Experiment Tutorial

When you are ready to begin your experiment, you should have already installed the PC2000, installed OOIChem, set up your light source/sample holder, and connected your fiber from the PC2000 to your light source/sample holder.

Now you are ready to take your measurements. Because of the components making up your CHEM2000, your system is ideal for absorbance, transmission and relative irradiance measurements. If, however, you wish to utilize your system for other measuring functions, additional products might be required. Contact an Ocean Optics Applications Scientist for options.

Absorbance Experiments

Absorbance spectra are a measure of how much light is absorbed by a sample. The software calculates absorbance ($A\lambda$) using the following equation:

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

where S is the sample intensity at wavelength λ, D is the dark intensity at wavelength λ, R is the reference intensity at wavelength λ.

Common applications include the quantification of chemical concentrations in aqueous or gaseous samples. To take an absorbance measurement:

1. Select **Scope** under **Mode of Operation** in the software display area. Make sure the signal is on scale. Adjust acquisition parameters so that the peak intensity of the reference signal is about 3500 counts. Take a reference spectrum by first making sure nothing is blocking the light path going to your spectrometer. The analyte you want to measure must be absent while taking a reference spectrum. Take the reference reading by clicking the **Reference** button in the software display area. (This command merely stores a reference spectrum. To save a spectrum, you must select **File | Save Spectral Values** from the menu.) Storing a reference spectrum is requisite before the software can calculate absorbance spectra.
2. While still in **Scope Mode**, take a dark spectrum by first completely blocking the light path going to your spectrometer. (If possible, do not turn off the light source. If you must turn off your light source to store a dark spectrum, make sure to allow enough time for the lamp to warm up before continuing your experiment.) Take the dark reading by clicking the **Dark** button in the software display area. (This command merely stores a dark spectrum. To save a spectrum, you must select **File | Save Spectral Values** from the menu.) Storing a dark spectrum is requisite before the software can calculate absorbance spectra.
3. Begin an absorbance measurement by first making sure the sample is in place and nothing is blocking the light going to your sample. Then select **Absorbance** under **Mode of Operation** in the software display area. Click on the **Scan** button in the display area to take a scan. If **Single** is selected, only one scan will be taken. If **Continuous** is selected, the spectrometer will continuously take scans until you click on the **Stop** button. To save the spectrum, select **File | Save Spectral Values** from the menu.

   If at any time any sampling variable changes -- including integration period, averaging, boxcar smoothing, distance from light source to sample, etc. -- you must store a new reference and dark spectrum.

Transmission Experiments

Transmission is the percentage of energy passing through a system relative to the amount that passes through the reference. Transmission Mode is also used to show the portion of light *reflected* from a sample. Transmission and reflection measurements require the same mathematical calculation. The transmission is expressed as a percentage (%Tλ) relative to a standard substance (such as air). The software calculates %Tλ (or %Rλ) by the following equation:

$$\%T_\lambda = \frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda} \times 100\%$$

where S is the sample intensity at wavelength λ, D is the dark intensity at wavelength λ, R is the reference intensity at wavelength λ.

Common applications include measurement of transmission of light through solutions, optical filters, optical coatings, and other optical elements such as lenses and fibers.

> For transmission of light through solutions, we offer a transmission dip probe with screw-on, removable tips in 2-mm, 5-mm or 10-mm path lengths. Contact Ocean Optics for more information.

To take a transmission measurement:

1. Select **Scope** under **Mode of Operation** in the software display area. Make sure the signal is on scale. Adjust acquisition parameters so that the peak intensity of the reference signal is about 3500 counts. Take a reference spectrum by first making sure nothing is blocking the light path going to your spectrometer. The analyte you want to measure must be absent while taking a reference spectrum. Take the reference reading by clicking the **Reference** button in the software display area. (This command merely stores a reference spectrum. To save a spectrum, you must select **File | Save Spectral Values** from the menu.) Storing a reference spectrum is requisite before the software can calculate transmission spectra.
2. While still in **Scope Mode**, take a dark spectrum by first completely blocking the light path going to your spectrometer. (If possible, do not turn off the light source. If you must turn off your light source to store a dark spectrum, make sure to allow enough time for the lamp to warm up before continuing your experiment.) Take the dark reading by clicking the **Dark** button in the software display area. (This command merely stores a dark spectrum. To save a spectrum, you must select **File | Save Spectral Values** from the menu.) Storing a dark spectrum is requisite before the software can calculate transmission spectra.
3. Begin a transmission measurement by first making sure the sample is in place and nothing is blocking the light going to your sample. Then select **Transmission** under **Mode of Operation** in the software display area. Click on the **Scan** button in the display area to take a scan. If **Single** is selected, only one scan will be taken. If **Continuous** is selected, the spectrometer will continuously take scans until you click on the **Stop** button. To save the spectrum, select **File | Save Spectral Values** from the menu.

