CellRaft AIR® System

One integrated platform to automatically image – identify – isolate

CELL Microsystems®

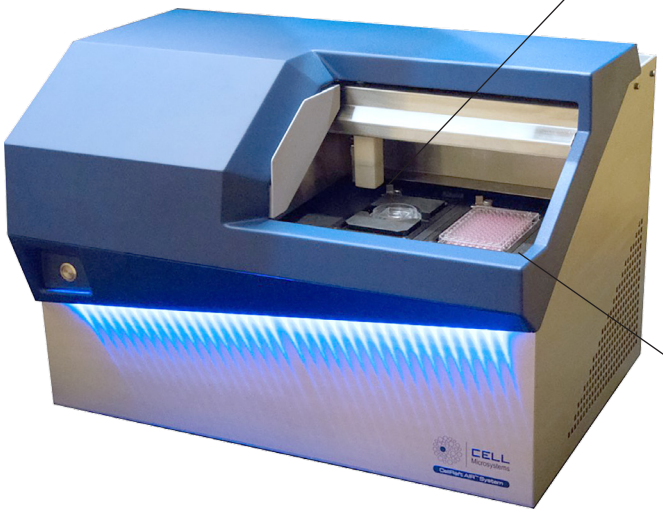
Rapid Imaging, Software-guided Identification, Automated Isolation

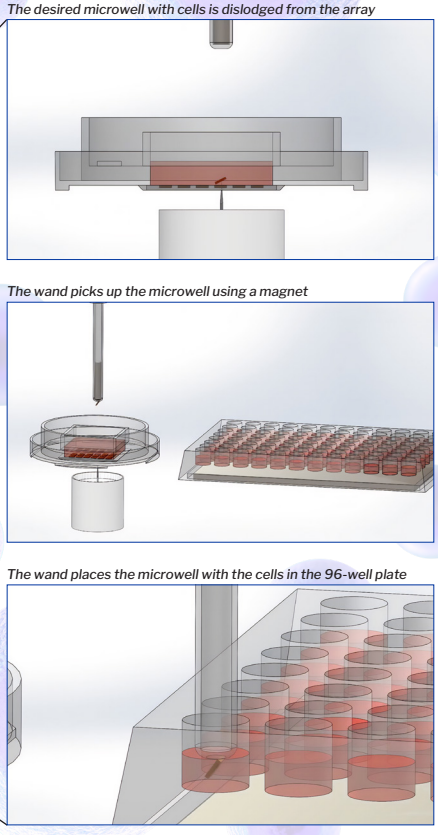
The CellRaft AIR® System is an integrated platform that uses proprietary CellRaft® technology and CellRaft® Arrays to maintain cells in an unperturbed state, leading to improved viability of single cells, highly proliferative colonies, and superior clonal outgrowth, that provides a dramatic increase in the number of clones available for downstream applications. With the system, you can:

- Obtain 10 - 50X more viable monoclonal colonies.
- Grow single cells in a flask-like environment, without physically separating them, eliminating perturbation to cell physiology and ensuring viability and vitality of single cells as they develop into clones.
- Identify cells of interest using powerful, label-free brightfield analysis software with user-defined parameters.
- Automatically and gently isolate CellRafts containing cells or colonies of interest for downstream endpoint analysis or clonal expansion.
- Create a complete image-based record of all clones to support regulatory submissions.

Common Applications

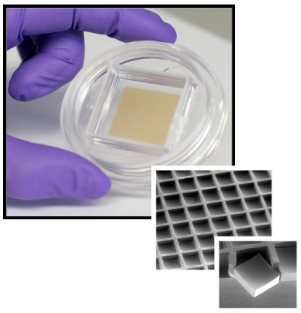
- Cell Line Development
- Stem Cells
- CRISPR
- Organoids
- Genomics

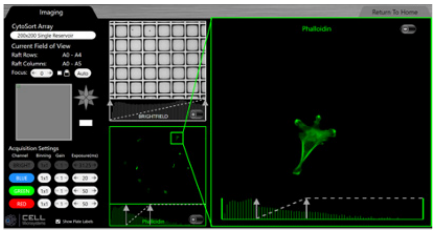




1. Seed Cells on CellRaft Array

Seeding cells on the CellRaft array is straightforward and comparable to seeding cells on any standard tissue culture dish. The dilute cell suspension is dispensed into the central reservoir, and cells settle into the microwells by gravity. The CellRaft Arrays are designed to yield a high number of single cells with the added benefit of shared media in a flask-like environment to increase viability and monoclonal outgrowth.



