overture

Software User's Guide
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Offices: **Ocean Optics, Inc. World Headquarters**
830 Douglas Ave., Dunedin, FL, USA 34698
Phone 727.733.2447
Fax 727.733.3962
8 a.m.– 8 p.m. (Mon-Thu), 8 a.m.– 6 p.m. (Fri) EST

E-mail:
Info@OceanOptics.com (General sales inquiries)
Orders@OceanOptics.com (Questions about orders)
TechSupport@OceanOptics.com (Technical support)

A HALMA GROUP COMPANY

Additional Offices:

Ocean Optics Asia

666 Gubei Road, Kirin Tower, Suite 601B, Changning District, Shanghai, PRC. 200336

Phone **86.21.5206.8686**
Fax **86.21.5206.8686**
E-Mail **Sun.Ling@OceanOptics.com**

Ocean Optics EMEA

Sales and Support Center
Geograaf 24, 6921 EW DUIVEN, The Netherlands

Phone **31-26-3190500**
Fax **31-26-3190505**
E-Mail Info@OceanOptics.eu

Regional Headquarters
Maybachstrasse 11
73760 Ostfildern
Phone **49-711 34 16 96-0**
Fax **49-711 34 16 96-85**
E-Mail **Sales@Mikropack.de**

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About This Manual

Document Purpose and Intended Audience

This document provides you with installation and operation instructions for your Overture software.

Document Summary

Chapter	Description
Chapter 1: *Introduction to Spectroscopy*	Provides an overview of spectroscopy and spectrometers.
Chapter 2: *Overture Software*	Lists installation procedures for Overture software.
Chapter 3: *Overture Software Icons and Menus*	Contains a list of the icons and menu items and their functions in Overture software.
Chapter 4: *Experiments*	Provides a variety of experiments to perform using Ocean Optics spectrometers and Overture software.
Chapter 5: *Applications*	Contains applications for Overture software.
Appendix A: *Sample Experiments*	Contains some possible experiments for Ocean Optics spectrometers and Overture software.
Appendix B: *Maintenance*	Provides suggested maintenance and a table of possible problems and suggested solutions.

Product-Related Documentation

You can access documentation for Ocean Optics products by visiting our website at http://www.oceanoptics.com. Select *Technical → Operating Instructions*, then choose the appropriate document from the available drop-down lists. Or, use the **Search by Model Number** field at the bottom of the web page.

You can also access operating instructions for Ocean Optics products on the *Software and Technical Resources* CD included with the system.

Engineering-level documentation is located on our website at *Technical → Engineering Docs*.

Upgrades

Occasionally, you may find that you need Ocean Optics to make a change or an upgrade to your system. To facilitate these changes, you must first contact Customer Support and obtain a Return Merchandise Authorization (RMA) number. Please contact Ocean Optics for specific instructions when returning a product.

Chapter 1

Introduction to Spectroscopy

Spectroscopy in a nutshell

A spectrometer is a device that breaks up light into different colors by spreading out, or dispersing different wavelengths. Rain does this by refraction of light, creating a rainbow that goes from violet through yellow and then red.

This spread of colors is called the visible spectrum. Humans can see light between 380nm (violet) and 780nm (deep red). Other creatures can see different ranges.

You can get the same effect by reflecting light with a CD. The very fine markings on a CD are so small that they are getting close to the wavelength of light and cause the diffraction of light into a spectrum. The CD surface is acting as a diffraction grating. In fact it is a reflecting diffraction grating.

If you want to find out how a motorcycle works, the best thing to do is take it apart to its individual pieces and see what it's made of. The same applies to light. If we can break it up using refraction or diffraction, we can see what is going on at each wavelength.

Note

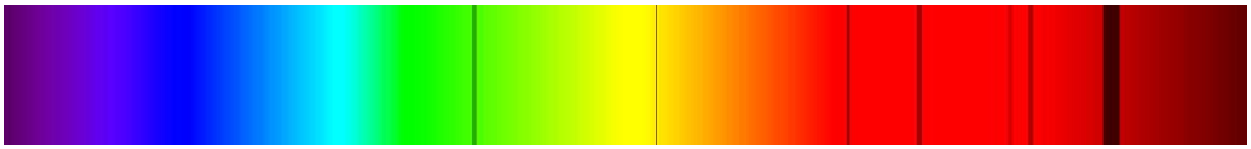
Try searching on the web for the words in blue.

Spectroscope views

Below are three spectra as seen through a traditional spectroscope.

This spectrum is of a tungsten filament lamp:

This is spectrum of sunlight. It is a similar continuous spectrum but it contains fine dark lines caused by absorption of certain wavelengths in the sun and earth atmospheres. These are called Fraunhofer lines.



This is an emission spectrum from a hydrogen gas discharge tube. It shows that the hydrogen gas only emits light at certain wavelengths. Excited hydrogen atoms emit a specific series of narrow spectral lines called the Balmer series.

The CCD array spectrometer

Looking at sunlight through the spectrometer will tell us a lot about how it works. If you have a pocket spectroscope you can compare the traditional spectroscope view with the Overture software display.

Sunlight enters the spectrometer through a 50 micron-wide slit. That is very narrow; 5/100ths of a millimeter.

In a conventional spectroscope you will see a spectrum and any absorbance lines will show as dark lines. These lines correspond to sharp dips in the Overture spectrum graph.

The light passes through an optical geometry of focusing mirrors and a reflection grating. The spectrum falls on a linear CCD array with 250 tiny sensors in a row so that each sensor (often called a pixel) in the array corresponds to one wavelength.

The number of photons hitting each pixel is converted to a voltage which is converted into a y-axis value on the graph. The x-axis is scaled to the pixel number, which indicates wavelength.

Optical Limitations

The Ocean Optics Spectrometers for Education can display peaks separated by less than 2nm, depending on the model. This is the limit of its resolution.

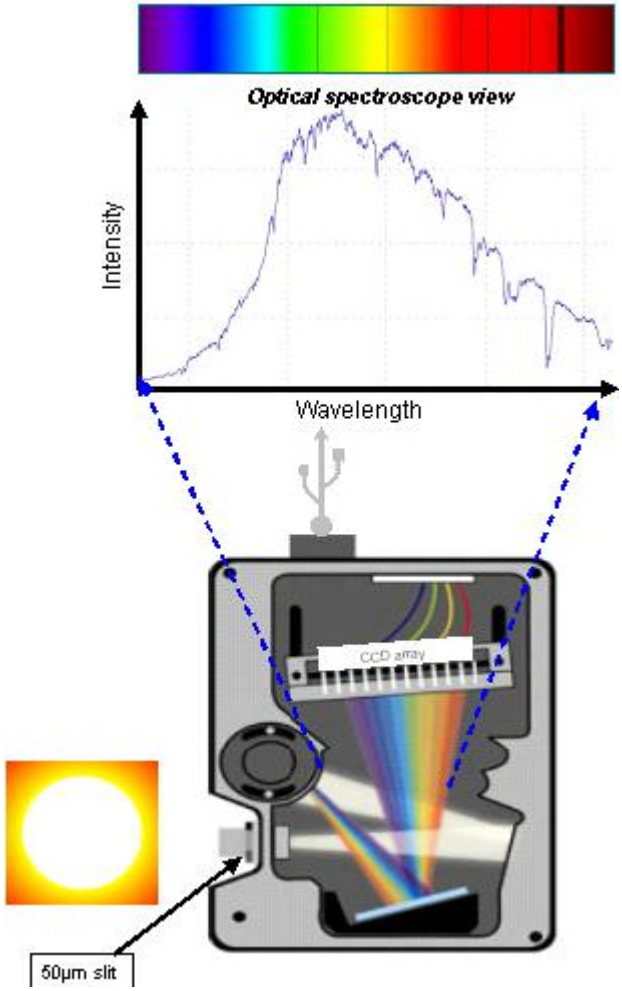
The spectrometer resolution is limited by a number of factors, including:

- Slit width
- Grating specification (lines per mm) and quality
- Number of pixels in the array
- Physical size of the system

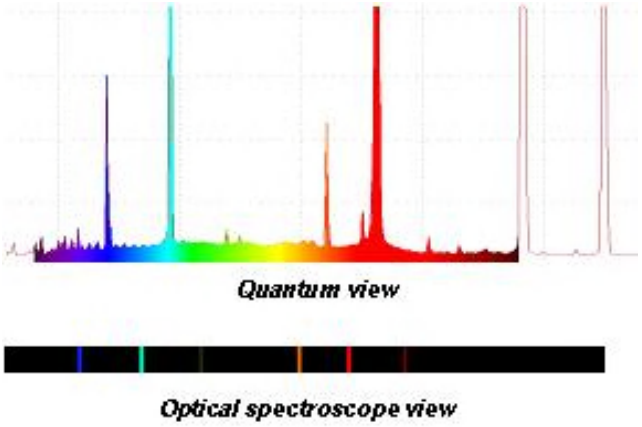
This causes an apparent spreading of emission and absorption lines in the Overture display so that they appear as sharp Gaussian peaks, but this is still remarkably high resolution for a compact instrument.

In chemistry applications this is not a problem as the absorption peaks are usually over a hundred nanometers wide. In fact you will deliberately “smooth” the spectrum by averaging the array output using Overture software.

For chemistry applications it is the sensitivity or dynamic range that is more important. This allows the spectrometer to detect small changes in absorption on the y-axis.



Here is what an emission spectrum looks like. This is from a Hydrogen lamp for observing Balmer lines:



Emission lines look like sharp peaks. It is possible to identify elements from their emission peaks. Notice that some peaks go beyond the visible range.

Chapter 2

Overture Software

Product Overview

Overture is a spectroscopy operating software platform for 32-bit and 64-bit Windows. The following operating systems are supported:

- Windows XP
- Vista
- Windows 7

This software can control the following Ocean Optics spectrometers:

- ADC1000-USB (including Deep Well)
- HR2000
- HR2000+
- HR4000
- Jaz
- Maya2000
- Maya2000Pro
- MMS-Raman
- NIR256
- NIR512
- NIRQuest
- QE65000
- STS
- Torus
- USB2000
- USB2000-FLG
- USB2000+
- USB4000

Overture Installation

Overture can be downloaded from the Ocean Optics Software Downloads site, or retrieved from the CD that you received with your purchase of an Ocean Optics spectrometer.

Retrieving from a CD

Your Overture software is shipped to you from Ocean Optics on a CD. The software is located either on the main **Software and Technical Resources** CD, or (in the case of the Windows 64-bit version) on a separate CD labeled **Overture Windows 64-bit Version**. You will need the password located on the jacket of the CD containing your SpectraSuite software to complete the installation.

