Research article / Article de recherche

SINGLE CELL SIZE ESTIMATION FROM BACKSCATTERED SPECTRUM BY USING SOME WEAK ACOUSTIC SCATTERING APPROXIMATIONS

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ABSTRACT

A method for the sizing of a cell in suspension by ultrasonic means is discussed. The technique uses frequency minima of the backscatter intensity pattern for an acoustically weak scatterer and provides a simple formula for scatterer size estimation in the framework of the Born approximation. The technique has been implemented here to examine performance of the Born approximation and a modified Born approximation in predicting size of a cell in a suspension. This was done by comparing the mean diameter of a cell obtained from optical microscopic measurements over many cells and that determined by employing these approximations only using the minima of measured high-frequency (10-65 MHz) ultrasonic backscatter spectra. Both approximations in estimating size of a scatterer worked with high accuracy (error < 3%) for scatterers like PC-3 cells ($ka \approx 0.55$-3.58) and sea urchin oocytes ($ka \approx 1.54$-10.03) where, k and a are the wave number of the incident wave and scatterer size respectively. This study suggests that this simple method can be used to estimate cell size.

RÉSUMÉ

Une méthode est présentée qui permet d'estimer la dimension d'une cellule par des techniques ultrasoniques. Cette méthode utilise les fréquences minimums dans la courbe de l'intensité de la rétrodiffusion acoustique pour des diffuseurs faibles et fournit une formule simple pour l'estimation de la dimension des diffuseurs dans le cadre de l'approximation de Born. Dans ce travail, on a comparé les résultats de l'approximation Born et une approximation modifiée de ce rapprochement pour l'estimation de la taille des cellules en suspension. Pour cela le diamètre moyen d'ensemble des cellules est obtenu par la microscopie optique. Ensuite ce valeur est comparé par les résultats de calcule en utilisant ces approximations sur les minimums du spectre de la rétrodiffusion acoustique à haute fréquence (10-65 MHz). Ces deux approximations ont montré une grande précision (erreur <3%) pour l'estimation de la dimension dans le cas de diffuseurs acoustiques comme les cellules PC-3 ($ka \approx 0.55$-3.58) et les ovocytes d'oursin ($ka \approx 1.54$-10.03) où les paramètres k et a indiquent respectivement le nombre d'onde pour l'onde incident sur l'objet et le taille du diffuseur acoustique. Cette étude suggère que la méthode proposée ci-dessus peut être utilisée pour estimer la taille des cellules.

1 INTRODUCTION

It was shown in recent publications [1-3] that it is possible to measure the backscatter signal for high frequency (10-65 MHz) ultrasound scattered from an individual cell. These studies examined the frequency dependent backscatter of nucleated and non-nucleated cells with known average size (radius $a < 80$ μm) and an approximately spherical shape. It was also shown that the analytical solution based on the Anderson's fluid sphere model [4] can be used to describe measured backscatter intensity patterns for a nucleated cell (PC-3 cells, a line of human prostate cancer cell with mean radius $a = 13$ μm) with nucleus to cell volume ratio 0.33 and also for a cell without nucleus (sea urchin oocyte with mean radius $a = 37.5$ μm). The mean size of a PC-3 cell and the approximate values of its acoustic properties were given as inputs to the model to generate a theoretical curve describing the cell backscatter assuming that the acoustic properties of the surrounding medium were known. Similarly for sea urchin oocytes its mean size and acoustic properties of the ambient medium were taken as inputs while density and speed of sound within the cell were found from the curve which provided the best fit to the experimental data. In both cases, complex calculations were required to generate the theoretical curves used and properties of both the cell and ambient medium were required to match the theoretical predictions with experimental data. It is believed that proper understanding of scattering process of ultrasound waves at the cellular level would be helpful to develop methods for the analysis of backscattered signals from cell ensembles and consequently to characterize their morphology which could then be related to clinical conditions such as malignancy [5, 6].

