Generation and Control of Light

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3.1 Introduction

Any photobiological experimental setup consists of three main parts: a light source, a light path, and a target. The biological object under investigation may form the light source, part of the light path, or, as in the most common case, the target. In the following, we shall treat the nonbiological components of the experimental setup.

3.2 Light Sources

3.2.1 The Sun

Almost all the natural light at the surface of the earth comes from the sun (this holds, of course, also for moonlight). The sun, on the whole, radiates as a glowing blackbody at a temperature of about 6,000 K. We have already mentioned the absence of some wavelength components from sunlight due to absorption in the outer cooler layers of the sun. Sunlight is further modified by the Earth's atmosphere before it reaches ground level. More about this and other natural light conditions will follow in Chaps. 6 and 7.

3.2.2 Incandescent Lamps

The light from an incandescent lamp originates at the surface of a glowing filament, which nowadays is, almost invariably, made from tungsten. It is heated by an electric current flowing through it. In order not to be destroyed (oxidized) by the oxygen in the air, the filament is enclosed in an envelope made of glass or quartz. The envelope is either evacuated (most commonly for small lamps) or filled with an inert gas or iodine vapor.

The spectral composition of the emitted light is strongly dependent on the temperature of the filament. As a first approximation, the spectrum varies with temperature as does that of blackbody radiation (Planck's law). However, due to the wavelength dependence of the emissivity of tungsten, more shortwave radiation is emitted than from a blackbody of the same temperature. The filament is, in most cases, coiled, which makes it somewhat "blacker" (more like a glowing cavity) than a smooth tungsten surface would be.

In most cases, it is desirable to obtain as much as possible of the radiation toward the short-wavelength end of the spectrum, i.e., to operate the lamp with as high a filament temperature as possible (the temperature can be increased by increasing the voltage). However, this results in a shorter life of the lamp, because the tungsten evaporates more quickly (and condenses again on the envelope, which blackens it). As a rule of thumb, an increase of the voltage by 10 % above the recommended voltage will decrease the lamp life to one third (i.e., by two thirds).

The lamp life at a certain filament temperature can be increased by making the filament thicker. Thereby, a certain power (wattage) is reached at a lower voltage (using a higher current). A further advantage of low-voltage lamps is, in many cases, that the filament is shorter. Quite often a small ("point-like") light source is desirable in optical systems.

A gas-filled envelope permits a higher filament temperature than an envelope with vacuum. A mixture of argon and nitrogen is the most common choice (old-fashioned household bulbs). The addition of iodine ("halogen lamps" or "quartz–iodine lamps") permits an even higher temperature. This is because the iodine vapor combines with the tungsten vapor. The compound is decomposed again when the molecules hit the hot filament, which is thereby continuously regenerated. For the regeneration to work properly, the temperature of the envelope must be so high that the tungsten iodide cannot condense on it. Therefore, such lamps are manufactured with a very small envelope made of quartz, which can withstand high temperature.

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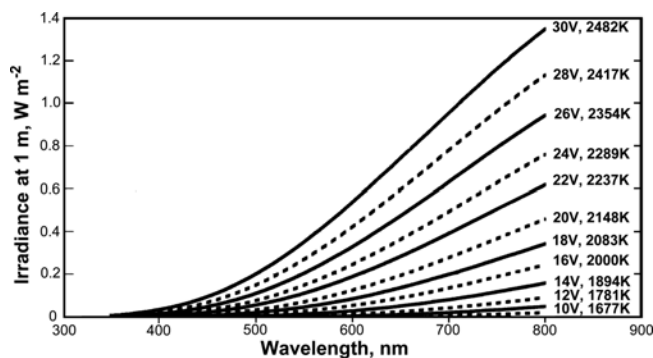e-mail: Lars_Olof.Bjorn@biol.lu.se

Fig. 3.1 Emission spectra of an incandescent lamp with a nominal rating of 24 V run at different voltages, with computed filament temperatures indicated. The lowest dashed curve corresponds to 8 V and 1,547 K

The advantages of incandescent lamps are their low cost, no requirement for complex electrical circuitry, and stability. The main disadvantage is that the emission at the short-wavelength end of the spectrum is low and the spectrum is strongly dependent on the current through the lamp and therefore difficult to keep absolutely constant. A method to estimate the spectrum of an incandescent lamp fed with direct current (within the spectral range where the glass or quartz envelope does not absorb appreciably) has been devised by Björn (1971). The principle is as follows: using a small current and measuring both the current through and the voltage across the lamp, the room temperature resistance of the lamp is measured (this is essentially the resistance of the tungsten filament). After the lamp has been powered up (with direct current), the current and voltage are measured again. From this, the "hot" resistance is computed. The temperature of the glowing filament can be computed from the ratio between room temperature resistance and "hot" resistance, and from this (using the Planck formula for a blackbody radiator and the known temperature- and wavelength-dependent emissivity of tungsten), the spectrum can be computed.

Using these principles, emission spectra for a lamp with a 24 V rating operated at various voltages were computed (Fig. 3.1) according to Björn (1971).

3.2.3 Electric Discharges in Gases of Low Pressure

In a gas discharge lamp, an electric current is flowing through a gas. The gas emits light, the spectral composition of which depends on the gas. When the gas has a low pressure, it emits a line spectrum, i.e., only light of certain wavelengths is represented (in contrast to the continuous spectrum emitted by an incandescent lamp).

