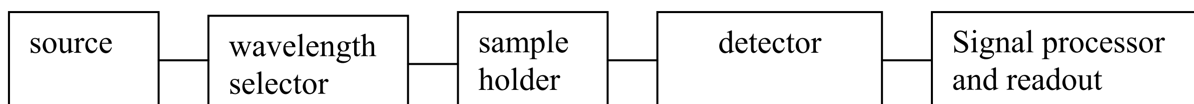
University of Wisconsin
Chemistry 524
Spectroscopic Components*

In journal articles, presentations, and textbooks, chemical instruments are often represented as block diagrams. These block diagrams highlight the important components of the instrument and show how the different components of the instrument are connected together. For example, a block diagram for a generic absorption spectrometer could be depicted as follows: (see text)



The specifics of each component depend on the particular application. In this activity, you will learn some of the operating and design details of the components used in a UV/Vis spectrometer. By looking carefully at the individual components, you will develop an understanding of what is actually taking place within many of the instruments used for chemical analysis.

The components that you will be working with are from an Acton Research Systems UV/Vis modular spectrometer. The modular design makes it easy to assemble and disassemble into a variety of arrangements. Although we want you get "hands on" experience with these systems, keep in mind that the monochromator mirrors and gratings are delicate optics so be careful when handling. **Do not touch any of the optical surfaces**! Also, the photomultiplier tubes can be destroyed if they are exposed to room light when powered. **Use the PMT shutter to prevent exposure of room light to the PMT!**

Exercise 1. The function of the monochromator

Attach the W/D_2 light source module to the entrance of the monochromator. Use the ¾ inch spacer between the source and the entrance slit to get the proper focus of the source light onto the entrance slit. **To avoid electrical shock, do not plug in the power until the module is attached and the source cover is replaced.** Turn on the tungsten lamp, the monochromator power, check to make sure the USB cable is connected between the monochromator and the computer and then start the SpectroPro monochromator control software. Adjust the slits to 3 mm, be careful not to exceed the 3 mm slit limit, and dim the room lights. Tape a small piece of lens paper to the exit slit so you can see the light coming out of the monochromator. Open the Operation routine within the software. Familiarize yourself with the monochromator controls. **What color is the light at the output slit when you set the monochromator to 0 nm, 425 nm, 520 nm, 640 nm, and 1040 nm respectively** with grating 1 selected? Scan the monochromator from 400 to 700 nm at 300 nm/minute while looking at the light at the exit slit. **Do you understand the function of the monochromator?**

Remove the exit slit by taking out the 4 mounting screws and reattach the lens paper the output of the monochromator. Using a wavelength of 525 nm, look at the output light of grating 2 compared to grating 1. Also compare the light when grating 2 is used at monochromator settings of 525 nm, 1050 nm, and 1575 nm, respectively. **How does the spacing between lines, d, affect the dispersion of the monochromator? How does the order, n, affect the dispersion?**

Exercise 2. Using CCD detection

A. Initial CCD set-up and calibration
Loosen the two set screws for the detector flange with your 3/32" hex head wrench. If the exit slit is still attached, remove the 4 long hex head screws with the 9/64" wrench and take off the exit slit assembly. Slowly pull out the PMT detector flange. Replace the PMT detector flange with the CCD flange assembly. Rotate the flange assembly to orient the CCD array horizontal to the detector axis. In its proper orientation, the Ames Photonics label should be nearly upright.

Connect the CCD to the Ames detector interface with the serial cable and connect the interface to the computer with a USB cable. Also connect the monochromator to another USB port and power up the monochromator. Open SpectraPro so you can control the monochromator. Open Spectra Array so you can read the data from the CCD. In Spectra Array, choose the real time acquisition and verify that your system is working. You should see a strong signal due to stray light entering the monochromator. You may have to open the entrance slit a little bit.

