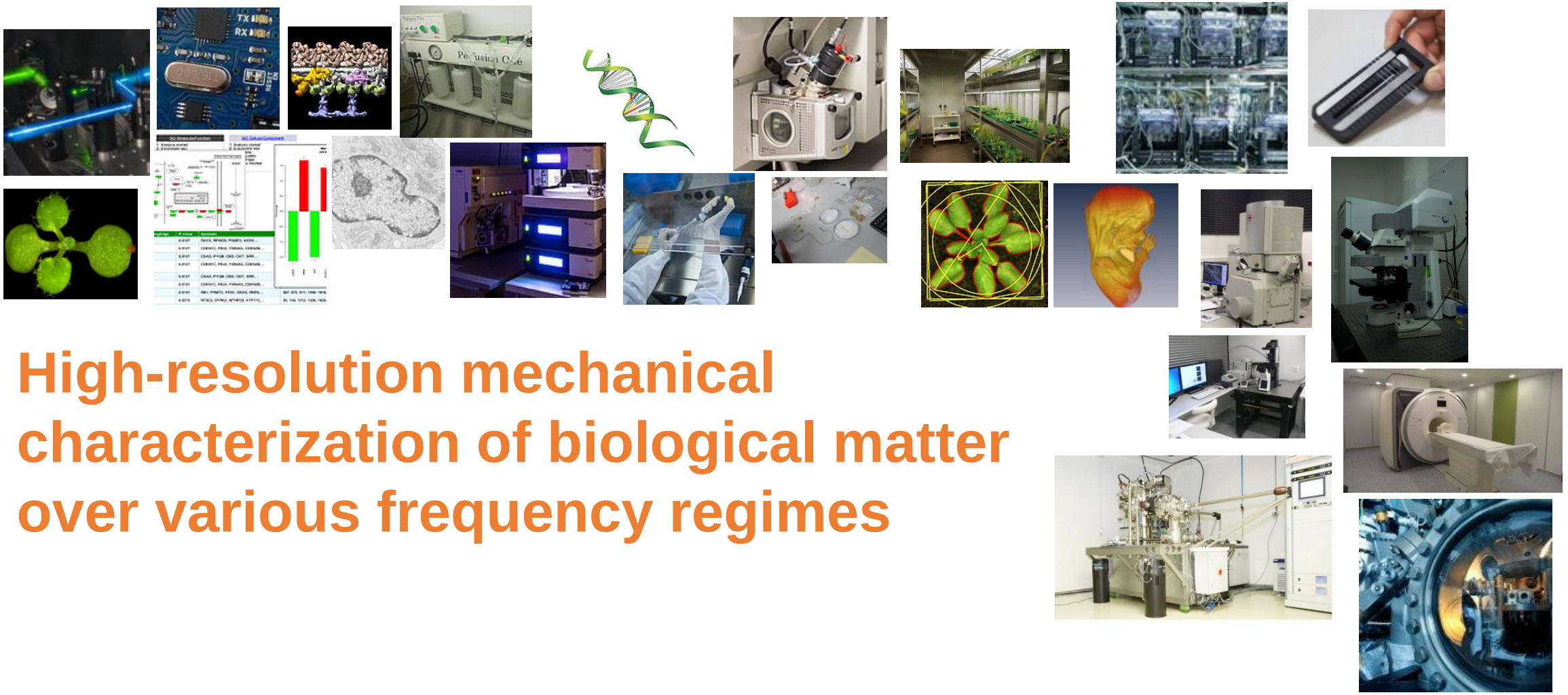

High-resolution mechanical characterization of biological matter over various frequency regimes

Kareem Elsayad (VBCF), Jan Přibyl (CEITEC-MU)

24. 09. 2018

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Pilot project introduction

Project partners:
Jan Prybl (CEITEC), Petr Skladl (CEITEC), Kareem Elsayad (VBCF), Carina Pleha (VBCF)

Goal:
*Connecting, correlating, and complimenting **AFM microscopy/spectroscopy** measured mechanical properties (CEITEC MU, CF NanoBiotechnology) and **Brillouin Microscopy** measured mechanical properties (VBCF Advanced Microscopy, Vienna).*

The two techniques provide complimentary information which together can tell us more about the mechanical properties of a sample:

Determine the type of systems/samples and conditions in which such measurements are best performed, make sense, and are most useful

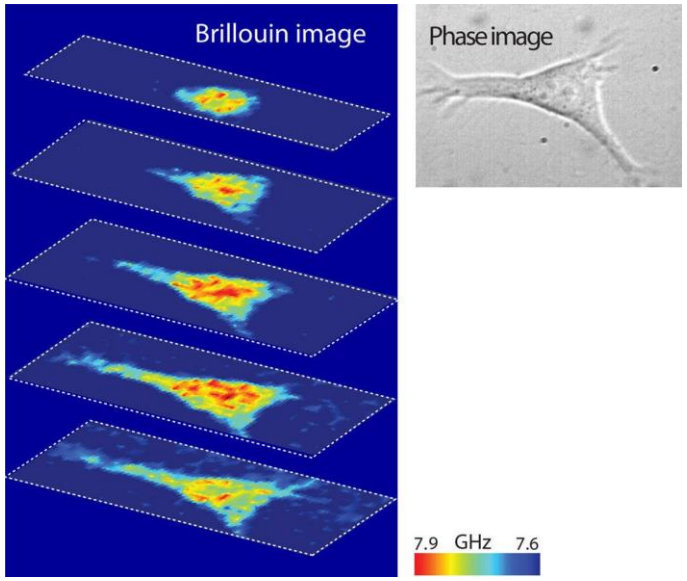
Establish a pipeline (combined service) where measurements on the same sample can be performed and interpreted using the two techniques most efficiently

Pilot project introduction

Brillouin Microscopy (VBCF)

Measures Longitudinal Modulus
Measures in GHz frequency-regime

3D confocal reconstruction—obtained via Brillouin microscopy (fibroblast cell)

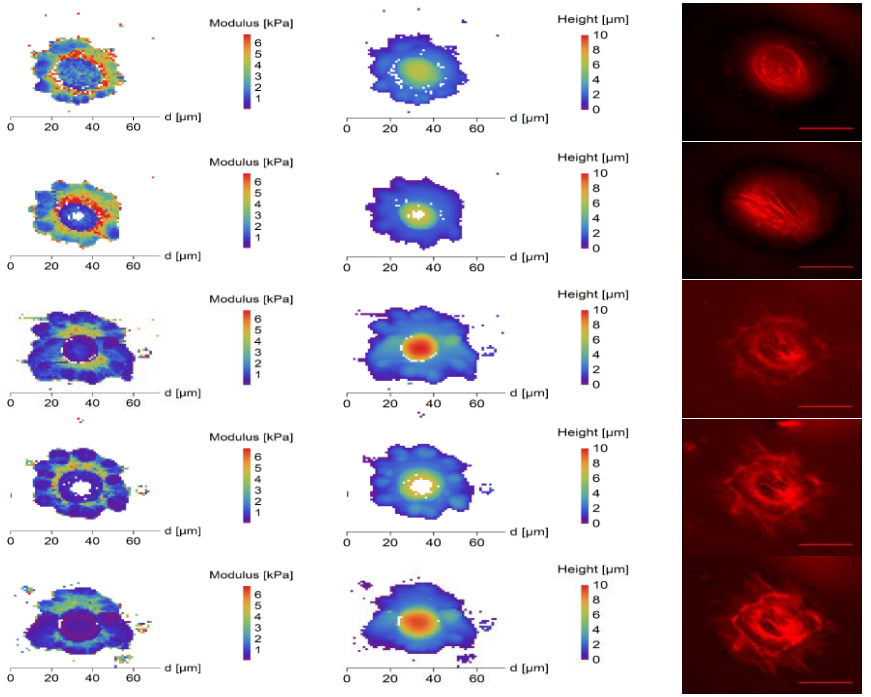


http://spie.org/newsroom/6698-all-optical-mapping-of-the-mechanical-properties-of-cells?SSO=1

Atomic Force Microscopy (CEITEC)

Measures Young's Modulus
Measures in <kHz frequency-regime

AFM - Young's Modulus map (left), height (in the middle) and fluorescence images of fibroblast cytoskeleton (right)



Front. Physiol. 9:804

High-resolution mechanical characterization of biological matter over various frequency regimes

Pilot project introduction

Brillouin Microscopy (VBCF)

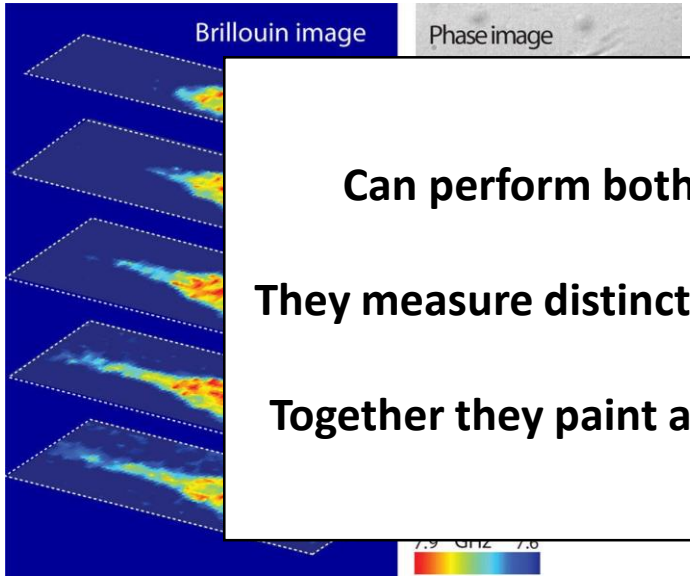
Measures Longitudinal Modulus
Measures in GHz frequency-regime

…related by "Poisson Ratio"

Atomic Force Microscopy (CEITEC)

Measures Young's Modulus
Measures in <kHz frequency-regime

3D confocal reconstruction—obtained via Brillouin microscopy (fibroblast cell)



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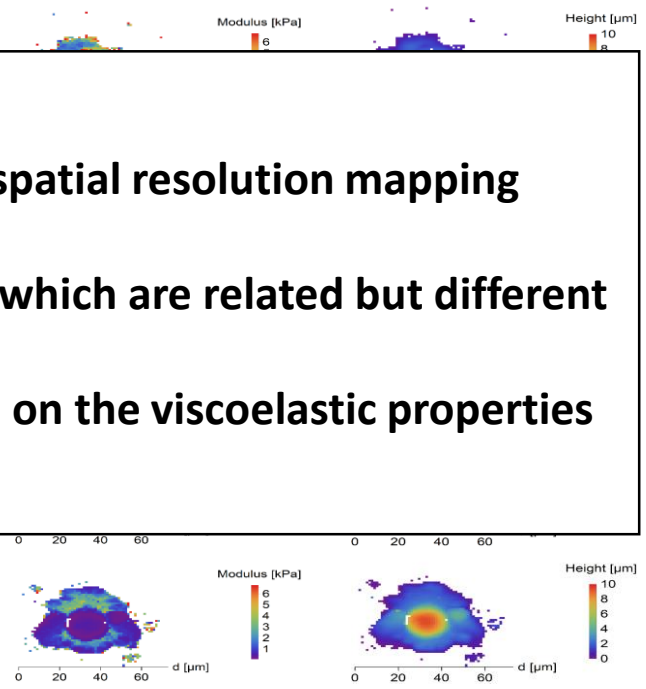
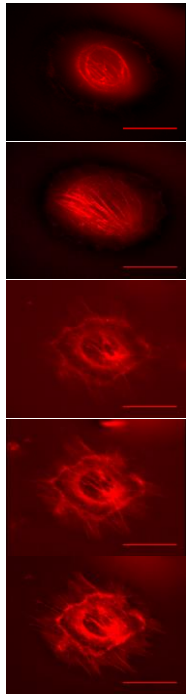




Front. Physiol. 9:804

Can perform both on live cells with high spatial resolution mapping

They measure distinct mechanical properties which are related but different

Together they paint a more complete picture on the viscoelastic properties

High-resolution mechanical characterization of biological matter over various frequency regimes

Pilot project introduction

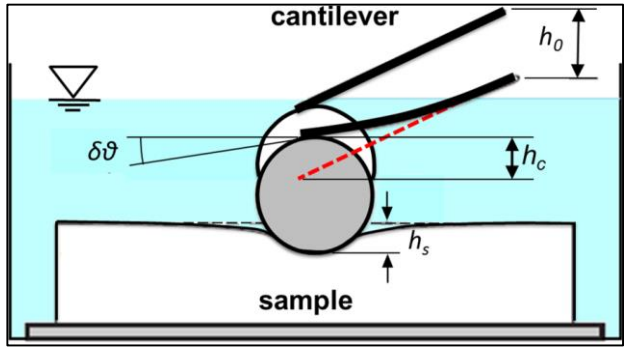
- Potential end-users:

