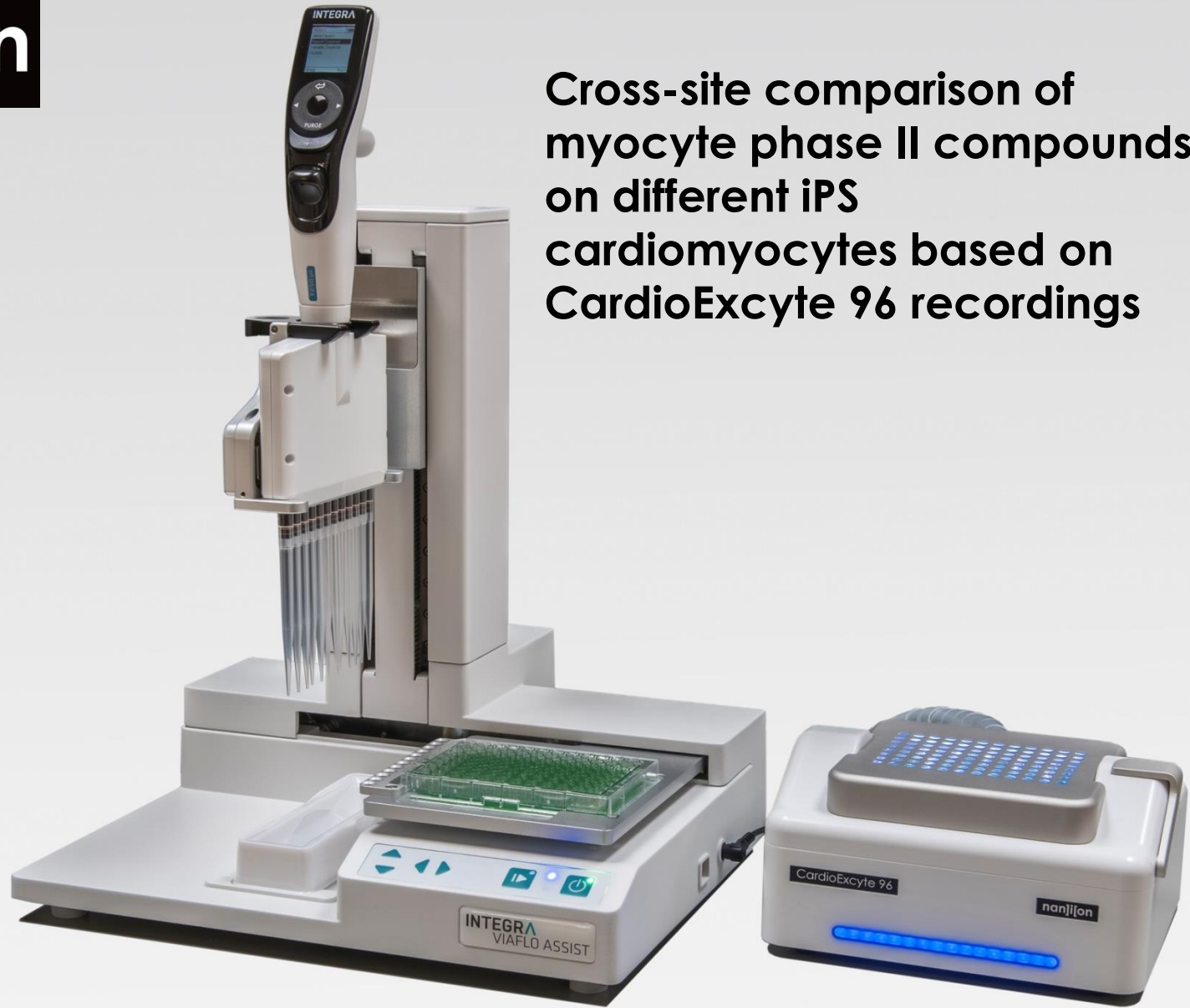


nan]i[on

**Cross-site comparison of
myocyte phase II compounds
on different iPS
cardiomyocytes based on
CardioExcyte 96 recordings**



Nanon Technologies

Nanon participation in CiPA

nan[i]on



CiPA

- ✓ Part of the leadership team in the HTS ion channel screening subteam:
5 cardiac ion channels recorded on automated patch clamp devices
(Patchliner & SyncroPatch384 PE and others)
- ✓ Participant in the myocyte phase II study:
hiPS cardiomyocytes on impedance/EFP system: the CardioExcyte96

CiPA power tools



Highlights

CiPA Myocyte II results:

- Data collection protocols
- Reported endpoints
- Cross-site evaluation for two CiPA sites and three cell types

Outlook:

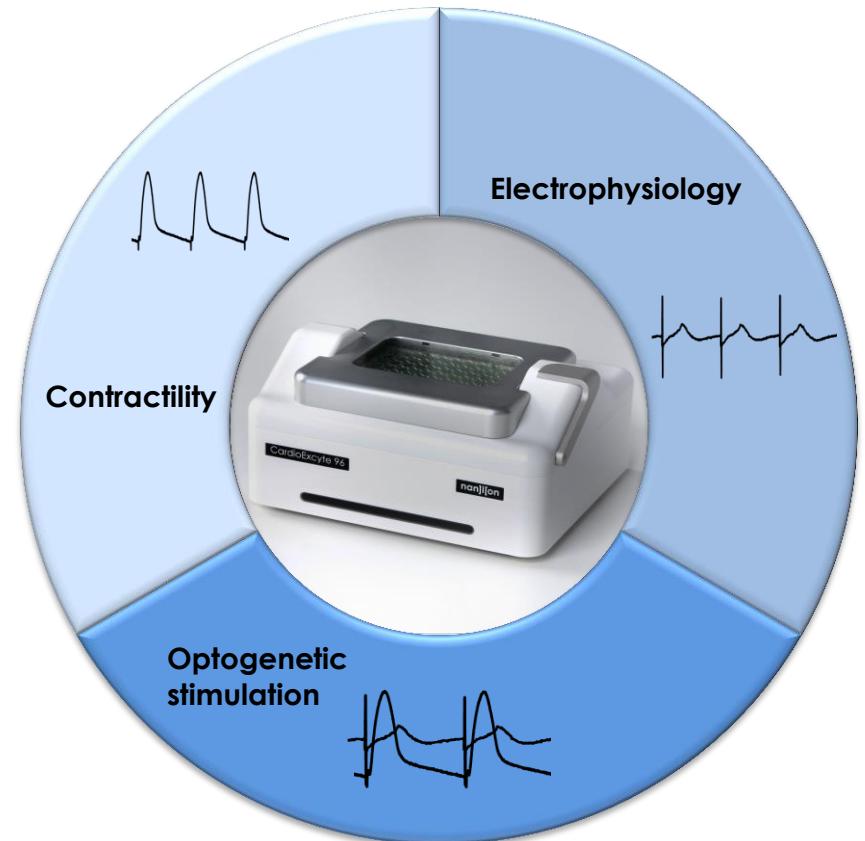
- CardioExcyte96 data from all sites will be compiled in a publication
- If other non-core sites are interested in comparing their data to ours, please contact us at info@nanion.de

Combined Impedance/**E**xtracellular **F**ield **P**otential (EFP) assay provides mechanistic electrophysiological endpoints in vitro

CiPA ✓ Established SOPs for
Impedance/EFP measurements
in frame of the CiPA studies



INTEGRA



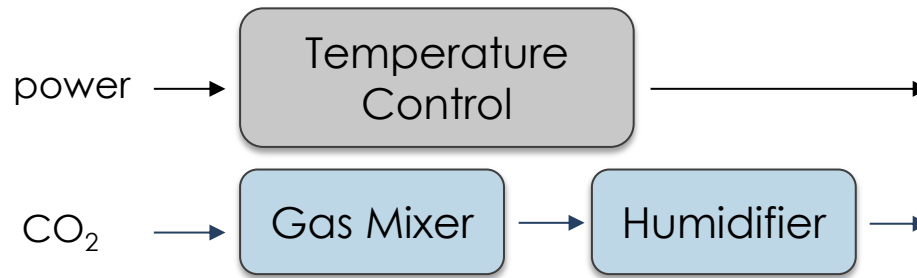
The CardioExcyte 96

- **96 wells** – 96 parallel sensors
- Efficient data analysis
- Automated liquid handling robot for cell seeding, medium exchange

Replacing the Incubator

CardioExcyte 96 Incubation System

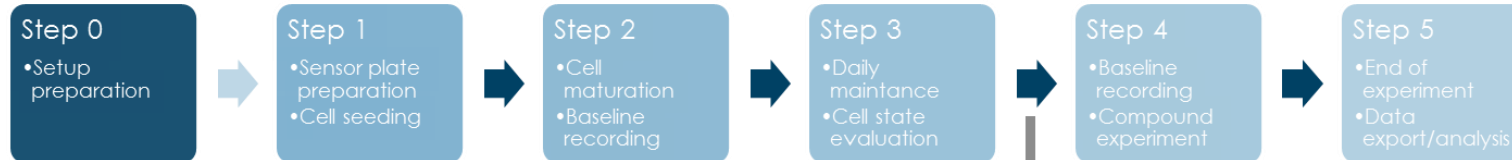
- Temperature control from RT to 60°C
- CO₂ Control Range 0.1 % - 20 %
- Humidity Control Range 20 % - 99 %



