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Tanev, Stoyan

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FDTD Simulation of Light Interaction with Cells for Diagnostics and Imaging in Nanobiophotonics

Stoyan Tanev

Integrative Innovation Management Unit, Department of Industrial and Civil Engineering, University of Southern Denmark, Niels Bohrs Alle 1, DK-5230 Odense M, Denmark

Wenbo Sun

Science Systems and Applications, Inc., USA

James Pond

Lumerical Solutions, Vancouver, BC, Canada

Valery V. Tuchin

Research-Educational Institute of Optics and Biophotonics, Saratov State University, Saratov, 410012, Russia, Institute of Precise Mechanics and Control of RAS, Saratov 410028, Russia

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This chapter describes the mathematical formulation of the Finite-Difference Time-Domain (FDTD) approach and provides examples of its applications to biomedical photonics problems. The applications focus on two different configurations - light scattering from single biological cells and Optical Phase Contrast Microscope (OPCM) imaging of cells containing gold nanoparticles. The validation of the FDTD

1 Formerly with the Technology Innovation Management Program in the Department of Systems and Computer Engineering, Faculty of Engineering and Design, Carleton University, Ottawa, ON, Canada.
approach for the simulation of OPCM imaging opens a new application area with a significant research potential — the design and modeling of advanced nanobiophotonic imaging instrumentation.

**Key words:** Finite-Difference Time-Domain (FDTD) method, light scattering, biological cell, gold nanoparticle, Optical Phase Contrast Microscope (OPCM) imaging, optical clearing effect, image contrast enhancement, nanobiophotonics.

### 2.1 Introduction

The development of non-invasive optical methods for biomedical diagnostics requires a fundamental understanding of how light scatters from normal and pathological structures within biological tissue. It is important to understand the nature of the light scattering mechanisms from micro-biological structures and how sensitive the light scattering parameters are to the dynamic pathological changes of these structures. It is equally important to quantitatively relate these changes to corresponding variations of the measured light scattering parameters. Unfortunately, the biological origins of the differences in the light scattering patterns from normal and pathological (for example, pre-cancerous and cancerous) cells and tissues are not fully understood. The major difficulty comes from the fact that most of the advanced optical biodiagnostics techniques have a resolution comparable to the dimensions of the cellular and sub-cellular light scattering structures [1–2]. For example, conventional Optical Coherence Tomography (OCT) techniques do not provide a resolution at the sub-cellular level. Although there are already examples of ultrahigh resolution OCT capabilities based on the application of ultra-short pulsed lasers [3–4], it will take time before such systems become easily commercially available. This makes the interpretation of images generated by typical OCT systems difficult and in many cases inefficient. Confocal microscopy provides sub-cellular resolution and allows for diagnostics of nuclear and sub-nuclear level cell morphological features. However, there are problems requiring a careful and not trivial interpretation of the images [5]. In its typical single photon form, confocal fluorescence microscopy involves an optical excitation of tissue leading to fluorescence that occurs along the exciting cone of the focused light beam, thus increasing the chances of photo-bleaching of a large area and making the interpretation of the image difficult.

In many nanobiophotonics diagnostics and imaging research studies optical software simulation and modeling tools provide the only means to a deeper understanding, or any understanding at all, of the underlying physical and biochemical processes. The tools and methods for the numerical modeling of light scattering from single or multiple biological cells are of particular interest since they could provide information about the fundamental light-cell interaction phenomena that is highly relevant for the practical interpretation of cell images by pathologists. The compu-
tational modeling of light interaction with cells is usually approached from a single particle electromagnetic wave scattering perspective which could be characterized by two specific features. First, this is the fact that the wavelength of light is larger than or comparable to the size of the scattering sub-cellular structures. Second, this is the fact that biological cells have irregular shapes and inhomogeneous refractive index distributions which makes it impossible to use analytical modeling approaches. Both features necessitate the use of numerical modeling approaches derived from rigorous electromagnetic theory such as: the method of separation of variables, the finite element method, the method of lines, the point matching method, the method of moments, the discrete dipole approximation method, the null-field (extended boundary condition) method, the T-matrix electromagnetic scattering approach, the surface Green’s function electromagnetic scattering approach, and the finite-difference time domain (FDTD) method [6].

The FDTD simulation and modeling of the light interaction with single and multiple, normal and pathological biological cells and sub-cellular structures has attracted the attention of researchers since 1996 [7–25]. The FDTD approach was first adopted as a better alternative of Mie theory [26–27] allowing for the modeling of irregular cell shapes and inhomogeneous distributions of complex refractive index values. The emerging relevance of nanobiophotonics imaging research has established the FDTD method as one of the powerful tools for studying the nature of light-cell interactions. One could identify number of research directions based on the FDTD approach. The first one focuses on studying the lateral light scattering patterns for the early detection of pathological changes in cancerous cells such as increased nuclear size and degrees of nuclear pleomorphism and nuclear-to-cytoplasmic ratios [7–17]. The second research direction explores the application of FDTD-based approaches for time-resolved diffused optical tomography studies [18–20]. A third direction is the application of the FDTD method to the modeling of advanced cell imaging techniques within the context of a specific biodiagnostics device scenario [21–25]. An emerging research direction consists in the extension of the FDTD approach to account for optical nanotherapeutic effects.

The present chapter will provide a number of examples illustrating the application of the FDTD approach in situations associated with the first two research directions. It is organized as follows. Section two provides a detailed summary of the formulation of the FDTD method including the basic numerical scheme, near-to-far field transformation, advanced boundary conditions, and specifics related to its application to the modeling of light scattering and optical phase contrast microscope (OPCM) imaging experiments. Examples of FDTD modeling of light scattering from and OPCM imaging of single biological cells are given in sections 3 and 4. The last section summarizes the conclusions.
2.2 Formulation of the FDTD Method

2.2.1 The basic FDTD numerical scheme

The finite-difference time domain (FDTD) technique is an explicit numerical method for solving Maxwell’s equations. It was invented by Yee in 1966 [28]. The advances of the various FDTD approaches and applications have been periodically reviewed by Taflove et al. [29]. The explicit finite-difference approximation of Maxwell’s equations in space and time will be briefly summarized following Taflove et al. [29] and Sun et al. [30–32].

In a source free absorptive dielectric medium Maxwell’s equations have the form:

\[ \nabla \times \vec{E} = -\mu_0 \frac{\partial \vec{H}}{\partial t}, \quad (2.1) \]
\[ \nabla \times \vec{H} = \varepsilon_0 \varepsilon_r \frac{\partial \vec{E}}{\partial t}, \quad (2.2) \]

where \( \vec{E} \) and \( \vec{H} \) are the vectors of the electric and magnetic fields, respectively, \( \mu_0 \) is the vacuum permeability and \( \varepsilon_0 \varepsilon_r \) is the permittivity of the medium. Assuming a harmonic \( [= \exp(-i\omega t)] \) time dependence of the electric and magnetic fields and a complex value of the relative permittivity \( \varepsilon = \varepsilon_r + i\varepsilon_i \) transforms Eq. (2.2) as follows:

\[ \nabla \times \vec{H} = \varepsilon_0 \varepsilon_r \frac{\partial \vec{E}}{\partial t} \Leftrightarrow \nabla \times \vec{H} = \omega \varepsilon_0 \varepsilon_r \vec{E} + \varepsilon_0 \varepsilon_i \frac{\partial \vec{E}}{\partial t} \Leftrightarrow \frac{\partial (\exp(\tau t) \vec{E})}{\partial t} = \frac{\exp(\tau t)}{\varepsilon_0 \varepsilon_r} \nabla \times \vec{H}, \quad (2.3) \]

where \( \tau = \frac{\omega \varepsilon_r}{\varepsilon_i} \) and \( \omega \) is the angular frequency of the light. The continuous coordinates \((x,y,z)\) are replaced by discrete spatial and temporal points: \( x_i = i \Delta x, \ y_j = j \Delta y, \ z_k = k \Delta z \), \( t_n = n \Delta t \), where \( i = 0, 1, 2, \ldots, I \), \( j = 0, 1, 2, \ldots, J \), \( k = 0, 1, 2, \ldots, K \), \( n = 0, 1, 2, \ldots, N \). \( \Delta s \) and \( \Delta t \) denote the cubic cell size and time increment, respectively. Using central difference approximations for the temporal derivatives over the time interval \([n \Delta t, (n + 1) \Delta t] \) leads to

\[ \vec{E}^{n+1} = \exp(-\tau \Delta t) \vec{E}^n + \exp(-\tau \Delta t/2) \frac{\Delta t}{\varepsilon_0 \varepsilon_r} \nabla \times \vec{H}^{n+1/2}, \quad (2.4) \]

where the electric and the magnetic fields are calculated at alternating half-time steps. The discretization of Eq. (2.1) over the time interval \([n - 1/2 \Delta t, (n + 1/2) \Delta t] \) (one half time step earlier than the electric field) ensures second-order accuracy of the numerical scheme. In a Cartesian coordinate system the numerical equations for the \( x \) components of the electric and magnetic fields take the form

\[ H^{n+1/2}_x(i, j + 1/2, k + 1/2) = H^n_{x_{1/2}}(i, j + 1/2, k + 1/2) + \frac{\Delta t}{\mu_0 \Delta s} \times [E^n_y(i, j + 1/2, k + 1) - E^n_y(i, j + 1/2, k) + E^n_z(i, j + 1/2, k + 1) - E^n_z(i, j + 1/2, k + 1/2)], \quad (2.5) \]
FDTD Simulation of Light Interaction with Cells

FIGURE 2.1: Positions of the electric- and the magnetic-field components in the elementary (Yee) cubic cell of the FDTD lattice.

\[ E^{n+1}_x(i + 1/2, j, k) = \exp[-\frac{\epsilon_e}{\epsilon_r(i + 1/2, j, k)} \omega \Delta t] E^n_x(i + 1/2, j, k) + \]
\[ \exp[-\frac{\epsilon_e}{\epsilon_r(i + 1/2, j, k)} \omega \Delta t/2] \frac{\Delta t}{\epsilon_e(i + 1/2, j, k)\Delta s} \]
\[ \times [H^{n+1/2}_y(i + 1/2, j, k - 1/2) - H^n_{y+1/2}(i + 1/2, j, k + 1/2) + H^{n+1/2}_z(i + 1/2, j + 1/2, k) - H^n_{z+1/2}(i + 1/2, j - 1/2, k)], \]  

where \( E_x, E_y, E_z \) and \( H_x, H_y, H_z \) denote the electric and magnetic field components, respectively. The numerical stability of the FDTD scheme is ensured through the Courant-Friedrichs-Levy condition [29]: \( c \Delta t \leq (1/\Delta x^2 + 1/\Delta y^2 + 1/\Delta z^2)^{1/2}, \) where \( c \) is the speed of light in the host medium and \( \Delta x, \Delta y, \Delta z \) are the spatial steps in the \( x, y \) and \( z \) direction, respectively. In our case \( \Delta x = \Delta y = \Delta z = \Delta s \) and \( \Delta t = \Delta s/2c. \) The positions of the magnetic and electric field components in a FDTD cubic cell are shown in Fig. 2.1.

2.2.2 Numerical excitation of the input wave

We use the so-called total-field/scattered-field formulation [29, 32] to excite the input magnetic and electric fields and simulate a linearly polarized plane wave propagating in a finite region of a homogeneous absorptive dielectric medium. In this formulation, a closed surface is defined inside of the computational domain. Based on the equivalence theorem [29], the input wave excitation within a given spatial domain can be replaced by the equivalent electric and magnetic currents located at the
FIGURE 2.2: Example of a closed rectangular surface separating the total fields and scattered fields. The graph also shows the configuration of the one-dimensional auxiliary FDTD grid that is used to calculate the input excitation fields [see the paragraph below Eq. (2.16)].

closed surface enclosing that domain. If there is a scatterer inside the closed surface, the interior fields will be the total fields (incident plus scattered) and the exterior fields are just the scattered fields. An example of geometrical configuration of such closed surface (in this case rectangular) is shown in Fig. 2.2.

