

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Abstract

Cell Microsystems, Inc. provides a broad range of products suited to isolating single cells for virtually any application and sample type. Recent observations indicated that when using small sample sizes (i.e., low cell titers), the CytoSort™ Array was providing a relatively small number of single cells available for isolation. Here we present a new cell seeding insert that dramatically increases the number of cells available for isolation when low cell titer samples are used. Our data indicates that samples containing 500 cells, when seeded on the CytoSort™ Array, result in approximately 79% of cells are available for single cell isolation. We have expanded this work to include samples of ~65 cells, where we observe roughly 90-95% of cells seeded available for single cell isolation. We also demonstrate the use of the cell seeding insert with more standard cell titers of 3,000 and 10,000 cells, which exhibit high percentages of single cell availability. When using the cell seeding insert with larger sample sizes, it is recommended to use the 100 micron CytoSort™ Array which comprises 40,000 microwells as opposed to 10,000 microwells on the 200 micron CytoSort™ Array.

The CellRaft™ Technology

The single cell isolation and recovery platform developed by Cell Microsystems allows for imaging, sorting, and isolation of living cells in a culture environment which closely replicates standard *in vitro* conditions. Currently, the CellRaft™ Technology yields optimal results when cells are seeded at a density roughly equal to one third to one half of the total number of microwells present (i.e., 3,000-5,000 cells seeded on an array with 10,000 microwells). The CytoSort™ Array comprises releasable polystyrene CellRafts™ and a small border to accommodate the magnetic wand used for isolation and retrieval on the CellRaft AIR™ System. Under normal conditions some cells are present on the border after cell seeding. Many applications of the CellRaft™ Technology are not limited by the number of cells in a sample, easily fullfilling the requirement for a few thousand cells; however, some applications are limited to a small sample of cells. For these applications, we developed a cell seeding insert to effectively guide the cells onto the array and minimize the number of cells present on the border, thereby increasing the total number of single cells available for isolation.

Cell Seeding on the CytoSort™ Array

Cells are seeded on the CytoSort™ Array in cell culture media (typically ~4 mL) and allowed to settle and adhere for several hours before imaging and isolation. The application of standard cell culture coatings to the array surface can assist in the seeding and recovery of non-adherent cells (see RaftNote on this topic). To test the current capabilities of the system for single cell counting, we seeded cells at varying titers- 500, 3K, and 10K on a CytoSort™ Array with 200 µm rafts (10K total rafts per array). After cells settled and adhered, they were stained with Hoechst and a full array scan was performed on the AIR™ System (See RaftNote on vital dyes and nuclear staining). Initial experiments indicated that only 15-25% of the total number of

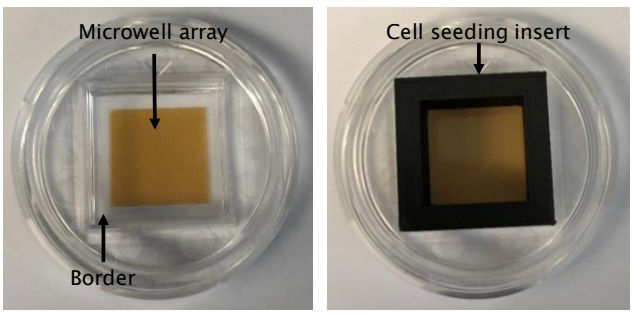


Figure 1. CytoSort™ Array shown with and without cell seeding insert. Black prototype of insert is shown for visualization (actual insert is clear polystyrene).

cells seeded were present on the array as single cells. This is particularly problematic for samples with low cell titers and revealed a need for improvement in the percentage of single cells available for isolation.

Design of the CellRaft™ Cell Seeding Insert

After further investigation, it was noted that a large percentage of the cells seeded on the CytoSort™ Array were on the border. This led to a design for a cell seeding insert to effectively occlude the border of the array and thereby decrease the percentage of cells present on the border (**Figure 1**).

Determining Single Cell Availability

To provide an unbiased means of counting single cells available for isolation with and without the insert, a custom MATLAB® program was developed to generate a heat map based on the manual counting of single cells on each CellRaft™ in every field of view present on the array. The CytoSort™ Array was seeded with cells at various titers and a full array scan was obtained using the AIR™ System to generate images for each field of view within a given array. The heat map is meant only for visualization purposes as it allows for easy interpretation of the distribution of cells across the CytoSort™ Array (**Figure 2**).