The basic parts of a gas discharge lamp are a gas enclosed in a transparent envelope and the two electrodes necessary

for the conduction of current to and from the gas. In addition to this, it is often necessary with other parts, such as heating filaments, to release enough electrons to start the current through the gas or to vaporize, e.g., mercury or sodium when vapors of these metals are to be used as emitting gas.

The electric resistance of an incandescent lamp increases when the current through it increases, since tungsten has a higher resistivity the higher the temperature. Thus, an incandescent lamp is self-regulating and burns in a stable way as long as the voltage is constant. In a gas discharge lamp, the reverse holds: its electric resistance decreases with increasing current. Therefore, such a lamp has to be connected to some kind of circuitry limiting the current. In the case of direct current, a series resistor is often used, and in the case of alternating current, a choke.

Gas discharge lamps containing mercury vapor of very low pressure emit most of their energy as ultraviolet radiation of wavelength 253.7 nm. This wavelength is close to the absorption maximum of nucleic acids, and the radiation is also absorbed by the aromatic amino acids in proteins and many other biological molecules. The photons are also energetic enough to initiate many chemical reactions, and therefore, this kind of radiation is very destructive for living matter. Low-pressure mercury lamps with quartz envelopes (which transmit this kind of radiation, in contrast to glass envelopes) are therefore used as sterilization (germicidal) lamps.

Fluorescent lamps are similar lamps, but with glass envelopes, which on the inner surfaces have a fluorescent layer converting the UV radiation to visible light (or, in certain cases, to UV radiation of longer wavelength than the original emission).

Glow lamps are a kind of low-pressure gas discharge lamp, usually containing neon. Lamps containing the element to be measured are used for atomic absorption spectroscopy. Low-pressure sodium lamps, emitting almost monochromatic light at 589 nm, have been used extensively as sources of outdoor working light, because they give more visible light per unit of energy input than any other type of lamp. To a large extent, they have been abandoned, mostly because our color vision cannot be used with monochromatic light.

Microwave radiation is used for the energy input in some other gas-filled lamps, such as "electrodeless" high-pressure xenon lamps.

3.2.4 Medium- and High-Pressure Gas Discharge Lamps

If the vapor pressure in a mercury lamp is increased, more and more of the emission at 253.7 nm is reabsorbed, and finally, very little of this radiation escapes from the vapor.

Instead, spectral lines of longer wavelength emerge (medium-pressure mercury lamps). At even higher pressures, the spectral lines are broadened to bands (high-pressure mercury lamps), and finally, a continuous spectrum results (superhigh-pressure mercury lamps).

Deuterium (heavy hydrogen) lamps of medium pressure are used as light sources for spectrophotometry in the ultraviolet region.

Lamps containing xenon of high pressure are used to obtain a strong continuous emission from 300 nm and into the infrared. Depending on the composition of the envelope, more or less of shorter wavelength, ultraviolet also escapes. Xenon lamps come in a great variety of types. We use lamps running at about 24 V on direct current (but ignited with about 2,000 V = danger!!) and wattages (rated powers) from 150 to 900 W. Xenon lamps of higher wattage are often water cooled. Electrodeless xenon lamps are also manufactured. They are powered by microwave radiation.

Because xenon lamps with UV-transparent envelopes cause conversion of oxygen to ozone, such lamps must be provided with exhausts to transport the ozone out of the building. The same holds for high-pressure mercury lamps with UV-transparent envelopes.

All high-pressure lamps are dangerous because they can explode. They must therefore never be operated without protective housing. Even when cold, they should be handled with care, using eye protection and other appropriate safety measures.

3.2.5   Flashlamps

Electronic flashes are xenon lamps through which a capacitor is discharged when a special triggering pulse has ionized the gas. The energy available is proportional to the capacitance of the capacitor and to the square of the voltage to which it has been charged. In many cases, it is desirable to have a short flash duration. This requires that the impedance of the circuit is low (short leads) and the capacitance low (high voltage has to be applied to the capacitor to get enough energy with a low capacitance). It is also necessary to prevent the circuit from oscillating and causing multiple flashes. Ordinary photographic flashes have a flash duration of about 1 ms. If they are "automatic," i.e., combined with a light-sensing photodiode and appropriate circuitry, the flash will be cut off when a certain amount of light energy has been emitted.

3.2.6   Light-Emitting Diodes

Light-emitting diodes (LEDs) are used in applications where very strong light is not needed, for instance, as indicator lights and displays. However, the maximum output power available from LEDs is increasing, and LEDs are the cheapest devices that can be modulated very rapidly: using an appropriate circuit, they can be switched on and off in a few nanoseconds. For this reason, they were used early as light sources for measuring variable fluorescence in plants. Initially, the trouble with this was that only red-emitting diodes were intense enough and their red light is not easy to efficiently separate from the chlorophyll fluorescence. Now sufficiently intense blue-emitting diodes are also available.

LEDs of several spectral emission types are presently manufactured: ultraviolet A, B, and C and blue, green, yellow, red, and infrared. It should be noted that they are not monochromatic light sources and especially the shortwave rated LEDs often have a broadband emission of longer wavelength than the nominal emission. For some types, the emission spectrum changes with operating current. LEDs are powered by a low-voltage source (e.g., a 1.5 V battery; some types need up to 5 V) in series with a resistor limiting the current to the rated value. Proper polarity should be observed.

