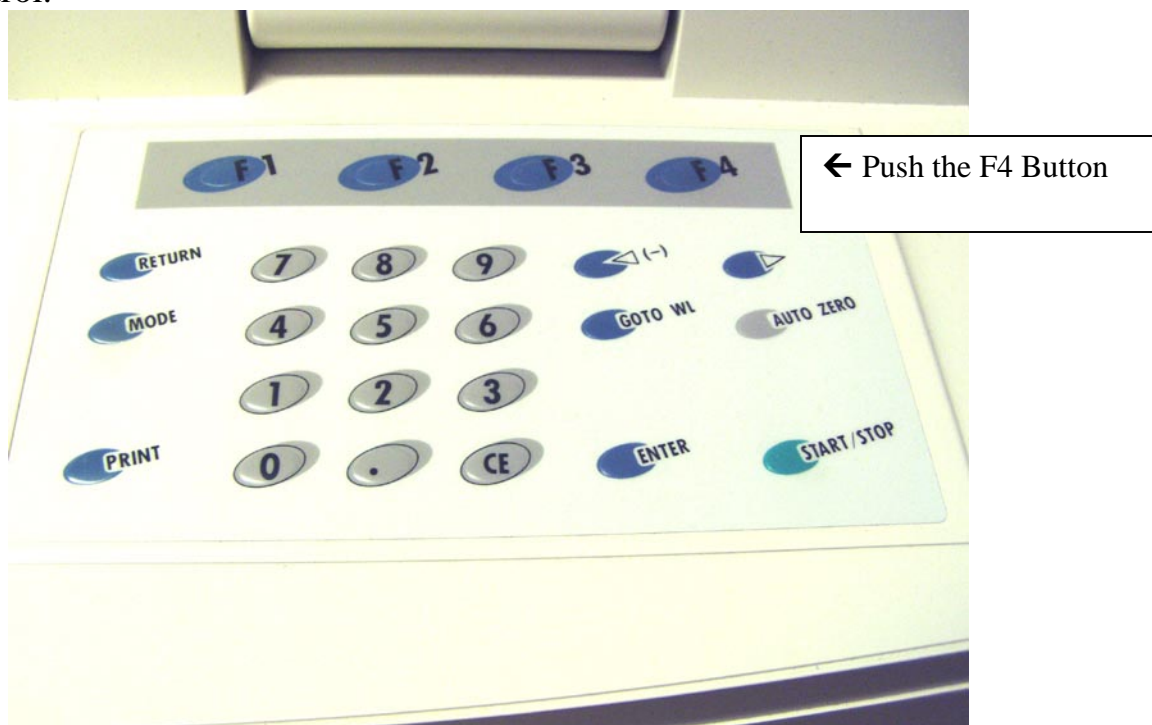
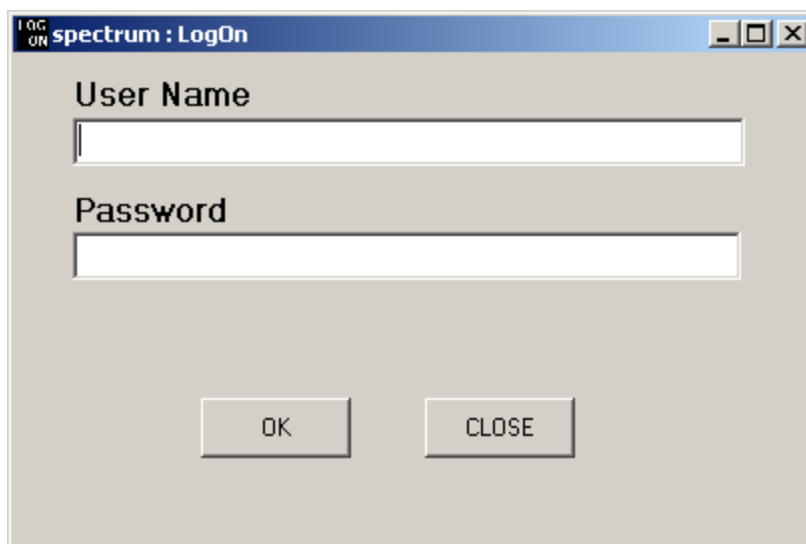


# Shimadzu UV-VIS User's Guide

- 1) Push the F4 button on the UV-VIS instrument keypad. This will enable PC control.



