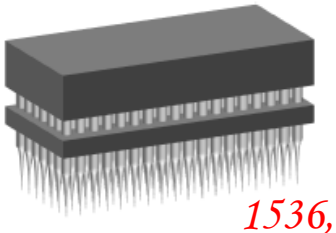
HAMAMATSU
PHOTON IS OUR BUSINESS

Overview of FDSS application:
With a focus on EFS assay

9th June 2016
Hamamatsu 12th European Functional Drug Screening Symposium

Natsumi KATO
Application Engineer
HAMAMATSU PHOTONICS K.K.

Imaging Plate Reader **FDSS** series



Add

1536,384,96wells
at a time

Read

Fluorescence
&
luminescence

FDSS7000EX

FDSS/µCELL

Temperature control

High speed acquisition

Analysis of waveform

expansion

Various options

Electric Field Stimulation (EFS)

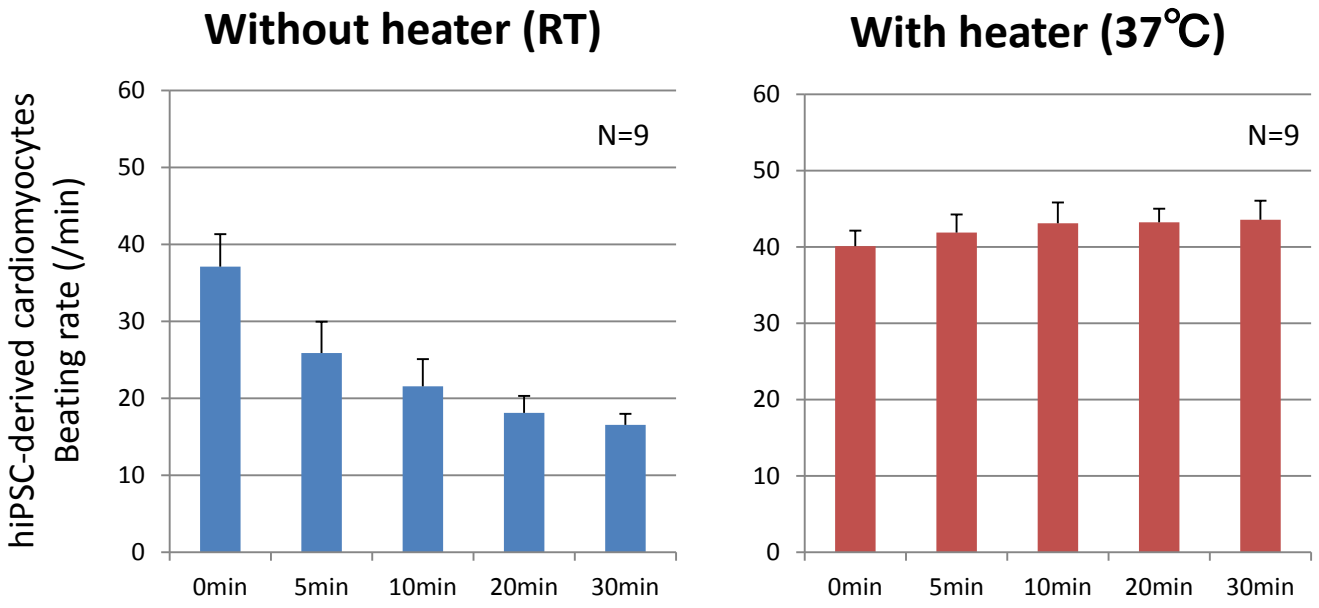
FDSS options

Temperature control

High speed acquisition

Analysis of waveform

Electric Field Stimulation (EFS)



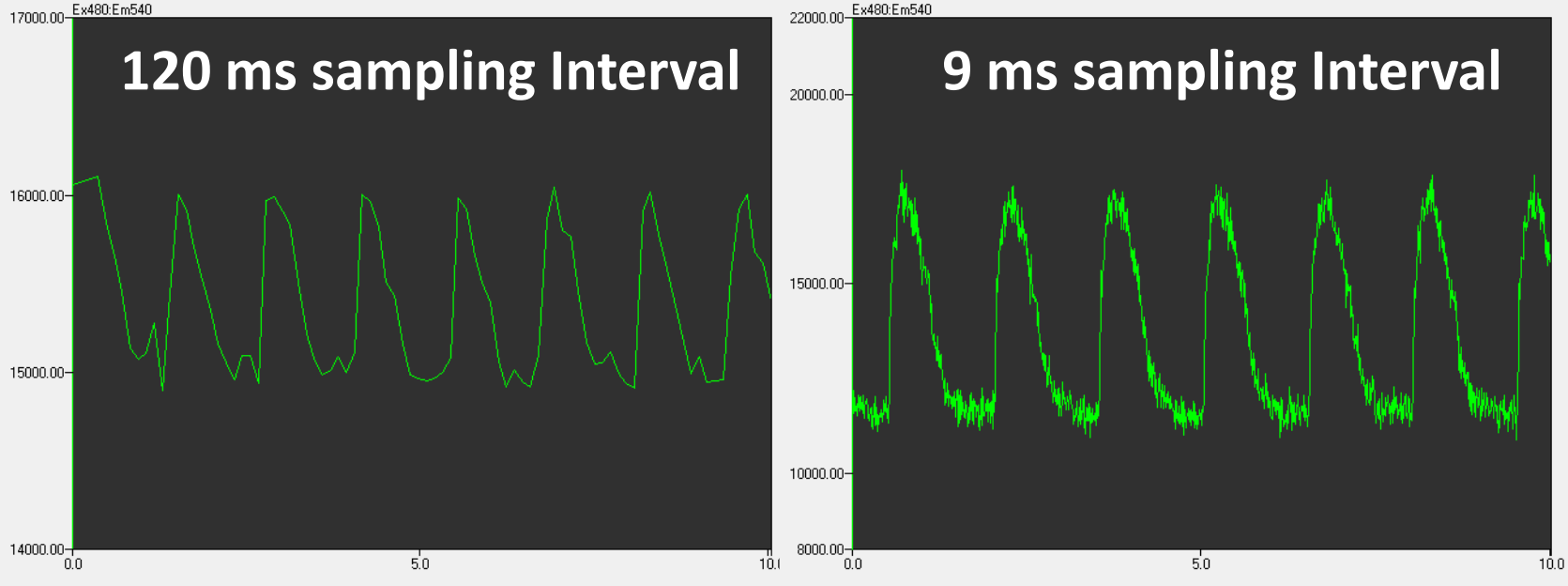
FDSS options

Temperature control

High speed acquisition

Analysis of waveform

Electric Field Stimulation (EFS)



FDSS options

Temperature control

High speed acquisition

Analysis of waveform

Electric Field Stimulation (EFS)

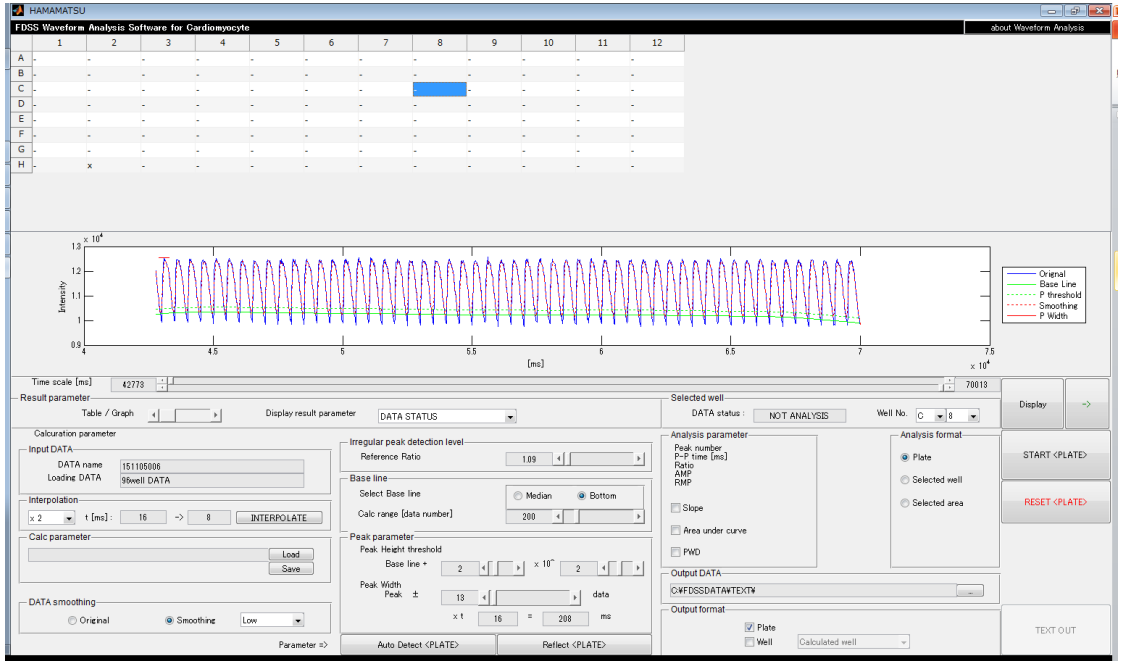


Fig1

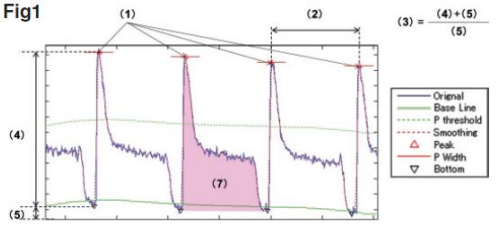
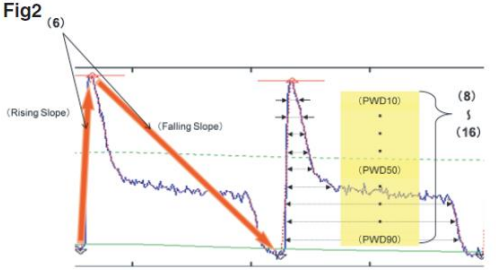


