The CellRaft AIR® System: A novel system enabling organoid imaging, identification, and isolation

Allysa Stern[1], Brandon Thompson[1], Keith Williams[1], Rob McClellan[1], Steven Gebhart[1], Jessica Hartman[1*]

[1] Cell Microsystems, Durham, NC 27713, USA

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***** Address correspondence to:

Jessica Hartman

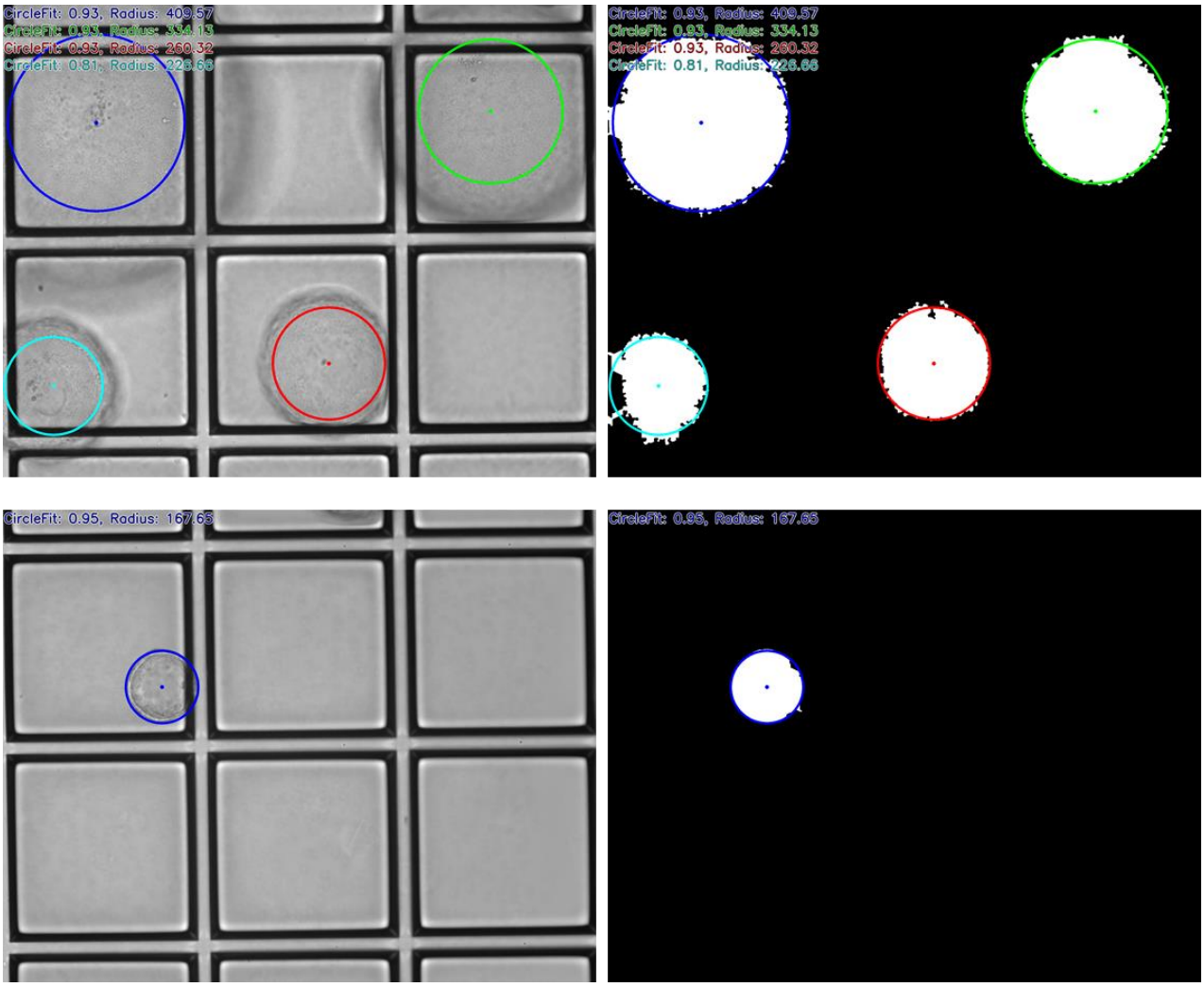
Cell Microsystems