Traditional LEDs contain inorganic semiconductors such as GaN, InGaN, SiC, and GaAsP (see Table 3.1). Very recently, several laboratories and companies have started to develop organic light-emitting diodes (OLEDs), which will probably widen the range of spectral types available. Roithner also markets a range of infrared-emitting diodes with emission peak wavelengths to greater than >4.5 μm. A LED emitting at 210 nm has been constructed (Taniyasu et al. 2006) but as of this writing not yet commercially available. An interesting new development is the construction of a LED that can generate a single photon at a time (Yuan et al. 2002).

3.2.7   Lasers

Laser is an acronym for light amplification by stimulated emission of radiation. Stimulated emission occurs when a photon causes a molecule in an excited state to emit a second photon. Stimulated emission as such requires no special equipment. It occurs regularly when photons of the proper energy encounter excited molecules. However, as a rule, excited molecules are very rare compared to molecules in the ground state (remember the equilibrium concentration formula, $N_y/N_x = \exp(E_x - E_y)/kT$). To get light amplification by stimulated emission, we must have more stimulated emission than light absorption, which means that we must have more molecules in the proper excited state than in the ground state. This can be achieved in different ways but never by "direct lift" from the ground state by absorption of light. Various lasers employ indirect "optical pumping" (sometimes by another laser), electrical energy, or chemical reactions. For a laser to work, photon losses must also be

Table 3.1 Examples of LEDs and where to obtain them

Peak wavelength (nm)	Semiconductors	Company and URL
237–365	AlGaN/GaN	Sensor Electronic Technology, Inc.
		www.s-et.com
370–390	GaN	Nichia America
460	GaN	www.nichia.co.jp/en
470, 505, 525	SiC/GaP	LEDtronics
574, 595, 611	InGaAlP	www.ledtronics.com
630	GaAsP/GaP	
660	GaAlAs/GAlAs	
660, 700, 720	GaAlAsP	Roithner Lasertechnik
780, 810, 905	GaAlAsP	http://www.roithner-laser.com/
375–1550	InGaN	Epitex
	AlGaAs/AlGaAs	http://www.epitex.com/
240–415	InGaAsP	Spectrecology
		http://www.spectrecology.com/
		(All accessed Aug. 17, 2013)

minimized by a suitable optical configuration, often involving mirrors.

Laser light has some unusual properties:

1. Laser light is coherent in the sense that the light constitutes very long wave trains, contrary to ordinary light, where each photon can be regarded as a limited wave packet independent of other photons.
2. Laser light can be made very collimated (all rays very parallel).
3. Laser light is usually very monochromatic (very narrow spectral bandwidth) or consists of a small number of such very narrow bands.
4. Laser light may be (but is not necessarily) plane polarized.
5. The light from some types of lasers is given off in extremely short pulses of extremely high power (energy per time unit). However, this does not hold for all lasers. Some lasers emit light continuously and have very feeble power.

Even lasers of low power, such as the helium–neon laser, should be handled with some caution. This is because the beam is so narrow, parallel, and monochromatic that if it hits your eye all its power will be focused onto a very small area of your retina and blind that particular spot.

One kind of laser that is in everyday use is the continuous helium–neon laser, emitting at 632.8 nm and a few infrared wavelengths. Dye lasers are advantageous in many cases because the wavelength can be selected over a wide range (within the fluorescence band of the dye used, and the dye can be changed if necessary). You may sometimes encounter a YAG laser. YAG is the acronym for yttrium aluminum garnet, containing trivalent neodymium ions in $Y_3Al_5O_{12}$. They are very powerful emitters of infrared radiation of 1,060 nm wavelength. For photobiological purposes, they are sometimes combined with frequency doublers made of potassium phosphate crystals, so that green light of 530 nm wavelength is obtained. The wavelength can be further changed either by letting the light undergo Raman scattering or by using it as a power source for a dye laser. Diode lasers are photodiodes emitting coherent light. They are now the most common lasers, used in CD players and other optical readout devices and laser pointers. They are available from 370 nm in the UV-A band to the long-wavelength red.

3.3 Selection of Light

In many cases, you do not want to use light as it comes from your light source. You may wish to remove some parts of the spectrum or select just a narrow spectral band or select light with a certain polarization, or you might wish to modulate the light in time, for instance, quickly change from darkness to light or obtain a series of light pulses. The first three sections below will deal with wavelength selection. A review of filters and mirrors of interest to biologists is provided by Stanley (2003).

3.3.1 Filters with Light-Absorbing Substances

The simplest devices for modifying the spectral composition of light are filters with light-absorbing substances that remove certain components from the spectrum. These color filters may be solid or liquid.

Cheap filters, which are quite useful for some purposes, consist of colored plastic (e.g., Plexiglas or cellulose acetate). Colored cellophane is not recommended as it is rather unstable, and cellulose acetate has to be used with great caution since it bleaches with time. It must also be realized that

all these substances transmit far-red light and infrared radiation freely. Thus, a piece of green Plexiglas does not transmit just green light. It is very instructive to put one, two, three, etc. sheets of green Plexiglas on an overhead projector and watch the effect (or look through the sheets toward an incandescent lamp). One or two sheets look clearly green, after which the color becomes indescribably dirty and finally shifts to deep red. This is because far-red light is transmitted even more freely than green light, and when practically all the green light (to which our eyes are most sensitive) is gone, we see the remaining far-red, which is otherwise hidden by the green. Remember that plants, contrary to our eyes, are more sensitive to far-red light than to green!

