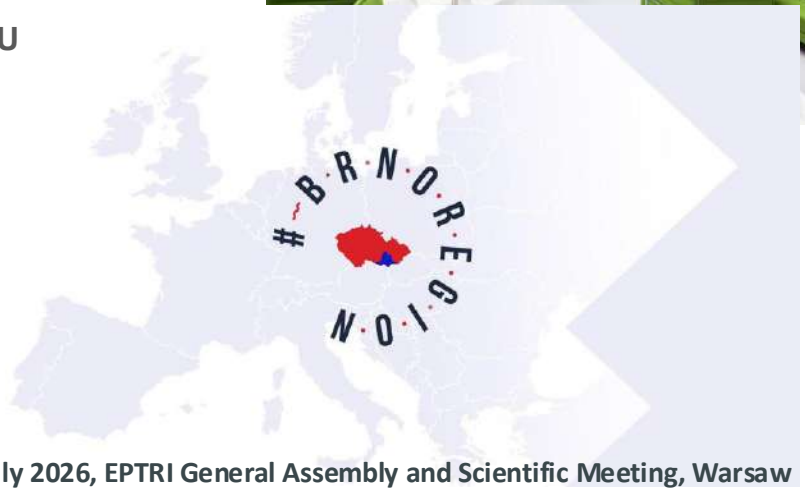
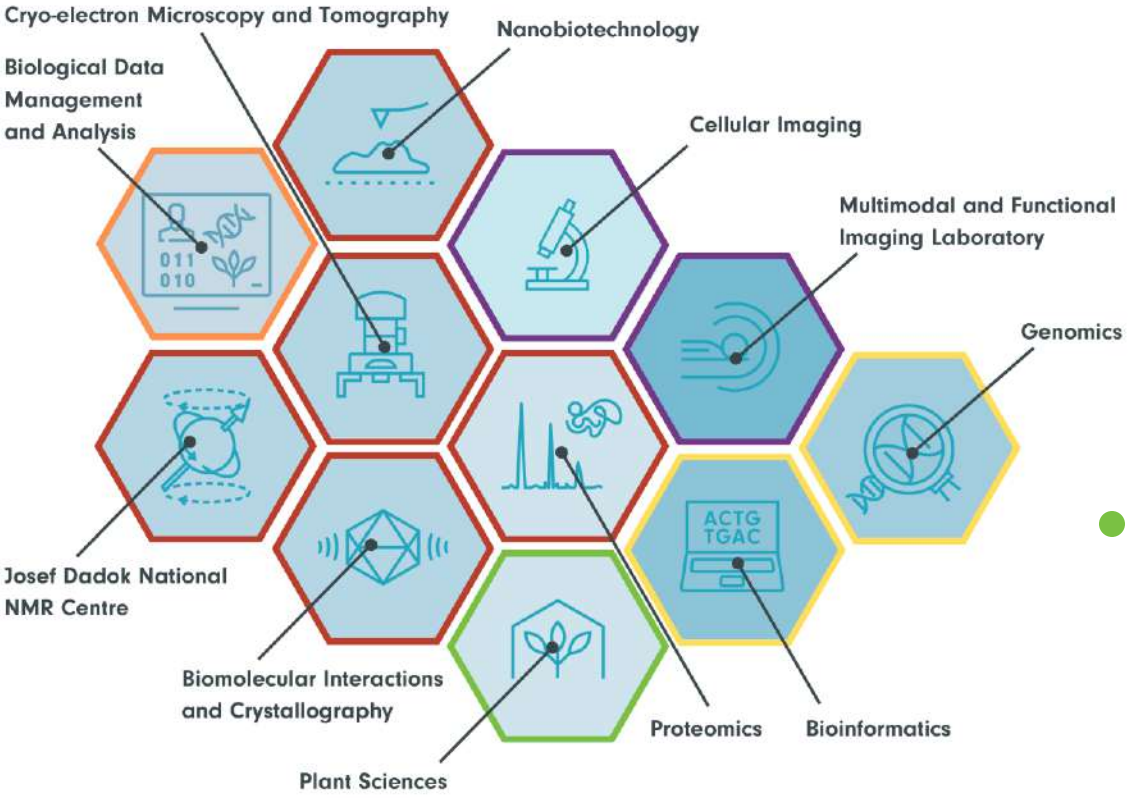
From Molecules to Disease Models: Correlative Microscopy Approaches and Integrated Structural Biology

Jan Přibyl, Radka Obořilová, Šimon Vrana, Jakub Máčala, Jakub Hruška
Core Facility NanoBiotechnology, CEITEC MU
Masaryk university, Brno, Czech Republic
E-mail: jan.pribyl@ceitec.muni.cz



