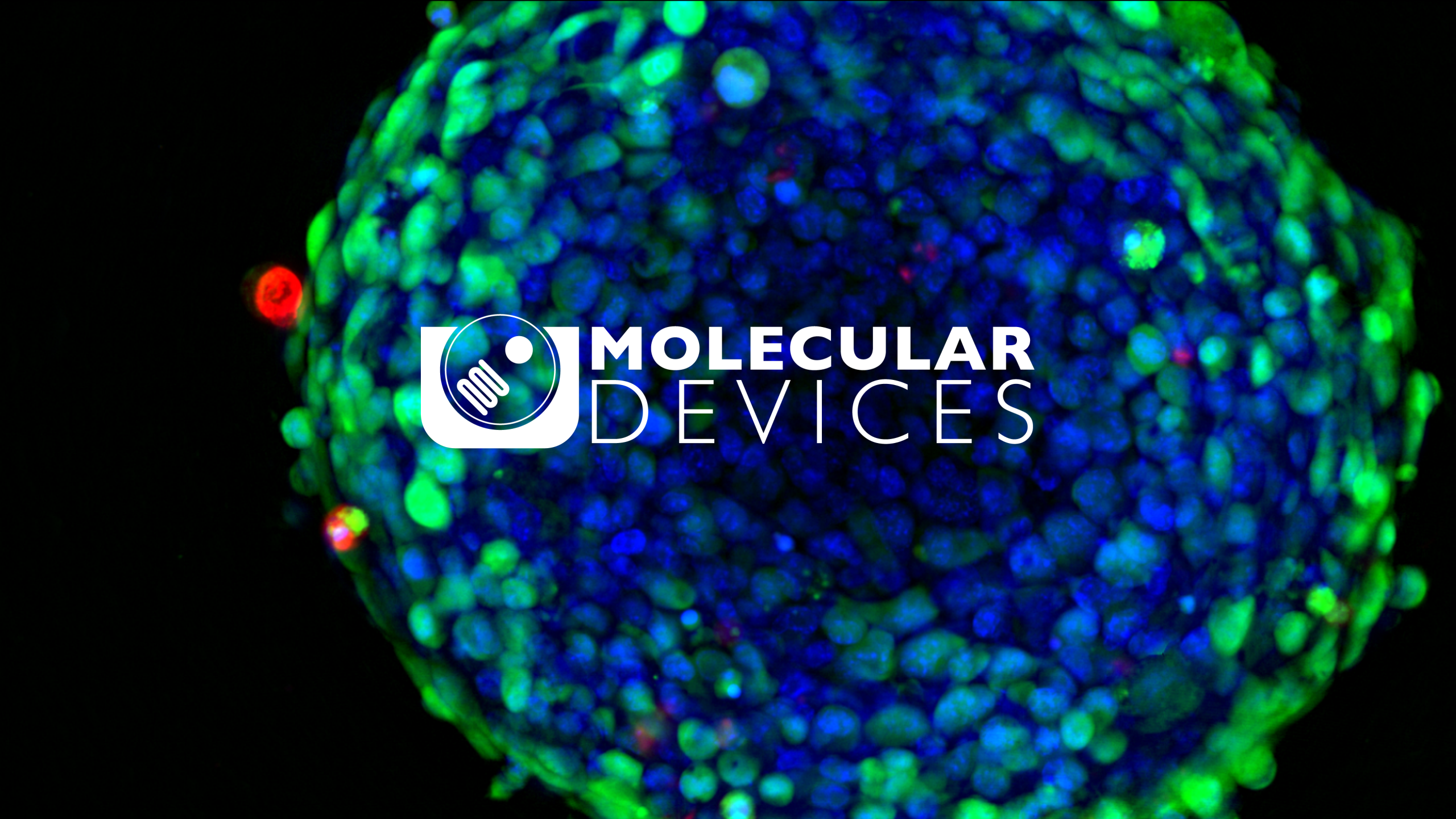

MOLECULAR
DEVICES

# Implementing 3D neural spheroids in drug discovery: phenotypic screening and compound profiling using structural and functional assays

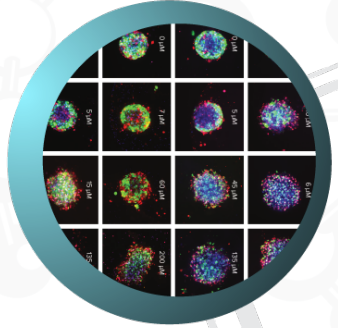
Carole Crittenden | Product Applications Scientist, Molecular Devices

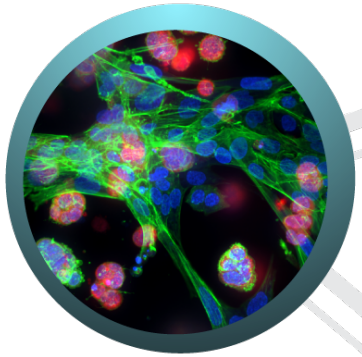
Blake Anson, Ph.D. | VP Business Development, StemoniX

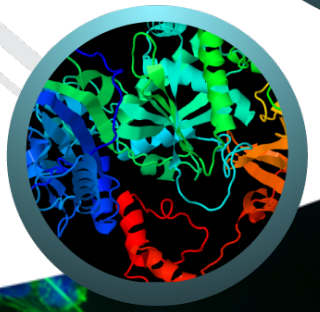
June 10, 2020

# Agenda

- Using microBrain® neuronal microspheroids
- Introducing the FLIPR® Penta High-Throughput Cellular Screening System
- Peak analysis with ScreenWorks® Peak Pro 2 software
- Calcium oscillations in neural spheroids
- Confocal imaging and 3D image analysis of compound treated neural spheroids
- Multi-parametric evaluation of neurotoxicity effects







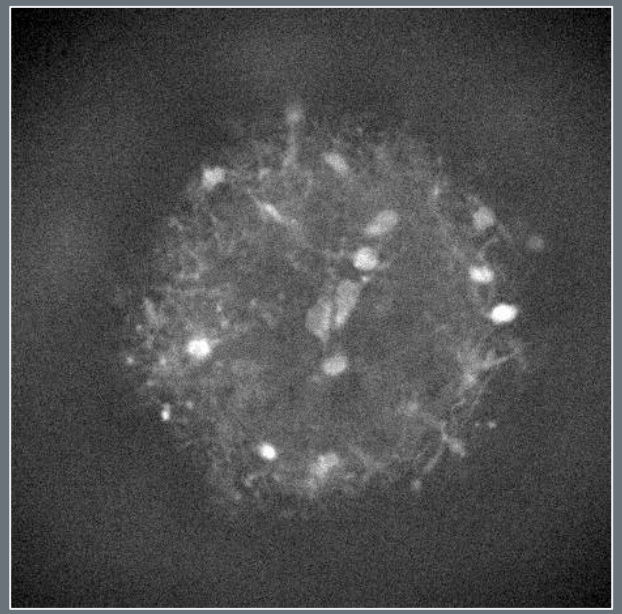
MOLECULAR DEVICES

# Neuronal microspheroids

## StemoniX microBrain® 3D activity can be monitored in high throughput format

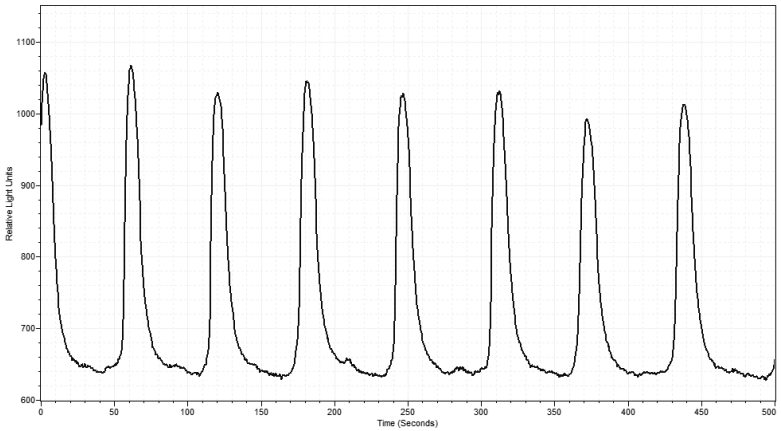
- Neuronal activity is monitored as calcium oscillations detected by high throughput kinetic fluorescence (FLIPR® Tetra and FLIPR® Penta Systems

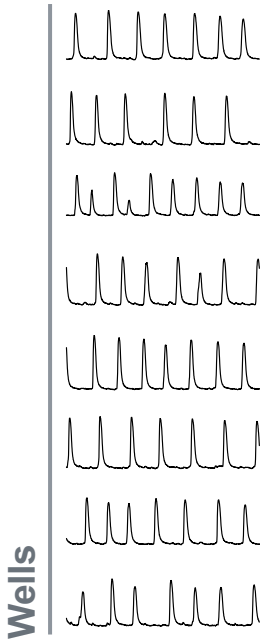
**Spontaneous neuronal activity**



**FLIPR Tetra System**

- Spontaneous activity on one well
- Detected oscillations correspond to synchronized calcium oscillation occurring on the sphere



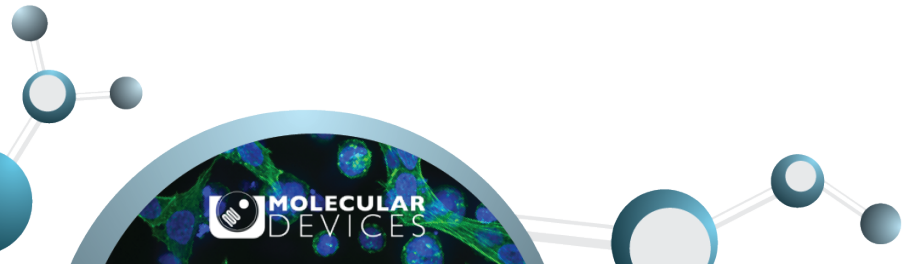

From StemoniX presentation at ISSCR 2019


StemoniX

# The FLIPR Penta High Throughput Cellular Screening System

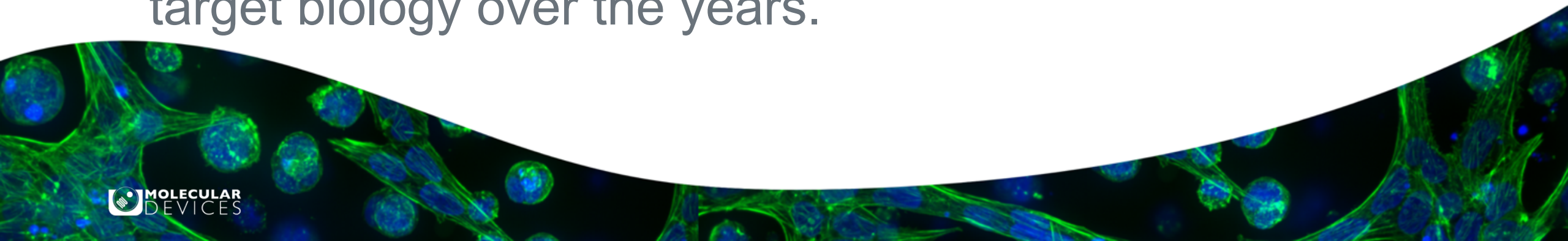
- Choice of camera
  - High Speed EMCCD
  - EMCCD
- Updated ScreenWorks 5.0
- Optional Peak Pro 2 Software Module
- 6,000 images per protocol
- iPSC derived Cardiomyocytes and Neuronal cell assays
- Same great original assays including GPCRs, MP, Potassium, and luminescence

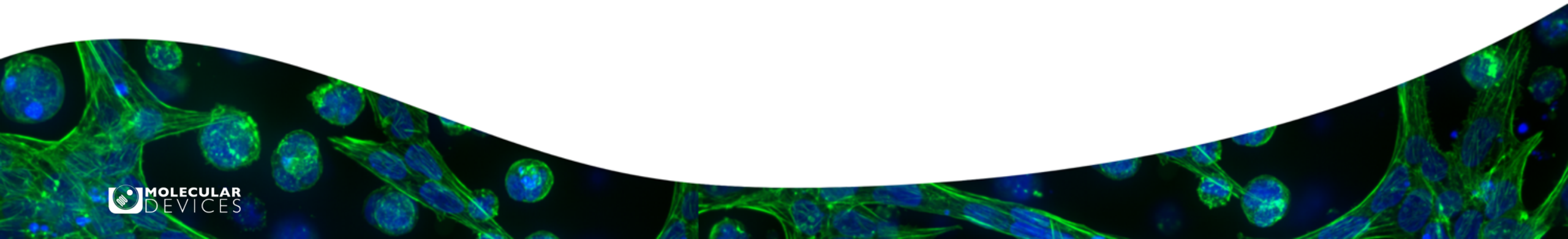
# Benefits of FLIPR Penta system

- 100 Hz camera enables new biology
  - Better understand iPSC cardiomyocyte and neuron calcium signaling biology with more signal detail provided by the HS EMCCD camera
- Greatly enhanced peak characterization of calcium oscillation assays with PeakPro 2.0 software
- All of the great technologies used for screening and target biology over the years.



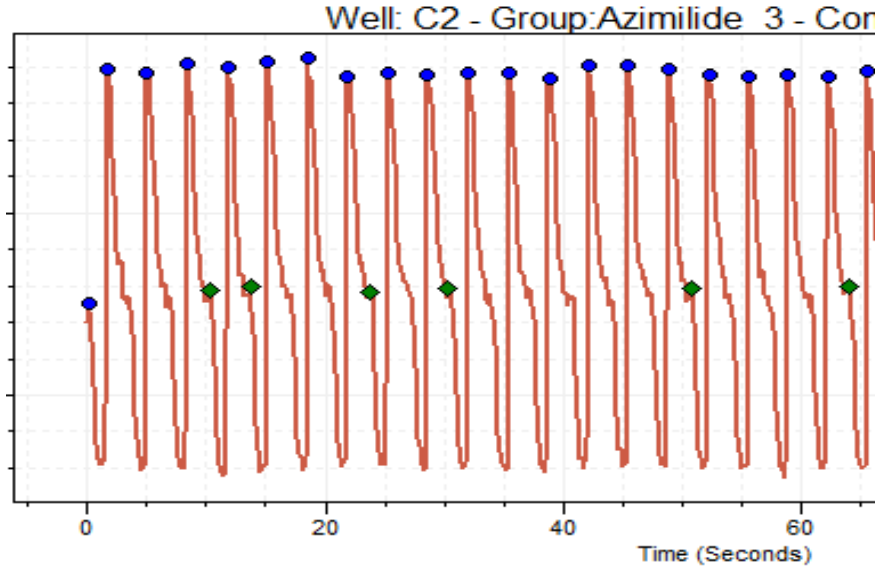
# Protocol

- microBrain microspheroids received in wells from StemoniX and cultured a few days
- Cells are incubated for 2 hours with FLIPR® Calcium 6 Assay Kit Dye
- Compounds are added and read on the FLIPR Penta System at intervals from 15 minutes to 24 hours



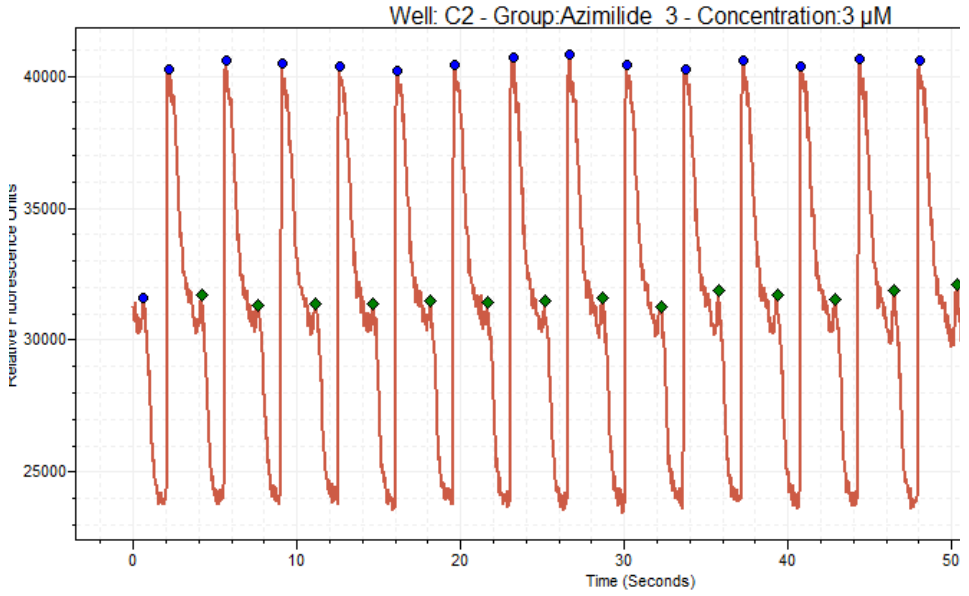
# Difference in temporal resolution

## Acquisition @8Hz




Not enough data to see and identify EAD-like events (green diamonds)

