The Measurement of Light

4

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4.1 Introduction

There are three principal types of light-sensitive devices in common use, based upon three different effects of light on matter: photothermal, photoelectric, and photochemical devices. We shall describe these and their uses and then go on to describe a more complex device, the spectroradiometer.

4.2 Photothermal Devices

Photothermal devices have slow response and low sensitivity. Their great advantage is that, unlike photoelectric and photochemical devices, they have the same response per energy unit throughout a very wide spectral range. Their principle of operation is that the light to be measured is allowed to be absorbed by a target. The temperature of the target is raised by the absorbed energy, and the temperature rise is taken as a measure of the amount of energy absorbed.

4.2.1 The Bolometer

In a bolometer, the target is a temperature-dependent resistor. The resistivity of all materials is temperature dependent. In the first bolometers, thin platinum foils, blackened with colloidal platinum for efficient absorption of light, were used. The resistivity of platinum rises with temperature. The platinum foils were freely suspended in the air, and these bolometers were very sensitive to air currents.

In the bolometers commonly used in photobiology laboratories today, the targets are thermistors, that is, semiconductor resistors with a large negative temperature coefficient. They are protected by a window made from sapphire, lithium fluoride, or other materials with a wide spectral transmittance range. The light target is part of a Wheatstone bridge, so that small changes in resistance can be recorded.

The setup is schematically depicted in Fig. 4.1. Of the four resistor arms of the bridge, one is variable, so that the bridge can be balanced (same potential at the top as at the bottom and no current flowing through the meter) with

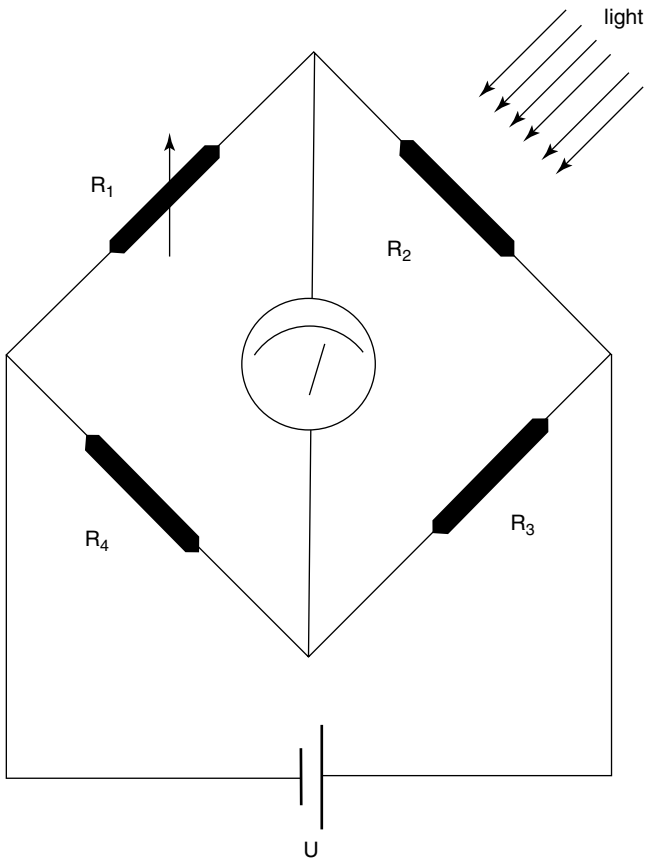

Fig. 4.1 Schematic diagram of a bolometer. It is connected to a voltage source with voltage U

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the target resistor shielded from light. In general, the potential difference between the top (R_1/R_2 junction) and bottom (R_3/R_4 junction) will be $U[R_2/(R_1+R_2)-R3/(R_3+R_4)]$, so that when the bridge is balanced $R_2/(R_1+R_2)=R_3/(R_3+R_4)$. We now remove the light shield and allow the light to be measured to fall on R_2. This resistor now heats up and changes its resistance by the amount ΔR_2. The concomitant change in potential between "up" and "down" will be $U\cdot\Delta R_2/(R_1+R_2)$, and change in current flowing through the meter will be proportional to the resistance change of the target.

Disregarding heating by the current flowing through it, the energy taken up by the target consists of the light to be measured plus heat radiation from the surroundings, assumed to be at absolute temperature T_a. The heat radiation received is proportional to T_a^4 (Stefan–Boltzmann's law). The radiation energy given off by the target (at absolute temperature T_t) is proportional to T_t^4. When equilibrium has been reached, we thus have the relationship for the irradiance of the light to be measured (k_1 and k_2 are constants):

$$\text{Irradiance} = k_1\cdot T_a^4 = k_1\cdot T_t^4$$

or

$$\text{Irradiance} = k_1\cdot\left(T_t^4-T_a^4\right)=k_1\cdot\left(T_t^2+T_a^2\right)\cdot\left(T_t+T_a\right)\cdot\left(T_t-T_a\right)\approx k_2\cdot\left(T_t-T_a\right)$$
$$=k_2\cdot\Delta T.$$

Thus, the irradiance is proportional to the temperature change of the target resistor and thus, as shown previously, proportional to the current flowing through the meter.

Irradiances down to about 1 W/m^2 can be measured with a standard bolometer. At lower irradiances, the drift problems become serious. When a low irradiance is to be measured, it is best to connect the bolometer to a strip-chart recorder or computer to keep track of the drift of the baseline. A suitable procedure is to expose the bolometer to the light for 30 s, then shield it for the same period for recording of the baseline, then expose it again, etc. Prolonged exposure decreases the reading, because the balancing resistors (not directly exposed to the light) heat up by heat conduction.

The calibration of a bolometer can be easily checked as described by Björn (1971). For highest accuracy, a special standard lamp should be used in the way specified in the directions supplied with it. For information regarding standard lamps, see the section on spectroradiometers.

4.2.2 The Thermopile

A thermocouple is a couple of junctions between two metals. Wherever two metals are in contact, a temperature-dependent potential difference exists. A thermopile consists of several thermocouples (each one with two junctions) connected in series, as shown in Fig. 4.2. Of each couple of junctions, one is shielded from and the other one exposed to the light to be measured. The sensitivity and speed of response are increased by attaching small light-absorbing (and heat-radiating) shields to the junctions, and these shields should be blackened for efficient absorption (and reradiation).

For optimal results, the input resistance of the current measuring meter should be matched to the resistance of the thermopile, which is of the order of 10–100 ohm. A thermopile is usable down to about the same irradiance as a bolometer. As for the bolometer, the output current is proportional to the irradiance.

4.2.3 Thermopneumatic Devices

The principle of pneumatic thermal radiation detectors is that the radiation heats a gas. The resulting expansion can be detected by the movement of an enclosing membrane. In one of the early devices, the Golay detector, the movement was detected optically. Sensors based on this principle, used principally for infrared radiation, are still being refined, and very small movements of the membrane can be detected interferometrically. Pneumatic detectors are often used in connection with chopped or pulsating light. In this case, the periodic expansion of the gas can be detected by a microphone. Many biologists have come in contact with this principle when measuring carbon dioxide, for instance, in the measurement of photosynthesis and respiration. An infrared gas analyzer (IRGA; Fig. 4.3) for such measurements often works in a differential mode.

