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 the CellRaft™ System for Inverted Microscopes

User Manual
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Notices

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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 instrument and tells you how to avoid the problem

Note: Bold text is primarily used for emphasis.

Revision History

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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.
- The CytoSort Array comprises materials that may be hazardous to one’s health if ingested. The CytoSort Array should be kept away from the eyes and mouth to avoid potentially irritating exposure.

3. Limited Warranty and Liability
Products sold by Cell Microsystems have been designed and tested to perform according to the published specifications. Cell Microsystems warrants that the products it manufactures and sells will conform to those specifications and be free from defects in workmanship and materials for a period of twelve (12) months form 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.

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4. Product Support and Service

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5. Features of the CytoSort Array

5.1. The CytoSort™ Array

The CytoSort™ Array comprises an elastomeric (polydimethylsiloxane or PDMS) microwell array with each well of the array filled with an individually releasable polystyrene element called a CellRaft™ (see Figure 1 bottom). The array is housed in a petri dish-sized 65 mm cassette (see Figure 1 top) with walls surrounding the array to create a reservoir for various media, buffers or other reagents. Cells are seeded on the CytoSort Array at recommended densities to ensure a high percentage of seeded cells reside in a microwell and on a CellRaft as individuals (see Figure 1 middle).

When used with the AIR™ System automated detection of single cells can be accomplished using fluorescent signals derived from a given cell's nucleus, or allows users to scan the array to identify individual cells of interest based on their own judgement. A list of array locations can also be determined during off-instrument imaging using the addresses imprinted on the array surface. This list can be imported as a spreadsheet for subsequent isolation of CellRafts.

The CytoSort Arrays can be surface-coated similar to any plastic cell culture consumable with extracellular matrix proteins or non-specific biological adhesives.

NOTE: The CytoSort Arrays are single-use consumables. Re-use of CytoSort Arrays 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 allow a more wettable array surface enabling liquids to flow easily into the wells of the array without forming large image-occluding bubbles. This coating cannot be re-applied for a second use and therefore the arrays are single use only.

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.

An image of the CellRaft address system is found in Figure 2. Each lettered row and column comprises 10 CellRafts which begin their numbering at 0 and end at 9 (i.e. A0 through A9 for the CellRafts in the “A” Row). Only the 0-designated CellRaft is labeled with its specific address. The 5th CellRaft in a given row or column is also labeled with a small dot in the middle of the CellRaft. This is depicted in more detail in Figure 3. These
CellRaft addresses are used throughout the operation of the system to give each CellRaft a unique identity.

It should also be noted that a user can image the CytoSort Array off the AIR System on another microscope and manually generate a list of addresses in a Microsoft Excel-style spreadsheet. The spreadsheet can be directly imported into the “Real Time” run mode. The method for importing is described in greater detail in the “Real Time” run mode.

![CytoSort Address System](image)

**Figure 3**: Field of view showing the CellRaft address system. F0/C0 is in the upper left corner. This represents the CellRaft in Row F0 and Column C0. Every 5th row or column is marked by a small fiducial dot on the CellRaft (see blue squares). An example CellRaft is also shown at position F7/C8 (row/column respectively).
6. Preparing the CytoSort Array

6.1. Storage
CytoSort Arrays should be stored at room temperature in a dry location.

6.2. Wetting the CytoSort Array Before Use
The CytoSort Array is covered with a proprietary biocompatible coating which is water-soluble and effectively prevents air bubble formation when adding liquid to the array. Only simple rinsing is required to completely remove the coating.

To remove the anti-bubble coating and prepare the CytoSort Array for cell seeding the following wash protocol should be followed:
1. Warm approximately 10 mL of buffer (such as phosphate buffered saline) or media to approximately 37°C.
2. Add 3 mL of buffer or media to the array.
3. Incubate at room temperature for 3 min
4. Aspirate the buffer or media
5. Repeat this wash protocol twice for a total of 3 washes.

