FDSS Applications

- Cardiovascular Disease
- Safety/Toxicology
- Cell Health and Energy Metabolism
- Protein-Protein Interaction
- GPCR
- Antibody
- Enzymatic Assay
- Ion Channel
- New Screening Technology
- Optogenetic
- New High Resolution Camera
- High Speed Acquisition

HAMAMATSU
PHOTON IS OUR BUSINESS
Cell Health and Energy Metabolism

Oxidative Stress Assay (ROS)
Assays for measuring real time production of ROS (Reactive oxygen species) as an indicator of cell heath or signalling events

Energy Metabolism Assay (NADPH)
NAD(P)H molecules are important cofactors for different enzymes involved in cellular pathways. Measurement of this molecule can determine metabolic activity for cells affected by disease.

Identification of small molecule modulators of reactive oxygen release in TNF-α primed primary human neutrophils.
In the test system freshly isolated primary human neutrophils are first incubated with TNF-α. The cells are then triggered with cytochalasin B. The superoxide anions produced are measured by a chemiluminescence technique based on isoluminol using the single photon counting camera of the Hamamatsu FDSS 7000.

Recording signal for 5 minutes

Protein-Protein Interaction (PPI)

Living-cell PPI assay is based on BRET (Bioluminescent Resonance Energy Transfer) screening technology

Therapeutic area
Research & Clinical application
- Inflammatory disease
- Monitoring inflammation of transplant before surgery
- Cancer (drug evaluation studies for Chemotherapy)

FDSS Screening technology
Amplex red (Thermofisher)/Luminol/ROS-Glo (Promega) NADPH-Glo/Pholasin®-based ABEL® (Knight Scientific)

Publication: Application Note Nr. 25 (CBCS, Karolinska Institutet, University of Gothenburg)
Any acute drug effect involved in primary or human iPSC-derived cardiomyocytes can be detected by monitoring \([\text{Ca}^{2+}]\) transients and electrical activity.

**FDSS Screening technology**

\([\text{Ca}^{2+}]\) sensitive dye (Cal520, ATT Bioquest)/Voltage sensitive dye (FluoVolt, Molecular probes)

**Therapeutic area**

Drug discovery, Safety pharmacology (Cardiac & Neuron), Assessment of cardiotoxicity and drug efficacy early in drug development process

**Application Note Pluricyte® Cardiomyocytes**

- 0.1 % DMSO
- 1 µM Diltiazem
- 1 µM Bay K 8644
- 100 nM E 4031
- 1 µM Nifedipine
- 100 nM E 4031
- 1 µM Isoproterenol

**Application Note COR.4U®**

- Baseline
- Isoproterenol (1 µM)
- E-4031 (1 µM)

**Presentation 3rd FDSS application Workshop, Barcelona, Stephane Bedut (ICM, Servier)**

Membrane potential activity hiPS-derived cardiomyocytes in 96 wells

- Ivabradine 1 µM
- Lidocaine 50 µM

Acquired 10 s (black) and 3 min. (red) after ouabain treatment (96 format, FluoVolt dye)
**GPCRs (G-protein-coupled receptors) are one of the most important classes of drug targets**

**Effect of GPCR pathway activation:**
- Intracellular Ca$^{2+}$, cAMP, Beta Arrestin

**Therapeutic area**
- Drug discovery

**FDSS Screening technology**
- [Ca$^{2+}$] sensitive dye (Cal520, ATT Bioquest)/Voltage sensitive dye (FluoVolt, Molecular probes), BRET, cAMP (Glow sensor, Promega), Beta Arrestin (Discoverx)

**Recording of Calcium transients using the Hamamatsu µCell: Response to neurotransmitter**

(a) Representative traces from calcium transients induced by various neurotransmitters. KCl as positive control and thyrode solution as negative control are shown. The traces are normalized to the baseline fluorescence.

(b) The peak of calcium transients are calculated and plotted against the neurotransmitter concentration.

**Publication: FDSS Application Nr. 23**

(a) Antagonism  
- Dopamine 1 µM  
- Haloperidol 10 µM +  Dopamine 1 µM  
- Haloperidol 10 alone

(b) Dose-response  
- Dopamine EC50=7.3

(c) Efficacies

The antagonist Haloperidol fully reverses the BRET signal induced by dopamine (a). The stimulation by dopamine is dose-dependent (b). Dopamine efficacy can be discriminated from other compounds activity (c).

