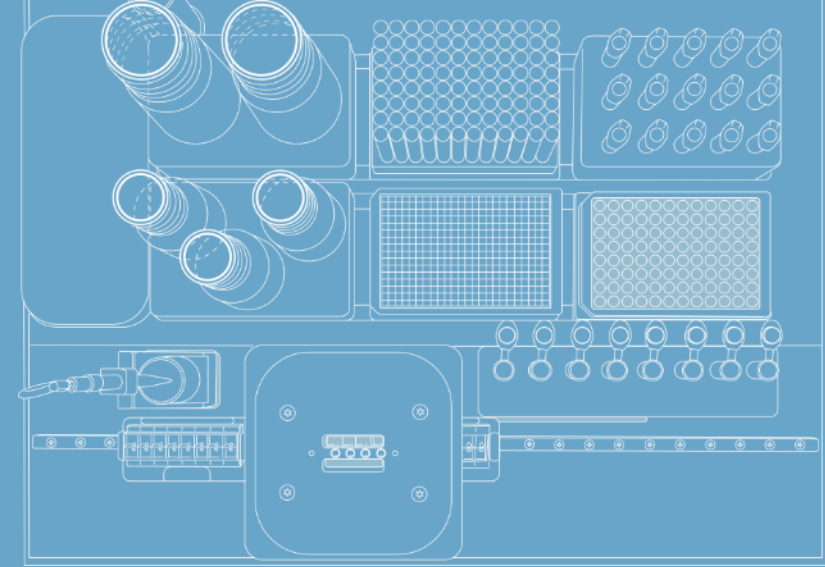


Nanon Technologies

SOT 2018 Exhibitor Session

San Antonio, March 12th 2018



Innovations for cell monitoring in safety and toxicity assays

Dr. Elena Dragicevic

Senior Scientist / Sales Manager at Nanon Technologies

nan]i[on

Agenda

Cell Monitoring Approaches:

- Cell proliferation monitoring
- Hepatotoxicity monitoring
- Cancer growth monitoring

Cardiomyocyte attachment and toxicity monitoring via impedance

SyncroPatch 384/768 PE
ion channels in cell lines and iPS CMs

The SyncroPatch 384/768 PE
with **Optical** Stimulation

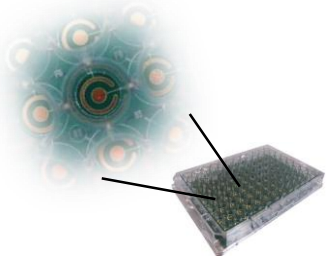


Long-term and short-term monitoring for chronic and acute effects

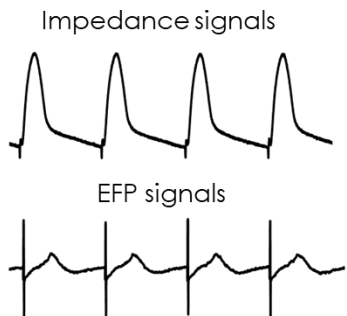
controlled environment



long-term stable consumables

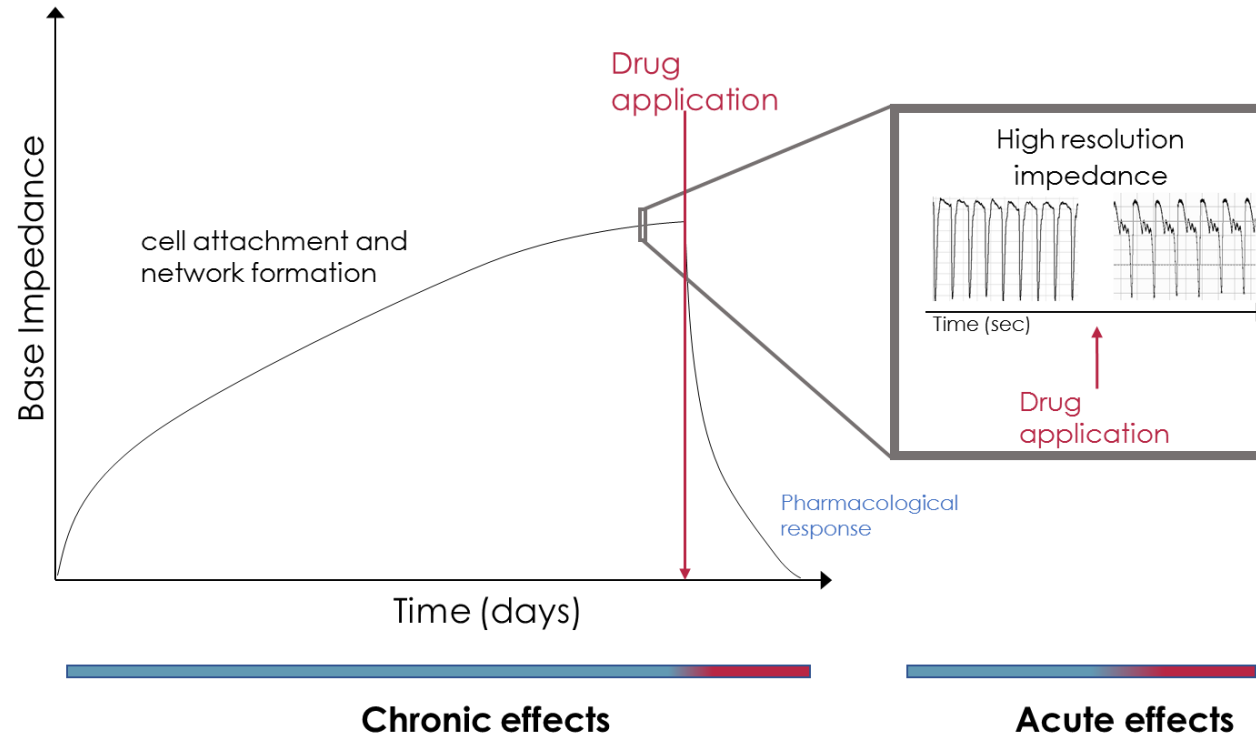
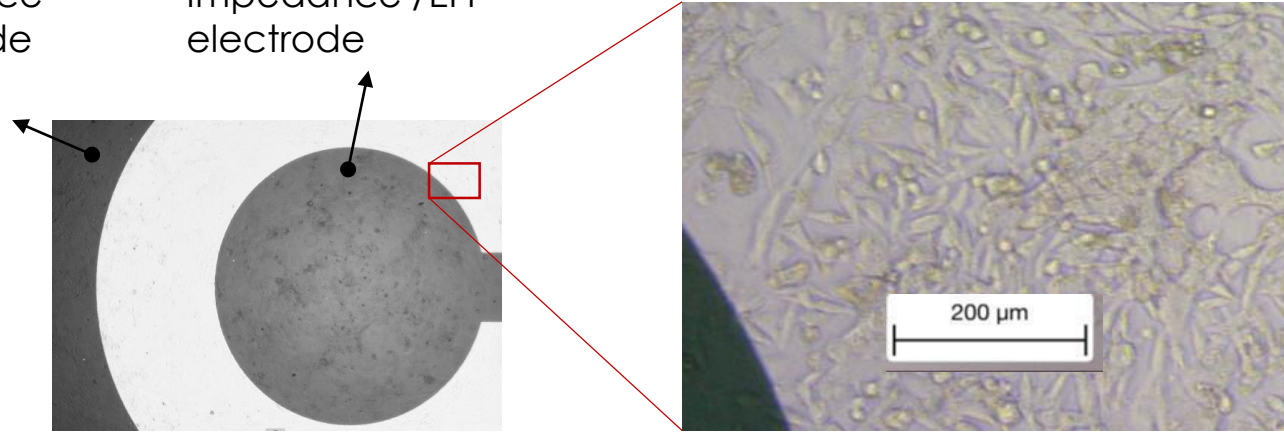


