INSTYTUT FIZYKI JĄDROWEJ
IM. HENRYKA NIEWODNICZAŃSKIEGO
POLSKIEJ AKADEMII NAUK

Novel methods for super-resolution imaging (based on nanomechanical properties) of liver sinusoidal endothelial cells

Krakow, Poland, 16 March 2023

dr Bartłomiej Zapotoczny

Department of Biophysical Microstructures, Institute of Nuclear Physics PAN

Liver Sinusoidal Endothelial Cells – LSEC

1. **Motivation of the research**
2. **Introduction to LSECs and Atomic Force Microscopy (AFM)**
3. **Fenestrations in live LSECs – fenestrae-associated cytoskeletal structures**
4. **Fenestrations in live LSECs – „*in vitro mechano*pharmacology on a single cell using AFM”**
5. **Morphomechanical studies of LSEC**

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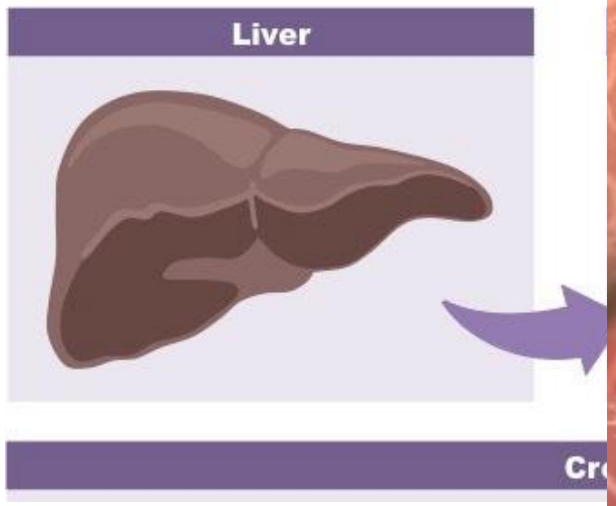
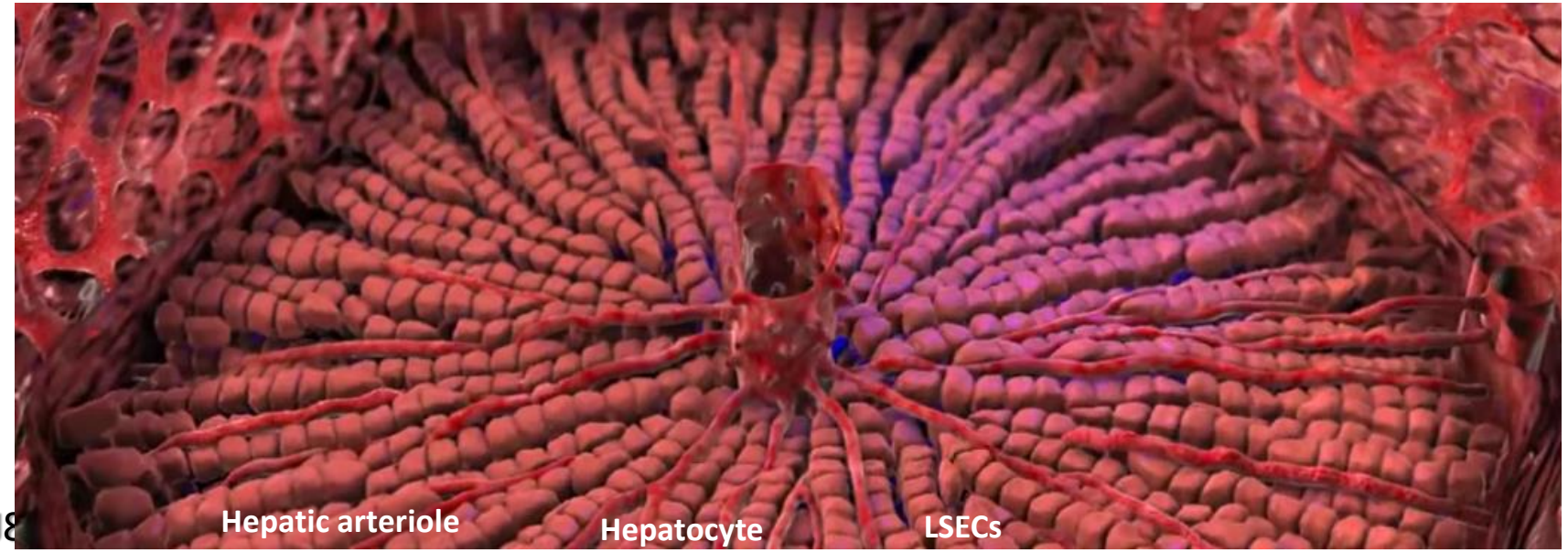
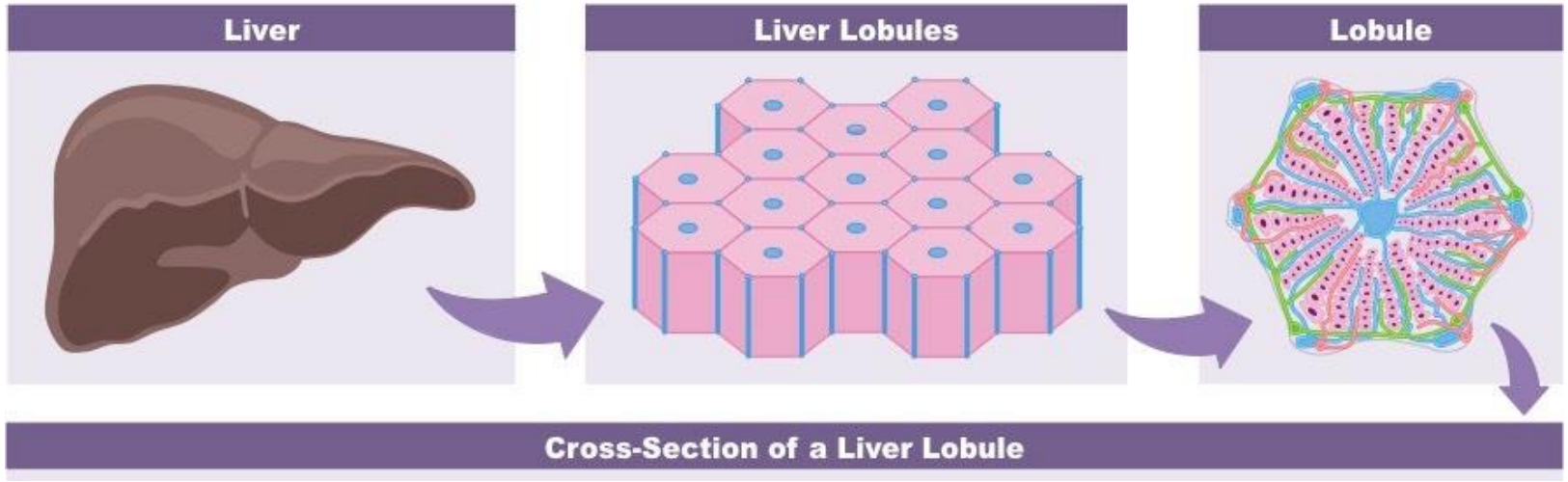
Motivation of the research

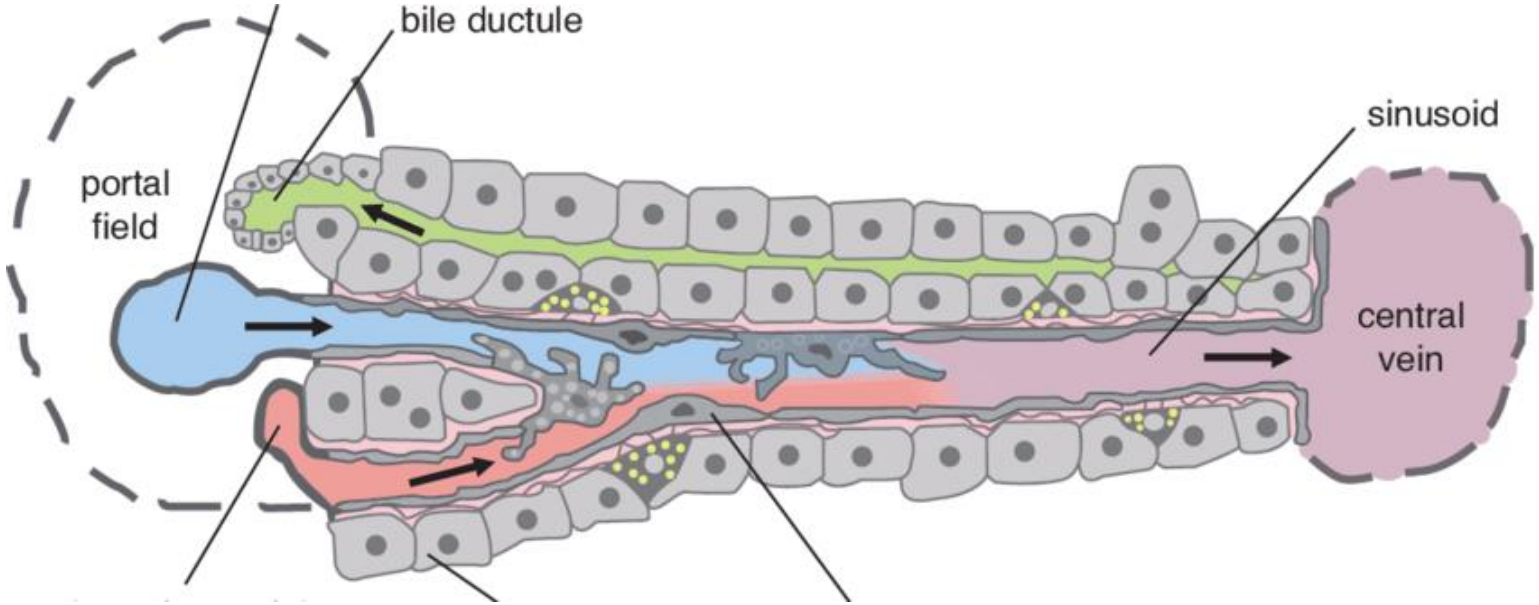




https://www.youtube.com/watch?v=fiJ8

Motivation of the research



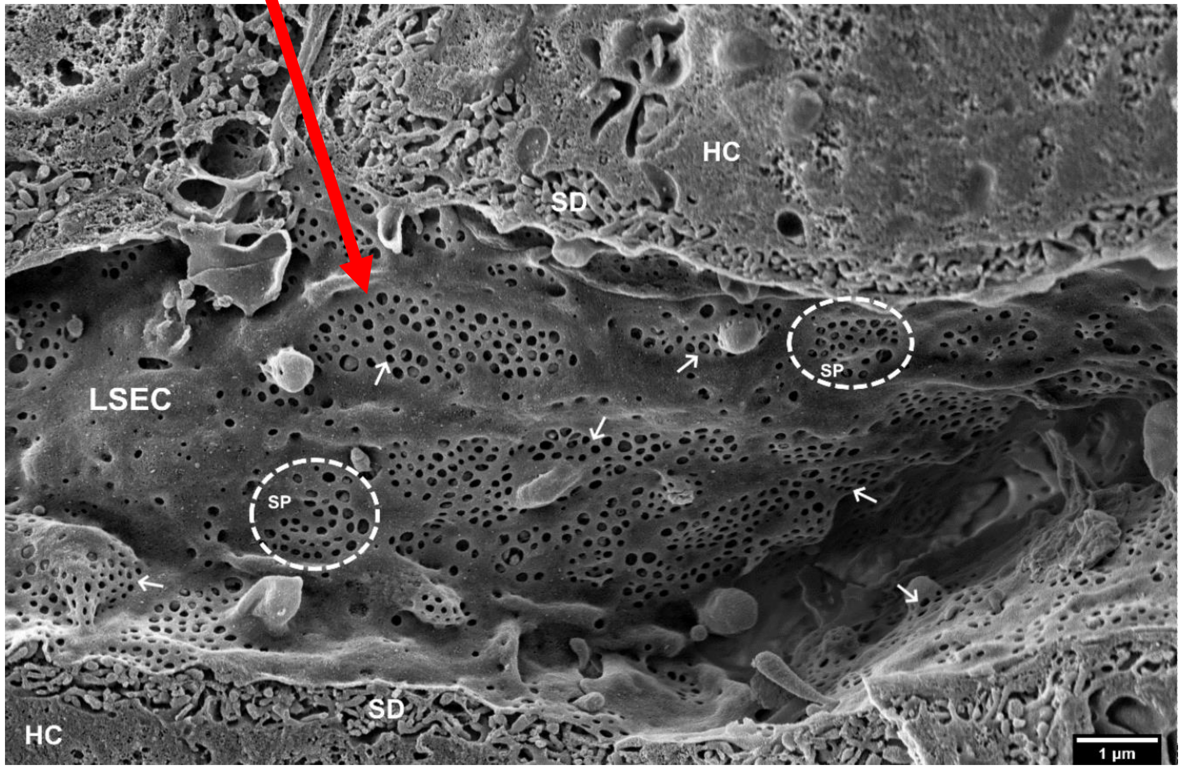


https://www.youtube.com/watch?v=fiJ80fBQQW4

Motivation of the research

Liver Sinusoidal Endothelial Cells – LSECs

Constitutes a physical and functional barrier between the vascular system and the hepatocytes (HC) via space of Disse (SD).
The transport is facilized through **fenestrations** – transcellular pores grouped in sieve plates (SP)



Szafranska K., [...], **Zapotoczny B.**, Frontiers in Physiology, 2021
Wisse E., et al., Hepatology, 1986

7 µm
typical diameter
of a single red blood cell !

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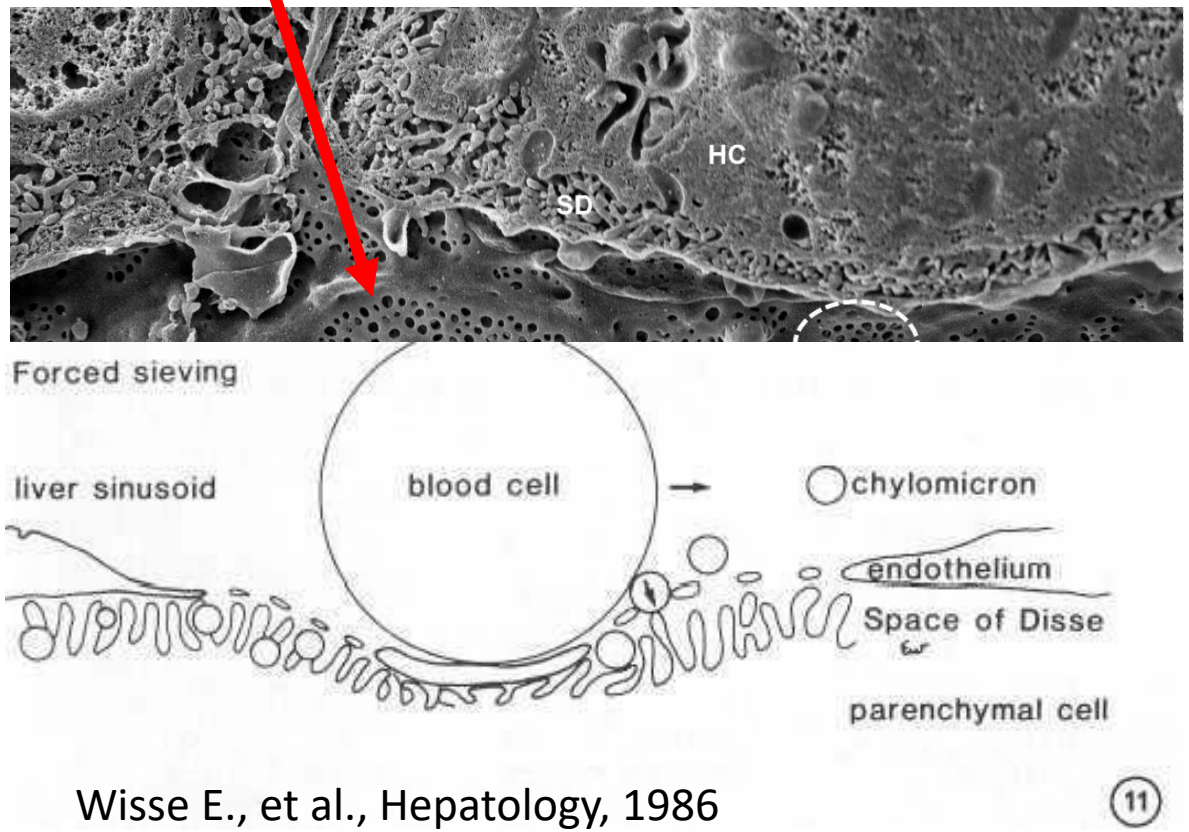

Wisse E., et al., Hepatology, 1986

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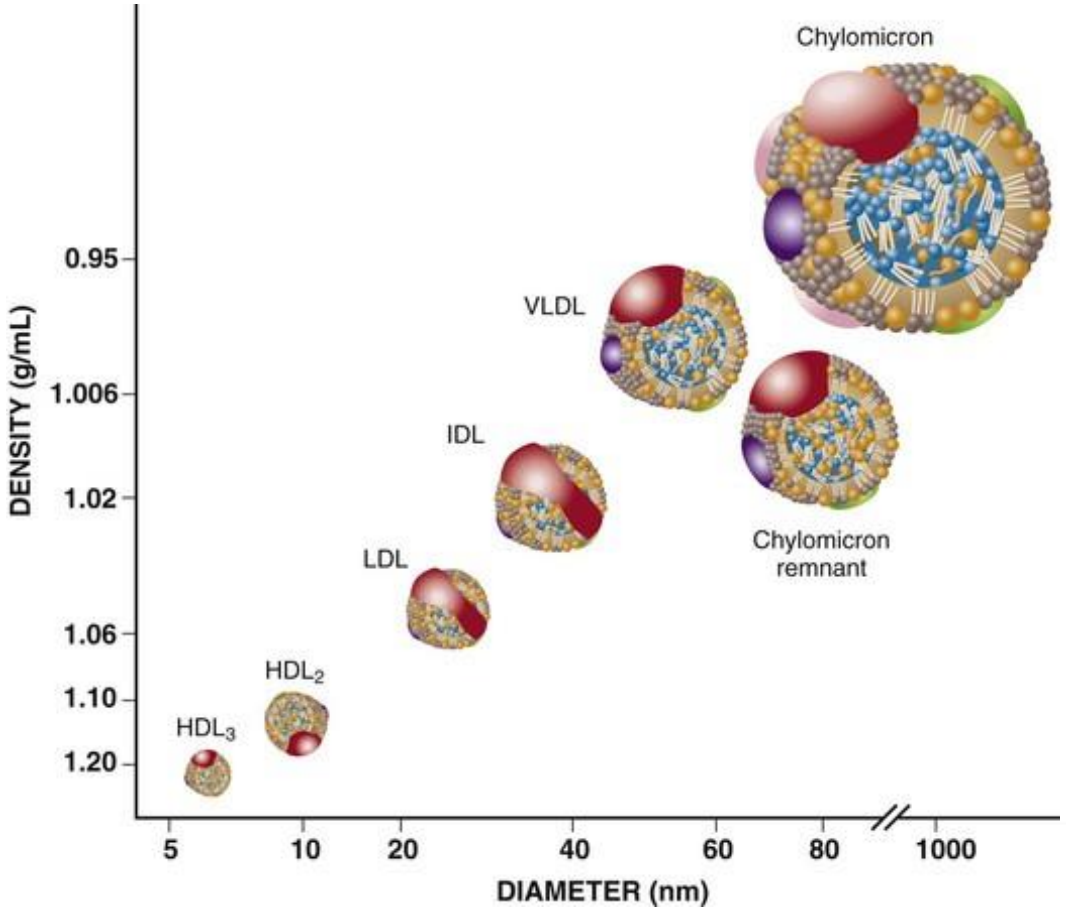
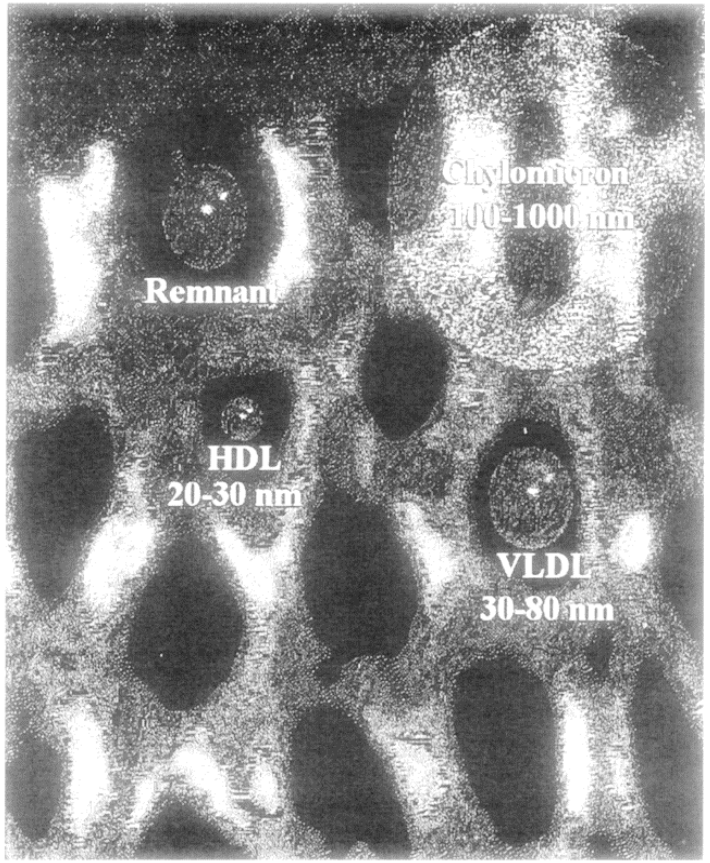
Motivation of the research

