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Introduction: Biophotonics – Visions for Better Health Care

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Since the late 1980s, natural scientists have introduced a multiplicity of new terms and definitions. We had to learn the difference between genes and proteins, we have been taken into the miniature world of viruses and prions, and the newspapers report on a great many new technologies, such as biotechnology, information technology and nanotechnology, and nowadays even on bionanotechnology. The development of those new technologies combines an increase of both the scientific and also the technological understanding and knowledge of, for example, life processes and has already led to economic profit as well as to enormous stock-exchange quotations. However, apart from undreamed-of possibilities for mankind, the new technologies also entail ethical risks and problems as we can conclude from the discussion on the first cloned sheep "Dolly" or the application of embryonic stem cells. Rather than entering into a detailed discussion, we will stop here, and instead introduce a fairly new discipline of natural science: Biophotonics.

Biophotonics deals with the interaction between light and biological systems. The word itself is a combination of the Greek syllables *bios* standing for life and *phos* standing for light. Photonics is the technical term for all procedures, technologies, devices, etc. utilizing light in interaction with any matter. Before we discuss Biophotonics in more detail, we focus first and foremost on the achievements of Photonic Technologies, which are often used synonymously with Photonics. The advanced control and manipulation of light now available make Photonics as powerful as Electronics. One major goal is to incorporate even more photonically driven processes into our daily lives. Therefore, Photonics is considered as a key technology of the twenty-first century.

Photonics encompasses the entire physical, chemical and biological laws of nature, together with technologies for the generation, amplification, control, manipulation, propagation, measurement, harnessing and any other type of utilization of light. This rather broad definition of Photonics emphasizes the huge importance of light for our modern human society. Photonics is a key technology for solving momentous problems in the domains of health

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care, food production and technology, environmental protection, transportation, mobility, etc. It is a pacemaking technology for other developments such as communication and production technology, biotechnology and nanotechnology. Just one brief example of what the near future holds: conventional lighting by light bulbs, neon lamps or fluorescent tubes will be a thing of the past. The new wonder is organic light-emitting diodes (OLED). Those innovative diodes provide more efficient and long-lasting illumination, with energy consumption reduced to a minimum. Thus, the wide-spread application of these diodes will allow our economy to save billion of Euros both on the cost of energy and also on consequential costs, such as, for example, the cost of environmental protection. Furthermore, the use of these diodes for displays will revolutionize graphic screen technology. In comparison to TFT (Thin Film Transistor) screens, an OLED display does not need background illumination. The display has the thickness of a plastic transparency, and its flexibility. Completely new applications, such as the use as "information wallpaper", are in the minds of scientists and advertising experts. However, in other fields of Photonics the future has already started. The information carried via the World Wide Web is mainly carried by light. From these few examples, we can recognize that photonic technologies are already pervading our entire life.

Now let us come back to another field in which Photonics is seen as a key technology for future scientific and economic progress – the field called "Biophotonics". What is special about Biophotonics? In order to answer this question we have to shed light on life as well as on light itself because, as already mentioned, Biophotonics deals with both light and life. Both phenomena will be important issues in this book. While the "life" aspect will be covered in the various chapters, a short summary of the properties of light, as well as on the interactions of light and matter, will be given later on in this introductory chapter. From time immemorial both phenomena have fascinated people. Despite life and light have being ubiquitous and self-evident, an understanding of the scientific bases of light and life was and is still a special challenge. As we can see from history, there was a long and roundabout journey from the first description of the nature of light in early antiquity to the photon theory of Albert Einstein. In a similar but no less complicated way, the phenomenon of life has occupied scientists and philosophers from Aristotle to the modern students of genomics in the twenty-first century.

It makes sense to put both life and light together. We can, indeed we must, learn from nature. Nature demonstrates how valuable and fruitful the interaction between light and biological systems, and thus Biophotonics as defined above, can be for life. Consider the "harnessing" of photons. Plants utilize light via photosynthesis as an energy source. In the process of vision light generates pictures of our environment in our brains by a quite complicated

but very efficient pathway. The scientist dealing with Biophotonics attempts to understand nature by mimicking the basic principles and taking advantage of the same tools. Using this approach, highly innovative future-oriented technologies can be brought into the real world. This makes the business quite thrilling. Biophotonics is not a science as an end in itself. In fact it opens undreamed-of possibilities for fundamental research, the pharmaceutical and food industries, biotechnology, medicine, etc.

The investigation of biological materials by means of innovative optical techniques has led to totally new optical technologies, including techniques capable of, for example, yielding snapshots of cellular conditions and monitoring dynamic processes. The ultimate goal of Biophotonics is to unravel life processes within cells, cell colonies, tissues, or even whole organs. Biophotonics seeks to provide a comprehensive multidimensional understanding of the various processes occurring in an organism. Therefore, Biophotonics combines all optical methods to investigate the structural, functional, mechanical, biological and chemical properties of biological material and systems. The optical phenomena exploited to gain all this information include all the interactions of electromagnetic radiation with living organisms or organic material, such as absorption, emission, reflection, scattering, etc. Other areas of Biophotonics use light as a miniaturized tool, e.g., optical tweezers or a laser scalpel.

Why do we use light? What are the advantages of applying light to the study of biological matter? The three major advantages are: (1) Light measures without contact, i.e., light allows processes taking place within a living cell to be studied without disturbing or affecting the biological activity. (2) Light measures more quickly and yields instantaneous information, i.e., the complex preparation needed in conventional methods to obtain, for example, a reliable diagnosis, which may take days, or even weeks, are no longer required or can be performed in a much shorter time. (3) Light measures more precisely, i.e., optical methods allow ultrasensitive detection, down to the single-molecule detection necessary for the life science sector.

As already indicated by the title "Biophotonics – Visions for better health care" the main focus of this contribution is on the topic "health care". However, the reader should bear in mind that Biophotonics is not limited to dealing with "health care". The final chapter of this book will mention other topics and challenges of Biophotonics. With the help of this new discipline we hope to get a precise understanding of the origin of diseases so that in the future we can prevent diseases or at least diagnose them more precisely and at an earlier stage so that we can treat them more efficiently. In principle, this looks quite simple. However, to be successful in achieving these ultimate goals scientists have to look beyond their own noses. This means that the developers of photonic technologies, mainly physicists, physical chemists, chemists and engineers, have to be in close contact with the possible users in biology, medicine,

the pharmaceutical and food industries and environmental research. Otherwise the great potential of Biophotonics cannot be applied. In various contributions to this book we shall see that to some extent the innovations of Biophotonics are based on precise observation of a natural phenomenon by a biologist or physician, which is then transformed with the help of photonic technologies into new diagnostic methods and technologies. Other progress is based on optical and spectroscopic innovation made by physicists and afterwards adapted to appropriate biological and medical problems. However, there is still a large gap between those scientists developing optical technologies and those scientists who like to use optical technologies. Thus, bringing together the various disciplines is one of the greatest challenges of Biophotonics.

1.1 The Situation of Biophotonics in Germany and Other Countries

The German government recognized the great potential of Biophotonics, not only as a key technology but also as a bridging technology, and installed a multidisciplinary operating research network. The main research framework Biophotonics, funded by the German Federal Ministry of Education and Research (BMBF), has two major common goals.

- Scientific goal: Shedding light on biological processes, i.e. understanding life processes on a functional as well as a molecular level. This will allow scientists to understand the origin of diseases and to invent new strategies to defeat them more effectively and will be the base for real prevention and effective therapies.
- Technological goal: Developing innovative light-based technologies to achieve the above-mentioned goal.

A prerequisite for reaching these two goals is that scientists from the various disciplines mentioned above should work closely together. This means that the potential user, e.g. the physician, has to be involved in the scientific and technical development from the very beginning. Another quite important requirement is that the industry, with all its technological knowledge and experience, cooperates right from the start. The involvement of both parties is seen as one important key to a successful conclusion.

This multi- and interdisciplinary research should make a strong impact on society. First of all, Biophotonics will lead to better health. Diseases, whether cancer or infectious diseases, will be defeated very effectively. Thus, the quality of life will be dramatically improved and the cost of health care considerably reduced. Areas in which Biophotonics is already operating successfully

include pathology, oncology, dermatology, cardiology, urology, ophthalmology, gastroenterology and dentistry. Secondly, in addition to scientific and technical progress, the research framework strives for the important goal of strengthening the position of Germany as a centre for technology by creating new employment and protecting highly qualified workers and sustainable jobs. As a consequence, Biophotonics will become as important as other leading technologies, such as nanotechnology, genomics and proteomics.

Germany is not the only country to have recognized the importance of Biophotonics. All over the world scientists from academia and industry are working on this topic. A short overview on the different activities of Germany, USA, France and England is given in **Table 1.1** which has been published in report on Biophotonics in Germany by Deloitte Consulting and Kraus Technology Consulting 2005.

