

## Ocean Optics Spec Protocol:

Written: 01/04/07 mengels; updated 09/05/08 wkoz

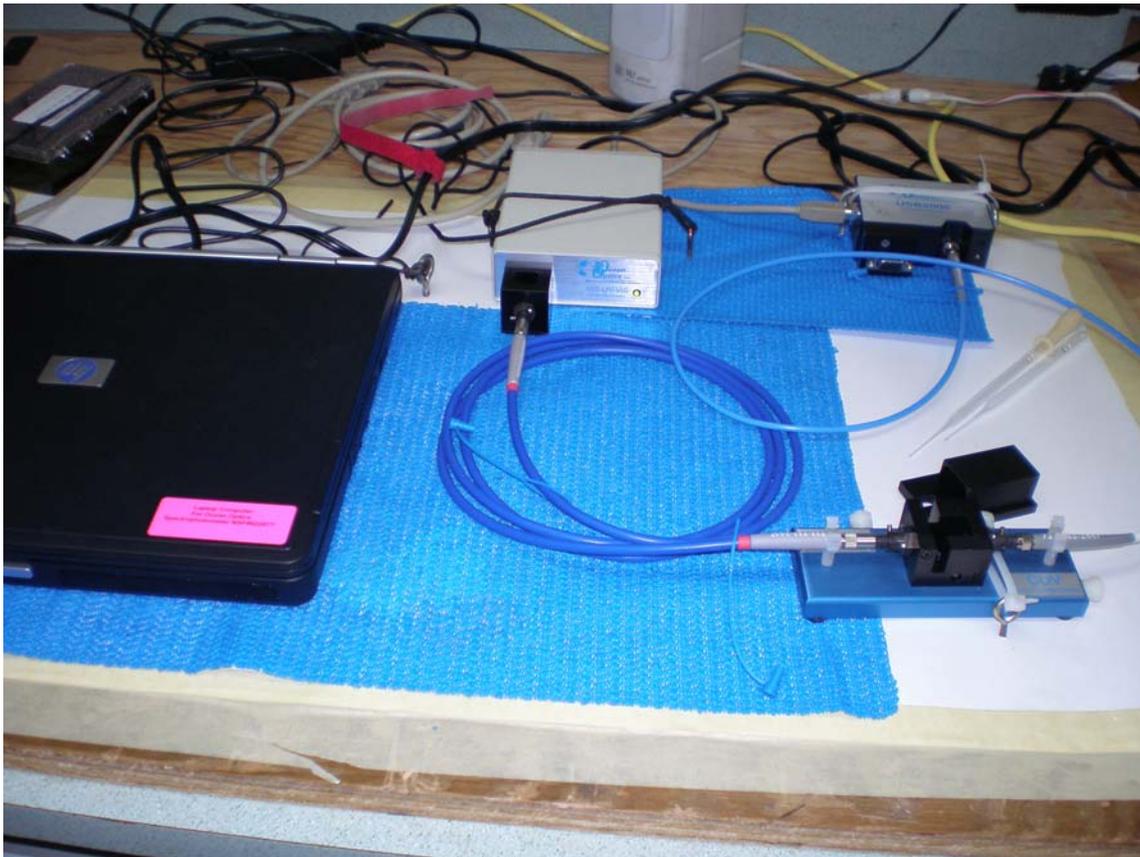
### Set up:

The Ocean Optics spectrophotometer should be set up in as indicated in the photo below.

The light source is the box labeled ISS-UV-VIS and the detector is the USB2000. Both of these have external ports covered with red covers. There are also port covers on the external ports on either side of the sample holder. Once removed make sure to keep the port covers together.

It is important to note that there are two different sized optical cables. The larger width cable (400 micron, large grey boot, small red band) goes between the light source and the input side of the spec sample holder. The smaller diameter cable (300 micron, large grey boot, small grey band) goes between the output side of the spec sample holder to the detector. Important: Make sure there are no kinks in the optical cables. These are the heart of this detection system and if mishandled can cause errors.

The detector connects to the back of the computer via USB cable or serial cable. This set up shows the USB type connection. There are two sample holders that come with this setup. One is fully adjustable and the other is specifically a 1-cm sample holder. The setup shown should work for either sample holder.



