

# **Cary WinUV**

## **Software Manual**



**Agilent Technologies**

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## Errata Statement

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A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

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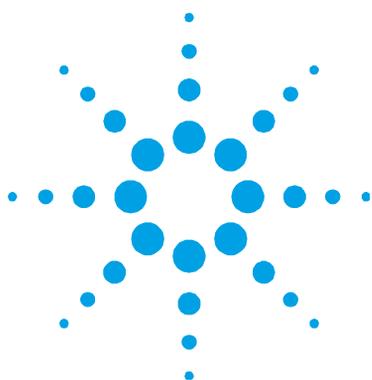
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## 1. Introduction

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### Applications

Agilent Cary 100/300/4000/5000/6000i instruments run using the easy-to-use Agilent Cary WinUV software. The Cary WinUV software consists of various applications, depending on the Cary package ordered (refer to Table 1). A brief description of each application is provided in Table 2. For detailed information about each application, refer to Chapter 3.

**Table 1.** Applications included with each Cary WinUV software package

Package	Applications
Analysis	ADL Shell, Advanced Reads, Align, Concentration, GLP Administration, Kinetics, Scan, Scanning Kinetics, Simple Reads, System Information, Validate
Bio	ADL Shell, Advanced Reads, Align, Concentration, Enzyme Kinetics, GLP Administration, Kinetics, RNA-DNA Estimation, Scan, Scanning Kinetics, Simple Reads, System Information, Thermal, Validate
Color	Color
Pharma	ADL Shell, Advanced Reads, Align, Concentration, Enzyme Kinetics, GLP Administration (not for CFR systems), Kinetics, RNA-DNA Estimation, Scan, Scanning Kinetics, Simple Reads, System Information, Thermal, Validate, Spectroscopy Configuration Manager (administrator use only), Varian Spectroscopy Database Administrator (administrator use only)

**Table 2.** Descriptions of each Cary WinUV software application

<b>Application</b>	<b>Description</b>	<b>Page</b>
ADL Shell	A pre-defined template for writing ADL programs.	22
Advanced Reads	For collecting absorbance readings for multiple samples at single and multi wavelengths (up to 6).	22
Align	For aligning various instrument lamps and accessories.	22
Color	For calculating color coordinates, determining color difference and graphically displaying both.	23
Concentration	For quantitative analysis.	24
Enzyme Kinetics	For determining various parameters of enzyme activity at single and multi wavelengths (up to 6).	24
GLP Administration	For restricting operator access, password-protecting each application.	24
Kinetics	For performing kinetics determinations at single and multi wavelengths (up to 6).	25
RNA-DNA Estimation	For collecting absorbance readings of nucleic acids.	25
Scan	For running and view data collections.	26
Scanning Kinetics	For performing kinetics determinations for a wavelength range.	26
Simple Reads	For taking absorbance readings at a single and multi wavelength (up to 6).	26
Spectroscopy Configuration Manager	For administrators to set 21 CFR Part 11 security and access privileges in Pharma software.	26
System Information	For recording your company and instrument details for use in reports, and so on.	27
Thermal	For temperature-based multicell measurements, typically DNA melts at single and multi wavelengths (up to 6).	28
Validate	For testing your instrument to ensure that it is working correctly.	28
Varian Spectroscopy Database Administrator	Provides a database environment for storing and maintaining your data.	28

## User documentation

You have been provided with the following documentation to help set up and operate your Cary system:

- A hardware manual, with safety practices and hazards information, instructions for installing and maintaining the components of the Cary and troubleshooting information.
- This software manual, with instructions for installing the Cary WinUV software, an overview of the software detailed 'How To' procedures and software-related troubleshooting information.
- Extensive Help (provided with the Cary WinUV software) containing context-sensitive Help, step-by-step instructions for frequently performed analyses and instructions for using any accessories you ordered. Refer to 'Using the Help' on Page 20.

## Conventions

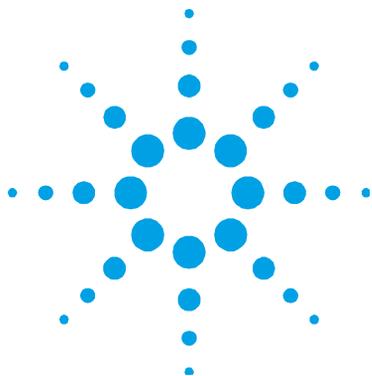
The following conventions are used throughout procedures in the documentation:

- Menus, menu items, buttons and check boxes have been typed in bold. For example, 'click **OK**' and 'From the **Edit** menu, choose **Copy**'.
- ALL CAPITALS indicate keyboard commands. For example, 'Press **ENTER**' and 'Press **SHIFT+F3**'.

A Note is used to give advice or information.

A Tip is used to give practical hints to help you achieve the best possible performance from your instrument.

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## 2. Installation

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This chapter assumes you have already set up the Cary instrument and computer, including installing the instrument interface card in the computer (if you supplied your own computer) and connecting the instrument and/or computer to the power supply as described in the appropriate Cary hardware documentation.

### Computer requirements

The Cary 100/300/4000/5000/6000i UV-Vis-NIR spectrophotometers were evaluated under Agilent's ISO9001 procedures using Cary WinUV software and a desktop computer. We strongly recommend a computer configuration that matches the system that was used during evaluations:

- HP Z210 SFF Workstation
- HP Single Unit (SFF) Packaging
- HP Energy Star 5.0 Enabled Configuration
- HP Z210 SFF 240W 90% Efficient Chassis
- MS Windows 7 Professional 32-Bit OS
- Intel Xeon E3-1225 3.1 6M 4C 95W GT2 CPU
- HP 4GB (2x2GB) DDR3-1333 nECC RAM

## Installation

- HP 250GB SATA 6Gb/s 7200 1st HDD
- Intel HD Graphics P3000
- HP No Keyboard Option
- HP USB Optical Scroll Mouse
- HP 16X DVD+-RW SuperMulti SATA 1st Drive
- HP 3-3-3 Warranty SFF
- HP Serial Port Adapter Kit
- HP Z210 Localization Kit
- SFF SATA HDD2 Cable

OR

- HP Compaq 8000 Elite Small Form Factor Desktop
- Intel Core 2 Duo E8500 Processor
- 2 GB PC3-10600 Memory (2x1 GB)
- 250 GB SATA 3.5 1st Hard Drive
- Integrated Intel Graphics Media Accelerator 4500
- Integrated Intel 82567LM Gigabit
- 16x SATA SuperMulti LightScribe DVD+/-RW
- Realtek ALC261 High Definition Audio Codec (all ports are stereo)
- Windows 7 Pro 32-bit
- One comm port if using the Agilent SPS 3 Sample Preparation System in conjunction with the Internal Routine Sampler
- Two comm ports if using the Agilent SPS 3 Sample Preparation System in conjunction with the External Sipper accessory

If upgrading from previous versions of Cary WinUV software, the computer configuration will need to include:

- IBM compatible
- Intel® Pentium® IV processor (or later)
- At least 1GB RAM
- At least 10 GB free space on hard drive
- Video card supporting 800 x 600 pixel resolution, high color (16 bit) mode (or better)
- Super VGA screen (or better)
- 20 x CD drive
- 16 bit sound card
- Windows® 101 key keyboard
- Microsoft® or compatible mouse
- Windows XP with Service Pack 3
- RS232 serial port
- One comm port if using the Agilent SPS 3 Sample Preparation System in conjunction with the Internal Routine Sampler
- Two comm ports if using the Agilent SPS 3 Sample Preparation System in conjunction with the External Sipper accessory
- Microsoft Internet Explorer version 6\* or later

PCI slot for IEEE \* The Cary WinUV software uses functionality provided by Microsoft Internet Explorer version 6.0. You do not need to use this as your Web browser. If your company rules prevent the installation of Internet Explorer version 6, you can use another browser, with some loss in functionality.

### Preparation

Before installing the Cary WinUV software, ensure that you have:

Preparation requirement	Reference
Installed Microsoft Windows 7 32-bit operating system with Service Pack 1 or Windows XP Service Pack 3 and checked that all devices, for example, sound card and CD drive, are working.	Windows operating system documentation.
Installed Windows Internet Explorer version 6 or later.	Documentation supplied with Internet Explorer.
Set the monitor screen resolution to at least 800 x 600 pixels and the color quality to at least High Quality.	Windows operating system documentation.
Logged on as an Administrator.	Windows operating system documentation.
Read the Late Breaking News.	Any Late Breaking News documents delivered with the Cary WinUV software.

#### NOTE

The Cary WinUV software will read Cary OS/2 and DOS data files. However, if you are upgrading to version 4.20 from Cary OS/2 or DOS, contact your local Agilent office, as a service call will be required.

#### NOTE

If you are installing 21 CFR Part 11 software, refer to the Cary WinUV Pharma Software Installation Instructions for 21 CFR Part 11 Environments.

21 CFR Part 11 software installation involves an administrator using the Agilent Spectroscopy Configuration Manager (SCM) to set up access privileges, and so on. In addition, SCM uses the Varian Spectroscopy Database Administration (VSDA) program for storing files. Refer to the SCM and VSDA Help for information about setting up 21 CFR Part 11 systems.

## New computer

### To install the Cary WinUV software on a new computer:

- 1 Log on to the instrument computer with Administrator privileges.
- 2 Insert the application software disk, select the preferred language and click **OK**.
  - If using Windows 7 operating system, a User Account Control dialog box will appear, click Yes for 'Do you want to allow the following program to make changes to this computer?'.

**NOTE**

If no installation screen appears, run 'CaryWinUVBioSetup.exe' from the CD directory.

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- 3 Follow the prompts on the screen until the 'Select Destination Location' window appears. Confirm the directory in which you would like to install the application. Alternatively, click Browse to choose a different location. Click Next.

**NOTE**

Agilent strongly recommends that the Varian\Cary WinUV folder and applications be installed in the recommended C:\Program Files directory.

---

- 4 The 'Folder Does Not Exist' dialog box may appear. Click **Yes** to create the folder.
- 5 Follow the prompts on the screen until the 'Ready to Install' window appears. Click **Install**.

**NOTE**

During the installation of the .Net Framework and GPIB driver, the computer may appear frozen and the 'Cancel' button is unavailable. This is correct. The installation can take 3 to 5 minutes. Do not try to exit the installation during this time.

---

- 6 If prompted to complete installation of Cary WinUV by restarting your computer, select **No, I will restart the computer later**. Click **Finish**.
- 7 Remove the application disk from the CD drive.

## Installation

- 8 Insert the disk labeled Help.
  - If using Windows 7 operating system, a User Account Control dialog box will appear, click Yes for 'Do you want to allow the following program to make changes to this computer?'.

### NOTE

If no installation screen appears, run 'Setup.exe' from the CD directory.

---

- 9 A list of Help files to be installed will be displayed. Click **OK**.
- 10 Follow the instructions on the screen to install the Help.
- 11 When the status indicates 'Finished', click **Close**.
- 12 Remove the Help disk from the CD drive.

### NOTE

At the end of the application software installation, you will need to turn off the computer in order to install the computer-instrument interface card or connect the computer-instrument interface converter and to complete the automatic detection and installation of the driver for the instrument interface card. Refer to the Cary 100/300/4000/5000/6000i Spectrophotometers Hardware Manual.

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- 13 Select the **System Information** application from the **Cary WinUV** folder on the Windows desktop. Enter the company details, instrument type and serial number. Click **OK**.
- 14 Restart the computer to complete the installation of the instrument interface card.

## Upgrade

**NOTE**

If you are upgrading 21 CFR Part 11 software, refer to the Cary WinUV Pharma Software Installation Instructions for 21 CFR Part 11 Environments.

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**To upgrade the Cary WinUV software:**

- 1** Log on to the instrument computer with Administrator privileges.
- 2** When upgrading Cary WinUV version 3.1 or earlier, proceed to Step 3. When upgrading Cary WinUV version 4.10 (build 464) or Cary WinUV version 4.10 (build 467), proceed to Step 4.
- 3** Right-click on the **System Information** icon on the Windows toolbar, and select **Shut Down System Information**.
- 4** Click **Start > Settings > Control Panel > Add/Remove Programs**.
  - If using Cary WinUV version 3.1 or earlier, click **NI-488.2.1.60** and then click **Change/Remove** and follow the prompts. When prompted, click **Yes** to remove all unused components.
  - If using Cary WinUV version 4.10 (build 464) or Cary WinUV version 4.10 (build 467), click **National Instruments Software** and then click **Change/Remove** and follow the prompts. When prompted, click **Yes** to remove all unused components.
- 5** Insert the application software disk, select the preferred language and click **OK**.
  - If using Windows 7 operating system, a User Account Control dialog box will appear, click Yes for ‘Do you want to allow the following program to make changes to this computer?’ to install the application software.

## Installation

### NOTE

If no installation screen appears, run 'CaryWinUVBioSetup.exe' from the CD directory.

---

- 6 Follow the prompts on the screen until the 'Select Destination Location' window appears. Confirm the directory in which you originally installed the application software. If required, click **Browse** to navigate to the correct location. Click **Next**.

### NOTE

Agilent strongly recommends the Varian\Cary WinUV folder and applications are installed in the recommended C:\Program Files directory.

---

- 7 The 'Folder Exists' dialog box will be displayed. Click **Yes**.
- 8 Follow the prompts on the screen until the 'Ready to Install' window appears. Click **Install**.

### NOTE

During installation of the Microsoft .Net Framework and GPIB driver, the computer may appear frozen and the 'Cancel' button is unavailable. This is correct. The installation can take 3 to 5 minutes. Do not try to exit the installation during this time.

---

- 9 If prompted to complete installation of Cary WinUV by restarting your computer, select **No, I will restart the computer later**. Click **Finish**.
- 10 Remove the application disk from the CD drive.
- 11 Insert the disk labeled Help.
  - If using Windows 7 operating system, a User Account Control dialog box will appear, click **Yes** for 'Do you want to allow the following program to make changes to this computer?'.

### NOTE

If no installation screen appears, run 'Setup.exe' from the CD directory.

---

- 12 A list of Help files to be installed will be displayed. Click **OK**.
- 13 Follow the instructions displayed on the screen to install the Help.

- 14 When the status indicates 'Finished', click **Close**.
- 15 Remove the Help disk from the CD drive.
- 16 Select the **System Information** application from the **Cary WinUV** folder on the Windows desktop; enter the Company details, Instrument type and Instrument Serial number. Click **OK**.
- 17 Restart the computer to complete the installation.

## Starting the software

### To start the Cary WinUV software:

- 1 Click the Windows **Start** button then **(All) Programs, Agilent and Cary WinUV**. Alternatively, double-click the **Cary WinUV** folder on the desktop.
- 2 Select the desired application. Refer to Chapter 3 for information about the available applications.
- 3 The first time the Cary WinUV software is open a Software Registration dialog will appear. Click **Next**.

#### NOTE

Ensure the software registration is completed by the user of the Agilent Cary UV-Vis-NIR spectrophotometer. For further information refer to the Software Registration Help.

- 
- 4 Complete all the fields on the 'Customer Details' page. Click **Next**.

#### NOTE

The Product Key is found on the cover of the Agilent Cary WinUV software CD case which was delivered with the instrument.

- 
- 5 Complete all the fields on the 'Product Details' page. Click **Next**.
  - 6 Complete all the fields on the 'Work Environment Details' page. Click **Register**.
  - 7 A dialog appears stating 'Your Agilent Software Registration has been successful'.

## Installation

### NOTE

If your computer is not connected to the internet, refer to the Software Registration Help for further information.

