

**CARY**

# **Cary WinUV**

## **Software manual**



**VARIAN**

85 101625 00  
May 1999

### **Varian offices**

Varian has offices in most countries. The major offices for optical spectroscopy products are listed below:

Varian Australia Pty Ltd (Manufacturing site)  
679 Springvale Road  
Mulgrave, Victoria 3170  
Australia  
International telephone: + 61 3 9560 7133  
International fax: + 61 3 9560 7950

Varian Instruments  
2700 Mitchell Dr  
Walnut Creek, CA 94598  
Telephone: 1 800 926 3000  
International telephone: +1.925.939.2400  
International fax: +1.925.945.2102

Varian Chrompack Benelux Analytical Instruments  
Boerhaaveplein 7, 4624 VT Bergen op Zoom  
Postbus 250, 4600 AG Bergen op Zoom, Netherlands  
International telephone: +31 0164 282800  
International fax: +31 0164 282828

### **Internet**

The Varian Internet home page can be found at:  
<http://www.varianinc.com>

Varian Australia Pty Ltd is the owner of copyright on this document and any associated software. Under law, the written permission of Varian Australia Pty Ltd must be obtained before either the documentation or the software is copied, reproduced, translated or converted to electronic or other machine-readable form, in whole, or in part.

First published October 1997 in Australia. Modified in Jan 1999 and May 1999. Comments about this manual should be directed to the Marketing Communications Manager, Varian Australia at the address above or email [uv@osi.varian.com](mailto:uv@osi.varian.com).

Varian Australia is ISO9001 certified.

© 1997 Varian Australia Pty Ltd (A.C.N. 004 559 540). All rights reserved.  
® Windows 95, 98 and NT are registered trademarks of Microsoft Corp.

# Contents

---

<b>1</b>	<b>Introduction</b>	<b>1-1</b>
1.1	Cary documentation	1-2
<b>2</b>	<b>Software installation</b>	<b>2-1</b>
2.1	PC requirements	2-1
2.1.1	Minimum computer requirements	2-1
2.1.2	Why do I need Internet Explorer 4?	2-2
2.2	Upgrading from version 1	2-3
2.3	Installing the software	2-3
2.3.1	Installing the Cary WinUV software:	2-4
2.3.2	Late breaking news	2-5
2.3.3	Changing the monitor resolution	2-5
2.4	Starting the Cary software	2-6
2.5	Using the online Help	2-6
2.5.1	Using Netscape Navigator instead of Internet Explorer	2-7
<b>3</b>	<b>Software overview</b>	<b>3-1</b>
3.1	Cary applications	3-1
3.1.1	ADL Shell	3-2
3.1.2	Advanced Reads	3-2
3.1.3	Align	3-2
3.1.4	Concentration	3-2
3.1.5	Dissolution (not available for Cary 400/500)	3-3
3.1.6	Enzyme Kinetics	3-3
3.1.7	Fabric Protection	3-4
3.1.8	GLP Administration	3-4
3.1.9	Kinetics	3-4
3.1.10	RNA/DNA	3-5
3.1.11	Scan	3-5
3.1.12	Scanning Kinetics	3-5
3.1.13	Simple Reads	3-6
3.1.14	Sunglasses	3-6
3.1.15	System Information	3-6
3.1.16	Thermal (not available for Cary 50)	3-7
3.1.17	Validate	3-7

3.2	Working with the Cary WinUV software	3-7
3.2.1	Menu line	3-8
3.2.2	Toolbar	3-8
3.2.3	Command buttons	3-8
3.2.4	Dialog boxes	3-9
3.2.5	Controls/settings	3-9
3.2.6	Hint text	3-11
3.2.7	Status line	3-11
3.2.8	File extensions	3-11
3.3	Online Help	3-13
3.3.1	Getting help	3-13
3.3.2	Navigating the Help	3-14
3.3.3	Searching for Help	3-15
3.3.4	Finding key words within a Help topic	3-16
3.3.5	Tracking 'favorite' Help topics	3-16
3.3.6	Printing the Help	3-17
<b>4</b>	<b>How to... 4-1</b>	
4.1	Advanced Reads	4-1
4.1.1	How to manually read samples	4-1
4.2	Concentration	4-5
4.2.1	How to perform a calibration and manually measure concentrations	4-5
4.3	Dissolution	4-10
4.3.1	How to perform a Dissolution run (Cary 100/300)	4-10
4.3.2	How to perform a Dissolution run (Cary 50)	4-16
4.4	Enzyme Kinetics	4-22
4.4.1	How to perform a temperature controlled run using the Multicell Holder (Cary 100–500)	4-22
4.4.2	How to perform a temperature controlled run (Cary 50)	4-29
4.5	Fabric Protection	4-33
4.5.1	How to perform a scan using the Cary 100/300/400/500 (Australian/New Zealand Standard)	4-34
4.6	Kinetics	4-35
4.6.1	How to perform a multi-wavelength, single cell, single rate Kinetics run (Cary 100–500)	4-35
4.6.2	How to perform a temperature controlled Kinetics run using the Multicell Holder (Cary 100–500)	4-40
4.6.3	How to perform a temperature controlled Kinetics run (Cary 50)	4-46
4.7	RNA/DNA	4-50