Biophotonics is also very important in other countries, e.g., Canada, Japan, China, Australia and all over Europe. There is almost no country in the world where scientists are not becoming aware of the possibilities and potential achievement of Biophotonics. The major focus of research activities lies on topics from medicine and biotechnology. To summarize all these activities is way beyond the scope of this book.

Table 1.1: Biophotonics in four major countries.

	Germany	USA	France	UK
Organized partnership and cooperation in network	yes	yes	Le Club Biophotonique [1]	not known
Annual statistics	no	yes	yes [2]	not known
Kick-off meeting and process of formation of opinion	Deutsche Agenda "Optische Technologien"[3]; Studie zur Biophotonik	Harnessing Light: Optical Science and Engineering for the 21st Century [4]	La Biophotonique française – Perspectives de développement 2003 [5]	not known
Coordinated Project executing organization	yes [6]	Center for Biophotonics – Science and Technology (CBST)	cooperation between various research centers in France	concentrated in Glasgow
Decided research center	no	CBST, founded in 2002	concentration of various partners around Paris	Institute of Photonics and Center for Biophotonics, University of Strathclyde
Decided research program	yes	yes	yes, but within existing research programs	not known

Table 1.1: (continued)

	Germany	USA	France	UK
Other scientific panels, organization, etc.	Deutsche Gesellschaft für angewandte Optik (DGaO), network of competence, scientific councils at universities	in collaboration with CBST	some collaboration between different groups from different disciplines from various universities	Work group Scottish Optoelectronics Association
Amount of public financial support p.a. (without military projects)	5 Million EUR p.a.	Million EUR p.a. for 10 Years	not known	not known
Programs for public relations, education and advanced training	general reports on the demand on qualified employees in the field of optical technology	public relation using the keyword "Biophotonics"	support by Optcis valley, annual symposia in Paris	not known

1) www.paris-biophotonique.org
2) Annuaire de la Biophotonique en Ile-de-France
3) bmbf, VDI-TZ
4) National Research Council
5) Opticsvalley et Grenoble® Evry En collaboration avec l'ADIT
6) VDI-TZ

1.2 The Interplay Between Light and Matter: Interactions Allowing Us to Understand Our Environment

Before we introduce the marvelous world of Biophotonics we shall first give a brief introduction into the basics of light–matter interactions. The interaction of light with matter, in particular with biological material, i.e., what happens if a light wave or photon hits matter, is an exciting topic. The various light–matter interaction phenomena enable us to observe our environment and are responsible for the existence of life on earth. What follows is a short and very general introduction into the manifold interplay (e.g. absorption, emission, scattering, reflection, refraction, diffraction, dispersion, polarization, etc.) between light and matter. The advanced reader may therefore skip the following pages.

Light propagates as electromagnetic waves. Electromagnetic waves are wavelike perturbations (invisible perturbations of the so-called force field) recurring periodically over a certain distance called the wavelength. Light waves are described as oscillating electric (E) and magnetic fields (H), which are perpendicular to each other. Time-dependent changes of the electric field are always combined with spatial changes of the magnetic field. Similarly, time-dependent changes of the magnetic field are connected with spatial changes of the corresponding electric field. Electromagnetic waves can propagate in a vacuum at a speed of $c = 299\,792.458\ \mathrm{km\,s^{-1}}$. The time-dependent electric field E can be described as: $E(z,t) = E_0 \cos 2\pi\nu(t - z/c)$ where $E(z)$ = electric field at position z; E_0 = maximum electric field; ν = light frequency; t = time; c = speed of light. It is just this oscillating electric field that can interact with matter and transfer energy to or from matter. The connection between frequency and wavelength is given by $\lambda\nu = c$. The spectroscopically more common unit wavenumber is defined by $\tilde{\nu} = 1/\lambda$.

The interaction of electromagnetic radiation with matter can polarize matter and induce a dipole moment. Matter, e.g., molecules, consists of atoms held together by electrons. If the binding electrons are distributed between the atoms, a covalent bond exists. If the binding electrons are located on an atom, ionic bonding is present. These two main models of bonding are extreme cases. The only covalent bonds which have no ionic character are those between identical atoms. However, molecules formed between different atoms may exhibit partial charges due to an asymmetric distribution of the binding electrons between the atoms. If no side of the molecule has more negative or positive partial charge than the other side, the molecule is nonpolar. However, molecules exhibiting an asymmetric partial charge distribution are polar. An example of a polar molecule is the water molecule, consisting of two hydrogen atoms and one oxygen atom held together by two common electron pairs. Since the oxygen atoms attract electrons more than the hydrogen atoms the

binding electrons in a water molecule are asymmetrically distributed, leading to a negative partial charge on the oxygen atom and positive partial charges on the hydrogen atoms. These partial charges make water act as a dipole. The interaction of polar molecules like water with electromagnetic waves of a certain frequency leads to an orientation of the water molecules.

To learn more about electromagnetic waves and their influence on matter, we look first at a plate-type capacitor whose polarity is changing with a certain frequency. Water molecules are aligned within a plate-type capacitor in such a way that the negative pole of the water molecules points towards the positively charged plate while the positive pole orients towards the negative plate. The water dipoles orient themselves according to the applied electric field of the capacitor, i.e., the water molecules become oriented. This phenomenon is called orientation polarization. Changing the polarity of the capacitor plates leads to a reorientation of the water molecules.

What happens if nonpolar molecules like nitrogen N_2, oxygen O_2 or carbon dioxide CO_2 are brought between the plates of a capacitor? Applying an external electric field leads to a distortion of the electrons compared to the positively charged atomic nuclei. This distortion creates partial charges, i.e., the electric field induces a dipole moment. This polarization effect is called distortion polarization. The more easily the electrons can be displaced compared to the positively charged nuclei the bigger the induced dipole moment. The induced dipole moment μ_{ind} is direct proportional to the applied electric field E where the proportionality constant α is called the molecule's polarizability: $\mu_{\text{ind}} = \alpha E$. α is a measure of how easily electrons can be moved or displaced within a molecule. Thus nonpolar molecules within a plate-type capacitor also become oriented owing to the induced dipole moments. Changing the polarity of the electric field changes the induced dipole moment accordingly. However, the nonpolar molecules are not reoriented; only the electrons will be moved in another direction.

The general form of the polarization depends on the frequency of the applied field. Radio frequency radiation (100 MHz) leads to an alignment of polar molecules according to the external electric field, i.e., a reorientation of the complete molecules (orientation polarization) takes place. The same is true for the electrons, which can follow the changing electric field much more easily owing to their low mass. However, higher frequencies lead to a distortion polarization of the electrons ("induced electric dipole moment") since molecules exhibiting a permanent dipole can no longer follow such rapidly oscillating fields. The induced dipole moment μ_{ind} oscillates with the same frequency as the exciting oscillating electric field E: $\mu_{\text{ind}} = \alpha E_0 \cos 2\pi\nu(t - z/c)$ where α is the molecule's polarizability. Since the oscillating induced dipole moment is simply an oscillating charge, matter radiates light of the same frequency as the initial exciting radiation field (secondary radiation). This is in total analogy

to a Hertzian dipole acting as a broadcasting and receiving antenna, which is based on the radiation of electromagnetic waves from a dipole. In the antenna, electrons are driven by a generator to the top or the bottom. This generates a charge distribution similar to a dipole.

The interplay of molecules with light, in particular with visible light, which polarizes molecules and leads to the emission of a secondary radiation of the same frequency as the polarizing field in all directions, is called elastic light scattering or Rayleigh scattering. It is precisely this undirected scattered radiation which enables us to observe our environment in the presence of sunlight or light from a lamp. The difference between the earth and outer space is that the earth has an atmosphere consisting of molecules which can be polarized. The darkness in outer space is due to the lack of an atmosphere. Rayleigh scattering scales with the fourth power of the light frequency (ν^4), i.e., short wavelengths or high-frequency blue light is scattered significantly more strongly than low-frequency red or infrared light. Hence the midday sky is blue and the sun appears more yellow or red than it really is. The ν^4 dependence becomes especially obvious when the path of the sunlight through the atmosphere is longest. Thus the rising or setting sun appears especially red, since then the less-scattered red light can better reach our eyes.

