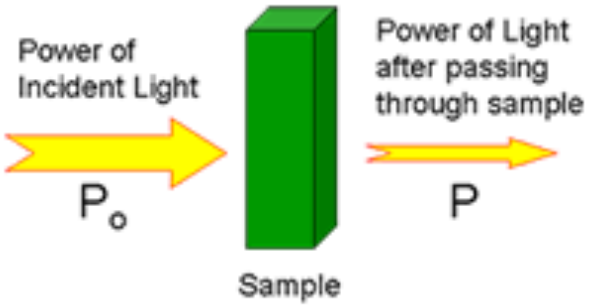
Using the Cary 50 for Experiment 4, Mn^{+2} Concentration Determination.

Background

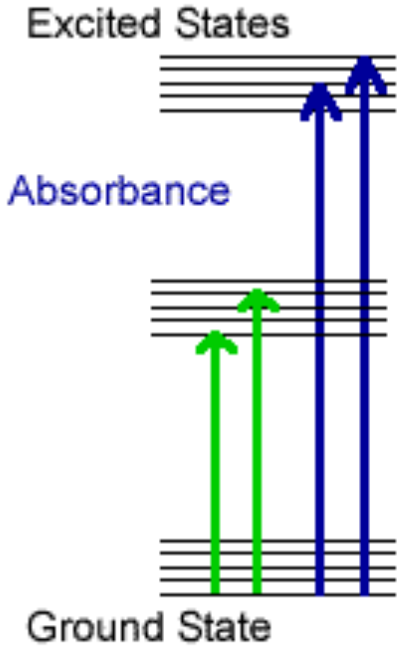
About UV-Vis Molecular Absorbance Spectroscopy

In molecular absorbance spectroscopy a beam of ultraviolet or visible light is directed through a sample. Some of the light may be transmitted through the sample. Light that was not transmitted through the sample was absorbed. Transmittance (T) is defined as the ratio of P/Po.

Absorbance (A) is defined as -log(T).



A molecule can absorb some of the light only if it can accommodate that additional energy by promoting electrons to higher energy levels. The energy of the light being absorbed must match the energy required to promote the electron. Therefore, not all wavelengths of light are absorbed equally by a sample. An absorbance spectrum depicts what wavelengths of light are absorbed by a sample. The UV-Vis absorbance spectrum below was obtained by passing different wavelengths of light through a solution of fabric dye, and measuring the intensity of light (P) passing through the solution. One can readily see what wavelengths of light are absorbed (peaks), and what wavelengths of light are transmitted (troughs).



Beer's Law

Quantitative Analysis using UV-Vis Spectroscopy

One very important relationship in absorbance spectroscopy is Beer's Law:

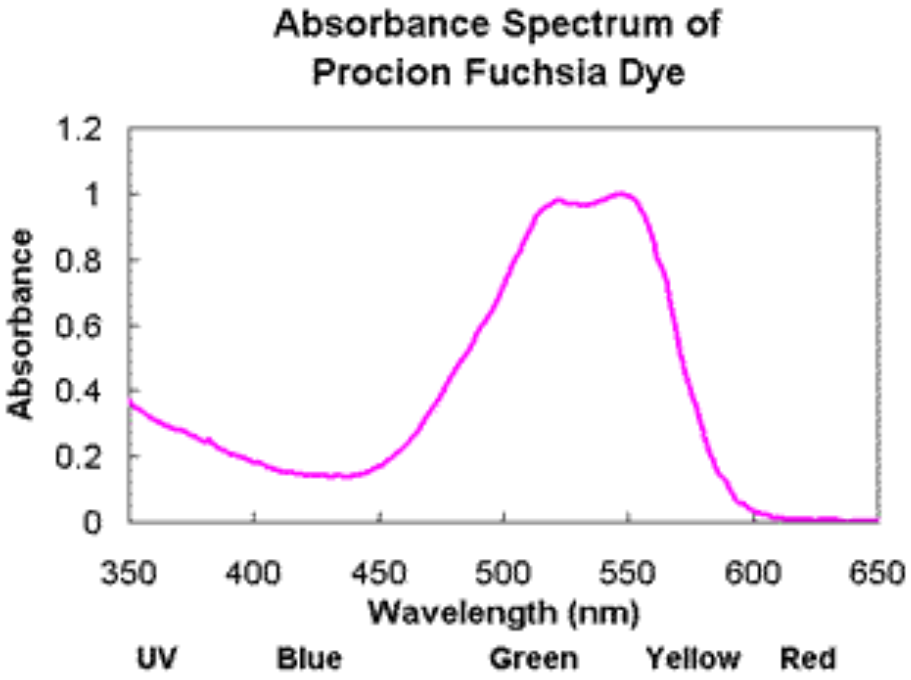
$$A=abc$$

where: A is absorbance

a describes the ability of a molecule to absorb radiation at a particular wavelength

