Operating instructions - Cary 100 Bio UV-visible Spectrophotometer

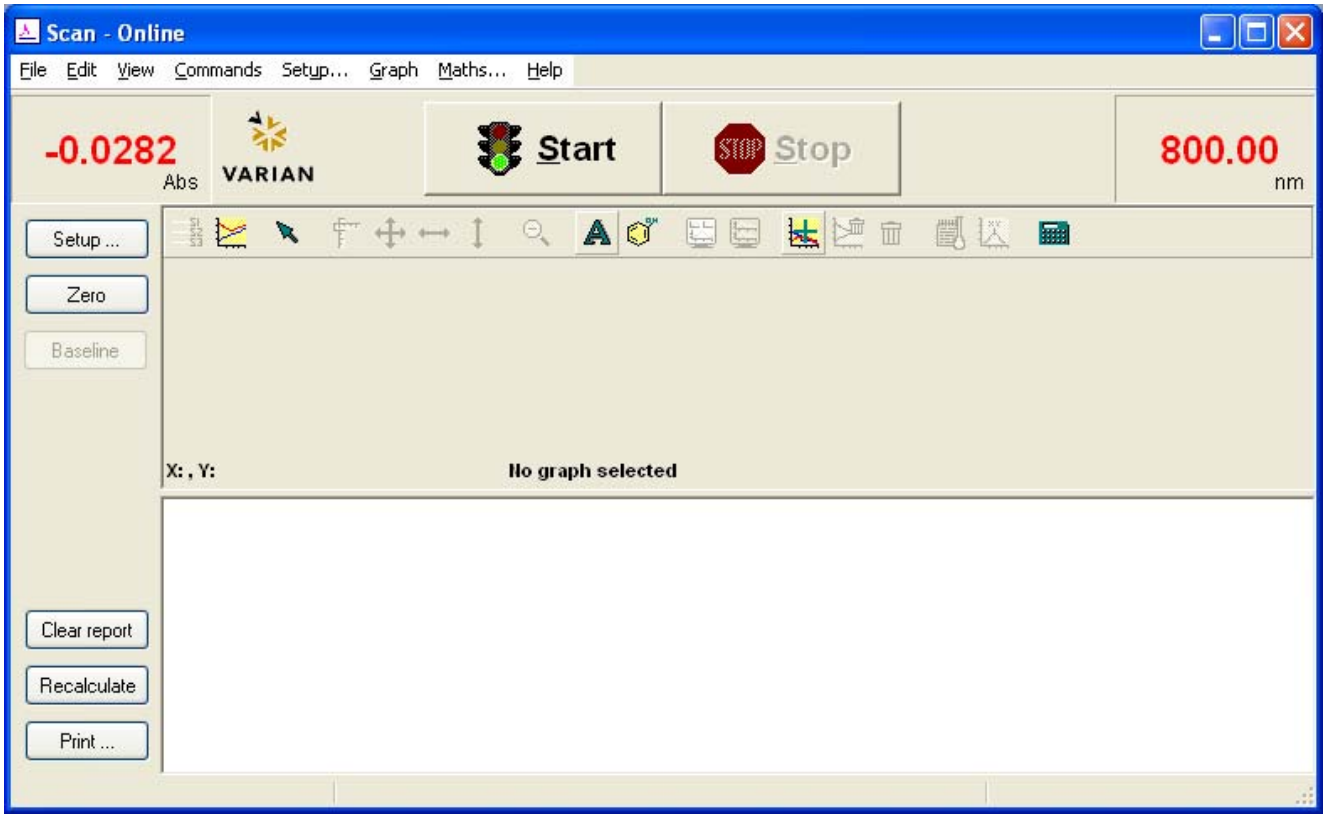
This document describes how to power up the spectrophotometer, set its measurement parameters, insert one or more of samples for analysis and collect data.

