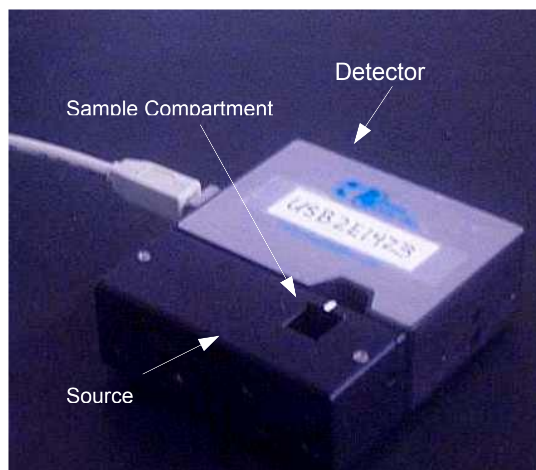
Diode Array Spectrometer

Jump to Spectrometer Operating Instructions

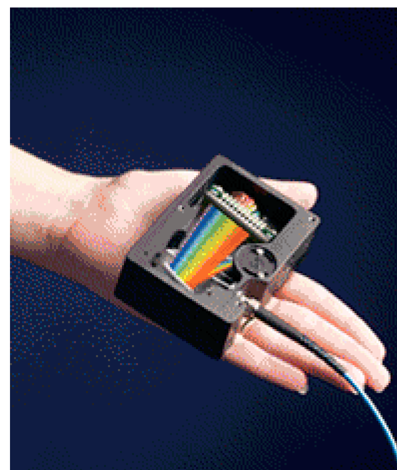
Visible light is electromagnetic radiation ranging in wavelength from about 350 to 750 nm. If a light source uniformly emits electromagnetic radiation in this range, the emitted light appears white. Some close approximations include light emitted from the sun and from incandescent light bulbs. White light is a composite of many wavelengths of light. This is readily shown by separating it into its component colors (wavelengths) by refraction or diffraction. Refraction or diffraction of white light produces the familiar rainbow of colors. (Indeed a rainbow is produced by the refraction of light by raindrops.)

A colored solution appears colored because something in the solution selectively absorbs visible light at one or more but not all wavelengths. Light that is not absorbed by the solution passes through (is transmitted by) the solution, and the solution takes on the color of the transmitted light.

When you observe a colored solution, ambient light serves as the light source that is incident on the solution and your eye detects the color of the solution. The diode array spectrometer operates similarly except that a tungsten lamp serves as the light source and 2048 pixel element linear CCD (Charge Coupled Device) array serves as the detector. Light from the source passes through the sample of interest and into a monochromator. Here it is collimated and reflected onto a diffraction grating that separates it into its component wavelengths. The resulting diffracted light is then projected onto the CCD detector. Data (photon flux) captured by the CCD array is transferred to a computer via an A/D card and displayed. Most surprisingly, the source and detector contain no moving parts and fit in the palm of your hand!



Diode Array Spectrometer with Integrated Visible Source and Sample Compartment



Cutaway View of Spectrometer

The detector is the same type of detector used in digital camera and scanners. And much like a camera, the CCD-array in the spectrometer takes a continuous real-time 'picture' of light impinging on it. (The associated spectrometer software calculates and displays this 'picture' in graphical form via a computer interface.)

The diode array spectrometers used in lab can detect both ultraviolet (UV) and visible light – from 200 to 850 nm. Hence, they can be used to quantitatively measure the amount of light at any wavelength in this range that is absorbed by a solution.

The most common display modes are transmittance and absorbance. Transmittance, T, is defined as the fraction of incident light of a particular wavelength transmitted by a solution:

$$T_\lambda = (I)_\lambda/(I_o)_\lambda$$

Where $(I_o)_\lambda$ is the intensity of incident light at a particular wavelength on a solution and $(I)_\lambda$ is the intensity of light transmitted by the solution. Percent transmittance is simply the transmittance times 100.

