CELL Microsystems

CellRaft® System for Inverted Microscopes

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

User Manual

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Notices

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

Revision History

Document Number: PD-051

Revision	Effective Date	Changes
00	24 FEB 2021	Initial Release

1. Intended Use Statement

The CellRaft® System for Inverted Microscopes is intended for use with other Cell Microsystems products, including CytoSort™ Arrays. The CellRaft® System for Inverted Microscopes is an accessory kit compatible with most benchtop inverted microscopes and can be 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 CellRaft® System for Inverted Microscopes sold by Cell Microsystems has 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. Overview of the CellRaft® System

The CellRaft® System for Inverted Microscopes and its associated consumables – the CytoSort™ Arrays – provide a cost-effective method to isolate and recover single cells for direct analysis or for the generation of clonal populations. The working principle is schematically depicted in **Figure 1A**.

The CytoSort™ Array is composed of 10,000 (200 × 200 μm) or 40,000 (100 × 100 μm) microwells, each containing a CellRaft that serves as an individually releasable culture site for single cells or small clonal colonies. Cells are plated on the CytoSort™ Array in the same manner as a standard tissue culture dish. The cells settle into the microwells and attach to the rafts within each microwell. The cells remain positioned on specific rafts while in culture so that single cells can be isolated or expanded into clonal colonies. In addition, analysis for target cell or colony identification can take place at single or multiple time points, dramatically expanding the potential criteria used for selection. For more information on the specifications or instructions for use of the CytoSort™ Arrays, please refer to the CytoSort™ Array User Manual at https://cellmicrosystems.com/resources/manuals/.

The CellRaft® Release Device is mounted onto a 4× or 10× objective of a common inverted lab microscope and contains a motorized needle capable of pushing a selected CellRaft out of its microwell. The needle is mounted within a transparent, acrylic window so as not to interfere with the imaging function of the microscope.

To isolate cells, the user first aligns the microwell of interest with the needle location, then presses a button on the controller to insert the release needle through the elastomeric substrate of the CytoSort™ Array to dislodge the CellRaft and its attached cell(s) (*e.g.* GFP^+ cells in **Figure 1B**). The released CellRaft is then collected using a magnetic wand (**Figure 1A**) and transferred to a 96-well plate or PCR tube.

This procedure achieves cell isolation and recovery with excellent viability (>95%) and purity (100%). The CellRaft® System for Inverted Microscopes offers a high degree of flexibility as cells may be selected using a wide variety of criteria, including temporal and spatial characteristics, and requires only a very small sample size to conduct an experiment. The recovered cells can be used for direct analysis (*e.g.* PCR, MALDI, SDS-PAGE), or clonal expansion (**Figure 1B**).

