

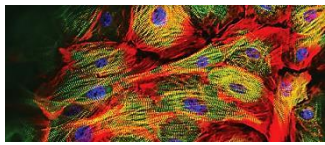
Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPSC-derived cardiomyocytes in 96 wells

Hamamatsu 3rd FDSS Workshop on 8th June 2016

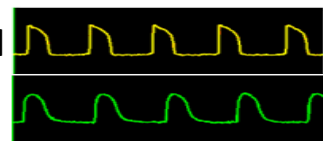


S. BEDUT

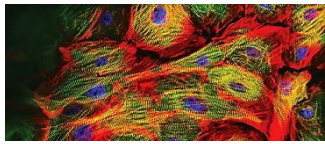




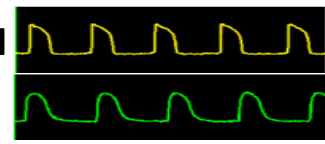
Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells



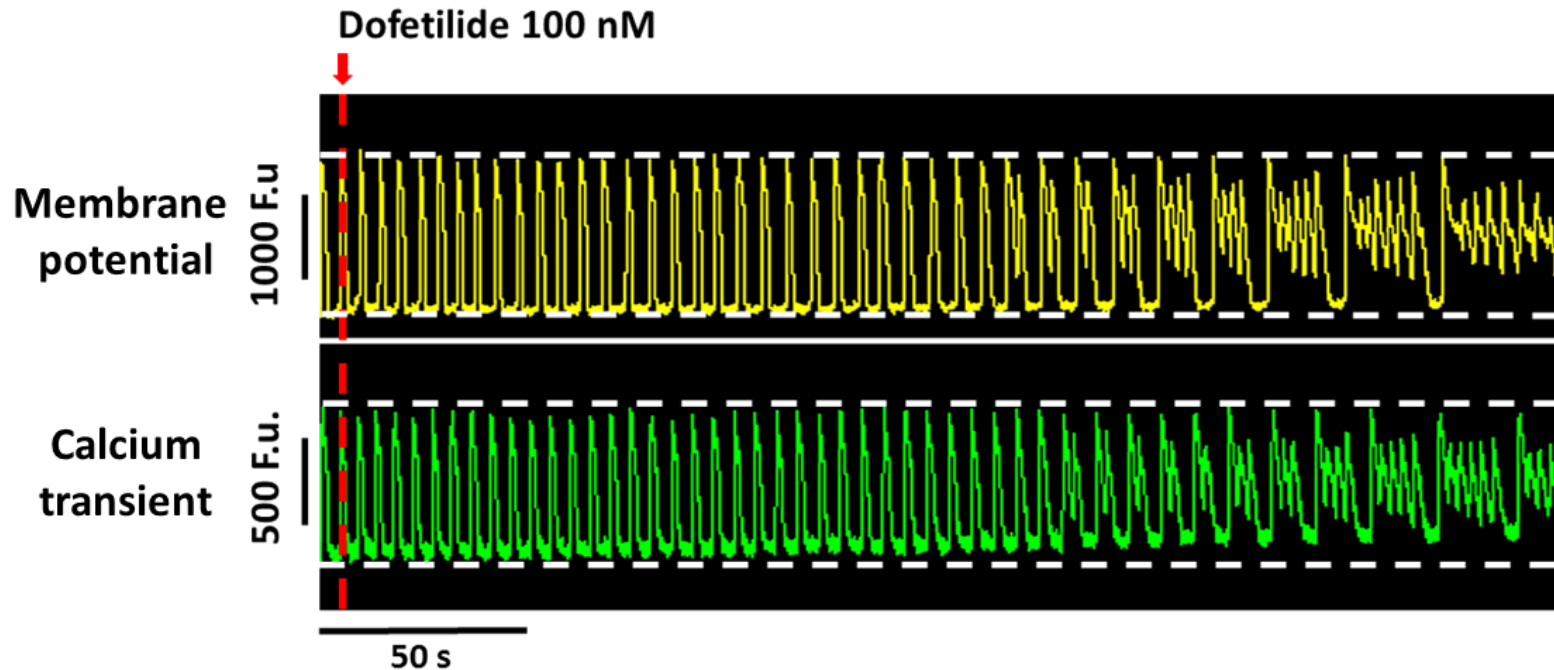
1. Parallel recording of voltage and calcium signals
2. FluoVolt dye
3. Cellular density (cells/well)
4. FluoVolt concentration
5. FluoVolt Toxicity
6. Washed-out calcium dyes

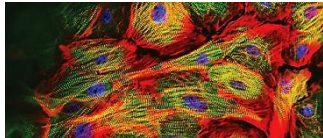


Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells

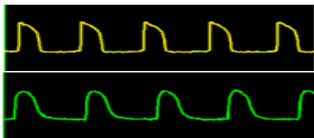


1. Paralell recording of voltage and calcium signals



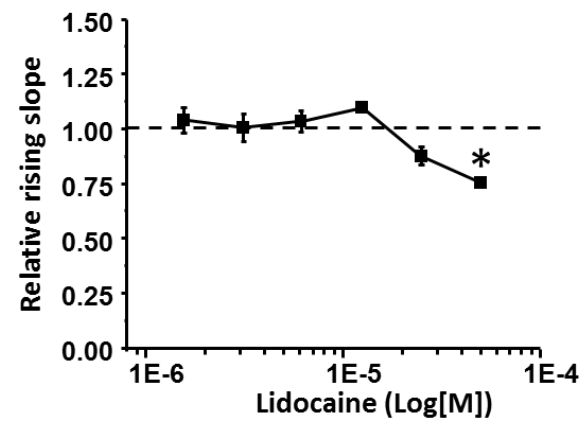
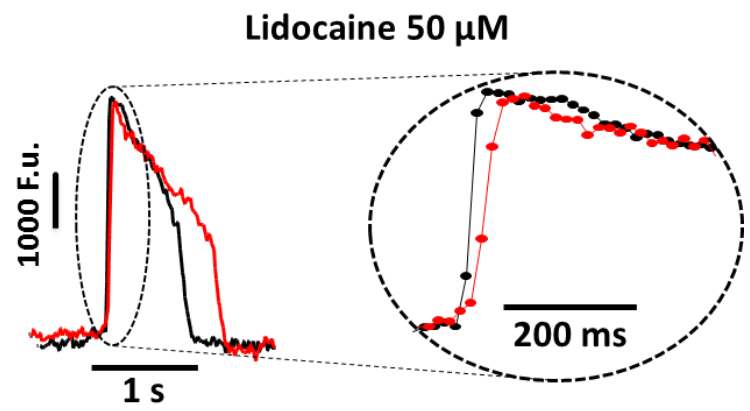


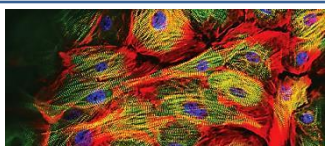
Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells



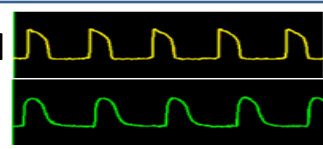
1. Paralell recording of voltage and calcium signals

Phase 0 alteration



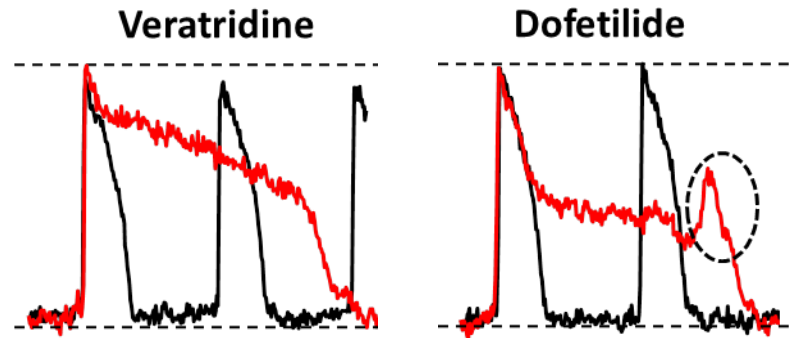


Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells

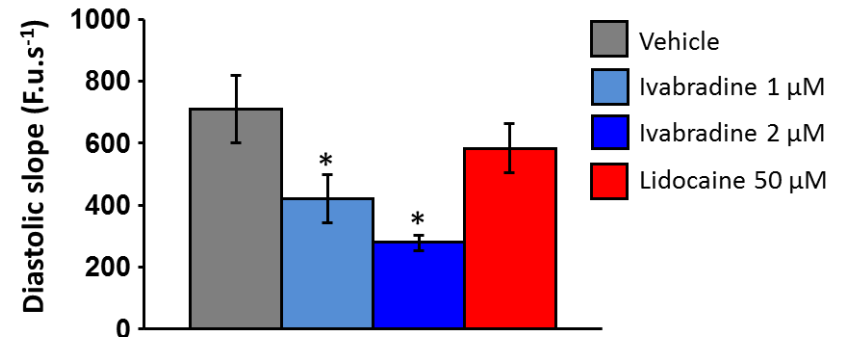
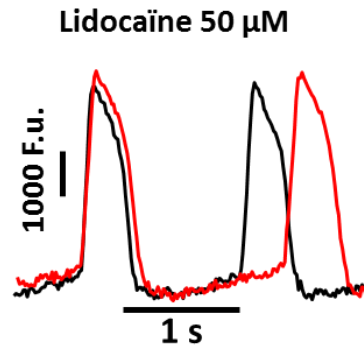
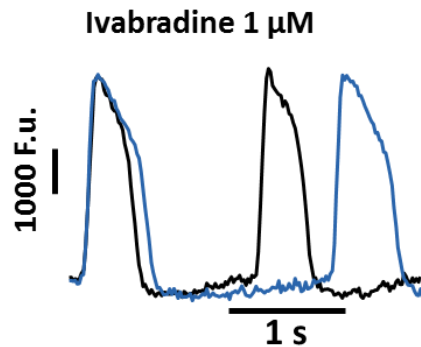


