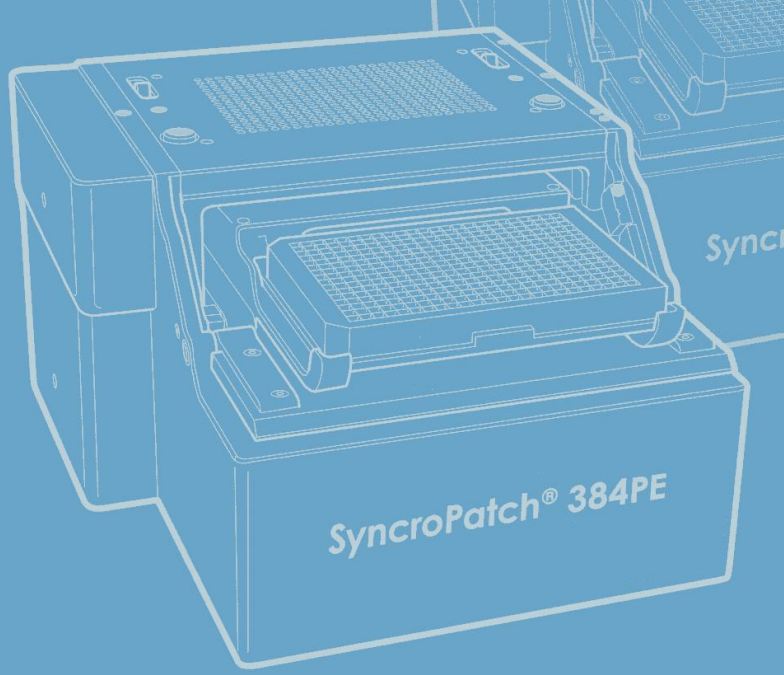
Nanion Technologies



Complementary HTS Technologies towards a more Rigorous Safety Screening Paradigm

George Okeyo, Ph.D.
Application Scientist
Nanion Technologies Inc.

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Agenda

- Company Background
  - Brief history
  - Technologies
- Comprehensive In Vitro Pro-arrhythmia assay
  - Goals
  - Relevant ion channels
- Recording platforms
  - Patchliner
  - CardioExcyte
  - Syncropatch 384/768

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Company History & Development

Continuous organic & dynamic growth since 2002

Final scale-up to HTS

Dedicated staff

Entering HTS

Versatile automation

First APC instrument

Seed investment

Management Buy-out

Long experience and dedicated customer service

Nanion:

13 years of patch clamping - and more to come

The SURFE²R.
Catch the wave for transporters.

- In-depth analysis of transporter protein activity and function
- Compatible with diverse membrane sources
- Multiple targets investigated with one sensor

The Patchliner.
Because quality does matter.

- Unlimited experimental freedom
- Best of all worlds: throughput, performance and versatility
- Press one button and walk away:
  - 48 cells in one run

The SyncroPatch 96.
Get more throughput.

- Cost-efficient ion channel screening
- Ligand- and voltage-gated channels
- High throughput and high data quality

The CardioExcyte 96.
Bump up your safety screening.

- Ultra-precise impedance measurements
- Real time access to beating parameters
- Quick experiments and long term observations

The Port-a-Patch.
The world's smallest patch clamp rig.

- Fast access to high quality patch clamp data
- Quick evaluation of cells and compounds
- Novel experimental possibilities

The Orbit 16.
Instant bilayer – just add protein.

- 16 parallel bilayers
- Low noise, high bandwidth recordings
- Compatible with your existing amplifier

The Vesicle Prep Pro.
Liposomes made easy.

- Quick and easy formation of GUVs
- Temperature control
- Stable bilayers for ephys recordings

Measure More Membrane

Smart tools for ion channel and transporter research and screening

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Ongoing HESI/FDA initiative: CiPA
(Comprehensive *In Vitro* Pro-arrhythmia assay)

CiPA

- A proposal to evaluate the pro-arrhythmic risk of compounds based on mechanistic electrophysiological understanding of this risk

FDA iPS CM play a major role; they express all 5: hERG, Nav1.5, Cav1.2 and KvLQT1, Kir2.1

Ongoing HESI/FDA initiative: CiPA
(Comprehensive *In Vitro* Pro-arrhythmia assay)

CiPA

- A proposal to evaluate the pro-arrhythmic risk of compounds based on mechanistic electrophysiological understanding of this risk

Goal

- Move the analysis and evaluation of pro-arrhythmia risk earlier in the discovery/development process
- Increase efficiency of development pathway
- Enhance accuracy of current or future drugs labeling
- Increase the number of compounds in development
- Revise ICH S7B guideline, and remove TQT study described in ICH E14 guideline
  - Proposed timelines: abandon E14 by July 2015, and revise S7B by July 2016

iPS CM play a major role; they express all 5: hERG, Nav1.5, Cav1.2 and KvLQT1, Kir2.1

HTS on the CIPA stipulated ion channels

(hERG, Nav1.5, Cav1.2 and KvLQT1, Kir2.1)

Voltage gated channels:

BK, **Cav1.2**, Cav2.2, Cav3.1, Cav3.2, Cav3.3, **hERG**, hEAG, K_{Ca}1.1, Kv1.3, Kv1.5, **KCNQ1**, Nav1.1, Nav1.2, **hNav1.5**, Nav1.7, hNav1.8, *Shaker I*, *Shaker II*

Ligand gated:

5-HT_3, ASIC ,CNG, $GABA_A$, hGlyR a1, HCN, hNAChR a7, NAChR a3β4, NMDA, P2X2/3, P2X7, TRPA1, TRPC1, TRPC3, TRPC5, TRPM2, TRPM3, TRPM7, TRPM8, TRPV1, TRPV3, TRPV4

Others:

Kir1.1, Kir7.1, **Kir2.1**, rGIRK, kNBCs-1 (NBCe1-A), ROMK, TPCN2

Bilayers:

Alamethicin, Bacterial Cytolysin, Connexins, Gramicidin, IP_3, KcsA, Kv1.2, McsL, NaChBac, OmpC, OmpF

Cell lines:

1321 N1, BHK, HEK293, CHO, COS, HeLa, IMR-32, Jurkat, L-tk, ND7-23, NG108-15, PC-12, RBL, S2, S9, SHS5Y5

Primary cells:

BY2 Protoplasts, DRG neurons, erythrocytes, hippocampal granule cells, human corneal endothelial cells**,** human sanoviocytes, human T-lymphocytes, human neutrophils, human vasacular smooth muscle cells, lysosomes, lymphoblasts, mesophyll protoplasts, mitochondria, mitoplasts, rat astrocytes

Stem cells:

hES (undifferntiated), hESC- derived cardiomyocytes (Axiogenesis, Cellectis, CDI, Geron/GE Health Care), mESC-derived cardiomycytes (Axiogenesis), Primary neuronal stem cells

Measured on Nanion APC

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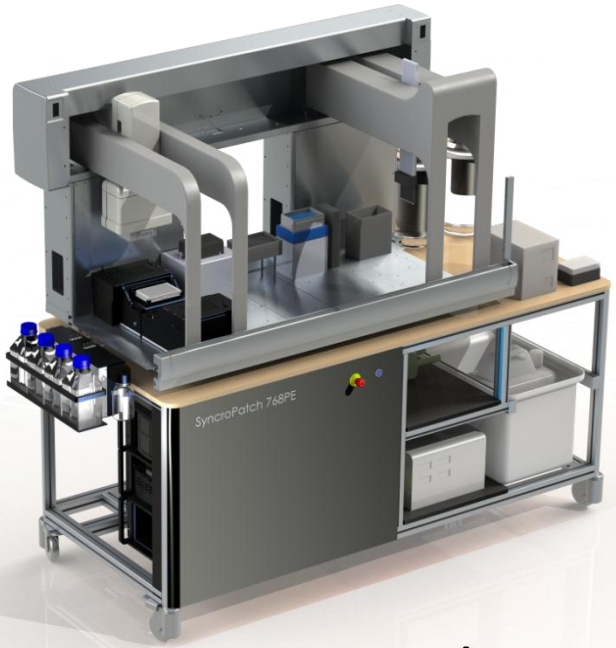
Is your safety program up-to-date?
Nanion's approach to CiPA….

CardioExcyte 96
Non-invasive impedance system
for beating cardiac networks

Patchliner
High quality whole cell patch clamp recordings



SyncroPatch 384/768
High quality patch clamp & 100% HTS

Patchliner

Automated patch clamp for safety screening

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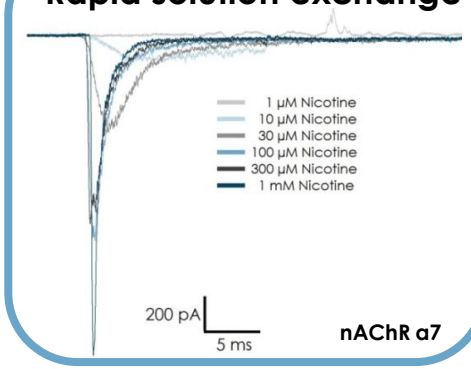
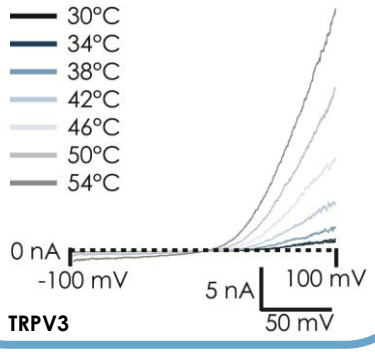
Patchliner®. Unlimited experimental freedom.

