Modelling of the Ultrasound Return from Albunex® Microspheres

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Abstract

A mathematical model that predicts the changes to the ultrasound frequency spectrum after passing through human tissue and Albunex® (a registered trademark of Molecular Biosystems Inc., San Diego, CA) microspheres is proposed. Changes in backscattered intensity and mean frequency of the reflected signal can be estimated as a function of imaging geometry, ultrasound frequency, and microsphere concentration and size distribution.

An important result is that the frequency shifts and the intensity variations are caused both by the microspheres in the path between the transducer and the region of interest, and by the reflection properties of the contrast agent in the region of interest.

The model enables one to explain and predict clinically observed intensity effects such as the shadow effect in the right ventricle, and the fact that received intensity increases with concentration up to a certain point where it falls off.

The effect of the removal of the larger microspheres in the lungs is so significant that concentrations that give positive frequency shifts with increasing concentration in the right ventricle will give negative shifts in the left ventricle. The frequency shift is only a good indicator of concentration for ultrasound frequencies below 4 MHz, while the intensity is very sensitive to Albunex® microsphere concentration and imaged depth also at the higher frequencies.

It is also found that the periodic variation in heart muscle thickness during the heart cycle will affect the intensity and mean frequency of the backscattered ultrasound observed in the left ventricle significantly, and in such a way as to give them a periodic variation over the heart cycle.

Suggested keywords:
medical ultrasound, contrast agent, Albunex®, radio frequency analysis, backscattering, frequency shift

1.0 Introduction

Enhanced ultrasound contrast due to small gas bubbles has been observed for almost as long as medical ultrasound equipment has been used clinically. The ultrasound contrast was first made by hand agitation of a liquid. More recently this has been replaced by sonification since this produces smaller bubbles with a more uniform size. Albunex® (a registered trademark of Molecular Biosystems Inc., San Diego, CA) is such an ultrasound contrast agent, produced by sonification of human serum albumin. It offers the advantage of a known size distribution, microspheres small enough for transpulmonary passage, and excellent storage stability.

Increased intensity in ultrasound images is the most striking effect of contrast. This effect has been utilized in several clinical studies [8], and it has been analysed using videodensitometric methods [4]. With the increased quality of the contrast agent, one has started using more advanced methods for detecting presence of contrast. They are based on analysis of digital radiofrequency (RF) data obtained directly from the front-end of the ultrasound equipment. RF-data opens up opportunities for analysis of changes in the frequency spectrum of the returned ultrasound signal.

So far only a few groups have reported such data. In [7] a frequency downshift in the ultrasound spectrum was reported. We have mainly observed upshifts in our analysis [9] of data recorded after intravenous injection of Albunex®. The best results were obtained for backscatter from blood in the ventricles. The data was recorded on a continuous basis with tracking of several heart cycles. In this way the changes with respect to the pressure variation during the heart cycle is shown. The ultrasound frequencies used were 2.35 and 3.25 MHz.

An example of frequency shifts is shown in Fig. 1. The mean frequency is estimated from data sampled at 10.67 MHz from a region of interest starting at a depth of 5.1 cm. The processing consists of extracting 128 samples (corresponding to a depth span of 0.92 cm) which are Hamming-windowed, Fourier transformed using an FFT, and then the power spectrum is found. The power spectra are averaged over 10

neighbouring beams (approximately 4.5 degrees or 0.43 cm in the lateral direction) and 5 consecutive frames are averaged in time (approximately 0.15 seconds). The mean frequency is found from the first order moment of the averaged power spectrum. The relatively large amount of averaging is necessary in order to get stable enough mean frequency estimates. The figure shows that the left ventricle mean frequency increases during active in-flow (before P-wave), fluctuates somewhat as pressure increases (between S- and T-waves), and then falls off (T-wave). The time for the fall-off relative to the ECG seems to vary somewhat from heart cycle to heart cycle. Although not shown in the figure, the intensity also shows an increase during active in-flow, but often falls off and rises again several times during the pressure phase.

1.1 Proposed Model

The purpose of the development of the mathematical model reported here is to create a tool for checking hypotheses on how intensity and frequency changes occur, and to learn how changes in various parameters affect the backscattered ultrasound signal. Such a model depends on reproducibility of the acoustic properties of the contrast agent. The main parameter to control is the size distribution. Since it is now possible to produce microspheres with a reproducible and stable size distribution, one has control of the acoustic absorption and reflection properties of the contrast agent. These properties have been measured in [5] and [6]. In [10] and [11] this has been taken one step further in that models for attenuation (i.e. absorption and scattering), and reflection have been proposed, and good agreement with measurements have been found.

In this work the attenuation and reflection models of [10] and [11] are incorporated into a model of the whole ultrasound signal path from the transducer through tissue and Albunex® microspheres and reflected back to the transducer. The purpose is to model the effects that the contrast agent has on the received ultrasound signal. Work is in progress to compare predicted values with observed *in-vitro* values.

The model as shown in Fig. 2 contains two parts that interact: a physiological model and an acoustic model. The physiological model (left-hand side) starts with Albunex® being injected in a vein, flowing to the right-hand side of the heart, passing through the lungs where the largest microspheres are filtered out, and then flowing into the left-hand side of the heart, and finally coming to the heart muscle.

The acoustic model (right-hand side) enables one to estimate changes in:

1. Backscattered intensity
2. Mean or modal (peak) frequency of backscattered signal
3. Other spectral shape parameters such as bandwidth (spectral standard deviation), skewness and kurtosis.

These factors depend on:

1. Parameters describing the region of interest, i.e. concentration and size distribution of Albunex® microspheres and pressure
2. Transmitted ultrasound frequency and bandwidth
3. Transmission path between transducer and region of interest, i.e depth of the scatterer, the width of the blood volume containing Albunex® microspheres that the ultrasound signal passes through, and the type of tissue between the scatterer and the transducer

Since the microsphere induced frequency shift is often compared to the Doppler frequency shift, it is useful to compare the rather complex model above to the relatively simple model for the Doppler shift:

$$f_D = 2f_0 v/c \qquad (1)$$

In this case the frequency change depends on:

1. A single parameter describing the region of interest: the velocity, v, of the reflecting blood
2. Transmitted ultrasound frequency, f_0

Thus the frequency shift is not at all affected by the transmission path between the transducer and the region of interest.

The interaction between the transmission path characteristics with the properties of the region of interest is the main reason why it is so difficult to get simple correlations between e.g. microsphere concentration and ultrasound intensity and mean frequency.

1.2 Model Assumptions

The simplest geometry to model is the cardiac long-axis apical view and that is what is done here. The model assumes that the first layer of tissue starting from the transducer (e.g. chest wall and ventricular wall) is homogenous and assigned specific parameters. The next layer can be the interventricular septum containing both muscular tissue, blood and Albunex® microspheres, or the left ventricle which contains blood and microspheres. Finally the model assigns parameters to describe the backscattering in the region of interest.

It is assumed here that the microspheres are present in the region of interest. Furthermore it is assumed that the backscattering is primarily determined by the contrast agent and that the backscattering from the tissue and the blood is negligible. This makes the model, at this stage of development, best suited for modelling ultrasound backscattering from the blood volume in the ventricles. In the ventricles one can safely assume that backscattering from the Albunex® microspheres dominate over the backscattering from the blood cells.

