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High Throughput (HTP) Fluorescence Imaging with Electric Field Stimulation (EFS): Phenotyping Human iPSC-derived Cardiomyocytes and Neurons

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Oct 18, 2016

HAMAMATSU PHOTONICS K.K.

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- Applications on FDSS

Part 2 What is new

- iPSC based cell based assay
- High speed data acquisition on whole plate
- Whole plate electric field stimulation

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Inspex Inc.
47 Manning Road
Billerica, MA 01821, U.S.A.
Telephone: (1)978-667-5500

Universal Spectrum Corporation

- A leader in optoelectronic components and advanced detector systems
- Founded 1953, based in Hamamatsu City, Japan
- Worldwide sales and service organisation, 4000 Employees
- Revenue about US$1.2 B (2015)

Photon
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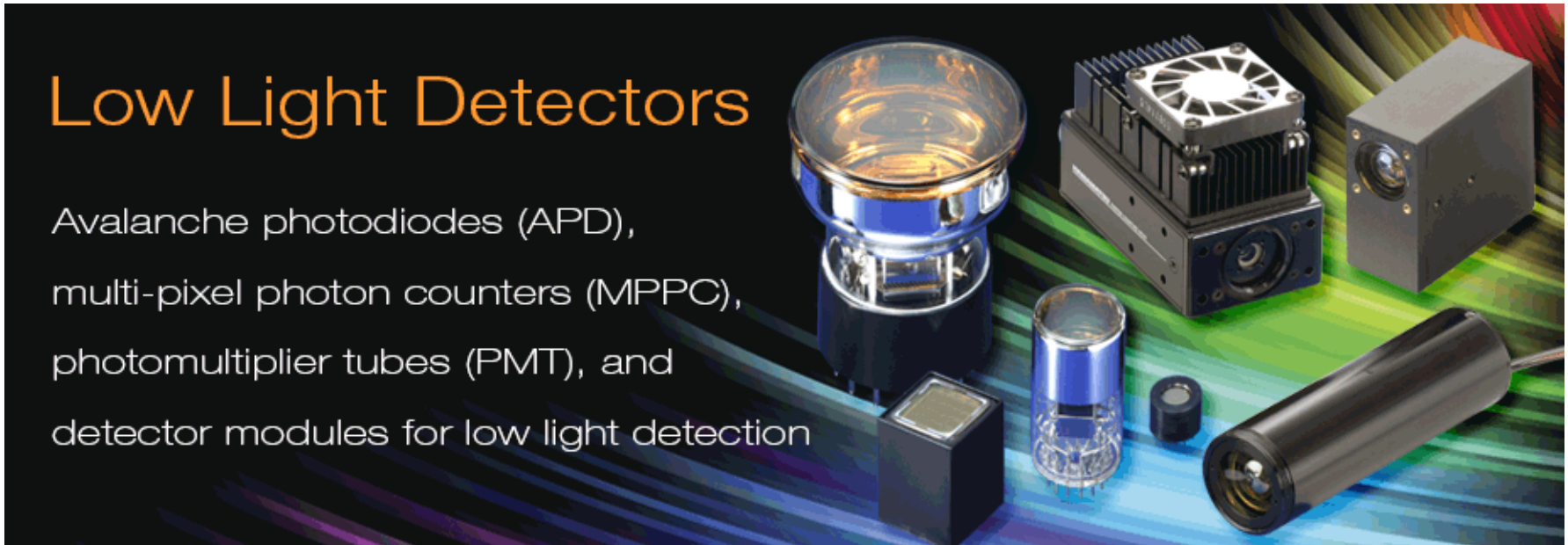
Hamamatsu's contribution to Nobel Prize in Physics in 2002 and 2015



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Hamamatsu PET center

Animal PET

Cyclotron

Perfusion
- [^{15}O]CO
- [^{15}O]CO2
- [^{15}O]O2
- [^{15}O]H2O

Dopamine
- [^{11}C]L-DOPA
- [^{11}C]DTBZ
- [^{11}C]SCH23390
- [^{11}C]NNC112
- [^{11}C]Raclopride
- [^{11}C]FLB457
- [^{11}C]NMPA
- [^{11}C]β-CFT
- [^{18}F]FE-CFT

Serotonin
- [^{11}C]5-HTP
- [^{11}C]WAY100635
- [^{18}F]MPPF
- [^{11}C]MDL100907
- [^{11}C]McN5652
- [^{11}C]DASB

Acetylcholine
- [^{11}C]4-MPB
- [^{11}C]3-MPB
- [^{11}C]3-EPB
- [^{11}C]3-PPB
- [^{11}C]5-Methyl-A85380
- [^{11}C]MP4A
- [^{11}C]HAPT
- [^{11}C]Me-SSR
- [^{11}C]Me-QAA

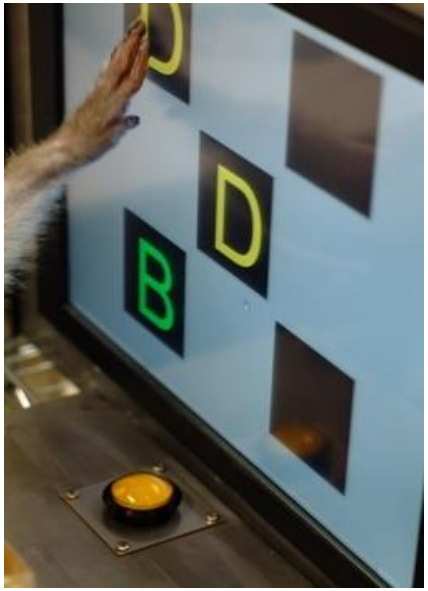
GABA/BZD
- [^{11}C]Ro15-1788
- [^{11}C]Ro15-4513
- [^{11}C]PK11195
- [^{11}C]Me-PK11195
- [^{11}C]Me-DAA116

Glutamate
- [^{11}C]Cyano-MK801
- [^{11}C]GMOM

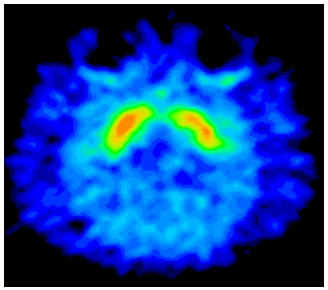
Sigma
- [^{11}C]SA4503
- [^{18}F]FM-SA4503

Metabolism
- [^{18}F]FDG
- [^{11}C]Methionin
- [^{11}C]D-CMT
- [^{18}F]D-FMT
- [^{11}C]Phenylalanine
- [^{11}C]Choline
- [^{18}F]FLT
- [^{76}Br]BFAU

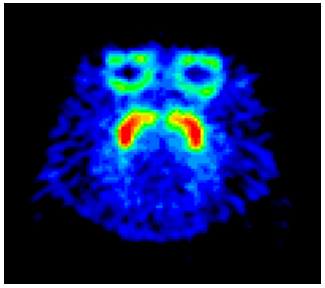
Others
- [^{11}C]PIB
- [^{11}C]FDDNP
- [^{11}C]Verapamil
- [^{18}F]FHBG
- [^{18}F]FBHB
- [^{18}F]FBMB
- [^{18}F]FIHB
- [^{18}F]FIMB
- [^{18}F]Lipids
- [^{18}F]Peptides
- [^{18}F]siRNA



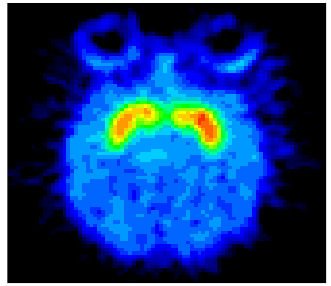
Conscious



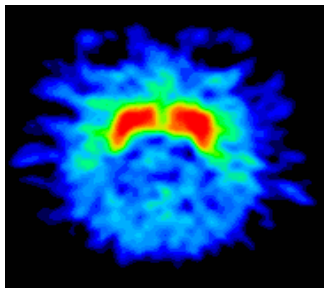
[^{11}C]SCH23390

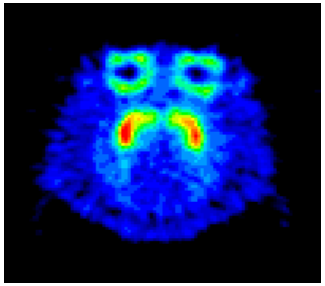


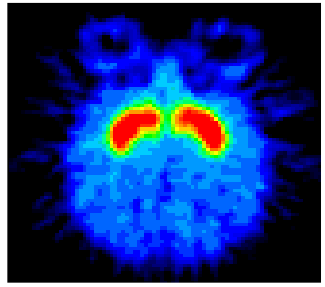
[^{11}C]Raclopride



[^{11}C]β-CFT

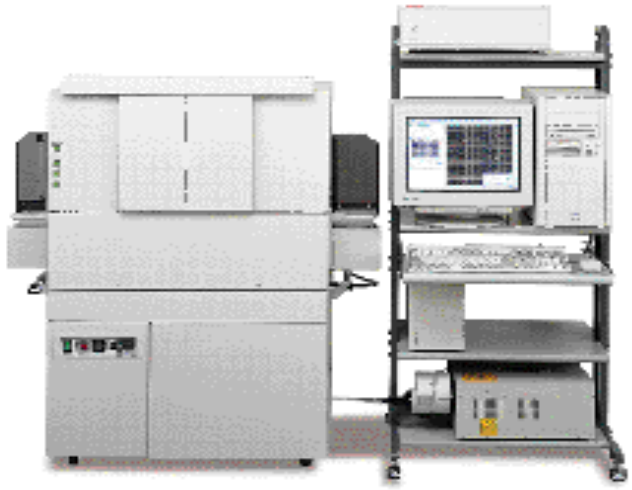






Ketamine anesthetized

FDSS system – Dispensing and Imaging

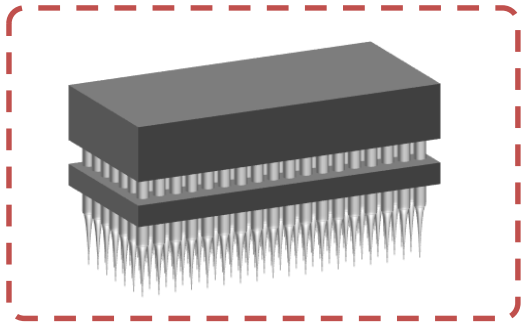


Kinetic or “flash”
fluorescence
or luminescence

FDSS μCell

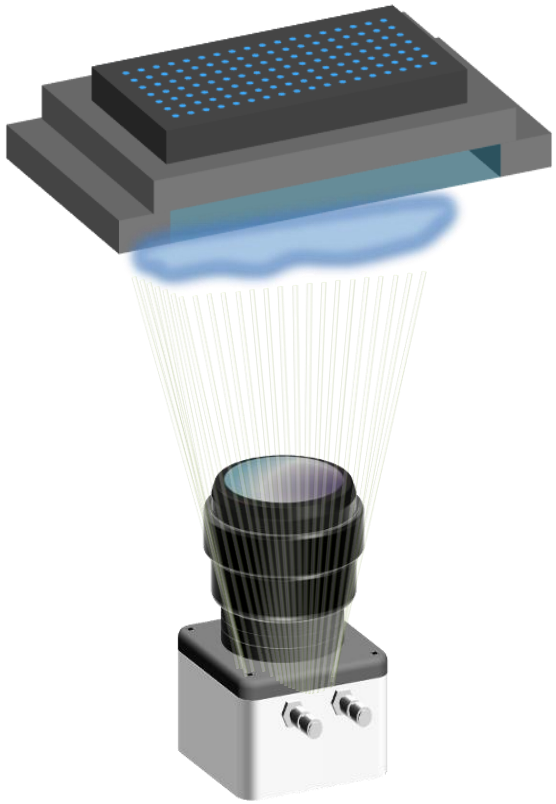
FDSS7000

What does Hamamatsu FDSS do?

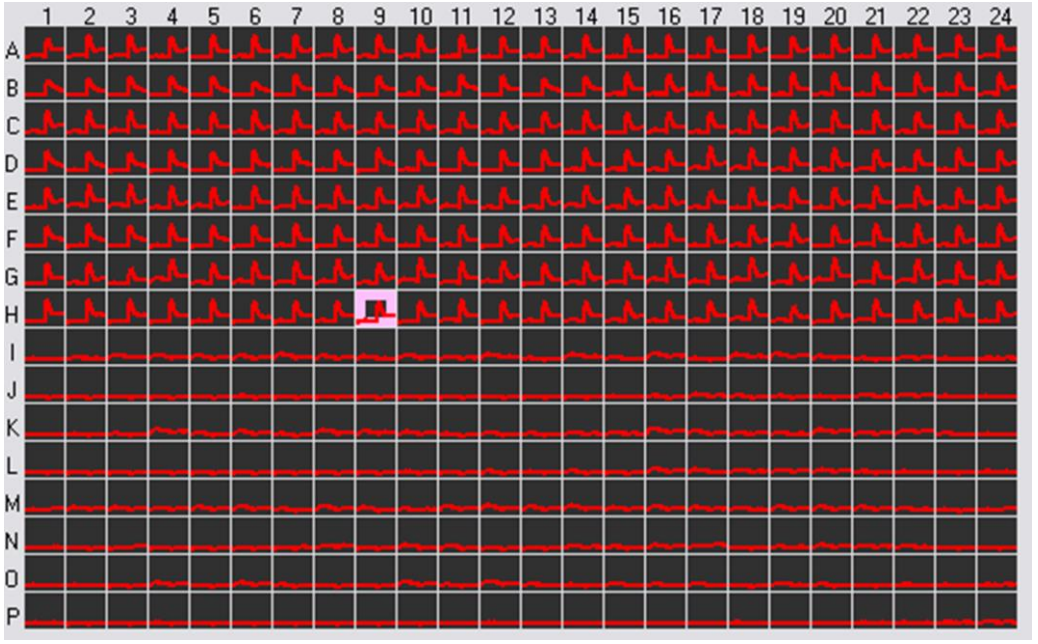


Add

96,384,1536 well s at a time



Microplate



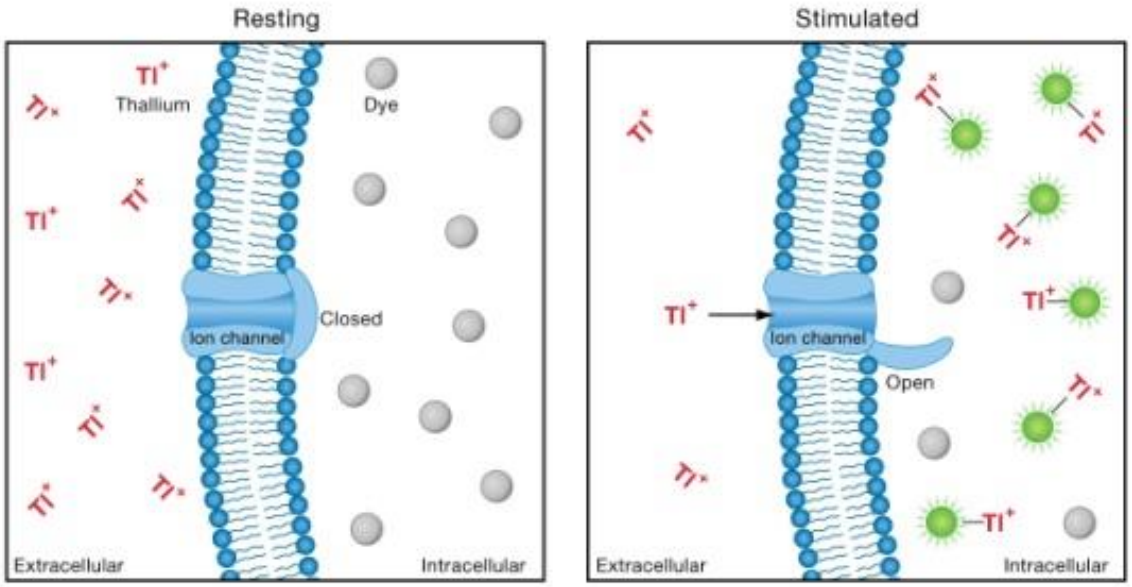
Read

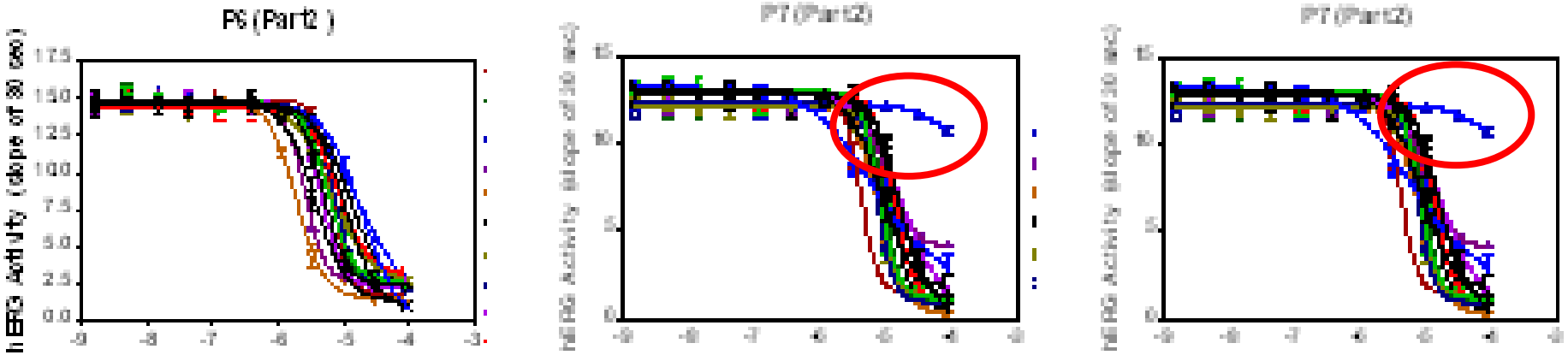
fluorescence & luminescence simultaneously

What has been done on FDSS?