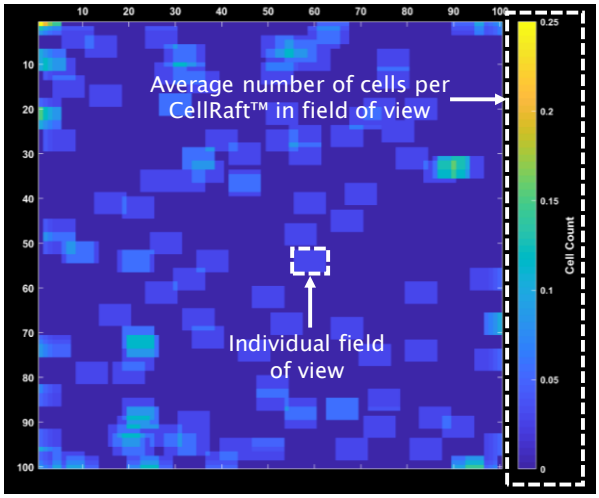


Figure 2. Heat map generated for visualization of the average number of cells per CellRaft™ in a given field of view on the CytoSort™ Array. Shaded fields of view indicate the presence and quantity of CellRafts™ containing single cells.

Isolation Efficiency using a Low Cell Titer (500 cells)

The cell seeding insert was first tested with a sample consisting of 500 cells (all experiments were performed using the 200 µm array with 10K individual CellRafts™). The CytoSort™ Array was prepared by rinsing three times with PBS (3 minutes per rinse). After the final wash, PBS was aspirated, and the seeding insert was placed in the array prior to cell seeding in 4 mL total volume. The full volume of media containing the cells was added dropwise to the center of the array, which was placed in an incubator for several hours. After cells settled and adhered to the array, Hoechst (1 µg/mL) was added directly to the array to stain the cells prior to a full array scan on the AIR™ System.

Figure 3 shows the heat maps for 500 cells seeded with and without the insert. Of the 500 cells seeded, 22% were singles without the insert versus 79% singles with the seeding insert. To test the lower limit of the insert, we seeded roughly 65 cells each on a 100 µm array (40K microwells) and a 200 µm array (10K microwells). Of the cells seeded on the array, 90-95% of those cells are present as singles, regardless of the size of the CellRafts™ (100 vs. 200 µm).

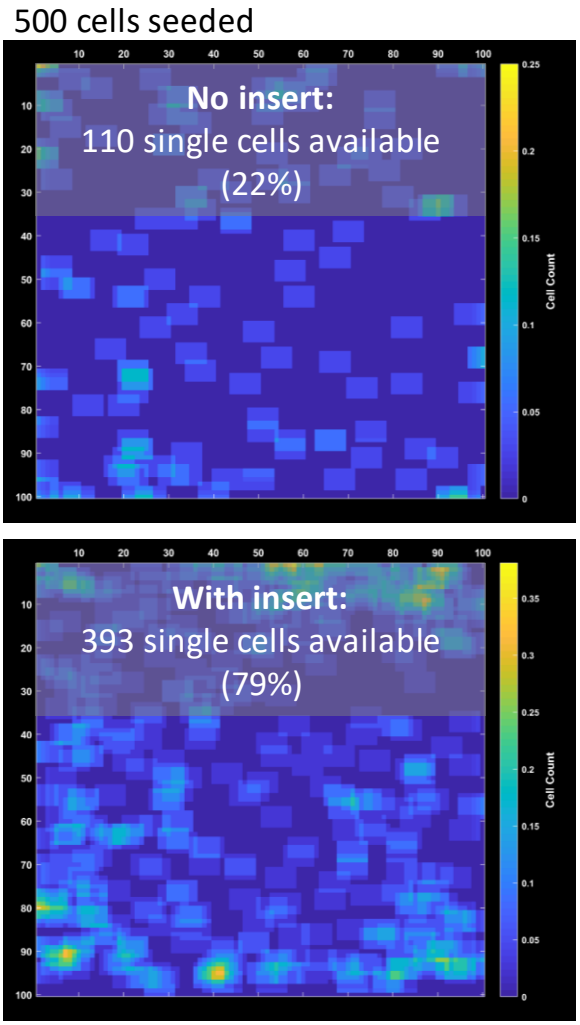


Figure 3. Heat maps generated from seeding 500 cells on the CytoSort™ Array. Results are shown without insert (top) and with insert (bottom).

Efficiency using Standard Cell Titers (3K and 10K)

We next performed the same experiment at a more standard cell density of 3,000 cells that is recommended for samples that are not limited in cell number. Heat maps for these results are shown in **Figure 4**, with 24% of the cells seeded present as single cells without the insert and 56% singles with the insert. The heat map for the seeding insert at this density begins to show a marked increase in the number of cells per CellRaft™ in each field of view (shown in yellow) compared to the heat map generated without the use of the seeding insert.

