Spectroscopy in a nutshell

A spectrometer is a device that breaks up light into different colours 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 colours 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 to 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.

Spectroscope views

On the right are three spectra as seen through a traditional spectroscope. The top one is of a filament lamp.

The next one is 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.

The next one is an emission spectrum from a hydrogen gas discharge tube. It shows that the hydrogen gas only emits light at certain wavelengths. This is an emission spectrum.
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 Quantum software display.

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

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

The light passes through an optical geometry of focussing mirrors and a reflection grating. The spectrum falls on a linear CCD array with hundreds of 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 educational spectrometers 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:
- The slit width
- The grating specification (lines per mm) and quality
- The number of pixels in the array
- The physical size of the system

This causes an apparent spreading of emission and absorption lines in the Quantum 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 nanometres wide. In fact you will deliberately “smooth” the spectrum by averaging the array output in Quantum.

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.
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<thead>
<tr>
<th>Icon</th>
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</table>
**Intensity mode**

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 fibre optic input.

**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 fibre and click the store dark spectrum icon. You will not see any change, but the Quantum software now has a zero or “dark” reading stored for every wavelength.

**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 than 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 there are too many photons, 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 bottom status bar.

**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 colourless 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.

**Colour fill**

Quantum can fill the space under the spectrum with an artificial display of spectral colour. The colour display covers the visible range of 380nm to 780nm. Outside that range is UV and NIR (near infrared) and of course we cannot see colours there.
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 and 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 \(\lambda_{\text{max}}\).

The Absorbance scale goes up to 3. Above three the solution is so dark that Absorbance is no longer possible to measure reliably. 2.5 is the normal maximum for reliable results.

Transmission and Absorption 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:
- \(S_{\lambda}\) = Sample intensity at wavelength \(\lambda\).
- \(D_{\lambda}\) = Dark intensity at wavelength \(\lambda\).
- \(R_{\lambda}\) = Reference intensity at wavelength \(\lambda\).

So now you can see why you need dark \(D\) and reference \(R\) readings. If you don’t have them, the maths won’t work!

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 Office application. The data is exported in two headed columns in I, A, and T modes. The first column is wavelength and the second depends on the mode.
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.

New graph window

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.

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.

Zoom

Zoom + lifts the highest peak to the full y axis scale. Zoom - cancels this. Numerical zoom allows you to set both the x and y ranges you want to display. It is useful to set the wavelength minimum to 400 in Absorbance and Transmission mode to eliminate the noisy signal that can often be seen at the short wavelength end of the scale. Numerical zoom can be used in all windows including kinetics.

Snapshots of LEDs show the link between wavelength and colour. With voltage data they can be used to derive Planck's constant.
Standard spectral lines are taken from NIST data. They are useful for identifying emission peaks from ionised gases or vapours. 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 and ADD or DELETE.

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.

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 colours, so the blue shield is not neon.
Amadeus sampling unit

For chemistry applications the sampling unit is required to provide constant illumination of solutions in cuvettes and elimination of ambient light. The sampling unit has a tungsten lamp to give a smooth broad spectrum reference light source. The inline fibre optic connector is for absorbance measurement. The side entry connector is for fluorescence measurement.

The Fluorescence fibre accessory connects to the side port. The fibre has a larger 400 micron diameter to collect the weaker florescence signal. The mirror helps to boost the fluorescence effect by reflecting light back into the sample.

Don’t lose this small accessory!
The L shaped mirror maximises light for fluorescence, but it should always be used as it holds the cuvette securely.

Turn the mirror through 90 degrees to block the light to take a dark reading.

To remove the bulb holder sub-assembly, use the Allen key provided to loosen the grub screw.
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 goes from 0 to 3. The ideal working range is between 0.5 and 2.5

**STEP 1**
Set up the integration time and smoothing

Put 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 will be asked to set it manually.

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

**STEP 2**
Store the dark spectrum

Put the reference cuvette into the sampling lamp. Click the bright lamp icon. The reference spectrum is now stored.