**Print screen FDSS software**

**GPCR assay:**
- 1536 assay – Ca$^{2+}$ Aequorin
- B2-Arrestin 2 Recruitment

**ACTELION**
- Full agonist vs. partial agonist: w/o full view  
  -> misleading
- Efficacy vs. AUC vs. Slow binders  
  -> kinetics
- Time course is key
Publication: YFP-halide assays for CFTR drug discovery using the FDSS/µcell

Thierry Christophe, PhD, Director - Biology, Galapagos NV
Hamamatsu 11th FDSS User Meeting 11 June 2015

**Detection of CFTR potentiators**

Cells over-expressing F508d-CFTR and YFP-H148/I152L

**Therapeutic area:**
Drug discovery

**FDSS Screening technology**

- **Na+/K+ channel:**
  - TEFLAB dye, Membrane potential dye (Molecular device)/FluxOR
  - Potassium Ion Channel Assay (Thermo Fisher)/ISBFI (Thermo Fisher)/FRET VSP dye (Invitrogen)
- **Cl- channel**
  - Fluorescence of Yellow Fluorescent Protein mutant (YFP-H148Q/I521L) quenched by halides

**Antibody (Functional Assay)**

**Therapeutic area**
Drug discovery (ion channel/GPCR)

**FDSS Screening technology**

- **Ca**²⁺ dye

**Enzymatic Assay**

(Isomerase Inhibitor Screening)

**Use of a Real-Time Fluorescence Monitoring System for High-Throughput Screening for Prolyl Isomerase Inhibitors**

Tadashi Mori, Selma Itami, Tomotaka Yanagi, Yota Tatara, Mari Takamiya and Takafumi Uchida

The Kₘ value of CypA with the peptide was 59 M. (C) Concentration dependence of CsA inhibition of CypA activity. Concentrations of CypA and CsA are expressed as follows: CypA none, CsA none (●); CypA 7 nM, CsA none (○); CypA 7 nM, CsA 12.5 nM (△); CypA 7 nM, CsA 50 nM (▽); CypA 7 nM, CsA 125 nM (★); CypA 7 nM, CsA 3.13 nM (▲); CypA 7 nM, CsA 50 nM (♦); and CypA 7 nM, CsA200 nM (★).
New Screening Technology

96-channel electrode array
- Stimulate all 96 wells simultaneously
- Cylindrical electrodes
- Stimulation voltage is changeable column by column

Therapeutic area
- Cardiac, CNS, Muscular diseases

FDSS Screening technology
- Ca²⁺ dye/(FDSS/µcell + EFS + High speed options)

High-Throughput Fluorescence Measurements of Ca²⁺ Transients in primary rat and in human iPSC-derived cardiomyocytes

a) Primary rat cardiomyocytes (Cosmo Bio) were cultured in 96-well plate. The above figures show the intracellular Ca²⁺ concentration changes for 5 s in 96 wells in a microplate. In primary cultured cardiomyocytes, cells in each well beat at different rates and with different timing (left). When electrical field stimulation was applied (1.0 Hz, voltage 5 V, duration 5 ms), the result was uniform Ca²⁺ oscillations between all wells resulting in synchronized beating of cells (right).

b) Effects of ion channel blockers on Ca²⁺ transients in iPS cardiomyocytes: Beating rate is dependent on Electric Field Stimulation frequency.

Electric Field Stimulation (EFS) of human iPSC-derived neuron using Hamamatsu FDSS/µCELL

The Ca²⁺ response is inhibited in the presence of a calcium channel blocker, Bepridil

Bepridil, a calcium channel blocker, was added to the wells at the indicated concentration and incubated for 20 min, and then EFS pulses (5 ms of pulse width, 40 Hz) were given. The figures on the right show intracellular Ca²⁺ concentration changes. At more than 15 µM, the Ca²⁺ response was completely inhibited. The IC₅₀ value was estimated to be 5.6 µM (graph on the left).

Presentation CDI 12th FDSS Users Meeting

iCell Skeletal Myoblasts

iCell SKM + EarlyTox calcium dye

Compound Treatment

Electric Field Stimulation (EFS)
**Optogenetic**

**Therapeutic area:**
Drug discovery

**FDSS Screening technology**
- **Detection system:**
  Ca^{2+} sensitive Fluo8 No Wash dye
- **Stimulus:**
  Depolarization induced by ChR2 activation with blue light pulses

- **Target:** human Cav1.3(α1/α2δ1/β3)
- **Recipient cell line:** HEK-293/Kir2.3
- **Detection system:** Ca^{2+} sensitive Fluo8 No Wash dye
- **Stimulus:** depolarization induced by ChR2 activation with blue light pulses
- **Cav1.3 Blocker:** Isradipine
- **HTS instrumentation:** Hamamatsu FDSS/µcell

**Other information:** FDSS/µCell & FDSS7000

**High Speed Acquisition & Fast Camera Option**

**Application:**
Enzymatic, Optogenetic, Cardiac assay...

**Speed of acquisition:**
200 Hz (96 well) 100 Hz (384 well)

**New High Resolution Camera (“Well Analysis”)**

Measurement 3D Cardiomyocyte (Spheroid)
- Kuraray spheroid microplate (cardiomyocyte)
  - 1 pixel around 1 spheroid
  - 1 pixel data showed Ca^{2+} oscillation of each spheroid.

Analyzed using Hamamatsu Software, AQUACOSMOS