Photobiology

Second Edition

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The Science of Life and Light

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Edited by

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Springer

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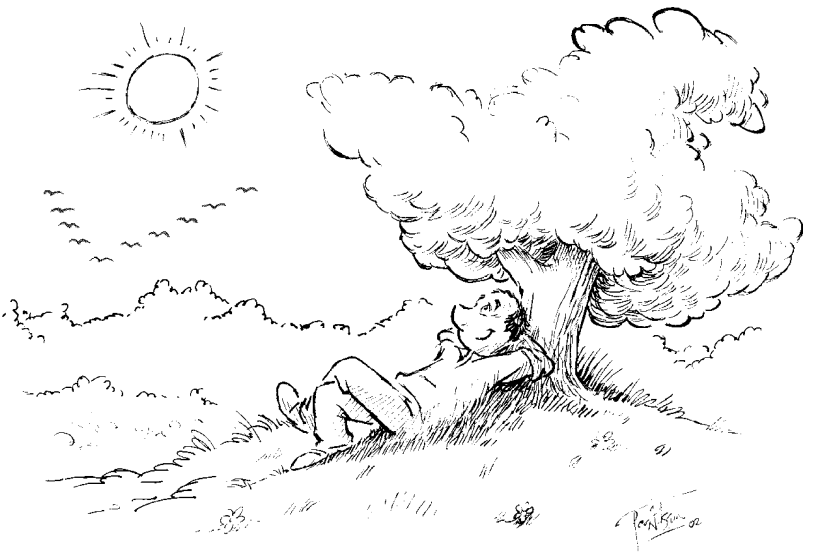

(Drawing by Per Nilsson)

Photobiology

I am lying on my back beneath the tree,
dozing, looking up into the canopy,
thinking: what a wonder!—I can see!

But in the greenery above my face,
an even greater miracle is taking place:
Leaves catch photons from the sun
and molecules from air around.
Quanta and carbon atoms become bound.
Life, for them, has just begun.

The sun not only creates life, it also takes away
mostly by deranging DNA.
Damage can be, in part, undone
by enzymes using photons from the sun.

Summer nears its end, already 'cross the sky
southward aiming birds are flying by.
Other birds for travel choose the night
relying on the stars for guiding light.

Imprinted in their little heads are Gemini,
Orion, Dipper, other features of the sky.
There is room for clocks that measure
day and night,
Correct for movement of the sky
and tell the time for flight

Deep into oceans, into caves
the sun cannot directly send its waves.
But through intricacies of foodweb's maze,
oxygen from chloroplasts, luciferin, luciferase,
at times, in place,
where night and darkness seem to reign,
solar quanta emerge as photons
once again.

L.O. Björn 2002

Preface

I started my first photobiological research project almost exactly 50 years ago, in the spring of 1957. My scientific interest ever since has been focused on photobiology in its many aspects. Because I have been employed as a botanist, my own research has dealt with the photobiology of plants, but throughout this time I have been interested in other aspects, such as vision, the photobiology of skin, and bioluminescence. A first edition of the present book was published in 2002, but this second edition is much expanded and completely updated. Several new authors have been recruited among my eminent colleagues.

It has not been possible to cover all aspects of photobiology in one volume, but I feel that we have managed to catch a fair and well-balanced cross section. Many colleagues promised to help, but not all lived up to their promises. To those who did, and who are coauthors to this volume, I direct my thanks; I think that they have done an excellent job.

Living creatures use light for two purposes: for obtaining useful energy and as information carrier. In the latter case organisms use light mainly to collect information but also (e.g., by coloration and bioluminescence) for sending information, including misleading information, to other organisms of their own or other species. Collection of free energy through photosynthesis and collection of information through vision or other photobiological processes may seem to be very different concepts. However, on a deep level they are of the same kind. They use the difference in temperature between the sun and our planet to evade equilibrium, i.e., to maintain and develop order and structure.

Obviously, all of photobiology cannot be condensed into a single volume. My idea has been to first provide the basic knowledge that can be of use to all photobiologists, and then give some examples of special topics. I have had to limit myself, and one of the interesting topics that had to be left out is the thermodynamics of processes in which light is involved.

Thus, this book is intended as a start, not as the final word. There are several journals dealing with photobiology in general, and an even greater number dealing with special topics such as vision, photodermatology, or photosynthesis. There are several photobiology societies arranging meetings and other activities. And last but not least, up-to-date information can be found on the Internet. The most important site, apart from the Web of Science and other scientific databases, is Photobiology Online, a site maintained jointly by the American and European Societies for Photobiology (ASP and ESP, respectively),

at http://169.147.169.1/POL.index.html or http://www.pol-europe.net/, where details about photobiology journals and books can be obtained.