► *Procedure*

1. Insert the CD containing your Overture software into your computer.
2. Select the Overture software for your computer's operating platform via the CD interface. Then follow the prompts in the installation wizard.

   Or,

   Browse to the appropriate Overture set-up file for your computer and double-click it to start the software installation. Set-up files are as follows:

   - Windows 32-bit: Overture-windows-x86-1.0.1-installer.exe
   - Windows 64-bit: Overture-windows-x64-1.0.1-installer.exe
3. Save the software to the desired location. The default installation directory is **\Program Files\Ocean Optics\Overture**. The installer wizard guides you through the installation process.

Downloading from the Ocean Optics Website

► *Procedure*

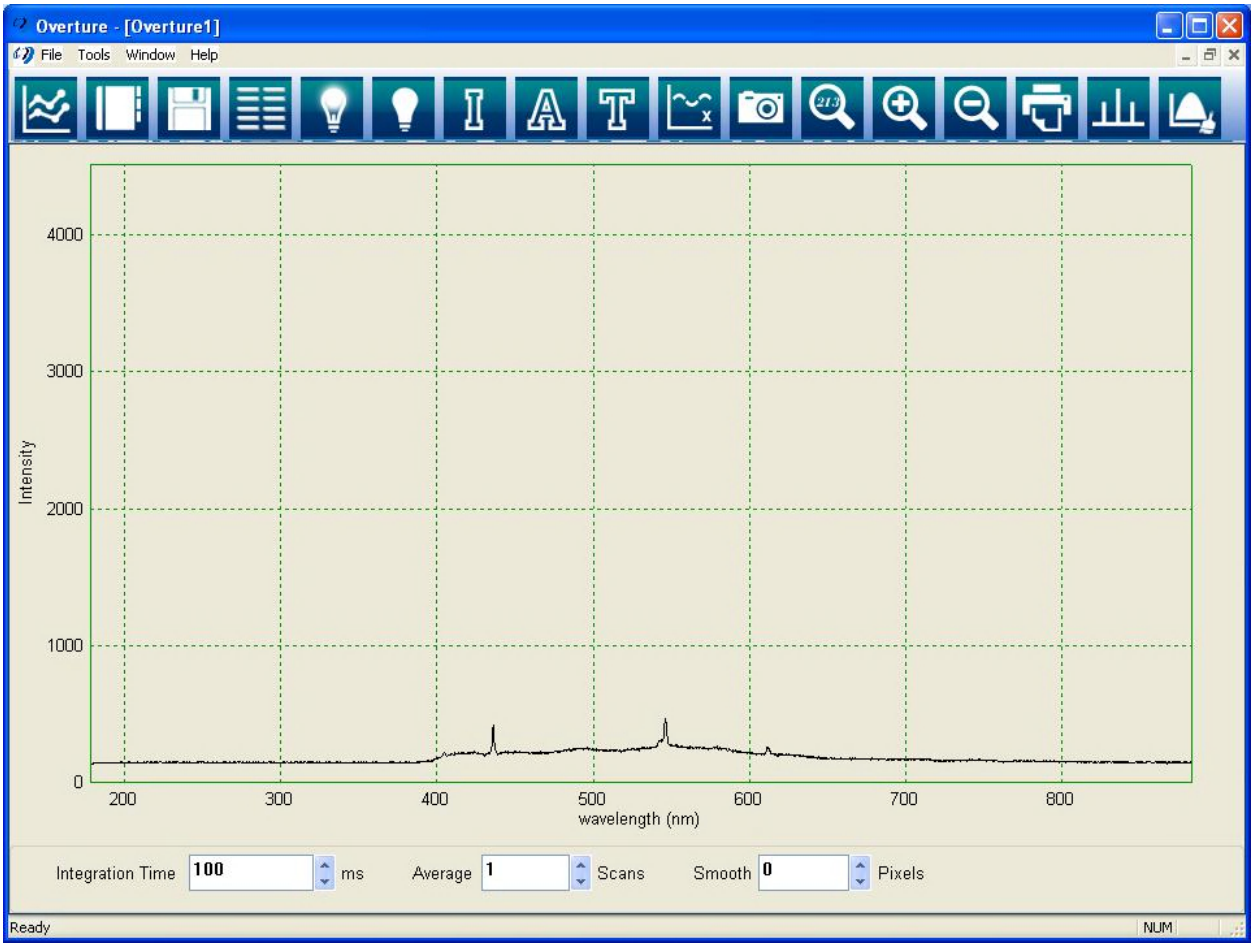
1. Close all other applications running on the computer.
2. Start Internet Explorer.
2. Browse to the Software Downloads page on the Ocean Optics website at http://www.oceanoptics.com/technical/softwaredownloads.asp.
3. Click on the Overture software appropriate for your Windows operating system.
4. Save the software to the desired location. The default installation directory is **\Program Files\Ocean Optics\Overture**. The installer wizard guides you through the installation process.

Chapter 3

Overture Software Icons and Menus

Overview

The following table provides a quick guide to the Overture Software icons. See the paragraphs following the table for information on how to use these icons when taking a measurement.



Overture Software Icons

Icon	Function
	New Graph
	Open Spectrum
	Save Spectrum
	Copy Data to Clipboard
	Store Reference Spectrum
	Store Dark Spectrum
	Intensity
	Absorbance
	Transmission
	Concentration
	Snapshot
	Wavelength Range
	Zoom In
	Zoom Out
	Print
	Reference Lines
	Color

Integration Time

Integration time is the exposure time for each pixel in the array. Each of the pixels is "read" in turn and the time between readings controls the amount of charge in each CCD sensor. The charge decreases with every photon that hits the sensor, so if there are not many photons around it takes a long time to reduce the charge. Too many photons will discharge the sensor completely in a short time.

If the time is too long, the sensors will "saturate" and the spectrum line will go off scale. This does not harm the sensor, but the data you collect will have no value.

For very dim sources a longer integration time is needed, but the penalty is more noise for less signal. The default integration time is 100 ms.

The integration time is set in the Integration Time filed in the status bar at the bottom of the screen.

Intensity 

Intensity is the default mode.

The y-axis reads Intensity, which is a count of how many photons have hit each pixel in the array during one integration time. It is a relative measurement.

Intensity mode is ideal for most physics-based applications using just the fiber optic input.

Store Dark Spectrum 

Before we can move on to any mathematical comparisons between sample spectra we have to tell the spectrometer where zero is. To do this, block any light entering the fiber and click the *Store dark spectrum* icon.

You will not see any change, but the Overture software now has a zero or "dark" reading stored for every wavelength.

Reference spectrum 

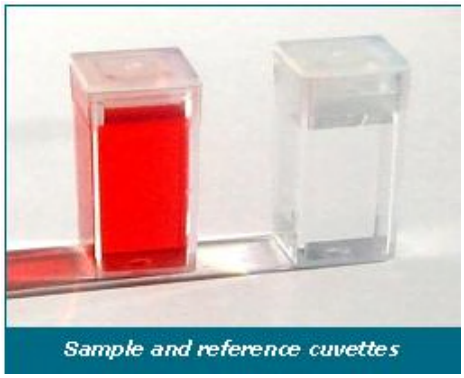
Next we have to tell the spectrometer about the source we are comparing to. This is the reference source. Typically this would be a cuvette containing a colorless solvent, but no dissolved sample.

Set up your reference sample so that the highest point on the intensity y-axis is about 85% of full scale.

Click the store *reference spectrum* icon. You will see no change, but your reference spectrum is now stored.

Be careful. If you change anything about your reference source now, you must store a new reference. You can click the icon to update the reference as many times as you like. If you change the integration time you will need to store a new reference reading.

Once you have stored dark and reference spectra, the Transmission and Absorbance modes are enabled.


Sample and reference cuvettes

Color

Overture can fill the space under the spectrum with an artificial display of spectral color. The color display covers the visible range of 380nm to 780nm. Outside that range is UV and NIR (near infrared) and of course we cannot see colors there.

Transmission

Note

Transmission and Absorbance modes are only enabled when dark and reference readings have been stored.

Transmission is the amount of light transmitted through the sample as a percentage of the light transmitted through the reference. When you select Transmission mode, the y-axis units change to percentage.

$$\text{Transmission \%} = \left(\frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda} \right) \times 100$$

Where:
S_λ = Sample intensity at wavelength λ
D_λ = Dark intensity at wavelength λ
R_λ = Reference intensity at wavelength λ

So now you can see why you need dark D_λ and reference R_λ readings. If you don't have them, the math won't work!

Absorbance

Note

Transmission and Absorbance modes are only enabled when dark and reference readings have been stored.

Absorbance is the amount of light absorbed by the sample compared to the reference.

Absorbance is the inverse of transmission, but on a log scale. When you select Absorbance mode the y-axis changes to a log scale of Absorbance number. So an absorbance number of 1 is 10 times less light getting through than reference. An absorbance number of two is 100 times less.

$$\text{Absorbance} = -\log_{10}\left(\frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda}\right)$$

Absorbance is normally measured at the wavelength of maximum absorption, called “Lambda Max”, written $\lambda_{max.}$ The Absorbance scale goes up to 3. Above 3 the solution is so dark that is no longer possible to measure Absorbance reliably.

Smoothing

Average scans

This averages over a number of complete scans. For low level light sources this can improve the signal to noise ratio. For bright sources it is normally not required.

Pixel smoothing

This technique averages a group of adjacent detector elements. A value of 6, for example, averages each data point with 3 points to its left and 3 points to its right. This average rolls along the array.

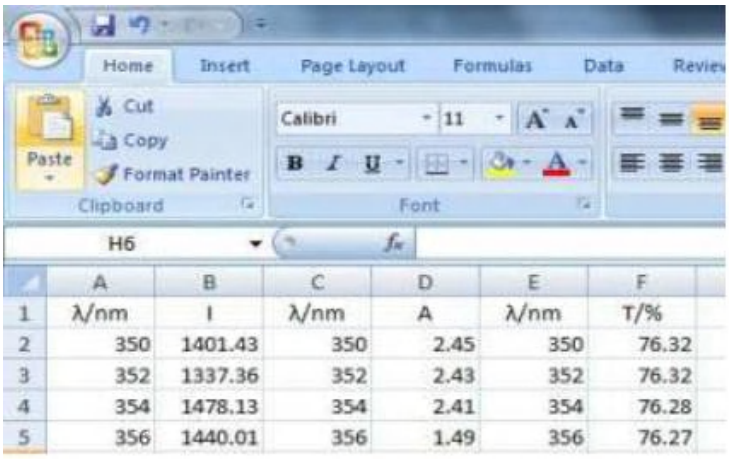
The greater this value, the smoother the data. For chemical absorbance experiments a default setting of 5 is set, but this can be changed.