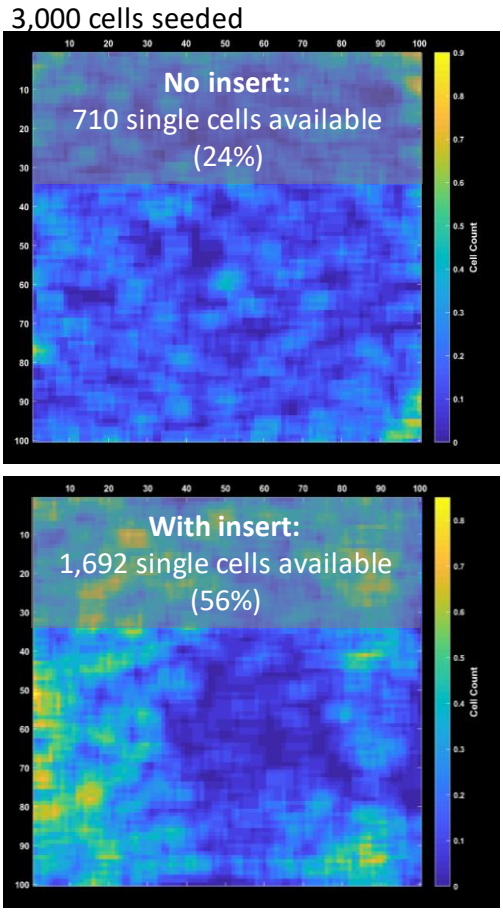


Figure 4. Heat maps generated from seeding 3,000 cells on the CytoSort™ Array. Results are shown without insert (top) and with insert (bottom).

Lastly, we tested the insert with 10,000 cells, which is equal to the number of CellRafts™ present on the array. Results from these experiments are shown in **Figure 5**, with 16% of cells seeded present as singles without the insert and 26% singles with the insert. The lower percentage of single cells even with the insert is likely due to the array being over-seeded at this cell titer, resulting in many CellRafts™ with more than 1 cell. For this cell titer, we would recommend the 100 µm CytoSort™ Array which comprises 40,000 microwells.

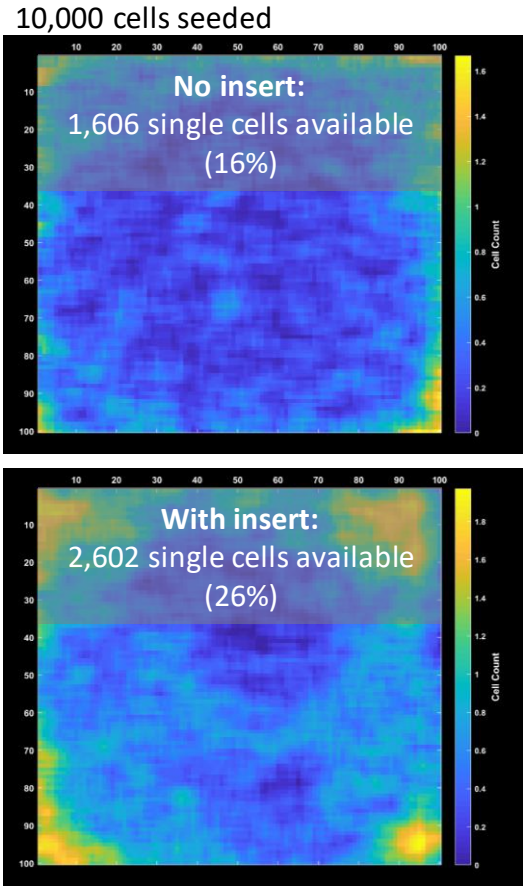
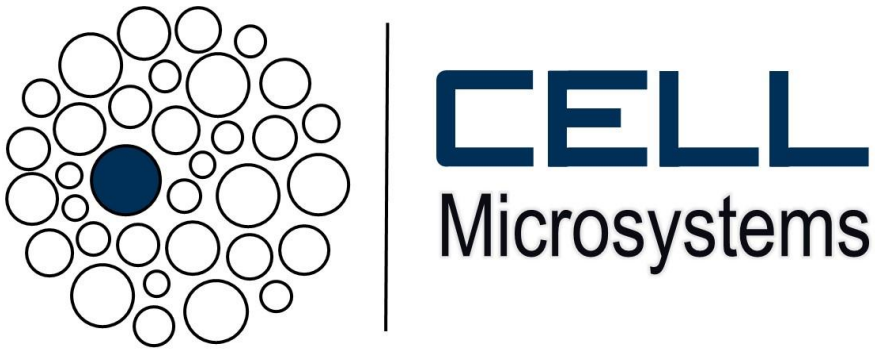


Figure 5. Heat maps generated from seeding 10,000 cells on the CytoSort™ Array. Results are shown without insert (top) and with insert (bottom).

Conclusions and Recommendations

Initial experiments indicated that 15-25% of the total number of cells seeded were present on the array as single cells based on manual counts of the cells on the CytoSort™ Array. Furthermore, the AIR™ System cell counting software culled around 20% of the single cells due to stringent default thresholding, leading to 12-20% of the total number of cells seeded residing as singles and available for isolation by the AIR™ System. The cell seeding insert was designed to occlude the border of the CytoSort™ Array and thereby increase the total number of single cells present on the array. For the 500-cell titer, the insert increased the percentage of single cells from 22% to 79%. It is recommended that when working with samples with a low cell number, the CellRaft™ cell seeding insert is used to improve the percentage of single cells available for isolation. While the data presented here was generated using a cell seeding insert designed for the single reservoir CytoSort™ Array, an insert is also under development for use with the quad reservoir CytoSort™ Array.



For more information:

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