# Multiple-scattering modelling of scattering by biological cells

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## Abstract

Past work on scattering by biological cells has been restricted to unrealistic over-simplified models of cells, or uses slow methods unsuited for calculations involving large numbers of cells or varying illumination. We consider the systematic use of fast, accurate methods for modelling light scattering in realistic complex biological cells. This will allow, for example, the study of light scattering by realistic ensembles of cells, which is immediately applicable to optical cancer detection, and remote sensing of microorganisms. It will also be possible to study light scattering by cells under constantly varying illumination, such as encountered in applications like flow cytometry.

## 1    Introduction

Light scattering by cells is of great practical interest, evidenced by its widespread use in laboratories for the analysis of cells. For example, flow cytometry [1], where cells pass one at a time through a focussed laser beam, allows rapid counting, identification, and sorting of cells. The saving in time and effort compared to manual counting using a microscope is enormous. However, the practical application of light scattering by cells is greatly in advance of theoretical developments. As a result, only very limited information about the size, shape, and optical properties of cells can be determined. There is no inherent reason why such analytical techniques cannot be made much more powerful by making greater use of the information contained in the scattered light, for example, to probe cell structure and properties [2, 3, 4, 5]. Light scattering can be used to study both scattering by individual cells, possibly confined using optical tweezers, or on populations of cells, such as phytoplankton in natural waters.

In particular, we note that light scattering is a potentially valuable tool for early cancer detection, since cancerous and pre-cancerous cells can exhibit changes in the sizes and refractive indices of their nuclei, both of which strongly affect the light scattering properties of the cell, altering both the spatial distribution of scattered light and its polarisation. For example, the nuclei of normal epithelial cells are typically 5–10 $\mu$m in diameter, while dysplastic nuclei (ie in cells with pre-cancerous changes) can be as large as 20 $\mu$m. This difference strongly affects the light scattering pattern of the cells. Light scattering is an attractive technology for cancer detection as it could allow rapid and inexpensive

scanning of large areas of surface tissue such as skin for early stages of cancer—currently histological analysis of biopsy specimens is required. While only surface tissues can be optically scanned (and internal surfaces are accessible via endoscopes), it should be noted that 85% of all cancers originate in the epithelium. Optical cancer detection by light scattering has recently been experimentally shown to be feasible [6, 7], and a proper theoretical understanding of the optical phenomena involved will be of great benefit to the practical development of this valuable diagnostic technique. The is especially important when considering the use of polarisation of scattered light as a diagnostic tool, since this is a purely wave effect that cannot be adequately dealt with using approximate methods, necessitating solution of Maxwell's equations.

## 2  Light scattering by cells

The eukaryote cell is a complex structure, composed of organelles such as a nucleus, mitochondria, chloroplasts, etc., contained within the cytoplasm, and enclosed within a cell membrane (and possibly a cell wall). The organelles have sizes on the order of a few wavelengths, for visible and near infrared light, and neither cells nor their organelles are spherical, precluding the use of Mie theory. Light scattering by cells has been calculated by solving Maxwell's equations using finite-difference methods [8, 9], but these methods are slow, especially when repeated calculations for varying illumination are required.

In comparison, other methods for computational light scattering, such as the $T$-matrix method, are much faster, but are restricted to homogeneous particles. In addition, the greatest benefits in speed only result when the scatterer is axisymmetric. These restrictions appear to make these methods inapplicable for modelling light scattering by cells. On the other hand, the individual organelles within a typical cell can mostly be considered to be homogeneous and axisymmetric, as a first approximation. While this is less realistic, the computational benefit is large—scattering by most individual organelles can be calculated using fast methods. An added benefit of axisymmetric organelles is the resulting sparsity of the $T$-matrix resulting it being diagonal w.r.t $m$ (that is, $T$-matrix elements coupling incident field modes $n_1, m_1$ to scattered field modes $n_2, m_2$ are only non-zero if $m_1 = m_2$).

Complex composite systems can be modelled as collections of individual simple scatterers, with the scattering properties of the combined system determined from the individual scattering properties, allowing the calculation to be broken down into manageable sub-problems. This has only recently become computationally feasible due to the growth of available computer power. Rapid development of new techniques is now taking place (for example, [10, 11]), and the application of these methods to realistic cases of practical interest appears feasible.

Scattering by organelles of simple shape and homogeneous structure can be readily calculated using methods such as the extended boundary condition method, giving the scattering properties of the organelle as a $T$-matrix. Individual organelle $T$-matrices can be combined using multiple scattering methods to obtain a $T$-matrix for the cell as a whole.

While some organelles might not be sufficiently homogeneous or of suitable shape for direct application of the $T$-matrix method, "hybrid" $T$-matrix methods where a general method of computational light scattering, such as finite-difference methods or the discrete-dipole approximation, is used to calculate the $T$-matrix for the scatterer [12, 13].

