

RAMAN E BRILLOUIN IMAGING APPLIED TO BIOLOGICAL MATTER:

Data interpretation and new methods for resolution improvement



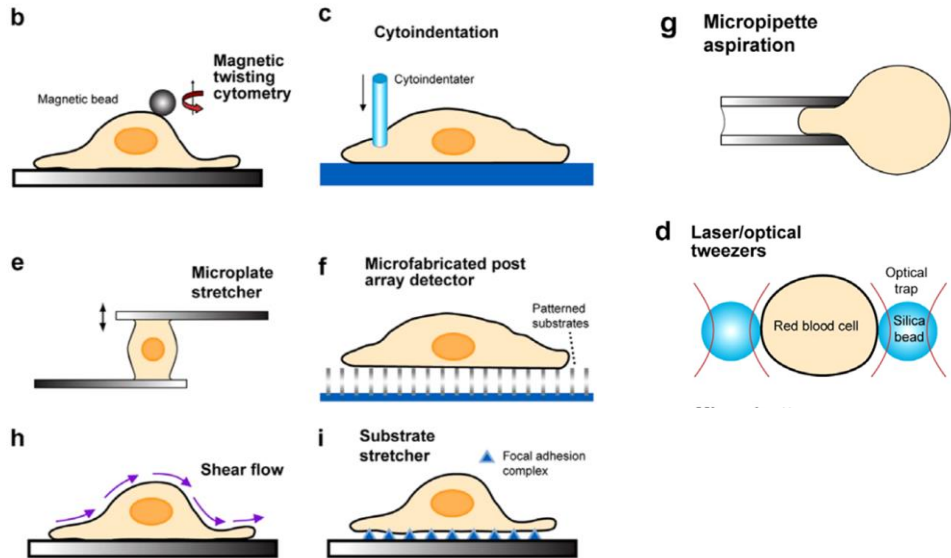
A.D. 1308
unipg
UNIVERSITÀ DEGLI STUDI
DI PERUGIA

PhD in Biotechnology, XXXVII cycle – First year report

Alessandra Anna Passeri

Supervisors: Silvia Caponi
Daniele Fioretto

AIM OF THE PROJECT: to investigate the relation between mechanical properties and biological function in cells

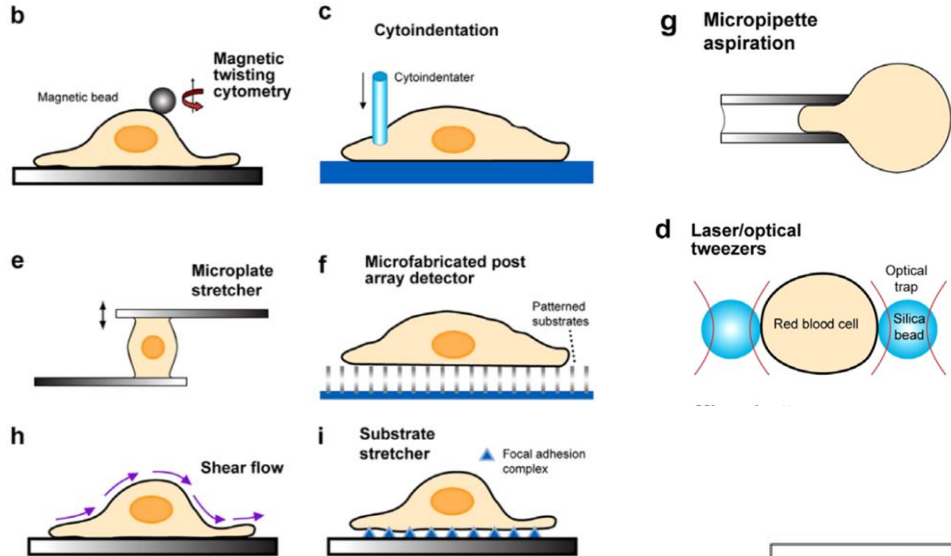


Several techniques can measure mechanical properties of cells

BUT

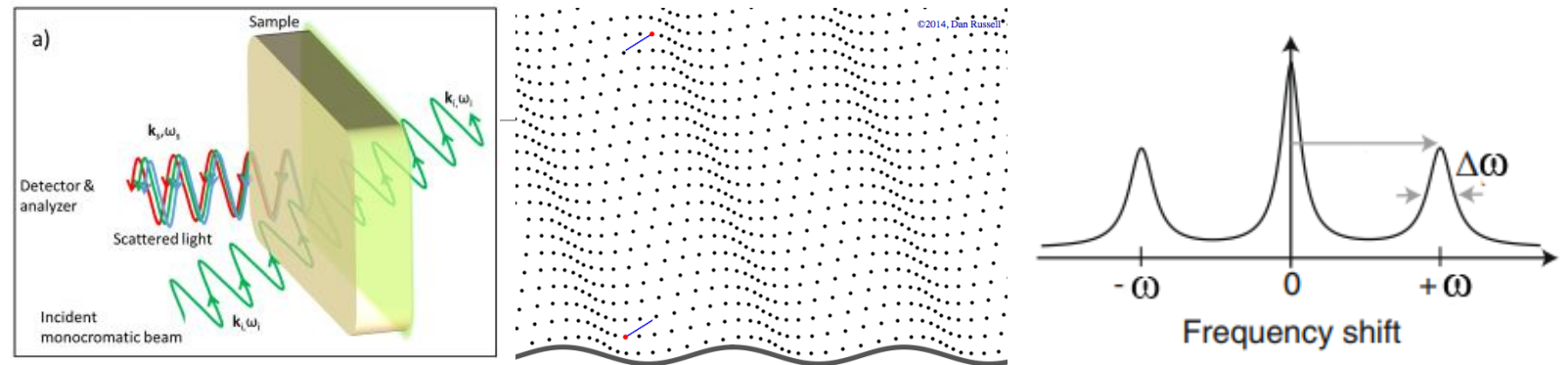
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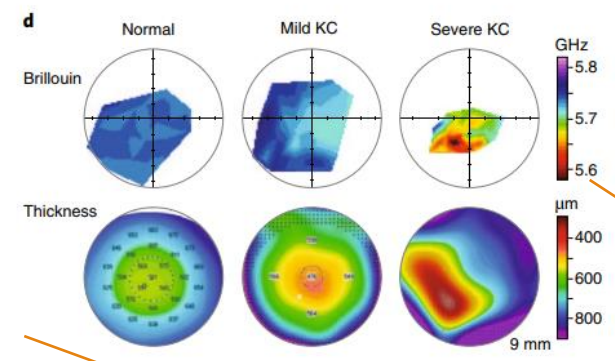
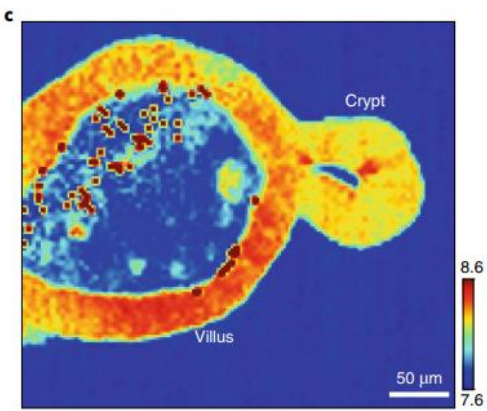


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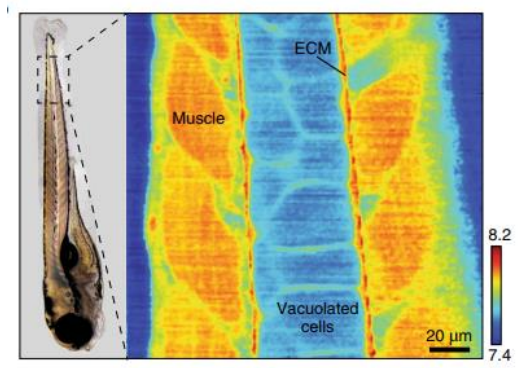
Brillouin micro-spectroscopy



Light interacts with spontaneous acoustic waves



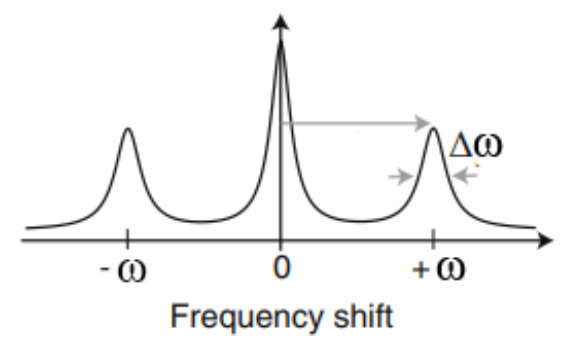
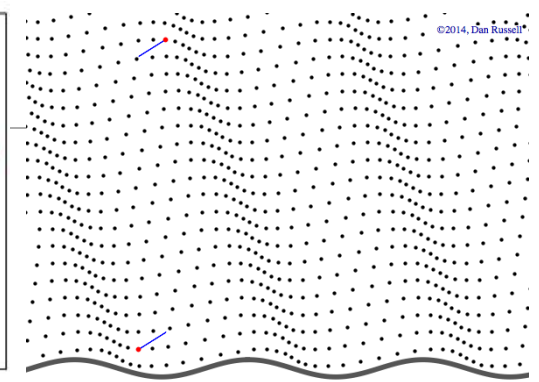
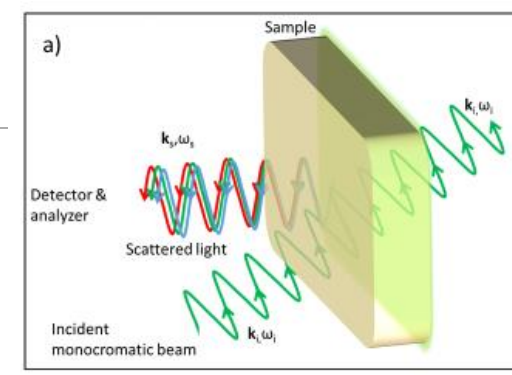
Eye retina



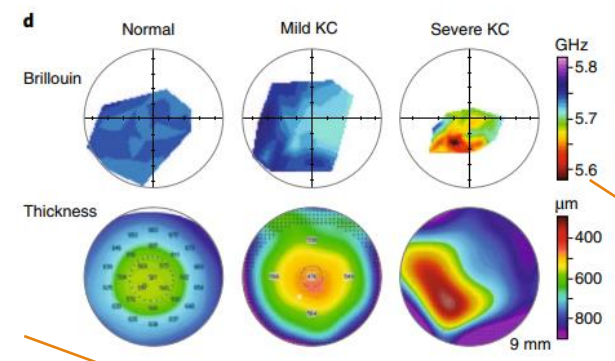
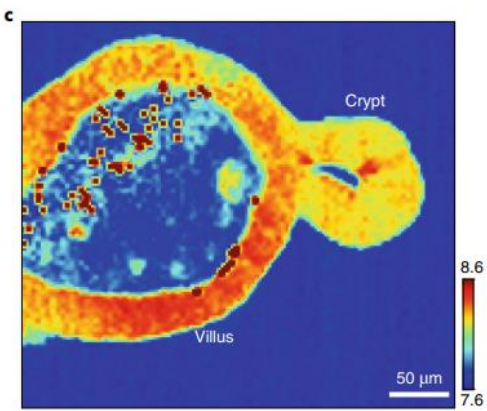
Zebrafish

