Chapter 14
The Photochemical Laboratory

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Abstract In this chapter we describe the basic photochemical instrumentation, instrument components and consumables, which make up a general photochemical laboratory. We consider factors such as sample preparation, optical properties of the sample, and contributions from background interferences, which can all affect the data obtained. We discuss the different accessories available, to optimise or perform more complex measurements such as fluorescence anisotropy and quantum yields. We do not consider in detail the more expensive systems required for specialised experiments, which are discussed in Chap. 15, although we do describe the general principles of these methods. Finally, we describe a Photochemical Library, a reference to useful books, journals, organisations, websites, programs, and conferences for researchers in the field.

14.1 Introduction

The success of any photochemical or photophysical experiment will depend on knowledge of the reaction or process under investigation, an understanding of the instrumentation, and consideration of the experimental details. The theoretical

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aspects of photochemistry are covered in Chap. 1 and also in several core textbooks (see Sect. 14.12); in this chapter we look at the experimental considerations and choice of instrumentation for photochemical experiments.

Although many of the requirements are the same, it is convenient to distinguish between what is necessary for synthetic applications of photochemistry and for spectral and photophysical studies. For the latter, sophisticated and fully-integrated steady-state and time-resolved instrumentation are now readily available and reasonably priced, making them accessible to many research laboratories. However, the simplicity of design and use means that these instruments are often treated as 'black boxes' and, consequently, inexperienced researchers can fail to take advantage of their full capacity. To get the best out of these instruments, it is important to understand the individual components and their limitations, to identify any potential artifacts or pitfalls that may distort the data, and to consider any sources of background interference. For photochemical synthesis the requirements are frequently less restrictive. However, it is vital to remember the First Law of Photochemistry (the *Grotthus–Draper law*)—the spectrum of the light source must have appropriate wavelengths that overlap with the absorption spectrum of the molecules of interest.

14.2 Controlling the Light

By definition, light plays a crucial role in any photochemical or photophysical experiment. The primary light source will be the excitation source (e.g., lamp, laser) used to irradiate the system under investigation to excite a molecule to an excited state, and a secondary monitoring beam might also be required. However, there will also be photons from the Sun or artificial lighting present and these must be eliminated or suitably minimised if controlled experiments are to be performed. In more complex optical arrangements, the light path will be controlled by a series of lenses, mirrors, slits and stops. Light management is therefore an important consideration at the start of any experiment.

14.2.1 Keeping the Light Out

Even on a dull day the intensity of both visible and near-UV light on the laboratory bench from windows and room lights is significant. A solution left out on the bench on a sunny day can easily receive more near-UV exposure than a sample under study in a fluorimeter or in an irradiation set-up. Aluminium foil is useful to wrap around flasks containing light sensitive solutions. For handling the most light-sensitive materials a darkroom with appropriate 'safe-light' is preferable.

14.2.2 Putting the Light In

The choice of excitation source will be determined by the sample and the nature of the experiment to be performed. Consideration must be given to the excitation wavelengths and source intensity required. These can be controlled by the use of monochromators and filters as necessary. Synthetic and steady-state experiments can be performed with continuous lamps or light-emitting diodes (LEDs). Time-resolved measurements, required for studying the kinetics of excited state reactions, need a pulsed light source such as a laser, pulsed LED, pulsed lamp or flash lamp, although for work in the ms–s range, a continuous lamp with a mechanical shutter can sometimes be used. The different light sources available are described below in Sect. 14.4.

14.2.3 Following the Light Beam

It is useful to see where the light beam travels in an optical arrangement. A piece of white paper will easily catch the path of the excitation beam of a fluorimeter in the sample compartment. In a darkened room, the monitoring beam of a spectrophotometer can similarly be followed if the instrument is set at 0 nm where the grating acts as a mirror, or even when set to pass green light, say 520 nm, to which the eye is most sensitive. A cell containing a fluorescent dye, or a plastic film or block of the same, is useful to follow a UV beam. A piece of white printing paper, which usually contains an optical brightener that will fluoresce blue under UV (See Chap. 4), will often suffice. Similarly, a solution, or plastic sheet, containing a fluorophore emitting in the window region of laser safety goggles can be used to safely see where a laser pulse is going while wearing the safety goggles. A piece of phosphorescent card, or paper painted with phosphorescent paint (available from many model shops), is a useful way to follow a pulse of light.

14.3 Sample Preparation

Photophysical and photochemical measurements can be performed on samples in a variety of forms, including: solutions, low-temperature glasses, powders, single crystals and thin films. The choice of sample form depends largely on the information one hopes to deduce from the experiment, or, for synthesis, the nature of the system. However, there are frequently specific considerations, such as the solubility or the amount of sample available. Measurements are perhaps most commonly made on solutions, and care must be taken not to introduce contaminants to the sample *via* the solvent. For solid samples, such as powders or films, light scattering from surfaces or crystallites can also introduce artefacts into the

spectrum. The main experimental considerations to take into account when preparing samples for photophysical characterisation are discussed below.

14.3.1 Solvents

Solvents come in various degrees of purity and cost. Fluorescence measurements are particularly sensitive to solvent impurities, so investing in a higher grade solvent may reduce time and frustration in the long run. The following general solvent grades (in increasing price) are commercially available (note that some manufacturers use different grade trade names).

1. **Reagent grade**. This is the lowest grade, suitable for general laboratory work.
2. **American Chemical Society (ACS) grade**. For general use requiring more stringent quality specifications.
3. **Analytical grade**. Prepared for analytical work with the level of specific important contaminants identified. However, higher chemical purity does not necessarily make the solvent more suitable for photochemical applications if there are still trace aromatic or other absorbing species. For example, 'water free' analytical grade ethanol is frequently obtained by azeotropic distillation with benzene, which absorbs light in the 254 nm region and is fluorescent.
4. **HPLC grade**. High purity, low residue on evaporation, and filtered, for use with HPLC systems incorporating absorbance detectors, with specified absorbances at specific wavelengths.
5. **Spectrophotometric**. Purified for spectrophotometric work. These are high-purity, low residue on evaporation, and with a clean UV spectrum with specified absorbances at specific wavelengths.
6. **Fluorimetric**. Some solvents are available specifically purified for fluorescence work.

Spectrophotometric and fluorimetric solvents can be very expensive; HPLC grades are often cheaper but with comparable optical specifications. Analytical grades can be cheaper still, and although not usually specified for absorbance, are of high general purity. Some solvents, e.g., chloroform, are supplied with stabiliser additives, so care should be taken to remove these [1] or to ensure they are unimportant for the work in hand. Some solvents are also available in anhydrous grades, but it is worth noting that these will pick up water very quickly if they are opened and handled under ambient conditions. Chemical suppliers' catalogues give detailed specifications of solvents; even so it is generally a prerequisite for any photochemical study to run a UV/Vis absorption spectrum of the solvent. For some photophysical studies, it may be advisable to purify solvents using standard procedures [1].

Judicious choice of solvent(s) is an important part of experimental design. The following are some important solvent parameters.

1. **Solvent cut-off wavelength**. Below this wavelength it is not possible to carry out any experiment that requires light to penetrate into or through the sample. The cut-off wavelengths for some common solvents are given in Table 14.1.
2. **Solvent polarity**. There are a variety of solvent polarity scales. Dielectric constant is probably the simplest bulk solvent physical parameter to consider, but measures of solvent polarity based on solvent–solute interactions at the microscopic molecular level, such as the interaction between solvent and solvatochromic dyes in the Reichart $E_T(30)$ scale [2], are also widely used. An increase in polarity will lower the energy of, and hence favour, all process involving charge separation, such as electron transfer, and charge transfer states, so a significant difference in behaviour in polar and non polar solvents indicates alternative charge transfer reaction routes or states (see Chap. 12).
3. **Solvent viscosity**. An increase in solvent viscosity will decrease the rate of molecular motion and reduce the rate of diffusion-controlled bimolecular interactions, such as those involved in dynamic quenching. For aqueous or alcoholic solutions, viscosity can easily be increased by use of poly-ols such as ethylene glycol or glycerol, either on their own or as part of a solvent mixture. These have the advantage that they are transparent through much of the UV/Vis. In addition, on freezing, they frequently form glasses, which are valuable for studying phosphorescence.
4. **Singlet oxygen lifetime**. This is highly solvent-dependent being particularly short in solvents containing −OH groups, and much longer in deuterated and halogenated solvents. Particularly long singlet oxygen lifetimes are observed in carbon tetrachloride. The use of deuterated solvents can be a very useful tool in singlet oxygen studies.

Ensuring homogeneous dissolution of material in solvents is important. Ultrasonic baths or continued stirring under gentle heating may be used to dissolve more obstinate materials, though care should be taken to minimise the possibility of sample degradation by these methods. In some cases, samples become dispersed as micro-crystallites rather than dissolving to a homogeneous solution. While such

Table 14.1 Cut-off wavelengths of some commonly used solvents

Solvent	λ (nm)
Alkanes	195
Water	195
Acetonitrile	195
Methanol	210
Ethanol	210
Dichloromethane	220
Chloroform	240
Carbon tetrachloride	250
Benzene	280
Toluene	280
Tetrahydrofuran	280

solutions can look perfectly transparent, the presence of micro-crystallites can be detected from the observation of light scattering if a narrow light beam is passed through the solution, and sometimes by a high UV/Vis absorption 'baseline' which shows increasing 'absorbance' towards shorter wavelengths. For any material that has proved difficult to dissolve one or other of these simple checks for micro-crystallites is worth doing.

Even for solutions that are free of micro-crystallites, it is important to be aware of the possibility of solute aggregation, especially since the photochemical characteristics of aggregated species are often quite different from their molecular counterparts. Many organic compounds will aggregate at moderate concentration and this is particularly important for: large planar aromatic molecules which can associate through π–π interactions, non polar organics in polar solvents, polar organics in non-polar solvents, polymers generally, and charge bearing organics in water. Aggregation can be detected by changes in absorption, excitation or emission spectra, and also changes in emission quantum yields. Spectral changes due to aggregation are often quite small, as little as a minor deviation from a linear Beer–Lambert law, but in some cases, such as J-aggregate formation in cyanine dyes, they can be dramatic, and lead to a significant shift in the absorption band. Aggregation of organics in aqueous solution can often be reduced by addition of an organic co-solvent such as ethanol or acetonitrile, or a surfactant, which may isolate solute molecules in micelles, although the changes in solute environment these additions make should always be borne in mind.

Even when solutes are present as monomolecular species, concentration can influence photochemical measurements through inner filter effects (see later), and also photochemical reactions through, for example, self-quenching, triplet–triplet annihilation, and excimer formation.

14.3.2 General Laboratory Equipment for Solution Preparation

Glassware. The precision and accuracy required in any experiment is determined by the nature of the experiment itself. The tolerances of class A standard volumetric glassware: volumetric flasks, burettes, and pipettes are typically a few tenths of a percent of the glassware volume (tolerances for class B glassware are typically about twice that of class A glassware), but precision is usually better than this. Piston micropipettes which give a wide range of delivery volumes in the μl–ml range are available from a variety of suppliers e.g., Eppendorf, Gilson, Hamilton, Finnpipette, and pipettes of either fixed, or variable, volume are available. These have, depending on supplier and model, accuracy and precision typically in the range of a half to two percent of the maximum delivery volume, with highest imprecision/inaccuracy (which can be as much as 10–20 %) when using variable volume pipettes at the low volume end of their useable range. Regular calibration

is essential to ensure the accuracy and precision of these pipettes are retained over time. Glass syringes for use in μl–ml range are also available from a number of manufacturers, such as Hamilton, and these have accuracy and precision of about a percent of the maximum delivery volume. Typically then, routine solution make-up should be precise to a percent or so. This is usually better than that for most photochemical measurements such as yields and rate constants, so experimental precision should not generally be limited by solution preparation. For accurate work it is crucial to use volumetric glassware correctly and it is well worth consulting standard texts on the subject [3, 4]. The time spent reading the manufacturer's specifications and instructions for the correct use of pipettes, burettes, volumetric flasks, micropipettes, syringes, and other precision equipment, is time well spent. Solutions need to be well mixed to ensure homogeneous dissolution of the solute at the molecular level. In particular, adding solvent to make a volumetric flask 'up to the mark' can result in the neck of the flask being filled with solvent only, and a cursory inversion of the flask will not be adequate for thorough mixing.

Balance. An analytical balance capable of weighing $\sim$100–200 g to 0.1 mg is suitable for most applications. For work where materials are limited, a six-figure balance may be useful, although the lower maximum weight, and increased care required for its use, mean that this is generally more an (expensive) addition to the lab rather than a replacement for a four figure balance. A four-figure balance is a precision instrument and requires treatment as such. For accurate work it is best placed on a vibration proof bench away from direct sun, drafts and thermal convection currents. When weighing to 0.1 mg, vessels should be handled with tongs rather than fingers because these can leave behind enough finger grease and moisture to add measurable weight to the vessel. Typically, repeat weighing on such an analytical balance can be made to $\sim$0.2 mg and so working with $\sim$20 mg of material gives $\sim$1 % precision; if significantly lower weights of material have to be used then it is important to be aware of the effect of this on the precision of subsequent experiments.

Optical determination of concentration. For a typical high absorber with a molar absorption coefficient, ε, of $\sim 10^4$ mol^{-1} dm^3 cm^{-1} and molar mass $\sim$200 g mol^{-1}, 20 mg will give enough for a 1 L solution with an absorbance of 1. For most studies this is far too much solution, and may be a waste of a very valuable compound. However, if the molar absorption coefficient is known, determination of the concentration from absorbance using the Beer–Lambert law (Chap. 1) is easier and more reliable than trying to weigh out small amounts of material. For photochemical studies, the optical properties of the solution are often more important than using a specific concentration, and it is possible to avoid having to deal with large volumes of solution simply by the addition of small quantities of material to the required volume of solvent until the required absorption (and hence concentration) is obtained. A capillary tube, such as used for melting point determinations, or the tip of a glass Pasteur pipette, gently pressed into a sample is a convenient and disposable, contamination free, way to pick up a few mg of solid which can then be dissolved in solvent.

Sample concentrations should be tailored towards the measurement being performed, striking a careful balance between a good signal to noise ratio (see Sect. 14.8.2), minimisation of artefacts and preferably working within the linear response range of the instrument. Standard UV/Vis absorption spectrometers exhibit a linear response up to 2–3 absorbance units and for best results determination of molar absorption coefficients is usually performed on a series of solutions with an absorbance in the range 0.2–1.5. For standard emission measurements with 1 cm path length cells, however, the optical density at the absorption maximum should generally be kept below $\sim$0.1 A to reduce gross inner filter effects (see Sect. 14.9). It is always advisable to run an absorption spectrum before and after any photophysical/photochemical experiment to see if sample degradation has occurred.

14.3.3 Optical Cells and Cuvettes

Gas and liquid samples are usually contained in optical cells or 'cuvettes'. Solid powders can also be held in such cells but are more commonly made into a compact optically diffusing pellet, or pressed into a hollowed out receptacle with an open surface which can be exposed to the light source. Note that with all optical components, care must be taken to avoid scratching or touching optical surfaces; fingerprints can lead to extensive light scattering. Cell windows can be cleaned with ethanol and lens cleaning tissue.

Three types of materials are commonly used in the manufacture of optical cells: plastic, glass (optical glass, Pyrex, special optical glass) and quartz (UV silica, spectrosil). For standard measurements, cells of square cross section with a 1 cm pathlength are used. For absorption work, the cells have two polished faces, while emission cells have all four faces polished. Plastic cells, usually made from polystyrene, which are available as 1 cm path length absorption and emission cells, are very cheap, disposable, and, depending upon the plastic used, can be optically transparent down to $\sim$280 nm. They are suitable for water and alcohol solutions but most other organic solvents dissolve the plastic. They are not made to the same tolerances as glass or quartz cells so should not be used for accurate absorption work, but they are excellent for non-demanding use in student laboratories. They are also good for preparing difficult samples, such as liquid crystals, which need a long time to stabilise. Glass and quartz differ in optical transmission properties; glass absorbs below about 320 nm, while UV grade silica has good transparency to $\sim$160–180 nm, depending upon thickness.

Quartz cells are significantly more expensive than those made of glass. Many different types of cell are available for specific applications: anaerobic, flow, micro, sipper, stirrer, stopcock, and others; examples of some of these cells are shown in Fig. 14.1. Cell manufacture catalogues are an excellent source of information about materials and what types of cell are commercially available [5]. Standard absorption cells typically have a 1 cm path length and square cross-

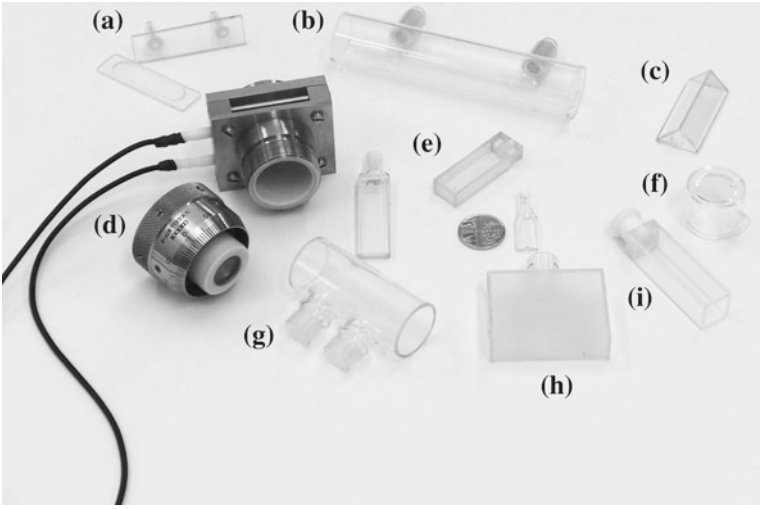

Fig. 14.1 Various absorption and emission cells. **a** Thin pathlength demountable flow cell; **b** 0 cm pathlength cylindrical absorption cell; **c** triangular emission cell for use with highly absorbing solutions; **d** variable path length absorption flow cell with stainless steel sleeve and screw; **e** standard 1 and 5 mm path length absorption cells, and micro cell; **f** 0.1 mm path length cylindrical cell (the 0.1 mm path length is behind the cell face placed down on the table); **g** 5 cm cylindrical absorption cell; **h** 4 cm absorption cell; **i** standard 1 cm emission cell. The 5p UK coin, included to give some idea of scale, has a diameter of 18 mm

section with a volume of about 3 ml, but a range of path lengths from 1 mm to 10 cm are readily available. A standard photochemical procedure is determination of the molar absorption coefficient from a Beer–Lambert plot of absorbance against concentration, and a collection of cells of different path lengths is very useful for this. Cells can be bought as 'matched pairs' with identical optical properties, for use as sample and reference in double beam spectrophotometers, but modern manufacturing methods and automatic background correction have reduced the need for such pairs somewhat.