28-29 July 2026, EPTRI General Assembly and Scientific Meeting, Warsaw

Core Facilities Involvement in Large Research Infrastructures

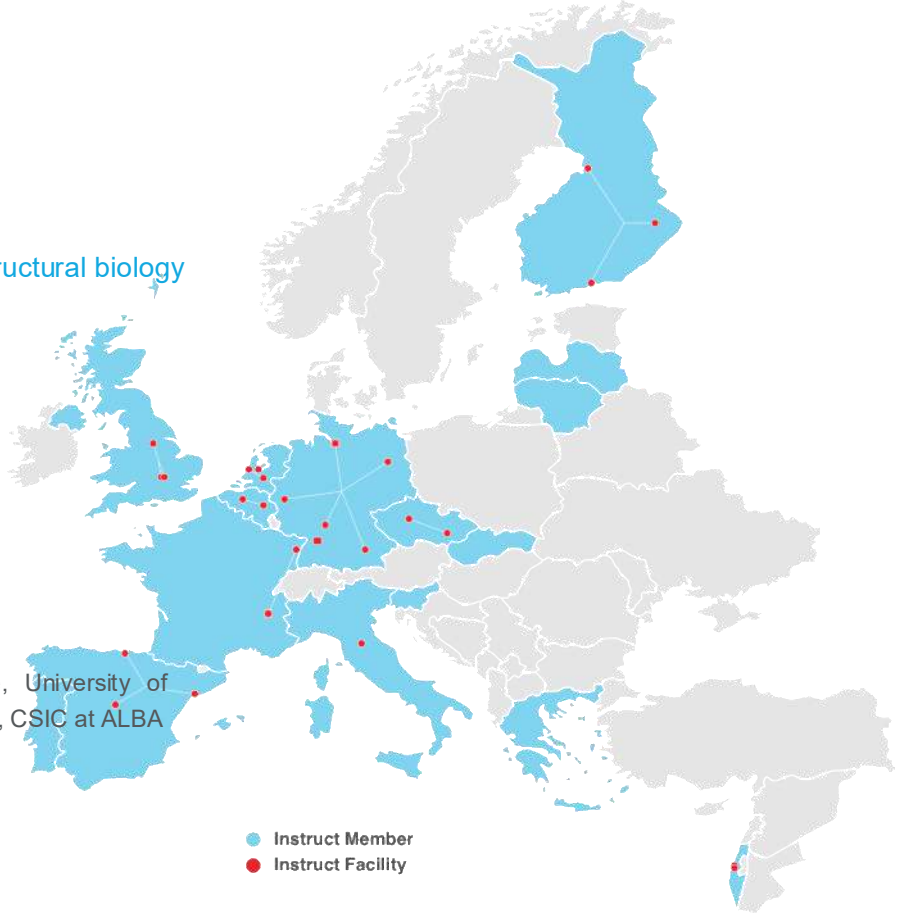


What is Instruct-ERIC? Centres

Instruct-ERIC is the single point of access to technology and expertise for structural biology research.

The Instruct consortium comprises **11 Instruct Centres** that offer access to **29 facilities** across Europe.

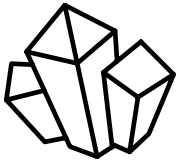
Instruct Centre BE	Nanobodies4Instruct, Robotein
Instruct Centre CZ	BIOCEV, CEITEC
Instruct Centre DE	GUF, BESSY at HZB, BNMRZ, FZJ, DESY, CSSB, European XFEL
Instruct Centre EMBL	EMBL Grenoble, EMBL Hamburg, EMBL Heidelberg
Instruct Centre ES	Instruct Image Processing Centre I2PC, CSIC-CNB, Fundacíon Biofisika, CIC-Biogune, University of Barcelona, ALBA, CSIC at ALBA
Instruct Centre FI	University of Eastern Finland, University of Helsinki, University of Oulu
Instruct Centre FR	IGBMC, IBS-ISBG
Instruct Centre IL	Centre for Bioinformatics Tel Aviv, ISPC (WIS) Israel
Instruct Centre IT	CERM
Instruct Centre NL	Bijvoet Centre, NeCEN, NKI
Instruct Centre UK	Astbury, Diamond, Harwell Campus, University of Oxford



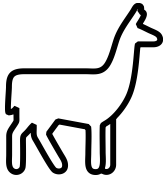
Instruct Offers a Full Range of Structural Biology Techniques

More than **95** Instruct services

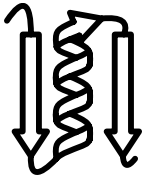
Sample preparation



Crystallisation



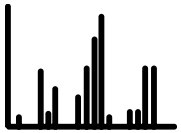
Nanobody Discovery



Protein Production

Biomolecular analysis

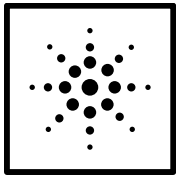
Imaging



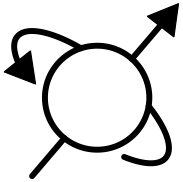
Mass Spectrometry

Molecular Biophysics

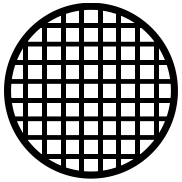
3D analysis



X-Ray Techniques



Magnetic Resonance



Electron Microscopy

Our laboratory

Core Facility Nanobiotechnology

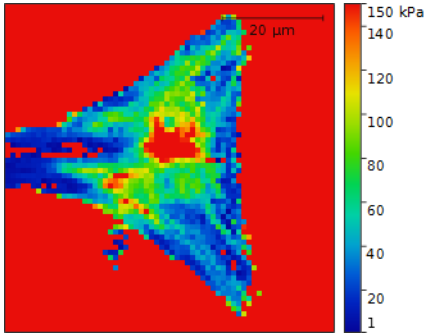
Imaging, mechanical mapping, Raman microscopy