External air passes through an optical cell ("reference cell"), then through the cell with the biological sample ("sample cell"), and finally through a second optical cell. A beam of infrared radiation passes alternatingly through one or the other of the cells. From there the radiation continues into an analyzing cell, partitioned by a flexible membrane. This cell contains the same kind of gas as the one being measured. Depending on how much radiation there remains after absorption in the reference cell and the sample cell, the gas in the two halves of the analyzing cell is heated more or less and temporarily expands in a corresponding way. Usually

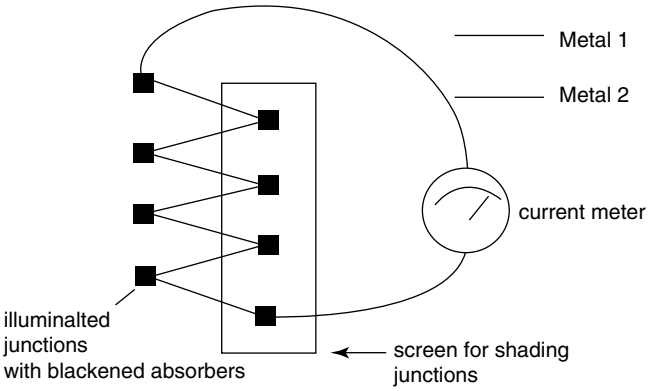

Fig. 4.2 Schematic diagram of thermopile with current meter

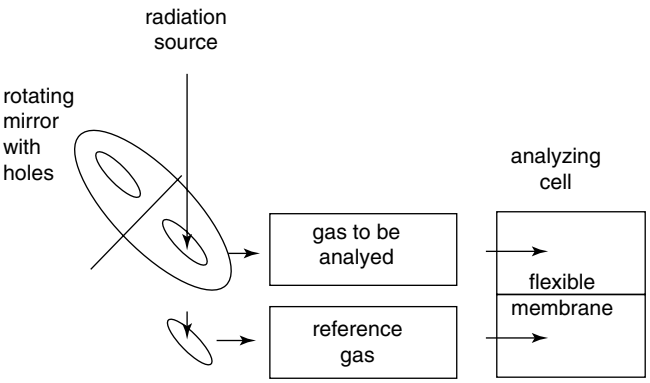

Fig. 4.3 Principle for an infrared gas analyzer (IRGA), very schematic. Infrared radiation passes alternatingly through a cell with reference gas, for instance, ambient air, and alternatingly through a cell with gas to be analyzed, for instance, air that has passed a compartment with plants taking up carbon dioxide. Depending on how much radiation remains to be absorbed in the two halves of the analyzing cell, the flexible membrane separating the two halves is deflected to a greater or lesser extent

the heating and expansion is different in the two halves, which causes the membrane to vibrate in relation to how the radiation is deflected through the reference cell and the sample cell. By making the membrane form one plate in an electrical capacitor, this vibration can give rise to an electrical signal proportional to the difference in gas concentration in the sample cell and the reference cell.

Evans (2005) has suggested that some beetles use the thermopneumatic principle for detecting infrared radiation.

4.3 Photoelectric Devices

A great number of photoelectric devices exist. They can be divided into two main categories, depending on whether they exploit an outer photoeffect (at a metal surface in vacuum or gas) or an inner photoeffect inside a solid semiconductor.

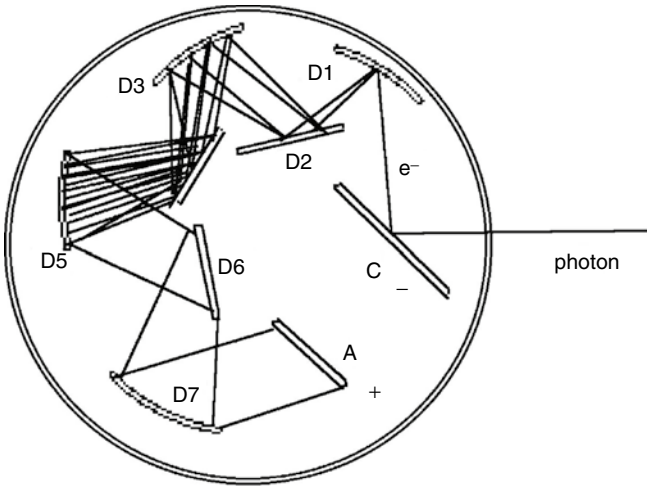

Fig. 4.4 Diagram of (side-on) photomultiplier

4.3.1 A Device Based on the Outer Photoelectric Effect: The Photomultiplier

Although many kinds of photocells (as well as television camera tubes, image intensifiers, etc.) utilize the outer photoeffect, we shall limit ourselves here to a description of the photomultiplier, a device extensively used by photobiologists. Figure 4.4 shows the basic principle. Inside an evacuated envelope of glass or quartz, there are a number of metal plates. The one marked C is the photocathode. It is held at a large negative potential relative to ground (wires connected to electrical circuitry not shown). The surface of the photocathode exposed to the light to be measured is covered with a layer of special metals. Usually a mixture of several metals, some of which are alkali metals, is used. Depending on the particular metal alloy, photomultipliers have different spectral responses.

When a photon hits the photocathode, an electron is released from the metal (as is known from chemistry, alkali metals are especially prone to losing electrons; they have a low *work function*). Because the photocathode is at a low electric potential, the electron does not return to the surface. Instead, it is accelerated toward another metal plate nearby, which is held at a higher potential, dynode 1 (D1). In flight the electron acquires such a velocity that when it hits the dynode, it releases two or three electrons from it. They travel on to dynode 2 (at an even higher potential) where further electrons are released. Photomultipliers are constructed with up to 12 dynodes in series, and at each dynode, more electrons are added. Finally, the electron swarm is collected at the final plate, the anode (A in Fig. 4.4). This is usually maintained close to ground potential. The electrons flowing to the anode represent an electrical current, which also flows

through the wire connected to the anode (not shown), and this can be recorded and used as a measure of the light incident on the photocathode.

Contrary to the thermoelectric devices described in the previous section, photomultipliers have different sensitivities to different kinds of light. Furthermore, they are rather unstable. Their great advantage lies in the high light sensitivity: even individual photons can be recorded by some photomultipliers under suitable conditions. Provided the electronic circuitry to which they are connected has a low time constant, photomultipliers also have a short response time (although different photomultipliers differ in this respect).

The diagram shows a so-called side-on photomultiplier. There are also other designs. A common one is the end-on photomultiplier, where the photocathode consists of a thin, semitransparent metal film on the inside of the flat end of the cylindrical envelope. In this type, the spectral response is also dependent on the thickness of the film, which usually varies somewhat over the surface. Photomultipliers require an operating voltage of 500–5,000 V; in many cases about 1,000 V is used. The different electrodes are given their proper voltages by a chain of resistors between the negative high voltage lead and ground. The output current is very strongly dependent on the operating voltage, which must therefore be held very constant (with N dynodes and operating voltage U, the output current is roughly proportional to U^N).