Other assumptions are that the Albunex® microspheres are homogeneously distributed in the blood volumes in the right and left ventricles, and that the size of the capillaries in the lungs is the only factor affecting the size distribution as the microspheres pass from the right to the left side of the heart. These assumptions are not verified clinically, but they are used anyhow because they result in a model which is tractable and which gives results that are correlated to clinically obtained results.

Despite the limitations, the model gives results that represent a first iteration on understanding the interaction of ultrasound signals and Albunex® microspheres in a clinically relevant setting.

In the next section the acoustic model is described in more detail. In section 3, typical geometries from the apical view of the left and right ventricles are modelled.

2.0 Acoustic Model

The model starts with a source spectrum generated by the ultrasound transducer. It is modified by the frequency-dependent attenuation of the tissue and the microspheres. In the region of interest the backscatter characteristics of the microspheres also modify the spectrum. Each of the factors that affect the intensity and the spectrum of the received signal are discussed here.

2.1 Attenuation through Tissue

The absorption loss is modelled as exponentially decaying with frequency, and with constants depending on the type of tissue [2]. The total attenuation depends on the distance the signals travel through:

$$I(d) = I(0)\, e^{-\alpha f^{\beta} d} \qquad (1)$$

I(0) is the intensity of the incident wave and *I(d)* is the intensity at depth *d*, α and β define the frequency dependence of the attenuation. Values for α and β for different soft tissues are given in [2]. A rule of thumb for average tissue is $\beta = 1$ (i.e. linearly increasing attenuation with frequency) and $\alpha = 0.5$ dB/cm/MHz, while for blood the values $\beta = 1.3$ and $\alpha = 0.1$ dB/cm/MHz are used [1]. The frequency dependency of the attenuation accounts for the decrease in mean frequency with increasing depth encountered in normal ultrasound imaging. This effect is also evident in some of the simulation results presented later.

2.2 Microsphere Size Distribution and Concentration

The size distribution of diluted Albunex® microspheres in water (concentration 1:10000) is taken directly from measurements. Filtering through the lungs is modelled according to the size distribution of pulmonary capillary segment diameters from [3] and [11]. Although the number of lung capillaries differs from subject to subject, the distribution of capillary sizes is assumed to be the same. The effect of the filtering is that microspheres of diameter 3-4 μm will pass the lungs with a 90%

probability. The probability to pass is 50% for microspheres of sizes 6-7 μm, and only 10% for sizes 10 μm. An original size distribution as expected in the right ventricle, and a filtered size distribution characteristic of the left ventricle are shown in Fig. 3.

The size distribution may change somewhat due to changes in the volume, *V*, of the gas bubbles, i.e. when the pressure, *p*, changes. Isothermal compression of the gas bubbles implies:

$$pV = (p + \Delta p)\ (V + \Delta V) \tag{2}$$

This is an approximation since the thin shell causes the volume to increase less with pressure than predicted from isothermal compression. In the model the above relation may be used to calculate the size distribution when the pressure changes, or the size distribution may be left unchanged.

Except for thermal compression, not enough is known about how the size distribution changes with pressure. It has however been observed that the number of large bubbles (diameter in the range 5-10 μm) is reduced more than the number of smaller bubbles after an increase in the pressure of 200-250 mmHg. In the simulations this can be accounted for either by changing the concentration, or by changing the size distribution during the heart cycle.

2.3 Attenuation through Tissue and Microspheres

The theory for absorption and scattering by microspheres is presented in [10], and the intensity of the acoustic field after passing through a cavity of thickness *d* is shown to be:

$$I(d) = \int_0^\infty e^{-\sigma_e(r)\, n(r)\, d} I(0)\, dr \tag{3}$$

where *I(0)* is incident intensity, *n(r)* is the concentration of scatterers with radius *r*, and σ_e is the extinction cross section. Data for the extinction cross section is found in

[10] and [11]. Attenuation through tissue or blood (1) is added to get the complete attenuation through Albunex® microspheres in this layer. Examples of the total absorption through a layer consisting of microspheres in blood is shown in Fig. 4 for both the right and left ventricles. Note that there is a major difference between the absorption characteristics caused by removal of the larger microspheres in the lungs. It should also be noted that above about 4 MHz the absorption characteristics flattens, implying that the layer of microspheres will only affect intensity and not frequency content of the passing ultrasound above this frequency.

2.4 Scattering from Albunex Microspheres

The scattered intensity from Albunex® microspheres is modelled in the same way as attenuation [11]. It is shown that the total scattered intensity from a small volume element of surface *S* and length *dz*, containing scatterers homogeneously distributed is:

$$dI_S = I_t \cdot dz \cdot \int_0^\infty \sigma_s(r) n(r) dr \qquad (4)$$

where $\sigma_s(r)$ is the scattering cross section and I_t is the total intensity passing through the surface. The length of the volume element is given by the length of the ultrasound pulse, which depends on the transducer bandwidth.

Examples of scattering for both the right and left ventricle are shown in Fig. 5 using data for $\sigma_s(r)$ from [11]. The curves show scattered intensity relative to applied intensity. It should be noted that the backscatter curves are essentially flat above 3 MHz. This implies that the backscatter will only affect intensity and not frequency content of the backscattered ultrasound above this frequency.

2.5 Received Spectrum and Parameter Estimation

The received spectrum is obtained by adding the source spectrum, the absorption losses and the scattering intensity in a logarithmic scale. An example using a

Gaussian input spectrum with centre frequency 3.07 MHz and relative -6 dB two-way bandwidth of 37% (52% one-way), is shown in Fig. 6 (curve a). This corresponds to measured values for the transducer used for the clinical trials. Fig. 6 also shows the two-way absorption in the first layer consisting of 2 cm tissue (b), the two-way absorption in the second layer consisting of Albunex® microspheres in 2 cm of blood (c), and the backscatter from the microspheres (d). The resulting received spectrum (e) is obtained as a sum of the source spectrum, the absorption functions and the backscatter, and shows a small downshift in frequency compared to the source spectrum. The filtered size distribution of Fig. 3 is used, scaled up by a factor of three. The scaling is done in order to achieve a concentration similar to what is expected in the ventricles with intravenous injection. It is mainly the absorption characteristics of the first layer of tissue and that of the second layer of microspheres in blood that cause the downshift in frequency to 2.95 MHz, a frequency shift of 120 kHz.

The received spectrum is analysed with respect to the spectral moments, m_n, of the returned spectrum, $S(f)$:

$$m_n = \int f^n S(f)\,df, \qquad n = 0, 1, \ldots \tag{5}$$

where f is the frequency. The main moments are m_0 which is intensity, m_1 which is related to the mean, and m_2 which is related to spectral bandwidth or standard deviation of the spectrum.

In the mathematical model, samples of the source spectrum are generated synthetically, and multiplied by the transfer functions and the backscatter characteristics (or added in the logarithmic domain). The spectral moments are found by numerical integration over the sampled values.

3.0 Modelling Results

In this section the model is used to simulate conditions resembling those in the left and right ventricles. It is assumed that the concentration of microspheres in the right ventricle is just a diluted suspension of Albunex®. The concentration is nominally 1:10000 dilution of Albunex® in water, but is scaled up with factors in the range 1 to 10 in order to achieve a concentration similar to what is expected in the ventricles with intravenously injected doses in the range 0.08 - 0.22 ml/kg. In the left ventricle it is assumed that the concentration and size distribution of microspheres is similar to that obtained after simulated lung filtering of these diluted suspensions of Albunex® microspheres.