---

4.7.1 How to perform a temperature controlled run using the Multicell Holder (Cary 100–500)	4-51
4.7.2 How to perform a run using the Multicell Holder (Cary 50)	4-56
4.8 Scan	4-60
4.8.1 How to perform a scan with baseline correction (Cary 100–500)	4-61
4.8.2 How to perform a scan in Signal-to-Noise mode (Cary 100–500)	4-65
4.8.3 How to perform a scan in Independent mode (Cary 500)	4-70
4.8.4 How to perform a scan with baseline correction (Cary 50)	4-75
4.9 Scanning Kinetics	4-79
4.9.1 How to collect data using the Multicell Holder with temperature control (Cary 100–500)	4-79
4.9.2 How to collect data (Cary 50)	4-85
4.10 Simple Reads	4-89
4.10.1 How to perform a measurement at a single wavelength	4-89
4.11 Sunglasses	4-91
4.11.1 How to perform a scan	4-91
4.12 Thermal	4-92
4.12.1 How to perform a run and determine T <sub>m</sub> using Derivative calculations	4-92
4.12.2 How to perform a run and determine T <sub>m</sub> using Hyperchromicity calculations	4-98
<b>5 Troubleshooting</b>	<b>5-1</b>
5.1 Instrument offline	5-1
5.2 Connect button instead of Start	5-2
5.3 Not enough memory	5-2
5.4 Poor display of videos and photographs	5-2
5.5 GLP log does not list some privilege changes	5-3
5.6 Report header and footer not being updated	5-3

*This page is intentionally left blank*

# 1 Introduction

---

The Cary 50/100/300/400/500 instruments run under Varian's easy-to-use Cary WinUV software. Depending on the type of system ordered, the software will consist of some or all of the following applications:

<b>ADL Shell</b>	A pre-defined template for writing ADL programs
<b>Advanced Reads</b>	Used to collect absorbance readings for multiple samples
<b>Align</b>	Used to align various lamps and accessories in the instrument
<b>Concentration</b>	Used for quantitative analysis
<b>Dissolution</b>	Used to perform tablet dissolution measurements (Cary 50/100/300 only)
<b>Enzyme Kinetics</b>	Used to determine various parameters of enzyme activity
<b>Fabric Protection</b>	Used to test solar radiation transmission through unstretched dry textiles
<b>GLP Administration</b>	Used to restrict operator access, password protecting each application
<b>Kinetics</b>	Used to perform kinetics determinations
<b>RNA/DNA</b>	Used to collect absorbance readings of nucleic acids
<b>Scan</b>	Used to run and view data collections
<b>Scanning Kinetics</b>	Used to perform kinetics determinations for a wavelength range
<b>Simple Reads</b>	Used to collect single wavelength absorbance readings
<b>Sunglasses</b>	Used to determine penetration of solar UV radiation through sunglasses and sunglare filters

<b>System Information</b>	Used to enter system information, such as company and instrument details
<b>Thermal</b>	Used to perform Thermal DNA analysis (Cary 100/300/400/500 only)
<b>Validate</b>	Used to verify the system's performance. These applications are discussed further in chapter 3.

- The Cary WinUV software runs under the Microsoft Windows® 95, Windows 98 or Windows NT operating systems.

## 1.1 Cary documentation

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

- Cary hardware manual, with Safety practices and hazards information, instructions for installing and maintaining various hardware components, and hardware-related troubleshooting information
- This software manual, with instructions for installing the Cary WinUV software, an overview of the software and software-related troubleshooting information
- Extensive online Help (provided with the Cary WinUV software) containing context-sensitive Help, step-by-step instructions for frequently performed analyses and instructions for using various accessories. Refer to section 3.3 for details on using the online Help.