Undyed cellulose acetate can be used as a cutoff filter to remove UV-C radiation but retain UV-A and UV-B. The exact absorption depends on the thickness and must be checked regularly because the filter changes, especially in front of a UV radiation lamp. A more stable alternative is a special type of uncolored Plexiglas, number FBL.2458 (from Röhm GmbH, ordinarily used in front of the UV radiators in Solaria).

A great variety of gelatin filters for photographic use are manufactured by Ilford, Kodak, and other companies. They may also be very unstable in strong light and also freely transmit far-red light.

From an optical viewpoint, some solutions are much better filters than anything that can be made in solid form, but solutions are in many cases inconvenient to use. Thick layers may be required for the optical properties you want, and the liquid filters may therefore become bulky. Furthermore, some of the most useful colored substances are, unfortunately, carcinogenic or toxic in other ways.

One useful substance, which is not particularly dangerous, is water (see Figs. 3.2 and 3.3). It can be used for removing infrared radiation and thus avoid heating by light from incandescent and xenon lamps. Addition of copper sulfate increases the absorption of far-red. Copper sulfate should be used only with distilled water, and the solution should be acidified with sulfuric acid to avoid precipitation of cupric carbonate.

Solutions of potassium dichromate (carcinogenic!) are very good for removing light of wavelength shorter than about 500 nm, as one might want to do in, e.g., studies of fluorescence. For this purpose, it is far superior to glass filters or any filters containing organic compounds because of the total lack of fluorescence. We use it for filtering away the blue excitation light when we study the red chlorophyll fluorescence from plants.

Containers (cuvettes) for solutions of copper sulfate or potassium dichromate can be made by gluing together pieces of ordinary clear Plexiglas. Be sure that the glue has dried thoroughly and throughout before you pour your solution into the cuvettes, or you will lose more time than you are
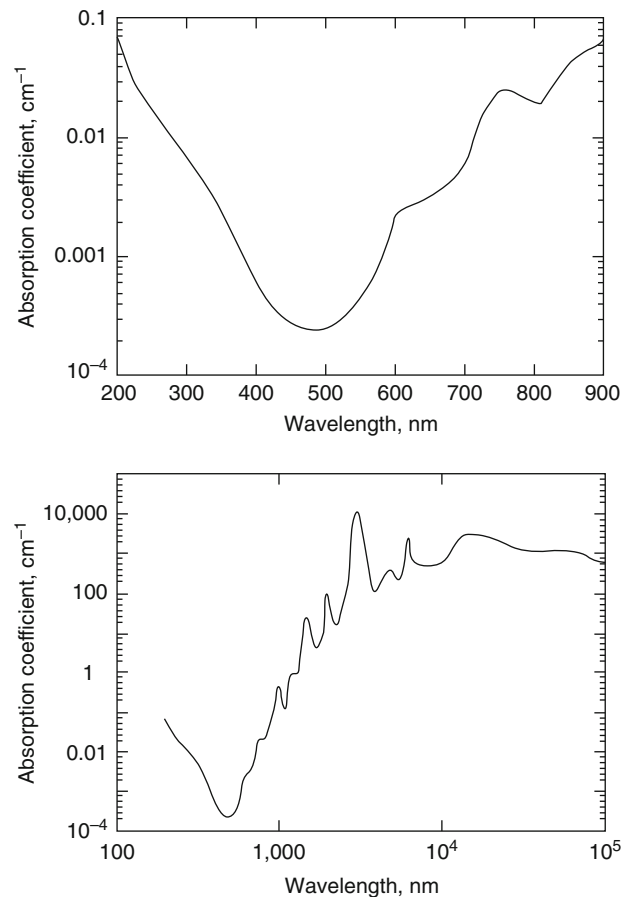

Fig. 3.2 The absorption spectrum of pure water plotted in two different ways (Data from Hale and Querry (1973), replotted)
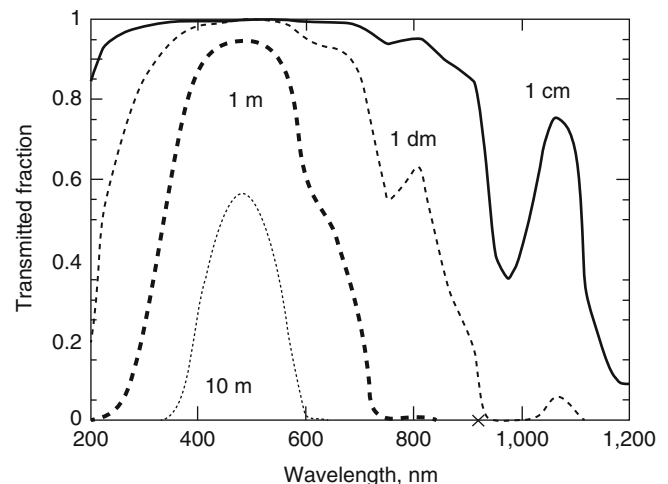

