CytoTronics

Electrical Imaging: Live Cell Characterization from Stem Cell Biology to Phenotypic Disease Models

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July 2024

Agenda for today

- **Technology**
- **Applications**
  - Disease modeling
  - Epithelial cells
  - Spheroids
  - Profiling
  - Multiplexed measurements
- **The Pixel system: built for scale**
- **Products**

Our team

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Electrical Engineering

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Co-founder & CBO
Executive with 25+ yrs
in Life Science tools

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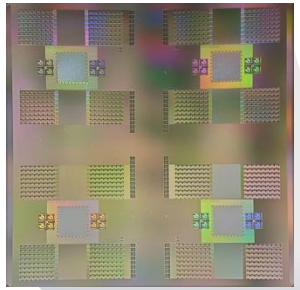
Leveraging semiconductors for cell-biology innovation

CytoTronics' microchip



CYTO tronics™

- ✓ Consolidation of functionalities
- ✓ >100× spatial resolution
- ✓ Scalability to unprecedented throughputs

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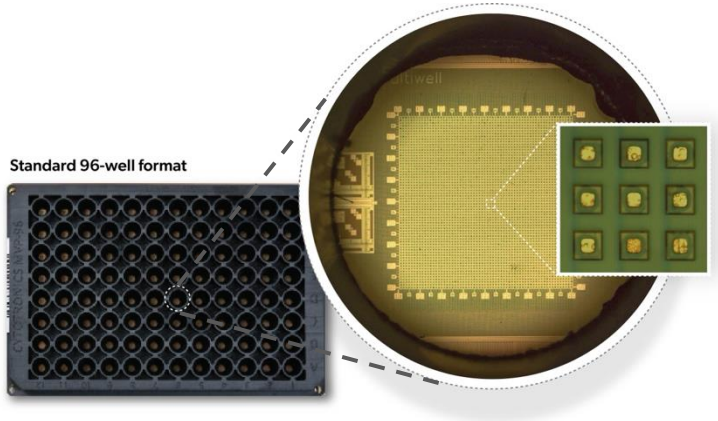
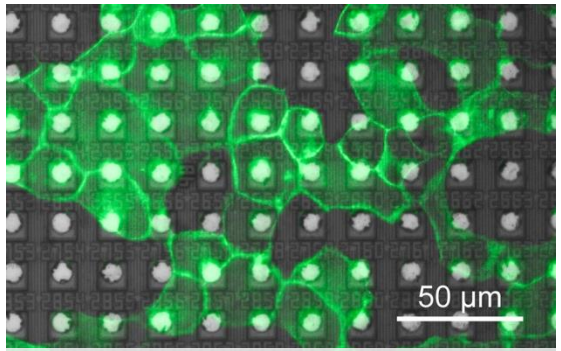
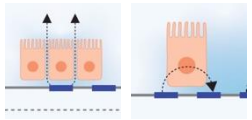
Harnessing the power of semiconductors



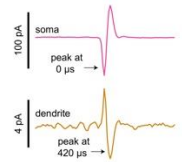


Cells grow on arrays of electrodes

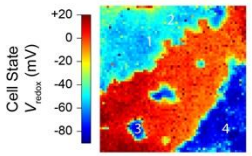
Impedance:
Morphology & live-cell dynamics



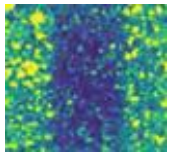
Electrophysiology:
Neurons & cardiac



Electrochemistry:
Redox/metabolic



Manipulation:
Stimulation and wounding



- ✓ **Multiplexed live-cell readouts**
- ✓ **Single-cell resolution**
- ✓ **Scale without compromise**
- ✓ **Seamless integration**

Nature Communications (2023), *Lab on a Chip* (2022), *IEEE Solid State Circuits* (2020), *Lab on a Chip* (2020), *Nature Biomedical Engineering* (2019), *Nature Nanotechnology* (2017)

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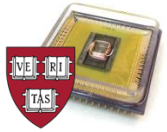
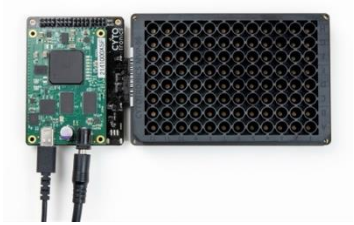
Technology development

Single-well devices at Harvard



2012-2021

96-well prototype



2022

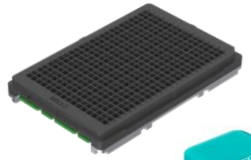
24 plate prototype platform

2023

First on-market products: The Pixel system

Plate 96

Plate 384



Octo

Primo

2024

Technology progression

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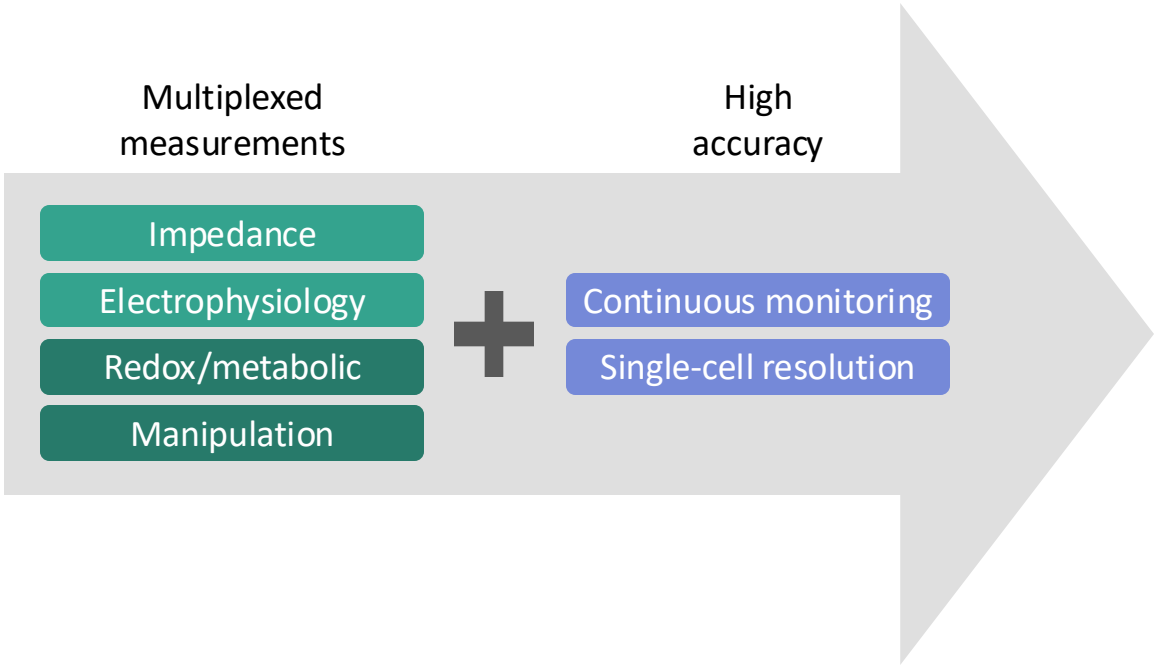
Combined modalities enable diverse applications

CytoTronics functional capability



Diverse cell-biology applications

Toxicology
- Cardiotoxicity
- Neurotoxicity

Differentiation
- iPSC differentiation
- Culture heterogeneity

Epithelial diseases
- Endothelial
- Infl. Bowel Dis. (IBD)

Profiling
- Compound profiling
- Cell death MOA

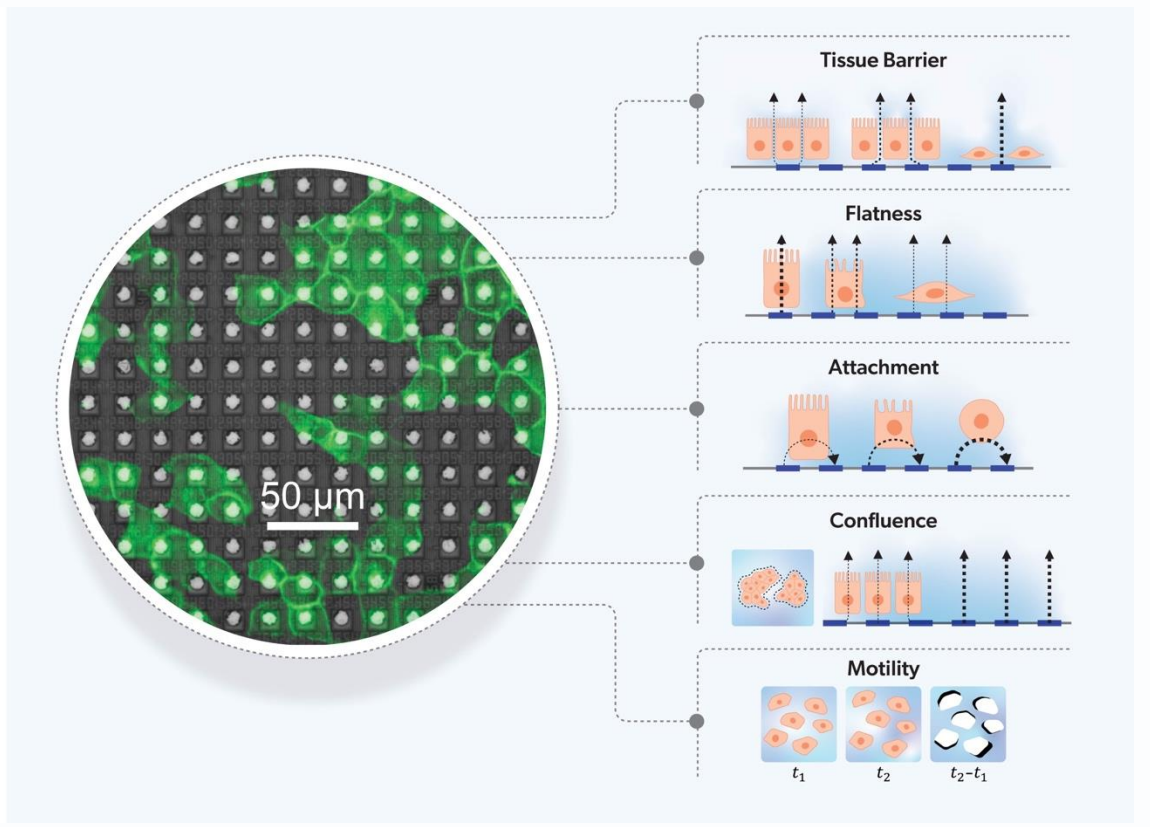
Oncology
- Immuno-oncology
- Epi.-Mes. Trans. (EMT)

