MODELLING POPULATIONS OF PROKARYOTIC CELLS: the n-Layered mRDG Approximation

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Abstract: In this paper, explicit expressions for the Scattering Amplitude elements of spherically symmetric inhomogeneous particles using the modified RDG approximation (mRDG) are derived. Computer simulation algorithms have been developed for the calculation of Scattered Light Intensity (full and backscattering) from a multi-layered sphere with an arbitrary number of layers. All quantities are estimated within the biological cell domain and in particular that of prokaryote. We have extended a previously proposed size distribution to account for the evident size asymmetry in nature. Simulation results show that the proposed model's rapid calculations are comparable in performance with that of Mie or RDG models. Finally, to the best of our knowledge, the included relative error study between these theories and for n-layered spheres is the first to appear.

Keywords: Light Scattering, Prokaryotic Cells, Asymmetric Populations, mRDG, Mie Scattering

1 INTRODUCTION

Light scattering measurements and in particular multi-angle (laser) light scattering has been of great interest in many fields of microscopic characterisation. In particular it has been indicated that laser scattering techniques will play a significant role in partial identification [Newman 1987], characterisation [Van de Merwe et.al. 1997] and clinical examination [Mourant et.al. 1998] of bacteriological samples. Optical data obtained from a circular array of photo detectors are usually interpreted by means of the Rayleigh-Gans-Debye (RDG) approximation [Wyatt 1993] or Mie theory homogeneous models, even though other theories have been developed (e.g. [Draine and Flatau 1994]).

However, most prokaryotic cells are of a complex makeup. In general the cell presents a structure that consists mainly of the cell wall, the plasma or cytoplasmic membrane, the cytoplasm and the nucleoid. Other morphological characteristics may also appear such as a slime layer (capsule) outside the cell wall or inclusions within the cell's cytoplasm (e.g. spores, granules). Therefore, in order to generate a more accurate representation of the cell, one would model it as having various compartments within its volume and within these compartments the refractive index is different from that of the surrounding objects. In cells where the overall morphology is that of a sphere (cocci), or if we allow for an approximate representative spherical model and for non-symmetric particles, each of the structures internal or external to the plasma membrane can be modelled as a different layer in an n-layered spherically symmetric inhomogeneous particle.

Biological particles, including bacteria, contain weakly scattering material, mainly because most of their bodies contain a high percentage (70% to 86%) [Schlegel 1997] of water. This alone supports the use of RDG. However conditions underlined in this approximation pose size restrictions and possible rise in the relative error. To accommodate for this, in [Shimizu 1983] an extension of RDG has been provided, also known as mRDG. In [Sloot and Fidgor 1986] this approximation has been generalised to a two-layered spherical particle and successfully applied for predictions in nucleated blood cells, but with no consideration of size variations in cell populations.

In this paper we extend the theory of mRDG to a spherically symmetrical particle/cell with an arbitrary number of layers and corresponding relative refractive indices. Population variations in size have also been accounted for and for asymmetry (positive or negative skewness of a size distribution) in nature. Intensity expression for the latter is provided within. Finally, from previous studies [Wyatt 1973, Volkov and Kovach 1990] we advocate the use of mRDG for biological, prokaryotic cells and provide a comparison of mRDG and Mie derived models for the n-layered sphere.

2 THE n-LAYERED SPHERE MODEL

As previously mentioned we consider the case of a spherical model for the prokaryotic cell as an inhomogeneous particle consisting of a multi-layered sphere with an arbitrary refractive index within each layer.