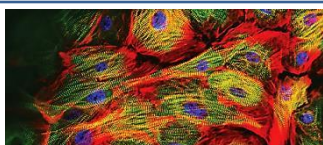
1. Paralell recording of voltage and calcium signals

Phase 1 and 3 alteration

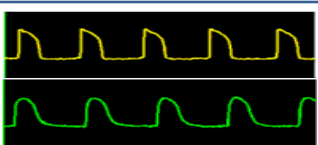


Phase 4 alteration

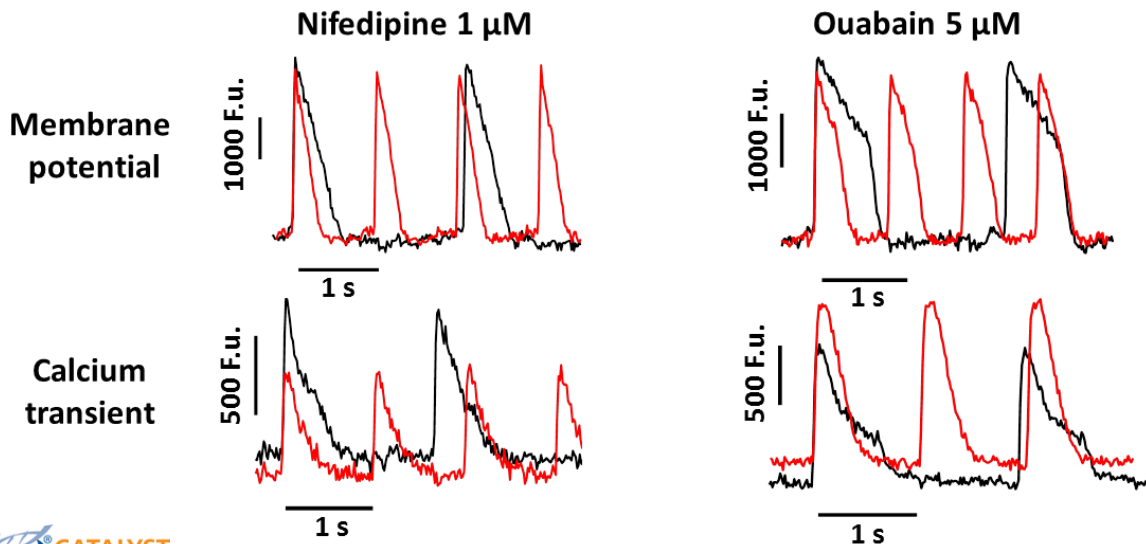
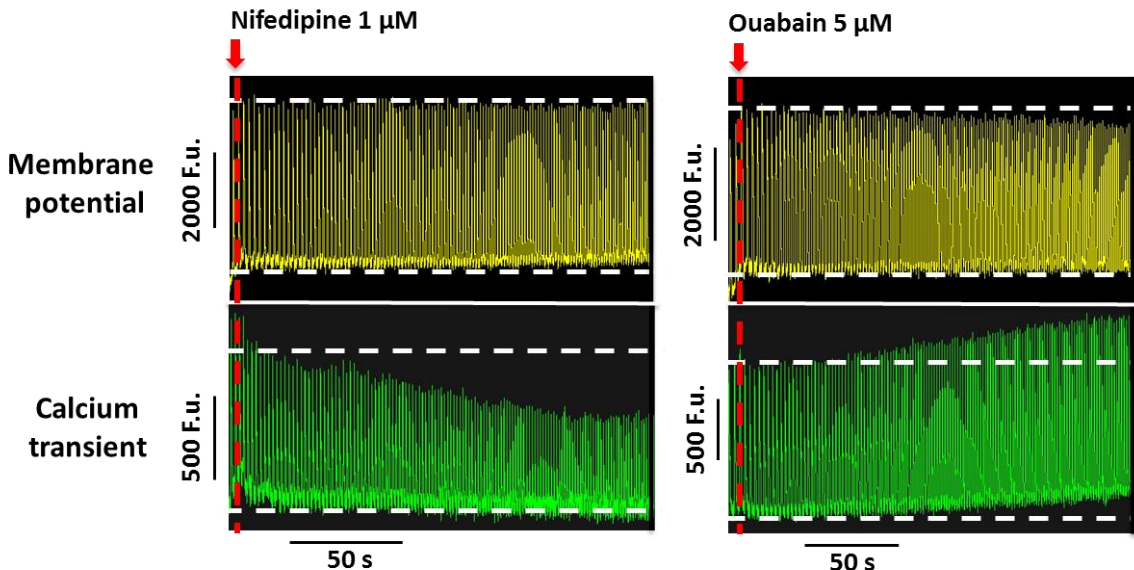


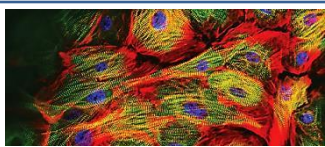


Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells

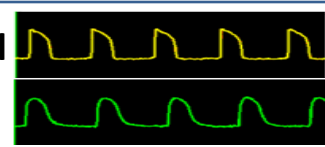


1. Paralell recording of voltage and calcium signals

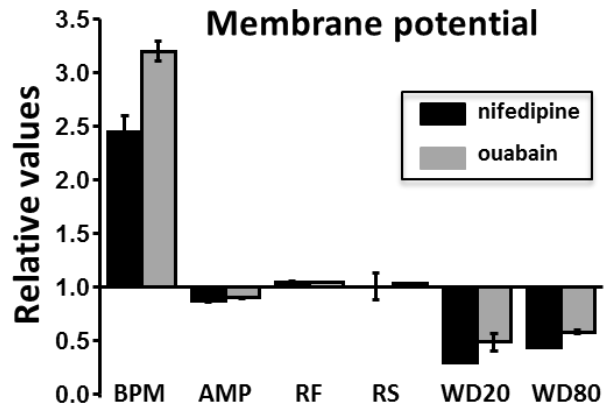
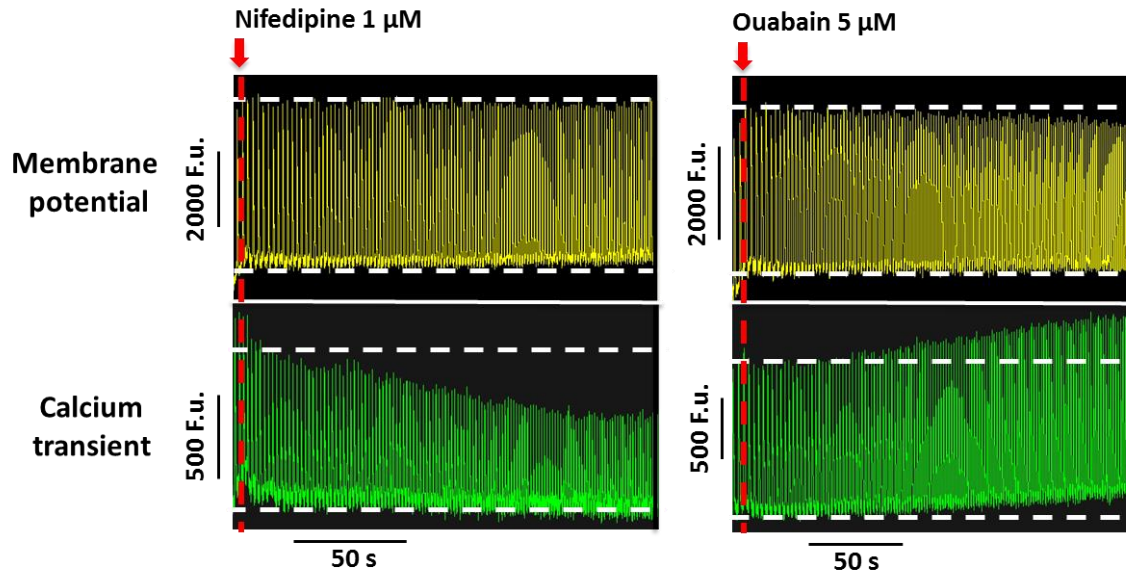