For absorbance measurements, high resolution is not required, so smoothing makes the spectrum easier to see and can reduce errors. Setting the pixels to average above 5 is not normally necessary.

Copy Data to Clipboard

The Copy Data tool takes a snapshot of the live spectral data for export as a CSV file into Excel or any Microsoft Office application.

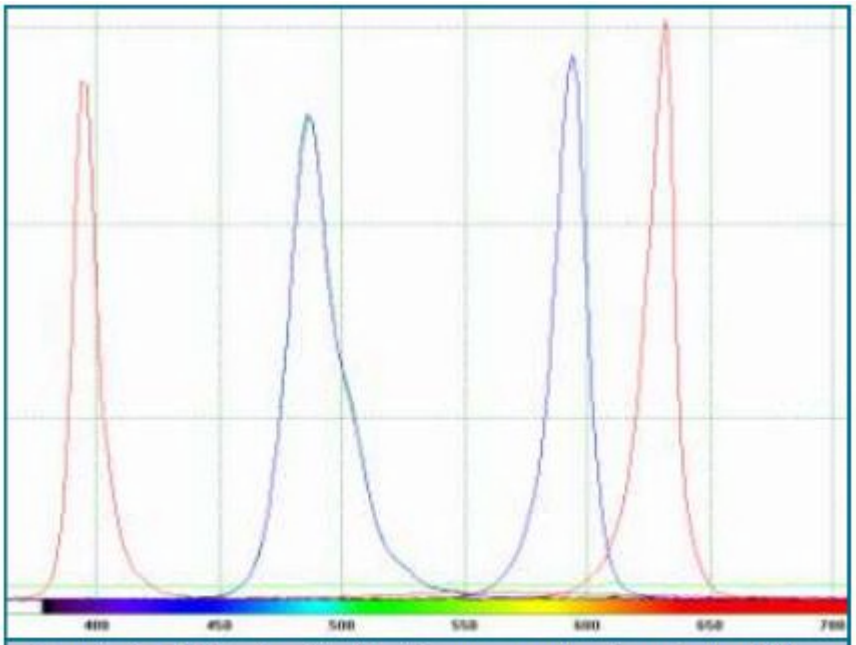
The data is exported into 2-headed columns in I, A an T modes. The first column is wavelength and the second column depends on the mode.



	A	B	C	D	E	F
1	λ/nm	I	λ/nm	A	λ/nm	T/%
2	350	1401.43	350	2.45	350	76.32
3	352	1337.36	352	2.43	352	76.32
4	354	1478.13	354	2.41	354	76.28
5	356	1440.01	356	1.49	356	76.27

Snapshot

Snapshot allows you to freeze as many spectral lines as you want to make comparisons. The frozen lines can be saved by printing, printing to file or print screen. They will not be saved as spectral data; only the live spectral line can be saved as a spectrum.



Snapshots of LEDs show the link between wavelength and color. With voltage data they can be used to derive Planck's constant

Zoom   

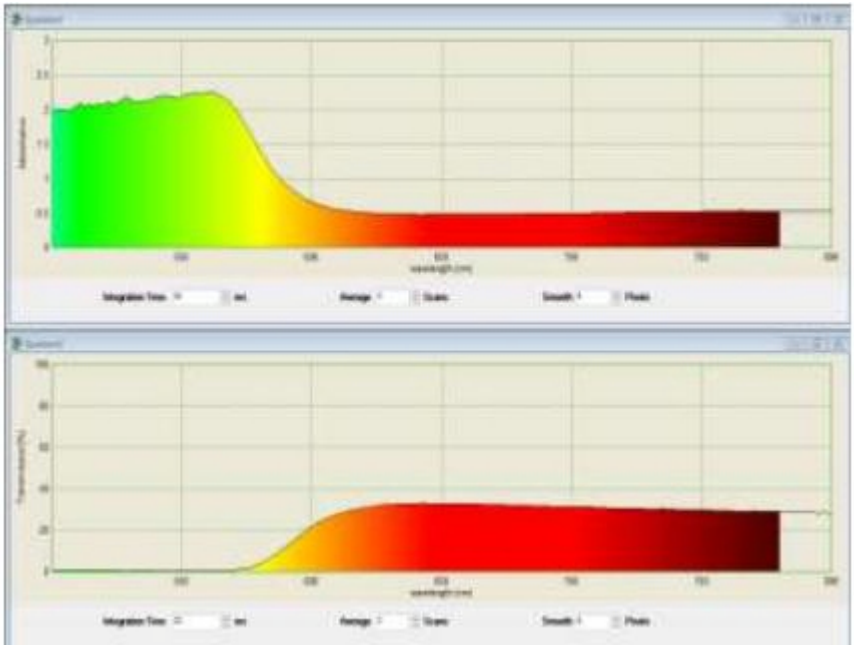
 Zoom In lifts the highest peak to the full y axis scale.

 Zoom Out cancels this.

 Wavelength Range allows you to set both the x and y ranges you want to display. Numerical zoom can be used in all windows. It is useful to set the wavelength minimum to 400 in Absorbance and Transmission modes to eliminate the noisy signal that can often be seen at the short wavelength end of the scale.

Open Spectrum 

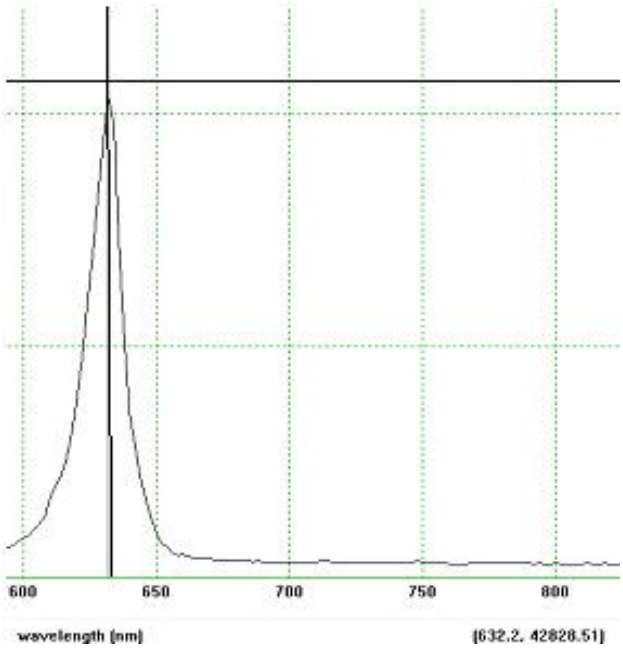
You can open up to two graphs at the same time and to show two different views, for example, Absorbance and Transmission at the same time as shown below.



Cursor

Click anywhere on the graph to launch the cross hairs cursor. The cursor x,y coordinates appear in the status bar at the bottom right of the screen.

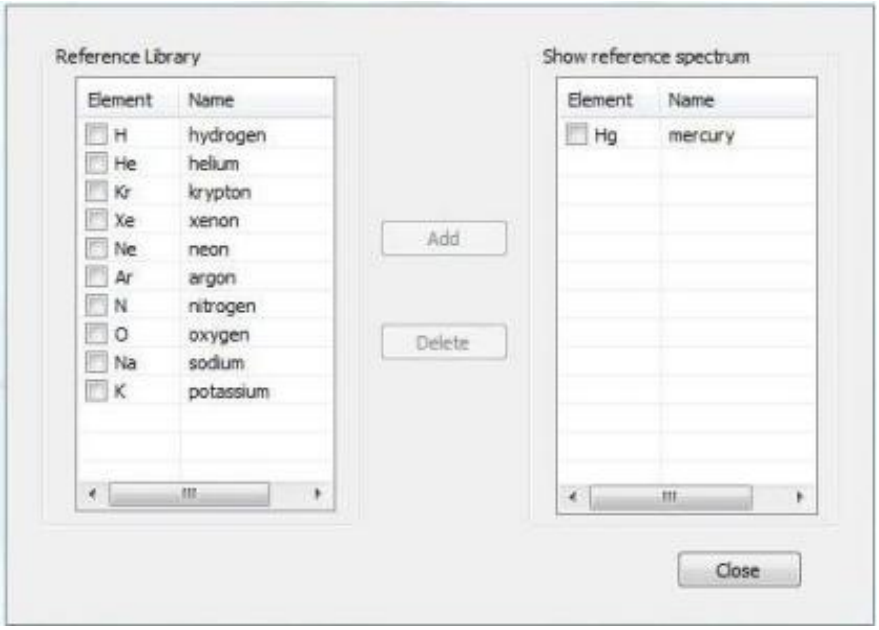
Press **ESC** to remove the cursor.



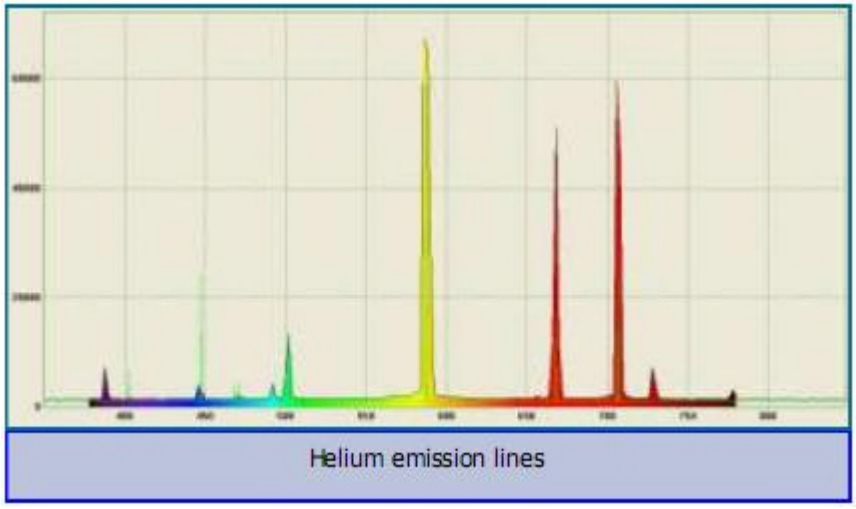
Reference Lines

Emission lines are referenced using the line spectra library.

Standard spectral lines are taken from NIST data. They are useful for identifying emission peaks from ionised gases or vapors. The line height is proportional to the relative probability of the transition causing the emission. Only the strongest lines are shown.