dual readout from the **SAME** cells



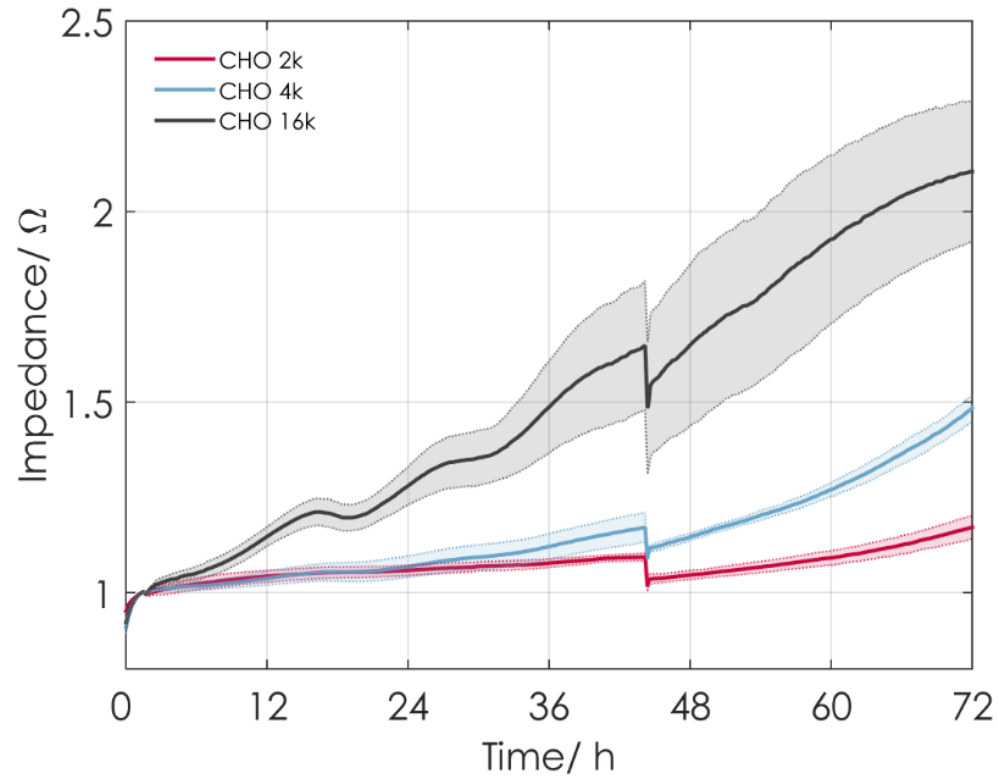
Reference electrode

Impedance /EFP electrode



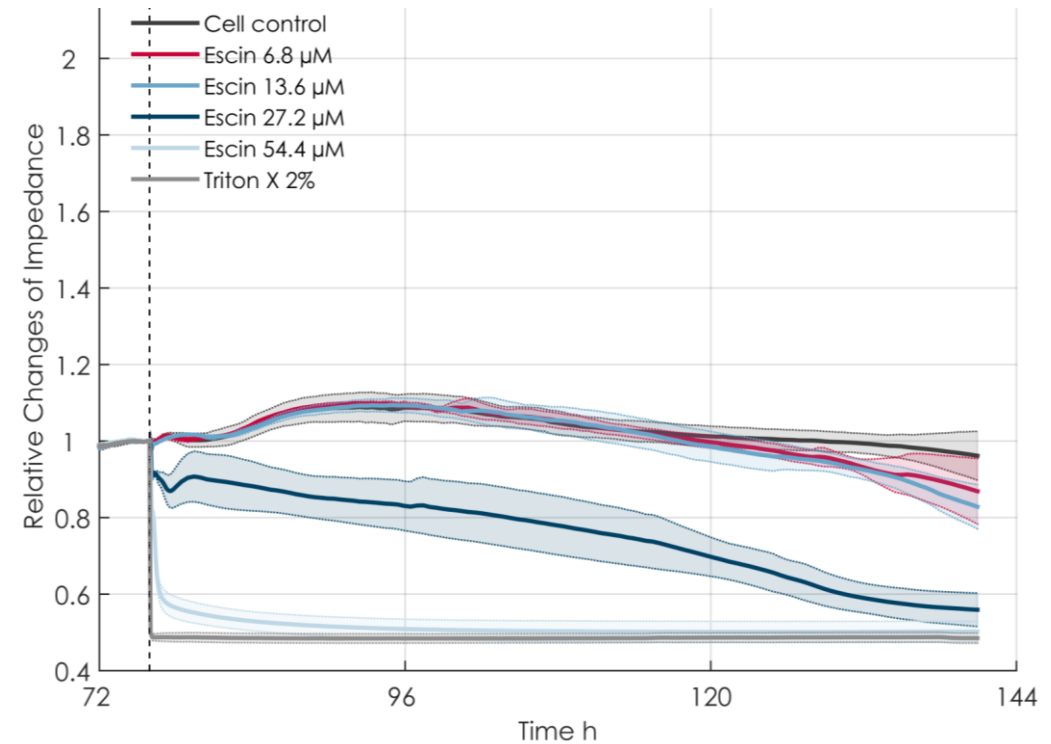
Cell proliferation monitored via impedance on CardioExcyte 96

Cell growth



CHO cells proliferation monitoring (72h)

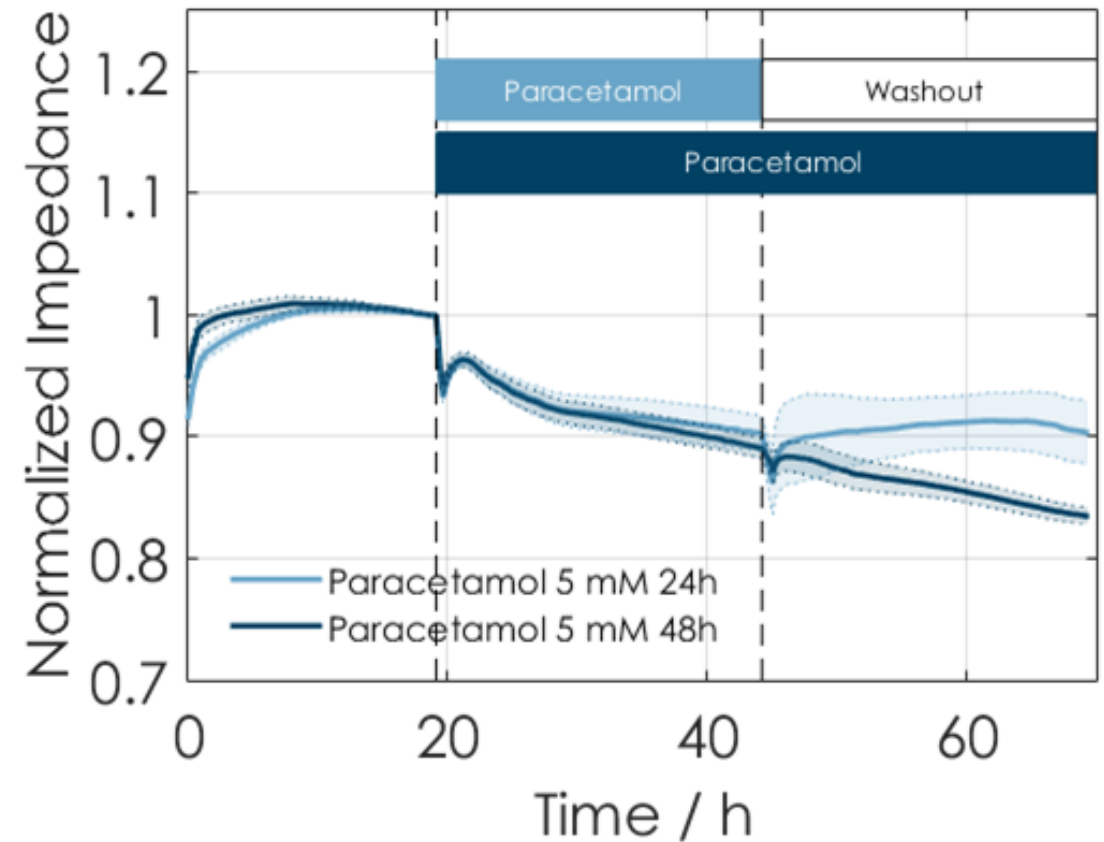
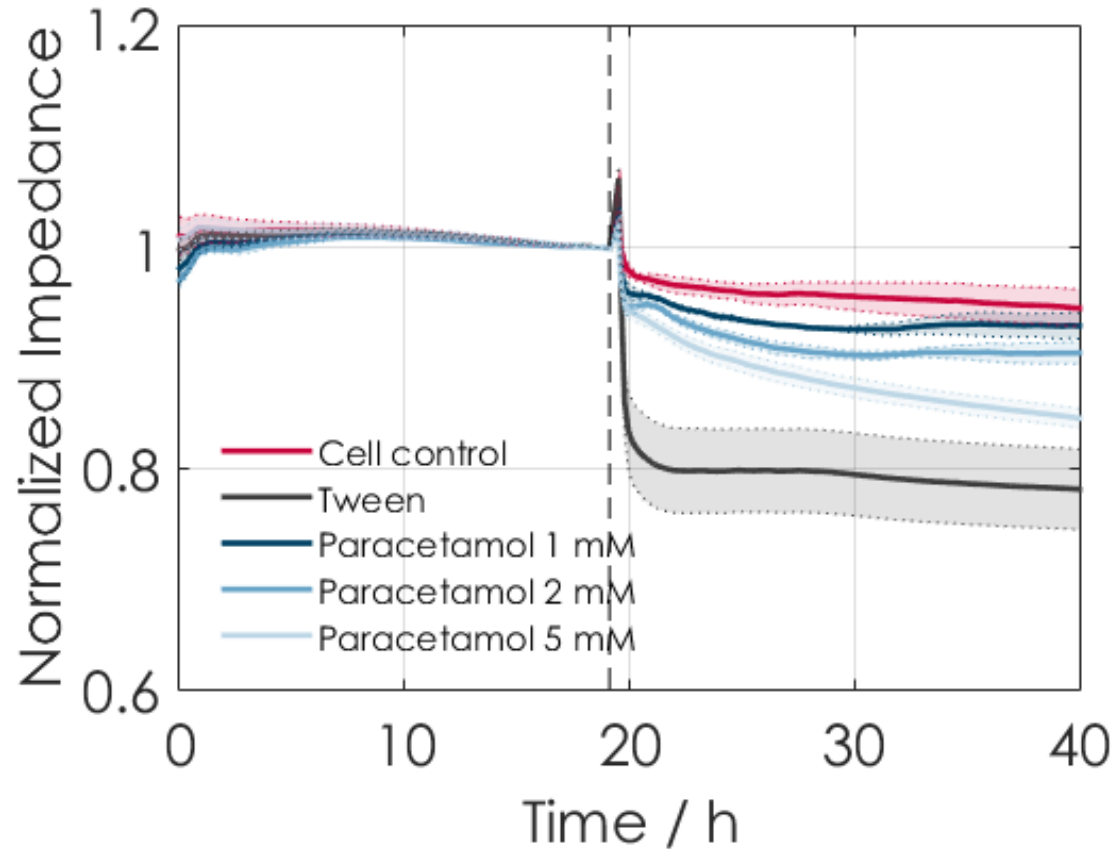
Cell death



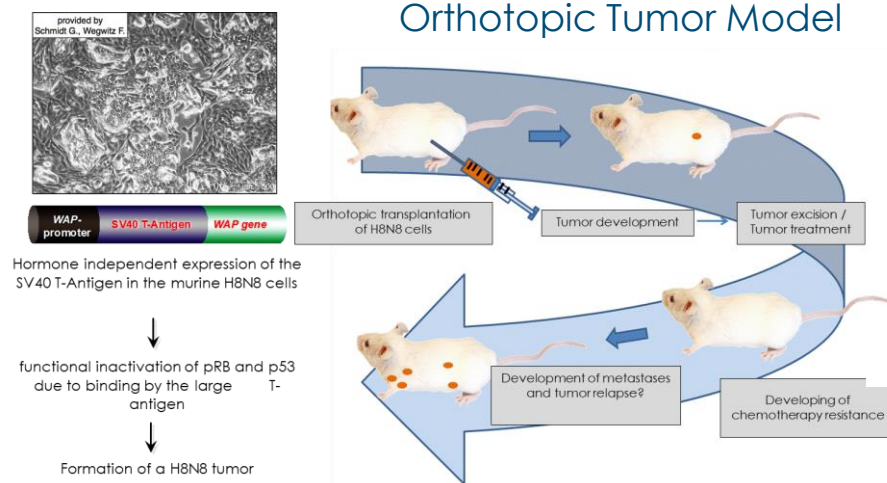
Effects of Escin and Triton X on CHO cells (>100h)

