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Picosecond ultrasonics signal in biological materials : comparison between predictions and experiments

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Picosecond ultrasonics is a non-destructive method for measuring mechanical properties such as velocity or stiffness coefficients for nanometric materials. This technique uses femtosecond laser pulses for generating and detecting acoustics waves from GHz to THz. Its resolution is about nanometers in depth and a few micrometers laterally. For transparent materials it allows generating the so-called Brillouin oscillations, which frequencies are determined by the material sound velocity, the optical index and the light beam wavelength. In this paper this technique is applied to biological cells. Investigations deal on a single *in-vitro* living vegetal cell and are non destructive for the cell. 1D mapping, composed of 6 measurement points, in an Allium Cepa cell with a lateral resolutions of 1 µm and an in depth resolution of 0.1 µm is presented. Velocities and attenuations of sound are deduced from the Brillouin oscillation frequencies which are 5.7±0.3 and 6.8±0.3 GHz in the vacuole and the nucleus of the cell respectively.

1 Introduction

The picosecond ultrasonic technique is used since the last 20 years[1] to characterize metals and semiconductors materials in the nano and micro domains. This technique is essentially applied in solid states physics and microelectronics. It presents several advantages like to be non-contact and non-destructive and has a nanometer in-depth resolution. Moreover it does not need coupling fluid or other additional material. This method is based on a femtosecond pump-probe technique. The pump produces a heat point source producing an acoustic deformation which is detected by the probe.

There is actually a powerful emergence of nanomedecine science and technology. In that way, an important step is to have a better understanding of the mechanical and optical properties of a single alive cell. Indeed, local optical index number may provide indication about local cell composition and structure and a better knowledge of cell mechanical properties should help to understand and model cellular and intracellular motility process[2]. It is now proved with *in vitro* nanomechanical experiments that cancer cells are elastically softer than healthy ones and recent measurements on cells from cancer patients suggest that this mechanical signature may be a powerful way to detect cancer in the hospital[3]. Moreover, mechanical properties of bone cells can be modified by the adhesion on biomaterials used for prosthesis[4].

Most of cell studies[5] are related with fluorescent marker[6], micromanipulation[7], microfluidic[8], AFM[9], Brewster angle microscopy[10], ellipsometry[11], optical and vibrational spectrometry[12], X ray reflectometry[13], mass spectroscopy[14], electric transport dynamic and characterization[15], acoustic microscopy[16]. All these techniques present many advantages and particularities but are limited by the measurements resolution, or by the need of a mechanical contact with the cell, external stress, or can not be applied to living cells.

In this paper, picosecond ultrasonics is proposed for optical and mechanical characterization of vacuole and nucleus allium cepa (common onion) cells. Onion cell mechanical properties are investigated in several papers: measurements of the Young modulus and the Poisson ratio[17, 18], studies of the membrane anisotropy[19]. To our knowledge, onion cell vacuole and nucleus acoustic velocities have never been measured.

2 Experimental setup and sample

A Ti: sapphire laser is used in the experiment which produces pulses of energy of 10 nJ, duration of 100 fs, with a 82 MHz repetition rate. The laser wavelength, at 800 nm, is used for the pump and the probe. The pump beam passes through a doubling crystal to obtain a 400 nm wavelength and has a sinusoidal modulation due to a 330 KHz opto-acoustic modulator in order to detect the probe signal by a lock-in amplification. An optical line is used to delay (0-12ns) the probe pulses. The two pulses are focused at normal incidence on a polish titanium substrate (Ti6A14V) through the cell with a 100x microscope objective (numerical aperture NA=0.8). The width at mid height of the space cross-correlation of the pump and the probe beams are 1 µm. The optical pulses can be scanned on the surface on the sample with a micrometer step. Temperature experiment is 21°C (temperature room).