- GPCR calcium influx assays
  - Fluo4 based
  - Fura2 based
  - Protein sensors such as Cameleon
  - Aequorin
- Ion Channel assays
  - Membrane potential: FMP, FluoVolt, VSP
  - potassium channel: Thalium
  - sodium channel: ANG2, SBFI
  - chloride channel: YFP
- Waveform analysis
  - calcium oscillation in cultured neurons
  - Characterizing the phenotype of iPS cardiomyocytes
- Enzymatic assays
  - Prolyl isomerase, LDHA, GTPase, Kvβ

Compound Profiling for hERG Channel Activities

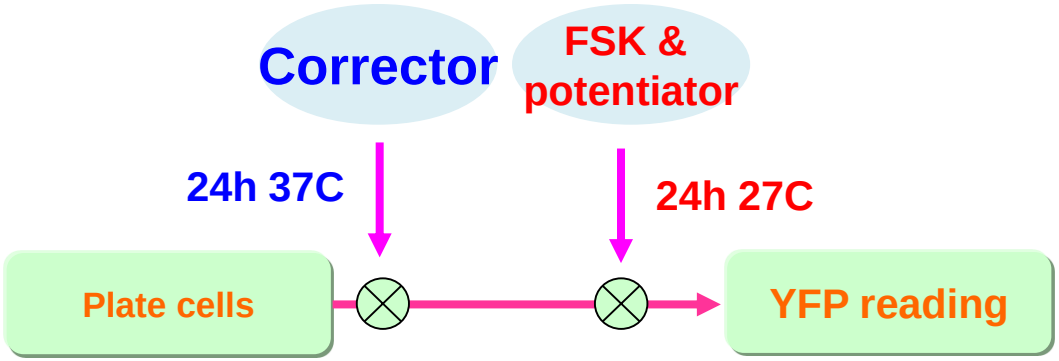


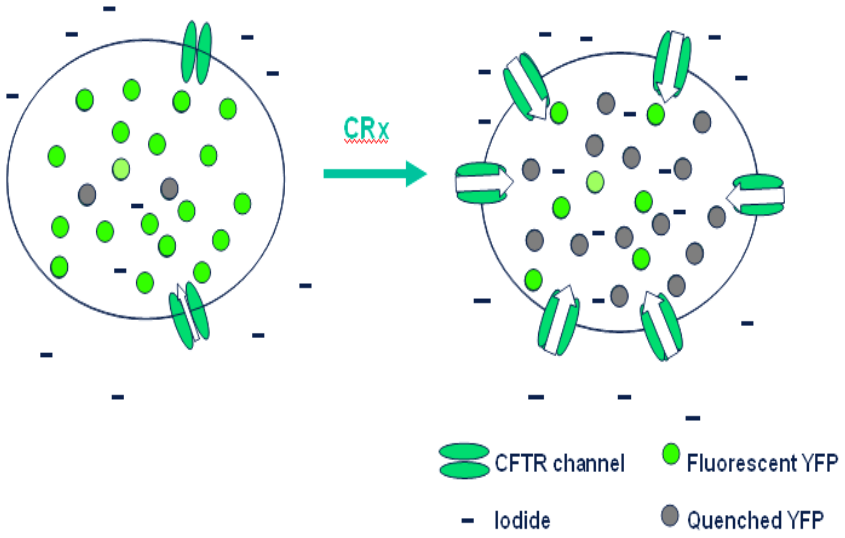


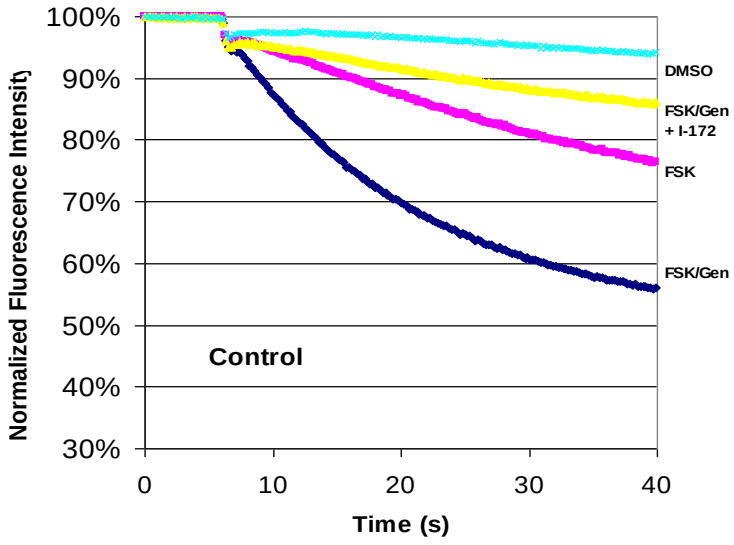
- 60 compounds per 1536 plate, 12 concentrations in duplicate

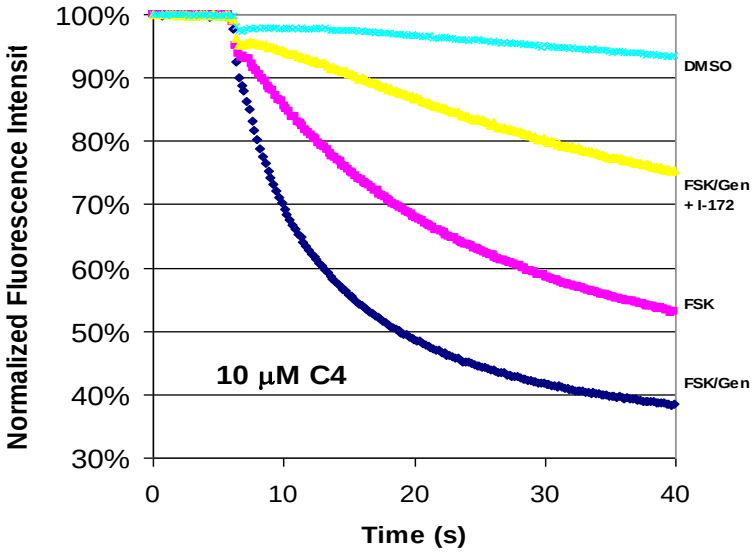
NCGC
NIH Chemical Genomics Center

YFP Assay to Identify Modulators of CFTR-ΔF508





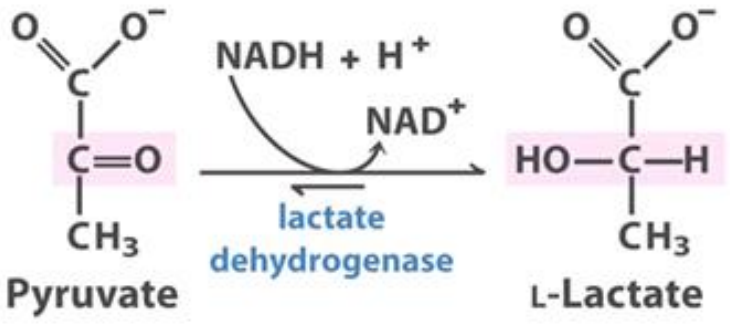




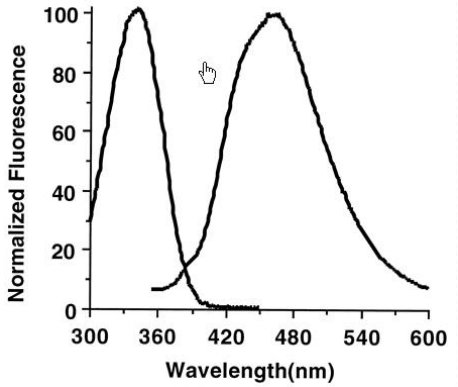
CombinatoRx

HTS Assay for Lactate Dehydrogenase

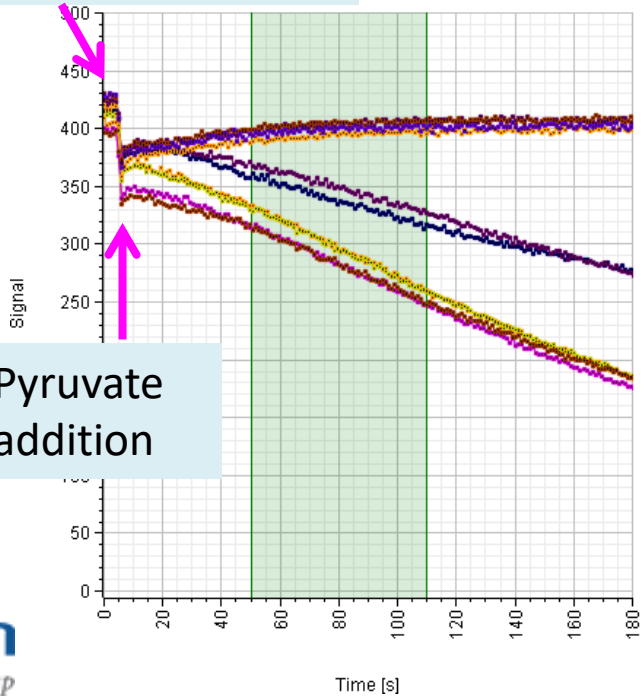
Reaction



Detection of NADH (Ex 340/Em 480)



Baseline fluorescence
LDHA + NADH + compound



No Enzyme

IC50 oxamate

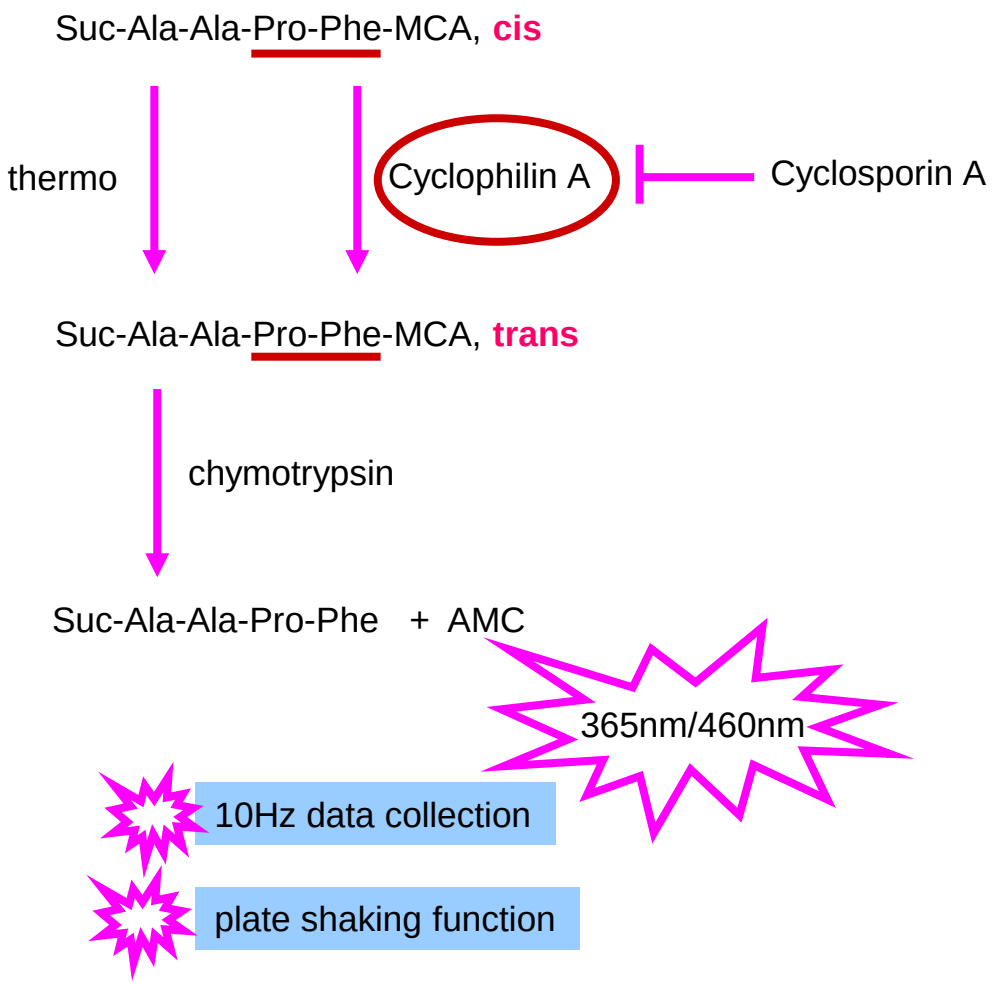
+LDHA

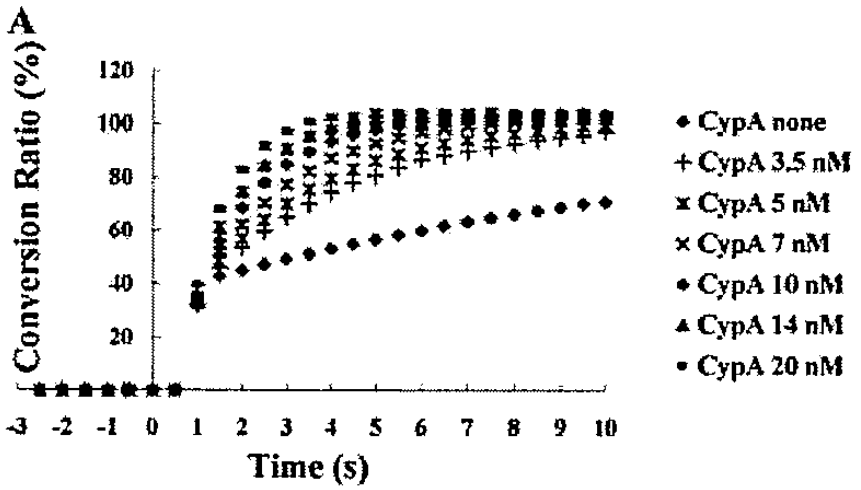
Pyruvate addition

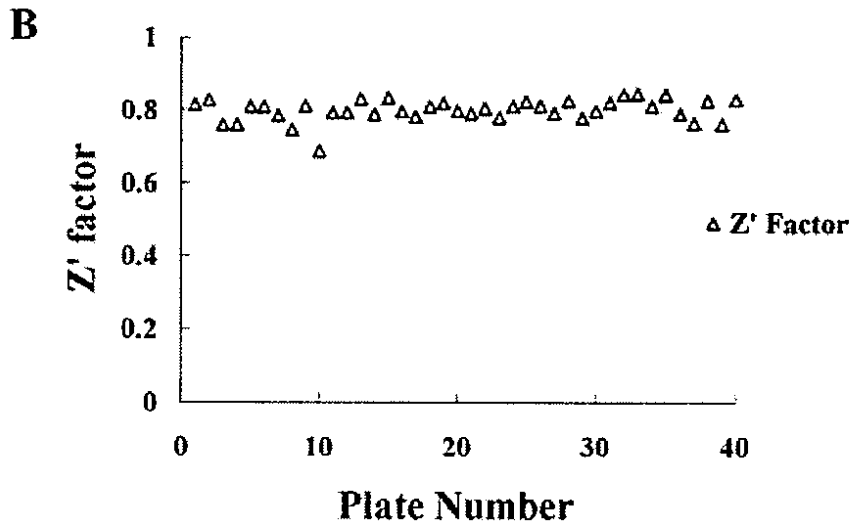
The more “traditional” approach of monitoring NADH fluorescence is prone to compound fluorescence interference. Kinetic assay screen allows elimination of fluorescent artefacts

