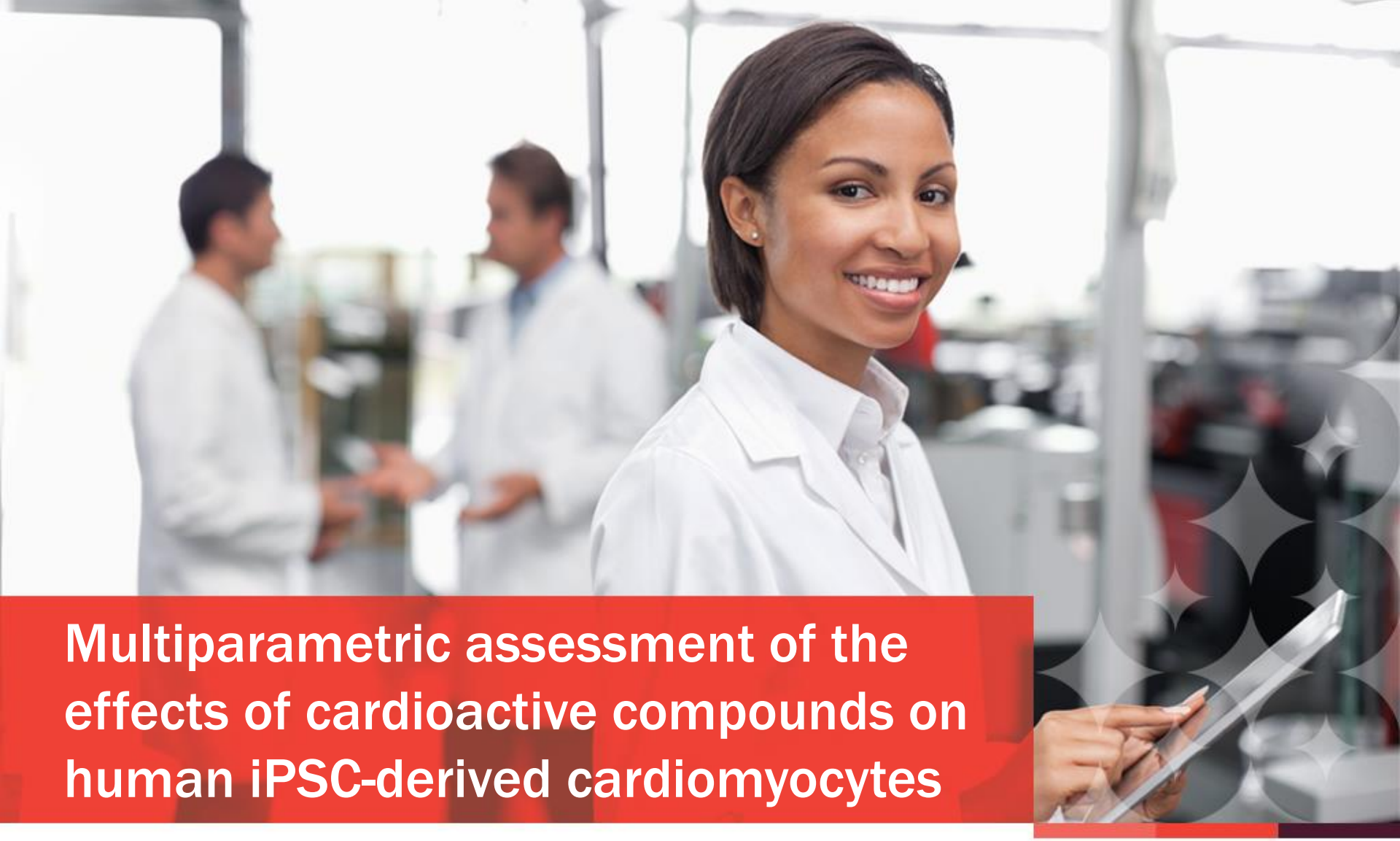


Multiparametric assessment of the effects of cardioactive compounds on human iPSC-derived cardiomyocytes

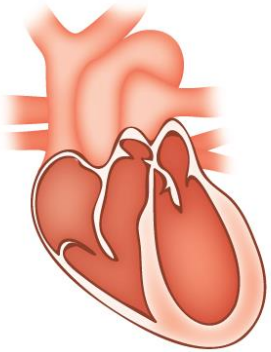
pluriomics
CLOSE TO THE HEART

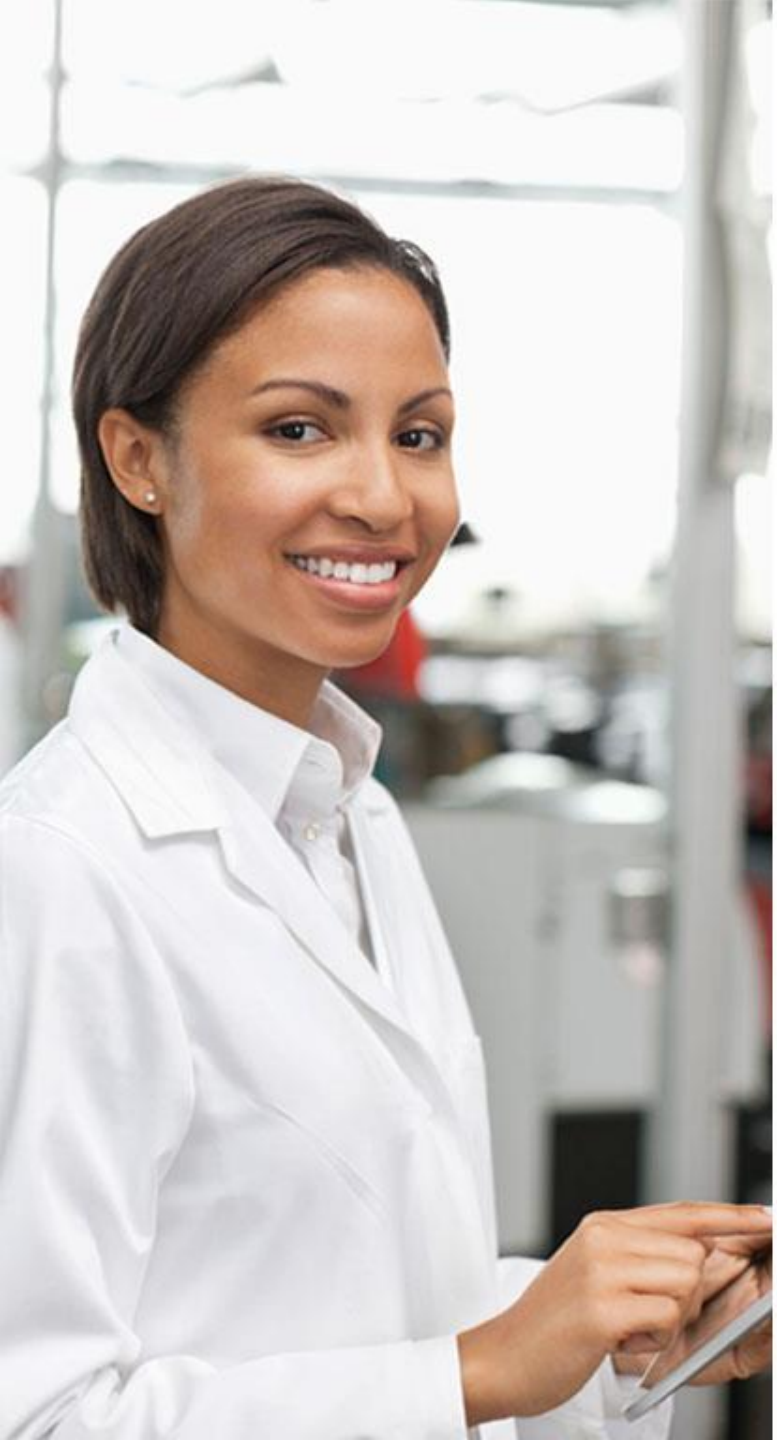
16th FDSS Users Meeting
June 9th, 2016

Marijn Vlaming, PhD
VP Technology
marijn.vlaming@pluriomics.com

About Pluriomics

- Operational since 2012
- R&D labs in the Leiden Bio Science Park (NL)
- Sales & Marketing in the Leiden Bio Science Park (NL)
- Production facilities in the Gosselies Biopark (BE)
  - QMS
  - Cell production
  - Medium production
- Currently 21 people





Our mission

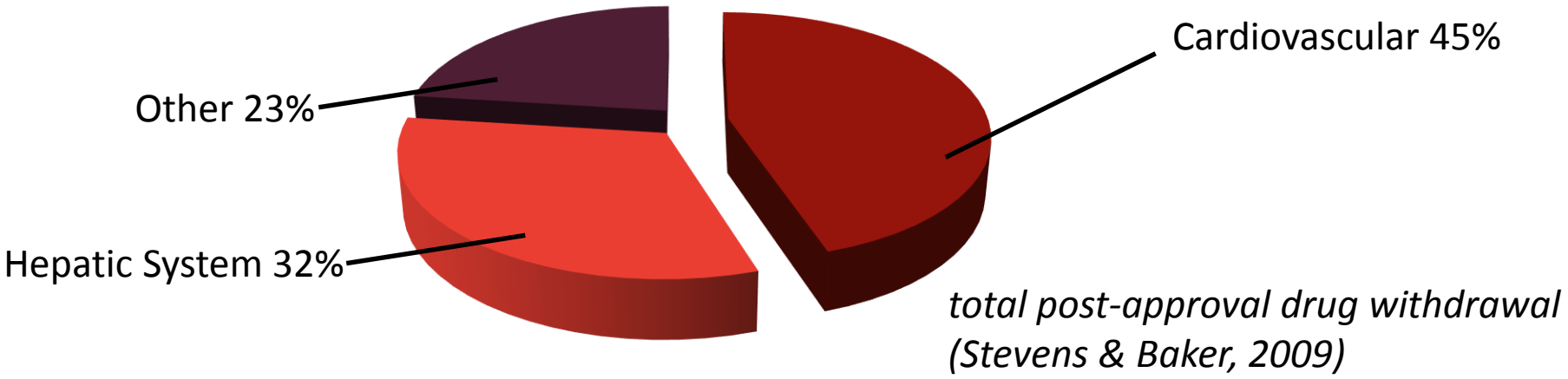
Implement human stem cell technology in biopharmaceutical R&D

- To improve efficiency of drug discovery and development by selecting for novel and safe drug candidates early on
- To significantly reduce animal experiments
- To personalize drug discovery and development

Cardiovascular toxicity in drug development

Cardiovascular toxicity:

- Represents most frequent serious adverse drug reaction *(Redfern et al., 2010)*
- Major cause of withdrawal of marketed drugs

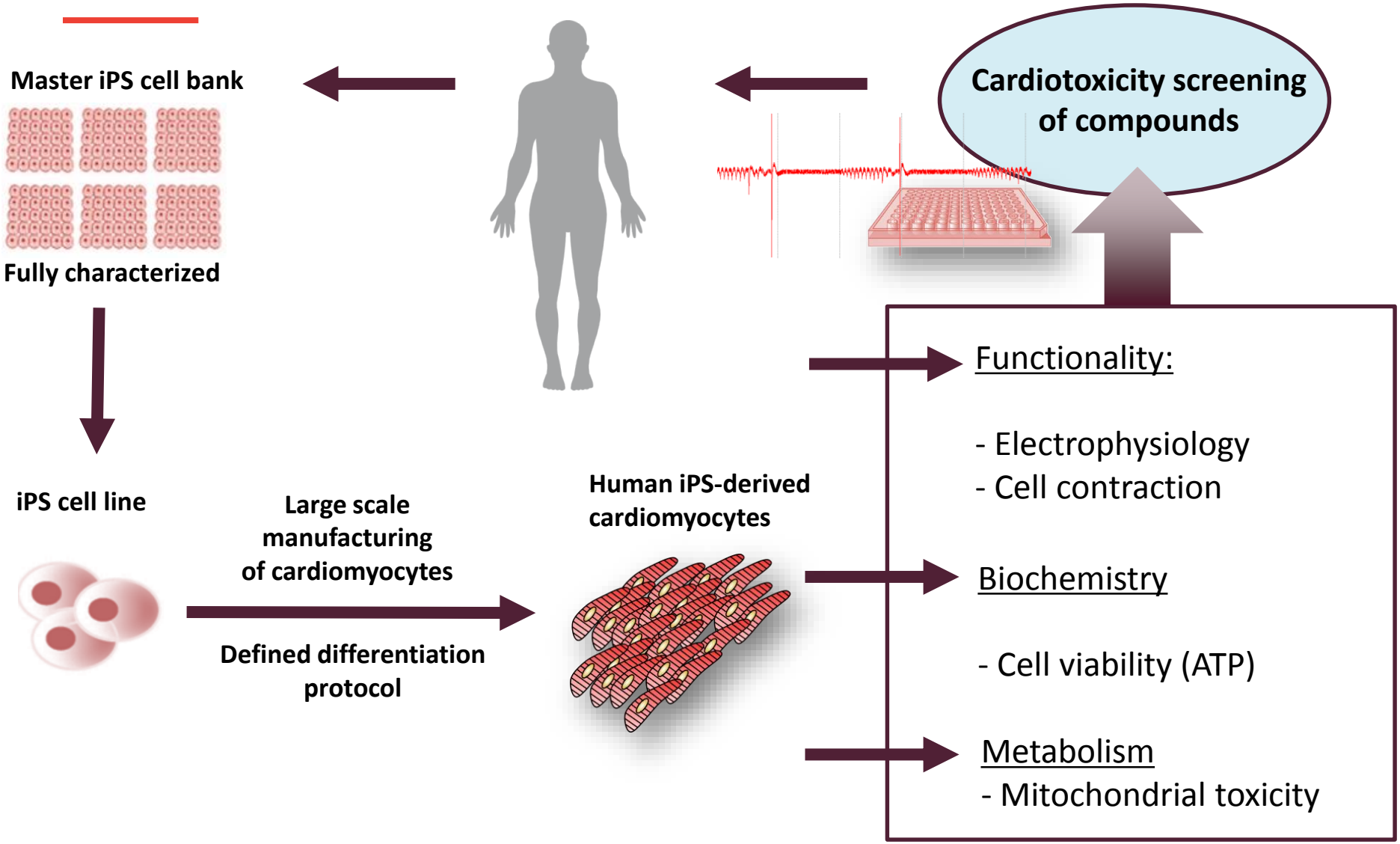


total post-approval drug withdrawal (Stevens & Baker, 2009)

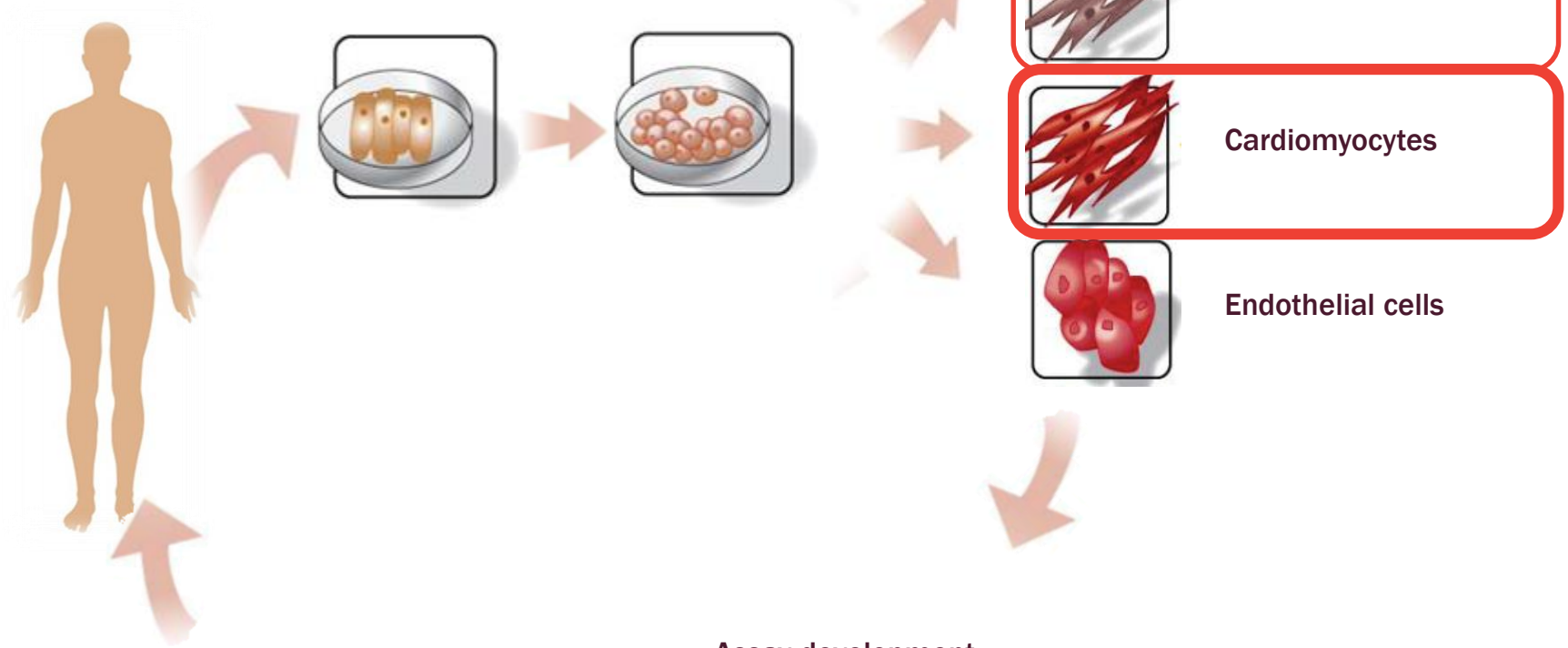
→ Urgent need for relevant *in vitro* models to screen for cardiotoxicity early in the drug development process

iPSC derived cardiomyocyte-based assays for cardiotoxicity screening

Pluriomics



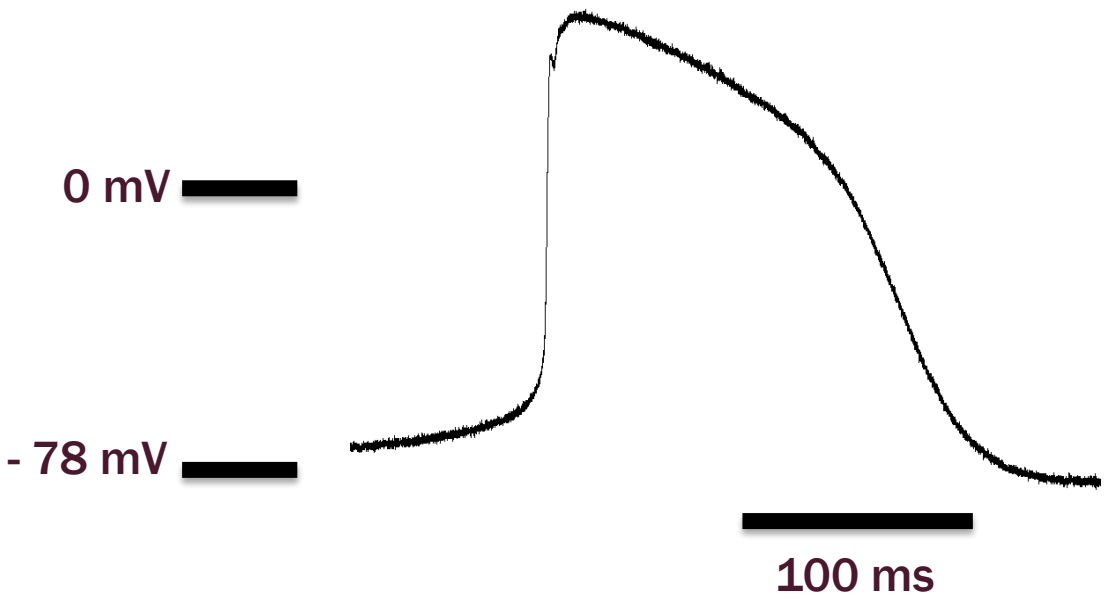
Pluriomics manufactures iPSC derived **functional cell types** and offers **cell-based assay services**

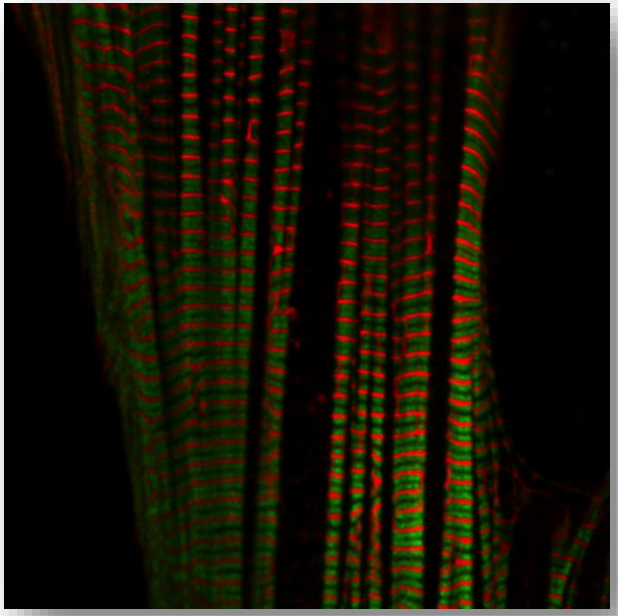


Assay development
- Electrophysiology
- Biochemistry
- Contraction

Drug development
Pharmacological research

Pluricyte® Cardiomyocytes exhibit a fast upstroke velocity and high degree of ultra-structural organization