Fig. 3.3 The fraction of light of various wavelengths absorbed by (pure) water layers of the thicknesses indicated. Ten-meter filters are not very practical in the laboratory, but 10 and even 100 m of water are natural light filters for many organisms. The transmission in natural waters differs from that in chemically pure water and will be dealt with in Chap. 7 (Calculated from data of Hale and Querry (1973))
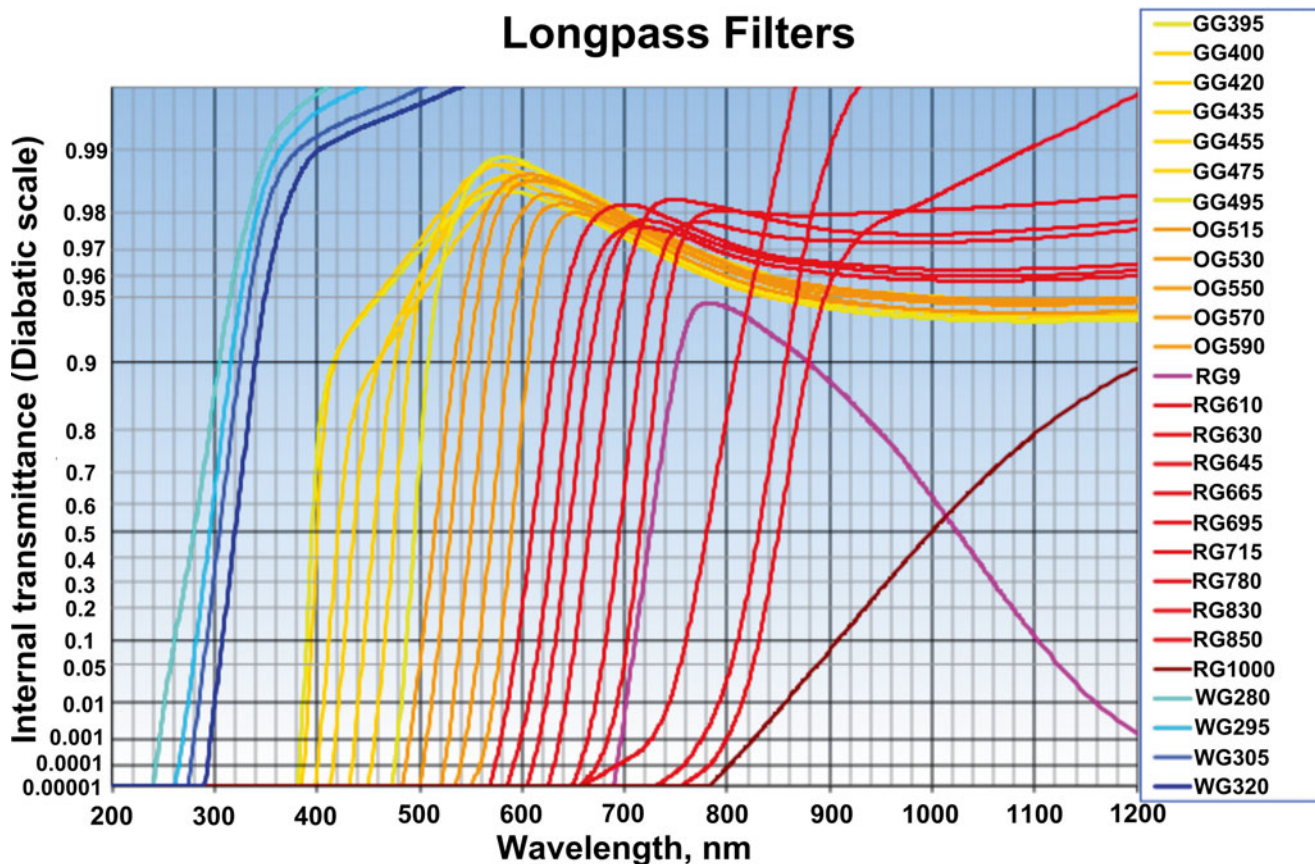
Fig. 3.4 Cutoff filters made of glass, manufactured by Schott & Gen., Mainz. Note that the vertical transmission scale is not linear. The filters absorb short-wavelength and transmit long-wavelength light. The diagram, drawn for 1-mm (WG 230 to GG 395)- or 3-mm (GG 400 to RG 1,000)-thick filters, does not take reflection (roughly 0.08 or 8 %) into account. The "diabatic" transformation of the vertical scale emphasizes the low- and high-transmittance portions. Mathematically, it means that scale is linear with the expression {1−log[log(1/transmittance)]}

trying to save. It is best to test for leaks using distilled water before you put your solutions in. Distilled water can be removed again from the leaks, but crystals cannot!

Cobalt chloride and nickel sulfate (nasty, carcinogenic, toxic, and in the case of nickel allergenic) dissolved in water or aqueous ethanol make very good broadband filters for the UV-B region, but because the substances are so dangerous, I do not recommend them unless you really know what you are doing and are sure that your cuvettes will not leak. Detailed descriptions of solution filters, especially for isolating lines of the mercury spectrum, can be found in Calvert and Pitts (1966), Rabek (1982), and Gahr et al. (1995).

Colored glass filters are made by several companies. My personal experience is mainly with filters manufactured by Schott & Gen. (Mainz, Germany) and Corning (USA). A large assortment of such filters is available (Fig. 3.4), so a catalog should be consulted before ordering. Of the filters from Schott, I have most often found BG12 and BG18 useful for isolating broadband blue light, and a series of cutoff filters, which cut off the short end of the spectrum (cutoff wavelengths from 250 to 780 nm) but transmit light of longer wavelength. For a particular kind of glass, the cutoff point

can be varied by having filters of different thickness or using several filters in tandem.

