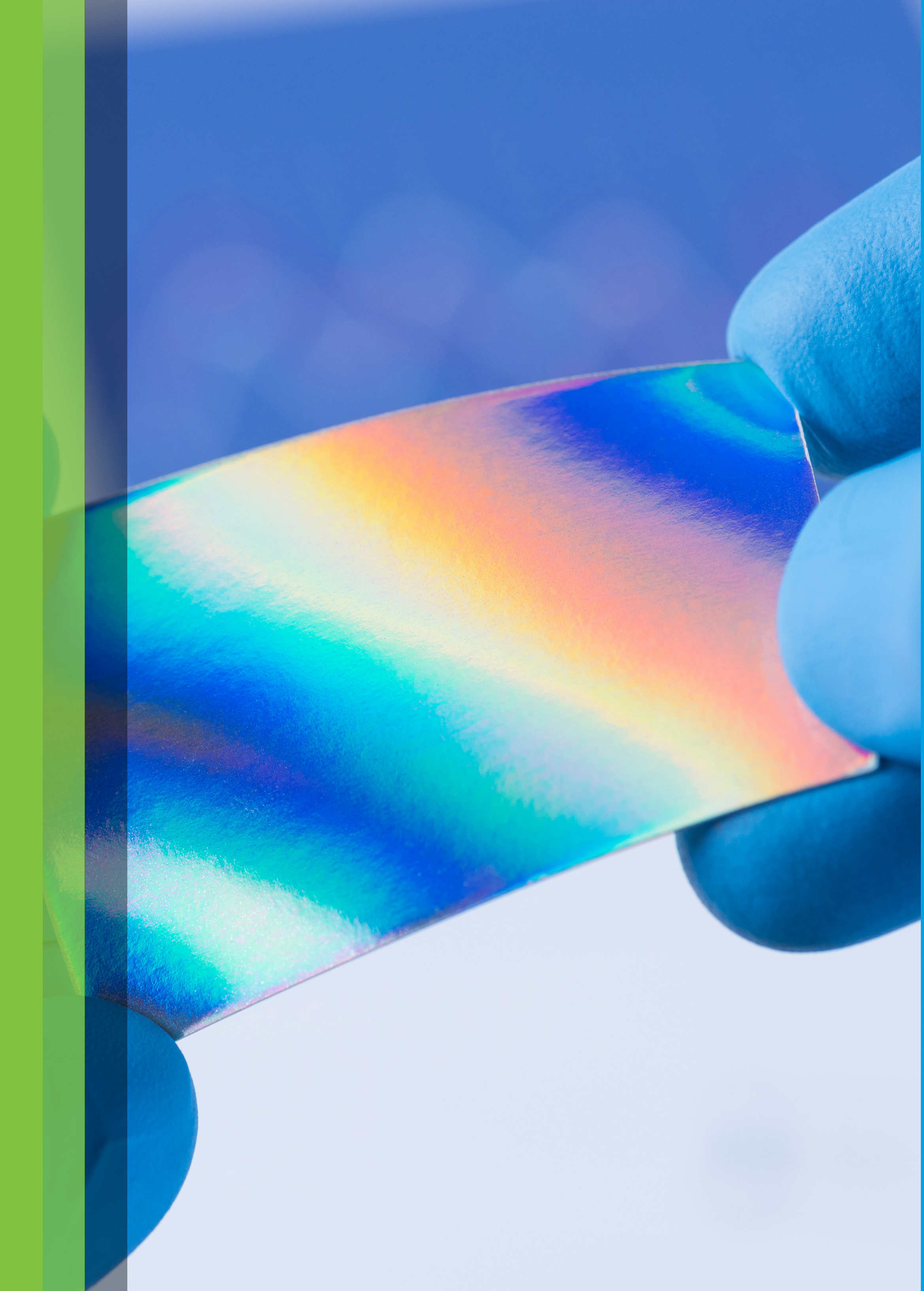
Agilent
Trusted Answers

The Basics of UV-Vis-NIR Spectrophotometry

A primer

Contents



1

Basic Principles of UV-Vis-NIR Measurements

1.1. The electromagnetic spectrum

Ultraviolet (UV) and visible (Vis) radiation are a small part of the electromagnetic spectrum, which includes other forms of radiation such as radio, infrared (IR), cosmic, and X-rays (Figure 1).

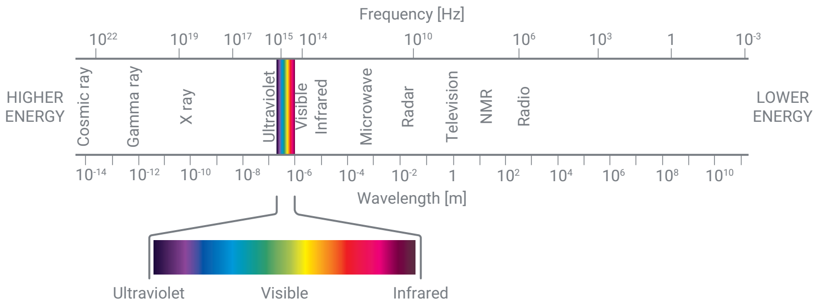


Figure 1. The electromagnetic spectrum, with the visible light section expanded.

The energy associated with electromagnetic radiation is defined as:

$$E = hv$$

where E is energy (in joules), h is Planck's constant (6.62×10^{-34} Js), and v is frequency (in seconds).

Spectroscopy enables the study of how matter interacts with or emits electromagnetic radiation. There are different types of spectroscopy, depending on the wavelength range that is being measured. UV-Vis spectroscopy uses the ultraviolet and visible regions of the electromagnetic spectrum, while infrared spectroscopy uses the lower-energy infrared part of the spectrum.

1.2. Wavelength and frequency

Electromagnetic radiation can be considered a combination of alternating electric and magnetic fields that travel through space in a wave motion. Because radiation acts as a wave, it can be classified in terms of either wavelength or frequency, which are related by the following equation:

$$v = c/\lambda$$

where v is frequency (in 1/seconds), c is the speed of light (3×10^{8} ms^{-1}), and λ is wavelength (in meters).

In UV-Vis spectroscopy, the wavelength is usually expressed in nanometers (1 nm = 10^{-9} m). It follows from the equation that radiation with a shorter wavelength has a higher energy, and, for UV-Vis spectroscopy, the low (short) wavelength UV light has the highest energy. Sometimes, this energy may be sufficient to cause unwanted photochemical reactions when measuring samples that are photosensitive.

UV-Vis spectroscopy is often used to study electronic transitions within atoms or molecules between different energy levels in the range of 10 to 750 nm. Near-infrared (NIR) spectroscopy covers frequencies that are lower than those of visible light and span the wavelength between 750 and 3000 nm. In the NIR region, molecular vibrations and overtones are typically observed which provide information about the movement of atoms within molecules. Both spectroscopic techniques provide valuable insight for the study of molecules and the properties of materials.

1.3. UV-Vis-NIR spectra

When radiation interacts with matter, several processes can occur, including reflection, scattering, absorbance, fluorescence/phosphorescence (absorption and re-emission), and photochemical reactions (absorbance and bond breaking).

Because light is a form of energy, absorption of light by matter causes the energy content of the molecules (or atoms) in the matter to increase. The total potential energy of a molecule is represented as the sum of its electronic, vibrational, and rotational energies:

$$E_{total} = E_{electronic} + E_{vibrational} + E_{rotational}$$

The amount of energy a molecule possesses in each form is not a continuum but a series of discrete levels or states. The differences in energy among the different states are in the order:

$$E_{electronic} > E_{vibrational} > E_{rotational}$$

In some molecules and atoms, incident photons of UV and visible light have enough energy to cause transitions between the different electronic energy levels.

The absorbed wavelength of light provides the energy needed to excite an electron from a lower energy level to a higher one. Figure 2 shows an example of electronic transitions in formaldehyde and the wavelengths of light that cause them.

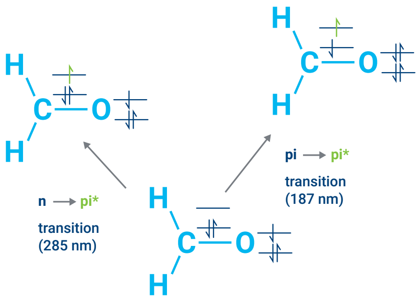


Figure 2. Electronic transitions in formaldehyde. UV light at 187 nm causes excitation of an electron in the C–O bond and light at the 285 nm wavelength causes excitation and transfer of an electron from the oxygen atom to the C–O bond.

These transitions result in very narrow absorbance bands at wavelengths that are highly characteristic of the difference in energy levels of the absorbing species. This phenomenon is also true for atoms, as depicted in Figure 3.

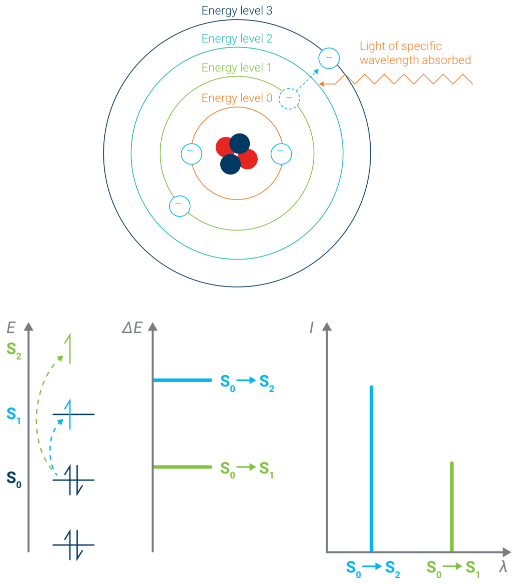


Figure 3. Incident light of a specific wavelength causes excitation of electrons in an atom. The type of atom or ion (i.e., which element it is) and the energy levels that the electron is moving between determines the wavelength of the light that is absorbed. Transitions can occur between multiple energy levels, with higher energy (i.e., shorter wavelengths of light) needed to move the electron farther from the nucleus.

However, for molecules, vibrational and rotational energy levels are superimposed on the electronic energy levels. Because many transitions with different energies can occur, the bands are broadened (see Figure 4). This broadening is even greater in solutions due to solvent—solute interactions.

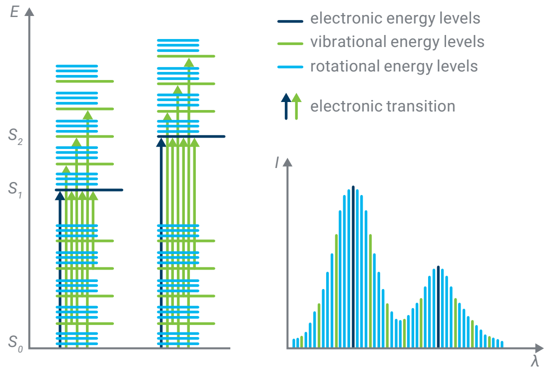


Figure 4. Electronic transitions and UV-visible spectra in molecules (I is intensity and λ is wavelength).

In NIR spectroscopy (750 to 3000 nm), the interplay between light and the sample causes molecular vibrations involving the stretching and bending of molecules. These vibrations manifest at specific frequencies, and their energy levels are intricately tied to the molecular configuration of the substance. The absorbed NIR light is then detected by the sample, generating a spectrum that provides insights into the molecular composition and material properties of a sample.

The molecular vibrations in NIR spectroscopy encompass overtones and combinations of fundamental vibrations (Figure 5). The NIR overtones and fundamental vibrations exhibit distinctive features in the spectrum for each molecule. NIR spectra are primarily the results of overtones and combination bands of groups such as O–H, C–H, N–H, and C=O. The NIR absorption bands are generally broader than UV-Vis due to multiple closely spaced vibrations that contribute to the overall absorption spectrum in the NIR range.

Test Methods for Solar Characteristics of Building Materials with UV-Vis-NIR Spectroscopy

In modern building design, material choices for facades, roofing, and paving greatly affect occupant comfort and energy efficiency. Key factors include roofing pitch and cladding color and texture. Understanding a material's optical properties in the solar radiation range is crucial for its application. Standard methods developed by national and international groups provide reproducible data on absorptance, reflectance, and transmittance of materials (300 to 2,500 nm). For instance, using ASTM standards and the Cary 5000 UV-Vis spectrometer with an external diffuse reflectance accessory, one can determine the total solar reflectance (TSR) and solar reflectance index (SRI) of painted aluminum samples.

[Read application note]

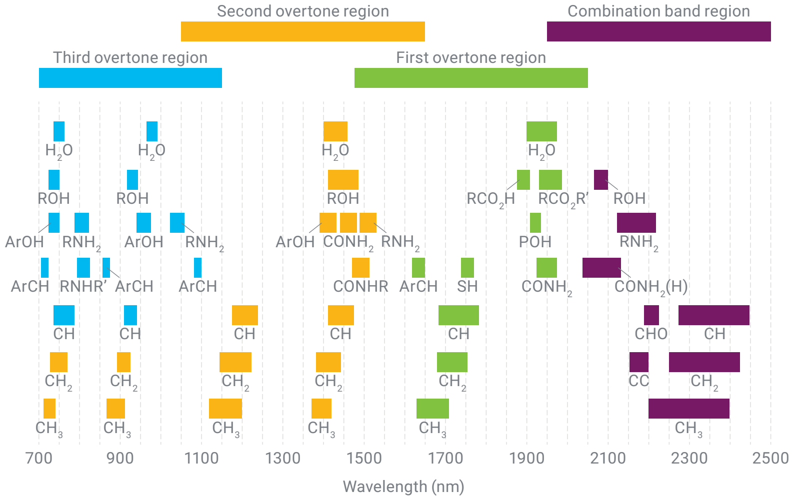


Figure 5. Characteristic overtones and combination vibrations in the near-infrared region. R and Ar (aromatics) represent other functional groups attached to the molecule.

1.4. Transmittance, absorbance, reflectance, and optical interference

When light passes through or is reflected from a sample, the amount of light that is absorbed is the difference between the incident radiation (Io) and the transmitted radiation (I). The amount of light absorbed is expressed as absorbance. Transmittance, or light that passes through a sample, is usually given in terms of a fraction of 1, or as a percentage, and is defined as follows:

$$T = I/Io \text{ or } \%T = I/Io \times 100$$

Absorbance is defined as follows:

$$A = -\log T$$

For most applications, absorbance values are used since the relationship between absorbance and both concentration and path length are normally linear. This linear relationship, which is known as the Beer–Lambert law, forms the foundation for quantitative analysis in UV-Vis-NIR spectroscopy.

The Beer–Lambert law states that the absorbance of a sample is directly proportional to the concentration (c) of the absorbing species, the path length (b) of the cuvette, and the molar absorption coefficient (ϵ) of the substance at a given wavelength:

$$A=\epsilon bc$$

Figure 6 shows a plot of absorbance against concentration.

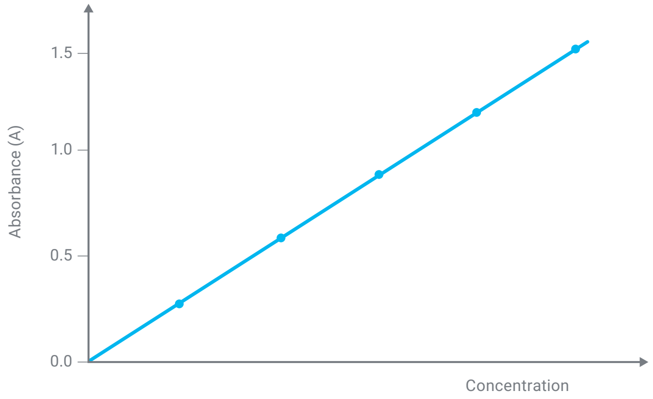


Figure 6. The Beer–Lambert law describes a linear relationship between absorbance of incident light and the concentration of the molecule.

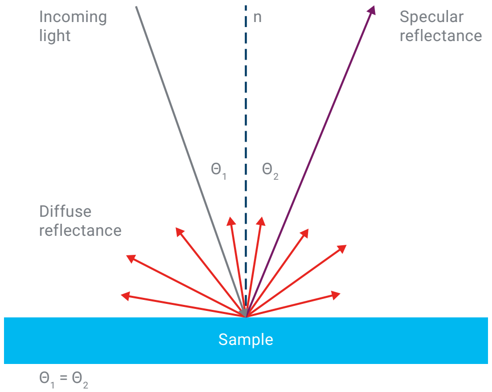


Figure 7. The two components of reflection: specular and diffuse reflection. n represents the surface normal, an imaginary line at 90° to the sample surface.

The traditional use of a UV-Vis-NIR spectrophotometer is to measure the absorbance or transmission of a clear or translucent liquid or solid.

In addition to this use, the inclusion of reflectance measurements expands the spectroscopic capabilities of the technique by enabling measurements beyond the portion of the beam that transmits through the sample. Reflection consists of two components: specular and diffuse. Specular reflectance is the mirror-like reflection off a sample surface. Diffuse reflectance occurs when the surface reflects light in many different directions, giving the surface a matt finish (Figure 7).

Therefore, the analysis is no longer limited to samples that are clear or translucent. UV-Vis-NIR diffuse reflectance accessories (DRAs) facilitate the measurement of absorbance or transmission in opaque, turbid, and reflective substances. Reflectance measurements in UV-Vis-NIR applications include characterizing solar materials, color measurement and characterization, and obtaining reflectance spectra of a painted surface, as described in Section 4 of this primer.

The transmission or reflection values obtained during a measurement may depend on the polarization of the radiation. The dependency is often more prevalent at angles of incidence (AOI) greater than 15 degrees onto the sample. For these reasons, separation of S (vertical) and P (horizontal) polarization state measurements via a polarizer is essential in UV-Vis-NIR spectroscopy. Using a polarizer enhances the quality of spectral data and enables the accurate characterization of materials.

Polarizers in UV-Vis-NIR spectroscopy serve several purposes including:

- The provision of separate results for each distinct polarization state: vertical (S) and horizontal (P). The unpolarized response can then be calculated (S + P)/2.
- Improving the photometric accuracy of the spectra. This improvement is crucial where the AOI exceeds 15° and if the S and P responses are markedly different.
- Controlling polarization effects: UV-Vis-NIR samples may have an inherent polarization response, and the use of polarizers allows researchers to control and manage these effects for specific sample types and experimental conditions.

Accurate material characterization using polarization is essential to ensure product quality, safety, and visual clarity. This concept is applied in everyday life, from eyewear (Figure 8) and photography to all display technologies and devices.