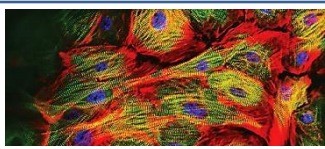


Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells

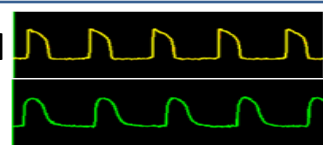


1. Paralell recording of voltage and calcium signals

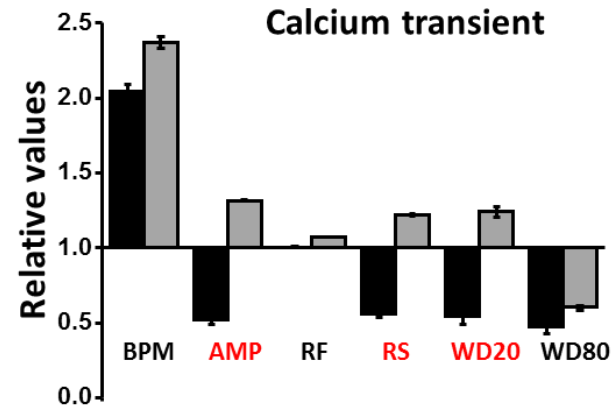
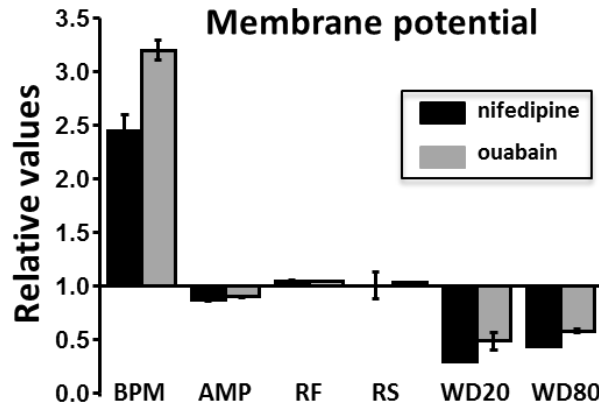
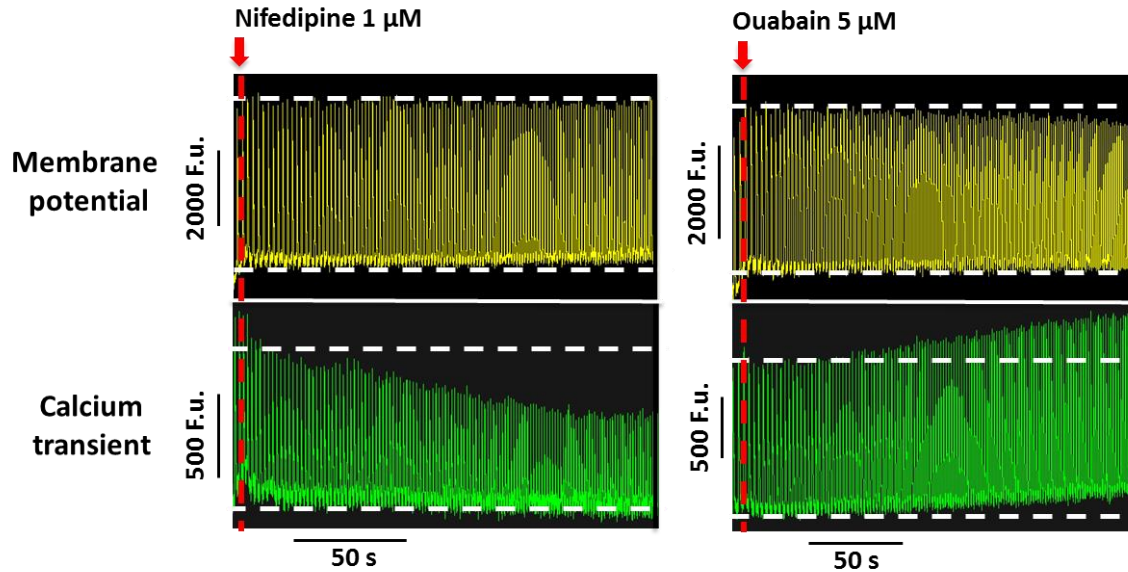


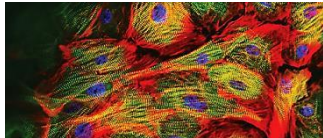


Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells

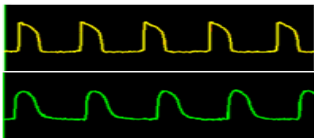


1. Paralell recording of voltage and calcium signals





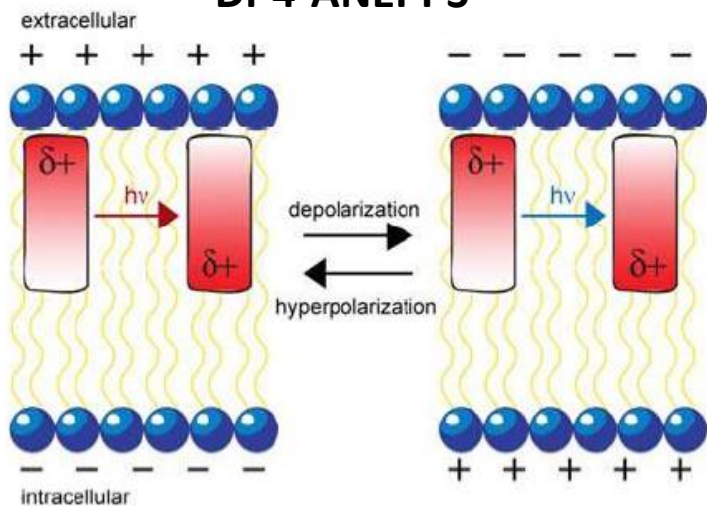
Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells



2. FluoVolt Dye

Electrochromic

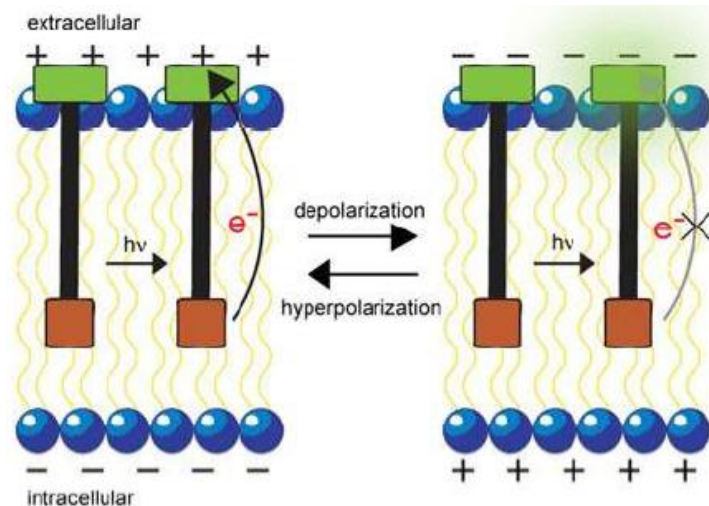
Di-4-ANEPPS



Shift of the excitation/emission spectra

$\Delta F/F \approx 6-10\%$ per 100 mV
Rapid phototoxicity

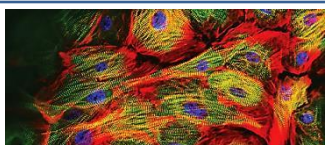
Photo-induced electron transfer



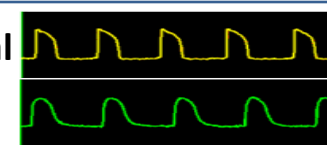
Modulation of the electron transfer rate

$\Delta F/F \approx 20-25\%$ per 100 mV
?

From Miller et al., PNAS 2012, 109 (6) : 2114-2119



Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells



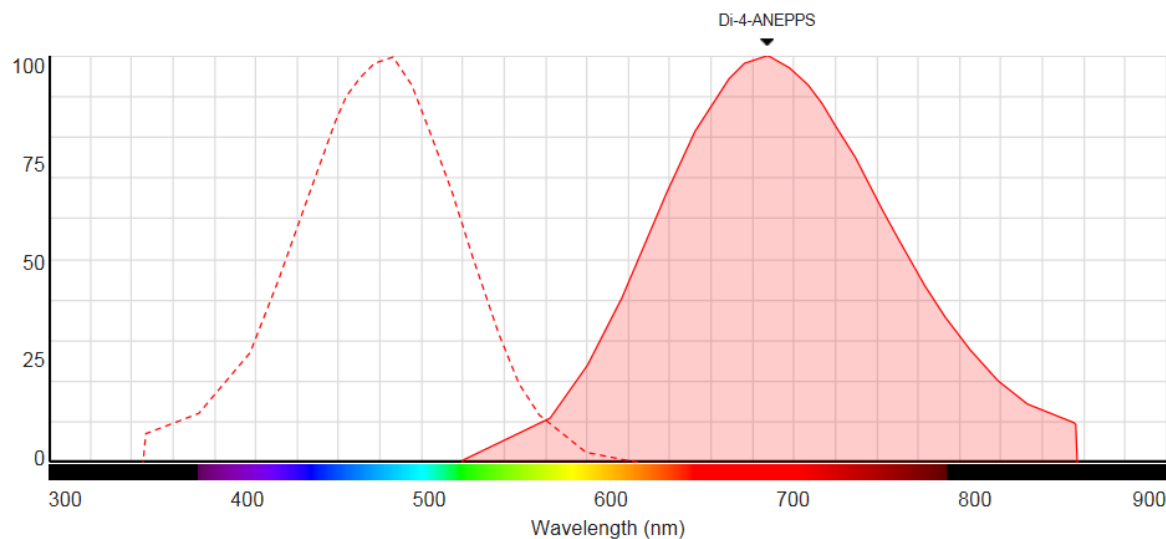
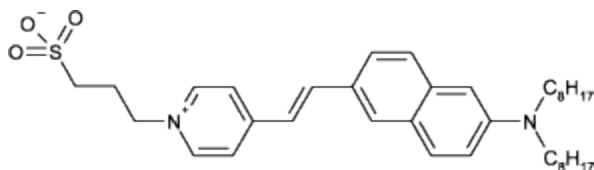
2. FluoVolt Dye