> If at any time any sampling variable changes -- including integration period, averaging, boxcar smoothing, distance from light source to sample, etc. -- you must store a new reference and dark spectrum.

Reflection Experiments

Reflection is the return of radiation by a surface, without a change in wavelength. The reflection may be:

- Specular, in which the angle of incidence is equal to the angle of reflection.
- Diffuse, in which the angle of incidence is not equal to the angle of reflection.

Every surface returns both specular and diffuse reflections. Some surfaces may return mostly specular reflection, others more diffuse reflection. The glossier the surface, the more specular the reflection. Reflection is expressed as a percentage (%Rλ) relative to the reflection from a standard substance (such as our WS-1 white reference for a diffuse reflection measurement):

$$\%R_\lambda = \frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda} \times 100\%$$

where S is the sample intensity at wavelength λ, D is the dark intensity at wavelength λ, R is the reference intensity at wavelength λ.

Common applications include measuring the reflection properties of mirrors, anti-reflection coatings, and measuring the visual properties of the color in paints, graphic arts, plastics, and food products.

> The components that came with the CHEM2000 will not allow the user to make reflection measurements. A reflection probe is necessary. We offer several components for reflection measurements such as a variety of reflection probes, a reflection probe holder, diffuse reflectance standards and an integrating sphere. Contact Ocean Optics for more information.

To take a reflection measurement:

1. Select **Scope** under **Mode of Operation** in the software display area. Make sure the signal is on scale. Adjust acquisition parameters so that the peak intensity of the reference signal is about 3500 counts. Take a reference spectrum by first making sure nothing is blocking the light path going to your spectrometer. The analyte you want to measure must be absent while taking a reference spectrum. Take the reference reading by clicking the **Reference** button in the software display area. (This command merely stores a reference spectrum. To save a spectrum, you must select **File | Save Spectral Values** from the menu.) Storing a reference spectrum is requisite before the software can calculate reflection spectra.
2. While still in **Scope Mode**, take a dark spectrum by first completely blocking the light path going to your spectrometer. (If possible, do not turn off the light source. If you must turn off your light source to store a dark spectrum, make sure to allow enough time for the lamp to warm up before continuing your experiment.) Take the dark reading by clicking the **Dark** button in the software display area. (This command merely stores a dark spectrum. To save a spectrum, you must select **File | Save Spectral Values** from the menu.) Storing a dark spectrum is requisite before the software can calculate reflection spectra.
3. Begin a reflection measurement by first making sure the sample is in place and nothing is blocking the light going to your sample. Then select **Transmission** under **Mode of Operation** in the software display area. The mathematics required to calculate reflection measurements are identical to those necessary to compute a transmission spectrum. Click on the **Scan** button in the display area to take a scan. If **Single** is selected, only one scan will be taken. If **Continuous** is selected, the spectrometer will continuously take scans until you click on the **Stop** button. To save the spectrum, select **File | Save Spectral Values** from the menu.

> If at any time any sampling variable changes -- including integration period, averaging, boxcar smoothing, distance from light source to sample, etc. -- you must store a new reference and dark spectrum.

Relative Irradiance Experiments

Irradiance is the amount of energy at each wavelength from a radiant sample. In relative terms, it is the fraction of energy from the sample compared to the energy collected from a lamp with a blackbody energy distribution, normalized to 1 at the energy maximum. Relative irradiance is calculated by the following equation:

$$I_\lambda = B_\lambda \left(\frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda} \right)$$

where $B\lambda$ is relative energy of the reference calculated from the color temperature in Kelvin, S is the sample intensity at wavelength λ, D is the dark intensity at wavelength λ, R is the reference intensity at wavelength λ.

Common applications include characterizing the light output of LEDs, incandescent lamps and other radiant energy sources such as sunlight. Also included in irradiance measurements is fluorescence, in which case the spectrometer measures the energy given off by materials that have been excited by light at a shorter wavelength.