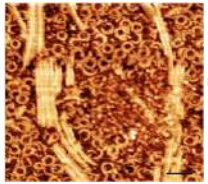
1. Cells – mechanical properties
2. Cells - imaging
3. Biomolecules - imaging
4. Nano-objects imaging
5. Raman-AFM combined microscopy
6. Raman microscopy
7. Electrochemical measurements
8. Nanodeposition system
9. SPR biosensor
10. Scanning of upconversion luminescence
11. Multielectrode array recording of cellular potential

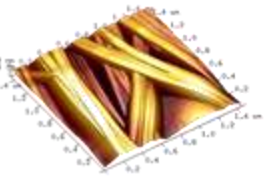
FULL SERVICE / MEASUREMENT only / DATA PROCESSING only

NANOINDENTATION

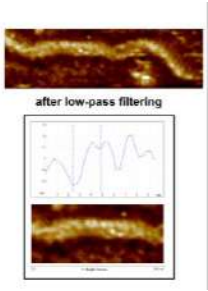
Stiffness mapping



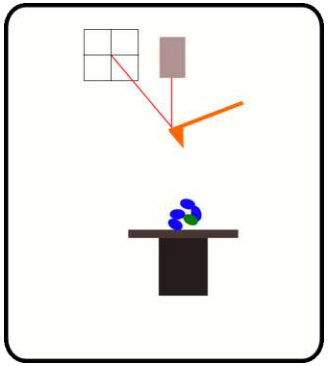




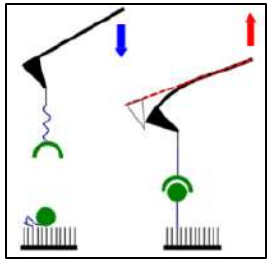
IMAGING
Proteins, DNA, Nanoobjects

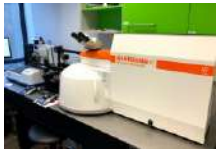


Single-molecule force spectroscope (SMFS)



AFFINITY INTERACTION





http://biomechanicalregulation-lab.org/smfs

Applications

Why this matters to a pediatric clinician

A biopsy can be tiny. The biological question is not.

PATIENT / MODEL → MEASURE → INTEGRATE → INTERPRET → TEST AN INTERVENTION

One clinical question, multiple complementary readouts

1 **Clinical question**

What differs—and does it matter?

2 **Structure**

Collagen, cells, ultrastructure

3 **Mechanics**

Stiffness, force, viscoelasticity

4 **Function**

Electrical, calcium, contractility

5 **Decision**

Mechanism, biomarker, therapy test

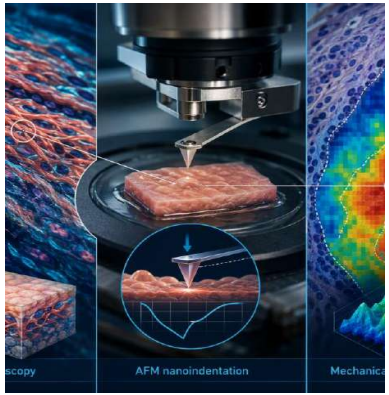
Case 1 — Relapsed congenital clubfoot

Microscopy and Microanalysis, 2023, 29, 265–272
https://doi.org/10.1093/micmic/ozac012
Advance access publication 20 December 2022
Original Article

Microscopy AND Microanalysis

Microstructural Analysis of Collagenous Structures in Relapsed Clubfoot Tissue

David Vondrášek[1,2,*,a], Daniel Hadraba[1,2,3,a], Jan Přibyl[4], Adam Eckhardt[2], Martin Oštádal[5], František Lopot[1,3], Karel Jelen[1], Martina Doubková[2,6], Jarmila Knitlová[2], Tomáš Novotný[7,8], and Jiří Janáček[2]



Medial vs lateral tissue

+34% — **Young's modulus**
stiffer medial tissue

+22% — **SHG collagen signal**
collagen-rich matrix

15% — **adipose area**
plus stronger collagen crimp

Clinical interpretation Altered extracellular-matrix tension may help sustain relapse—and provides a measurable mechanistic endpoint.

Microscopy and Microanalysis, 2023, **29**, 265–272
https://doi.org/10.1093/micmic/ozac012
Advance access publication 20 December 2022
Original Article

Microscopy AND Microanalysis

Microstructural Analysis of Collagenous Structures in Relapsed Clubfoot Tissue

Fiber orientation by polarized microscopy

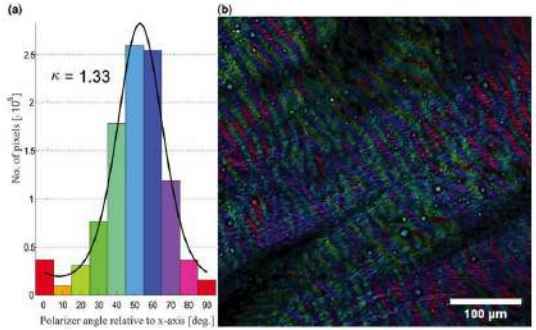


Fig. 7. (**a**) Distribution of individual polarization angles within a representative sample image. (**b**) Polarization image of the medial side with HSV color-code (each color-angle pair represents the same color-angle pair in (**a**).

