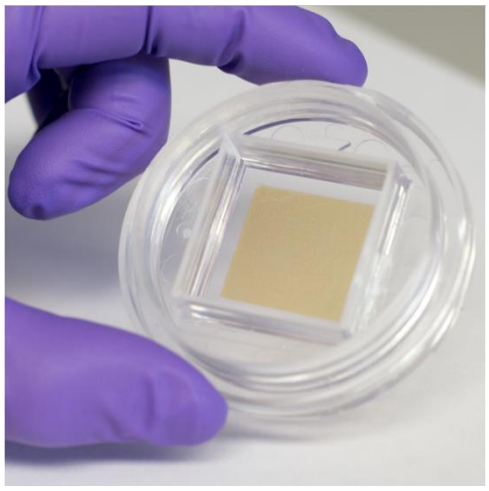
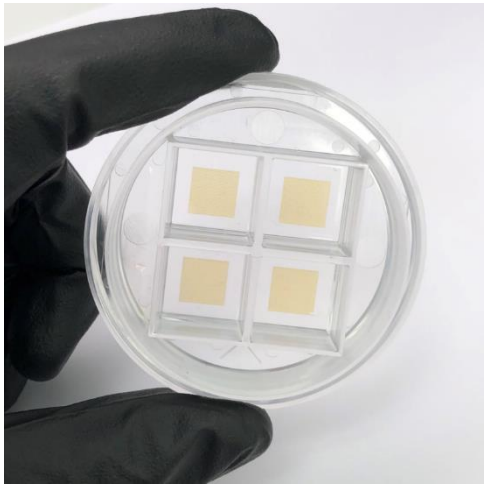
CELL Microsystems





CytoSort™ Array

Microwell Array with Releasable CellRafts for Cell Isolation

Single cell screening, isolation, and retrieval using CellRaft™ Technology

For use with the CellRaft AIR™ System and CellRaft™ System for Inverted Microscopes

User Manual

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Notices

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For general information about Cell Microsystems, contact info@cellmicrosystems.com.

Document Conventions

This icon calls attention to important safety notes

Warning! A warning indicates the potential for bodily harm and tells you how to avoid the problem.

Caution A caution indicates potential damage to the product and tells you how to avoid the problem.

Note: **Bold** text is primarily used for **emphasis**.

Revision History

Document Number: PD-015

Revision	Effective Date	Changes
00	14 JAN 2019	Initial Release

1. Intended Use Statement

CytoSort™ Arrays are intended for use with other Cell Microsystems products including the CellRaft Automated Isolation and Retrieval (AIR)™ System and the CellRaft™ System for Inverted Microscopes. The CytoSort™ Array is a single-use consumable used to image cells seeded on the array for subsequent isolation and retrieval using a magnetic wand. **The system is not intended for use in diagnostic or other clinical applications.**

For Research Use Only.
Not for use in diagnostic procedures.

2. Warnings and Precautions

- **For Research Use Only. Not for use in diagnostic procedures.**
- Use standard lab precautions when handling and using the product.
- Physiological samples of all types from any source should be treated as biohazardous according to your organization's approved procedures.
- Performance can be adversely affected by extraneous contaminants such as dust, dirt, adhesive, etc. on the CytoSort Array. Make sure CytoSort Arrays are clean and free of debris before use.

3. Limited Warranty and Liability

The CytoSort Arrays sold by Cell Microsystems have been designed and tested to perform according to the published specifications. Cell Microsystems warrants that these products will conform to those specifications and be free from defects in workmanship and materials for a period of twelve (12) months from the date of shipment. Defective products will be repaired or replaced, at Cell Microsystems option, during this period provided the purchaser has used the products under conditions of normal and proper use, but not for damage caused by the purchaser.