On the closed surface the electric and magnetic field incident sources are added as follows:

\[
\vec{H} = \vec{H} - \frac{\Delta t}{\mu_0 \Delta s} (\vec{E}_{inc} \times \vec{n}),
\]

\[
\vec{E} = \vec{E} - \frac{\Delta t}{\varepsilon_0 \varepsilon \Delta s} (\vec{n} \times \vec{H}_{inc}),
\]

where \(\vec{E}_{inc}\) and \(\vec{H}_{inc}\) are the incident fields and \(\vec{n}\) is the inward normal vector of the closed surface [29]. Eqs. (2.5ab) take different forms at the different interfaces of the closed rectangular surface \(O_1O_2O_3O_4O'_1O'_2O'_3O'_4\) shown in Fig. 2.2.

At the interface \((i = i_a, \ldots, i_b; j = j_a - 1/2; k = k_a + 1/2, \ldots, k_b - 1/2)\):

\[
H_s^{n+1/2}(i, j_a - 1/2, k) = \{H_s^{n+1/2}(i, j_a - 1/2, k)\}_{(1a)} + \frac{\Delta t}{\mu_0 \Delta s} E_{z,inc}^n(i, j_a, k); \quad (2.9)
\]

at the interface \((i = i_a, \ldots, i_b; j = j_b + 1/2; k = k_a + 1/2, \ldots, k_b - 1/2)\):

\[
H_s^{n+1/2}(i, j_b + 1/2, k) = \{H_s^{n+1/2}(i, j_b + 1/2, k)\}_{(1a)} - \frac{\Delta t}{\mu_0 \Delta s} E_{z,inc}^n(i, j_b, k); \quad (2.10)
\]
at the interface \((i = i_a, \ldots, i_b; j = j_a + 1/2, \ldots, j_b - 1/2; k = k_a - 1/2)\):

\[
H^{n+1/2}_x(i, j, k_a - 1/2) = \{H^{n+1/2}_x(i, j, k_a - 1/2)\}_{(1a)} - \frac{\Delta t}{\mu_0 \Delta x} E_{x,inc}^n(i, j, k_a); \quad (2.11)
\]

at the interface \((i = i_a, \ldots, i_b; j = j_a + 1/2, \ldots, j_b - 1/2; k = k_b + 1/2)\):

\[
H^{n+1/2}_x(i, j, k_b + 1/2) = \{H^{n+1/2}_x(i, j, k_b + 1/2)\}_{(1a)} + \frac{\Delta t}{\mu_0 \Delta x} E_{x,inc}^n(i, j, k_b); \quad (2.12)
\]

at the interface \((i = i_a + 1/2, \ldots, i_b - 1/2; j = j_a; k = k_a, \ldots, k_b)\):

\[
E^{n+1}_x(i, j_a, k) = \{E^{n+1}_x(i, j_a, k)\}_{(1b)} - \exp \left[ -\frac{\mu_0 \omega \Delta t}{2} \right] \frac{\Delta x}{\epsilon_0 \mu_0 (i, j_a, k)} H^{n+1/2}_z,inc(i, j_a - 1/2, k); \quad (2.13)
\]

at the interface \((i = i_a + 1/2, \ldots, i_b - 1/2; j = j_b; k = k_a, \ldots, k_b)\):

\[
E^{n+1}_x(i, j_b, k) = \{E^{n+1}_x(i, j_b, k)\}_{(1b)} + \exp \left[ -\frac{\mu_0 \omega \Delta t}{2} \right] \frac{\Delta x}{\epsilon_0 \mu_0 (i, j_b, k)} H^{n+1/2}_z,inc(i, j_b + 1/2, k); \quad (2.14)
\]

at the interface \((i = i_a + 1/2, \ldots, i_b - 1/2; j = j_a, \ldots, j_b; k = k_a)\):

\[
E^{n+1}_x(i, j, k_a) = \{E^{n+1}_x(i, j, k_a)\}_{(1b)} + \exp \left[ -\frac{\mu_0 \omega \Delta t}{2} \right] \frac{\Delta x}{\epsilon_0 \mu_0 (i, j, k_a)} H^{n+1/2}_y,inc(i, j_a - 1/2); \quad (2.15)
\]

at the face \((i = i_a + 1/2, \ldots, i_b - 1/2; j = j_a, \ldots, j_b; k = k_b)\):

\[
E^{n+1}_x(i, j, k_b) = \{E^{n+1}_x(i, j, k_b)\}_{(1b)} - \exp \left[ -\frac{\mu_0 \omega \Delta t}{2} \right] \frac{\Delta x}{\epsilon_0 \mu_0 (i, j, k_b)} H^{n+1/2}_y,inc(i, j, k_b + 1/2). \quad (2.16)
\]

The incident fields \(E_{y,inc}^n, E_{z,inc}^n\) and \(H_{y,inc}^{n+1/2}, H_{z,inc}^{n+1/2}\) in Eqs. (2.9)–(2.12) and (2.13)–(2.16) are calculated by means of a linear interpolation of the fields on an auxiliary one-dimensional linear FDTD grid. This auxiliary numerical scheme pre-simulates the propagation of an incident plane wave along a line starting at the origin of the 3D grid \(m = 0\), passing through the closest corner of the closed rectangular surface located at \(m = m_0\), and stretching in the incident wave direction to a maximum position \(m = m_{\text{max}}\), as shown in Fig. 2.2. The incident wave vector \(k_{\text{inc}}\) is oriented with a zenith angle \(\theta\) and an azimuth angle \(\phi\). \(m_{\text{max}}\) is chosen to be half of the total number of simulation time steps for the incident wave propagation in the entire absorptive dielectric medium. Since it is impossible to use a transmitting boundary condition for the truncation of the one-dimensional spatial domain, the selected \(m_{\text{max}}\) value needs to ensure that no numerical reflection occurs at the forward end of the one-dimensional grid before the 3D FDTD simulation ends. A Gaussian-pulse hard wave source [29] is positioned at the \(m = 2\) grid point in the form
\[ E_{inc}^n(m = 2) = \exp \left[ -\left( \frac{t}{30\Delta t} - 5 \right)^2 \right]. \]  

(2.17)

By using the hard wave source rather than a soft one at \( m = 2 \), the field at the grid points \( m = 0 \) and 1 will not affect the field at the grid points \( m > 2 \). Therefore, there is no need of boundary conditions at this end of the auxiliary one-dimensional FDTD grid.

Assuming that a plane wave is incident from the coordinate origin to the closed rectangular surface between the total- and scattered-fields as shown in Fig. 2.2, the one-dimensional FDTD grid equations become

\[ H_{inc}^{n+1/2}(m + 1/2) = H_{inc}^{n-1/2}(m + 1/2) + \frac{\Delta t}{\mu_0 \Delta s \left[ \frac{\nu_p(0,0)}{\nu_p(\theta,\phi)} \right]} \left[ E_{inc}^n(m) - E_{inc}^n(m + 1) \right], \]  

(2.18)

\[ E_{inc}^{n+1}(m) = \exp \left[ -\frac{\varepsilon_i(m)}{\varepsilon_r(m)} \omega \Delta t \right] E_{inc}^n(m) + \exp \left[ -\frac{\varepsilon_i(m)}{\varepsilon_r(m)} \omega \Delta t \right] \frac{\Delta t}{\varepsilon_0 \varepsilon_r(m) \Delta t \left[ \frac{\nu_p(0,0)}{\nu_p(\theta,\phi)} \right]} \left[ H_{inc}^{n+1/2}(m - 1/2) - H_{inc}^{n+1/2}(m + 1/2) \right], \]  

(2.19)

where \( \varepsilon_i(m) \) and \( \varepsilon_r(m) \) denote the imaginary and real relative permittivity of the host medium at position \( m \), respectively. The equalization factor \( \left[ \nu_p(0,0)/\nu_p(\theta,\phi) \right] \leq 1 \) is the numerical phase velocity ratio in the 3D FDTD grid [29].

### 2.2.3 Uni-axial perfectly matched layer absorbing boundary conditions

The FDTD numerical scheme presented here uses the Uni-axial Perfectly Matcher Layer (UPML) suggested by Sacks et al. [33] to truncate the absorptive host medium in the FDTD computational domain. The UPML approach is based on the physical introduction of absorbing anisotropic, perfectly matched medium layers at all sides of the rectangular computational domain. The anisotropic medium of each of these layers is uni-axial and is composed of both electric permittivity and magnetic permeability tensors. To match a UPML layer along a planar boundary to a lossy isotropic half-space characterized by permittivity \( \varepsilon \) and conductivity \( \sigma \), the time-harmonic Maxwell’s equations can be written in forms [34–35].

\[ \nabla \times \bar{H}(x,y,z) = (i\omega \varepsilon_0 \varepsilon + \sigma)\bar{E}(x,y,z), \]  

(2.20)

\[ \nabla \times \bar{E}(x,y,z) = -i\omega \mu_0 \bar{H}(x,y,z). \]  

(2.21)

The diagonal tensor \( \bar{s} \) is defined as follows

\[ \bar{s} = \begin{bmatrix} s_x^{-1} & 0 & 0 \\ 0 & s_y^{-1} & 0 \\ 0 & 0 & s_z^{-1} \end{bmatrix} = \begin{bmatrix} s_x & 0 & 0 \\ 0 & s_y & 0 \\ 0 & 0 & s_z \end{bmatrix} = \begin{bmatrix} s_x s_y s_z^{-1} & 0 & 0 \\ 0 & s_x s_y & 0 \\ 0 & 0 & s_x s_y s_z^{-1} \end{bmatrix}, \]  

(2.22)
where $s_x = \kappa_x + \sigma_x \omega \varepsilon_0$, $s_y = \kappa_y + \sigma_y \omega \varepsilon_0$, and $s_z = \kappa_z + \sigma_z \omega \varepsilon_0$.

The UPML parameters $(\kappa_x, \sigma_x)$, $(\kappa_y, \sigma_y)$, and $(\kappa_z, \sigma_z)$ are independent on the medium permittivity $\varepsilon$ and conductivity $\sigma$, and are assigned to the FDTD grid in the UPML as follows: i) in the two absorbing layers at both ends of the computational domain in the $x$-direction, $\sigma_y = \sigma_z = 0$ and $\kappa_y = \kappa_z = 1$; in the two layers at both ends of the $y$-direction, $\sigma_x = \sigma_z = 0$ and $\kappa_x = \kappa_z = 1$; in the two layers at both ends of the $z$-direction, $\sigma_x = \sigma_y = 0$ and $\kappa_x = \kappa_y = 1$; ii) at the $x$ and $y$ overlapping dihedral corners, $\sigma_z = 0$ and $\kappa_z = 1$; at the $z$ and $x$ overlapping dihedral corners, $\sigma_y = 0$ and $\kappa_y = 1$; iii) at all overlapping trihedral corners, the complete general tensor in Eq. (2.22) is used. To reduce the numerical reflection from the UPML, several profiles have been suggested for incrementally increasing the values of $(\kappa_x, \sigma_x)$, $(\kappa_y, \sigma_y)$, and $(\kappa_z, \sigma_z)$. Here we use a polynomial grading of the UPML material parameters [34–35]. For example,

$$\kappa_x(x) = 1 + (x/d)^m(\kappa_{x,\text{max}} - 1),$$  \hspace{1cm} (2.23)

$$\sigma_x(x) = (x/d)^m \sigma_{x,\text{max}},$$  \hspace{1cm} (2.24)

where $x$ is the depth in the UPML and $d$ is the UPML thickness in this direction.