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Suppose that there are n layers, such that the ith layer has outer radius r_i and relative refractive index m_i. Thus for a radially changing $m(r)$,

$$m(r) = \begin{cases} m_1, & r \in (0, r_1] \\ m_2, & r \in (r_1, r_2] \\ & \vdots \\ m_n, & r \in (r_{n-1}, r_n] \end{cases} \quad (1)$$

It is known [Bohren and Huffman 1998] that in the RDG regime the scattering amplitude S of a cell of volume V at scattering angle θ and for perpendicular polarisation to the scattering plane (and hence the subscript $\perp$ in $S_\perp$ below) can be expressed as follows:

$$S_\perp(\theta) = \frac{\jmath k^3}{2\pi} \int_V (m(r) - 1) \exp\left(\jmath 2kr \sin\frac{\theta}{2}\right) dV \quad (2)$$

In Equation 2, $S_\perp(\theta)$ is a complex number and $\jmath$ denotes $\sqrt{-1}$, whilst k is the propagation constant in the water medium ($k = 2\pi/\lambda$, where λ is the wavelength of the incident light). For a spherical cell, the integrand in Equation 2, in polar coordinates, depends only on the distance r from the origin, and consequently the triple integral can be replaced by a single integral, with the volume element $dV = 4\pi r^2 dr$. We have:

$$S_\perp(\theta) = \jmath 2k^3 \int_0^{r_n} r^2 (m(r) - 1) \exp\left(\jmath 2kr \sin\frac{\theta}{2}\right) dr \quad (3)$$

In the RDG approximation it is assumed that the applied field inside the particle equals that in the medium. Hence, the propagation constant in and out of the particle's region is unchanged. Shimizu [1983] has extended the RDG by altering the propagation constant to accommodate for the contributions resulting from the field inside the particle. As a result, within the phase lag expression the particle's refractive index is taken into account so that now k is replaced by $km(r)$. With hindsight and using the method of slices [Wyatt 1973] Equation 3 is replaced by

$$S_\perp(\theta) = \jmath 2k^3 \int_0^{r_n} r^2 (m(r) - 1) \frac{\sin\left(2km(r) r \sin\frac{\theta}{2}\right)}{2km(r) r \sin\frac{\theta}{2}} dr \quad (4)$$

Evaluating Equation 4 in the region $r \in [0, r_n]$ and using Equation 1 we now get

$$S_\perp(\theta) = \jmath 2k^3 \left((m_1 - 1) \int_0^{r_1} r \frac{\sin\left(2km_1 r \sin\frac{\theta}{2}\right)}{2km_1 \sin\frac{\theta}{2}} dr + \cdots \right.$$
$$\left. + (m_n - 1) \int_{r_{n-1}}^{r_n} r \frac{\sin\left(2km_n r \sin\frac{\theta}{2}\right)}{2km_n \sin\frac{\theta}{2}} dr \right)$$

resulting in

$$|S_\perp(\theta)| = k^3 \sqrt{2\pi} \left(K_{1,1} J_{3/2}(2km_1 r_1 \sin\frac{\theta}{2}) + \cdots \right.$$
$$+ (K_{n,n} J_{3/2}(2km_n r_n \sin\frac{\theta}{2}) -$$
$$\left. - K_{n,n-1} J_{3/2}(2km_n r_{n-1} \sin\frac{\theta}{2})) \right) \quad (5)$$

where $J_{3/2}$ is the Bessel function of order $\frac{3}{2}$, we write $r_0 = 0$, and, for $i, \ell \in \mathbb{N}$,

$$K_{i,\ell} = (m_i - 1) \sqrt{\left(\frac{r_\ell}{2km_i \sin\frac{\theta}{2}}\right)^3} \quad (6)$$

For a more compact model we write

$$G_{i,\ell}(\theta) = J_{3/2}(2km_i r_\ell \sin\frac{\theta}{2}) \quad (7)$$

so that Equation 5 now becomes

$$|S_\perp(\theta)| = k^3 \sqrt{2\pi} \sum_{i=1}^{n} (K_{i,i} G_{i,i}(\theta) - K_{i,i-1} G_{i,i-1}(\theta)) \quad (8)$$

bearing in mind that $K_{i,0} = G_{i,0} = 0$. The expression in Equations 6, 7 and 8 predicts amplitude of light scattered from a single cell and it is the n-layered sphere extension model. It can be applied to any population of n-layered spheres and would lead to better approximations of light scattered phenomenon on real cells by simulated models. In effect its physical meaning corresponds to the fact that a cell of n layers will scatter light proportional to the sum of n homogeneous spheres of corresponding r_n and m_n, by subtraction of contributions arising from the $(n-1)$ homogeneous spheres of corresponding r_{n-1} but having the same refractive index, that is m_n.