And absorbance, $A = -\log T = -\log (I)_\lambda/(I_o)_\lambda = \log (I_o)_\lambda/(I)_\lambda$.

The spectrometer software calculates $(I_o)_\lambda$ and $(I)_\lambda$ from three intensity measurements at each wavelength: 1) reference (blank), R; 2) dark, D; 3) sample, S. These are obtained as follows:

$$(I_o)_\lambda = R_\lambda - D_\lambda$$

$$(I)_\lambda = S_\lambda - D_\lambda$$

Absorbance is directly proportional to the concentration of absorbing species in solution. This relationship, known as the Beer-Lambert Law, can be expressed as:

$$A = abc$$

where A is the absorbance, *a* is the absorbtivity which is a constant at a given wavelength and is characteristic of the absorbing species, *b* is the length of the absorbing medium, and *c* is the absorbing species concentration. Both *a* and *b* are constants and the product of these two values is also a constant, k; hence, the Beer-Lambert Law can be rewritten as:

$$A = kc$$

The most common application of the Beer-Lambert law is concentration determination. In this situation, A is measured using a spectrophotometer and *k* is known. While *k* is usually known, its value is determined by experiment. Typically, a series of solutions of differing but known concentration (of the species of interest) are prepared. Absorbance measurements are then made and *k* evaluated from the collected data. This evaluation is frequently made and presented in form of a graph of absorbance versus concentration – a calibration curve; the resulting curve is a straight line with a slope equal to *k*. A more precise but less visual way of evaluating *k* is to compute a series of individual *k*s from each (A, *c*) pair and then take the average.

