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# Cary 100 Bio UV-Vis Operating Instructions

***09/25/2012 S.V.***

**Location:** **1240 Hach Hall**
**Contact:** **Steve Veysey, 1234 Hach Hall**

## Safety

All researchers working in 1240 Hach Hall must complete the EH&S course: *"Fire Safety and Extinguisher Training"*. When preparing samples in this room, please wear all appropriate personal protective equipment. Aprons, safety glasses, and rubber gloves are available in 1238A Hach Hall. If solvents are involved, consider preparing your sample in room 1238A.

Properly dispose of glass pipettes and plastic pipette tips in the containers provided. Waste solvents can be disposed of in the waste containers provided in 1238A. All of the computers in this lab have direct links from the desktop to MSDS sheets, the EH&S Laboratory Safety Manual and to the CIF Safety Manual.


## Introduction

The Cary 100 Bio UV-Vis instrument is a powerful double-beam spectrophotometer capable of quickly acquiring data in the spectral range from 200 to 900 nanometers. The configuration includes a thermostatic multicell sample transport and a Harrick diffuse reflectance unit. The instrument was originally purchased in 2008 and was moved to CIF in July, 2012.


**Cary 100 Bio UV-Vis Spectrophotometer**

The instrument is available for both reservations and walk-on use. As part of your training, a login account will be established for you on the acquisition PC.

## Instrument Startup

**1**. Log on to the computer using the username and password established during your training. The username should be same as your ISU netID.

**2**. Turn on the instrument. The switch is at the lower left corner, below the "Cary 100 Bio" logo. Wait at least two minutes for the instrument to complete hardware initialization steps BEFORE starting the WinUV Scan program.

**3**. Double-click on the Cary WinUV program folder icon 

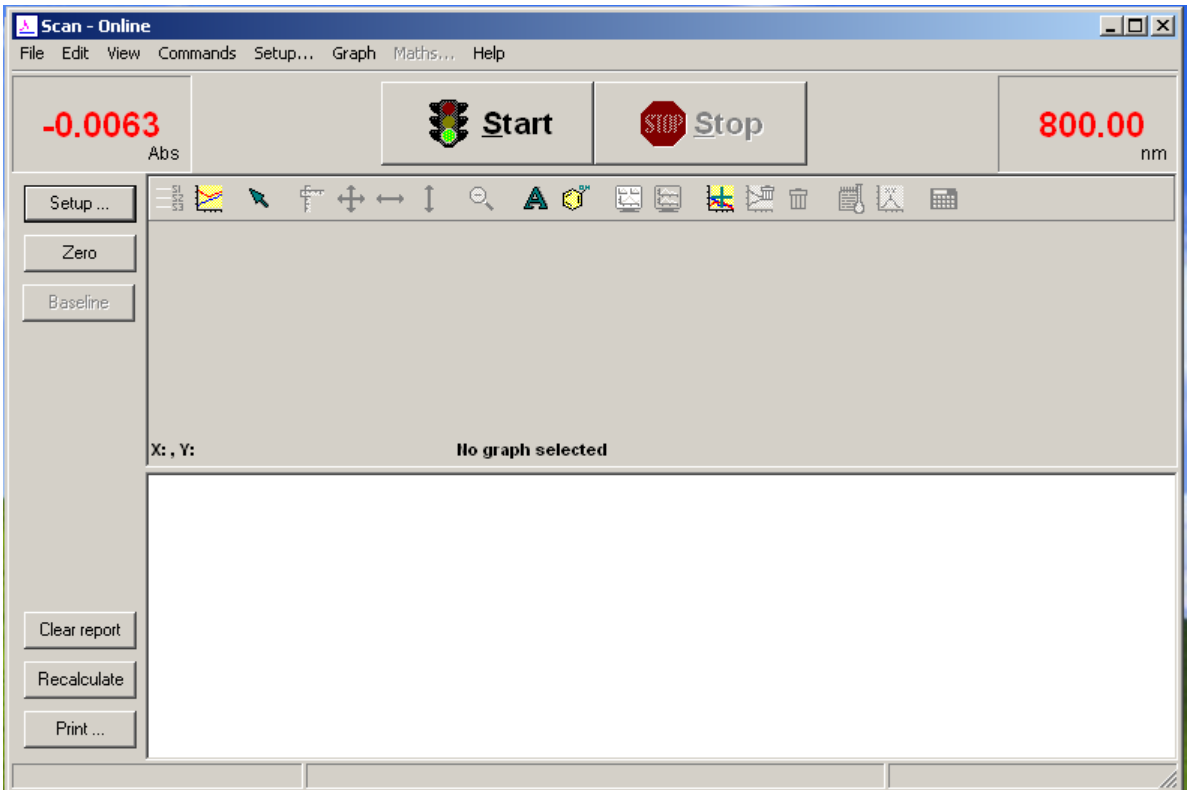
. A folder will open showing all of the acquisition and processing programs included in the Cary 100 Bio suite.