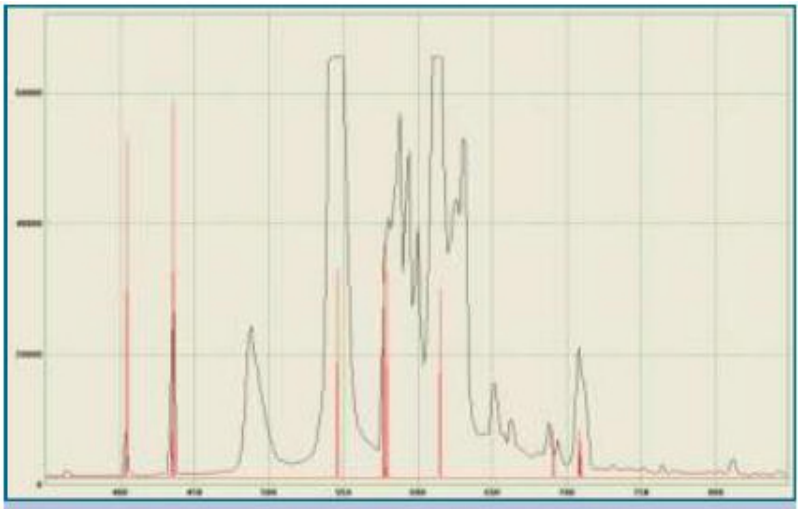


Select the lines you want to display using the check boxes, then click Add or Delete to add them to or delete them from the list.



The fine green lines are He reference lines. The first thing to notice is that the spectrometer is correctly calibrated; the lines match the peaks.

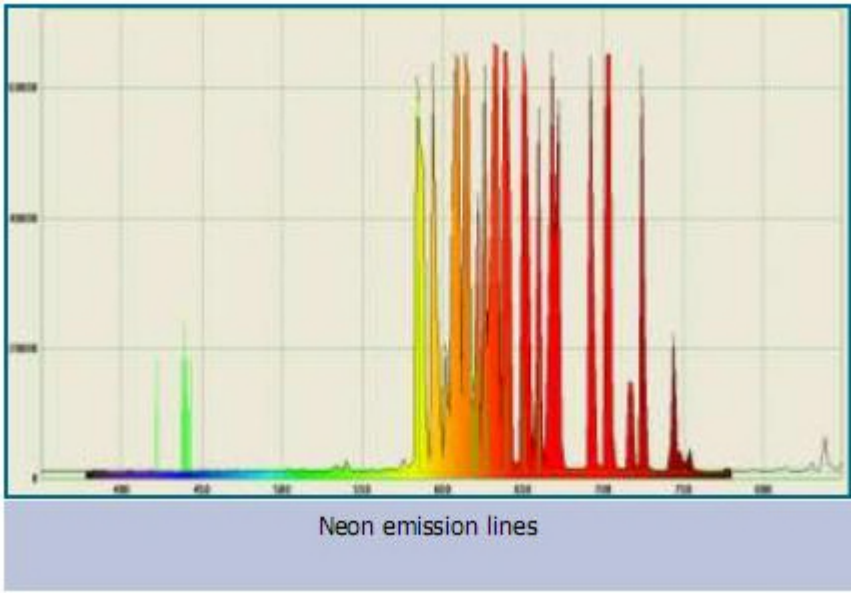
The reference lines are sharp and the real emission lines appear to be not so sharp. The reason for the broadening is the resolution limits of the spectrometer.



Fluorescent lamp with mercury reference lines, showing that the lamp contains mercury vapor.

You can use the reference lines to identify elements such as mercury in lamps and metallic elements in flame tests.

Neon emission lines are closely packed in the yellow and red end of the spectrum. This is why traditional neon signs have a warm red glow. Different gases produce different colors, so the blue shield is not neon.



Menu Functions

This section details the various functions available from the Overture menu.

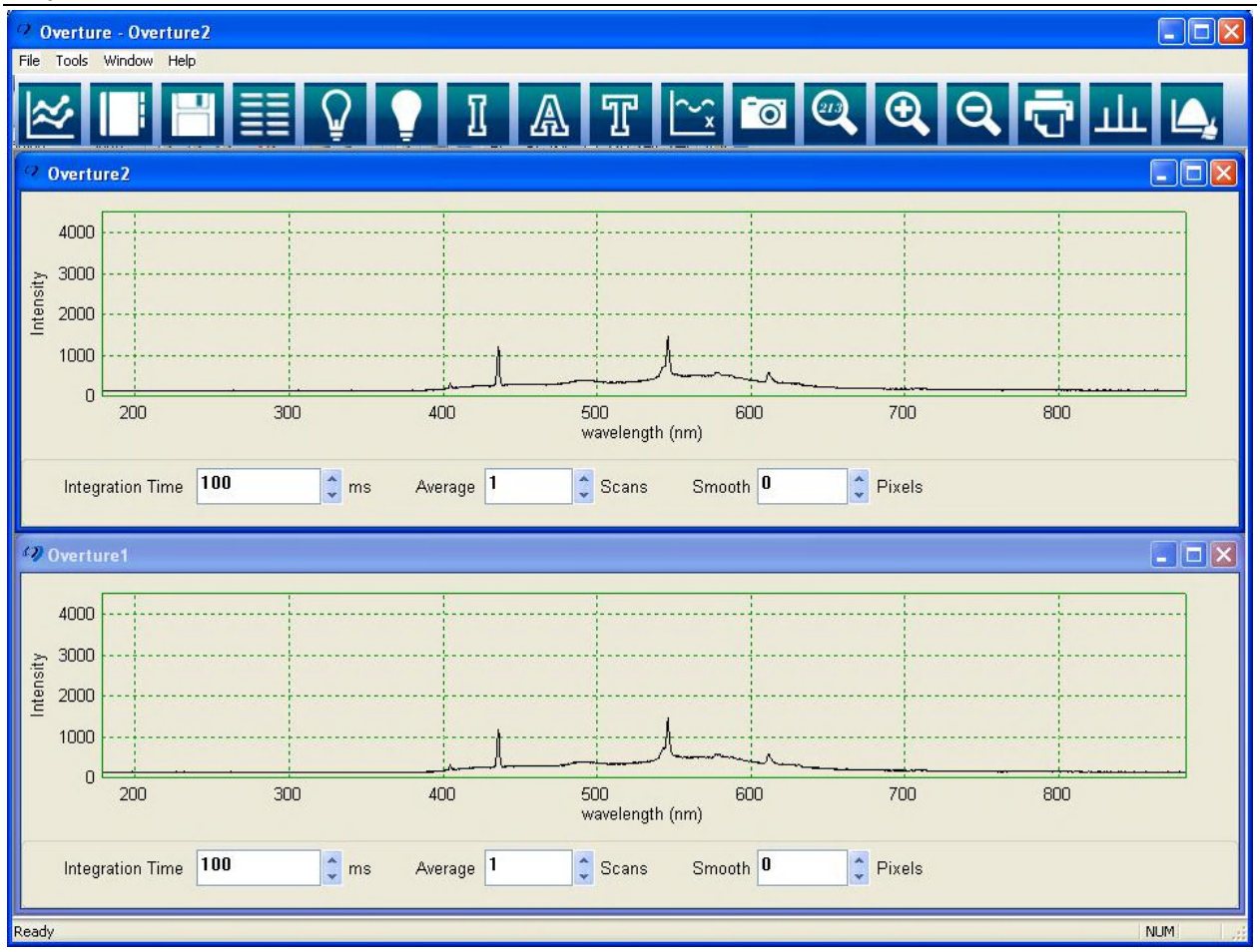
File Menu

The File menu offers the following functions:

- New – Opens the current
- Open
- Save Spectrum
- Print
- Print Setup
- Exit

New

Select this menu item to open the Overture graph in a new screen:



Open

This menu item allows you to choose whether to show data in a new window or to show data in the current window.

Save Spectrum

Choose **File | Save Spectrum** to save a .spec file to a folder on your computer.

Print

Use this menu selection to print your graph. This is the same function that is invoked with the print icon  .

Print Setup

Select Print Setup to choose options for the printer to print your graph.

Exit

This menu selection allows you shut down Overture.

New

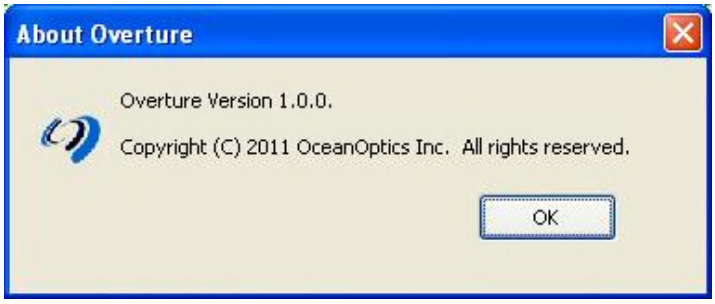
This menu item has one selection: Language. Select **Tools | Language** to choose to display the Overture interface from the displayed list.

Window

This menu item provides two options for displaying the graph: Cascade and Tile.

Help

Displays information about Overture.



Chapter 4

Experiments

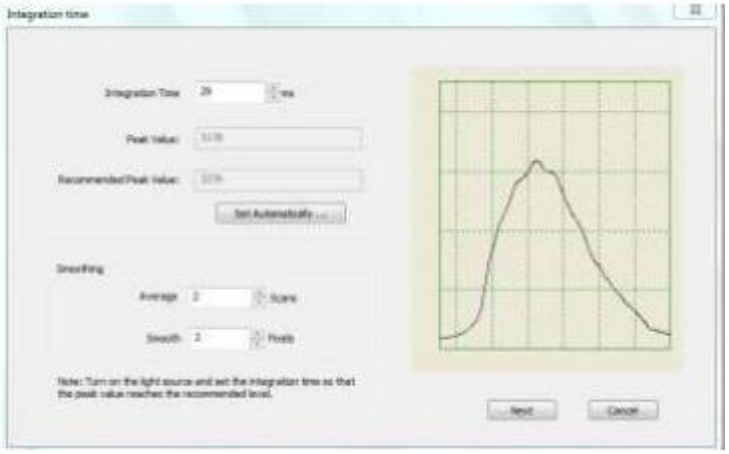
Concentration wizard

The concentration wizard guides you through the process of measuring absorbance at different concentrations and plotting a calibration curve to use the Beer-Lambert law to measure unknown concentrations with the spectrometer system.