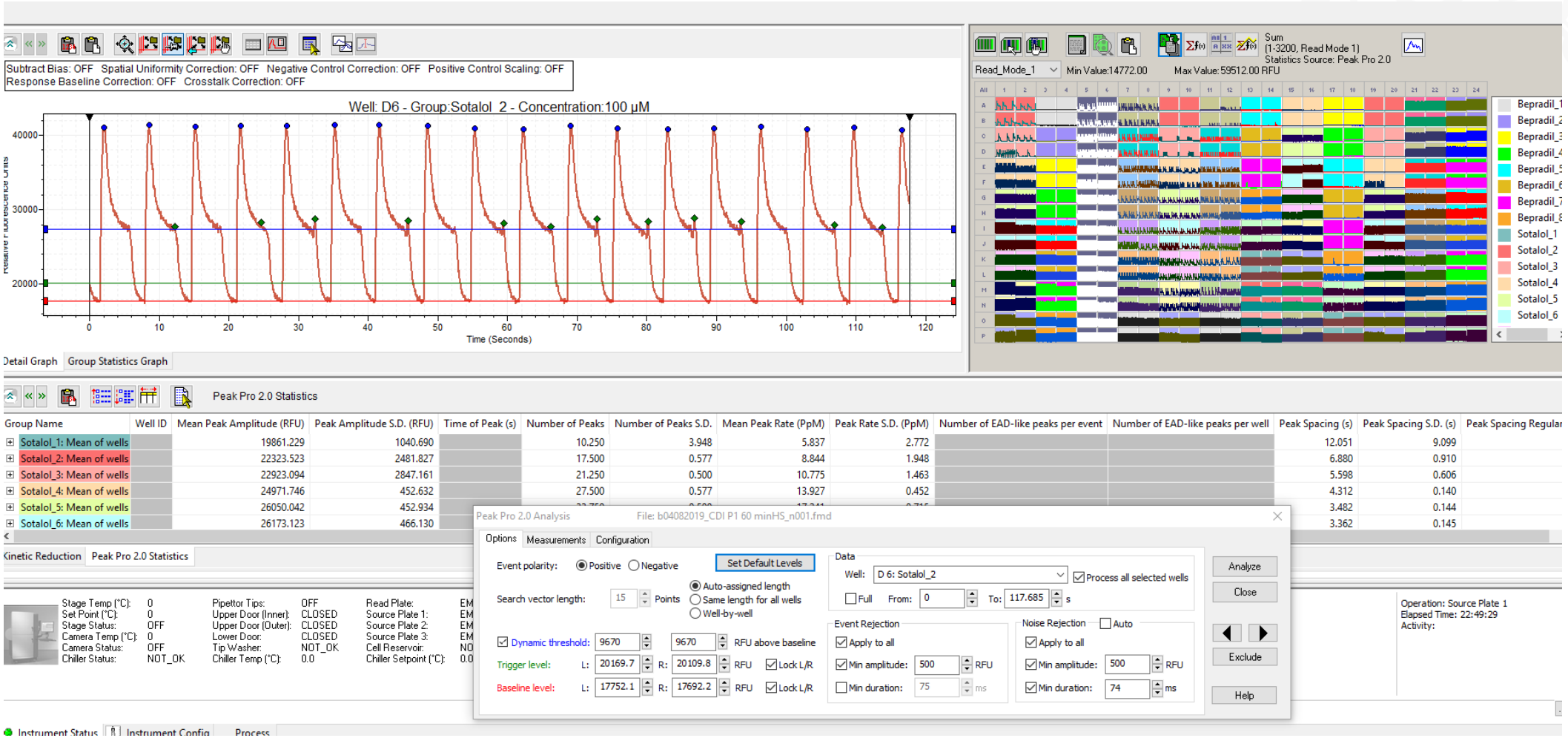
## Acquisition @30Hz




EAD-like events easily visible and identified by Peak Pro 2 software

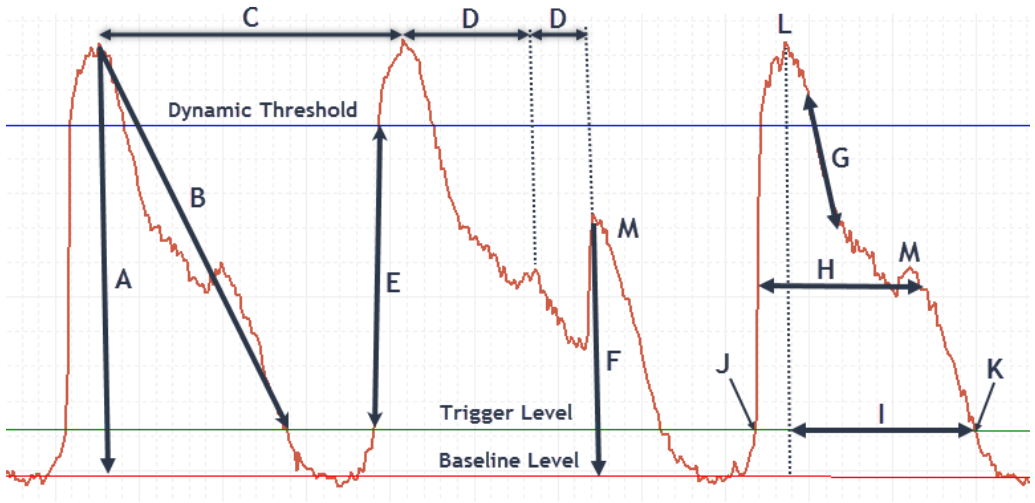
 

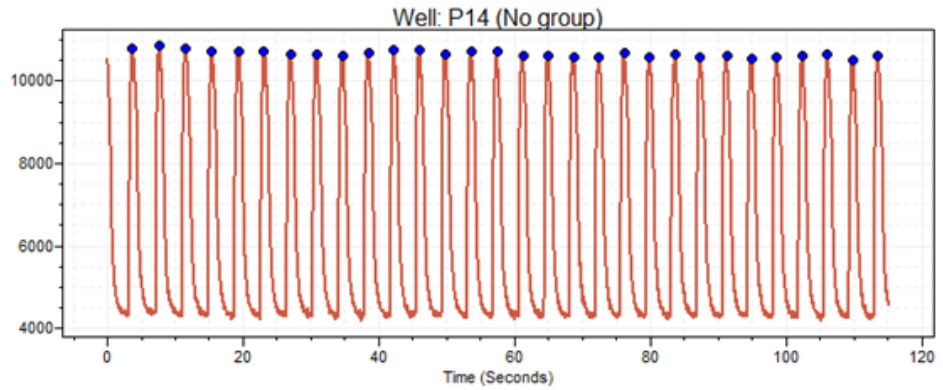
# ScreenWorks 5.0 Peak Pro 2 Software



# Characterization of oscillation patterns

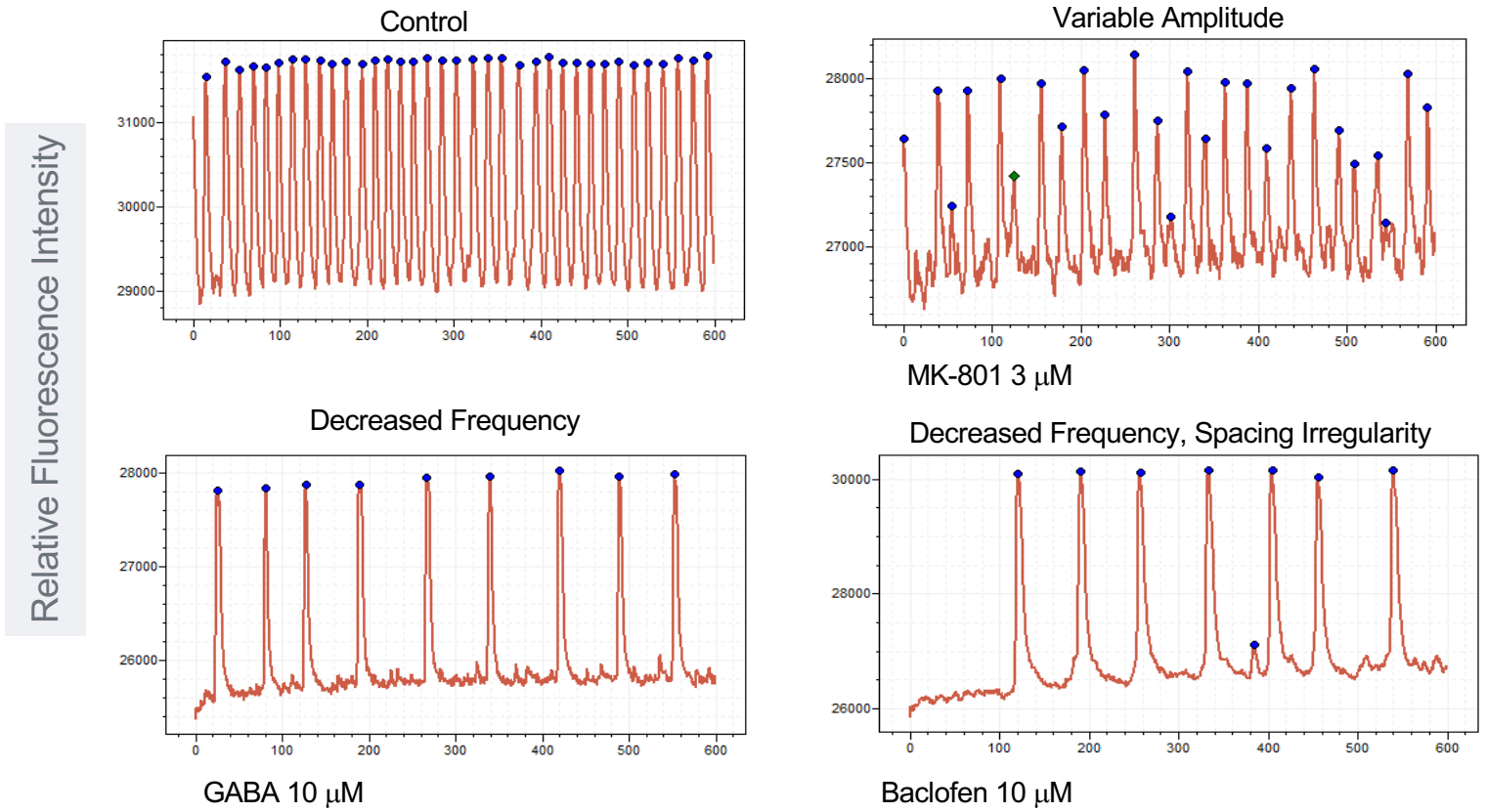



| Measurement Terminology Illustrated | |
|---|---|
| Item | Description |
| A | Main peak amplitude |
| B | Linear decay slope |
| C | Main peak interval used to calculate the peak rate, expressed in peaks per minute (PpM) |
| D | Early afterdepolarization-like event (EAD-like) intervals used to calculate the EAD-like event rate, expressed in peaks per minute (PpM) |
| E | Rise slope |
| F | EAD-like event amplitude |
| G | Decay slope |
| H | Calcium transient duration (CTD) |
| I | Calcium transient duration from peak position (CTDP90) |
| J | Start of an event |
| K | End of an event |
| L | Main peak |
| M | Early afterdepolarization-like event (EAD-like) |

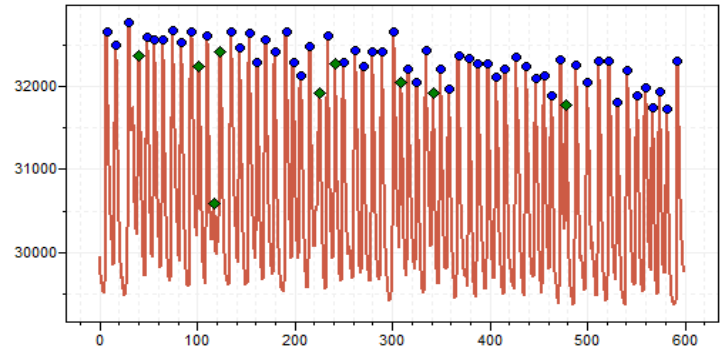
# Oscillation Patterns in neuronal spheroids



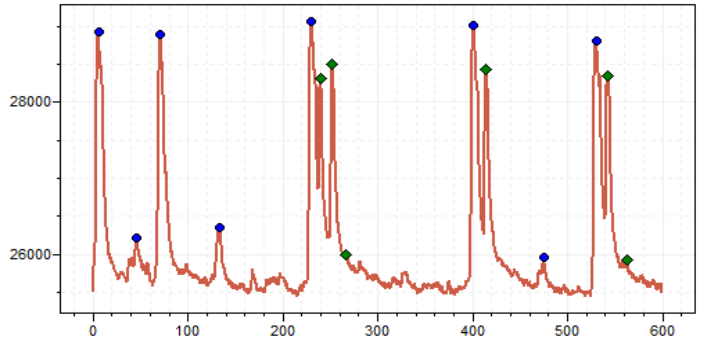
# Oscillation patterns in neuronal spheroids

Increased Frequency, secondary peaks




4-Aminopiridine 30 μM

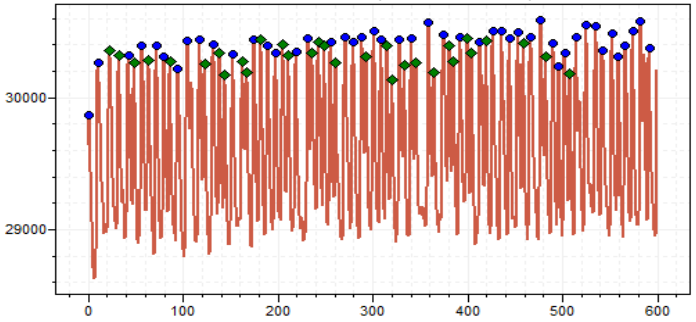
Decreased Frequency, Secondary peaks




Valinomycin 0.3 μM

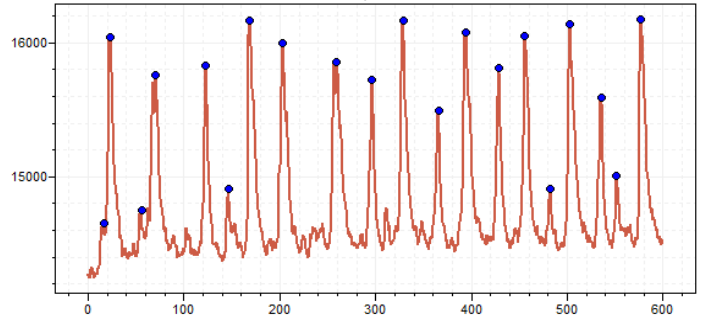
Relative Fluorescence Intensity

Increased Frequency, Secondary peaks




Kainic acid 1 μM

Decreased Frequency, Variable Amplitude

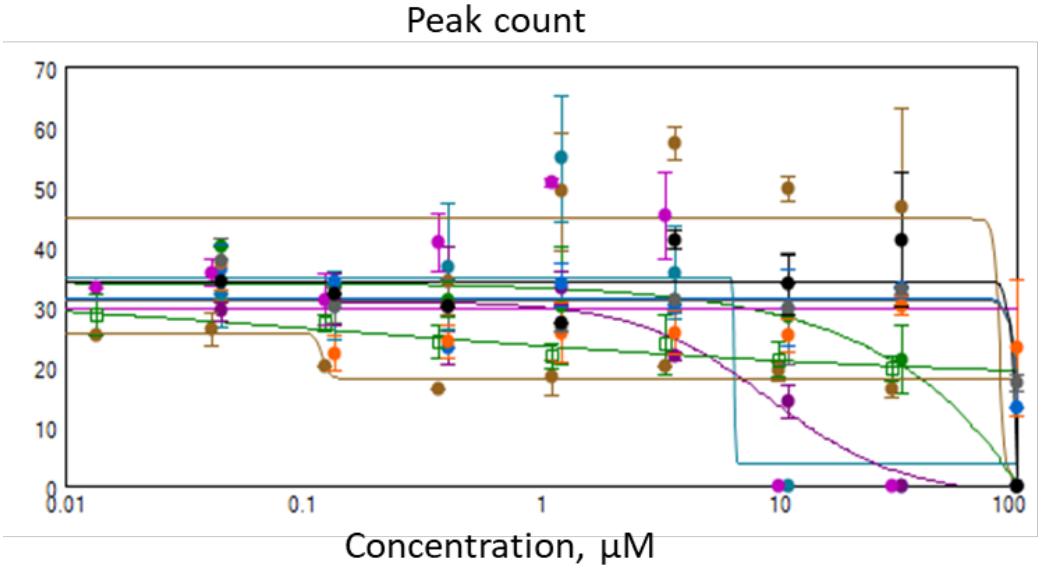


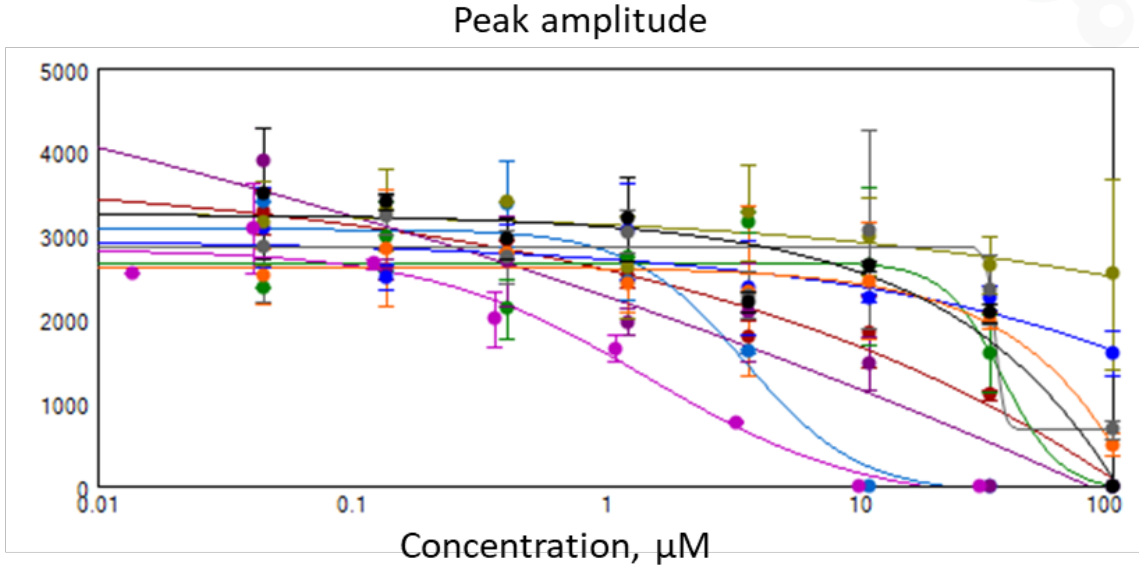

Tamoxifen 30 μM

Time, seconds

# Concentration dependencies




**Concentration-dependencies for changes in peak counts and amplitudes were calculated using 4-parametric curve fits and $EC_{50}$ values were determined.** Other readouts did not have monotonous changes, therefore minimal concentrations causing indicated phenotypes (secondary peaks, amplitude irregularities, spacing irregularities) were recorded. 4-paramentiric curves indicated for the following compounds:

tamoxifen- light blue
amiodarone- pink
pindolol- brown
stilbestrol- green
dopamine- green square
cytarabine- yellow
lipopiridine- orange
cephalosporin- blue
Taxol- purple
carbamazepine- black
tetraethyl thiuram- gray

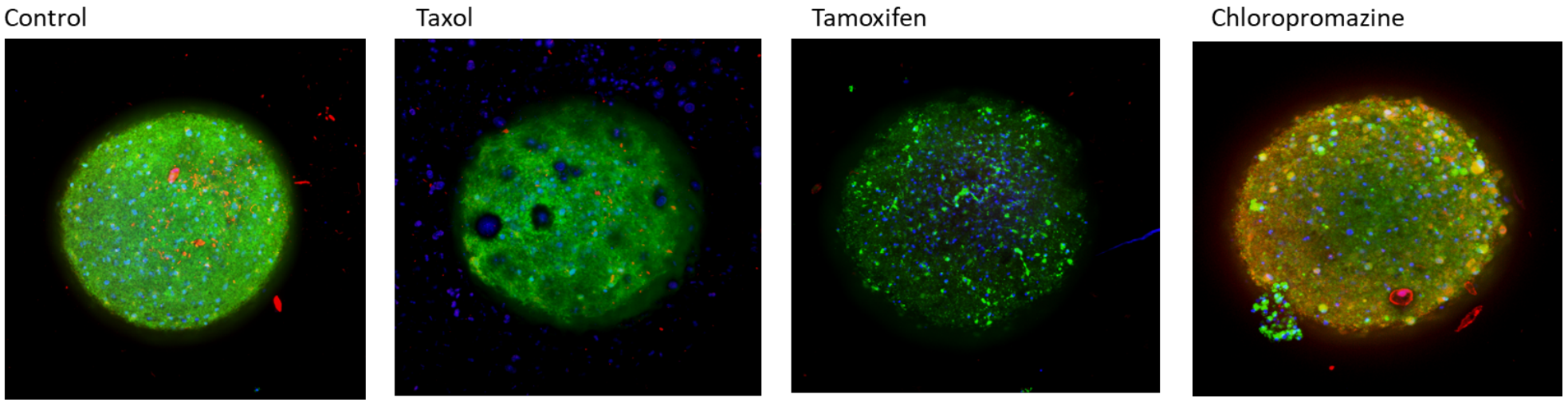
 

# Viability studies using the ImageXpress® Micro Confocal system

- Confocal imaging and 3D image analysis
- Characterize compound effects on morphology and viability of 3D neural spheroids
- Spheroids treated with 30 μM drug for 24 hours
- Cells stained with DAPI, Calcein AM, and Mitotracker Orange