This generalised expression correctly predicts the effect of removing layers. Putting $m_{k-1} = m_k$ will result in a multi-layered sphere where the $(k-1)$th layer will disappear. This is true since the previous $(k-1)$th and kth layers will merge to a new layer with $m_{\text{new}} = m_k = m_{k-1}$, of thickness[1] t_{new} such that $t_{\text{new}} = t_k + t_{k-1}$. Furthermore, if $m_k = 1$ then the k^{th} layer becomes redundant, which is true since this layer becomes transparent to incoming light and as such does not contribute to the scattering amplitude.

The light intensity from such a cell, and for perpendicular incident polarisation, can be expressed in terms of $S_\perp$ using the following expression:

$$I(\theta) = \frac{I_0}{2(kr)^2} |S_\perp(\theta)|^2 \quad (9)$$

where $r = r_n$ is the overall radius of the spherical cell and I_0 is the intensity of the incident light.

[1] Note that, for example, $t_k = r_k - r_{k-1}$

3 SINGLET POPULATION

For cells that appear alone, that is, where there is no binding of cells together, and for low densities so that multiple scattering is avoided, the average scattering pattern can be calculated using a size distribution. The term "size" in the current context should be interpreted as the radius of the cell, but in general would be thought of as the length of the minor or major axis of an ellipsoid form (e.g. rod like cells). The cell size is denoted by s. We use a probability density function $P(s)$ for the size, and assume that we have N size ranges with mid-points $s_1, s_2, \ldots, s_N$. The relative frequencies of the cell samples in the ranges are approximated by the density function at the mid-points, so that the mean light intensity at scattering angle θ is given by

$$\langle I(\theta) \rangle = \frac{\sum_{i=1}^{N} I(\theta)\rfloor_{r=s_i} P(s_i)}{\sum_{i=1}^{N} P(s_i)} \tag{10}$$

Multiple scattering is a problem that cannot be addressed using Equation 10. However, Equations 6–8 are used for modelling an aggregate's discrete scattering elements of any bounded configuration with no multiple scattering.

Often a Gaussian distribution of cell sizes is assumed. However, the normal distribution has long tails, which is rather unrealistic since, in the bacteria domain, sizes do not exceed a specific range. Moreover, from a variety of sources of variability, usually only a few are dominant. This results in a positively or negatively skewed distribution, which does not resemble the familiar Gaussian symmetry. Consequently, we have adopted a distribution first proposed in [Wyatt 1973], but here we have allowed for κ in Equation 12 to be assigned independently at the left and right of the mode. The density function is proportional to

$$P(s) = \begin{cases} (1-z^2)^4 & \text{for } z \in [-1,1] \\ 0 & \text{for } z \notin [-1,1] \end{cases} \tag{11}$$

where

$$z = \begin{cases} 1.084(s-s_0)/(\kappa_{\text{left}} s_0) & \text{for } s \leq s_0 \\ 1.084(s-s_0)/(\kappa_{\text{right}} s_0) & \text{for } s > s_0 \end{cases} \tag{12}$$

The spread of the distribution is dictated by the constant κ which is assigned independently at the left and right of the mode s_0, resulting in an asymmetric distribution that avoids long tails. It should be evident that for $\kappa_{\text{left}} = \kappa_{\text{right}}$ the distribution is symmetric and s_0 becomes the mean; whilst κ is approximately equal to $3\hat{\sigma}/s_0$ with $\hat{\sigma}$ being the variability measure (standard deviation) of the symmetric distribution. It is known that in any non-synchronised culture and in nature we expect a variation in size of at least 30% ($\kappa_{\text{left}} + \kappa_{\text{right}} \geq 0.30$). The latter applies not only to singlet spheres but also to any other configuration of cocci bacteria.

