Research article

Apparent attenuation by opto-acoustic defocus in phonon microscopy

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A B S T R A C T

Understanding the mechanical properties of biological cells is a challenging problem for the life sciences partly because there are limited methods for mapping elasticity with high resolution. Phonon microscopy is a form of Brillouin light scattering which uses coherent phonons for imaging with elasticity-related contrast, phonon resolution and without labels. It can measure material properties such as sound velocity, acoustic impedance and attenuation. To use it as a contrast mechanism in microscopy, high numerical aperture (NA) lenses are key to high resolution. However, increasing NA induces apparent attenuation, a premature decay of the detected signal. To reduce signal decay and quantify the sound attenuation coefficient in cells, it is necessary to understand the mechanisms that affect signal decay. Here we define opto-acoustic defocus as a signal decay mechanism and propose methods to achieve quantitative sound attenuation measurements, and to optimise in-depth imaging at high resolution which is crucial for cell imaging.

1. Introduction

Elasticity is emerging as an important parameter for the characterisation of biological materials. Numerous works have demonstrated the ability to use elasticity measurements as a way to characterise biophysical parameters [1–5]. Among the available techniques for elasticity characterisation, those based on Brillouin light scattering (BLS) are particularly promising because they offer a non-invasive measure of the product of the refractive index and the speed of sound with optical resolution [6–11].

Phonon microscopy is a novel technology that uses coherent phonon fields to image biological cells with contrast provided by BLS [9,12]. Through the use of ultra-fast pump-probe methods [13], the technique provides access to the phenomena of BLS in picosecond time scales (time-resolved Brillouin scattering (TRBS)). An interesting consequence of the high temporal resolution phonon microscopy provides, is that material properties such as sound velocity, acoustic impedance and sound attenuation can be measured directly in the time domain with sub-optical resolution provided by the phonon wavelength [14].

Sound attenuation is the loss of energy of a propagating sound wave and is a relevant characteristic of materials [15–19]. For a non-diffracting plane wave, this loss is related to properties of the medium such as viscosity, inhomogeneity and the frequency of the wave. The relationship of these properties with the attenuation of sound are not easy to model, especially for the case of heterogeneous biological materials. Nevertheless, in cancer research sound attenuation has been used as a biomarker to characterise cells and tissue; for instance, it has been used to image uterine [20,21] and breast tumours [22], as well as to identify cancer in fluid [5].

Imaging cells at high resolution with contrast given by sound attenuation offers an opportunity for applications in cell biology at the single cell level. However, imaging with high lateral resolution requires high numerical aperture (NA) lenses to reduce the size of the illumination spot. However, increasing NA has the potential to introduce a parasitic source of signal decay which is unrelated to the elastic properties of the specimen. Such an effect would have two important implications for phonon imaging: reduction of measuring depth and errors on the quantification of the sound attenuation coefficient $\alpha_0$.

The effect of NA has been reported extensively for spontaneous Brillouin spectroscopy (SBS) [23,24], however for TRBS, the conditions are different. Whereas in SBS, the symmetry of the Brillouin frequency spectrum is in part determined by the range of spatial frequencies produced by the NA of the objective lens, in TRBS this is less relevant as the strong coherence of the longitudinal phonons enhances scattering of the lower spatial frequencies (parallel to the optical axis). Nevertheless, effects of NA in TRBS have been reported [16] yet not thoroughly investigated, and have been avoided by either using a pulse-echo approach [25] or using low NA lenses [26]. More recently, their cause has been attributed to acoustic diffraction [27].

In this article we report the loss of signal with increasing NA and the
measurement of the attenuation coefficient as performed by TRBS. Based on model and experimental results, it is concluded that, contrary to previous reports, a main cause leading to an apparent increase in the attenuation is *opto-acoustic defocus* which occurs when the coherent phonon field propagates beyond the Rayleigh range of the optical system. The implications of this conclusion are discussed within the context of cell imaging.

