# Dual cardiomyocyte cluster atomic force microscopy detection: the novel analysis type of drug arrhythmogenic potential

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# Rhythm diseases

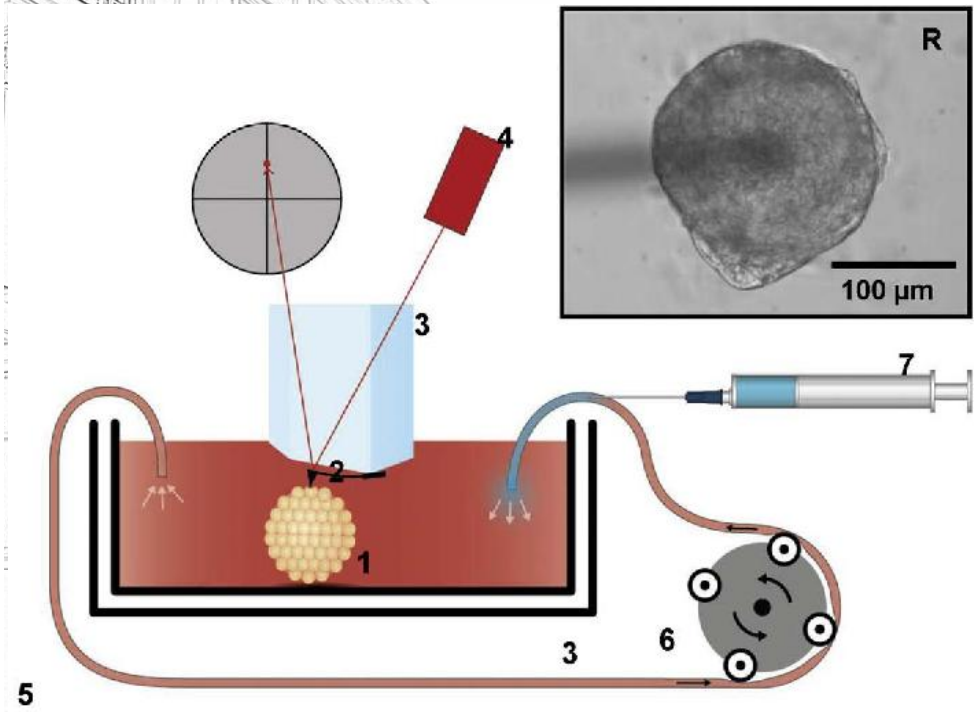
Focal activation

- single cell model
- animal models
- multicellular clusters

Propagation

- cannot be studied on single cells
- partially relevant in animal models,
- limited in small cell clusters

# Disease modelling: CM-iPSc




CMs derived from stem cell „wild type“ lines / lines from patient biopsies

inherited cardiomyopathies
- muscle diseases
- neuromuscular diseases
- cardiomyopathies
- channelopathies

drug testing (iatrogenic cardiomyopathy)
- novel drugs adverse effect screening
- drugs „in use“ combination effect

methods combination - Ca imaging, MEA

*Jelinkova S, et al. 2020*

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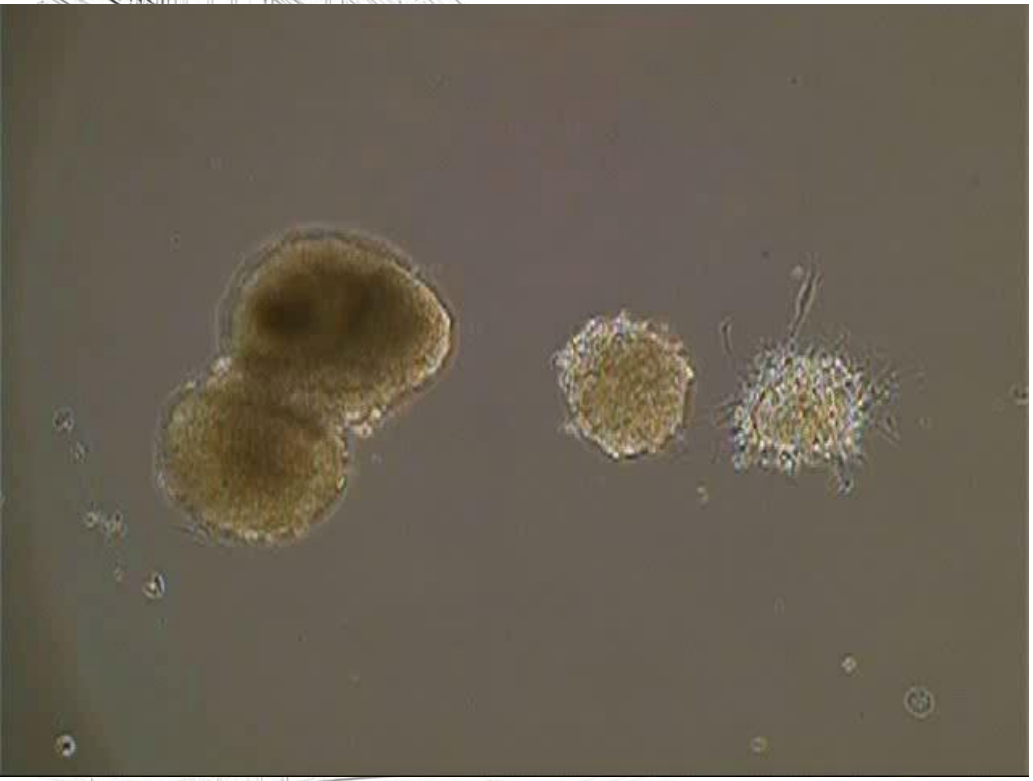