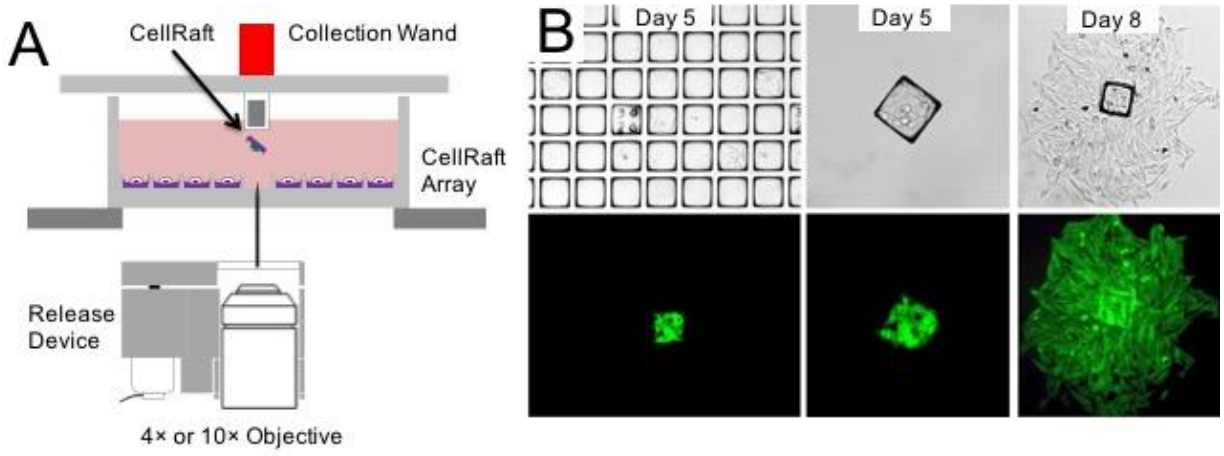

Figure 1. CellRaft® System for Inverted Microscopes. (A) Schematic of the working principle of the CellRaft® System. (B) Example of sorting GFP-H1299 cells out of a mixture of cells. A mixture of GFP-H1299 cells and wild-type H1299 cells were plated as single cells on the CytoSort™ Array. At day 5, a clonal fluorescent colony was identified and released from the CytoSort™ Array. The recovered colony was expanded in a separate Petri dish for an additional 3 days.

5. Contents of the CellRaft® System

1. Pelican Kit Case
2. Controller and Power Supply
3. Motorized Release Device (Type O or Type N) (both shown)
4. Release Device Cable
5. Magnetic Collection Plate
6. Magnetic Collection Wands (2)
7. Replacement Needles (fixed to acrylic windows) (3) (not visible)
8. Torx driver and screws for replacing windows (4) (not visible)
9. SBS-Format Adapter Plate (for 65-mm CytoSort™ Arrays) (not visible)

6. Setting up the CellRaft® System

6.1. Attaching the Release Device to Your Microscope

NOTE: Due to the 5mm protrusion of the CellRaft® release needle from its top surface, the Motorized Release Device should only be mounted on an objective with at least a 10mm working distance. If you are unsure of the working distance of your objective, please contact support@cellmicrosystems.com with the model number or a picture of the objective.

To set up the universal mount Motorized Release Device on your microscope (**Figure 2**):

1. Remove the Motorized Release Device from the Pelican case with the release device cap still attached.
2. Prepare microscope:
    a. Remove any stage inserts present atop the microscope stage.
    b. Lower the objective turret to its bottom position using the microscope focus knob (or drive).
    c. Position the objective intended to house the release device in the active position on the turret.
    d. Align the opening in the microscope stage for easiest access to the objective.
3. Feed the free end of the cable attached to the Motorized Release Device through the opening in the microscope stage and out the side of the microscope where you plan to position the Controller.
4. Slide the Motorized Release Device over the microscope objective and position it as far down the microscope objective as possible.
5. Holding the base of the Motorized Release Device with one hand, rotate the tightening right clockwise with the other hand to clamp the release device to the microscope objective.
6. Remove the cap of the Motorized Release Device by sliding it vertically to avoid damage to the release needle underneath.
7. Check to ensure that the release device is positioned as far down the microscope objective as possible and with the top surface of the objective centered within the opening in the top of the release device.

    NOTE: When complete, always replace the cap when the release device is not in use to prevent accidental damage to the release needle.