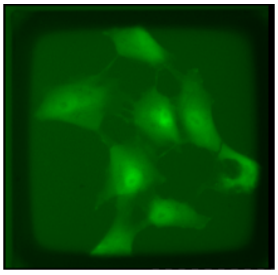


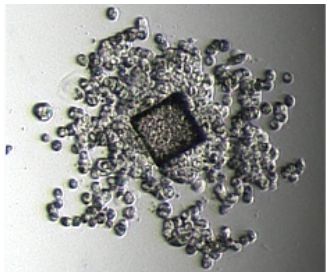
2. Image using CellRaft AIR System

Brightfield and fluorescence scanning provides single-cell resolution imaging in as little as seven minutes. The keyed orientation of the CellRaft arrays means that CellRafts can be scanned over the days or weeks needed to identify samples of interest without monopolizing the instrument with a single experiment.

3. Identify Cells of Interest in Software

CellRaft Cytometry™ allows for image-based verification of single cells to ensure monoclonality and analysis of a variety of parameters over time, ranging from morphology to gene expression. Users can easily define the characteristics of the target cells or colonies and map them for software-guided CellRaft selection for downstream isolation.



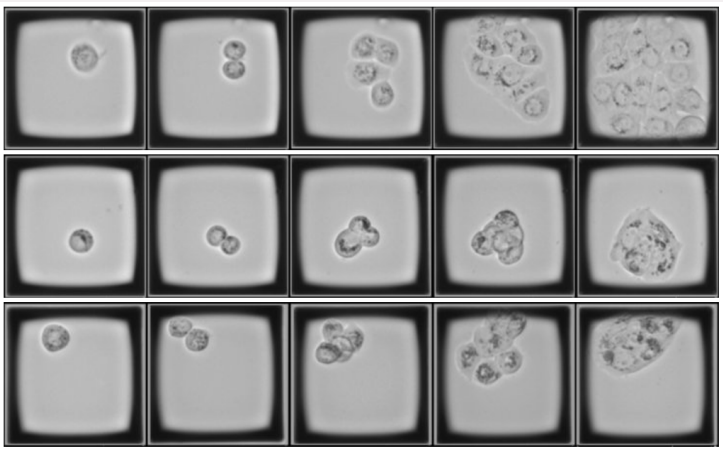


4. Automated Isolation to 96-well Plate

After identification, the CellRafts are gently dislodged from the CellRaft Array by a release needle and retrieved from the array by a magnetic wand to transfer the CellRafts into 96-well plates for downstream analysis. The fully automated CellRaft AIR System enables gentle, dissociation free isolation of fully validated, intact clones for propagation without dissociation or fluidics.

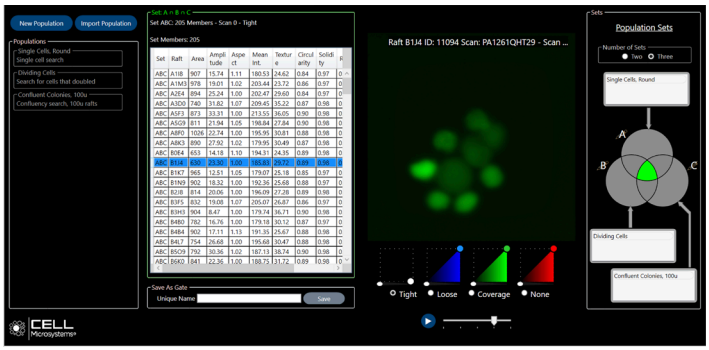
Time-Course Imaging for Track and Traceability for Audit trail

Time-course imaging is a key feature of the CellRaft AIR System. After cells are seeded on the CellRaft Array, the array is serially scanned at the desired frequency to capture complete growth records of clonal development. All scans are automatically stored in a single data record for easy image analysis and permanent record keeping.



