CRISPR/Cas9 Genome Editing using the CellRaft AIR™ System

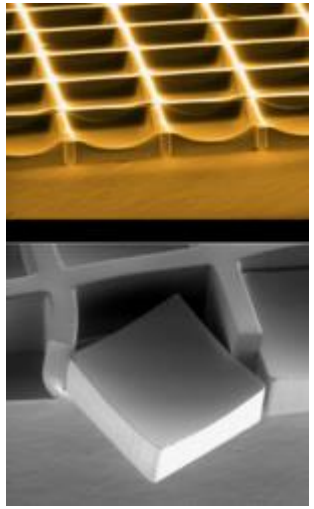
Nick Trotta, PhD and Steve Gebhart, PhD
Cell Microsystems, Inc.
Product Applications - ntrotta@cellmicrosystems.com
Engineering - sgebhart@cellmicrosystems.com

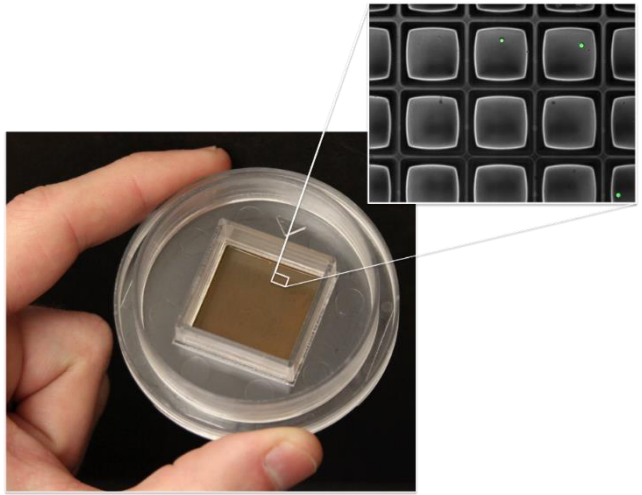
CellRaft Technology

- Straightforward means of imaging, sorting and isolating single cells
- Employs a microwell array which replicates standard culture conditions
  - Eliminates minimum sample input requirements
  - Imaging-based sorting has advantages over FACS and other methods to see cell phenotypes prior to isolation
- Multiple products available tailored to specific experimental and throughput needs

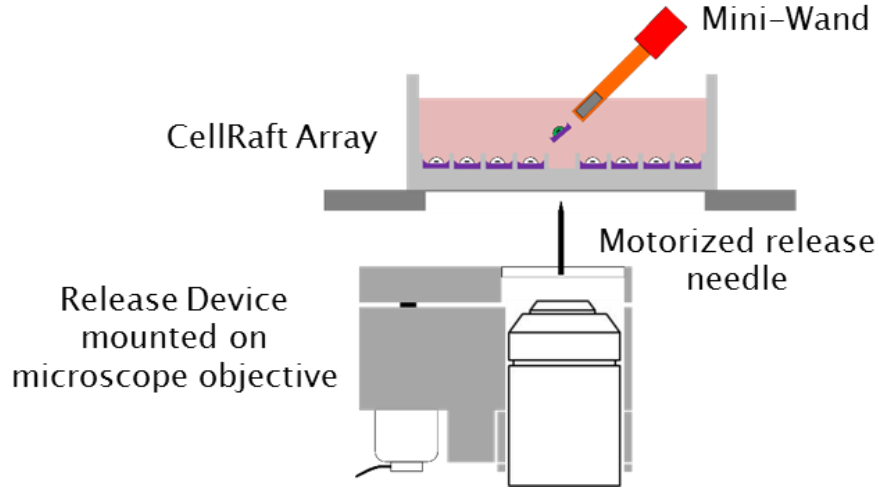
CellRaft Technology - Components

- CytoSort Array
  - Microwell array
  - Releasable CellRaft in each microwell
  - 100 or 200 micron CellRafts
  - Elastomer array
  - Polystyrene CellRaft