The subtitle of this book may be somewhat misleading. There is only one science. But I wanted to point out that the various disciplines dealing with light and life have more in common than perhaps generally realized. I hope that the reader will find that the same principles apply to seemingly different areas of photobiology. For instance, we have transfer of excitation energy between chromophores active in photosynthesis, in photorepair of DNA, and in bioluminescence. Cryptochromes, first discovered as components in light-sensing systems in plants, are involved in the human biological clock, and probably in the magnetic sense of birds and other animals, and they have evolved from proteins active in DNA photorepair. The study of the photomagnetic sense of birds has, in turn, led to new discoveries about how plants react to a combination of light and magnetic fields.

Many colleagues have been helpful in the production of this book. Two of my coauthors—Professors Helen Ghiradella and Anders Johnsson—who are also close friends, have earned special thanks, because they have helped with more chapters than those who bear their names. Helen has also helped to change my Scandinavian English into the American twist of the islanders' tounge, but we have not changed the dialect of those who are native English speakers. Professor Govindjee has contributed not only with his knowledge of photobiology, but also with his great experience in editing. Drs. Margareta Johnsson and Helena Björn van Praagh have helped with improvements and corrections, and Professor Allan Rasmusson at our department in Lund has been very helpful when I and my computer have had disagreements. I have enjoyed the friendliness and help of other colleagues in the department. The staff of our biology library has been very helpful and service-minded.

Many others have also helped, but special thanks go to my wife and beloved photobiologist Gunvor, who has supported me during the work and put up with paper and books covering the floor in our common home; to her I dedicate those chapters of the book that bear my name.

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The Nature of Light and Its Interaction with Matter

Lars Olof Björn

Abstract: This chapter provides a physical background to the following ones. It describes the particle and wave properties of light, and the diffraction, polarization, refraction, reflection, and absorption of light, statistics of photon emission and absorption. Planck's law of heat radiation is described in various mathematical and graphical ways. One section is devoted to a simplified description of the propagation of light in absorbing and scattering media. The final sections are devoted to interactions between light and matter: spectra of and energy levels in atoms and molecules, the relation between absorption and emission spectra, the molecular geometry of absorption and emission, and the transfer of electronic excitation energy between molecules, including the Förster mechanism, triplet states, and the photobiologically important properties of the dioxygen molecule.

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 colored filter should be used to limit the light to a narrower waveband. 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 $\alpha$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., $d.\sin \alpha = n.\lambda$, 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., $d.\sin \alpha = (n + 1/2).\lambda$. 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

