The Nature of Light and Its Interaction with Matter

1

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1.1 Introduction

The behavior of light when it travels through space and when it interacts with matter plays a central role in the two main paradigms of twentieth-century physics: relativity and quantum physics. As we shall see throughout this book, it is also important for an understanding of the behavior and functioning of organisms.

1.2 Particle and Wave Properties of Light

The strange particle and wave properties of light are well demonstrated by a modification of Young's double-slit experiment. In Young's original experiment (1801), a beam of light impinged on an opaque screen with two parallel, narrow slits. Light passing through the slits was allowed to hit a second screen. Young did not obtain two light strips (corresponding to the two slits) on the second screen but instead a complicated pattern of several light and dark strips. The pattern obtained can be quantitatively explained by assuming that the light behaves as waves during its passage through the system.

It is easy to calculate where the maxima and minima in illumination of the last screen will occur. We can get some idea of the phenomenon of *interference* by just overlaying two sets of semicircular waves spreading from the two slits (Fig. 1.1), but this does not give a completely correct picture.

For the experiment to work, it is necessary for the incident light waves to be in step, i.e., the light must be spatially coherent. One way of achieving this is to let the light from a well-illuminated small hole (in one more screen) hit the screen with the slits. The pattern produced (Fig. 1.2) is a so-called interference pattern or, to be more exact, a pattern produced by a combination of *diffraction* (see the next section) in each slit and *interference* between the lights from the two slits. It is difficult to see it if white light is used, since each wavelength component produces a different pattern. Therefore, at least a

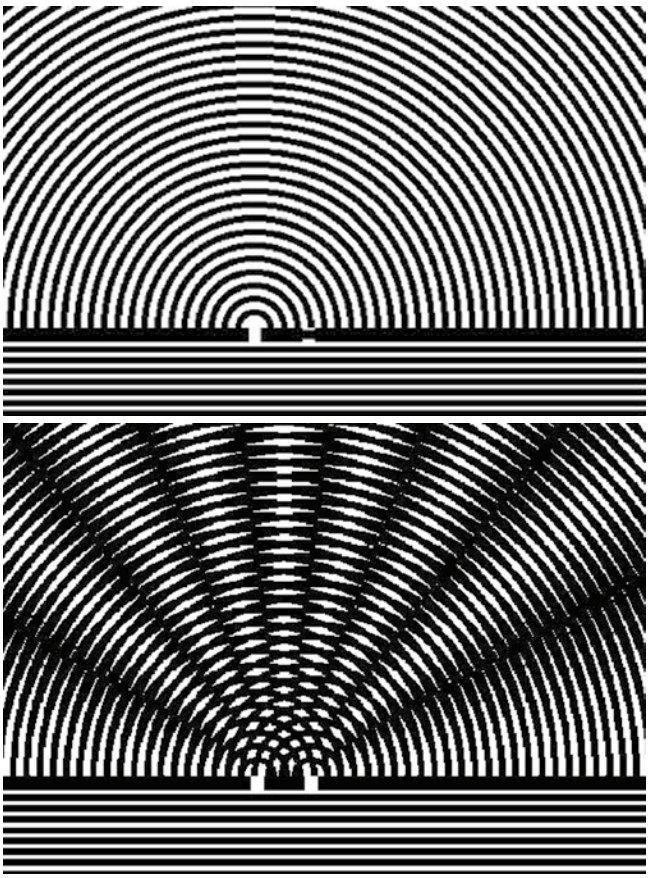

Fig. 1.1 (*Top*) Light waves impinge from below on a barrier with only one slit open and spread from this in concentric rings. (*Bottom*) Light waves impinge from below on a barrier with two slits open. The two wave systems spreading on the other side interfere and in some sectors enhance, in others extinguish one another. The picture is intended only to simplify the understanding of the interference phenomenon and does not give a true description of the distribution of light

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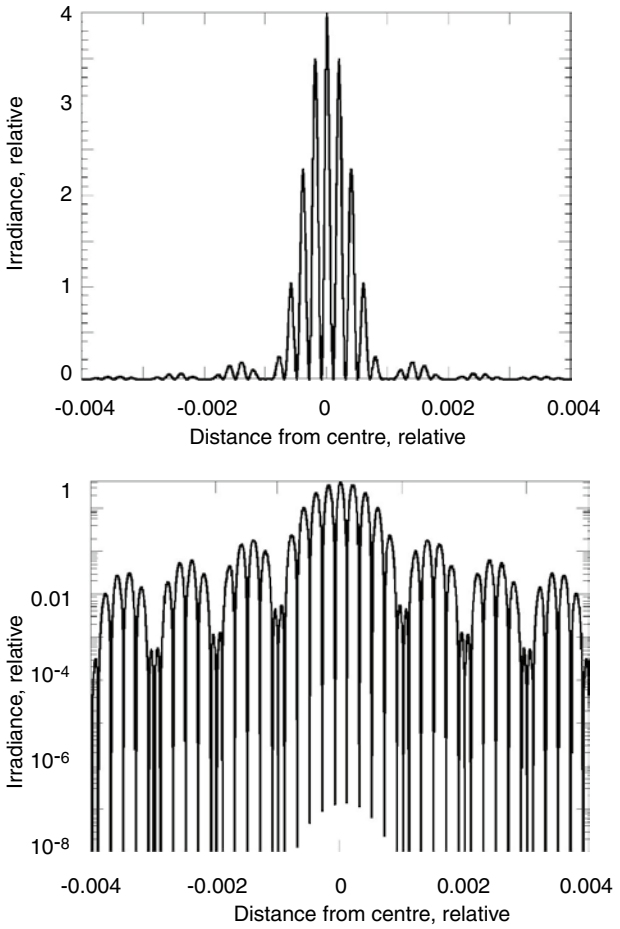

Fig. 1.2 Interference pattern produced in Young's double-slit experiment (computer simulation). The width of each slit is 1 mm, the distance between slit centers 4 mm, and the wavelength 0.001 mm (1 μm). The distance from the center of the screen is along the horizontal axis and the irradiance ("light intensity") along the vertical axis, both in relative units. Note that the vertical scale is linear in the upper diagram and logarithmic in the lower one

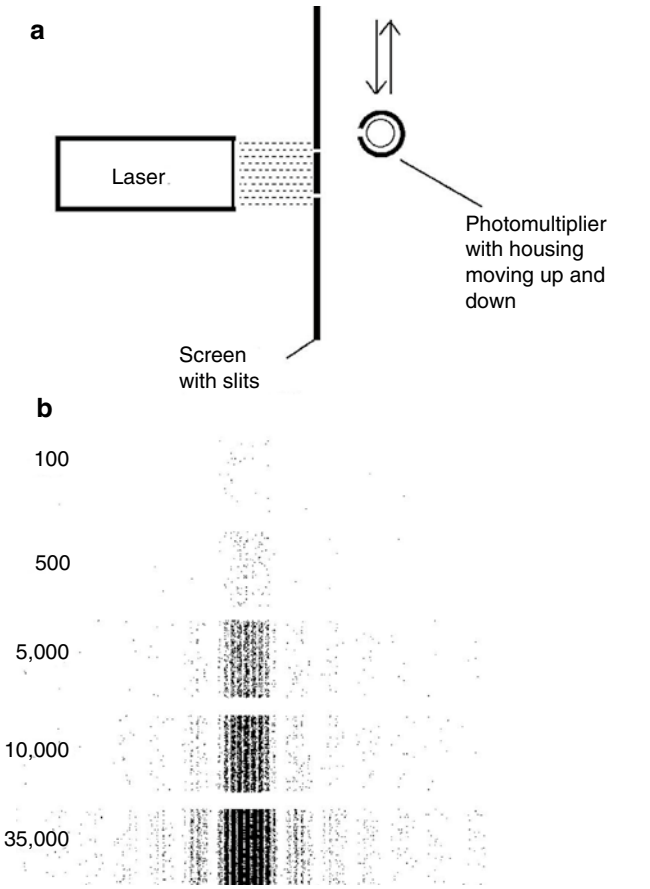

Fig. 1.3 (**a**) Double-slit experiment set up to count single photons. The sketch is not to scale. In a real experiment, the distance of the photomultiplier from the screen with slits would be greater, and the opening in the photomultiplier housing smaller. (**b**) Simulation of the pattern of photon hits on a screen behind a double slit arranged in the same way as in Fig. 1.2. The number of photons is indicated for each experiment. Although the photon hits take place randomly and cannot be predicted, the interference pattern emerges more and more clearly with increasing number of photons

colored filter should be used to limit the light to a narrower wave band. The easiest way today (which Young could not enjoy) is to use a laser (a simple laser pointer works well), giving at the same time very parallel and very monochromatic light, which is also sufficiently strong to be seen well.

In a direction forming the angle α with the normal to the slitted screen (i.e., to the original direction of the light), waves from the two slits will enhance each other maximally if the difference in distance to the two slits is an integer multiple of the wavelength, i.e., $d \cdot \sin \alpha = n \cdot \lambda$, where d is the distance between the slits, λ the wavelength, and n a positive integer (0, 1, 2, …). The waves will cancel each other completely when the difference in distance is half a wavelength, i.e., $d \cdot \sin \alpha = (n + 1/2) \cdot \lambda$. To compute the pattern is somewhat more tedious, and we need not go through the details. The outcome depends on the width of each slit, the distance between the slits, and the wavelength of light. An example of a result is shown in Fig. 1.2.

So far so good—light behaves as waves when it travels. But we also know that it behaves as particles when it leaves or arrives (see later). The most direct demonstration of this is that we can count the photons reaching a sensitive photocell (photomultiplier).

But the exciting and puzzling properties of light stand out most clearly when we combine the original version of Young's experiment with the photon counter. Instead of the visible diffraction pattern of light on the screen, we could dim the light and trace out the pattern as a varying frequency of counts (or, if we so wish, as a varying frequency of clicks as in a classical Geiger counter) as we move the photon counter along the projection screen (Fig. 1.3a). Since we count single photons, we can dim the light considerably and still be able to register the light. In fact, we can dim the light so much that it is very, very unlikely that more than *one photon at a time* will be in flight between our light source and the photon counter. This type of experiment has actually been

performed, and it has been found that a diffraction pattern is still formed under these conditions. We can do the experiment also with an image-forming device such as a photographic film or a charge-coupled diode (CCD) array as the receiver and get a picture of where the photons hit. A computer simulation of the outcome of such an experiment is shown in Fig. 1.3b.

If you think a little about what this means, you will be very puzzled indeed. For the diffraction pattern to be formed, we need *two* slits. But we can produce the pattern by using only one photon at a time. There can be no interaction between two or more photons, which have traveled different paths, e.g., one photon through one slit and another photon through the other slit. The experiment shows that each photon "must be aware" of both slits, or, in other words, must have traveled through both slits. I know of no other physics experiment that demonstrates more clearly than this one that light is not waves or particles. The wave and the particle are both *models*, incomplete pictures or imaginations of the nature of light. The limitations of our senses and our brain prevent us from getting closer to reality than this, simply because it has not made sense during our evolution to get closer to reality. This limitation does not prevent us from using our models very successfully as long as we use them in a correct way.

Not only light behaves in this way but also electrons, atoms, and molecules. Arndt et al. (1999) passed a beam of fullerene (C_{60}) through a double slit and got a similar pattern, and Sclafani et al. (2013) used a diatom frustule to diffract phthalocyanine molecules.

Let us take one more example to make clear how "weird" (i.e., counterintuitive) the scientific description of the behavior of light is. When I was younger I used to watch the Andromeda galaxy using my naked eyes (now it is difficult, not only because my vision has worsened, but because there is so much electric light around where I live). I could see the galaxy because atoms in it had emitted light about 2 million years earlier. The photons, after having traveled through empty space, interacted with rhodopsin molecules in my eyes. But no photon started on its course following a straight line toward the earth. It traveled as an expanding wave. Just before interacting with the rhodopsin molecule in my eye, the photon was *everywhere* on a wavefront with a radius of 2 million light years. The energy of the photon was not localized until it came into contact with my eye.

1.3 Light as Particles and Light as Waves and Some Definitions

When we are dealing with light as waves, we assign a wavelength to each wave. Visible light has wavelengths in a vacuum in the range 400–700 nm (1 nm equals 10^{-9} m), while ultraviolet radiation has shorter and infrared radiation longer waves.

