Biological and Medical Physics, Biomedical Engineering

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D. Mathur

Ultrafast Biophotonics

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Preface

This book discusses contemporary and emerging techniques of ultrafast science which are helping to open entirely new vistas for probing biological entities and processes. These include the use of femtosecond lasers to facilitate time-resolved imaging, multiphoton microscopy, single molecule studies, laser surgery and, also, to create plasma channels in aqueous media that help detect stress marker proteins and probe DNA damage induced by slow electrons and radicals. We review some of the topics that are presently on the horizon, like the use of coherent control, squeezed light, frequency combs, terahertz imaging, the possibility of mimicking biological systems and, perhaps surprisingly to many, invoking quantum mechanical effects such as coherent superposition, radical pair production and tunneling to rationalize phenomena like photosynthesis, avian navigation in the earth's magnetic field and respiration. Also discussed is the role played by ultrafast biophotonics in developing biomimetic devices whose quantum functionalities may be "engineered" for applications in light-harvesting, solar energy conversion, magnetic field sensing, photonic devices and single biomolecular electronics.

It is hoped that the interdisciplinary contents of this book will be of benefit to physical scientists and life scientists alike.

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Mumbai, India

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Chapter 1
Introduction and Overview

Abstract This chapter presents an overview of the prospects of present and anticipated experimental and theoretical advances in ultrafast biophotonics discussed in this book. The areas include multiphoton and multidimensional microscopy, ultrafast single molecule studies, femtosecond laser surgery, quantum biology, biomimetic devices, tunneling in biological entities, cavitation in aqueous biological media by thermal as well as plasmonic means, coherent control of biochemical processes and probing biological entities beyond the quantum limit. Emerging techniques that are likely to play a major role in the development of the subject are also discussed; they include ultrafast multi-dimensional spectroscopy, generation of femtosecond-long X-ray pulses from free electron lasers for ultrashort imaging of biological materials without causing photodamage, application of terahertz radiation for imaging and spectroscopy, uses of frequency comb Fourier transform spectroscopy, and exciting advances in the emerging area of biomimetic technology.

What is photonics?

A succinct definition might be: *photonics is the science and technology of light, with emphasis on applications*. One of the distinguishing features of the science of photonics lies in the natural and apparently seamless linkages that the subject provides between fundamental scientific studies and technological applications in diverse areas of contemporary and futuristic importance. The extension of photonics to *biophotonics* is a rather obvious one: it concerns, on the one hand, the use of optical science to gain insights into biological phenomena and, on the other, utilizing optical science to drive the development of new biological and biomedical methodologies and technologies. The mutually beneficial dialectical relationships between experiment, theory, and applications that are a hallmark of biophotonics are serving to accelerate developments within the field. The prognosis for the near future is exciting!

As can be imagined, the canvas that becomes available on the basis of the above definition of biophotonics is immensely wide. This book focuses on only that subset of the canvas that deals with, or relies on, ultrafast phenomena and processes. The term *ultrafast* is used in reference to timescales that are prevalent in dynamics that

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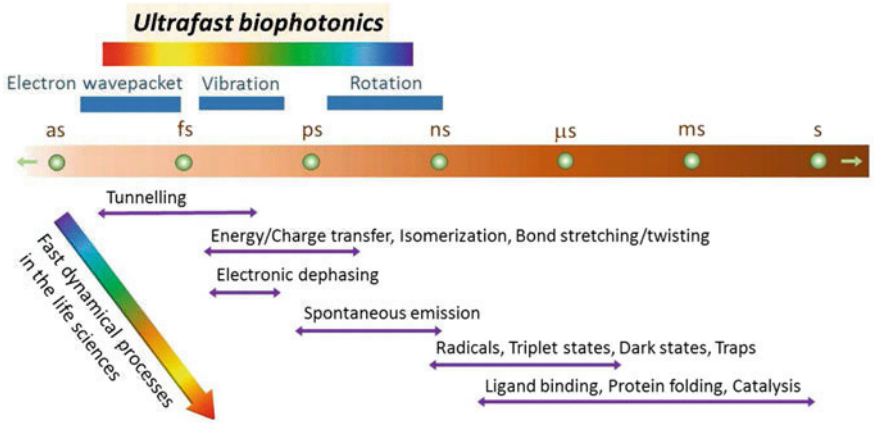

