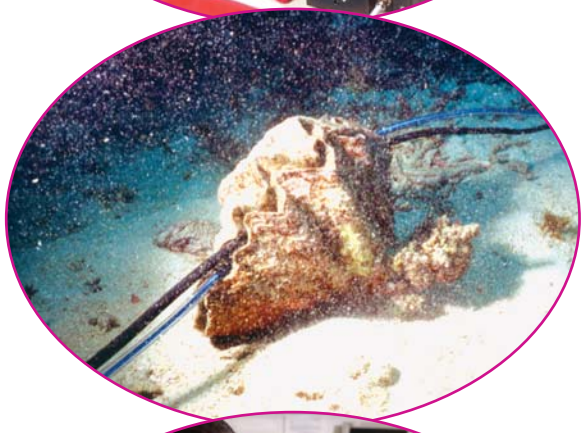
Resources



Overview: Resources

Our Know-how = More Resources for You

Find out more about our products, experience and support through the following:

Live Demonstrations
Each year we exhibit at nearly 100 tradeshows around the world (OceanOptics.com/Tradeshows.asp). At home, we conduct formal seminars and can customize training sessions to your requirements.

Science Curricula
Our *Educational Spectroscopy Grant Program* rewards educators and researchers for their use of spectroscopy in curricula or research. Information about grant-winning projects is posted at OceanOptics.com/Applications/GrantWinners.asp.

R&D Services
Our Applications Group will take ownership of your most challenging application needs. The Group provides optical and electronic design services, software engineering and spectral modeling, testing and validation, and rapid prototyping capabilities.

Reference Library
We have amassed nearly 500 technical papers featuring our spectrometers and accessories. Citations are on our website at OceanOptics.com/Applications.asp.

Technical Information on the Web

We believe in easy access to information. That's why we don't hide our prices and that's why we provide easily accessible technical documentation on our website, so that you can view manuals before you buy the instrument. We also include the manufacturer's name and the model number for components that go into our instruments. We want to provide you with all of the information you need not only to make the right purchasing decision, but also to get the best performance out of your Ocean Optics products.

- **OceanOptics.com/Technical.asp.** Choose the TECHNICAL button on our website to view and download information about our products and technology, including manuals and operating instructions, software downloads and system specifications.
- **Operating Instructions.** We provide hundreds of pages of easy-to-access operating instructions and specifications of our products so that you can read before you buy at OceanOptics.com/Technical/OperatingInstructions.asp.
- **Software Downloads.** Easily download the latest operating and application software, device drivers and code, utility programs and microcode at OceanOptics.com/Technical/SoftwareDownloads.asp.
- **Spectrometer System Specifications.** Spectrometer system performance depends on a host of factors, such as the detector, optical bench, grating, entrance aperture size and sampling optics, just to list a few. To help you understand how to configure spectrometer systems, visit OceanOptics.com/Technical/SystemSpecifications.asp.
- **Applications Database.** Choose the APPLICATIONS button from any Ocean Optics webpage to view an up-to-date bibliographic listing of journal and magazine articles that reference our products. Visit OceanOptics.com/Applications/References.asp.

85,000+ Spectrometers, 1,000s of Applications

We've sold over 85,000+ Ocean Optics optical-sensing systems since 1992, which has provided us with a body of applications knowledge that is unmatched in the industry. Our spectrometers are used in applications such as these:

- Air and soil in situ monitoring
- Astronomy
- Biological and chemical warfare agent detection
- Biotechnology
- Blood oximetry
- Cancer detection
- Chemistry
- Color measurement
- Crystal growth
- Display technologies
- Dissolved oxygen
- Elemental analysis
- Endpoint detection
- Exhaust emission analysis
- Flow injection analysis
- Fluorescence of corals
- Food processing
- Forensics
- Gemstone grading
- General R&D
- Headspace monitoring
- Laser characterization
- LED quality control
- Life sciences
- Manufacturing
- Medical research
- Non-destructive testing
- Optical filter transmission
- pH monitoring
- Pharmaceuticals
- Physics/Optics
- Physiological applications
- Plasma monitoring
- Process control
- Radiometry
- Raman spectroscopy
- Reaction kinetics
- Semiconductor processing
- Shelf life of food and beverages
- Stack emissions
- Thin film thickness
- Tissue composition

The ABCs of Absorbance

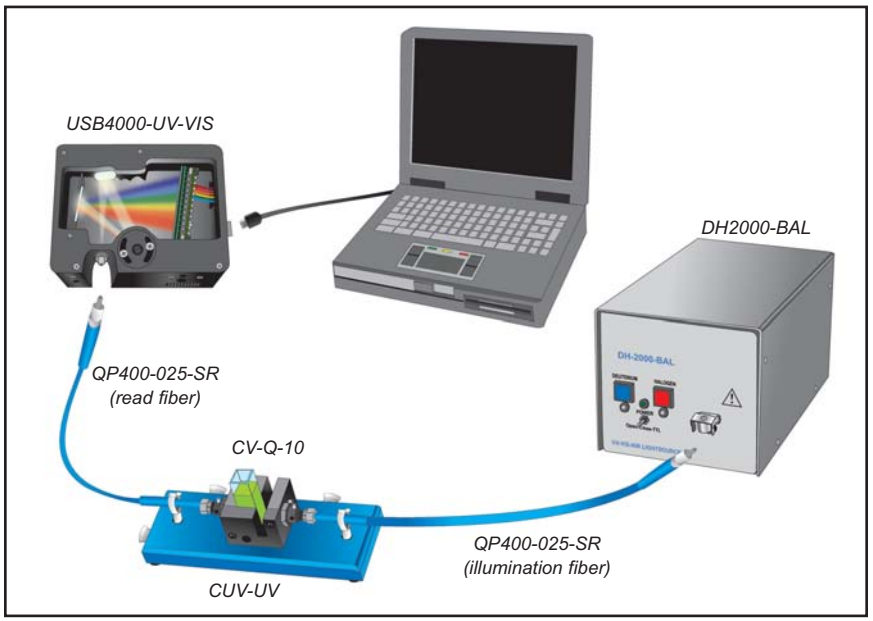
Like thousands of other educators, chemists at Miami (Ohio) University have equipped their labs with Ocean Optics spectrometers and accessories for basic spectroscopic measurements such as solutions absorbance.

Of particular interest is a PC-based setup for measuring the UV-VIS absorption spectrum of iodine crystals from 500-580 nm. This experiment is readily performed using an S2000 Spectrometer, LS-1 Tungsten Halogen Light Source, fiber optic patch cords and a 10-cm pathlength cuvette holder. Substitute a USB4000 Spectrometer (see drawing at right) to eliminate the external A/D card that completes the Miami University system.

Another option is the CHEM4-UV-VIS Lab Spectrophotometer, which consists of a 200-850 nm USB-interface spectrometer, a combination deuterium tungsten halogen light source and 1-cm cuvette holder, high-speed electronics and software.

Solutions absorbance experiments are not limited to cuvette holder setups. Flow cells, on-line dip probes and other sampling optics are available, with the latter especially useful for in situ applications. For example, one Ocean Optics customer uses a UV-VIS spectrometer and dip probe to measure the absorbance of vanadium oxytrichloride (VOCl3), a potentially toxic liquid used in the production of rubber (the absorptivity of VOCl3 relates to its stability). Because the VOCl3 reacts with moisture in the air and forms vanadic and hydrochloric acids, it must be measured in a moisture-free environment. In situ measurements eliminate the need for potentially risky sample collection.

Setup: Solutions Absorbance



Overview

Absorbance measurements are used to quantify the concentration of gases and solutions (the latter is described here) that absorb light in a media that transmits light. The signal in absorbance units is proportional to the molar absorptivity, pathlength and concentration of the sample (see Beer's Law, page 178).

Spectrometer

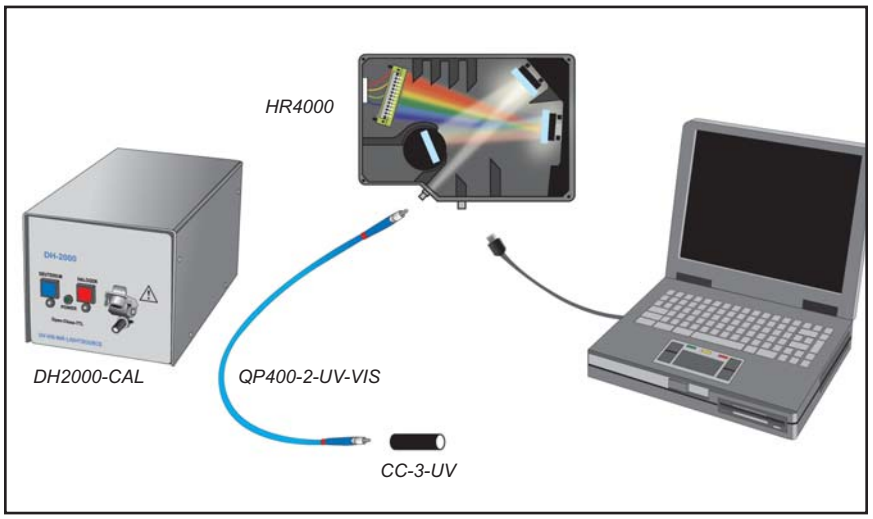
The USB4000-UV-VIS Spectrometer is ideal for absorbance measurements from 200-850 nm. The spectrometer is configured with Grating #1, which has peak efficiency at 300 nm. This configuration provides adequate resolution (~1.5 nm FWHM) for most solutions absorbance measurements. The built-in OFLV-200-850 Order-sorting Filter eliminates second-and third-order effects that otherwise yield false peaks in absorbance spectra. The preferred light source is the DH2000-BAL Deuterium Tungsten Halogen Light Source. The DH2000 is a less expensive source, but lacks the filtering technology that eliminates problems associated with the D-alpha line in the deuterium source.