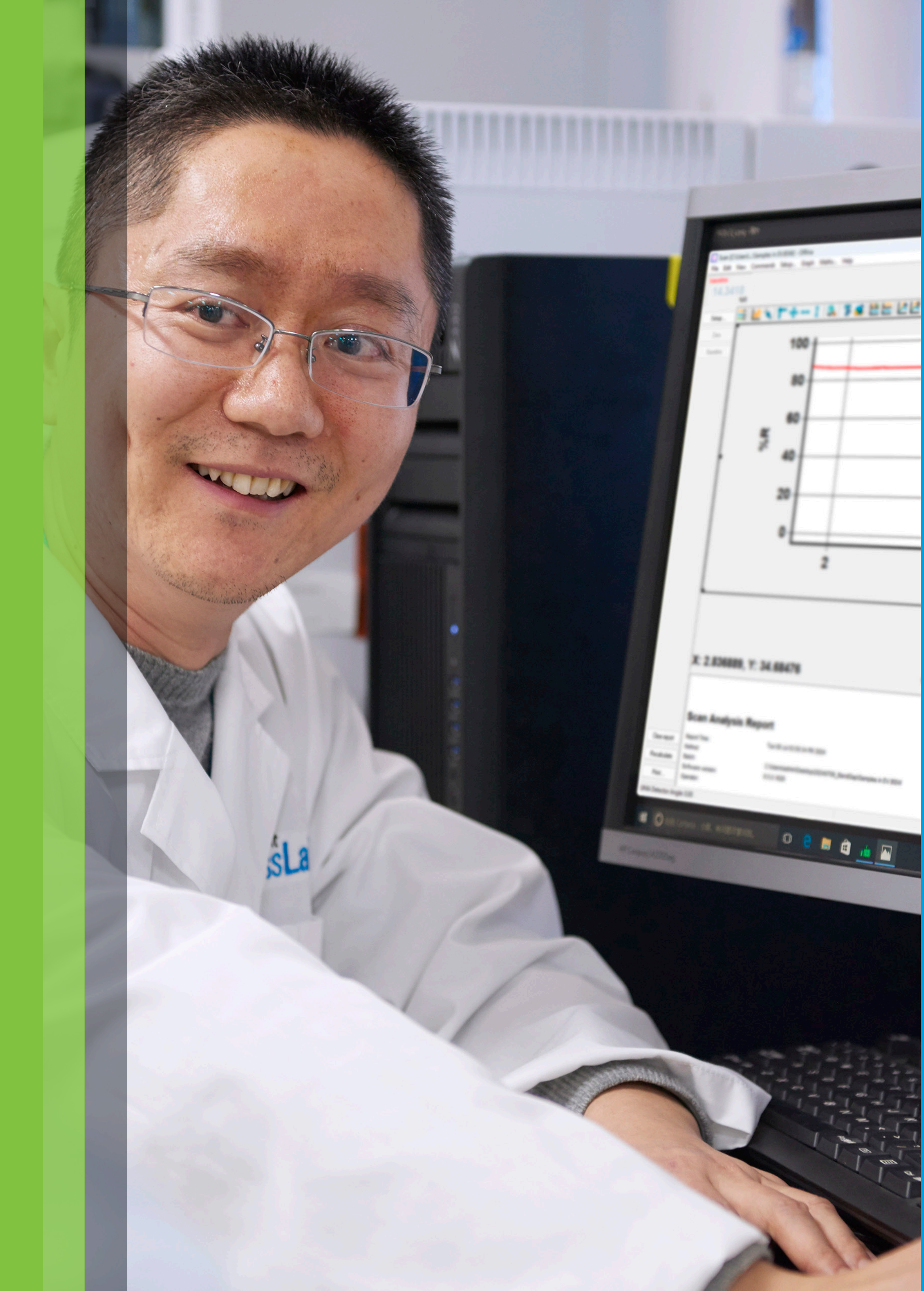
Figure 8. Illustration of the optical principle used by polarized sunglasses designed to reduce glare. The strong horizontally (S) polarized light reflected from the water is blocked by the vertically (P) oriented polarizer in the lens.

In instances where the diffuse scattering (in %T or %R) of a sample is low and the sample comprises one or more thin layers, optical interference can occur. Optical interference strengthens or diminishes the amplitude of the incident light, especially when the film thickness is of the same order of magnitude of the wavelength of incident light. Thin film interference is most commonly evident in the colors of the skin of soap bubbles (Figure 9). When the interference is analyzed spectroscopically, the results can provide information on the thickness of the film and the refractive index of the underlying material.

Figure 9. Spectral interference in the skin of soap bubbles.

1.5 Summary

- UV, visible, and infrared light are part of the electromagnetic spectrum.
- In UV-Vis-NIR spectroscopy, wavelength is expressed in nanometers (nm).
- Light can be reflected, scattered, transmitted, or absorbed from matter, and can cause photochemical reactions to occur.
- Energy from incident light causes electrons to transition to different energy levels. Electronic transitions also occur between the vibrational and rotational energy levels of molecules.
- Absorbance of light is used for most UV-Vis spectroscopy applications. It is defined as $A=-\log T$, where T is transmittance.
- Reflectance measurements expand the capabilities of spectroscopy by allowing analysis beyond the portion of the beam that penetrates the sample.
- Some NIR spectroscopy measurements require the use of polarizers/depolarizers based on the specific characteristics of the samples being analyzed and the experimental conditions.
- Optical thin film interference can be used to control the spectral response of samples that have been designed for specific applications and products.



2

How Does a Modern UV-Vis-NIR Spectrophotometer Work?

Ultraviolet visible (UV-Vis) spectrophotometers use a light source to illuminate a sample with light across the UV to the visible wavelength range (typically 190 to 900 nm). The instruments then measure the light that has been absorbed, transmitted, or reflected by the sample at each wavelength. Some spectrophotometers have an extended wavelength range, into the near-infrared (NIR) region (typically 800 to 3300 nm).

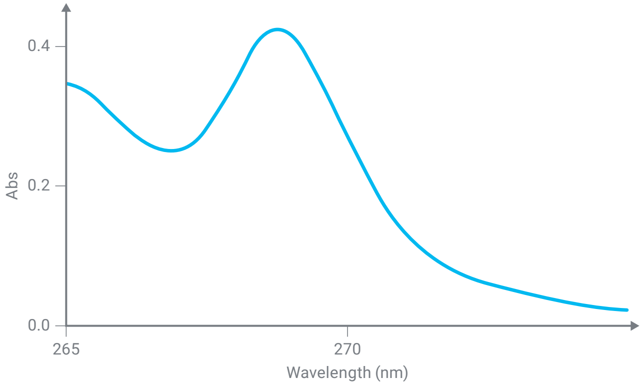


Figure 10. A UV absorbance spectrum, showing an absorbance peak at approximately 269 nm.

From the spectrum obtained, such as the one shown in Figure 10, it is possible to determine the chemical or physical properties of a sample.

In general, it is possible to:

- Identify molecules in a solid or liquid sample.
- Determine the concentration of a particular molecule in solution.
- Characterize the absorbance or transmittance through a liquid or solid—over a range of wavelengths.
- Characterize the reflectance properties of a surface or measure the color of a material.
- Study chemical reactions or biological processes.

Various types of measurements can be performed by combining different accessories and sample holders with the UV-Vis or UV-Vis-NIR spectrophotometer.

A range of accessories is available to accommodate various measurement capabilities and sample types, such as solids and liquids, as well as different measurement conditions (Figures 11 and 12).

Figure 11. A fiber-optic probe accessory can be fitted to a UV-Vis spectrophotometer to measure liquid samples in various containers such as a vial.

Figure 12. A solid sample, like this polycrystalline photovoltaic solar cell, can also be measured using a UV-Vis spectrophotometer.

2.1 Instrumental design

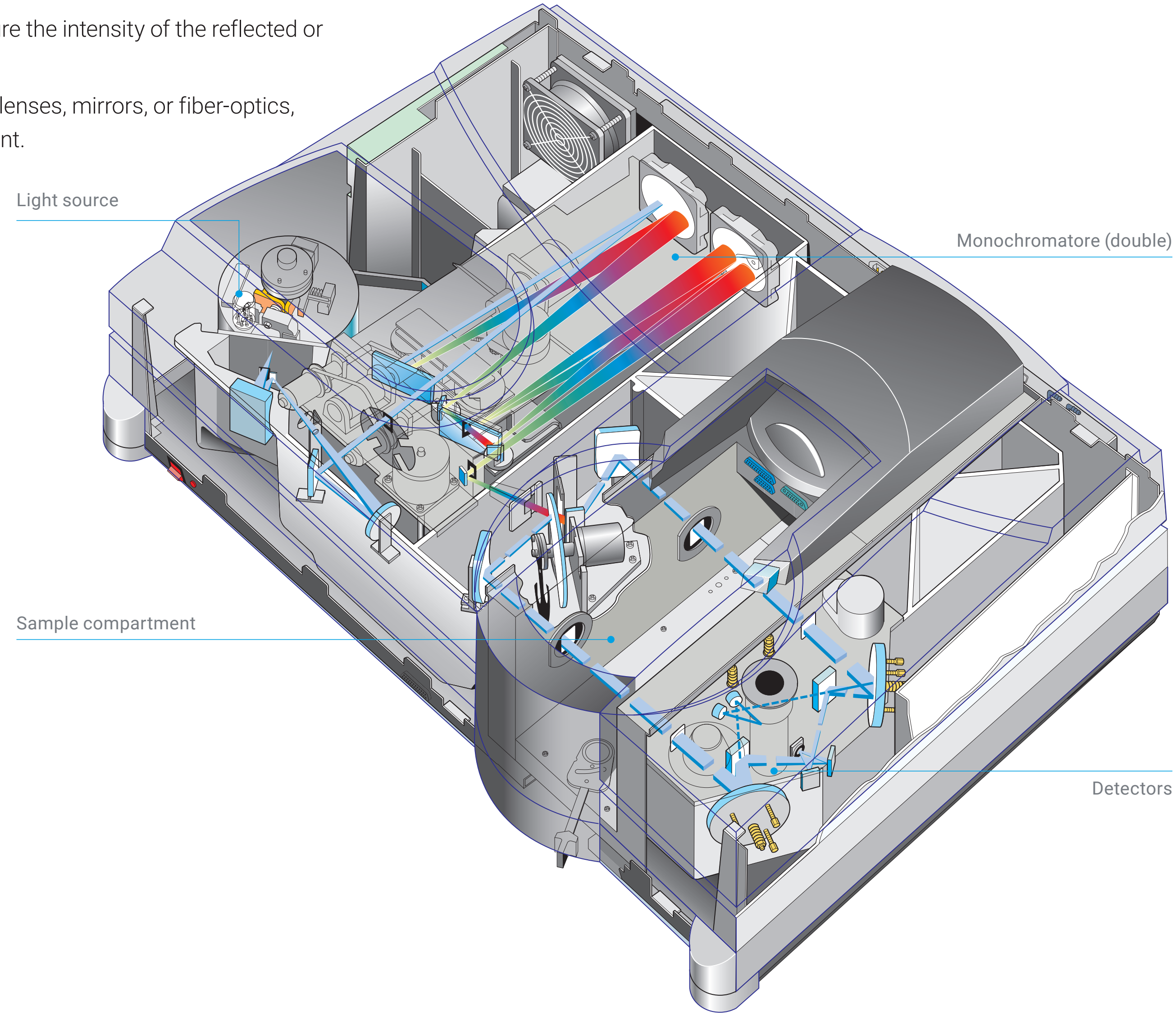
Components

The key components of a spectrophotometer (Figure 13) include:

- A light source that generates a broad band of electromagnetic radiation across the UV-Vis and NIR spectrum.
- A monochromator that separates the broadband radiation into discrete wavelengths (spectral bandwidths).
- A sample area, where the light passes through or reflects off a sample.
- One or more detectors to measure the intensity of the reflected or transmitted radiation.

Other optical components, such as lenses, mirrors, or fiber-optics, that relay light through the instrument.

Figure 13. Schematic of the internal layout of an Agilent Cary 5000 UV-Vis-NIR spectrophotometer, showing the main components. Note that this is a high-performance instrument. UV-Vis spectrophotometers used for routine measurements have a simpler optical design.



2.1.1 Light sources

The ideal light source would yield a constant intensity over all wavelengths with low noise and good long-term stability of the output. Unfortunately, such a source does not exist. Two different light sources have historically been used in UV-Vis-NIR spectrophotometers:

- The deuterium arc lamp provided a good intensity continuum in the UV region and useful intensity in the visible region.
- The tungsten-halogen lamp yielded good intensity over the visible and NIR range.

More recently, a single xenon flash lamp has been used more widely for UV-Vis systems. Using a single light source has significant advantages over the use of the two conventional lamps.

2.1.1.1 Deuterium arc lamp

Deuterium (D_2) arc lamps use an arc discharge from deuterium gas and yield a good intensity continuum in the UV region and useful intensity in the visible region, 185 to 400 nm (see Figure 14). Although modern D_2 arc lamps have low signal noise, noise from the lamp is often the limiting factor in overall instrument noise performance. Over time, the intensity of light from a D_2 arc lamp decreases steadily. Such a lamp typically has a half life (the time required for the intensity to fall to half of its initial value) of approximately 1,000 hours. This short half life means that D_2 lamps need to be replaced relatively frequently.

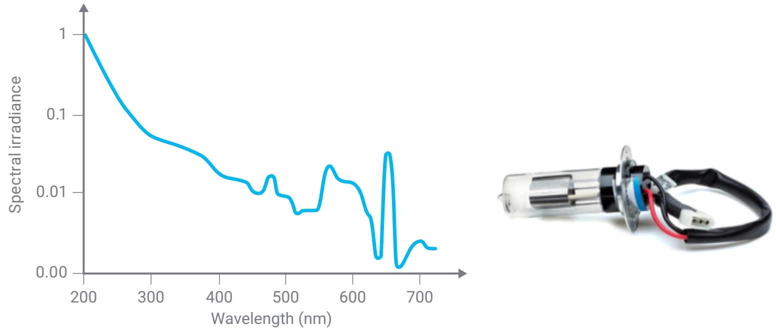


Figure 14. Intensity spectrum of a deuterium arc lamp.

2.1.1.2 Tungsten-halogen lamp

Tungsten-halogen lamps use a tungsten filament and halogen gas inside the bulb. When a current is passed through the filament, it becomes heated and emits light (see Figure 15). The lamp yields a good intensity over part of the UV spectrum and over the entire visible and NIR range (350 to 3300 nm). This type of lamp has very low noise and low drift and typically has a functional life of 10,000 hours.

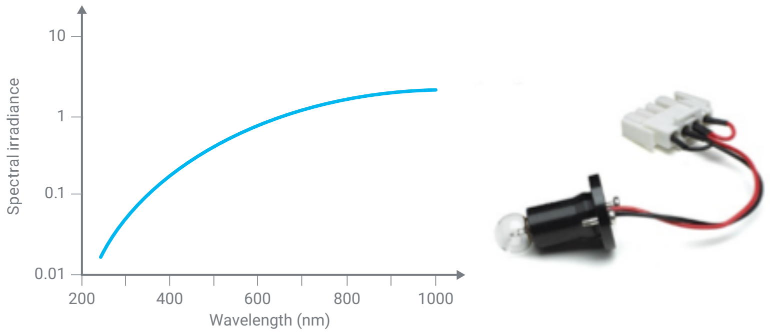


Figure 15. Intensity spectrum of a tungsten-halogen lamp.

In UV-Vis spectrophotometers using both a D_2 and a tungsten-halogen lamp, either a source selector is used to switch between the lamps as appropriate, or the light from the two sources is mixed to yield a single broadband source.

2.1.1.3 Xenon flash lamp

Unlike the D_2 or tungsten-halogen lamps, which provide a constant light source, a xenon flash lamp emits light for an extremely short time, in flashes. Since the lamp emits only for a short time and only during sample measurement, it has a long life. The sample is only irradiated with light at the time of measurement. This short illumination time makes the xenon flash lamp suitable for measuring samples that may be sensitive to photobleaching. Photobleaching can be observed on sensitive samples when exposed to a constant long exposure by a continuous light source. The xenon flash lamp emits high-intensity light from 185 to 2500 nm, which means no secondary light source is required (Figure 16). The xenon flash lamp may be used for many years before requiring replacement, which makes it a popular choice compared to systems using D_2 or tungsten-halogen lamps. An extra benefit is that it does not require any warmup time, unlike D_2 or tungsten-halogen lamps.

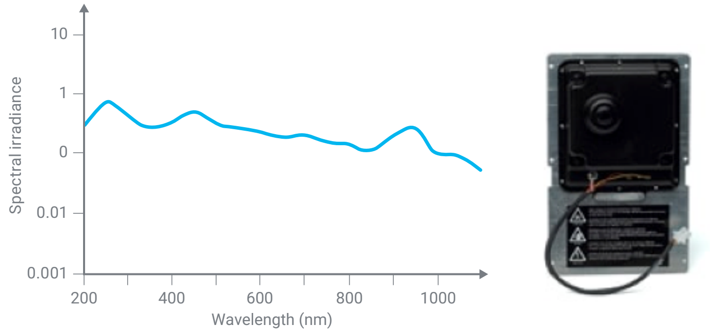


Figure 16. Intensity spectrum of a xenon lamp.

2.1.1.4 Mercury lamp

Mercury lamps emit a unique UV-Vis spectrum, making them suitable for UV-Vis spectroscopy (Figure 17). The spectral output of a mercury lamp is not limited to the UV range but also extends to the visible and infrared regions. The lamp efficiently emits mercury atomic emission lines that are used for spectrophotometer wavelength calibration and validation. Hence, the lamps are valuable light sources for measurement design experiments that are used to verify spectrophotometer performance. However, mercury lamps have several limitations in their use as a broad spectral light source, including light intensity variation over time and the emission of discrete spectral lines that can affect measurement reproducibility. Mercury lamps continue to be used in spectrophotometers due to their validation effectiveness and fine spectral output.

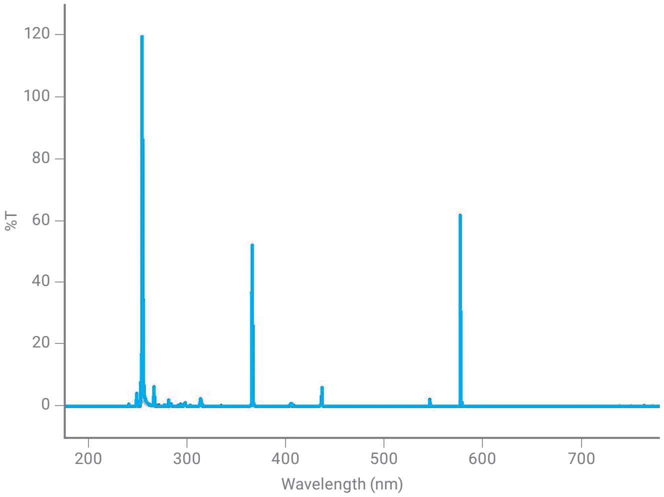


Figure 17. Intensity spectrum of a mercury lamp.

2.1.2 Monochromator

All the light sources produce a broad-spectrum of white light. To narrow the light down to a selected wavelength band, the light is passed through a monochromator, which consists of:

- An entrance slit.
- A dispersion device, to spread the light into different wavelengths (like a rainbow) and allow the selection of a nominated band of wavelengths.
- An exit slit where the light of the nominated wavelengths passes through and onto the sample.

An easy way to think about a monochromator is to think of a room with the sun shining in through a small gap in the window. On hitting a prism, the white light separates into a rainbow. The rainbow then falls onto a physical slit on the opposite side of the room. As the prism is rotated, light of different colors i.e., different wavelengths, pass through the slit at narrow-wavelength intervals.

Ideally, the output from a monochromator is light of a single wavelength. In practice, however, the output is always a band of wavelengths. Many spectrophotometers on the market today use holographic gratings as the dispersion device. These optical components are made from glass, with extremely narrow grooves precisely imprinted on their surface. The dimensions of the grooves are of the same order as the wavelength of light to be dispersed. Finally, an aluminum coating is applied to create a reflective surface.

Interference and diffraction of the light falling on the grating is reflected at different angles, depending on the wavelength. Holographic gratings yield a linear angular dispersion with wavelength and are temperature insensitive. However, they reflect light in different orders, which overlap (see Figure 18). As a result, filters must be used to ensure that only the light from the desired reflection order reaches the detector. A concave grating disperses and focuses light simultaneously.

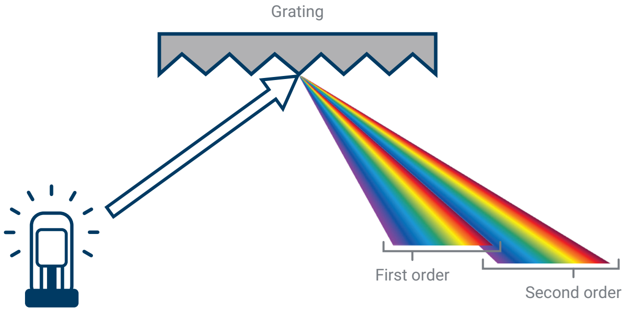


Figure 18. How a holographic grating disperses white light into light of different wavelengths.

