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Picosecond ultrasonics in a single biological cell

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The picosecond ultrasonic technique is applied for the non-invasive evaluation of sound velocity at a sub-micron scale in living onion cells. Velocity and attenuation of hypersound at 5.7 GHz in cells are measured by a femtosecond laser pump-probe technique. A nanometric co-polymer layer deposited between the cell and the substrate has been used to improve the photoacoustic signal. Comparison of the measured signals with the photoacoustic responses calculated according to thermoelastic generation mechanism and reflectometric detection shows high sensitivity to the cell adhesion on substrate. In addition to single cell imaging with the high lateral resolution provided by optics (ie $\approx 1\mu$m), the sensitivity of the measurements to cell compressibility suggests promising perspectives in the field of biology.

1 Introduction

The picosecond ultrasonic technique[1] was developed during last 20 years due to permanent interest in the study of mechanical and thermal properties of structures of metals and semiconductors in nano and micro domains used essentially in solid state physics and microelectronics. This technique relies on generation and detection of nanometric ultra-short acoustic waves by the use of femtosecond laser pulses and it is based on the well-known pump-probe technique. Important advantages are the absence of direct contact to the sample and very high frequency range. The bandwidth is limited only by the dynamics of the photoacoustic response of the medium under study and/or by the duration of the laser pulse. The present paper demonstrates that picosecond ultrasonics techniques can be used to measure the mechanical properties of single alive cells which are related to their interfacial and environmental surrounding.

The mechanical properties of cells have been studied with various techniques: cell indentation with a flat-ended glass fiber[2], micropipette aspiration[3], dynamic reflection interference contrast spectroscopy[4], laser tweezers[5], magnetometry[6], atomic force microscopy[7]. Most of these methods, however, provide average cell properties over relatively large areas and hence have very modest spatial resolution. Moreover, an external stress or strain must be applied and they are either destructive, or they require a mechanical contact with the cell. Studying cell deformability is not an easy task: a cell is a composite viscoelastic structure the description of which requires many parameters. Further, applying mechanical forces to cells will trigger a network of signaling events[8]. Thus, the measurements cited above of the mechanical properties of cells perturb the measured properties; *i.e.* the properties are modified by their measurement.

Conventional acoustical imaging of cells has made it possible to obtain direct information about the mechanical properties of their internal structure. But, acoustic microscopy is difficult to apply due to following drawbacks: first the presence of direct contact to the cell which distorts acoustical properties, and second technical limitation of frequency range. The main techniques are the scanning acoustic microscopy[9] and the scanning laser acoustic microscopy[10]. In spite of demonstration of 8 GHz operation frequency in cryogenic acoustic microscope[11], these techniques have not been widespread for biological and medical tasks because they are limited to the study of fixed cells and due to complicated design demanding liquid helium cooling.

2 Samples and experimental set-up

In this paper, the picosecond acoustic technique is applied to the non-invasive evaluation of ultrasound velocity and attenuation in the vacuole of alive single Allium cepa cells (common onion). Most mature plant cells have one or several vacuoles that can occupy as much as 90% of the volume for certain cell types and conditions (the case of the Allium cepa cells).

Slices of alive cells are deposited directly on the surface of a polished titanium alloy (Ti6Al4V) substrate. The thickness and the lateral size of a cell are measured by optical microscopy as ~5 _m and 50-100 _m, respectively. In addition, another polished titanium alloy substrate is coated by an optically transparent 0.25 μm layer made of polyethyleneimine (PEI) as a model molecule[12]. All the experiments are performed at the room temperature of 21°C without any treatment or fixation of the alive cells.

Well known reflectometric pump-probe experimental set-up is used[1] to generate and detect acoustic waves in the cells. The radiation of a femtosecond laser (wavelength 0.795 μm, pulse duration 100 fs, energy of the pulse 10 nJ) is divided in two beams to provide the pump and probe beams. Optical delay line (0-12 ns) is introduced in the probe beam path. The pump beam is modulated at a frequency of 300 kHz to provide a reference signal for lock-in amplification. Intensity of the reflected probe beam is detected by a photodiode. Such a set-up allows the detection of reflectivity relative variations at the level as low as 10^{-7}. Both beams are focused on the sample surface by a same objective with a 50x magnification. The width at mid height of the space cross-correlation of the pump and probe beams is approximately 2 μm.