Sampling Optics

For absolute absorbance measurements, use the 1-cm pathlength CUV-UV Cuvette Holder and the CV-Q-10 Quartz Cuvette. For relative absorbance, direct-attach USB accessories, dip probes and flow cells are available. We recommend QP400-025-SR Premium-grade Solarization-resistant Optical Fibers as illumination and read fibers. Use NIST-traceable STAN-ABS Photometric Absorbance Standards to provide certifiable results.

Components	Page	Price
1. USB4000-UV-VIS General Lab Spectrometer	34	$2,649
25 µm Slit as entrance aperture	15	included
Grating #1, 200-850 nm range	16	included
DET4-200-850 Detector with UV4 Detector Window Upgrade and OFLV-200-850 Order-sorting Filter	17	included
2. DH2000-BAL Deuterium Tungsten Halogen Light Source	122	$3,588
3. (2) QP400-025-SR Premium-grade SR Assemblies	142	$238
4. CUV-UV Cuvette Holder	90	$399
5. CV-Q-10 Quartz Cuvette	93	$75
6. STAN-ABS-UV Photometric Absorbance Standards	93	$370
7. SpectraSuite Spectroscopy Operating Software	80	$199
8. ASP Annual Service Package	62	$250
	Total:	**$7,768**

Setup: Upwelling/Downwelling



Overview

Upwelling radiation is radiation -- either reflected solar or emitted terrestrial -- that is directed upward from the earth's surface. Downwelling radiation is radiation that is directed toward the earth's surface from the sun or atmosphere. The relationship between the two (albedo) can be used to derive spectral information from vegetation, forest canopies, seabeds and more.

Spectrometer

An HR4000 Spectrometer with an HC-1 grating provides an elegant solution for upwelling and downwelling measurements. The HC-1 is a variable-blazed grating that covers the 200-1050 nm wavelength range; optical resolution is ~1.5 nm (FWHM) with a 50 µm slit as the entrance aperture. An OFLV-200-1100 Order-sorting Filter eliminates second- and third-order effects.

Sampling Optics

The spectrometer connects to a patch cord that screws into the CC-3-UV Cosine Corrector. The CC-3-UV can be used as part of a configuration for measuring absolute spectral irradiance. You'll need a DH2000-CAL (or LS-1-CAL for 300-1050 nm only) to calibrate the absolute spectral response of the system and SpectraSuite Spectroscopy Operating Software to calculate spectral intensity and photopic data in lumens, lux or candela. An alternative to the CC-3-UV is a Gershun tube, which has fixtures for adjusting the area of light from 1° to 28° and attaches directly to the spectrometer or to an optical fiber.

Components	Page	Price
1. HR4000 High-resolution Spectrometer	21	$3,999
50 µm Slit as entrance aperture	22	$150
Grating HC-1, 200-1050 nm range	23	$600
DET4-200-1100 Detector with OFLV-200-1100 Order-sorting Filter and UV4 Detector Window Upgrade	24	$400
2. QP400-2-UV-VIS Premium-grade Patch Cord Assembly	142	$169
3. CC-3-UV Cosine Corrector	104	$129
4. DH2000-CAL Radiometric Calibration Standard	132	$3,275
5. SpectraSuite Spectroscopy Operating Software	80	$199
6. ASP Annual Service Package	62	$250
	Total:	**$9,171**

Measuring Mining Effects

In the small Pacific island of New Caledonia, a multinational team of researchers has used Ocean Optics spectrometers to measure the effects of strip mining on coastal erosion, sea grass growth and coral reef health.

The team focused on the relationship between above-water reflectance and turbidity profiles. The latter relates to fluxes in the presence of metals and various pollutants -- and thus, to sea grass growth and coral reef health.

A USB4000 Spectrometer set from 360-1100 nm measures reflectance and irradiance. The USB4000 connects to a patch cord that screws into a Gershun Tube, which has fixtures for adjusting the area of light entering the fiber -- in this case, to reduce the field of view to 3°. Upwelling irradiance and downwelling radiance measurements -- the spectral distribution of the underwater light field -- add valuable data.

The researchers also have measured the concentration of chlorophyll pigment in coastal waters and the reflectance of sand and mud collected at Caribbean, Mediterranean and Pacific beaches. The sand application used a dual-channel spectrometer for visible (410-900 nm) reflectance measurements of various natural sands. Reflectance spectra were deduced from successive measurements of upwelling irradiance using a Spectralon plate and downwelling radiance captured under natural light.

Ultimately, researchers will use satellite monitoring, spectroradiometric measurements and numerical models to better understand the nature of particulate transport in coral reef lagoons, especially as it relates to erosion rates in coastal areas.

O_2 Medical Diagnostics

Researchers at two Irish universities have monitored dissolved oxygen in cellular media in order to validate the optimum gassing technique to induce hypoxia in irradiated cells.

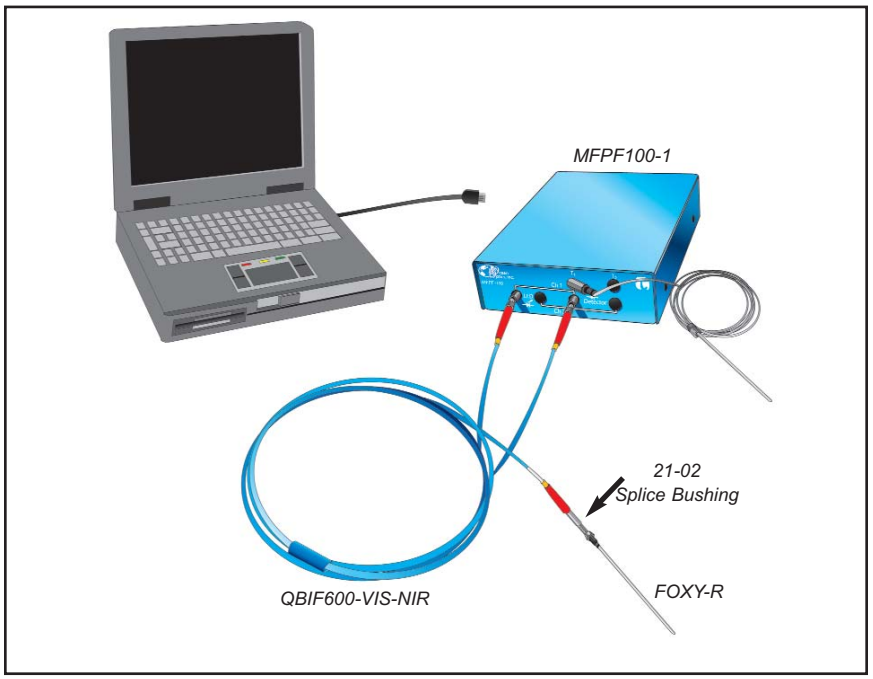
Scientists from University College Cork and Cork University Hospital measured irradiated HeLa cells -- a strain of human cells used for biological studies -- under both oxic (rich in oxygen) and hypoxic (lacking oxygen) conditions. With oxygen present, the irradiation injury to the cells was greater than when optimum levels of

hypoxia (~90%) were reached. To induce hypoxia, and thus mitigate any oxygen-enhancement injury, the cells were gassed with nitrogen. This hypoxia was confirmed with a FOXY Fiber Optic Oxygen Sensor and a USB2000 Spectrometer.

The FOXY Sensor has been used for other hypoxia experiments, including an application where clinicians determine how much of a diseased human limb targeted for amputation can be saved; the presence of oxygen correlates to tissue health. Monitoring dissolved oxygen in both human and animal tissue is a common application for the FOXY Sensor, which offers the advantages of being minimally invasive, not consuming the sample, and working well in viscous media.

Ultimately, the cellular hypoxia researchers determined that oxygen measurements of the cellular environment made with the FOXY Sensor matched the predicted hypoxic saturation values, depending on the amount and duration of nitrogen flushed through the sample chamber. The FOXY Sensor proved to be a valuable tool in confirming the desired level of hypoxia.

Setup: Oxygen Sensing



Overview

Oxygen is sensed by measuring the decrease in fluorescence intensity of a fluorophore bound to the tip of an optical fiber. The sensor responds to the partial pressure of oxygen in gases, liquids and even viscous samples.

Spectrometer

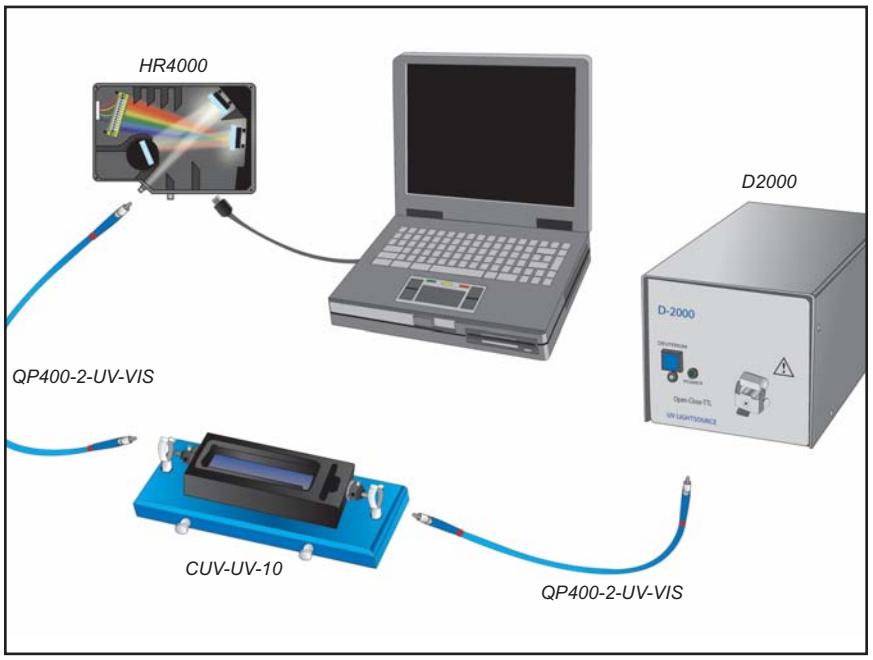
Used with Ocean Optics Fiber Optic Oxygen Sensors and custom probes, the MultiFrequency Phase Fluorometer (MFPF), manufactured by TauTheta, is a flexible platform for measurement of luminescence lifetime, phase and intensity. This frequency-domain luminescence monitor uses LED excitation and avalanche photodiode detection with filter-based wavelength selection for easy experimental set-up and control. The MFPF is especially useful for oxygen sensing applications where sensitivity to drift is important and where sample set-ups must be undisturbed for long periods of time. Because it utilizes phase-shift technology, it is invariant to fiber bending and stray light, has a wide dynamic range of optical intensity, and has low optical and electronic crosstalk as well as low drift and phase noise.

