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Fernando Pérez-Cota, Richard J. Smith, Emilia Moradi, et al.

Non Destructive Evaluation of Biological Cells

Fernando Pérez-Cota[1,a)], Richard J. Smith[1], Emilia Moradi[1], Kevin F. Webb[1] and Matt Clark[1]

[1]*Optics and Photonics, Faculty of Engineering, University of Nottingham, Nottingham, UK.*

[a)]Corresponding author: fernando.perez-cota@nottingham.ac.uk

Abstract. Regenerative medicine promises to be the next revolution in health care. This technology, which will be the first systematic manufacturing of biological parts for human consumption, requires non destructive evaluatioin (NDE) thechniques for the inspection and quality assurance of products such as tissue and organs. Ultrasound is the technology of choice due to its low invasiveness however state of the art is limited in resolution and not able to inspect single cells. In this paper we present a novel NDE technique applicable for single cells that uses sound, offers contrast provided by mechanical properties, does not require toxic chemical labels and can achieve optical or higher resolution. This will be required for tissue manufacturing as the current technologies used for research on the life-sciences heavily depends on toxic chemicals and radiation. This will stablish ultrasound as a single platform for the inspection of biological products at all scales.

INTRODUCTION

Non destructive evaluation (NDE) is traditionally aimed to test the quality of manufactured goods, typically metallic parts, without compromising their integrity. This is a simple yet powerful concept that has allowed to increase the useful life and reliability of critical components of industries that are at the core of modern society[1].

Manufacturing with inert materials it is the norm, however things are changing. Recent developments in stem-cell biology are making possible the manufacturing of parts for human consumption that are based on living materials in what is commonly referred to regenerative medicine[2]. Figure 1 illustrates the concept of regenerative medicine. Stem cells (or mother cells which can become any type of cell) are extracted from the user, these then will be cultured and used to manufacture a new organ/tissue wich then is going to be re-implanted into the user. Such parts for human consumption will require NDE to ensure they are suitable for human use. This is critical as the health implications of defective parts can be counter-productive.

Most cell and tissue characterisation techniques rely on a number of invasive or destructive methods which are acceptable for research in the life sciences. For instance, the chemical toxicity of fluorescent labels widely used in cell biology[3] are not suitable for re-implantation. The photon toxicity of autoflorescence techniques can produce significant damage[4] and other forms of radiation such as x-ray are simply unacceptable.

In this landscape ultrasound offer great advantages compared to optical techniques: first it can image the tissue without damaging it: ultrasound is the only acceptable means to image a living human embrio. Ultrasound can also image the tissue at wide scales: from tissue to full organ. Light is severy hampered as the thickness of the tissue reaches a approximately 1mm while at the same time is harmful as the the part approaches a single cell, particularly for short wavelengths[5].

Current ultrasound technology can address the larger side of the scale with technologies reaching resolutions from a few to several hundred microns and penetration depths from a few milimiters to tens of centimetres. However these technologies are not suitable for single cell applications.

In this paper we present an ultrasound technology that is suitable for the NDE of single cells. This technology, wich we call phonon microscopy, overcomes the limitations of the state of the art ultrasound for single cell applications by the use of a novel all-optical aproach for the generation and detection of GHz ultrasound (or phonons).