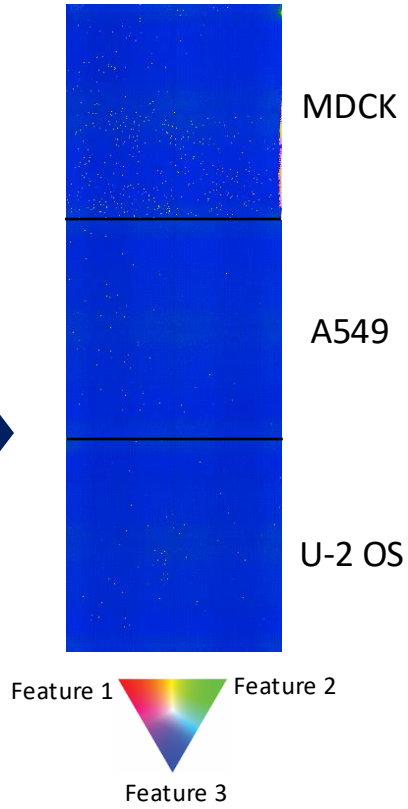
High-Throughput Ephys.
- Cardiac functional
- Neuro functional

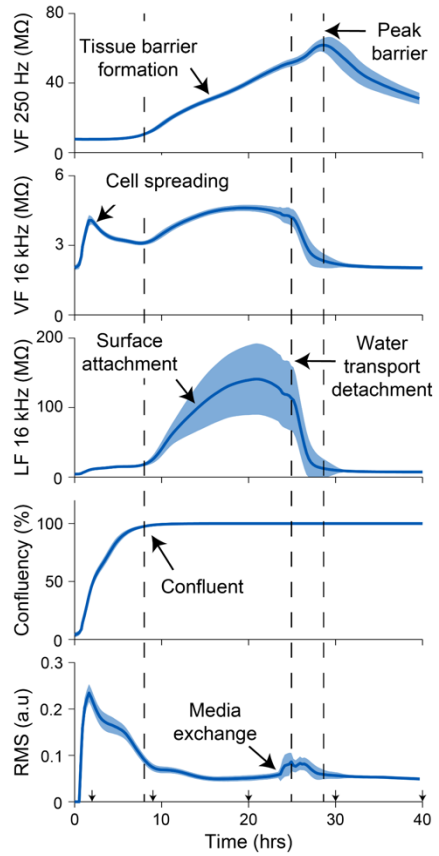
Next gen. areas
- Metabolic studies
- CRISPR Screens

Subset of *CytoTronics* application development roadmap

Field-based impedance: “electrical imaging”







Cells grow over electrodes in the electronic microplate

Scanning with different fields & frequencies create multiple “images” at each time-point

Magnitude and spatial parameter extraction: <u>20+ parameters measured over time</u>

A semiconductor 96-microplate platform for electrical-imaging based high-throughput phenotypic screening, Nat. Commun. (2023)

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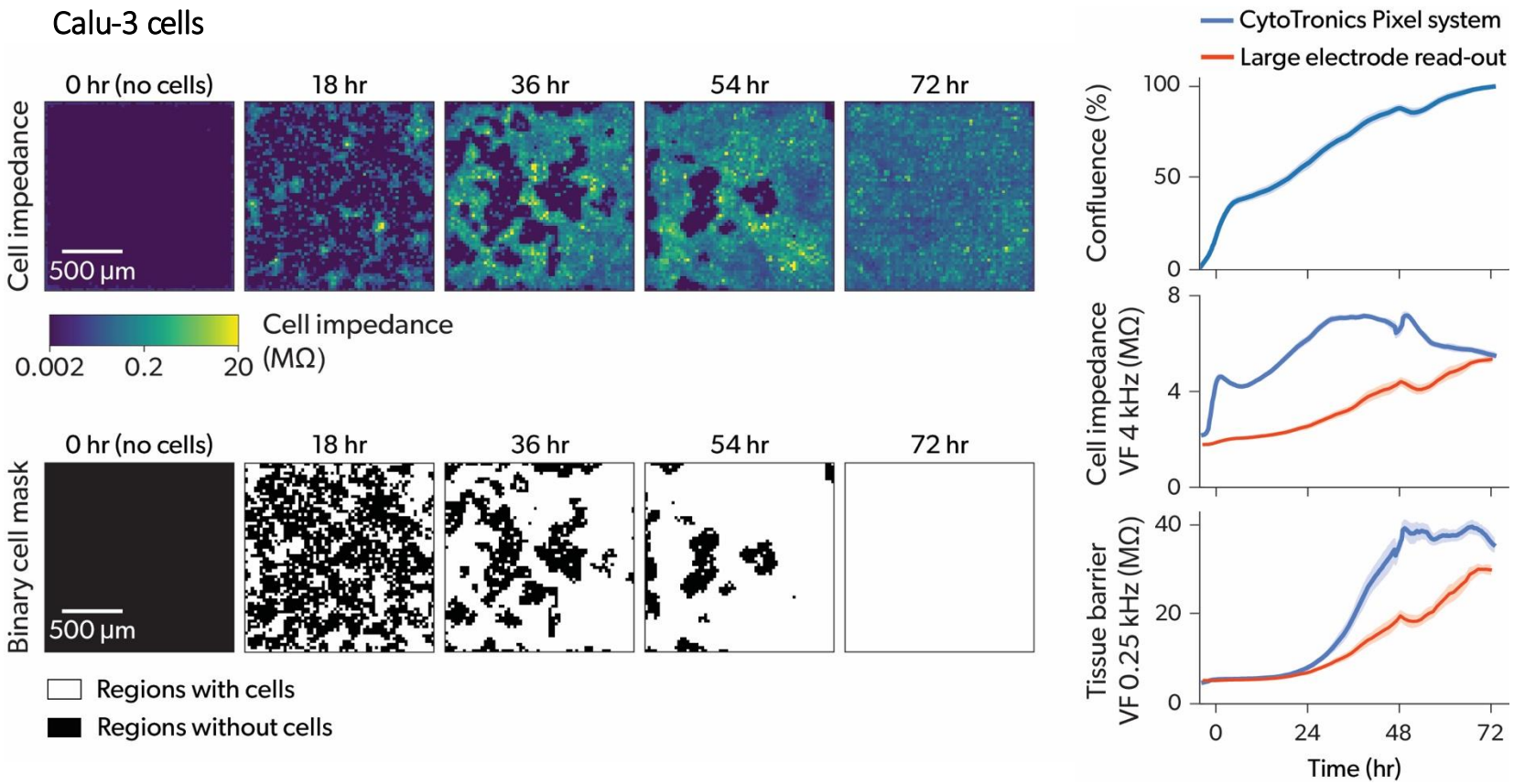
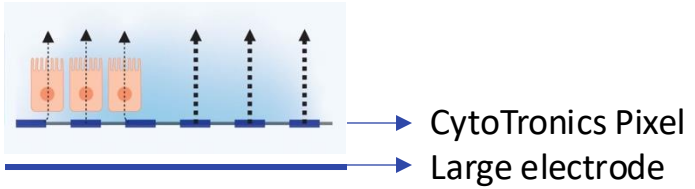
Unparalleled sensitivity and accuracy



- **High sensitivity and dynamic range**
  - Single electrode vs well aggregate
  - Small number of cells vs large number of cells
- **Accurate measurements at low confluence**
  - Cell mask ensures empty electrodes do not dominate the signal