+

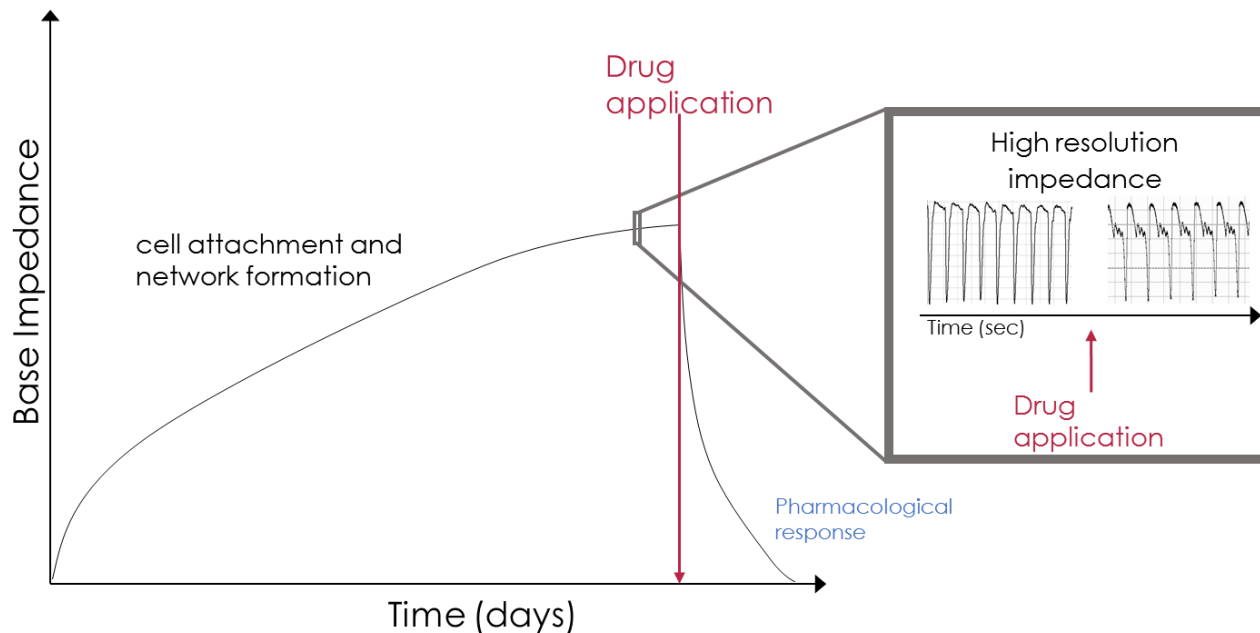


CIPA
workflow



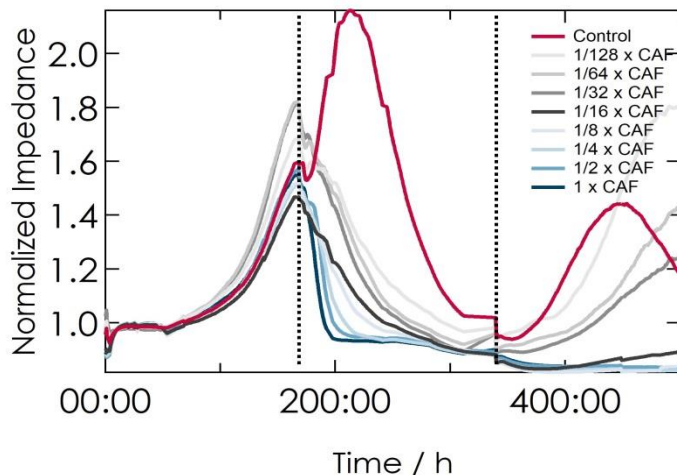
If cells did not pass the evaluation, please repeat Step 2 or check another plate if available.

Long and Short-term Recordings for Chronic or Acute Pharmacology



Chronic effects

Acute effects

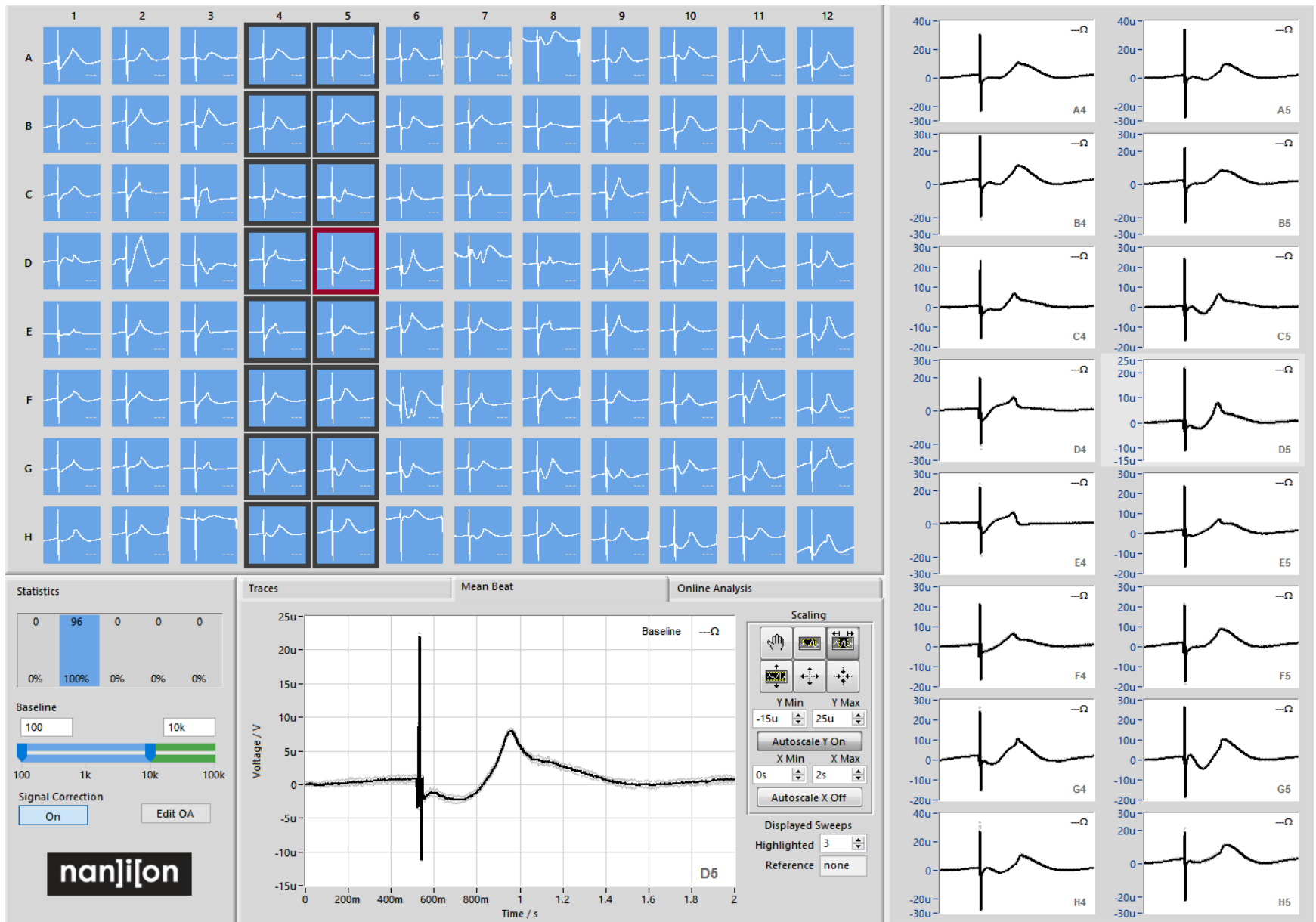


NSP[®]-96

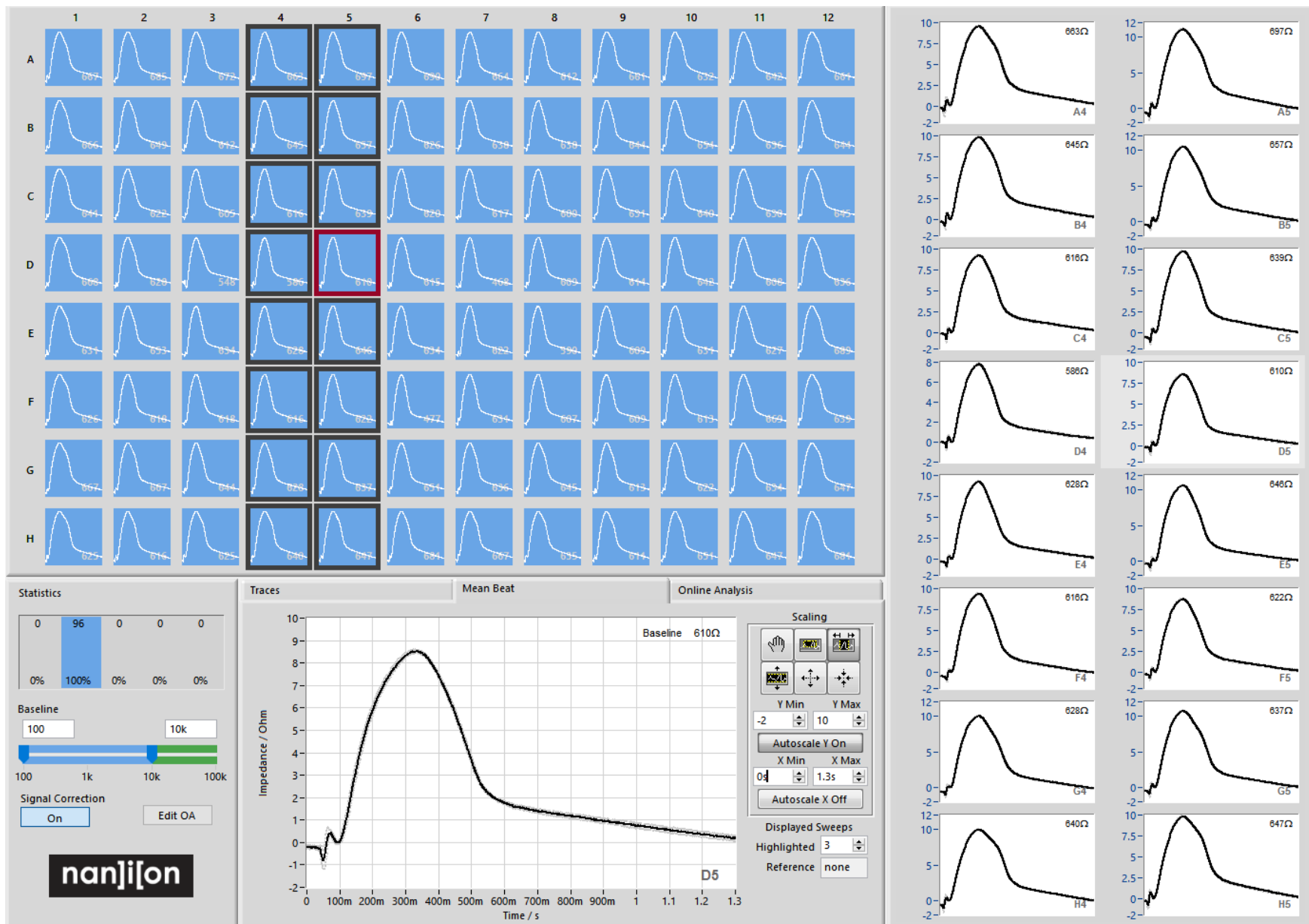
Sensor plates
allow stable
recordings
over weeks