All the filters absorbing the unwanted light convert the absorbed energy to heat. If the light to be filtered is strong, the filters may become overheated and be destroyed. Organic substances may be decomposed, plastic may melt or burn, solutions may boil, and glass may crack. The risk for these unwanted effects is considerably less in the case of interference filters.

3.3.2 Interference Filters

An interference filter removes the unwanted radiation from a beam not by absorption, but by reflection. It does not contain any colored substances but instead a number of partially reflecting and partially transmitting interfaces. Some interference filters contain very thin metal films; others are made from alternating layers of transparent compounds of high and low refractive indices. The complete theory for interference filters is complicated. However, its essence is that when the spacing between the layers is a quarter of a wavelength, destructive interference will occur in the
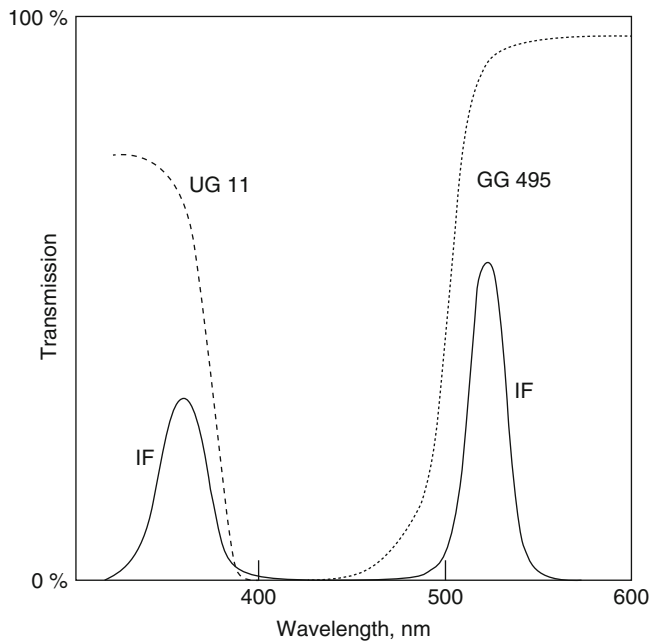
Fig. 3.5 The transmission spectrum for an interference filter (*IF*) with two transmission peaks. The ratio between the two peak wavelengths in this case is 3:2. Either one of the transmission bands can be selected by combining the interference filter with suitable glass filters: UG 11 for the short-wavelength band or GG 495 for the long-wavelength band

The half-band width of a spectral band is defined as the difference in wavelength (or frequency) between the two points in the spectrum where the band is half the maximum height. Photobiologists often use interference filters with half-band widths of about 15 nm. This gives a reasonable compromise between spectral purity and amount of light transmitted. For some purposes, filters with half-band widths up to 50 nm are useful. There are also interference filters with half-band widths as small as a fraction of a nanometer. They are used, e.g., by astronomers for photographing the sun using light emitted by a single kind of atom.

Continuous interference filters (also called spectral wedges) transmit light of different wavelengths at different points on the filter. They are usually oblong, with different wavelengths along their length. Also, circular spectral wedges have been manufactured.

There are interference filters other than the narrow band type. One useful type is Calflex (see http://www.linos.com/pages/no_cache/home/shop-optik/planoptik/filter/?sid = 12664&cHash = c8035311a6). One version of it transmits almost the full visible range and reflects ultraviolet and infrared (including far-red).

3.3.3 Monochromators

For high spectral purity of light, yet flexibility in the choice of wavelength, a monochromator is the device of choice. A very simple monochromator can be made from a continuous interference filter between two slits (Fig. 3.6). Light of different wavelength is obtained simply by sliding the interference filter. However, this arrangement is not suitable when a small half-band width (high-purity light) is required.

In earlier times, most monochromators contained a prism as the dispersing element (i.e., the component deflecting the light differently depending on wavelength). Gratings were too difficult to make and hence expensive. New methods, however, allow the mass production of high-quality gratings, and nowadays, practically all monochromators for the near infrared, visible, and ultraviolet regions use a reflection grating as the dispersing element.

The basic theory for a grating is best understood as an extension of Young's double-slit experiment. Using a computer, we can investigate the effect of increasing the number of slits more and more. An essential part of a program for this consists of the three equations relating wavelength (λ), deflection angle (θ), and relative fluence rate (I) to the width (b), number (n), and distance (a) of the slits:

reflected beam, so no light is reflected for this particular wavelength. Instead, light of this wavelength is transmitted. Light with twice or three times (or any integer times) the basal wavelength will also be transmitted, since there will be layer distances corresponding to a quarter of these wavelengths. The reader is referred to Sect. 9.10, which deals with a very similar topic.

Interference filters of the type just described will thus allow several narrow spectral bands to pass through, with wavelengths 4, 8, 12, … times the distance between interfaces. By combining interference and glass filters, one of the bands can be selected (Fig. 3.5). When using such combination filters, it is essential (at least if strong light is to be filtered) that the interference part of the filter faces the incident light. If the absorbing glass part is hit by the unfiltered light, the filter might become overheated.

For the filter to function properly, the light to be filtered must be nearly perpendicular to the filter, or the transmitted band will be broadened and shifted to longer wavelength. Thus, only collimated (parallel) light, not diffuse light or light from an extended light source (e.g., a fluorescent tube), can be efficiently filtered by an interference filter.