Photobiologists divide the ultraviolet part of the spectrum into ultraviolet A (UV-A) with 315–400 nm wavelength, UV-B with 280–315 nm wavelength, and UV-C with <315 nm wavelength. You may see other limits for these regions in some publications, but these are supported by the Comité Internationale de l'Eclairage (CIE), which introduced the concepts. Just as everybody should use the same internationally agreed-upon length of the meter, everybody should honor the definitions of UV-A, UV-B, and UV-C; otherwise, there is a risk for chaos in the scientific literature. Plant photobiologists, for whom the spectral region 700–750 nm is especially important, call this radiation "far-red light." They also call the region 400–700 nm "photosynthetically active radiation," or PAR, rather than visible light. Just as radiation outside this band is perfectly visible for some organisms such as some insects, birds, and fish (and some light in the range 400–700 nm invisible to many animals), so radiation with wavelengths shorter or longer than "photosynthetically active radiation" is photosynthetically active to many organisms.

Natural light never has a single wavelength but can rather be regarded as a mixture of waves with different wavelengths.

When we characterize light by its wavelength, we usually mean the wavelength in a vacuum. When it travels through a vacuum, the velocity of light is always *exactly* 299,792.4562 km/s, irrespective of wavelength and the movement of the radiation source in relation to the observer. The reason that this value is exact is that the velocity of light in a vacuum links our definitions of the meter and the second. This velocity is usually designated c and wavelength λ (the Greek letter lambda). A third property of light which we should keep track of is its frequency, i.e., how many times per time unit the wave (the electric field) goes from one maximum (in one direction) to another maximum (in the same direction). Frequency is traditionally designated ν (Greek letter nu), and in a vacuum we have the following relation between the three quantities just introduced: $c = \lambda \cdot \nu$, or $\lambda = c/\nu$, or $\nu = c/\lambda$. When light passes through matter (such as air or water or our eyes), the velocity and wavelength decrease in proportion, and frequency remains unchanged. Sometimes the wave number, i.e., $1/\lambda$, is used for the characterization of light. It is usually symbolized by ν with a line (bar) over it, and a common unit is cm^{-1}.

When we think of light as particles (photons), we assign an amount of energy (E) to each photon. This energy is linked to the wave properties of the light by the relations $E = h \cdot \nu$, where h is Planck's constant, 6.62617636 J·s (joule-seconds). It also follows from the preceding that $E = h \cdot c/\lambda$. We can never know the exact wavelength, frequency, or energy of a single photon.

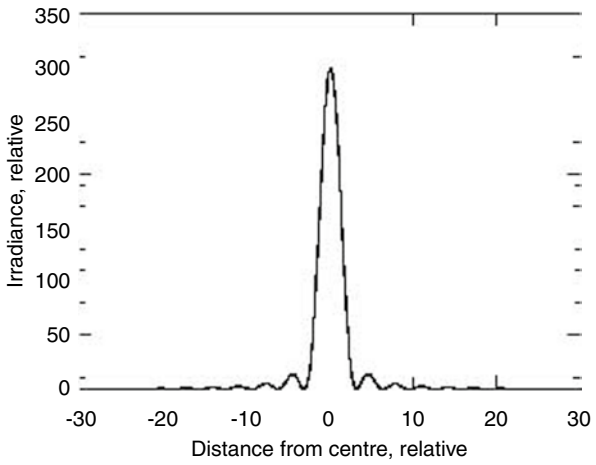

Fig. 1.4 Diffraction pattern in a single slit (the pattern from a round hole looks similar but is slightly different)

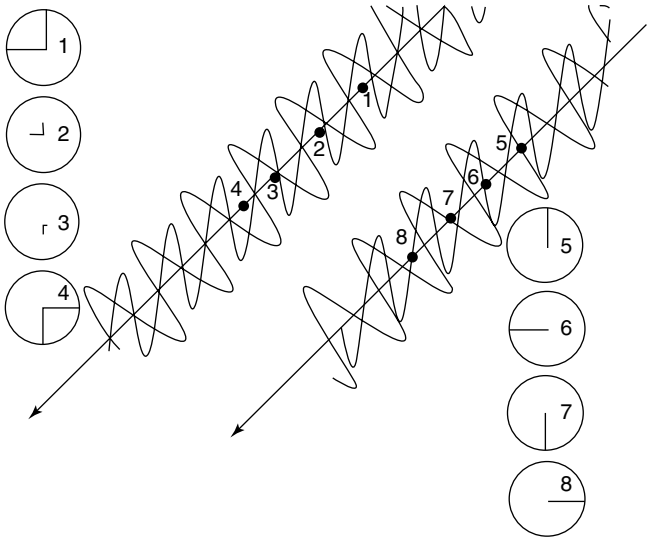

Fig. 1.5 In the *upper left part* of the figure, a plane-polarized light beam, composed of one vertically and one horizontally polarized component, is depicted in perspective and also "head-on" at different points (or at one point at different moments). Numbered points in the perspective drawing correspond to the numbers on the "head-on" drawings. Only the electric components of the electromagnetic fields are shown (*wavy lines* in the perspective drawing, *straight lines* in the "head-on" drawings). In the *lower right part* of the drawing, the same is shown for a circularly polarized beam

1.4 Diffraction

We usually think of light traveling in straight lines if there is nothing in its way. We have seen in Young's double-slit experiment that it does not always do that. In fact, the great physicist Richard Feynman has shown that its behavior is best understood if we think of it as always traveling every possible way at the same time and components traveling those different ways interfering with one another at every possible point.

We do not have to have two slits to show how the light "bends" near edges. This "bending" is called diffraction in scientific terminology. It is very important to take diffraction into account to understand some biological phenomena, such as the vision of insects (see Chap. 15). Light is diffracted in any small opening and also near any edge. To compute the diffraction pattern, we can make use of something called Huygens' principle (sometimes the Huygens-Fresnel principle). It states that we can think of propagating light as a sum of semispherical waves emanating from a wavefront. If the wavefront is flat, the semispherical waves emanating from it add up to a new flat wavefront. But if something stops some of the semispherical waves, the new wavefront is no longer flat. In Fig. 1.1 (top) we illustrate this in one plane. Flat waves impinge from below on a screen with an opening. Many semicircular waves start out from the opening. Along a line from the middle of the opening, the resulting wavefront is flat, but at the edges the semicircular waves produce a bent pattern. We have calculated this pattern more exactly in Fig. 1.4.

1.5 Polarization

Light waves are *transverse*, i.e., the oscillation is perpendicular to the direction of wave propagation and the direction of the light (this is in contrast to sound waves, in which particles vibrate in the line of wave propagation). In the case of light, there are no vibrating particles but a variation in electric and magnetic fields. The electric and magnetic fields are both perpendicular to the direction of propagation but also perpendicular to one another. When the electric fields of all the components of a light beam are parallel, the beam is said to be *plane-polarized*. The *plane of polarization* is the plane that contains both the electrical field direction and the line of propagation.

If we add two beams which travel in the same direction and are both plane-polarized and have the same *phase* (i.e., the waves are in step) but different planes of polarization, the resulting light is also plane-polarized with its plane of polarization at an intermediate angle.

Light can also be circularly polarized, in which case the electrical field direction spirals along the line of propagation. Since such a spiral can be left- or right-handed, there are two kinds of circular polarization, left-handed and right-handed (Fig. 1.5).

Circularly polarized light can be regarded as the sum of two equally strong plane-polarized components with right angles between the planes of polarizations and a 90° *phase difference* between the components. On the other hand, plane-polarized light can be regarded as a sum of equally strong left- and right-handed components of circularly polarized light. There are several animations available on the internet that explain this better than a stationary illustration can, e.g.:
http://en.wikipedia.org/wiki/Circular_polarization
http://ja01.chem.buffalo.edu/~jochena/research/opticalactivity.html

http://www.photophysics.com/tutorials/circular-dichroism-cd-spectroscopy/1-understanding-circular-dichroism
http://www.enzim.hu/~szia/cddemo/edemo7.htm
(accessed August 16, 2013)

Natural light, such as direct sunlight, is often almost unpolarized, i.e., a random mixture of all possible polarizations. After reflection in a water surface, the light becomes partially plane-polarized. Skylight is a mixture of circularly and plane-polarized light, which we call elliptically polarized light. We cannot directly perceive the polarization of the light we see. Insects do and often use the polarization of skylight as an aid in their orientation. Plants in many cases react differently to plane-polarized light depending on its plane of polarization. This holds for chloroplast orientation in seed plants, mosses, and green algae and also for growth of fern gametophytes. A good treatise on the subject (in German) is provided by W. Haupt (1977).

1.6 Statistics of Photon Emission and Absorption

Usually the members of a population of excited molecules can be expected to emit photons independently of one another, i.e., the time of emission of one photon does not depend on the time of emission of another photon. One exception to this rule occurs when stimulated emission becomes significant, as happens in a laser. Another exception is when there is cooperation between different parts of a cell (e.g., when a dinoflagellate flashes), between different cells in an organism (e.g., when a firefly flashes), or between different individuals in a population (e.g., when fireflies in a tree send out synchronized flashes). The examples in the last sentence are very obvious. However, careful study of the statistics of photon emission offers a very sensitive way of detecting cooperation between different parts of a biological system, and we shall therefore dwell a little on this subject, which also has a bearing on the reliability of measurement of weak radiation in general.

When photons are emitted independently of one another, the distribution of emission events in time is a Poisson distribution, just as in the case of radioactive decay. This means that if the mean number of events in time Δt is x, then the probability of getting exactly n events in the time Δt is $p = e^{-x} \cdot x^n / n!$. In this formula, n! stands for factorial n, i.e., $1 \cdot 2 \cdot 3 \cdot 4 \ldots$ n. Thus 1! = 1, 2! = 2, 3! = 6, 4! = 24, and so on. By definition 0! = 1.

We are familiar with the Poisson distribution of events from listening to a Geiger–Müller counter. That events are Poisson-distributed in time means that they are completely randomly distributed in time. When one event takes place does not depend on when a previous event occurred. One might think that there cannot be much useful information to be extracted from such a random process, but such a guess is wrong. The reader is probably already familiar with some of the useful things we can learn from the random decay of atomic nuclei. We can, in fact, use our knowledge of how Poisson statistics work for determining the number of photons required to trigger a certain photobiological process. The remarkable thing is that we can do this even without determining the number of photons we shine on the organism that we study.

The principle was first used by Hecht et al. (1942) to determine how many photons must be absorbed in the rods of an eye to give a visual impression. Their ingenious experiment was a bit complicated by the fact that our nervous system is wired in such a way that several rods have to be triggered within a short time for a signal to be transmitted to the brain (thereby avoiding false signaling due to thermal conversion of rhodopsin). We shall demonstrate the principle with a simpler example, an experiment on the unicellular flagellate *Chlamydomonas* (Hegemann and Marwan 1988). This organism swims around with two flagella, and it reacts to light by either stopping ("stop response") or by changing swimming direction ("turning response").

All one has to do is to take a sample of either light-adapted or dark-adapted *Chlamydomonas* cells, subject them to a flash of light, and note which fraction of the cells either stop or turn. The experiment is then repeated several times, with the flash intensity varied between experiments. The absolute fluence in each flash need not be determined, only a relative value. If one possesses a number of calibrated filters, no light measurement at all need be performed. Then the fraction of reacting cells for each flash is plotted against the logarithm of the relative flash intensity. It turns out that (for dark-adapted cells) the curve so obtained, if plotted on a comparable scale, has the same shape as the curve labeled $n = 1$ in Fig. 1.6. This holds for both stop response and for turning response, and it means that both responses can be triggered by a single photon. If the experiment is carried out within 20 min of removing the cells from strong light, the stop-response curve has a shape similar to the curve labeled $n = 2$ in Fig. 1.6, meaning that in this case two photons are required.

The curves in Fig. 1.6 have been computed in the following way (let, as before, x be the average number of events recorded in a large number of trials): the curve for $n = 1$ is the probability (p) of absorption of at least one photon, which is one minus the probability for absorption of no photon or $p = 1 - e^{-x} \cdot x^1 / 1! = x / e^x$. The curve for $n = 2$ follows the formula $p = 1 - e^{-x} \cdot x^1 / 1! - e^{-x} \cdot x^2 / 2!$, the curve for $n = 3$ follows the formula $p = 1 - e^{-x} \cdot x^1 / 1! - e^{-x} \cdot x^2 / 2! - e^{-x} \cdot x^3 / 3!$, etc.

