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.
Content

Part 1 Overview
- Hamamatsu Photonics
- FDSS7000 and FDSS/uCell
- 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
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 Is Our Business

Hamamatsu’s contribution to Nobel Prize in Physics in 2002 and 2015

Low Light Detectors

Avalanche photodiodes (APD), multi-pixel photon counters (MPPC), photomultiplier tubes (PMT), and detector modules for low light detection
Hamamatsu PET center

Animal PET  Cyclotron

Perfusion
- \(^{18}O\)CO
- \(^{18}O\)CO2
- \(^{18}O\)O2
- \(^{18}O\)H2O

Dopamine
- \(^{11}C\)L-DOPA
- \(^{11}C\)DTBZ
- \(^{11}C\)SCH23390
- \(^{11}C\)NNC112
- \(^{11}C\)Raclopride
- \(^{11}C\)FLB457
- \(^{11}C\)NMPPA
- \(^{18}F\)β-CFT
- \(^{18}F\)FE-CFT

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

Metabolism
- \(^{18}F\)FDG
- \(^{11}C\)Methionin
- \(^{11}C\)CD-CMT
- \(^{18}F\)FD-FET
- \(^{11}C\)Phenylalanine
- \(^{11}C\)Choline
- \(^{18}F\)FIT
- \(^{29}Br\)BFAU

GABA/BZD
- \(^{11}C\)Ro15-1788
- \(^{11}C\)Ro15-4513
- \(^{11}C\)PK11195
- \(^{11}C\)Me-PK11195
- \(^{11}C\)Me-DAA116

Serotonin
- \(^{11}C\)5-HTP
- \(^{11}C\)WAY100635
- \(^{18}F\)MPPF
- \(^{11}C\)MDL100907
- \(^{11}C\)McN5652
- \(^{11}C\)DASB

Glutamate
- \(^{11}C\)Cyano-MK801
- \(^{11}C\)GMOM

Others
- \(^{11}C\)PIB
- \(^{11}C\)FDN
- \(^{11}C\)Verapamil
- \(^{18}F\)FBG
- \(^{18}F\)FBMB
- \(^{18}F\)FHB
- \(^{18}F\)FIMB
- \(^{18}F\)Lipids
- \(^{18}F\)Peptides
- \(^{18}F\)siRNA

Conscious

- \(^{11}C\)SCH23390
- \(^{11}C\)Raclopride
- \(^{11}C\)β-CFT

Ketamine anesthetized

- \(^{11}C\)SA4503
- \(^{18}F\)FM-SA4503
FDSS system – Dispensing and Imaging

1996

Kinetic or “flash” fluorescence or luminescence

FDSS μCell

2000

FDSS7000

2008

2012
What does Hamamatsu FDSS do?

Add
96,384,1536 wells at a time

Microplate

Read
fluorescence & luminescence simultaneously
What has been done on FDSS?

• **GPCR calcium influx assays**
  – **Fluo4 based**
  – Furaz 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
YFP Assay to Identify Modulators of CFTR-ΔF508

Corrector
FSK & potentiatior

Plate cells → YFP reading

24h 37°C → 24h 27°C

DMSO
FSK/Gen
+ I-172
FSK
FSK/Gen

CFTR channel
Fluorescent YFP
Iodide
Quenched YFP

Control

FSK & potentiator

10 μM C4

Normalized Fluorescence Intensity

Time (s)
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

Suc-Ala-Ala-Pro-Phe-MCA, **cis**

Cyclophilin A

Thermo

Suc-Ala-Ala-Pro-Phe-MCA, **trans**

Chymotrypsin

Suc-Ala-Ala-Pro-Phe + AMC

365nm/460nm

10Hz data collection

Plate shaking function

![Graph A](image1)

*Conversion Ratio (%) vs. Time (s)*

- CypA none
- CypA 3.5 nM
- CypA 5 nM
- CypA 7 nM
- CypA 10 nM
- CypA 14 nM
- CypA 20 nM

![Graph B](image2)

*Z’ Factor vs. Plate Number*

Journal of Biomolecular Screening. 14(4) p419-422. 2009
Aequorin-based Functional Assay for GPCR

Agonist

Aequorin + Ca^{++}

Inositol Phosphates

PLC_{3}

GTP

GDP

Apoaequorin

+ Coelenterazine

FLASH LIGHT
(10 to 30 seconds)

E. Le Poul et al., J. Biomol. Screening 2002; 7(1) 57-66
Kinetically Analysis by cAMP GloSensor
Use FDSS to optimize GCaMP sensor

Akerboom et al.,
Springer
Neuromethods 2012
What is new?

- iPSC based cell based assay
- High speed data acquisition on whole plate
- Whole plate electric field stimulation
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)
Neuronal calcium oscillations for preclinical seizure risk evaluation
Spontaneous calcium oscillation in neurons

5 μM DNQX

iCell DopaNeurons

20 μM d-AP5

iCell GlutaNeurons

Baseline
+ 0.2 mM Mg^{2+}
+ 2% FBS
+ 10K AST (no FBS)
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.