NOTE: Once washed, the CytoSort array must be kept wet. Do not allow the CytoSort array to become dry or bubbles may form in the microwells thereby impacting image quality and CellRaft release efficiency.

6.3. Applying Coatings to the CytoSort Array
The CytoSort Array contains materials which are similar to tissue culture vessels (e.g. Petri dish, culture flask, multiwell plate) and are therefore compatible with adherent cells cultured in a standard tissue culture dish without a coating.

If the cells to be cultured require a coating such as laminin, fibronectin, RGD peptides, collagen, Matrigel, polylysine, etc., these should be applied to the CytoSort Array using standard coating protocols provided by the coating’s manufacturer or based on cell type specific protocols familiar to the user.

Typically, 1.5 mL of coating solution is sufficient to cover the entire surface area of the CytoSort Array. Otherwise, standard coating procedures normally used should be followed to apply coatings to the CytoSort Array. At the end of the coating incubation period, the coating solution should be removed and cells can be seeded on the CytoSort Array.

NOTE: During the coating application protocol, the CytoSort array must be kept wet. Do not allow the CytoSort array to become dry or bubbles may form in the microwells thereby impacting image quality and CellRaft release efficiency.
6.4. Staining Cells for Imaging on the CytoSort Array

Most vital dyes and fluorophores are compatible with the CytoSort Array. Generally, it is advisable to stain cells prior to seeding cells on the CytoSort Array. This prevents excess dye from adsorbing into the materials of the CytoSort Array which can lead to additional fluorescent background signal and reduced imaging quality. In some cases, staining of cells directly on the CytoSort Array will not impact imaging if the signal is quite strong and can be resolved over background. These issues should be empirically determined by the user on a dye-to-dye basis and a cell type-to-cell type basis.

6.5. Plating Cells on the CytoSort Array

It is recommended that prior to seeding a suspension of cells, they be counted for density using a hemocytometer or other method.

The cell distribution on the CytoSort Array follows a Poisson distribution (Figure 4). To achieve the maximal number of sites on the array containing a single cell, the following guidelines are suggested:

1. Seed cells at a cell:CellRaft ratio between 1:2 or 1:3. The CytoSort Arrays contain 10,000 (200 × 200 µm CellRafts) or 40,000 (100 × 100 µm CellRafts) individual sites, so plating approximately 3,000-5,000 or 15,000-20,000 cells, respectively, is generally recommended.
2. Adjust the cell density of the suspension by aliquoting and/or dilution, and add the desired number of cells to the CytoSort Array in 3-5 ml of media.
3. Allow cells to recover on the CytoSort Array for roughly 1 hour. At this point cells can be examined for appropriate morphology and indicators of viability. Different cell types can require different times for adhesion and recovery and this should be determined empirically on a cell type to cell type basis.

At this point, cells can be imaged on the CytoSort Array. In subsequent sections of this manual, methods for using the CytoSort Array with CellRaft release and isolation systems (i.e. the automated AIR System or the CellRaft System for Inverted Microscopes) are described.
6.6. Imaging Cells on the CytoSort Array

While most microscopy methods are compatible with the CytoSort Array, 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 that prevents the use of higher magnification (i.e. 20X, 40X etc.) objectives. The AIR System employs a 10X objective and generates brightfield images as well as fluorescent images in 3 channels, with pre-determined excitation/emission specifications (see AIR System literature and user manual for details and recommended dyes/fluorophores). 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, record the CellRaft addresses for cells of interest and release those CellRafts. The CellRaft address system on the CytoSort Array is described above. Using the CellRaft System for Inverted Microscopes, the specific addresses can be found and their CellRafts released. Using the automated AIR System, the CellRaft addresses can be imported into the system in a spreadsheet format and automatically collected.
7. Using CytoSort Arrays with CellRaft Isolation Systems

There are two devices currently marketed by Cell Microsystems which allow the release and retrieval of individual CellRafts for downstream analysis or expansion culture: the CellRaft System for Inverted Microscopes and the automated AIR™ System. Use of the CytoSort Array with these release and retrieval systems is described below.