1.7 Heat Radiation

The term heat radiation is sometimes (erroneously) used synonymously with infrared radiation. We shall use it here as the energy emitted when the energy of the random heat

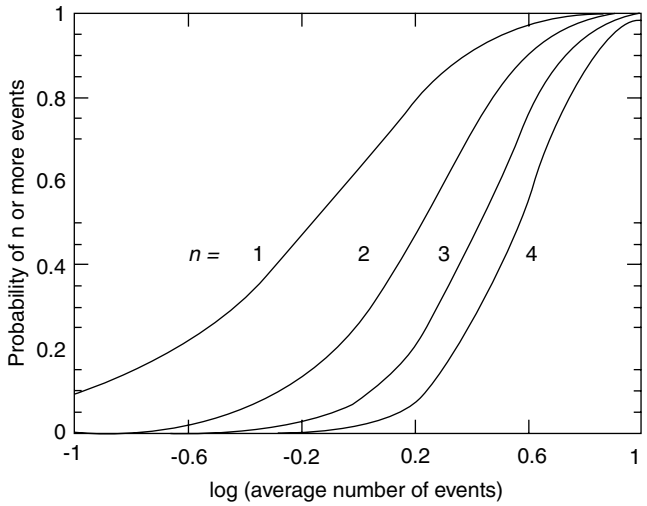

Fig. 1.6 The probability that at least a certain number (n) of absorption events will occur during a sampling time plotted against the logarithm of the average number of events that would occur during a large number of similar samplings. It is seen that the shape of the curves depends on the value of n. If at least n absorption events are necessary for inducing a process, one can determine the number n by plotting the frequency of successful inductions against the logarithm of fluence and compare the shape of the curve obtained with the above diagram

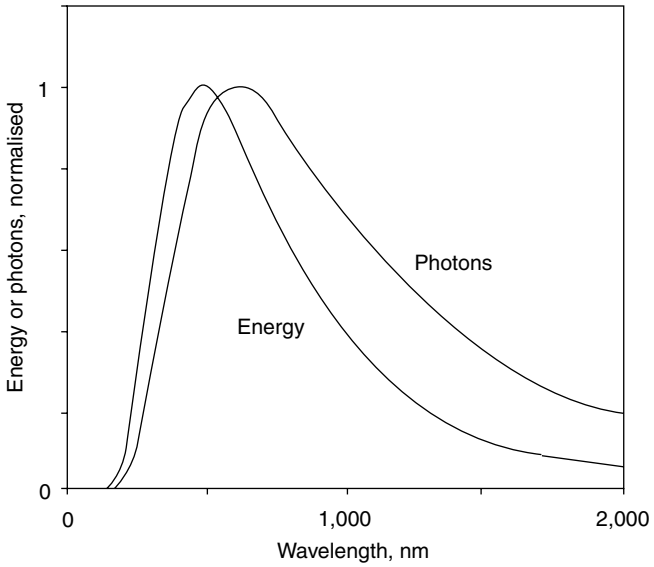

Fig. 1.7 Blackbody radiation (5,000 K) plotted as photons per wavelength interval and as energy per wavelength interval

$$\text{Energy density per frequency interval} = \left(8\pi h / c^3\right) \cdot \nu^3 / \left(\mathrm{e}^{h\nu/kT} - 1\right)$$

$$\text{Photon density per frequency interval} = \left(8\pi / c^3\right) \cdot \nu^2 / \left(\mathrm{e}^{h\nu/kT} - 1\right)$$

$$\text{Energy density per wavelength interval} = 8\pi hc \cdot \lambda^{-5} / \left(\mathrm{e}^{h\nu/kT} - 1\right)$$

$$\text{Energy density per wavelength interval} = 8\pi\lambda^{-4} / \left(e^{h\nu/kT} - 1\right)$$

movement of the particles in condensed matter (solids, liquids, or compressed gases) is converted to radiation. It is easiest to think of heat radiation as the glow of a hot body (lamp filament or the sun), but our own bodies also emit heat radiation, as does, in fact, a lump of ice or even a drop of liquid nitrogen. A body that is cooler than its environment absorbs more radiation than it emits, but still it radiates according to Planck's radiation law, to be described below. Heat radiation may be infrared, visible, or ultraviolet and, if we go to exotic objects in the cosmos, even outside this spectral range.

The starting point of the quantum theory was the attempt to explain the spectrum of the radiation emitted by a glowing body. To derive a function that matched the observed spectrum, Planck had to assume that the radiation is emitted in packets (quanta or photons) of energy $h{\cdot}\nu$, where ν stands for frequency (which is also the velocity of light divided by the wavelength) and h is a constant, Planck's constant = $6.62620{\cdot}10^{-34}$ J·s. Planck's radiation law was derived for an ideal blackbody, best approximated by a hollow body with a small hole in it. With modifications it can be used for other bodies as well. The sun radiates almost as a blackbody.

Planck's formula can be written in different ways, depending on whether we consider radiation per frequency interval or per wavelength interval and whether we express the radiation as power (energy per time) or number of photons (per time). Furthermore, we may be interested in the radiation density inside a hollow body (mostly for theoretical purposes) or the radiation flux leaving a body (for most applications).

These functions are mostly plotted with ν or λ as the independent variable and T as a parameter. It should be noted that even for the same T, the functions all have maxima at different values of ν or λ (see Fig. 1.7, which shows the plots of energy per wavelength interval and photons per wavelength interval for 5,000 K).

These examples are shown merely as an illustration of the fact that the maxima occur at different locations depending on which principle you use for plotting the spectra. This is not only true for heat radiation; it holds for all emission spectra, also for fluorescence emission spectra for instance. The most common sin of people publishing about fluorescence is that they do not understand this. They write "fluorescence, relative" on their vertical axis without further specification and do not realize that not even the shape of their spectrum, nor the positions of maxima, will be defined in such graphs. The second most common way of sinning is to spell fluorescence incorrectly.

You can see from Fig. 1.8 that the maxima occur at longer wavelengths when the temperature is lower and also that the total radiation is less in that case. In fact, the wavelength of the maximum is inversely proportional to the absolute temperature (Wien's law), while the total photon emission is proportional to the third power of the absolute temperature (i.e., to T^3) and the total energy emission to the fourth power (T^4, Stefan–Boltzmann's law). Wien's and Stefan–

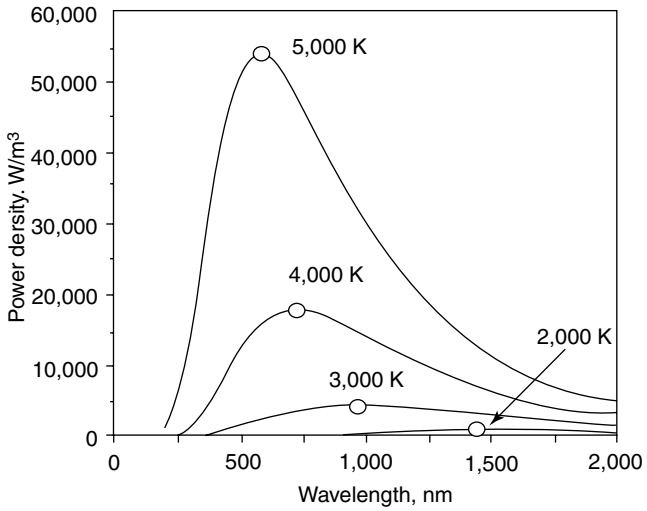

Fig. 1.8 Blackbody radiation plotted as power per wavelength interval for different temperatures. Note that since the graphs show power (i.e., energy per time) per area and per wavelength interval, the dimension is power per volume and the unit W/m^3. The maximum of each curve is indicated by a *circle*

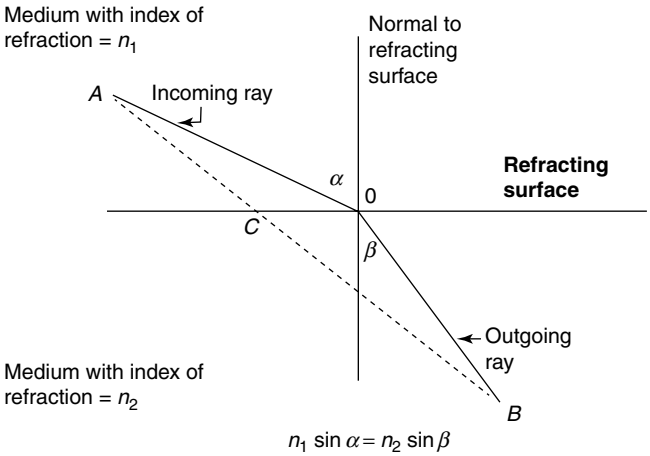

Fig. 1.9 Refraction of light in a plane interface between transparent materials

Boltzmann's laws can both be derived from Planck's radiation formula but were found experimentally before Planck did his theoretical derivation.

The formulas shown above refer to radiation density in a closed cavity with radiating walls. The *fluence rate*, or amount of radiation per unit of time and unit of *cross-sectional* area falling from all directions on a sphere in this cavity, is obtained by multiplying the radiation density by the velocity of light. (Do not worry if you have some difficulty with this here. We shall return later to the concept of fluence rate, which is quite important in photobiology and often misunderstood.) Suppose that the sphere in the cavity is ideally black (absorbing all the radiation falling on it) and has the same temperature as the walls. The second law of thermodynamics states that (assuming that no heat energy is generated or consumed in the sphere) the sphere must stay at the same temperature as the walls and it must radiate the same amount of radiation (distributed in the same way across the spectrum) as it receives. Therefore its *excitance* (radiation given off per unit of time and unit of *surface* area) is the energy density given by the formulas above multiplied by the velocity of light and divided by 4 (since the *surface* area of the sphere is four times the *cross-sectional* area).

To obtain the excitance of a non-blackbody (such as a glowing tungsten filament in a light bulb or your own body), the excitance computed for a blackbody should be multiplied by the *emissivity*. The emissivity varies quite a lot with wavelength, so the multiplication must be carried out separately for each wavelength value in which you are interested. The emissivity also varies somewhat with temperature. The *absorptivity*, or the ability to absorb radiation, is identical to the emissivity; otherwise, the second law of thermodynamics would be violated.

It may seem that this is a little too much physics for a biology book, but an understanding of the basic physical principles is very helpful when it comes to the experimental work in photobiology. What has just been described can be used for calibrating measuring equipment in the photobiology laboratory.

1.8 Refraction of Light

From school you should be familiar with Snell's law. This describes how light is refracted at an interface between two media with different indices of refraction (refractive indices), say n_1 and n_2. Figure 1.9, in which we assume $n_1 < n_2$, will serve as a reminder. If you need further explanation, you will have to look in other books.

The refractive index can be regarded as the inverse of the relative velocity of light in the medium in question, i.e., it is the velocity in a vacuum divided by that in the medium. It can be shown that Snell's law is equivalent to the statement that the light takes the fastest path possible between any two points on the rays shown. Compared to a straight line (dashed in Fig. 1.9) between point A on the upper ray and point B on the lower ray, you can see that the light goes a longer distance (solid line) in the medium with refractive index n_1 (lower index, higher velocity) than in the medium with refractive index n_2 (higher index, lower velocity), i.e., AO > AC and OB < CB. The refractive index is a pure number (no unit, as it is the ratio of two velocities). As we have used it here, it is a real number (the usual type of number we use in most calculations, represented as a decimal number). In more advanced optical theory, the refractive index is a complex ("two-dimensional") number.

As for the values of α and β in relation to one another, the figure looks the same if the light direction is reversed. However, this does not hold any longer when reflection is taken into account or when we consider the amount of light in the beams.

Throughout most of the spectrum the refractive index decreases with wavelength, but there are spectral regions (where absorption bands occur) where it increases steeply with wavelength; this phenomenon is, for historical reasons, called *anomalous dispersion*, although it is quite normal. In general, the change in refractive index with wavelength is called *dispersion*.

In some crystals and many biological materials, the refractive index is different depending on direction and plane of polarization of the light. Such a medium is termed *birefringent*. Birefringence occurs in plant cell walls and other structures where elongated molecules are arranged in a certain direction. Measurement of birefringence has been an important method in elucidating the arrangement of molecules in such cases. Media that are originally *isotropic* (with the same properties in different directions and thus not birefringent) may become birefringent by stretching or squeezing, application of electric fields, or other treatments.

When light passes through a birefringent medium of suitable thickness, it becomes circularly or elliptically polarized because of the phase difference that develops between the components of different plane polarization.

1.9 Reflection of Light

Reflection may be *specular* (from a shiny, smooth surface or interface) or *diffuse* (from a more or less rough surface or interface). Diffuse reflection is very important in biology, but we shall limit ourselves here to specular reflection at interfaces between dielectric (nonmetallic) media.