Fig2



FDSS options

Temperature control

High speed acquisition

Analysis of waveform

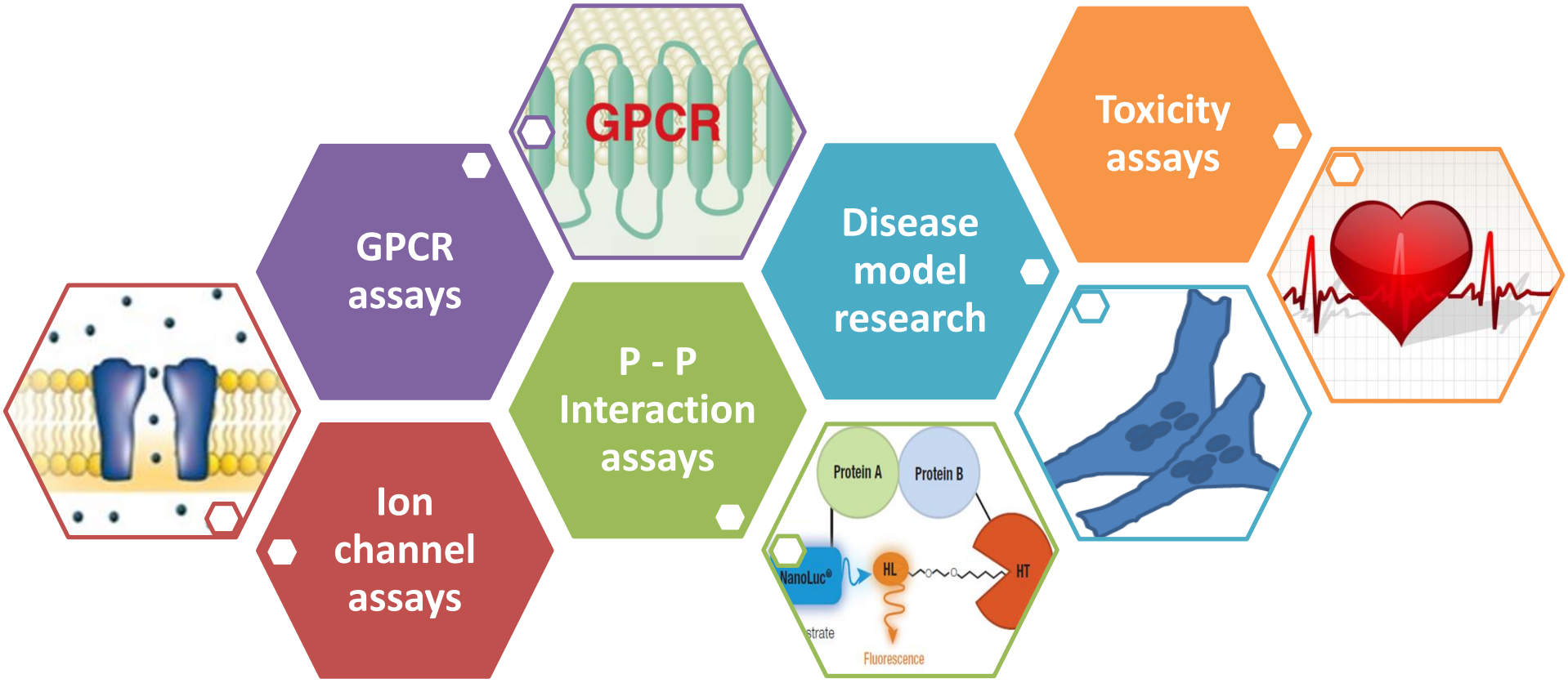
Electric Field Stimulation (EFS)

96-channel electrode array

- ✓ stimulate all 96 wells simultaneously
- ✓ cylindrical electrodes
- ✓ Stimulation voltage is changeable column by column

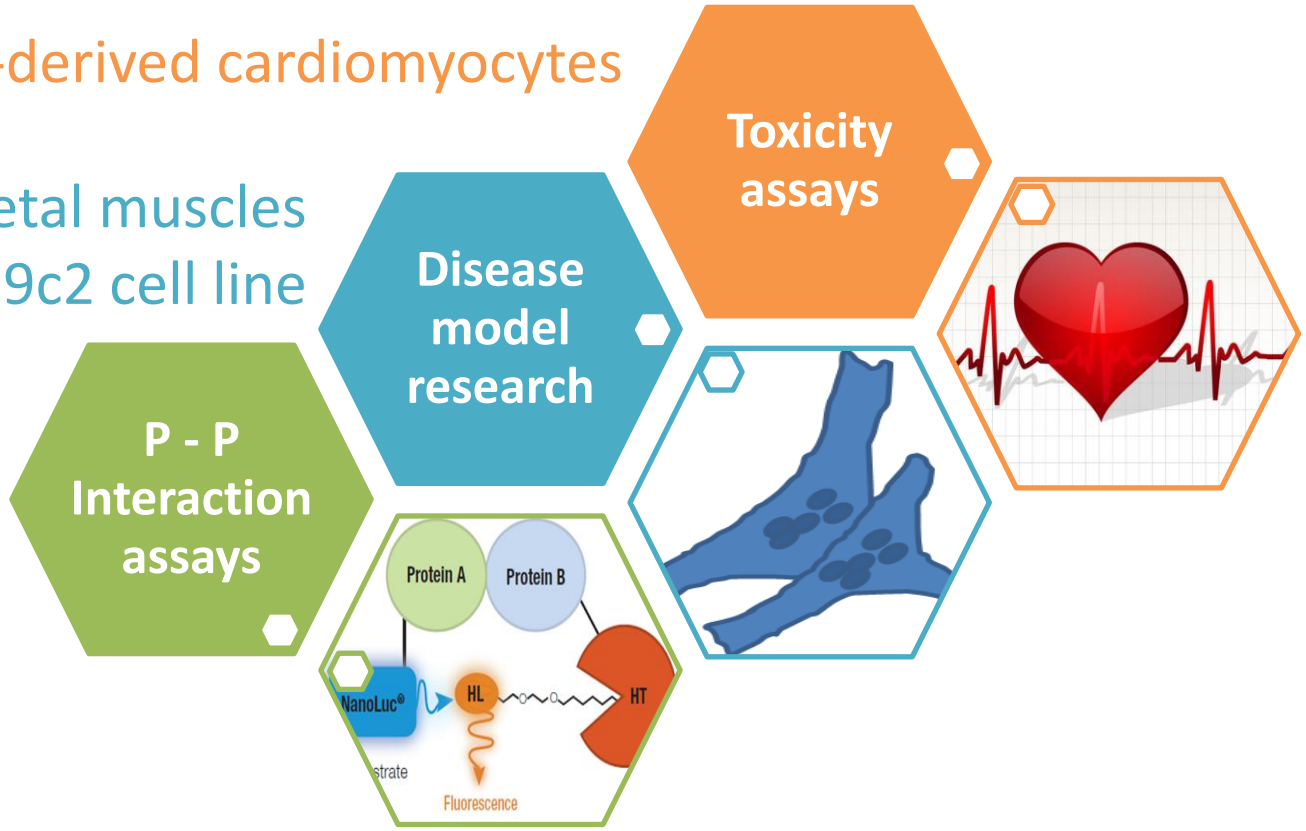
* Only FDSS/µCELL

FDSS applications



Today's topics

1. iPSC-derived cardiomyocytes
2. iPSC-derived skeletal muscles
3. H9c2 cell line
4. nanoBRET



Today's topics

1. iPSC-derived cardiomyocytes

- iPSC-derived cardiomyocytes toxicity assay & EFS availability
- From 2D to 3D culture cells & potential of high resolution camera (well analysis)

Recent information

Journal of Pharmacological and Toxicological Methods

Available online 21 May 2016

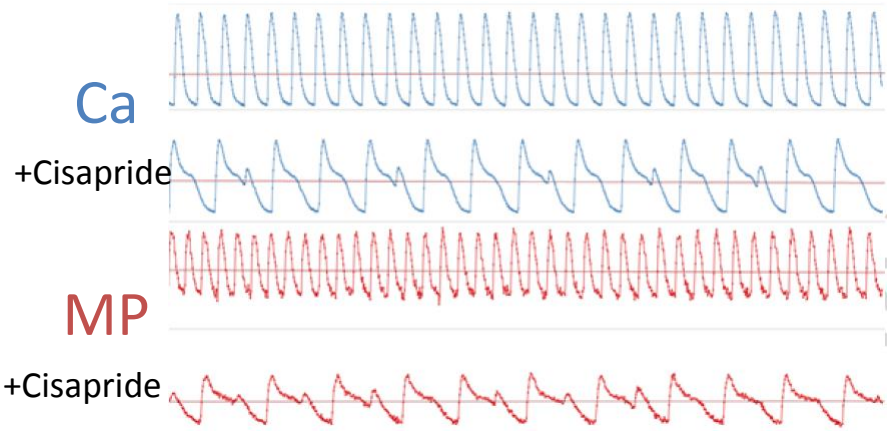
In Press, Accepted Manuscript — Note to users

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 Roman, Edward Lis, Armando Lagrutta, Frederick Sannajust