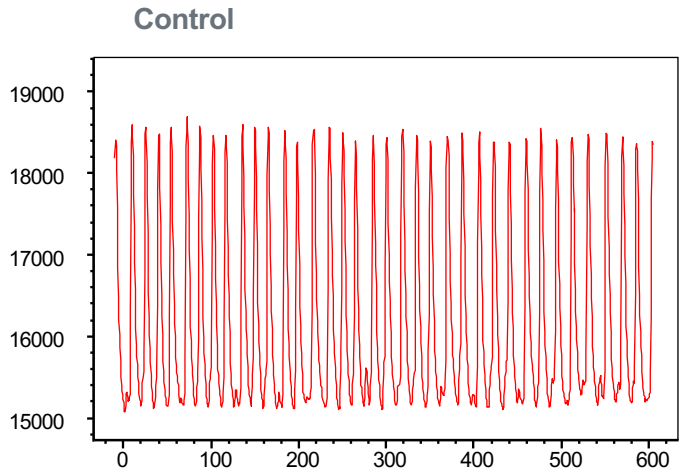
# Assessment of spheroid morphology and viability by high-content imaging

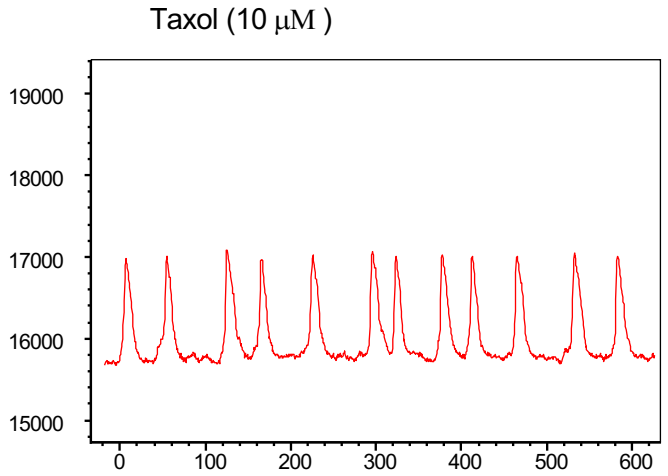


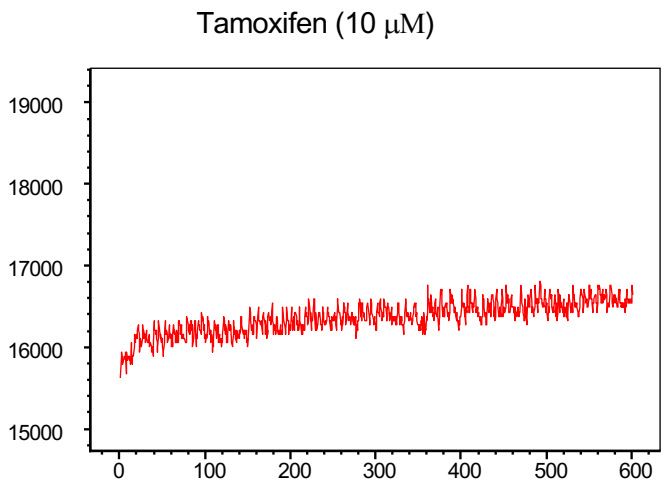

- Projection images analyzed using the Custom Module Editor and Cell Scoring Algorithms
- The images shows spheroid nuclei (blue), viable cells stained with Calcein AM (green), and viable mitochondria (orange)

# Calcium oscillation patterns

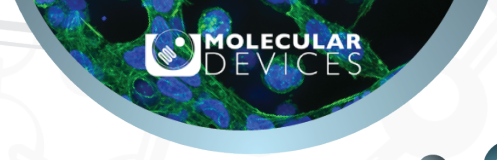


# Multi-parametric evaluation of neurotoxicity effects




**Neurotoxic drugs**

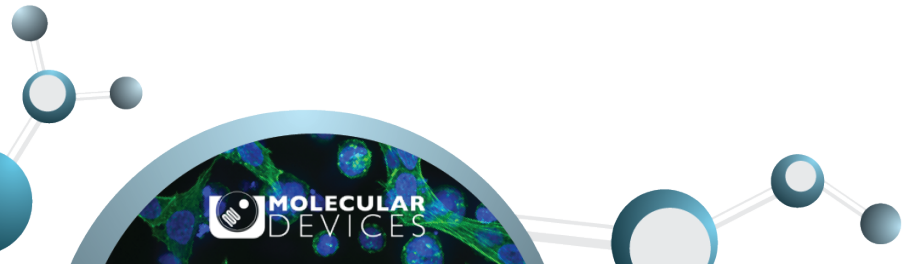
| | Compounds | Peak Count[1] | Amplitude (decrease)[1] | Oscillation Frequency | Fibrillations* | Oscillation Stop* | Amplitude Irregularity* | Peak spacing Irregularity* | Secondary Peaks* | Cytotoxicity* | Mitochondria toxicity* | Max concentration | Description |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Pindolol | 57.3 | 30 | up/down | | 100 | 3 | 3 | 3 | | | 100 | Beta-blocker |
| 2 | Diethylstilbestrol | >100 | 39.3 | down | | 100 | 10 | 10 | 10 | | 100 | 100 | Estrogen agonist |
| 3 | Tamoxifen | 6.43 | 3.59 | down | | 100 | 30 | 30 | | 100 | 100 | 100 | Estrogen receptor modulator |
| 4 | Taxol | 9.15 | >30 | down | | 30 | 10 | 3 | 3 | 100 | 100 | 100 | Anti cancer |
| 5 | Cefepime HCl | | | | | | | | | | | 100 | Antibiotic |
| 6 | Ciprofloxacin | | | | | | | | | | | 100 | Antibiotic |
| 7 | Cephalosporine | | ~100 | | | | 100 | 30 | 30 | | | 100 | Antibiotic |
| 8 | Gentamycin Sulfate | | | | | | | | | | | 100 | Antibiotic |
| 9 | Isoniazid | | | | | | | 100 | | | 100 | 100 | Antibiotic |
| 10 | Berberine (HCl) | ~30 | no effect | down | | 30 | 10 | 10 | 10 | | 30 | 30 | Alkaloid, antibiotic |
| 11 | ß estradiol | 202 | | down | | | 30 | 30 | 30 | | | 100 | Hormone, osteoporosis |
| 12 | Amiodarone | 8 | 1.48 | up/down | | 30 | 3 | 10 | | | | 100 | Anti-arrhythmic drug |
| 13 | Oxotremorine M | | | | | | 10 | | | | 30 | 30 | Anti tremor |
| 14 | Pentylenetetrazole | | | | | | | | | 1000 | 1000 | 1000 | Respiratory stimulant |
| 15 | Pilocarpine (HCl) | | | | | | | | | | | 31 | Glaucoma drug |
| 16 | 6-hydroxydopamine hydrobromide | >100 | | down | | | | | | 30 | 30 | 30 | Neurotoxin induces a reduction of dopamine levels in the brain |
| 21 | Linopiridine (HCl) | | 100 | | | | 3 | 10 | 10 | | | 100 | Cognition-enhancing drug |
| 22 | Tetraethylthiuram disulfide | 200 | 34 | down | | | 100 | 30 | | 100 | 100 | 100 | Anti-alcohol abuse |
| 23 | Carbamazepine | 101 | >30 | up/down | | 100 | 30 | 10 | 30 | | | 100 | Anti-epileptic drug |
| 24 | Amoxicillin | no effect | no effect | no effect | | no effect | no effect | no effect | no effect | no effect | no effect | 100 | Negative control |

$EC_{50}$ values (1) or lowest concentrations (μM) that cause specific changes(*) are indicated for different read-outs. No changes indicated with blank cells.

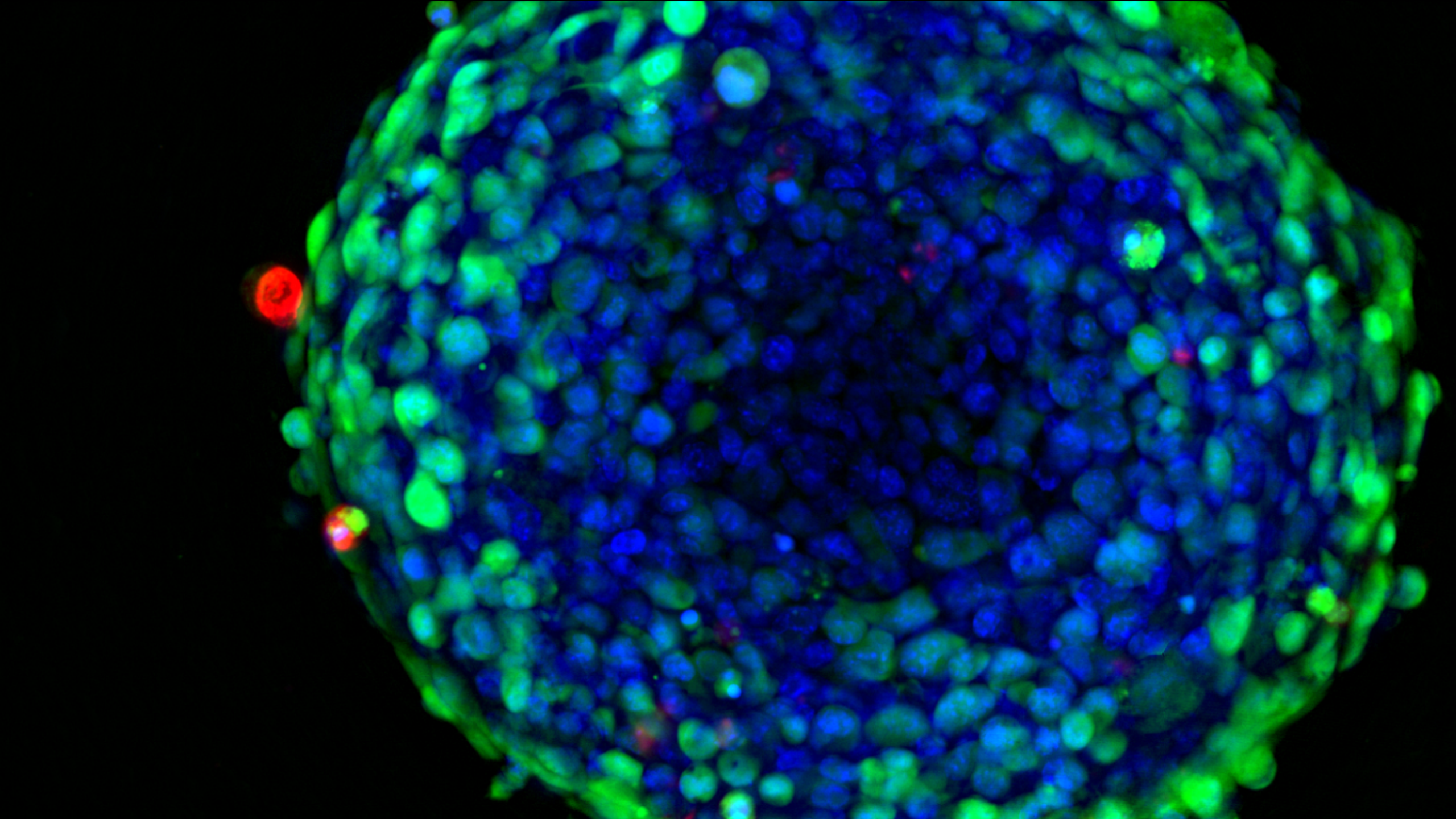
 

# Summary

- Multi-Parametric studies combining calcium oscillation peak analysis on the FLIPR Penta system and viability imaging analysis on the ImageXpress Micro Confocal system
- Data quickly shows the effects of neuro-mediators and neuro-active drugs so that they can be readily detected and studied
- Inducers of seizure can be detected (predictive value)
- Sensitivity to neurotoxic drugs can be evaluated
- New Peak Pro 2 Software features enable better analysis and more read-outs useful for characterization of compound effects on neural activity also prediction of neurotoxicity and seizure-inducing effects



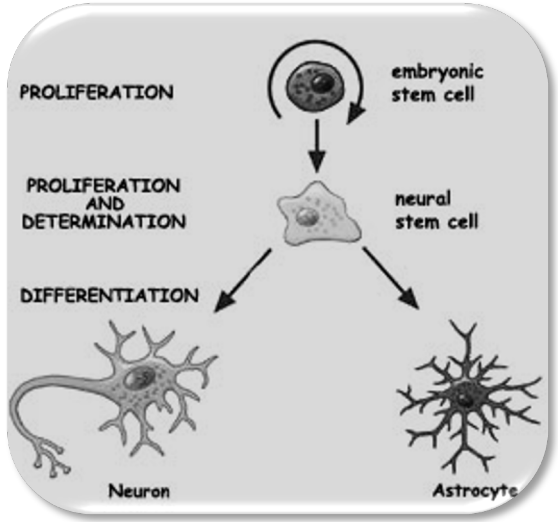

 



# Nervous System Function

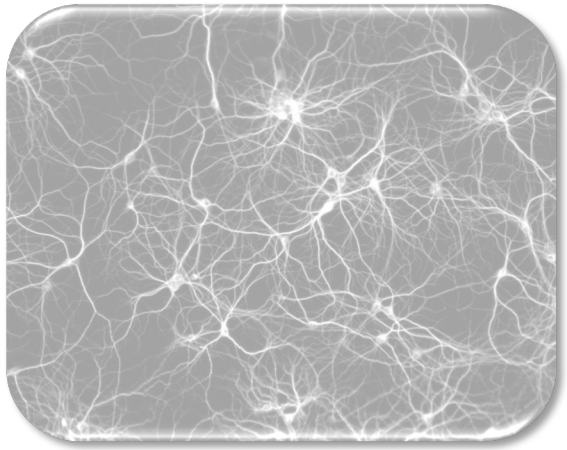
*The complex interplay of multiple developmental and operational processes*

Cell Development



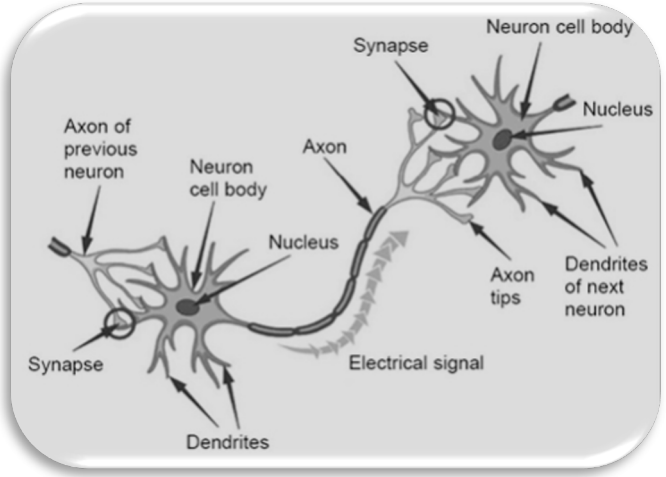

https://thebrain.mcgill.ca/flash/d/d_09/d_09_cl/d_09_cl_dev/d_09_cl_dev.html

Network Formation



https://upload.wikimedia.org/wikipedia/commons/e/e6/R%C3%A9seau_de_neurones.jpg

Functional Neural Activity




http://www.humanagingcentral.com/brain_page.html

Integrated Organ

WebMD.com

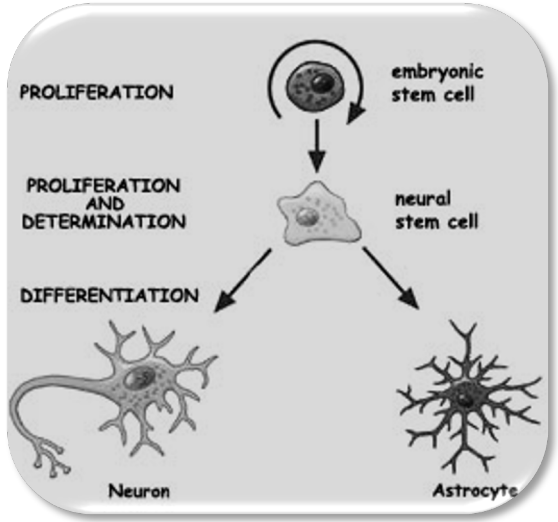
Developmental, operational, and mature integrated function have bi-directional influence

Functional activity of the integrated organ acts as a downstream biomarker for all the upstream processes

# Organoid / Spheroid Function

*The complex interplay of multiple developmental and operational processes*

Cell Development

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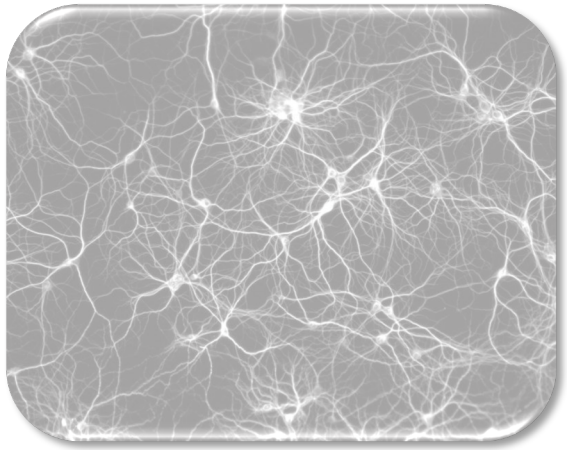
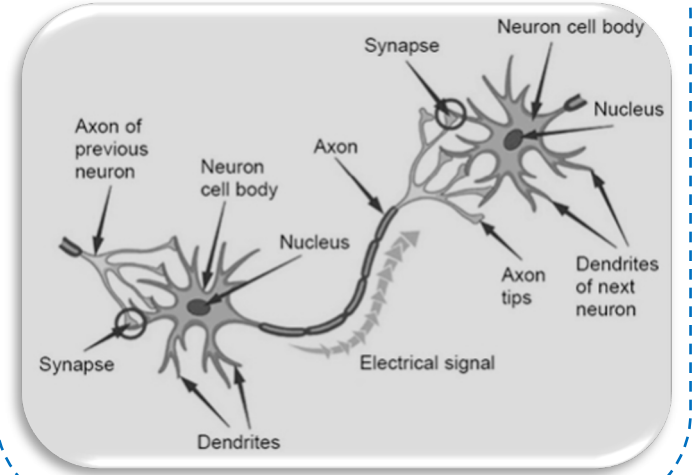
Network Formation



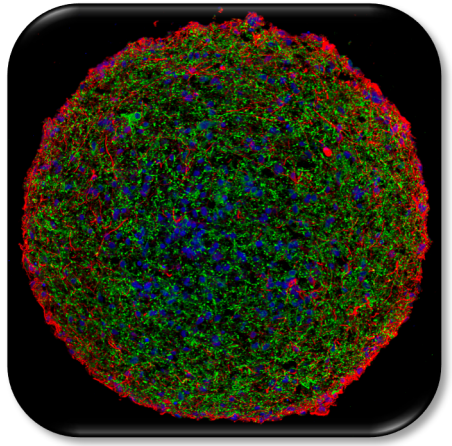
https://upload.wikimedia.org/wikipedia/commons/e/e6/R%C3%A9seau_de_neurones.jpg

Functional Neural Activity




http://www.humanagingcentral.com/brain_page.html

Integrated Organoid / Spheroid



Organoids and spheroids generated from stem cells reflect native ontogeny and function.