The parameter $m$ is a real number [35] between 2 and 4. $\kappa_{x,\text{max}}$ and $\sigma_{x,\text{max}}$ denote the maximum $\kappa_x$ and $\sigma_x$ at the outmost layer of the UPML. For example, considering an $x$-directed plane wave impinging at an angle $\theta$ upon a PEC-backed UPML with the polynomial grading material properties, the reflection factor can be derived as [35]

$$R(\theta) = \exp\left[-\frac{2 \cos \theta}{\varepsilon_0 c} \int_0^d \sigma(x) dx \right] = \exp\left[-\frac{2 \sigma_{x,\text{max}} d \cos \theta}{\varepsilon_0 c (m + 1)} \right].$$  \hspace{1cm} (2.25)

Therefore, if $R(0)$ is the reflection factor at normal incidence, $\sigma_{x,\text{max}}$ can be defined as

$$\sigma_{x,\text{max}} = -\frac{(m + 1) \ln|R(0)|}{2d/(\varepsilon_0 c)}. \hspace{1cm} (2.26)$$

Typically, the values of $R(0)$ are in the range between $10^{-12}$ to $10^{-5}$ and $\kappa_{x,\text{max}}$ is a real number between 1 to 30.

The UPML equations modify the FDTD numerical scheme presented by Eqs. (2.5) and (2.6). The modified UPML FDTD numerical scheme is then applied to the entire computational domain by considering the UPMLs as materials in a way no different than any other material in the FDTD grid. However, this is not computationally efficient. The usual approach is to apply the modified scheme only to the boundary layers in order to reduce the memory and CPU time requirements. In the non-UPML region, the unmodified FDTD formulation (Eqs. (2.5), (2.6)) is used. The derivation of the modified UPML FDTD numerical scheme is not trivial at all. To explicitly obtain the updating equations for the magnetic field in the UPML, an auxiliary vector field variable $\vec{B}$ is introduced as follows [35]
\[ B_x(x,y,z) = \mu_0 \left( \frac{\omega}{\kappa_x} \right) H_x(x,y,z), \quad B_y(x,y,z) = \mu_0 \left( \frac{\omega}{\kappa_y} \right) H_y(x,y,z), \quad B_z(x,y,z) = \mu_0 \left( \frac{\omega}{\kappa_z} \right) H_z(x,y,z). \]  

(2.27)

Then Eq. (2.21) can be expressed as

\[
\begin{bmatrix}
\frac{\partial E_x(x,y,z)}{\partial z} - \frac{\partial E_y(x,y,z)}{\partial y} \\
\frac{\partial E_x(x,y,z)}{\partial x} - \frac{\partial E_z(x,y,z)}{\partial z} \\
\frac{\partial E_y(x,y,z)}{\partial y} - \frac{\partial E_z(x,y,z)}{\partial x}
\end{bmatrix} = i\omega \begin{bmatrix} s_x & 0 & 0 \\
0 & s_y & 0 \\
0 & 0 & s_z
\end{bmatrix} \begin{bmatrix} B_x(x,y,z) \\
B_y(x,y,z) \\
B_z(x,y,z)
\end{bmatrix}. \tag{2.28}
\]

On the other hand, inserting the definitions of \( s_x, s_y \) and \( s_z \) into Eqs. (2.27) leads to

\[
(i\omega \kappa_x + \frac{\sigma_x}{\varepsilon_0}) B_x(x,y,z) = (i\omega \kappa_x + \frac{\sigma_x}{\varepsilon_0})\mu_0 H_x(x,y,z), \tag{2.29}
\]

\[
(i\omega \kappa_y + \frac{\sigma_y}{\varepsilon_0}) B_y(x,y,z) = (i\omega \kappa_y + \frac{\sigma_y}{\varepsilon_0})\mu_0 H_y(x,y,z), \tag{2.30}
\]

\[
(i\omega \kappa_z + \frac{\sigma_z}{\varepsilon_0}) B_z(x,y,z) = (i\omega \kappa_z + \frac{\sigma_z}{\varepsilon_0})\mu_0 H_z(x,y,z). \tag{2.31}
\]

Now applying the inverse Fourier transform by using the identity \( i\omega f(\omega) \rightarrow \frac{\partial f(t)}{\partial t} \) to Eqs. (2.28) and (2.29)–(2.31) gives the equivalent time-domain differential equations, respectively

\[
\begin{bmatrix}
\frac{\partial E_x(x,y,z,t)}{\partial x} - \frac{\partial E_y(x,y,z,t)}{\partial y} \\
\frac{\partial E_x(x,y,z,t)}{\partial y} - \frac{\partial E_z(x,y,z,t)}{\partial z} \\
\frac{\partial E_y(x,y,z,t)}{\partial x} - \frac{\partial E_z(x,y,z,t)}{\partial y}
\end{bmatrix} = \frac{\partial}{\partial t} \begin{bmatrix} \kappa_x & 0 & 0 \\
0 & \kappa_y & 0 \\
0 & 0 & \kappa_z
\end{bmatrix} \begin{bmatrix} B_x(x,y,z,t) \\
B_y(x,y,z,t) \\
B_z(x,y,z,t)
\end{bmatrix} + \frac{1}{i\omega_0} \begin{bmatrix} \sigma_x & 0 & 0 \\
0 & \sigma_y & 0 \\
0 & 0 & \sigma_z
\end{bmatrix} \begin{bmatrix} B_u(x,y,z,t) \\
B_v(x,y,z,t) \\
B_w(x,y,z,t)
\end{bmatrix}, \tag{2.32}
\]

\[
\kappa_x \frac{\partial B_x(x,y,z,t)}{\partial t} + \frac{\sigma_x}{\varepsilon_0} B_x(x,y,z,t) = \mu \kappa_x \frac{\partial H_x(x,y,z,t)}{\partial t} + \mu \frac{\sigma_x}{\varepsilon_0} H_x(x,y,z,t), \tag{2.33}
\]

\[
\kappa_y \frac{\partial B_y(x,y,z,t)}{\partial t} + \frac{\sigma_y}{\varepsilon_0} B_y(x,y,z,t) = \mu \kappa_y \frac{\partial H_y(x,y,z,t)}{\partial t} + \mu \frac{\sigma_y}{\varepsilon_0} H_y(x,y,z,t), \tag{2.34}
\]

\[
\kappa_z \frac{\partial B_z(x,y,z,t)}{\partial t} + \frac{\sigma_z}{\varepsilon_0} B_z(x,y,z,t) = \mu \kappa_z \frac{\partial H_z(x,y,z,t)}{\partial t} + \mu \frac{\sigma_z}{\varepsilon_0} H_z(x,y,z,t). \tag{2.35}
\]

After discretizing Eqs. (2.32) and (2.33)–(2.35), we can get the explicit FDTD formulations for the magnetic field components in the UPML [29, 32]:
\[ B_{n+1/2}^{x}(i, j + 1/2, k + 1/2) = \left( \frac{2\varepsilon_0 \kappa_n - \sigma_1 \Delta t}{2\varepsilon_0 \kappa_n + \sigma_1 \Delta t} \right) B_{n}^{x}(i, j + 1/2, k + 1/2) \]
\[ + \left( \frac{2\varepsilon_0 \Delta t / \Delta x}{2\varepsilon_0 \kappa_n + \sigma_1 \Delta t} \right) [E_{n}^{y}(i, j + 1/2, k + 1) \]
\[ - E_{n}^{y}(i, j + 1/2, k) + E_{n}^{z}(i, j, k + 1/2) - E_{n}^{z}(i, j + 1, k + 1/2) ] , \]  

(2.36)

\[ H_{n+1/2}^{y}(i, j + 1/2, k + 1/2) = \left( \frac{2\varepsilon_0 \kappa_n - \sigma_2 \Delta t}{2\varepsilon_0 \kappa_n + \sigma_2 \Delta t} \right) H_{n}^{y}(i, j + 1/2, k + 1/2) \]
\[ + \left( \frac{1/\mu}{2\varepsilon_0 \kappa_n + \sigma_2 \Delta t} \right) [(2\varepsilon_0 \kappa_n + \sigma_2 \Delta t) B_{n}^{x+1/2}(i, j + 1/2, k + 1/2) \]
\[ - (2\varepsilon_0 \kappa_n - \sigma_2 \Delta t) B_{n+1/2}^{x}(i, j + 1/2, k + 1/2) ] . \]  

(2.37)

Similarly, for electric field in the UPML, two auxiliary field variables \( \vec{P} \) and \( \vec{Q} \) are introduced as follows [32, 35]

\[ P_{x}(x, y, z) = \left( \frac{\kappa_{x}^{y} \kappa_{z}^{y}}{\kappa_{x}^{y}} \right) E_{x}(x, y, z) , \]  

(2.38)

\[ P_{y}(x, y, z) = \left( \frac{\kappa_{x}^{y} \kappa_{z}^{y}}{\kappa_{y}^{y}} \right) E_{y}(x, y, z) , \]  

(2.39)

\[ P_{z}(x, y, z) = \left( \frac{\kappa_{x}^{y} \kappa_{z}^{y}}{\kappa_{z}^{y}} \right) E_{z}(x, y, z) , \]  

(2.40)

\[ Q_{x}(x, y, z) = \left( \frac{1}{\kappa_{y}^{y}} \right) P_{x}(x, y, z) , \]  

(2.41)

\[ Q_{y}(x, y, z) = \left( \frac{1}{\kappa_{z}^{y}} \right) P_{y}(x, y, z) , \]  

(2.42)

\[ Q_{z}(x, y, z) = \left( \frac{1}{\kappa_{x}^{y}} \right) P_{z}(x, y, z) . \]  

(2.43)

Inserting Eqs. (2.38)–(2.40) into Eq. (2.20), simply following the steps in deriving Eq. (2.36), leads to the updating equations for the \( \vec{P} \) components:

\[ P_{n+1}^{x}(i + 1/2, j, k) = \left( \frac{2\varepsilon_0 \kappa_n - \sigma_3 \Delta t}{2\varepsilon_0 \kappa_n + \sigma_3 \Delta t} \right) P_{n}^{x}(i + 1/2, j, k) \]
\[ + \left( \frac{2\varepsilon_0 \Delta t / \Delta y}{2\varepsilon_0 \kappa_n + \sigma_3 \Delta t} \right) [H_{n}^{y+1/2}(i + 1/2, j + 1/2, k + 1/2) \]
\[ - H_{n+1/2}^{y}(i + 1/2, j, k + 1/2) - H_{n+1/2}^{y+1/2}(i + 1/2, j + 1/2, k) ] . \]  

(2.44)

From Eqs. (2.41)–(2.43), in an identical way to the derivation of Eq. (2.37), leads to the updating equations for the \( \vec{Q} \) components:

\[ Q_{n+1}^{x}(i + 1/2, j, k) = \left( \frac{2\varepsilon_0 \kappa_n - \sigma_3 \Delta t}{2\varepsilon_0 \kappa_n + \sigma_3 \Delta t} \right) Q_{n}^{x}(i + 1/2, j, k) \]
\[ + \left( \frac{2\varepsilon_0 \kappa_n - \sigma_3 \Delta t}{2\varepsilon_0 \kappa_n + \sigma_3 \Delta t} \right) [P_{n+1}^{x}(i + 1/2, j, k) - P_{n}^{x}(i + 1/2, j, k) ] . \]  

(2.45)
Inserting Eqs. (2.38)–(2.40) into Eqs. (2.41)–(2.43) and also following the procedure in deriving Eq. (2.37), leads to the electric field components in the UPML:

\[
E_{n+1}^x(i + 1/2, j, k) = \left( \frac{2\varepsilon_0 \kappa_z - \sigma_z}{2\varepsilon_0 \kappa_z + \sigma_z} \right) E_{n}^x(i + 1/2, j, k) + \left( \frac{1}{2\varepsilon_0 \kappa_z + \sigma_z} \right) \times \left[ (2\varepsilon_0 \kappa_x + \sigma_x \Delta t) Q_{n+1}^x(i + 1/2, j, k) - (2\varepsilon_0 \kappa_x - \sigma_x \Delta t) Q_{n}^x(i + 1/2, j, k) \right].
\]

### 2.2.4 FDTD formulation of the light scattering properties from single cells

The calculation of the light scattering and extinction cross sections by cells in free space requires the far-field approximation for the electromagnetic fields [29, 36]. The far-field approach has been also used [37] to study scattering and absorption by spherical particles in an absorptive host medium. However, when the host medium is absorptive, the scattering and extinction rates depend on the distance from the cell. Recently, the single-scattering properties of a sphere in an absorptive medium have been derived using the electromagnetic fields on the surface of the scattering object based on Mie theory [38–39]. Here we derive the absorption and extinction rates for an arbitrarily-shaped object in an absorptive medium using the internal electric field [32]. The absorption and extinction rates calculated in this way depend on the size, shape and optical properties of the scattering object and the surrounding medium, but do not depend on the distance from it. The single particle scattering approach is perfectly applicable to studying the light scattering properties from single biological cells.