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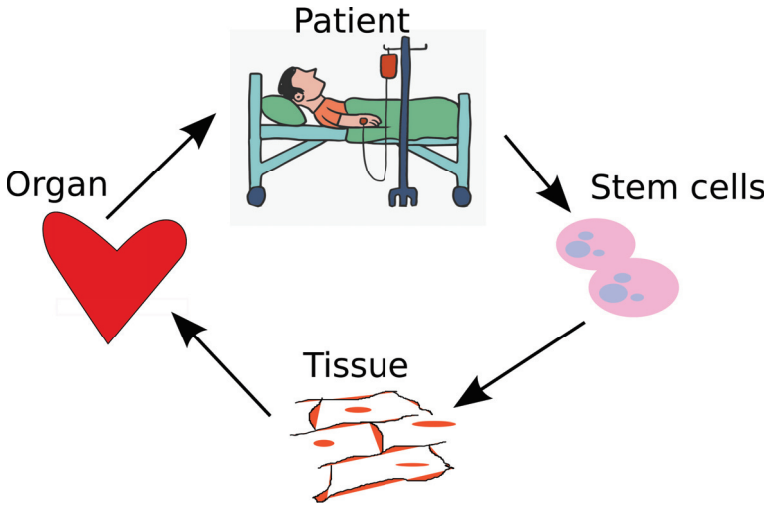

FIGURE 1. Regenerative Medicine cycle. Stem cells are extracted from the patient, then these are differentiated into a specific type of cells. These special cells, for instance cardiac muscle, are used to manufacture a new organs which finally will be reimplanted to the patient. Regenerative medicine promises to be the next revolution in medicine and when possible NDE of biology will be required.

How to NDE a single cell

The requieremnts to be able to image a single cell with ultrasound are challenging. The resolution needed to see sub-cellular structures is in the order of 500nm. This means that the frequency of the ultrasound is in the order of approximately 4-10GHz and this frequency brings with it some complications. First, the attenuation of sound at this frequency is very strong (for instace water at 4.4GHz is 1900dbm^{-1}[6]) : this means that the source of sound needs to be as as close as possible to the sample. At this regime even an acoustic lens with 15μm focal length[7] would not be suitable to detect the sound scattered back from a cell in a water medium at room temperature.

To address this problem we have proposed the arquitecture shown in fig. 2a. An opto-acoustic transducer (OAT) generates sound upton excitation from a pulse of laser light. In this way propagation losses are reduced since the cell is cultured on the OAT effectively moving the source of sound as close as it is physically possible. More over the signals, are detected by Brillouin scattering which are an interaction between ligth and sound. This reduces even further the need of long propagation distance since the sound is probed as it propagates rather than a fixed detector at a long distance away[8]. At this regime, the operational acoustic wavelength can be shorter than that of light offering the potential for super-resolution.

The OAT is a key element because allows to image the cells safely: it is designed to protect the cells against pump light and heat while allowing transmission of probe light for detection. At the same time it acoustically resonates at a specific frequency to increase signal to noise ratio.

This addresses the high attenuation, however brings with it a second complication: The signal, now in the multi-GHz range, it has to be captured also optically. A multi-GHz optical detection chain is susceptible to noise and since the modulation depth expected from these signals is in the order of 10^{-10} to 10^{-6}, it becomes challenging. To solve this, a pump-probe[9, 10, 11] configuration was implemented (see figure 2b). Here two pulsed lasers are used for generation and detection of sound. The length of the pulses, which is approximately 150fs, allows to generate and sample extremely high frequencies. In this modality the probe pulse is delayed with respect to the pump pulse, this delay is sweep allowing reconstruction of the signals at much slower rate and higher modulation depth[10, 12].

The detected transmission probe light carries the acoustic signal. The frequency (f_B, or Brillouin frequency) and amplitude (A) of the signal reveals the position and amplitude of the acoustic wavefront which encodes the sound velocity (v=$f_B\lambda/2n$) and attenuation (α_0 see fig. 2c). These are relevant mechanisms for contrast and used to build images. Altogether we call this technique, phonon microscopy [13, 14].