Hepatotoxicity monitoring via impedance on CardioExcyte 96

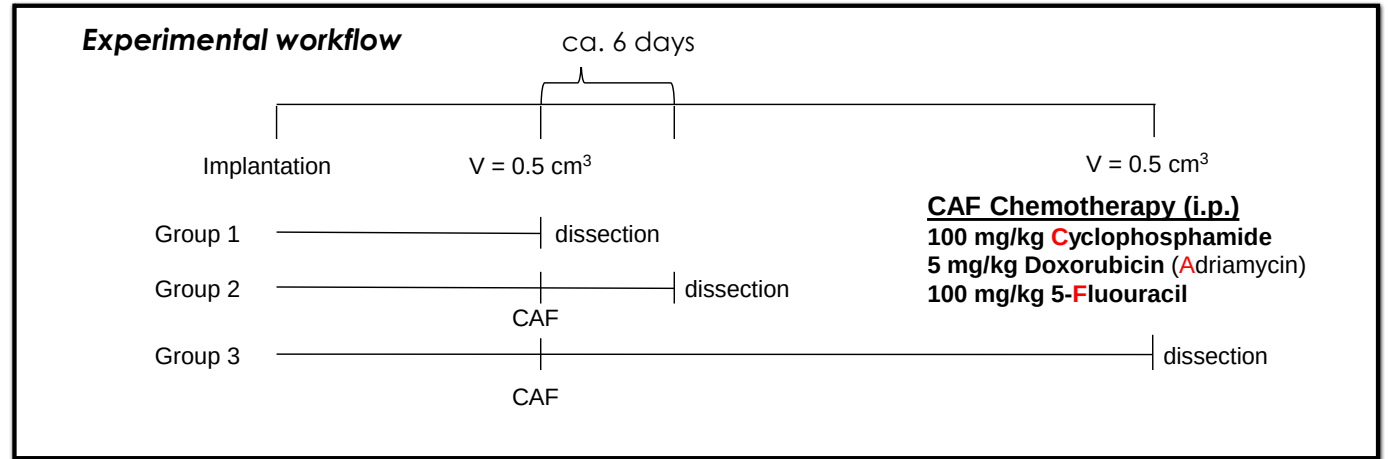
Effects of increasing concentrations of paracetamol on base impedance of monocyte-derived hepatocyte-like (MH) cells (>80h monitoring)



Investigating therapy response/resistance of mammary carcinoma cell lines *in vitro*



Murine H8N8 mammary carcinoma cells exhibit a plastic epithelial or mesenchymal phenotype, validated by *in vitro* immunofluorescence stainings.



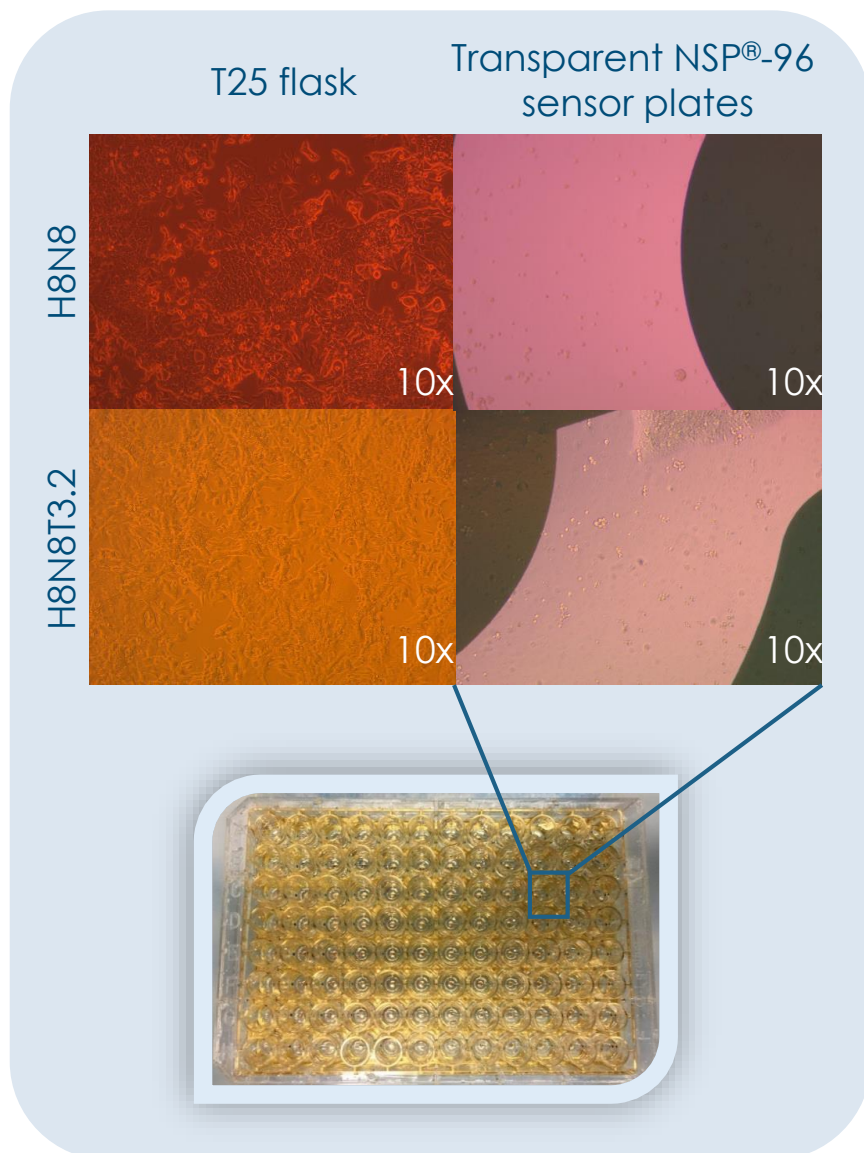
G1 - Untreated: Untreated Control; dissected after tumor growth to 0,5 cm³

G2 - Residual: Treated with CAF; dissected at minimal tumor volume

G3 - Relapse: Treated with CAF; dissected after tumor regrowth to 0,5 cm³

	Animal	Tumor	Cell Line	Shortcut
Untreated	1124	6163	pH8N8T1.1	T1.1
	1194	6164	pH8N8T1.2	T1.2
	1195	6165	pH8N8T1.3	T1.3
	1214	6166	pH8N8T1.4	T1.4
Residual	1099	6172	pH8N8T2.1	T2.1
	1112	6174	pH8N8T2.2	T2.2
	1104	6173	pH8N8T2.3	T2.3
	Insufficient cell isolation			
Relapse	1098	6228	pH8N8T3.1	T3.1
	1149	6230	pH8N8T3.2	T3.2
	1106	6229	pH8N8T3.3	T3.3
	1177	6231	pH8N8T3.4	T3.4

Investigating therapy response/resistance of mammary carcinoma cell lines *in vitro*



Murine H8N8 cells: immortal mammary carcinoma cell line with tumor stem cell properties.

H8N8 T3.2 cells represent a recurrent tumor variant. H8N8 T3.2 cells were established from a solid breast tumor that received a CAF clinical regimen *in vivo*.

CAF Chemotherapy (i.p.)
100 mg/kg Cyclophosphamide
5 mg/kg Doxorubicin (Adriamycin)
100 mg/kg 5-Fluoracil

Poster P374: 'In Vitro Investigations of Adjuvant Chemotherapy for Prevention of Breast Cancer Tumor Recurrence' on **Tuesday March 13th**. Data courtesy of Dr. Oliver Reinhardt, Dr. Frauke Alves, Translational Molecular Imaging Group, Max-Planck Institute of Experimental Medicine, Göttingen, Germany

Cancer growth monitoring via impedance on CardioExcyte 96

Impedance readout (CardioExcyte 96)

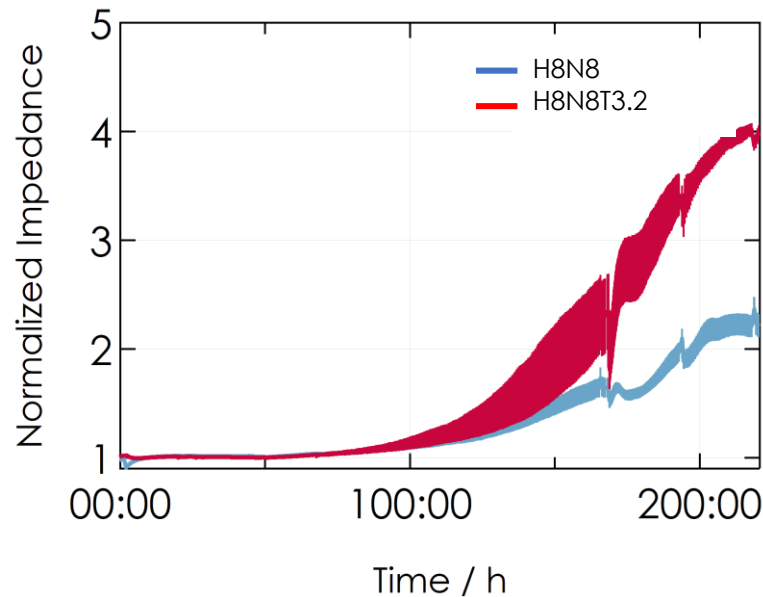
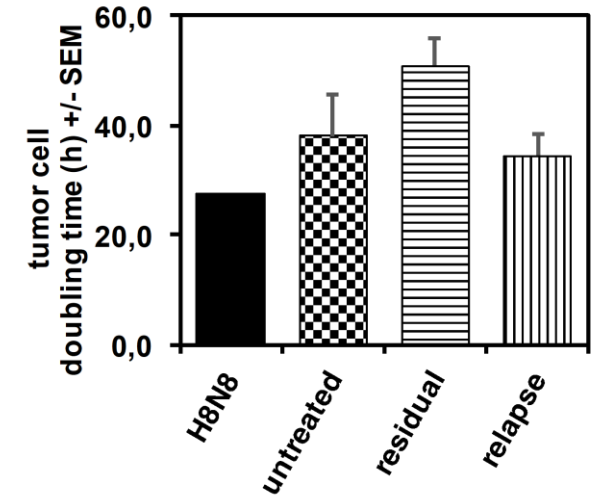
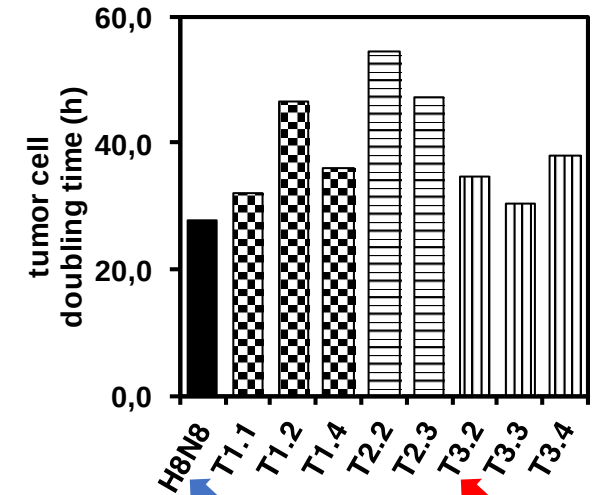
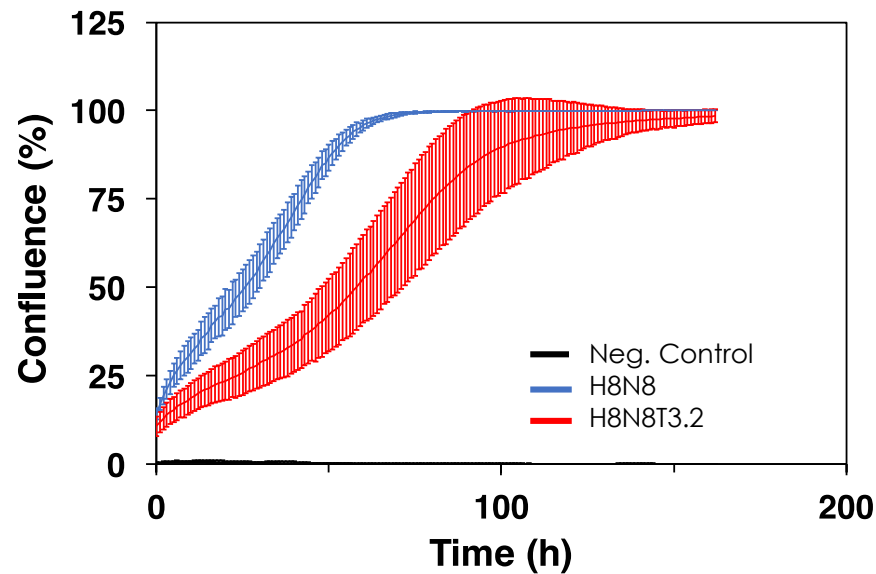


