Two color probing of the ultrafast photo-acoustic response in a single biological cell

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Abstract The measurement of the mechanical properties of single biological cells with a nanometer depth resolution using only coherent light is proposed. A pump-probe set-up based on an ultrafast laser (100 fs pulses) is used to excite and detect acoustic frequencies in the GHz range. Experiments are performed on single fixed mouse MC3T3 cells adhering on titanium alloy substrate. Using two different probe wavelengths, the contributions to the optical detection resulting from the cell interface displacements and from interactions between acoustic waves and the laser light are identified. Semi-analytical calculations allow the determination of acoustic celerities and thicknesses in cells thinner than 150 nm.

1. Introduction

Since the 80’s, [1] interest in the picosecond acoustics technique has been growing owing to its wide range of applications in non-destructive control and solid-state physics. By this technique high frequency acoustic waves are both generated and detected with laser light pulses (duration<1 ps), allowing the measurement of the optical, thermal, or mechanical properties of submicrometric materials. [2] Some applications of picosecond acoustics are thickness measurement and bonding control at the nanometer scale, or studies of material microstructures. Experiments can be performed in opaque or transparent media.

In this paper, the picosecond acoustics technique is used in biological cells, the smallest unit of life that is classified as a living thing. Innovative experiments have already been performed in vegetal allium cepa cells and the potentialities of the picosecond acoustics technique to improve current cell imaging resolution have been demonstrated. [3-4] The method is now applied to single mouse MC3T3 fixed cells adhering on a titanium alloy substrate. Acoustic celerities are measured in cell thinner than 150 nm. In the second section of the paper, the optical detection in a cell of finite thickness adhering on an opaque substrate is proposed. We use two different probe wavelengths at the same point in the cell in order to identify the different contributions to the opto-acoustic detection. Simulation results and measurements are compared in the third section to determine the acoustic celerities and thicknesses of the cells.

2. Picosecond acoustics detection in a thin viscoelastic film adhering on a half-space substrate

The picosecond acoustics technique has already been used to study thin transparent films, from nanometric to micrometric thicknesses. [5-7] The cell is modeled as a thin transparent elastic film of
thickness $d$ adhering on an opaque substrate. A short light pulse is absorbed in the vicinity of the substrate and the subsequent thermal expansion generates a short strain pulse. The continuity of displacement and stress at the cell/substrate interface launches two acoustic pulses, one propagating in the cell and one propagating in the substrate. The acoustic wavelength of the acoustic pulse propagating in the cell is dictated by the acoustic celerity and the optical penetration depth in the titanium alloy substrate, [1] around 5.8 nm/ps and 20 nm, respectively. This acoustic wavelength is about 5 nm in the present study. We impose a zero stress boundary condition at the cell/air interface. The acoustic pulse propagates in the cell and is reflected at the cell interfaces. The period of the first harmonic of the acoustic resonances in the cell is:

$$T_d = \frac{4d}{v},$$

(1)

where $v$ and $d$ are the acoustic celerity and the cell thickness of the cell, respectively.

The optical detection of the acoustic response is the sum of the interfaces displacements and the acousto-optic interaction between the acoustic pulse and the probe laser radiation. [6] The acousto-optic interaction leads to the so-called Brillouin oscillation. [5] Their period for a normal probe incidence is:

$$T_B = \frac{\lambda}{2nv},$$

(2)

where $\lambda$ and $n$ are the laser wavelength and the optical index of the medium. The amplitude of these oscillations is proportional to the piezo-optic coefficient. [6]

Figure 1. Picosecond acoustics of a thin biological cell. For each plot, calculated signals labelled with squares and stars markers represent the acousto-optic contribution and the interface displacement contribution, respectively. We compare cells where (a) the acoustic frequency is higher than acousto-optic frequency and (b) the inverse situation. Unlabelled plots are the sum of the acousto-optic and interface displacement contributions.

Figure 1 presents the relative reflectivity changes calculated for different cell thicknesses compared to the acousto-optic interaction period $T_B$. Plots are function of the dimensionless times $4vT_d$ (down abscissa) and $vT_B$ (up abscissa). Plots with squares and stars represent the acousto-optic and the interface displacement contributions to the detection, respectively. The square-like modulation is caused by the step-like acoustic displacement of the interface. Frequencies of interface displacement and acousto-optic interactions are dictated by equation 1 and equation 2, respectively. On figure 1(b),
\( \lambda \ll d \), that is \( T_B \ll T_A \); Brillouin oscillation arise in the cell. On figure 1(a), \( \lambda \gg d \) and Brillouin oscillations cannot arise.

Let us now compare experimental data obtained in submicrometric cells with the modeling presented above.

