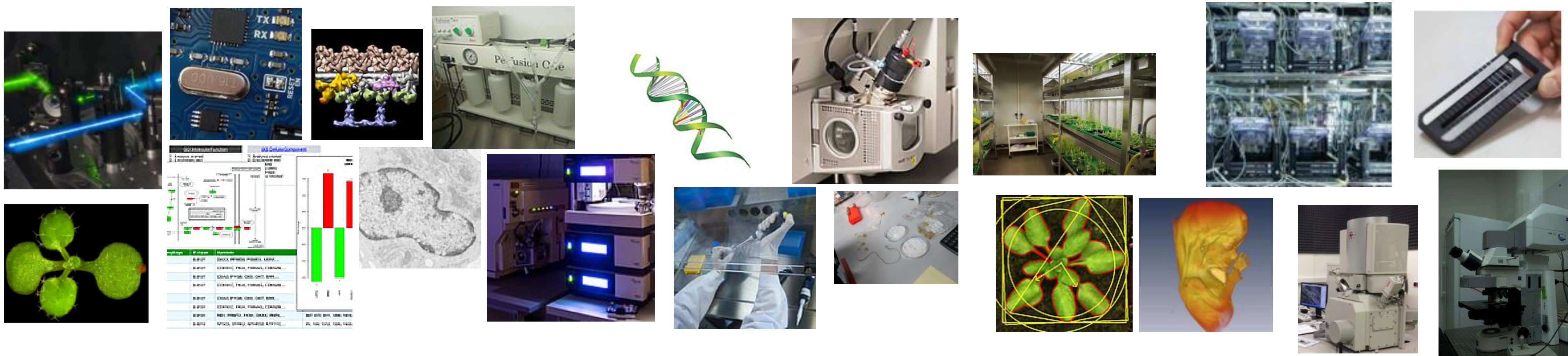

High-resolution mechanical characterization of biological matter over various frequency regimes

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

24. 09. 2018

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European Regional Development Fund

Pilot project introduction

Project partners:
Jan Pribyl (CEITEC), Petr Skladal (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).*

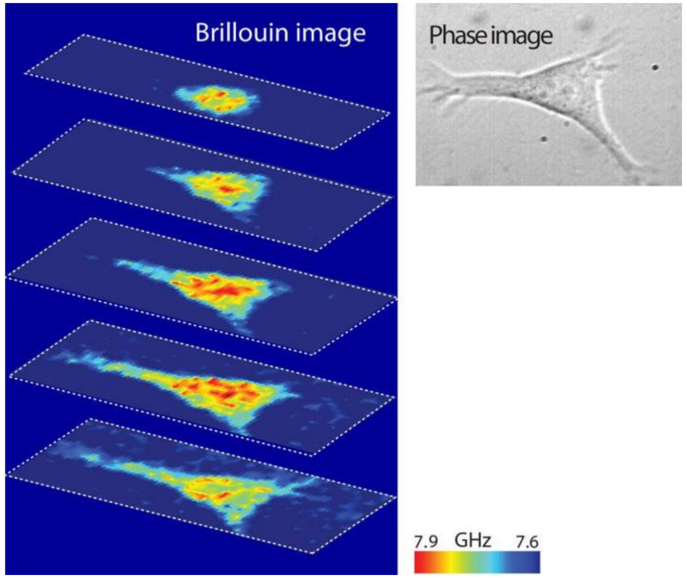
The two techniques provide complimentary information which together can tell us more about the mechanical properties of a sample.

Pilot project introduction

Brillouin Microscopy (VBCF)

Measures Longitudinal Modulus
Measures in GHz frequency-regime

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

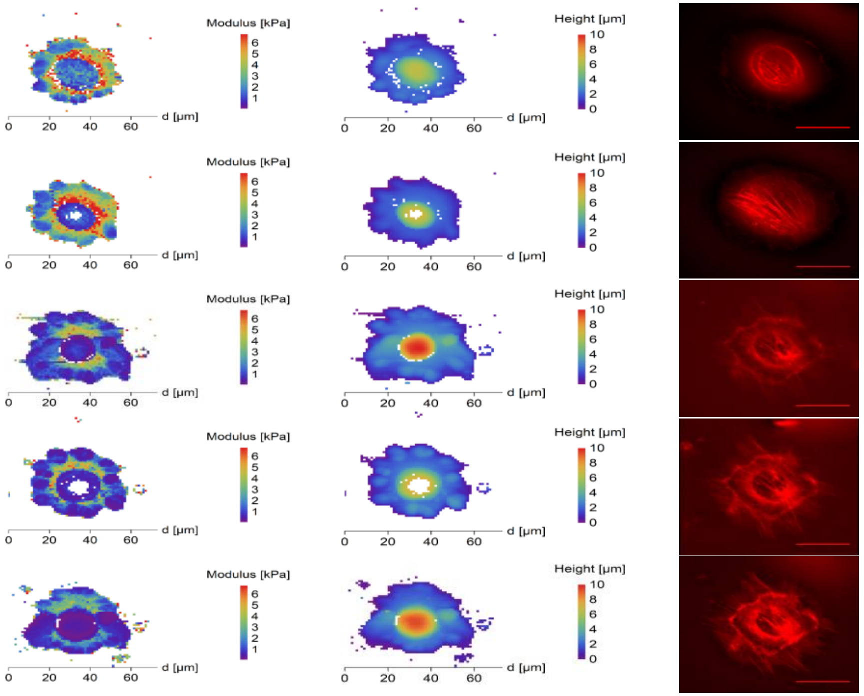


SPIE Newsroom 10.1117/2.1201611.006698

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)



Golan M. Et al., Front Physiol. 2018 Jun 29;9:804

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

Pilot project introduction

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European Regional Development Fund

Brillouin Microscopy (VBCF)

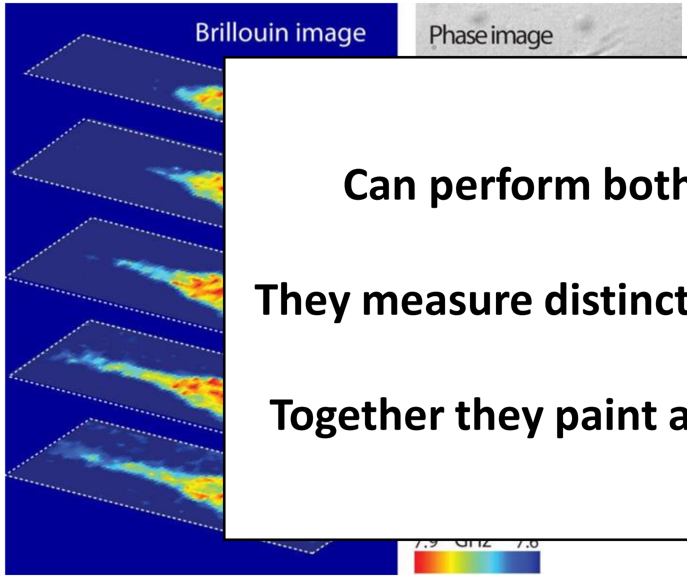
Measures Longitudinal Modulus
Measures in GHz frequency-regime

…related by
"Poisson Ratio"

Atomic Force Microscopy (CEITEC)

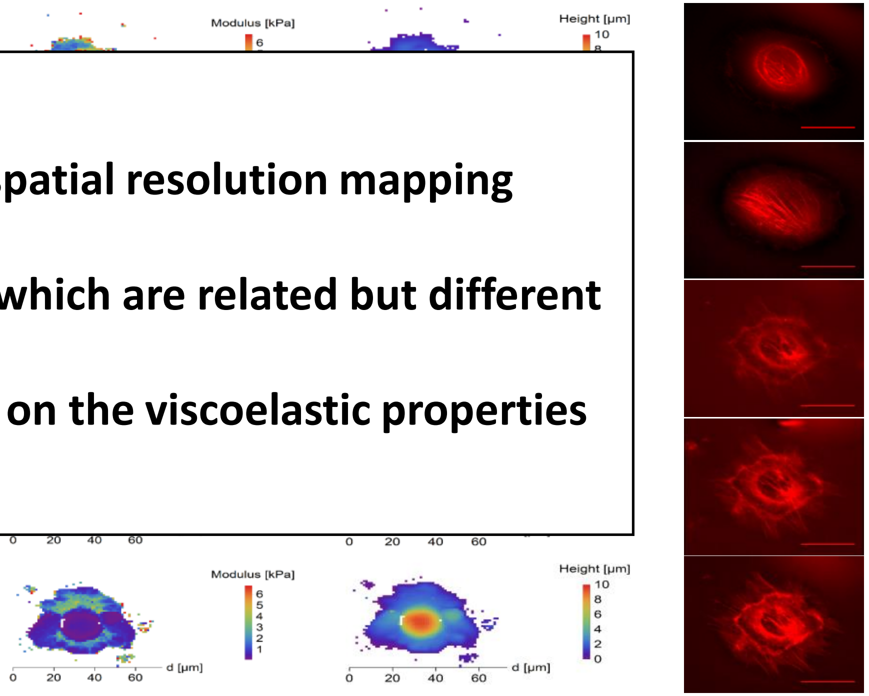
Measures Young's Modulus
Measures in <kHz frequency-regime

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



SPIE Newsroom 10.1117/2.1201611.006698

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



Golan M. Et al., Front Physiol. 2018 Jun 29;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

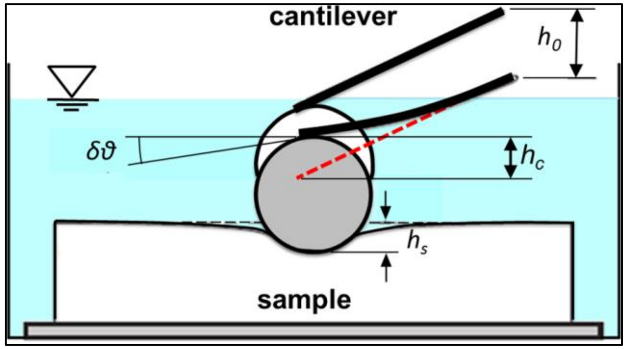
- Potential end-users:

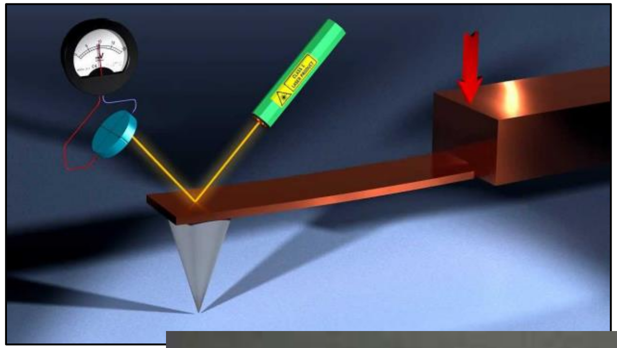
Mostly academic users – possible candidates:

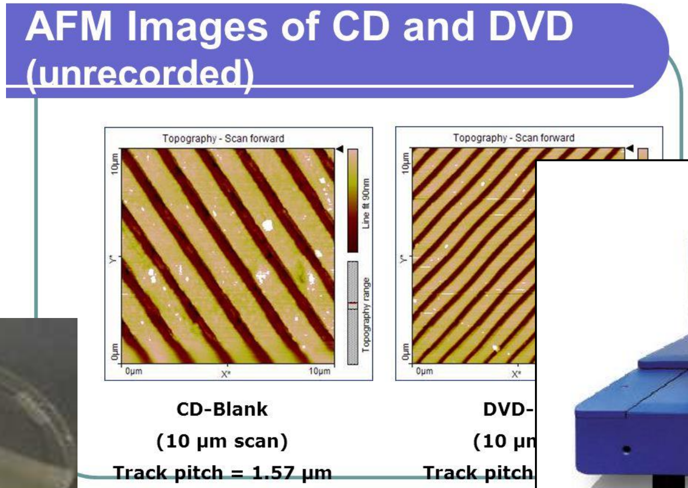
- *Giancarlo Forte, ICRC Brno – dECM samples*
- *Eva Benkova, IST Austria – plant tissues*
- *Jan Hejátko, CEITEC MU - plant tissues*
- *Daniel Hadraba, Institute of Physiology CAS*
- *Irena Kratochvilova, Institute of Physics CAS*
- *Vladimir Rotrekl, Faculty of Medicine, MU*
- *Daniel Gerlich, IMBA,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