The output is not always measured as a current. As the incident irradiance is lowered, the discontinuous nature (quantization) of light becomes more and more apparent. At very low light levels, it becomes advantageous to record individual photons by counting the pulses of current flowing to the anode. The measurement of very weak light is further dealt with in Sect. 4.7.

Photomultipliers are made with different cathode layers for different spectral ranges from the ultraviolet to the near infrared (to about 900 nm). Photomultipliers sensitive to light of long wavelength generally have a higher dark current (dark noise) than others. The noise level can be decreased (to achieve a better signal-to-noise [S/N] ratio) by lowering the temperature. Liquid nitrogen, dry ice, or Peltier coolers are generally used for this. Cooling must be combined with precautions to avoid dew on the optical components.

4.3.2 Devices Based on Semiconductors (Inner Photoelectric Effect)

In the inner photoelectric effect, absorption of a photon inside a solid semiconductor results in the separation of a positive charge from a negative one.

Photoconductive cells with a semiconductor as the light-sensitive element have been used extensively in the past. Among these lead sulfide cells for measurements in the near infrared, for which no photomultipliers or other suitable photocells were available, and cadmium sulfide cells for photographic exposure meters can be mentioned. Photoconductive cells are variable resistors and require an external source of voltage for creation of a current that can be measured. They have a slow response and are not used much for scientific measurements.

Photodiodes and phototransistors, on the other hand, can be made to have a very rapid response. For very long wavelengths (>1,000 nm), they also compete with photomultipliers in terms of sensitivity. The so-called avalanche photodiode can be regarded as a semiconductor equivalent to the photomultiplier. Photodiodes are often preferred in applications where photomultipliers could also be used, because photodiodes are small, cheap, and rugged and do not require high voltages. In combination with special electronics, it is nowadays possible to detect single photons of 1,550 nm wavelength using an avalanche photodiode (Namekata et al. 2006), and this is possible also with special, cooled photomultipliers (Skovsen et al. 2006). Photodiode arrays have become popular for recording a whole spectrum at one time (each diode in the array measures one spectral band).

Two main types of barrier layer cells are in current use: the selenium cell and the silicon cell. They are similar in principle but differ in their spectral response: the selenium cell is most sensitive to green and blue light, and the silicon cell, to red and far-red light. The general principle is shown in Fig. 4.5.

Because selenium barrier layer cells have a spectral sensitivity somewhat resembling that of the human eye, they are used in photometers (lux meters) for measurement of visible light at, for example, working places. By combination with suitable filters, the spectral sensitivity curve can be made to almost completely match the curve for scotopic vision (which defines illuminance). In earlier days, such cells were also used for photographic exposure meters, where they have now been replaced by cadmium sulfide photoconductive cells and, more recently, by photodiodes.

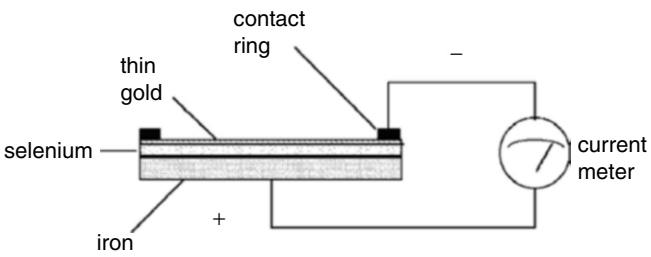

Fig. 4.5 Selenium barrier layer cell with required circuitry. Note that the device generates its own operating voltage and does not require any battery or other external voltage source

4.4 Photochemical Devices: Actinometers and Dosimeters

Chemical systems for measurement of light and ultraviolet radiation are called actinometers. (The best known photochemical device for recording light is photographic film. This has also been used for quantitative measurements, but it will not be further discussed here. Other chemical systems are usually better suited for quantitative measurements of radiation.)

Actinometers have the advantage of not having a need for calibration by the user and thus do not require the purchase of an expensive standard lamp with an expensive power supply. Standardization has usually been taken care of by those who have designed the actinometer. Another advantage is that the geometry can more easily be adjusted to the measurement problem. The shape of a liquid actinometer can easily be made to correspond to the overall shape of the irradiated object under study. In many cases, it is of interest to study a suspension or solution that can be put in an ordinary cuvette for spectrophotometry or fluorimetry, and the actinometer solution can be put into a similar cuvette.

A large number of actinometers have been devised. Kuhn et al. (1989) list and briefly describe and give references to 67 different systems involving gaseous, liquid, and solid phases. Of these they recommend five. In general, actinometers are sensitive to short-wave radiation and insensitive to long-wave radiation. Insensitivity to long-wave radiation can be both a drawback and an advantage, but by choosing the best actinometer for the purpose, one can avoid the disadvantages. One advantage of using an actinometer insensitive to long-wave radiation is that one can work under illumination visible to the human eye, without disturbing the measurement. We shall give an introduction below to a few different actinometers, not all of which are mentioned by Kuhn et al. (1989), and then go on to describe more in detail the most popular one for ultraviolet radiation—the potassium ferrioxalate or potassium iron(III) oxalate actinometer:

1. The potassium iodide actinometer (Rahn 1997) is sensitive primarily to UV-C radiation (wavelength <280 nm) and with slight sensitivity also for short-wave UV-A. It is suitable for determining the 253.7 nm radiation from low-pressure mercury lamps (bactericidal lamps), since the contribution from other spectral lines of the lamp will be negligible (but the ferrioxalate actinometer works almost as well for this purpose). It can be handled in ordinary incandescent light (not light from unshielded quartz–iodine lamps). The reaction on which this actinometer is based is the oxidation of iodide ion by iodate ion to form iodine, or rather triiodide ion (I_3^-).
2. An actinometer sensitive to visible light (photosynthetically active radiation) has been described by Wegner and Adamson (1966). It works up to above 700 nm and is based on potassium tetrathiocyanatodiamminechromate (III), $K[Cr(NH_3)^2(SCN)_4]$. The latter can rather easily be prepared from the commercially available Reinecke's salt, that is, $NH_4[Cr(NH_3)_2(SCN)_4]$. Irradiation causes the uptake of water and release of thiocyanate, which can be measured spectrophotometrically after addition of an Fe(III) salt.
3. Two more actinometers that have recently been used in biological contexts are the 2-nitrobenzaldehyde actinometer (Allen et al. 2000) and the oxalic acid/uranyl sulfate actinometer (Mirón et al. 2000). A different version of the latter one preferable for small radiation doses is among those recommended by Kuhn et al. (1989).
4. As already mentioned, the most popular actinometer for ultraviolet radiation (and for violet and blue radiation as well) is the ferrioxalate actinometer. The description below will be sufficient for the experimenter starting in the field. For more detailed information, one should consult Parker (1953), Hatchard and Parker (1956), Lee and Seliger (1964), and Goldstein and Rabani (2007). Complete recipes have also been published by, for example, Seliger and McElroy (1965) and Jagger (1967).