Functional activity can be used to assess;

1. Neurodevelopmental disorders (and treatment)
2. Compound (drug) activity on neural function

# StemoniX

*Accelerating the Discovery of New Medicines*

**Implementing 3D neural spheroids in drug discovery: Identifying new and safe compounds through phenotypic screening and compound profiling using functional and structural assays.**

- StemoniX Overview
- microBrain® 3D
  *hiPSC-derived neural spheroids*
- Case Studies
  *Toxicity Testing*
  *Screening*
  *Disease modeling*

# StemoniX Key Differentiators

## Leader in Industrialized Human microOrgans

***How:***

Leverage core competencies in iPSC biology, high-tech manufacturing and engineering to create scale, consistency and mature functionality

***Why?***

Provides a richer and more relevant context for better answers. Demonstrated utility in both drug discovery and toxicity testing.

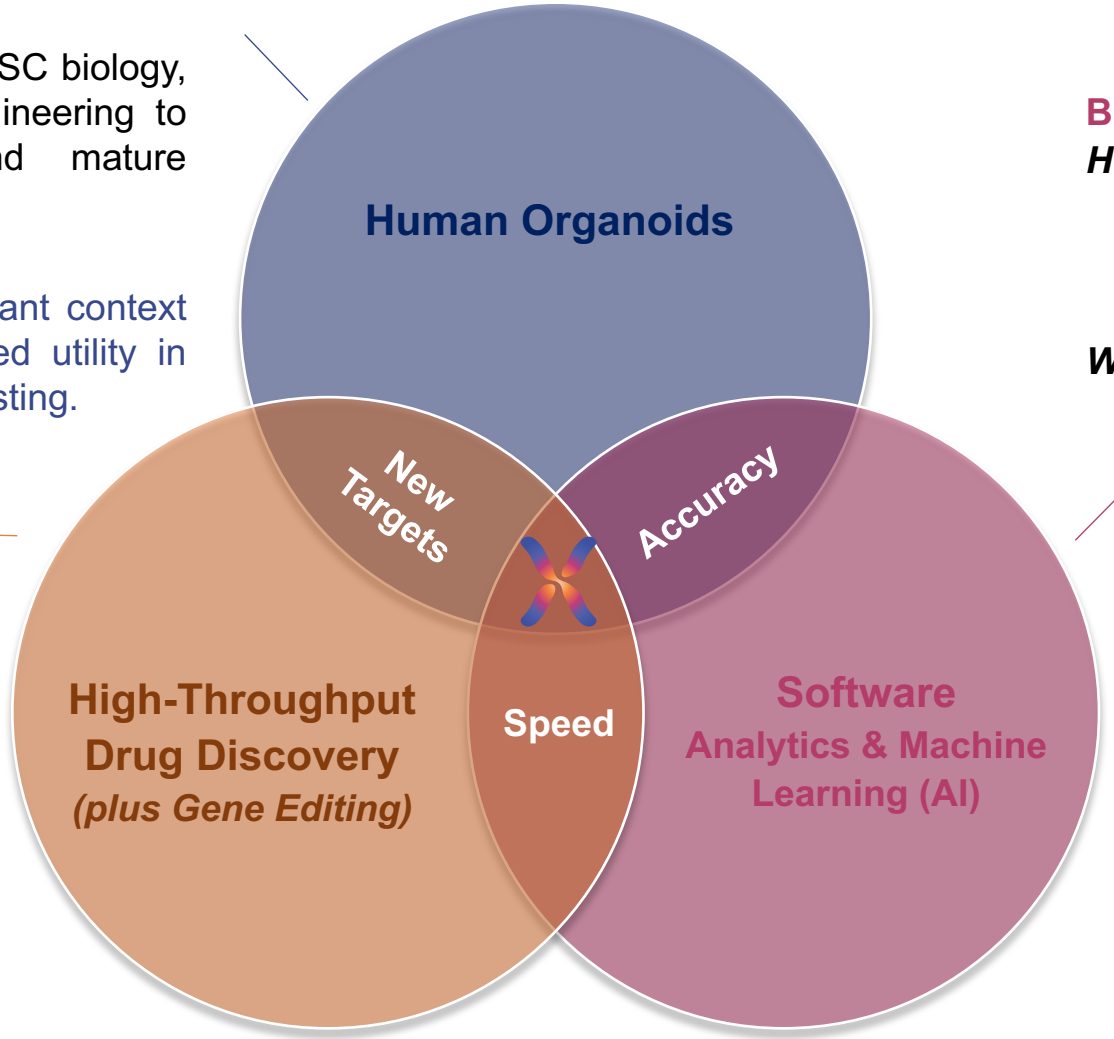
## Novel Therapeutic Assets

***How:***

Identify, optimize and produce new medicines discovered through human microOrgan technology and AI drug discovery

***Why?***

Optimizes the Discovery pipeline, saving front end time and dollars and downstream patent life.




## Biology-Tuned Analytics

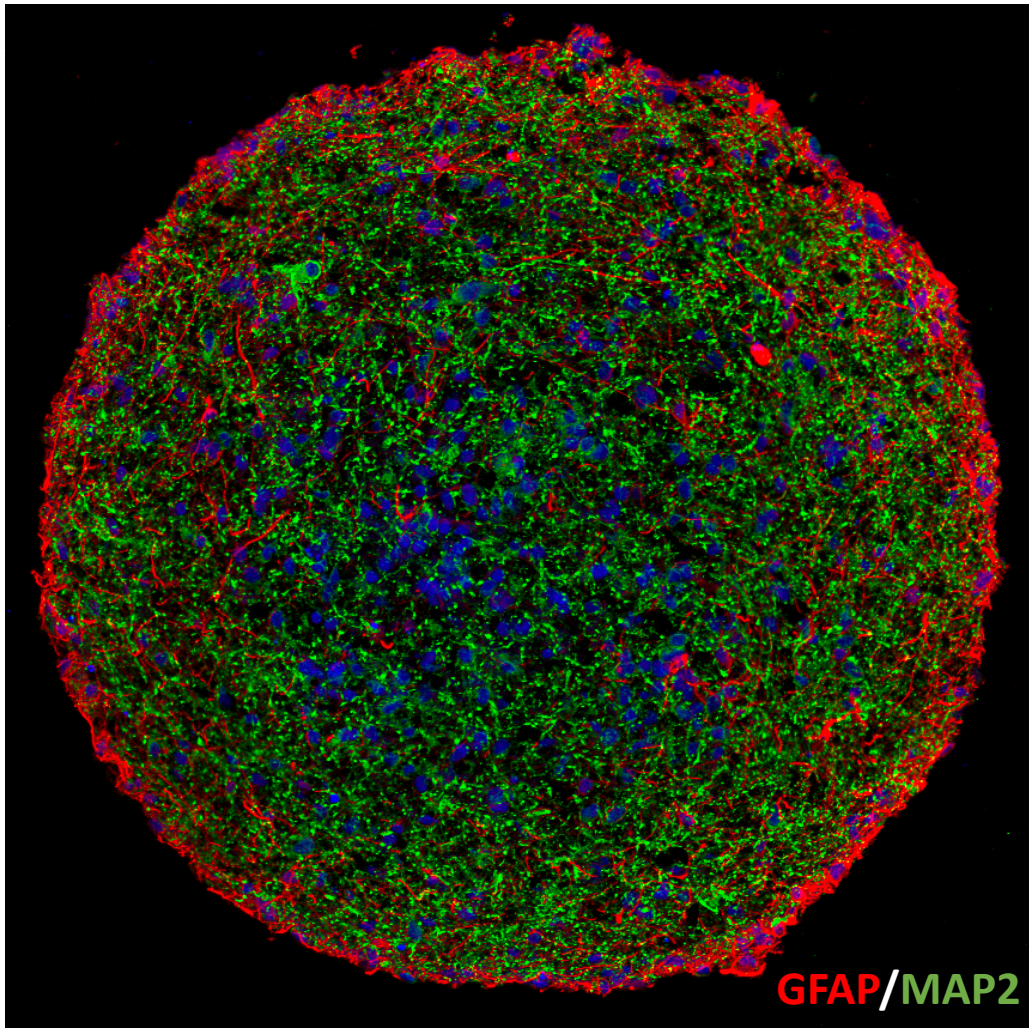
***How:***

Data science applied to functional biology matured to demonstrate key disease characteristics

***Why?***

More accurate, robust, and bias-free data extraction lays a stronger foundation for higher-order analytics such as hierarchical clustering, machine learning, and AI.

# microBrain® 3D



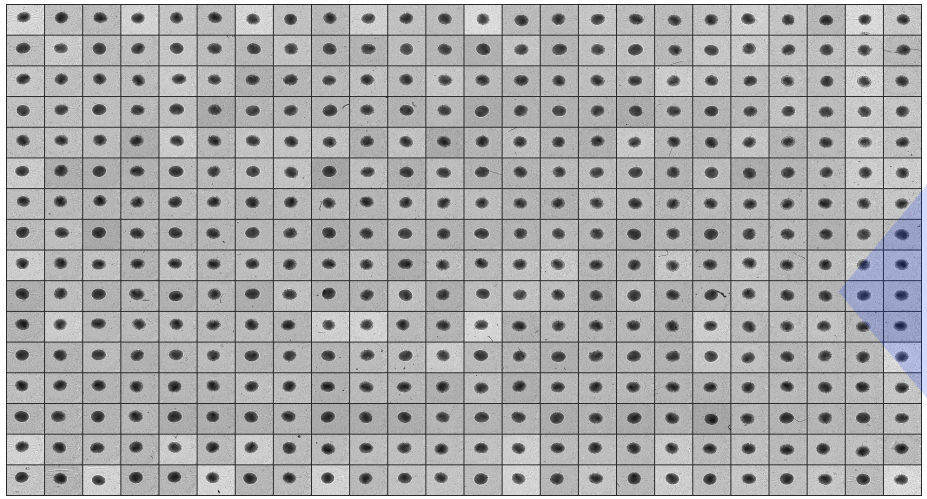

## Main Features

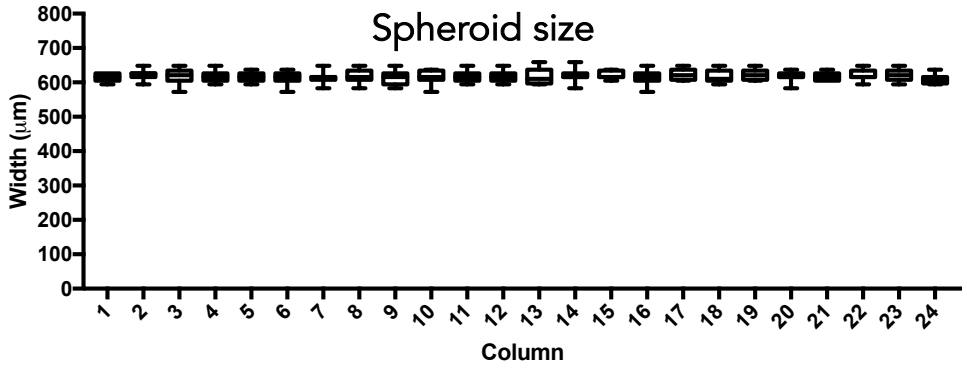
- Single donor human iPSC line
- Co-culture of neurons and astrocytes
- Key neuronal and astrocytes markers
- Spontaneous synchronized activity
- Amenable to HTS (384-well format)

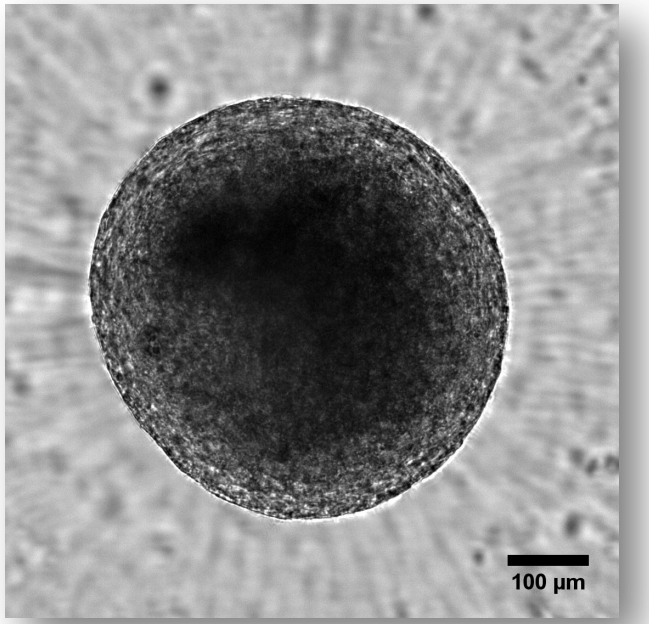
# Pre-plated spheroids

*Robust, easy to use, rapid workflow*

384w microBrain 3D plate






Features:

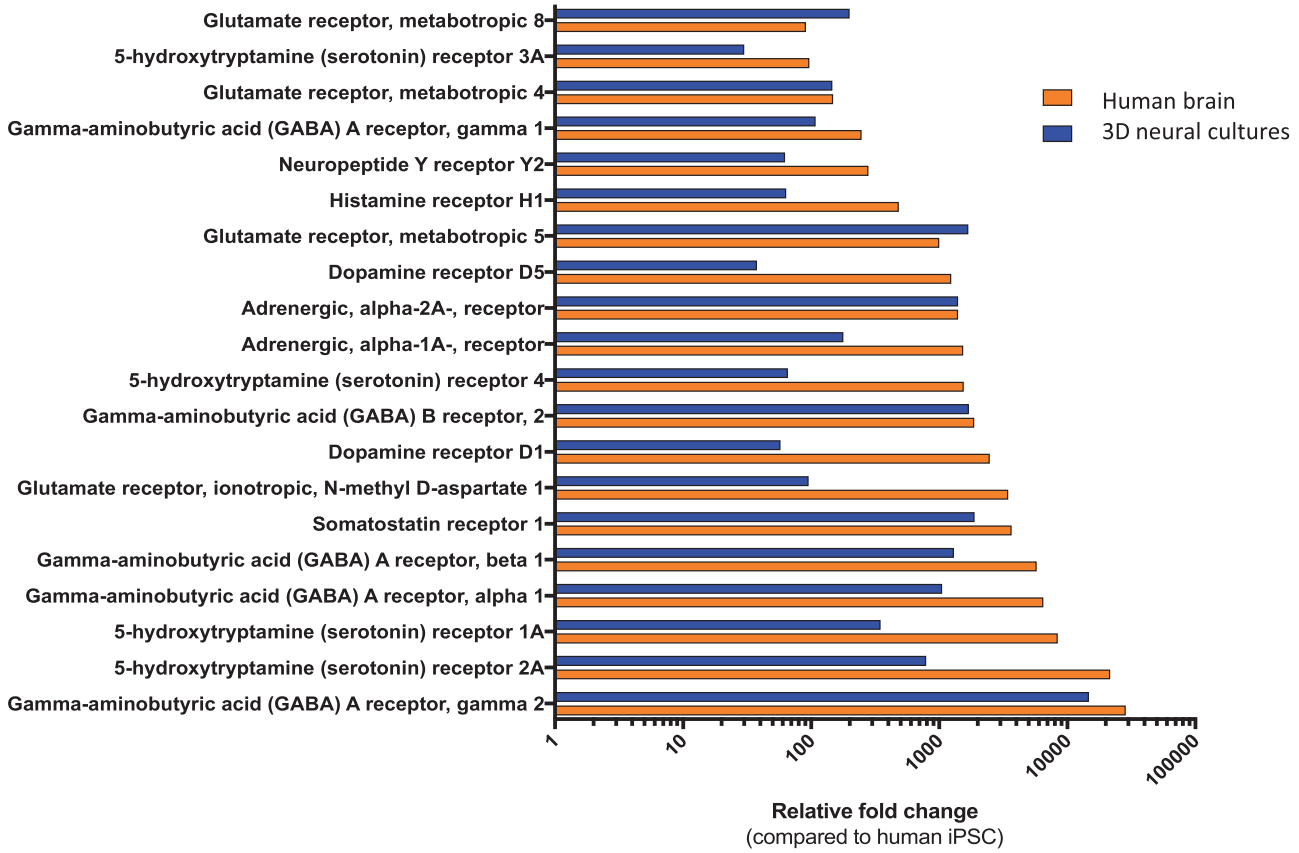
- 1 spheroid per well
- Consistent size across the plate
- *Able to use within one week of receipt*
- Amenable to acute and chronic (weeks) exposures