Stiffness mapping by AFM

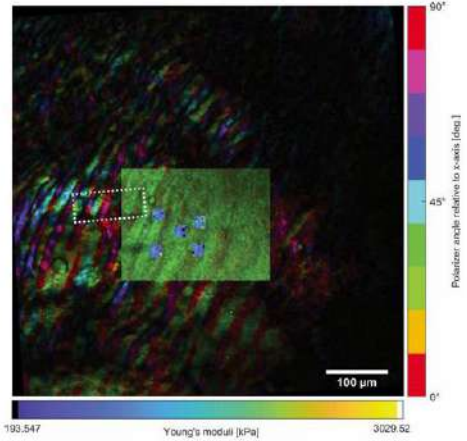


Fig. 2. Co-registration of Young's moduli and optical images.

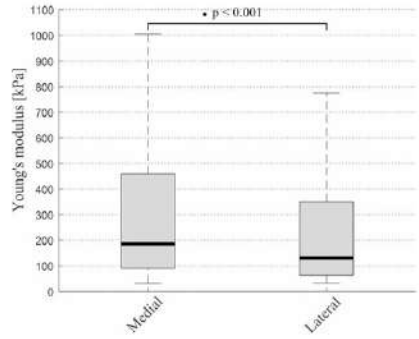
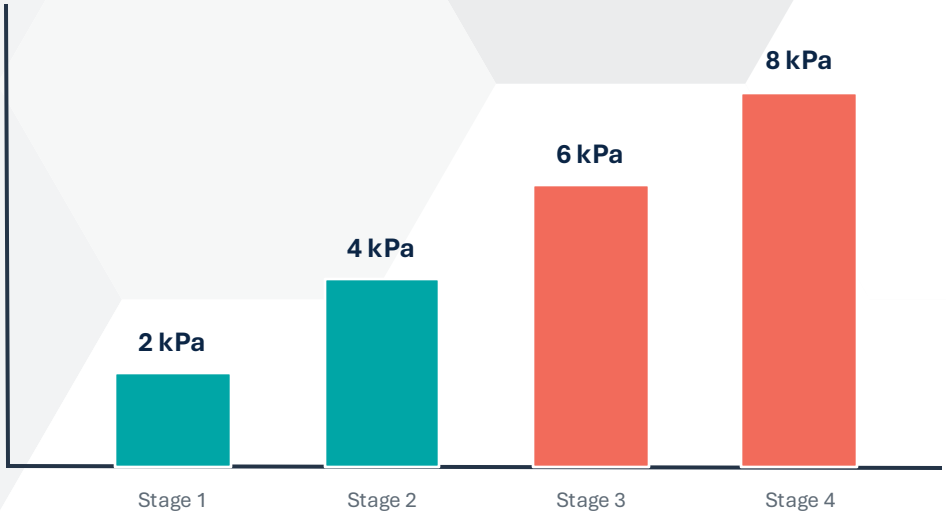


Fig. 4. Mechanical properties of medial and lateral relapsed clubfoot tissue. The Young's moduli represent the elastic properties of the medial and lateral side of the relapsed clubfoot tissue.

Case 2 — Fibrosis becomes measurable heterogeneity

Collagen-rich septa stiffen stepwise across fibrosis stages



Local stiffness ≠ one average value

What clinicians gain

- Spatially resolved disease burden
- A bridge between histology and mechanics
- Candidate endpoints for progression or response

jove

Measurement of Liver Stiffness Using Atomic Force Microscopy Coupled with Polarization Microscopy

Srikant Ojha[1], Jan Pribyl[2], Simon Klimovic[2,3], Daniel Hadraba[4], Marketa Jirouskova[1], Martin Gregor[1]

[1] Laboratory of Integrative Biology, Institute of Molecular Genetics of the Czech Academy of Sciences [2] CEITEC MU, Masaryk University [3] Department of Biochemistry, Faculty of Science, Masaryk University [4] Laboratory of Biomathematics, Czech-BioImaging IPHYS, Institute of Physiology of the Czech Academy of Sciences

eLife

RESEARCH ARTICLE

Dynamics of compartment-specific proteomic landscapes of hepatotoxic and cholestatic models of liver fibrosis

Marketa Jirouskova[1]*, Karel Harant[2], Pavel Cejnar[3], Srikant Ojha[1,4], Katerina Korelova[1], Lenka Sarnova[1], Eva Sticova[5,6], Christoph H Mayr[7], Herbert B Schiller[7,8], Martin Gregor[1]*

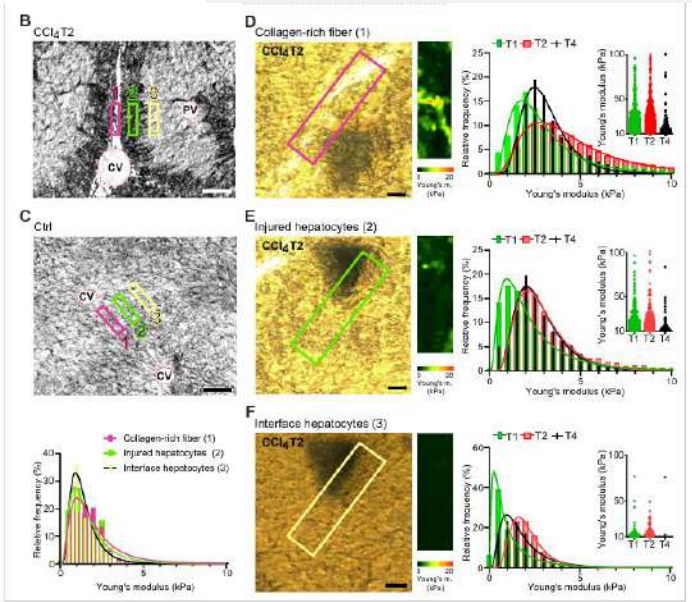


Figure 7. Atomic force microscopy (AFM) stiffness mapping reveals changes in the local mechanics of liver tissue upon hepatotoxic injury. (**A**) The line plot shows the dynamics of 32 myocardin-related transcription factor (MRTF) targets (highlighted in black) significantly enriched in extracellular matrix (ECM)-enriched cluster I of carbon tetrachloride (CCl_4) Total proteome (**Figure 2C**) identified by Fisher's exact test (p=0.01; Benjamini-Hochberg false discovery rate = 3%). (**B, C**) Representative polarized microscopy images of CCl_4-treated (T2, B) and untreated control (Ctrl, C) liver sections with