1. Software configuration

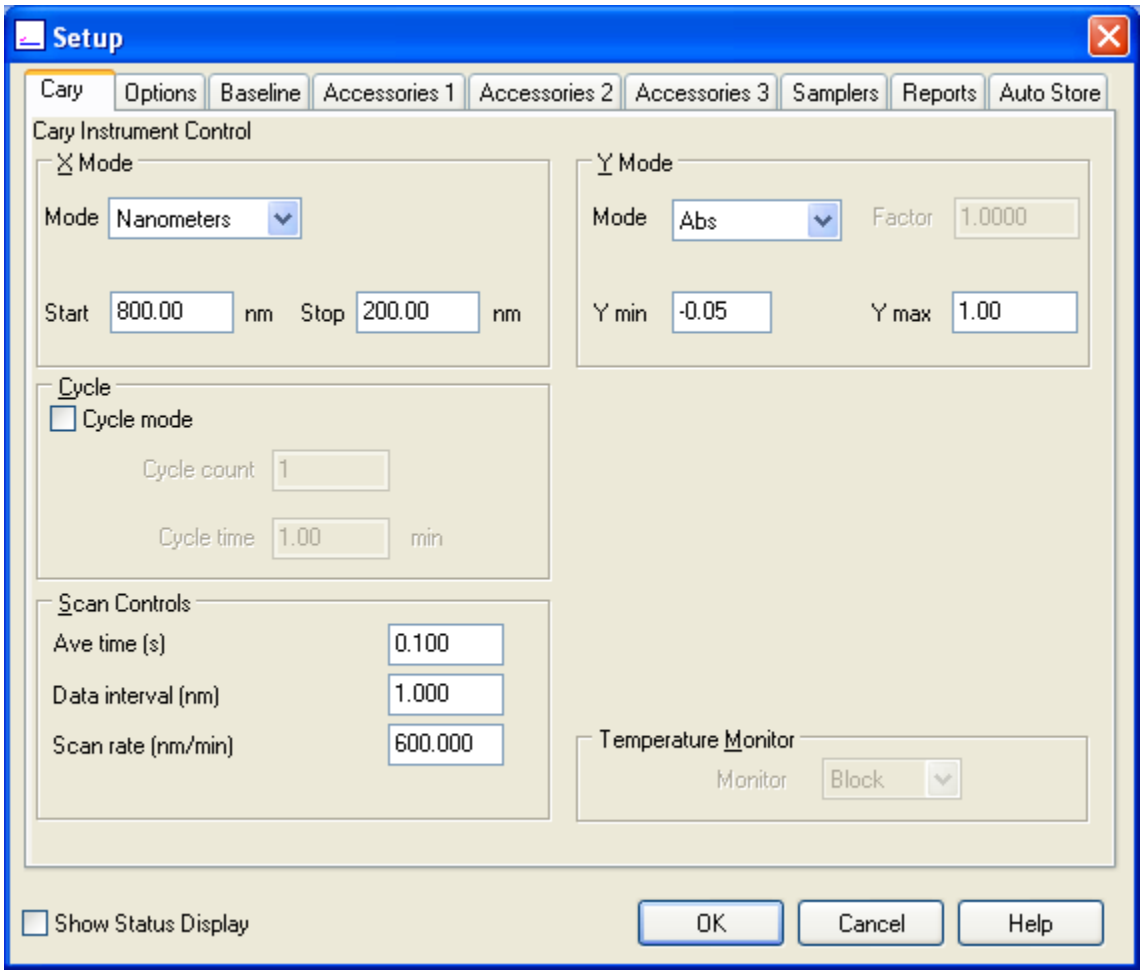
1.1. Turn on the spectrophotometer (using the power switch located directly beneath the Cary 100 Bio logo) and log into the computer terminal with your N-number.
1.2. Open the Cary WinUV folder on the computer's desktop and start launch the "Scan" program. The Varian scan software should launch. Before proceeding, wait one minute for the software to finish establishing its connection to the spectrophotometer.

2. Physical Preparations

2.1. Obtain two clean cuvettes. If desired mark one non-transmitting face of each as the "front" (when placing them in the instrument, orient those faces towards the beam). Rinse them with the solvent you used to prepare your solutions.
2.2. Fill each cuvette to at least ¾ capacity: one with only the solvent (this is your "blank"), the other will contain a solvent to first obtain the baseline and then the solution of your sample.
2.3. Wipe the cuvettes' transmitting sides with a Kimwipe®, while holding the top of the cuvette
2.4. Use 8x6 cell changer (if needed) and this assumes double mode norms



2.5. Access the Setup controls (using the "Setup…" button at left) and set the following parameters to suit your experiment.



2.5.1. Under the "Cary" tab:

2.5.1.1. X Mode (sets the scan range to be marked in nanometers, wave numbers or angstroms)

2.5.1.2. X Start and Stop (sets the region of the spectrum to be scanned)

375 to 700 nm is a typical range using a plastic cuvette

2.5.1.3.