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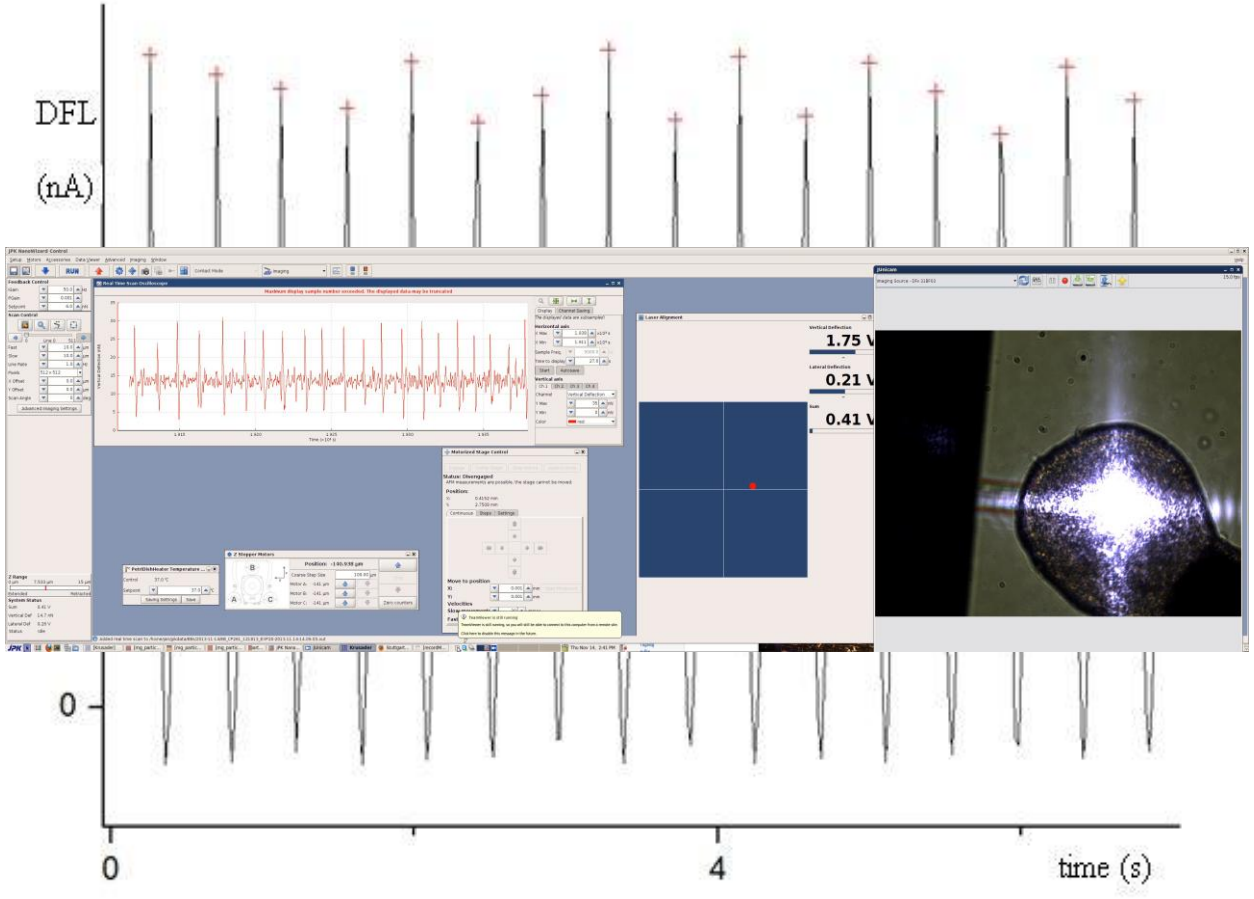
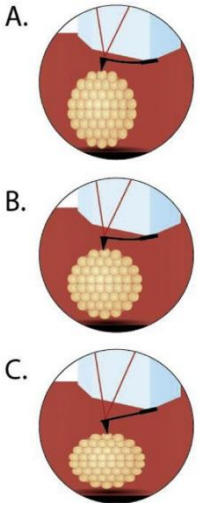
# Cardiomyocyte phenotyping by AFM