### 1.1. Sound attenuation and signal decay in phonon microscopy

TRBS is measured experimentally with a pump-probe system which uses femtosecond lasers pulses to resolve picosecond phenomena [13,28–30]. Sound is produced by focusing a pulsed pump laser into a metallic transducer layer which launches a strain pulse due to thermal expansion (see Fig. 1). A time-delayed pulsed probe laser is focused at the same point as the pump (see Fig. 1a) and light is scattered from the wavefront of the strain pulse as it propagates axially away from the source (see Fig. 1b). The acoustic signal arises from the interference of the sound-scattered (object) light with the directly propagating (reference) light at a detector which produces an oscillatory change in the amplitude and phase using Fourier-Bessel angular spectrum propagation (FBASP) [31].

![Fig. 1](image)

Fig. 1. Typical decay observed in time resolved Brillouin scattering in glass using different NA lenses. (a) Typical TRBS experimental configuration. (b) Interaction of light and sound in TRBS where the phase difference between the object and reference beams is dictated by the Bragg condition. (c) Typical detected signals in glass. An increase in NA gives an apparent increase in attenuation.

Acoustic diffraction (divergence of the acoustic field) has been previously attributed as a leading cause of apparent attenuation [27]. However, we believe this is an incomplete conclusion since the near field distance of the optical wavelength actually matched that of the acoustic beam, and therefore the decay of the scattered optical intensity cannot be neglected. To individually test the contributions of the optics and acoustics to apparent attenuation, their near field distances should be varied independently. In order to achieve this, separate objective lenses with unique NA are used to generate the sound and optical fields.

We propose that apparent attenuation also arises in TRBS by *opto-acoustic defocus* which occurs when the phonon wavefront (originated at the beam waist) propagates beyond the Rayleigh range of the optical system. Here, the optical beam that carries the signal, drops in intensity at the detector plane (due to the divergence of the optical beams) which causes loss of interference contrast and could be mistaken as sound attenuation (see Fig. 1c). As NA increases, this effect becomes more aggressive producing signal decay comparable, or even greater than, that produced by the combination of sound attenuation and acoustic diffraction.

### 2. Modelling

Acoustic diffraction (divergence of the acoustic field) has been previously attributed as a leading cause of apparent attenuation [27]. However, we believe this is an incomplete conclusion since the near field distance of the optical wavelength actually matched that of the acoustic beam, and therefore the decay of the scattered optical intensity cannot be neglected. To individually test the contributions of the optics and acoustics to apparent attenuation, their near field distances should be varied independently. In order to achieve this, separate objective lenses with unique NA are used to generate the sound and optical fields.

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The amplitude of the scattered object beam (\(U_{obj}(r, z)\)) can be modelled as being proportional to the product of the amplitudes of the probe field (\(U_{probe}(r, z)\)) and the sound field (\(U_{sound}(r, z)\)) as derived by Matsuda et al [32]. Determining the phase of the object beam (\(\phi_{obj}(r, z)\)) can be simplified by using the Bragg condition. Consequently the object photons which partake in TRBS will have phases that are a function of...
twice the acoustic path length \( L_{\text{sound}} \), see Fig. 1b):

\[
\phi_{\text{obj}}(r, z) = 2n_{\text{probe}}k_{\text{sound}}L_{\text{sound}}(r, z) = 2n_{\text{probe}}k_{\text{sound}}^{\text{obj}}(r, z)
\]

where \( k_{\text{probe}} \) and \( k_{\text{sound}} \) are the optical and acoustic wavenumbers respectively and \( \phi_{\text{sound}}(r, z) \) is the phase of the acoustic field. Therefore, the object field at every position in space can be written as:

\[
u_{\text{obj}}(r, z) = U_{\text{obj}}(r, z)e^{i\phi_{\text{obj}}} = U_{\text{probe}}(r, z)U_{\text{sound}}(r, z)e^{i\phi_{\text{probe}}}
\]

The object beam is then propagated from each \( r, z \) position through the optical system to the detector \((z_{\text{det}})\). The objective and tube lenses were modelled by simply adding a spherical phase function to the incident wavefront. At the detector, the object and reference fields are then interfered and integrated as a function of the radius \( r \). Finally, the signal amplitude is calculated as the modulation depth of the interference between the reference and object beams:

\[
S_{\text{amp}} = \frac{2U_{\text{ref}}U_{\text{obj}}}{l_{\text{ref}} + l_{\text{obj}}} = \frac{2U_{\text{ref}}U_{\text{obj}}}{l_{\text{ref}} + l_{\text{obj}}}
\]

where \( l_{\text{ref}} \) and \( l_{\text{obj}} \) are the intensities of the object and reference beams respectively at the detector and \( U_{\text{ref}} \) and \( U_{\text{obj}} \) their respective amplitudes.