3. Experimental evaluation of the acoustic celerity and thickness in submicrometric cells

![Cell #1](image1.png)

![Cell #2](image2.png)

**Figure 2.** Experimental data (unlabelled plots) obtained in two different cells. On the left-hand side plots, detection is done using blue probe, and on right-hand side plots, detection is performed at the same point in the cell with a red probe. Triangles are corresponding calculated signals, sum of the acousto-optic detection (squares) and the interface displacement detection (stars).

The experimental set-up is a classical pump-probe setup used to generate and to detect acoustic waves in a single MC3T3 fixed cell adhering on a Ti6Al4V substrate. [8] Red pulses (800 nm) of 100 fs duration are generated by a mode-locked Ti:sapphire laser, of repetition rate 82 MHz. A polarizing beam splitter divides the laser beam in pump and probe beams. The pump beam passes through an acousto-optic modulator (330 kHz) to provide the reference signal for lock-in amplification. One of the laser beams is converted into blue light using a BBO crystal and the other beam passes through a
delay line to provide a tunable time delay between the pump and the probe. Both beams propagate through the transparent cell and are focused at a normal incidence with a X20 microscope objective at the Ti6Al4V surface and can be used either as pump or probe. The width at half-height of the space cross-correlation of the pump and probe beams is approximately 5 µm. Reflectometric measurements are presented.

Four experimental results obtained on two different cells, one cell per line, and corresponding simulations are presented on figure 2. Unlabelled plots are experimental detection. On the left plots, signals are detected using blue probe while on right plots the detection is done using red probe at the same point of the cell. The experiments are led on the vacuole across the nucleus, the cytoplasm and the cytoskeleton of the cell. Lines with triangles are the corresponding simulation. Physical parameters are identical for each cell. The piezo-optic coefficients are adjusted to match the ratio of the acousto-optic to interface displacement contributions to the change of reflectivity. For each cell, the signal detected using the blue probe allows the measurements of the mechanical properties of the inspected cell. Assuming a cell optical index equal to 1.4, [9] the acoustic celerity is measured using the acousto-optic detection (square plots) equal to 4.4 and 3.7 nm/ps in cells 1 and 2, respectively, corresponding to 33 and 26 GHz acousto-optic frequency signal, respectively. Taking a cell density equal to 1100 kg.m⁻³, the measured stiffness of the cell nucleus is evaluated between 15 to 21 GPa. Comparable rigidity has already been measured in osteoblasts cells on titanium alloy substrate. [10] The interface displacement contribution (star plots) allows the measurements of the cell thicknesses, 300 and 140 nm for cell 1 and 2, respectively. Data obtained using the red probe do not permit the mechanical evaluation of the cell for two reasons. For cell 1, the signal is very low. This may be attributed to a weak piezo-optic coefficient in the MC3T3 cell at the probe wavelength. The cell 2 is very thin compared to the red probe optical wavelength and the probe is almost not sensitive to the acousto-optic effect. It is not possible to distinguish the acoustic celerity from the cell thickness value in the detection.

4. Conclusion
Optical detection of acoustic frequencies higher than 30 GHz has been successfully performed in MC3T3 cells allowing the determination of acoustic celerity and thickness in single cells thinner than 150 nm. This technique suggests promising perspectives in single cells imaging and biomedical applications, as various as cancer studies or cell adhesion on biomaterials.

References
[1] Thomsen C, Grahn T C, Maris H J and Tauc J 1986, Phys. Rev. B 34 4129
[2] Antonelli G, Perrin B, Daly C D, Cahill D G 2006, Mater. Res. Bull. 31 607
[3] Rossignol C, Chigarev N, Ducousso M, Audoin B, Forget G, Guillemot F and Durrieu M C 2008, Appl. Phys. Lett. 93 123901
[4] Audoin B, Rossignol C, Chigarev N, Ducousso M, Forget M, Guillemot F and Durrieu M C 2010, Ultrasonics 50 202
[5] Rossignol C, Perrin B, Laborde S, Vandenbulcke L, De Barros M I and Djemia P 2004, J. Appl. Phys. 95 4157
[6] Wright O B 1992, J. Appl. Phys. 71 1617
[7] Côte R and Devos A 2005, Rev. Sci. Instrum. 76 053906
[8] Chollet C, Lazare S, Guillemot F and Durrieu M C 2010, Colloid. Surface. B 75 107
[9] Beuthan J, Minet O, Helfman J, Herrig H M and Müller G 1995, Phys. Med. Biol. 41 369
[10] Saruwatari L, Aita H, Butz F, Nakamura H, Ouyang J, Yang Y, Chiou W A and Ogawa T 2005 J. Bone Miner. Res. 20 2002