Rapid solution exchange



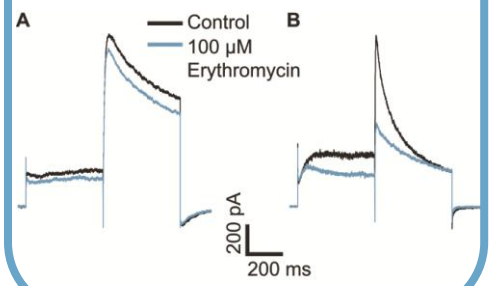
nAChR α7

Heatable pipette

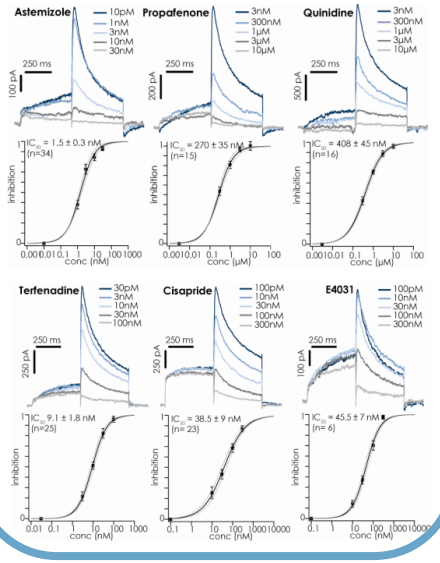


TRPV3

Physiological temperature

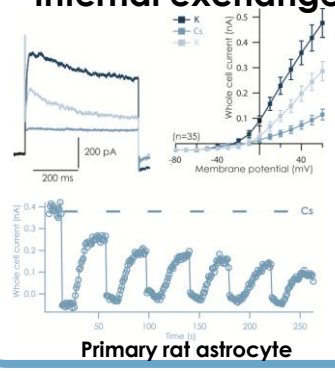


Efficient CRC generation



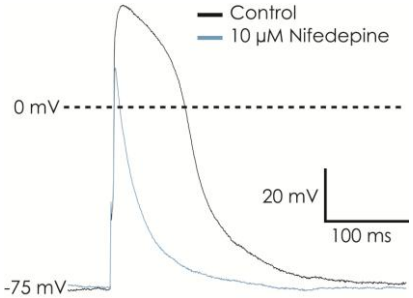
+ accurate pharmacology!

Internal exchange



Primary rat astrocyte

Current clamp recordings



Stem cell derived cardiomyocytes

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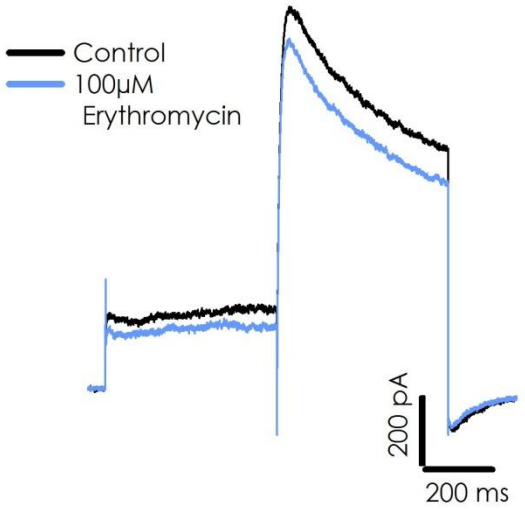
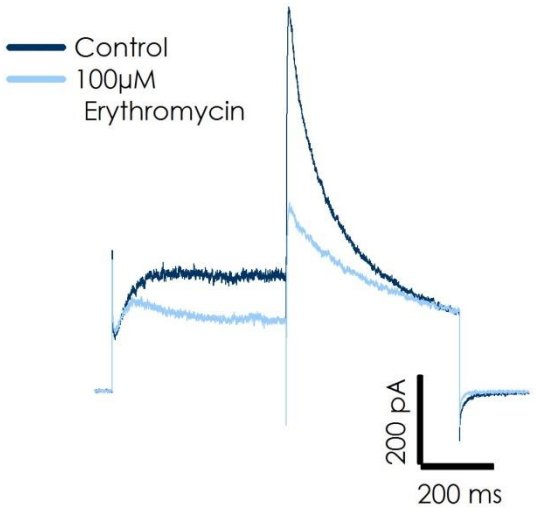
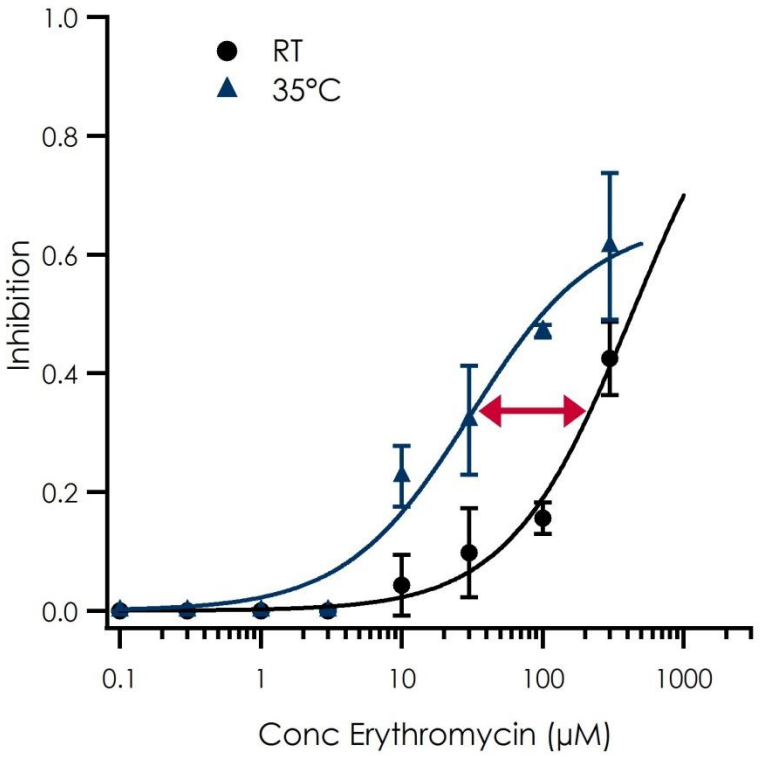
Pharmacology of hERG at physiological temperature







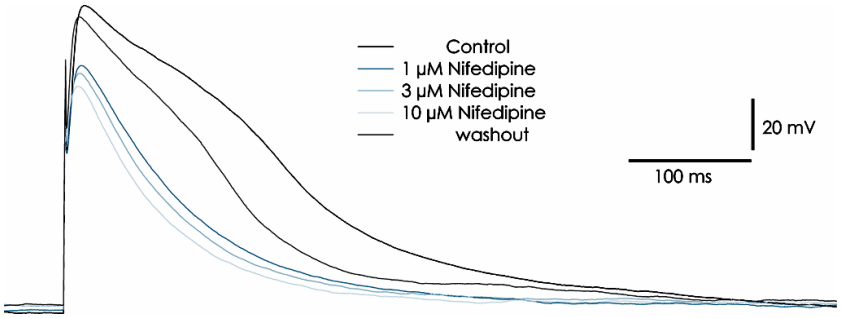
Pharmacology can be altered at physiological temperature

Erythromycin is 10X more potent at physiological temperature *vs* RT

Polunchuk, L. *Frontiers in pharmacology*, 2012,Vol 3, 1-7

Patchliner offers all required features for screening of stem cell-derived cells

- **Current clamp** – automated current clamp recordings
- **GigaOhm seals even with primary /stem cells**
- **Temperature control** – stable physiological temperatures or temperature jumps (<70°C)
- **Minimized cell usage** – higher cost efficiency
- **Internal solution exchange** – allows modulation of the ion channels on the cytosolic membrane side
- **Fast solution exchange** - >20 ms solution switch time
- **Brief compound exposure** – allows short compound exposure times down to 500 ms



Action potential are shortened by the presence of nifedepine, an L-type calcium channel blocker.

CardioExcyte 96

label-free cardiac safety screening

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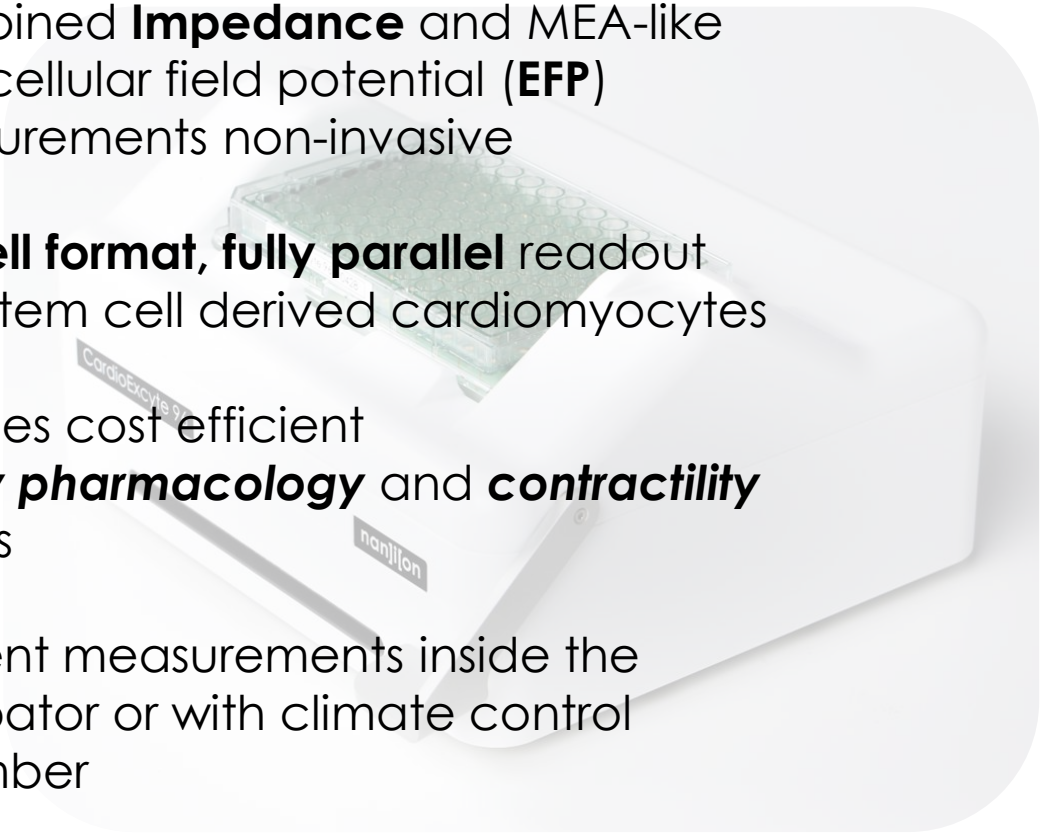
CardioExcyte96