CellRaft Technology

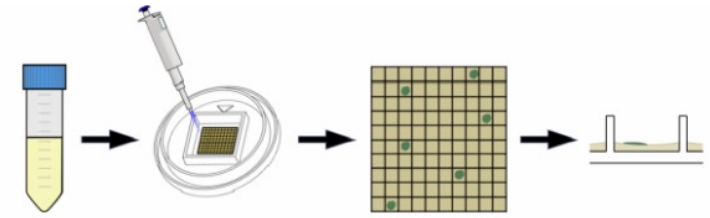
The CellRaft technology is an entirely new and seamless way of enabling cell culture, analysis, and isolation that goes beyond trypsinization and dispensing a single cell into a well. This unique method for developing monoclonal colonies from single cells uses three components (1) a CellRaft Array comprised of CellRafts (2) CellRaft Cytometry™ software and (3) CellRaft AIR, an integrated platform that incorporates rapid imaging, software-guided identification, and automated isolation.



What is a CellRaft?

The key to the technology is the CellRaft Array, a novel proprietary tissue culture dish containing thousands of microscale cell culture growth surfaces called CellRafts. Each CellRaft is housed within an individual microwell, allowing for gravity-based separation of a population into single cells.

Despite being spatially separated in the microwells, the novel design of the array allows the entire cell population to share a contiguous media volume, which leads to improved viability and proliferation of single cells compared to other single-cell culture methods. The cells are cultured while attached to the CellRaft for the entire duration of the experiment, with no need for dissociation or sorting to recover the validated clones.



Prepare Cell Suspension

Dispense Cell Suspension into CellRaft Array

Cells Settle into Microwells via Poisson-like Distribution

Cells Attach to Polysterene CellRaft

Software-Guided Identification and Automated Isolation

Finding those precious clones is easy using the CellRaft AIR System and the CellRaft Cytometry™ software. The arrays are rapidly scanned on the instrument in brightfield and three-color fluorescence in as little as seven minutes*.

The arrays are maintained in standard tissue culture incubators when not being scanned or isolated, freeing up the instrument for other experiments. Using software analysis tools, CellRafts can be analyzed for a wide range of parameters for expression or function, morphology, and time or a combination of all three to identify the desired phenotypes. After the automated identification of CellRafts with the cells or colonies of interest, the rafts can be easily mapped for isolation.

Isolation of the CellRafts is fully automated. The CellRafts are released from the array and isolated using a magnetic wand. The process is rapid, gentle, and efficient, yielding a 96-well plate filled with viable, validated clones.

*time dependent on experimental conditions

"One of the **biggest bottlenecks** in our lab is the ability to **produce clones at a fast rate**. The CellRaft AIR is uniquely placed in that **cells are able to survive,** as they share the media, and it is very gentle. We **are able to really speed up** the process using the CellRaft AIR System."

— Associate Director, Cell Engineering at a Pharma Company

More Viable Clones – Faster

Generate phenotypically-verified single cell-derived colonies in as little as 72 hours, with just 15 minutes of hands-on time, and 10 to 50x the number of clones compared to traditional methods. Cells share and enrich a common culture media while remaining separated. This contiguous media approach is much more favorable and dramatically increases cell viability.*

Eliminate the need for trypsin, fluidics, or limiting dilution while getting more clonal colonies. Our system also uses less plasticware and media than competitors.

*compared to FACS into a 96-well culture plate

Applications

Cell Line Development

Traditionally, a limiting dilution method is used to isolate and generate single cell-derived clones, which is both time and cost prohibitive due to low efficiency of success and the manual labor input required to successfully generate a useful cell line. Several automated technologies exist that aim to improve the efficiency of single cell or colony isolation, such as FACS, colony pickers, and droplet dispensers, but they often reduce cell viability due to harsh manipulation and stress on cells that are forced through fluidic systems or to grow alone in a large volume of media.

The CellRaft technology allows for streamlined and efficient cell line development. The AIR system offers an automated solution to an otherwise labor-intensive workflow and maintains cell health, provides time-course imaging for clonal verification, and delivers automated isolation for downstream propagation, altogether providing a superior platform that is more time, labor, and cost-efficient for cell line development.

