Picosecond ultrasonics in single cells: Interface step motion for thin animal cells and Brillouin scattering for thick vegetal cells

M Ducousso, T Dehoux, B Audoin
Université de Bordeaux, CNRS, UMR 5469, Talence, F-33405, France

O Zouani, C Chollet, M C Durrieu
Université de Bordeaux, INSERM, U 577, Bordeaux, F-33000, France

mathieu.ducousso@u-bordeaux1.fr

Abstract. The measurement of the mechanical properties of single biological cells using a picosecond laser–ultrasonic method is proposed. A pump-probe set-up based on ultrafast laser (100 fs pulses) is used to generate and detect acoustic frequencies in the GHz range in a cell on a metallic substrate. The time resolution is about 1 ps and the laser focusing allows a 1 µm lateral resolution. We carry out experiments in both animal and vegetal cells. A semi-analytical simulation model of the physical phenomena involved in experiments is presented. The coupled heat and stress equations are solved including a thermal boundary resistance at the cell/substrate interface and strong acoustic absorption. The optical detection resulting from the interaction between the acoustic wave and the laser light is also modelled. Simulations allow the analysis of experimental signals in both vegetal and animal cells. The results support the potentialities of the non-invasive technique for bioengineering and medical applications.

1. Introduction

Cell is the smallest unit of life that is classified as a living organism, and is often called the building block of life. Cell biomechanics seems to be important in cell growth, differentiation, motility and apoptosis, in some diseases proliferation, or in the adhesion mechanics of cell/implant for instance. For this reason, studies of the cell mechanics, or even of subcellular components mechanics, are of growing interest, favored by new technologies, more sensitive instruments, and improvements in bioimaging.

The use of lasers to generate acoustic waves provides a contactless and non-invasive tool to study biological tissues. Ultrasound at MHz frequencies can be generated by taking advantage of the optical absorption of nanosecond laser pulses in the tissue itself. [1] The contrast of the imaging is thus provided by the contrast in tissue absorption. However, many important cell subcomponents have dimensions less than 500 nm and therefore fall below resolution of this technique and of most nondestructive imaging systems. [2]

The picosecond ultrasonics technique [3] uses the absorption of femtosecond laser pulses in a solid transducer to generate acoustic frequencies up to 1 THz. Since 1984, interest in this 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<1ps), allowing the inspection of submicrometric materials. Main applications of picosecond acoustics are thickness measurement [4-5] and bonding control at the nanometer scale, [6] or studies of material microstructures. [7-10] Experiments can be performed in opaque or transparent media. Their specific optical responses permit to determine optical, thermal, or mechanical properties of the sample.

Picosecond acoustics has already been used to study vegetal cells (allium cepa) and the potentialities of this technique to improve current cell imaging resolution have been demonstrated. [11-12] The method is applied in this paper to single mouse MC3T3 fixed cells.

In the second section of the paper, a thermo-mechanical model describing a cell of finite thickness, sandwiched between two semi-infinite media is proposed. The third section concerns the modelling of the detection, and a detailed analysis of the influence of the cell thickness on the opto-acoustic interaction is proposed. A comparison between experimental data and simulations is presented in the fourth section.

2. Picosecond acoustics in a viscoelastic film sandwiched between two half-spaces.

In picosecond ultrasonics experiments a short light pulse is absorbed in a material. Because of the thermal expansion, a short strain pulse is generated. Picosecond acoustics has already been used to study thin films, [3-6,8] from nanometric to micrometric thicknesses. A detailed analysis of the thermal diffusion and of the acoustic propagation in a viscoelastic thin film is proposed in this section. The model couples the temperature diffusion equation and the wave motion equation. [13] Equations are analytically solved in the frequency domain and time results are calculated using a numerical inverse Fourier transform. Figure 1 shows the considered geometry. The cell is modelled as a thin transparent viscoelastic film of thickness \( d \) sandwiched between two semi-infinite half spaces, the first one being the opaque substrate and the second one being a transparent physiological serum. Indices \( i = 0, 1, 2 \) refer to the substrate \((z>0)\), the cell \((0>z>-d)\), and the transparent half space \((z<-d)\), respectively.