Fig. 1.1 Schematic depiction of some fast and ultrafast dynamical processes encountered in contemporary biology

occur within individual atoms and molecules, the constituents of all living matter. These timescales vary from tens and hundreds of picoseconds that single molecules take to rotate, to tens of femtoseconds that molecules take to vibrate to, finally, tens and hundreds of attoseconds—the time taken for electronic motion within atoms and molecules (Fig. 1.1). In the context of biophotonics, this book shall explore biological phenomena that occur on such short timescales and, also, show how photonics techniques based on ultrafast interactions can enable these phenomena to be probed.

It is not difficult to appreciate that tools, methods and concepts initially developed for diverse endeavours in physics in general, photonics in particular, are playing an increasingly important role in biology; indeed, some of them have become an accepted feature of many a contemporary life sciences laboratory. Raman and fluorescence spectroscopy, especially multidimensional and multiphoton varieties, are examples that come readily to mind. It is also not unexpected that biology itself is starting to stimulate physical scientists to develop afresh methods, tools, and concepts that, of necessity, induce close participation of life scientists: "super-resolution" microscopy, multiphoton imaging, and the computational modeling of single-molecule trajectories are amongst several contemporary examples.

In the course of this book recourse has been taken to discuss processes that appear to occur on picosecond, or even nanosecond, timescales. Whenever we refer to such "long" timescales, they will pertain to the composite of several sub-processes, each of which occur on ultrashort, usually femtosecond, timescales. A challenge that remains to be tackled is to experimentally "deconvolute" these ultrafast sub-processes from the currently observable process.

1.1 Living Systems

Living systems have been around on earth for at least 4 billion years. In order to distinguish living systems from inanimate matter, we articulate the following features: Living entities show

(i) the ability to self-reproduce,
(ii) the ability to harvest energy;

Furthermore,

(iii) they evolve, and
(iv) they manifest complexity.

Complexity is one distinguishing feature: even a single living cell comprises over 1000 different molecules. While complexity is also found in inanimate objects, it remains a fact that as far as the other three features are concerned, there are no analogues to be found in the physical sciences that possess such characteristics.

There are also similarities that should be articulated. As is known from several examples, the physical sciences are witness to length scales and time scales that span very many orders of magnitude: from time periods of billions of years since the primordial big bang to electron dynamics within atoms and molecules that occur on attosecond (10^{-18} s) time scales. Biological entities and processes also span many orders of magnitude. In terms of size and parameter space, life scientists readily deal with a single molecule of water on the one hand, and the effect on the ecosystem of the dynamic energy budget of the earth's atmosphere on the other.

It is known that a single living cell—such as that of *E. coli*—can transcribe as many as 5×10^6 genes in the course of $\sim$2000 s or less. This implies that the real-time information processing capabilities of a single simple cell are far in advance of any practical computational devices that physical scientists and engineers can envision at present. A single cell can process about 10 Gb of information per hour while occupying a volume of only a few μm^3! We note that there is nothing special about a cell of *E. coli*; it is a relatively simple cell, coded by only about five million base pairs. One can extrapolate to multi-cellular systems and it becomes very obvious that biological entities present very remarkable information capabilities, much in excess of what even the most advanced silicon-based non-living systems can hope for at present.

1.2 Energy Scales

Important entities that one encounters in the life sciences are DNA, RNA, and proteins. The covalent bonds that constitute the backbone of such polymers have evolved to be quite robust and, typically, their binding energies are $\Delta G \geq 1$ eV. However, forces that act in a direction that is perpendicular to the polymer backbones can be

considerably weaker. But, surprisingly (from the perspective of a physical scientist), these weak forces often influence, in major fashion, the form, dynamics, and functions of many biomolecules. Hence, one frequently encounters forces in the life sciences that are very much smaller than ΔG—of the order of $k_B T$—and these tiny forces often prove to be important. Here, T denotes the temperature (either the laboratory temperature in the case of a process or the body temperature in the case of a living entity) and k_B is the Boltzmann constant.