Initially it was expected that the received mean integrated backscatter, intensity, and mean frequency would vary with:

- Isothermal compression.
- Scaling of the concentration.
- Filtering of the size distribution.

However, it was found that changes in the size distribution due to isothermal compression as in equation (2), hardly changes the attenuation or the backscatter, and the received signals are practically unchanged. This holds for pressures corresponding to those in the left ventricle during the heart period (10 - 130 mmHg). Isothermal compression is therefore neglected in the simulations.

3.1 The Nature of the Contrast Agent Frequency Shift

Fig. 7 shows the result of scaling the nominal size distribution in the left ventricle by factors of 5 and 10. The assumed geometry is a first layer of 2 cm tissue, and a second layer of 2 cm microspheres in blood. Curve b) shows how the attenuation in the second layer increases with increased concentration. Curve c) shows that the backscatter also increases. The final result is curve d) which shows how the received spectrum is shifted to lower frequencies as the concentration increases. This reflects

itself in a down-shift in mean frequency. The intensity scale is relative to the transmitted spectrum, i.e. the source spectrum's peak is 0 dB.

It is a common misunderstanding that the frequency shift associated with the Albunex® contrast agent and that of conventional Doppler are similar in nature. This is however not the case as there is a large difference in the spectra transmitted. In the Doppler technique, a narrow line-spectrum is transmitted (i.e. a relatively long pulse) and that line is shifted due to the velocity. Thus, in this modality, a new frequency is created. Contrast agents are used with a wide spectrum (i.e. short pulses typical of B-mode imaging), and no new frequencies are created as shown in Fig. 7. The effect is only that the centre of gravity of the spectrum is tilted towards lower or higher frequencies.

3.2 Modeling of the Left Ventricle Return Signal

The results from modelling the left ventricle return signals are shown in Fig. 8. Curve a) shows the nominal size distribution with some of the largest microspheres missing due to the lung filtration. The intensity relative to transmitted intensity as a function of the scaling of the concentration is shown in curve 8b). The logarithmic abscissa shows clearly that as the concentration increases, the backscattered intensity also increases up to a certain point, where it starts to fall off. Theoretically the scattering from the microspheres increases linearly with concentration (3), and the attenuation increases exponentially (4). Therefore the received intensity from the microspheres will increase linearly with concentration up to a certain limit, then decrease exponentially. This is also consistent with typical systems for grading the degree of opacification in clinical tests using contrast, i.e. 1 - none, 2 - faint opacification, 3 - suboptimal, 4 - optimal, and 5 - attenuated. Note also that with no transmission path through the contrast agent, there is no attenuation effect, and that the deeper layer 2 is, the more pronounced is the attenuation effect. The intensity curve shows that there are two regions: a low-concentration region where the backscatter characteristics

dominate, and a high-concentration region where the transmission path effect dominates.

The mean frequency shift shown in curve 8c) shows that the frequency falls with increased concentration and with increased depth. When layer 2 (Albunex® microspheres in blood) disappears and there is no transmission through the microspheres, the frequency shift is invariant with concentration. Since the backscatter characteristics is flat above 2.5-3 MHz (Fig. 5), the frequency shift is primarily an effect determined by the microsphere-induced attenuation. The last curve (8d) is equivalent to curve b) except for a linear abscissa and is included to show the correlation between the mean frequency variation (8c) and the intensity variation. The curves are correlated except when there is no transmission path through the microspheres.

3.3 Modeling of the Right Ventricle Return Signal

The result from modelling the right ventricle is shown in Fig. 9. In the intensity curve (curve b), the main difference from the left ventricle results of the previous figure, is that in the high-concentration (scaling factor larger than five) transmission path-dominated region, the attenuation increases to more than 100 dB compared to about 50 dB in the left ventricle. This is consistent with the shadow often observed clinically in the right ventricle. It is an effect caused mainly by the presence of the larger microspheres.

Another important result is that the frequency shift (curve 9c) increases with increased concentration and increased depth. Thus the frequency shift and the intensity have a negative correlation (curves 9c and d). This can be explained by reference to Fig. 4 which shows that for frequencies above 1.5 MHz the attenuation characteristics of filtered microspheres has a negative slope with respect to frequency while the unfiltered microspheres give a positive slope. Since the attenuation characteristics dominate in causing the frequency shift, this explains the positive and negative frequency shifts in the right and left ventricles, respectively. It should be

noted that for high concentrations and 4 cm distance through microspheres in blood, the frequency shift is so high that it can no longer be reliably estimated. Therefore the 4 cm curve in 9c) ends at a normalised concentration of 8.

3.4 Effect of Removing the Larger Microspheres

The difference between the right and left ventricles is also shown in Fig. 10. In these simulations, the nominal size distribution is first scaled by a factor of three. This is believed to be close to concentrations used clinically, and implies that one operates in the high-concentration region where the transmission path effect dominates. The size distribution is then cut-off so that only the microspheres smaller than the cut-off diameter are retained (Fig. 10a). Thus above 10-15 μm cut-off, the size distribution is similar to that in the right-ventricle, and the frequency shift increases with depth. For a cut-off below about 8 μm, the frequency shift falls with depth as in the left ventricle.

One of the hypotheses for what takes place in the left ventricle during the heart cycle is that the size distribution varies with time. Curves c) and d) of Fig. 10 can also be used to find the effect of that. If for instance the smaller microspheres arrive before the larger ones, the frequency shift (curve c) may change from negative to positive with time. The curves for intensity relative to transmitted intensity (curve d) also show that the larger microspheres contribute most to the attenuation.

Curve b) is included to show the effect on the shape of the received spectrum. The three curves are all for a depth of 4 cm and correspond to the smallest and largest cutoff diameters and the one which gives the smallest mean frequency. These curves show that the received spectra are no longer symmetric, but may consist of two peaks with a valley in between. The location of the valley is related to the frequency of maximum attenuation (solid curve of Fig. 4) as it varies with the cutoff diameter. The asymmetric shape of the spectra also indicates that centre frequency estimates from independent trials should be compared with caution since different estimators such as

mean frequency (5), peak frequency, and average of -6 dB frequencies will give different values.

3.5 Effect of Varying the Ultrasound Frequency

It is evident from the attenuation and backscatter characteristics (Fig. 4 and Fig. 5) that as the frequency increases to 4-5 MHz, the curves become almost flat. Thus one would expect that the frequency shifts will be reduced compared to frequencies in the range 2-3 MHz. This is also brought out in Fig. 11, which shows a simulation of frequency shift in the left ventricle at a centre frequency of 4.0 MHz. All other parameters including relative bandwidth are as in Fig. 8. There is only a small variation in centre frequency with concentration and at a depth of 4 cm there is both an upshift and a downshift depending on the concentration. It seems safe to conclude that at these frequencies and above, the frequency shift is not a good indicator of the presence of microspheres. The intensity variation is however comparable to that at lower frequencies.

3.6 Periodic Variation of Mean Frequency Observed Clinically

Several hypothesis for the explanation of the periodic variation of left ventricle mean frequency shown in Fig. 1 can be postulated:

1. Periodic variation in concentration
2. Periodic variation in size distribution
3. Periodic movement of the heart

Let us now examine each of these hypothesis. The first is that the concentration may vary with the heart cycle. According to Fig. 8, an upshift is associated with a decrease in concentration. However, since the upshift occurs at the time of the active in-flow this does not seem to be a likely explanation.