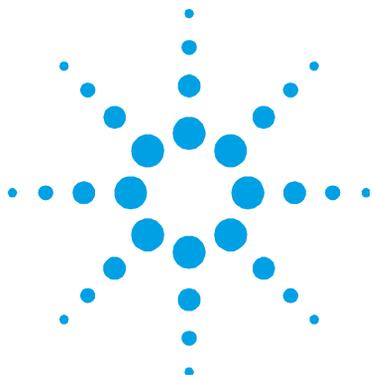
---

**8** The application will now open, and you can collect your data.

### TIP

To familiarize yourself with the Cary WinUV software, browse the Help after installing the software. See 'Using the Help' on Page 20.

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### 3. Software Overview

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This chapter provides a brief introduction to the Cary WinUV software and the individual applications to help you familiarize yourself with its use. For more detailed information about the applications and their settings, refer to the extensive Help (see Page 20 for information about using the Help).

### ADL Shell

The Applications Development Language (ADL) is a built-in spectroscopy language supplied with the Cary WinUV software. The ADL Shell gives you a pre-defined template for writing ADL 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. In other words, you do not have to design your own interface – you can use the ADL Shell to provide the basic functionality and build on that.

### Advanced Reads

The Advanced Reads application can be used to read multiple samples in a single run at a single or multi wavelength (up to 6). You can use various accessories to take multiple sample solution and aliquot readings in absorbance, percent transmittance, Abs\*F or percent reflectance mode, and find the mean.

### Align

The Align application is used to align the lamp(s) in the instrument and various accessories. It enables you to set instrument parameters such as the beam mode and wavelength. It also allows you to keep track of operating hours by lamp type.

The 'Lamps' page of the Alignment window allows you to monitor the energy of the lamp(s). Instrument parameters can be changed on the 'Cary' page.

Align can also be used to configure the Series 1 and Series II 6 x 6 and 8 x 6 cell changer (Cary 100/300), the cell changer for microcells (Cary 100/300/4000/5000/6000i), and the Series I and II Rear Beam Attenuator (Cary 100/300/4000/5000/6000i).

## Color

The Color application is used to measure and calculate the transmitted or reflected color of samples. The following extensive color calculations are supported:

- Tristimulus
- Chromaticity
- CIE Lab
- CIE LUV
- Hunter Lab
- Whiteness
- Yellowness (ASTM E 313-00)
- Tint (ASTM E 313-00)
- Gardner Color (ASTM D 6166-97 and DIN EN1557)
- Haze (ASTM D1003-61)
- Choice of 21 illuminants, including 2 user-specified illuminants
- Choice of 6 observers, including 2 user-specified observers
- Calculation interval of 1, 5 or 10 nm
- Thickness correction to calculate the color of the same sample in varying thicknesses, rather than having to measure the samples
- Graphical display of color spaces
- Color difference calculations – Delta E, FMC-2, CMC and BFD
- Color matching tolerance circle using DE Lab

### Concentration

The Concentration application is used to calibrate the system for quantitative analysis. You can select from several fit types for your calibration: linear, direct linear and quadratic. Based on the fit type, the Concentration application will calculate the coefficients of the fit equation and the correlation coefficient. Alternatively, you can define your own equation for the calibration.

### Enzyme Kinetics

The Enzyme Kinetics application uses Michaelis-Menten principles to calculate the maximum rate ( $V_{max}$ ) and substrate concentration that gives half the maximum rate ( $K_m$ ) of enzyme-catalyzed reactions.

Accurately obtaining  $V_{max}$  and  $K_m$ , requires you to perform numerous Enzyme Kinetics runs at different substrate or inhibitor concentrations to create a series of absorbance versus time curves. The software determines the initial velocity ( $V_0$ ) of each curve, and then you enter the substrate and inhibitor concentrations for each cuvette.

The software uses the  $V_0$ , [S] and [I] values to plot traces representing absorbance versus time curves with a common [S] or [I]. It is from these graphs that the software determines  $V_{max}$  and  $K_m$ .

Enzyme kinetics measurements can be performed at single or multi wavelengths (up to 6).

### GLP Administration

The GLP Administration application is used to protect the system from unauthorized use, enabling application-specific privileges to be turned on or off by the system administrator.

If this application is operational, users will need to be registered, have a user name and a valid password before they can access the various privileges.

## Kinetics

The Kinetics application is used to obtain absorbance versus time data to enable you to determine the rate of reaction. Kinetics measurements can be performed at single or multi wavelengths (up to 6).

The Kinetics application allows:

- Calculation of Zero Order, First Order and Second Order reaction rates from absorbance versus time data.
- Entry of activity factors for multiple cells.
- Overlay of the best-fit line on raw data.
- Auto or manual estimates for the first order and second order Marquardt fitting.

## RNA-DNA Estimation

The RNA-DNA Estimation application is used to calculate the following parameters, used in determining the amount, type and purity of nucleic acid samples:

- Absorbance of samples at selected wavelengths.
- $A(260)/A(280)$  ratios with or without background correction at 320 nm.
- Absorbance ratios at your own nominated wavelengths with or without background correction.
- Average ratio values for replicate samples.
- Protein and nucleic acid concentrations using Warburg Christian coefficients.
- 260 nm Factor Parameters,  $A(260) * F$ .

### Scan

The Scan application enables you to set up and run wavelength scans, with the collected scans displayed in the 'Scan' window.

### Scanning Kinetics

The Scanning Kinetics application allows you to perform cyclic scans 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 kinetics curves can then be used to calculate Zero Order, First Order and Second Order reaction rates.

You can choose an automatic or manual estimate for the First Order and Second Order Marquardt fitting.

This application also enables you to correct samples for a baseline during the scan. You can choose a 100%T baseline, or you can select from other baseline options such as a zero baseline correction that will apply both a 100%T and a 0%T baseline correction to your sample scans.

### Simple Reads

The Simple Reads application is used to perform quick absorbance readings of samples at single wavelengths. To take a reading, click 'Zero' to zero the instrument and then click 'Read'.

Simple Reads measurements can be performed at single or multi wavelengths (up to 6).

### Spectroscopy Configuration Manager

The Spectroscopy Configuration Manager (SCM) provides the system administrator with a tool to manage your 21 CFR Part 11 environment. SCM provides the means to create, configure and maintain data in relation to system security, user management and data paths.

The Privileges and Profiles software controls which applications/functions may be run by a particular user. It also establishes the level of authority a user may have with regard to signatures and accessing certain parts of an application

Agilent uses the SCM for security and permission rights. These security functions provide:

- Access controls and authority checks via the use of user identification codes and passwords.
- Electronic record security via the use of databases.
- Time and date stamped audit trails.

The use of user identification codes and passwords enables control over who can log on to the system and who can perform particular functions within the Agilent application software. It also provides the mechanism to allow electronic signing of electronic records. The use of databases coupled with SCM, prevents all unauthorized users from changing or deleting files. The SCM event logs augment the audit trails resident in the application software. The SCM administrator must set up the required users. It is important that a number of simple requirements are followed when this is done to ensure that compliance with the 21 CFR Part 11 rule is maintained.

## System Information

The System Information application allows you to enter company and instrument information, and specify headers, footers and bitmap or icon files to be used in reports (for example, your company logo).

System Information also allows you to specify the default settings for the 'Hint' text, such as the Hint Pause (the length of the delay before the hint appears when the pointer is moved over a control) and Hint Hide Pause (the time the hint stays visible for if the pointer is not moved off the control). These settings will be used by the other Cary WinUV applications.

### Thermal

The Thermal application is used to perform thermal DNA analysis, enabling you to calculate  $T_m$  using the various thermoelectric Cary accessories. Once the data is collected, you can choose to calculate the  $T_m$  value by either the derivative or hyperchromicity methods.

Thermal measurements can be performed at single or multi wavelengths (up to 6).

### Validate

The Validate application enables you to carry out a number of performance tests to verify that the system is performing according to specification. Included are the validation tests needed to satisfy the requirements of the British Pharmacopoeia, European Pharmacopoeia, US Pharmacopoeia and Therapeutic Goods Association of Australia.

An optional Validation package is also available from Agilent, providing detailed information on the functional specification and development process of the Cary system. It also contains detailed DQ/IQ/OQ documentation to assist your initial and on-going validation activities.

### Varian Spectroscopy Database Administrator

The Varian Spectroscopy Database Administrator (VSDA) is designed for system administrators to set up and maintain the databases that are used by the application software to store data. VSDA uses Microsoft SQL Server 2005 for database operations.

Companies can use VSDA together with the Agilent Spectroscopy Configuration Manager (SCM), the application software and their own Standard Operating Practices to form a 21 CFR Part 11 capable environment for controlling their Agilent instruments.

VSDA allows the data collected by Agilent instruments to be stored locally (on the same computer as the application software), or remotely in a Client/Server arrangement.

Configuration must be performed by the system administrator, or a person with administration rights to run VSDA.

## File name extensions

The various Cary WinUV software files, such as method files, report files and graphic files are saved with a three-letter file name extension that represents the type of file, and the application that created the file.

The file name extensions for each file type are:

File type	File name extension
Methods	.m**
Data	.d**
Report	.r**
Graph Template	.g**
Settings	.s**
Baseline	.c**
Batch	.b**
ASCII	.csv
Rich Text Format	.rtf
Cary OS/2	.dat
Cary DOS	data.
Grams	.spc
ADL	.adl

where \*\* equals the Cary WinUV application code, used to distinguish between different applications.

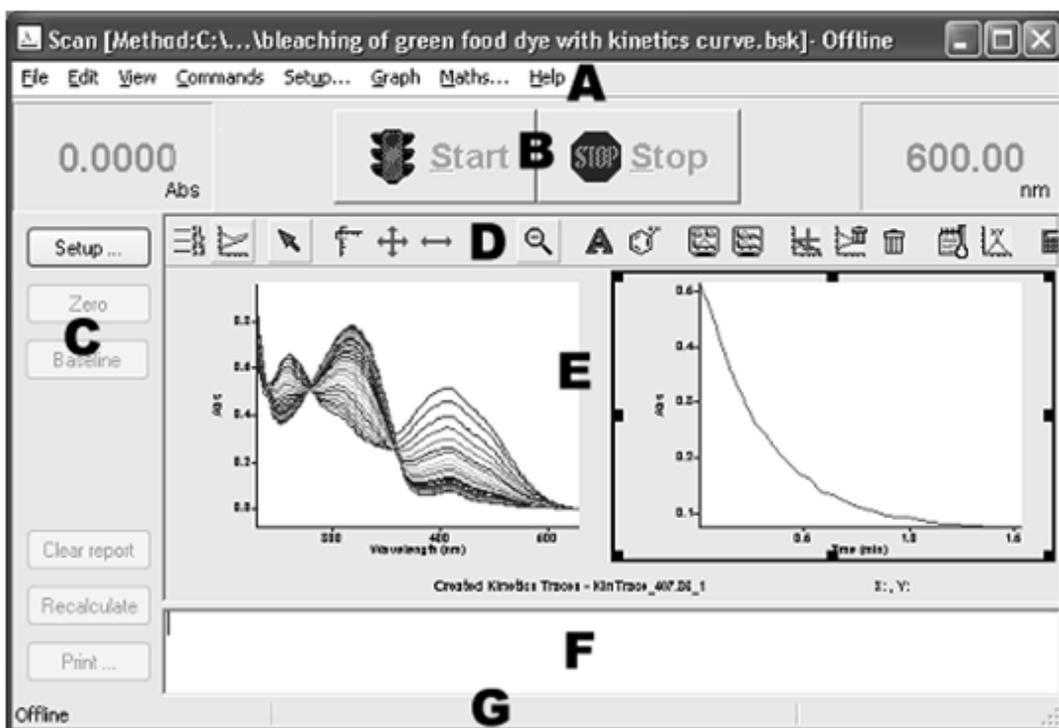
The application codes are:

<b>Application</b>	<b>File name extension</b>
Advanced Reads	.*ab
Color	.*cl
Concentration	.*cn
Enzyme Kinetics	.*ek
Fabric Protection	.*fp
Kinetics	.*kn
RNA-DNA	.*dn
Scan	.*sw
Scanning Kinetics	.*sk
Simple Reads	.*sr
Sunglasses	.*sg
Thermal	.*tm
Validate	.*vo

For example, a method file from the Concentration application would have the file name extension “\*.mcn”.

## Interface

A typical Cary WinUV software screen consists of a Menu line ('A' in **Figure 1**), Instrument buttons (B), Command buttons (C), Toolbar (D), Graph area (E), Report area (F) and Status line (G).



**Figure 1.** A typical Cary WinUV software screen

- |               |                       |                    |            |
|---------------|-----------------------|--------------------|------------|
| A. Menu line  | B. Instrument buttons | C. Command buttons | D. Toolbar |
| E. Graph area | F. Report area        | G. Status line     |            |

### Hint text

You can obtain 'Hint text' for a particular control or field by positioning the pointer over the control/field name for a short time. After a short delay, a brief hint will be displayed describing what the control or field does. If you hold the pointer over a numeric entry field, the valid range for that field will be displayed.

If these hints do not appear, choose 'Hints' from the 'View' menu to enable them. Conversely, if the hint text is enabled and you wish to turn it off, choose 'Hints' from the 'View' menu.

### NOTE

You can alter the properties of the hints, such as the length of the delay before hints appear, on the 'Hints' page in the System Information application.

---

## Using the Help

The Cary WinUV software includes extensive Help, which should be your primary source of information on how to use your Cary system. It contains descriptions of the various application windows, dialog boxes and fields that make up the software, as well as step-by-step instructions to help you perform various tasks. It also includes 'Tips and Tricks', 'Troubleshooting' and 'Contacts' sections in case you encounter difficulties using the software.

Since the Help system is so extensive, it is advisable to familiarize yourself with the contents of the Help by viewing the Help Home page. There are a number of ways to open the Help Home page:

- Click the Windows 'Start' button, '(All) Programs', 'Agilent', 'Cary WinUV', 'Cary Help'.
- Double-click the 'Cary WinUV' folder on the desktop and double-click 'Cary Help'.
- Click 'Help Topics' on the 'Help' menu when in an application to go to the Help Home page.

You can also make the Help open on the appropriate page containing information relevant to the currently open application or dialog box:

- Click '(Application) Help' (for example, 'Scan Help') on the 'Help' menu when in an application to go to the Home page for that application.
- Click the 'Help' button on a dialog box to go to information about that dialog box.
- Press F1 to go to information about the current dialog box.

## Navigating

You can move around the Help using the 'Contents' on the left of the Help window.

### NOTE

To display the Contents, click the 'Show' button at the top of the Help window.

---

The Contents sections are accessed by clicking the icons and associated text:

- To expand/contract a section in the Contents list, double-click the 'folder' icon for that section, or click the '+'/'-' symbol next to the icon.
- To display a topic, click the text associated with that topic.

The topics in the Help are often hyperlinked to other related information. To return to the previous topic after clicking a hyperlink, click the 'Back' button at the top of the Help window.

## Searching

You can quickly search the Help system for specific information using key words.

### To search for information on a particular subject:

- 1 Click the **Search** tab (next to the 'Contents' tab).
- 2 Type the word(s) you want to search for and click the **List Topics** button or press ENTER to list all the relevant pages containing the search word/s.

The number of topics found will be displayed. You can view the topics in alphabetical order by clicking 'Title' at the top of the list, or in the order they appear in the Help by clicking 'Rank'.

- 3 Select the desired topic from the list by highlighting it then clicking the **Display** button. Alternatively, double-click the topic. All occurrences of the keyword will be highlighted.

- 4 If this does not provide the information you require, enter a more specific word or additional words in the key words field and try again. Click the **right arrow** symbol to the right of the keywords field and select any of these words AND, OR, NEAR and NOT to place between your keywords.