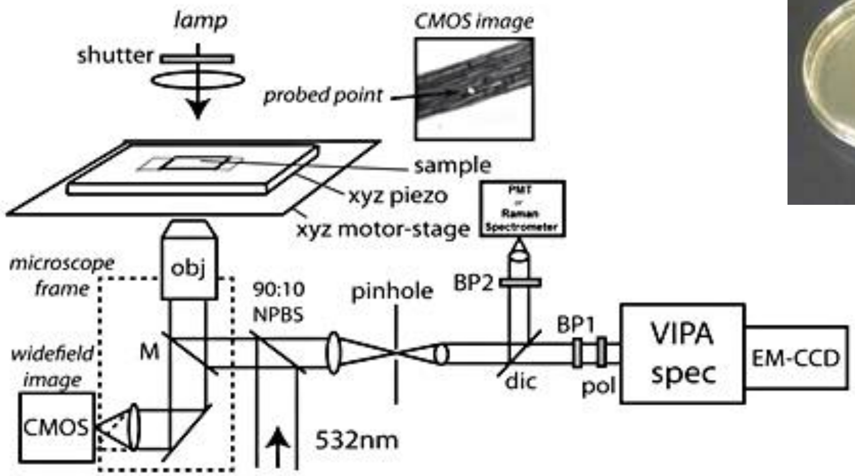
Project implementation

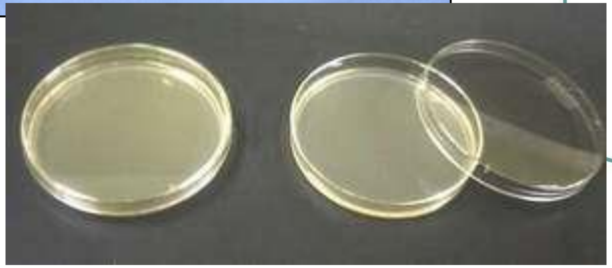
- *Approach/methodology*

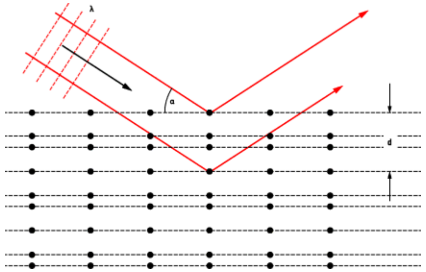


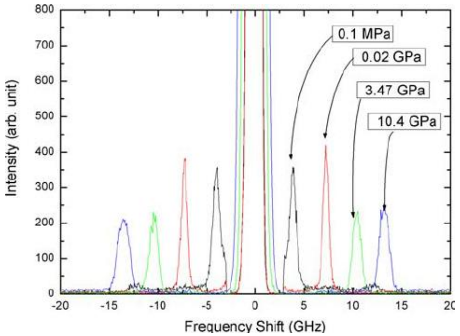


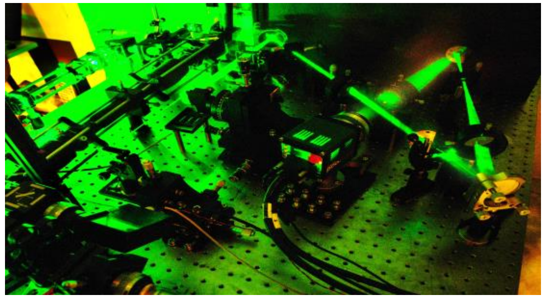












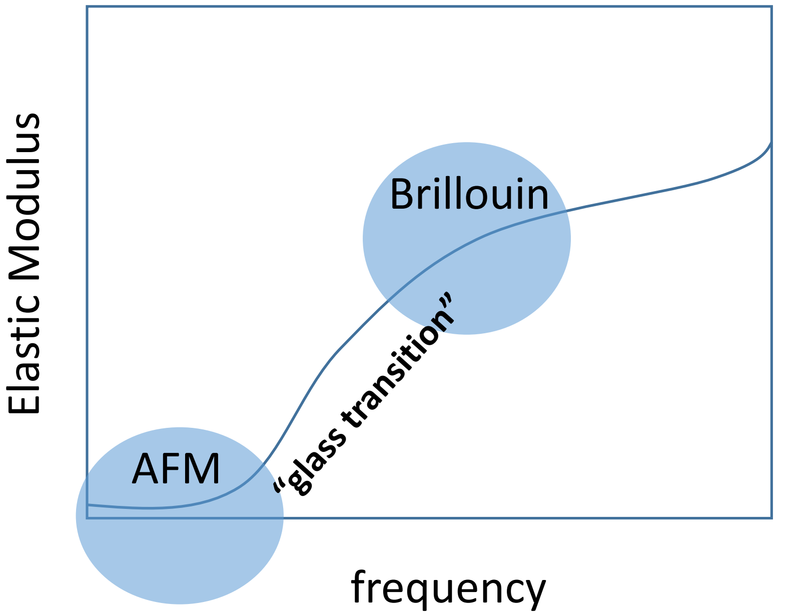
There is no budget for staff exchanges.
We are cooperating by exchanging the samples and results („remote control“).

J Biomech Eng 137(7), 071005
www.jpk.com
https://www.myce.com/review

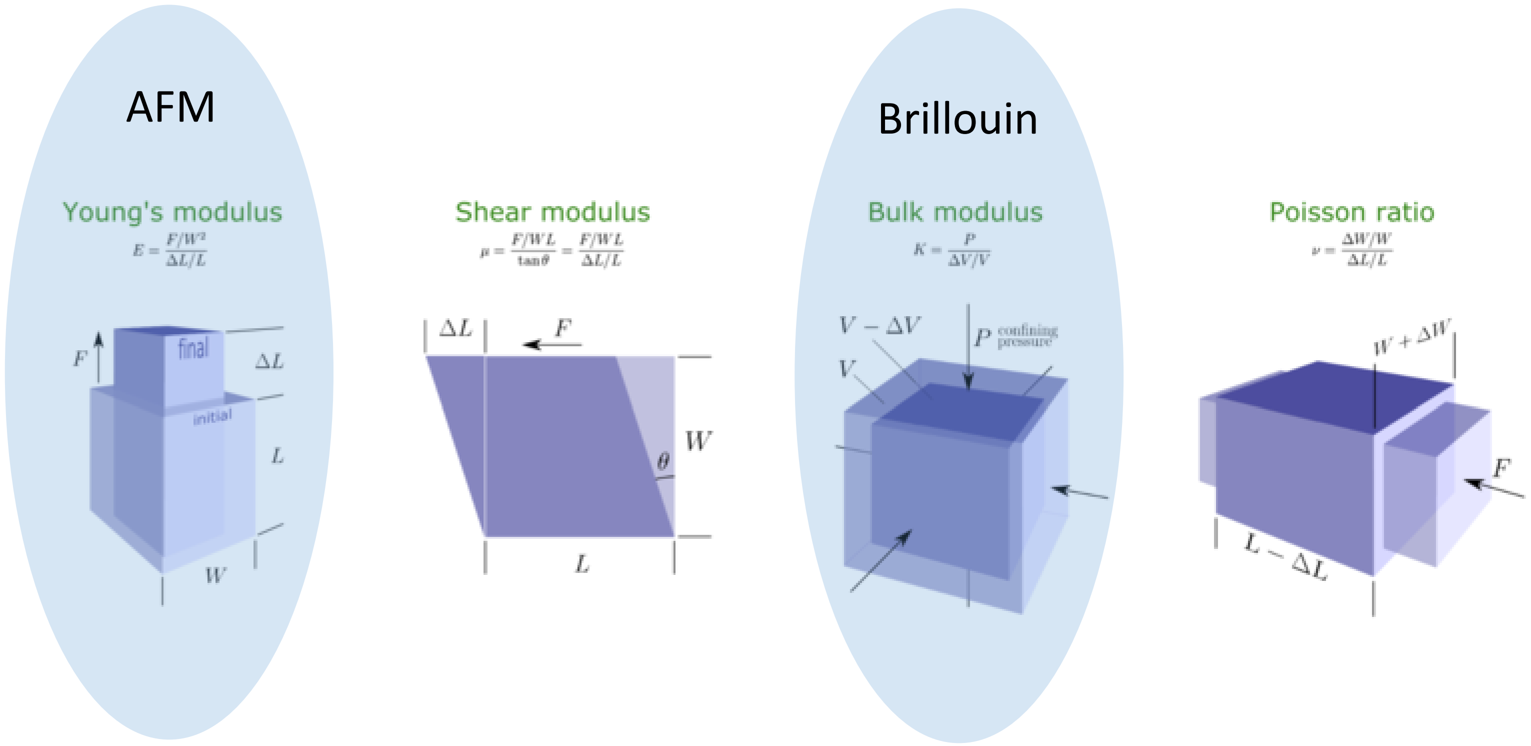
Project results

How do the measurements even compare?

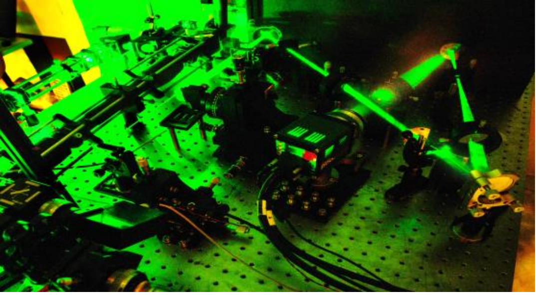
Different relaxation mechanism(s)

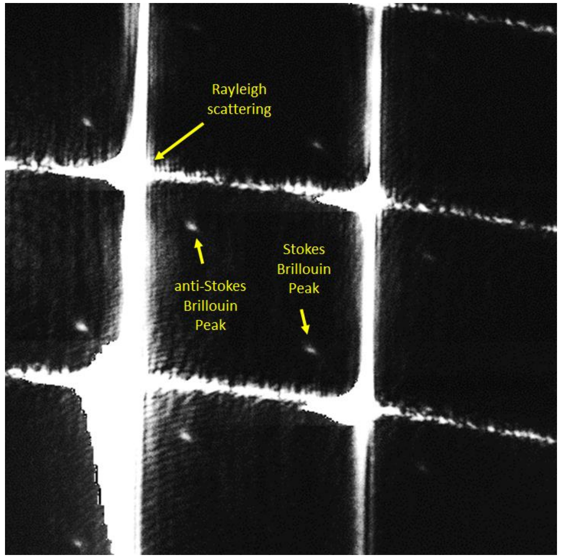


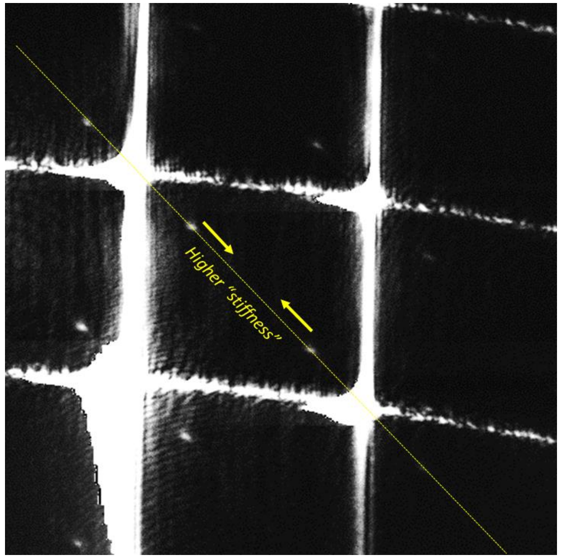
Different boundary conditions

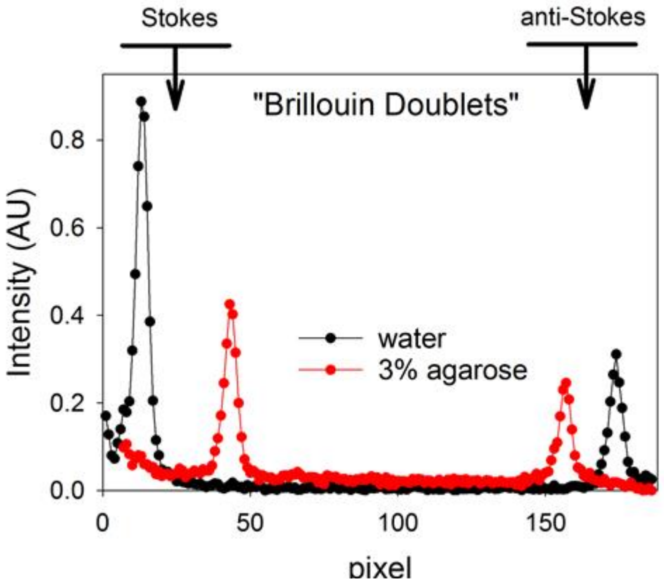


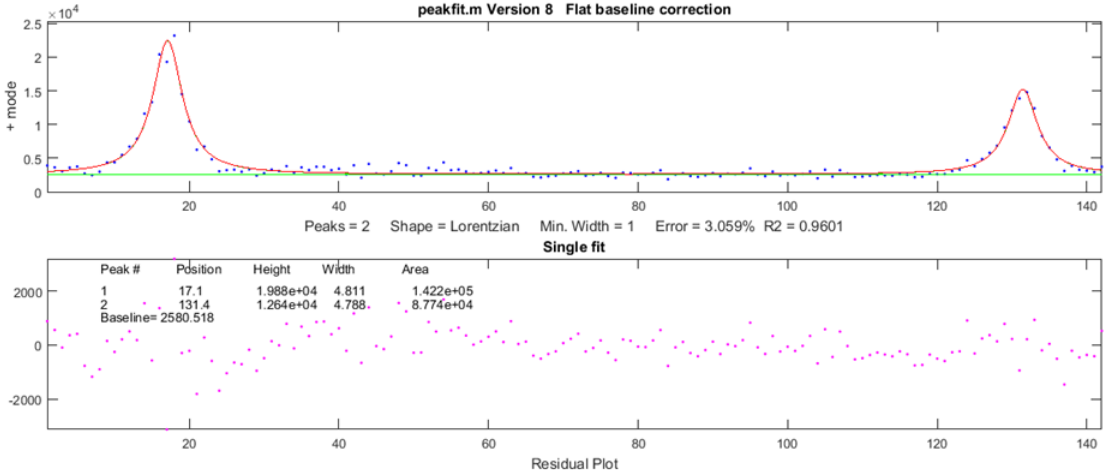
Different Moduli important for different processes