2.5.1.4. Y Mode (sets the output data to be reported as absorbance, % transmittance or other parameters)

2.5.1.5. Y min and max (sets the upper and lower bounds on the graph the Cary system will use to display the collected data)

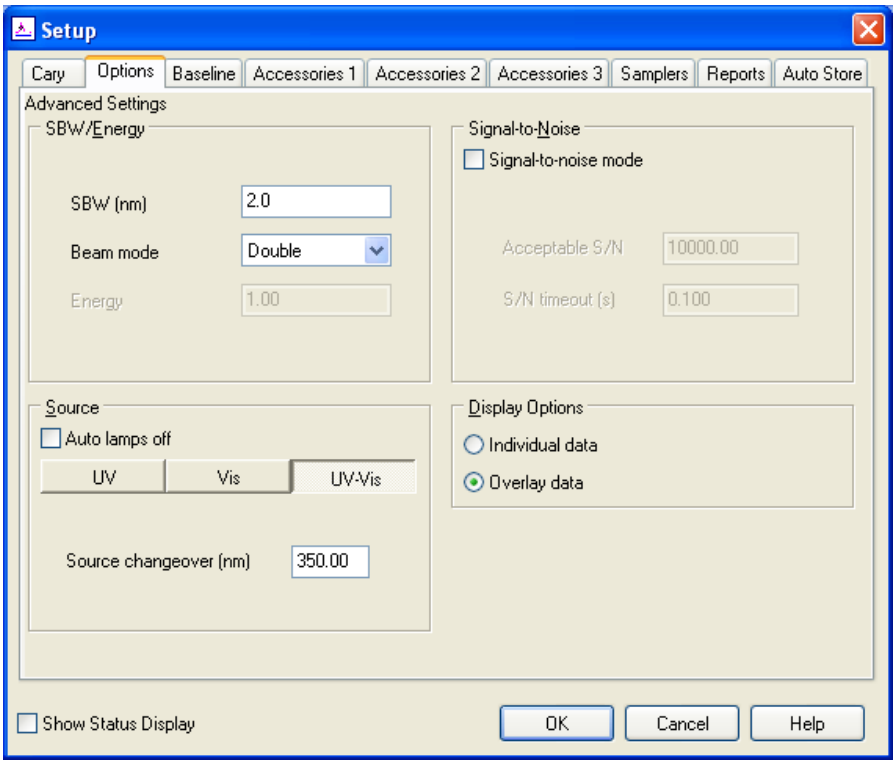
2.5.1.6. Cycle parameters (instructs the Cary system to take multiple scans of the same sample at a specific time separation)