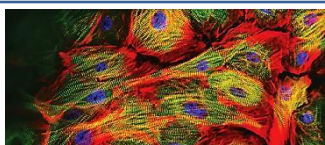
Electrochromic

Di-4-ANEPPS

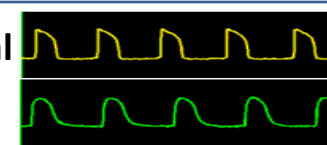
Photo-induced electron transfer

4-[2-[6-(Diocetylamino)-2-naphthalenyl]ethenyl]-1-(3-sulfopropyl)-pyridinium





Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells



2. FluoVolt Dye

Electrochromic

Di-4-ANEPPS

4-[2-[6-(Diocetylamino)-2-naphthalenyl]ethenyl]-1-(3-sulfopropyl)-pyridinium

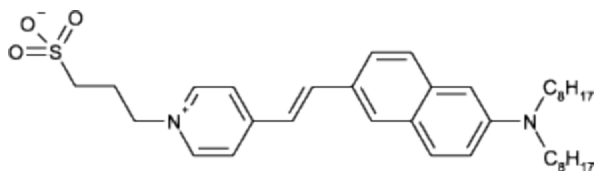
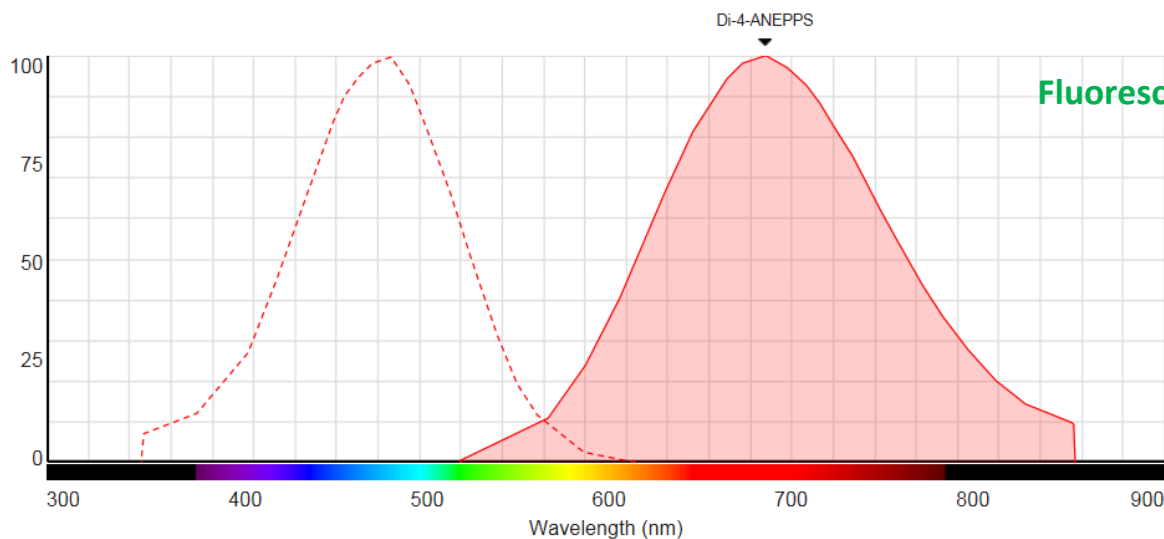
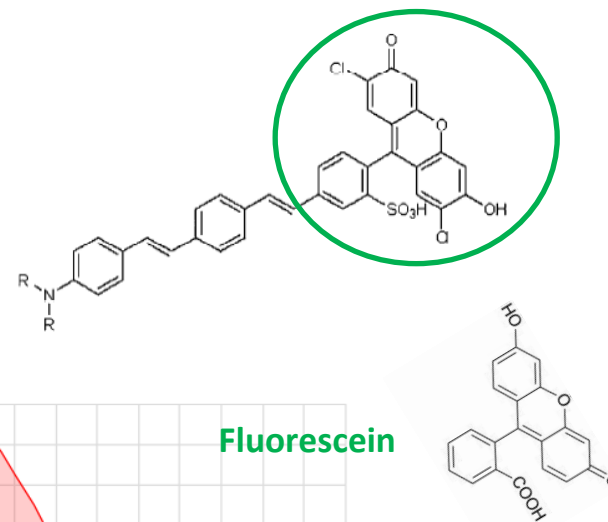
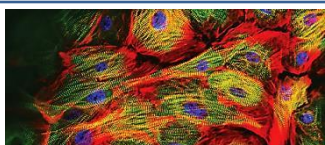


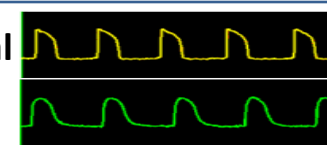
Photo-induced electron transfer

FluoVolt





Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells



2. FluoVolt Dye

Electrochromic

Di-4-ANEPPS

4-[2-[6-(Diocetylamino)-2-naphthalenyl]ethenyl]-1-(3-sulfopropyl)-pyridinium

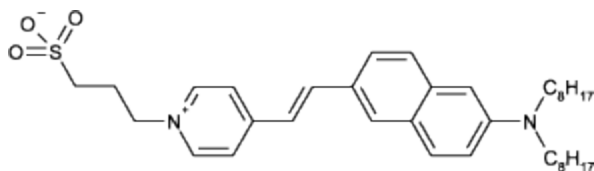
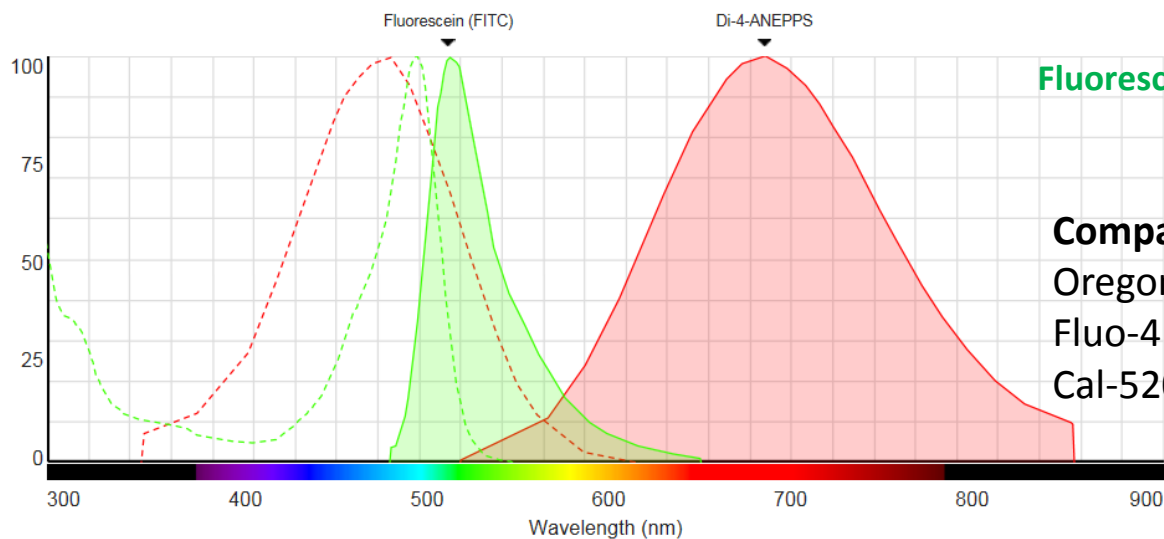
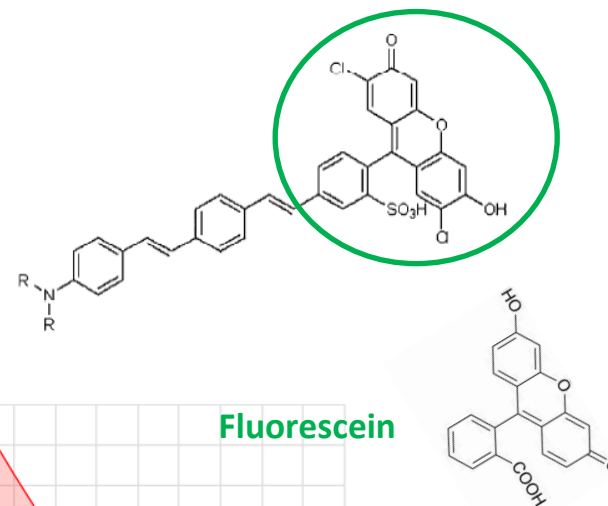


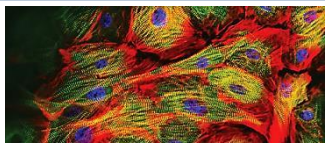
Photo-induced electron transfer

FluoVolt

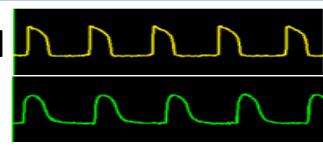


Fluorescein

Compatible with :
Oregon green (OGB1 & 2)
Fluo-4
Cal-520



Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells



2. FluoVolt Dye

96 wells

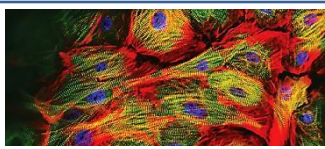
**Experimental media: HBSS + 20 mM HEPES, pH 7.4 with NaOH
(serum-free, phenol red-free)**

Temp: 37°C

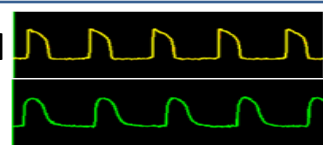
Loading time: 15 mn (50 µl, 37°C)

Rinsed twice (2 x 100 µl)