801 Capitola Dr. Ste 10

Durham, NC 27713

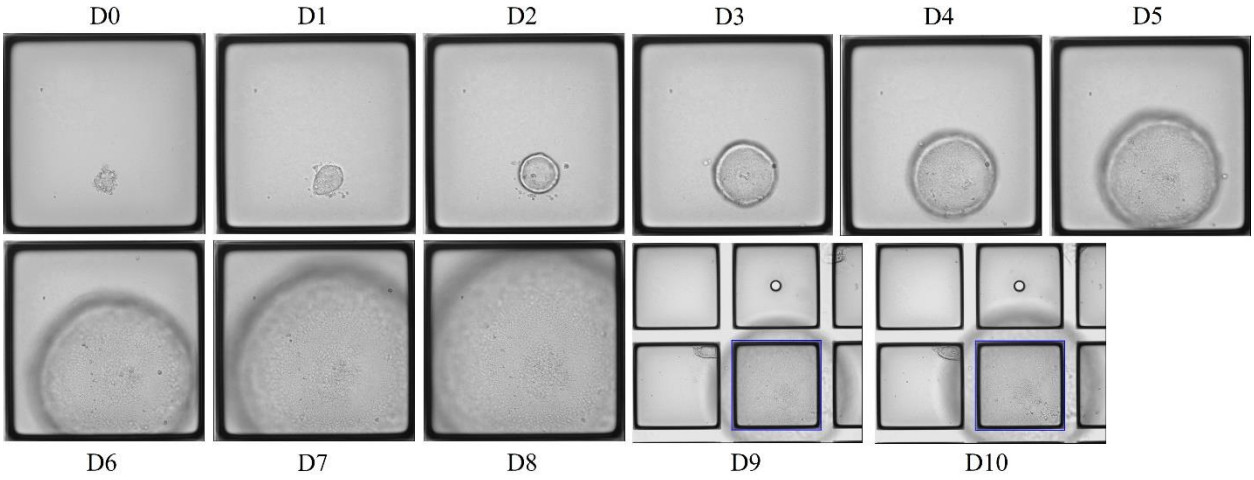
U.S.A

Email: jhartman@cellmicrosystems.com

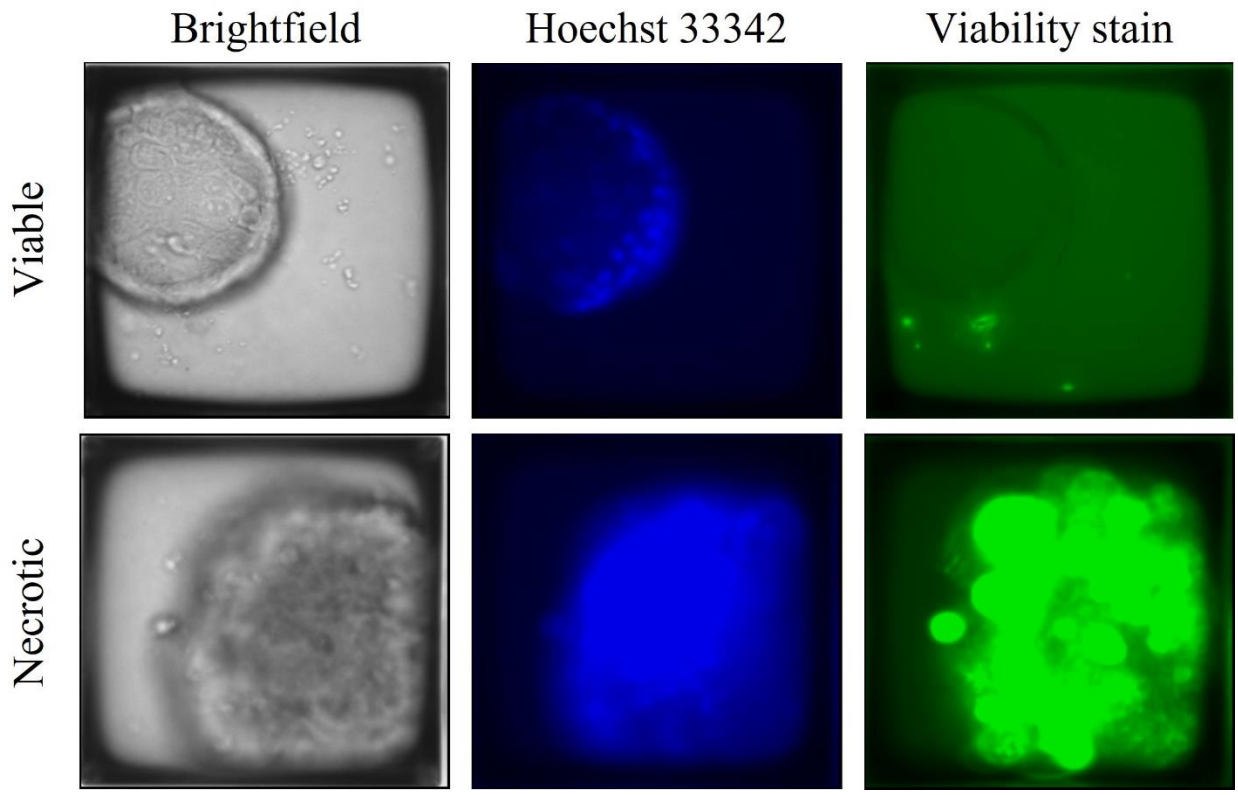


Supplemental Figure 1. Brightfield segmentation algorithms analyze every 2D single focal plane image captured during full array scans. The black and white masks made during segmentation are then used to identify organoids on the 3D CytoSort Array. The centers of different areas of interest within the mask are found, which are then used to assign organoids to a particular CellRaft, even if the organoids have grown larger than the 500 x 500 µm CellRaft footprint. A circle is fit to each area of interest, allowing for calculation of the radius (in pixels) and CircleFit. These calculated data allow for the user to build a population in the Cytometric Image Analysis function in Off The AIR