2.5.1.7. Scan controls (note: these parameters are interdependent)

2.5.1.7.1. Ave time (controls the length time the Cary will collect a signal for each data point in the X range)

2.5.1.7.2. Data interval (sets the distance between data points in the X range)

2.5.1.7.3. Scan rate (sets how quickly the Cary will scan the X range)

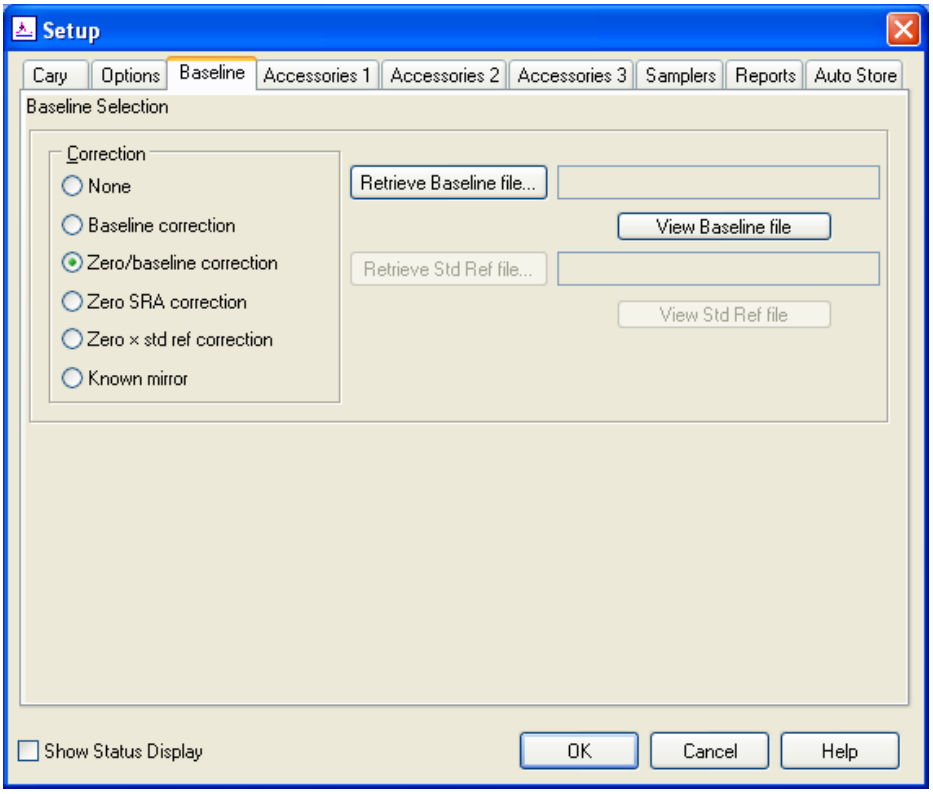


2.5.2. Under the "Options" tab:

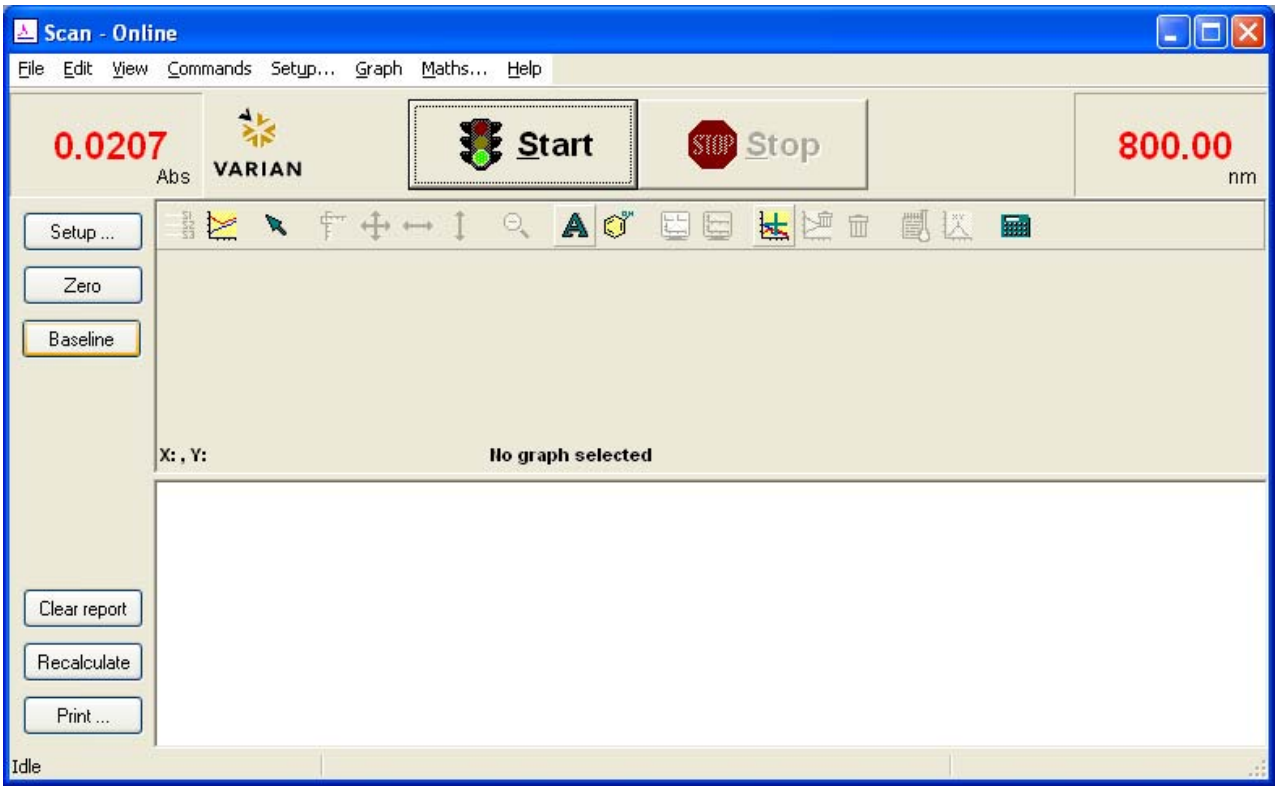
2.5.2.1. Spectral bandwidth (the width in nm of the light at half peak height; can be used to increase the signal to noise ratio or the spectral resolution of the instrument.)