Analytical Biochemistry, 441(2) p115-122, 2013

HTS to identify Prolyl Isomerase Inhibitors



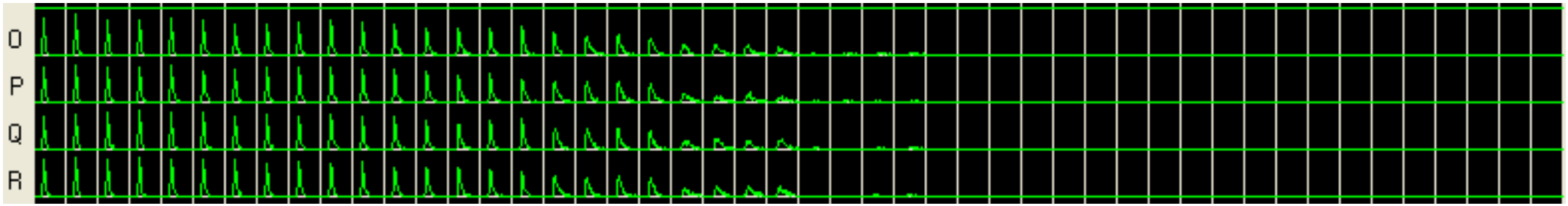


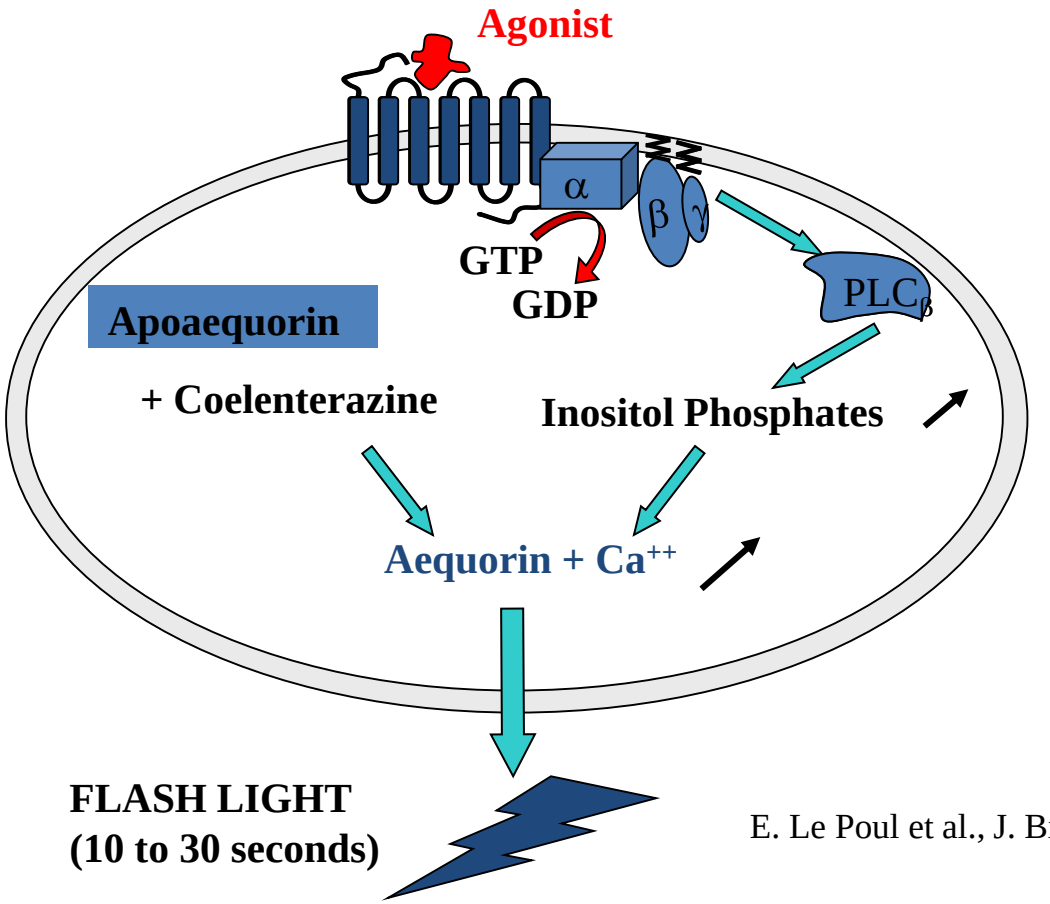


Journal of Biomolecular Screening. 14(4) p419-422. 2009

Mitsubishi Tanabe Pharma

Aequorin-based Functional Assay for GPCR





E. Le Poul et al., J. Biomol. Screening 2002; 7(1) 57-66

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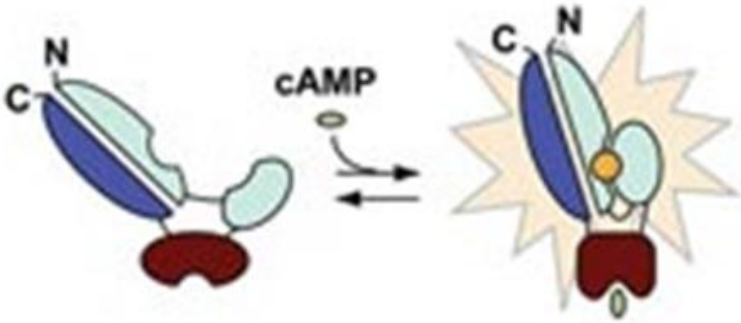
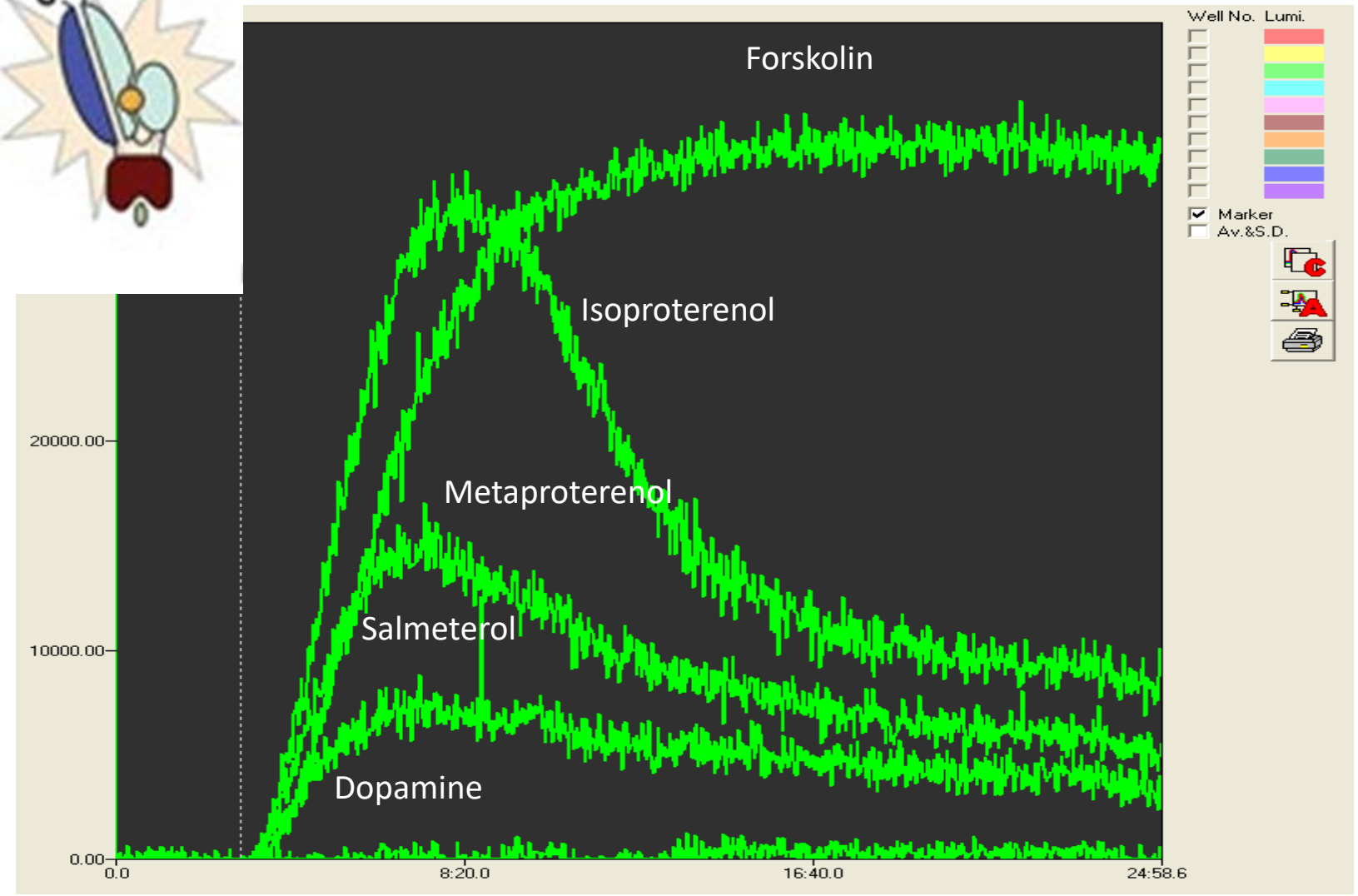
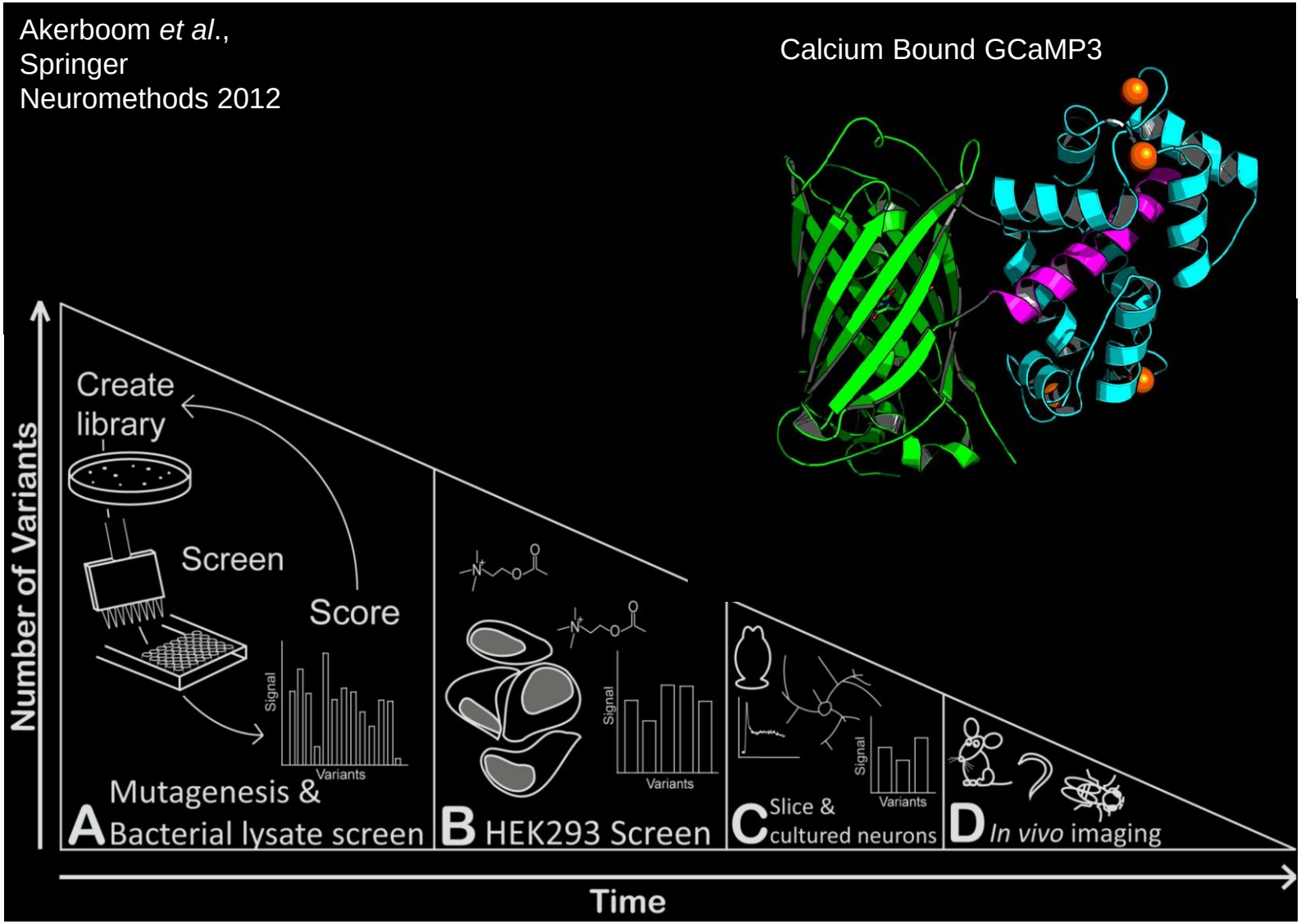
Kinetically Analysis by cAMP GloSensor





Promega

Use FDSS to optimize GCaMP sensor



What is new?