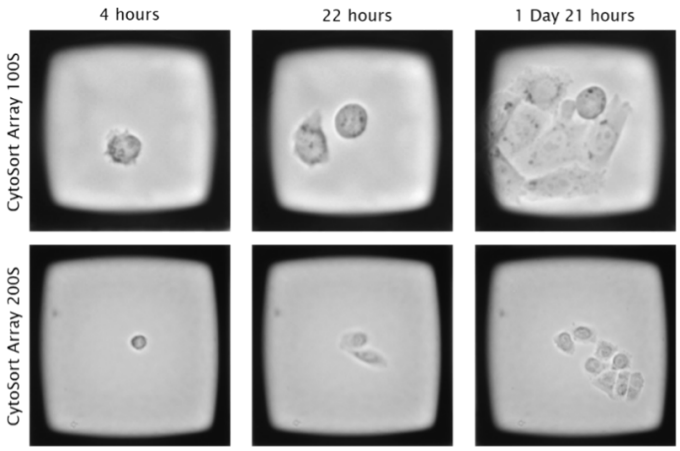


Figure 1. CHO cells seeded on the CellRaft Array are imaged and traced over time to monitor clonal outgrowth prior to isolation.

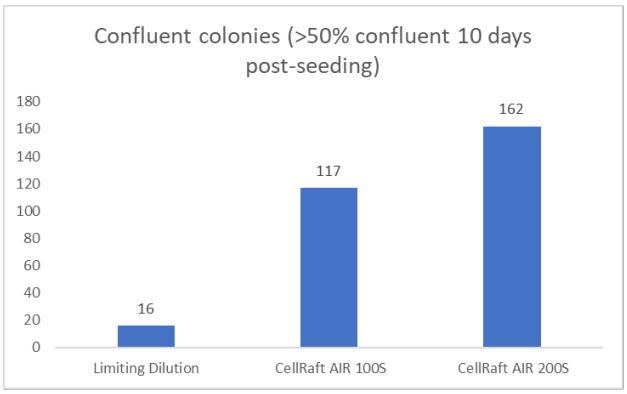


Figure 2. Clonal outgrowth efficiency of CHO-K1 cells in n=3 96-well plates using limiting dilution, Cell Raft Air 100S and 200S formats.

Stem Cell Culture

The research benefits of using iPSCs are often outweighed by the challenges of working with them. Stem cells require specialized media and growth conditions and are notoriously difficult to grow from single cell culture. With the CellRaft AIR® System, stem cells plated on the CellRaft Array show improved viability due to shared media, low culture volumes, and isolation of intact clonal colonies from single iPSCs. With the CellRaft technology, expensive media, extracellular matrix, and viability enhancers are significantly reduced, leading to cost and consumable savings as well.

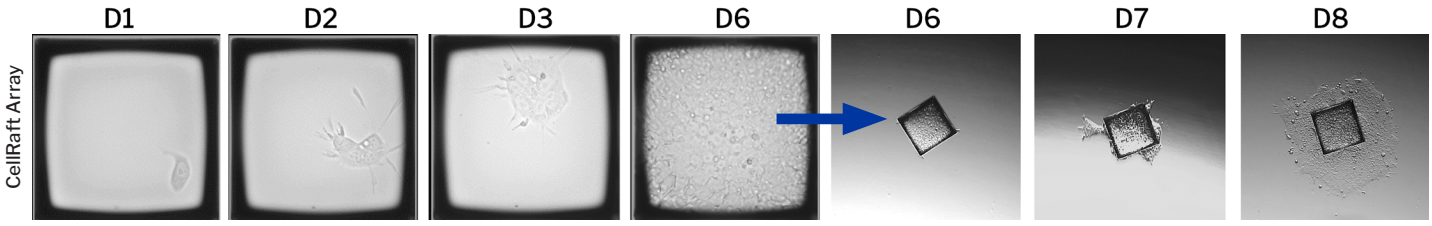


Figure 3. iPSCs grown on a the CellRaft Array. The CellRaft is isolated on Day 6 into a 96-well plate.

CRISPR

The process of cellular cloning and sorting is a major bottleneck in the CRISPR/Cas workflow. The imaging capabilities of the CellRaft AIR® System reduces this bottleneck by providing the user with Day 0 scans to visually connect CRISPR edits to desired phenotype expression at the single cell level. The user can identify clones of interest and confirm that clonal colony propagation began from a single cell.