Selectively assess electrodes occupied by cells

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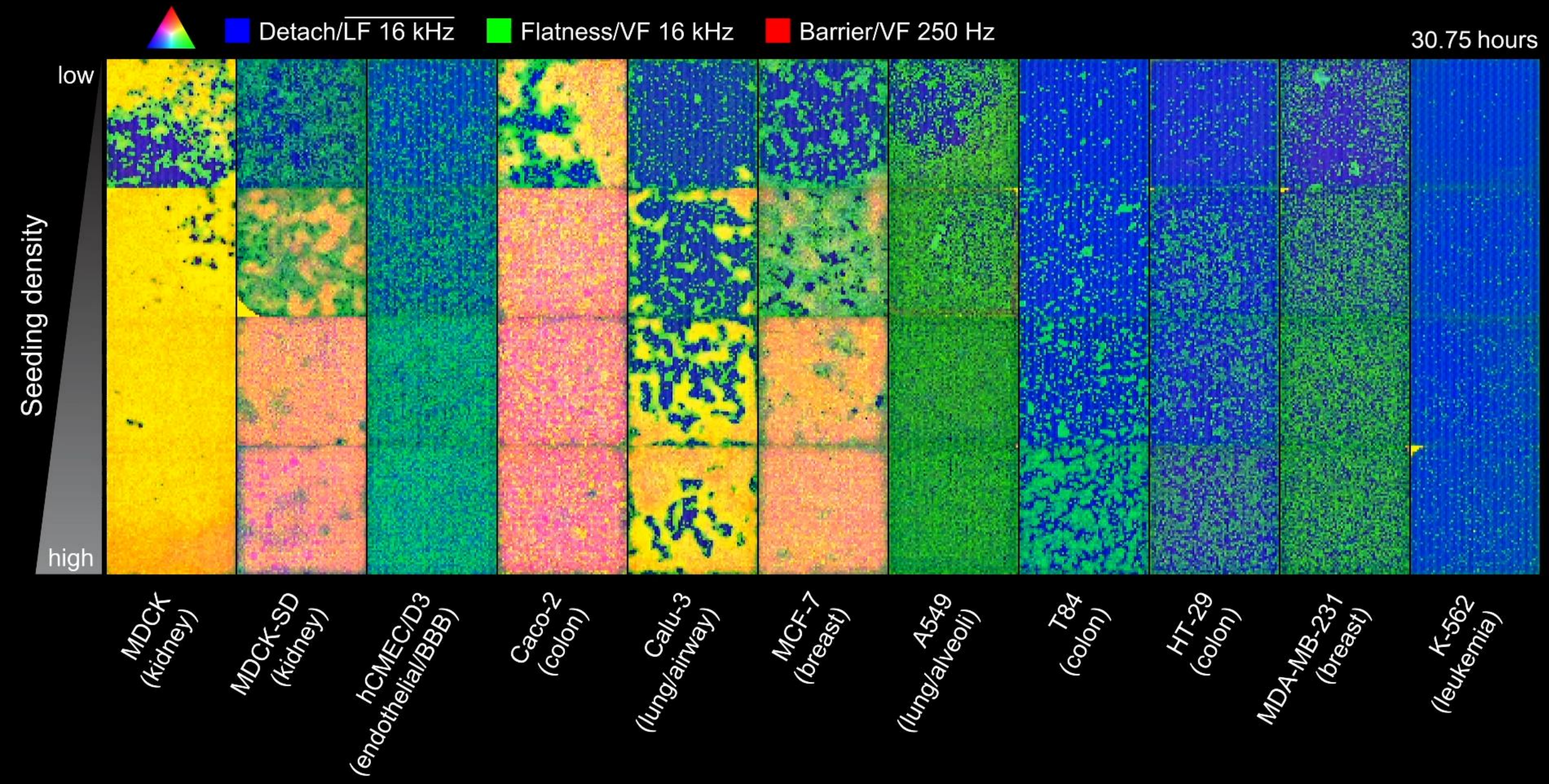
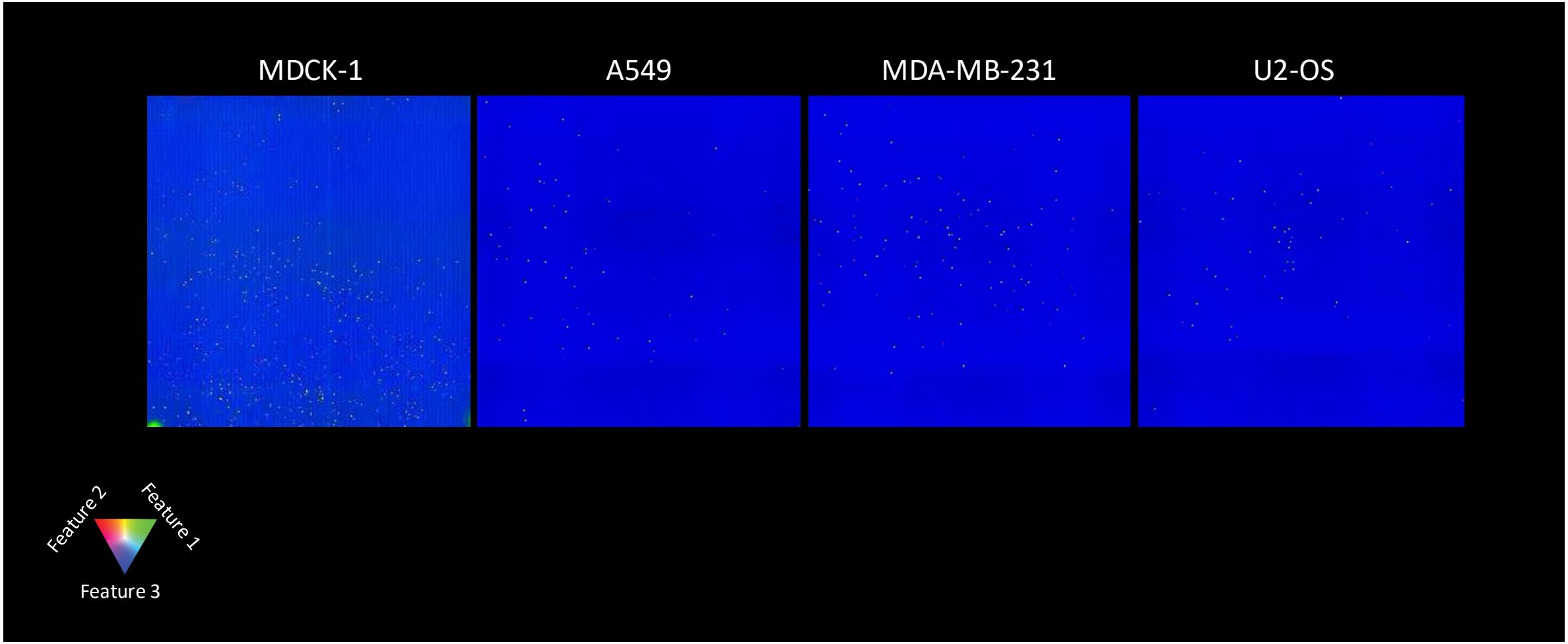
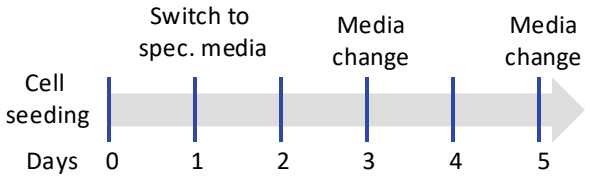


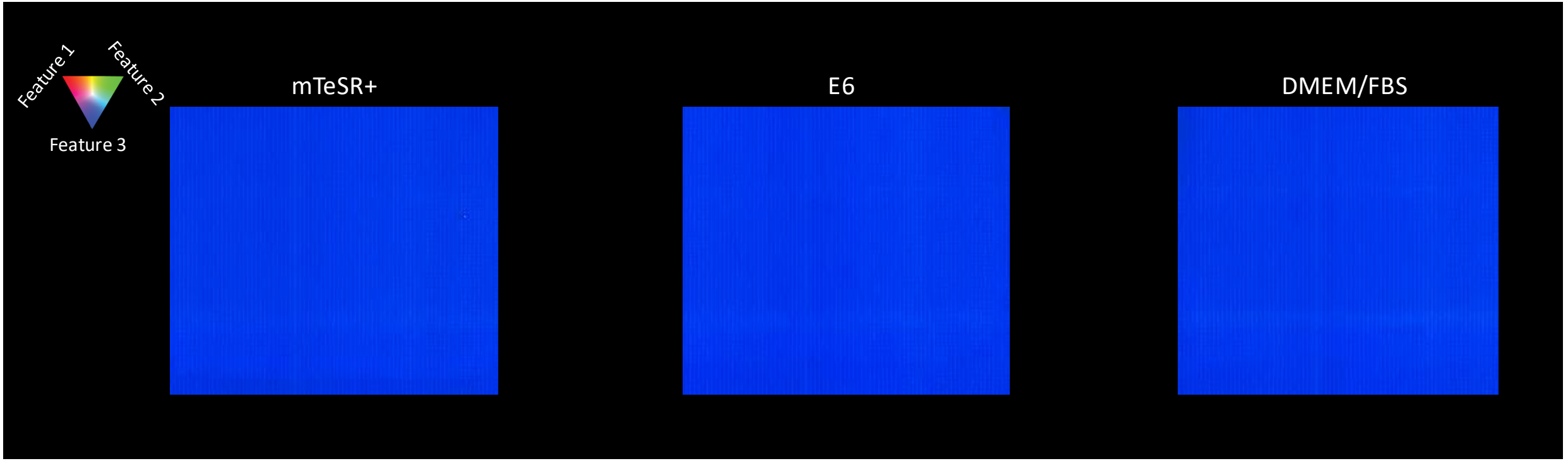
Electrical imaging : ability to measure any cell line



Live-cell monitoring of pluripotency and differentiation



- iPSCs grown in mTeSR+ to maintain pluripotency
- After cell attachment, media changed to mTeSR+, E6 (differentiation primed media) or DMEM/FBS (differentiation induction media)
- Electrical imaging performed every 15 min for duration of experiment to monitor change in phenotype



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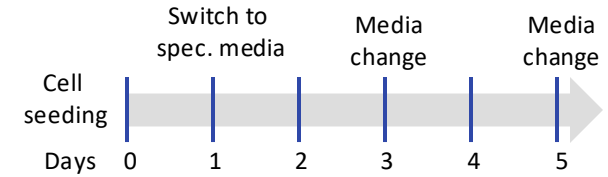
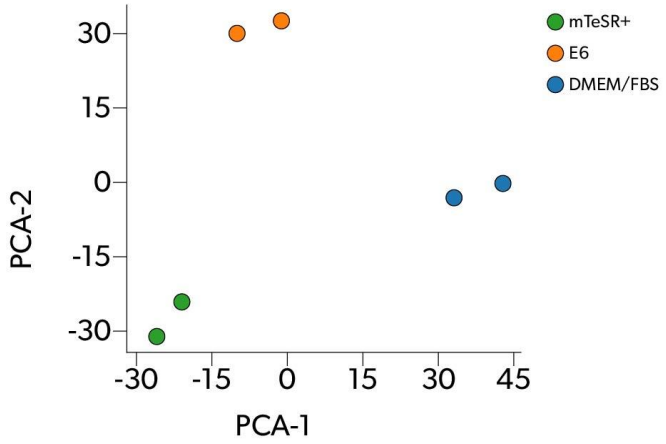
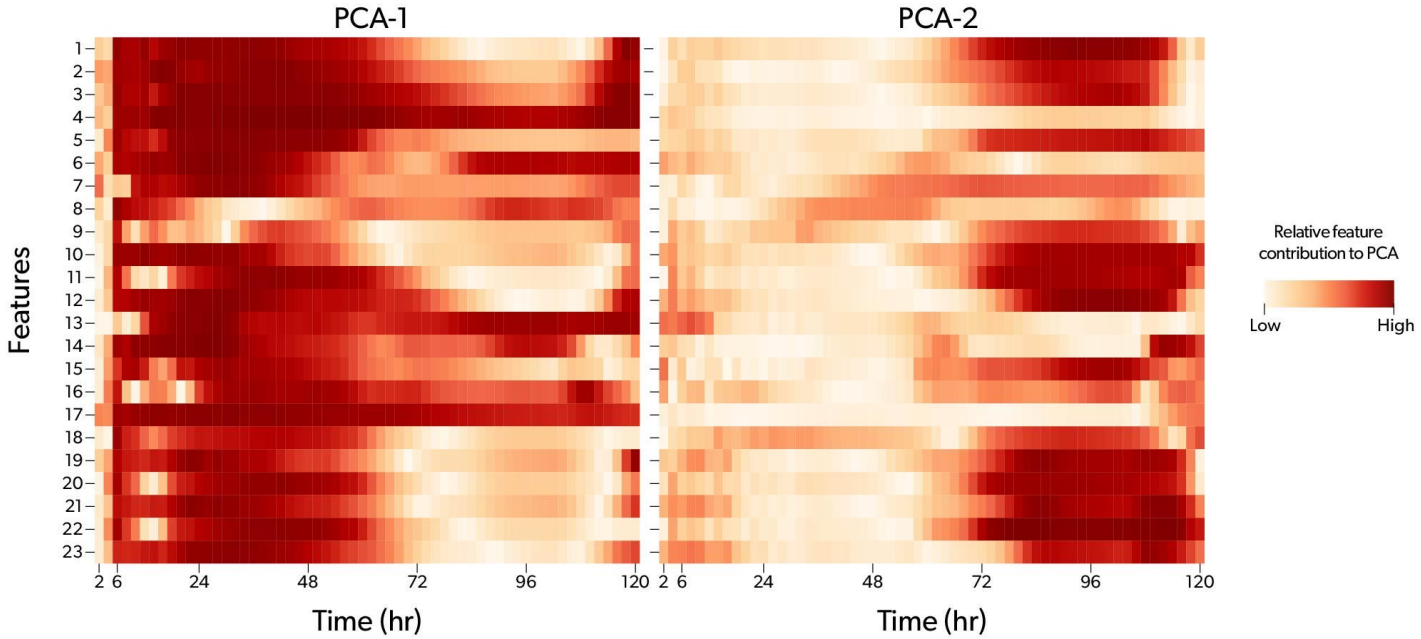
Identification of dynamic phenotypes