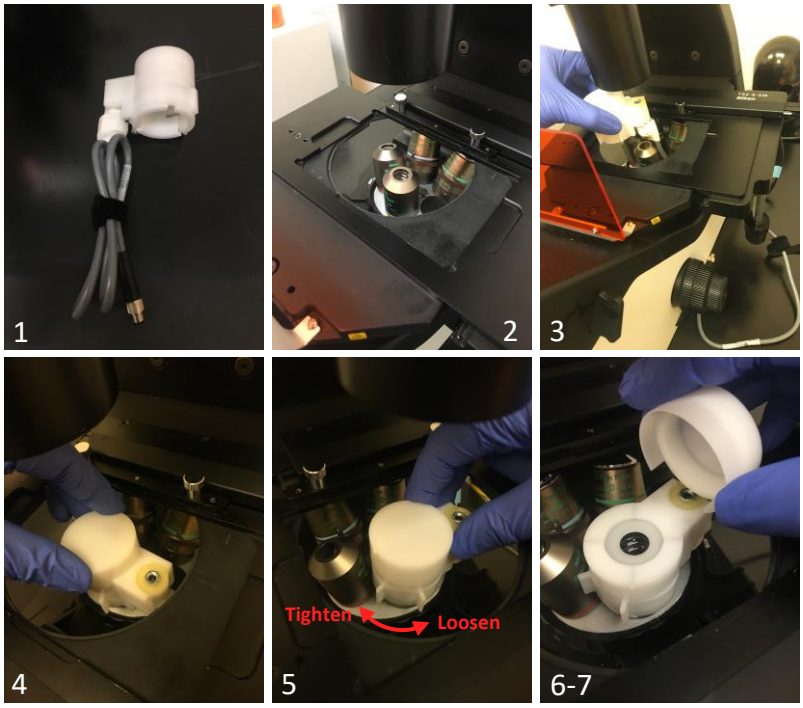

Figure 2. Installation of the Motorized Release Device.

NOTE: There are two ways to remove the Motorized Release Device:

1. While holding the base of the Motorized Release Device with one hand, rotate the tightening ring counter-clockwise and slide the entire assembly from the objective.
2. The Motorized Release Device can also be removed while the tightening collar remains fixed to the microscope objective (**Figure 3**). Re-install the cap on the release device and lift the release device vertically such that the main body of the release device detaches from the tightening collar. This feature enables quick removal from and attachment to the microscope to keep it uncluttered for other users. To reattach the release device to the tightening collar, simply align the three tabs on the inside of the release device with the corresponding grooves on the tightening collar and slide it on.

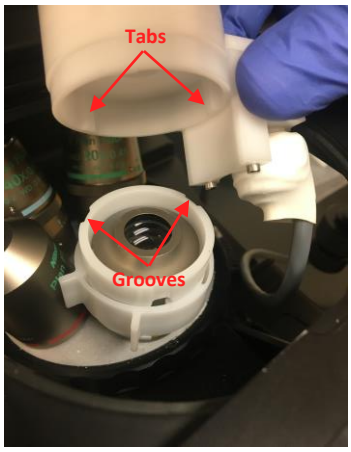

Figure 3. Removal of release device with collar fixed to objective.

6.2. Setting up the Controller

1. Remove the Controller from the Pelican case and set it on a flat surface near your microscope.
2. Check that the power switch on the rear of the Controller is in the OFF position.
3. Remove the Controller Power Supply from the Pelican case.
4. Plug the barrel connector on the Controller Power Supply into its socket on the rear of the Controller.
5. Connect the plug on the Controller Power Supply into a standard wall outlet.
6. Remove the Release Device Cable from the Pelican case.
7. Connect the larger four-pin connector on the Release Device Cable to its socket on the rear of the Controller, ensuring to fully tighten the connection.
8. Connect the smaller four-pin connector on the Release Device Cable to its mating connector on the Motorized Release Device cable, ensuring to fully tighten the connection.

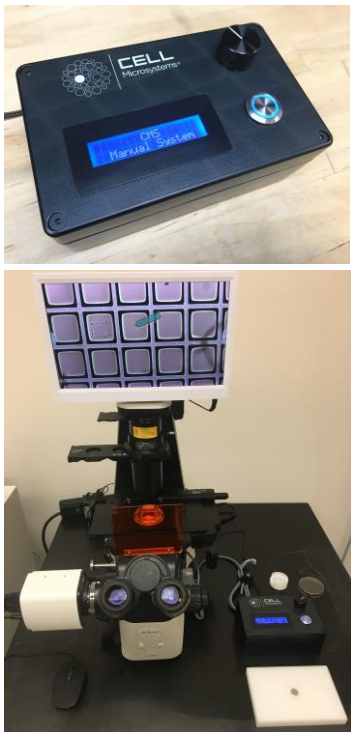

Figure 4. Controller and full CellRaft® System setup.

6.3. Calibrating the CellRaft® System

NOTE: The Controller operates in two modes: Alignment Mode and Release Mode.