In the context of energy scales, it is pertinent to compare the vastly different energy scales that are encountered throughout the life sciences compared to those that are routinely encountered in physics, chemistry and engineering. Molecular interactions that drive most biological processes work on k_BT energy scales. As $k_B N_A = R$, where R is the gas constant and N_A denotes Avogadro's number, 1 k_BT = 0.62 kcal/mole at a temperature of 300 K. It is also instructive to compare k_BT = 25 meV at room temperature with energy levels in the prototypical hydrogen atom, $\sim$10 eV, and covalent bonds and binding energies of atoms in metals, $\sim$1 eV. The comparison becomes even more stark on a macroscopic level: an object that would be considered tiny in the context of the physical sciences—one weighing, say, 1 mg—being displaced with a speed of 1 cm s^{-1} has an energy of 10^9 eV!

1.3 Overview of the Book

We begin the book by presenting, in Chap. 2, an overview of some basic concepts related to light, nonlinear optics, and ultrashort pulses on which many of our explorations of ultrafast biophotonics will be based. We introduce, in pedagogical fashion, concepts like the intensity of laser light, ponderomotive energy, nonlinear optics, frequency conversion; also introduced are methods of generating and characterizing ultrashort pulses, and how such pulses propagate through matter.

Structural studies have acted as significant precursors of many contemporary developments in the life sciences, with structure determination of biological entities often being the precursor to the discovery of tangible links to their biological function. Chapter 3 deals with a well-established use of ultrafast nonlinear optics for structure determination: biophotonic microscopy. The essential physics behind such microscopy is that of multiphoton excitation using pulses of intense infrared light which enable greater depth penetration to be achieved, an important consideration in microscopy. This chapter also discusses the successes of super-resolution microscopy, which is based essentially on the "manipulation" of molecular spectra, and the anticipated successes of 4D electron microscopy in unravelling ultrafast biological phenomena. Among the biophotonic applications discussed in this chapter is one that concerns protein fibrillization, specifically the potential involvement of amyloid fibrils in diseases like Parkinson's and Alzheimer's diseases which share fibril formation as the common symptom. Unfortunately, for long it has been the case that Alzheimer's disease (AD) could only be diagnosed upon analysis of postmortem tissue; such analysis invariably reveals the existence of extensive

neurofibrillary tangles and plaques of beta-amalyoid (βA). Multiphoton microscopy offers in vivo imaging of βA plaques in intact brains, with much superior spatial resolution than competing methods like positron emission tomography, magnetic resonance imaging, and fluorescence imaging. Tangible benefits of superior spatial resolution include the ability to image objects like dendritic spines, the post-synaptic apparatus of excitatory synapses as well as the onset and subsequent growth of amyloid plaques, and Ca^{2+} transients. Second harmonic microscopy is more complex than multiphoton microscopy as account has to be taken of a coherent optical process that demands phase matching from all parts of the nonlinear focal volume. However, retention of phase information permits useful information to be obtained from signal directionality, a facet of particular importance in samples like type I collagen fibrils (whose diameters are almost the same as the wavelength of visible light). This has allowed in vivo studies of tissues under dynamical conditions like the healing of wounds, development, and malignancy. "Super-resolution" microscopy has allowed imaging of cultured hippocampal neurons from neonatal rats, with images revealing the existence of nanometre-sized protein clusters. 3D mapping of neurofilaments in differentiated neuroblastoma cells has also been accomplished!

In the last two decades, the detection and spectroscopy of individual molecules has begun to find widespread applications in the life sciences. While conventional time-resolved experiments continue to provide information about ensemble-averaged properties, ultrafast single molecule techniques are able to track the photodynamics of individual molecules, revealing their unique transient intermediates. Chapter 4 presents an overview of single molecule techniques like surface enhanced coherent anti-Stokes Raman scattering, Förster resonance energy transfer, pump-probe spectroscopy, and pulse-shaping; these can not only identify but also provide information about electronic/vibrational wavepacket interferences and relaxation mechanisms at the level of individual molecules. As an illustration of their relevance in ultrafast biophotonics, a study of persistence of coherence in an individual photosynthetic complex under physiological conditions is presented.