### Running Blanks:

Make sure that the light source is fully warmed up (at least half an hour). There are three settings on the light source (ON, OFF and REMOTE). The green led on the front of the light source will be on as long as the light source is plugged in. Start up OOIBASE on the Spec computer. Insert a sample of acetone (blank) in the spec sample holder. The orientation of the sample container is important; make sure it is the same each time. The amount of acetone (or sample) need only cover the light path so about one glass pipettes worth is sufficient. Cover the sample with the sample cover to eliminate any ambient light from reaching the sample.

On start-up the screen will be in Scope (S) mode. And the graph will display wavelength vs. counts. With the blank in the sample holder the height of the highest point of the graph should be ~3500 counts. You can change the intensity of the counts by changing the Integration Time, located just above the graph. Bigger IT numbers = more counts. You can think of this like the shutter speed. Next set Average to what you think might be appropriate. Average indicates the number of discrete samples will be averaged together in order to determine a spectrum. Bigger Average numbers equal better signal to noise ratios. Good numbers seem to be something between 25-50. Finally there is the boxcar setting. This is a smoothing filter that averages adjacent detector elements. The bigger the number, the smoother the data, but, if too big, resolution will be lost. Something under 10 usually works well.

With all the settings adjusted properly and the blank in the detector click once on the Store Global Reference (indicated by a light bulb). Note: There are two light bulbs on this screen, make sure you are using the Store Global Reference on the first line and not the Store Reference on the second line. Next completely block the light path using the sample cover. Slide the cover toward you until the spectrum goes completely to nothing. Now click once on the Store Global Dark (indicated by a dark bulb). Again, first line only, the second line is Store Dark (for individual samples). Now you have your references. Note, if you change the IT, Average or Boxcar you will need to re-store the reference.

- Note that if there are multiple channels designated in the spec setup, you can run separate references for each (the second lightbulb)... if only one master channel is designated, you can use just the Global Reference.

Next, with the light path uncovered click into Absorbance mode (A on the tool bar). The absorbance spectrum should be zero except perhaps at the very start of the spectrum. Now you are ready to run samples.

### Running Absorbance Samples:

With the room dark, pipette sample into the 1-cm spec cell and place into detector. The spectrum should come up on the screen. Give the spectrum about 30 sec to settle out before saving. You can zoom in on one part of the spectrum by using the View/Spectrum Scale menu; once your y-axis is defined in that dialog box, you can also just use the autoscale button to adjust the x-axis as needed. You can also find one particular point on the spectrum using the cursor line. Go to View/Cursor to enable the cursor. Once enabled the cursor can be moved along the spectrum with a double-click of the mouse or by using the arrow keys. In the lower left corner of the screen (depending on setup – but they are often found there) are three numbers that change as the cursor scrolls along. These numbers indicate wavelength, pixel

number (used in instrument calibration), and Absorbance (when in absorbance mode) at the cursor. You can read a specific absorbance at a given wavelength with the cursor method, or save a whole spectrum for later analysis in excel.

### **Saving/Exporting Data:**

There are at least two ways to save data that we found:

1. From the File menu, use Save. This saves as OOI proprietary format... which means you'll need OOIBase to read it later on.
2. From the Edit menu, Copy Spectral Data. You can select all channels or just selected channel (if you have only one set up, both options copy the same data), then you can simply select a cell in Excel and paste to get two columns of data – wavelength and absorbance (or Transmittance if that's what you're reading). Note that if you use this method you get no header or configuration information with your data, but it sure is easy.

### **Miscellaneous Notes from LMG0701:**

1. There is a small screw driver in each case, but it is not small enough to move the “bumpers” in the flow cells – ET shop has smaller flathead screwdrivers that you can be borrowed if you need to adjust for different cell types (ie quartz low volume cells are slightly bigger than the 1cm cells included with the specs)
2. Instrument NSF # 022877 (lightsource FATS # 00010467, s/n USB2G5004)
  - a. No manuals
  - b. Outside of the Storm Case was mislabeled as 022876 – corrected 02/09/07
3. Instrument NSF #022876 (lightsource FATS #0010468, s/n USB2G1015)
  - a. Exhibited significant drift over time
  - b. Relative to 022877 instrument required twice the Integration Time to achieve 3500 Intensity Counts for blanking.
  - c. Leading us to wonder if perhaps the lamp on this instrument was old and needed to be changed. No actual calibration was done to confirm this (directions in manual), but expected lamp life only 800-1000 hours, so it is possible that both issues listed above might be a result of an old lamp.
4. Leading us to make the following suggestion:

*Both instruments should have maintenance/use logs kept in the cases so users can monitor lamp usage hours, lamp changes and purchase dates of spare lamps (usually these have a limited shelf life)!*