Gratings can be made using various methods, with the most common types being holographic and ruled gratings. Holographic gratings are created using a laser interference pattern to produce grooves in a photosensitive material, resulting in smooth, regular patterned grooves. This smoothness leads to lower stray light, high signal quality, but relatively low efficiency. On the other hand, ruled gratings are produced by mechanically ruling grooves with tools, which can result in higher stray light due to imperfections or irregularities in the grooves. Such imperfections can be mitigated by careful pairing of gratings in double monochromator systems.

The efficiency and performance of both types of gratings are characterized by their groove density—measured as the number of lines or grooves per millimeter—which affects the grating's ability to disperse light. The optimal choice of grating type must be made with consideration of the entire optical system and its requirements, including wavelength range, maximum optical resolution, and dynamic range. High-quality order sorting filters can control stray light variations and counter efficiency differences.

2.1.2.1 Single monochromator spectrophotometers

A single monochromator spectrophotometer is used for general-purpose spectroscopy and can be integrated into a compact optical system.

Figure 19 shows a schematic diagram of a single monochromator optical system. A single monochromator spectrophotometer cannot select the wavelengths of light as narrowly as a double monochromator system. However, this ability may not be required for many applications, for example when measuring samples that have broad absorption peaks.

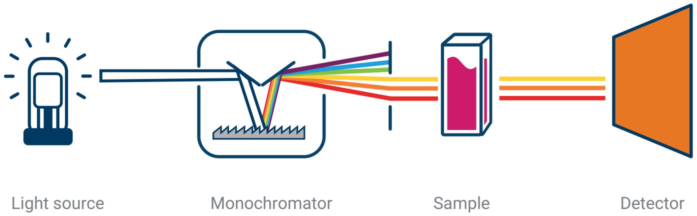


Figure 19. Schematic diagram of a single monochromator spectrophotometer.

2.1.2.2 Double monochromator spectrophotometers

A double monochromator is typically found in high-performance instruments. The two monochromators are arranged in series. The light source is split by the first monochromator and then further split by the second. This design ensures that stray light (light that leaks into the system) is reduced, while the spectral accuracy (the ability to accurately select a particular wavelength) is increased. Figure 20 shows a schematic diagram of a double monochromator optical system.

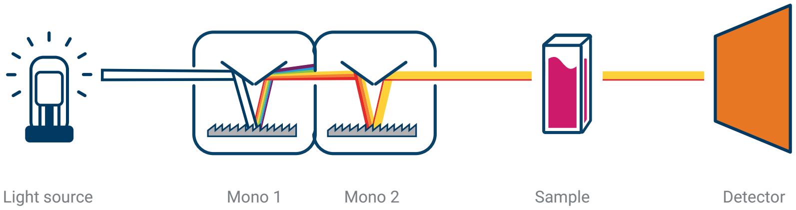


Figure 20. Schematic diagram of a double monochromator spectrophotometer.

2.1.2.3 Choppers

Choppers are rotating mechanical devices used in UV-Vis-NIR spectroscopy to modulate the light beam into sample and reference channels. Modulating the light beam enhances the signal-to-noise ratio and helps reduce the background noise by compensating for any short-term source intensity fluctuations. The chopper function is to periodically interrupt the light beam, generating a modulated signal that can be easily distinguished from the continuous background noise. As depicted in Figure 21, the chopper is divided into three sections. One section is mirrored and reflects the light beam, another section is 'cut out' and allows the light beam to pass through, and the third section is matt black to provide a dark, zero signal, reference level. This design allows spectrophotometers to alternatively direct light to the sample and reference beam channels.

The non-measurement (or dark) phase of the chopper allows the spectrophotometer to move the grating to the next wavelength and correct for 'dark current' at the detector. Moving the grating only during the non-measurement phase of the chopper eliminates the sideways shift of spectra that occurs on conventional spectrophotometers when higher scan speeds are used.

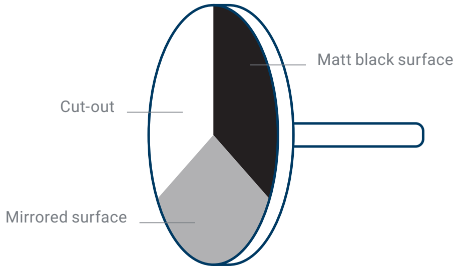


Figure 21. Illustration of a chopper used in UV-Vis-NIR spectrophotometers.

A three-segment chopper allows the spectrophotometer more time to measure each phase since less time is lost switching through three segments than four. This higher usable duty cycle results in more accurate and stable signal acquisition compared to a four-segment chopper.

2.1.3 Sample compartment

In the sample compartment, the sample is placed in the path of the sample beam, allowing the monochromator's light to pass through it. For the measurement of absorbance, liquid samples would typically be held in a cuvette with a known, fixed path length. Cuvettes are rectangular liquid holders, as shown in Figure 22, which are made from glass, quartz, plastic, or another material that transmits UV or visible light. Standard cuvettes have a 10 mm path length and are made from quartz, to ensure good transmittance of UV wavelengths. Cheaper plastic cuvettes can also be used, but generally do not transmit UV light so are only useful if measurements in the visible wavelength region are required. A multitude of cuvettes for special applications is available—from cuvettes that hold micro volumes of liquids through to cuvettes that have much longer path lengths, for use with very dilute samples.

Figure 22. Cuvettes for measuring liquid samples. From left to right: A standard 10 mm path length, 3 mL cuvette, an ultra-micro cell for measuring very low volumes, and a long path length cuvette for dilute solutions.

The sample compartment of a UV-Vis spectrophotometer is typically a black-colored box with a closing lid. The matt-black interior of the compartment helps to absorb stray light that may enter the compartment or be reflected from the sample. In the sample compartment, the sample is positioned to allow the beam from the monochromator to pass through the sample. As discussed previously, glass, plastic, or quartz cuvettes (Figure 22) are used for liquid samples. Solid samples are held in position by a holder attached to the floor of the sample compartment. The light can also be taken outside of the sample compartment using fiber optics. Fiber optics are useful when measuring very large, hot, cold, radioactive, or other hazardous samples. As shown in Figure 11, fiber optics can take the light from the spectrophotometer through a fiber-optic probe, to measure solutions outside of the sample compartment. Alternatively, a fiber optic device that allows the measurement of light reflectance, fluorescence, or transmission through a solid sample can be used.

A wide range of accessories can be fitted in the sample compartment of UV-Vis-NIR spectrophotometers to characterize various sizes and types of solid and liquid samples. For solid-, powder-, and paste-type samples, accessories such as Brewster angle holders, diffuse reflectance accessories (DRAs), polarizers and depolarizers, relative or absolute fixed, and variable angle specular reflectance accessories (SRAs) can be fitted in the sample compartment. For liquid samples, the sample compartment can accommodate a variety of single- and multicell holders, with or without temperature control.

Solid samples can be held in place for simple transmission measurements. They can also be rotated to be measured at various angles of incidence. For more scattering measurements, like diffuse reflectance or scattered transmission, other accessories may be installed into the sample compartment that are purposefully designed to capture the indirect scattered light.

2.1.4 The detector

A detector converts the light from the sample into an electrical signal. Detectors should give a linear response over a wide wavelength range, with low noise and high sensitivity. Spectrophotometers normally contain either a photomultiplier tube detector or a photodiode detector. Other specialized detectors are found on high-performance systems to improve wavelength coverage or sensitivity. Each detector has a different sensitivity and wavelength range. For systems with multiple detectors, the system will switch to the detector corresponding to the required wavelength range for the measurement.

2.1.4.1 Photomultiplier tube (PMT)

The photomultiplier tube (Figure 23) combines signal conversion with several stages of amplification within the body of the tube. The nature of the cathode material determines spectral sensitivity. A single PMT yields good sensitivity over the entire UV-visible range from 200 to 900 nm. A PMT detector provides high sensitivity at low light levels. For dilute samples, most of the light hitting the sample will pass through to the detector. To accurately detect small differences between blank and sample measurements, the detector must have low signal noise at these high light intensity levels.

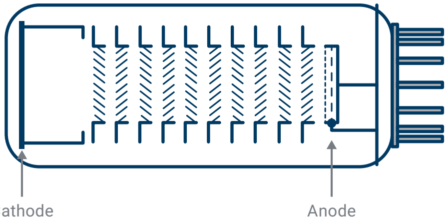


Figure 23. A photomultiplier tube detector.

2.1.4.2 Silicon diode (Si)

Silicon photodiode detectors (Figure 24) are extensively used as detectors in modern spectrophotometers. Photodiode detectors have a good dynamic range and are more robust to beam shifts than PMT detectors. In a photodiode, light falling on the semiconductor material allows electrons to flow through it, depleting the charge in a capacitor connected across the material. The amount of charge needed to recharge the capacitor at regular intervals is proportional to the intensity of the light. The limits of detection for silicon-based detectors are approximately 190 to 1100 nm.

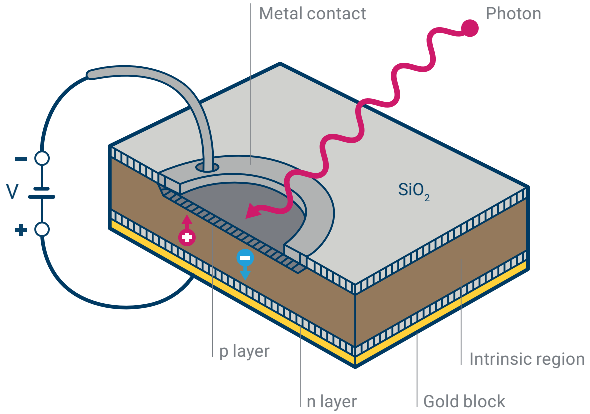


Figure 24. A silicon photodiode detector.

2.1.4.3 Indium gallium arsenide (InGaAs) photodiode detector

The InGaAs detector is a specialized infrared detector that provides excellent performance for the NIR wavelength range. InGaAs detectors are available in high sensitivity narrowband (800 to 1800 nm) versions and wideband (800 to 2500 nm) options. These detectors are useful for their linear response and sensitivity in the NIR region. The detectivity of wideband InGaAs detectors is similar to that of PbS detectors but is approximately one-hundredth of the detectivity of narrowband InGaAs detectors (Figure 25).

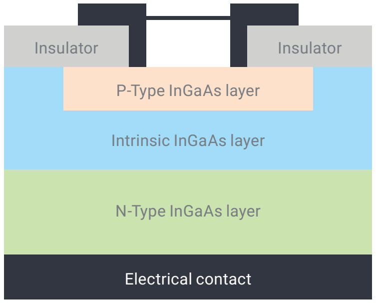


Figure 25. Indium gallium arsenide (InGaAs) photodiode detector.

2.1.4.4 Lead sulfide (PbS) detector

The most common NIR detector used in spectrophotometers is the PbS detector. This detector is sensitive between 800 and 3300 nm (Figure 26). In high-performance, wide-wavelength range spectrophotometers, the PbS detector is often combined with a PMT detector for UV-visible coverage. Where high sensitivity is required at the low NIR frequencies, a PbS detector may be combined with a narrowband InGaAs detector. PbS detectors are more susceptible to system- and sample-specific phenomena such as dark current, black body radiation, and linear response. However, optimal PbS performance can be achieved using appropriate electronic and algorithmic corrections to minimize these effects.

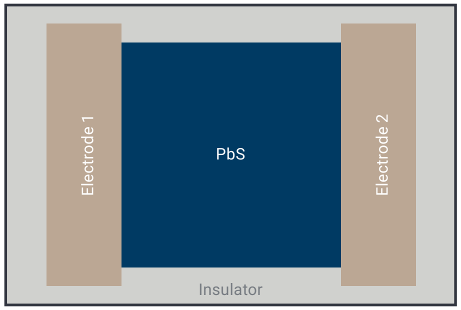


Figure 26. Lead sulfide (PbS) detector.

2.1.4.5 Two-color detector (Si/InGaAs)

Two-color detectors, which cover a very wide spectral response range of 190 to 2500 nm, consist of a Si photodiode overlapped with an InGaAs PIN photodiode along the same optical axis (Figure 27). These detectors typically include a built-in thermoelectric cooler that maintains a constant temperature during operation, enabling precise measurements with an enhanced signal-to-noise ratio. The broad wavelength range of this detector simplifies the design of UV-Vis-NIR spectrophotometry.

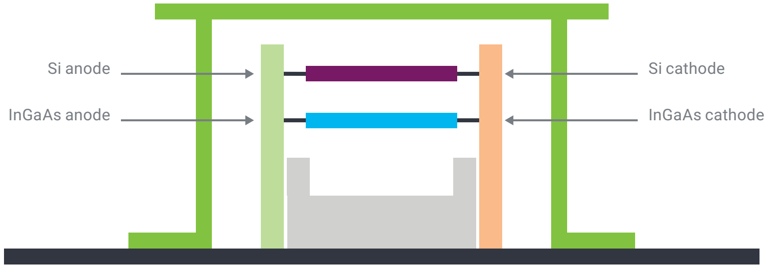


Figure 27. Two-color detectors (Si/InGaAs) for UV-Vis-NIR spectrophotometry.

Measuring the Performance of Compact Visual Displays

The prevalence of smart devices that use visual displays continues to increase in popularity as their size and weight requirements face growing demands for reduction. Longer battery life simultaneously puts pressure on the power allocated to their displays. Device mobility in both handheld and head-worn devices is driving this. LED and LCD technologies are widely used in mobile phones, laptops, TVs, and virtual/augmented reality headsets. Manufacturers of such devices strive for thinner display packages, with backlighting (using CCFL or LED) illuminating the display and liquid crystals controlling the image. Solid light guides, often made from transparent polymers, can be used for backlighting, and are enhanced with back reflectors to improve display electrical efficiency. High reflectivity (>98%) of the back reflectors is crucial for effective light transport, as even small losses can significantly reduce available light.

Read application note

2.2 Optical configurations of spectrophotometers

2.2.1. Single beam spectrophotometer

The simplest UV-Vis spectrophotometer has a single beam optical system. In a single beam system, the light from the monochromator passes through the sample and then to the detector. This simple design means that less optical components are used, making it possible to reduce the size and cost of the instrument.

However, before a sample can be measured, a baseline or blank sample must be measured. For liquid measurements, the baseline reading is taken to account for any absorbance of the cuvette and solvent used. With a single beam system, the baseline needs to be measured separately from the sample. The separate readings mean that if there is any variation of light intensity (e.g., instrument drift), or system optical performance, between the baseline and sample being read, the measurement may be less accurate. This inaccuracy is a concern for sample measurements that take a long time, or where the blank may vary over time. In practice, this means that a baseline/blank measurement should be run frequently and regularly during a measurement session if using a single beam system.

2.2.2 Double beam spectrophotometer

Many UV-Vis-NIR systems use a double beam optical system. In the double beam type, the light emitted from the monochromator is split into two beams: a reference beam and a sample beam (Figure 28). The light is usually split with an optical component, such as a chopper (Figure 21), which has a mirrored segment or a half-silvered mirror called a beam splitter. Each beam enters the sample chamber through separate optical paths. Since two beams of the same wavelengths are available, the reference/blank and sample can be measured at the same time. This dual-measurement capability means that the sample measurement can be corrected for any instrument fluctuations in real time. This real-time correction ensures highly accurate measurements.

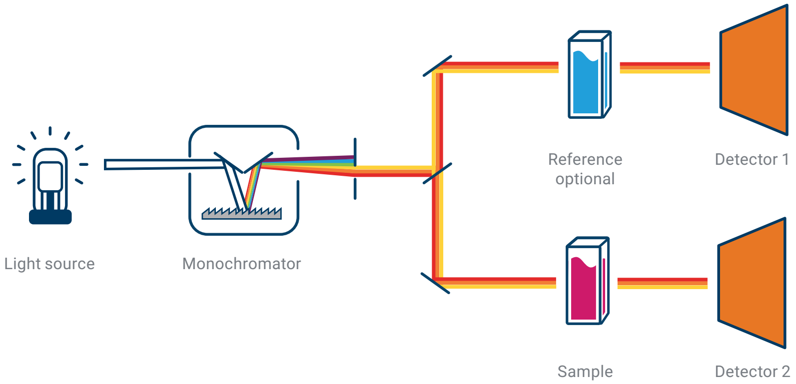
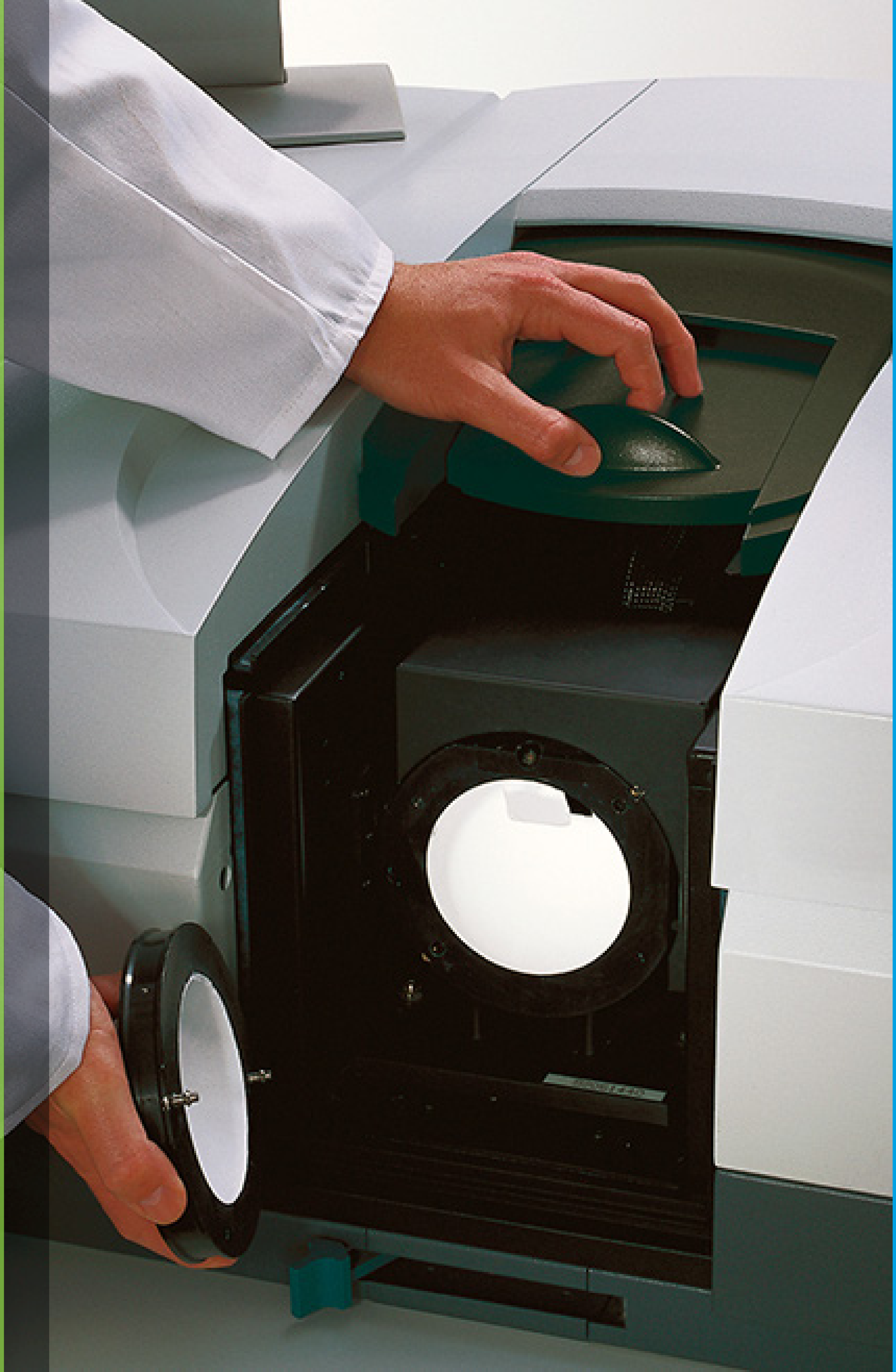


Figure 28. Schematic diagram of a double beam optical system, with dual detectors.

