Mechanical characterization of temperature-sensitive objects using picosecond ultrasonics

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Abstract. Biological objects are exquisitely sensitive to temperature variations and their mechanical characterization is often a challenge when using the picosecond ultrasonics technique. To reduce the laser-induced temperature rise, we place single biological cells on a thin metal transducer and we focus the laser beam that generates the acoustic waves at frequencies \( \leq 150 \) GHz on the rear side of the transducer. The acoustic waves propagate through the transducer and are partially transmitted to the cell to create the so-called Brillouin oscillations. The frequency of these oscillations provides a direct measurement of the sound velocity. The simultaneous measurement of the acoustic reflection coefficient at the transducer/cell interface allows the determination of both the density and the compressibility of the cell.

1. Introduction
Temperature-sensitive materials (TSM) include materials with a phase transition close to the room temperature, or objects that can be damaged by temperature variations, such as water-based gels or biological media. The mechanical characterization of TSM is often a technical challenge when using a laser-based setup such as the picosecond ultrasonics technique (PU).

PU is an optical pump-probe technique [1] which has proven useful for the non-invasive mechanical characterization of viscous liquids,[2] Langmuir-Blodgett films,[3] or liquid mercury.[4] The measurement of the acoustic reflection coefficient from a Al/water interface has been used to design an acoustic microscope with nanometer resolution.[5] Sound velocity \( v \) has also been determined in water by measuring the frequency of Brillouin oscillations.[6]

PU has recently been applied to the mechanical characterization of biological cells deposited on a metallic half-space. [7] The approach used by the researchers has been to generate acoustic waves through the absorption of laser pulses at the cell-substrate interface. The first drawback of this configuration is that the temperature rise is maximal at the interface, thus imposing to limit the fluence of the laser to avoid damage to the cell. Obviously, the same problem would arise in any TSM. The second drawback is that the presence of organelles, diffusing the pump light, and the strong acoustic absorption require high pumping fluence. These irreconcilable hindrances hamper the optimization of the signal-to-noise ratio (SNR) in cells.

The acoustic reflection coefficient, which can yield the acoustic impedance \( Z \) of the TSM, and \( v \) have only been measured in separate configurations. However, to fully characterize the mechanical properties of a soft material one needs to measure simultaneously \( Z \) and \( v \) to obtain...
the density $\rho = Z/v$ and the compressibility $\chi = 1/Zv$. In the present paper we place single allium cepa cells on a thin metal transducer. We focus the pump beam on the rear side of the transducer to reduce the laser-induced temperature rise in the cell. We measure simultaneously $\rho$ and $\chi$ of the cell using acoustic echoes in the film and Brillouin oscillations in the cell.

**2. Transducer design: Reduction of thermal stress in the cell and increase in SNR**

Titanium-based alloys are commonly used as structural biomaterials for the replacement of hard tissues in artificial joints and they display the most suitable biomedical properties. For this reason we sputtered a polycrystalline Ti film on a silica supporting layer transparent at both pump and probe wavelengths (figure 1). To define the optimum Ti thickness, consider the diffusion length $\mu = (\kappa/\rho C_p \pi f_m)^{1/2} = 660$ nm at the modulation frequency $f_m = 1$ MHz of the laser, where $\rho = 4500$ kg m$^{-3}$ and $C_p = 500$ J kg$^{-1}$ K$^{-1}$ are the density and specific heat capacity of the Ti film, respectively.[7] The thermal conductivity of the film $\kappa = 3.1$ W m$^{-3}$ K$^{-1}$ is assumed to be 1/7 of the single-crystal value.[8] In order to avoid thermal confinement at $f_m$ and to keep a thickness within rf sputtering capabilities, we chose a thickness $d = 500$ nm.