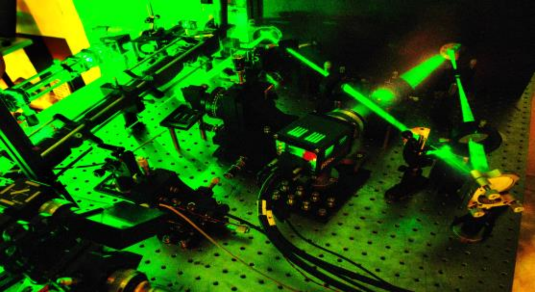


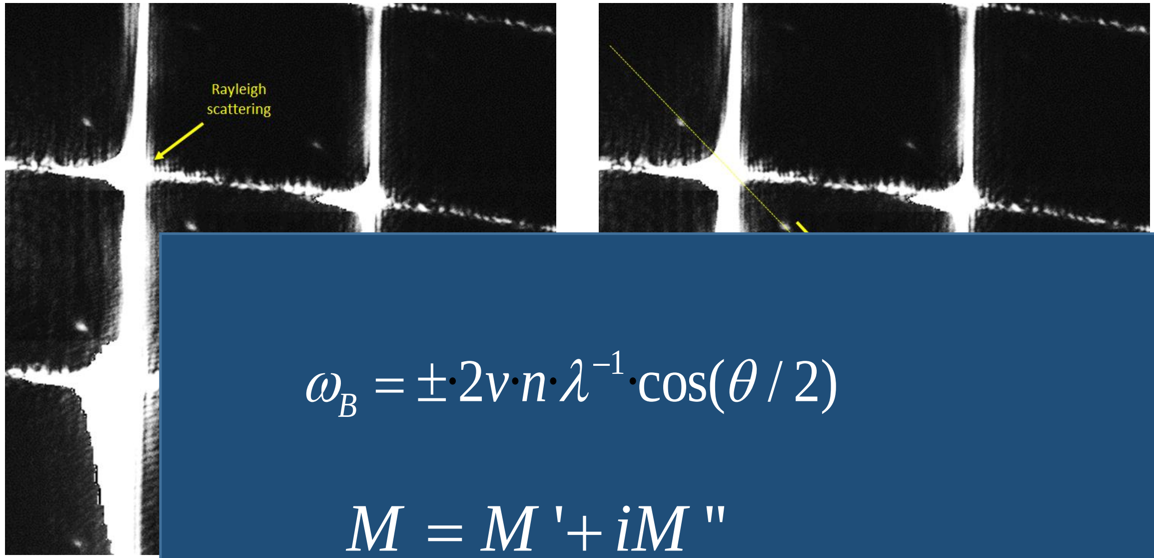

Rayleigh scattering
anti-Stokes Brillouin Peak
Stokes Brillouin Peak

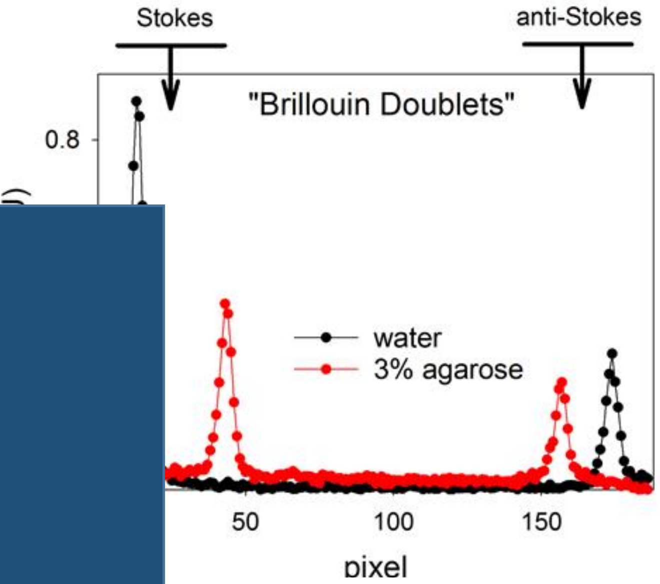

Higher "stiffness"

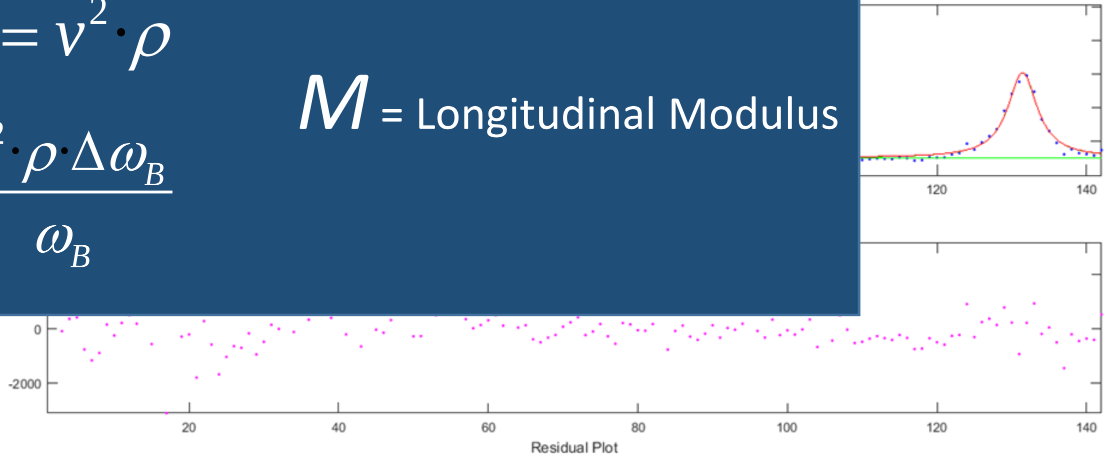

Stokes
anti-Stokes
"Brillouin Doublets"
Intensity (AU)
pixel
water
3% agarose


peakfit.m Version 8 Flat baseline correction
+ mode
Peaks = 2 Shape = Lorentzian Min. Width = 1 Error = 3.059% R2 = 0.9601
Single fit
Peak # Position Height Width Area
1 17.1 1.988e+04 4.811 1.422e+05
2 131.4 1.264e+04 4.788 8.774e+04
Baseline= 2580.518
Residual Plot









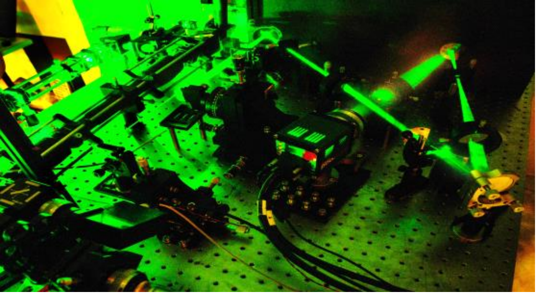
$$\omega_B = \pm 2 v \cdot n \cdot \lambda^{-1} \cdot \cos(\theta / 2)$$

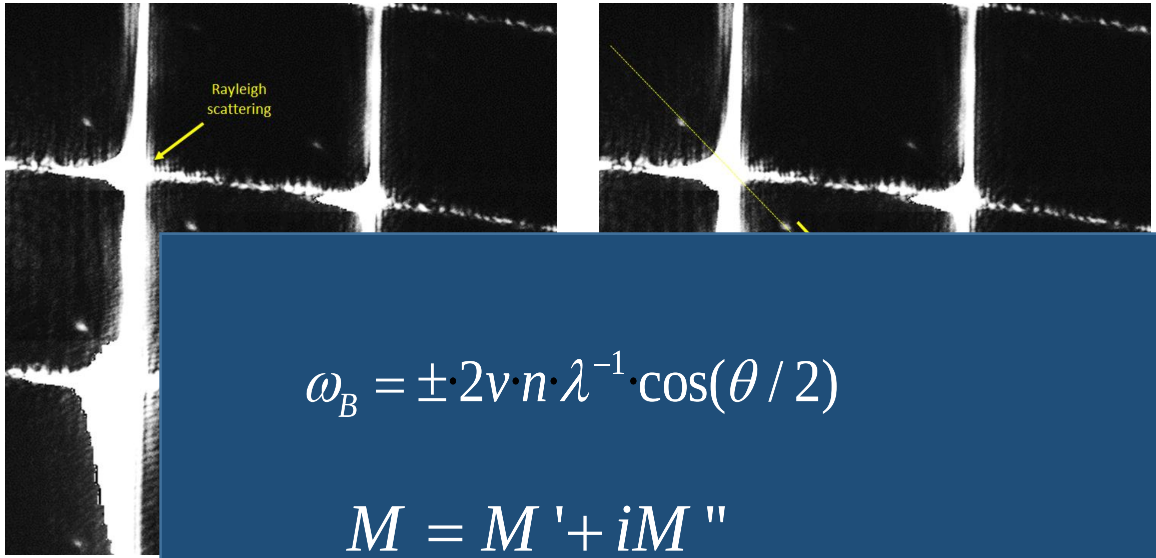
$$M = M' + iM''$$

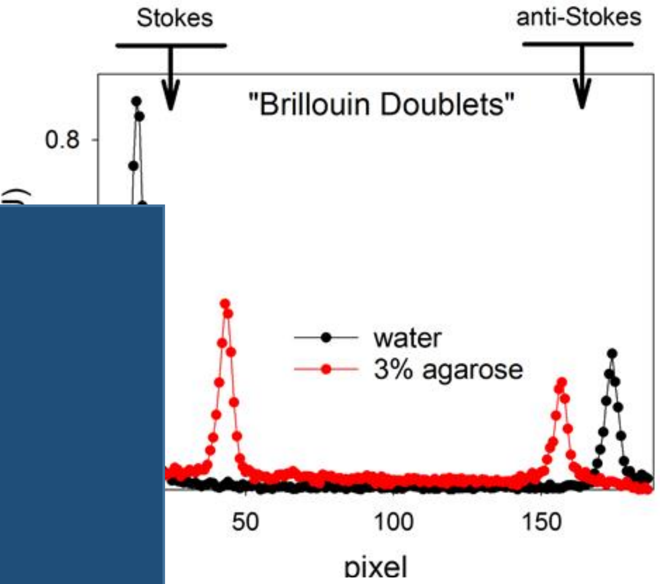
$$M' = v^2 \cdot \rho$$

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

M = Longitudinal Modulus







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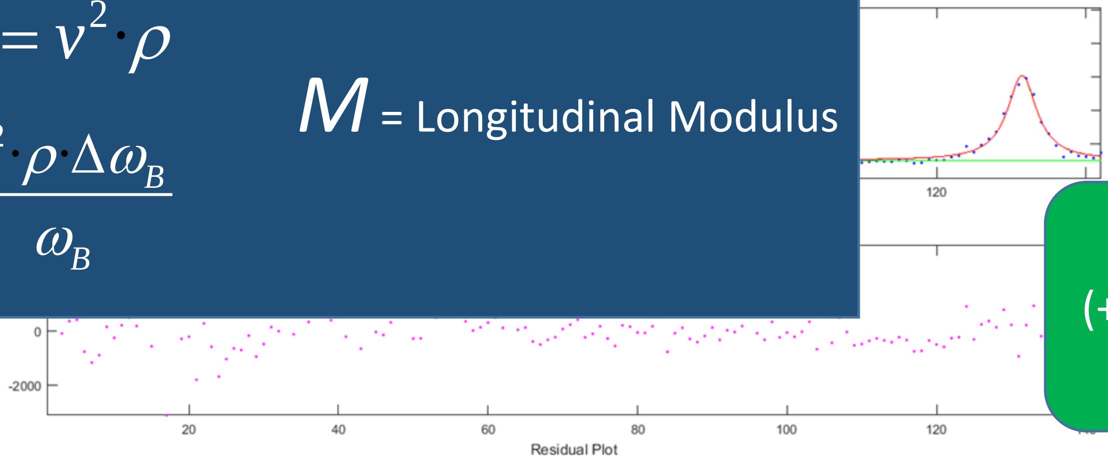
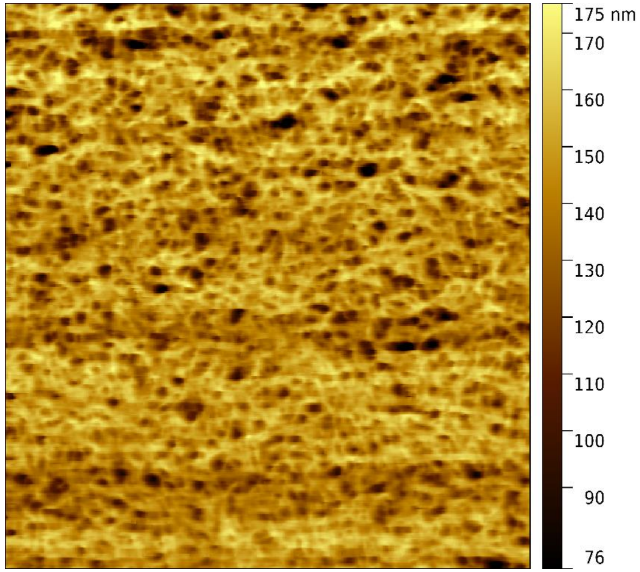


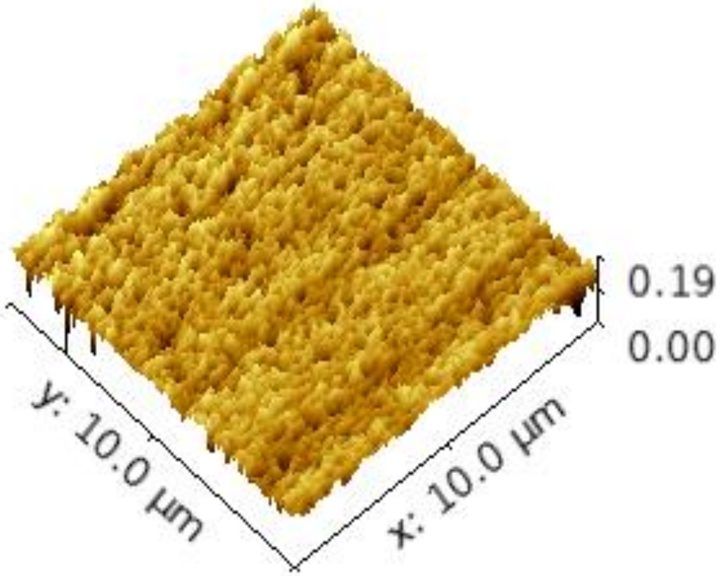
(+) 3d spatial mapping possible

Project results

How do the measurements compare?