The array is scanned and imaged over time, allowing the user to easily identify which cells to target and track through the initial colony growth phase.

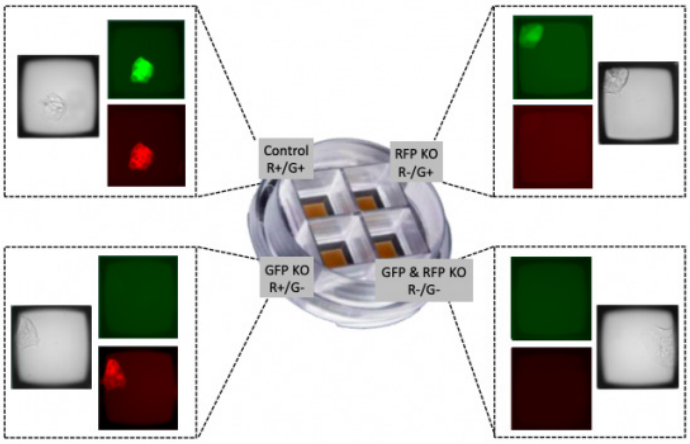


Figure 4. Use of the CytoSort QuadArray to isolate clonal colonies from four distinct CRISPR gene editing processes. Using the GFP/RFP-expressing model system, all four conditions were processed in parallel to generate both experimental and control gene editing cell lines in a single cell culture consumable. Given the 500 µL volume of each QuadArray well, reagent consumption was also reduced by 50-100 fold.

Genomics

Even with the use of highly advanced FACS platforms, enriched cell populations often contain dead cells, expression negative cells, doublets, debris, or other artifacts leading to false positives and negatives. The imaging capabilities of the CellRaft AIR System provide the user with initial scans to not only see the quality and phenotype of the cells, but to choose only the best cells.

Eliminate doublets and debris with automated selection of the best single cells that maintain their native phenotype for downstream genomic analysis. The system's unique design allows users to connect downstream nucleic acid and protein profiles to a specific individual cell, providing greater value to each experiment.

Organoid Development

In typical 3D cultures, the organoids overlap, requiring multifocal imaging with expensive equipment. They grow at different sizes and rates, leading to heterogeneity in viability and phenotype, resulting in confusing and uninterpretable data. Organoids grown in bulk culture are stuck in viscous extracellular matrix and are difficult to retrieve intact.

With this platform, CellRafts containing 3D structures of interest can be easily identified using CellRaft Cytometry™. The system enables visual verification of single cell clonality, morphology, and growth, providing critical data points for workflows requiring track and traceability for audit purposes. Identified organoids can be further evaluated and selected for phenotypic characteristics, such as organoid diameter, circularity, and fluorescence intensity.

The CellRaft AIR System offers a solution with a fully automated imaging and isolation workflow for organoid culture. Capture images in brightfield and fluorescence channels throughout the full height of the organoid with z-stack imaging. The images showing detailed phenotypic characterization are easily exported for the desired use.

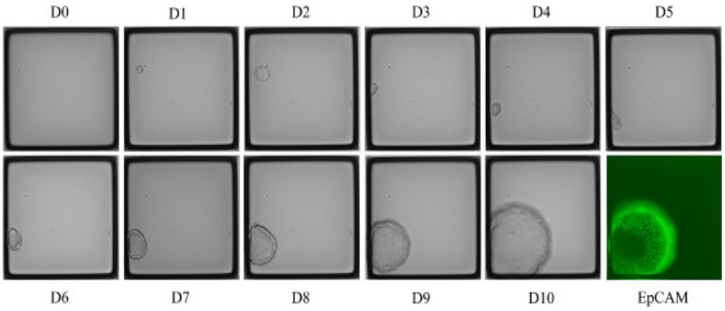


Figure 5. Organoid grown on a CellRaft for ten days and serially imaged.