CardioExcyte96

- Combined **Impedance** and MEA-like extracellular field potential (**EFP**) measurements non-invasive
- **96-well format, fully parallel** readout from stem cell derived cardiomyocytes
- enables cost efficient ***safety pharmacology*** and ***contractility*** assays
- Efficient measurements inside the incubator or with climate control chamber



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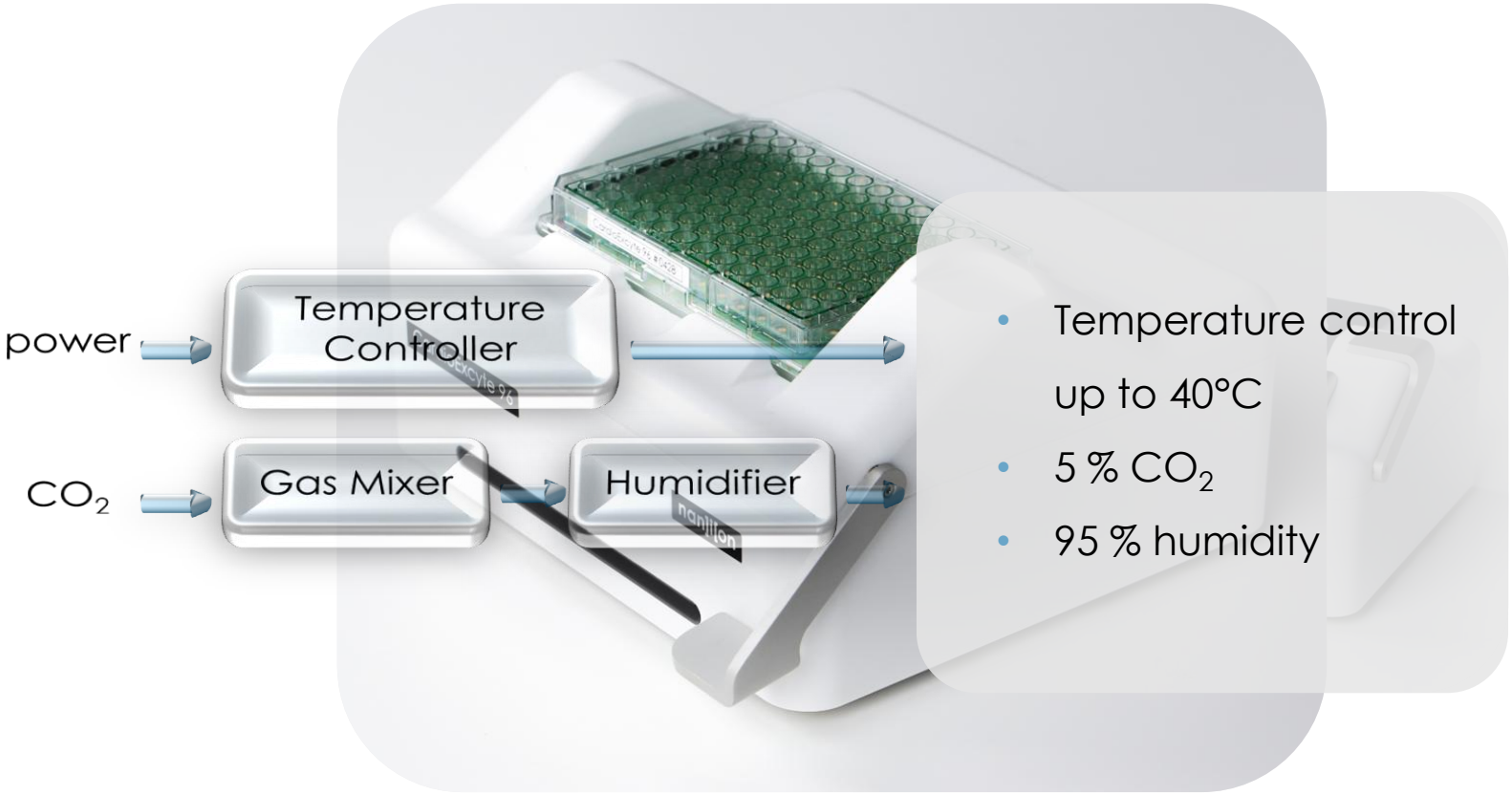
Replacing the Incubator – CardioExcyte Incubation chamber

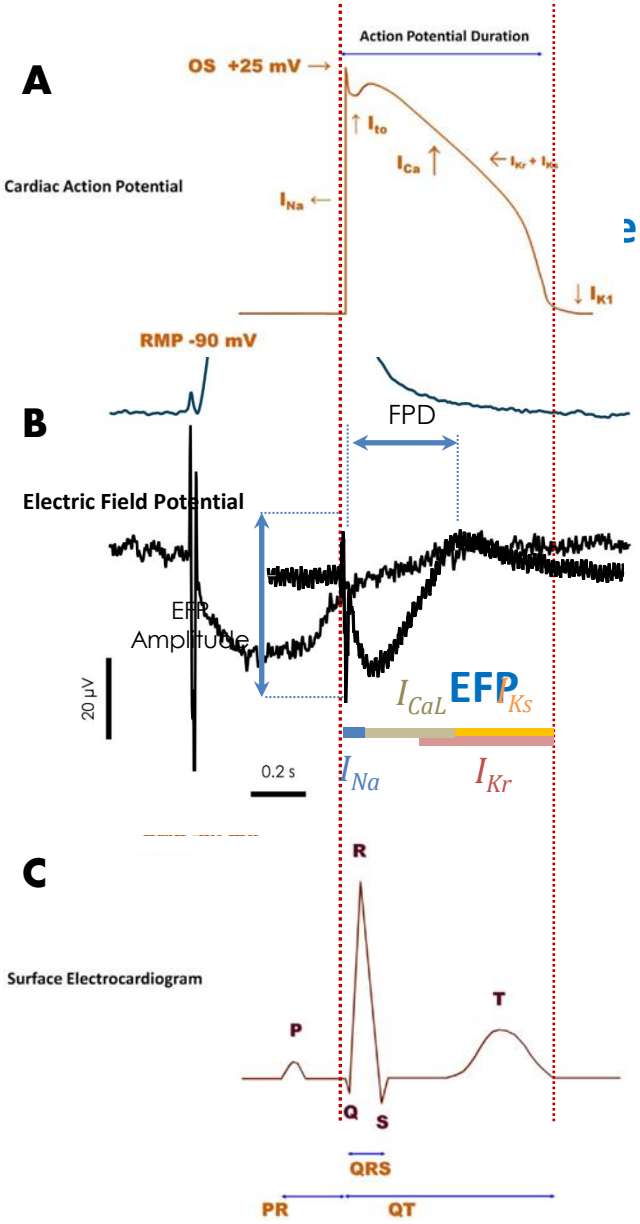


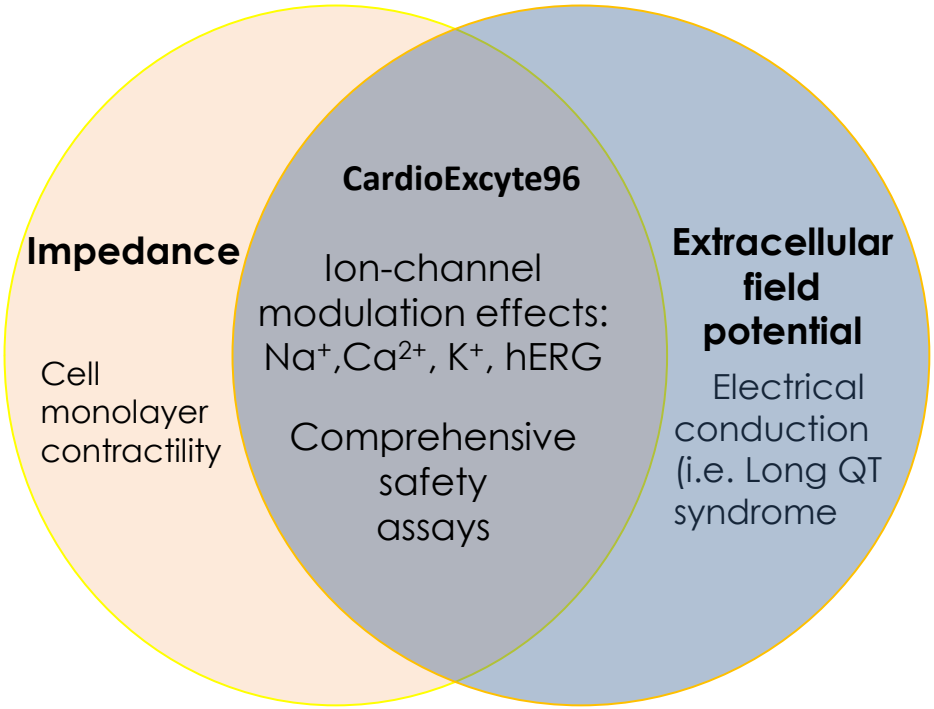
- Temperature control up to 40°C
- 5 % CO_2
- 95 % humidity

→ Minimal space requirements

→ Easy experimental setup for assessment of temperature-dependent effects

Impedance and Extracellular Field Potential (EFP) measurements recorded in a combined mode





A. Human ventricular action potential [1]

B Extracellular electric field potential (EFP) and cardiac ion channel currents that contribute to iPSC-CM potentials.