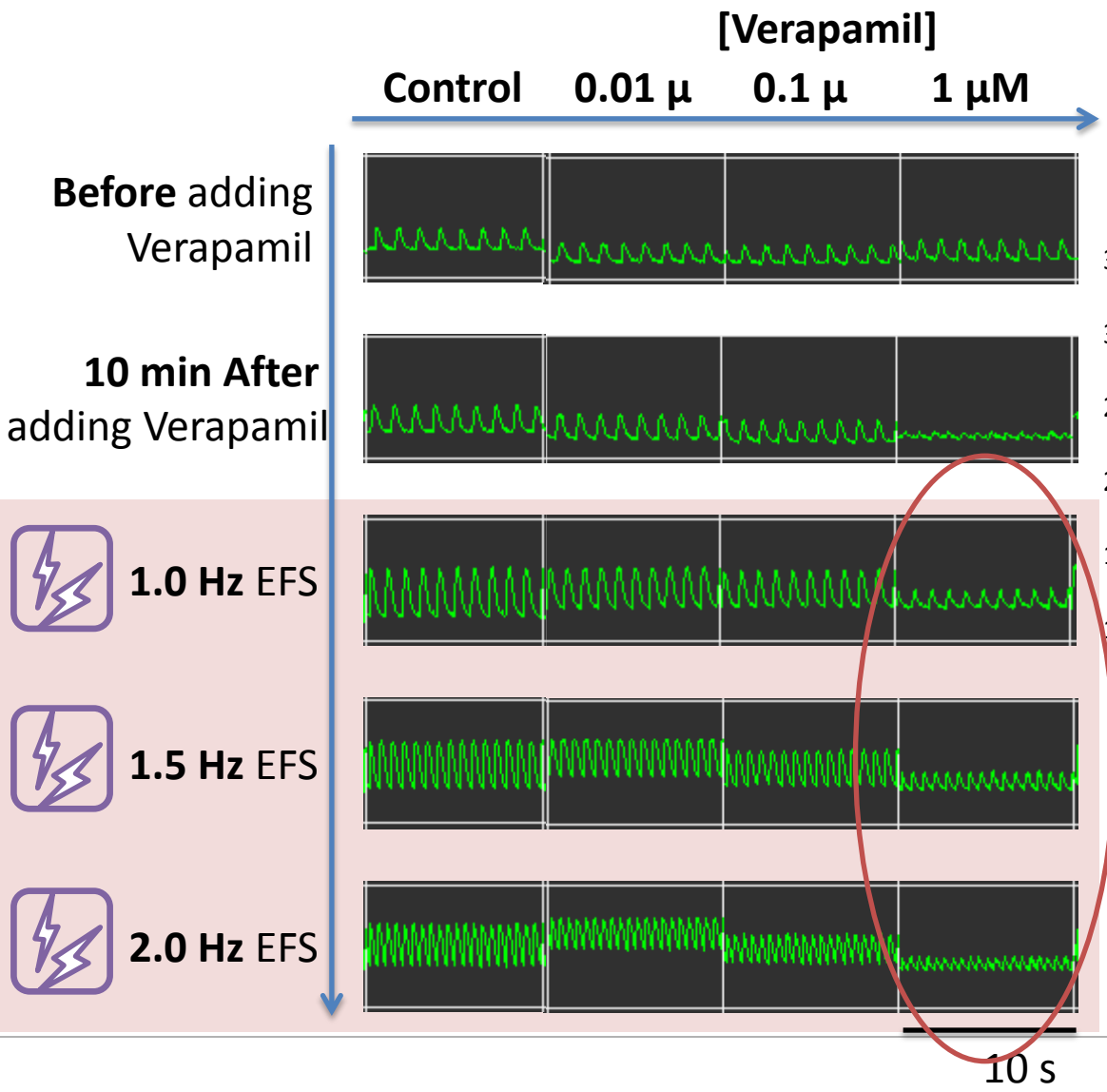
SALAR, Safety & Exploratory Pharmacology Department, Merck Research Laboratories, West Point, Pennsylvania, 19486, U.S.A.

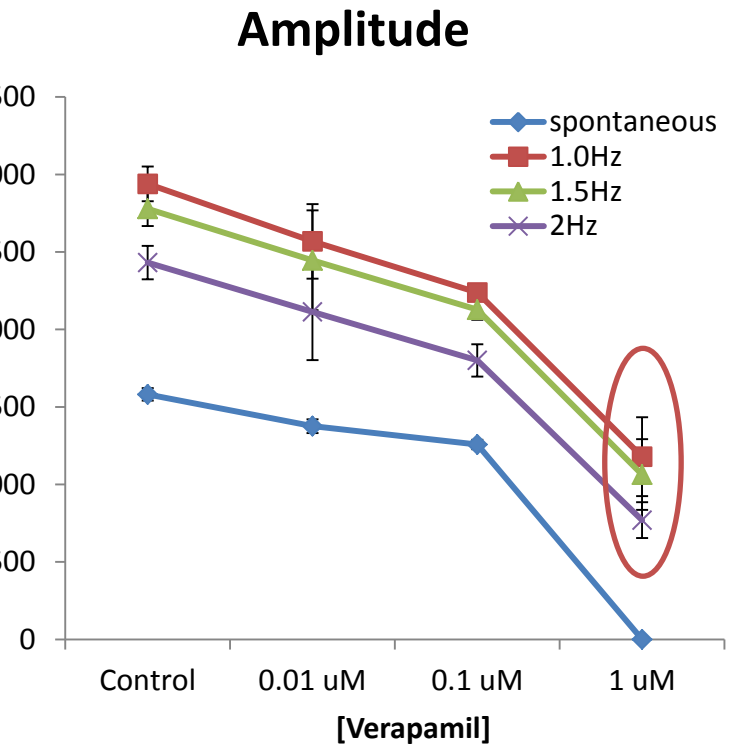
Received 29 February 2016, Revised 6 May 2016, Accepted 18 May 2016, Available online 21 May 2016



…… Our data suggest that **the membrane potential and intracellular Ca^{2+} signal are tightly coupled**, supporting the idea that the EAD-like signals reported are the accurate representation of an EAD signal of the cardiac action potential. Finally, the EAD-like Ca^{2+} signal was well correlated to clinically-relevant concentrations where Torsade de Pointes (TdPs) arrhythmias were noted in healthy volunteers treated orally with some of the compounds we tested, as reported in PharmaPendium®

Human iPSC-derived cardiomyocyte (Cor.4U)
Verapamil (Ca^{2+} channel blocker)

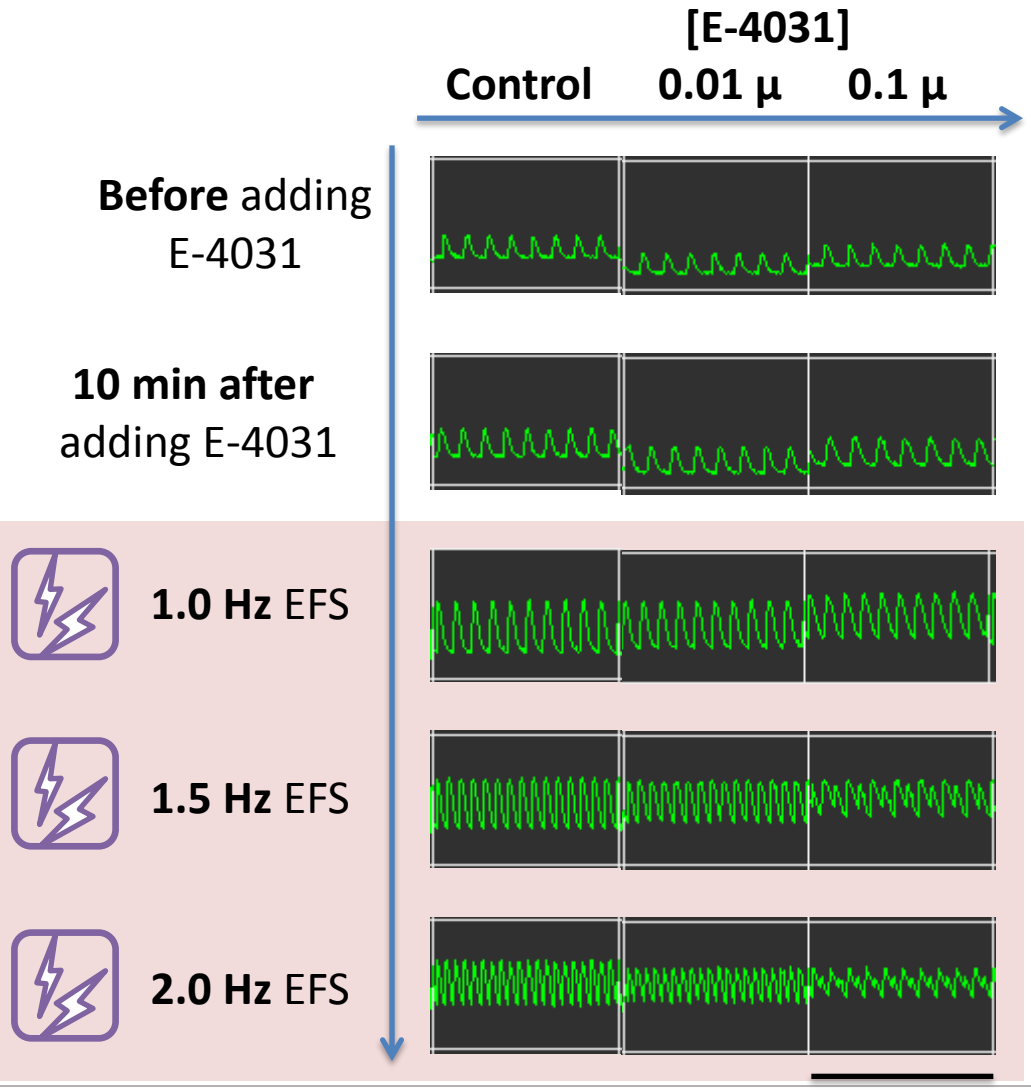


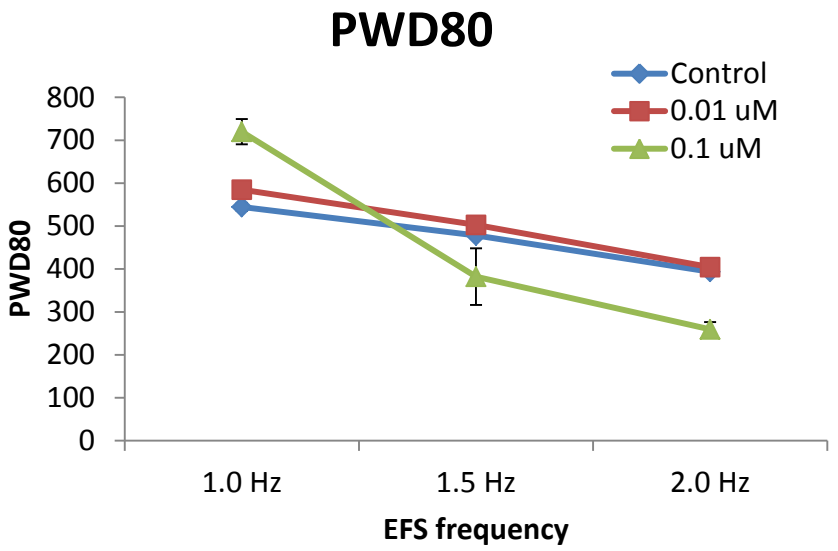


Cell: Cor. 4U (Axiogensis)
Dye: Cal-520 AM(AATbioquest)

