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● *Original Contribution*

A MODEL BASED UPON PSEUDO REGULAR SPACING OF CELLS COMBINED WITH THE RANDOMISATION OF THE NUCLEI CAN EXPLAIN THE SIGNIFICANT CHANGES IN HIGH-FREQUENCY ULTRASOUND SIGNALS DURING APOPTOSIS

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Abstract—Recent ultrasound (US) experiments on packed myeloid leukaemia cells have shown that, at frequencies from 32 to 40 MHz, significant increases of signal amplitude were observed during apoptosis. This paper is an attempt to explain these signal increases based upon a simulation of the backscattered signals from the cells nuclei. The simulation is an expansion of work in which a condensed sample of cells, with fairly regular sizes, could be considered as an imperfect crystal. Thus, destructive interference could occur and this would be observed as a large reduced value of backscattered signals compared with the values obtained from a similar, but random, scattering source. This current paper explores the possibility that simple changes in the nuclei, such as their observed condensation or the small loss of nuclei scatterers from cells, could cause a significant increase in the observed backscattered signals. This model indicates that the greater backscattered signals can be explained by further randomisation of the average positions of the scattering sources in each cell. When these "microechoes" are added together, so that the destructive interference is reduced, a large increase in the signal is predicted. The simplified model strongly suggests that much of observed large increases of the backscattered signals could be simply explained by the randomisation of the position of the condensed nuclei during apoptosis, and the destruction of the nuclei could produce further signal amplitude changes due to disruption of the cloud of backscattered waves. (E-mail: hunt@uhnres.utoronto.ca) **© 2002 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Ultrasound microscopy, Apoptosis, Modelling, Simulation.**

INTRODUCTION

For many years, numerous ultrasound (US) groups have explored the complex scattering from tissues whose structures are separated by less than the wavelength of the excitation pulse. These studies had considerable success in the analysis of the backscattered signals for defined, randomised structures. However, a collection of single cells have special properties. In many cases, the cells have similar sizes, thus, if they are packed together, they are not in a random arrangement, but are more organized. A striking finding was observed by Mo and Cobbold (1992), Shung et al. 1984 and Lim and Cobbold (1999), where a "black" region was found in their B-scan backscatter images (ie. loss of signal amplitude) of high concentration, more packed solutions of blood cells (erythrocytes). Logically, they have explained this reduction of the backscattered signals as a result of the more regular separations in the high concentration of red blood cells during laminar flow, plus the scattering properties from cellular clusters. In addition, Sherar et al. (1987) and Berube et al. (1992) were able to show large changes in ultrasound microscopy images of cellular spheroids during various treatments.

Packed cells were further investigated by Hunt *et al.* (1995) using simulations that were closely linked to the physical properties of a focused US source. Normal mammalian cells should not be modelled by a random

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array of mini scatterers[1], but by a more regular distribution. These simulations predicted that there would be a large reduction of the backscattered signals if the scattering sources were regularly spaced. This model also indicates that backscattered signals are very sensitive to the value of the randomisation.

During recent US microscopy studies on apoptosis as a result of chemotherapy treatments by Czarnota et al. (1997, 1999), and Kolios et al. (1999), highly significant changes have been observed both in their backscattering amplitudes and in their frequency spectra. Their studies also strongly support the model that the nucleus is the major scattering source of these cells. What is novel in these experiments is that the backscattered signal from the cells shows significant kinetic changes during various stages of the apoptotic process in the same samples. The question posed in this paper is if the backscattered scattered signals from fairly regularly spaced healthy cells can be greatly increased by the more random position of the nuclei during their simple condensation and fragmentation during apoptosis.

Most previous backscattering studies were based upon complex amplitude and frequency signals, and linked to randomised structures below the resolution of the acoustic beam (Mottley and Miller 1990; Insana et al. 1990, 1992; Silverman et al. 1994; Lizzi et al. 1986; Molthen et al. 1998). Lizzi (1996) described the theories in the US spectrum analysis. Extension of these models to using quasiperiod spacing of the scatterers with non-Rayleigh statistics has been explored (Shankar et al. 1996; Shankar 2000) with good success in distinguishing between benign and malignant tissues. These approaches have considerable potential, but they do not seem to have a close link between the physical changes that occur inside the cells and the statistical analysis. This paper combines the fairly regular spacing of packed cells (Hunt et al. 1995) with the observed physical changes of the nuclei. Thus, if there is an ensemble of cells with regular separations between scatterers, the backscattered signals will be small. Packed cells can be thought of like an imperfect crystal. Subtle changes in the cells can be amplified by distorting the ensemble of microechoes detected by the acoustic receiver.

Earlier computer simulations carried out by Bamber and Dickinson (1980), Foster et al. (1983), Bly et al. (1986) and Kerr and Hunt (1992), considered randomised scatterers whose separations are much smaller than the acoustic wavelength, λ. These simulations were able to show a close relationship between the observed speckled B-scan images and the beam produced by the focused acoustic source (*i.e.*, the beam width, the excitation pulse, and the transducer geometries). The simulated images closely agreed with the observed images for different acoustic beam features. These new simulations are designed to evaluate which subtle physical changes in the cells may have major effects in the backscattered data.