3 Hypersound generation and detection

Let us now consider a transparent biological cell on the surface of an optically polished metallic substrate (Fig. 1). The pump and probe beams are focused on the substrate through the cell. Absorption of the pump laser beam in the substrate provides a local change of the temperature, which induces thermal and acoustic strains. The so generated sound pulse propagates in the cell and in the substrate. The strain field induces a transient local change in the optical index of the cellular vacuole propagating with longitudinal sound velocity. This change provides a second reflection of the probe beam scattered from the acoustic pulse and interfering on the detector with the beam reflected from the interface with the metallic substrate. So called Brillouin

scattering oscillations are the result of this interference. Their frequency at normal incidence of probe beam is[13]:

$$f_B = \frac{2nv}{\lambda} \quad (1)$$

where λ is the wavelength of probe light, and v is the velocity of sound propagation in the medium. If the optical index n of the medium is known it is straightforward to obtain the value of sound velocity from relation (1). The coefficient of sound attenuation α at the frequency f_B could also be evaluated from the attenuation time of the oscillations τ as $\alpha = (v\tau)^{-1}$.

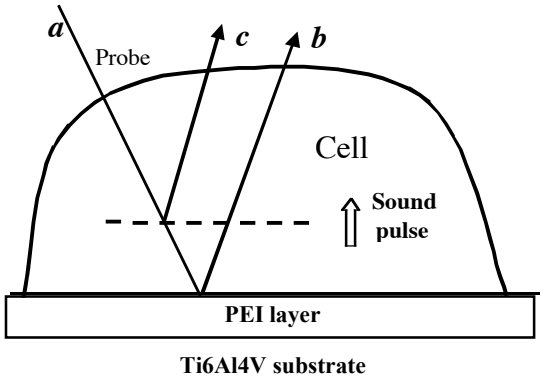

Fig.1 Principle of the Brillouin scattering oscillations in the alive single cell. Laser pulses are labeled as following: incident (*a*), reflected by the substrate (b), scattered by the acoustic pulse (*c*).

4 Results

Quantitative estimation of the temperature rise in the cell has been obtained with picosecond ultrasonic experiments performed with a droplet of water on a titanium alloy substrate. The ultrasound velocity in water is measured at 1.49 µm/ns from the frequency of Brillouin scattering oscillations. This value corresponds to a water temperature nearby the substrate of 22°C[14]. Assuming that heat capacity of water and cell vacuole are similar, this result means that heating of the biologic samples is not significant in our experiments. The increase of temperature due to the laser heating is then equal to +1°C.

Typical signal of the reflectivity variation in a cell on a titanium alloy substrate is shown in the inset of Fig. 2. It consists mainly of Brillouin scattering oscillations superposed on a thermal decay due to the diffusion of the heat. The first peak is a consequence of the absorption of the pump pulse by the substrate. The thermal background is subtracted from the experimental signal (curve 1 in Fig. 2). The acoustic signal is detected also in an alive cell on a titanium alloy substrate after PEI deposition (curve 2 in Fig. 2). Several results are derived from the analysis of signals in Fig. 2.

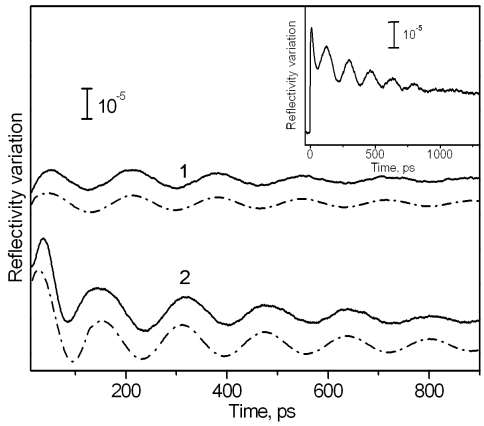

Fig.2 In inset: the total reflectivity variation signal measured *in vitro* in the vacuole of the cell on titanium alloy substrate without the polymer layer. Brillouin scattering oscillations in Allium Cepa alive single cell on titanium alloy substrates without (up) and with (down) polyethyleneimine (PEI) intermediate layer (experiment in solid lines, calculation in dashed lines). The measured frequencies and attenuation time of Brillouin scattering oscillations are f_B=5.7±0.1 GHz and _=0.4±0.15 _s in the cell and f_B=7.5±0.1 GHz in the PEI (_ not measured) respectively.