Gels

Brillouin micro-spectroscopy

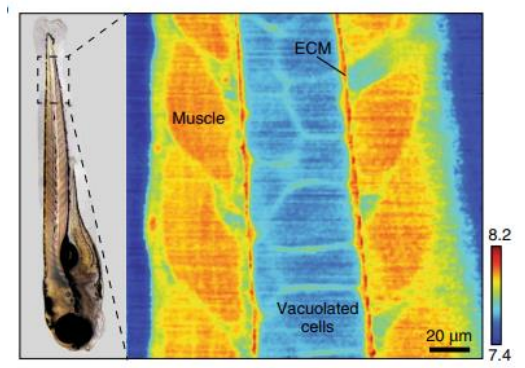


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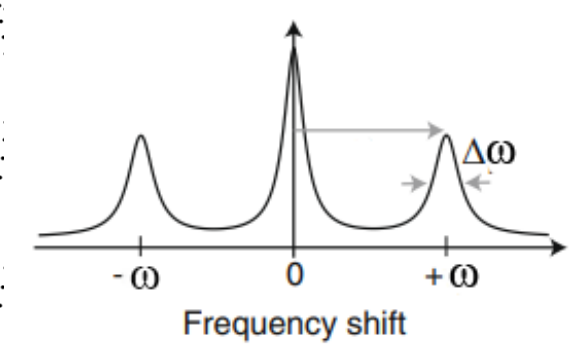
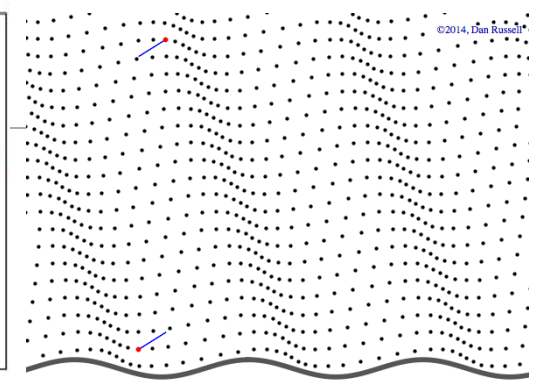
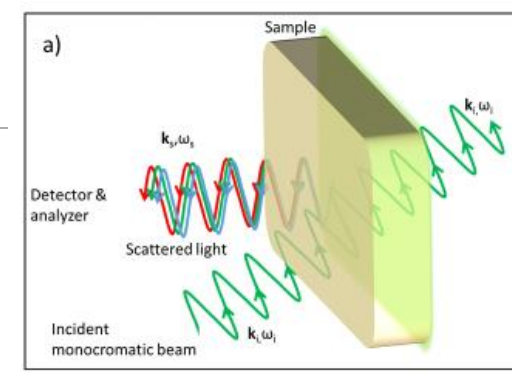
Light interacts with spontaneous acoustic waves



that are related to the mechanical properties of the sample
(longitudinal module [GPa])

$$M' = \frac{\rho \omega^2}{q^2} \quad M'' = \frac{\rho \omega \Delta \omega}{q^2}$$

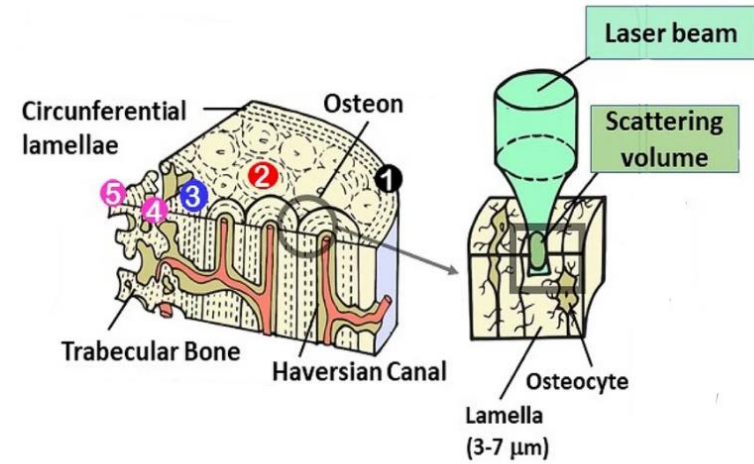
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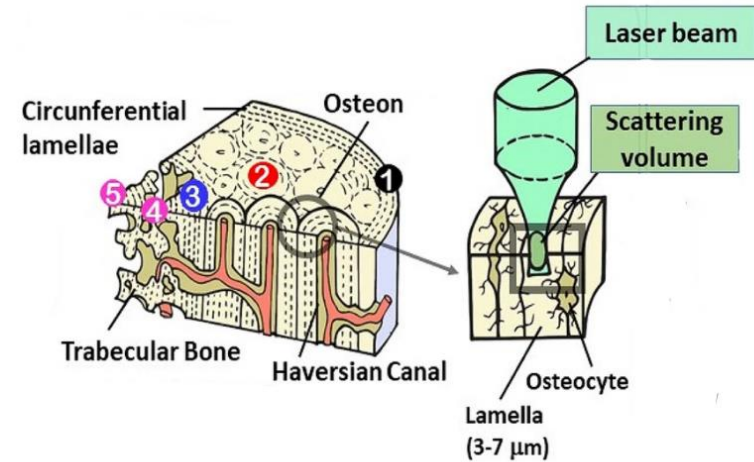
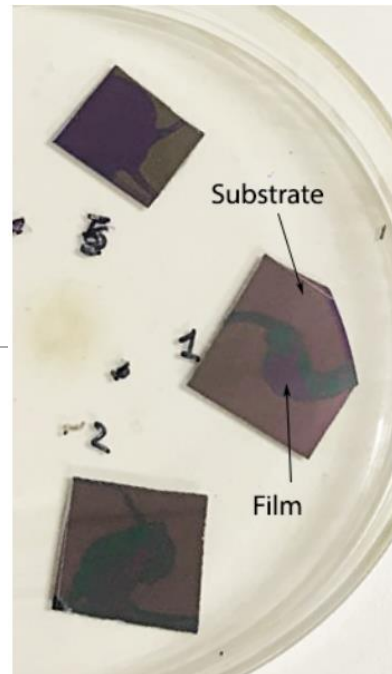


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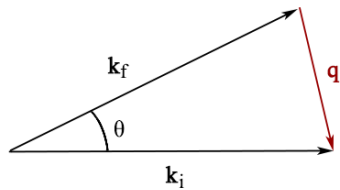
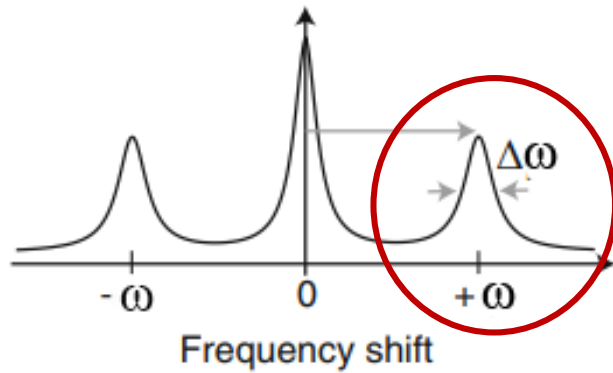
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Follow up from master thesis:

BLS on thin films



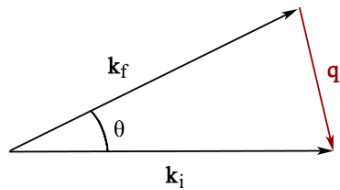
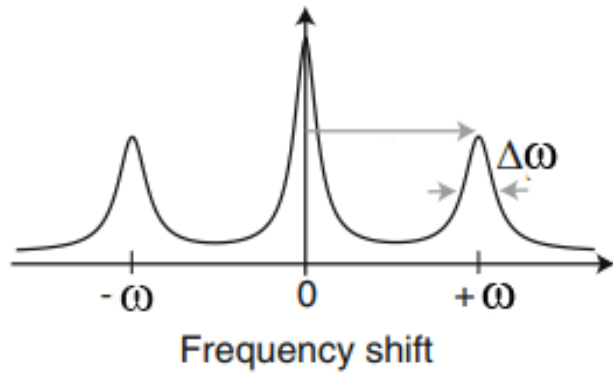
Bulk Samples:



$$I(f) = \frac{I_0}{\pi} \frac{4\Delta\omega \omega^2}{(f^2 - \omega^2)^2 + 4(\Delta\omega)^2 \omega^2}$$

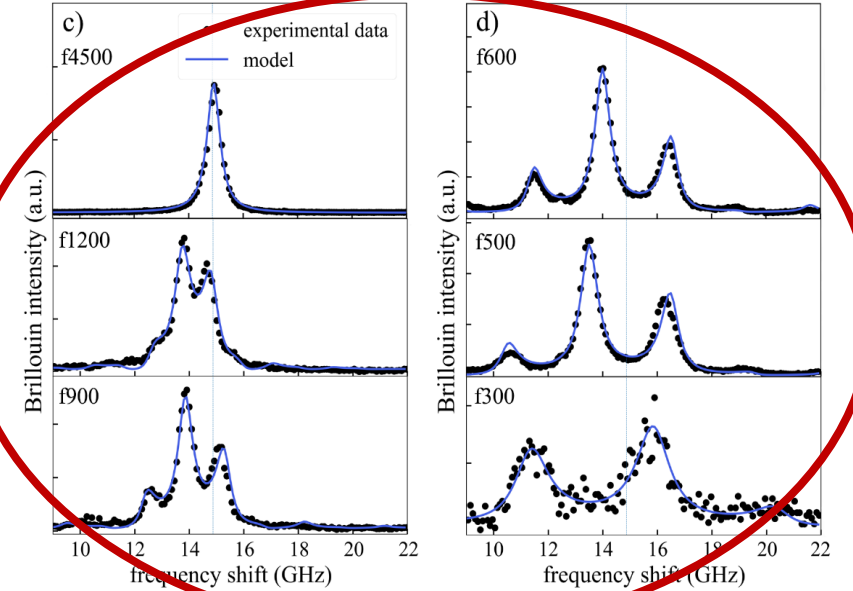
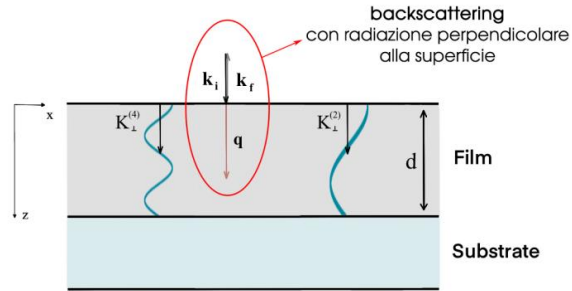
A.A. Passeri; A. Di Michele; I. Neri; F. Cottone; D. Fioretto; M. Mattarelli and Silvia Caponi, **Size and environment: the effect of phonon localization on Micro-Brillouin imaging** (Submitted)

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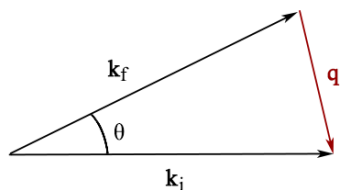
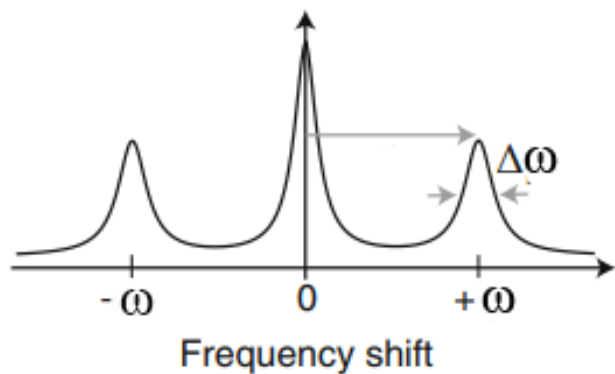


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Thin films:

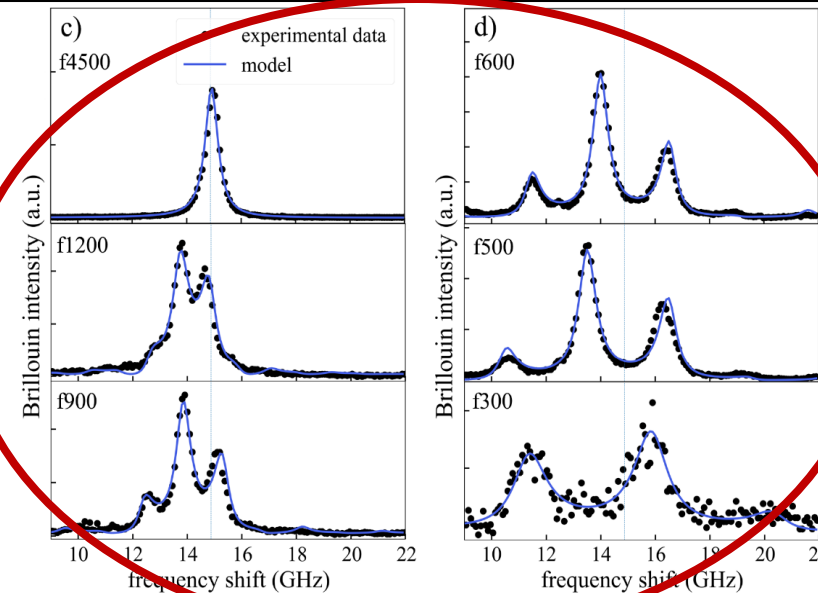
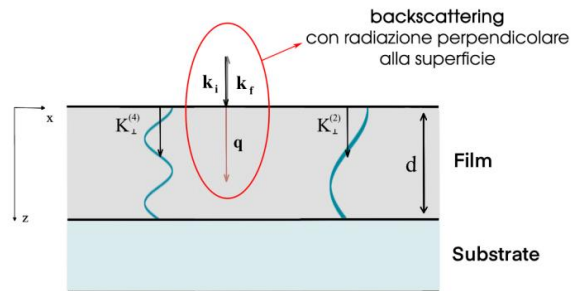


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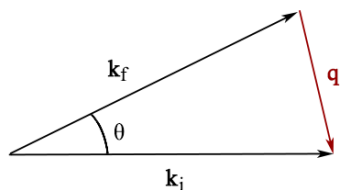
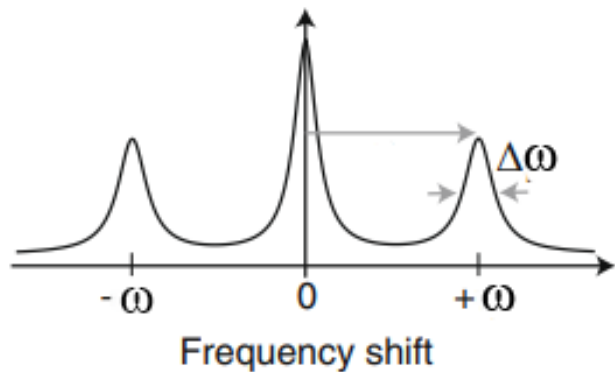
Thin films:



(i) Brillouin intensity depends on the film thickness...

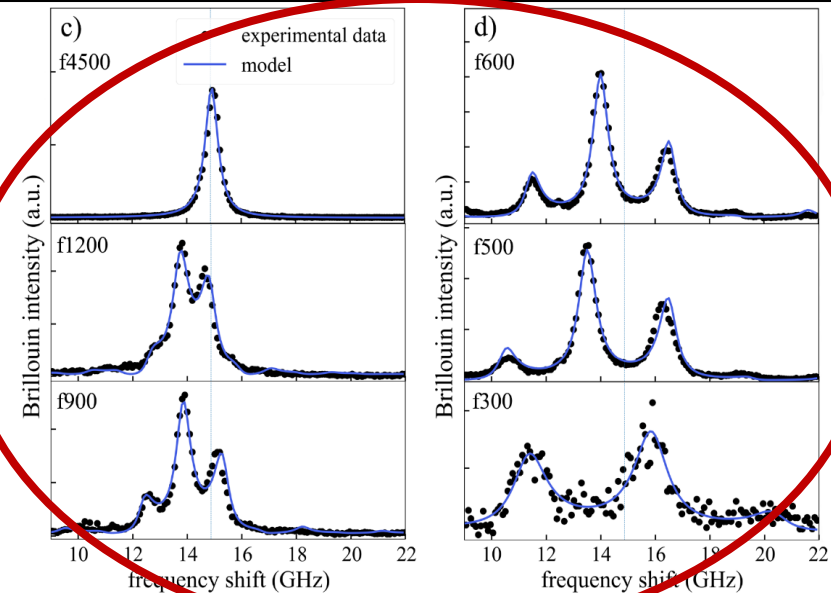
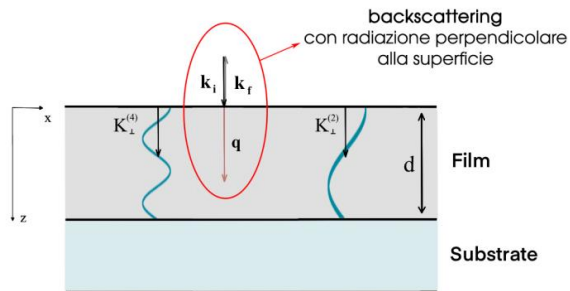
$$I_m(x) = c + I \sum_m \frac{\Gamma}{\Gamma^2 + \left(x - \frac{(2m-1)\Delta f}{2}\right)^2} \text{sinc}^2 \left(\frac{1}{2\Delta f} \left(x - \frac{4nd\Delta f}{\lambda_i}\right) \right)$$

Bulk Samples:



$$I(f) = \frac{I_0}{\pi} \frac{4\Delta\omega \omega^2}{(f^2 - \omega^2)^2 + 4(\Delta\omega)^2 \omega^2}$$

Thin films:



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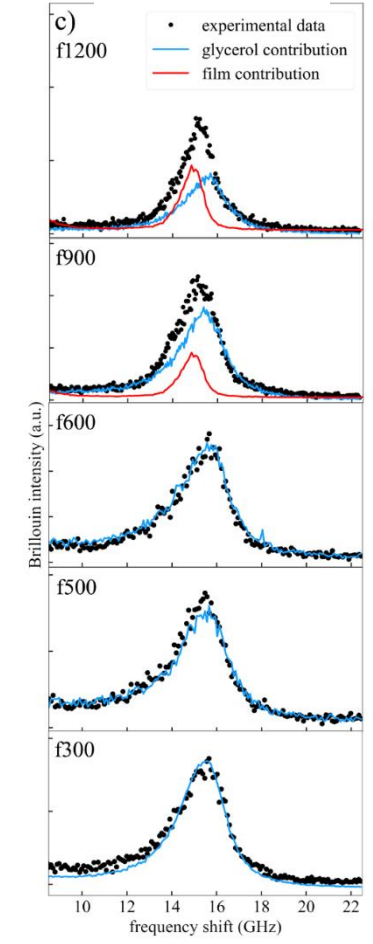
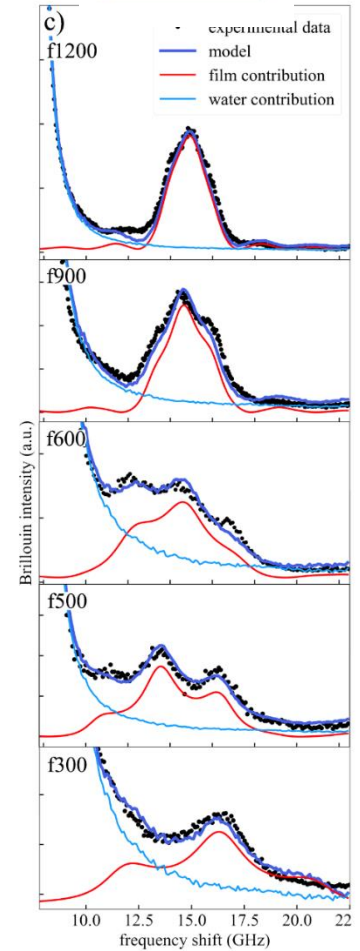
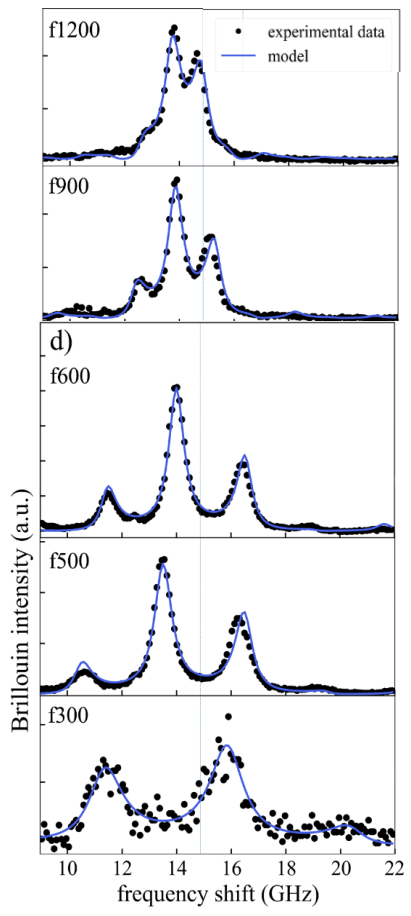
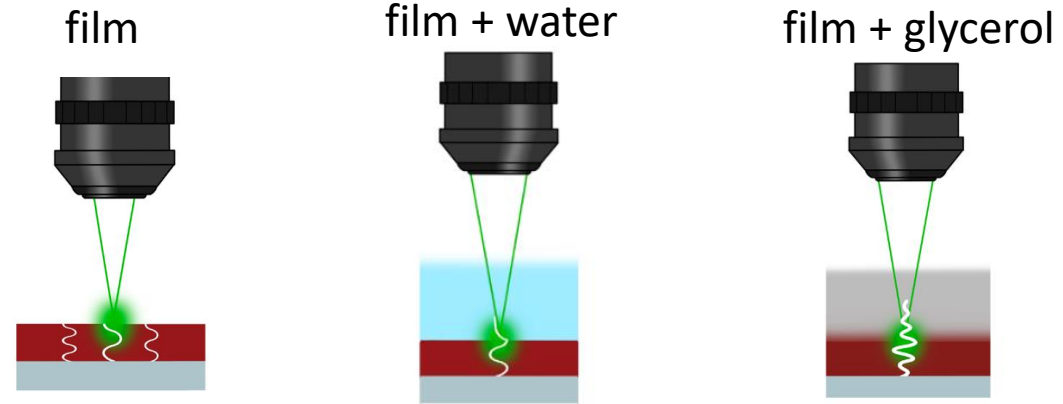
Fitting procedure allows the characterization of the thin film

Thickness: d

Sound speed: $v = \Delta f * 2d$

(ii) and on acoustic mismatch between media

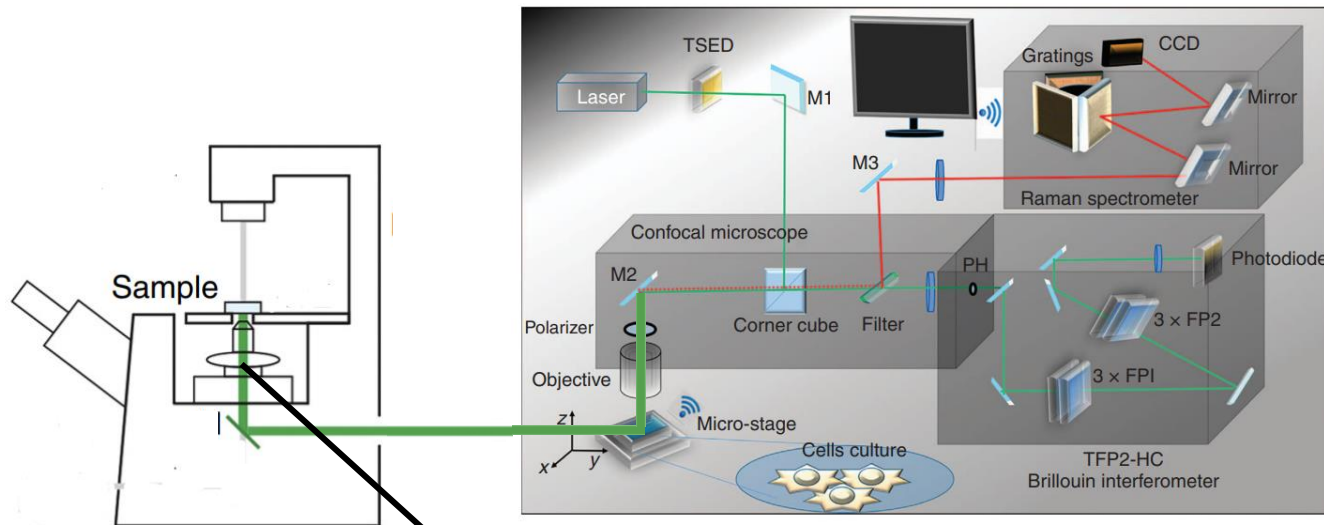
Interface	Acoustic mismatch (Z_R)	Transmittance	Reflectance
PMMA + air	$8.8 \cdot 10^3$	0	1
PMMA + Silica	6	0.47	0.53
PMMA + H_2O	2	0.89	0.11
PMMA + Glycerol	0.8	0.99	0.01