Structural
MYH6, MYH7, MYL2, TNNI, ACTN2

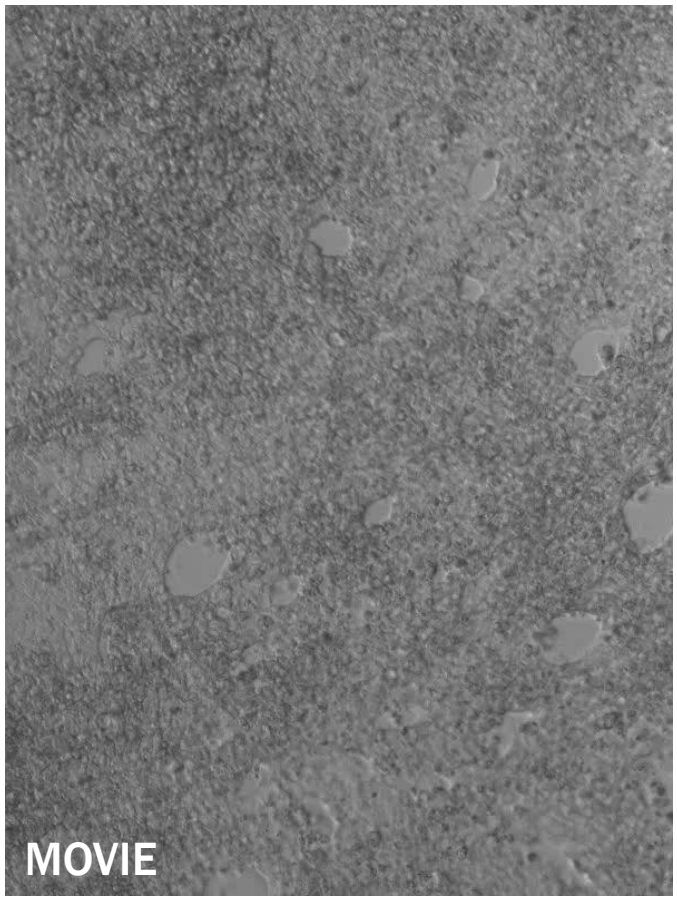
Ion channels
I_{Na} $I_{Ca,L}$ $I_{Kr,}$ I_{Ks} $I_{to,}$ I_{k1}

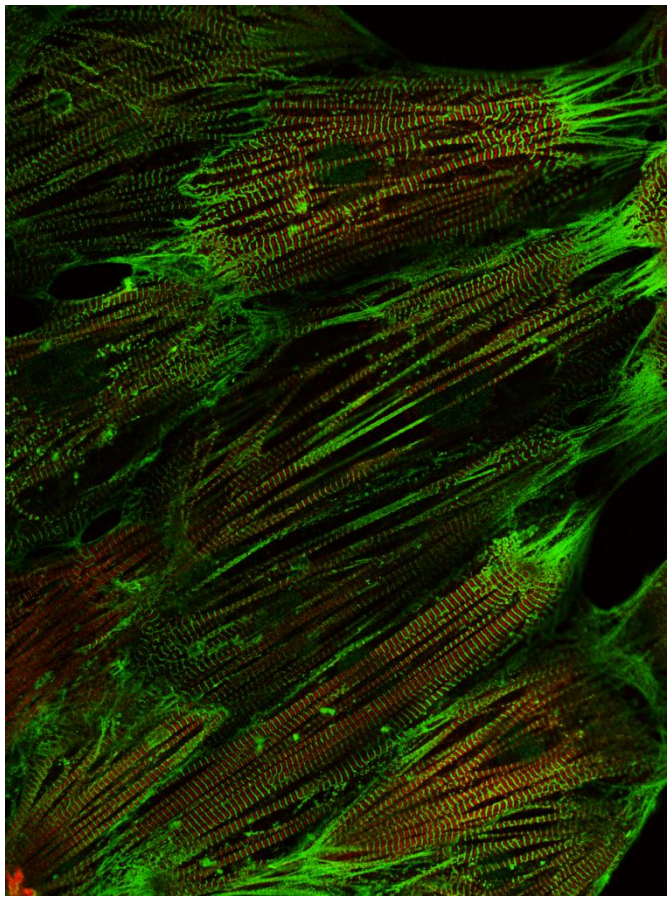
Calcium handling
CSQ2, PLN, RYR2

Gap Junctions
Connexin 40, 43

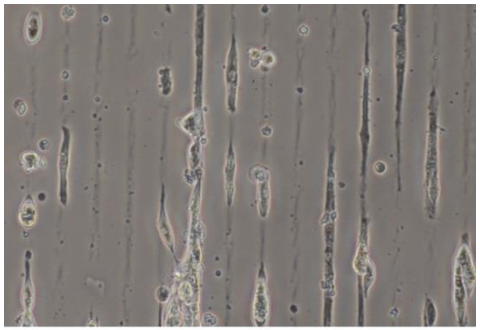
Transcription factors
Nkx2-5, MEF2c, GATA4

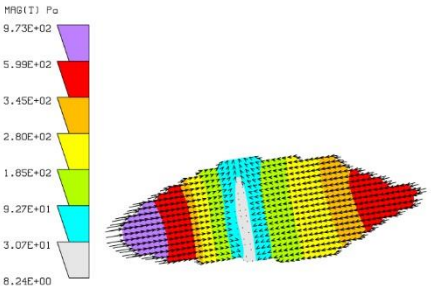
Synchronically beating Pluricyte® Cardiomyocytes and ultra-structural organized sarcomeres

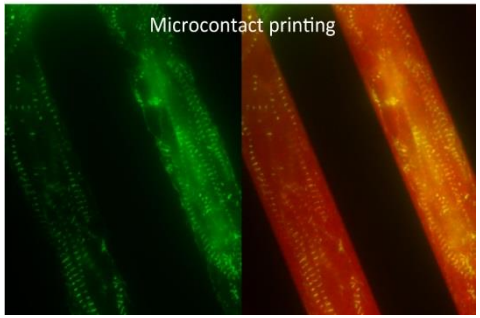


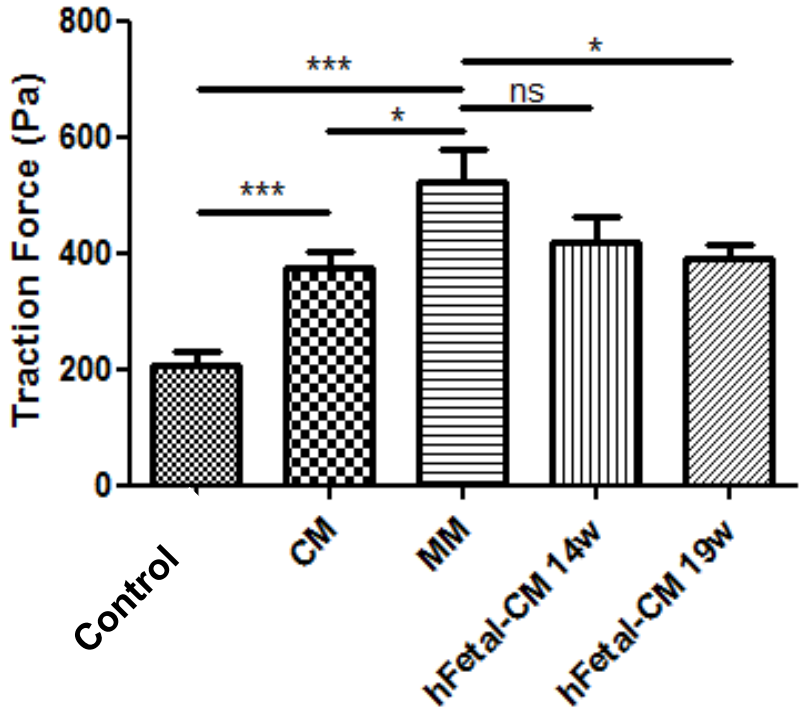


Pluricyte® Cardiomyocyte Medium increases force of contraction









Control = LUMC standard medium
CM = Previous generation medium
MM = Pluricyte® Cardiomyocyte Medium
hFetal = human fetal cardiomyocytes

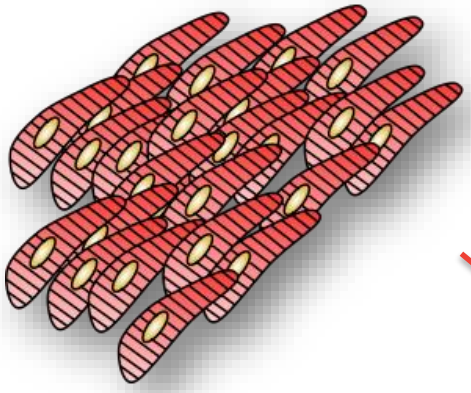
Ribeiro MC Biomaterials. 2015 May;51:138-50. doi: 10.1016/j.biomaterials.2015.01.067.