During the basic training session all users are shown how to use the "Scan" program and the "Cary Help" program. The other programs are discussed briefly at the end of this document in Appendix A, but operational details are not included here. You may request advanced training to use any of the other programs.

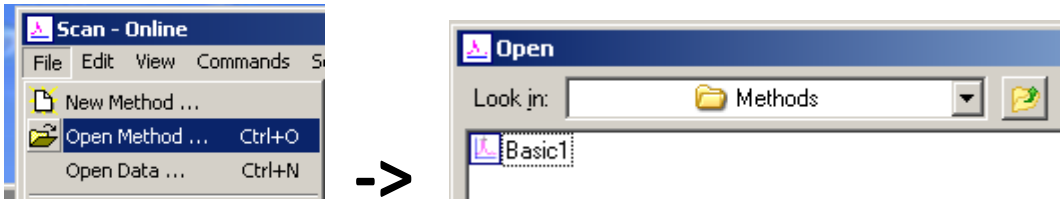
**4**. Double-click on the Scan icon. 

The software should now open in the standard view. The view should clearly indicate that the instrument is "Scan - Online" 

and the "Start" traffic light button should be enabled (not greyed out). If the program indicates "Scan – Offline", then exit the program and start the Scan program again.



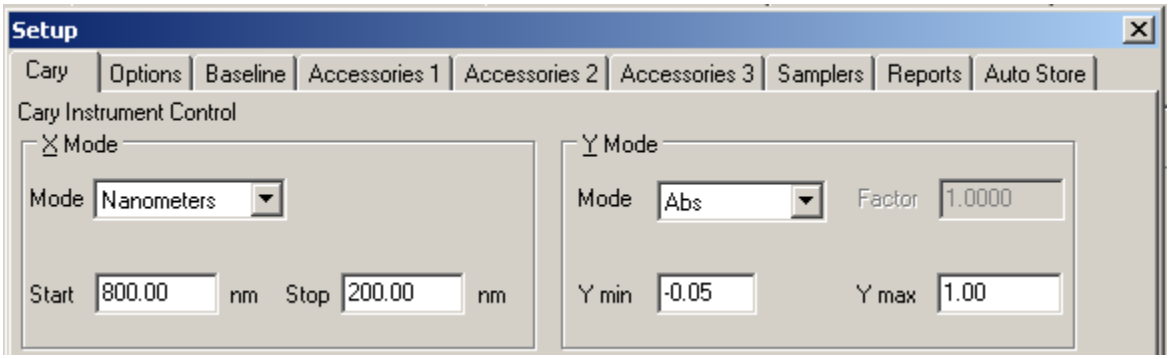

**5**. From the File pull-down menu, select "Open Method". Navigate to the Methods folder, then double-click to select the method you would like to use. In this example, it is "Basic1".



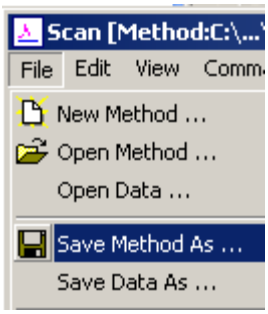

You will be returned to the Scan window.

**6.** Use the Setup button  to access and modify all of the method parameters.





Note that the changes you make to the method are only temporary unless you save the modified method.



