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Cell imaging by sub-optical wavelength phonon microscopy

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Abstract

The mechanical properties of cells are largely unknown, but they play an important role in cell function and behaviour. Living cells are particularly challenging for mechanical imaging techniques as cells are very fragile and easy to damage unless the method is non invasive. This paper presents recent developments that have enabled the use of laser generated phonons (ultrasound) with sub-optical wavelengths to look inside living cells. The use of phonons gives contrast from changes in the mechanical structure of the cell. The phonons are generated and detected with laser beams which allows high resolution images to be taken. Additionally because the time of flight of the phonons can be recorded it is possible to section the images with resolution that depends on the phonon wavelength. This technique can provide a novel modality for the analysis of individual cell components in a non-destructive way.

Mechanical cell imaging

Variation in the mechanical properties of a cell such as stiffness often provide little or no optical contrast when illuminated under visible light. However, the mechanical information is directly related to specific cell characteristics such as cell mobility, adherence or division. Thus, there is an interest in measuring these properties with the aim of revealing underlying mechanisms. Current methods for this purpose include acoustic microscopy [1], atomic force microscopy [2], Brillouin microscopy [3] and photoacoustic microscopy [4] where the last two, for single cell applications, are still in their infancy. Given the dimensions of a cell, extraction of useful mechanical information is challenging – cells are very sensitive to environmental stimulus and they are very difficult to measure without causing an undesired reaction. In that sense, conventional mechanical imaging systems often struggle while at the same time their resolution often remains below that of conventional optical microscopy. Despite this, mechanical characteristics of cells have produced important contributions. For instance, in the field of cancer research, diseased cells where found to have different mechanical properties compared to healthy cells [5].

In this landscape, laser-generated phonons offers several advantages over existing technologies. Unlike conventional acoustic microscopes [6], it has the capacity to work at the high frequencies necessary for high resolution imaging (∼5GHz). The obtained measurements are independent of the optical absorption profile of the sample as in the case of opto-acoustics [7].

The measurements are also taken through the cell (not just near the surface as in the case of atomic force microscopy [5]). There is no need for chemical labels and, unlike Brillouin microscopy, where the Brillouin frequency is measured on a given optical volume, [8;3], additional information such as sub-optical sectioning, attenuation [9] and impedance [10] can be obtained.

The phonon microscope

In a phonon microscope, a pulse of laser light is absorbed in a metal film to generate a phonon field. The sound is then detected by a second pulse of light in a pump-probe configuration [11] which gives access to the GHz region without the need for extremely fast electronics and detectors. The detected signal could be a traditional pulse-echo signal or an interference signal, the so-called Brillouin oscillation [12] – which is a direct measure of the refractive index and the speed of sound. Brillouin oscillations result from the interference of light scattered from the phonon wavefront with the unscattered light, (see figure 1a). As the wavefront travels into the sample, the phase of the scattered component changes and so the signal oscillates at a specific frequency, f_B. If the phonon field travels from one material to another the f_B changes accordingly. The Brillouin frequency f_B can be measured in three dimensions by applying signal processing techniques to the time-resolved trace to track the changes in frequency as the sound propagates through different materials in the sample. The lateral resolution is determined by optical diffraction while the axial resolution is determined by the acoustic wavelength $\lambda_{acoustic} \sim \lambda_{probe}/2n$ [13] (see figure 1b).

There are significant challenges to be addressed to allow this technology to be suitable for live cell imaging: direct exposure of the cells to light and heat, low signal–to–noise ratio (SNR) and low acquisition speed. Exposure to light induces damage by absorption in the cell itself and by the heat generated by the absorption occurring in the generation film. Low SNR due to weak phonon-to-photon interactions is commonly compensated by large photon intensities. Low acquisition speeds given by a mechanical delay line means that exposure to light is often unnecessarily long. All these factors decrement the ability to image living or even fixed cells using conventional phonon techniques.

Our solution to the challenges of cell phonon imaging are given by a novel opto-acoustic transducer, measurement configuration and electronic pump-probe method. A schematic of our microscope is shown in figure 1. A three-stack thin film transducer [14] is specially designed to absorb the pump beam while transmitting the probe beam. As both beams approach the transducer via the substrate, the cell is shielded, by the transducer itself, from pump light and the probe light is used efficiently. This manages the amount of light exposure for the cells. For the substrate, we use sapphire due to its high thermal conductivity which helps to dissipate the heat generated at the transducer thus reducing thermal load applied to the cells. The mechanical resonance of the transducer increases the acoustic amplitude at the frequency of interest ($\sim$ 5GHz) thus improving SNR. Finally, an asynchronous optical sampling (ASOPS) pump-probe system produces an electronic sweep across two pulsed lasers eliminating the need for a delay line. This increases acquisition speed, in our case, to 1-2 measurements every second depending on averaging. The combination of all those allowed to image both fixed and living cells within biologically relevant time scales [13].