Lipoproteins in blood plasma





Blood plasma

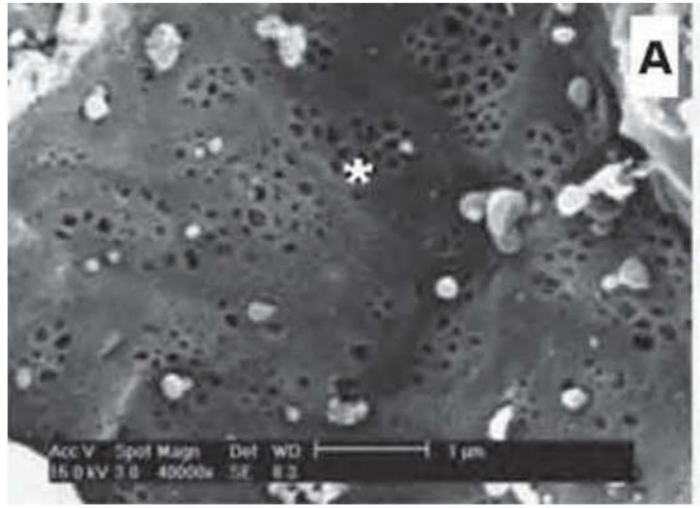
after heavy meal

fasted patient

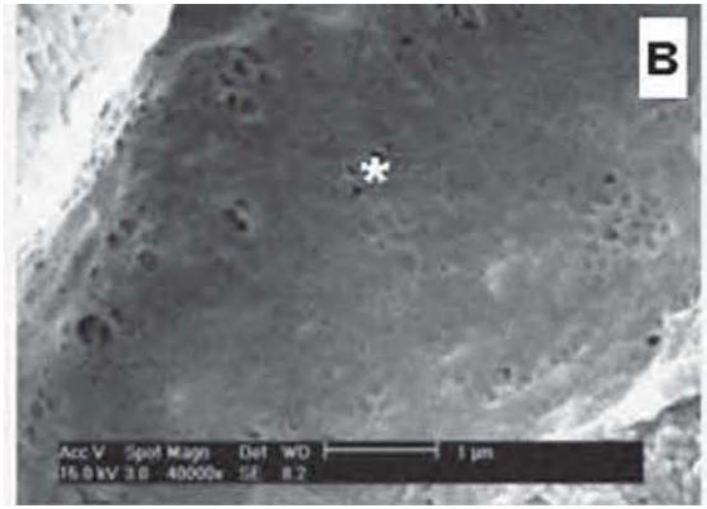
Fraser R. et al., Hepatology 1995

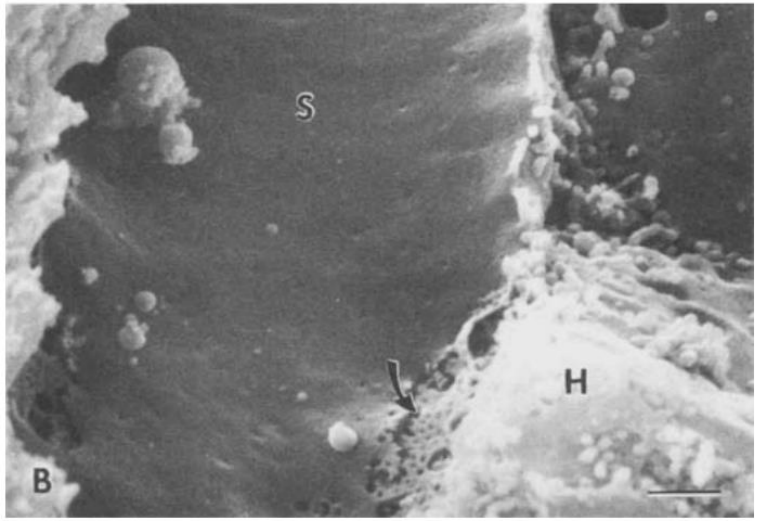
Motivation of the research

young mouse

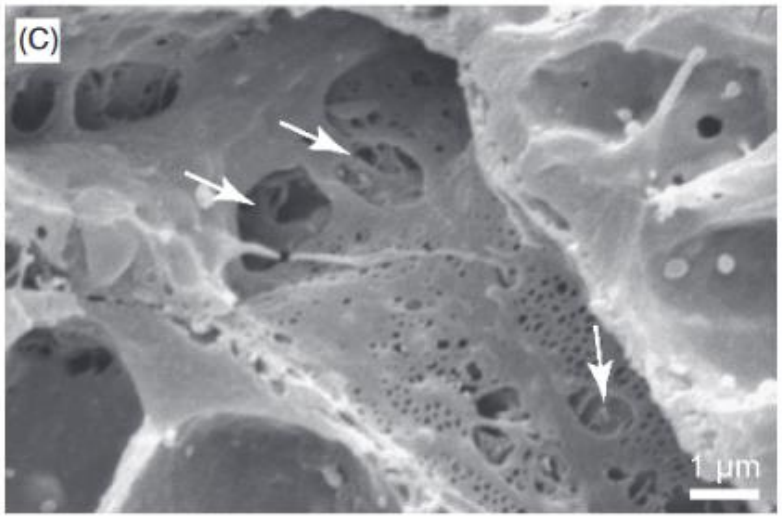


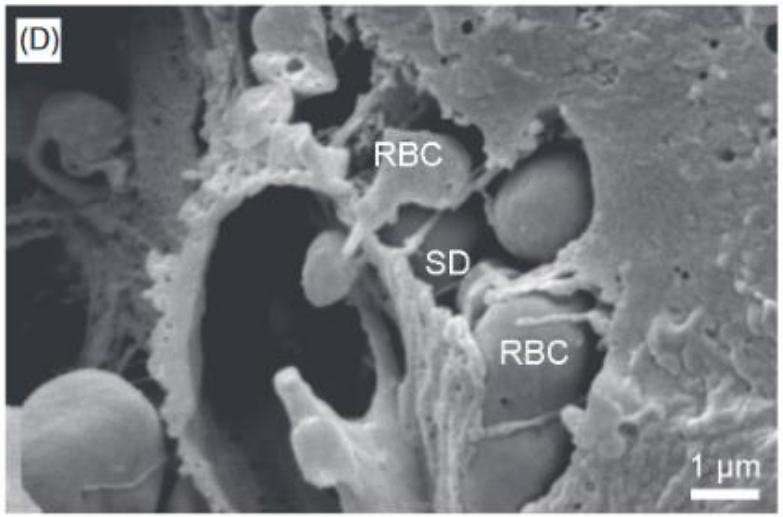
old mouse





Alcohol abuse





Paracetamol overdose

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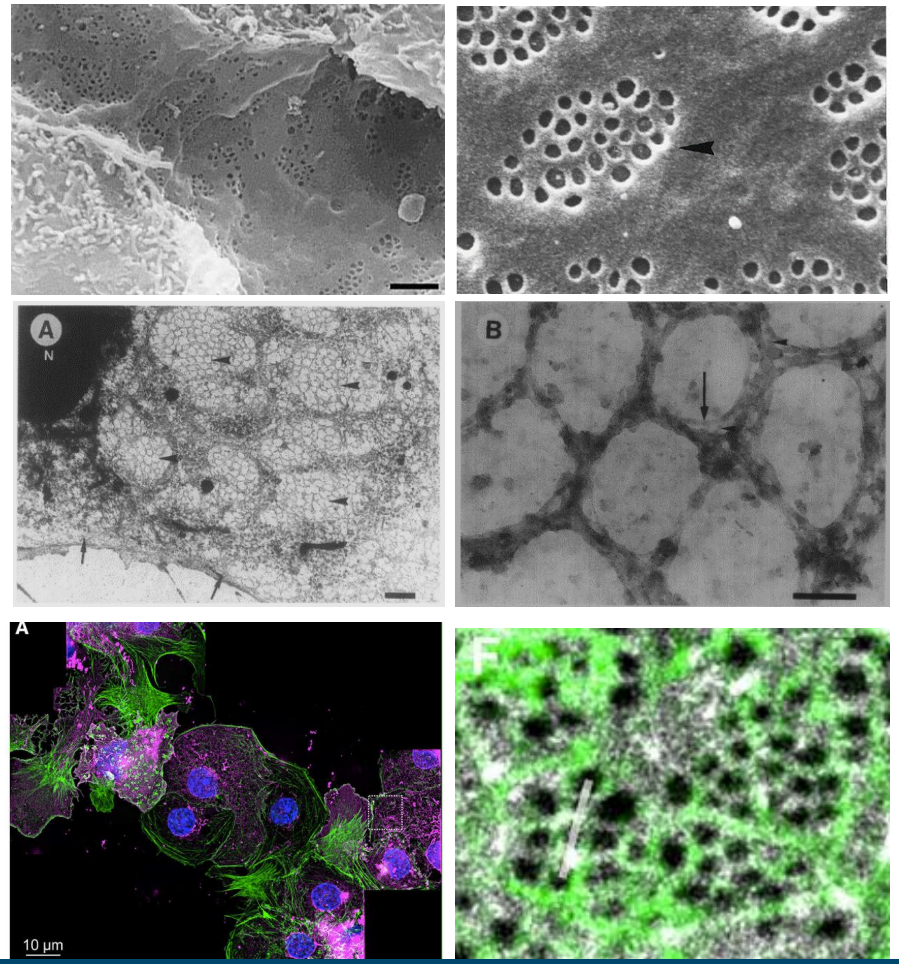
Atomic Force Microscopy

Imaging

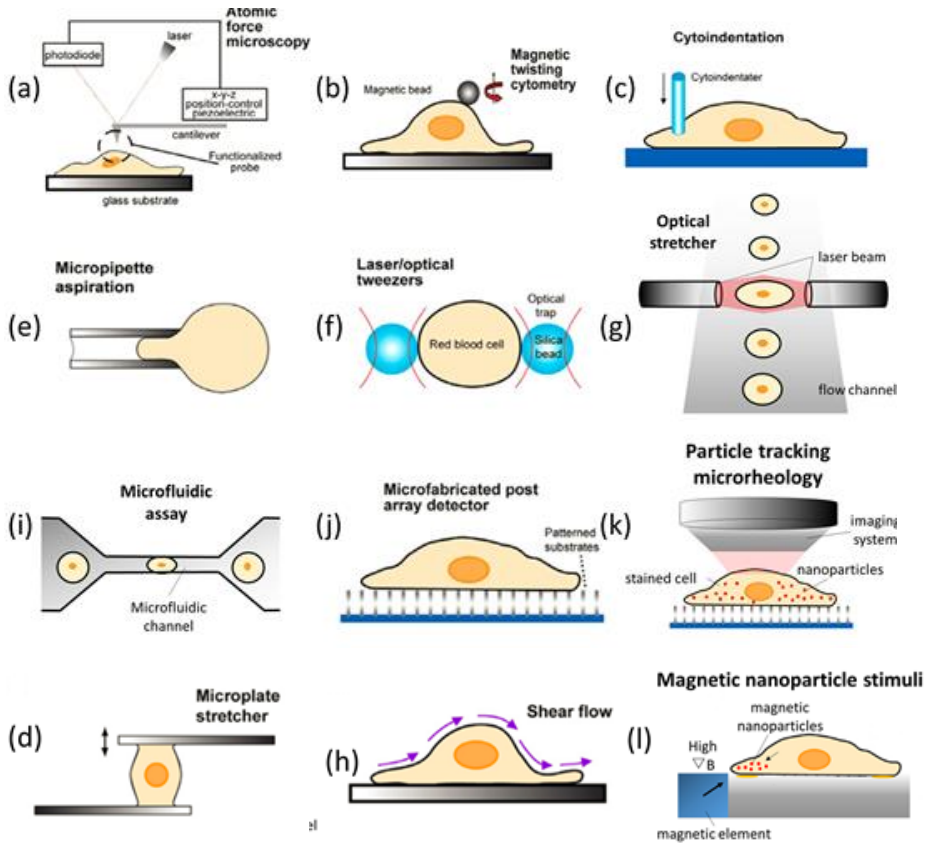
SEM

TEM

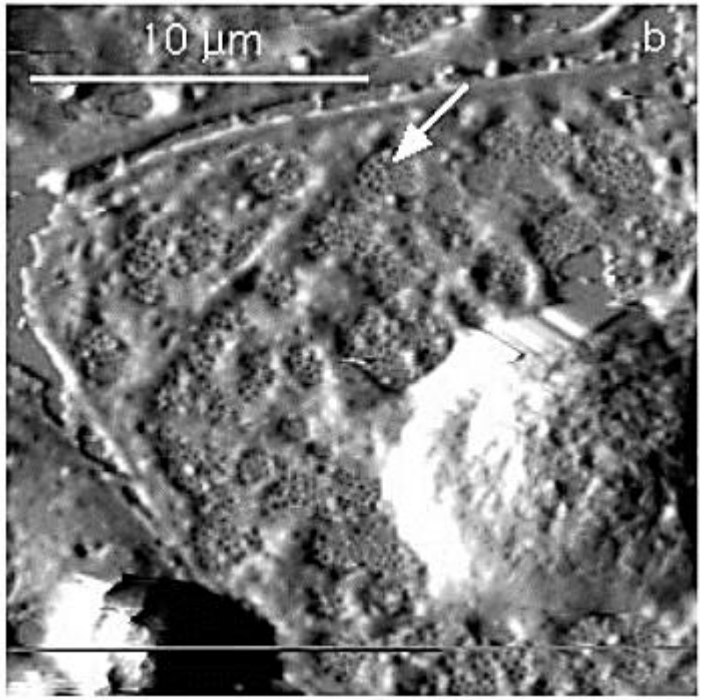
3D-SIM



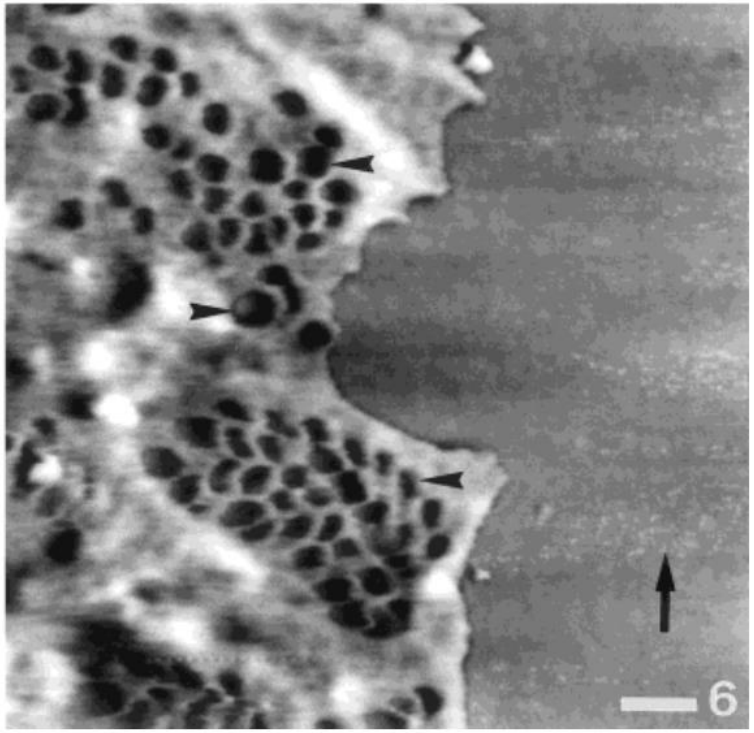
Nanomechanical properties



AFM and LSEC – literature overview

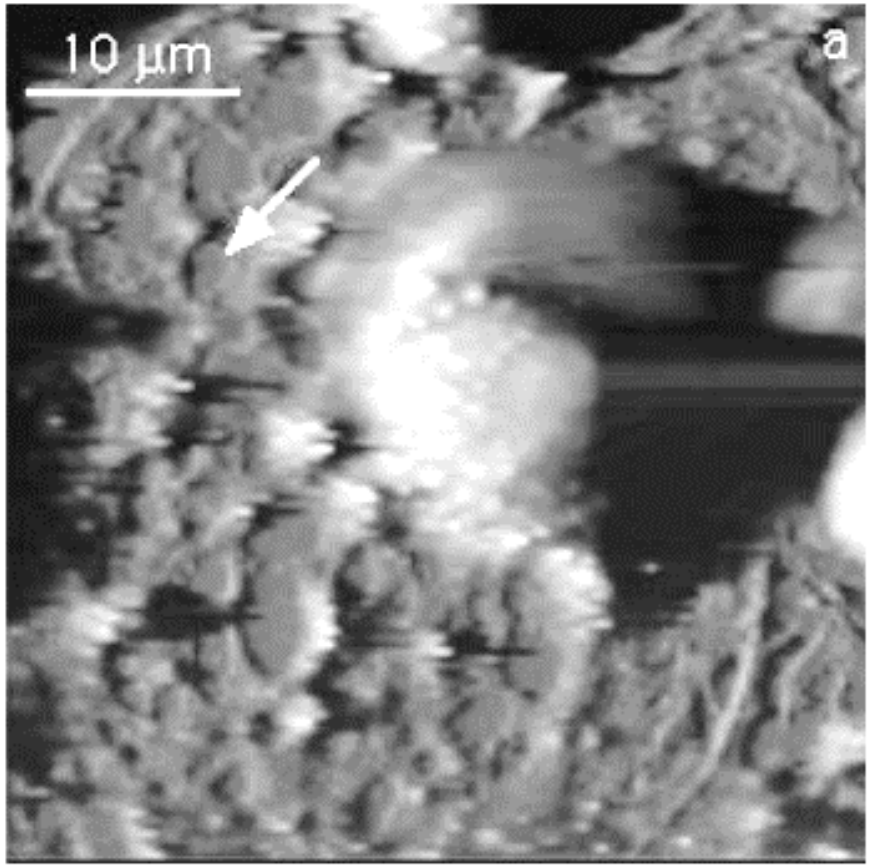


Chemically fixed cells with aldehydes



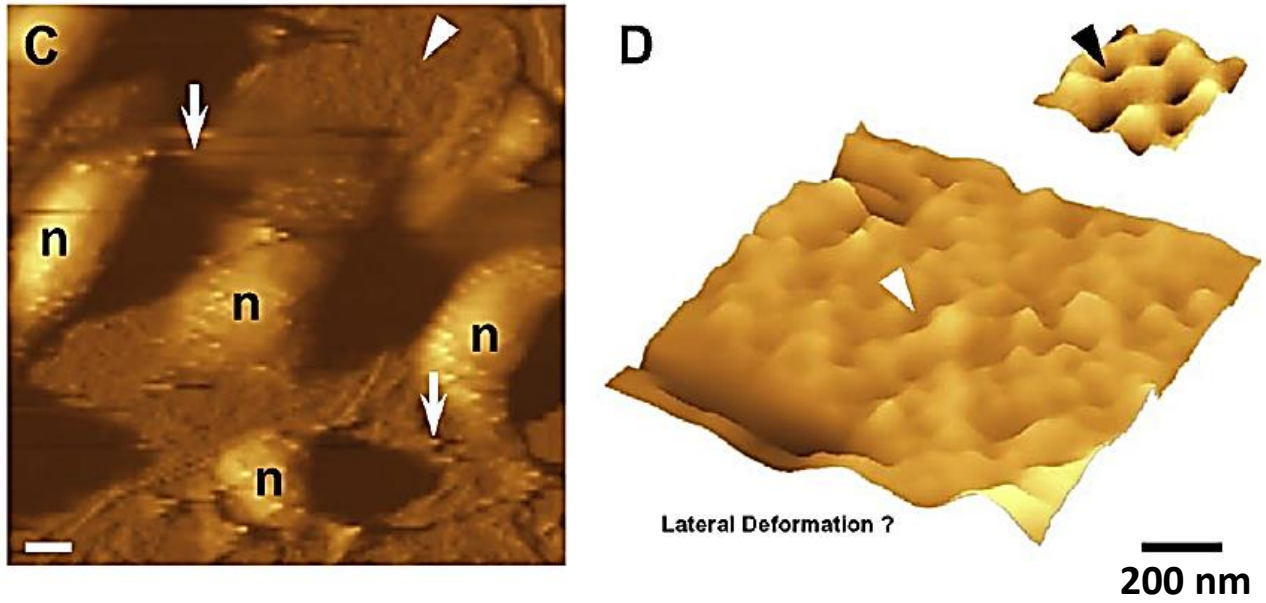
Braet et al., 1996 Int. J. Im. Sys. Techn.
Braet et al., 1998 Applied Physics A