Sampling Optics

The MFPF is embedded with LED excitation sources and transmits light at ~475 nm to one leg of a QBIF600-VIS-NIR Bifurcated Optical Fiber Assembly. The bifurcated assembly connects to the oxygen sensor probe via a 21-02 SMA Splice Bushing. If the excited formulation at the probe tip encounters an oxygen molecule, the fluorescence signal decreases. The fluorescence is collected by the probe and is transmitted to the spectrometer via the other leg of the bifurcated assembly. OOISensors Software calculates partial pressure of the oxygen from this signal. For more on sensor operation, see page 65.

Components	Page	Price
1. MFPF100-1 MultiFrequency Phase Fluorometer	67	$5,000
2. QBIF600-VIS-NIR Premium-grade Bifurcated Fiber Assembly	72	$369
3. 21-02 Splice Bushing	72	$13
4. FOXY-R Fiber Optic Oxygen Sensor Probe	71	$499
5. OOISensors Software	75	$199
6. ASP Annual Service Package	62	$250
	Total:	**$6,330**

Setup: Gas Absorbance



Overview

Absorbance measurements are used to quantify the concentration of solutions and gases (as described here) that absorb light in a media that transmits light. The signal in absorbance units is proportional to the molar absorptivity, pathlength and concentration of the sample. (See more on Beer's Law on page 178.)

Spectrometer

A setup for measuring benzene gas, for example, would call for an HR4000 High-resolution Spectrometer with an H7 grating and a 200-300 nm wavelength range. Optical bench accessories include an L4 Detector Collection Lens for increased light throughput, and a UV4 Detector Upgrade to transmit light in the UV. With a 5 μm slit, optical resolution of ~0.07 nm (FWHM) is possible. The preferred light source for work in the ultraviolet is the D2000 Deuterium Light Source.

Sampling Optics

The 10-cm pathlength CUV-UV-10 Cuvette Holder, the CV-Q-10 Cylindrical Cell and QP400-025-SR Premium-grade Solarization-resistant Optical Fibers (one fiber illuminates, the other reads signal) comprise the system's sampling optics. For applications requiring shorter pathlengths or open-air monitoring (see sidebar), use an optical fibers-and-collimating lenses configuration.

Components	Page	Price
1. HR4000 High-resolution Spectrometer	21	$3,999
Grating H7, 2400 lines per mm, 200-300 nm range	23	included
5 µm Slit as entrance aperture	22	$150
L4 Detector Collection Lens	24	$150
DET4-UV Detector with UV4 Detector Upgrade	24	$150
2. D2000 Deuterium Light Source	126	$2,172
3. CUV-UV-10 Cuvette Holder	90	$549
4. CV-Q-100 Cylindrical Cell	93	$165
5. (2) QP400-2-UV-VIS Premium-grade Patch Cord Assemblies	142	$338
6. SpectraSuite Spectroscopy Operating Software	80	$199
7. ASP Annual Service Package	62	$250
	Total:	**$8,122**

Volcano Emissions

Active volcanoes emit various gases including sulphur dioxide (SO_2), a colorless, pungent gas that can irritate the skin and the mucous membranes of the eyes, nose and throat. Volcanologists regularly monitor SO_2, which absorbs in the UV.

For example, on the Caribbean island of Montserrat, researchers use three S2000 Spectrometers to collect UV absorbance (from 245-380 nm) of SO_2 in gas emissions. The spectrometers are set up at three plume sites, each of which is about 3.5 km from the volcano's dome. The spectrometers are small, making them simple to transport and deploy at the volcano site. The entire setup costs less than $10,000, within most budget limits and almost "disposable" (this is a volcano, after all).

The Montserrat researchers configured a system that makes efficient use of light-collection optics and provides good optical resolution (~3.5 nm FWHM). Each spectrometer is connected to a 1000 μm optical fiber, which screws into a telescope mount.

At the Montserrat Volcano Observatory (www.mvo.ms) sampling sites, spectra are collected every 4-6 seconds and transmitted to researchers at the observatory via modem; one complete scan of the plume takes 4-6 minutes. Depending on wind direction, data from two of the three spectrometers is used to calculate plume height, by comparing the angles at which peaks in the SO_2 plume are measured.

All That Glitters . . .

By some accounts, fluorescence of minerals has been observed for more than a century. For early miners, fluorescence of minerals such as calcite helped to target drilling operations to the richest bodies of ore. For amateur geologists, mineral fluorescence is a more esoteric pursuit: samples that fluoresce simply look really cool.

Consider genthelvite, an opaque mineral that fluoresces bright green under UV radiation and remains phosphorescent for a short period. In 2003, mineralogists Earl Verbeek and Herb Yeates measured fluorescence of both genthelvite and willemite (another fluorescent mineral) found in deposits at a site in New Jersey.

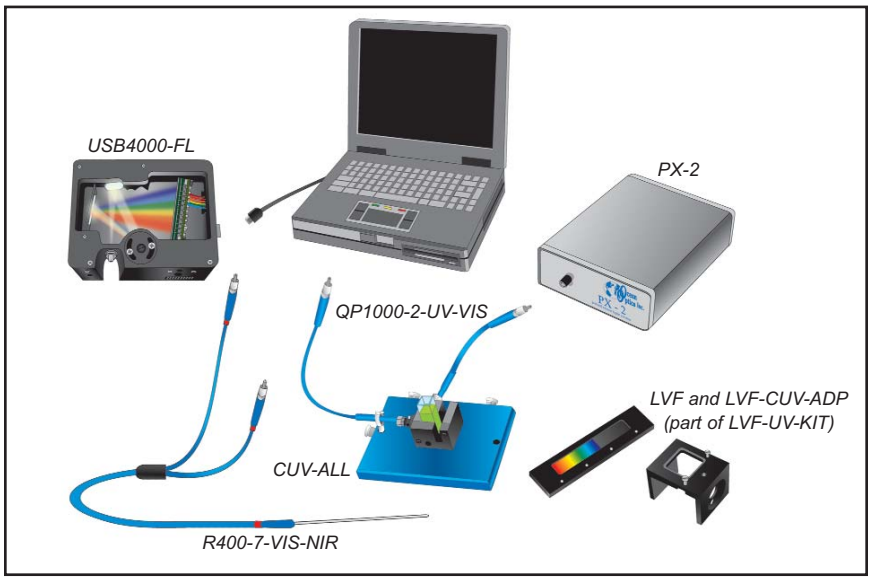
In a paper submitted to the Franklin-Ogdensburg Mineralogical Society, Verbeek and Yeates described using a USB2000-VIS-NIR Spectrometer (350-1000 nm), a high-power UV excitation source and a 600 μm probe to observe emission peaks of 511 nm for genthelvite and 528 nm for willemite.

Spectrometer integration times were set for 1000 ms to measure the dim (although visible to the naked eye) genthelvite fluorescence, compared with a 10 ms integration to measure the brighter willemite fluorescence.

To ensure that the light emitted from the samples came from the minerals themselves, Verbeek and Yeates measured the samples in a light-tight enclosure and filtered out excitation source wavelengths and ambient light.

Why does genthelvite fluoresce? Verbeek and Yeates identified the source as divalent manganese -- a substitute for zinc in the genthelvite structure that is also responsible for the color in amethyst.

Setup: Fluorescence



Overview

Fluorescence measurements require a sensitive detector and an effective filter for discriminating between powerful excitation source wavelengths and weak spectral emissions from the sample.

Spectrometer

We offer several spectrometers useful for fluorescence, but recommend the high-sensitivity, preconfigured USB4000-FL Spectrometer for most general fluorescence applications. The USB4000-FL is set to 360-1000 nm and comes with a 200-μm slit and an L4 Detector Collection Lens for increased light throughput.

Sampling Optics

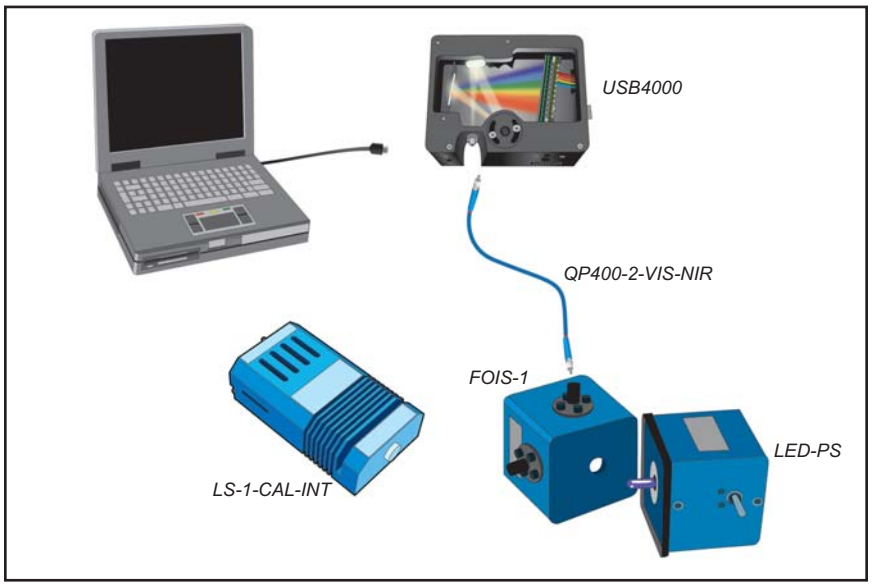
Your standard excitation source option is our PX-2 Pulsed Xenon Source. Our proprietary LVF Linear Variable Filters are excellent tools for spectrally shaping the excitation energy from broadband sources used for fluorescence. Various sampling optics are available for detecting picomolar-range concentrations of fluorophores from surfaces and in solutions and powders.

