Cary 6000i UV-Vis-NIR --- User's Guide

Version 2.0

Edited by Dr. Hartmut G. Hedderich

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The following guide describes the use of the Cary 6000i UV-Vis-NIR spectrometer. The guide is intended to assist instrument users after the initial training session.

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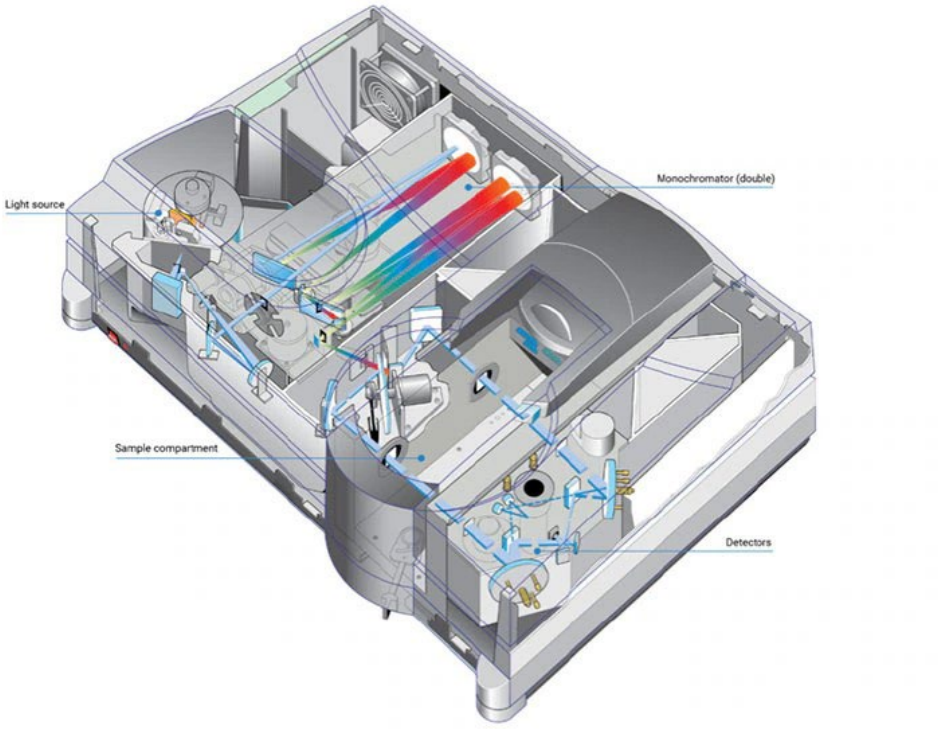
The Cary 6000i UV Vis-NIR spectrometer is a research grade instrument offering dual beam capabilities with high-wavelength accuracy. The wavelength range is 175 to 1800nm. The instrument is controlled by Agilent's WinUV software package.

Users are responsible for providing cuvettes and preparative materials. Samples should be prepared prior to arriving at RIC.

1. Hardware

The Cary UV-Vis-NIR spectrometer has two light sources – a deuterium lamp for the UV and a tungsten lamp for the visible and near-IR regions of the spectrum. The spectrometer has two gratings for the UV-Vis and NIR spectral ranges, respectively. On the detector end there is a PMT for the UV and visible and a PbS detector for the NIR.

Lamp	Deuterium to Tungsten	350 nm
Grating		800 nm
Detector	PMT to PbS	800 nm



The default sample holder is a multi-cell changer. It can hold up to six cuvettes in the front and back beam, respectively. The multi-cell changer can be temperature controlled. The temperature range is -10°C to 100°C. Spectra can be collected at a fixed temperature only. Temperature scans are not possible!

A standard single cuvette holder (no T-control) is also available. Furthermore, a sample holder for thin film transmission measurements can be installed.

The DRA-1800 is a diffuse reflectance accessory that has sample holders for solid and liquid samples.

2. Cuvettes

The standard configuration of the UV-Vis-NIR spectrometer uses cuvettes. Users need to provide their own cells for their measurements. At least one far-UV quartz cuvette with stopper or screw cap is needed. **Cuvettes with flat tops or plastic cuvettes are not allowed!**

RIC purchases cuvettes from Starna Cells (www.starnacells.com). There are two options – users who know they will only need UV-Vis measurements and users who also want to use the Edinburgh fluorometer for fluorescence spectroscopy. In the first case standard cuvettes with 2 windows and 2 frosted sides can be used; in the second case fluorescence cells with 4 windows are the better choice since they can be used with both instruments and are more cost efficient.

Here are the order numbers for Starna Cells. For micro cells please check their webpage.