Agarose samples of different concentrations (0-2%)





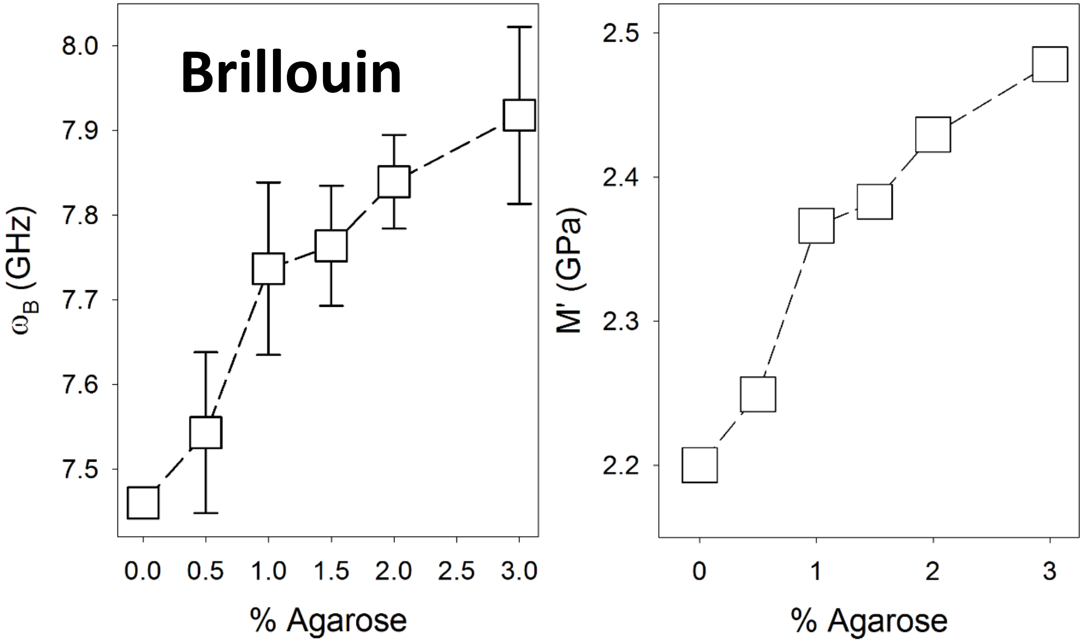
10*10µm topography
2% Agarose w/v

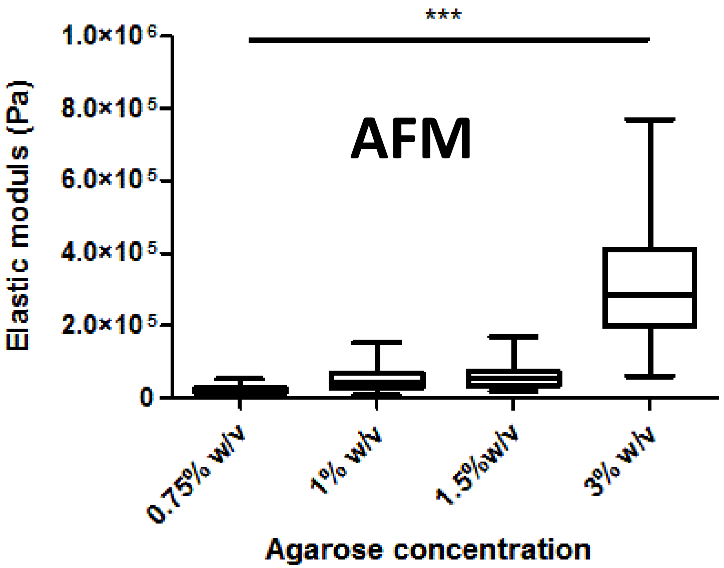
Surface imaging (with HYDRA-ALL B)

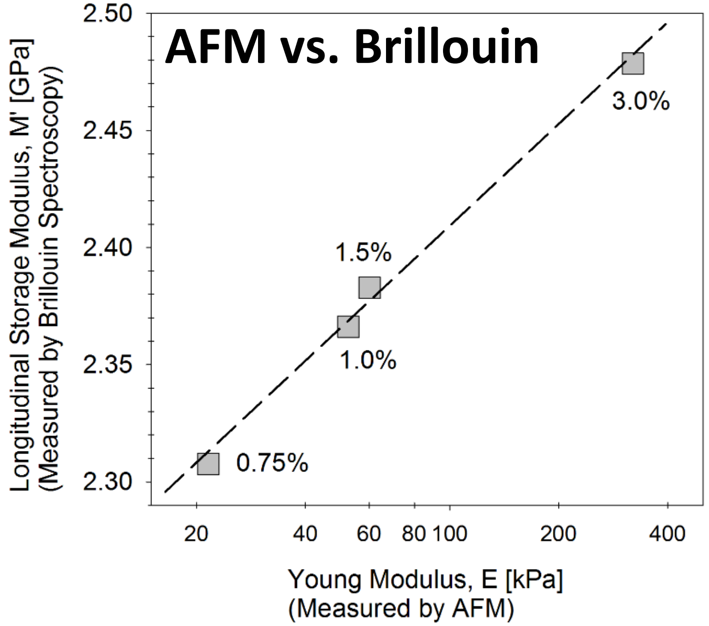
Project results

How do the measurements compare?

Agarose samples of different concentrations







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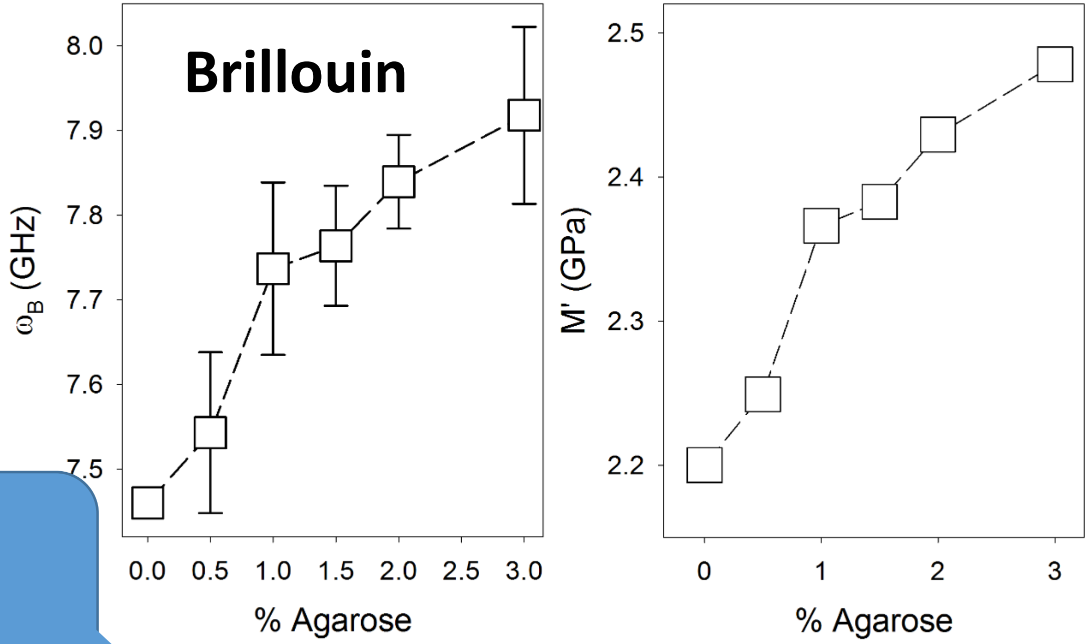
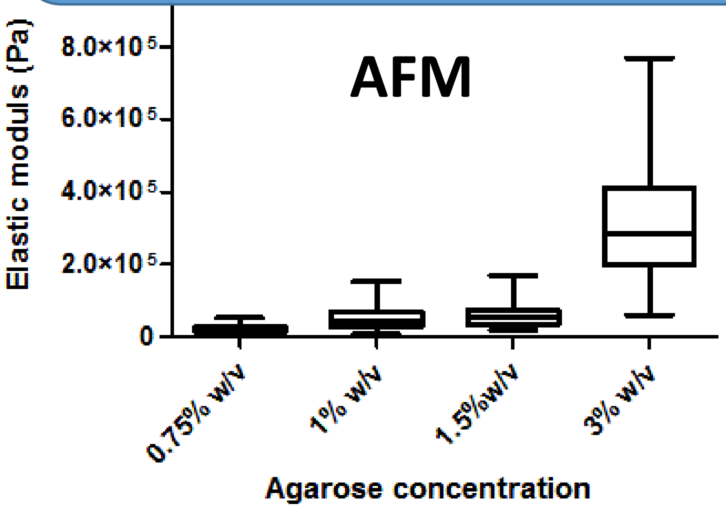
Project results

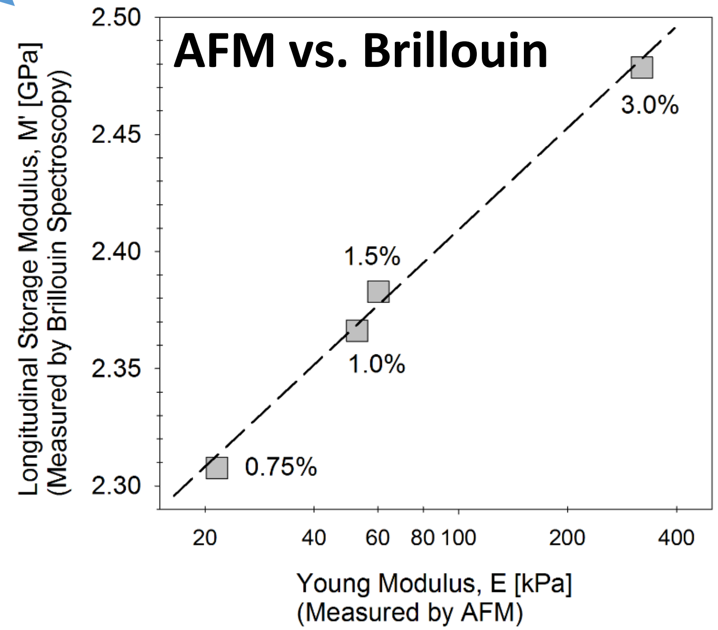
How do the measurements compare?