Human iPSC-derived cardiomyocyte (Cor.4U)

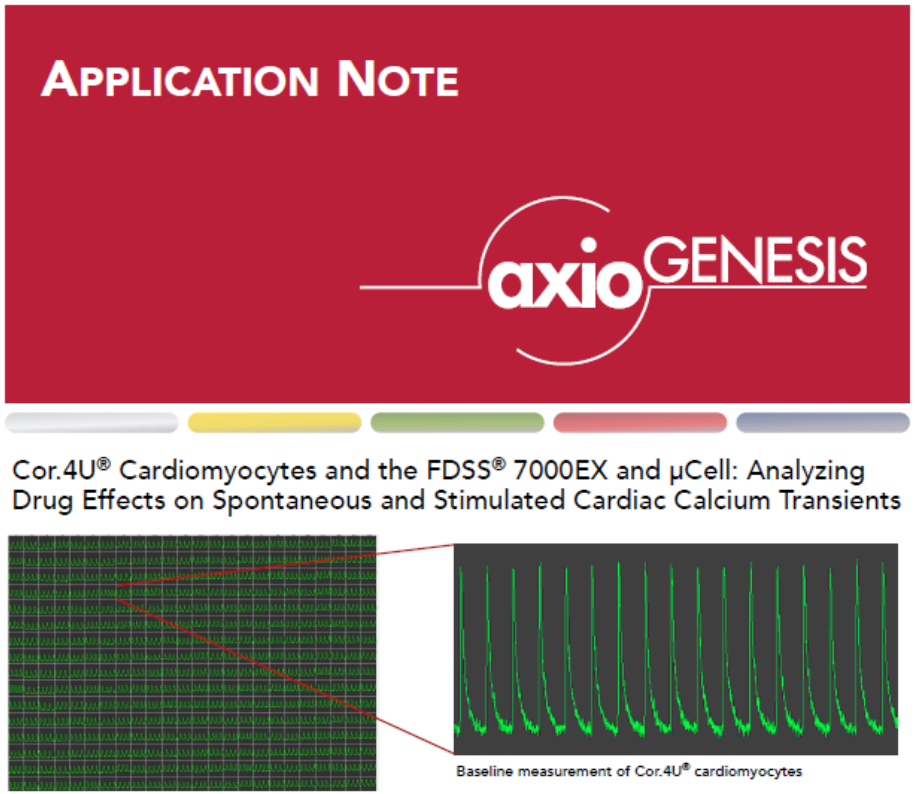
E-4031 (hERG channel blocker)



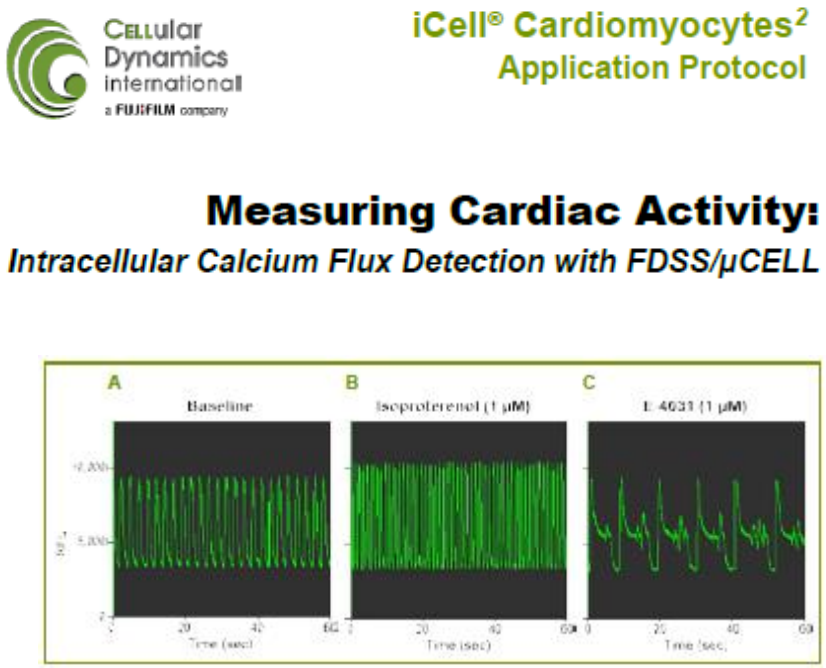


Cell: Cor. 4U (Axiogensis)
Dye: Cal-520 AM(AATbioquest)

Application note & protocol



http://axiogenesis.com/images/phocadownload/application_notes/AppNote_Cor4U_FDSSuCELL.pdf



https://cellulardynamics.com/assets/CDI_iCellCardiomyocytes2-FDSS_AP.pdf

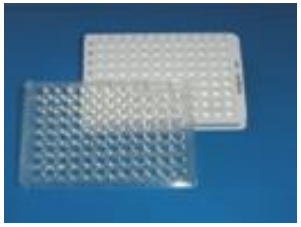
Today's topics

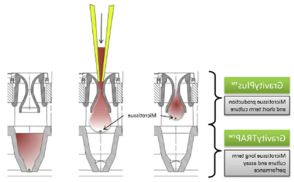
1. iPSC-derived cardiomyocytes

- iPSC-derived cardiomyocytes toxicity assay & EFS availability
- From 2D to 3D culture cells & potential of high resolution camera (well analysis)

Various 3D cell culture microplate

Corning	SUMITOMO BAKELITE	Greiner	Kuraray	InSphero
Coster 3D	PrimeSurface	CELLSTAR®	Elplasia™	GravityPLUS™ GravityTRAP™
black wall, clear U bottom	Clear wall, clear U/V bottom	clear wall, clear U/V bottom	black wall, clear bottom	clear wall, clear bottom
384/96	384/96	394/96/6	384/96/24	96
Hydrogel	Ultra Hydrophilic polymer		Plasma treatment	Hunging drop SureDrop™

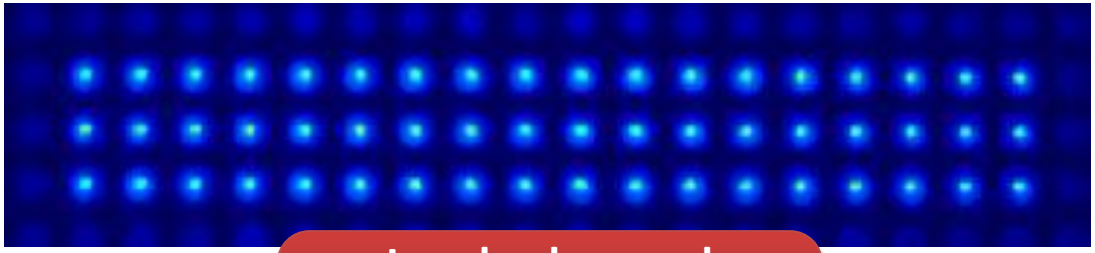



Corning spheroid microplate x FDSS

Corning 96 & 384 well spheroid microplates

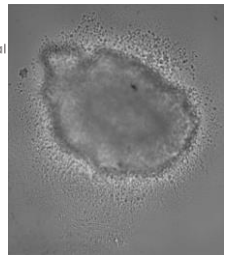
- U shape bottom plate
- Black plate → low background

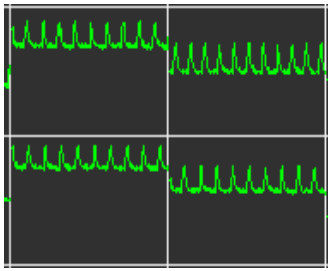
FDSS7000EX



Low background

Cellular Dynamics international



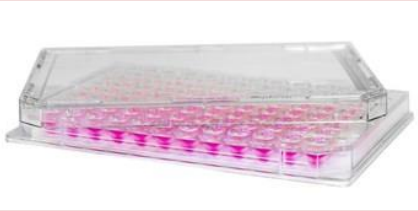


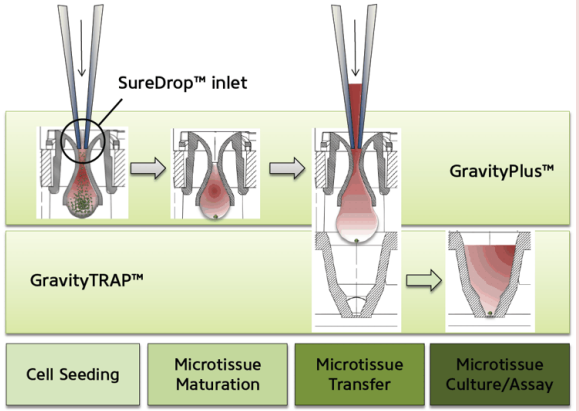
Cells: iCell cardiomyocyte (CDI)
Dyes: Cal-520 AM(AATbioquest)