Multiparametric approach to study cardioactive effects in Pluricyte® Cardiomyocytes

Viability / metabolism

Trans membrane
Action potentials*

Calcium
transients*



Ion channel
Currents

Impedance*

Contraction*

Field potential*

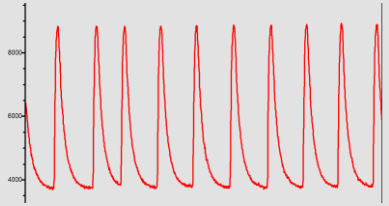
* Possible in combination with pacing

Assay platforms for multiparameter safety and efficacy testing in Pluricyte® Cardiomyocytes – 2 step approach

1. Ca^{2+}-flux assays

FDSS/µCell

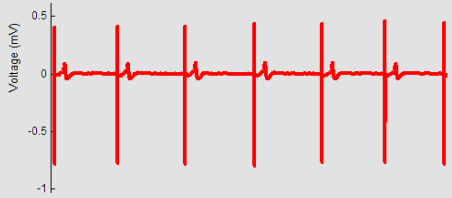
- 96/384/1536 wells
- Calcium flux-based
- ***High throughput***
- Pacing



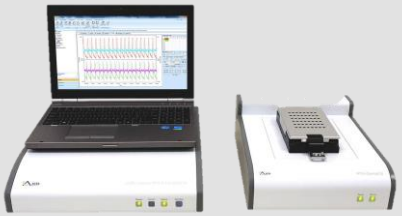
2. Multielectrode array (MEA) assays

Maestro

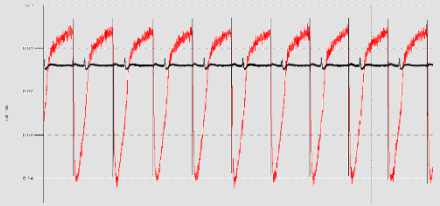
- 48/96 wells
- Electrophysiology-based
- 768 electrodes
- High resolution
- Pacing



Cardio ECR

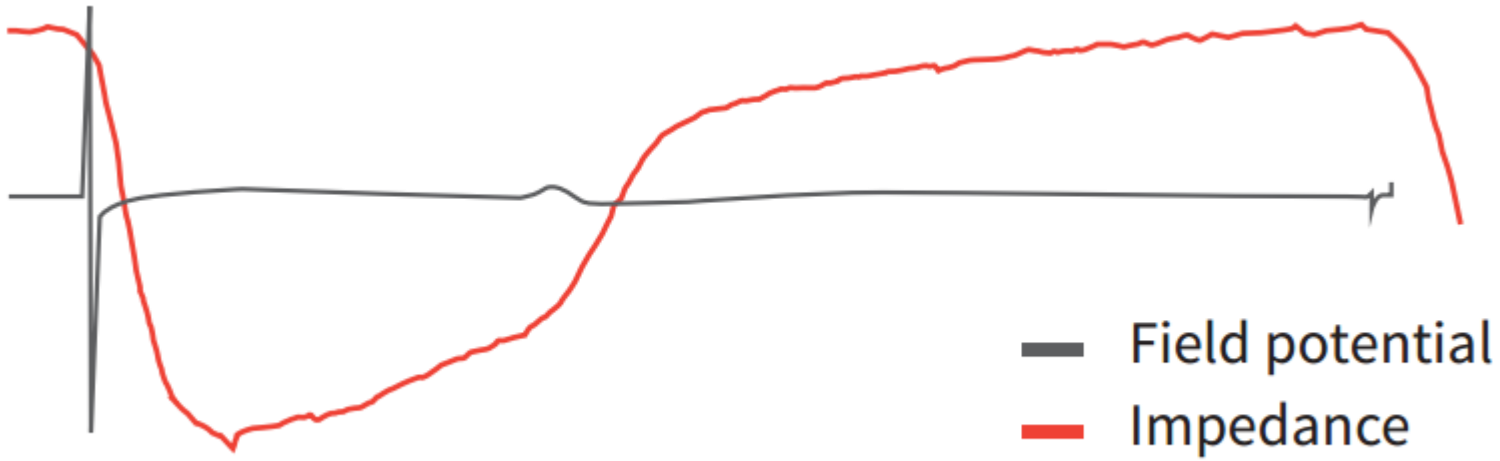


- 48 wells
- Electrophysiology- and contraction-based
- Pacing



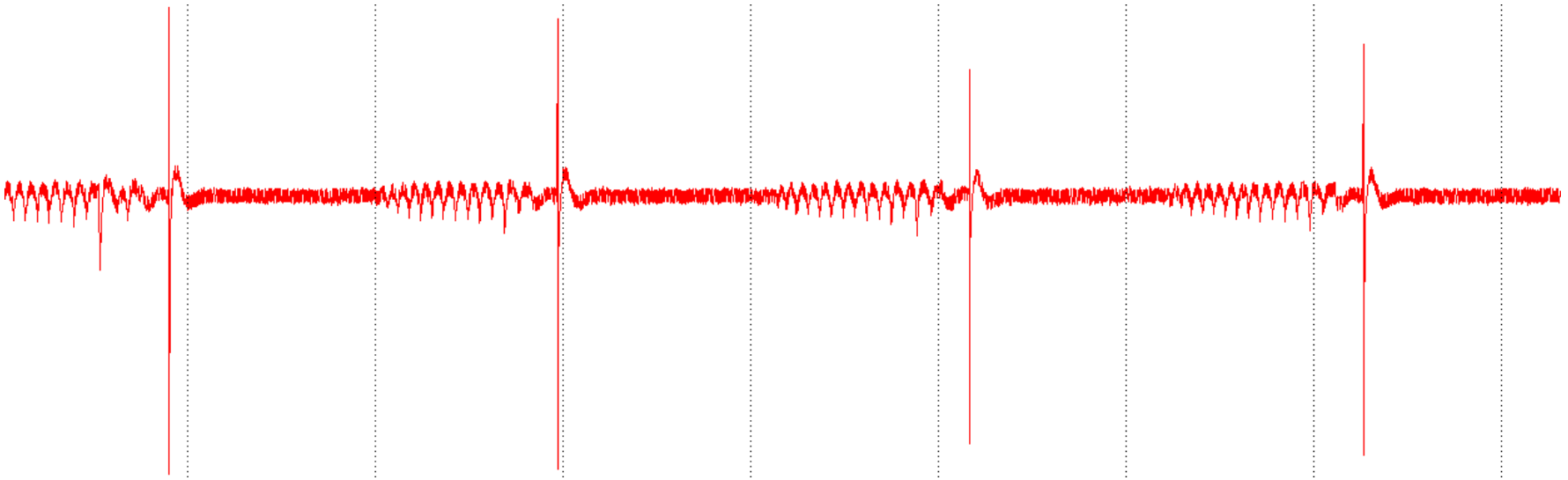
Link between electrophysiology and contraction– CardioECR analysis

Cardiomyocyte excitation-contraction coupling



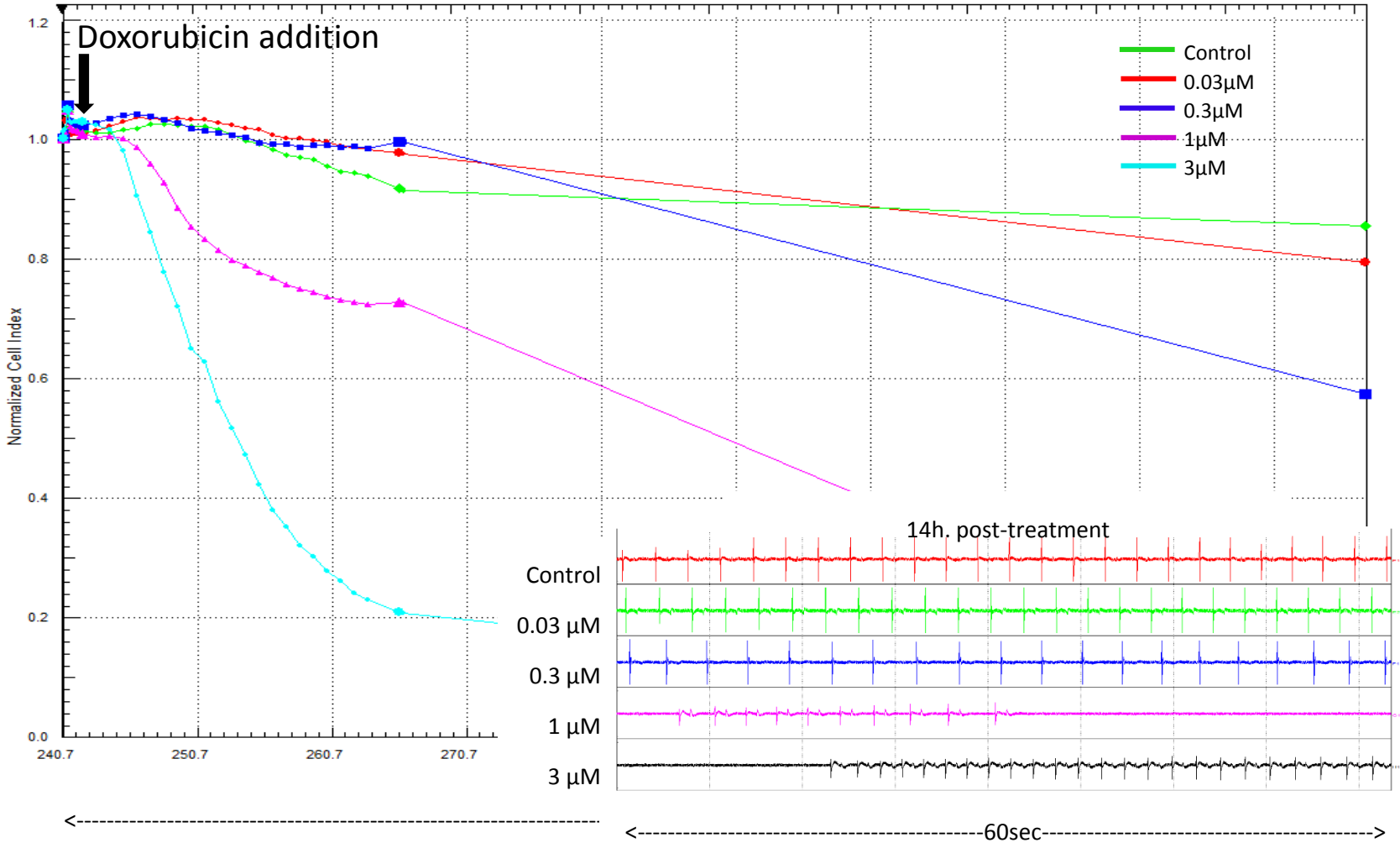
A typical single field potential and impedance waveform of Pluricyte® Cardiomyocytes

Using MEA assays to investigate arrhythmic effects of hERG channel blockers on Pluricyte® Cardiomyocytes