C Surface electrocardiogram (ECG).[1]

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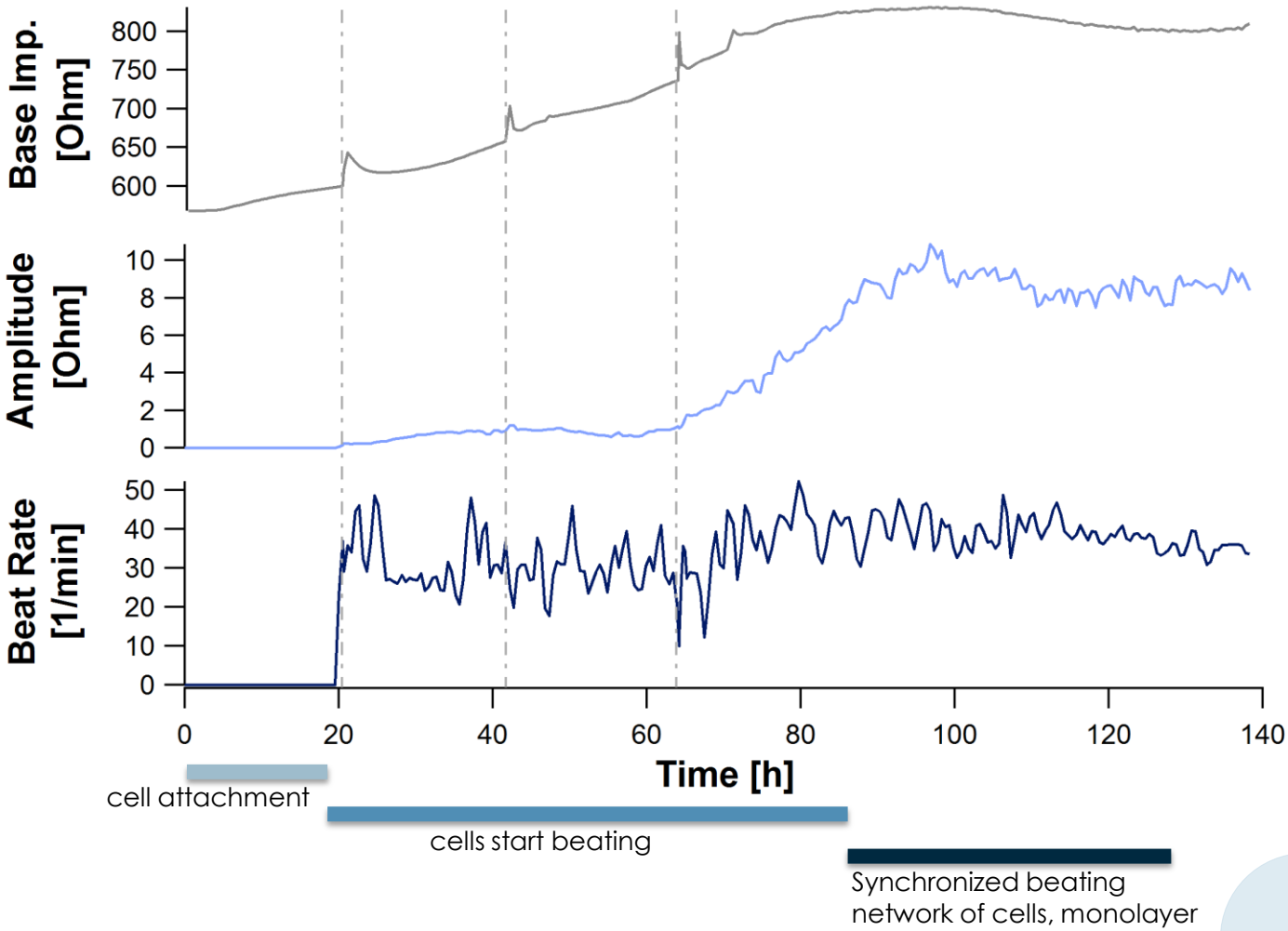
1. Front. Pharmacol., 2011 doi: 10.3389/fphar.2011.00078

Extended Applications: cell adhesion and initiation of rhythmic activity



Base Impedance, Amplitude and Beat Rate during the attachment/growth of cardiac cells (iCell® Cardiomyocytes) (T = 0: cell seeding, dotted lines: Medium exchange).

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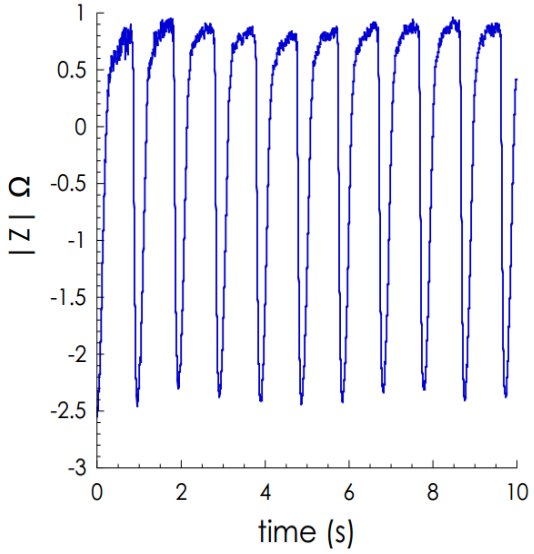
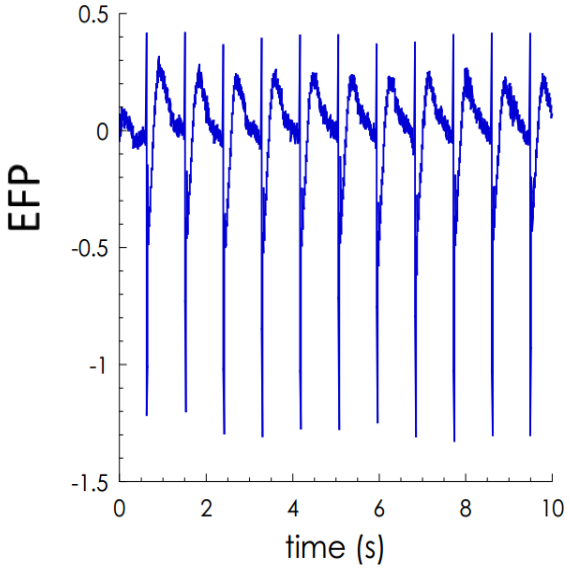
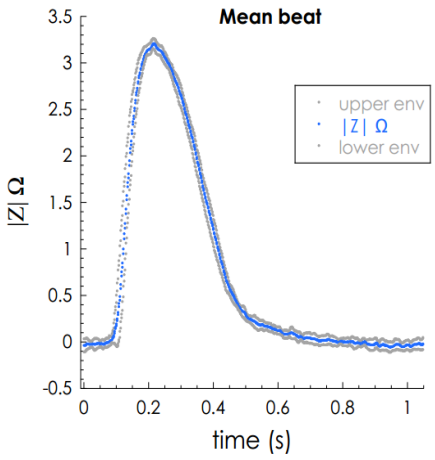
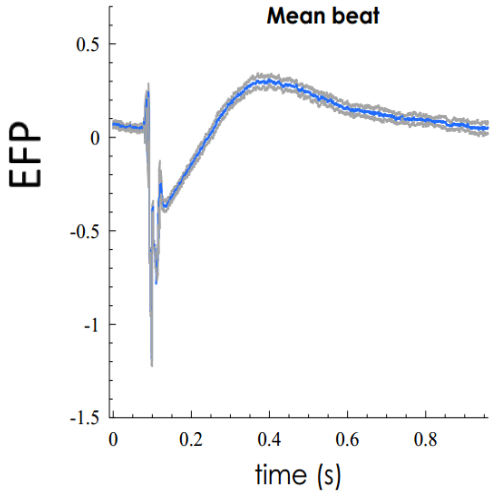
CardioExcyte96