b is the length of sample through which the light beam passes

c is the concentration of the absorbing species

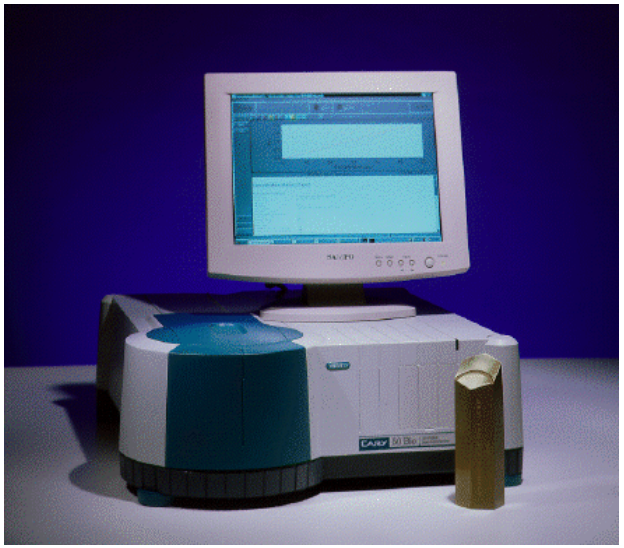


This relationship is the basis of all quantitative work in absorbance spectroscopy. It allows one to determine the concentration of an absorbing species simply by measuring its absorbance.

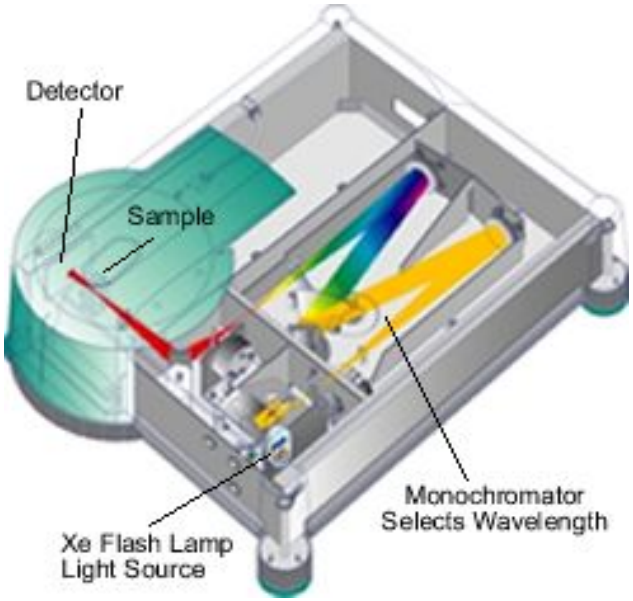
Varian Cary 50

Cary 50 Spectrophotometer

The Cary 50 design is simple, yet elegant. Its fast-scanning monochromator collects spectra in seconds, and the Xe flash lamp is pulsed to avoid interference from room light. The Cary 50 is controlled by intuitive software which allows students who are unfamiliar with spectrophotometers to begin collecting data without extensive software training.



We have purchased several accessories for use with this instrument that include a peltier thermostat cell holder to rapidly change sample temperature between 0 to 100 °C, sample temperature probes to accurately determine the temperature of the sample inside the cuvette, and a stopped flow (rapid kinetics) accessory to measure kinetics of fast reactions (less than a second).



Getting started.

