Non-contact mechanical and chemical analysis of single living cells investigated by micro-spectroscopic techniques

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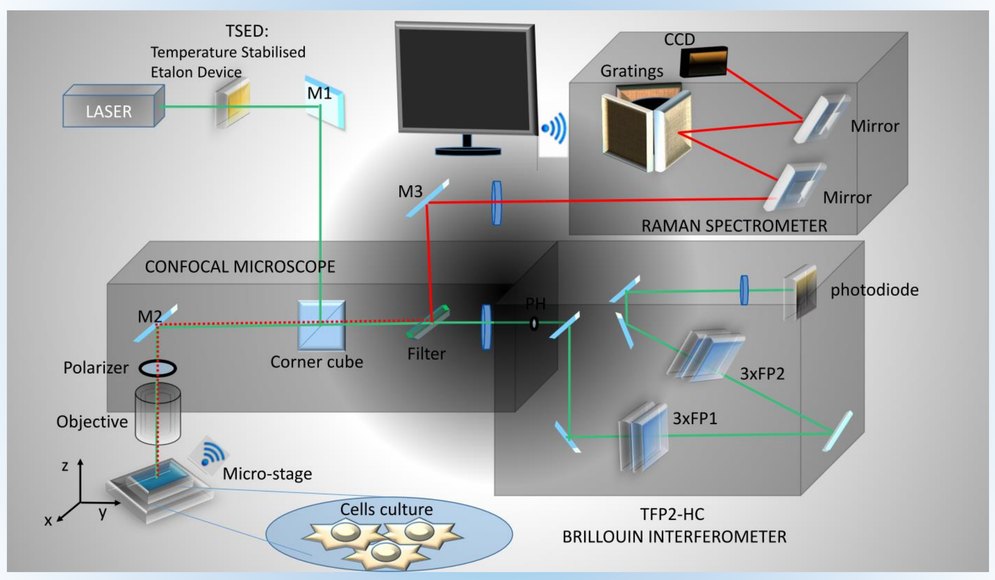
Abstract:

We developed an innovative method that can simultaneously characterize the elastic and chemical properties of biological materials with sub-cellular spatial resolution [1-2]. The technique use Raman and Brillouin spectroscopies, established techniques for nondestructive contactless and label free readout of materials properties. In a proof-of-principle experiment, the ability of the set-up to characterize subcellular compartments distinguish cell status was successfully tested.
From the Brillouin signal, the elastic properties of single living cells immersed in buffer solution are analyzed [2]. The existence of mechanical heterogeneity inside the cell has been point out: a 20% increase is observed in the elastic modulus passing from the plasmatic membrane to the nucleus as distinguished by comparison with the Raman spectroscopic marker. Brillouin line shape analysis is even more relevant for the comparison of cells under physiological and pathological conditions. Following oncogene expression, cells show an overall reduction in the elastic modulus (15%) and apparent viscosity (50%) showing how the mechanical and chemical properties of cells are intimately connected and their imbalances can be symptoms or effects of pathologies.