THE FOREGOING WARRANTY IS THE SOLE AND EXCLUSIVE WARRANTY MADE BY CELL MICROSYSTEMS. CELL MICROSYSTEMS MAKES NO OTHER WARRANTY OR GUARANTEE OF ANY KIND, EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION IMPLIED WARRANTIES OF FITNESS FOR A PARTICULAR PURPOSE OR MERCHANTABILITY. CELL MICROSYSTEMS' TOTAL LIABILITY AND PURCHASERS EXCLUSVIE REMEDY FOR ANY CLAIM OR LIABILITY ASSOCIATED WITH THE PRODUCTS SOLD, WHETHER BASED IN TORT, CONTRACT, STRICT LIABILITY OR ANY OTHER LEGAL THEORY IS EXPRESSLY LIMITED TO, AT CELL MICROSYSTEMS OPTION, REPLACEMENT, REPAIR, OR REWORK, AS APPLICABLE, OF NONCONFORMING PRODUCT OR PAYMENT IN AN AMOUNT NOT TO EXCEED, IN THE AGGREGATE, THE PURCHASE PRICE OF THE SPECIFIC PRODUCT FOR WHICH DAMAGES ARE CLAIMED. IN NO EVENT SHALL CELL MICROSYSTEMS BE LIABLE FOR ANY OTHER DAMAGES, LOSSES OR EXPENSES, INCLUDING, WITHOUT LIMITATION, INDIRECT, INCIDENTAL, PUNITIVE, CONSEQUENTIAL, EXEMPLARY, OR SPECIAL DAMAGES.

4. Quick Start Guide for the CytoSort™ Array

NOTE: The CytoSort™ Array should be prepared in accordance with standard aseptic technique in a sterile hood or environment.

1. Remove CytoSort™ Array from sterile packaging. Rinse with 3 mL of warm buffer (such as PBS) or media; let sit for three minutes. Aspirate the liquid and repeat rinse procedure twice for a total of three rinses. **Do not let CytoSort™ Array dry completely at any point during the preparation process**. If using the CellRaft™ seeding insert, place insert in the array after the last rinse but before seeding cells.

2. Prepare the appropriate aliquot of cell suspension for seeding the array. To maximize the gross number of single cells on the CytoSort™ Array, it is recommended to seed a total number of cells equal to approximately ⅓ to ½ the number of microwells present on the CytoSort™ Array. For example:
    - 200-micron single reservoir array has 10,000 microwells. Seed 3,000 – 5,000 cells.
    - 100-micron single reservoir array has 40,000 microwells. Seed 13,000 – 20,000 cells.
    - 200-micron quad reservoir array has 1,600 microwells per reservoir. Seed 500 – 800 cells.
    - 100-micron quad reservoir array has 6,400 microwells per reservoir. Seed 2,000 – 3,200 cells.

3. Add the aliquot of cells to 5 mL of media for cell seeding on the single reservoir array or 1 mL per reservoir for the quad reservoir array. For example:
    - If the cell suspension contains 500 cells/µL and you want to seed 5,000 cells total onto a single-reservoir array, add 10 µL of cell suspension to 5 mL of media for cell seeding. Media should contain at least 10% serum protein, such as fetal bovine serum (FBS).

    Seed the cells dropwise in the center of the CytoSort™ Array. Incubate the array and allow cells to settle and adhere. Time for this step will be dependent upon cell type and culture conditions, and cells can be imaged (either on the CellRaft AIR™ System or benchtop microscope) to confirm they have settled and adhered to the CytoSort™ Array.

NOTE: If the CellRaft™ Seeding Insert is used, cells should be seeded dropwise in 4 mL total volume for the single reservoir array or 500 µL per reservoir for the quad reservoir array. Prior to use with the AIR™ System, the insert must be removed, and media should be added to the CytoSort™ Array for a total volume of 5 mL for the single reservoir array or 1 mL per reservoir for the quad reservoir array.

5. Features of the CytoSort™ Array

5.1. The CytoSort™ Array

The CytoSort™ Array comprises an elastomeric (polydimethylsiloxane, PDMS) microwell array filled with individually releasable polystyrene elements called CellRafts™ (**Figure 1A**). The polystyrene CellRafts™ are loaded with iron oxide nanoparticles, facilitating isolation and collection with a magnetic wand. The array is housed in a petri dish-sized 65 mm cassette with walls surrounding the array to create a reservoir for media, buffer, or other reagents (**Figure 1B**). Cells are seeded on the CytoSort™ Array at a recommended density to ensure a high percentage of cells reside as a single cell in a microwell (**Figure 1C**).