A.A. Passeri; A. Di Michele; I. Neri; F. Cottone; D. Fioretto; M. Mattarelli and Silvia Caponi, **Size and environment: the effect of phonon localization on Micro-Brillouin imaging** (Submitted)

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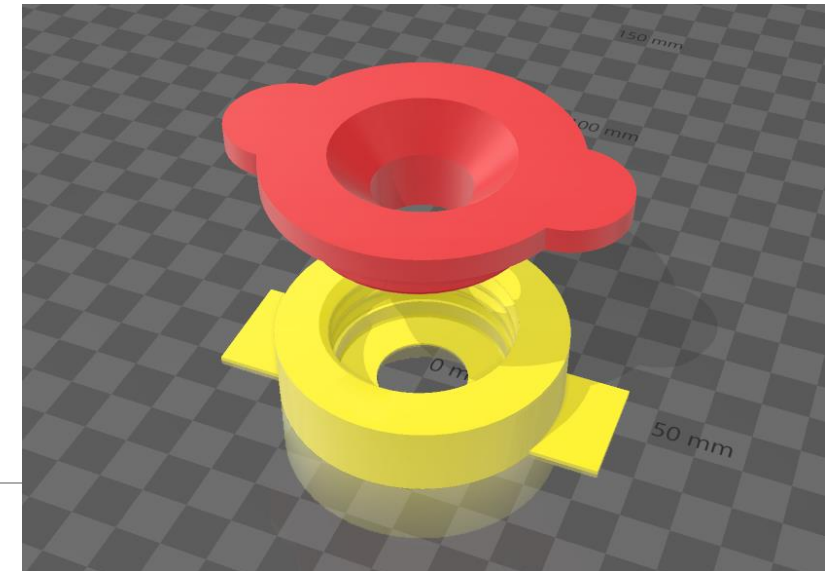


**Inverted
microscope**

XYZ stage (micron resolution)

Possibility to acquire maps

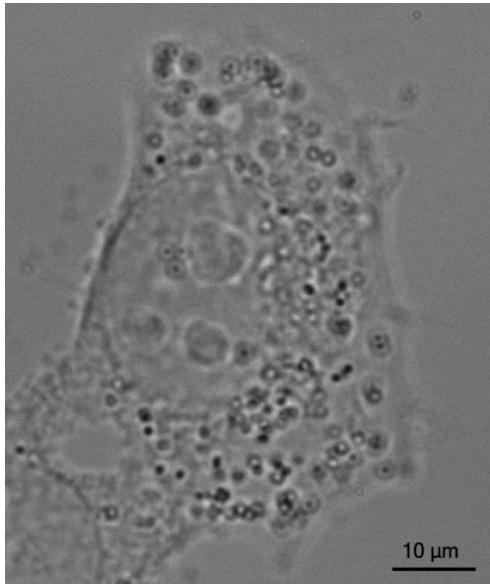
Sample Holder for cells – design



Thanks to Giulio Capponi
(Dep. Of Physics UNIPG)

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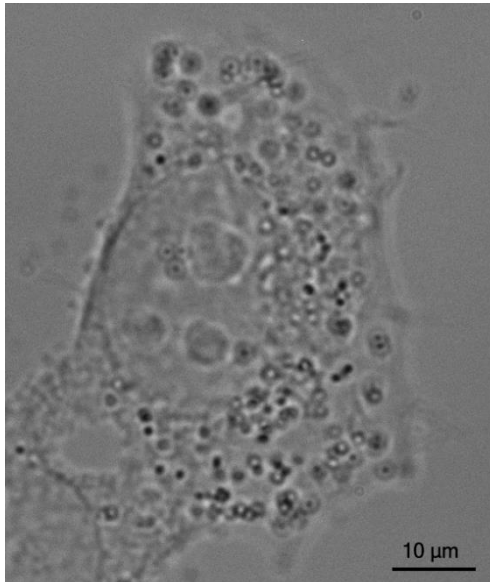


Probing cells is not easy!

- Micrometric dimension
- Consisting of different compartments
- Limited lifetime

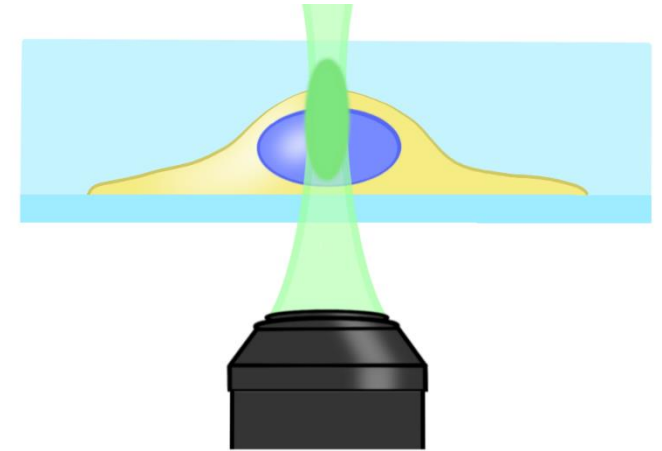
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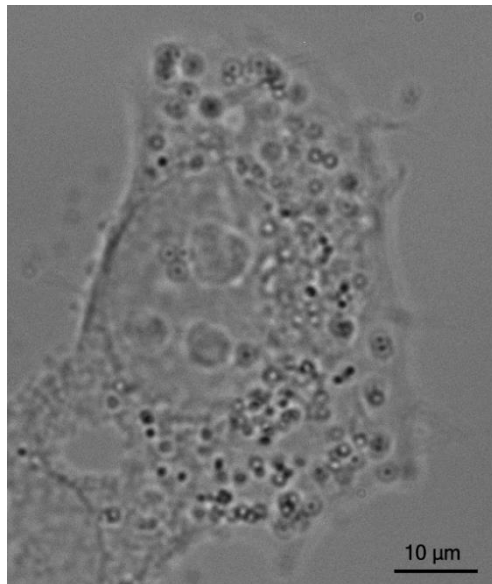
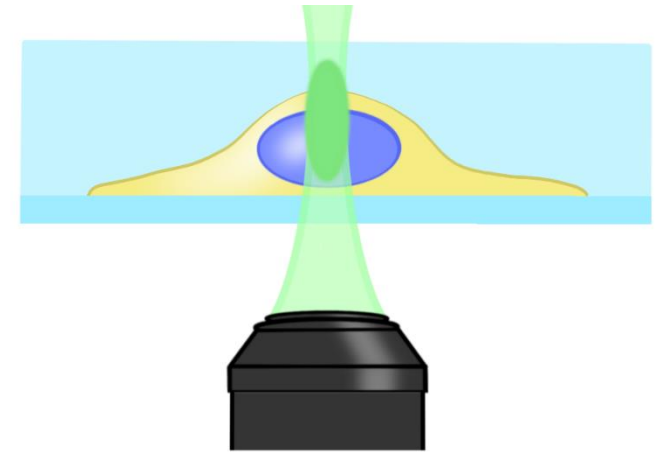
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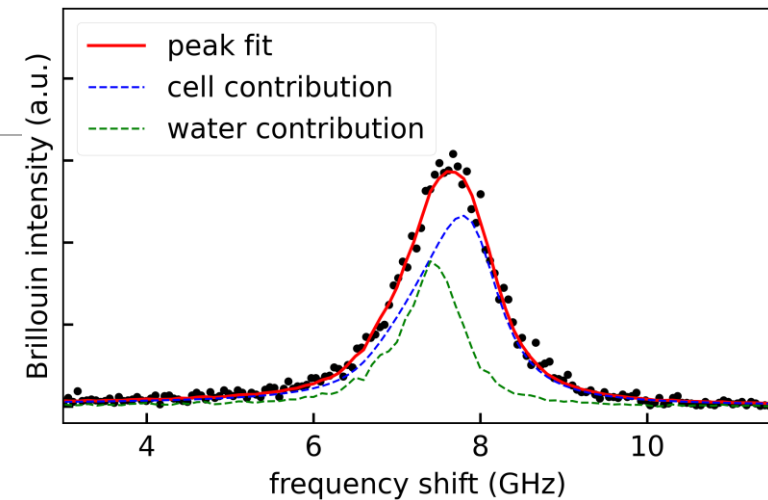
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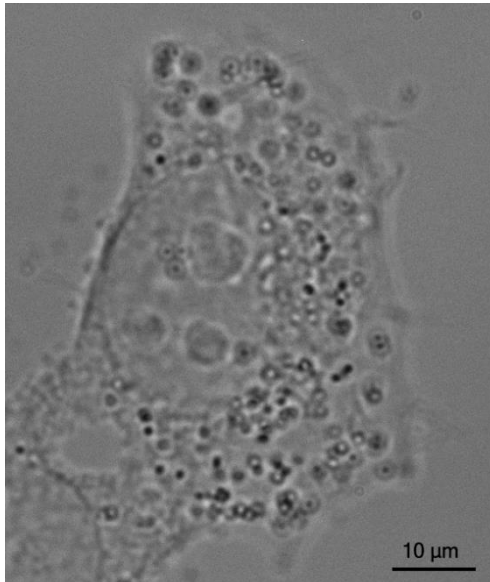
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We implemented a function fit able to separate water and cell contribution