The spectrometer can detect very small changes in concentration. The Absorbance number y-axis ranges from 0 to 3. The ideal working range is between 0.5 and 2.5

► *Procedure*

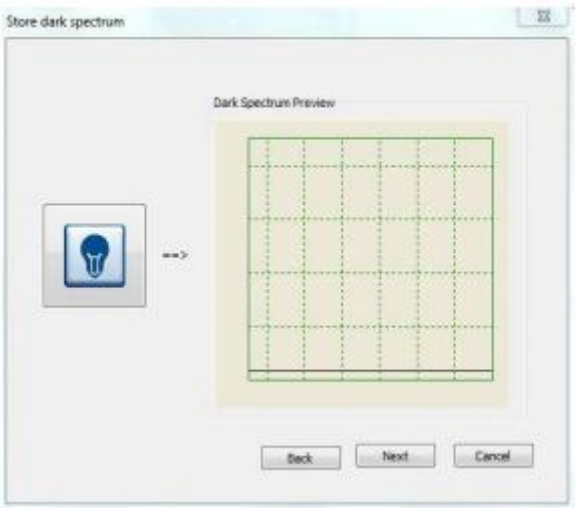
1. **Set the integration time and smoothing for the reference cuvette.** Insert the reference cuvette into the sampling lamp. Set the integration time so that the peak is at about 85% of maximum. The *Set Automatically* button will do this for you unless the signal is too far above or below the recommended peak value. In that case, you are asked to set it manually.



Note

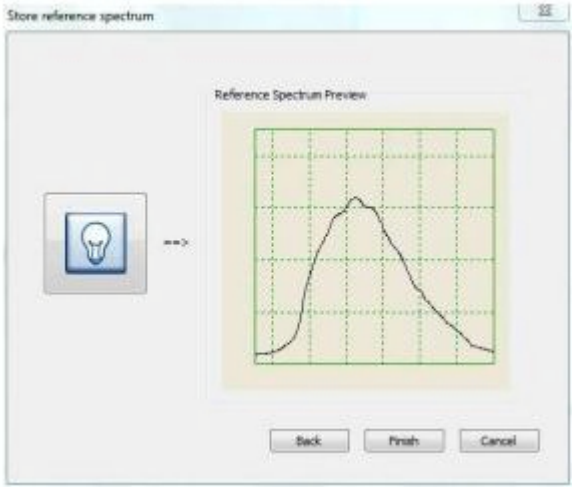
If you change the integration time or anything else in the experiment setup, you will need to store new dark and reference readings.

2. **Store the dark spectrum.** Block the light by turning the mirror. The spectrum line will be flat and close to the baseline. Click the dark spectrum bulb icon ( ). The dark spectrum is now stored.



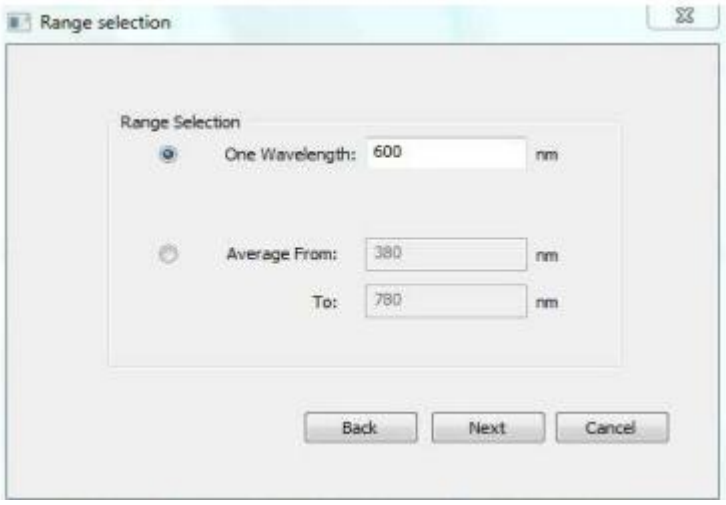
3. **Store the reference spectrum.** Insert the reference cuvette into the sampling lamp. The reference cuvette should contain only the solvent, usually water or an organic solvent. With a compound in solution, the absorbance will reduce the peak. Then, click the Reference bulb icon ( ).

   The line can be displayed in 1st or 2nd order and forced through the origin. Values for the molar extinction coefficient and R^2 are calculated.

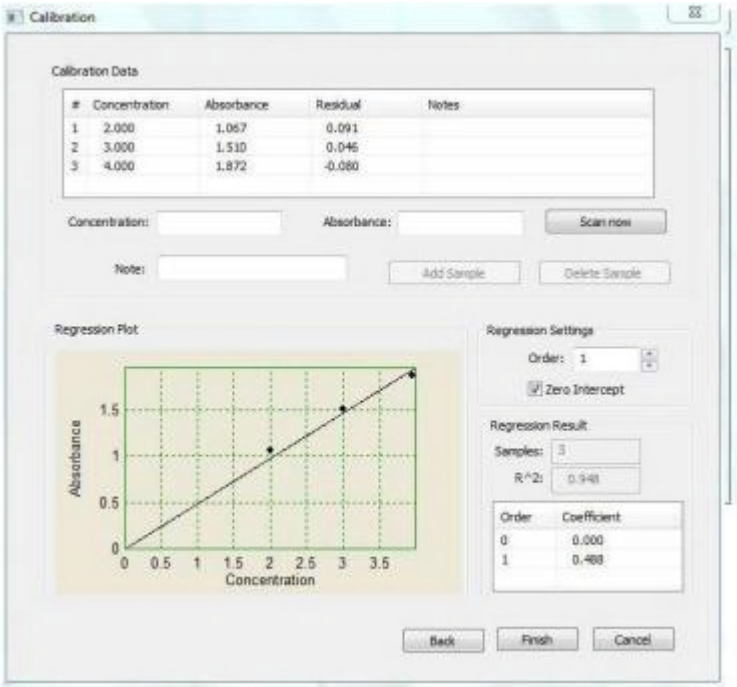


4. **Choose Beer-Lambert Law or Calibration.** To use the Beer-Lambert Law option you need to know the molar absorption (extinction coefficient ε) for the compound you are using. If you are trying to find ε you need to choose the option "Calibrate from solutions of known concentration."

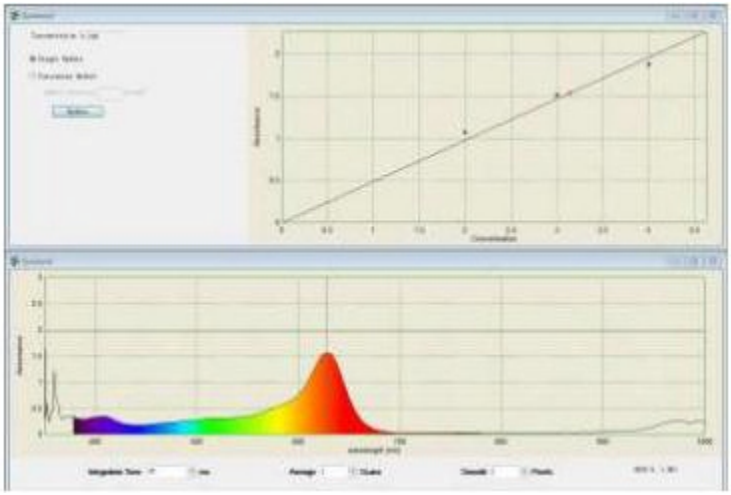
5. **Wavelength range selection.** Overture software needs to know which wavelength to measure for absorbance. Select one (Lambda max) or a range around Lambda max. You can see the absorbance spectrum behind the dialog box.



6. **Create the calibration curve.** Take at least three samples of known concentration. Either enter the Absorbance by hand from your notes or scan for it using the *Scan now* button. When you enter the third sample a regression line will be plotted. The line can be displayed in first or second order and forced through the origin. Values for the molar extinction coefficient and R^2 are calculated.



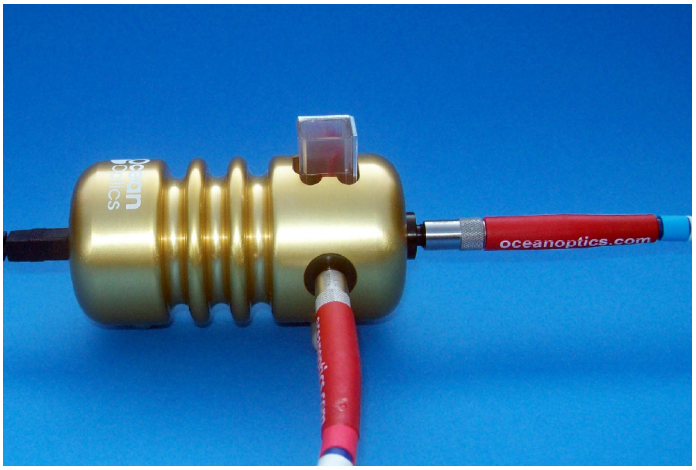
7. **Concentration meter.** Now you can place any unknown concentration of your compound into the cuvette holder and Overture software will give you an instant reading of the concentration and show where it sits on the calibration curve with a red diamond marker. No units are shown next to the reading because you have to know what units you are working in. You can make notes in Step 6 of the units you are working in.



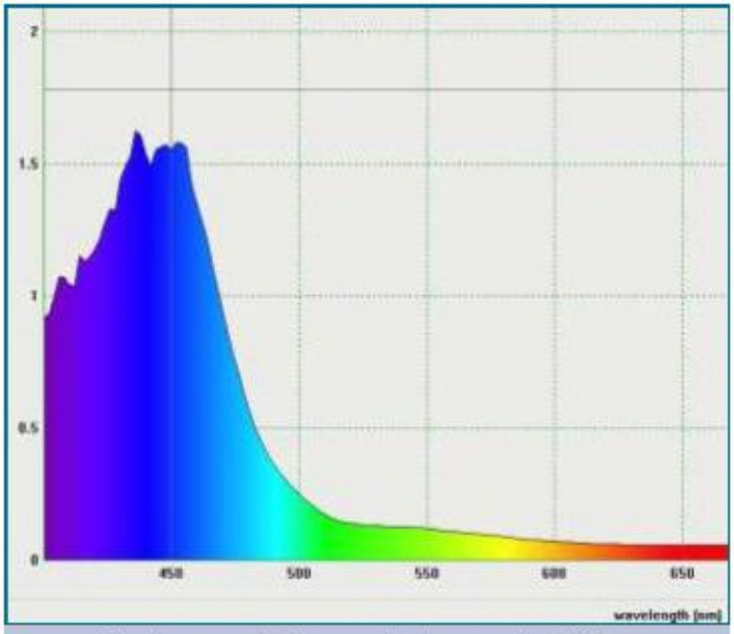
Fluorescence

The sampling unit can be set up to measure fluorescence from a side port at 90 degrees to the light path. Light coming from the sample at 90 degrees has either been scattered or is fluorescence from the sample. The intensity of fluorescent light is much lower than the in-line light used for absorbance or transmission. As a result, you need a fiber optic probe with a larger diameter to collect more light. The mirror also helps to redirect light into the sample to increase the fluorescence.