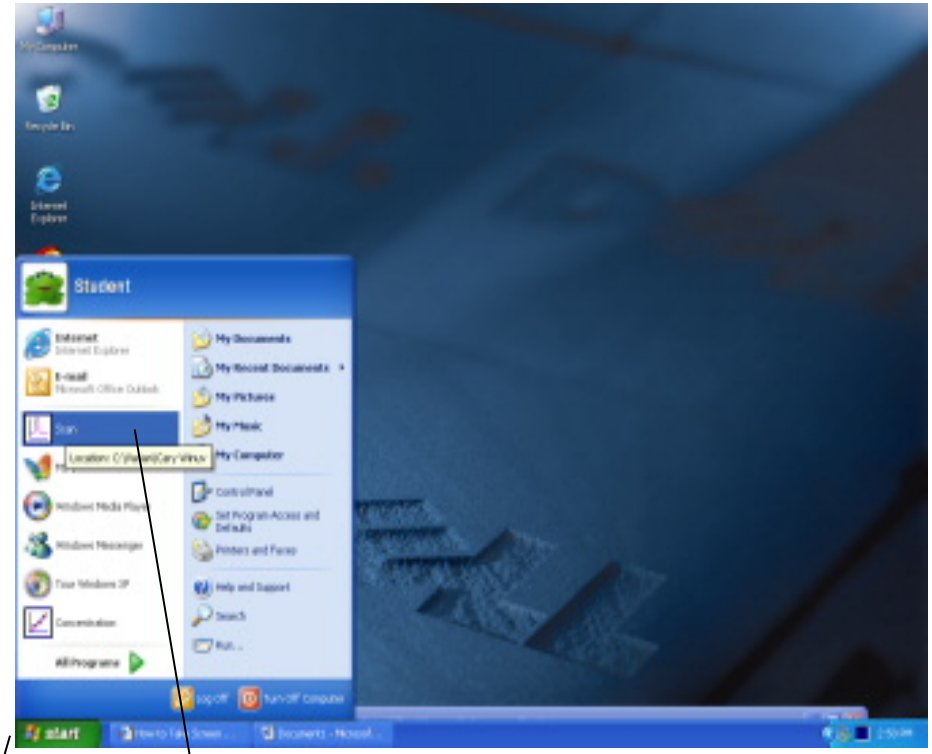
Turn on the Cary50 Dell computer

Choose the user "Student"
Type the password "Student"

After the computer has warmed up navigate to the "start" button on the bottom left corner

Click and then move to "Scan"

Allow the computer to sync with the Cary-50 spectrometer

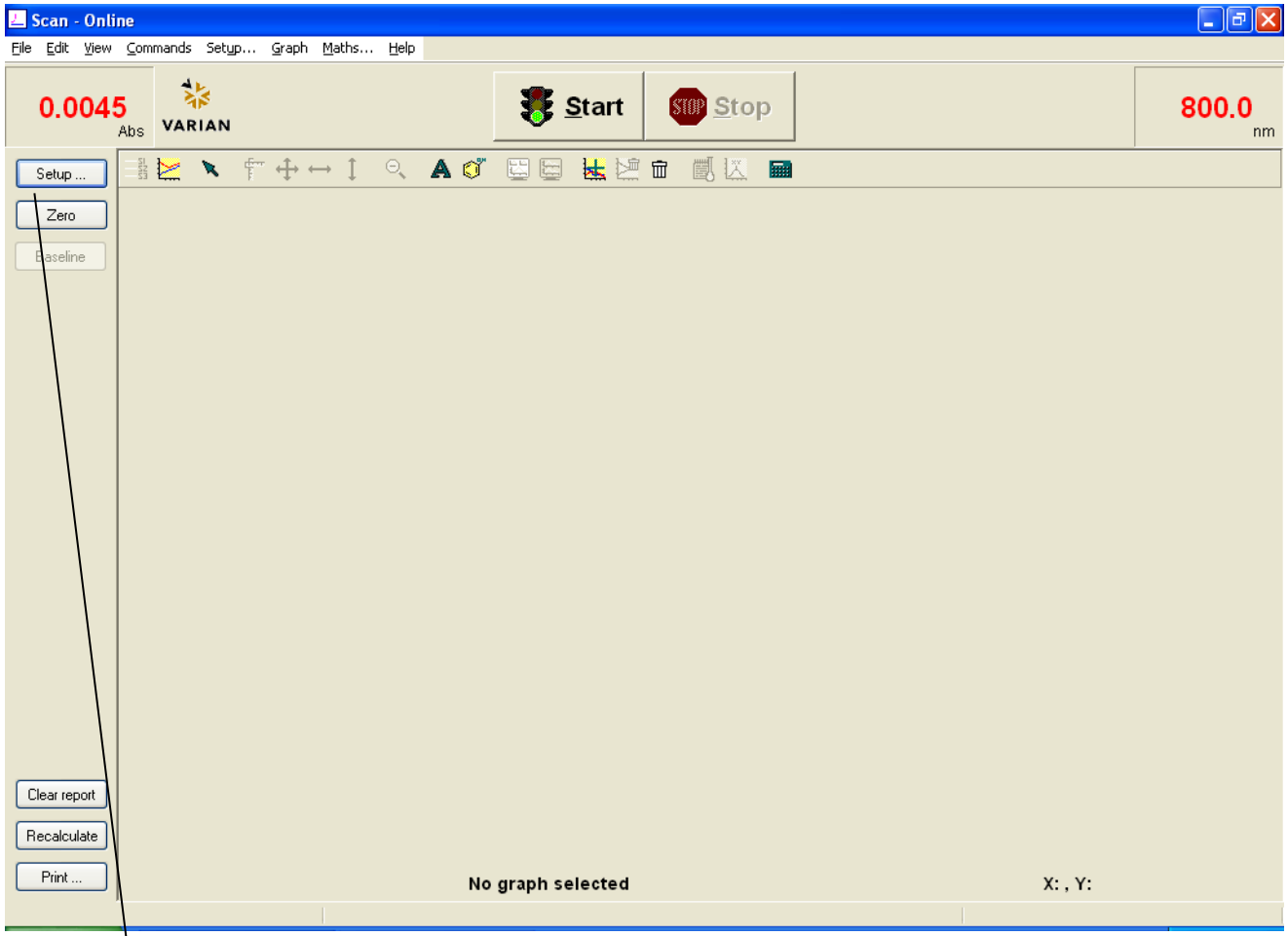


1. Click on start

2. Navigate to scan

Setting up Cary-50 for Scan.