For a description of microscopic systems like atoms or molecules, quantum effects need to be considered. The term quantum (Latin, "how much") refers to discrete units assigned to certain physical quantities, such as the energy of an atom or molecule. This means that a physical quantity that appears macroscopically continuous appears in the microscopic world only in well defined values that cannot be further divided. The energy of a microscopic system is quantized, i.e., divided into well defined energy portions. An illustration of this phenomenon might be a staircase, in which each step marks an energy portion. Molecules exhibit certain movement patterns, i.e., the molecular degrees of freedom can be classified into translation, rotation and vibration. The energy of all these degrees of freedom is quantized. Similarly, the electron energy in an atom or molecule is quantized. What happens if light interacts with such a quantized rotating and vibrating molecule? The energy of the molecule's degrees of freedom are quantized, and the energy of light is quantized too. Light can exhibit properties of both waves and particles. This phenomenon is known as wave–particle dualism. Einstein postulated the existence of photons, which are quanta of light energy with particle character. By postulating these photons, Einstein was able to explain the photoelectric effect which cannot be explained by the wave theory of light. The photoelectric effect describes the emission of electrons from matter after the absorption of high-energy ultraviolet light due to a collision with the particle-like photon. Each photon possesses the energy $E = h\nu$ where h is Planck's constant ($6.626 \times 10^{-}3$ J sec) and ν the frequency of the light, i.e., electromagnetic radi-

ation of the frequency ν can only carry energy of $0, h\nu, 2h\nu, \ldots$. Only photons possessing energy above a certain threshold lead to an ejection of electrons. According to the wave theory, the electromagnetic field E exerts an oscillating force on the electrons within the matter. This, however, would mean that electrons are ejected with increasing amplitude and not frequency, which is contrary to experiment. Since the energy of matter, i.e., molecules, and electromagnetic radiation cannot be varied continuously, light can only promote matter from one discrete energy level to an energetically higher one if the photon possesses an energy matching the energy difference between two quantum states of the system. This process is called absorption.

Depending on the light wavelength, rotations, vibrations or electrons can be excited within a molecule. Microwaves can excite a transition between two rotational energy states, infrared light is necessary to promote a molecule into a higher vibrational state and visible or ultraviolet light transfers electrons from one electronic state in an energetically higher electronic quantum state. An excited system, however, can relax into the lowest energy state by releasing the additional energy in terms of heat via collisions with the surrounding environment or even by the emission of light. This special form of light–matter interaction is the basis for modern molecular diagnostic procedures in life sciences and medicine. In the following we will concentrate on the vibrational and electronic excitation of molecules, since the excitation of rotations in condensed matter plays no significant role for diagnostic purposes.

A polyatomic molecule exhibits a multitude of vibrations, but of interest are the so-called normal mode vibrations; an N-atomic molecule has $3N - 6$ normal modes. How can one derive this little relationship? The molecular degrees of freedom can be divided into translation, rotation and vibration. Atomic motion through space can be described by the three directions in space x, y and z. As already mentioned, these three coordinates are therefore enough to describe the translation motion of an atom, i.e., an atom has three translational degrees of freedom. Rotations and vibrations do not exist for an atom. If we consider a molecule consisting of three atoms, e.g., water H_2O, the three atoms could move independently through space if they were not connected via a chemical bond. This independent motion would result in nine translational degrees of freedom. However, we know of course that the atoms of a molecule cannot move independently through space but only the whole molecule as an entity. Therefore we need to subtract the three degrees of freedom describing the collective motion of the whole molecule in space from the totality of motions, i.e., of the nine degrees of freedom six remain. By taking into account that a molecule can rotate along the three axes of the coordinate system we need to subtract three more degrees of freedom for the rotational motion. From the original nine degrees of freedom only three remain. These remaining degrees of freedom can be assigned to the vibrational degrees of

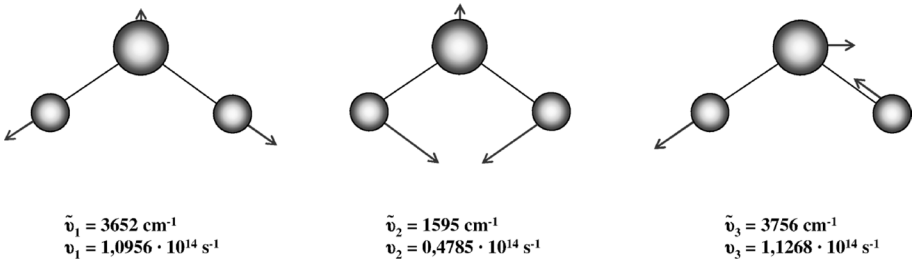

Figure 1.1 Three normal modes of water. The atoms move along the arrows. These three vibrational motions exhibit different vibrational frequencies and can be excited independently.

freedom, i.e., the normal modes of water. The three normal modes of water are shown in **Figure 1.1**. These vibrational motions differ from each other by different atomic displacements. One can differentiate between pure stretching (chemical bonds are stretched or compressed) or pure bending (bond angles are changed) vibrations and mixed forms exhibiting both stretching and compression of chemical bonds and extension or reduction of the bond angles. The easiest way to describe such a vibration is by approximating or describing the chemical bond as atoms held together by a spring. The vibrational frequency then depends on the atomic mass and the spring force constant. The atoms move during a vibrational period towards or away from each other. This movement is repeated periodically and can be easily described by a harmonic oscillator. A harmonic oscillator is a system performing periodic vibrations about its equilibrium position where the restoring force F is directly proportional to the displacement x. **Figure 1.2** shows such a vibrational motion in a harmonic parabolic potential. The minimum of the potential corresponds to the equilibrium distance, i.e., the nuclear distance exhibiting the lowest energy. According to quantum theory, the vibrational energy cannot be continuously but quantized. This quantum harmonic oscillator is symbolized by the horizontal lines inside the parabolic potential. The vibrating quantum mechanical system possesses a zero point energy, i.e., molecules are always vibrating and are never at rest. If the displacement from the equilibrium position is minimal, which is true for small vibrational quantum numbers, the harmonic oscillator model is well suited to describe a vibrational absorption process. However, for large displacements, i.e., for high vibrational states, the model of the harmonic oscillator model is problematic: Firstly, it is impossible to put an arbitrary amount of energy into the system without destroying the molecule. However, it is known that chemical bonds can break, i.e., dissociate, or molecules can fall apart if they are heated too much. To allow for dissociation to take place the harmonic oscillator model has been refined by the anharmonic oscillator model. The anharmonic oscillator model considers

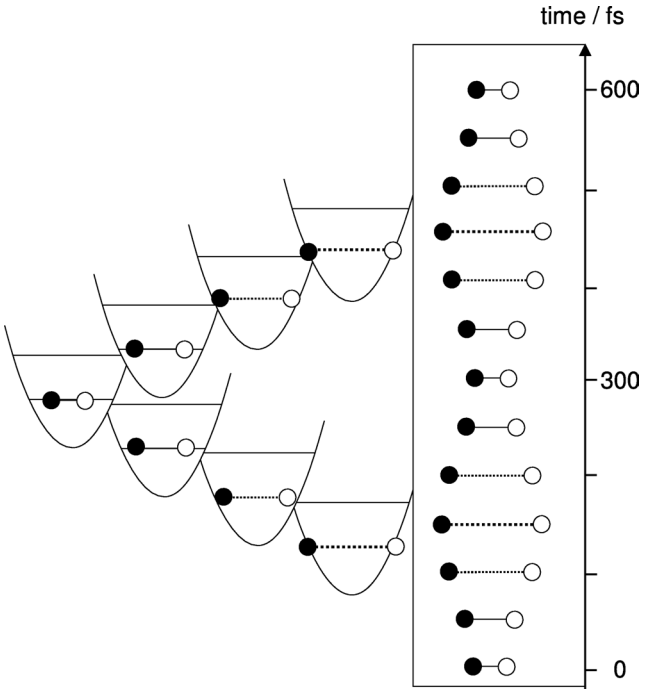

Figure 1.2 Harmonic Oscillator Model. The molecule performs a harmonic vibration about its equilibrium position, i.e., the restoring force is direct proportional to the displacement of the atoms from their equilibrium position (right side). The molecule is modelled as balls connected by a spring. The vibrational frequency depends on the mass of the balls and the spring force constant. The left side depicts the vibrational motion in a harmonic parabolic potential. According to quantum theory the vibrational energy is quantized which is symbolized by the horizontal lines within the harmonic potential.

the fact that molecules dissociate at high vibrational energies, i.e., the spring between the atoms breaks. While for the harmonic oscillator the energy difference between the quantized vibrational states is always the same, this difference for the anharmonic oscillator decreases with increasing energy until a continuum is reached where all vibrational states have almost the same energy. Within this continuum dissociation occurs, i.e., the atoms of the molecule can leave the molecule's force field (anharmonic potential see **Figure 1.3**).

