Ocean Optics OOIBase32
(from Dr. David Chesney)

Set-up and positioning of the Ocean Optics Spectrophotometer

1. 1. On the top shelf (where the computers are) lay out the following components:
    a. a. USB2000
    b. b. USB cable for the USB2000 (white)
    c. c. CHEM2000
    d. d. Power supply for the CHEM2000
    e. e. Fiber-optic cable (blue)
2. 2. Connect the fiber optic cable between the CHEM2000 and the USB2000. Do not bend or stress the cable in any way. Lay out the components such that there is minimal bend in the fiber optic or stretch them out in a linear fashion.
3. 3. Connect the power supply to the CHEM2000. Turn the unit on using the small switch in the back.
4. 4. Connect the USB cable to the USB2000 module. Plug the other end into the USB port in the front of your computer.
5. 5. Start up and log in to the computer.
6. 6. From the START menu, choose Programs/Ocean Optics/OOIBase32
7. 7. You should get a display that looks like the one here. Expand the outer window so that the inner (spectrum) window is completely exposed. The inner window should be displaying some non-zero signal.

Operation

1. 1. Position your cursor over each of the buttons in the top button bar of the inner (spectrum) window. NOTE: The buttons are identified by icon on page 32 of the OOIBase32 Spectrometer Operating Software Manual. The buttons of immediate importance are highlighted in the list below. From the left these are:
    a. a. Store Dark
    b. b. Store Reference
    c. c. Snapshot
    d. d. Data Acquisition
    e. e. Emergency Reset
    f. f. Subtract Dark Spectrum

g. g. **Scope Mode**
h. h. Absorbance Mode
i. i. Transmission Mode
j. j. Irradiance Mode
k. k. Configure Spectrum and View
l. l. Autoscale
m. m. Set Scale
n. n. Unscale
o. o. Toggle Cursor
p. p. Cursor Peak Left
q. q. Cursor Big Left
r. r. Cursor Left
s. s. Cursor Right
t. t. Cursor Big Right
u. u. Configure Cursor

2. 2. We will ignore the second row of buttons for now.
3. 3. Directly above the spectrum display area is a row of response boxes. We are only concerned with the first three for now. From the left:

   Integ. Time (Integration Time)
   Average
   Boxcar
4. 4. With the spectrometer in **Scope Mode** (blue S showing), adjust the **Integration Time** to cause the highest peak in the spectrum to read about 3500 counts on the y-axis. See this spectrum.
5. 5. Adjust the value in the **Average** box to change the number of discrete spectral scans to accumulate before showing a spectrum. Note that the higher the value, the smoother the resulting spectrum. Also, the longer it takes to refresh the spectrum. The signal-to-noise ratio of the spectrum improves by the square root of the number of spectra averaged. A value of 32 is a suitable compromise.
6. 6. Adjust the value in the **Boxcar** box. Note that the higher the value, the smoother the resulting spectrum. However, if the value entered becomes too large, a significant loss in spectral resolution results. A value of 10-12 is reasonable for UV-Vis spectra.
7. 7. Depress the **Store Reference** button.
8. 8. Block the light beam. The spectrum should be just a straight line along the bottom of the display. Depress the **Store Dark** button.
9. 9. Un-block the light beam. You should have a spectrum similar to what you had previously in the display window.

What you did: The fiber optic cable transmits different wavelengths of light to varying degrees. (Note that the "spectrum" in the display is not of uniform intensity from left to right.) You need to subtract this "cable spectrum" from your analytical spectra.

The dark spectrum corrects for any signal output which does not originate at the cuvette holder.

HOWEVER, the "cable spectrum" will change if you vary the position of the cable or any of the modules of the spectrometer system. Hence, **it is very important that you do not move any part of the spectrometer system once you have stored a reference (cable) spectrum. If you do move the components, you need to take and store another reference and dark spectrum.**

Of course, the "cable spectrum" also changes if you change the acquisition parameters (Integ. Time, Average and Boxcar). **If you change any of these parameters, you must take and store a new reference and dark spectrum before proceeding.**

Absorbance Measurements

1. 1. In **Scope Mode** with an empty cuvette in place in the cuvette holder, adjust the acquisition paramters (Integ. Time, Average and Boxcar) to produce a suitable signal. Take and store a reference spectrum.

   NOTE: In many cases, you need a reference spectrum of your solvent. In that case, take the reference spectrum at this point with clean solvent in your cuvette.

2. 2. Remove the cuvette and block the light beam. Take and store a dark spectrum. Do not change any data acquisition parameters (Integ. Time, Average or Boxcar) from this point on without taking new reference and dark spectra.
3. 3. Replace the empty cuvette.
4. 4. Click on **Absorbance Mode** in the button bar. Your spectrum should have zero absorbance across the selected wavelength range.
5. 5. Adjust scale parameters with the **Set Scale** button.