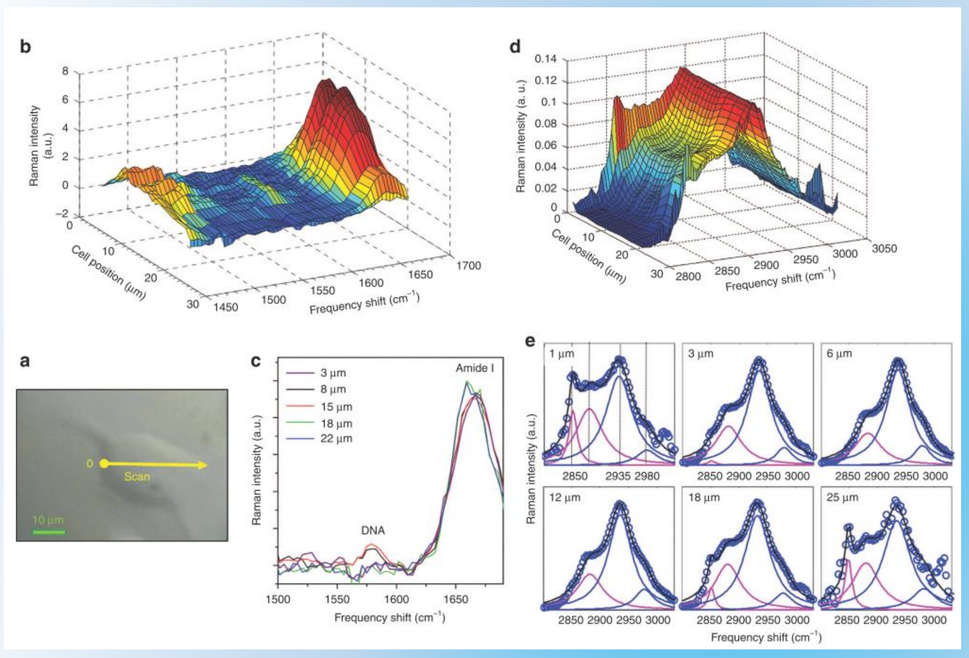
Experimental set-up



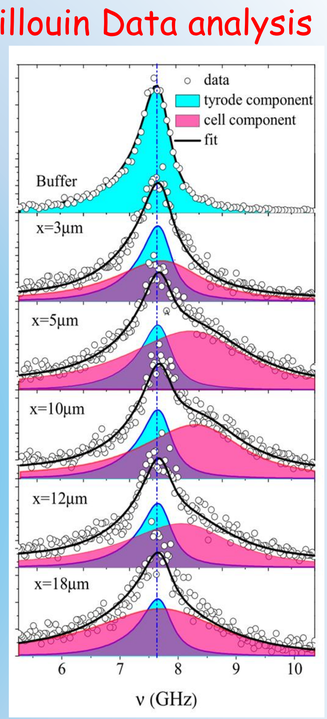
Layout of the experimental setup for the simultaneous Brillouin-Raman characterization.

Linear scan on the cell

Raman Data analysis



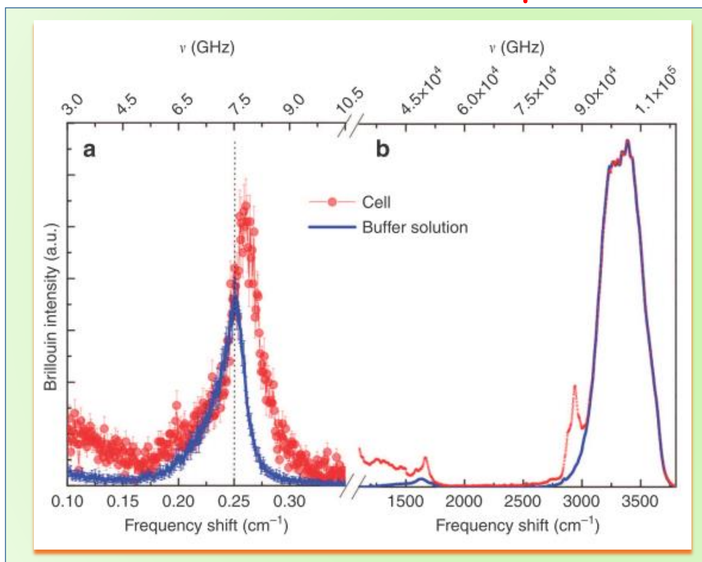
Brillouin Data analysis



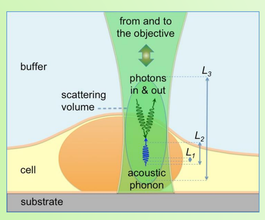
Thanks to the high chemical specificity of the Raman spectroscopy and its sensitivity to the local environment, the distribution of the chemical species and their supramolecular structural arrangement can be correlated with the modulation of the biomechanical properties as we move across different cell compartments.

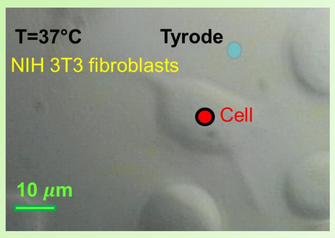
The frequency of the acoustic excitation and the viscoelasticity of different parts of the cell can be obtained fitting the Brillouin data by the convolution of the measured resolution function R(q) with two DHO functions. The first peak is assigned to the spectral contribution of the buffer component that is always present in the scattering volume. The second one changes while probing different cell parts, highlighting its sensitivity to the local cell elastic moduli.

Characteristic cellular spectrum



- Label free analysis
- Conctact less measurements
- Physiological conditions



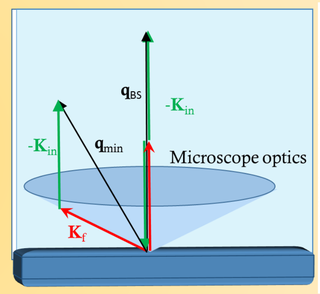


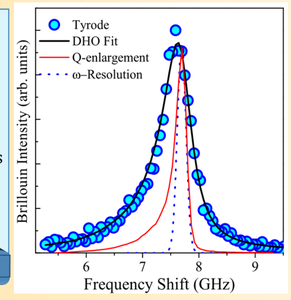
Brillouin line shape

The use of a high numerical aperture (NA) microscope objective for the focusing of the laser and collection of the scattered radiation induces a relevant q indeterminacy in a backscattering configuration. This contribution is relevant for the 60 × immersion objective in our setup where $\Delta q/q_{BS}$ is greater than 10%.