After all this theory the question remains, "What can we learn from vibrating molecules?" Most normal modes if they are not degenerated (degeneracy = same energy) exhibit different vibrational frequencies. The vibrational frequencies of the three normal modes of water are: $\nu_1 = 3652\ \mathrm{cm}^{-1}$ (symmetrical stretch motion), $\nu_2 = 1595\ \mathrm{cm}^{-1}$ (symmetrical bending motion) and $\nu_3 = 3756\ \mathrm{cm}^{-1}$ (asymmetrical stretch vibration). Light with the appropriate frequency can be absorbed by the ensemble of vibrating molecules, promoting them from the vibrational ground state into the first excited vibrational state.

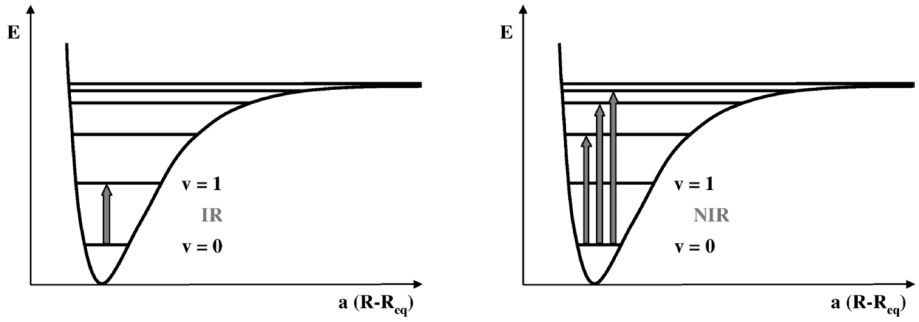

Figure 1.3 Comparison between harmonic (left) and anharmonic oscillator (right). The anharmonic oscillator considers the fact that molecules can dissociate for high vibrational energies. For the harmonic oscillator the energy distance between the vibrational levels is constant. This is in contrast to the anharmonic oscillator, where the energy difference decreases for increasing energy until a continuum is reached where dissociation takes place.

During the course of the absorption process only the amplitude of the vibrational motion out of the rest position changes, while the vibrational frequency stays constant. The light frequencies required to directly excite vibrational absorptions covers the spectral range from 2.5 µm to 1 mm or in spectroscopic wavenumber units 400–4000 cm^{-1}. This frequency range is also called the far-infrared region. The spectral area below 400 cm^{-1} called terahertz radiation has been opened recently for vibrational spectroscopy. Terahertz radiation is extremely promising for Biophotonics, since the penetration depth of this radiation into biological tissue is extremely large.

We shall now consider IR absorption, i.e., the direct absorption of IR radiation by vibrating molecules. What can vibrating molecules tell us about the molecules themselves or the surroundings in which they are embedded? The answer is a lot, since both the number and the type of vibrations depend directly on the atoms present in the molecule and, in particular, how these atoms are chemically bonded to each other. Absorption of IR radiation with the appropriate frequency promotes the molecule from its vibrational ground state into the first excited vibrational molecular state. This absorption process decreases the transmitted intensity with respect to the incident intensity. A plot of the transmitted intensity versus the radiation frequency yields an IR spectrum. For this reason the molecule provides detailed information about itself via interaction (absorption) with an appropriate electromagnetic field (e.g. IR radiation). Moreover, the energy of the vibrational transition depends on the chemical environment the molecules are embedded in. Hence, vibrational spectroscopy provides a key to the molecular environment of the molecules. In addition to the transition from the vibrational ground state to the first excited vibrational state (the fundamental transition), direct absorption

processes can also promote the molecule into the second, third or even higher vibrational state, although with much less probability than the fundamental transition. These higher transitions are called overtones. The energy required to excite overtones moves from the IR region into the mid- (2.5–50 μm) or even the near-IR (800 nm to 2.5 μm) spectral region. Overtone vibrational spectroscopy is an important well established method in quality control, but plays only a minor role in the field of health care.

Vibrational transitions can also take place via an inelastic light-scattering process. We have shown already that light can polarize molecules. For the visible wavelength region, the main contribution to the induced polarization comes from electrons, whose distribution relative to the atomic nuclei is distorted by the interacting electromagnetic field. Thus this type of light–matter interaction induces an electric dipole moment within the molecules. The polarizability α is a measure of how easily the electron distribution can be distorted within a molecule. The induced dipole, oscillating with the frequency of the electromagnetic field, emits an electromagnetic wave in all directions. If the polarizability does not change with time, the frequency of the emitted secondary wave corresponds to the frequency of the oscillating induced dipole, i.e., the frequency of the external electromagnetic wave inducing the dipole (= Rayleigh scattering). However, since molecules are always vibrating, the polarizability α is not constant over time, but changes according to the different vibrational frequencies of the molecule's normal modes. Therefore, the induced dipole moment and thus the emitted secondary radiation are also modulated by the different vibrational frequencies. Consequently, the secondary radiation emitted by the molecule is a superposition of the exciting frequency and the various vibrational frequencies of the molecule. Dispersing this secondary radiation into its frequency components yields beside the strong Rayleigh scattering also weak sidebands. The distance between the Rayleigh wavelength and the wavelength of the sidebands corresponds to the vibrational frequencies of the molecule. The appearance of these sidebands arising from an inelastic light-scattering process was first discovered in 1928 by C.V. Raman. This so-called Raman Effect marks an indirect approach to the excitation of molecular vibrations. The Raman Effect can be interpreted quantum mechanically as an inelastic collision between photons and vibrating molecules. Photons can be scattered from molecules. This scattering process corresponds to a molecular transition into an extremely short-lived transition state, the so-called virtual level (= collective quantum energy state of the entity molecule and photon). The molecule can subsequently relax from this virtual level into the original molecular state or to an energetically excited molecular state. If the scattering process starts from the vibrational ground state and ends up in a vibrationally excited state via a transition into the virtual state it is called Stokes–Raman scattering. If the molecules are initially already in a vi-

brationally excited state and are transferred by the scattering process into the vibrational ground state one refers to as anti-Stokes Raman scattering. Since at room temperature the vibrational ground state is significantly more populated than the vibrationally excited states, the Stokes–Raman spectrum of a sample is more intense than the anti-Stokes Raman spectrum. For Rayleigh scattering, the state before and after the scattering process is the same. These scattering processes are classified as two-photon processes since two photons are involved.

The two vibrational spectroscopic methods Raman and IR absorption spectroscopy are complementary methods based on two different light–matter interaction phenomena and thus exhibit different selection rules. Selection rules determine which vibration of a molecule can be excited by what method. In the case of IR absorption, one photon directly promotes the molecule into a higher vibrational state while the Raman scattering process involves two photons. In order for a molecular vibration to absorb an IR photon, the dipole moment of the molecule has to change during the course of the vibration, i.e., only those vibrations which give rise to an oscillating dipole are IR active. The polarizability has to change during the vibration so that a molecular vibration can be promoted via an inelastic scattering process into a higher vibrational state.

Both Raman and IR absorption spectra can be considered as molecular fingerprints of the molecules existing in a biological sample. The important role of vibrational spectroscopy, and in particular Raman spectroscopy, in Biophotonics can be found in Chapter 3, where representative examples of IR absorption, Raman spectra and a more detailed introduction to Raman spectroscopy (theory, instrumentation, etc.) is given.

Light scattering can take place over the complete electromagnetic spectrum, although the scattering power scales with the fourth power of the exciting frequency, i.e., short-wavelength radiation is scattered strongly while long-wavelength radiation is only scattered poorly. Direct absorption of microwaves or IR radiation can excite rotations or vibrations, respectively. However, the interaction of matter with visible or UV light can also lead to an absorption of this radiation. In this case, light–matter interaction leads to electronic excitation. We can differentiate between two spectral regions: the region between 200 and 380 nm is called the ultraviolet (UV) region, while the area between 380 and 700 nm spans the visible (VIS) wavelengths. What happens if UV-VIS radiation is absorbed by a molecular system? So far, the description of IR absorption and Raman scattering has been limited to the electronic ground state, which was described as a harmonic or anharmonic oscillator (parabolic potential curves, see also **Figures 1.2** and **1.3**). **Figure 1.4** displays the electronic ground state, denoted S_0, as well as the first electronically excited state S_1. In order to simplify the presentation, the harmonic or anharmonic poten-