The Fluorescence fiber (P400-2-VIS/NIR) can be obtained from your supplier.

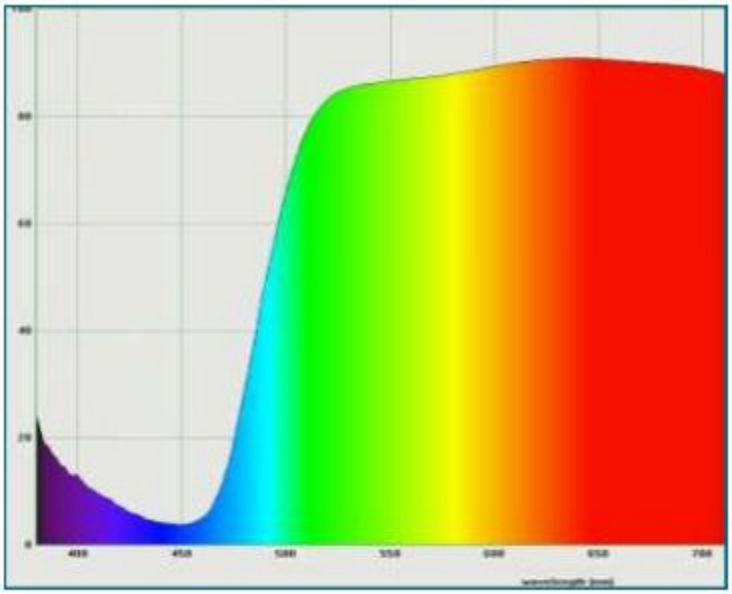


The following spectra were taken from a fluorescein dye used in a domestic floor cleaner.



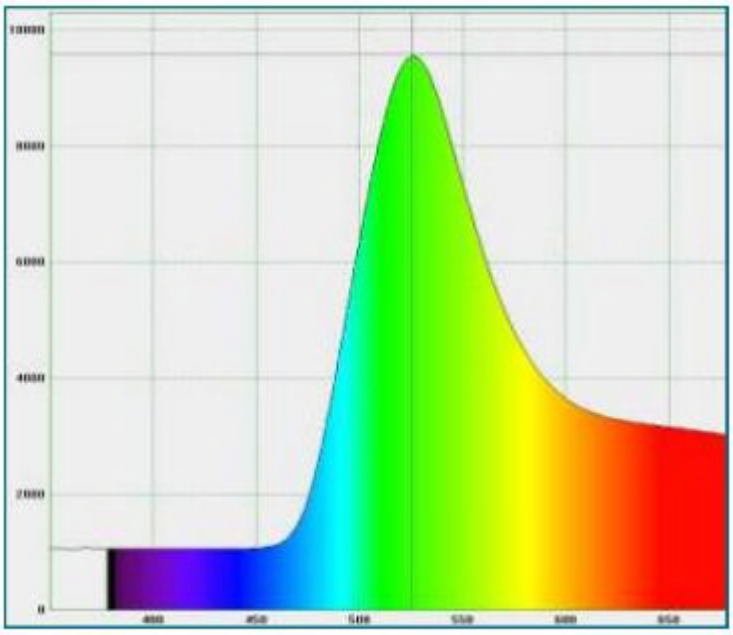
Absorbance mode shows an absorbance peak at 450nm

In transmission mode the same absorbance (now a dip) is seen. We know that light energy is being absorbed in the 450nm region.



Transmission mode shows the equivalent absorbance dip at 450nm

The spectrum below is taken from the side port using the fluorescence fiber. It shows a peak at 520 nm . That is 70nm longer wavelength than the absorption peak. The difference between the absorbance and fluorescence peaks is about 70nm. The dye is re-emitting light at a longer wavelength. This is called the Stokes shift.



The signal from the side port in Intensity mode show light with a sharp peak at about 510nm—a Stokes shift of 60 nm.

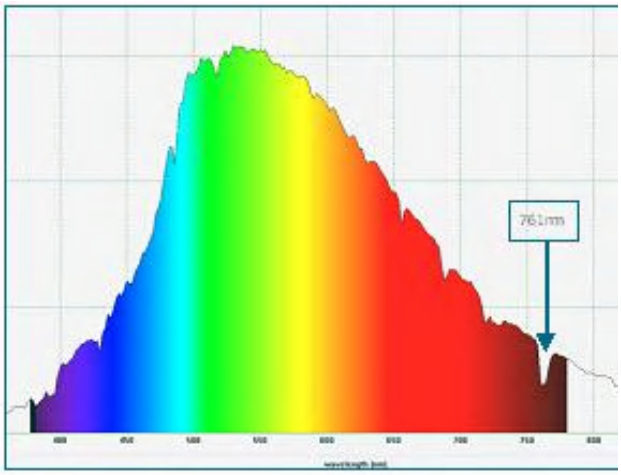
Chapter 5

Applications

Physics applications

The sun

The spectrometer can resolve the strongest Fraunhofer lines. These can be seen in all weather conditions.



Observed lines nm	Known lines nm	Element
382	382.5891	Fe I
393	393.0308	Fe I
393	393.3682	Ca II^2
404	404.5825	Fe I
486	486.1342	H
590	588.9973	Na I(D_2)
590	589.594	Na I(D_1)
656	656.2808	H
761	761	O

Most of these lines originate from the sun's chromosphere, but the strong 761nm absorption dip is caused by Oxygen in our own atmosphere.

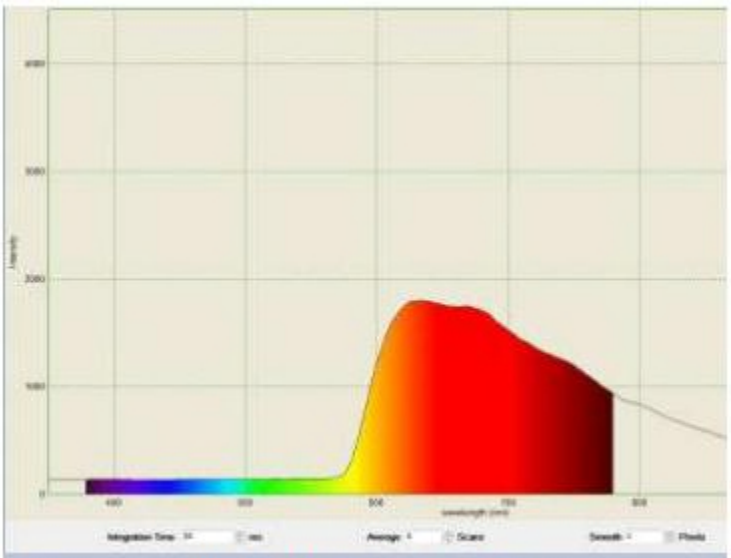
Match the Balmer emission lines from the line spectra library to hydrogen absorption lines.

Fluorescent lamps

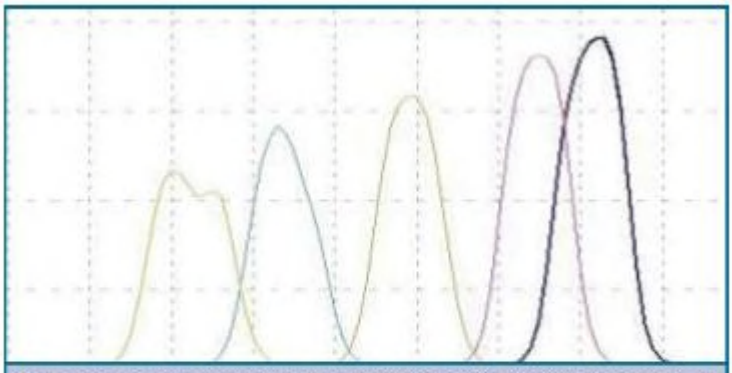
Strip and compact fluorescent lamps show mercury spectral lines and fluorescence. Use the line spectra library to identify the mercury

Filters, solutions and glasses

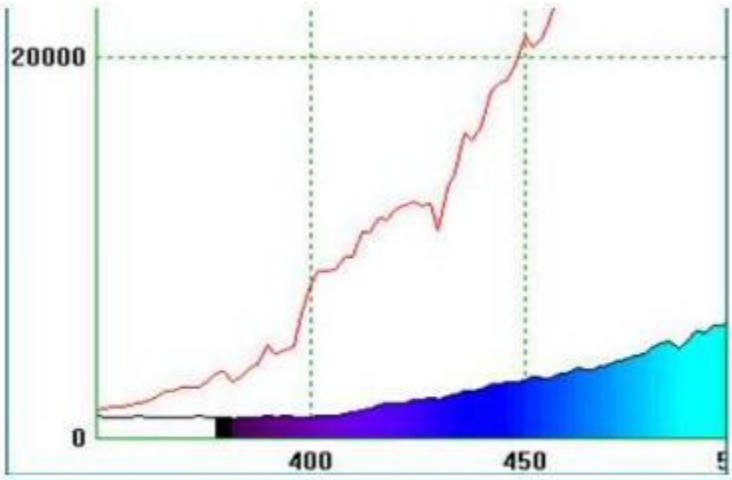
The absorption of near UV rays through sunglasses, optical filters and interference filters. The change in transmitted wavelength is caused by changing the incident angle of a collimated white light source through an interference filter.



The sharp cut off of a polymer filter
in a rear light cluster of am automobile.



These snapshot lines were taken by rotating a thin film interference filter through 20 degrees and viewing the transmission of a collimated beam of white light at 5 degree intervals. The optical path through the thin film coating gets longer as the incident angle increases and the thin film interference maximum changes wavelength.



The snapshot red line is daylight. The live spectrum shows the light transmitted through sunglasses. Note the zero transmission below about 400nm, hence the label UV400.