- iPSC based cell based assay
- High speed data acquisition on whole plate
- Whole plate electric field stimulation

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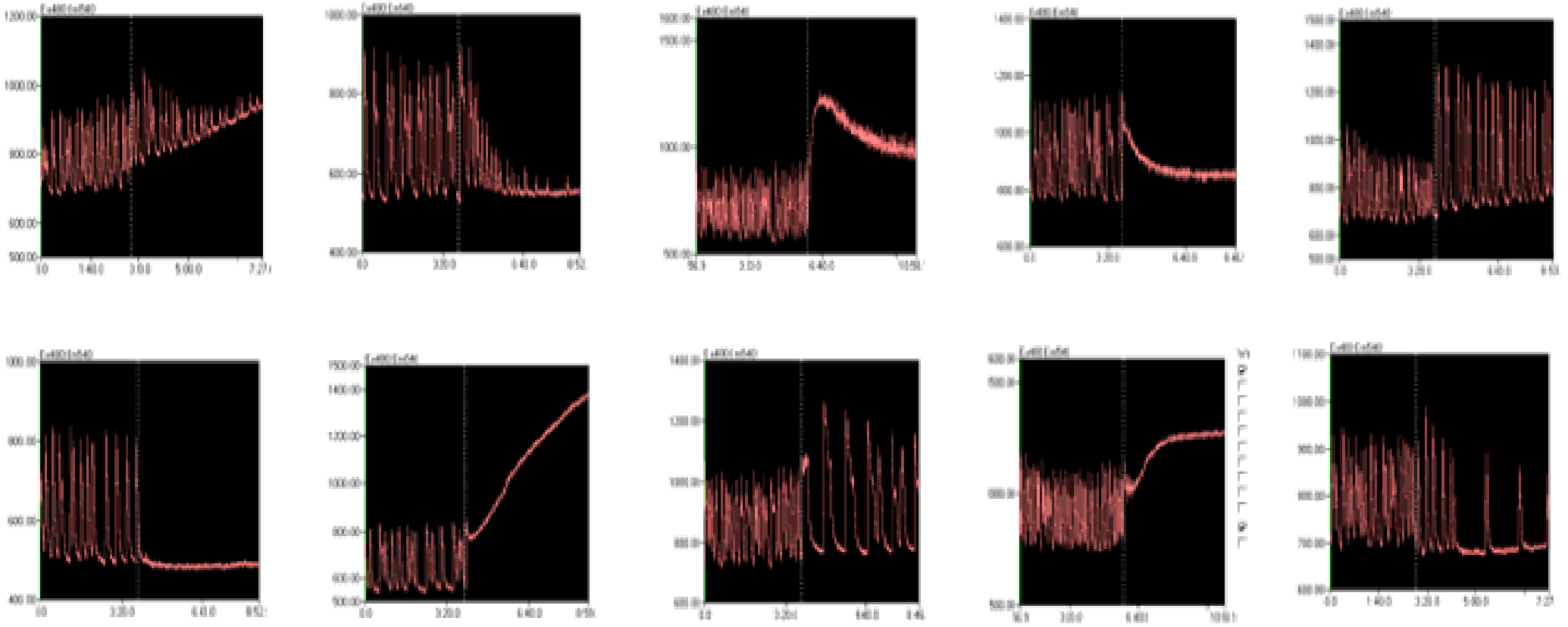
Low Mg^{2+}-induced synchronized calcium oscillation in cultured neurons

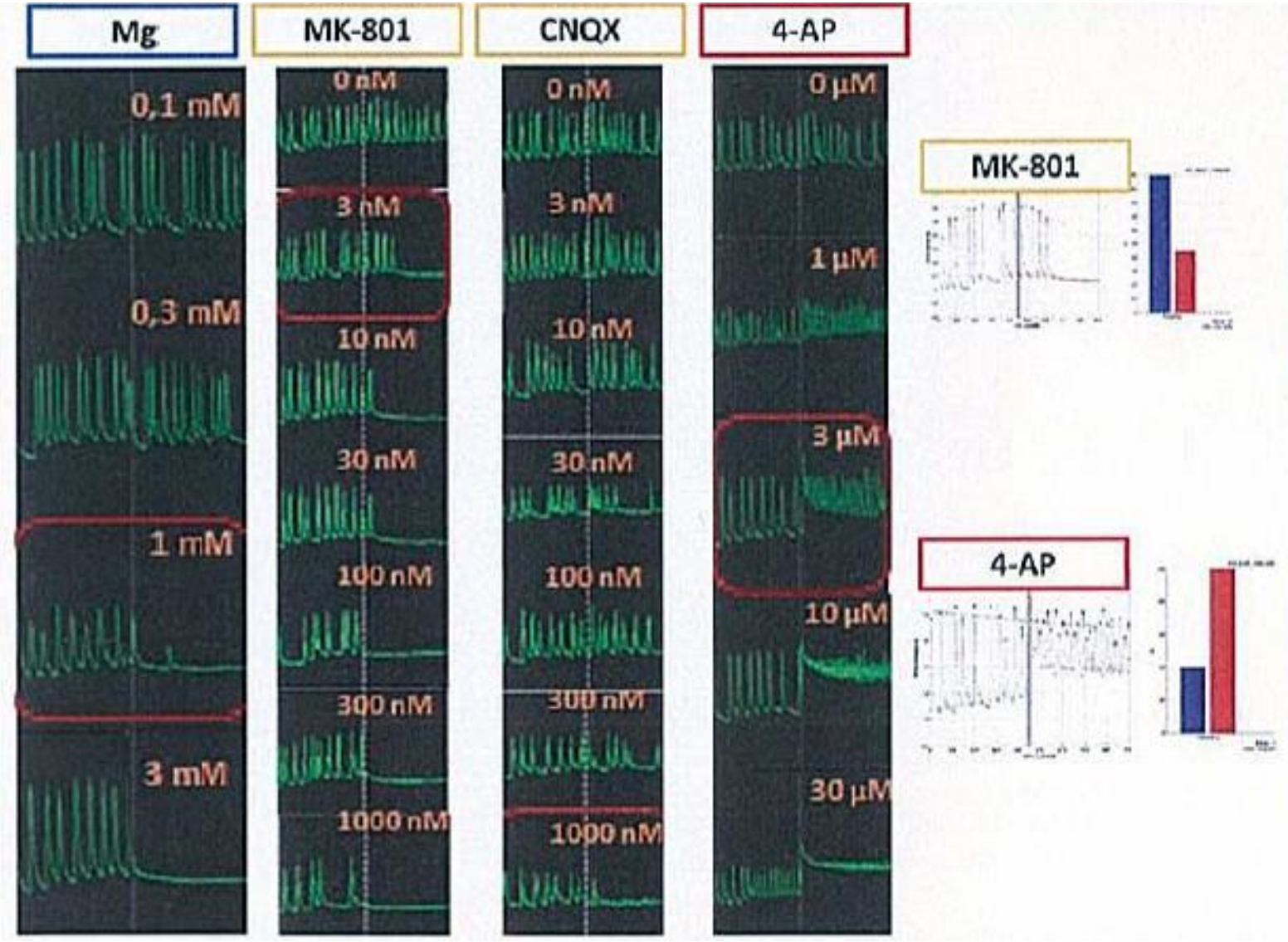
40 ref & 28,000 cmpds @ 10 µM

150 cmpds selected for acute in vivo antiepileptic effect (MES @ 30 mg/kg ip Mouse)



sanofi aventis
L'essentiel c'est la santé.

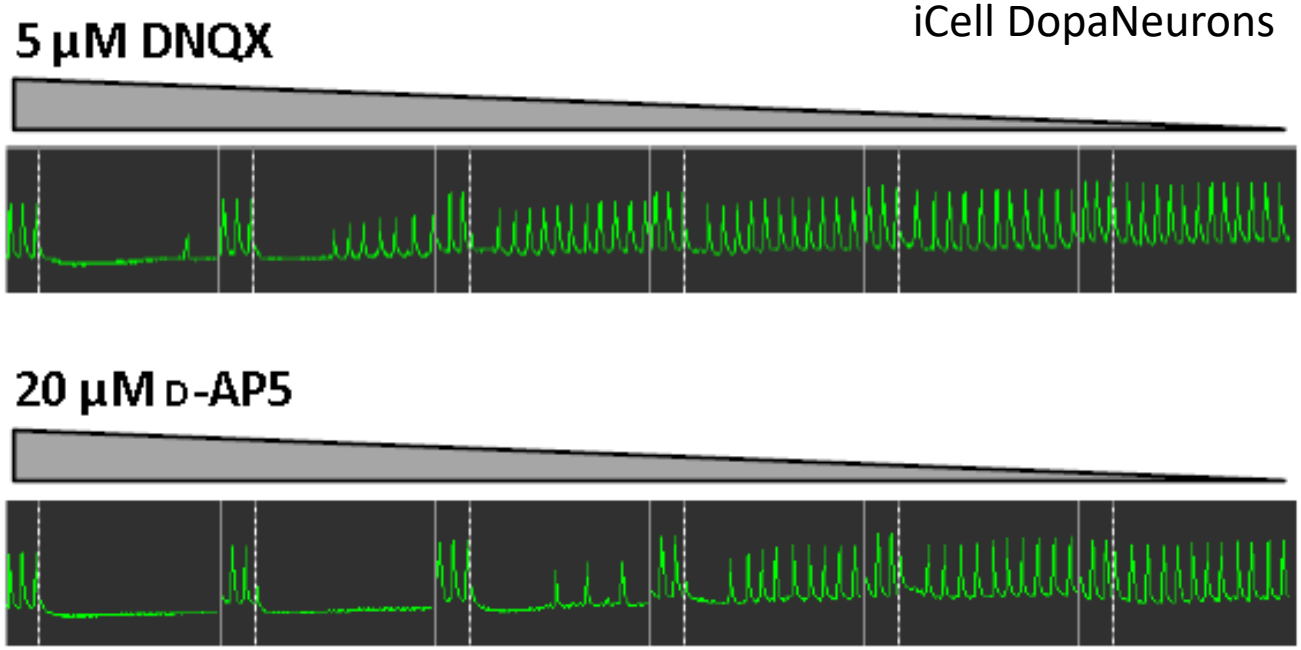
Neuronal calcium oscillations for preclinical seizure risk evaluation



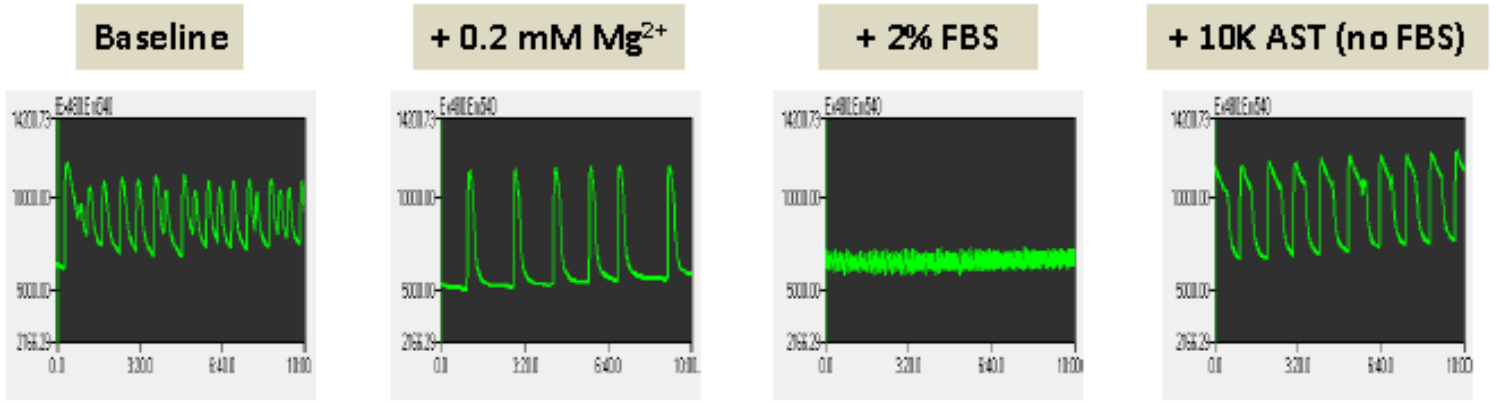
SANOFI

Spontaneous calcium oscillation in neurons

iCell DopaNeurons



iCell GlutaNeurons



Cellular Dynamics international
a FUJIFILM company

Development of High Throughput Phenotypic Screening Assays for Pain

Linda Kitching*, Peter Stacey*, Emma Impey*, Paul Karila#, Anna Karlsson#, Charlotta Blom#, Susanne Lardell#, Magda Bictash* & Darren Cawkill*

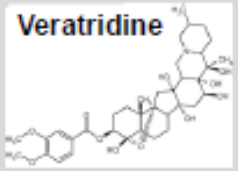
*Neusentis, The Portway Building, Granta Park, Great Abington, Cambridge CB21 6GS, UK #Cellectricon AB, Flöjelbergsgatan 8C, SE-431 37, Mölndal, Sweden

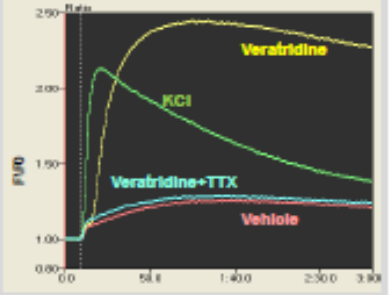
Neusentis
A Pfizer research unit

Case Study 2: hiPSC-Derived Sensory Neuron Excitability Assay

Assay Concept / Method

Veratridine-based 384 well Calcium flux screen: Veratridine (right) is a non-selective Na_V channel opener. We have used this as a chemical stimulant to cause membrane depolarisation and trigger action potential transduction in the hiPSC-sensory neurons. This mimics the excitability induced by a noxious substance.

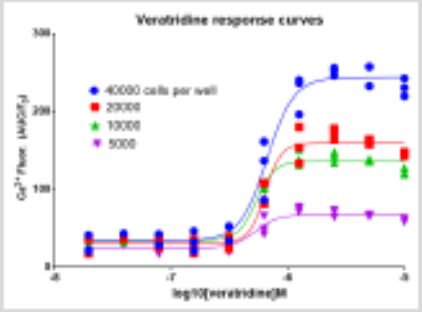


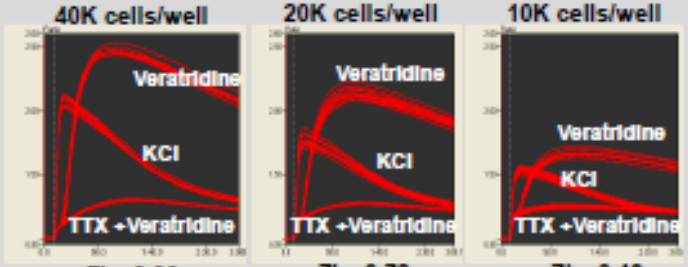


1. Cryopreserved neurons recovered into 384 well plate and maintained for required timeframe (up to 28 days)
2. Cells loaded with Ca-5 indicator dye
3. Test compounds pre-incubated with cells for 15 mins
4. Depolarisation induced with 5 µM veratridine
5. Whole well fluorescence recorded for 3 mins using FDSS6000
6. Data analysis performed using AUC / baseline

Assay Development

Cell Density Optimisation: Experiments were performed to ensure optimal data quality while reducing the requirements for cell production to minimise cost and logistical complexity. 20K to 40K cells per well produced good assay quality.

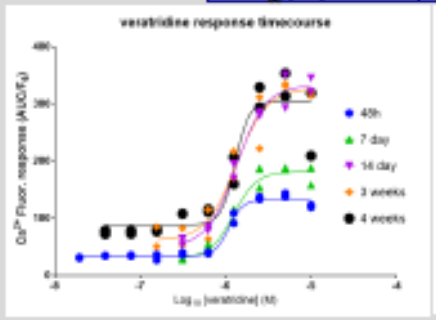


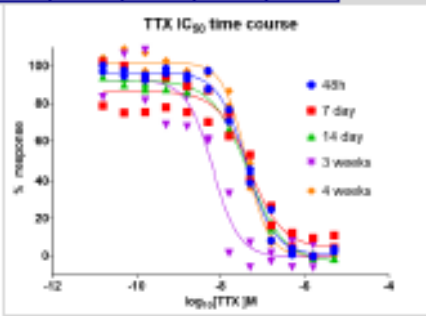


Z' = 0.86 Z' = 0.72 Z' = 0.43

Optimisation of cell maturation: Functional characterisation of hiPSC-sensory neurons demonstrated maturation changes as they are maintained in culture[2,3]. Therefore we optimised the assay to enable screening of cells maintained up to 28 days in 384-well plates.