This paper is an expansion of the earlier simulation paper by Hunt et al. (1995), in which fairly regular-spaced cells were simulated, but now with changes in the scattering properties inside the cells that could explain the large, backscattered signals during treatments that cause apoptosis.

BACKGROUND

During backscattering studies using focused 40- and 32-MHz beams (Czarnota et al. 1999), chemotherapy treatments showed changes in the nuclei including their condensation, and their fragmentation into smaller pieces. Although observing histological changes, they observed only small changes in the cell average diameter during the treatment. It appears that a major factor producing the observed backscattered signal changes is linked with the scattering from the cell nuclei, because digestion of the DNA with DNAse greatly reduces the backscattered signals (Czarnota et al. 1999). As noted in the previous section, highly significant changes occur in the nuclei sizes and positions inside the cells. These appear to be linked closely to the backscatter signals. Using scanning acoustic microscopy (SAM) operating at 100–200 MHz, nuclei were clearly observed inside the endothelial cells (Saijo et al. 2000). Other studies using an atomic force microscopy system have shown large elastic differences between the nucleus and the cell body (Mathur et al. 2000; Sato et al. 2000). Other investigators have also explored cytoskeleton and elasticity changes during apoptosis processes (DeMeester et al. 1998). These studies are in general agreement with our model that the nuclei may be a major source of the scattering. Therefore, this paper will explore the major factors that may produce such large signal changes during apoptosis, by using a detailed simulation model related to the scattering from the nucleus.

In a previous paper (Hunt et al. 1995), the fairly regular scatterers were used to mimic the average diameter of the cells. A single scattering point in the centre of each cell was modelled the backscattering signals that varied with different randomisations for the cell positions. When cells are much smaller than the acoustic wavelength and, if there is a regular separation of scatterers, the reflecting signal microechoes interfere so the observed backscattered signal detected from the receiver will be very small. Based upon an extension of previous

[1] The small wavelets from a single cell are considered in this simulation, and these are added together to produce complete waves to form the backscattered signals detected by the receiver. Because the word "wavelets" is often used in a different context in the literature, this paper will define these as "microechoes."

simulation analysis (Hunt et al. 1995), an attempt is made to explain how subtle physical changes of the nucleus (Czarnota et al. 1999) can produce such large changes of the backscattered signals.

To test this postulate, a 2-D simulation of the backscattered signals has been investigated. These simulations are based upon regular and more random distributions of scatterers for A-scan and B-scans, as described in detail by Hunt and colleagues. This paper, an expansion of the previous paper, now considers the nucleus to contain multiple scatterers with different sizes, arrangements and randomisations. This simulation explores our premise that the observed microscopic US images of apoptotic cells in the frequency range from 32 to 40 MHz can be explained by the physical changes of the nuclei linked to its randomisation inside the cells.

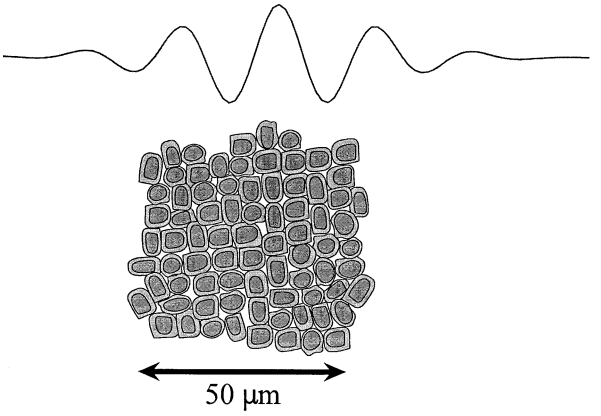


Fig. 1. The relationship between a typical 40.0-MHz pulse and the ensemble of the 6-μm average diameter cells.

EXPERIMENTS

Comparison: the experimental and simulation model

The previous model, based upon simulations described by Hunt et al. (1995), assumes a focused US beam and that each cell consists of a single scattering source. The properties of the US beam are defined by an average frequency, pulse length and beam width akin to spherically shaped, high-frequency transducers (Czarnota et al. 1999; Kolios et al. 1999). The positions of packed cells are assumed to be a result of randomisation, in a linear way, as a fraction of the average cell diameters. As one increases randomisation of the position of scatterers, the backscattered signals increase rapidly (Hunt et al. 1995) and reach the maximum when the scattering distribution becomes random. However, the previous model does not simulate the subtle changes that occur during apoptosis (Czarnota et al. 1997, 1999).