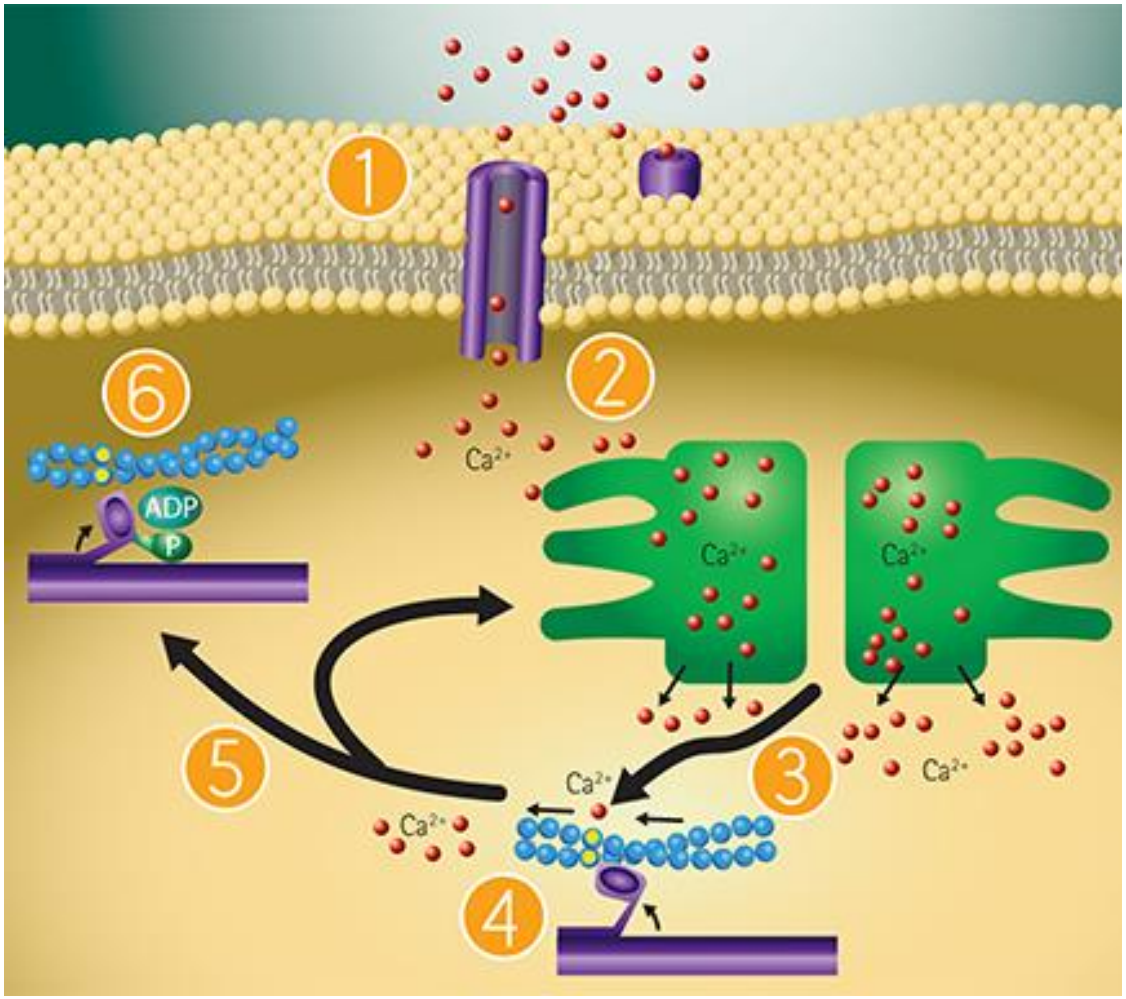
30 nM E4031

Testing chronic effects of compounds on Pluricyte® Cardiomyocytes using the CardioECR



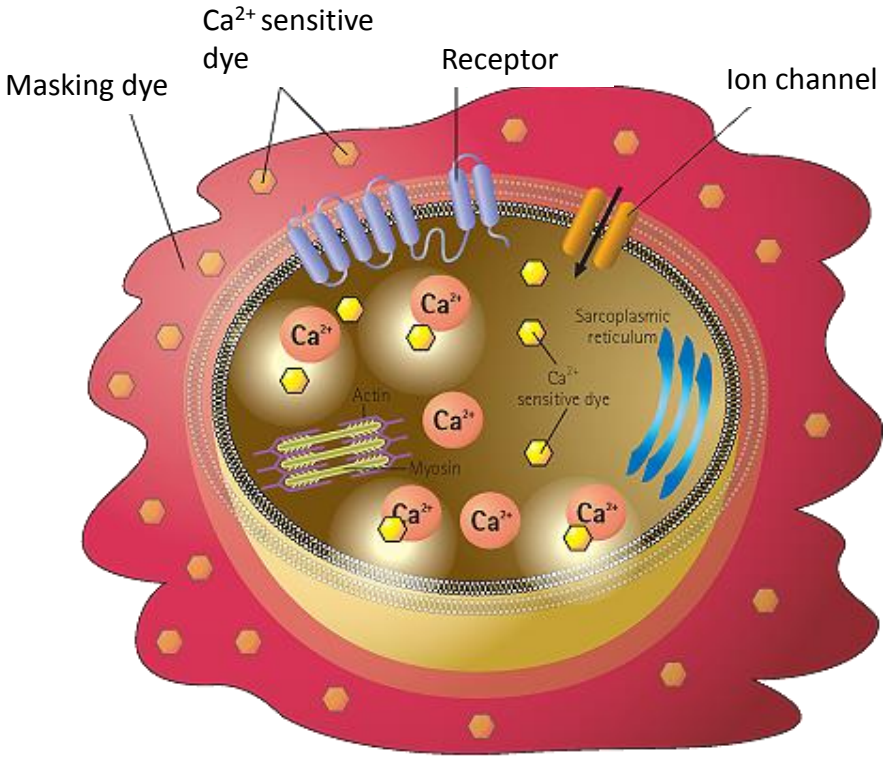
Combining the FDSS/µCell system and Pluricyte® Cardiomyocytes for high-throughput assays to study compound effects

Role of Calcium in Cardiomyocyte Contraction



1. Membrane depolarization → Ca^{2+} influx
2. Release of Ca^{2+} from sarcoplasmic reticulum
3. Binding of cytoplasmic Ca^{2+} to troponin → sarcomere activation
4. ***Contraction***
5. Removal of Ca^{2+} into SR and out of cell

Measuring calcium transients in Pluricyte® Cardiomyocytes using the FDSS

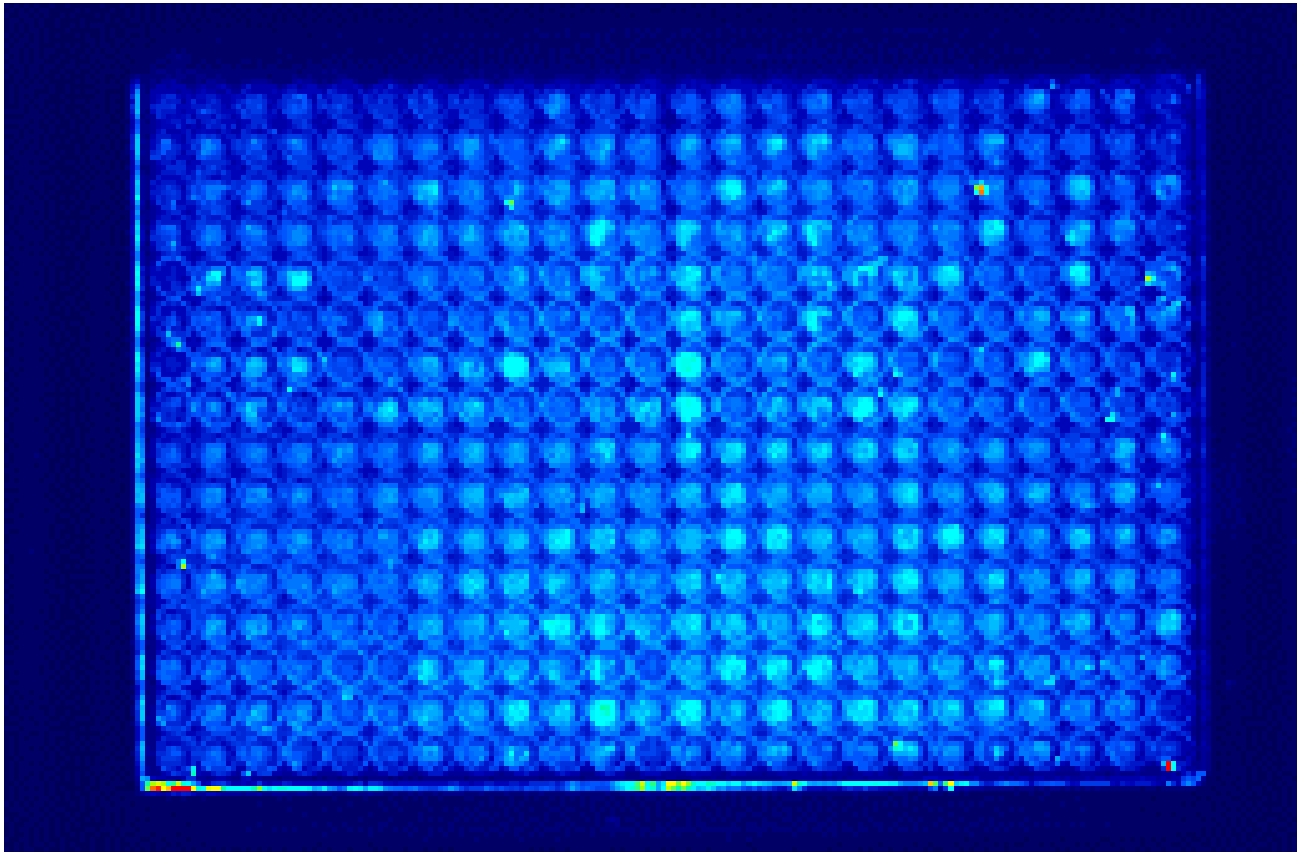


Calcium sensitive dyes, e.g. Calcium-6 (Molecular Devices)