Days in culture	2	7	14	21	28
Veratridine EC_{50} (µM)	1.10	1.24	1.33	1.34	1.30
TTX IC_{50} (nM)	40.6	49.6	43.7	8.44	40





Stem Cell Research 17 (2016) 306–317

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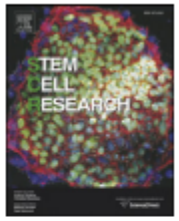
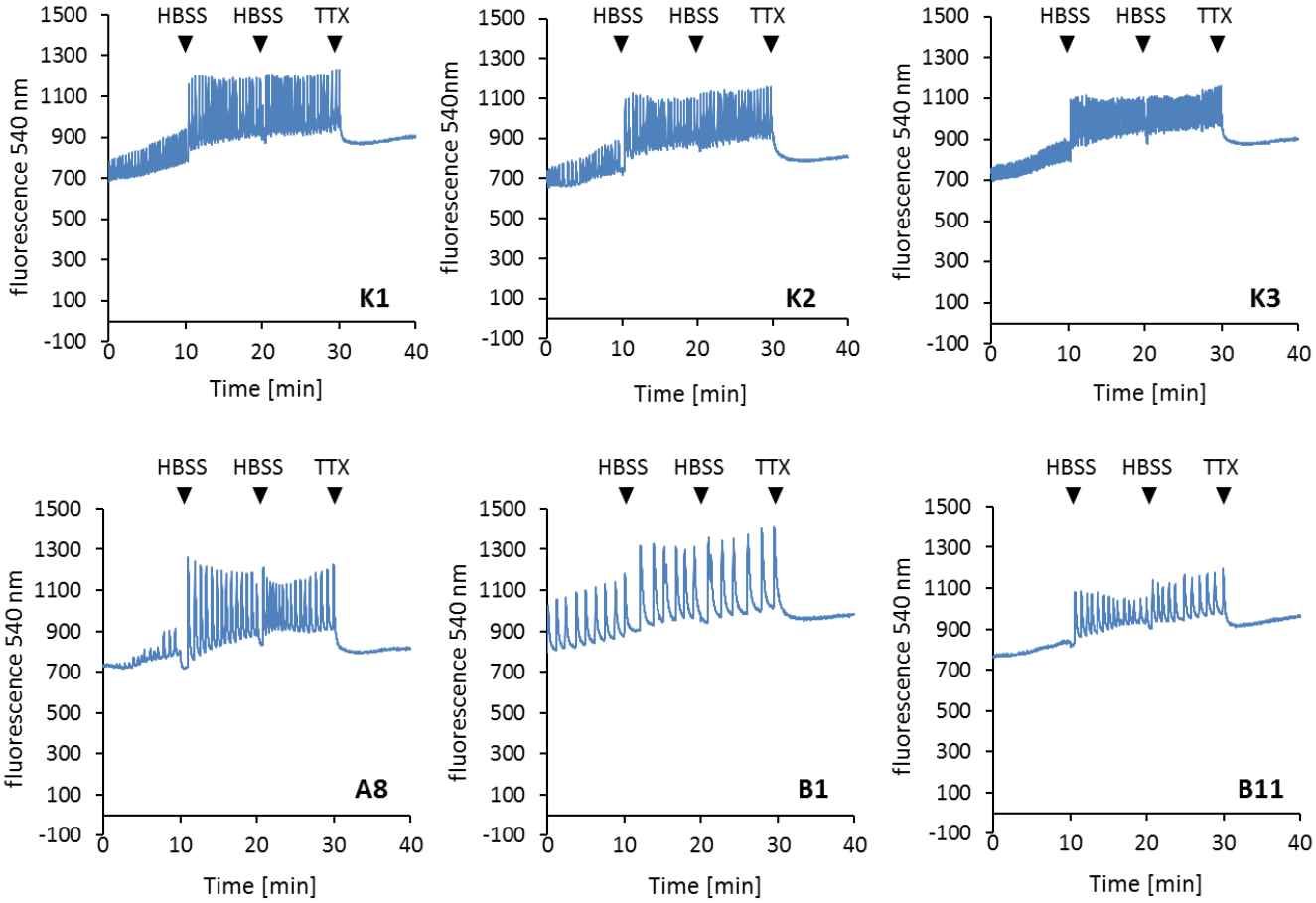


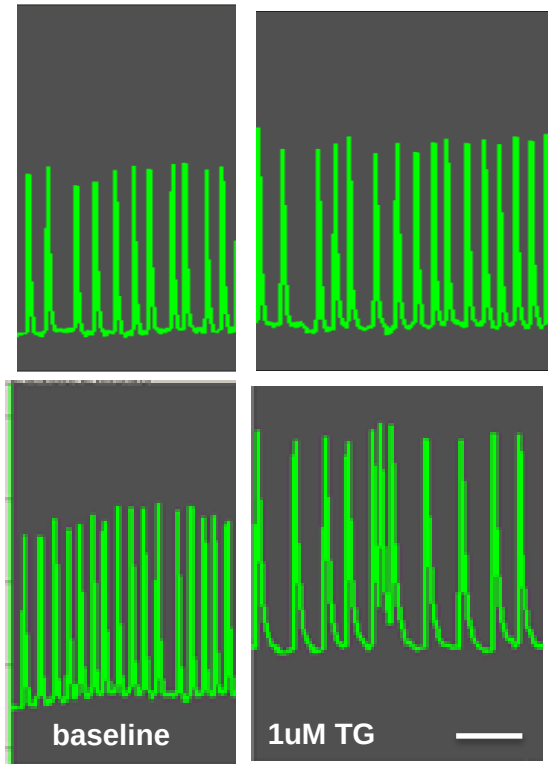
Induced pluripotent stem cell - derived neurons for the study of spinocerebellar ataxia type 3

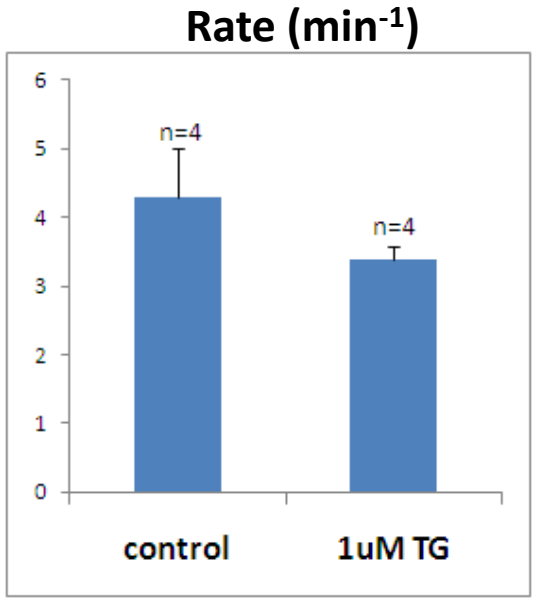
Susanne K. Hansen [a,b,*], Tina C. Stummann [b], Helena Borland [b], Lis F. Hasholt [c], Zeynep Tümer [d], Jørgen E. Nielsen [c,e], Mikkel A. Rasmussen [a,1], Troels T. Nielsen [e], Justus C.A. Daechsel [b], Karina Fog [b], Poul Hyttel [a]

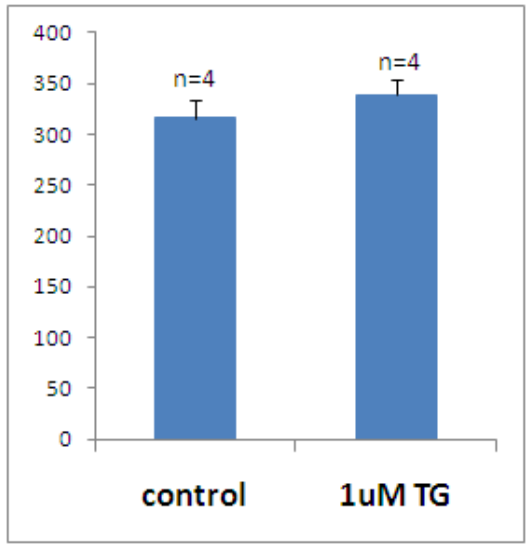
[a] Department of Veterinary Clinical and Animal Sciences, University of Copenhagen,
[b] H. Lundbeck A/S, Ottiliavej 9, Valby 2500, Denmark
[c] Institute of Cellular and Molecular Medicine, University of Copenhagen, Blegdamsv
[d] Applied Human Molecular Genetics, Kennedy Center, Department of Clinical Genet
[e] Neurogenetics Clinic & Research Laboratory, Danish Dementia Research Centre, Rig



High speed is more desirable for iPS cardiomyocytes







Decay

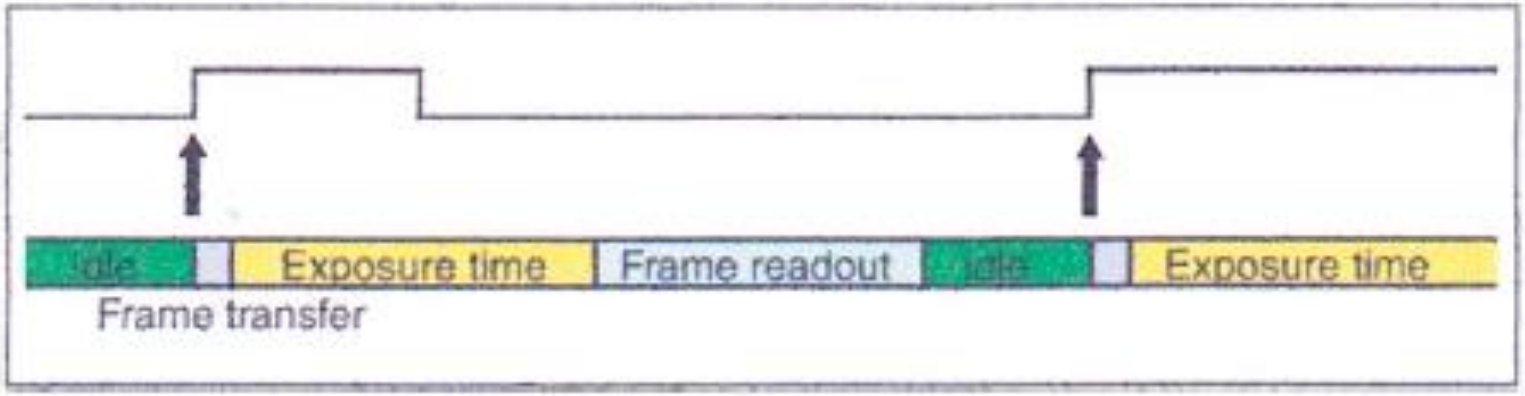
Baseline: τ = 0.93s (n=4)

TG: τ = 1.97s (n=4; p<0.005)

TOXICOLOGICAL SCIENCES **131(1)**, 292–301 (2013)
doi:10.1093/toxsci/kfs282
Advance Access publication September 14, 2012

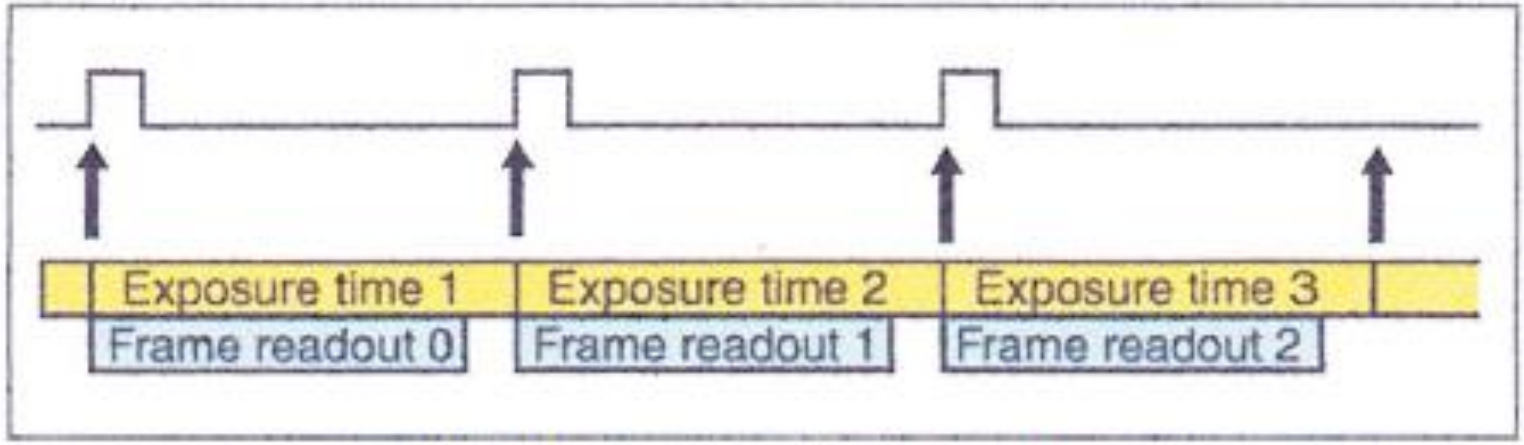
Realization of high speed acquisition

8hz



Standard mode

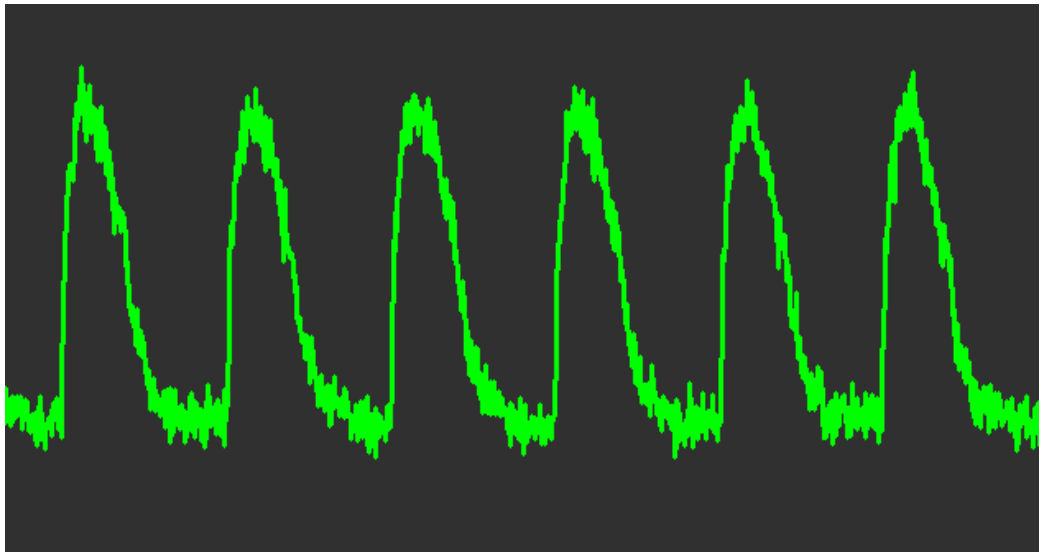
100hz



High speed mode

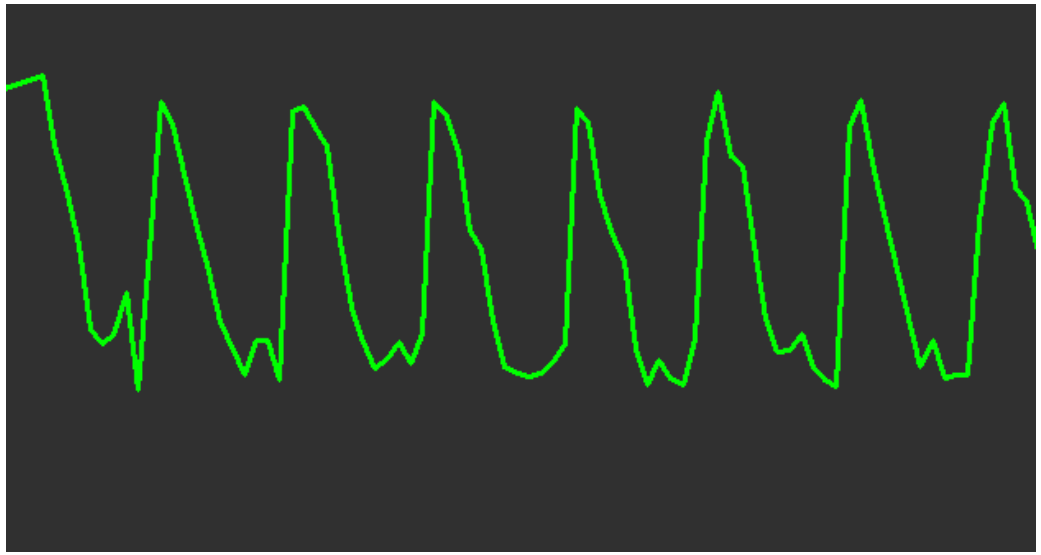
Impact of different data acquisition speed