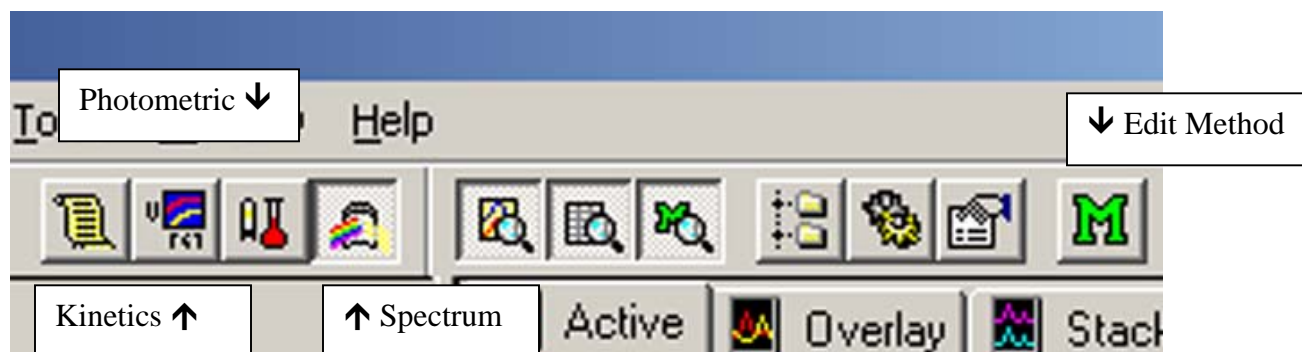
- 2) Log into the UV-VIS software with your username and password.



- 3) After the UV-Probe software comes up, click on the “Connect” button near the bottom of the screen to connect with the spectrometer.

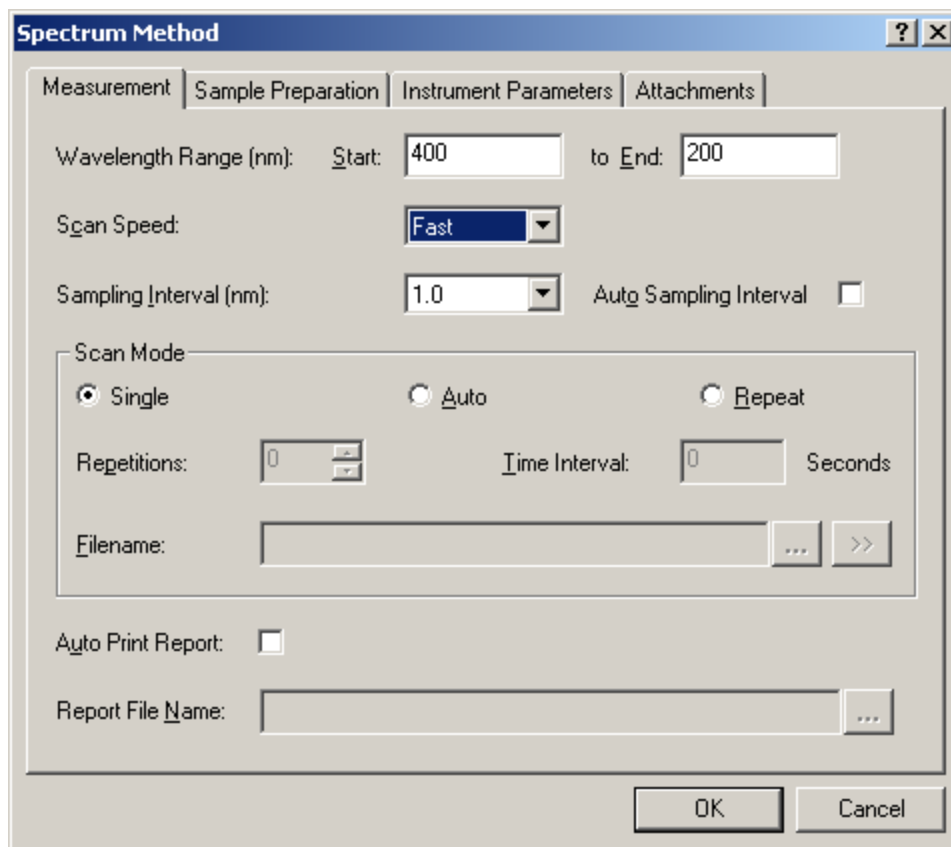


- 4) Choose what type of experiment mode you need.
- ❖ Spectrum mode is for wavelength scanning over a continuous wide range.
  - ❖ Photometric mode is for quantitative analysis, measuring absorbance at single wavelengths.
  - ❖ Kinetics mode will collect absorbance data at a particular wavelength versus time.
- 5) Choose the mode you need by clicking on one of the buttons on the toolbar.