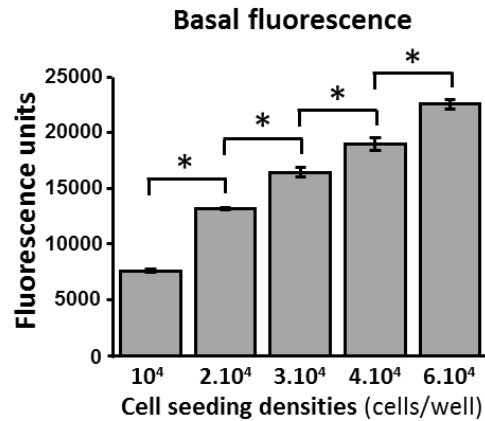
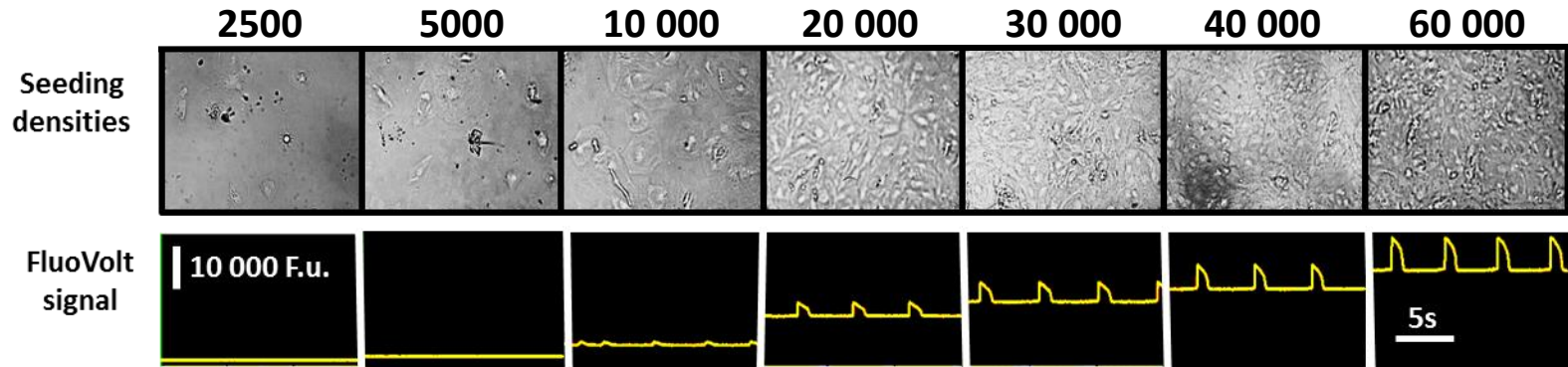
Sampling interval: 16 ms

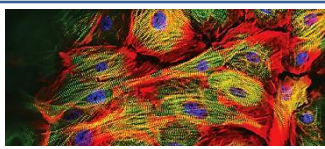


Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells

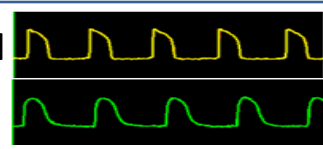


3. Cellular density (cells/well)

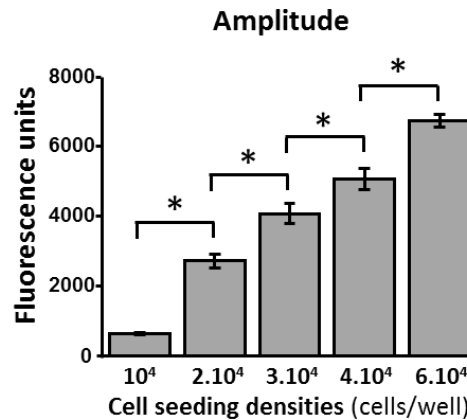
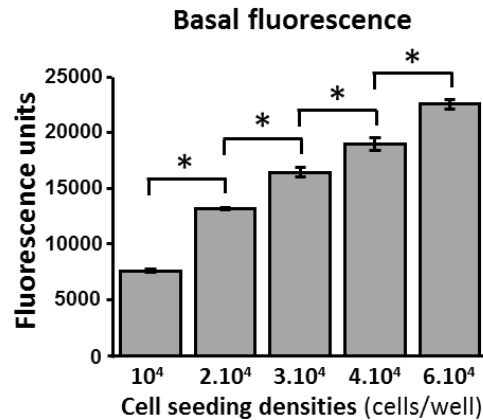
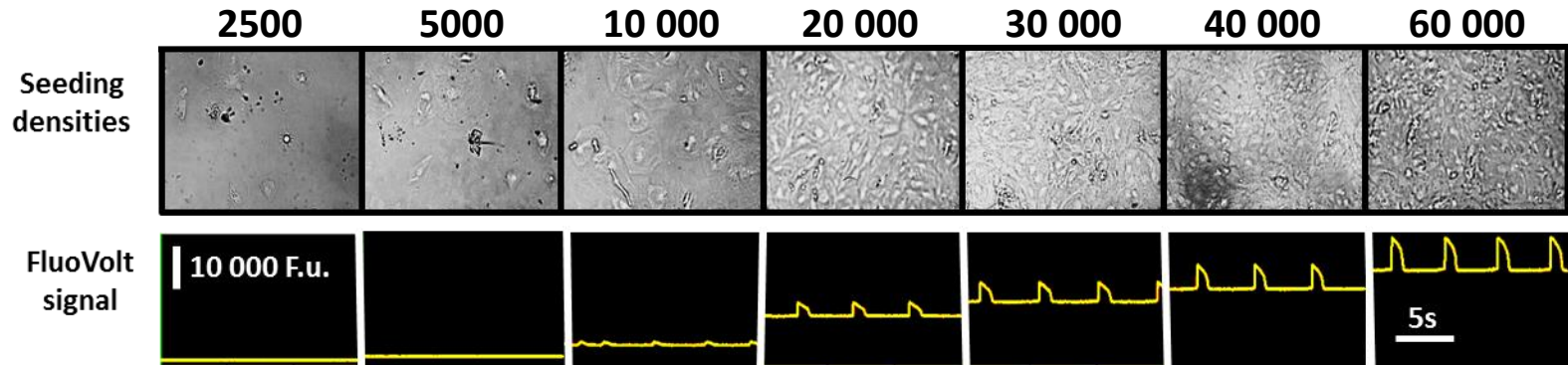


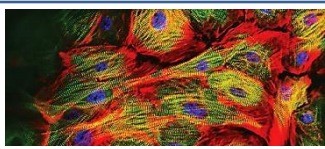


Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells

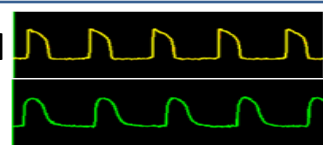


3. Cellular density (cells/well)

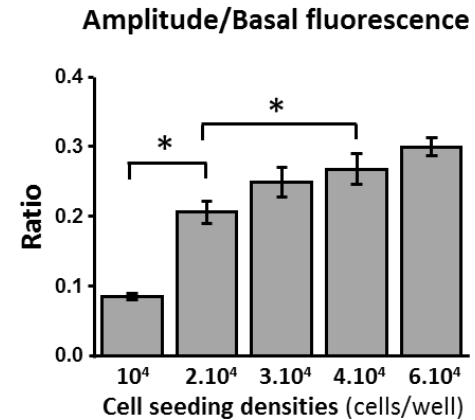
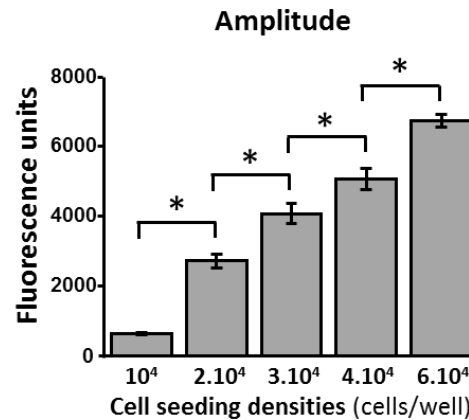
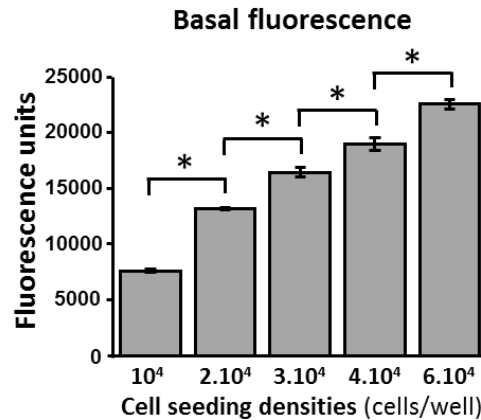
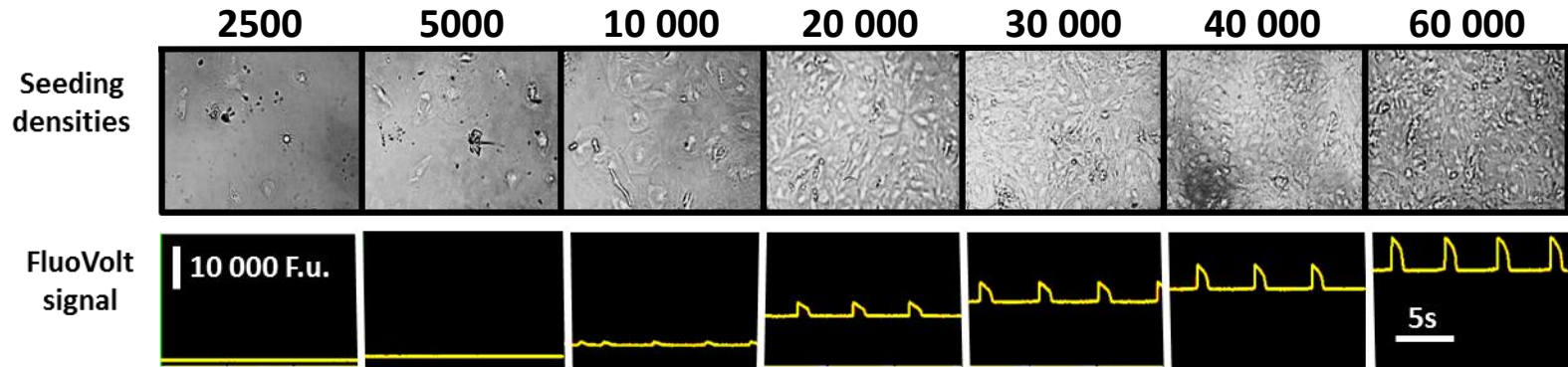


