

RFA 07-01: CIRM Shared Research Laboratory Grants and Stem Cell Techniques Course



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- The objectives of the Shared Research Laboratory Grant Program are twofold:
 - To create dedicated laboratory space for the culture and maintenance of hESCs, in particular the hESC lines that fall outside the federal guidelines, by supporting the creation of core laboratories to be used by multiple investigators conducting research in the home institution and neighboring research institutions, and
 - To provide an environment through CIRM-funded space and equipment for the conduct of scientific research on hESCs without regard to federal limits.

RFA 07-01: CIRM Shared Research Laboratory Grants and Stem Cell Techniques Course

- The objective of the Stem Cell Techniques Course Program is:
 - To train scientists and technical staff in the growth and maintenance of hESCs by funding a number of hands-on courses teaching hESC culture techniques to be given several times a year for California investigators.
- Three investments: capital projects to build lab and purchase equipment, 3 years' annual operations budget, stem cell course. Operations budget was then extended an additional 5 years.
- RFA originally invested ~\$50M, ultimately invested ~\$68.5M.

Berkeley/CHORI's CIRM hESC Shared Research Facility

Mary West, PhD CTAF/HTSF
Director

David Schaffer, PI Academic
Supervisor

- 43 PIs and 100 researchers used facility in 2021.
- Majority in Stanley Hall, but cell sorter in Li Ka Shing with rest of campus flow cytometry core.



Li Ka Shing Center



Stanley Hall



Stem Cell Culturing Facility



3 x 6' BL-2 Level Laminar Flow Hoods, 4 CO₂ incubators, Zeiss epifluorescence microscope, liquid nitrogen, 4°C and -20°C degree storage



Milltenyi gentleMAX cell dissociator for disruption of tissue and isolation of stem cells from tissue, also does DNA, RNA isolation, protein extraction

- Initially used for non-registry hESC lines, like UCSF lines.
- During Obama, transitioned into being used by physical sciences and engineering labs lacking cell culture space.
- Berkeley didn't have a course, many attended UCSF's.