The reference cuvette will contain only the solvent, usually water or a solvent such as alcohol. With a compound in solution the absorbance will reduce the peak. If you change the integration time or anything in the experimental setup, you will need to store new dark and reference readings.
**STEP 4**
Choose Beer Lambert or Calibration

To use the Beer Lambert law option you need to know the molar absorptivity, $\varepsilon$, for the compound you are using. If you are trying to find $\varepsilon$ you will need to choose the option “Calibrate from solutions of known concentration.”

**STEP 5**
Wavelength range selection

Quantum 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 dialogue box.

**STEP 6**
Create 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.

**STEP 7**
Concentration meter

Now you can place any unknown concentration of your compound into the cuvette holder and Quantum 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 given 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.
Kinetics wizard

The Kinetics wizard guides you through the set up for measuring absorbance against time for reaction kinetics. Before you start you should run trials on the absorbance and reaction times of your experiment to find out
1. How long does the reaction take.
2. What is lambda max for absorption

**STEP 1**
Set up the integration time for the reference cuvette

[Image of integration time setup]

With a reference cuvette in the sampling unit, use the Set Automatically button to set the integration time to the optimum value. If the signal is outside the automatic setting range you will be asked to set the integration time manually.
Set the pixel smoothing to 3 or 4. Average scans can stay at 1.

**STEP 2**
Store the dark reading

[Image of dark reading storage]

**STEP 3**
Store the reference reading

**STEP 4**
Kinetics settings

The update rate controls how frequently the absorbance vs time line is re-drawn. For most reactions one update per second is about right.
Set the update rate you want to take and the wavelength range you want to monitor for absorbance.

The Range selection is the wavelength or wavelength range around lambda max. In this case the range is set for 620 to 635nm.
STEP 5

Get ready

The default kinetics screen opens two windows. The graph of absorbance vs time at the top and the absorbance spectrum at the bottom. Set the time axis in the drop down box to match your expected reaction time. Start the reaction and click on the green start button.

Tips for successful kinetics experiments

- Set up the wet bench chemistry a safe distance from the spectrometer.
- Check the absorption spectrum of your sample first. If the absorbance is greater than about 2.5, consider diluting the sample.
- Run a trial reaction to see how fast it goes. This will help you select the right kinetics settings.
- If you want to run a second reaction, close the absorbance vs time graph window and click on the kinetics icon. This will save you having to redo the dark and reference readings.
- Try using the cursor and a stop clock to create your own kinetics curve from the absorption spectrum window. This will help you understand how the absorbance vs. time graph is made.
- Use numerical zoom to set the ideal y-axis range. If your absorbance range is below say, 1.5 then set 1.5 as the y axis range to improve the view.

STEP 5

Stop the recording when the reaction is finished

Maximise the top graph window to get a better view. Use the print command to print out the graph.

As the absorbance peak for the sample drops you see the absorbance vs time line in the top graph fall.

Finished. The absorbance peak at lambda max has flattened as the reaction progressed.
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 fibre optic probe with a larger diameter to collect more light. The mirror also helps to re-direct light into the sample to increase the fluorescence.

The Fluorescence fibre, part No. P400-2-VIS/NIR can be obtained from your supplier.

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

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 fibre. It shows a peak at 520nm. 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.

Absorbance mode shows an absorbance peak at 450nm
Physics applications

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

<table>
<thead>
<tr>
<th>Observed lines nm</th>
<th>Known lines nm</th>
<th>Element</th>
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</thead>
<tbody>
<tr>
<td>382</td>
<td>382.5891</td>
<td>Fe I</td>
</tr>
<tr>
<td>393</td>
<td>393.0308</td>
<td>Fe I</td>
</tr>
<tr>
<td>393</td>
<td>393.3682</td>
<td>Ca II^2</td>
</tr>
<tr>
<td>404</td>
<td>404.5825</td>
<td>Fe I</td>
</tr>
<tr>
<td>486</td>
<td>486.1342</td>
<td>H</td>
</tr>
<tr>
<td>590</td>
<td>588.9973</td>
<td>Na I(D₂)</td>
</tr>
<tr>
<td>590</td>
<td>589.594</td>
<td>Na I(D₁)</td>
</tr>
<tr>
<td>656</td>
<td>656.2808</td>
<td>H</td>
</tr>
<tr>
<td>761</td>
<td>761</td>
<td>O</td>
</tr>
</tbody>
</table>