In the ferrioxalate actinometer, the following photochemical reaction is exploited:

$$\tfrac{1}{2}\left(COO^-\right)_2 + Fe^{3+} + \text{photon} \rightarrow CO_2 + Fe^{2+}$$

or

$$\text{Oxalate ion} + \text{Fe(III) ion} + \text{light} \rightarrow \text{carbon dioxide} + \text{Fe(II) ion}.$$

The quantum yield for this reaction (i.e., the number of iron ions reduced per photon absorbed) is slightly wavelength dependent but close to 1 in the spectral region, 250–500 nm, where the ferrioxalate actinometer is used. Usually a 1-cm layer of 0.006 M ferrioxalate solution is used. Quantum yield and the fraction of the radiation (perpendicular to the 1 cm layer) absorbed are shown in Table 4.1.

The quantum yields for 0.15 M actinometer solution are 0.952 of the above values. Irradiation of the side walls of the

Table 4.1 Quantum yield and the fraction of radiation (perpendicular to 1-cm layer) absorbed

Wavelength	Quantum yield	Fraction absorbed	Quantum yield × fraction absorbed
253.7	1.26	1	1.260
300.0	1.26	1	1.260
313.3	1.26	1	1.260
334.1	1.26	1	1.260
365.6	1.26	1	1.260
404.7	1.16	0.92	1.067
435.0	1.11	0.49	0.544
509.0	0.85	0.02	0.017

cuvette should be avoided, that is, the beam should be smaller than the cross section of the cuvette.

The amount of Fe(II) formed can be measured spectrophotometrically after addition of phenanthroline, which gives a strongly absorbing yellow complex with Fe(II) ions.

The ferrioxalate for the actinometer is prepared by mixing 3 volumes of 1.5 M $(COOK)_2$ with 1 volume of 1.5 M $FeCl_3$ and stirring vigorously. This step and all the following involving ferrioxalate should be carried out under red light (red fluorescent tubes). The precipitated $K_3Fe(C_2O_4)_3 \cdot 3H_2O$ should be dissolved in a minimal amount of hot water, and the solution allowed to cool for crystallization (this crystallization should be repeated twice more).

Following is a recipe for the three solutions required for carrying out actinometry (see Goldstein and Rabani 2007 for a different procedure and other quantum yields):

Solution A: Dissolve 2.947 g of the purified and dried Fe(III) oxalate in 800 ml distilled water, add 100 ml 0.5 M sulfuric acid, and dilute the solution to 1 l. This gives 0.006 M actinometer solution, which is suitable for measurement of ultraviolet radiation. For visible light, which is only partially absorbed, it may be advantageous to use 0.15 M Fe(III) oxalate instead, that is, 73.68 g per liter solution.

Solution B: The phenanthroline solution to be used for developing the color with Fe(II) ions should be 0.1 % w/v 1:10 phenanthroline monohydrate in distilled water.

Solution C: Prepare an acetate buffer by mixing 600 ml of 0.5 M sodium acetate with 360 ml of 0.5 *M* H_2SO_4.

Solution A is irradiated with the light to be measured. The geometries of the container and of the light are important and must be taken into account when evaluating the result. The simplest case is when the light is collimated, the container a flat spectrophotometer cell, the light strikes one face of the cell perpendicularly, and no light is transmitted. Even in this case, one has to distinguish whether the cell or the beam has the greater cross section and correct for reflection in the cell surfaces. The irradiation time should be adjusted so that no more than 20 % of the iron is reduced.

After the irradiation, two volumes of the irradiated solution are mixed with two volumes of solution B and one volume of solution C and then diluted to 10 volumes with distilled water. After 30 min, the absorbance at 510 nm is measured against a blank made up in the same way with unirradiated solution A.

Example of calculation: 4 ml of 0.006 M actinometer solution are irradiated in a flat quartz container by parallel rays of UV-B impinging at right angles to one surface (and not able to enter any other surface). The radiation cross section intercepted by the solution is 2 cm^2. Five minutes of irradiation produces 0.6 µmol Fe(II).

Throughout the UV-B region, the quantum yield is 1.26. Reflection from the surface is estimated to be 7 % (by application of Fresnel's law). None of the radiation penetrates the solution to the rear surface, since the solution thickness is well over 1 cm. Therefore, 0.6 mmol corresponds to $0.6/(1.26 \cdot 0.93)$ µmol = 0.512 µmol radiation incident on 2 cm^2 in 5 min, and the photon irradiance (quantum flux density, in this case equal to the photon fluence rate, since the rays are parallel and at right angles to the surface) is $0.512/(2 \cdot 5)$ µmol/cm^2/min^1 = 5.10 nmol/cm^2/min^1 or $5.12 \cdot 10^4/60$ nmol/m^2/s^1 = 853 nm/m^2/s^1.

The great limitation of the ferrioxalate actinometer is that it is not sensitive to long wavelength light (in many cases this is also an advantage; one reason being that red working light can be used without interference with the measurements). Several actinometers sensitive to longer wavelengths have been designed. Warburg, for instance, used one based on chlorophyll. A modern, red-sensitive actinometer has been described by Adick et al. (1989).

Chemical or biological systems, mostly in the solid state, for recording light, and ultraviolet radiation in particular, are widely employed for estimating exposure of people, leaves in a plant canopy, and other objects which for various reasons are not easily amenable to measurements with electronic devices. These chemical devices are generally referred to as *dosimeters* rather than actinometers, even if there is no defined delimitation between these categories. Construction, calibration, and use of chemical and other dosimeters have been the subject of frequent reviewing (Bérces et al. 1999; Horneck et al. 1996; Marijnissen and Star 1987). Their radiation-sensitive components can be either chemical substances (natural like DNA or provitamin D or artificial) or living cells (e.g., various spores and bacteria). A critical evaluation of two kinds of dosimeters was recently performed by Seckmeyer et al. (2012).

4.5 Fluorescent Wavelength Converters ("Quantum Counters")

As stated earlier, photomultipliers have the advantage of being very sensitive as well as the disadvantage of having wavelength-dependent sensitivity. Fluorescent wavelength converters or "quantum counters" are solids or solutions, usually used in conjunction with photomultipliers, to obtain devices which are sensitive yet have a sensitivity per photon that is independent of wavelength over a certain interval. The idea is to use a solution that has an absorbance high enough that all photons (except those reflected) will be absorbed and that has a high fluorescence yield. Incident light of any wavelength distribution within certain limits is then converted, photon for photon, to light of a fixed wavelength distribution (the fluorescence spectrum of the "quantum counter"), to which the photomultiplier has a fixed sensitivity. One of the major uses of "quantum counters" is calibration of excitation units of spectrofluorometers. The "quantum counter" most widely used consists of a concentrated solution of rhodamine B in ethylene glycol. It is useful for wavelengths up to 600 nm.

4.6 Spectroradiometry

4.6.1 General

A spectroradiometer is an apparatus with which you can measure the spectrum of light, that is, either the spectral irradiance, the spectral fluence rate, or the spectral radiance as a function of wavelength (or frequency, which is equivalent but less commonly used by biologists). It consists of three main parts: (1) input optics, different for spectral irradiance, spectral fluence rate, or spectral radiance; (2) a monochromator or, preferably, a double monochromator; and (3) a transducer for converting the light signal to an electrical signal. The latter may be, in some cases, a photodiode but is usually a photomultiplier. In some spectroradiometers, instead of a monochromator, there is a spectrograph that projects a whole spectrum, and the transducer is a diode array, charge-coupled device (CCD, see Sect. 4.8), complementary metal–oxide–semiconductor (CMOS), or a multichannel plate, which samples the whole spectrum at once. The latter arrangement has the advantage of speed and synchronous sampling of all spectral channels, but is not always suitable. In particular, it is very unsuitable for measuring ultraviolet radiation in daylight, in which case stray light problems must be minimized by use of a double monochromator.