AFM-based biosensor setup
stem cell derived CMs cluster




contraction rate

relaxation time

displacement / deflection

computed contraction force

*Pesl et al. Biosens. Bioelectron 2016*

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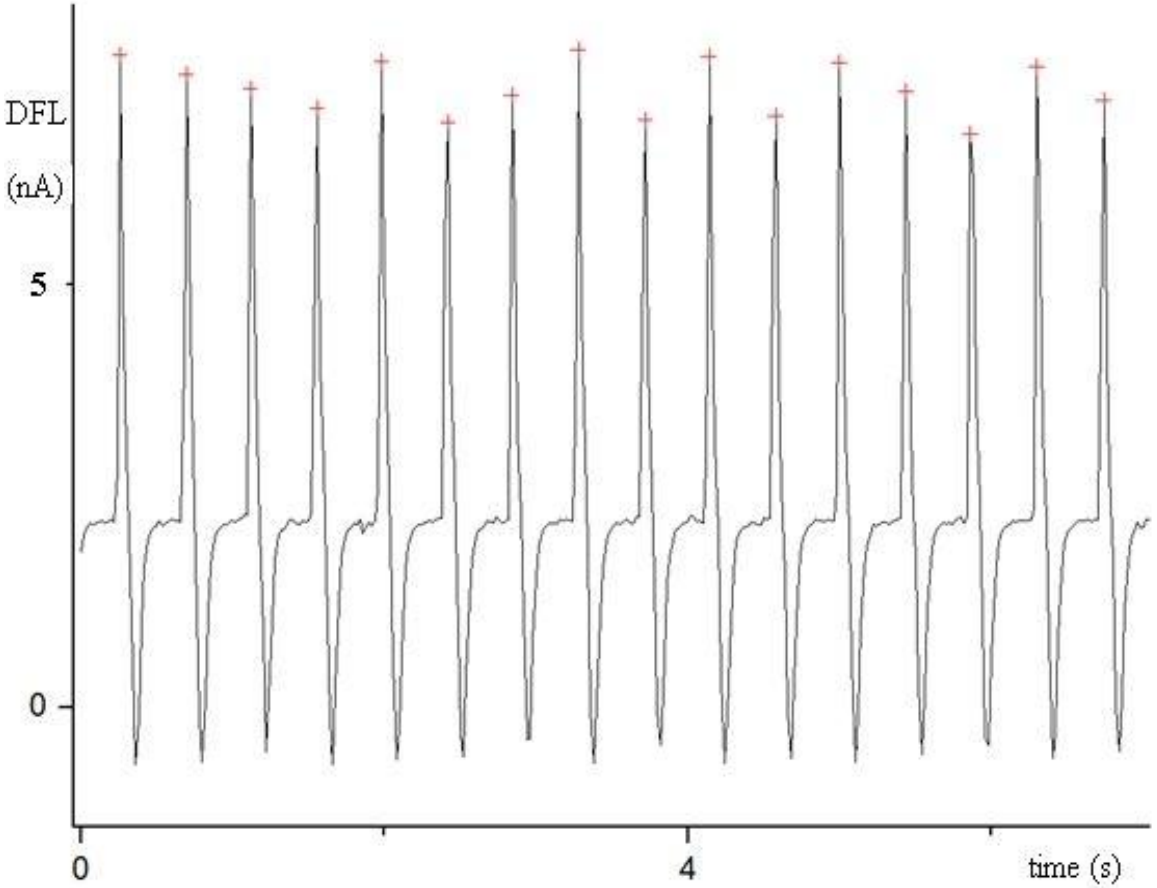


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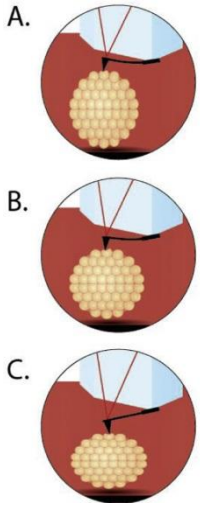


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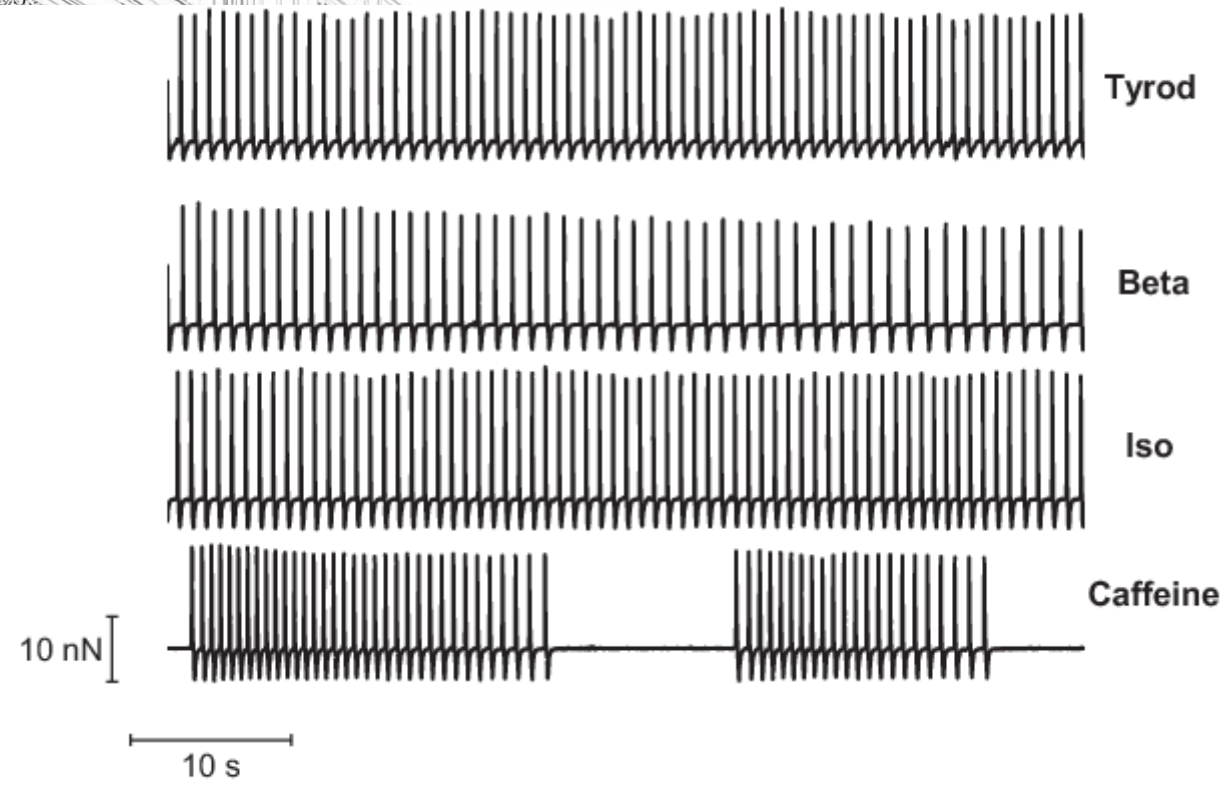
# Drug effects and related adverse events