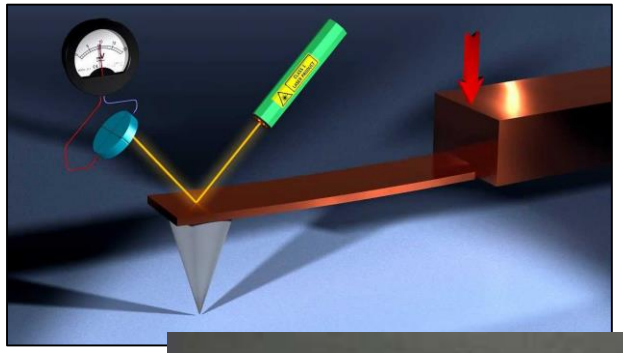
Mostly academic users – possible candidates:

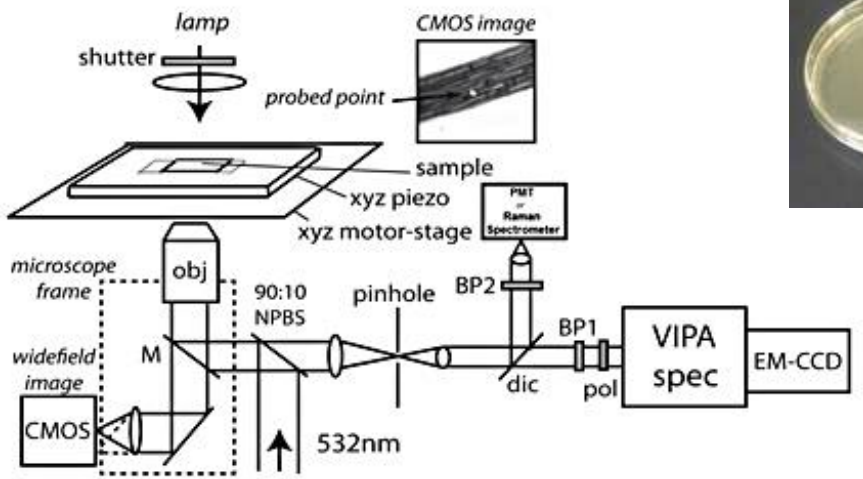
- *Giancarlo Forte, ICRC Brno – dECM samples*
- *Eva Benkova, IST Austria – plant tissues*
- *Jan Hejátko, CEITEC MU - plant tissues*
- *Daniel Hadraba, Institute of Physiology CAS*
- *Irena Kratochvilova, Institute of Physics CAS*
- *Vladimir Rotrekl, Faculty of Medicine, MU*
- *Daniel Gerlich, IMBA, Vienna*
- *Youssef Belkhadir GMI, Vienna*
- *Josef Penninger IMBA, Vienna*
- *Ulrich Technau, University of Vienna*
- *Sabine Eichinger, Medical University of Vienna*
- *…*

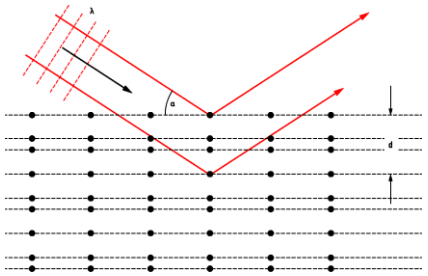
Project implementation

- *Approach/methodology*

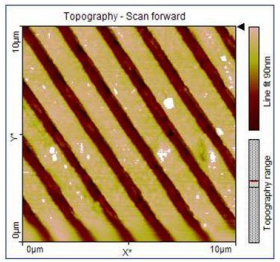




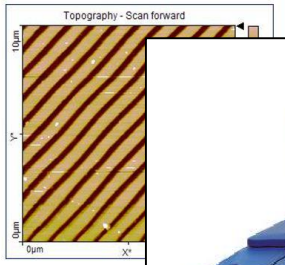




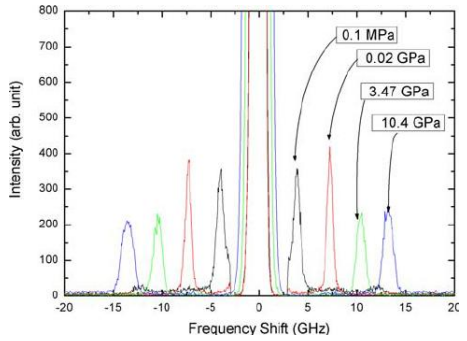
AFM Images of CD and DVD (unrecorded)

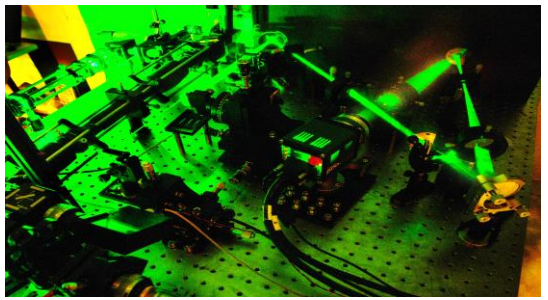


CD-Blank
(10 µm scan)
Track pitch = 1.57 µm



DVD-
(10 µm
Track pitch



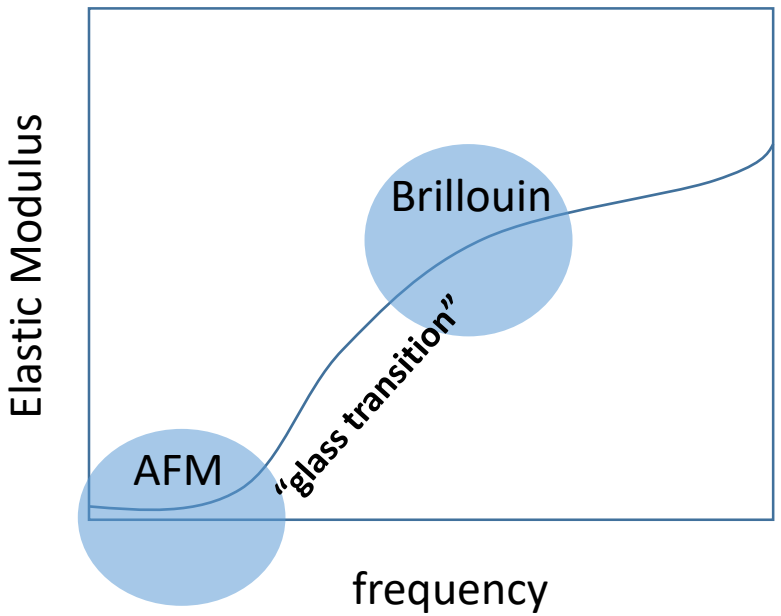


There is no budget for staff exchanges.
We are cooperating by exchanging the samples and results („remote control“).

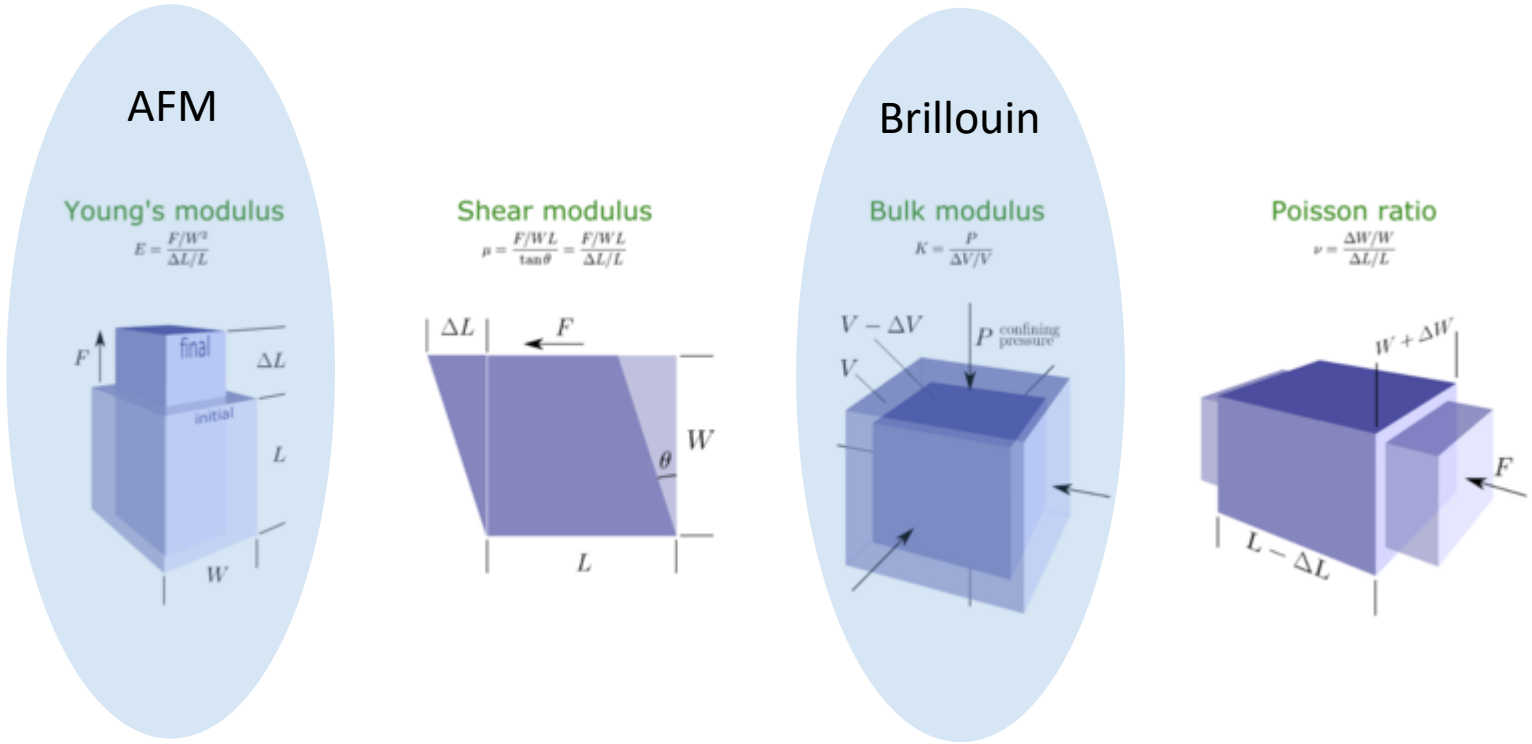
Project results

How do the measurements even compare?

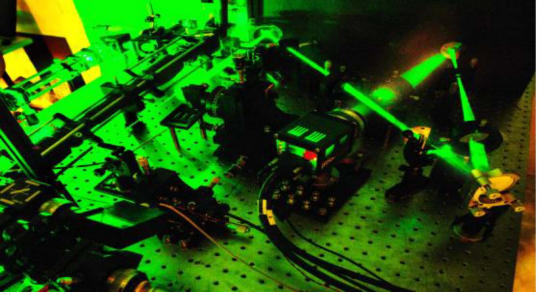
Different relaxation mechanism(s)



Different boundary conditions



Different Moduli important for different processes



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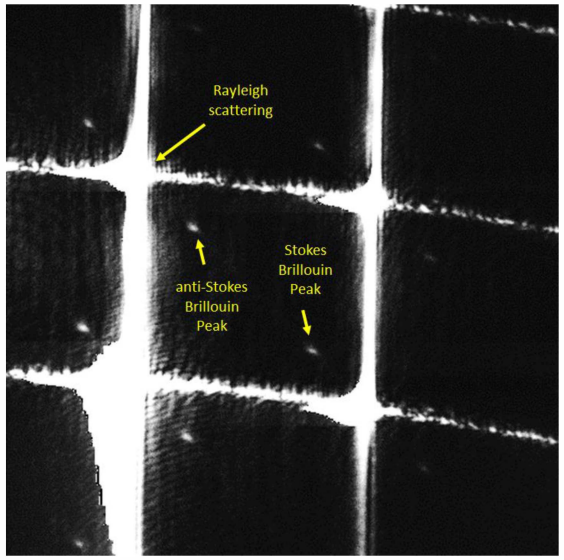

