How to Use the NANSLO Remote Spectrometer (Absorbance)

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You can also watch a video about the Absorbance Spectrometer:
http://denverlabinfo.nanslo.org/NANSLO-Lab-Info/video/absorbance_spectroscopy.html

A – INTRODUCTION TO THE MAIN CONTROL PANEL

When you access your Absorbance Spectrometry lab through the NANSLO Remote Web-based Science Lab (RWSL), you will see an interface that looks like this (Figure 1). You take control of the equipment by right-clicking on the screen and selecting Request Control of VI. You release control by right-clicking and selecting release control of VI. If you have a Macintosh computer, you may need to ctrl-click, cmd-click, or maybe two-finger tap on your touch-pad in order to “right-click” and take control of the interface. Only one person may be in control of the equipment at one time, you must release control before anyone else can take control of the equipment.
You must call into a voice conference to communicate with your lab partners and with the Lab Technicians. This is very important because only one person can be in control of the equipment at any one time, so you will need to coordinate and collaborate with your lab partners.

You may join the voice conference with your telephone or by using your computer if you have a microphone and speakers. Click on the yellow button at the bottom of the screen (you may need to scroll down to see it), and you will see this information in a pop-up (Figure 2). Follow these directions to join the voice conference. If you can’t join the voice conference for some reason, just email the Lab Technician at the address shown on the main control panel.
Figure 2: Voice Conference Information

To Join the NANSLO Voice Conference:

1. Copy this address:
   https://global.gotomeeting.com/meeting/join/123456789
2. Open a browser window using your favorite browser.
3. Click in the address bar and select Edit and then Paste (or just press CTRL-V or CMD-V).
4. Press Enter to go to the GoToMeeting site.
5. Follow the instructions to join the meeting.
6. Once GoToMeeting has started up, you can choose how you want to join the voice conference.
The controls on the right side of the screen are for controlling the camera (Figure 1 Blue dotted outline). The preset positions allow you to quickly zoom in to a different part of the setup, but you can also pan, tilt and zoom the camera using the keypad controls on the screen.

On the left side of the screen, you can see the tabs for the equipment controls that are used in this experiment (Figure 1 Red Solid outline). One piece of equipment is called the Qpod, and it is a device into which a cuvette containing a sample is placed so that light can be shined through it in order to measure absorbance. All of our cuvettes have a path length (distance that the light travels through them) of 1.00 cm.

![Figure 3: Cuvette](http://cuvette.net/)
For clarity, here is a labeled picture of the spectrometry activity equipment (Figure 3)

![Figure 3: Absorbance Spectrometer Equipment](image)

The Qpod is controlled by the Qpod controller, the Qpod also connects to a water bath to regulate its temperature. The Spectrometer produces light which passes through fiber-optic cables into the Qpod and then back out of the Qpod through another fiber-optic cable and back into the Spectrometer. Some of the fiber optic cabling that the light flows through is not visible in the photo, but you probably get the idea. The light is produced by a Xenon strobe inside the spectrometer. The light passes through whatever sample is contained in the cuvette inside the Qpod, and then returns to the sensing unit in the spectrometer.

**B - CONTROLLING THE QPOD:**

The first thing to do is turn on the Qpod’s temperature control system and ensure that it is set to 25.00 °C, which is the standard temperature for most Absorbance measurements. You do this by gaining control of the interface and clicking the button labeled “Temperature Controller” (see Figure 4a). Watch the temperature curve for a few minutes to ensure that the temperature of the Qpod is adjusted to 25.00 °C +/- 0.05 °C. If your procedure calls for it, you can also turn the Stirring control on or off from this tab. If your procedure calls for it, in order to keep the solutions in the cuvettes properly mixed.
The Cuvette Selection tab allows you to rotate the carousel that holds the six cuvettes. They are numbered 0 through 5 (see Figure 4b dotted blue box). From this tab, you can also add liquids to the cuvettes, if that is enabled for your activity. There can be up to six pumps available, in which case, you will see a pump drop-down menu in the Volume Delivery Controls section (Figure 4 Red solid box).
If the currently selected cuvette is not already full, you can choose to add liquid to it (for dilutions, etc.) depending on your lab procedure instructions. The system will not allow you to over-fill the cuvette and you can monitor how much is currently in it on this screen. Just change the Volume to be added to whatever quantity you wish to add (note: the minimum amount you can add is 0.1 ml), select a pump and then click Add Volume (see Figure 5). Which pump you select will depend on what you are trying to add to the cuvette and there will be more information in your specific lab procedure.

The temperature Ramp Controls are on the third sub-tab (see Figure 6) under the cuvette Holder tab and allow you to set temperature points and lengths of time so you can run through a pre-set temperature profile if your lab procedure calls for it.
C - BASIC FUNCTIONS OF THE SPECTROMETER:

After the temperature of the Qpod has been set properly, you are ready to proceed with taking Absorbance measurements. Click the “Spectrometer” tab to proceed.