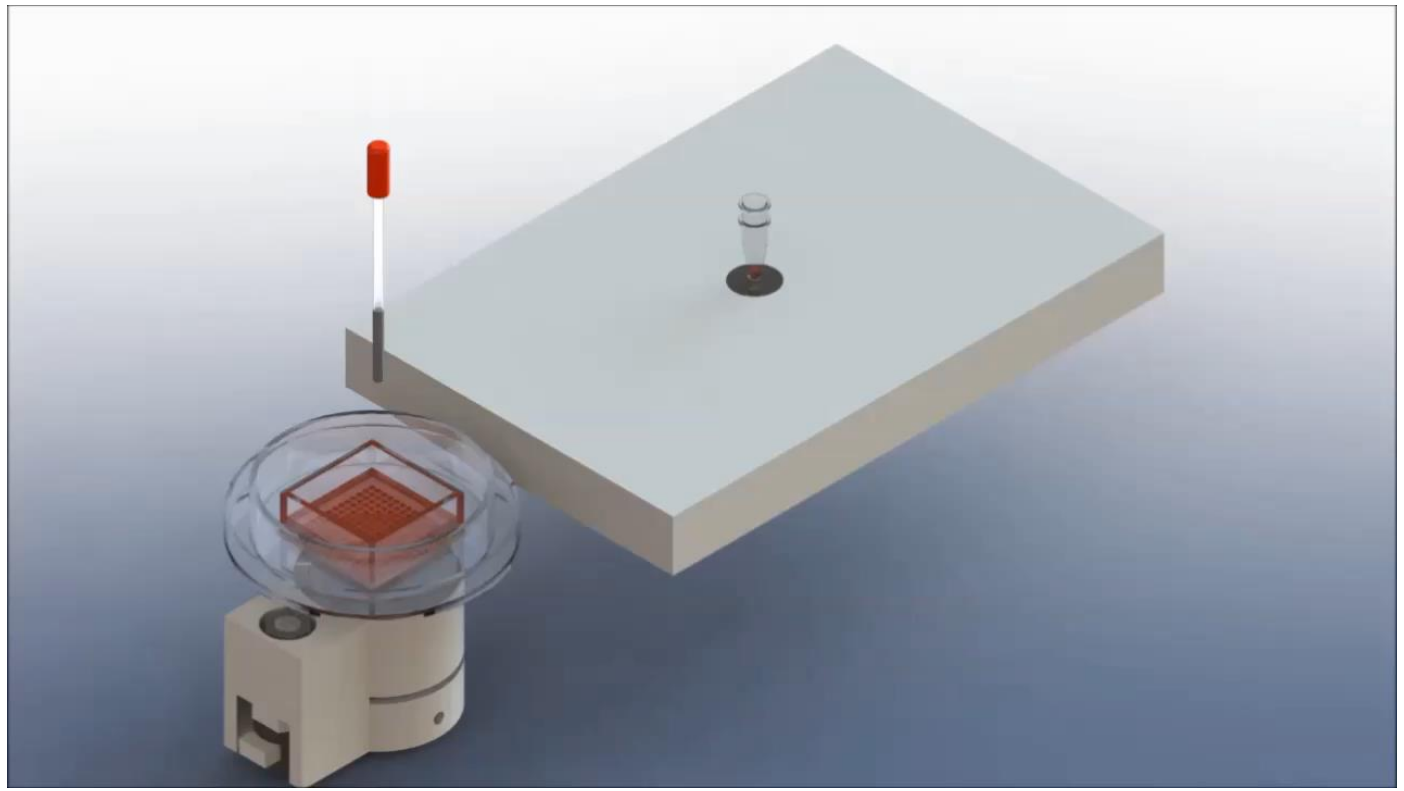




- Release/Transfer Devices
  - Needle for release of CellRaft from array
  - Magnetic wand for CellRaft recovery and transfer to plate or tube
  - Manual and automated versions



CellRaft Technology - Operation



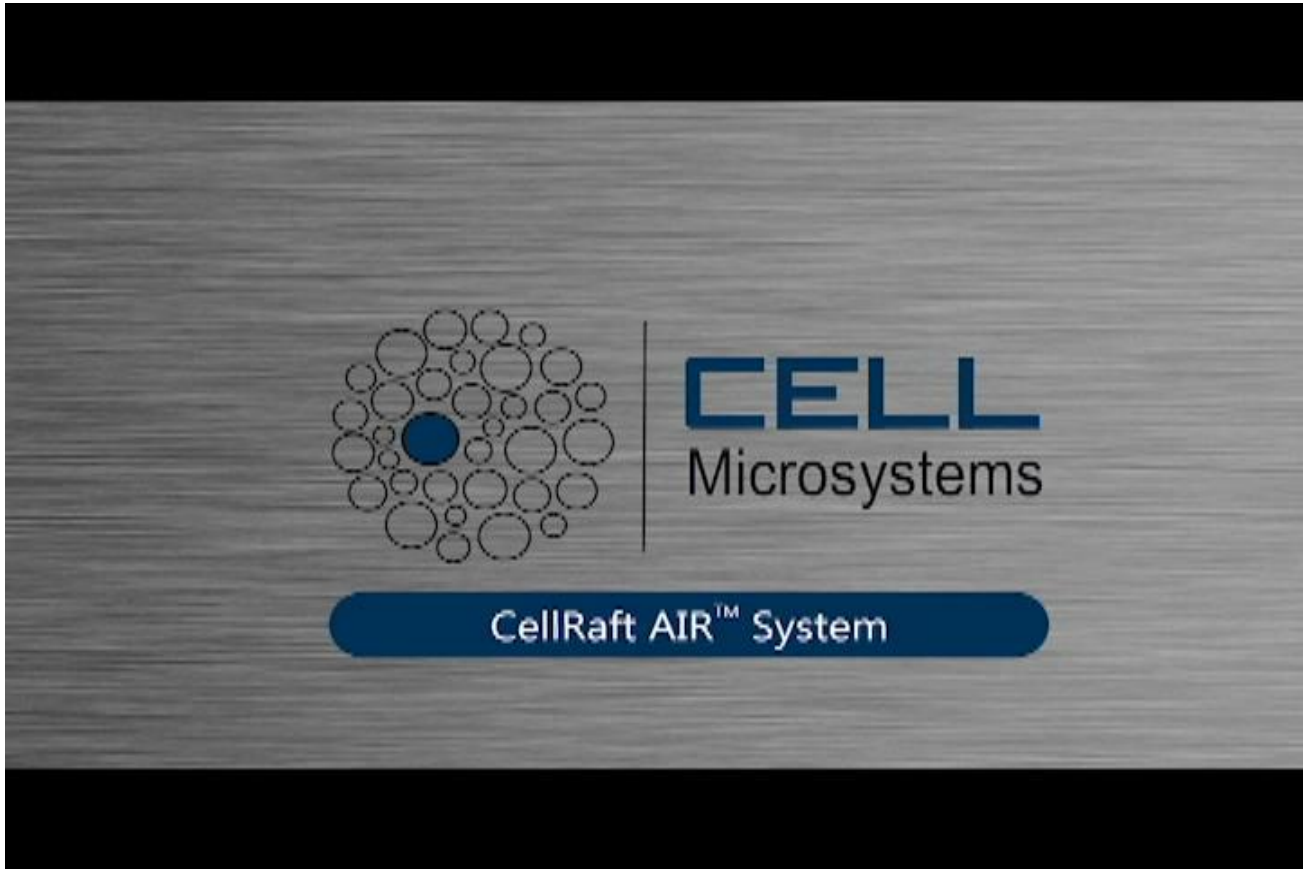
Video can be viewed here: https://cellmicrosystems.com/cellraft-technology/