In another recent paper [7] it was shown that the size of a weak spherical scatterer in the Mie regime with size parameter, $ka > 1$, can be determined by employing the Born approximation (or the modified Born approximation) from the measured backscattered intensity using its frequency minima. Here, k and a indicate the wave number of the incident wave and scatterer size, respectively. Essentially the knowledge of orders of minima of the backscatter intensity pattern and wave numbers of incident waves corresponding to those minima can provide an estimation of the size of the scatterer. It is a much simpler approach because there is no need to run an optimization algorithm to fit the experimental data with the theoretical model. This approach is also capable of providing accurate estimation of scatterer size.

The expression for the scattering amplitude of an incident plane wave by a spherical object in the modified Born approximation can be derived by approximating the unknown pressure field inside the scatterer as $\exp(im\vec{k}\cdot\vec{r})$, (rather than $\exp(i\vec{k}\cdot\vec{r})$ as in the case of Born approximation), where m is the ratio of wave number within scattering object to that of surrounding medium and is analogous to the refractive index in optics. In this manner acoustic properties of the scatterer can be incorporated into the expression of pressure field inside the scatterer. One then solves the integral equation for pressure field using the Green's function method [8]. Alternatively one can also derive the same mathematical expression for the scattering amplitude from the Anderson's model in the weak scattering limit [8]. This simple modification was found to increase the validity domain of the Born approximation in the forward scattering direction [8]. Moreover, both approximations work at their best to reproduce backscattered intensity pattern when $ka < 1$. However, for size estimation alone, they can even be used for scatterers with $ka > 1$. This technique may be useful to monitor the time evolution of the size of a target cell/region embedded in a tissue medium for applications ranging from tissue engineering to the monitoring of response to treatment in situations where the power spectrum exhibits the frequency minima.

This methodology relies on the fact that for a Mie scatterer the measured backscattered intensity passes through zeros or minima when plotted as a function of incident wave vector k. Physically, a zero or a minimum arises due to the destructive interference of scattered signals originating from the secondary sources within the scatterer. In the Born approximation this behavior can be mathematically attributed to the spherical Bessel function, which appears in the analytical expression of scattering amplitude for a homogeneous weak spherical scatterer. For the case of backscattering, the argument of the spherical Bessel function becomes $2ka$. The square of the spherical Bessel function reaches its minima (viz, its zeros) at some particular values of its argument. For example, the first three minima occur at $2ka$ = 4.49, 7.72 and 10.90 respectively, and the n^{th} zero (or minimum) at $2ka = \sqrt{(n+0.5)^2\pi^2 - 2}$ [9]. Therefore, the estimated size of a spherical scatterer in the Born approximation by using the n^{th} minimum is given by [7]:

$$a^{b}_{es} = \frac{\sqrt{(n+0.5)^2\pi^2 - 2}}{2k_n}, \tag{1}$$

where, a^{b}_{es} is the estimated radius of the spherical scatterer and k_n is the wave number of interrogating wave corresponding to n^{th} zero or minimum of the measured backscattered power spectrum. The subscript *es* represents the estimated size whereas the superscript *b* indicates the Born approximation. Similarly by using the modified Born approximation one can arrive at [7]:

$$a^{mb}_{es} = \frac{\sqrt{(n+0.5)^2\pi^2 - 2}}{(1+m)k_n}, \tag{2}$$

because in this derivation for backscattering the argument of the spherical Bessel function becomes $(1+m)ka$. The superscript *mb* refers to the modified Born approximation. Note that the knowledge of m is a prerequisite for the implementation of Eq. (2). The accuracies of these approximate methods have been numerically assessed in detail in [7] for spherical and cylindrical targets with a wide range of the size parameter ka (ranging from 3 to 75).