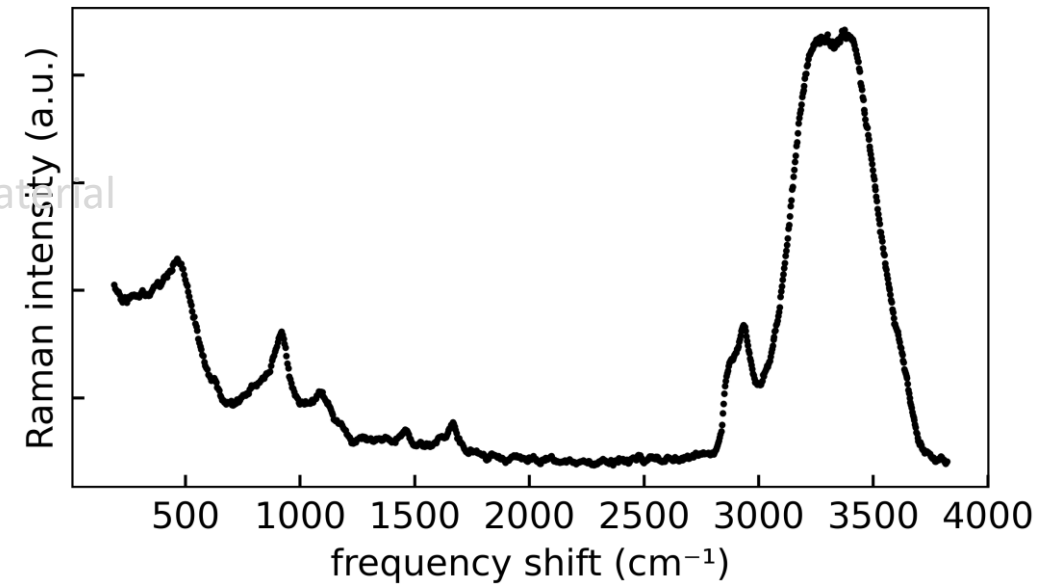
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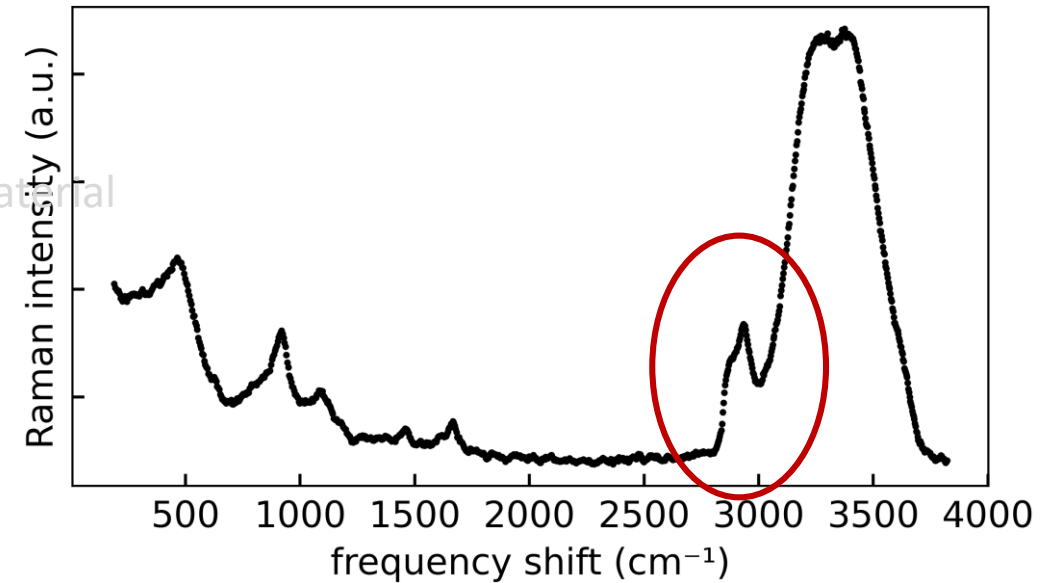
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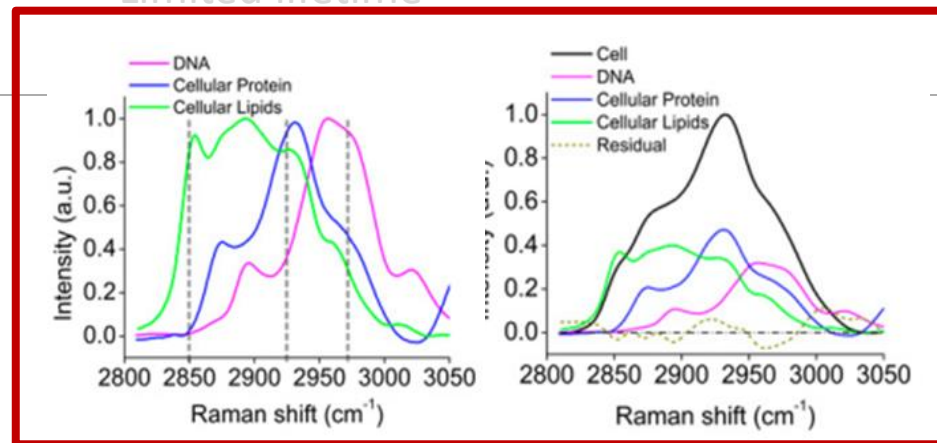
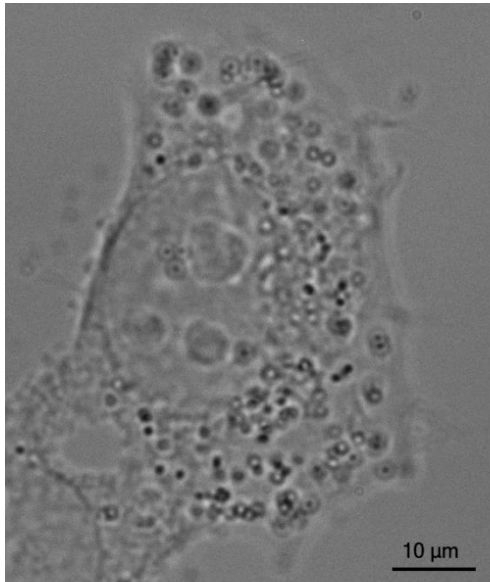
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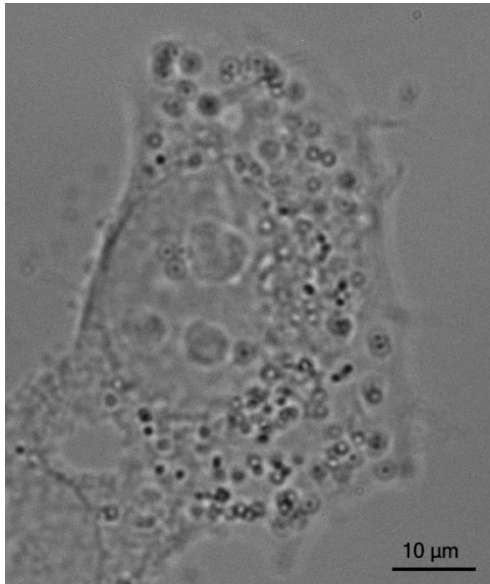
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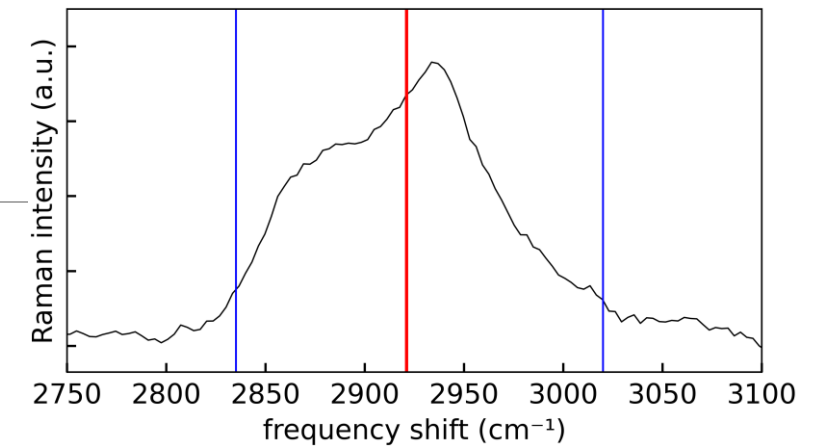
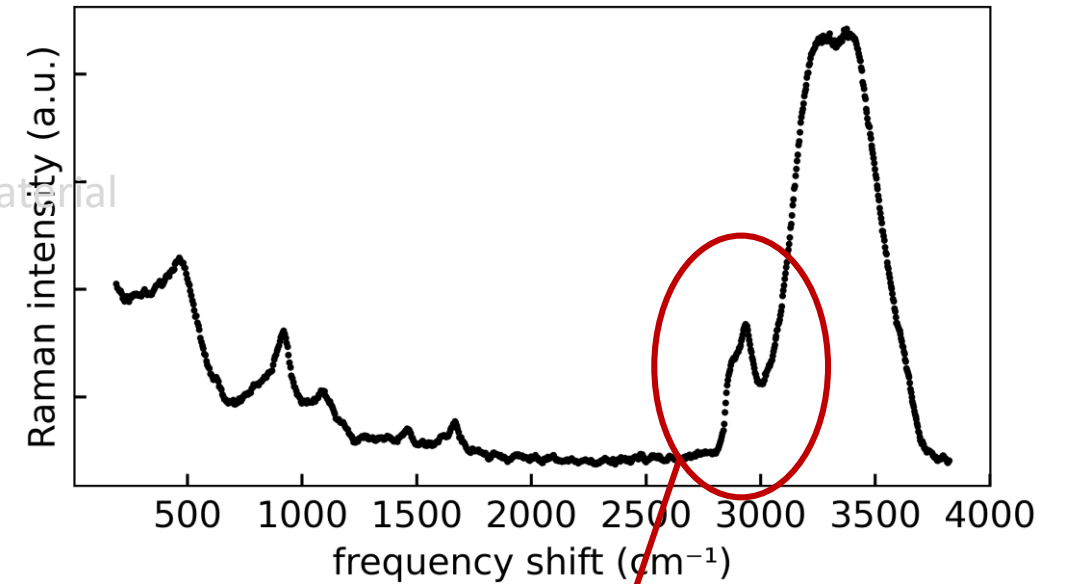
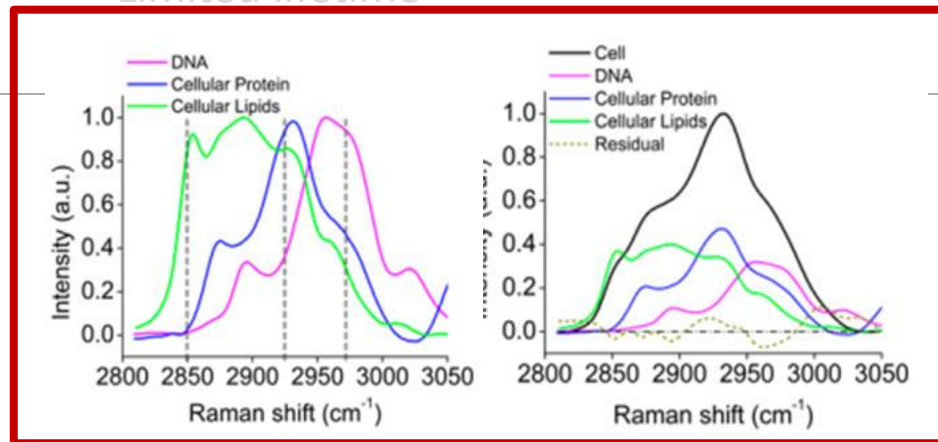
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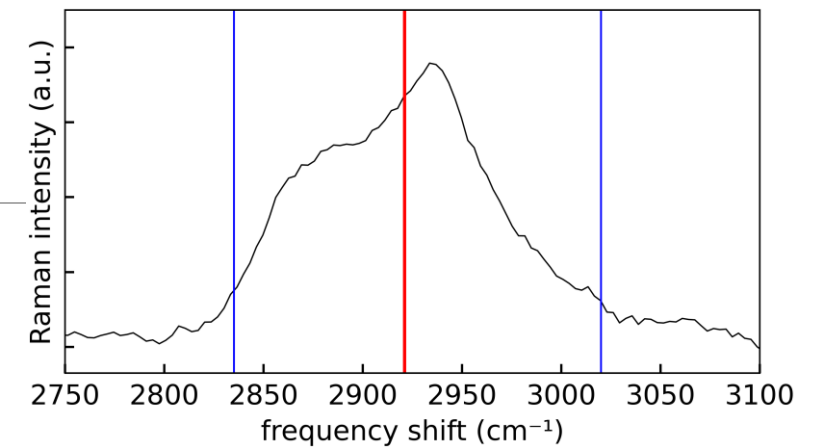
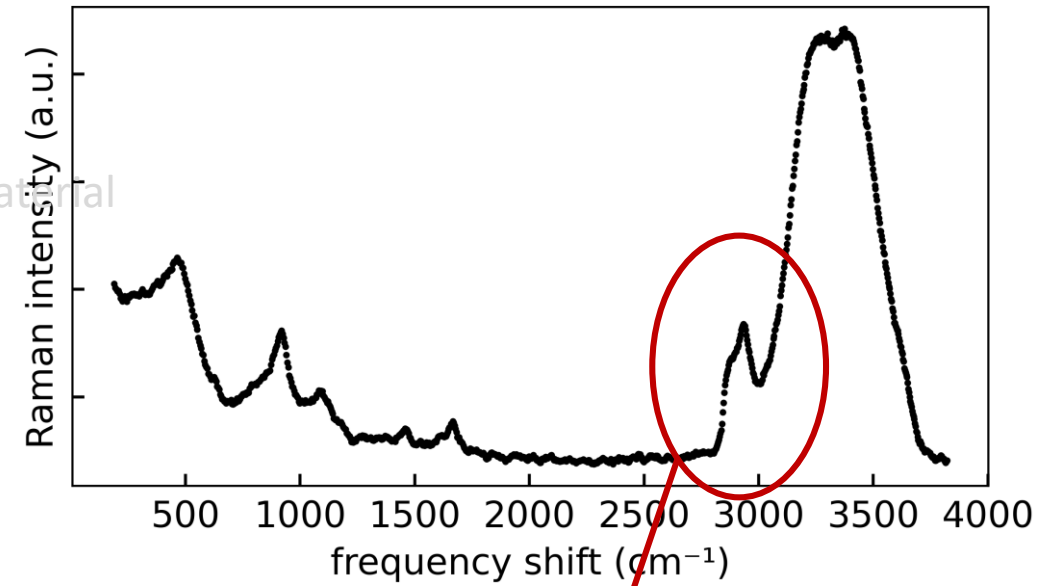
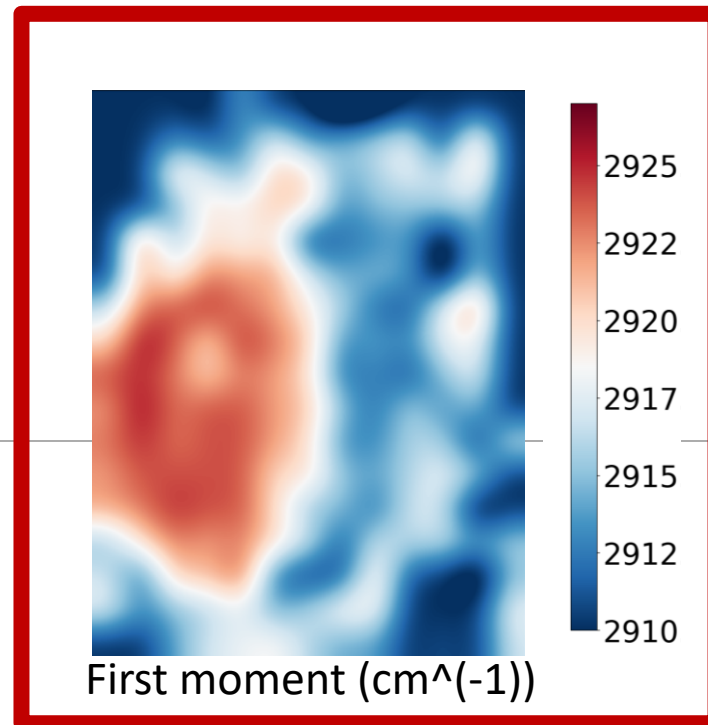
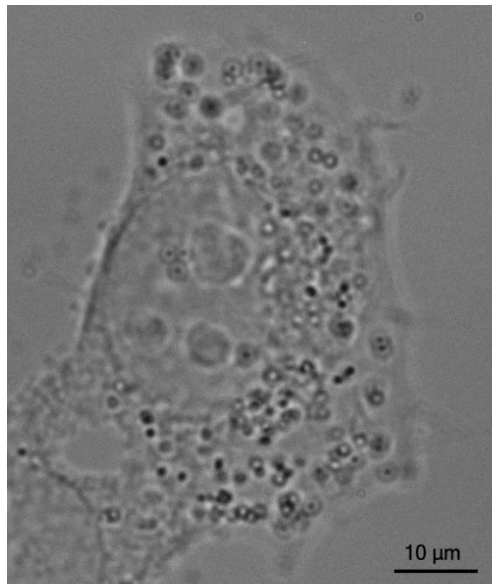
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Raman analysis allows us to distinguish nucleous from cytoplasm