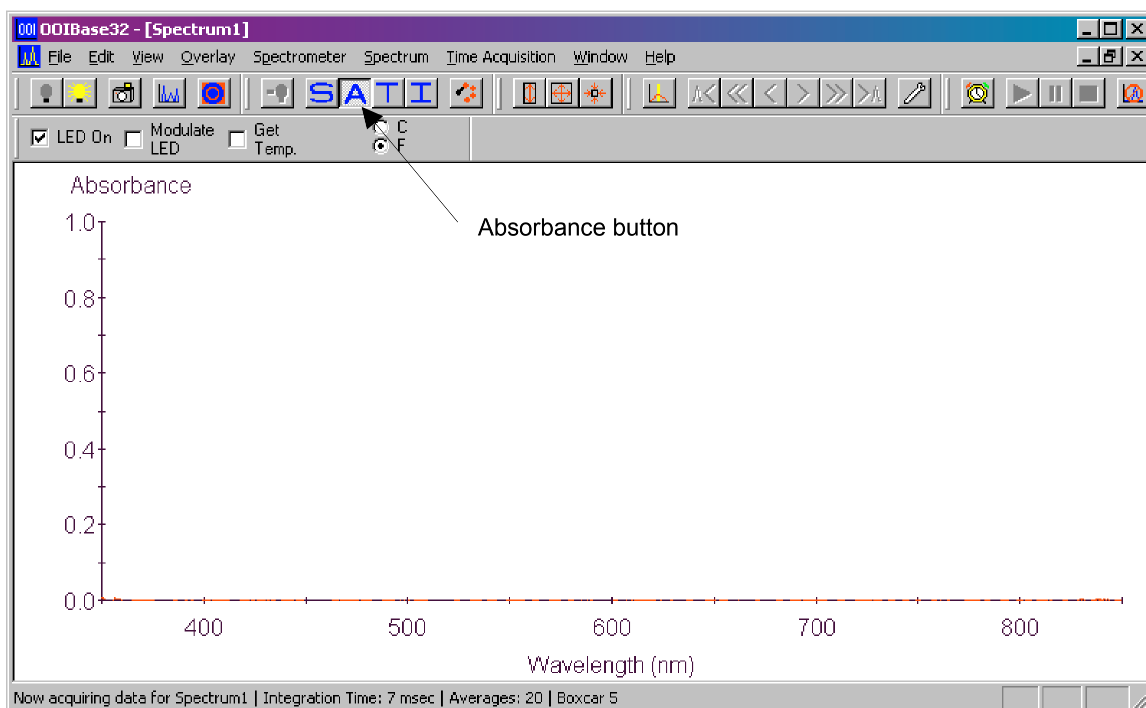
Using the Diode Array Spectrometer

Obtaining an absorbance reading involves three steps: 1) acquiring and storing a reference spectrum using a blank – typically the solvent used to dissolve the substance of interest; 2) acquiring and storing or alternatively loading a previously saved dark spectrum; your instructor or stockroom personnel will take care of this step in advance of lab; 3) acquiring and displaying the absorbance spectrum of the sample and recording absorbance at a specified wavelength.

<u>Getting Started</u>:

1. Bring solutions, deionized water wash bottle, and 250-mL beaker to a nearby instrument.

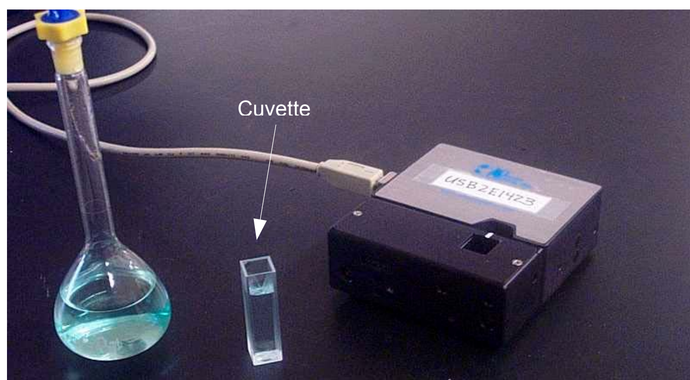
2. The computer and spectrometer should be on, and the spectrometer operating software (OOIBase32) should be running. If it is, the main spectrometer window will be evident and it will look similar to the one shown (next page).
3. The spectrometer should be configured and ready to use. If so, the spectrometer will be in absorbance mode – **A**(bsorbance) button depressed. In addition, the spectrometer number and acquisition parameters displayed at the bottom left of the spectrometer window – the main status bar – will match those posted on the monitor frame.