Cary50 scan menu



Click on "Setup"

Setup Parameters.

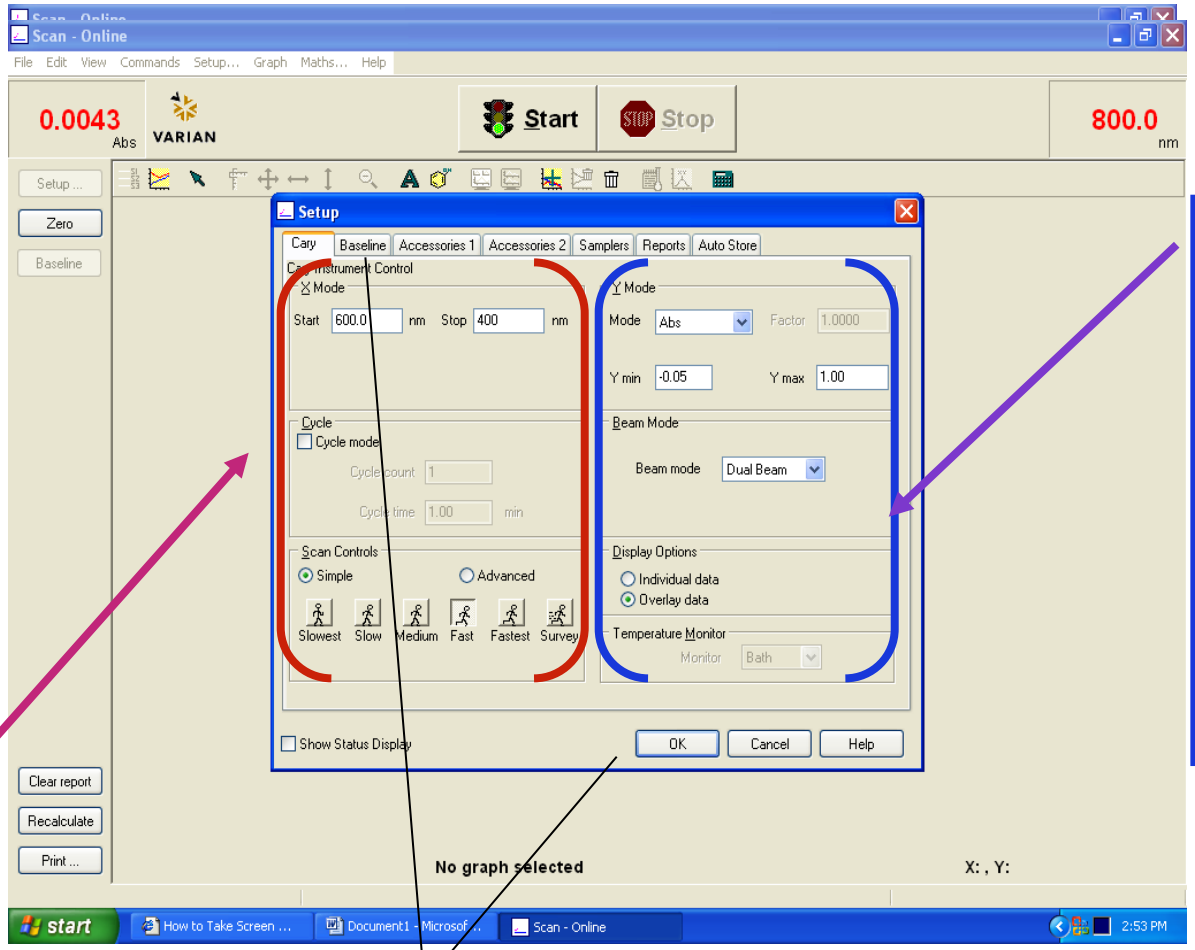
Cary50 Setup menu

Cary Instrument Control

X Mode
Start 600 nm
Stop 400 nm

Cycle
-

Scan Cycle
Fast



Y Mode
Mode Abs

Ymin -
Ymax -

Beam Mode
Dual Beam

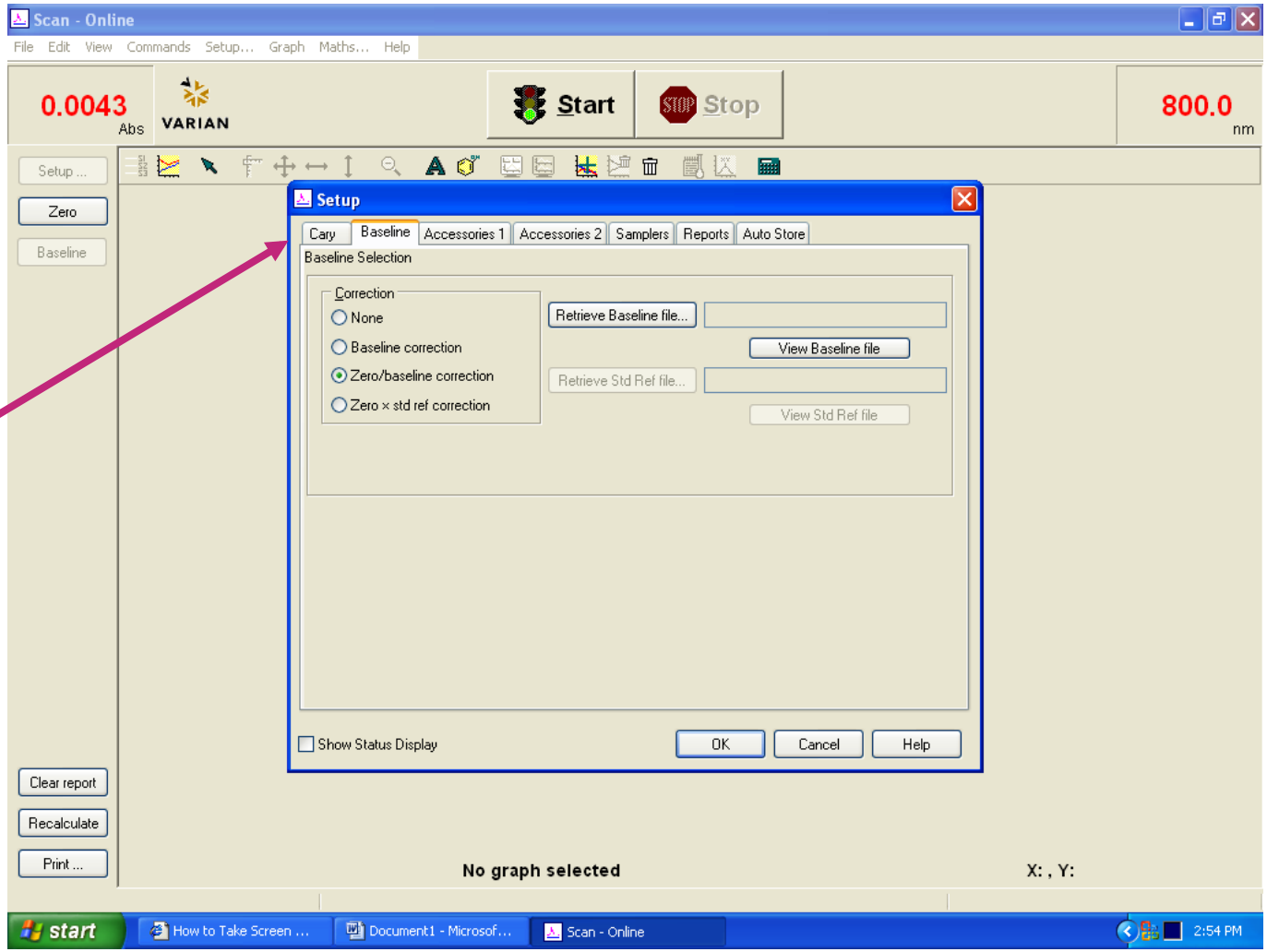
Display Option
Overlay data

Click on Baseline upon completion of Cary menu

Click " OK"

Setup Parameters.

Baseline Setup menu



Baseline

Baseline Selection

Zero / Baseline Correction

Zero and Baseline

Click on "Zero"