Shorter path lengths down to 0.08 mm can be obtained using cells where the faces are demountable and a spacer of known thickness can be interposed between the cell faces, or where cell faces are brought together with one face on a screw thread and with path length read from a dial. Many spectrophotometers have the option of introducing a 'beam mask' to cut down the area of the light beam incident on the cell, so that small volume cells can be used, and many instrument manufacturers offer cell designs allowing measurement down to volumes of a few μl. The non-transmitting portion of cells may be made of black glass to prevent scatter and stray light.

Standard emission cells are also 1 cm square, and since the normal 90° optical arrangement is designed to pick up emission from the centre of the cell, both the excitation beam and the emission beams travel through *ca.* 0.5 cm of solution before being incident on the sample or collected by the detector. For anything

other than optically dilute solutions, absorption within these optical path lengths can distort excitation and emission spectra (so called *inner filter effects*). Emission cells of shorter pathlength for standard 1 cm cell holders must either be made with the solution held in the middle of the cell, e.g., 1 mm square wells in a 1 cm block, or held in an adapter which does this. Again many instrument manufacturers offer a variety of emission cells and cell holders, allowing measurement down to volumes of a few μL.

Low temperature phosphorescence measurements are most easily carried out at the boiling point of liquid nitrogen, 77 K, in a frozen organic glass. The sample is dissolved in an organic solution, which forms a transparent glass upon freezing, and the solution placed in a *ca.* 15 cm long tube of optical quartz (or glass if the near UV—visible spectral region is all that is required) of a few mm diameter. This is then immersed into liquid nitrogen held in a specially designed low temperature quartz Dewar which is equipped with a short protruding stem at the bottom into which the quartz tube fits (see Fig. 14.2). A reasonably snug fit is desirable for good repeatability of measurement, but if the fit is too tight there is risk of shattering the Dewar as the tube expands upon freezing. Excitation and emission measurements can then be made through the sides of the Dewar and tube. Two common problems with this arrangement are interference from nitrogen bubbles through the excitation or emission beams and a build-up of ice crystals in the liquid nitrogen. Bubbling can be reduced by a preliminary cooling and rinsing of the Dewar with liquid nitrogen followed by rapid filling immediately after the first rinse is discarded. Ice build-up can be minimised by keeping the liquid nitrogen sources covered. Care must be taken to ensure that the quartz tubes are warmed from the top down when being brought back up to room temperature. If warmed bottom first, expansion, as the solution in the bottom of the tube melts, may shatter the tube and/or eject solid material from the top of the tube. The glass tubes used for NMR are relatively cheap, easy to buy, and suitable for the visible spectral region. Quartz phosphorescence tubes are also available for the UV region.

Fig. 14.2 Silvered quartz Dewar and phosphorescence tube for low temperature work. The sample is placed in the narrow tube, which is then inserted into the vertically held liquid nitrogen filled Dewar. Excitation and emission is carried out through the unsilvered nipple on the Dewar. The 5p UK coin, included to give some idea of scale, has a diameter of 18 mm

Phosphorescence spectra and lifetimes are easy to measure using this arrangement but quantum yields are rather more difficult because of difficulty in repeatable positioning of the sample, the many curved surfaces in the light beams, and often a lack of knowledge of low temperature absorption spectra. The latter problem can be addressed, in part at least, if the sample fluoresces, since relative low temperature fluorescence and phosphorescence yields can be obtained from the individual and total emission spectra. Relative low temperature and room temperature fluorescence yields can then be obtained from emission spectra with the sample left in the same position, with allowance for temperature dependent changes in absorption spectra made by comparing excitation spectra at both temperatures. As a first approximation excitation spectra can be normalised assuming the area of the lowest energy absorption band is constant, *i.e.*, a temperature independent transition probability, and then allowance made for contraction of the solvent upon freezing to calculate the relative absorbances at the two temperatures. If it is possible to hold the Dewar in an absorption spectrometer then, provided the monitoring beam is narrow enough and aligned properly with the sample, reasonable low temperature spectra can be recorded. There are a number of solvents and solvent mixtures which form glasses at 77 K [6], but perhaps the easiest to work with for moderately polar systems is EPA (diethyl ether:isopentane:ethanol at 5:5:2 (v/v)), because it gives a relatively soft glass which does not shatter, while for non-polar compounds, 3-methylpentane, methylcyclohexane or isooctane all give good glasses. A more detailed discussion is given in Chap. 15.

14.3.4 Removing Oxygen

At a concentration of $\sim 2 \times 10^{-4}$ mol dm^{-3} in air-equilibrated water and $\sim 2 \times 10^{-3}$ mol dm^{-3} in non-polar organic solvents, oxygen is a major reactive contaminant. In particular, it is a very effective triplet quencher. Oxygen can be removed from solution in one of two ways: (i) purging the solution with an inert gas, either oxygen–free nitrogen or argon; or (ii) *via* a series of freeze–pump–thaw cycles, with evacuation of the gas above the solid sample using a vacuum pump when the solution is frozen. In most cases inert gas purging is easiest, and it has replaced the freeze–pump–thaw method in most laboratories, although there is the suspicion that freeze–pump–thaw is most effective at removing the last traces of residual oxygen, and for studying long-lived triplet states it may still be the better method, particularly where information on lifetimes is needed.

Inert gas purging can be carried out with the sample *in situ*, with the gas delivered by a long syringe needle or thin Teflon tubing, using a fine needle valve on the cylinder to control gas flow, and with an adequate safety valve in the gas line, such as a balloon on a T piece, in case of blockage of gas flow. About 15–20 min purging is usually adequate, and it is a simple check to repeat the measurement in question before and after a further 5 min purge, to ensure

maximum oxygen removal. Results of the effect on fluorescence lifetimes from such purging are given in Chap. 15. Once purging is complete the system can be sealed or gas flow continued but with the rate turned down to a lower level to ensure no return of oxygen into the sample. Care should be taken, particularly with volatile solvents, that purging does not transfer unacceptable levels of solvent vapour into the laboratory, and also that any change in concentration due to solvent loss is not critical to the experiment. Solvent loss can be minimised by pre-bubbling the gas through the solvent first to saturate it. Because of the risk of loss of solvent by purging, if comparison between aerated and oxygen free solution is required it is best to make measurements on the purged solution first and then shake the sample thoroughly in air before measuring under air equilibrated conditions.

14.4 Light Sources

Light sources are required to probe both the ground and excited state properties of a photoactive system, and also as a source of photons for photochemical reactions. The light source may be an integrated module of an instrument or a stand-alone component.

UV/Vis absorption measurements are used to probe and measure electronic transitions of ground and excited states and other short-lived species. Ground state absorption is usually obtained using continuous illumination lamps emitting in the UV and visible spectral regions. However, absorption measurements of excited states and other short-lived species may require a high intensity pulsed monitoring beam, (in addition to the excitation pulse required to generate the transient species in the first place), because continuous lamps cannot provide a high enough photon flux for adequate signal-to-noise ratio (S/N) for measurements over very short timescales.

Steady-state photoluminescence measurements use constant illumination and detection. The sample is irradiated with a continuous beam of light and the emission spectrum (or single-wavelength intensity) recorded. (In practice the excitation source may be ‘chopped’ at a few tens of Hz using a rotating disc chopper in the excitation beam with detection locked into the chopped signal in a method sometimes referred to as ‘AC mode’. This can be used to give a better background zero and more stable response since the portion of the beam ‘chopped out’ can be used with a reference detector to correct for wavelength, or time, dependent variations in excitation intensity. Providing the chopping frequency is much less than the excited-state lifetime, the steady-state excited-state concentration is the same as that for continuous irradiation.) The steady-state concentration of excited-states is reached on a timescale of a few excited-state lifetimes and since fluorescence typically occurs on nanosecond timescales, the fluorescent steady-state is reached essentially immediately upon excitation. However, because of their longer lifetimes, it may take a few seconds to reach the steady-state

concentration of phosphorescent excited-states. For time-resolved absorption and emission measurements a pulsed excitation light source must be used. This is discussed in more detail in Chap. 15.

Steady-state and time-resolved measurements are related. The steady-state concentration of excited-states of a lumophore of lifetime τ is $R_{abs}\phi\tau$ where R_{abs} is the rate of absorption of photons, in E dm^{-3} s^{-1} (E is the Einstein, which corresponds to 1 mole of photons), and ϕ the quantum yield for excited-state formation. The total steady-state emission intensity is $R_{abs}\phi\tau/\tau_{rad}$ where τ_{rad} is the radiative lifetime. The instrument response depends on instrument optics and detector sensitivity, but for a given lumophore and constant R_{abs}, it is proportional to τ, and the introduction of any process, such as quenching, which reduces τ reduces instrument response proportionately, providing of course the instrument is operating in the linear range of the detector.

Working the other way, from decay curves to intensities, for a single lumophore decaying exponentially the integrated intensity across the decay (I_{ss}) is related to the excited state decay time by:

$$I_{SS} = \int_0^\infty I_0 e^{-t/\tau} dt = I_0\tau \tag{14.1}$$

where I_0 is the emission intensity at time, $t = 0$, and τ is the corresponding emission decay time. So integrating the area under an emission decay curve gives a value proportional to the total number of emitted photons, *i.e.*, the total emission intensity. For a system of a number of lumophores or independent decay processes, the single term, $I_0\tau$, is replaced by a summation of such terms for each lumophore or process.

For photochemical reactions, as opposed to physical measurements, the key requirement of the lamp is high intensity, and, for maximum versatility, emission across a wide spectral range. In addition, for quantum yield measurements the light flux should be reasonably constant.

14.4.1 Continuous Light Sources

Spectral profiles and photographs of the most commonly encountered continuous light sources are shown in Fig. 14.3. The Newport Corporation website (www.newport.com) is an excellent source of data on lamps and how to use them.

14.4.1.1 Tungsten Halogen Lamps

Tungsten iodide (also called tungsten halogen) lamps give a continuous spectrum with useful intensity from ~350 nm out into the near IR (Fig. 14.3a). The exact

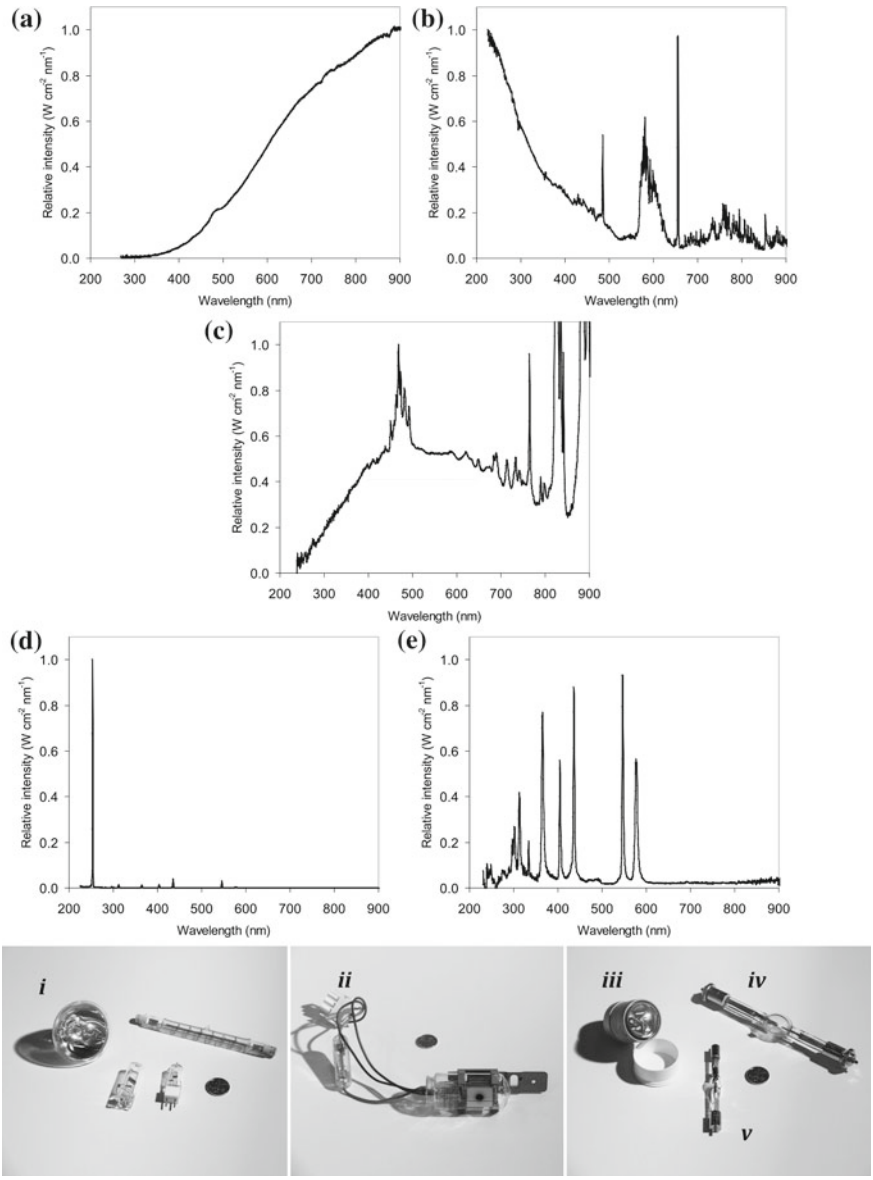

Fig. 14.3 Typical spectral profiles of **a** tungsten halogen, **b** deuterium, **c** compact continuous Xe arc, **d** low pressure and **e** medium pressure Hg lamps recorded with an Ocean Optics calibrated spectrometer. Note that the spectra are each individually normalised to a maximum of 1 and comparison of relative intensities between lamps cannot be made. Note also that when using a detector bandwidth wider than the emission line bandwidth, the measured relative intensities of lines compared to continuum varies with detector bandwidth, with the ratio of line-emission to continuum increasing with decreasing bandwidth. The lower panel shows photographs of commonly encountered lamps: **i** a variety of tungsten lamps, **ii** deuterium, **iii** compact xenon, **iv** a short arc xenon arc and **v** super pressure mercury lamp. The 5p UK coin, included to give some idea of scale, has a diameter of 18 mm

spectral distribution, which approximates to that of a black body, shifts to the blue as the temperature is increased, but the filament lifetime decreases rapidly with increasing operating temperature. Lamps ranging in electrical rating from mW to kW are readily available; those most commonly used in the laboratory are in the *ca*. 50–1000 W range. With a stabilised power supply the output from tungsten lamps can be very steady, and the combination of a spectral continuum and excellent output stability make these lamps ideal as visible light spectrophotometric monitoring sources. Since the total lamp output is varied by varying the size of the filament, then, for the same optical arrangement, increasing output does not directly correspond to increased intensity at the irradiation area, and for a small irradiation area, e.g., a spectrophotometer slit or small irradiation vessel, a 100 W lamp can be as effective as a 1000 W lamp.

Much of the input electrical energy ends up as heat, so the lamp housing can get very hot. For small power lamps, up to $\sim$100 W, a well-ventilated housing may suffice, but for higher power lamps a fan is recommended. Due to the large amount of IR radiation it is useful to include either a filter of a few cm of water, or IR absorbing glass, or a 'hot mirror', between the source and sample (A 'hot mirror' is a dielectric filter that selectively reflects IR. Some hot mirrors must be placed at 45° to the source-sample axis while others require a 90° angle. Some may also reflect UV light, and if made of glass will also absorb UV. It is always worth measuring the absorption spectrum of any material placed in the light beam).

In dichroic tungsten lamps, a dichroic reflector which reflects visible light but absorbs IR helps increase the forward intensity of visible light while reducing the heating effect of IR radiation. Commercial irradiation sources for microscopy using dichroic lamps coupled to a fibre optic and a filter arrangement are useful reasonably high intensity, small area, visible light irradiation sources [7].

14.4.1.2 Arc Lamps

The highest intensity sources, other than lasers, are arc lamps, in which an electrical discharge is passed through a gas in a transparent, usually quartz, envelope. While lasers are brighter, the laser spot area is usually small and arc lamps are the brightest continuous large area illumination sources generally available, and as such are ideal irradiation lamps for synthesis and photodegradation studies. The gas and its pressure determine the emission spectrum. Mercury, xenon and deuterium are the most common gases employed. Xenon lamps are almost all high pressure ($\sim$30 atm) while mercury lamps may have a gas fill at low ($\sim 10^{-5}$ atm), medium ($\sim$2–5 atm) or high ($>$ 100 atm) pressure, deuterium lamps are low pressure lamps.

Deuterium lamps are commonly used for UV spectroscopy. They give a moderately intense continuum of UV radiation from 360 nm down to 160 nm, with a weaker pseudo-continuum with strong line emission superimposed throughout the visible (Fig. 14.4b). Deuterium lamps with quartz envelopes generate ozone, a toxic chemical hazard formed *via* ionisation of O_2 molecules,

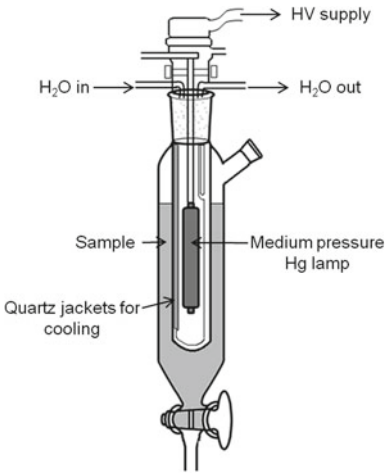

Fig. 14.4 Immersion well batch photochemical reactor

primarily from the radiation below 170 nm and ozone free deuterium lamps use a window material which absorbs below 180 nm. The combination of a deuterium and tungsten halogen lamp gives a continuum right through the UV/Vis/near-IR spectra region. Most UV/Vis spectrometers will use this arrangement, and there is usually an automatic lamp change as the instrument switches between lamps. Deuterium lamps can also be used as UV irradiation sources for the deep UV, in photolithography for example.

Continuous xenon (Xe) arc lamps are very high intensity visible light emitters with good yield of UV which decreases in intensity from about 400 nm downwards; emission also extends out into the near IR (Fig. 14.3c). Emission is a continuum with some high intensity emission lines from xenon, particularly around 420–480 nm and in the near IR, superimposed. Most fluorimeters use Xe-arc lamps as excitation source and these emission lines can be used for internal wavelength calibration e.g., the line at 467 nm can be used to calibrate the position of the excitation monochromator in a fluorimeter. Xe-arc lamps also make excellent high intensity irradiation sources for photochemical reactors. When fitted with a suitable 'Air Mass' filter they give a reasonable approximation to the solar spectrum and are widely used in solar simulators.