When used with the CellRaft AIR™ System, automated detection of single cells can be accomplished using the fluorescent signal derived from the nucleus of a given cell in Cytometric Image Analysis Mode. The AIR™ System also allows users to scan the array to identify individual cells of interest in User Navigation and Selection Mode. A list of array locations (i.e., individual CellRaft™ addresses) can also be determined during off-instrument imaging, and this list can be imported as a spreadsheet for subsequent isolation of CellRafts™.

The CytoSort™ Array can be surface-coated similar to any plastic cell culture consumable with extracellular matrix proteins or non-specific biological adhesives. See Section 6.5 for more information regarding surface coating protocols.

NOTE: The CytoSort™ Array is a single-use consumable. Re-use of a CytoSort™ Array will significantly decrease the efficiency of cell seeding and adherence, as well as release and retrieval of CellRafts™. At the time of manufacturing, a coating is applied to the surface of the array to increase wettability and allow fluid to flow into the microwells without forming large image-occluding bubbles. This coating cannot be re-applied or recovered, rendering the CytoSort™ Array single-use.

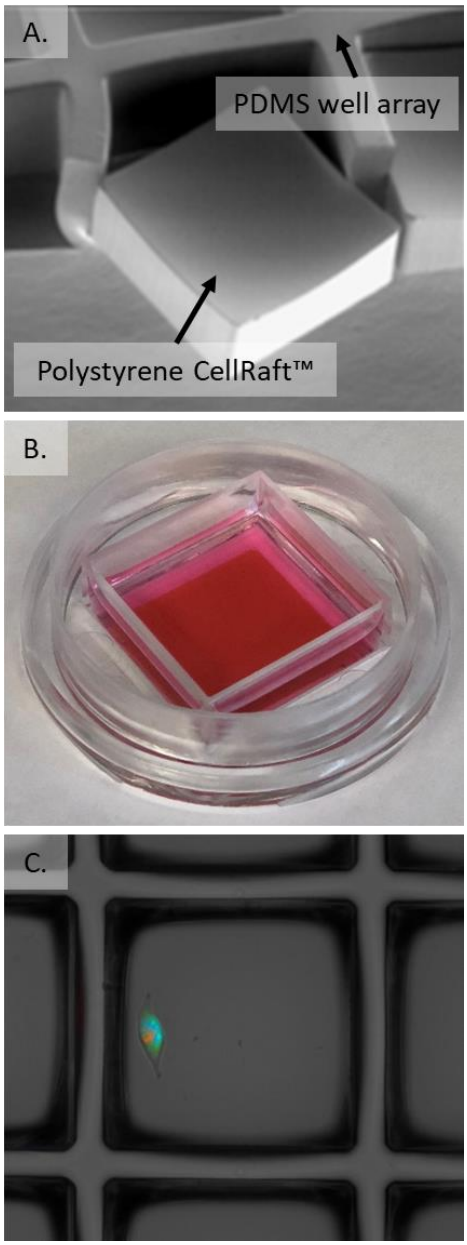

Figure 1. The CytoSort™ Array comprises a PDMS well array and polystyrene CellRafts™ (A). Cells are seeded on the array for imaging and isolation in a contiguous volume of media (B). Single cell on a CellRaft™ (C).

5.2. CellRaft™ Addresses Within the CytoSort™ Array

Each CellRaft™ within the CytoSort™ Array has a unique address describing its row and column position within the array (**Figure 2**). Each lettered row and column consist of 10 individual CellRafts™ which begin their numbering at 0 and end at 9 (i.e., CellRafts™ in the "A" Row comprise A0 through A9). Only the 0-designated CellRaft™ (i.e., A0A0) is labeled with its specific address. The 5th CellRaft™ in a given row or column is labeled with a small dot in the center of the CellRaft™. This is depicted in more detail in **Figure 3.**

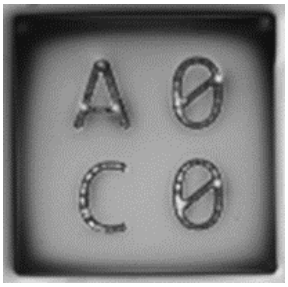

Figure 2: CellRaft addresses represent a row and column position within the CytoSort™ Array. This CellRaft is in row A and column C.