Click on Baseline

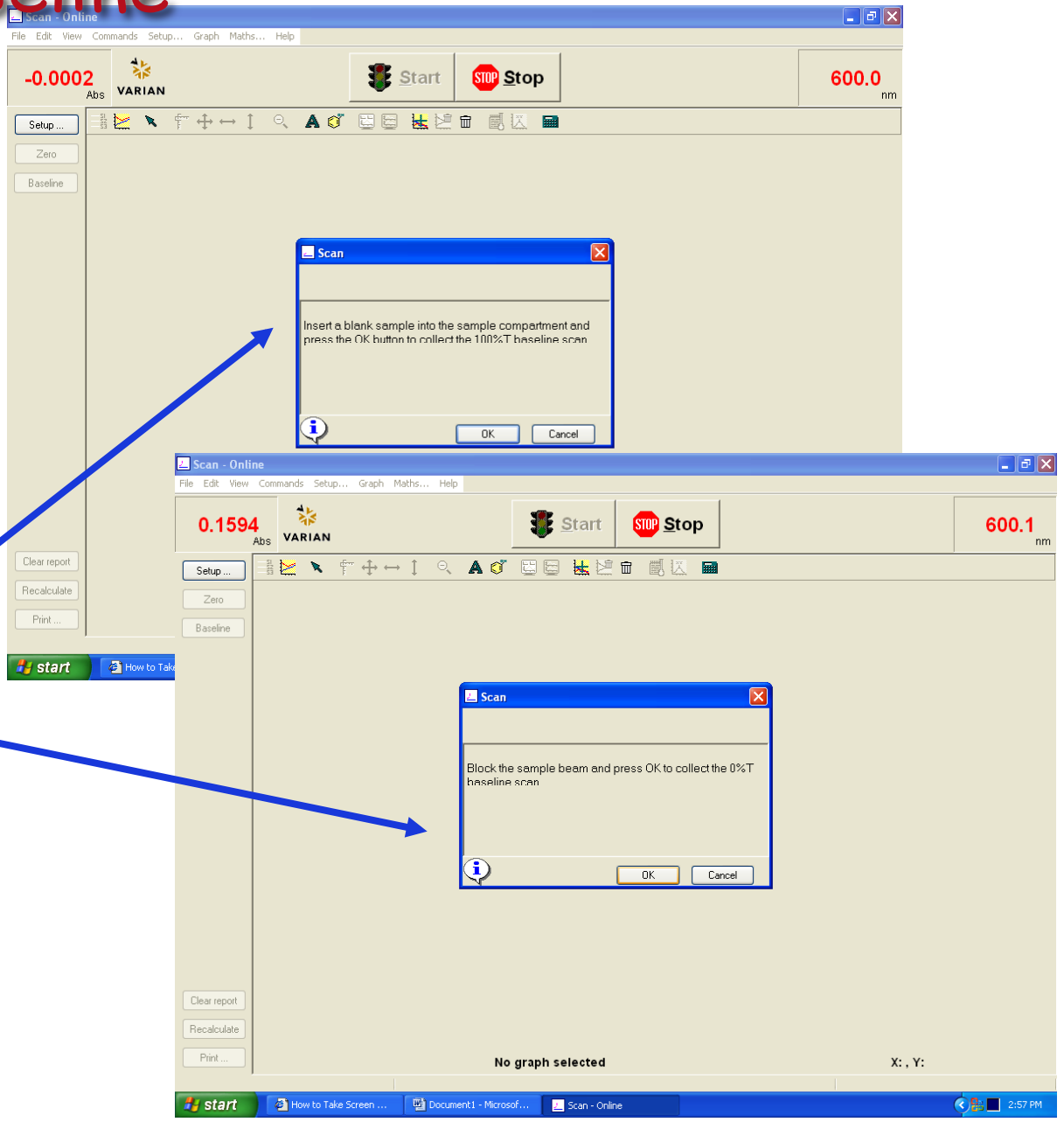
The spectrometer will prompt you to insert a blank.

Take a cuvette and pre-rinse the inside with your blank solution, then fill 3/4 with the blank reagent.

Insert the cuvette with the blank solution and click on "OK".