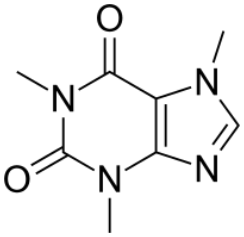



standard pharmacological indicators of different phenotypic features

beta adrenergic blocking
Metoprolol

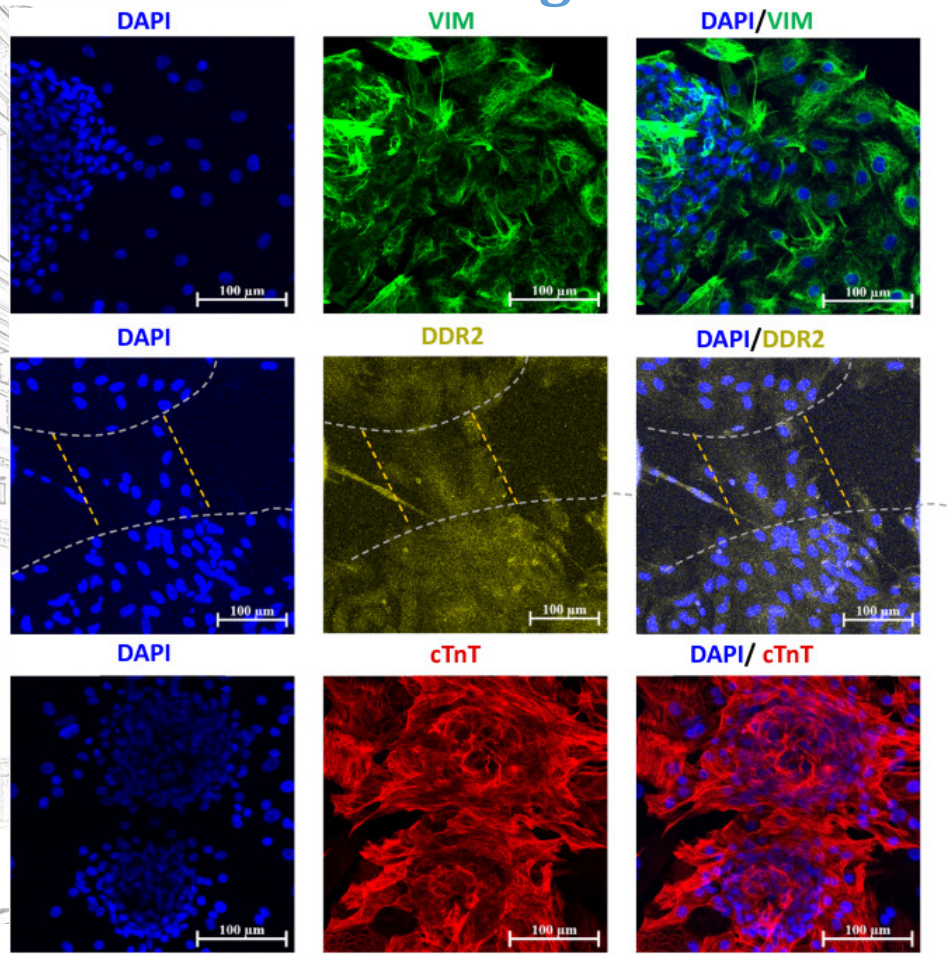
beta adrenergic stimulation
Isoproterenol / Adrenalin