Even if interference filters do not heat up as easily as absorbing filters, care should be taken so that their temperature does not rise by conduction from other parts of your apparatus. They should also be protected from moisture. When not in use, they should be kept in a desiccator with dry silica gel.

$$\alpha = (a \cdot \pi / \lambda) \cdot \sin \theta$$
$$\beta = (b \cdot \pi / \lambda) \cdot \sin \theta$$
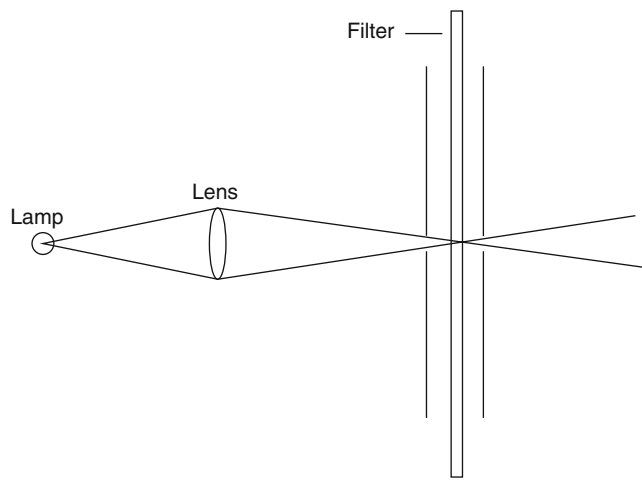$$I = 4\left[\sin \beta \cdot \sin(n \cdot \alpha) / (\alpha \cdot \beta) \right]$$

Fig. 3.6 Simple monochromator consisting of an interference filter between two slits. It is here combined with a simple illuminator consisting of a lamp and a lens, providing light which is almost perpendicular to the filter, which is essential for proper function

Fig. 3.7 (**a**) Computed diffraction patterns from n slits ($n=1$, 10, or 100). With increasing slit number, the peaks become sharper. The slit width is 0.3 mm, the slit distance 1 mm, and the wavelength 1,000 nm. (**b**) As in (**a**), but with slit number kept constant and the wavelength varied. With increasing wavelength, the deflection angle increases

The highest central peak (nondeflected light) in Fig. 3.7a is called the first-order light. With mixed input light, this includes all wavelengths. The two peaks on each side of it is the first-order light and so on.

Comparing the last three printouts (Fig. 3.7b), we see that the deflection angles increase with wavelength. We can see that the second-order light with wavelength 500 nm is deflected to the same angle as the first-order light with wavelength 1,000 nm. Likewise, fourth-order light of 500 nm wavelength, second-order light of 1,000 nm wavelength, and first-order light of 2,000 nm wavelength are all deflected to the same angle. As will be explained later, this has important consequences for photobiological experimentation.

Transmission gratings in the form of multiple slits are seldom used, except as here for teaching the theory of gratings. Instead, mirrors with grooves, called reflection gratings, are made in many variants, usually as replicas, i.e., copies molded in plastic from very expensive originals. The principle of operation is essentially the same, except that the light exits on the same side of the grating where it enters.

Modern reflection gratings have the added refinement of blaze. This means that the grooves are not symmetrical but shaped in such a way that the direction of specular reflection coincides with the diffraction direction for a certain wavelength—the blaze wavelength. That makes a grating particularly efficient for this wavelength. If you have a choice, you should select a grating with a blaze wavelength near the wavelength you are particularly interested in or for which it is difficult to get sufficiently strong light. For a lamp–monochromator combination like the one shown in Fig. 3.8, it is usually preferred to have a low blaze wavelength to compensate for the lower lamp output in the UV and blue regio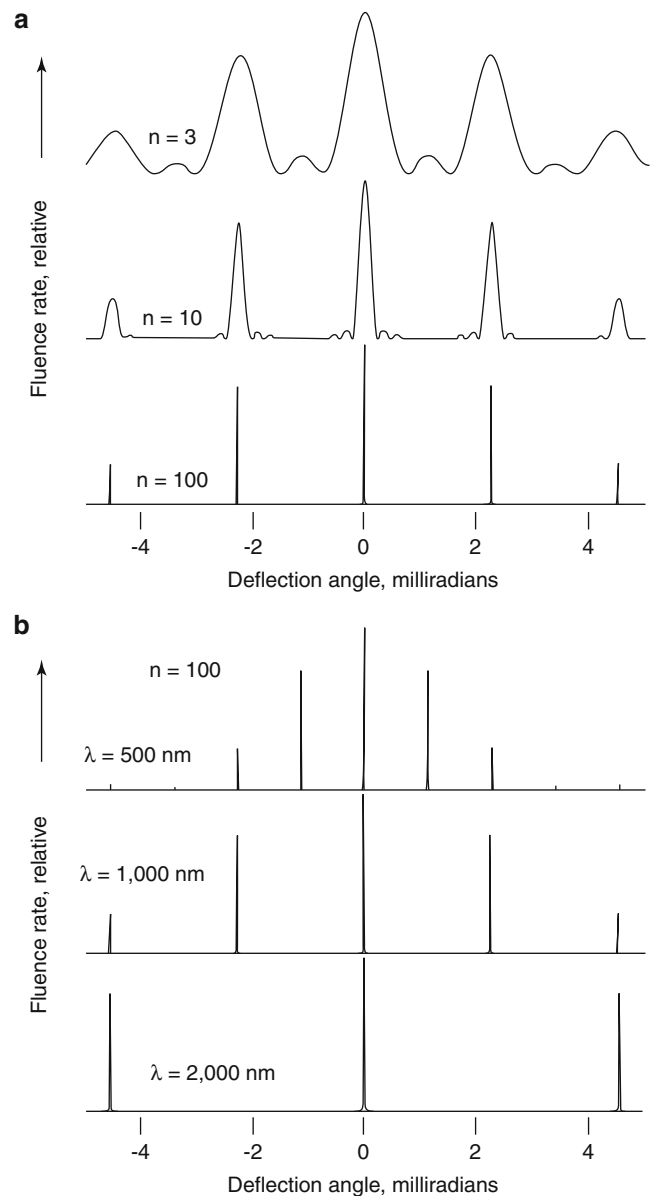ns. On the other hand, in equipment for the analysis of light (monochromator–photomultiplier combinations in spectroradiometers or the emission units of spectrofluorometers), it may be advantageous to use a high blaze wavelength to compensate for the lower sensitivity of the photocell for long-wavelength light.