Transparent sensor plates



CardioExcyte96 experimental view of mean extracellular field potential signals recorded in stem cell derived cardiomyocytes. Random selection of multiple wells (black squares) allows the display of the impedance recordings on the right. Data from cell type 1



CardioExcyte96 experimental view of mean impedance beat signals recorded in stem cell derived cardiomyocytes. Random selection of multiple wells (black squares) allows the display of the impedance recordings on the right. Data from cell type 1

Endpoints can be monitored live, while system is recording, or can be further processed when recording is finished, offline

Beat Detection and Analysis

I) Choose OA functions

Base Imp
Ampl
Beat Rate
BR CoV
PW 30%
Falling T
#primary
#secondary
Temp

Area under Beat
Amplitude
Beat Rate
Beat Rate Regularity
Pulsewidth
Rising Time
Falling Time
Shape Regularity Index
Base Impedance
Primary Beats
Secondary Beats
Sensor Temperature
Upstroke Velocity
Total RMS
Stimulus Parameter

II) Adjust additional

Parameters available to process
Impedance data

Beat Detection and Analysis

I) Choose OA functions

Ampl
Beat Rate
FPD(max)

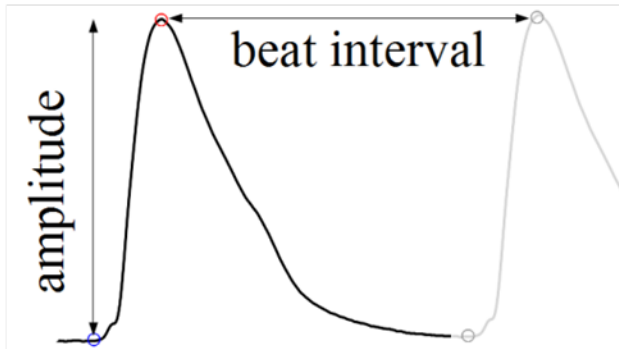
Amplitude
Beat Rate
Beat Rate Regularity
Shape Regularity Index
Primary Beats
Secondary Beats
Sensor Temperature
Field Potential Duration
Total RMS
Stimulus Parameter

II) Adjust additional set

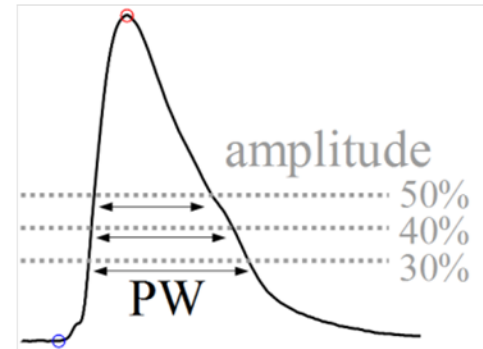
Parameters available to process
EFP (voltage) data

Online Analysis - Parameters

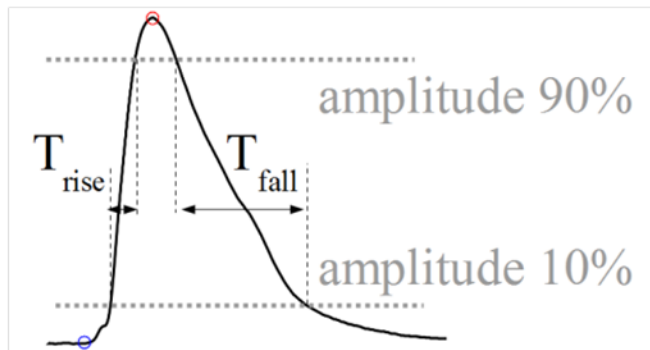
Amplitude & Beat Rate



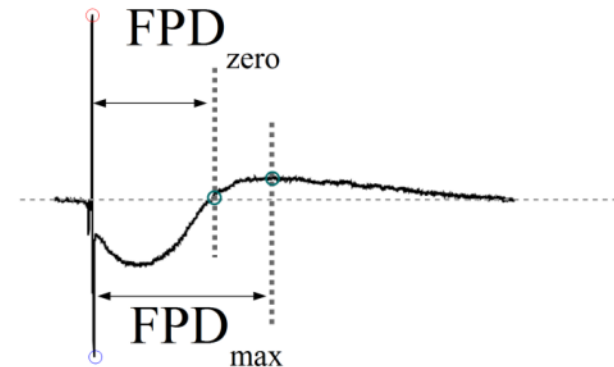
Pulsewidth



Rise Time & Fall Time

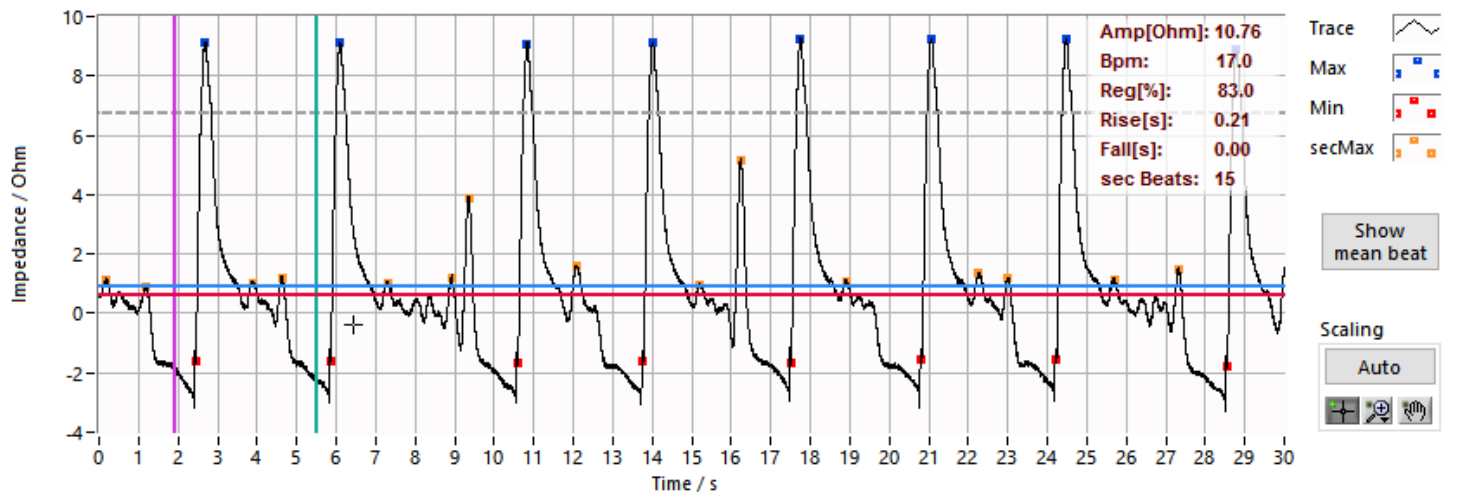
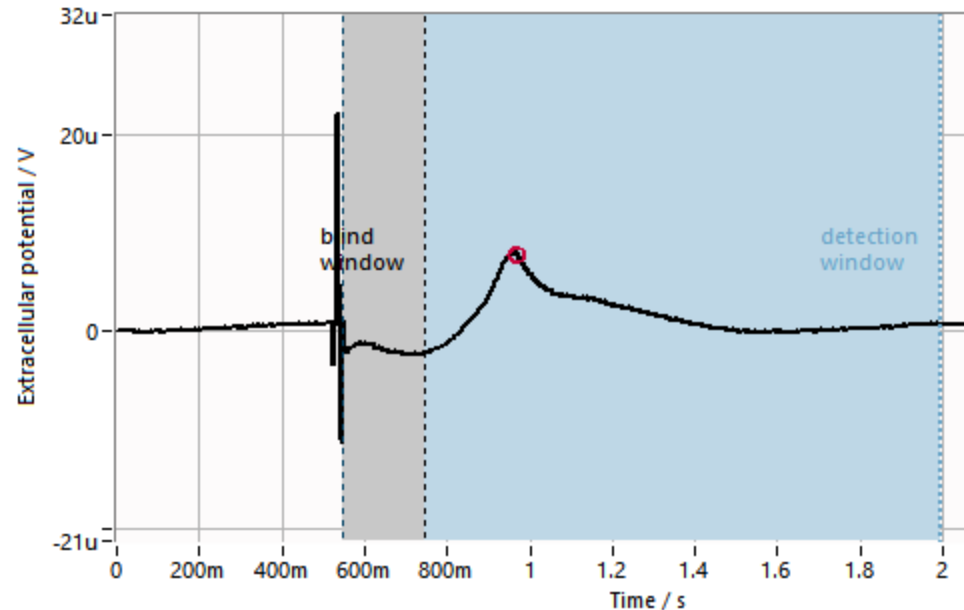


Field Potential Duration (EFP)



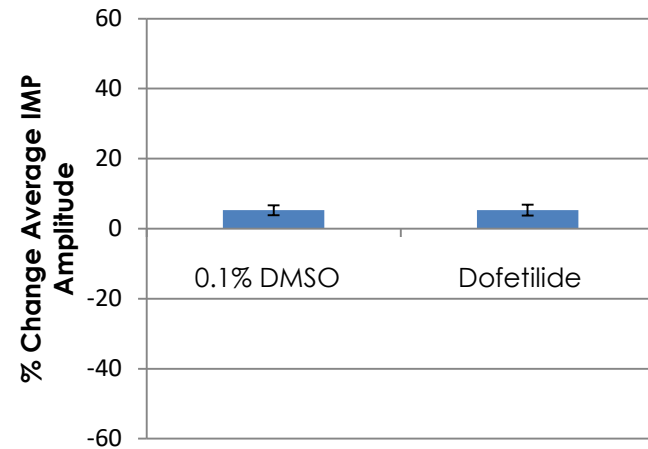
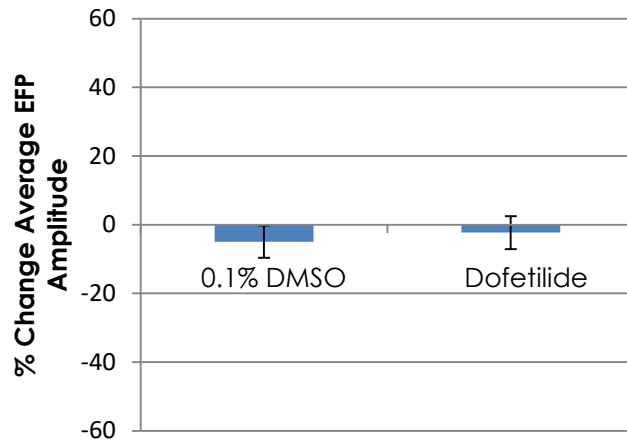
Online Analysis - Parameters

Automated detection and calculation of Field Potential Duration.