Continuous Xe-arc lamps are also used as high intensity monitoring beams for pulsed absorption work such as nanosecond flash photolysis, where, because of the short duration of measurement, a very high photon flux is required for adequate signal-to-noise ratios. Signal-to-noise ratio is proportional to the square root of the light intensity so the lamp is often pulsed to give a millisecond burst of even brighter illumination than that achievable in constant running.

A Xe-arc lamp requires an initial high voltage pulse of a few tens of kV to break down the gas between the electrodes to give electrical conduction. This pulse is intense enough to cause serious damage to sensitive electrical equipment close to the lamp or lamp power cables. It is therefore usual procedure to 'arc' the

Xe lamp before switching on any other electrical equipment in that section of the laboratory. Far UV emission through fused quartz will generate ozone and ozone-free Xe-arc lamps use fused quartz doped with titanium dioxide to block deep UV radiation (< 254 nm).

Mercury lamps are particularly rich in the UV, but also show line emission in the visible region. Low pressure lamps show narrow atomic lines, whereas emission from medium and high pressure lamps is subject to pressure broadening, although none give anything approaching a true continuum over a wide range of wavelengths. The intensity from high pressure lamps is significantly greater than for a low pressure lamp, and medium and high pressure mercury lamps are excellent intense UV sources. As shown in the spectra in Fig. 14.3d, e, they have a few very intense peaks (particularly the ones at 254, 312 and 365 nm). These can be isolated using appropriate filters to obtain nearly monochromatic light for quantum yield measurements.

A combined Xe–Hg lamp is also available. This gives a spectrum which is richer in the UV than a Xe lamp alone, but which also shows much greater line structure. The Xe–Hg lamp is used as a fluorimeter source when intense excitation in the mid/far UV is a particular requirement.

All of these lamps become very hot in operation and some cooling arrangement, such as water coils, a heat sink, a fan, or effective convective cooling is needed. Care is always required in operating high intensity lamps because of the intense UV and visible light, high voltages, and the very high temperature and pressure of the bulbs when running.

14.4.1.3 Spectral Lamps

Low pressure lamps with different gas fills, either rare gases, or reasonably volatile metals, e.g., Zn, Cd, Hg lamps, are available. They give emission spectra made up of a number of lines, characteristic of the element(s) used, and depending upon the element, lines can be found right across the UV/Vis/near IR range (see Fig. 14.3d for the spectrum of an Hg lamp). Spectral lamps are useful for wavelength calibration, and sodium lamps are used as a standard monochromatic source in a number of instruments, e.g., polarimeters and refractometers. Spectral lamps can also be used as excitation sources and irradiation sources for photochemical reactions. The company UVP provide them in a variety of sizes right down to a light length of 1.5 mm, and powers outputs, 2–30 W electrical energy and 0.04–20 mW cm^{-2}, under the trade name of PenRay® lamps [8].

14.4.1.4 Hand Held UV lamps

A UV lamp operating at 365 and 254 nm, as used for chromatographic visualisation, is a useful source for a quick check of luminescence from a sample. More powerful hand held ‘Black Light’ lamps, which emit at around 365 nm, are also

available, and these also make a useful lamp for a check on sample emission for samples which absorb at this wavelength.

14.4.1.5 Irradiation Reactors

Photochemical syntheses, as well as photodegradation studies, are usually performed in irradiation reactors; a variety of different designs are commercially available, depending on the specific application requirements. For synthetic photochemistry, immersion well reactors are commonly used (Fig. 14.4). The source, typically a low or medium pressure mercury lamp, is housed in a double-walled quartz jacket, which allows water-cooling and/or filtering of excitation radiation. The solution to be irradiated surrounds the lamp source, enabling homogenous irradiation.

14.4.1.6 LED Light Sources

LEDs (light-emitting diodes) are semiconductor devices that emit photons around a specific peak wavelength when an electrical current is passed through them. The colour of the emitted light depends on the composition of the semiconducting material, and a variety of emission wavelengths spanning the near UV, visible and NIR spectral regions, with typical emission bandwidths of a few tens of nm are available. Since LED light sources do not generate significant infrared radiation, an additional heat filter is not required. LED sources offer the additional advantages of low power consumption and long lifetime and are particularly useful as miniature light sources for instrument building. With the introduction of increasing legislation restricting the use of mercury vapour lamps, LEDs are likely to increase in importance as photochemical light sources in the future. They have the added advantage that they can often be tailored for specific applications, e.g., as flat-bed photoreactors, which are likely to be useful in areas such as water effluent treatment.

14.4.2 Pulsed Light Sources

14.4.2.1 Millisecond Pulses

A mechanical shutter or rotating disk with a continuous light source can be used to give pulses down to $\sim$ ms timescales. Repetition rates of a few Hz can readily be obtained with a camera shutter, and up to about hundred Hz with a rotating disk. However, since a continuous source is used, the energy in a ms pulse will be quite weak. (Note that there is a risk that prolonged high intensity illumination will thermally distort fast shutter blades.) Photographic flash units are ms pulsed Xe

flash lamps. They will typically give ~10 μs–ms pulses of up to a few hundred joules (electrical energy) across the visible and near IR, and, if any plastic cover is removed, possibly into the UV depending upon the transmission characteristics of the bulb. Operated normally flash units give repetition rates of around 1–10 Hz or so, with higher repetition rates available from stroboscopic flash units.

14.4.2.2 Microsecond Pulses

High intensity μs Xe flash lamps are available. Emission is a continuum covering the UV/Vis and near IR, similar to a continuous Xe lamp, but the continuum is weaker with a much stronger line structure superimposed. Two types of lamps are commonly used: those used as excitation sources for lifetime measurements in the μs range, which give short duration pulses, typically 0.2–2 μs, but which are only a few tenths of a Joule electrical energy; and those used in μs flash photolysis, which give longer pulses, of around 10 μs duration, but with much higher energies, typically 10–1000 J.

14.4.2.3 Nanosecond Pulses

ns flash lamps. An electrical spark in a gas generates a ns pulse of light. The spectrum is typically a mixture of line and continuum across the UV/Vis/near IR, and is determined by the gas use. These ns flash lamps are most commonly used in time-correlated single photon counting (TCSPC) and are described in detail in Chap. 15. They are not used as sources for many other instruments but they can be used to study emission in the ns–μs range using a fast photomultiplier with signal averaging over many pulses to compensate for the low pulse energy.

Pulsed diode lasers and pulsed LEDs. Diode lasers and LEDs can be pulsed or modulated and used as excitation sources for time-resolved measurements. LEDs can be operated with pulse rates up to ~50 MHz and pulse widths of ~1–2 ns, enabling lifetimes of > 10 ns to be readily determined, and the good stability and pulse characteristics of these sources can allow the measurement of even shorter fluorescence lifetimes. A similarly wide range of emission wavelengths is available for pulsed LEDs as their continuous counterparts. Laser diodes differ from LEDs in that they emit monochromatic radiation (LEDs typically have a broader spectral profile). Pulsed laser diodes can achieve a repetition rate of up to several GHz, enabling rapid data acquisition. Pulse widths are shorter than for LEDs, typically ~50 ps, enabling facile determination of ns decay times. Currently available laser diode wavelengths include: 375, 390, 405, 420, 440, 473, 500, 510, 531, 596 nm and a number of wavelengths above 635 nm. Currently available LEDs wavelengths include: the full range from 250–380 in *ca.* 5–10 nm intervals, 450, 460, 475, 500, 600 nm. This is such a fast developing field that the introduction of many other pulsed laser diode and pulsed LED wavelengths can be expected in the near future.

14.4.2.4 Lasers

Lasers emit light through the process of stimulated emission. Both continuous wave (CW) and pulsed lasers are available, with pulsed lasers emitting radiation over timescales short enough to monitor even the fastest photochemical processes [9, 10]. The basic requirements for the generation of laser radiation are:

1. **Laser medium**. The laser medium contains a chemical system that exhibits a relatively long-lived excited state (*metastable* state), N_m, capable of undergoing stimulated emission. Stimulated emission requires the presence of a photon of energy $h\nu$ to induce radiative relaxation of the excited state to the ground state N_n:

$$N_m + h\nu \rightarrow N_n + 2h\nu \tag{14.2}$$

   The emitted photon travels in phase with and in the same direction as the initial photon. These two photons can similarly interact with additional excited states, stimulating the emission of further photons, resulting in light amplification (*laser* is an acronym for **l**ight **a**mplification by **s**timulated **e**mission of **r**adiation), For stimulated emission to occur, the probability (or rate) of stimulated emission must exceed that of absorption. At thermal equilibrium, the relative population of the ground and excited state in a two-level system has $N_n > N_m$. As discussed in Chap. 1, for stimulated emission to dominate absorption, a population inversion is required, such that $N_n < N_m$. To create this population inversion an energy source is needed and is provided in the form of an external *pumping* mechanism (see below). Solid-state, gaseous and liquid laser media are available.
2. **Optical resonance cavity**. To sustain laser action, the laser medium must be placed within an optical resonance cavity, which is confined by two highly reflecting mirrors. The mirrors are coated by alternate layers of high and low dielectric materials, such as TiO_2 and SiO_2, to give almost total reflection at the laser wavelength. The photons emitted by the laser medium are reflected by these mirrors, confining them within the cavity and enabling the photon flux to sustain the conditions necessary for stimulated emission to occur. However, the mirrors, in fact, differ slightly in their reflectivity; the output mirror being specifically coated to allow around 1–10 % to leave the laser cavity forming the laser beam.
3. **Pumping mechanism**. In order to generate an excited-ground state population inversion, the laser medium must be pumped with energy—this may be provided by a light source, an electrical discharge or a chemical reaction.

Lasers offer four main advantages for time-resolved measurements over conventional light sources:

1. **Monochromatic emission**. Lasers emit light of a clearly defined single wavelength, or very narrow band of wavelengths, the energy of which is determined by the lasing medium. This enables selective excitation and observation of specific electronic transitions. Frequency doubling (or second harmonic generation), where the high laser intensity allows the conversion of two photons of frequency ν into a single photon of frequency 2ν when passed through a medium exhibiting nonlinear optical properties, such as a crystal of KH_2PO_4, may be used to produce a wavelength that is one half of the fundamental wavelength of the laser. This can be subsequently combined, in a similar way, with the fundamental frequency to give a wavelength one third that of the fundamental, and frequency doubling the doubled beam gives a wavelength one quarter that of the fundamental.
2. **Very short pulses**. Pulses as short as 10 fs can be generated relatively easily, enabling ultrafast photochemical reactions and processes to be studied. Over the last decade, attosecond (10^{-18} s) laser pulses have been reported. However, their applications in photochemistry are limited to a few specific systems since Heisenberg's uncertainty principle dictates that these short pulses have relatively broad spectral bandwidths.
3. **High power**. The laser power is the amount of energy delivered by the laser per unit time in Watts (W = J/s). Pulsed lasers compress the emitted radiation into very short timescales, generating peak powers in the order of 10^4–10^9 W (10^9 W = 1 GW, the output of several power stations!). This enables excitation of even very weak transitions.
4. **Low divergence**. The laser beam is highly collimated, resulting in a low divergence angle (typically less than 1/100th of a degree). This allows laser radiation to travel long distances without spreading. Moreover, the laser beam can be focused onto a small, precisely located spot, enabling small sample volumes and high power densities.

Pulsed lasers are used to monitor the time-evolution of excited states, the formation of photochemical products and the kinetics of photoprocesses. The high intensity of the laser beam also enables very weak transitions to be excited, so that vibrational photochemistry and multiphoton transitions, which exhibit different selection rules, can be studied. Chap. 15 provides further details about some of the experiments that may be performed using pulsed laser sources. Table 14.2 gives details of the most commonly used lasers in photochemistry today. The key properties to consider for any particular application are: wavelength(s), pulse duration, and power. Simplicity of operation is another important factor.

Table 14.2 Properties of some pulsed lasers commonly used as excitation sources in time-resolved photoluminescence measurements. The reader is directed to Refs. [9, 10] for further information on lasers

Laser	Laser medium	Pumping mode	Wavelength(s)	Pulse duration	Power
Nd:YAG	Solid-state Nd^{3+} ions embedded in yttrium aluminium garnet ($Y_3Al_5O_{12}$)	Flash lamp, laser diode	Fundamental $\lambda = 1064$ nm Harmonic generation: 533 nm (2nd), 355 nm (3rd), 266 nm (4th)	$\sim$10 ns for Q-switched	2–50 W, continuous MW-GW, Q-switched
Dye	Liquid Organic dye in a non-absorbing solvent. Dye circulated from a reservoir to avoid problems from photodegradation and triplet state formation	Nitrogen laser Excimer laser Nd:YAG laser	Depends on dye: Stilbene (390–435 nm) Coumarin 102 (460–515 nm) Rhodamine G (570–640 nm)	Ring-dye laser—0.1 ps	1 MW
Ti:sapphire	Solid-state Ti^{3+} doped aluminium oxide	Q-switched Nd:YAG CW argon	Broad absorption band of Ti^{3+} enables tuning between 700–900 nm. Frequency doubling leads to wavelengths in 350–450 nm range	$\sim$10 ns Mode-locking generates $\sim$10–100 fs pulse	$\sim$3 W (for mode-locked oscillators) 50 GW for chirped-pulse

14.5 Selecting the Wavelength

For anything but a monochromatic source it is useful to have some wavelength selection, and this is achieved with filters or a monochromator.

14.5.1 Filters

Apart from filters for specific applications, it is useful to have a set of general use cut-off filters in the laboratory to remove damaging UV radiation in irradiation systems, and to block excitation light and low wavelength light to reduce scatter in detection systems. In many cases the judicious use of filters can significantly improve experiment design. Some instruments have integral absorption filters, which can be put into the excitation or emission beams to minimise stray light, second order effects (see below), or reduce light intensity. If internal filters are not available, filters can still be used, placed in the sample compartment, before and/or after the sample. There is a very wide range of filters of different characteristics available, which generally fall into three classes: (i) absorption filters, (ii) dielectric filters and (iii) neutral density filters. A selection of different filter types can be seen in Fig. 14.5.

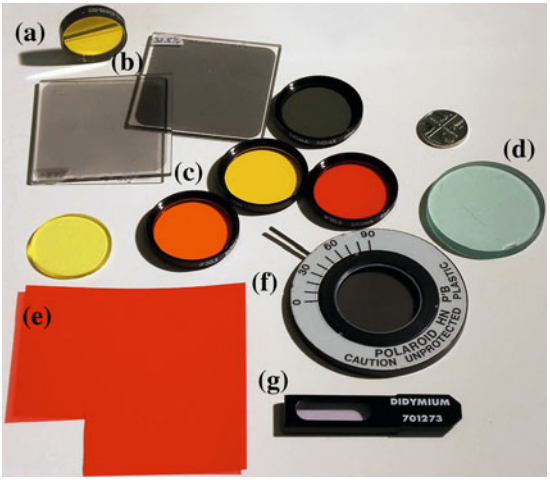

Fig. 14.5 A variety of typical filters. **a** An interference bandpass filter showing the typical mirrored surface of these types of filter; **b** two square silvered glass neutral density filters and a round neutral density filter of a colloidal dispersion in glass; **c** a series of round, coloured absorption 'cut-off' filters, and **d** a pale blue glass heat filter; **e** a 'Wratten' filter of dyed gelatin, which is flexible and easily cut to shape; **f** a polariser; **g** a didymium absorption wavelength standard. The 5p UK coin, included top left to give some idea of scale, has a diameter of 18 mm

Absorption filters are made using molecular or semiconductor materials dispersed in a support which is usually either glass, quartz, plastic or gelatin [11]. In these filters the incident energy not transmitted is absorbed within the filter, and care is required to ensure irradiation is not so intense as to cause the filter to shatter, or, in the case of plastic or gelatin filters, to melt. Care should also be taken when using such filters to ensure the filter itself is not luminescent, or, if it is, that any filter luminescence will not interfere with the experiment being performed. A luminescent filter on the detection side of an emission experiment can be a source of spurious results and consequent embarrassment!

Dielectric filters are made from a series of layers of thin dielectric coatings deposited onto a transparent, usually glass or quartz, support [12]. The structure of the dielectric coatings is such that interlayer reflections interfere constructively or destructively in the forward or backward directions, resulting in a filter which either reflects or transmits selected wavelengths of light. Since they operate on the principle of interference, the wavelengths transmitted/rejected depend on the angle of incidence the radiation makes upon the filter. If the instrument set-up means that light is incident upon the filter across a range of angles, then the filter performance can be significantly shifted from that with perpendicular irradiation. (This can be demonstrated easily by looking through a dielectric band-pass filter held up to a white light while shifting the angle of the filter with respect to the eye-to-source axis, a range of colours will be seen depending upon angle.) In these filters most of the rejected light is reflected, and they have a mirrored appearance. A range of dielectric filters are available: band-block, IR reflecting (hot) mirrors, visible reflecting (cold) mirrors, and band-pass filters. When selecting a band-pass filter it is important to consider the band-width required since a narrow bandwidth will result in lower transmission intensity. The best sources of information for these are manfacturers data books and catalogues [12].

Neutral density (ND) filters have a nominally constant absorption across the spectrum, and therefore reduce the light intensity for all wavelengths by the same fixed amount. They are either silvered glass or quartz, or colloidal carbon or metal in plastic or gelatin. They are very useful when determining the dependence of photochemical reaction yield on photon flux, which can be important in mechanistic studies. For example, the dependence of a steady-state yield, or transient absorption in flash photolysis, on the light intensity can show whether monophotonic, biphotonic or other processes are involved. ND filters are also useful to generally reduce light intensities in experimental arrangements to give matched transmission values or reduce intensities to keep within the linear range of detectors. Metal mesh filters are some times used as neutral density filters, but their effect depends upon where they are placed in the optical train. For example, when placed immediately before an irradiated sample, mesh filters reduce the area illuminated but do not reduce the intensity of the light on those areas which remain illuminated; therefore it can be expected that the apparent result of such an experiment will always be a linear dependence upon light intensity, even if the true dependence, as obtained using a true ND filter, is non-linear. If placed earlier in the optical train, e.g., immediately after the irradiation source, and before any beam

focussing, they may, depending upon the exact optical arrangement, act as reasonably uniform attenuators.

For some applications it is useful to have a filter solution rather than a solid filter. These are not commercially available but a list of useful solution filters is given in the literature [13]. These may be used in conjunction with absorbance filters. Often it is possible to use the solvent cut-off absorption wavelength (see Table 14.1) and/or one of the reaction components themselves to make up a filter to remove specific wavelengths relevant to the experiment. However care should be taken to check that such a filter solution remains effective throughout the experiment and is not subject to photochemical, thermal, or chemical, degradation. Special care should always be taken when interpreting spectra recorded using filters because light transmitted at the edge of the filter cut-off curve can result in a signal which looks like the edge of an absorption or emission band from the sample.