# How to Work in Spectrum Mode

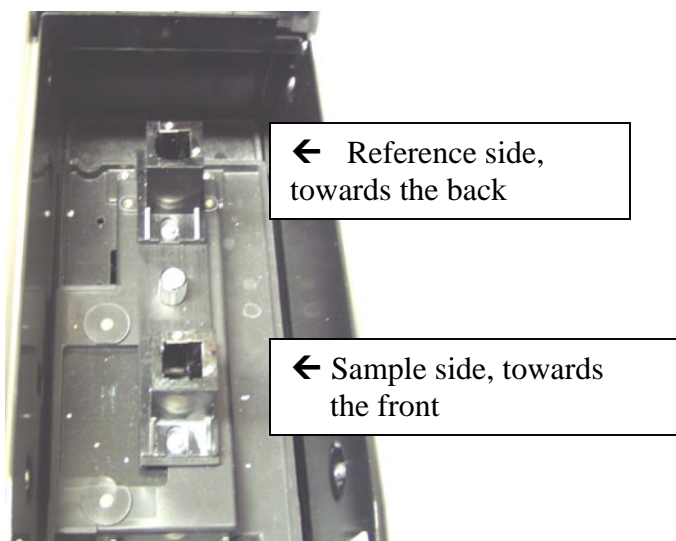
- 1) After choosing the Spectrum mode of operation, click on the “M” button to set up your acquisition parameters.
- 2) Enter your wavelength range and scan speed (usually fast), and click on OK. The maximum allowable wavelength range is 1100 nm to 190 nm.



- 3) It's good practice to zero the baseline by clicking on the “Baseline” button near the bottom of the screen. Be sure that there are no samples in either the sample or the reference side while correcting the baseline. (And the cover must be closed, of course).



- 4) Insert your sample into the sample compartment. If you want to, you can insert a cuvette with blank solution into the reference side.



- 5) Click on Start to start collecting the spectrum.

**Please Note:**

**After your spectrum has been collected, the program will prompt you for a filename. However, this does not automatically save your spectrum to disk! You must then do a “File, Save As” in order to permanently save the spectrum to a disk file.**

To change the appearance of your spectrum, such as the display limits, or the background color, right-click the mouse on the spectrum graph, and left-click on Customize...

**Please Note:**

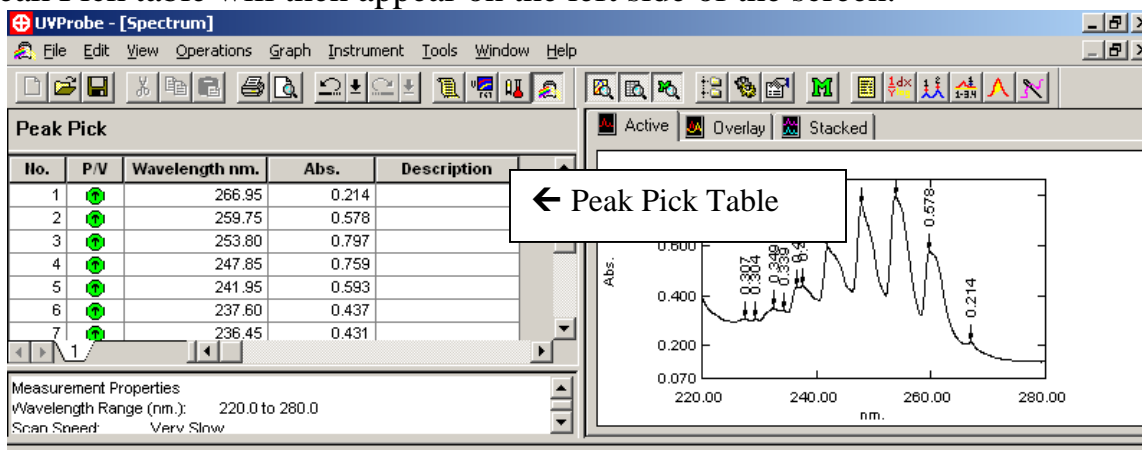
**If your peaks of interest are greater than 2 absorbance units, you should dilute the sample, and then re-run it.**

## How to label peaks on your spectrum:

Click on the Peak Pick button on the toolbar.



The Peak Pick table will then appear on the left side of the screen.



Right-click the mouse on the Peak Pick table, and left-click on Properties.

You can then change parameters, such as the threshold for labeling peaks, and whether the graph is labeled with the peak table number or the actual absorbance value, and whether peaks or valleys are labeled.