   Typically, the Absorbance axis ranges from 0 – 1 A.U.

(Absorbance Unit), and the wavelength axis is truncated to show only the peak(s) of interest.

6. 6. Display the cursor using the **Toggle Cursor** button. You should see a vertical green line show up in the spectrum display. Also, the six cursor movement buttons light up with yellow arrows.

   Experiment with moving the cursor. The mouse is used for gross movement; The button bars are used for progressively finer adjustments. Note that the wavelength and absorbance information is displayed in the <u>lower left hand corner</u> of the display window.

   A typical display reads: M: 435.18 nm—M: 702, 0.0025
   The first number is the wavelength in nanometers; The second number is the pixel number (ignore it); And the last number is the signal in absorbance units corresponding to this wavelength.

7. 7. Remove the cuvette; Place your sample in the cuvette and replace it in the cuvette holder. NOTE: Always remove the cuvette from the CHEM2000 unit when changing cuvette contents to avoid spills and contamination of the spectometer system.
8. 8. Save your sample spectrum by clicking File/Save/Processed. Type in a file name and choose a folder in which to store it.
9. 9. To take subsequent spectra, just change the cuvette contents and replace the cuvette. Save the spectrum as in step 8.

<u>Displaying Spectra</u>

To display a stored spectrum, click File/Open/Processed. Choose the spectrum file to display by highlighting it and clicking Open. Active data acquisition halts while you display the saved spectra (note the camera icon is depressed).

<u>Overlaying Spectra</u>

To overlay spectra on top of one another so that a series of spectra can be compared to one another, select Overlay from the menu bar and then choose a **Select to add overlay** slot. When the drop-down menu appears, choose a saved spectrum file for the overlay. To clear an individual file, select one of the files from the drop-down window. You can clear all the overlay files by selecting **Clear All** in the drop-down window.

Copying Spectra (as displayed)
Click on the copy icon or select Edit/Copy Graphical Spectra from the menu bar. The graphic spectrum is copied to the clipboard and can then be pasted into any application that accepts a Windows metafile (MS Word, etc.).

Copying Spectral Data (as numerical data)
There may be times when having the actual numerical data (signal vs. wavelength) would be necessary. Select Edit/Copy Spectral Data from the menu bar. The spectral data is copied to the clipboard and can be pasted directly into MS Excel or a similar application. The spectral data is tab-delimited and includes a header to allow for easy identification of the spectrum.

Transmittance Measurements

1. 1. In **Scope Mode** with an empty cuvette in place in the cuvette holder, adjust the acquisition paramters (Integ. Time, Average and Boxcar) to produce a suitable signal. Take and store a reference spectrum.

   NOTE: In many cases, you need a reference spectrum of your solvent. In that case, take the reference spectrum at this point with clean solvent in your cuvette.

2. 2. Remove the cuvette and block the light beam. Take and store a dark spectrum. Do not change any data acquisition parameters (Integ. Time, Average or Boxcar) from this point on without taking new reference and dark spectra.
3. 3. Replace the empty cuvette.
4. 4. Click on **Transmission Mode** in the button bar. Your spectrum should have 100% transmittance across the selected wavelength range.
5. 5. Adjust scale parameters with the **Set Scale** button.

   Typically, the Transmittance axis ranges from 0 – 100% T (percent Transmittance), and the wavelength axis is truncated to show only the peak(s) of interest.

6. 6. Display the cursor using the **Toggle Cursor** button. You should see a vertical green line show up in the spectrum display. Also, the six cursor movement buttons light up with yellow arrows.

Experiment with moving the cursor. The mouse is used for gross movement; The button bars are used for progressively finer adjustments. Note that the wavelength and transmittance information is displayed in the lower left hand corner of the display window.

A typical display reads: M: 435.18 nm—M: 702, 100.0025
The first number is the wavelength in nanometers; The second number is the pixel number (ignore it); And the last number is the signal in percent transmittance corresponding to this wavelength.

7. 7. Remove the cuvette; Place your sample in the cuvette and replace it in the cuvette holder. NOTE: Always remove the cuvette from the CHEM2000 unit when changing cuvette contents to avoid spills and contamination of the spectometer system.
8. 8. Save your sample spectrum by clicking File/Save/Processed. Type in a file name and choose a folder in which to store it.
9. 9. To take subsequent spectra, just change the cuvette contents and replace the cuvette. Save the spectrum as in step 8.
10. To display a stored spectrum, click File/Open/Processed. Choose the spectrum file to display by highlighting it and clicking Open.

Displaying Spectra

To display a stored spectrum, click File/Open/Processed. Choose the spectrum file to display by highlighting it and clicking Open. Active data acquisition halts while you display the saved spectra (note the camera icon is depressed).

Overlaying Spectra

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