**Consistent, reproducible structure in ready-to-use format**

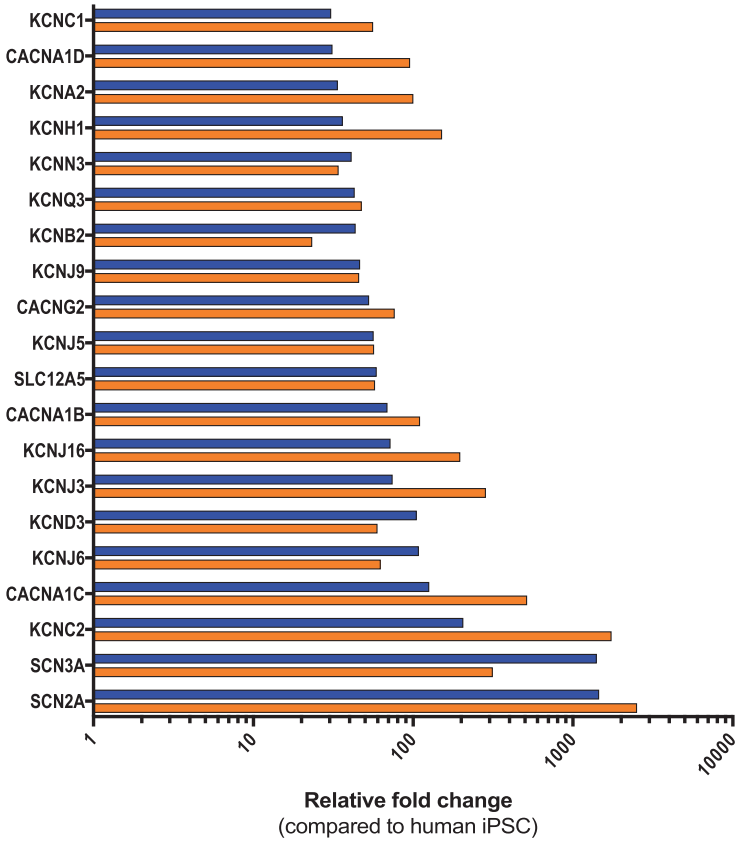
# microBrain 3D - Gene Expression

*Neurotransmitter receptor qPCR Array panel*

## Neurotransmitter receptor profiling

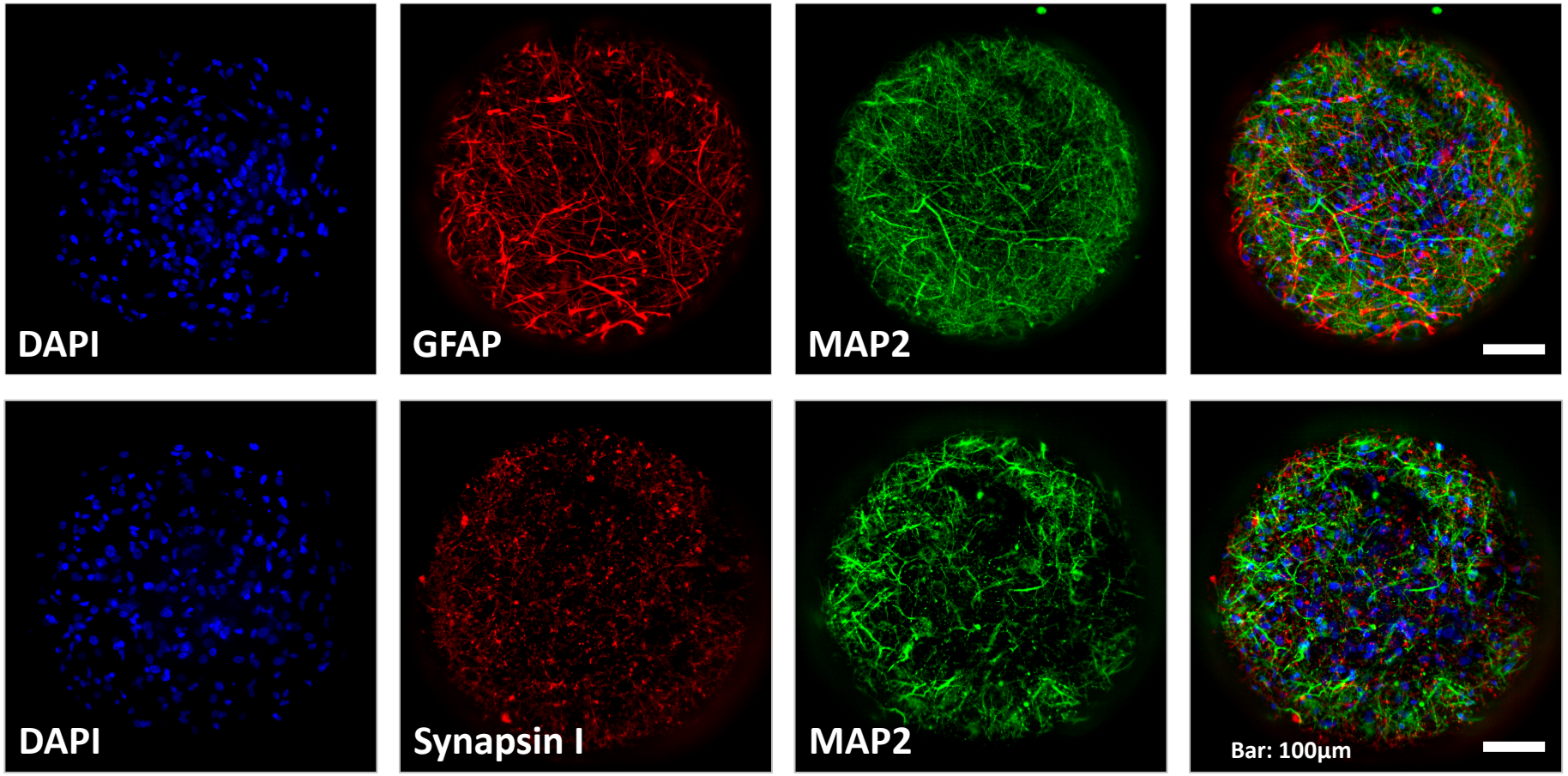



## Ion channel profiling




## Gene expression recapitulates native condition

# microBrain 3D - Relevant mixture of astrocytes and neurons

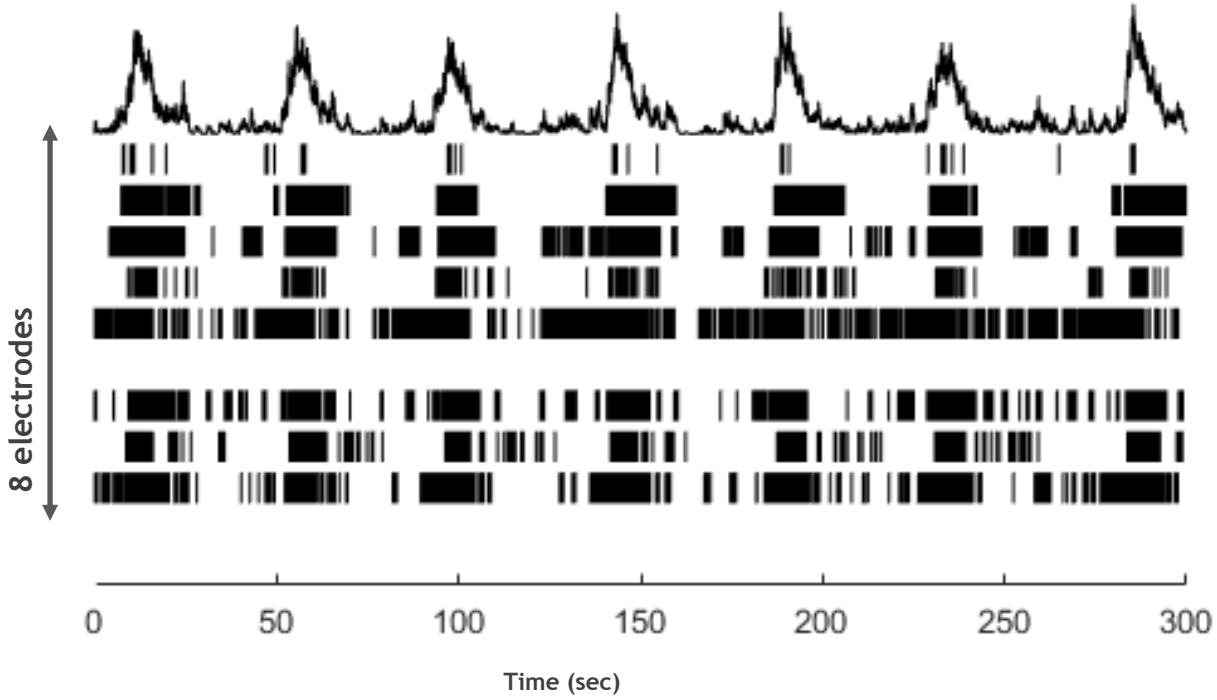



**Co-culture enables interrogation of complex biology**

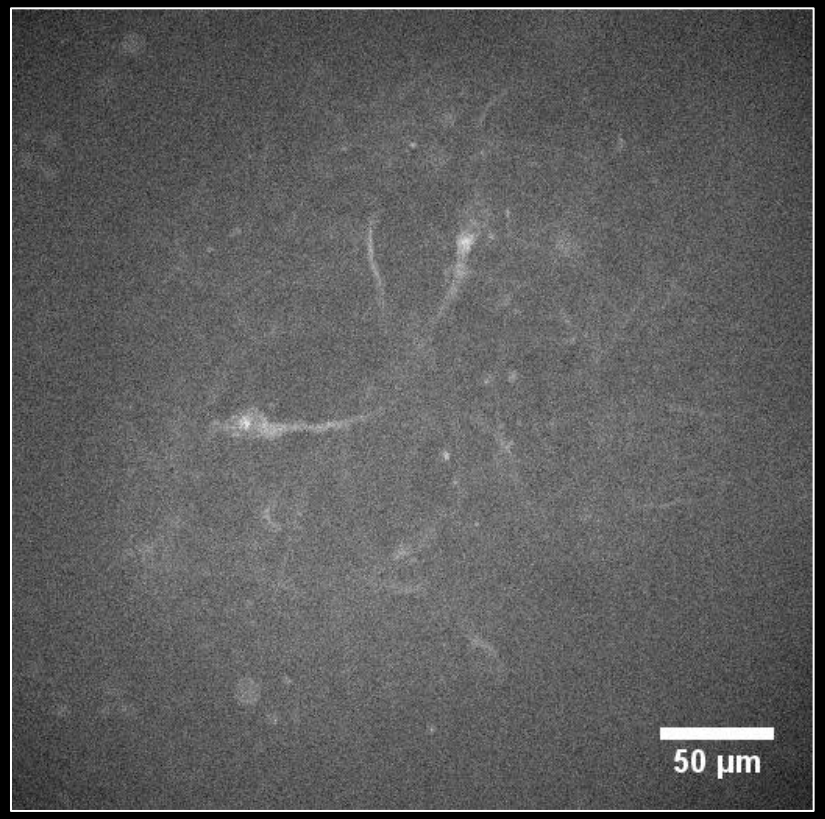
# microBrain 3D demonstrates spontaneous synchronized neural activity

- Spheroid electrical activity is confirmed via MEA recordings
- Neuronal origin of activity is confirmed via synapsin-targeted $Ca^{2+}$ measurements with high-speed CFM

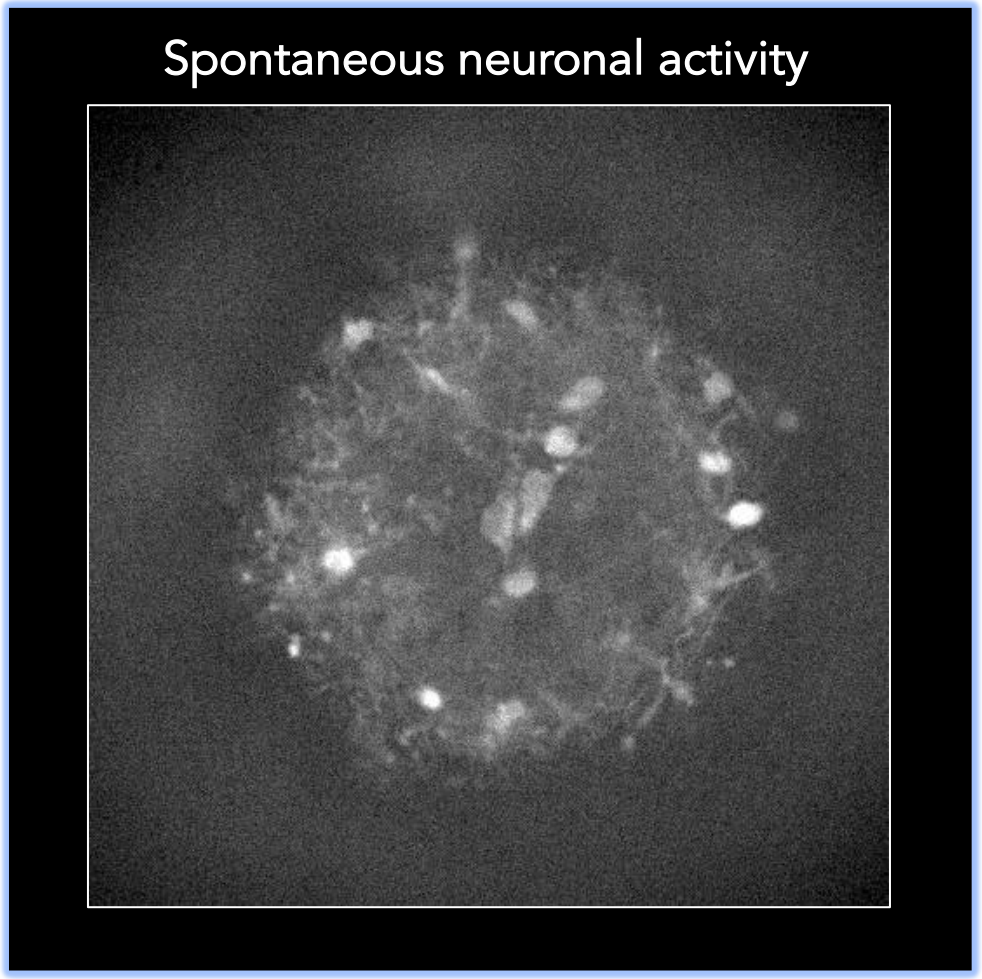
**Plated microBrain 3D on MEA (Maestro)**



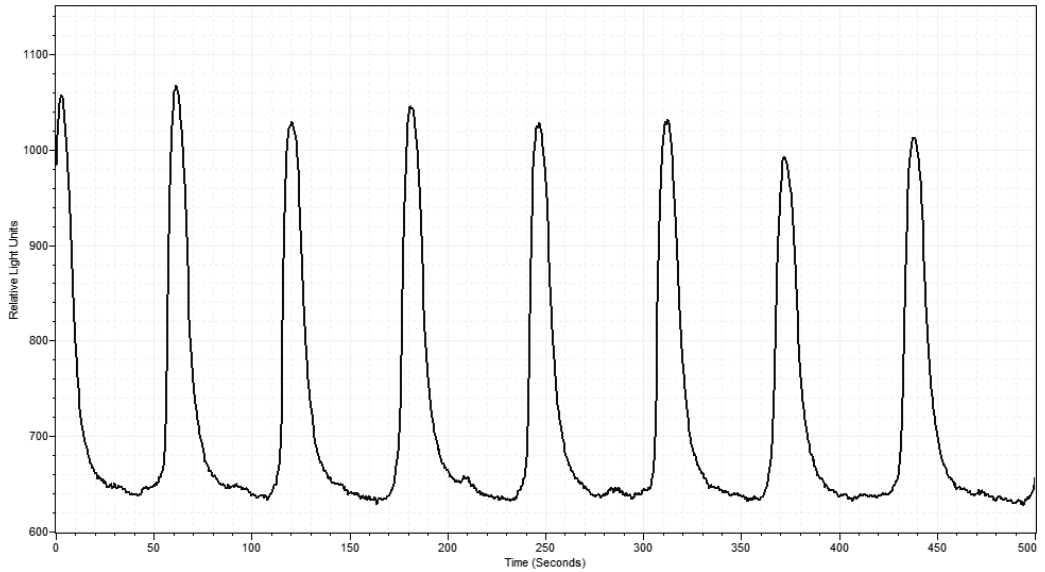

**Synapsin driven $Ca^{2+}$ indicator**

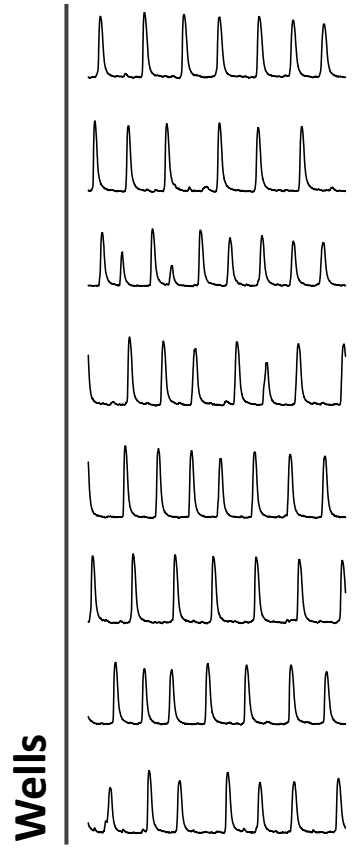


# microBrain 3D – Functional Output




- Spontaneous $Ca^{2+}$ oscillations
- Oscillations correspond to synchronized $Ca^{2+}$ waves



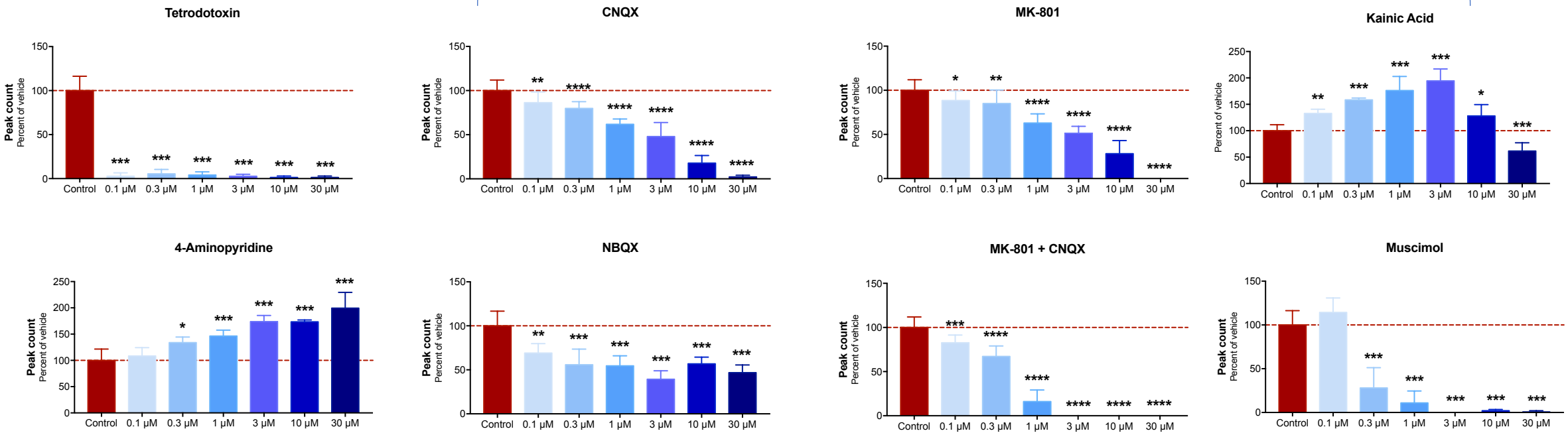




$Ca^{2+}$ oscillations provide a phenotypic readout of underlying functional neuronal activity

