# Elastic light scattering by cells: from Mie scattering to fractal scattering

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## ABSTRACT

A unified theory for light scattering by biological cells is presented. It is shown that Mie scattering from the bare cell and the nucleus dominates cell light scattering in the forward directions. The random fluctuation of the background refractive index within the cell, behaving as a fractal random continuous medium, dominates light scattering by cells in other angles. The theory is validated by experimental angular light scattering spectra of epithelial cells for scattering angles from 1.25 to 173.8 degrees and in the spectral range from 400nm to 700nm.

**Keywords:** elastic light scattering, Mie scattering, fractal scattering, random fluctuation of refractive index, cells, optical biopsy

## 1. INTRODUCTION

Light interaction with small particles is the foundation for biomedical optical spectroscopy and imaging. Elastic light scattering by biological cells has been extensively investigated to probe pre-cancerous and cancerous tissue states both *in vivo* and *ex vivo*, and to assess the presence and concentration of biochemicals for diagnostic purposes. [1,2] Modeling light scattering by a complex medium such as biological tissue and cells is one essential issue in biomedical optics.

The optical property of tissue and cells is determined by their microstructures and local refractive index variations. Mammalian cells are $10-30\mu m$ in diameter. Microstructures in tissue and cells range from organelles $0.2-0.5\mu m$ or smaller, mitochondria $1-4\mu m$ in length and $0.3-0.7\mu m$ in diameter, and nuclei $3-10\mu m$ in diameter. The refractive index variation is about $0.04-0.10$ with a background refractive index $n_0 = 1.35$.[3] Due to the complex structure of biological tissue and cells, the computation of light scattering by such media is both difficult and resource demanding. Various techniques including the finite difference time domain (FDTD) method have been applied. [4–7] These methods are computationally expensive. Furthermore, the effect of the presence of inclusions (such as nuclei inside a cell) on the its light scattering property is hard to quantify.

It was found that a fractal model describes well both the wavelength dependence and angular patterns in larger scattering angles ($\gtrsim 5-10$ degree) of light scattering by biological tissue and cells. [8,9] Experimental results also demonstrate that light scattering by cells at smaller scattering angles ($< 5$ degree) is similar to that from Mie scatterers. Either fractal scattering or Mie scattering alone is incomplete in describing light scattering by biological cells across all the scattering angles.

In this contribution, we provide a unified analysis of light scattering by cells in both small and large scattering angles. We will show both Mie scattering and fractal scattering emerge naturally from an anomalous diffraction modeling (ADT) of light scattering by biological cells. The small angle light scattering behavior is determined by the bare cell and the nucleus (the most significant inclusion in the cell) whereas the large angle light scattering behavior is dominated by the fluctuation of the background refractive index within the cell. Based on this unified understanding of light scattering by cells, we will clarify some misconceptions about light scattering by biological tissue and cells, including the origin of the power law about the reduced scattering of light in tissue.

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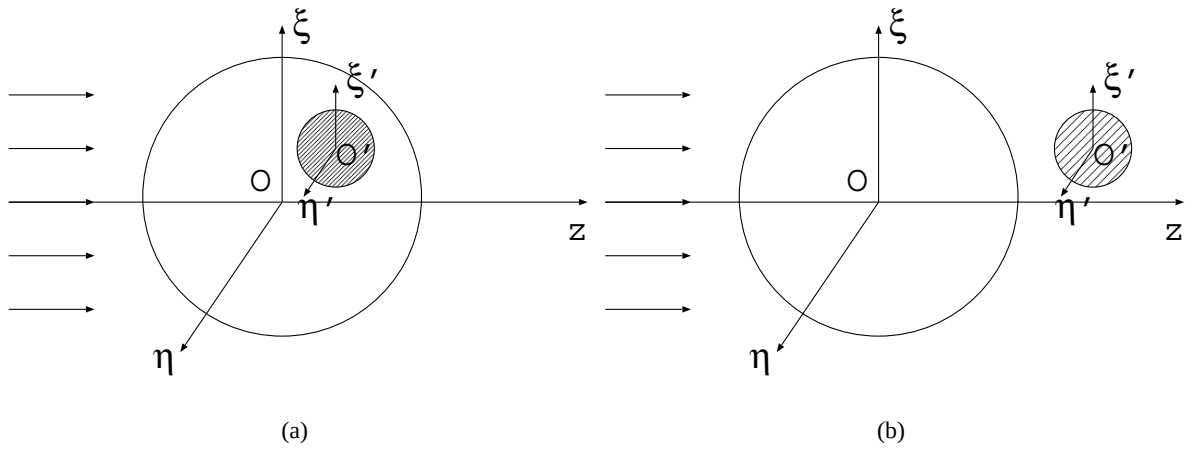