**Amplitude scattering matrix**

For electromagnetic waves with time dependence \(\exp(-i\omega t)\) propagating in a charge-free dielectric medium, we can write Maxwell’s equations in the frequency domain as follows

\[
\nabla \times \vec{D} = 0, \quad \nabla \times \vec{H} = 0, \quad \nabla \times \vec{E} \rightarrow = i\omega \mu_0 \vec{H}, \quad \nabla \times \vec{H} = -i\omega \vec{D}. \tag{2.46}
\]

The material properties of the host medium are defined by the background permittivity \(\varepsilon_h\) and the electric displacement vector is defined as

\[
\vec{D} = \varepsilon_0 \varepsilon_h \vec{E} + \vec{P} = \varepsilon_0 \varepsilon \vec{E}, \tag{2.47}
\]

where here \(\vec{P}\) is the polarization vector. Given Eq. (2.47), the first and last equations in Eqs. (2.46) lead to

\[
\nabla \cdot \vec{E} = -\frac{1}{\varepsilon_0 \varepsilon_h} \nabla \cdot \vec{P}, \tag{2.48}
\]

\[
\nabla \times \vec{H} = -i\omega (\varepsilon_0 \varepsilon_h \vec{E} + \vec{P}). \tag{2.49}
\]
Combining the third equation in Eqs. (2.46) and Eqs. (2.48), (2.49) yields a source-dependent form of the electromagnetic wave equation

\[ (\nabla + k_h^2)\vec{E} = -\frac{1}{\varepsilon_0 \varepsilon_h} [k_h^2 \vec{P} + \nabla (\nabla \cdot \vec{P})], \tag{2.50} \]

where \( k_h = \omega \sqrt{\mu_0 \varepsilon_0 \varepsilon_h} \) is the complex wave number in the host medium. Using the unit dyad \( \vec{II} = \vec{x} \cdot \vec{x} + \vec{y} \cdot \vec{y} + \vec{z} \cdot \vec{z} \) (where \( \vec{x}, \vec{y} \) and \( \vec{z} \) are unit vectors in the \( x, y \) and \( z \) direction, respectively), we can rewrite Eq. (2.50) in the form

\[ (\nabla + k_h^2)\vec{E} = -\frac{1}{\varepsilon_0 \varepsilon_h} (k_h^2 \vec{II} + \nabla \nabla) \cdot \vec{P}. \tag{2.51} \]

Eq. (2.47) leads to

\[ \vec{P} = \varepsilon_0 (\varepsilon - \varepsilon_h) \vec{E} \]

which means that \( \vec{P} \) is nonzero only in the region inside the cell. The general solution of Eq. (2.51) is given by a volume integral equation [38]:

\[ \vec{E}(\vec{R}) = \vec{E}_0(\vec{R}) + \int_V G(\vec{R}, \vec{\xi}) (k_h^2 \vec{II} + \nabla \nabla) \times (\vec{P}/(\varepsilon_0 \varepsilon_h)) d^3 \vec{\xi}, \tag{2.52} \]

where \( \vec{E}_0(\vec{R}) \) can be any mathematical solution of \( (\nabla^2 + k_h^2)\vec{E} = 0 \) but, in practice, the only nontrivial solution here is the incident field in the host medium. The integration volume \( v \) is the region inside the particle and \( G(\vec{R}, \vec{\xi}) \) is the 3D Green function in the host medium:

\[ G(\vec{R}, \vec{\xi}) = \frac{\exp(ik_h|\vec{R} - \vec{\xi}|)}{4\pi |\vec{R} - \vec{\xi}|}. \tag{2.53} \]

The scattered field in the far-field region can then be derived from Eq. (2.52) [36]:

\[ \vec{E}_s(\vec{R}) \bigg|_{k_h R \to \infty} = \frac{k_h^2 \exp(ik_h R)}{4\pi R} \int_V \left[ \frac{\varepsilon(\vec{\xi})}{\varepsilon_h} - 1 \right] \left\{ \vec{E}(\vec{\xi}) - \vec{P}/(\varepsilon_0 \varepsilon_h) \right\} \exp(-ik_h \vec{R} \cdot \vec{\xi}) d^3 \vec{\xi}. \tag{2.54} \]

To calculate the amplitude scattering matrix elements, the incident and the scattered fields are decomposed into their components parallel and perpendicular to the scattering plane (Fig. 2.3).

The incident field is decomposed in two components along the unit vectors \( \vec{e}_\alpha \) and \( \vec{e}_\beta \) both laying in the \( X - Y \) plane and defined as parallel and perpendicular to the scattering plane, respectively:

\[ \vec{E}_0 = \vec{e}_\alpha \vec{E}_{0,\alpha} + \vec{e}_\beta \vec{E}_{0,\beta}. \tag{2.55} \]

\( \vec{E}_{0,\alpha} \) and \( \vec{E}_{0,\beta} \) are related to the \( x \)-polarized and \( y \)-polarized incident fields used in the FDTD simulation with

\[ \begin{pmatrix} \vec{E}_{0,\alpha} \\ \vec{E}_{0,\beta} \end{pmatrix} = \begin{bmatrix} \vec{\beta} \cdot \vec{x} & -\vec{\beta} \cdot \vec{y} \\ \vec{\beta} \cdot \vec{y} & \vec{\beta} \cdot \vec{x} \end{bmatrix} \begin{pmatrix} \vec{E}_{0,y} \\ \vec{E}_{0,x} \end{pmatrix}. \tag{2.56} \]
FIGURE 2.3: Incident and scattering wave configurations. The incident wave is propagating in the $Z$-direction. The unit vectors corresponding to the three coordinate axes are: $x, y, z$. The scattering direction is defined by the vector $\vec{R}$ with a unit vector $\vec{r} = |\vec{R}|/\vec{R}$. The $z$ coordinate axis and the vector $\vec{R}$ define the scattering plane. The unit vector $\vec{\alpha}$ is in the scattering plane and is perpendicular to $\vec{R}$ and $\vec{r}$. The unit vector $\vec{\beta}$ is perpendicular to the scattering plane and $\vec{\beta} \times \vec{\alpha} = \vec{r}$. The unit vectors $\vec{e}_\alpha$ and $\vec{e}_\beta$ are in the $X-Y$ plane and are, respectively, parallel and perpendicular to the scattering plane. All vectors in the figure are in bold.

The scattered field is decomposed in two components along the unit vectors $\vec{\alpha}$ and $\vec{\beta}$:

$$\vec{E}_s(\vec{R}) = \vec{\alpha}E_{s,\alpha}(\vec{R}) + \vec{\beta}E_{s,\beta}(\vec{R}).$$

It is important to note that the incident and scattered fields are specified relative to different sets of basis vectors. The relationship between the incident and scattered fields can be conveniently written in the following matrix form

$$\begin{pmatrix} E_{s,\alpha}(\vec{R}) \\ E_{s,\beta}(\vec{R}) \end{pmatrix} = \begin{pmatrix} \exp(ik_R) & S_2 & S_3 \\ -ik_R & S_1 & S_4 \end{pmatrix} \begin{pmatrix} E_{0,\alpha} \\ E_{0,\beta} \end{pmatrix},$$

where $S_1$, $S_2$, $S_3$, and $S_4$ are the elements of the amplitude scattering matrix and, in general, depend on the scattering angle $\theta$ and the azimuth angle $\phi$. The combination of Eqs. (2.54) and (2.58) leads to the following expressions for amplitude scattering matrix:
The quantities $F_{\alpha,x}$, $F_{\beta,x}$ and $F_{\alpha,y}$, $F_{\beta,y}$ are calculated for $x$- and $y$-polarized incident light, respectively, as follows [36–38]:

for $x$-polarized incidence:

$$
(F_{\alpha,x}, F_{\beta,x}) = i k_h^3 4\pi \int_V \left[ 1 - \frac{e^*(\xi)}{\epsilon_h} \right] \left( \alpha \cdot E(\xi) \beta \cdot E(\xi) \right) \exp(-i k_h r \cdot \xi) d^3 \xi,
$$

(2.60)

for $y$-polarized incidence:

$$
(F_{\alpha,y}, F_{\beta,y}) = i k_h^3 4\pi \int_V \left[ 1 - \frac{e^*(\xi)}{\epsilon_h} \right] \left( \alpha \cdot E(\xi) \beta \cdot E(\xi) \right) \exp(-i k_h r \cdot \xi) d^3 \xi,
$$

(2.61)

where $k_h = \omega \sqrt{\mu_0 \epsilon_0}$ and $\epsilon_h$ is the complex relative permittivity of the host medium. When $\epsilon_h = 1$, Eqs. (2.60), (2.61) will degenerate into a formulation for light scattering by cells in free space.