Living cells



AFM and LSEC – literature overview

Contact mode AFM
Living LSEC



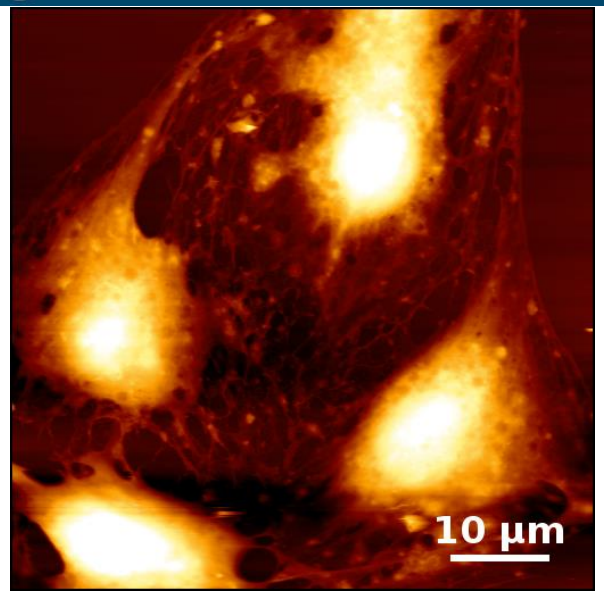
„Conversely, we might still have to wait for future AFM improvements. Near ‘forceless’ scanning could be the answer to the existing challenge herein”.

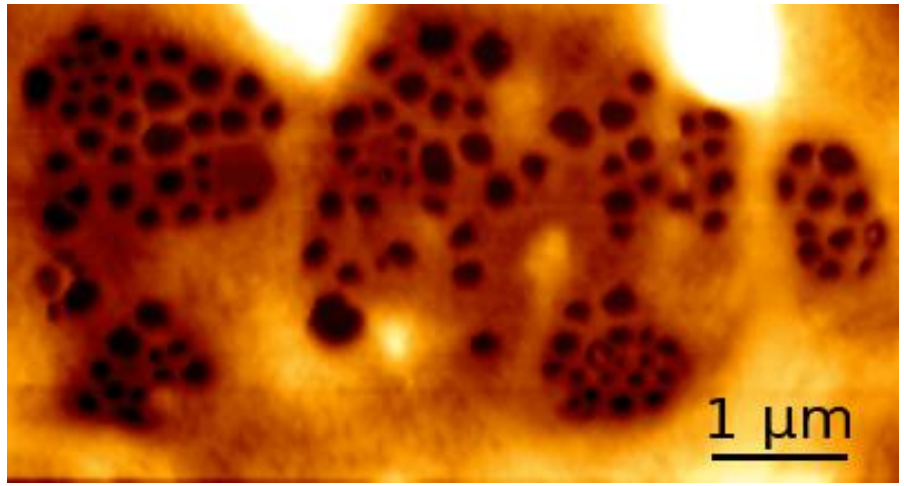
„Especially, the unique softness of LSECs hampered our initial approaches to successfully record their fenestrae in vitro. We have to wait until AFM cantilevers with softer spring constants become available to disclose their ‘presence’ and dynamics in the living state”.

F. Braet, E. Wisse, *AFM imaging of fenestrated liver sinusoidal endothelial cells*, Micron 43 (2012) 1252–1258.

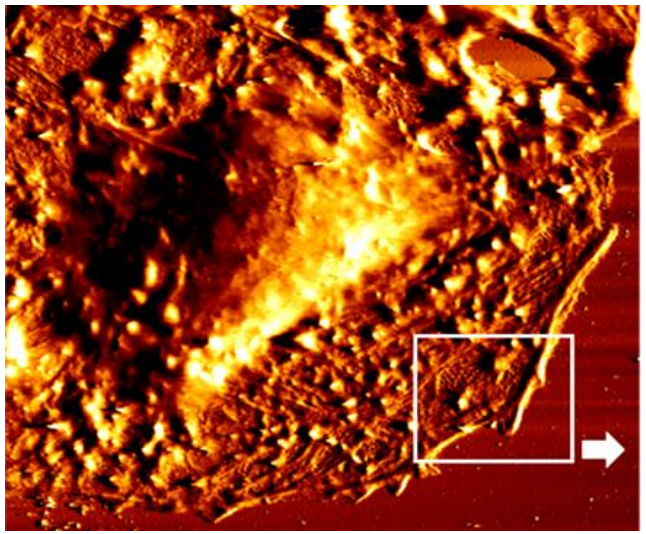
New „approach” to contact mode AFM

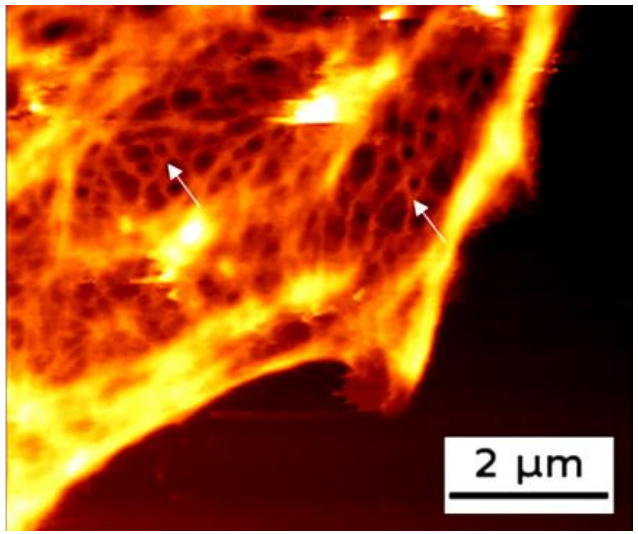
1% glutaraldehyde





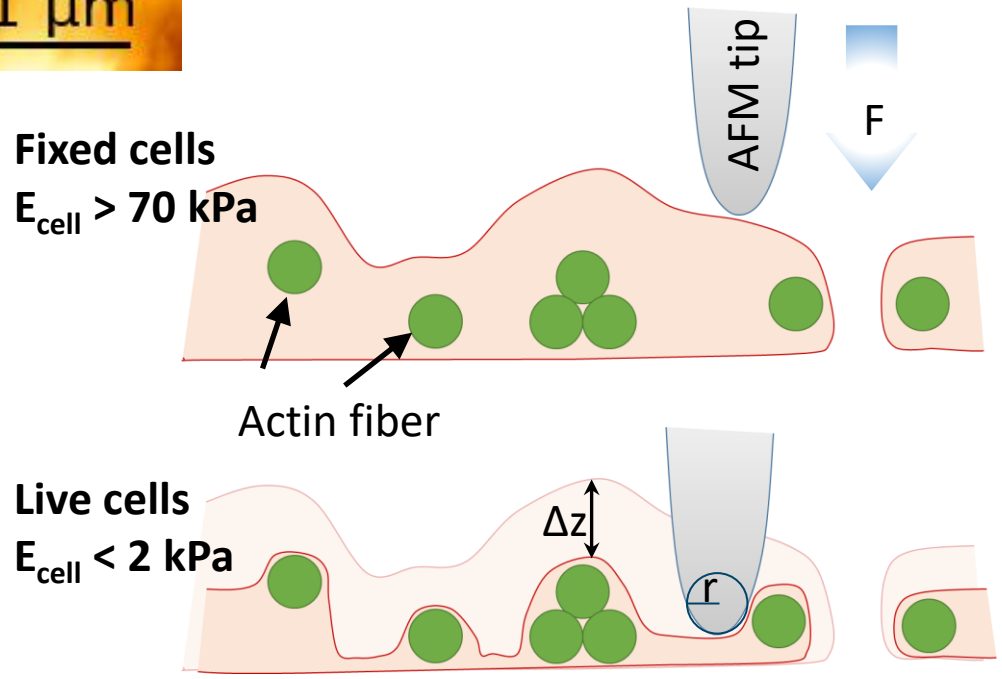
Live cells





Hertz model of contact mechanics

$$F(\Delta z) = \frac{4}{3} \cdot \frac{E_{cell}}{1-\mu_{cell}^2} \cdot \sqrt{r} \cdot \Delta z^{\frac{3}{2}}$$



Zapotoczny B. et al., 2017 J. Mol. Recogn.

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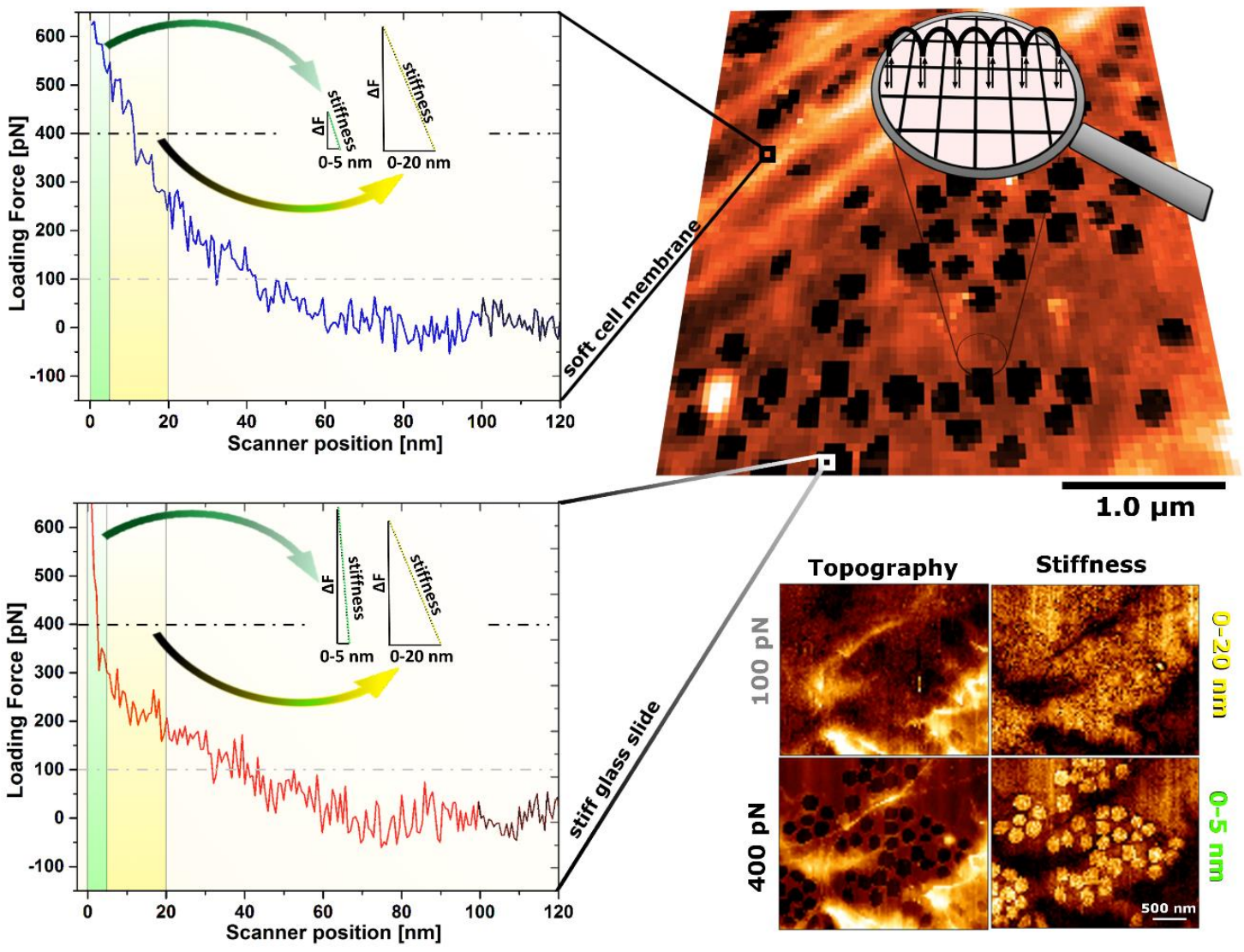
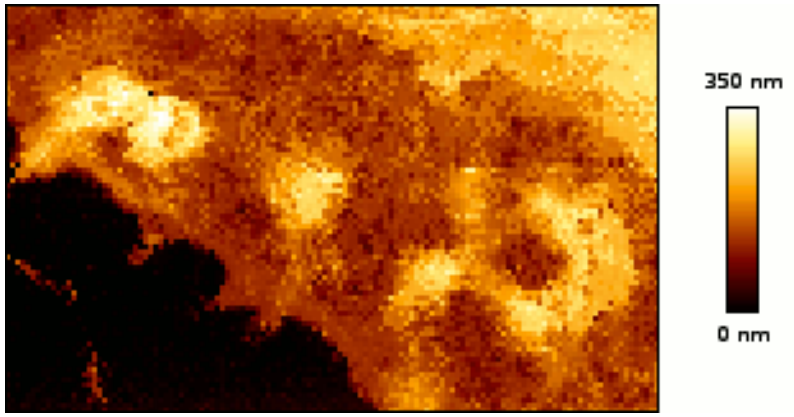
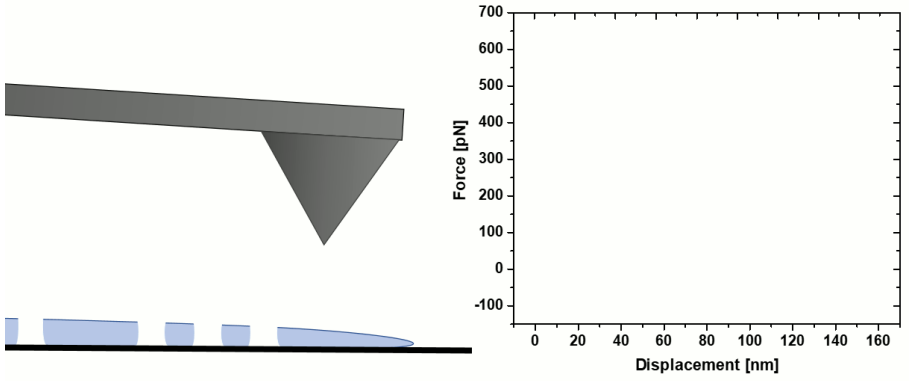
New approach to AFM imaging based on force-distance curves

QI AFM



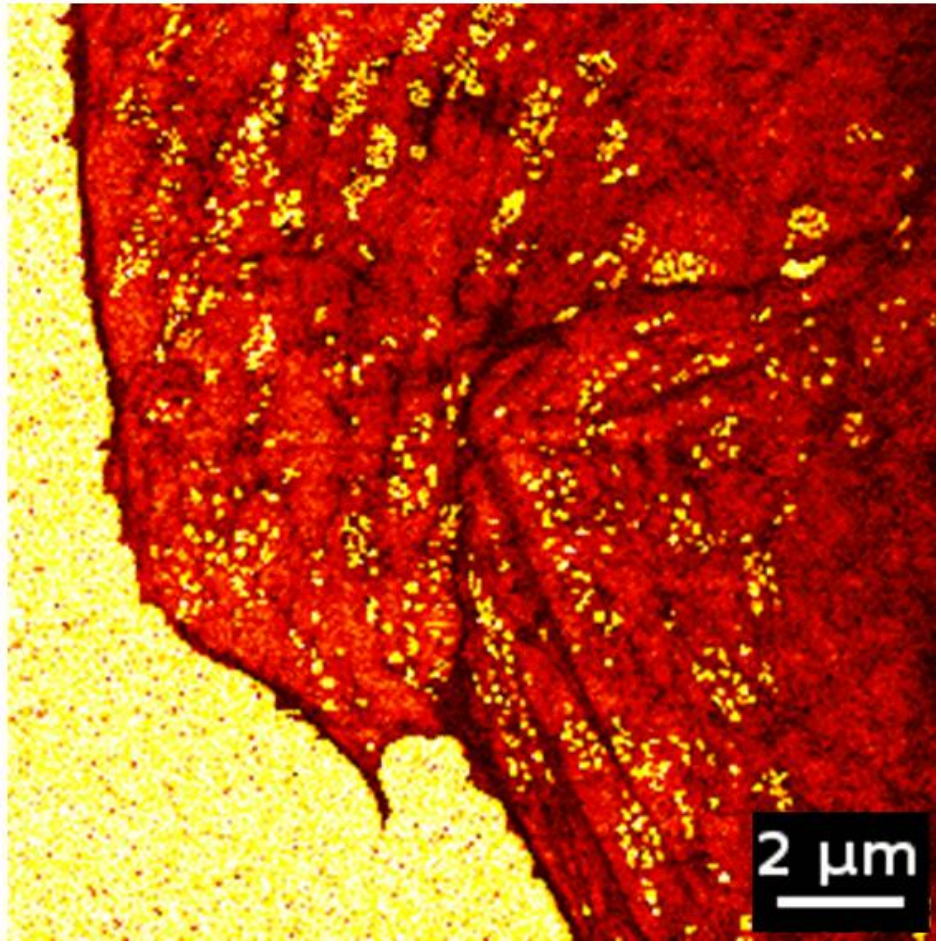




Zapotoczny B. et al. 2017 Scientific Reports

AFM imaging of fenestrations in live LSEC

Tracking fenestrae dynamics in LSECs



4 hours long experiment (**15min/frame**)