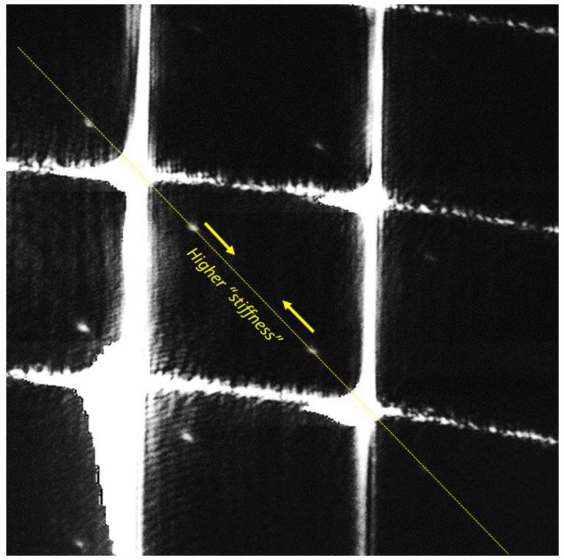

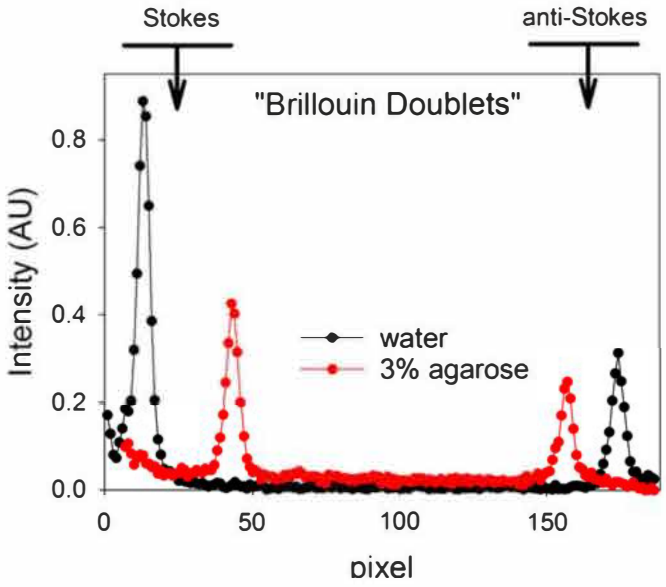

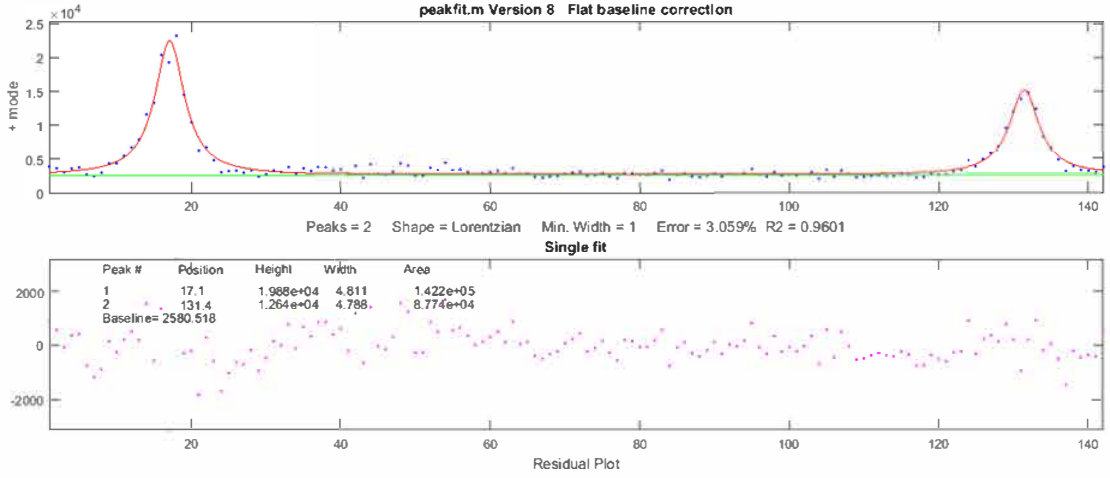

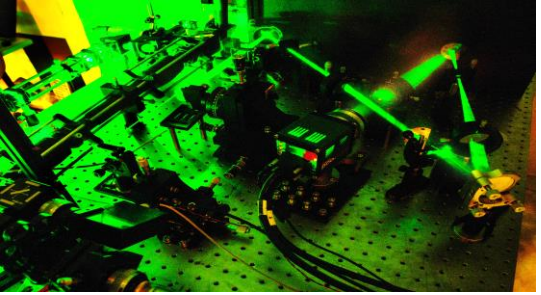

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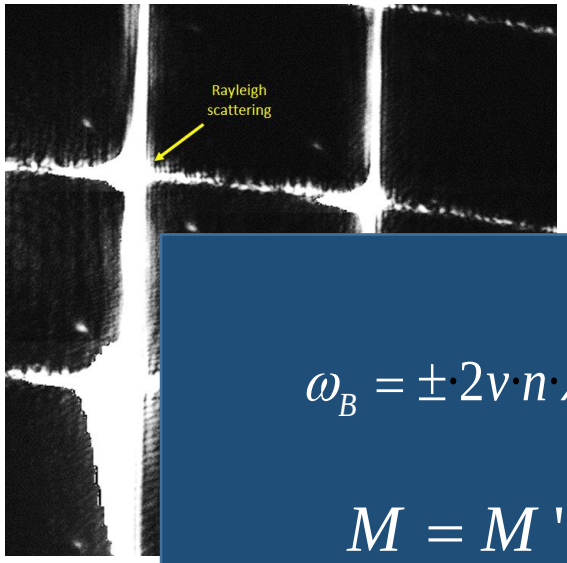
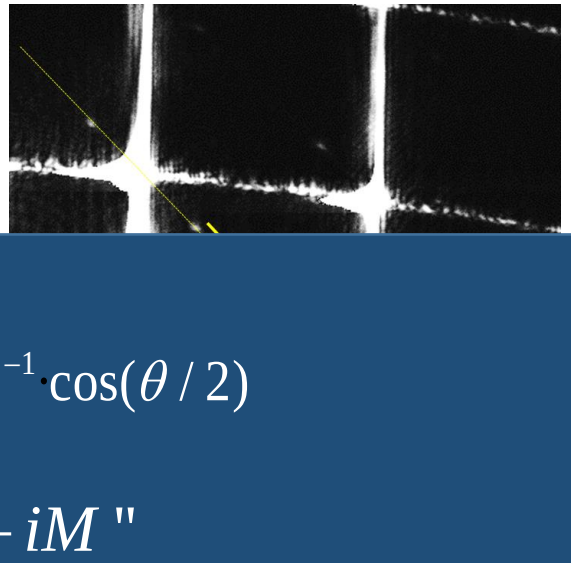
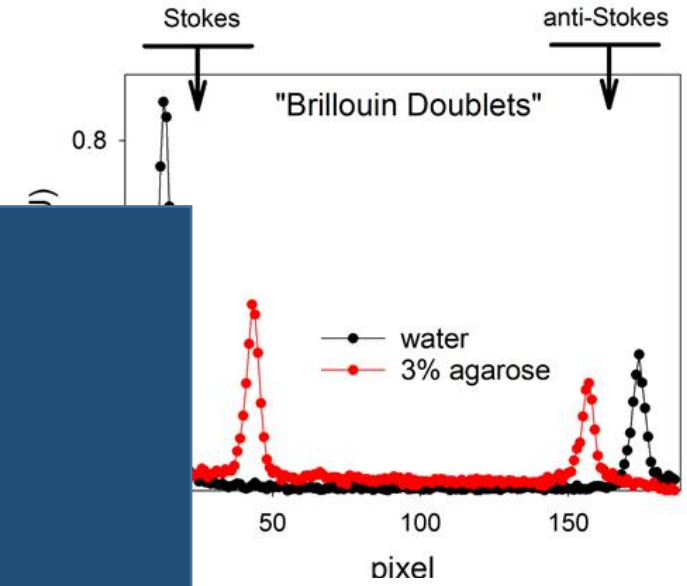






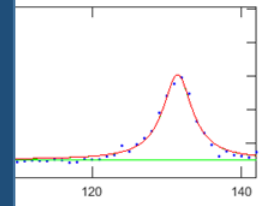
$$\omega_B = \pm 2v \cdot n \cdot \lambda^{-1} \cdot \cos(\theta/2)$$

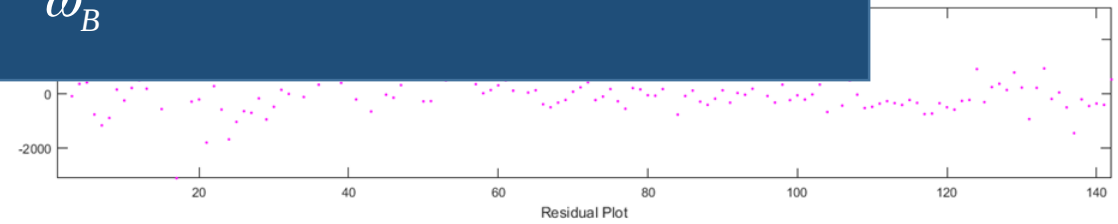
$$M = M' + iM''$$

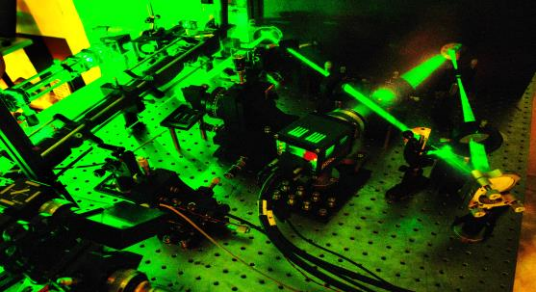
$$M' = v^2 \cdot \rho$$

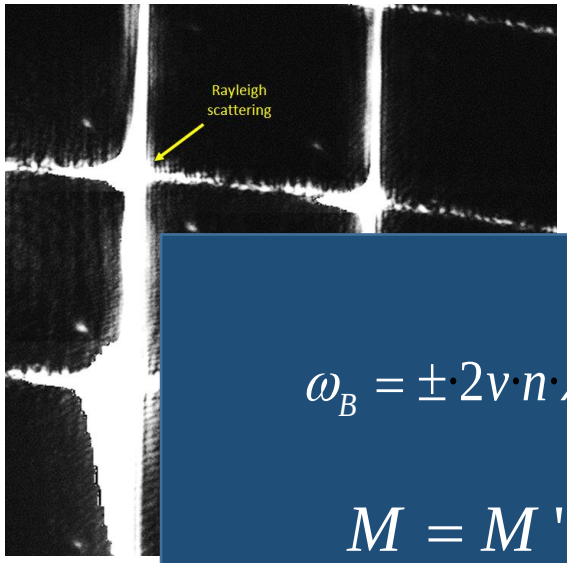
$$M'' = \frac{v^2 \cdot \rho \cdot \Delta\omega_B}{\omega_B}$$

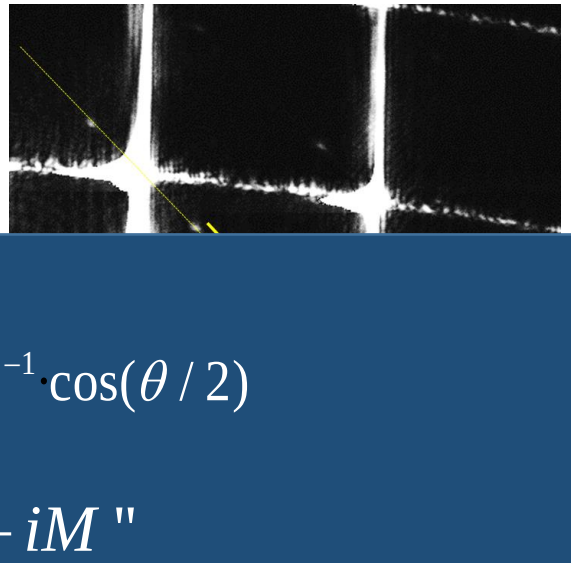
M = Longitudinal Modulus

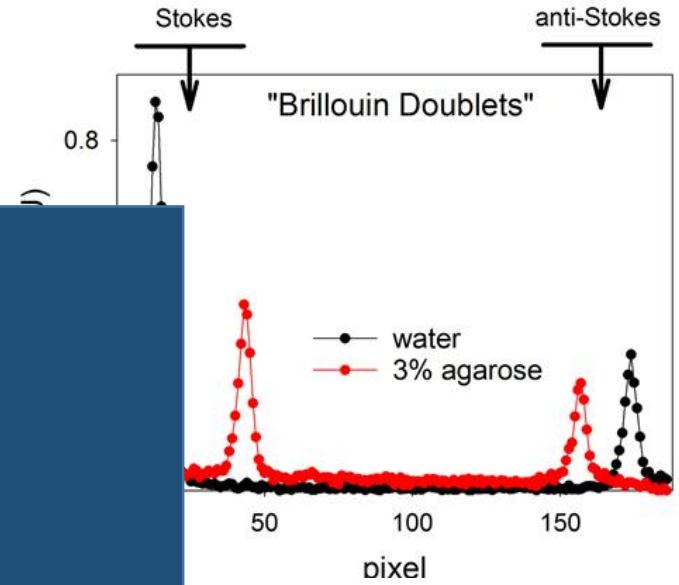












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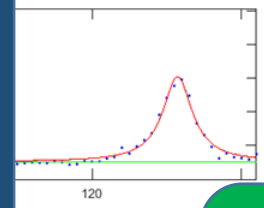
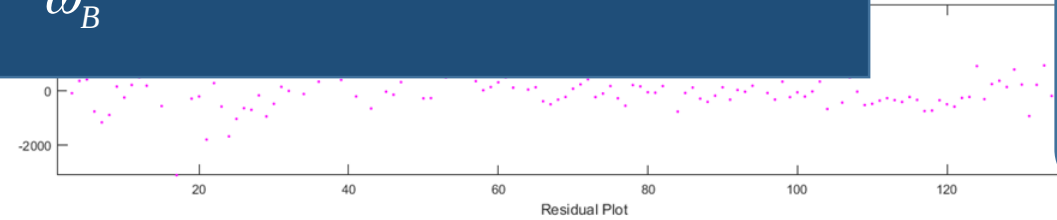