7.1. CellRaft System for Inverted Microscopes

7.1.1. System Contents
1. Sterile CytoSort Array in Sealed Pouch (2)
2. Plastic Carrying Case
3. Multiwell Collection Plate (CP-MW)
4. Motorized Release Device with Controller and Power Supply (MRD)
5. MiniWand (CW-MW) (2)
6. Replacement Needle Fixed to Acrylic Window (3) (RN-WM)
7. Screwdriver for Mounting Windows
8. Post-It® Arrow Flags (5)
9. PCR strip tube holder (1)

7.1.2. Setting Up the Release Device on an Inverted Microscope

The CellRaft System for Inverted Microscopes comes in a kit format in a small black carrying case (**Figure 5**). The components listed above are used at various points in the workflow. The installation of the release device onto an objective on an inverted microscope is described below.

Before installing the CellRaft System on a microscope, a few key specifications should be checked on the microscope to ensure compatibility:

1. Inverted microscope (i.e. the objective is positioned below the stage).

2. "Moveable" stage – the stage must be able to move in X and Y directions (relative to the Z direction which is represented by the focal path of the objective). This feature is used to position the CytoSort Array for both imaging purposes as well as centering the release needle under the CellRaft of interest. Given that CellRafts are either 100X100 or 200X200 microns, a knob- or motor-actuated stage is required because moving a CytoSort Array by hand with this level of precision is quite difficult.

**Figure 5**: Manual CellRaft System.
3. Microscope objective with > 10 mm "working distance" – this is typically satisfied by most 4X objectives and some 10X objectives. Working distance information can be found in the objective specifications from the manufacturer, or call Cell Microsystems customer support for assistance. Cell Microsystems' sales representatives will assist in identifying an appropriate objective for a given microscope.

To setup the Universal Mount CellRaft Release Device on a microscope, remove the system (release device, controller and power supply) from the plastic carrying case and follow the following steps:

1. Place the controller (small box with a red button and Cell Microsystems sticker) on a flat surface near your microscope.

   ![NOTE: Do not plug in power supply at this time!!]

2. Remove any stage inserts present on the microscope stage and lower the microscope turret to its lowest position. (Figure 6A)

3. Insert the electrical connector attached to the control box from under the underside of the microscope stage up through the stage (Figure 6B).

4. Connect the control box to the release device (Figure 6C).

5. Place the release device over the microscope objective (Figure 6D). Objectives with a magnification of 4× or 10× with a working distance ≥ 10 mm are required to visualize the array while avoiding damage to the needle. (See compatibility specifications listed above)

6. Rotate the release device to an appropriate position allowing it to sit as far down the microscope objective as possible. Holding the two small tabs on the tightening ring with one hand and the release device with the other hand (Figure 6D), rotate the tightening ring clockwise to clamp the release device to the microscope objective.

   ![NOTE: To remove the release device, hold the two small tabs on the tightening ring with one hand and the release device with the other hand and rotate the tightening ring counter-clockwise. The release device can also be removed while the tightening collar remains fixed to the microscope objective by reinstalling the cap and lifting the release device vertically (Figure 6F). This feature enables quick removal and attachment. To reattach the release device to the tightening ring, align the three tabs on the inside of the release device with the corresponding grooves on the tightening collar (Figure 6F) and press down vertically (Figure 6G).]
7.1.3. **Calibrating the Position of the CellRaft Release Device Needle.**

The release device controller has two modes: “Setup” and “Release”. To calibrate the needle position the system must be in “Setup” mode. To enter setup mode:

1. Plug the power supply into a standard wall outlet (120V). Initialization will automatically occur that lowers the needle to its lowest position. Once this initialization has completed, the green LED in the top right corner of the control box will turn on and remain solid.