Eq. (2.58) is now fully defined and can be rewritten in a vectorial form:

$$
\vec{E}_s = \vec{S} \cdot \vec{E}_0,
$$

(2.62)

where $S_k = S_k(\theta, \phi)$, $k = 1, 2, 3, 4$. In actual experiments the measured optical signal is proportional to quadratic field combinations [40]. Therefore, to describe the monochromatic transverse wave one introduces four Stokes parameters which in the case of the scattered wave take the form [37]

$$
I_s = \left\langle E_{s,\alpha}^* E_{s,\alpha} + E_{s,\beta}^* E_{s,\beta} \right\rangle,
$$

$$
Q_s = \left\langle E_{s,\alpha}^* E_{s,\alpha} - E_{s,\beta}^* E_{s,\beta} \right\rangle,
$$

$$
U_s = \left\langle E_{s,\alpha}^* E_{s,\beta} + E_{s,\beta}^* E_{s,\alpha} \right\rangle,
$$

$$
V_s = \left\langle E_{s,\alpha}^* E_{s,\beta} - E_{s,\beta}^* E_{s,\alpha} \right\rangle.
$$

(2.63)

The relation between the incident and the scattered Stokes parameters is given by the Mueller scattering matrix (or simply the scattering matrix) which is defined as follows

$$
\begin{pmatrix}
I_i \\
Q_i \\
U_i \\
V_i
\end{pmatrix} =\frac{1}{k_h^2 R^2} \begin{pmatrix}
P_{11} & P_{12} & P_{13} & P_{14} \\
P_{21} & P_{22} & P_{23} & P_{24} \\
P_{31} & P_{32} & P_{33} & P_{34} \\
P_{41} & P_{42} & P_{43} & P_{44}
\end{pmatrix} \begin{pmatrix}
I_s \\
Q_s \\
U_s \\
V_s
\end{pmatrix},
$$

(2.64)
where

\[ P_{11} = \frac{1}{2} \left( |S_1|^2 + |S_2|^2 + |S_3|^2 + |S_4|^2 \right), \quad P_{12} = \frac{1}{2} \left( |S_2|^2 - |S_1|^2 + |S_4|^2 - |S_3|^2 \right), \]

\[ P_{13} = \text{Re}(S_2S_3^* + S_1S_4^*), \quad P_{14} = \text{Im}(S_2S_3^* - S_1S_4^*), \]

\[ P_{21} = \frac{1}{2} \left( |S_2|^2 - |S_1|^2 - |S_4|^2 + |S_3|^2 \right), \quad P_{22} = \frac{1}{2} \left( |S_2|^2 + |S_1|^2 - |S_4|^2 - |S_3|^2 \right), \]

and

\[ P_{23} = \text{Re}(S_2S_3^* - S_1S_4^*), \quad P_{24} = \text{Im}(S_2S_3^* + S_1S_4^*), \]

\[ P_{31} = \text{Re}(S_2S_4^* + S_1S_3^*), \quad P_{32} = \text{Re}(S_2S_4^* - S_1S_3^*), \]

\[ P_{33} = \text{Re}(S_1S_2^* + S_3S_4^*), \quad P_{34} = \text{Im}(S_1S_2^* + S_3S_4^*), \]

\[ P_{41} = \text{Im}(S_2S_4^* + S_1S_3^*), \quad P_{42} = \text{Im}(S_4S_2^* - S_1S_3^*), \]

\[ P_{43} = \text{Im}(S_1S_2^* - S_3S_4^*), \quad P_{44} = \text{Re}(S_1S_2^* - S_3S_4^*). \]

The \( P \) matrix elements contain the full information about the scattering event. In non absorptive media the elements of the Mueller matrix [Eq. (2.64)] can be used to define the scattering cross-section and anisotropy. The scattering cross-section \( \sigma_s \) is defined as the geometrical cross-section of a scattering object that would produce an amount of light scattering equal to the total observed scattered power in all directions. It can be calculated by the integration of the scattered intensity over all directions. It can be expressed by the elements of the scattering matrix \( P \) and the Stokes parameters \((I_0, Q_0, U_0, V_0)\) of the incident light as follows [40]

\[
\sigma_s = \frac{1}{k^2 l_0} \int_{4\pi} [I_0 P_{11} + Q_0 P_{12} + U_0 P_{13} + V_0 P_{14}] d\Omega. \tag{2.65}
\]

In the case of a spherically symmetrical scattering object and non polarized light the relationship (2.65) is reduced to the usual integral of the indicatrix with respect to the scattering angle:

\[
\sigma_s = \frac{2\pi}{k^2} \int_0^\pi P_{11}(\theta) \sin(\theta) d\theta. \tag{2.66}
\]

The anisotropy parameter \( g \) is defined as follows [40]
A positive (negative) value of $g$ corresponds to a forward (backward) dominated scattering. The isotropic scattering case corresponds to $g = 0$.

In an absorptive medium, the elements of the Mueller scattering matrix [Eq. (2.64)] depend on the radial distance from the scattering object and cannot be directly related to the scattering cross-section as given above by Eq. (2.65). In this case the different elements of the matrix are used individually in the analysis of the scattering phenomena. In practice, their values are normalized by the total scattered rate around the object in the radiation zone, which can be derived from the integral of $P_{11}$ for all scattering angles.

Absorption, scattering and extinction efficiencies

The flow of energy and the direction of the electromagnetic wave propagation are represented by the Poynting vector:

$$\vec{s} = \vec{E} \times \vec{H}^*,$$  

(2.68)

where the asterisk denotes the complex conjugate. To derive the absorption and extinction rates of a particle embedded in an absorptive medium, we can rewrite the last equation in Eq. (2.46) as

$$\nabla \times \vec{H} = -i\omega\epsilon_0 (\epsilon_r + i\epsilon_i) \vec{E}. \quad (2.69)$$

Combining the third equation in Eqs. (2.46) with Eq. (2.69) leads to

$$\nabla \cdot \vec{s} = \nabla \cdot (\vec{E} \times \vec{H}^*) = \vec{H}^* \cdot (\nabla \times \vec{E}) - \vec{E} \cdot (\nabla \times \vec{H}^*)$$

$$= i\omega (\mu_0 \vec{H} \cdot \vec{H}^* - \epsilon_0 \epsilon_r \vec{E} \cdot \vec{E}^*) - \omega \epsilon_0 \epsilon_i \vec{E} \cdot \vec{E}^* \quad (2.70)$$

For the sake of convenience in the following presentation, we will define the real and imaginary parts of the relative permittivity of the scattering object as $\epsilon_r$ and $\epsilon_i$, and those for the host medium as $\epsilon_{r,0}$ and $\epsilon_{i,0}$, respectively. The rate of energy absorbed by the object is

$$w_a = -\frac{1}{2} \text{Re} \left[ \int \vec{n} \cdot \vec{s}(\xi)d^3\xi \right] = -\frac{1}{2} \text{Re} \left[ \int \vec{n} \cdot \vec{s}(\xi)d^3\xi \right]$$

$$= \epsilon_0 \frac{\epsilon_i}{\epsilon_r} \left[ \epsilon_{r,0}(\xi) \vec{E}(\xi) \cdot \vec{E}^*(\xi) \right] d^3\xi, \quad (2.71)$$

where $\vec{n}$ denotes the outward-pointing unit vector normal to the surface of the object. The surface and volume integrals are defined by the volume of the scattering object. When electromagnetic waves are incident on an object, the electric and magnetic field vectors $\vec{E}$ and $\vec{H}$ can be taken as sums of the incident and scattered fields. Therefore the scattered field vectors can be written as

$$\vec{E}_s = \vec{E} - \vec{E}_i, \quad (2.72)$$
where $\vec{E}_i$ and $\vec{H}_i$ denote the incident electric and magnetic field vector, respectively. Therefore the rate of energy scattered by the object can be expressed as

$$w_s = \frac{1}{2} \text{Re} \left\{ \oint_S \vec{n} \cdot (\vec{E}_s \times \vec{H}_s^*) d^2 \xi \right\} = \frac{1}{2} \text{Re} \left\{ \oint_S \vec{n} \cdot \left[ (\vec{E} - \vec{E}_i) \times (\vec{H}^* - \vec{H}_i^*) \right] d^2 \xi \right\}. \quad (2.74)$$

Because both absorption and scattering remove energy from the incident waves, the extinction rate of the energy can be defined as

$$w_e = w_s + w_a = \frac{1}{2} \text{Re} \{ f_s \vec{n} \cdot [(\vec{E} - \vec{E}_i) \times (\vec{H}^* - \vec{H}_i^*)] d^2 \xi \} - \frac{1}{2} \text{Re} \{ f_s \vec{n} \cdot (\vec{E} \times \vec{H}_s^*) d^2 \xi \} \quad (2.75)$$

Using Eqs. (2.70) and (2.75), similar to the derivation of Eqs. (2.71), we can obtain

$$w_e = w_a + w_s = \varepsilon_0 \frac{4\pi}{3} \int_V \left[ \varepsilon_{tr} (\vec{r}) + \varepsilon_{hr} (\vec{r}) \right] \text{Re} [\vec{E}_s(\vec{r}) \cdot \vec{E}^* (\vec{r})] d^3 \vec{r}$$

$$- \varepsilon_0 \frac{4\pi}{3} \int_V \left[ \varepsilon_{tr} (\vec{r}) - \varepsilon_{hr} (\vec{r}) \right] \text{Im} [\vec{E}_s(\vec{r}) \cdot \vec{E}^* (\vec{r})] d^3 \vec{r}. \quad (2.76)$$

Assuming the rate of energy incident on a particle of arbitrary shape is $f$, then the absorption, scattering and extinction efficiencies are $Q_a = w_a / f$, $Q_s = (w_s - w_a) / f$ and $Q_e = w_e / f$, respectively. Consequently, the single scattering albedo is $\sigma = Q_s / Q_e$.

In an absorptive medium, the rate of energy incident on the object depends on the position and intensity of the wave source, the optical properties of the host medium, and the objects size and shape. For spherical objects, if the intensity of the incident light at the center of the computational domain is $I_0$, the rate of energy incident on a spherical scatterer centered at the center of the 3D computational domain is

$$f = \frac{2\pi a^2}{\eta^2} I_0 [1 + (\eta - 1) e^\eta], \quad (2.77)$$

where $a$ is the radius of the spherical object, $\eta = 4\pi n_{hr}/\lambda_0$, $I_0 = \frac{1}{2} \left( \frac{n_{hr}}{n_{hi}} \right) |E_0|^2$ is the intensity of the incident light at the center of the computational domain, $\lambda_0$ is the incident wavelength in free space, $n_{hr}$ and $n_{hi}$ are the real and imaginary refractive index of the host medium, respectively. $|E_0|$ is the amplitude of the incident electric field at the center of the 3D computational domain. For non-spherical objects, the rate of energy incident on the object is calculated numerically.
2.2.5 FDTD formulation of optical phase contrast microscopic (OPCM) imaging

The 3D FDTD formulation provided here is based on a modified version of the total-field/scattered-field (TFSF) formulation that was described earlier [29, 32]. It could be more appropriately called total-field/reflected-field (TFRF) formulation. The 3D TFRF formulation uses a TFSF region which contains the biological cell and extends beyond the limits of the simulation domain (Fig. 2.4). The extension of the transverse dimension of the input field beyond the limits of the computational domain through the UPML boundaries would lead to distortions of its ideal plane wave shape and eventually distort the simulation results. To avoid these distortions one must use Bloch periodic boundary conditions (Fig. 2.4) in the lateral x- and y-directions which are perpendicular to the direction of propagation z [29].

Bloch boundary conditions are periodic boundary conditions which take into account the phase effects due to the tilting of the input plane waves incoming at periodic structures, i.e. what we are actually modeling is a periodic row of biological cells. The near scattered fields, however, are calculated in the transverse planes located in the close proximity to the cell where the coupling effect due to waves scattered from adjacent cells is negligible. This effect can be further minimized or completely removed by controlling the lateral dimension of computational domain by using a large enough period of the periodic cell structure. The larger is this period, the smaller is
the coupling effect. In the 3D TFRF formulation the location in the computational domain corresponding to the forward scattered light is positioned within the total field region (Fig. 2.4). The OPCM simulation model requires the explicit availability of the forward scattered transverse distribution of the fields. The phase of the scattered field accumulated by a plane wave propagating through a biological cell will be used in the FDTD model of the OPCM that will be described in the next section.

**FDTD OPCM principle**

Phase contrast microscopy is utilized to produce high-contrast images of transparent specimens such as microorganisms, thin tissue slices, living cells and sub-cellular components such as nuclei and organelles. It translates small phase variations into corresponding changes in amplitude visualized as differences in image contrast. A standard phase contrast microscope design is shown in Fig. 2.5a, where an image with a strong contrast ratio is created by coherently interfering a reference (R) with a diffracted beam (D) from the specimen.