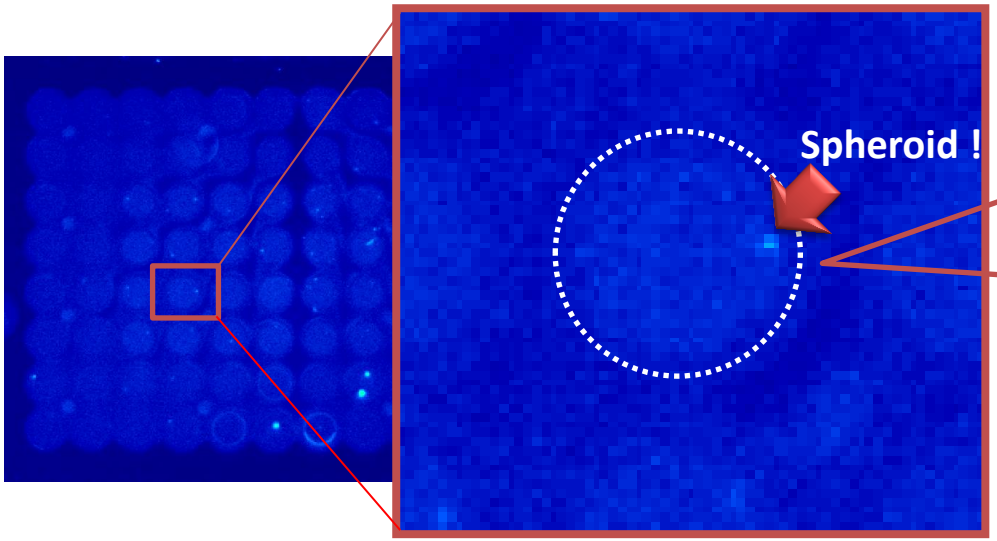
inSphero spheroid microplate x FDSS

GravityPlus™ & GravityTRAP™ (inSphero)

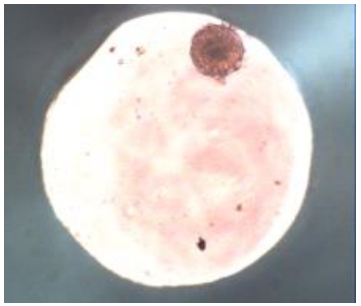
- Hanging drop
- Small number of cells







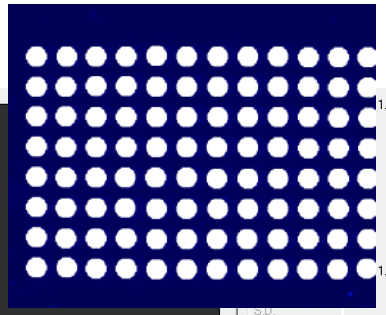
Spheroids transferred from GravityTRAP plate to normal flat-bottom black plate



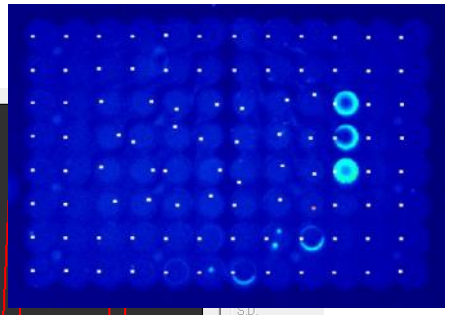
- Neonatal rat primary cardiomyocytes
- Average diameter 217.4um±7.1um

inSphero spheroid microplate x FDSS

ROI 24 (Normal)



ROI 5

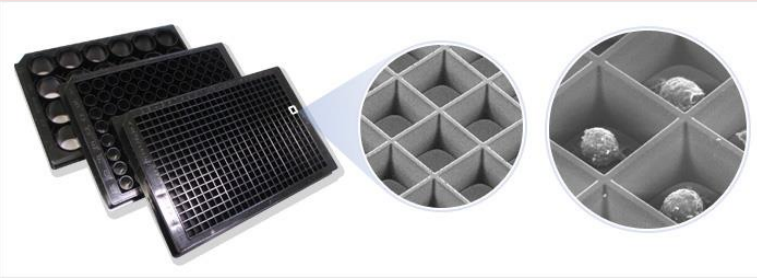


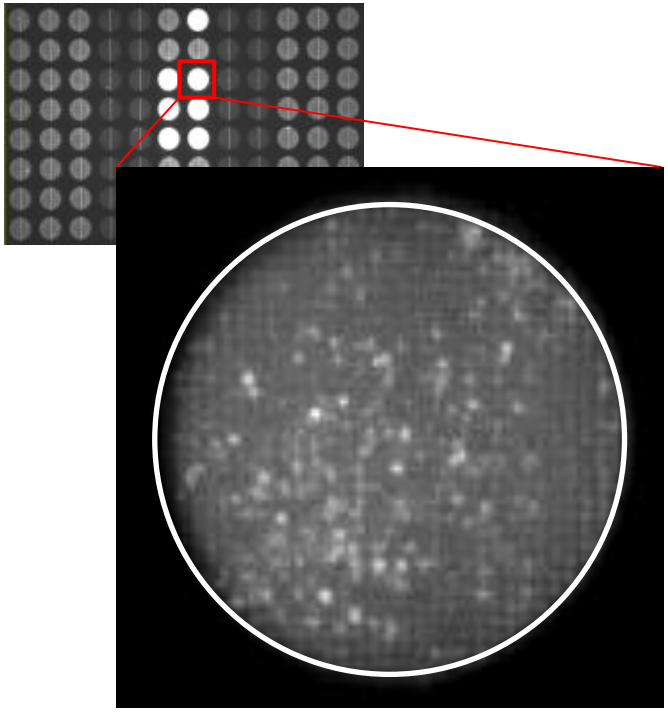
Small ROI is necessary to measure spheroids placed in the edge of the well.

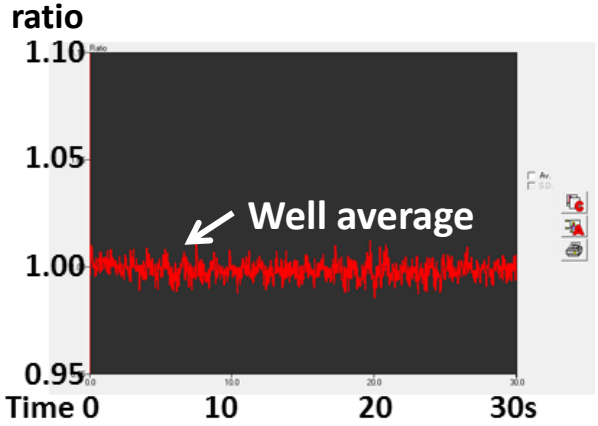
Kuraray spheroid microplate x FDSS

Elplasia™ SQ 200 100 (Kuraray)

- Suitable for high-throughput screening
- SBS 96-, 384-well plate format
- A number of micro-spaces that are divided by wall