The CellRaft® System should be calibrated to determine the position of needle release within the microscope field of view every time the Motorized Release Device is removed from the microscope or there is reason to believe its position on the objective was disturbed since last use.

1. Start by turning on the Controller via its power switch. An initialization will automatically occur that lowers the needle on the Motorized Release Device to its lowest position.
2. Once initialization has completed, the LCD Screen will display “CMS Manual System.”
3. Pressing the illuminated Release Button will dismiss the welcome message and put the Controller into Release Mode with a travel distance (0-100%) determined by the position of the Poke Height Knob.
4. To enter Alignment Mode, turn the Poke Height Knob fully counter-clockwise, then press and hold the illuminated Release Button for 3 seconds.
5. After releasing the illuminated Release Button, the LCD Screen will indicate Alignment Mode. In this mode, the Motorized Release Device will move the vertical position of its release needle to track the position of the Poke Height Knob.

   NOTE: Before raising the needle position, ensure that cap has been removed from the release device and the microscope stage area above the needle is free of obstructions, as damage to the needle will occur if it contacts any hard surface.

6. To determine the position of needle release within the microscope field of view, slowly rotate the Poke Height knob clockwise while monitoring the microscope field of view (in trans-illumination brightfield mode) through the eyepiece or camera monitor, until a small dot – the tip of the needle – comes into the field of view (**Figure 5**).

   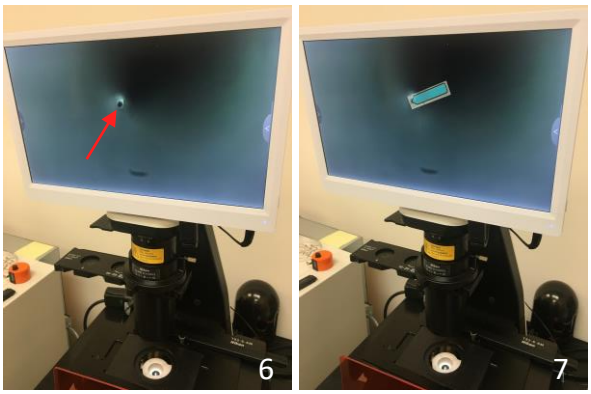

   Figure 5. Determining the release needle position within the microscope field of view.

   NOTE: A camera with a monitor system is recommended for use with the CellRaft® System to enable marking the needle release position within the field of view for subsequent CellRaft release steps. An alternative but less desirable option is an ocular with a grid reticle installed.

   NOTE: It is acceptable and common for the needle release position not to be perfectly centered within the field of view.

   TROUBLESHOOTING: If you reach the 100% distance limit of the Poke Height Knob and the tip of the needle is not visible, it is possible that the needle release position is outside the microscope field of view. Manually adjust the angle of the Motorized Release Device on the objective (taking care not to contact the release needle) until the needle tip comes into view.

7. Mark on the monitor (or make note of within the ocular reticle grid) the needle release position to indicate the CellRaft release site (**Figure 5**). Five Post-It® Arrow Flags are generally provided in the kit for this purpose. During subsequent CellRaft release steps, CellRafts will need to be aligned with this position in order to dislodge them from the CytoSort™ Array.
8. Exit Alignment Mode by pressing and releasing the Release Button on the Controller.

   NOTE: It is recommended to leave the Poke Height Knob set to the position in which the needle was first visible within the field of view. It does not need to be at 0% to exit Alignment Mode.

   NOTE: Upon exiting Alignment Mode, the Motorized Release Device will automatically revert to its lowest position and the LCD Screen on the Controller will return to the message “Align or Travel Distance:” with the current position of the Travel Distance Knob.

7. Isolating CellRafts with the CellRaft® System

7.1. Releasing CellRafts from CytoSort™ Arrays

NOTE: The CellRaft release process can be performed with the CytoSort™ Array lid in place to help prevent contamination. The lid must be removed prior to collecting CellRafts as outlined in section 7.2.