Spectrometer Components	Page	Price
1. USB4000-FL Spectrofluorometer	46	$2,499
200 µm Slit as entrance aperture	15	included
Grating #3, 380-1000 nm range	16	included
L4 Detector Collection Lens	17	included
SAG+UPG Mirrors	16	$250

Components for Use with Solutions	Page	Price
2. PX-2 Pulsed Xenon Source	127	$769
3. CUV-ALL-UV 4-way Cuvette Holder	90	$809
4. LVF-UV-KIT Linear Variable Filter Kit	114	$999
6. (2) QP1000-2-UV-VIS Premium-grade Patch Cord Assemblies	142	$718
7. (2) 74-MSP Mirrored Screw Plugs	90	$198
8. SpectraSuite Spectroscopy Operating Software	80	$199

Components for Use with Solids	Page	Price
2. PX-2 Pulsed Xenon Source	127	$769
3. R400-7-VIS-NIR Reflection/Backscattering Probe	148	$499
4. SpectraSuite Spectroscopy Operating Software	80	$199

Setup: LED Analysis



Overview

To measure the absolute spectral intensity and color of LEDs, specify the configuration described here or see page 56.

Spectrometer

We suggest a USB4000 Spectrometer with a 25 µm Slit and Grating #2 (350-1000 nm). An L4 Detector Collection Lens increases light-collection efficiency and reduces stray light. An OFLV-350-1000 Order-sorting Filter eliminates second- and third-order effects. This optical bench configuration maximizes system sensitivity, mitigating the light loss inherent with use of an integrating sphere -- the sampling optic of choice for most LED applications. (You also can collect LED signal with a CC-3-UV Cosine Corrector and fiber.)

Sampling Optics

The LED is mounted in the NIST-traceable LED-PS-NIST LED Power Supply, which provides a white background for the LED and a controlled drive current to characterize LED output. The FOIS-1 Integrating Sphere is placed over the LED-PS-NIST and collects the LED output. The attached optical fiber collects the light energy from the LED and transmits it to the spectrometer. The power and color of the LED is determined by comparing the LED to a radiant standard -- the LS-1-CAL-INT Calibrated Source, which fits into the sample port of the FOIS-1. SpectraSuite Spectroscopy Operating Software calculates absolute irradiance and spectral features such as dominant, central and centroid wavelength; hue, chroma and saturation, X,Y,Z; L*, a*, b*; xyz; u'v'w'; CCT and more.

Components	Page	Price
1. USB4000 Plug-and-Play Spectrometer	14	$2,199
Grating #2, 350-1000 nm range	16	included
25 µm Slit as entrance aperture	15	$150
L4 Detector Collection Lens	17	$150
DET4-350-1000 Detector with OFLV-350-1000 Order-sorting Filter	17	$150
2. LS-1-CAL-INT Tungsten Halogen Calibrated Light Source	133	$749
3. LED-PS LED Power Supply	104	$499
4. FOIS-1 Integrating Sphere for Emission	105	$499
5. QP400-2-VIS-NIR Premium-grade Patch Cord Assembly	142	$169
6. SpectraSuite Spectroscopy Operating Software	80	$199
7. ASP Annual Service Package	62	$250
	Total:	**$5,014**

QC of LED Curing Lights

High-output LEDs may be a viable alternative to other light sources for curing ceramic materials used in dentistry, according to researchers from the University of Manchester in England.

As researchers Adrian Bennett and David Watts suggested in a 2003 article submitted to the journal *Dental Materials*, LEDs have longer lifetimes, are less prone to degradation and temperature effects, and require less power than tungsten halogen curing units.

To assess LED performance, Bennett and Watts used a radiometrically calibrated USB2000 Spectrometer to measure the absolute spectral output and irradiance of three LED curing units. The spectrometer was radiometrically calibrated using the LS-1-CAL Tungsten Halogen Light Source; a FOIS-1 Integrating Sphere collected the LED output and funneled it to an optical fiber coupled to the spectrometer. The spectral range of the LEDs also was measured.

By most criteria, Bennett and Watts concluded, the LED curing units compared favorably with the tungsten halogen curing units. However, longer curing times may be necessary with LEDs, which have lower irradiance than the tungsten halogen sources.

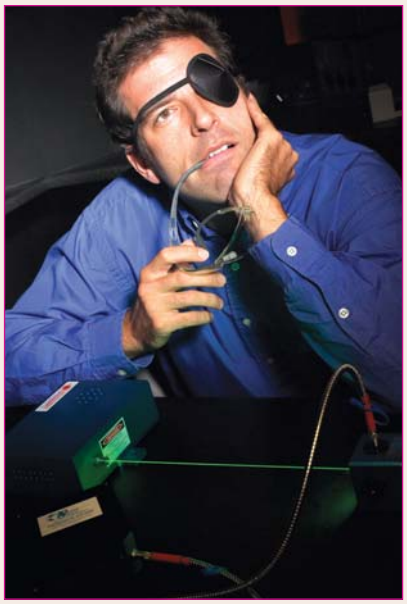
Similar studies also have been performed at the Indiana University School of Dentistry.

Whatever their ultimate application, LEDs can be analyzed for color and absolute spectral intensity very easily and inexpensively with Ocean Optics spectrometers and accessories.

Laser Plume Analysis

Ocean Optics spectrometers and accessories are useful tools for measuring the spectral output and power of lasers, with configurations as simple as the setup shown at right.

But we also provide components for applications involving what happens after the laser fires. Consider laser welding, which is now common to a number of industries. An Ocean Optics customer has used our PC Plug-in Spectrometer and an optical fiber to measure the plume created by a CO_2 laser used in welding metals such as copper and stainless steel alloys. Researchers were particularly interested in the processes related to welding of dissimilar materials.

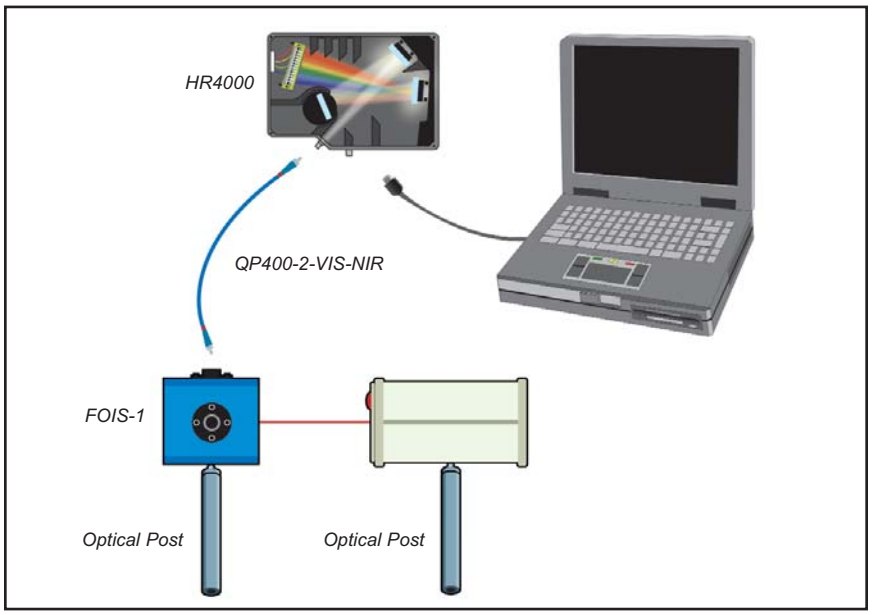


By measuring the concentration of elements within the laser weld plume, as well as the plume temperature, the researchers were able to determine the efficiency of the weld. Species identification is useful in controlling the welding of dissimilar alloys; plume temperature can be correlated to laser power and speed.

The UV-VIS spectrometer used in the study had a wavelength range of 263-523 nm. One leg of a bifurcated optical fiber carried light from a diode laser to the weld site; the other leg sampled the plume emission.

Ultimately, real-time monitoring of the laser weld plume makes it far simpler to correct process problems before large numbers of parts are affected. This increases manufacturing yields and speeds up inspection processes.

Setup: Laser Analysis



Overview

Our HR4000 High-resolution Spectrometer is ideal for measuring the spectral characteristics and intensity of continuous-wave and pulsed lasers. For high-power lasers, an integrating sphere or cosine corrector attenuates the light to avoid saturating the CCD array.

Spectrometer

The HR4000 Spectrometer uses the "HR" Optical Bench, which was designed to yield high optical resolution for resolving fine spectral features. For laser characterization, we recommend a grating with a high groove density, such as the H6 1200 mm-1 grating set to a 750-925 nm wavelength range and with a 5 μm Slit as the entrance aperture. This configuration provides ~0.12 nm resolution (FWHM). For better resolution consider an 1800 mm-1 or 2400 mm-1 grating.