The angle of incidence is always equal to the angle of reflection, but the amount of light reflected (as opposed to refracted) depends on the polarization of the light. The plane in which both the incident and the reflected rays (and the normal to the reflecting surface) lie is called the *plane of incidence*. The component of the light with an electric field parallel to this plane is designated by //; that with an electric field perpendicular to the plane of incidence by +. The fractions, *R*// and *R*+, of the irradiance of these components that are reflected are given by Fresnel's equations in which α is the angle of incidence (equal to the angle of reflection) and β the angle of transmission (see Fig. 1.9 in the section on refraction):

$$R// = \left[\tan(\alpha-\beta)/\tan(\alpha+\beta)\right]^2$$

$$R+ = \left[\sin(\alpha-\beta)/\sin(\alpha+\beta)\right]^2$$

The reflected fraction of unpolarized light is the mean of the two ratios. For normal incidence ($\alpha = \beta = 90°$) another set of equations has to be used, since with the equations above, divisions by zero would occur. In this case there is no distinction between *R*// and *R*+:

$$R = \left[(n_1 - n_2)/(n_1 + n_2)\right]^2$$

As an example of use of the last equation, let us consider the reflection in a glass plate ($n_2 = 1.5$) in air ($n_1 = 1$). When light strikes the glass plate (perpendicularly), $R = [(1-1.5)/(1+1.5)]^2 = [-0.5/2.5]^2 = 0.04 = 4$ %. When the light strikes the second interface (from glass to air), the value of *R* comes out the same again, because since the expression is squared, it does not matter in this case which of the indices you subtract from the other one. Thus 96 % of the 96 % of the original beam, or 92.16 %, will be transmitted in this "first pass." It can be shown that after an infinite number of passes between the two surfaces, the reflected fraction will be $R[1+(1-R)/(1+R)] = 2.0.04/(1+0.04) = 7.69$ % and the transmitted fraction 92.31 %. For most practical purposes, we may estimate a reflection loss at normal incidence of about 8 % in a clean glass plate or glass filter, but if the refractive index is exceptional, this value may not hold. If the glass is not clean, it certainly does not.

The multiple internal reflection is not of much effect in a single glass plate, but I wanted to mention it here, because the effect is taken advantage of in so-called interference filters to be described in a later section.

Going back to the case of $\alpha < 90°$, we find by division, member by member, of the equations above that *R*// divided by *R*+ is $[\cos(\alpha-\beta)/(\cos(\alpha+\beta)]^2$. This ratio will always be > 1, so *R*// > *R*+, or, in other words, the component of light with the electric field perpendicular to the plane of incidence and parallel to the interface will be more easily reflected than the other component. The interface can act as a polarizing device. It can be shown that the reflected beam becomes completely polarized when $\tan\alpha = n_2/n_1$, because none of the light polarized parallel to the plane of incidence is reflected (Fig. 1.10). The angle $\alpha = \arctan(n_2/n_1)$ is called the *Brewster* angle.

If a beam strikes a flat interface obliquely from the side where the refractive index is highest, the outgoing beam will have a greater angle to the normal than the ingoing (according to Snell's law). If the angle of incidence is increased more and more, an angle will eventually be reached when the outgoing beam is parallel to the interface. At greater angles of incidence, there will be total reflection, i.e., all light will be reflected, and none transmitted. The smallest angle of incidence at which total reflection occurs is called the *critical angle*.

If an object with higher refractive index immersed in the medium with lower refraction index comes very close to the reflecting interface (at a distance less than a wavelength), then light energy can "tunnel" through and interact with that object. It is a principle exploited for, e.g., fingerprint readers and some special kinds of microscopy (see Chap. 5).

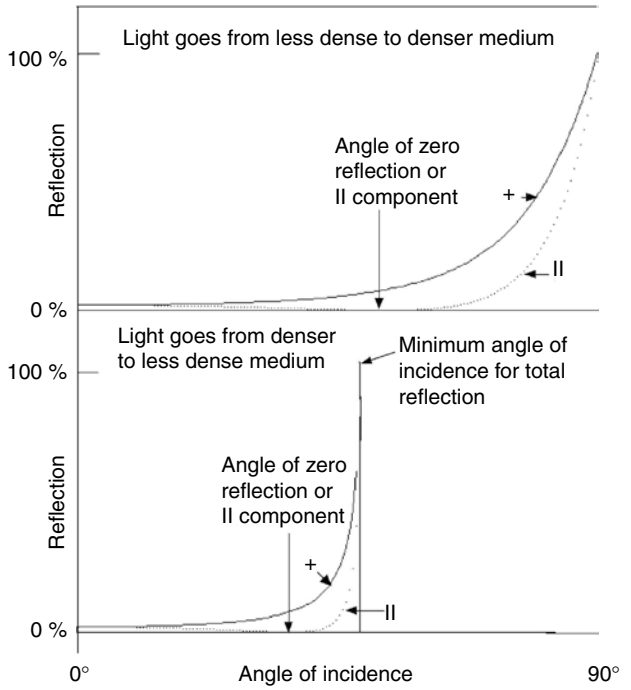

Fig. 1.10 Percent of light reflected for different angles of incidence for light going from air ($n=1$) to water ($n=1.33$, *top*) and from water to air (*bottom*) and for light polarized with the electric vector in the plane of incidence (II) or perpendicular to the plane of incidence (+). For II-polarized light, no light is reflected for a certain angle of incidence (the Brewster angle). For light going from the denser medium (water) to the less dense medium, total reflection occurs for angles of incidence larger than the critical angle

1.10 Scattering of Light

Although, strictly speaking, reflection and refraction are also a result of scattering (absorption and reemission of electromagnetic energy by material oscillators), we do, in practice, use the term scattering in a more restricted sense for processes that tend to change the propagation of light from an ordered way to a random one. We can distinguish three types of scattering named after three distinguished scientists: Mie scattering, Rayleigh scattering, and Raman scattering.

Mie scattering is caused by particles larger than the wavelength of the light and having a refractive index different from that of the continuous phase in which they are suspended. Typical examples are water droplets (clouds, fog) or dust in the atmosphere, or the result of mixing a solution of fat in acetone with water. Almost any animal or plant tissue is a strong Mie scatterer due to the boundaries between cells and between different parts of the cells and, in the case of plant tissue, between cells and intercellularies. Mie scattering is nothing other than repeated reflection and refraction at numerous interfaces. As we have seen, light of different wavelengths is not reflected or refracted in exactly the same way, but most of these differences cancel out in Mie scattering, and there is no strong wavelength dependence of this phenomenon.

Rayleigh scattering is caused by the interaction of light with particles smaller than the wavelength of the light. The particles may even be individual molecules or atoms. In this case there are no interfaces at which reflection or refraction can take place. However, the closer the wavelength of the light is to an absorption band of the scattering substance (i.e., the closer the frequency of the light is to a natural oscillating frequency in the matter), the more strongly the electrons in the matter "feel" the light and the greater is the probability that the electromagnetic field is disturbed when it sweeps by. Most substances have their strongest absorption bands in the far ultraviolet. Therefore, in the infrared, visible, and near ultraviolet regions, Rayleigh scattering increases very steeply toward shorter wavelength. Ultraviolet is scattered more strongly than blue, which in turn is scattered more strongly than red. The blue color of the sky is due to more blue than red light being scattered out of the direction of the direct sunlight. To be more precise, Rayleigh scattering is inversely proportional to the fourth power of the wavelength, i.e., proportional to $1/\lambda^4$.

In Rayleigh scattering the direction of the electrical field is not changed. If, for instance, a horizontal beam, vertically polarized (i.e., with the electric field vertical), is scattered, the electric field remains vertical. But light can never propagate in the direction of its electric field (remember, it is a transverse wave). This means that the light is not scattered up or down, only in horizontal directions. If, on the other hand, the incident light is not polarized, it is scattered in all directions but with different polarizations.

In both Mie and Rayleigh scattering, the wavelength of the light remains unchanged. In Raman scattering, on the contrary, either part of the photon energy is given off to the scattering particles (which in this case are molecules) or some extra energy is taken up from the particles. The amount of energy taken up or given off corresponds to energy differences between vibrational levels in the molecule. Raman scattering can be used as an analysis method and is also a source of error in fluorescence analysis, but we do not need to consider it in photobiological phenomena, since it is always very weak.

1.11 Propagation of Light in Absorbing and Scattering Media

We shall consider here first the simplest case: a light beam (irradiance I_o) that perpendicularly strikes the flat front surface of a homogeneous nonscattering but absorbing object. The most common objects of this kind that we deal with in the laboratory are spectrophotometer cuvettes and glass filters. A small fraction of the incident light is specularly reflected at the surface according to Fresnel's equation (see Sect. 1.9). For simplicity, we disregard this in this section. In spectrophotometry, reflection is taken care of by comparing a sample with a reference cuvette having approximately the same reflectivity as the sample cuvette.

At depth x within the object, the irradiance (see Chap. 2 for definitions of irradiance and other terms) will be $I_x = I_o \cdot e^{-Kx}$, where K is the linear absorption coefficient. The relationship is known as Lambert's law and follows mathematically from the conditions that (1) the light is propagated in a straight line and (2) the probability of a photon being absorbed is the same everywhere in the sample.

In spectrophotometry we also make use of Beer's law, which states that under certain conditions, K is a product of the molar concentration of the absorbing substance and its molar absorption coefficient (or, in the case of several absorbing substances, the sum of several such products).

However, we are concerned now not with spectrophotometry, but with the propagation of light in living matter. Almost invariably, we will be facing complications caused by intense scattering. A general quantitative treatment of scattering is so complicated as to be useless for the photobiologist. All it would lead to would be a system of equations with mostly unknown quantities.

A simplified theory, which has been found very useful as a first approximation, is the Kubelka–Munk theory (Kubelka and Munk 1931). It should be observed that this theory is valid only for "macro-homogeneous" objects, i.e., those that on a macroscopic scale are uniform and isotropic, with absorption and scattering coefficients that can be determined. Seyfried and Fukshansky (1983) have shown how the theory can be modified for an object consisting of several macro-homogeneous layers. Specular reflection at the surfaces has to be dealt with separately. Uncertainty in the specular reflection leads to uncertainties in the absorption and scattering coefficients if they, as proposed by Seyfried and Fukshansky, are determined from overall reflection and transmission by the object. In any case, the method is good enough to demonstrate here the general features of light propagation in media that both absorb and scatter light.

Suppose that we can determine, with sufficient confidence, the reflectance R (except for specular reflectance) and transmittance T of our sample. The linear absorption coefficient K and the linear scattering coefficient S, as well as the fluence rate at any point inside the sample, can then, with some effort, be computed from the system of equations:

$$
\begin{aligned}
R &= 1/\left[a + b \cdot \coth\left(bSd\right)\right] \\
T &= \mathrm{b}/\left[a \cdot \sinh\left(bSd\right) + b \cdot \cosh\left(bSd\right)\right] \\
a &= \left(S + K\right)/S \\
b &= \sqrt{\left(a^2 - 1\right)} \\
I_x &= I_\mathrm{o} \cdot T \cdot \left[\begin{matrix}\left\{\left(a+1\right)/b\right\} \cdot \sinh\left\{bS \cdot \left(d - x\right)\right\} \\ +\cosh\left\{bS \cdot \left(d - x\right)\right\}\end{matrix}\right].
\end{aligned}
$$

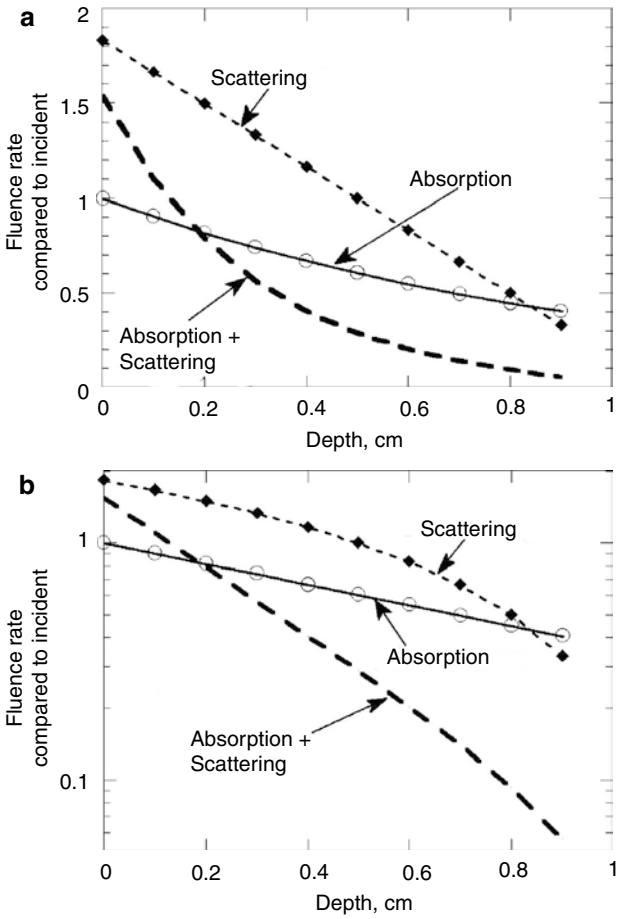

Fig. 1.11 Decrease of fluence rate with depth. The decrease of fluence rate with depth of penetration in a 1-cm-thick slab of a medium with absorption only (linear absorption coefficient 1 cm^{-1}), scattering only (linear scattering coefficient 5 cm^{-1}), and one with both absorption (1 cm^{-1}) and scattering (5 cm^{-1}). The values were computed using the Kubelka–Munk theory and assuming isotropic incident light. In the upper frame, the fluence rate scale is linear, and in the lower one, logarithmic. Note that in a scattering medium, fluence rate can exceed the fluence rate of the incident light (**a** is a linear plot; **b** has a logarithmic vertical scale)

Here I_o is the fluence rate incident from one side, and I_x the fluence rate at depth x of a sample of overall thickness d. The so-called hyperbolic operators sinh, cosh, and coth are defined by the following relationships: $\sinh(y) = (e^y - e^{-y})/2$; $\cosh(y) = (e^y + e^{-y})/2$; $\coth(y) = \cosh(y)/\sinh(y)$. If light is incident from both sides, the last equation has to be modified.