The temperature fields \( T_i \) are determined solving the Fourier heat diffusion equation for each medium \( i \):

\[
\rho_i C_p^i \frac{\partial T_i(z,t)}{\partial t} - \kappa_i \frac{\partial^2 T_i(z,t)}{\partial z^2} = \left\{ \begin{array}{ll}
\beta_0 L_0 \delta(t) e^{-\beta_0 z}, & i = 0 \\
0, & i = \{1,2\}
\end{array} \right.,
\]

where \( \rho_i, C_p^i, \kappa_i \) are the density, the specific heat capacity and the thermal conductivity, respectively. \( \beta_0 \) is the optical absorption coefficient of the substrate and \( L_0 \) stands for the electromagnetic intensity absorbed in the substrate. Since media 1 and 2 are transparent, the optical generation results only from the absorption of the pump laser beam radiation in the substrate, in the vicinity of the film/substrate interface. No source term is considered in the heat diffusion equation for media 1 and 2. The temperature rise in these media results from the continuity of the temperature and heat flux at the interfaces. A thermal boundary resistance is considered to account for the thermal mismatch at the 0/1 interface.

The thermal parameters of the cell are assumed to be close to that of water. Simulations allow quantifying the maximum temperature rise \( \sim 1 \) K at the 0/1 interface for one laser pulse with the
considered experimental fluence, around 16 mJ/m². Since the thermal diffusivity of the titanium alloy is thirty times higher than that of the cell heat essentially diffuses in the substrate.

The instantaneous temperature rise creates a thermal stress which acts as a source term in the wave equation:

\[
C_i \frac{\partial^2 U_i(z,t)}{\partial z^2} - \rho_i \frac{\partial^2 U_i(z,t)}{\partial t^2} = C_i \alpha_i \frac{\partial T_i(z,t)}{\partial z},
\]

where \( C_i, U_i \) and \( \alpha_i \) are the stiffness coefficients, the displacements and the thermal dilatation, respectively. The product of \( \alpha_i \) and \( C_i \) defines the thermal rigidity parameter. [14] The boundary conditions are the continuity of the stress and of the displacement at each interface.

Figure 2: Cell thickness variation as a function of time \( t \) with (solid) or without (dash) source term in Eq. (2). The cell thickness changes when \( t \) is an integer multiple of the acoustic travel time \( t_1 = d/v_1 \) in the film. \( v_1 \) is the velocity in the film.

| Medium | \( C_i \) [GPa] | \( \rho_i \) [kg/m³] | \( \alpha_i \) [1/K] | \( \beta_i \) [1/m] |
|--------|-----------------|-----------------|-----------------|------------------|
| TiAl4V | 155             | 4500            | 9×10⁻⁶          | 5.2×10⁻⁷         |
| MC3T3  | 2.9 + j0.2ω     | 1100            | 3.5×10⁻⁴        | -                |

Table 1: Physical parameters used in the simulations shown in Fig. 2.

The difference between the acoustic displacements calculated at the two interfaces 0/1 and 1/2 yields the dynamics of the cell thickness. It is plotted with solid lines in Fig. 2 using data shown in table 1 where medium 0 is TiAl4V and medium 1 is MC3T3 cell. Acoustic attenuation in the cell is modelled by a complex stiffness coefficient. This approach, similar to previous works on ultrasonics attenuation measurements in water, [15] leads to an attenuation factor proportional to the square of the frequency. [16] The signal shows periodic step-like changes with large amplitude caused by the displacement of the 1/2 interface. Periodic step-like changes with smaller amplitude are overlaid, resulting from the smaller displacement (downward arrows) of the 0/1 interface. The periods of both the small and large amplitude contributions is the back and forth propagation time through the cell \( 2t_1 = 2d/v_1 \), where \( v_1 \) is the velocity in the cell. In addition, the substrate dilatation creates a negative displacement of the 0/1 interface and a compressive wave in the cell at \( t=0 \).

The dynamics of the cell thickness has also been calculated when the source term in Eq. (2) is omitted for media 1 and 2. The result is plotted with dashed lines in Fig. 2 using data in table 1. This signal has the same shape with smaller amplitude as the signal plotted with solid lines. This comparison shows that, despite the temperature rise in the cell is small and confined over a distance of a few nanometer from the substrate, the source term can not be neglected since the thermal dilatation of the cell is much higher than that of the substrate.