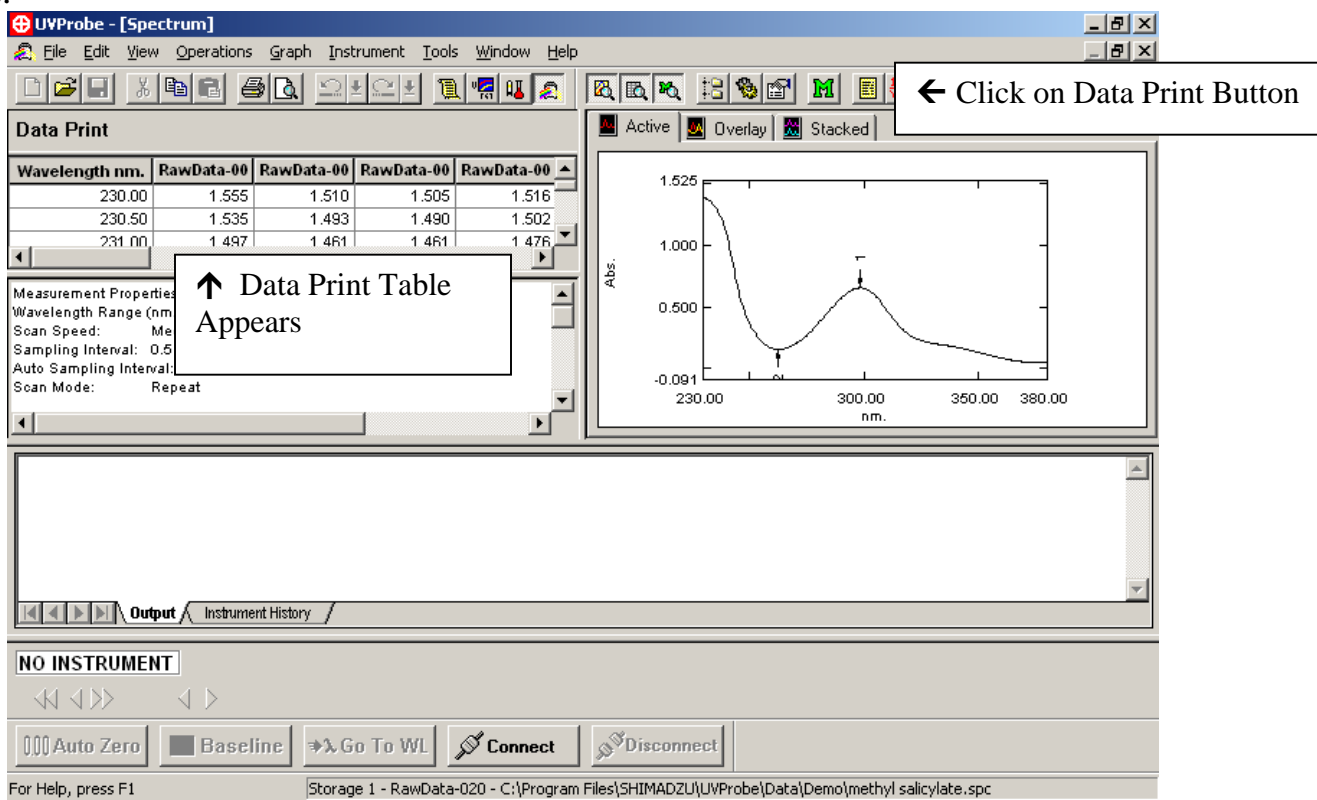
After you have set up the Peak Pick table the way that you want, click on the Peak Pick table to make it the active window. Then click on File, Print Preview. The report generator will show you how the printout will appear. If it looks ok, then click on Print.

### Please Note:

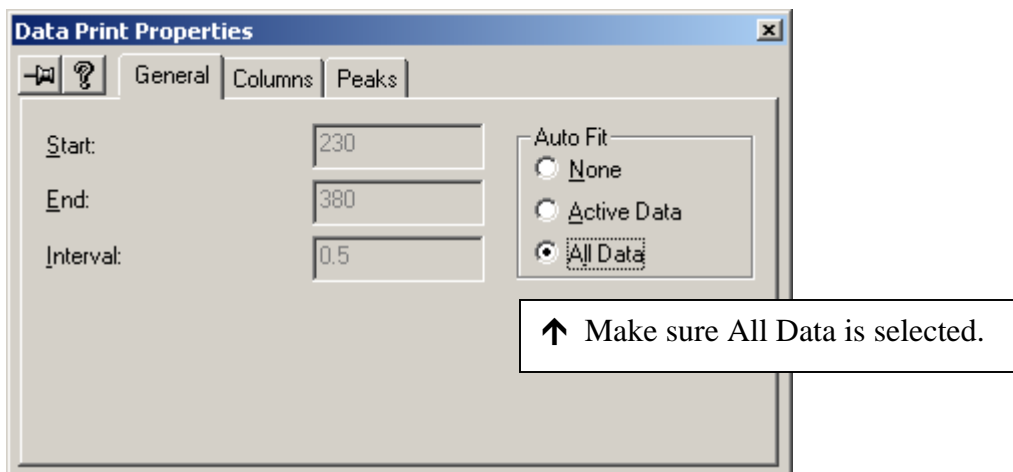
**If the spectrum graph is selected as the active window, you will get a different looking report than if the peak table is selected as the active window, prior to clicking on Print Preview.**

# How to Convert Spectra to ASCII Text Format

Click on the “Data Print” button. A Data Print table will appear, listing the raw data points.