Double beam spectrophotometer design uses a dual-beam optical layout with a sample and reference detector. The reference detector is used to correct any lamp brightness fluctuations for each measurement. The solvent or blank (in the case of a solid sample) is measured in the sample position and then subtracted from the sample spectrum after collection.

Some double beam systems don't provide access to the reference beam, this design keeps the measurement process simple and reduces the chance of user error due to mismatched cuvettes or incorrect sample placement.



3

Selecting the Optimum Accessory and Parameters for Your UV-Vis-NIR Measurements

Selecting the most suitable accessory, sample holder, and instrument parameters is critical for the success of your UV-Vis-NIR measurement.

3.1 Measurement mode

UV-Vis-NIR spectroscopy is widely used for the characterization of solid samples. Example applications include assessing the performance of solar materials, checking the visual appearance of samples through color measurements, and quantifying the reflectance of optical components. These applications can be performed by measuring reflection or transmission, as summarized in Figure 29.

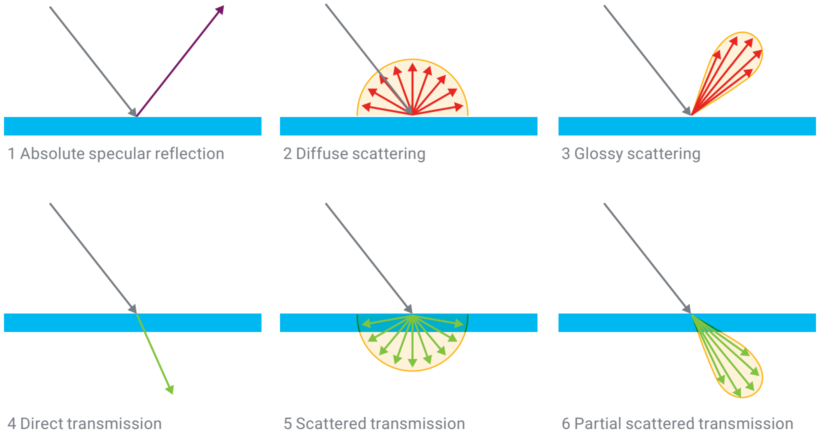


Figure 29. Modes of measurement used in UV-Vis-NIR spectroscopy. Specular reflection (purple), Diffuse scattering (red), Transmission and Scattered Transmission (green).

As illustrated in Figure 7, Chapter 1, the incident light that strikes a sample may result in:

- Transmission
- Diffuse reflectance, which occurs when the surface reflects light in many different directions giving the surface a matt appearance.
- Specular reflectance, which is the mirror-like reflection off a sample surface.

The reflection of light from a sample can be measured in either absolute %R values or relative %R, depending on the measurement mode and accessory used.

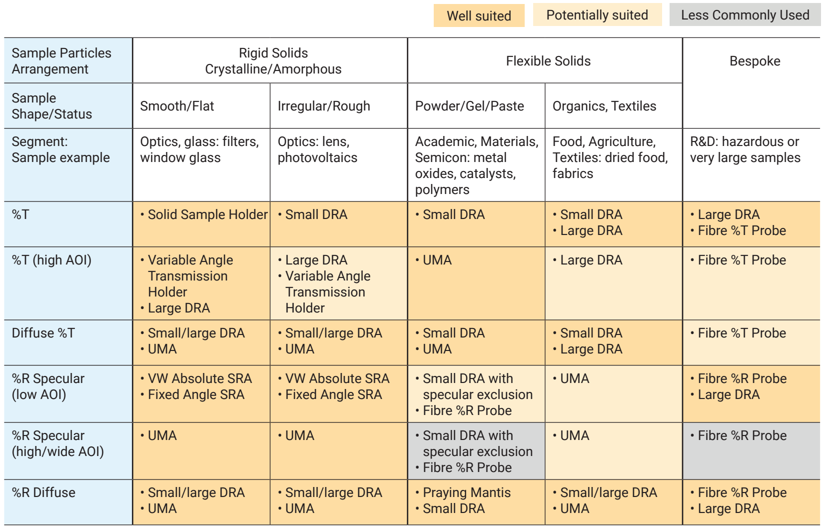
Relative reflectance is measured by calculating the proportion of reflected light collected from a sample surface, relative to the amount of reflected light measured from a reference material (RM). Polytetrafluoroethylene (PTFE), a barium sulfate ($BaSO_4$) plate, or an aluminum mirror are typically used as RMs. (Standard reference materials (SRMs) are also available that provide certified values for optical properties such as transmission or reflection.) The RM is used to establish a baseline, based on the assumption that the RM has 100% reflection. Relative reflectance is calculated using the following equation:

$$\textit{Relative reflectance (R\%)} = \frac{\textit{Light reflected from the sample}}{\textit{Light reflected from the reference}} \times 100$$

On the other hand, absolute reflectance is measured by calculating the proportion of reflected light relative to the amount of light measured directly from a light source, without using a reference sample (see the following equation). This measurement accurately determines the true reflectance of samples.

$$\textit{Absolute reflectance (R\%)} = \frac{\textit{Light reflected from the sample}}{\textit{Instrument light}} \times 100$$

Depending on the type of sample, its size, and the analytical requirements of the study, an appropriate UV-Vis-NIR accessory can be employed, as illustrated in Figure 30.



🟧 Well suited 🟨 Potentially suited ⬜ Less Commonly Used

Sample Particles Arrangement	Rigid Solids Crystalline/Amorphous		Flexible Solids		Bespoke
Sample Shape/Status	Smooth/Flat	Irregular/Rough	Powder/Gel/Paste	Organics, Textiles	
Segment: Sample example	Optics, glass: filters, window glass	Optics: lens, photovoltaics	Academic, Materials, Semicon: metal oxides, catalysts, polymers	Food, Agriculture, Textiles: dried food, fabrics	R&D: hazardous or very large samples
%T	🟧 • Solid Sample Holder	🟧 • Small DRA	🟧 • Small DRA	🟧 • Small DRA • Large DRA	🟧 • Large DRA • Fibre %T Probe
%T (high AOI)	🟧 • Variable Angle Transmission Holder • Large DRA	🟨 • Large DRA • Variable Angle Transmission Holder	🟧 • UMA	🟨 • Large DRA	🟨 • Fibre %T Probe
Diffuse %T	🟧 • Small/large DRA • UMA	🟧 • Small/large DRA • UMA	🟧 • Small DRA • UMA	🟧 • Small DRA • Large DRA	🟨 • Fibre %T Probe
%R Specular (low AOI)	🟧 • VW Absolute SRA • Fixed Angle SRA	🟧 • VW Absolute SRA • Fixed Angle SRA	🟨 • Small DRA with specular exclusion • Fibre %R Probe	🟨 • UMA	🟧 • Fibre %R Probe • Large DRA
%R Specular (high/wide AOI)	🟧 • UMA	🟧 • UMA	⬜ • Small DRA with specular exclusion • Fibre %R Probe	🟨 • UMA	⬜ • Fibre %R Probe
%R Diffuse	🟧 • Small/large DRA • UMA	🟧 • Small/large DRA • UMA	🟧 • Praying Mantis • Small DRA	🟧 • Small/large DRA • UMA	🟧 • Fibre %R Probe • Large DRA

Figure 30. V-Vis-NIR accessory selection guide based on sample type, size, analytical requirements, and measurement mode. DRA (Diffuse Reflectance Accessory), SRA (Specular reflectance Accessory), UMA (Universal Measurement Accessory).

3.1.1 Transmission measurements (%T)

UV-Vis-NIR spectroscopy is used to study the optical properties of solid samples designed to transmit light, including filters, glass, polymers, and other transparent solid materials.

The technique involves measuring the transmission of light through a sample across the UV-Vis-NIR spectral regions. The sample holder is one of the most critical components to consider when performing transmission measurements of solid samples (Figure 30). The holder is designed to facilitate transmission measurements of various solid samples. It holds the sample securely in the path of the incident light beam, allowing accurate measurement of the transmitted light. The holder is versatile and can accommodate different types of solid materials, including filters, glass, and polymers, as mentioned previously.

Other transmission holders can enhance the functionality of the solid sample holder by enabling transmission measurements at various angles of the incident light. The Brewster angle holder (Figure 31) fits into the solid-sample holder slide and allows measurement of the transmission of light through the sample at different angles of incidence. This capability is useful for examining optical properties that are angle-dependent.

The variable angle transmission accessory is another useful holder. It is designed for the examination of thick samples and optical coatings at any angle using transmission spectroscopy. When light impinges on a thick sample at an angle, refraction occurs causing a slight shift in the transmitted light. This shift can alter the measured transmittance. The variable angle transmission accessory addresses this issue by allowing both the sample and a blank (with matching thickness and refractive index) to be placed in the beam at the same angle but in opposing directions. This arrangement ensures that the transmitted beam is shifted back to its original position, providing accurate measurements.

The variable angle transmission accessory also includes a holder for an optional polarizer. A polarizer is beneficial for optimizing measurement performance of samples that polarize the beam due to the material properties or a high angle of incidence. The polarizer can be rotated 90° around the beam axis, making it suitable for Fourier Transform (FT)-IR or UV-Vis transmission studies of samples up to 6 or 10 mm thick, depending on the sample holder.

Figure 31. UV-Vis-NIR transmission holder for solid samples (left) and adjustable angle transmission holders (right).

Liquid sample measurements using UV-Vis-NIR spectroscopy involve analyzing the absorption of UV-Vis-NIR light by a liquid solution to determine its chemical properties and concentration. Typically, the sample is placed in a cuvette, a vessel typically made of quartz glass that holds the liquid without absorbing light transmitted at the wavelengths of interest. The spectrophotometer directs a beam of light through the sample and measures the intensity of light before and after it passes through the liquid. The difference in light intensity at specific wavelengths reveals the absorbance, which can be correlated to the concentration of the absorbing species in the solution.

This method is widely used for its simplicity, accuracy, and rapid results, making it ideal for applications in chemistry, biology, environmental science, and industrial processes. When measuring liquid samples, it is important to consider the path length of the cuvette, the absorbance properties of the solvent, and the concentration range of the sample. These factors help avoid deviations from the Beer–Lambert law, ensuring reliable and reproducible results.

3.1.2 Diffuse/total reflection measurements (%R)

Diffuse Reflectance Accessory (DRA)

Traditionally, an integrating sphere accessory has been used to measure relative diffuse reflectance (Figure 32). Integrating spheres have also been effective for measuring the transmission of turbid, translucent, or opaque refractory materials. Standard techniques are unsuitable for the analysis of these sample types because the materials scatter light, leading to significant light loss. However, integrating spheres help capture and measure this scattered light, providing more accurate results.

Figure 32. Diffuse reflectance accessory with a white integrating sphere.

Diffuse reflectance measurements are made by mounting a sample in line with the hole in the sphere wall. This alignment ensures an efficient collection of a high proportion of diffusely reflected radiation from the sample. Although commonly referred to as a diffuse reflectance accessory (DRA), options are available to exclude or include the specular component of the reflected radiation, providing the choice of either diffuse-only or total-reflectance modes.

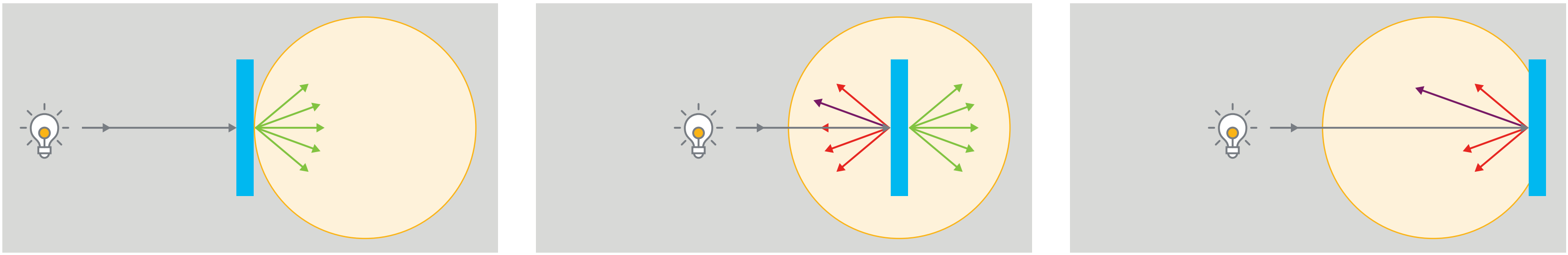
The functionality of a DRA is not limited to reflectance measurements. Integrating spheres can also be used to measure transmittance by mounting the sample at the entrance port or at the center of the sphere. The center mount, augmented by rotational control over the sample axis, complements the straight diffuse measurement mode (Figure 33).

DRAs are available in various sizes, typically with a 60 to 150 mm internal diameter, and are usually coated with PTFE, which exhibits high reflectivity across the UV, visible, and NIR range. To accommodate different sample types and sizes, the DRA can be fitted with a liquid or powder cell holder.

Factors affecting the accuracy or precision of measurements when using DRAs

When using DRAs, some of the reflected light escapes through the ports, which reduces the signal-to-noise ratio, and therefore the precision of the measurement.

- **Port-to-sphere ratio:** This is the ratio of the total area of all ports to the internal area of the integrating sphere. The value of the ratio should be maintained at <10% to avoid excessive light from escaping through the port. Maintaining a low port-to-sphere ratio also ensures that the introduction of the sample itself does not change the reflectance properties of the sphere wall outside of the direct area being illuminated and measured (called substitution error).
- **Coating non-uniformity, aging, or contamination:** Integrating spheres are coated with PTFE via a unique process that ensures both a uniform coating on the inside of the sphere and the correct powder density. PTFE is durable and does not yellow. The reflectivity of PTFE coatings is above 96% between 200 and 2500 nm, and greater than 99% between 350 and 1800 nm. The sphere will maintain its reflectivity indefinitely, provided it is not exposed to smoke or other contaminants.
- **Incorrect sample placement:** Theory assumes that the placement of the sample will coincide with the inside of the sphere wall. The sample is normally placed at a port on the outside of the sphere wall. Spacing between the sample and the sphere wall can lead to large errors due to loss of reflected light.



Transmission

Center

Figure 33. Schematics showing transmitted and reflected light. Diffuse reflection (red), specular reflection (purple), and diffuse transmission (green). The incident light is shown in black, and the sample is shown in blue.

- **Sample recess:** Theory assumes that the sample is placed coincidently with the inside of the sphere wall; however, in practice, the sample is placed against the outside of the sphere wall. The porthole edges have a finite thickness, and some part of the beam that is reflected at wide angles may be intercepted by the sphere wall. This effect can be minimized by beveling the sphere wall.
- **Reference beam attenuation:** Reference beam attenuation is important when the accessory or sample in the sample beam significantly attenuates the light beam. In these situations, attenuation of the reference beam will decrease noise and greatly increase the dynamic range of the instrument, as the detector is not 'seeing' two dramatically different signals. If reference beam attenuation is required, metallic mesh screens are recommended.
- **Stray light:** If the irradiating beam overfills the reflectance port, a proportion of the sphere-wall reflectance signal mixes with the sample reflectance signal. If this effect is not consistent between the baseline and the sample measurement, it will give a false-high reading. To account for this potential inconsistency, the baseline signal can include a zero-signal level reading by mimicking an infinitely absorbing sample. This reading corrects for overfilling ports when small samples are measured with port reducing apertures.
- **Gloss trap error:** A gloss trap error is produced when the gloss trap is unable to completely absorb the specular component. In the measurement of diffuse-only reflectance, a 'gloss trap' is used to extinguish/absorb the specular component. Gloss traps are typically glossy black pyramidal light traps, mat, black-coated cavities, or razor blade Fresnel light traps. However, the reflectance of glossy samples can broaden the specular reflection, which cannot be excluded by standard-sized light traps.
- **Differences between the standard and sample:** It is important that the reflectivity and properties of the RM are similar to those of the sample. Otherwise, major errors can be introduced, if, for example, a poorly reflecting material is measured relative to a highly reflective PTFE reference plate. Table 1 lists the appropriate RMs that are recommended for use with different sample types.

Table 1. Recommended reference materials for use with different sample types.

Type of Sample Surface	Reflectivity	Reference Material
Matt	High	PTFE reference plate
Matt	Low	Low %R diffuse reflectance standard
Glossy	High	Protruding PTFE reference plate
Glossy	Low	NIST SRM 2021

- **Inaccuracy in the reference material:** If the RM that is used to calculate a value is not accurate, this will hamper the determination of the reflectance of the sample. Great care must be taken to keep RMs clean and scratch-free.

Praying Mantis

The Praying Mantis (Figure 34) is another accessory that is used to measure relative diffuse reflection, especially of small volumes of powders and pastes. It uses two large hemispherical mirrors that gather light reflected from the crystalline or amorphous samples. The requirement for sample volumes as small as 3 mm in diameter enables researchers who are developing new compounds to perform precise analyses. The diffuse reflection values measured with the Praying Mantis are relative to a PTFE RM.

High- and low-temperature chemical reaction chambers can also be integrated into the Praying Mantis during diffuse reflection measurements. These versatile sample holders allow control over the temperature, pressure, and gas environment of the sample during measurement. This versatility makes them ideal for material science analysis in academic and government research laboratories, as well as in inorganic chemistry and physics laboratories.

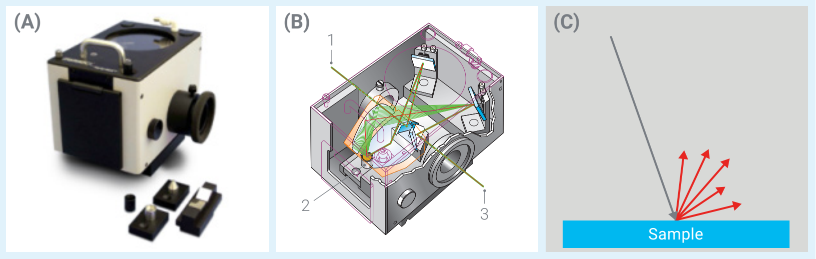


Figure 34. (A) UV-Vis-NIR Praying Mantis diffuse reflectance accessory used for the measurement of small volumes of powders or pastes. (B) Optical diagram of the Praying Mantis accessory: 1. Incoming light, 2. Sample cup, 3. Outgoing light. (C) The incident light (black) hits the sample, and the Praying Mantis accessory collects light that is reflected from the sample surface (red).

3.1.3 Specular reflection measurements (%R)

Specular reflection is measured when light reflects off a smooth, shiny surface at a specific angle, maintaining the angle of incidence equal to the angle of reflection. The light remains organized and does not scatter in various directions, a characteristic of surfaces like mirrors, gloss paints, and polished materials. In UV-Vis-NIR spectroscopy, absolute specular reflection can be measured using an absolute Specular Reflectance Accessory (VW SRA) or a relative SRA. These measurements can be made near to the sample normal (a line that is orthogonal to the sample surface) or at any other acute angle up to 90°.