The CytoSort™ Array can be imaged off the AIR™ System on an inverted microscope and the user can manually generate a list of addresses in a Microsoft Excel® spreadsheet. The spreadsheet can be imported directly into the AIR™ System software. For more information on use with the AIR™ System software, please refer to the AIR™ System User Manual.

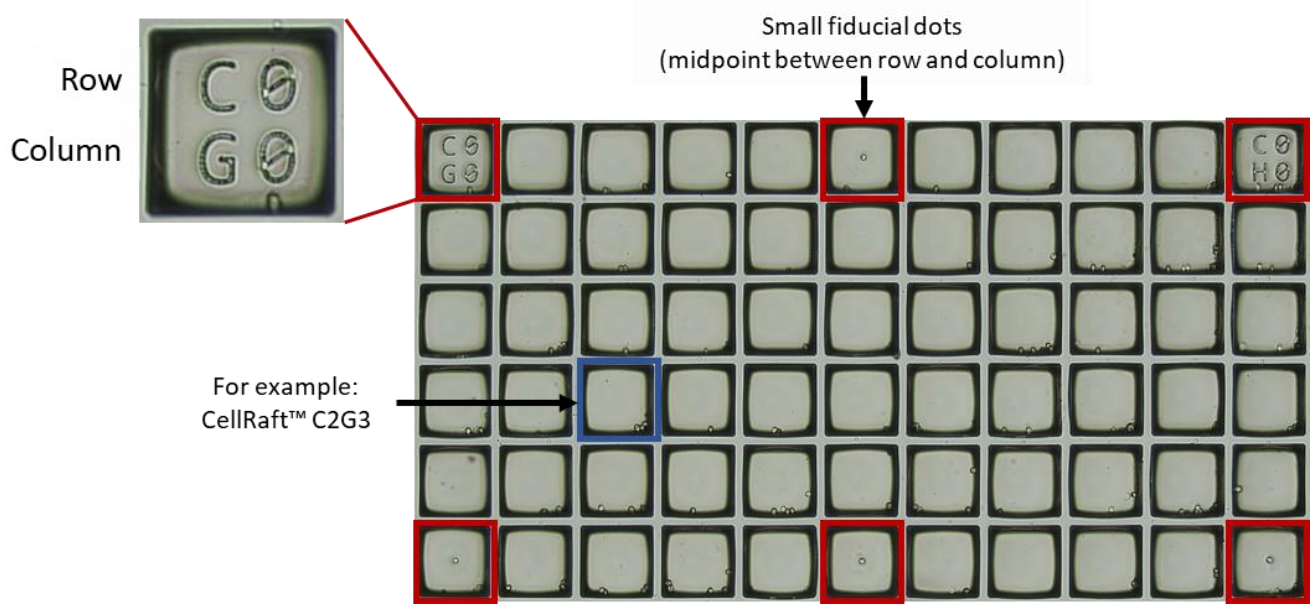

Figure 3: Field of view showing the CellRaft™ address system. C0G0 is in the upper left corner. This represents the CellRaft™ in Row C0 and Column G0. Every 5th row or column is marked by a small fiducial dot on the CellRaft™.

6. Preparing the CytoSort™ Array

6.1. Storage

Prior to use, CytoSort™ Arrays should be stored in their packages at room temperature in a dry location.

6.2. Wetting the CytoSort™ Array Before Use

The CytoSort™ Array is covered with a proprietary water-soluble biocompatible coating which prevents air bubble formation when adding liquid to the array. A simple rinsing procedure is required to completely remove the coating just prior to cell seeding on the CytoSort™ Array.