Zapotoczny B., et al., Hepatology, 2019

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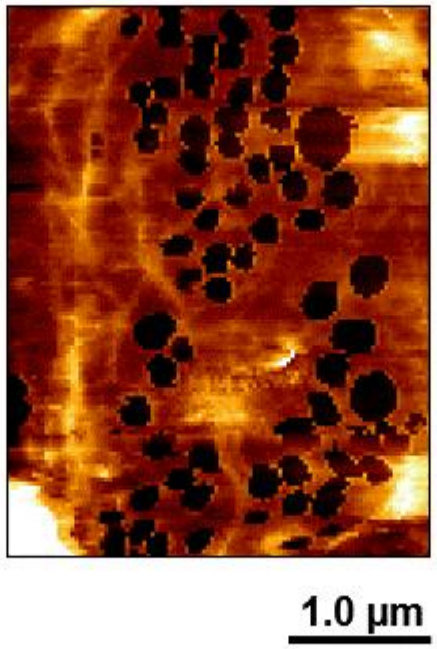
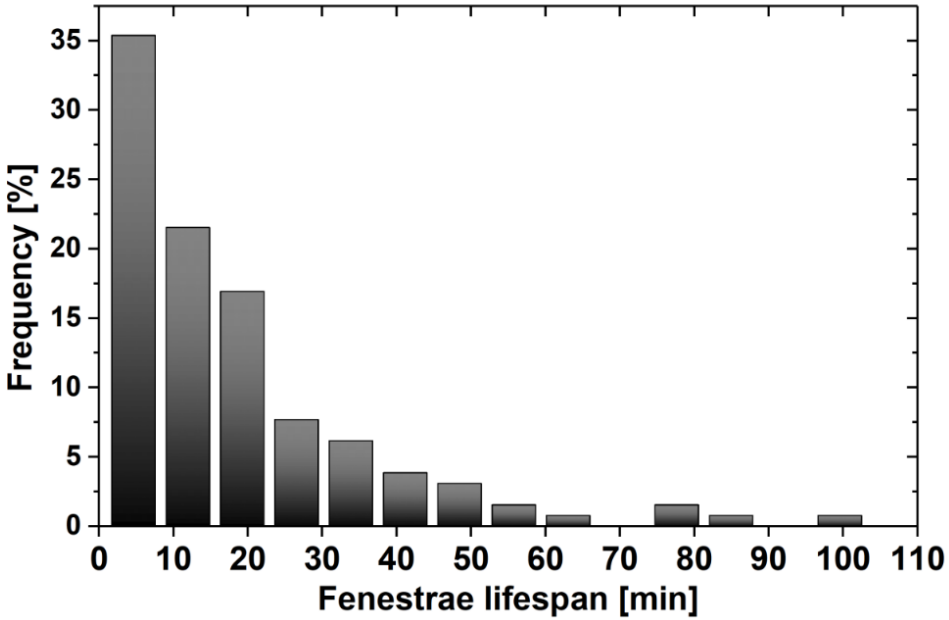


0 min

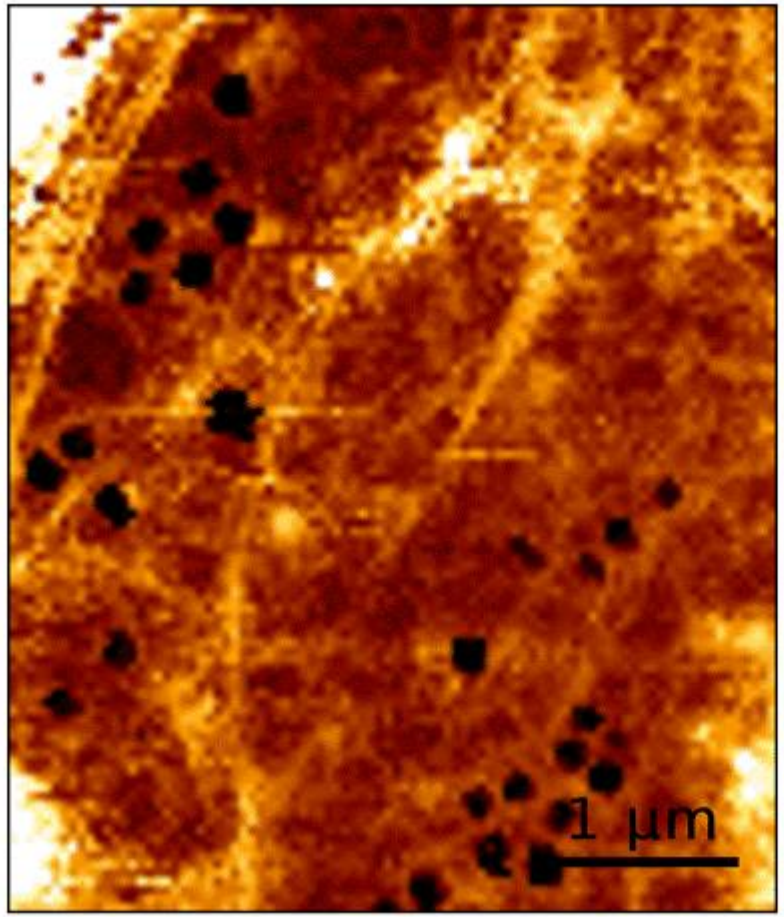


> 3h long experiment (**60sec./frame**)

Zapotoczny B., et al., Hepatology, 2019

AFM imaging of fenestrations in LSEC

Tracking fenestrae dynamics in LSECs



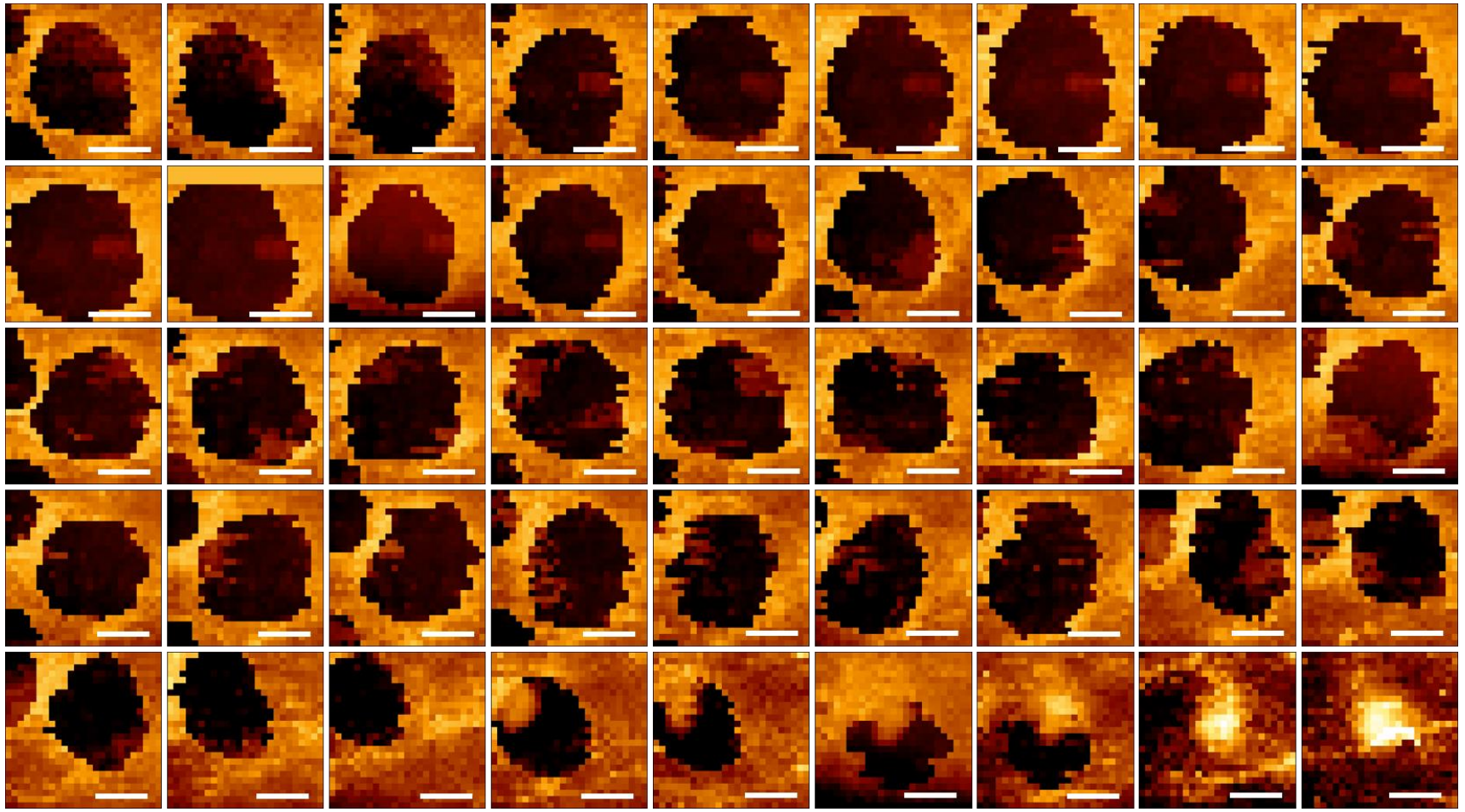
0 min

16 min long experiment (**up to 9 sec/frame**)

Zaptoczny B., et al., Bioph Rev., 2020
Zapotoczny B., et al., Traffic, 2019

AFM imaging of fenestrations in LSEC

Tracking fenestrae dynamics in LSECs



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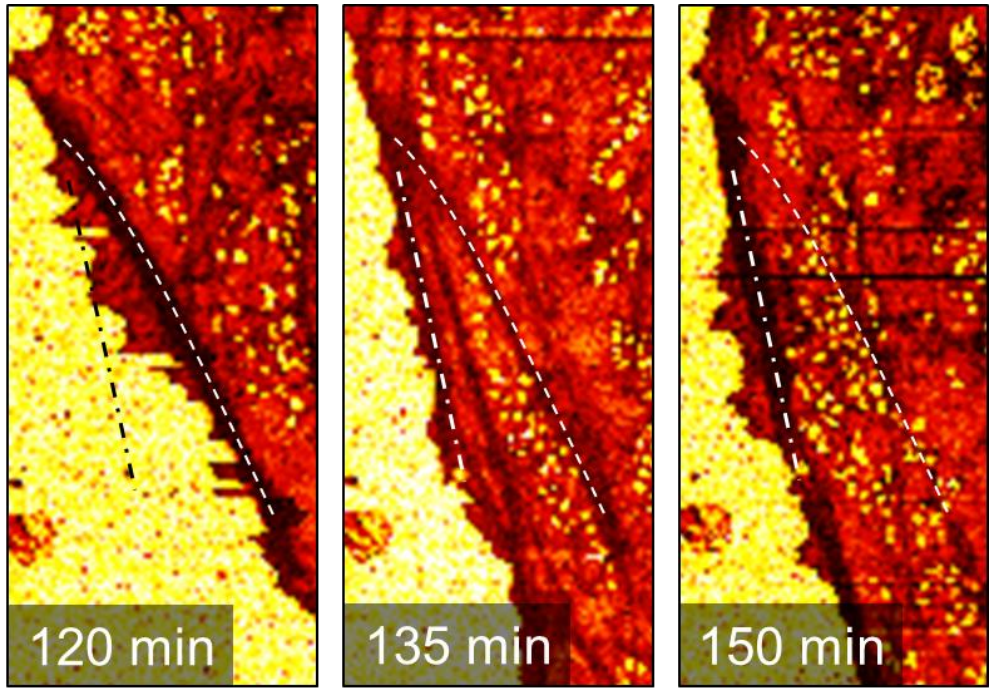
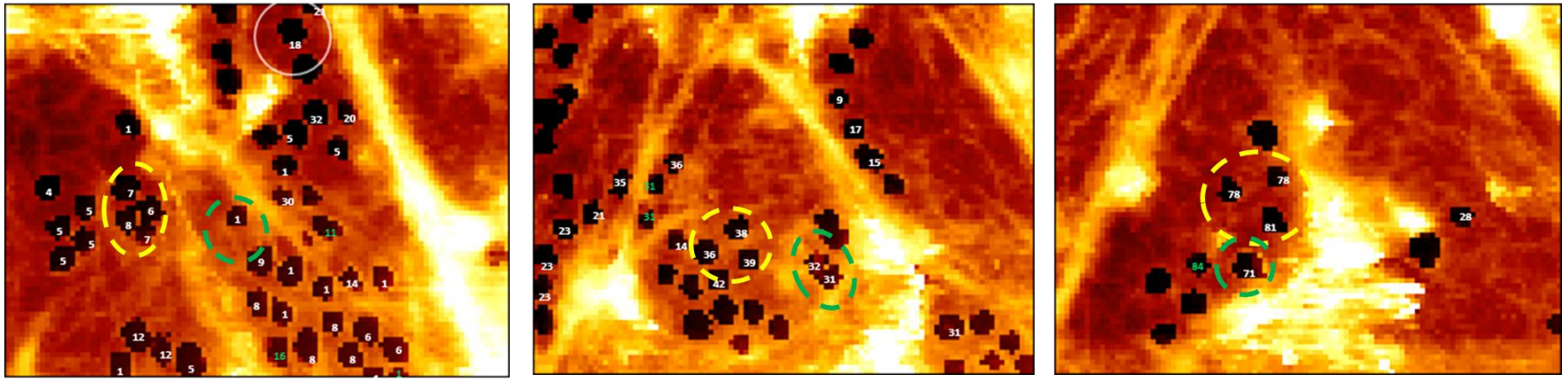
Zaptoczny B., et al., Bioph Rev., 2020
Zapotoczny B., et al., Traffic, 2019

AFM imaging of fenestrations in LSEC

Fenestrae-associated cytoskeletal structures:

- FACR – fenestrae-associated cytoskeleton ring
- **SACR – sieve-associated cytoskeleton ring**
- FFC – fenestrae-forming center
- DFC – defenestration center
- Gaps – >400nm holes in sieve plates





1 h 42 min | 2 h 18 min | 3 h 7 min

Zapotoczny B. et al. 2019 Hepatology

AFM imaging of fenestrations in LSEC

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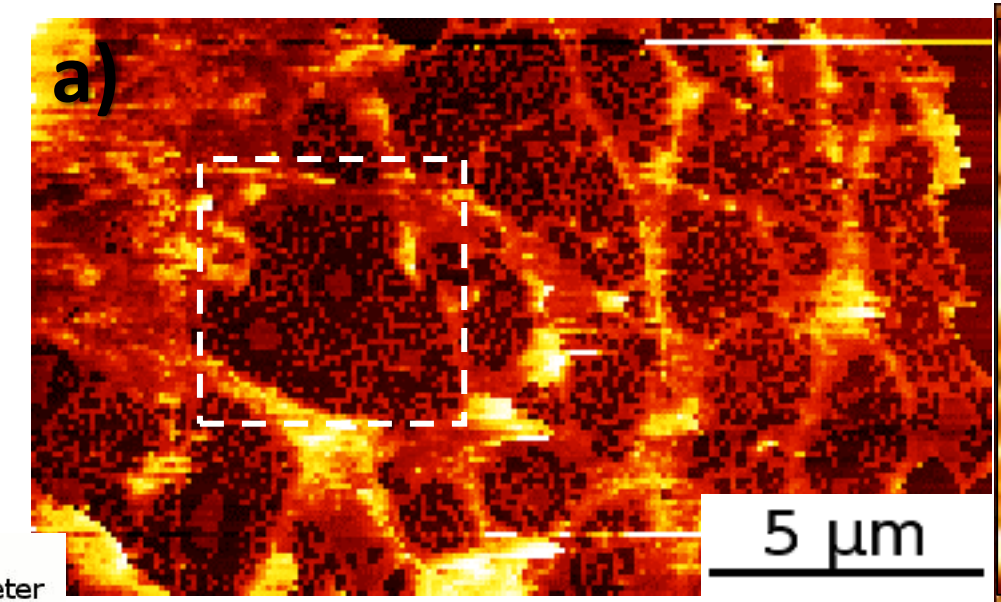
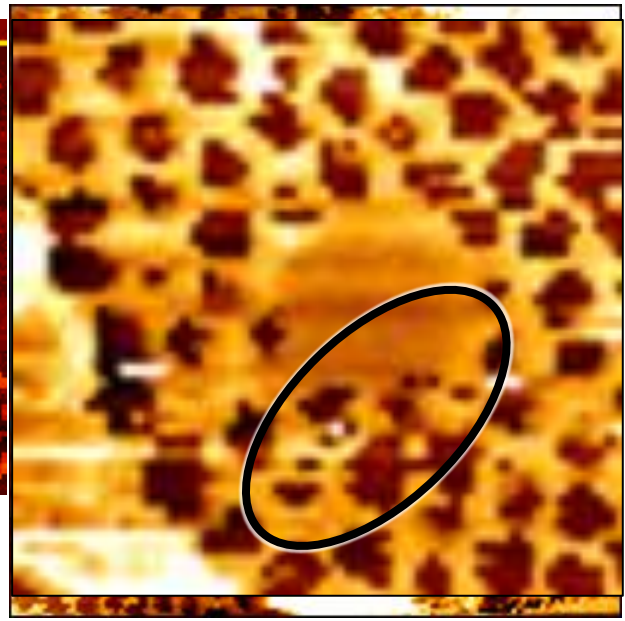
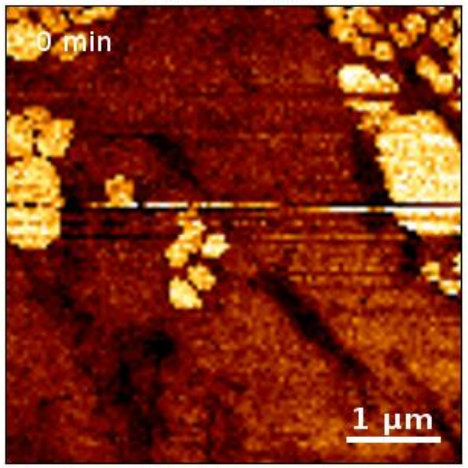
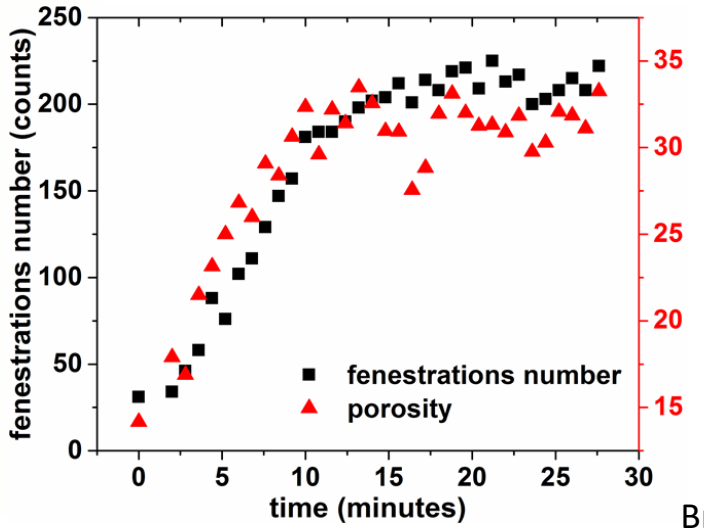