Agarose samples of different concentrations



Power law or logarithmic relation





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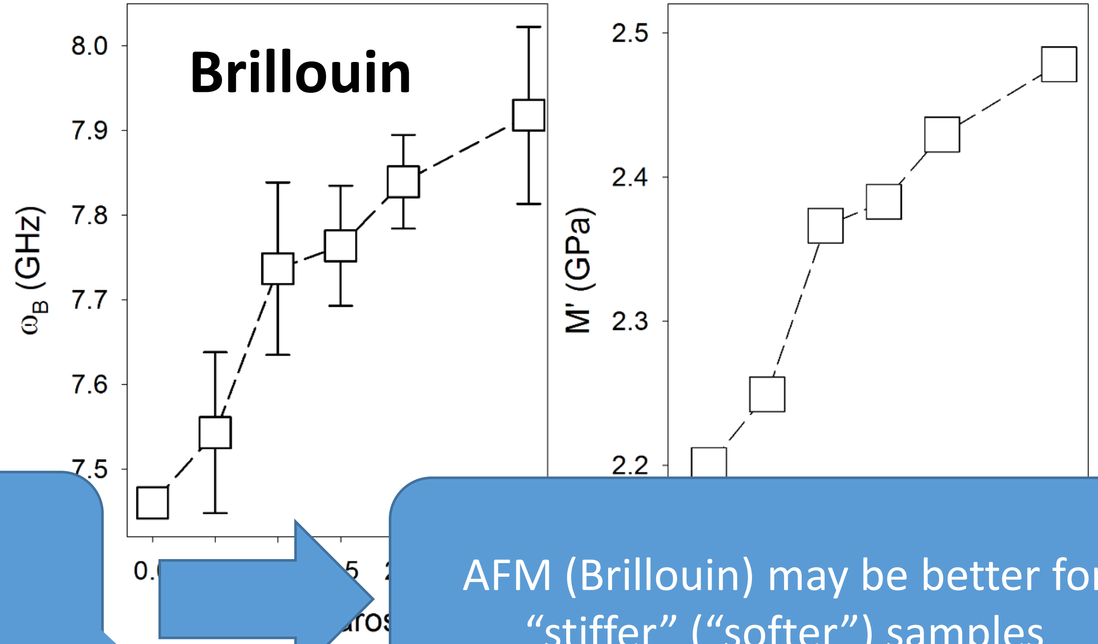
Project results

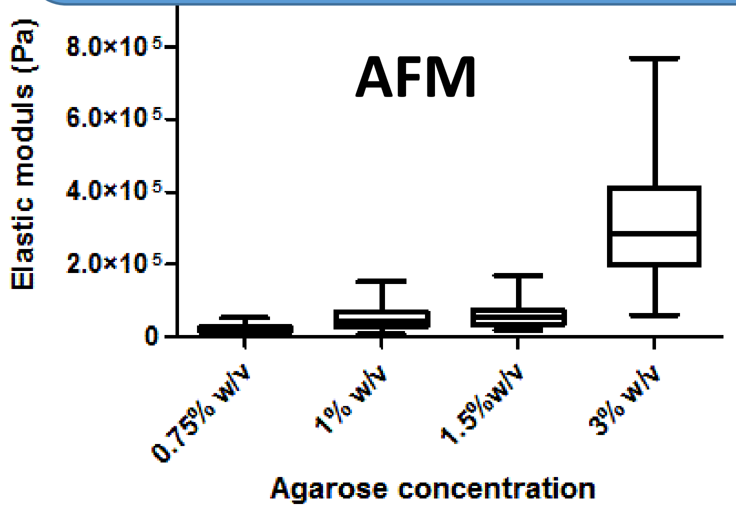
How do the measurements compare?

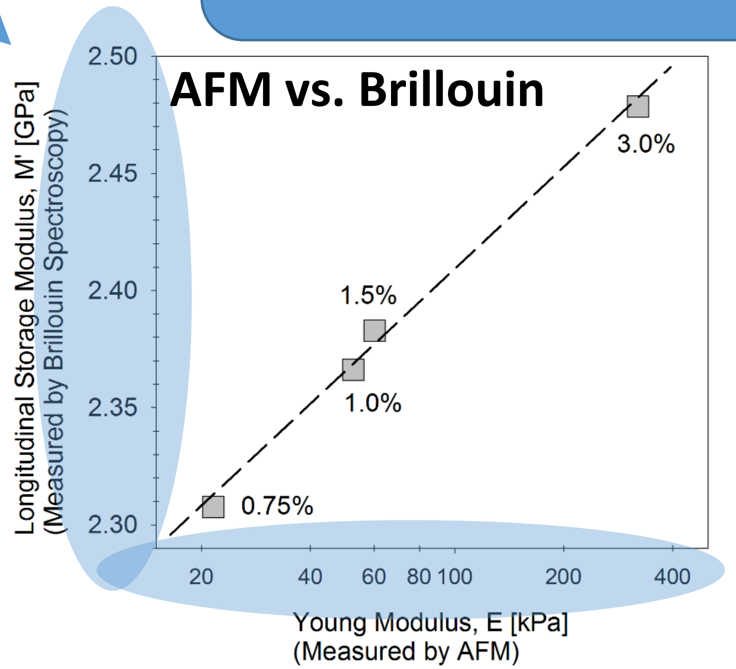
Agarose samples of different concentrations



Power law or logarithmic relation

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





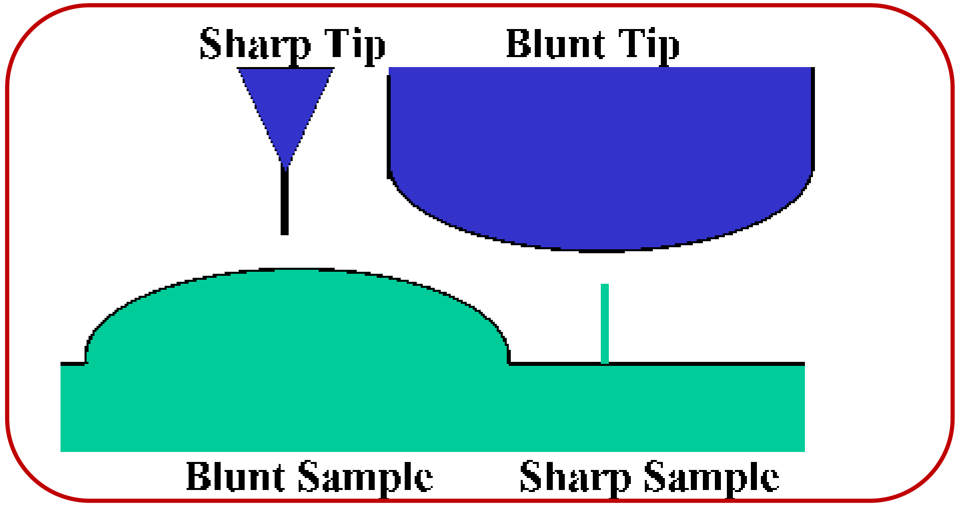
Project results

What about structural features?

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

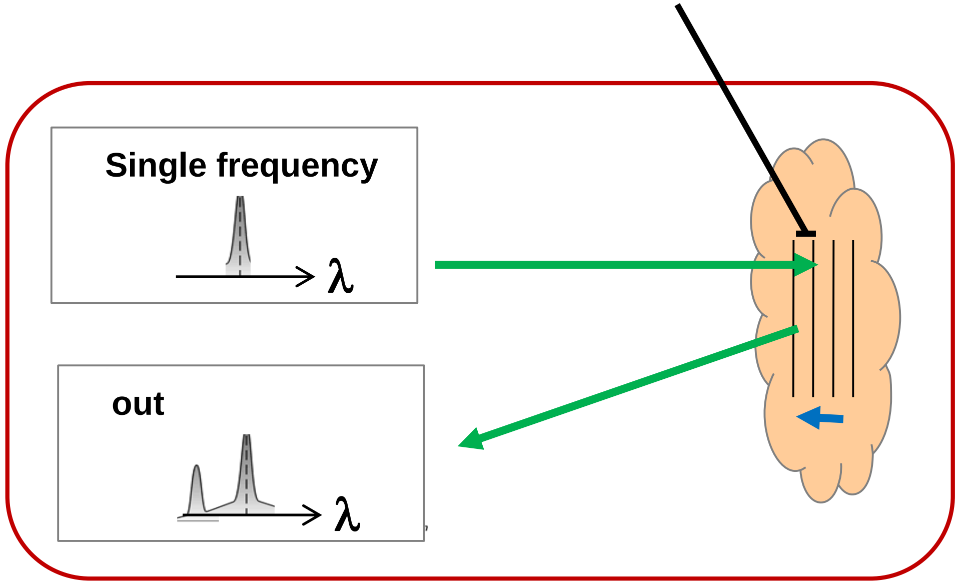
AFM

probes an area the size of the tip



Brillouin

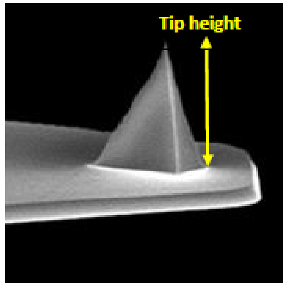
probes area on the size of acoustic wavelength (~100-200nm)

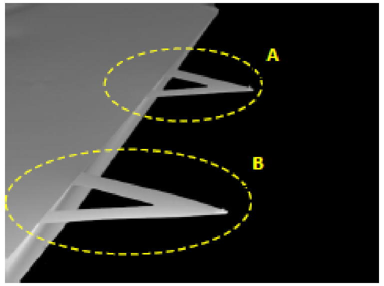


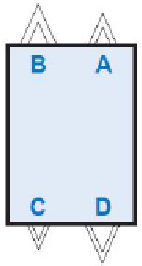
FIB milling of AFM tips
Plan

Modify size of AFM probing area

Bruker SNL10 A+B

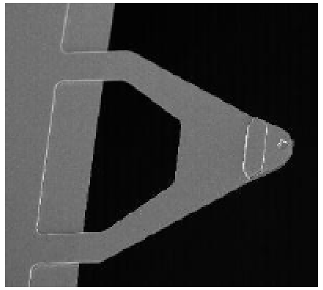


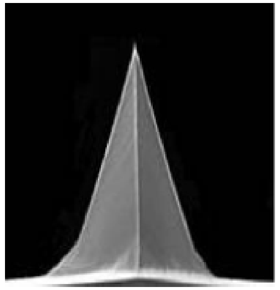


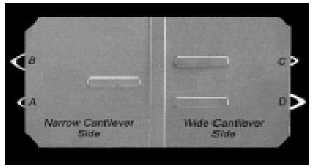


- **Silicon / silicon nitride tip** on silicon nitride cantilever
- Cantilever spring constant **0.080 – 0.200 N/m**
- Tip height **4-8 µm**

AppNano Hydra 100N-6V







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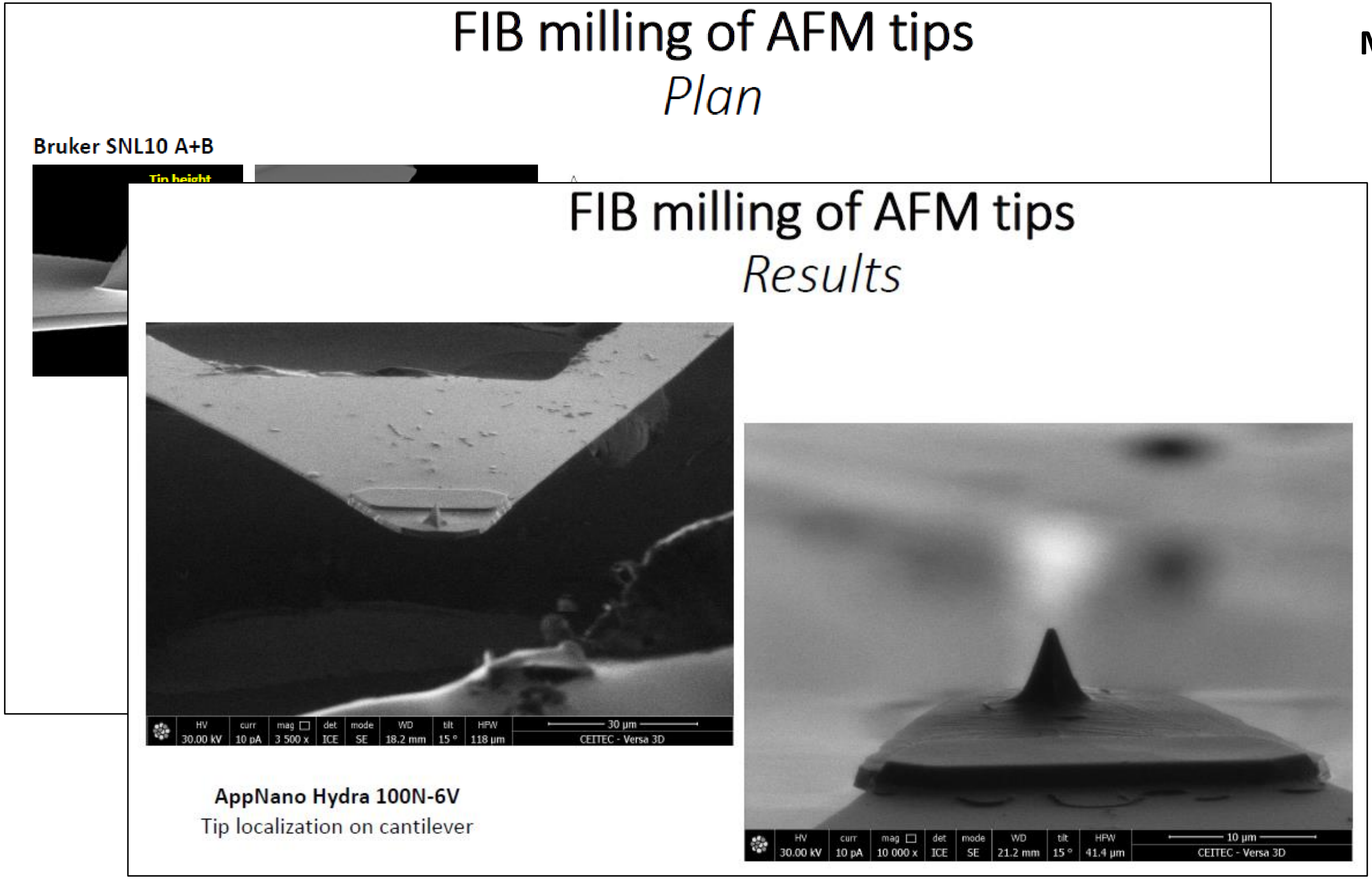
http://www.appnano.com/product/category/silicon-probes