The rise time \( \tau = 1/v_0\beta_0 \) of each step depends mainly on the optical absorption length \( 1/\beta_0 \) in the medium 0. The influences of the thermal diffusivity in the substrate and of the viscoelastic properties
of the film (medium 1) have negligible influence on \( \tau \). The acoustic wavelength in the cell \( d = v_1 \tau \) (about 5 nm in our configuration) at frequency \( \tau^{-1} \), will play a key role in the analysis of the reflectivity changes, as discussed in the following section.

3. Interface motion and optical scattering contributions to the acousto-optic detection.

The last step in the modelling is to represent the detection, i.e. the change of reflectivity caused by thermo-optic and acousto-optic effects. Several papers deal with the detection in ultrafast acoustics, in either opaque or transparent films, with oblique or normal incidence taking into account 3D acoustic diffraction, for sample thicknesses ranging from a few nanometers to half spaces. \([6, 8, 17-21]\) If the optical penetration depth is smaller than the acoustic wavelength, the detection results essentially from the surface displacement. If the sample is transparent, the acousto-optic interaction can no longer be neglected. Part of the probe beam is sensitive to the transient change of the dielectric permittivity induced by the transient perturbation. If the transparent sample is thick enough, the so-called Brillouin oscillation appears. \([6-8, 19]\) This dynamic optical process can be understood in terms of Stokes or Raman scattering. \([22]\) We verified using our simulation that the acousto-optic contribution of the semi-infinite titanium alloy substrate is negligible since the optical penetration depth is around 20 nm. Therefore the acousto-optic detection is considered in the transparent media only. Let us now detail the detection mechanism.

Both thermal and acoustic perturbations create a local modification of the dielectric permittivity \( \varepsilon_i' \) in the medium. Since this perturbation is small, the dependence of \( \varepsilon_i' \) on temperature \( T \) and on the acoustic strain \( \eta \) is supposed to be linear:

\[
\varepsilon_i'(z) = \frac{\partial n_i}{\partial T} T_i(z) + \frac{\partial n_i}{\partial \eta} \eta_i(z) .
\]

\( \frac{\partial n_i}{\partial T} \) and \( \frac{\partial n_i}{\partial \eta} \) are piezo-optic and thermo-optic coefficients, respectively.

In the Fourier domain, the nonlinear Maxwell equation in the perturbed medium is:

\[
\frac{\partial^2 E_i(z, \omega)}{\partial z^2} + q^2 \varepsilon_i(z, \omega) E_i(z, \omega) = 0 ,
\]

where \( \varepsilon_i(z, \omega) \), \( E_i(z, \omega) \) and \( q \) are the dielectric permittivity, the electric field of the medium \( i \), and the optical wave vector in vacuum, respectively. Considering a small transient perturbation, the electric field and the dielectric permittivity can be written as the sum of a homogeneous part and of a perturbed part (noted with superscripts \( h \) and \( s \), respectively). \([21]\) Equation (4) is linearized and two Maxwell equations are obtained: One without source term for the homogeneous part of the electric field and one for the scattered field where the perturbed dielectric permittivity appears as a source term: \([21]\]

\[
\frac{\partial^2 E_i^h(z, \omega)}{\partial z^2} + q^2 \varepsilon_i^h(z, \omega) E_i^h(z, \omega) = 0
\]

\[
\frac{\partial^2 E_i^s(z, \omega)}{\partial z^2} + q^2 \varepsilon_i^s(z, \omega) E_i^s(z, \omega) = -q^2 \varepsilon_i^s(z, \omega) E_i^h(z, \omega)
\]

The homogeneous refractive index \( n_i^h \) is obtained using Maxwell formula \( \varepsilon_i^h = (n_i^h)^2 \) and the homogeneous field can be written as:

\[
E_i^h(z, \omega) = M_i^he^{-i\omega z} + N_i^he^{i\omega z} ,
\]

where coefficients \( M_i^h \) and \( N_i^h \) are determined by expressing optical boundary conditions. The solution of Eq. (6) is: \([23]\)
Finally, the total electric field is the sum of the homogeneous and the scattered fields. The integral equation of potential scattering is thereby obtained. The relative intensity variation is finally deduced from the electric field. The homogeneous field is represented by Eq. (7). The continuity of the electric and magnetic fields at each interface is considered. Since the interface displacement due to the transient wave is considered, Eq. (7) describes the optical detection of the interface motion.