Second, if the size distribution becomes wider (more large microspheres) during in-flow, Fig. 10, shows that there will be an upshift in frequency. If this is followed by a removal of the large microspheres, for instance due to pressure effects, the

frequency will again fall. This seems at first to be a reasonable explanation, but the first upshift will take microspheres larger than about 10 μm to create the upshift. These microspheres are supposedly not present in the left ventricle due to the size of the lung capillaries.

The final hypothesis, that of a periodic movement of the heart combined with a thickening of the heart muscle during the pressure phase seems to be the most likely explanation. As the wall thickens, the size of layer 2 diminishes. According to Fig. 8, this will give an upshift, which depending on the concentration may be up to 300 kHz per cm thickening. This seems fairly consistent with the observed frequency shift.

4.0 Conclusion

It has been shown that the intensity and frequency changes observed from the Albunex® microspheres are complex functions of imaging geometry, contrast agent parameters such as concentration and size distribution, and ultrasound frequency and bandwidth. An important result is that the frequency shifts and the intensity variations are caused both by the microspheres in the path between the transducer and the region of interest, and by the reflection properties of the contrast agent in the region of interest. This is in contrast to the Doppler frequency shift which depends only on the velocity in the region of interest. The consequence is that frequency shifts generated by the presence of microspheres are much harder to interpret than the Doppler shifts which the medical community is so used to interpret.

The model proposed here is able to explain the shadow-effect usually observed in the right ventricle. It is caused by the large concentration of microspheres that attenuates so much that hardly any ultrasound intensity at all passes through. The model is also able to predict at what concentrations this occurs and why it occurs much more seldom in the left than in the right ventricle.

Another result is that an increase in microsphere concentration gives an increase in backscattered intensity up to a certain point where the intensity slowly starts to fall off with increase in concentration. This saturation effect is more pronounced at larger depths, an effect which has also frequently been observed clinically.

The change in size distribution as the microspheres pass through the lungs is so significant that the concentration which gives an upshift in frequency in the left ventricle gives a down-shift in the right ventricle.

The frequency shift method is only of use for ultrasound frequencies below 4 MHz, while the intensity is very sensitive to microsphere concentration and imaged depth also at the higher frequencies. This implies that variations in frequency shift can only be used as an indicator of the presence of Albunex® microspheres at frequencies

typical of cardiac trans-thoracic ultrasound imaging of adults (2.0 - 3.5 MHz), and not for paediatric or trans-oesophageal imaging (5.0 MHz and above). At these higher frequencies only intensity gives a good correlation with presence of microspheres.

The most likely explanation for the periodic frequency shifts observed in-vivo in the left ventricle in the apical view is the periodic movement and thickening of the heart muscle. Due to the dependence on the transmission path, this effect is strong enough to account for several hundreds of kHz frequency shift.

Further research is underway in order to compare the theoretical prediction of the acoustical model with *in-vitro* measurements.

Acknowledgment

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Figure Captions

Fig. 1: Experimentally observed ECG signal and ultrasound mean frequency in the left ventricle. Data is taken from the 4-chamber apical view at depth 5.5 cm, where distance from transducer surface to ventricle cavity varies between 1 and 2 cm.

Fig. 2: Physiological model (left-hand side) and acoustic model (right-hand side) of ultrasound imaging through tissue and Albunex® microspheres.

Fig. 3: Measured size distribution of Albunex® microspheres after 1:10 000 dilution in water is shown in solid line. The dashed line shows the distribution as expected in the left ventricle after simulated lung filtering.

Fig. 4: Estimated attenuation through a 2 cm layer consisting of Albunex® microspheres in blood. Solid curve in the right ventricle, dashed curve in the left ventricle. The size distributions of Fig. 3 are used.

Fig. 5: Estimated backscatter from Albunex® microspheres. Solid curve from the right ventricle, dashed curve from the left ventricle. The size distributions of Fig. 3 are used.

Fig. 6: Illustration of the nature of the frequency shift as the spectrum of the ultrasound source (curve a) is modified by two-way attenuation through tissue (b), two-way attenuation through microspheres in blood in the left ventricle (c), and backscatter characteristics from Albunex microspheres (d). The resulting downshifted received spectrum is curve e).

Fig. 7: Left ventricle simulation showing change in attenuation of 2 cm Albunex® microspheres in blood (b), change in backscatter (c), and change in received spectra (d) with scaling of size distribution (changing concentration). The signals also pass a 2 cm layer of tissue. The numbers in each of the plots are the scaling factors of a lung filtered size distribution (a).

Fig. 8: Left ventricle simulation showing change in received intensity (b: versus a logarithmic, and d: versus a linear concentration) and mean frequency (c) as a function of microsphere concentration at an ultrasound frequency of 3.07 MHz. Distance through tissue is 2.0 cm. Parameter is distance through blood and microspheres.

Fig. 9: Right ventricle simulation showing change in intensity (b: versus a logarithmic, and d: versus a linear concentration) and mean frequency (c) as a function of microsphere concentration at 3.07 MHz. Distance through tissue is 2.0 cm. Parameter is distance through blood and microspheres.

Fig. 10: Simulation showing the effect of removing the larger microspheres. Change in mean frequency (c) and intensity (d) as a function of microsphere size at 3.07 MHz. Only microspheres smaller than the cut-off diameter are kept. Distance through tissue is 2.0 cm. Parameter is distance through blood and microspheres. Curve (b) shows the received spectra at 4 cm for three values of cutoff diameter.

Fig. 11: Left ventricle simulation at an ultrasound frequency of 4.0 MHz showing change in intensity (b: versus a logarithmic, and d: versus a linear concentration) and mean frequency (c) as a function of microsphere concentration. Distance through tissue is 2.0 cm. Parameter is distance through blood and microspheres.