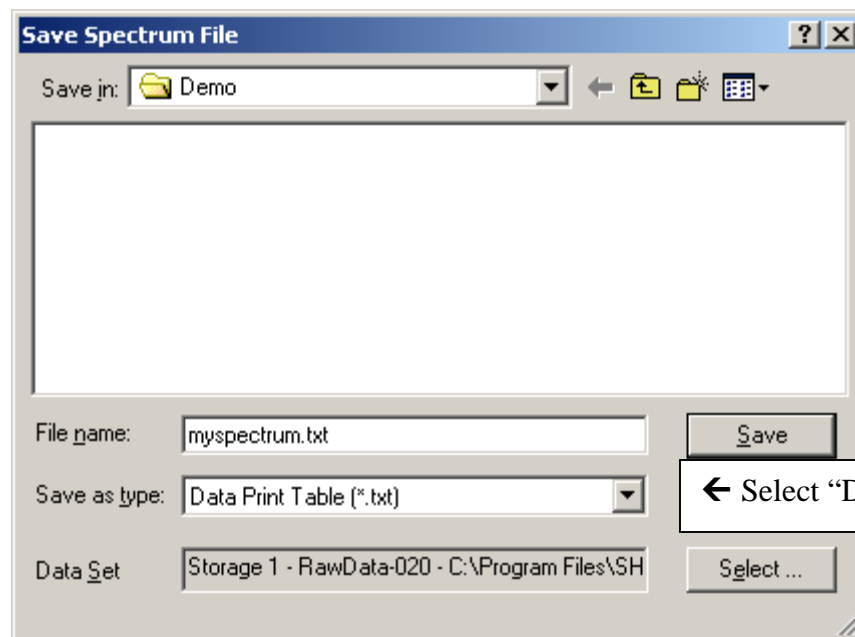


Right-click the Data Print table, and left-click “Properties”. Make sure that the option “All Data” is clicked on, otherwise all of the data might not be included in the ASCII file.



Click on the File menu, and Save As. Navigate to the folder where you want to save the data.

For Save As Type, click on the drop down menu and select Data Print Table (\*.txt). Give it a file name, and click on OK.



# How to Shut Down the Instrument When You Are Finished

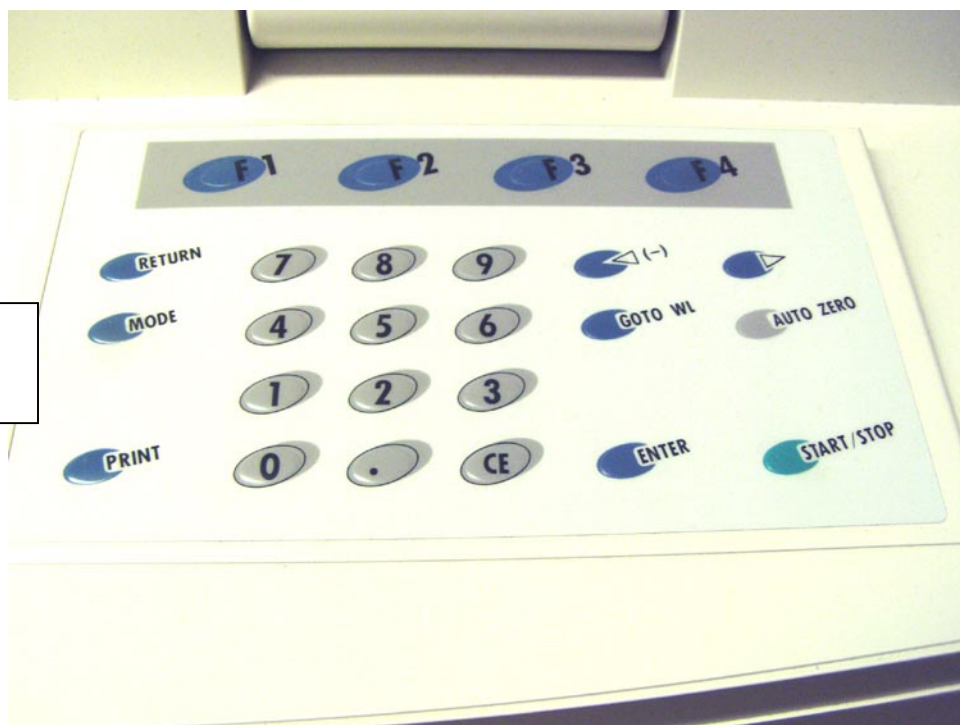
Be sure to save all of your data.