Chapter 5 reviews two other well-established applications of ultrafast biophotonics: femtosecond laser surgery and cell manipulation. The essential physics that drives these applications is discussed, including quantitative considerations of parameters that affect biophotonic applications. At the tissue level, ablation induced by ultrafast pulsed lasers has allowed the "cutting" of minute volumes of tissue and to facilitate transection of cells: sub-micrometre surgery of neuronal and vascular entities are now routinely performed as are various types of eye surgery. On smaller scales—at the cellular level—light pulses have been used to activate membrane channels and membrane pumps so as to induce changes of electric potential across cell membranes; this has opened prospects of enabling neural circuitry. On even smaller scales—at the sub-cellular level—it is becoming feasible to toggle individual biomolecules from an active to a passive state, and vice versa, by photoswitching of fluorescent labels. The use of femtosecond-duration laser pulses results in high instantaneous peak powers that enable multiphoton (nonlinear) absorption while, concomitantly, avoiding heat damage via single-photon (linear) absorption. Hence, ultrafast laser pulses have also begun to be of utility in a gamut of non-surgical applications: multiphoton laser

scanning microscopy with utility in the neurosciences, high harmonic imaging of cells, tissues and organisms, coherent anti-Stokes Raman spectroscopy, and nonlinear imaging of biological entities in general. Intense laser light also has associated with it radiation pressure, and this has been utilized for laser pressure catapulting of dissected samples into appropriate containers for polymerase chain reaction (PCR) investigations. Such laser beams also carry out cell lysis and subsequent catapulting of the contents of the lysed cell into a micropipette for time-resolved capillary electrophoresis, for fusing together of cells and for in-vitro fertilization. Less vigorous applications include the use of ultrafast laser pulses to gently transfect genes into specific cells, with a tightly focused laser beam acting like a sharp but contact-less "needle".

Ultrashort pulses of intense laser light affect matter with which they interact and, in turn, matter also affects the light pulses. Chapter 6 considers both "laser-matter" and "matter-laser" interactions from the biophotonic perspective. Propagation of intense light through matter gives rise to visually spectacular phenomena like supercontinuum generation and filamentation which have helped to probe damage induced in DNA by low-energy interactions involving electrons and OH-radicals. They have also provided high sensitivity diagnostic of stress markers in human saliva. There are openings for similar biophotonics applications without the need for very high-intensity lasers, and possible ways of achieving this are discussed.

Conventional wisdom dictates that quantum mechanical processes do not manifest themselves in large systems, like biological complexes, at room temperature. However, ultrafast time-resolved spectroscopic investigations have begun to yield contradictory evidence: long-range quantum mechanical effects *do* occur in biological systems under physiological conditions. Some topics relevant to the functional role of quantum processes in ultrafast biophotonic phenomena in plants and animals, along with their experimental investigations, are discussed in Chaps. 7 and 8, respectively. The concept of quantum mechanical superposition is briefly presented as are some descriptions of important ultrafast spectroscopy techniques that may be used to explore coherent effects in biology. Chapter 7 considers how coherent energy transfer involving entangled states is much more efficient than classical incoherent energy hopping, and such coherence is utilized by nature in the energy transport mechanism in light-harvesting complexes in plants and in photosynthetic bacteria. One intriguing and counterintuitive manifestation of quantum effects in biology is that of avian navigation in the earth's magnetic field. It appears that such navigation depends on exploiting the earth's magnetic field to tune radical pair production that aids both orientation and navigation. The possibility of complex biological systems performing what is essentially a kind of spin resonance experiment in order to navigate is fascinating; it is discussed in Chap. 8.

The focus in contemporary biology has begun to discernibly shift from morphological explorations and phenotypic probing of organisms to seeking quantitative insights into underlying mechanisms at molecular levels. For instance, protein dynamics are now computationally modelled in terms of trajectories on energy landscapes. These landscapes are multidimensional "cousins" of conventional potential energy surfaces—the variation of potential energy as a function of internuclear

coordinates—that physicists and chemists have long used to understand molecular dynamics. As protein trajectories evolve on such landscapes, processes have been discovered that can only be rationalized using quantum mechanical effects like tunneling, and in terms of transitions that occur in non-adiabatic fashion. All these concepts have been creatively adapted by nature so as to be make them useful to the biological milieu. Chapter 9 provides a working overview of such ultrafast processes in proteins like myoglobin.