Background is significantly reduced by masking extracellular solution

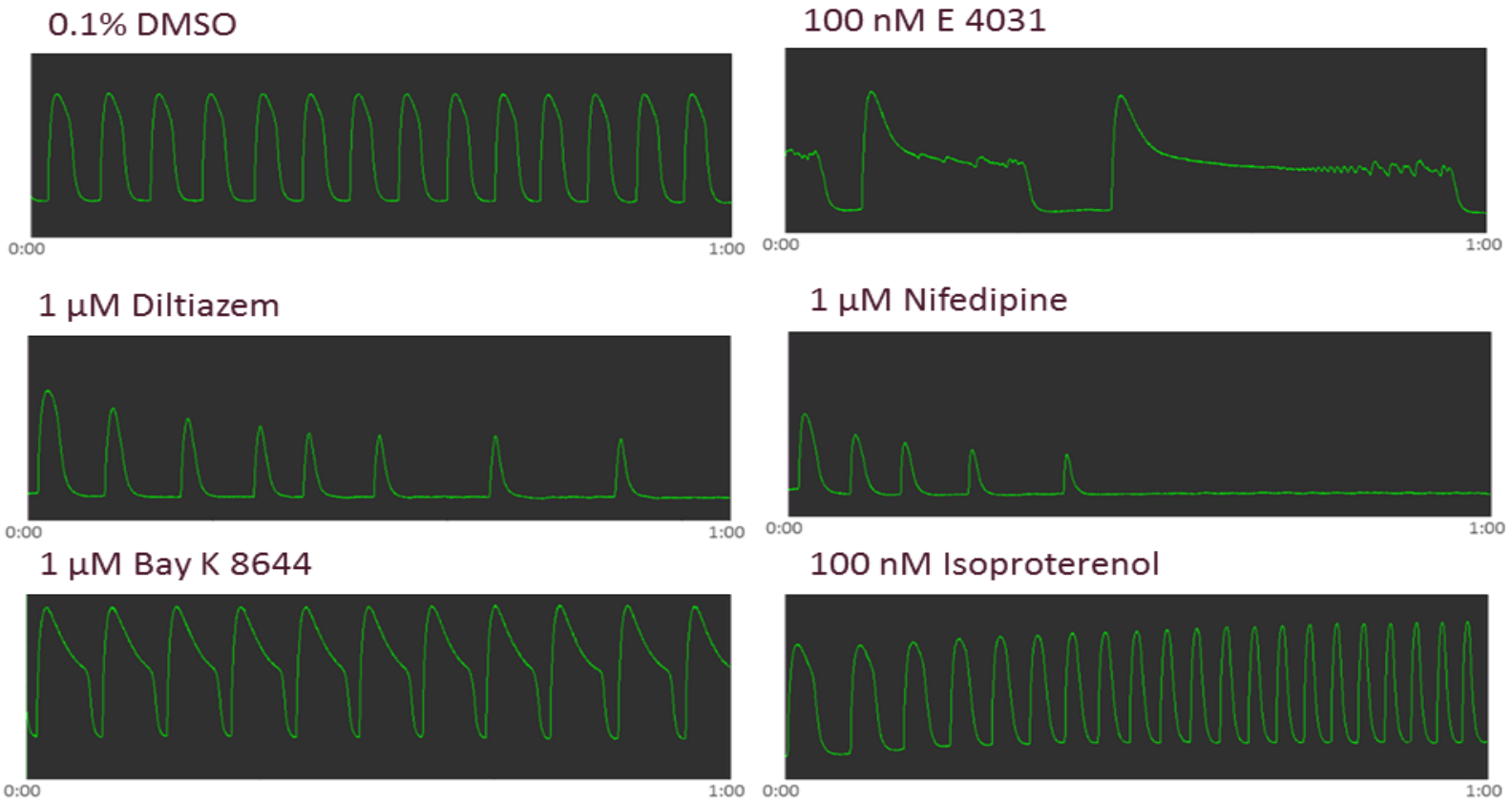
Contraction = Increase in cytosolic Ca^{2+}
Relaxation = Decrease in Ca^{2+}

1. Calcium flux in Pluricyte® Cardiomyocytes – 384 well format



Data recorded with FDSS/µCell with Molecular Devices Calcium 6 dye

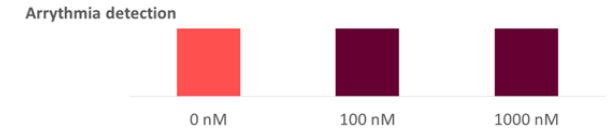
Testing cardioactive effects of test compounds in Pluricyte® Cardiomyocytes



Data recorded with Hamamatsu Photonics FDSS/µCell 4:30-5:00 (30s) after compound addition

Screening for cardioactive effects in Pluricyte® Cardiomyocytes using FDSS/µCell

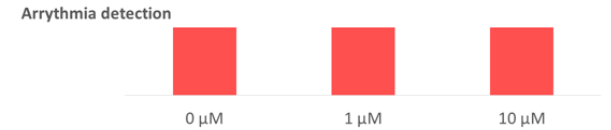
E4031



Calcium peak analysis (Percentage change)

	0 nM	100 nM	1000 nM
Peak frequency	0%	N.A.	N.A.
Amplitude	0%	N.A.	N.A.

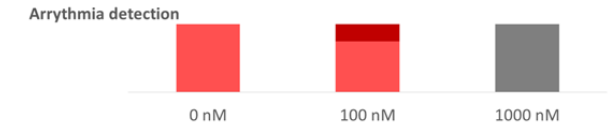
Bay K 8644



Calcium peak analysis (Percentage change)

	0 µM	1 µM	10 µM
Peak frequency	0%	- 25%	- 11%
Amplitude	0%	+ 20%	+ 25%

Nifedipine



Calcium peak analysis (Percentage change)

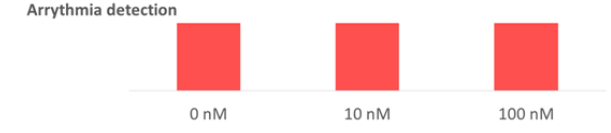
	0 nM	100 nM	1000 nM
Peak frequency	0%	- 43%	N.A.
Amplitude	0%	- 10%	N.A.

Diltiazem

Calcium peak analysis (Percentage change)

	0 nM	100 nM	1000 nM
Peak frequency	0%	- 44%	- 62%
Amplitude	0%	- 14%	- 26%

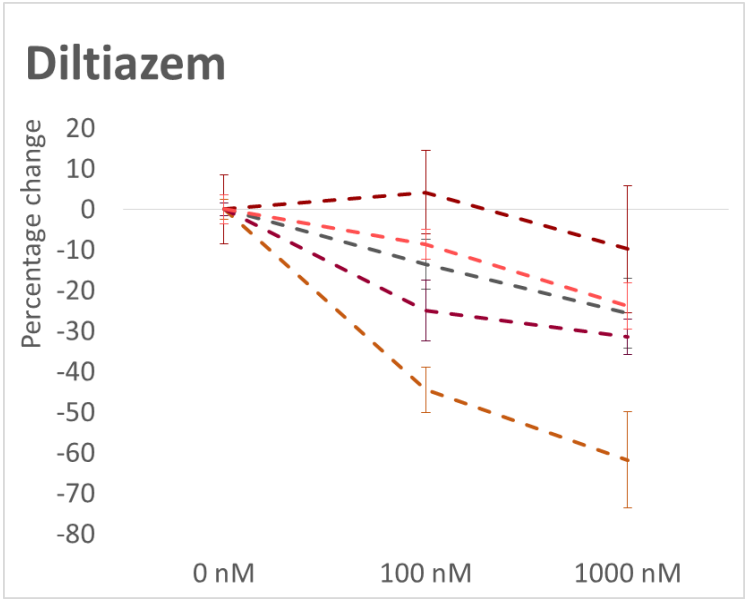
Isoproterenol

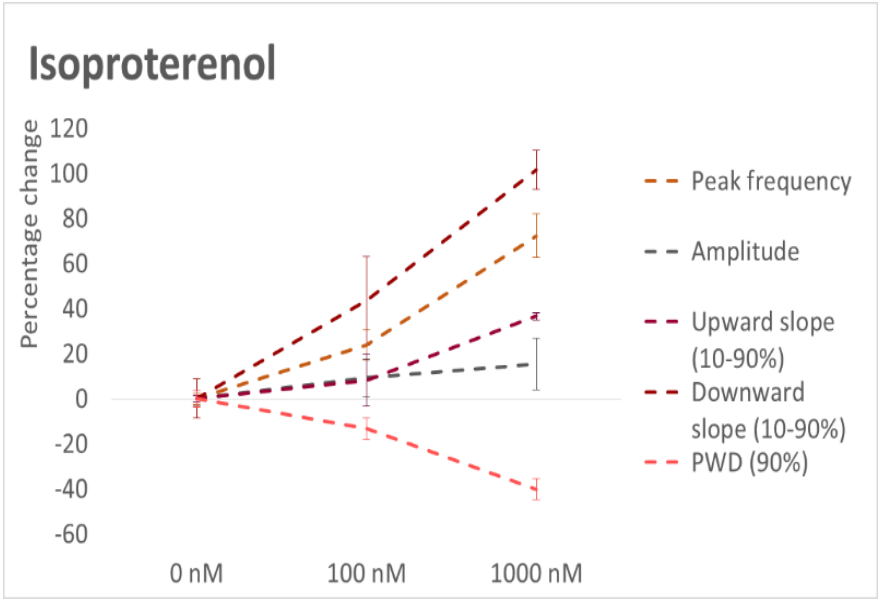


Calcium peak analysis (Percentage change)

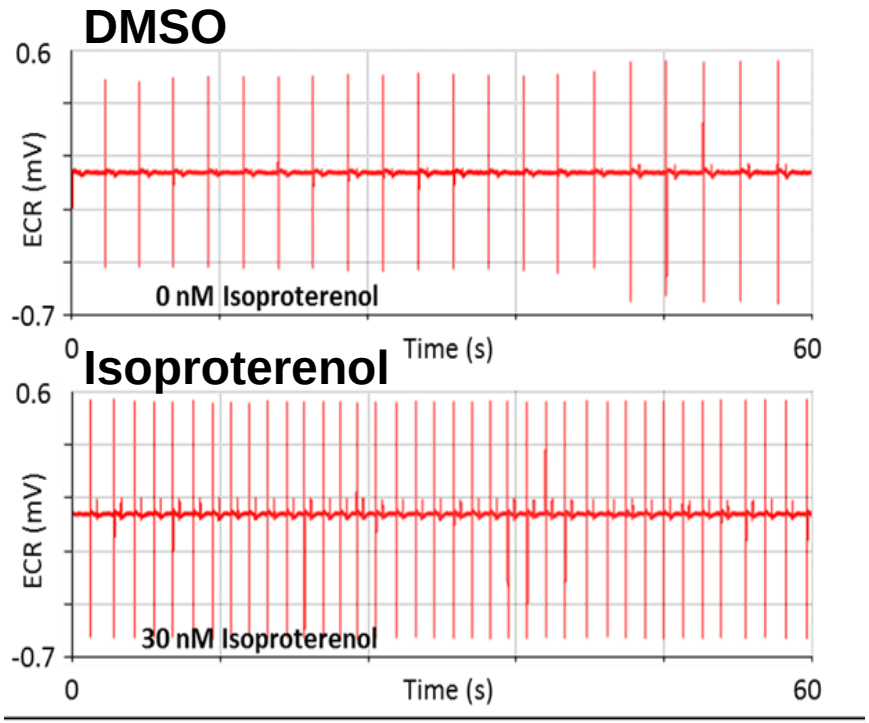
	0 nM	10 nM	100 nM
Peak frequency	0%	+ 24%	+ 72%
Amplitude	0%	+ 9%	+ 15%