To demonstrate, without too much computation, the effect of scattering, we shall assume that we have determined K and S. For any sample thickness, d, we can then compute I_x as a function of x.

Note the following features in the examples of computer outputs (Figs. 1.11 and 1.12):

1. When S is given a low value (0.01), the Kubelka–Munk curve coincides with the Lambert curve (and is therefore invisible).

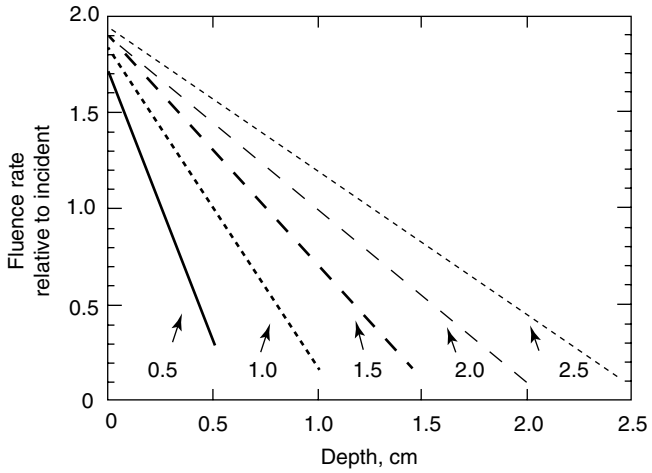

Fig. 1.12 Decrease of fluence rate in layers of the indicated thickness (in cm) of a purely scattering medium (no absorption); the linear scattering coefficient is 5 cm^{-1} in all cases. Note that the fluence rate decreases more quickly in a thin scatterer because there is less backscatter of light

2. When *S* has a value similar to or higher than *K*, i.e., when scattering is appreciable compared to absorption, the fluence rate in the sample near the illuminated side is higher than the incident fluence rate. This is no violation of the law of energy conservation; the sample does not create any new light. However, the concentration of photons is increased by their bouncing back and forth.

The expediency with which the Kubelka–Munk equations can be evaluated using a computer must not cause us to forget the limitations of the Kubelka–Munk theory. One severe restriction is that only diffuse incident light or light with an incidence angle of 60° is considered. We need only enter three constants, *K*, *S*, and thickness of the scattering medium, to describe the scattering object. A more complete description would give more realistic results, but apart from the difficulty in choosing the correct constants, the equations and algorithms would rise in complexity very fast. More complete theories are described by Star et al. (1988) and Keijzer et al. (1988).

1.12 Spectra of Isolated Atoms

We shall deal in this section with isolated atoms (which are not part of di- or polyatomic molecules and also not close to one another for other reasons, such as high pressure). They can increase their internal energy by absorbing photons and also give off energy by emitting photons. They can absorb or emit only very particular photons, whose energy corresponds very exactly to differences between energy levels in the atom. The simplest case is the hydrogen atom, and it has been found that its energy levels are inversely proportional to $1/n^2$, where n represents positive integers (1, 2, 3, …). The possible energy

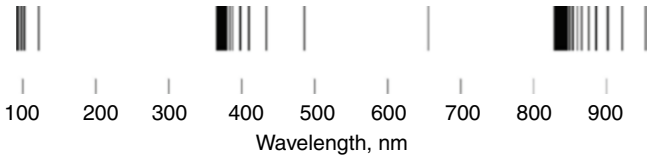

Fig. 1.13 Spectrum of atomic hydrogen (computer simulation)

jumps are then proportional to the energy differences $1/n^2–1/m^2$, where n = 1, 2, 3, …and m = n + 1, n + 2, etc. Since, according to the relationships $E = h\nu$ and $\lambda = c\nu$, the energy (E) of a photon is inversely proportional to wavelength (λ), the wavelengths of light which can be absorbed or emitted by a hydrogen atom are given by $1/\lambda = R\cdot(1/n^2–1/m^2)$ (see Fig. 1.13). The proportionality constant is called the reduced Rydberg constant. It is slightly dependent on the mass of the atom's nucleus, and for ordinary hydrogen, it amounts to 0.0109677581 nm^{-1}.

In ordinary hydrogen gas, the atoms are combined in pairs. However, when an electric current runs through the gas, the pairs are split and photon emission from energized (excited) free hydrogen atoms takes place. In the laboratory we use lamps containing heavy hydrogen (deuterium), for instance, in the spectrophotometer. We use the continuous part of the spectrum in the ultraviolet (as well as continuous emission in the ultraviolet arising from molecular deuterium) for measuring ultraviolet absorption of samples. We can use the two first lines of the Balmer series, for which $n = 2$ (Hα at 656 nm and Hβ at 486 nm), for wavelength calibration. In the program on which Fig. 1.13 is based, an approximate Rydberg constant in between that for light and heavy hydrogen was used. In nature, Hα, Hβ, and some other hydrogen lines appear in the spectrum from the sun as absorption lines (Fraunhofer lines), because of the presence of nonexcited hydrogen atoms in the atmosphere of the sun outside the glowing photosphere. Light of these particular wavelengths is therefore almost absent in the daylight spectrum. The absence of Hα light from daylight should make it possible to measure other light (e.g., fluorescence) at this wavelength in full daylight. However, the chlorophyll fluorescence from plants is weak at such a short wavelength.

One other case where the photobiologist is concerned with the spectrum of isolated atoms is when he or she uses low-pressure mercury lamps. We shall return to this in Chaps. 3 and 25.

1.13 Energy Levels in Diatomic and Polyatomic Molecules

The energy relations immediately become much more complex when we proceed from single atoms to molecules consisting of two atoms each, i.e., diatomic molecules. The

simplest example of such a molecule (if we disregard the hydrogen molecular ion H_2^+) is the hydrogen molecule, H_2.

In the molecule we have, in addition to the electronic energy described for the atom, vibrational and rotational energy. In diatomic molecules, the bond between the atoms, mediated by the electrons, can be regarded as an elastic string or spring, which stretches and contracts. At one instant the nuclei of the two atoms move toward one another. When the positively charged nuclei come close enough, their mutual electric repulsion becomes strong enough to reverse the motion, and the distance between the nuclei starts to increase. The nuclei move apart until the attractive force from the negatively charged electrons becomes strong enough to reverse the motion again.

This oscillating movement of the nuclei has some resemblance to that of a pendulum, but one difference is that it is asymmetrical. The force on the nuclei is not proportional to the distance from a symmetry point, and therefore, the molecule is an inharmonic oscillator rather than a harmonic one.

The changes in energy due to changes in oscillating movement are smaller than (the largest) energy jumps due to electronic transitions (changes in electronic energy).

In molecules consisting of more than two atoms each, there are also oscillations due to the bending of bonds, but we shall disregard this in the following.

The molecule can also absorb or emit energy by changing its state of rotation. In diatomic molecules, only rotation around an axis perpendicular to the bond contributes to the rotational energy, but in more complicated molecules, we must consider three axes of rotation, all perpendicular to one another.

All these energy changes are quantized, i.e., only certain energy changes are possible. However, because the vibrational and rotational energy amounts are much smaller than the electronic energy amounts and are combined with them, the molecules have apparently continuous absorption and emission bands rather than lines. At equilibrium, the number of molecules (N_x, N_y) "occupying various energy states" as the jargon goes, i.e., having various amounts of energy (E_x, E_y), is related to the energy differences between the states by the formula $N_x / N_y = e^{(E_y - E_x)/(kT)}$.

We shall now restrict the discussion to the stretching vibrations and their interaction with the electronic energy transitions. At one point in the stretching oscillation, the force acting on the nuclei is zero (the repulsive and attractive forces compensate one another exactly). All the vibrational energy is then kinetic (translational) energy. In contrast, when the distance between the nuclei is either minimal or maximal, i.e., at the inner and outer turning points, the velocity is zero, and therefore, the kinetic energy is zero. All the vibrational energy is then potential (positional) energy. In between, the kinetic and potential parts of the energy change in such a way that their sum is constant.

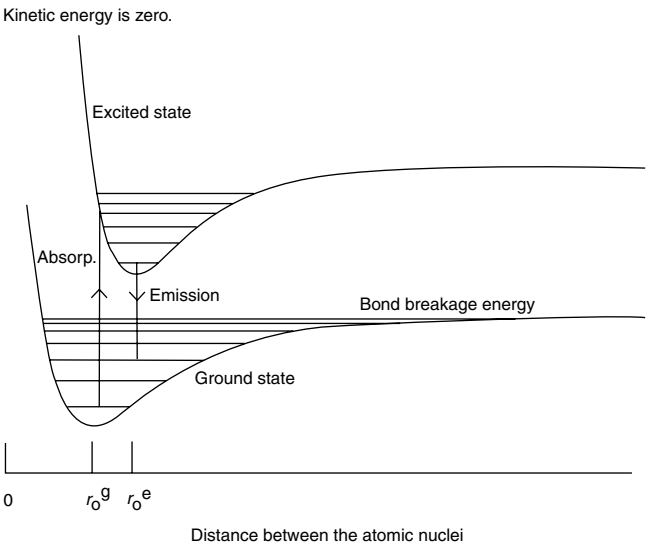

Fig. 1.14 The potential energies (vertical coordinate) of the electronic ground state and the first excited state are shown by the *curves* as functions of the distance between the atomic nuclei in a diatomic molecule (horizontal coordinate). The equilibrium distances (lowest potential energy) for the ground and excited states are denoted by r_o^g and r_o^e, respectively. At this distance the potential energy is minimal, and the kinetic energy (distance between *curves and horizontal lines*) is maximal. However, the molecule never comes to rest at this position. Even at zero absolute temperature, the vibrations continue (*lowest horizontal lines on the curves*)

In Fig. 1.14, the distance between the atomic nuclei is plotted in the horizontal direction (lowest values to the left) and the energy of the molecule in the vertical direction (lowest values at the bottom). The curved lines show the potential energy for various distances and for two different electronic states of the molecule. The various horizontal lines within the curved lines show the total energy for various vibrational states and for the two electronic states. The turning point in the oscillating movement of the nuclei is where these horizontal lines reach the curves. For these interatomic distances, the kinetic energy is zero.

Looking at Fig. 1.14 and the lengths of the vertical lines in it, we get some understanding of why absorption maxima occur at shorter wavelengths (higher photon energy) than emission maxima (this difference is referred to as the "Stoke's shift"). The maxima, which we can determine experimentally, of course, correspond to the wavelengths and photon energies of the most likely transitions. Later we will see how one can look at the same phenomenon from quite a different point of view.