Block the beam with an index card and then click on "OK".

You are now ready to record a Absorption spectrum of your solution.



Writing Filename for Data in Computer

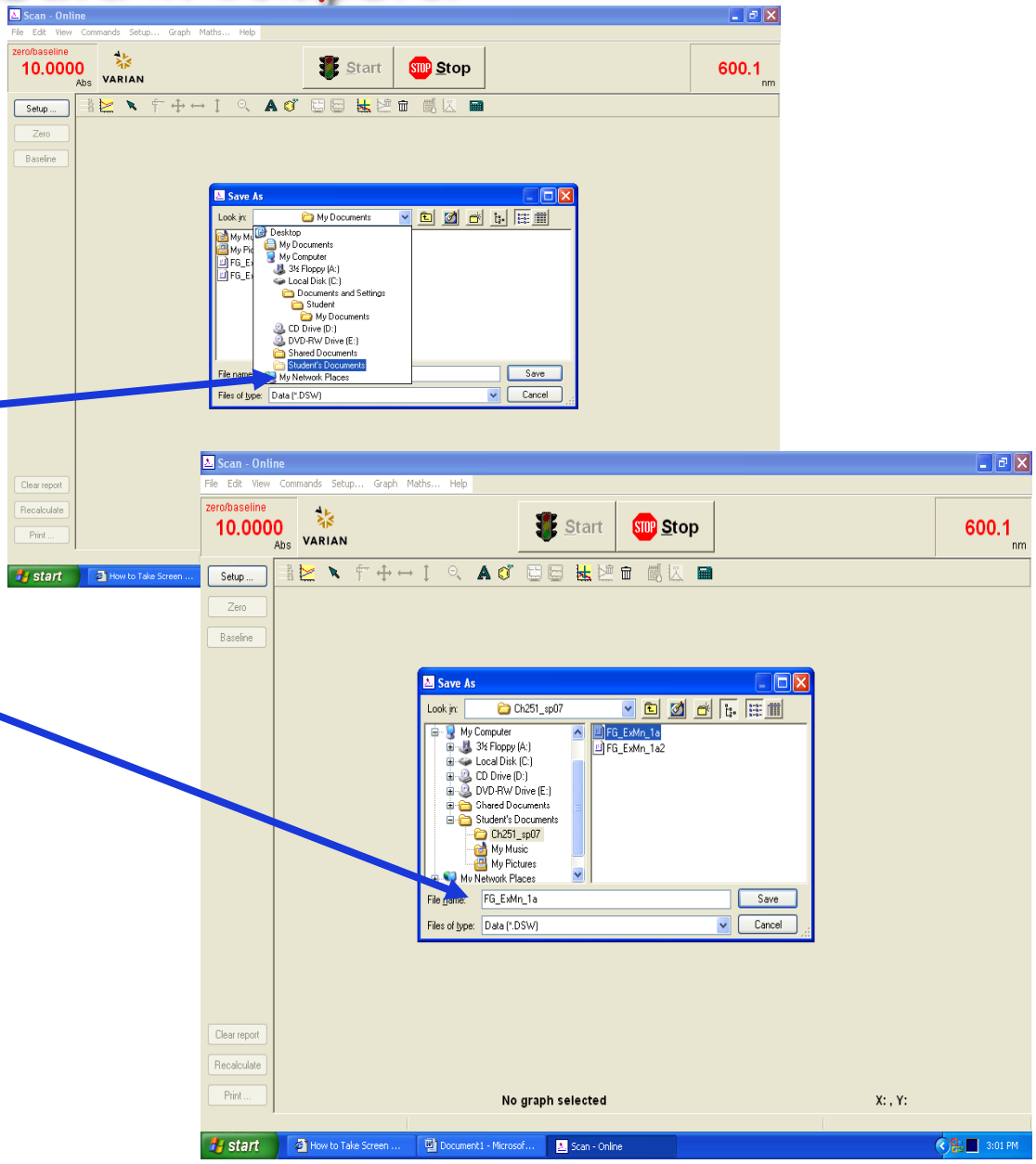
Click on "Start"