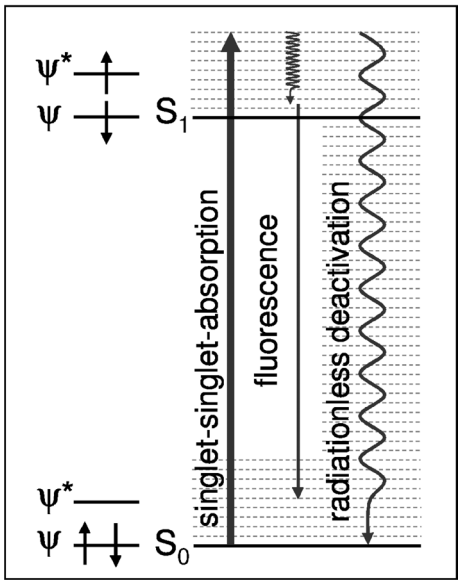

Figure 1.4 Electronic energy diagram. The horizontal lines represent the electronic energy at the equilibrium position. The S_0 state is the electronic ground state of the molecule. A UV or a VIS photon can promote the molecule from its electronic ground state S_0 into the first electronic excited state S_1. The excitation takes place from the vibrational ground state of the electronic ground state into a vibrationally excited state of the electronic excited state. The molecule can lose its additional vibrational energy within the S_1 state via collisions with, for example, solvent molecules to relax into the vibrational ground state of the S_1 state. From there the molecules can relax to the electronic ground state by emission of fluorescence light. Besides the radiative decay processes, radiationless decay processes from the S_1 into the S_0 state also exist.

tial curves are not shown and the electronic states are depicted as horizontal lines, reflecting the electronic energy at the equilibrium geometry of the relevant molecule. S stands for a singlet state. Two electrons are involved in a chemical bond. As mentioned before in a covalent bond, the two binding electrons are mainly located between the two atomic nuclei. If the electrons are mainly located more or less on one side the chemical bond is classified as polar or an ionic bond. Without going into too much detail we need to consider that electrons possess a negative charge as well as a spin. This means in the figurative sense that the electrons spin on their own axes. Depending on the rotating direction we differentiate between electrons having α-spin (clockwise spin) and β-spin (counter-clockwise spin). Why is this quantum mechanical detail of importance? So far electrons have been considered as particles but wave–particle dualism also applies to electrons, i.e., an electron can also be described as a wave. If electrons are moving around atomic nuclei in the form of a wave the wave must reproduce itself on successive circuits. Thus a waveform in which after one or more circuits a wave peak meets a

wave valley is not allowed since then it would interfere destructively with itself and would not survive. This simple picture reveals that only special, discrete waves are acceptable to describe the electronic motion. The spatial distributions of these special waves (in the strict sense the square modulus of theses discrete waves) are called orbitals. The discrete solutions lead to quantization, and physicists denote the possible single-valued electron waves with quantum numbers. Wolfgang Pauli found that electrons occupying a single orbital are not allowed to be identical, i.e., the existence of two electrons exhibiting exactly the same quantum numbers is not allowed. Thus an orbital can be occupied by maximally two electrons differing in their spin state, i.e., their intrinsic angular momentum. In the case of chemical bonds, the quantized electron waves occupied by the bonding electrons are called molecular orbitals. According to the Pauli principle, electrons need to be paired in a chemical bond, i.e., one electron has α-spin and the other β-spin. In **Figure 1.4** the electron having α-spin corresponds to arrow up ($\uparrow$) and the electron exhibiting β-spin is denoted by a down arrow ($\downarrow$). Paired electrons within the S_0 state are denoted by ($\uparrow\downarrow$). The absorption process promotes an electronic excitation, i.e., an electron from the S_0 state is transferred into an energetically higher lying orbital, the S_1 state, while the spin state is conserved. This excitation proceeds in analogy to the aforementioned IR absorption process via direct absorption of an appropriate photon. Thus, electronic excitation takes place from the vibrational ground state ($v = 0$) of the electronic ground state into a vibrational state v' of the first excited electronic state. Which vibrational states within the electronic excited states are populated depends on the geometrical rearrangement taking place upon electronic excitation. If we measure, as described above for IR absorption spectroscopy, the ratio between the initial light intensity I_0 and the transmitted intensity I vs. the light wavelength it is possible to determine the concentration of absorbing molecules within a sample via the so-called Lambert–Beer law: $E(\nu) = \epsilon(\nu)cd$. The attenuation of light due to an electronic absorption is described by $I(\nu) = I_0 \times 10^{-E}$. $\epsilon(\nu)$ is the molar decade absorption coefficient, E corresponds to the absorption, c is the desired concentration and d represents the thickness of the sample cell.

Now the questions arise, "What happens to electronically excited molecules? What is the residence time of the molecules in the excited state?" An excited molecule tends to release its additional energy in any form to get back into the lowest energy state, i.e., the most stable ground state. What happens in detail? If the electronic excitation generated vibrationally excited molecules within the excited electronic state, the molecule rapidly releases the additional vibrational energy via collisions with the surroundings (e.g. solvent molecules) to pass into the vibrational ground state of the excited electronic state. The time-scale of this ultrafast vibrational relaxation is 10^{-14}–10^{-12} s. In polyatomic molecules, vibrational relaxation can also take place without the

presence of solvent molecules via a redistribution of the additional vibrational energy from one specific mode populated upon electronic absorption to other vibrational modes.

From the vibrational ground state of the excited electronic state the molecule can spontaneously relax to the electronic ground state by emission of light. This radiational transition is called fluorescence. The time-scale for fluorescence to take place is 10^{-9}–10^{-8} s. Since the time-scale for vibrational relaxation is orders of magnitudes shorter than that for the emission of fluorescence light, the emission of molecules in condensed phases or solid state always starts from the vibrational ground state of the first excited electronic state S_1. However, the emission of fluorescence light following an electronic absorption is not the most common electronic relaxation mechanism but rather an exception. In nature, radiationless transitions are the dominant form of electronic relaxation. Collisional deactivation processes lead to a decay of the excited electronic state S_1 directly back into the S_0 state. The color of plants, fruits, etc. are not due to the emission of fluorescence following the electronic absorption of light but rather a consequence of light absorption and reflection (vide infra). White light results from a superposition of all wavelengths in the UV-VIS electromagnetic spectrum. A tomato appears red under irradiation with white light because it absorbs all colors from the white light spectrum except red. Thus the color red is reflected from a tomato.

Besides fluorescence and vibrational relaxation, several other electronic relaxation mechanisms exist. However, a detailed description of these is beyond the scope of this general introduction.

So far this short and general introduction of light–matter interactions has mainly concentrated on single molecules. However, matter is generally not present in the form of single molecules but rather as molecular aggregates. The aggregates can exhibit different spatial dimensions and might range from a few associated molecules called clusters via nano- and microparticles to large molecular aggregates, e.g., crystals, visible to the naked eye. Nanoparticles are aggregates of a few hundreds of molecules or atoms forming discrete units with a size in the nanometer range. When light interacts with matter whose particles are larger than the light wavelength, i.e. larger than 300 nm, beside absorption and scattering new light–matter-interaction phenomena occur. Such new light–matter interactions are, for example, reflection and diffraction of light. These mechanisms play an important role in the interplay of light with biological matter, e.g., united cell structures or tissues. For these phenomena the aforementioned molecular polarization is of particular importance. In the case of extended matter, with spatial dimensions greater than the light wavelength, the polarizability is described as the sum of the molecular properties, i.e., as an averaged value. In total analogy to the molecular polarizability, the bulk polarizability describes the dipole moment

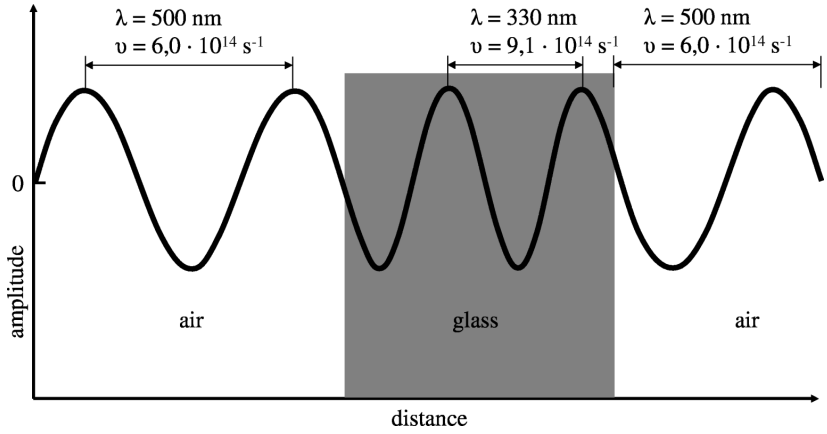

Figure 1.5 Light wave of a wavelength of 500 nm ($\nu = 6.0 \times 10^{14}$ s^{-1}) travelling through air hits a glass medium ($n = 1.5$). Because of the refractive index of glass, light no longer travels at almost 300 000 km s^{-1} but only with a reduced speed of 198 000 km s^{-1}. Since the frequency of the light wave remains constant while travelling through matter the wavelength is reduced from 500 nm to about 330 nm.

induced into bulk matter by an external electric field. Put simply, a value can be derived from the bulk polarizability indicating how fast light travels through matter. This value is called the refractive index. If no matter is present, i.e., in a vacuum, the speed of light is $c_0 = 3 \times 10^8$ m s^{-1}. If, however, light travels through matter the speed of light c is reduced. The ratio of the two speed values determines the refractive index: $n(\nu) = n(\lambda) = c_0/c$. Like the polarizability the refractive index depends on the light frequency ν or the wavelength λ. If a light beam hits matter, e.g., a piece of glass, the light beam experiences a refractive index difference from n_1 to n_2. The speed of light reduces from around 300 000 km s^{-1} to about 198 000 km s^{-1} if the glass has a refractive index of $n(500\text{ nm}) = 1.515$. The refractive index of the vacuum is by definition 1. Since frequency (ν), wavelength (λ) and speed of light (c) are related by the equation $\nu = c/\lambda$ and the speed of light c is a function of the refractive index, the following equation results: $\lambda = c_0/(\nu n)$. This equation raises the question, "Which value stays constant when light passes through matter: the wavelength or the frequency?" This question can be easily answered by taking into account the effect a visible-frequency electromagnetic wave causes within matter. The oscillating electric field polarizes the molecule and induces a dipole moment oscillating with the same frequency as the external field. The evolving secondary wave has the same frequency as the exciting one. Thus the frequency remains constant, while the wavelength decreases (see **Figure 1.5**).