Relative to the reference beam, the diffracted beam has lower amplitude and is retarded in phase by approximately $\frac{\pi}{2}$ through interaction with the specimen. The main feature in the design of the phase contrast microscope is the spatial separation of the R beam from D wave front emerging from the specimen. In addition, the amplitude of the R beam light must be reduced and the phase advanced or retarded by another $\pm\frac{\pi}{2}$ in order to maximize the differences in the intensity between the specimen and the background in the image plane. The mechanism for generating relative phase retardation has two-steps: i) the D beam is being retarded in phase by a quarter wavelength (i.e., $\frac{\pi}{2}$) at the specimen, and ii) the R beam is advanced (or retarded) in phase by a phase plate positioned in or very near the objective rear focal plane (Fig. 2.5a). This two-step process is enabled by a specially designed annular diaphragm – the annulus. The condenser annulus, which is placed in the condenser front focal plane, is matched in diameter and optically conjugated to the phase plate residing in the objective rear focal plane. The resulting image, where the total phase difference is translated by interference at the image plane into an amplitude variation, can have a high contrast ratio, particularly if both beams have the same amplitude.

Fig. 2.5a illustrates the part of the microscope that will become the subject of FDTD modeling combined with Fourier optics. Fig. 2.5b provides a visual representation illustrating the major steps in the FDTD OPCM model. The phase contrast microscope uses incoherent annular illumination that could be approximately modeled by adding up the results of eight different simulation using ideal input plane waves incident at a given polar angle (30 deg), an azimuthal angle (0, 90, 180 or 270 deg), and a specific light polarization (parallel or perpendicular to the plane of the graph). Every single FDTD simulation provides the near field components in a transverse monitoring plane located right behind the cell (Fig. 2.4).

The far field transformations use the calculated near fields right behind the cell and return the three complex components of the electromagnetic fields far enough from the location of the near fields, i.e. in the far field [29]: $E_r(u_x, u_y)$, $E_\theta(u_x, u_y)$
FIGURE 2.5: a) Schematic representation of an OPCM. b) 2D visual representation of the FDTD OPCM model using incoherent illumination by two plane waves at a polar angle of 30 deg. For each of the two plane waves the propagation of light is modeled as a combination of two parallel wave phenomena: i) propagation of the reference (R) beam without the cell, and ii) propagation of the diffracted (D) beam due to the cell.

and \( E_\phi(u_x,u_y) \), where \( r, \theta \) and \( \phi \) refer to the spherical coordinate system shown in Fig. 2.3, and the variables \( u_x \) and \( u_y \) are the \( x \) and \( y \) components of the unit direction vector \( \vec{u} \). The unit direction vector \( \vec{u} \) is related to the angular variables \( \theta \) and \( \phi \):

\[
\begin{align*}
  u_x &= \sin(\theta) \cos(\phi), & u_y &= \sin(\theta) \sin(\phi), & u_z &= \cos(\theta), \\
  u_x^2 + u_y^2 + u_z^2 &= 1.
\end{align*}
\]

The in-plane wave vectors for each plane wave are given by \( k_x = ku_x \) and \( k_y = ku_y \), where \( k = \frac{2\pi}{\lambda} \). What is important here is to note that in this case the near-to-far field transformation must take into account the Bloch periodic boundary conditions in the lateral dimension and is calculated only at angles that correspond to diffracted orders of the periodic structure such as defined by the Bragg conditions. This is
done by calculating the direction cosines of all the diffracted orders that meet the Bragg condition, and interpolating the previously far-field distributions onto those specific directions. The near-to-far field projection therefore provides the electric field amplitude and phase corresponding to each diffracted order. The zeroth order, i.e. the light travelling through the scattering object without any deviation in angle, is the reference beam and the phase contrast microscope is designed to provide a phase delay to this order.

The amplitudes and the phases of the calculated far-field components can now be used to do Fourier optics with both the scattered and reference beams. We can assume an ideal optical lens system that could be characterized by a given magnification factor. This simple model could be easily extended to include the numerical equivalent of the two lenses together with an additional model to take into account any aberrational effects. The magnification was implemented by modifying the angle of light propagation, i.e. by multiplying the transverse components of the direction cosines, \( u_x \) and \( u_y \) by the inverse value of the desired magnification factor \( M \):

\[
U_x = \frac{u_x}{M} \quad \text{and} \quad U_y = \frac{u_y}{M}.
\]

In any other circumstances the modification of the direction cosines would lead to complications because of the vectorial nature of the \( \vec{E} \) field. In our case, however, working in spherical coordinates (\( E_r, E_\theta \) and \( E_\phi \)) leads to the advantage that the vectorial components do not change when \( u_x \) and \( u_y \) are modified because they are part of a local coordinate system that is tied to the values \( u_x \) and \( u_y \). The factor \( M \) is applied to the far fields before the interference of the diffracted (D) and reference (R) beams at the image plane.

It was also possible to apply the effect of a numerical aperture NA which clips any light that has too steep an angle and would not be collected by the lens system. This means that all the light with \( U_x^2 + U_y^2 > (NA)^2 \) is being clipped. The effect of the aperture is defined by applying the last inequality to the corrected aperture angles \( \theta' \) and \( \phi' \):

\[
\sin(\phi') = \frac{U_x}{U_{xy}}, \quad \cos(\phi') = \frac{U_y}{U_{xy}}, \quad \cos(\theta') = U_z, \quad \sin(\theta') = U_{xy}, \quad (2.79)
\]

where \( U_{xy} = \sqrt{U_x^2 + U_y^2} \), \( U_z = \sqrt{1 - U_{xy}^2} \) and the “sqrt” labels a square root mathematical operation. The magnified field components will then have the following form:

**Diffracted (D) beam:**

\[
E_{x-D}(k_x,k_y) = -E_{\phi-D} \sin(\phi') + E_{\theta-D} \cos(\phi') \cos(\theta'),
\]

\[
E_{y-D}(k_x,k_y) = E_{\phi-D} \cos(\phi') + E_{\theta-D} \sin(\phi') \cos(\theta'),
\]

\[
E_{z-D}(k_x,k_y) = -E_{\theta-D} \sin(\theta').
\]  

**Reference (R) beam:**

\[
E_{x-R}(k_x,k_y) = -E_{\phi-R} \sin(\phi') + E_{\theta-R} \cos(\phi') \cos(\theta'),
\]

\[
E_{y-R}(k_x,k_y) = E_{\phi-R} \cos(\phi') + E_{\theta-R} \sin(\phi') \cos(\theta'),
\]

\[
E_{z-R}(k_x,k_y) = -E_{\theta-R} \sin(\theta').
\]
$E_{y-R}(k_x, k_y) = E_{\phi-R} \cos(\phi') + E_{\theta-R} \sin(\phi') \cos(\theta')$, \hspace{1cm} (2.81)

$E_{z-R}(k_x, k_y) = -E_{\theta-R} \sin(\theta')$, 

where $E_{\phi-R}$ and $E_{\theta-R}$ are the far field components of the reference beam.

The fields given above are then used to calculate back the Fourier inverse transform of the far field transformed fields leading to the distribution of the scattered and the reference beams in the image plane:

**Diffracted (D) beam:**

$E_{x-D} = \sum (E_{x-D}(k_x, k_y) \exp(ik_x x + ik_y y + ik_z z)),$

$E_{y-D} = \sum (E_{y-D}(k_x, k_y) \exp(ik_x x + ik_y y + ik_z z)),$ \hspace{1cm} (2.82)

$E_{z-D} = \sum (E_{z-D}(k_x, k_y) \exp(ik_x x + ik_y y + ik_z z)).$

**Reference (R) beam:**

$E_{x-R} = \sum (E_{x-R}(k_x, k_y) \exp(ik_x x + ik_y y + ik_z z)),$

$E_{y-R} = \sum (E_{y-R}(k_x, k_y) \exp(ik_x x + ik_y y + ik_z z)),$ \hspace{1cm} (2.83)

$E_{z-R} = \sum (E_{z-R}(k_x, k_y) \exp(ik_x x + ik_y y + ik_z z)),$

where the summation is over all angles.

The OPCM images at the image plane are calculated by adding up the scattered and the reference beam at any desired phase offset $\Psi$:

$I = \text{abs}(E_{x-D} + aE_{x-R} \exp(i\Psi))^2 + \text{abs}(E_{y-D} + aE_{y-R} \exp(i\Psi))^2 + \text{abs}(E_{z-D} + aE_{z-R} \exp(i\Psi))^2. \hspace{1cm} (2.84)$

The coefficient $a$ and the phase $\Psi$ are simulation parameters corresponding to the ability of the OPCM to adjust the relative amplitudes and the phase difference between the two beams.

### 2.3 FDTD Simulation Results of Light Scattering Patterns From Single Cells

#### 2.3.1 Validation of the simulation results

To simulate the light scattering and absorption by single biological cells, we use a C++ computer program that is based on the FDTD formulation described in section

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**FDTD Simulation of Light Interaction with Cells**

25
A Mie scattering program was also used to provide exact results for the validation of the FDTD simulations. Details about the specific Mie scattering formulation can be found in [27] and [42].

Figure 2.6 shows some preliminary FDTD simulation results for the phase function of a simple spherical biological cell containing only a cytoplasm and a nucleus both embedded in a non-absorptive extra-cellular medium. The phase function represents the light scattering intensity as a function of the scattering angle in a plane passing through the center of the cell. It is defined by the angular dependence of the $P_{11}$ element of the Mueller scattering matrix normalized by the total scattered rate - the integral of $P_{11}$ in all possible directions around the cell. The cell membrane is not taken into account. The nucleus has a size parameter $2\pi R_c/\lambda_0 = 7.2498$, where $R_c$ is the radius of the nucleus and $\lambda_0$ is the incident wavelength in free space. The refractive index of the nucleus is 1.4, of the cytoplasm 1.37 and of the extra-cellular material 1.35. The size parameter of the whole cell is 19.3328. The FDTD cell size is $\Delta s = \lambda_0/30$ and the UPML parameters are $\kappa_{\text{max}} = 1$ and $R(0) = 10^{-8}$ [see Eqs. (2.12) and (2.13)]. The number of mesh points in all three directions is the same: 209. The number of simulation time steps is 10700. These preliminary results were compared with the exact solutions provided by Mie theory. The relative error of the FDTD simulation results is $\sim 5\%$. Some absolute values of cell parameters can be derived as follows. If $\lambda_0 = 0.9\mu m$, the FDTD cell size $\Delta s = \lambda_0/30 = 0.03\mu m$, the nucleus' radius $R_c = 7.2498\lambda_0/2\pi = 1.0385\mu m$ and the cytoplasm radius $R_c = 19.3328\lambda_0/2\pi = 2.7692\mu m$. These dimensions are more typical for relatively small cells or bacteria which have no nucleus.

The extinction efficiency $Q_e$, absorption efficiency $Q_a$ and anisotropy factor $g$ for the cell associated with Fig. 2.6 with the parameters given above are listed in Table 2.1. Other $P$ matrix elements are shown in Fig. 2.7. To complete the validation of the UPML FDTD scheme we compared the numerical and exact solutions for the light scattering patterns from biological cells embedded in an absorptive extra-cellular medium.

**TABLE 2.1:** Comparison between FDTD simulation results and analytical solutions provided by Mie theory for the extinction efficiency $Q_e$, scattering efficiency $Q_s$, absorption efficiency $Q_a$ and anisotropy factor $g$ of a cell with a cytoplasm and a nucleus in non-absorptive extra-cellular medium.