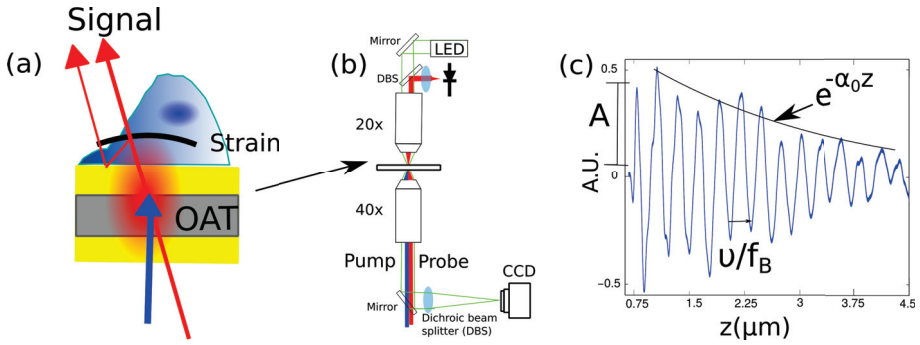

FIGURE 2. How to NDE a single cell. Cells are cultured in a transducer which converts light into GHz sound while protecting the cells against pump light (a). A back to back microscope configuration is used to capured the transmitted ligth (b). The sound is detected from the transmitted ligth which provides the signal in (c). This signal contains information about the amplitude of sound, its velocity and attenuation.

RESULTS

Figure 3 shows results obtained on living cells using phonon microscopy. The cells did not have any preparation, staining and were in buffer medium. The figure presents pairs of images with optical (shown in gray scale) and acoustic images (shown in colour) . Compared to the optical, acoustic images show little contrast for the inner features of the cell. This arises simply for the fact that the cells are alive. Compared to inert objects, live cells, react easily to exposure to light, heat and they move. All these characteristics of living objects pose additional challenges to NDE for biology at the single cell level: acquisition speed, exposure to light, heat and adequate environment must be carefully addressed to reduce the invasiveness of the imaging process to the levels required for regenerative medicine.

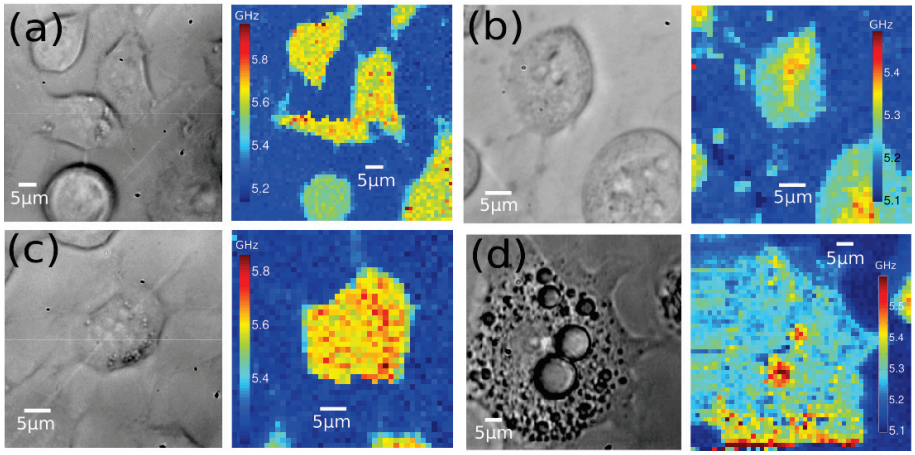

FIGURE 3. Example of single cell NDE of living cells. Images are presented in pairs with optical (gray) and acoustical (colour). All images have a with 1μm resolution given by scanning steps. (a - (c, 3T3 fibroblast cells. d) Stem cell differentiating into a fat cell. In all cases there is loss of detail due to blur.

In our case, the lack of detail in the inner cell is due to two main reasons: acquisition speed and resolution. The resolution in the pictures from fig 3 is 1μm but since acquisition took approximately 20min, the mobility of the cell caused blur of adjacent pixels. Increasing the resolution will increase acquisition time even further increasing blur. So to prevent blur then acquisition speed has to be improved which has a few limiting factors. First, point by point scanning requires moving mechanical elements. Second, averaging is necessary (5-10k) as signal-to-noise ratio (SNR) is low. Third, a time trace for each pixel needs to be processed and finally pump-probe measurements, which allow to measure very high frequencies, slows down acquisition of a single trace to 100μs for our particular configuration. All

these factors are technological but not fundamental limitations that can be addressed.