In this paper we discuss the accuracy of this technique when employed in extracting information on the size of single cells from measured backscattered spectra of nucleated and non-nucleated cells. This is done by comparing the mean diameter available from direct optical microscopic measurements of a cell with those predicted by the Born approximation and modified Born approximation from measured frequency dependent backscattered spectra. This method is simple and easy to implement. Our results show that the method is quite accurate and reliable for weak scatterers of ultrasound waves. Hence, it can be used in practice when size information is not known a-priori.

2 RESULTS

The left panel of Fig. 1 illustrates frequency dependent backscattering cross-section [8] calculated using the Anderson, Born and modified Born approximation methods for a PC-3 cell ($ka \approx 0.55$ to 3.58 for the incident waves between 10-65 MHz) with phosphate buffer solution (PBS) as the ambient medium. Corresponding ka values are also presented. The numerical values of the parameters were taken from [1] to generate the theoretical curves. Although the error associated with intensity prediction in the Born approximation becomes more than 10% after 34.5 MHz ($ka \approx 1.90$), the position of minimum is reproduced reasonably well (error < 3.6%). The error decreases slightly in case of modified Born approximation. Therefore, the size of a scatterer with regular shape can be determined by using these approximations because the positions of minima are defined by the size parameter *ka*. The right panel of Fig. 1 (taken from [1]) shows a comparison between theoretical and experimental results. In this figure the backscatter transfer function, which is the ratio of magnitude of the Fourier transform of backscatter signal and that of the average reference signal, has been plotted in dB scale as a function of insonifying frequency. Backscattered echo signals from a flat polished SiO_2 crystal (Edmund Industrial Optics Inc., part 43424; ρ=2200 kg/m^3, c=5720 m/s) placed at the transducer focus in seawater were recorded to obtain the reference signal. It is evident from this figure that both are in good agreement. The measured minimum occurs at about 41.5 MHz, and for that incident wave Eq. (1) and (2) give $a_{es}^{b} \approx 12.8$ µm and $a_{es}^{mb} \approx 12.9$ µm respectively for $n = 1$. The numerical value for the *m* parameter in Eq. (2) was obtained from the values of speed of sound in the ambient medium and within the scatterer. Similar plots are displayed in Fig. 2 (right panel taken from [2]) for the scattering of ultrasound waves by sea urchin oocytes ($ka \approx$ 1.54 to 10.03) suspended in artificial seawater. It is clear from the left panel of Fig. 2 that the positions of minima are more accurately reproduced by the modified Born approximation. In this case also we choose the numerical values of parameters similar to that used in [2] to obtain the curves. The estimated average values of size are $a_{es}^{b} \approx 36.4$ µm and $a_{es}^{mb} \approx 37.0$ µm in the two approximations respectively. Here, we used the orders $n = 1$ to 5 and the corresponding positions of intensity minima at 15, 26, 36.6, 46.5 and 57 MHz respectively as shown by the experimental curve. In both cases estimations are quite accurate (error < 3%). The accuracy of the modified Born approximation is even better than the Born approximation. However, information regarding m (the ratio of wave numbers inside and outside the scatterer) is required [see Eq. (2)] in the case of the modified Born approximation.