### TIP

Another way to narrow your search is to use the check boxes at the bottom of the Search page. For example, selecting 'Search titles only' will only list those Help topics containing the key word in the title.

---

### Within a page

You can skip to a section of interest in a Help page by searching for a relevant word.

#### To find a key word within a Help page:

- 1 Click anywhere on the Help page.
- 2 Press CONTROL+F to open the 'Find' dialog box.
- 3 In the 'Find what:' field, enter the word you wish to look for.
- 4 If required, limit the Find by selecting an option such as 'Match case'. (This option will only find the words that appear in the same letter case (for example, all lower case) as the entered key word.
- 5 Select the **Direction** of the Find. Select 'Up' to search for the key word from the bottom of the page upwards, or 'Down' to start the Find from the top of a page.
- 6 Click the **Find Next** button or press ENTER to begin the Find. The first occurrence of the designated word will be highlighted.
- 7 Click the **Find Next** button or press ENTER repeatedly to jump to other occurrences of the key word. When all instances of the word have been found, the message 'Finished searching the document' will be displayed.

### Tracking 'favorite' topics

You can keep a list of useful Help topics using the 'Favorites' option.

If you come across a topic you may wish to refer to again:

- 1 Click the **Favorites** tab. The title of the page currently visible will be present in the 'Current topic:' field at the bottom of this tab.
- 2 Click the **Add** button to include this topic to the Favorites list.

If you would like to view this page later on, you can simply click the 'Favorites' tab and highlight the topic of interest by clicking it, then click the 'Display' button. (Alternatively, double-click the topic.)

If a topic is no longer required in the Favorites list, highlight it then click the 'Remove' button.

### Printing

You can print one or more Help topics for a particular application.

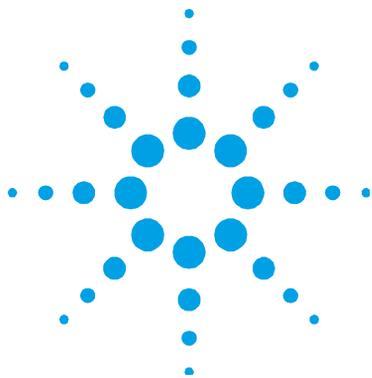
#### To obtain a hard copy of the current Help page:

- 1 Click the **Print** button at the top of the Help window. The 'Print' dialog box will be displayed.
- 2 Click **Print**.

#### To obtain a hard copy of multiple Help pages:

- 1 Right-click on the topic in the 'Contents' page, and choose **Print**.
- 2 Click **Print the selected heading and all sub-topics**.

*This page is intentionally left blank.*



## 4. How To...

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This chapter provides step-by-step instructions on how to perform common operating procedures using your Cary instrument and various software applications. For more detailed information on the software applications or your Cary instrument and accessories, refer to the extensive Help (see Page 20 for information on using the Help).

### Start an application

#### To start an application:

- 1 Click the Windows **Start** button, then **(All) Programs, Agilent, Cary WinUV** and click the application that you wish to run. Alternatively, double-click the application icon in the 'Cary WinUV' folder on the desktop.
- 2 Select your instrument type if necessary and click **OK** to open the application.

**NOTE**

If you are running a GLP system, you will be prompted to enter a password before accessing the application.

---

**NOTE**

If you are using 21 CFR Part 11 software, you will be prompted to enter a user identification, select the appropriate Group and Project and enter a password before accessing the application.

---

## Manually read samples using Advanced Reads

This procedure describes how to read samples in the Advanced Reads application using no accessories.

### 1 Set up data collection parameters

*Setup dialog box*

In Advanced Reads, click the **Setup** button or choose **Setup** from the Menu line to display the 'Setup' dialog box and specify the method parameters for a new method.

### 2 Set up instrument parameters

*Setup dialog box | Cary page*

- a** In the 'Wavelength' field, enter the relevant wavelength.
- b** In the 'Ave. (averaging) Time' field, enter the required value. A good starting point is 0.1 seconds.
- c** In the 'SBW' field, enter the required spectral bandwidth. Unless your method specified another value, use the maximum setting.
- d** Select 'Replicates' or 'Sample Averaging'. For Replicates, enter the number of replicates of each sample that you would like read. For Sample Averaging, enter '2' for duplicate aliquots of the sample, and so on.

**NOTE**

If using a microcell, select a smaller spectral bandwidth.

- e Select the ordinate mode you require from the drop-down list in the 'Y Mode' field. Enter a 'Factor' value if you have selected 'Abs\*F'.

### 3 Set up lamp options

*Setup dialog box | Options page*

- a Select **Auto lamps off** to automatically turn off the lamps at the end of the collect. This option is especially useful when performing reads overnight or unattended for long periods of time. In some Cary instrument models, you may also choose to use a third lamp, such as a mercury lamp (if installed).
- b Click the **UV/Vis** button to use both lamps.
- c Enter the wavelength at which you would like the source lamp to change from the ultraviolet to the visible/near-infrared lamp. The recommended changeover is 350 nanometers for lamps with an ultraviolet cutoff. If you are using a Cary 5000/6000i, also enter the detector and grating changeover wavelengths (800 nanometers is recommended for both).
- d Under 'Beam Mode', select the beam mode that you require. In most cases this should be **Double Beam** and **Normal**. If you select 'Single Beam' you also need to enter a value in the 'Energy' field.

### 4 Ensure no accessories are selected

*Setup dialog box | Accessories pages*

Make sure no accessories are selected on the 'Accessories 1' and 'Accessories 2' pages.

### 5 Set up your samples

*Setup dialog box | Samples page*

- a Enter the 'Number of Samples'. The table below expands or contracts to match your choice.

- b** In the 'Samples' table, enter the name of each sample. You can enter up to 20 characters for each name.

If the samples have the same name with a different numeric extension, enter the name in the first sample position and then click the 'Increment' button.

You can click the 'Import' button to use the names of samples stored in a text file.

### **6 Set up reporting and printing requirements**

*Setup dialog box | Reports page*

- a** Enter your name in the 'Name' field.
- b** Enter a comment relating to your experiment in the 'Comment' field.
- c** Set up your report style by selecting the appropriate check boxes under 'Options'. For example, select 'AutoPrint' to automatically obtain a printout of your report. Select 'Parameters' to include your experimental parameters in the report.

#### **NOTE**

If 'AutoPrint' is selected, the system will send the report information to the specified printer as well as the Report area. However if 'AutoPrint' is not selected, the report will only be sent to the Report area.

---

### **7 Set up storage of collected data**

*Setup dialog box | Auto Store page*

Select **Storage off**. The method, collected data and report will not be automatically saved. However, you can manually save it all at the end of the collection.

### **8 Set up visual system monitoring**

Select **Show Status Display** on any of the Setup pages, or from the **View** menu, to display information about your current reaction.

### **9 Finish setup**

Once you are satisfied with your method setup, click **OK** to confirm any changes you have made and close the 'Setup' dialog box.

#### **10 Zero the instrument**

- a** Click **Zero**. Alternatively, choose **Zero** from the **Commands** menu.
- b** Place a blank in the sample compartment and click **OK**.

#### **11 Read samples**

- a** Click the **Start** button, press F9 or choose **Start** from the **Commands** menu. The 'Sample Selection' dialog box will be displayed.
- b** Select the samples you would like to read, then click **OK**.
- c** The 'Present Sample' dialog box will prompt you to place the appropriate sample in the sample compartment. Click **OK** to read the sample.
- d** Repeat for the remaining samples.

#### **12 Save your data**

- a** On the **File** menu, click **Save Data As**.
- b** Enter the 'File name' for this Concentration run.
- c** Click **Save**. The data will be stored as a Batch file.

#### **13 Export your data**

- a** On the **File** menu, click **Export report (\*.csv)**.
- b** Enter the 'File name' for this read.
- c** Click **Save**. The data will be stored as an ASCII spreadsheet, with a \*.csv file name extension.

## Perform a calibration and manually measure concentrations using Concentration

This procedure describes how to perform a multi-standard calibration and measure sample concentrations in the Concentration application using no accessories.

### 1 Set up data collection parameters

*Setup dialog box*

In Concentration, click the **Setup** button or choose **Setup** from the Menu line to display the 'Setup' dialog box and specify the method parameters for a new method.

### 2 Set up instrument parameters

*Setup dialog box | Cary page*

- a** In the 'Wavelength' field, enter the relevant wavelength.
- b** In the 'Ave. (averaging) Time' field, enter the required value. A good starting point is 0.1 second.
- c** Enter the required spectral bandwidth in the 'SBW' field. Use the maximum setting unless your method specifies another value.

#### NOTE

If using a microcell, select a smaller spectral bandwidth.

- d** Select 'Replicates' or 'Sample Averaging'. For Replicates, enter the number of replicates of each sample that you would like read. For Sample Averaging, enter '2' for duplicate aliquots of the sample, and so on.
- e** Select the 'Y mode' you require. Click 'Abs' to specify Absorbance mode or 'Emission' if you are measuring fluorescence.
- f** Enter an upper range and lower range value in the 'Y min.' and 'Y max.' fields to specify the displayed ordinate range. These are starting values only. The Cary WinUV software will automatically rescale the calibration graph as the standards are measured.

### 3 Set up the calibration

*Setup dialog box | Standards page*

- a Click the **Standards** tab to set up the standards and their parameters associated with the data collection.
- b Select **Calibrate During Run** to perform a calibration when the 'Start' button is clicked.
- c Set the appropriate units for your standards for reporting purposes.
- d Set the 'Standards' field to the number of standards that you are using. The table below will expand or contract to match your choice.
- e In the 'Standards' table, enter the concentration of each standard in the 'Conc.' column.
- f Under 'Fit Type', select the type of curve fitting required for your calibration.
- g Enter the required  $R^2$  value or correlation coefficient in the 'Min  $R^2$ ' field. The closer the number is to 1.000, the better the fit. Typically, 0.95 is used.

### 4 Set up lamp options

*Setup dialog box | Options page*

- a Select **Auto Lamps Off** if you want to automatically turn off the lamps at the end of the collect. This option is especially useful when performing Concentration runs overnight or unattended for long periods of time.
- b Click the **UV/Vis** button to use both lamps.
- c Enter the wavelength at which you would like the source lamp to change from the ultraviolet to the visible/near-infrared lamp. The recommended changeover is 350 nanometers for lamps with an ultraviolet cutoff. If you are using a Cary 5000/6000i, also enter the detector and grating changeover wavelengths (800 nanometers is recommended for both).

- d** Under 'Beam Mode', select the beam mode that you require. In most cases this should be **Double Beam** and **Normal**. If you select 'Single Beam', you also need to enter a value in the 'Energy' field.

## **5 Ensure no accessories are selected**

*Setup dialog box | Accessories page*

Click the **Accessories** tab and make sure that no accessories are selected.

## **6 Set up your samples**

*Setup dialog box | Samples page*

- a** Enter the number of samples in the 'Number of Samples' field. The table below expands or contracts to match your choice.
- b** In the 'Samples' table, enter the name of each sample. You can enter up to 20 characters for each name.

If the samples have the same name with a different numeric extension, enter the name in the first sample position and then click the 'Increment' button.

You can click the 'Import' button to use the names of samples stored in a text file.

## **7 Set up reporting and printing requirements**

*Setup dialog box | Reports page*

- a** Enter your name in the 'Name' field.
- b** Enter a comment relating to your experiment in the 'Comment' field.
- c** Set up your report style by selecting the appropriate check boxes under 'Options'. For example, select 'AutoPrint' to automatically obtain a printout of your report.

**NOTE**

If 'AutoPrint' is selected, the system will send the report information to the specified printer as well as the Report area. However, if 'AutoPrint' is not selected, the report will only be sent to the Report area and can be viewed by choosing 'Report' from the 'View' menu.

---

## 8 Set up weight and volume correction

*Setup dialog box | Samples page*

- a Under 'Weight/Volume Corrections', select **Corrections** to activate the correction facility.
- b Enter the theoretical sample weight in the 'Method Weight' field. This is the weight of the sample specified in your method.
- c Enter the weight units in the 'Units' field.
- d Enter the theoretical sample volume in the 'Method Volume' field. This is the volume to which the method tells you to make the sample.
- e Enter the volume units in the 'Units' field.
- f In the 'Samples' table, enter the actual weight and volume for each sample.

## 9 Set up storage of collected data

*Setup dialog box | Auto Store page*

Select **Storage off**. The method, collected data and report will not be automatically saved. However, you can manually save it all at the end of the collection.

## 10 Set up visual system monitoring

Select **Show Status Display** on any of the Setup pages, or from the **View** menu, to display information about your current reaction.

## 11 Finish setup

Once you are satisfied with your method setup, click **OK** to confirm any changes you have made and close the 'Setup' dialog box.

## 12 Zero the instrument

- a Click **Zero**. Alternatively, from the **Commands** menu, choose **Zero**.
- b Place a blank in the sample compartment and click **OK**.

## 13 Perform the calibration

- a Click the **Start** button or, from the **Commands** menu, choose **Start**. The 'Standard/Sample Selection' dialog box will be displayed.
- b Select the standards and samples to be used in the analysis. By default all standards and samples are selected.
- c Click **OK**.
- d The 'Present Standard' dialog box will prompt you to place the appropriate standard in the sample compartment. Click **OK** to measure the standard.
- e Repeat until you have measured all of the standards. The Cary will calculate the calibration and the correlation coefficient.

### NOTE

If the set correlation coefficient ( $R^2$ ) value is not met, the Cary will prompt you with 'Min  $R^2$  test failed'. When you click 'OK', the Cary will then prompt you with 'There is no valid calibration. Proceed in Abs (or Emission)?' If you click 'Cancel', the Concentration run will finish. If you click 'Yes', the Cary will measure the absorbance or emission of any presented samples, but will not generate a concentration.

---

## 14 Measure sample concentration

- a Once all the standards have been read, the 'Present Sample' dialog box will prompt you to place the appropriate sample in the sample compartment. Click **OK** to measure the sample and calculate its concentration. (If replicates have been nominated, the concentration is calculated after the final sample replicate is read.)
- b Repeat for the remaining samples.

**15 Save your data**

- a** On the **File** menu, click **Save Data As**.
- b** Enter the 'File name' for this Concentration run.
- c** Click **Save**. The data will be stored as a Batch file.

**16 Export your data**

- a** On the **File** menu, click **Export report (\*.csv)**.
- b** Enter the 'File name' for this read.
- c** Click **Save**. The data will be stored as an ASCII spreadsheet, with a \*.csv file name extension.

## Enzyme Kinetics

### Perform a temperature-controlled Enzyme Kinetics run using the Multicell Holder

This procedure describes how to perform a multicell, multi-rate Enzyme Kinetics run at 37 °C using the Temperature Controller accessory with the Multicell Holder accessory (Cary 100/300/4000/5000/6000i).

**1 Set up data collection parameters**

*Setup dialog box*

In Enzyme Kinetics, click the **Setup** button or select **Setup** from the Menu line to display the 'Setup' dialog box and specify the method parameters for a new method.

**2 Set up the Multicell Holder accessory**

*Setup dialog box | Accessories page*

As various options do not become available until the appropriate accessories are selected, you need to select these first.

- a** Select **Use Cell Changer** to enable the accessory.
- b** For Cary 100/300 instruments, choose the type of Multicell Holder you are using (6 x 6 or 8 x 6). Ensure that you have this accessory installed before starting the run.

**NOTE**

If you are using a Series I 6 x 6 (Cary 100/300), you must calibrate the cell changer using the Align application before starting experiments.

---

- c** Choose **Select Cells** and select the cells you require from the available cells under 'Use Cells'.