Image-based live-cell analysis readout

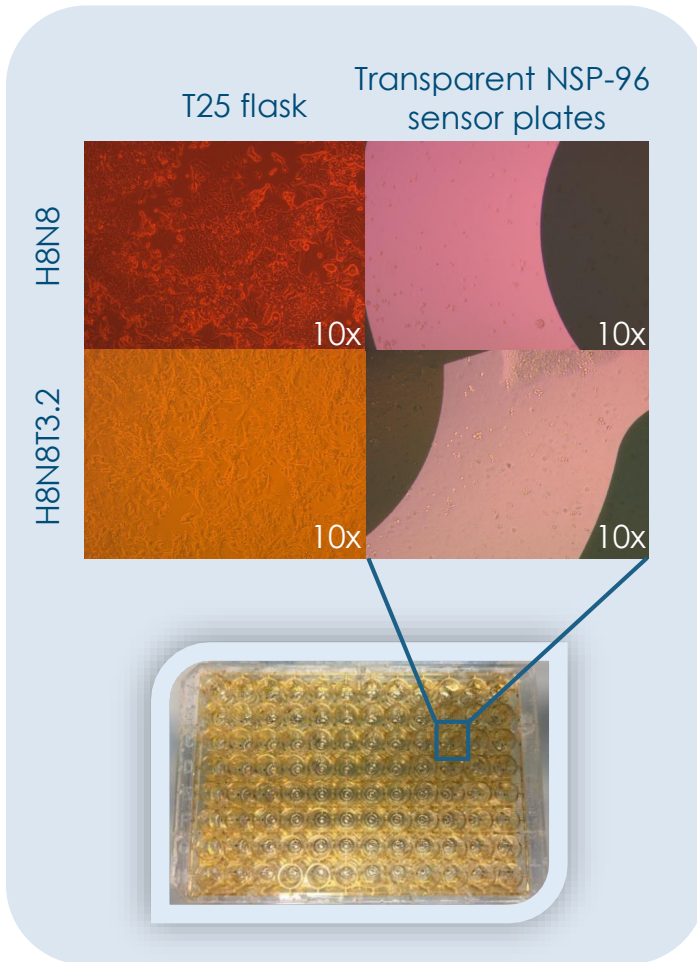


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GÖTTINGEN

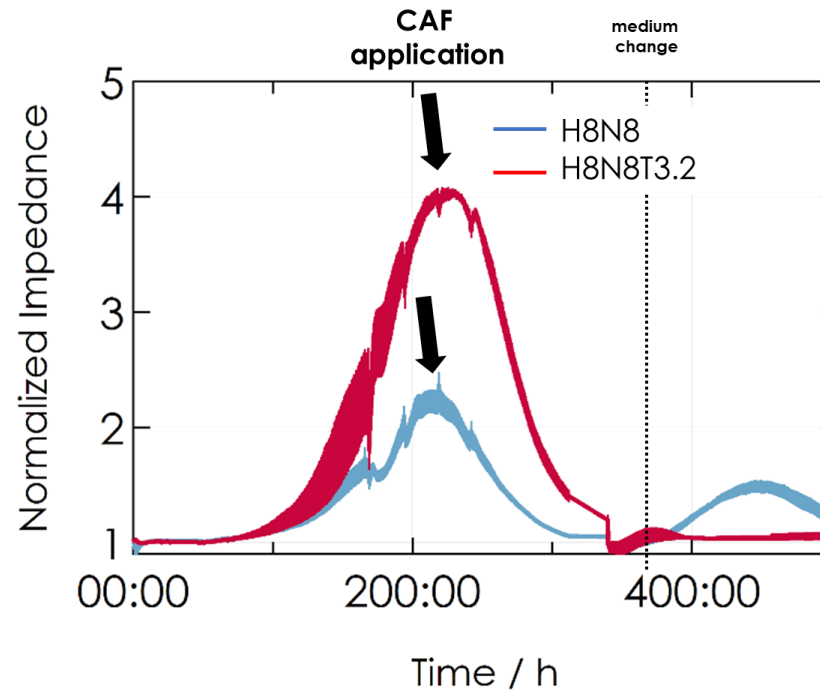


Translational Molecular Imaging
Prof. Dr. Frauke Alves
Oliver Reinhardt

Investigating therapy response/resistance of mammary carcinoma cell lines *in vitro*

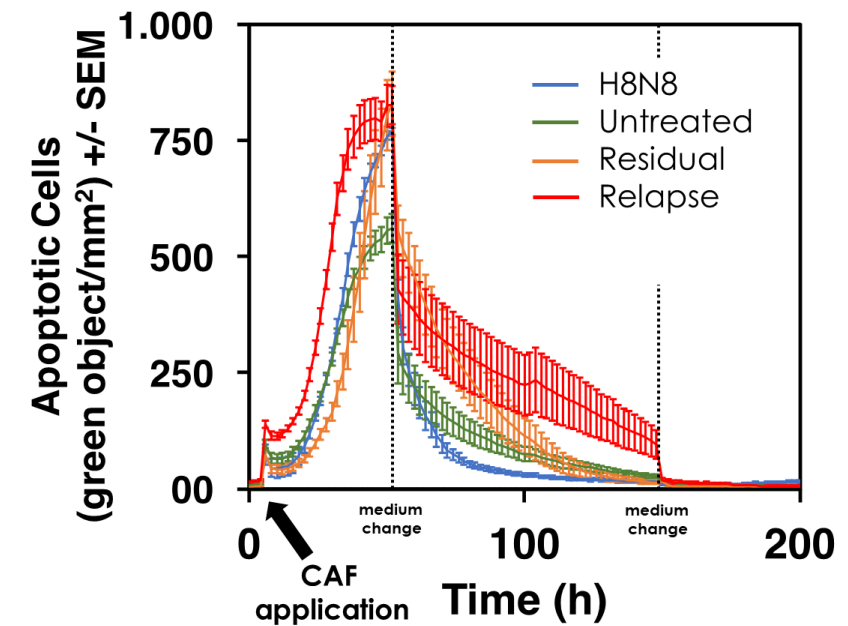


Impedance readout (CardioExcyte 96)



CAF mix applied:
10 µg/ml Cyclophosphamide;
0,05 µg/ml Doxorubicin; 10 µg/ml 5-Fluouracil

Image-based live-cell analysis readout



CAF mix applied:
0,64 µg/ml Cyclophosphamide;
0,032 µg/ml Doxorubicin; 0,64 µg/ml 5-Fluouracil

Agenda

Cell Monitoring Approaches:

- Cell proliferation monitoring
- Hepatotoxicity monitoring
- Cancer growth monitoring

Cardiomyocyte attachment and toxicity monitoring via impedance

SyncroPatch 384/768 PE
ion channels in cell lines and iPS CMs

The SyncroPatch 384/768 PE
with **Optical** Stimulation

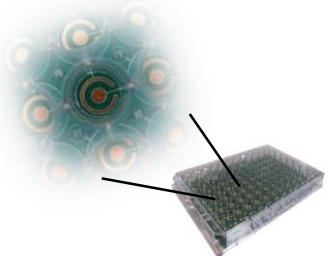


CardioExcyte 96 Hybrid Measurements: Mode of Operation

controlled environment



long-term stable
consumables

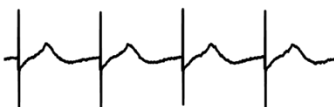


dual readout from
the **SAME** cells

Impedance signals



EFP signals

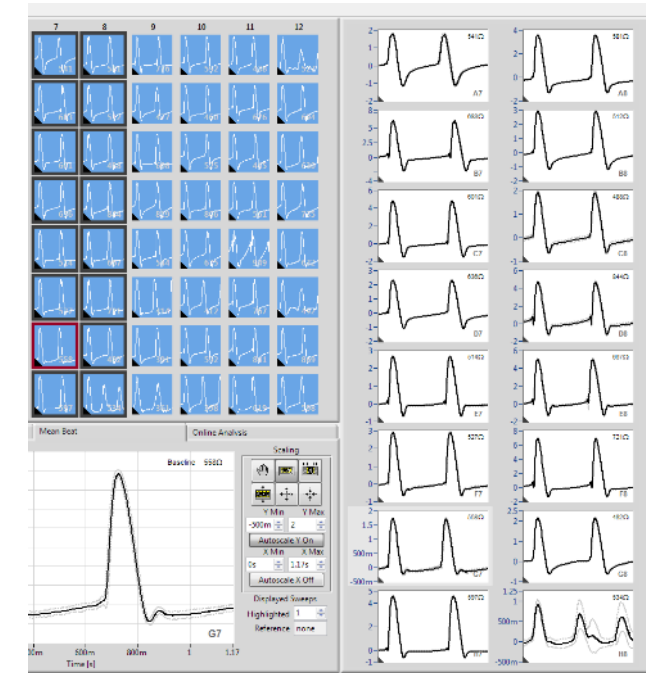
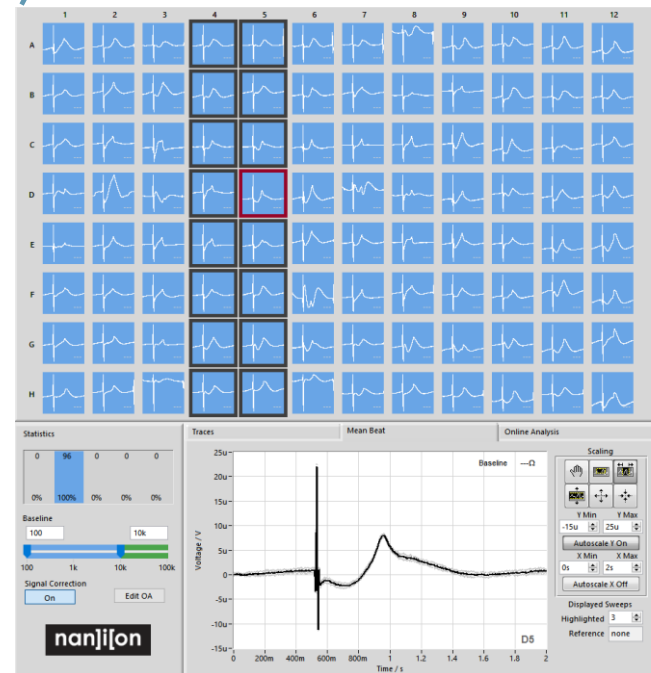