**Figure 1.** (a) A composite scatterer illuminated by an incident beam along the $z$-axis. (b) The equivalent configuration with an outside nucleus shadowed by the host particle. The shadowed nucleus has a modified refractive index.

## 2. THEORY

The starting point of the analysis of light scattering by a cell is the superposition rule for light scattering by soft particles. [10] Light scattering by a soft particle can be modelled by the anomalous diffraction theory (ADT) of van de Hulst. [11–13] The scattering amplitude function in ADT is given by

$$S(\mathbf{q}) = \frac{k^2}{2\pi} \int \{1 - \exp[-ip(\xi,\eta)]\}\, e^{-i(\xi q_\xi + \eta q_\eta)} d\xi d\eta \tag{1}$$

where the integration of $\xi$ and $\eta$ is over the area of the projection of the particle in the direction ($z$ axis) of the incident light, $p(\xi,\eta) = k\int dz[m(\xi,\eta,z) - 1]$ is the phase delay of the ray piercing the particle at the position $(\xi,\eta)$, $\mathbf{q} = (q_\xi, q_\eta, 0) = q(\cos\phi, \sin\phi, 0)$ is the wave vector transfer with a magnitude $2k\sin\frac{\theta}{2}$, $k = 2\pi n/\lambda$ is the wave number with $n$ the refractive index of the background medium and $\lambda$ the wavelength of the incident beam in vacuum, and $\theta,\ \phi$ are the polar and azimuthal angles of scattering, respectively. The superposition rule asserts that the scattering amplitude function $S(\mathbf{q})$ for a composite particle can be approximated by the superposition of those of the host particle and the inclusion such as nucleus (see Fig. 1):

$$S(\mathbf{q}) = S_0(\mathbf{q}) + \exp(-i\mathbf{r}_c \cdot \mathbf{q} - i\bar{\rho}) S_n(\mathbf{q}) \tag{2}$$

where $\mathbf{r}_c$ is the center of the inclusion and $\bar{\rho}$ is the average phase delay seen by the inclusion due to the host particle. The superposition rule is valid as long as the maximum phase delay seen by the nucleus $(\Delta\rho)_{\max} < 1$. The shadowed nucleus is same as the real nucleus except that its refractive index is replaced by $m_1 - m_0 + 1$ where $m_0$ and $m_1$ are the relative refractive indices of the host and the inclusion, respectively. The rule (2) can be straightforwardly extended to multiple inclusions.[10] The same rule can also be applied to the scattering amplitude matrix [11] in which $S$ in Eqs. (2) is a $2 \times 2$ matrix.[10] After obtaining the scattering amplitude of a composite particle, its scattering properties such as optical efficiencies, phase function, and Muller matrix will be fully determined. [11]

The superposition rule provides a simple means to analyze light scattering by a soft complex particle and quantify the contributions from each individual inclusion. For a typical biological cell of radius $a \sim 10\mu m$ and refractive index $m_0 \sim 1.01$, the value of the maximum phase delay difference can be estimated to be $(\Delta\rho)_{\max} \sim 0.4$ for incident light of wavelength $500nm$. Hence, the superposition rule is, in particular, suited to investigate light scattering by a biological cell with internal structures (nucleus, mitochondria and other organelles).