BD Influx Cell Sorter

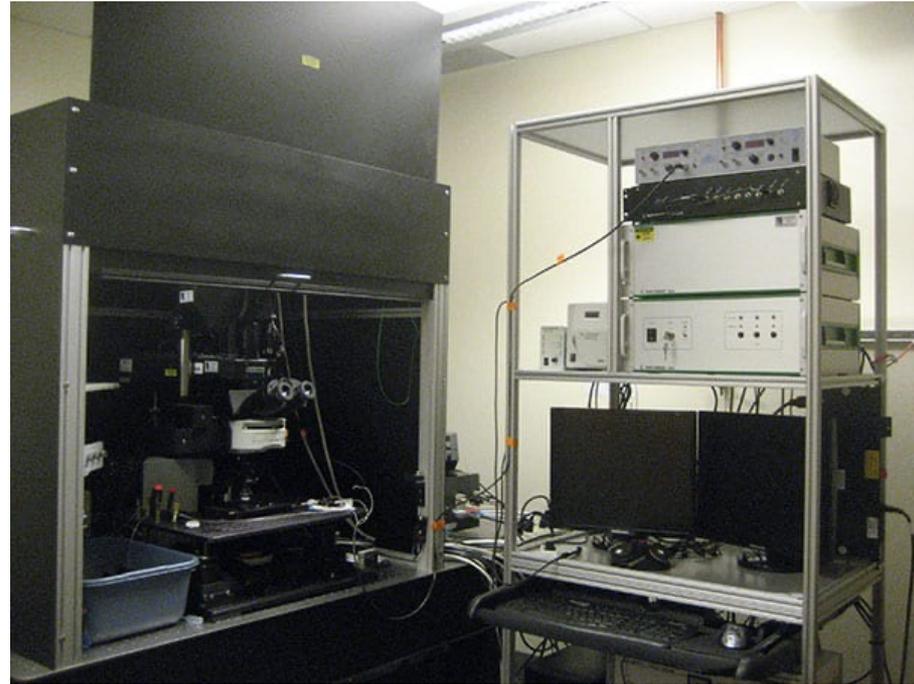


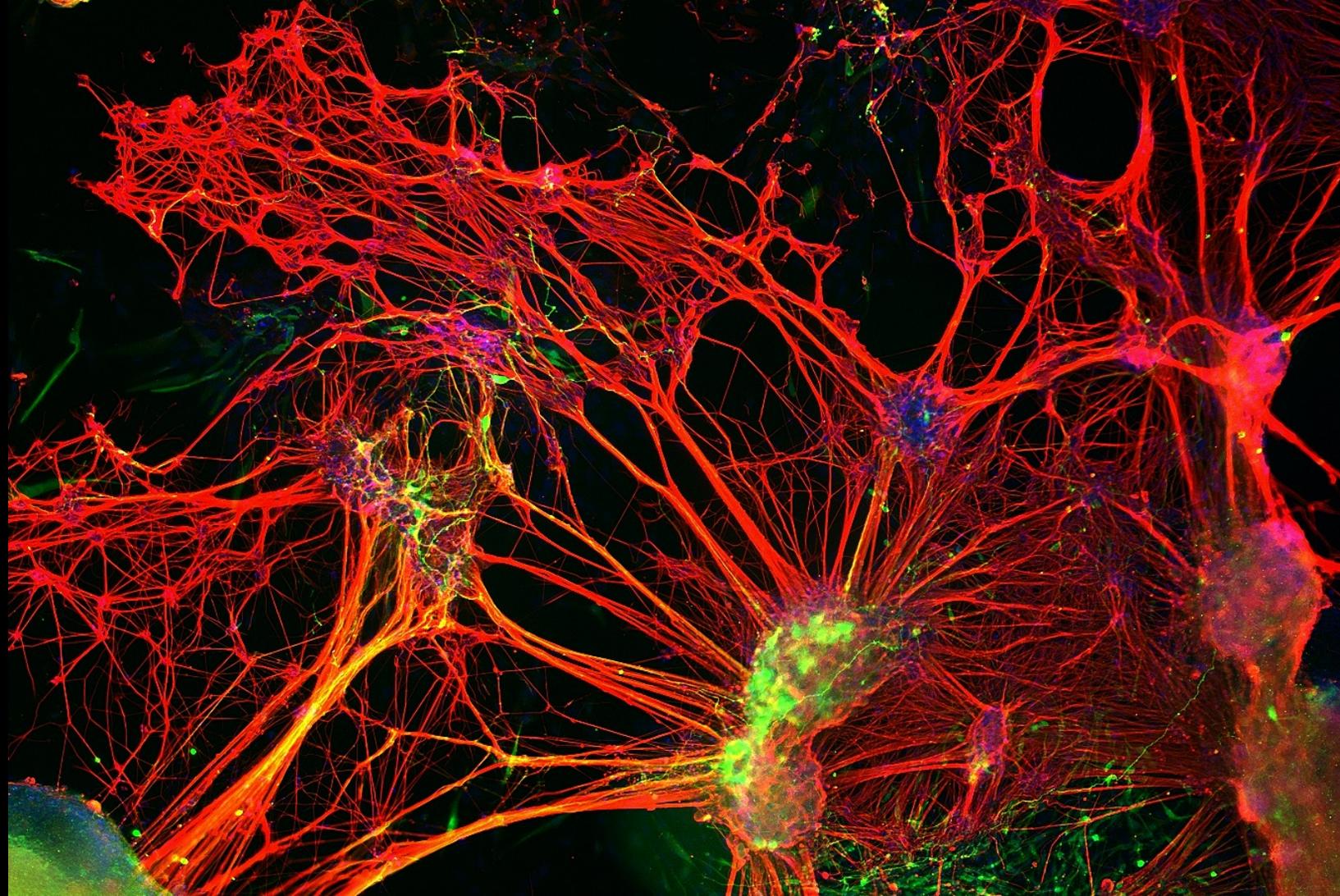
Laser	Parameter	Filters	Fluorochromes
488 nm	FL1 FL2	550 LP 530/40 BP 550 LP 710/40 BP	FITC, GFP, YFP, CFSE PerCP Cy 5.5, PI
405 nm	FL8 FL9	480 LP 460/50 BP 480 LP 550/50 BP	Pacific Blue Pacific Orange, AmCyan
UV	FL6 FL7	550 LP 460/50 BP 550 LP 670/30 BP	Hoechst, DHPI Red Hoechst
561 nm	FL3 FL3 FL5	645 LP 593/40 BP 700 LP 670/30 BP 700 LP 750 LP	PE, M-Cherry, RFP PECy5 PECy7
640 nm	FL10 FL11	700 LP 660/20 BP 700 LP 50 LP	APC APC-Cy7