Modify size of AFM probing area



SEM images by Dr. J. Novacek – CryoEM CF CEITEC MU

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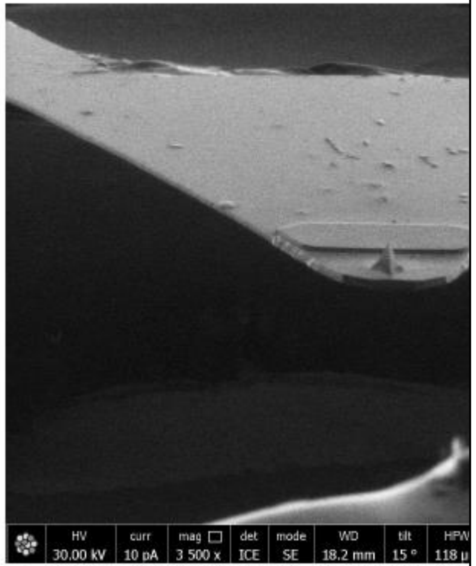
Modify size of AFM probing area

FIB milling of AFM tips
Plan

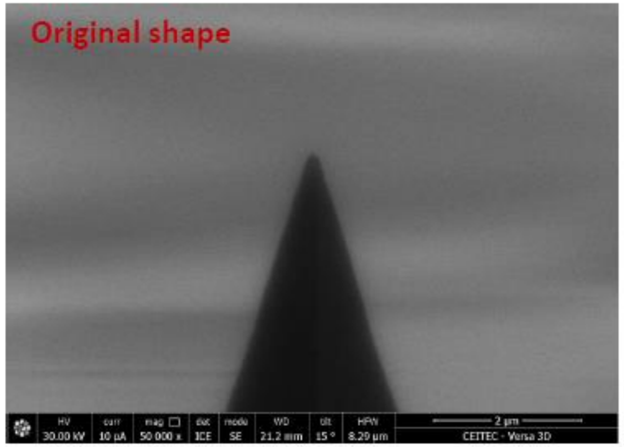
Bruker SNL10 A+B

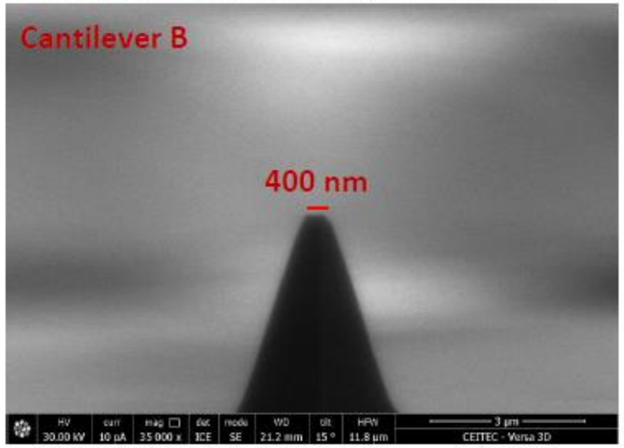
FIB milling of AFM tips



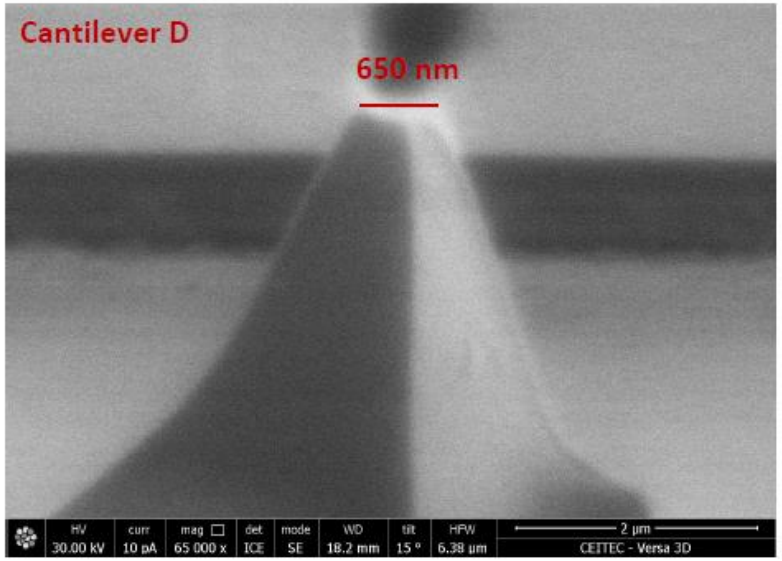
AppNano Hydra 100N-6V
Tip localization on cantilever

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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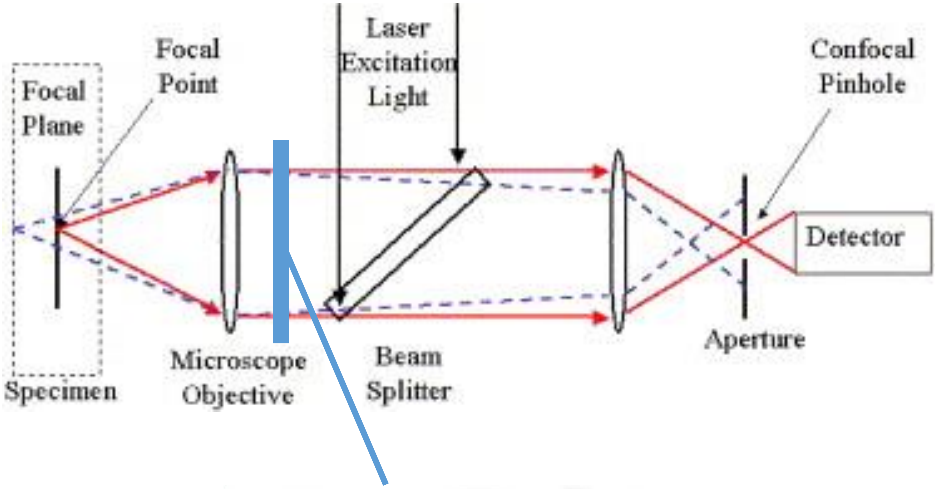
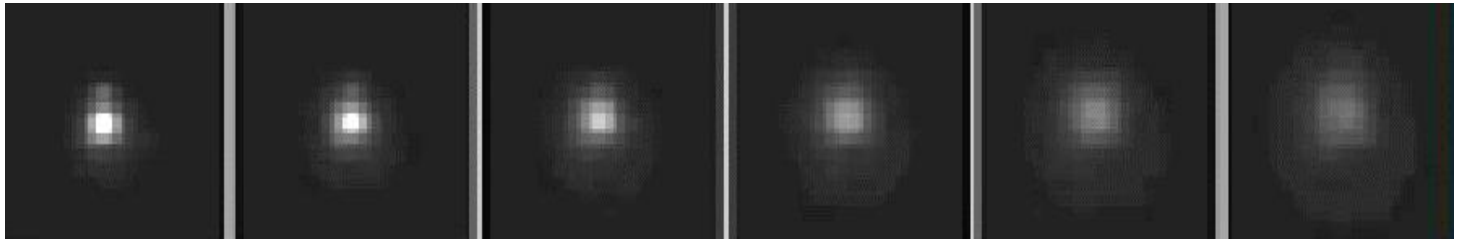
SEM images and FIB milling by Dr. J. Novacek – CryoEM CF CEITEC MU

Modify probing volume in Brillouin



(spectrometer)

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



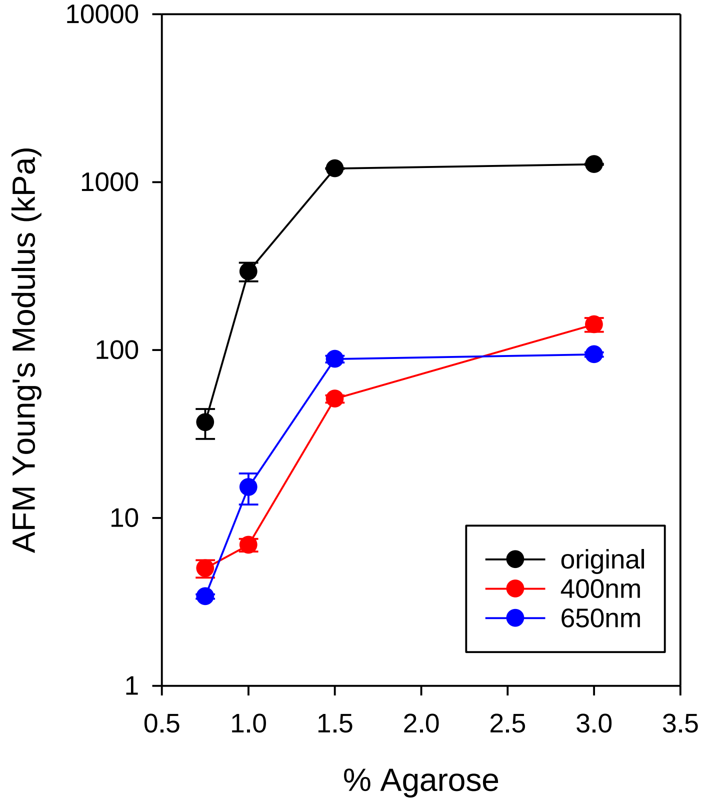
FWHM~200nm

FWHM~600nm

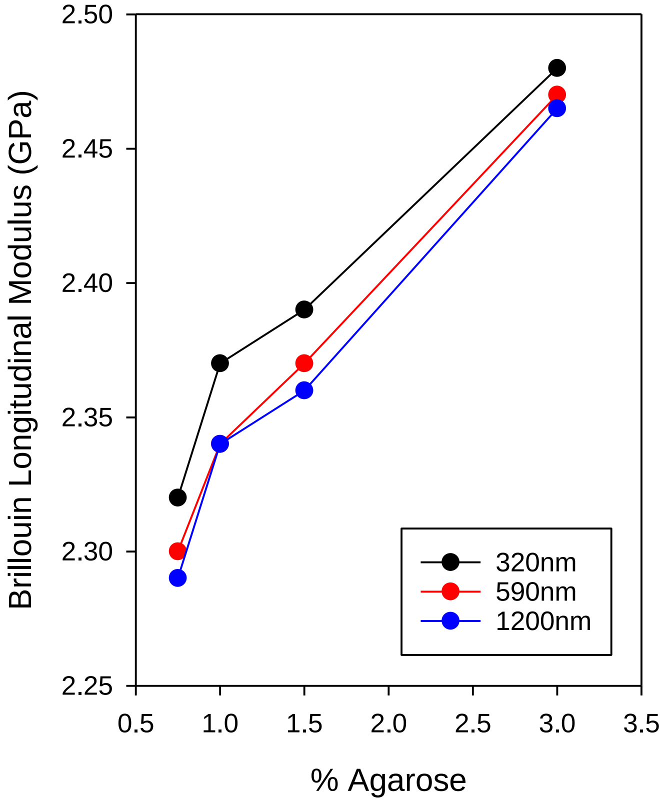
*Effective Point Spread Function (PSF) = **probing volume***

Dependence on probing volume (agarose series)