Fixed Angle Specular Reflectance Accessories

Basic SRAs are designed for relative specular reflectance measurements, often at fixed angles and with horizontal sample placement (Figure 35). A near-normal SRA, which operates at angles typically less than 10° from normal is suitable for UV-Vis-NIR reflectance assessments of materials like films on metal bases, epitaxial layers, and both antireflection and reflective coatings, especially for opaque samples. Other common non-normal angles used for measurements, depending on the specific accessory, include 12.5°, 22.5°, 30°, 45°, and 60°. For reflection measurements, an RM, such as a mirror, is initially positioned on top of the accessory to establish the baseline data. The RM is then replaced by the test sample, which is positioned face-down, so that a relative specular reflection measurement can be obtained. Samples can be positioned using an exchangeable mask with diameters of 3, 6, or 13 mm, accommodating small sample sizes or targeted areas of larger samples.

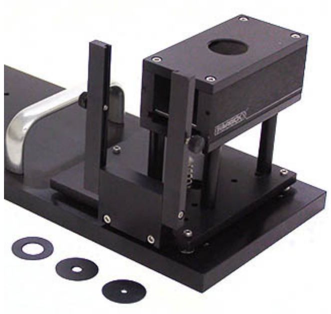


Figure 35. Fixed angle specular reflectance accessory for relative UV-Vis-NIR measurements.

VW absolute specular reflectance accessory

The VW absolute SRA (Figure 36) is named after the shape of the light paths through the accessory: “V” for baseline calibration measurements and “W” for sample measurements. The accessory is designed to measure near-normal (7º) mirror-like reflectance off sample surfaces, such as high-reflectance mirrors or low %R antireflection coatings. Applications include determining the degree of polishing of an optical surface and measuring the film thickness of thin coatings.

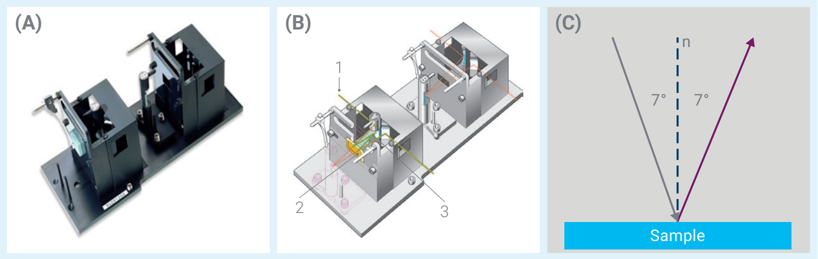


Figure 36. (A) UV-Vis-NIR absolute specular reflectance measurement accessory. (B) Optical diagram of a VW SRA accessory: 1. Incident light, 2. Movable spherical mirror, 3. Sample. (C) The accessory measures light reflected from a sample surface at a near-normal (7°) angle of incidence.

The accessory features a dual ‘VW’ configuration, with one spherical mirror (S1) and two toroidal mirrors (T1 and T2) per beam (Figure 37). The movable mounting of the spherical mirrors allows them to be used for both baseline calibration and sample measurement. Hence, the same optical components are in the light path during both baseline and sample measurements. When a sample is mounted, the only change in the system is due to the reflectivity of the sample, enabling an absolute value of the reflectance to be obtained.

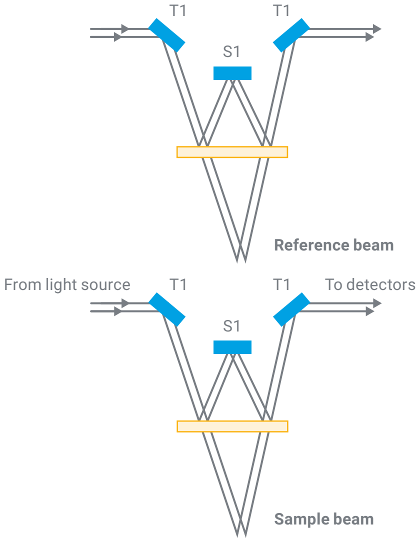


Figure 37. Optical design of the VW Absolute Specular Reflectance accessory.

Universal Measurements Accessory (UMA)

Advancements in UV-Vis-NIR technology have facilitated the development of innovative accessories that enhance material characterization and provide comprehensive information. An example is the UMA, a versatile, multi-angle UV-Vis-NIR tool that allows for the measurement of direct transmission and specular reflectance off a sample surface at user-defined angles (Figure 38). The UMA can also measure scattered transmission and reflection, thanks to the independent control of sample rotation and detector position.

The UMA can perform transmission and reflection measurements without moving the sample or altering the incident light. Motorized accessories enable automated data collection to be performed unattended.

The UMA offers unique measurement capabilities, allowing users to automatically measure absolute specular reflectance, transmission, and scattering at a wide range of angles of incidence and different polarizations.

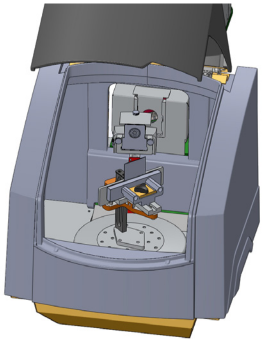


Figure 38. A schematic of the Universal Measurement Accessory.

3.1.4 Fiber optic based remote reflection measurements

Measuring the reflection of solids can be challenging depending on the size, shape, or location of a sample. Often, large samples that must remain intact cannot fit inside an instrument's sample compartment. This is the case, for example, when measuring the reflectance on a freshly painted car or the surface of a painting. For these types of applications, measurements that are performed outside of an instrument's sample compartment are preferred. The fiber-optic coupler, which includes various fiber-optic probes and accessories for either liquids or solids, allows for remote measurements. Remote fiber optic specular and diffuse reflectance accessories (as shown in Figure 39) extend an instrument's capabilities. However, these accessories do have limitations, including a wide angular range that affects accuracy, making them suitable only for relative measurements. Also, these accessories cannot be used with polarizers/depolarizers.

Figure 39. A remote diffuse reflectance accessory being used to measure the color of paint directly on the surface of a canvas.

3.1.5 Use of a polarizer/depolarizer

The unpolarized light emitted by the lamp of a spectrophotometer will become partially polarized by the time it reaches the sample. The optical components within the instrument, such as the grating and each mirror, introduce plane polarization to the light. The alignment, age, selected spectral bandwidth, and photomultiplier used will all affect the polarizing characteristics of a spectrophotometer. For these reasons, it is difficult to predict the degree of plane polarization of the light in the sample compartment.

The transmission or reflection values, especially those measured at non-normal angles, may vary depending on the polarization of the incident radiation. A polarizer is used to correct for this effect. Polarizers are designed to transmit only one polarized component of an incident light beam, which can be characterized as having two oppositely polarized components, S and P.[1]

A depolarizer scrambles polarized light, transmitting an incident light beam with the minimum degree of plane polarization. Essentially, it converts any plane polarization into a mixture of polarizations (Figure 40).

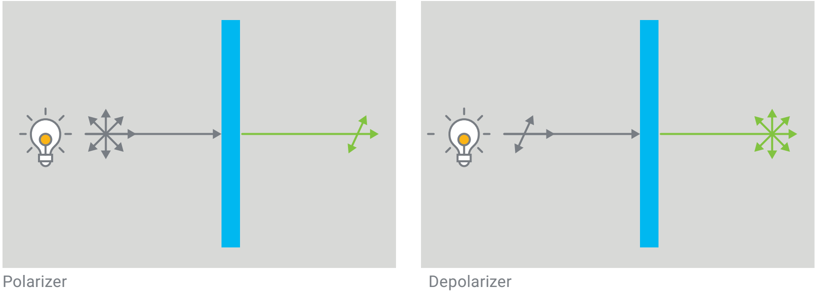


Figure 40. Schematic diagram of a polarizer and depolarizer on an incident light beam.

Controlling the plane polarization of the light beam in a spectrophotometer is necessary for many measurements. Polarizers and/or depolarizers should be used in the following situations:

- **Material properties:** If the sample being measured is sensitive to plane-polarized light, for example, liquid crystals, the transmission of the sample will change as the plane of polarization changes. It is important, therefore, to control the plane of polarization of the incident beam for these types of materials.
- **Polarizing samples:** If the sample itself polarizes the incident light, the transmission results will be affected by the polarization state of the incident beam. It is important, therefore, to control the plane of polarization of the incident beam by polarizing it in a known way (S or P) or completely depolarizing the beam.
- **High Angles of Incidence:** When performing reflectance or transmission measurements on non-scattering samples at angles greater than 10°, the results will depend on the angle due to Fresnel phenomena. A polarizer should therefore be placed before the sample.
- **Polarimetry:** When performing polarimetry measurements to determine the concentration of optically active compounds, a polarizer should be used to control the polarization of the incident beam.
- **Anisotropy:** When measuring anisotropic materials such as single crystals or liquid crystals, a polarizer should be used to control the polarization of the incident beam.

3.2 Photometric parameters selection

3.2.1 Optimum spectral bandwidth

When measuring a sample, consideration should be given to the measurement resolution that is required. Most solid or liquid samples analyzed by UV-Vis spectroscopy have naturally broad peaks, in the order of 20 nm or more, from side to side. It is good practice to use an instrument with a spectral bandwidth (SBW) setting of approximately one-tenth of the natural bandwidth of the analyte. The SBW of the instrument is defined as the width of the band of light at one-half the peak maximum (as shown in Figure 41), and sometimes referred to as 'full width at half maximum' (FWHM). The SBW of a UV-Vis spectrophotometer is related to the physical slit width of the monochromator design.

---

[1] *P-polarized (German: parallel) light has an electric field polarized parallel to the plane of incidence, whereas S-polarized (German: senkrecht) light has an electric field polarized perpendicular to the plane of incidence.*

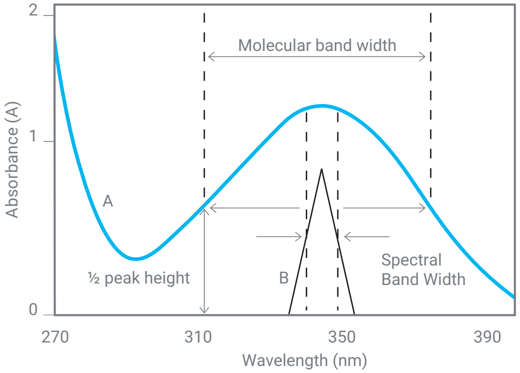


Figure 41. Spectrum A shows a peak maximum close to 345 nm. The spectral bandwidth is shown. The spectral slit width of the UV-Vis spectrophotometer will always be narrower than the required spectral bandwidth.

Depending on the design of the spectrophotometer, the width of the physical slit can be either fixed or variable. For most midrange UV-Vis spectrophotometers, a fixed SBW of 1.5 nm is common and sufficient for resolving the peaks of most liquid and solid samples. Using a larger SBW allows more light through the sample and can give better quality data and less noise, but will not resolve narrow or closely spaced sample peaks. Using a smaller SBW will provide better resolving power but can result in increased data collection times to achieve the same data quality due to less light reaching the sample.

High-performance or research-grade spectrophotometers are more frequently designed to allow the user to select the slit width, which adjusts the resolution of the system. This is useful when measuring more challenging sample types. The slit width can be maximized to allow greater light throughput in highly absorbent samples where high resolution of the peak is not necessary. Greater light throughput to the detector allows better method repeatability, accuracy, and precision of the results. When high resolution is required, the slit width can be reduced, as shown in Figures 42 and 43.

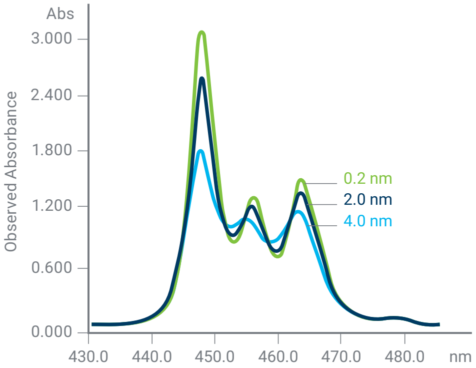


Figure 42. Three overlaid scans, each measured using a different instrument slit width. As the slit widens, the signal-to-noise ratio improves, but resolution decreases.

When optimizing spectral resolution, the data interval of the collection also needs to be considered. A minimum of 10 data points across the peak should be collected. While a smaller data interval can provide better resolution, there will be a trade-off between how long the data takes to collect and the data interval.

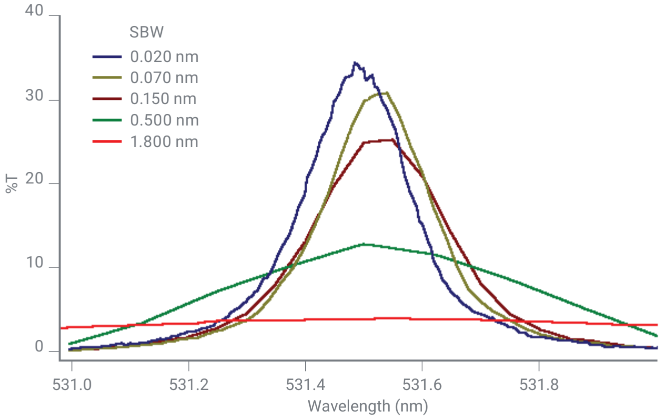


Figure 43. These scans demonstrate the effect on resolution of varying the SBW. At an SBW of 1.80 nm (red), little structural detail is observed in the spectrum. At an SBW of 0.02 nm (dark blue), the peaks are more defined.

The SBW plays a critical role in the instrument's spectral resolution and its signal-to-noise ratio. A wider SBW delivers a variety of wavelengths to the sample, which compromises the instrument's capacity to differentiate nearby spectral peaks, though it ensures a robust signal with higher light throughput. In contrast, a narrower SBW enhances the ability to resolve closely spaced peaks but results in decreased light delivery to the detector, increasing signal 'noise'. In auto- or servo-mode, the slit width will vary automatically during data collection to maintain the electrical output of the detector at a constant level. This action is also known as Fixed Energy Level mode. Auto-mode secures the broadest dynamic range of the detector. If the sample's transmission varies significantly throughout the scanning range, auto-mode will also guarantee an accurate measurement capability and consistent signal-to-noise ratio across the wavelength range.

The alternative mode, known as fixed-SBW mode, conserves a uniform SBW across all wavelengths and adjusts the detector electrical output/gain as needed to maintain a steady signal. Fixed-SBW mode sustains a consistent level of resolution throughout the scan but sometimes achieves a lower signal-to-noise ratio in the NIR region of the NIR detectors.

3.2.2 Stray light

Stray light, or stray radiant energy (SRE), is defined as the percentage of radiation with wavelengths outside of the user-selected SBW that reaches the detector. It can be caused by poor instrument design (light leaking into the instrument from the laboratory lights or daylight through windows, or the light not being well separated by the monochromator) or from damage to the instrument. Most systems are provided with instrument performance checks that identify stray light issues. This test is done using a solution that is non-transmitting at a known wavelength (but transmits at other wavelengths). These properties ensure that any observed transmittance is due to stray light only (Figure 44).

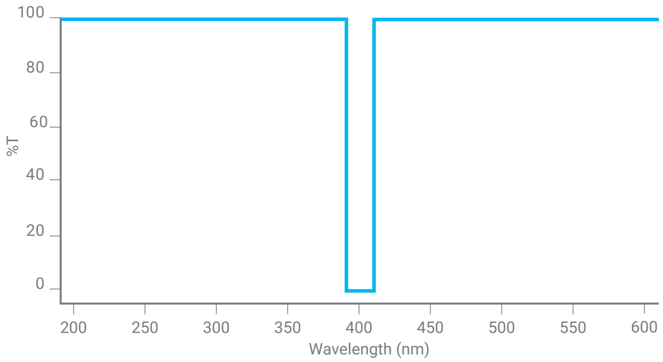


Figure 44. An ideal spectrum for a stray light filter.

Stray light causes decreased absorbance readings and changes the observed peak shape (as shown in Figure 45). As a result, stray light causes deviation from the Beer–Lambert law (as shown in Figure 46), making concentration measurements unreliable. The stray light performance of a UV-Vis-NIR instrument also determines the maximum absorbance that the instrument can reliably measure.

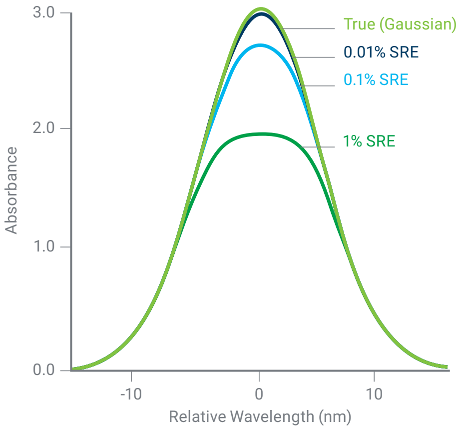


Figure 45. Showing the effects of stray light: as the level of SRE in the instrument increases from 0.01 to 1%, the maximum absorbance of the peaks reduces, and the curves flatten.

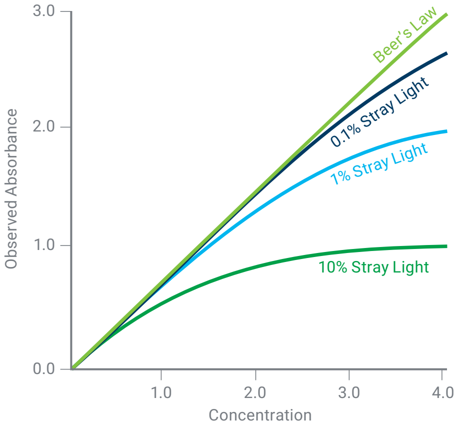


Figure 46. A UV-Vis-NIR system with poor stray light performance will show deviations from the Beer–Lambert law. This deviation makes concentration calculations unreliable.

Pharmacopeia tests require the measurement of four cutoff filters—potassium chloride (KCl), sodium iodide (NaI), sodium nitrite ($NaNO_2$), and acetone—to qualify the stray light levels within an UV-Vis instrument (Figure 47). USP Chapter <857> specifies another procedure to measure the limit of stray light. This method involves producing a differential spectrum by subtracting the spectrum of a 5-mm path length cell from that of a 10-mm cell, both containing the same filter solution (e.g., KCl, NaI, $NaNO_2$, and acetone).

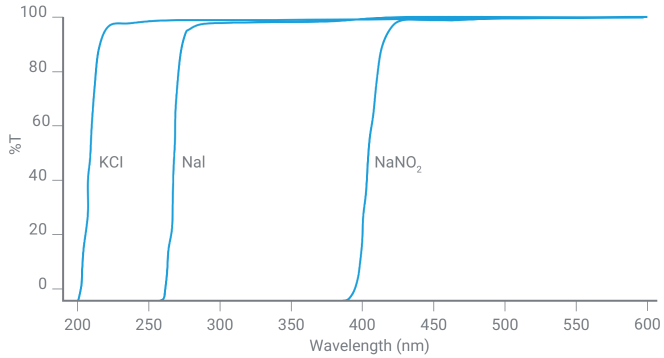
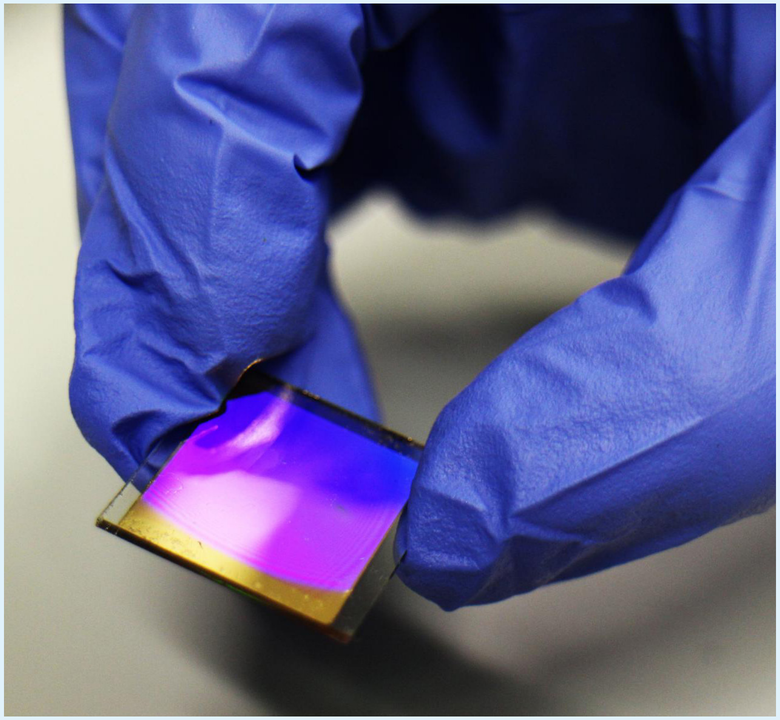


Figure 47. Spectra of three cutoff filters: potassium chloride (12 g/L), sodium iodide (10 g/L), and sodium nitrite (50 g/L) in water.