- High-dimensional phenotype features distinguish pluripotent iPSCs from differentiation primed and differentiated cells
- Cells exposed to serum show distinct phenotype as early as **5 hours** after media change (PCA-1)
- Cells in E6 medium change phenotype, at later time points, **starting 72 hours** after media change (PCA-2)

Live cell electrical imaging can identify dynamic change in phenotypes

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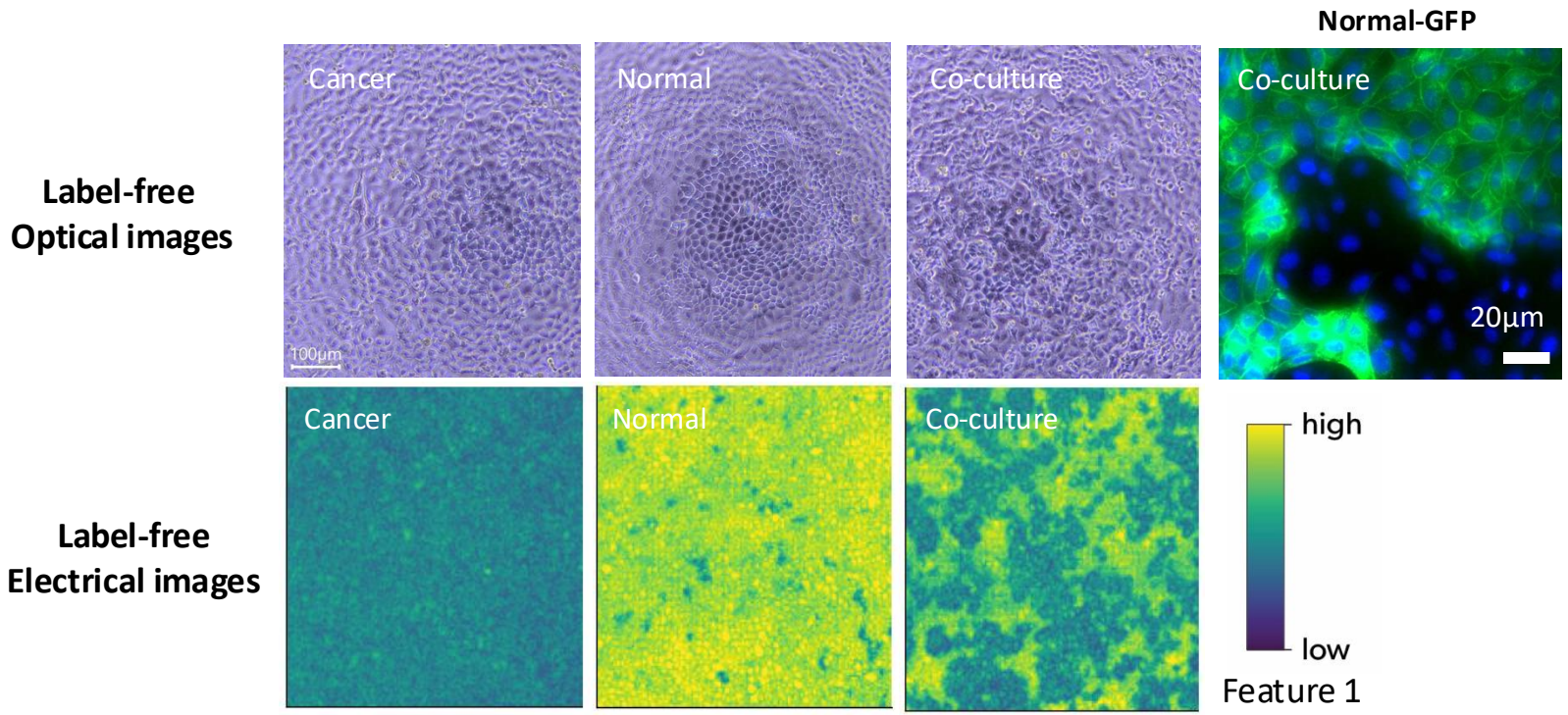
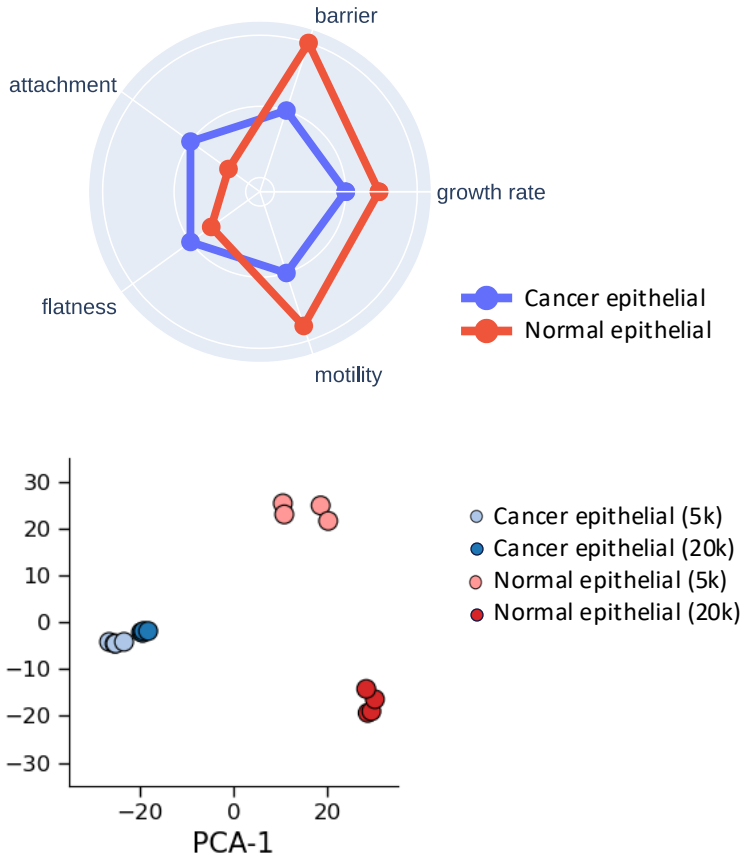
Disease Modeling and Heterogenous Cell Populations

- Interactions of multiple cell types within a tissue often play a role in disease models
- Electrical imaging can distinguish cell populations based on their phenotype





Label free tracking of heterogenous cell populations

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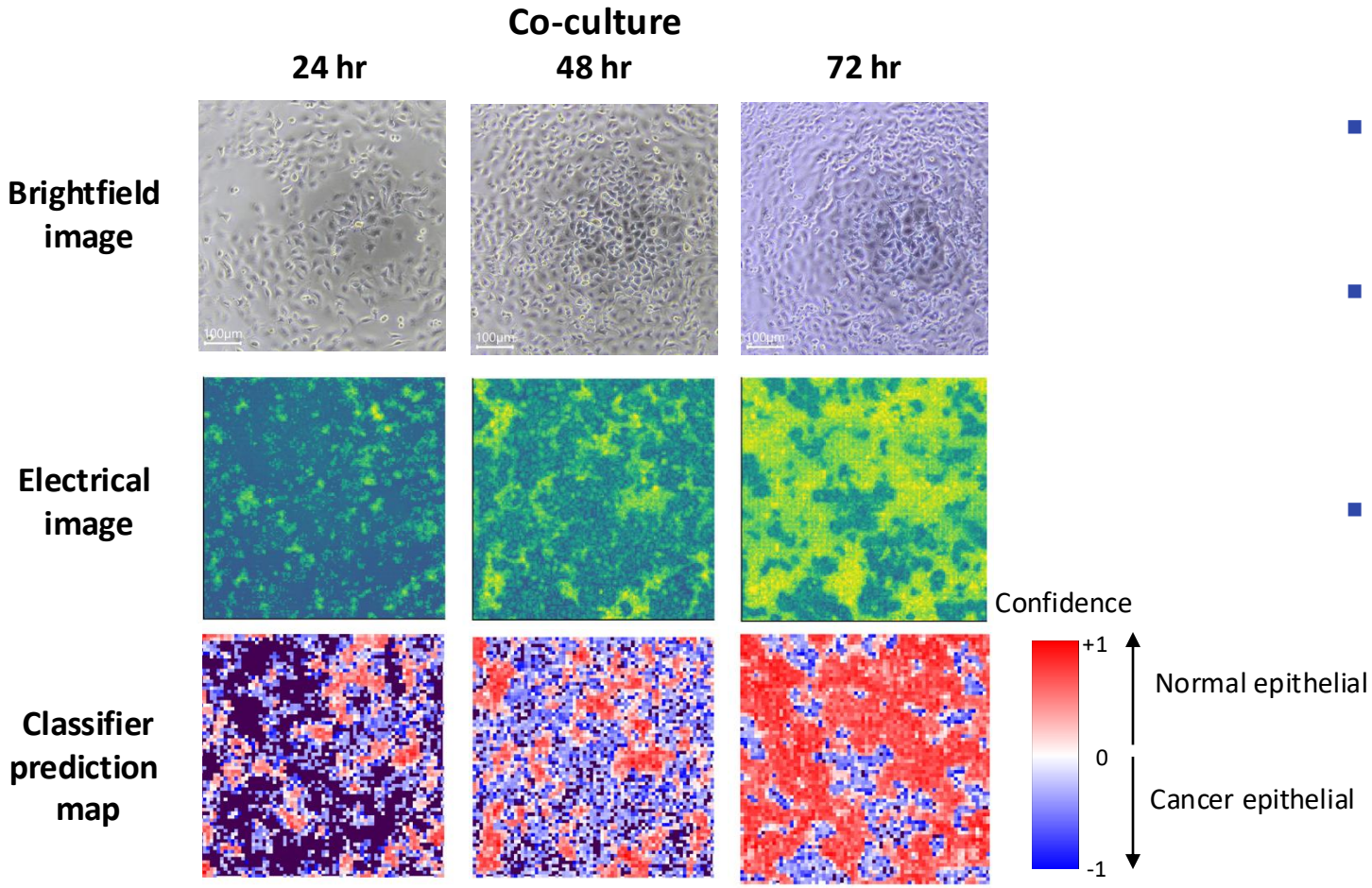
Disease Modeling and Heterogenous Cell Populations