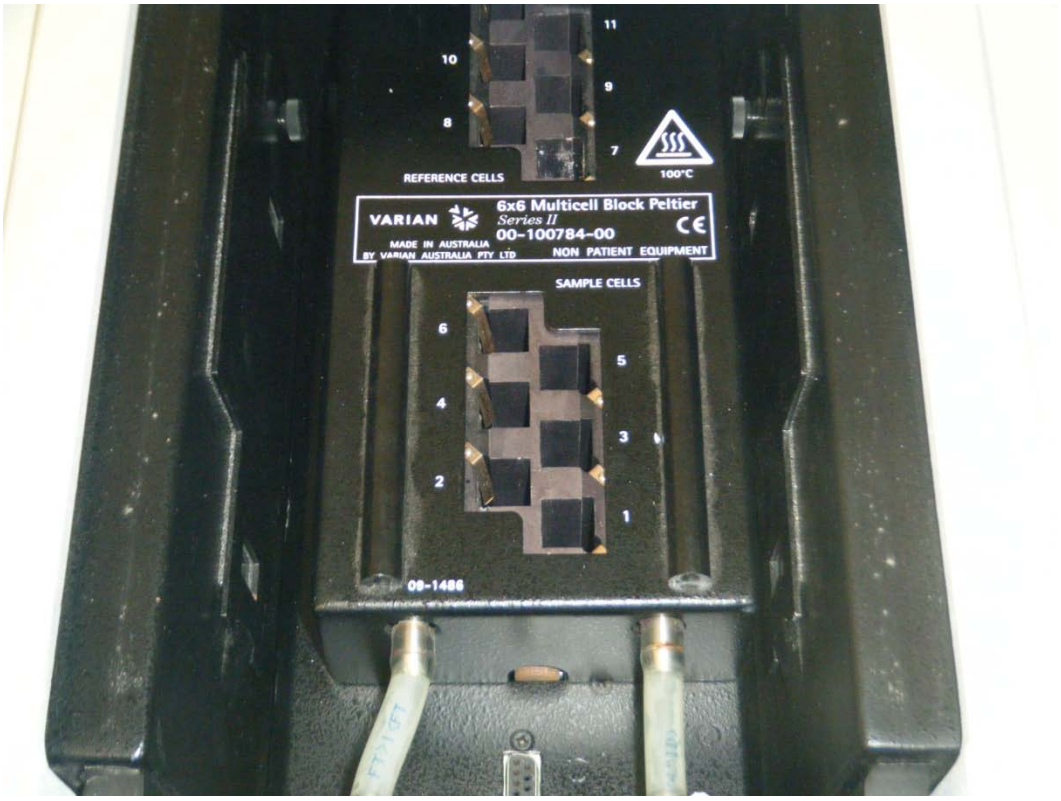

Your methods MUST begin with your username, but you may extend the name with more descriptive elements if you wish. Inappropriately named methods will be deleted.

| | | |
|---|---|---|
| Example: | *sveysey_kinetics-polyphenols* | Legal method name. |
| | *kinetics-polyphenols* | Illegal method name! |

Methods must be saved in the Methods folder, or they will be deleted. A description of all of the various parameter settings on the nine Setup tabs is included in Appendix B.

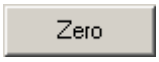
## Acquiring Data

A double-beam instrument can be operated in several ways. In the protocol below, the "reference" and "sample" files are acquired sequentially in Position 1 of the multi-cell holder. Each file is ratioed to the second beam passing through the empty Position 7. This allows the "reference" ratioed file to be subtracted from the "sample" ratioed file. If the subtraction constant is 1, this is exactly equivalent to dividing the Sample file by the Reference file – the definition of a transmittance spectrum. If the subtraction constant is varied, then features common to the sample and reference can be minimized, leaving the difference spectrum.