$$I(\omega) = \int \frac{I_0}{\pi} \frac{(Vq)^2 Dq^2}{\left(\omega^2 - (Vq)^2\right) + \omega^2 (Dq^2)^2} R(q)\,dq$$

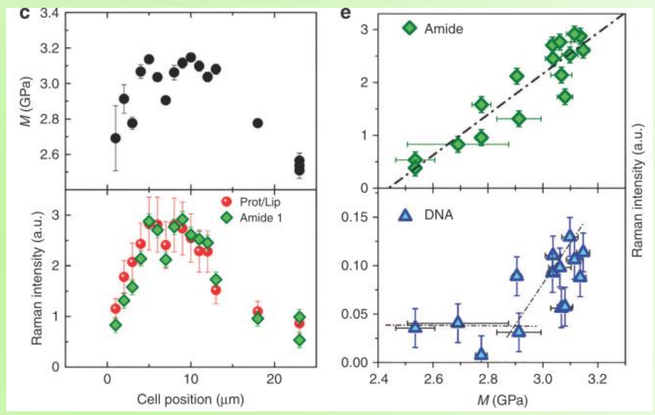
The collected intensity can be considered as a sort of convolution between the q-dependent spectrum and the q-dependent optical response. Therefore, measuring the spectrum of a material with a negligible intrinsic broadening, approximated to a Dirac delta function, allows for the direct evaluation of the whole response of the optical setup, R(q).





Biomechanical and biochemical correlation

The longitudinal elastic modulus $M=\rho V^2$ as a function of the cellular position is obtained from the fitting procedure of the Brillouin peak. It shows a strong increase of ~20% in the elastic modulus in the central region of the cell.
To correlate this evolution with the corresponding biochemical composition, we analyzed the relative variation of selected Raman bands. In particular, the area of the amide 1 peak and the proteins vs lipids ratio, which can both be considered as spectroscopic measures of the protein concentration, appear strictly correlated with the cell elasticity.



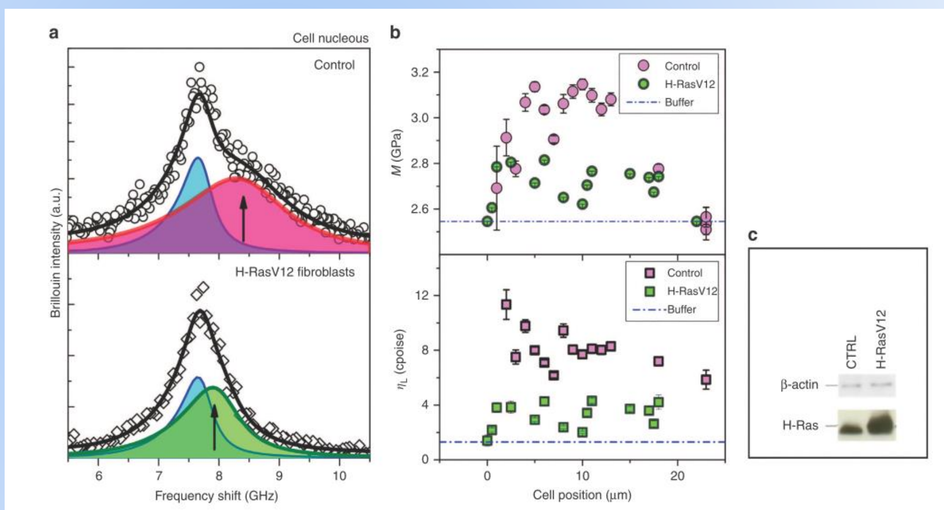
A linear dependence was observed. This purely spectroscopic result confirms the key role of protein structures in conferring rigidity to the cell. Moreover, by analyzing the DNA signal vs. the elastic modulus, we found that the nucleus occupies the stiffer cell zone

Evidence of different elastic properties in transfected cells

We compared the Brillouin data acquired in the control cells (NIH/3T3) with those obtained in the same cells after transfection (H-RASV12). In fact, NIH/3T3 are known to undergo oncogenic transformation upon the expression of constitutively active H-Ras by activating multiple downstream signaling pathways.

While probing the nucleus of the cell a clear decrease in the peak frequency accompanied with a decrease in its width comparing healthy and cancerous cells has been found.
The transfected cells present a generally lower longitudinal apparent viscosity and lower elastic moduli values with respect to healthy cells.

These properties are in agreement with the invasive potential observed in cancer cells; their increase in deformability enhances their squeezing ability through narrow spaces of the extracellular matrix, favoring *in vivo* dissemination and metastasis.



Funded by the Horizon 2020 Framework Programme of the European Union

References

[1] F. Scarponi et al. PRX 7, 031015 (2017); [2] S. Mattana et al. Nature Light: Science and Applications 7, 17139 (2018)