Study of light scattering by a granulated coated sphere – a model of granulated blood cells

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Abstract

We performed extensive simulations of light scattering by granulated coated sphere model using the discrete dipole approximation and varying model parameters in the ranges of sizes and refractive indices of granulated blood cells. We compared these results with predictions of Maxwell-Garnett effective medium theory and independent scattering approximation. Extinction efficiency and Mueller matrix element S_{11}, averaged over azimuthal angles, are accurately described by one of the approximations, except for the intermediate range of polar angles. We discuss dependence of S_{11} on granule diameter for any fixed angle, showing a distinct maximum, with respect to the solution of the inverse light scattering problem.

1 Introduction

Many naturally occurring particles from biological cells to astrophysical dust can be characterized by a matrix with multiple inclusions (granules). Rigorous simulation of light scattering by such particles requires computationally intensive algorithms: superposition T-matrix method [1] (applicable only to certain geometries), the finite difference time domain method (FDTD) [2] or the discrete dipole approximation (DDA) [3]. A number of approximate theories also exist, such as effective medium theories (EMTs) [4-7]. Most researchers who studied granulated particles are concerned with astrophysical or atmospheric applications [1,5-8], while our main application – light scattering by biological cells – is a much less studied field.

Biological cells, when suspended in liquid, have an important advantage with respect to the light scattering simulation [9]. Their relative refractive index is close to unity. This accelerates the rigorous methods and improves the accuracy of the EMTs and other approximate theories. FDTD simulation of light scattering by biological cells was performed in a number of manuscripts by Dunn and coworkers (summarized in [10]). However, only the dependence on the volume fraction and not on the size of the granules was studied. Recently we have performed a systematic study of light scattering by a granulated sphere in the size and refractive index range of human granulated leucocytes (granulocytes) [11]. We explained the difference of depolarization side scattering signals measured by a flow cytometer between different subtypes of granulocytes and proposed approximate methods to quickly calculate these signals.

Granulocytes are the most numerous type of leukocytes, consisting of three subtypes: neutrophils, eosinophils, and basophils. They participate in protection of the host against infectious agents including parasites, inflammations, and allergy conditions [12]. Their morphology, e.g. number or volume fraction of granules, can be an indicator of certain pathological conditions. The Scanning flow cytometer (SFC) allows fast measurement of angle-resolved light scattering patterns (LSPs) [13], which potentially contains enough information to characterize cell morphology.

In this paper we extend our previous simulations [11] including a cell nucleus. We perform extensive DDA simulations varying all parameters of the model. To analyze results we concentrate on simulated LSPs and test the accuracy of two approximate methods: Maxwell-Garnett EMT [14] and independent scattering approximation (ISA).

2 Methods

An optical model used for simulations is shown in Fig. 1. It consists of spherical cytoplasm with concentric spherical nucleus. The remaining cytoplasm is randomly filled with spherical granules up to volume fraction *f*. Diameters and refractive indices of cytoplasm, nucleus and granules are D_c, D_n, D_g and m_c, m_n, m_g respectively. All values of model parameters (except D_g) used for simulations are given in Table 1. Wavelength λ corresponds to the wavelength of 0.66 μm semiconductor laser in buffer saline (refractive index 1.337). All refractive indices are taken relative to the same medium. Values of m_c and m_g are the same as used in our previous study [11], and values of m_n correspond to literature data [15]. Imaginary part of all refractive indices is assumed to be zero. Values of *f*, D_c and D_n/D_c cover the range of all leukocytes, including monocytes and lymphocytes. For non-granulocytes, granules in our model may represent either cell organelles, e.g. mitochondria, or real granules that appear under certain physiological conditions. We have changed a single parameter at a time, fixing all other at their default values. In total 13 sets of parameters, given in Table 1, were used. For each of these sets we performed simulations for all D_g from the list: 0.075, 0.1, 0.125, 0.15, 0.175, 0.2, 0.225, 0.25, 0.275, 0.3, 0.325, 0.35, 0.375, 0.4, 0.45, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.2, 1.4, 1.6, and 2 μm, except those that cannot fit into the cytoplasm with specified *f* (e.g., for the default set of parameters D_g up to 0.8 μm were used).