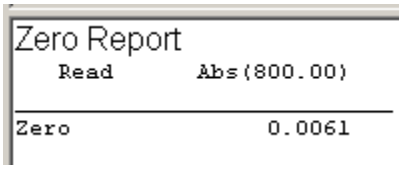



**CAUTION: Do not spill solvents inside the cell compartment. Report any spills.**

**1.** Fill a clean quartz sample cell about 2/3 full with distilled water or solvent as a blank. Place the **blank in "Position 1 – Sample Cells" in the sample compartment**. The clear sides of the cuvette must be facing left and right (a frosted side towards you). Close the spectrometer lid.

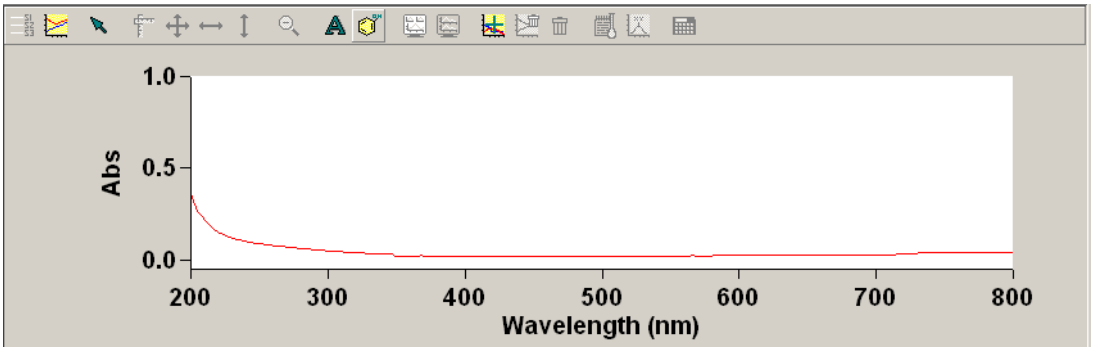
**2.** Click Zero 

to set the absorbance to approximately 0.00 absorbance units.




**3**. Click Baseline. 

Make sure your solvent-filled cell is in place; click OK. The instrument runs and displays a baseline spectrum. The instrument will subtract this baseline spectrum from each subsequent spectrum during your session. Wait until the baseline measurement is complete and the traffic light is green.

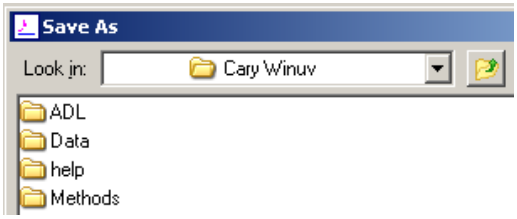


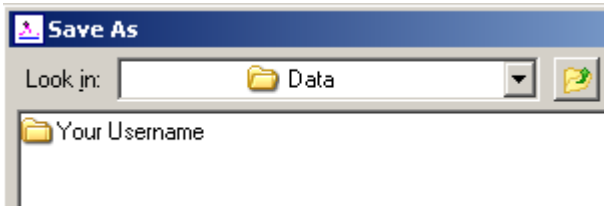

**4**. Using the eject button, carefully remove the solvent-filled cell. Empty it, and then refill it with your sample solution. Place the cell in "Position 1 – Sample Cells" and close the lid.

**5**. Click Zero 

. Click Start (traffic light) 

to begin the scan. The "Save As" dialog box will open if the *Setup -> Auto Store -> Storage ON* feature is enabled in your method parameters. Navigate to the Data folder, and then to the folder with your Username.



->


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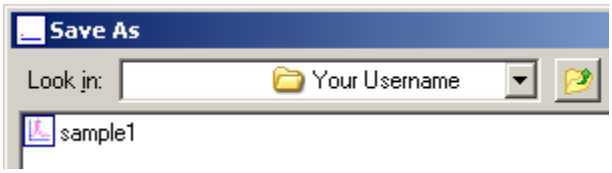
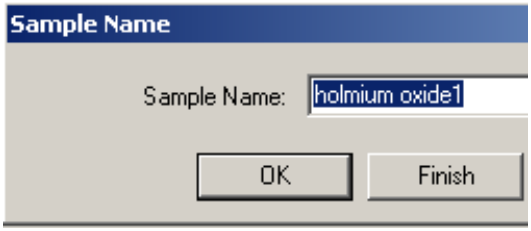



You may name your data files as you wish, as long as they are stored in YOUR username folder.