CellRaft Systems

- CellRaft System – Manual
  - Fits on virtually any inverted microscope
  - Very flexible and cost-effective
  - Compatible with any type/size of CytoSort Array
  - Approximately 50 cells in 2-4 hours
  - Manual collection into any vessel
  - Comes with 2 CytoSort Arrays

- AIR™ System – Automated
  - Fully integrated microscope and release/transfer hardware
  - 3-channel fluorescence
  - "Real-time" or "Cytometric" sorting capabilities
  - 96 cells in approximately 1.5 hr;
  - 30 min for each subsequent 96 cells
  - Collect into
    - 96-well plates (~150-200 µL volume)
    - PCR strip tubes (minimum 2.5 µL volume).

AIR System in Operation



CellRaft Technology for CRISPR

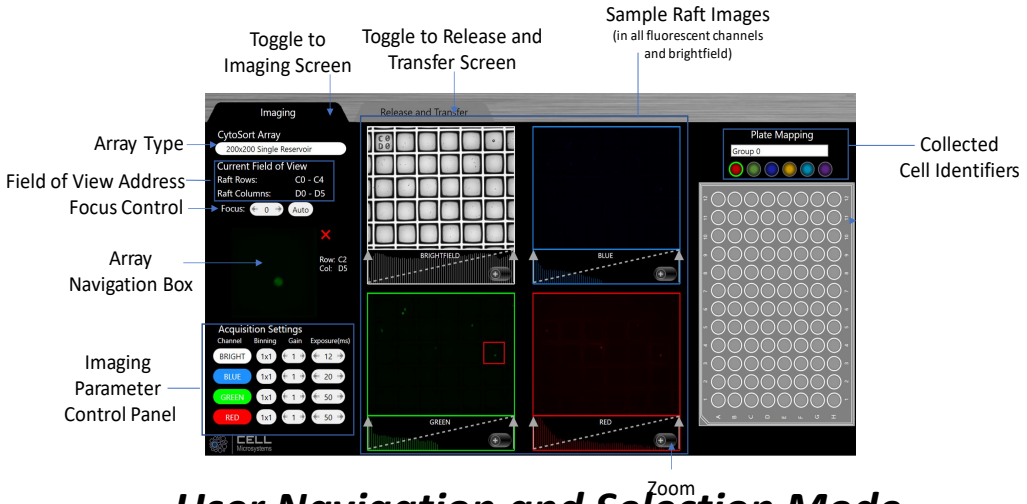
- Allows imaging-based sorting of single cells and colonies
  - Seed a cell suspension on the CytoSort Array
  - Image on the AIR™ System (or your microscope)
  - Isolate cell/colony by release of a single CellRaft from the array

- Technology supports the growth of clonal colonies
  - Monitor colony growth over time and release individual colonies without disturbing the growth and viability of neighbors
  - ***Laboratories observe a significant increase in efficiency by growing clones on the array prior to transferring to expansion culture***