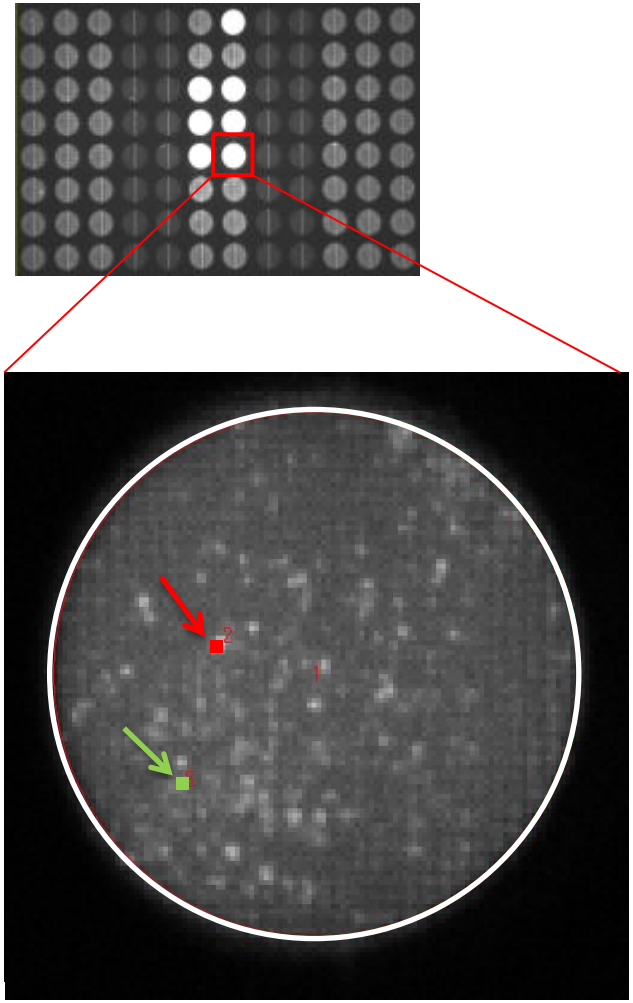


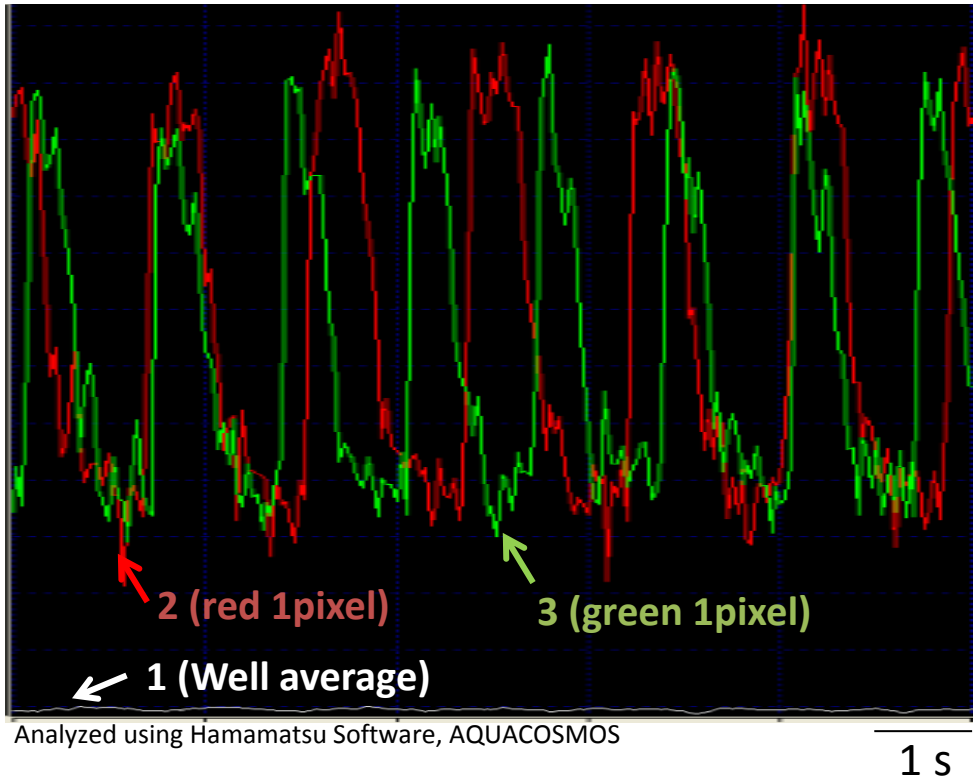
Cells:
Cor. 4U (Axiogensis)
Dyes:
Cal-520 AM(AATbioquest)

- There are thousands of spheroids in a well, each of which beats at each different timing.
- This situation results in that the whole-well measurements show no apparent waveform of Ca^{2+} transients. (Upper picture)

Under Development

Kuraray spheroid microplate x FDSS with **high-resolution camera** (“well analysis”)





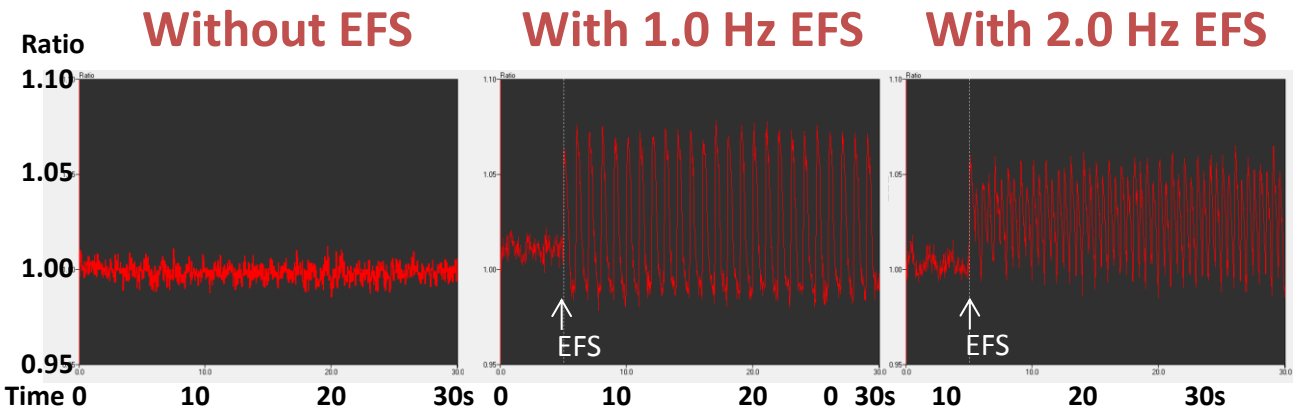
Analyzed using Hamamatsu Software, AQUACOSMOS

1 s

- camera one pixel ≒ one Speroid
- One pixel data shows a Ca^{2+} oscillation of each spheroid

Kuraray spheroid microplate x FDSS **EFS system**

3D microplate (Elplasia)



- Each spheroid in a well beats at each different timing

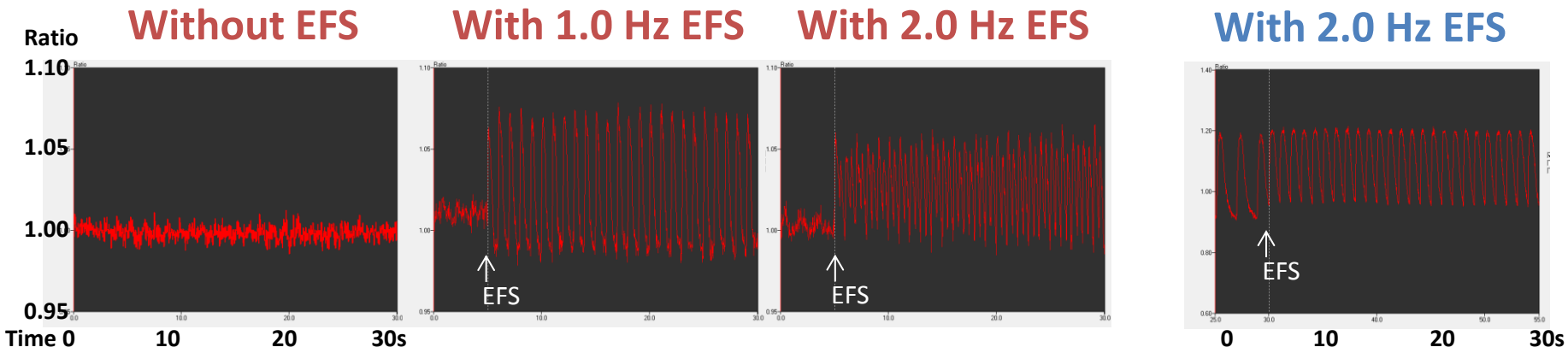
+ EFS

- The beatings of almost all of spheroids in a well were synchronized by electric stimulations

Kuraray spheroid microplate x FDSS EFS system

3D microplate (Elplasia)

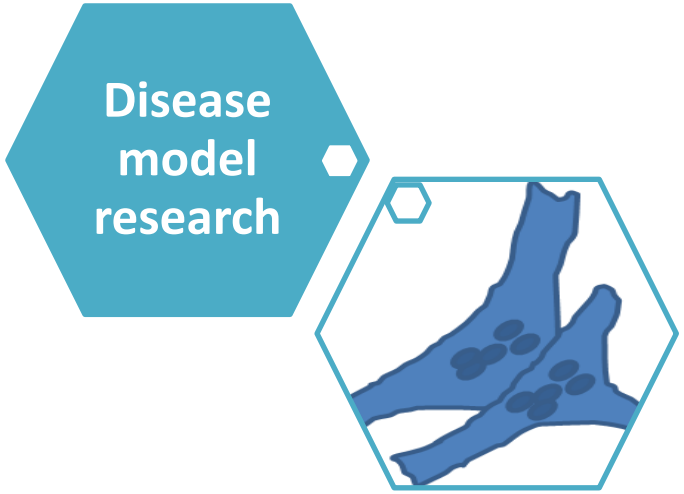
Flat-bottom microplate (2D)



- some differences between 2D and 3D
  - ✓ 3D cells can be paced at 2 Hz
  - ✓ baseline of Ca^{2+} oscillation

Today's topics

2. iPSC-derived skeletal muscles
3. H9c2 cell line



iPS lab (CiRA) Kyoto University, Dr. Sakurai

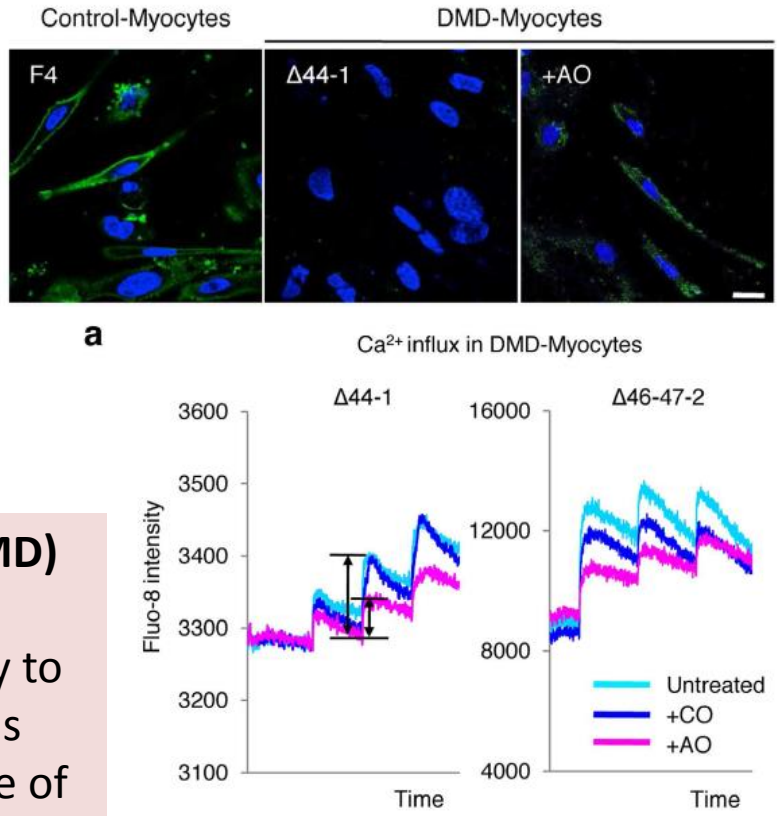
SCIENTIFIC REPORTS

OPEN

Early pathogenesis of Duchenne muscular dystrophy modelled in patient-derived human induced pluripotent stem cells