## 3  Implementation

The rapid and efficient calculation of scattering by realistic cells is a formidable computational task. However, the basic tool—calculation of the $T$-matrix for a homogeneous axisymmetric scatterer—is readily available. Existing codes are accurate and very fast for axisymmetric scatterers with sizes, shapes, and refractive index constrast typical of organelles. A brief recipe for the entire calculation:
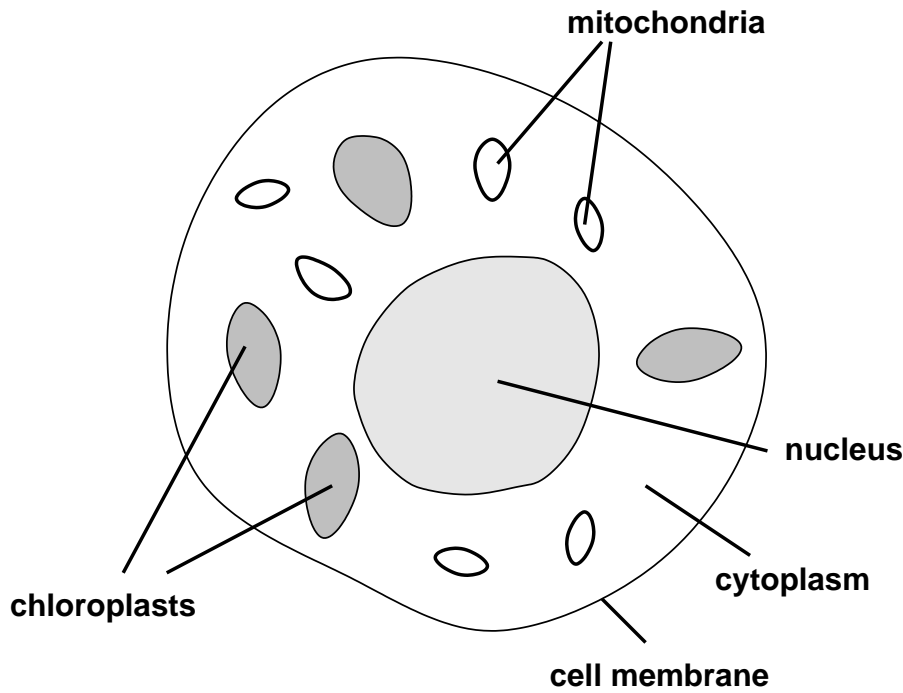
1. Generate cell

Figure 1: A typical eukaryote cell is composed of simpler organelles.

    (a) determine total number of organelles of each type

    (b) generate individual organelles

2. Calculate $T$-matrix for cell

    (a) calculate $T$-matrix for organelle (using hybrid methods if necessary)

    (b) combine individual organelle $T$-matrices to determine whole cell $T$-matrix

3. Calculate scattering by whole cell

shows that the main required remaining components are (i) methods for generating cells, and (ii) a multiple scattering code. While multiple scattering codes exist, the number of high-quality publicly available codes is very small. Accordingly, we are focussing on these two key ingredients in our current project to computationally model light scattering by cells.

Generation of realistic cells is the simpler of these two tasks; while appropriate choice of methods and data structures is important, and correct input data is needed, this is primarily an exercise in programming. Two elements of this part of the project deserve further mention: care should be taken to maximise re-use of particular organelles (allowing direct re-use of $T$-matrices for them, which is especially important for "difficult" organelles requiring slower hybrid $T$-matrix methods, and model cells checked for potential difficulties with convergence or applicability of the multiple scattering methods (such as when circumscribing spheres about organelles touch or overlap).

A key component of the multiple scattering portion of the code is the efficient calculation of translation and rotation matrices for the re-expansion of multipole fields about new origins, or the translation or rotation of organelle $T$-matrices. Noting that

- multipole rotation matrices are diagonal w.r.t. $m$, and

- multipole translation matrices along the $z$-axis are diagonal w.r.t. $n$,

it can readily be seen that rotation–axial translation–rotation takes on the order of $N_{\max}$ less storage and time compared to direct translation. We also note that axial translation matrix elements can

be calculated directly using recurrence relations, without the need to calculate Gaunt coefficients, Wigner $3j$ symbols, or Clebsch–Gordan coefficients.

A final task would be consider scattering by multiple cells—tissue or a suspension of cells. One of our goals is to determine the accuracy of single-scattering approximations in such cases.

Our implementation of a multiple-scattering cell scattering code is in progress. Our successes, surprises, and problems will be reported.

## 4 Conclusion

While the calculation of light scattering by realistic cells remains a difficult and demanding computational task, multiple scattering methods can be used to model cells as collections of individually simple scatterers which can be readily modelled using well-known methods. We presented a recipe for the practical implementation of this process, and considered some key elements of the method.

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