2. Turn the “Travel Distance” knob to its lowest (left-most) setting.

3. Press and hold the red “Release” button until the LED begins to flash (note: the controller will not go into “Setup” mode unless the “Travel Distance” knob is at its lowest setting).

4. Once the LED begins to flash, the “Travel Distance” knob will control the vertical position of the needle. Before raising the needle position, ensure that the area above the needle is free of obstructions, as damage to the needle will occur if it contacts any hard surface.

5. After ensuring that no obstructions exist, carefully remove the cap from the release device to expose the release needle (Fig. 5E). Again, remove the cap cautiously to avoid contact with the needle. Lifting the cap straight up and off of the needle release device will best avoid needle...
damage. Should the needle come into contact with a hard surface, it will need to be replaced with one of the provided replacement needles.

**NOTE:** Use caution when removing the cap from the release device and exposing the needle as this can cause bodily harm due to puncture risks.

6. Once the release device is in place and the cap has been removed, it is important to ensure that the needle will release CellRafts within the microscope’s field of view. To examine the field of view, first look into the microscope eyepiece.

7. While looking into the eyepiece, slowly increase the vertical position of the needle by increasing the “Travel Distance” knob (turning clockwise). Again, ensure no obstructions are present that may damage the needle. Continue increasing the needle position until a small dot comes into the field of view (indicated by white arrow in Figure 6). This dot is the tip of the needle.

8. If this circle can be seen while looking through the eyepiece, switch to examining the field of view with a camera and monitor system (a camera with a monitor system is suggested to facilitate the CellRaft release process).

9. Determine if the white dot is still visible within the camera’s field of view. The needle may not be found exactly in the center of the field of view. This will not impact CellRaft release efficiency, as long as the dot still remains somewhere within the field of view.

10. If the dot is outside the field of view, adjust the release device angle by holding the release device and manually adjust the pitch slightly until the white dot comes into the field of view.

11. Once the needle position is determined, it is important to mark this position on the monitor to indicate the CellRaft release site. To do this, place one of the provided Post-It® Arrow Flags on the monitor at the center of the needle dot. Position CellRafts at this point using the moveable stage of the microscope to put the CellRaft of interest over the needle release location.

12. After the release site has been marked, the setup process is complete.

13. To exit “Setup” mode, press and release the “Release” button. Do not adjust the “Travel Distance” knob (it does not need to be at its lowest setting to exit “Setup” mode). Leaving it at the position used for setup will facilitate CellRaft release.
14. After pressing the “Release” button, the release device will automatically revert to the lowest vertical position and the LED indicator will stop flashing and remain solid. The solid LED light indicates that the controller is now in release mode.

7.2. Using CytoSort Arrays with the AIR™ System

The AIR™ System employs the same proprietary needle-based release and magnet-based collection strategy as the manual CellRaft System for Inverted Microscopes described above. The AIR™ System will move the CytoSort Array over a motorized microneedle. Once in position the needle is actuated upward, penetrating the resealable PDMS microwell floor and pressuring the hard polystyrene CellRaft out of the microwell. The system will detect successful release from the microwell and repeat the needle actuation process up to 10 times until the CellRaft is free. If the CellRaft is somehow not freed from the microwell array, the system will report the error to avoid downstream analytical issues with suspected empty wells or doublets (should the CellRaft be collected in subsequent operations). Once free, the CellRaft is collected by actuating a magnetic “wand” over the CellRaft location. The wand then deposits the CellRaft into a collection vessel (96-well plate). Once all CellRafts have been collected in the wells of the 96 well plate, the plate can be retrieved by the user.