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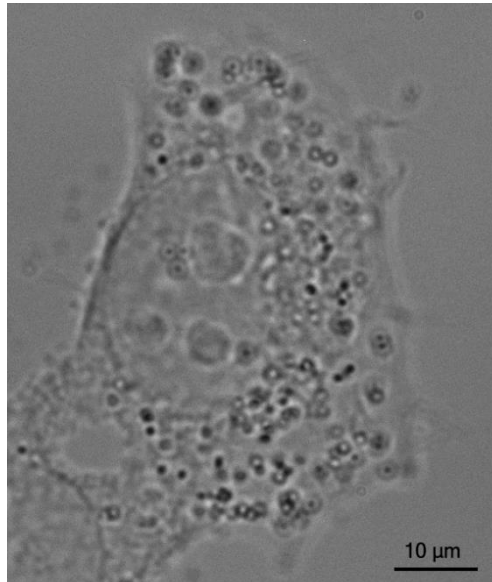
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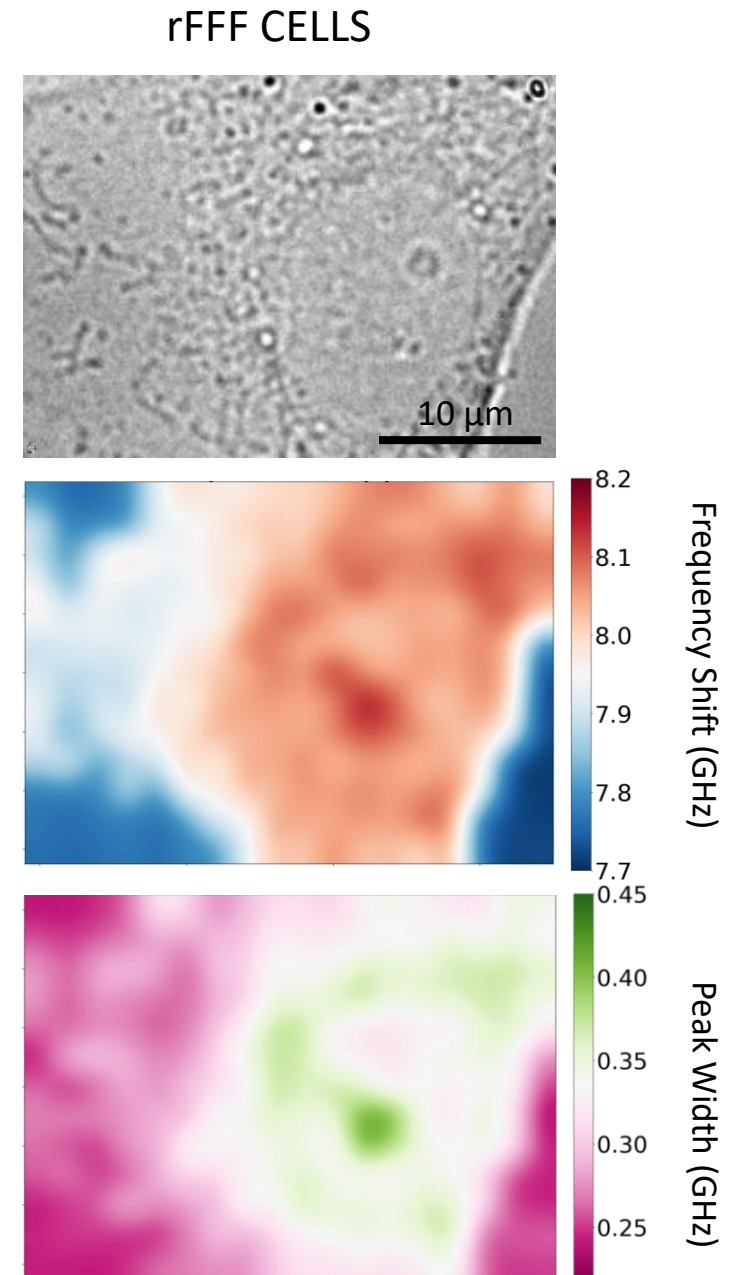
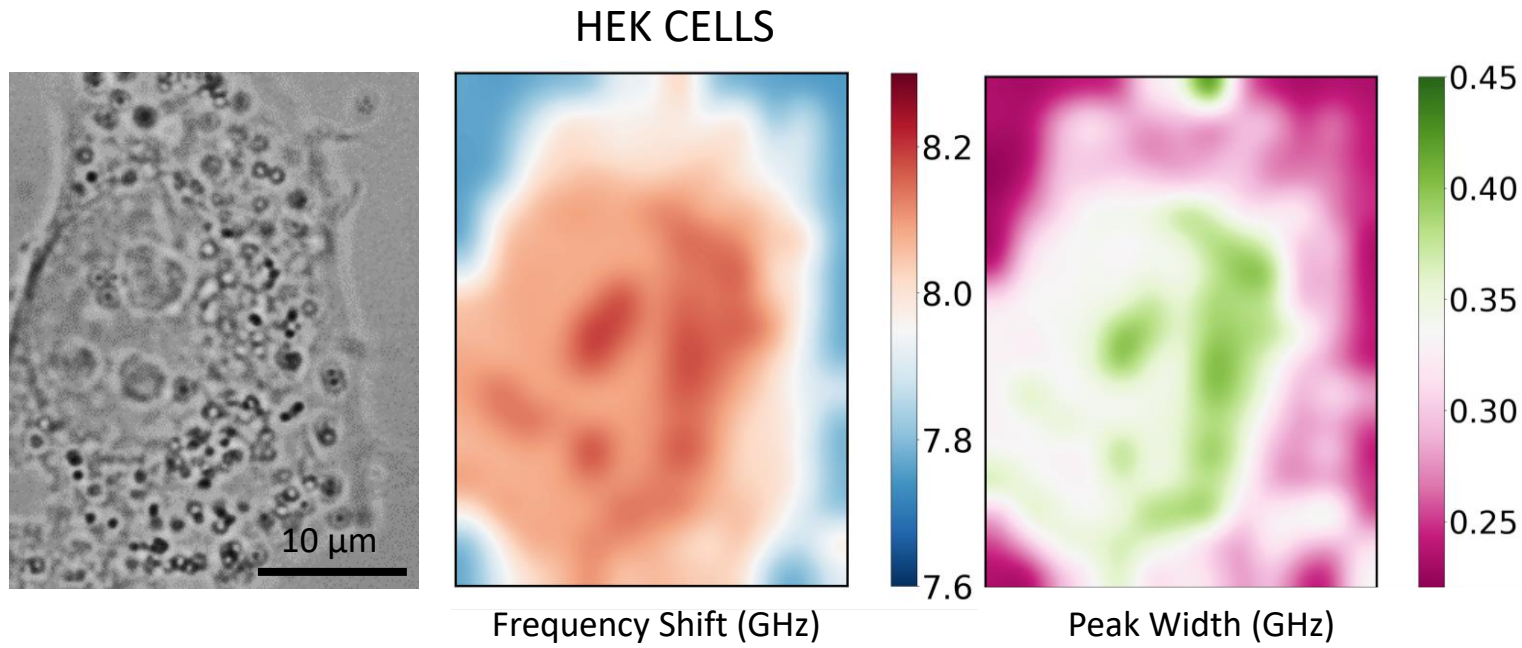
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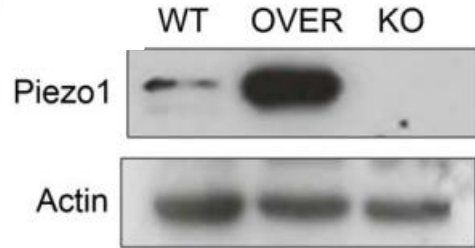
Fresh cells:
Linear maps

4% PFA fixed cells:
XY maps

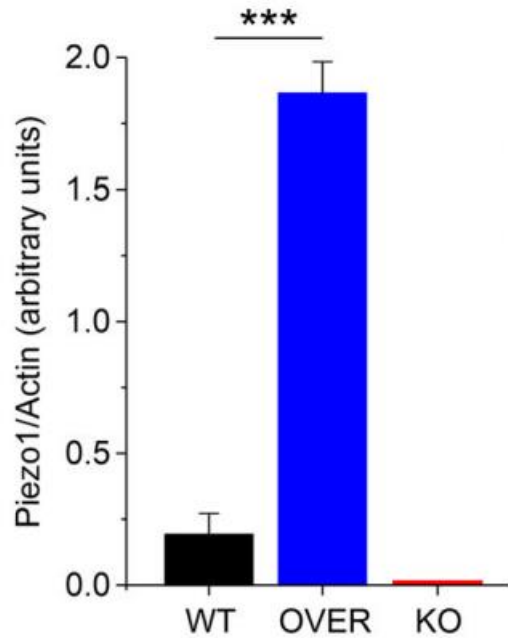
We acquired mechanical maps on different type of cells:



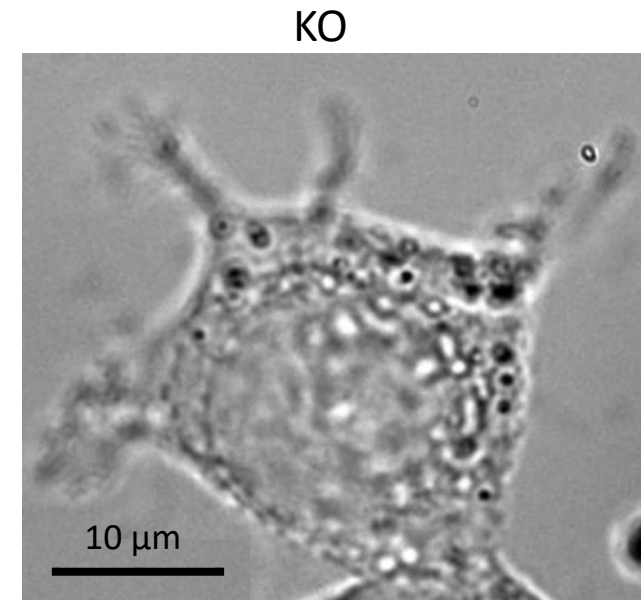
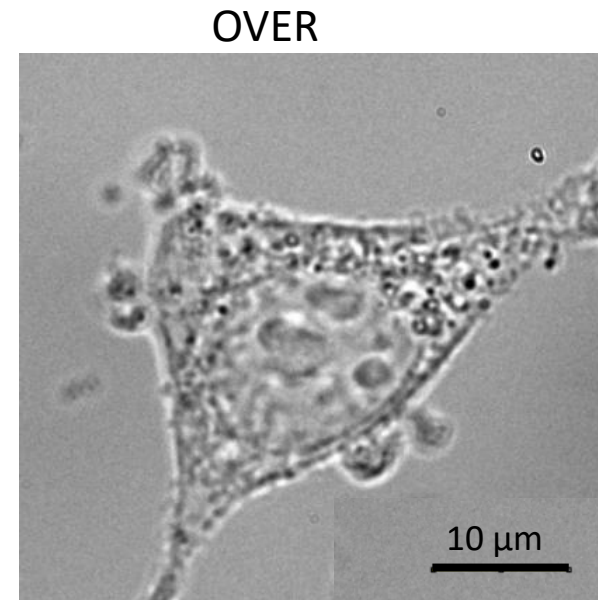
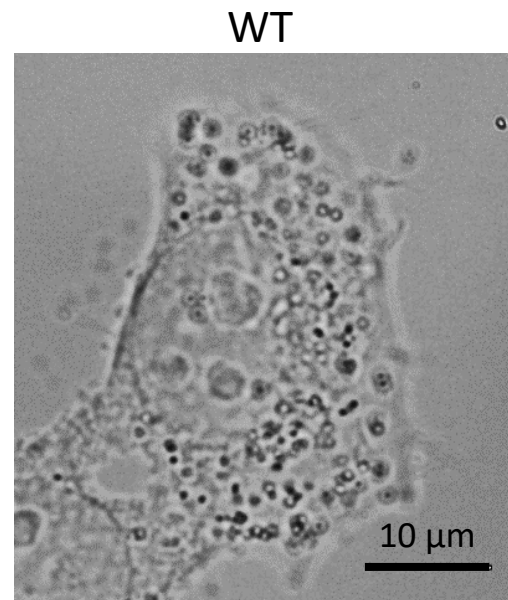
Preliminary results on HEK293T cell line presenting mechanosensitive ion channel Piezo1:



- Wild-type (WT) HEK293T cells
- Piezo1 overexpressing (OVER) HEK293T cells
- Piezo1 knock-out (KO) HEK293T cells

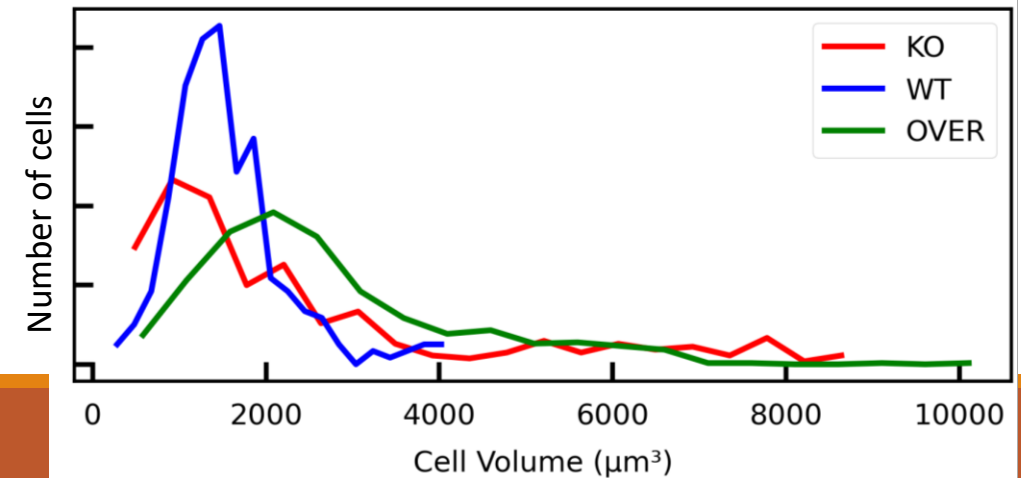
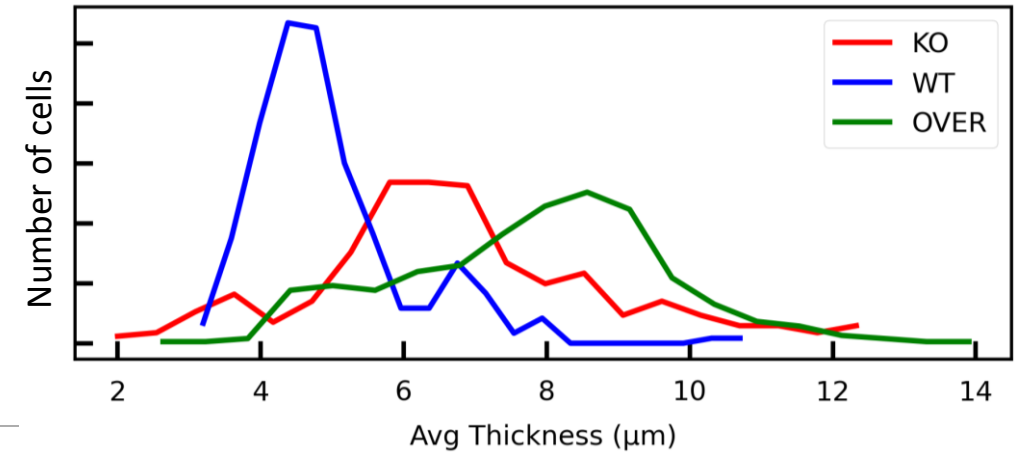
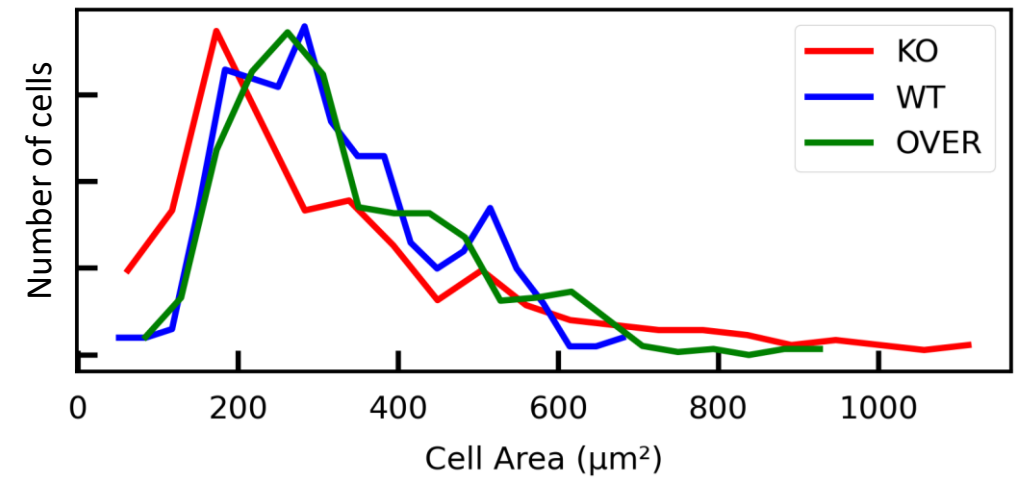
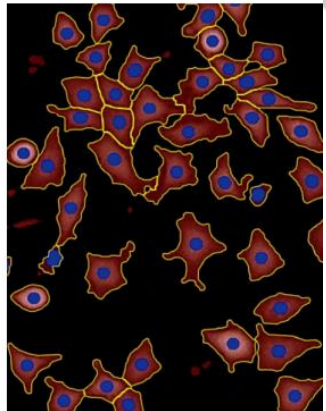
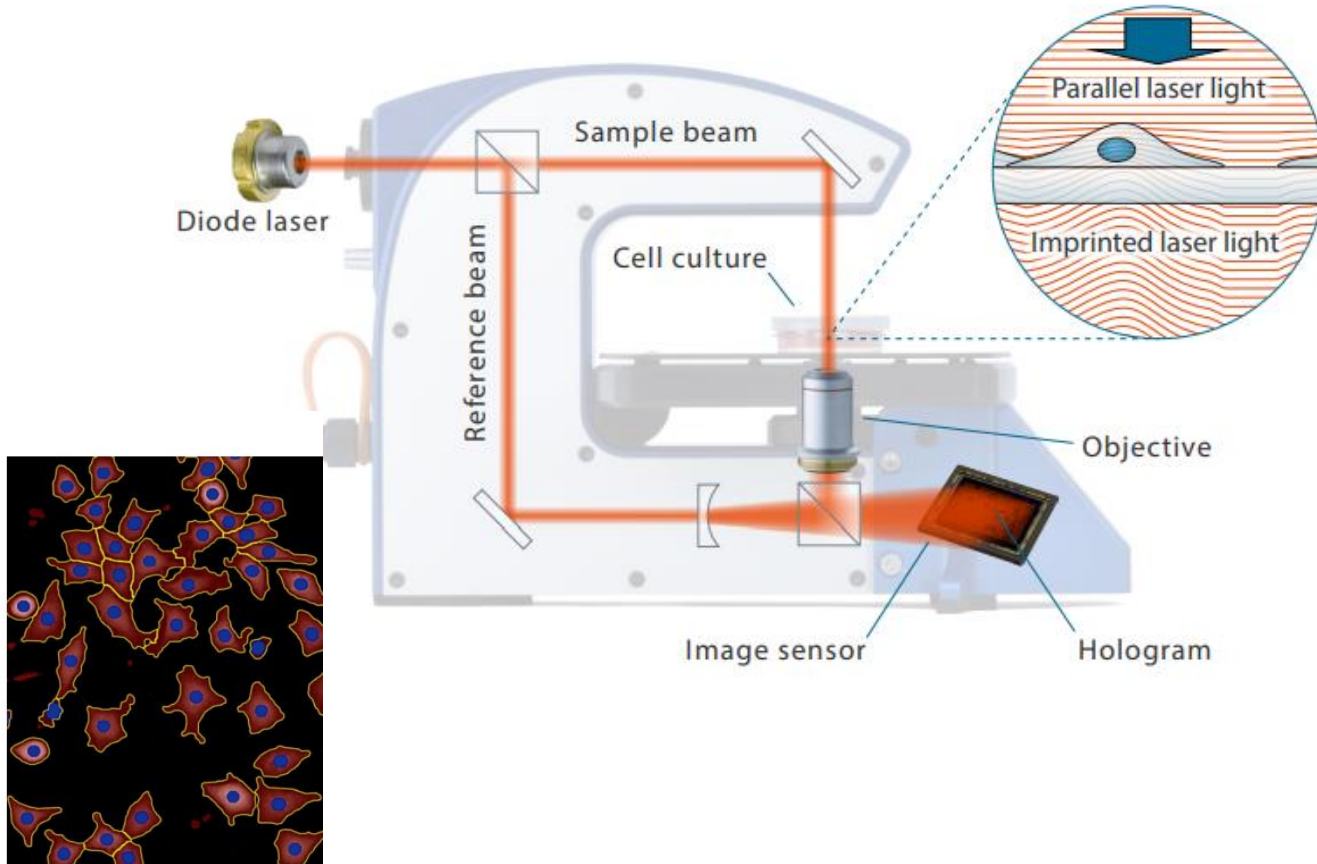


Sforna L. et al. J Cell Physiol. 237(3) (2022).