**NOTE**

For Front Beam analysis, select 'Cell 1–Cell 6' (6 x 6) or 'Cell 1–Cell 8' (8 x 6). This will ensure that all front cell positions in the Multicell Holder will be measured during your enzyme kinetics analysis.

---

- d** Select **Multi Zero** to turn on the 'Multi Zero' facility.
- e** Ensure that **Blank Correction** is not selected.

**3 Set up accessories for reaction temperature control and temperature display**

- a** If you are not using a Peltier-controlled accessory (for example, the water-thermostatted 8 x 6), ensure that you have the Temperature Controller accessory installed before starting the run, and,

- (i) Select **Automatic Temperature Setting** and select **Temperature Controller** to enable the accessory.

- (ii) Set the monitoring temperature by entering the Block temperature as 37 °C. (The monitoring device is selected in Step 4d).

- b** Under 'Temperature Display', select **Block** and **Probe 1** to view the temperature of the Multicell Holder block and one temperature probe in the 'Status Display' window.

**4 Set up instrument parameters**

*Setup dialog box | Cary page*

- a** Enter the 'Wavelength', 'SBW' (spectral bandwidth) and 'Ave. (averaging) Time' you require in the corresponding fields.
- b** Select the ordinate mode you require. Click 'Abs' to specify Absorbance mode or '%T' to specify percent Transmittance.

**NOTE**

The '%T' mode is used when performing fluorescence kinetics measurements using the Total Fluorescence accessory or the Fluorescence Fiber Optic Probe.

---

- c Enter an upper range and lower range value in the 'Y min.' and 'Y max.' fields to specify the displayed ordinate range.
- d In the 'Monitor' field, choose where you are going to monitor the temperature.

**5 Set up rate parameters**

- a Under 'Collect Timing', select **Advanced Collect**.

**NOTE**

The 'Advanced Collect' facility enables you to collect data more frequently during the crucial stages of your reaction, and to collect data less frequently where you know there will be little activity.

---

- b Enter the number of different reaction rates that you require in the 'Number of Stages' field. The number you set here will be reflected in the table below.
- c Vary the number of data points collected per cell per run by setting the 'Dwell time' for each rate stage.
- d Specify how long the Cary will wait after reading each cell before it starts another reading cycle, by setting the 'Cycle time' for each rate stage.
- e Specify the duration of the measurement by setting the 'Stop time' for each rate stage.

**6 Set up lamp and graphics options**

*Setup dialog box | Options page*

- a Select **Auto Lamps Off** if you want to automatically turn off the lamps at the end of a collect. This is especially useful when performing data collections overnight or unattended for long periods of time.
- b Click **UV/Vis** if you want both lamps on during the run.

- c** Enter your required 'Source Changeover' and 'Detector Changeover' (Cary 5000 only) wavelengths in the corresponding fields. If you are using a Cary 5000/6000i, also enter the detector and grating changeover wavelengths (800 nanometers is recommended for both).
- d** Set the 'Slit Height' to full or reduced (Cary 4000/5000/6000i only).
- e** Under 'Display Options', choose 'Individual Data' to display the collected data of each sample in individual graph boxes. Choose 'Overlay Data' to superimpose the collected data of each sample in the Enzyme Kinetics run in one graph box.

## 7 Set up $V_o$ calculation

*Setup dialog box | Analyze page*

- a** Set the start and stop times for your  $V_o$  calculation.
- b** Enter the required product absorptivity and cell pathlength for your reaction.

## 8 Set up calculations for the maximum rate ( $V_{max}$ ) and substrate concentration that gives half the maximum rate ( $K_m$ )

- a** Under 'Analyze', select the method by which the data obtained from your selection under 'Plot/Fit' will be calculated. Choose 'Linear Least Square' or 'Marquardt'.
- b** Choose the inhibitor model for your analysis.
- c** Select the Plot/Fit type/s that will be used to determine  $V_{max}$  and  $K_m$  values.
- d** Select **Auto Calculate** to automatically perform enzyme kinetics calculations at the end of each run.

## 9 Set up reporting and printing requirements

*Setup dialog box | Reports page*

- a** Enter your name in the 'Name' field.
- b** Enter a comment relating to your experiment in the 'Comment' field.
- c** Set up your report style by selecting the appropriate items under 'Options'. For example, select 'Auto Print' to automatically obtain a printout of your report. Select 'Graph' to include a graph in the generated report.

**NOTE**

If 'Auto Print' is selected, the system will send the report information to the specified printer as well as to the Report area.

---

- d** Select the Autoconvert option you require. If you choose 'Select for ASCII (csv)' or 'Select for ASCII (csv) with Log', the system will automatically generate a report and store the data in the Cary format as well as ASCII XY pairs format in the current folder.

## **10 Set up storage of collected data**

*Setup dialog box | Auto Store page*

Select **Storage On (Prompt at Start)**, to set up the Cary to prompt you for a file name before the start of an Enzyme Kinetics reaction.

## **11 Set up visual system monitoring**

Select **Show Status Display** on any of the Setup pages, or from the **View** menu, to display information about your current reaction.

## **12 Finish setup**

Click **OK** to save any changes you have made and close the 'Setup' dialog box. Depending on the cells selected in the Multicell Holder, the Cary may inform you that it will perform a dual single beam calibration. Click **OK**.

## **13 Zero the instrument**

- a** Click **Zero** or choose **Zero** from the **Commands** menu. A 'Cell Loading Guide' will be displayed.
- b** If you like, change the names of the blank samples.
- c** Place the blank solution(s) in the correct cell positions and click **OK**. The system will perform an instrument zero on the blank solution(s).

**NOTE**

If you had chosen not to use the 'Multi Zero' facility in Step 2d, the system will prompt you to enter the blank solution into the instrument. You **must** make sure that you place the blank solution in the cell position that is currently in the light path. Once you click 'OK', the system will perform a zero on the cell position in the light path, that is, the system will not reset the Multicell Holder to position 1.

---

**14 Start the run**

- a** Click **Start** or choose **Start** from the Commands menu. *Do not add your active reagent at this time.* The system will display a 'Cell Loading Guide'.
- b** If you like, change the names of the samples.
- c** Place the sample solution(s) in the correct cell positions and click **OK**. The system will set up the Graphics area and display the 'Save File' dialog box.
- d** Enter the file name and click **Save**. The 'Sync Start' dialog box will be displayed.
- e** Reset the Multicell Holder to position to cell 1 by clicking the **Reset Slide** button. Add your active reagent just before the countdown reaches 0:00, or commence the data collection by clicking **OK**.

**15 Enter substrate and inhibitor concentrations (two options)**

Once the run has started:

- a** Open the 'Setup' dialog box by clicking the **Setup** button or by choosing **Setup** from the Menu line.
- b** Click the **Samples** tab. In the 'S' column, enter the Substrate concentration in micromoles. In the 'I' column, enter the Inhibitor concentration in micromoles.

**NOTE**

If no information has been entered during the collect, no calculation is performed.

---

You can also use the 'User Data Form' to enter the Inhibitor and Substrate concentrations:

- a Open the 'User Data Form', by right-clicking in a graph box and clicking **User Data Form** from the menu, or by choosing **User Data Form** from the **Graph** menu.
- b The table that appears has Data Names and may have  $V_0$  values already entered in the first two columns. In the third and fourth columns, enter your values for [S] and [I] in micromoles per liter.
- c Click **OK**. Your [S] and [I] values are now ready to be used in calculations, and the Cary will perform the calculations at the end of the run.

## Kinetics

The following Kinetics procedures are described:

- Performing a temperature-controlled Kinetics run using the Multicell Holder
- Performing a multi-wavelength, single cell, single rate Kinetics run

### Perform a temperature-controlled Kinetics run using the Multicell Holder

This procedure describes how to perform a multicell, multi-rate Kinetics run at 37 °C using the Temperature Controller accessory with the Multicell Holder accessory (Cary 100/300/4000/5000/6000i).

#### 1 Set up data collection parameters

*Setup dialog box*

Click the **Setup** button or choose **Setup** from the Menu line to display the 'Setup' dialog box and specify the method parameters for a new method.

#### 2 Set up the Multicell Holder accessory

*Setup dialog box | Accessories page*

As various options do not become available until the appropriate accessories are selected, you need to select these first.

- a Select **Use Cell Changer** to enable the accessory.
- b For Cary 100/300 instruments, choose the type of Multicell Holder you are using (6 x 6 or 8 x 6). Ensure that you have this accessory installed before starting the run.

### NOTE

If you are using a Series I 6 x 6 (Cary 100/300), you must calibrate the cell changer using the Align application before starting experiments.

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- c Click **Select Cells** and select the cells you require from the available cells under 'Use Cells'.

For Front Beam analysis, select 'Cell 1 to Cell 6' (6 x 6) or 'Cell 1 to Cell 8' (8 x 6). This will ensure that all front cell positions in the Multicell Holder will be measured during your Kinetics analysis.

- d Select **Multi Zero**.
- e Ensure 'Blank Correction' is not selected.

### 3 Set up accessories for reaction temperature control and temperature display

- a If you are not using a Peltier-controlled accessory (for example, the water-thermostatted 8 x 6), ensure that you have the Temperature Controller accessory installed before starting the run, and,

(i) Select **Automatic Temperature Setting** and select **Temperature Controller** to enable the accessory.

(ii) Set the monitoring temperature by entering the Block temperature as 37 °C. (The monitoring device is selected in Step 4e).

- b Under 'Temperature Display', select **Block** and **Probe 1** to turn on monitoring of the Multicell Holder block and one temperature probe.

#### 4 Set up instrument parameters

*Setup dialog box | Cary page*

- a Enter the 'Wavelength', 'SBW' (spectral bandwidth) and 'Ave. (averaging) Time' you require in the corresponding fields.
- b Select the ordinate mode you require. Click 'Abs' to specify absorbance mode or '%T' to specify percent transmittance.
- c Enter an upper range and lower range value in the 'Y min.' and 'Y max.' fields to specify the displayed ordinate range.
- d Select 'Min.' or 'Sec.' to set the abscissa (X).
- e In the 'Monitor' field, choose where you are going to monitor the temperature. The 'Start' button will not be enabled until the temperature of the selected monitor is within 0.5 °C of the block temperature set on the 'Accessories' page (Step 3a (ii)).

#### 5 Set up rate parameters

- a Under 'Collect Timing', select **Advanced Collect**. This enables you to set up different data collection procedures for the multiple rates in your reaction.

#### NOTE

The 'Advanced Collect' facility enables you to collect data more frequently during the crucial stages of your reaction, and to collect data less frequently where you know there will be little activity.

- b Enter the number of different reaction rates that you require in the 'Number of Stages' field. The number you set here will be reflected in the table below.
- c Vary the number of data points collected per cell, per run, by setting the 'Dwell time' for each rate stage.
- d Specify how long the Cary will wait after reading each cell before it starts another reading cycle by setting the 'Cycle time' for each rate stage.
- e Specify the duration of the Kinetics run by setting the 'Stop time' for each rate stage.

## 6 Set up lamp and graphics options

*Setup dialog box | Options page*

- a** Select **Auto Lamps Off** if you want to automatically turn off the lamps at the end of a collect. This is especially useful when performing Kinetics data collections overnight or unattended for long periods of time.
- b** Click the **UV/Vis** button if you want both of the lamps on during the run.
- c** Enter your required 'Source Changeover' and 'Detector Changeover' (Cary 5000 only) wavelengths in the corresponding fields. If you are using a Cary 5000/6000i, also enter the detector and grating changeover wavelengths (800 nanometers is recommended for both).
- d** Set the 'Slit Height' to full or reduced (Cary 4000/5000/6000i only).
- e** Under 'Display Options' choose 'Individual Data' to display the collected data of each sample in individual graph boxes. Choose 'Overlay Data' to superimpose the collected data of each sample in the Kinetics run in one graph box.

## 7 Set up analysis parameters

*Setup dialog box | Analyze page*

- a** Select **Auto Calculate** to automatically perform a rate calculation at the end of each run.
- b** Select **Advanced Calculate** to set up multiple rate calculations for the Kinetics run.
- c** Enter the number of different rate calculations that you require in the 'Number of Stages' field. The number you set here will be reflected in the table below.
- d** Set up the stage 'Start' and 'Stop' times and select the reaction order for each of these reaction stages.

**NOTE**

If you select a 'first order' or 'second order' Simple Calculate rate calculation, you can use the items under 'Manual Guess' to manually enter the parameters:  $A_0$ ,  $A_{inf}$  and Rate ( $k$ ).

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- e** Enter a value in the 'Factor' field to calculate enzyme activity. This multiplication factor is applied to the absorbance.
- f** If you are performing a second order reaction, enter the initial concentration of substrate before reaction.
- g** Select **Display Fit** to automatically overlay the calculated lines of best fit onto the plotted data.

**8 Set up reporting and printing requirements**

*Setup dialog box | Reports page*

- a** Enter your name in the 'Name' field.
- b** Enter a comment relating to your experiment in the 'Comment' field.
- c** Set up your report style by selecting the appropriate check boxes under 'Options'. For example, select 'Auto Print' to automatically obtain a printout of your report. Select 'Graph' to include a graph in the generated report.

**NOTE**

If 'Auto Print' is selected, the system will send the report information to the specified printer as well as to the Report area.

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- d** Select **Include X-Y Pairs Table** to view a list of abscissa values and their corresponding ordinate values.
- e** Select the 'Autoconvert' option you require. If you select 'ASCII' or 'ASCII with Log', at the end of the data collection, the system will automatically generate a report and store the data in the Cary format as well as ASCII XY pairs format in the current folder.

## 9 Set up storage of collected data

*Setup dialog box | Auto Store page*

Select **Storage On (Prompt at Start)** to set the Cary to prompt you for a file name, before the start of a Kinetics reaction.

## 10 Set up visual system monitoring

Select **Show Status Display** on any of the Setup pages, or from the **View** menu, to display information about your current reaction.

## 11 Finish setup

Click **OK** to save any changes you have made and close the 'Setup' dialog box.

## 12 Zero the instrument

- a Click **Zero** or from the **Commands** menu, choose **Zero**. A 'Cell Loading Guide' will be displayed.
- b If you like, change the names of the blank samples.
- c Place the blank solution(s) in the correct cell positions and click **OK**. The system will perform an instrument zero on the blank solution(s).

## 13 Start the run

- a Click the **Start** button or from the **Commands** menu, choose **Start**. *Do not add your active reagent at this time.* The system will display the 'Save As' dialog box.
- b Enter the 'File name' of this Kinetics run and click **Save**. The system will display a 'Cell Loading Guide'.
- c If you like, change the names of the samples.
- d Place the sample solution(s) in the correct cell positions and click **OK**. The system will set up the Graphics area and then the 'Sync Start' dialog box will be displayed.
- e Reset the Multicell Holder position to cell 1 by clicking the **Reset Slide** button.
- f Add your active reagent just before the countdown reaches 0:00, or commence the data collection by clicking **OK**.

## Perform a multi-wavelength, single cell, single rate Kinetics run

This procedure describes how to perform a multi-wavelength, single cell Kinetics run at ambient temperature (Cary 100/300/4000/5000/6000i).

### 1 Set up data collection parameters

*Setup dialog box*

In Kinetics, click the **Setup** button or choose **Setup** from the Menu line to display the 'Setup' dialog box and specify the method parameters for a new method.

### 2 Set up instrument parameters

*Setup dialog box | Cary page*

- a In the 'Wavelength' field, enter the relevant wavelengths.
- b In the 'SBW' field, enter the required spectral bandwidth.
- c In the 'Ave. (averaging) Time' field, enter the required value.
- d Select the ordinate mode you require. Click 'Abs' to specify the absorbance mode or '%T' to specify percent transmittance.
- e Enter an upper range and lower range value in the 'Y min.' and 'Y max.' fields to specify the ordinate range.
- f Select 'Min.' or 'Sec.' to set the abscissa (X).