Cell imaging using phonons

Figure 2 shows two examples of cell imaging using phonons. Figures 2a–d show the images obtained from a fixed adipose cell differentiated from a stem cell. The optical image is shown in figure 2a where some fat droplets are clearly visible. Figures 2b–d show a subsection (560nm) of the result presented in figure 2b as it moves in the axial direction. Here each picture corresponds to a 400nm step forward in z starting at 600nm (lens NA is 0.42, and depth of focus $\sim 8\mu$). As the section moves, the fat droplet marked by a circle is revealed to be approximately situated at 1 μm away from the substrate and its thickness is approximately that of one section. Figures 2e and 2f show the example of a cardiac cell where the sarcomeres, a mechanically relevant structure, are clearly visible. Finally figure 2g shows a representative experimental trace.

Figure 3 shows further examples of this method. Figures 3a-b show the example of an acanthamoeba where the voids in the amoeba are revealed to be cell medium. This particular fact is more difficult to deduce with optical imaging as optical contrast does not gives enough information, even using fluorescence labels. Figures 3c-j show examples of living cells. Living 3T3 cells are shown on figures 3c–h. The acoustic image of the cells show good correlation with their optical counterparts, their resolution is limited in this case by acquisition speed due to cell mobility. However, this opens the opportunity to image the cells dynamically. Figures 3i–j show a live adipose cell. Here the large fat droplets show good contrast with respect to the rest of the cell. However, due to sound attenuation, the acoustical penetration depth is smaller than the optical depth of focus making the droplets appear smaller in size.

Discussion

We have introduced a label free, high resolution acoustic live-cell imaging method using phonons. This method has the ability to resolve an object based on the contrast of its mechanical properties. The lateral resolution is limited by optical diffraction however the axial resolution is limited by the acoustic wavelength. It was also shown that the method is compatible with living cells. This technique offers a label-free alternative for live-cell imaging and mechanical characterisation. For the cell types presented in this paper, the change in the measured Brillouin frequency within a cell is predominantly related to changes in the acoustic velocity. The ability to measure the response of the speed of sound on a living cell dynamically, under a given stimulus, offers great potential to study the mechanical response of cells.

In principle, the highest axial resolution achievable using this method is $\lambda_{probe}/2n$, which is $\sim$280nm in cells at λ_{probe} =780 nm. This is significantly better than the optical axial resolution of the optical system used to take the measurements, (NA of 0.42 and depth of focus of $\sim 8\mu$m) and is higher than that achievable with a typical oil immersion confocal system. In practise the axial resolution is currently limited by SNR and contrast.

Most other sectioning techniques require re-scanning at a different axial position, however time-resolving the acoustic signal allows sectioning of the measured volume by post-processing. Therefore, the cell only receives one dose of light during the imaging process. The time to acquire the signal is $\sim$1–2 seconds per point due to averaging, which typically yields 5–10 voxels per point if the data is sectioned.

Mechanical imaging of cells is an important field of research. However, the challenges presented to measure cells mechanically are significant. The method presented here provides

a new tool for mechanical imaging and characterisation of cells which is also the only new alternative to the photon for three dimensional imaging of live cells since the invention of the optical microscope.

