A NOVEL METHOD for Live Staining, Imaging, and Clonal Isolation of 2D & 3D Cellular Systems

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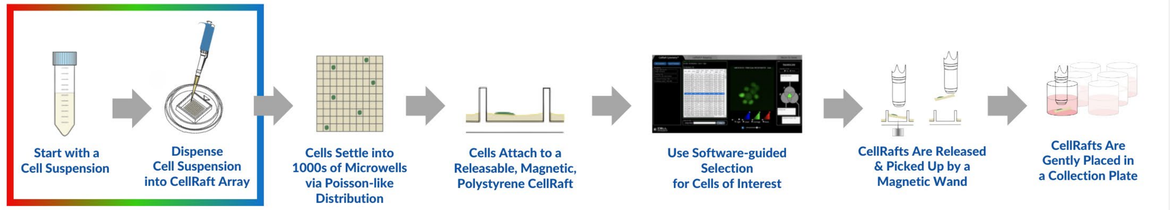
Background

- Cell staining has existed throughout the world since Joseph Von Gerlach in 1858 used ammoniacal carmine to successfully stain cerebellum cells [1]. Over time, protocols have been developed to stain nearly any cellular component, enhancing the visualization of a cell under microscopic conditions [2].
- Often, live staining takes place in a culture dish where a bulk population of cells is visualized under a microscope to identify characteristics of interest. While the cells may display the qualities that are important to the researcher, it is impossible to retrieve those cells without assistance from FACS or another type of sorting. This process often involves trypsinizing cells, which alters cell morphology, expression of certain genes, and affects the viability of the cell population.
- The CellRaft AIR® System is a powerful tool that can be leveraged to overcome these obstacles. This system offers the unique ability to use fluorescent staining to examine cellular marker expression and phenotypically characterize adhered cells in real time in their native state. The CellRaft AIR System can recover the population of interest without disturbing the colony; there are no dissociation reagents and no microfluidics involved, unlike with FACS.
- Using CellRaft® Arrays, staining protocols commonly used in 2D and 3D workflows can be easily adapted to identify populations of interest for isolation and recovery into expansion plates with high cell viability for further downstream characterization.

CellRaft® Workflow

Figure 1: Three options for staining cells for use with the CellRaft Array, which is then placed on CellRaft AIR System.

Stain Cells: Option 1: In a dish, then seed on the Array. **Option 2:** In a tube, then seed on the Array. **Option 3:** Directly on the Array.



Live Staining for Confirmation of Pluripotency

Figure 2: Reprogramed iPSCs and a stable iPSC line stained using pluripotency marker. (LEFT A) BJ cells reprogrammed into iPSCs using the Epi 5 kit on a 200µm Quad Array stained with (1:100) Anti-TRA-1-60-FITC or (LEFT B) Anti-TRA-1-60-Alexa Fluor 594 conjugated undifferentiation marker on D15 of reprogramming. (RIGHT) β-Actin tagged GFP iPSCs on a 200µm Single Array stained with (1:50) Anti-TRA-1-60 Alexa Fluor 594 and NucBlue Live Ready Probes nuclear stain (2 drops/mL).

Live Cell Painting Using Non-Toxic Nuclear, Mitochondria, and Cell Membrane Dyes

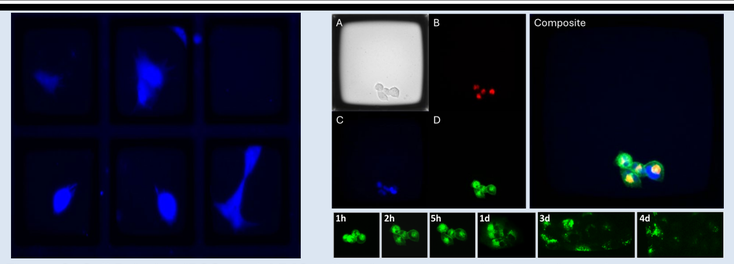


Figure 3: Cell Painting iPSCs and A549 cells using nuclear, mitochondria, and membrane dyes. (LEFT) KYOU iPSCs on a 200µm Quad Array stained with CytoPainter Live Cell Labeling Kit – Blue Florescence following manufacturer recommendations (1:25,000). (RIGHT) A549 cells stained with (500nM) MitoTracker Red FM and (1:200) CellBrite Green membrane before seeding on a 200µm Quad Array then stained on the array with (2µg/mL) Hoechst 33342 staining solution. A549 cells stained with (1:200) CellBrite Green membrane before seeding on a 200µm Quad Array.