# microBrain 3D – Phenotypic Output Reflects Pathway Activity

## Ion Channels

## Complex Receptor Biology




Tetrodoxin: sodium channel blocker

4-Aminopyrydine: Non-selective $K_V$ channel blocker

CNQX: competitive AMPA/kainate receptor antagonist

NBQX: AMPA receptor antagonist

MK-801: NMDA receptor antagonist

Kainic Acid: Kainate receptor agonist

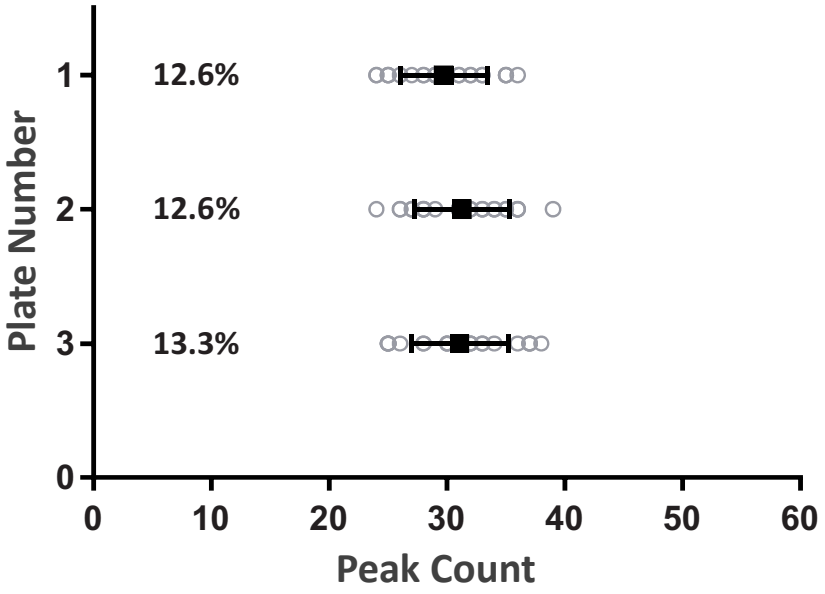
Muscimol: potent GABA agonist

**microBrain 3D provides a human system for physiologically relevant interrogation of druggable pathways**

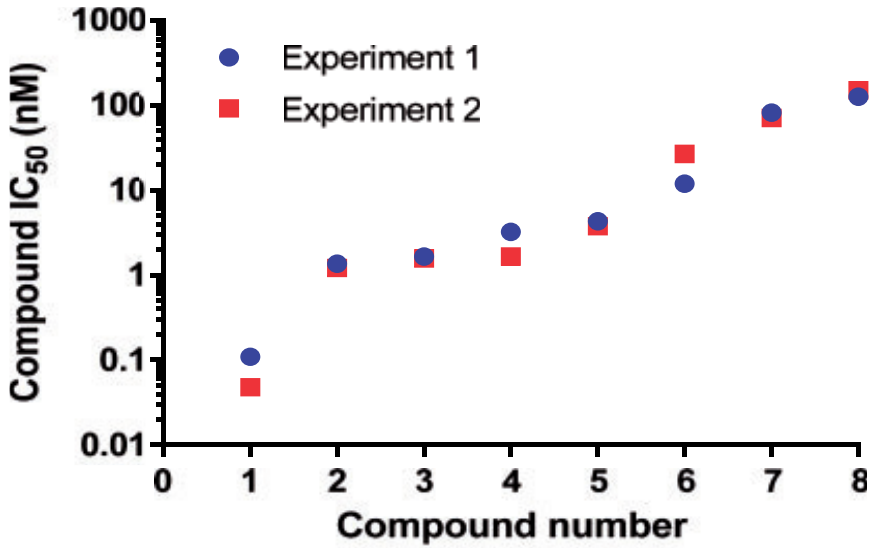
# microBrain 3D activity is reproducible

- **Inter-plate reproducibility of spontaneous neuronal activity**
- **Each experiment number correspond to a different microBrain 3D plate**

**Inter-plate baseline behavior**




**Inter-plate compound response comparison**




**Compound Legend:**

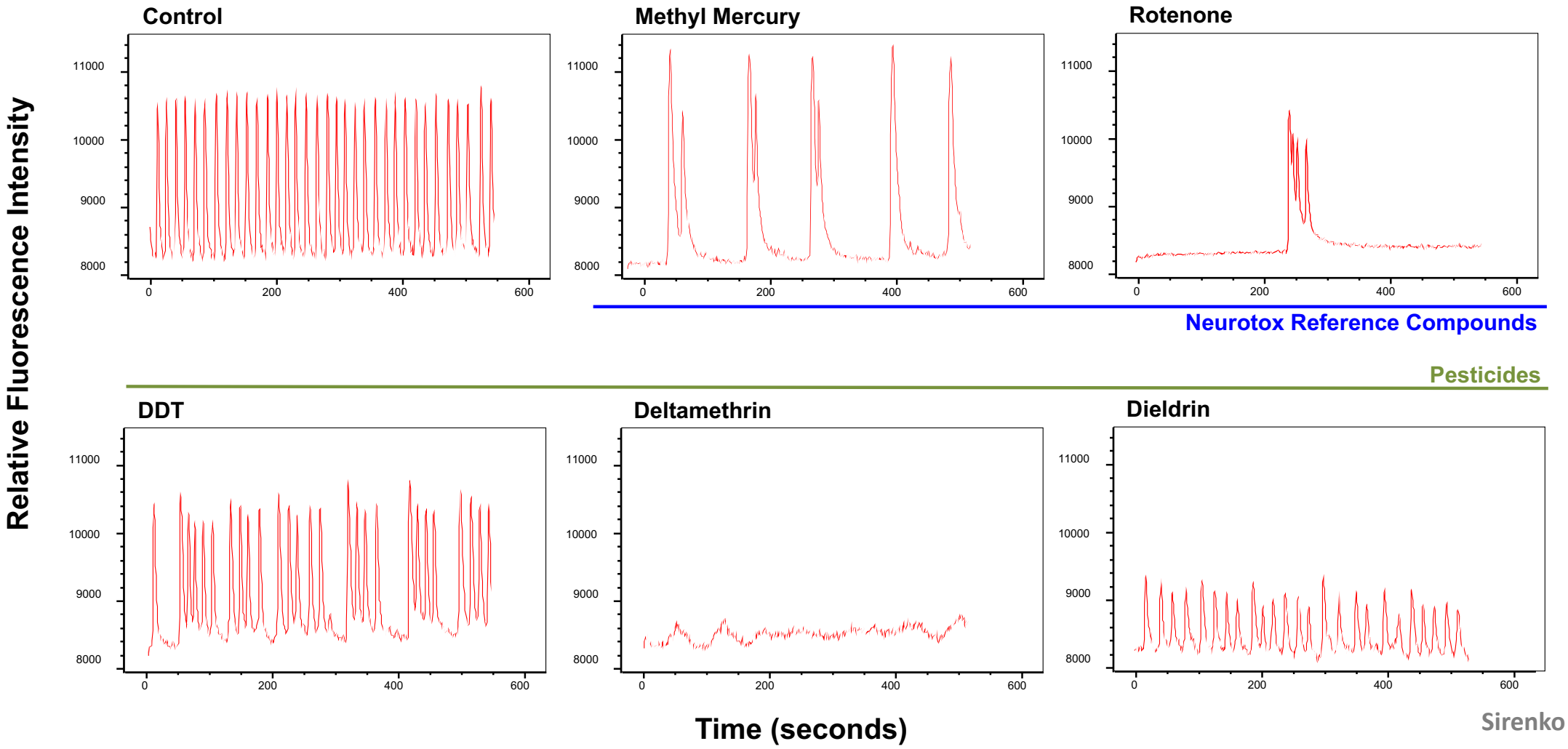
[1]Rotenone
[2]2-Ethylhexyl diphenyl phosphate
[3]Methyl mercuric (II) chloride
[4]Isodecyl diphenyl phosphate
[5]2,2',4,4',5-Pentabromodiphenyl ether
[6]2,2',4,4'-Tetrabromodiphenyl ether
[7]Lead (II) acetate trihydratre
[8] 1-Ethyl-3-methylimidazolium diethylphosphate

Sirenko et al. 2018

**Reproducible baseline and drug response behavior**

# Case Study 1 Toxicity Testing with  with microBrain 3D

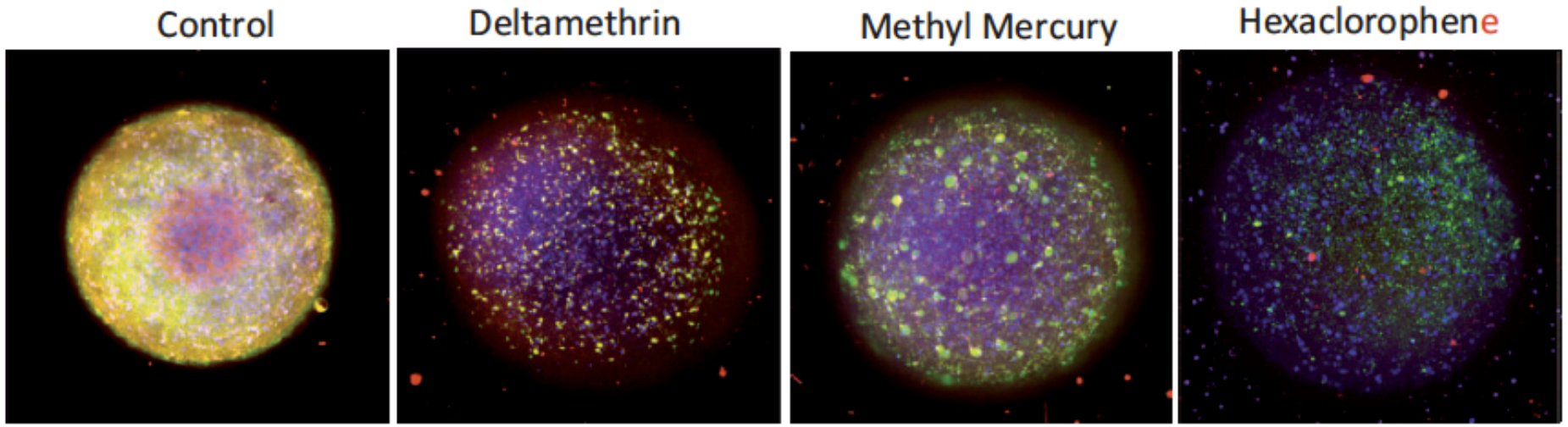
*Toxic compounds interfere with microBrain 3D activity*




Sirenko et al. 2019

# microBrain 3D in Toxicity Studies

*Endpoint specific and terminal toxicity detection*




Nuclei- Hoechst nuclear stain, blue
Viability- Calcein AM, green
Mitochondria- MitoTracker Orange, red

***Sirenko et al., 2019***

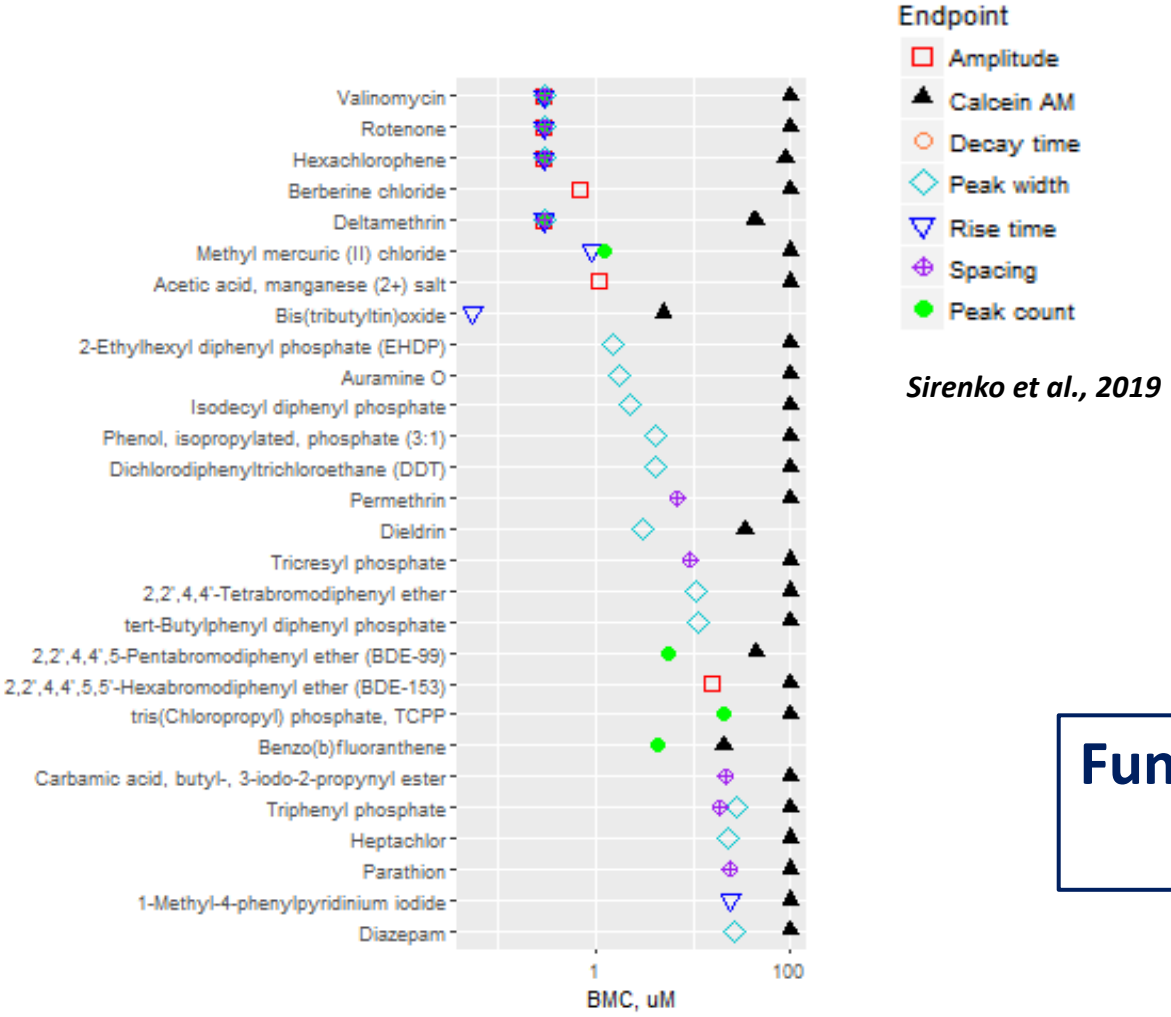
- **Mitochondrial function is assessed with MitoTracker Orange**
- **Viability is assessed with Calcein AM**

- **Control spheroids show extensive overlap of mitochondrial and viability markers (yellow-brown)**
- **Compound incubation shows loss of both mitochondrial function and loss of viability**

# Case Study: Readout Sensitivity

*Chemical library from NTP demonstrates the sensitivity of functional readouts*

**BMC concentrations for select compounds**




*Sirenko et al., 2019*

- **microBrain 3D spheroids were exposed to an NTP library of diverse chemicals**
- **$Ca^{2+}$ oscillation endpoints were compared to endpoint viability assay**
- **Bench Mark Concentration (BMC) were calculated across endpoints**

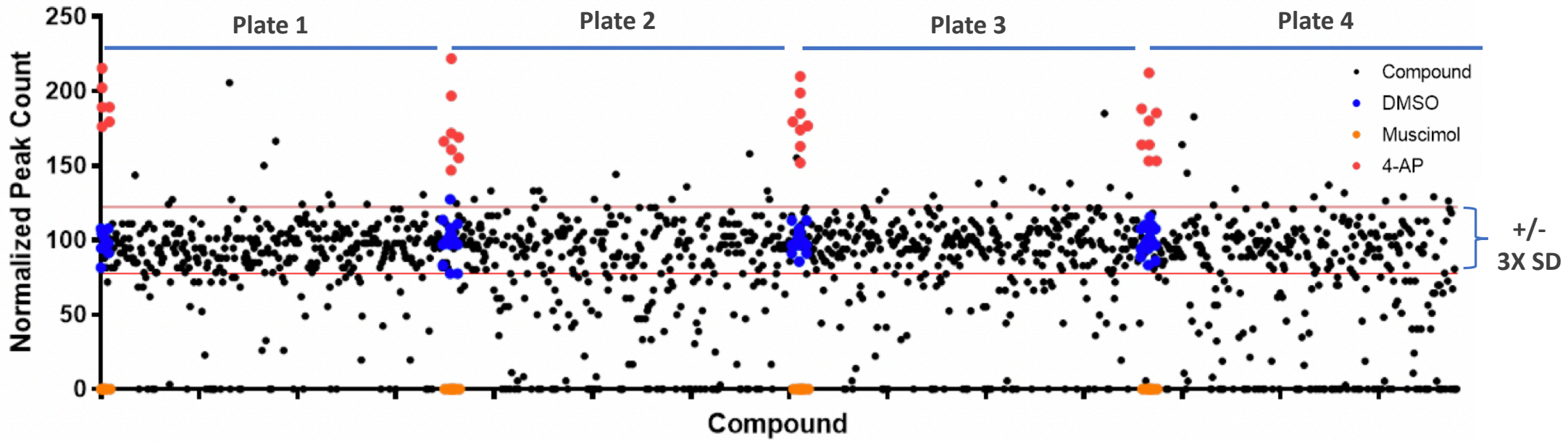
**Functional endpoints are more sensitive than terminal assay endpoints**

# Case Study 2: Screening with microBrain 3D

*SelleckChem and Lopac[1280]*

## Example Screen

- ✓ FDA Approved Compound library (SelleckChem), 10µM single dose, 384 well format
- ✓ Bidirectional modulation; increased and decreased activity
- ✓ Reproducible control responses with large assay window enables stringent hit criteria