Then click on the Disconnect button near the bottom of the screen.

Exit from the UV-Probe software.

**Important! Please go to the UV-VIS instrument keypad, and push the Mode button to return local control to the spectrometer. This allows the instrument to turn off the deuterium lamp, which will make the lamp last longer.**

Push the Mode →  
Button





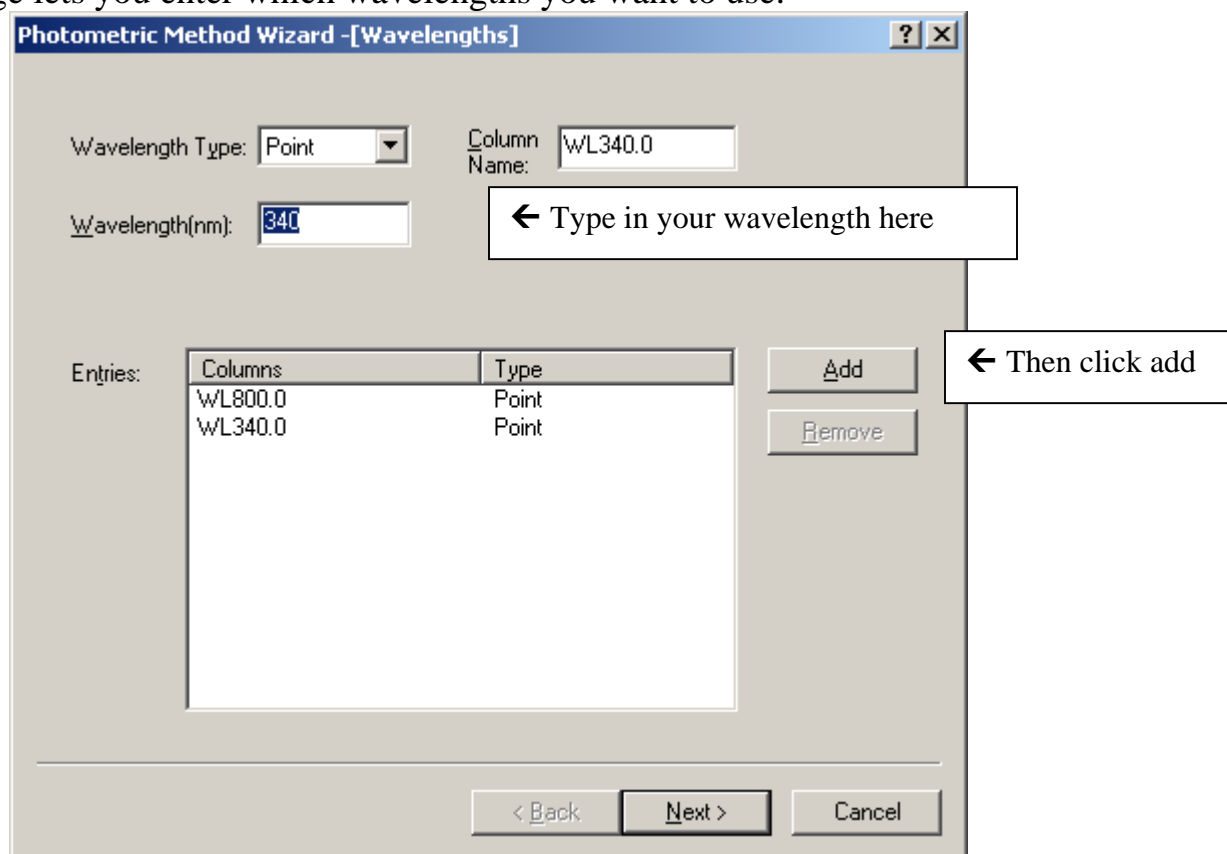
# How to Work in Photometric Mode

To operate in photometric mode, first click the photometric button on the toolbar. Then click on the “M” button to change your method parameters.



The following pages will come up to set up your experiment.

The first page lets you enter which wavelengths you want to use.



The next page lets you set the concentration units, and the type of standard curve fit.

**Photometric Method Wizard -[Calibration]**

Type: **Multi Point** Column Name:

Formula: **Fixed Wavelength** Units: **mg/l**

WL1: **WL800.0** ← Choose the wavelength to use for calibration

Parameters

☒ **Abs = f (Conc)** ☐ **Conc = f (Abs)**

$Abs = K1 * (Conc) + K0$

Order of Curve: **1st** ← Set standard curve fit order

☐ **Zero Interception**

< Back Next > Cancel

The next page lets you enter measurement parameters for your standards. Be sure to click on “Data Acquired By: Instrument”.