Start up

EFP

switch

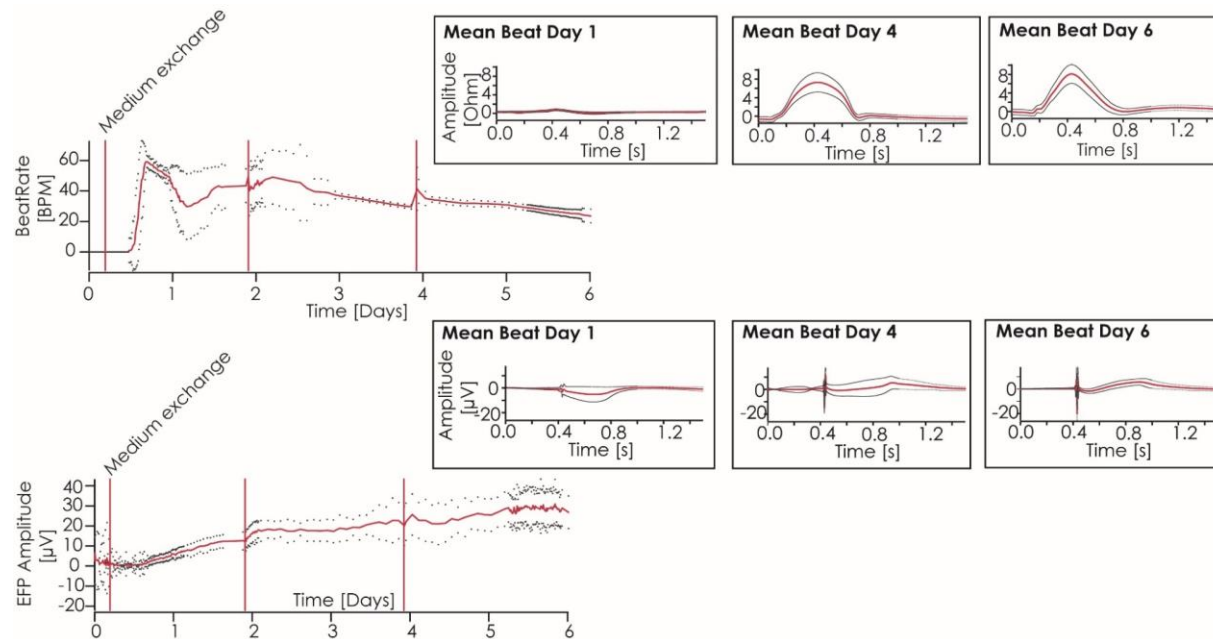
Impedance



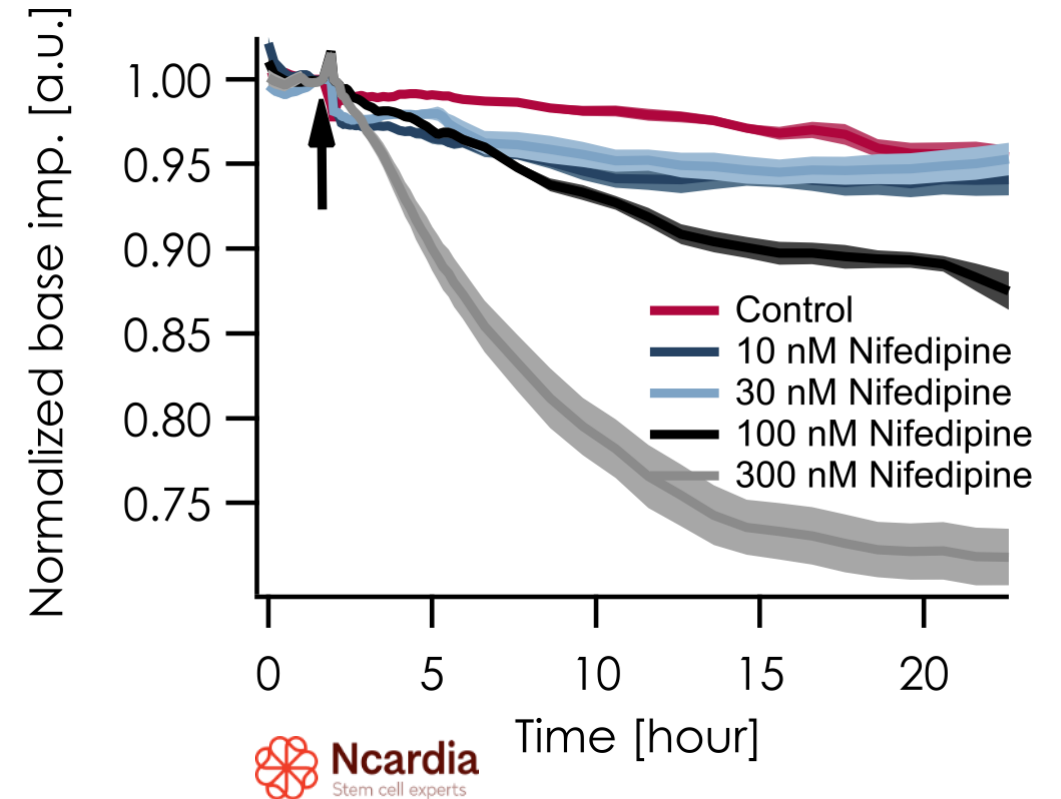
Independent data files for each recording mode.
EFP and IMP recordings of **the same cells**.

Cardiomyocyte attachment and toxicity monitored via impedance on CardioExcyte 96

Development of beat rate and EFP amplitude of iPSC CMs



Chronic effect of Nifedipine (>24 h)



Agenda

Cell Monitoring Approaches:

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Cardiomyocyte attachment and toxicity monitoring via impedance

SyncroPatch 384/768 PE
ion channels in cell lines and iPS CMs

The SyncroPatch 384/768 PE
with **Optical** Stimulation



Automated patch clamp technology

from manual work to high throughput state-of-the-art automation

Port-a-Patch

50

data points / day



6-20

compounds / day

Patchliner

600

data points / day



75-300

compounds / day

SyncroPatch 384/768PE

20,000 / 40,000

data points / day



2.5k-10k / 5k-20k

compounds / day

The SyncroPatch 384/768 PE

the 4th generation APC

Giga-seal patch clamping

384 or 768 recording channels

Up to 20,000/40,000 data points per day

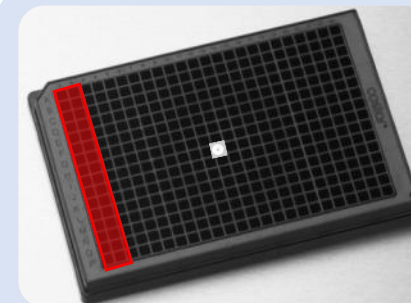
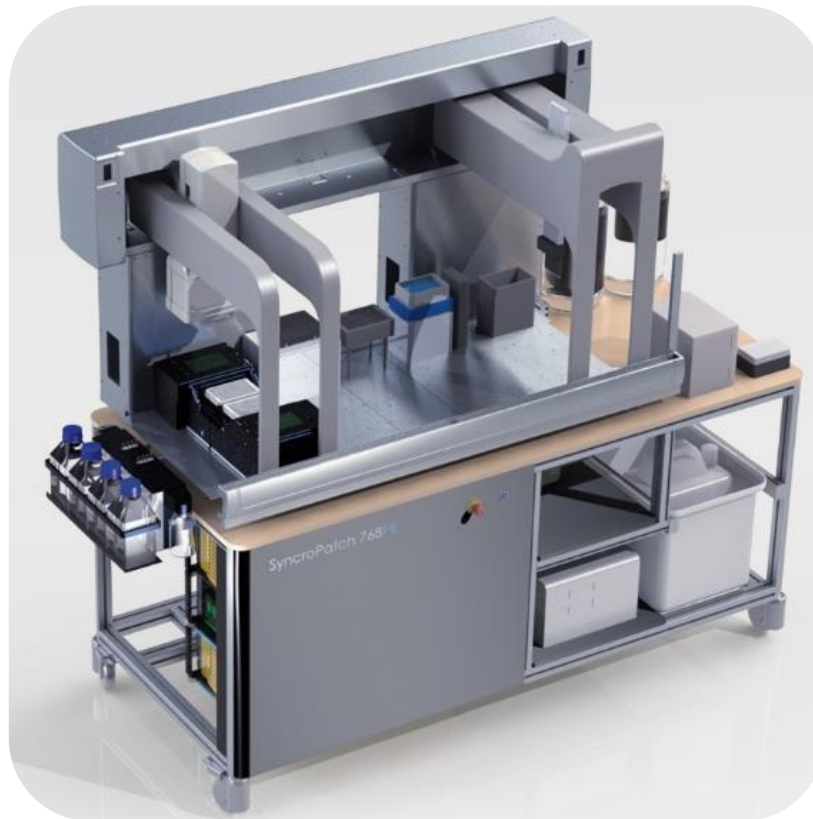
Unattended operation 4-6 hours

€ 0,16 - 0,65 per data point

Ultra flexible: Can be applied in ALL phases of drug discovery

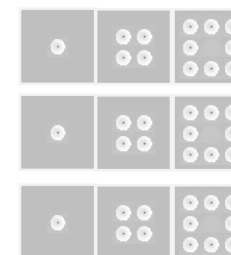


Preferred technology
HTStec APC report 2016



Made in Germany @ nanji[on]