8 ms or 100 hz



10 s

120 ms or 8 hz



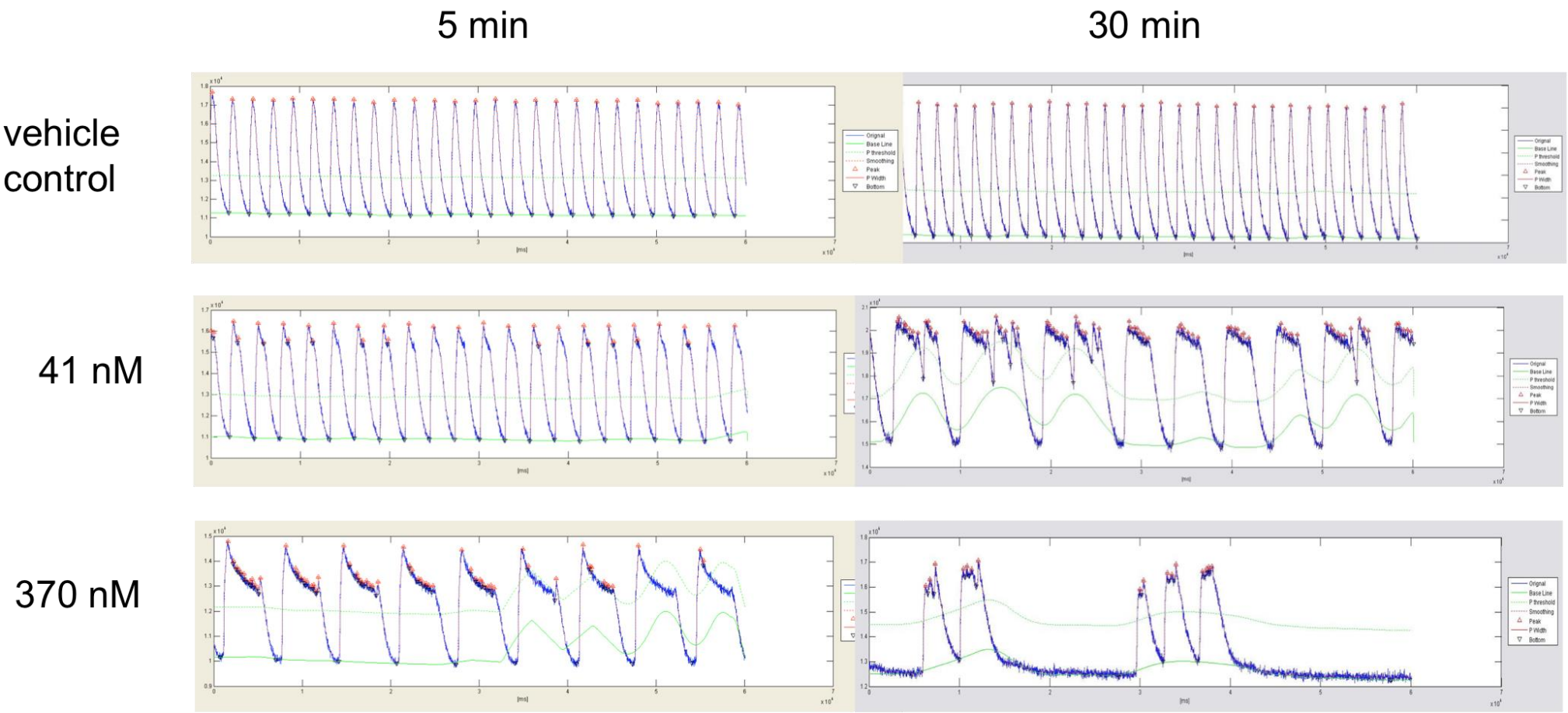
10 s

Amplitude

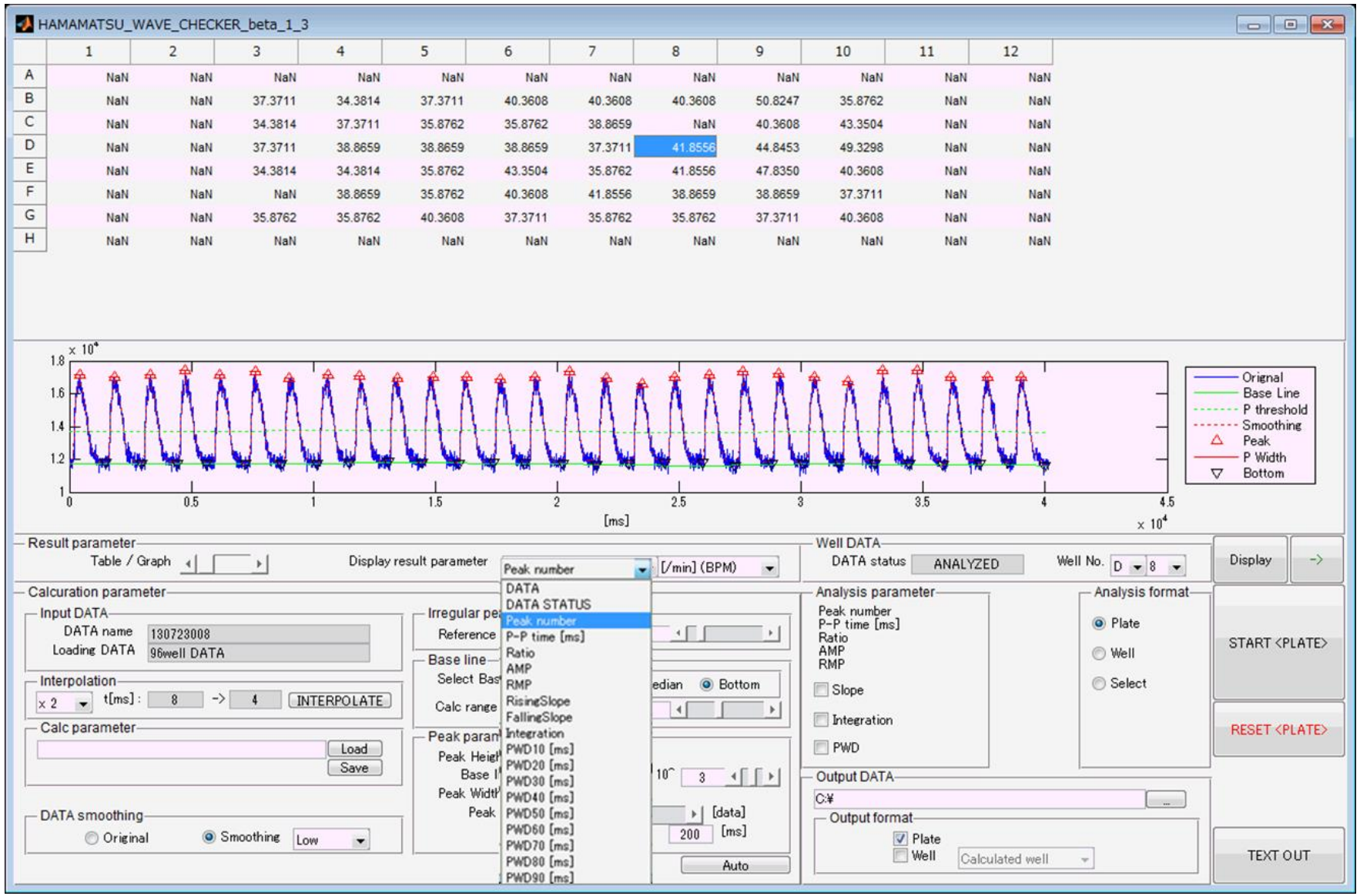
sampling rate	**8 ms**	**120 ms**
CV (%)	2.96	8.20

High speed acquisition

5 vs 30 min: Effect of hERG/I_{Kr} blocker Astemizole

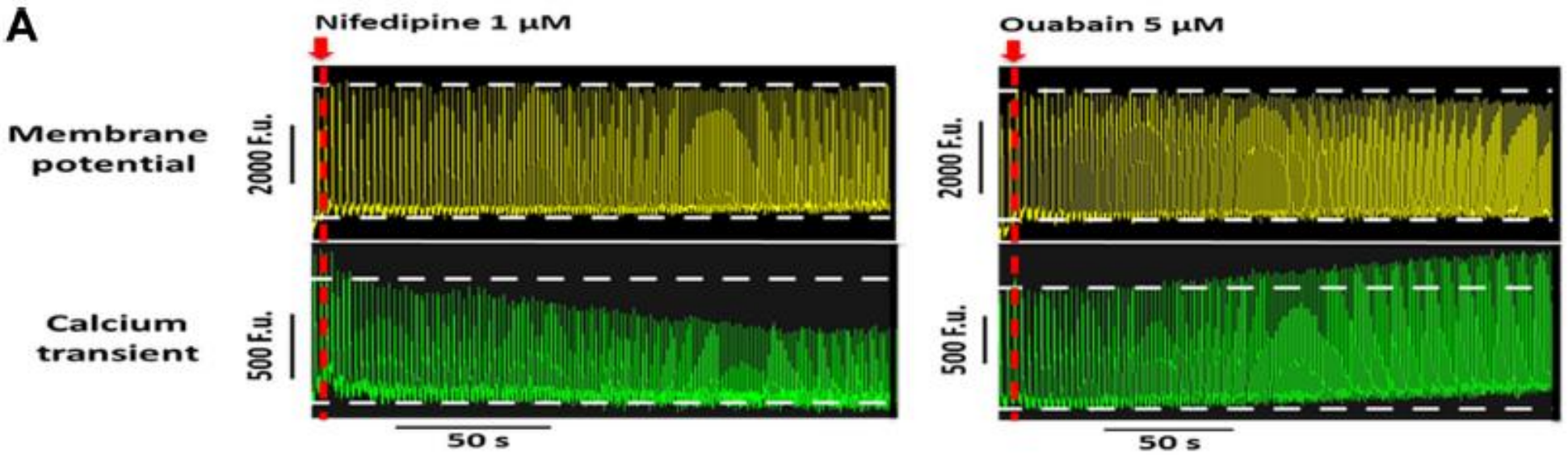


FDSS waveform analysis software



Heat map of cmpds due to their parameters

Voltage and calcium probes might have different profile



Am J Physiol Heart Circ Physiol 311: H44–H53, 2016.
First published May 3, 2016; doi:10.1152/ajpheart.00793.2015.

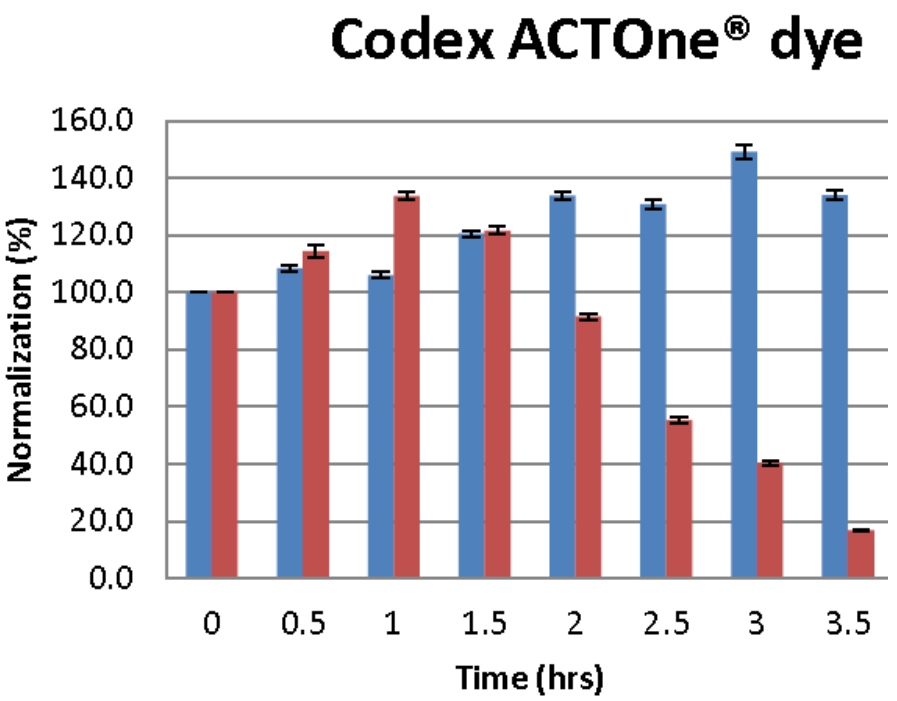
High-throughput drug profiling with voltage- and calcium-sensitive fluorescent probes in human iPSC-derived cardiomyocytes

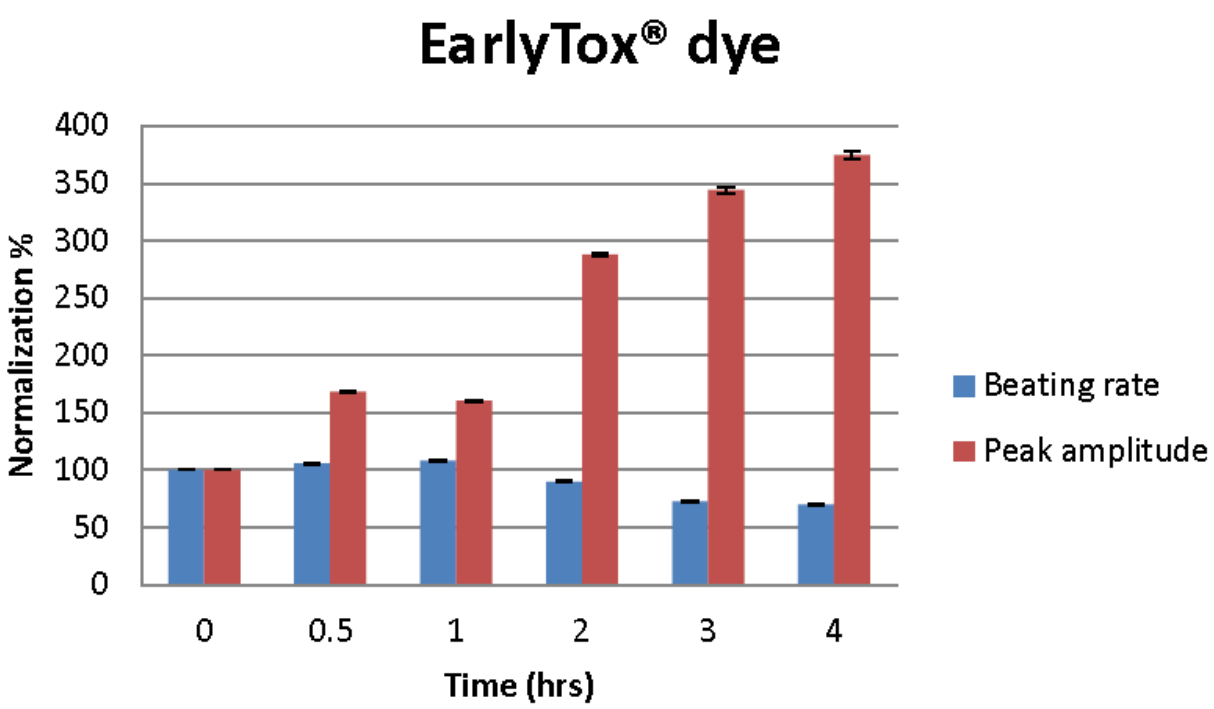
Stephane Bedut,[1,2] Christine Seminatore-Nole,[1] Veronique Lamamy,[2] Sarah Caignard,[2] Jean A. Boutin,[2] Olivier Nosjean,[2] Jean-Philippe Stephan,[2] and Francis Coge[2]