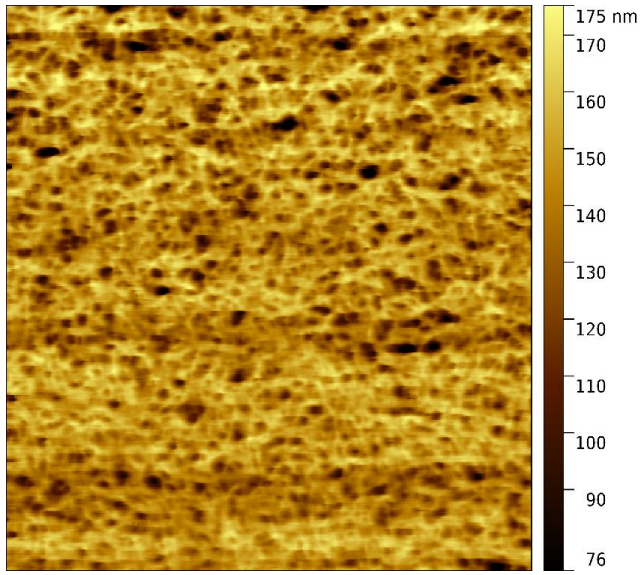


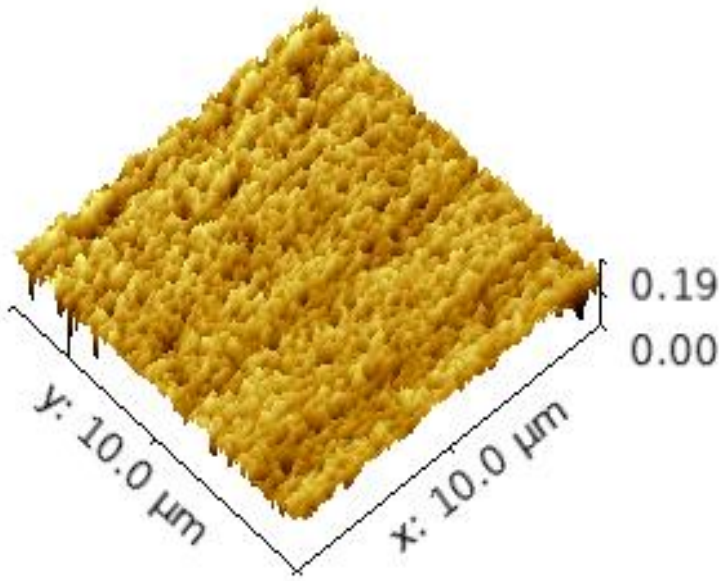
(+) 3d spatial mapping possible

Project results

How do the measurements compare?

Agarose samples of different concentrations (0-2%)





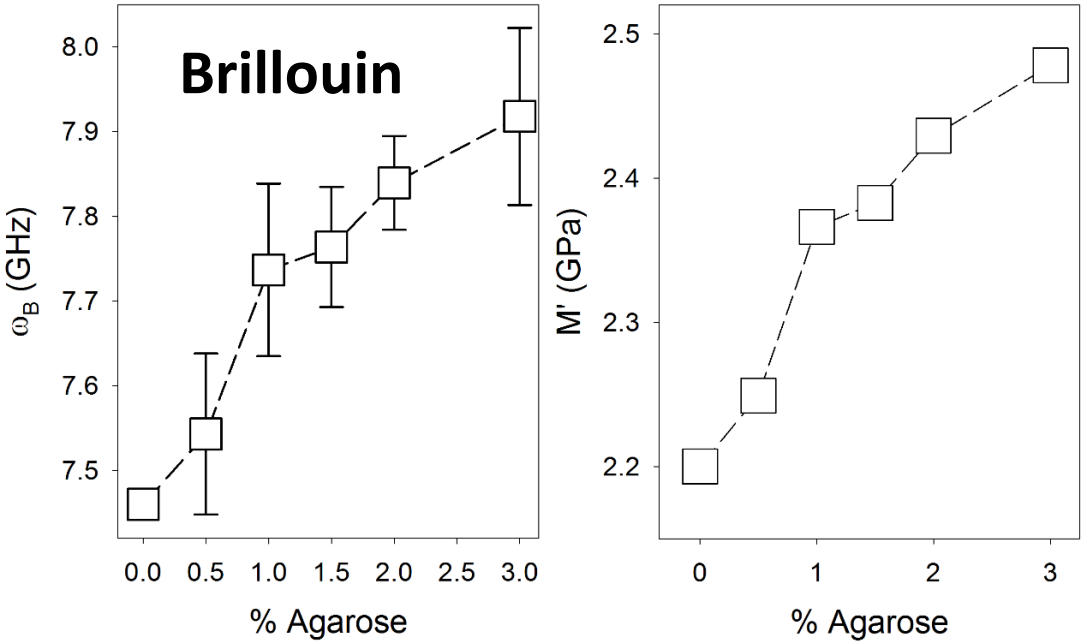
10*10µm topography
2% Agarose w/v

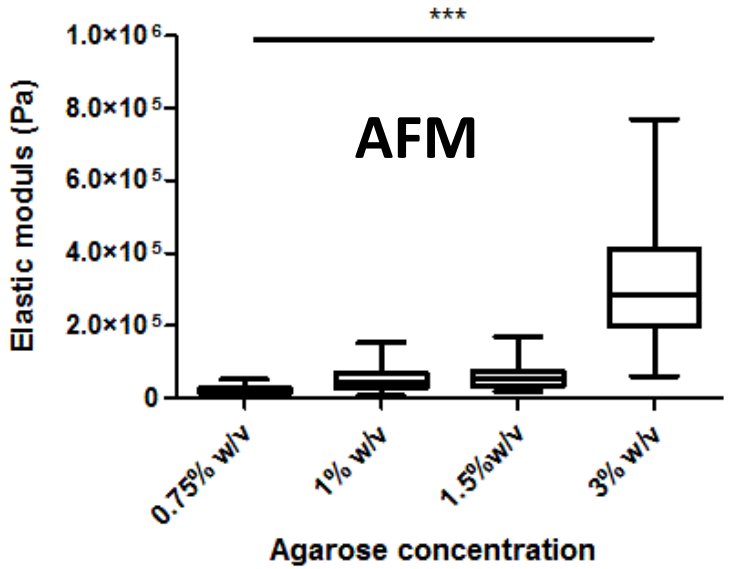
Surface imaging (with HYDRA-ALL B)

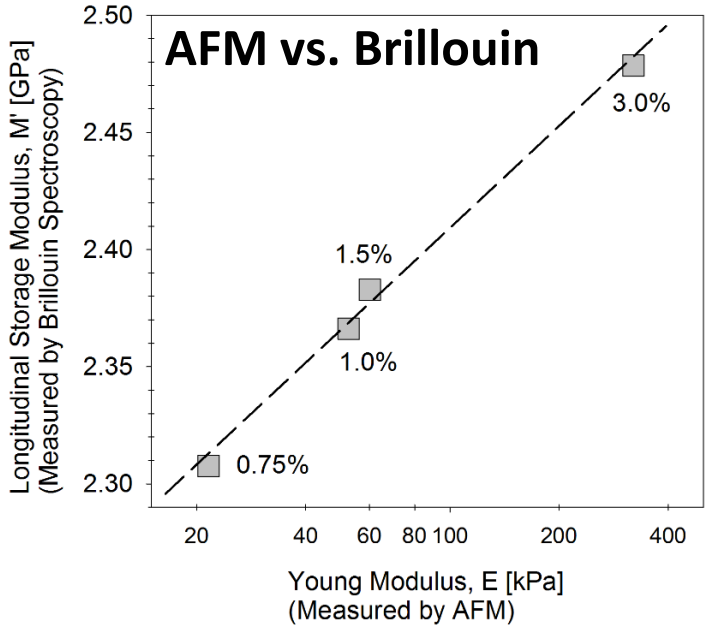
Project results

How do the measurements compare?

Agarose samples of different concentrations







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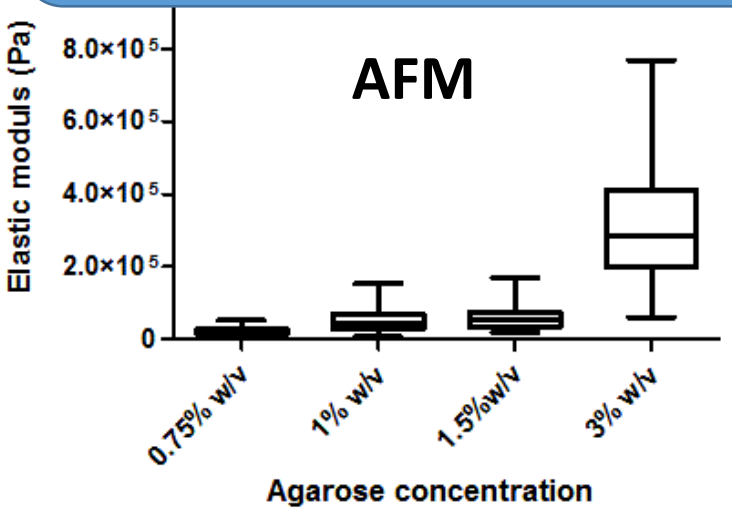
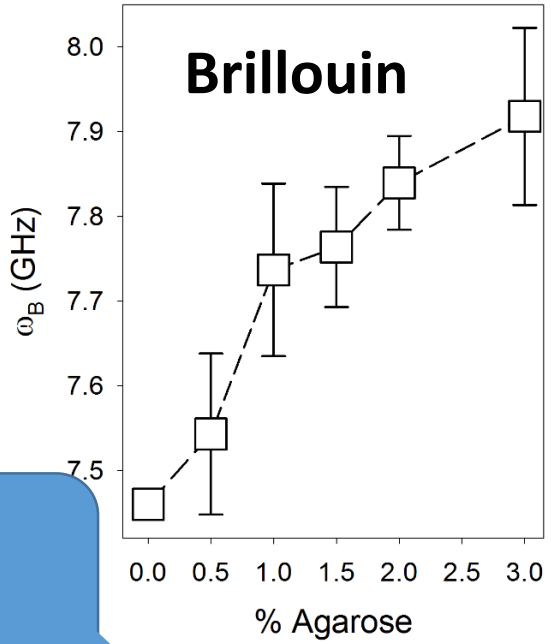
Project results

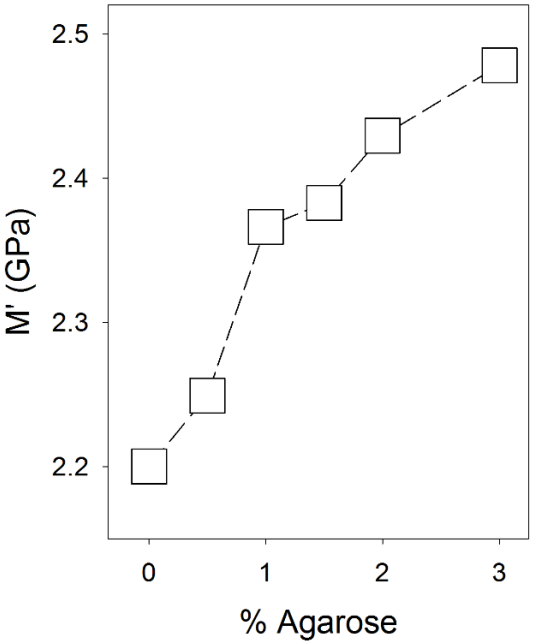
How do the measurements compare?