What opportunities do quantum processes offer for the development of ultrafast artificial systems that succeed in mimicking their natural counterparts? An important question in ultrafast biophotonics, thus, is whether it is possible to incorporate quantum effects into bio-inspired synthetic systems in order to develop devices that possess quantum-enhanced functionality. Chapter 10 explores opportunities of ultrafast photonic functionalities of some proteins in plants and higher organisms, and their potential role in designing new devices that mimic natural systems possessing quantum-enhanced efficiency and adaptability. This chapter discusses the role of ultrafast biophotonics in developing biomimetic devices whose quantum properties can be "engineered" for applications in light-harvesting, solar energy conversion, magnetic field sensing, photonic devices and single-biomolecular electronics.

We end the book by presenting in Chap. 11 an overview of the prospects of forthcoming experimental and theoretical advances in ultrafast biophotonics. The areas discussed include tunneling in biological entities, cavitation in aqueous biological media by thermal as well as plasmonic means, probing biological entities beyond the quantum limit. Emerging techniques that are likely to play a major role in the development of the subject include coherent control of biological processes, femtosecond-long X-ray pulses from free electron lasers for ultrashort imaging of biological materials without causing photodamage, application of terahertz radiation for imaging and spectroscopy, and uses of frequency comb Fourier transform spectroscopy.

Chapter 2
Ultrashort Pulses and Nonlinear Optics: Nuts and Bolts

Abstract In this chapter we summarize some basic concepts related to light, nonlinear optics, and ultrashort pulses that will be employed in our explorations of ultrafast biophotonics. Concepts like the intensity of laser light, ponderomotive energy, nonlinear optics, frequency conversion as well as the characterization and propagation of ultrashort pulses that we discuss here will be frequently encountered in this book.

2.1 Conceptual Aspects

Ultrafast photonics is related to the study of photon-induced phenomena that occur on very short timescales. It is, therefore, but natural that ultrashort pulses of light will form an essential tool for such studies. The basic principle of an ultrashort pulse is quite simple: it is a burst of light energy consisting of only a few cycles of oscillating electromagnetic field [1, 2]. Such pulses are generally characterized by their space- and time-varying electric field $\widetilde{E}(\mathbf{r}, t)$.[1] For the purpose of this book, the quantum mechanical properties of the light field are not important, hence it is sufficient to use the classical approach or Maxwell's equations of electrodynamics [3, 4]. A concise outline of some relevant concepts is provided in the following, with a view to laying the conceptual foundations of what follows in succeeding chapters.

2.1.1 Maxwell's Equations

An appropriate place to start are Maxwell's equations in a homogeneous medium with electric charge density ρ and the electric current density $\mathbf{j}$ which are given by

[1]

- Throughout the book, we use bold letters to represent vector quantities and tilde ($\sim$) to denote quantities rapidly varying in time. Constant or slowly varying quantities are written without the tilde.
- Except where otherwise noted, we use the SI (MKS) system of units.

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$$\nabla \cdot \mathbf{E} = \rho, \tag{2.1}$$

$$\nabla \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial t}, \tag{2.2}$$

$$\nabla \cdot \mathbf{B} = 0, \tag{2.3}$$

and

$$\nabla \times \mathbf{B} = \frac{\partial \mathbf{D}}{\partial t} + \mathbf{j}. \tag{2.4}$$