Solid-state reflectors and absorbers are often useful additions to an experimental set-up. MgO and $BaSO_4$ powders are widely used as white reflectance standards, (see Fig. 14.6), which reflect $\sim$ 95 % of light between 400–700 nm and $>$ 95 % of light 340–1400 nm, respectively [14]. A material that absorbs uniformly across the spectrum is also useful as a matt black paint coating to prevent scattering of light in optical instruments, and also as a uniform absorber for use in thermopiles and calorimeters which rely on the conversion of radiant energy to thermal energy. In thermopiles and surface calorimeters all of the incident energy is converted into heat in a very thin surface layer and care must be taken to limit the incident irradiation intensity to prevent damage to the absorbing surface. This is particularly important in calorimetric pulsed laser meters, where a single pulse may be sufficient to cause serious damage to a surface coated calorimetric detector. For

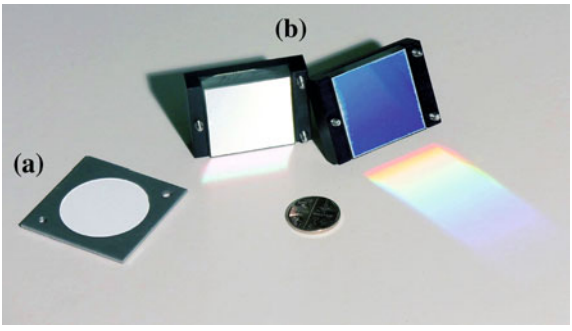

Fig. 14.6 **a** A diffuse reflectance plate used as a reference plate in an integrating sphere; **b** two diffraction gratings showing the effect of number of lines per mm on dispersion. The one in the *middle* of the photograph shows low dispersion because it has only 150 lines per mm (it is for use in the IR), while that on the *right*, showing better dispersion, is ruled with 600 lines per mm for use across the UV/Vis and near infrared (the *blue* colouring of the face of the grating is a consequence of the angle between camera, grating and light source). The 5p UK coin, included in the *middle* of the photograph to give some idea of scale, has a diameter of 18 mm

laser intensity measurements the absorber is often designed so that absorption occurs in the body of the detector element rather than just at the surface.

14.5.2 Monochromators and Spectrographs

The key elements of most monochromators are the diffraction grating, which disperses the radiation, and the entrance and exit slits. Some earlier systems used a prism for wavelength dispersion instead of diffraction gratings. A diffraction grating is a flat, or curved, surface which has a set of very closely spaced (100–1000's of lines per mm) parallel lines cut, etched, or deposited on it. When white light is incident upon the grating, interference between the light reflected from each line results in each wavelength of the incident beam being reflected at a different angle. Thus, the grating separates out the wavelengths in the original white light source and spreads then out into a fan of coloured light, a spectrum. The angular dispersion is proportional to the number of lines per mm on the grating, so a grating with a lot of lines per mm spreads the spectrum out across a wider beam than one with a few lines per mm (see Fig. 14.6). In the monochromator, part of this dispersed spectrum from the grating is made to fall upon the exit slit, which therefore only lets a certain band of wavelengths through. The wavelength of light passed through the monochromator is chosen by varying the angle the grating makes to the excitation and emission beams. This is usually achieved using a screw thread arrangement with wavelength measured directly from the number of turns of the screw. A diffraction grating does not diffract all wavelengths with equal efficiency, but rather shows a smooth curve of efficiency against wavelength. The blaze angle is the angle at which the grooves are cut into the grating, and by choice of this angle the wavelength at which the diffraction grating operates at maximum efficiency, the blaze wavelength, can be set. The transmission efficiency of a monochromator falls off either side of the blaze wavelength. Typically in a fluorimeter an excitation monochromator will be blazed at a lower wavelength than an emission monochromator. It is worth checking the blaze wavelength of any grating used.

Monochromators usually operate with equal input and exit slits. The light from a monochromator with input and exit slits of equal width illuminated by a spectrally uniform source is not strictly monochromatic, but is rather a range of wavelengths, which if plotted as intensity *vs* wavelength gives an isosceles triangle of light; the width at half height of this triangle of light is termed the *bandwidth* (or *bandpass)*. The bandwidth of a spectrophotometer is determined by the instrument geometry, the number of lines per mm on the diffraction grating, and the slit widths on the monochromator. For monochromators with adjustable slits, the slit width is usually measured or given in mm, and the bandwidth, in nm, is then obtained by a conversion factor for the particular monochromator and diffraction grating used. A typical value for a 'fast' monochromator, *i.e.*, one which lets a lot of light through, would be about 4–5 nm per mm, but each instrument will have a

specific value. As slit width is narrowed spectral resolution is increased, but this is at the cost of reduced transmitted intensity.

The use of a monochromator often involves the compromise between best spectral resolution and best signal-to-noise-ratio (S/N). Because of the monochromator bandwidth, the measured absorption line is always broader than the true line. The degree of instrumental line broadening is determined by the ratio between the instrument bandwidth and the true bandwidth. An instrument bandwidth of $\sim$1/5th the true bandwidth will give a measured bandwidth only a few percent higher than the true bandwidth. Such a bandwidth ratio is acceptable for most applications and there is usually no advantage in using a bandwidth that is much narrower, since this results in lower S/N without any increase in spectral information (see Fig. 14.12). Monochromators have either a set of fixed slits or continuously variable slits—typically giving bandwidths in the range 0.1–10 nm. Narrow bandwidths are used in absorption studies in the gas phase; typical bandwidths for absorption studies in the gas phase are 0.1–2 nm; bandwidths for general solution phase emission spectroscopy are often wider, typically 1–20 nm; for time-resolved studies, S/N considerations usually mean relatively wide bandwidths, typically in the 2–30 nm range, are used. When using wide bandwidths care should be taken to make allowance for any deviation from the Beer–Lambert law arising from the wide bandwidth.

For a lamp-monochromator combination as an irradiation source for photochemical reactions the compromise is between maximum photon flux and spectral selectivity. Due to the need for a high photon flux, irradiations for photochemical reactions are usually carried out using wider bandwidths than those used in absorption or emission spectrometers. Bandwidth is a particularly important consideration when using sources that contain some line emission such as Hg or Xe lamps, since line emission within the bandwidth can dominate even though it is not the wavelength shown on the monochromator setting or readout.

A monochromator set at wavelength λ nm, will also transmit wavelengths of $\lambda/2$, $\lambda/3$, $\lambda/4$… nm, produced by second order, third order, fourth order, etc. dispersion. Thus, in the absence of any filtering, an excitation monochromator set at say 500 nm will also let through unwanted 250 nm radiation from the source. Similarly, scanning an emission monochromator to obtain the emission spectrum for a sample excited using say 225 nm radiation will show bands at 450 and 675 nm due to the second and third orders of the 225 nm excitation wavelength. If these artifacts become troublesome then judicious use of filters can usually remove them.

A monochromator is never perfect and there will always be some residual stray light transmitted for all wavelengths incident on the entrance slit. Stray light is particularly important when trying to measure intensities of wavelengths where the detector has poor sensitivity, e.g., using a photomultipler tube (PMT) to measure near IR radiation. In such a case, stray light may be only a very low fraction of the total energy but if it falls in a region of high PMT sensitivity it may swamp the PMT response to the radiation of interest. Again, judicious use of filters can reduce the problem significantly. Some high-resolution instruments use two

monochromators, one after the other, in a double monochromator arrangement, to reduce stray light.

A spectrograph has an entrance slit but no exit slit because the output is a long strip of light where the spectrum is spread out in a controlled manner and made incident on an array of discrete detector elements, or *pixels*, *i.e.*, a CCD or photodiode array. A spectrograph bandwidth refers to the entire wavelength range incident on the detector array. The optics of a spectrograph differ from those of a monochromator because of the need to bring all of the wavelengths to focus on the flat surface of the detector. The resolution of a spectrograph, *i.e.*, the ability to separate two spectral lines which are close together, is, for a given diffraction grating, determined by the entrance slit width, until this is reduced to the width of a single pixel when the spectrograph is at its limiting resolution with that particular grating and array. Thus increasing the amount of light incident on the detector by opening the entrance slit leads to a loss of resolution in a similar way that widening entrance and emission slits on a scanning monochromator gives a loss of resolution because of the increase in bandwidth.

14.6 Measurement of Light Intensity

Light intensity can be measured by a physical device, a *radiometer*, probably the simplest of which is a calorimetric detector which converts radiant energy into heat (see Fig. 14.7), or by a chemical change, an *actinometer*. A number of chemical actinometers, for both the solution and gas phase, are available, and the standard methodologies for their use are described in detail in the Handbook of Photochemistry (see Sect. 14.12.2). Potassium ferrioxalate is commonly used as a chemical actinometer in the UV-green spectral region ($\sim$250–500 nm). A significant advantage of a chemical actinometer over a radiometer is that it can be placed in exactly the same optical position as a solution sample. In addition, relatively low intensities can be measured simply by integrating over a long time. The physical parameter measured differs for the two devices: actinometers give a response proportional to the photon flux, whereas many radiometers respond to the energy flux. If irradiation is over even a moderate spectral range, then, unless the spectral distribution is known, precise inter-conversion between photon and energy flux is not straightforward. If the spectral distribution is known, interconversion between energy and photon fluxes can be made using the average photon energy.

In a typical application using the potassium ferrioxalate actinometer, aqueous solutions of $K_3[Fe(C_2O_4)_3]$ are photolysed with monochromatic light, leading to reduction to iron(II) through the overall reaction:

$$2\left[Fe(C_2O_4)_3\right]^{3-} + h\nu \rightarrow 2Fe^{2+} + 2CO_2 + 5C_2O_4^{2-} \qquad (14.3)$$

The resulting Fe^{2+} is then determined spectrophotometrically as its 1,10-phenathroline complex, and the total number of photons absorbed by the irradiated

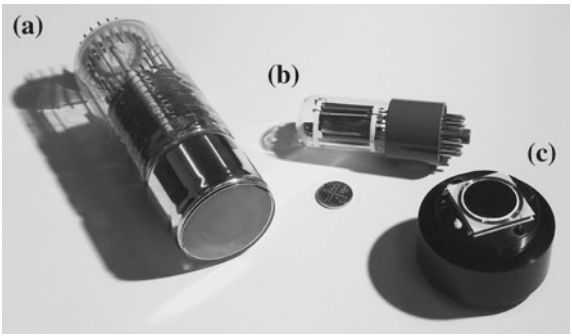

Fig. 14.7 **a** Front face PMT. The photosensitive surface, the photocathode, is at the front, at the back can be seen the pins used in the circuit to fix the dynode voltages. **b** A side window PMT. Here the photocathode is behind the grid towards the left centre of the device. **c** The thermoelectric detector element of a calorimetric laser power meter, the laser pulse is made incident on the thin black disc in the centre of the device, absorption converts the photon energy into thermal energy, which is subsequently measured using the thermoelectric effect in a thermopile immediately behind the disc. When in use the unit shown is held in a thermally isolated chamber which is screwed over the top of the detector element. The 5p UK coin, included in the *middle* of the photograph to give some idea of scale, has a diameter of 18 mm

solution per unit of time is then calculated from this, the irradiation volume, irradiation time and ferrioxalate decomposition quantum yield [6]. The ferrioxalate actinometer is simple to use and has excellent sensitivity over a wide wavelength range. Other systems, such as Reinecke's salt ($NH_4[Cr(NH_3)_2(SCN)_4]$), are available for long wavelengths, while reusable photochromic systems have also been proposed as convenient chemical actinometers. These, and other systems for specific applications, are discussed in Ref. [6].

Chemical actinometers, such as these, are very convenient for determination of quantum yields of photochemical reactions. Typically, the amount of product formed in the reaction of interest on photolysis with monochromatic light is compared with the extent of reaction of the actinometer under the same conditions (irradiation time, etc.). The quantum yield is obtained using the product ratio and the quantum yield of the actinometer [13]. Corrections can be made for differences in absorbance, irradiation time or reaction media.

14.7 Detectors

The most important requirements of a detector are sensitivity and selectivity. The sensitivity determines the lowest signal that can be measured by the detector. Selectivity is achieved when the detector elicits a strong response to a specific input. All real measurements are affected to some extent by noise. The signal-to-noise ratio (S/N) is a measure of the desired signal against the background noise

(for example, one definition takes the S/N ratio as the difference of peak and background signal, divided by the square root of the background signal). Although various methods, such as the Savitzky–Golay algorithm [15] have been used to smooth data points, the overall sensitivity of the system is limited by the S/N ratio. Background noise includes electronic noise (random fluctuation of an electrical signal), but also external effects that may influence the measurement, such as vibrations, temperature or humidity fluctuations. Performing the experiment under controlled conditions may eliminate some of these external influences, but electronic noise will always be present. If the characteristics of the noise are known it may be possible to filter it or to reduce it by signal processing; for example, if the background noise fluctuates much more rapidly than the signal of interest, introducing an electronic filter with appropriate time constant, or post-collection averaging of data points over time will help reduce noise.

14.7.1 Photodiodes

Photodiodes are *p–n* or *p–i–n* junction semiconductors that generate a current or voltage upon irradiation with light that is proportional to the rate of photon absorption [16]. When light of energy greater than the band gap, E_g, strikes the photodiode, an electron is promoted from the valence band to the conduction band, leaving behind a hole. If this process occurs in the depletion (charge carrier free) zone of the junction, application of an external voltage sweeps the electrons and holes towards the cathode and anode respectively, generating a photocurrent. The current generated by the photodiode is proportional to the incident light power and the sensitivity is limited to one electron per photon absorbed ($\sim$0.5 A/W for $\sim$2 eV photons, *i.e.*, $\sim$620 nm). However, photodiodes are capable of measuring fairly high light intensities ($\sim$1 mW) with fast response times ($\sim$0.01 ns), making them suitable for measurement of laser powers, for example. Sensitivity can be improved using avalanche photodiodes, which operate under high reverse bias to enable multiplication of the charge carriers created by the initial electron–hole pairs created by photon absorption. This avalanche action enables the gain of the photodiode to be increased by several orders of magnitude. The wavelength sensitivity is determined by the semiconductor from which the photodiode is constructed: silicon photodiodes typically cover the $\sim$400–1000 nm region and InGaAs photodiodes the region $\sim$900–2500 nm.

14.7.2 Photomultiplier Tubes

Photomultiplier tubes (PMTs) (Fig. 14.7) have a light-sensitive cathode from which photoelectrons are ejected when illuminated. These primary photoelectrons are then accelerated by an electric field and made incident on a secondary emissive

layer which ejects many electrons for each, now high energy, incident photoelectron. These secondary electrons are, in turn, accelerated and made incident on another secondary emissive layer which ejects many more electrons. Repetition of this process at a number of secondary emissive layers (*dynodes*), leads to amplification of the current by as much as 10^8 electrons per photon. This current is collected at the photomultiplier anode as the output of the PMT, (although it is measured and recorded as a voltage across a resistor). Both the photomultiplier response and background noise depend on the photocathode and dynode voltages, and increasing the operating voltage significantly increases both sensitivity and noise. Photomultipliers need a very stable variable high voltage power supply that provides a few hundreds of volts, or a kV or so, to produce stable photocathode and dynode voltages. PMTs can be used in DC mode, in AC mode with a chopped light source which is most useful for low light levels, or in single photon counting mode. Photomultiplier tubes are the most sensitive and commonly used detectors for the 180–850 nm range. The wavelength sensitivity of some PMTs extends out to 1700 nm but for operation above *ca.* 850 nm photomultipliers must normally be cooled to *ca.* −80 °C to reduce thermal noise to an acceptable level. The time resolution of a conventional PMT is typically a few hundred ps but a change in design of the way electron amplification occurs has led to development of Multi-Channel Plate photomultiplier tubes, MCP-PMTs, which have time resolution in the tens of ps range. In ordinary PMTs amplification is brought about using a series of discrete dynodes whereas in MCP-PMTs amplification occurs within a honeycomb of capillaries each 6–20 μm diameter and coated with an electron emissive surface so that the whole capillary surface acts as an electron amplifier. The photocathode is placed a few mm from the multichannel plate so that the primary photoelectrons are caught in the capillaries of the plate and the electron current is amplified by secondary emission of electrons as the electron stream bounces back and forth from the walls of the capillary. MCP-PMT are the fastest photomultiplier tubes available but they are expensive and can be easily damaged by excessive light levels. Hamamatsu, a major manufacturer of photomultipliers, has an excellent photomultiplier handbook available online [17].

14.7.3 Charge-Coupled Devices

Charge-coupled devices (CCDs) are silicon-based imaging detectors containing a two-dimensional array of accumulating wells, or *pixels*. Each pixel is composed of a $Si–SiO_2$ metal–oxide–semiconductor (MOS) capacitor, which operates as a photodiode and a storage device, accumulating electric charge in proportion to the number of photons striking the depletion zone of each individual well. CCDs typically contain up to 500,000 pixels, and sensitivity can be several orders of magnitude better than a PMT at low light intensities. CCDs are commonly encountered in fluorescence microscopy—the charge at each pixel is read out after a specific time to construct a two-dimensional image. Peltier-cooled CCDs can

achieve dark noise counts of less than one electron per pixel per day, giving rise to high contrast low background noise images. CCDs can detect photons in the 400–1100 nm spectra range, with peak sensitivity normally in the range of 550–800 nm with quantum efficiencies of 40–60 % [16], and are currently proving attractive as detectors in reasonably priced miniature spectrometers and fluorimeters [18].

14.8 Data Collection, Analysis, and the CIE Representation of Colour

14.8.1 Digitisation

A detector operating in single photon counting mode provides a digitised response directly. Analog signals from detectors are usually digitised using an analog-to-digital (A-to-D) converter. For time resolved studies, in which a response is measured over a period of time, a digital oscilloscope is a very convenient A-to-D converter, but there are also plug-in A-to-D converters for PCs which, with appropriate software, can be used to convert a PC to a virtual oscilloscope. The two most important features of the oscilloscope, or signal recorder, are time resolution, and signal, *i.e.*, voltage, resolution. For ns flash photolysis a time resolution in the region of a few ns per data point is required, while for work with μs pulsed lamps, pulsed Xe arc or flash lamps, resolution in the μs range per point is required. A range of 1024–4096 data points per recorded event is typical.