The NA of the pump and probe lenses are set during the calculation of the Airy disks and for the probe reference beam, this NA is preserved. However, the acoustic field can be smaller than the probe field (due to \( \lambda_{\text{pump}} < \lambda_{\text{probe}} \)) and can introduce additional spatial frequencies into the object beam. Consequently, it is necessary to enforce the probe NA by introducing an aperture function at the objective lens.

We have considered a single acoustic wavelength model since the probe beam only interacts with the acoustic wavelength that satisfies the Bragg condition (see equation 1) despite the generation of a broadband pulse. Additionally, the broadening of \( \lambda_{\text{sound}} \) given by the incident angle in equation 1, is small (~10% in our case for NA = 0.7) and does not change \( u_{\text{sound}} \) significantly. More rigorous models have been implemented [28], however in the context of probing the causes of apparent attenuation, a single wavelength model is sufficient to approximate signal decay.

In the context of using this model to test the sources of signal decay, we have not considered the effect of the limited coherence length; femtosecond laser pulses typically used in TRBS have coherence distance longer than the propagation distance of GHz sound. Additionally, the coherence distance is not a function of NA.

The model was executed with \( \text{NA}_{\text{pump}} \) and \( \text{NA}_{\text{probe}} \) as the input variables. In order to investigate how the choice of probe NA affects signal decay (opto-acoustic defocus), \( \text{NA}_{\text{pump}} \) was fixed to be low (0.1) while \( \text{NA}_{\text{probe}} \) increases. Conversely, to investigate how the choice of pump NA affects signal decay (acoustic diffraction), \( \text{NA}_{\text{pump}} \) was fixed (0.1) while \( \text{NA}_{\text{probe}} \) increases. The output of each simulation \( (S_{\text{amp}}) \) was normalised to unity at \( z = 0 \) for comparison with the experimental results.

The resultant \( S_{\text{amp}} \) traces calculated for the different combinations of NA are shown in figure 3 Figure 3. The relative change in \( S_{\text{amp}} \) due to opto-acoustic defocus is shown in figure 3a where a clear decrease is seen with \( z \) especially for \( \text{NA}_{\text{probe}} > 0.13 \). The Rayleigh range for each probe NA (indicated as a coloured dashed line) occurs before the Fraunhofer distance of the sound field (black dashed line) and appears.
to correspond with the position at which the decay of rate of $S_{\text{amp}}$ is maximum.

Conversely, figure 3b reveals the signal decay ($S_{\text{amp}}$) due primarily to acoustic diffraction. Even though the sound leaves the acoustic near field (Fraunhofer distances shown in the coloured dashed lines) much earlier than the optical near field (Rayleigh range beyond the plot limit), this does not appear to affect the overall signal decay. In order to verify that the sound fields are indeed diffracting, yet the effect on the signal is not dominant, the acoustic intensity ($I_{\text{sound}}$) as a function of $z$ is presented in figure 3c. This set of simulations also represents the typical NA and propagation distances seen in previously reported TRBS contributions.

3. Results

In order to replicate the method employed by the model, a similar strategy of varying the pump-delivery and probe-delivery/collection NA was used to experimentally probe the sources of apparent attenuation (see Fig. 4). In all cases the sample under study is a glass coverslip which is coated with 20nm of gold to act as an opto-acoustic transducer and is placed at the beam waist (Fig. 4a). The traces are obtained using a pump-probe system in an ASOPS configuration [29], with a pump wavelength of 415nm and a probe wavelength of 780nm, which corresponds to an acoustic wavelength (in glass) of $\sim$250nm. TRBS detection takes place by collecting the reflected signal as usually observed in previous works [13,33,34], however the pump beam is delivered from the opposite side with another objective. This allows changing NA probe while maintaining NA sample and vice versa (see Fig. 4). The position of the sample at the beam waist was corroborated by simultaneous optical brightfield illumination.

Experimental measurements of the TRBS signals, modulated by apparent attenuation, are presented in figure 5. Figure 5a corresponds with the configuration shown in figure 4b, and figure 5b with that of figure 4c. The implications of these experimental results will be discussed in the following section, but initially the contribution of opto-acoustic defocus appears to outweigh that of solely acoustic diffraction.