### 3 Set up rate parameters

- a Under 'Collect Timing', select **Simple Collect** to set up a single rate for your reaction.
- b Specify how long the Cary will wait after reading each cell before it starts another reading cycle by setting the cycle time.
- c Specify the duration of the Kinetics run by setting the stop time.

#### 4 Set up lamp and graphics options

*Setup dialog box | Options page*

- a** Select **Auto Lamps Off** if you want to automatically turn off the lamps at the end of a collect. This is especially useful when performing Kinetics data collections overnight or unattended for long periods of time.
- b** Click the **UV/Vis** button if you want both lamps on during the run.
- c** Enter your required 'Source Changeover' and 'Detector Changeover' (Cary 5000 only) wavelengths in the corresponding fields. If you are using a Cary 5000/6000i, also enter the detector and grating changeover wavelengths (800 nanometers is recommended for both).
- d** Set the 'Slit Height' to full or reduced (Cary 4000/5000/6000i only).
- e** Under 'Display Options', select 'Individual Data' to display the collected data of each sample in individual graph boxes. Select 'Overlay Data' to superimpose the collected data of each sample in the Kinetics run in one graph box.

#### 5 Set up accessories

*Setup dialog box | Accessories page*

Click the **Accessories** tab and ensure that no accessories are selected.

#### 6 Set up analysis parameters

*Setup dialog box | Analyze page*

- a** Select 'Auto Calculate' to automatically perform a rate calculation at the end of each run. Or select 'Simple Calculate' to perform one rate calculation for the entire Kinetics run.
- b** Set up the reaction start and stop times and select the reaction order.
- c** If you select a 'first order' or 'second order' Simple Calculate rate calculation, you can use the 'Manual Guess' items to manually enter the parameters:  $A_0$ ,  $A_{Inf}$  and Rate (k).

**NOTE**

If you do not choose 'Manual Guess', the Cary system will automatically calculate the values:  $A_0$ ,  $A_{inf}$  and Rate when the 'Start' button is clicked.

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- d** Enter a value in the 'Factor' field to calculate enzyme activity. This multiplication factor is applied to the absorbance.
- e** If you are performing a second order reaction, enter the initial concentration of substrate before reaction.
- f** Select **Display Fit** to automatically overlay the calculated lines of best fit onto the plotted data.

## 7 Set up reporting and printing requirements

*Setup dialog box | Reports page*

- a** Enter your name in the 'Name' field.
- b** Enter a comment relating to your experiment in the 'Comment' field.
- c** Set up your report style by selecting the appropriate check boxes under 'Options'. For example, select 'Auto Print' to automatically obtain a printout of your report. Select 'Graph' to include a graph in the generated report.

**NOTE**

If 'Auto Print' is selected, the system will send the report information to the specified printer as well as the Report area.

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- d** Select **Include X-Y Pairs Table** to view a list of abscissa values and their corresponding ordinate values.
- e** Select the autoconvert option you require. If you select 'ASCII' or 'ASCII with Log', at the end of the data collection, the system will automatically generate a report and store the data in the Cary format as well as ASCII XY pairs format in the current folder.

## 8 Set up storage of collected data

*Setup dialog box | Auto Store page*

Select **Storage Off**. The method, collected data and report will not be saved. Or, select 'Storage On Prompt at Start' to set the Cary to prompt you for a file name before the start of a run; or 'Storage On Prompt at End' to set the Cary to prompt you for a file name at the end of a run.

## 9 Set up visual system monitoring

Select **Show Status Display** on any of the Setup pages, or from the **View** menu, to display information about your current reaction.

## 10 Finish setup

Once you are satisfied with your method setup, click **OK** to confirm any changes you have made and close the 'Setup' dialog box.

## 11 Zero the instrument

- a Click **Zero**. Alternatively, choose **Zero** from the **Commands** menu. A 'Loading Guide' will be displayed.
- b If you like, change the name of the blank.
- c Place the blank solution in the sample compartment and click **OK**. The system will perform an instrument zero on the blank solution(s).

## 12 Start the run

- a Click the **Start** button, or choose **Start** from the **Commands** menu. *Do not add your active reagent at this time.* The system will display a 'Loading Guide'.
- b If you like, change the names of the sample.
- c Place the sample solution in the front cell holder and click **OK**. The system will set up the Graphics area and then display the 'Sync Start' dialog box.
- d Add your active reagent just before the countdown reaches 0:00, or commence the data collection by clicking **OK**. The Cary will start the Kinetics data collection.

### 13 Save your data

- a On the **File** menu, and click **Save Data As**.
- b Enter the 'File name' for this Kinetics run.
- c Click **Save**. The data will be stored as a Batch file.

## RNA-DNA Estimation

### Perform a temperature-controlled RNA-DNA run using the Multicell Holder

This procedure describes how to perform a multicell RNA-DNA run with multi zero and multi baseline at 37 °C using the Temperature Controller accessory with the Multicell Holder accessory (Cary 100/300/4000/5000/6000i).

#### 1 Set up data collection parameters

*Setup dialog box*

In RNA-DNA, click the **Setup** button or choose **Setup** from the Menu line to display the 'Setup' dialog box and specify the method parameters for a new method.

#### 2 Set up the Multicell Holder accessory

*Setup dialog box | Accessories 1 page*

As various options do not become available until the appropriate accessories are selected, you need to select these first.

- a Select **Use Cell Changer** to enable the accessory.
- b For Cary 100/300 instruments, choose the type of Multicell Holder you are using (6 x 6 or 8 x 6). Ensure that you have this accessory installed before starting the run.

#### NOTE

If you are using a Series I 6 x 6 (Cary 100/300), you must calibrate the cell changer using the Align application before starting experiments.

- c** Click **Select Cells** and select the cells you require under 'Use Cells'. For double beam analysis, select 'Cell 1 through to the Cell 6' (6 x 6) or 'Cell 1 through to Cell 8' (8 x 6).
- d** Select **Multi Zero**.

The 'Multi Baseline' facility will not be accessible until completing Step 4.

### **3 Set up accessories for reaction temperature control and temperature display**

- a** If you are not using a Peltier-controlled accessory (for example, the water-thermostatted 8 x 6), ensure that you have the Temperature Controller accessory installed before starting the run, and,
  - (i) Select **Automatic Temperature Setting** and select **Temperature Controller** to enable the accessory.
  - (ii) Set the monitoring temperature by entering the block temperature as 37 °C. (The monitoring device is selected in Step 4).
- b** Under 'Temperature Display', select **Block** and **Probe 1** to view the temperature of the Multicell Holder block and one temperature probe in the 'Status Display' window.

### **4 Set up instrument parameters**

*Setup dialog box | Cary and Baseline pages*

- a** Enter the first and second wavelengths at which you would like to measure your sample/s.
- b** If required, select **Background Correction** and enter a background wavelength in the corresponding field.
- c** Choose the desired temperature monitoring device in the 'Monitor' field. The 'Start' button will not be enabled until the temperature of the selected Monitor is within 0.5 °C of the Block temperature set on the 'Accessories 1' page (Step 3a (ii)).
- d** To perform a wavelength scan, select **Scan Samples** and set the 'Start', 'Stop' and 'Rate' for the scan. The 'Baseline' tab will be displayed.
- e** Click the **Baseline** tab.

- f Select **Baseline correction**. To use a previously stored baseline, click the 'Baseline' button and browse for the appropriate file. Otherwise, you can perform your baseline correction at the beginning of the run. To perform a 'Multi Baseline', you can now go back to the 'Accessories 1' page and select 'Multi Baseline'.

## 5 Set up source, SBW and display options

*Setup dialog box | Options page*

- a Select **Auto Lamps Off** if you want to automatically turn off the lamps at the end of a collect. This is especially useful when performing data collections overnight or unattended for long periods of time.
- b Click **UV/Vis** if you want both lamps on during the run.
- c Enter your required 'Source Changeover' and 'Detector Changeover' (Cary 5000 only) wavelengths in the corresponding fields. If you are using a Cary 5000/6000i, also enter the detector and grating changeover wavelengths (800 nanometers is recommended for both).
- d Enter the spectral bandwidth of your instrument in the 'SBW' field.
- e Set the appropriate 'Slit Height' (Cary 4000/5000/6000i only).
- f Under 'Display Options', select 'Individual Data' to display the collected data of each sample in individual graph boxes. Choose 'Overlay Data' to superimpose the collected data of each sample in the run in one graph box.

## 6 Set up your samples

*Setup dialog box | Samples page*

- a Enter the number of samples that you are going to use in the 'Number of Samples' field. The 'Sample Names' list below will expand or contract to match your choice.
- b In the 'Sample Names' list, enter the name of each sample. You can enter up to 20 characters for each name.

If the samples have the same name with a different numeric extension, enter the name in the first sample position and then click the 'Increment' button.

Click the 'Import Names' button to use names from a stored text file.

- c** Clear the red tick for any samples not required.
- d** If you would like multiple readings of the same aliquot, select **Replicates** and enter the number of replicates required.

## 7 Set up analysis parameters

*Setup dialog box | Analyze page*

If you would like to calculate any Warburg Christian coefficients or 260 nm Factor parameters, make your selections here.

## 8 Set up reporting and printing requirements

*Setup dialog box | Reports page*

- a** Enter your name in the 'Name' field.
- b** Enter a comment relating to your experiment in the 'Comment' field.
- c** Set up your report style by selecting the appropriate check boxes under 'Options'. For example, select 'Auto Print' to automatically obtain a printout of your report. If you have selected 'Scan Samples' on the 'Cary' page, select 'Graph' to include a graph in the generated report.

### NOTE

If 'Auto Print' is selected, the system will send the report information to the specified printer as well as to the Report area.

- d** Select the 'Autoconvert' option you require. If you choose 'Select for ASCII (csv)' or 'Select for ASCII (csv) with Log', at the end of the data collection, the system will automatically generate a report and store the data in the Cary format as well as ASCII XY pairs format in the current folder.

## 9 Set up storage of collected data

*Setup dialog box | Auto Store page*

Select **Storage On (Prompt at Start)**, to set the Cary to prompt you for a file name before the start of the RNA-DNA run.

## Set up visual system monitoring

Select **Show Status Display** on any of the Setup pages, or from the **View** menu, to display information about your current reaction.

## 10 Finish setup

Click **OK** to save any changes you have made and close the 'Setup' dialog box.

## 11 Zero the instrument

- a Click **Zero** or choose **Zero** from the **Commands** menu. A 'Cell Loading Guide' will be displayed.
- b Place the blank solution(s) in the correct cell positions and click **OK**. The system will perform an instrument zero on the blank solution(s).

### NOTE

If you have chosen not to use the 'Multi Zero' facility in Step 2d, the system will prompt you to enter one blank solution into the instrument. In this case, it is important to ensure that you place this blank solution in the cell position that is currently in the light path. On clicking 'OK', the system will perform a zero on the cell position in the light path, that is, the system will not reset the Multicell Holder to position 1.

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## 12 Perform a baseline correction (if not using a stored baseline)

If you have selected to perform a baseline correction (Step 4f) and are not using a stored baseline, take a baseline reading now by following the steps below. Otherwise, proceed to Step 13.

- a Click **Baseline**. A 'Cell Loading Guide' will be displayed.
- b Load the blank/s as shown.
- c Click **OK**. The system will collect a baseline for each cell in use and the word 'baseline' will be displayed above the ordinate instrument status reading.

## 13 Start the run

- a Click the **Start** button to start a data collection. Alternatively you can choose **Start** from the **Commands** menu. The 'Save As' dialog box will be displayed.
- b Enter the 'File name' for this RNA-DNA run and click **Save**. The system will display a 'Cell Loading Guide'.
- c Place the sample solution(s) in the correct cell positions and click **OK**. The system will then begin reading the samples and printing the results to the Report area.

## Scan

The following Scan procedures are described.

- Performing a scan with baseline correction (Cary 100–6000i)
- Performing a scan in Signal-to-Noise mode (Cary 100-6000i)
- Performing a scan in Independent mode (Cary 5000/6000i)

### Perform a scan with baseline correction

This procedure describes how to perform a wavelength scan from 800 nm to 200 nm with baseline correction (Cary 100/300/4000/5000/6000i).

#### 1 Set up data collection parameters

*Setup dialog box*

In Scan, click the **Setup** button or choose **Setup** from the Menu line to display the 'Setup' dialog box and specify the method parameters for a new method.

#### 2 Set up instrument parameters

*Setup dialog box | Cary page*

- a Set the appropriate abscissa mode in the 'X Mode' field.
- b Set the wavelength range by entering the values you require in the 'Start'/'Stop' fields.
- c In the 'Y Mode' field, select the ordinate mode.
- d Enter an upper and lower range in the 'Y min.' and 'Y max.' fields to specify the displayed ordinate range.
- e Set the speed of the data collection by setting the 'Ave. Time' and 'Data Interval'. The Data Interval is the wavelength increment between data points. The 'Scan Rate' will automatically update when selected.
- f Make sure 'Cycle Mode' is not selected.

### 3 Set up SBW, lamp and graphics options

*Setup dialog box | Options page*

- a** Set the 'SBW' (spectral bandwidth).
- b** Set the 'Beam Mode' for the run (usually 'Double').
- c** For Cary 5000/6000i, the fixed SBW is used to automatically alter the Energy level and maintain a constant signal level.
- d** For Cary 4000/5000/6000i, set the 'Slit Height' to **Full**.
- e** Click the **UV/Vis** button if you want both lamps on during the run.
- f** Select **Auto Lamps Off** if you want to automatically turn off the lamps at the end of a collect. This is especially useful when performing Scan runs overnight, or unattended for long periods of time.
- g** Do not select 'Signal-to-Noise Mode'.
- h** Under 'Display Options', select 'Individual Data' to display the collected data of each sample in individual graph boxes. Choose 'Overlay Data' to superimpose the collected data of each sample in the Scan run in one graph box.

*Setup dialog box | Independent page*

If you are using a Cary 5000/6000i, do not change any options on the 'Independent' page.

### 4 Set up the baseline correction

*Setup dialog box | Baseline page*

Select **Baseline Correction** to perform a baseline correction on each sample data point.

#### NOTE

You can use a stored baseline by clicking 'Baseline' and opening the saved \*.csw baseline file.

## 5 Ensure no accessories are selected

*Setup dialog box | Accessories 1 page*

Make sure that no options are selected on this page and that no accessories are installed.

*Setup dialog box | Accessories 2 page*

Make sure that no options are selected on this page and that no accessories are installed.

*Setup dialog box | Accessories 3 page*

Make sure that no options are selected on this page and that no accessories are installed.

## 6 Set up reporting and printing requirements

*Setup dialog box | Reports page*

- a Enter your name in the 'Name' field.
- b Enter a comment relating to your sample in the 'Comment' field.
- c Set up your report style by selecting the appropriate check boxes under 'Options'. For example, select 'AutoPrint' to automatically obtain a printout of your report. Select 'Graph' to include a graph in the printed report.

### NOTE

If 'AutoPrint' is selected, the system will send the report information to the specified printer as well as the Report area.

- d Set up the 'Peak Table' reporting.
  - (i) Select **Peak Labels**.
  - (ii) Click the **Peak Information** button and choose the 'Peak Type', the 'Labels Type', and set the 'Peak Threshold'. Click **OK**.
  - (iii) Select **Maximum Peak** to report the peak with the largest peak threshold that exceeds the Peak Threshold value.