What other effects can be seen if a light beam hits a glass plate? As is well known, the light beam is reflected or refracted at the glass surface. These

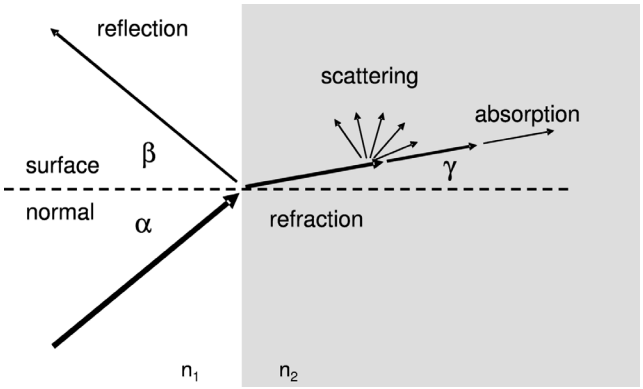

Figure 1.6 Illustrative description of the optical processes: reflection, refraction, scattering and absorption taking place if a light beam passes from one material to an optically different material.

phenomena can be easily explained by geometrical optics. Depending on the nature of the material, a certain fraction of the incident light is reflected, and the angles from the normal of the incident and reflected waves are identical. Refraction of the wave into the medium takes place if the medium does not absorb the radiation. **Figure 1.6** summarizes the light–matter interactions of reflection, refraction, scattering and absorption. All these processes are of special importance while investigating biological cells or tissue by means of optical methods.

The change of the propagation angle of the light beam depends on the refractive angle and can be described by Snell's law: $n_1 \sin\alpha = n_2 \sin\gamma; n_1 < n_2$. In case $n_1 > n_2$, i.e., n_1 is the optically more dense medium, the light is refracted away from the normal and not towards the normal. Before dealing with the interaction of light with biological matter in more detail another important phenomenon, total reflection, needs to be explained. The phenomenon of total reflection is depicted in **Figure 1.7**. Total reflection means the complete reflection of the light beam. A prerequisite for total reflection to take place is the light beam needs to travel from an optically more dense to an optically less dense medium. If the angle of incidence is larger than the threshold angle θ_c, total reflection takes place. The threshold angle θ_c is defined as $\theta_c = \arcsin(n_2/n_1)$ where $n_2 < n_1$.

If light hits biological tissue it may be partly reflected from the surface or it will be in parts refracted into the tissue. Within the tissue light can be absorbed or scattered. The proportion of light refracted increases as the angle between the incident light and the surface increases (see **Figure 1.8**), i.e., as the angle of incidence α (**Figure 1.6**) decreases. If as much light as possible is to penetrate into the tissue the light must hit the tissue at a right angle. Since biological tissue is rather inhomogeneous, the various light–matter effects occur

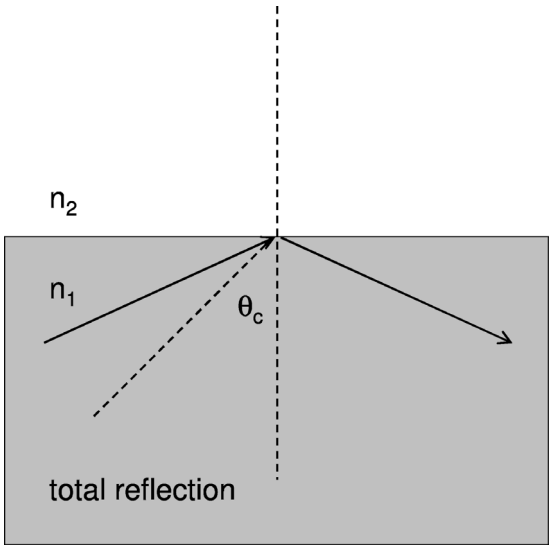

Figure 1.7 If a light beam coming from an optical thicker medium hits an interface to an optical thinner medium ($n_1 > n_2$) the light beam can only pass over into the optically thinner medium, if the incident normal angle is smaller than a critical angle θ_c. In case the incident normal angle is bigger than θ_c total reflection occurs.

in different proportions. Biological tissue is usually a strongly light-scattering material. Depending on the tissue constituents, we can differentiate between elastic light (Rayleigh) scattering and Mie scattering. Rayleigh scattering occurs predominantly from cell constituents smaller than the light wavelength. If the size of the tissue constituents is the same as the light wavelength, a new scattering phenomenon occurs, the so-called Mie scattering discovered by Gustav Mie 1908. In contrast to Rayleigh scattering, Mie scattering shows a less pronounced wavelength dependence and the light is mainly scattered in the forward direction. Both light scattering and absorption lead to an attenuation of the light beam. The absorption of light originates from the multitude of molecules present in biological tissue. The attenuation can be described by the Lambert–Beer law $I(z) = I_0 \exp[-(\alpha(\nu) + \alpha_s)z]$. $I(z)$ characterizes the light intensity at the position z within the biological tissue, z equals the penetration depth, $\alpha(\nu)$ is the absorption coefficient and α_s denotes the scattering coefficient. Both coefficients describe the loss of light intensity within the tissue.

Because of the multitude of molecular constituents which can be found in biological tissue, e.g., proteins, peptides, Desoxyribonucleic Acid (DNA) and Ribonucleic Acid (RNA), haemoglobin, melanin and water, biological tissue absorbs over a wide spectral range. **Figure 1.9** shows various wavelength regions as well as absorption data of typical components of biological tissue,

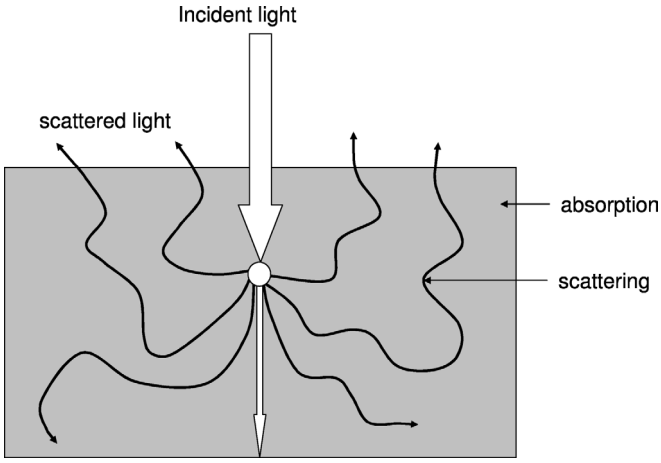

Figure 1.8 If a light beam strikes perpendicularly on biological tissue the proportion directly reflected from the surface becomes minimal. The light penetrating into the tissue is absorbed more or less depending on the wavelength. Besides absorption, the straightforward propagation of light is hindered within biological tissue by Rayleigh and Mie scattering. Depending on the biological tissue, only a minimal intensity of the originally applied electromagnetic radiation reaches through the tissue.

such as blood, melanosome and epidermis. Owing to the high absorption of biological tissue over a relatively broad spectral range, the penetration depth of light strongly depends on the light wavelength used. To some extent, light can only penetrate by a fraction of a millimeter before it is totally absorbed or scattered. Blue and green light will be absorbed strongly while red light is almost not absorbed by biological tissue. Furthermore, short-wavelength light is scattered strongly (ν^4 dependency). Overall this means that only red light can travel through biological tissue without experiencing too many losses. Near-IR light exhibits the highest penetration depth of 2 to 5 mm. Green light will be totally absorbed or scattered after a penetration depth of 0.5 to 2 mm.