1. Once the CellRaft® System has been set up and calibrated, place a CytoSort™ Array on the microscope stage.

   NOTE: It is recommended to immobilize the CytoSort™ Array on the stage during use, either through the use of array clips as shown in **Figure 6A** or by mounting the array in the SBS-Format Adapter Plate provided in the Pelican case.

2. After placement on the stage, the array can be screened for cells of interest. When a target cell or colony is identified, align the corresponding CellRaft containing the cell or colony with the needle release position marked during calibration.

   NOTE: Release will be most efficient when the CellRaft is centered over the needle release position.

3. Press and release the illuminated Release Button to actuate the release needle and pierce the CytoSort™ Array. The LCD Screen on the Controller will indicate "Traveling" to confirm the release request was registered by the controller.

   NOTE: Upon contact by the release needle, the CytoSort™ Array will go out of focus, then back into focus as the needle withdraws from the array.

4. Examine the CytoSort™ Array under the microscope to determine whether the identified CellRaft was successfully dislodged from the CytoSort™ Array (**Figure 6C**).

   TROUBLESHOOTING: In the event that the CellRaft was not successfully dislodged:

   - If there was no apparent movement of the CellRaft within its microwell, increase the needle poke height by 5-10% (by turning the Poke Height Knob clockwise) and repeat the release process.

     NOTE: Successful release may require multiple increases in poke height when first attempting to release CellRafts from an array, particularly after a new calibration.

     NOTE: While increasing the poke height is the first step in troubleshooting incomplete CellRaft release, the release process will be most efficient when the poke height is set to the minimum distance necessary to successfully release a CellRaft.

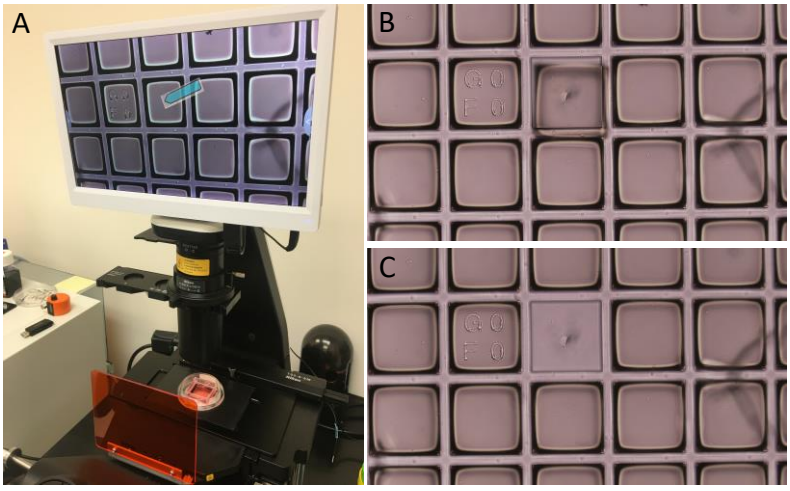

Figure 6. CellRaft Release. (A) Alignment of target CellRaft with needle release position. (B) Target CellRaft dislodged from its microwell. (C) CytoSort™ Array after collection of target CellRaft.

- If the CellRaft tipped within its microwell but did not fully release, adjust the stage position to align the new position of the CellRaft with the needle release position and repeat the release process.

  NOTE: Successful full release from the microwell may require multiple release attempts at different locations within the microwell depending on how the CellRaft is positioned after each attempt.

5. Once successfully dislodged, the CellRaft and its accompanying cell/colony are now ready for collection and deposit.

7.2. Collecting CellRafts and Depositing within a Collection Plate

The Magnetic Collection Wands can be used in combination with the Magnetic Collection Plate to collect individual CellRafts and deposit them into tissue culture (TC) plates, PCR tube strips, or PCR plates (**Figure 7**).

1. Remove the Magnetic Collection Plate and a Magnetic Collection Wand from the Pelican case.

   NOTE: Use caution when handling the Magnetic Collection Plate, as it contains a strong magnet. Keep clear of any metal surface. If the plate must be used near metal surfaces, use extreme caution as to avoid losing control of the plate.