Stray light levels in the IR region are normally measured using chloroform ($CHCl_3$ at 2365 nm), dibromomethane (CH_2Br_2 at 1690 nm), and water (H_2O at 1,420 nm) as filters. Liquid-based optical filters that are suitable for assessing stray light levels in the NIR region are shown in Figure 48.

The significant causes of stray light in the instrument include defects and contamination of optical components in the monochromator section of the engine. These defects would affect all cuvette positions equally, so it is only necessary to test one cuvette position.



Determination of Band Gap in Metal Oxides Using UV-Vis Spectroscopy

The band gap of semiconducting materials is key to their electronic and optoelectronic performance. UV-Vis spectroscopy evaluates these band gaps, offering insights crucial for improving materials in photocatalysis and solar energy conversion. This technique measures absorbance or reflectance across wavelengths, with electrons transitioning between valence and conduction bands. The application note details accurate band gap measurements using the Agilent Cary 5000 spectrophotometer and WinUV software.

Read application note

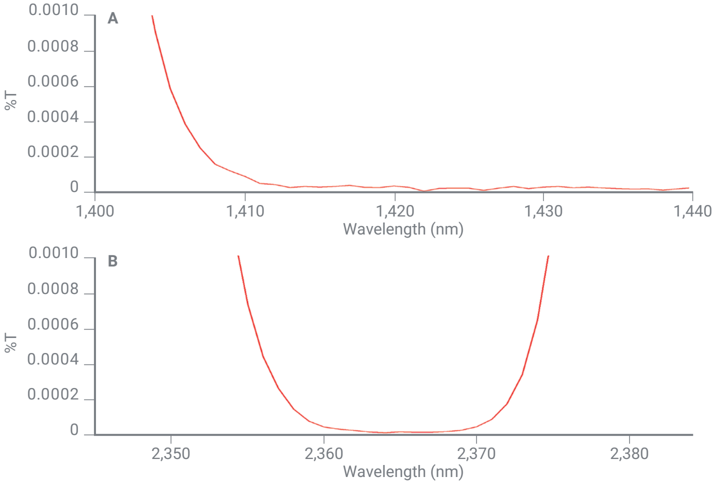


Figure 48. NIR stray light filter spectra measured by a UV-Vis-NIR spectrophotometer using a 10 mm cuvette, 3 Abs Rear Beam Attenuator (RBA), and auto SBW: (A) water (1420 nm) and (B) chloroform (2365 nm).

3.2.3 The linear range of a UV-Vis-NIR instrument

Both instrument design and the selected measurement parameters will determine the maximum absorbance that an instrument can measure at a specific wavelength. At high absorbance, very little light reaches the detector, which decreases the signal-to-noise ratio (see the characteristic 'fringe' on the spectrum in Figure 49).

Understanding the limits of a system allows the operator to avoid measuring samples or performing calibrations which are outside the capabilities of the instrument. For liquid samples, diluting the sample is one way to bring the measurement into the linear range of the instrument. Alternatively, a short path length cuvette can be used.

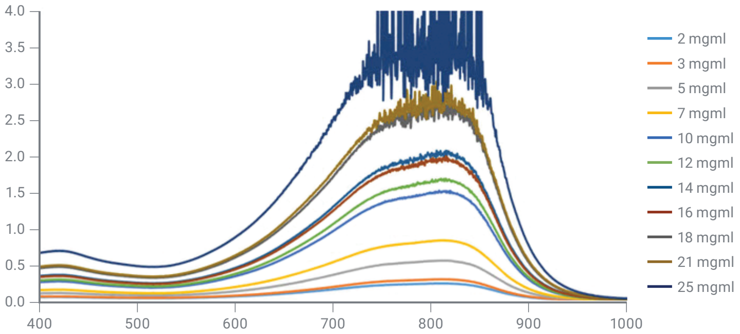


Figure 49. As sample absorbance increases, progressively less light reaches the detector. This increases noise in the results and a spiky signal will be observed in scanning mode.

Addition of filters

The usable linear dynamic range of an instrument can be tested using the Addition of Filters technique. The absorbance of two filters is measured individually. The sum of these results is then compared to the simultaneous measurement of the two filters when they are measured together in the instrument.

The principal of the test is that the mathematical addition of two filters (A + B) should equal the absorbance of the combined measurement of the two filters (AB), as follows:

(Abs of Filter 1 and Filter 2) – ((Abs of Filter 1) + (Abs of Filter 2)) = Measured Tolerance

Metal-on-quartz filter reference materials

To achieve high-quality measurements, the photometric accuracy of a spectrophotometer can be verified using CRMs. These measurements are usually performed in the UV-Vis region, as required by global pharmacopeias. However, photometric accuracy (absorbance) in the NIR region can be evaluated using three metal-on-quartz filter RMs (Figure 50).

Solid filter RMs with different transmission properties can be used to evaluate the photometric accuracy in the NIR region (e.g., a metal-on-quartz NIR reference set comprising 1%T (~2 Abs), 3%T (~1.5 Abs), and 50%T (~0.3 Abs) filters). Filter RMs are traceable to NIST SRM 2031a (for the UV-Vis wavelengths) and National Research Council of Canada (NRC) primary CRMs for the NIR wavelengths. The filters are orientated at 0 degrees to the incident beam in the spectrophotometer and are constructed using an optical sandwich configuration. This design minimizes the fringing that is often caused by internal reflection effects from metal-on-quartz filters.

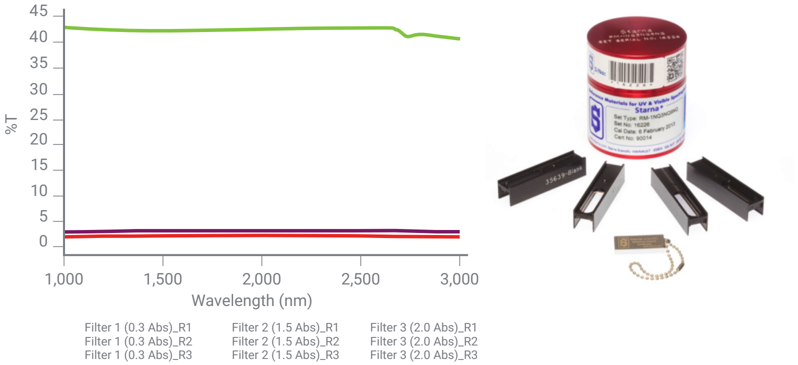


Figure 50. Three metal-on-quartz filters measured using a UV-Vis-NIR system in transmission mode. Values at 1100, 1700, 2210, 2500, and 2800 nm of each filter were recorded and compared to the certified reference values.

The Rear Beam Attenuator (RBA)

The detector in a double beam UV-Vis-NIR views the sample and reference beams typically between 30 to 250 times per second. If the sample is highly absorbing, the flicker (bright, dark, bright, dark etc.) that is detected adds noise to its response—similar to when someone moves quickly from a brightly lit room to a dark room or vice versa. To avoid this effect, the rear/reference beam signal level can be reduced (attenuated) using a Rear Beam Attenuator (RBA) to balance the signal levels and reduce noise at the detector.

An RBA is suitable for tasks that call for constant, broad-spectrum attenuation of an optical beam. Examples of typical use include assessing the transmittance of heavy-duty optical filters, balancing out interference from sample holders and devices that impede the light beam, and evaluating samples with considerable absorbance (low transmittance).

RBA is most effective when the sample beam intensity has been reduced significantly by an accessory, sample, or an aperture. In such cases, applying attenuation to the rear beam can greatly extend the dynamic range of the instrument because the detector will not be exposed to widely fluctuating signals.

It is recommended to set the RBA at a level that is half of the maximum absorbance units (Abs) of the sample. RBA can be used to adjust the attenuation of the rear beam from 0 to approximately 6 Abs. Various detectors exhibit different responses to RBA depending on the scanning modes used. Table 2 provides guidance for users on the appropriate use of RBA for samples with absorbance values above 4.

Some RBAs are controlled by software and operator via a stepper motor, which can be configured to achieve a specified level of attenuation, a designated photometric reading, or a particular angle.

Table 2. Guide for using RBA with samples exceeding 4 Abs.

Spectral Bandwidth Mode	UV-Vis (PMT)	UV-Vis (Si)	NIR (PbS/InGaAs)
Fixed	Yes	No	No
Auto	No	No	Yes

3.2.4 Wavelength accuracy

Wavelength accuracy is used to ensure that the wavelength axis of the UV-Vis-NIR spectrum is accurate (correct and within acceptable limits) across the intended operational range. Poor wavelength accuracy can produce low quantitative results, as the measurement may have been taken on the side, rather than at the top of a peak. This test monitors how close the indicated wavelength is to the actual wavelength.

Confirmation of UV-Vis wavelength accuracy is recommended to be tested using atomic line spectra from xenon, mercury, or deuterium light sources. Atomic line spectra from deuterium light occur in both the visible (486 and 656.1 nm) and NIR (1312.2 and 1968.3 nm) regions and can be used to qualify NIR wavelength accuracy.

Rare earth oxides that yield well-characterized absorption bands can also be used, as they enable the comparison of the UV-Vis spectrophotometer wavelength readings to published values. The rare earth oxide solutions—holmium oxide in perchloric acid (from 200 to 600 nm), didymium (from 700 to 860 nm), and cerium sulfate solution (200 to 300 nm)—are well established and widely available as CRMs. These solutions yield well-characterized peaks across the UV-Vis range of the spectrum. Alternatively, glass filters, prepared by fusing a rare earth, such as holmium, into a base glass matrix, can be used for wavelength verification tests.

3.2.5 Other useful information

Absorbance (A or Abs) is frequently measured in UV-Vis spectroscopy due to the linear relationship between concentration and absorbance as described by the Beer–Lambert law. For other applications, the percentage of light transmitted or absorbed may be more meaningful. When comparing the optical properties of a material, for example, it may be more useful to compare the percent transmission or absorbance difference. Most UV-Vis spectrophotometers will enable you to convert your collected data between the commonly used parameters. The relationship between these parameters can be difficult to understand without a clear explanation of the underlying principles and mathematical conversion. Table 3 summarizes the corresponding values and shows that a sample measuring 7 Abs transmits just 0.00001% of the light through the samples.

Table 3. The relationship between percent transmission (%T) and absorbance (Abs).

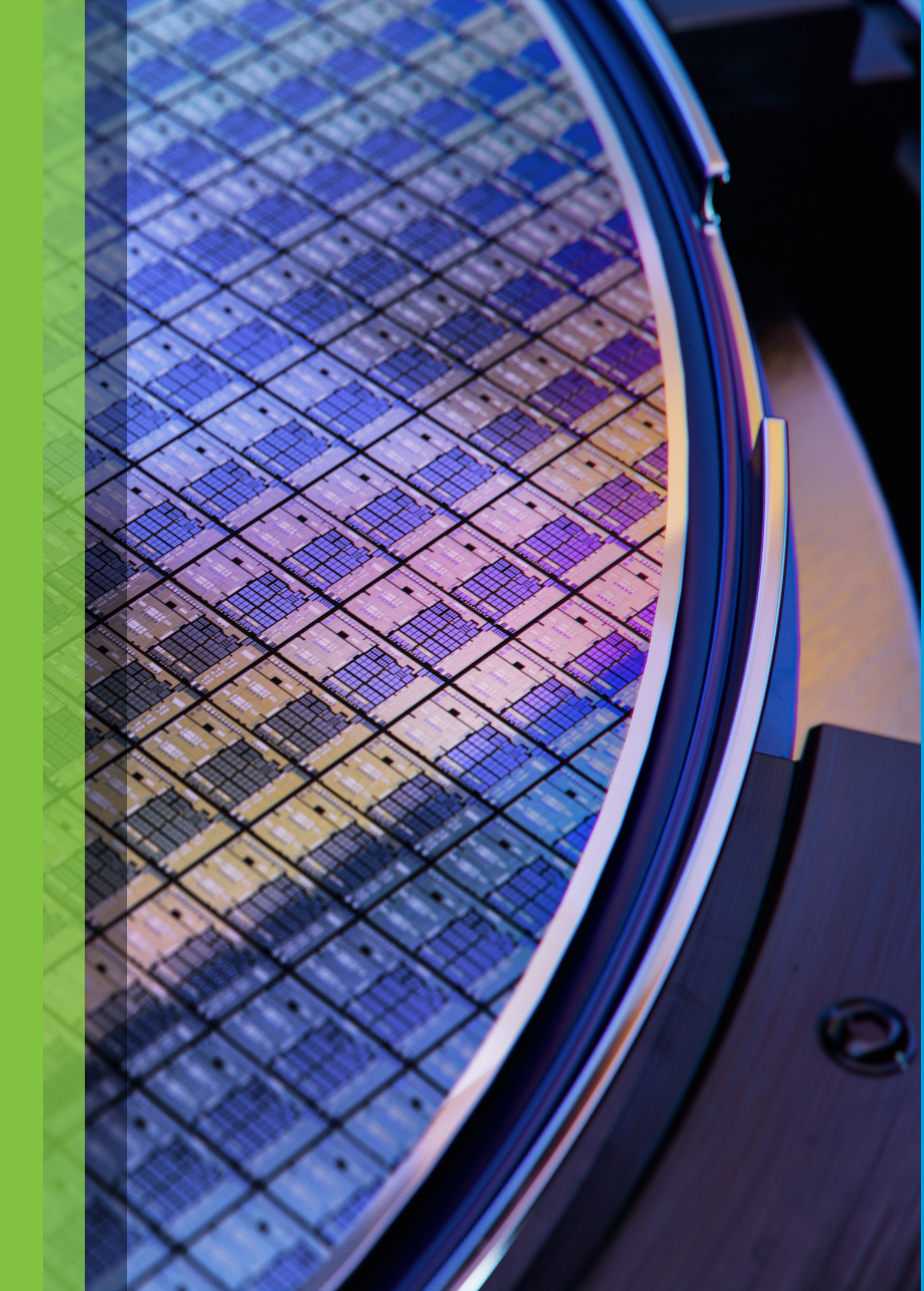
%T	T	Abs
100	1.0	0
50	0.5	0.3
10	0.1	1
1	0.01	2
0.1	0.001	3
0.01	0.0001	4
0.001	0.00001	5
0.0001	0.000001	6
0.00001	0.0000001	7

3.2.6 Wavelength or inverse centimeters

Most UV-Vis measurements are reported against wavelengths measured in nanometers (1×10^{-9} m), but in some older literature, the reciprocal length or wavenumber (cm^{-1}) is used. Wavenumber is often used in IR spectroscopic measurements. Using a wavenumber scale is useful as it conveys the change in energy levels of the incident radiation. A lower wavelength gives a larger wavenumber and a higher energy (as shown in Table 4). The use of wavenumber for IR spectroscopy also allows for easier visualization of spectral differences as the wavelength gets progressively shorter. For UV-Vis spectroscopy, wavelength is often preferred as a convenient way to visualize the displayed spectrum over a spectral range. Most UV-Vis spectrophotometers facilitate the collection of a spectrum in either wavelength or wavenumber format.

Table 4. Conversion between wavelength (nm) and wavenumber (cm^{-1}).

λ nm	cm^{-1}
3300	3030
3000	3300
2500	4000
2000	5000
1500	6666
1000	10000
600	16667
400	25000
200	50000
175	57143



4

Overview of Common UV-Vis-NIR Applications

UV-Vis-NIR spectroscopy provides valuable insights into a sample's properties by capturing light across a broad spectrum, from UV (175 nm) to NIR (3300 nm). This non-invasive technique supports various measurement methods, enabling the analysis of diverse sample types. By revealing molecular vibrations and atomic electronic transitions through spectral signatures, UV-Vis-NIR spectroscopy serves as an effective tool for investigating the properties of samples across a wide range of industries and scientific fields. Some example applications of the technique are outlined in this chapter.

4.1 Material characterization (optics, photonics, and coatings)

Given its versatility, UV-Vis-NIR spectrophotometers are widely used in applied research and development laboratories and production processes, from early prototype evaluation to QA in manufacturing.[1] The technique is used across various high-tech industries, such as optics, semiconductors, solar, and personal safety gear, to examine the properties of materials and devices.

Optical materials

Optical materials are integral to numerous technologies, from everyday lighting and visual displays to smartphones and monitors, as well as specialized areas such as telecommunications via fiber-optic cables, solar energy solutions, and specialized building materials. Optics require components like mirrors, lenses, light sources, and detectors, each serving a purpose to manipulate, generate, or detect light at specific wavelengths. UV-Vis-NIR spectrophotometers are available that can evaluate the transmission, reflective, and scattering attributes of these types of optics. These instruments can also accommodate different component sizes, offer multiple measurement modes, and deliver the precise photometric capabilities needed in this field.

Multilayer optical coatings

Multilayer optical coatings are essential for devices that harness the qualities of light in the UV to IR range. Researchers and manufactures of these types of coatings require reliable information on refractive indices, absorption coefficients, and thin-film thickness. UV-Vis-NIR spectroscopy enables such detailed assessments, as demonstrated by the transmission spectra in Figure 51 obtained for a nanocomposite coating on a quartz substrate. The thickness measurements (d) for the sample transmission spectra were obtained at two arbitrary, unpolarized light incidence angles, which, in this case, were at normal incidence φ1 = 0° and φ2 = 20°.

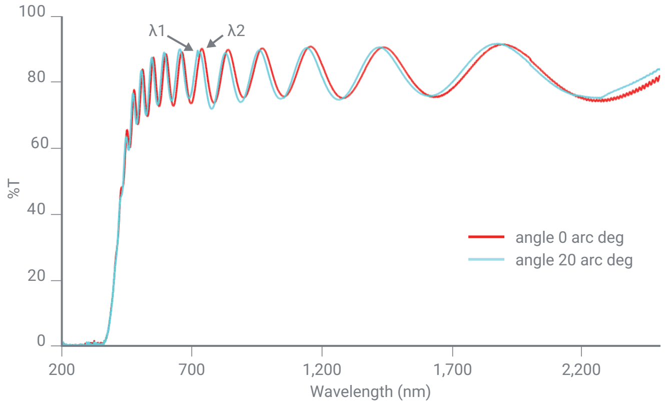


Figure 51. Transmission spectra of a Zr-Si-B-(N) nanocomposite coating on a quartz substrate at normal incidence (λ2) and at 20° (λ1).

Semiconducting materials: Coated wafer mapping

Semiconducting materials control the flow of electricity in analog or digital circuits. The electrical conductivity of a semiconductor, which lies between an efficient conductor (metal) and strong insulator (glass), changes predictably under light and temperature exposure. Semiconductor materials are often made from silicon (integrated circuits, photovoltaics), silicon carbide (power devices), or gallium arsenide (high-frequency transistors). These materials facilitate the performance of digital tasks at a micro scale in microelectronics and integrated circuits (transistors) or light conversion tasks at the macro scale of light-emitting diodes (LEDs) and photovoltaics.