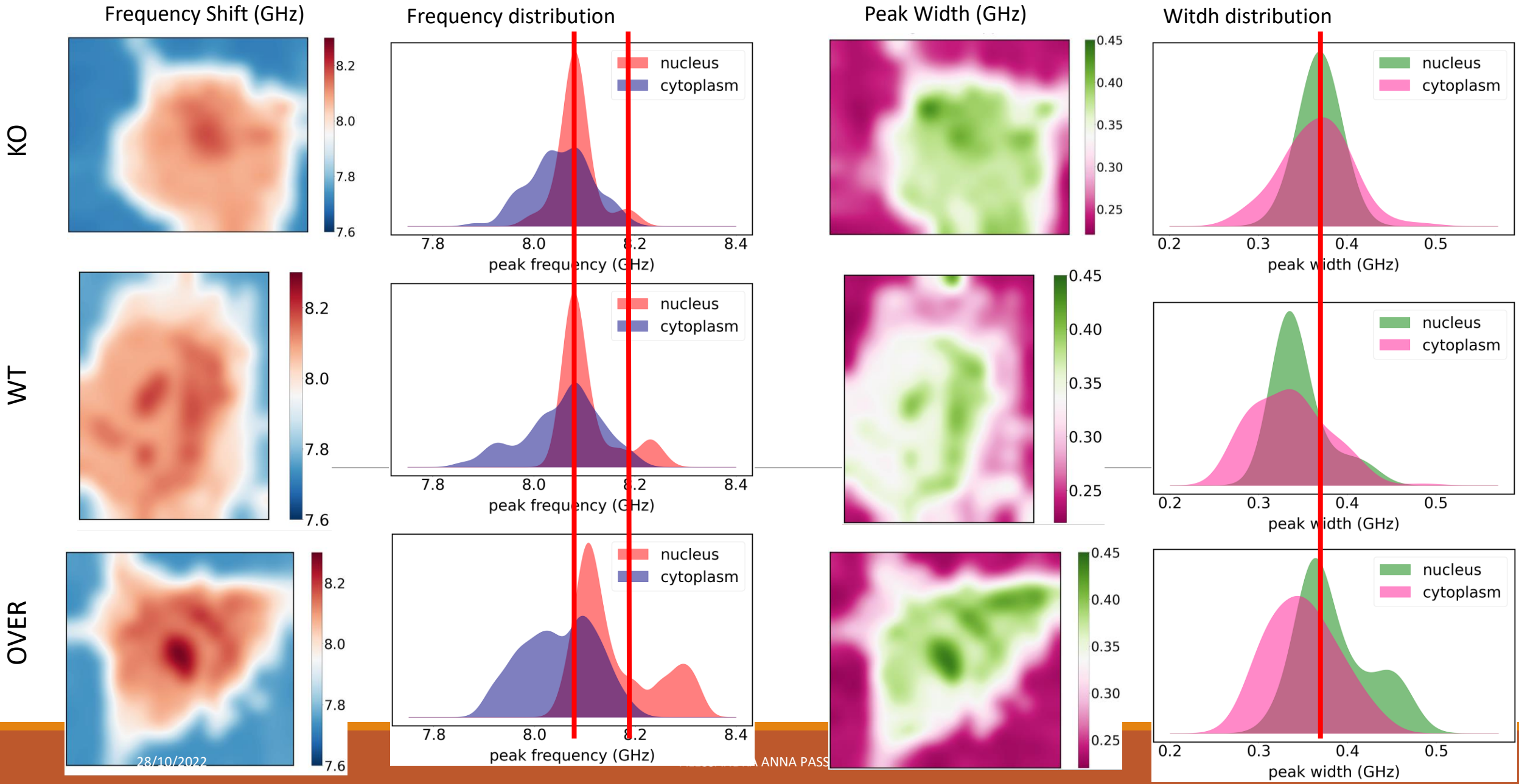


Morphology measurement of HEK cells (University of Glasgow – August 2022)

By *Quantitative phase imaging*



Brillouin maps on HEK cells



Recap:

- We optimized the Brillouin-Raman micro-spectroscopy setup to investigate cells
- We were able to describe the mechanical properties of microstructured samples
- We successfully acquired mechanical maps on different type of cells

Next Steps:

- Mapping analysis of HEK cell -> to understand the relation between the mechanical properties and the channel expression
- Mechanical analysis on fresh living cells and comparison with data acquired on fresh cells

Cell incubator on the way!

Courses:

- Nanosystems and advanced materials;
- Introduction to Data analysis and data formats;
- Introduction to basic programming in Biotechnologies;
- Femtobiology;
- Two-Photon Absorption for Bio-Applications;

International activity:

- Research activity at University of Glasgow – August 2022

Articles:

- S. Caponi, A.A. Passeri, G. Capponi, D. Fioretto, M. Vassalli, M. Mattarelli “Non-contact elastography methods in mechanobiology: a point of view”, *Eur Biophys J* 51, 99–104 (2022)
- M. Mattarelli, G. Capponi, A.A. Passeri, D. Fioretto, and S. Caponi, Disentanglement of Multiple Scattering Contribution in Brillouin Microscopy, *ACS Photonics* 9 (6), 2087-2091 (2022)
- A.A. Passeri; A. Di Michele; I. Neri; F. Cottone; D. Fioretto; M. Mattarelli and S. Caponi, Size and environment: the effect of phonon localization on Micro-Brillouin imaging (Submitted)

Schools:

- BBM5 (5th BioBrillouin Meeting) *Virtual Training School* – Online
- Winter school of Biotechnology

Conferences:

- 5th BioBrillouin Meeting (BBM5) - Online
- XXVI Congresso Nazionale SIBPA – San Miniato (PI)

Collaborations:

Dep. Physics, Geology (UNIPG) and CNR:

- Silvia Caponi (CNR), Maurizio Mattarelli, Martina Alunni Cardinali, Daniele Fioretto, Igor Neri

Dep. Chemistry, Biology and Biotechnology (UNIPG):

- Chiara Argentati, Ilaria Tortorella, Sabata Martino

Dep. Of Biomedical Engineering (University of Glasgow):

- Massimo Vassalli, Sabah Sardar, Camilla Romagnoli

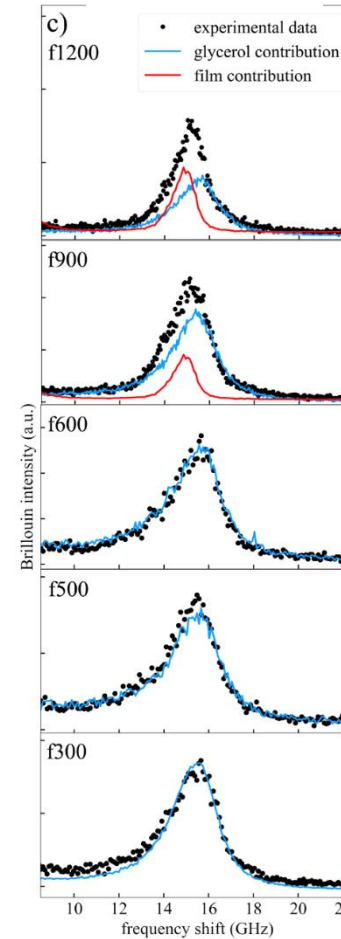
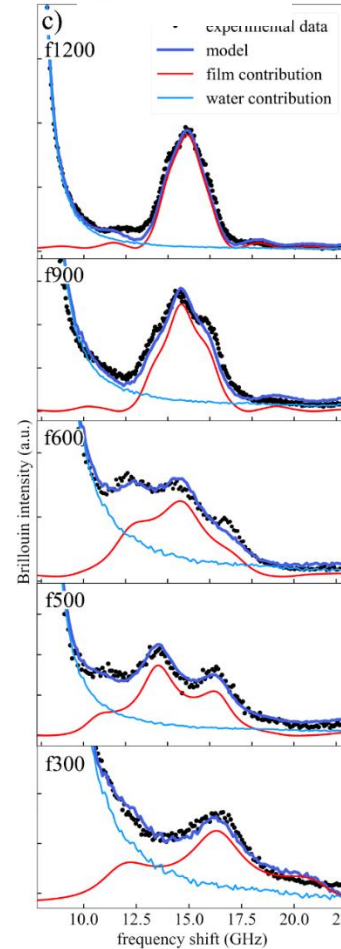
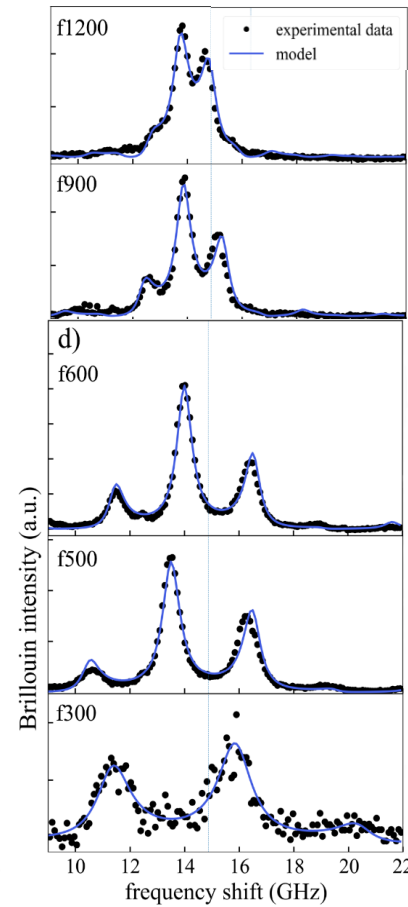
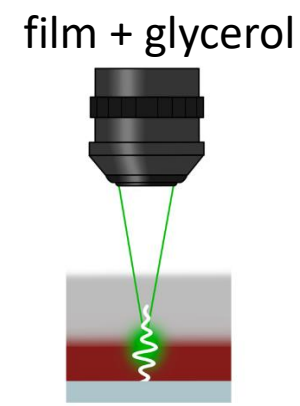
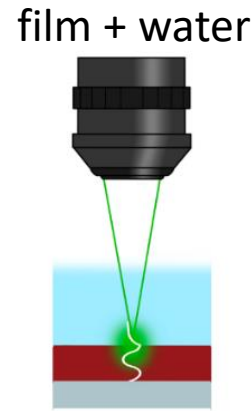
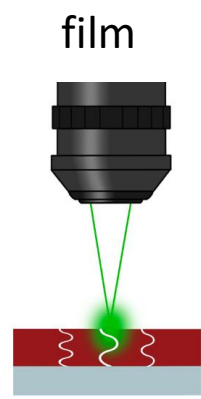
Dep Physics (University of Genova):

- Claudio Canali, Sajedeh Kerdegari

Thank you for your attention!

(ii) and on acoustic mismatch between media

Interface	Acoustic mismatch (Z_R)
PMMA + air	$8.8 \cdot 10^3$
PMMA + Silica	6
PMMA + H_2O	2
PMMA + Glycerol	0.8



Acoustic mismatch is related to the peak width:

$$\Gamma_R = \frac{-\ln(R_{AB}R_{BC})}{2\pi t_{RT}}$$