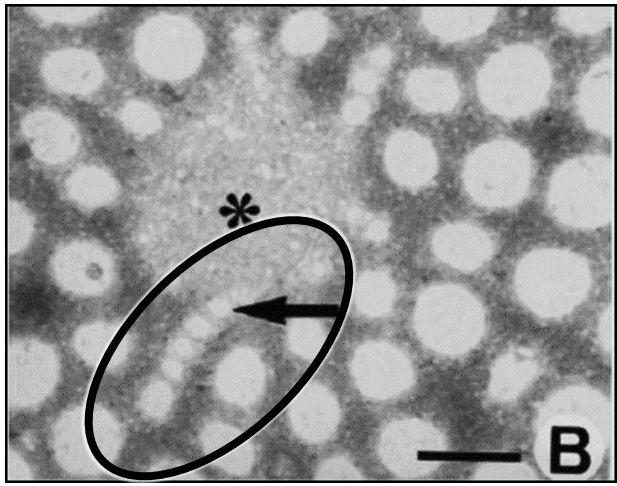


Cytochalasin B
Living LSEC membrane visualized with QI AFM. Stiffness parameter is presented



Images size and resolution: 5.0×5.0 µm, 100×100 lines





Braet F et al., 1998 PNAS
Zapotoczny B. et al. 2019 Hepatology

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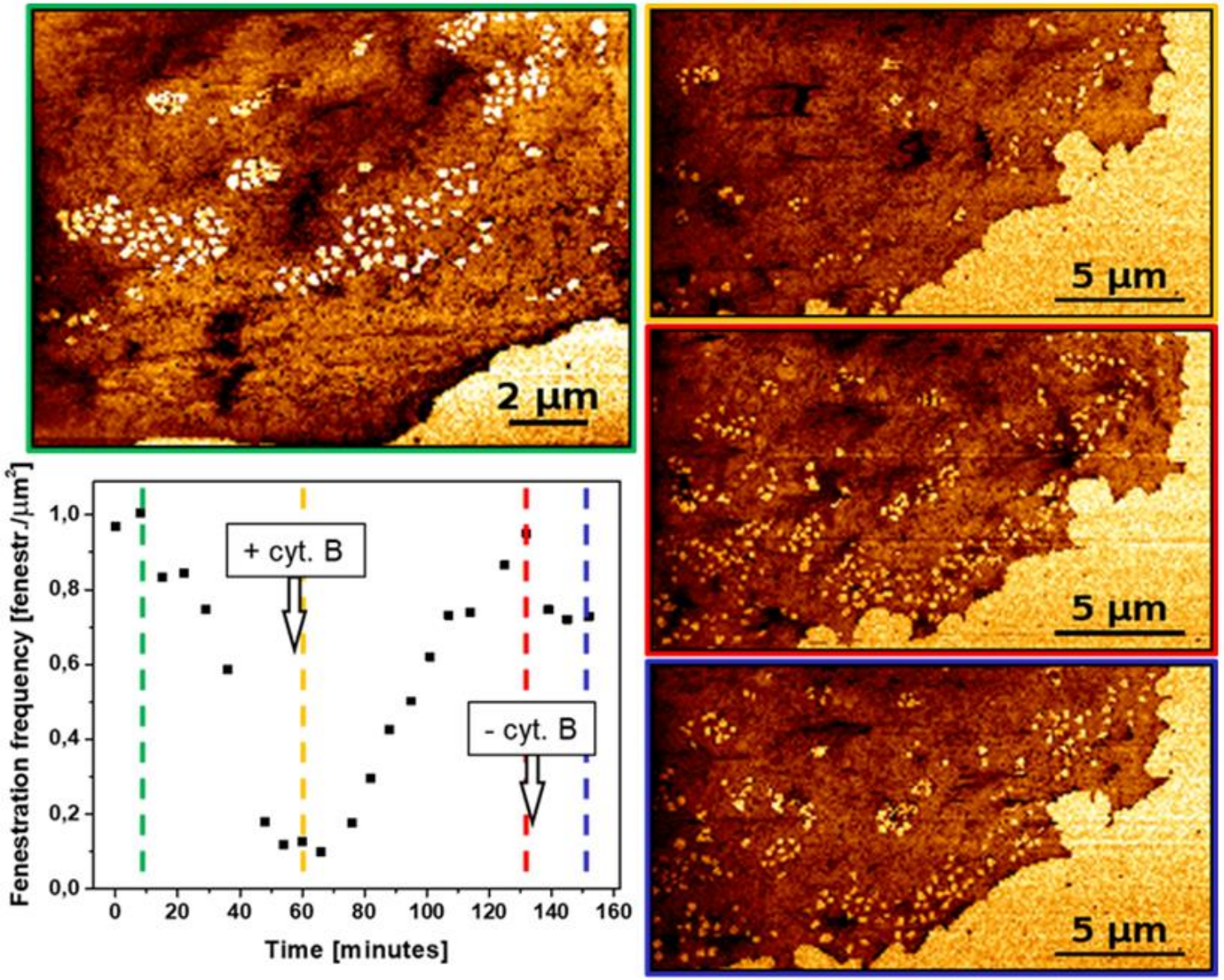
AFM imaging of fenestrations in LSEC

Multiparametic investigation by AFM covers

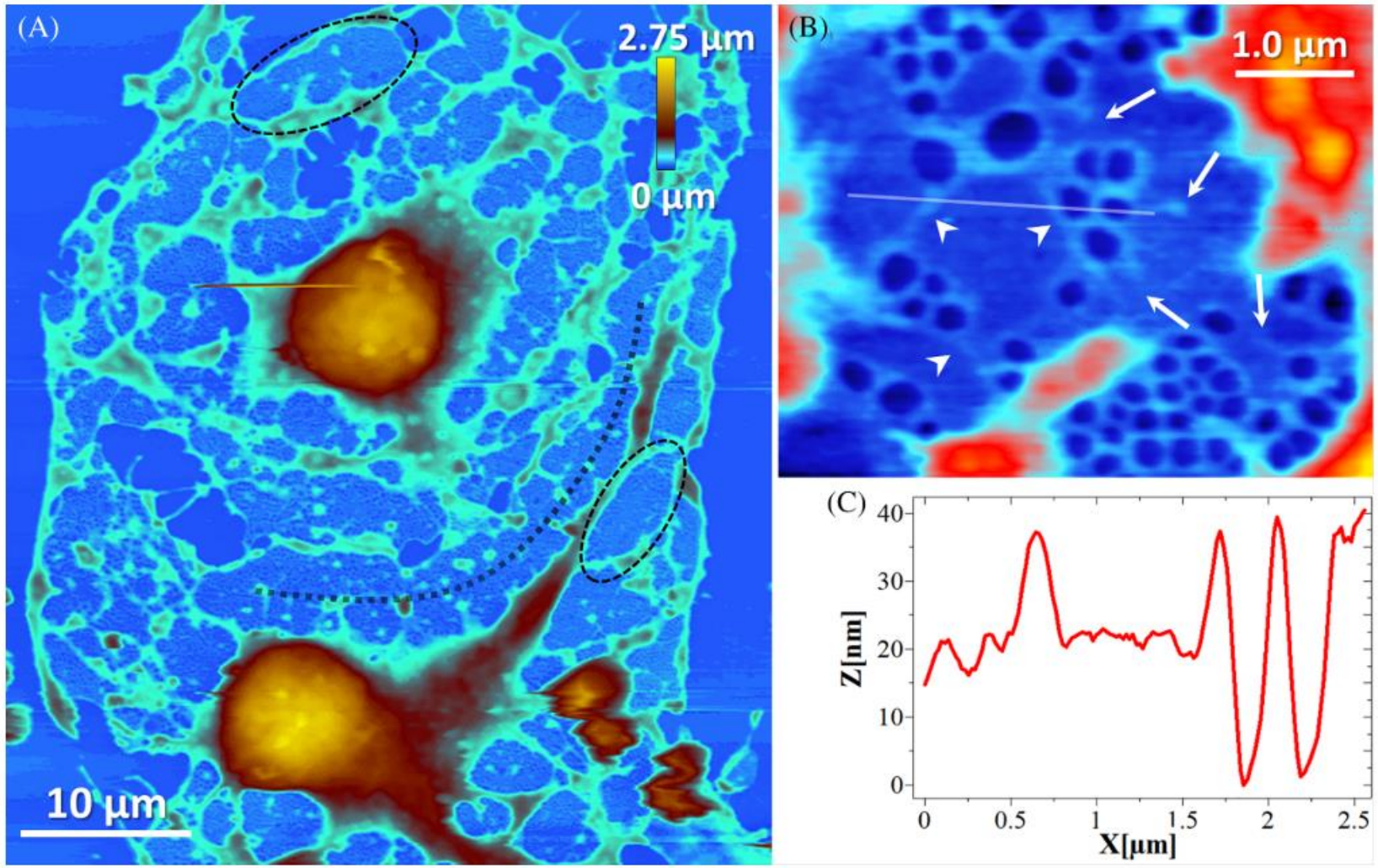
- Topography – number of fenestrations, size of fenestrations, porosity
- Deformability - load force dependent tomography
- Elasticity (modulus) / stiffness parameter

...and their changes with time
and with response to drugs

Reversibility of defenestration



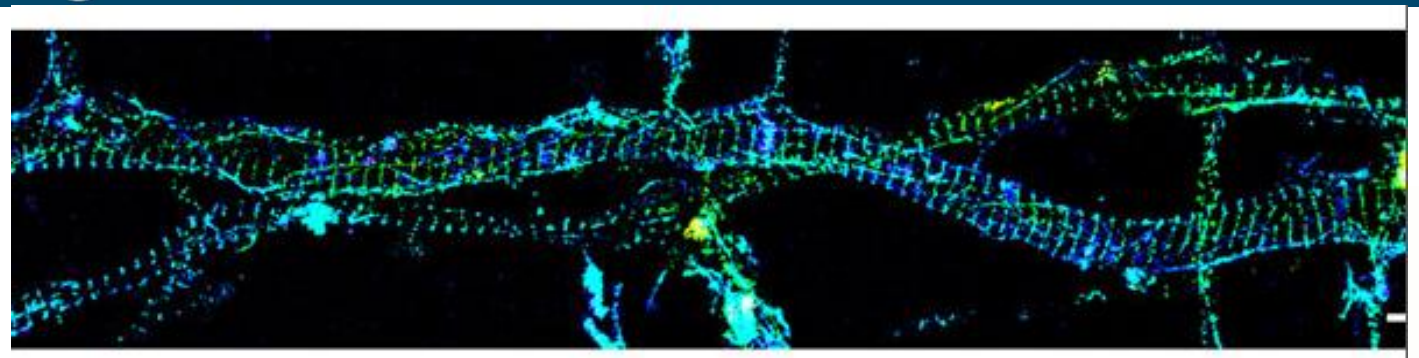
High-resoultion imaging of glutaraldehyde fixed LSEC

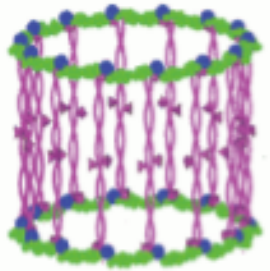


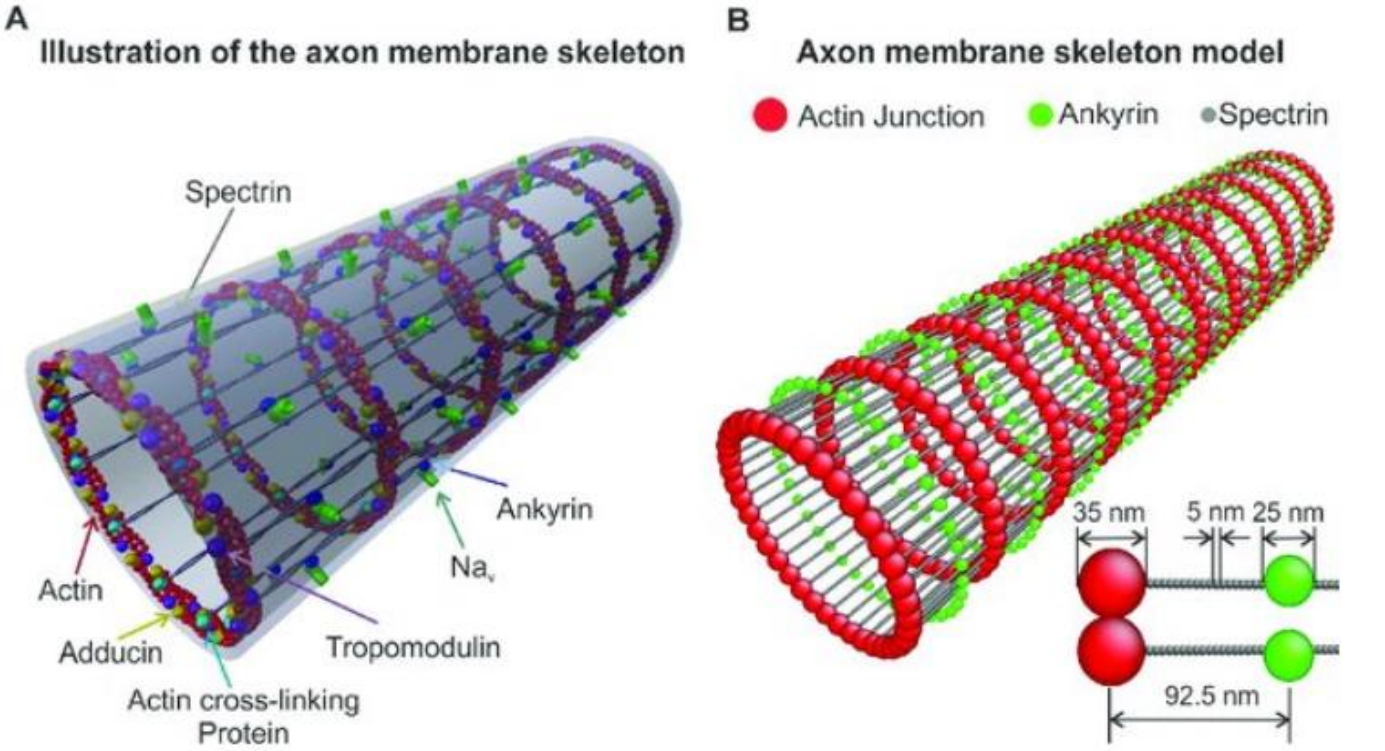
Volume 20, Issue 12
Pages: 881-982
December 2019

Zapotoczny B. et al. Traffic, 2019

Spectrin-actin hypothesis

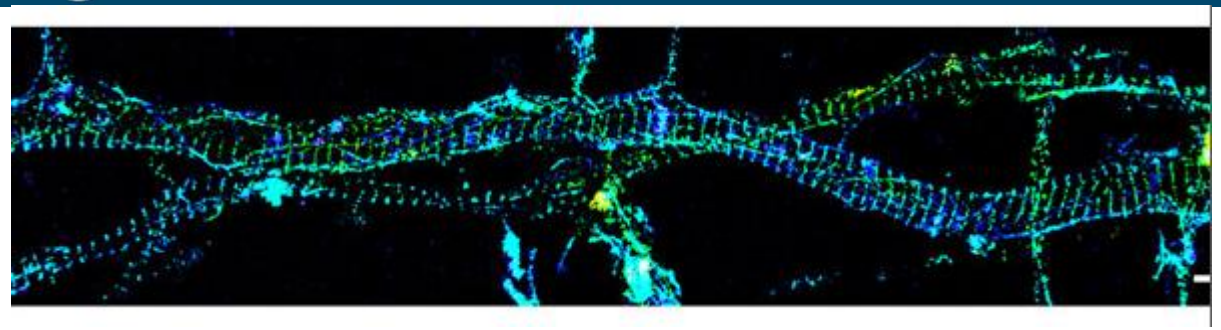


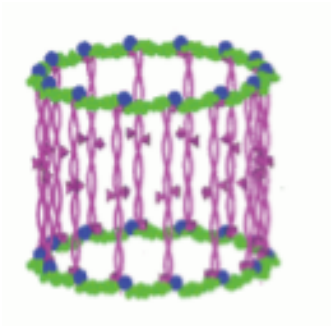


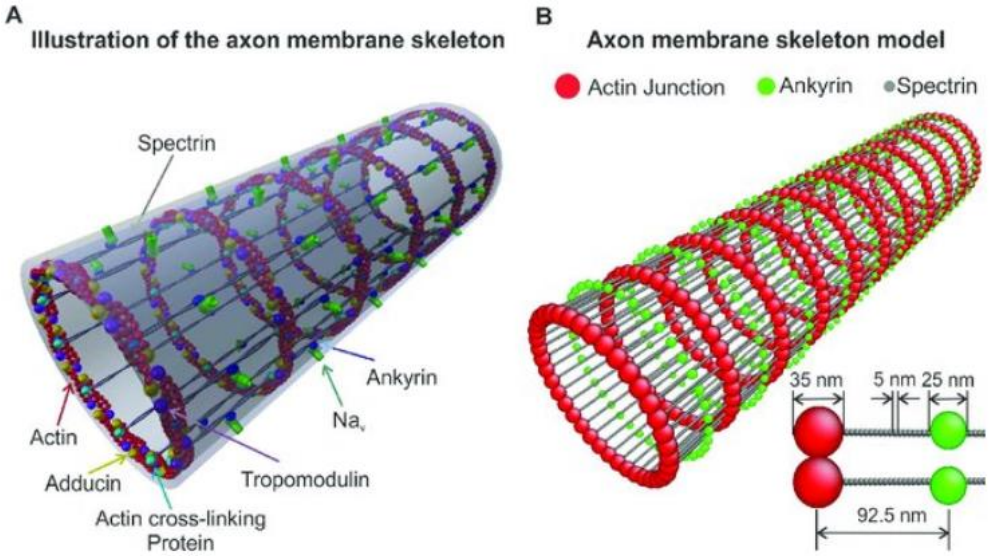


Xu et al, Science 339: 452-456 (2013)