To take a relative irradiance measurement:

1. Select **Spectrometer | Spectrometer Configuration** from the menu. Next to **Color Temp**, make sure the color temperature in Kelvin of the reference lamp you are going to use is entered here. The color temperature of the tungsten halogen light source that came with your CHEM2000 has a color temperature of 3100 Kelvin. Click **OK**.
2. Select **Scope** under **Mode of Operation** in the software display area. Make sure the signal is on scale by adjusting acquisition parameters. Take a reference spectrum of your reference lamp. Take the reference reading by clicking the **Reference** button in the software display area. (This command merely stores a reference spectrum. To save a spectrum, you must select **File | Save Spectral Values** from the menu.) Storing a reference spectrum is requisite before the software can calculate relative irradiance spectra.
3. While still in **Scope Mode**, take a dark spectrum by first completely blocking light from going to your spectrometer. Take the dark reading by clicking the **Dark** button in the software display area. (This command merely stores a dark spectrum. To save a spectrum, you must select **File | Save Spectral Values** from the menu.) Storing a dark spectrum is requisite before the software can calculate relative irradiance spectra.
4. Begin a relative irradiance measurement by first positioning the fiber at the light or emission source you wish to measure. Then select **Relative Irradiance** under **Mode of Operation** in the software display area. Click on the **Scan** button in the display area to take a scan. If **Single** is selected, only one scan will be taken. If **Continuous** is selected, the spectrometer will continuously take scans until you click on the **Stop** button. To save the spectrum, select **File | Save Spectral Values** from the menu.

   If at any time any sampling variable changes -- including integration period, averaging, boxcar smoothing, distance from light source to sample, etc. -- you must store a new reference and dark spectrum.

Concentration Experiments

The absorbance of a solution is related to the concentration of the species within it. The relationship, known as Beer's Law, is:

$$A_\lambda = \varepsilon_\lambda c l$$

where A is the absorbance at wavelength λ, ε is the extinction coefficient of the absorbing species at wavelength λ, c is the concentration and l is the optical pathlength.

Concentration is the amount of a specified substance in a solution. Graphs of absorbance vs. concentration are known as Beer's Law plots. These are prepared by measuring the light absorbed by a series of solutions with different *known* concentrations. The length of the sample -- such as the path length of our cuvette holder -- and the wavelength chosen for monitoring the amount of light absorbed are constants. A linear plot from taking scans of these standard solutions with known concentrations is then obtained. The plot is then used to determine the *unknown* concentrations of substances in solutions.

In order to discover the unknown concentration of a substance in a solution, you must first take spectral scans of a series of solutions with different known concentrations of the same substance. You begin this process by taking an absorbance spectrum of the solution with the highest known concentration.

1. Select **Scope** under **Mode of Operation** in the software display area. Make sure the signal is on scale. Adjust acquisition parameters so that the peak intensity of the reference signal is about 3500 counts. Take a reference spectrum by first making sure nothing is blocking the light path going to your spectrometer. The solution with the highest known concentration you want to measure must be absent while taking a reference spectrum. Take the reference reading by clicking the **Reference** button in the software display area. To save the spectrum, select **File | Save Spectral Values** from the menu.
2. While still in **Scope Mode**, take a dark spectrum by first completely blocking the light path going to your spectrometer. (If possible, do not turn off the light source. If you must turn off your light source to store a dark spectrum, make sure to allow enough time for the lamp to warm up before continuing your experiment.) Take the dark reading by clicking the **Dark** button in the software display area. To save the spectrum, select **File | Save Spectral Values** from the menu.
3. Take the solution with the highest known concentration and put it in the cuvette holder. Make sure nothing is blocking the light going to your sample. Then select **Absorbance** under **Mode of Operation**. Click on the **Scan** button in the display area to take a scan. Make sure **Single** is selected. To save the spectrum, select **File | Save Spectral Values** from the menu.
4. Now select the wavelength for monitoring the concentration of your solutions by choosing **Spectrometer | Select Concentration Wavelength** from the menu. Move the cursor to the highest absorbance peak of the spectrum of the solution with the highest known concentration and choose **Select**.
5. Remove the solution with the highest known concentration. Select **Spectrometer | Calculate a Calibration Curve** from the menu. The Calculate Calibration Curve dialog box opens. Now you will begin taking scans of the rest of your series of standard solutions with known concentrations, from the lowest known concentration to highest, all while working in this dialog box.
6. If you wish, you can take a new reference and a new dark scan for each solution by choosing **Scan | Dark** and **Scan | Reference** from the menu of this dialog box. However, in this case, it is not necessary. If no reference or dark scan is taken at this point, the software will use the reference and dark scans taken in Steps 1 and 2 to calculate absorbance.
7. Take the solution with the lowest known concentration and put it in the cuvette holder. Enter the known concentration of the standard solution in the chart in the **Concentration** column, next to **Solution #1**.
8. Click the **Scan** button or select **Scan | Solution** from the menu. The absorbance value will appear next to the concentration for Solution #1. At any point, you can select **Edit | Clear** from the menu to clear the dialog box of all data.
9. Take Solution #1 out of the cuvette holder and put in another standard solution with the next highest known concentration. Enter the known concentration of the standard solution in the chart in the **Concentration** column, next to **Solution #2**.
10. Click the **Scan** button or select **Scan | Solution** from the menu. The absorbance value will appear next to the concentration for Solution #2.