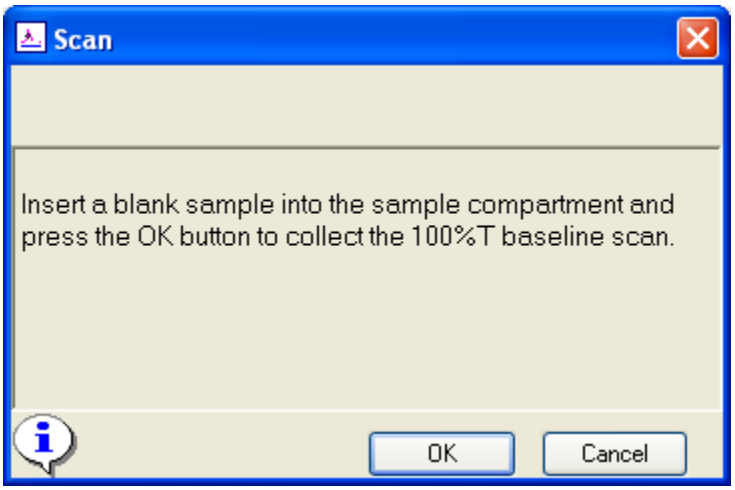
2.5.2.2. Beam mode ("Double" is recommended, as it will scan the sample and the reference solution simultaneously to generate a spectrum that is independent of the solvent and cuvette's aborptivity; these instructions assume you are using "Double" mode)

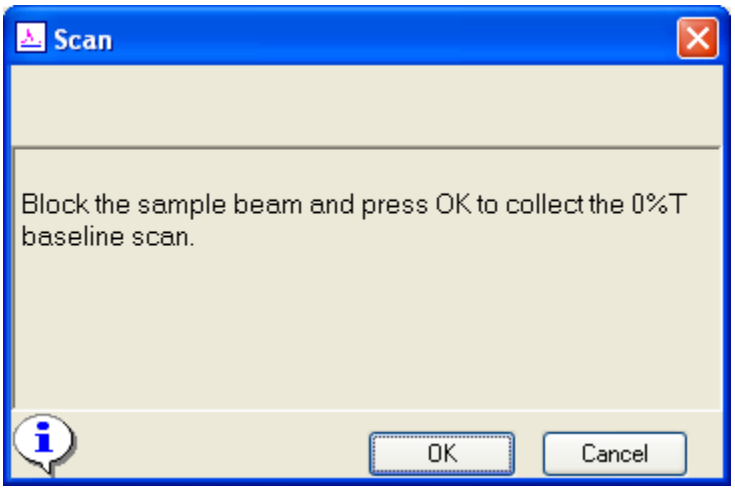
2.6. Under the "Baseline" tab:



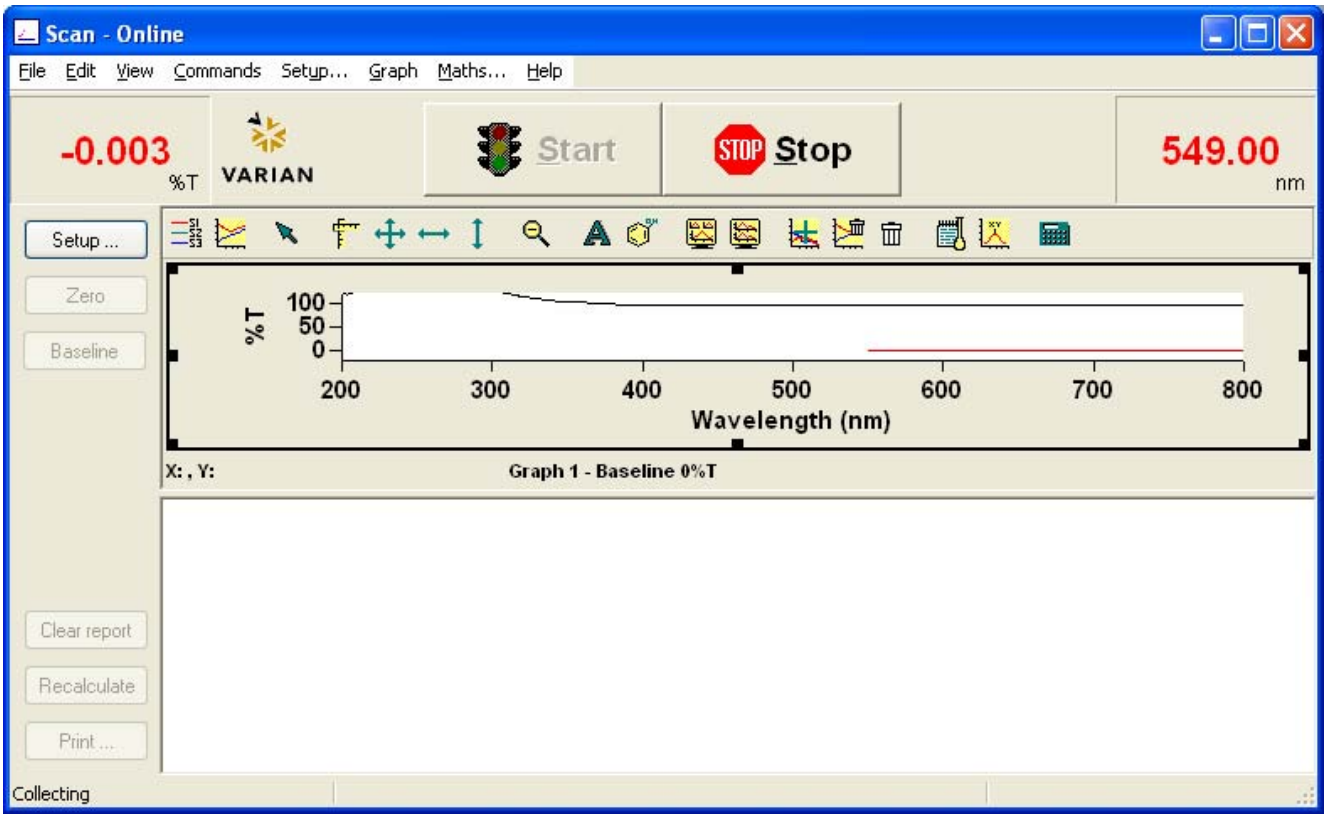
2.6.1. Execute a Zero/baseline correction with DI water in cell holder 1 and 9. Press OK, then baseline.