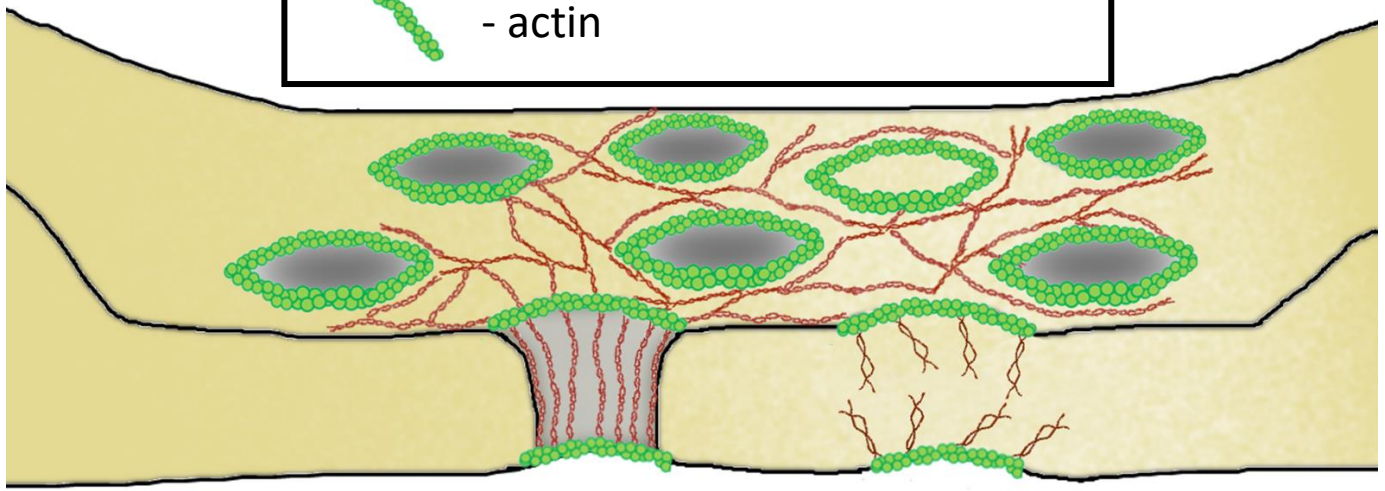
Spectrin-actin hypothesis







- destabilized spectrin
- intact spectrin
- actin



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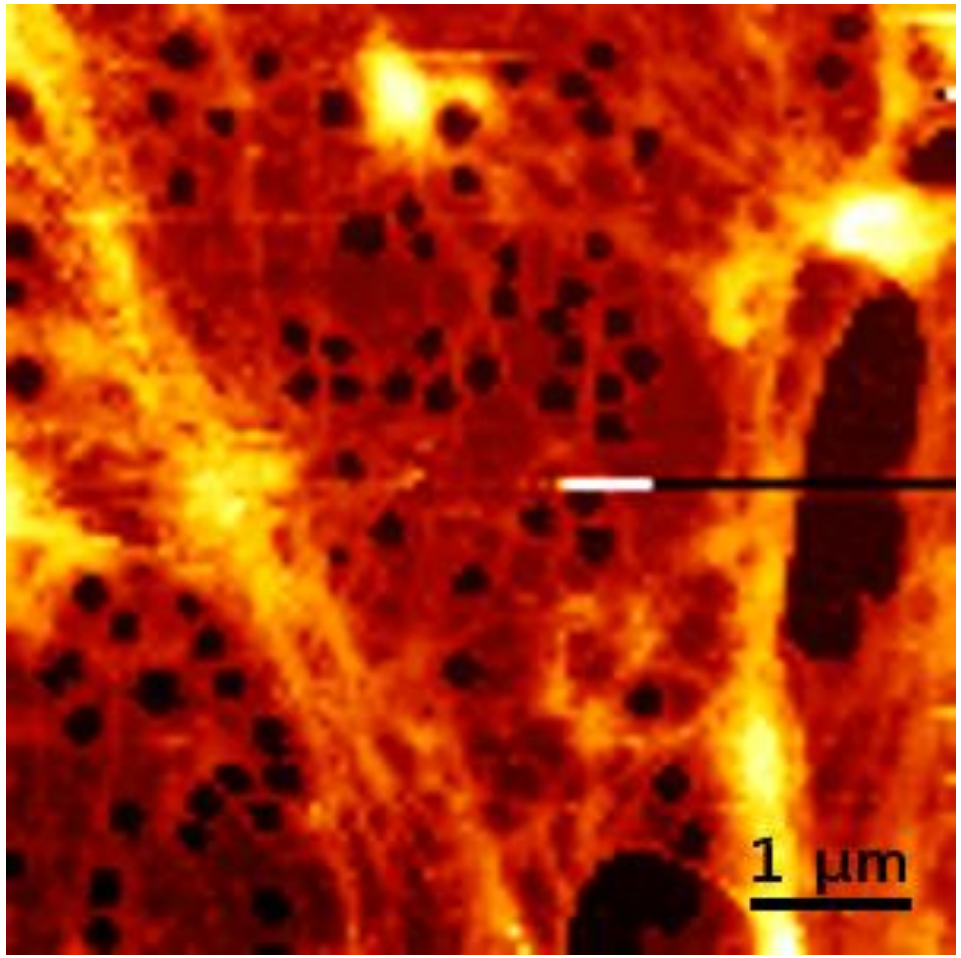
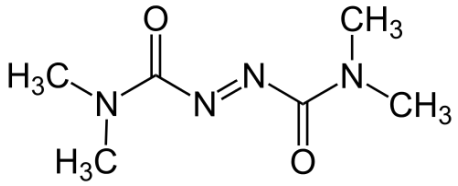
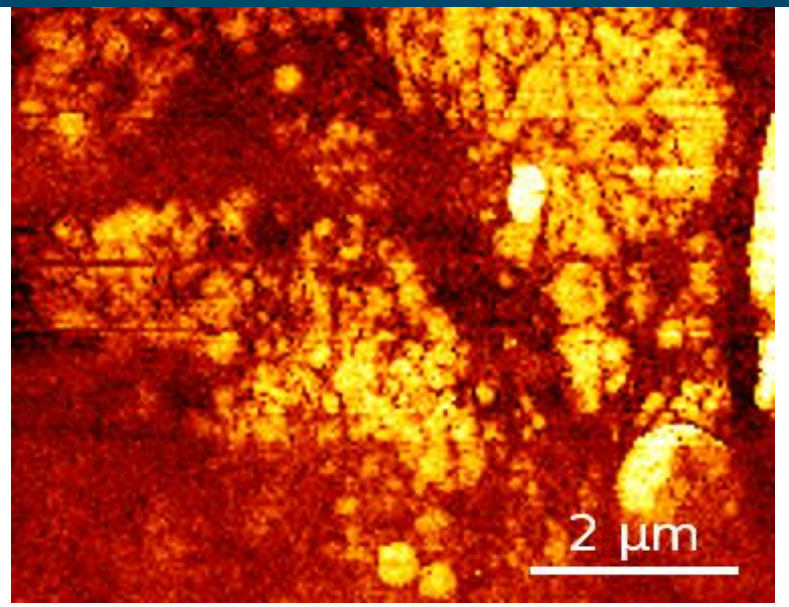
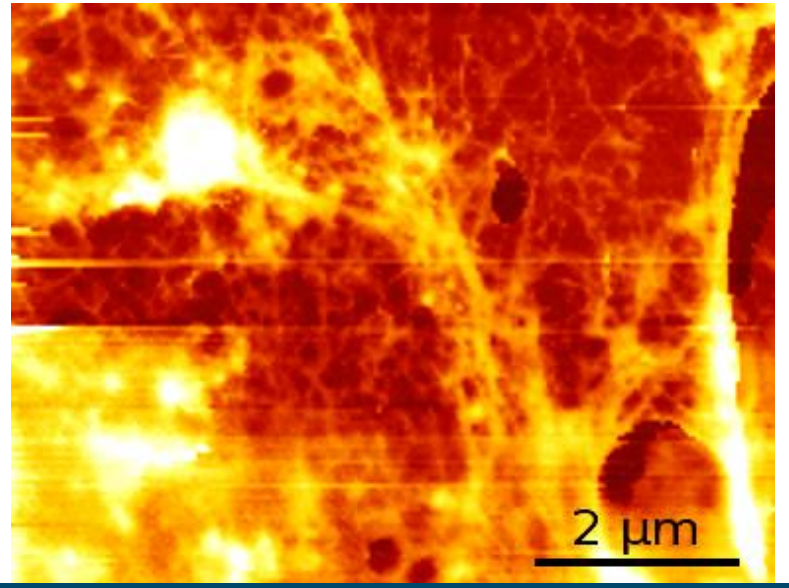


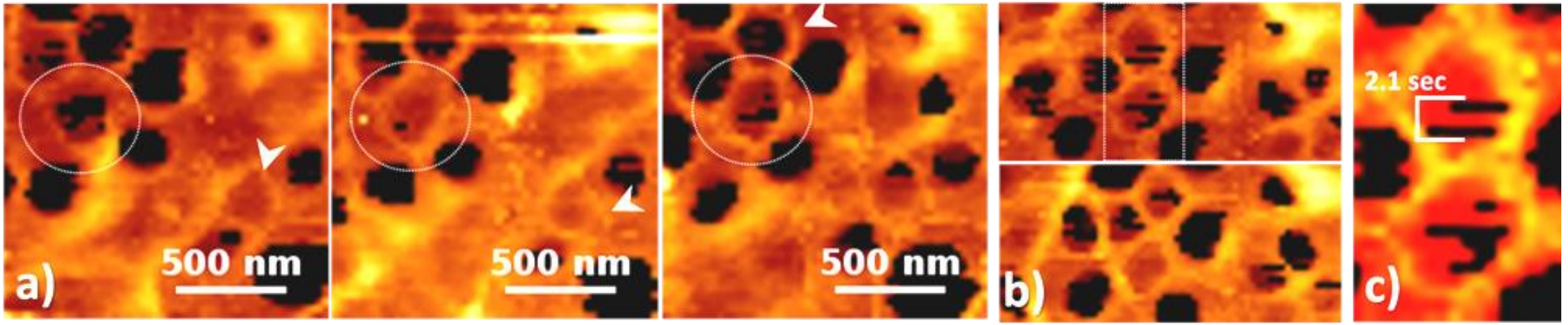
+ Diamide
500 µM





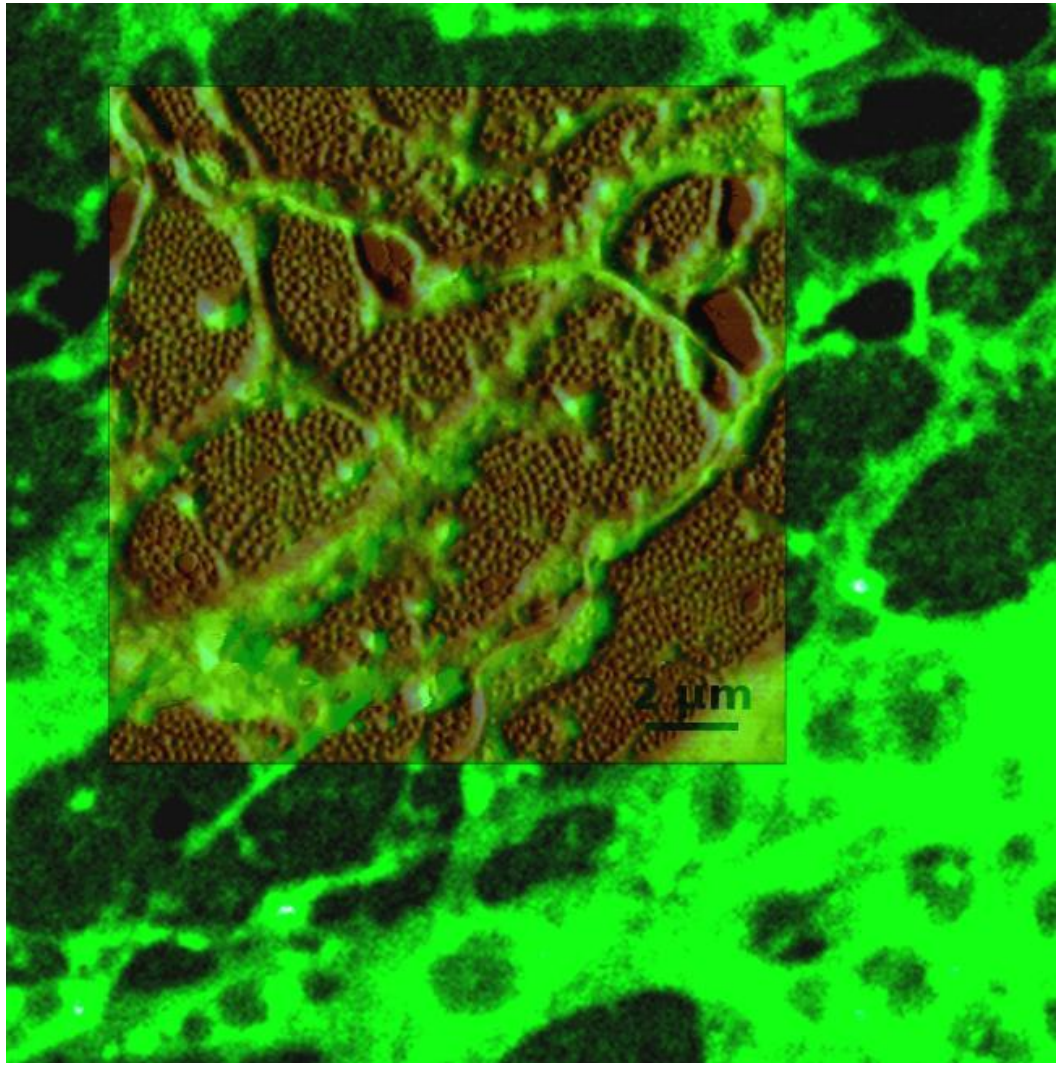


Spectrin-actin hypothesis



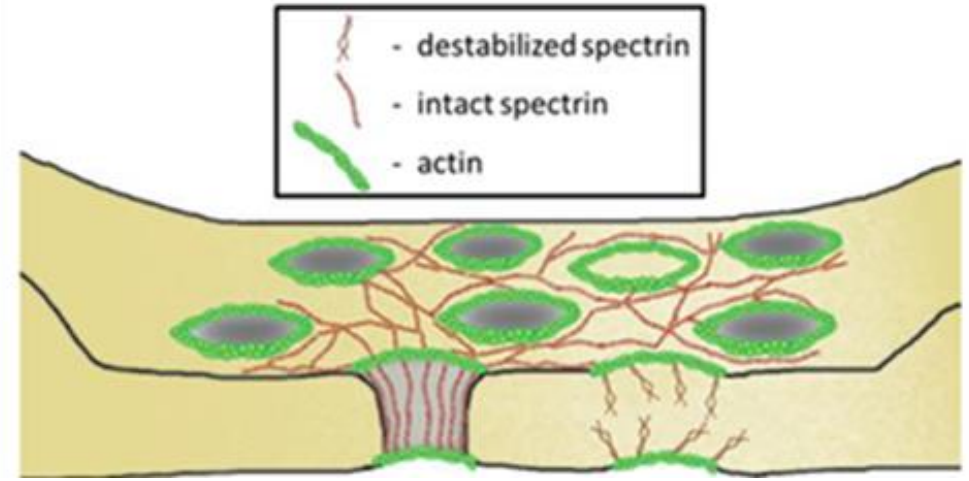
Zapotoczny B., et al., Traffic, 2019

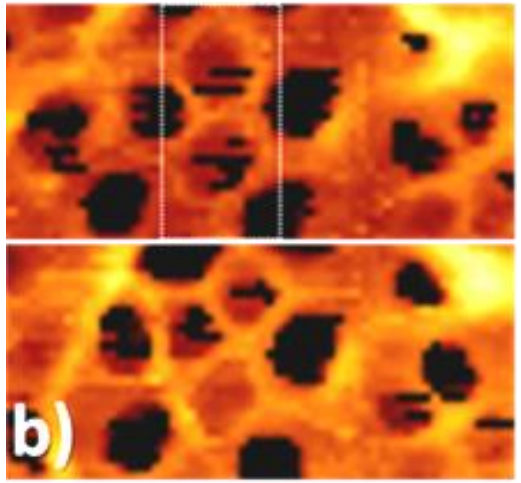
Why do we need super-resolution imaging?

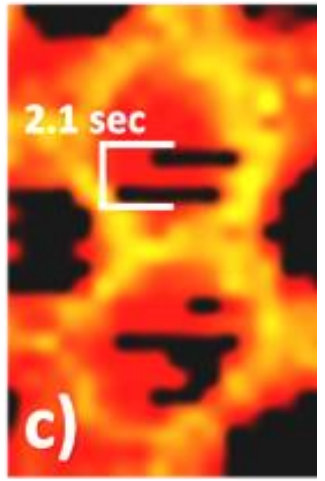


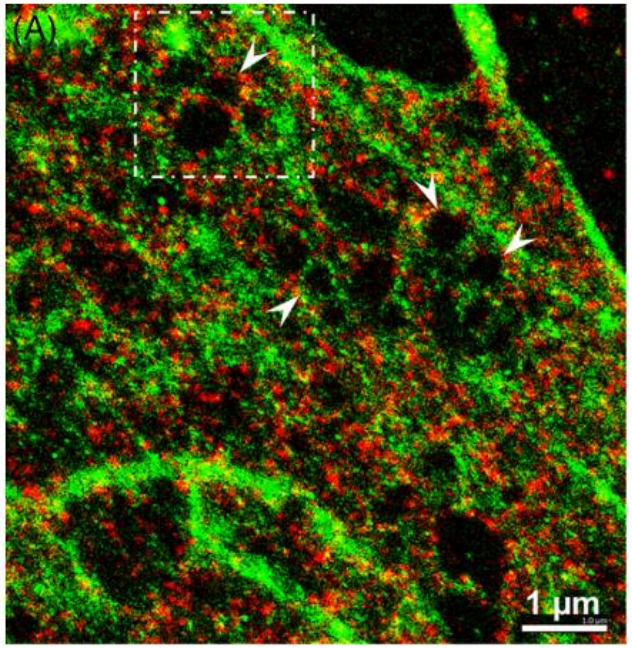
Phalloidin-Atto488 confocal FL + AFM contact mode

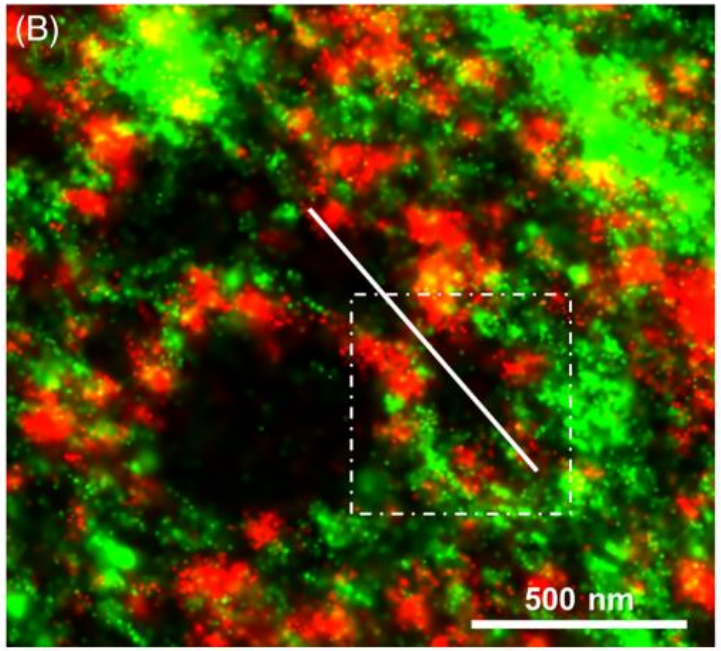
Comparative imaging of fenestrations in LSEC