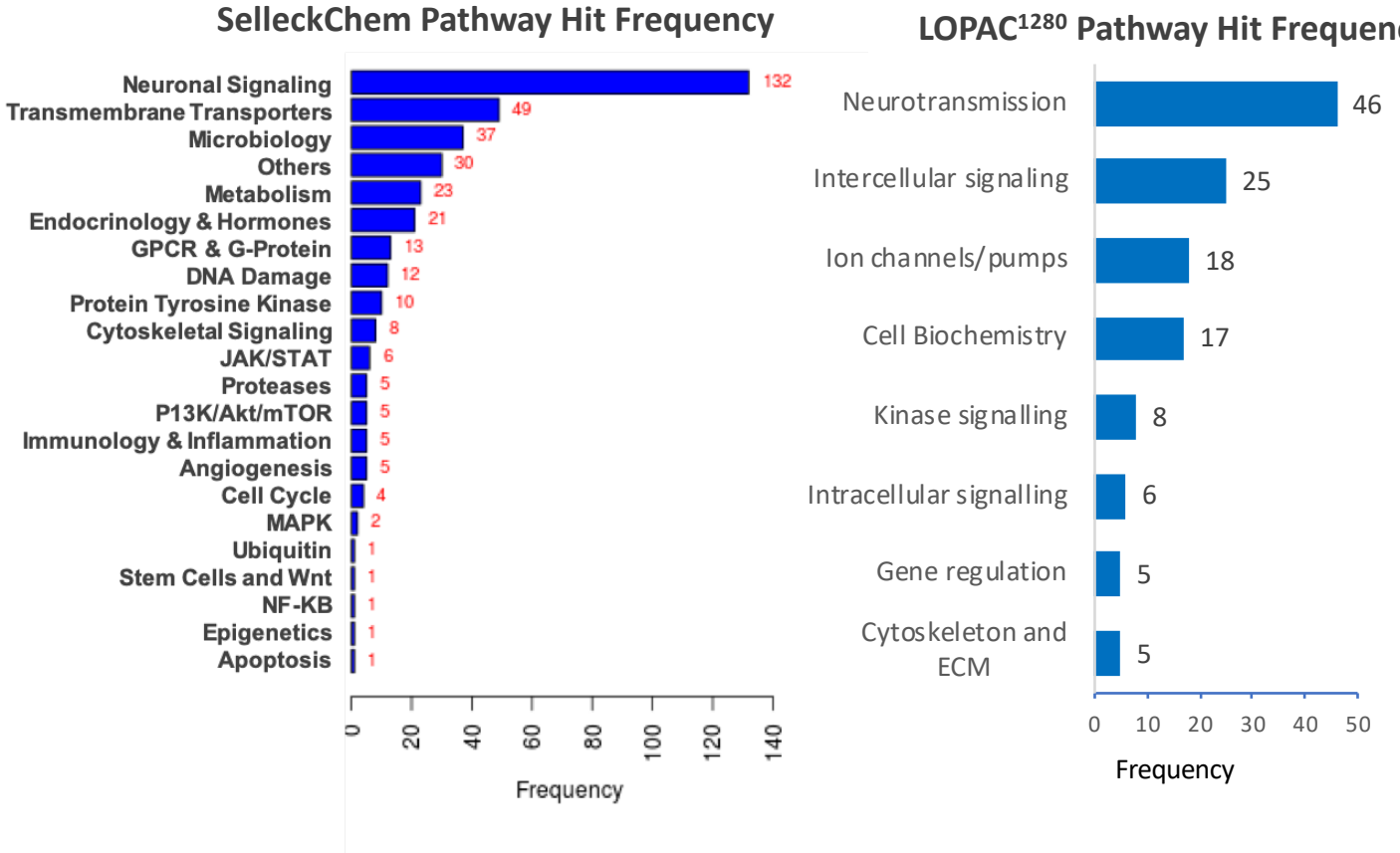



microBrain 3D provides a robust, easy to use screenable platform for interrogating human biology

# Screening with microBrain 3D

*Broad-based and granular interrogation*

## Identifying hits across classes







**Synaptic Transmission**
- Glutamatergic, GABAergic, Cholinergic, Serotonergic, Dopaminergic

**Ion Channels and Transporters**
- $Na^+$, $K^+$, $Ca^{2+}$, ATPases

**Adrenergic and Adenosine signaling**

**Neurological Disorders**
- Convulsions, Anxiety, Depression, Addiction

**Cell Biology**
- Homeostasis, Cytoskeleton, Kinases, 2nd messengers, etc.

microBrain 3D enables drug discovery and interrogation across a broad base of pathways and classes

# Screening with microBrain 3D

*Examining intra-family specificity and practical implementation*

## Opioid Receptor Hits from LOPAC$^{1280}$ Screen

| Compound Name | Compound Class | Action | Receptor Selectivity | % Inhibition (Peak Frequency) | % Inhibition (Avg. Peak Amplitude) |
|---|---|---|---|---|---|
| | | | | microBrain-3D | microBrain-3D |
| Loperamide hydrochloride | Opioid | Ligand (Ag) | (mu) | 85.1 | -36.5 |
| (-)-trans-(1S,2S)-U-50488 hydrochloride | Opioid | Agonist | kappa | 30.5 | -5.8 |
| U-62066 | Opioid | Agonist | kappa | 29.7 | -11.8 |
| ICI 204,448 hydrochloride | Opioid | Agonist | kappa | 27.4 | -35.4 |
| GR-89696 fumarate | Opioid | Agonist | kappa | 19.6 | 24.5 |
| (±) trans-U-50488 methanesulfonate | Opioid | Agonist | kappa | 19.2 | 25.5 |
| Carbetapentane citrate | Opioid | Ligand | sigma1 | 16.5 | -1.5 |
| (+)-Cyclazocine | Opioid | Antagonist | (?) | 13.0 | 28.0 |
| SNC80 | Opioid | Agonist | delta | 2.6 | 1.4 |
| Naltrexone hydrochloride | Opioid | Antagonist | (?) | 0.1 | -35.4 |
| PRE-084 | Opioid | Agonist | sigma1 | -1.8 | 0.5 |
| L-687,384 hydrochloride | Opioid | Agonist | sigma1 | -3.4 | 16.2 |
| Noscapine hydrchloride | Opioid | Ligand (Ag) | (sigma) | -5.4 | 25.8 |
| U-69593 | Opioid | Agonist | kappa | -7.6 | 11.4 |
| Naloxone hydrochloride | Opioid | Antagonist | (?) | -11.2 | 1.1 |
| Naltrindole hydrochloride | Opioid | Antagonist | delta | -17.8 | 31.6 |

Granular dissection of intra-family specificity

## StemoniX – NCATS CRA: NIH HEAL Initiative

*Helping to End Addiction Long-term*


Identify signature opioid response

- *Identify potentially addictive compounds*
- *Prioritize library and med chem efforts*
- *Prescreen drug candidates prior to more expensive, low-throughput experiments*

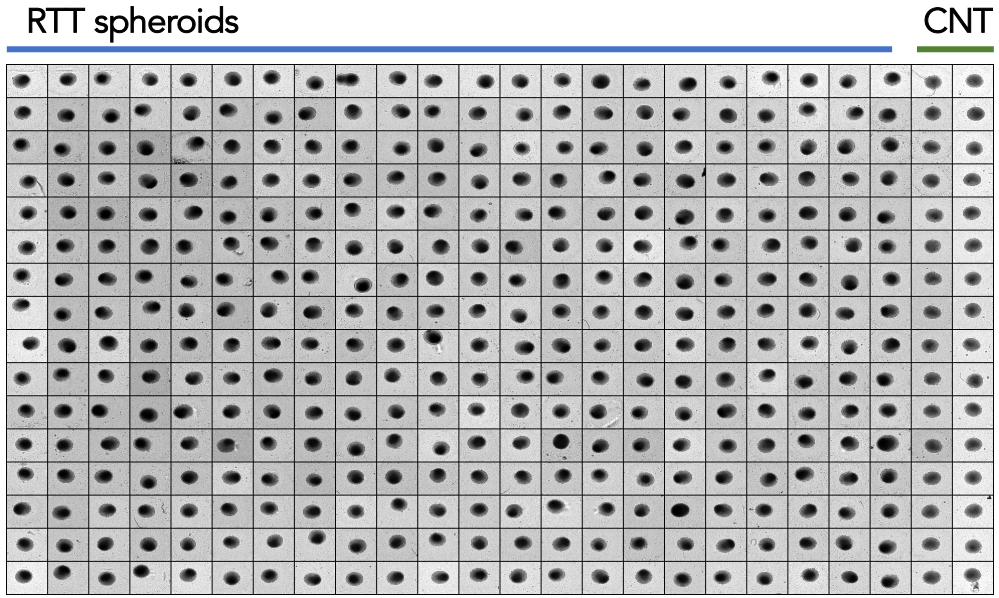
https://www.fiercebiotech.com/biotech/nih-taps-stemonix-s-organ-a-chip-for-opioid-addiction-research

# Case Study 3: Disease Modeling

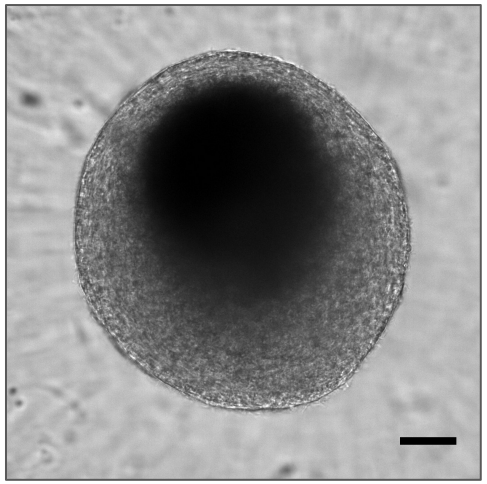
*Rett Syndrome using microBrain 3D*

- Affect primarily females (X-linked disease). Males are severely affected;
- Early onset (6-12 months);
- Autism-like behaviors, seizures and loss-of-language;
- Mutations in the MECP2 gene present in >95% of the cases;
- MeCP2 is a transcription regulator, binding throughout the whole genome;
- Disease Line: Rett Syndrome (RTT) hiPSC
  - Control line (WT): Parental control
  - Disease line (RTT): male, nonsense MeCP2 mutation (Q83X)

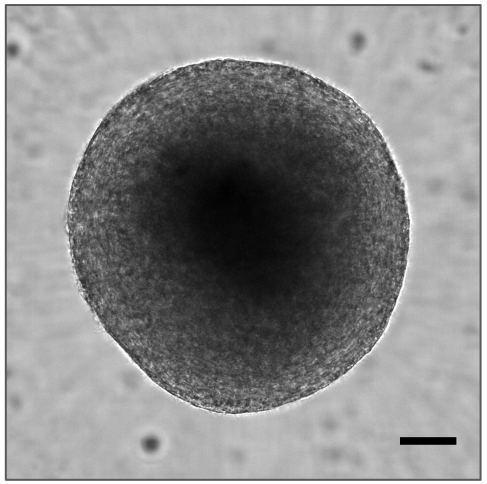
# microBrain 3D and microBrain 3D-RTT show good reproducibility



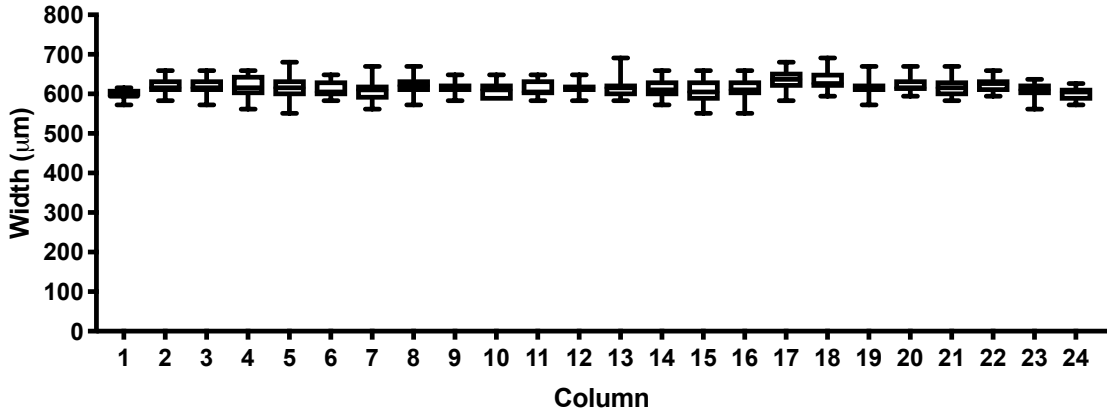

RTT microBrain spheroid



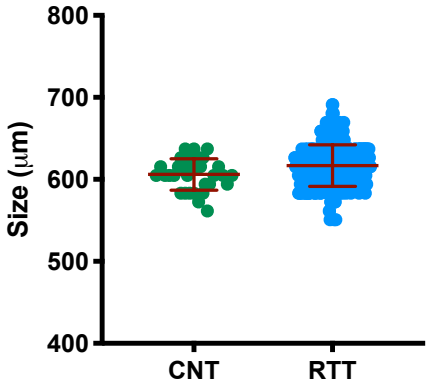
Control microBrain spheroid



Maximum and minimum distribution of spheroid size




Distribution of Width Values

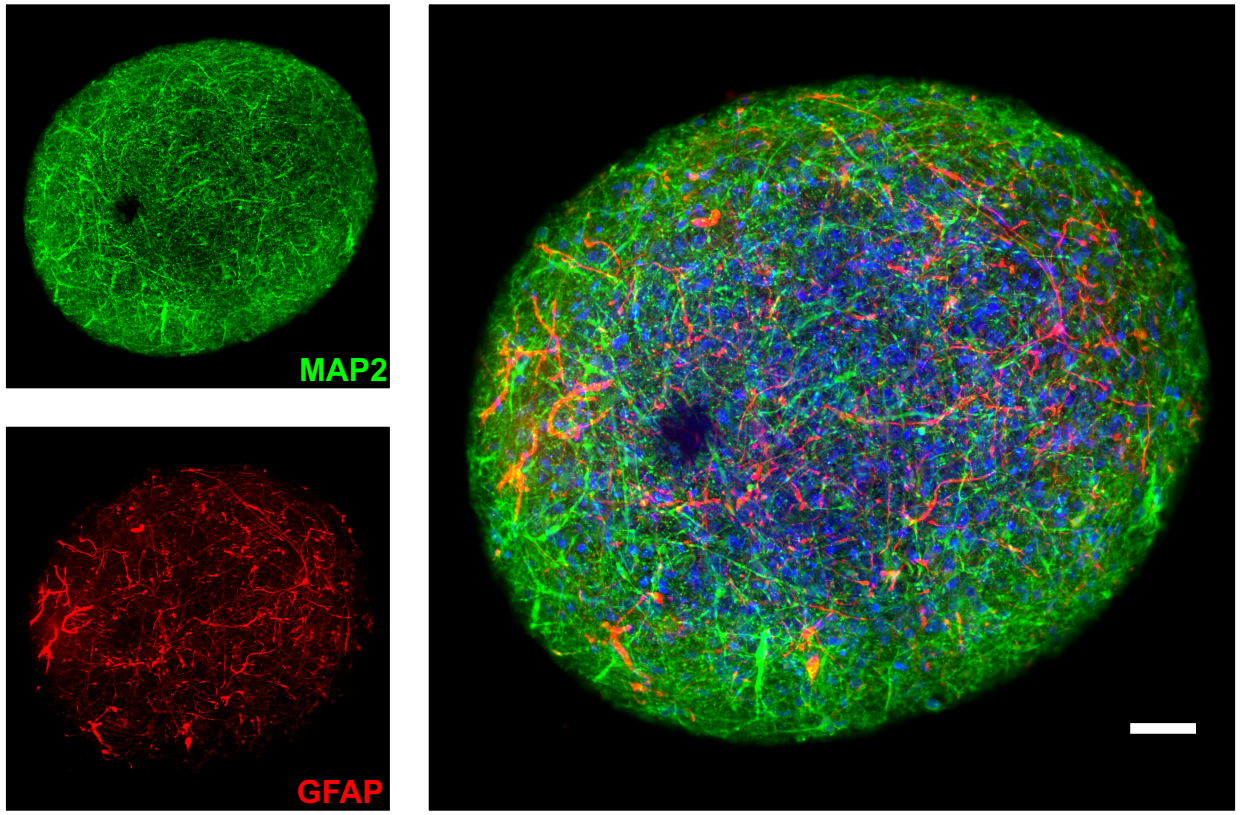



CoV: 3.16%. 4.10%

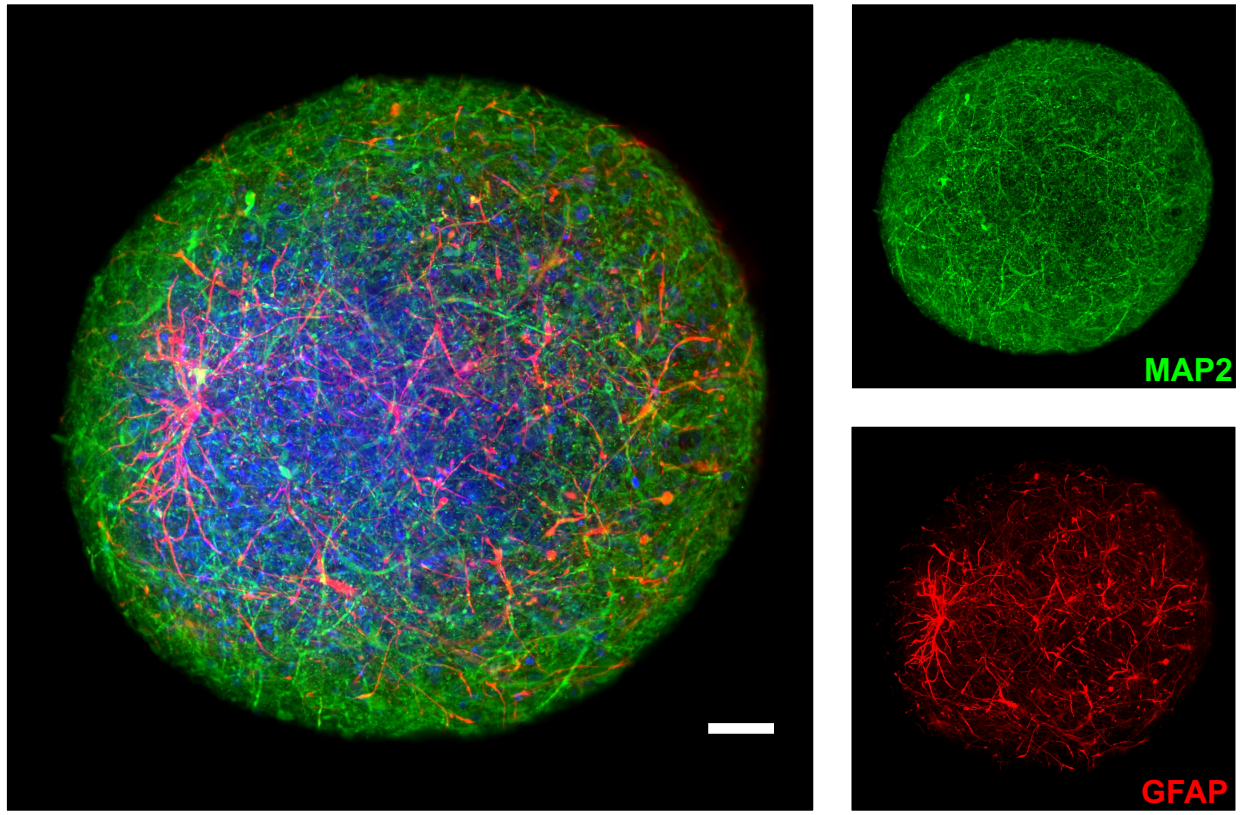
# microBrain 3D and microBrain 3D-RTT have similar cellular composition

- Neuronal and glial cell populations in 3D control and 3D RTT microBrain spheroids