Consider now light scattering by one biological cell. The amplitude scattering function for the cell is given by the superposition of those of the host (the bare cell), the nucleus, and random fluctuations of the background refractive index within the cell, i.e.,

$$S_{\text{cell}}(\mathbf{q}) = S_0(\mathbf{q}) + \exp(-i\mathbf{r}_c \cdot \mathbf{q} - i\bar{\rho}_c) S_n(\mathbf{q}) + \sum \exp(-i\mathbf{r}_i \cdot \mathbf{q} - i\bar{\rho}_i) S_i(\mathbf{q}) \tag{3}$$

where the nucleus is centered at $\mathbf{r}_c$ and sees a phase delay $\bar{\rho}_c$ due to the shadow of the host, and the third term presents the summation of all contributions from the random fluctuation of refractive index $\delta m(\mathbf{r}_i) = m(\mathbf{r}_i) - m_0$ at position $\mathbf{r}_i$ with a corresponding phase delay $\bar{\rho}_i$. The intensity of light scattered into direction $\mathbf{q}$, normally measured in the light scattering measurement, is given by the configurational average of the squared scattering amplitude function $\overline{|S(\mathbf{q})|^2}$ over all possible size, shape, and orientation of the cell and the relative position of its inclusions. The squared scattering amplitude function can be approximated simply by

$$\overline{|S_{\rm cell}(\mathbf{q})|^2} = |S_0(\mathbf{q})|^2 + |S_n(\mathbf{q})|^2 + |S_{\rm bg}(\mathbf{q})|^2 \tag{4}$$

where $|S_{\rm bg}(\mathbf{q})|^2 = 2\pi k^6 V \hat{R}(q)$ is the fractal scattering term, $V$ is the volume of the cell, and $\hat{R}(q)$ is the power spectrum of the random fluctuation of the background refractive index $R(|\mathbf{r}_1 - \mathbf{r}_2|) = \langle \delta m(\mathbf{r}_1)\delta m(\mathbf{r}_2)\rangle$, after performing the configuration average. Among the three terms in Eq. (4), the first two terms are the Mie scattering of the bare cell (the uniform cell without any internal structure) and the nucleus, dominating forward light scattering by a cell; the third term originates from the fluctuation of the background refractive index within the cell, dominating light scattering at other angles. The fractal random continuous medium model has been shown to describe well the random fluctuation of the background refractive index for biological tissues and cells.[8,9]

To account for the polydispersity of cells, the radius of the bare cell and that of the nucleus are assumed to follow a lognormal distribution,

$$f(x) \quad = \quad \frac{1}{\sqrt{2\pi}\delta_i} x^{-1} \exp\left[ -\ln^2(\frac{x}{a_{m_i}})/2\delta_i^2 \right], \tag{5}$$

with $i = 0$ standing for the bare cell and $i = 1$ for the nucleus, respectively. The first two terms in Eq. (4) can be computed using a regular Mie scattering code weighted by the lognormal size distribution. The third term in Eq. (4) can be expressed as

$$|S_{\rm bg}(\theta)|^2 \propto \int_0^{kl_{\max}} \frac{k^{D_f-1}x^{6-D_f}}{2\pi[1+2(1-\cos\theta)x^2]^2} dx. \tag{6}$$

where $D_f$ is the fractal dimension of the medium and $l_{\max}$ is the cutoff correlation length.[8,9] The measured angular spectra of light scattering versus the scattering angle $\theta$ and the wavelength $\lambda$ can then be used to fit for the size distribution ($a_{m_0}$ and $\delta_0$) and the relative refractive index $m_0$ of the bare cell, the size distribution ($a_{m_1}$ and $\delta_1$) and the relative refractive index $m_1$ of the nucleus, and the fractal dimension $D_f$ and the cutoff correlation length $l_{\max}$ of the background refractive index fluctuation.