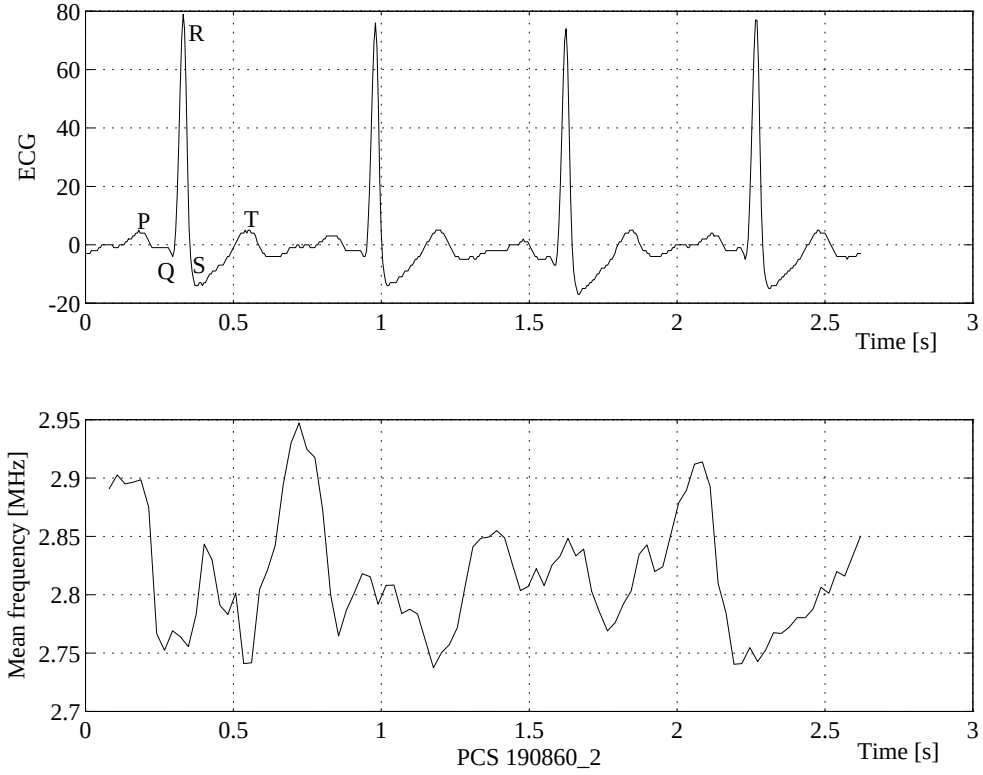

Fig. 1

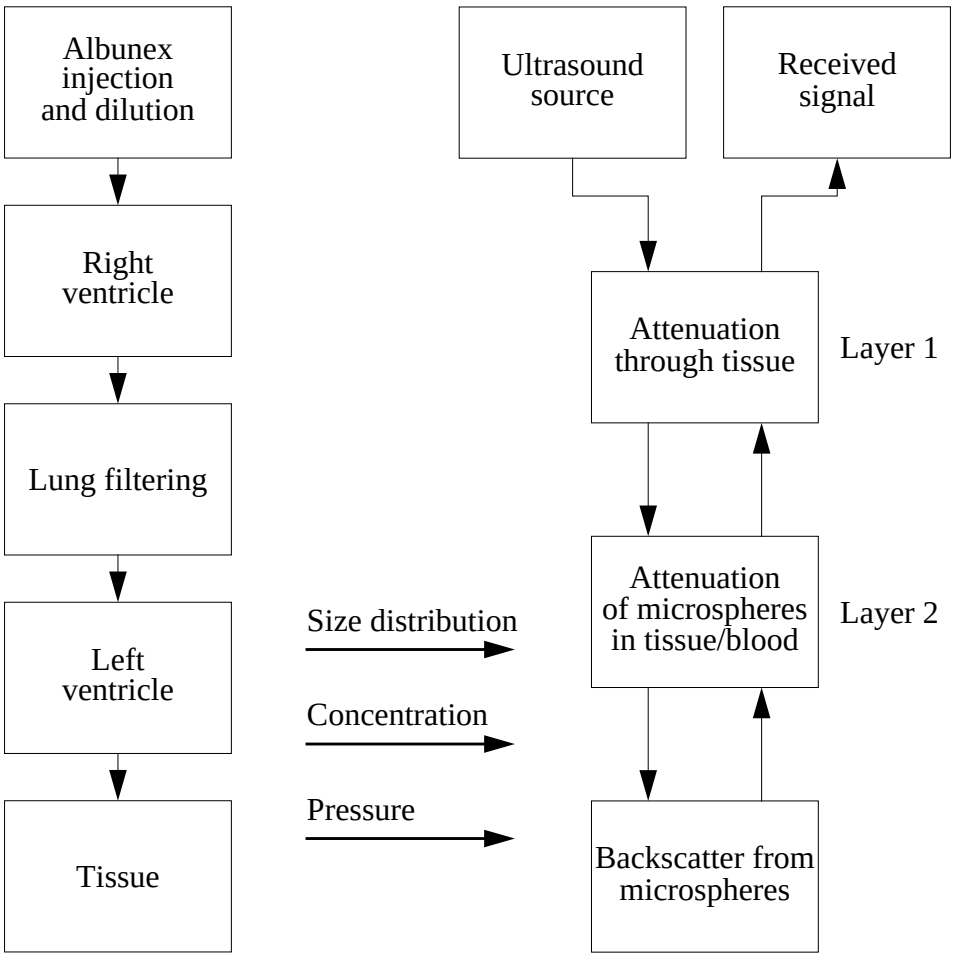
Albunex injection and dilution
Right ventricle
Lung filtering
Left ventricle
Tissue
Size distribution
Concentration
Pressure
Ultrasound source
Received signal
Attenuation through tissue
Layer 1
Attenuation of microspheres in tissue/blood
Layer 2
Backscatter from microspheres

Fig. 2

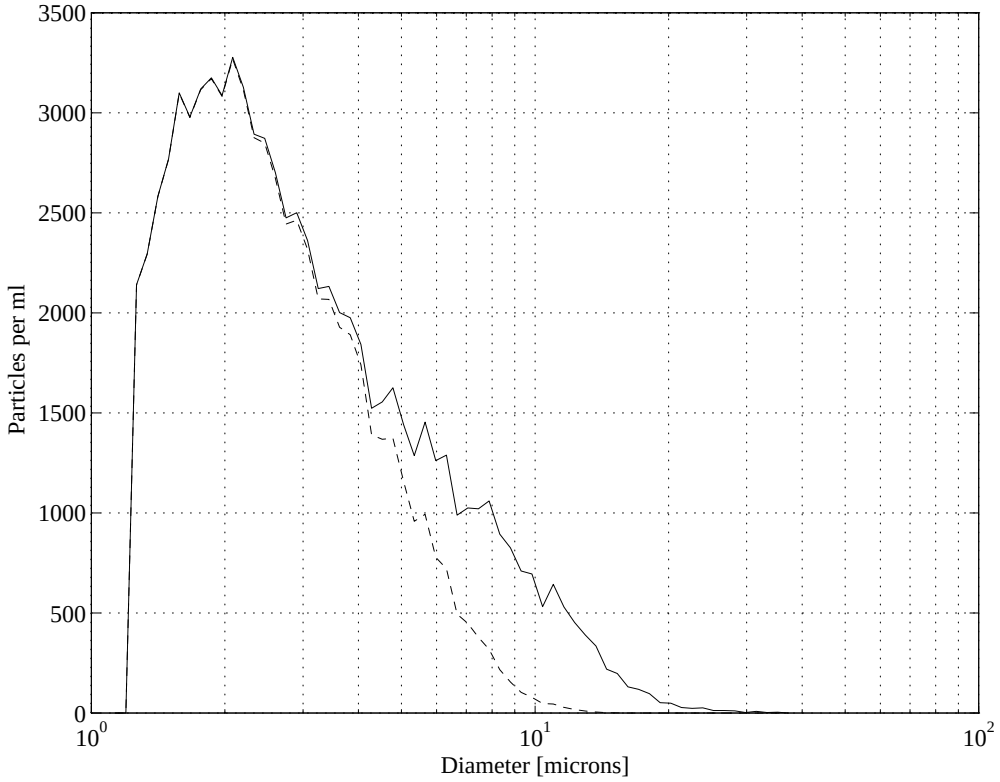
3500
3000
2500
2000
1500
1000
500
0
Particles per ml
10^0
10^1
10^2
Diameter [microns]