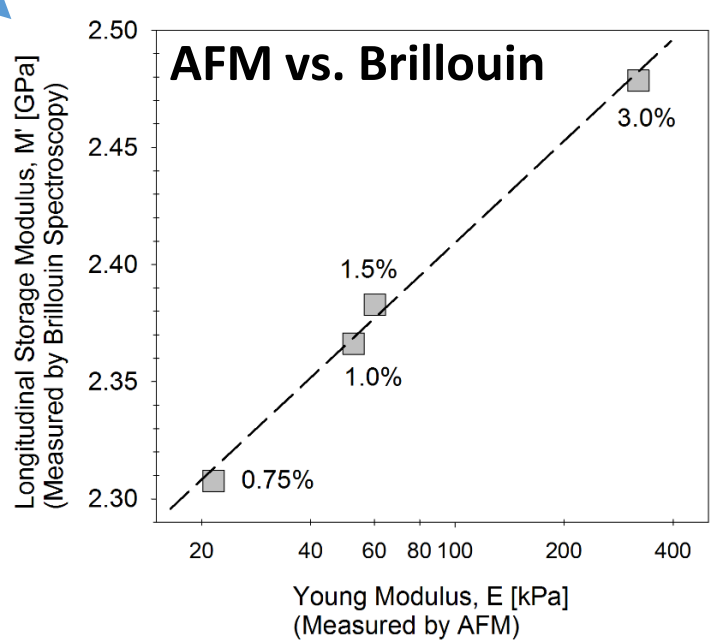
Agarose samples of different concentrations

Power law or logarithmic relation









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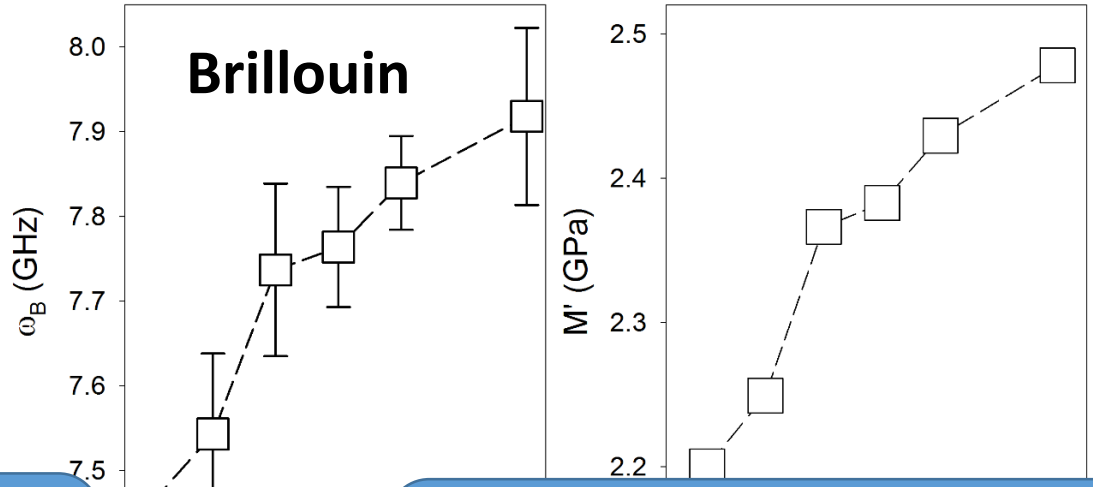
Project results

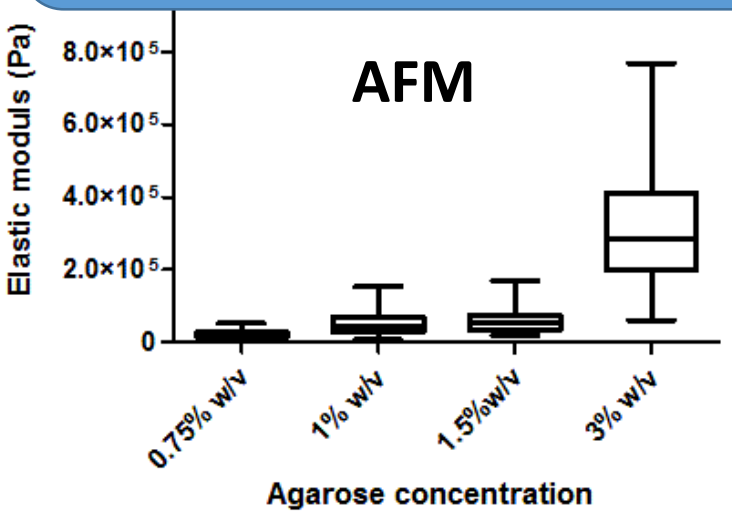
How do the measurements compare?

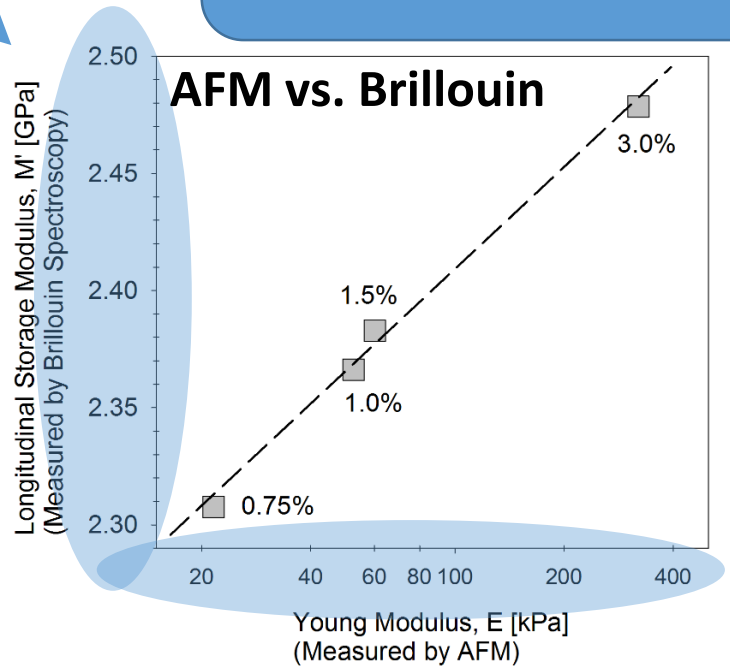
Agarose samples of different concentrations



Power law or logarithmic relation

AFM (Brillouin) may be better for "stiffer" ("softer") samples





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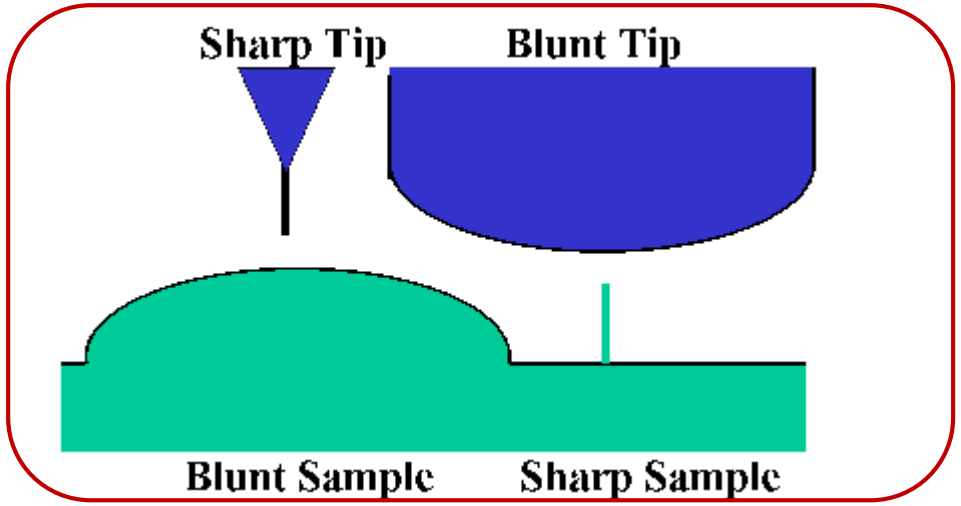
Project results

What about structural features?

“real” biological samples are not homogeneous on sub-micron/micron scales

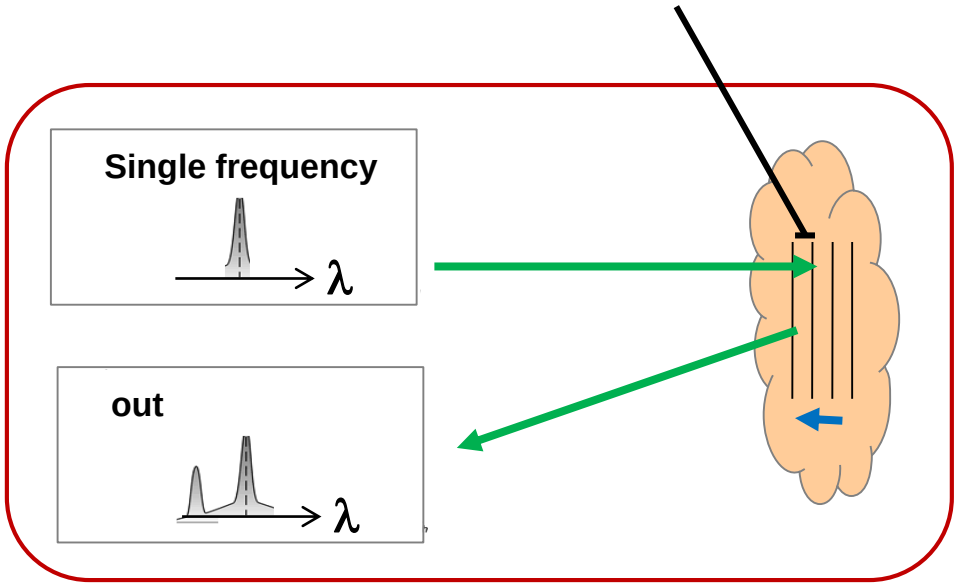
AFM

probes an area the size of the tip



Brillouin

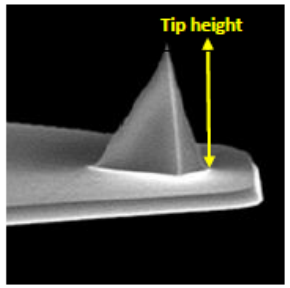
probes area on the size of acoustic wavelength (~100-200nm)

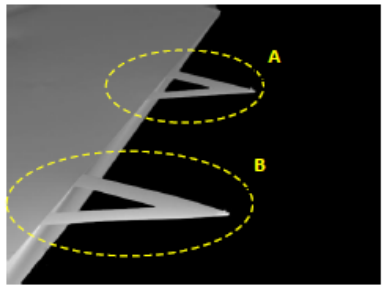


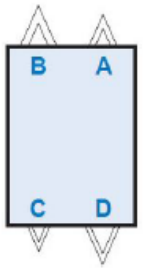
FIB milling of AFM tips

Plan

Bruker SNL10 A+B

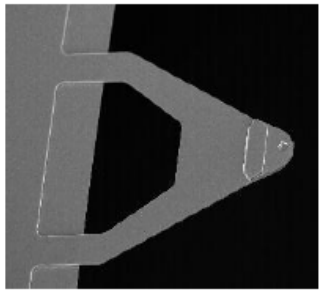


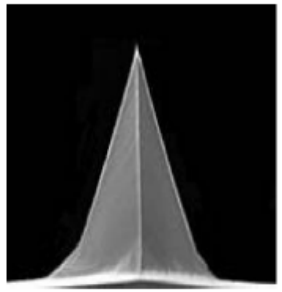


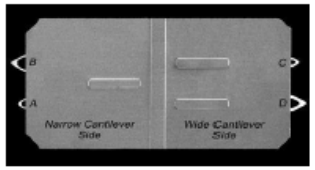


- **Silicon / silicon nitride tip** on silicon nitride cantilever
- Cantilever spring constant **0.080 – 0.200 N/m**
- Tip height **4-8 µm**

AppNano Hydra 100N-6V







Modify size of AFM probing area

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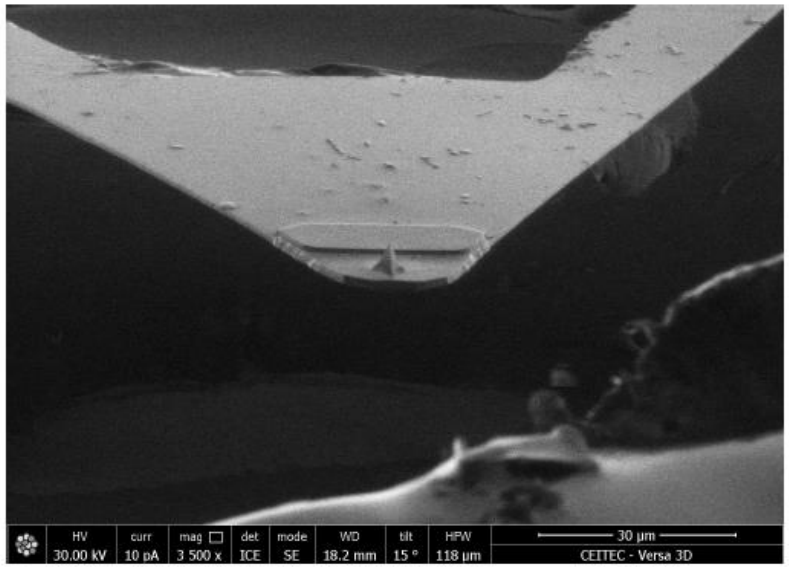
FIB milling of AFM tips
Plan