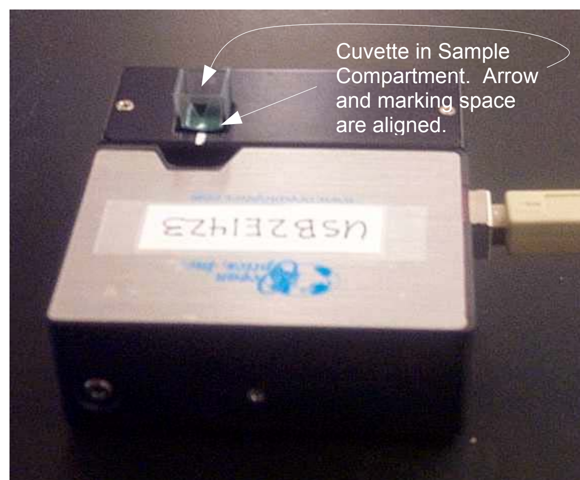


Main status bar

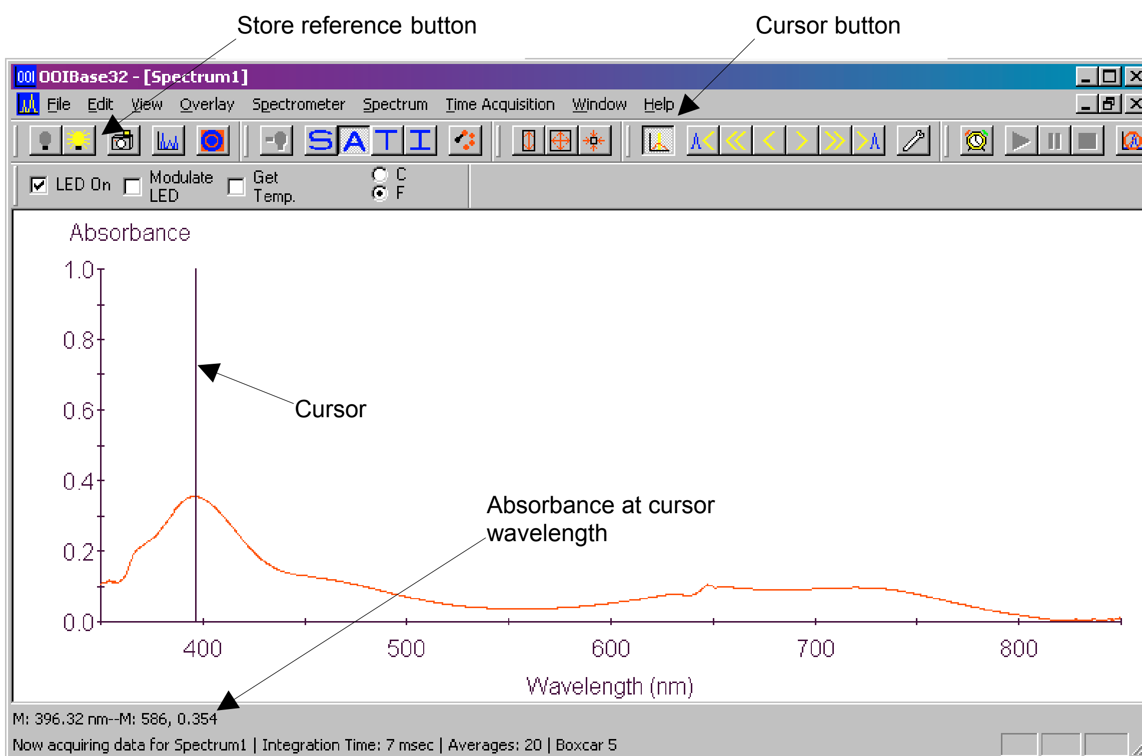
Making Measurements:

1. Obtain a rectangular cuvette. Two sides of the cuvette are frosted and two are transparent. There is an alignment arrow at the top of one of the transparent sides. Handle the cuvette by the frosted sides only. Always wipe the transparent sides with a Kimwipe prior to inserting the cuvette into the sample compartment.
2. Fill the cuvette two-thirds full with deionized water. Insert the cuvette into the sample compartment and orient it so that the arrow on the cuvette window and the white line on the sample compartment are aligned. Press firmly to ensure that the cuvette is properly seated – the top half-inch will protrude from the sample compartment.





3. Acquire and store a reference spectrum by pressing the illuminated light bulb button.
4. Remove the cuvette from the sample compartment, discard the water, and shake out the excess onto a Kimwipe. Rinse the cuvette with a small portion of the solution of interest – the most dilute if sequential measurements are being made. Discard the rinse. Fill the cuvette two-thirds full with a fresh portion of this solution. Wipe it with a Kimwipe and place it in the sample compartment. Align marks.
5. The entire visible spectrum of the sample is displayed, but only the absorbance at λ_{max} is required. When the cursor is active, the absorbance at the cursor position is displayed in the main status bar – last value on the first line. Position the cursor at the correct wavelength and record the absorbance. The cursor should be active and positioned at the correct wavelength. If not activate the cursor by pressing the cursor button and position it at the wavelength of interest using the mouse pointer, arrow keys or cursor control buttons. These items are highlighted in the screen shot of the absorbance spectrum of a Ni^{2+} (aq) below.



6. Return the solution in the cuvette to its original container and shake out the excess onto a Kimwipe. Rinse the cuvette with the next solution of interest and discard. Fill the cuvette two-thirds full with a fresh portion of this solution and determine the absorbance.
7. Determine the absorbance of remaining solutions by repeating the previous step.
8. When you finish take all your items with you. Leave OOIBase32 running. Do not exit this program or turn the computer off.