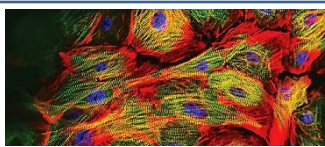


Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells

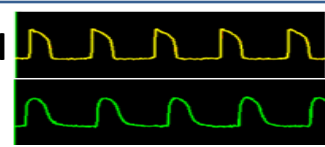


3. Cellular density (cells/well)





Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells



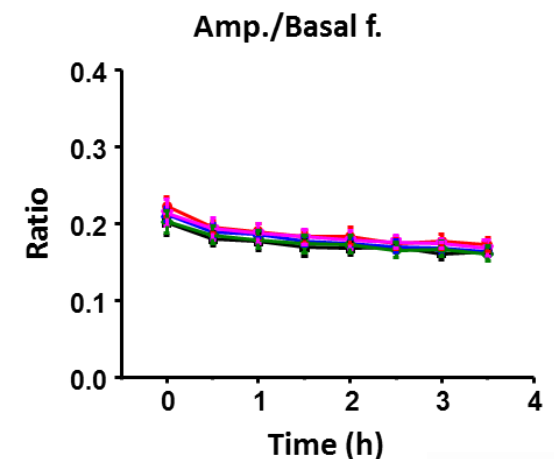
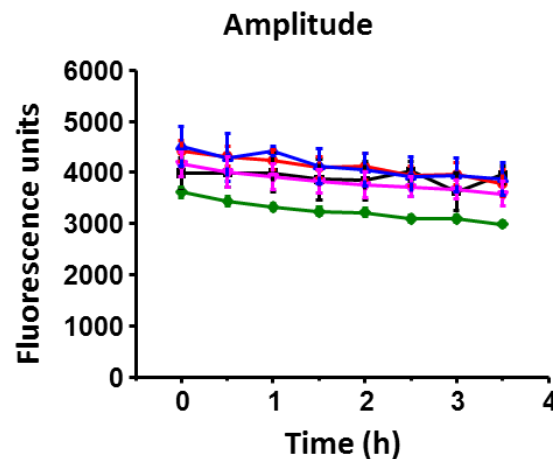
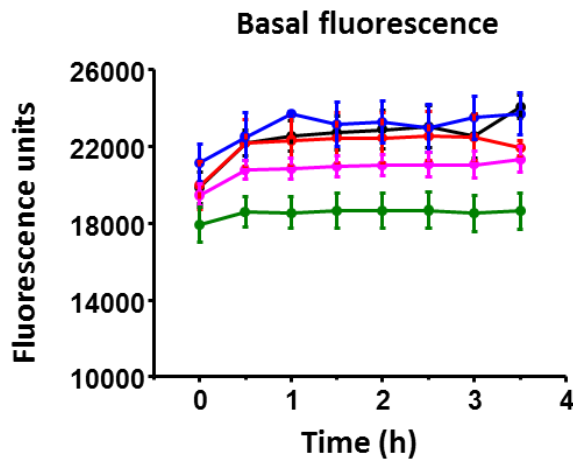
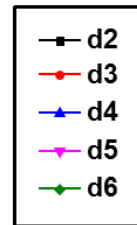
4. FluoVolt concentration

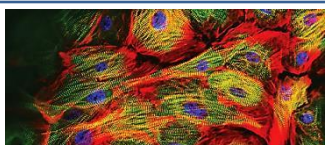


50 μ l + 500 μ l Powerload

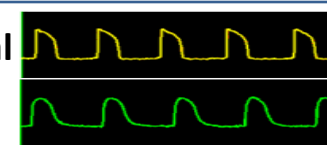


In 10, 20, 30, 40, 50 and 60 ml HBSS (d1 to d6)
d1, d2, d3, d4, d5 and d6





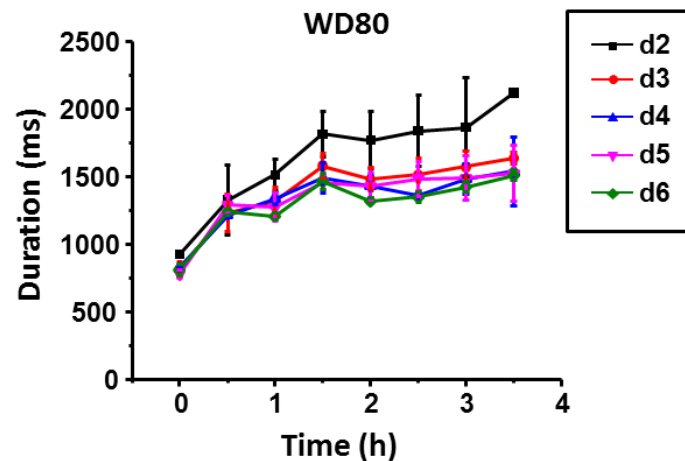
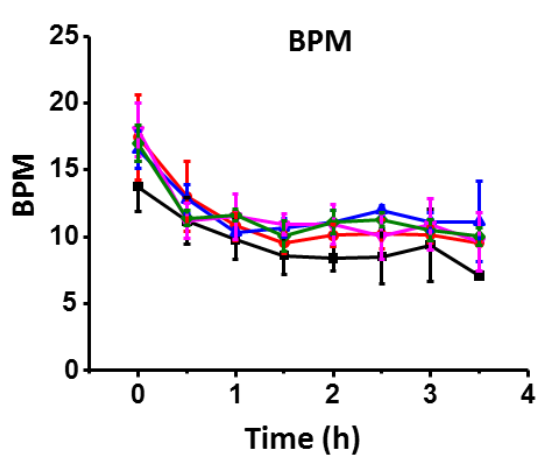
Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells

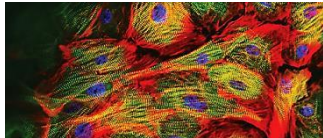


5. FluoVolt toxicity

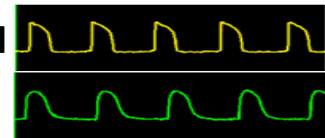


In 10, 20, 30, 40, 50 and 60 ml (d1 to d6)
d1, d2, d3, d4, d5 and d6





Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells



6. Washed-out calcium dyes

For an easy to operate assay, the same protocol as FluoVolt is expected

96 wells

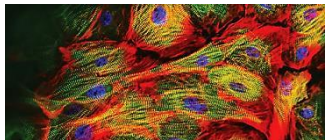
Experimental media: HBSS + 20 mM Hepes, pH 7.4 with NaOH

Temp: 37°C

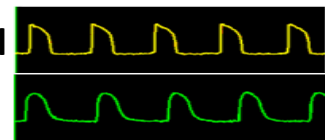
Loading time: 15 mn (50 μ l, 37°C) ~~Oregon green~~

Rinsed twice (2 x 100 μ l) **Dye extrusion**

Sampling interval: 16 ms

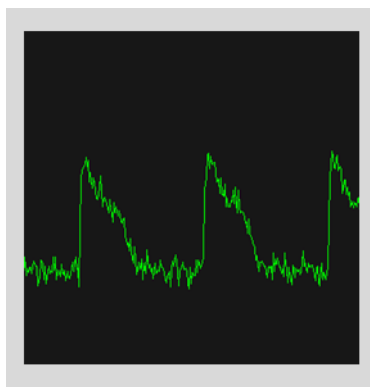
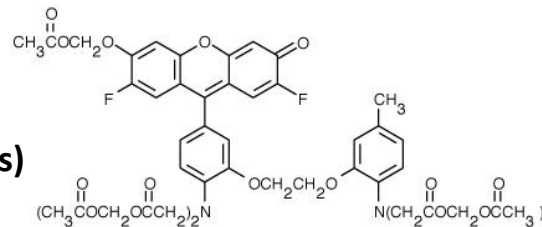


Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells

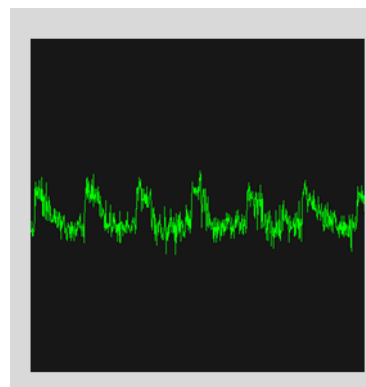


6. Washed-out calcium dyes

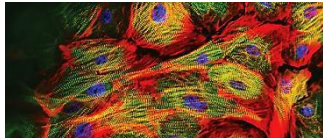
Fluo-4
(Molecular probes)