To remove the anti-bubble coating and prepare the CytoSort™ Array for cell seeding:

1. Warm buffer (such as phosphate buffered saline, PBS) or media to approximately 37°C.
2. Add 3 mL of buffer or media to the array.
3. Incubate at room temperature for 3 minutes.
4. Aspirate the buffer or media.
5. Repeat this rinse protocol twice for a total of 3 rinses.

NOTE: Once rinsed, the CytoSort™ Array must be kept wet. Do not allow the CytoSort™ Array to become dry as bubbles may form in the microwells, which can negatively impact cell seeding, image quality, and CellRaft™ release efficiency.

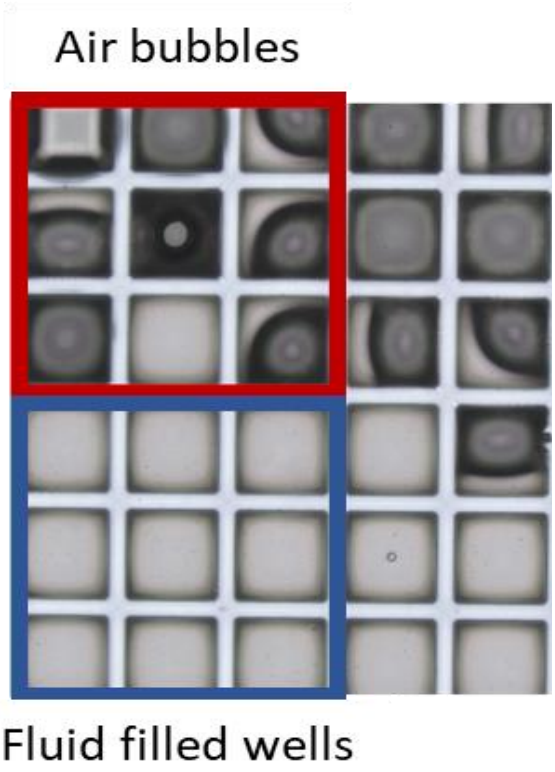

Figure 4. Air bubbles form when anti-bubble coating is not fully dissolved. Fluid fills wells after coating is removed completely.

6.3. Seeding Cells on the CytoSort™ Array

Cells should be counted via hemocytometer or other cell counting method prior to seeding on the CytoSort™ Array. The cell distribution on the CytoSort™ Array roughly follows a Poisson distribution. To maximize the number of single cells on the array, it is recommended to seed a total number of cells equal to approximately ⅓ to ½ the number of microwells present on the CytoSort™ Array. For example:

- 200-micron single reservoir array has 10,000 microwells. Seed 3,000 – 5,000 cells.
- 100-micron single reservoir array has 40,000 microwells. Seed 13,000 – 20,000 cells.
- 200-micron quad reservoir array has 1,600 microwells per reservoir. Seed 500 – 800 cells.
- 100-micron quad reservoir array has 6,400 microwells per reservoir. Seed 2,000 – 3,200 cells.

Prepare the appropriate aliquot of cell suspension for seeding the array. Add the aliquot of cells to 5 mL of media for cell seeding on the single reservoir array or 1 mL per reservoir for the quad reservoir array. For example:

- If the cell suspension contains 500 cells/µL and you want to seed 5,000 cells total onto a single-reservoir array, add 10 µL of cell suspension to 5 mL of media for cell seeding.

Seed the cells dropwise in the center of the array. Incubate the CytoSort™ Array and allow cells to settle and adhere. Time for this step can vary from 30 minutes to several hours and will be dependent upon cell type and culture conditions; cells can be imaged (either on the CellRaft AIR™ System or benchtop microscope) to confirm they have settled and adhered to the CytoSort™ Array.

6.4. Seeding Cells using the CellRaft™ Seeding Insert

Samples with very few cells (<500) may require the use of the CellRaft™ seeding insert to increase the percentage of microwells containing a single cell. The CellRaft™ seeding insert can also be used with samples of any cell titer to increase the overall number of single cells available for isolation (**Table 1**).