A macroscopic pendulum moves most slowly near the turning points. If it were possible to get snapshots of the molecule at random times, one would therefore expect most of the snapshots to show the atoms near the turning points. However, the quantum physics is more complicated than that. For the lowest vibrational state, the zero state, it is quite

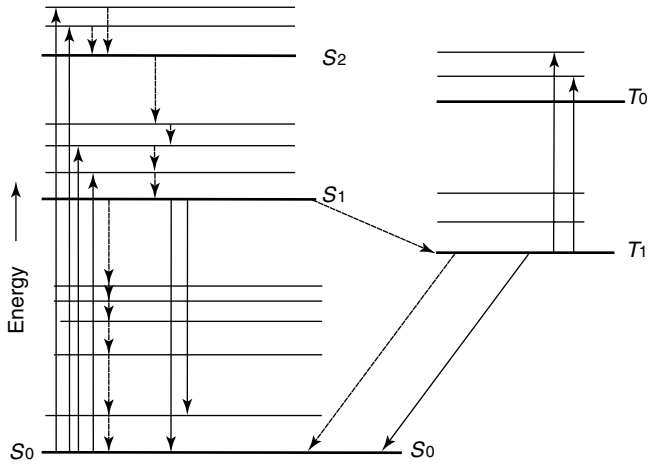

Fig. 1.15 Jablonski diagram: the *thick horizontal lines* indicate electronic energy states, the *thin horizontal lines* above each thick line vibrational substates. Associated with each electronic state, and indicated by *thinner lines* above the *thick lines*, are several vibrational energy levels. The higher up in the diagram, the higher energy the *lines* indicate. The *solid upward arrows* represent absorption of photons (light energy), and the *down pointing solid lines*, emission of photons (either spontaneous or stimulated emission). The *wider arrow* to the far *left* indicates that energy increases upward in the diagram. T_o the *left* in the figure the system of singlet states (S_o, S_1, S_3, with only paired electrons), to the *right* the system of triplet states (T_1, T_2, containing unpaired electrons). *Arrows with short dashes* indicate radiationless transitions within each state system. *Arrows with long dashes* indicate intersystem transitions (intersystem crossing). In the singlet system, radiative de-excitation (fluorescence) can take place only from the lowest excited state, since all other states are very short-lived. The transition from a triplet state to the ground level (S_o) does not take place easily, since an electron has to change spin. Thus the T_1 state (the lowest triplet state) is long-lived, and the radiative de-excitation from T_1 results in phosphorescence. This Jablonski diagram is not intended to depict energy relations in a particular molecule, but only general principles. A Jablonski diagram for the oxygen (O_2) molecule would, however, look radically different, since for this molecule the lowest-lying level represents a triplet state

the opposite, and the probability is greatest that the molecule will be near the state of zero potential energy and maximum kinetic energy. Thus, when for some reason a molecule changes electronic state, in most cases, the transition will occur from near the midpoint of the line for the lowest vibrational state. The vertical line to the left in Fig. 1.14 shows a likely transition from the lower electronic state to the higher electronic state, and the line to the right shows a likely transition from the higher electronic state to the lower one. The upward transition could be associated with absorption of photons, and the downward one by emission of photons.

In order to illustrate the various energy levels and transitions between them, it has become customary to use a kind of diagram named after Aleksander Jabłonski (pronounced Jabwonski). In such a diagram (see Fig. 1.15), the electronic energy levels (energy states of the molecule) are indicated by thick horizontal lines, the overlayered vibrational states by thinner horizontal lines, and state transitions by arrows.

Note that the downward pointing solid arrows (light emission) are generally shorter than the upward pointing ones (light absorption) corresponding to the fact that light emission is generally of longer wavelength (lower photon energy) than light absorption. This is, however, a matter of statistical distribution, and in stimulated emission, the wavelength is the same for absorbed and emitted light.

Stimulated emission is a phenomenon theoretically stipulated by Einstein but observed much later. According to Einstein, a molecule can be transformed by light absorption in two ways: either from a lower to a higher energy state, as we have described before, or from a higher to a lower state. In the latter case two photons are emitted (stimulated emission) for each one absorbed, and all three photons are of the same wavelength. The reason that it took a long time for stimulated emission to be observed is that the concentration of molecules in sufficiently high energy states is usually low, and the probability for stimulated emission therefore low. The transitions are described using so-called Einstein coefficients, B_{12}, B_{21}, and A_{21}. If, in a certain radiation field of energy density $r(\nu)$ for the frequency (ν), the number of molecules in the lower energy state is N_1, in the upper state N_2, then the probability of upward transitions is $B_{12}{\cdot}\rho(\nu){\cdot}N_1$, and the probability of downward stimulated transitions is $B_{21}{\cdot}\rho(\nu){\cdot}N_2$, but there are also spontaneous downward transitions with a probability $A_{21}{\cdot}N_2$. Because of the spontaneous downward transitions, N_1 is generally much larger than N_2. When light intensity is increased more and more, N_2 gradually approaches N_1 but cannot be caused to exceed it by light absorption alone (see Fig. 1.16).

The most important applications of stimulated emission are the laser (acronym for *l*ight *a*mplification by *s*timulated *e*mission of *r*adiation) and some types of high-resolution optical microscopy, to be described later.

Apart from the changes in vibrational and rotational energy, there are other causes of the "broadening" of spectra mentioned above (from line spectra to band spectra). More complicated molecules are usually (and the biomolecules always) in a condensed phase (liquid or solid) rather than in a low-pressure gas.

The different molecules in the phase affect one another in complicated ways so that the energy levels of one molecule are not the same as those of its neighbors. Finally, the different molecules are not identical (as a collection of isolated atoms of the same kind are) since they, even if they correspond to a single chemical formula, may have different *conformations*, e.g., an extended or folded chain of atoms. This results in continuous absorption and emission spectra.

Because, at ordinary temperatures, transitions between different conformational states take place readily, we do not experience molecules with different conformations as different kinds of molecules. By greatly lowering the temperature, we may prevent the transitions between different

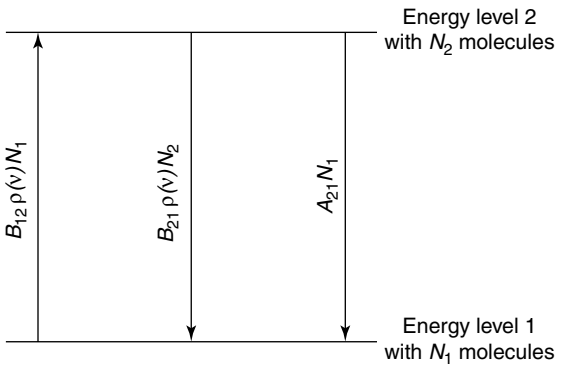

Fig. 1.16 Simplified energy level diagram (Jablonski diagram) for a molecule to explain stimulated emission. The *arrows* symbolize transitions between the two levels, by (from *left* to *right*) absorption, stimulated emission, and spontaneous emission. The probability of a transition is proportional to the population (N_1 or N_2) of molecules from which the transition takes place. Absorption and stimulated emission are also proportional to the energy density, $\rho(\nu)$, at the frequency (ν), which corresponds to the energy difference ($\Delta E = h\nu$) between the levels. (The energy density, in turn, is the product of the speed of light and the fluence rate.) For each one of these transition probabilities, there is also a proportionality factor (called an Einstein coefficient), i.e., B_{12} for absorption, B_{21} for stimulated emission, and A_{21} for spontaneous emission. Einstein showed that $B_{12} = B_{21}$ and $A_{21} = B_{21} \cdot 8\pi \cdot h\nu^3/c^3$. In the steady state, the upward and the total downward transition rates are the same, i.e., $B_{12} \cdot \rho(\nu) \cdot N_1 = B_{21}\rho(\nu) \cdot N_1 = B_{21} \cdot \rho(\nu) \cdot N_2 + A_{21} \cdot N_2$, from which follows $N_2/N_1 = \rho(\nu)/[\rho(\nu) + 8\pi \cdot h\nu^3/c^3]$. It follows from this that for low energy densities (weak light), N_2/N_1 and also N_2 increase proportionally to the light but also that N_2/N_1 cannot exceed 1 even if the light is very strong. A so-called inverted population of molecules with $N_2/N_1 > 1$, which is necessary for laser action, can only be obtained in other ways than by simple light absorption from one energy state to another one

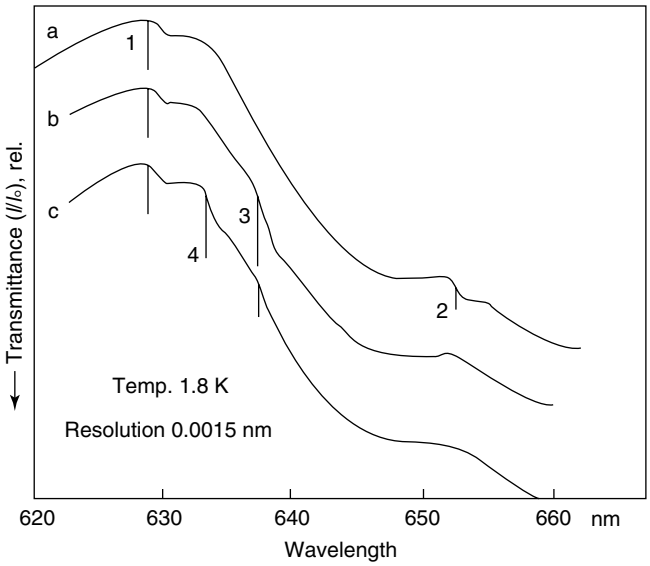

Fig. 1.17 A solution of C-phycocyanin was irradiated with a strong laser beam. Various wavelengths were used in the order indicated by the numbers and the *vertical lines* on the curves. These *lines* are, in fact, narrow dips or "holes" in the absorption spectra caused by depopulation of specific molecular states by the laser light. Note that irradiation with light of shorter wavelength following one with light of longer wavelength causes a repopulation of the less energetic state (corresponding to the longer wavelength). For instance, irradiation 3 practically cancels the effect of irradiation 2 (but not that of irradiation 1 at an even shorter wavelength than 3). Similarly, irradiation 4 partly cancels the effect of irradiation 3 (From Friedrich et al. 1981, modified)

conformational states as well as between different vibrational and rotational states, and it becomes possible to selectively deplete one state by monochromatic light from a laser. In this way one may "burn holes" in an absorption spectrum and see which portion of a spectrum is associated with a particular state (Fig. 1.17).

Even at a temperature of absolute zero, the oscillation continues with all molecules in the lowest vibrational state, the zero state. In fact, even at room temperature, the majority of the molecules are in this state, but a substantial fraction is in higher states.

Even at moderately lowered temperatures, absorption spectra (as well as fluorescence spectra) are sharpened (Fig. 1.18). This effect is often taken advantage of in spectroscopic investigations of biological samples containing several substances with similar spectra, such as cytochromes or chlorophyll proteins.

It should be understood that a molecule can appear in an electronically excited state for reasons other than having absorbed light or ultraviolet radiation. In rare cases, the collisions with other molecules can give a molecule sufficient energy for transition to an excited state. Chemical reactions may also produce reactants in electronically excited states, which can lose their energy by emission of light. This is how chemiluminescence works, and bioluminescence, which will be described later, is biochemical chemiluminescence.