The first thing to do is to take a “dark spectrum”, which is merely a measurement of what the spectrometer is measuring when there is no light present (see Figure 7). This establishes a level of baseline “noise” in the instrument, which will be automatically subtracted out later in the process.

First, on the Spectrometer tab of the interface, click the green button labeled “Start” the button text will change to Pause and the color will change to dark yellow. This enables the spectrometer to operate. You take and store the dark spectrum by ensuring that the “Light” is not on, and then clicking the “Store Dark” button. There will be no indication that anything happened, so if you’re not sure you clicked this button, just click it again – you won’t hurt anything by storing another dark spectrum (see Figure 7).
Figure 7: Store Dark Spectrum
At this point, turn on the spectrometer’s light source by clicking the “Light” button (Blue box Figure 8), which will then turn green. You should now see a spectrum on the screen that looks like this (Figure 8).

Figure 8: Light On
Now you need to collect and store the spectrum of the “reference sample”. The reference sample is just a cuvette full of distilled water. Selecting this cuvette in the Qpod and clicking the “Store Ref” button (Figure 9) will store a spectrum where light is being absorbed by the cuvette and by water. Having this spectrum stored allows it to be subtracted out from your later sample measurements, thus allowing you to measure the Absorption of light that is only due to the material you are interested in.

Figure 9: Store Light Spectrum

Now you are ready to measure the absorbance of whatever chemical you are interested in. Switch the currently selected cuvette to one containing a chemical of interest, and then click the “Show Absorbance” Spectrum button to view the ratio of the currently selected cuvette to the previously stored Reference Spectrum. After you click the “Show Absorbance” Spectrum Button your graph may appear blank. See section D to learn about resizing your graph. See paragraph F - Finding Lambda-max: for more information about the Absorbance Spectrum.
D - ZOOMING IN AND OUT ON THE SPECTRUM:

There will be times when you want to examine different parts of a spectrum more closely, or to view an entire spectrum full-scale. Here are the steps for doing this:

A. Click on the Zoom controls button at the lower right of the graph, shown below in Figure 10a.

B. This brings up a small sub-menu of six other buttons. The only two that are useful to you are the two left-most buttons in the top and bottom rows (See Figure 10b). You can play around with the others if you want to. Select the left-most button in the bottom row to view the entire spectrum.

C. Select the left-most icon in the top row to select specific parts of the spectrum to “zoom in” on and view more closely. After clicking this button, you use the mouse to draw a box around the area that you want to zoom in on. Be sure you draw the box so that it includes some area past the top of the peak you are interested in, or else it will chop off the top of it in the viewing window.

D. If you accidentally zoom in too far or on the wrong part of the spectrum, just zoom out and start over again.

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**Figure 10: Spectrum Manipulation**

10a: Zoom Controls

10b: Zoom In and Out
E - SMOOTHING OUT THE SPECTRUM:

When you first turn on the spectrum (after you clicked the start button) do you notice how much the spectrum is jumping around? This is due to noise in the data. Look at the Spectrometer Tab (Figure 11). See the fields called “Integration Time”, “Boxcar Width”, and “# Spectra to Average” (Figure 11 Blue box)? These are variables that you can adjust to “clean up” the noise in the spectrum. The integration time is how many milliseconds the spectrometer will wait before it stores a spectrum, and you should leave it set to 50000. The Boxcar Width is how many sequential points in the spectrum will be averaged to produce one point on the curve. The “# Spectra to Average” variable tells the spectrometer how many spectra to average before it reports a result. Just like any other measurement that contains random error (“noise”), averaging several measurements can average out the noise and “clean up” the signal. Play around with these settings to see what effect they have on the spectrum. Once you find a setting that gives you results that you think are good, stick with them for the rest of the experiment.
**F - FINDING LAMBDA-MAX:**

This is merely identifying the tallest peak in the Absorbance spectrum, and you only need to do this once for a chemical, no matter how many different solution concentrations you measure. Use the Cuvette Selection controls to select a cuvette containing a solution you wish to measure, with the light turned on, and the cursor enabled, click the “Show Absorbance Spectrum” button and then zoom all the way out on the spectrum. You will now see the absorbance spectrum of the sample, as in Figure 12. Click the Cursor Control button (any time you move the cursor you need to click this button) and move the cursor, clicking on the point where the green line (cursor) crosses the spectrum, to the top of the tallest peak using the mouse.
You can ignore the noisy parts of the spectrum on both ends. There may be one peak as shown in Figure 12, or there may be more than one. Always use the tallest absorbance peak. With the curser on top of the tallest peak, you can read the wavelength in the “Cursor Location Information” line. In this case, the tallest peak is at 395.6 nm. This is \( \lambda_{\text{max}} \), and the absorbance at \( \lambda_{\text{max}} \) is shown in the Absorbance at Wavelength box. Remember, \( \lambda_{\text{max}} \) does not change as long as you are measuring the absorbance of the same chemical so you do not need to adjust the cursor location after you have it set it.