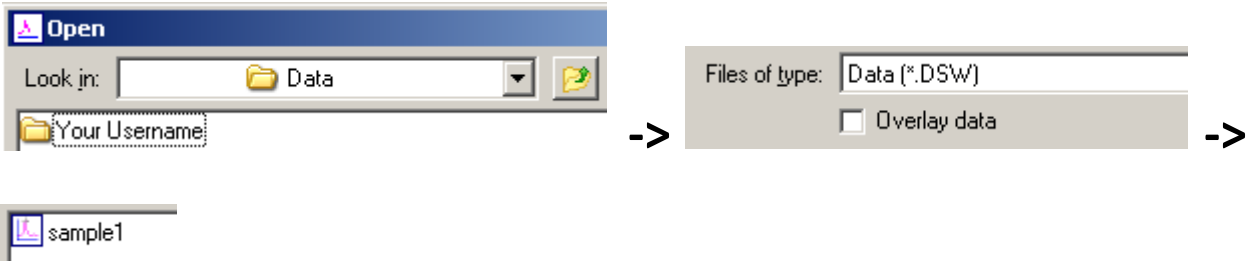



After designating a filename for the data, you will be requested to provide a sample name. This can be as descriptive as you like.

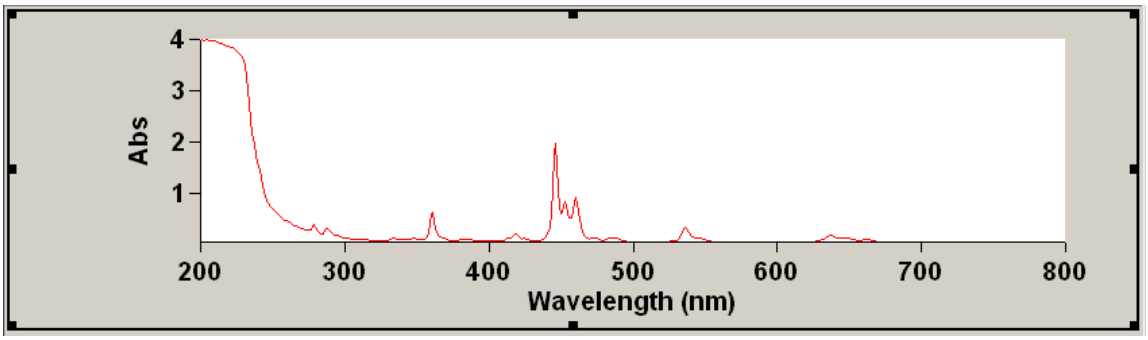
Data acquisition will then proceed. The spectrum will typically appear in the upper half of the screen, followed by calculation of peak positions and intensities (absorbance) and generation of a report in the lower half of the screen. The exact view format will depend upon the *Setup -> Report*s settings in your method parameters.

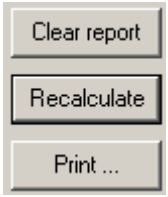
## Processing Data

**1.** The current run data will be displayed automatically. You may also retrieve previously acquired data from your data folder (*File -> Open Data…*). Navigate to your data directory. Be sure to specify file type .DSW.




**2.** Newly acquired data will show both the graphical spectrum and the report. Previously acquired data will show the graphical spectrum, but will not show a report or peak list.



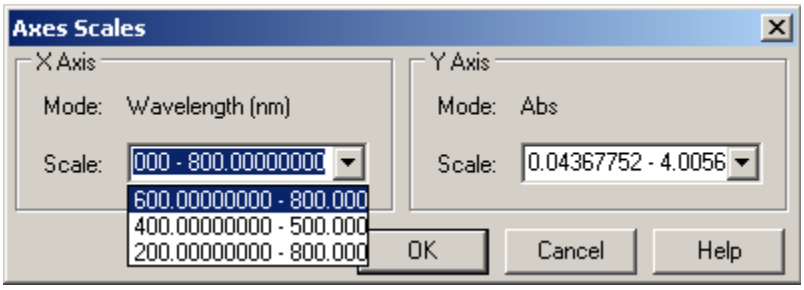

**3.** To generate a report (based upon your *Setup -> Reports* method parameters) left-click in the graph area so that the black outline and handles are now displayed. From the action buttons on the left, 

select "Recalculate" . The report will now be displayed.