The acousto-optic interaction is described by Eq. (8) in which four integral terms are identified. An optical phase reference is defined when there is no perturbation, i.e. when the interface motion is not taken into account. The terms containing the phase shift $\pm 2q_n x z$ with respect to the phase reference result from the backscattering induced by the acoustic perturbation. These terms may be understood as Stokes and anti-Stokes scattering modes. If the cell thickness is larger than $d_o = 1/2q_n$ , i.e. the half optical wavelength in the cell $\lambda_0/2$, Brillouin oscillations arise and their frequency for a normal probe incidence is: \[ f_i = \frac{2n_i v_i}{\lambda} \] where $\lambda$ is the probe wavelength. The other terms, in phase with the reference, do not create interferences but are sensitive to the sign and the amplitude of the perturbation.

Relative reflectivity changes are calculated for several cell thicknesses $d$ and they are plotted in Fig. 3 where medium 0 is TiAl4V and medium 1 is the cell. The influence of the cell thicknesses compared to the critical distances $d_A$ and $d_o$, assuming $d_A < d_o$, is studied. In Fig. 3.(a)-3.(c) the cell has a finite thickness while it is semi-infinite in Fig. 3(d). The signals labelled with stars represent the intensity changes calculated with the solution of the homogeneous Maxwell equation, Eq. (7). The signals labelled with rounds are calculated with the solution accounting for acousto-optic effect in media 1, Eq. (8). Distinguishing these two contributions allows a better understanding of the total signal, plotted with unlabelled lines in Fig. 3. For illustration, medium 2 is assumed to be air.

For films such that $d < d_A < d_o$, Fig 3.(a), the acoustic pulse generated in the substrate acts as a source exciting a resonant layer. Since the film thickness is slightly less than the acoustic wavelength $d_A$, the fundamental resonant frequency of the thin film is excited whereas the harmonics are beyond the acoustic spectrum. Thus the homogeneous solution describes the acoustic ringing of the film at a frequency $f_i = v_i/4d$. The attenuation is a result of the cell viscoelastic behaviour and of the acoustic losses due to the successive reflections at the cell/substrate interface. In this situation, the film permittivity is quasi-homogeneous within the film and the acousto-optic contribution is modulated at the film resonant frequency. For larger film thicknesses, the acoustic wavelength $d_A$ in the cell at frequency $\tau^{-1}$ is less than the film thickness and step like waveforms are observed. Successive contributions have opposite signs owing to acoustic impedance ratio between the considered media and the substrate. These features are reproduced in Fig. 3.(b) and Fig. 3.(c) by the waveforms representing the solution of the homogeneous Maxwell equation. For a film thickness $d_A < d < d_o = \lambda_0/2$, the evolution of the reflectivity is modulated by the strain integral along the film thickness. The square-like modulation is caused by the step-like changes in the displacement, Fig. 2. Although the thickness of the cell is too small in Fig. 3.(a) and Fig. 3.(b) to observe Brillouin oscillations, these oscillations arise significantly when the cell thickness is larger than $d_o$, Fig. 3.(c).
Figure 3.(d) illustrates the limit case where the film thickness is semi-infinite. Only the acousto-optic interaction contributes to the change of reflectivity and produces Brillouin oscillations with a larger amplitude.

![Graphs showing different cases of reflectivity change](image)

Figure 3: Relative reflectivity change calculated in 4 different cases. (a): \( d < d_\lambda < d_\varnothing \), (b): \( d_\lambda < d < d_\varnothing \), (c): \( d_\lambda < d_\varnothing < d \) and (d): \( d \to \infty \). For each plot, signals labelled with round and star markers represent the acousto-optic contribution, Eq. (8), and the interface displacement contribution, Eq. (7), respectively. Unlabelled plot is the sum of the acousto-optic and interface displacement contributions.