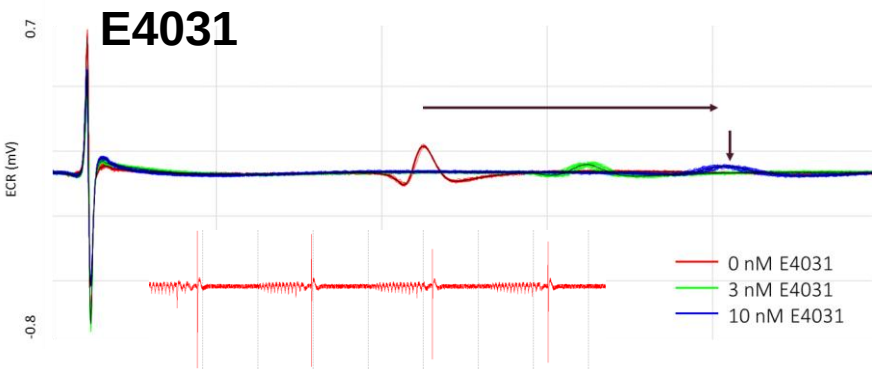
Detailed analysis of compound effects in Pluricyte® Cardiomyocytes using FDSS/µCell

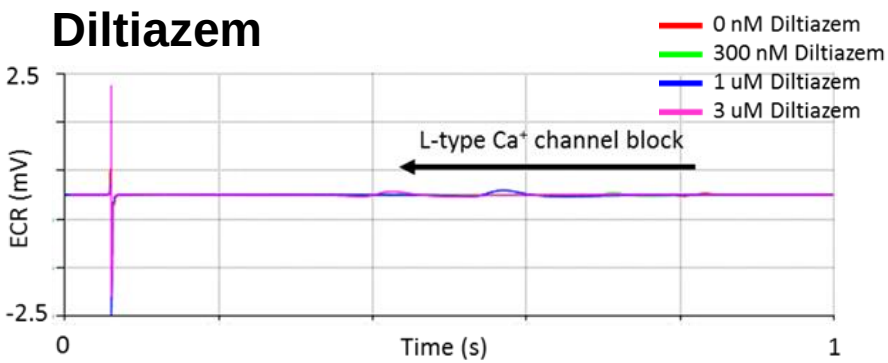


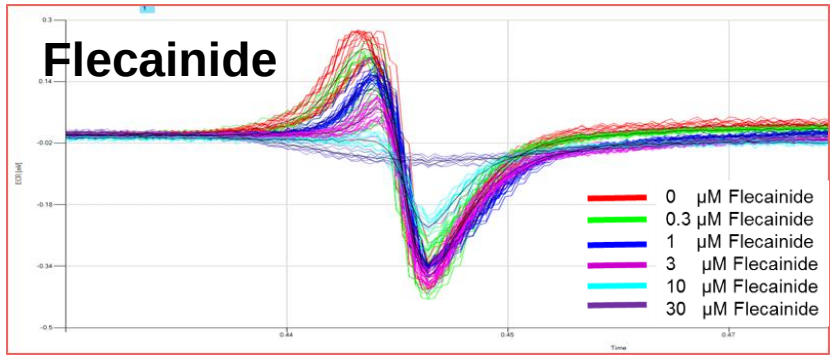


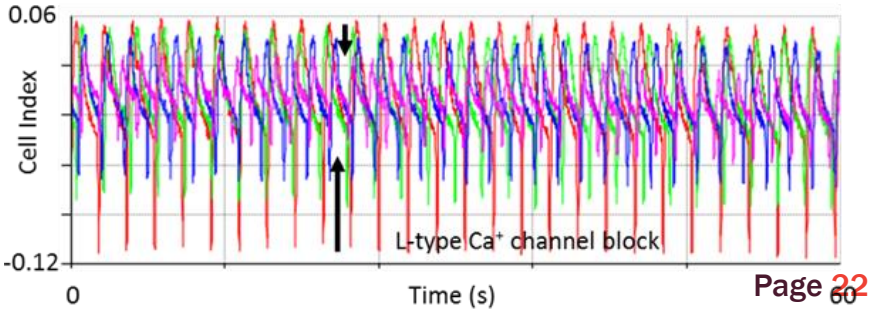
2. Detailed assessment of compound effects in MEA-based assays









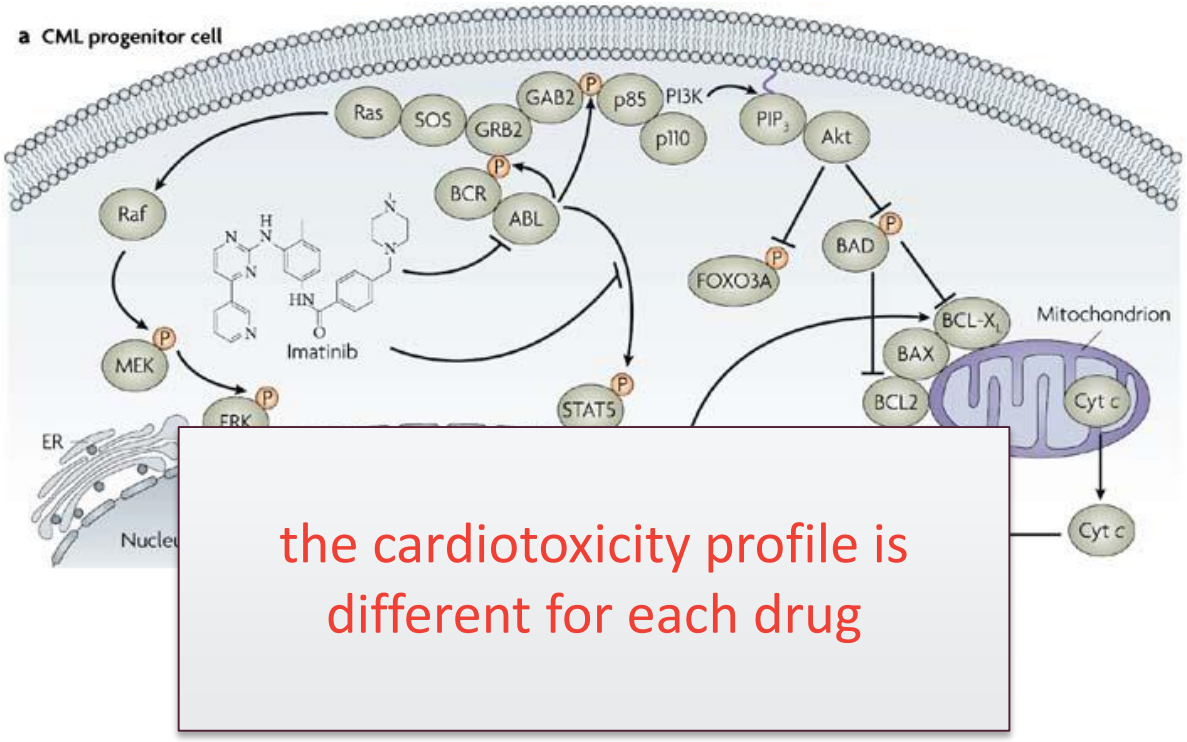


Case example: effects of Tyrosine Kinase Inhibitors on Pluricyte® Cardiomyocytes

Tyrosine kinase inhibitors (TKIs) do not only target cancer cells

Tyrosine Kinase Inhibitors:

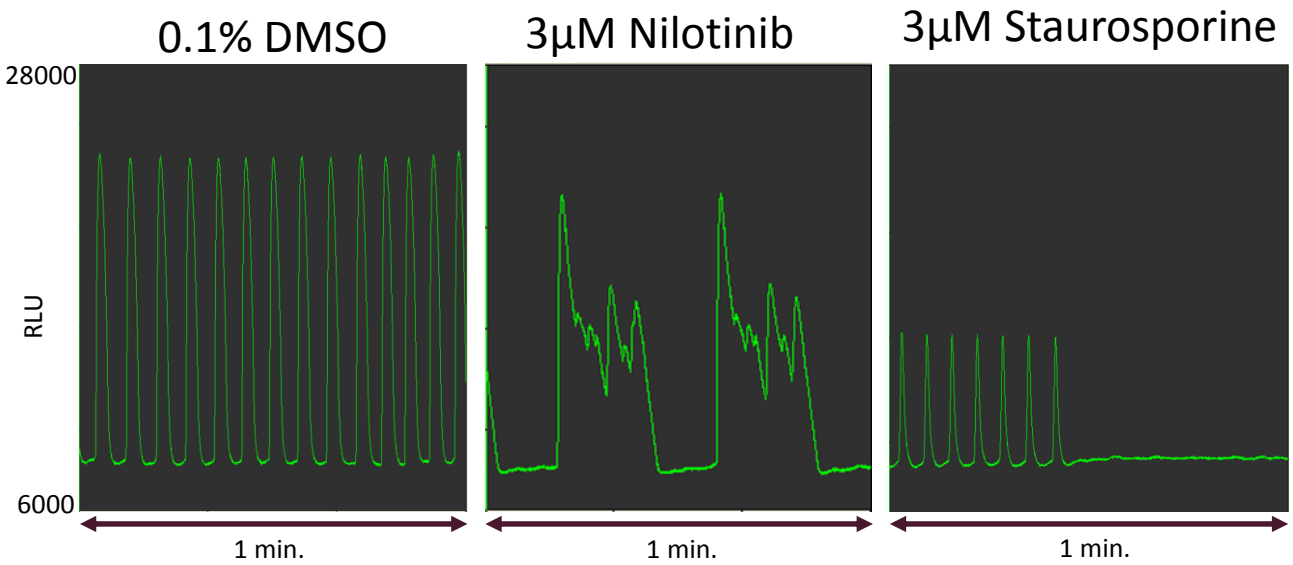
- improved antitumor efficacy and have fewer toxic side-effects, compared to traditional chemotherapy,
- have been **associated with (severe) cardiotoxicities**.
- presence of identical molecular pathways in cardiomyocytes



the cardiotoxicity profile is different for each drug

Nature Reviews Cancer **7**, 332-344 (May 2007)