1.3 A Fascinating Tour Across Biophotonics

After this very brief introduction into light–matter interactions we will come back to the question of the scope of this book. Our aim is to take you on a fascinating tour across Biophotonics. We will tell you nine different stories, which result from nine projects funded within the Biophotonics research framework of the German Federal Ministry of Education and Research (BMBF) since the year 2001. Each chapter describes the basic principles, methods and results of a single network project, showing the long journey from a scientific idea, via constant improvements for various scientific and business applications, to a

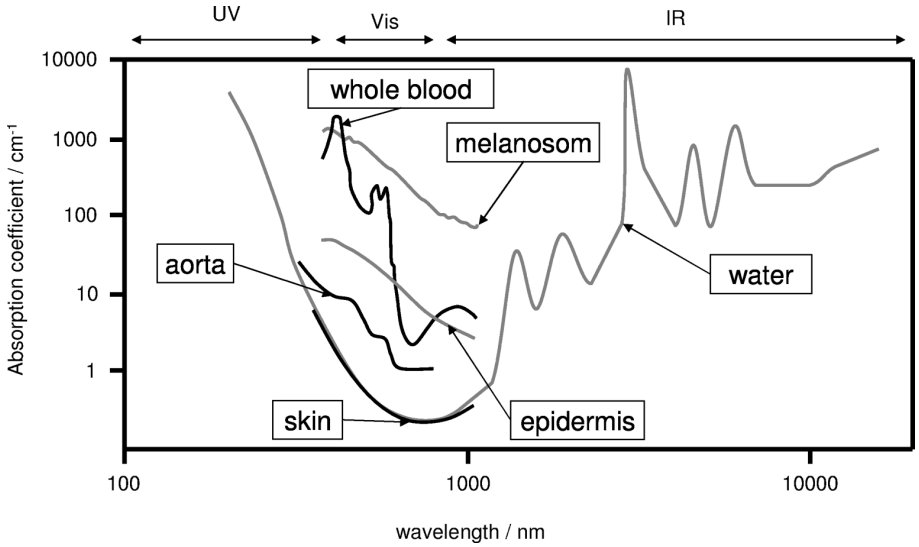

Figure 1.9 The main constituents of the absorption spectrum of biological tissue: (1) Absorption in the UV is increased by proteins, DNA and other molecules. (2) In the IR the absorption increases for longer wavelengths because of the water (75%) present in biological tissue. (3) In the red spectral region as well as the near-IR the absorption of all molecular constituents of biological tissue is minimal. This frequency region is therefore called the diagnostic or therapeutic window since using these wavelengths allows one to penetrate deeply into biological tissue. (4) Blood is a strong absorber in the red/NIR region. However, since blood is only present to a small percentage within biological tissue the average absorption coefficient is not influenced to a great extent. However, if a photon hits a blood vessel it will be absorbed, i.e., the spatially varying absorption properties of biological tissue determine the light–tissue interactions and the average absorption properties determine the light transport through biological tissue. (5) Melanosomes are strong absorbers. However, they are only present in a small percentage in the epidermis, i.e., the local interaction of light with melanosomes is great, but the contribution of melanosomes to the average absorption coefficient is rather low, i.e., the light transport is only slightly influenced by these molecules.

product which will very soon be ready to be brought to the market.

Themed by the key phrase "Light for better physical health", the projects deal with bio-processes, cell–cell communication and biological interactions in the whole organism. As mentioned before, the intention is to gain a deeper understanding of the processes that lead to the outbreak of common diseases. To achieve this end will take time, and as in an old saying so also in Biophotonics, even the longest journey begins with a single small step. So our first purpose has to be to learn more about the correlation between the various influences from outside and inside the human body shown in **Figure 1.10**, which draw the distinction between health and illness. Internal influences are based on the genetic profile of the individual, and are responsible for changes in protein production and metabolism, which can have severe effects no matter

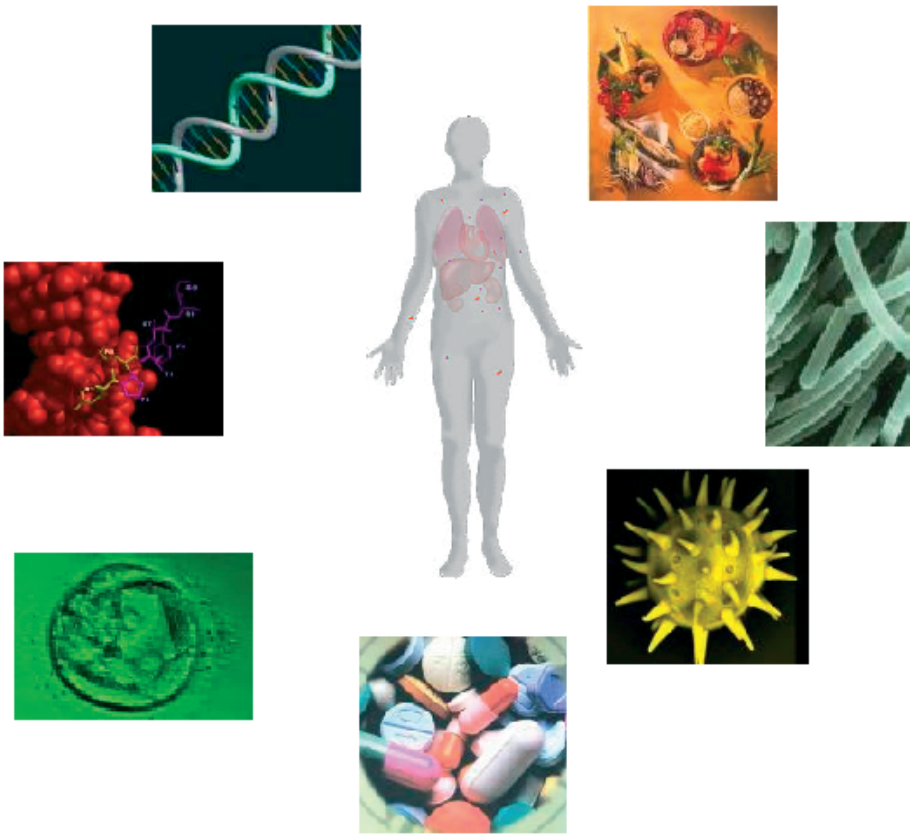

Figure 1.10 The individual projects of the German main research topic Biophotonics work on different biological levels. Some deal with the genomic level, i.e., DNA mutations, others with the interaction of proteins or changes in the metabolism of a single cell. Other projects concentrate on influences from outside, such as airborne microorganisms or pollen grains or the effects of food and drug ingredients. However, always at center stage stands the human being and its physical health.

how marginal they might be. External parameters can also be found inside the body, such as microorganisms living in our gut. But despite that, and because our organism is an open system, there is a continuous intake and excretion of substances that also influence our state of health, e.g., food substances, drugs and airborne biotic and abiotic particles. You will find this variety of influencing agents reflected in the topics of Chapters 2 to 10, which highlight the so far undreamed-of possibilities being provided by the fast-emerging field of Biophotonics. In the following we will give a short overview of the content of the various Biophotonics projects.

A better quality of life for the millions of allergic persons is the ambitious goal of the network project "Online Monitoring of Airborne Allergenic Par-

ticles" (OMNIBUSS). To allow people suffering from hay fever or asthma a nearly normal way of life and to avoid unnecessary intake of pharmaceuticals, a continuously and routinely updated knowledge of pollen concentration in the air is essential. Until now, pollen forecasting has employed manual microscopy techniques, which are not only time-consuming and labor-intensive but also provide results of undefined and unsatisfactory quality. As described in Chapter 2, p. 31ff., OMNIBUSS has developed a new microscope-based fully automated monitoring method, which is characterized by high temporal resolution, excellent reproducibility, detection limit, recall and precision, and which therefore meets the strong public demand for absolutely reliable of pollen concentration data. The device the project has led to combine continuous sampling and automatic preparation of aerosol, automated particle imaging and automated identification of pollen grains based on mathematical fingerprints.

Just as in allergy prevention, the presence of airborne biotic particles plays a very important role in connection with clean-room processes. In industrial food or pharmaceutical production, such bioaerosol may lead to fatal consequences. To drastically reduce the time needed for quality assurance of life science products, traces of contamination need to be tracked down reliable and rapidly. Chapter 3, p. 89ff., "Online Monitoring and Identification of Bioaerosols", shows how the new approach of the research network OMIB can make a decisive contribution to the monitoring process for a rapid detection of aerosol and an identification of airborne microorganisms without loss of time. For standard microbiological tests, the microorganisms are collected on a growth medium, bred and eventually counted. Under certain conditions even the combination of several microbiological tests leads only to an ambiguous identification. The authors of Chapter 3 describe how they developed totally new equipment, which combines differentiation of biological from non-biological particles by means of fluorescence detection with an identification step by vibrational spectroscopy, in particular Raman spectroscopy. This method offers an enormous time gain compared to the conventional methods applied until now.