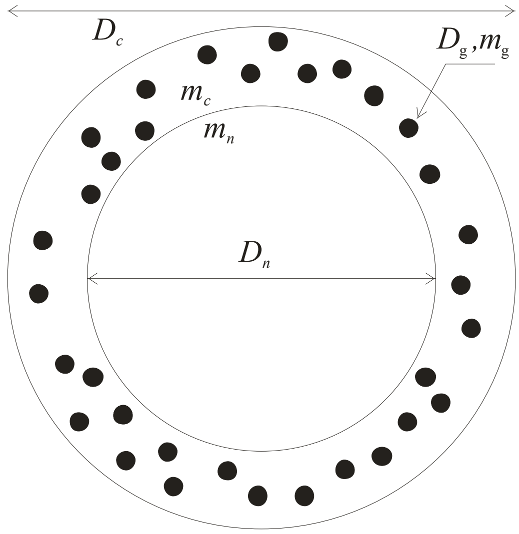

Fig. 1. A granulated coated sphere model. Shown parameters are diameters and refractive indices of cytoplasm, nucleus, and granules.

Table 1. Parameters of the model used for simulations. Values of D_g are given in the text.

f	D_c, μm	D_n/D_c	m_g	m_n	m_c	λ, μm
0.02	6	0.4	1.1	1.05	1.015	0.4936
0.05	8*	0.6	1.15	1.08*		
0.1*	11	0.8*	1.2*			
0.2	14					
0.3						

* default value for the parameter.

For each unique set of parameters including D_g we have performed 10 simulations for different random placements of the granules. Afterwards we computed mean and standard deviation (SD) of each scattering quantity. In this contribution we analyze extinction efficiency Q_{ext} and angle dependencies of Mueller matrix element S_{11}. The latter is first averaged over the azimuthal angle, so it corresponds to the LSP measured by SFC [13] as a function of polar angle θ. All simulations were performed with ADDA v.0.77 using built-in granule generator, 12 dipoles per wavelength, and the default settings of the iterative solver [16]. All simulations were run on the Dutch compute cluster LISA.*

We have also employed approximate methods to model light scattering by the same particles. First, we used Maxwell-Garnett EMT to replace granulated cytoplasm by a homogeneous spherical layer. Second, we used ISA, i.e. take the sum of scattering quantities (such as Mueller matrix or extinction cross section) of the coated sphere without granules and all granules, considered independently. Both approximate models are easily simulated using the Mie theory.

3 Results and discussion

Here we present only part of the results of analysis of the simulated data and our first conclusions. More details will be presented at the conference. Fig. 2 shows the dependence of Q_{ext} on D_g for different *f*, including EMT result. Also Q_{ext} do deviate from EMT result with increasing D_g, the difference is within 10% for all shown points. On contrary, ISA is completely wrong for prediction of Q_{ext} showing much steeper dependence on D_g (data not shown).

* http://www.sara.nl/userinfo/lisa/description/

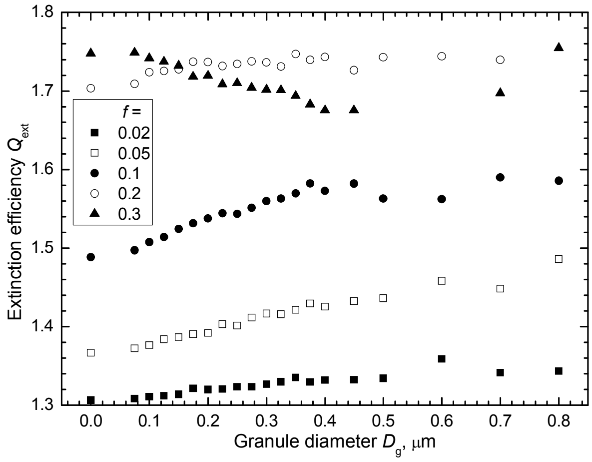

Fig. 2. Q_{ext} versus D_g for different f. Other parameters are set to the default values (see text). Values for $D_g = 0$ are computed using EMT.

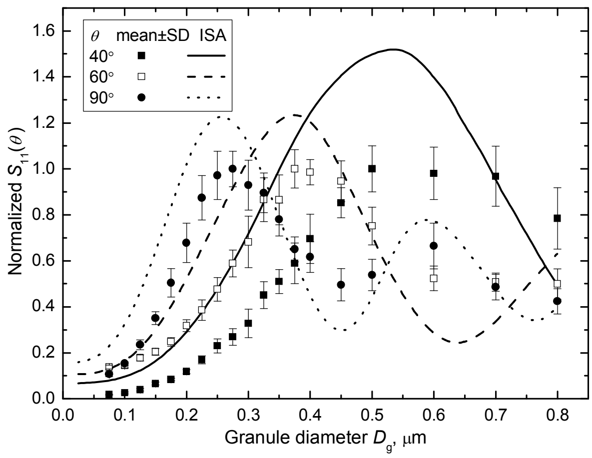

Fig. 3. $S_{11}(\theta)$ (averaged over φ and normalized over maximum DDA result for this θ) versus D_g for different θ. Other parameters are set to the default values.