[1]*Laboratoire SERVIER de Chemogénétique, Institut du Cerveau et de la Moelle, Hôpital de la Salpétrière, Paris, France;*
[2]*Pôle d'Expertise Biotechnologie, Chimie & Biologie, Institut de Recherches SERVIER, Croissy-sur-Seine, France*

Submitted 14 October 2015; accepted in final form 20 April 2016

Impact of different calcium dyes on cardiomyocytes





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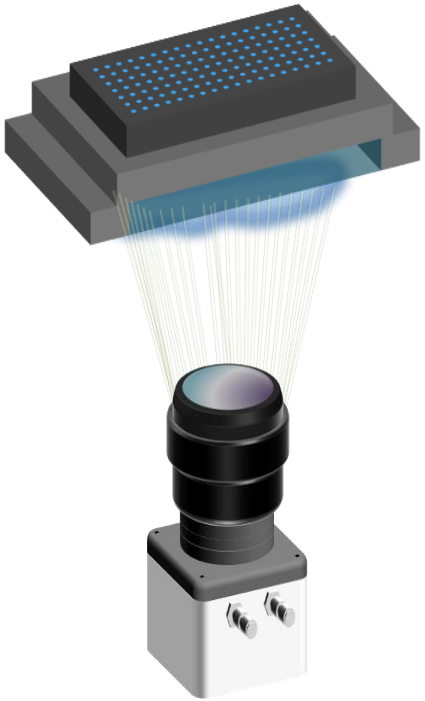
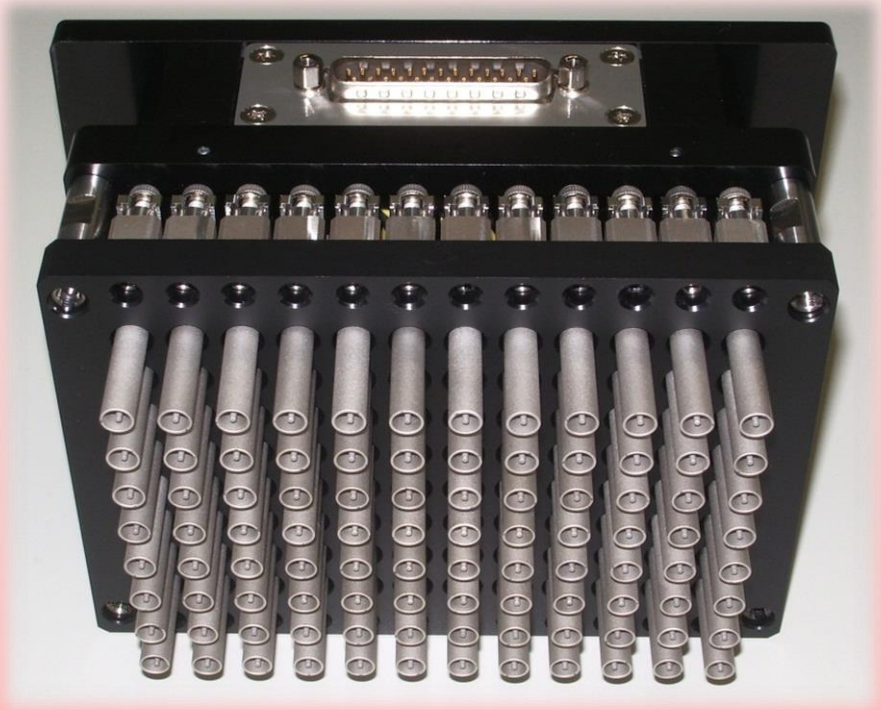
Research article

Use of FDSS/μCell imaging platform for preclinical cardiac electrophysiology safety screening of compounds in human induced pluripotent stem cell-derived cardiomyocytes

Haoyu Zeng *, Maria I. Roman, Edward Lis, Armando Lagrutta, Frederick Sannajust
SAIAR, Safety & Exploratory Pharmacology Department, Merck Research Laboratories, West Point, PA 19486, USA

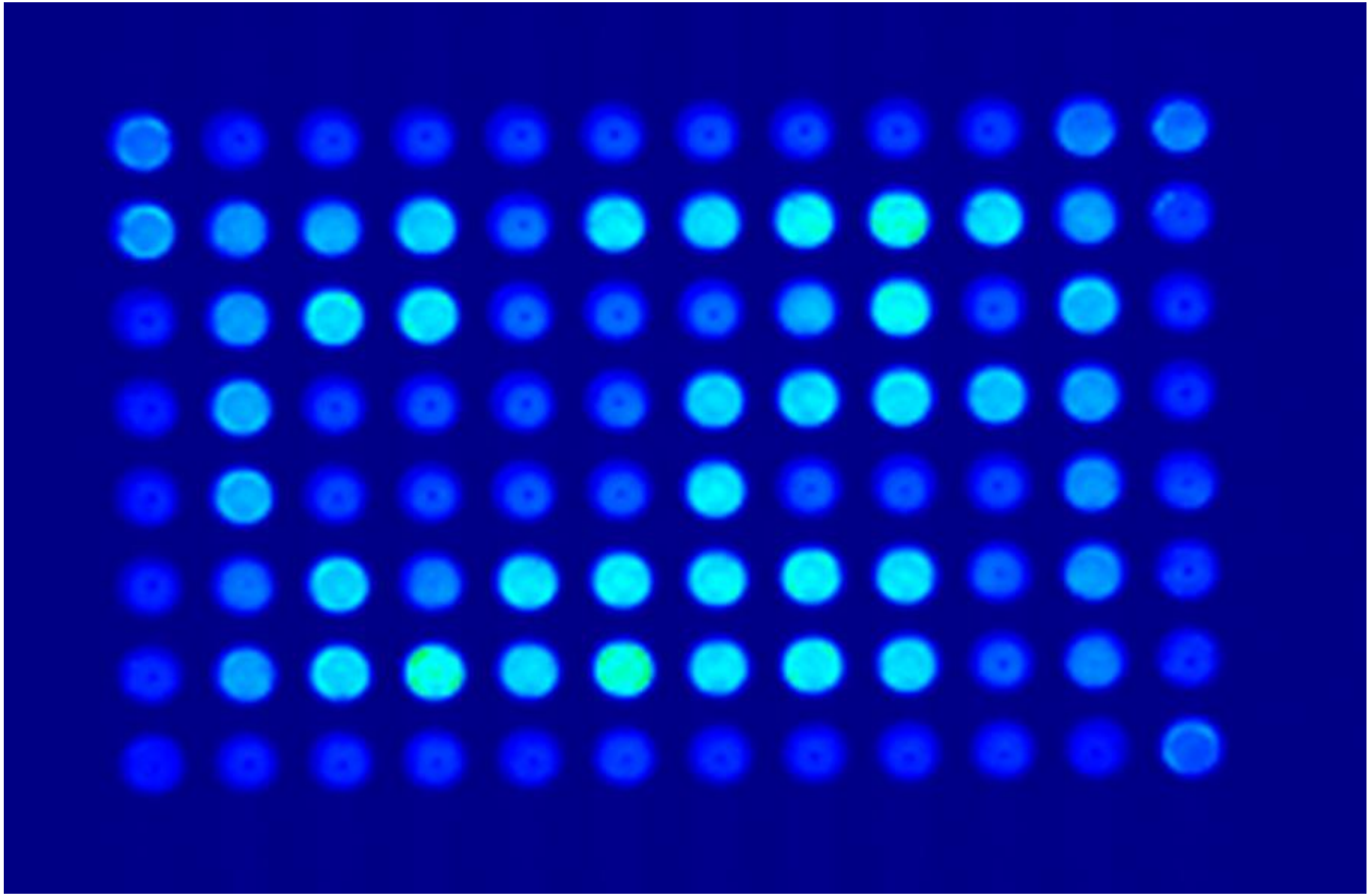
Electrode array to stimulate cells





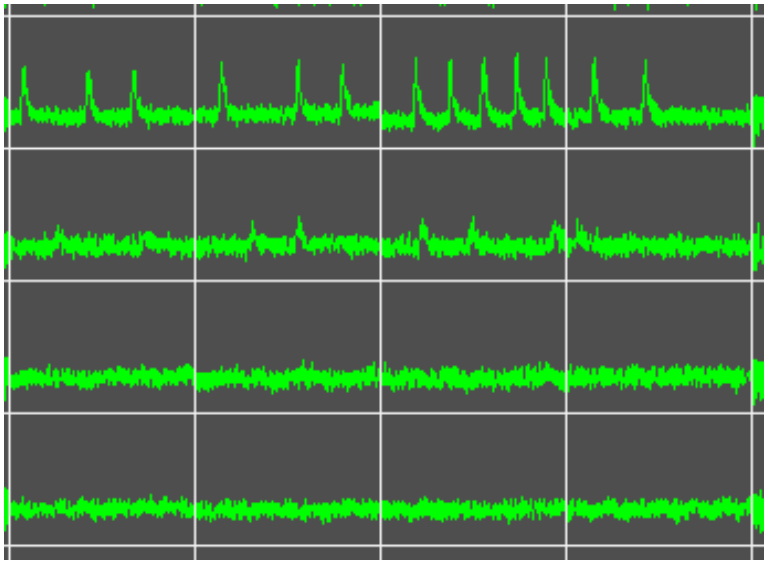
- Stimulate all 96 wells simultaneously
- Cylindrical electrodes
- change stimulation voltages by column
  (Patent pending)