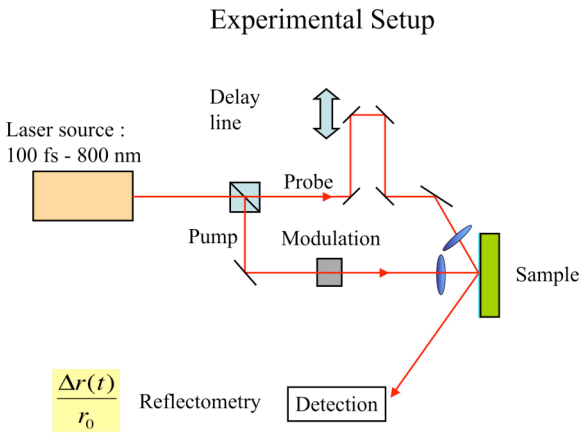

Figure 1: Experimental setup.

Cells studied are allium cepa (common yellow onion) cells. Onion cells are known to be large and robust. The cells are deposited on a polish titanium alloy surface (Ti6A14V). The size of the onion cell allows several experiments in the same single living cell.

3 Theory

Picosecond ultrasonics technique permits to obtain Brillouin oscillations for transparent material[1], resulting from the electromagnetic interferences between the reflected beam on the acoustic deformation and the

reflected beam on the substrate surface. Its frequency is determined with the following relation:

$$f(\lambda) = \frac{2n(\lambda)v}{\lambda}\cos(\theta), \qquad (1)$$

$n(\lambda), v, \lambda$ and θ are the optical index number, the acoustic celerity, the wavelength and the incidence angle of the probe respectively.

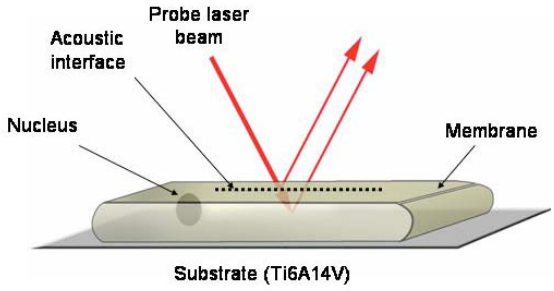

Figure 2: Experiment principles. Brillouin oscillations come from the interferences between two reflected laser beams: the first from the substrate surface and the second from the acoustic wave.

4 Results

Preliminary measurement permits to assume that these measurements are non destructive for the cell. Indeed, an experiment has been realized in a droplet of pure water and the Brillouin frequency measured permits to estimate the ultrasound velocity in water: 1.49 µm/ns. This value corresponds to the acoustic velocity in water nearby 22°C. The temperature elevation during experiments is then close to +1°C.

Onion cell vacuole is composed approximately of 90% of water so, assuming that the heat capacity between onion cell and water are closed, one can suppose that the temperature elevation is the same: +1°C which is biologically acceptable. Moreover, two hours duration experiments on the same measurement point show no significant variations in Brillouin frequency. Then no significant changes in the cell composition and vitality during these experiments are noticed.

All the cell extractions have been done with the same protocol, removing the thin, transparent membrane from the inner surface to an onion piece and putting it on the substrate. The adhesion is done mechanically, applying a pressure on the cell. With a 5x magnification microscope objective several cells can be seen including cell components: membranes, vacuoles and nucleus (cf. fig.3). Typical dimensions of onion cells are 50-200 µm laterally, 2-20 µm thickness; its diameters nucleus 5-15 µm. These dimensions permit to measure different points of an unique cell and to have a typical response for the different components (membrane, nucleus and vacuole) of the cell. Characteristic Brillouin oscillation signals are obtained in the vacuole and in the nucleus. Brillouin oscillations signals are not obtained for measurements in the membrane.