Analysts use molecular spectroscopic techniques to calculate the bandgap of doped semiconductors and quantify operating efficiencies under light exposure, typically after purpose-designed optical coatings have been applied to the semiconductor. Also, the spectral transmission and reflection of semiconductor wafers of varying sizes, from 2.54 to 20.32 cm (1 to 8 inch) diameters, can be measured using the spatial mapping features of a UV-Vis-NIR spectrophotometer fitted with a solids autosampler.

UV-Vis-NIR is employed for advanced wafer mapping through spectral reflection and transmission measurements across the UV-Vis-NIR range. This approach uses Multi-angle Photometric Spectroscopy (MPS) to assess the optical properties of materials without requiring sample movement, enhancing data quality and consistency.

In the following example, this technology was used to map the band gap energy of a zinc tin oxide (ZTO) layer on a sapphire substrate. A UV-Vis-NIR spectrophotometer equipped with a Universal Measurement Accessory and a solids autosampler collected transmission spectra across the wafer's diameter, revealing variations in band gap energy due to changes in the concentration of zinc (Figure 52). This measurement capability is pivotal for characterizing and optimizing thin-film substrates and coatings, offering significant potential for applications that require precise material properties, such as flexible displays and organic photovoltaics.

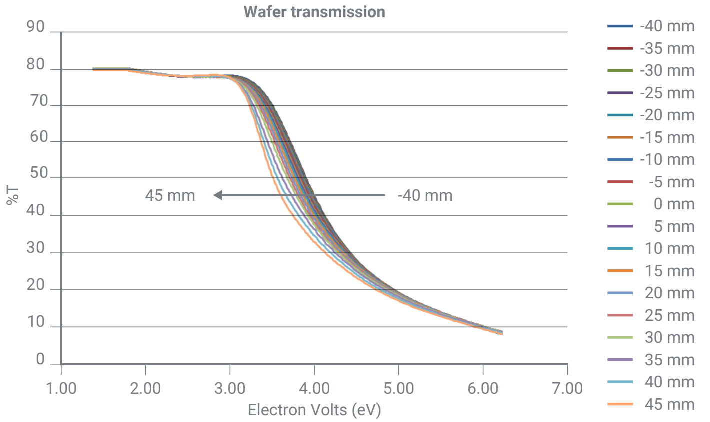


Figure 52. Transmission spectra of a ZTO coated wafer obtained at 11 positions (at 5 mm increments) across its diameter.

Privacy screens of electronic devices

The quality of privacy screens developed for electronic devices can be assessed using UV-Vis transmission spectroscopy. By conducting transmission assessments at various angles of incidence, scientists can evaluate how effective the privacy screen is in blocking unsanctioned viewing of an electronic device. As shown in Figure 53, the results for the privacy screen included in the study demonstrated a clear correlation between the angle of incidence and the level of transmission. As expected for a privacy device, increasing the angle of incidence corresponded to a reduction in transmission, ensuring that the view from wide angles (±20 to 30°) was obstructed and privacy was maintained.

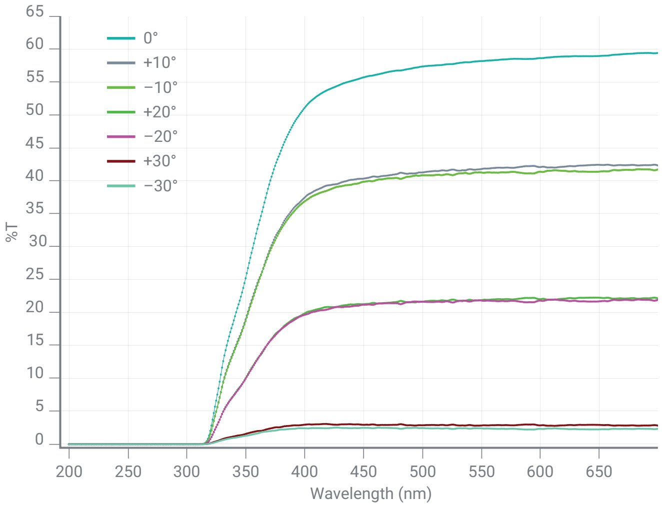


Figure 53. Privacy screen transmission of depolarized light at various angles of incidence.

Safety and fashion eyewear

Ensuring the efficacy and safety of sunglasses and protective eyewear is critical for their intended use. The QC of lenses and optical coatings are typically performed using UV-Vis-NIR spectroscopy, which provides the data needed to assess the optical properties, durability, and protective performance of eyewear.

As shown in Figure 54, UV-Vis-NIR spectroscopy was used to measure the transmission through the lenses of three types of sunglasses: two types of safety glasses and a pair of prescription eyeglasses. This test assesses the effectiveness of the UV coating on the lenses to block harmful UV radiation while allowing visible light to pass through for clear vision. For laser and welding goggles, UV-Vis-NIR can verify that the coatings or materials provide adequate protection against the intense light from laser emissions or welding arcs, helping to prevent eye injuries. The technique can also be used to assess the consistency of antireflective, scratch-resistant, and antiglare coatings on eyewear lenses.

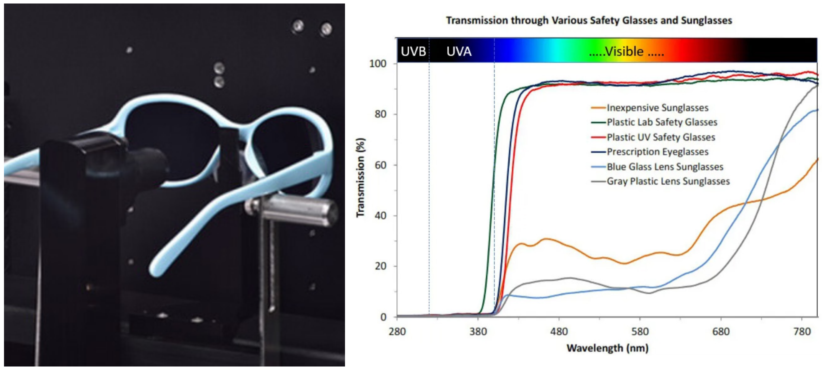


Figure 54. Left: A UV-Vis sunglasses holder accessory. Right: The UV blocking efficiency of sunglasses and other eyewear. The more expensive sunglasses (blue glass and gray plastic lenses) performed slightly better than the less expensive glasses. Graph source: uv.substack.com

4.2 Quality control of solar cells

The QC of solar cells is another important use of UV-Vis-NIR. Solar cells are composed of various semiconducting materials, with the majority (>95%) made primarily of silicon. To produce a solar cell, the semiconductor is "doped", which involves the intentional introduction of elements to obtain a surplus of either positive (p-type) or negative (n-type) charge carriers within the semiconductor material. When two differently doped semiconductor layers are combined, a "p–n junction" forms at the boundary of the layers. This junction creates an electric field that separates the charge carriers that are released by light. Metal contacts can then draw this electric charge, and if the outer circuit is closed, a direct current will flow. Silicon solar cells are typically 10 × 10 cm in size and feature a transparent antireflection (AR) coating to protect the cell and decrease reflective losses on the cell surface. Figure 55 shows a schematic diagram of a typical photovoltaic cell.

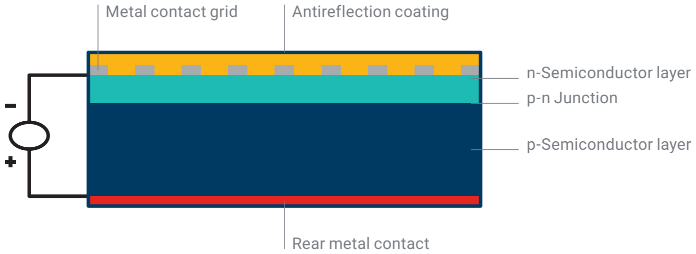


Figure 55. Schematic diagram of a typical solar cell.

In any photovoltaic cell, the intensity of the current increases with higher light flux. While many factors can affect the efficiency of a particular cell, reflectance of light at the cell's surface is an important parameter. To perform these types of reflectance measurements, a high-performance UV-Vis-NIR spectrophotometer equipped with an integrating sphere is required. Integrating spheres are designed to collect reflected radiation (diffuse or total) from a solid surface (such as a photovoltaic cell). Using the appropriate sampling geometry, integrating spheres can also be used for the measurement of the diffuse transmission properties of solar cells.

Figure 56 demonstrates how the reflectance properties of a solar cell and its precursors can be measured at various stages of the manufacturing process using a UV-Vis-NIR spectrophotometer equipped with an external DRA.

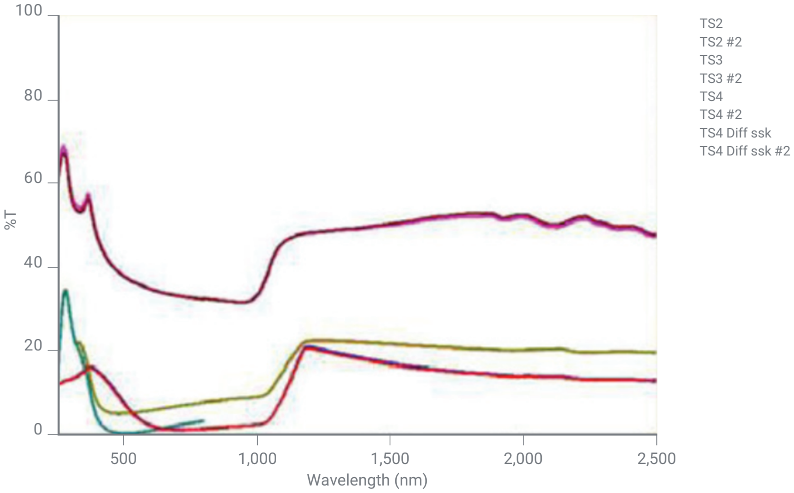


Figure 56. UV-Vis-NIR diffuse reflectance spectra of solar cells.

4.3 Nanomaterial characterization

UV-Vis-NIR spectroscopy is a versatile technique employed to investigate a broad spectrum of materials in both solid and liquid states. Its importance for analyzing nanomaterials, such as metal nanoparticles, semiconducting quantum dots, carbon nanotubes, and two-dimensional (2D) materials like graphene, is also increasing.

Typically, these nanomaterials are suspended in a solvent—often water—as they are more easily analyzed in a dispersed state rather than as a dense powder. UV-Vis-NIR spectroscopy not only examines the optical characteristics of nanomaterials but also evaluates factors that influence these properties, such as particle size, shape, and stability.

The absorption of light in the UV and visible ranges by nanomaterials can be attributed to the excitation of their electrons to higher energy states. NIR wavelengths can be absorbed in a similar way, although they may also lead to molecular vibrations that cause the material to heat up. This heating arises from the unique bond absorptions at different NIR wavelengths.

Spectroscopy can offer insights into the nanomaterial's composition. For instance, the absorption profile for metal nanoparticles typically shows a bell-shaped curve with a peak indicating maximum absorption (as shown in Figure 57). Knowing the location of the peaks for specific nanoparticles allows UV-Vis-NIR spectroscopy to discern relative sizes of particles in different samples, as smaller particles absorb light at shorter wavelengths.

Moreover, the technique can assess multiple characteristics of metal nanoparticle solutions. The sharpness of an absorption peak can indicate particle uniformity; narrow peaks suggest homogeneity, while wider peaks denote a variety of particle sizes absorbing a broader wavelength range (see Figure 58).

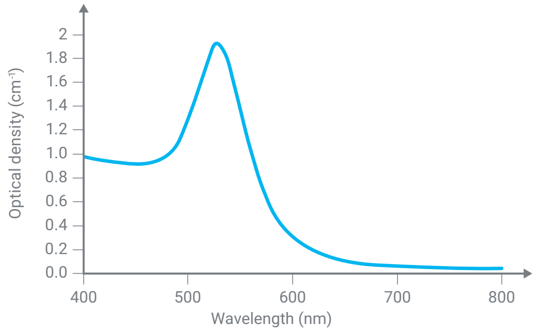


Figure 57. Absorption spectrum for 50 nm gold nanospheres.

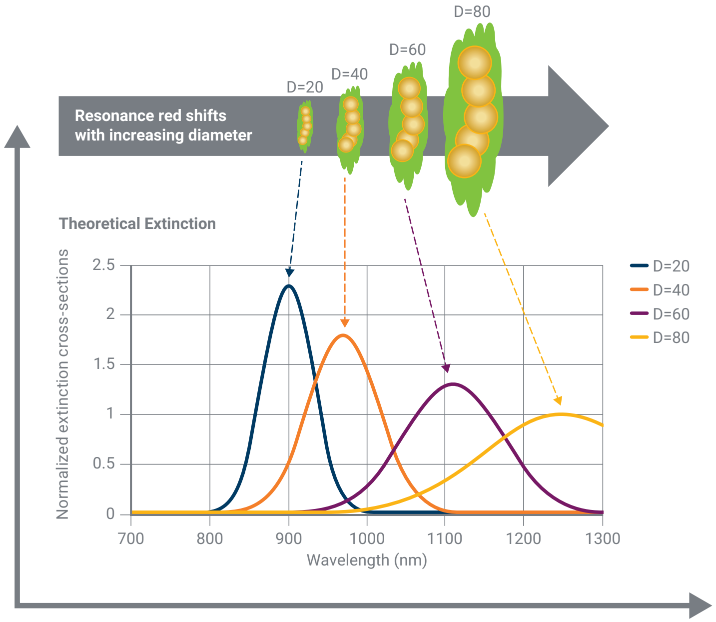


Figure 58. Absorption spectrum for silver nanoparticles as they transition from a well-dispersed state to an aggregated state following the addition of a concentrated salt solution.

Recent research employed UV-Vis spectroscopy to investigate the impact of both temperature and ionic strength on the swelling state of poly N-isopropylacrylamide (PNIPAM)-coated gold nanoparticles. Temperature and ionic strength are two parameters that can influence the swelling state of the polymers. The swelling state can dictate the extent of the steric stability imparted by the PNIPAM polymer coating. The polymers may become more strongly attracted to other neighboring polymer chains than to the surrounding water molecules (they become more hydrophobic) at a certain temperature or ionic strength. This effect is referred to as the cloud point of a polymer. If the cloud point is met or exceeded, the polymer coating resides closer to the gold nanoparticle, decreasing its hydrodynamic radius. The reduced hydrodynamic radius results in closer interactions with neighboring particles and aggregation due to the overwhelming Van der Waals (VdW) forces. As the polymer-coated particles aggregate, they attenuate incident light. Therefore, the cloud point can be measured as a function of iconic strength or temperature using UV-Vis spectroscopy. This measurement is done by monitoring the transmission of light (measured as absorbance) at a specific wavelength.

Wavelength scans of AuPNIPAM suspensions at incremental temperatures are shown in Figure 59. The increase in absorbance signal with temperature indicates deswelling of the polymer's coating.

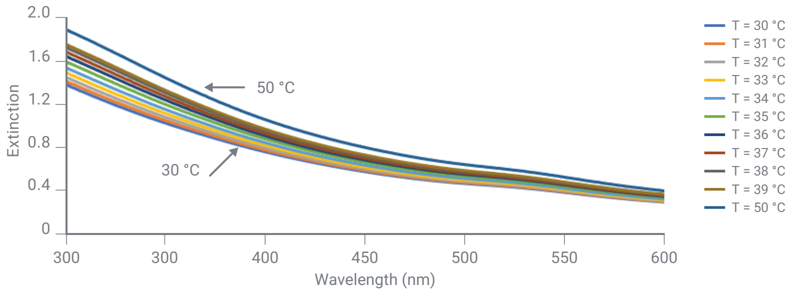


Figure 59. Wavelength scans of AuPNIPAM suspensions at incremental temperatures. The increase in the extinction with temperature indicates deswelling of the polymer's coating.

By monitoring changes in light absorbance, researchers were able to determine the cloud point of the nanoparticles—where they transition from a swollen to a deswelled state—due to temperature variations. The study revealed that increasing temperature and ionic strength led to higher aggregation temperatures, with significant aggregation observed at approximately 35.7 °C for higher salt concentrations. This approach underscores the utility of UV-Vis spectroscopy in assessing the stability and behavior of nanoparticle systems, crucial for optimizing their applications in various fields.

4.4 Advanced materials (bandgap measurements)

Determining the bandgap of semiconductors is a crucial attribute that determines their electrical behavior and compatibility for different uses, including semiconductor devices, solar energy technology, and optoelectronic components. UV-Vis-NIR spectroscopy is a suitable tool for measuring band gap energy and providing insights into the electronic structure of materials. The technique measures the absorbance or reflectance of materials over a range of wavelengths. When the energy of incident photons matches or exceeds the band gap energy, electrons transition from the valence band to the conduction band, leading to distinctive absorption characteristics.

It is possible to assess the diffuse reflection properties of small amounts of sample, such as germanium dioxide (GeO_2), across various wavelengths using a DRA or Praying Mantis accessory. The resulting reflection spectrum typically shows a distinctive rise at wavelengths that correspond to the material's band gap energy. Techniques such as the Tauc plot method are frequently applied to determine a material's band gap energy, as illustrated in Figure 60 for GeO_2.

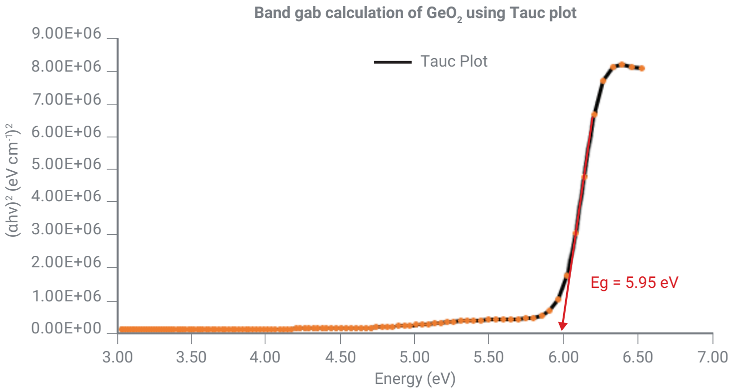


Figure 60. Band gap energy measurements and calculations for a GeO_2 sample.

4.5 Catalysis

Catalysts are compounds that increase the rate of chemical reactions. They occur naturally in living systems (e.g., enzymes). Synthesized catalysts have a range of uses such as boosting food production, driving yields of fine or bulk chemicals, or improving energy-producing reactions. Catalysts in powder form comprise either coarse sand grain-sized particles or nanoparticles. Light that is diffusely reflected from the surface of the particles can be measured by UV-Vis-NIR. The spectral data provides insights into the molecular and electronic structure of the catalyst, as well as its effectiveness. In cases where temperature or atmospheric gases play a significant role in catalysis, efficacy control over these variables also becomes essential. UV-Vis-NIR accessories that facilitate studies of catalysis reactions include DRAs and the Praying Mantis accessory equipped with a high-temperature reaction chamber (Figure 61).