Figure 4 shows phonon imaging of fixed cells. In this case the resolution of the acoustic and optical images is approximately matched to 500nm. Removing the limitations posed by living biology, phonon imaging offers improved contrast compared to bright field imaging. The features in the cells are clearer because the intrinsic mechanical characteristics of the cells show greater variation than the optical ones (refractive index).

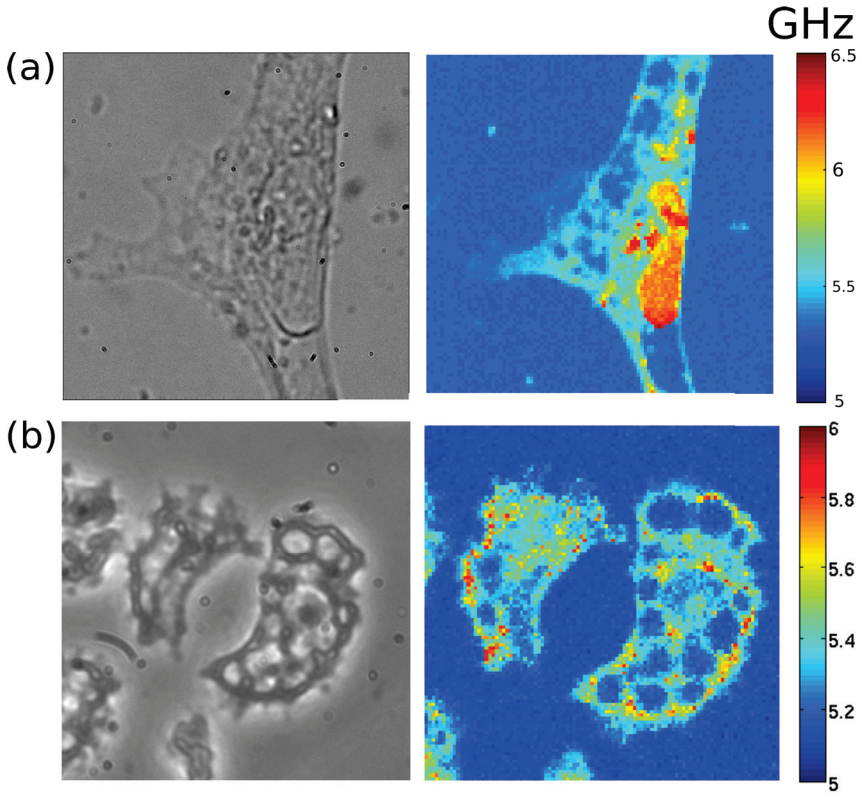

FIGURE 4. Example of NDE on fixed cells. Images are presented in pairs of optical (gray) and acoustical (colour). All images have a with 500nm resolution given by optical imaging system. (a Colo-rectal mammal cells. b) Acanthamoeba tropozoide. In all cases there is additional contrast seen in the acoustics with respect to the optics.

DISCUSSION

Regenerative medicine is the next revolution in health care and when finally arrives, will be the its important development since the arrival of antibiotics. At single cell level, traditional imaging methods are not compatible with it simply because they rely heavily from toxic chemical labels and radiation. Ultrasound offers a great alternative for this application as it does not cause damage and offers the potential for greater contrast and resolution.

Imaging single cells with ultrasound is very challenging. Here we propose a method that can achieve this with optical resolution or greater. This method overcomes the limitations of traditional piezo-electric based technology and offers a viable alternative for regenerative medicine.

There are still many challenges to address in signal-to-noise and acquisition speed. However these challenges are of technological nature and we expect that high speed, high resolution phonon imaging of livings cells will be a reality in the near future.

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