4 SIMULATION RESULTS

Bacteria sizes vary considerably, from half micrometer up to several micrometers. In particular, cocci (spherical morphology) would be said to have a radius r within the range $0.5\mu\text{m} \leq r \leq 1.2\mu\text{m}$ with a few exceptions such as *Sarcina ventriculi* with a 4μm radius and spore inclusions. In scattering experiments, cells are usually suspended in water based media and so the relative refractive index m is close to unity and the cytoplasm's refractive index value is close to 1.35, resulting in a selected range for m in the studies reported here as $1 < m < 1.3$.

Following the criteria set by [Hoekstra and Sloot 2000], we present a relative error study for values of relative refractive index and radius as discussed. However, since we are dealing with multiple layers, the examination of single particle scattering is introduced in more detail. Hence, for each cell size defined by an overall radius, the thickness of each layer is defined by the use of uniform random numbers. The relative error is estimated over an average of R runs, where for each run a corresponding random relative refractive index value has been provided within the range of interest. In the analysis, only the average refractive index m of the cell is illustrated for each value of radius r.

The error metric E_R is equivalent to [Hoekstra and Sloot 2000] but here we examine the light scattering intensity as opposed to the phase matrix relations. In particular, the error is a measure of the difference between intensities estimated by Mie and mRDG models and is normalised as:

$$E_R = \frac{\sum_{i=0}^{N} |logI^{Mie}(i\Delta\theta) - logI^{mRDG}(i\Delta\theta)|}{(N+1)(logI^{Mie}(0) - logI^{Mie}(\theta_o))} \tag{13}$$

The values used in the simulations were $N = 91$, $R = 30$ and $\Delta\theta = \pi/N$. Moreover, at a scattering angle θ_o the light intensity of the Mie scattering function (I^{Mie}) is at minimum. Figure 1 depicts typical light intensity patterns for the Mie and mRDG models which are the basis for error evaluation through Equation 13.

Many authors including [Hoekstra and Sloot 2000] have concluded that for a homogeneous sphere the mRDG model covers a significant part of the domain; particularly if one allows for error of 12% as compared to Mie scattering. However, we have found that in the case of multi-layered spheres this relative difference doubles. In particular, Figure 2 depicts the error map between Mie scattering model and mRDG for two layer spheres. The gray scale represents the average relative error from 0% (white) to 33% (black). Generally speaking, in Figure 2 the error does not exceed the limit of approximately 23%, even though small areas of 33% do appear. The latter can be verified by consulting

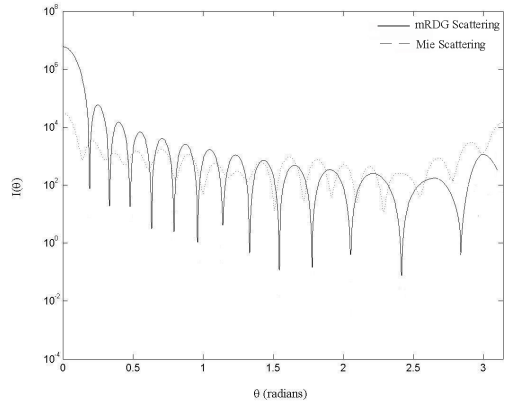

Figure 1: Layered mRDG and Mie light scattering patterns for a two layer concentric sphere.