Methylxanthie
Caffeine




*Klimovic et al. Front Pharmacol.2022*
*AFM Biomed Munster, 2019*

# Methods: combining two clusters




seeding clusters in agarose mask

200um apart

within 96h after seeding forming conductive „bridge“

synchronizing contraction pattern

DDR2 Discoidin Domain Receptor
cTnT Cardiac Troponin T
VIM Vimentin

*Pivato et al. ACB 2022*
*AFM Biomed Naogya-Okazaki 2022*

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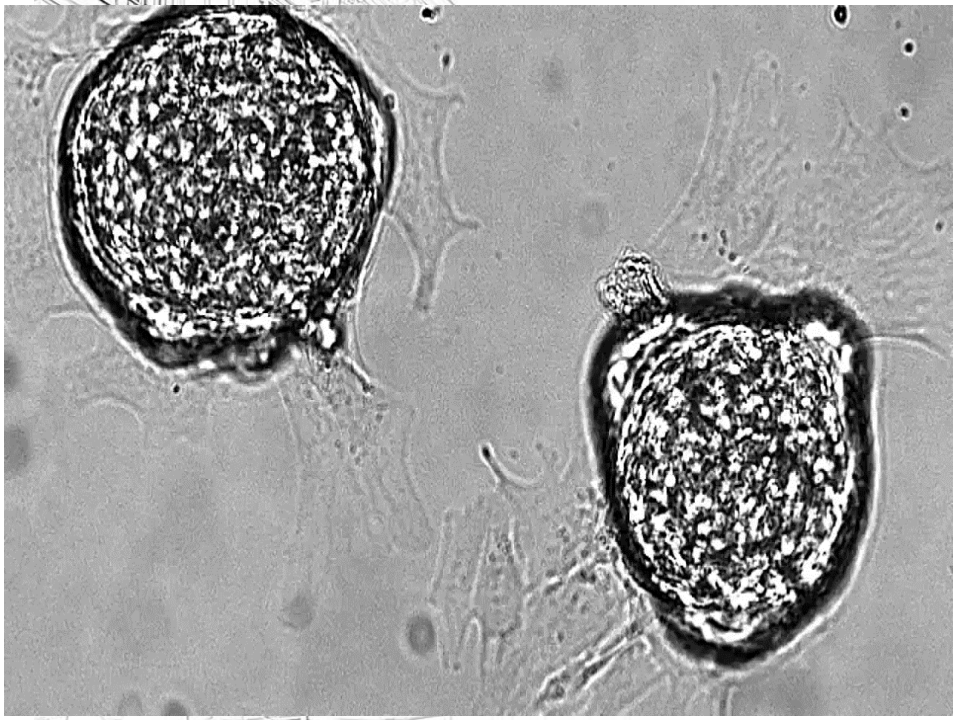
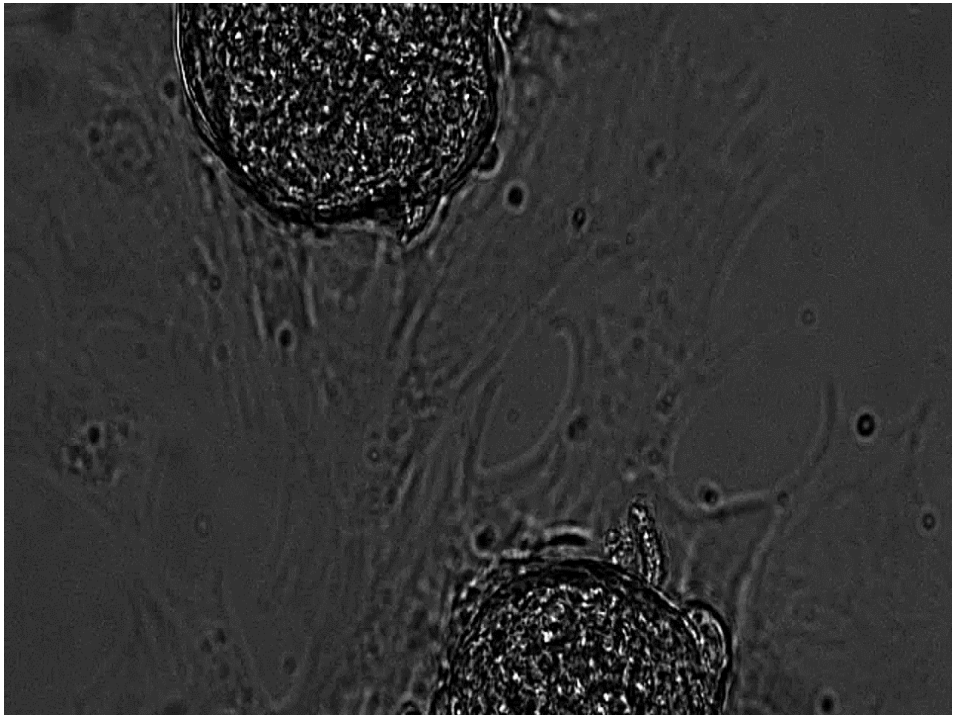


synchronized twins



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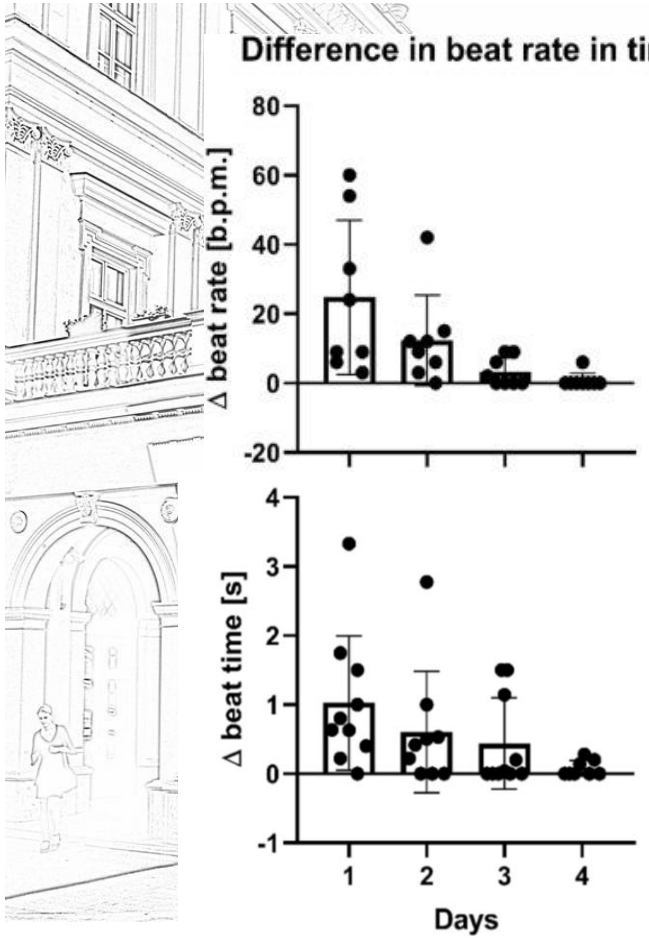