Sampling Optics

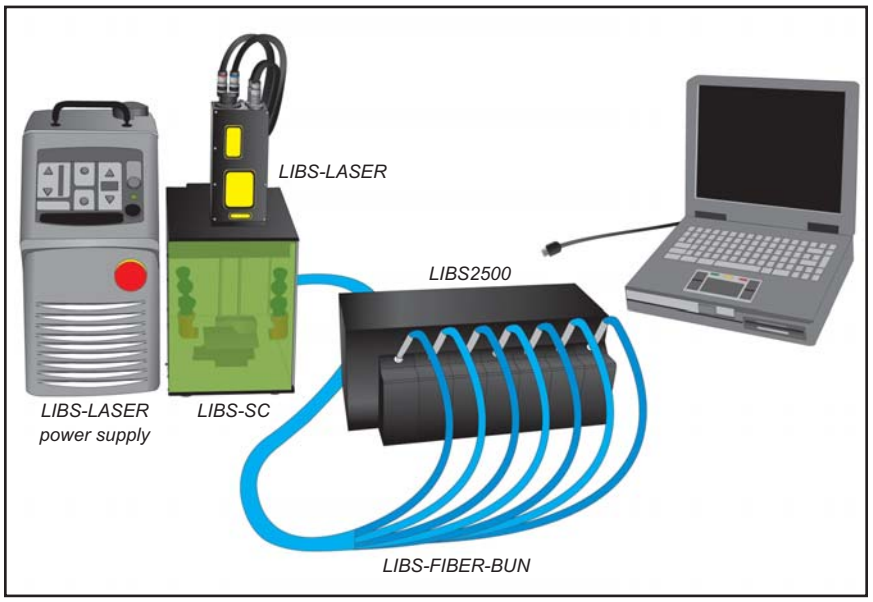
There are several possible sampling setups: a CC-3-UV Cosine Corrector with an optical fiber; FOIS-1 Integrating Sphere with a fiber; or fiber assembly coupled to the laser. Optical posts are used to hold fixtures in place.

Measurements

Our operating software can detect the laser wavelength peak; SpectraSuite Spectroscopy Operating Software obtains peak, centroid and central wavelength values, and full-width half-maximum values.

Components	Page	Price
1. HR4000 High-resolution Spectrometer	21	$3,999
Grating #H6, 750-925 nm range	23	included
5 µm Slit as entrance aperture	22	$150
DET4-VIS Detector	24	Free
2. FOIS-1 Integrating Sphere for Emission	105	$499
3. OPM-3 Three-inch Optical Post (2)	89	$30
4. QP400-2-VIS-NIR Premium-grade Patch Cord Assembly	142	$169
5. SpectraSuite Spectroscopy Operating Software	80	$199
6. ASP Annual Service Package	62	$250
	Total:	**$5,296**

Setup: LIBS



Overview

The LIBS2500 Broadband Spectrometer is a detection system for real-time elemental analysis in solids, solutions and gases. This high-resolution system provides full spectral analysis from 200-980 nm, with optical resolution of ~0.1 nm (FWHM).

Principle of Operation

An Nd:YAG pulsed laser beam is focused on the sample area. The energy of the laser generates a plasma, in which a trace amount of the sample has been ablated. As the plasma decays or cools, the plasma emits light of wavelengths that are distinct to each element. The emission is collected by a 7-fiber bundle and sent to the spectrometers for analysis.

Spectrometers

The LIBS2500-7 uses seven high-resolution spectrometers, which connects to a PC via one USB port. All seven spectrometers acquire data simultaneously; software displays the results. However, you may require a system with less than seven spectrometer channels. See page 48 for options on all LIBS2500 Systems.

Sampling Optics

The LIBS-LASER is a 50 mJ CFR Nd:YAG laser for metal and thin film samples and sells for $14,500. The LIBS-LAS200MJ is a 200 mJ CFR Nd:YAG laser for most all other materials and is priced at $22,500. Both lasers are manufactured by Big Sky Laser. The LIBS-SC Sample Chamber has a manual x-y-z stage and a remote laser safety lock. Signal is collected by a fiber bundle comprising (7) 600 µm UV-VIS patch cords, each with a collimating focusing lens built into the fiber termination.

Measurements

OOILIBS Software allows users to set operating parameters such as the laser Q-switch delay (the time between the firing of the laser and the beginning of spectral acquisition) and signal averaging of laser pulses.

Components	Page	Price
1. LIBS2500-7 7-Channel Laser-induced Breakdown Spectrometer	48	$29,999
2. LIBS-BUN-7 Optical Fiber Bundle	48	$985
3. LIBS-LASER Nd:YAG 50 mJ Laser (from Big Sky Laser)	49	$14,500
4. LIBS-SC Sample Chamber	49	$9,800
5. OOILIBS Software	48	$500
	Total:	**$55,784**

LIBS for Defense

In an earlier LIBS application, closely related spores of the genus *Bacillus* were deposited on silver membrane filters for analysis using broadband Laser-induced Breakdown Spectroscopy (LIBS). The observed spectral differences among the spores -- *Bacillus subtilis*, *Geobacillus stearothermophilus* and *Bacillus pumilus* -- provide evidence of the power of Ocean Optics' Laser-induced Breakdown Spectrometer in resolving complex biological samples.

The presence of the spores' unique spectral lines, as well as different combinations of spectral lines, provide many opportunities for discrimination. While most of the unique peaks occurred in the *G. stearothermophilus* spectrum, spectral differences were observed in the spectra for all the spores. Spore characteristics such as surface profile and coat mineralization may account for these differences.

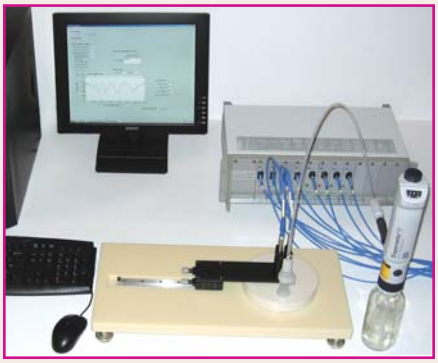
The results reported for the Bacillus spores, along with others obtained for biological molecules including nucleic acids and proteins, provide exciting evidence of the discriminating capability of our LIBS system. In fact, we are now collaborating with others to develop a man-portable LIBS system for field detection of chemical and biological warfare agents. The system will be able to make a complete analysis every one to two seconds, be small enough to carry in a backpack, and require very little power to operate.

Thin Film Thickness

Product developer Thickness Detection Systems (TDS) of Salt Point, N.Y., has integrated an Ocean Optics multichannel spectrometer into a broadband dissolution rate monitor (DRM) for analyzing very thin resist films used in the semiconductor and optics industries.

DRMs help to determine the thickness of thin film layers and the rate at which the film resist material dissolves -- important parameters in controlling thin film production processes. In its initial testing, Thickness Detection Solutions focused on applications involving films of <300 nm thickness, where existing monochromatic and polychromatic interferometric testing methods have had limited effectiveness.

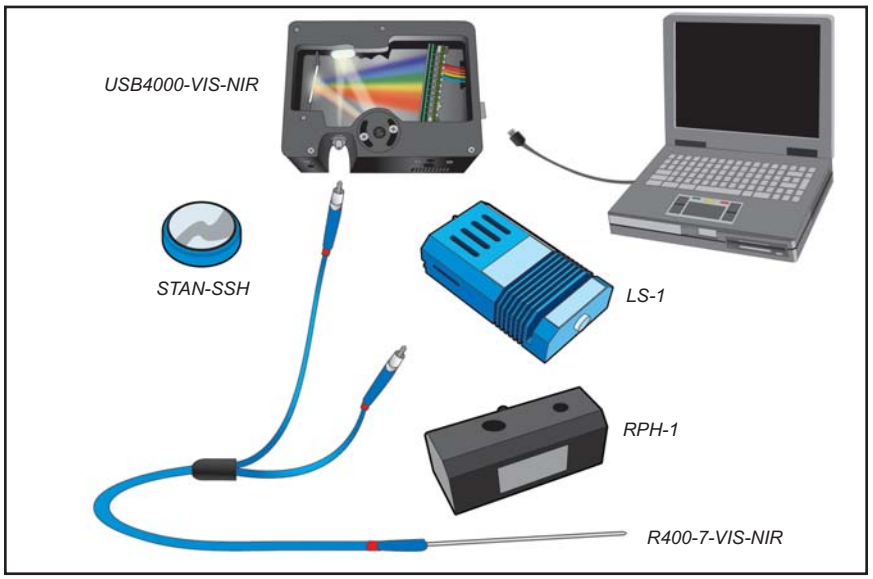
In testing, TDS used an SD2000 Dual-channel Spectrometer. Reflection measurements were performed with an R-series Reflection Probe. As TDS reports on its website, results indicated that multi-wavelength DRMs would be able to determine film thicknesses at discrete time intervals, to monitor photoresist phenomena that are difficult to separate with traditional DRMs, and to provide additional value to the researcher "by eliminating the need for discrete, static optical thickness measurement tools."



Today, TDS offers 1-, 2-, 4- and 8-channel configurations. TDS just recently announced the commercial release of its L-Series DRM product line for photoresist R&D, formulation studies, photoresist manufacturing QC, and polymer resin manufacturing QC.

The L-series line includes multiwavelength and multilayer analysis algorithms, which enable discrete thickness measurements to zero film thickness and provide accurate data of non-linear dissolution rate phenomena. For more details, visit www.thicknessdetection.com.

Setup: Metrology



Overview

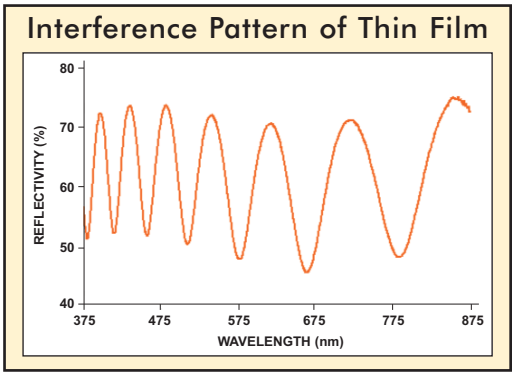
A thin film on a substrate can act as an etalon, creating an interference pattern superimposed on the surface reflectivity when viewed in reflection. The spacing of the pattern's sinusoidal peaks, when combined with the refraction index of the material, can be used to calculate the thickness of the material.

Spectrometer

The USB4000-VIS-NIR (350-1000 nm) is ideal for reflectometry of thin films. The spectrometer is preconfigured with Grating #3, which is blazed at 500 nm; an OFLV-350-1000 Filter to eliminate second- and third-order effects; and a 25 μm slit for optical resolution of ~1.5 nm (FWHM).