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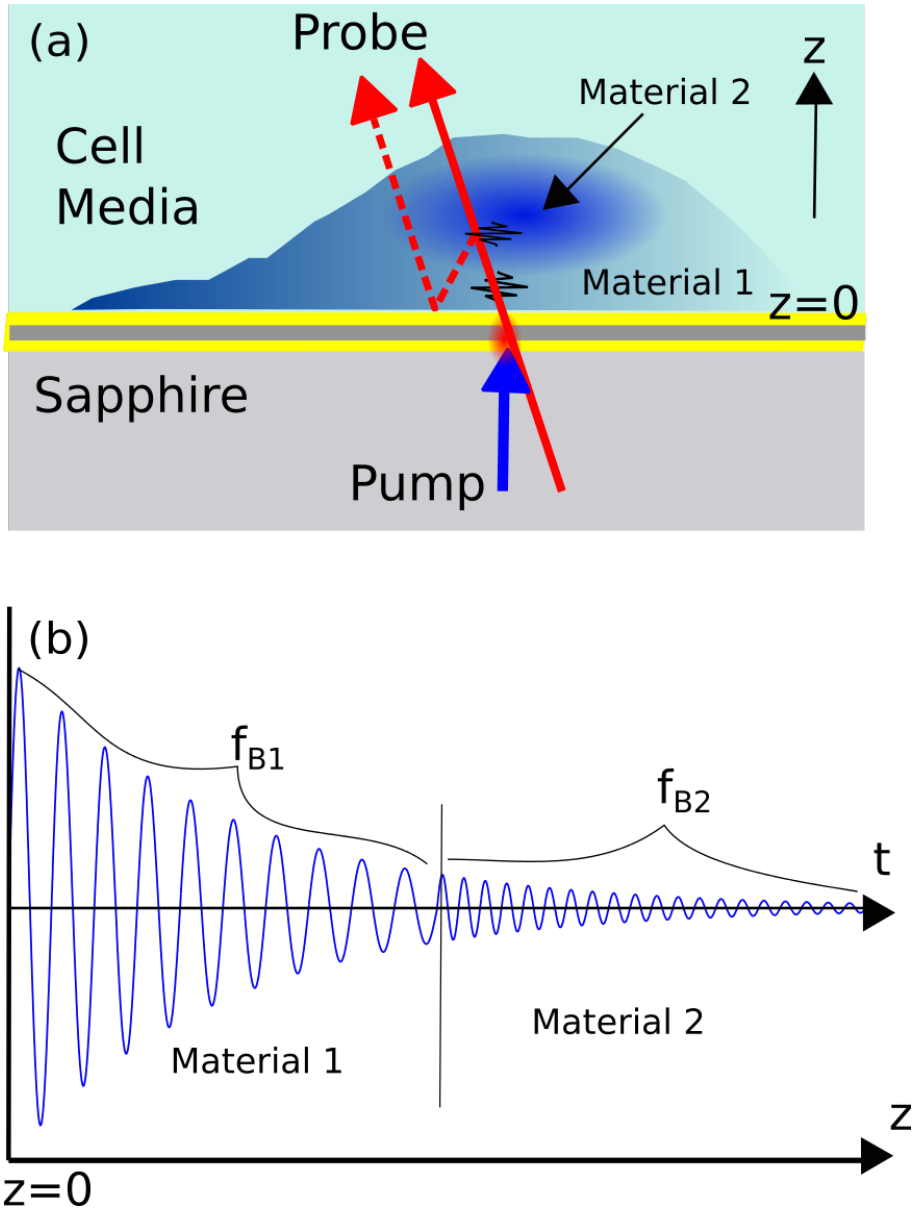

Figure 1: Simplified diagram of three dimensional phonon imaging of cells. In (a), a light pulse (pump) is used to thermoelastically generate a coherent acoustic field which is probed by a second light beam (probe, shown in angle for clarity). The interference of the direct (solid) and scattered (dashed) probe beams induces an oscillation in the detected probe light intensity. The frequency of this oscillation f_B is a function of the speed of sound. As the phonon field travels from material 1 to another material 2 (frequency change is exaggerated for clarity), the detected frequency changes from f_{B1} to f_{B2} as graphically shown in (b). As the spatial position z is related to the time information by the speed of sound, it is possible to section the optical volume by post-processing without the need of further acquisition, mechanical positioning or change of focal position.