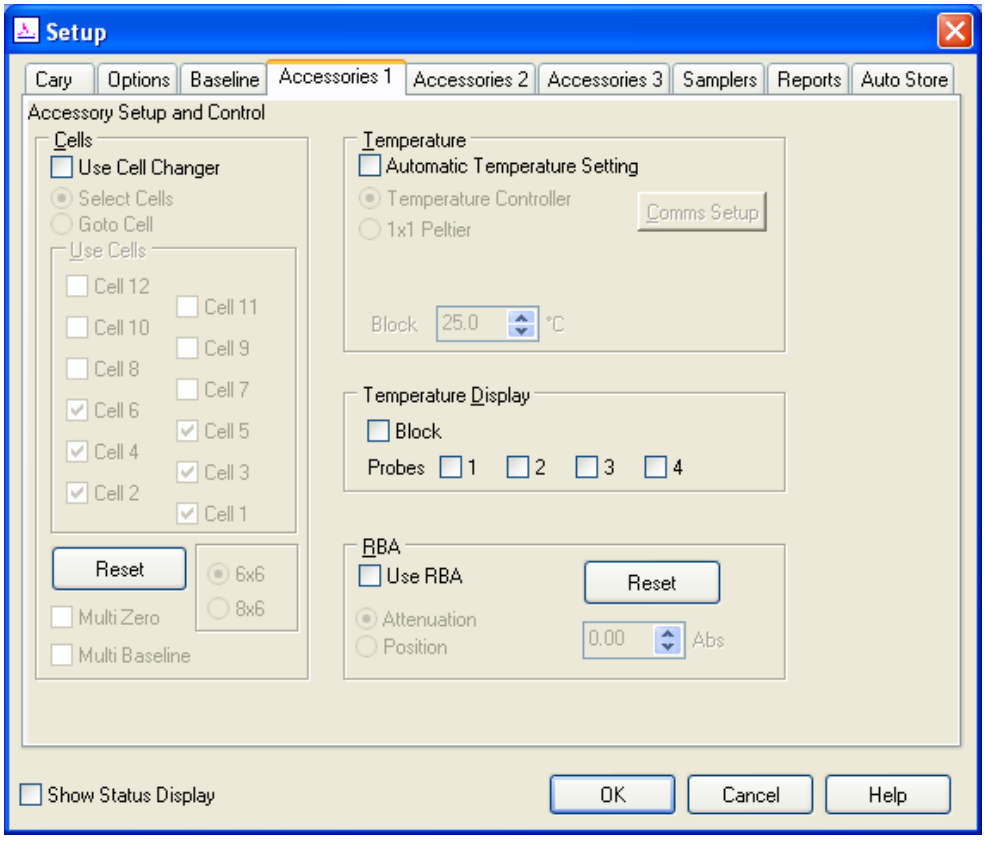


Block sample beam (e.g. floppy disk)



Remove, then click zero.