2. Inspect and clean the tip of the Magnetic Collection Wand.

   NOTE: If its magnets are not visible within the wand tip, give the wand a gentle shake. Avoid excessive shaking as impact of the magnets with the tip can cause the glass tip to break.

   NOTE: The glass tip of the wand can be cleaned with a 70% ethanol wipe or soak but avoid exposure of the plastic handle to ethanol as it can discolor and degrade over time.

3. Once a CellRaft of interest has been successfully released from its microwell, lower the glass tip of the Magnetic Collection Wand into the media and bring the wand into proximity of the released CellRaft (**Figure 7A**).

   NOTE: Use the oculars or camera monitor to assist in bringing the wand into proximity of the released CellRaft based on the shadow cast by the tip of the wand (**Figure 7B**).

   NOTE: It is recommended to position the wand tip laterally above the released CellRaft prior to moving it down toward the array surface, to avoid accidental contact with the array surface and disturbance of cells within the microwell array.

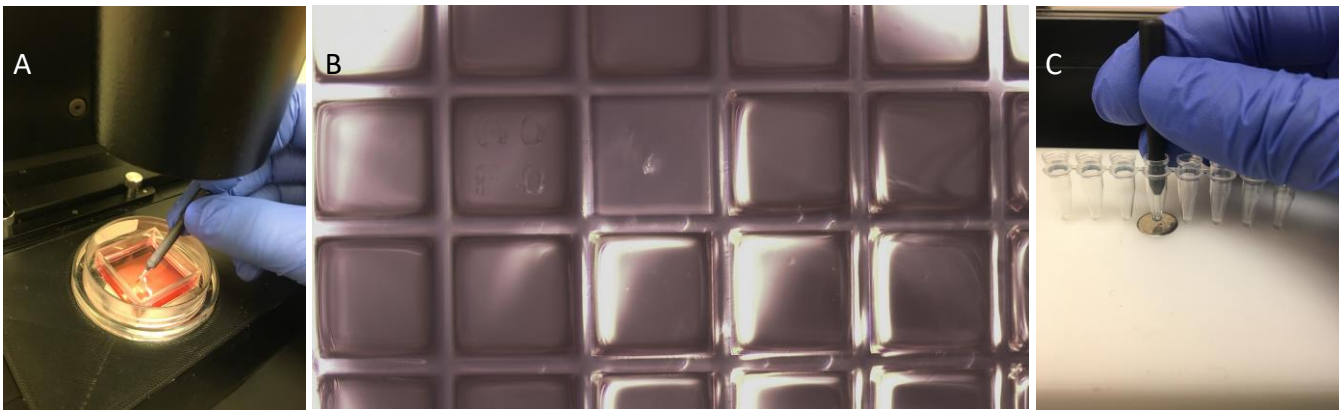

Figure 7. CellRaft Collection and Deposit. (A) Magnetic Collection Wand inserted into media to collect a released CellRaft. (B) Wand tip shadow indicating alignment with released CellRaft. (C) CellRaft deposit into a PCR tube using the Magnetic Collection Plate.

4. Once nearby, the CellRaft should be attracted to and collected on the tip of the wand, as evidenced by the CellRaft floating out of the microscope field of view.

   NOTE: It is recommended to wait five seconds after disappearance of the CellRaft from the field of view before withdrawing the wand to ensure that it has been successfully collected.

   NOTE: Physical contact between the wand tip and released CellRaft is generally not required for successful collection – often 2-3mm proximity is sufficient to attract CellRafts to the wand – but contact will not harm the cells on the CellRaft.

5. Slowly lift the wand vertically out of the media.

   NOTE: It is recommended for the media within the CytoSort™ Array to contain at least 10% serum to facilitate CellRaft collection. Lack of protein in the media can cause released CellRafts to adhere to the PDMS walls or be pulled off the tip of the wand by surface tension in the meniscus as the wand is withdrawn from the media.

   NOTE: Once removed from the CytoSort™ Array, the tip of the Magnetic Collection Wand may be rinsed without loss of the attached CellRaft by slowly dipping the wand tip into a 1.5 mL centrifuge tube containing a rinsing agent. Avoid contact with the tube walls if possible.