The simulation is based upon packed cultures of acute myeloid leukaemia AML-3 cells. The healthy cells have a fairly regular average diameter (SD = ± 8%), whose nucleus average diameter fills 90% to 70% of the cell diameters. During treatment with cisplatin, apoptotic death occurs. Visually, changes include the condensation of the nucleus to about 40% of the cell average diameter, the breakdown of the nucleus into fragments and the loss of DNA in many cells as time progresses. During these treatments, the US backscatter signal in the frequency range from 32- to 40-MHz had observed increased amplitudes for up to seven-fold. In addition, experiments with DNAse (to digest the DNA) strongly support the premise that DNA is the major backscattering source in the cells (Czarnota et al. 1999).

Simulation design

Our new simulation model is based upon the paper of Hunt et al. (1995) but with the major difference being that the cell does not consist of a single, weak scatterer. Instead, each cell contains multiple scattering sources that mimic the size and position of the nucleus inside the cell. Thus, a large nucleus will have little randomisation in its position inside the cell, but a smaller nucleus will result in increasing the position randomisation. More subtle changes will occur in the phase of signals from different parts of the nucleus. Several simulations have been carried out. The first example mimics the condensation of the nucleus and the fragmentation of the nuclei into smaller pieces, but using the same scattering strengths. Thus, this assumes that the scattering material in the nuclei does not change but, instead, positions of the scatterers change in a defined way. A second example models the random loss of scattering strength in the nuclei. In addition, we have explored the signal amplitudes when different values of 64, 16 or 2 nuclear scatterers are assumed inside each cell. In these experiments, the relative signal amplitudes were comparable and, because the 64 scatterers were found to require too long a computation time, detailed simulations were not performed. The highly simplified model using only two scatterers; one on the front and one on the back of the nucleus, is an excellent way of modelling signals from an intact nucleus, but is not useful for evaluating the fragmentation of the nucleus. Thus, as a compromise, most of the analyses used 16 scatterers per cell, which mimics the crucial aspects of the breakdown of the nucleus. In the simplified model in these simulations, all the arrays have average separations of $< 0.1\lambda$. Figure 1 shows the relation between a typical pulse and 6-μm average diameter cells tightly packed into a sample. In addition, two other models were explored. One assumes a uniform nucleus, where there was a reversed polarisation from the waves from the front to the back of the nucleus, and the second assumes equal scattering from each discrete DNA scattering source.

Table 1. Models used to simulate backscatter signals for packed cells

Models	Scatterer properties	Separation of the scatterers	Changes of the backscattered signal amplitudes	References
Random scatterers	Random positions	Scatterers $\ll \lambda$, $\lll$ resolution volume	Not measured	1, 2
Single scatterer from an ensemble of cells with variable random positions	Scatterers separation various randomisation	Scatterers $\ll \lambda$, $\lll$ resolution volume	Large range of signal amplitudes	3, 4
Clusters of scatterers in variable random positions in an ensemble of cells	Cells containing clusters of miniscatterers	Cells separation $\ll$ to $< \lambda$ Microscatterer separation $\lll \lambda$	Large range of signal amplitudes due to the changes in the cell	Present paper

1–Bamber and Dickinson (1980); 2–Foster et al. (1983); 3-Bly et al. (1986); 4–Hunt et al. (1995).

The general simulation has been designed to better mimic the more complex properties inside the cells. Accurate simulations of complex structures in a nucleus cannot be modelled here. Instead, specific simplifications are made. The differences in the previous papers are summarised (Table 1) and show the specific changes in the model that better mimic the physical properties of the normal and abnormal cells. The basic parameters used in the simulations are as follows:

1. Figure 2 shows the basic properties of the acoustic beams being considered in these simulations.
2. The cells are weak scatterers and it is assumed that this scattering is due to the nuclei. Thus, possible reflections from other components of the cell and from gaps between the cells are disregarded.
3. A value of 1.50×10^3 m/s is used as the speed of sound.
4. Attenuation is ignored.
5. Due to computing limitations, only a 2-D analysis has been considered. A 200-MHz computer required about 4 h to obtain one experimental simulated value.
6. The nucleus is mimicked by an array of miniscatters. The number of scatterers and their properties remains constant as the nucleus is condensed.

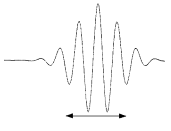
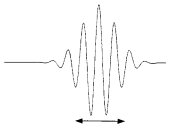
Beam properties	32 MHz		40 MHz	
Beam f/number	4	2	4	2
Effective beam width (FWHM), μm	360	180	290	145
Excitation pulse features				
Pulse length, μm	FWHM ~150		FWHM ~120	
Wavelength, μm	46.9		37.5	

Fig. 2. Simulation parameters.

7. Large changes, such as the loss of the nucleus or a mixture of different cell types, can be modelled in this simulation.