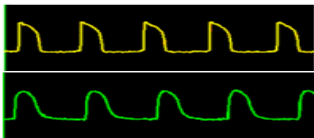
Start



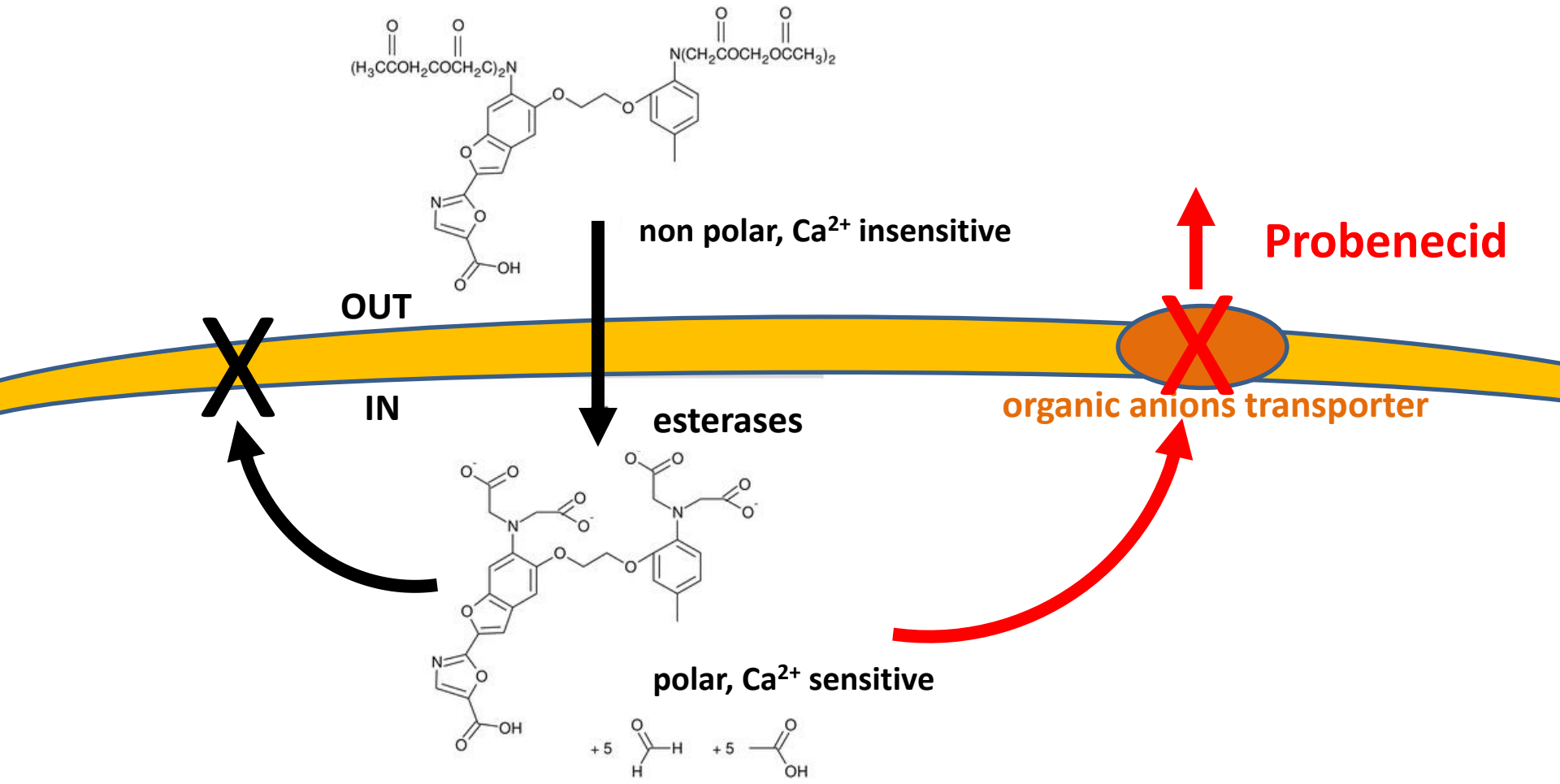
30 mn



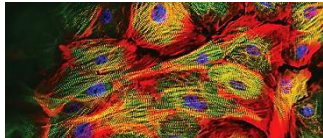
Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells



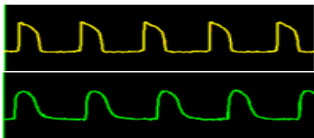
6. Washed-out calcium dyes



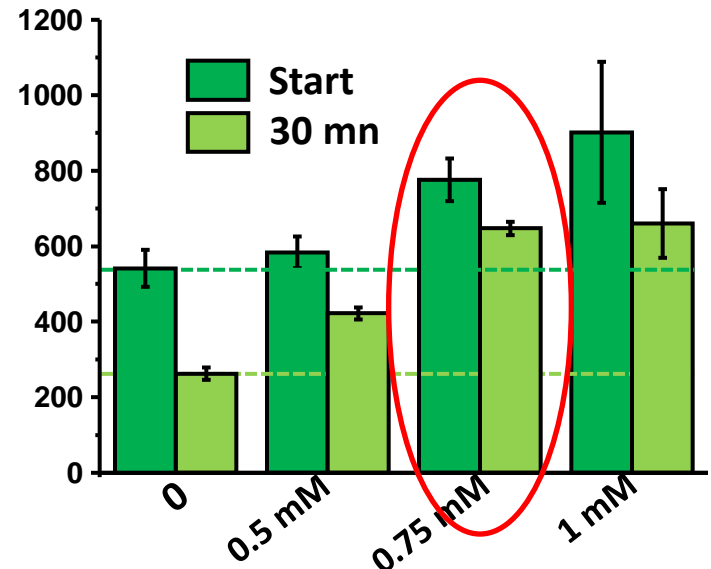
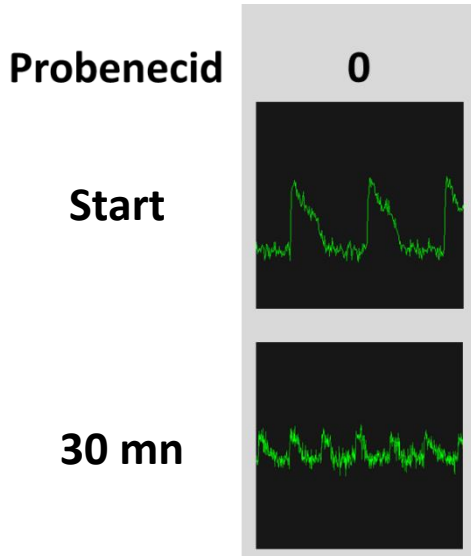
FLUO-4

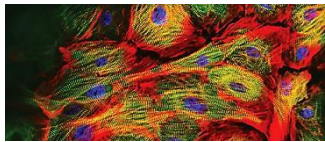


Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells

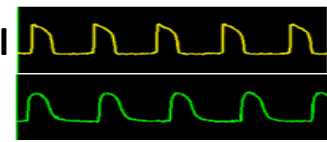


6. Washed-out calcium dyes



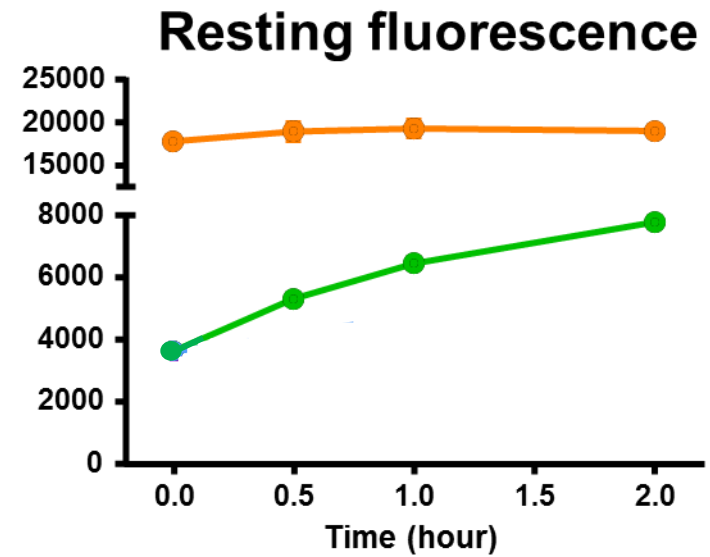
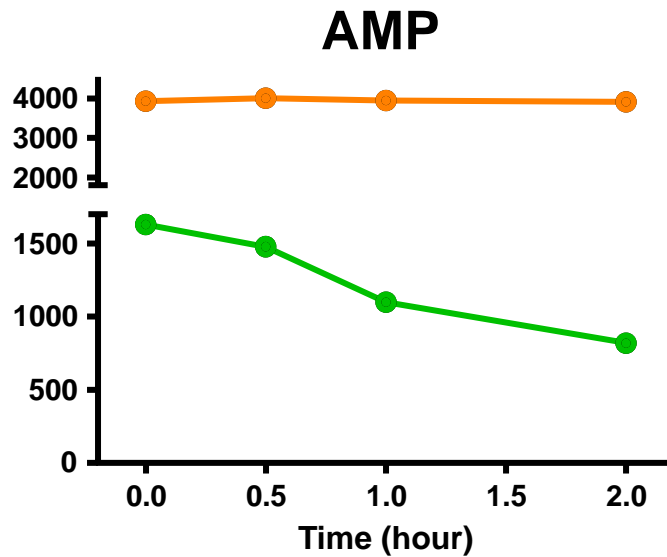


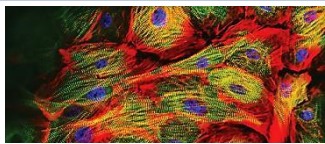
Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells



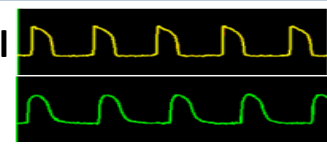
6. Washed-out calcium dyes

—●— FluoVolt
—●— Fluo-4 + 0.7 mM P.