- Cell types cannot be distinguished using brightfield imaging
- Electrical imaging identifies distinct cell populations in co-cultures and their dynamic changes over time
- Predictions at 72 hr correlate well with end-point fluorescent imaging using cell-specific markers

Label free tracking of heterogenous cell populations

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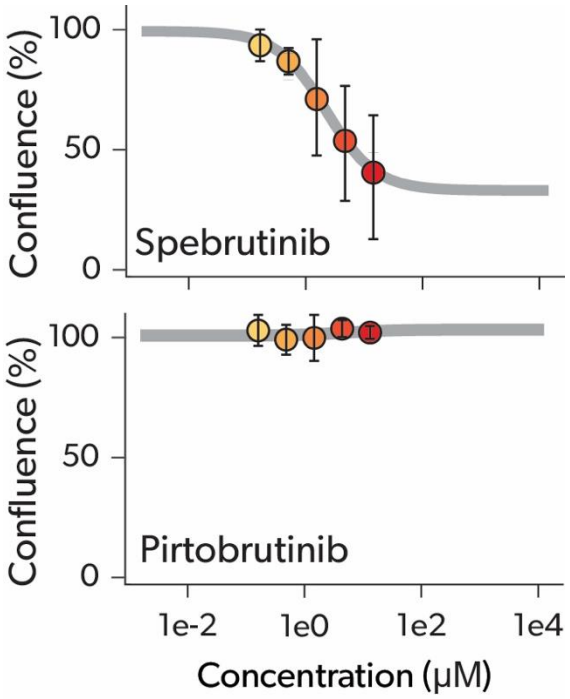
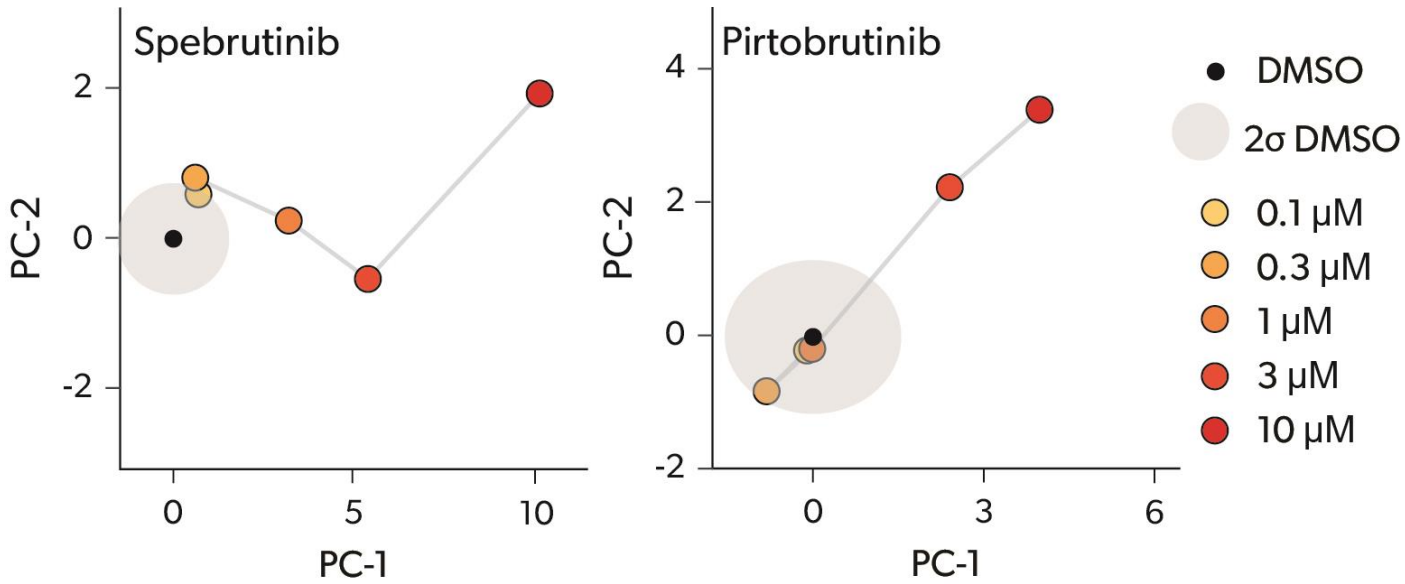
Cellular responses: Multiparametric readouts

BTK inhibitors: irreversible and reversible

Compound response in a cell death assay



Compound response in an electrical imaging assay



Beyond IC_{50}: Dose response in high-dimensional space

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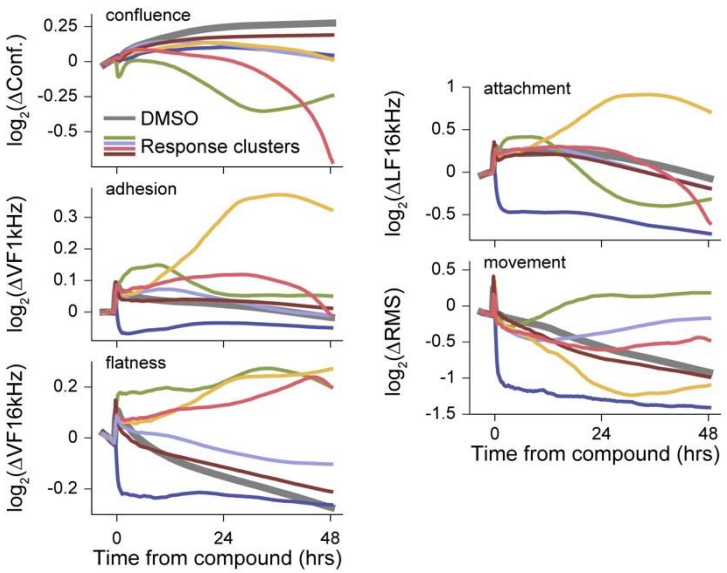
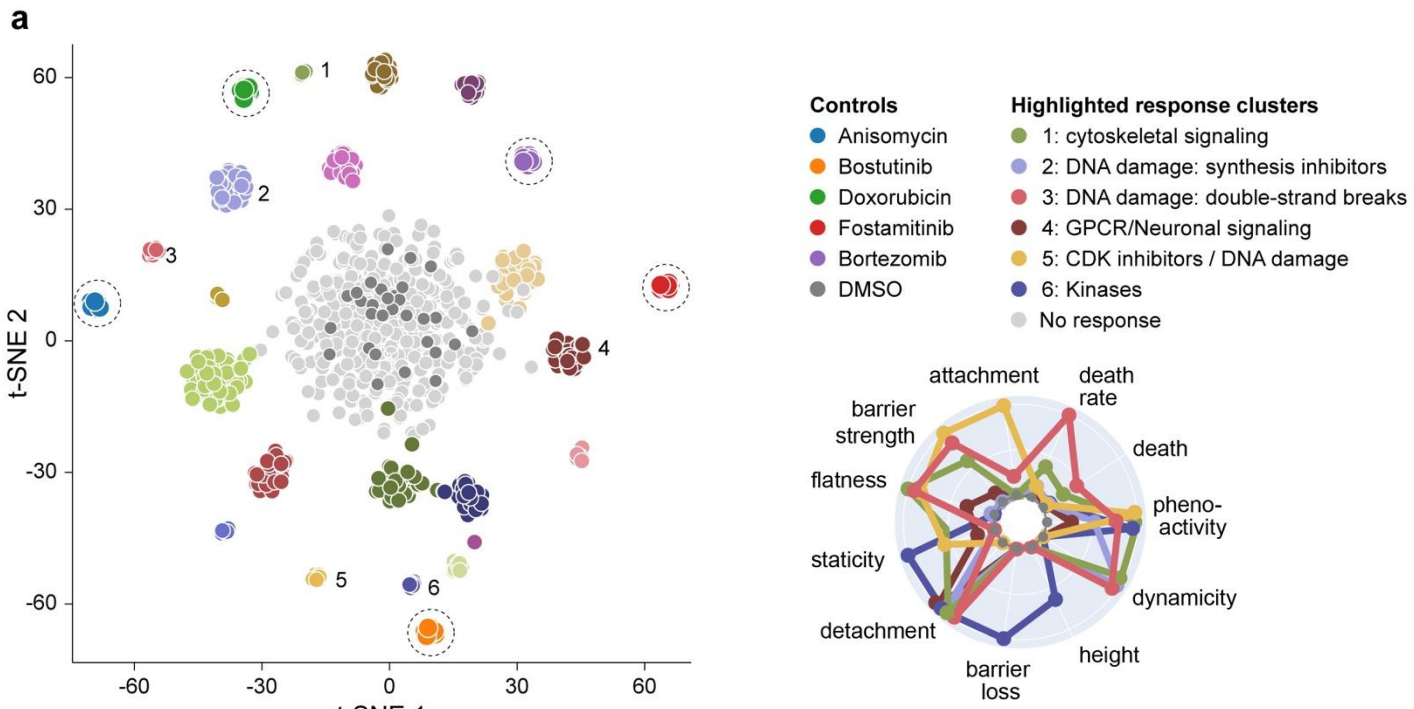
A 900+ proof-of-concept screen reveals 20+ distinct responses