Power up the mercury lamp and direct its light into the monochromator. Using SpectroPro select the 300 lines/mm grating and set the wavelength to 500 nm. Using Spectra Array, set the x and y axis to full scale. Use the zoom tool for this by setting the range for x from 0 to 2048 and y from 0 to 4000. While looking at the mercury spectrum, slightly adjust the CCD flange (rotation and forward and backward) to optimize the spectrum. When you are satisfied with the alignment, use the set screws to hold the flange in place.

To calibrate the CCD, determine the pixel number for the mercury transitions at 254 nm, 405nm, 436 nm, and 507 nm. These should be the 4 strongest transitions. Use the calibration routine in Spectra Array to calibrate the CCD and display the x axis in nm. **Save and Print the Hg emission spectrum**.

B. Examination of various light sources
Using the CCD**,** collect the emission spectrum of the tungsten lamp and the D_2 lamp. **Why are both lamps found in most UV/Vis spectrometers?**

Attach the fiber optic flange and fiber optic to the entrance slit flange of the monochromator. Be careful with the optical cable, **Do not bend it harshly!!** Use the fiber optic to collect the emission spectrum of the fluorescent ceiling lights. Also collect the emission from your flashlight. **Explain how the fluorescent light give you white light.**

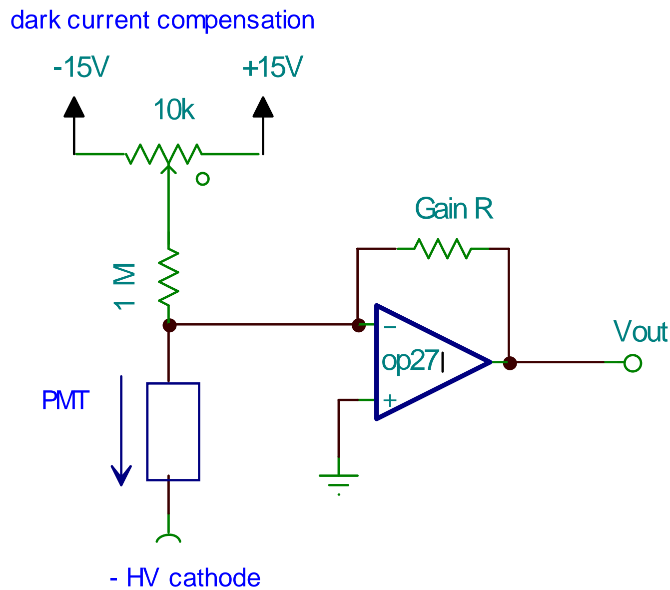
Remove the fiber optic and attach the white LED to the fiber optic flange. Power the LED with a 10 V source. Acquire the spectra of the White, Blue, Red and Green LEDs. When you are finished with this exercise, reassemble the monochromator with the PMT flange and exit slit.

Exercise 3. S/N and absorption measurements
The Acton components can easily be configured into an absorption spectrometer. Absorbance measurements are widely used for quantitative measurements of solutions with an unknown concentration of analyte. The quantitative relationship between absorbance signal and concentration is given by Beer's Law, A=εbc, where ε is the molar absorbtivity, b is the cell path length, and c is the species concentration. Remember that absorbance is calculated from the transmittance, A=-logT, where $T = \frac{P}{P_0}$ is the ratio of light power through the sample to the light power through the blank, so the instrument must measure P and P_0 in order to determine the Absorbance.

A. Setting up the absorption spectrometer
Attach the W/D_2 light source module to the entrance of the monochromator. Use the ¾ inch spacer between the source and the entrance slit to get the proper focus of the source light onto the monochromator entrance slit. The TAs can show you how to do this. **To avoid electrical shock, do not plug in the power until the module is attached and the source cover is replaced.** Connect a USB cable between the monochromator and computer. Turn on the tungsten lamp and monochromator power. Start the *Acton Set Wavelength.vi* in LabView. Run the program using the run continuous arrow on the upper left of the screen and use the control button to set the wavelength of the monochromator to 525 nm. Set both slits to 3 mm. **3 mm is the maximum opening of the slits; do not force the micrometer screw any further than the 3 mm settting.** Verify that you are getting green light the exit slit of the monochromator. Use a small piece of index card to "track" the light.