Most of these lines originate from the sun’s chromosphere, but the strong 761 nm absorption dip is caused by Oxygen in our own atmosphere. For more lines see the Appendix page. Match the Balmer emission lines from the line spectra library to hydrogen absorption lines.

Filters, Solutions and Glasses
The absorption of near UV rays through sunglasses, optical filters and interference filters.

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

The sharp cut off of a polymer filter in a rear light cluster of an 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 400 nm, hence the label UV400.
Reflection of Light from Coloured Surfaces
The spectral emission of scattered light from surfaces shows how surface colour 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 Vapours
Spectrum tubes and lamps produce line spectra. The lines can be used with Planck’s constant to investigate transition energies and show elementary quantum 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.

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

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 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 with LEDs, interference and gel filters.
These are just some of the colour related experiments that can be carried out with the spectrometer system.

**Spectral Signatures**
Chlorophylls and food colourings 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, Absorption and the Concept of Absorbance Number**
Transmission % = \( \frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda} \times 100 \)

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

**Beers Law using KMnO₄**

**Determination of the pKa of bromocresol green**
Finding the isosbestic point using the snapshot tool.

**Kinetics of Methylene Blue Reduction by Ascorbic Acid**
Determining whether reactions are first or second order by using the kinetics plot of absorbance against time.

**The Kinetics of a Bleach Reaction**
Determine the rate law and order of a reaction between food coloring and commercial bleach. Analyze the absorbance vs. time graphs to determine the order of the reaction. Write the rate law for the reaction.

**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 colour 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.
Application Notes 1 - Beer’s Law analysis of KMnO₄

You will need:
Ocean Optics CCD array spectrometer with lamp/cuvette holder unit.
Equipment for making up aqueous solutions of different concentrations of KMnO₄.
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.

Set the spectrometer in A (Absorbance) mode. Click on the concentration wizard icon before setting dark and reference readings. The wizard will help you set the correct integration time.

From a stock solution of known concentration in Moles per litre 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. Try these out in A mode. Potassium permanganate has a very high molar absorbance coefficient (ε) and so solutions have to be very dilute. As Absorbance numbers reach 3, Beers Law starts to become less reliable.

Find \( \lambda_{\text{max}} \). For potassium permanganate this should be about 534nm (you will see two peaks at 524 and 544).

In the Concentration Wizard. Select “Calibrate from solutions of known concentration”. In the Range selection set the wavelength at \( \lambda_{\text{max}} \), or average across the peak.
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.
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 Erythrosin B in Maraschino cherries.
- Use water quality indicator reagents to measure concentrations of nitrates in water samples.
Application Notes 2 - Kinetics of a bleach reaction

You will need:
Ocean Optics CCD array spectrometer with lamp/cuvette holder unit.
Food colouring and domestic bleach
Cuvettes
Flasks, beakers and pipettes for preparation.

OBJECTIVES
Food colouring reacts with bleach over time to lose most of its colour. The rate of reaction is shown in the Quantum kinetics tool, by displaying a graph of absorbance against time. From the graph the reaction order can be found.

Click on the kinetics wizard icon before setting dark and reference readings. The kinetics wizard will help you set the correct integration time.

Set the reference reading using water.
Set up dilute solutions of food colouring with Absorbance numbers < 2.5
Find \( \lambda_{\text{max}} \) for the colouring. You will need this to set up the kinetics wizard.