Live Staining Targeted Antibody Selection

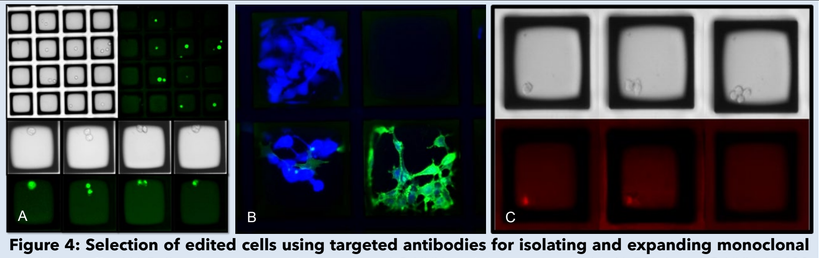


Figure 4: Selection of edited cells using targeted antibodies for isolating and expanding monoclonal positive cells. (A) Raji cells engineered to express AG-XXX and then stained with (1:50) Anti-AG-XXX-AF488 before seeding on a 100µm Quad Array. (B) Polyclonal eBFP2 and HER2 engineered NIH-3T3 cells on a 200µm Single Array live stained with (1:50) Anti-HER2-FITC. (C) Polyclonal Jurkat cells transfected with SR-XXX and then stained with Anti-SR-XXX-AF594 (10µg/mL) before seeding on a 100µm Single Array.

Visualize Mitotic States with Long-lasting and Non-toxic DNA Labeling Probes

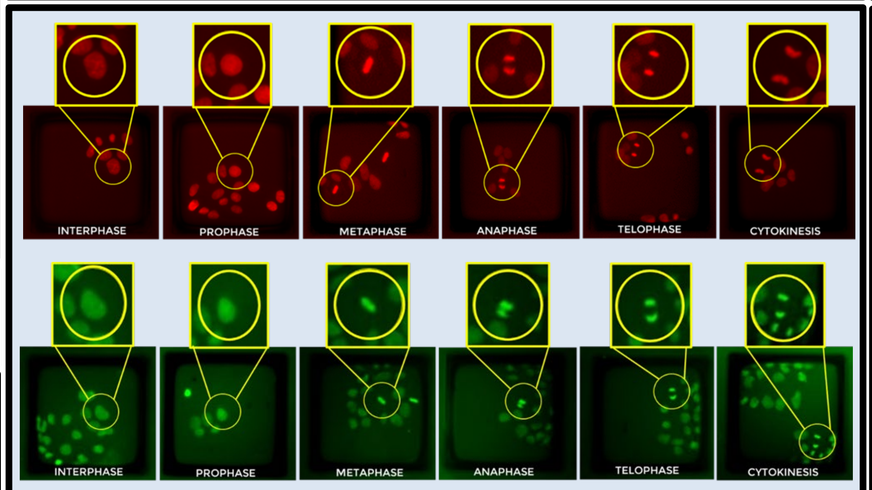


Figure 5: Staining iPSCs with nuclear DNA labeling probes for visualization of low contrast cells and mitotic phases within the cell. KYOU iPSCs stained with either (1:1000) SPY594-DNA (TOP) or (1:1000) SPY505-DNA (BOTTOM) live cell fluorogenic DNA labeling probe on a 200µm Quad Array.