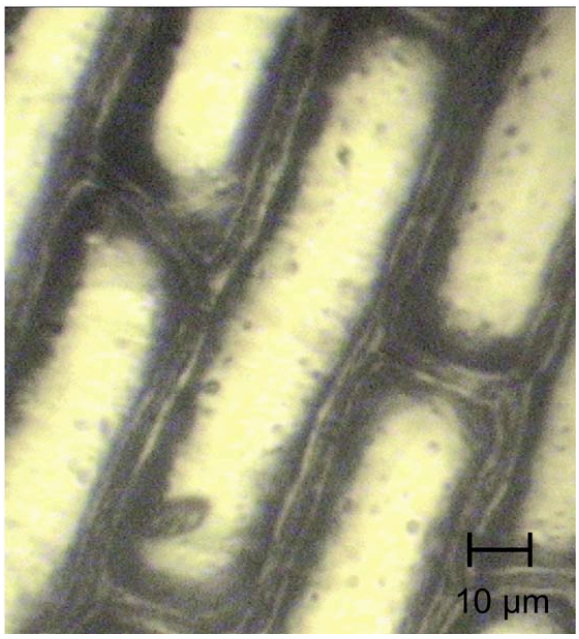

Figure 3: Photography of onion cells with a 5x microscope objective. Classical dimensions of an onion cell are 50-200 µm laterally for the cell, 5-15 µm diameter for the nucleus.

Experimental signals obtained can be described with:

$$g(A, f, \varphi, \alpha) = A\sin(2\pi ft + \varphi)e^{-\alpha t}, \qquad (2)$$

A, f, φ, α represent respectively the amplitude, the frequency, the phase and the attenuation of the Brillouin oscillation signal. An example of a Brillouin signal in the vacuole is given in fig.4. One can verify the very good correlation between the calculated fit (red) and the Brillouin oscillation signal (black).

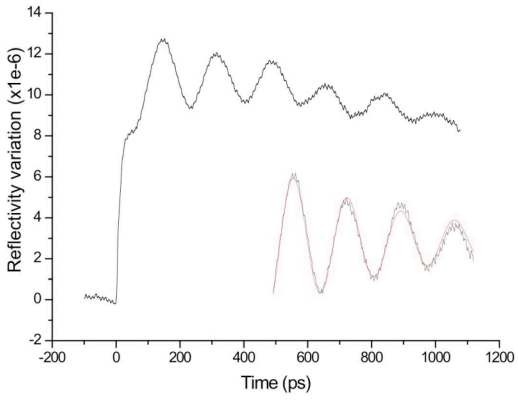

Figure 4: Brillouin picosecond signal in the vacuole of an onion cell. Brillouin frequency obtained is equal to 5.82 GHz. Attenuation value is 2.14 ns^{-1}.

A is related to the amplitude of the acoustic wave in the vacuole. This amplitude can be related to the efficiency of the opto-acoustic generation in the substrate and to the quality of the adhesion between the cell and the surface.

Brillouin frequency oscillations, f, is function of the optical index number and the acoustic celerity see Eq.(1). For the vacuole, the Brillouin frequency is equal to 5.7 ±0.3GHz. Taking a typical optical index close to that typical cells[20], as n=1.4, the average velocity of ultrasound in vacuole is obtained as 1.6 ±0.1 µm/ns.

The phase of the oscillation, φ, is related to the optical index number. If the material is transparent, the optical index is real and the phase value is equal to 0±π. If the material is semi-transparent, the optical index is complex, and the phase is different of 0±π and no straightforward expression can be given[21]. The Brillouin phase oscillation is measured equal to 172° (closed to 180° value for transparent media).

The acoustic attenuation, α, limits, for an acoustic amplitude given, the thickness of the cell from the substrate, studied by the picosecond ultrasonics technique. Indeed, the Brillouin oscillations end at 1.2 ns corresponding to 2 µm scan in depth using the acoustic velocity previously measured (for comparison, water have an acoustic attenuation value which permits to scan more than 5 µm depth in the same conditions[22]). The measured acoustic attenuation is at 1.7 ±0.5 ns^{-1}.

Picosecond ultrasonics experiments in the nucleus give Brillouin frequency equal to 6.8 ±0.3 GHz, phase 70° and attenuation 1.9±0.5 ns^{-1}. Taking the same real part of index optical value as in the vacuole, the frequency corresponds to a 1.9 ±0.1 µm/ns acoustic celerity and the phase leads to a semi-transparent media (i.e. complex value of the optical index). The amplitude of the Brillouin signal is appreciatively lower than the amplitude signal in the vacuole because of 2 main reasons: the nucleus is semi-transparent and is not directly in contact with the substrate.