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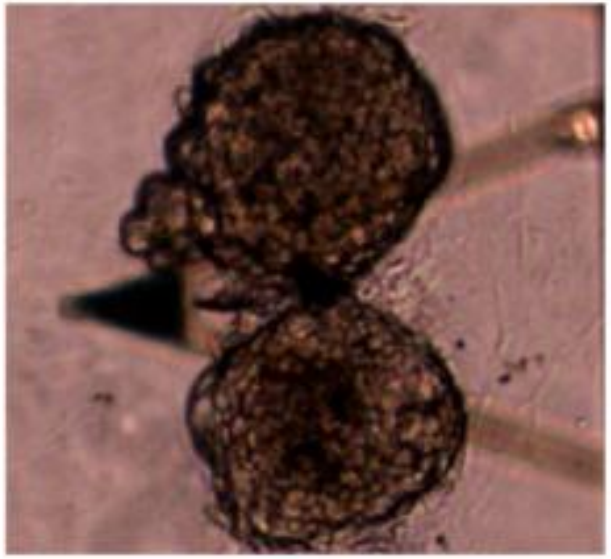

ImageJ/Fiji macro Musclemotion

*Pivato et al. ACB 2022*
*AFM Biomed Naogya-Okazaki 2022*

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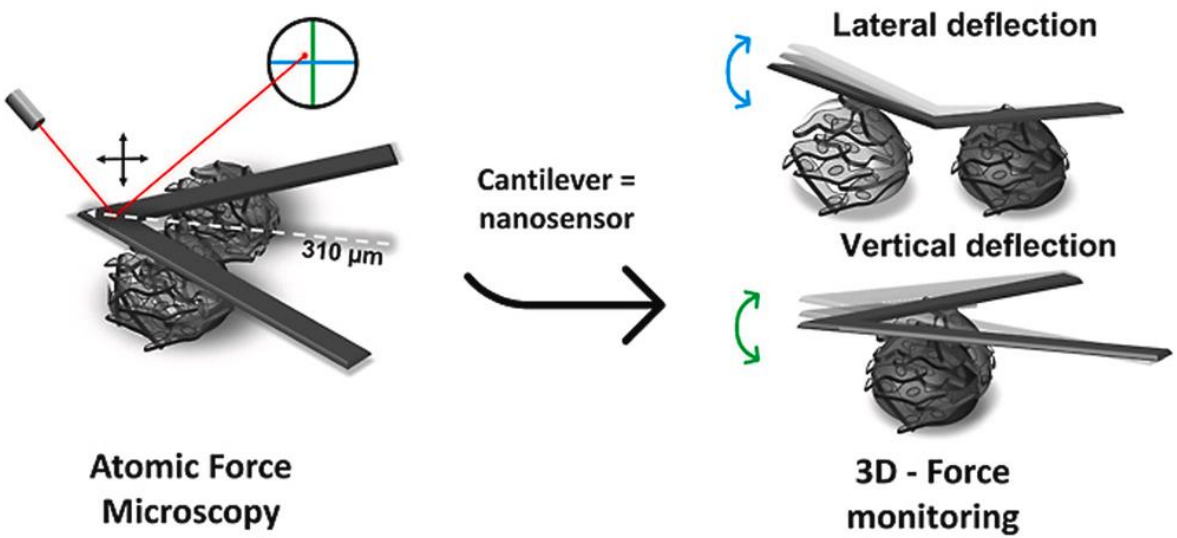



AFM-based biosensor setup

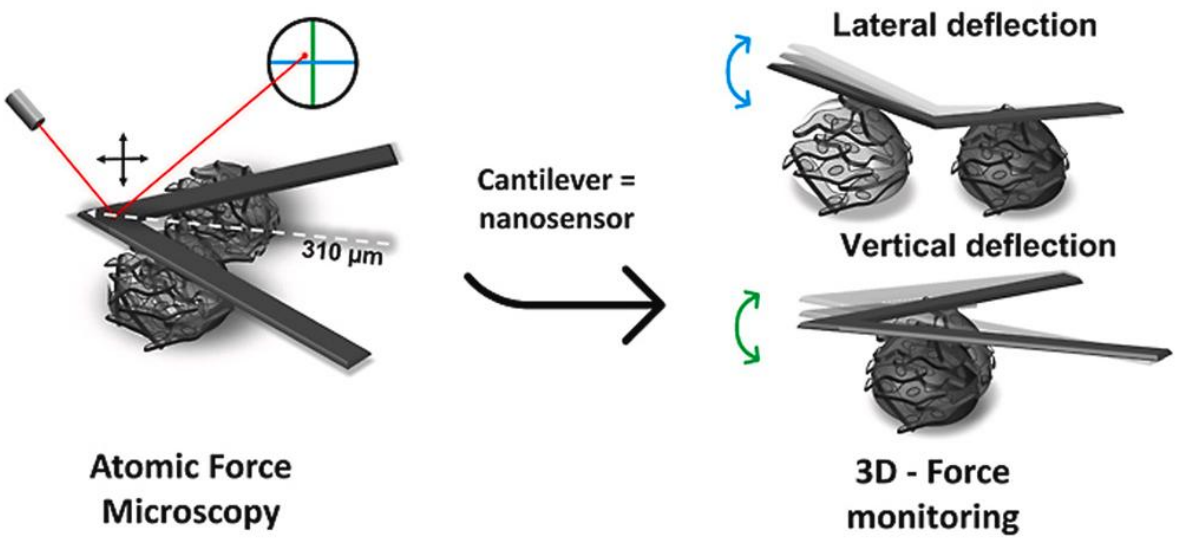
JPK/Bruker Nanowizard 3
MLCT-C cantilever Bruker
- partially coated silicon nitride cantilever

force spectroscopy mode
1 nN setpoint (active mode)