Fig. 3

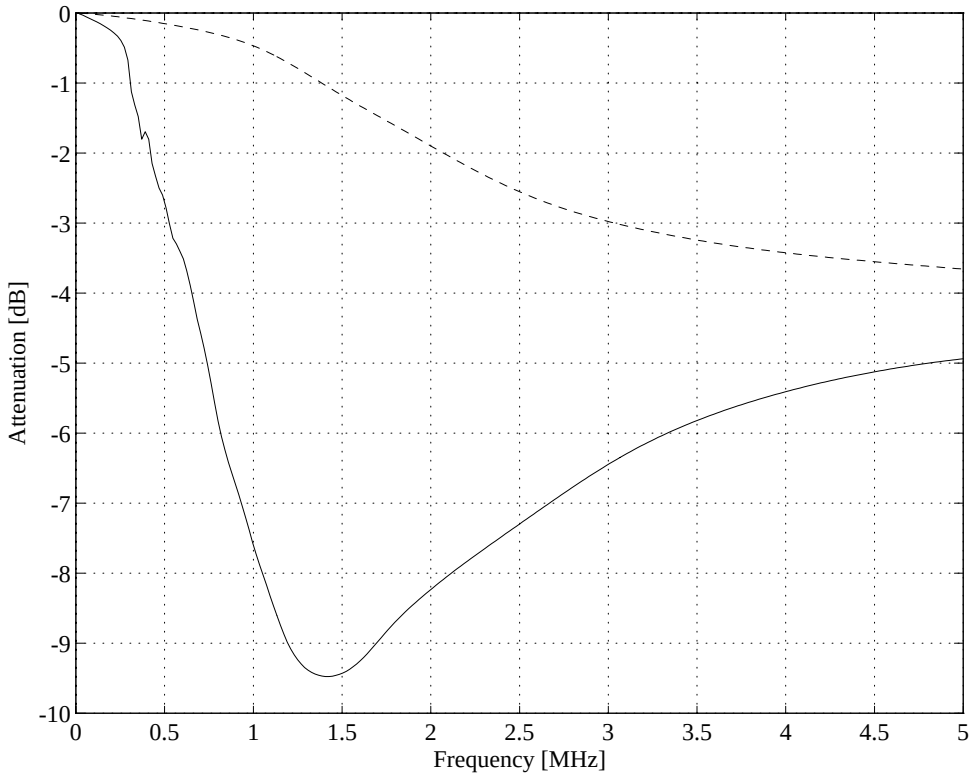
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
Attenuation [dB]
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
Frequency [MHz]

Fig. 4

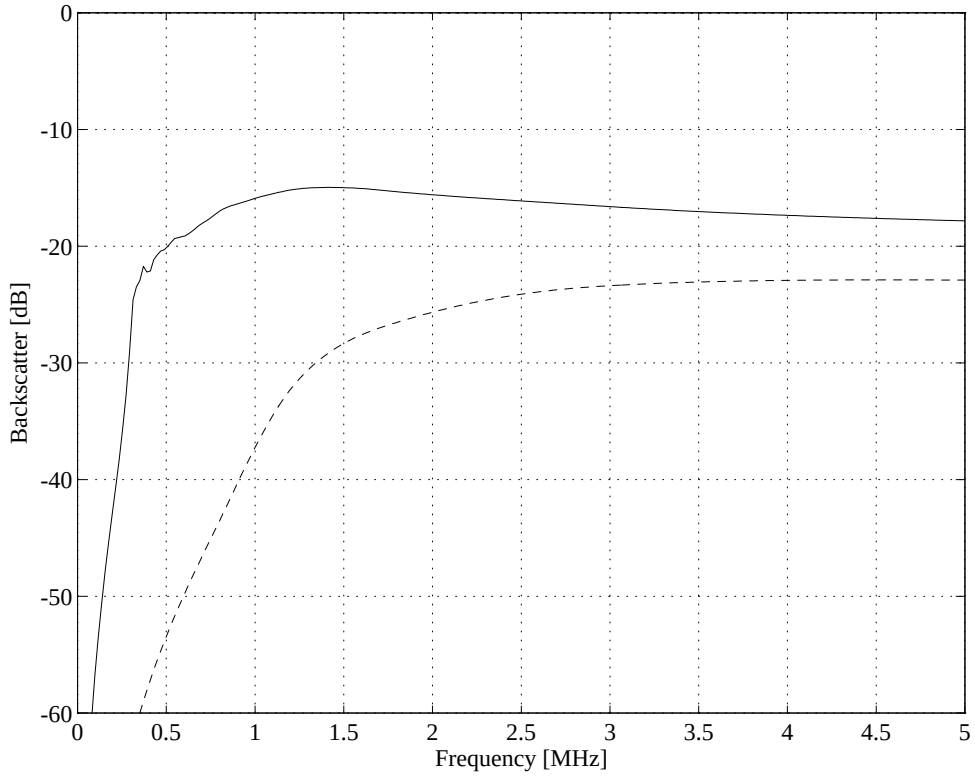
0
-10
-20
-30
-40
-50
-60
Backscatter [dB]
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
Frequency [MHz]

Fig. 5

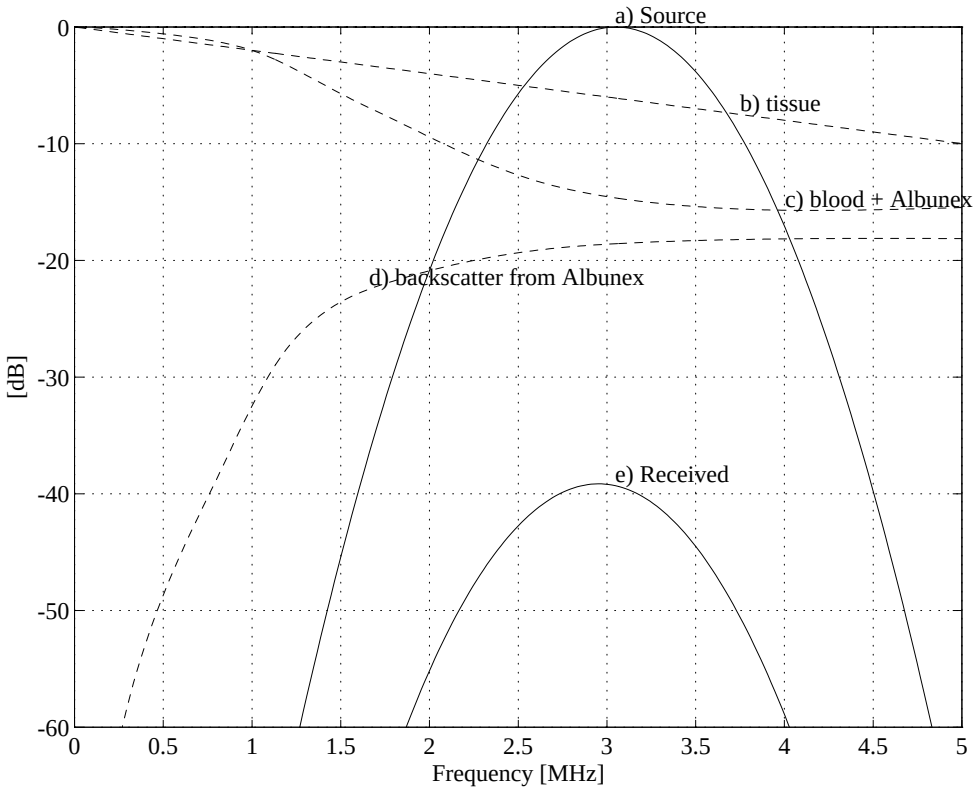
a) Source
b) tissue
c) blood + Albunex
d) backscatter from Albunex
e) Received
[dB]
Frequency [MHz]
0
-10
-20
-30
-40
-50
-60
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5