First, fitting the experimental data with a sine function damped by an exponential decay, averaged values of the frequency and attenuation time of Brillouin scattering oscillations have been evaluated as f_B=5.7 ± 0.1 GHz and τ=0.40 ± 0.15 µs, respectively. These values are obtained as a result of the measurements of f_B and τ at ten different locations inside the same vacuole. Similar periods and attenuation times are obtained either with or without the polymer coating. Choosing the optical index close to that in typical cells[15] as n=1.4, the averaged velocity of ultrasound in vacuole is obtained from (1) as 1.60 ± 0.10 µm/ns. Note that the sound velocity in water at this temperature of 22°C is equal to 1.49 µm/ns[14] which is different from the experimental value in vacuole. Similar increase of sound velocity has been observed in human keratinocyte cell (HaCaT) by 1 GHz scanning acoustic microscopy[16]. It should be pointed out that the accurate evaluation of local value of the optical index of cell would be necessary for measurement of accurate value of sound velocity. The sound attenuation coefficient in vacuole at the frequency of Brillouin scattering is obtained from the attenuation time and sound velocity as α=1.5 ± 0.1 µm^{-1}. Corresponding value of ultrasound penetration depth in the cell is α^{-1}=0.7 µm which is significantly less than the typical thickness. For comparison, attenuation of 5.7 GHz sound in water at 20°C provides an estimation of the corresponding penetration depth of 1.2 µm[14]. Same increase of sound absorption in cell cytoplasm are observed in HaCaT cells at lower frequency[16].

Second, calculations of Brillouin scattering oscillations in the cell with and without interlayer have been performed according to the model described in Ref. 17. These calculations account for wave generation and reflectometric detection in the 3 layers structure (cell, PEI, and substrate). They allow estimation of the values of the physical properties of the polymer interlayer. Using the parameters

measured previously for the cell, the best calculated fit of the experimental results is obtained with the following values for the polymer layer: L=0.255 µm (thickness), v_l=2 µm/ns ρ_l=10^3 kg/m^3, and n=1.5. These values are typical for polymers[18]. Very good description of phase and frequency of Brillouin scattering oscillations with/without the polymer is obtained with the mentioned parameters (Fig. 2). Because the vacuole is a cavity filled only by liquids the presence of the PEI layer has no influence on its mechanical properties *i.e.* on its sound velocity or Brillouin oscillation frequency. The area of fast transition (0-0.1 ns) in reflectivity variation signal with polymer in Fig. 2 corresponds to Brillouin scattering in PEI. The corresponding Brillouin frequency is measured equal to 7.5 GHz. The attenuation time of Brillouin scattering oscillations has not been evaluated in the PEI layer since less than one period can be measured. It is clear from Fig. 2 that the Brillouin frequencies in the PEI and the cell are different. When the acoustic wave generated in the Ti substrate, propagating through all the PEI layer, reaches the interface with the cell, the Brillouin frequency suddenly changes according to Eq. (1). The spatial depth resolution is then better than the thickness of the PEI layer (0.25 µm). The change in frequency provides easy monitoring of both the longitudinal sound velocity and the thickness of the PEI intermediate layer. The 200 degrees phase shift of the Brillouin scattering oscillations in the cell due to the polymer layer is also correctly reproduced by the simulation. It is due to the delay of the sound pulse propagation in the PEI interlayer. Indeed, assuming a sound velocity in the polymer typically of 2 µm/ns provides a time delay of 0.12 ns which approximately corresponds to half a period of Brillouin oscillation in the cell vacuole.

Third, Fig. 2 shows an increase of the oscillations amplitude of about 70%, in the experiment with the interlayer. The magnification of wave amplitude provided by the acoustic matching caused by the polymer layer can be calculated using the physical parameters estimated above. An improvement of the photoacoustic signal of 30% only is predicted. The additional magnification observed in the second experiment could suggest that adhesion of the cell on the titanium alloy substrate or on the polymer coating is different. Indeed, cell adhesion is a complicated and intricate phenomenon related to the geometrical, physical, chemical properties of the surface such as roughness, porosity, surface tension, etc. This detailed study is beyond the scope of this paper.