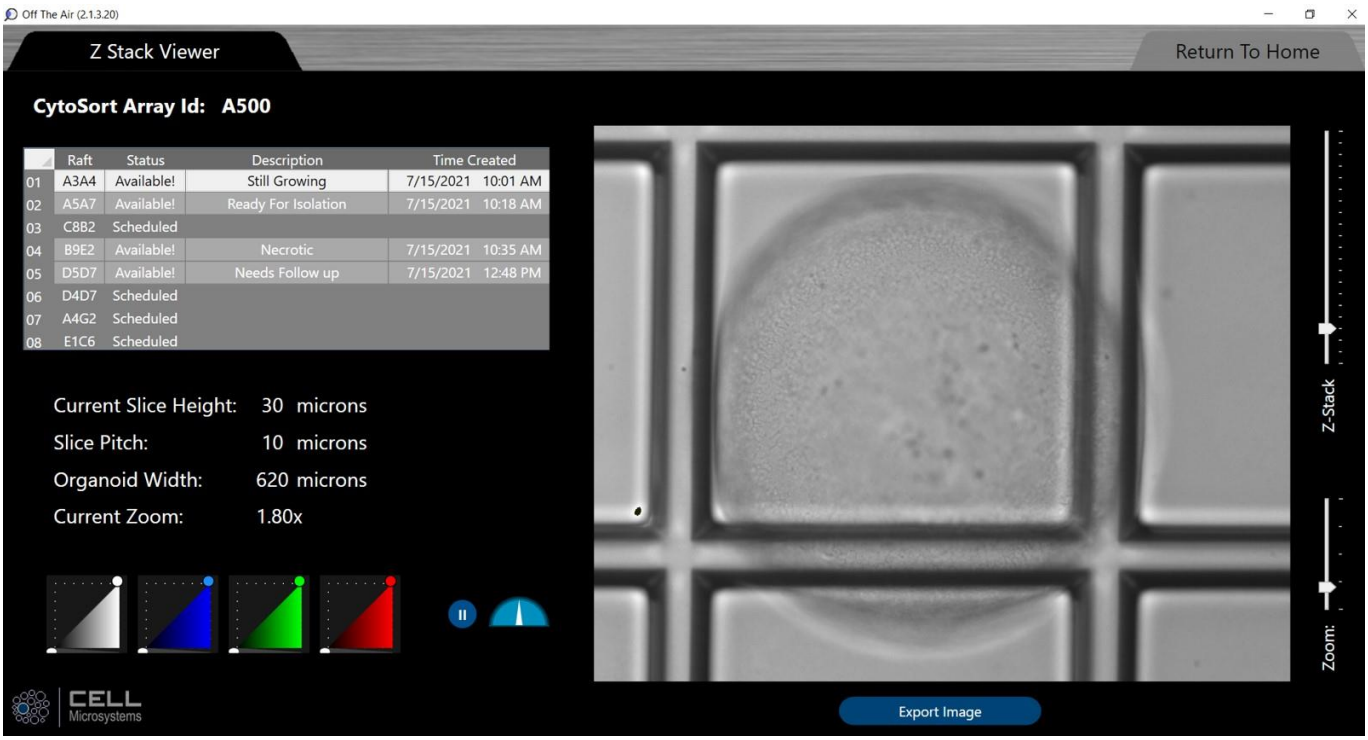
software to identify CellRafts with a single area of interest (AOI count) or organoid, that has a specified diameter (in microns) and roundness.



Supplemental Figure 2. Temporal imaging of mouse pancreatic organoids on the 3D CytoSort Array. Mouse pancreatic organoids were mechanically dissociated into small fragments and seeded on the 3D CytoSort Array in dilute Matrigel media. Serial scans of the array were performed every 24 hours for 10 days to monitor organoid development.



Supplemental Figure 3. Using the brightfield and fluorescence imaging capabilities of the CellRaft AIR system, mouse pancreatic organoids were assessed for viability using the ReadyProbes Blue/Green Cell Viability kit (Invitorogen). Individual organoids are easily imaged and assessed for viability, including necrotic cores.



Supplemental Figure 4. The CellRaft AIR System software, and companion Off The Air data analysis software, provide a user-friendly, intuitive interface to automatically acquire and explore z-stack images of organoids of interest. After the user defines the z-stack slice pitch and brightfield and fluorescence exposures, the software acquires the images across the full organoid height. After image acquisition, the user can easily view each image that was captured within the stack. The software reports the organoid diameter (width), allows the user to zoom in and out of each image to visualize the organoid at single-cell resolution, and provides tools to modify the relative contrast of each imaging channel for composite display. The software also allows the user an area to write in a “description” of each organoid of interest that was imaged, providing a complete data catalog of individual organoids.

Supplemental Video 1. Using the CellRaft AIR System fitted with the concentric needle design, isolation of a CellRaft and its attached organoid from the 3D CytoSort Array is dynamically performed to achieve rapid CellRaft release and collection.

Supplemental Video 2. Using the CellRaft AIR System, brightfield z-stack images were captured every 10µm throughout the height of the organoid. Z-stack images were exported and used to create videos for full organoid display.

Supplemental Video 3. Using the CellRaft AIR System, green and blue fluorescence z-stack images were captured every 10µm throughout the height of the organoid. The overlay z-stack images were exported and used to create a video of the composite cell staining for full organoid display.