**Photometric Method**

Measurement Parameters(Sample) Equations Pass/Fail

Method Summary Instrument Parameters Attachments

Wavelengths Calibration Measurement Parameters(Standard)

**STD**

Data Acquired By: ☐ **User Entry** ☒ **Instrument** ← Be sure Instrument is clicked on.

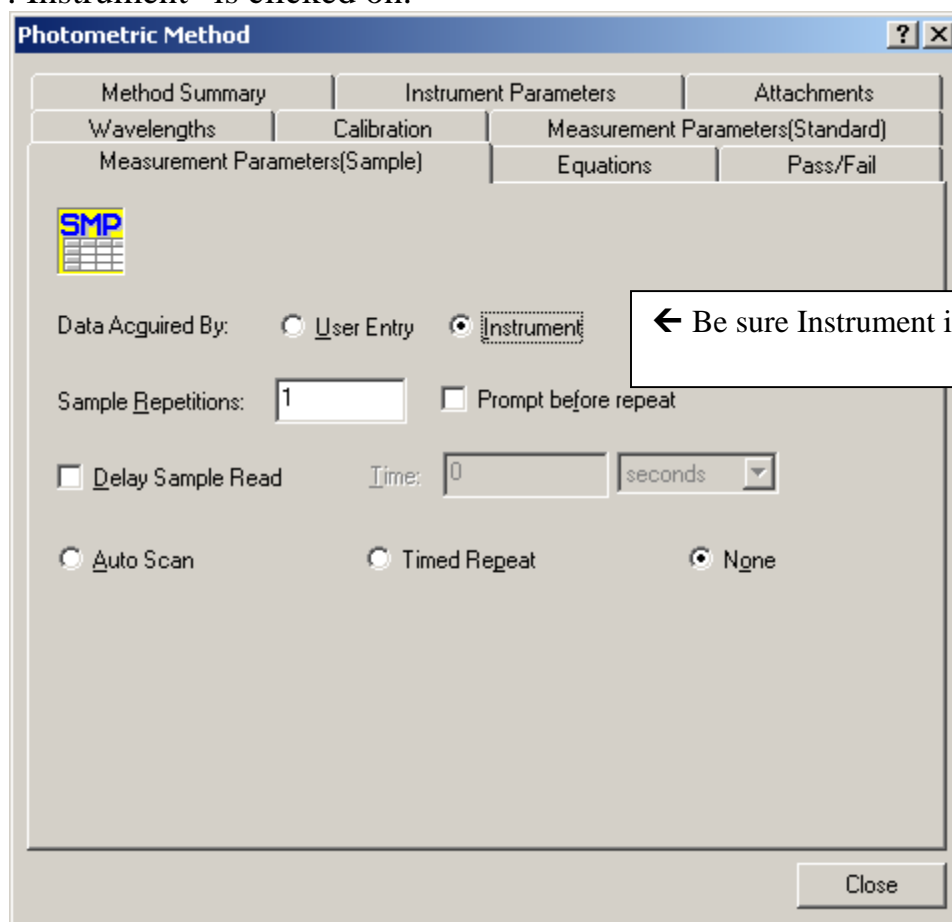
Sample Repetitions: **1** ☐ **Prompt before repeat**

☐ **Delay Sample Read** Time: **0** seconds

☐ **Auto Scan** ☐ **Timed Repeat** ☒ **None**

Close

The next page lets you set sample measurement parameters. Again, make sure that “Data Acquired By: Instrument” is clicked on.



The image shows a software dialog box titled "Photometric Method". It has a tabbed interface with the following tabs: "Method Summary", "Instrument Parameters", "Attachments", "Wavelengths", "Calibration", "Measurement Parameters(Standard)", "Measurement Parameters(Sample)", "Equations", and "Pass/Fail". The "Measurement Parameters(Sample)" tab is currently selected. Inside this tab, there is an "SMP" icon with a grid. Below the icon, the "Data Acquired By:" section has two radio buttons: "User Entry" (unselected) and "Instrument" (selected). A callout box points to the "Instrument" radio button with the text "← Be sure Instrument is clicked on." Below this, the "Sample Repetitions:" is set to "1" in a text box, and the "Prompt before repeat" checkbox is unchecked. The "Delay Sample Read" checkbox is also unchecked. The "Time:" is set to "0" in a text box, and the unit is "seconds" in a dropdown menu. At the bottom, there are three radio buttons: "Auto Scan" (unselected), "Timed Repeat" (unselected), and "None" (selected). A "Close" button is located at the bottom right of the dialog box.