If the CellRaft™ seeding insert (**Figure 5**) is to be used, place the insert in the CytoSort™ Array after the array preparation rinses (see Section 6.2), prior to cell seeding. The cell suspension should be added dropwise to the center of the array in 4 mL total volume for the single reservoir array or 500 µL per reservoir for the quad reservoir array.

Total # Cells Seeded	Total # Single Cells		% Single Cells
500	No insert	110	22%
	Insert	393	79%
3,000	No insert	710	24%
	Insert	1692	56%
10,000	No insert	1606	16%
	Insert	2602	26%

Table 1. Cell seeding data with and without the use of the CellRaft™ seeding insert. Percentage of single cells refers to the percentage of cells seeded that are residing as a single cell in a microwell on the CytoSort™ Array.

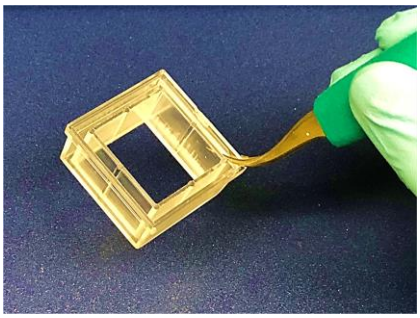

Figure 5. The CellRaft™ seeding insert can be used to increase the percentage of single cells, especially for samples with a low cell titer.

NOTE: Cell seeding insert must be removed prior to use with the CellRaft AIR™ System. After insert is removed, media should be added to the CytoSort™ Array for a total volume of 5 mL for the single reservoir array or 1 mL per reservoir for the quad reservoir array.

Additional details regarding use of the CytoSort Array seeding insert can be found in the RaftNotes section of the Cell Microsystems website under Support and Resources.

6.5. Applying Coatings to the CytoSort™ Array

The CytoSort™ Array contains materials (PDMS and polystyrene) which are commonly found in tissue culture vessels (e.g., Petri dish, culture flask, multi-well plate, etc.) and are therefore compatible with adherent cells without the requirement for a coating. For non-adherent cells, a cell culture coating can be applied to the CytoSort™ Array to render the surface compatible.

Table 2 summarizes the cell culture coatings that have been specifically tested with the CytoSort™ Array and gives recommended conditions for each coating. These protocols are intended only as a basic guideline; coatings should be optimized with specific cell lines and culture conditions before use with the CytoSort™ Array. Coatings should be applied prior to seeding cells on the array.

Coating	Manufacturer	Recommended concentration	Incubation	Wash steps
Poly-L-lysine	Sigma	1.5 mL of 0.01% solution	1 hour, 37˚C	Sterile water (x1)
Cell-Tak	Corning	31.5 ug/mL (in PBS)	1 hour, 37˚C	Sterile water (x3)
Fibronectin	Corning	10 ug/mL (in PBS)	1 hour, 37˚C	PBS (x2)
Gelatin	Sigma	1 mL of 2% solution	1 hour, 37˚C	None
RGD peptides	Selleckchem	10 ug/mL (in PBS)	1 hour, 37˚C	Sterile water (x1)
Matrigel	Corning	1 mL of 1% solution	1 hour, 37˚C	PBS (x2)
Collagen type IV	Sigma	10 ug/mL (in PBS)	1 hour, 37˚C	PBS (x2)

Table 2. Coating information and application conditions for use with CytoSort™ Arrays.

NOTE: During the application of cell culture coatings, the CytoSort™ Array must be kept wet. Do not allow the CytoSort™ Array to become dry as bubbles may form in the microwells, which can negatively impact cell seeding, image quality, and CellRaft™ release efficiency.

Additional details regarding use of the coatings on the CytoSort Arrays can be found in the RaftNotes section of the Cell Microsystems website under Support and Resources.