Together with the Lorentz force law that relates the force $\mathbf{F}$ acting on a particle with charge q moving with velocity $\mathbf{v}$,

$$\mathbf{F} = q(\mathbf{E} + \mathbf{v} \times \mathbf{B}), \tag{2.5}$$

Maxwell's equations summarize the theoretical content of classical linear electrodynamics and optics. Here, the relation between the $\mathbf{E}$-field and the $\mathbf{D}$-field associated with light is given by

$$\mathbf{E} = \frac{1}{\epsilon_0}(\mathbf{D} - \mathbf{P}), \tag{2.6}$$

with $\mathbf{P}$ being the dipole moment per unit volume or the macroscopic polarization. Similarly, the $\mathbf{B}$-field and the $\mathbf{H}$-field are related by the magnetization $\mathbf{M}$ as

$$\mathbf{B} = \mu_0(\mathbf{H} + \mathbf{M}). \tag{2.7}$$

For topics relevant to this book or for nonmagnetic materials in general, $\mathbf{M} = 0$ is a valid condition, simplifying (2.7) to $\mathbf{B} = \mu_0\mathbf{H}$.

In the case of conventional—or linear—optics, we have

$$\mathbf{P} = \epsilon_0\chi\mathbf{E}, \tag{2.8}$$

where the complex function, χ, is the linear optical susceptibility. The relation between the macroscopic polarization and the $\mathbf{E}$-field, (2.6) simplifies to $\mathbf{D} = \epsilon_0\epsilon\mathbf{E}$, with the relative dielectric function, $\epsilon = 1 + \chi$.

The real part of the susceptibility describes the physical process of refraction whereas its imaginary part is related to the absorption coefficient, α.

The well-known wave equation for the $\mathbf{E}$-field associated with light can be derived from Maxwell's equations as

$$\nabla^2\widetilde{\mathbf{E}} - \frac{1}{c_0^2}\frac{\partial^2\widetilde{\mathbf{E}}}{\partial t^2} = \mu_0\frac{\partial^2\widetilde{\mathbf{P}}}{\partial t^2}, \tag{2.9}$$

or, using (2.6), as

$$\nabla^2\widetilde{\mathbf{E}} - \frac{1}{c^2}\frac{\partial^2\widetilde{\mathbf{E}}}{\partial t^2} = 0, \tag{2.10}$$

with $c = \frac{c_0}{n}$ being the speed of light in a medium. This is lower than the speed of light in vacuum, $c_0 = \frac{1}{\sqrt{\epsilon_0\mu_0}} = 2.998 \times 10^8$ m s^{-1}, by a factor n—the refractive index—which, for a nonmagnetic material, is given by

$$n = \sqrt{\epsilon}. \tag{2.11}$$

As with the optical susceptibility or the dielectric function, the refractive index is also a complex function. For topics discussed in this book, we confine our attention to harmonic, linearly polarized, transverse plane wave solutions of (2.10) with angular frequency ω traveling through the medium in the direction along $\mathbf{k}$:

$$\widetilde{\mathbf{E}} = \mathbf{E_0}e^{i(\mathbf{k}\cdot\mathbf{r}-\omega t)} + cc. \tag{2.12}$$

Here, $\mathbf{E_0}$ is the complex $\mathbf{E}$-field amplitude and the magnitude of the propagation vector $\mathbf{k}$ is related to the wavelength of light in vacuum by $k = \frac{2\pi n}{\lambda}$. The corresponding $\mathbf{B}$-field amplitude of the light wave is given by

$$\mathbf{B_0} = \frac{1}{c_0}\widehat{\mathbf{k}} \times \mathbf{E_0}. \tag{2.13}$$

Since Maxwell's equations do not involve higher order field terms, (2.10) is a linear, second-order partial differential equation. Hence the superposition principle governing the interference and diffraction of light waves holds. As we see later, interference plays a fundamental role in the generation of ultrashort pulses. Though described here only for linear polarization, the theoretical treatment can be extended to include other polarization states.

Polarization is an important parameter for investigating biological systems. It plays a key role in several fields of biophotonics, like biological nonlinear optics. It is also noteworthy that the range of wavelengths of electromagnetic radiation span a very wide range, from 10^5 m (radio waves) to $\leq 10^{-12}$ m (gamma rays): the wave equation is applicable over the entire range! This scale invariance of classical electromagnetic theory is really remarkable.