Live Cell Painting Using Proliferation Dyes for Cell Tracking

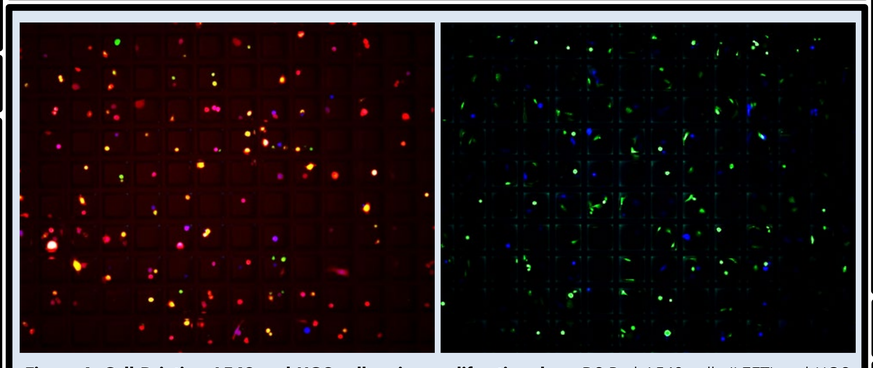


Figure 6: Cell Painting A549 and HOS cells using proliferation dyes. DS-Red A549 cells (LEFT) and HOS cells (RIGHT) stained with either (2.5µM) ViaFluor SE 488 or (5µM) ViaFluor SE 405 before seeding on a 100µm Quad Array.

Live 2D and 3D Staining for Examining Cell Morphology and Viability

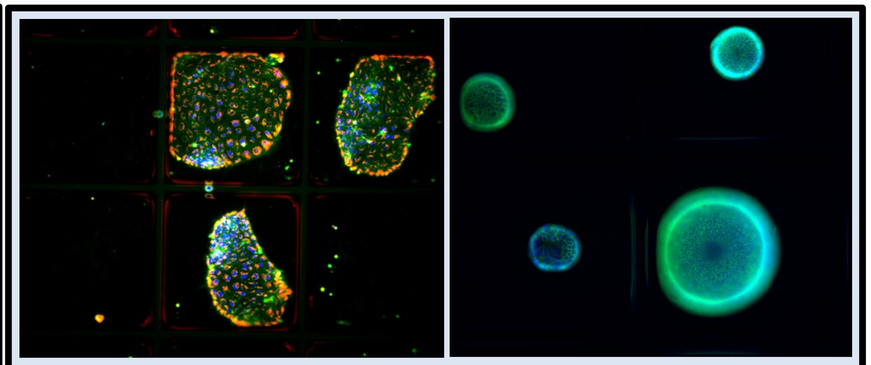


Figure 7: iPSC derived lung progenitor cells and mouse hepatic organoids stained for examining cell junctions and structure. (LEFT) iPSC derived Lung Progenitor Cells on 500µm Single Array. NucBlue Live Ready Probes nuclear stain (2 drops/mL), MitoTracker Red FM mitochondrial stain (250nM), and CellBright Green membrane stain (1:200). (RIGHT) Mouse hepatic organoids on a 500µm Single Array stained with (1:200) EpCAM-FITC and NucBlue Live Ready Probes nuclear stain (2 drops/mL).

Summary

- Staining protocols for 2D cells and 3D organoids can be easily adapted for use with the Arrays and CellRaft AIR System, allowing for live staining to occur on the consumable.
- The CellRaft AIR System is compatible with viability dyes to monitor proliferation and cell health, cell surface antibodies that can identify subpopulations of interest, and subcellular dyes to label cell parts for studying expression or monitoring mitotic changes within the cell.
- The CellRaft AIR System provides time-course imaging to visualize real-time expression measured over time within the CellRaft Arrays. The data is captured automatically and is housed within the same file for easy access and interpretation.
- While cells are live-stained on the CellRaft Array, the CellRaft AIR System can be used to automatically retrieve populations of interest without trypsinizing or using microfluidics that often harm cell viability, morphology, and expression levels in cells.
- Cells retrieved using the CellRaft AIR System are located on individual growth surfaces called CellRafts. These can be left in culture and do not interfere with downstream -omic applications, qPCR results, or imaging under the microscope.
- Cells are more viable, healthier, and never physically manipulated using the CellRaft AIR System compared with FACS or other cell sorters.

References

1. Alturkistani, Hani A, et al. “Histological Stains: A literature review and case study.” Global Journal of Health Science, vol. 8, no. 3, 25 June 2015, p. 72, https://doi.org/10.5539/gjhs.v8n3p72.
2. Bruckner, Monica Z. “Microbial Life.” Microscopy, 27 Feb. 2024, serc.carleton.edu/microbelife/research_methods/microscopy/index.html#:~:text=The%20most%20basic%20reason%20that,dead%20cells%20in%20a%20sample.