1x 4x 8x



R: low

R: medium

R: high

SyncroPatch 384 PE – consumables NPC-384

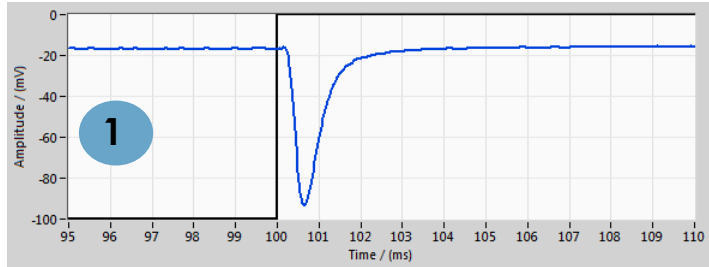
Variable hole number and size

Partial run (any 32 wells per run)

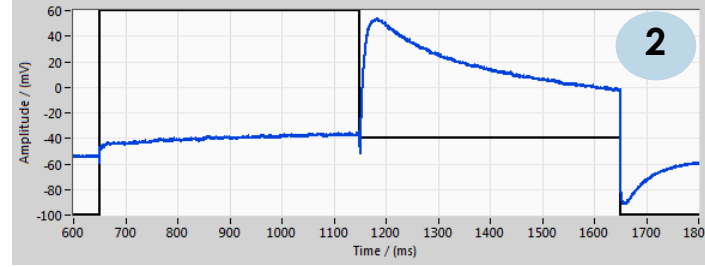
Partial plates can be run without using the whole chip

One protocol takes care of many ion channels

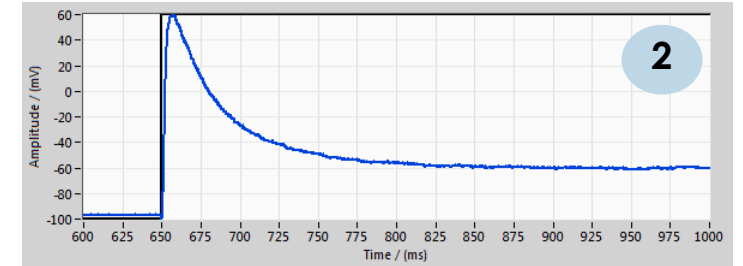
$\text{Na}_v1.5$ („I_{Na}“)



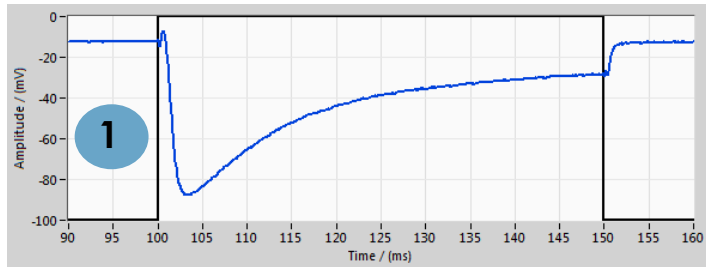
$\text{K}_v11.1$ (hERG – „I_{Kr}“)



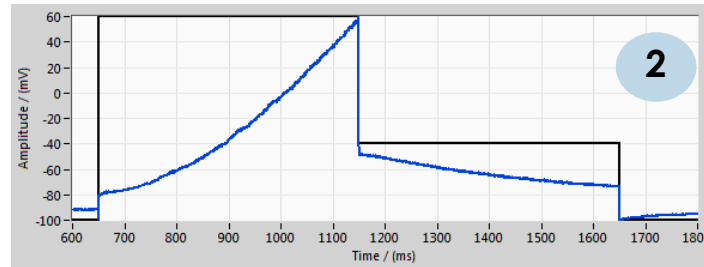
$\text{K}_v4.3\text{-K}_{\text{Chip}2.2}$ („I_{to}“)



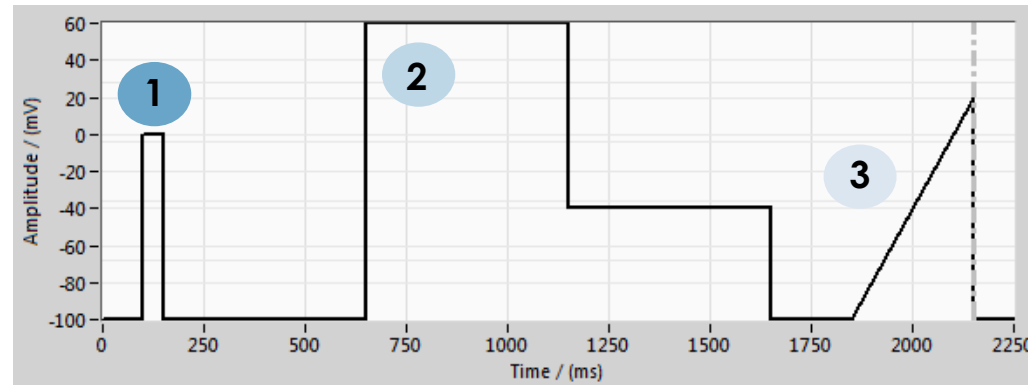
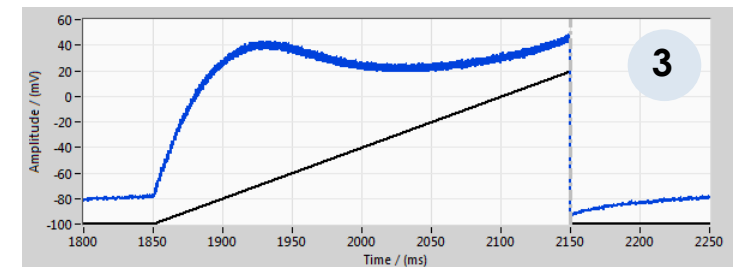
$\text{Ca}_v1.2/\text{b}2/\text{a}2\text{d}$ („I_{Ca}, I“)



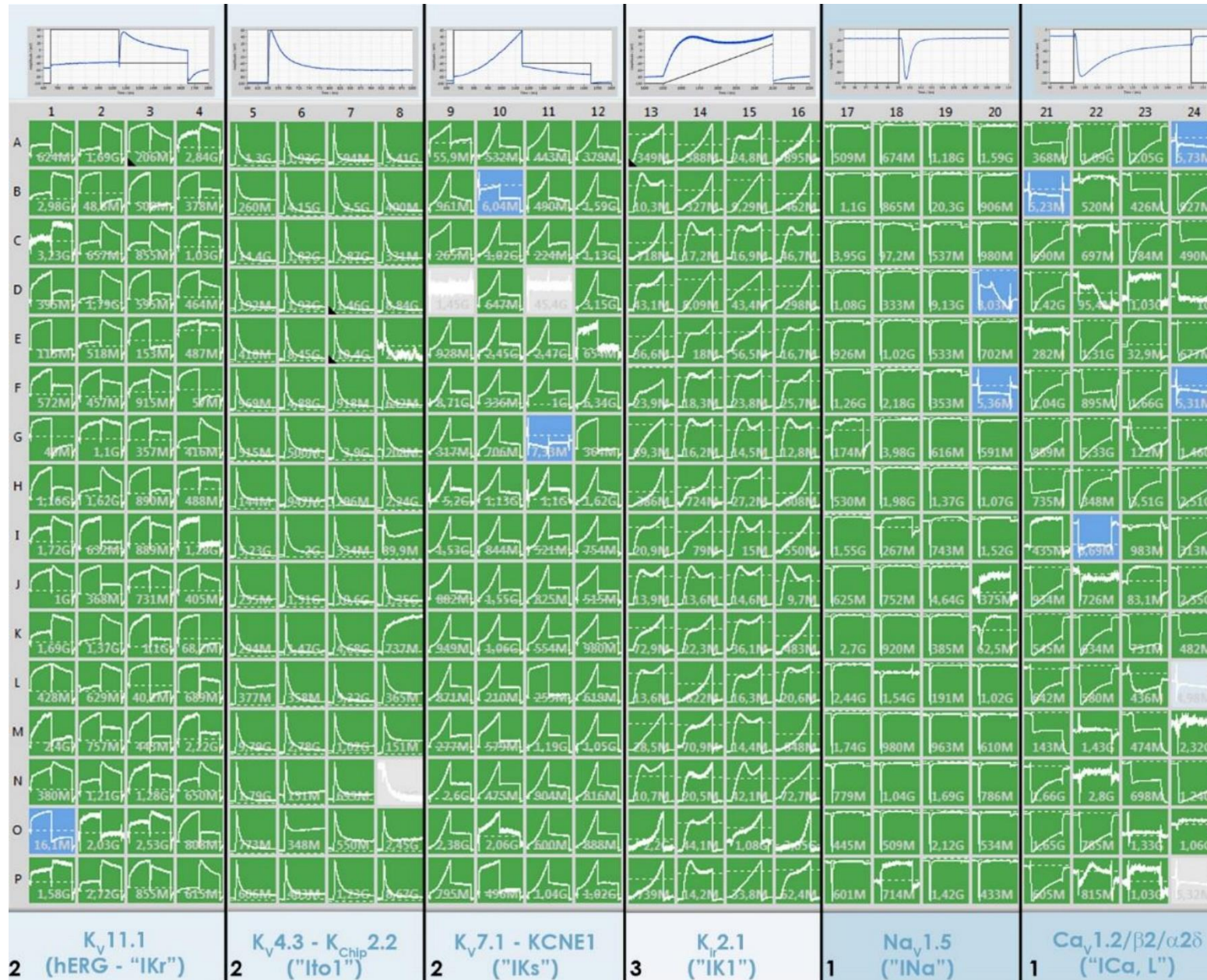
$\text{K}_v7.1\text{-KCNE}1$ („I_{Ks}“)



$\text{K}_{ir}2.1$ („I_{K1}“)