**4.** Use the tools on the ribbon bar to edit and/or annotate the spectrum.

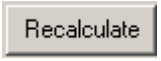
The graph must be "selected" for some of the tools to be operable. Note that there is no "zoom in" tool. The standard left mouse click-and-drag action is used to zoom in.

**5.** You may use the Scale Graph button  to expand or contract the scan to fit the graphic area and to set and select preset ranges for plotting.




**6**. If you acquire dilution data in order to get the peaks of interest within a quantifiable concentration range, you will probably want to overlay the graphs. When you have a satisfactory scan, click the Trace Preferences button  and uncheck all scans (traces) except the one to be printed. Click OK. Only one scan is visible.

**7**. Use the Peak Labels button  to adjust the peak labels as desired.

**8**. If more than one scan was acquired, click Clear Report  to remove all trace reports, and then click Recalculate  to rewrite a report for just the scan(s) visible.

**9**. Look over the report. Use the menu command Edit: Edit Report,  and edit as required. Click anywhere off of the report to exit the edit mode.

**10**. When the report is satisfactory, click Print  .

## Instrument Shut Down

**1.** Remove your sample.

**2.** Exit from the Scan Program.

**3.** Turn off the instrument. The switch is at the lower left corner, below the "Cary 100 Bio" logo.

**4.** Log out of the computer.

**5.** Sign in the log book.

## APPENDIX A – Program Descriptions


The ADL Shell gives you a pre-defined template for writing ADL (Advanced Data Language) programs. Rather than needing to write the code for basic functions such as graphing and filing, the ADL Shell has a number of these commands already implemented.


The Advanced Reads application allows you set up methods to read multiple samples in a single run. Features include finding the mean of multiple readings of the sample solution, finding the mean of multiple sample aliquots of the sample solution, et cetera.


This program is only to be used by service personnel.


The Applications Development Language (ADL) is a BASIC-like spectroscopy language that is built into the Cary software. you can write your own Cary interface to set up the instrument, collect and store data, calculate results and create reports.


Help is available for every program included in the Cary UV-Vis software suite.


The Concentration application is used to determine the concentration of an absorbing sample, using up to a 30-point calibration for quantitative analysis. The application enables you to select from several curve fit types for the calibration.


The Enzyme Kinetics application enables you to set up various parameters of the instrument and accessories, perform an Enzyme Kinetics run, and perform calculations on your results.


The Kinetics application is used to calculate reaction rates from absorbance versus time data. You can also calculate emission versus time data for fluorescence measurements.


This application enables you to scan samples across a wavelength or wavenumber range and manipulate the collected data. You can choose various display modes for the collected data depending on the type of sample you are measuring and the Cary accessories that you are using.


The Scanning Kinetics application enables you to scan samples across a wavelength or wavenumber range. From the resultant absorbance versus wavelength data, an absorbance versus time (kinetics) curve can be obtained for any wavelength in the range.


The Simple Reads application is used to perform simple absorbance readings of samples. In addition, simple mathematical operations can be performed (via the User Collect option) on readings performed at multiple wavelengths.


The RNA-DNA application can be used to collect data and calculate the various parameters used in determining the amount, type and purity of nucleic acid samples.


This application enables you to enter your laboratory details, to select the Cary instrument, and store the instrument's serial number. The information stored here is then made available to the reporting functions of all the other applications.


The Thermal application enables you to perform thermal analyses on DNA using one of the thermostatted Cary accessories. Once the data is collected, you can choose to calculate the melting temperature, Tm, by either the Derivative or Hyperchromicity method.


The Validate application enables you to optimize the settings and validate the accuracy of the Cary 100 by executing a number of pre-defined tests. The tests are preset with default parameters that comply with international standards for Good Laboratory Practices.