Combined impedance and EFP recordings









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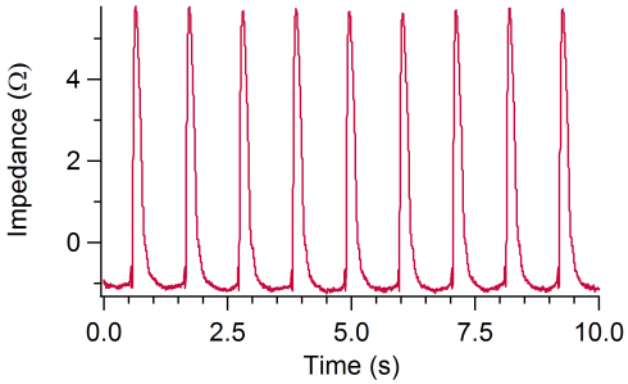
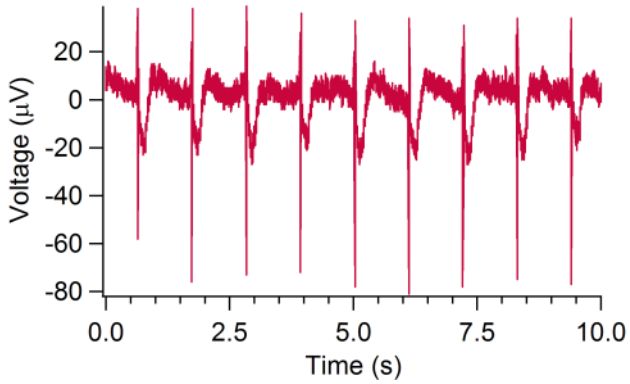
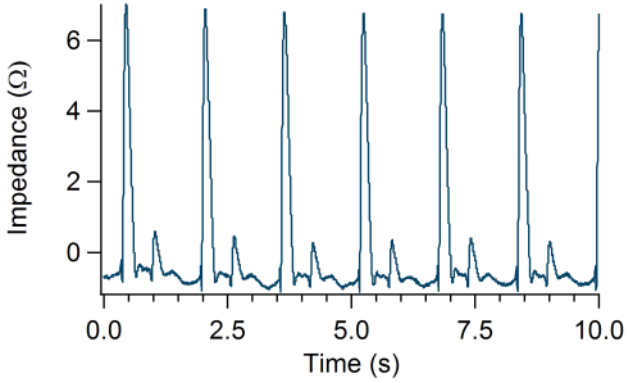
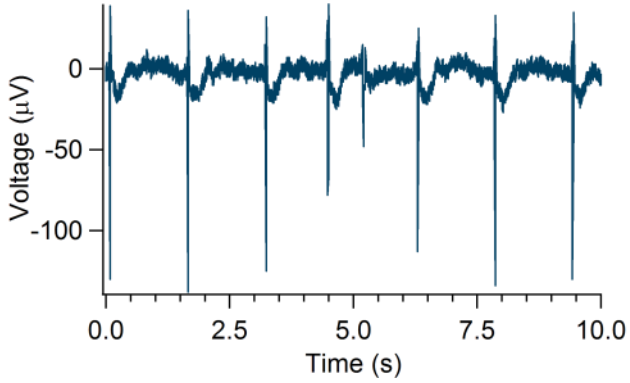
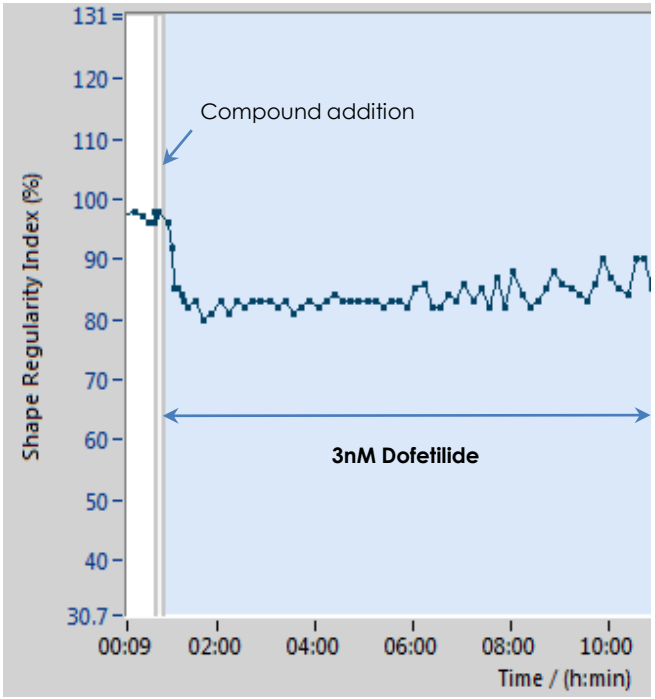
Cardiac Pharmacology: Dofetilide – single point addition

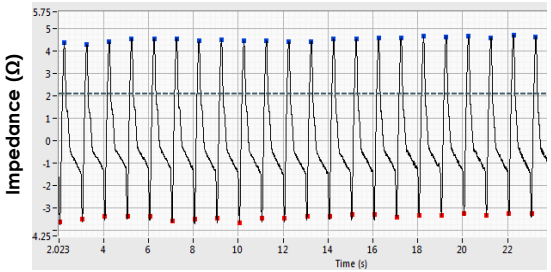
	Impedance	EFP
Baseline		
100 nM Dofetilide		

Detection and quantification of secondary beats

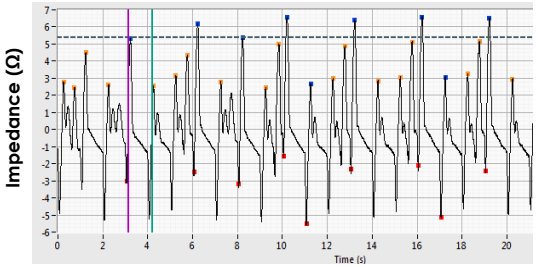
Impedance recordings



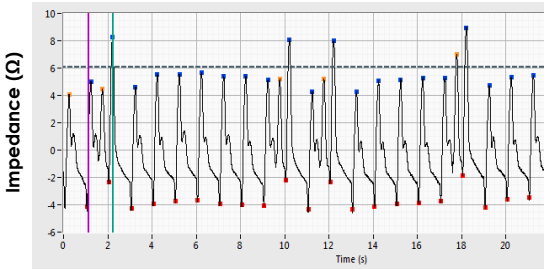
~35 min pre-addition



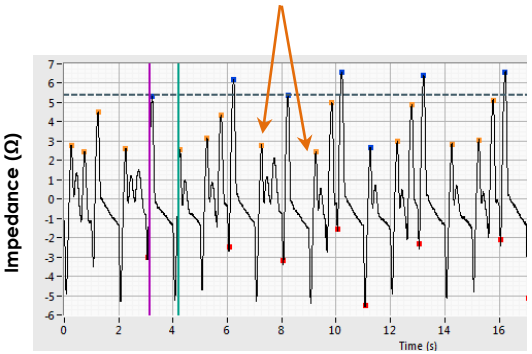
~1h 45 min post-addition



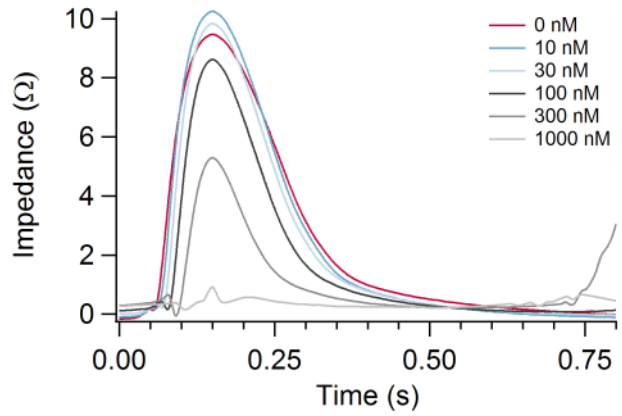
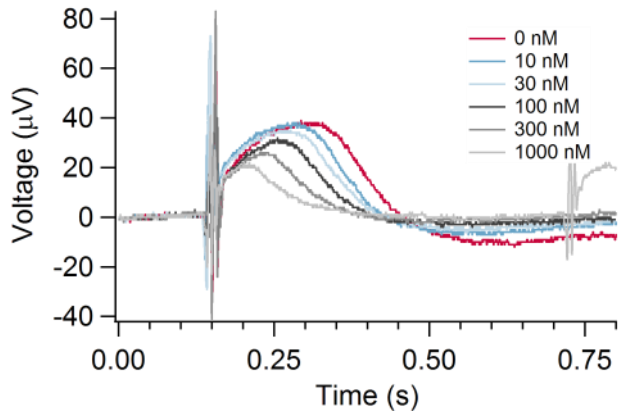
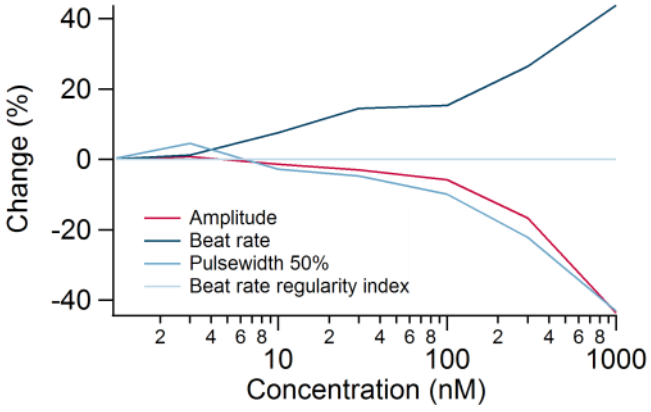
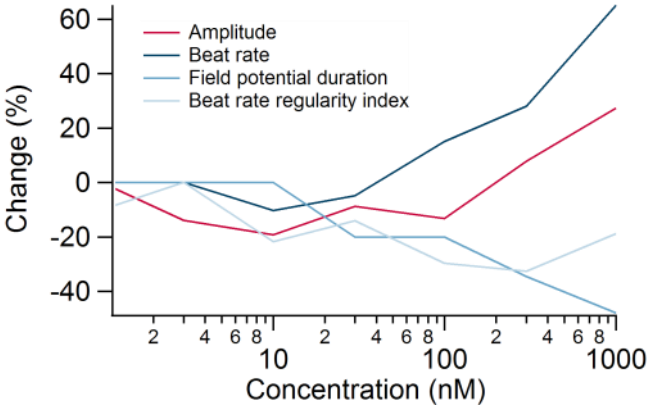
~9h 45 min post-addition