Now attach the sample compartment to the exit slit of the monochromator. Again use a ¾ inch spacer between the sample compartment and monochromator to get proper focus of the light source. With the small index card, look at the light at the sample location and right after the sample compartment. **Is the light focused or defocused at these locations?** Set the monochromator to get red, orange, yellow, green, blue, and violet light to the sample. R**ecord the wavelengths of these colors on your report sheet.**

The absorption spectrometer will use a photomultiplier tube (PMT) as the detector. The photomultiplier tube (PMT) provides a current that is proportional to the intensity of light striking the photocathode. The output of the PMT is a very low (10^{-5} A to 10^{-7} A) current signal. This small current is converted to a voltage and amplified with the following op amp circuit. The circuit is already constructed for you on the printed circuit board but you will have to add the appropriate feedback resistor. Start with a 100 K resistor in the feedback loop but realize that you can change the gain of the circuit by changing the value of this feedback resistor.



Attach the photomultiplier (PMT) housing to the sample compartment. Connect the high voltage supply to the PMT and the PMT signal to your current to voltage converter. Adjust both the entrance and exit slits to 100 µm. Connect the monochromator to the computer with a USB cable. To interface the detection circuit to the computer, connect your current to voltage output to pin AI0+ on the USB-6008 A to D converter. Connect the A to D converter to the computer with a USB cable.

Make sure the lid is on the sample compartment and monochromator. Turn on the power supplies for all components**. When the power is on, always be sure the PMT shutter is closed before opening the sample compartment!**

Test the detection system as follows: 1) Set the wavelength of the monochromator to 525 nm using the *Acton Set Wavelength.vi*. Now you can use the control buttons to set the wavelength or read the voltage from the A to D. 2) Adjust the detector voltage to - 400 V. 3) Adjust the dark current compensator for a small positive voltage near zero volts. This will verify that your I to V and A to D converters are working. 4) Open the PMT shutter. You should see a voltage signal that diminishes when the shutter is closed or the slit width is made smaller. **If necessary, change the gain resistor to provide about a 1 V output for signal.**

Now use the *Acton Acquisition 2007.vi* to collect the spectrum of the tungsten source from 300 to 800 nm. Use 1 nm steps, 100 samples per point at an acquisition rate of 10k samples per second. **Save the data and plot the lamp output spectra in Excel. What would be the useful wavelength range for an instrument based on your setup? Which components determine the high frequency limit and the low frequency limit of the spectrometer?**

B. The absorption of fluorescein
Set the slits to 50µm. You can again check that your system is working by using the LabView program Acton Set Wavelength vi to control the monochromator and measure the voltage. Set the wavelength to 525 nm and open and close the shutter to see if you get a voltage response from your detection system.

Set the slits to 50 µm and collect a background spectrum with a cuvette filled with water in the sample compartment. This time collect the spectrum from 350 to 700 nm using 1 nm steps, 100 samples per point at an acquisition rate of 10k samples per second. Save the data and plot the background in Excel.

Collect a sample spectrum over the same range with the fluorescein solution in the cuvette and plot this in excel. **Using Excel, calculate and plot the absorbance spectrum of fluorescein. From the concentration of the fluorescein, calculate the molar absorbtivity for fluorescein at its wavelength of maximum absorption**.

C. Measuring and improving the detection limit
Tune your monochromator to the wavelength of fluorescein's maximum absorbance. With distilled water in the sample cell, use the *S to N calculator.vi* to monitor the signal. Collect about 50 measurements and **record the average and standard deviation of the signal and the Signal to Noise Ratio. Use this to calculate the detection limit of fluorescein in your spectrometer with this configuration.**

Signal averaging Signal averaging is often used to reduce the noise level relative to the signal level. Open *S to N w avg.vi* and collect about 50 points of data using 10, 100, and 1000 samples per measurement respectively. **Record the signal to noise ratio in each case. Does the signal to noise improve as you would expect from a statistical model?**