- Veratridine response curves
  - 40K cells/well
  - 20K cells/well
  - 10K cells/well

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

- Veratridine EC_50 (μM): 1.10, 1.24, 1.33, 1.84, 1.30
- TTX IC_50 (nM): 40.6, 48.6, 40.7, 6.44, 40

- Veratridine response timecourse
- TTX IC_50 time course

- 2 days
- 28 days
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 Tuner d, Jørgen E. Nielsen e,f, Mikkel A. Rasmussen a,1, Troels T. Nielsen 2, Justus C.A. Daechsel b, Karina Fog b, Poul Hyttel a

a Department of Veterinary Clinical and Animal Sciences, University of Copenhagen, b Lollandbrock A/S, Ottiliarej 9, Vakby 2500, Denmark c Institute of Cellular and Molecular Medicine, University of Copenhagen, Blegdamsvej d Applied Human Molecular Genetics, Kennedy Center, Department of Clinical Genetics e Neurogenetics Clinic & Research Laboratory, Danish Dementia Research Centre, Rigshospitalet, Copenhagen University Hospital, Denmark
High speed is more desirable for iPS cardiomyocytes

**Rate (min⁻¹)**

- **Baseline:** $t = 0.93s$ (n=4)
- **TG:** $t = 1.97s$ (n=4; p<0.005)

**Amplitude (a.u.)**

- **Baseline:**
- **TG:** $\tau = 1.97s$ (n=4; p<0.005)

**Decay**

Baseline: $\tau = 0.93s$ (n=4)
TG: $\tau = 1.97s$ (n=4; p<0.005)

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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

### Amplitude

<table>
<thead>
<tr>
<th>Sampling Rate</th>
<th>8 ms</th>
<th>120 ms</th>
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</thead>
<tbody>
<tr>
<td>CV (%)</td>
<td>2.96</td>
<td>8.20</td>
</tr>
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</table>
5 vs 30 min: Effect of hERG/\(I_{Kr}\) blocker Astemizole

vehicle control

5 min

30 min

41 nM

370 nM
FDSS waveform analysis software
Heat map of cmpds due to their parameters

<table>
<thead>
<tr>
<th>BPM</th>
<th>AMP</th>
<th>RMP</th>
<th>Rising Slope</th>
<th>Falling Slope</th>
<th>Integration</th>
<th>PWD 50%</th>
<th>time dependence</th>
<th>Beating arrest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

- Astemizole
- Cisapride
- Dofetilide
- Quinidine
- Haloperidol
- Sotalol
- E-4031
- Erythromycin
- 4-Aminopyridine
- Veratridine
- Ketokonazol
- ZD7288
- Ivabradine
- Alfuzosin
- Ciprofloxacin
- S-BayK8644
- Amiodarone
- TTX
- Lidocaine
- Mexiletine
- Chlorpromazine
- Clomipramine
- Nifedipine
- Verapamil
- Isoproterenol
- Dobutamine
- Phenytoin
- Serotonin
- Histamine
- Aconitine
- Digitoxin
- Ouabain
- Carbamazepine
- NS1643
- Nicorandil
- SN6
- SB 202190
- Carbachol
- Aspirin
- Atropin

**Legend:**
- Decrease
- No effect
- Increase
Voltage and calcium probes might have different profile

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

Stephane Bodet,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 Cege2

1Laboratoire SERVIER de Chimie génétique, Institut du Cerveau et du cerveau, Hôpital de la Salpêtrière, Paris, France.
2Pôle 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

Codex ACTOne® dye

![Graph showing impact of Codex ACTOne® dye on cardiomyocytes.]

EarlyTox® dye

![Graph showing impact of EarlyTox® dye on cardiomyocytes.]

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

Hanyu Zeng *, Maria L. Roman, Edward Liu, Armando Lagutta, Frederick Sannajest

SALK Institute & Salk Institute Pharmacy Department, Alere Research Laboratories, West Hollywood, CA, 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

<table>
<thead>
<tr>
<th>Voltage</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1V</td>
<td>3x10^4 cels/well</td>
</tr>
<tr>
<td>2V</td>
<td>2x10^4 cels/well</td>
</tr>
<tr>
<td>3V</td>
<td>1x10^4 cels/well</td>
</tr>
<tr>
<td>4V</td>
<td>0.5x10^4 cels/well</td>
</tr>
</tbody>
</table>

After

<table>
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<th>Count</th>
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<td>1V</td>
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</tr>
<tr>
<td>3V</td>
<td>1x10^4 cels/well</td>
</tr>
<tr>
<td>4V</td>
<td>0.5x10^4 cels/well</td>
</tr>
<tr>
<td>5V</td>
<td>3x10^4 cels/well</td>
</tr>
<tr>
<td>6V</td>
<td>2x10^4 cels/well</td>
</tr>
<tr>
<td>7V</td>
<td>1x10^4 cels/well</td>
</tr>
<tr>
<td>8V</td>
<td>0.5x10^4 cels/well</td>
</tr>
</tbody>
</table>

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

without pacing  
with pacing

untreated
dofetilide

E-4031
Motor neurons have matured electrophysiologically
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