Automatic detection of secondary beats



Cardiac Pharmacology: Nifedipine

	Impedance	EFP
Raw data		
Dose-response		

Compound profiles

		Impedance				EFP				
		Amp	Rate	PW50	BRRI	Amp	Rate	FPD	BRRI	
Dofetilide	100 nM									hERG blocker
Cisapride	1 uM									hERG blocker
E-4031	1 nM									hERG blocker
Sotalol	1 uM									hERG blocker
Astemizole	1 nM									hERG blocker
Quinidine	30 uM									hERG,Ca,Na blocker
Terfenadine	1 uM									hERG,Na blocker
Isoproterenol	30 uM									β-adrenergic agonist
Nifedipine	300 nM									Ca blocker
BAYK8644	1 nM									Ca activator

increase

decrease

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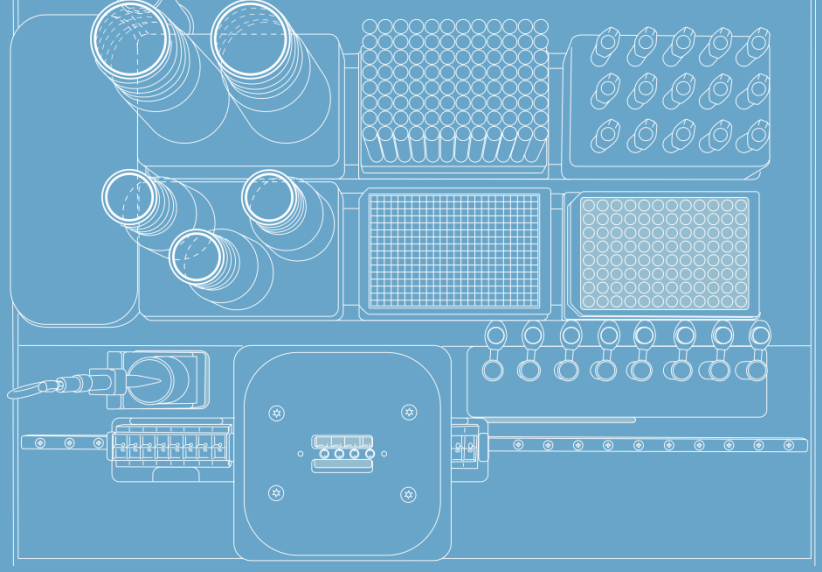

SyncroPatch 384 & 768 PE

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The PatchEngine – 100 % integration in HTS environment:

The PatchEngine –
the core of the SyncroPatch 384PE

- ✓ Fits into commercially available liquid handler
- ✓ Up to two **PE**'s per robot
- ✓ Open design allows integrations into fully robotic environments
- ✓ Used successfully with
  Beckman Coulter's Biomek
  Cybio's Felix

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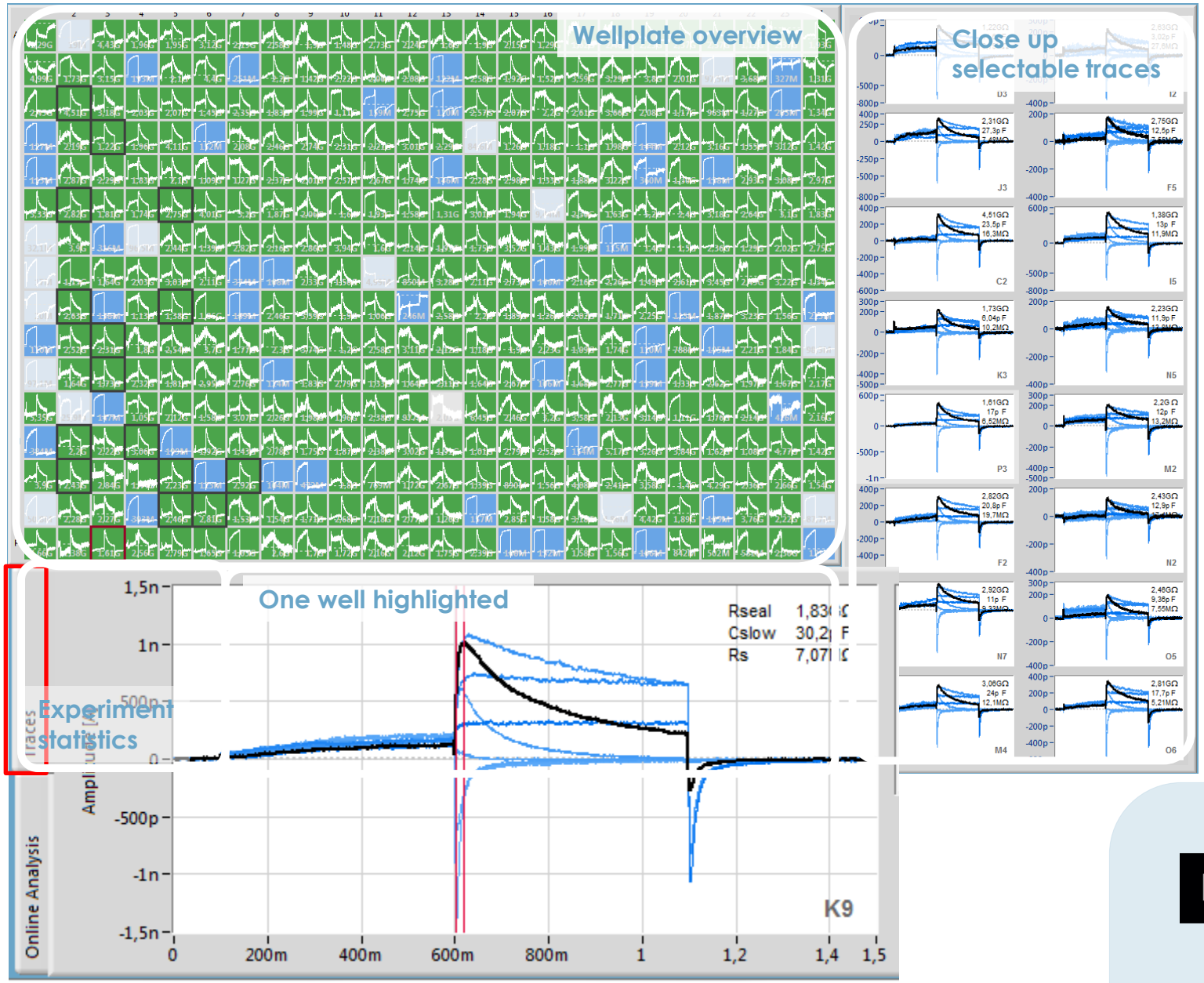
Current-Voltage (I/V) Relationship of hERG (CHO)



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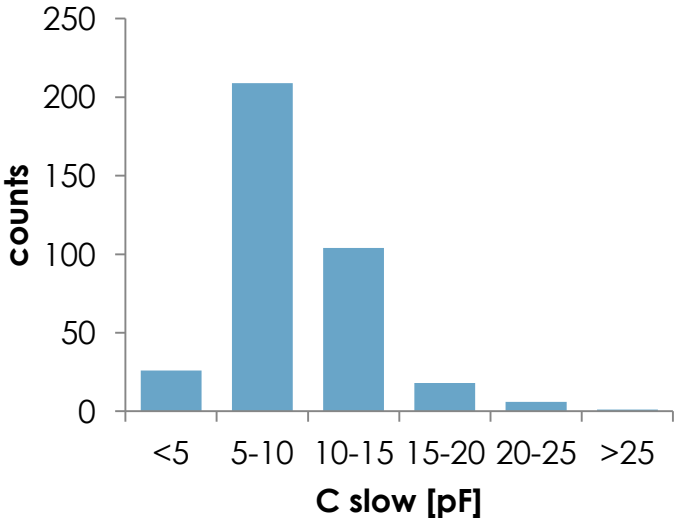
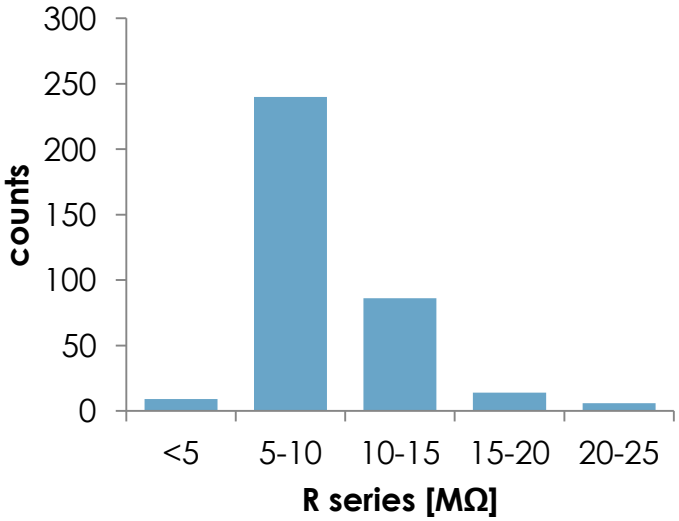
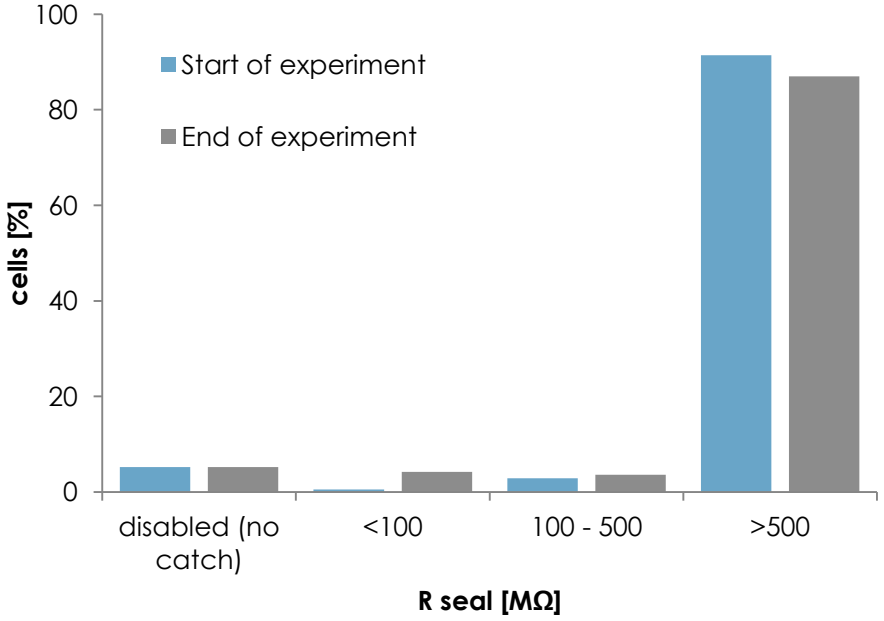
Success rates of hERG expressed in CHO cells







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Application Note

Channels: hERG, $Na_v1.5$, $Ca_v1.2$, $K_{ir}2.1$, $K_v4.3$, $K_v7.1$
Cells: HEK, CHO
Tools: SyncroPatch® 384/768PE

Simultaneous Assessment of CiPA Stipulated Ion Channels on the SyncroPatch® 384PE

The electrophysiology team at Nanion Technologies GmbH, Munich.
Cells kindly provided by ChanTest.