EFS to pace cardiomyocytes



EFS: assay optimization

Before



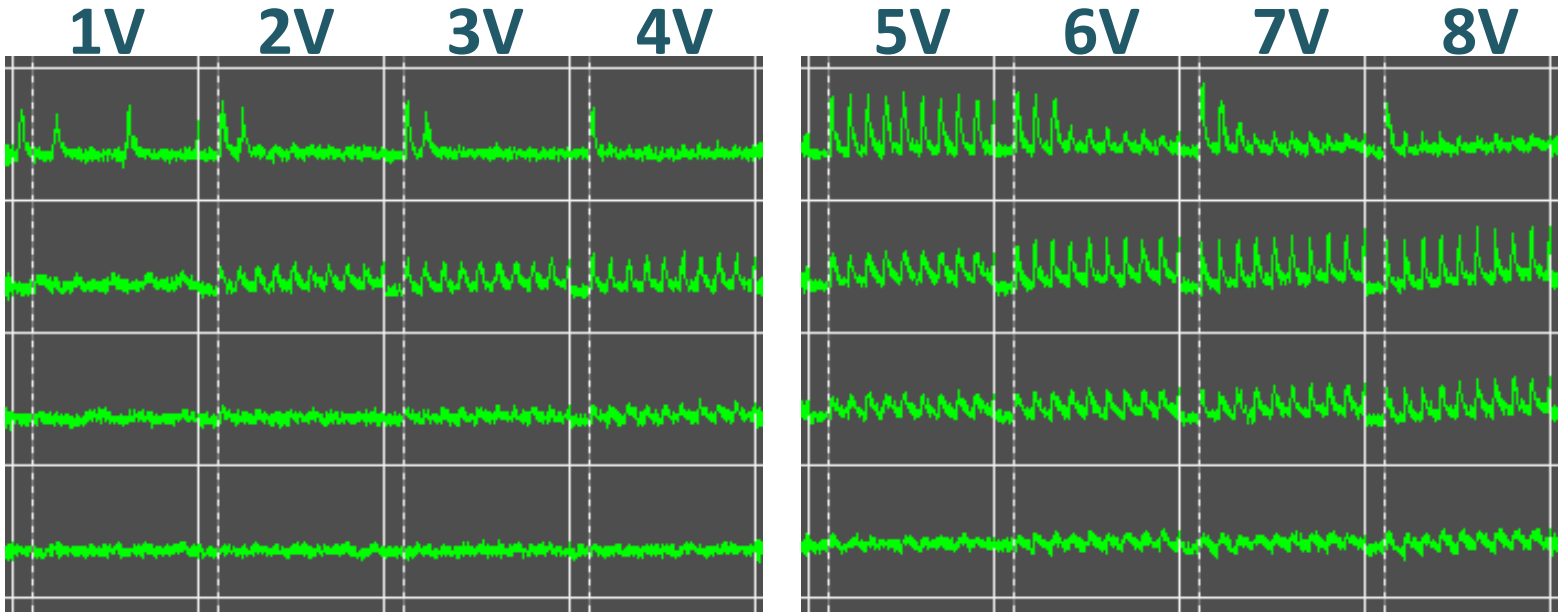
3x10⁴cels/well

2x10⁴cels/well

1x10⁴cels/well

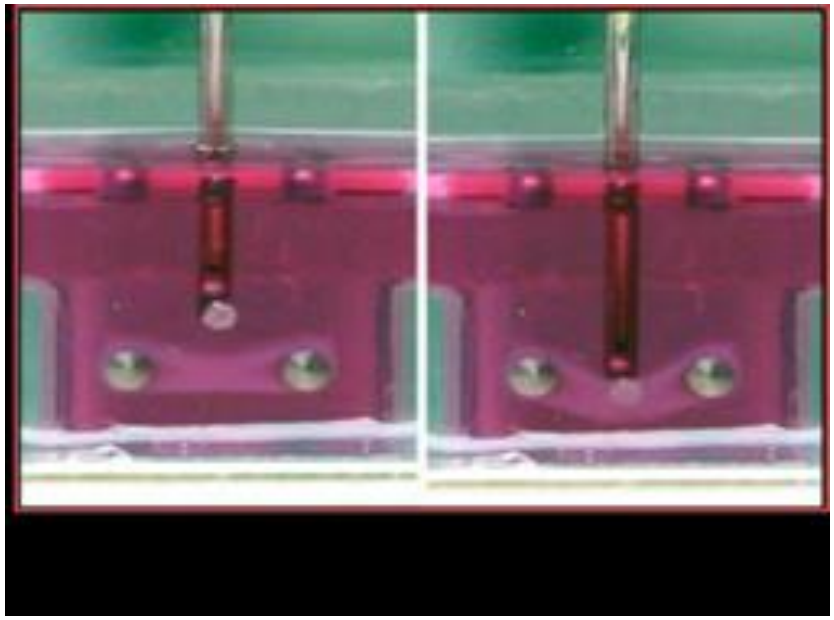
0.5x10⁴cels/well

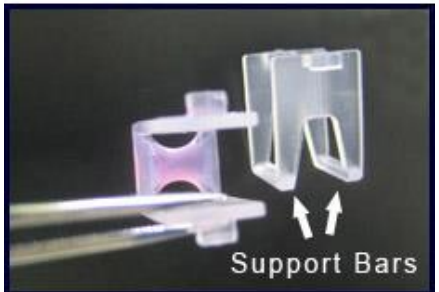
After

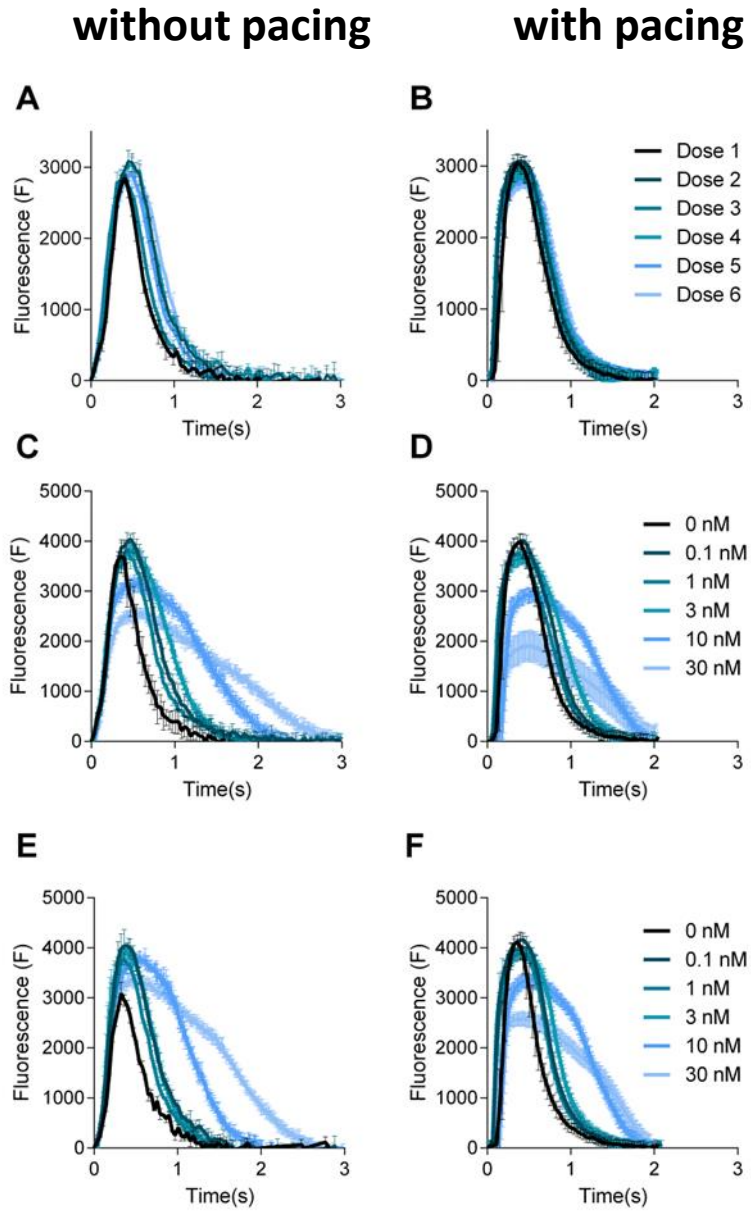


130909044/048/049
10秒間
S.U.あり(959-1917)
8ms
10ms duty
0.5mm

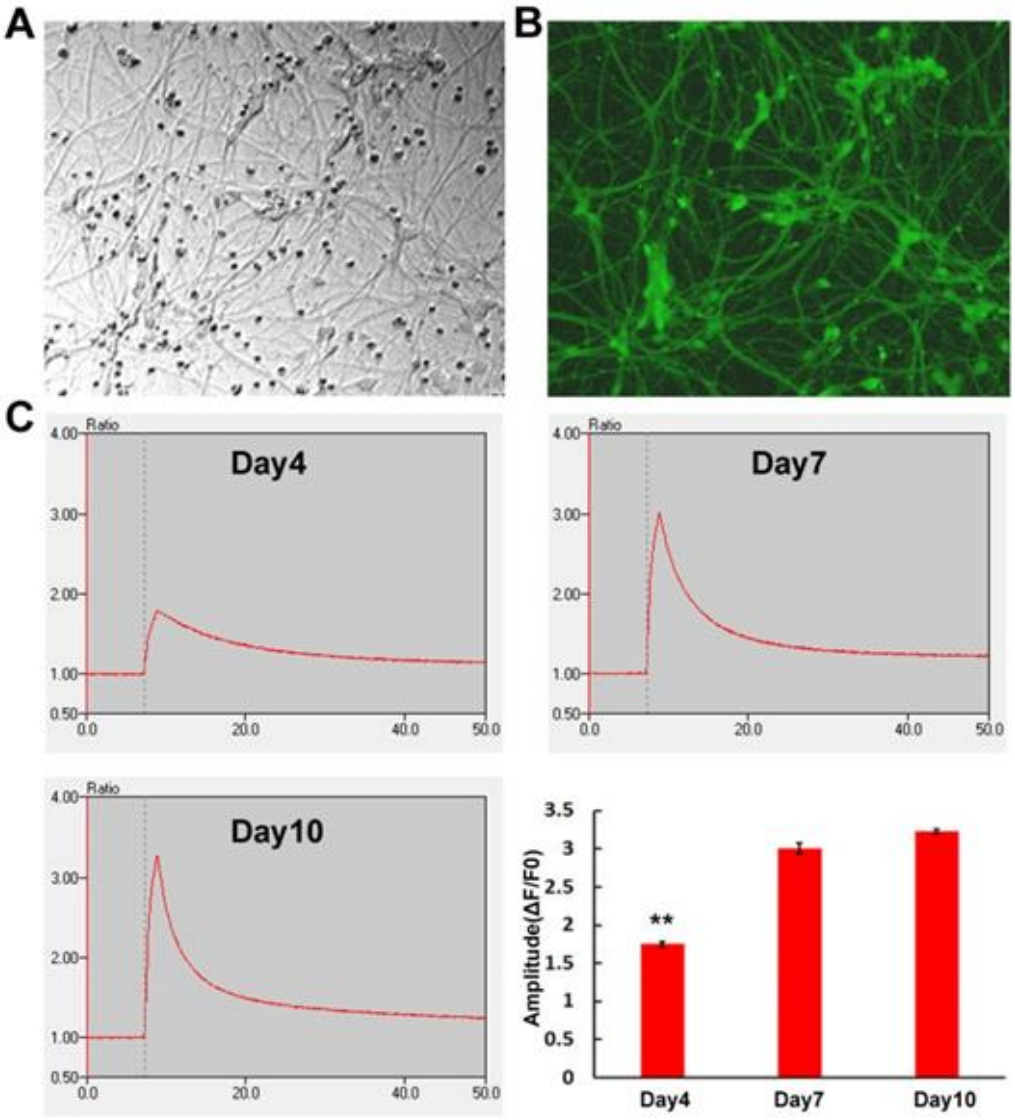
Force measurement with cardiomyocytes tissue

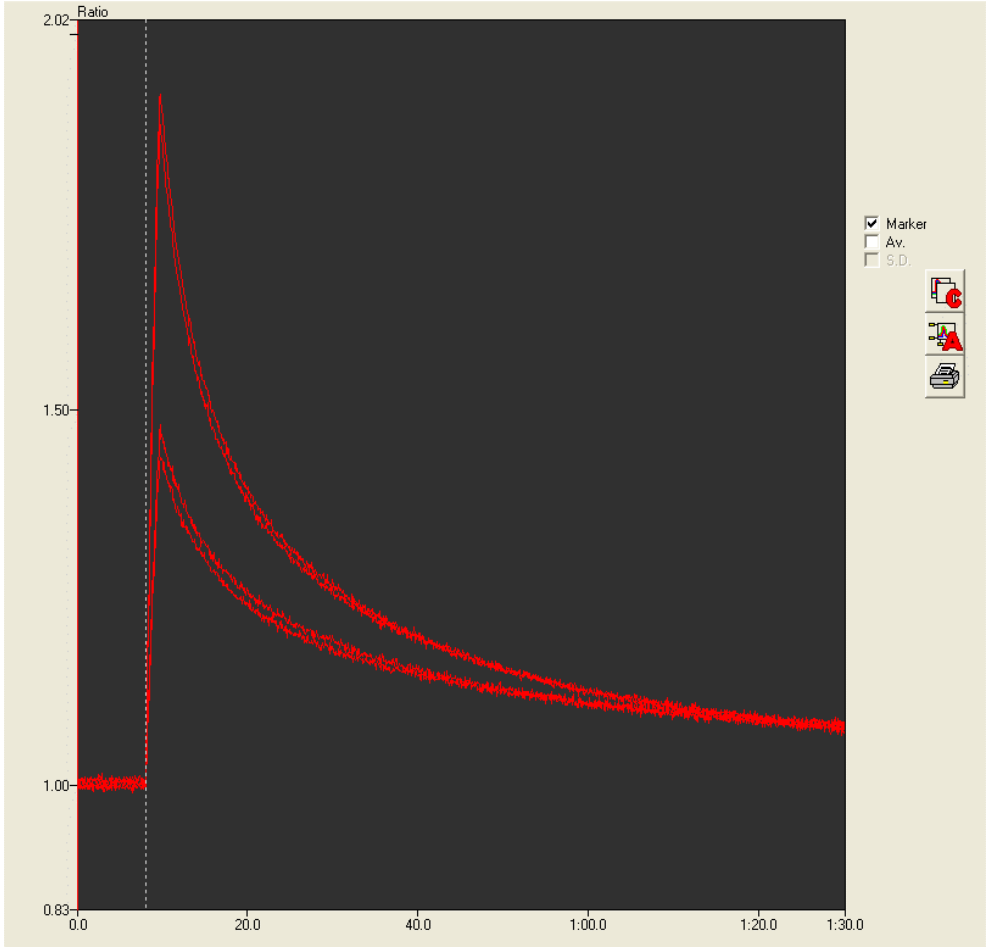






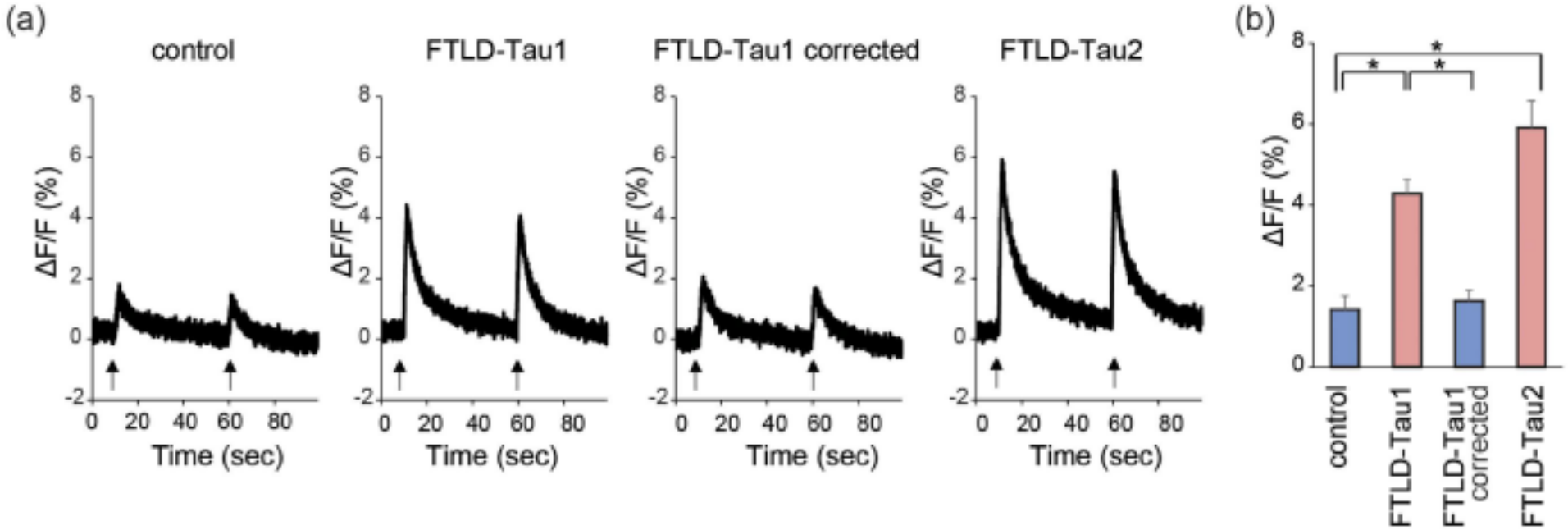
Motor neurons have matured electrophysiologically





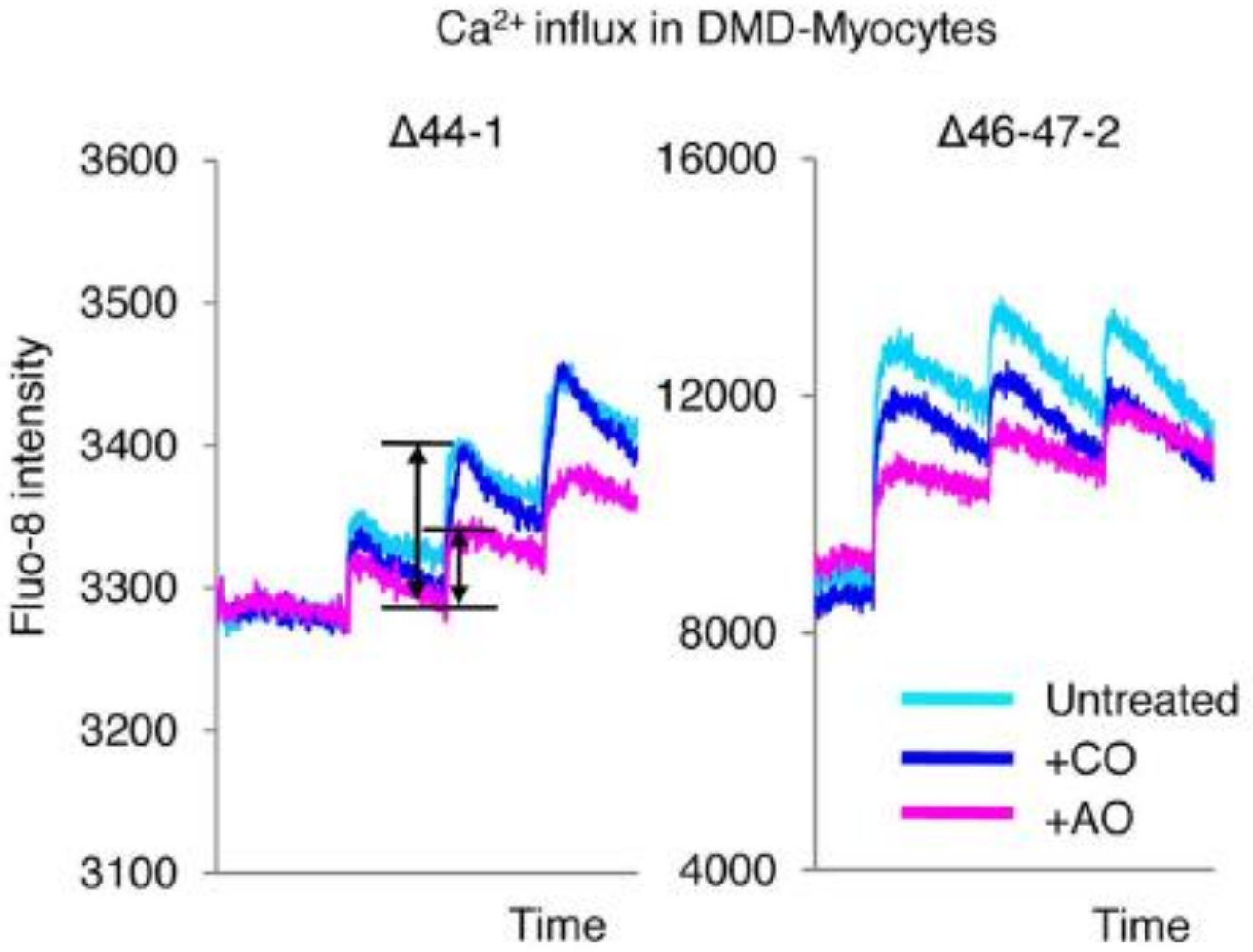
BrainXell
Neurons are our business.

Calcium dysregulation contributes to neurodegeneration in FTLD patient iPSC-derived neurons



Source: http://www.nature.com/articles/srep34904

Early pathogenensis of DMD modelled in patient-derived Human iPSC cells



Source: http://www.nature.com/articles/srep12831