**Photometric Method** [?] [X]

Method Summary | Instrument Parameters | Attachments

Wavelengths | Calibration | Measurement Parameters(Standard)

Measurement Parameters(Sample) | Equations | Pass/Fail

**SMP**

Data Acquired By: ☐ User Entry ☒ Instrument

Sample Repetitions: 1 ☐ Prompt before repeat

☐ Delay Sample Read Time: 0 seconds

☐ Auto Scan ☐ Timed Repeat ☒ None

Close

Next, you need to fill in the information in the Standard Table, and the Sample Table. The Standard table needs concentration information filled in, otherwise the Read Standard button will be grayed out.

**Standard Table - [ Active ]**

	Sample ID	Type	Ex	Conc	WL 800.0	WL 340.0	Wgt.
1 *	std1			1.000			
2	std2			2.000			
3	std3			3.000			
4	std4			4.000			
5							

← Be sure to enter the concentrations of your standard solutions. Otherwise, the Read Standard Button will not be active.

**Sample Table**

	Sample ID	Type	Ex	Conc	WL 800.0	WL 340.0	Wgt.
1 *	Sample A						
2	Sample B						
3							

↑ Be sure to enter sample names, otherwise the Read Sample Button will not be active.

800.000 nm 0.001 Abs.

Cell Blank Auto Zero Baseline Go To WL Read Std. Disconnect

For Help, press F1 Filename: File\_040525\_084141.pho

It is a good practice to click on the Baseline button, with nothing in either the reference or the sample side of the compartment. (Be sure that the sample lid is closed). This will set the absorbance to zero at every wavelength.

Then, put cuvettes with blank solution in both the sample and reference sides, and click on Cell Blank (with the lid closed). This will read the absorbance of the blank cells. This blank absorbance reading will be subtracted from the absorbance readings of all standards and samples measured after it.

After your data is collected, you must save the data by doing a File, Save As.

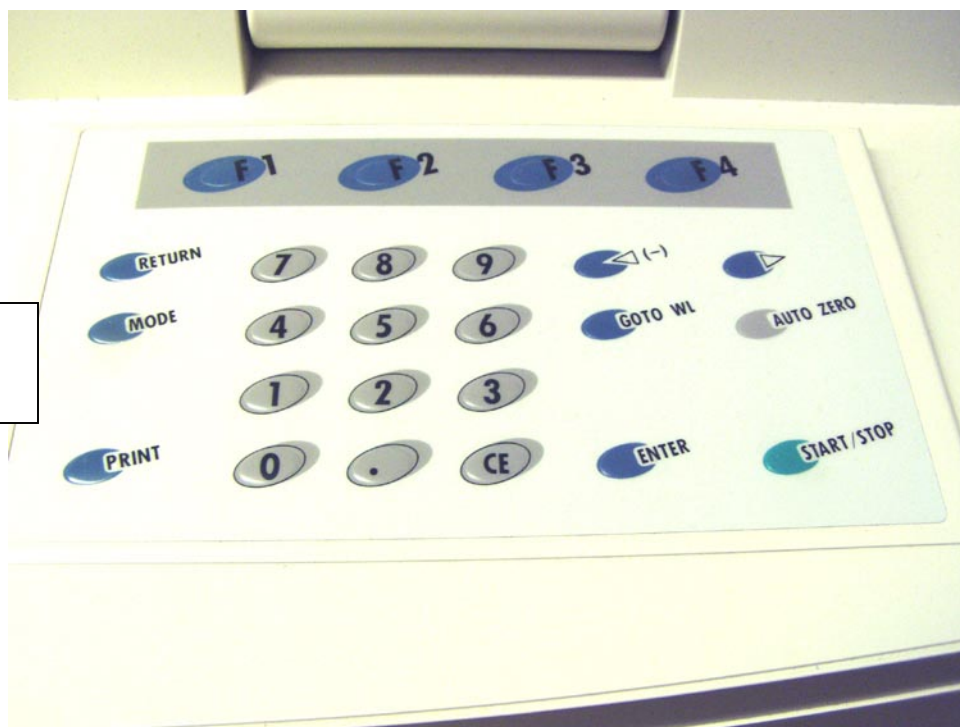
# How to Shut Down the Instrument When You Are Finished

Be sure to save all of your data.

Then click on the Disconnect button near the bottom of the screen.

Exit from the UV-Probe software.

**Important! Please go to the UV-VIS instrument keypad, and push the Mode button to return local control to the spectrometer. This allows the instrument to turn off the deuterium lamp, which will make the lamp last longer.**



Push the Mode →  
Button