ChanTest™
a charles river company

Summary

The cardiac action potential is defined by multiple voltage-dependent ion channels (see Fig. 1). A drug candidate's capacity to interact with the ion channels involved in the depolarization or repolarization phases of the cardiac action potential is important for drug safety assessment. Until now, safety testing has focussed on interaction with the hERG channel and QT prolongation which can lead to potentially fatal torsades de pointes (TdP). Although this approach has been largely successful in preventing new drugs reaching the market with unexpected potential to cause TdP, it is also possible that potentially valuable therapeutics have failed due to this early screening. A new paradigm, the Comprehensive In-vitro Proarrhythmia Assay (CiPA), was introduced in 2013 to provide a more complete assessment of proarrythmic risk[1,2]. An assessment of a multitude of cardiac ion channels, in addition to hERG, should provide a more accurate prediction of the proarrythmic risk of a compound compared with testing on hERG alone.

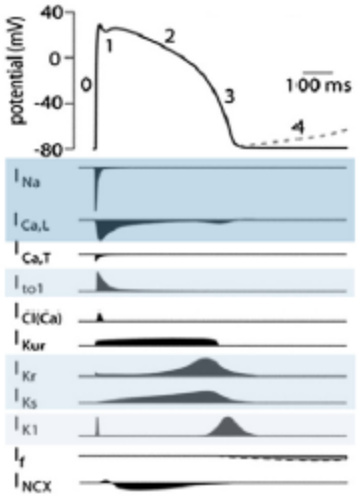


Figure 1: Cardiac action potential and underlying currents (reproduced from Ref. 2).

Results

A combined voltage step-ramp protocol (Fig. 2) was applied simultaneously to HEK or CHO cells expressing different cardiac ion channels. The first segment was used to elicit $Na_v1.5$ or $Ca_v1.2$ (1) followed by a classical hERG-like segment to activate $K_v4.3$, $K_v11.1$ and $K_v7.1$ (2) and finally a ramp protocol to elicit inwardly rectifying $K_{ir}2.1$ (3).

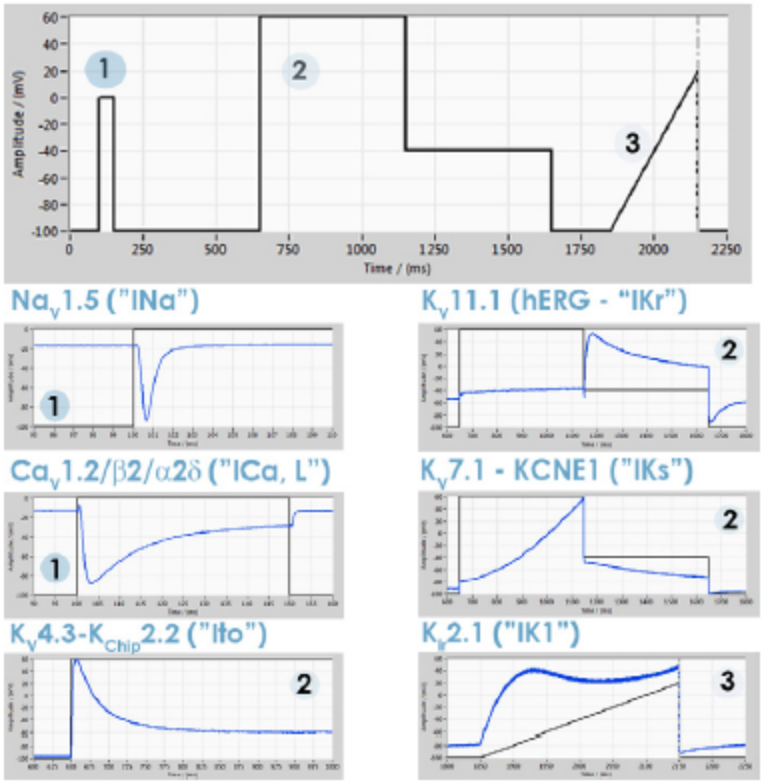


Figure 2: A voltage protocol was used to activate 6 distinct cardiac ion channels. The top panel shows the voltage protocol used on all wells to simultaneously activate the 6 distinct cardiac ion channel currents shown in the bottom panels.

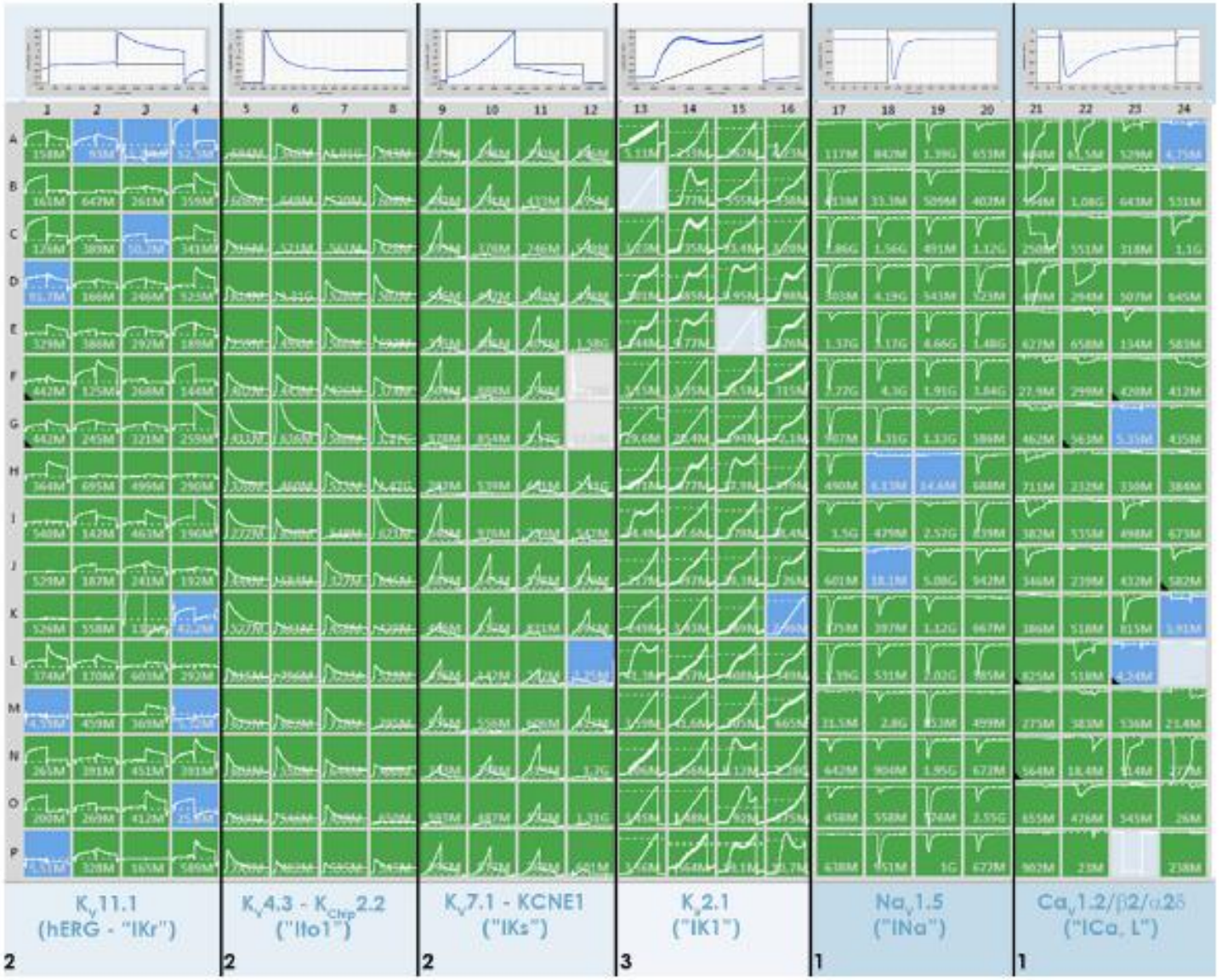
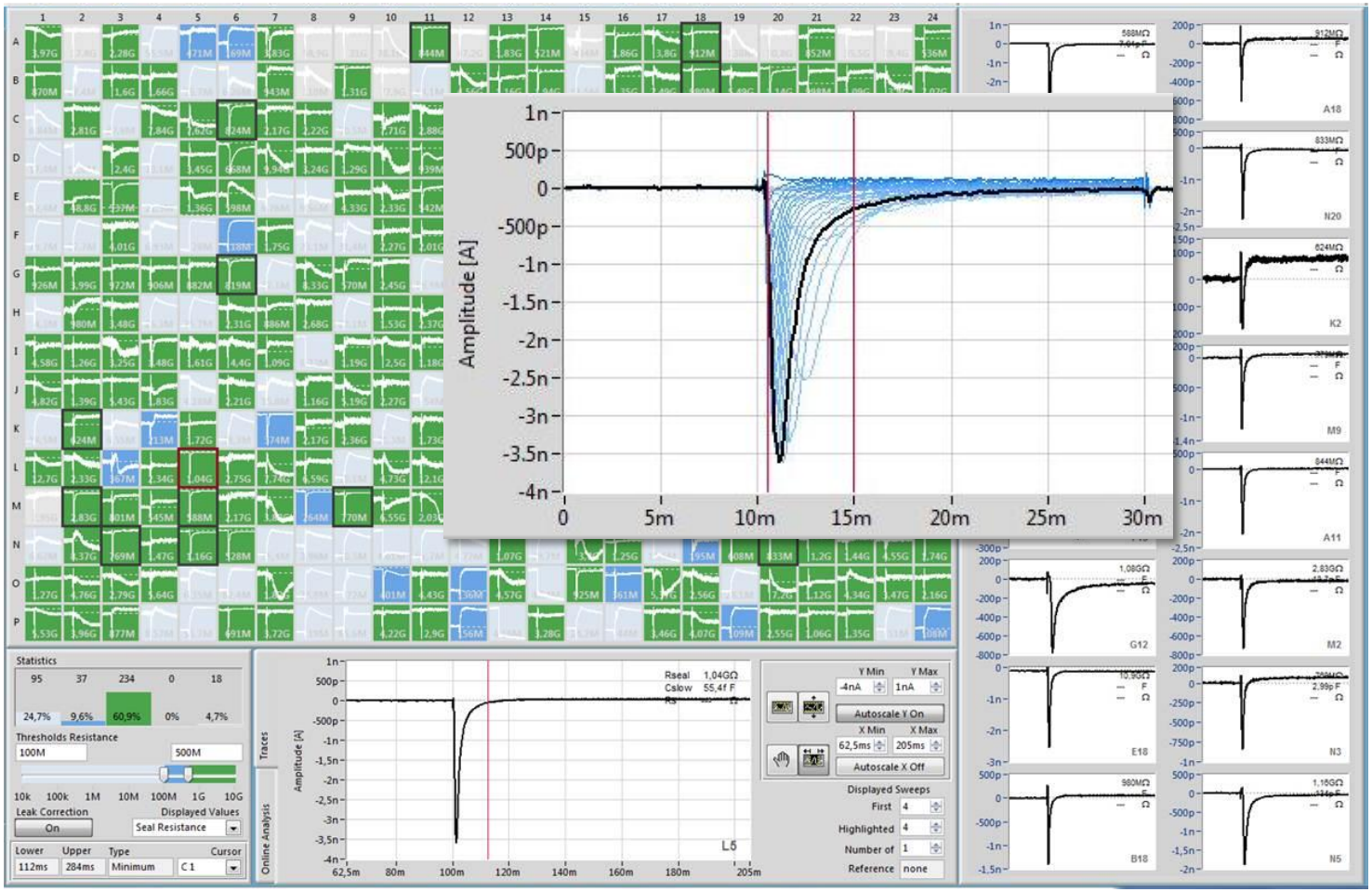