- (iv) Select **All Peaks** to report all peaks meeting the Peak Style criterion and exceeding the Threshold value.
- e** Set up 'X-Y pairs' reporting, if required. You can use the actual Data Interval by which the data was collected, or you can make the Cary interpolate the points to a new Interval.
- f** Select the 'Autoconvert' option you require. If you select 'Select for ASCII (csv)' or 'Select for ASCII (csv) with Log', at the end of the data collection the system will automatically generate a report and store the data in the Cary format as well as ASCII XY pairs format in the current folder.

## 7 Set up storage of collected data

*Setup dialog box | Auto Store page*

Select **Storage On (Prompt at Start)**.

## Set up visual system monitoring

Select **Show Status Display** on any Setup page, or from the **View** menu, to display information about your current reaction.

## 8 Finish setup

Once you are satisfied with your method setup, click **OK** to confirm any changes you have made and close the 'Setup' dialog box.

## 9 Zero the instrument

Click **Zero** or choose **Zero** from the **Commands** menu to perform a zero.

## 10 Measure a baseline

- a** Click **Baseline** or choose **Baseline** from the **Commands** menu.
- b** When prompted, insert the blank sample into the sample compartment front beam and click **OK**. After the baseline is collected, the word 'baseline' will be displayed in red in the ordinate status box, indicating that you are in baseline correction mode and have a valid baseline file for the correction.

**NOTE**

If the word 'baseline' is gray and in italics, the baseline file is still valid. The gray and italics indicate that the Cary is idling outside the abscissa range of the baseline file.

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## 11 Start the run

Click the **Start** button or choose **Start** from the **Commands** menu to start the Scan run.

## 12 Name the data file and samples

- a When you click **Start**, the 'Save As' dialog box will be displayed. Enter the appropriate 'File name' for the data and click **Save**.
- b The 'Sample Name' dialog box will be displayed. Enter the appropriate name for your sample and click **OK**. The Scan run will commence and the corrected trace will be displayed in the Graphics area. At the end of the run, the Cary will create the report and also print it, if 'AutoPrint' has been selected on the 'Reports' page of the 'Setup' dialog box.

## Perform a scan in Signal-To-Noise mode

This procedure describes how to perform a wavelength scan from 800 nm to 200 nm in signal-to-noise mode (Cary 100/300/4000/5000/6000i).

### 1 Set up data collection parameters

*Setup dialog box*

In Scan, click the **Setup** button or choose **Setup** from the Menu line to display the 'Setup' dialog box and specify the method parameters for a new method.

### 2 Set up instrument parameters

*Setup dialog box | Cary page*

- a Set the appropriate abscissa mode for the scan in the 'X Mode' field.

- b** Set the wavelength range for the scan by entering the values you require in the 'Start' and 'Stop' fields.
- c** In the 'Y Mode' field, select the ordinate mode.
- d** Enter an upper and lower range in the 'Y min.' and 'Y max.' fields to specify the displayed ordinate range. You can click the 'Autoscale' button during the data collection to automatically scale the display.
- e** Set the speed of the data collection by setting the 'Ave. Time' and 'Data Interval'. The Data Interval is the wavelength increment between data points. The 'Scan Rate' will automatically update when selected.
- f** Make sure that 'Cycle Mode' is not selected.

### 3 Set up SBW, lamp and graphics options

*Setup dialog box | Options page*

- a** Set the 'SBW' (spectral bandwidth).
- b** If you are using a Cary 100/300/5000/6000i, set the 'SBW' for the run. A good starting point is 2 nanometers if your method does not specify a SBW.
- c** Set the 'Beam Mode' for the run (usually 'Double').
- d** For Cary 5000/6000i, the fixed SBW is used to automatically alter the Energy level and maintain a constant signal level.
- e** For Cary 4000/5000/6000i, set the 'Slit Height' to **Full**.
- f** Click the **UV/Vis** button if you want both the lamps on during the run.
- g** Select **Auto Lamps Off** if you want to automatically turn off the lamps at the end of a collect. This option is especially useful when performing Scan runs overnight or unattended for long periods of time.
- h** Check **Signal-to-Noise Mode**, and set the required values for the Acceptable S/N and the S/N Timeout.
- i** Under 'Display Options', select 'Individual Data' to display the collected data of each sample in individual graph boxes. Choose the 'Overlay Data' to superimpose the collected data of each sample in the Scan run in one graph box.

*Setup dialog box | Independent page*

On the 'Independent' page, make sure 'Independent Control' is not selected.

#### **4 Set up the baseline correction**

*Setup dialog box | Baseline page*

Select 'Baseline Correction' to perform a baseline correction on each sample data point.

#### **NOTE**

You can use a stored baseline by clicking 'Baseline' and opening the saved \*.csw baseline file.

#### **5 Ensure no accessories are selected**

*Setup dialog box | Accessories 1 page*

Make sure that no options are selected on this page and that no accessories are installed.

*Setup dialog box | Accessories 2 page*

Make sure that no options are selected on this page and that no accessories are installed.

*Setup dialog box | Accessories 3 page*

Make sure that no options are selected on this page and that no accessories are installed.

#### **6 Set up reporting and printing requirements**

*Setup dialog box | Reports page*

- a** Enter your name in the 'Name' field.
- b** Enter a comment relating to your experiment in the 'Comment' field.
- c** Set up your report style by selecting the appropriate check boxes under 'Options'. For example, select 'AutoPrint' to automatically obtain a printout of your report. Select 'Graph' to include a graph in the generated report.

**NOTE**

If 'AutoPrint' is selected, the system will send the report information to the specified printer as well as the Report area.

---

- d** Set up the 'Peak Table' reporting.
  - (i) Select **Peak Labels**.
  - (ii) Click the **Peak Information** button and choose the 'Peak Type', the 'Labels Type', the and set the 'Peak Threshold'. Click **OK**.
  - (iii) Select **Maximum Peak** to report the peak with the largest peak threshold that exceeds the Peak Threshold value.
  - (iv) Select **All Peaks** to report all peaks meeting the Peak Style criterion and exceeding the Threshold value.
- e** Set up 'X-Y pairs' reporting, if required. You can use the actual Data Interval by which the data was collected or you can make the Cary interpolate the points to a new Interval.
- f** Select the 'Autoconvert' option you require. If you select 'Select for ASCII (csv)' or 'Select for ASCII (csv) with Log', at the end of the data collection the system will automatically generate a report and store the data in the Cary format as well as ASCII XY pairs format in the current folder.

### 7 Set up storage of collected data

*Setup dialog box | Auto Store page*

Select **Storage On (Prompt at End)**.

### 8 Set up visual system monitoring

Select **Show Status Display** on any Setup page, or from the **View** menu, to display information about your current reaction.

### 9 Finish setup

Once you are satisfied with your method setup, click **OK** to confirm any changes you have made and close the 'Setup' dialog box.

## 10 Zero the instrument

Click **Zero**, or choose **Zero** from the **Commands** menu to perform a zero.

## 11 Measure a baseline

- a Click **Baseline** or choose **Baseline** from the **Commands** menu.
- b When prompted, insert the blank sample into the sample compartment front beam and click **OK**. After the baseline is collected, the word 'baseline' will be displayed in red in the ordinate status box, indicating that you are in baseline correction mode and have a valid baseline file for the correction.

### NOTE

If the word 'baseline' is gray and in italics, the baseline file is still valid. The gray and italics indicate that the Cary is idling outside the abscissa range of the baseline file.

---

## 12 Start the run

Click the **Start** button or choose **Start** from the **Commands** menu.

## 13 Name your samples

Once you click 'Start', the 'Sample Name' dialog box will be displayed. Enter the appropriate name for your sample and click **OK**. The Scan run will commence and the corrected trace will be displayed in the Graphics area.

If for a particular point, the set Acceptable S/N cannot be met in the set 'S/N Timeout' time, the Cary will collect the point as normal (using the S/N Timeout as the Ave. Time) and display the message SNR Timeout in the hardware status area.

## 14 Save your data

When the Cary has measured the sample, the 'Save As' dialog box will be displayed. Enter the appropriate name for your sample and click **Save**. The Cary will then create the report and print it, if 'AutoPrint' has been selected on the 'Reports' page of the 'Setup' dialog box.

## Perform a scan in Independent mode (Cary 5000)

This procedure describes how to perform a wavelength scan from 2200 nm to 400 nm in Independent mode (Cary 5000 only).

### 1 Set up data collection parameters

*Setup dialog box*

In Scan, click the **Setup** button or choose **Setup** from the Menu line to display the 'Setup' dialog box and specify the method parameters for a new method.

### 2 Set up instrument parameters

*Setup dialog box | Cary page*

- a** Set the appropriate abscissa mode for the scan in the 'X Mode' field.
- b** Set the wavelength range for the scan by entering the values you require in the 'Start' and 'Stop' fields.
- c** In the 'Y Mode' field, select the ordinate mode.
- d** Enter an upper and lower range in the 'Y min.' and 'Y max.' fields to specify the displayed ordinate range. You can use the 'Autoscale' button during the data collection to automatically scale the display.
- e** Do not set any Scan Controls, such as 'Ave. Time' or 'Data Interval', as the settings in Independent mode will override these.
- f** Make sure that 'Cycle Mode' is not selected.

### 3 Set up SBW, lamp and graphics options

*Setup dialog box | Options page*

- a** Do not set the 'SBW' (spectral bandwidth) or Energy, as the settings in Independent mode will override these.
- b** Set the 'Beam Mode' for the run (usually 'Double').
- c** Set the 'Slit Height' to **Full**.
- d** Click the **UV/Vis** button if you want both lamps on during the run.

- e Select **Auto Lamps Off** if you want to automatically turn off the lamps at the end of a collect. This option is especially useful when performing Scan runs overnight or unattended for long periods of time.
- f Under 'Display Options', select 'Individual Data' to display the collected data of each sample in individual graph boxes. Choose 'Overlay Data' to superimpose the collected data of each sample in the Scan run in one graph box.

#### 4 Set up the signal level controls (SBW/energy) for the scan

*Setup dialog box | Independent page*

- a Select **Independent Control**.
- b Select the **Auto** 'Measurement Mode'. This will mean that the Cary will use a Fixed SBW (spectral bandwidth) in the UV-Vis region and a fixed Energy level in the near infra-red region.
- c Under 'UV-Vis', set the parameters you require in the UV-Vis region. Good starting values are: Ave. Time = 0.1, Data Interval = 1, Scan Rate = 600, SBW = 2.
- d Under 'NIR', set the parameters you require in the near infra-red region. Good starting values are: Ave. Time = 0.1, Data Interval = 4, Scan Rate = 2400, Energy=1.

#### 5 Set up baseline correction

*Setup dialog box | Baseline page*

Select **Baseline Correction** to perform a baseline correction on each sample data point.

#### NOTE

You can use a stored baseline by clicking 'Baseline' and opening the saved \*.csw baseline file.

#### 6 Ensure no accessories are selected

*Setup dialog box | Accessories 1 page*

Make sure that no options are selected on this page and that no accessories are installed.

*Setup dialog box | Accessories 2 page*

Make sure that no options are selected on this page and that no accessories are installed.

*Setup dialog box | Accessories 3 page*

Make sure that no options are selected on this page and that no accessories are installed.

## **7 Set up reporting and printing requirements**

*Setup dialog box | Reports page*

- a** Enter your name in the 'Name' field.
- b** Enter a comment relating to your experiment in the 'Comment' field.
- c** Set up your report style by selecting the appropriate check boxes under 'Options'. For example, select 'AutoPrint' to automatically obtain a printout of your report. Select 'Graph' to include a graph in the generated report.

### **NOTE**

If 'AutoPrint' is selected, the system will send the report information to the specified printer as well as the Report area.

- d** Set up the 'Peak Table' reporting.
  - (i) Select **Peak Labels**.
  - (ii) Click the **Peak Information** button and choose the 'Peak Type', the 'Labels Type', and set the 'Peak Threshold'. Click **OK**.
  - (iii) Select **Maximum Peak** to report the peak with the largest peak threshold that exceeds the Peak Threshold value.
  - (iv) Select **All Peaks** to report all peaks meeting the Peak Style criterion and exceeding the Threshold value.
- e** Set up 'X-Y pairs' reporting, if required. You can use the actual Data Interval by which the data was collected or you can make the Cary interpolate the points to a new Interval.

- f Select the 'Autoconvert' option you require. If you select 'Select for ASCII (csv)' or 'Select for ASCII (csv) with Log', at the end of the data collection the system will automatically generate a report and store the data in the Cary format as well as ASCII XY pairs format in the current folder.

## 8 Set up storage of collected data

*Setup dialog box | Auto Store page*

Select **Storage On (Prompt at End)**.

## 9 Set up visual system monitoring

Select **Show Status Display** on any Setup page, or from the **View** menu, to display information about your current reaction.

## 10 Finish setup

Once you are satisfied with your method setup, click **OK** to confirm any changes you have made and close the 'Setup' dialog box.

## 11 Zero the instrument

Click **Zero** to zero the system. Alternatively, choose **Zero** from the **Commands** menu to perform a zero.

## 12 Measure a baseline

- a Click **Baseline** or choose **Baseline** from the **Commands** menu.
- b When prompted, insert the blank sample into the sample compartment front beam and click **OK**. After the baseline is collected, the word 'baseline' will be displayed in red in the ordinate status box, indicating that you are in baseline correction mode and have a valid baseline file for the correction.

### NOTE

If the word 'baseline' is gray and in italics, the baseline file is still valid. The gray and italics indicate that the Cary is idling outside the abscissa range of the baseline file.

## 13 Start the run

Click the **Start** button or choose **Start** from the **Commands** menu.

#### 14 Set up sample names

When you click **Start**, the 'Sample Name' dialog box will be displayed. Enter the appropriate name for your sample and click **OK**. The Scan run will commence and the corrected trace will be displayed in the Graphics area.

#### 15 Save your data

When the Cary has measured the sample, the 'Save As' dialog box will be displayed. Enter the appropriate name for your sample and click **Save**. The Cary will then create the report and print it, if 'AutoPrint' was selected on the 'Reports' page of the 'Setup' dialog box.

## Scanning Kinetics

### Collect data using the Multicell Holder with temperature control

This procedure demonstrates how to perform a multicell, multi-stage wavelength scan with baseline correction from 600 to 500 nm at 37 °C using the Temperature Controller accessory with the Multicell Holder accessory (Cary 100/300/4000/5000/6000i). You can then use the data to create kinetics continuums in order to calculate reaction rates.

#### 1 Set up data collection parameters

*Setup dialog box*

In Scanning Kinetics, click the **Setup** button or choose **Setup** from the Menu line to display the 'Setup' dialog box and specify the method parameters for a new method.

#### 2 Select the baseline correction type

*Setup dialog box | Baseline page*

Select **Baseline Correction**. This will force the Cary to use a baseline scan to perform a baseline correction on the sample data. The correction will be performed on each point before it is displayed.

**NOTE**

You can use a stored baseline. To do this, click the 'Retrieve Baseline File' button and open the saved \*.csk baseline file.

---

### 3 Set up the Multicell Holder accessory

*Setup dialog box | Accessories page*

- a Ensure that you have the appropriate accessories installed before starting the run.
- b Select **Use Cell Changer** to enable the Multicell Holder accessory.
- c Choose the type of Multicell Holder you are using (6 x 6 or 8 x 6).

**NOTE**

If you are using a Series I 6 x 6x (Cary 100/300), you must calibrate the cell changer using the Align application before starting experiments.

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- d Click **Select Cells** and select the cells you require under 'Use Cells'.

For Front Beam analysis, select 'Cell 1–Cell 6' (6 x 6) or 'Cell 1–Cell 8' (8 x 6). This will ensure that all front cell positions in the Multicell Holder will be measured during your Scanning Kinetics analysis.