We use an optical pump-probe technique described in details in Ref. [1]. Longitudinal acoustic pulses are thermoelastically generated at the Ti-SiO$_2$ interface with optical pump pulses of duration 100 fs, repetition frequency $f_r = 80$ MHz, wavelength $\lambda_{pp} = 790$ nm from a Ti:Sapphire mode-locked laser. This light is chopped at $f_m = 1$ MHz for lock-in detection. The reflectivity change is measured with frequency-doubled probe pulses of wavelength $\lambda_{pb} = 395$ nm as a function of the pump-probe time delay $t$. The pump beam focus is focused either on the top or rear side of the Ti film, as discussed later. Each pump pulse is initially absorbed in the metal layer over a depth comparable to the optical skin depth of $\sim 15$ nm.[7] Conduction band electrons are excited and diffuse over a depth of $(\kappa/g)^{1/2} = 1$ nm during their thermalization with the lattice, where $g = 35 \times 10^{17}$ W m$^{-3}$ K$^{-1}$ is the electron-phonon coupling constant in Ti.[9] Therefore, when the pump is focused on the rear-side of the Ti film, neither the pump radiation nor the overheated electrons reach the top Ti surface where the cell is deposited.

We place an allium cepa cell on top of the transducer to characterize the efficiency of acoustic generation. The probe beam is focused at the Ti/cell interface (top curve) using a $\times 60$ objective.
lens (NA 0.7). The measured change of reflectivity is plotted in figure 2. The pump beam is alternatively focused at the Ti/cell interface (top curve) through the same objective lens as the probe, or at the rear-side of the Ti film (bottom curve) through a ×50 objective lens (NA 0.8). The latter configuration is depicted in figure 1. The probe beam is partially reflected from the Ti surface and partially scattered by the acoustic wavefront propagating in the cell. Interferences arise from these two probe contributions causing the so-called Brillouin oscillations visible on both curves in figure 2.[7] Their frequency is \( f_b = 2n_c^r v/\lambda_{pb} \), where \( \lambda_{pb} \), \( n_c^r \), and \( v_c \) are the probe wavelength, the real part of the refractive index and the sound velocity in the cell, respectively. The amplitude of the Fourier spectrum is shown in the inset of figure 2. The ratio of the peaks observed at \( f_b = 5.6 \text{ GHz} \) in the two configurations is \( H = 0.25 \).

A slow decay is observed on the top curve due to thermal diffusion and overheated electrons relaxation. As \( d \) is larger than the diffusion length at \( f_r \), these effects do not show on the bottom curve. The thermal stress in the cell is thus reduced importantly when the pump is focused on the rear-side of the Ti film. An offset is also observed at \( t < 0 \) resulting from the temperature rise at the modulation frequency. This temperature is five times lower when the pump beam is focused on the rear-side of the Ti film. The pump fluence could therefore be increased by a factor of 5 to obtain the same temperature rise in both configurations. The acoustic amplitude would also be increased by the same factor, resulting in an increase in SNR by a factor \( 5H = 1.25 \) when changing the position of the pump from the top to the rear-side of the Ti film.

3. Simultaneous measurement of the density and compressibility of the TSM

We present in figure 3 the amplitude of the reflectivity change \( |\Delta R/R(t)| \) for bare Ti (a), Ti/ethanol (b) and Ti/cell (c). \( |\Delta R/R(t)| \) measured in Ti shows three acoustic echoes arising from successive reflections in the Ti film. The amplitude of the Fourier spectra of the echoes plotted in the inset of figure 3 is centered around 50 GHz. In the case of Ti/ethanol, Brillouin oscillations measured at 3.8 GHz from Fourier analysis allows the determination of the sound velocity \( v_e = 1120 \text{ m/s} \) in ethanol assuming a refractive index of \( n_e = 1.36 \).[10] The value of \( v_e \) agrees closely with literature.[11] In the case of Ti/cell, we measured a frequency of 5.6 GHz which gives a sound velocity in the cell of \( v_c = 1610 \text{ m/s} \), the expected value for such a cell.[7]

