Cell Imaging by Phonon Microscopy

Sub-Optical Wavelength Ultrasound for Non-Invasive Imaging

The mechanical properties of cells play an important role in cell function and behavior. 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 phonons reveal contrast from changes in the elasticity of the cell and can provide high resolution three dimensional images.

Mechanical Cell Imaging

Variation in the mechanical properties of a cell such as stiffness often provides little or no optical contrast when illuminated with light. Mechanical information is directly related to cell characteristics such as cell mobility, adherence or division and 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 characterization of cells has been shown to be significant. For instance, in the field of cancer research, diseased cells were found to have different mechanical properties compared to healthy cells [5].

Laser-generated phonons offer 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 (~5 GHz), the measurements are independent of the optical absorption profile of the sample (as in the case of photoacoustics [7]) and there is no need for chemical labels. The measurements are also taken through the cell (not just near the surface as in the case of atomic force microscopy [5]) and unlike Brillouin microscopy, where the resolution is optical [3,8], additional information such as sub-optical sectioning,
attenuation [9] and impedance [10] can be obtained using the acoustic time of flight.

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 is 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, (fig.1a). As the wavefront travels into the sample, the phase of the scattered component changes so the detected intensity oscillates at a specific frequency $f_B$. If the phonon field travels from one material to another, $f_B$ changes accordingly. The Brillouin frequency $f_B$ can be measured in three dimensions. The lateral resolution is determined by optical diffraction while the axial resolution is determined by the acoustic wavelength $\lambda_{\text{acoustic}} = \lambda_{\text{probe}} / 2n$ [13] (fig. 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.

Our solutions to the challenges of live-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] was 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. For the substrate, we use sapphire due to its high thermal conductivity which aids heat dissipation. The mechanical resonance of the transducer increases the acoustic amplitude at the frequency of interest (~5GHz) thus improving SNR.
Finally, an asynchronous optical sampling (ASOPS) pump-probe system increases acquisition speed, in our case, to 1-2 measurements every second depending on averaging. The combination of those has allowed imaging of 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 (560 nm) 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 600 nm (lens NA is 0.42, and depth of focus ~8μm). As the section moves, the fat droplet marked by a circle is revealed to be approximately situated at 1μm 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 vacuoles in the amoeba are revealed to be cell medium. Figures 3c-j shows 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 fat droplets can be seen in the Brillouin shift (see fig. 3j) and also in the measurement of the sound attenuation (not shown).

**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, while 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 characterization. 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 $\frac{\lambda_{\text{probe}}}{2n}$, which is $\sim$280nm in cells at $\lambda_{\text{probe}} = 780$ 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 $\sim$ 8μm) and is higher than that achievable with a typical oil immersion confocal system. In practice 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 characterization 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.

Acknowledgments

This work was supported by the Engineering and Physical Sciences Research Council [grant numbers EP/K021877/1, EP/G061661/1] (EPSRC). F.P.C and A.L.L. acknowledge Conacyt for sponsoring their studies. We are grateful to the EU-CASCADE fellowship scheme funded by the EU’s 7th FP PCOFUND-GA-2012-600181 (K.V.) and to Prof Harold Sacks (CRF, Los Angeles, USA) for their support.

References


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