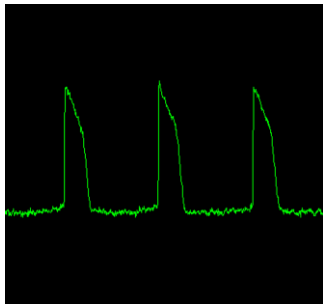


Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells

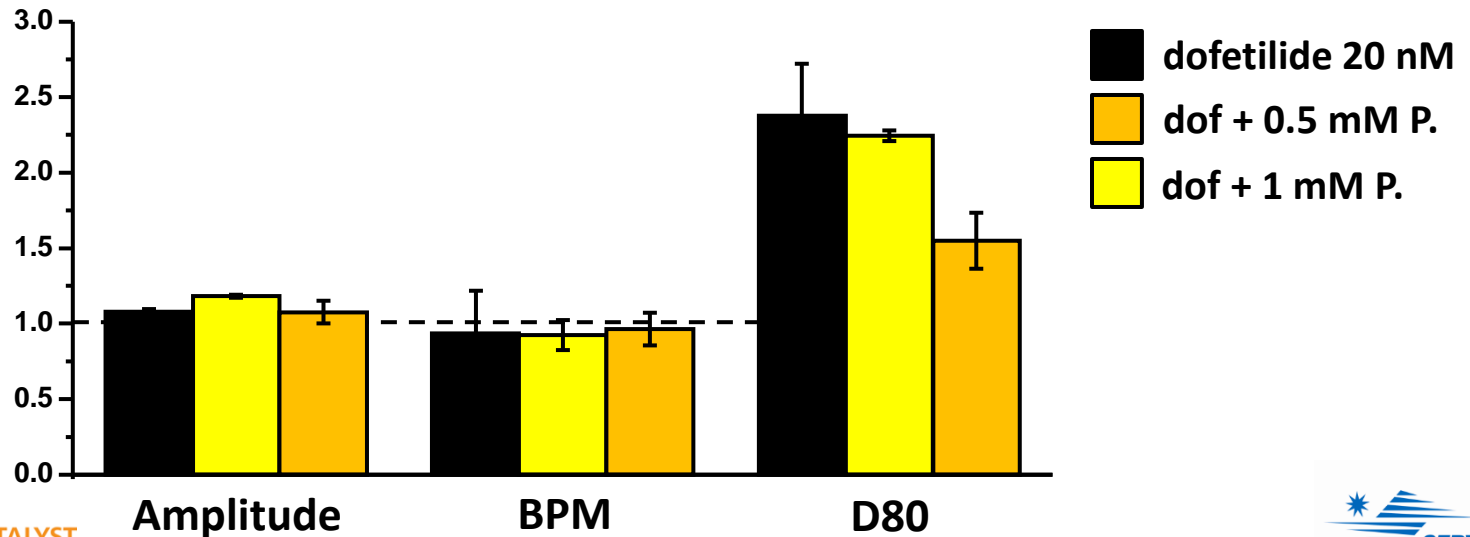
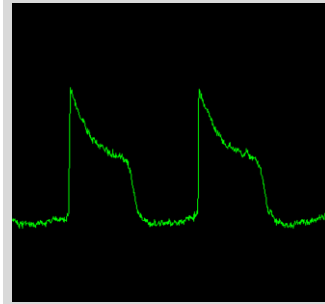


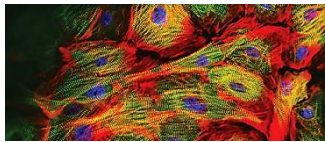
6. Washed-out calcium dyes

vehicle

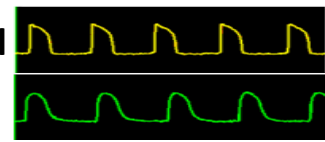


dof. 20 nM





Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells

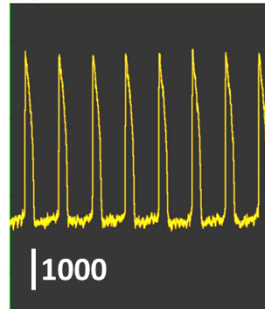


6. Washed-out calcium dyes

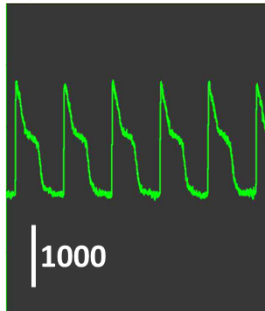
Cal-520

(AAT bioquest)

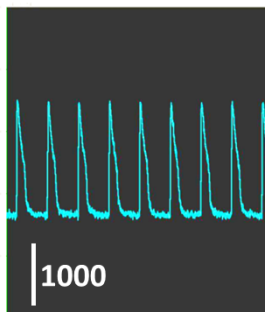
FluoVolt

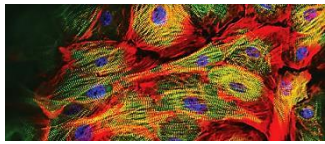


Fluo-4
+ 0.7 mM P

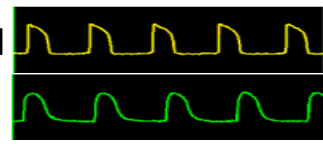


Cal520



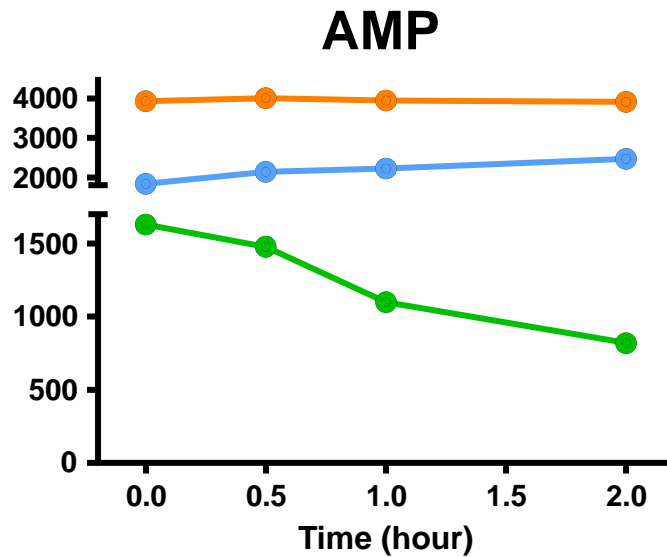


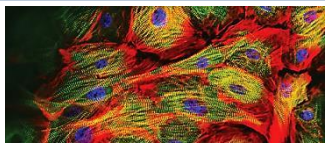
Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells



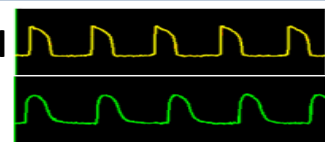
6. Washed-out calcium dyes

- FluoVolt
- Fluo-4 + 0.7 mM P.
- Cal520



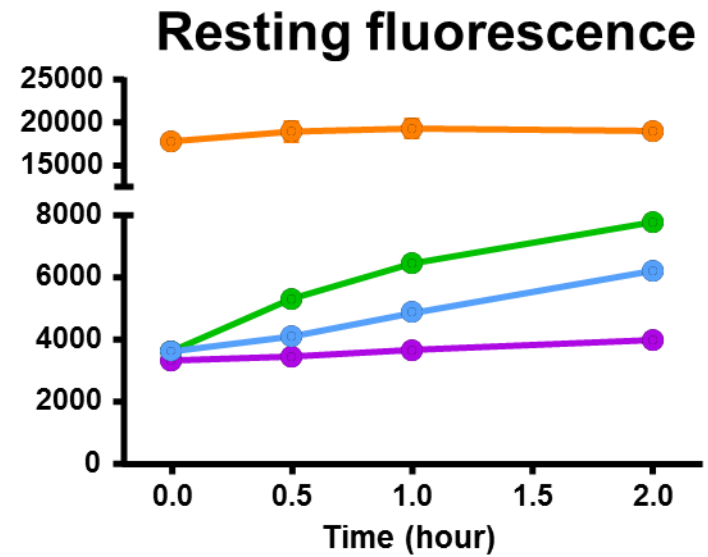
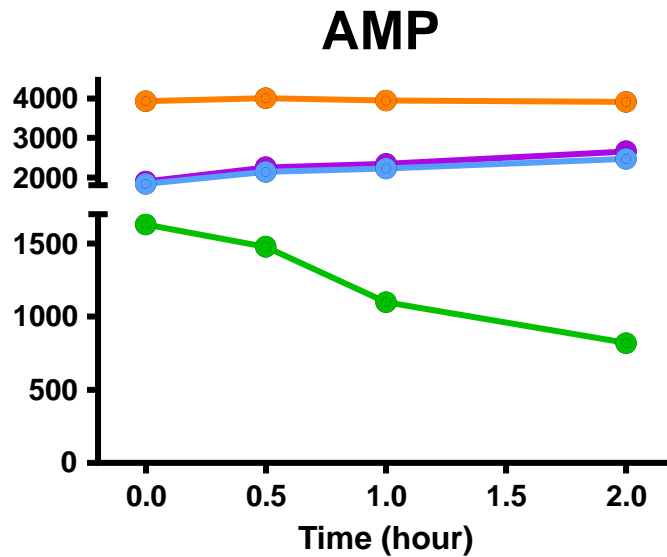


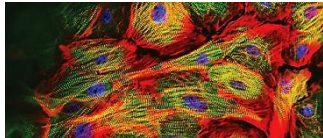
Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells



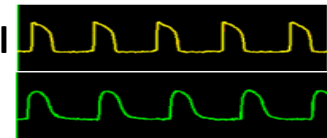
6. Washed-out calcium dyes

- FluoVolt
- Fluo-4 + 0.7 mM P.
- Cal520
- Cal520 + 0.7 mM P.





Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells



7. Conclusions

No serum, no phenol-red (HBSS + Hepes)

No probenecid

High cellular density

37°C

16 ms sample interval

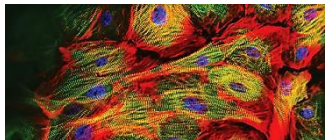
FluoVolt

Cal-520

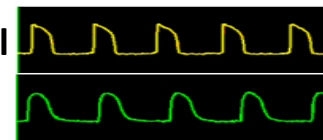
15 mn loading time

2 wash-outs

About 3 hours of recording capacity



Experimental parameters for an efficient recording of membrane potential and calcium activity from hIPS-derived cardiomyocytes in 96 wells



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V. LAMAMY
N. VILLAIN

Thank you for your attention