Computer will prompt your for a filename

Use the following form:

firstinital Lastinital_Expt#_Trial#

example: **FG_Expt4_Trial1a**



Collecting UV-Vis Absorption Spectra

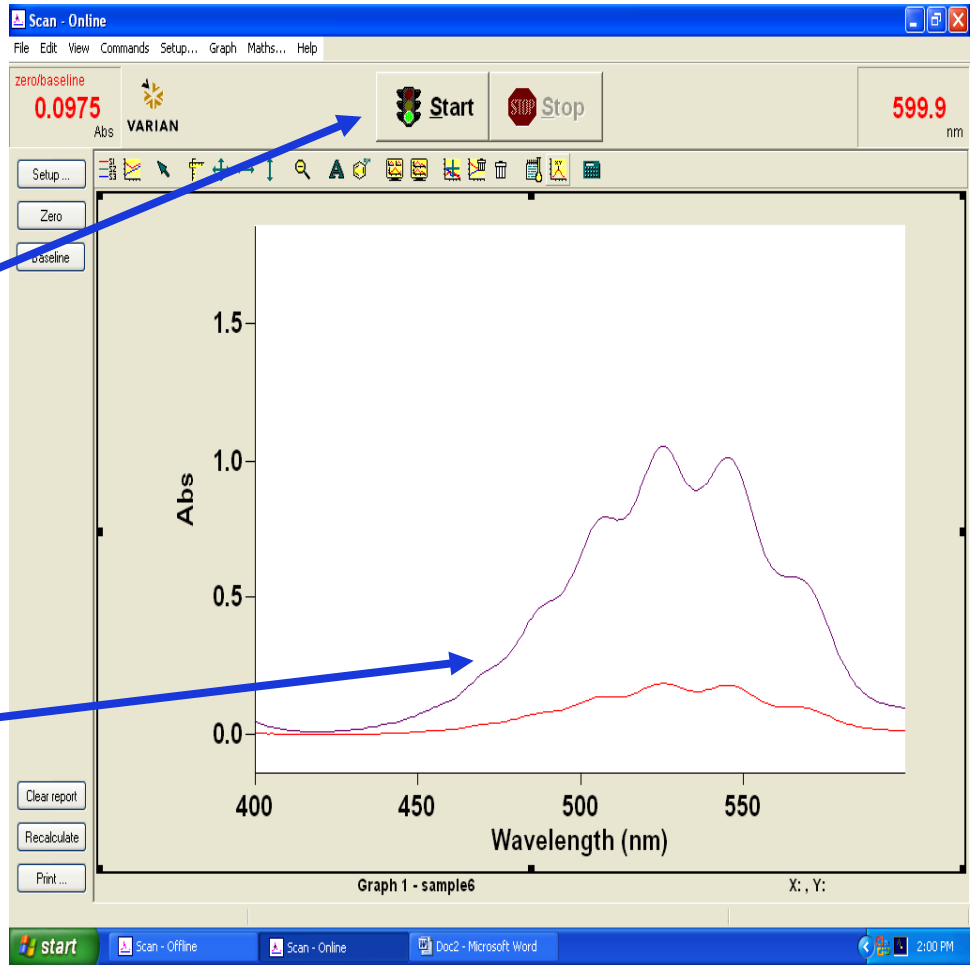
Remove cuvette with blank

Pre-rinse cuvette with solution to be analyze

Insert cuvette in spectrometer with clear window of cuvette cell properly align

Click on start:
The Cary 50 will scan from 600 to 400 nm and record the absorption spectra.