A complete spectroradiometer system also requires some facility for frequent recalibration, as especially photomultipliers have very bad long-term stability.

4.6.2 Input Optics

Before deciding on input optics, we need to decide what quantity we wish to measure. For spectral radiance, we need input optics with which we can sample a very narrow solid angle. This can be some kind of telescope, but for most purposes, it is sufficient to have a tube with a terminal stop that determines the sampling angle and a few internal baffles and an inner matte black surface to avoid reflections inside the tube to reach the monochromator entrance slit Fig. 4.6.

For spectral fluence rate, we need a device that samples all directions with equal sensitivity. This is an ideal that cannot really be fulfilled, but it can be approached. Using input optics for irradiance, it is possible to combine several measurements to obtain the fluence rate (Björn 1995; Björn and Vogelmann 1996).

For spectral irradiance measurements, we need input optics, which has a "cosine response," meaning that the sensitivity, or the efficiency of sampling, for a certain direction should be proportional to the cosine of the angle between that direction and the optical axis. The concept of "cosine response" is graphically explained in Fig. 4.7.

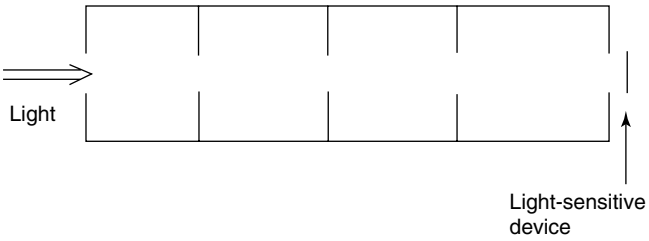

Fig. 4.6 Input optics for radiance (narrow-angle) measurements, consisting of a tube with stops and internal baffles, and inside painted dull black to prevent internal reflections

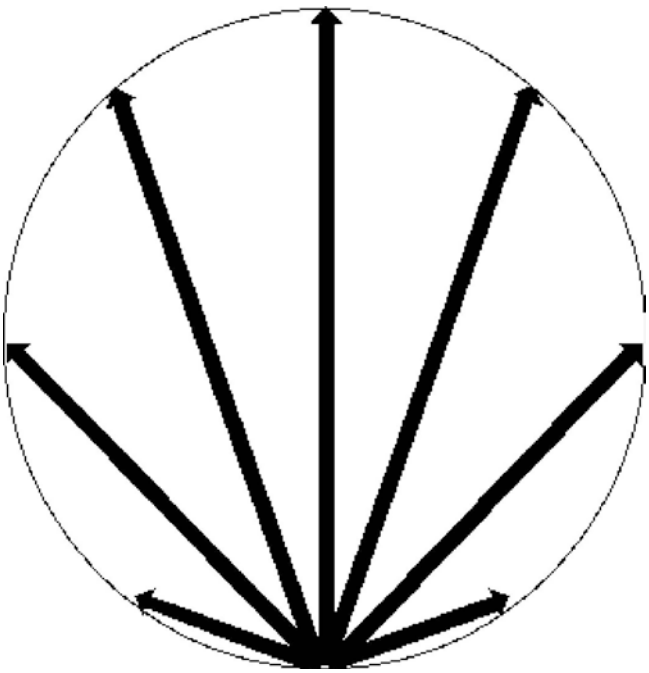

Fig. 4.7 Cosine sensitivity of a receiver. The sensitivity is greatest straight up in this case and decreases proportionally to the cosine of the angle to the vertical. In the horizontal direction, the sensitivity is zero

As a first crude device to reach this situation, one could let the light to be measured strike a strongly scattering but translucent plate above the entrance slit of the monochromator. Suitable materials for this include ground quartz or fused silica or Teflon, depending on the spectral range. A flat plate is, however, not very satisfactory, especially for large deviations from the optical axis. For measuring irradiance of light from an extended light source, for instance, an overcast sky, light at these large angles is very important, since the "amount of sky" corresponding to a certain angular deviation from the optical axis is proportional to the sine of the angle. Somewhat better results are obtained using a hemispherical scattering dome over the slit. The only device that works well is an integrating sphere (Fig. 4.8), and to work well it must be well designed. Details on this subject are provided by Optronic Laboratories (1995, 2001); Schneider and Young 1998).

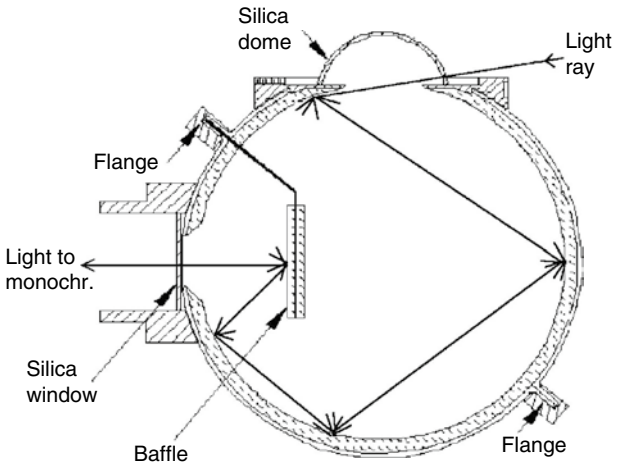

Fig. 4.8 Integrating sphere used as input optics for spectroradiometric irradiance measurements. On top is a silica dome, which can be omitted if the instrument is to be used only indoors or in good weather conditions. *Below* that is the opening in the sphere that defines the area over which the irradiance measurement is taken and the direction of the reference surface. A light ray has been drawn that strikes this at a low angle. It is important that the walls of the sphere taper off to an edge to allow rays at such low angles to enter the sphere. The ray strikes the inner diffusely reflecting wall. From the point where it strikes, light is scattered in all directions. We have followed one possible path through the sphere, but the little "brushes" at each scattering point are meant to indicate that there are many possibilities. Eventually the light strikes the backside of the baffle, which serves as the direct light source for the monochromator (see Fig. 4.9)

In some cases, we are more interested in the shape of a spectrum than in the absolute light level, and then the angular sensitivity function is less important. We may also be interested in measuring light in places where it is not easy to put the spectroradiometer itself (such as underwater or inside the mouth). In that case, the best choice may be to use fiber optics at the input end of the spectroradiometer. Even then one may add, for instance, a small scattering device at the tip of the fiber optic conductor to collect light from different directions. Single light-conducting fibers may even be used to measure light inside plant or animal tissues (Vogelmann and Björn 1984).

4.6.3 Example of a Spectroradiometer

We show here (Fig. 4.9), as an example of a spectroradiometer often used by biologists, the construction of Model 754 from Optronic Laboratories.