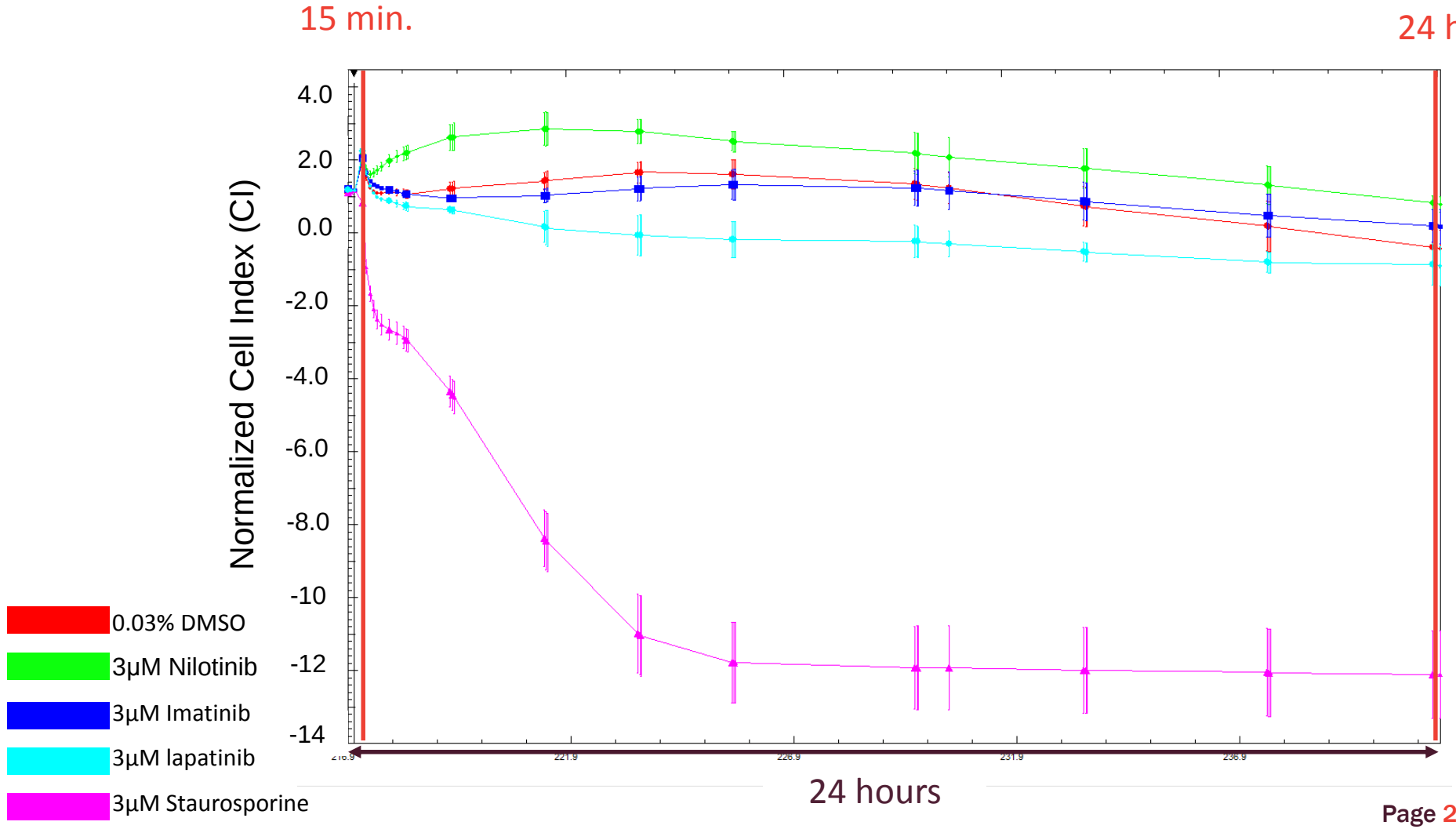
1. TKI-induced alterations in calcium transients of Pluricyte® Cardiomyocytes



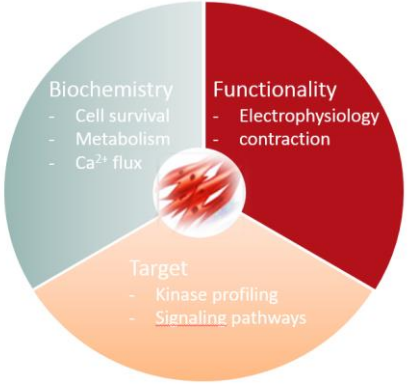
Nilotinib has an hERG IC50 of 0.66 µM

Data recorded with Hamamatsu Photonics FDSS/µCell directly after compound addition

2. Further analysis of TKI effects using CardioECR MEA-based assays



Nilotinib shows arrhyhtmic like-events in electrophysiology of hiPSC-cardiomyocytes



Before compound addition | t=15min. | t=24h.

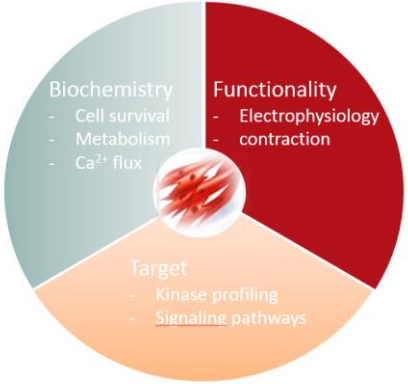
3µM Nilotinib

3µM Imatinib

3µM Lapatinib

Not shown: Staurosporine (cells irreversibly stopped beating directly after compound addition)

Nilotinib at different time points



Before compound addition

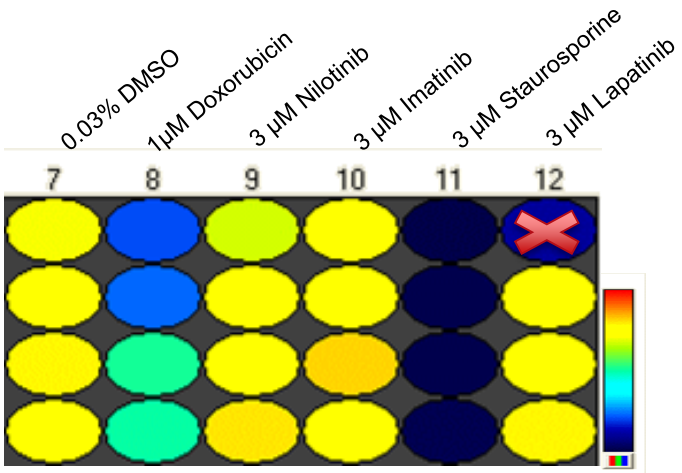
t=15min.

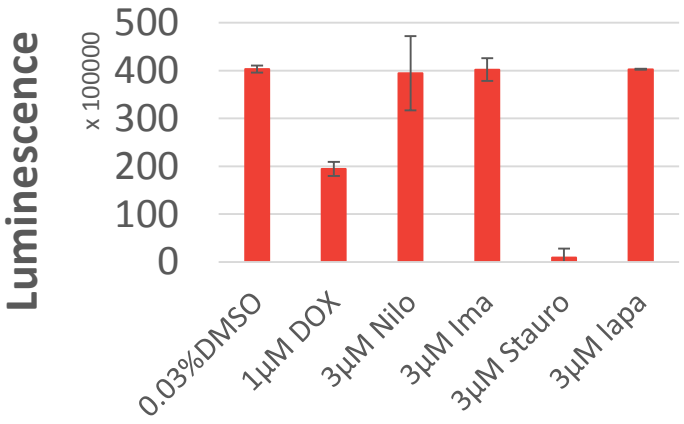
t=20min.

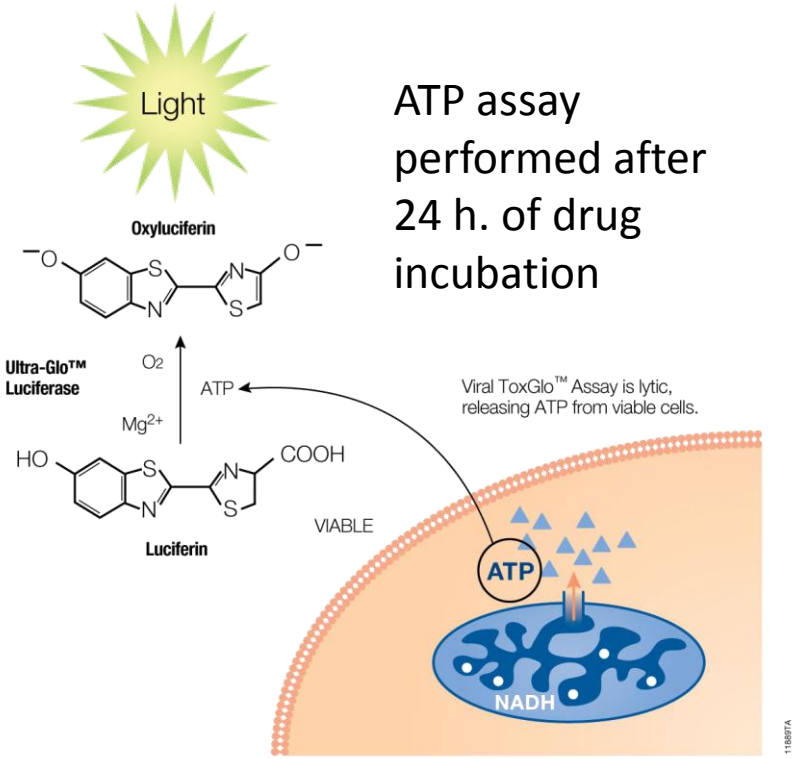
t = 16h.

t=24h.

3. Other parameters: ATP assays to study effects of tyrosine kinase inhibitors







ATP assay performed after 24 h. of drug incubation

Summary

- Pluricyte® Cardiomyocytes combined with the Hamamatsu Photonics FDSS/µCell system provide a very useful assay platform for screening of cardioactive effects at an early stage in drug development.

- Combination of high-throughput dye-based assays with medium throughput MEA based assays to further assess cardioactive effects provides a complete overview of cardioactive effects of (candidate) drugs on hiPSC-derived cardiomyocytes and will help to predict potential cardiotoxicities.

- Further development of high-throughput multiparametric assays to study safety (and efficacy) of cardioactive compounds will contribute to:
  - More efficient, and therefore cost- and time-effective, decision making early in drug discovery & development
  - Reduction of animal experiments

Acknowledgements

Hamamatsu Photonics

Jean Marc D'Angelo
Emmanuel Pirson
Thomas Niedereichholz

Pluriomics BV

Peter Nacken
Fleur Stevenhagen
Tessa de Korte
Sabine den Hartogh
Arie Reijerkerk
Stefan Braam

Coming soon: application note for assessing Ca^{2+} flux in Pluricyte® Cardiomyocytes using the FDSS/µCell system!

CONTACT:

support@pluriomics.com
www.pluriomics.com