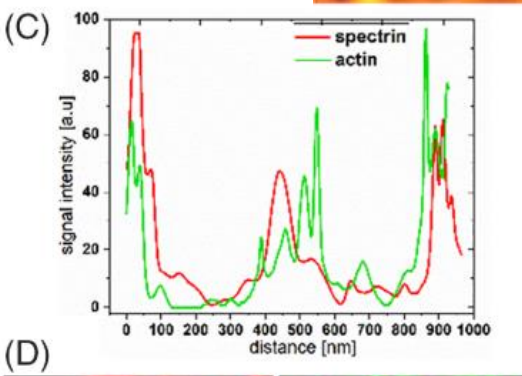


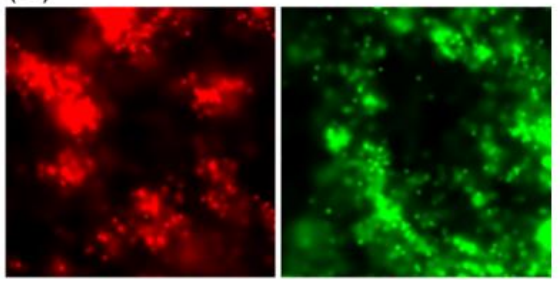




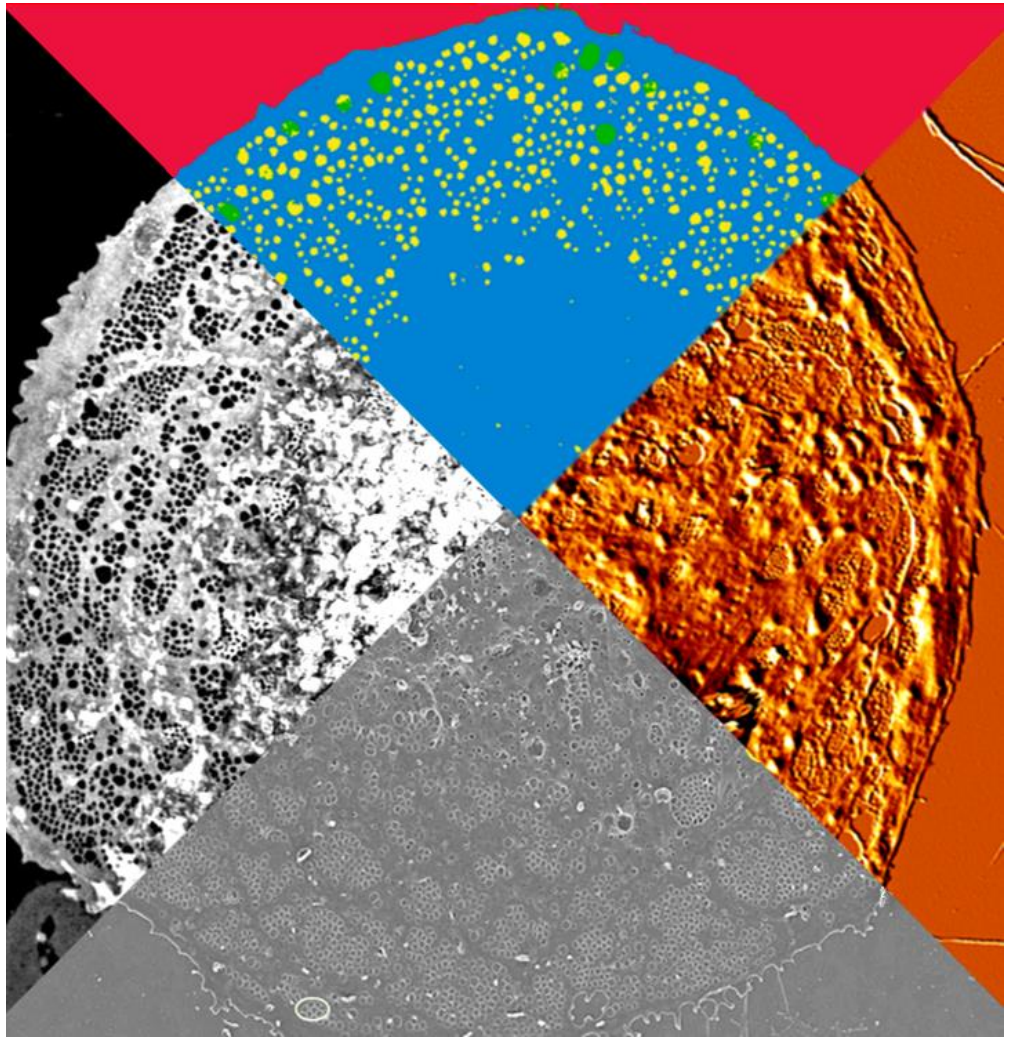








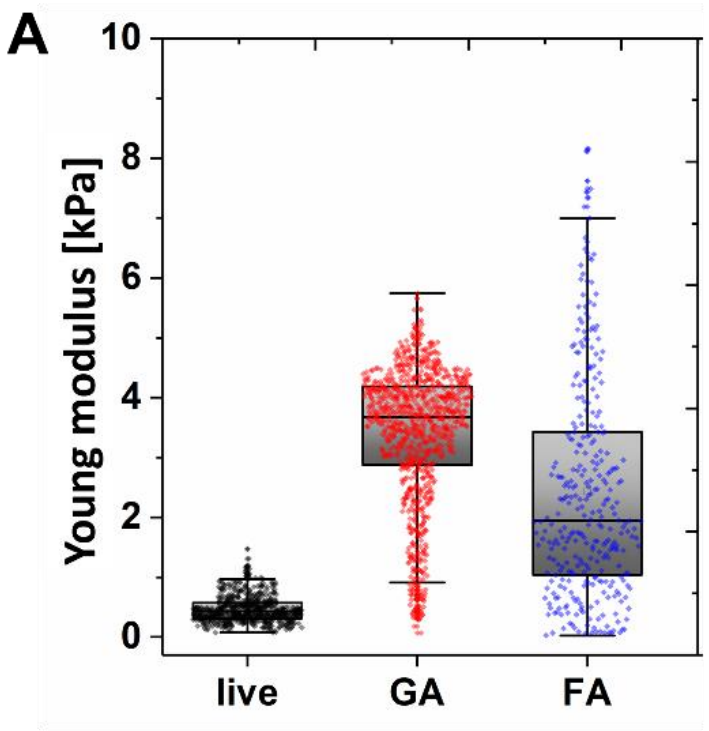
Zapotoczny B., et al., Traffic, 2019

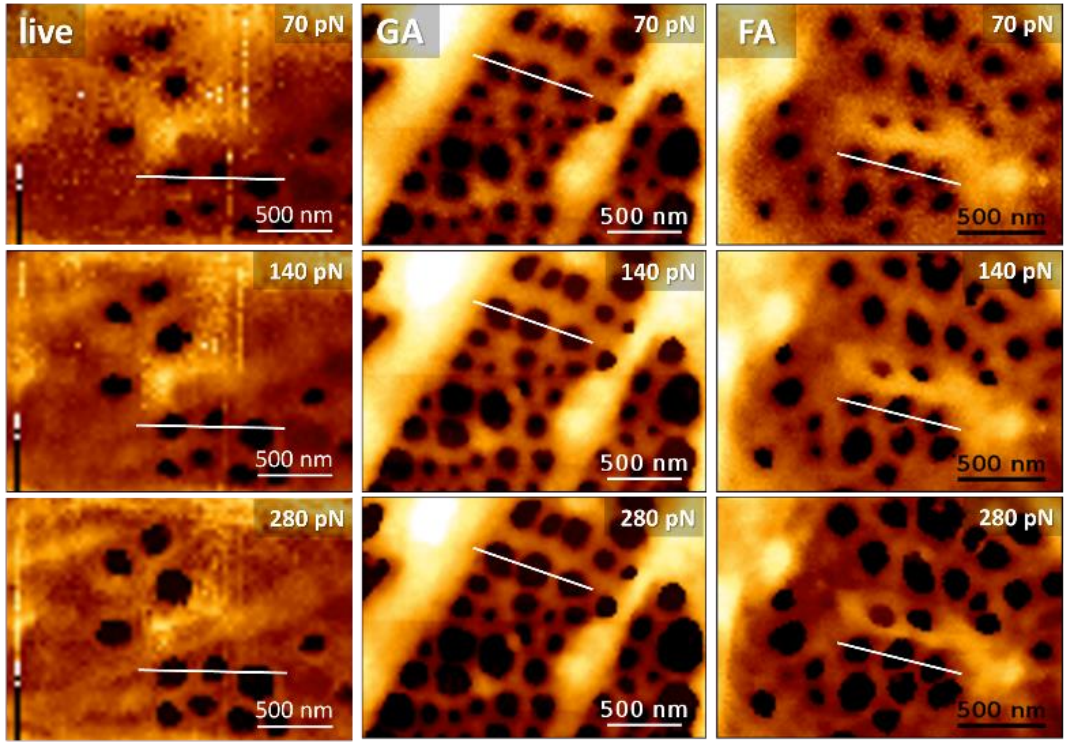


Quantitative Correlative Light, Atomic Force and Electron Microscopy

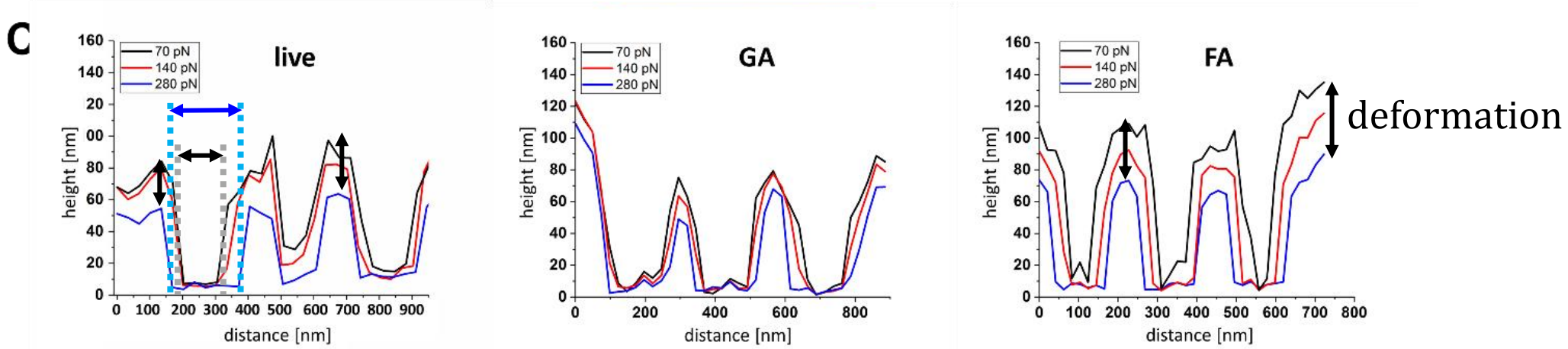
Szafranska K., [...],. **Zapotoczny B.**, Nanophotonics, 2022

Quantitative Correlative Light, Atomic Force and Electron Microscopy

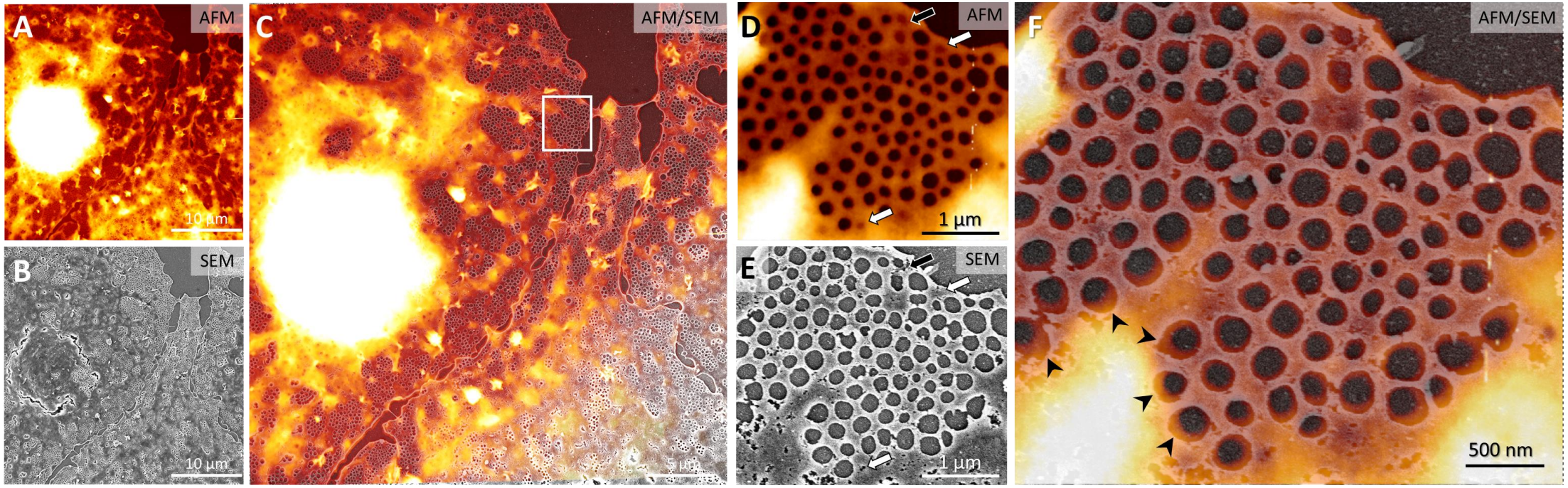


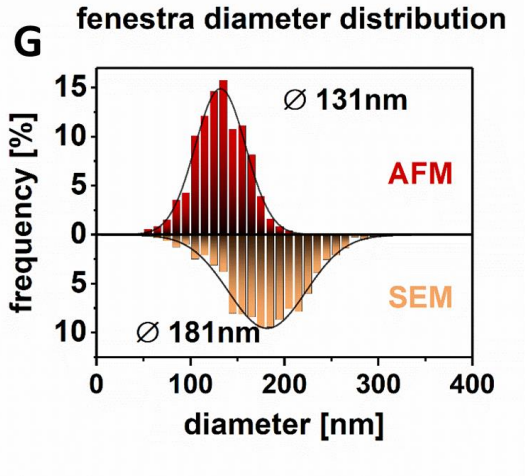


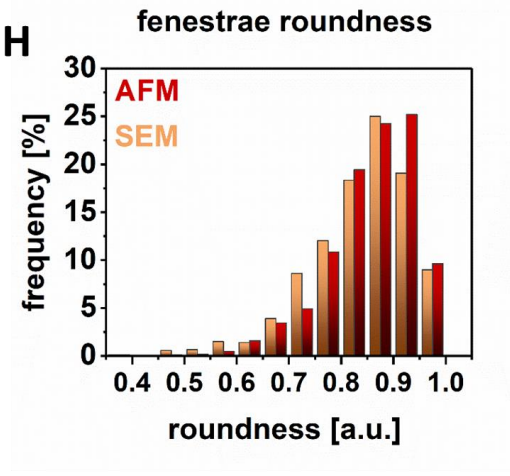
increesing load force

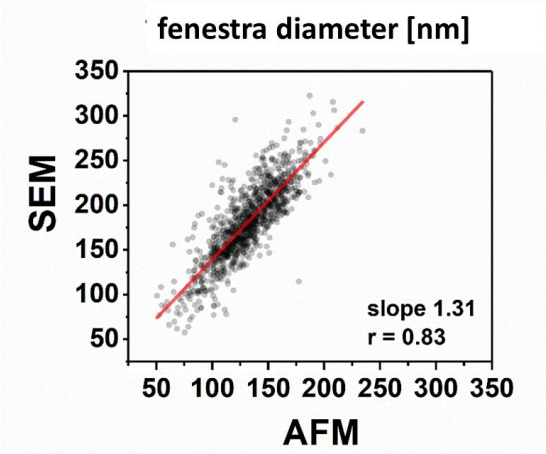


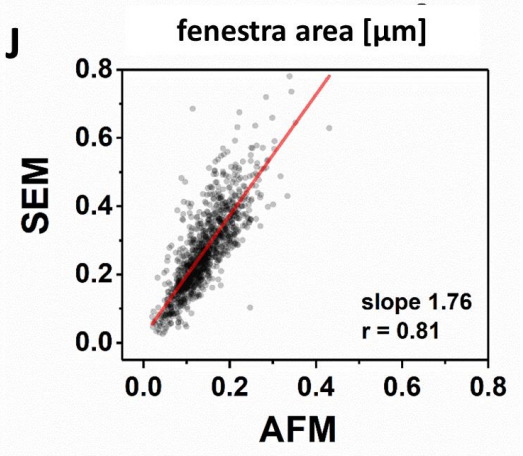
Quantitative Correlative Light, Atomic Force and Electron Microscopy





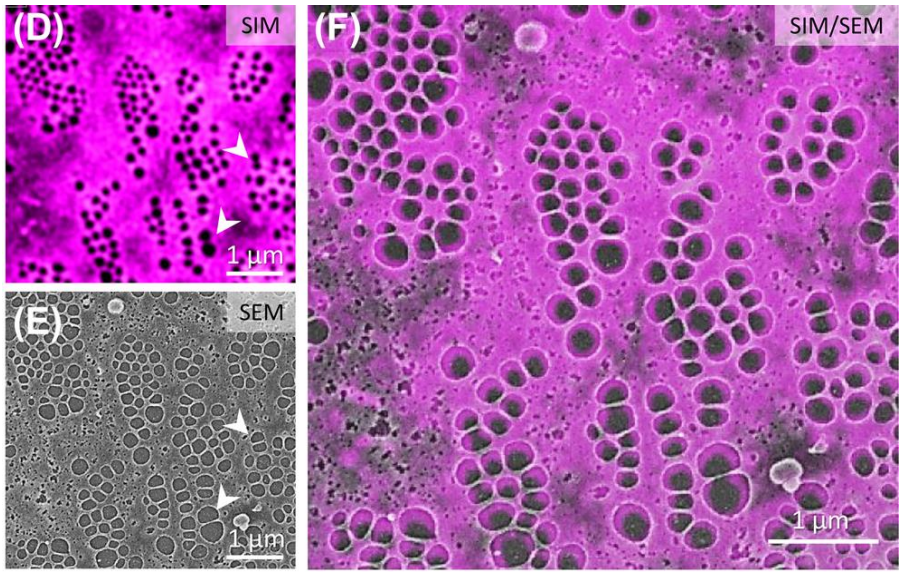


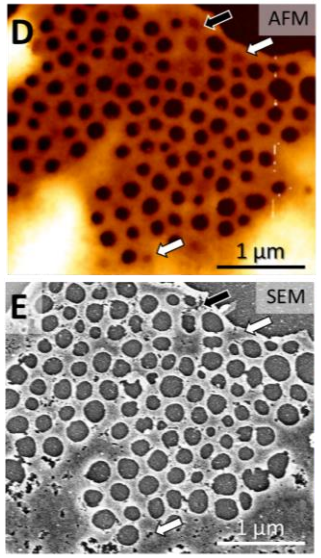


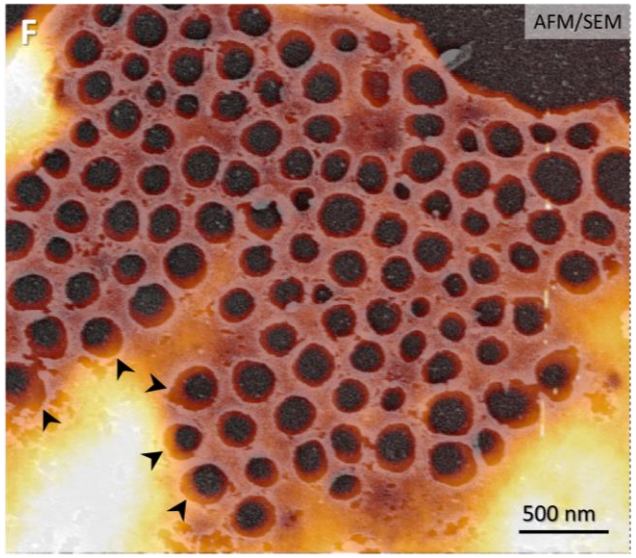


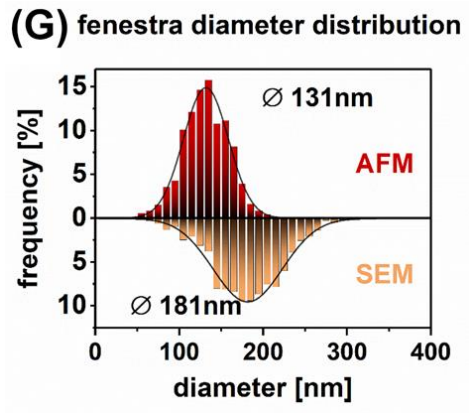
Szafranska K., [...],. **Zapotoczny B.**, Nanophotonics, 2022