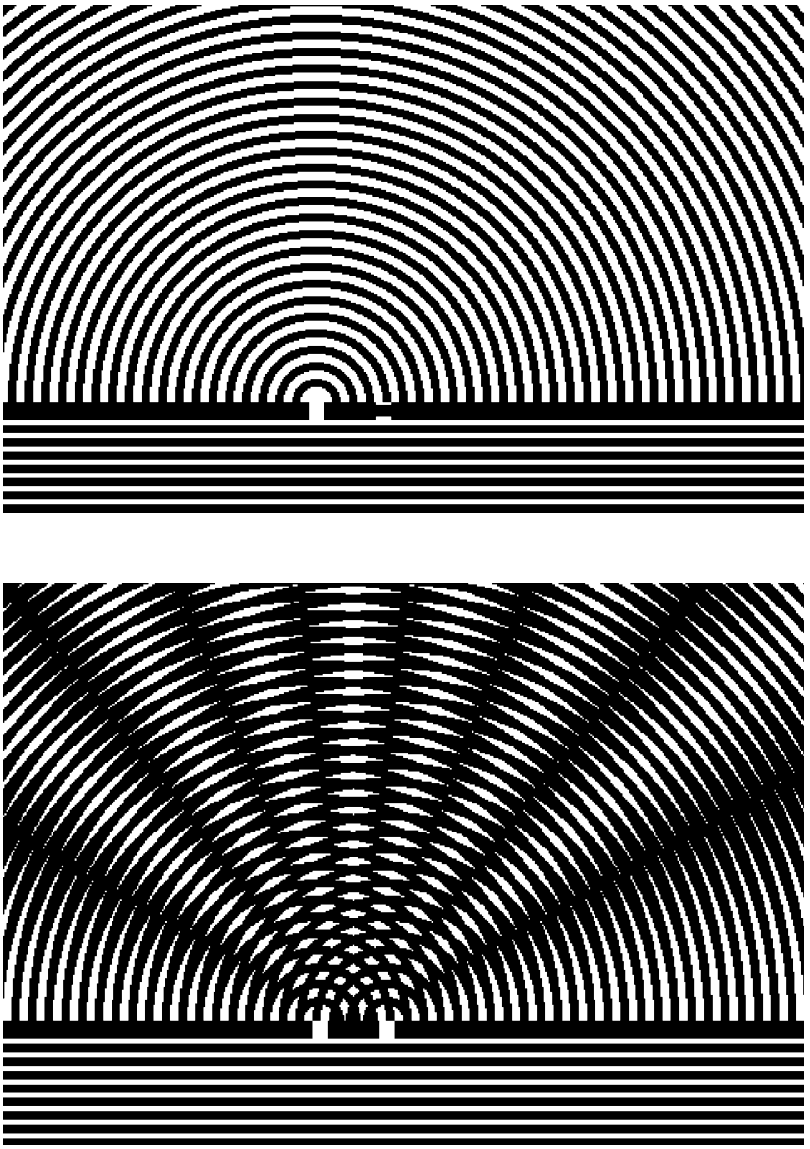

FIGURE 1.1. **(Top)** Light waves impinge from below on a barrier with only one slit open and spread from this in concentric rings. **(Bottom)** Light waves impinge from below on a barrier with two slits open. The two wave systems spreading on the other side interfere and in some sectors enhance, in 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.

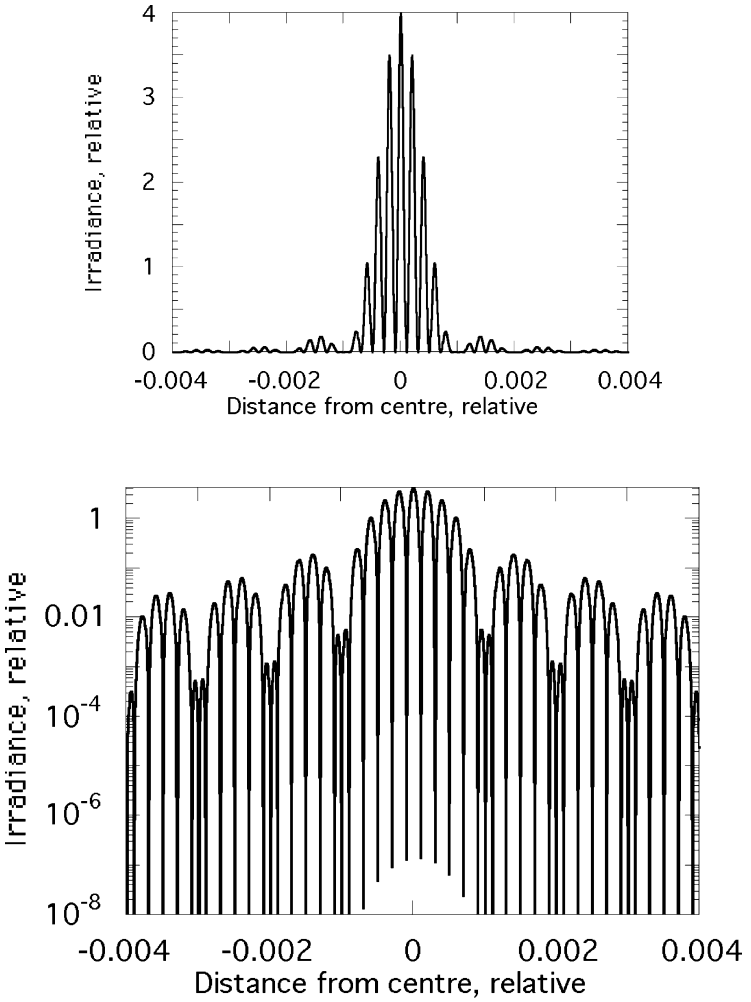

FIGURE 1.2. Interference pattern produced in Young's double slit experiment (computer simulation). The width of each slit is 1 mm, the distance between slit centers 4 mm, and the wavelength 0.001 mm (1 μm). The distance from the center of the screen is along the horizontal axis and the irradiance ("light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