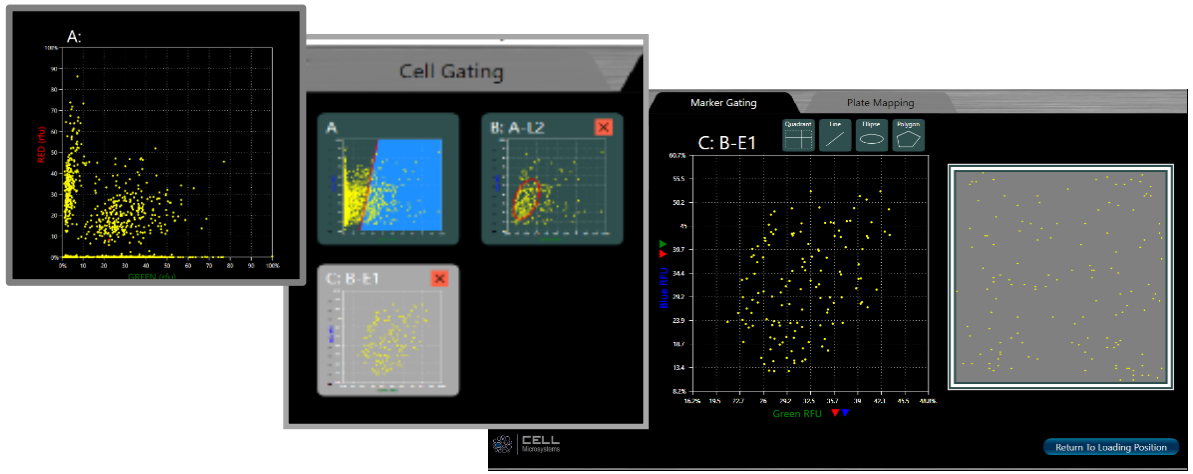
AIR System Run Modes

- Multiple ways to conduct an experiment based on sorting requirements

Feature	User Navigation and Selection	Full Array Scan + User Selection	Full Array Scan + Cytometric Mode	Multiple Full Array Scans + User Selection
Sorting Modality	User navigates the array by mouse clicks and empirically identifies cells of interest	After full array scan user navigates the array images by mouse clicks and empirically identifies cells of interest	Fluorescent intensities plotted on XY axes; set thresholds and gates on the histogram	After full array scan user navigates the array images and movies by mouse clicks and empirically identifies cells of interest
Array Position	CytoSort Array must remain on system	Array can be removed from the AIR System after Full Array Scan concludes	Array can be removed from the AIR System after Full Array Scan concludes	Array can be removed from the AIR System after each Full Array Scan concludes
Imaging Parameters	Imaging parameters can be adjusted in real time	Set at beginning of scan	Set at beginning of scan	Set at beginning of each scan
Applications	Identify single cells for molecular analysis	Sensitive cells	Sorting of subpopulations with varying marker expression	CRISPR; Clonal colony tracking; time-course analysis



User Navigation and Selection Mode

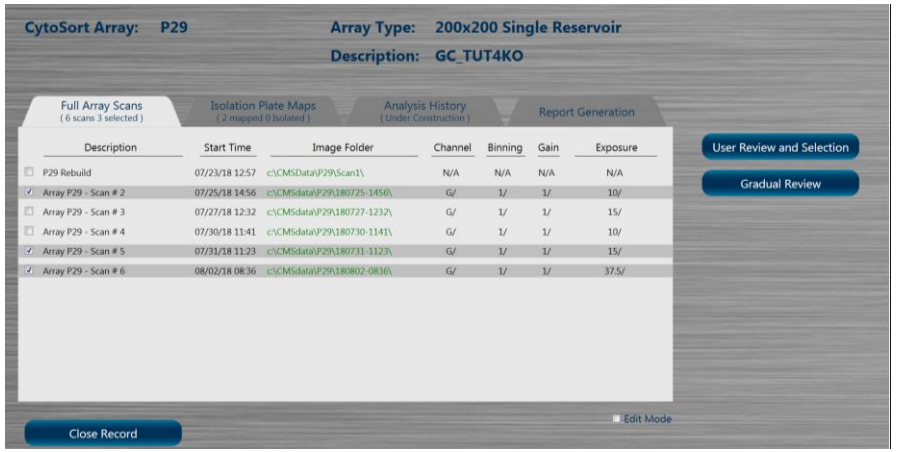


Cytometric Sorting Mode

Clonal Colony Tracking

- **Multiple Full Array Scans + User Selection Mode**
- Once cells are seeded, software has been developed to monitor cells over time as clonal colonies grow
- Colonies can then be isolated at any time without disturbing neighboring colonies
- Multiple array scans can be viewed simultaneously to watch movies of colony growth

Off the AIR Software: Database software allows multiple full array scans to be viewed simultaneously