Figure 7 continued on next page

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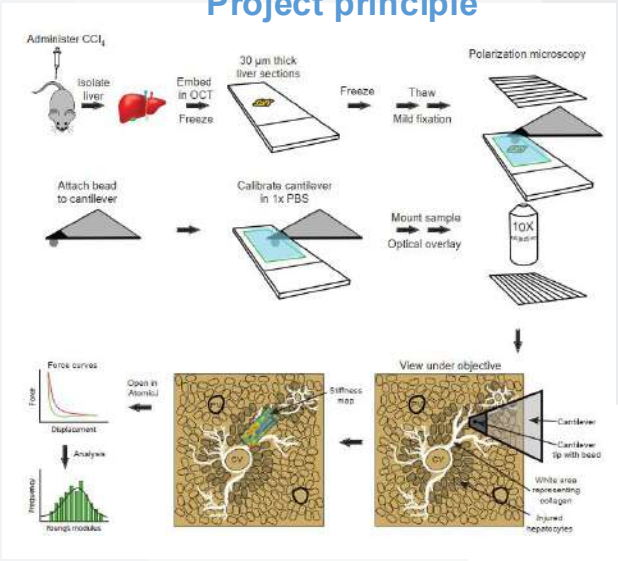
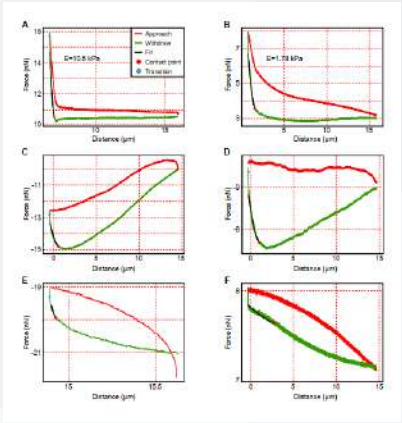
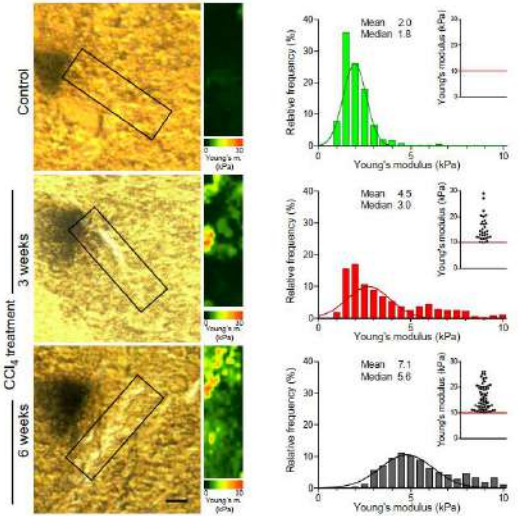
Project principle