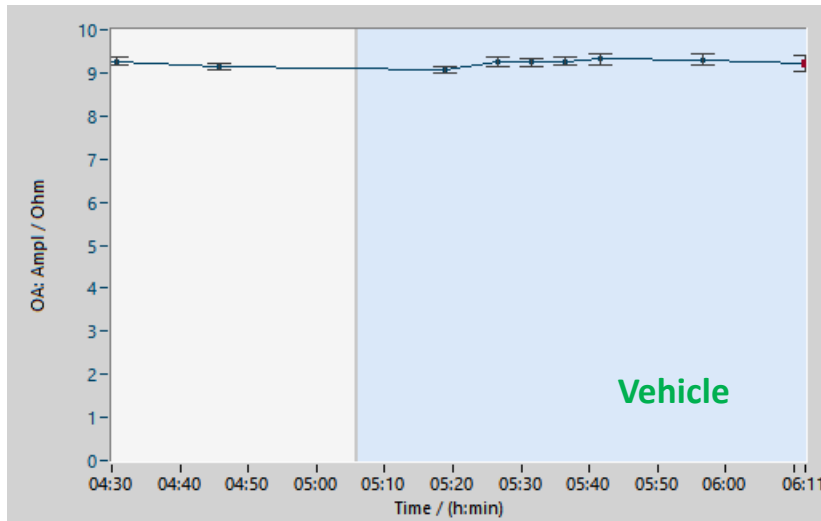


Secondary beat detection allows quantification of number of arrhythmic events for each recorded sweep

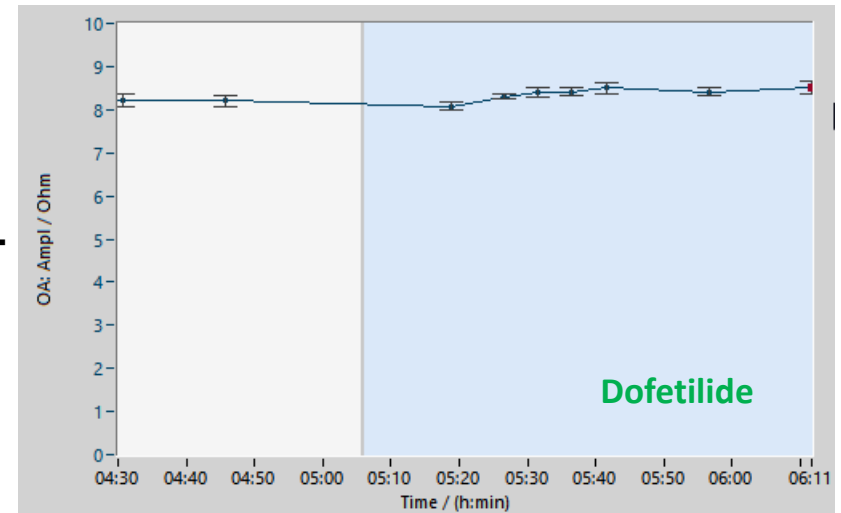
Long-term Stability of Vehicle control and Dofetilide



IMP Amplitude

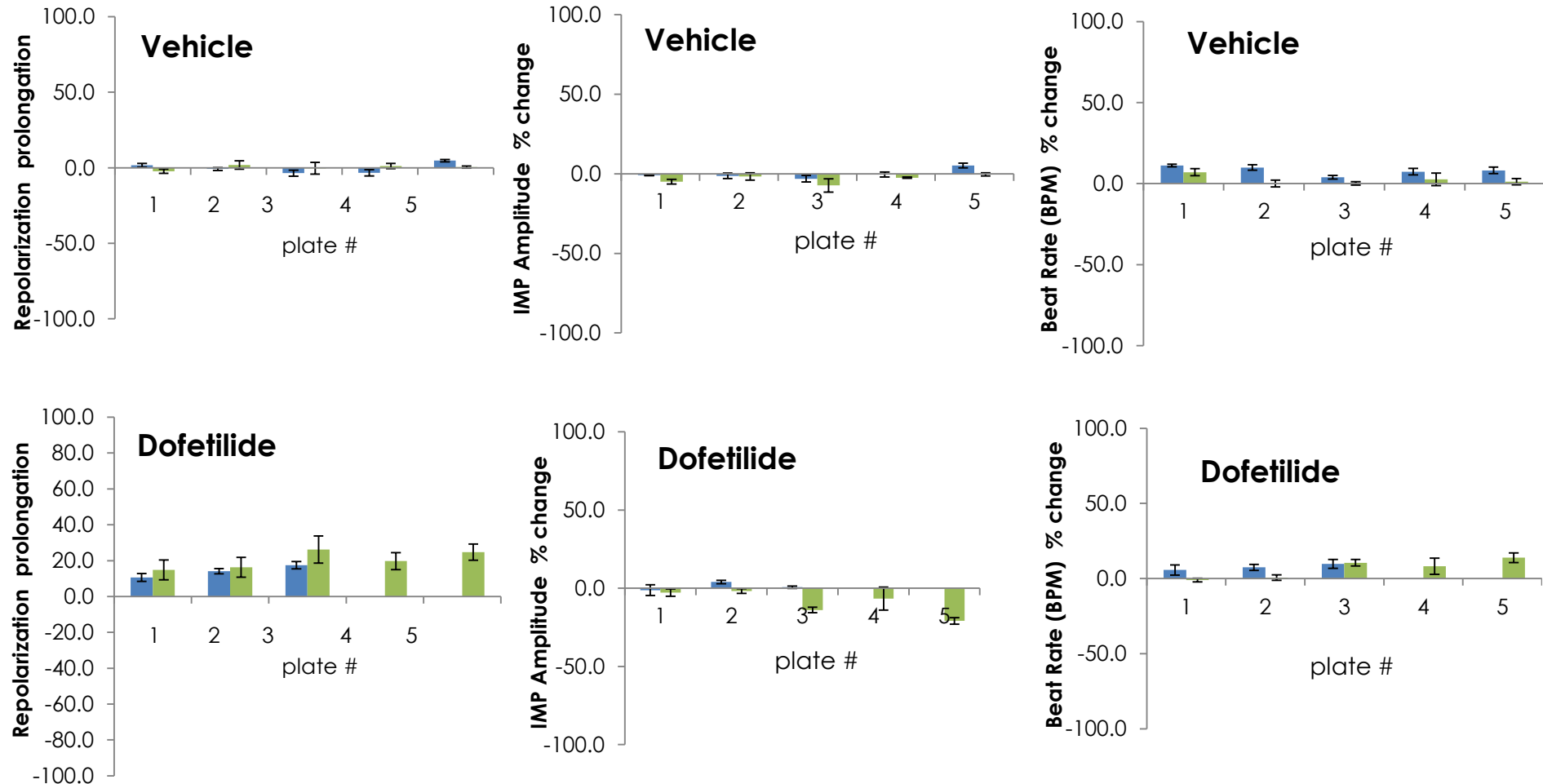


IMP Amplitude



No significant amplitude changes are present in either impedance or EFP modes when comparing addition of vehicle vs CiPA recommended concentration of Dofetilide. Bottom graphs represent average beat rate calculations for a representative plate treated. Data from cell type 1

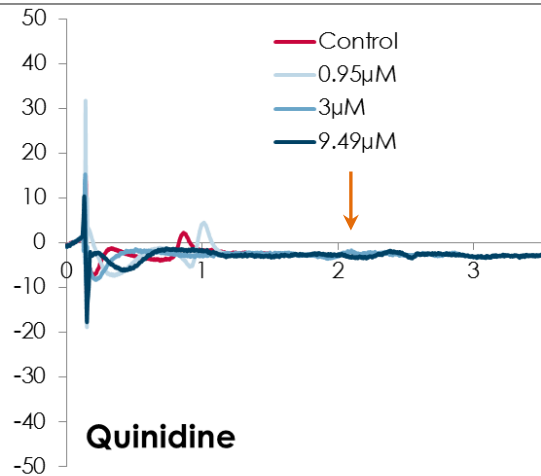
Control Experiments: Vehicle vs Dofetilide



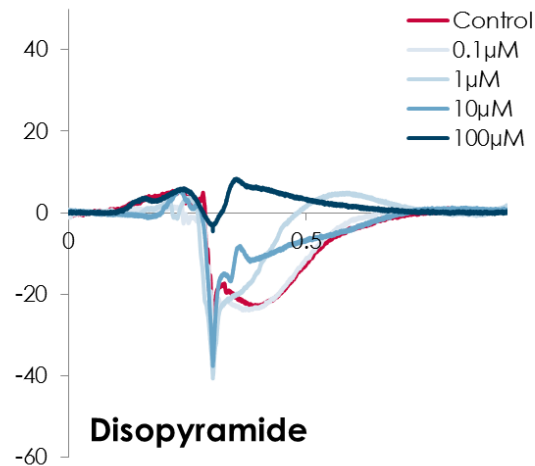
An increase in repolarization prolongation is observed post application of Dofetilide, when comparing to vehicle control. No significant changes are present in beat rate, when comparing Dofetilide to vehicle control.