Only a coincidence peak[1] is measured on cell membranes without Brillouin oscillations. Two reasons could be given: the membranes are 10 nm thick and they are not directly in contact with the substrate in the frontier between 2 cells.

Consequently, it is possible to identify at least 3 different onion cell organisms from their acoustic signatures with picosecond ultrasonics.

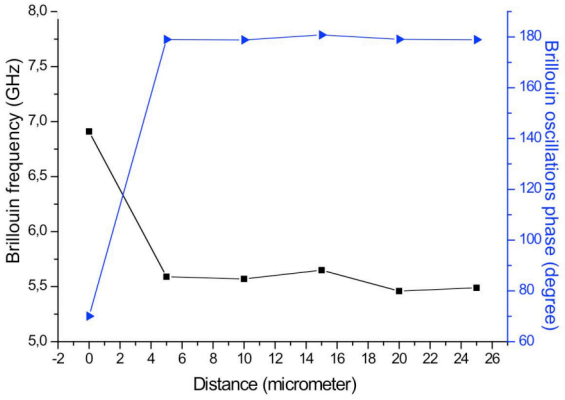

Figure 5: Frequency (Black) and phase (Blue) from Brillouin oscillations from a 1D scan in a single onion cell with a 5 µm lateral step. The first point corresponds to the measurement on the cell nucleus, the other 5 points are in the vacuole.

Fig.5 and fig.6 present 1D experiments scan with a 5 µm lateral step.

The Fig.5 shows that the measured frequencies and phases of the Brillouin oscillations are very similar for all the scanned part of the vacuole (25 µm). This means that the acoustic velocities and the optical index are also very similar in all the vacuole.

However, the fig.6 shows that the measured amplitudes and attenuations of the Brillouin oscillations have a larger range in the scanned vacuole. These variations can be related to the quality of the local mechanical adhesion of the cell on the substrate.

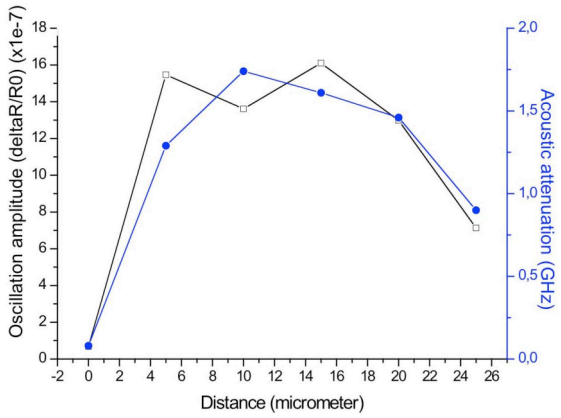

Figure 6: Acoustic amplitude (Black) and attenuation (blue) from the same 1D scan as in fig.5.

5 Conclusion

To conclude, it is possible to use picosecond ultrasonic technique in biological sample. This technique appears to be non-destructive for *in-vitro* vegetal cell because of the limited temperature increase during the experiments. Mechanical and optical characterization of the cell with a 1 µm lateral resolution, less than the nucleus diameter, are obtained using the frequency, the phase, the amplitude and the attenuation of the Brillouin oscillations of the acoustic signature. This values are 5.7 GHz, 180°, 13e-7 and 1.4 GHz respectively in the vacuole; 6.8 GHz, 70°, 1e-7 and 0.1 GHz in the nucleus; respectively too.
To our knowledge, these picosecond ultrasonic experiments are the first in vegetal single cell. Promising perspectives are open, particularly on animal or human cells. It should permit to assist biomaterials studies with its possibility to quantify cell adhesion onto a substrate, or moreover, in cancer researches to give mechanical information in function of cell vitality.

Acknowledgments

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