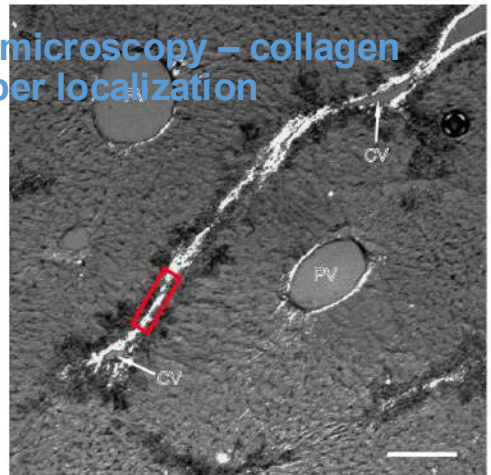




Time lapsed mechanical properties



Polarized microscopy – collagen fiber localization



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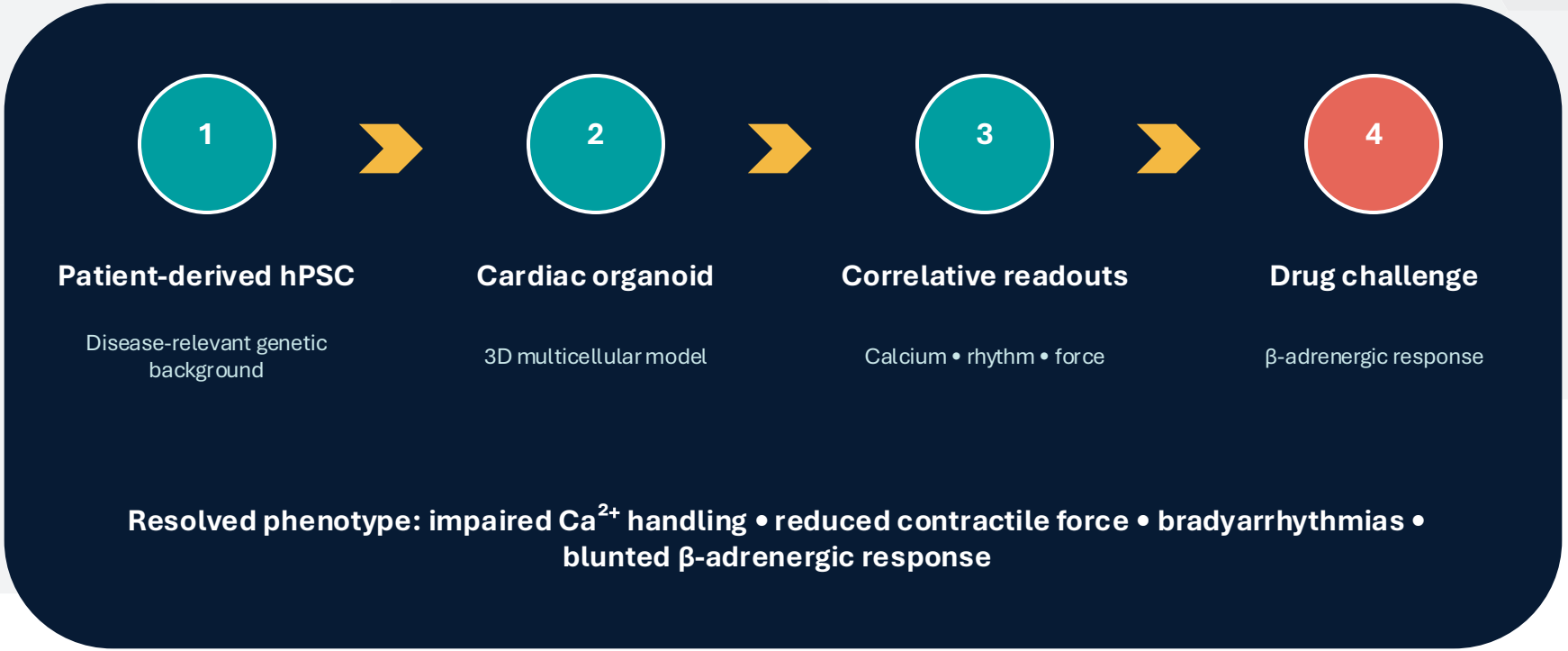
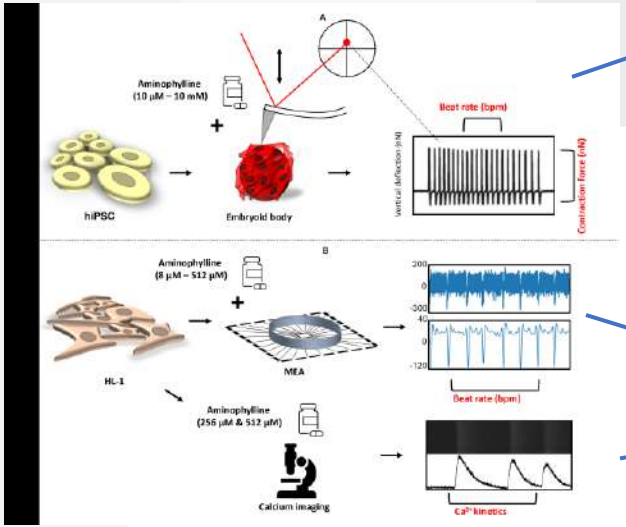
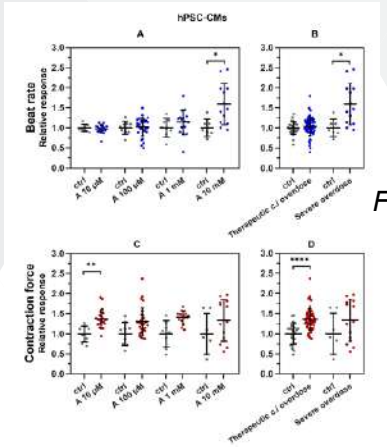
Case 3 — Cardiac organoids connect genotype to function