Day 2

Day 4

Day 8

CRISPR/cas9 Clonal Colony Propagation

Transfect Cells with Cas9 + sgRNAs

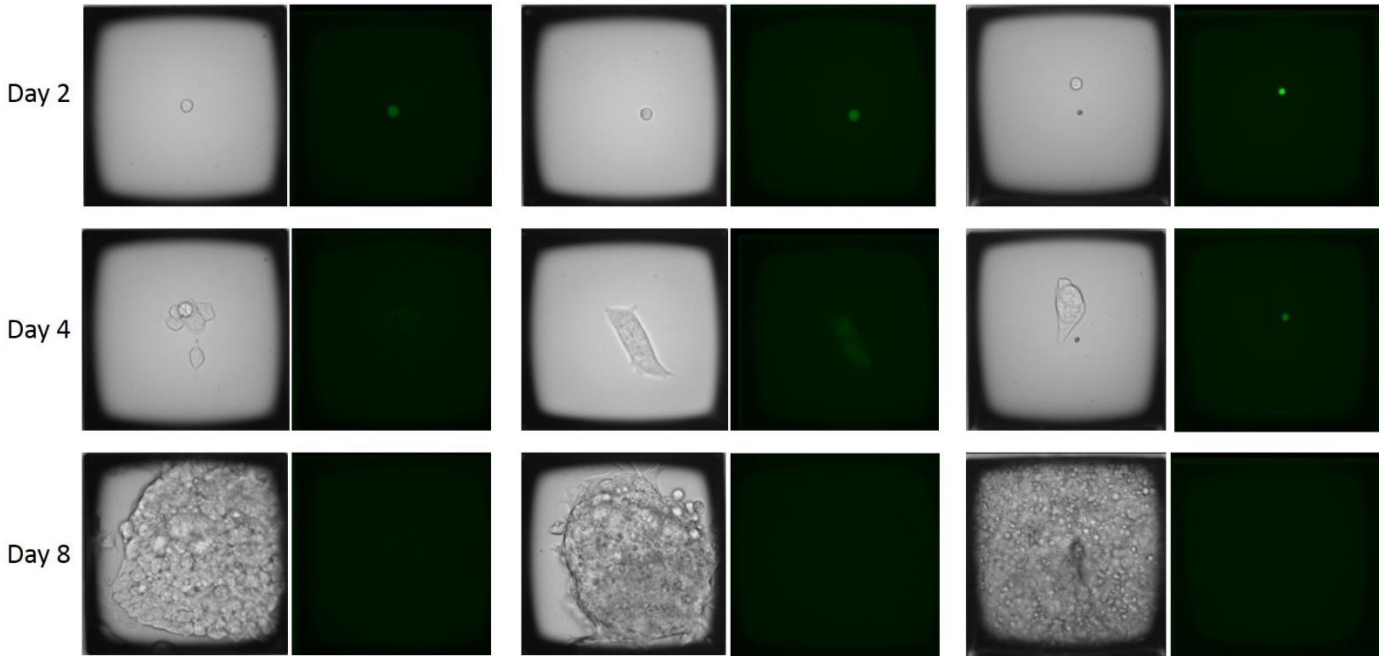
Seed CytoSort Array (~5-20K cells)

Day 1
Screen for transfection positive single cells

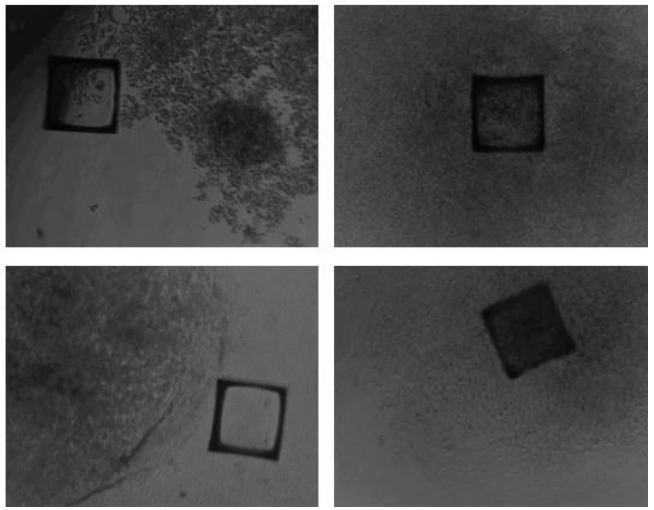
Day 2
Screen single cell microwells for colony growth

Day 4-beyond
Isolate clonal colonies; expand in 96-well plate

Characterize
Analyze expanded colony for genome edit



Expansion culture in 96-well plate after 19 days



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Data generated for TUT7 KO; Courtesy of William Marzluff, PhD UNC Chapel Hill

Double KO – TUT7 + 3'hExo

- Using the same workflow a double KO was generated
  - TUT7 – uridylates 3' of histone mRNAs
  - 3'hEXO – binds and degrades histone mRNAs at appropriate times of cell cycle
  - Two double KO cell lines generated

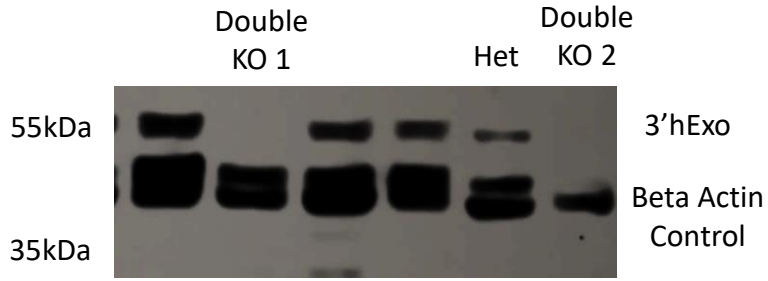
```
Allele #1    GGATCCAAGT--------------GCGAGTGACTTCAGTCACCCG
Wild-type    GGATCCAAGTTCATGACCTCCAGTGCGAGTGACTTCAGTCACCCG
Allele #2    GGATCCAAGTTCATGACCTT  GTGCGAGTGACTTCAGTCACCCG
```