Real-time compound responses across 20+ parameters



900+ compounds on 13 plates

Distinguish 20+ unique responses using a classifier



A single screening step = MOA + toxicity + off-target effect profiling

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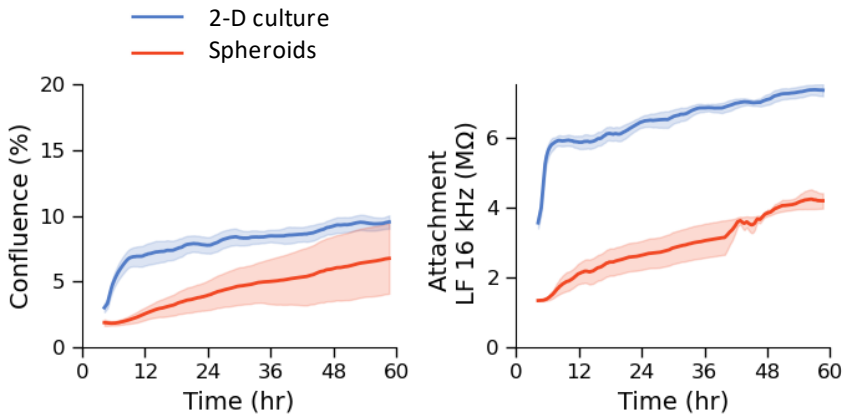
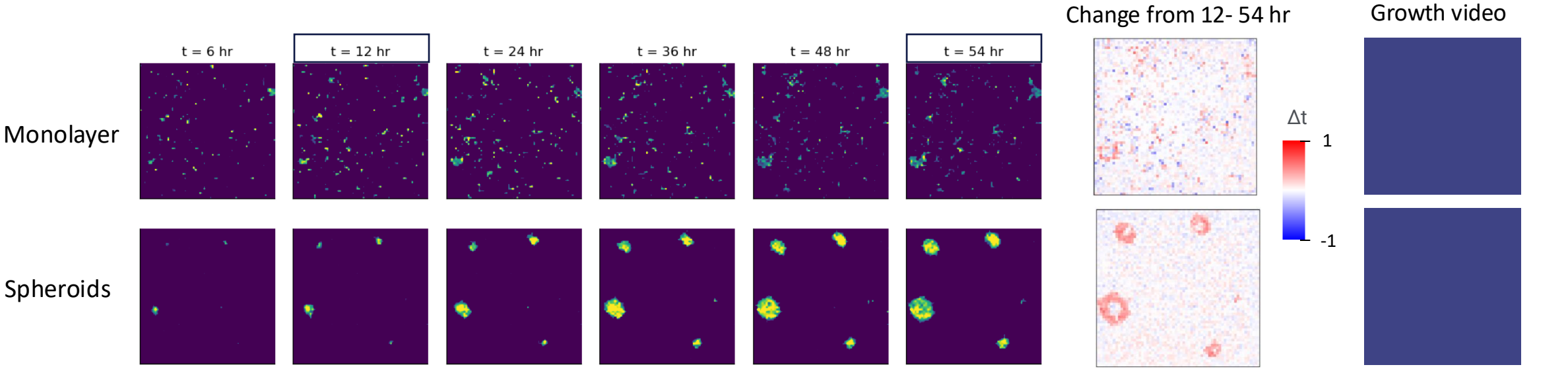
Monitoring 3D structure: HepG2 spheroids

- **Differentiate between 2D and 3D growth**
  - Monitor increase in confluence
  - Cells in 2D grow across well, while spheroids show radial growth over time
- **Functional properties of cells in 2D versus 3D**
  - Attachment of spheroids is much lower than monolayer of cells
  - Changes in various functional properties can be monitored over time to identify phenotypes

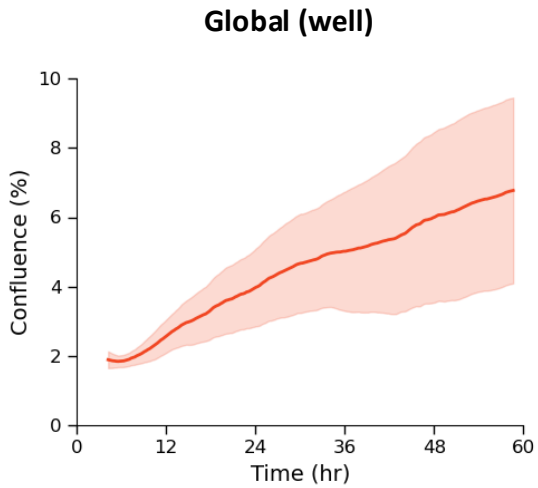


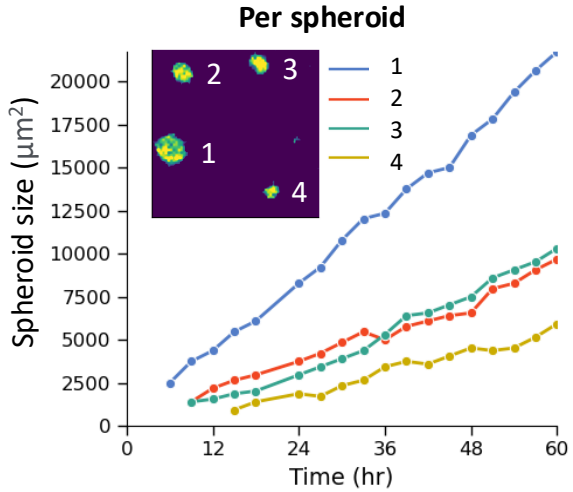


Spatial resolution differentiated two types of growth

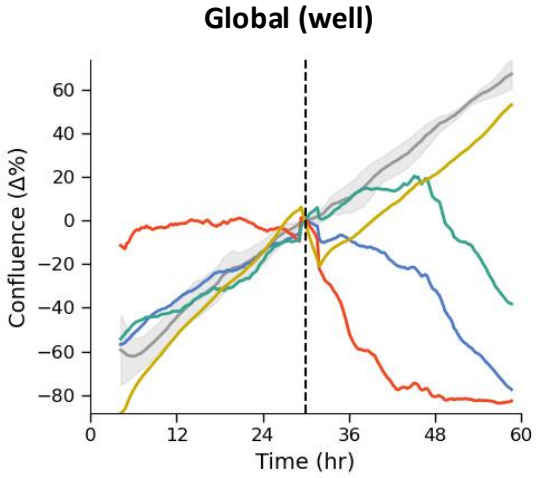
Growth and death in individual spheroids

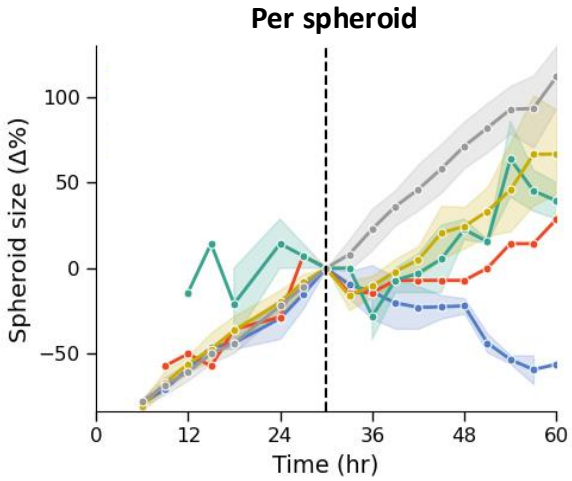
- **Track growth per spheroid**
  - More sensitive than global measurements of confluence
  - Highlights inter-spheroid variability





- **Accurate measurements of spheroid death**
  - Dose dependent effect of Doxorubicin on spheroid size can be measured
  - Monitor changes in functional properties of spheroids upon compound treatment





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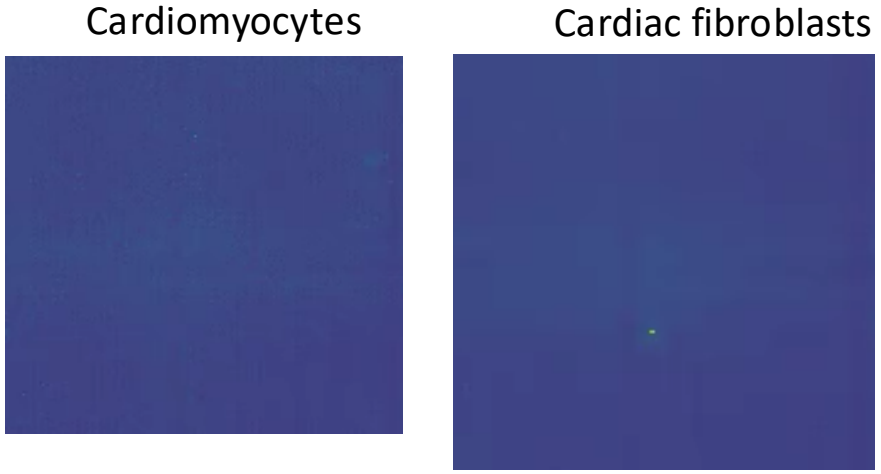
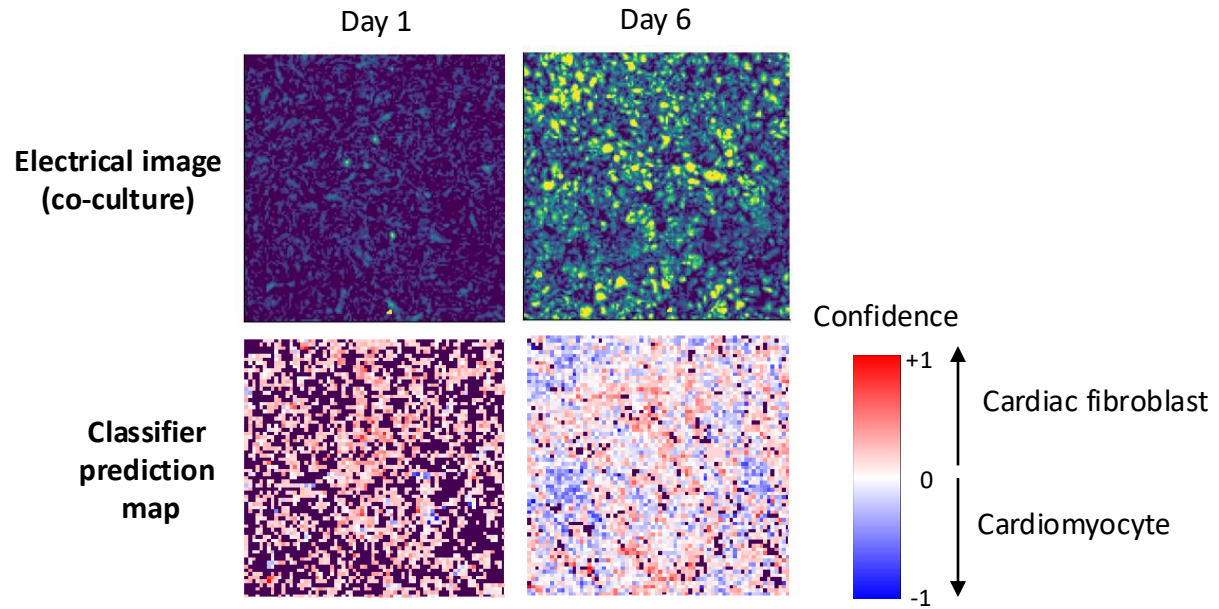
Multiplexed measurements in electrogenic cells