Fig. 6

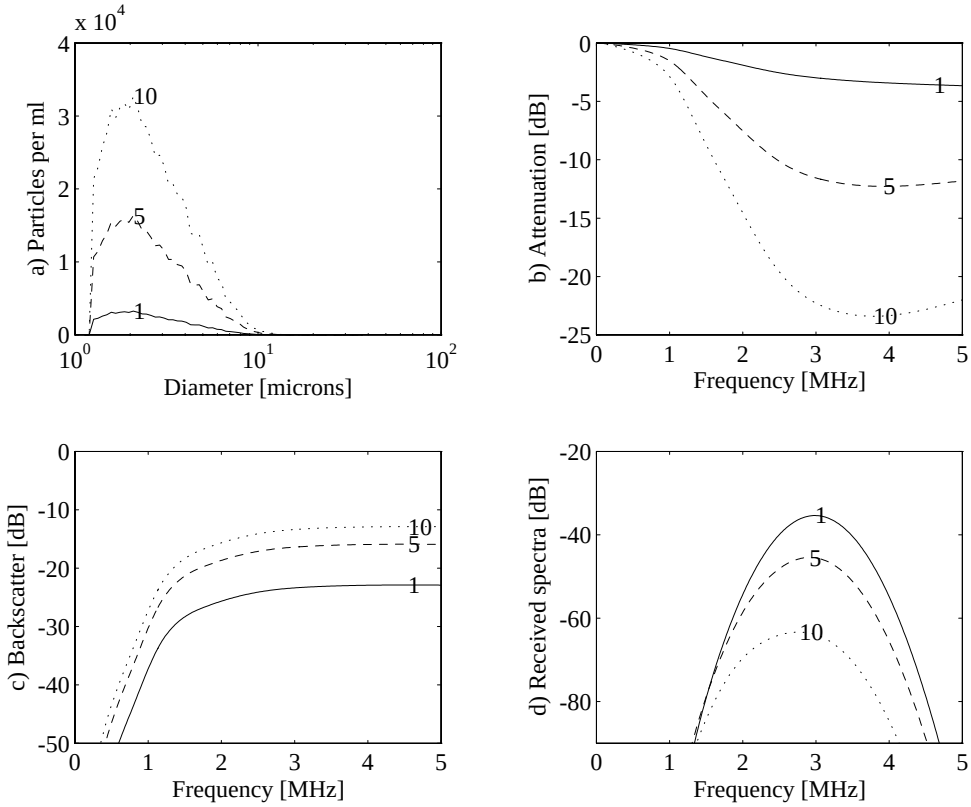
x 10^4
a) Particles per ml
Diameter [microns]
10
5
1
b) Attenuation [dB]
Frequency [MHz]
c) Backscatter [dB]
Frequency [MHz]
d) Received spectra [dB]
Frequency [MHz]

Fig. 7

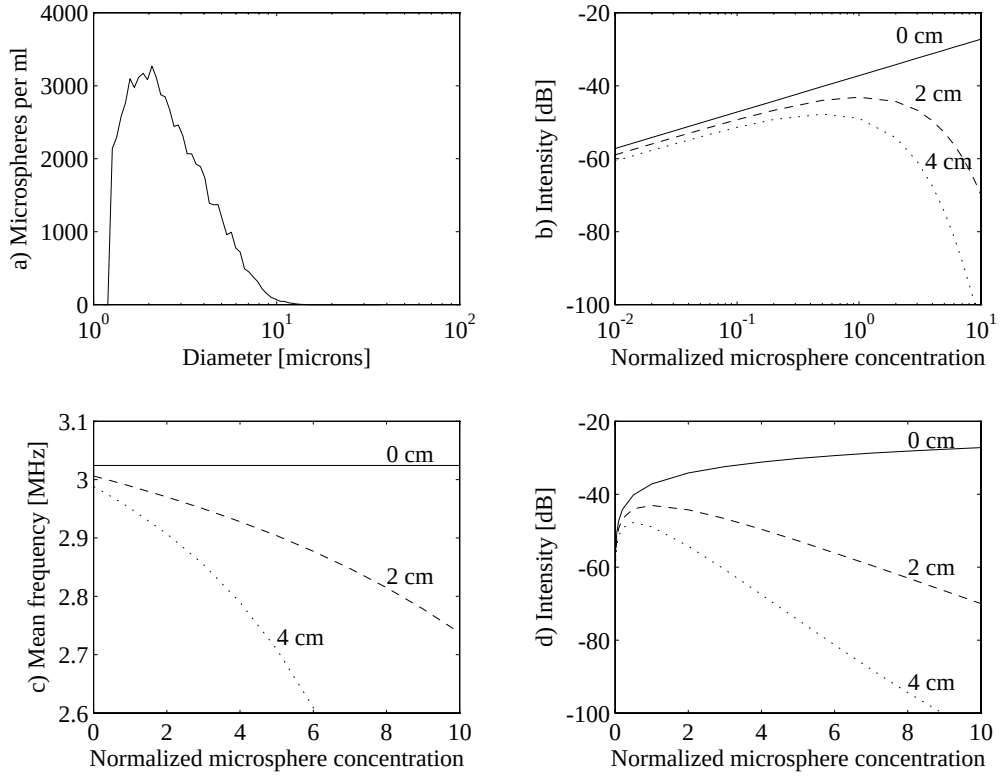
a) Microspheres per ml
Diameter [microns]
b) Intensity [dB]
Normalized microsphere concentration
0 cm
2 cm
4 cm
c) Mean frequency [MHz]
Normalized microsphere concentration
d) Intensity [dB]
Normalized microsphere concentration