Parameter 6 is important for this simulation. If the nucleus is condensed, the number of scatterers is constant. This assumes that the nucleus is distributed into a tighter distribution or a linear average change of the density. These miniscatterers form the "nucleus" or its "fragments." The actual number of miniscatterers is not crucial in each simulation family (*e.g.*, 16 miniscatterers/cell); what is important is the arrangement of the scatterers in the cell. Of course, in later stages of apoptosis, when the nucleus is slowly digested (*i.e.*, loss of the nuclear regions noted in parameter 7), the loss of the scatterers can also be modelled. Thus, if one has a uniform distribution of cells, the cloud of microechoes backscattered should cancel effectively, and very little net backscatter signals will be observed. However, any change in cell properties will disturb this cloud and will result in significant increases of the signal amplitudes.

Physical changes of the cells that are modelled

As noted earlier, simulated backscattered signals are very sensitive to various values of randomisation for the cell position that produce the destructive interference of the microechoes. Figure 3 shows typical A-scans for the 0%, 5%, 10% and 20% randomisation of central positions of the cell while all other properties are constant. However, the cell line we explored has a very small variability of cell average diameter and there was no significant difference between the normal and apoptotic cells average diameters.

These more complex simulations are an attempt to better mimic the experimental situation. First, there is some randomisation of the cell average diameter. It appears that the SD of the cellular average diameters was approximately 15%, so a range from 10% to 20% was explored. Because the average diameter of the nucleus is much less than the wavelength (λ) of the ultrasound

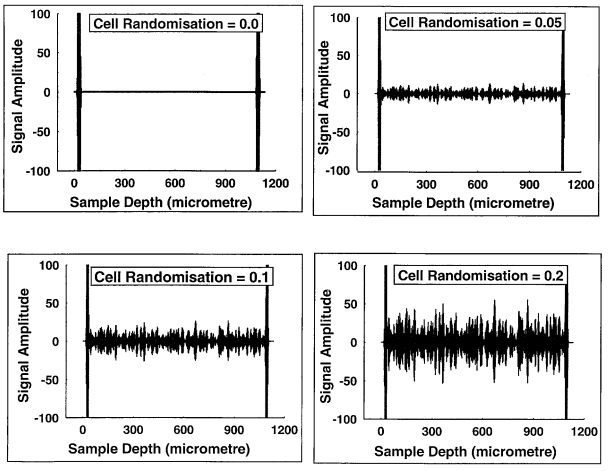


Fig. 3. The simulation for a 40-MHz source for 0%, 5%, 10% and 20% for the randomisation for the cell position, and no randomisation for the nucleus (*i.e.*, the centre of the cell). The intense signals at the beginning and end of the treatment field are related to the building up, and the decay, of the destructive interference effects at the sample boundaries. All of the simulations are selected only in the regions well inside of these boundaries.

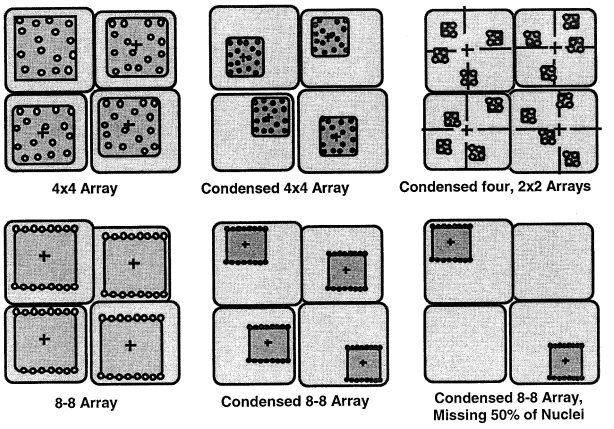


Fig. 4. Two simplified concepts are considered. The top section is for the scattering from 16 sources randomised through the cell, and the lower section assumes a uniform nucleus, with reflection from the front, and also reversed polarity reflection from the back, but higher acoustic impedance for the nucleus; thus, positive. In addition, the loss of the nucleus is also considered, as suggested in the lower section.

beams, one could simplify it as a single scatterer. If a healthy nucleus fills 90 to 70% of the cell, one could allow only 10 to 30% position randomisation within the cell. Similarly, if the nucleus is condensed to 40% of the cell average diameter, a position randomisation of 60% could occur inside the cell. However, a model from a single scatterer will not simulate the physical size of the nucleus, the nucleus fragmentation into smaller pieces, or the fractional loss of the cellular nucleus.

Two ways of simulating the backscattered signals for more complex situations are shown in Fig. 4, in which two different point scatterer arrays may be used to simulate the microechoes scattered from the nucleus. The basic model consists of 16 scatterers that mimic the nucleus. Electron microscopic studies have shown that the nucleus is not uniform, but has dense regions containing the DNA. Thus, simulating the nucleus as a slightly random, 4×4 array would be closer to the real situation. The second model argues that, even though the DNA is not uniform throughout the nucleus, the structure spacings in the nucleus are $\ll \lambda/20$, so that a uniform distribution is a good approximation. According to Huygens's principle, the microechoes can be approximated by one set of tightly separated scatterers at the front face of the nucleus and a second at the back. Note, however, that there is a reversed polarisation of the microechoes at the back of the nucleus. In the limit, both models should produce similar backscatter changes while the nucleus condenses and breaks into small fragments. A more simplified model using four and two scatterers (and allowing much shorter computation times) is not able to mimic breakdown of the nucleus.