Bruker Multi-photon/Confocal Microscope

2 PMTs for simultaneous acquisition from a single high powered ultra-fast (femto-second pulse) Ti:Sapphire Chameleon Ultra II laser (Coherent). Z-series, T-series, and targeted area laser exposures for FRAP, FRET, ablation, photoactivation, and photobleaching are easy to setup.

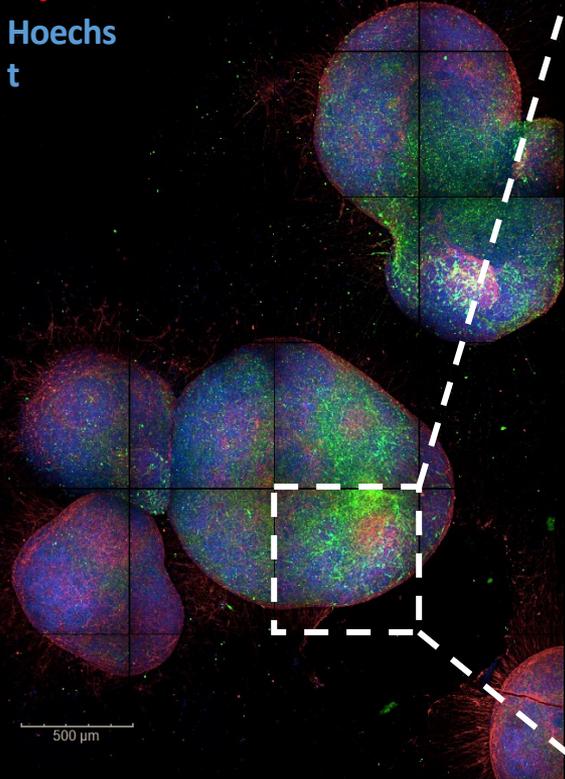
The upright microscope configuration is specially optimized for water immersion microscopy techniques. Selected objectives give high quality images for tissue slices, cell colonies, or monolayers of cells on different bioengineered materials or traditional culture dishes and slides.