4. Discussion

Glass was used instead of water for model validation because: (a) reports of the attenuation coefficient for glass are more widely available in the literature and (b) the acoustic wavelength is similar to that of water ($\sim$8% variation, see supplement). The attenuation of sound for a wave $S$ at a position $x$ is described as an exponential decay $S(z) = S_0 e^{-\alpha z}$ where $S_0$ is the initial amplitude and $\alpha$ the attenuation coefficient. To validate the model and determine the attenuation coefficient of the glass sample, simulated ($S_{\text{amp}}$) and experimental results (envelopes of $\Delta R/R$) were all fitted to exponential curves to obtain their mean value was found to be $\alpha_0 = 2.5 \times 10^5 \text{m}^{-1}$. The attenuation coefficient for glass was then added to the simulated results for comparison. The dotted line (NA2) represents an approximation to the change in depth of focus and follows the same trend as the $\alpha_{\text{diff}}$.
Figure 6 shows the comparison of the apparent attenuation due to opto-acoustic defocus (red) and diffraction (blue) obtained from simulation (solid lines) and experiment (stars). Equation 5 was calculated for each set of experimental NAs, the average of these was taken to be the attenuation coefficient for glass, $\alpha_0 = 2.5 \times 10^5 m^{-1}$, which is comparable to previous reports ($10^5 m^{-1}$ [35,36]). In order to compare simulation with experiment, the calculated $\alpha_0$ was added to the simulated apparent attenuations (which did not include material attenuation contributions). Both simulation and experiment, for the case of opto-acoustic defocus, follow the shape of the inverse depth of focus (NA$^2$, dotted line) corroborating that opto-acoustic defocus, an optical effect, is a significant contributor to apparent attenuation.

Based on the measurement of $\alpha_0$ (glass), it was possible to produce a simulated TRBS signal using the known refractive index of glass ($n=1.45@780nm$ [37]), for normal incidence and the measured Brillouin frequency of the form:

$$TRBS(z, NA) = S_{amp}(z, NA) \cos(2\pi f_B \frac{z}{\lambda_{sound}}) e^{-\alpha_0 z}$$

as shown in Fig. 7, here the simulated TRBS signal agree well with the experimental (||$\Delta R/R$||) results presented in figure 5.

In all experimental and simulated traces, there is a clear attenuation in intensity of the TRBS signal as a function of depth ($z$). Aside from the effects of material attenuation ($\alpha_0$), it is reasonable to assume that a heavily diffracting sound field would have a detectable effect on the signal amplitude. However, it is clear from simulation (Fig. 3) and experiment (Fig. 5), that apparent attenuation appears to be dominated by an alternative effect: opto-acoustic defocus. The lack of contribution by acoustic diffraction can be understood by considering the case of optical plane wave detection (low probe NA) and a heavily diffracting sound field (high pump NA):

Insidethe acoustic focal volume: there are no evanescent components and therefore no near field diffraction, hence sound amplitude and optical intensity remain constant.

Outsidethe acoustic focal volume: acoustic diffraction starts to occur, hence sound amplitude decreases proportional to $z$. However, since the illumination is approximately collimated and constant, the TRBS signal remains constant. This is because with a low NA optical system, only the light that interacts with the area of the acoustic wavefront that is flat (area of the first Fresnel zone, $F_0$) reaches the detector and contributes to the signal. As the area of this zone increases proportional to $z$, the signal loss due to acoustic diffraction is compensated. A figure of merit for this effect on the TRBS signal can be described with $TRBS \propto F_0 \times U_{sound} \propto z^{-1}$. Furthermore, this condition is only valid while $F_0$ is smaller than the area of illumination; however, in

Fig. 7. Simulated TRBS signals from glass with $\lambda_{probe}=780nm$. (a) Shows the simulated effects of opto-acoustic defocus with NA. (b) Shows the simulated effects of acoustic diffraction with NA. There is a good match in the signal decay between simulation and experiment (compare with figure 5) which indicates defocus is the main cause of apparent attenuation.

$$\alpha_0 = \alpha_{def} - \alpha_{diff} = \alpha_{def} - \alpha_{diff}$$

(5)
In practice, it is difficult to observe the effects of acoustic diffraction using TRBS, as it would require a small yet collimated probe spot, and a larger diffracting acoustic spot; circumstances which are contradictory within the Bragg condition.