<table>
<thead>
<tr>
<th></th>
<th>$Q_e$</th>
<th>$Q_s$</th>
<th>$Q_a$</th>
<th>$g$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mie theory</td>
<td>0.359057</td>
<td>0.359057</td>
<td>0.00</td>
<td>0.993889</td>
</tr>
<tr>
<td>FDTD</td>
<td>0.358963</td>
<td>0.358963</td>
<td>0.00</td>
<td>0.993914</td>
</tr>
<tr>
<td>(FDTD-Mie)/Mie</td>
<td>0.026 %</td>
<td>0.026 %</td>
<td>-</td>
<td>0.0025 %</td>
</tr>
</tbody>
</table>

Accounting for this absorption effect requires a special attention since not all types
of boundary conditions can handle absorptive materials touching the boundary of the simulation domains. One of the advantages of the UPML boundary conditions considered here is that they can handle that [17]. To study the effect of the absorption of the extra-cellular medium we assume that the refractive index of the extra-cellular medium has a real and an imaginary part: \( n + ik \). Figure 2.8 demonstrates the good agreement (~5% relative error) between the FDTD and the exact (Mie theory) results for the normalized light scattering patterns in the case when the refractive index the extra-cellular medium is \( 1.35 + i0.05 \).

The extinction efficiency \( Q_e \), scattering efficiency \( Q_s \), absorption efficiency \( Q_a \) and anisotropy factor (g) for a cell in an absorptive medium (the same as in Fig. 2.8) are listed in Table 2.2.

### 2.3.2 Effect of extra-cellular medium absorption on the light scattering patterns

This section describes the FDTD simulation results for the effect of absorption in the extra-cellular medium on the light scattering patterns from a single cell [17]. We consider two different cell geometries: the one considered in the previous section and another one with a shape of a spheroid (both cytoplasm and nucleus) which can be described by the surface function

\[
\frac{x^2}{a^2} + \frac{y^2}{b^2} + \frac{z^2}{c^2} = 1, \tag{2.85}
\]
FIGURE 2.7: Angular distributions of the normalized scattering matrix elements $P_{12}, P_{33}, P_{43}$, and $P_{44}$ calculated by the FDTD method. Cell parameters are the same as for Fig. 2.6. There is a very good agreement between exact (Mie theory) and numerical results with a relative error of the FDTD results of approximately 5%.

TABLE 2.2: Comparison between FDTD simulation results and the analytical solutions provided by Mie theory for the extinction efficiency $Q_e$, scattering efficiency $Q_s$, absorption efficiency $Q_a$ and anisotropy factor $g$ of a cell with a cytoplasm and a nucleus in a non-zero imaginary part of the refractive index of the extra-cellular medium $k = 0.05$ and cell parameters as described above in this section.

<table>
<thead>
<tr>
<th>Results</th>
<th>$Q_e$</th>
<th>$Q_s$</th>
<th>$Q_a$</th>
<th>$g$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mie theory</td>
<td>0.672909</td>
<td>0.672909</td>
<td>0.0</td>
<td>0.992408</td>
</tr>
<tr>
<td>FDTD</td>
<td>0.676872</td>
<td>0.676872</td>
<td>0.0</td>
<td>0.992440</td>
</tr>
<tr>
<td>(FDTD-Mie)/Mie</td>
<td>0.589 %</td>
<td>0.589 %</td>
<td>-</td>
<td>0.0032 %</td>
</tr>
</tbody>
</table>

where $a$, $b$, and $c$ are the half axes of the spheroid with $a = 2b = 2c$.

Figure 2.9 illustrates the light scattering configuration in the case of a cell with a spheroid shape. The light is incident in the x-direction along the long axis of the spheroid. The size parameters of the cytoplasm are defined by $2\pi R_a/\lambda_0 = 40$ and $2\pi R_{b,c}/\lambda_0 = 20$, where $R_a = a/2$ and $R_{b,c} = b/2 = c/2$. The size parameters of the
FIGURE 2.8: Normalized light scattering intensity distribution with scattering angle in the case of absorptive extra-cellular medium. Cell parameters are the same as for Fig. 2.6 and Fig. 2.7, except a non-zero imaginary part of the refractive index of the extra-cellular medium.

FIGURE 2.9: Coordinate system and geometry of a cell with the shape of a spheroid with half axes $a$, $b$, and $c$, where $a = 2b = 2c$. The light is incident in the positive $x$-direction, along the long axis $a$.

nucleus are defined by $2\pi r_a/\lambda_0 = 20$ and $2\pi r_b, r_c/\lambda_0 = 10$, where $r_a$ and $r_b$ are the large and small radii of the nucleus, respectively. The cell refractive indices are: cytoplasm: 1.37; nucleus: 1.40, extra-cellular medium: 1.35, and 1.35+0.05i. The FDTD cell size is $\Delta z = \lambda / 20$. The number of mesh point are $N_x = 147$, $N_y = 147$ and $N_z = 275$. The number of simulation time steps is 14000. Figure 2.10 shows: i) the effect of cell size on the light scattering patterns, and ii) the effect of the absorption of the extra-cellular medium on the phase function of spheroid cells [16–17].

A more detailed analysis of the two graphs shown in Fig. 2.10 leads to some interesting findings. First, absorption in the extra-cellular material of spherical cells (Fig. 2.10, right graph) increases the intensity of the light scattering up to one order in the angle range between $90^\circ$ (transverse scattering) and $\Psi = 180^\circ$ (backward scattering). The same light scattering feature was also found in the case of spheroid cells.
Second, the influence of absorption in the extra-cellular material is relatively more pronounced in the case of spherical as compared to spheroid cells (Fig. 2.10, left graph). Third, in the case of exact backward scattering and absorptive extra-cellular material, the light scattering intensity is approximately equal for both cell shapes. However this is not true for non-absorptive cell surroundings. This last finding could be highly relevant for OCT and onfocal imaging systems, especially for studies in the wavelength ranges within the hemoglobin, water and lipids bands.

These findings show that the analysis of light scattering from isolated biological cells should necessarily account for the absorption effect of the surrounding medium. It could be particularly relevant in the case of optical immersion techniques using intra-tissue administration of appropriate chemical agents with absorptive optical properties [43-45]; however, this relevance has not been studied before. It should be pointed out that whenever there is a matching of the refractive indices of a light scatterer and the background material, the scattering coefficient goes to zero and it is only the absorption in the scatterer or in the background material that will be responsible for the light beam extinction. The results presented here provide some good preliminary insights about the light scattering role of absorption in the background material; however, it needs to be further studied.

2.4 FDTD Simulation Results of OPCM Nanobioimaging

2.4.1 Cell structure

This section describes the 3D FDTD modeling results of OPCM imaging of single biological cells in a number of different scenarios. The results are based on the FDTD
OPCM model described in subsection 2.2.5 [46]. The optical magnification factor $M = 10$ and the numerical aperture $NA = 0.8$. The cell is modeled as a dielectric sphere with a realistic radius $R_c = 5 \mu m$ (Fig. 2.4). The cell membrane thickness is $d = 20 \text{nm}$ which corresponds to effective (numerical) thickness of approximately 10 nm. The cell nucleus is also spherical with a radius $R_n = 1.5 \mu m$ centered at a position which is $2.0 \mu m$ shifted from the cell center in a direction perpendicular to the direction of light propagation. The refractive index of the cytoplasm is $n_{\text{cyto}} = 1.36$, of the nucleus $n_{\text{nuc}} = 1.4$, of the membrane $n_{\text{mem}} = 1.47$ and of the extracellular material $n_{\text{ext}} = 1.33$ (no Refractive Index Matching – no RIM) or 1.36 (RIM).

### 2.4.2 Optical clearing effect

The RIM between the cytoplasm and the extra-cellular medium leads to the optical clearing of the cell image. The optical clearing effect leads to the increased light transmission through microbiological objects due to the matching of the refractive indices of some of their components to that of the extra-cellular medium [42–45]. If a biological object is homogenous, matching its refractive index value by externally controlling the refractive index of the host medium will make it optically invisible. If the biological object contains a localized inhomogeneity with a refractive index different from the rest of the object, matching the refractive index of the object with that of the external material will make the image of the object disappear and sharply enhance the optical contrast of the inhomogeneity. In the case of biological cells the refractive index of the extra-cellular fluid can be externally controlled by the administration of an appropriate chemical agent [42–45].

Figure 2.11 shows the cross-sections of two cell images for different values of the phase offset $\Psi$ between the reference and diffracted beam of the OPCM: $180^\circ$ and $90^\circ$. The images illustrate the nature of the optical clearing effect and the value of its potential application for the early detection of cancerous cells by a careful examination of their nucleus size, eccentricity, morphology and chromatin texture (refractive index fluctuations) [14]. At no RIM conditions in both cases ($\Psi = 180^\circ$ and $\Psi = 90^\circ$) the image of the nucleus is represented by a dip in the cell image. At RIM conditions the image contrast of the cell is drastically reduced to zero levels and it is only the image of the nucleus that remains sharply visible. The image of the nucleus is represented by a nice peak associated with the 3-dimensional optical phase accumulation corresponding to its perfectly spherical shape and homogeneous refractive index distribution. A finer analysis of the two graphs shown in Fig. 2.11 will show that the diameter of the nucleus (the full width at the half-height of the nucleus image contrast peak) depends on the phase delay $\Psi$. At $\Psi = 180^\circ$ and no RIM conditions the diameter of the nucleus is estimated at value of $\sim 2.3 \mu m$ as compared to RIM conditions where it’s value is $3.3 \mu m$ (the estimation accounts for the optical magnification factor $\times 10$ of the system). At $\Psi = 90^\circ$ and no RIM conditions the diameter of the nucleus is estimated at a value of $\sim 3.05 \mu m$ as compared to RIM conditions where its value is $3.75 \mu m$ (the cell model used in the FDTD simulations has a nucleus with a diameter $3.0 \mu m$). This shows that the OPCM should be preliminary set-up at a given optimum phase delay and the OPCM images should be used
FIGURE 2.11: Cross-sections of FDTD-generated OPCM images of a single cell illustrating the optical clearing effect for different values of the phase offset $\Psi$ between the reference and diffracted beam of the OPCM: 180° (on the left-hand side) and 90° (on the right-hand side). Matching the refractive index value of the extracellular material with that of the cytoplasm enhances the optical contrast and leads to a finer view of the morphological structure of the nucleus.

for relative measurements only after a proper calibration. The analysis of the graphs, however, shows an unprecedented opportunity for using the optical clearing effect for the analysis of any pathological changes in the eccentricity and the chromatin texture of cell nuclei within the context of OPCM cytometry configurations. This new opportunity is associated with the fact that at RIM the cell image is practically transformed into a much finer image of the nucleus.

2.4.3 The cell imaging effect of gold nanoparticles

Optical properties of gold nanoparticles (NPs)

Gold NPs have the ability to resonantly scatter visible and near infrared light. The scattering ability is due to the excitation of Surface Plasmon Resonances (SPR). It is extremely sensitive to their size, shape, and aggregation state offering a great potential for optical cellular imaging and detection labeling studies [47–51]. Our FDTD approach [21, 24] uses the dispersion model for gold derived from the experimental data provided by Johnson and Christy [52] where the total, complex-valued permittivity is given as:

$$\varepsilon(\omega) = \varepsilon_{\text{real}} + \varepsilon_L(\omega) + \varepsilon_P(\omega). \quad (2.86)$$

Each of the three contributions to the permittivity arises from a different material model. The first term represents the contribution due to the basic, background permittivity. The second and third terms represent Lorentz and plasma contributions:

$$\varepsilon_L(\omega) = \frac{\varepsilon_{\text{Lorentz}}}{\omega_0^2 + (\omega \delta_0 - i \omega \delta_0)^2}, \quad \varepsilon_P(\omega) = \frac{\omega^2}{i \omega \nu_C + \omega^2}, \quad (2.87)$$
where all material constants are summarized in Table 2.3.