1.14 Quantum Yield of Fluorescence

It has been already mentioned that a molecule in the excited state may lose its energy in various ways as light by radiative de-excitation. This process is called fluorescence if the transition is from a singlet excited state to a singlet ground state, and phosphorescence if the transition is from a triplet excited state to a singlet ground state or from a singlet excited state to a triplet ground state (the most important example of the latter is phosphorescence of singlet oxygen). It can also lose energy as vibrations to neighboring molecules (thermal de-excitation). Singlet excited states can disappear by "intersystem crossing" to produce triplet states; this happens, e.g., sometimes with chlorophyll molecules. And finally, energy may be lost through chemical reactions. Thus, the total rate of disappearance of singlet excitation can be described as the sum of the rates for the different de-excitation "pathways." In most cases, each molecule "acts on its own," so the kinetics of disappearance of singlet excitate states is of first order (in contrast, de-excitation of singlet oxygen is mixed first and second order at higher concentrations). It can thus be described by a

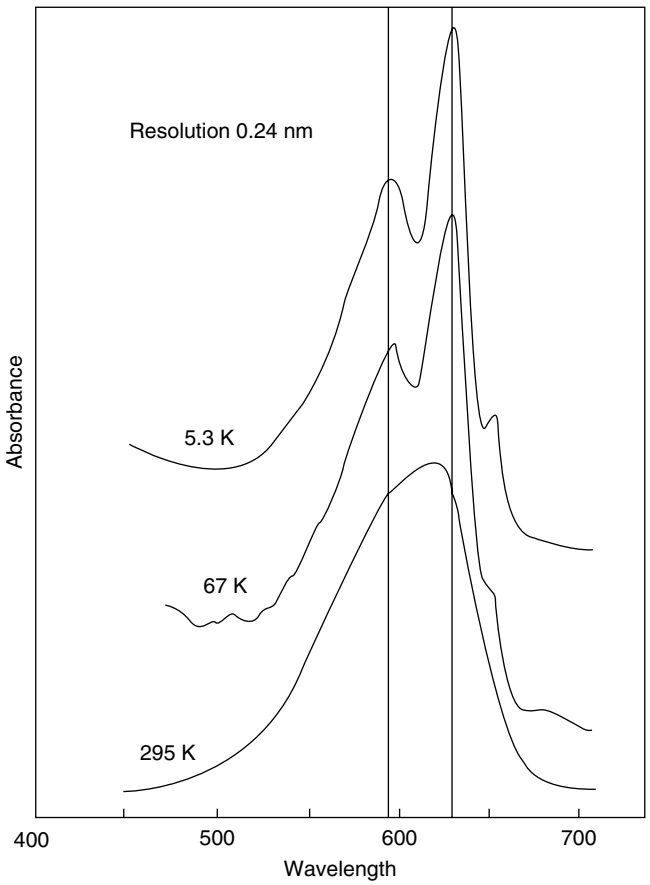

Fig. 1.18 Absorption spectra of C-phycocyanin at various temperatures (Redrawn from Friedrich et al. 1981)

first order rate constant k, which is a sum of the rate constants for the different pathways:

$$k = k_{\mathrm{f}} + k_{\mathrm{th}} + k_{\mathrm{ic}} + k_{\mathrm{ch}},$$

where f stands for radiative de-excitation (usually fluorescence), th for thermal de-excitation, ic for intersystem crossing, and ch for chemical de-excitation. Under steady illumination, a steady state develops, so the rate of excitation by absorption of photons equals the total rate of de-excitation. Therefore, the ratio of the number of photons emitted as fluorescence to the number of photons absorbed will be

$$\phi_f = k_{\mathrm{f}} / k = k_{\mathrm{f}} / \left(k_{\mathrm{f}} + k_{\mathrm{th}} + t_{\mathrm{ic}} + k_{\mathrm{ch}}\right).$$

The quantity φ_{f} is called the quantum yield of fluorescence. In the same way, we have a quantum yield for each de-excitation path; for example, also for the chemical deactivation,

$$\phi_{\mathrm{ch}} = k_{\mathrm{ch}} / k = k_{\mathrm{ch}} / \left(k_{\mathrm{f}} + k_{\mathrm{th}} + t_{\mathrm{ic}} + k_{\mathrm{ch}}\right).$$

The different pathways compete with one another. Therefore, the chlorophyll fluorescence from a plant, which is usually weak and invisible, increases if we add a poison that stops photosynthesis, the main pathway for chemical de-excitation. The fluorescence from chlorophyll becomes even stronger and clearly visible if we extract the chlorophyll and illuminate it dissolved in an organic solvent as acetone. Then we have not only completely stopped chemical de-excitation but also decreased thermal de-excitation.

Studying the changes of fluorescence from chlorophyll is an important way to investigate the functioning of the photosynthetic apparatus. In this context, one often uses the terms photochemical quenching and nonphotochemical quenching, respectively, for the chemical and thermal de-excitations competing with fluorescence.

1.15 Relationship Between Absorption and Emission Spectra

A simple relationship between absorption and emission spectra of even very complicated molecules was first hinted at by E. H. Kennard (1918) and later elaborated upon mostly by B. I. Stepanov. The relationship is most commonly referred to as the Stepanov (1957) relationship. The basic idea is that it is of no consequence to the future behavior of a molecule in which way it reached a certain state. From this it follows that any emission from an excited (energy-rich) electronic state of any kind of molecule in thermal and conformational equilibrium with its surroundings must have the same spectrum, whether the molecule reached the energy-rich state by collisions with its neighbors, or by absorbing a photon, or as a result of a chemical reaction. More specifically, the shape of the fluorescence spectrum (excited state reached by absorption of photons) is identical to the shape of the heat radiation spectrum from that kind of molecule. The heat radiation spectrum follows Planck's law for a blackbody, modified by the emissivity of the substance. But the emissivity, as was already mentioned, is the same as the absorptivity, which has the same spectral dependence as the experimentally measured absorption coefficient. Thus (fluorescence spectrum) = (absorption spectrum) × (blackbody spectrum). The multiplication sign here stands for *convolution*, i.e., multiplication of pairs of values throughout the spectrum. The fluorescence spectrum will then be expressed as photons per wavelength interval, energy per frequency interval, etc., depending on how the blackbody spectrum is entered into the equation.

The Stepanov relationship breaks down when heat energy cannot easily diffuse away from the emitting molecule. This happens in solid media or liquids of high viscosity and is always the case at low temperatures. Conversely, by comparing a fluorescence and an absorption spectrum, it can be found out whether or not the molecules in the excited state are in thermal and conformational equilibrium with the surroundings at the time of photon emission. An example of the

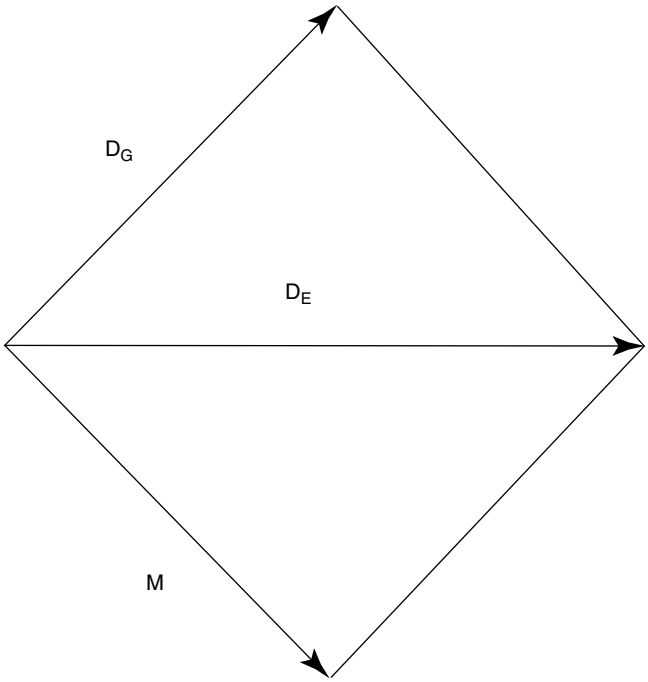

Fig.. 1.19 The transition moment **M** for a transition from the ground state to an excited state is the vectorial difference between the dipole moments of the molecule in the excited state ($\mathbf{D_E}$) and in the ground state ($\mathbf{D_{EG}}$)

application of these ideas to a biological system is provided by Björn and Björn (1986).

1.16 Molecular Geometry of the Absorption Process

In a molecule, the center of positive charge (associated with the nuclei) may, or may not, coincide with the center of negative charge (associated with the electrons). If the center of positive charge does not coincide with the center of negative charge, the molecule is a dipole. Unless molecules are symmetrical, as are CCl_4 or CH_4, they are more or less strong dipoles.

A dipole is characterized by a dipole moment. This is a vector having direction and magnitude. The magnitude is the distance between positive and negative charge centers times the amount of charge. The direction of the dipole moment is from the negative to the positive charge center. Like other vectors, the dipole moment is often symbolized by an arrow (Fig. 1.19).

When a molecule is electronically excited, the negative charge is generally displaced in relation to the positive charge, i.e., there is a change in dipole moment. This change in dipole moment is called the transition moment and often symbolized by **M.** Like the dipole moment, it is a vector; in fact, it is the dipole moment of the excited state ($\mathbf{D_E}$) minus the dipole moment of the ground state ($\mathbf{D_G}$). Symbolizing the vectors by arrows, we may describe the subtraction as shown in Fig. 1.19.

The magnitude of the transition moment can be estimated from the absorption spectrum of the compound in question. Denoting the molar absorption coefficient by ε, the refractive index of the solvent by n, and light velocity divided by wavelength (frequency, c/λ) by ν, the oscillator strength (the square of the magnitude of the transition moment) is approximately $1.0222 \times 10^{-62} n \, \mathrm{x} \int[\varepsilon(\nu)/\nu] \, \mathrm{d}\nu$ $\mathrm{C^2\,m^2}$, where integration is carried out over the absorption band. If we let ε instead stand for molecular cross section in $\mathrm{m^2}$, the formula becomes $2.673 \times 10^{-25} n$ x $\int[\varepsilon(\nu)/\nu] \, \mathrm{d}\nu$ $\mathrm{C^2}$ $\mathrm{m^2}$. C is the symbol for coulomb. For further details, see Knox (2003).

In most cases of excitation by light absorption, the probability of absorption is proportional to the square of the component of the transition moment in the direction of the electric field of the light. (There are cases of interaction between electrons and the magnetic field of the light rather than the electric field, but these cases are of little interest in photobiology.) Expressed in another way, the probability is proportional to $|\mathrm{M}|^2\cos^2\alpha$, where α is the angle between the transition moment and the direction of the electric vector of the light wave, i.e., the direction in the plane of polarization which is perpendicular to the direction of light propagation.

To those of you who think this is hard to follow, remember that the probability of absorption depends on how the molecule is oriented in relation to the direction of the light and (for plane-polarized light) the plane of polarization. For absorption of light by molecules in ordinary solutions, this is of no consequence, since the molecules (except in special cases) have random directions. For absorption of light by molecules in living cells, it is sometimes very important, since these molecules may be very accurately aligned. In such cases, light polarized in a direction parallel to the transition moment of the absorbing molecule is more strongly absorbed than light polarized in other directions. This phenomenon is called (absorption) dichroism.

In the same molecule there may be transition moments with different directions. For example, in the chlorophyll molecule, two transition moments are nearly at right angles to one another. The transition moment for emission of fluorescence may have a direction different from that for excitation of fluorescence. By measuring the polarization of fluorescence from molecules irradiated by polarized light, one can gain information about the angle between the transition moments.

1.17 Transfer of Electronic Excitation Energy Between Molecules

Transfer of electronic excitation energy from compound A to compound B may be symbolized as $\mathrm{A^* + B \rightarrow A + B^*}$. The energy quantum to be transferred must have a size such that it can be given off by A, i.e., corresponding to the energy of

a photon within the fluorescence band of A. Furthermore, it must be of a size that can be taken up by B, i.e., correspond to the energy of a photon within the absorption band of B. There are a few photobiological phenomena in which this energy transfer is actually mediated by a photon. As an example, we may mention the transfer of energy from luciferin in the lantern of a firefly female to the rhodopsin in the eye of a firefly male. However, in the majority of cases, the radiation transfer is radiationless, a process that is much more efficient at short range. Very few of the photons emitted by firefly females happen to be absorbed in rhodopsin molecules of firefly males. The advantage of energy transfer by photons is that it can take place over distance. We also all depend on the energy transfer taking place directly between atoms in the sun and chlorophyll molecules in plants, but also, this is a very wasteful process in the sense that a very small fraction of the photons emitted by the sun end up in chlorophyll molecules. On the other hand, once the quantum has been caught by a chlorophyll molecule (or a molecule of phycoerythrin or phycocyanin), it is channeled from molecule to molecule with an efficiency of practically 100 % by radiationless energy transfer (Fig. 1.20).

There are two main mechanisms for radiationless energy transfer: *exciton coupling* and the *Förster mechanism*. Exciton coupling occurs in a pure form in the photosynthetic antennae of green photosynthetic bacteria like *Chloroflexus*. The so-called chlorosomes of these bacteria contain rods made up of bacteriochlorophyll and carotenoid molecules. The pigment molecules are so tightly packed together that the whole rod behaves almost as a single pigment molecule; the energy is delocalized. This phenomenon, called *exciton coupling*, provides very fast transfer of the energy to the reaction center.

In other cases, chromophores may just pairwise be close enough to share energy and form what is called *exciplexes*. When exciplexes are formed, the energy levels split up.

The other mechanism, the Förster mechanism or resonance transfer, or dipole–dipole interaction, takes place in the phycobilisomes, pigment antennae of cyanobacteria and red algae composed of phycoerythrin, phycocyanin, allophycocyanin, and linker peptides holding the complex together (phycoerythrin is not always present, and in some cases, there are also other phycobiliproteins, such as phycoerythrocyanin). Only a few of the chromophores in the phycobilisomes are close enough to form exciplexes. A special section will be devoted to the Förster mechanism because it is so important.