5 Conclusion

Very promising abilities of the picosecond ultrasonic technique for the non-invasive study of biological alive single cells have been demonstrated in this paper. The application of contactless and very high frequency picosecond ultrasonic technique will improve significantly the space resolution of acoustic imaging of alive cells. It will also provide more information about mechanical properties of the cell. Values of the longitudinal sound velocity 1.6 ± 0.1 µm/ns and attenuation, 1.5 ± 0.1 µm^{-1} of 5.7 ± 0.1 GHz acoustic waves in a vegetal cell vacuole have been obtained *in vitro*. From the velocity, cell compressibility nearby the cell-substrate interface can now be mapped with a resolution better than 2 µm laterally and 0.25 µm in depth. In addition to single cell elastography, the sensitivity of the measurements to cell adhesion suggests promising perspectives for the imaging of the physiological cell functions or the cell in function of its surroundings or its health

References

[1] C. Thomsen, J. Strait, Z. Vardeny, H.J. Maris. J. Tauc, J.J. Hauser, "Coherent phonon generation and detection by picosecond light pulses", *Phys. Rev. Lett*. **53**, 989 (1984)

[2] N. Peterson, W. McConnaughey, and E. Elson, "Dependence of locally measured cellular deformability on position on the cell, temperature, and cytochalasin", *B. Proc. Natl. Acad. Sci*. **79** 5327–5331 (1982)

[3] A. Young, and E. Evans, "Cortical shell-liquid core model for passive low of liquid-like spherical cells into micropipettes", *Biophys*. J. **56** 139–149 (1989)

[4] A. Zilker, H. Engelhardt, and E. Sackmann, "Dynamic reflection interference contract (RIC) microscopy: a new method to study surface excitations of cells and to measure membrane bending elastic moduli", *J. Phys.* **48** 2139 –2151 (1987)

[5] A. Ashkin, and J. Dziedzic, "Internal cell manipulation using infrared laser traps", *Proc. Natl. Acad. Sci*. **86** 7914 –7918 (1989)

[6] P. Valberg, and H. Feldman, "Magnetic particle motions within alive cells Measurement of cytoplasmic viscosity and motile activity", *Biophys. J.* **52** 551–561 (1987)

[7] M. Radmacher, M. Fritz, C. Kacher, J. Cleveland, and P. Hansma, "*Measuring the viscoelastic properties of human platelets with the atomic force microscope*", *Biophys. J.* **70** 556–567 (1994)

[8] M. Horoyan, AM Benoliel, C. Capo, P. Bongrand, "Localization of calcium and microfilament changes in mechanically stressed cells", *Cell Biophysics* **17** 243 (1990)

[9] J. Hildebrand, D. Rugar, R. N. Johnston, and C. F. Quate, "Acoustic microscopy of alive cells", *Proc. Natl. Acad. Sci.* **78** 1656–1660 (1981)

[10] A. Korpel, L. W. Kessler, P. R. Palermo, "Acoustic microscopy operating at 100 MHz", *Nature* **232** 110 (1971)

[11] B. Hadimioglu, J.S. Foster, "Advances in superfluid helium acoustic microscopy", *J. Appl. Phys*. **56** 1976 (1984)

[12] P.-L. Kuo, S. K. Ghosh, W.-J. Liang, Y.-T. Hsien, "Hyperbranched polyethyleneimine architecture onto poly(allylamine) by simple synthetic approach and the chelating characters", *Journal of Polymer Science: Part A: Polymer Chemistry* **39** 3018 (2001)

[13] L.J. Shelton, F. Yang, W.K. Ford, H.J. Maris, "Picosecond ultrasonic measurement of the velocity of phonons in water", *Phys. Stat. Sol. (B)* **242**, 1379 (2005)

[14] V.A. Grosso, C.W. Mader "Speed of Sound in Pure Water", *J. Acoust. Soc. Am.* **52** 1442 (1972)

[15] J. Beuthan, O. Minet, J. Helfmann, M. Herrig, and G. Muller, "The spatial variation of the refractive index in biological cells", *Physics Med. Biol.* **41** 369 (1996)

[16] T. Kundu, J.P. Lee, C. Blase, and J. Bereiter-Hann, "Acoustic microscope lens modeling and its application in determining biological cell properties from single- and multi-layered cell models", *J. Acoust. Soc. Am*. **120** 1646 (2006)

[17] C. Rossignol, B. Perrin, S. Laborde, L. Vandenbulcke, M.I. De Barros, and P. Djemia, "Nondestructive evaluation of micrometric diamond films with an interferometric picosecond ultrasonics technique", *J. Appl. Phys.* **95** 4157 (2004)

[18] A.G. Every, A. McCardy, "Numerical data and fundamental relationships in science and technology", *New Series, Group III of Landolt-Bornstein*, vol. 29A, Springer Berlin (1992)