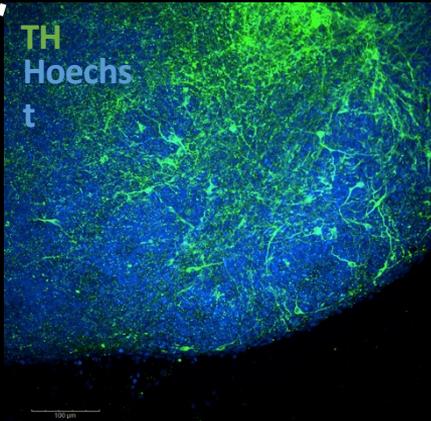


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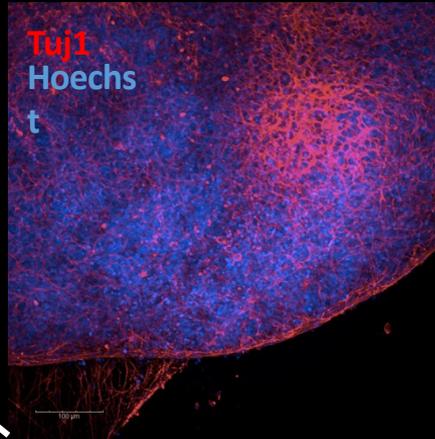
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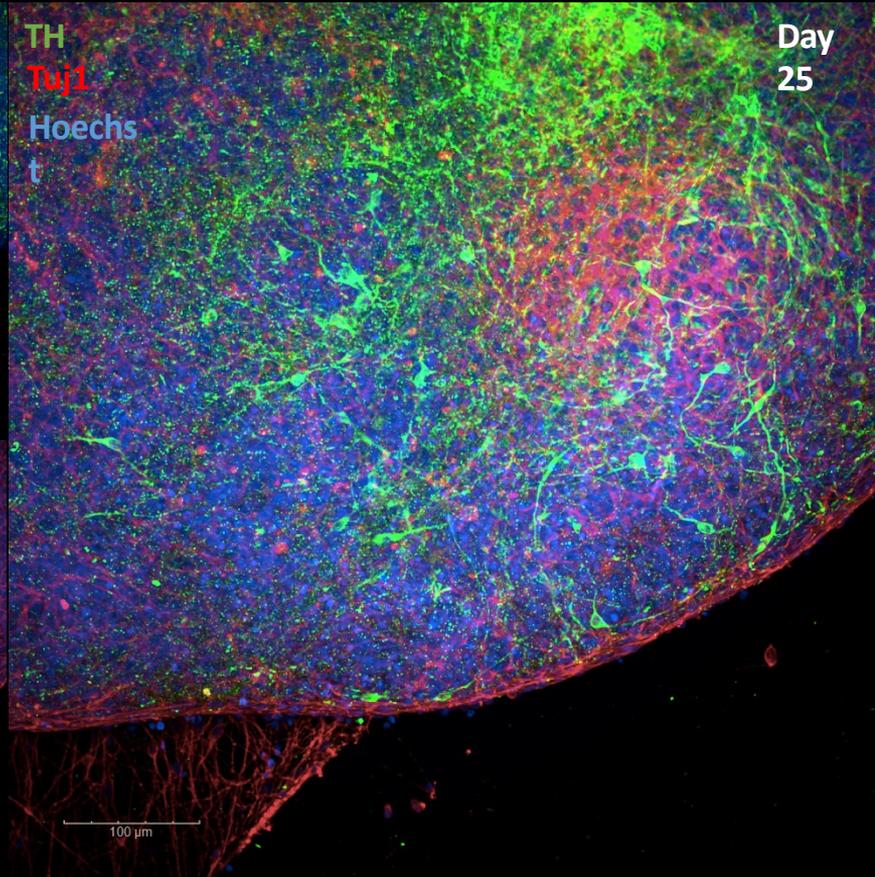
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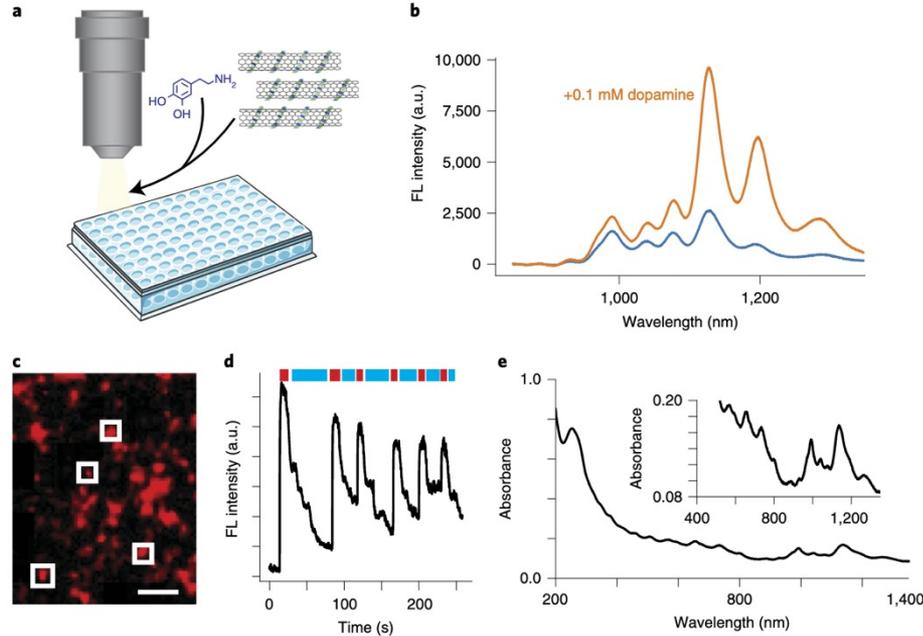