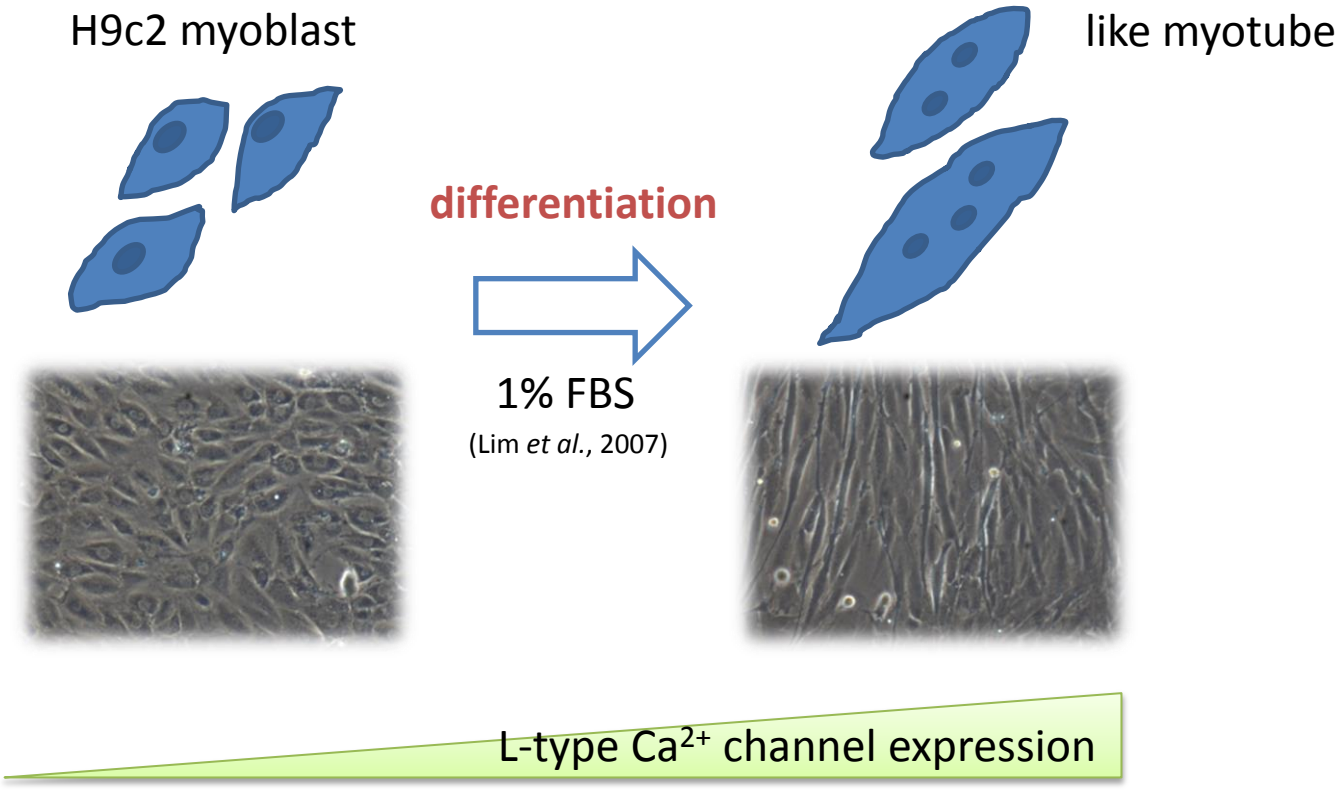
Received: 12 October 2014
Accepted: 11 May 2015
Published: 20 August 2015

Emi Shoji[1,2], Hidetoshi Sakurai[1], Tokiko Nishino[1], Tatsutoshi Nakahata[1], Toshio Heike[3], Tomonari Awaya[3], Nobuharu Fujii[5], Yasuko Manabe[5], Masafumi Matsuo[4] & Atsuko Sehara-Fujisawa[2]



- They made a **Duchenne muscular dystrophy (DMD)** cell model induced from patient-derived iPSCs.
- They stimulated their DMD cell model electrically to simulate muscle cell contraction, finding that cells from DMD patients shows the significant increase of Ca^{2+} influx.
- Such DMD cell models from patient-derived iPSCs have a great potential to develop and evaluate novel drugs for DMD.

H9c2 cells as a disease model



H9c2 cells

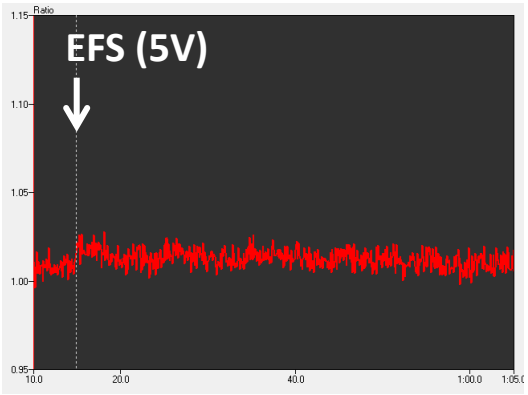
- ✓ rattus myoblast
- ✓ Sometimes used as an alternative for cardiomyocytes

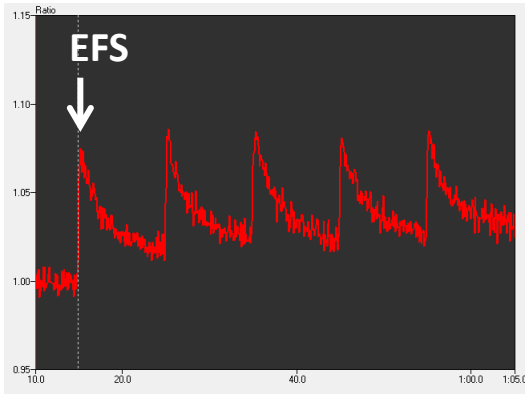
H9c2 cells as a disease model

H9c2 myoblast

differentiated H9c2
(like myotube)

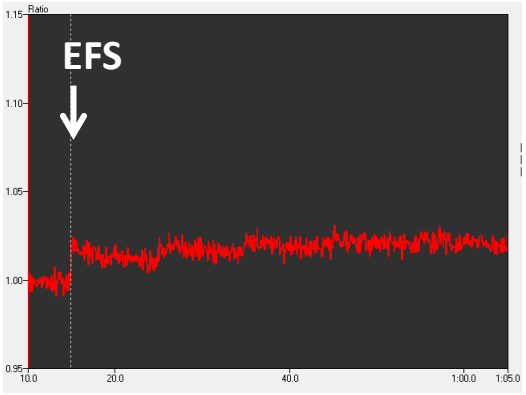
[Ca^{2+}] Ratio
1.15
0.95

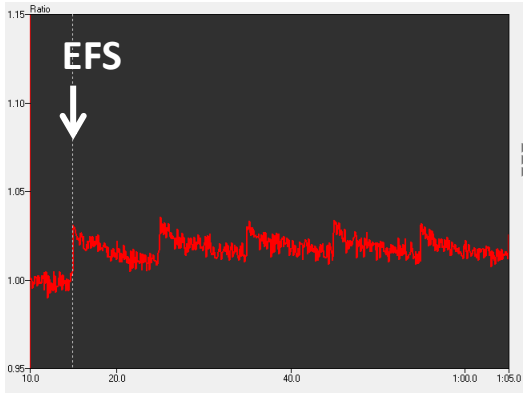




10 s

+ 10 µM Nimodipine
(Ca^{2+} channel blocker)

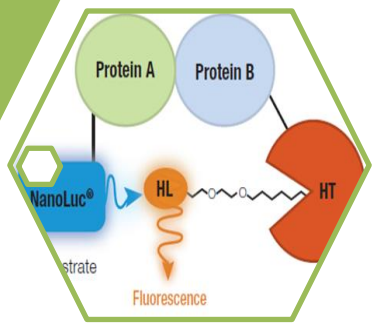




Today's topics

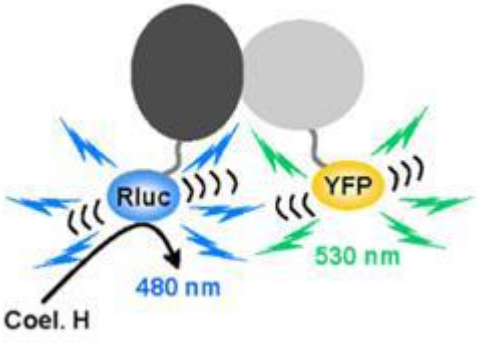
4. nanoBRET

P - P Interaction assays

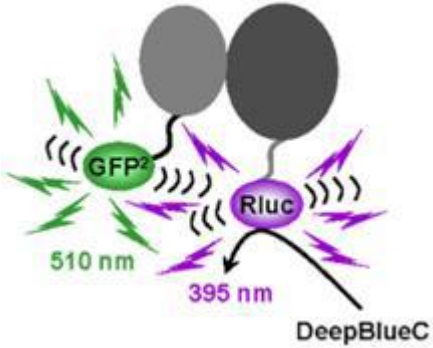


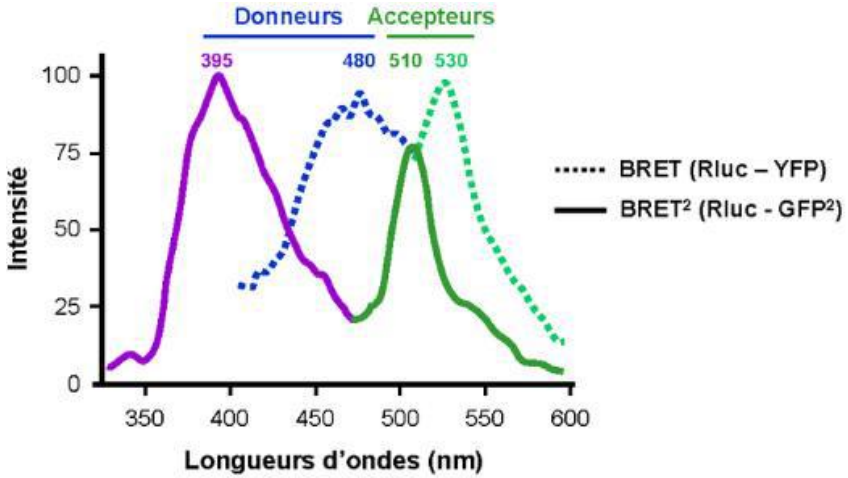
BRET assay

BRET[1]