Sampling Optics

The R400-7-VIS/NIR Reflection Probe positioned at 90° measures specular reflectance from surfaces such as thin films. An LS-1 Tungsten Halogen Lamp and a STAN-SSH High-reflectivity Specular Reflectance Standard complete the sampling setup.

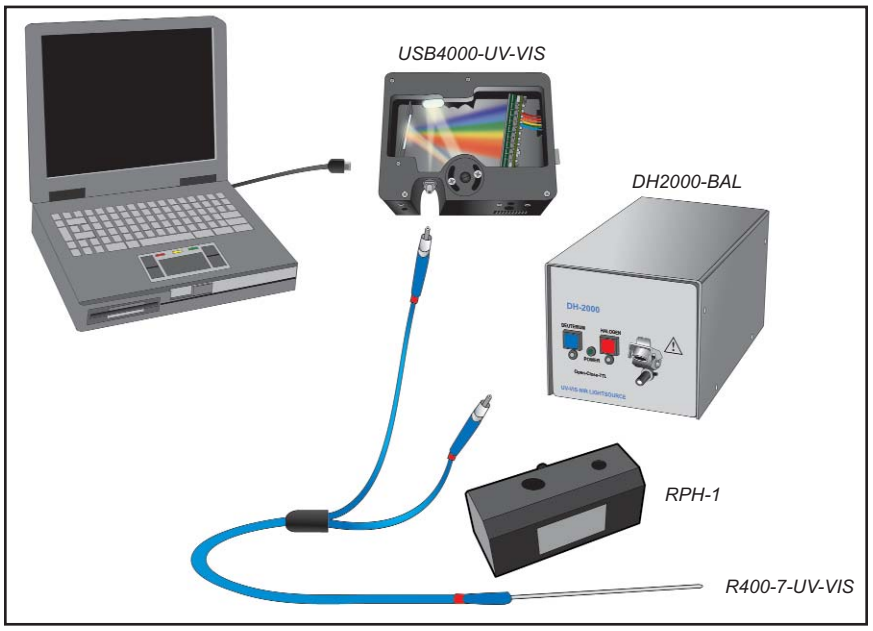


Measurements

Spectra observed in our operating software (see above) reveal oscillations caused by optical interference within the layers of the thin film substrate. Analysis of the wavelength position of the minima or maxima can determine either the thin film's thickness (with the known refractive index of the film) or its refractive index (with the known film thickness). Keep in mind that the thickness of samples may not be uniform; we recommend measuring several locations on the film.

Components	Page	Price
1. USB4000-VIS-NIR General-purpose Spectrometer	34	$2,499
Grating #3, 600 lines per mm, blazed at 500 nm	16	included
25 μm Slit as entrance aperture	15	included
DET4-350-1000 Detector with OFLV-350-1000 Order-sorting Filter	17	included
2. LS-1 Tungsten Halogen Light Source	128	$499
3. R400-7-VIS-NIR Reflection/Backscattering Probe	148	$499
4. RPH-1 Reflection Probe Holder	157	$75
5. STAN-SSH High-reflectivity Specular Reflectance Standard	108	$499
	Total:	**$4,071**

Setup: UV-VIS Reflection



Overview

Diffuse reflection measurements can be used to determine information about the chemical content or color (see page 177) of a sample.

Spectrometer

The USB4000-UV-VIS (200-850 nm) is ideal for most UV-VIS reflectometry. The spectrometer is preconfigured with Grating #1, which is efficient in the deep UV; an OFLV-200-850 Order-sorting Filter to eliminate second- and third-order effects; and a 25 µm slit for optical resolution of ~1.5 nm (FWHM).

Sampling Optics

The R400-7-UV-VIS Reflection Probe measures diffuse or specular reflectance from surfaces, or backscattering from translucent materials and fluids. The RPH-1 Probe Holder positions the R400-7 at either 45° for diffuse reflection or 90° for specular reflection. (For reflection measurements with an integrating sphere, see page 106.) For illumination, we recommend the DH2000-BAL Deuterium Tungsten Halogen Light Source. If your application requires portability, use the smaller DT-MINI-2 Deuterium Tungsten Halogen Light Source. (Because the DT-MINI-2 is a low-power source, configure your spectrometer with a 50 µm Slit and an L4 Detector Collection Lens.)

Measurements

Reflectance standards include the WS-1 Diffuse Reflectance Standard (page 107) for diffuse measurements and the STAN-SSH Specular Reflectance Standard (page 108) for specular measurements. Use our software to correct data for deviations from 100% reflectivity of standards, field tiles or NIST-traceable materials.

Components	Page	Price
1. USB4000-UV-VIS General Lab Spectrometer	34	$2,649
Grating #1, 200-850 nm range	16	included
25 µm Slit as entrance aperture	15	included
DET4-200-850 Detector with OFLV-200-850 Order-sorting Filter	17	included
2. DH2000-BAL Deuterium Tungsten Halogen Light Source	122	$3,588
3. R400-7-UV-VIS Reflection Probe	148	$499
4. RPH-1 Reflection Probe Holder	157	$75
5. SpectraSuite Spectroscopy Operating Software	80	$199
6. ASP Annual Service Package	62	$250
	Total:	**$7,260**

Plants and Reflectance

Spectral reflectance measurements of fruits, vegetables and other plants have long been performed using Ocean Optics spectrometers, light sources and fiber optic probes, with applications in the lab and in the field.

For example, researchers at the University of Arkansas at Little Rock have measured spectral reflectance of rice seedlings (pictured) in relation to soil salinity and to the chlorophyll content of individual rice leaves -- two factors related to rice yield. The experiment setup included an S2000 Spectrometer, LS-1 Tungsten Halogen Light Source and R-series Fiber Optic Reflection Probe.

One of our favorite plant applications is a high school science fair-winning project covering similar territory. Then-student Naomi Levine used one of our old S1000 Spectrometers, a tungsten halogen source, and a fiber optic probe to measure the reflection at 90° of philodendron plant leaves. Naomi believed that correlating reflectance to fertilization levels could be useful in detecting over-fertilization in crops.

What Naomi discovered was that plant reflectance at wavelengths >700 nm was insensitive to the stress of over-fertilization (samples were fertilized at 4x the recommended amount), while the peak within the 530-630 nm range was noticeably sensitive to stress (manifest as increased leaf reflection). She concluded that the latter related to a decrease in chlorophyll and to the effects of osmosis. Osmosis caused water to collect between the cell membrane and cell wall and exposed more of the leaf surface, thus increasing reflectance.

As for Naomi, she graduated from Princeton University in 2003.

Nice Asp!

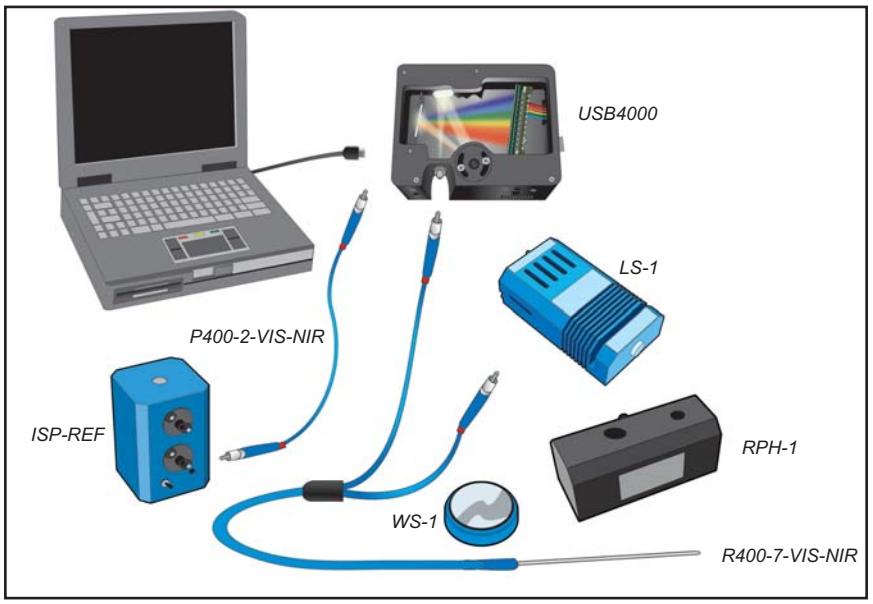
No, it's not an asp, but we couldn't resist. Dr. Ted Rohr -- a wildlife biologist and lecturer at RMIT University in Melbourne, Australia -- is actually holding an Australian Copperhead, which is one of the most venomous snakes in the world.

The Australian Copperhead is a front-fanged snake restricted to the cooler parts of Southeastern Australia. It preys on frogs, lizards, snakes and small mammals.

Rohr is studying the capacity of these snakes to undergo rapid color change -- from several shades of brown or green to black -- on the dorsal surface. Using a USB2000 Spectrometer and a fiber optic probe with a custom shield on its end (the shield helps to maintain a fixed distance to the sample point of interest), Rohr measured the reflectance of individual snake scales, both in the field and in the laboratory.

According to Rohr, the snake's ability to change body color makes sense in a cool-temperate environment, where thermal conditions can change many times during the season and even throughout the day. Changing colors is a perfect mechanism for adapting to fluctuations in temperature. However, body coloration is also important for camouflage. Being black may be great in order to absorb solar radiation, but it makes the snake more obvious to birds of prey -- and wary researchers!

Setup: Reflected Color



Overview

Color measurement involves determining the reflection spectrum of a sample and applying it to a standard illuminant. The amount of light energy the sample reflects is manipulated and reduced to tristimulus values X, Y and Z. These values correspond to the physiological response of the three types of color receptors in the human eye. X, Y and Z values are combined into uniform colorspace values such as L*, a* and b*.

Spectrometer

A USB4000 with a 25 μm Slit and Grating #2 (350-1000 nm) works well for color analysis. For those using an integrating sphere as the sampling optic, we recommend an L4 Detector Collection Lens to improve sensitivity.