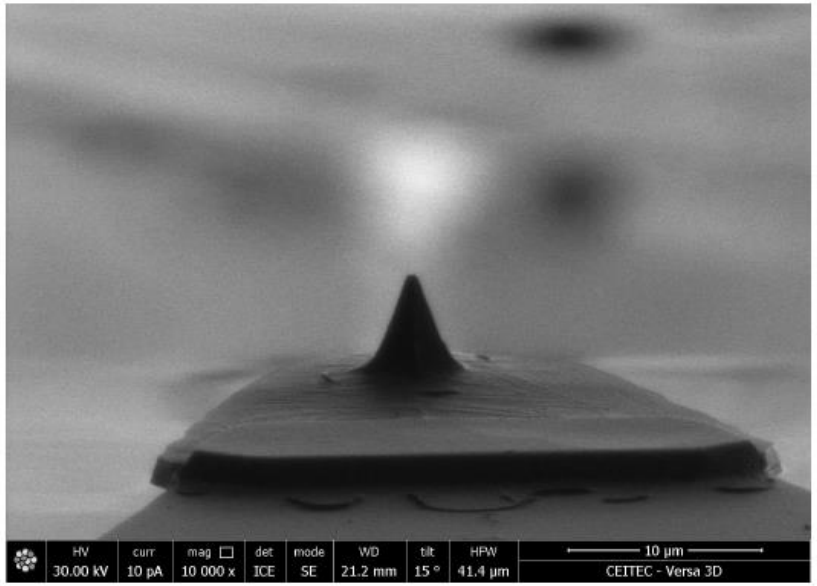
Bruker SNL10 A+B

FIB milling of AFM tips
Results



AppNano Hydra 100N-6V

Tip localization on cantilever



Modify size of AFM probing area

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FIB milling of AFM tips
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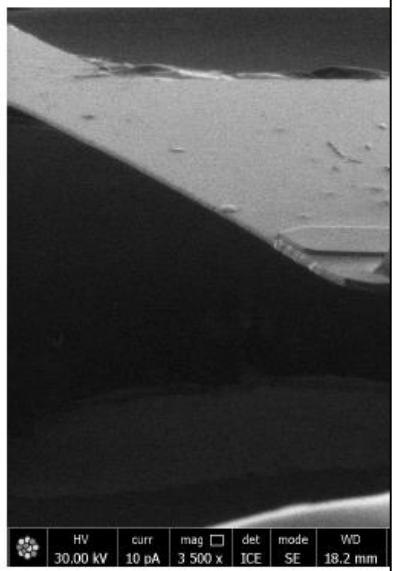
Bruker SNL10 A+B

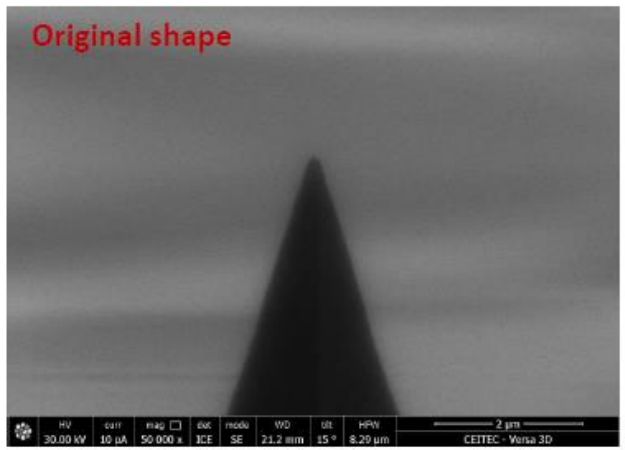
FIB milling of AFM tips
Results

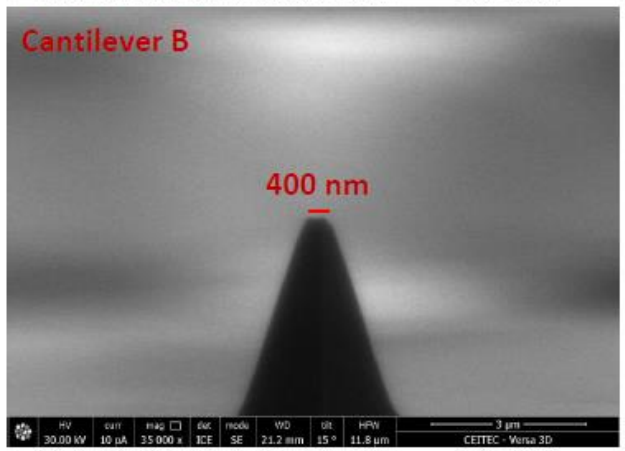


AppNano Hydra 100N-

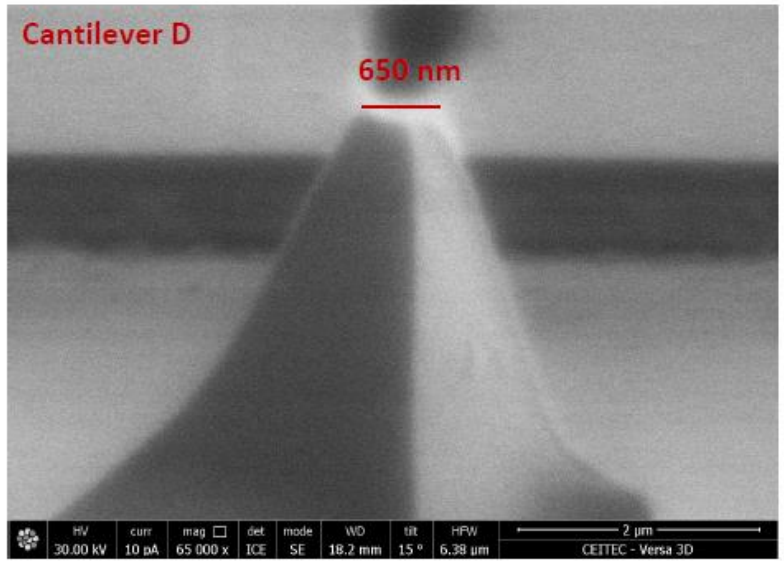
Tip localization on cantil

AppNano Hydra 100N-6V





FIB milling of AFM tips
Results



- Silicon nitride cantilever is very soft → bending in the stream of ions (FIB)
- Even low energy FIB has high energy for gentle milling

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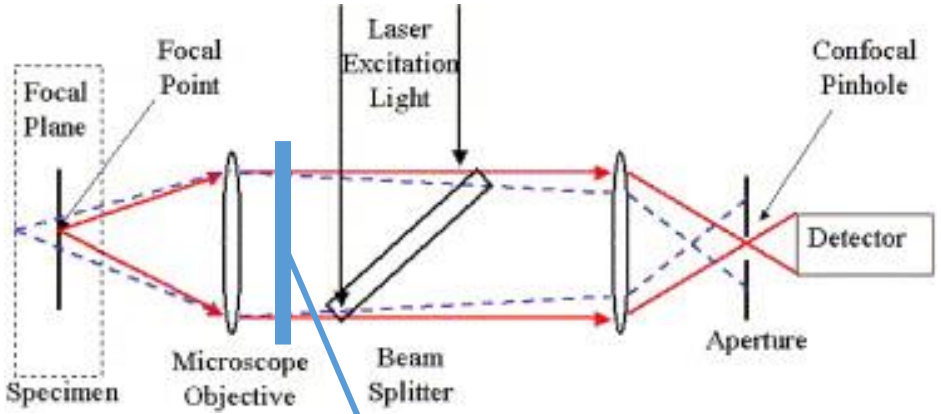
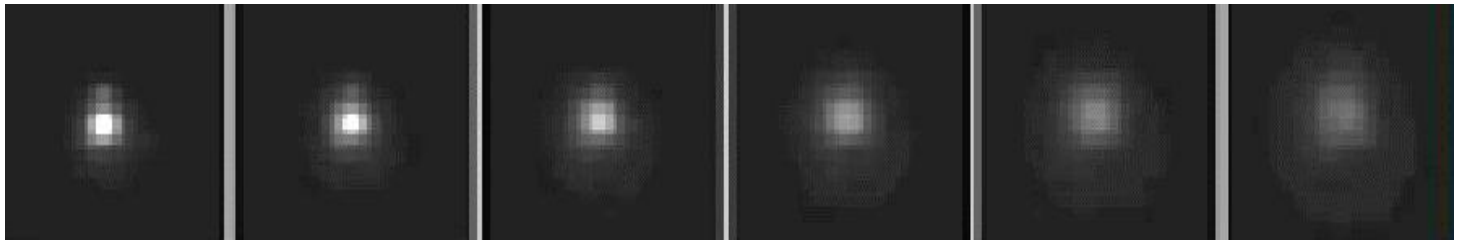
Modify probing volume in Brillouin



(spectrometer)

…by adjusting iris opening
-changes numerical aperture
-changes probing volume



FWHM~200nm

FWHM~600nm

Effective Point Spread Function (PSF) = ***probing volume***

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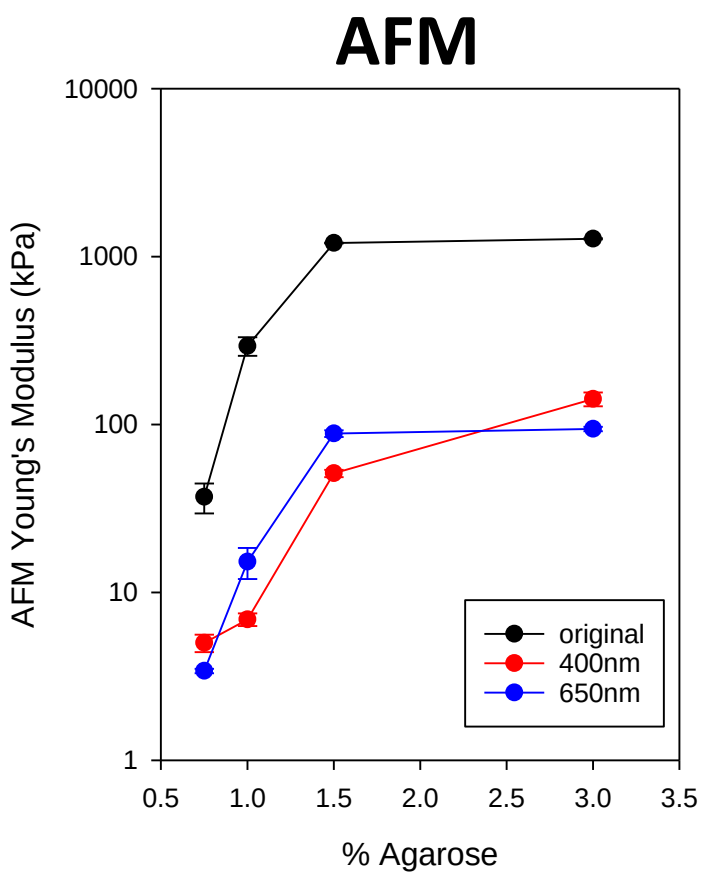
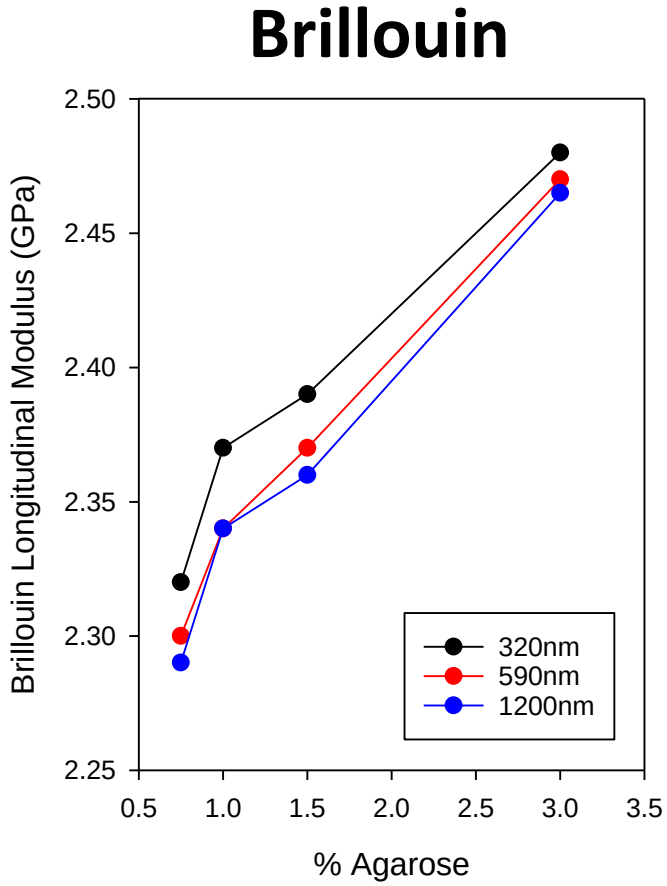
Dependence on probing volume (agarose series)





