# MEASURING SCATTERING IN APOPTOTIC CANCER CELLS USING ULTRA HIGH FREQUENCY ACOUSTIC MICROSCOPY

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### 1. INTRODUCTION

Apoptosis, or programmed cell death, is a method of cellular self-disassembly to minimize damage to nearby cells [1]. The cell breaks into apoptotic bodies to contain the toxic intracellular components, which are then removed by phagocytes. Apoptosis is triggered by toxins, ionizing radiation or physical damage. Proteins begin breaking down the cell, and eventually the cell collapses onto itself. Morphological features such as membrane blebbing and protrusions from the cell are typically observed. Once initiated, the entire apoptotic process can take less than an hour, and is reversible until collapse occurs.

It is important to understand how apoptosis occurs, as deregulation of apoptosis can result in a variety of diseases, such as Alzheimer's, AIDS, autoimmune disorders and tumours. Of particular importance is the study of cancer cells, which have mutated to prevent apoptosis from occurring. Understanding apoptosis is important to treating cancer and other diseases related to apoptotic dysfunction.

High frequency ultrasound (10-60 MHz) has been used to study apoptosis, where an increase in the ultrasound backscatter intensity was observed for cells treated with a chemotherapeutic agent to induce apoptosis, compared to untreated cells [2]. However individual cells cannot be resolved at these frequencies, and the source of increased backscatter could not be determined. Acoustic microscopy uses ultra-high frequencies (100+ MHz) with resolutions approaching 1  $\mu$ m, which is able to resolve cellular and subcellular features [3]. Ultrasound can be used to image the mechanical properties of the cell, which may give insight into the non-visible changes that are occurring within the cell, an advantage over optical observations.

## 2. METHOD

MCF-7 breast cancer cells were grown in 175 cm<sup>2</sup> cell culture flasks (Sarstedt, Newton, NC, USA) using Dulbecco's modified eagle's medium (ATCC, Manassas, VA, USA) with 10% fetal bovine serum and 0.1% insulin. Cells were incubated at  $37^{\circ}$ C with 5% CO<sub>2</sub> and passed every 3 days to maintain exponential growth. Cells were dissociated using trypsin and transferred to Lab-Tek II chambers (Nunc, Germany). 15 hours prior to experimentation, the medium was replaced with a solution consisting of the DMEM cell culture medium (without serum or insulin), 3 mg/mL of caffeine and 20 ng/mL of paclitaxel to induce apoptosis. During experimentation, cells were kept at a constant temperature of 36°C with 5% CO<sub>2</sub>.

The acoustic microscope (Kibero GmbH, Saarbrücken, Germany) is equipped with an acoustic and optical component, which allows for simultaneous optical and acoustic measurements. The transducer can be visually positioned over a region of interest for a precise alignment of the transducer and the sample. The electronics consist of a monocycle pulse generator at 300 MHz with a 100% bandwidth, 10  $V_{pp}$  amplitude and a 500 kHz pulse repetition rate. The RF signals are sampled at 8 GHz, amplified by a 40 dB amplifier and filtered to remove noise. The transducer used in this study had a center frequency of 375 MHz with a -6dB bandwidth of 42% and a F-number of 1.

Cells were imaged in two different states; before apoptotic collapse (denoted normal) and after collapse (denoted apoptotic). The transducer was positioned over a selected cell, and the ultrasound RF signal was recorded every 10 seconds for a period of 900 seconds. This process was repeated for four cells in each state.

### 3. **RESULTS**

An a-scan showing a single RF-signal measurement as a function of ultrasound propagation time is shown in figure 1. The ultrasound backscatter from the cell (A) and the substrate (B) are clearly visible and separated in time. The region from approximately 1500 to 1520 ns, the area including the cell signal but excluding the substrate signal, was integrated to give the backscatter intensity over the cellular region. This calculation was done for each measurement for each cell. The integrated backscatter intensity for a typical normal and apoptotic cell as a function of measurement time is shown in figure 2.