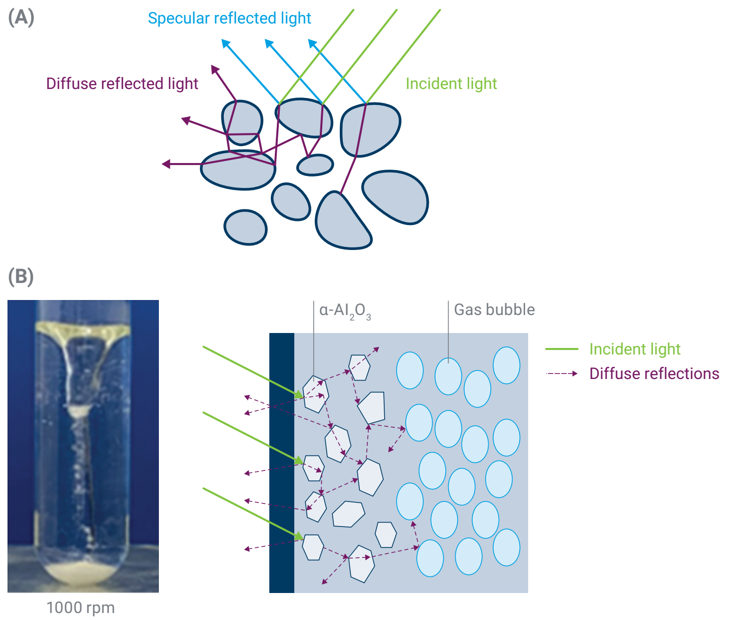


Figure 61. (A) Visualization of the interaction between the incident light and a rough surface. (B) Suspension of α-Al_2O_3 in a stirred liquid medium to enable diffuse reflectance mode measurements.[2]

Figure 62 shows the thermal transformations of $Ni[H_2O]_6(NO_3)_2/Al_2O_3$ at a series of temperatures between 20 and 250 °C. Ni^{2+} ions in an octahedral geometry exhibit three absorption bands: two in the visible region (around 400 and 600 nm) and a broader one in the NIR region (around 1,200 nm).

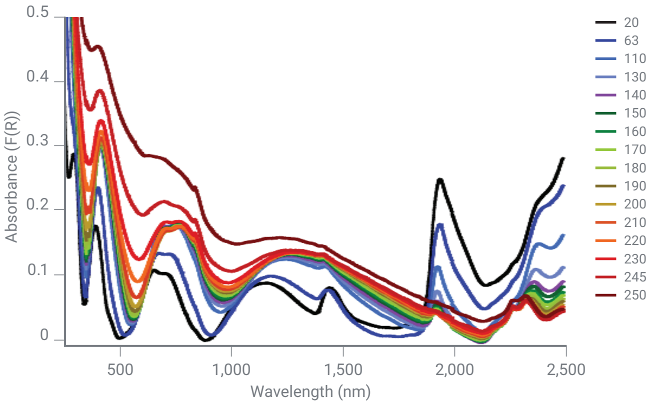


Figure 62. Spectral results of the thermal transformations of $Ni[H_2O]_6(NO_3)_2/Al_2O_3$ that occur between 20 and 250 °C measured using a UV-Vis-NIR spectrophotometer fitted with a Praying Mantis accessory equipped with a reaction chamber. Results are presented in Kubelka–Munk (F(R)) units versus wavelength in nanometers. F(R) units are a mathematical transformation of the %R to aid in the correlation between reflectance and concentration.

Shifts of the 390 nm band with temperature up to 110 °C are evidence of the changes around the nickel ions upon transformation of hydrated nickel nitrate to nickel hydroxynitrate, while NiO forms above 230 °C.

4.6 Color measurements

Color is an important property of a material. The color of matter is related to its absorptivity or reflectivity of specific wavelengths of light. The human eye sees the complementary color of the light that is absorbed, as shown in Figure 63 and Figure 64.

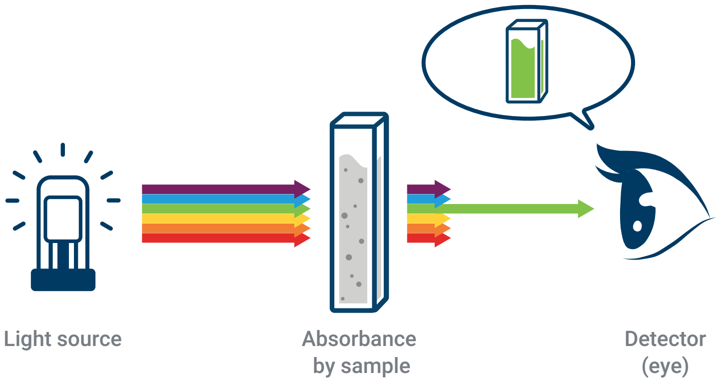
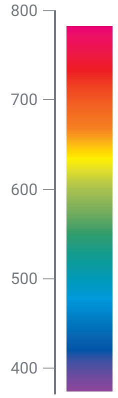


Figure 63. Transmission and color. Much like a spectrophotometer's detector, the human eye sees the light that is transmitted through or bounced off a surface. We perceive this light as the color of the object.



Wavelength (nm)	Absorbed color		Complementary Color
650-780	red	🟥	🟩 blue-green
595-650	orange	🟧	🟦 greenish blue
560-595	yellow green	🟩	🟪 purple
500-560	green	🟩	🟪 red-purple
490-500	bluish green	🟩	🟥 red
480-490	greenish blue	🟦	🟧 orange
435-480	blue	🟦	🟨 yellow
380-435	violet	🟪	🟩 yellow-green

Figure 64. The wavelengths of light associated with different colors (left) and the color of absorbed light and the associated complementary color that the human eye sees (right).

In practice, both the generation and sensation of color are highly complex and depend on many factors, including:

- The spectrum of the light falling on an object (consider the difference in colors seen at sunset compared to the middle of the day).
- The surface structure of a solid material (the scales of a fish or the feathers of a bird are two examples where the physical structure of the surface changes the perceived color).
- The viewing angle (some surfaces such as pearlescent paints change color depending on the viewing angle of the surface).

Specialized color measurement systems, such as the CIE L*a*b color model, along with instruments for precise color analysis, have been developed. When equipped with the appropriate software, most spectrophotometers can be used to measure color. Color perception is also influenced by the surface and its ability to produce specular (mirror-like) reflectance or diffuse (scattering) reflectance. Because of these factors, color measurement may require special accessories which allow specular and diffuse reflectance to be collected and observed at different viewing angles.

A color measuring instrument will take the UV-Vis spectrum of a sample and convert it into three color coordinates that locate the color in a three-dimensional color space. The three coordinates define the sample's lightness, chroma, and hue. Lightness is a measure of how light or dark a color appears, chroma is a measure of 'color purity', and hue is the dominant spectral color, similar to the colors seen in a rainbow.

As well as being used for color matching measurements, e.g., measuring paint colors on a manufactured item, a UV-Vis spectrophotometer can also be used to measure a change of color in a solution. UV-Vis measurements are often used for this purpose to assess whether a reaction has taken place or is proceeding, without the need for a visual inspection. Color-based assays are one of the widest used applications of UV-Vis spectrophotometry.

Spectral information is processed after choosing the illumination source (lighting conditions), observers field of view, and the preferred color space. This enables precise color measurement. These measurements are essential in many industries that conduct QC testing to ensure product color uniformity for branding, appearance, and clarity standards. Industries that use UV-Vis color testing include food and beverages, pharmaceuticals, textiles, automotive, plastics, paints, electronics, personal care products, and more. Also, as a rapid and non-invasive method, UV-Vis spectroscopy is employed in art restoration to accurately match paint hues.

Can the narrow red bands of dragonflies be used to perceive wing interference patterns?

Researchers have applied UV-Vis-NIR spectroscopy and various optical techniques to explore how dragonflies (Odonata) might use narrow red spectral bands in their vision to perceive conspecifics through wing interference patterns (WIPs). The researchers investigated the optical properties of Odonata wings using a range of tools including hyperspectral imaging, ellipsometry, and laser profiling. The results of the study suggested that the narrow red spectral bands from the wings present in some dragonflies may enable them to detect members of the same species. The study concluded that these bands likely aid in conspecific identification, proposing further behavioral and electrophysiological tests to validate the hypothesis.[3]

Read the research results

4.7 Characterization of biomolecules

UV-Vis-NIR spectroscopy is widely used for the characterization of biomolecules such as proteins, nucleic acids, and other macromolecules. The technique uses the interaction of light with matter to provide valuable insights into the quantity, quality, stability, and conformational changes of biomolecules.

Protein and nucleic acid quantification

Proteins absorb UV light primarily due to the presence of aromatic amino acids such as tryptophan, tyrosine, and phenylalanine. By measuring absorbance at 280 nm, the concentration of proteins in a sample can be determined using the Beer–Lambert law, as detailed in Chapter 1. This method is simple, rapid, and nondestructive, making it ideal for routine analysis and QC testing. Nucleic acids such as DNA and RNA absorb UV light at 260 nm due to the presence of nucleotide bases (adenine, thymine, guanine, and cytosine). The absorbance at 260 nm can be used to determine the concentration of nucleic acids, while the ratio of absorbance at 260 and 280 nm (A260/A280) provides information about the purity of the sample. A ratio of ~1.8 indicates pure DNA, while a ratio of ~2.0 indicates pure RNA.

Protein and nucleic acid melting temperature

As mentioned in the previous paragraph, proteins absorb UV light due to aromatic amino acids. The tertiary structure of tryptophan, tyrosine, and phenylalanine affects the electronic environment of the amino acids. By heating or using chemical denaturants, proteins unfold, altering their electronic environment and causing spectral changes. DNA absorbs UV light primarily due to its bases (adenine, thymine, guanine, cytosine), peaking at 260 nm. Heating DNA breaks the hydrogen bonds between bases, unwinding the helix and increasing absorbance. This process, which is called thermal melt, is tracked by measuring absorbance at 260 nm while increasing the temperature, resulting in a melting curve. The midpoint of this curve, the Tm value, depends on the G–C pair percentage in the sample, each of which contains three hydrogen bonds (in contrast, each A–T pair contains two hydrogen bonds). The higher the percentage of G–C pairs in the sample, the higher the observed Tm (Figure 65).

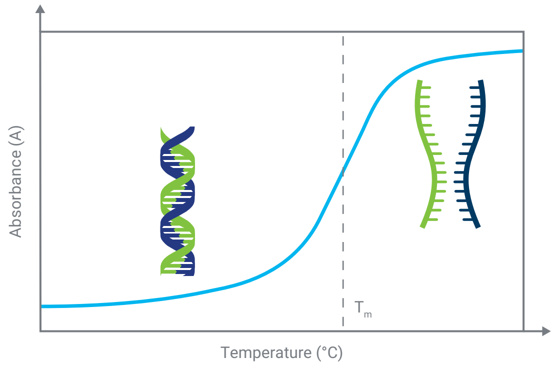


Figure 63. Measuring the absorbance at 260 nm, while increasing the temperature results in this characteristic graph of a DNA 'melt'. The change in absorbance indicates multiple transitions as the DNA helix unwinds.

Enzyme activity

UV-Vis spectroscopy is useful for investigating enzyme reactions. The UV-Vis measures changes in absorbance over time as the reaction progresses. For example, if the substrate or cofactor absorbs light, the decrease in absorbance can be monitored over time as the substrate is consumed in the reaction. Conversely, a product of the reaction may absorb light, and the level of absorption will increase over time as the reaction proceeds.

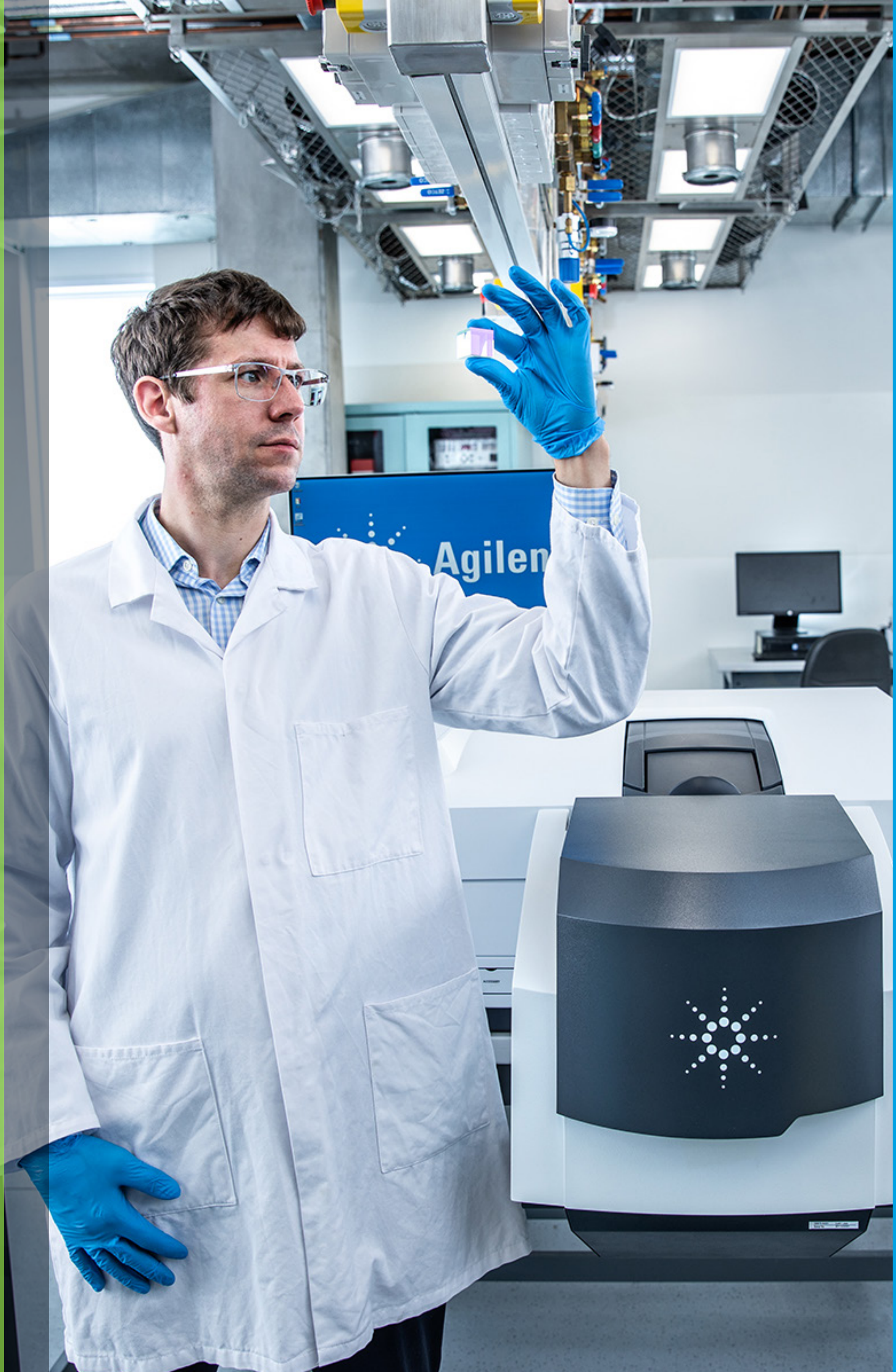
The absorbance data can be used to determine the reaction rate constant and catalyst efficiency, and to characterize reaction inhibitors. Enzymatic activity studies can be done by performing repeated wavelength scans and monitoring changes in absorbance. A simpler way to perform these studies is by measuring the absorbance change at a single wavelength.

References

1. Applications of UV-Vis-NIR, Optical Characterization of Materials Using Spectroscopy, Application Compendium, Agilent Technologies, 2023, 5994-5621EN

2. Li, R.; Kodaira, T.; Kawanami, H. In situ formic acid dehydrogenation observation using a uv-vis-diffuse-reflectance spectroscopy system, *Chem. Commun.* (Camb) **2022**, 58, 11079–11082

3. Brydegaard, M.; Jansson, S.; Schulz, M.; Runemark, A. Can the narrow red bands of dragonflies be used to perceive wing interference patterns? *Ecol Evol.* **2018**, 8; 8(11):5369-5384



5

Glossary of Terms Widely Used in UV-Vis-Nir Spectrophotometry

absorbance: 1. Characteristic of a substance to absorb light. 2. Unit for light absorbance, represented as A or Abs.

angle of Incidence: the angle formed between an incoming light ray and the perpendicular vector to the surface it strikes.

arc (lamp): Creates light by an electric or voltaic arc through an inert gas.

baseline: A measurement collected under the same parameters as the sample measurement, but without the sample in place. A blank is usually used for a baseline measurement as this allows the contributions of the instrument, the solvent, the cuvette etc. to be subtracted from the final sample measurement.

blank: A solvent or substrate of the sample, without the absorbing species. When measuring liquid samples, this will be a solvent (often water) in a cuvette. The absorbance of the blank can then be subtracted from the absorbance of the sample to determine the absorbance that is due to the sample.

chromophore: Part of a molecule that absorbs light.

CRM: *abbreviation.* Certified reference material. Substances that have been certified to a primary standard for comparison purposes for use in calibration and/or to validate analytical measurement methods.

cuvette: Commonly referred to as a cell, the cuvette is a container that holds liquid samples. Cuvettes are available with different volumes and path length options. The cuvette material determines its optical transparency.

diffuse reflectance: Occurs when the surface reflects light in many different directions giving the surface a matt appearance.

dispersion: In optical design, dispersion refers to the ability of the optical device to split light into its constituent wavelengths, e.g. white light on a prism creates a rainbow effect through dispersion.

fluorescence: A form of luminescence and characteristic of some molecules to absorb light at a frequency and emit short-lived light of another wavelength.

GMP: *abbreviation.* Good manufacturing practice. Commonly used in pharmaceutical and other regulated manufacturing industries.

holographic (optics): Holographic optics are created by etching an interference pattern on an optic surface. Holographic optics can be used in place of lenses, mirrors, and other optical devices. Their design makes them easy to replicate accurately. They are also small and light.

noise: In spectrophotometry, noise refers to the background electrical signal that is contributed by the instrument itself. If the level of noise is too great, it can affect the measurement signal from the sample, making it difficult to differentiate between the two signals. An easy way to understand noise is to consider looking at the stars from a city compared to a remote location. In an urban setting, the city lights create background 'noise', which makes it difficult to see the stars. In contrast, the relatively low background light in remote areas allows the stars to be seen more clearly.

pharmacopeia: Regulatory documents listing details of pharmaceuticals and required or recommended testing procedures for the pharmaceutical industry.

phosphorescence: A form of luminescence related to fluorescence. It is characteristic of certain molecules to absorb light at one frequency and emit light of another wavelength after a time delay.

photochemical (reactions): Chemical reactions caused by the absorption of light.

photosensitivity: Sensitivity of a substance to react when exposed to light.

QA/QC: *abbreviation.* Quality control and/or quality assurance.

qualitative (measurements): Measurements that provide non-numerical information about the sample, e.g. the identification of a molecule in solution.

quantitative (measurements): Measurements that resulting in a numerical value e.g., concentration.

rare earth oxides: Holmium, didymium, and samarium oxides are referenced by standards' organizations and pharmacopoeia to be used for wavelength validity measurements.

reflection: Describes the path of light as it strikes sample which is deflected at the angle of incidence.

scattering: Effect of light bouncing from a surface at random angles.

SOP: *abbreviation.* Standard operating procedure. Document written to ensure that measurements can be formed safely and repeatedly.

spectrum: plural, spectra. Range of wavelengths. The electromagnetic spectrum. Also refers to a graphical output of wavelength vs. intensity (or absorbance, as measured by a spectrophotometer).

Specular reflectance: Mirror-like reflection off a sample surface.

transmittance: Percentage of incident light that is transmitted through a sample.

zero: This is equivalent to the 'tare' function on a set of scales—it sets the instrument reading to 0 Abs.

Advanced Molecular Analysis with Cary Spectrophotometers for UV-Vis-NIR

Cary 5000 UV-Vis-NIR Spectrophotometer

Cary 6000i UV-Vis-NIR Spectrophotometer

Cary 7000 Universal Measurement Spectrophotometer (UMS)

Further Information

Agilent Cary WinUV Software for UV-Vis-NIR Applications

Agilent Cary UV-Vis Spectrophotometer Accessories

Agilent Praying Mantis DRA for Cary UV-Vis-NIR Instruments

UV-Vis Spectroscopy and Spectrophotometer FAQs

Learn more:
www.agilent.com/chem/uvvis-uvvisnir

DE-008956

Published in the USA, August 18, 2025
5994-7919EN

Agilent
Trusted Answers