Instruments usually digitise voltage signals into 8, 10, or 12, (or even 16) bit signals, *i.e.*, the voltage signal range is split into 256, 1024, or 4096 digital values. The sensitivity of the recorder is usually given by the size of the signal voltage that will give a full-scale deflection. Oscilloscope voltage ranges are usually specified as volt/div. with ten divisions (div.) full scale. Typical maximum sensitivities for oscilloscopes are 1–5 mV per div. *i.e.*, 10–50 mV full-scale, while low cost A-to-D converters are generally less sensitive. An increase in time resolution usually has an associated loss in finesse of digitisation, so most ns work is at 8 bit digitisation, while ms work, or wavelength or other scanning, can easily be 12 bit.

14.8.2 Signal to Noise Considerations

Signal-to-noise in photophysical measurements comes down to the ratio between the signal generated by photons incident on a detector, and the background noise of the detector and instrument electronics. Experimentally, S/N is usually increased by: increasing photon absorption by increasing sample concentration (although this is often fixed by the experiment); changing instrument settings to

increasing the number of photons incident on the detector per second; decreasing background noise by increasing the time constants of the electronics which results in effectively integrating the signal over a longer time; cooling the detector to reduce noise; or signal averaging. Increasing the number of photons incident per second usually has the associated penalty of reducing spectral resolution since the increase in photon flux is usually achieved by widening instrument monochromator slits. Increasing instrument time constants usually has the associated penalty of reducing the time resolution of the experiment, which may not be acceptable in fast time-resolved studies. Since the S/N ratio for an averaged signal improves as the square root of the number averaged, it is often time effective to average four signals, which doubles S/N. But it requires the average of 16 experiments to get a 4-fold improvement in S/N, so it is always best to optimise S/N instrumentally before having to rely on signal averaging to improve S/N. Furthermore, some experiments are not suitable for averaging, for example if there is a risk of photodegradation of the sample. Most experimental arrangements end up being a compromise between S/N, spectral resolution and time resolution. Knowing what is required from the experiment and how these three factors are related helps in making the optimum choice of experimental/instrumental variables.

14.8.3 Data Analysis

Most instruments have associated software for data analysis, but it is useful to be able to curve fit data independently of any particular software restrictions, so a method to export data as a number file, such as an ASCII file or similar, to general curve fitting programs (e.g., Table Curve, IGOR Pro, Origin) is useful. When decay curve fitting from lifetime studies, it is important to select the relevant section of the data for fitting. To give a common example; if the time over which data is collected is much longer than the decay itself then curve fitting can be dominated by the noise on the tail of the curve where the signal is essentially zero. The quality of a curve fit is always improved by addition of more parameters, but care has to be taken to ensure that the parameters are physically meaningful. A plot of the residuals, *i.e.*, the difference between experiment and theoretical data, is one of the most useful ways to evaluate the quality of the curve fit, a good curve fit should have residuals evenly distributed around zero for the whole curve. Examples are given in Chap. 15. Most curve fitting programs also give the standard deviations for the various parameters used to create the curve, and these should be given along with the parameters themselves when reporting results.

14.8.4 Perception of Colour and Colour Representation

For many technological applications, the *colour* and *luminance* of the emitted light can be extremely important. Luminance is often used to characterise emission or reflection from a flat, diffuse surface and is an indicator of how much luminous power is perceived by the human eye looking at the surface from a particular angle of view, *i.e.*, how bright the surface will appear. Luminance is the luminous intensity per unit area projected in a given direction (in candela per square metre).

Colour perception in humans is initiated by the absorption of light by three different spectral classes of *cone cells* present in the retina, conveniently referred to as blue, green and red. Each class exhibits a different but overlapping spectral sensitivity, with maximum values at *ca.* 419, 531 and 558 nm respectively. The sum of the differing sensitivities is called the *photonic response* and displays a maximum value at 555 nm. However colour perception can be very subjective, and the description of colour differences can be quite challenging. Colorimetry and the trichromatic perception of colour are based on Grassmans' laws [19, 20]:

1. Any colour may be matched by a linear combination of three other primary colours, provided that none of these may be matched by a combination of the other two.
2. A mixture of any two colours can be matched by linearly adding together the mixtures of any three other colours that individually match the two source colours.
3. Colour matching persists at all luminances.

In practice, experiments on the additive mixture of light prove that there are no three colours which when mixed additionally can duplicate all spectral colours. Whilst the mixture may exhibit the required spectral hue, it generally fails to duplicate the required saturation for that colour. The only approach to obtain a perfect match is the addition of a 'negative' colour, to desaturate the spectral hue. To overcome this problem in 1931 the CIE (Commission Internationale de l'Éclairage) defined a system based on colour coordinates to characterise the colour properties of light [19, 20]. The primary colours (*X*, *Y* and *Z)* in this system are theoretically defined super-saturated colours, which lie outside the bounds of the spectral locus, eliminating the need for 'negative' colours. In order to standardise this system, the CIE defined a secondary standard observer based on the differing sensitivity of the three classes of human cone cells. Consequently any colour, *C,* of wavelength, λ, may be expressed as:

$$C_\lambda = \bar{x}_\lambda X + \bar{y}_\lambda Y + \bar{z}_\lambda Z \tag{14.4}$$

where *X, Y, Z* are the system primaries (known as *tristimulus values)* and $\bar{x}_\lambda$, $\bar{y}_\lambda$ and $\bar{z}_\lambda$ are the colour-matching functions. The luminance and CIE colour response are obtained by determining the spectral energy distribution of a sample using a spectroradiometer and subsequently processing the data using appropriate software

containing the CIE colour matching curves. Alternatively, since values for the colour-matching functions as a function of wavelength are available in the literature [19, 20], the *X, Y* and *Z* tristimulus values may also be determined from properly corrected photoluminescence data in the visible region according to:

$$\left\{\begin{array}{l} X = \sum_{\lambda=380}^{700} \bar{x}_\lambda E_\lambda(\Delta\lambda) \\ Y = \sum_{\lambda=380}^{700} \bar{y}_\lambda E_\lambda(\Delta\lambda) \\ Z = \sum_{\lambda=380}^{700} \bar{z}_\lambda E_\lambda(\Delta\lambda) \end{array}\right\} \tag{14.5}$$

where E is the emission intensity at wavelength, λ. To simplify the calculations, sampling wavelength bands ($\Delta\lambda$) at 5 or 10 nm apart is adequate. The *chromaticity* of a colour is specified by two parameters, x and y, known as *chromaticity* or *colour* coordinates, which are functions of the *XYZ* tristimulus values, given by:

$$x = \frac{X}{X+Y+Z} \qquad y = \frac{Y}{X+Y+Z} \tag{14.6}$$

The x and y chromaticity coordinates are typically plotted in a two-dimensional grid known as the CIE (x,y)-chromaticity diagram (Fig. 14.8). The curve is made of the pure spectral colours from the blue to the red, covering the entire visible

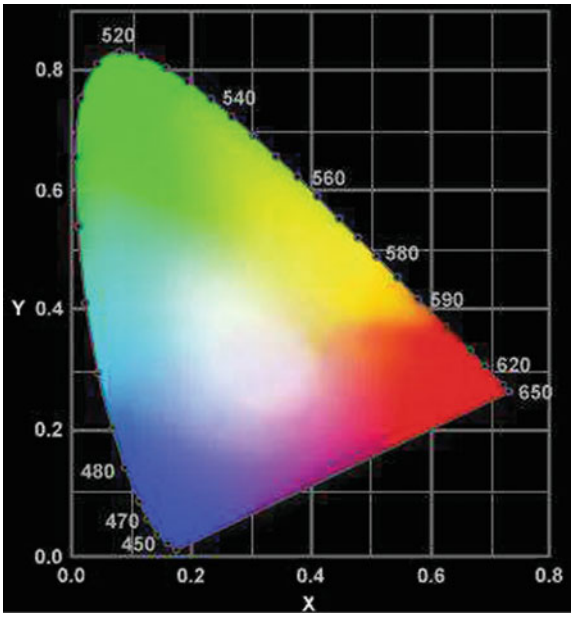

Fig. 14.8 The CIE (x,y)-chromaticity diagram

spectrum (380–770 nm) and is known as the *spectral locus.* The two extremes of the spectral locus are connected by a straight line, the *purple boundary,* which represents colours which cannot be formed from any single part of the spectrum, but which must include a mixture of at least the two extremes of the visible spectrum. Consequently, the colours represented by the purple boundary are not pure spectral colours. The centre of the diagram is taken as the *white point,* whose coordinates are designated as (0.33, 0.33). The area restricted by the diagram, the spectral locus and the purple boundary encloses the domain of all colours.

14.9 General Instrumentation and Techniques

14.9.1 UV/Vis/NIR Spectrophotometer

A scanning UV/Vis spectrophotometer is essential; a schematic representation is shown in Fig. 14.9. Moderately priced instruments typically cover the wavelength range *ca.* 190–800 nm, but interest, and technological advances in detectors, have pushed the range available into the NIR, although at increased cost. Full wavelength instruments either scan each wavelength independently as a grating is rotated within a monochromator, or use a detector array with a spectrograph to give simultaneous measurement across the full spectral range. The latter are often referred to as 'diode array' spectrophotometers, and are particularly useful for kinetic studies where full spectra recorded at the same instant are preferred.

A typical scanning UV/Vis will use a tungsten lamp for ~320–800 nm (and NIR if applicable), and a deuterium lamp for ~190–320 nm, with an automatic lamp change, the position of which can usually be altered, within the ~300–340 nm range. Internal wavelength calibration on start-up is often carried out using the 486.0 and 656.1 nm lines of the deuterium lamp. There are also a number of automatic filter changes as the spectrum is recorded, as filters are interposed in the light beam to reduce stray light and second order light. The detector is typically a photomultiplier tube for the UV and visible regions; and a cooled PbS detector for the NIR region. High-specification UV/Vis/NIR

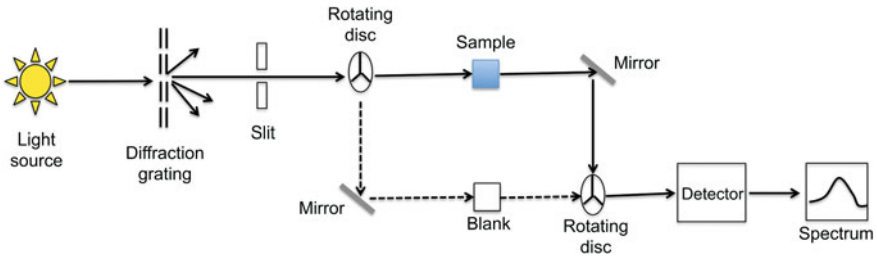

Fig. 14.9 Schematic of a dual-beam UV/Vis absorption spectrophotometer

spectrophotometers also incorporate an InGaAs detector to bridge the spectral gap between the PMT-PbS switching wavelength, thus ensuring high sensitivity across the entire measured range.

Most low to mid-range standard laboratory instruments have a fixed bandwidth of *ca.* 1–2 nm. More expensive instruments include variable slits, usually in fixed sizes but sometimes continuously variable, typically across the range 0.1–10 nm. Narrow slits are used for gas phase studies and narrow solution lines (such as in lanthanides, see Fig. 14.12), while wide slits are useful for matching absorption spectra to emission excitation spectra which are often recorded using a bandwidth wider than 2 nm. High specification instruments working with very narrow bandwidths may use a double monochromator arrangement to minimise stray light.

The design of the instrument sample compartment determines what can be studied. Typically, use of cells of up to 10 cm path length with a heating/cooling block for temperature control is easy, and some instruments allow the whole sample compartment to be removed so a custom built sample compartment can be inserted, or unusual samples studied. If the sample compartment lid needs to be removed for any samples of unusual shape, then a few layers of black cloth generally reduces stray light enough for measurement, although it is also a good precaution to dim the room light to the minimum convenient level, and to also check the effect of removing all room light completely.

Absorption of solids is usually measured using diffuse reflectance. Diffuse reflectance measurements typically use an integrating sphere, which replaces the normal spectrophotometer sample compartment. Diffuse reflectance relies upon the focused projection of the spectrometer beam onto the sample where it is reflected, scattered and/or absorbed. Both specular and diffuse reflectance will be generated by the sample. By placing either a diffusely reflective panel or a light absorbing cup at the angle of specular reflectance two different spectra can be recorded, *i.e.*, total reflectance or diffuse reflectance respectively. Reflectance, R, and concentration, c, are not linearly related, but, under certain circumstances the Kubelka–Munk function, $f(R)$ (Eq. 14.7), which assumes infinite sample dilution in a non-absorbing matrix, a constant scattering coefficient, s, and an infinitely thick sample, is linearly related to c:

$$f(R) = \frac{(1-R)^2}{2R} = \frac{k}{s} = \frac{Ac}{s} \tag{14.7}$$

where R is the absolute reflectance of the sample, k is the extinction coefficient and A is the absorbance. It is often convenient, therefore, to present diffuse reflectance spectra in terms of the Kubelka–Munk function. k is the imaginary part of the complex index of refraction (the real part is given by the refractive index, n), which is related to the molar absorption coefficient by:

$$\varepsilon = \frac{4k}{\lambda} \tag{14.8}$$

Universal reflectance accessories to measure the absolute specular reflectance of polished surfaces and films are also available.

Absorption instruments can be single or double beam—the latter having a second light beam for a reference, or blank, which should contain everything except the compound of interest (e.g., solvent, buffer etc.). Due to absorption by cell materials and solvents, it is a useful check to run a preliminary spectrum of the blank against air, so that the cut-off wavelength of high blank absorbance, below which absorption measurements are meaningless, can be identified. Single beam variable wavelength, but non-scanning, UV/Vis spectrophotometers are relatively cheap instruments which are very useful for single wavelength kinetic studies or studies of absorbance changes arising from system response to external variables, such as sensor response studies. They are also excellent for optically matching solutions for relative quantum yield measurements.

Solution and gas phase spectra are usually presented in absorbance mode but can easily be transformed to transmittance using the Beer–Lambert law. Solid state spectra are presented as diffuse reflectance, the Kubelka–Munk function, or % reflectance, as described above. However, first, second, and even high, derivative spectra are also quite common. These higher derivative spectra are useful in picking out structure in spectra, showing vibrational shoulders etc., or components in mixtures [21].

Without doubt the most common error when first beginning to work with absorption spectroscopy is that of attempting to measure spectra of samples with absorbances which are too high for accurate measurement. A typical general laboratory instrument is usually reliable up to an absorbance of about 2–3, *i.e.*, with the sample transmitting at least 1–0.1 % of the incident light; although the range may have to be reduced in regions of the spectrum where lamp intensity or detector sensitivity is low, or if narrow slits are used. With such an instrument it is best, if possible, to work within the absorbance range of $\sim$0.2–1.7, but it is a relatively simple procedure to evaluate spectrophotometer performance using a solution which obeys the Beer–Lambert law at a range of concentrations, or the same solution in different path length cells, to cover a wide absorbance range. Stray light is much reduced by using a double monochromator arrangement and some double monochromator instruments claim a useful absorbance range of up to 8.

14.9.2 Steady-State Photoluminescence Spectroscopy

Figure 14.10 shows a schematic of modular components for emission spectroscopy. There is a wide range of fluorimeters available ranging from cheap non-scanning filter instruments to scanning instruments with very high specifications. A moderate price range scanning instrument will typically contain the following components: (i) a 150 W xenon lamp as excitation source; (ii) an excitation monochromator with variable slits with a diffraction grating blazed for maximum output in the UV; (iii) an emission monochromator with variable slits, and grating

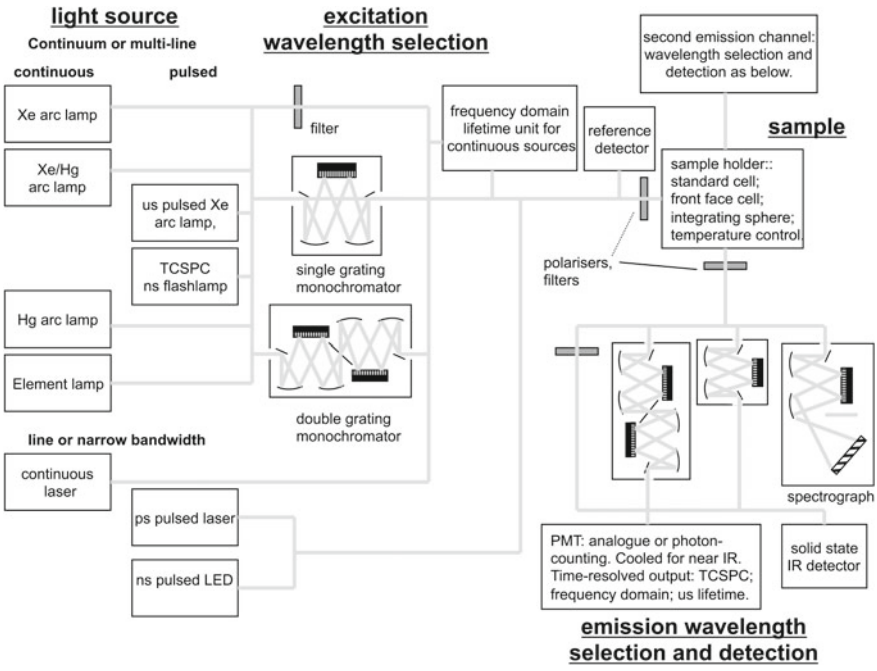

Fig. 14.10 Schematic of modular components for emission spectroscopy. The standard fluorimeter arrangement is: a Xe arc lamp as source; single grating monochromators for excitation and emission selection; and analogue or photon counting PMT as detector

blazed for the visible spectral region; and (iv) a red-sensitive photomultiplier, operating in either analogue, or perhaps photon counting, mode, as detector. Since xenon arc lamps are not very stable, a second reference detector is usually present to monitor and correct for variations in the excitation source intensity. More expensive instruments may use higher intensity lamps such as a 450 W xenon lamp, or have the facility to use an Hg or Hg/Xe lamp with their UV-rich line emission, and will use photon counting detection. Photon counting detection offers advantages over analogue measurements of both improved signal stability and improved signal to noise.

Polarisation studies are common in fluorescence spectroscopy. In the more expensive instruments internal polarisers may be automatically switched between horizontal and vertical alignments, otherwise polarisers placed in the sample compartment require manual switching. Plastic polarisers are relatively inexpensive but will absorb and be bleached by light below about 300 nm and so are unsuitable for UV work.

The instrument sample compartment once again determines what can be studied. Typically, right-angle geometry is used with standard 1 cm square cells for optically-dilute, transparent solutions. In other words, the sample emission is detected at 90° relative to the incident beam; this configuration minimises the

amount of incident light reaching the detector. A heating/cooling block for moderate temperature control is also quite standard. Smaller path length cells, across either the excitation beam, or emission beam, or both, are usually quite easily fitted into the 1 cm standard holder using an appropriate adaptor. Some instruments allow the whole sample compartment to be removed so a custom-built sample compartment can be inserted, or unusual samples can be studied. Again, a few layers of thick black cloth over any unusual samples in a dimly lit room generally reduces stray light enough for accurate measurement, unless the sample signals are particularly weak.