Ion Channels of the CIPA Initiative – on ONE plate

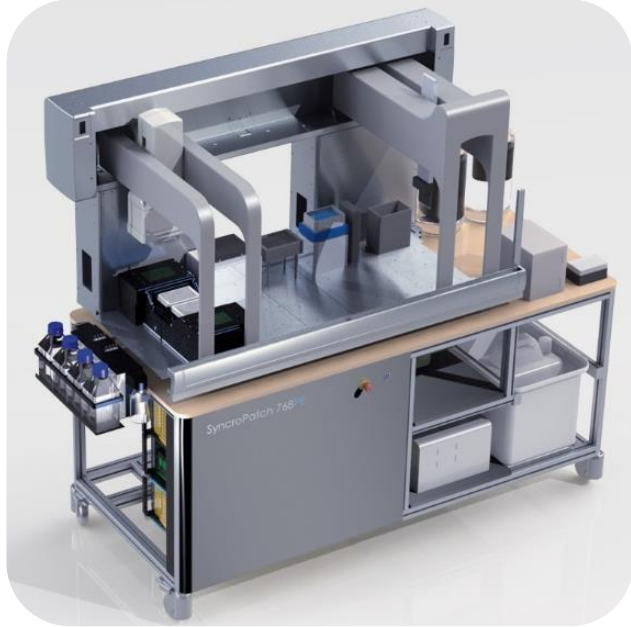


Different (iPS CM) cells on one 384-well plate

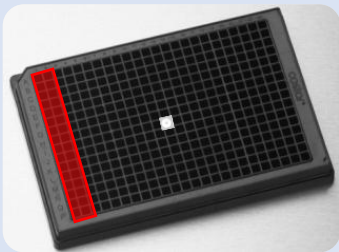
Vendor iPSC-CMs

Ncardia Cor.4U

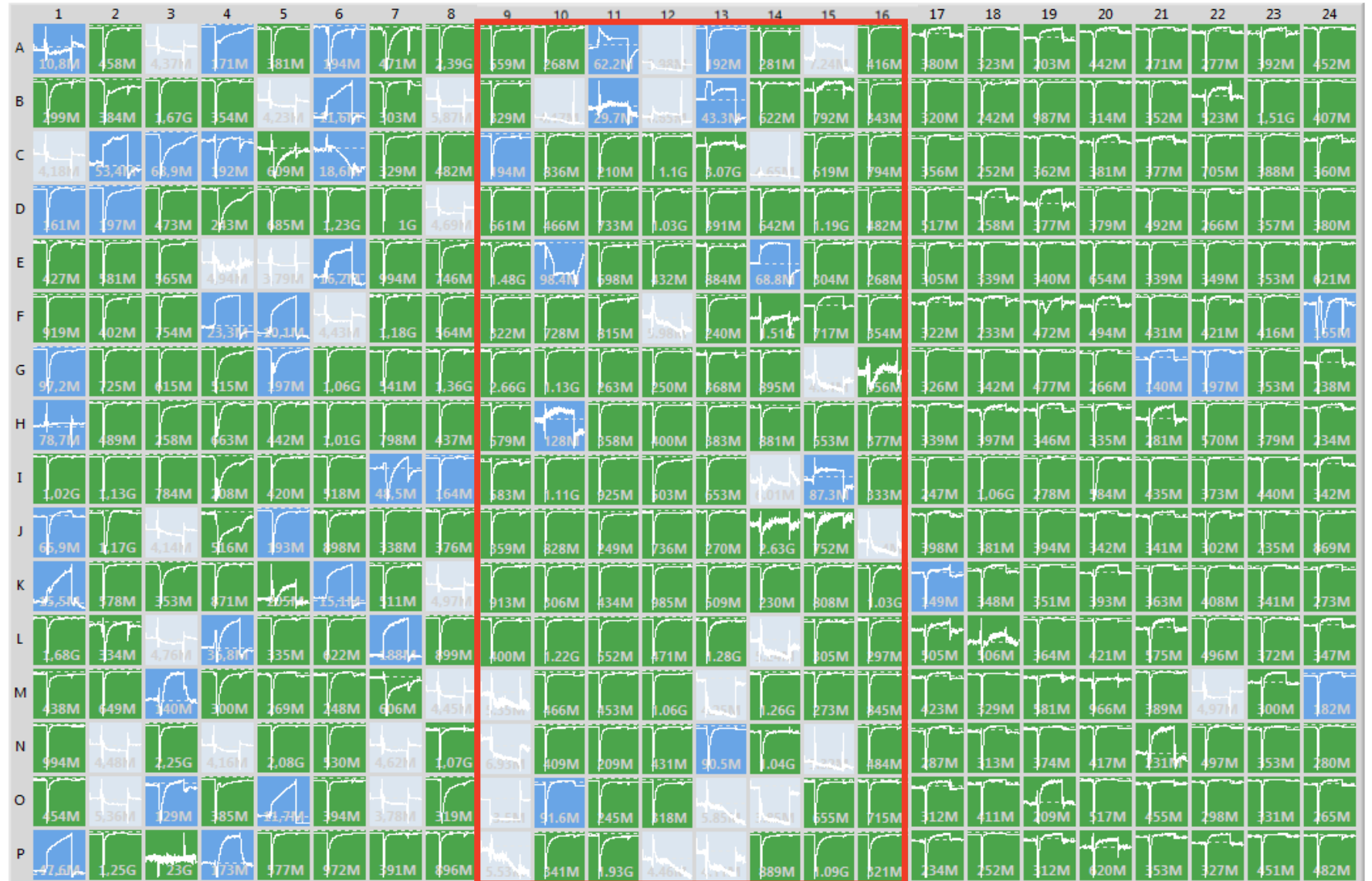
CHO Nav1.7



The SyncroPatch 384/768 PE
the 4th generation APC



Made in Germany @ nanion



Inter-platform and Inter-site Comparison

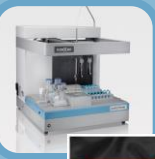


- CiPA cpds were tested on medium and high throughput APC devices
- 3 Nanion sites contribute IC50 data on 6 ion channels

Exemplary results for 3 cpds on hERG and Nav1.5

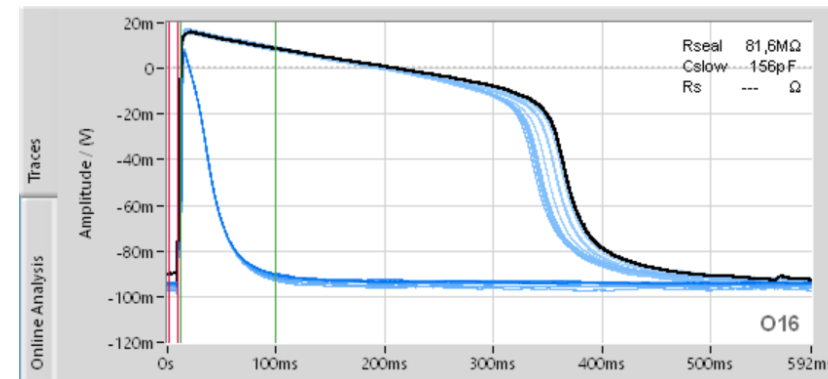
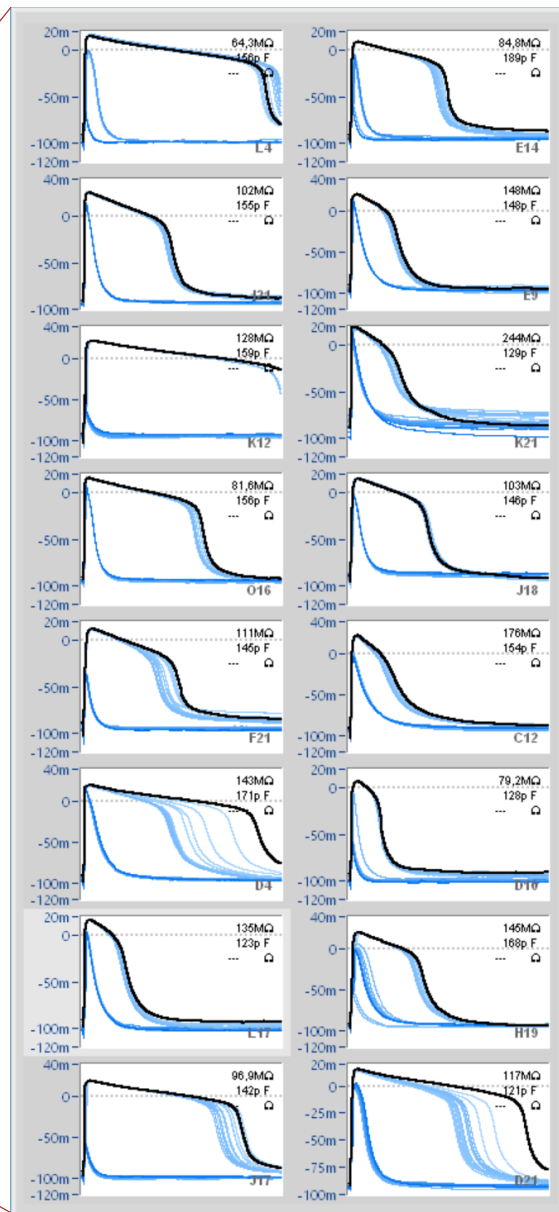
* Risk of TdP

** Chantest

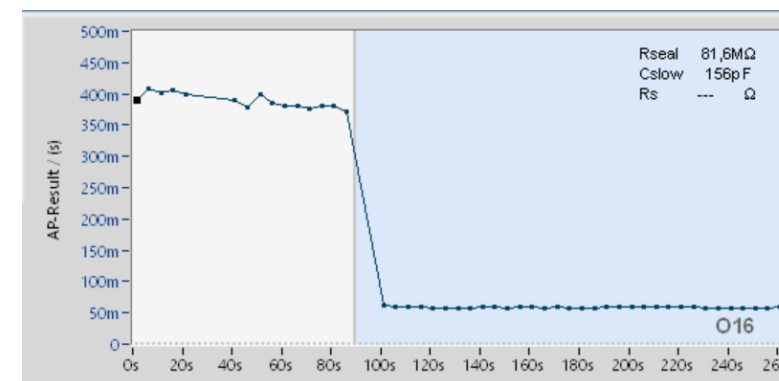
Channel	Cpd	IC50 Literature** [μM]	Highest test conc. [μM]	 Ambient temp. IC50 [μM]	 Physiol. temp. IC50 [μM]	 Ambient temp. IC50 [μM]	 Ambient temp. IC50 [μM]	 Physiol. temp. IC50 [μM]
hERG	Sotalol*	111	300	160	151	192	217	71.4
	Diltiazem	13.2	100	13.9	15.6	4.1	8.4	5.7
	Quinidine*	0.72	10	1.02	0.92	1.54	1.02	0.78
Nav1.5	Sotalol*	1180	300	>300	>300	>300	>300	>300
	Diltiazem	22.4	100	44.4	44.5	25.2	13.3	22.0
	Quinidine*	14.6	10	>10	>10	>10	>10	>10