High Risk: Extracellular Field Potential Pharmacology

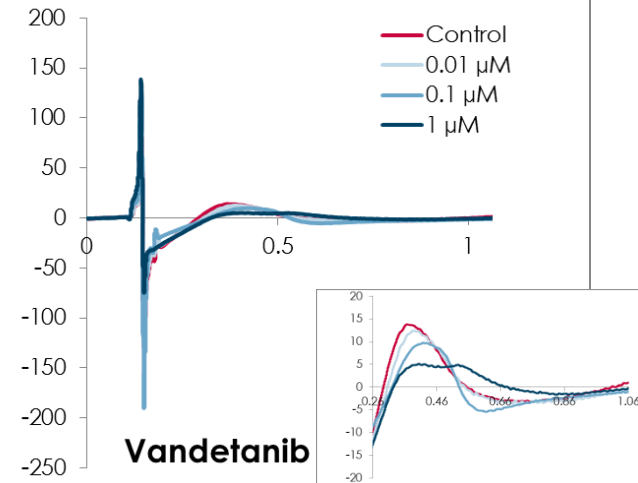
cell type 1



cell type 2



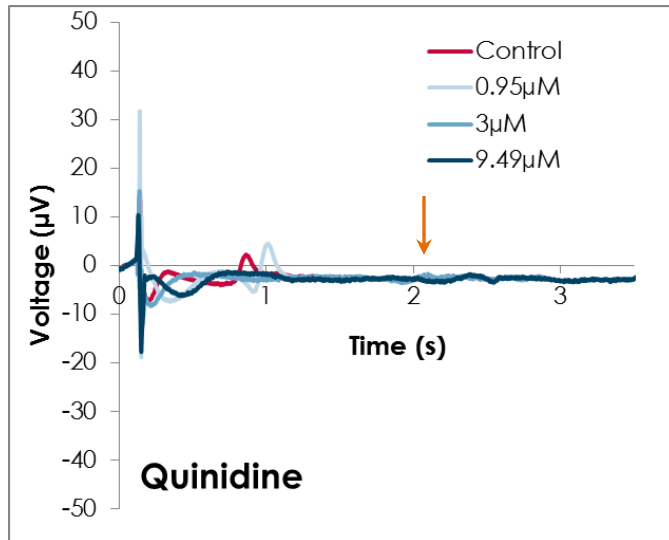
cell type 3



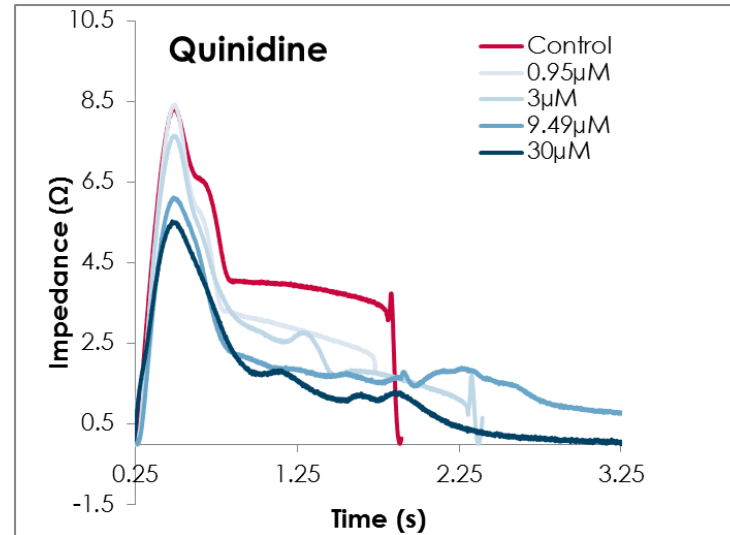
Extracellular field potential is prolonged post application of different high risk compounds.
Raw data examples showing one high risk compound for each cell type.

High Risk: Quinidine Pharmacology

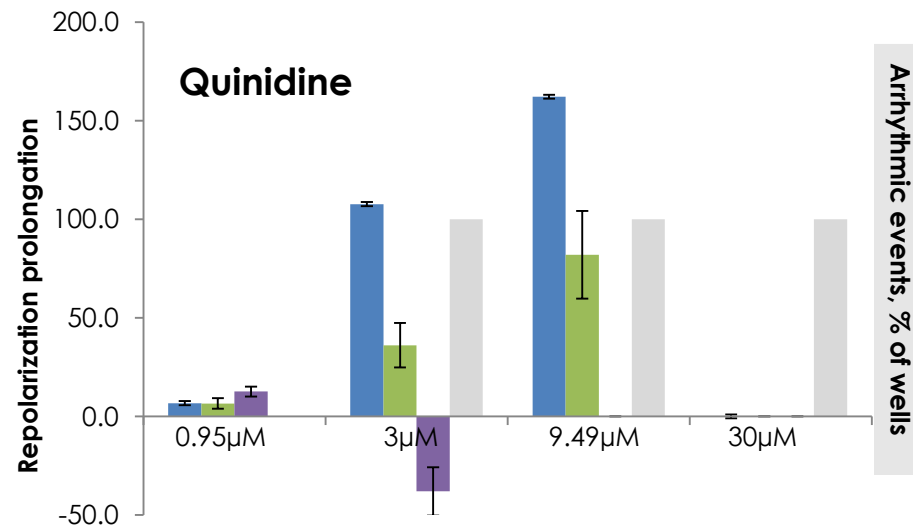
cell type 1



cell type 1



Extracellular field potential is prolonged post application of Quinidine. Compound induced arrhythmic events, that are also visible in impedance traces (right panel). Statistical comparison of repolarization prolongation (bottom left, **cell type 1 (blue)**, **cell type 2 (green)**, **cell type 3 (purple)**)).



CE96

Repolarization prolongation

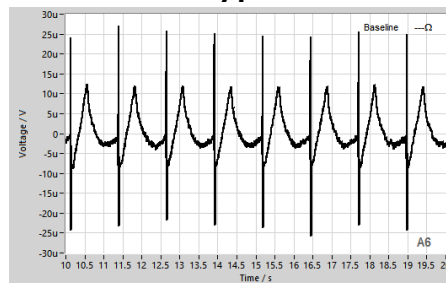
d, l Sotalolol

High Risk

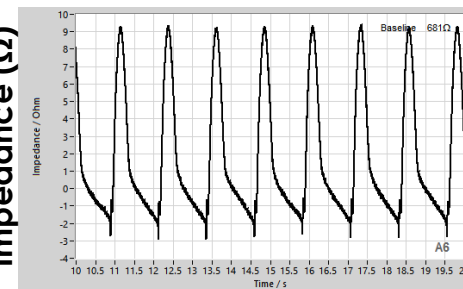
Arrhythmic events, % of wells

Voltage (μ V)

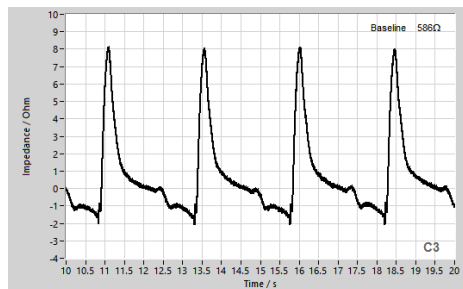
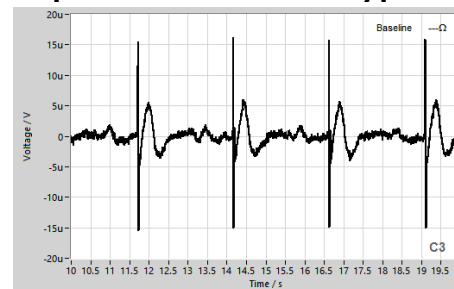
Control, cell type 1



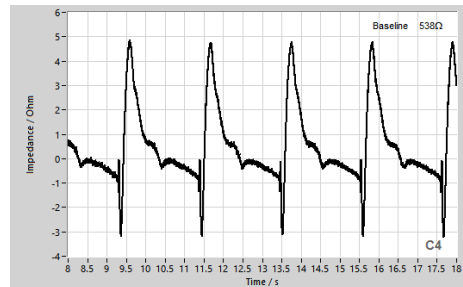
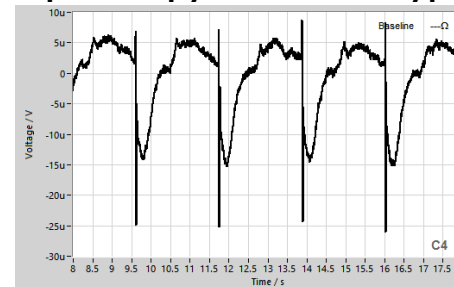
Impedance (Ω)



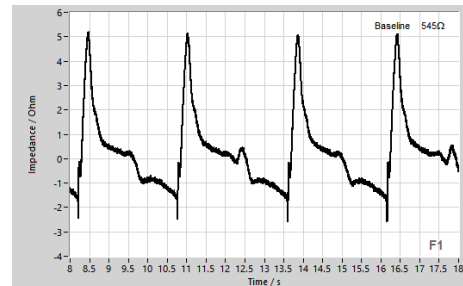
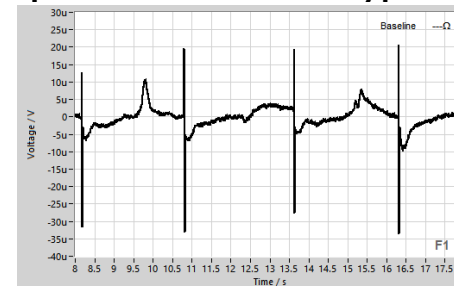
10 μ M d, l Sotalolol, cell type 1