- allows tracing of cantilever/ cluster drift and loss of contact
- mechanical response natural to cardiac cells - as preload and afterload

*Klimovic et al. Front Pharmacol 2022*

# Methods




vertical and lateral deflection mechanocardiograms (MCG) collected in 100 Hz sampling frequency in a pilot group

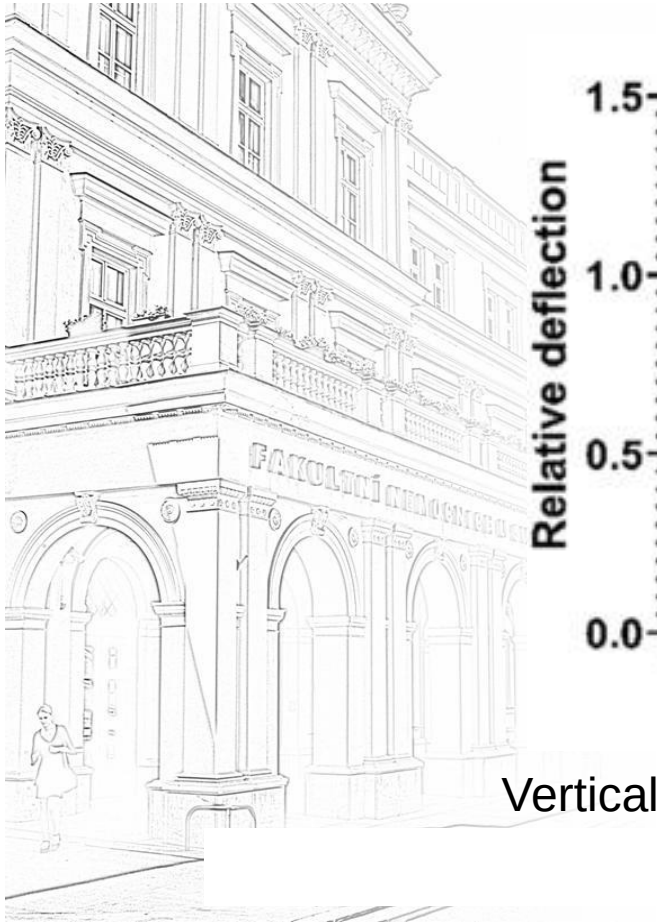
vertical deflection = contraction frequency (R-R interval)

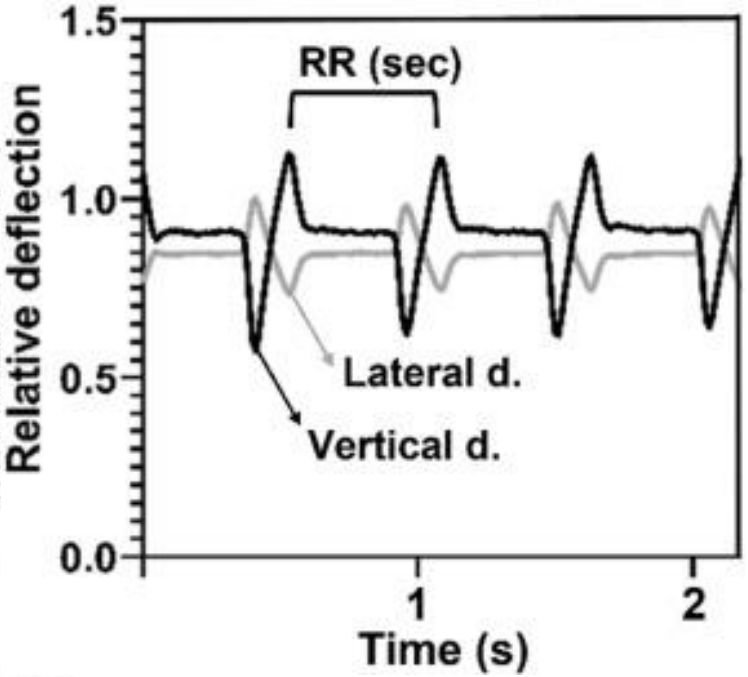
lateral (horizontal) deflection remains stable (repeating curve) until desynchronization