Sampling Optics

When taking reflective-color measurements, your data depends on sampling geometry. The R400-7-VIS-NIR Reflection Probe provides illumination and detection from the same direction. If you use the probe at a 45°, it measures diffuse reflection. If you use the probe at a 90°, it measures specular reflection. The distance from the probe to the surface determines the sample size. An alternative is the ISP-REF Integrating Sphere, which provides 180° illumination and detection from flat surfaces for measuring specular and diffuse reflection.

Measurements

Reflectivity is measured against a reference standard such as the WS-1 Diffuse Reflectance Standard. SpectraSuite Spectroscopy Operating Software calculates a variety of colorspace values from the reflection spectra.

Components for Color Measurements	Page	Price
1. USB4000 Plug-and-Play Spectrometer	14	$2,199
Grating #2, 350-1000 nm range	16	included
25 µm Slit as entrance aperture	15	$150
L4 Detector Collection Lens	17	$150
DET-4-350-1000 detector with OFLV Order-sorting Filter	17	$150
2. WS-1 Diffuse Reflectance Standard	107	$299
3. SpectraSuite Spectroscopy Operating Software	80	$199
4. LS-1 Tungsten Halogen Light Source	128	$499
5. R400-7-VIS-NIR Reflection Probe	149	$499
6. RPH-1 Reflection Probe Holder	157	$75
7. ASP Annual Service Package	62	$250
	Total:	**$4,470**

Spectral Identity

It's not uncommon for our customers to be unfamiliar with the absorbing or emitting wavelength or wavelength range of their analytes. In the next few pages, we've provided absorbance and emission data for many analytes. Our Applications Scientists are another good resource for this information -- after all, we've configured nearly than 85,000 spectrometers -- as are Internet searches and commercial ventures specializing in spectral data.

Absorption Wavelength Bands for Chromophores

Chromophore	System	Max. Absorption in nm	Absorb. Intensity
Acetylide	—C≡C—	175-180	6 000
Aldehyde	—CHO	210	strong
		280-300	11-18
Amine	—NH_2	195	2 800
Azido	>C=N—	190	5 000
Azo	—N=N—	285-400	3-25
Bromide	—Br	208	300
Carbonyl	>C=O	195	1 000
		270-285	18-30
Carboxyl	—COOH	200-210	50-70
Disulfide	—S—S—	194	5 500
		255	400
Ester	—COOR	205	50
Ether	—O—	185	1 000
Ethylene	—C=C—	190	8 000
Iodine	—I	260	400
Nitrate	—ONO_2	270 (shoulder)	12
Nitrile	—C≡N	160	—
Nitrite	—ONO	220-230	1 000-2 000
		300-400	10
Nitro	—NO_2	210	strong
Nitroso	—NO	302	100
Oxime	—NOH	190	5 000
Sulfone	—SO_2—	180	—
Sulfoxide	>S=O	210	1 500
Thiocarbonyl	>C=S	205	strong
Thioether	—S—	194	4 600
		215	1 600
Thiol	—SH	195	1 400
	—$(C=C)_2$— (acrylic)	210-230	21 000
	—$(C=C)_3$—	260	35 000
	—$(C=C)_4$—	300	52 000
	—$(C=C)_5$—	330	118 000
	—$(C=C)_2$— (alicyclic)	230-260	3 000-8 000
	C=C—C≡ C	219	6 500
	C=C—C=N	220	23 000
	C=C—C=O	210-250	10 000-20 000
		300-350	weak
	C=C—NO_2	229	9 500
Benzene		184	46 700
		204	6 900
		255	170
Diphenyl		246	20 000
Naphthalene		222	112 000
		275	5 600
		312	175
Anthracene		252	199 000
		375	7 900

Beer's Law

Beer-Lambert Law, more commonly known as Beer's Law, states that the optical absorbance of a chromophore in a transparent solvent varies linearly with both the sample cell pathlength and the chromophore concentration. Beer's Law is the simple solution to the more general description of Maxwell's far field equations describing the interaction of light with matter. In practice Beer's Law is accurate enough for a range of chromophores, solvents and concentrations, and is a widely used relationship in quantitative spectroscopy.

Absorbance is measured in a spectrophotometer by passing a collimated beam of light at wavelength λ through a plane parallel slab of material that is normal to the beam. For liquids, the sample is held in an optically flat, transparent container called a cuvette. Absorbance (A_λ) is calculated from the ratio of light energy incident passing through the sample (I_0) to the energy that is incident on the sample (I):

$$A_\lambda = -\log (I/I_0)$$

Beer's Law follows:

$$A_\lambda = \varepsilon_\lambda bc$$

- ε_λ = molar absorptivity or extinction coefficient of the chromophore at wavelength λ (the optical density of a 1-cm thick sample of a 1 M solution). ε_λ is a property of the material and the solvent.
- b = sample pathlength in centimeters
- c = concentration of the compound in the sample, in molarity (mol L^{-1})

In an absorbance experiment, light is attenuated not only by the chromophore, but also by reflections from the interface between air and the sample, the sample and the cuvette, and absorbance by the solvent. These factors can be quantified separately, but are often removed by defining I_0 as the light passing through a sample "blank" or "baseline" or reference sample (for example, a cuvette filled with solvent but zero concentration of the chromophore is used as the blank).

Many factors can affect the validity of Beer's Law. It is usual to check for the linearity of Beer's Law for a chromophore by measuring the absorbance of a series of standards. This "calibration" can also remove errors in the experiment, the equipment and the batch of reagents (such as cuvettes of unknown pathlength).

Spectral Identity

Determining Optical Resolution

The optical resolution, measured as Full Width Half Maximum (FWHM), of our spectrometers depends on the groove density of the grating and the width of the entrance aperture (slit width or fiber diameter).

In selecting these components, consider two trade-offs. First, the optical resolution improves as the groove density of the grating increases, but at the expense of spectral range and signal strength. Second, the resolution improves as the slit width or diameter of the fiber decreases, but at the expense of signal strength. The formula for calculating the optical resolution follows:

Step 1
Choose a Grating from the Grating Selection Chart. See the table below to locate the page for the grating choices for your spectrometer. Note the value in the Spectral Range column in the chart. Check the number of pixel elements in the spectrometer's detector. Divide the Grating's Spectral Range by the total number of Detector Elements in the detector. This is your Dispersion.

Step 2
Choose a Slit. See the table below to find the page on slit choices for your spectrometer. Note the value in the Pixel Resolution column in the slit chart. Multiply the Dispersion (nm/pixel value from Step 1) x Pixel Resolution of your entrance aperture. This is your Optical Resolution (in nm).

Example
Here is an example of how to calculate optical resolution of a USB4000 Spectrometer using Grating #3 and a 10- μm slit. With this data, you can obtain the approximate optical resolution.

Step 1 650 nm ÷ 3648 = 0.178
Step 2 0.178 x 5.7 = 1.015 nm
FWHM = ~1.02 nm

Finding Your Values

Spectrometer	Grating Spectral Range	Entrance Aperture Pixel Resolution
USB2000:	website	website
USB4000:	page 16	page 15
HR2000:	website	website
HR2000+:	page 23	page 22
HR4000:	page 23	page 22
QE65000:	page 28	page 27
NIR-512:	pages 32	page 32
NIR256-2.1:	pages 32	page 32
NIR256-2.5:	pages 32	page 32

Absorption Wavelength Bands for Chromophores

Chromophore	Max. Absorption in nm	Absorb. Intensity
Phenanthrene	251	66 000
	292	14 000
Naphthacene	272	180 000
	473	12 500
Pentacene	310	300 000
	585	12 000
Pyridine	174	80 000
	195	6 000
	257	1 700
Quinoline	227	37 000
	270	3 600
	314	2 750
Isoquinoline	218	80 000
	266	4 000
	317	3 500

Absorption Wavelength Cutoffs for Solvents*

Solvent	Wavelength
Acetic Acid	260
Acetone	330
Acetonitrile	190
Benzene	280
1-Butanol	210
2-Butanol	260
Butyl acetate	254
Carbon disulfide	380
Carbon tetrachloride	265
1-Chlorobutane	220
Chloroform (stabilized with ethanol)	245
Cyclohexane	210
1,2-Dichloroethane	226
Diethyl ether	218
1,2-Dimethoxyethane	240
N,N-Dimethylacetamide	268
N,N-Dimethylformamide	270
Dimethylsulfoxide	265
1,4-Dioxane	215
Ethanol	210
2-Ethoxyethanol	210
Ethyl acetate	255
Ethylene chloride	228
Glycerol	207
Heptane	197

Solvent	Wavelength
Hexadecane	200
Hexane	210
Isobutyl alcohol	230
Methanol	210
2-Methoxyethanol	210
Methylcyclohexane	210
Methylene chloride	235
Methyl ethyl ketone	330
Methyl isobutyl ketone	335
2-Methyl-1-propanol	230
N-Methylpyrrolidone	285
Nitromethane	380
Pentane	210
Pentyl acetate	212
1-Propanol	210
2-Propanol	210
Pyridine	330
Tetrachloroethylene (stabilized with thymol)	290
Tetrahydrofuran	220
Toluene	286
1,1,2-Trichloro-1,2,2-trifluoroethane	231
2,2,4-Trimethylpentane	215
o-Xylene	290
Water	191