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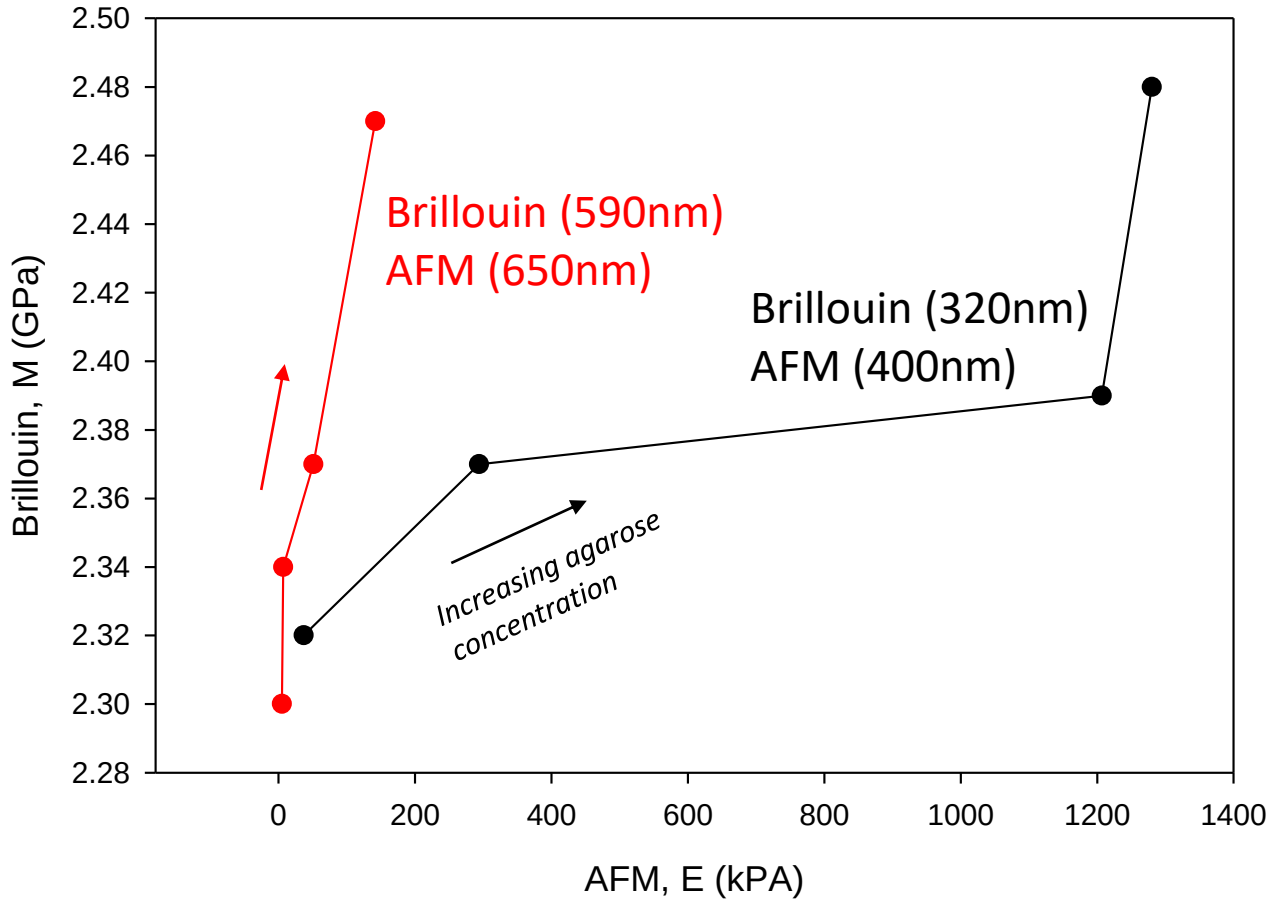
Dependence on probing volume (agarose series)



While AFM is very sensitive to probing volume
Brillouin is largely insensitive in the measured range

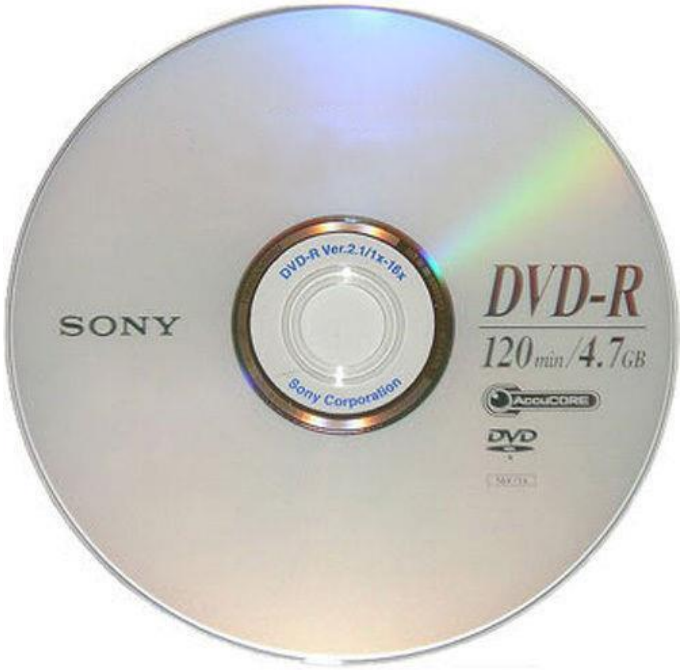
Conclusion:

Chosen probing volume in Brillouin is not critical (likely defined by acoustic length), whereas in AFM it is.

Comparative studies should account for this

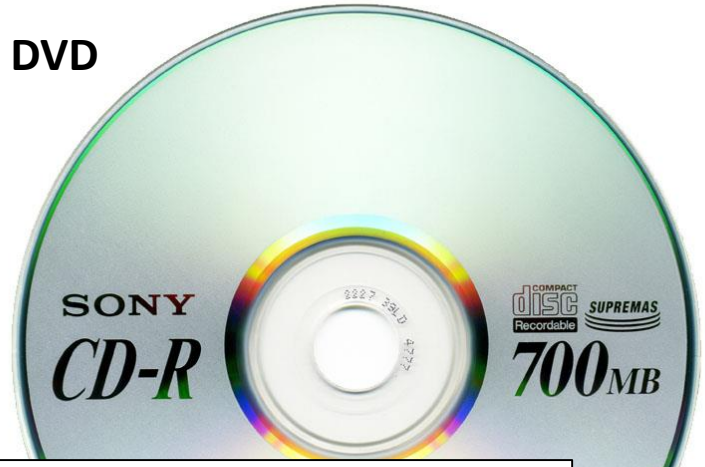
This affects the spatial sampling distances/probes that should be chosen for comparative AFM and Brillouin measurements

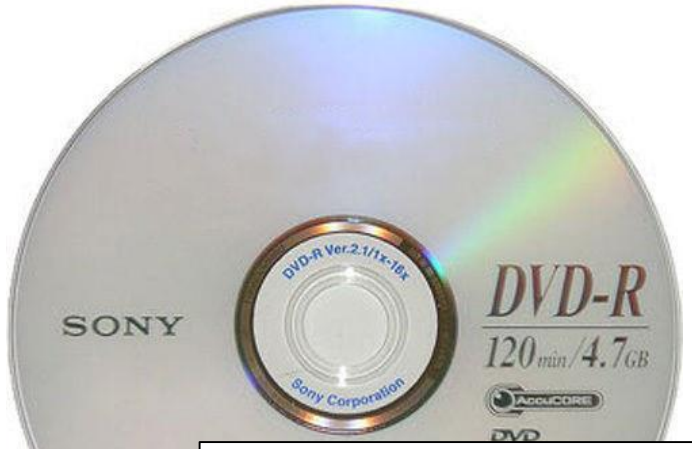
Systematic investigation into structured samples



Systematic investigation into structured samples

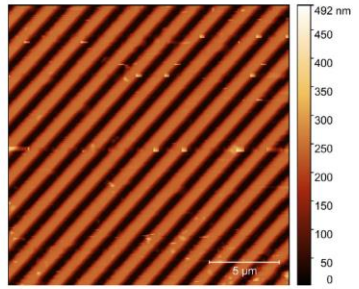
Use un-written CD and DVD as “stamp” on agarose

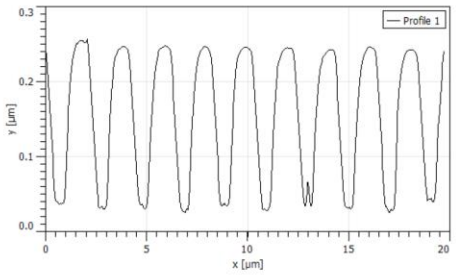


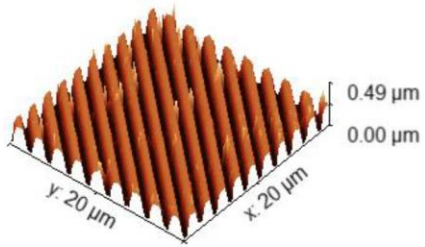


CD-ROM Verbatim

Average height 210 nm
Pitch to pitch distance 1.5 µm

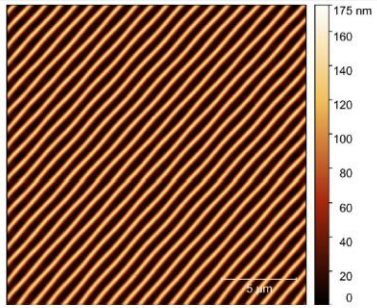


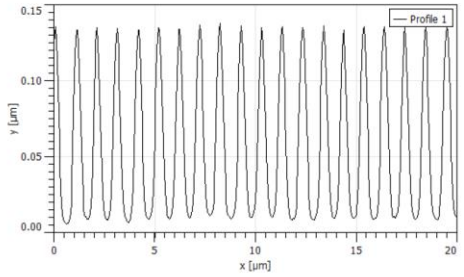


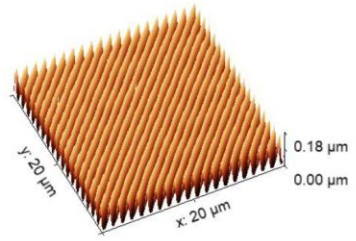


DVD Verbatim

Average height 115.2 nm
Pitch to pitch distance 0.78 µm

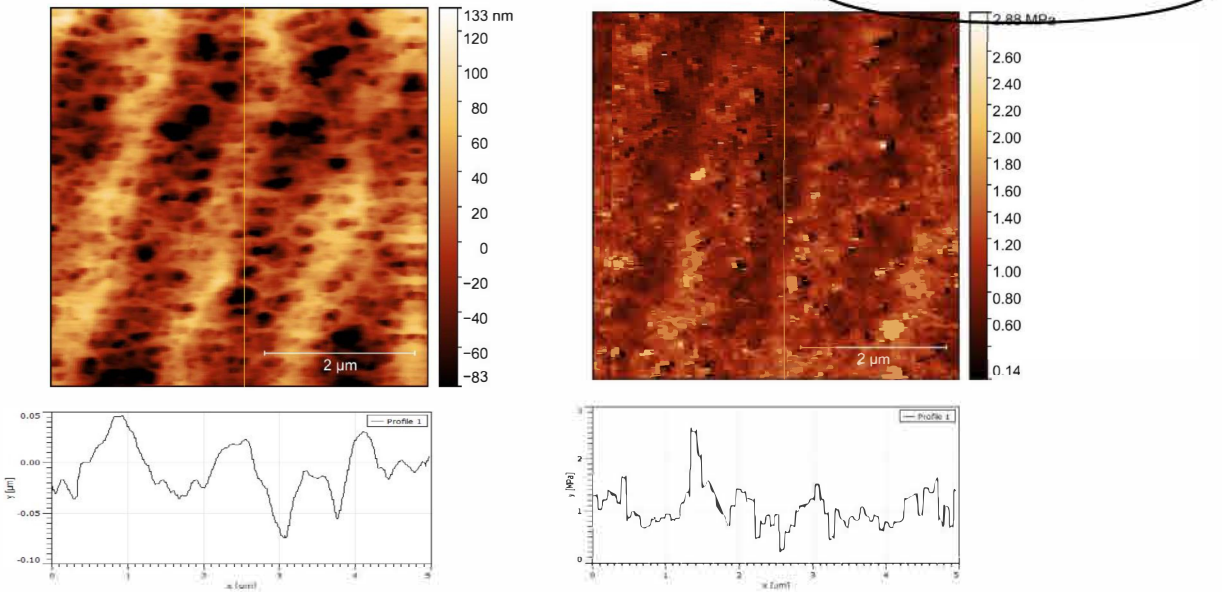






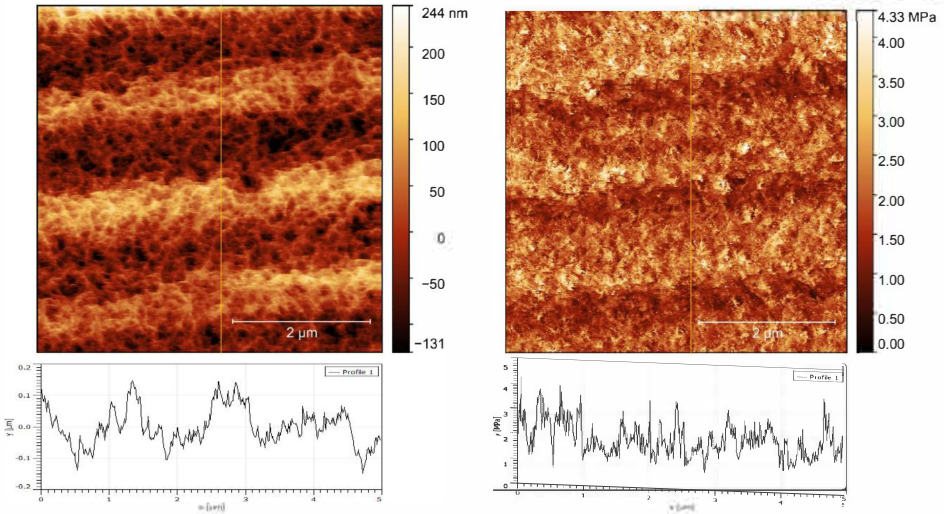
Agarose 3%, CD Kodak stamp

Average height 50.7 nm
Pitch to pitch distance 1.55 µm
Average stiffness: 956 ± 241 kPa