The correlation coefficient of the RF-signal was calculated by comparing the first RF-line, restricted to the cellular backscatter region only, to the other RF-lines at the same location but at different measurement times for that particular cell. The correlation coefficient is a measure of the similarity of two signals; identical signals have a correlation coefficient of 1. The correlation coefficient as a function of measurement time is shown in figure 3 for a typical normal and apoptotic cell. The normal cells had an average correlation coefficient of  $0.93 \pm 0.05$ , compared to  $0.68 \pm 0.17$  for the apoptotic cells.

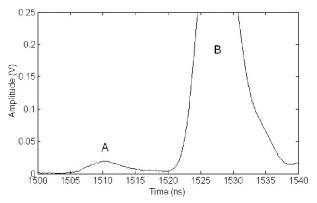


Figure 1. A single RF signal showing the ultrasound backscatter from the cell (A) and substrate (B).

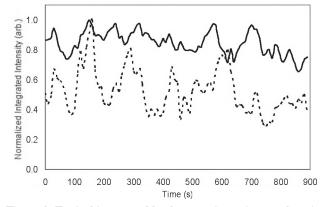


Figure 2. Typical integrated backscatter intensity as a function of time for a normal cell (solid line) and apoptotic cell (dotted).

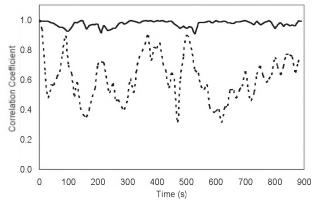


Figure 3. Typical correlation coefficient as a function of time for a normal cell (solid line) and apoptotic cell (dotted).

Table 1. Transient analysis results of MCF-7 cells.

Measurement	Normal Cells	Apoptotic Cells
Correlation Coefficient	$0.93\pm0.05$	$0.68\pm0.17$
Integrated Intensity	$0.75\pm0.10$	$0.57\pm0.14$
Maximum Amplitude	$0.529 \pm 0.003$	$0.498 \pm 0.004$

In addition to the cellular backscatter, the maximum amplitude from the substrate was also measured which is an indication of the attenuation in the cell. Table 1 summarizes the average correlation coefficient, integrated intensity and maximum amplitude for four cells measured in each state.

#### 4. **DISCUSSION**

The variations of the integrated backscatter intensity were larger for normal cells compared to apoptotic cells, as shown in figure 2. The average normalized backscatter integrated intensity was  $0.75 \pm 0.10$  for normal cells, compared to  $0.57 \pm 0.14$  for apoptotic cells. The larger variations and higher standard deviation for apoptotic cells indicate an increase in activity within the cell during the measurement period.

A more thorough method of measuring the variations in the cell is by looking at the correlation coefficient. An average correlation coefficient of  $0.93 \pm 0.05$  was calculated for normal cells, indicating a small amount of variation of the RF signal over time. For apoptotic cells, an average correlation coefficient of  $0.68 \pm 0.17$  was calculated, which is lower than for normal cells. In addition, the standard deviation is higher, indicating extensive variations are occurring within the cell (Figure 3).

The maximum amplitude from the substrate was 0.529  $\pm$ 0.003 for normal cells compared to  $0.498 \pm 0.004$  for apoptotic cells. The low standard deviation suggest the attenuation in the cell is not changing. However variations in the backscatter signal from the apoptotic cells indicate extensive activity occurred within the cell over a time period of only seconds. Morphological changes of apoptotic cells were observed optically in real time, which correlates with the activity observed acoustically. Therefore in addition to surface variations, the interior of the cell also undergoes extensive structural changes during apoptosis. The variation in ultrasound backscatter but not attenuation is expected since backscatter is primarily influenced by changes in structure whereas attenuation by changes in composition. In this work, we have been able to measure these variations in mechanical properties using acoustic microscopy.

#### ACKNOWLEDGEMENTS

This research was undertaken, in part, thanks to funding from the Canada Research Chairs Program, Canada Foundation for Innovation, the Ontario Ministry of Research and Innovation and Ryerson University. The authors would like to acknowledge Min Rui, Eike Weiss and Arthur Worthington for technical support during this study.

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