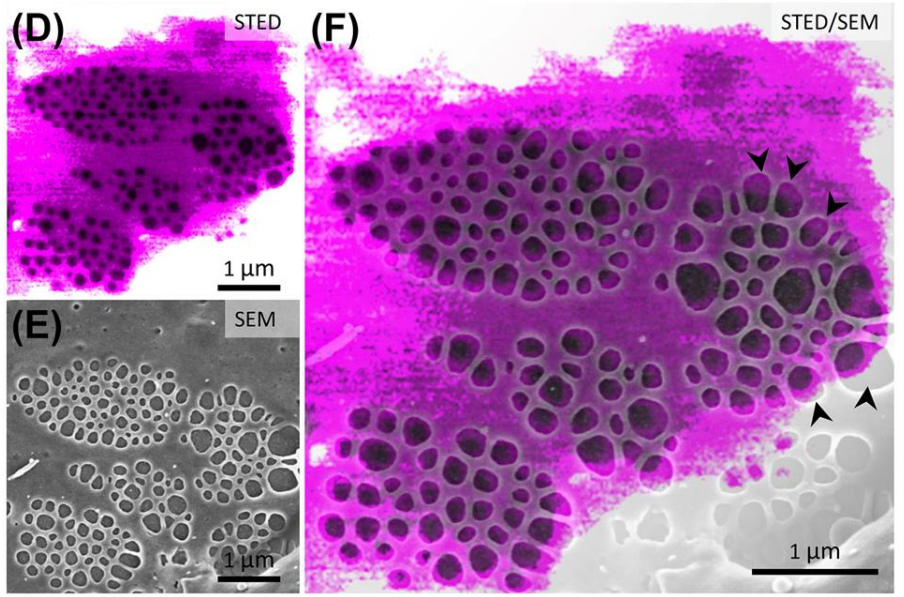
Future outlook – correlation with optical nanoscopy

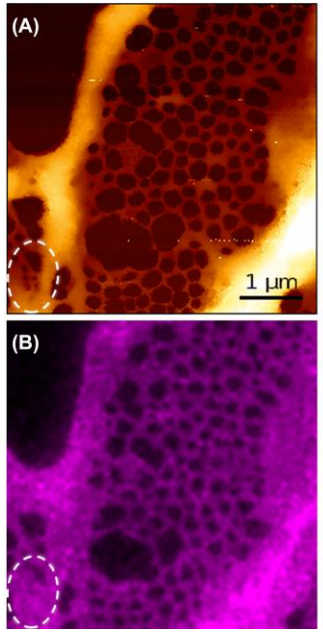


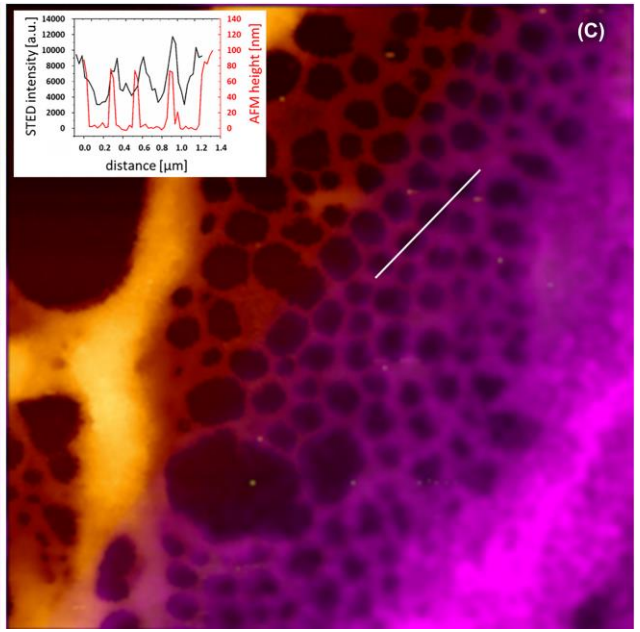


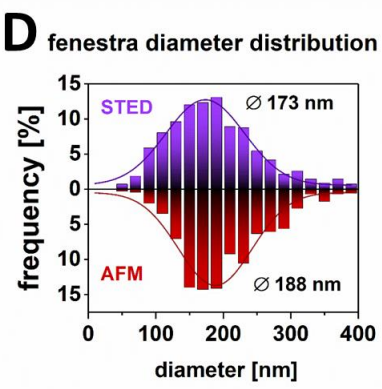








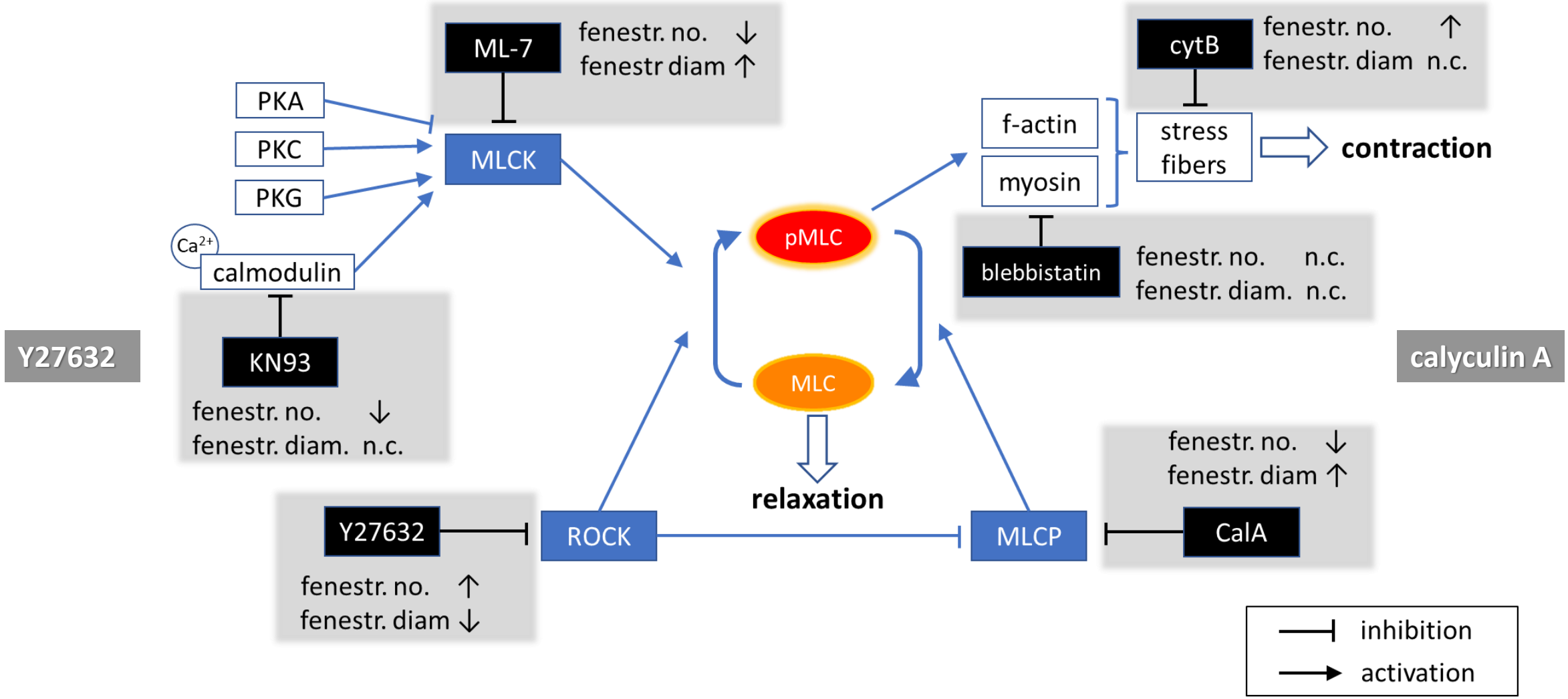




Szafranska K., et al., Nanophotonics, 2022

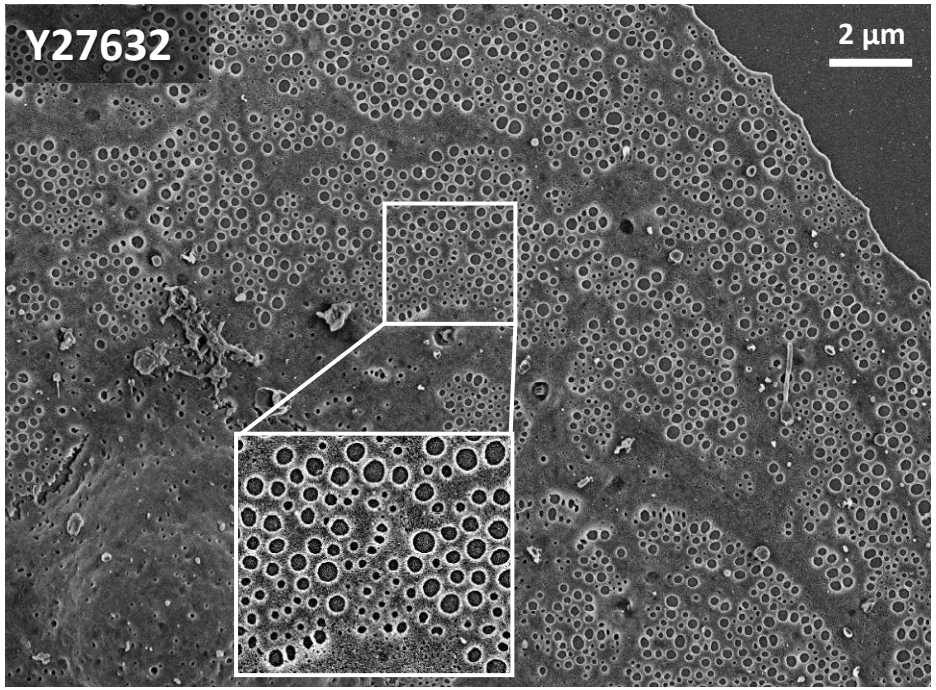
Super-resolution techniques - SEM

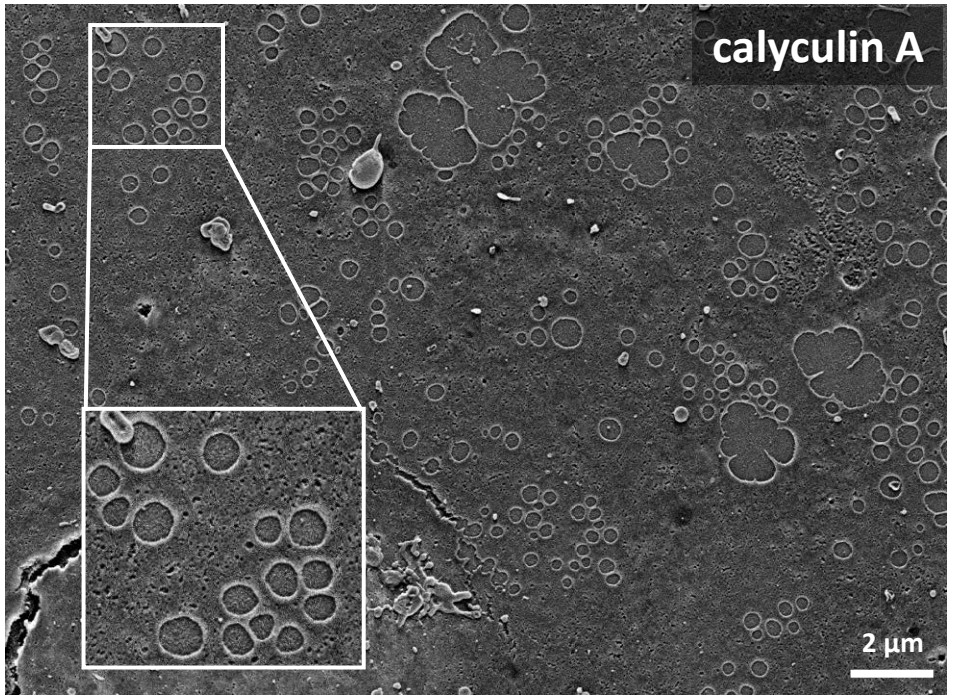
Zapotoczny B et al., IJMS, 2022



Super-resolution techniques - SEM

Zapotoczny B et al., IJMS, 2022

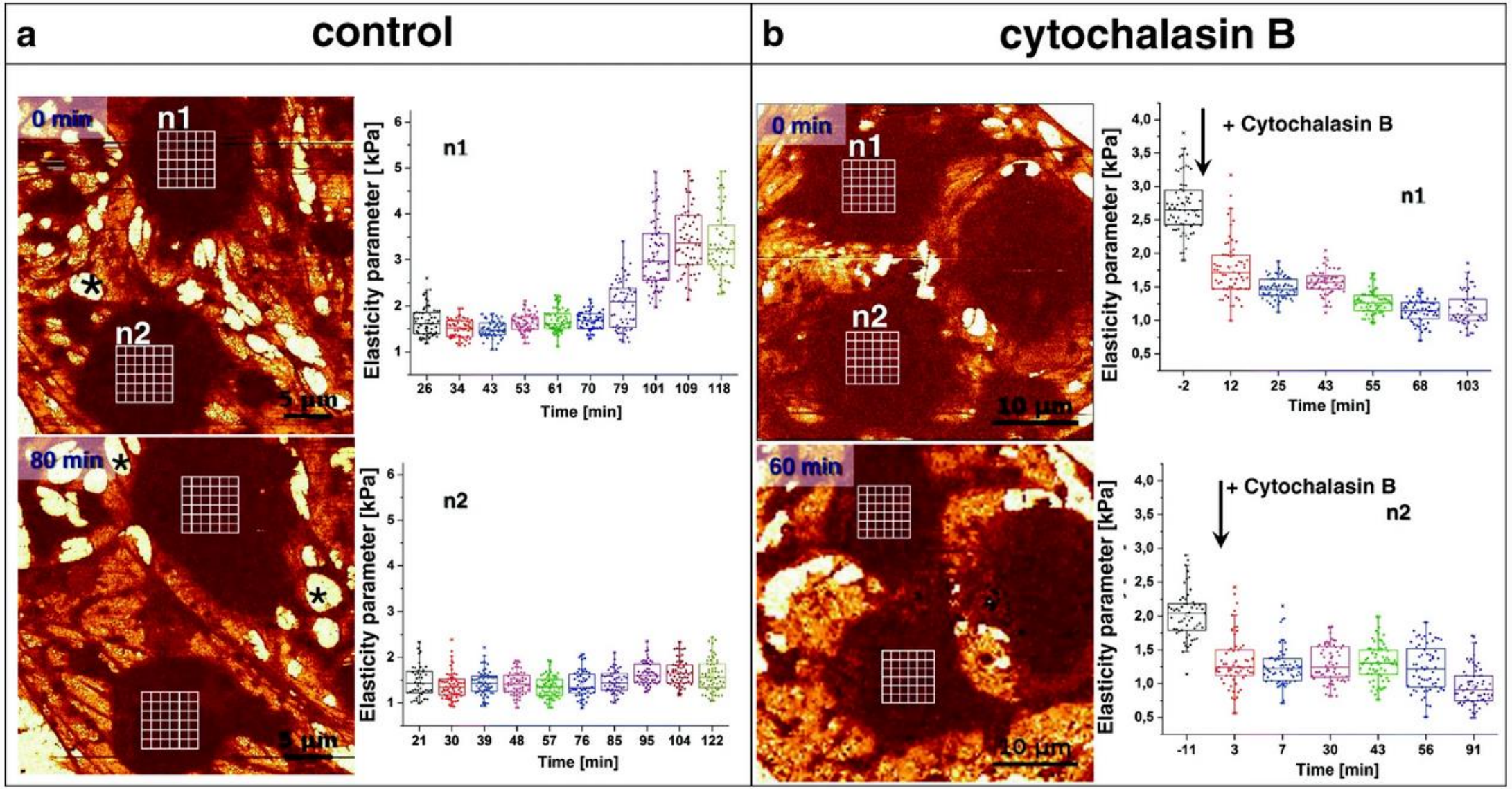




Liver Sinusoidal Endothelial Cells – LSEC

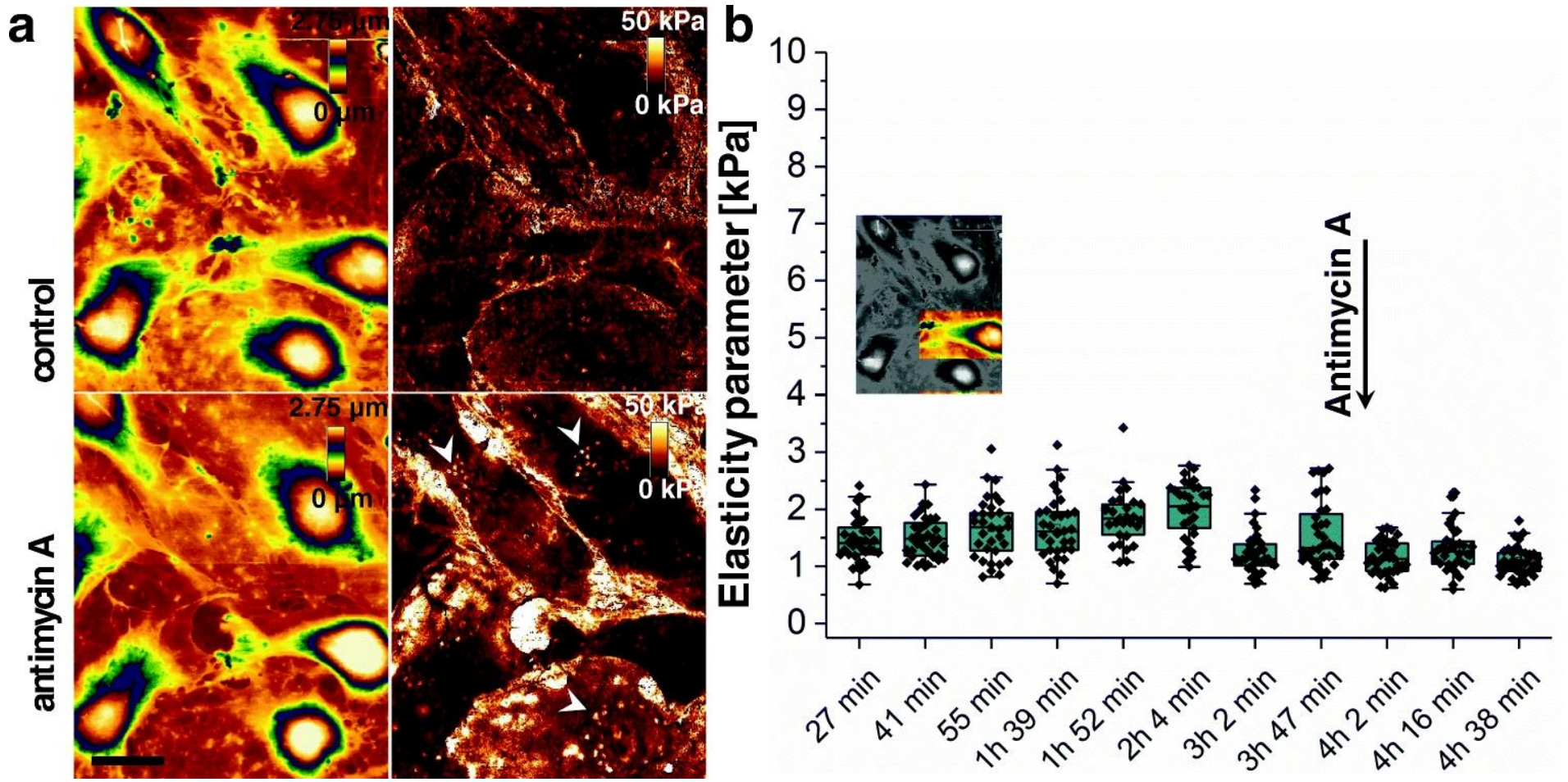
1. Motivation of the research
2. Introduction to LSEC and Atomic Force Microscopy (AFM)
3. Fenestrations in live LSEC – fenestrae-associated cytoskeletal structures
4. Fenestrations in live LSEC – „*in vitro* pharmacology on a single cell using AFM”
5. **Morphomechanical studies of LSEC**

Morphomechanical studies of LSEC



Zapotoczny B. et al. Biophysical Reports, 2020

Morphomechanical studies of LSEC



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