Another feature of the experimental observations during chemotherapeutic treatments is that some of the cells appear to be missing their nuclei, although there is an increase the backscattered signals. Therefore, this simulation assumes, in a random way, that an increasing fraction of the cells lose their nuclei. The typical distribution of packed cells is shown in Fig. 5, containing the 40% normal cells and 60% that have lost their nuclei. The loss of the nuclei actually can produce large signal increases because the fairly regular backscatter distribution of microechoes is disturbed. Eventually, when a large fraction of the nuclei scatterers are lost, the amplitude of the backscattered signals is reduced.

Based upon histological studies by Czarnota et al. (1999), Fig. 6 is a schematic model, starting with a normal cell, followed by the apoptosis process with the condensation, fragmentation and digestion of the DNA complex in the nucleus. The second stage denotes the observed changes of the backscattered signal amplitude observed in different experiments. The third stage denotes the simplified model describing the development of the nuclear changes in our simulation model.

RESULTS

Simulation of the signal amplitude produced by 16 miniscatterers as a 4×4 array

The first analysis assumes the simple condensation of the nucleus in the early stages of apoptosis. Figure 7 shows simulated increases of average signal amplitudes

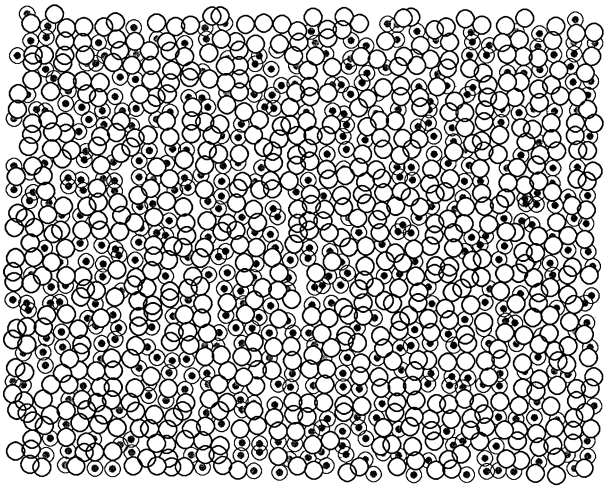


Fig. 5. A typical random array of cells containing (◉) normal cells and others (○) that have lost their nuclei. These arrays are used to simulate the measured average signal amplitudes.

as the nuclei are condensed inside the cells. The average fraction for the nucleus compared to cell average diameter (FNCD) is ≈ 75%, although noted in the *Simulation design* section, normal nuclei diameters can range from 90% to 70% (Czarnota et al. 1999) of the cell diameter 6.0 ± 0.4 μm. The value of FNCD shrinks to ≈ 0.4 in the midstages of the apoptosis in the chemotherapy treatment (≈12 h). Thus, there are three different randomisation values of the cell separation to mimic the different

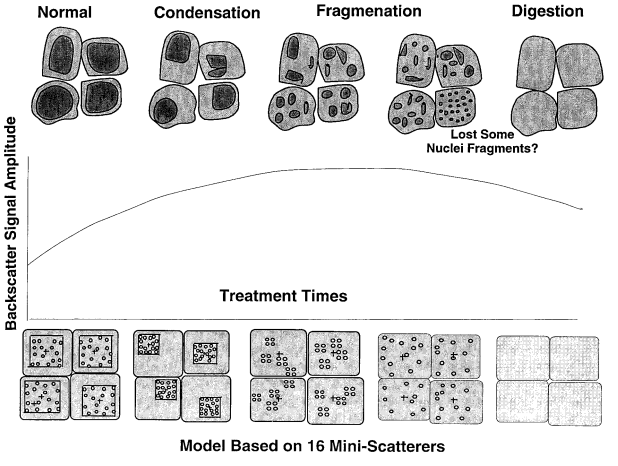


Fig. 6. The top panel is a simplified sketch of observed changes in the cells during apoptosis, showing the nuclear condensation, fragmentation and DNA digestion. The second panel shows typical observed changes. The bottom panel is the simulated arrays of the backscattered signals during the chemotherapeutic treatments, in an attempt to mimic the nuclear changes.