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Landry Lab: Near Infrared Imaging of Dopamine



- Carbon nanotube based sensor for dopamine fluoresces in near infrared range.
- Using to image dopamine in healthy and diseased brain sections.

Molecular Devices ImageXpress Micro XLS



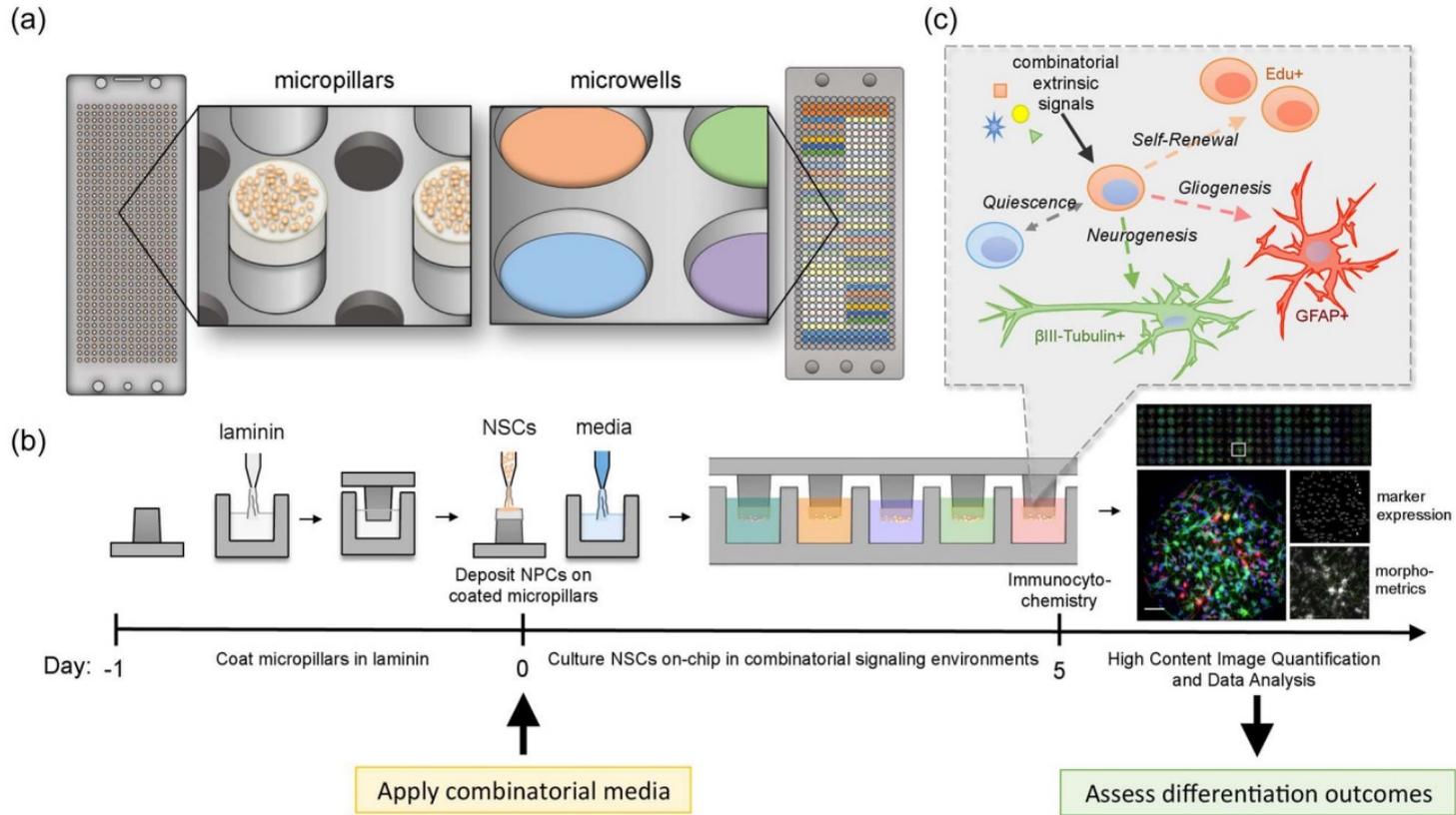
The ImageXpress Micro™ cellular imaging system by Molecular Devices is a fully-integrated hardware and software system for high throughput automated epifluorescent cell-based imaging. Great for multiwell plates and slides.

