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4pBA1. Acoustic and photoacoustic imaging of spheroids
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Acoustic and photoacoustic high frequency imaging (50-100 MHz) can be used to generate images of cell constructs and spheroids with good spatial resolution and contrast. Here we demonstrate how co-registered acoustic and photoacoustic imaging can be used for imaging spheroids. Spheroids are widely used in cancer research and biology since they emulate a 3-dimensional environment such as that experienced in tumors. Spheroids were made by the hanging-drop method using the MCF-7 cancer cell line. To generate photoacoustic contrast, MCF-7 cells were incubated with optical absorbing nanoparticles (e.g. gold nanorods, 780nm absorption) for 24 hours and mixed with native MCF-7 cells prior to spheroid formation. The spheroids were between 0.5 mm and 1mm in diameter. Imaging was performed with the VisualSonics VEVO 770 (25-55 MHz) and a high-resolution SASAM acoustic/photoacoustic microscope for frequencies over 80 MHz (Kibero GmbH, Germany). The spheroid was imaged first using pulse echo ultrasound, then with photoacoustics immediately after. The necrotic core of the spheroid had a 20dB increase in ultrasound backscatter compared the viable cells surrounding the core, and the ultrasound/photoacoustic images of the spheroid were co-registered showing the distribution of the optical absorbing agents.

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INTRODUCTION

Ultrasound imaging can be used to obtain cross-sectional images that represent the echogenicity of the sample imaged. The spatial and contrast resolution of the imaging device are highly dependent on the frequency of the ultrasound used to perform the imaging[1]. The ultrasound frequency dictates the relevant length-scales for which the interactions of the incident ultrasound waves and the object that scatter the sound occur and how the spatial distribution of the ultrasound scattering structures influence the overall backscatter[2]. While it is well understood that the axial and lateral resolution of ultrasound imaging improves with increasing ultrasound frequency, less well understood is how the contrast generated while imaging tissues changes according to the frequency change used in the imaging[2]. An example of the significant change that the ultrasound frequency can cause involves red blood cells. For ultrasound in the frequency range between 1-10MHz, the blood within blood vessels appears on an ultrasound image as black cylinders with very low echogenicity compared to the tissue surrounding the vessel[3]. In this case, the scattering from small micron sized red blood cells does not significantly scatter the ultrasound waves from the interrogating beam. On the other hand, for high frequency ultrasound (> 30MHz), the scattering from blood is often comparable to that of the surrounding tissues. At even higher frequencies, the echogenicity from within the vessel surpasses the echogenicity of surrounding the tissue. However, this increased spatial and contrast resolution comes at the expense of imaging depth. It is also known that the spatial distribution of the red blood cells also plays an important role in the overall scattering strength and therefore contrast resolution of the imaging system[4]. The ultrasound backscatter from blood, for the ultrasound frequencies discussed, is dependent on the degree of spatial randomization of red blood cells in the medium – the greater the degree of spatial randomization, the greater the backscatter strength. Therefore, it is of great interest to image at the relevant length scales that can interrogate cell structure with wavelengths approaching the size of a cell. It is expected at these frequencies the ultrasound backscatter to be sensitive to the changes in cell structure that may occur with cell death. Understanding the frequency dependence of scattering from sub-resolution scattering structures is used in ultrasound techniques and form part of what is known as quantitative ultrasound (QUS)[5]. These techniques allow the differentiation of tissues based on non-resolvable cellular structures that modify primarily the frequency dependence of the scattered ultrasound.

Another imaging modality that can be used to obtain cross-sectional images, this time representative of the optical absorption of tissue structures, is photoacoustic imaging. In photoacoustic imaging, short laser pulses illuminate the sample, and the thermo-elastic expansion of the target absorbers causes pressure waves, of ultrasonic frequencies, to be produced[6]. These ultrasound waves can then be detected by conventional ultrasound instrumentation to produce photoacoustic images. High spatial resolution can be achieved by tightly focussed light illumination, or very high frequency ultrasound detection, or both. By selecting the appropriate ultrasound detection frequency images can be generated of high spatial resolution (sub-micron), or of lower spatial resolution but greater penetration depth, in a way similar to acoustic microscopy.

In this work we show how acoustic and photoacoustic high frequency imaging (50-200 MHz) can be used to generate cross sectional mages of cell constructs and spheroids with good spatial resolution and contrast.

METHOD

A SASAM acoustic / photoacoustic microscope was used for all measurements[7, 8]. For the photoacoustic measurements, samples were irradiated with a 1064 nm laser focused to a 5-10 µm spot size using a laser power of up to 30 nJ per pulse. Photoacoustic measurements were done using 80 or 200MHz transducers. Acoustic measurements of spheroids were also done using 80 or 200 MHz transducers in pulse-echo mode. For the photoacoustic measurements, MCF-7 cells were incubated with gold nanorods (780nm absorption) for 24 hours and mixed with native MCF-7 cells (number ratio of 6:1). A schematic of a close up of the experimental system is shown in figure 1.
FIGURE 1: Close up of the SASAM acoustic microscope for which the optical objective and ultrasound transducer are co-aligned. The laser illuminates the sample through the optical objective.

RESULTS AND DISCUSSION

Ultrasound images of the MCF7 spheroid, acquired at 80MHz, are shown in Figure 2. The image to the left shows an acoustic attenuation image of the spheroid, with the necrotic core in the middle of the spheroid demonstrating greater attenuation compared to the viable periphery. A C-scan backscatter image of the same spheroid is shown in the middle panel of the figure. In this case, the necrotic core of the spheroid shows an increased scattering, at the same location for which the increased attenuation is measured. Finally, a B-scan through a cross-section of the spheroid is shown in the right panel. Consistent with the other two images, an increased backscatter is measured at the necrotic core. It is clear that at these length scales that the ultrasound backscatter and attenuation can be used to detect changes in cell structure that are due to cell death, even though individual cells cannot be resolved.

FIGURE 2. Acoustic attenuation (80MHz), backscatter C-scan and B-scan ultrasound backscatter images of MCF7 spheroids in their early stages of growth (diameter ~120 mm). Formation of necrotic core (confirmed in H & E histology) evident in images.

A photoacoustic image of the mixed MCF7 spheroid containing gold nanorods, acquired at 80MHz, is shown in the left panel of Figure 3. The clusters of the gold nanorods at various locations within the spheroid are clearly visible. For the same section, an ultrasound acoustic microscopy image is shown, acquired at 200 MHz. In this spheroid, that was grown using a different method in order to introduce the gold nanorods in the cells before the spheroid was made, there was no necrotic core evident. Areas of increased attenuation seemed to correlate with locations of increased photoacoustic signal, indicating that the clusters of the gold nanorods that were internalized in the cells potentially increased the ultrasound attenuation at those locations. The optical image of the same spheroid is shown in the rightmost panel of Figure 3.
CONCLUSION

In summary, we have shown how acoustic and photoacoustic microscopy can be used to generated images of the interior of spheroids. In the acoustic images, the location of the necrotic core of the spheroids is clearly visible, due to the endogenous contrast that is produced during the process of cell death. For the photoacoustic images, the spheroid images provide information about the location of the gold nanorods within the interior of the spheroid. The approach pursued in this work also allows axial (depth) imaging of absorbing structures due to the high bandwidth and focussed ultrasound detection. This is similar to the high axial resolution of acoustic microscopy, and is an advantage of the technique over photoacoustic microscopy that relies on highly focussed optical illumination to achieve lateral resolution. Future work will focus on more detailed co-registered photoacoustic and acoustic microscopy of spheroids.

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