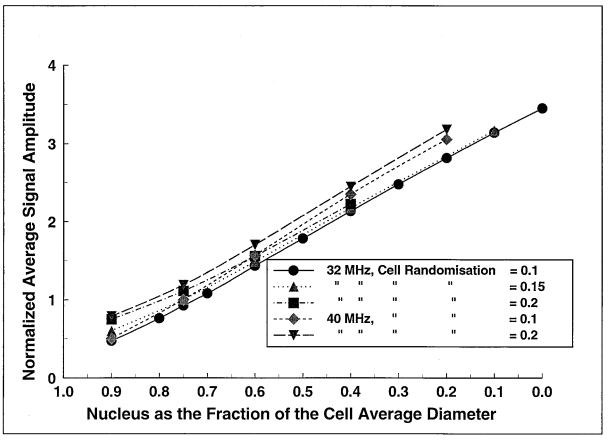


Fig. 7. The changes in simulated signal amplitudes as the nuclei were condensed from a value of 90% to a point scatterer of 0% compared to the normal cell average diameter. The nuclei are randomised throughout the cell. The frequencies of 32 MHz and 40 MHz are simulated, and different randomisation of the central cell positions (10% to 20%). These are normalised to a nucleus filling a fraction of the cell average diameter, 0.75. Note the fairly linear increase of the signal amplitude from a nucleus average diameter from 0.9 to smaller nuclei when the cell position randomisation changed from 10% to 15%, and only significant changes for 20% when the nuclei filled the cell.

cell average diameters in the range from 10% to 20%. As shown in Fig. 7, except for nuclei filling 90% of the cell average diameter, the major changes are insensitive to the random cell separation in the range from 10% to 20%. As expected, the higher frequency of 40 MHz generates a greater slope, but this slope is only 15% greater than generated by the 32-MHz simulations. The simulation indicates that a more important factor is the nuclear average diameter. Our simulation indicates that a modest nuclear condensation produces a very large change in the average signal amplitude. If the normal nucleus, which fills 75% of the cell, is condensed to 40% during the apoptotic treatment, the signal should significantly increase by about 2.4 times for a 32-MHz US source, and by a similar value, 2.3, for a 40-MHz source. Thus, the simulation strongly suggests that, in a fairly regular cellular milieu, any small changes of the nuclear properties should produce very large changes of the backscattered signals.

Should one model the nucleus as multiple scatterers or model the nucleus as a uniform region?

Two models based on Fig. 6 are considered that may best mimic the scattering in the nucleus. The first section mimics 16 single scattering point sources randomly positioned in the nucleus. The second section models for uniform nuclei, with reflections from the front and back faces of these nuclei. Multiple equal and tight-

Table 2. Average backscattered signal amplitudes for the condensed nuclei without and with randomisation of nucleus inside the cells

	Randomised scatterers in a 4 × 4 array		Uniform nucleus as two lines of scatterers as an 8–8 array	
Cell conditions	32 MHz	40 MHz	32 MHz	40 MHz
Healthy cell	1.0	1.0	1.0	1.0
Condensed nuclei to 40% of cell, no randomisation	0.78	0.75	0.79	0.71
Same as above, but nuclei randomisation	4.5	4.7	4.4	4.4

Normalized signal amplitudes from a healthy nucleus average diameter of 90% for the cell average diameter.

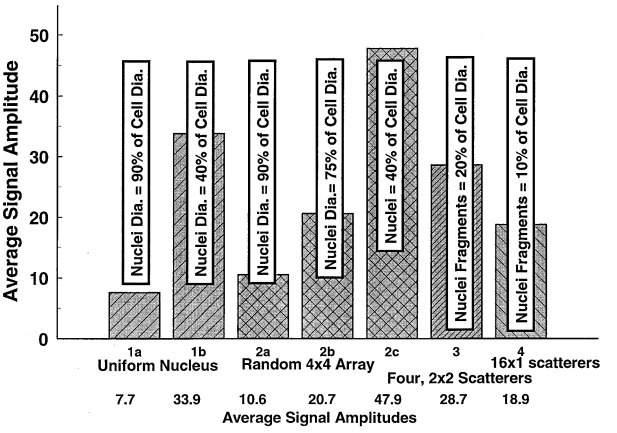


Fig. 8. The comparison of the predicted signal amplitude during two basic models of the condensation and fragmentation of the nucleus. The first two bars are for uniform nuclei; the second three bars are for the condensation of the nucleus containing 16 random scatterers; the third section is of the nucleus broken into four fragments; and the last section is broken into 16 fragments. Note that the largest signals are predicted when the complete nucleus is condensed, and further fragmentation will produce smaller backscattered signals.

ly-spaced scatterers at the front and the back are used to model the scattering microechoes. To compare signal amplitudes from both models, the same number of microechoes is considered. This is called the "8–8 model." As expected, the microechoes from the 8–8 array produce destructive interference between the front and back of the nuclei. This might affect the signals when the nuclei condense. The alternative model, 4 × 4, is of 16 scatterers in a pseudorandom square array[2]. However, as shown in Table 2, the difference between the two models is not large, so either model could explain the results. The lack of significant differences between the signal amplitudes generated in the two models is probably due to the complex summations and subtraction of microechoes from the large number of cells in the resolution volume. However, both models show a lack of sensitivity to the shape of the nuclei. What is important is the maximum randomisation of the nucleus position, which shifts the phase of the microechoes from each cell. The smaller the size of the nucleus, the more randomisation of the position of the cluster of scatterers in the cell, and the more randomisation of the phases.