Repeat the procedure for the second trial, you will see the second absorption spectrum trace over layed the first spectrum



Labeling Peaks and Printing Spectrum

To label the peak maxima, click on the "Peak Label" icon.

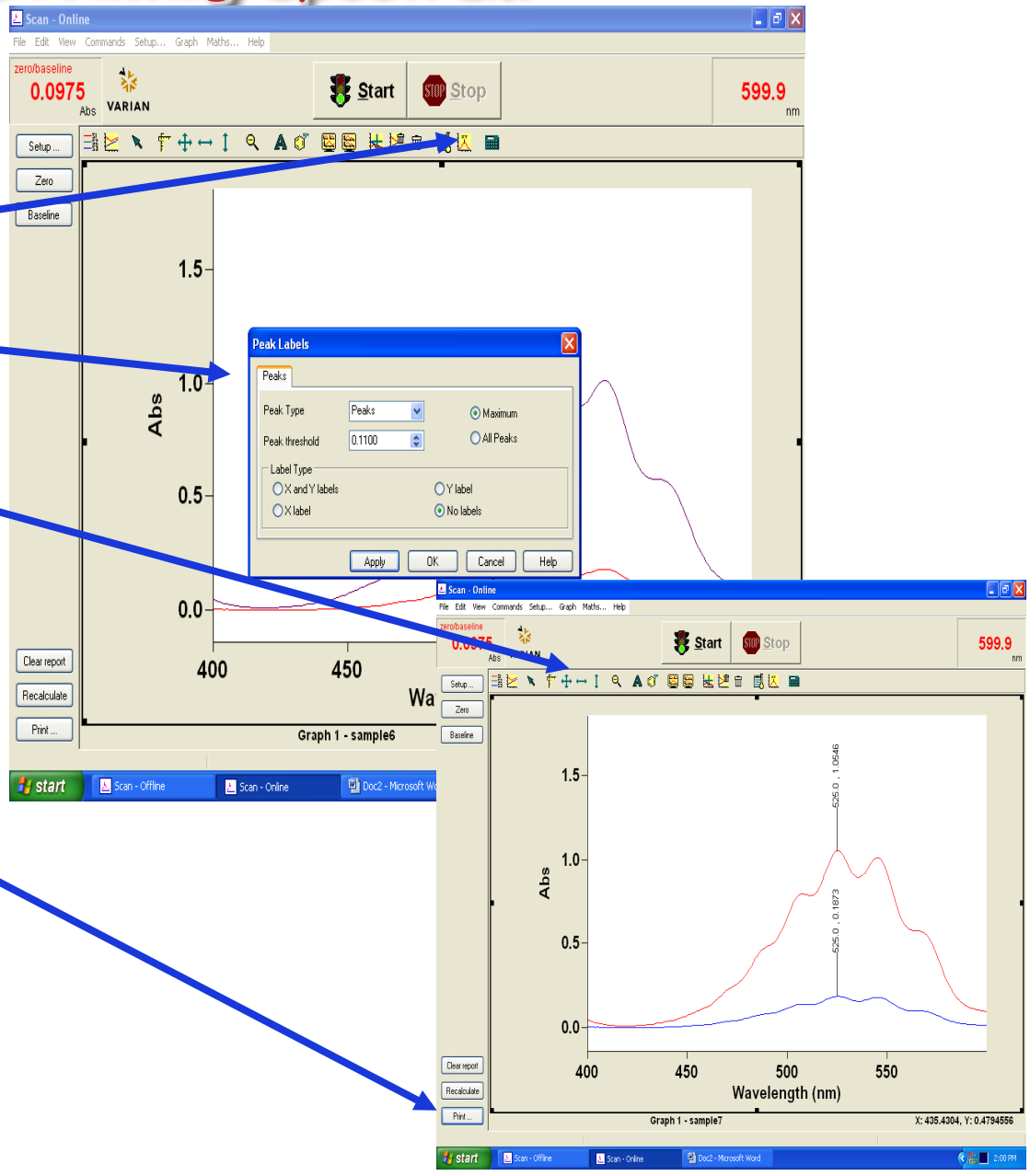
The menu "Peak Label" will appear. Choose maximum and "X and Y label".

Click on "Apply" and then "OK"

The spectra will be labeled. Click the up-down arrow to get the labels in the window.

Click on "Print".

Remove cuvette and clean the workstation and the bench area.



Summary

After printing your data, carry out the analysis by "Standard Addition" as discussed in experiment 4.