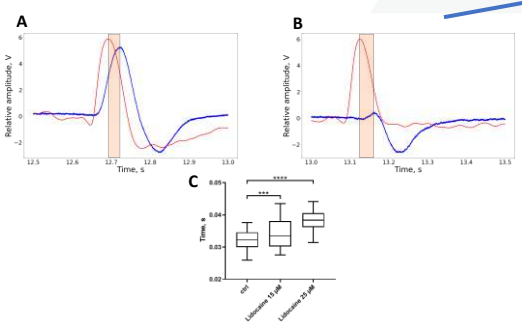


Complex characterization of cardiomyocytes



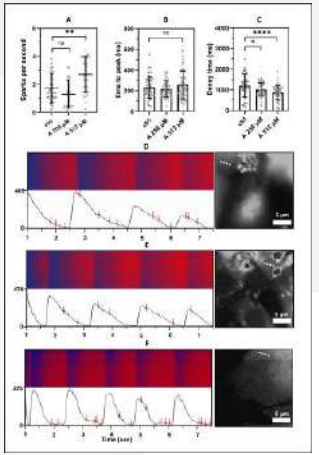


AFM
Force and BR changes



AFM-MEA
Mechano-electrical feedback

Fluorescence
– Calcium sparklings



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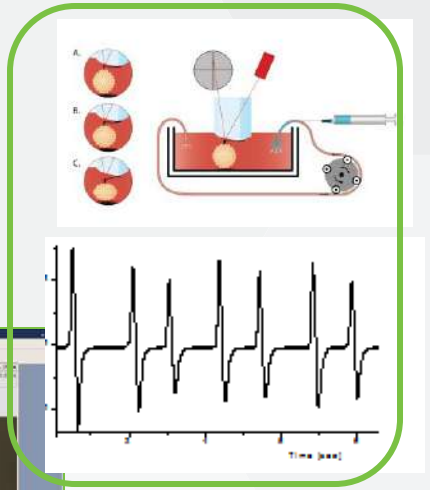
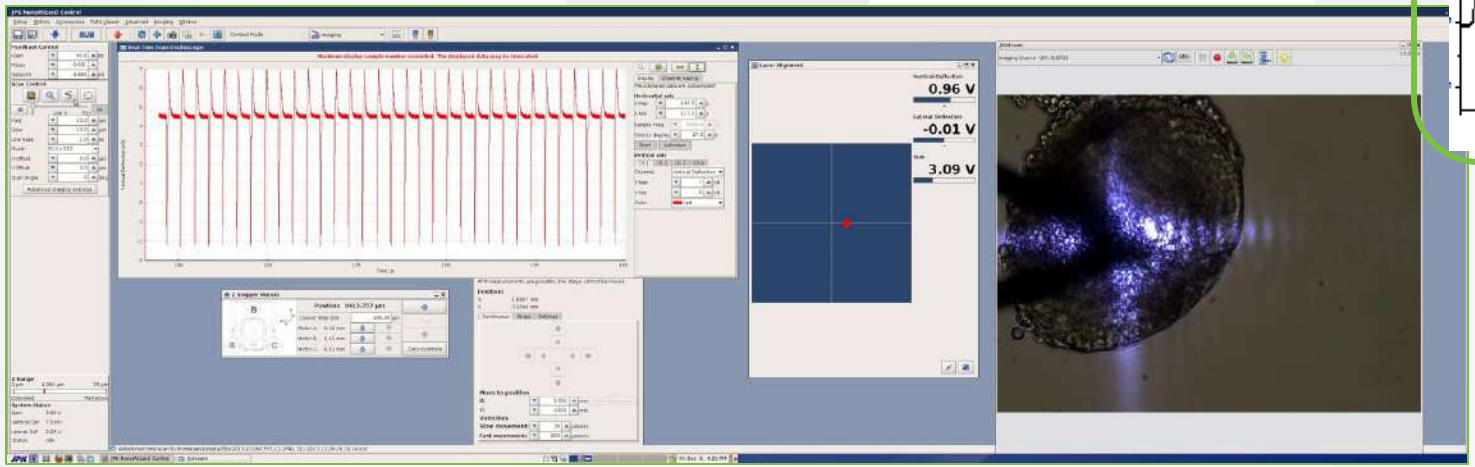
Other applications of AFM-based biomechanics

Setup scheme



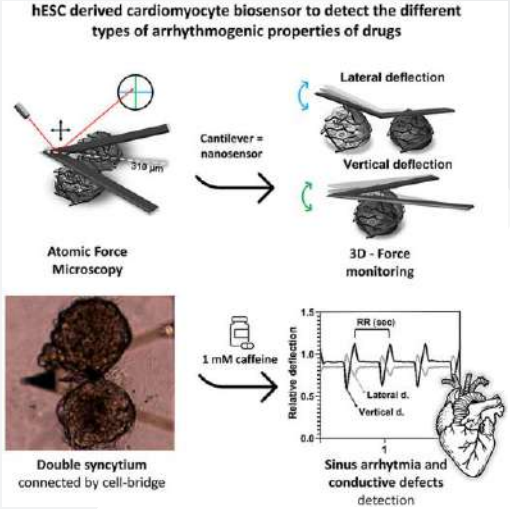
Cardiac cells biomechanics

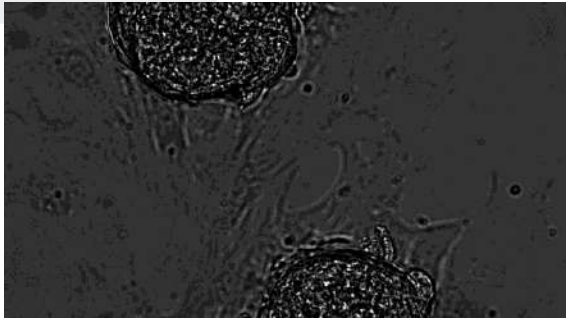


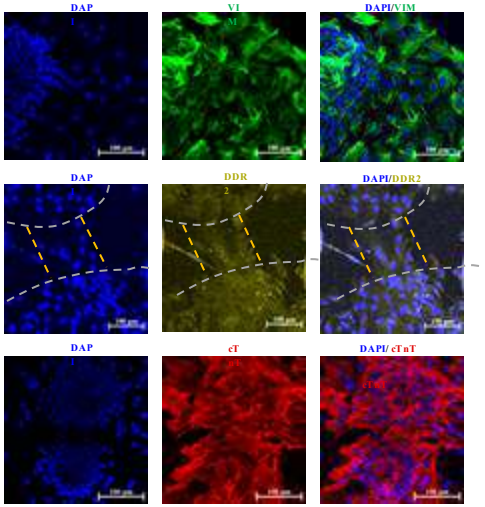
Pesl M, Pribyl J, et al. 2016 *Biosensors and Bioelectronics* **85** 751–7
Pesl M, Pribyl J, et al. 2016 J Mol Recognit n/a-n/a
Pesl M, Acimovic I, Pribyl J, et al. 2014 Heart Vessels 29 834–46