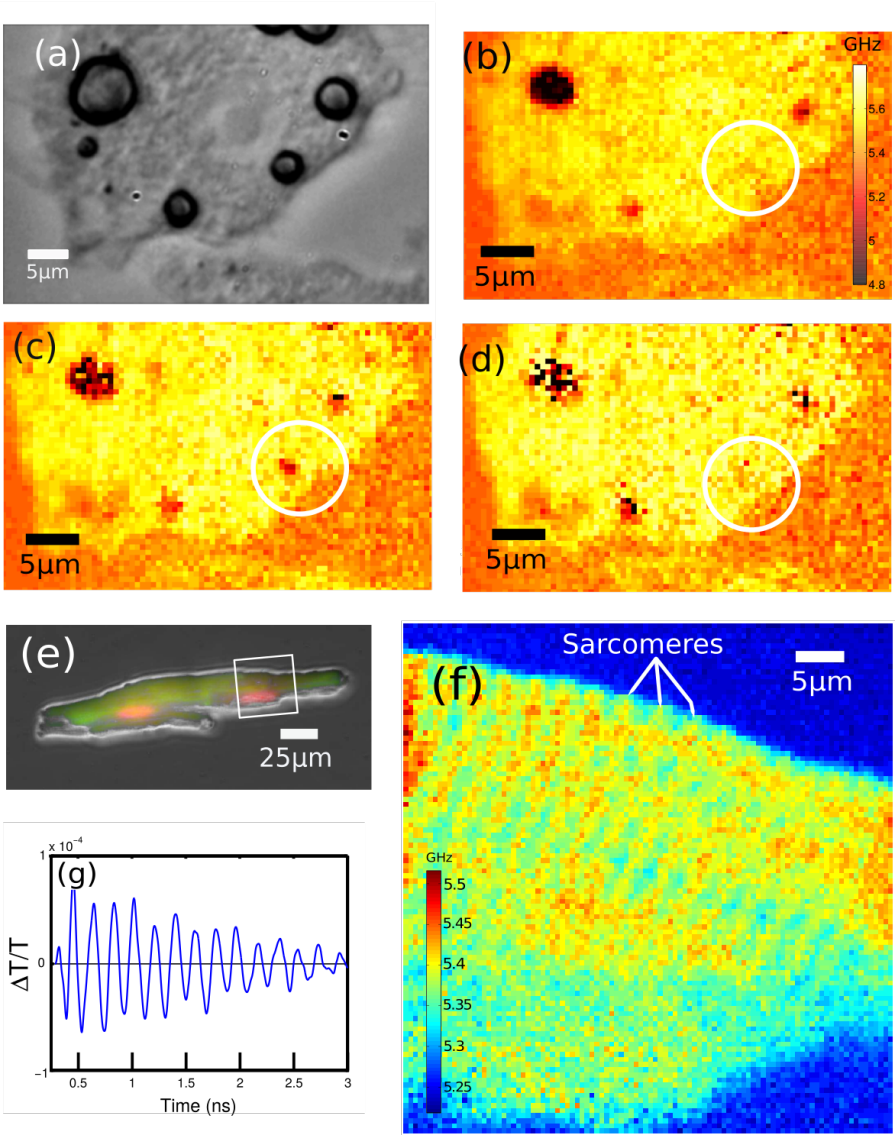

Figure 2: Phonon imaging of fixed cells. Images (a–d) show a stem cell differentiated into an adipose cell. (a) is an optical picture, (b–d) Show 800nm sections extracted by post-processing, here a fat droplet is contained within a single section. (e–f) Example of acoustic imaging of a cardiac cell. (e) Optical image, (f) Acoustic image, where the sarcomere structures are clearly visible. (g) Representative detected signal.

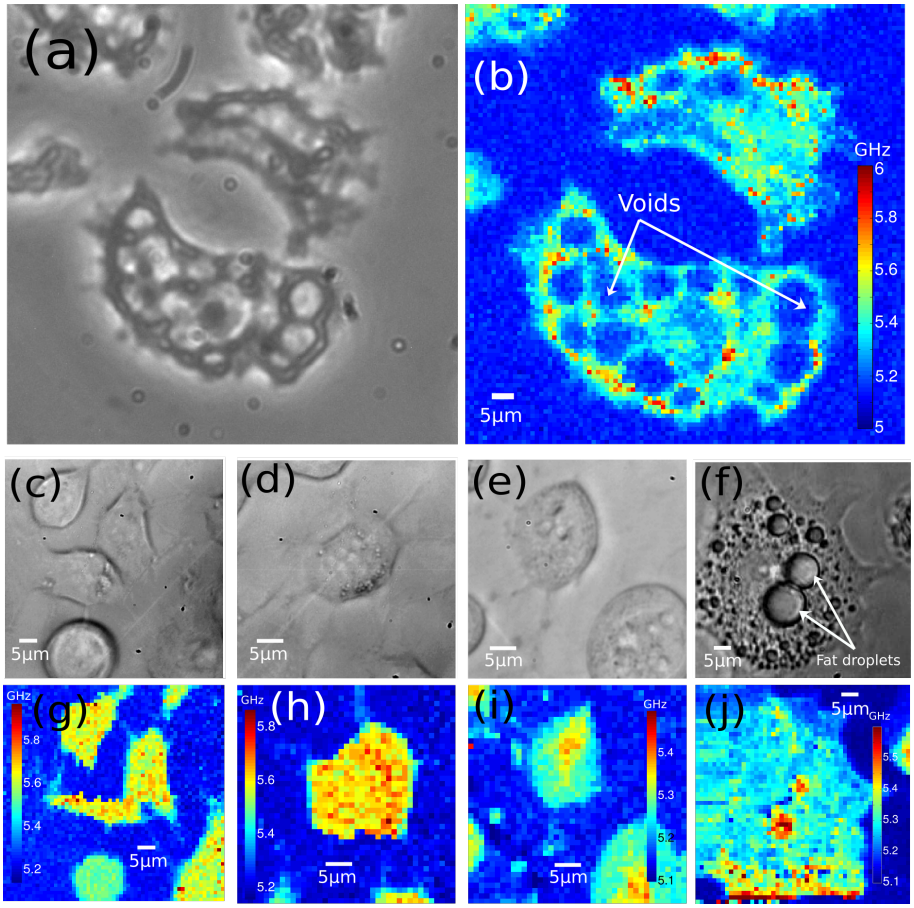

Figure 3: Laser ultrasound imaging of fixed and living cells. Images (a–b) shows *Acanthamoeba*. The holes in the *Acanthamoeba* clearly show the same Brillouin frequency as the medium. (c–h) Live 3T3 fibroblasts. where the resultant image shows good correlation. (i–j). Live adipose cell. The large fat droplets show good contrast compared to the rest of the cell.