4. Experimental results for thick vegetal cells and thin animal cells.

4.1. Picosecond acoustics experiments

The experimental arrangement is a classical pump-probe setup used in this paper to generate and to detect acoustic waves in a single cell adhering to a TiAl4V substrate. Red pulses (790 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. The pump beam is converted into blue light using a BBO crystal. Its energy is reduced to 0.5 pJ per pulse. A lower energy probe 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 X50 microscope objective at the TiAl4V surface. The measured sound velocity is an
average value over the micrometric probe spot. The full width at half-maximum of the space cross-correlation of the pump and probe beams is approximately 2 \( \mu \text{m} \).

4.2. Thick vegetal cell.

![Figure 4: Experimental and calculated (stars) signals in the vacuole of an *allium cepa* cell. The Brillouin attenuation is due to the cell viscoelasticity.](image)

We carry out experiments on *allium cepa* (common onion) cells. The ultrasonic attenuation prevents in-depth inspection above 3\( \mu \text{m} \) from the 0/1 interface and the cell thickness is about 5 \( \mu \text{m} \). So this experiment must be compared to the simulated signal shown in Fig.3.(d). The attenuation of the Brillouin oscillations is caused by the viscous properties of the cell only. Figure 4 shows experimental results obtained in the vacuole of a white onion cell and a comparison with the calculated signal. Acoustic data are obtained by fitting the simulation presented in sections 2 and 3 to the experimental curves. The thermal decay is suppressed by subtraction of a polynomial. The simulation result is fitted to the remaining acoustic data (i.e., the Brillouin oscillations) by adjusting the complex stiffness coefficient of the cell to minimize the least mean square residue. [12] Assuming a fixed value for the optical index of the cell \( n_1 = 1.4 \), the fitting procedure allows obtaining the velocity with an accuracy about 0.1 \( \text{nm/ps} \). Piezo-optic and thermo-optic coefficients are adapted from ref. 25. The local inspection in the cell permits to distinguish the vacuole acoustic velocity from the nucleus acoustic velocity and the white onion nucleus velocity from the yellow onion nucleus velocity (See table 2).

[^12]:

| Organelle | Vacuole | Nucleus |
|-----------|---------|---------|
| Variety   |         |         |
|           | White   | Yellow  |
| \( v \) [\text{nm/ps}] | 1.6 \( \pm \) 0.1 | 1.8 \( \pm \) 0.1 | 2.0 \( \pm \) 0.1 |

Table 2: Measured velocity in vacuole and nucleus for white and yellow onion cells. [12]

4.3. Thin MC3T3 mouse cell.

A RGD peptide has been added at the surface of the TiAl4V substrate to allow a better cell adhesion on the substrate, resulting in cell spreading and creation of focal adhesion. [26]

Figure 5 presents an optical image of a MC3T3 cell. Optical profilometry along a line across the nucleus of the cell indicates a thickness in other areas than the nucleus less than 200 nm: The experimental condition corresponds to a cell thickness between \( d_A \) and \( d_O \). This optical scan has been performed in several cells and reproducible results were obtained. An experiment performed on such a cell is compared with a simulation in Fig. 6. The thermal background of the experimental signal has been removed to analyze the acoustic part of the signal only. The interface motion and the acousto-optic interaction arise in the detected signal but the cell is too thin to detect Brillouin oscillations. The
main contribution in the signal is the interface motion, which allows determining the acoustic travel time $t_1=d/v_1=65$ ps in the cell. The values of $d$ and $v_1$ cannot be measured independently. However, the couple of values $d=105\text{nm}$, consistent with the optical profilometry, and $v_1=1.6\text{nm/ps}$, agreeing with the values found in literature, [27] gives a good fit to the data. Compared to measurements performed on onion cells, the signal to noise ratio is degraded in MC3T3 cells. This can be attributed to two main reasons. Firstly, the vacuole of MC3T3 cell is more heterogeneous than that of the *allium cepa* vacuole. This may affect both acoustic and optic wavefronts. Secondly, the MC3T3 upper surface may be rougher than that of the onion cell.

Figure 5: Optical image of a MC3T3 cell. A thickness scan (not shown) along the white line by optical profilometry indicates a thickness in other areas than the nucleus less than 200 nm.

Figure 6: Comparison between the data and the simulation results (stars).