Based on recent electron microscopy analysis (unpublished data), it is suggested that multiple scattering sources will be closer to a real situation, as modelled in the 4 × 4 array with some randomisation of scatterers in the nucleus. However, the major signal differences are not due to the details of the organization of scatterers in the nucleus but, instead, are due to the randomisation position of the scattering cluster in the nucleus. Figure 7 shows the predicted signal amplitudes for this random array of scatterers during condensation in apoptosis. Large signal increases are predicted as the nuclei condenses. There are only minor differences between the 40-MHz and 32-MHz sources. It appears that the increased freedom of the randomisation of the nuclei position in the cell can explain the large increases for the backscattered signals.

Modelling of the fragmentation of the nucleus

The bar chart in Fig. 8 shows a comparison of the signal amplitudes for the uniform nucleus model, 8–8, and for the random scattering in the nucleus, 4 × 4, for different values of condensation. The uniform nucleus model predicts a slightly smaller signal for the random 4 × 4 array (see the average signal amplitudes in the bottom of the figure). However, for both models, the ratios of the signal amplitudes when the nuclei condense produce similar values. The fragmentation of the nucleus was also modelled by smaller clusters; four 2 × 2 arrays, or 16 × 1 single scatterers. Here, the fragmentation of the nuclei is assumed to be reduced in proportion to the area of the nuclei. If the nucleus, as defined by the 4 × 4 array, is condensed to 40% of the cell width and length, the fragments for four 2 × 2 array should each only have a size 20% of the cell width and length. Thus, more freedom of scattering would occur inside the cell. Figure 8 shows that further breakdown from the condensed

[2] The relative amplitude signals from the 8–8 array that mimics the uniform nuclei are fairly similar to the 4 × 4 models of multiple small scatterers inside the nuclei (see Fig. 3) and for this analysis, act as point scatterers. However, the frequency response is only correct for the 8–8 model where there is reversed polarisation between the front and back of the nuclei. New programmes are being explored that combine both models.

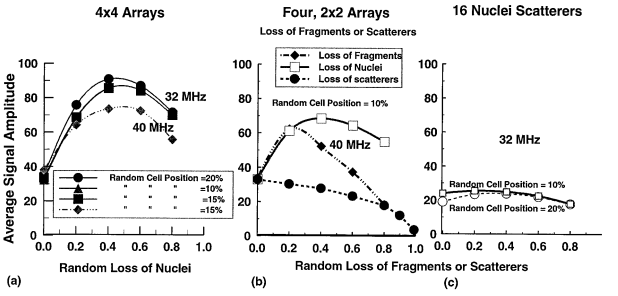


Fig. 9. The predictions of the average signal amplitude if, in a random way throughout the cell, a fraction of the nuclei, or its fragments, have disappeared during apoptosis. (a) 4 × 4 arrays of nucleus; (b) four 2 × 2 fragments of the nucleus, with a fraction of the fragments lost; (c) a fraction of the 16 nuclei fragments have been lost. The smaller signal amplitude is similar for a nucleus totally filling the cell. Note the large signal increase if a fraction of the cells are missing their nuclei, or their fragments, as shown in (a) and (b).

intact nucleus into smaller fragments throughout the cell reduces the signal amplitude. This can be explained by a uniform cloud of microechoes from each cell, and this ensemble of echoes will again produce destructive interference at the US receiver.

Modelling of the random loss of nuclei scatterer sources

Another potential large change of the backscattering signals could occur from the random loss of the scattering source in the cells. This could occur by the digestion of the DNA into small segments or the actual loss of the material out of the cell itself. Figure 9 shows the random total loss of microechoes from nuclei in the cells, or the loss of a fraction of the fragments. Figure 9a is the simulation for a 4 × 4 array of scatterers, with different cell fractions having a loss of nuclei. The simulation predicts a dramatic signal increase (≈ 3 times) even though a small fraction of scattering nuclei is missing. There appear to be two conflicting factors that produce these changes. First, the fairly regular ensemble of microechoes, where strong interference will occur, will be distorted by loss of the scattering nuclei. Thus, the signals will be increased. Second, when a larger fraction of the nuclei scatterers are lost, loss of microechoes become the major factor, and the average signal amplitudes will be reduced.

The loss of nuclei fragments also demonstrates some increase of the signals, but this is much less sensitive. Figure 9b is the case for four fragments of the nuclei, each of 2 × 2 scatterers, where a fraction of the nuclei fragments have lost their scattering properties (for example, are digested), and Fig. 9c is simulated for a random fraction of cells that have total scattering loss.