1 NT MUTATION 2 NT DELETION

CCT is the PAM site: red box shows the guide RNA

```
Wild-type    GGATCCAAGTTCATGACCTCCAGTGCGAGTGACTTCAGTCACCCG
Both alleles GGATCCAAGTTCATGA-----AGTGCGAGTGACTTCAGTCACCCG
```

5 NT DELETION



3'hEXO KO western

Data courtesy of William Marzluff, PhD; UNC Chapel Hill

Other Applications of the CellRaft Technology

- Capable of both replacing FACS and enabling novel experiments

- Single cell genomics
  - Single cell RNA-Seq (Welch et al., 2016)
  - Single cell DNA-Seq (SNP analysis; Milholland et al., 2017; Dong et al., 2017)
  - Single nucleus sequencing (Weirman et al., 2017)

- Clonal colony propagation
  - Stem cell niche co-cultures (Gracz et al., 2015)
  - Organoid culture (Williamson, et al., 2018)

- Time-course screening for imaging-based phenotypes
  - Cytotoxic T-Cell screening for cancer immunotherapy (Attayek et al., 2016)

See our website for a comprehensive list of publications:
https://cellmicrosystems.com/publications/

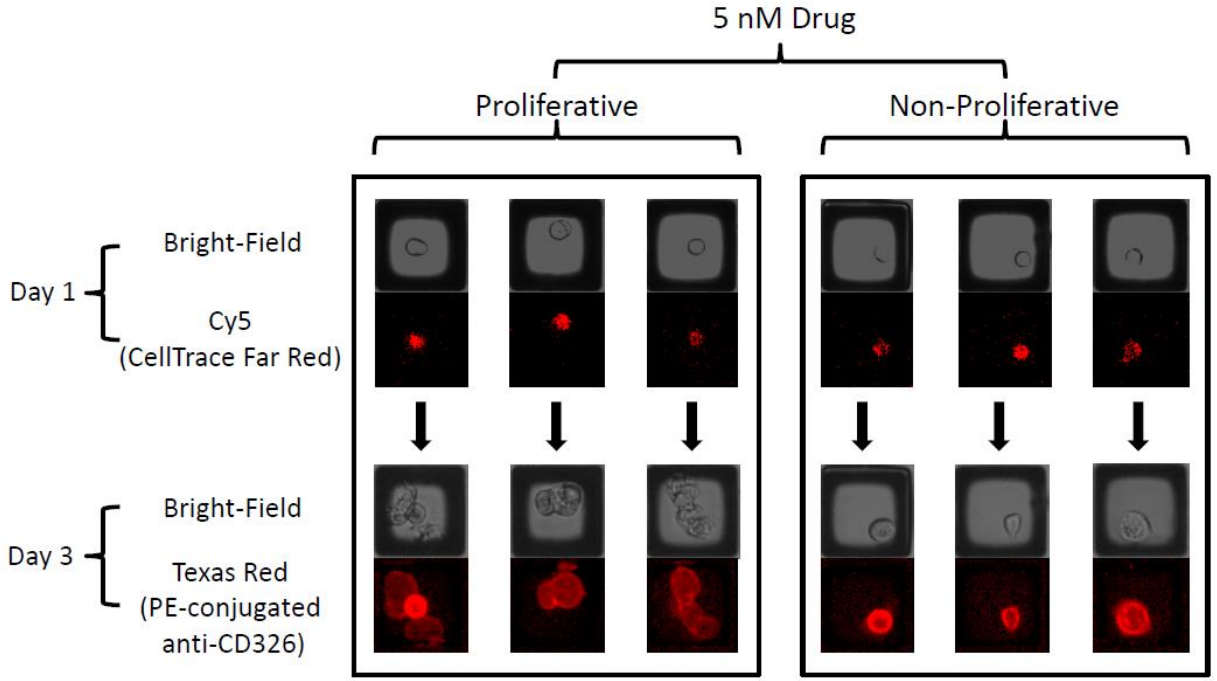
CellRaft Technology compared to FACS

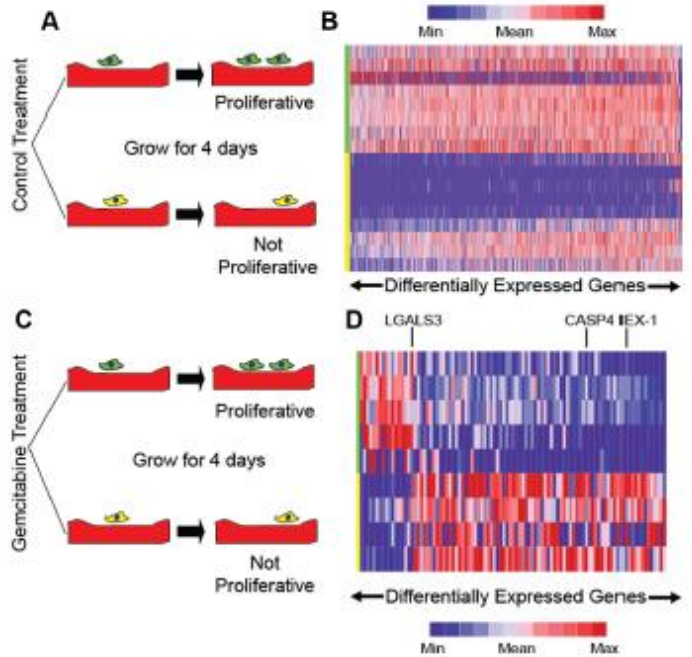
- Some workflows are uniquely supported by the CellRaft Technology
- Some workflows enabled by FACS are also supported