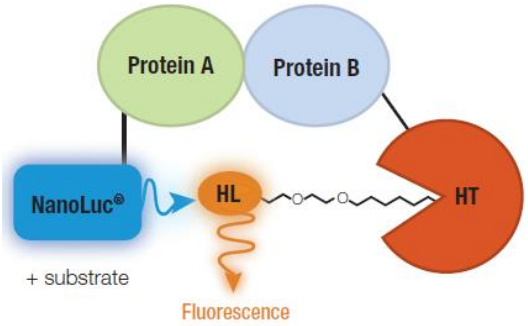


BRET[2]

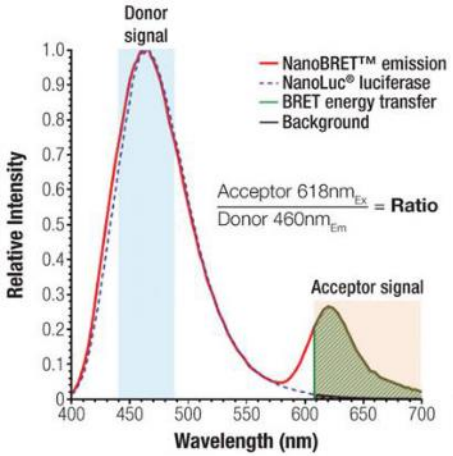




Promega nanoBRET™



HL: HaloTag® NanoBRET™ 618 Ligand **HT:** HaloTag® protein



Promega Technical Manual "NanoBRET™ KRas/BRaf Interaction Assay"

https://fr.wikipedia.org/wiki/Bioluminescence_Resonance_Energy_Transfer

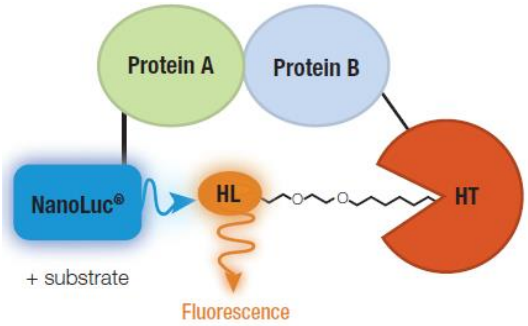
nanoBRET assay

- Very Bright
- Small size (19kDa)
- High emission intensity
- Relatively narrow spectrum (460 nm peak intensity)
- Spectral separation (>175 nm)

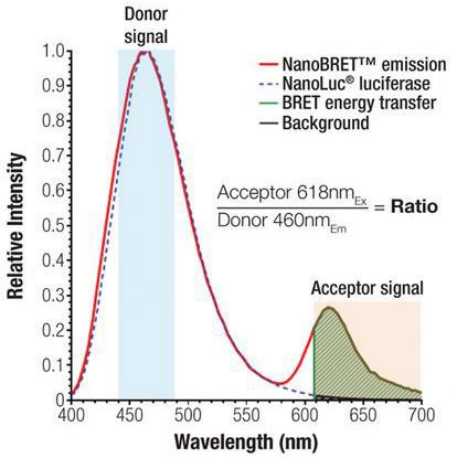
(*ACS Chem. Biol.* 10; 1797, 2015)

Promega

nanoBRET™



HL: HaloTag® NanoBRET™ 618 Ligand **HT:** HaloTag® protein



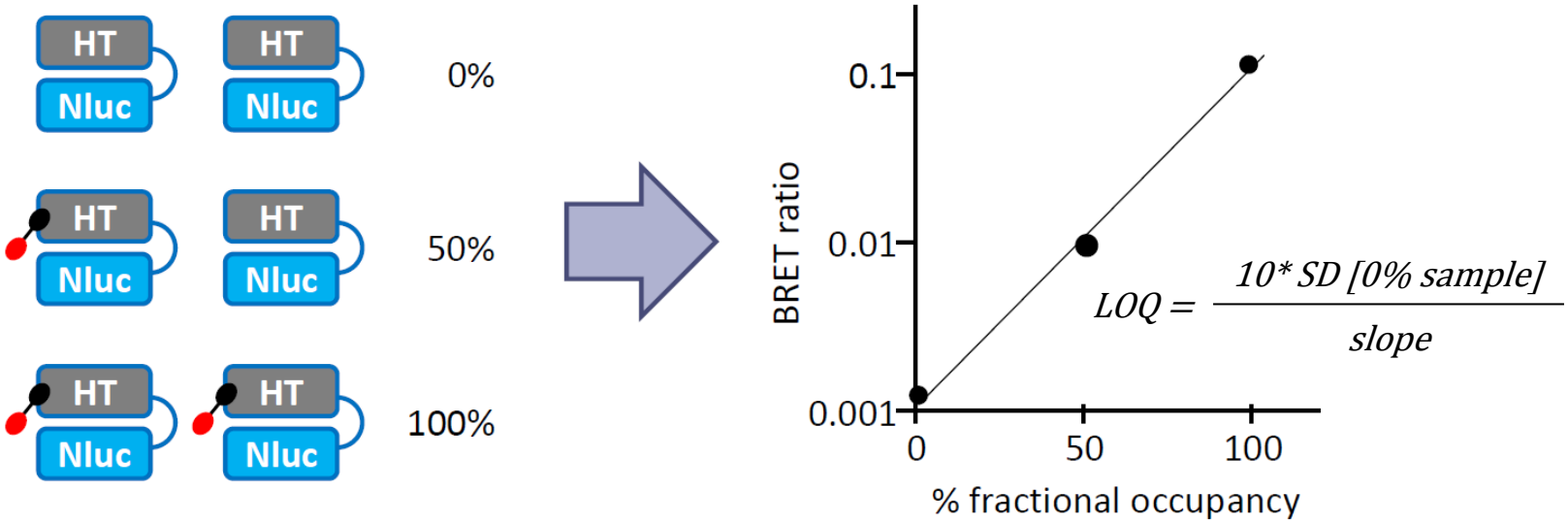
Promega Technical Manual "NanoBRET™ KRas/BRaf Interaction Assay"

NanoBRET™ assay using FDSS luminescence

NanoBRET™ Control Protein Calibration Panel

- NanoLuc-HaloTag fusion protein + NanoBRET™ Control protein
- 5 types of NanoBRET™ Control protein panel

  0%, 0.1%, 1%, 10%, and 100% NL-HT NanoBRET fractional occupancy
- Limit of quantitation (LOQ) represents the minimum percentage of BRET pairs relative to the total donor population



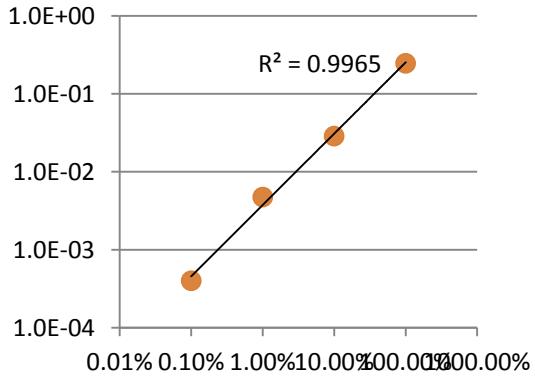
Detection limit of FDSS was validated by using the NanoBRET™ Control Protein

NanoBRET™ assay using FDSS luminescence

Detection of LOQ

FDSS7000EX

LOQ = 0.23378%



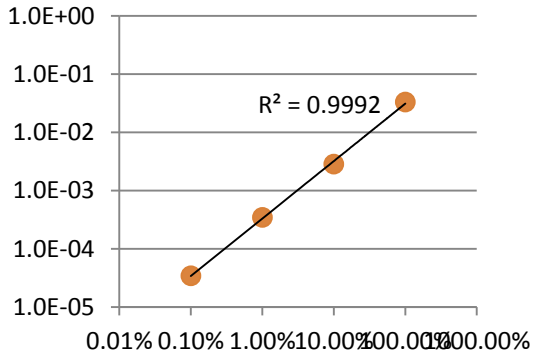
Detector EM-CCD camera
-80℃ (with water cooling OP)
4x4 binning (for 96 well)

Camera Exposure time 1 sec (Ave Lumi)

Filter 456(Donor), 610 (Acceptor)

GloMax®

LOQ = 0.34920%



Detector Photomultiplier tube (PMT)

Data provided by Promega

Summary

- FDSS with the EFS systems would be useful for cardiac toxicity assay and disease model research
- FDSS can measure Ca^{2+} transients in various 3D spheroids of cardiomyocytes
- Spatial analysis inside a well in the FDSS data, which is possible using a new software and a high-resolution camera (under development), could provide another useful information in 3D spheroid experiments.
- FDSS has the equal LOQ to PMT-based microplate reader in measuring nanoBRET

Acknowledgements

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Thank you for your attention

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