7.2.1. CytoSort Array Stage Adapter

The CytoSort Array is held in place on the deck of the instrument by an adapter (Figure 8). The Array should be placed with the Keying Arrow’s flat side toward the back of the adapter plate. Matching the flat side of the adapter plate to the flat side of the array standardizes the placement of the array from run-to-run. A spring-loaded pin is located at the front of the adapter to apply light pressure to the array to stabilize its location within the adapter. Once the array is positioned on the adapter, the entire assembly can be placed on the deck of the AIR™ System. Alternatively, the adapter can be placed on the deck first followed by placing the CytoSort Array into the adapter.

7.2.2. Placing the CytoSort Array Stage Adapter on the AIR™ System Deck

The AIR™ System deck has two recessed holders which are similar in size to a standard 96-well plate. The left position is intended to house the CytoSort Array within the adapter plate (described above). The right position is intended to house the 96-well plate for collection of CellRafts (see Figure 9).
When placing both the array/adapter and 96-well collection plate on the deck, care should be taken to not spill any media or other liquid reagents onto the underlying components as this can damage the function of those components.

Prior to unlocking the door of the instrument, the system moves the deck into the “loading” position, moving both array and collection plate positions to the front of the system, and moving the white light brightfield illuminator and retrieval wand assembly to the back of this area.

Again, care should be taken not to bump or otherwise perturb the position of the white light illuminator and/or retrieval wand to avoid suboptimal performance.

The remaining steps of the AIR System workflow can be found in the AIR System User Manual.

8. Troubleshooting

8.1. Bubbles forming in the wells 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 cells being seeded on the CytoSort Array repeat the wash protocol described in this manual to ensure full dissolution of the coating. The coating is fully biocompatible and poses no threat to the health and viability of cells seeded on the CytoSort Array.

If bubbles are observed AFTER cells have been seeded on the CytoSort Array, add 1-3 extra mL of media to the array and incubate the CytoSort Array for 1 hour. Slight agitation (e.g. rotation) may help gently dissolve the remaining anti-bubble coating without harming the seeded cells.

8.2. Replacing the Release Needle
In the event that the release needle on the release device becomes bent or otherwise out of alignment with a perpendicular approach to the CytoSort Array underside surface, it is recommended that the needle be replaced.

NOTE: The needle can only be replaced by the user on the CellRaft System for Inverted Microscopes. The needle on the AIR System can only be replaced by a Cell Microsystems service technician or engineer. At no point should a user attempt to disassemble the AIR System in an attempt to replace the needle as this presents risk for significant bodily injury.
Minor damage to the needle will result in the inability to consistently puncture the CytoSort Array, leading to failures in CellRaft release from the array. Gloves should be worn while replacing the needle to prevent fingerprints from obscuring the clear window.

To replace the needle:

1. Ensure the release device is at the lowest position and remove the release device from the microscope objective and place it on a flat surface.

2. Using the screwdriver provided in the CellRaft Manual System Kit, loosen but do not remove the four set screws from the sides of the window holder.

3. While holding the release device over a countertop or a sharps container, invert the release device. The window should drop from the window holder without added pressure. Should the window not fall on its own, pass the screwdriver through the collar used to mount the device to the microscope objective and apply pressure to the back of the clear plastic window to dislodge the window from the holder.

4. Dispose of the needle in a sharps waste container.

5. Once the damaged needle has been removed, obtain a replacement needle from the CellRaft Kit (Figure 7).

6. Remove the needle from the dish by first removing the tape on the outside of the dish.

7. Open the dish carefully (avoid touching the needle) and use tweezers to remove the tape while holding down the edge of the window. Do not grab the new needle with tweezers, as this will damage the needle.

8. Once the tape is removed, carefully remove the window from the dish by grabbing the window by the edge.

9. Place the new clear plastic window in the holder and tighten all four set screws until finger tight. Do not over tighten the screws.

8.3. Cells appear unhealthy on the CytoSort Array

If cells appear unhealthy after seeding on the CytoSort Array, they may require more time to equilibrate after seeding. Allow the cells extra time (2-4 hours) on the CytoSort Array and monitor their appearance via imaging. 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.