- e Select **Multi Zero**.

### 4 Set up accessories for reaction temperature control and temperature display

- a If you are not using a Peltier-controlled accessory (for example, the water-thermostatted 8 x 6), ensure that you have the Temperature Controller accessory installed before starting the run.
- b Select **Automatic Temperature Setting** and click **Temperature Controller** to enable the accessory.

- c Set the monitoring temperature by entering the block temperature as 37 °C. (The monitoring device is selected in Step 6d.)
- d Under 'Temperature Display', select **Block** and **Probe 1** to view the temperature of the Multicell Holder block and one temperature probe in the 'Status Display' window.

## 5 Set up instrument parameters

*Setup dialog box | Cary page*

- a Set the appropriate abscissa mode for the scan in the 'X Mode' field.
- b Set the wavelength range for the scan by entering the values you require in the 'Start' and 'Stop' fields.
- c In the 'Y Mode' field, select the ordinate mode you require.
- d Enter an upper and lower range value for 'Y min.' and 'Y max.' to specify the displayed ordinate range.
- e Set the speed of the data collection by setting the 'Ave. Time' and 'Data Interval'. The Data Interval is the wavelength increment between data points. The 'Scan Rate' will automatically update when selected.

## 6 Set up rate parameters

Under 'Collect Timing', select **Advanced Collect** to set up different data collection procedures for the multiple rates in your reaction.

### NOTE

The 'Advanced Collect' facility enables you to collect data more frequently during the crucial stages of your reaction, and to collect data less frequently where you know there will not be much activity.

- 
- a Enter the number of different reaction rates that you require in the 'Number of Stages' field. The number you set here will be reflected in the table below.
  - b Specify how long the Cary will wait after reading each cell before it starts another reading cycle by setting the 'Cycle time' for each rate stage.

- c Specify the duration of the Scanning Kinetics run by setting the 'Stop time' for each rate stage.
- d Choose the desired temperature monitoring device in the 'Monitor' field. The 'Start' button will not be enabled until the temperature of the selected monitor is within 0.5 °C of the block temperature set on the 'Accessories' page (Step 4c).

## 7 Set up spectral bandwidth or energy and lamp options

*Setup dialog box | Options page*

- a Set the 'SBW' (spectral bandwidth) for the run.
- b Set the 'Slit Height' to **Full**.
- c Select **Auto Lamps Off** if you want to automatically turn off the lamps at the end of a collect. This is especially useful when performing data collections overnight or unattended for long periods of time.
- d Click the **UV/Vis** button if you want both lamps on during the run.
- e Enter your required 'Source Changeover' wavelength in the corresponding field. If you are using a Cary 5000/6000i, also enter the detector and grating changeover wavelengths (800 nanometers is recommended for both).

## 8 Set up analysis parameters

*Setup dialog box | Analyze page*

This page is used for post-run calculations.

## 9 Set up reporting and printing requirements

*Setup dialog box | Reports page*

- a Enter your name in the 'Name' field.
- b Enter a comment relating to your experiment in the 'Comment' field.
- c Set up your report style by selecting the appropriate check boxes under 'Options'. For example, select 'Auto Print' to automatically obtain a printout of your report. Select 'Graph' to include a graph in the generated report.

**NOTE**

If 'Auto Print' is selected, the system will send the report information to the specified printer as well as to the Report area.

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- d** Select **Include X-Y Pairs Table** to view a list of abscissa values and their corresponding ordinate values.
- e** Select the 'Autoconvert' option you require.
- f** If you choose 'Select for ASCII (csv)' or 'Select for ASCII (csv) with Log', at the end of the data collection the system will automatically generate a report and store the data in the Cary format as well as ASCII XY pairs format in the current folder.

#### **10 Set up storage of collected data**

*Setup dialog box | Auto Store page*

Select **Storage On (Prompt at Start)**, to set the Cary to prompt you for a file name before the start of the Scanning Kinetics run.

#### **11 Set up visual system monitoring**

Select **Show Status Display** on any of the Setup pages, or from the **View** menu, to display information about your current reaction.

#### **12 Finish setup**

Click **OK** to save any changes you have made and close the 'Setup' dialog box. Depending on the cells selected in the Multicell Holder, the Cary may then inform you that it will perform a dual single beam calibration. Click **OK**.

#### **13 Measure a baseline**

- a** If you do not have a valid baseline file, the Cary will prompt you to click **Baseline** to set up the baseline collection.
- b** The system will display a 'Cell Loading Guide'. If you like, change the name of the blank.
- c** Insert blank samples into the cell changer to collect the 0Abs/100%T baseline scans and click **OK**.

The system will set up the Graphics area and the Cary will collect the baseline scan. After the collection, the word 'baseline' will be displayed in red in the ordinate status box, indicating that you are in baseline correction mode and you have a valid baseline file for the correction.

If you want to use the baseline again with other samples, save the method using 'Save Method As' on the 'File' menu. Then, when you re-open the method, the baseline will also open and be ready to use. It is preferable to save the baseline with the method, rather than a baseline file, as that way you can be sure that all your collection parameters are exactly the same for the new Scanning Kinetics runs. However, Good Laboratory Practice recommends that you collect a new baseline for each laboratory session.

#### 14 Zero the instrument

- a Click **Zero** or choose **Zero** from the **Commands** menu.
- b If you like, change the name of the blank/s.
- c Insert blank samples into the appropriate positions of the Multicell Holder and click **OK**.

#### 15 Start the run

- a Click the **Start** button to start a data collection. Alternatively you can choose **Start** from the **Commands** menu. *Do not add your active reagent at this time.*

#### NOTE

At this point, the system will display the 'Save File' dialog box if you have selected 'Storage On (Prompt at Start)' on the 'Auto Store' page of the 'Setup' dialog box. If so, enter the file name for this Scanning Kinetics run in the 'File name' field and click 'Save'.

- b The system will display a 'Cell Loading Guide'. If you like, change the names of the samples.
- c Place the sample solution(s) in the correct cell positions and click **OK**. The 'Sync Start' dialog box will be displayed.
- d Add your active reagent just before the countdown reaches 0:00, or commence the data collection by clicking **OK**.

- e At the end of the run, determine the actual Stop Time by observing the last value in the 'Time' column of the 'User Data Form'.

## Perform a Simple Reads measurement at a single wavelength

This procedure describes how to perform a Simple Reads measurement at a single wavelength.

### 1 Set up instrument parameters (wavelength, Y mode)

- a In Simple Reads, click the **Setup** button or choose **Setup** from the Menu line to display the 'Setup' dialog box.
- b Select **Read at Wavelength** and enter the required wavelength.
- c Under 'Y Mode', select the ordinate mode you require. The ordinate mode determines the way in which the photometric value is measured and displayed in your report.
- d Click **OK**. The instrument will change to the new wavelength.

### 2 Zero the instrument

Make sure that the sample compartment is clear (or you have a blank in position) and click **Zero**. (Wait for the ordinate reading to reach '0'.)

### 3 Read the sample

- a Insert the sample into the sample compartment. Wait while the instrument changes to the specified wavelength. The current wavelength is displayed in the Abscissa status display at the top right of the application window.
- b Click the **Read** button to perform photometric measurements on the sample at the specified wavelength. The result appears in the Report area and includes the ordinate reading obtained and the wavelength at which the reading was measured.

### 4 Print the results

You can print the contents of the Report area by clicking the **Print** button.

## Thermal

The following Thermal procedures are described:

- Performing a run and determine  $T_m$  using Derivative calculations
- Performing a run and determine  $T_m$  using Hyperchromicity calculations

### Perform a run and determine $T_m$ using derivative calculations

This procedure describes how to perform a Thermal run using a single temperature rate and automatically calculate  $T_m$  from the first derivative.

#### 1 Set up data collection parameters

*Setup dialog box*

In Thermal, click the **Setup** button, or choose **Setup** from the Menu line to display the 'Setup' dialog box and specify the method parameters for a new method.

#### 2 Set up instrument parameters

*Setup dialog box | Cary page*

- In the 'Wavelength' field, enter the wavelength that you would like to monitor.
- In the 'SBW' field, enter the required spectral bandwidth.
- In the 'Ave. (averaging) Time' field enter the required value.

#### NOTE

For slow changes in temperature (that is, slow ramp rates), it is recommended that a long Ave. Time (for example, 2 to 3 seconds) be set.

- Enter the ordinate values in the 'Y min.' and 'Y max.' fields.

#### 3 Set up rate parameters

- Specify the 'Collect Temperatures' parameters:
- In the 'Start °C' field, enter the temperature at which you want to start the data collection.

**NOTE**

Once you click 'OK' to close the 'Setup' dialog box, the Cary will drive the temperature to the specified Start Temperature.

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- c** In the 'Return °C' field, enter the temperature to which you want the Temperature Controller to drive at the end of the data collection.
- d** Use 'Monitor' to specify whether the temperature will be measured at the block or the probe.
- e** Select **Simple Collect** to set a single rate of temperature change for the entire Thermal run.
- f** Enter the 'Data Interval', 'Ramp Rate', 'End Temperature' and 'Hold time'. Select the stages where you want to collect data in the 'Collect Data' column.

**4 Set up the lamp source operation and data display options**

*Setup dialog box | Options page*

- a** Select **Auto Lamps Off** if you want to automatically turn off the lamps at the end of a collect. This is especially useful when performing collections overnight or unattended for long periods of time.
- b** Click **UV/Vis** if you want both lamps on during the run.
- c** Under 'Display Options', select 'Individual Data' to display the collected data of each sample in individual graph boxes. Select 'Overlay Data' to superimpose the collected data of each sample in the run in one graph box.

**5 Ensure no accessories are selected**

*Setup dialog box | Accessories page*

Click the **Accessories** tab and make sure that no accessories are selected.

**6 Set up the analysis to reduce measurement interference**

*Setup dialog box | Analyze page*

Select **Smoothing** and nominate the smoothing 'Interval' and 'Filter Size' to control the number of measurement points to be averaged.

## 7 Set up a derivative method of analysis

- a Select **Derivative** to calculate the temperature at which 50% of the DNA strands have separated ( $T_m$ ).
- b Select **Autocalculate** to automatically perform a derivative calculation at the end of each run.

**NOTE**

You can manually perform the derivative calculation, once the run is complete.

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- c Define the range over which to calculate and plot the first derivative of the selected scan.
- d Enter the Interval at which data is sampled in order to perform a derivative calculation. A derivative calculation involves the collection of points from a plot. Selection of these points is dependent upon the data interval chosen. The data points are then used to generate a separate trace from which a derivative calculation is performed. The selection of the Derivative Interval is dependent on the rate of data collection. If data is collected every 0.1 °C then a derivative interval of 0.1 °C will give the most accurate result.
- e Enter the Filter Size you require for the derivative calculation. The derivative uses a Savitzky Golay technique where a number of points surrounding an individual point are averaged to produce a new, smoothed point. For example, a five point derivation uses the two data points before and after each data point.

**NOTE**

The larger the filter size, the more data points that are included in the filter for smoothing.

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- f Enter the temperature that you want to start the calculation in the 'Low Calculation Limit' field.
- g Enter the temperature where you want to end the calculation in the 'High Calculation Limit' field.

## 8 Set up other recalculations and normalization

*Setup dialog box | Reports page*

- a Select **Calculation** and enter or select an equation in the field to perform a calculation.
- b Select **Correction** and enter the 'Correction Temperature' to perform a Thermal Expansion Correction.
- c Select **Normalize** and enter the temperature at which you would like the data to be normalized.

## 9 Set up reporting and printing requirements

- a Enter your name in the 'Name' field.
- b Enter a comment relating to your experiment in the 'Comment' field.
- c Set up your report style by selecting the appropriate items under 'Options'. For example, select 'Auto Print' to automatically obtain a printout of your report. Select 'Graph' to include graphics in the generated report.

### NOTE

If 'Auto Print' is selected, the system will send the report information to the specified printer as well as the Report area.

---

- d Select the 'Autoconvert' option you require. If you select 'ASCII' or 'ASCII with Log', at the end of the data collection the system will automatically generate a report and store the data in the Cary format as well as ASCII XY pairs format in the current folder.

## 10 Set up storage of collected data

*Setup dialog box | Auto Store page*

Select **Storage Off**.

## 11 Set up visual system monitoring

Select **Show Status Display** on any of the Setup pages, or from the **View** menu to display information about your current reaction.

## 12 Finish setup

Once you are satisfied with your method setup, click **OK** to save any changes made and close the 'Setup' dialog box. The Cary will start to drive the temperature to the start temperature.

## 13 Zero the instrument

- a Click **Zero** or choose **Zero** from the **Commands** menu. A 'Loading Guide' will be displayed.
- b Place a blank solution in the cell that is in the light beam in sample compartment and click **OK**. The system will perform an instrument zero on the blank solution.

## 14 Equilibrate the sample

Make sure that the Cary has reached the start temperature and has been at this temperature for 2 to 3 minutes. If you entered a hold time in the 'Stage 1 Hold' field (Cary page), the system will wait and equilibrate at this temperature for the hold time before proceeding with the ramp. It is not necessary to wait to click the 'Start' button.

## 15 Start the run

- a Click the **Start** button or choose **Start** from the **Commands** menu. The system will display a 'Loading Guide'.
- b Load your sample into the front cell holder.
- c Enter the name of your sample then click **OK**. The Cary will wait for the specified hold time and then perform the run.

## 16 After the run

At the end of the run, depending on what you have set in previous steps, the 'User Data Form' may appear. If it does, fill in the appropriate entries in the columns provided for the sample and click **OK**. The calculation equation will be displayed in the report.

Also, since 'Autocalculate' (under 'Derivative') was selected on the 'Analyze' page of the 'Setup' dialog box, a derivative calculation will be performed at the end of the data collection.

The sample under investigation, with corresponding  $T_m$  values, will be displayed in the Report area as well as parameters used to generate the selected data file, temperature profile, and the method used to contain the  $T_m$  value.

A plot will be displayed in the Graphics area indicating the original temperature data and a plot of the first derivative of the selected scan. The point at which the derivative plot peaks (that is, maximum or minimum gradient on the original plot) is reported as the  $T_m$  value and the original plot is marked with an arrow to indicate this point.

To view a plot of the collected data, choose **Graph** from the **View** menu.

### 17 Save your data

- a From the **File** menu choose **Save Data As**.
- b In the 'Files of Type' field, click **Batch**.
- c Enter the file name for this run in the 'File name' field.
- d Click **Save**.

### Perform a run and determine $T_m$ using hyperchromicity calculations

This procedure describes how to perform a Thermal run with multiple temperature ramps and determine  $T_m$  using a hyperchromicity calculation.

#### 1 Set up data collection parameters

*Setup dialog box*

In Thermal, click the **Setup** button or choose **Setup** from the Menu line to display the 'Setup' dialog box and specify the method parameters for a new method.

#### 2 Set up instrument parameters

*Setup dialog box | Cary page*

- a In the 'Wavelength' field, enter the wavelength that you would like to monitor.
- b In the 'SBW' field, enter the required spectral bandwidth.
- c In the 'Ave. (averaging) Time' field, enter the required value.

#### NOTE

For slow changes in temperature (that is, slow ramp rates), it is recommended that a long Ave. Time (for example, 2 to 3 seconds) be set.

- d Enter the ordinate values in the 'Y min.' and 'Y max.' fields.

### 3 Set up the collect temperature parameters

- a In the 'Start °C' field, enter the temperature at which you want to start the data collection.

#### NOTE

Once you click 'OK' to close the 'Setup' dialog box, the Cary will drive the temperature to the specified Start Temperature.