6.6. Staining Cells for Imaging on the CytoSort™ Array

Many vital dyes and fluorophores are compatible with the CytoSort™ Array. Cells can be stained either in suspension prior to seeding, or after settling and adhering to the CellRafts™. Several red dyes have been found to increase background fluorescence when stained on-array and should be used for suspension staining only. Some general recommendations are made in **Table 3**. Dye concentrations and incubation times should be optimized for individual cell types and cell culture conditions. An example of a triple-stained NIH/3T3 (ATCC® CRL-1658™) mouse cell is shown in **Figure 6**.

Additional details regarding staining cells on the CytoSort Array for use with the CellRaft AIR System can be found in the RaftNotes section of the Cell Microsystems website under Support and Resources.

6.7. Imaging Cells on the CytoSort™ Array

Most microscopy methods are compatible with the CytoSort™ Array; however, the release and isolation systems provided by Cell Microsystems may preclude some microscopy modes. The CellRaft™ System for Inverted Microscopes requires the use of an inverted microscope with an objective having at least 10 mm working distance, which can prevent the use of higher magnification (i.e., 20X, 40X, etc.) objectives.

The CellRaft AIR™ System employs a 10X objective and generates brightfield and fluorescence images in 3 channels (for information on specific fluorescent channels and excitation/emission spectra, please refer to the AIR™ System User Manual).

If different magnifications and/or microscopy modes are needed for a given experiment, it is recommended to image cells on the CytoSort™ Array using a microscope that supports the desired imaging settings. The user can then record the CellRaft™ addresses for cells of interest and release those CellRafts on either the CellRaft™ System for Inverted Microscopes or the automated CellRaft AIR™ System. Please see Section 5.2 for more details on the CytoSort™ Array address system.

For guidance on CytoSort Array™ compatibility with various microscopy methods, contact Cell Microsystems, Inc. using the contact information on page 3 of this manual.

Dye	Concentration	Time
CellTracker™ Green CMFDA	5 µM	30 min
CellTracker™ Blue	5 µM	30 min
CellTracker™ Red	5 µM	30 min
SYTO® 13 Green	5 µM	30 min
SYTO® 41 Blue	5 µM	30 min
SYTO® 17 Red	5 µM	30 min
Hoechst 33342	1.6 µM (1 µg/mL)	30 min
MitoTracker® Red FM	500 nM	30 min

Table 3. Vital dyes and recommended conditions for use with CytoSort™ Arrays. Dye concentrations reflect the final concentration used on-array or in suspension.

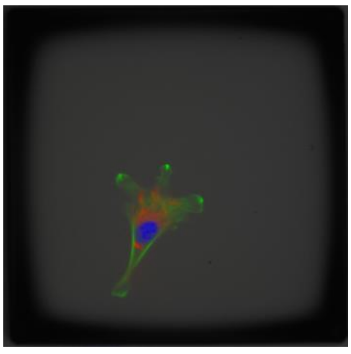

Figure 6. Example of a NIH/3T3 mouse cell stained with Alexa Fluor 488® Phalloidin (actin), MitoTracker® Red FM (mitochondria), and Hoechst 33342 (nucleus).

7. Troubleshooting

7.1. Bubbles Forming in the Microwells of the CytoSort™ Array

If large bubbles are observed in the microwells of the CytoSort™ Array, this is likely because the anti-bubble coating was not fully dissolved. If bubbles are observed prior to cell seeding, repeat the wash protocol described in Section 6.2 to ensure full dissolution of the coating.

If bubbles are observed after cells have been seeded on the CytoSort™ Array, slight agitation of the array may help gently dissolve the remaining anti-bubble coating without harming the seeded cells.

7.2. Cells Appear Unhealthy on the CytoSort™ Array

If cells appear unhealthy after seeding on the CytoSort™ Array, additional equilibration time may be needed. Allow cells to settle and adhere for an additional 2-4 hours and monitor via imaging, if desired. Overnight equilibration is required for some particularly sensitive cell types. If extended recovery time does not improve the appearance of the cells, fresh media should be applied. Cell culture coatings can also be applied to improve the viability of cells on the CytoSort™ Array. Please refer to Section 6.5 for more information on coatings.

For additional troubleshooting or technical support questions, contact Cell Microsystems Technical Support at support@cellmicrosystems.com.


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