Fig. 1.20 Demonstration of energy transfer in phycobilisomes prepared from a cyanobacterium. The chemical composition is exactly the same in the two test tubes, except for the concentration of the phosphate buffer in which the phycobilisomes are suspended. The test tubes are illuminated with *green light* (absorbed mainly by phycoerythrin), and the *yellow* and *red color* is due to fluorescence of the phycobiliproteins. In the test tube to the *left*, the phosphate concentration is low, the phycobilisomes are dissociated with large distances between the various phycobiliproteins, and the fluorescence is directly emitted by phycoerythrin. In the test tube to the right, the phosphate concentration is high (0.15 M), the phycobiliproteins are close together in undissociated phycobilisomes, and energy is transferred from phycoerythrin, via phycocyanin, and finally emerges as red fluorescence from allophycocyanin. Such "fluorescence resonance energy transfer" (FRET) is nowadays exploited in a number of applications, such as a method for DNA sequencing (Phycobilisome preparation by Dr. Gunvor Björn, photo by the author)

1.18 The Förster Mechanism for Energy Transfer

Thus, the transition of the molecule from one electronic energy state to another causes a change in the electrical field around it. Conversely, a change in the electric field can cause the transition from one energy state to another one. The field change caused by the transition in one molecule can cause the opposite transition in a neighboring molecule. This is the essence of energy transfer by dipole–dipole interaction, the Förster mechanism for energy transfer.

Just as the field change from the transition taking place in one molecule (the donor) drops off with the third power of the distance, so the sensitivity of the other molecule (the acceptor) to a field change drops off with the third power of

the distance. The combined effect is a sixth power relationship: the rate of dipole–dipole energy transfer between two molecules is inversely proportional to the sixth power of the distance.

We are now ready to have a look at a simplified Förster's formula:

$$\text{Energy transfer rate} = \text{factor } \phi \left(\text{overlap initegral}\right) \cdot \cos^2 \alpha / \left(r^6\right).$$

Here φ is the fluorescence quantum yield in the absence of the acceptor (see Sect. 1.15), α is the angle between the transition moments of the molecules, r is the distance, and the overlap integral is the convolution (pointwise product) of the donor fluorescence spectrum by the acceptor absorption spectrum, integrated over the whole spectral region in common.

1.19 Triplet States

Our description of molecular energy states so far has been aimed primarily at explaining the properties and processes associated with so-called singlet states. A molecule is said to be in a singlet state when all its electrons are grouped in pairs, so that the two electrons in each pair have opposite *spins*. Spin is a property of an electron or other charged particle that makes it act like a small magnet to produce a magnetic field. Positively charged particles such as atomic nuclei also have spin.

Because all electrons in a molecule in a singlet state occur in pairs, and the electrons in each pair have opposite spins, the electrons produce no net magnetic field. Most molecules like to be in a singlet state, so usually the ground state, the most stable state, having the lowest electronic energy, is a singlet state. A notable exception is the dioxygen molecule (making up ordinary oxygen in the air), which we shall come back to later.

However, it can occasionally happen that when a molecule has been excited from its ground (singlet) state to an excited singlet state, an electron "flips over," i.e., changes spin. Let us take a concrete and important example—the chlorophyll a molecule (Fig. 1.18). Like other chlorophyll forms, chlorophyll a has two prominent absorption bands corresponding to two electronic transitions with high probability. For chlorophyll a, these absorption bands are in the blue and red parts of the spectrum. In a collection of chlorophyll a molecules, be it in the plant or in solution, most of the molecules are in the ground state. Absorption of a photon of red light transforms a ground state molecule to the first excited singlet state. Absorption of a photon of blue light transforms a molecule from the ground state to the second excited singlet state. A molecule in the second excited singlet state very rapidly transfers some of its electronic energy to vibrational energy (heat) and lands in the first excited singlet state. Then various things can happen. The most "exciting" (pardon the expression) of the possibilities is that an electron completely leaves the molecule. This is the key step in photosynthesis and the key step in the whole living world. Another possibility is that the molecule "shakes off" more energy, heats its environment even more, and returns to the ground state. A third possibility is that it emits a photon, which carries away the excess energy and also returns the molecule to the ground state. A fourth possibility, which is realized in only a small fraction of the cases, is that the molecule is transferred from the first excited singlet state to the first (excited) triplet state. Although this happens after only a small fraction of excitations, it is important, and if plants were not specially equipped to handle such events, they would not survive.

A change from a singlet to a triplet state, which involves a spin change, a "flip" of an electron, is sometimes referred to as "intersystem crossing," because singlet and triplet states can be considered to be two types of states. Intersystem crossings are still sometimes also called "forbidden transitions," because early theories did not include the rare occasions when they occur.

Also, the change from the excited triplet state to the (singlet) ground state is "forbidden." In fact, it does occur (as many forbidden things do in our society). In any case, it does not take place quickly, or, in other words, the excited triplet state has a long lifetime. A triplet molecule does not easily react with a singlet molecule, but if it meets another triplet molecule, things are different. The magnetic fields created by the unpaired electrons interact. Even if the triplet molecules should not react chemically, they can exchange energy and become two singlet molecules. But because creation of a triplet state is in most cases a rare event, most triplet molecules are in low concentration, and the chance that two will meet is not great. We shall now come to a very important exception to this rule, already mentioned above.

1.20 The Dioxygen Molecule

The molecules of ordinary oxygen that we breathe have very remarkable properties. The most remarkable, important, and unusual of them all is that dioxygen molecules have a triplet ground state. From what will follow, the reader might get the impression that this is very unfortunate, because it makes oxygen a bit difficult to handle for organisms and imposes many threats. We shall deal with some of these in the chapters on phototoxicity and photosynthesis. But as is the case with many properties of the surprising and exciting world which we inhabit, if things were not exactly as they are, we would not be around. Just think for a moment that if the dioxygen in the air were in the singlet state, what would happen? We all know that oxygen under certain circumstances can react with organic

matter such as wood or our own bodies. Not only single houses or trees, but whole towns and forests have sometimes burned down. When oxygen oxidizes organic matter, large amounts of energy are released as heat. Processes that release energy usually take place quite easily. But for a house to catch fire, something has to get hot to start with. Once fire has started, other things get hot, and the fire is not easy to extinguish. Why is it that the fire does not start spontaneously?

The answer is that dioxygen consists of triplet molecules, and triplet molecules do not easily react with singlet molecules. Only after things get hot and some of the organic molecules get into states with lone electrons does a reaction with oxygen take place. When this happens, heat is released, more organic molecules acquire unpaired electrons and can react with oxygen, and so on.

Since we know that electrons like to join to pairs, one would expect that two oxygen atoms combine to a dioxygen molecule by joining two unpaired electrons to a pair. Instead, they combine to form a molecule with two lone electrons, a diradical.

1.21 Singlet Oxygen

Singlet dioxygen exists, but its lowest electronic state has more energy than the triplet ground state. Singlet oxygen can be produced by reaction of ordinary triplet oxygen with another compound in the triplet state, provided the energy of that other molecule is high enough. As we can see from Fig. 1.21, the energy of triplet chlorophyll (in relation to the singlet ground state of chlorophyll) is high enough to transfer oxygen from its triplet ground state to an excited singlet state called $^1\Delta g$, according to the following scheme:

$$\text{Chlorophyll}\ (\text{excited triplet}) + O_2\ (\text{ground state}) \rightarrow \text{Chlorophyll (ground state)}$$

The singlet so created is very reactive and can attack various other singlet molecules in the cell. If the plant did not have special systems both for preventing as much as possible the formation of singlet oxygen (this is the role of carotene in the plant cell) and for ameliorating the effects of it if it is formed, the plant could not survive for long, as shown in mutants lacking these protective systems.

In addition to chlorophyll, many other pigments, when illuminated, can form triplet states and generate singlet oxygen. We shall deal with this further in the chapter on phototoxicity.

The electronic configurations of various O_2 molecules and O_2 ions are shown schematically in Fig. 1.22.

1.22 Some Aspects of Light Recently Attracting Increased Interest

In this section, we shall briefly mention aspects of light that have recently attracted increased interest and provide references for further study.

1.22.1 Surface Plasmons

Surface plasmons constitute a form of energy which can be described as intermediate between light and electrical energy. Plasmons occur in the interface between an electrically conducting medium and an electrical insulator. They are not known to have direct relevance for biology but are used in biological research, e.g., for nano-tweezers (see Chap. 5, Juan et al. 2012; Dionne et al. 2012). For further information about plasmons, see Lakowicz (2006) and Berweger et al. (2012).

1.22.2 Orbital Angular Momentum of Light

This should not be confused with the ordinary angular momentum of photons due to their spin, neither should light having orbital angular momentum be confused with circularly polarized light. It can be generated only using special methods. So far, it has been important mostly for manipulation of very small particles (Grier 2003). Possibly, it can have relevance for the origin of chirality of biological molecules, but there is no evidence for this.

1.22.3 Coherence

Coherent light is light in which the waves corresponding to different photons are in step, and such light is best known as emitted by lasers. Recently, there has been much discussion concerning whether coherent energy transport might occur in photosynthetic systems even after absorption of incoherent light and of what importance that may be. We refer to Fassioli et al. (2012) and Kassal et al. (2013) for an introduction to the literature.

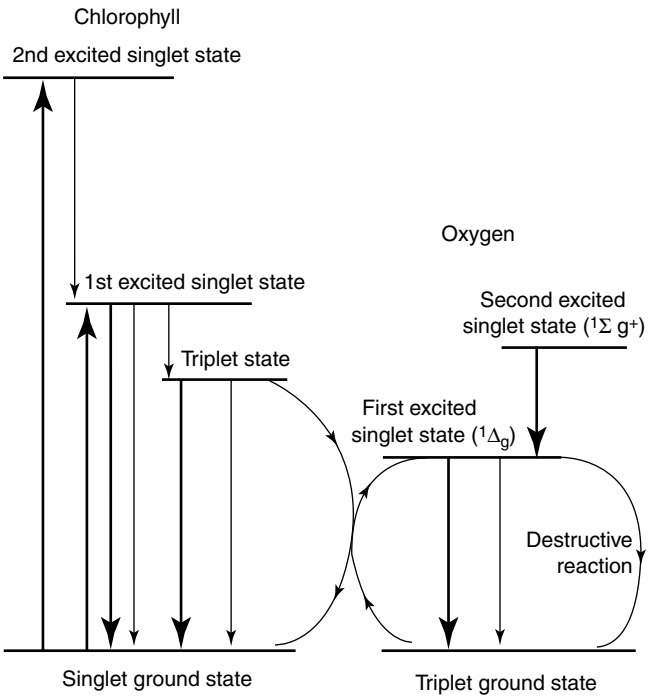

Fig. 1.21 The various energy states (*horizontal lines*) of chlorophyll (*left*) and molecular oxygen (*right*) and their energy transitions (*arrows*). Energy is plotted upward, i.e., a *high horizontal line* depicts a high energy state. Only the most important electronic levels are indicated, and the vibrational levels have been omitted. *Thicker lines* depict energy transitions associated with absorption (*upward arrows*) or emission (*downward arrows*) of light. The *long upward arrow* from the ground state of chlorophyll to the second excited state represents absorption of blue light; the *shorter upward arrow* from the ground state to the first excited state absorption of red light. *Thin arrows* represent radiationless transitions, in which energy is either transformed to heat (*straight arrows*) or reaction with another molecule (*curved arrows*) takes place. Emission of light can take place either as fluorescence (rapid light emission from singlet to singlet state) or as phosphorescence (slow light emission associated with change from singlet to triplet, as in chlorophyll at low temperature, or from triplet to singlet—in oxygen gas even at room temperature)

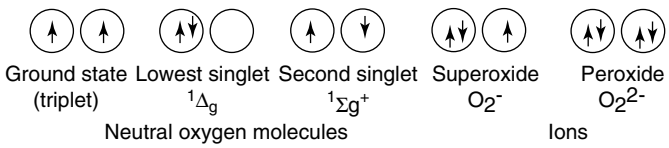

Fig. 1.22 The electronic configurations in various forms of neutral dioxygen (*left*) and dioxygen ions of biological importance (*right*). Only the "antibonding" (π^*) electrons are shown, since all lower orbitals are similar (completely filled) for all the species. *Arrows* of opposite directions represent electrons of opposite spin

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