Spectroradiometers for measurement of UV-B radiation in daylight do not work well with a single monochromator, mainly because spectral irradiance in the UV-B region changes very rapidly with wavelength. Very small amounts of radiation outside the intended band can therefore ruin the measurement. When two monochromators are used in tandem, it is of course very important that their wavelength settings agree throughout the scan. The only way to achieve this

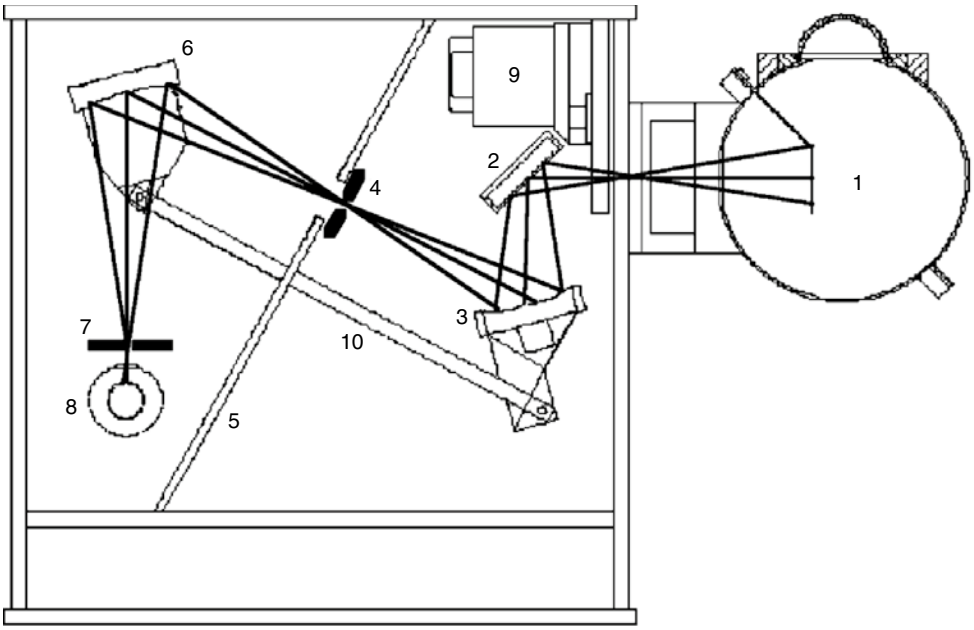

Fig. 4.9 Diagram of spectroradiometer (Optronic Laboratories model 754), simplified to enhance the important optical parts. Light from the integrating sphere (*1*, see Fig. 4.8) enters the light-tight box through a slit (entrance slit for the first monochromator, not specifically shown) and is deflected by the mirror (*2*) to the first grating (*3*). The chosen wavelength band leaves this first monochromator unit through the exit slit (*4*) in the wall (*5*) separating the two monochromators. This slit (4) is also the entrance slit for the second monochromator. The second grating (*6*) again disperses the light and deflects the chosen wavelength band onto a slit (*7*) in front of the photomultiplier (*8*). A stepper motor (*9*) turns the grating to change the wavelength. The rotating grating supports are connected by a bar (*10*), thus assuring that they follow one another with high fidelity

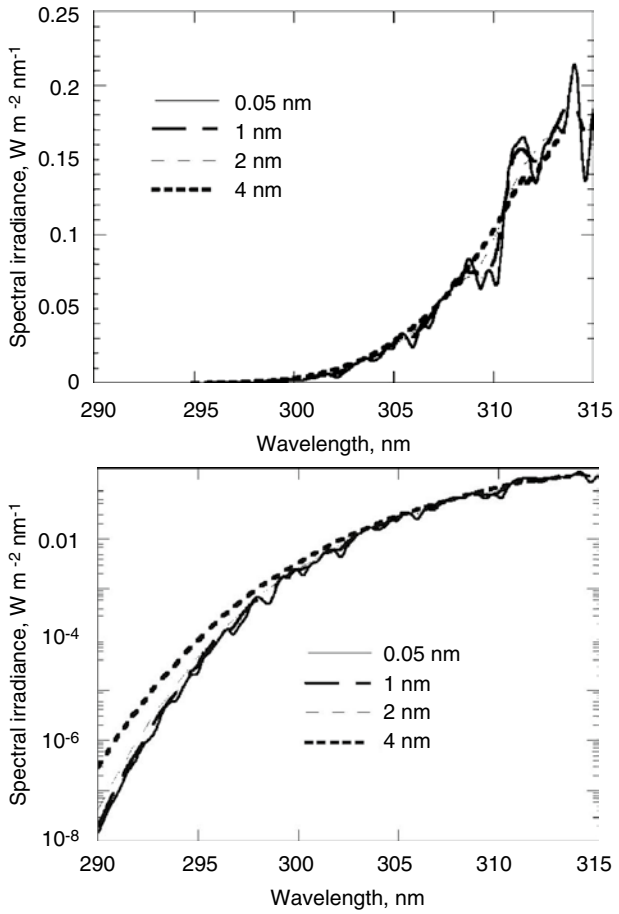

Fig. 4.10 A portion of the ultraviolet daylight spectrum in Lund at noon on June 15, plotted with three different half-bandwidths (modeled values). Left frame with linear, right frame logarithmic spectral irradiance scale. In the *linear plot*, we can see how the fine structure is gradually smoothed out, while the semilogarithmic plot shows more clearly how systematic positive errors develop in the short-wavelength part. With 4 nm bandwidth, the error is more than an order of magnitude for part of the spectrum

is to have both built on a single baseplate and be driven by a single wavelength drive mechanism.

Because biological chromophores have rather broad spectra, the fine structure of the daylight spectrum or the spectrum of any other light is not of great importance. Still, it is important that the bandwidth of the measuring system is not too great, that is, that the slits in the monochromator are not wide when daylight UV-B radiation is to be measured. This is because the spectrum is so steep in this region. Figure 4.10 illustrates this: when the bandwidth is increased above 1 nm, values in the short-wavelength part of the UV-B band start being too high. Errors are also introduced in the calibration (see below) if the bandwidth is too great.

Information about other types of spectroradiometers of interest for biologists is available from the following websites (accessed on August 17th, 2013):

http://www.oceanoptics.com/products/usb2000uvvis.asp
http://www.eoc.csiro.au/instrument/html/terrestrial/asd_fieldspec.htm
http://www.spectralevolution.com/portable_spectroradiometer.html

A relatively new development in spectroradiometer construction is to use a diode array to capture the whole spectrum simultaneously, instead of a photomultiplier in combination with a mechanical scanning system. These instruments have the advantage of speed and lack of any mechanical problems and also consume less energy (which is of importance for field equipment). However, the problems with stray light are larger than for the more conventional instruments. Thus they are generally not useful for daylight measurements below 300 nm, and below 350 nm a stray light correction must often be applied to reach an acceptable accuracy.

4.6.4 Calibration of Spectroradiometers

4.6.4.1 Wavelength Calibration with Lamps

This is the simple part but important. If the wavelength is not correct, then everything else will be wrong, too. The wavelength error should be less than 1 nm; for measurements of daylight UV-B radiation, it should preferably be much less.