- Pixel combines electrical imaging with electrophysiological measurements in electrogenic cells and non-electrogenic cells
- Electrical imaging can identify structural characteristics of cardiomyocytes and cardiac fibroblasts in co-cultures





Electrical imaging monitors structural features of multiple cell types

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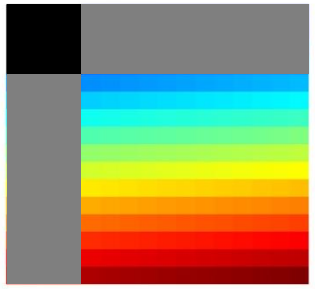
Multiplexed measurements in electrogenic cells

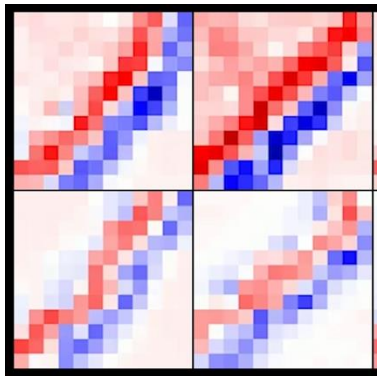


Array configuration

Stimulation

Grounds

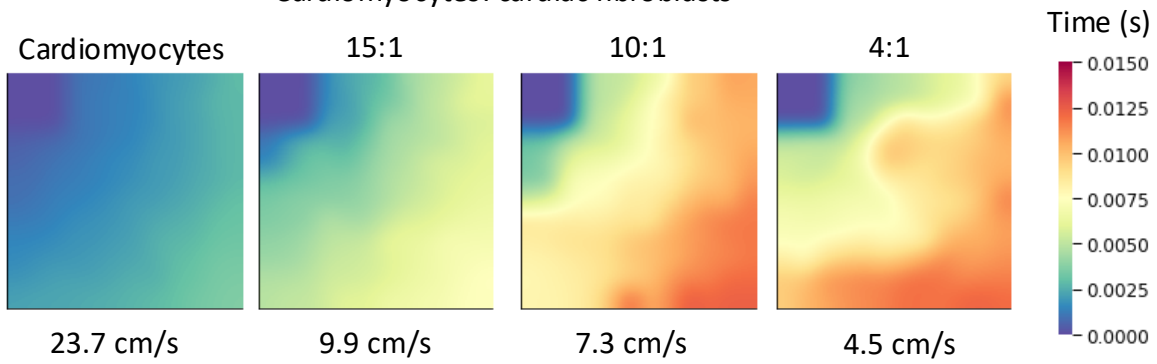
144 contractility + field potential recording electrodes

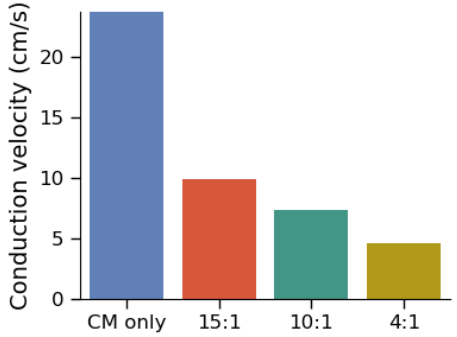


- Cardiac fibroblasts modulate electrical function of cardiomyocytes
- Cells can be paced using any electrode to stimulate
- Increasing the number of cardiac fibroblasts decreases conduction velocity across the cell sheet

Conduction maps

Cardiomyocytes: cardiac fibroblasts





Multiplexed measurements relate structure and function

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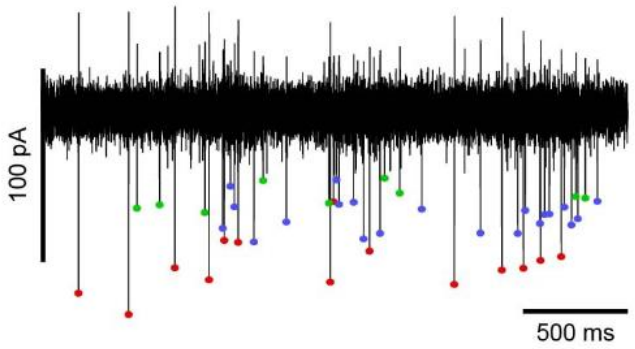
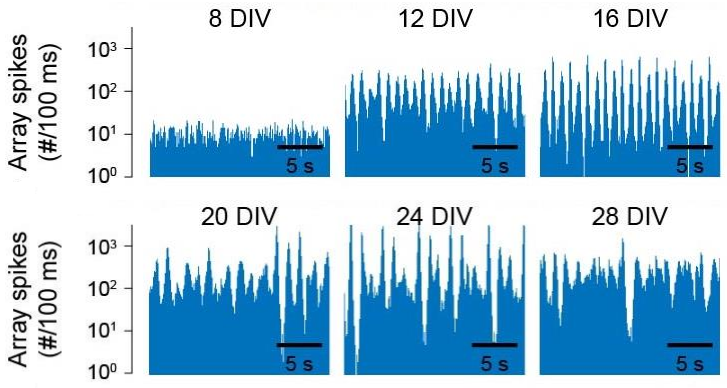
Neuro electrophysiology – translatable capabilities from research

Spontaneous recordings

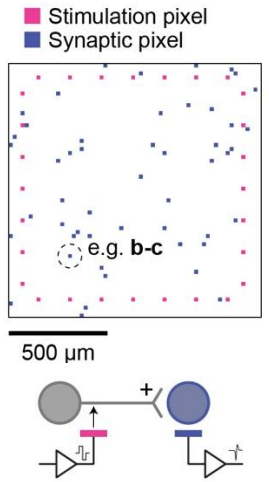
Spike detection

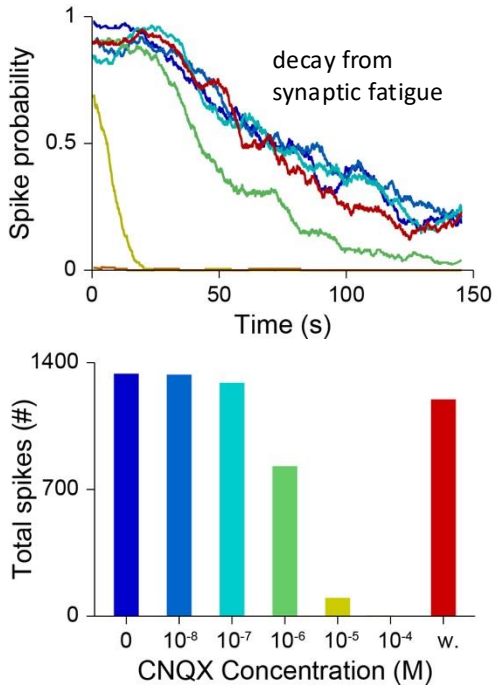


Network activity



Stimulated synapses





Extracellular recording of direct synaptic signals with a CMOS-nanoelectrode array, Lab on a Chip (2020)

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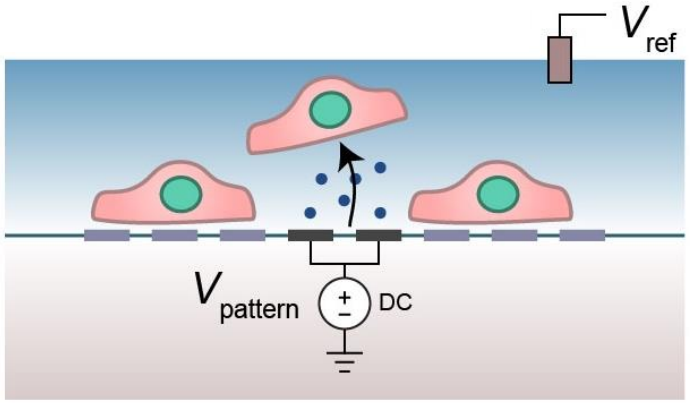
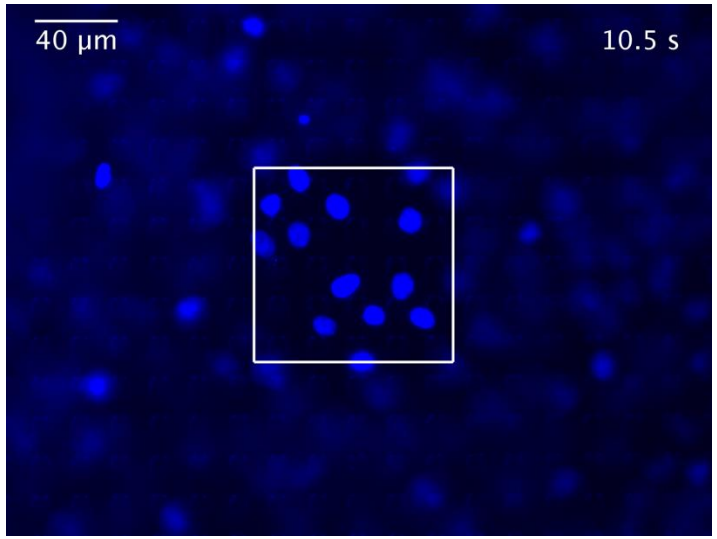
Manipulation – translatable capabilities from research

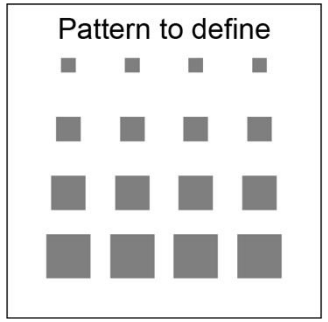
- Patterning via electrode-based gas generation
  - Co-culture boundary generation
  - Wound healing & cell migration
  - Removal of cell heterogeneity
- Cardiac & neuron stimulation