2.1.2 Light Intensity and Ponderomotive Energy

One of the most significant properties of electromagnetic waves is that they carry energy and momentum. The light emitted by the nearest stars (other than our sun) travels for millions of kilometres through vacuum to reach the earth, carrying sufficient energy to initiate a chain of photochemical reactions within our eyes. Our eye—and most other light detectors—are not sensitive to rapidly oscillating **E**-fields or **B**-fields but to the cycle-average of the energy density carried by the light waves. The flow of electromagnetic energy per unit time and across unit area is represented by the Poynting vector

$$\widetilde{\mathbf{S}} = \frac{1}{\mu_0}\widetilde{\mathbf{E}} \times \widetilde{\mathbf{B}}. \tag{2.14}$$

Applying (2.14) to plane waves in vacuum, the modulus of the Poynting vector, or the energy density per unit time transported by an electromagnetic wave, is given by

$$\widetilde{S} = |\widetilde{\mathbf{S}}| = \sqrt{\frac{\epsilon_0}{\mu_0}}|\widetilde{\mathbf{E}}|^2, \tag{2.15}$$

which is a time varying quantity. Its cycle-average is the light intensity, I,

$$\langle\widetilde{\mathbf{S}}\rangle = I = \frac{1}{2}\sqrt{\frac{\epsilon_0}{\mu_0}}E_0^2. \tag{2.16}$$

This is also known as the radiant flux density or the optical power density, representing radiant energy incident on—or exiting from—a surface. For a point source, it is proportional to $\frac{1}{r^2}$.

As optical detectors are sensitive to intensity, the phase information about the **E**-field is generally lost in a measurement unless care is taken to employ special techniques based on interference. Like wavelength, the light intensity also spans a wide range of values, as shown in Table 2.1. Ranging from extremely low values ($\sim 10^{-23}$ W cm^{-2}) to extremely high ones ($\sim 10^{30}$ W cm^{-2}), it covers more than fifty orders of magnitude [2]. The lower bound here corresponds to the intensity of the visible part of blackbody radiation at room temperature (300 K), whereas the upper bound corresponds to the Schwinger intensity limit at which the energy content in the light field is sufficient to spontaneously generate an electron-positron pair from vacuum. For comparison, the sun's light intensity on the earth is $\sim$0.1 W cm^{-2} whereas that generated by focussing (to 1 mm^{-2}) light from a reasonably powerful ($\sim$10 mW) continuous-wave laser is $\sim$1 W cm^{-2}. Focusing, pulsed (femtosecond) lasers readily produces intensities in the 10^{15}–10^{16} W cm^{-2} range; at the time of writing this book there are several national facilities across the world which host large laser systems capable of routinely producing intensities of the order of 10^{19}–10^{22} W cm^{-2}.

Table 2.1 Some illustrative order-of-magnitude values of light intensity, the corresponding values of the **E**-field, and the average kinetic energy acquired by an electron due to the ponderomotive potential

Intensity (I) (W m^{-2})	Intensity (I) (W cm^{-2})	Field amplitude (E_0) (V m^{-1})	Cycle average electron kinetic energy at $\lambda = 1.06\ \mu$m (U_p) (eV)	Typical light-matter interaction
10^{34}	10^{30}	10^{18}	10^{17}	Schwinger intensity limit, spontaneous generation of electron-positron pair
10^{24}	10^{20}	10^{13}	10^{7}	Light-induced nuclear fission
10^{22}	10^{18}	10^{12}	10^{5}	Light-induced particle acceleration for cancer therapy
10^{20}	10^{16}	10^{11}	10^{3}	Atomic unit of intensity for an H-atom
10^{18}	10^{14}	10^{10}	13.6	Electron tunneling from an atom
10^{10}	10^{6}	10^{6}	10^{-7}	Typical intensities at which nonlinear effects are observed
10^{6}	10^{2}	10^{4}	10^{-11}	A continuous wave laser intensity that can cause serious burn injuries
10^{3}	10^{-1}	10^{3}	10^{-14}	Total intensity of the sun on the earth's surface
10^{2}	10^{-2}	10^{2}	10^{-15}	Thermal radiation from a human body
10^{-6}	10^{-10}	10^{-2}	$<10^{-15}$	Total intensity of the cosmic background radiation at 2.8 K
10^{-11}	10^{-15}	10^{-4}	$<10^{-15}$	Vision threshold of the human eye
10^{-18}	10^{-22}	10^{-8}	$<10^{-15}$	Intensity of the visible part of blackbody radiation at 300 K