Filtering Changing the time constant of the measurement should also decrease the noise level relative to the signal level. Observe this effect by building a low pass filter with a 1K resistor and 1 uF capacitor and add the filter to your current to voltage converter output. The resistor needs to go into location C2, the capacitor into R2, and a jumper goes from I to V out to Signal in. The TAs can help you set this up on the printed circuit board. The signal is measured after the filter and a voltage follower at TP4. **Compare the Signal to Noise ratio before and after the low pass filter. By what factor was the detection bandwidth decreased?**

Light intensity The amount of light from the source will also affect the signal to noise ratio. Using "S to N calculator" measure the S/N ratio and voltage signal while changing the slits between 10 and 300 µm. Use 1000 samples for each S/N measurement and make these measurements without the low pass filter. **Plot S/N vs Signal and fit the plot to a power function in Excel. Does the S/N increase as you would expect in a system that is limited by shot noise? Explain.**

Play around with your acquisition system to obtain the highest signal to noise ratio. **Calculate the new detection limit for the fluorescein absorbance in your optimized set up and compare to your original set up.**

Exercise 4. A closer look at the monochromator

Remove the top of the monochromator and identify the slits, the folding, collimating and focusing mirrors, and the grating. **Identify these components in the diagram on the report sheet.**

A. Grating spacing measurement

A laser can be used to measure the distance between grooves on the gratings. Carefully remove the grating holder from the monochromator and place it on the support flange. Direct a laser beam onto the grating and look for the diffracted beams. Play around with different angles of incidence. Make appropriate measurements so you can **use the grating equation,**

$$n\lambda = d(\sin\phi_i + \sin\phi_d)$$

to determine the distance between grooves, d, for each of the diffraction gratings. Also calculate the number of lines per mm etched in each of the grating surfaces and the total number of lines illuminated, N. The width of the grating is 30 mm.

Return the grating mount to the monochromator and put the monochromator top back on.

B. Wavelength calibration using a mercury lamp

Set up the Hg lamp so its light is directed into the monochromator. Use the LabView program "Acton Acquisition 2007" to collect the Hg spectrum from 240 nm to 600 nm in 0.1 nm steps. Use 100 samples at an acquisition rate of 10,000 samples per second. Note that the LabView Acquisition program and the SpectraPro monochromator program cannot run at the same time so be sure to close "SpectraPro". After the spectrum is taken, save the data and open it in Excel. **Plot the spectrum within Excel and compare the measured peak wavelengths to the literature values found in the following table. Comment on the wavelength calibration of your instrument.** If the wavelength calibration is off, notify the TAs and they will help you recalibrate the monochromator. **One of the strongest spectral lines you see is near 507 nm. Where does this line come from?**

TABLE Mercury Lamp Spectrum and <u>Approximate</u> Intensities

Wavelength (nm)	Appr. Intensity
253.65	Very strong
296.73	Weak
302.15	Weak
312.57*	Strong
334.15	Weak
365.02*	Strong
365.48	Strong
404.65	Strong

407.78	Weak
435.84	Strong
546.07	Strong
576.96	Weak
579.07	Weak

*denotes doublet

C. Measurement of slit function, resolution, and dispersion

Set up the collection parameters to zoom in on one of the mercury lines so that you can accurately measure the shape of the mercury line. Do not use a doublet. Decrease the step size so that you get a good representation of the lineshape. Collect spectra for ten different slit widths between 300 µm and 10 µm. Start at the larger slit. In Excel, plot all of your spectra on the same chart and **measure the full width at half maximum of the spectral lines. Plot FWHM vs slit width and use the slope of the linear region to calculate the reciprocal linear dispersion, D^{-1}, for the monochromator. What is the best resolution ($\lambda/\Delta\lambda$) that you can achieve? How does this compare to the ultimate resolution given by nN?**

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* This Lab was written in its current form by R. McClain on 4/9/2009. D. Sykes, M. Sanders, J. Wright, J. Taylor, D. Besemann, M. Wendt, and R. McClain have made contributions to the activities associated with this lab.