Data extraction performed by in-house made python script

*Pivato et al. ACB 2022*
*AFM Biomed Naogya-Okazaki 2022*

# Results






Vertical and lateral deflection „synchronized“

*Pivato et al. ACB 2022*
*AFM Biomed Naogya-Okazaki 2022*

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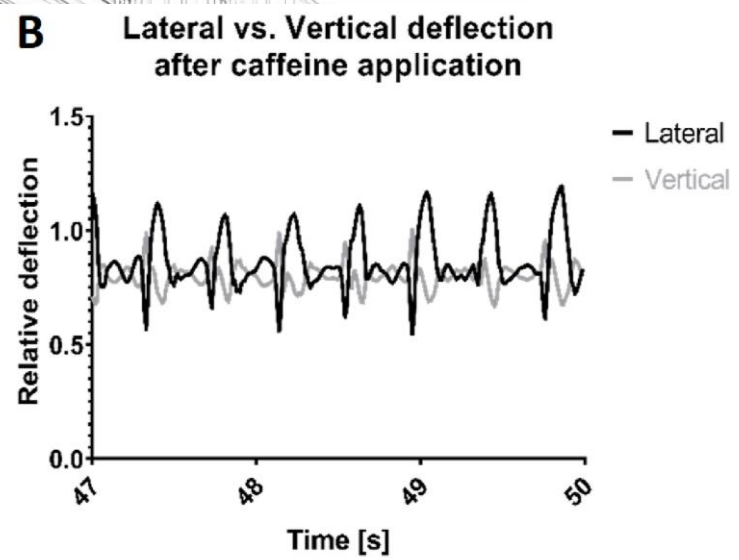
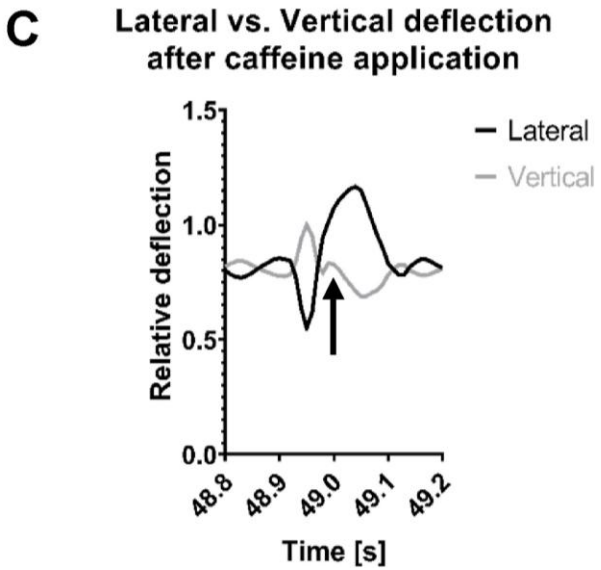
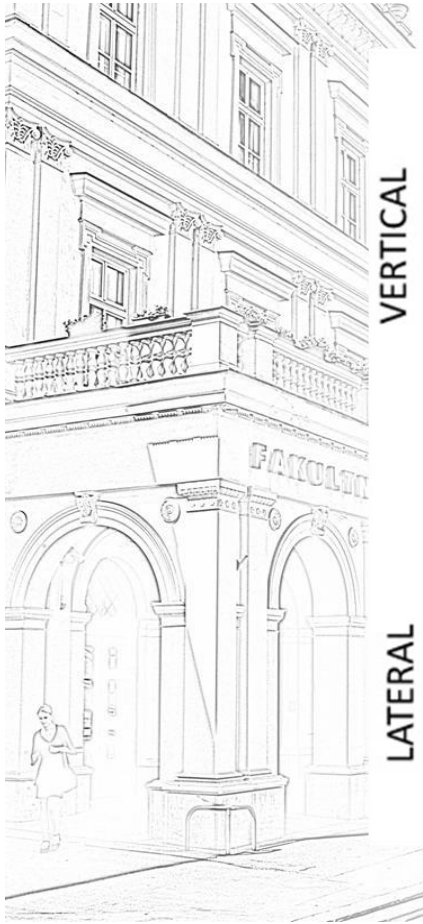


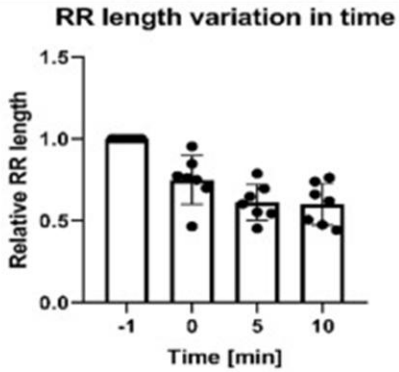

Vertical and lateral deflection „de-synchronized“

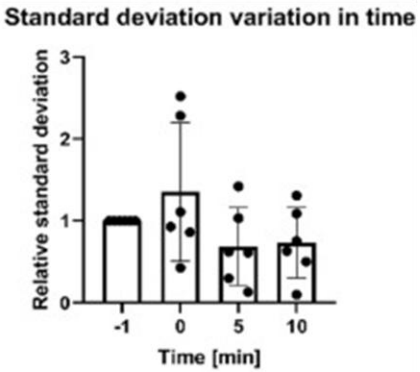
*Pivato et al. ACB 2022*
*AFM Biomed Naogya-Okazaki 2022*

# Results

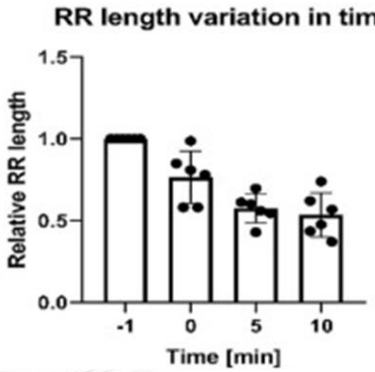


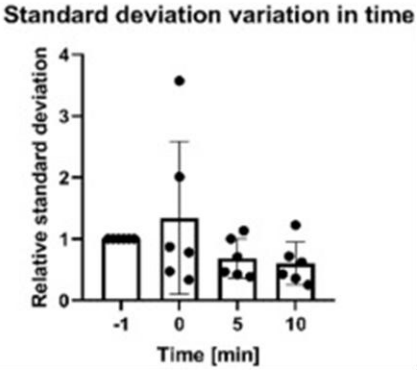
VERTICAL




LATERAL

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*AFM Biomed Naogya-Okazaki 2022*

# CONCLUSIONS

The lateral and vertical deflection peaks were synchronized in dual CMs cluster model.

Caffeine affected the synchronization of vertical and lateral displacement and produced independent lateral and vertical deflections.

Defects in signal spreading through the bridge resulting in the irregular beat of the two clusters is novel model for advanced drug screening and disease-drug interaction.

# Thanks for your interest!

contact: pesl@fnusa.cz

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