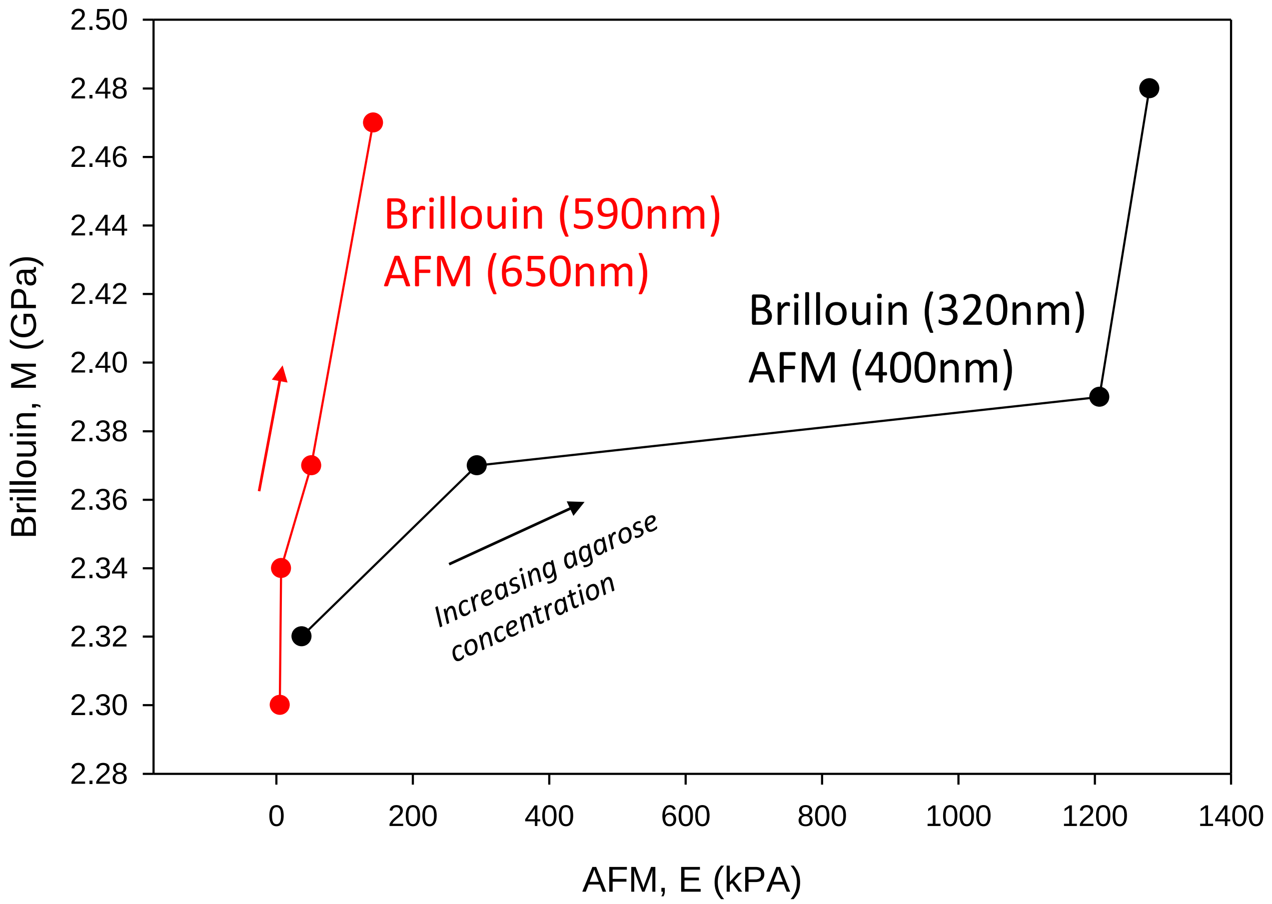
AFM



Brillouin



Dependence on probing volume (agarose series)



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

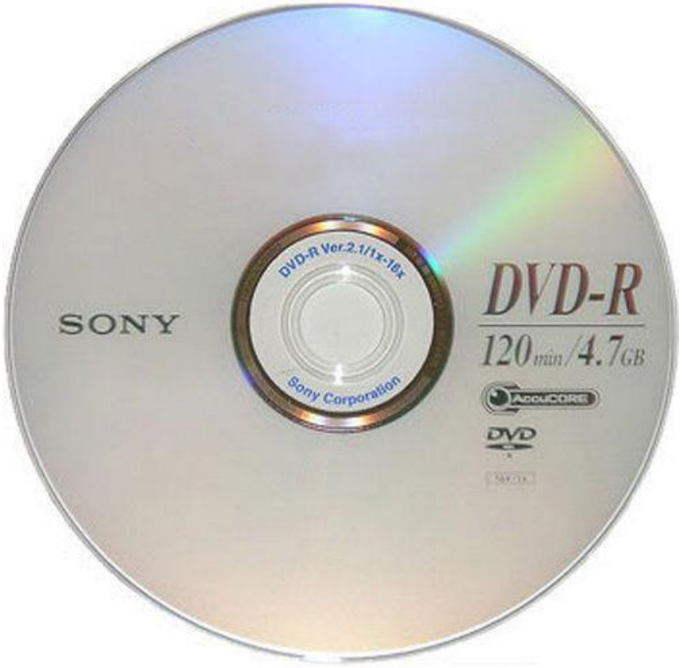
Conclusion:

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

Comparative studies should account for this

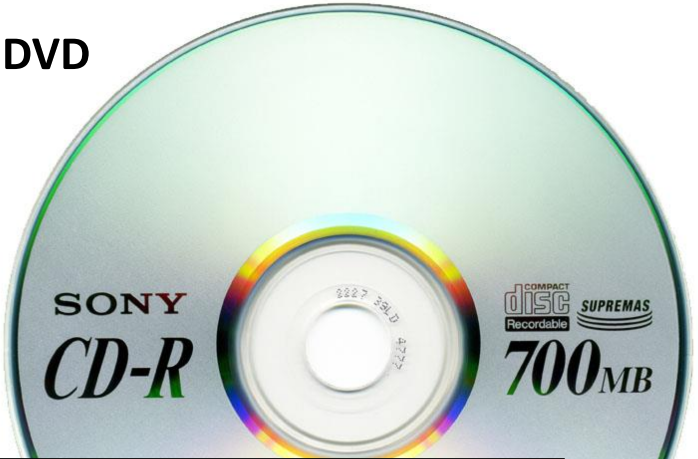
This affects the spatial sampling distances/probes that should be chosen for comparative AFM and Brillouin measurements

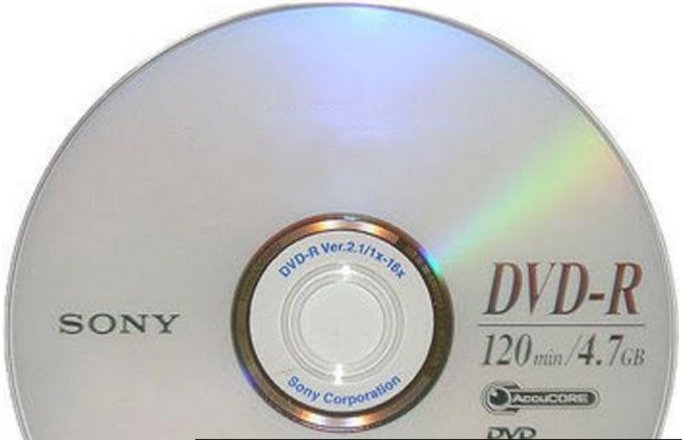
Systematic investigation into structured samples



Systematic investigation into structured samples

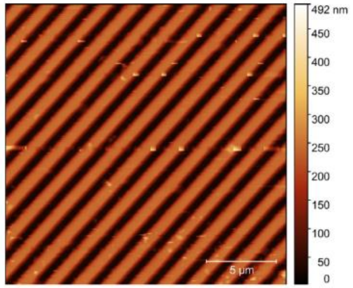
Use un-written CD and DVD as “stamp” on agarose

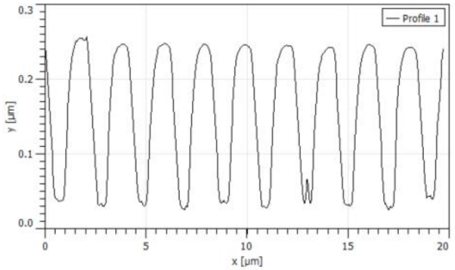


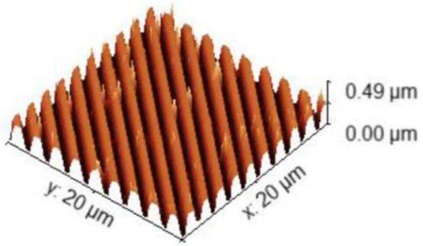


CD-ROM Verbatim

Average height 210 nm
Pitch to pitch distance 1.5 µm

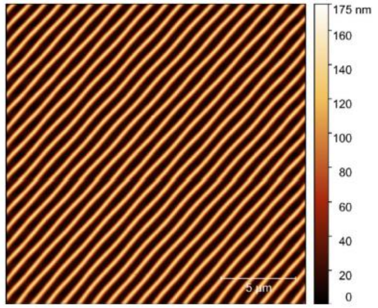


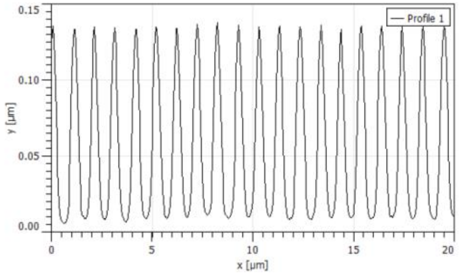


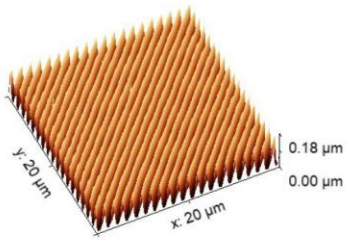


DVD Verbatim

Average height 115.2 nm
Pitch to pitch distance 0.78 µm







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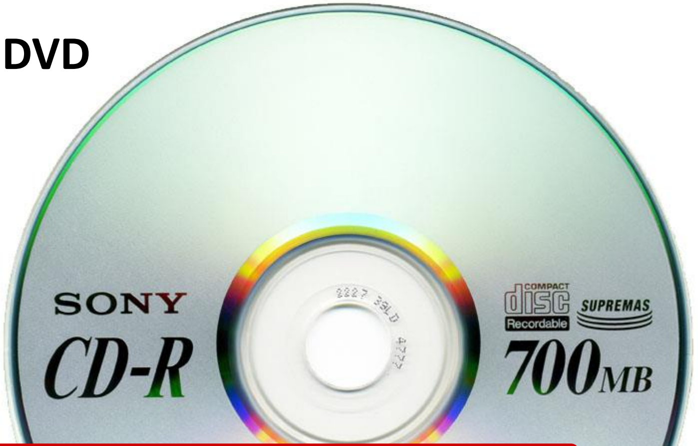
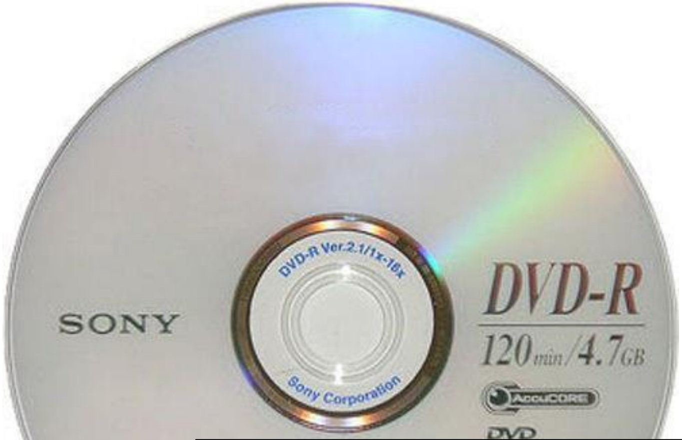
Systematic investigation into structured samples

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





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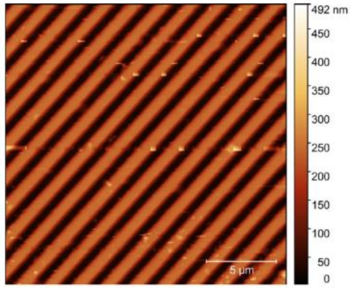

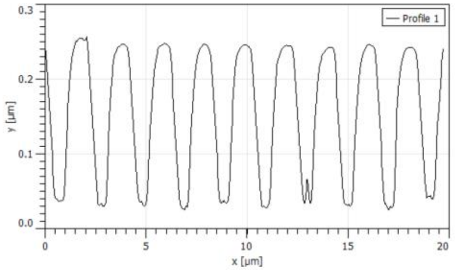

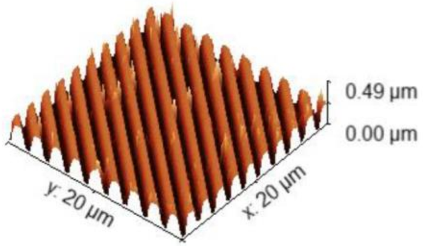

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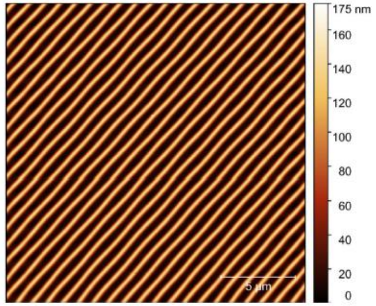
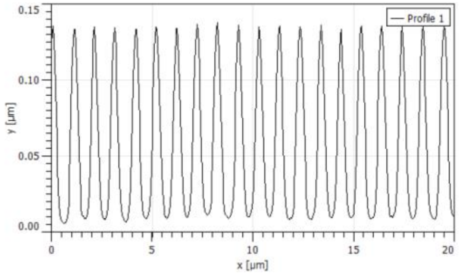
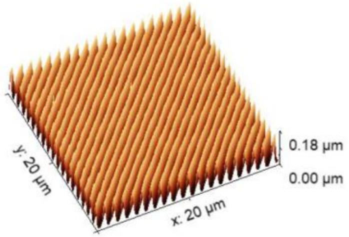
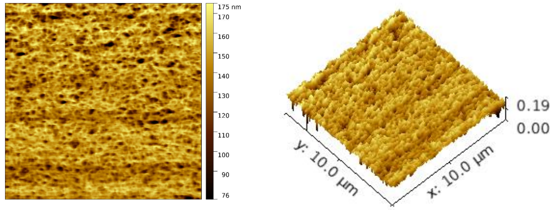
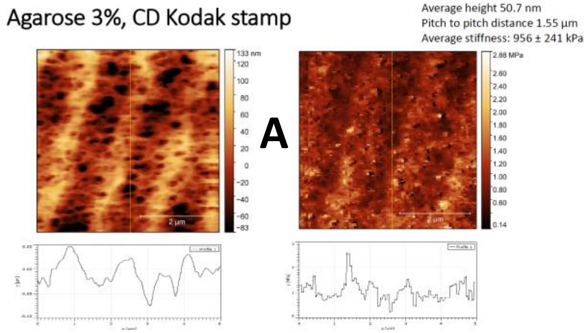