Light is absorbed and emitted in discrete numbers of photons, but even from a very low intensity source, like our Sun, light reaching us on earth has such a large photon flux ($\sim 10^{10}$ photons s^{-1} m^{-2}) that its inherently discrete nature is totally obscured: what is generally observed—and considered by the classical theory of electrodynamics—is a continuous phenomenon. An alternate viewpoint to estimate the strength of the light field is to consider the amplitude of the associated **E**-field. Here, a useful benchmark is that of the Coulomb field, E_{at} within a hydrogen atom whose Bohr radius a_0 is 0.53×10^{-10} m when the electron is in its ground state:

$$E_{at} = \frac{e}{4\pi\varepsilon_0 a_0^2} \sim 5 \times 10^{11}\ \text{V m}^{-1}. \tag{2.17}$$

The Coulombic field experienced by this electron maps to an intensity of $\sim 10^{16}$ W cm^{-2}. At this intensity value, as depicted in Fig. 2.1, a non-resonant light field is sufficiently strong to ionize a hydrogen atom. Strong optical fields can distort (or "dress")

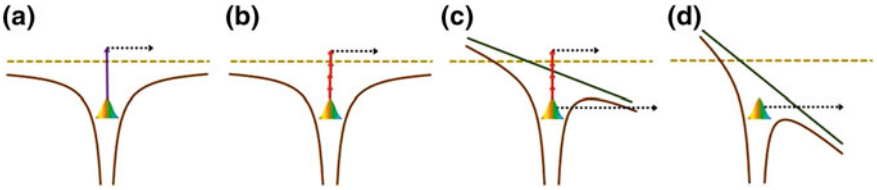

Fig. 2.1 **a** Direct (one-photon resonant), **b** multiphoton, **c** tunnel and **d** over-the-barrier ionization of an electron. At sufficiently high intensity, field dressing of the atomic potential, as schematically depicted in (**c**) and (**d**), becomes the dominant effect

the Coulombic field such that tunneling through the Coulomb barrier can occur. Indeed, ionization of matter is inevitable under strong-field (high laser intensity) conditions.

The electron that tunnels through the dressed potential (Fig. 2.1) carries signatures of the extent to which the strong laser light has distorted the Coulombic potential and, hence, electron spectroscopy proves to be an important tool in gaining insights into strong field ionization. The theoretical underpinnings of strong field ionization were laid by Volkov as long ago as 1935, decades before the advent of strong optical fields. Volkov solved the Dirac equation in the presence of a plane wave [5] and, along with subsequent pioneering theoretical considerations of electron motion in intense, time-varying electromagnetic fields [6–11], helped lay the foundations on which modern strong field science has developed. We shall have recourse to using some of these strong field concepts and techniques in discussions pertaining to ultrafast biophotonics in later chapters. Today, pulse durations and laser intensities are readily available that can easily generate fields much larger than E_{at}. Whenever the **E**-field associated with light is comparable to E_{at}, the material response does not follow (2.8); in fact, it becomes dependent on higher powers of the **E**-field. This is the domain of nonlinear optics and strong field science [12]. We shall discuss several novel and unusual effects that arise due to nonlinear optical responses and explore their applications in ultrafast biophotonics. During the course of this book, we shall mostly encounter intensity values in the range 10^6–10^{15} W cm^{-2}. We shall also see that ultrashort pulses are essential to reach these intensity values.

As any light-matter interaction involves motion of electrons, it is useful for our study of nonlinear effects to understand electron dynamics in a strong field. Unless the electron is moving at a very high speed, approaching c_0, (2.5) and (2.13) confirm that the influence of the magnetic field associated with light is negligible. For a linearly polarized **E**-field given by (2.12), the displacement **x** of a free electron of mass m_e can be written as

$$m_e \ddot{\mathbf{x}} = -e\widetilde{\mathbf{E}}, \tag{2.18}$$

giving the cycle-averaged kinetic energy of an electron as