Concentration Experiments (continued)

11. You may continue to scan solutions with known concentrations. You must scan at least 2 in order to achieve a calibration curve.
12. When you have completed taking scans of your solutions with known concentrations, click the **View Calibration Curve** button. You will then have the **Intercept** and **Slope** of your curve. The Slope is the ε necessary to compute Beer's Law and to find the *unknown* concentration of a solution.
13. At this time, you may also select a label for your concentration values, such at **Moles per Liter**, in the **Concentration Units** box. This is only a label and does not affect the data in any way.
14. Select **Edit | Show Legend** from the menu to display the legend for the calibration curve. The legend allows you to choose a plot design, point style, line style, line width and plot color. Select **Edit | Show Palette** from the menu to display a variety of options for configuring the curve. The palette provides features such as autoscaling, graph formatting, value precision, mode mapping, and graph positioning.
15. You can select **File | Print** from the menu for the dialog box to print the dialog box. To save the current calibration curve data, select **File | Save** from the menu for the dialog box.
16. Select **File | Close** from the menu for the dialog box to close this dialog box and return to the main window.
17. When a message box asks if you would like to use this calibration curve when calculating concentration values, select **Yes**.
18. Now that you are back in the main display window, place the solution with the *unknown* concentration of a substance in the cuvette holder.
19. Under **Mode of Operation**, select **Concentration**. Click the **Scan** button to receive your concentration values.

Kinetics Experiments

Select **Spectrometer | Kinetics Configuration** from the menu to configure and establish the parameters for a kinetics experiment. In the **Kinetics Configuration** dialog box, you can collect spectral data as a function of time, from up to 4 single wavelengths and up to two mathematical combinations of these wavelengths.

A kinetics experiment will not be displayed in the graph unless you choose **Spectrometer | Spectrometer Configuration** from the menu and choose **Spectrum & Kinetics** next to **Graph and chart display mode**. This way, not only will your kinetics experiment be displayed in the bottom half of the graph area, you will also still see a spectrometer channel's real-time spectra in the top half of the graph area.

To run a kinetics experiment:

1. In the graph area, select acquisition parameters, such as integration period, averaging, and boxcar smoothing values. Do not change these parameters for the duration of the kinetics experiment.
2. Select **Spectrometer | Kinetics Configuration** from the menu to open the Kinetics Configuration dialog box.
3. Enter a **Preset Duration** value to set the length of time for the entire kinetics process. Be sure to select hours, minutes and seconds. The duration of your kinetics experiment cannot exceed 24 hours.
4. Enter a **Preset Sampling Interval** value to set the frequency of the data collected in a kinetics process. Be sure to select hours, minutes and seconds. Data from a timed acquisition is stamped with a time that is accurate to 1 millisecond.
5. Under **Wavelength**, enter the single wavelengths from which you wish to collect data. You can collect data from up to 4 single wavelengths, characterized as **a**, **b**, **c**, and **d**.
6. If you want the data graphed from these single wavelengths, or from the mathematical calculations (described in the next step), select the **Display** box to the right of your values.
7. In the boxes next to **x =** and **y =**, you have the opportunity to perform calculations on the data collected from the single wavelengths you specified as **a**, **b**, **c**, and **d**. In the **y =** box, you can also use **x**, which represents the calculation used in **x =**.
8. Click **OK** to confirm the parameters and close the dialog box.
9. Click the **Scan** button to begin the kinetics experiment. Make sure that **Continuous** is selected. The top half of the graph displays a real-time full wavelength spectrum. The bottom half of the graph displays the data for the selected wavelengths and their derived arithmetic calculations. Each data set is stored with a time stamp.
10. Click the **Stop** button to stop the experiment. However, if a **Preset Duration** time was selected, the experiment will automatically stop after the designated time has passed.
11. Select **File | Save Kinetics** from the menu to save a tab-delimited ASCII file with the spectrometer's serial number, active channel and acquisition parameters in a header. This file can be opened with any text or spreadsheet editor.