Sample cells with mirrors can enhance sensitivity by reflecting excitation light to give a double pass excitation, and by redirecting otherwise uncollected emission to the detector.

Opaque samples, films and solids are usually studied in the front-face configuration. In this arrangement, the incident beam is focused on the front surface of the sample and the emission is collected from the same region at an angle that minimises reflected and scattered light (typically 22.5° when the sample is orientated perpendicular to the excitation beam). For many instruments it is possible to buy solid-state sample holders which enable sample orientation angle to be altered; depending on the sample type and form, spectra may be improved by changing the sample orientation to an angle of 30° or 60° to the incident beam.

There are a number of common problems and artifacts in fluorescence spectroscopy (see also Chap. 15).

1. **Solvent Raman lines**. When operating even at moderate sensitivity, a fluorimeter will pick up the Raman lines from the solvent. Therefore the solvent blank will show both the scattered Rayleigh band, at the excitation frequency, and lower intensity Raman bands which are shifted from the excitation line by an energy corresponding to the solvent vibrational energy. Raman lines can be identified by: (a) their presence in the emission from the solvent blank alone; (b) the band shape, which is essentially the same as the excitation band, and therefore varies with excitation band width; (c) the position of a Raman band, which varies with excitation wavelength—shifting the excitation wavelength by 10 nm results in a *ca.* 10 nm shift in the position of the Raman band. Some solvents give stronger Raman bands than others, with high energy vibrations, such as OH and CH being particularly troublesome since these are shifted most from the excitation wavelength. If Raman bands are a significant problem then using CCl_4 as solvent which, because of the high atomic masses, has only low frequency Raman bands lying close to the excitation wavelength, may help. Raman bands, however, can be very useful for monochromator calibration and checking the S/N ratio of fluorimeters.
2. **Distorted excitation and/or emission spectra—inner filter and self absorption effects**. It is important to be aware of the optical geometry of the instrument and the absorption characteristics of the sample in the cell used. The excitation optics are usually arranged to focus the beam into the centre of the cell. In practice this gives a thin beam of excitation light, the width of which

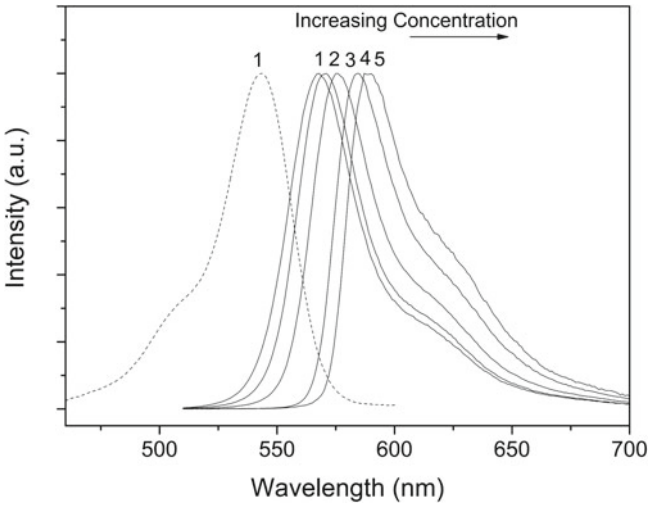

Fig. 14.11 The effect of concentration on the normalised emission spectrum of Rhodamine B in ethanol ($\lambda_{ex} = 500$ nm). The concentrations are: **1** 1×10^{-6} mol dm^{-3}; **2** 4×10^{-6} mol dm^{-3}; **3** 8×10^{-6} mol dm^{-3}; **4** 2×10^{-5} mol dm^{-3}; **5** 4×10^{-5} mol dm^{-3}. The UV/Vis absorption spectrum is also shown for (**1**) (*Dashed line*)

increases somewhat with slit width. Since the Beer–Lambert law indicates an exponential dependence of light transmittance upon optical pathlength, emission intensity is only proportional to absorption extinction coefficient at very low absorbances across the cell. If absorption is high then excitation light is absorbed even before it reaches the centre of the cell. At very low absorbance, the fraction of light absorbed is given by $2.303 \times A$; so even with an absorbance as low as 0.02 in a 1 cm cell, with emission measured from the centre of the cell, the incident light has been attenuated by 2.3 % by the time it reaches the middle of the cell, and a 2.3 % correction to intensity for the excitation spectrum is required. The optimum absorbance depends upon a number of factors, and sample concentration may be fixed by a variety of experimental requirements, but for typical preliminary fluorescence measurements using 1 cm cells, the optical density at the absorption maximum should be kept below 0.1 to reduce gross inner filter effects, and if precise excitation spectra are required it should, emission quantum yield allowing, be much less. In the most commonly used right-angle geometry the detector lens is arranged to collect light from the centre of the cell, and therefore any emission must pass through the sample solution between the centre and edge of the cell. With standard 1 cm cells this will be a 0.5 cm path length. If there is any significant absorption within this path length across the emission band then the emission band shape will be distorted by this 'self-absorption'. This is most notable at the high energy side of a fluorescence band for fluorophores with a small Stokes' shift. Figure 14.11 illustrates the problem of self-absorption in the emission spectrum

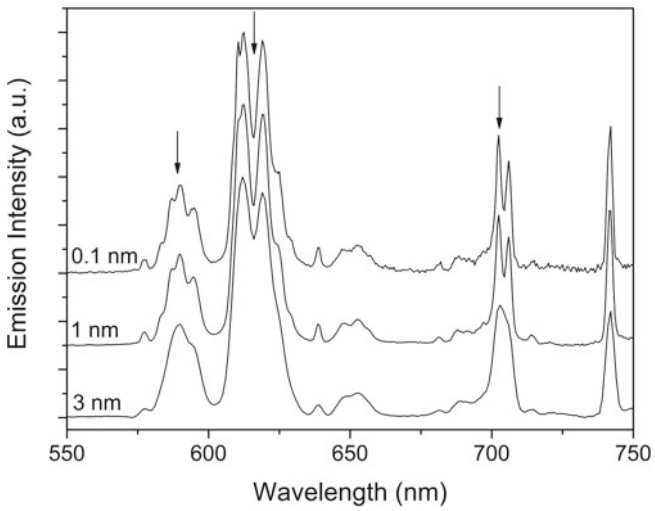

Fig. 14.12 The effect of varying the emission bandwidth on the photoluminescence spectrum of europium (III) (in $Na_{1.08}K_{0.5}Eu_{1.14}Si_3O_{8.5}{\cdot}1.78H_2O$, $\lambda_{ex} = 393$ nm). As the bandwidth increases from 0.1 to 3 nm, a gradual loss in the spectral resolution is observed, but S/N increases

of the laser dye Rhodamine B. As the concentration increases the emission spectrum shifts to longer wavelengths due to reabsorption of the higher energy emission, which results in a gradual shift in the fluorescence colour from green to orange to red. If emission 'self-absorption' is a problem, then either a lower concentration sample or a cell with a narrower path length along the emission path should be used.

3. **Second order transmission by monochromators**. As discussed earlier monochromators containing diffraction gratings will transmit light of wavelength $n\lambda$, where n is an integer. If these different order spectra become troublesome then a filter cutting off below the excitation wavelength on the excitation side, and a filter cutting off just above the excitation wavelength on the emission side will remove them.

14.9.2.1 Recording of Excitation and Emission Spectra

For an emission spectrum, the spectral resolution is provided by the emission monochromator, and so if spectral resolution is important the bandwidth of this monochromator should be set suitably narrow. The excitation bandwidth can be much wider, consistent with a suitably low level of scattered light and no ovelap of emission and excitation bandwidths. Figure 14.12 illustrates the effect of varying the emission slit width on the resolution of the photoluminescence spectrum of a microporous europium(III) silicate ($Na_{1.08}K_{0.5}Eu_{1.14}Si_3O_{8.5}{\cdot}1.78H_2O$). Lanthanide(III) ions typically exhibit sharp, line emission spectra, since their optical

transitions take place predominantly within the 4*f* manifold, where the electrons are largely shielded from crystal field effects by the filled 5*s* and 5*p* shells. If the emission slits are opened too widely, the fine-structure of the emission lines is poorly resolved. Reducing the slit width decreases the spectral bandwidth, thereby improving the resolution of closely spaced emission peaks, but with the penalty of decreasing S/N. Inevitably for weakly emitting samples, a compromise between spectral resolution and sufficient emission intensity must be made. In this instance, with standard detection electronics, longer integration times may be used to improve the S/N ratio.

For a full emission spectrum, the excitation wavelength is usually chosen to be on the high energy side of the longest wavelength absorption band. This is usually a better choice than the absorption maximum because it allows the full emission spectrum, which usually partly overlaps the absorption spectrum, to be recorded. Exactly how far away from the absorption maximum depends upon the Stokes shift, and the bandwidths required for suitable S/N.

The reverse arrangement of monochromator bandwidths applies for an excitation spectrum, and the emission wavelength is usually set to be on the low energy side of the highest energy emission band, for the same reasons as described above.

For both spectra the raw signal provided by the spectrometer is the wavelength dependence of the detector response to the light falling on it. For emission spectra this is not the same as the wavelength dependence of the emission from the sample; the two values differ because both the detector sensitivity and the efficiency of the optics between sample and detector vary with wavelength. For excitation spectra it is not the same as the relative efficiency of conversion of incident light into emitted light; here the two differ because the lamp output is not constant across the spectrum, and the efficiency of the optics between lamp and sample are wavelength dependent. Most spectrophotometers provide manufacturers correction factors, and spectra are usually automatically corrected using these correction factors. If these are not available then spectra can be corrected using the methods described below (Sect. 14.10.1).

14.9.2.2 Phosphorimetery

Fluorimeters which use a pulsed Xe lamp as the excitation source can be used for phosphorescence measurements down to lifetimes of about 10 μs using electronic gating of the detection system, with both gate delay and gate width as variable operator set parameters. The sum of these must be less than the time between pulses unless mechanical shutters are used to isolate individual excitation pulses from the pulse train. In the absence of a single pulse facility, decay curves across *ca.* 10 ms are typically possible; if single pulses can be isolated then much longer decay curves can be obtained.

Fluorimeters which use a continuous light source can usually be adapted for phosphorescence work by addition of a phosphorimeter attachment, which is usually either a pair of mechanical shutters on the excitation and emission

monochromators which can be used in single shot or repetitive chopping mode, or, as in older phosphorimeters, a high speed "rotating can" with a slit in it, placed around the sample, so the slit alternately sweeps past the excitation and detection optics. The time resolution of these phosphorimeters is typically in the ms–s range.

14.9.2.3 Portable and Microvolume Spectrometers

There are a number of small portable spectrometers available, at reasonable cost, in which a fibre optic collector is attached to a small spectrograph-array spectrometer [18]. They are available with fibre optic of different diameters, which control light collection efficiency, and, in part, spectral resolution, and also different gratings and blazes for enhance sensitivity across particular spectral ranges. These are particularly useful for emission from unusual samples, or emitters fixed in particular experimental arrangements. When coupled to a white light source they can also be used for absorption measurements. Recently, spectrometers and fluorimeters have been developed which can be used with μl volumes of samples [22]. These are proving particularly valuable for fluorescence measurements on biological samples.

14.9.3 Near Infrared Luminescence and Steady-State Singlet Oxygen Luminescence Studies

The near IR ($\lambda > 700$ nm) spectral region is becoming increasingly important in the study of a wide range of inorganic and organic lumophores. Photomultipliers are available with sensitivity up to about 1700 nm, while solid state photodiodes can extend the spectral response of detectors to several μm. A particularly important application is in the detection and quantification of singlet oxygen. Many of the expensive fluorimeters can be fitted with an additional special monochromator and detector designed to measure the very weak emission from singlet oxygen centred at 1270 nm. An example is given Chap. 15. The monochromator is blazed for this wavelength range, and since the emission band is quite broad a wide bandwidth can be used. A cut-off filter is used to remove all lower emission wavelengths especially since visible light from sample emission in the second, or third, order spectra may interfere. Either a highly sensitive liquid nitrogen cooled photomultiplier or solid-state device is used as the detector. The presence of the characteristic emission band at 1270 nm is evidence of singlet oxygen production and the yield is determined in the same way as a fluorescence yield but this time using a known singlet oxygen generator as standard (see Chap. 15).

14.9.4 Time-Resolved Measurements

14.9.4.1 Time-Resolved Microsecond Emission Using Pulsed Xenon Lamp Excitation

Pulsed xenon lamps are available which give white light emission with pulse durations of a few 10 μs, and pulse frequencies of a few tens of Hz. The lamp intensity is high enough for emission work. Combination with an emission monochromator, excitation monochromator, and moderately fast detector gives a time resolved emission spectrometer operating over the UV/Vis range with a time resolution of a few μs, and at reasonable price. A number of commercial fluorimeters/phosphorimeters use pulsed xenon lamps with gated detection, rather than continuous lamps, as the excitation source. Fluorescence is detected with simultaneous gate and pulse; while longer lived emission can be isolated from fluorescence by delaying the detector gate. If gated detection is replaced by continuous monitoring then the full emission decay curve can be recorded. Averaging over a large number of pulses can be used to improve signal-to-noise for weak signals.

14.9.4.2 Microsecond Flash Photolysis

This is the classic photochemical time-resolved method developed by Porter and Norrish [23, 24]. Although now generally replaced in most laboratories by ns flash photolysis (see below) it is still the superior method for transient absorption studies at timescales longer than about 50 μs. The fundamental principles of the flash photolysis technique are discussed in Chap. 8. Flash lamps with outputs typically of ~10–100 J and pulse durations of a few μs are used as the excitation source. It is worth noting that the pulse duration depends on its energy, and that lower energy, but shorter, pulses are preferred for many applications. The lamp emission spectrum is a white light continuum with atomic lines superimposed; the exact lamp wavelength output is determined by the choice of inert gas fill, with xenon being most commonly used. A tungsten lamp operated from a stabilised power supply makes a very stable monitoring beam for the visible and near UV. After travelling through the sample the monitoring beam is passed through a monochromator, and/or filters, for wavelength selection, before being incident on a moderately fast photomultiplier. A typical solution phase optical arrangement will have the sample held in 10 cm cylindrical path length cell, with two slightly longer flash lamps on either side, all in a reflective cylinder with aperture stops either side to help discriminate between monitoring beam and flash lamp pulse. The long path length means that relatively dilute solutions can be used which helps minimise second order processes for long-lived transients, such as triplet–triplet annihilation. A solution filter jacket around the sample, or gelatin sheet filters, can be used to give wavelength selection to the excitation light, and a thermostatted jacket can

be used for temperature dependent experiments such as the determination of reaction activation energies.

14.9.4.3 Nanosecond Absorption/Emission Using Laser Excitation (ns Flash Photolysis)

A schematic diagram of the typical experimental configuration for ns-flash photolysis experiments is shown in Fig. 14.13. A ns pulsed laser is used as the excitation source, with a fast kinetic spectrometer as the detection system. Most absorption systems use a 150 W xenon lamp as the monitoring source. Even this lamp, when operating in normal continuous mode, generates too few photons per nanosecond for good S/N across the ns–μs range, and it is usual to provide an electrical pulse into the lamp which increases the output by a factor of ~ 50, to give a relatively stable high intensity monitoring beam for ~ 100 μs. The xenon arc pulse is synchronised with the laser system, so the laser pulse is timed to arrive during a flat portion of the monitoring beam (The temporal 'shape' of the monitoring pulse depends on the electrical pulse applied and can be altered somewhat using ferrite cores in the pulsing system). For times longer than a few hundred μs the Xe lamp can be operated in non-pulsed mode, although, because of the poor stability of Xe lamps, for times longer than a hundred μs, a stabilised tungsten lamp may well give a better signal. Aperture stops are set in the monitoring beam, before and after the sample, to ensure that the monitoring beam traverses that part of the sample exposed to the laser pulse, and also to reduce stray and scattered light. A shutter on the monitoring beam is also usually synchronised with the laser pulse to limit exposure of the sample to the intense xenon arc beam. Photodegradation of the sample can be a problem in ns flash photolysis, and regular checks on sample integrity are always worth carrying out. After passing through the sample the monitoring beam goes through a monochromator and then usually onto

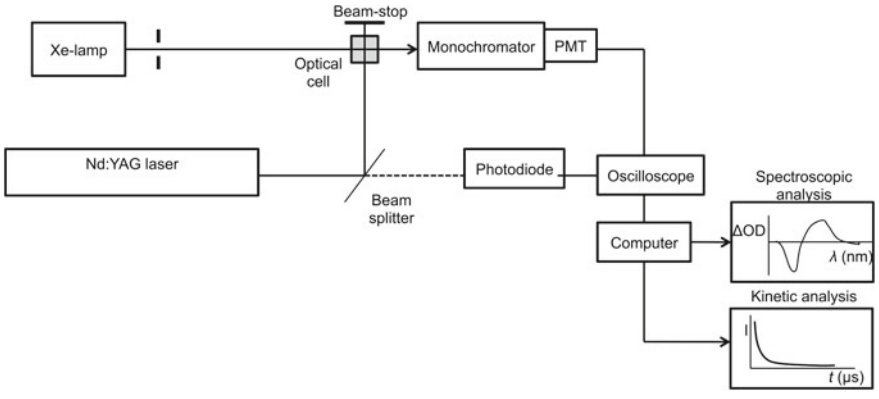

Fig. 14.13 Schematic of typical ns-flash photolysis instrumentation

a fast photomultiplier. Signal to noise considerations generally require a somewhat wider spectral bandwidth than is used in a conventional absorption spectrophotometer, but care must also be taken to prevent too much light being incident on the photomultiplier tube, since an excessive photomultiplier current is a major source of instrumental artifacts such as spurious signals, oscillating signals ("ringing") and non-linear response. Most recording devices have an input impedance of 1 MΩ and for ns timescales the photomultiplier output signal must be terminated with a 50 Ω load to limit current drain on the photomultiplier. However, as the time scales of interest increase a larger termination impedance can be used to increase the size of the signal voltage, and improve S/N.

The choice of laser determines which excitation lines are available. Any pulsed laser operating with high enough pulse energies, typically in the mJ range, can be used. The most commonly used is probably the Nd/YAG laser, which, with frequency doubling, tripling and quadrupling, gives lines at 1064, 532, 355, 266 nm, with pulse powers in the mJ range readily available (one Einstein of 355 nm radiation is 336 kJ, so a 10 mJ 355 nm pulse in 1 cm^{-3} is *ca.* 3×10^{-5} E dm^{-3} which is usually adequate for generation of measurable concentrations of transient species). Q-switched lasers generate pulses of a few ns lifetime, which generally limits the time resolution of the apparatus to a few tens of ns.