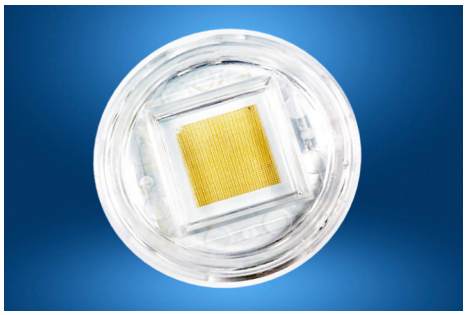
CellRaft® Arrays

Available in seven formats to optimize specific application workflows and outcomes.

CellRaft Single Array

Our most versatile array, suitable for any application.

Sizes:
100 x 100 microns microwells - 40k CellRafts Per Array
200 x 200 microns microwells - 10k CellRafts Per Array



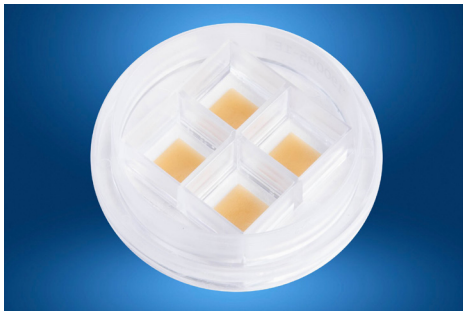
CellRaft Quad Array

Four individual reservoirs make this array the best choice for:

- Clonal expansion and colony selection for parallel gene editing and cloning
- Multivariable treatments and differing media applications

Sizes:

100 x 100 microns microwells - 25,600 CellRafts Per Array (6.4k per reservoir)
200 x 200 microns microwells - 6,400 CellRafts Per Array (1.6k per reservoir)



CellRaft HexaQuad™ Array

Up to 24 individual reservoirs make this array the best choice for:

- High throughput CRISPR screening, clonal expansion and colony selection
- On-array functional assays and dosing studies

Sizes:

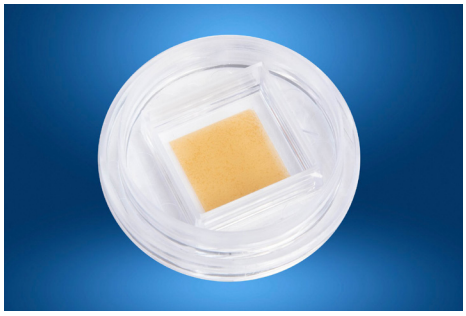
100 x 100 microns microwells - 153,600 CellRafts Per Array (6.4k per reservoir)
200 x 200 microns microwells - 38,400 CellRafts Per Array (1.6k per reservoir)

3D CellRaft Array

Large surface areas that allows cell growth up to 1 mm in diameter make this array best for:

- 3D structures, including organoids

Size:
500 x 500 microns microwells - 2,114 CellRafts Per Array



System Specifications

IMAGING

Fluorescence excitation-emission specifications

Channel	Representative Dyes	Excitation	Emission
Blue	DAPI, Hoechst	379 – 401 nm	412 – 453 nm
Green	FITC, GFP	461 – 489 nm	498 – 548 nm
Red	mCherry, Texas Red	559 – 591 nm	603 – 805 nm

SPECIFICATIONS

Size	20” High, 20” Deep, 27” Wide
Weight	125 lbs
Camera	2752 x 2192 resolution (6MP) 4.54 x 4.54 µm pixels 8-bit depth
Magnification	1.2 x 1.4 mm field of view 0.57 µm/pixel* **On par with 20X magnification using a typical scientific microscopy camera*
Computer and Monitor	Included
Data Storage	0.5 TB on computer, 0.5 TB external USB drive, network capable (ethernet, wi-fi)
Stage Top Incubator	Optional
Power	125/250 VAC, 10A, 60/50 Hz
Certification	ISO 61010, FCC emissions, CE Mark

SOFTWARE MADE FOR YOUR WORKFLOWS

The CellRaft AIR System software, CellRaft Cytometry, is powerful and intuitive enabling:

- Brightfield, label-free identification of CellRafts containing desired cells
- 3-color fluorescent analysis of cells, colonies, or organoids
- User customizable parameters to identify cells of interest
- Saving and importing analytical parameters for time, function, and morphology across experiments and cell types
- Leveraging CellRaft coverage metrics to identify the ideal time to isolate your viable clones
- Analyzing CellRafts over time to ensure clonality and monitor phenotype
- Queuing CellRafts meeting your experimental criteria directly for isolation

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