Has 2x, 4x, 10x, 20x, 40x, and 60x oil objectives, and filter sets for DAPI, CFP, GFP, FITC, YFP, TRITC, Cy3, TxRed, Cy5.

The CTAF Facility's system has environmental control of temperature and 5% CO₂ and single-channel pipetting for kinetic experiments.

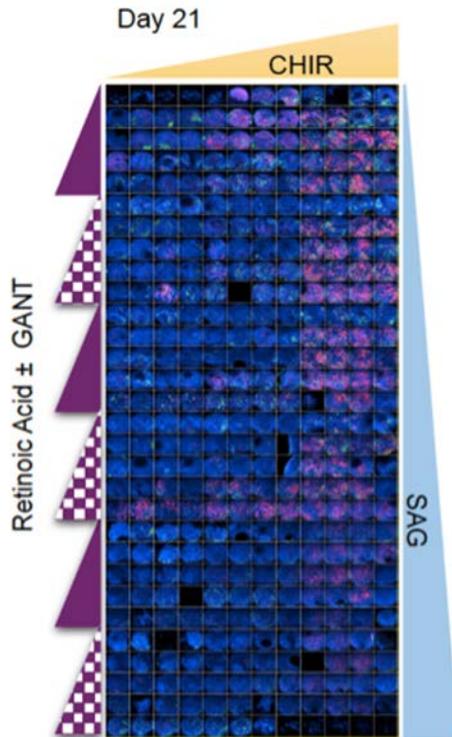
The Shared Facility also has licensed the MetaXpress® cellular image analysis software for high content analysis.