Run a trial using bleach in different concentrations to find out which concentration will give a complete reaction in about one or two minutes. If the reaction is too fast you will not have time to put the reactants into a cuvette before the reaction goes too far.

Mix the reactants in glassware and transfer to the cuvette with a beral pipette. Always do the wet chemistry away from the cuvette holder.

When you are ready to run the experiment transfer the reactants to the cuvette, put the cuvette in the lamp unit and press START.
You will need:
Ocean Optics CCD array spectrometer withlampcuvette holder unit.
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.

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.

Take reference and dark readings and set Quantum to Absorbance mode. If necessary, use numerical zoom to cut off the noisy signal below 400 nm.

Visible absorbance peaks for chlorophyll are at: 413, 454, and 482 nm, 631 and 669 nm. See how many you can detect.
You will need:
Ocean Optics CCD array spectrometer with 400 micron fibre optic cable.
Stand and clamp
Flame test wire loop
Banana or dried banana chips
Salts: LiCl, NaCl, SrCl₂, CuCl₂, BaCl₂, CaCl₂
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.

Set up the spectrometer in Intensity mode.
Use a 400 micron fibre optic or remove the fibre optic cable.

WARNING
If you use the spectrometer without a fibre optic cable you should take steps to prevent chemicals entering the entrance port. Use a small piece of Clingfilm to cover the port.

Hold the fibre in a laboratory clamp about 30cm from the flame.
Be careful not to have fluorescent lamps in the line of sight of the fibre. This will cause mercury spectral lines. Use the Mercury reference lines to check for these.
Use the snapshot tool to freeze spectral lines. Then overlay the reference lines to identify the metal elements in the 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 recognise 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 colours. The spectrometer does not have that problem because every wavelength is detected and displayed separately.
Fibre care

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

If the fibre ends become dirty, use a lens wipe and alcohol to clean gently. Always keep the protective caps on when not in use.

Spectrophotometric Analysis of a Buffer Solution – Isosbestic Point

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

![Graph showing isosbestic point and absorbance vs. wavelength.](image)

Fluorescence and Stokes Shift

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

![Diagram showing absorption, emission, and Stokes shift.](image)

Spectrometer care

Ocean Optics spectrometers are precision instruments designed as light, portable devices.

- 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
**Trouble shooting**

**The icons are greyed out.**
Close Quantum
Check the USB connection
Re-start Quantum

**The trace is very low.**
Increase the integration time.
Try zoom+ or numerical zoom on the y-axis.
Position the fibre to receive more light.

**The trace is going off the intensity scale.**
You have too much light or zoom+ is set.
Reduce the integration time
Click zoom -
Position the fibre to receive less light.

**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 A and T mode the signal to noise ration is too low to give a reliable signal. Use numerical zoom to start the x-axis at 450nm

**There are sharp peaks in the spectrum that I was not expecting to see.**
Check for fluorescent lamps in the lab. These emit sharp mercury spectral lines.

**The fluorescent signal from the side fibre connector is very weak.**
Make sure you have the P-400-2-VIS-NIR 400 micron fibre connected to the side port.

**Kinetics**
**The kinetics line is very flat.**
Use numerical zoom on the y-axis to display only the absorbance range you are working in.

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**FAQs**

**How can I get software upgrades?**
These are available from your distributor. Software upgrades are free and also posted on oceanoptics.eu/quantum

**How strong is the fibre optic cable?**
The fibre core is made of glass. Do not bend the fibre 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?**
Use the hex key to remove the lamp module.
Clean the lamp with a soft damp cloth.
Wash out the cuvette holder with de-ionised water.
Air dry. Reassemble.

**Where can I buy the 400 micron Fluorescence fibre?**
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 fibre. This lets in about ten times as much light as the 50 micron fibre.
Alternatively use without the fibre, but keep the dust cap on when not in use.

**How do I calibrate my spectrometer?**
Red Tide and Amadeus 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.