Comparison to Flow Sorting	
No minimum input	Isolate at least 96 cells from samples with as few as 500 cells
Minimal set-up	Cell culture consumable goes into the system; no specific set-up for cell size or type
Flexible collection	Collect in 96-well plates or PCR strip tubes in as low as 2.5 μL
Efficient	95% in 96-well plates and 90% in PCR strip tubes (2.5 μL volume)
Dynamic Collection	Can isolate individual colonies without disturbing neighboring colonies not yet ready for collection
High Effciency	Colonies grow beyond “critical period” prior to isolation; 85-90% colony survival compared to 10-15% from single cells from FACS

Gemcitabine sensitivity of CFPAC-1 Cells

- Cells were exposed to drug which should eliminate proliferation
- Drug-treated cells which remain proliferative may be expressing drug-resistance genes

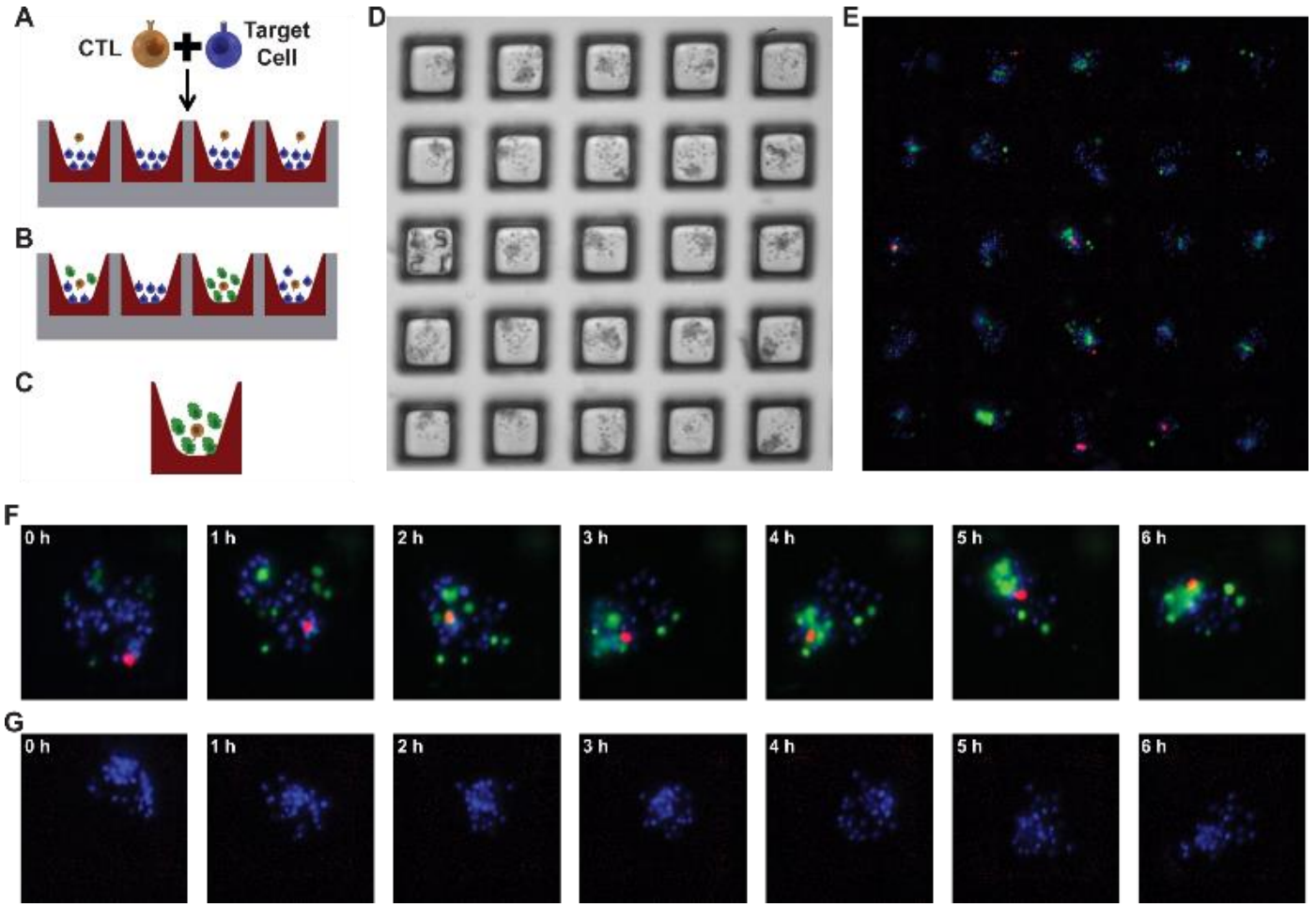




Functional Assay ⟶ Genomic Assay

Welch et al., Nucl Acid Res 2016

Cancer Immunotherapy



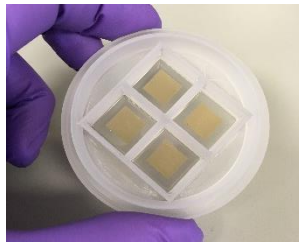
- Monitor cytotoxic T-cells over time for tumor cell killing
  - Kinetics can be monitored
  - T-Cells can be cloned to identify TCR sequences

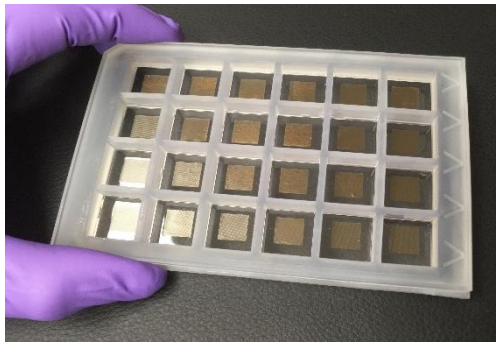
Clone	M1p Tetramer	TCRα	TCRβ