Arrhythmia screening on double-organoid system

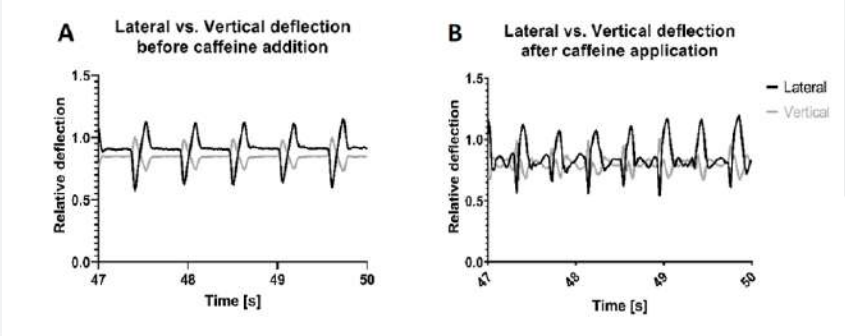
Principle – double organoid synchronization measured by AFM







Immediate action of caffeine on de-synchronization



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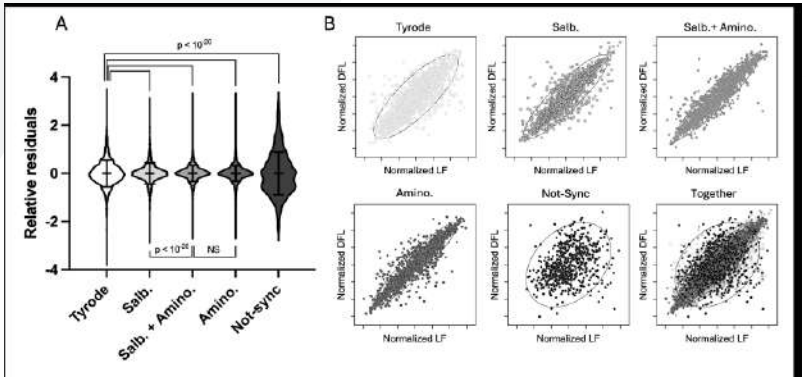
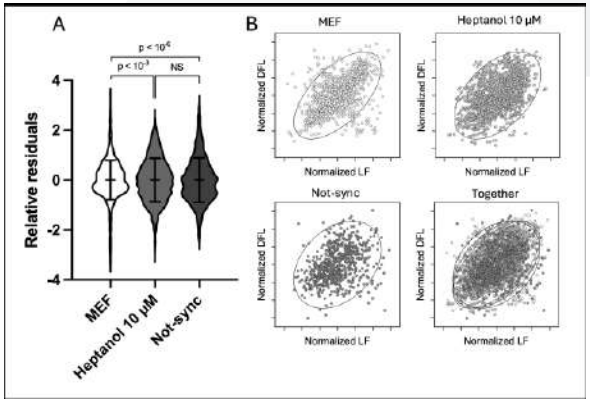
Stochastic (random) fluctuations in unsynchronized clusters





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Design: sample availability, not the instrumentation, is the limit

Start with the decision the study must support.

Question	Diagnosis, mechanism, stratification or treatment response?
Material	Biopsy, section, cells, organoid, biofluid or purified target?
Constraints	Amount, viability, fixation, biosafety, timeline, metadata?
Primary endpoint	One pre-specified readout that answers the question.
Correlative layer	Add only modalities that reduce uncertainty.

Bring us the question; we assemble the workflow

Molecular cause	Genomics • proteomics • NMR • interactions
Cell / tissue phenotype	Imaging • BioAFM • Raman • multimodal imaging
Ultrastructure	Cryo-EM / tomography • X-ray methods
Functional consequence	MEA • calcium • contractility • biomechanics
Integrated evidence	Bioinformatics • biological data management

Full service • Measurement only • Data processing only • Collaborative method development

RESEARCH INFRASTRUCTURE

Instruct-ERIC turns local expertise into European access

instruct ERIC

1 Submit one scientific proposal

Select one or several complementary services.

2 Expert review and workflow matching

Facilities and specialists help refine feasibility.

3 Access, training and pilot support

Eligible projects may receive instrument, consumable and travel contributions.

Open to researchers worldwide • funded access depends on member-country eligibility

CEITEC

CIISB
Czech Infrastructure for Integrative Structural Biology

instruct Integrating Biology

(LM2023042)

EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research, Development and Education

MINISTRY OF EDUCATION, YOUTH AND SPORTS

OP VVV CZ.02.1.01/0.0/0.0/18_046/0015974

CarDia Project reg. number: LX22NPO5104

MUNI Grant Agency

JAROSLAV KOČA BRIDGE FUND

Thank you for your attention!

BRNO 2027

The 12th AFM BioMed CONFERENCE will be held in BRNO, Czech Republic, April, 2027

Dr. Martin Pešl

Masaryk University, Brno

Dr. Jan Přibyl

CEITEC Masaryk University, Brno

are the organizers of the conference.

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April 19-21 2027

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