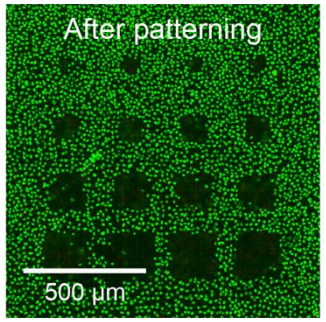


Patterning applied outside of white box



Across full array





Multi-parametric functional imaging of cell cultures and tissues with a CMOS microelectrode array, Lab on a Chip (2022)

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The Pixel Octo: built for scale

Plate 96

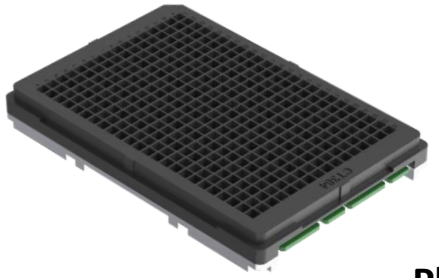
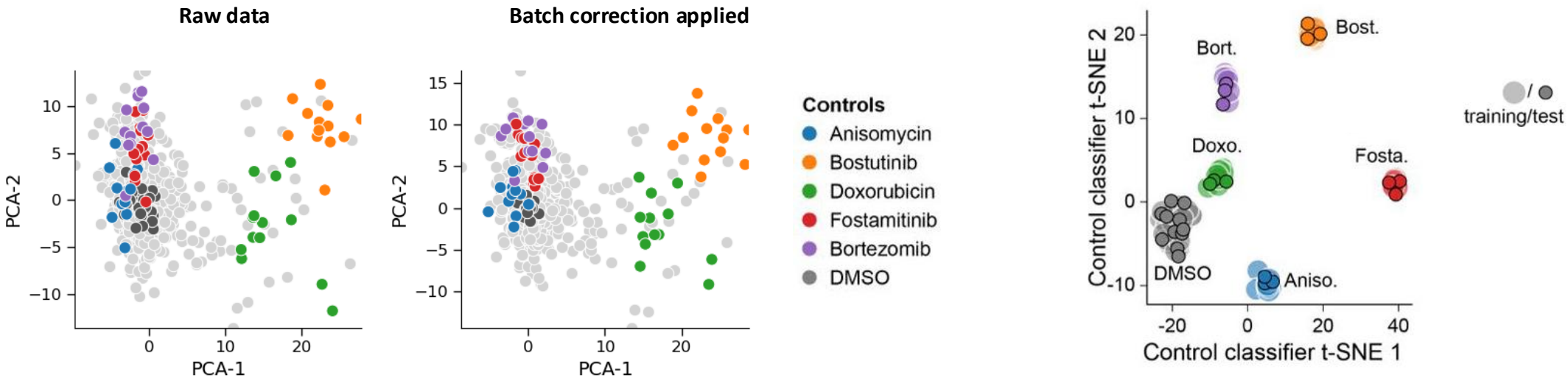


Plate 384

- ✓ Multiplexed live-cell readouts
- ✓ Low variability
- ✓ Scale without compromise
- ✓ Automation compatible

Label-free technology reduces variability



- Positive and negative controls used to assess batch effects
- No strong batch effects are observed from plates run across different days
- Phenotypes of the negative and positive controls are highly reproducible across plates

Electrical imaging provides robust quantitative measurements of phenotype

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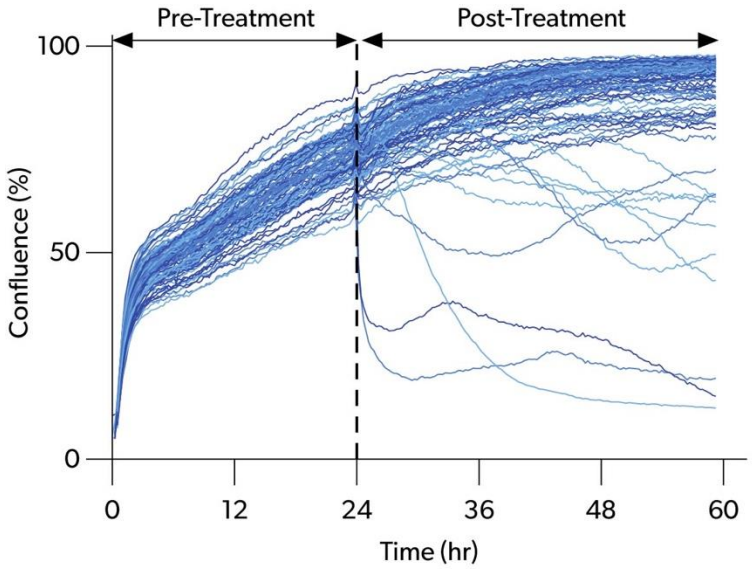
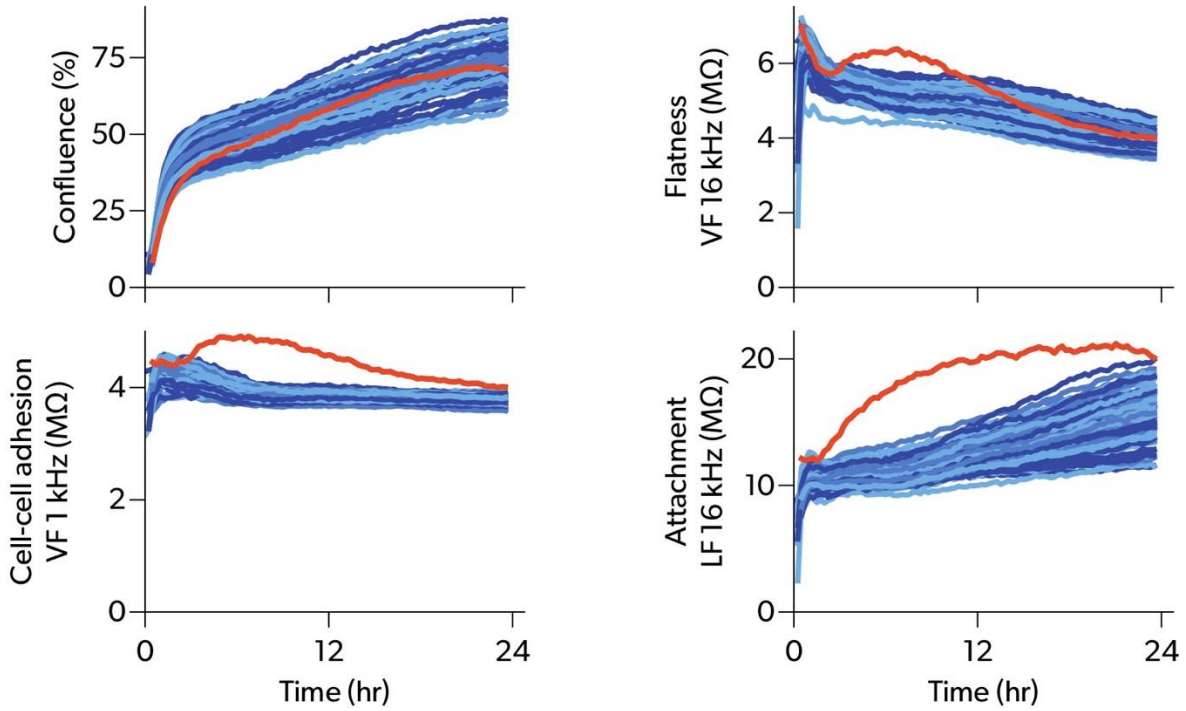
Real-time readiness assessment

- Live cells are monitored before and after compound/modulator addition
- Outlier wells with aberrant phenotypes are easily identified
- Improves interpretability of treatment related phenotypes



Single feature



Multiple features

The Pixel system

Software and data modules

- **Electrical imaging/impedance**, (Beta, Q1)
- Cardiac, (Beta, Q2)
- Neuronal, (Beta, Q3)
- Redox (metabolism), TBD
- Manipulation (patterning), TBD

Plate 96
(Beta, Q1)

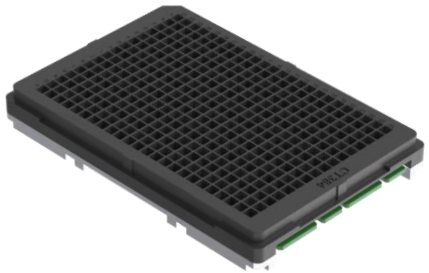


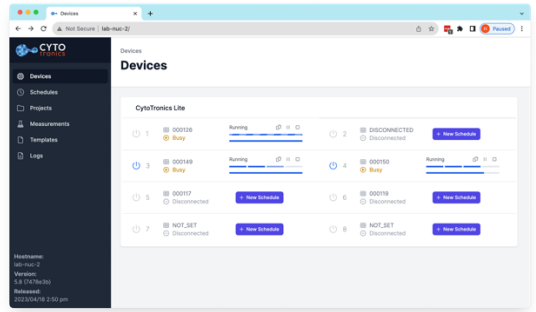
Plate 384
(Beta, Q3)

Primo
(Beta, Q1)

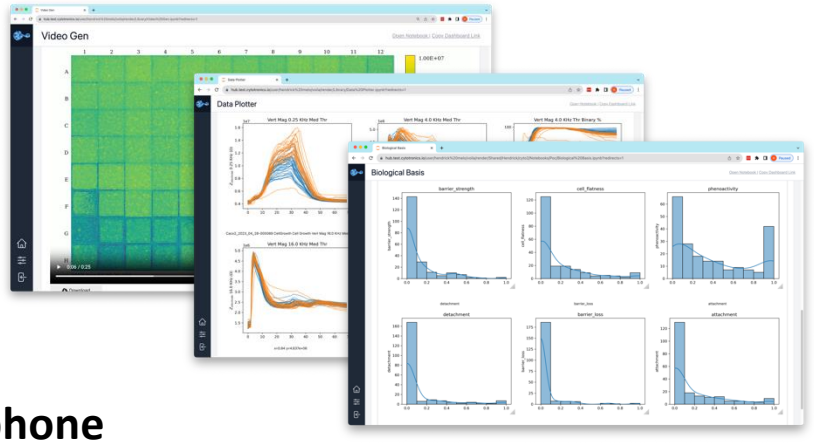
Octo
(Beta, Q4)

Cloud-centric software design

Web-based remote control



Visualize, analyze, and export



- ✓ **Control & monitor experiments remotely using a computer or cellphone**
- ✓ **Securely access, visualize, and analyze your data**
- ✓ **Customize data analysis with Python**

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Thank you!

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