High-Throughput Screening Capabilities

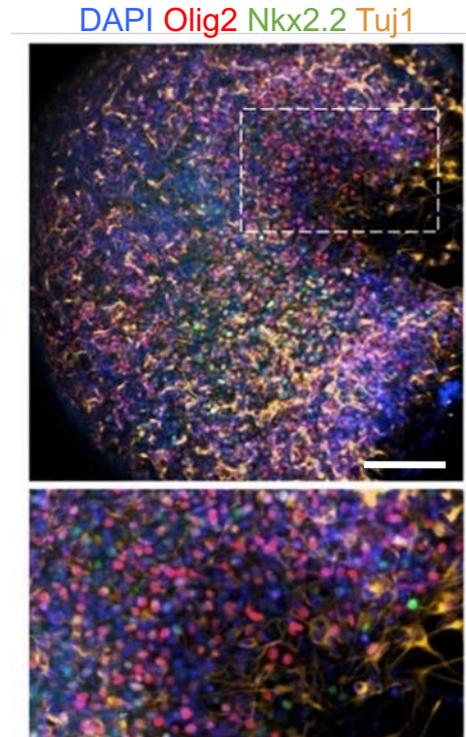


High-Throughput Screening Capabilities

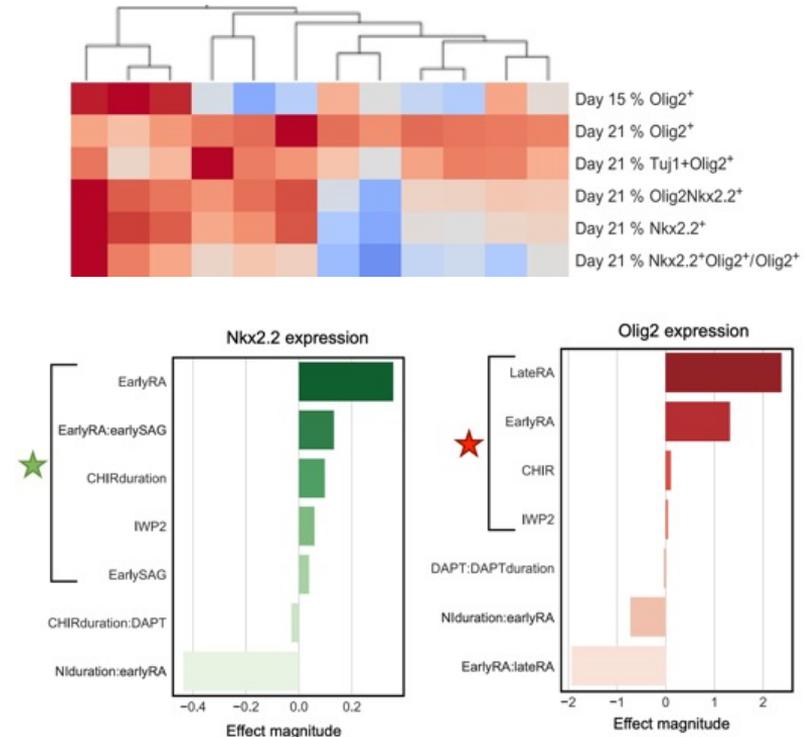
>1000 3D cultures systematically screened



Multiplexed high-content biomarker data



Statistical modeling for data driven optimization



BioSpherix Hypoxia Chambers



The two Hypoxia Chambers are single shelf and two shelf for optimization experiments and scale up with separate controls for both O₂ and CO₂ levels. Provides researcher with longterm constant hypoxia conditions as well as fast (within 3 minutes) recovery when removing cells for media changes.

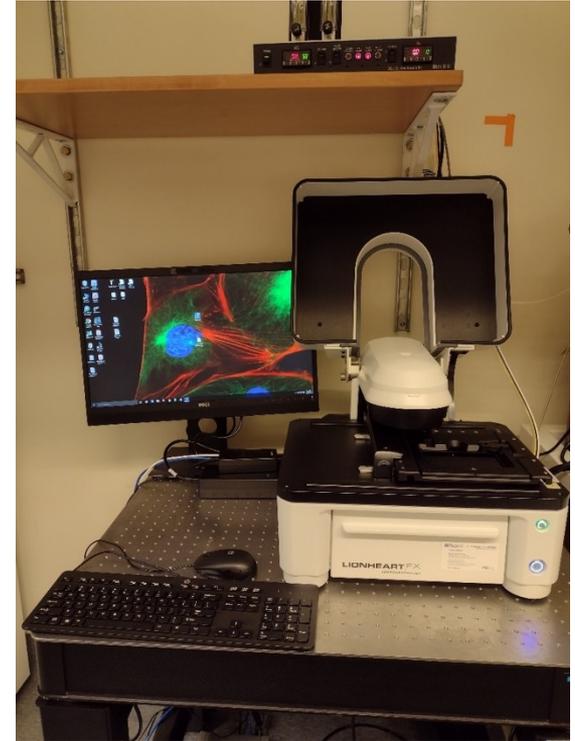
Cells can be imaged in the Lionheart microscope under similar hypoxia conditions.