For such calibration, any medium pressure mercury lamp works well, even an ordinary fluorescent lamp. Easily recognizable spectral lines occur at 253.7, 265.2, 312.6 + 313.2, 334.1, 365.0, 365.4, 366.3, 404.7, 435.8, 491.6, 496.0, 546.1, 577.0, 579.1, 623.4, and 690.7 nm. The short-wavelength bands do not penetrate the envelopes of most fluorescent lamps.

4.6.4.2 Irradiance Calibration with Standard Lamps

Usually, a spectroradiometer is calibrated using a lamp with a known output at different wavelengths. The most commonly used lamp is a 1 kW tubular quartz–iodine incandescent lamp. The reason to use such a powerful lamp is to obtain sufficient output in the ultraviolet region, and a lamp of this power can be used down to 250 nm. (For shorter wavelengths usually deuterium standard lamps are used.)

Using transfer standards, these standard lamps are ultimately calibrated against cavity radiators held at a well-determined temperature and designed so they follow as closely as possible the theoretical Planck blackbody radiation formula (see Chap. 1). When you purchase such a lamp, you will obtain a table of the spectral irradiance obtainable at certain wavelengths at certain distance using a specific geometry and also information about the accuracy. You will be surprised at (1) the wide uncertainty limits compared to most other kinds of physical measurements (typically 3.5 %

at 250 nm) and (2) the high price of the lamp. After all, it looks almost like the lamp you have in the overhead projector in your lecture hall. What you should consider at this moment of surprise is that the lamp has been preburned and selected to be particularly stable and the effort and cost involved in calibrating it as accurately as possible. You should buy a second similar but uncalibrated lamp at a much lower cost, calibrate this as a working standard against your expensive lamp, and only occasionally use your expensive lamp to check your working standard.

The disadvantage of using a 1 kW lamp (apart from the heat it produces in your usually small calibration room) is that it requires a direct current of 8 A to run at 125 V, and quite a big power supply is needed to produce this with good accuracy. It is very important that you really run it at the specified current at which it was calibrated. An error of 0.1 % in the current produces a 1.2 % error in the spectral irradiance at 250 nm and a 0.6 % error at 400 nm. It is important that the current is as ripple-free (has as little ac component) as possible. The best way of measuring the current is to measure the voltage across a precision resistor of, say, 0.1 ohm in the lamp circuit with a good digital voltmeter.

Calibration of a spectroradiometer is a tricky thing. When you calibrate it with your standard lamp, you usually put the standard lamp on the optical axis. Suppose that you calibrate two spectroradiometers in this way with the same standard lamp in the same setup, so that they show the same result when you measure light from a lamp in the laboratory. Then you take the spectroradiometers outdoors and measure the daylight. You will then likely find that the two spectroradiometers show different results. This can have different causes, but the two major ones are probably that (1) the temperature is different and the two spectroradiometers have different temperature dependencies and (2) they have different off-axis sensitivities, and you are now measuring a very extended light source rather than an almost point-like standard source.

It is recommended that you recalibrate a spectroradiometer about once a month. This interval can be modified according to the experience you obtain over time with your particular instrument under your particular working conditions. If you move your instrument around, you should perform a rather easy wavelength check at each new location.

4.6.4.3 Irradiance Calibration with an Improvised Standard Lamp

Björn (1971) devised a method to use an ordinary tubular incandescent lamp as a standard lamp without prior optical calibration of the lamp, just relying on electrical measurements. The basic idea is that the temperature of the lamp filament is calculated from the increase in electrical resistance when it heats up from room temperature; calculate the spectrum of the glowing filament from its temperature using Planck's radiation formula with appropriate corrections for the (temperature and wavelength-dependent) emissivity of tungsten. This method is not recommended if calibrated standard lamps are obtainable for the experimenter.

4.6.4.4 Calibration Without a Standard Lamp

Considering the cost of standard lamps, their instability, and the difficulty of ensuring the same standard everywhere in the world, it would be good if a radiation source were available free of charge so that everyone could use the same radiation source. There is such a radiation source: the sun.

4.6.4.5 Wavelength Calibration Using Daylight

The sun is essentially a heat radiator, thus radiating an essentially continuous spectrum, while a line spectrum is more suitable for wavelength calibration. However, some of the outer layers of the sun have a temperature that is low enough to reabsorb light from the inner layers and still hot enough to contain single atoms, not united to molecules. They produce the so-called Fraunhofer lines in daylight, which are absorption spectral lines of these free atoms. The wavelengths of these Fraunhofer lines can be looked up in various sources. Here we just mention the two due to hydrogen, which are most suitable for wavelength calibration in the visible region: 486.1344 nm (Fraunhofer F line) and 686.9955 nm (Fraunhofer C line).

4.6.4.6 Irradiance Calibration Using the Sun

Surprisingly, the sun can also be used for irradiance calibration. This is surprising because we have the variable terrestrial atmosphere between ourselves and the sun, and this atmosphere is not the same everywhere. If we are on a mountain, there is less atmosphere between us and the sun than at sea level, and other factors also contribute to different attenuation of the sunlight at different places and times. However, these difficulties can be circumvented, provided the atmosphere is reasonably clear and stable over a day.

Consider first two (unrealistically) simple cases (Fig. 4.11). We have no clouds in the sky. In the first case the sun is directly overhead, and the light is attenuated by, as the jargon in the field goes, one air mass. At a certain wavelength, the spectral irradiance from the direct sunlight can be written as $I_1 = I_e \cdot e^{-a}$, where I_e is the corresponding extraterrestrial spectral irradiance, that is, the spectral irradiance just outside the terrestrial atmosphere. The spectral irradiances we consider here are in the direction of the sun (light falling on a plane perpendicular to the direction to the sun). We consider only the direct sunlight, that is, not that scattered in the atmosphere or reflected from the ground. In the second case, the sun is at an angle of 60° to the vertical, that is, the zenith angle of the sun is 60°. In this case, the light path through the atmosphere is twice as long as in the first case. The irradiance then must be (provided the Lambert–Beer law is valid)

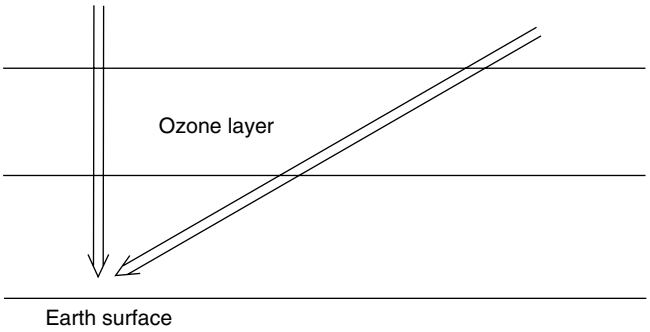

Fig. 4.11 Effect of atmosphere on sunlight from different directions. If scattered light is excluded so only the direct beam is considered, the length of the path of light through the atmosphere is longer for obliquely incident light, in proportion to the cosine of the incidence angle

$I_2 = I_e.e^{-2a}$, since the light is now attenuated by two air masses. Only when the scattered light is excluded by a narrow-angle input does the Lambert–Beer law hold (see Sect. 1.11). The ratio between the two irradiances below the atmosphere is then $I_2/I_1 = e^{-a}$, and this ratio we should be able to determine without absolute calibration of the spectroradiometer. But knowing e^{-a}, we can now calibrate the spectroradiometer against the extraterrestrial irradiance using the relationship $I_1 = I_e \cdot e^{-a}$.