10 μ M Disopyramide, cell type 1



1 μ M Vandetanib, cell type 1



Repolarization prolongation

Disopyramide

Arrhythmic events, % of wells

Vandetanib

Arrhythmic events, % of wells

Repolarization prolongation

0.01 μ M

0.1 μ M

1 μ M

10 μ M

0.1 μ M

1 μ M

10 μ M

100 μ M

0.1 μ M

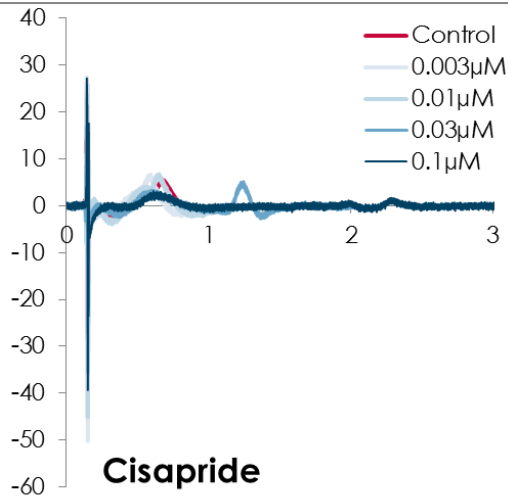
1 μ M

10 μ M

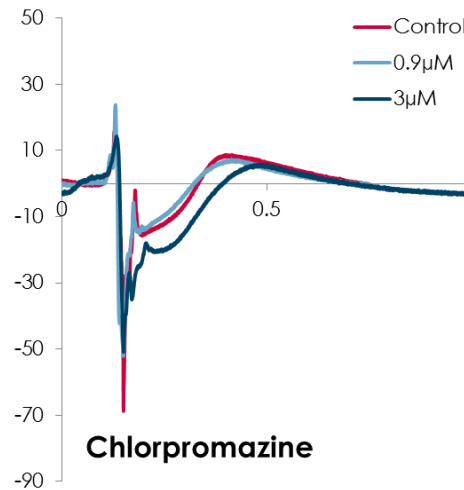
100 μ M

Intermediate Risk: Extracellular Field Potential Pharmacology

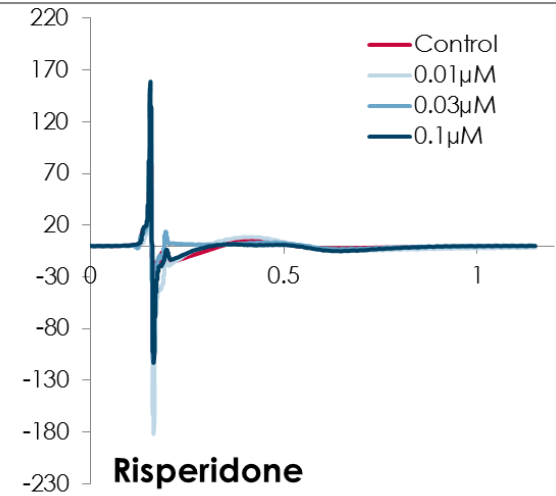
cell type 1



cell type 2



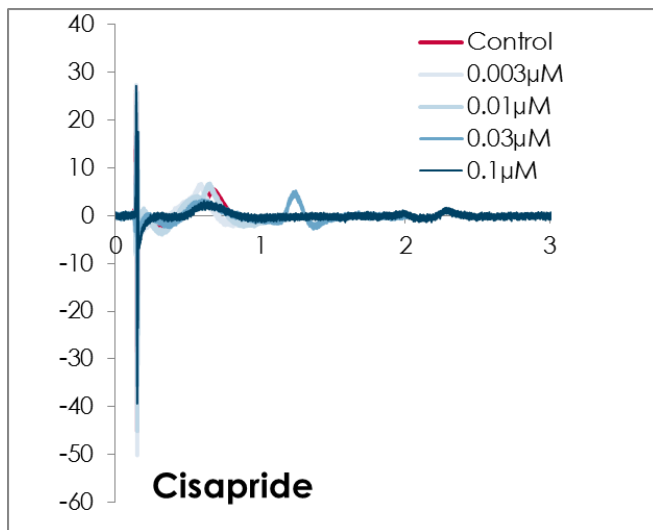
cell type 3



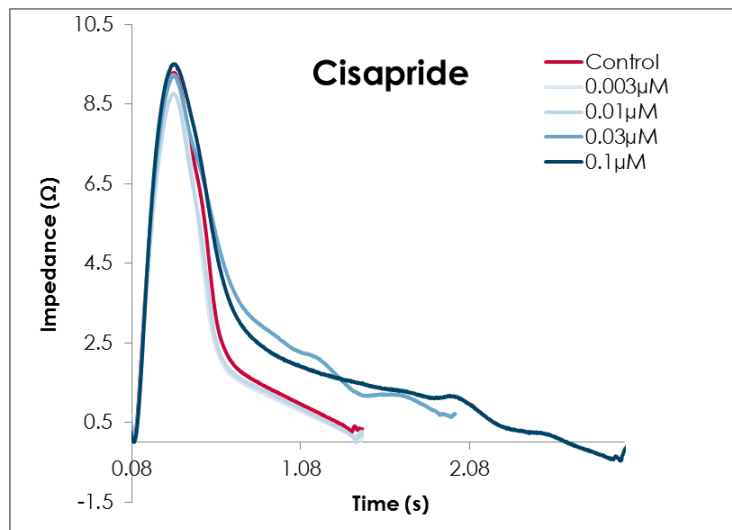
Effects of different intermediate risk compounds on extracellular field potential. Raw data examples showing one intermediate risk compound for each cell type.

Intermediate Risk: Cisapride Pharmacology

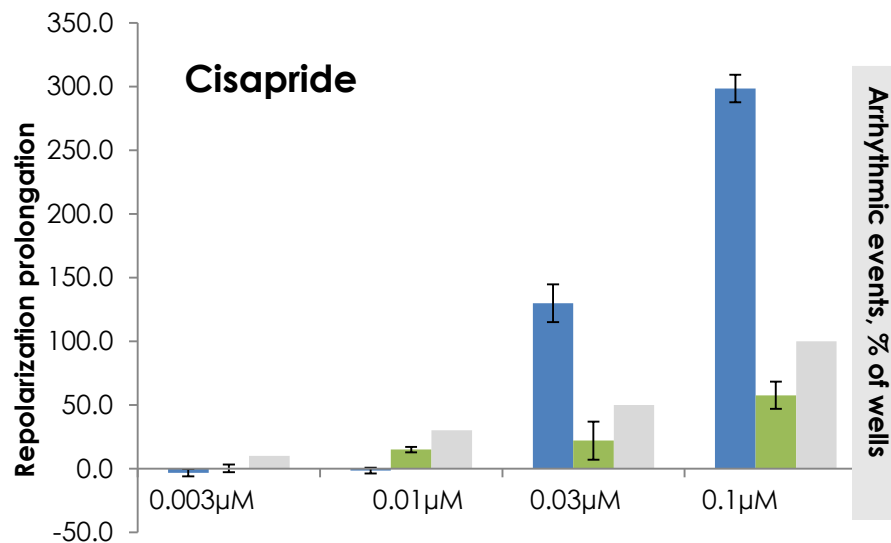
cell type 1



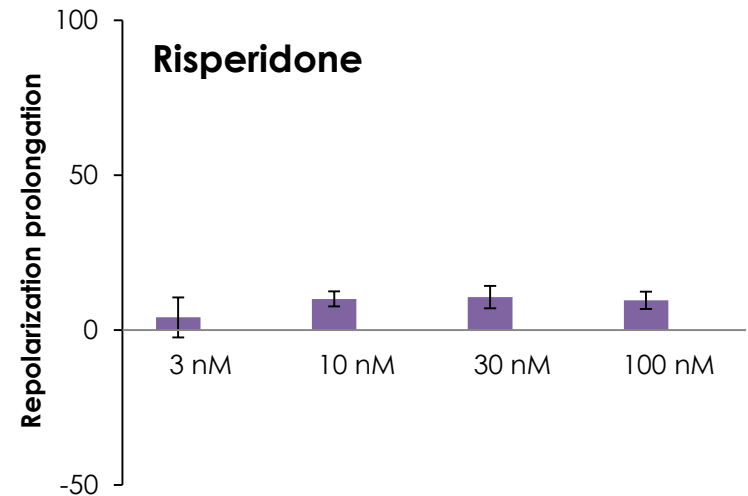
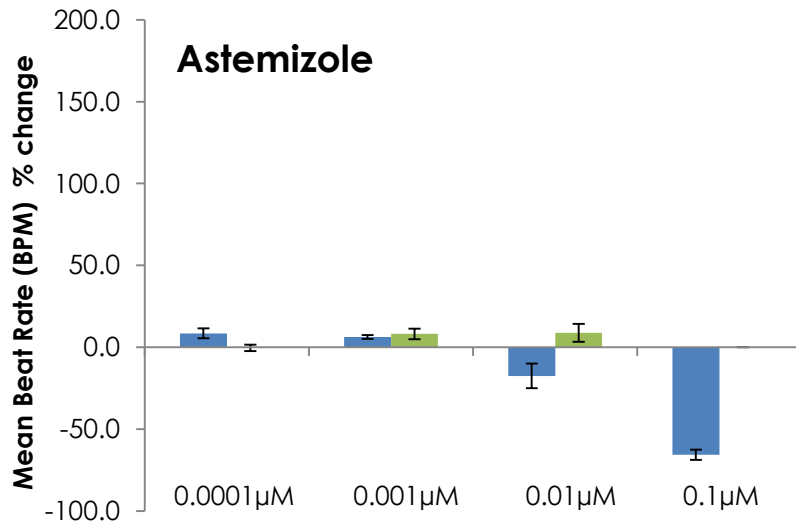
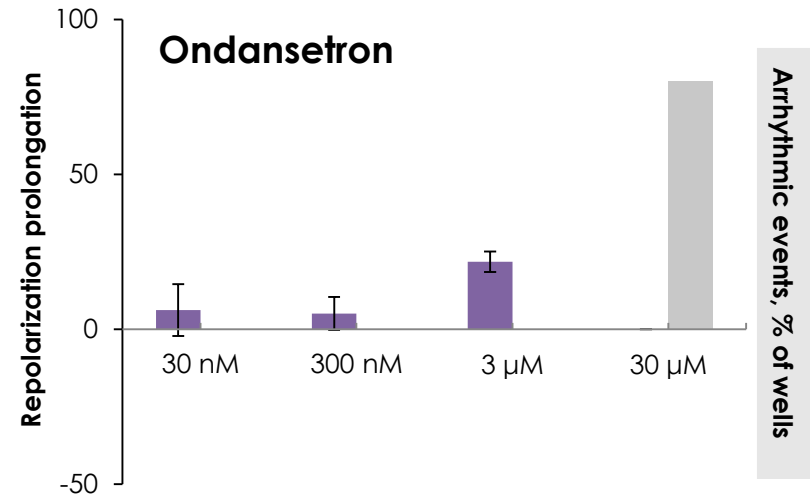
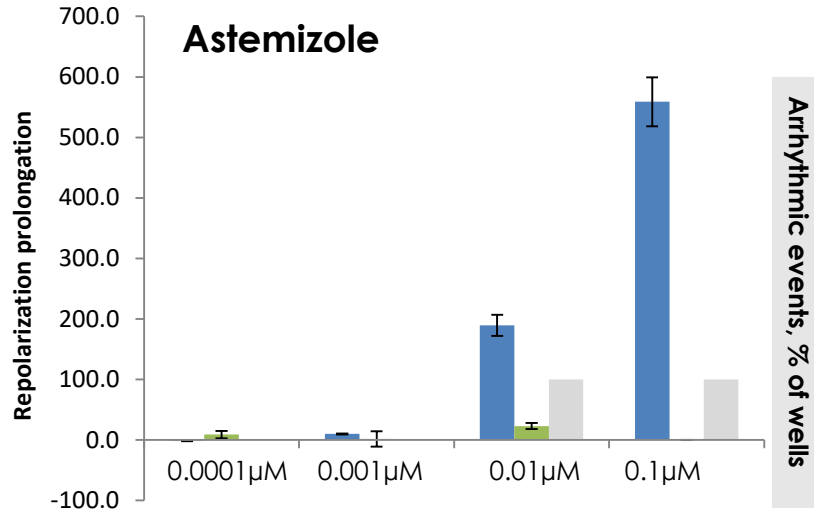
cell type 1