Computer-controlled equipment is available which will automatically record transient spectra across a specified spectral and temporal range. Often, a number of "shots" are made at each wavelength and the signals averaged. In addition, it is usually best to run the spectra at a series of random wavelengths (e.g. 490, 570, 420, 460, 530, 430 nm…) rather than at regular increasing or decreasing wavelengths (420, 430, 449, 450 nm…) to check for any problems associated with photodegradation of solutions. A preliminary examination would usually include such measurements but then a detailed examination of transient curves at specific wavelengths of interest should also be carried out to make sure that the timescales of transient spectra recorded are such to include all major spectral changes following absorption.

The usual optical arrangement has the sample in a 1 cm cell with the monitoring beam at 90° to the excitation pulse but narrower cells can be used, and it is also possible to arrange the laser beam and monitoring beam to be approximately collinear down the cell so both shorter and longer path length cells can be used.

ns-laser emission studies are generally easier than absorption. The same kinetic spectrometer can be used, but there is no need for the monitoring beam, the aperture stop on the emission side of the sample can be widened to allow more light through. Unless the emission is particularly strong, the monochromator bandwidth may need to be widened to collect enough light for an acceptable signal.

14.9.4.4 Time-Resolved Singlet Oxygen Studies

The lifetime of singlet oxygen is ideal for studies using laser generation on a ns timescale. Unfortunately the emission yield is very low, but there are fast detectors designed to measure the very weak emission from singlet oxygen centred at 1270 nm. A filter is used to select the wavelength range to avoid interference from second, or third, order emission spectra. Either a highly sensitive liquid nitrogen cooled photomultiplier, or solid state device is used as the detector. Yields can be obtained by comparison with the signal produced by a standard singlet oxygen generator in the same solvent and with the same absorbance at the excitation wavelength, preferably over a range of excitation pulse energies (see Chap. 15). Since the lifetime of singlet oxygen is highly solvent dependent, and the signal quality significantly improved by separating the timescale of the laser pulse from that of singlet oxygen decay, it is worth trying to work in solvents which give relatively long lifetimes; acetonitrile is a good polar solvent for singlet oxygen work, and hydrocarbons are suitable non polar solvents.

14.9.4.5 Time Correlated Single Photon Counting (TCSPC)

Instrumentation for TCSPC is described in Chap. 15 and will not be discussed in detail here. A wide range of instruments is available, with cost generally increasing with: increasing time resolution arising from choice of excitation source *i.e.*, ns flash lamps, ns pulsed LEDs, or ps lasers; decreasing wavelength of excitation source; complexity of the detection equipment: filters, monochromator, polarisers; and time resolution and wavelength range of the photomultiplier used.

14.9.4.6 Phase Modulated (or Frequency Domain) Fluorimeter

This technique measures the phase shift, and change in degree of modulation, between a rapidly modulated excitation source and the subsequent modulated emission, which arises because of the time interval between excitation and emission in the sample (Fig. 14.14). The modulation in the emission intensity is measured for a number of excitation modulation frequencies. Best lifetime precision, and multi-component resolution, is achieved if measurements are made over a range of frequencies spread around $\sim 1/(2\pi\tau)$ Hz, where τ is the emission lifetime. Data from frequencies far removed from this value gives little information since: if the frequency of excitation modulation is much lower than this the emission modulation exactly matches that of excitation; while if it is much higher there is no measurable modulation in emission intensity. So the range of available modulation frequencies which can be generated, or measured, determines the range of lifetimes the instrument can be used for.

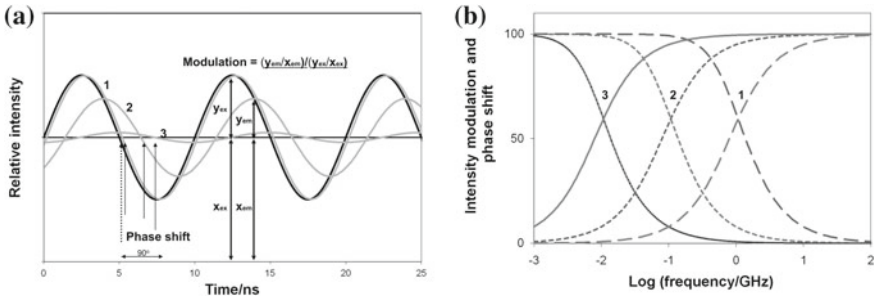

Fig. 14.14 **a** Modulation and phase shift of emission for lifetimes of: **1** 0.2 ns, **2** 2 ns, and **3** 20 ns (*grey lines*) in response to 0.1 GHz modulated excitation (*black line*). **b** Modulation and phase shift response as a function of frequency of intensity modulation. Modulation (decreasing with frequency) and phase shift (increasing with frequency) for lifetimes of: *large dashed line*, **1** 0.2 ns, *short dashed line*, **2** 2 ns, and *solid line*, **3** 20 ns. Note that as the excitation modulation frequency increases modulation decreases from a maximum of 1 to a minimum of 0, while at the same time phase shift increases from a minimum of 0 to a maximum of 90°, but these changes are most commonly shown as % of full range, as on the vertical axis in **b**

Either, or both, excitation source modulation frequency or detector response can limit instrument capabilities. Most standard commercial instruments have modulation frequencies up to a maximum of a few hundred MHz and are best suited for relatively long-lived fluorophores with high quantum yields. The excitation source is typically a modulated LED or laser diode, or for a wide wavelength range, light from a continuum steady-state source, such as a Xe arc lamp modulated with an electro-optical cell, such as a Pockels cell; detection is typically a fast photomultiplier, or multichannel plate photomultiplier. Polarisers are commonly used accessories. Such an instrument is ideally suited for lifetimes of a few ns, but will also measure, albeit with lower precision, lifetimes in range of 100's ps. GHz modulation frequencies are obtained with mode locked lasers with a fast multichannel plate photomultiplier and these allow lifetime measurements in the range of tens of ps.

A particular advantage of frequency domain lifetime measurements is that, by measuring a number of frequencies simultaneously, it is possible to make lifetime measurements very quickly, in as short a time as a few ms. Thus changes in excited-state lifetimes during chemical reactions can be studied in real time across the ms time domain. This matches that of the most commonly used chemical and biochemical fast reaction technique, stopped-flow, and stopped-flow accessories are available for commercial frequency domain fluorimeters. Because the measurements are quick, frequency domain measurements over a wide spectral range also provide an attractive method for obtaining time-resolved fluorescence spectra.

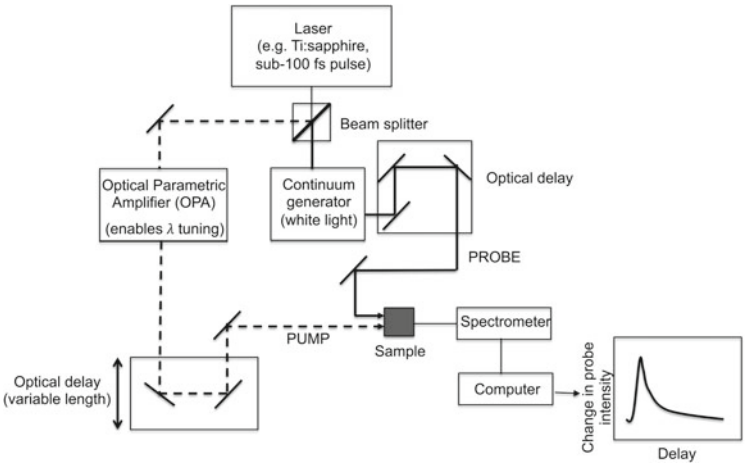

Fig. 14.15 Femtosecond pump-probe system

14.9.4.7 Ultrafast ps/fs Pump Probe Absorption Spectroscopy

Continuous monitoring at times much less than a nanosecond is limited by the response time of electronic components (particularly the photomultiplier) and poor S/N for continuous light sources on sub-ns timescales. However, many photochemical and photophysical processes occur on the sub-nanosecond timescale, including isomerism, internal conversion, energy transfer and electron transfer. The pump-probe method overcomes these limitations enabling reactions on the pico- and femtosecond timescale to be investigated. In pump-probe spectroscopy, two light pulses are generated from a single ultrafast (50–100 fs) laser source, which is typically a Ti:sapphire laser pumped by a diode or argon-ion laser (see Fig. 14.15). The first pulse is used to excite or *pump* the sample, the second to *probe* the changes induced in the system at a given time after excitation. The Ti:sapphire laser output is split into two pathways using a partially-reflecting mirror or beam splitter. Although the two laser pulses leave the beam splitter at the same time, they travel along different pathways so that they arrive at the sample at different times. The time resolution of the technique now no longer depends on the electronics but instead on the pathlength (using the speed of light, we can calculate that a 1 mm delay corresponds to 3.33 ps). The laser output is normally amplified and the wavelengths of both exciting and probe pulses can be tuned using an optical parametric amplifier (OPA). The monochromatic laser pulse may be transformed into a broad band continuum pulse covering the UV, visible and IR regions using various nonlinear optical effects. For example, this can be achieved simply by focusing the pulse into a cell containing a suitable liquid (e.g., water, D_2O, tetrachloromethane). The white light continuum pulse can then be used as analysing light to obtain the transient spectrum. The pump-probe method is

especially useful to monitor the dynamics of population of vibrationally excited states, however, the low absorption cross-section in the IR spectral region means that a high pump intensity ($\sim 10^{11}$ W cm^{-2}) is required. Similar methods involving splitting and introducing a time delay in laser pulses are also used to study ultrafast excited state emission processes in the technique of time-gated fluorescence upconversion [25]. The pump pulse is used to excite the fluorescence in the sample and then combined with the probe pulse in a nonlinear optical crystal (such as potassium dihydrogen phosphate, KDP) through sum-frequency generation to give the detected signal. This is then studied as a function of the optical delay to give time-resolved fluorescence with resolution of tens of fs.

14.9.5 Fluorescence Imaging

There is a wide range of commercially-available fluorescence microscopes available to meet all research budgets. Lower end models enable the observation of fluorescence from biological or materials samples at the micrometer level, with image capture if a camera is available. A larger budget will allow probing of sample depth, fluorescence lifetime measurements and high-end photography and video capture. Refurbished, second-hand and ex-demonstration microscopes are a good option for those with a limited budget. Optical microscopes tend to be extremely robust, so it is often possible to obtain a higher-specification instrument for a fraction of the price of a new microscope, and spare parts are relatively easy to find on the internet. It is also possible to convert a regular transmission microscope into a fluorescence microscope using LEDs as the excitation source—the Cheaposcope website gives an excellent description of how to achieve this [26].

Conventional wide-field fluorescence microscopes use vertical or *epi*-illumination, where the excitation source is typically a xenon arc or mercury lamp and the sample is irradiated from above. Excitation wavelength selection is achieved by first passing the light through a wavelength selection excitation filter. The transmitted light is then reflected from a dichroic mirror, through the microscope objective, where it is focused onto the sample. If the sample fluoresces, the emitted photons are collected by the same objective, passed back through the dichroic mirror, and are subsequently filtered by an emission filter, which removes unwanted excitation or background wavelengths. In a properly configured microscope, only the emission light should reach the eye or the detector. The limits of detection are therefore governed by the darkness of the background. Since the excitation light intensity is typically several orders of magnitude greater than the emitted fluorescence, an effective emission filter is crucial for good S/N.

The excitation filter, dichroic mirror and emission filter are typically housed in a single optical unit known as a *filter cube*. The filter cube provides a convenient means to change the excitation/emission wavelength range without direct handling of either the mirror or filters. The role of the dichroic mirror is to separate the excitation and emission light paths. Each dichroic mirror has a set wavelength

value at which it transmits 50 % of the incident light. The mirror reflects wavelengths below and transmits wavelengths above this value. To enable efficient separation of the excitation and emission light, the transition value should lie at a wavelength somewhere in between. Since the dichroic mirror is not 100 % effective, excitation and emission filters are used to minimise the effects of stray light. These filters are typically interference filters, which exhibit an extremely low transmission outside of their characteristic band pass. They are therefore very effective at selecting the excitation and emission wavelength range. Filter cubes containing various combinations of excitation and emission filters and dichroic mirrors suitable for use with most common fluorophores are available. Optical spectra for different filter cube types may be found in microscope manufacturer's catalogues.

The depth of field of an epifluorescence microscope is 2–3 μm and the maximum resolution is approximately equal to half the excitation wavelength (*i.e.* $\sim$0.2 μm for $\lambda_{ex} = 400$ nm) [27]. If the sample is thicker than the depth of field, then out-of-focus fluorescence may result in blurred images. This problem may be overcome using *confocal fluorescence microscopy*. In contrast to conventional widefield microscopy, where the entire sample is irradiated, in a confocal microscope, a focused beam of light, typically from a laser, is scanned across the sample. The focused beam forms a series of diffraction-limited spots on the specimen, effectively dissecting it into optical sections. The fluorescence emitted by each optical section is separated from the incident beam by a dichroic mirror and focused by the objective lens through a pinhole aperture to a photomultiplier. The excitation intensity rapidly falls off above and below the plane of focus as the beam converges and diverges. This essentially confines the sample illumination to a specific area and depth, inhibiting excitation of the sample outside of the focal plane under examination. The optical path of the confocal microscope is additionally configured so that any fluorescence resulting from out-of-focus planes is blocked by the aperture, thus further attenuating any background interference. With certain fluorophores, two-photon fluorescence can also provide an excellent method for achieving depth profiling in imaging, particularly with biological samples. In this case, electronic excited states are produced by simultaneous absorption of two photons. For fluorophores emitting in the UV and visible this requires excitation in the near infrared, which provides the advantages for biological samples that this radiation is transmitted by tissues and that it is not normally damaging to biomolecules. Two-photon excitation needs a high photon flux, usually delivered by a pulsed laser. This has the advantage that the laser can be focused into a very small volume, providing high resolution. Chromophores for two-photon fluorescence possess certain requirements in terms of their structure and symmetry. However, a number of good two-photon fluorophores are now available. The area has been extensively reviewed [28, 29].

A number of more sophisticated fluorescence imaging techniques are available. **Fluorescence lifetime imaging microscopy (FLIM)** exploits the fact that the excited state lifetime is sensitive to its microenvironment to provide a contrast mechanism for imaging. By imaging the fluctuations of an excited-state lifetime

across a sample it is possible to obtain complementary information on local physical and chemical parameters such as viscosity, polarity and pH. **Fluorescence correlation spectroscopy (FCS)** measures the temporal fluctuation of fluorescence intensity due to translational motion, which may be correlated to physical parameters such as translational or rotational diffusion coefficients, molecular weights or aggregate size, or rate constants. An excellent discussion of the theory and applications of these and related fluorescence imaging methods may be found in the literature [30]. For FCS to work, it is important that solutions are very dilute and that the observation volume is very small. At the limit, it is possible to observe the fluorescence from a single molecule through the use of various advanced optical and detection methods. The behavior of one isolated molecule can be very different from that of an ensemble, and single-molecule spectroscopy and microscopy [31] are now important techniques for studying both chemical and biological systems.

The optical resolution (the distance between two points which can be distinguished) in microscopy is normally considered to be limited by the diffraction of light. Ernst Abbé showed in 1873 for optical devices that this diffraction limit is the wavelength of light divided by twice the numerical aperture of the imaging lens. This was thought for a long time to provide a limit on the resolution in optical microscopy, which with visible light corresponds to around a couple of hundred nm. However, over the last decade, a number of microscopic techniques, mainly based on photochemical processes, have been developed which allow sub-diffraction limited resolution. These are generally classified as super-resolution microscopy. The area is developing rapidly, and we will just mention three of these methods.

Near-field scanning optical microscopy (SNOM) overcomes the optical diffraction limit enabling very high spatial resolution at the sub-micron level and even single molecule detection. This uses a light source which is of nanometer dimensions obtained by the light passing through the tip of a nm dimension optical fibre [32]. In **stochastical optical reconstruction microscopy (STORM)**, photoswitchable fluorophores are used [33]. These are normally non-fluorescent but a certain percentage are transformed into emissive species upon photoswitching. These are then observed by single-molecule imaging and the centroid positions determined. The fluorophores are subsequently deactivated, a second group of photoswitchable fluorophores being converted into their emissive forms. These are imaged and the process repeated. In **stimulated emission depletion microscopy (STED)** [34], deactivation of excited states by stimulated emission at the edge of a fluorescent spot is used to inhibit the spontaneous emission of a probe. The overall effect is that it is possible to image samples on length scales smaller than the diffraction limit. Typically, two separate laser pulses are used, one to induce spontaneous emission and the other for stimulated emission depletion. However, it is possible to perform STED experiments using continuous wave lasers. With both STORM and STED, the use of dyes having appropriate photophysical properties is of fundamental importance, and much research effort is being employed in the design and synthesis of appropriate systems.

14.9.6 Simple Test Rigs

With the wide availability of commercial instruments custom building of optical test rigs is less important than previously. However, a variety of simple optical rigs can be made with just a few components. A tungsten lamp with a stabilised power supply can act as either a very stable monitoring beam, or a moderate intensity irradiation source for the visible region. A xenon lamp is an excellent general purpose irradiation source for the UV/Vis region, and though less stable than a tungsten lamp, a beam splitter and photodiode detector can be inserted in the beam to monitor intensity variations when used as a monitoring beam for absorption or emission work. The light beams can be controlled using glass, or, for UV, quartz lenses, and iris apertures, or using fibre optics. A water filter, glass IR filter, or hot mirror will remove unwanted IR radiation, and either filters or a monochromator, will give wavelength selection as required for irradiation, excitation or absorption work. A photomultiplier running from a stabilised power supply is probably still the most convenient high sensitivity general purpose detector, but solid state detectors are also available. Cheap analogue-to-digital recording devices can be used to output signals from PM tubes and other recording devices directly in computers for data recording. There is a range of optical rails, stands, lenses and sample holders available from a number of commercial suppliers. A useful sample holder for irradiation of multiple samples is a "merry-go-round" in which samples are rotated in front of the beam, thus ensuring all samples are exposed under the same irradiation conditions irrespective of variations in lamp output.