DISCUSSION AND CONCLUSIONS

Summary of the simulated average amplitude of the backscattered signals

1. The destructive interference of the backscattering from an ensemble of fairly regular spacing of the cells is crucial to explain the large changes of the signals (Fig. 3 based on Hunt et al. 1995).
2. If the normal nuclei, filling 90% of the cell average diameter, are condensed to 40%, we predict a signal increase of up to 4.7 times (Fig. 7). This is in general agreement with experimental results (Czarnota et al. 1997, 1999).
3. This simplified nuclei condensation model is based on the assumption that the backscattered wavelets from each cell have similar scattering values (Experimental section *Simulation design*, parameter 6).
4. For a condensed cell, the random total loss of scattering from specific cells might actually produce a further increase of the signal amplitude of up to 2.6 times (Fig. 8).
5. The model predicts that further breakdown of the nucleus into smaller fragments would reduce the signal amplitude (Fig. 9).

An explanation why randomisation of the nuclei inside the cells can markedly change the signal amplitudes

In the example where there is no loss of scattering from the nucleus, and the wavelength is ≫ greater than cells average diameter, the summation of the multiple microechoes from each cell will be similar (although not identical, due to minor phase differences of the arrays). Practically, this would be the case in which the nuclear changes are linked to fluid loss inside the nuclei. Thus, the basic acoustic scattering inside the nuclei does not change, but the same scatterers are just closer packed inside the cell. Thus, this model suggests that the major changes of average backscattered signal amplitudes could be closely linked to the randomisation of the nucleus inside the cell, and these signal amplitude changes can be linked to the randomisation of the average phase shift of the microechoes from each cell. Thus, for normal cells, the nuclei will almost fill the cell, so little spatial randomisation would occur. Therefore, the phases from the microechoes from cell to cell will be more regular and large destructive interference will occur. Therefore, the ensemble of cells will produce a fairly small signal amplitude. As the nuclei condense during apoptosis, the spatial randomisation increases, and also the signal amplitude increases. Most of the signal changes occur along the direction of the beam (*i.e.*, z-axis).

Further changes of backscattered signals will be predicted due to the breakdown of DNA into fragments.

When these small fragments (*i.e.*, 16 single scatterers) are simulated, filling a large fraction of the cell, the average phases from the microechoes from each cell will produce more regular phases from cell to cell, and the signal would be reduced (Fig. 9). Thus, the backscattered signals from a normal cell in which the nucleus fills a large fraction of the cellular space, should have similar values from the small fragments. This is shown in Fig. 9a and c. This simulation is in general agreement with our experimental studies (Czarnota et al. 1997, 1999).

An explanation why a small loss of nuclei scatterers can increase and not decrease the signals

Another factor may considerably change our signals: the potential random loss of all or some of the nuclear material from the cells. There are two conflicting components producing these changes of the signals. From our simulations, if a small fraction of the nuclei are lost, we predict a large signal increase. This is due to the further distortion of the cloud of destructive interference of the microechoes (*i.e.*, in certain aspects, this is a further distortion of the scattering from an imperfect crystal). As shown in Fig. 9, even a 10% random loss of nuclei could produce an increase of the signal by 3 times in certain conditions. However, when there is a greater loss of the nuclei (*i.e.*, $\approx > 50\%$), the number of microechoes will be considerably less, and the average signal amplitude will be less. Thus, it appears that a small disruption of a fairly uniform cloud of microechoes may have the potential of greatly amplifying the signal changes during apoptosis. It is possible, as well, that the heterogeneous distribution of cells at different stages of apoptosis may also show an amplification of the signals.

Simplifications have been made by this model

1. This is a 2-D model. The actual number of cells producing the cluster of microechoes will be considerably larger if a 3-D simulation were carried out. This could decrease the signal difference between normal and apoptotic cells. For practical reasons, (*i.e.*, long computation times), this has not yet been explored. In addition, the cell and the nuclei are assumed to be square, although the differences should be minor.
2. The actual transducers used for recent experiments have a lower f-number (f/2) compared to the models used in these simulations (f/4). The f/2 suggests there will be a larger difference between normal and apoptotic cells. However, simulation for a f/4 was considered as a compromise until a 3-D analysis can be carried out.
3. Many assumptions were made about the physical properties of the cell that could change their backscattering properties. This model assumes that all the scattering occurs in the nucleus, and there is no scattering from other parts of the cell. Because this model assumes that there is a linear relationship between the nucleus average diameter, essentially similar backscattered signals occur for each cell. However, different scattering values could occur from each cell, that are linked to changes in the acoustic properties of the cellular components, such as the speed of sound, stiffness and density. In addition, when intense high-frequency sources are used, nonlinear properties of the tissues may alter the signals.
4. In all the simulations, we assume that the cells are well packed, with no separation between the cells. Practically, there will be gaps between the cells, with an acoustic mismatch between the cells and the medium that must produce some backscattering.
5. The frequency analysis of the simulation signals should agree with experimental analysis. This is being explored at this time.
6. Preliminary evaluations also suggest that changes in the scattering properties from cell to cell could further increase the backscattered signals.

Even though many simplifications were made in this paper, this model is consistent with the experimental data. The model appears to be an excellent starting point to explain how such small physical changes in a cell could produce such large changes of the observed backscattered signals between a normal and apoptotic cell. Further experimental and theoretical evaluations are needed to determine if these basic concepts are correct.

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