This is the principle of the Langley calibration method. In practice, it is a bit more complicated than described here. One has to take spectra of the sun over at least 1 day and preferable over several days when the weather is stable and the sky without clouds. For the Lambert–Beer law to be valid, one has to measure the *direct* sunlight and exclude skylight as well as possible. One way of doing this is to measure the sunlight through a narrow baffled tube (Fig. 4.6) following the sun. Another way is to take a difference reading, that is, the difference between the total daylight and the daylight when shadowing the sun. One then plots the logarithm of the reading against the air mass (the air mass is proportional to one over the cosine of the zenith angle). This gives a nearly straight-line relationship, which can be extrapolated to zero air mass, corresponding to the reading that would have been obtained outside the atmosphere. For high accuracy one must apply various corrections (especially for large zenith angles), for instance, for the refraction (bending) of the light rays in the atmosphere and for the curvature of the earth and for the fact that different attenuators in the atmosphere do not have the same height distribution (especially for the fact that ozone, absorbing at short wavelengths, is higher than most attenuators). One must also take into account the variation of the sun–earth distance over the year, but this is easy. With decreasing wavelength, the difficulties increase (e.g., due to the rapidly changing ozone attenuation, the strong wavelength dependence of ozone absorption, less constant output of the sun at short wavelengths, and the smaller signal in relation to instrument noise). Below 300 nm this method cannot be used at all. Details of the method with different variations and comparisons with other calibration methods can be found in Schmid and Wehrli (1995), Wilson and Forgan (1995), Schmid et al. (1998), Slusser et al. (2000), Adler-Golden and Slusser (2007), and Chen et al. (2013).

4.7 Special Methods for Measurement of Very Weak Light

4.7.1 Introduction

Only methods based on photomultipliers will be reviewed here, but photomultipliers can be used in different ways. We shall not touch upon imaging of very weak light, which is important in many contexts from astronomy to biology. Photomultipliers can be used in the following main ways.

4.7.2 Direct Current Mode

This is the "classical way" described in Sect. 4.3.1. The dc component of the anode current is measured. If the light is steady or varies only slowly, an amplifier with a long time constant can be used to obtain a "smoothed" or averaged value of the current; alternatively, this averaging can, of course, be achieved using a computer. Then the light is shut off and the dark current is measured in the same way. With the photomultiplier connected to suitable electronic circuitry, the difference between "light" and "dark" currents is proportional to the light to be measured.

If the light is very weak, this does not work well. This is because the difference between "light" and "dark" currents will be a small difference between two larger terms, and a small relative error in any of them will result in a larger relative error in the difference. Furthermore, for a weak light, a long time will be required to get a reliable value of the light current, and in the meantime the dark current might drift.

4.7.3 Chopping of Light and Use of Lock-In Amplifier

In this mode, for instance, a rotating shutter or mirror is used to cut the light into short pulses separated by darkness of similar duration. A special amplifier amplifies the current during the light and dark periods separately, and the difference is continuously computed, or electric charges from the two sets of periods are stored for integration of the difference over time. In this way, the effect of drift with time of the dark current is minimized. In this way, much weaker light can be measured than is possible in the direct current mode. It is, for

example, easy to measure the light emitted from a plant leaf (as a reversal of the photosynthetic process; see Sect. 26.8) for tens of minutes after the leaf has been placed in "darkness." However, "ultraweak luminescence" (Sect. 26.9) can hardly be measured with this method.

4.7.4 Measurement of Shot Noise

Shot noise is the "noise" of the dc signal from the photomultiplier arising from the quantized nature of light, that is, the current pulses generated by the single photons. Yoh-Han Pao et al. (1966) suggested that the shot noise, treated as an ac signal, could be used as a measure of the light. Later experiments indicate that the signal-to-noise ratio for this method is somewhat better than for the lock-in method. However, because of the advantages of the method to be described below, this method has not been used much.

4.7.5 Pulse Counting

The dominating technique today for measuring very weak light is to count the current pulses generated in a photomultiplier by single photons. All pulses of the anode current, however, are not due to photons. Electrons are also released both from the photocathode and from the dynode surfaces by thermal energy. This is what gives the dark current in the dc mode. One great advantage of the pulse counting method is that pulses due to thermal emission from the dynodes can be filtered off, because they are smaller than those coming from the photocathode, since they have gone through fewer amplification steps Alfano and Ockman (1968). To achieve this, the pulses from the anode go to a pulse discriminator, which allows only pulses above a certain amplitude to pass. The pulses passing through are then shaped to a uniform amplitude and pulse shape, so they can be more accurately counted.

However, this cannot be done in a perfect way. Some photon-induced pulses are discarded, and some spurious pulses are passed. Some photons do not give rise to any pulses at all, because the quantum efficiency of the photocathode is lower than one (even in the spectral region where it is highest). And there is no way by which thermal pulses arising in the photocathode can be distinguished from light-induced pulses. Therefore, a photomultiplier can never be used as an absolute photon-counting device. To estimate the true number of photons arriving at the photocathode, the photomultiplier has to be calibrated, and a correction has to be made for "dark pulses."

Just as in a Geiger counter, there is a certain minimum time necessary for two pulses to be counted separately. Since the pulses are Poisson distributed in time, the counting efficiency starts to decline gradually when the photon absorption rate increases over a certain limit. The absolute standard error of the number of pulses recorded, according to Poisson statistics, is proportional to the square root of the number of pulses counted and the relative error proportional to one over this square root. With absolute standard errors of e_l for the light count and e_d for the dark count, the standard error of the difference between light and dark counts is the square root of $(e_l{}^2 + e_d{}^2)$.

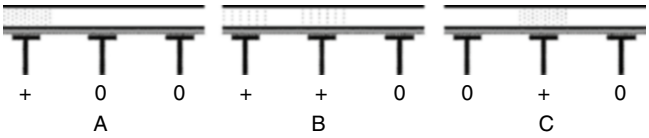

Fig. 4.12 The principle for a charge-coupled device (CCD). A small part of the CCD is shown at three different times (**a**–**c**). Electrodes are shown as **T**. In A, light has released electrons (shown as *dots*) in a doped silicon plate near the leftmost electrode. A wave of positive electrical potential (shown with the *plus signs*) is passed along the electrodes from *left* to *right*, and the electrons follow this positive wave through the silicon and can finally be collected sequentially from each location at a collection electrode at the right end (or edge, if it is a two-dimensional CCD)

4.8 A Sensor for Catching Images: The Charge-Coupled Device

There are different kinds of electronic sensors for recording images, but only the most commonly used one will be described here: the charge-coupled device (CCD). Most readers have probably used a CCD already as part of a digital camera. But a CCD is useful everywhere when you wish to record light in one or two dimensions. In Chap. 29, we show how it can be used for recording spectra. The principle for a CCD is shown in Fig. 4.12.

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