14.9.7 Access to Infrastructure

The high costs associated with specialist ultrafast laser techniques can make their purchase prohibitive to many university research laboratories. However, centralised national and international research infrastructures hosting a variety of large scale sophisticated laser facilities are available to researchers. In Europe access to these facilities is currently obtained either *via* successful application to Laser Lab Europe (a European Union Research Initiative) [35] or directly to the research facility. Calls for proposals are launched at least annually and instrument time is allocated to the research on the basis of peer-reviewed evaluation of the proposal. Each facility hosts a variety of exotic techniques, enabling photoactive systems to be probed across a variety of timescales in different dimensions. For example, the STFC Central Laser Facility at the Rutherford Appleton Laboratory (UK) is home to optical tweezers, femtosecond pump-probe spectroscopy, time-resolved stimulated and resonance Raman spectroscopy, time-resolved linear and non-linear infrared transient spectroscopy, to name just a few techniques [36].

14.10 Reference Materials, Temperature Control, and Computer Programs

14.10.1 Reference materials: Absorption, Emission, Scattering

Although most modern spectrophotometers undergo automatic wavelength calibration upon start-up, using the emission lines from the deuterium lamp, and some fluorimeters may offer the same facility using the lines in the xenon lamp, reference materials are useful. For absorption spectrophotometry solid state filters, made of holmium and didymium oxide in a glass, with narrow reference absorption lines are available (See Fig. 14.5). For the UV, benzene vapour is an excellent standard for both wavelength and spectral resolution. The vapour pressure is sufficient that it is only necessary to put one drop of liquid benzene in a stoppered cuvette to get a good absorption spectrum.

To obtain corrected emission spectra, it is necessary to know the wavelength-dependent efficiency of the detector. The determination of correction factors can be a time-consuming process and several approaches are possible. For many commercial instruments, the manufacturer will supply correction files for emission and excitation spectra. While these are an invaluable assistance, it is advisable to exert caution when using them and make independent checks on their validity from time to time. One reliable method of doing this is to compare the experimental emission spectrum and known corrected emission spectrum of a standard emitter. Sets of standard emitters spanning the UV and visible regions are commercially available and their corrected emission spectra are available in the literature. If correction factors are not supplied by the instrument manufacturer, then they may be determined by measuring the wavelength-dependent photon output from a calibrated light source. Standard lamps for this purpose, and their spectral profiles, are available commercially. However, it is important to note that the spectral output of a lamp will vary with age. Alternatively, the spectral lamp output of the Xe lamp excitation source can be determined *in situ* using a *quantum counter* and a scatterer. A quantum counter emits with a quantum yield that is independent of the excitation energy over a defined spectral region. For example, concentrated rhodamine 6B solutions are often used for the spectral range between 300–600 nm. By scanning the excitation wavelength with the quantum counter in place, the relative photon output of the lamp can be obtained. A standard scatterer, such as barium sulfate or magnesium oxide, is then placed in the sample holder and a synchronous scan of the excitation and emission monochromators is performed. The resulting spectral output is then divided by wavelength dependent photon output of the lamp to yield the wavelength dependent sensitivity factors for the detection system (emission monochromator and detector). For further details on this method see the literature [37]. Fluorescence reference materials for

molecular spectroscopy in which the fluorophores are incorporated in poly(methyl methacrylate) blocks are available commercially, e.g., from Starna [5].

Magnesium oxide and barium sulfate are commonly used as scattering (diffuse reflectance) standards in the UV/Vis/NIR spectral regions as they scatter all wavelengths in this region efficiently and with reasonably uniform efficiency. However, there are some difficulties associated with using these materials including variation in reflectivity over time and on exposure to UV light. Moreover, the angular distribution of the scattered light can also show some wavelength dependence. To overcome the latter problem, integrating spheres have been developed, both for measurement of diffuse reflectance (absorption) spectra, and more recently, for the determination of emission quantum yields. An integrating sphere is typically a spherically shaped enclosure containing a hollow cavity that is coated with a highly reflective material (e.g., $BaSO_4$) which uniformly scatters light. Any photons which are incident with the highly reflective surface of the sphere wall are, by multiple scattering reflections, distributed equally to all other points in the sphere before eventually hitting the detector. This has the effect of eliminating the spatial and directional information of the scattered light. The use of an integrating sphere to determine emission quantum yields is described in Chap. 15.

14.10.2 Temperature Control Units, and Cryostats

Most instrument manufacturers will make temperature control units for use at around a few tens of degrees from ambient, with either thermoelectric (Peltier) temperature control, or more simply a cell holder which allows water, or other liquid, circulation from an external temperature controlled bath. Cryostats are also available for precise very low temperature control, easily down to 77 K using liquid nitrogen cooling, less easily down to 4 K using liquid helium, and even to lower temperatures if required. A hot air blower, such as a hair dryer, is a convenient way to raise the temperature of a sample up to a few tens of degrees above ambient for the occasional experiment and a thermocouple or thermistor a convenient way to measure sample, or cell holder, temperature.

A quartz Dewar (Fig. 14.2) as described in Sect. 14.3.3 is a relatively cheap alternative for low temperature work. 77 K is the most convenient temperature to work at, but thermostatting at various other temperatures down to 113 K is possible using solid CO_2 or liquid nitrogen slush baths [1, 38]. If a thermocouple or thermistor can be placed in the Dewar the temperature can be monitored, and measurements made, as the whole assembly warms to ambient temperature.

14.10.3 Computer Programs

Molecular modelling. Molecular orbital modelling has reached the stage where it is possible to carry out useful electronic state modelling on a PC. It is mathematically convenient to use *Gaussian* equations rather than hydrogen like atomic orbitals for these calculations, and Gaussian programs are widely used in photochemical studies to give some theoretical insight into the nature of the transitions under investigation, and to calculate transition energies (wavelengths), and oscillator strengths, for comparison with those observed experimentally [39, 40]. Calculations for isolated molecules in the gas phase are most straightforward, but the effect of solvent can also be incorporated, most easily by considering the molecule of interest to be in the centre of a sphere of uniform dielectric constant but there are other models. Where a good match is obtained, the Gaussian molecular orbitals, and the atomic orbital coefficients used to generate them, can be used to help visualise the nature of the electronic transition. These programs can generate useful information such as the transition dipole, degree of charge transfer in the transition, and changes in atomic electron densities. Knowledge of those atoms of the molecular structure most involved in the orbitals of the transition, and also any atoms only slightly involved, is very useful in understanding substituent effects on transition energies [40].

Curve fitting programs. Most instruments have associated software for data analysis, but it is also useful to have some curve fitting programs available to explore custom designed models and models not included in the manufacturers software. We have found Table Curve from Jandel Scientific to be useful and fairly easy to use.

14.11 Safety

Aside from general laboratory precautions, the Photochemistry Laboratory has some more specific safety concerns that should be considered before undertaking any experiment.

High pressure lamps. Mercury and xenon arc lamps have high internal pressures even when not in operation. Follow the manufacturer's guidelines when changing and disposing these bulbs to avoid accidental breakages. In the event of breakage of a mecury lamp, the workplace safety protocol for a mercury spill should be followed. A mercury spill kit is a useful addition to any laboratory. Mercury lamps emit dangerous levels of UV radiation. It is important that if the lamp is not enclosed protective gloves and eyewear are used. If you are using a Hg lamp as the source in a fluorescence microscope, always ensure that appropriate filters are in place before looking down the eyepiece! Some Hg and Xe arc lamps produce ozone, which is toxic at relatively high concentration levels. Ozone-producing lamps should be used in lamp housings equipped with exhaust systems

in a well-ventilated room. Care should be taken with the high voltage start pulse of Xe lamps. Water cooling of any electrical equipment introduces another potential hazard.

Laser radiation. A laser produces an intense, highly directional beam of light. If directed, reflected, or focused upon an object, laser radiation will be partially absorbed, raising the temperature of the surface and even the interior layers of the object, potentially resulting in material deformation. The human body is vulnerable to laser radiation and exposure can result in serious tissue damage in the eye and skin. It is therefore essential that anyone working with a laser receives appropriate training and is familiar with the safe operating procedure. Most universities run a laser safety course which is a requirement for anyone intending to use lasers in their research.

Lasers are divided into a number of classes depending on the power of the beam and the wavelength of the emitted radiation. The weakest beams are designated Class 1, and are generally safe under all circumstances—these include laser pointers. Mode-locked Ti:sapphire, Q-switched Nd:YAG and dye lasers are all designated as Class 4 lasers, meaning they constitute a significant hazard if safety procedures are not strictly followed. Viewing of the beam and of specular reflections or exposure to diffuse reflections can cause eye and skin injuries.

Avoiding inadvertent exposure to the laser beam is an essential part of laser safety. Wherever possible, the laser optical path should be horizontal and well below eye level and ideally the beam path should be fully or at least partially enclosed. A beam dump should be inserted to terminate the beam path at some appropriate point and should be made from a material capable of absorbing the full intensity of the laser beam. All work with class 3B and four lasers should be carried out in a designated laser room, which should be clearly identified with a suitable warning notice and separate from the main laboratory. Lasers may also be required to have beam shutters or key-controlled interlocks to prevent operation if the laser casing or room door is open.

Laser protective eyewear fitted with appropriate filtering optics can protect the eyes from exposure to direct, reflected or scattered laser light and should always be worn if the experimental configuration involves an open beam or if there is a risk of accidental exposure. Laser goggles must be selected for the specific type of laser, to block or attenuate in the right wavelength range. Since laser goggles are subject to damage and deterioration, periodic inspection of these items should be part of the routine maintenance procedure.

High voltage electrical circuits. Many lasers are high voltage devices, typically 400 V upward for a small 5 mJ pulsed laser, and exceeding many kilovolts in higher powered lasers. This, coupled with high pressure water for cooling the laser and other associated electrical equipment, can create a greater hazard than the laser beam itself. Electrical equipment should generally be installed above ground level to reduce the electrical hazard in the case of flooding. Optical tables, lasers, and other equipment should be well grounded electrically.

Chemicals and laser dyes. The chemicals used in photochemical experiments, including laser dyes and solvents, may be harmful to health and should be handled appropriately in an adequately ventilated workspace.

14.12 The Photochemical Laboratory Library

While the following list of resources and useful information reflects our own research interests, with, for example, an emphasis on solution phase photochemistry, it should provide a good starting point for those interested in most aspects of photochemical research.

14.12.1 Books and Reviews

1. **Reference handbooks**

Montalti M, Credi A, Prodi L, Gandolfi MT (2006), Handbook of photochemistry, 3rd edn. CRC Press, Boca Raton. An essential reference book containing data tables for a wide range of compounds, and a variety of reference materials including: quantum yields, lifetimes, quenching rate constants, electrochemical potentials and solvent properties; as well as information on standard procedures used in chemical actinometry, determination of emission and excitation spectra correction factors, and quantum yield measurements; and also information on equipment such as lamps and filters.

Haynes WM (ed) (2011) CRC Handbook of chemistry and physics, 92nd edn. CRC Press Boca Raton, USA. Usually referred to as the Rubber Handbook in reference to the publisher of earlier editions, this is the first point of call when searching for physical or chemical constants, conversion factors, parameters, potentials, affinities, radii etc.

2. **Photochemistry**

Turro NJ (1991) Modern molecular photochemistry, University Science Books, California; Turro NJ, Ramamurthy V, Scaiano JC (2010) Principles of molecular photochemistry: an introduction, University Science Books, California; Turro NJ, Ramamurthy V, Scaiano JC (2010) Modern molecular photochemistry of organic molecules, University Science Books, California. The classic *Modern Molecular Photochemistry* recently underwent a comprehensive revision and is now available under the title *Modern Molecular Photochemistry of Organic Molecules*. It provides a detailed description of the

fundamental principles of molecular photochemistry, focusing in particular on organic photochemistry. The related primer *Principles of Molecular Photochemistry: An Introduction*, by the same authors, contains the introductory chapters of the main textbook.

Wardle B (2009) Principles and applications of photochemistry, Wiley. This book includes some excellent chapters on fluorescence sensors and probes, as well as a detailed description of more advanced fluorescence spectroscopy and imaging techniques.

3. Fluorescence and fluorescence spectroscopy

Lakowicz JR (2006) Principles of fluorescence spectroscopy, 3rd edn. Springer, Singapore. The big blue reference book for fluorescence spectroscopy and its applications. Detailed information provided on fundamental principles and theory, instrumental techniques and applications, and state-of-the-art applications.

Valeur B (2001) Molecular fluorescence: Principles and applications, Wiley. An excellent introductory textbook to the fields of photochemistry and photophysics and their applications.

4. Single photon counting

Becker W (2005) Advanced time-correlated single photon counting techniques, Springer. A detailed account of the principles and applications of time-correlated single photon counting.

5. Ultrafast processes

El-Sayed MA, Tanaka I, Molin Y (ed) (1995) Ultrafast processes in chemistry and photobiology, Blackwell. Some of the leading research workers in the field present brief accounts of ultrafast studies of reactions of interest in photochemistry and photobiology.

6. General spectroscopy

Banwell CN, McCash EM (1994) Fundamentals of molecular spectroscopy, 4th edn. McGraw-Hill, UK. An excellent easy to read undergraduate introductory text.

Hollas JM (2004) Modern spectroscopy, 4th edn. John Wiley and Sons Ltd, UK. This textbook contains an excellent chapter on lasers and laser spectroscopy.

7. **Physical chemistry**

Atkins P, de Paula J (2010) Physical chemistry, 9th edn. Oxford University Press, UK.

Winn JS (2001) Physical chemistry, Harper Collins, USA.
Two very good undergraduate texts, which differ in style.

8. **Molecular quantum mechanics**

Atkins P, de Paula J, Friedman R (2009) Quanta, matter and change: A molecular approach to physical chemistry, Oxford University Press, UK

Atkins PW, Friedman RS (2011) Molecular quantum mechanics, 5th edn. Oxford University Press, UK.

9. **General chemistry, analytical chemistry, statistics**

Mendham J, Denney RC, Barnes JD, Thomas MJK (2000) Vogel's quantitative chemical analysis, 6th edn. Pearson Education Ltd, UK. A comprehensive and detailed description of apparatus and methods used in quantitative chemistry and chemical analysis.

Skoog DA, West DM, Holler FJ, Crouch SR (2003) Fundamentals of analytical chemistry, 8th edn. Thomson Brooks/Cole, USA. An excellent standard undergraduate text, with more emphasis on instrumental methods than the above.

Armarego WLF, Chai, CLL (2003), Purification of laboratory chemicals, 5th edn. Elsevier. Procedures and processes for purifying organic, inorganic and organometallic chemicals.

Chatfield C. (1999) Statistics for technology, 3rd edn. (revised), CRC Press, Boca Raton, USA. Relatively easy to read and with plenty of illustrative examples.

10. **Review articles**

Glossary of terms in photochemistry (IUPAC Recommendations 2006), Prepared for publication by Braslavsky SE (2007) Pure Appl Chem 79:293–465. This gives detailed descriptions of the most important terms and concepts used in photochemistry.
Bonneau R, Wirz J, Zuberbuhler AD (1997) Methods for the analysis of transient absorbance data. Pure & Appl Chem 69:979–992. An excellent review of flash photolysis methods and common pitfalls in their use.

Wilkinson F, Helman WP, Ross AB (1995) Rate constants for the decay and reaction of the lowest electronically excited singlet state of molecular oxygen in solution. An expanded and revised compilation. J Phys Chem Ref Data 24:663–677. An excellent collection of data. The previous compilation: Wilkinson F, Brummer JG (1981) J Phys Chem Ref Data 10:809–999, also identified their preferred values, which helps when trying to decide which values to use from the many values given in the tables.

14.12.2 Websites

Some useful discussion of a wide variety of topics in photochemistry and photobiology can be found at dedicated websites such as that from the American Society of Photobiology (http://www.photobiology.info)/ and the Outreach site from the Center for Photochemical Sciences, Bowling Green State University (http://www.photochembgsu.com/main.html).

14.12.3 Journals

Scientific journals specifically publishing fundamental research in photochemistry/photophysics include:

- Photochemical and Photobiological Sciences (RSC)
- Journal of Photochemistry A: Chemistry, B: Biology, C: Reviews (Elsevier)
- Photochemistry and Photobiology (Wiley)
- Journal of Luminescence (Elsevier)
- Journal of Fluorescence (Springer)
- International Journal of Photoenergy (Hindawi)
- Sensors and Actuators B: Chemical (Elsevier)

However, as we have seen throughout this book, the applications of photochemistry and photophysics are hot topics in the scientific community and as such, research in this field is often published in many of the more general high-impact chemistry, physics and materials journals, including:

- Journal of the American Chemical Society
- Nature Photonics, and Nature Materials
- Angewandte Chemie
- Advanced Materials, and Advanced Functional Materials
- Chemical Communications, Chemical Science and RSC Advances
- Inorganic Chemistry
- Dalton Transactions
- Physical Chemistry Chemical Physics
- Journal of Physical Chemistry A, B and C

14.12.4 Instrument and Chemical Catalogues

Several instrument and chemical manufacturers produce extremely useful detailed reference catalogues, including:

The Molecular Probes® Handbook
Johnson I, Spence MTZ, The molecular probes handbook-A guide to fluorescent probes and labeling technologies, 11th edn. Life Technologies.

This provides a comprehensive guide of commercially-available fluorescence probes and labeling methods (including protocols), with particular emphasis on biological and biotechnological applications.

Hamamatsu Opto-semiconductor handbook
http://jp.hamamatsu.com/sp/ssd/tech_handbook_en.html (accessed May 2012)
Detailed information on semiconductor based light sources and detectors.

The Book of Photon Tools (2001, Oriel Instruments)
Unfortunately it is extremely difficult to obtain a copy of this excellent catalogue. If you don't own one already, it is possible to obtain some individual chapters *via* the Newport Corporation website (www.newport.com)—try using "Oriel Product Training" as your search term.

14.12.5 Professional Bodies and Conferences

The major continental professional bodies for photochemists are:

- European Photochemistry Association (EPA)
- Inter-American Photochemical Society (I-APS)
- Asian and Oceanian Photochemical Association (APA)
- The Japanese Photochemistry Association (JPA)

Similar groups exist for photobiology, including:

- American Society for Photobiology
- European Society for Photobiology

Partner members of each of these bodies may also have their own special interest groups e.g., Royal Society of Chemistry Photochemistry Group, German Group of Photochemistry (Fachgruppe Photochemie), Grupo Especializado de Fotoquímica (Real Sociedad Española de Química), Photobiology Association of Japan etc.

Some of the more specific photochemistry-related conferences series are listed below. Again, photochemistry/photophysics and their applications will also be key topics in more general conferences not listed below and new or one-time symposia

and summer schools in the field also frequently appear. Application-specific conferences are also not listed here.

- IUPAC Symposium in Photochemistry
- International Conference on Photochemistry
- Gordon Research Conference on Photochemistry
- Central European Conference on Photochemistry
- Asian Photochemistry Conference

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