## 3. RESULT

In this section, the cell light scattering model presented in Eq. (4) is used to analyze angular light scattering spectrum of epithelial cells within the spectral range from $400\text{nm}$ to $700\text{nm}$ and the scattering angles from 1.25 to 173.8 degree. The details of the experimental setup and the analysis of other experimental data are presented in a companion paper.[14]

Fig. 2 displays the fitting of light scattering spectra at 12 forward scattering angles (1.25, 1.4, 1.55, 1.8, 2.0, 2.3, 2.5, 2.7, 3.0, 3.25, 3.5, and 3.7 degrees) with Eq. (4). The first two terms (Mie scattering from the bare cell and the nucleus) dominate at these scattering angles. The fitting yields $a_{m_0} = 6.75\mu m$, $\delta_0 = 0.127$ and $m_0 = 1.028$ for the bare cell, and $a_{m_1} = 5.07\mu m$, $\delta_1 = 0.189$ and $m_1 = 1.060$ for the nucleus. The lognormal size distribution of parameters $a_m$ and $\delta$ attains its peak at $a_m/\exp(\delta^2)$ and a FWHM of the size distribution to be $2\sinh(\sqrt{2\ln 2}\delta)a_m/\exp(\delta^2)$. This gives a typical radius $6.63\mu m$ and dispersion $1.99\mu m$ for the bare cell, and a typical radius $5.25\mu m$ and dispersion $2.36\mu m$ for the nucleus. The refractive index is $1.37$ for the bare cell and $1.41$ for the nucleus.

Fig. 3 displays the fitting of light scattering spectra at three large scattering angles ($32.2$, $90.0$, and $173.8$ degrees) with Eq. (4). For scattering angles ($\gtrsim 5^{\rm o} - 10^{\rm o}$), the third term due to the random fluctuation of the background refractive index dominates. The fitting yields the fractal dimension $D_f = 4.45$ and the cutoff correlation length $l_{\max} = 0.53\mu m$.