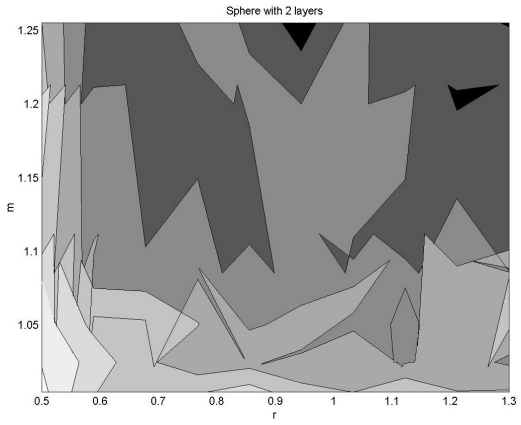

Figure 2: $n = 2$. Error map between mRDG and Mie scattering for a two layer spherical model.

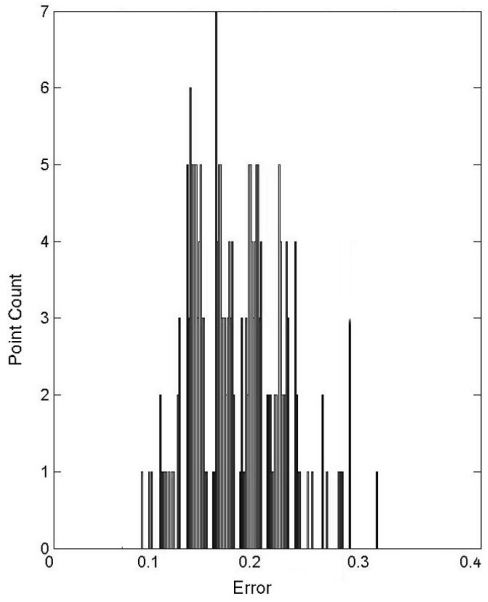

Figure 3: Error histogram for a two layered sphere.

the error histogram of Figure 3 which shows that most difference between the two models lie between 15 and 23%. This error or difference is consistent throughout the two models either for 2, 3, 4 or 5 layers. The mRDG model is, in fact, just an alternative representation for Mie scattering in this context. Moreover, if one considers that Mie algorithms are at least 100 times slower (or more depending on programming skills), as opposed to their RDG or mRDG counterparts, there are significant advantages in using the alternative representation of mRDG model as proposed here.

In Figures 4 to 6 it must be emphasized that as the number of layers increases the maximum relative error margin slightly shifts towards higher r values and covering a larger m value margin. As a matter of fact [Volkov and Kovach 1990] state that for near index particles (high water content) the key factor in the Mie scattering behaviour is the thickness of the layers. As such, it may seem rather surprising that the relative error increases not due to the r values but due to the average refractive index as it is evident in Figure 5. This may mean that Mie theory is not particularly sensitive to changes in refractive index for larger values of radius. This indeed may have given rise to the relative error not attributed to the mRDG approximation. Returning to the earlier rare example of *Sarcina ventriculi*, in an experiment of $r = 4\mu$m and for various m values, the average relative error was found to be in the region of 3 to 27%; with the latter arising as $m \to 1.3$. Finally, within the domain of Prokaryotic cells such large m-values are rarely found and, as such, the use of mRDG model as proposed here is justified.

Testing the relative error of bacteria populations, as introduced in Section 3, has been performed using the same procedure. The population analysis yields very similar results and so further illustrations are not included. We must however highlight the fact that as the spread of the size distribution increases the relative error remains within the same margins. Therefore, the apparent smoothing of sharp maxima (or minima) in the scattering intensity does not indicate degradation in performance of the n-layer mRDG model.

5 CONCLUSIONS

In this paper we have derived a new model for the multilayer sphere problem based on the mRDG approximation and used it to simulate light scattering phenomena in bacteria cells. In order to assess the performance of the model, computer algorithms were developed in Matlab and compared with the equivalent Mie scattering model. An error parameter was defined based on a measure of the difference between Mie and mRDG scattering. All simulations have been conducted using sizes and refractive indices in accordance with values found in bacteria cells.