3 CONCLUSION

The Born approximation and its modified form can be used to estimate the size of a cell with weak scattering properties and size parameter *ka* > 1 (Mie scatterer), if its frequency dependent backscattering pattern is measured. New ultrasound technologies have the capability to display, in real time, the ultrasound backscatter power spectra (for example the VEVO series from VisualSonics, Toronto, Ontario); therefore, simple calculations based on the values of frequency minima and equations 1 and 2 can be used to rapidly assess the size of the scatterers examined. The accuracy of these approximations in determining the size of a cell has been assessed in this paper. This was done by comparing direct optical microscopic measurements with those obtained by employing these approximations from the measured backscattered spectra. The orders (n) of frequency minima of the backscatter intensity pattern and wave numbers of incident waves corresponding to those minima were required to implement the scheme. In this study for this purpose we used backscattered spectra for PC-3 cells ($ka \approx$ 0.55 to 3.58) and sea urchin oocytes ($ka \approx$ 1.54 to 10.03) for the incident waves between 10-65 MHz. This method is simple and the computation of scatterer size is straight-forward. In case of the Born approximation knowledge of the acoustic properties of the scattering cell are not required as inputs to the model and thus it is completely geometrical. However, in case of the modified Born approximation knowledge of *m* (the analogue of refractive index) of the scatterer is needed to implement the scheme. Our results show that this technique works quite accurately (size estimation error < 3%) and thus may be used to monitor time evolution of scatterer size in a non-invasive manner. This size information can further be used to obtain acoustic properties (speed of sound and density) of that cell while finding the best fit to experimental data by the analytical expression of backscattered spectrum based on the Anderson's model. In this case it would be an optimization for two parameters not for three parameters (size, speed of sound and density) and intuitively that is a simpler approach. Therefore size as well as the acoustic properties of a scatterer can be determined by combining approximate methods and the Anderson's model.

ACKNOWLEDGEMENTS

We like to thank Dr. Behrouz Soroushian of Ryerson University for helping us to prepare the French version of the abstract. We also thank Prof. Binayak Dutta-Roy of Satyendra Nath Bose National Centre for Basic Sciences for careful reading of the manuscript.

REFERENCES

[1] R E Baddour and M C Kolios, "The fluid and elastic nature of nucleated cells: Implications from the cellular backscatter response", J. Acoust. Soc. Am., **121**(1), EL16-22, 2007.

[2] O Falou, R E Baddour, G Nathanael, G J Czarnota, J C Kumaradas and M C Kolios, "A study of high frequency ultrasound scattering from non-nucleated biological specimens", J. Acoust. Soc. Am., **124**(5), EL278-283, 2008.

[3] R E Baddour, M D Sherar, J W Hunt, G J Czarnota and M C Kolios, "High frequency ultrasound scattering from microspheres and single cells", J. Acoust. Soc. Am., **117**(2), 934-943, 2005.

[4] V C Anderson, "Sound scattering from a fluid sphere", J. Acoust. Soc. Am., **22**(4), 426-431, 1950.

[5] T E Doyle, A T Tew, K H Warnick and B L Carruth, "Simulation of elastic wave scattering in cells and tissues at the microscopic level", J. Acoust. Soc. Am., **125**(3), 1751-1767, 2009.

[6] T E Doyle, K H Warnick and B L Carruth, "Histology-based simulations for the ultrasonic detection of microscopic cancer *in vivo*", J. Acoust. Soc. Am., **122**(6), EL210-216, 2007.

[7] R K Saha and S K Sharma, "*Determination of accuracy of particle size by acoustic scattering using a modified Born approximation*", Indian Journal of Pure and Applied Physics, **46**(11), 761-770, 2008.

[8] S K Sharma and R K Saha, "On the validity of some new acoustic scattering approximations", Waves in Random Media, **14**(4), 525-537, 2004.

[9] S K Sharma and D J Somerford, "Light scattering by optically soft particles theory and applications", Springer-Praxis:Chichester, UK, pp-93, 2006.