Agilent/Biotek LionHeart Live-Imaging System for Fluidic Devices

A fully-integrated hardware and software system for medium throughput automated cell-based imaging-- Wide-field, Brightfield, phase, digital phase, color (like H&E staining) for fixed/live cells with capabilities for movies, fluidic devices, slides, and other setups.

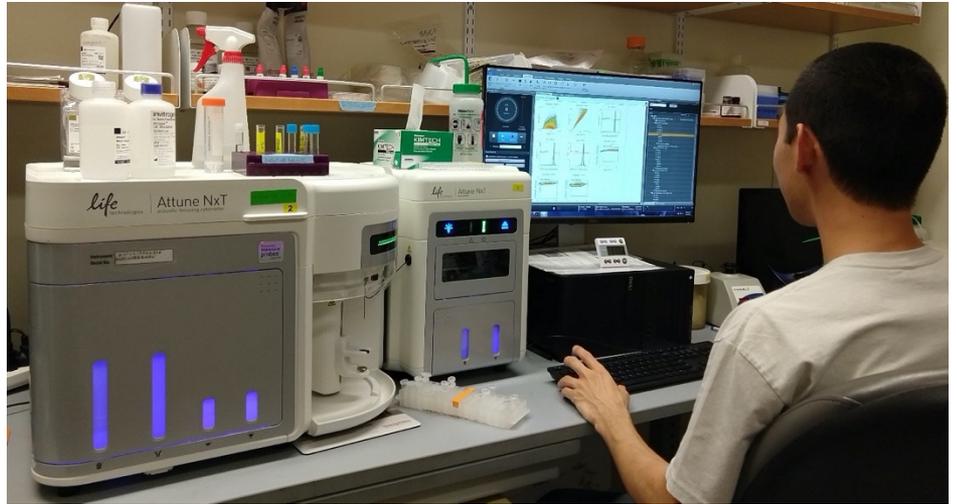
The CTAF's system has environmental control of temperature, CO₂ and O₂, can do wound healing analysis.

Has 2x, 4x, 10x, 20x, and 40x, and filter sets for DAPI, GFP, FITC, TRITC, TxRed, and Cy5.



Flow Cytometry: Thermo Fisher Attune Acoustic Focusing Cytometer (blue/red/violet/yellow)

The CTAF ThermoFisher Attune lasers are 405 nm, 488nm, 561nm, and 635nm. Researchers can measure up to 14 colors simultaneously. This cytometer operates either in a single tube mode or using the autosampler (96 and 384 well, including deep well for around 2 ml/sample). It has specially engineered features to analyze 1) very dilute dilute samples 2) larger size particles, and 3) blood constituents without the processing of whole blood. Sample size can vary between 200 ul - 2ml.



Bio-Plex MAGPIX Multiplex Reader

The Bio-Plex MAGPIX multiplex reader is a compact, robust system for magnetic bead-based immunoassays. This multiplex reader is capable of reading assays designed on magnetic xMAP (MagPlex) beads. Read up to 50 analytes per sample (as little as 25 ul), and can be used to quantify levels of phosphoproteins and cytokines that are present in cell or tissue lysates, serum or plasma samples.

Researchers can quickly evaluate analytes by using pre-validated assays available in the areas of cancer, cardiovascular, cell signaling, cellular metabolism, immunology, metabolism and endocrinology, neuroscience, stem cells, and toxicity.



hESC Shared Research Facility: Current Status

- Facility fulfilled RFA's goal on Berkeley/CHORI campuses: heavily used for registry and non-registry hESC culture.
- New smaller equipment has been added using Berkeley campus funds.
- In general, core facility expenses (staff, service contracts) are higher than recharge income (campus and private), so need subsidies.
- With CIRM funding sunset in 2015, QB3 took on facility subsidy. Facility was renamed QB3 Cell and Tissue Analysis Facility in 2018.
- Staff person part time, equipment run by campus "super-users," and large equipment aging, but still up and running!