2.7. Under the “Accessories 1” tab:



2.7.1. Use cell changer enables or disables the automated motor for testing multiple solutions in a single run; these instructions assume you are using the cell changer)

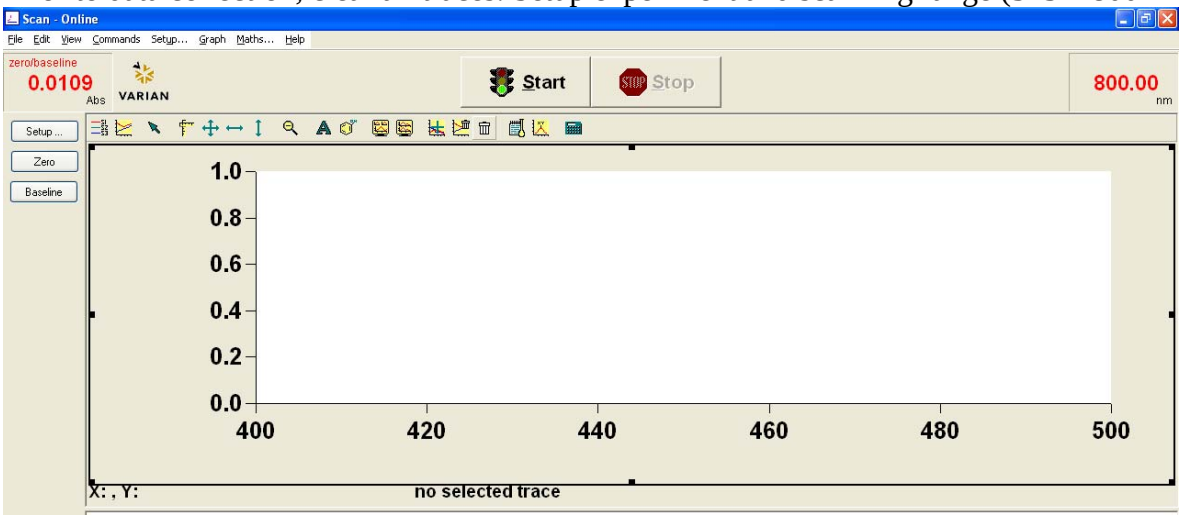
2.7.2. Select cells allows you to select which cells the spectrophotometer will scan; select any cells 1-6 which will contain a sample during your experiment (n.b: selecting any cell 7-14 will force the spectrophotometer into single-beam mode)