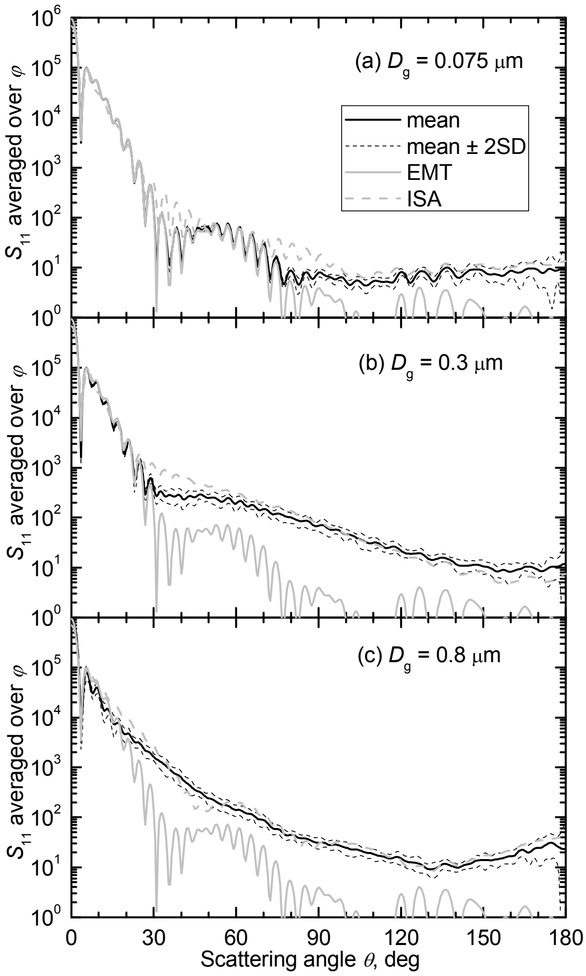

Fig. 4. S_{11} (averaged over φ) versus scattering angle θ simulated using DDA (mean values and ± 2SD range are shown), EMT and ISA for three values of D_g. Other parameters are set to the default values.

Three representative examples of LSPs are shown in Fig. 4 for the default set of parameters and $D_g = 0.075$, 0.3, and 0.8 μm. One can see that EMT is a good approximation for relatively small angles, and ISA is within 2×SD from DDA simulations for relatively large angles. However, there always exist a range of angles, where none of the tested approximations are accurate enough. This range moves towards smaller angles with increasing D_g. In other words, interaction between granules and coated sphere is negligible for large angles and is well described by EMT for small angles. In the intermediate range a more sophisticated approximation should be used. A possible candidates is Rayleigh-Debye-Gans approximation [14], which differs from ISA only in consideration of interference of fields scattered by different constituents. The interference may decrease overestimation of scattered intensity by ISA for intermediate angles.

Another interesting feature of light scattering by granulated particles is dependence of S_{11} on D_g for any fixed θ, showing a distinct maximum. An example is shown in Fig. 3 for the default set of parameters and $\theta = 40°$, 60°, and 90°, where DDA results (mean ± SD) is compared with ISA. One can see that ISA is overall rather inaccurate, but it well describes the position of the peaks, i.e. D_g for which $S_{11}(\theta)$ reaches maximum. This means that this position is largely determined by the dependence of single granule scattering intensity on size, although there is significant interference decreasing the peak amplitude. The fact that peak position decreases with increasing θ agrees with general notion of diffraction. Moreover, in combination with satisfactory accuracy of ISA it potentially allows "deconvolution" of contribution of granules with different D_g to LSP, which is a subject of future research.

4 Conclusion

We performed extensive DDA simulations of light scattering by granulated coated sphere model varying model parameters in the ranges of sizes and refractive indices of granulated blood cells. We compared these results with predictions of Maxwell-Garnett effective medium theory (EMT) and independent scattering approximation (ISA). EMT predicts values of extinction efficiency with accuracy within 10%, and accurately describes values of averaged over azimuthal angles Mueller matrix element S_{11} (light scattering pattern measured by a scanning flow cytometer) for relatively small polar angles θ. On contrary, ISA is a good approximation of $S_{11}(\theta)$ for relatively large θ. Intermediate range of θ, which moves towards smaller θ with increasing granule diameter (at fixed volume fraction), is not described accurately by any of the two approximations.

Dependence of S_{11} on granule diameter for any fixed θ contains a peak, which position decreases with increasing θ. This allows one to approach inverse problem of determining granule sizes from light scattering patterns. Moreover, ISA accurately predicts positions of these peaks, but not their amplitudes.

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