21-Q-10 Standard Cell, Spectrosil® Far-UV Quartz windows, range 170 to 2700nm, 3.5ml

23-Q-10 Fluorometer Cell, Spectrosil® Far-UV Quartz windows, range 170 to 2700nm, 3.5ml

Cuvettes with screw caps are only useful if the user wants to add reactive components over time since they can be purchased with a septum screw cap.

3. Startup

Turn on the power to the spectrometer. If needed, start the temperature controller also. Start the nitrogen purge if measurements below 200nm are required. This will eliminate the UV spectrum of oxygen from your data. Also, turn on the purge for measurements below 20°C to avoid condensation in the sample chamber and on the cuvette. The purge valve is on the main gas manifold. The valve at the spectrometer should not be changed at all. It is set to a fixed flow!

Login into the Cary computer. The spectrometer must be powered up before starting any program! Open the Cary *WinUV* folder. Double-click on the *Scan* icon.

For best results, let the spectrometer stabilize for about 10-15 minutes.

4. Software

The software needs to connect to the spectrometer. It will run through an initialization. Depending on the status of the instrument that can take from a few seconds up to a minute or so. All buttons and menus will be greyed out during this time. Please do not start clicking buttons or menus until the screen turns to colored displays.

The main screen has two windows. The top one will graph data, the bottom one shows a report which contains all settings of your setup. On the left side are the command buttons:

setup, ***zero*** and ***baseline***. The top of the screen shows from left to right: Abs, %T or %R, Start and Stop buttons and wavelength.

Click the *Setup* button. A menu will open that contains the following choices:

Instrument Parameters:

(a) Cary Options:

Set *X-mode* (usually nanometers) and wavelength range (max. is 1800nm, min. is 175nm). Start is always the higher(!) wavelength. Set *Y-mode*; either Abs (absorbance), %T (transmittance) or %R (reflectance). Also define *average time* (per data point), *data interval* (resolution) and *scan rate*.

(b) Advanced Settings:

Default slit width (SBW) is 2.00nm, default energy is 1.00. Slit height depends on the experiment. It is *full* for standard setups and *reduced height* for micro cells and the DRA-1800 setup.

Beam mode has 4 options: single front, single back, double and reverse double. The single modes are for using one cuvette and measuring baseline and sample separately, double modes are for using two cuvettes – sample and reference.

Source/Detectors has settings for turning the correct light source(s) on and off. By default, all light sources will be turned on during startup of the spectrometer. If the UV light source is not needed (all measurements are above 350nm) please click the **Vis** button. This will turn off the deuterium lamp which will extend its lifetime. When using the complete UV-Vis-NIR region both light sources will be used. Please push the **UV-Vis** button.

***(c) Independent UV-Vis and NIR*:**

The independent control allows separate settings for the UV-Vis and NIR regions of the spectrum. This can be useful to collect i.e.: higher resolved data for the NIR, have increased S/N ratio for NIR (NIR detectors display more noise than a PMT), etc.

(d) Baseline:

How to apply baseline corrections – for the double beam mode corrections don't need to be applied since we do measure the sample and reference (usually solvent) together. If the cuvettes are from the same material this will ensure auto-corrected data. Setting: *None*

In the single beam mode setup baseline and zero line need to be collected. Setting: *Zero/baseline correction*.

Baseline is a measurement of the cuvette filled with solvent. It will return the maximum signal for each wavelength over the complete spectral range. The beam needs to be blocked to obtain the zero line. When baseline/zero correction is applied a window will open and guide the user through the correct steps.

Accessories:

A long list of accessories will be displayed. The spectrometer has access to the multi cell changer and the temperature controller. The DRA-1800 is NOT displayed in the accessories' menu. Please see below how this accessory is accessed.

(a) Cell Change:

check *Use Cell Changer* if you plan to measure multiple samples automatically. Select the cell positions you will be using. Default positions (if cell changer option is not used) for standard measurements are positions 1 and 7.

(b) Temperature Controller:

check *Automatic Temperature Setting* and then enter your desired temperature (-10°C to 100°C). The Temperature Display should be set to *Block*.

Warning: when the temperature controller is in use, all options will be greyed out. Access is regained after the desired temperature is reached. The screen will turn back to colored display. Do not push buttons or menus during this process, it might crash the program.

Reports:

If desired, please fill in *Operator* name and any experimental comments. Click on *Options* on what data to save in the report.

The only important part is the choice of the correct *autoconvert* function. Always select "**Select for ASCII (csv)**". This will produce a standard ASCII/CSV text file that can be read in Excel, MathCAD, MATLAB, etc. A save file in the WinUV format will be generated automatically, too.