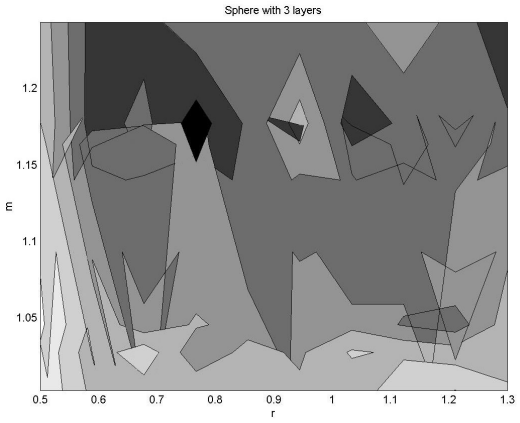

Figure 4: $n = 3$. Error map between mRDG and Mie scattering for a three layer spherical model.

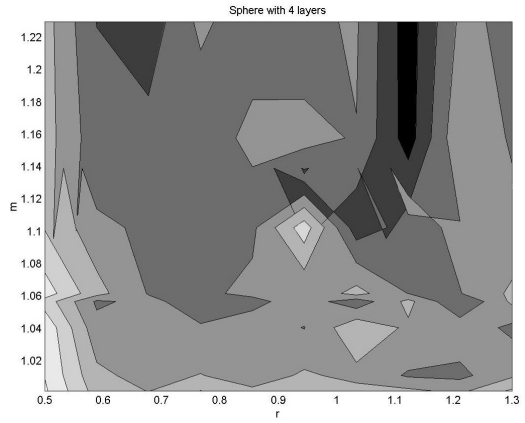

Figure 5: $n = 4$. Error map between mRDG and Mie scattering for a four layer spherical model.

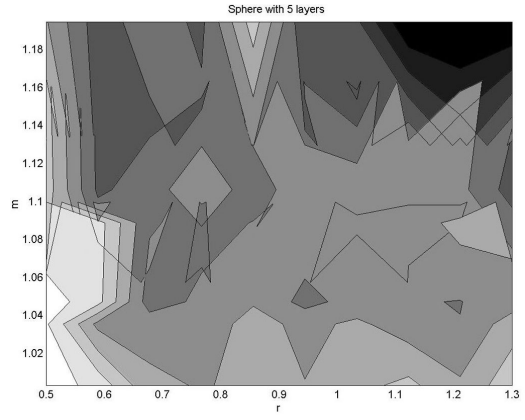

Figure 6: $n = 5$. Error map for a five layer spherical model between mRDG and Mie scattering

It appears that the difference between the two models is at its maximum at about 25%. We have used the term *relative error*, which does not necessarily portray the expected error under true experimental conditions. In particular, one has to bear in mind the much faster computation of the mRDG models as opposed to the Mie equivalents. This can be explained as follows. Calling t the number of terms to be calculated in the Mie series and n the number of layers, and l_{mie} the scattering coefficients, there would be a minimum of $(l_{mie}nt)$ calculations. The equivalent number for mRDG scattering would be $(l_{mrdg}(2n-1))$. In our implementation of both models on the same platform, Mie models were at least 100 times slower than the RDG or mRDG counterparts. As a result, for real time or time critical applications the mRDG approximation is expected to be favoured over other more complex theories.

The consistency of errors throughout the two models indicate that the mRDG is a convenient alternative representation for light scattering phenomena and its superior computational performance brings obvious advantages to cell characterisation.

Further research include relative error studies with other theories that can be applied in the domain of interest such as Anomalous Diffraction scattering (AD) and variants of this approach, higher order RDG, Discrete Dipole Approximation (DDA) and Physical Optics (PO) to name just a few. The most important further development of this work would be the generation of true scattering patterns from benchmark prokaryotic cells, and consequently, compare the mRDG, AD and PO calculations with rigorous numerical methods such as the DDA. Research is underway and will be reported in the near future.

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BIOGRAPHY

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