Infectious agents are gaining ground again – and they can not only cause industry a loss of time and money but each one of us a loss of life. Germs such as the tuberculosis-causing *Mycobacteria* are horrifying the world, not only by their rapid spread around the globe but also by their ability to cope with antimicrobial drugs. One new powerful weapon is presented in Chapter 4 called "Novel Singly Labeled Probes for Identification of Microorganisms, Detection of Antibiotic-resistant Genes and Mutations, and Tumor Diagnosis". "Smart Probes" (Chapter 4, p. 167ff.) are fluorescently labelled DNA-hairpin structures, which have the potential to open a new avenue in molecular diagnostics by their ability to discriminate between wild type and

resistant bacteria. Besides a detailed description of Smart Probes applications in the detection of antibiotic-resistant genes and mutations as well as in tumor diagnosis, the chapter gives a competent general survey of single-molecule fluorescence spectroscopy and 3D fluorescence nanoscopy.

The abbreviation PLOMS (Chapter 5, p. 231ff.) conceals an innovative way to detect cancer of the colon in its very early stages, thus dramatically increasing healing rates simultaneously cutting the expenses in the health system. The authors of Chapter 5 captioned their contribution "Early Diagnosis of Cancer". They give a very detailed introduction to the epidemiological and biological background of cancer, the mechanisms of carcinogenesis and the impact of early cancer diagnosis before outlining their new approach for very early detection of the first stages of tumor growth in the colon. This new approach is based on the fact that the structure of the glycocalyx of normal mucosa cells changes as they degenerate into cancer cells. Like an old blanket becoming threadbare, the glycocalyx forms small holes, which allow the scientists of the network project PLOMS to discriminate between healthy and degenerated cells in the colon via sophisticated labelling strategies.

Chapter 6, p. 301ff., entitled "New Ways for Marker-free Live Cell and Tumor Analysis" also deals with early tumor diagnosis. MIKROSO is a network project developing digital holographic microscopy as a very new approach for label-free quantitative imaging of living cells and therefore as a useful tool for seeing the signs that reveal even marginal pathological changes in cells and tissue, and for watching very closely the behavior of healthy and diseased cells. The authors point out the many advantages of digital holographic microscopy in comparison with standard methods of cell microscopy, and describe the various application possibilities of combinations of digital holographic microscopy with other techniques, such as phase-contrast and fluorescence imaging or laser micromanipulation. In addition, the article provides an introduction to optical coherence tomography as well as to minimally invasive holographic endoscopy.

The aim of "Regenerative Surgery" is to heal diseased tissue by full or partial reconstruction and to support the regeneration of organs if not actually to substitute them. Chapter 7, p. 361ff., gives an account of the medical and biological background not only of regenerative surgery but also of tissue engineering, with a strong emphasis on cell and tissue culture technologies. The network project MeMo designs novel techniques to improve such cell and tissues cultures by laser optical on-line monitoring. Innovative biophotonic technologies have been developed for a more efficient evaluation of tissues. Taking as an example the replacement of human cartilage tissue and chondrocytes, the authors outline the advantages of recent methods like three-dimensional laser-scanning microscopy, fluorescence lifetime measurements and parallelized two-photon measurement systems for rapid high-resolution tissue imaging.

Chapter 8, p. 405ff., brings us from the surgery back to the laboratory, and in particular to the "lab on a chip". Microarrays have been one of the enabling technologies of the 1990s, and have greatly increased the possibilities not only for basic research in molecular biology but also for the identification and validation of drug targets. But as a technique which can perform "thousands of reactions on a small chip" as indicated by the chapter title, microarrays demand efficient, reliable and comprehensive analysis methods. In this regard, optical systems provide a wide choice of techniques. The scientists of the MOBA network project concentrate on terahertz spectroscopy to obtain very rapid high-quality results, previously unknown. The binding of DNA and other biomolecules can be analyzed to learn more about the genetic profile of the scanned samples. As well as the specific adjustment of drugs for individual gene profiles, further applications lie in the areas of bioweapon analysis and telemedicine. An overview of fluorescence and label-free techniques completes this chapter.

A deeper understanding of the processes of life from the molecular structure to the whole organism demands methods that provide very high resolution in time and space. Therefore, analytical tools are required which cope with the need for minimally invasive measurement techniques featuring not only high precision and selectivity but also the ability to process a large number of samples at the same time. Chapter 9, p. 477ff., called "Hybrid Optodes" describes the work of the research network HYBOP aiming for the development of a novel fluorescence-based hybrid technology which will be employed in spatiotemporal high-resolution bioprocess analysis. This new technology is based on indicator-specific polymer surface coatings which supply two kinds of information at the same time by means of optoelectronic measurements. Thus, in each case two parameters, e.g., temperature and oxygen or carbon dioxide and pH, can be measured with high precision simultaneously. The chapter gives an overview of the principles of hybrid optodes and their applications and perspectives in biotechnology.

With the vision of an early detection of diseases and tailor-made therapies the ODMS network project is consistent with the aims of the main research topic "Biophotonics" in general. As described in Chapter 10, p. 519ff., entitled "Digital Microscopy", ODMS has developed an "Ocularless Digital Microscope System" for the on-line *in vivo* measurement of biological or biomedical parameters. There are three main areas of application to which the device will be adapted. One is the target assay development on the cell-culture level, providing a comprehensive visualization of the pharmaceutical effects of a new drug. Telepathology, that is the transfer of histological medical findings in the form of digitalized data ("virtual slides"), is another application for ODMS. This technology reduces the time-consuming dispatch of samples to different consultants. Thirdly, ODMS will aid cell and developmental biological stud-

ies. The aims are in particular to minimize stress for the biological probe and to use light to its full capacity.

We hope that with these short summaries we have aroused your curiosity so that you continue to read. But first we should like to thank the Ministry of Education and Research and the Association of German Engineers (VDI) for their financial support of the work of all the network projects and their excellent cooperation over the years. In addition, the editors would like to thank all the people who have contributed to this book. Above all, we should like to thank Andreas Thoss and the team at Wiley for providing us with the idea for this project. We appreciate the confidence and patience they have shown us very much indeed. To cope with the task of editing a book like this cannot be done without many helping hands. So our gratitude goes to the authors of the single chapters of this book for all their efforts to make this volume a valuable one for scientists all over the world. A truly special word of thanks goes to our colleagues at the Institute of Physical Chemistry in Jena, who provided us with invaluable help in the matter of technical support and were able to restore us to a good mood at any time. We must mention by name Kathrin Strehle, Ute Uhlemann, Dana Cialla and Reinhold Gade, but our thanks go also to all the other members of our working group. On a very personal note we should like to thank our families for being so understanding during all the days and nights we were constrained by the work on this book.

1.4
Links and Literature about Biophotonics

The following list is far from complete and is only intended to provide the interested reader with further insight into the rapidly growing subject of biophotonics:

Journal:

- "Biophotonics International", Laurin Publishing Company (www.photonics.com)

Selected "Special Issues":

- Special Issue on Biophotonics, Journal of Physics D: Applied Physics, Volume 36, Number 14, 21 July 2003
- Special Issue on Biomedical Optics, Journal of Physics D: Applied Physics, Volume 38, Number 15, 7 August 2005

- "Biophotonics Micro- and Nano-Imaging", Progress in Biomedical Optics and Imaging, Vol. 5, No. 33, in: Proceedings of SPIE, Volume 5462, 2004.

Books:

- Faupel, M., Brandenburg, A., Smigielski, P. and Fontaine, J. (Eds): Biophotonics for Life Sciences and Medicine, FontisMedia, Lausanne/Formatis, Basel, in press.
- Marriott, Gerard (Ed): Biophotonics, Academic Press, San Diego, 2003.
- Prasad, Paras N.: Introduction to biophotonics, Wiley-Interscience, Hoboken, New Jersey, 2003.
- Shen, X. and van Wijk, R. (Eds): Biophotonics: Optical Science and Engineering for the 21st Century, Springer, Berlin, 2006.
- Wilson, B.C., Tuchin, V.V. and Tanev, S. (Eds): Advances in Biophotonics, NATO Science Series: Life and Behavioural Sciences, Volume 369, IOS Press, Amsterdam, 2005.

Selected Initiatives in Germany:

- Main research topic "Biophotonics" funded by the German Federal Ministry of Education and Research: http://www.biophotonik.org
- OptecNet: Network consisting of several local initiatives dealing with photonics and biophotonics, respectively: http://www.optecnet.de
- Another local network, the "Biotech/Life Science Portal Baden Württemberg" offers a number of articles in the field of biophotonics: http://www.bio-pro.de