➤ Strong consistency across platforms and sites

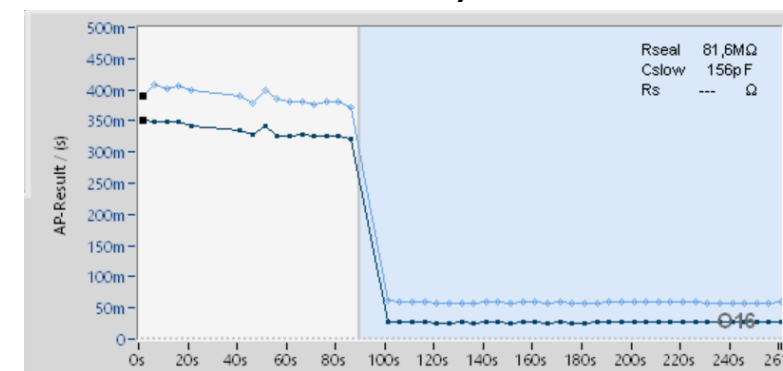
HTS pharmacology: current-clamp on iPSC Cardiomyocytes



AP raw trace: CTR vs Nifedipine



Online Analysis APD 90



Online Analysis APD90 and APD50

Agenda

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- Cell proliferation monitoring
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Cardiomyocyte attachment and toxicity monitoring via impedance

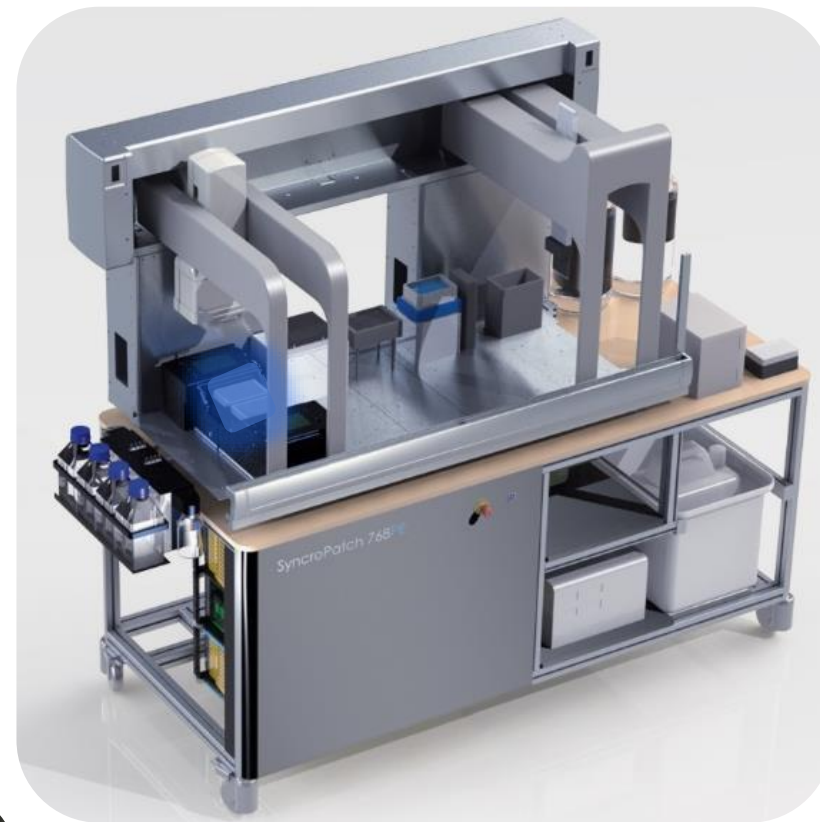
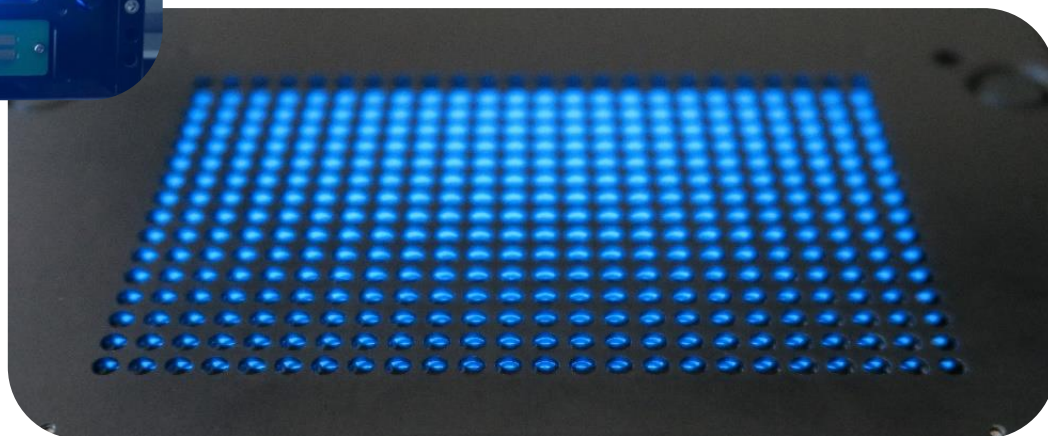
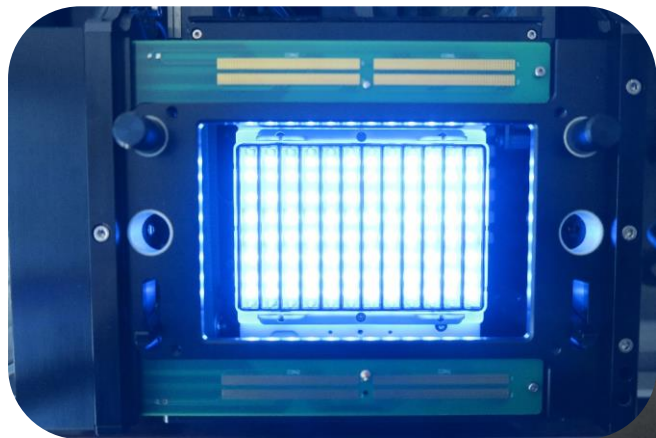
SyncroPatch 384/768 PE
ion channels in cell lines and iPS CMs

**The SyncroPatch 384/768 PE
with **Optical** Stimulation**



The SyncroPatch 384/768 PE with Optical Stimulation

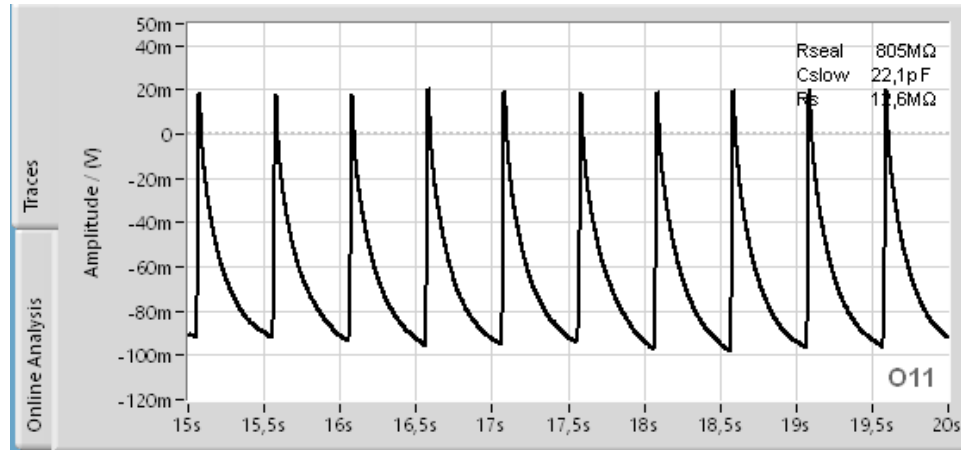
- First Prototype:
 - 96 blue LEDs (470 nm) below the Chip
 - Constant stimulation at selected intensity possible
 - Stimulation at selected frequency (e.g. 1 Hz) possible



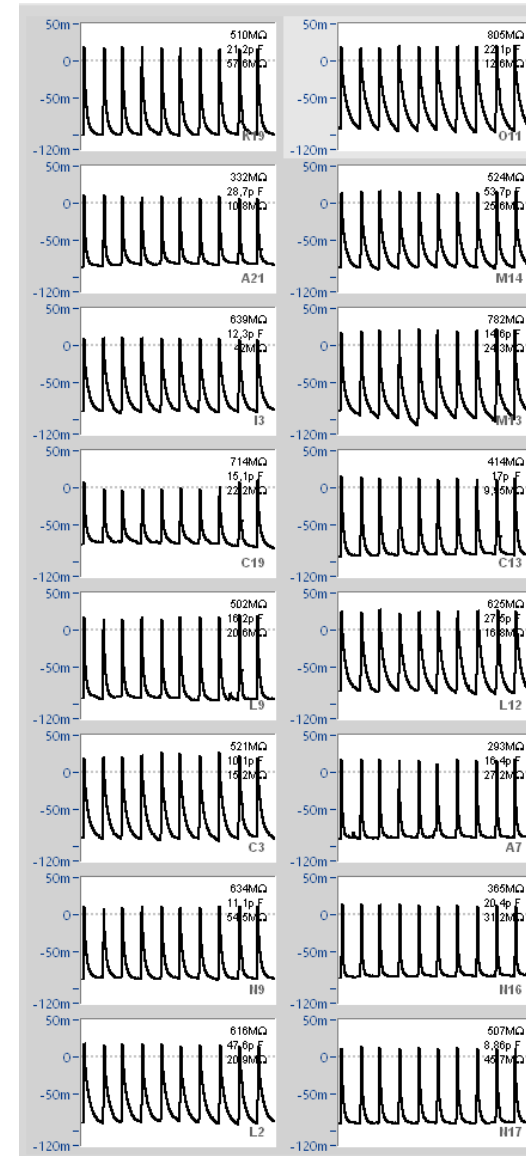
The SyncroPatch 384/768 PE with Optical Stimulation

Depolarization Via ChR2: Nav1.5-ChR2

- Proof of Concept:
 - CHO cells expressing ChR2 and Nav1.5 were used
 - Cells were measured in Current Clamp (CC)
 - -90 mV were achieved by LFVC (Low Frequency Voltage Clamp)
 - 1 ms Light Pulse at 2Hz (470 nm) was used for stimulation



Example Traces



Thank you!

SyncroPatch 384/768PE.



Nanion PE team

The CardioExcyte 96.



Nanion CE team

UNIVERSITÄTSMEDIZIN GÖTTINGEN : UMG



Translational Molecular Imaging
Prof. Dr. Frauke Alves
Oliver Reinhardt

Cell
Providers



Nanion Booth #534

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- **P374:** 'In Vitro Investigations of Adjuvant Chemotherapy for Prevention of Breast Cancer Tumor Recurrence' on **Tuesday March 13th**

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