A grating monochromator, in addition to the grating, consists of an entrance slit, an exit slit, and optics, which forms an image of the entrance slit at the exit slit via reflection in the grating. In Fig. 3.8, we see a schematic diagram of one type of monochromator suitable for photobiological use. In this case, a plane grating is combined with a concave
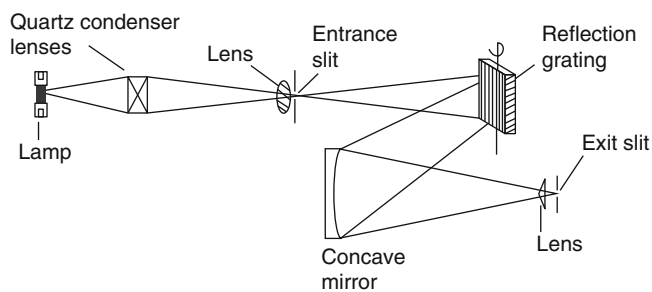
Fig. 3.8 Schematic diagram for a monochromator used for photobiology experiments

Fig. 3.9 Images of the entrance slit for different wavelengths in relation to the exit slit (*above*), and the ideal transmission function, neglecting diffraction in entrance and exit slits. If the slit is narrow, with a width approaching the wavelength, diffraction will take place (see Fig. 3.4)

mirror. Another solution is to use a concave grating, which focuses the light without any concave mirror. The wavelength of light leaving the exit slit is changed by rotating the grating. The monochromator is shown in combination with an illumination unit consisting of a lamp and a lens.

When using a grating monochromator, one should be aware of the fact that despite the name, with a certain setting, it transmits light of more than one wavelength:

First, we have the problem of light of various orders (first, second, etc.) mentioned above. The wavelength scale on the monochromator is valid for only a certain order, usually the first. When the dial is set at 300 nm, second-order light of 150 nm wavelength may also be transmitted.

Second, the spectral composition of the light within one spectral transmission band depends on the size of the slits. A compromise has to be made in choosing the slits: The wider they are, the more light is transmitted (Fig. 3.9), but the narrower they are, the higher the spectral purity (which is, in many cases, important). The best compromises are found when the image of the entrance slit (in monochromatic light) just covers the exit slit. In this case, the spectral transmission band shape would be triangular were it not for diffraction at the slits and imperfections in the construction. In reality, the spectral band transmitted will be of a somewhat rounded triangular shape (see Chap. 4).

An interesting new development is the construction of acousto-optic tunable interference filters (see Tran 1997 for a review). Such a filter is in a way intermediate between an interference filter and a grating. The basic principle is that a sound wave in a crystal creates regions of alternating low and high refractive index, which causes diffraction of a light beam. In this way, the unit functions as a grating. But unlike a grating, it need not be rotated for changing the wavelength of ligh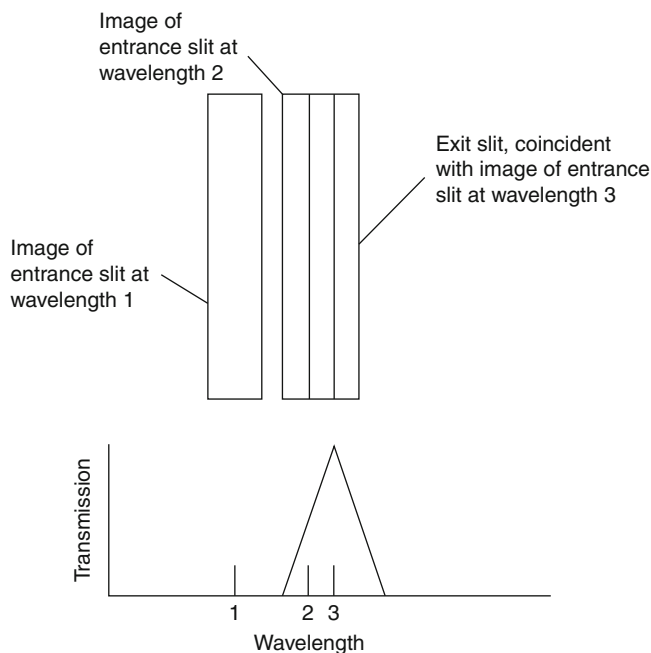t exiting in a certain direction. This can be done by changing the frequency of the sound wave, which is generated piezoelectrically. This makes possible very rapid scanning over a spectral range by changing the frequency of the driving voltage.

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