CTL3-MR-B8	Positive	V19-CALSEAGTGGSYIPTF-J6	V19-CASSMFVGQPQHF-J1-5
CTL3-MR-D10	Positive	V41-CAVSVEETSGSRLTF-J58	V19-CASSFFHNNEQFF-J2-1
CTL3-MR-F9	Positive	ND	V19-CASSIRSSYEQYF-J2-7
CTL3-LD-B2	Negative	ND	ND
CTL3-LD-C4	Negative	ND	ND
CTL3-LD-C8	Negative	ND	ND
CTL3-LD-G3	Negative	ND	ND

Attayek et al. (2016) Integrative Biol. 8(12):1197-1314

New CytoSort Arrays

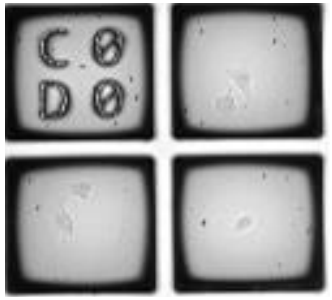
- **CytoSort-Quad**
  - Four reservoirs each featuring a CellRaft Array
  - ~1,600 CellRaft microwells in each reservoir's array
  - Seed 100-1000 cells and allow colonies to grow
  - No minimum and capable of growing hundreds of clonal colonies

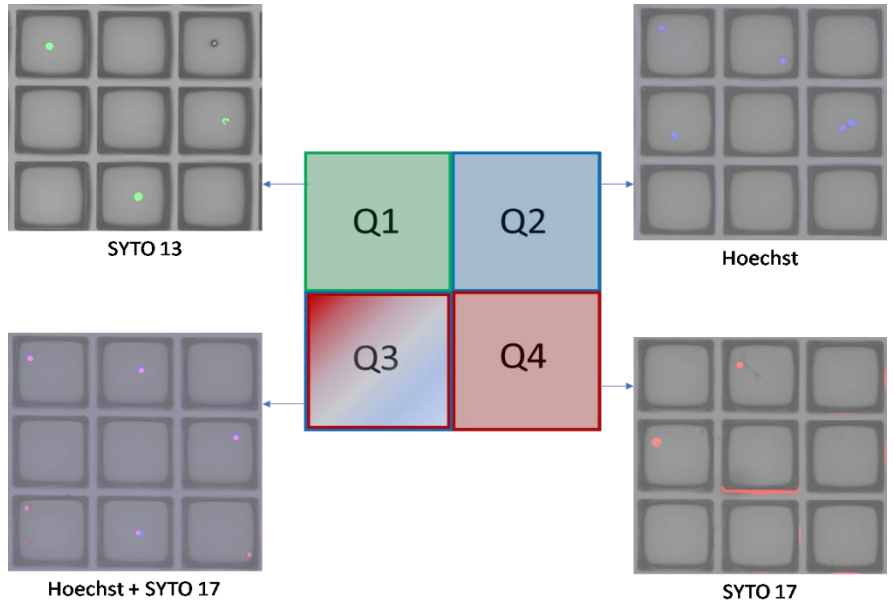




- **HexaQuad Array**
  - Same principle as Quad arrays
  - 24 reservoirs
  - Array is on SLAS-standard footprint to allow compatibility with liquid handling robots

- **High resolution imaging arrays**
  - Plastic of the CellRaft is replaced by a glass-like material
  - Improved refractive index complementarity and less "shadowing"





RaftNotes – Application White Papers from CMS

- Please check our website and twitter for updates
- New RaftNotes, publications, webinar and workshop announcements
- Technology descriptions and other literature