Figure 3: Six different cardiac channels recorded simultaneously on the SyncroPatch® 384PE. Shown is a screenshot of the data acquisition and analysis software used on the SyncroPatch® 384PE showing an experiment recording six different cardiac channels at once. A representative image of the current recording for each ion channel is shown at the top.

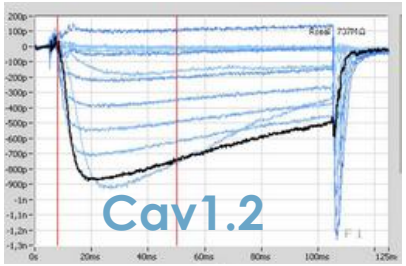
Ongoing project: HTS screening of CDI/Cor.4U cells!

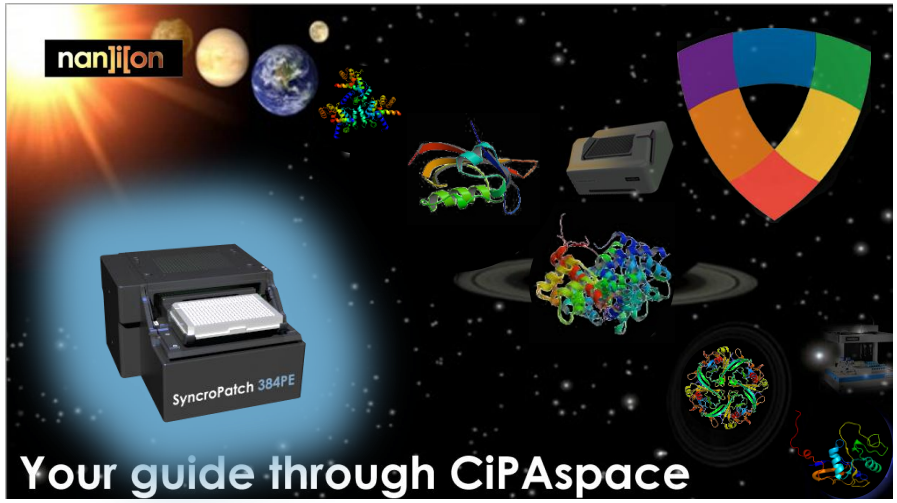


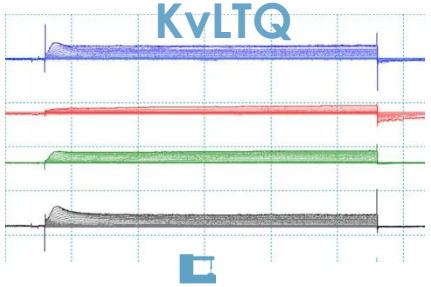
- ✓ Cor.4U iPSC Derived Human Cardiomyocytes
- ✓ Low Cell consumption: ~300 cells per well
- ✓ **One T-75 Flask with 1.5 Mio cells is sufficient for 10 x 384-patch clamp plates**

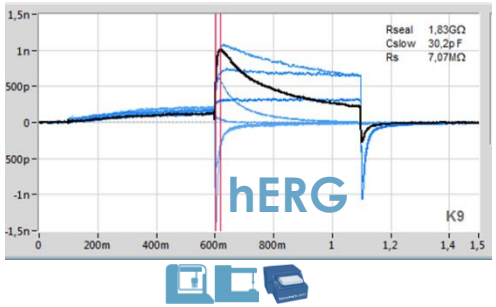
nan]i[on

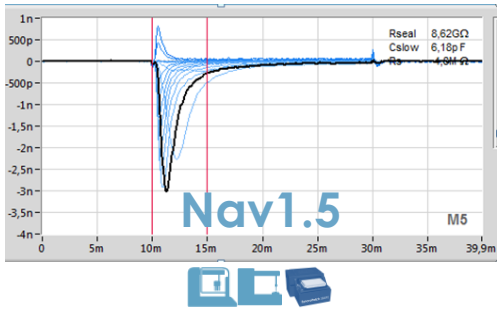
Summary: HTS on the CIPA stipulated ion channels

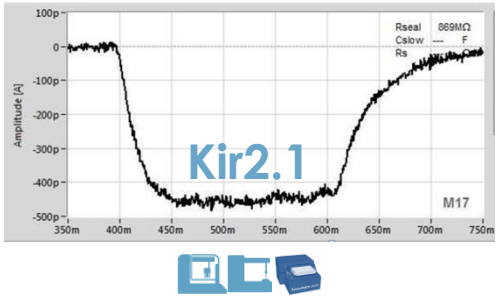


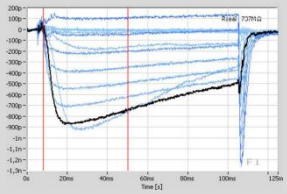








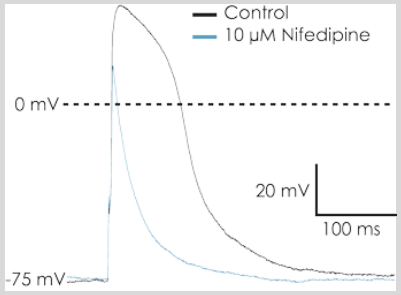




IV on SyncroPa

- ✓ Cor.4U iPSC Derived Human Cardiomyocytes
- ✓ All 6 targets recorded with high success rate
- ✓ We have cooperations with Millipore, ChanTest and Anaxon – choose your cell line!

Current clamp



Thank you!

- Company intro
- Overall platforms
- CIPA intro
- PL
- CE
- Syncro
- Relate to CIPA

CIPA | Calendar | CIPA Proposal | CIPA Project Documents | References & Background | Members

ILSI EXTRA > HESI > Science > Cardiac Safety Committee > CIPA

Libraries

CIPA

Comprehensive In Vitro Proarrhythmia Assay (CIPA)

The objective of the CIPA initiative is to facilitate the adoption of a new paradigm for assessment of clinical potential of TdP that is not measured exclusively by potency of hERG block and not at all by QT prolongation. The new CIPA paradigm will be driven by a suite of mechanistically based in vitro assays coupled to in silico reconstructions of cellular cardiac electrophysiologic activity, with verification of completeness through comparison of predicted and observed responses in human-derived cardiac myocytes. It is envisioned that the CIPA initiative will ultimately require the modification or replacement of the existing ICH S7a/b guidelines and elimination of E14 guidelines, although progress can be made in the short term under the existing regulatory construct. Read more about the proposal.

Anticipated Final O
CIPA will eliminate
negative dataset ba
profiling, in silico a

CIPA PARTNERS:
US FDA, HESI, C

New data Cardiomyos CIPA panel user meeting

HESI

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202.659.3306

7/8/2014

COMPOUNDS SELECTED FOR CIPA PROARRHYTHMIA TESTING

Compounds Identified as High Risk for Manifesting Human TdP

- Azimilide
- Bepridil
- Dofetilide
- Ibutilide
- Quinidine
- Vandetanib
- Methadone
- D,l Sotalol

Compounds Identified as Intermediate Risk for Manifesting Human TdP

- Astemizole
- Chlorpromazine
- Cisapride
- Clarithromycin
- Clozapine
- Domperidone
- Droperidol
- Terfenadine
- Pimozide
- Risperidone
- Ondansetron

Compounds Identified as No or Very Low Risk for Manifesting Human TdP

- Diltiazem
- Loratadine
- Metoprolol
- Mexiletine
- Nifedipine
- Nitrendipine
- Ranolazine
- Tamoxifen
- Verapamil
- Flecainide