A closer look at \( |\Delta R/R(t)| \) measured in ethanol shows at least two echoes with the same arrival time as in the case of bare Ti. In the case of the cell, there is apparently no echo. Consider a Morlet wavelet centered on the center frequency of the acoustic echoes in Ti defined by \( W_m(t) = \cos(4\alpha t) \exp(-\alpha t^2/2) \), with \( \alpha = 0.04 \). The amplitude of the Fourier spectra of the wavelet is plotted in the inset of figure 3(a) (dash). The convolutions of \( |\Delta R/R(t)| \) with \( W_m(t) \) are plotted on figure 4. The density of acoustic energy resulting from the convolution reveals a first echo at around 137 ps and a second echo at around 288 ps in all three cases.

The ratio of the second to the first echo yields the acoustic reflection coefficient \( R_{ts} = (Z_t - Z_s)/(Z_t + Z_s) = 0.22 \) at the Ti/silica interface in the case of bare Ti, where \( Z_t \) and \( Z_s \) are the acoustic impedances of Ti and silica, respectively. The ratio of the second echo measured in the case of Ti/ethanol (and in the case of Ti/cell, respectively) to the second echo measured in Ti yields the acoustic reflection coefficients \( R_{te} = 0.91 \) and \( R_{tc} = 0.77 \) at the Ti/ethanol and Ti/cell interfaces, respectively. \( Z_s = 12.7 \text{ MPa} \text{ s}^{-1} \text{ m}^{-1} \) has been obtained from a previous measurement, so \( Z_t = 19.5 \text{ MPa} \text{ s}^{-1} \text{ m}^{-1} \) can be deduced from \( R_{ts} \). We obtain the impedances of ethanol \( Z_e = 0.9 \text{ MPa} \text{ s}^{-1} \text{ m}^{-1} \) and of the cell \( Z_c = 2.5 \text{ MPa} \text{ s}^{-1} \text{ m}^{-1} \) similarly from \( Z_t \), \( R_{te} \) and \( R_{tc} \).

The particularity of the present experiment is that we measure simultaneously \( v \) and \( Z \). So we can obtain the compressibility \( \chi_c = 1/Z_e v_e = 1.0 \text{ GPa}^{-1} \) and the density \( \rho_e = Z_e/v_e = 805 \text{ kg} \text{ m}^{-3} \) for ethanol, values in close agreement with literature. Similarly we obtain \( \chi_c = 1.0 \text{ GPa}^{-1} \) and \( \rho_c = 1550 \text{ kg} \text{ m}^{-3} \) for the cell. The value of the \( \rho_c \) is larger than that commonly found in the literature \( \sim 1100 \text{ kg} \text{ m}^{-3} \). The cumulated errors on \( v_e \) and on \( R_{te} \) are not likely to explain this discrepancy since we measured reasonable values for ethanol. However,
Figure 3. $|\Delta R/R|$ for (a) bare Ti, (b) ethanol on Ti and (c) cell on Ti. The amplitude of the Fourier spectra of $|\Delta R/R|$ in the case of bare Ti and of the Morlet wavelet (dash) used to extract the acoustic echoes in figure 4 are plotted as an inset.

there might be an influence of the frequency on the determination of $R_{tc}$ due to the imperfect contact at the Ti/cell interface and to the viscosity of the cell. It should also be noted that the value given in literature is an average value since the cell is composed of different compartments, notably the vacuole and the first and secondary cell walls. Indeed $R_{tc}$ might be sensitive to the wall, which exhibits a higher density, rather than to the vacuole.

4. Conclusion

Using a transducer composed of a thin Ti film we have measured simultaneously the density and the compressibility of a single biological cell by means of the PU technique. Focusing the pump beam on the rear side of the Ti film we have removed completely the pump radiation, the diffusion of overheated electrons and the thermal diffusion on a nanosecond time scale, thereby reducing the cell stress and improving the SNR. Further investigation of the sensitivity of our measurements to cell wall and contact quality should pave the way towards mechanical characterization of subcellular components and should enlighten the mechanics of cell adhesion.

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