For the case of opto-acoustic defocus, the problem can be described within the context of geometrical optics: if the acoustic wavefront is considered an object in an infinity corrected microscope, as that object moves its image will no longer converge at the detector plane. As higher NA lenses are used, this deviation of the image plane will magnify. Within a TRBS trace, the effect of opto-acoustic defocus can be evaluated by comparing the Rayleigh range of the probe objective with the propagation distance of the signal (as seen in Fig. 3a).

In an imaging context it is often desirable to maximise resolution, however this requires increasing the NA of the objective lens thereby increasing apparent attenuation. Using the methodology presented in this report, the errors introduced by apparent attenuation can be removed by first modelling \( \alpha_{\text{def}} \) and subtracting it from the experimental measurement (\( \alpha_{\text{def}}^{\prime} \)) to recover \( \alpha_0 \) (see equation 5). Despite being able to compensate for the error on measuring \( \alpha_0 \), the ability to measure the Brillouin frequency in-depth will still be compromised by opto-acoustic defocus.

Alternatively, a better compromise between resolution an imaging depth can be achieved by a mismatched pump-probe NA configuration: maximise resolution (high \( \text{NA}_{\text{pump}} \)) while minimising opto-acoustic defocus (low \( \text{NA}_{\text{probe}} \), see Fig. 6). However, this compromises signal-to-noise ratio (SNR) due to the following reasons: the effective cross section at which pump and probe spots interact is significantly reduced, the drop in power density due to enlarging spots and the difficulty of alignment of opposing objectives.

In previous works \( [9,12] \), we have proposed an alternative optical arrangement (presented in Fig. 8) where one objective (high NA) is used to deliver pump and probe light and a second objective (low NA) is used to collect probe light in transmission. Preliminary experiments show this minimises the effects of opto-acoustic defocus (Fig. 8b), while also simplifying alignment and optimising the power density of the probe for a constant average input power. In this configuration, probe light which originates from a high NA objective, and is collected with a low NA objective, will suffer a reduction in SNR in the following way: the highest probe spatial frequencies will have angles that are beyond the light-cone of the low NA objective and will miss the detector. Overall the trade-offs offered by this configuration enhances the applicability of phonon microscopy, however, further modelling and optimisation are required.

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**Fig. 5.** Experimental results. (a) Time traces of TRBS signals \( \Delta R/R \) obtained from glass at fixed \( \text{NA}_{\text{pump}} \) and increasing \( \text{NA}_{\text{probe}} \) to observe apparent attenuation due to opto-acoustic defocus. (b) Time traces of TRBS signals obtained from a glass sample at fixed \( \text{NA}_{\text{pump}} \) and increasing the \( \text{NA}_{\text{pump}} \) to observe apparent attenuation due to acoustic diffraction. These results confirm that opto-acoustic defocus is the strongest contribution to apparent attenuation.

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**Fig. 8.** Alternative configuration of phonon microscopy. By reducing the NA of the collection lens it is possible to reduce the apparent attenuation by opto-acoustic defocus. (a) Shows the experimental setup: delivery NA remains fixed so that the resolution available for imaging remains constant as it is governed by the pump spot size. (b) Resulting TRBS signals obtained with different collection: the lowest NA shows longer lasting signals however resolution remains the same, leading to a better compromise between imaging depth and resolution compared to matched NA.
5. Summary

We have isolated, in simulation and experiment, the effects of acoustic diffraction and opto-acoustic defocus in TRBS signals as NA increases. By doing so, we have identified opto-acoustic defocus as a strong cause of premature decay of TRBS signals which we call apparent attenuation. By understanding the mechanisms of signal decay, we have removed ambiguities and errors related with the NA of the system allowing us to accurately measure the sound attenuation coefficient. Additionally we proposed an alternative experimental configuration for phonon microscopy which offers a better compromise between resolution and imaging depth. This opens the possibility to use the sound attenuation coefficient as an additional contrast mechanism which could aid in identifying new biomarkers in cell biology.

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Appendix A. Supplementary Data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.pacs.2020.100180.

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