5. Conclusion

Picosecond acoustics technique has been presented theoretically in this paper for thin viscoelastic cells. The detection has been described using Maxwell equation and the fundamental processes of the detection have been identified in terms of acousto-optic and interface motion contributions. For the optical detection in the cell, the two detection processes have been studied and acoustic and acousto-optic critical thicknesses have been determined. Experiments have been led in onion and mouse cells with thicknesses respectively larger and smaller than the acousto-optic critical thickness. Good agreement between the simulation and the data is found. The Brillouin oscillations allow the measurement of the acoustic velocity in the onion cell whereas an acoustic travel time is measured in the thin animal cell. Ultrafast acoustics should offer promising perspectives for quantitative cell adhesion measurement on implants or for measurement of cell adhesion during cell cycle and motility.

References

[1] M. Xu and L. V. Wang, *Rev. Sci. Instrum.* **77**, 041101 (2006)
[2] Tsien R Y 2003 *Nat. Rev. Mol. Cell Biol.*, 4 16
[3] Thomsen C, Strait J, Vardeny Z, Maris H J, Tauc J and Hauser J J 1984, *Phys. Rev. Lett.* **53** 989
[4] Lin H N, Stoner R J and Maris H J 1990, *J. Nondestr. Eval.* **9** 239
[5] Côte R and Devos A 2005, *Rev. Sci. Instrum.* **76** 053906
[6] Rossignol C, Perrin B, Laborde S, Vandenbulck L, De Barros M I and Djemia P 2004, *J. Appl. Phys.* **95** 4157
[7] Shelton L J, Yang F, Ford W K and Maris H J 2005 *Phys. Status Solidi B* **242** 1379
[8] Kashiwada S, Matsuda O, Baumberg J J, Li Voti R and Wright O B 2006 *J. Appl. Phys.* **100** 073506
[9] Wright O B, Perrin B, Matsuda O and Gusev V E 2008 *Phys. Rev. B: Condens. Matter* **78**
024303

[10] Ségur D, Shuvalov A L, Audoin B and Pan Y D 2010 J. Acoust. Soc. Am \textbf{127} 181

[11] Rossignol C, Chigarev N, Ducousso M, Audoin B, Forget G, Guillemot F and Durrieu M C 2008, Appl. Phys. Lett. \textbf{93} 123901

[12] Audoin B, Rossignol C, Chigarev N, Ducousso M, Forget M, Guillemot F and Durrieu M C 2010 \textit{Ultrasonics} \textbf{50} 202

[13] Antonelli G, Perrin B, Daly C D, Cahill D G, 2006, \textit{Mater. Res. Bull.} \textbf{31} 607

[14] Scruby C B and Drain L E 1990 \textit{Laser Ultrasonics Techniques and Applications} (Adam Hilger)

[15] Rouch J, Lai C C, Chen S H 1976 \textit{J. Chem. Phys.} \textbf{65} 4016

[16] Auld B A 1990 \textit{Acoustic fields and waves in solids, vol.1} (Krieger Publishing Compagny)

[17] Thomsen C, Grahn T C, Maris H J and Tauc J 1986 \textit{Phys. Rev. B: Condens. Matter} \textbf{34} 4129

[18] Lin H N, Stoner R J, Maris H J and Tauc J 1991 \textit{J. Appl. Phys.} \textbf{69} 3816

[19] Wright O B 1992 \textit{J. Appl. Phys.} \textbf{71} 1617

[20] Wright O B 1995 \textit{Opt. Lett.} \textbf{20} 632

[21] Dehoux T, Chigarev N, Rossignol C and Audoin B 2007, \textit{Phys. Rev. B: Condens. Matter} \textbf{76} 024311

[22] Dil J G 1982 \textit{Rep. Prog. Phys.} \textbf{45} 285

[23] Jeffrey A and Dai H H 2008 Handbook of mathematical formulas and integrals 4\textsuperscript{th} edition (Elsevier: Academic press)

[24] Born M and Wolf E 2005 \textit{Principles of optics} 7\textsuperscript{th} (expanded edition) (Cambridge)

[25] Saubade C 1984 \textit{J. Phys. C: Solid State Phys.} \textbf{17} 3507

[26] Hersel U, Dahmen C and Kesser H 2003, \textit{Biomaterials} \textbf{24} 4385

[27] Weiss E C, Anastasiadis P, Pilarczyk G, Lemor R M, Zinin P V 2007 \textit{IEEE Trans. Ultrason. Ferroelectr. Freq. Control} \textbf{54} 2257