** Solvents are transparent at wavelengths greater than the stated cutoff.*

Spectral Identity

Absorption/Emission for Fluorophores

Fluorophore	Absorption in nm	Emission in nm
1,5 IAEDANS	336	490
4-Methylumbelliferone	385	502
5-Carboxynapthofluorescein (pH 10)	512/598	563/668
5-Carboxytetramethylrhodamine (5-TAMRA)	542	568
6-Carboxyrhodamine 6G	518	543
6-CR 6G	518	543
6-JOE	520	548
7-Amino-4-Methylcoumarin	351	430
7-Aminoactinomycin D (7-AAD)	546	647
7-Hydroxy-4-methylcoumarin	360	449,455
Acridine Orange +DNA	502	526
Alexa Fluor 350™	346 342	442 441
Alexa Fluor 430™	431	540
Alexa Fluor 488™	495,492	519,520
Alexa Fluor 532™	531,532	553,554
Alexa Fluor 546™	556,557	572,573
Alexa Fluor 568™	577,578	603
Alexa Fluor 594™	590,594	617,618
Alexa Fluor 633™	632	650
Alexa Fluor 647™	647	666
Alexa Fluor 660™	668	698
Alexa Fluor 680™	679	702
Allophycocyanin (APC)	630,645	655,660
AMCA (Aminomethylcoumarin)	345 347	425 444
AMCA-X	353	442
ATTO-TAG™ FQ	486	591
BCECF (high pH)	492,503	520,528
BCECF (low pH)	482	520
Bodipy 505/515	502	510
Bodipy 558/568	558	569
Bodipy 564/570	564	570
Bodipy 576/589	579	590
Bodipy 581/591	584	592
Bodipy 630/650-X	625	642
Bodipy 650/665-X	647	665
Bodipy 665/676	605	676
Bodipy FI	504,505	511,513
Bodipy TMR	542	574
Bodipy TR	589	617
Calcein	494	517
Calcein Blue	373	440
Calcium Crimson™	588,589	611,615
Calcium Green	501,506	531
Calcium Green-1 Ca2+ Dye	506	531
Calcium Orange	549	575
Calcofluor White	385,395,405	437,440,445
Cascade Blue™	377 398 399	420 423
CFP - Cyan Fluorescent Protein	430,433,436,(453)	474,475,476,(501)

Non-linearity & CCDs

All CCD detectors exhibit a non-linearity in their response to light; i.e., doubling the number of photons received during the sample interval results in slightly less than a doubling of the voltage output. The effects of non-linearity, if left uncorrected, will be slight but detectable errors in the calculation of normalized values (absorbance, transmission or irradiance).

The non-linearity is a consequence of the R-C circuit used to read out the electrons that are left on the CCD capacitor (the charge well). The effect is independent of light level, integration time and optics. It depends only on the charge in the charge well.

The pattern of non-linearity is different for the various detector models used in our spectrometers. The magnitude of the linearity varies from detector to detector, but fortunately it is the same for all pixels in the detector. This makes it possible to 1) measure the linearity, and 2) correct for the errors in software.

For example, the ILX511 has a maximum response at 2000 counts (half well capacity). It drops to ~94% at 4000 counts and near zero counts. We can establish this curve precisely using an automated program that varies the integration time to precisely control the amount of light being sampled. This program (OOINLCorrect) is available for free download at our website at OceanOptics.com/Technical/Software Downloads.asp.

The linearity is captured from the experiments as a plot of normalized counts/sec versus counts for a constant light source observed at a series of integration times. The data is fit to a 7th order polynomial. The inverse of this function is stored in the software and/or on the EEPROM. When the linearity correction feature is turned on, all spectra are multiplied by the stored coefficients. Uncorrected spectra are linear to ~92%. Corrected spectra are linear to >99.8%.

Collimating Lenses

The 74-UV and 74-VIS Collimating Lenses screw onto the end of SMA 905-terminated fibers and other sampling optics to convert divergent beams of radiation (light) into a parallel beam. The optical fibers we sell have a field of view (FOV) of ~25° -- an acceptance angle that may not be appropriate for some experiments. Collimating lenses are adjustable, providing FOV angles from collimation (near 0°) to ~45°. Without the collimating lenses, the light would disperse more than is required for efficient transmission and collection of the signal.

Focus the Lamp's Collimating Lens

In order to obtain accurate data, the light entering and exiting a sample by means of a fiber/collimating lens assembly must be well collimated. Here are instructions for adjusting the focus of the collimators in a typical spectrometer setup.

1. Connect to the light source the fiber that you're going to use as the illumination fiber in your setup. The female SMA 905 Connector of the fiber must be screwed all the way into the male connector of the lamp.
2. Turn on the lamp and inspect the beam emitted from the other end of the fiber by pointing the fiber at a white piece of paper. The distance is not too critical but should be at least 3 inches from the surface.
3. Loosen the setscrew on the fiber barrel of the light source with an Allen wrench.
4. Slide the inner barrel of the collimating lens until you see an even intensity across the beam spot. The spot should be uniform in intensity and color.
5. Once the inner barrel is positioned so that a well-focused, uniform spot is obtained, tighten the setscrew. Don't put down the fiber and then tighten the setscrew as you may lose the focus.

Focus the Next Collimating Lens

6. The illumination fiber is still connected to the lamp and the lamp is on. Take the second collimating lens in your setup (removed from a cuvette holder, for example) and screw it securely onto the other end of the fiber. Point this end of the fiber at least 2 meters from a wall.
7. Repeat Steps 3, 4 and 5. Then remove the lens from the end of the fiber and install it back into your setup (back into a cuvette holder, for example).
8. Continue to adjust the focus of the other collimating lenses in your setup.

Absorption/Emission for Fluorophores (continued)

Fluorophore	Absorption in nm	Emission in nm
CL-NERF (Ratio Dye, pH)	504/514	540
Cy2™	489	506
Cy3.5™	581	598
Cy3™	514	566
Cy5.5™	675	695
Cy5™	649	666
Cy7™	710,743	767,805
Dabcyl	453	
Dansyl Cadaverine	335	518
DAPI	359	461
Di-4-ANEPPS	496	705
Di-8-ANEPPS (non-ratio)	488	605
	498	713
DiA (4-Di-16-ASP)	456	591
DIDS	341	415
DiI (DiIC18(3))	549,551	565
Dinitrolphenol	349	
DiO (DiOC18(3))	484,487	501,502
DM-NERF (Ratio Dye, high pH)	497/510	540
ELF 97	345	530
Eosin	524	545
Erythrosin	529,532	554,555
Ethidium Bromide	510,523	595,605
Ethidium homodimer -1 (EthD-1)	528	617
Europium (III) chloride	337	613
Fast Blue	360	440
Fluo-3	480-506,506	520,527
Fluo-4	494	516
Fluorescein (FITC)	490,494	520,525
Fluoro-Gold (Hydroxystilbamidine)	361	536
FluorX	494	520
FM 1-43™	479	598
Fura Red™ (high pH)	572	657
Fura-2, high calcium (Excitation ratio dye)	335	505
Fura-2, low calcium (Excitation ratio dye)	363	512
GFP (S65T)	498	516
Hoechst 33258	345	487
Hoechst 33342	347	483
JC-1	514	529
JO-JO-1	530	545
JO-PRO-1	532	544
Lucifer Yellow	425,428	528,536,540
Lyso Tracker Green	504,534	511,551
Mag-Fura-2 (Ratio Dye, Ca2+)	369/329	508
Mag-Fura-2 (Ratio Dye Mg2+)	369/330	511/491
Mag-Fura-5 (Ratio Dye, Ca2+)	369/330	505/500
Mag-Fura-5 (Ratio Dye, Mg2+)	369/332	505/482
Magnesium Green	506,507	531
Marina Blue	362	459
Mitotracker Green FM	490	516

Spectral Identity

Absorption/Emission for Fluorophores (continued)

Fluorophore	Absorption in nm	Emission in nm
Mitotracker Orange	551	576
NBD	466	539
Nile Red	515-555,559	590,640
Oregon Green™	503	522
Oregon Green™ 488	490,493	514,520
Oregon Green™ 500	497	517
Oregon Green™ 514	506	526
PKH26 (Sigma)	551	567
POPO-3	533	574
PO-PRO-3	539	567
Propidium Iodid (PI)	(305), 536,538	617
Pyrene	360	387
QSY 7	560	591
Rhod-2	552	576
Rhodamine 110	496,497	520
Rhodamine 123	507	529
Rhodamine 6G	525	555
Rhodamine B	540	625
Rhodamine Green	502	527
Rhodamine Phalloidine	542	565
Rhodamine Red	570	590
R-phycoerythrin (PE)	565	578
SITS (Ion Channels)	336	436
SNAFL-1 (Ratio Dye, pH)	508/540	543/623
SNARF1 Excitation and emission ratio dye	576/548	635/587
Sodium Green Na+, K+	506,507	532
SpectrumGreen (Vysis)	497/30, 509/31	538/44,524/56
SpectrumOrange (Vysis)	559/38,560	588/48
SPQ (6-methoxy-N-(3-sulfopropyl)	344	443
SYTO 11Dye for DNA, RNA	508,510	527,530
SYTO 13Dye for DNA, RNA	488,491	509,514
SYTOX Green (Nucleic Acid Stain	504	523
SYTOX Orange (Nucleic Acid Stain	547	570
Tetramethylrhodamine (TRITC)	555	576
Texas Red™	595	620
TO-PRO-1	515	531
TOTO-1	514	531,533
YFP (Yellow Fluorescent Protein)	513,520	527,532
YO-PRO-1	491	506
YOYO-1	491	508,509

Phosphorescence & Fluorescence

Phosphorescence and fluorescence are closely related subcategories of luminescence. The difference between the two is in the nature of a material's ground and excited states.

In a singlet excited state, the higher-energy orbital electron spins opposite the lower-energy orbital. The two electrons are considered "paired." In a triplet state, the electrons are "unpaired," and spin in the same direction. A return to the ground state from a singlet excited state does not require one of the electrons to change its spin orientation; a return from a triplet state to the ground state does require an electron's spin orientation to change.

Fluorescence is the photonic emission that occurs when the higher-energy electron in a singlet state returns to the lower-orbit electron. The laws of quantum mechanics permit this rapid transition at a rate near 10^{-8} second.

The fluorescence lifetime is the average period of time that a fluorophore remains in the excited singlet state. By comparison, phosphorescence emission occurs as the electronically excited condition of a material in the triplet state returns to the singlet ground state. Again, the laws of quantum mechanics prevail, and the probability of this transition is lower. The lifetime of an excited triplet state is much longer than that of an excited singlet state, producing phosphorescence lifetimes that range from milliseconds to seconds.