Reflection of light from colored surfaces

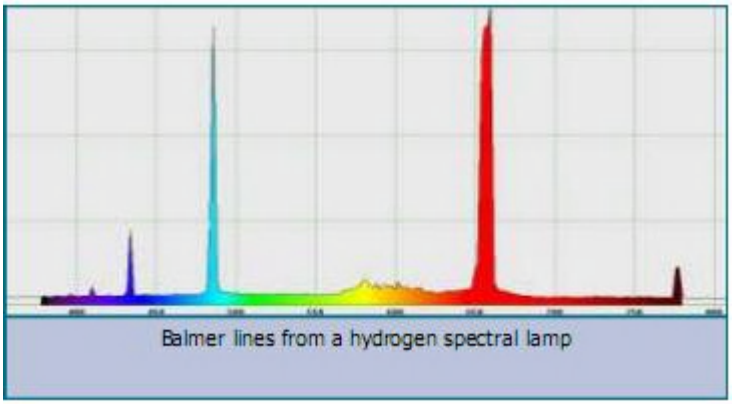
The spectral emission of scattered light from surfaces shows how surface color depends on absorbed and reflected light. Illuminate at 90 degrees to the surface and angle the probe at 45 degrees or vice versa.

Ionized gases and metal vapors

Spectrum tubes and lamps produce line spectra. The lines can be used with Planck's constant to investigate transition energies and show elementary Overture physics in action. Automotive HID lamps give a Xenon line emission spectrum.

Hydrogen spectrum Balmer lines

Calculation of the Rydberg constant from the Balmer series lines. Match to Fraunhofer lines.

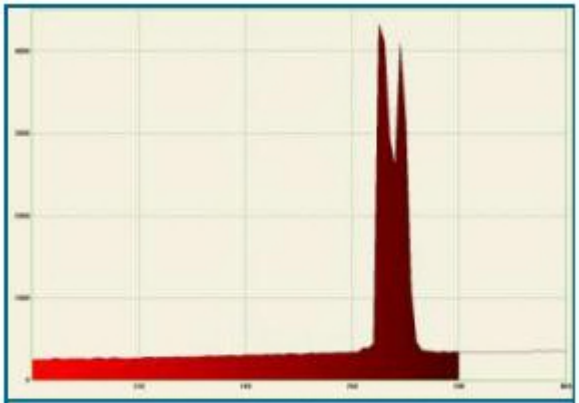


Balmer lines from a hydrogen spectral lamp

Flame emissions

Heating foods that contain sodium or potassium with a hot blue Bunsen flame produces line spectra. Try potato snacks and a banana. Many foods contain potassium. Now you can find out which ones by spectroscopic analysis.

A hot torch flame makes the banana glow red hot. The spectrometer detects a strong potassium line spectrum.

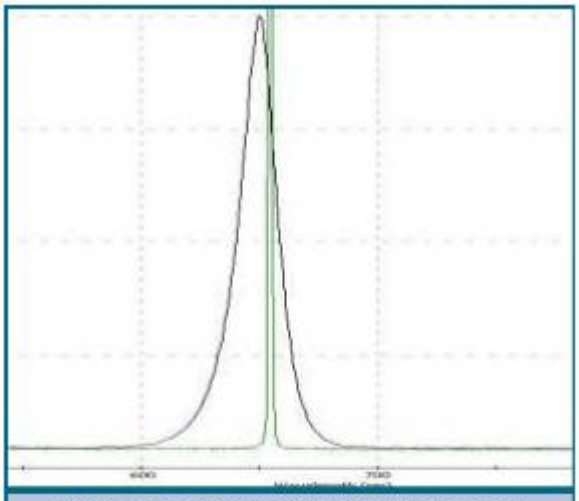


Potassium K doublet from a hot flame applied to a banana

Standard flame test compounds can be used to show how all the metallic elements produce line spectra. Sodium contamination is less of a problem as the Na line can be identified and does not affect other lines. The spectrometer "sees" discrete wavelengths, so even if you can't see a line behind a yellow sodium glow, the spectrometer can.

Lasers

Lasers have a near-monochromatic line spectrum (sharp Gaussian). Some of the Gaussian spread is caused by the spectrometer. The sharper the line the more monochromatic the light. Compare this with LEDs, interference and gel filters.



A snapshot comparison of a laser diode and LED. Which is the most monochromatic?

Chemistry applications

Following are some of the color-related experiments that can be carried out with your spectrometer system.

Spectral signatures

Chlorophylls and food colorings in solution can be identified by their absorbance 'fingerprint'.

Olive oils of different quality show very different absorption depending on the amount of chlorophyll they contain.

Flame tests

The spectrometer detects sharp emission peaks even when these are invisible to the human eye.

Multiple elements can be observed. This reinforces understanding of advanced spectroscopic techniques that show multiple peaks.

Common foods like potato snacks and dried banana chips show distinct emission peaks when heated. Bananas show a potassium doublet. Salted snacks show sodium and some containing LoSalt (Potassium Chloride) show a strong potassium line.

The problem of sodium contamination is removed because the spectrometer is a polychromator and sees all wavelengths at the same time. The sensitivity and speed of the spectrometer allows momentary emission spectra to be recorded using the snapshot tool.

Transmission, absorbance and the concept of Absorbance Number

Transmission % = (Sλ – Dλ / Rλ – Dλ) x 100

Absorbance = - log10 (Sλ – Dλ / Rλ – Dλ)

Beers law using $KMnO_4$

Determination of the pKa of bromocresol green

Finding the isosbestic point using the snapshot tool.

Spectrophotometric analysis of commercial aspirin

Acetyl Salicylic Acid (ASA) complex ion is formed by hydrolyzing the ASA or aspirin sample in NaOH solution and complexing it with Fe3+ ion in acid solution to bring out the color with a maximum absorption at a wavelength of 530 nm.

Spectrophotometric determination of an equilibrium constant

Investigating the reaction between aqueous solutions of iron (III) nitrate, Fe(NO3)3, and potassium thiocyanate, KSCN. The reaction produces the blood-red complex FeSCN2+. The reaction also establishes an equilibrium allowing the equilibrium constant to be obtained by sampling multiple reactions using different concentrations.

Water quality testing using indicator reagents

Phosphate and Nitrate concentrations can be accurately measured by analysis of the absorbance of solutions using commercial testing reagents.

Appendix A

Sample Experiments

Beer's law analysis of $KMnO_4$

Equipment Needed:

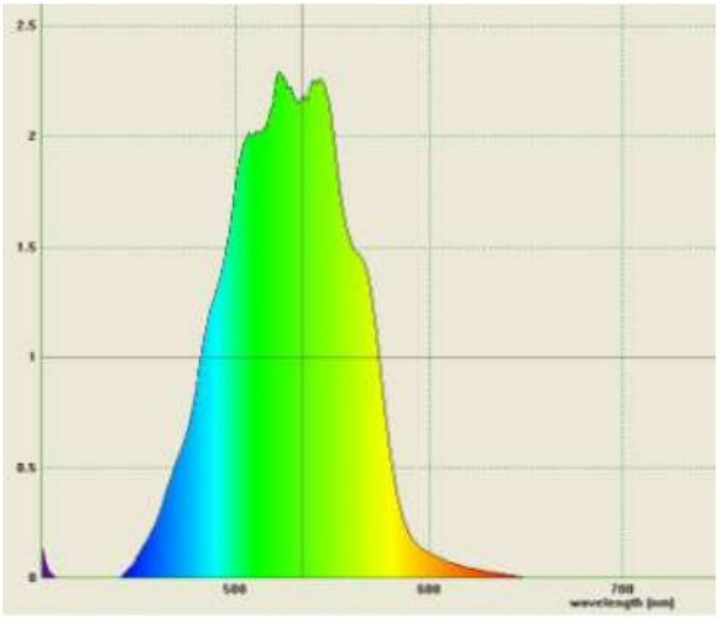
- Ocean Optics CCD array spectrometer with lamp/cuvette holder
- Equipment for making aqueous solutions of different concentrations of $KMnO_4$
- Cuvettes

Objectives:

- To find the molar absorbance coefficient (ε) for potassium permanganate, potassium manganate (VII), by plotting a calibration curve using the Beer Lambert Law.
- Using the calibration to find the concentration of an unknown sample.

Procedure:

1. Set the spectrometer in A (Absorbance) mode.
2. Click on the concentration wizard icon before setting dark and reference readings. The wizard will help you set the correct integration time.
3. From a stock solution of known concentration in Moles per liter, make up a range of dilutions of at least three precisely known concentrations. Use solutions which have absorbance numbers between 0.5 and 2.5.
4. Measure these solutions in A mode. Potassium permanganate has a very high molar absorbance coefficient (ε), so solutions have to be very dilute. As Absorbance numbers reach 3, Beers Law starts to become less reliable.
5. Find $\lambda_{max.}$ For potassium permanganate this should be about 534nm (you will see two peaks at 524 and 544nm).



6. In the Concentration Wizard, select “Calibrate from solutions of known concentration”.
7. In the Range selection, set the wavelength at λ_{max} or average across the peak.
8. Enter at least three concentration values and scan for Absorbance in each case. On the third data point the wizard will plot a regression line. Add more samples if you have them.
9. Clicking Finish will convert the spectrometer into a concentration meter that uses the calibration line that you have made. Use the meter to measure unknown concentrations.

Other Beers Law Experiments:

- Use food dyes and measure unknown concentrations in food products. For example, measure Erythrosin B In Maraschino cherries.
- Use water quality indicator reagents to measure concentrations of nitrates in water samples.

Chlorophyll detection in olive oils

Equipment Needed:

- Ocean Optics CCD array spectrometer with lamp cuvette holder
- Three grades of olive oil
- Cuvettes

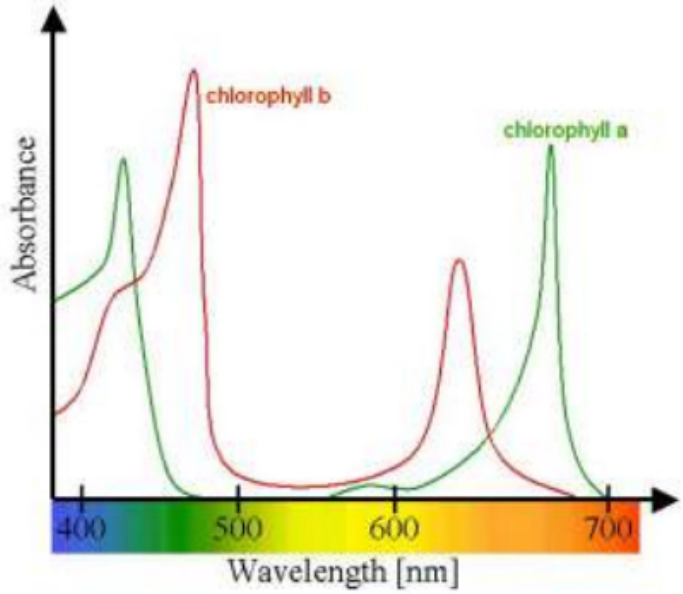
- Flasks, beakers and pipettes for preparation

Objectives:

Olive oils contain varying amounts of chlorophyll, depending on the grades: extra virgin, standard and light. This experiment will show qualitatively how the grades compare and can be extended to compare with chlorophyll extracted from green leaves with isopropanol.