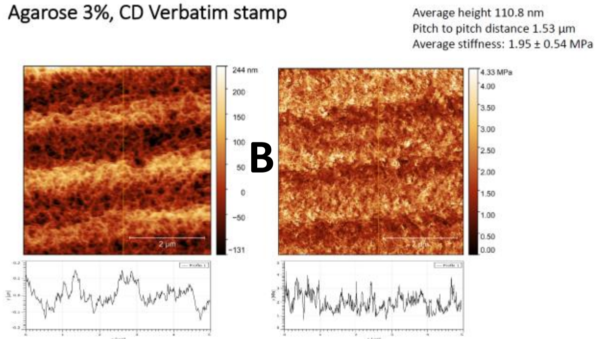


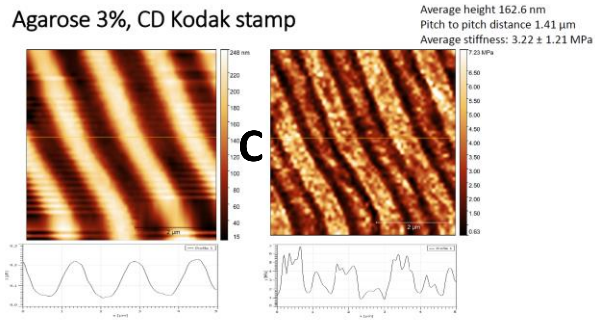


Brillouin: M~2500kPa | M~2500kPa | M~2500kPa | M~2500kPa



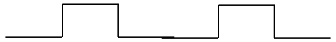






AFM:

z=0nm



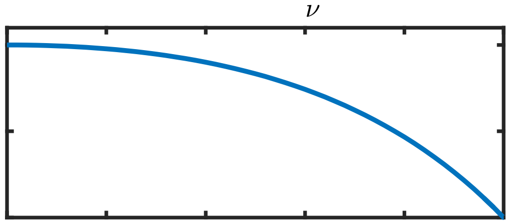
z=50nm

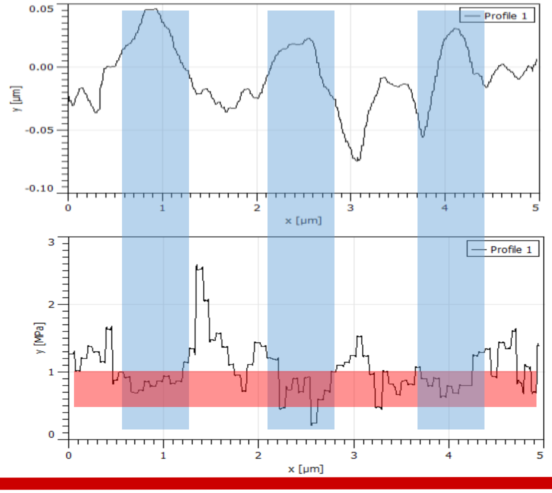
z=110nm

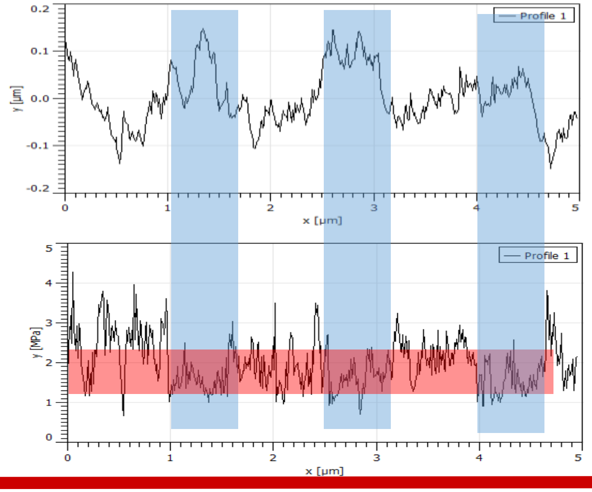
z=160nm

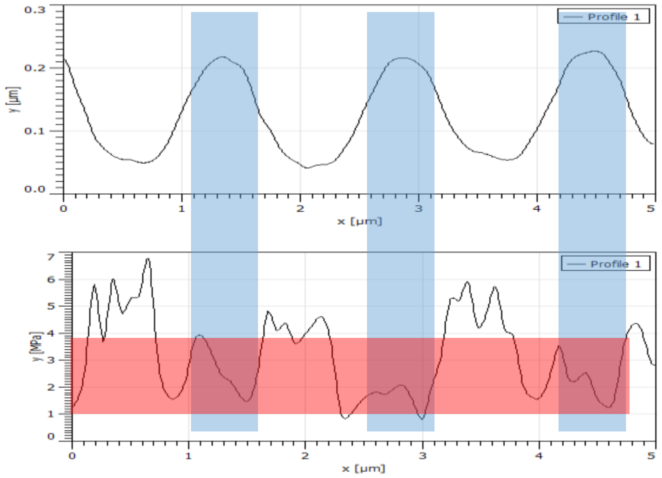
E~300kPa | E~900kPa | E~1500kPa | E~2000kPa

$$E = \frac{M(1+\nu)(1-2\nu)}{(1-\nu)}$$

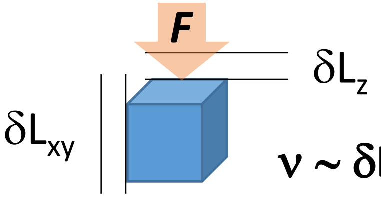




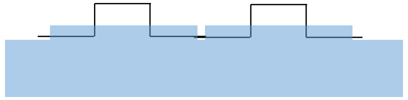


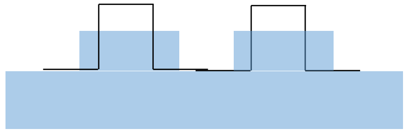


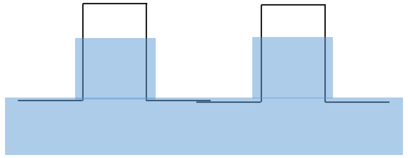
$\Rightarrow$ $\nu_A > \nu_B > \nu_C$



$\nu \sim \delta L_{xy}/\delta L_z$







$\delta L^{(A)}_z = \delta L^{(B)}_z = \delta L^{(C)}_z$ $\delta L^{(A)}_{xy} > \delta L^{(B)}_{xy} > \delta L^{(C)}_{xy}$

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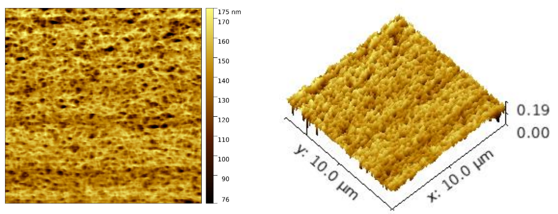
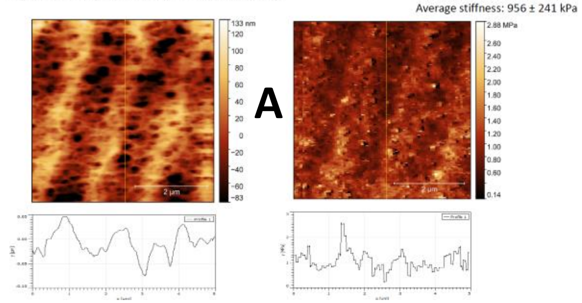
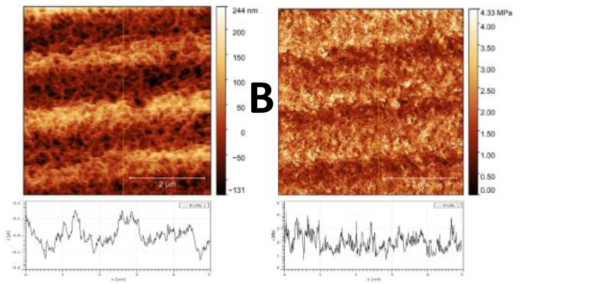
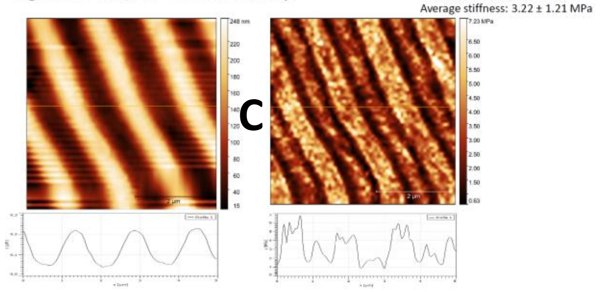
Brillouin: M~2500kPa | M~2500kPa | M~2500kPa | M~2500kPa





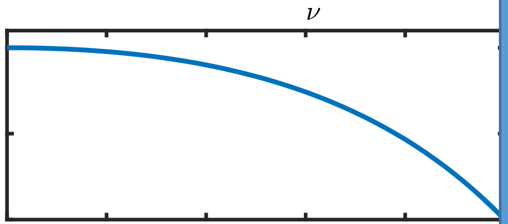


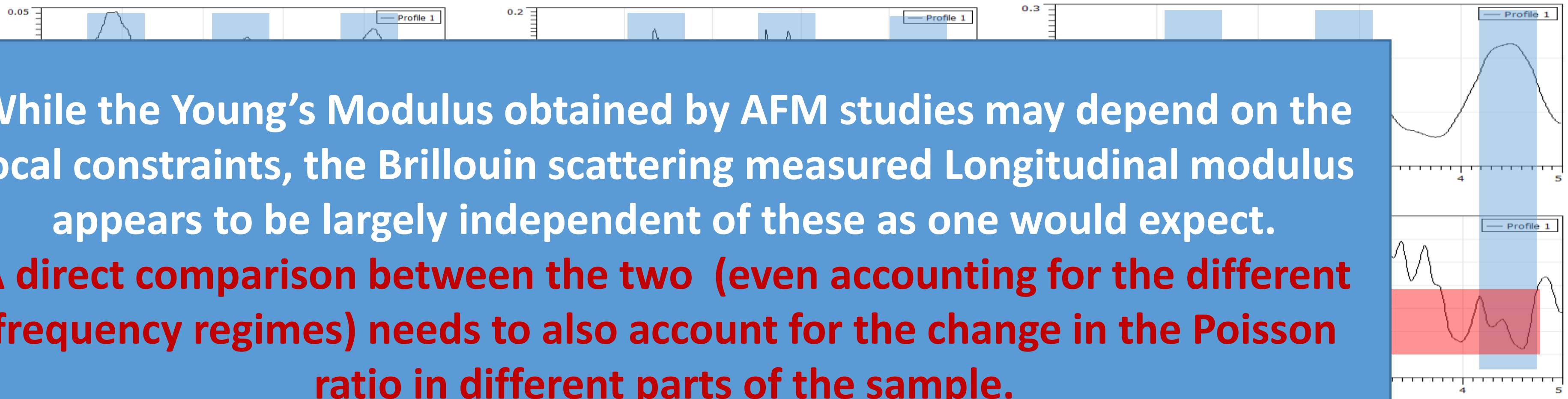


AFM:

E~300kPa | E~900kPa | E~1500kPa | E~2000kPa

$$E = \frac{M(1+\nu)(1-2\nu)}{(1-\nu)}$$

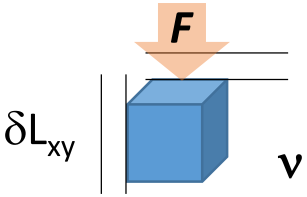




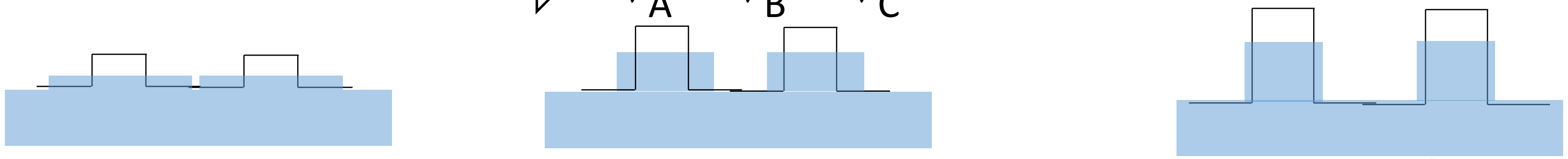
While the Young’s Modulus obtained by AFM studies may depend on the local constraints, the Brillouin scattering measured Longitudinal modulus appears to be largely independent of these as one would expect.

A direct comparison between the two (even accounting for the different frequency regimes) needs to also account for the change in the Poisson ratio in different parts of the sample.

$\nu_A > \nu_B > \nu_C$



$\nu \sim \delta L_{xy}/\delta L_z$



$\delta L^{(A)}_z = \delta L^{(B)}_z = \delta L^{(C)}_z \qquad \delta L^{(A)}_{xy} > \delta L^{(B)}_{xy} > \delta L^{(C)}_{xy}$

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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**