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

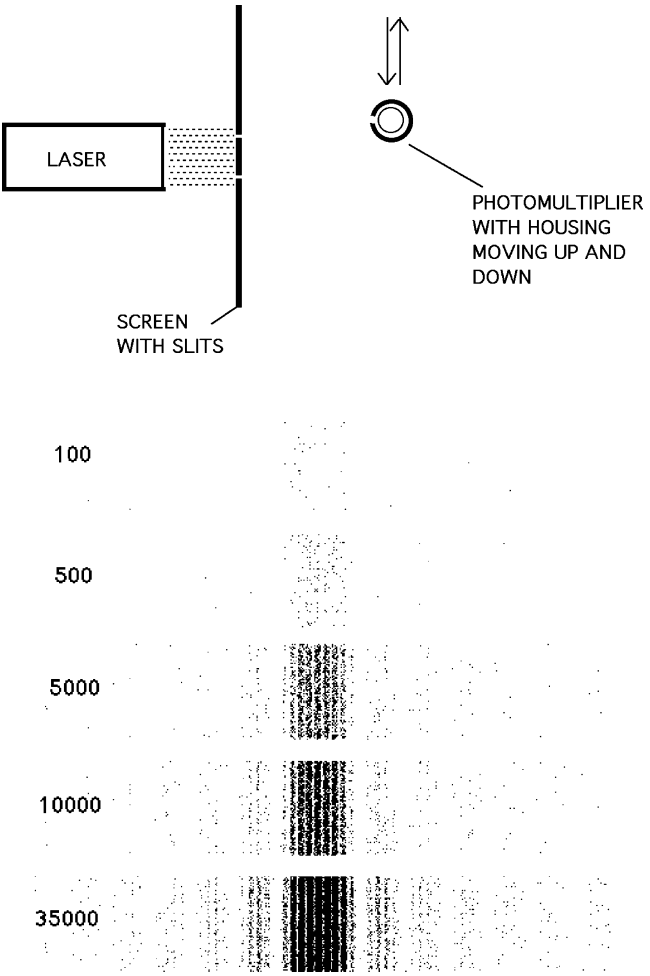

FIGURE 1.3. **(Top)** Double slit experiment set up to count single photons. The sketch is not to scale. In a real experiment the distance of the photomultiplier from the screen with slits would be greater, and the opening in the photomultiplier housing smaller. **(Bottom)** Simulation of the pattern 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.

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.

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 towards 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* 299792.4562 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 wavenumber, 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.

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 Chapter 9). 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.

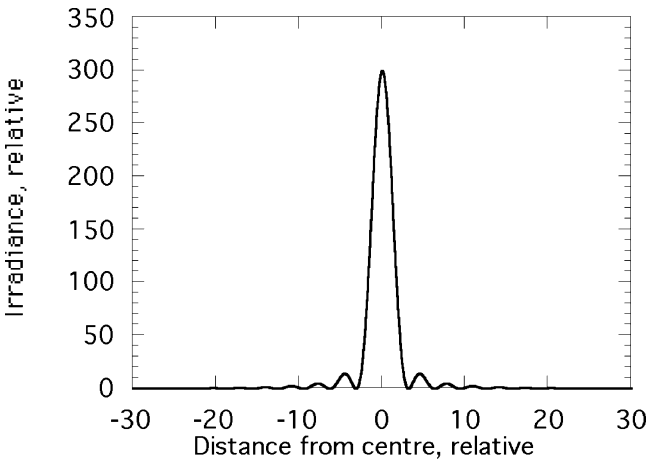

FIGURE 1.4. Diffraction pattern in a single slit (the pattern from a round hole looks similar in one dimension, but is slightly different). The horisontal axis shows the sine of the deviation angle in units of the ratio beween wavelength and slit width.

1.5. Polarization

Light waves are *transverse*, i.e., the oscillation is perpendicular to the direction of wave propagation, 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 degree *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.

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

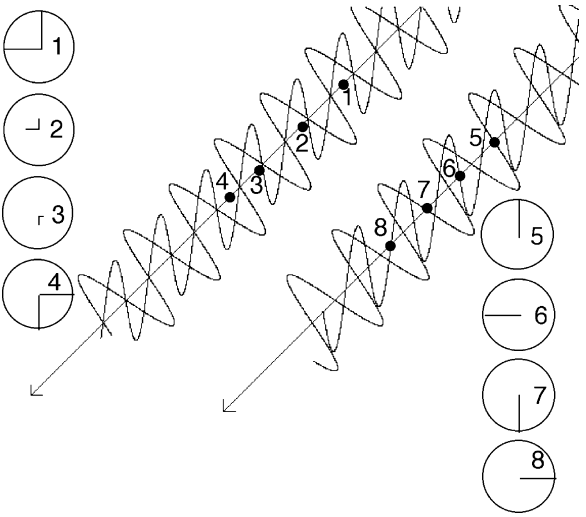

FIGURE 1.5. In the upper left part of the figure a plane-polarized light beam, composed of one vertically and one horisontally polarized component, is depicted in perspective and also "head on" at different points (or at one point at different moments). Numbered points in the perspective drawing correspond 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.

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., $1 \cdot 2 \cdot 3 \cdot 4 \ldots \cdot n$. 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 minutes of removing the cells from