Extracellular field potential is prolonged post application of Cisapride. In impedance mode, Cisapride induced arrhythmic events as well, together with a prolongation of the pulse width.



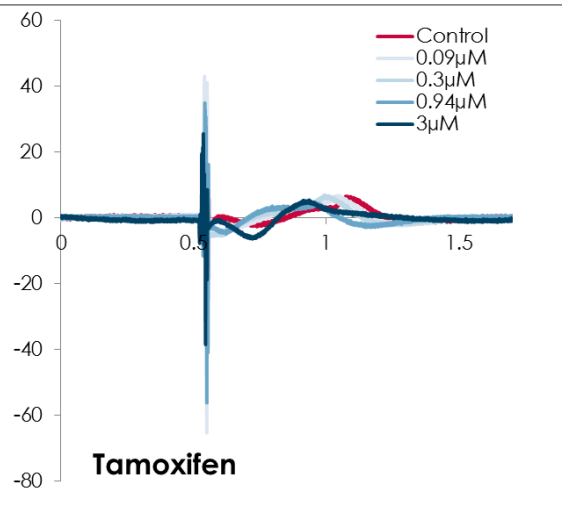
Intermediate Risk Compounds



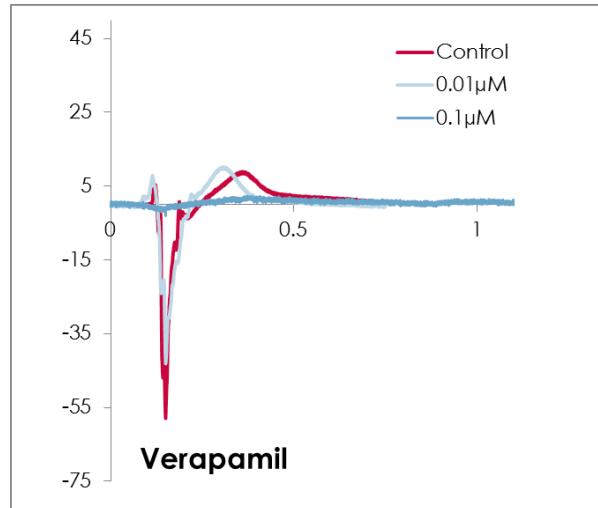
Intermediate risk compounds prolonged the repolarization of the field potential at different rates, or produced changes in beat rate.

Low Risk: Extracellular Field Potential Pharmacology

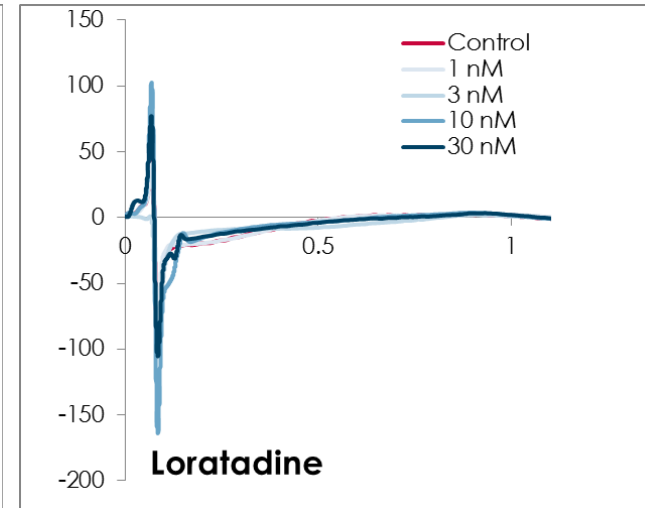
cell type 1



cell type 2

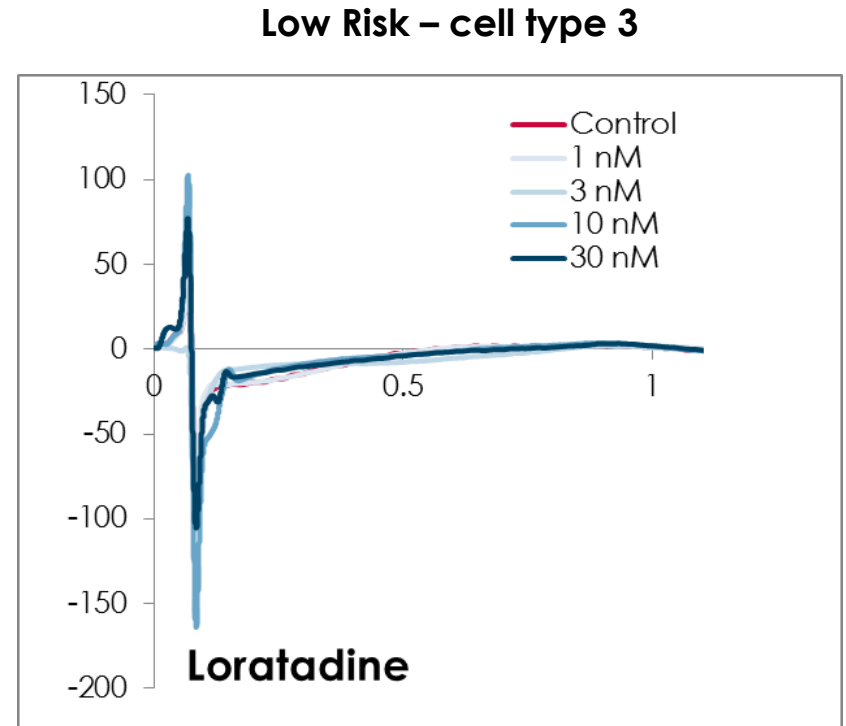
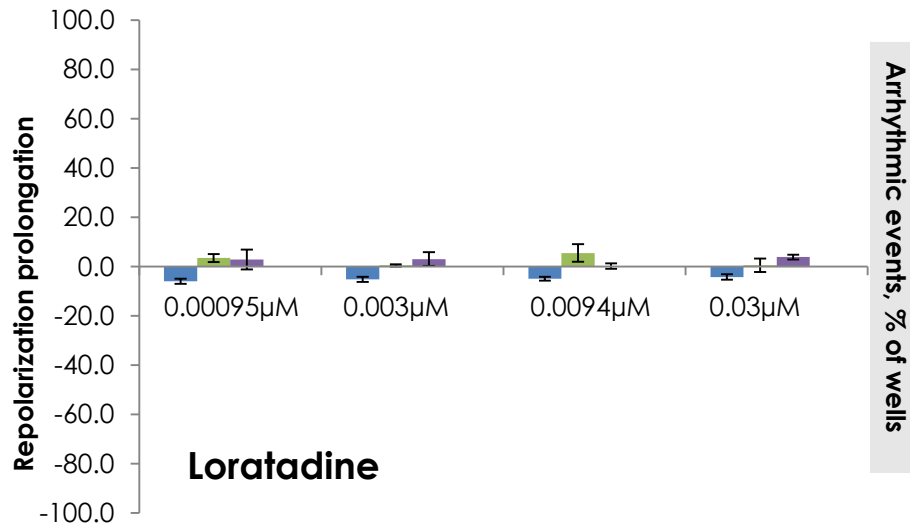


cell type 3



Effects of different low risk compounds on extracellular field potential. Raw data examples showing one low risk compound for each cell type.

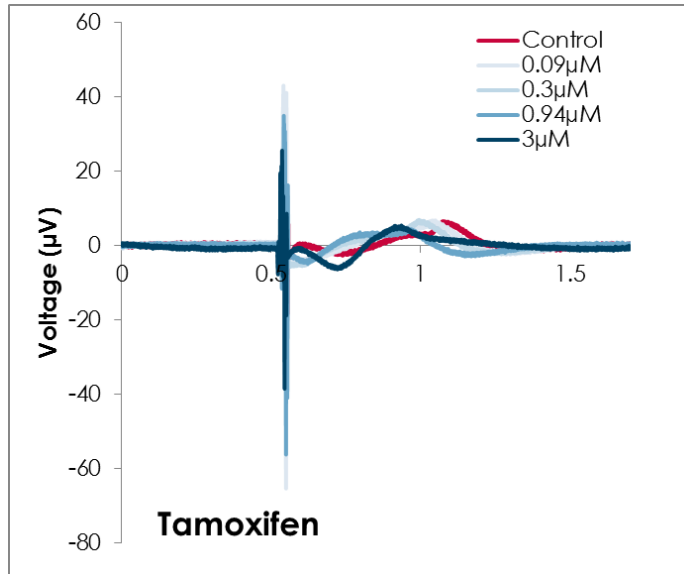
Low Risk: Loratadine Pharmacology



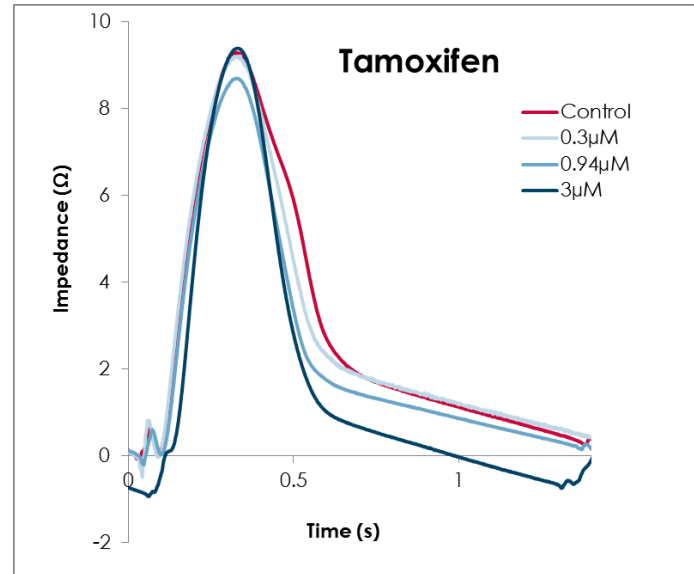
No significant extracellular field potential changes are observed post application of Loratadine.