6. Center the destination TC well, PCR plate well, or PCR tube above the silver magnet in the Magnetic Collection Plate (**Figure 7C**).

   NOTE: Depending on the format, it is recommended that the destination well or tube contain sufficient fluid volume to make reliable contact between the wand tip and fluid without contacting the vessel walls. Transfer into PCR tube volumes as small as 1 μL are possible, but 5 μL are recommended.

7. Lower the tip of the Magnetic Collection Wand (with CellRaft attached) into the liquid within the destination well or tube (**Figure 7C**).

   NOTE: The magnets in the collection wand should rise within the wand upon insertion into the vessel and proximity to the magnet in the Magnetic Collection Plate. Examine the location of the magnets to ensure they have risen, as CellRafts will not be deposited otherwise.

8. Insert and withdraw the Magnetic Collection Wand into and out of the liquid in the destination vessel 3-to-5 times to deposit the CellRaft, avoiding contact with the sidewalls and maintaining the position of the vessel above the Magnetic Collection Plate magnet.

   NOTE: It is recommended to inspect the destination vessel and the Magnetic Collection Wand tip under a microscope to ensure successful deposit of the CellRaft.

   NOTE: Once the wand is removed from proximity of the collection plate magnet, the wand magnets should fall back to the tip. If not, shake the wand to reposition the magnets for the next isolation. Avoid excessive shaking as impact of the magnets can cause the glass tip to break.

   NOTE: After successful CellRaft deposit, the tip of the wand can be rinsed in PBS or ethanol to prevent contamination between isolations or to remove lytic agents, but avoid exposure of the plastic wand handle to ethanol as it can discolor and degrade over time.

8. Troubleshooting

8.1. Replacing the Window and Needle Assembly

In the event that the release needle does come into contact with any hard surface, replacement of the window and needle assembly is recommended. Minor needle damage will result in the inability to consistently puncture the CytoSort™ Array and effect CellRaft release. Gloves should be worn while replacing the needle to prevent fingerprints from obscuring the optical window.

To replace the window and needle assembly:

1. Ensure the Motorized Release Device is at its lowest position, attach its cap, disconnect its cable, and remove it from the microscope objective.
2. Remove the cap from the Motorized Release Device and loosen (but do not remove) the four set screws from the sides of the window holder using the Torx driver provided in the kit.

   NOTE: Additional screws (4) are included in the CellRaft® Kit should any be lost during the replacement process.
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.

   TROUBLESHOOTING: 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. Obtain a replacement needle from the Pelican case.
5. Remove the tape from the outside of the petri dish, then carefully remove its lid without contacting the needle.
6. Use tweezers to remove the tape from the edges of the window while holding down the edge of the window with your thumb.

   NOTE: Do not grab the new needle with tweezers, as this will damage the needle.

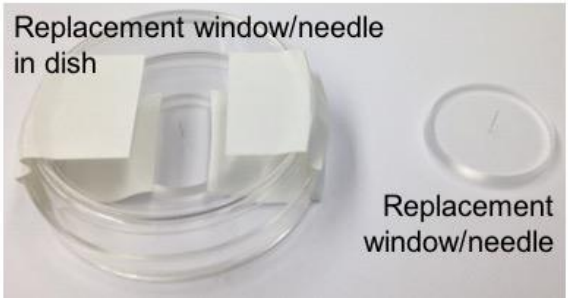

Figure 8. Replacement window and needle assembly.

7. Carefully remove the window from the dish by grabbing the window at its edges.
8. Place the new window and needle assembly in the window holder of the Motorized Release Device and lightly tighten all four set screws.

   NOTE: Do not over-tighten the screws as that can cause stripping of the plastic threads along the screw holes.

   NOTE: Attempt to position the window as centrally as possible within the window holder by tightening the screws symmetrically.
9. Attach the cap to the Motorized Release Device and re-install on the microscope objective as described in section 6.1. Reconnect its cable as described in section 6.2. Re-calibrate as described in section 6.3.


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