Procedure:

1. For a reference spectrum, water can be used. There is a discussion point about what a reference should be for an experiment with oils, since there is no solvent as such.
2. Take reference and dark readings and set Overture to Absorbance mode. If necessary, use numerical zoom to cut of the noisy signal below 400 nm.
3. Visible absorbance peaks for chlorophyll are at: 413, 454, and 482 nm, 631 and 669 nm. See how many you can detect.



Absorbance peaks of chlorophyll. Source:Wikipedia

Flame tests

Equipment Needed:

- Ocean Optics CCD array spectrometer with 400 micron fiber optic cable

- Stand and clamp
- Flame test wire loop
- Banana or dried banana chips
- Salts: LiCl, NaCl, $SrCl_2$, $CuCl_2$, $BaCl_2$, $CaCl_2$
- 1M HCl for cleaning the loop

Objectives:

- To show that flames test emissions are line spectra that can be identified by comparison with reference line spectra.
- To use potassium salts and the reference library of emission lines to identify potassium in bananas.

Procedure:

1. Set the spectrometer to Intensity mode. Use a 400 micron fiber optic or remove the fiber optic cable.

Caution

If you use the spectrometer without a fiber optic cable, you should take steps to prevent chemicals from entering the entrance port. Use a small piece of plastic wrap to cover the port.

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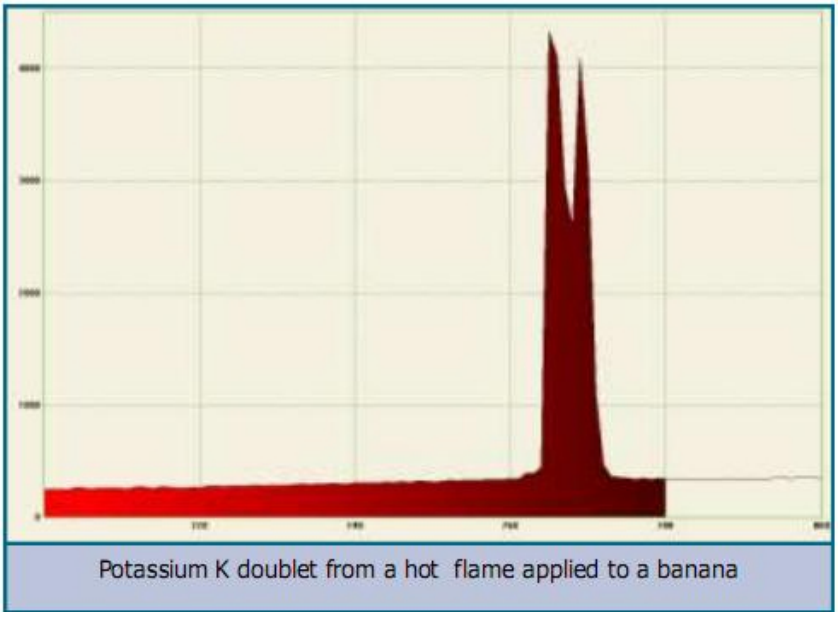
2. Hold the fiber in a laboratory clamp about 30 cm from the flame.
3. Be careful not to have fluorescent lamps in the line of sight of the fiber. This will cause mercury spectral lines. Use the mercury lines to check for these.
4. Use the Snapshot tool to freeze spectral lines.
5. Overlay the reference lines to identify the metal elements in salts.

Sodium contamination will show up as a line at 589nm.You can try to clean the flame test loop with HCl to eliminate it, or just recognize it as contamination. Sodium contamination is a problem when looking at flames by eye because the yellow is so bright that it masks other fainter colors. The spectrometer does not have that problem because every wavelength is detected and displayed separately.

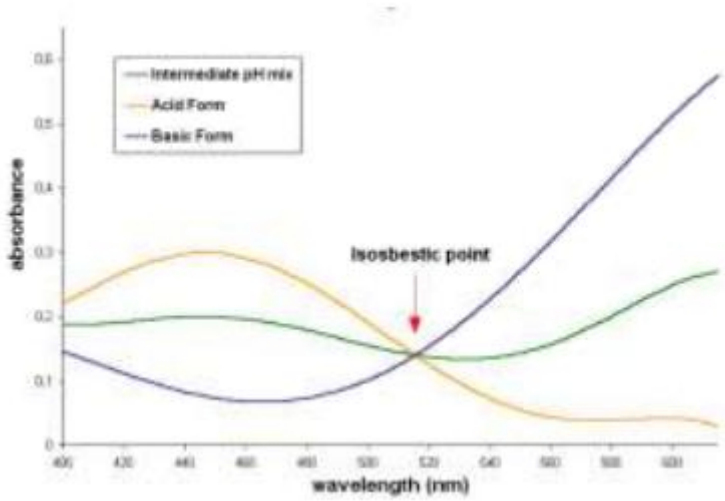


Potassium K doublet from a hot flame applied to a banana

Spectrophototmetric analysis of a buffer solution – Isobestic point

Procedure:

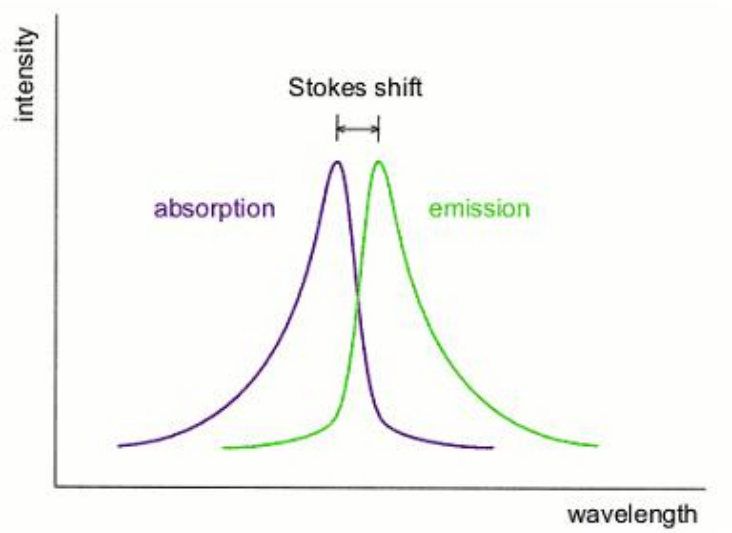
1. Measure and analyze the visible light absorbance spectrum of an acetate buffer solution containing bromocresol green indicator.
2. Compare the spectra of a sample of acetate buffer that has been treated with acid to a sample of the buffer treated with base.
3. Use test results to calculate the pH of the buffer solution.



Fluorescence and Stokes shift

Objectives:

Using the side port of the sampling unit the Stokes shift of fluorescent compounds in solution can be measured.



Procedure:

1. Using an open bench set up and a UV lamp you can detect fluorescence by pointing the fiber at the side of the cuvette.
2. Visible fluorescence of fluorescein from UV excitation by a UV led can be measured. This phenomenon is used by opticians to show up scratches on the cornea and is the basis of confocal microscopy used to view biological samples.

Appendix B

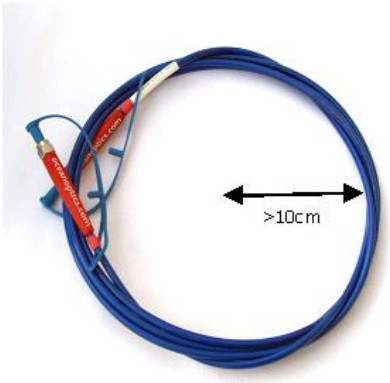
Maintenance

Fiber care

The fiber optic cables have a glass core. They should not be bent with a radius of less than 10cm.

If the fiber ends become dirty, use a lens wipe and alcohol to clean gently.

Always keep the protective caps on when not in use.



Spectrometer care

Ocean Optics spectrometers are precision instruments designed as light, portable devices. Take the following precautions:

- Avoid shocks and drops
- Avoid extremes of temperature
- If exposed to sub zero temperatures allow to reach room temperature and wait for one hour before operating
- Avoid prolonged exposure to direct sunlight
- Always replace dust caps when not in use

Troubleshooting

Problem	Possible Cause(s)	Suggested Solution(s)
The icons are grayed-out	USB cable is not firmly connected.	Close the Overture software, recheck the USB connection, then re-open the software.
The trace is very low	Too little light Zoom – is set.	Increase the integration time. Position the fiber to receive more light. Try Zoom+ or numerical zoom on the y-axis.
The trace is off the intensity scale	Too much light Zoom + is set	Reduce the integration time. Position the fiber to receive less light. Click Zoom –
The low wavelength trace in Absorbance mode or Transmission mode is spiky and jumpy.	At below 400nm the sampling unit lamp has a low intensity. In Absorbance and Transition modes, the signal to noise ratio is too low to give a reliable signal.	Use numerical zoom to start the x-axis at 450nm.
There are unexpected sharp peaks in the spectrum.	There is a fluorescent lamp in the vicinity.	Remove or turn off fluorescent lamps in the lab. These emit sharp mercury spectral lines.
The fluorescent signal from the side fiber connector is very weak.	Incorrect fiber is being used.	Make sure you have the P-400-2-VIS-NIR micron fiber connected to the side port.

FAQs

How can I get software upgrades?

These are available from your distributor. Software upgrades are free.

How strong is the fiber optic cable?

The fiber core is made of glass. Do not bend the fiber sharply with a radius of less than 10cm.

Can I use the spectrometer outside?

Yes, but only in dry, warm conditions. Do not expose the case to direct sunlight. The spectrometer is a laboratory instrument and not designed to operate in a harsh environment.

I have spilled chemicals in the cuvette holder. What should I do?

1. Use the hex key to remove the lamp module.
2. Clean the lamp with a soft damp cloth.
3. Wash out the cuvette holder with de-ionized water.
4. Air dry. Reassemble.

Where can I buy the 400 micron Fluorescence fiber?

These are available from your distributor.

I am looking at low level light sources. How can I get more light into the spectrometer?

Use the 400 micron fiber. This lets in about ten times as much light as the 50 micron fiber. Alternatively use without the fiber, but keep the dust cap on when not in use.

How do I calibrate my spectrometer?

Ocean Optics spectrometers are factory calibrated. A severe shock could cause a calibration error, but in normal use the spectrometer should never require re-calibration. You can check calibration against the library of emission spectra.
Contact you distributor if you have a problem.

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