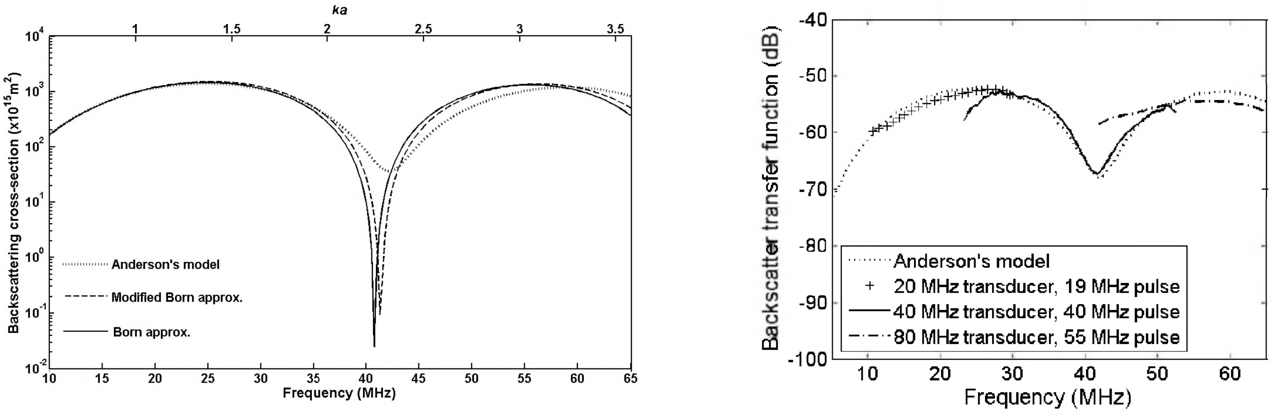

Fig. 1: Left panel: Theoretical (fluid sphere model), modified Born approximation and the Born approximation results for a scatterer (fluid sphere) with d=26 μm, c=1523 m/s, ρ=1180 kg/m^3 surrounded by a medium with c=1483 m/s, ρ=1000 kg/m^3. Right panel: Normalized theoretical and measured backscatter frequency response for a PC-3 cell (a line of human prostate cancer cells) in phosphate buffered saline (PBS) solution subject to three incident pulses from three transducers: 20, 40 and 80 MHz excited at 19, 40 and 55 MHz respectively (reproduced from [1]).

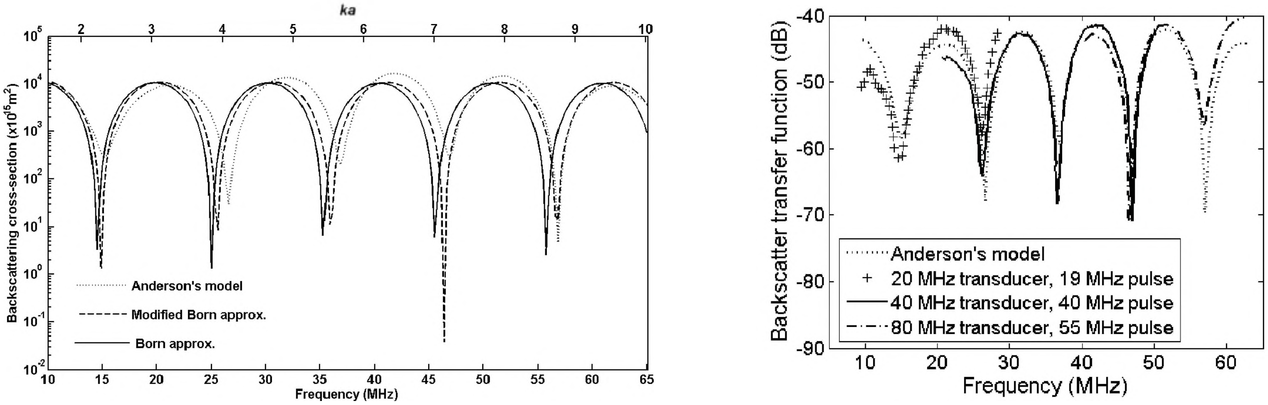

Fig. 2: Left panel: Theoretical (fluid sphere model), modified Born approximation and the Born approximation results for a scatterer (fluid sphere) with d=75 μm, c=1573 m/s, ρ=1198 kg/m^3 surrounded by a medium with c=1527 m/s, ρ=1025 kg/m^3. Right panel: Normalized theoretical and measured backscatter frequency response for single sea urchin oocyte in artificial seawater. Same transducers as given in Fig. 1 were used during measurements (reproduced from [2]).