- b In the 'Return °C' field, enter the temperature to which you want the Temperature Controller to drive at the end of the data collection.
- c Use 'Monitor' to specify whether the temperature will be measured at the block or at the probe.
- d Select **Advanced Collect**.
- e Enter the number of changes in temperature rate that you require in the 'Number of Stages' field.

#### NOTE

An increase or decrease in the temperature must be a separate stage.

- f Enter the 'Data Interval', 'Ramp Rate', 'End Temperature' and 'Hold time'. Select the stages where you want to collect data in the 'Collect Data' column.

### 4 Set up the lamp source operation and the data display options

*Setup dialog box | Options page*

- a Select **Auto Lamps Off** if you want to automatically turn off the lamps at the end of a collect. This is especially useful when performing Thermal data collections overnight or unattended for long periods of time.
- b Click **UV/Vis** if you want both lamps on during the run.
- c Under 'Display Options', select 'Individual Data' to display the collected data of each sample in individual graph boxes. Or select 'Overlay Data' to superimpose the collected data of each sample in the run in one graph box.

## 5 Set up the Multicell Holder accessory

*Setup dialog box | Accessories page*

- a Ensure that you have the Thermostatable 6 x 6 Multicell Holder accessory installed before starting the run.
- b Select **Use Cell Changer** to enable the accessory.
- c Click **Select Cells**, and select the cells you require from the available cells under 'Use Cells'.
- d Select **Multi Zero** to turn on the 'Multi Zero' facility.
- e Clear **Blank Correction**.

## 6 Set up temperature display

Under 'Temperature Display', select **Block** and **Probe 1** to turn on monitoring of the Multicell Holder block and one temperature probe.

## 7 Set up the RBA accessory

- a Under 'RBA', select **Use RBA**.

### NOTE

If you are using a Series I RBA, you must configure it using the Align application before starting any experiments.

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- b Select either 'Attenuation' and enter the Abs value or select 'Position' and enter the angle position.

## 8 Set up the analysis to reduce measurement interference

*Setup dialog box | Analyze page*

Select **Smoothing** and nominate the smoothing 'Interval' and 'Filter Size' to control the number of measurement points to be averaged.

## 9 Set up your method of analysis

- a Select **Hyperchromicity** to calculate the temperature at which 50% of the DNA strands have separated ( $T_m$ ).

### NOTE

The hyperchromicity calculation is performed once the run is completed.

- b Select **Van't Hoff Calculation** to calculate a Van't Hoff plot ( $\ln(K(T))$  vs  $1000/T(\text{deg K})$ ) and activate the 'Hyperchromicity' items.
- c Select 'Self Complementary' if the DNA molecule being analyzed consists of identical strands. Otherwise, select 'Non Self Complementary'.
- d Enter the concentration in the 'Total Conc of Strands' field.
- e Enter the 'Molarity' (that is, the number of strands) of the hybrid. For example, enter '2' if the DNA molecule being analyzed consists of two strands.
- f Select  **$\Delta G$**  to perform  $\Delta G$  (free energy) and K (equilibrium constant) calculations.
- g In the 'Number of Temperatures' field, specify the number of temperatures at which you would like to perform the  $\Delta G$  and K calculations. The number set here is reflected in the 'Temperature' table below this field.
- h Enter the required temperature in each cell of the 'Temperature' table.

## 10 Set up other recalculations and normalizations

*Setup dialog box | Calculations page*

- a Select **Calculation** and enter or select an equation to perform a calculation.
- b Select **Correction** and enter the 'Correction Temperature' to perform a Thermal Expansion Correction.
- c Select **Normalize** and enter the temperature at which you would like the data to be normalized.

## 11 Set up your reporting and printing requirements

*Setup dialog box | Reports page*

- a** Enter your name in the 'Name' field.
- b** Enter a comment relating to your experiment in the 'Comment' field.
- c** Set up your report style by selecting the appropriate items under 'Options'. For example, select 'Auto Print' to automatically obtain a printout of your report. Select 'Graph' to include graphics in the generated report.

### NOTE

If 'Auto Print' is selected, the system will send the report information to the specified printer as well as the Report area..

- d** Select the 'Autoconvert' option you require. If you select 'ASCII' or 'ASCII with Log', at the end of the data collection the system will automatically generate a report and store the data in the Cary format as well as ASCII XY pairs format in the current folder.

## 12 Set up storage of collected data

*Setup dialog box | Auto Store page*

Select **Storage On (Prompt at End)**.

## 13 Set up visual system monitoring

Select **Show Status Display** on any of the Setup pages, or from the **View** menu, to display information about your current reaction.

## 14 Finish setup

Once you are satisfied with your method setup, click **OK** to save any changes made and close the 'Setup' dialog box.

## 15 Zero the instrument

- a** Click **Zero** or choose **Zero** from the **Commands** menu. The system will display a 'Cell Loading Guide'.
- b** Place the blank solutions in the correct positions in the holder then click **OK** to zero the specified cells.

## 16 Equilibrate the samples

Ensure that the start temperature has been reached and held at this temperature for 2 to 3 minutes. If you entered a hold time in the 'Stage 1 Hold' field (on the 'Cary' page), the system will wait and equilibrate at this temperature for the hold time before proceeding with the ramp, and it is not necessary to wait to click the 'Start' button.

## 17 Start the run

Click the **Start** button or choose **Start** from the **Commands** menu to start the Thermal run. The 'Save As' dialog box will be displayed.

## 18 Set up file name and trace names for the data

- a Enter the appropriate name for your run and click **Save**. The system will display a 'Cell Loading Guide'.
- b Place the solutions in the correct cell positions in the holder.
- c Enter the name of each sample in the corresponding field then click **OK**.

The Cary will wait for the specified hold time and then perform the Thermal run.

## 19 After the run

At the end of the run, depending on what you have set in previous steps, the 'User Data Form' may appear. If it does, fill in the columns for each sample trace and click **OK**. The calculation equation will be displayed in the report.

## 20 Hyperchromicity calculations

The Cary WinUV software will now prompt you to use the Thermal ruler to define various limits for the hyperchromicity calculations.

- a A prompt will be displayed asking you to select the Associated DNA limits using the Ruler. Click **OK**. The cursor changes to ruler mode.
- b Position the mouse pointer on the Thermal curve at one end of the linear region of the Associated DNA part of the curve (before transition). Click and drag along the curve and release at the other end of the linear region.

## How To...

The software will automatically calculate a linear least squares line between the selected points and extrapolate to both ends of the melting curve.

- c** An 'Associated DNA Limits' dialog box will be displayed, displaying the upper and lower limits that you selected with the Thermal Ruler. Click **OK** to accept the calculated line.
- d** A prompt will then appear asking you to select the 'Deassociated DNA limits' using the Ruler. Click **OK** on this prompt. The cursor changes to ruler mode.
- e** Position the mouse pointer on the Thermal curve at one end of the linear region of the Deassociated DNA part of the curve (after the transition). Click and drag along the curve and release at the other end of the linear region.

The software will automatically calculate a linear least squares line between the selected points and extrapolate to both ends of the melting curve.

- f** A 'Deassociated DNA Limits' dialog box will be displayed displaying the upper and lower limits that you selected with the ruler. Click **OK** to accept the calculated line.

### NOTE

The Associated and Deassociated DNA Limits dialog boxes allow you to re-select your limits if you are not satisfied with the linear least squares lines drawn in the graph box. To do this, click 'Retry' and select another region. If you wish to exit from the calculation mode, click 'Cancel'. The ruler trace is removed from the graph.

The Linear Region Associated and Deassociated correction points are displayed in the Report area.

### NOTE

If the two extrapolated lines cross at any point, a warning will be displayed. Click 'Cancel' and repeat steps (a) to (f) above and re-select the linear regions. Click 'Ignore' to proceed with the calculation using the regions you have selected.

Using the lines calculated in the previous steps, the software will automatically create a second graph box and display the calculated Alpha curve.

- g** A prompt will be displayed asking you to select the range on the Alpha curve that will be used for the  $T_m$  calculation. Click **OK** to make your selection.
- h** Position the mouse pointer on the alpha curve at one end of the linear region. Click and drag along the curve and release at the other end of the linear region.
- i** An 'Alpha  $T_m$  Limits' dialog box will be displayed displaying the selected limits. Click 'Retry' to reselect the limits, if required. Click **OK** to accept the selected ranges. A linear least squares line is drawn between the selected points and the value of temperature where  $\varphi = 0.5$  is calculated to give the  $T_m$  value. (The  $T_m$  value is automatically reported in °C and K.)

**If you have chosen the Van't Hoff Calculation method to calculate  $\Delta G$  and K:**

- j** Using the data calculated in Step (i) above, the system automatically creates a third graph box displaying a Van't Hoff plot. A prompt will be displayed asking you to select the required range on the Van't Hoff plot to calculate  $\Delta G$  and K. Click **OK**.
- k** Position the mouse pointer on the Van't Hoff curve at one end of the linear region. Click and drag along the curve and release at the other end of the linear region.
- l** A 'Van't Hoff Limits' dialog box will also appear, displaying the upper and lower limits that you selected with the ruler. Click **OK** to accept the calculated line. Alternatively, click 'Retry' to reselect the limits using the ruler.

**NOTE**

If there is an error in the Van't Hoff calculation, you will be prompted to reselect the linear region.

A linear least squares line is calculated between these points and is extrapolated to both ends of the Van't Hoff plot. The slope and intercept of the calculated line gives the enthalpy and entropy of the hybrid formation ( $\Delta H^\circ$  and  $\Delta S^\circ$  respectively).

The software will automatically send the results, using the calculated alpha curve, to the Report area including:

- $K(T_m)$ experimental
- $K(T_m)$ from first principles
- $K(T_m)$ experimental/ $K(T_m)$ from first principles

21 Step 20 will be repeated for each sample trace measured.

### Set up tests for validation

The Validate application enables you to carry out a number of performance tests to verify that the system is performing according to specification. This procedure describes how to set up your instrument to perform a validation test.

#### 1 Set up test parameters

- a** In the Validate application, click the **Tests** menu or the **Tests** button to display the 'Configure' page.
- b** Select the type of instrument performance tests that you require. The various tests that are available will be displayed as tabs across the top of this dialog box.

#### NOTE

If your access privileges are restricted by the GLP application, you may find that fields and buttons in this dialog box are not accessible.

- c** Click each tab and select the tests you require to be performed.
- d** For each test, change any parameters that you require to be changed to suit your laboratory.

**NOTE**

If you wish to save the tests you have set up for future use, choose 'Save Method As' from the 'File' menu to display the 'Save As' dialog box and save the method (with the file name extension '\*.mvo').

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## 2 Set up automated runs

If you are using either the Multicell Holder or the Auto Double Aperture accessory to automate the testing procedure:

- a Click **Setup** to display the 'Setup' dialog box.
- b Click the **Analyze** tab.
- c Select the appropriate accessory.

**NOTE**

Check with your local Agilent office regarding the availability of this accessory.

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## 3 Start the run

- a Click the **Start** button or click **Start** on the **Commands** menu to commence the testing procedure.
- b If you set up for an automated run with the Multicell Holder in the previous step, then the 'Cell Loading Guide' will be displayed. Place the specified solutions and/or filters exactly as shown in this dialog box and click **OK**.

Alternatively, follow the instructions on the prompts that appear and click **OK** to continue with the performance tests.

Click **Cancel** at any time to stop the Validate run.

### NOTE

For manual runs (without the use of automated accessories), the Validate application will automatically arrange the tests so that tests requiring your intervention (for example, placing filters or solutions into the sample compartment) are performed early in the Validate run. Tests that do not require your intervention will be performed later in the run. The system will advise you when you are no longer required. However, if you require, you can change the order of the tests.

---

The results for each test are displayed in the Report area immediately after the test is completed.

#### **4 After the run**

At the end of the Validate run, the Cary system automatically generates a report file. The report file is stored in the Cary WinUV directory.

The Report file will be saved with the following format:

DATE TIME.RVO

For example:

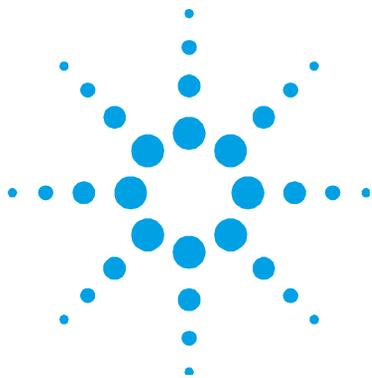
16 Apr 97 3:57:48 PM.RVO

The date and time will be displayed in the same form that is set in the Windows Regional Settings Properties dialog box, accessed from the Windows Control Panel.

### NOTE

At the end of the Validate run, you can also save your data. To do this, choose 'Save Data As' from the 'File' menu to display the 'Save As' dialog box and save the current method, data and report in a batch file (BVO).

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## 5. Troubleshooting

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If you are having problems with your software, check the information in this chapter to see if there is a solution to your problem. You may also find a solution to your problem in the:

- ‘Troubleshooting’ section in the hardware manual supplied with the instrument.
- ‘Troubleshooting’ section of the Help. To view this, click the Windows ‘Start’ button, then ‘(All) Programs’, ‘Cary WinUV’, and ‘Cary Help’. Click ‘Troubleshooting’ and follow the links.
- Late Breaking News documents that were shipped with the Cary WinUV software.

If you still have not found the solution to your problem, contact your local Agilent office or representative.

### Instrument offline

#### Problem

When I start the Cary WinUV software, the application reports that the instrument is 'Offline'.

#### Solutions

- Check the connection of the main instrument cable (IEEE cable) attaching the computer to the instrument.
- Ensure the instrument has completed its initialization tests before you start the Cary WinUV software.
- Contact your local Agilent office.

For more information, refer to Chapter 4 in the Hardware manual provided with the instrument.

### Connect button instead of Start

#### Problem

When I start the Cary WinUV application, I want to use the 'Start' button but it has changed to a 'Connect' button – why?

#### Solution

When you first start the Cary WinUV software, the System Information application has control of the instrument while it initializes. Until the initialization has finished, any other application that you start will not have control of the instrument; hence the 'Connect' button. Wait until the 'Connect' button changes to 'Start'. If it does not change, check to see if you have any other Cary applications running. If they are not collecting data, you can just click the 'Connect' button to get control of the instrument.

## Not enough memory

### Problem

After installing version 3 of the Cary WinUV software, I get an error saying that there is not enough memory and the Cary WinUV software will not run.

### Solution

Check that you have version 6.0 or later of Microsoft Internet Explorer installed. (To check the version, start Internet Explorer and choose 'About Internet Explorer' from the 'Help' menu. The version number will be displayed).

## Poor display of videos and photographs

### Problem

The graphics and video display appears fuzzy, grainy or in weird colors.

### Solution

Increase your display settings to a higher resolution.

### To do this:

- 1 Click the Windows **Start** button then (**Settings**), **Control Panel** and double-click **Display**.
- 2 Click the **Settings** tab and adjust the color quality and screen resolution to a higher setting (that is, 16 bit or 32 bit color), providing your monitor and display adapter allow it.
- 3 Click **OK**.

**NOTE**

You may have to restart your computer for the settings to take effect.

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## GLP log does not list some privilege changes

### Problem

I have changed multiple privileges using the GLP application, but not all of them appear in the log file that the application creates. What has happened?

### Solution

You need to click the 'Apply changes now' button on the 'Privileges' page each time you change privileges for a user or application. You cannot make multiple changes and then click the 'Apply Changes now' button – the changes are actually made but they don't appear in the log.

## Report header and footer not being updated

### Problem

I have changed the report header and footer information in the System Information application, but it is not being updated when I generate a report – why?

### Solution

You need to be logged on as an Administrator to make the changes in System Information.