Control microBrain 3D



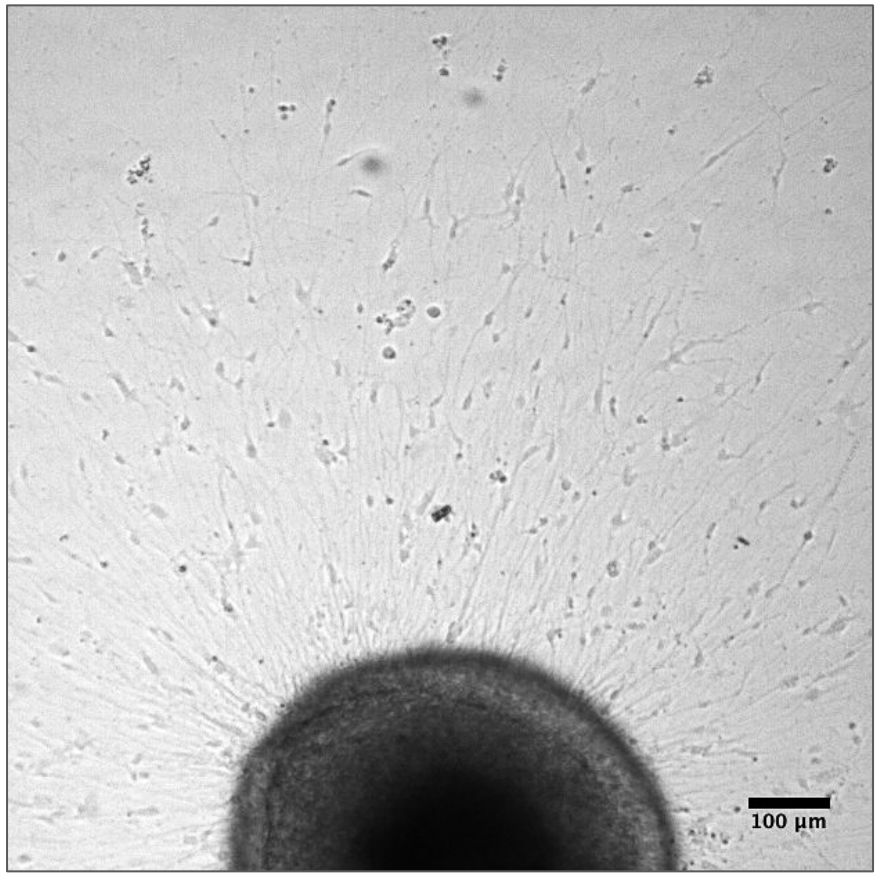

microBrain 3D-RTT



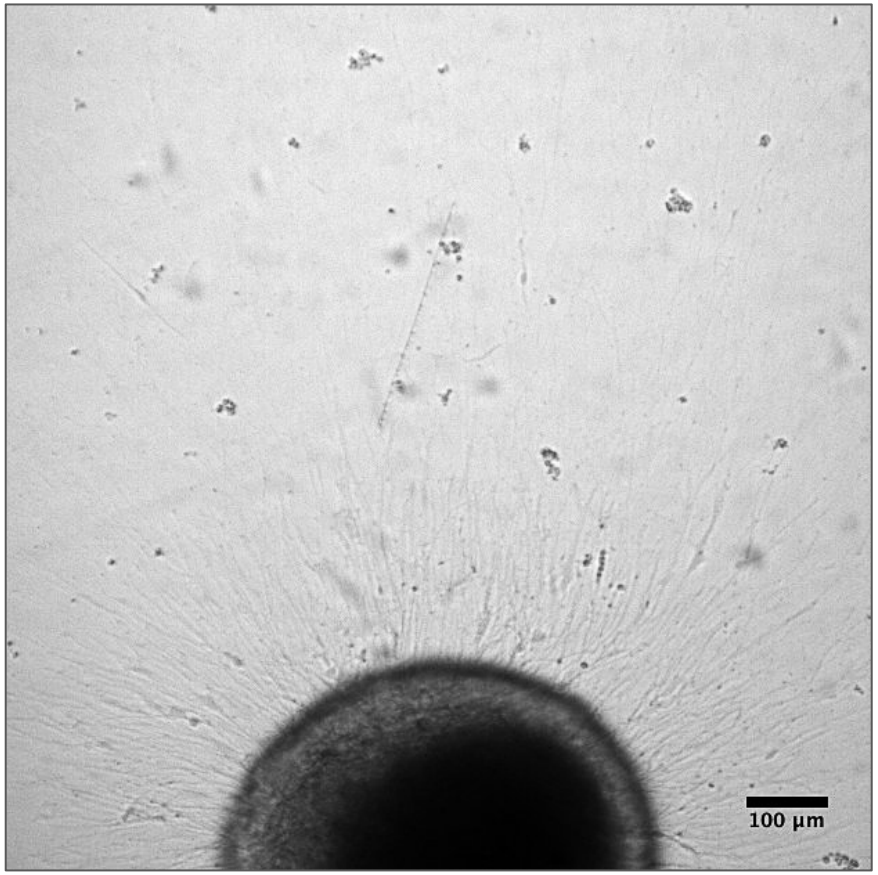
# microBrain 3D-RTT shows altered neurite outgrowth

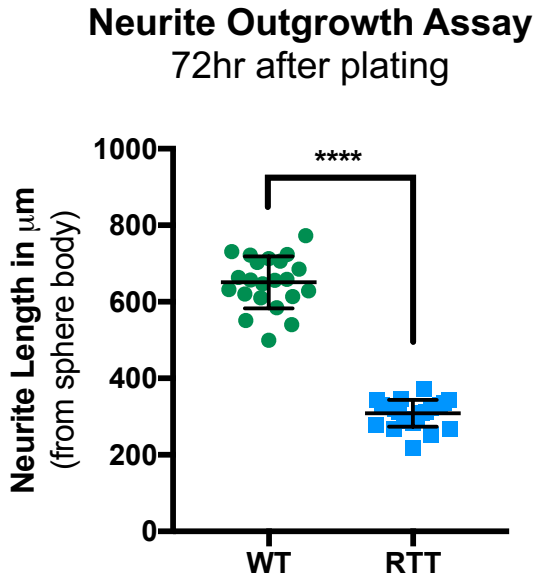
- microBrain 3D and microBrain 3D-RTT were plated on Matrigel coated plates
- Brightfield images were acquired 72hr after plating

**WT**



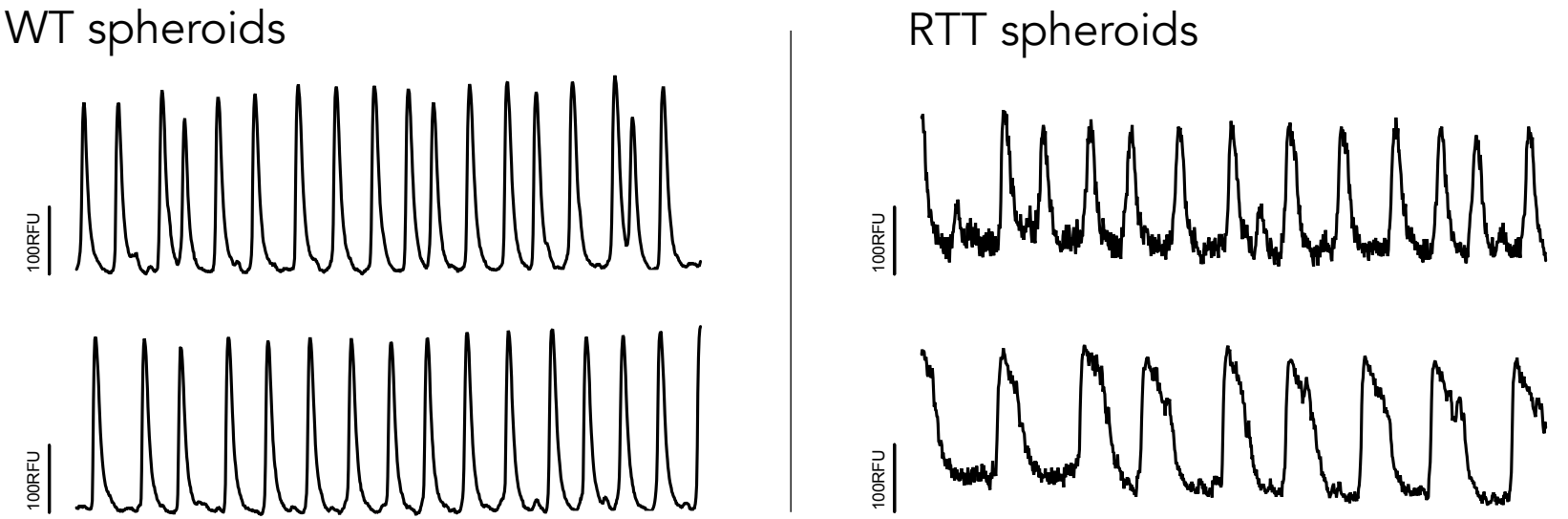

**RTT**

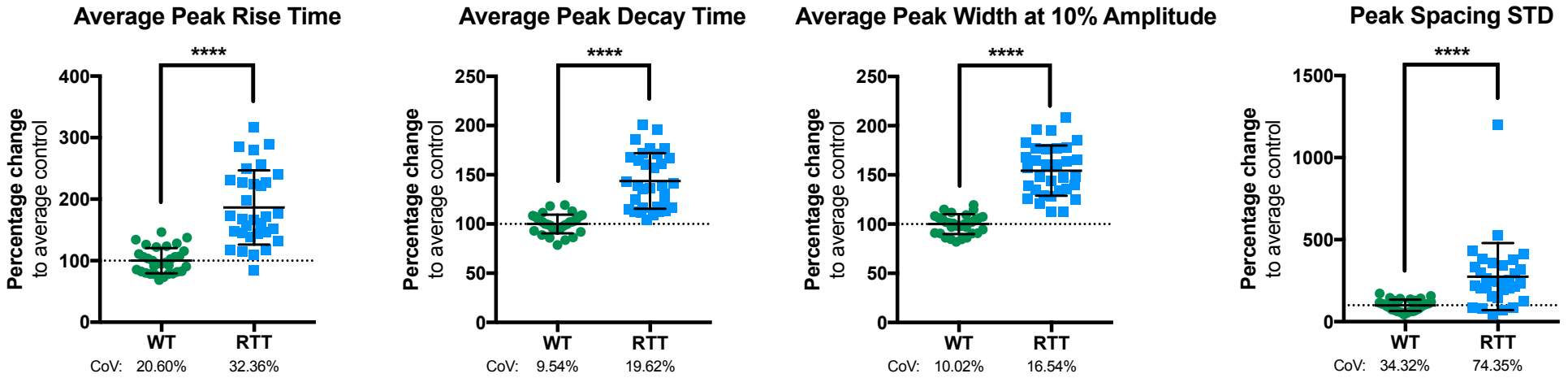


# microBrain 3D-RTT displays altered electrophysiology phenotype

- Calcium tracings from spontaneous calcium activity recorded using FLIPR Tetra®



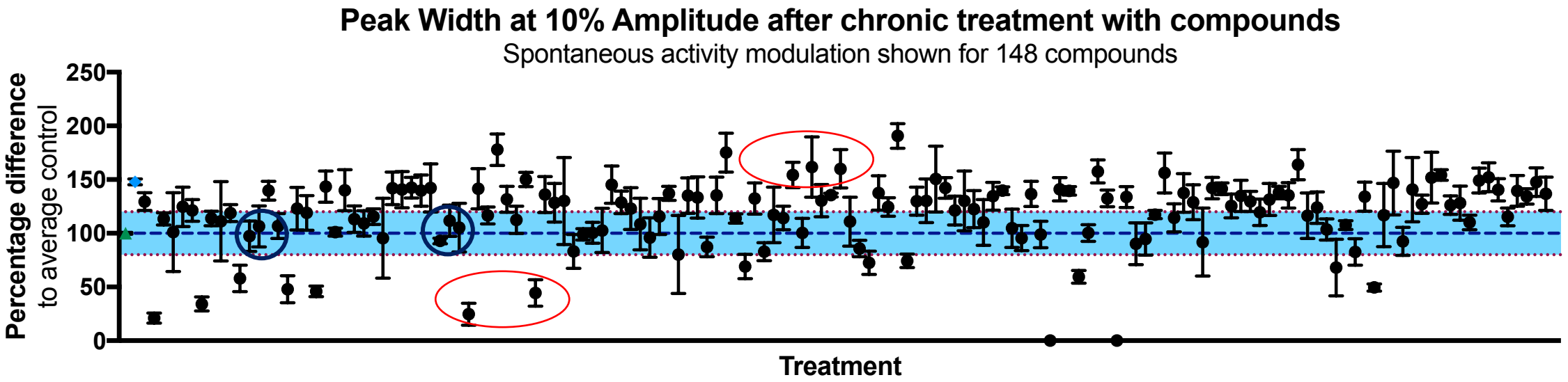



# Primary screening using the SMART Library

- SMART (Selected Molecular Agents for Rett Therapy) library of compounds
  - Vetted collection of compounds (using bioinformatics methods) tightly focused on Rett syndrome and its biological causes;
- Modulators for many key pathways misregulated in Rett Syndrome;
- Screening summary:
  - 296 compounds at 1 µM;
  - Four replicates on independent plates;
  - Vehicle control: DMSO;
  - Two weeks of chronic treatment.

# microBrain 3D-RTT identifies compounds that ‘rescue’ the iPSC-RTT phenotype

- Single endpoint results from screening the SMART library

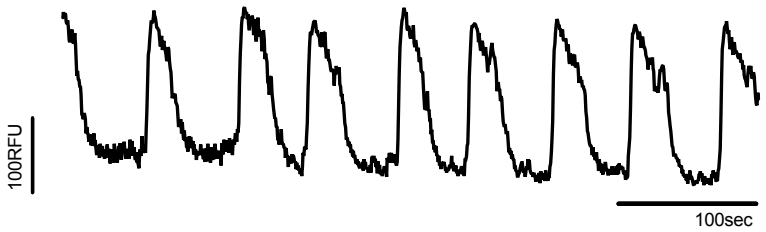


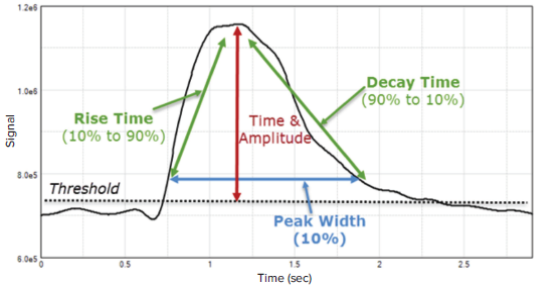

- Many compounds rescue the measured RTT phenotype
- Many compounds do not rescue the phenotype
- Data are messy……

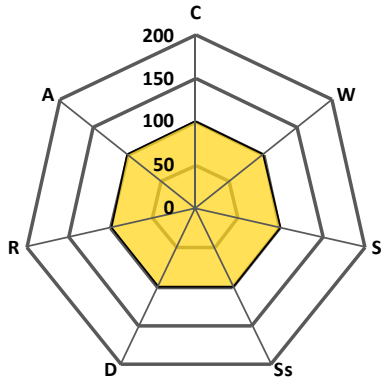
# Multiparametric Analysis 'Cleanly' Identifies Rescue

*Multiple parameters identify rescue while phenotypic screening identifies multiple target pathways*

## Functional readout is a parametric endpoint



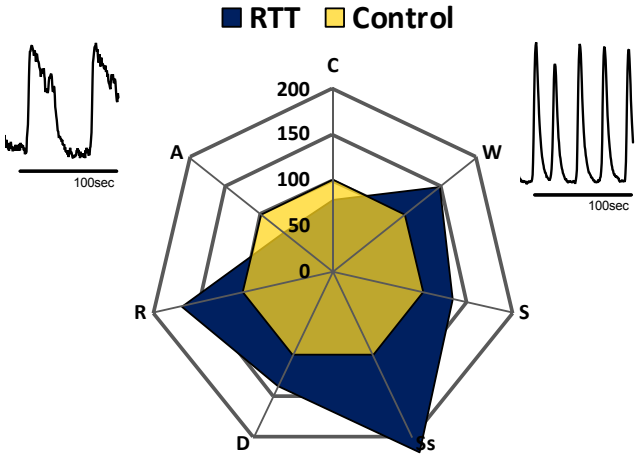




Radar plot enables multiparametric display for a phenotypic 'fingerprint'

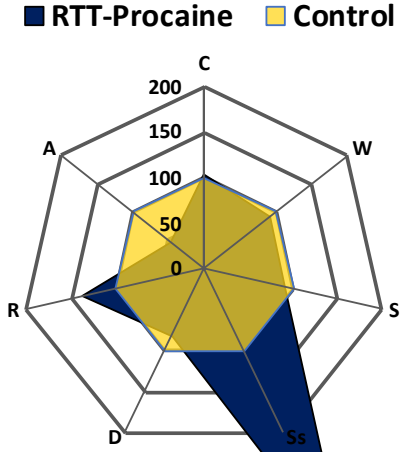
## Multiparametric analysis provides more clear representation of rescue

### No Treatment

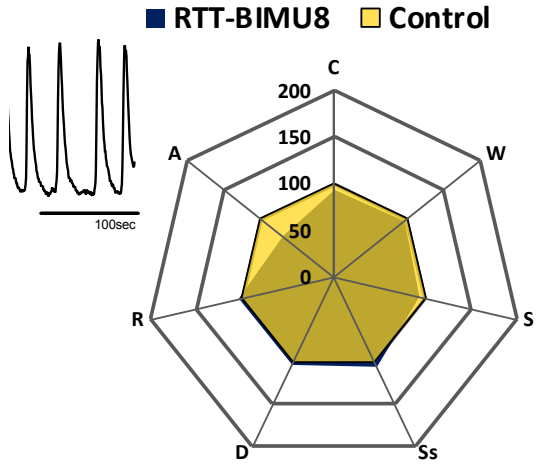



Visualize differences

### Partial Rescue

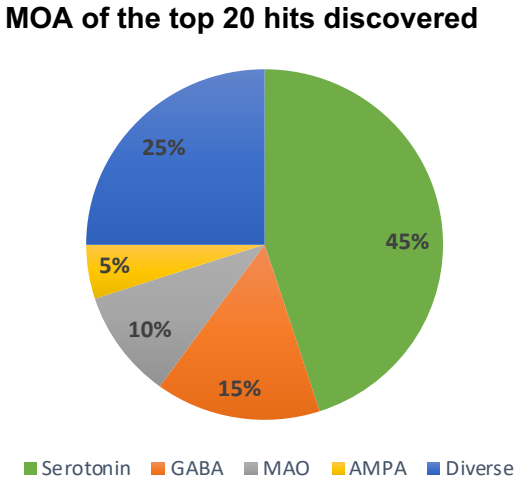



### Near-Complete Rescue




Visualize and stratify results

### Phenotypic Screening Identifies Multiple Target Pathways




Uncover multiple pathways

# Summary

- microBrain 3D - human-based tool for CNS modeling *in vitro*
  - ✓ HTP, relevant, complex neuronal culture system with a reduced workflow burden
- Functional and structural toxicity prioritization
- Can be used to screen diverse neurobiology endpoints
- Demonstrated ability for human disease modeling and target pathway identification