2.8. Under the "Reports" tab:

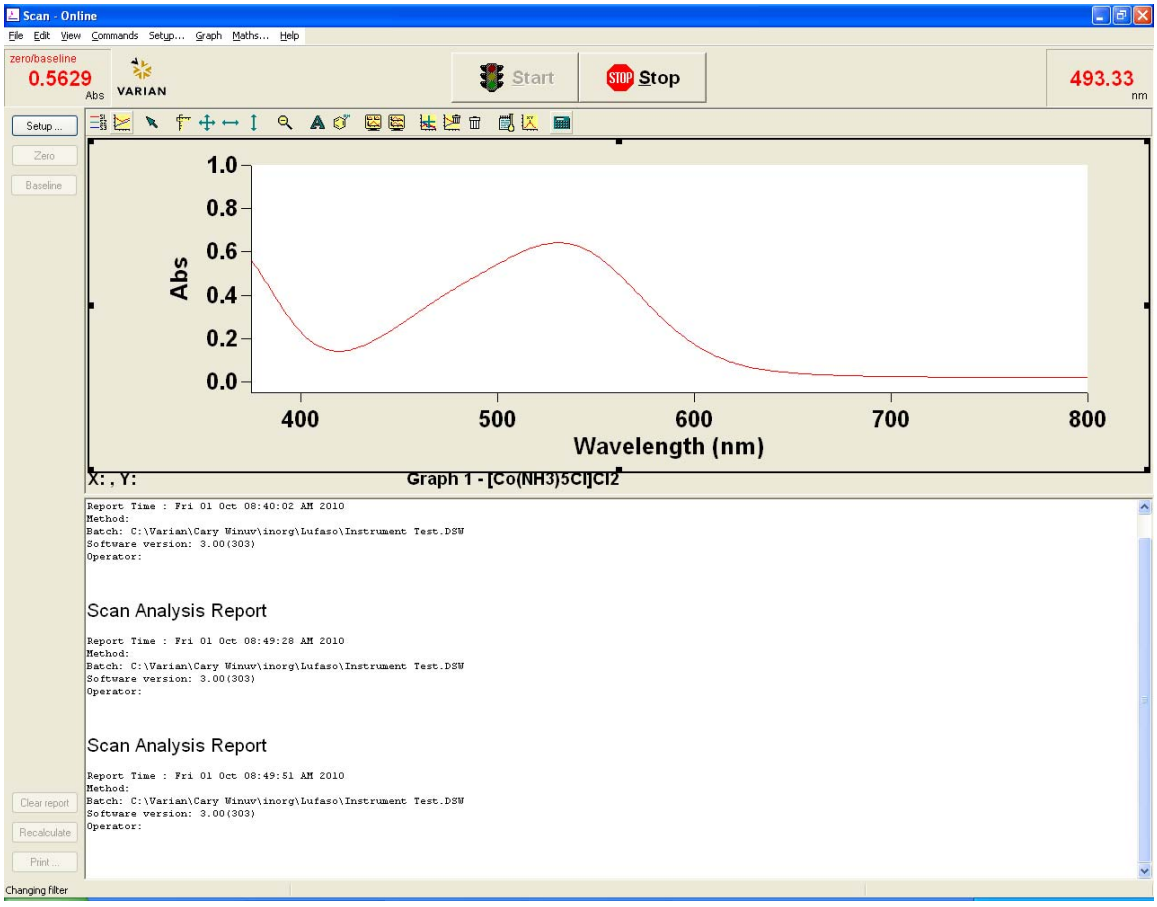
2.8.1. Save data as ascii, e-mail to yourself, for use in later plotting in Excel or equivalent program.

3. Data Collection

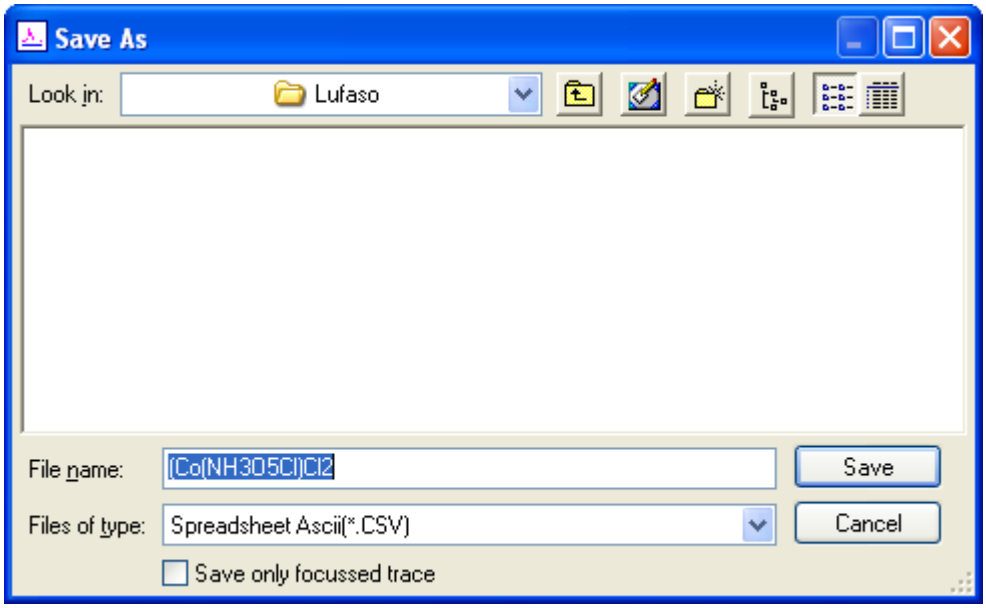
Prior to data collection, clear all traces. Setup experiment and scanning range (375 – 800 nm).



Click start, type in sample name and filename.



Finish collecting and note wavelength of maximum absorbance and absorbance in your notebook. Print the report. If needed, save the data as a Spreadsheet ACSII file for later retrieval and plotting in Excel.



3.1. If needed, create batch file for your data. Go to the "File" dropdown menu, then "Save Data As…", then use the "Look in:" dropdown menu to open the My Computer/C:/Documents and Settings directory. Open the folder with your N-number as its title, and then open its Desktop subfolder. Give your batch file a descriptive filename and click "Save".