File Storage:

select how you would like to save the raw data.

After all settings and options have been chosen, select ***OK*** to transfer all the parameters to the instrument.

If you are using a single beam setup, follow the instructions on the screen to obtain baseline and zero-line data. Then put your sample in position 1 of the multi-cell changer.

If you are using two matched cuvettes: place a cuvette containing solvent in the reference compartment (position 7) and place a cuvette containing your sample in the corresponding sample compartment (position 1).

Click ***Start*** to collect a spectrum.

The data will be automatically saved in the internal file format as well as in any format that was chosen (usually ASCII/CSV). The report file is NOT automatically saved! To save the report, choose *export report as*, choose the preferred format and save the file.

Push the ***clear report button*** before collecting the nest data set.

5. DRA-1800 Diffuse Reflectance Accessory

The DRA-1800 is an accessory which comes with its own set of detectors. It allows measurements on non-transparent samples in reflection. Sample holders for cuvettes, powders and flat samples are available. The range of the DRA is 200 to 1800nm.

Please give the person-in-charge for the instrument at least 24-hour notice since it takes some effort to change the Cary 6000i to the DRA setup.

The correct setup setting for DRA-1800 calibration are listed in the table below.

A PTFE sample disc is used for *baseline* measurements. The PTFE powder sample is 6mm thick and has a density of 1g/cm^3. This is the optimum thickness and density for accurate calibration measurements. Please do NOT touch or scratch the surface of the PTFE disc!

For the *zero* measurement remove the PTFE disc and perform a measurement with the backend of the DRA open. The black material of the cover box will absorb the light and thus give us the correct zero line.

DRA-1800 Setup Parameters for Cary Options	**UV-Vis:**	**UV-Vis-NIR:**
X-mode: Mode	Nanometers	Nanometers
X-mode: Start	700	1800
X-mode: Stop	400	300
Y-mode: Mode	%R	%R
Y-mode: Y min	-5.00	-5.00
Y-mode: Y max	110.00	110.00
Scan controls: Ave time (s)	0.100	
Scan controls: Date interval (nm)	1.000	
Scan controls: Scan rate (nm/min)	600.00	
Advanced Settings		
SBW/Energy: Fixed SBW	ON	
SBW/Energy: SBW (nm)	2.00	
SBW/Energy: Beam mode	Double	Double
SBW/Energy: Slit height	Reduced	Reduced
Source: lamps	UV-Vis	UV-Vis

Source: Source change (nm)	350.0	350.0
Source: Detector change (nm)		800.00
Independent UV-Vis and NIR		
Independent control		ON
Measurement mode		Auto
UV-Vis controls		
Ave time (s)		0.1
Data interval (nm)		1.000
Scan rate (nm/min)		600.00
SBW (nm)		2.00
NIR controls		
Ave time (s)		1.000
Date interval (nm)		2.000
Scan rate (nm/min)		120.00
Energy level		3.00
Baseline		
Correction	Zero/baseline correction	Zero/baseline correction
File Storage		
File Storage: Storage	ON (prompt at start)	ON (prompt at start)

Cuvette holder:

Powder holder:

6. Cary WinUV software suite

The Cary WinUV software is a suite of programs. The programs are listed below with a short description. The basic setup of all those programs is essentially the same. Depending on the program some extra input is needed (i.e.: temperature, kinetic constants, etc.). Please see the person-in-charge for more details if needed.

- *Cary Help* is the online user's manual.
- *Advanced/Simple Reads* are used to make measurements at fixed wavelengths.
- *Concentration* is used, with calibration standards, to determine the concentration of a sample.
- *RNA/DNA* is used to measure the amount, type, and purity of nucleic acid samples.
- *Scan* is used to scan samples across a wavelength range and manipulate the collected data.
- *Kinetics* is used to measure absorbance as a function of time at a single wavelength and to calculate reaction rate constants. Calculation of Zero, First and Second order reaction rate constants with best-fit simulations is provided.
- *Enzyme Kinetics* is used to measure absorbance changes as a function of time and to calculate various rate-related constants.
- *Scanning Kinetics* is used to measure absorbance changes as a function of time over a scanned wavelength range.
- *Thermal* is used to measure the absorbance of a sample as the temperature is varied.

7. Turn Off/Shut Down Cary 6000i

After finishing all experiments, close the program. Turn off the Cary 6000i and if used the temperature controller. Remove your samples and cuvettes from the instrument. Turn off the purge gas!

8. Contacts

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9. Notes