Low Risk: Tamoxifen Pharmacology

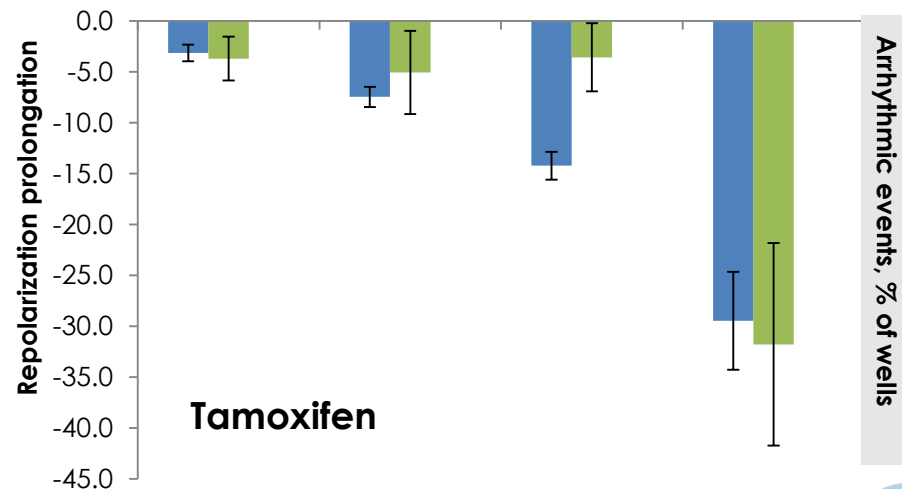
cell type 1



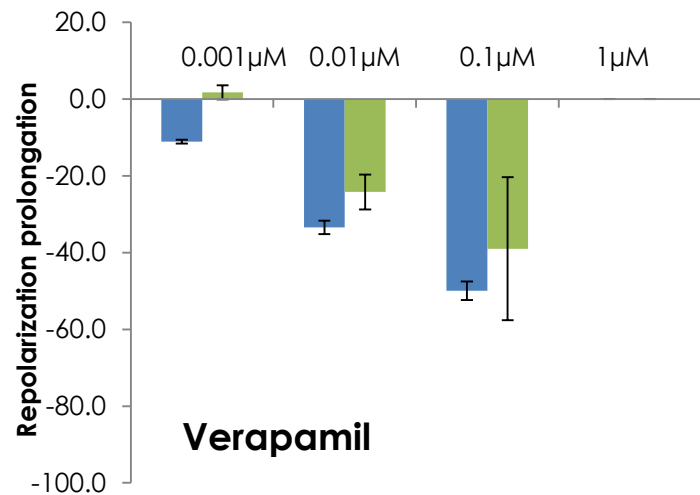
cell type 1



Extracellular field potential is shortened post application of Tamoxifen. In impedance mode, Tamoxifen produced a shortening the pulse width (bottom right graph).



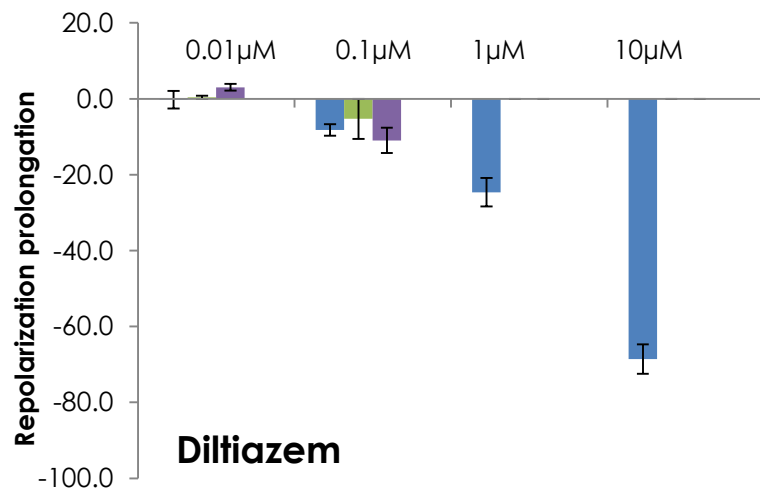
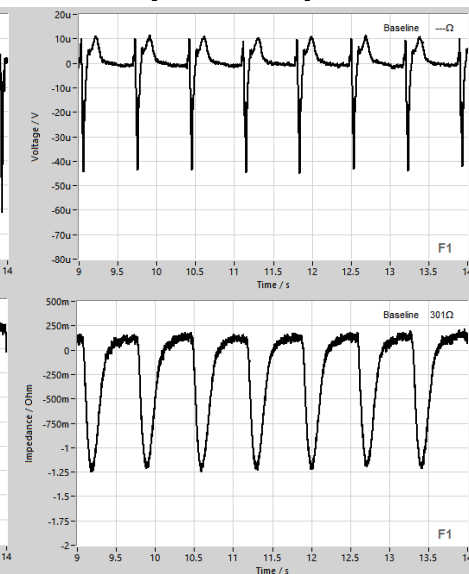
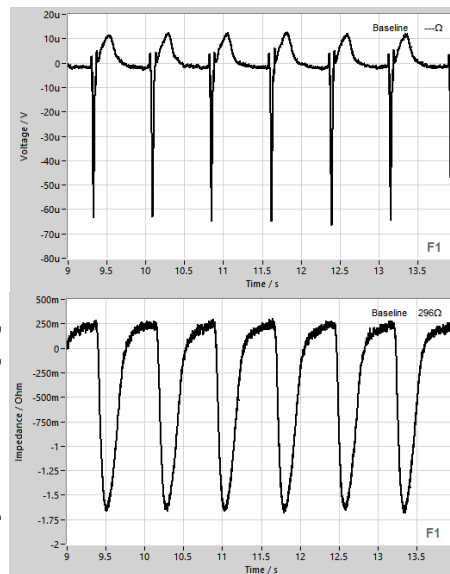
Low Risk compounds



Arrhythmic events, % of wells

Voltage (μ V)Impedance (Ω)

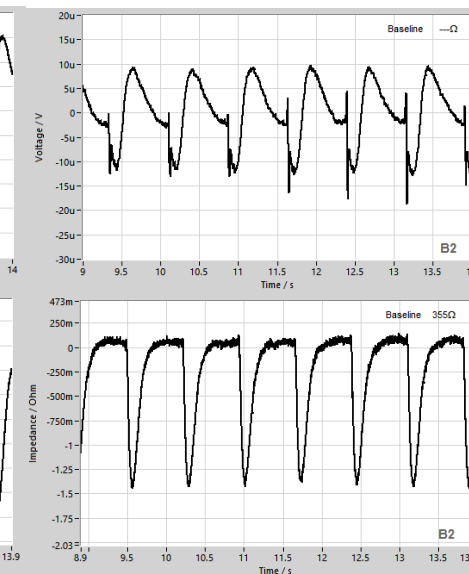
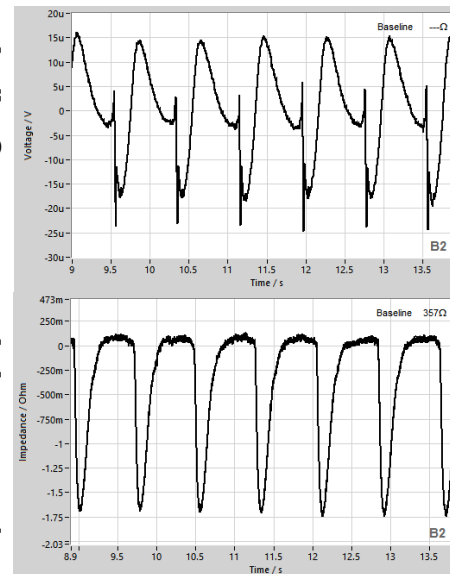
Control, cell type 2

0.1 μ M Verapamil

Arrhythmic events, % of wells

Voltage (μ V)Impedance (Ω)

Control, cell type 2

0.1 μ M Diltiazem

Outlook: Optical Stimulation with CardioExcyte 96 SOL



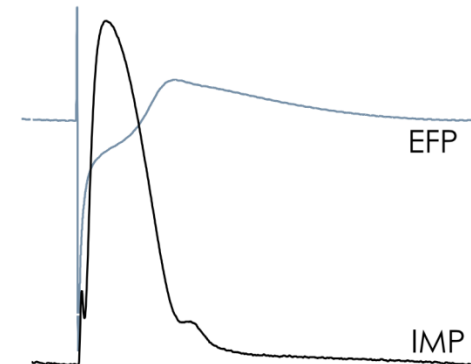
Unique CardioExcyte 96 feature:

Impedance and EFP recordings from the same cells.

Optical stimulation allows for an exact overlay of the signal, providing in-depth **mechanistic understanding of the contractility and electrophysiology** of iPS CM.

CardioExcyte96 SOL

- for in vitro optogenetic assays
- 96 high power LEDs - each well is stimulated by an individual LED
- Software controlled adjustment of stimulus intensity, pulse length and pace rate



Timed stimuli allow parallelized IMP and EFP mean beat overlays



Special thank you to the stem
cell providers for the great cells!