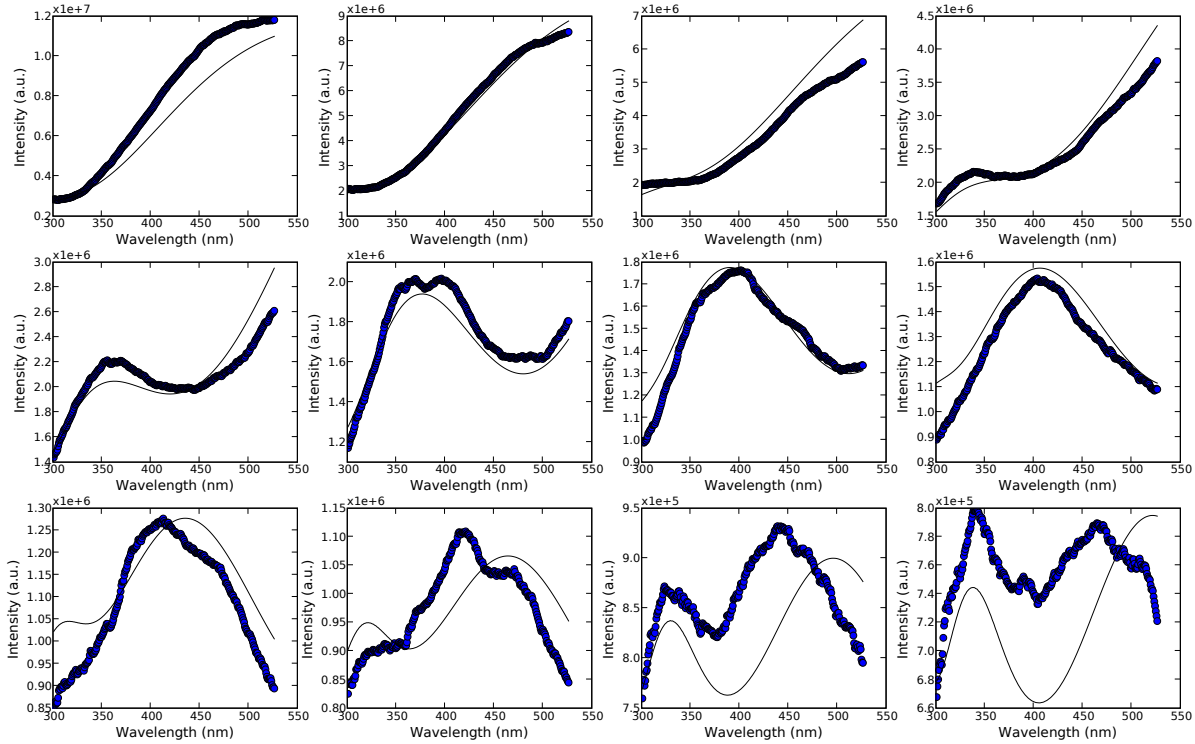


**Figure 2.** Fitting for light scattering by the epithelial cell suspension at scattering angles of 1.25, 1.4, 1.55, 1.8, 2.0, 2.3, 2.5, 2.7, 3.0, 3.25, 3.5, and 3.7 degrees.

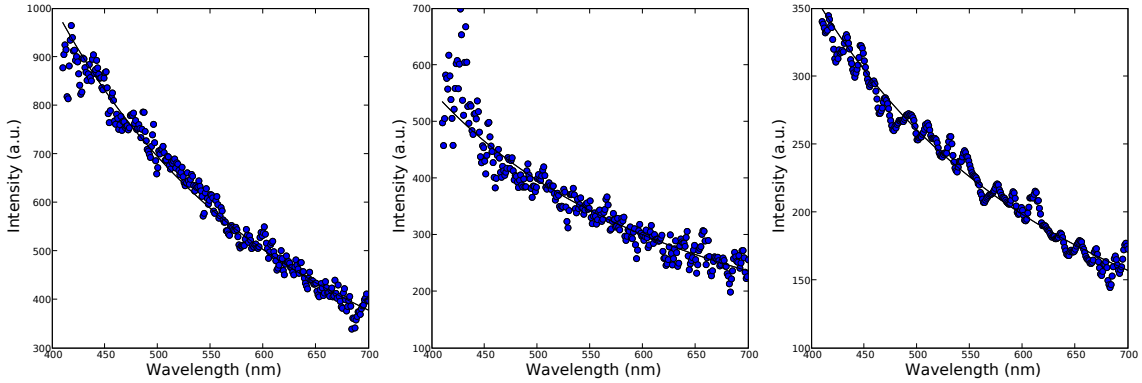


**Figure 3.** Fitting for light scattering by the epithelial cell suspension at scattering angles of 32.2, 90.0 and 173.8 degrees.

## 4. DISCUSSION AND CONCLUSION

The significance of this unified model for light scattering by biological cells is its capability to fit reasonably well the angular light scattering patterns from cells across all scattering angles and within the whole investigated spectral range (400nm-700nm). It is also worth pointing out that the reduced scattering coefficient being probed in optical diffuse tomography is proportional to $\int (1-\cos\theta)\overline{|S_{\rm cell}(\theta)|^2} d\cos\theta \simeq \int (1-\cos\theta)\,|S_{\rm bg}(\mathbf{q})|^2\, d\cos\theta$ and depends mainly on the random fluctuation of the background refractive index. The Mie scattering component concentrates in the forward angles and is much suppressed by the $(1-\cos\theta)$ factor. This is the reason why the fractal random continuous medium model works well for biological tissues and cells in the context of diffuse imaging. This is also the origin of the power law relation between the reduced scattering and the wavelength of the probing light despite the presence of Mie scattering in single light scattering by a cell.

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