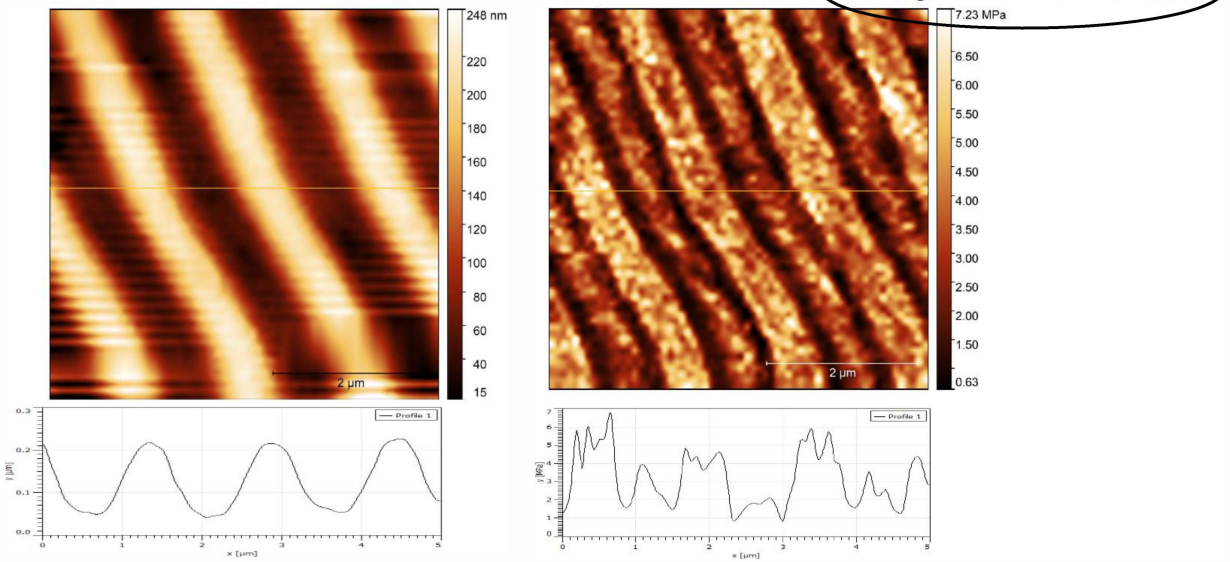
Agarose 3%, CD Verbatim stamp

Average height 110.8 nm
Pitch to pitch distance 1.53 µm
Average stiffness: 1.95 ± 0.54 MPa



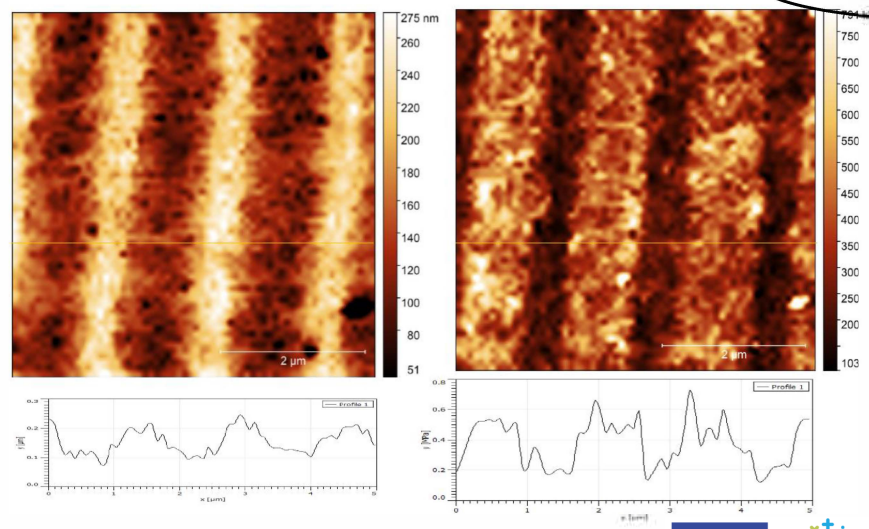
Agarose 3%, CD Kodak stamp

Average height 162.6 nm
Pitch to pitch distance 1.41 µm
Average stiffness: 3.22 ± 1.21 MPa



Agarose 3%, CD Verbatim stamp

Average height 127.4 nm
Pitch to pitch distance 1.47 µm
Average stiffness: 357 ± 109 kPa



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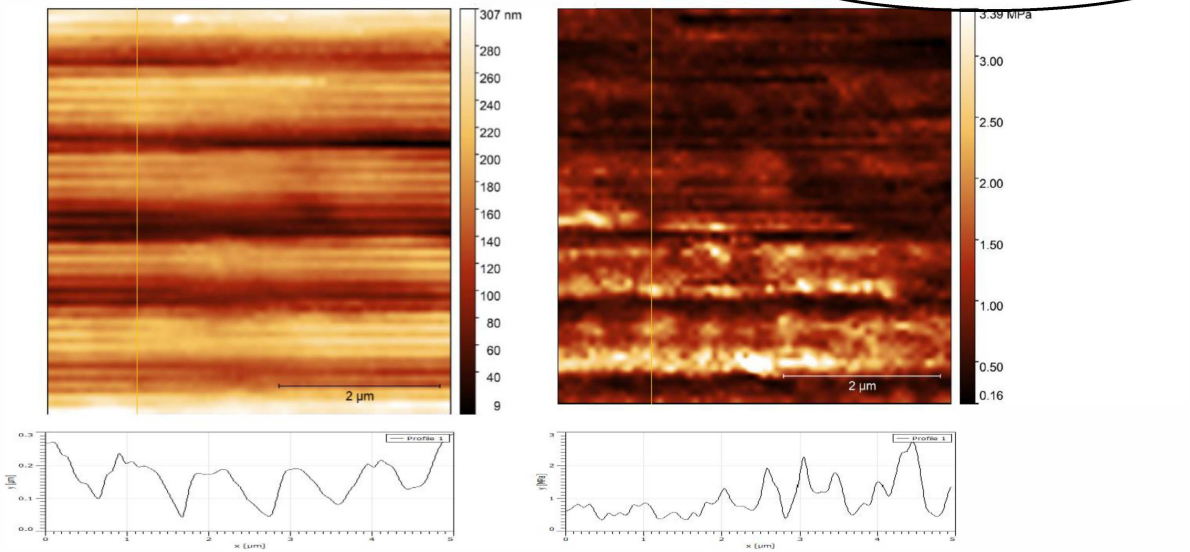
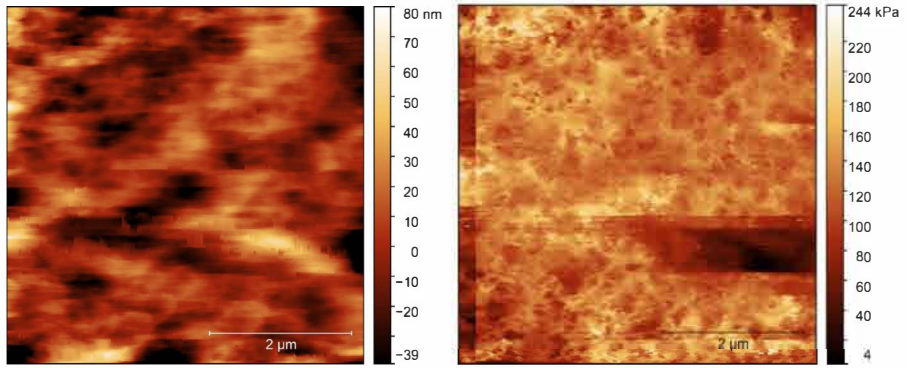
Agarose 3%, DVD Verbatim stamp

Average height 121.6 nm
Pitch to pitch distance 0.987 µm
Average stiffness: 984 ± 442 kPa



Agarose 3%, DVD Verbatim stamp

~~Average height~~
~~Pitch to pitch~~
Average stiffness: 121 ± 23 kPa



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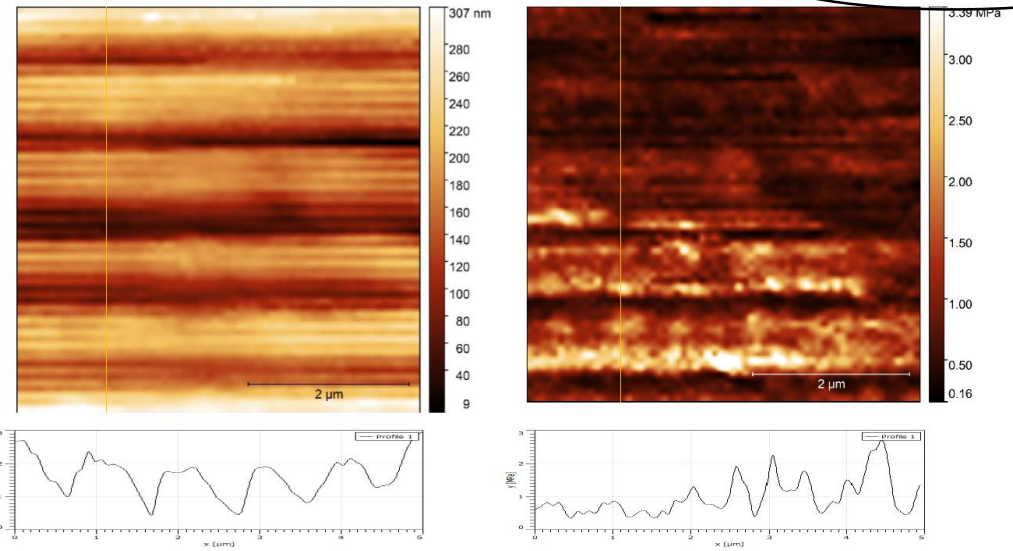
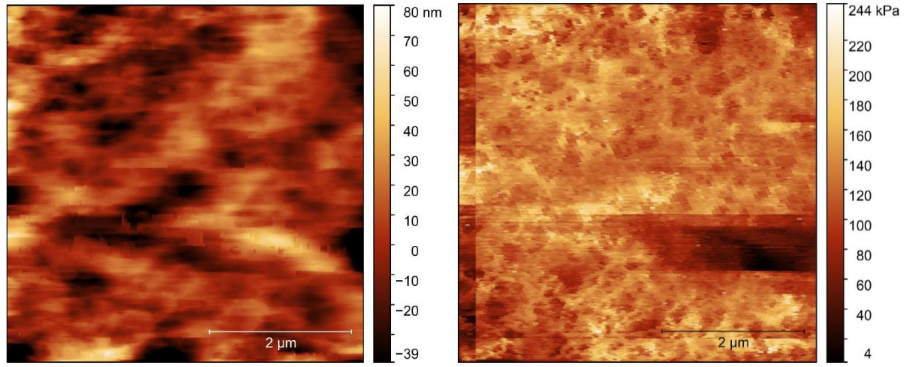


Agarose 3%, DVD Verbatim stamp

~~Average height~~
~~Pitch to pitch~~
Average stiffness: 121 ± 23 kPa



Brillouin scans with series of probing volumes performed last week and currently being analyzed

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Conclusions

- Set up the basis for correlative AFM – Brillouin studies
- Established details on sample mounting and how to perform efficient site-matched studies
- Ongoing work on details of interpretation of data in light of different measurement modalities
- Next step should be proof-of-principle studies on real (live) biological samples, but current funding/resources are limiting this.
- **Can now perform select open access projects**