Fig. 8

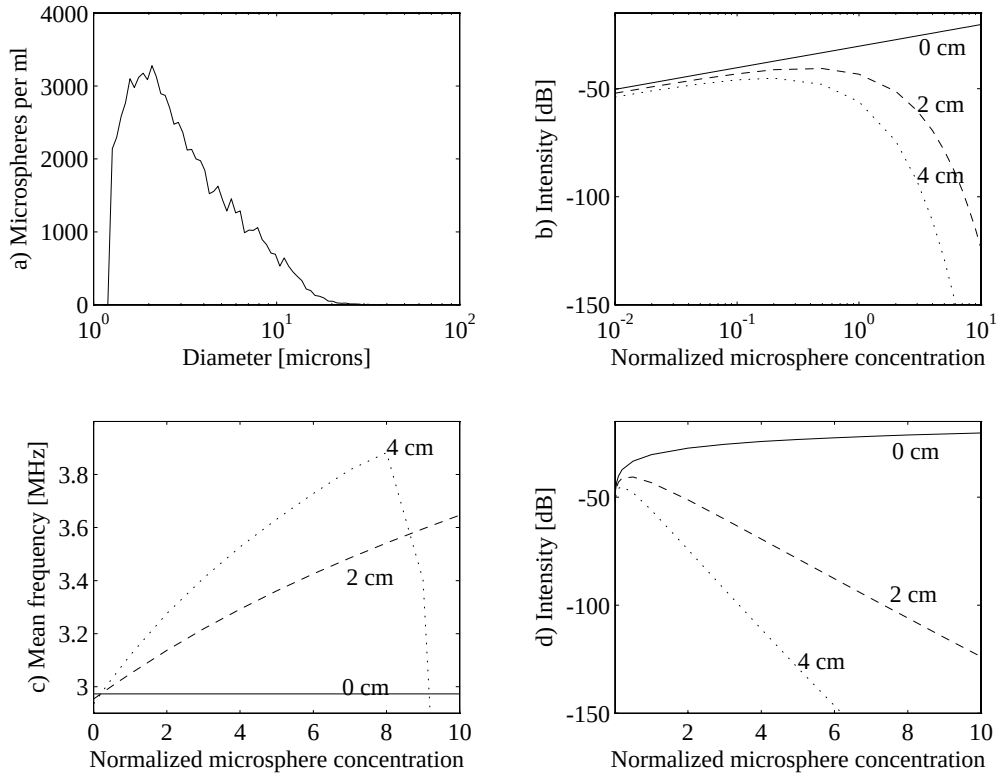
a) Microspheres per ml
Diameter [microns]
b) Intensity [dB]
Normalized microsphere concentration
0 cm
2 cm
4 cm
c) Mean frequency [MHz]
Normalized microsphere concentration
d) Intensity [dB]
Normalized microsphere concentration

Fig. 9

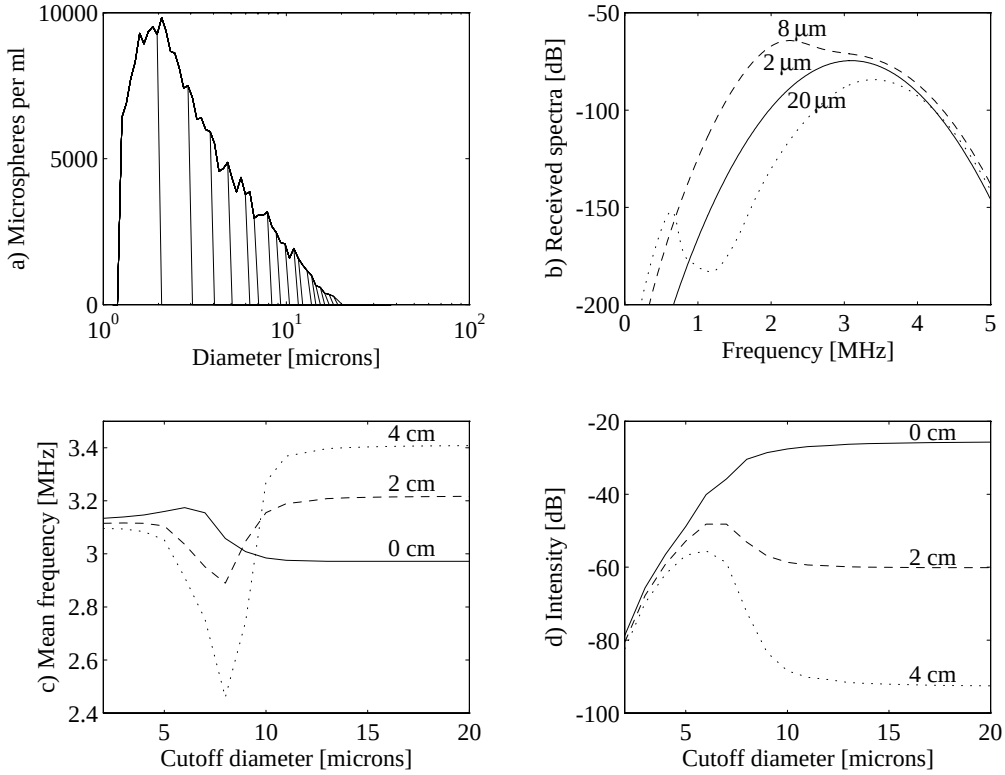
a) Microspheres per ml
10000
5000
0
10^0
10^1
10^2
Diameter [microns]
b) Received spectra [dB]
-50
-100
-150
-200
8 µm
2 µm
20 µm
0
1
2
3
4
5
Frequency [MHz]
c) Mean frequency [MHz]
3.4
3.2
3
2.8
2.6
2.4
4 cm
2 cm
0 cm
5
10
15
20
Cutoff diameter [microns]
d) Intensity [dB]
-20
-40
-60
-80
-100
0 cm
2 cm
4 cm
5
10
15
20
Cutoff diameter [microns]

Fig. 10

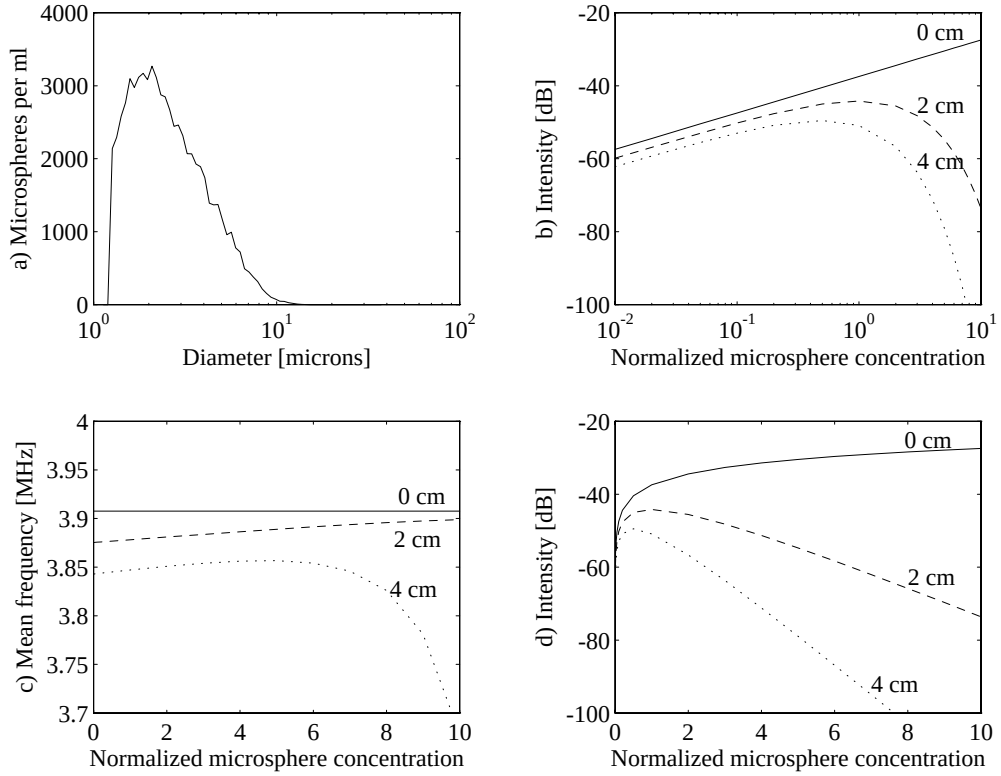
a) Microspheres per ml
Diameter [microns]
b) Intensity [dB]
Normalized microsphere concentration
0 cm
2 cm
4 cm
c) Mean frequency [MHz]
Normalized microsphere concentration
d) Intensity [dB]
Normalized microsphere concentration

Fig. 11