**TABLE 2.3:** Optical material constants of gold [52]

<table>
<thead>
<tr>
<th>Background permittivity</th>
<th>Lorentz dispersion</th>
<th>Plasma dispersion</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\varepsilon_{\text{real}} = 7.077$</td>
<td>$\varepsilon_{\text{Lorentz}} = 2.323$</td>
<td>$\omega_p = 1.391 \times 10^{16} \text{Hz}$</td>
</tr>
<tr>
<td>$\omega_0 = 4.635 \times 10^{15} \text{Hz}$</td>
<td>$\delta_0 = 9.267 \times 10^{14} \text{Hz}$</td>
<td>$\nu_C = 1.411 \times 10^7 \text{Hz}$</td>
</tr>
</tbody>
</table>

We have modeled both the resonant and non-resonant cases. The ability to model these two different cases, together with the effect of optical clearing effect, provides the opportunity to numerically study the possibility for imaging the uptake of clusters of NPs – a scenario which needs to be further studied [21]. We have also used the FDTD technique to calculate the scattering and absorption cross-sections over a 400–900 nm wavelength range for a single 50 nm diameter gold NP immersed in a material having the properties of the cytoplasm ($n_{\text{cyto}} = 1.36$) and resolution $dx = dy = dz = 10 \text{ nm}$. The scattering cross-section is defined as

$$\sigma_{\text{scat}} = \frac{P_{\text{scat}}(\omega)}{I_{\text{inc}}(\omega)},$$

(2.88)

where $P_{\text{scat}}$ is the total scattered power and $I_{\text{inc}}$ is the intensity of the incident light in W / m$^2$. It was calculated by applying the total-field/scattered-field FDTD formulation described in subsection 2.2.2. We have used the GUI features of the FDTD Solutions software to create 12 field power monitoring planes around the nanoparticle in the form of a box: 6 in the total field region and 6 in the scattered field region. The total scattered power was calculated by summing up the power flowing outward through 6 scattered field power monitors located in the scattered field region.

The absorption cross-section is similarly defined as

$$\sigma_{\text{abs}} = \frac{P_{\text{abs}}(\omega)}{I_{\text{inc}}(\omega)},$$

(2.89)

where $P_{\text{abs}}$ is the total power absorbed by the particle. The power absorbed by the particle is calculated by calculating the net power flowing inward through the 6 total field power monitors located in the total field region.

The extinction cross-section is the sum of the absorption and scattering cross-sections

$$\sigma_{\text{ext}} = \sigma_{\text{scat}} + \sigma_{\text{abs}},$$

(2.90)

Figure 2.12a shows that the extinction cross-section has a maximum of 3.89 at around 543.0 nm corresponding to one of the radiation wavelengths of He-Ne lasers. Here we also present the results for $\lambda = 676.4 \text{ nm}$ (a Krypton laser wavelength).
FIGURE 2.12: a) Extinction cross-section of a 50 nm gold nanoparticle immersed in material having the optical properties of the cytoplasm \( n = 1.36 \) (left). The gold optical properties are described by Eqs. (2.86), (2.87) with the parameters given in Table 2.3. b) Positioning of a cluster of gold nanoparticles randomly distributed within a spatial sphere at a cell location symmetrically opposite to the center of the nucleus.

which corresponds to the non-resonant case (extinction cross-section value 0.322, ~12 times smaller than 3.89). The FDTD results are compared with the theoretical curve calculated by Mie theory. The slight discrepancy between the theoretical and FDTD results for the extinction cross-section is due to the finite mesh size. The consistency of the results could be visibly improved by reducing the mesh size.

OPCM images of gold nanoparticle clusters in single cells

The OPCM cell images are the result of simulations using non-uniform meshing where the number of mesh points in space is automatically calculated to ensure a higher number of mesh points in materials with higher values of the refractive index [53]. Fig. 2.12b visualizes the schematic positioning of a cluster of 42 nanoparticles in the cytoplasm that was used to produce the simulation results presented in this section. The cell center is located in the middle \((x = y = z = 0)\) of the computational domain with dimensions 15 \(\mu m \times 12 \mu m \times 15 \mu m\) (Fig. 2.12b). The center of the nucleus is located at \(x = -2 \mu m, y = z = 0 \mu m\). The cluster of gold nanoparticles is located at \(x = 2 \mu m, y = z = 0 \mu m\). The realistic cell dimensions (including both cell radius and membrane) require a very fine numerical resolution making the simulations computationally intensive. The numerical resolution of the nanoparticles was hard-coded to \(dx = dy = dz = 10 \text{ nm}\) to make sure that their numerically manifested optical resonant properties will be the same as the ones shown in Fig. 2.12a. This lead to additional requirements for the CPU time and memory (~120 Gbs RAM) requiring high performance computing resources. The time
step used during the simulation was defined by means of the Courant stability limit:
\[ c \Delta t = 0.99 \times \left( \frac{1}{\Delta x^2} + \frac{1}{\Delta y^2} + \frac{1}{\Delta z^2} \right)^{-1/2}. \]

Based on the fact that RIM enhances significantly the imaging of the cell components, we have used the FDTD OPCM model to create the OPCM images of the cell components.

**FIGURE 2.13:** (Color figure follows p. 34.) OPCM images of a single cell for different values (a: $-150^\circ$, b: $-90^\circ$, c: $+90^\circ$, d: $+180^\circ$) of the phase offset $\Psi$ between the reference and diffracted beam of the OPCM at RIM (optical immersion) conditions including a cluster of 42 gold NPs located in a position symmetrically opposite to the nucleus. The arrows indicate the position of the cluster. The left column corresponds to a cell without NPs. The other columns correspond to a cell with NPs at resonant (right) and non-resonant (middle) conditions.
FIGURE 2.14: Comparison of the geometrical cross sections at $y = 0 \, \mu m$ of the three OPCM images corresponding to a phase offset $\Psi = 180^\circ$ between the reference and the diffracted beam (bottom row in Fig. 2.13) in terms of optical contrast. The right hand-side graph illustrates the optical contrast enhancement due to the effect of the gold NP resonance at $\lambda = 543.0 \, nm$.

FIGURE 2.15: Optical contrast due to the gold NP cluster as a function of the phase offsets between the reference (R) and the diffracted (D) beams of the optical phase microscope.

FIGURE 2.16: A cluster of 42 Gold NPs randomly distributed on the surface of the cell nucleus. The NP size on right hand graph is slightly exaggerated.
FIGURE 2.17: OPCM images of the cell for different values of the phase offset $\Psi$ (a: $-90^\circ$, b: $-30^\circ$, c: $+30^\circ$, d: $+90^\circ$) between the reference and diffracted beam of the OPCM at optical immersion, i.e. refractive index matching, conditions without NPs (left) and including 42 Gold NPs (middle – at no resonance, right – at resonance) randomly located at the surface of the cell nucleus.

At optical immersion conditions including the cluster of 42 gold NPs (Fig. 2.13) and for different values of the phase offset $\Psi$ between the reference beam and the scattered beam [assuming $a = 1$, see Eq. (2.62)]. The two graphs in Fig. 2.14 compare the geometrical cross sections ($y = 0\mu m$) of the three OPCM images shown at the bottom of Fig. 2.13. The right-hand side graph shows the relevant half of the image where the gold NP cluster is located.

At resonance the optical contrast of the gold NP peak is $\sim$2.24 times larger than the one at no resonance and 24.79 times larger than the background optical contrast.
FIGURE 2.18: Cross-sections of the images shown in Fig. 2.17c (corresponding to phase offset $\Psi = +30^\circ$) expressed in terms of optical contrast. The right-hand side graph provides in finer details an enlarged portion of the left-hand side one. The specific fragmentation of the nucleus’ image is due to the presence of the Gold NPs at resonant condition.

corresponding to the case when there are no nanoparticles. The enhanced imaging of the gold NP cluster at resonant conditions is clearly demonstrated. It however needs to be further studied as a function of particular phase offset $\Psi$ between the reference beam and the scattered beam.

Figure 2.15 visualizes the optical contrast due to the gold nanoparticle cluster as a function of the phase offsets between the reference (R) and the diffracted (D) beams of the optical phase microscope [21]. It shows that the enhancement of the optical contrast due to the nanoparticle resonance changes significantly from minimum of 0.0 ($\Psi = 0^\circ$) to a maximum of 3.60 ($\Psi = -150^\circ$). This finding should be taken into account in real life OPCM imaging experiments.

OPCM images of gold nanoparticles randomly distributed on the nucleus of a cell

Figure 2.16 visualizes the positioning of a cluster of 42 Gold NPs on the surface of the cell nucleus. This was the NP configuration used to produce the FDTD-based simulation results presented in this section [24].

It should be pointed out that the optical wave phenomena involved in the simulation scenario considered here are fundamentally different from the ones considered in the previous section where the gold nanoparticles are randomly distributed within the homogeneous material of cytoplasm and their presence is manifested by means of their own absorption and scattering properties. In the present case the NPs are located at the interface of the nucleus and the cytoplasm which is characterized by a relatively large refractive index difference ($\Delta n = 0.04$) and which is, therefore, expected to largely dominate and modify the visual effect of the NPs.

A close examination of the OPCM images in Fig. 2.17 provides an illustration of this fact. The OPCM cell images are for different values of the phase offset $\Psi$
First, the images of the cell without the Gold NPs are hardly distinguishable from the images including the Gold NPs at no resonance conditions ($\lambda = 676.4$ nm). Second, the visual effect of the Gold NP presence at resonant conditions ($\lambda = 543.0$ nm) depends significantly on the phase offset $\Psi$. Third, the presence of the Gold NPs at resonant conditions can be identified by a specific fragmentation of the image of the nucleus for specific values of the offset $\Psi$ (see the right-hand side graphs in Figs. 2.17c and 2.18). This last finding indicates the importance of the ability to adjust the offset $\Psi$ between the reference and diffracted beam of the OPCM in practical circumstances. It is expected to be of relevance for the development and calibration of similar optical diagnostics techniques by medical photonics researchers and clinical pathologists.

2.5 Conclusion

In this chapter we provided a detailed summary of the mathematical formulation of the FDTD method for application in medical biophotonics problems. We have then applied the FDTD approach to three different modeling scenarios: i) light scattering from single cells, ii) OPCM imaging of realistic size cells, and iii) OPCM imaging of gold NPs in single cells. We have demonstrated the FDTD ability to model OPCM microscopic imaging by, first, reproducing the effect of optical immersion on the OPCM images of a realistic size cell containing a cytoplasm, a nucleus and a membrane. Second, the model was applied to include the presence of a cluster of gold NPs in the cytoplasm at optical immersion conditions as well as the enhancing imaging effect of the optical resonance of the nanoparticles. Third, we have studied the imaging effect of gold NPs randomly distributed on the surface of the cell nucleus. The results do not allow analyzing the scaling of the NP imaging effect as a function of the number of the NPs. However, the validation of the model provides a basis for future research on OPCM nanobioimaging including the effects of NP cluster size, NP size and number, as well as average distance between the NPs. Another future extension of this research will be to study the capability of the model to provide valuable insights for the application of gold NPs in optical nanotherapeutics.

We believe that the shift from the modeling of the light scattering properties of single cells to the construction of OPCM images of cells containing gold NPs represents a major step forward in extending the application of the FDTD approach to biomedical photonics. It opens a new application area with a significant research potential – the design and modeling of advanced nanobioimaging instrumentation.
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References


[44] B.A. Fikhman, Microbiological Refractometry, Medicine, Moscow (1967).


[46] The simulations were performed by the FDTD Solutions™ software developed by Lumerical Solutions Inc., Vancouver, BC, Canada: www.lumerical.com.


[53] Non-uniform meshing is a standard feature of the FDTD Solutions™ software.