### Conventions

The following conventions have been used throughout this manual:

- Italics* indicate menu items, menu options and field names (e.g. select *Copy* from the *Edit* menu).
- Keyboard and mouse commands have been typed in **bold** (e.g. press the **F1** key).
- Double quotes (" ") are used to signify the buttons appearing throughout the software (e.g. select "OK").
- ALL CAPITALS indicate text you must type in from the keyboard (e.g. type SETUP at the prompt).

## 2 Software installation

---

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

### 2.1 PC requirements

#### 2.1.1 Minimum computer requirements

The minimum configuration represents the absolute minimum you can run the software on. This PC configuration may be out of manufacture, but you may want use a PC you already have. The recommended configuration is that which you would buy new.

Minimum	Recommended
IBM compatible	IBM compatible
Intel Pentium processor	Intel Pentium II processor
16 M RAM	64 M RAM
150 MB free space on hard disk	500 MB free space on hard disk
Video card supporting 800 x 600 resolution, high color (16 bit) mode	Video card supporting 800 x 600 resolution, high color (16 bit) mode
Super VGA screen	Super VGA screen
4 x CDROM drive	10 x CDROM drive
16 bit sound card	16 bit sound card
Windows 101 key keyboard	Windows 101 key keyboard

Continued...

Minimum	Recommended
Microsoft or compatible mouse	Microsoft or compatible mouse
One spare AT bus 16 bit ISA expansion slot	One spare AT bus 16 bit ISA expansion slot
Microsoft Windows 95®, Windows 98® or Windows NT® (including service pack 4 or later)	Microsoft Windows 95®, Windows 98® or Windows NT® (including service pack 4 or later)
Microsoft Internet Explorer*4 or later if using Windows 95 or NT	Microsoft Internet Explorer *4 or later if using Windows 95 or NT
<b>Cary 50 only</b>	
1 Spare PC power supply connector	1 Spare PC power supply connector
Power Supply rating 220 Watts.	Power Supply rating 220 Watts.
1 Spare Slot for the accessory cable connector	1 Spare Slot for the accessory cable connector

\*The Cary WinUV software uses functionality provided by Microsoft's Internet Explorer 4.0 (IE4). You do not need to use IE4 as your web browser. If your company's rules prevent the installation of IE4 you can use another browser, with some loss in functionality (Refer to section 2.5.).

### 2.1.2 Why do I need Internet Explorer 4?

In order to use all the features of the online Help, you should have Microsoft Internet Explorer 4.0 installed on your PC. The unique features include:

- an expandable hierarchy of all Help contents;
- a search facility to display a list of topics containing specific key words;
- the ability to find words within a displayed topic;
- an editable 'Favorites' tab to list those Help pages that you would like to easily redisplay;
- instructional videos to visually demonstrate selected procedures.

You do not need to be connected to the Internet and you do not need to use IE4 as your browser. The Cary WinUV instrument software also uses some of the functionality provided by IE4.

You can download a free copy of IE4 from Microsoft's website at [www.microsoft.com](http://www.microsoft.com). Make sure that you download the correct language version for your version of Windows. If you are not connected to the Internet you can purchase IE4 on CDROM from your local software supplier.

Refer to section 3.3 for more information on using the special features of the online Help.

## 2.2 Upgrading from version 1

If you have received an upgrade package then you can follow the instructions in section 2.3 to install the software. Note the following points:

- Install version 2 of the WinUV software into the same directory as you installed version 1 to replace version 1 of the software. Your data files will be kept as they are and can be used with version 2.
- The upgrade packages include three Help & Videos CDs. Select the one appropriate for your instrument. You can discard the other two if you wish.

## 2.3 Installing the software

Use the following checklist to install the Cary WinUV version 2 software.

A Windows operating system is installed and all devices e.g. sound card, CDROM are working.	Refer to the documentation supplied with your Windows software.
Internet Explorer 4 or later has been installed.	Refer to the documentation supplied with Internet Explorer.
You have set your PC screen desktop area to at least 800 x 600 and have set the color palette to at least High color.	Refer to section 2.3.3
You have installed the instrument interface card in the PC and connected the PC to the Cary instrument	Refer to the Hardware manual supplied with the instrument.

Continued...

You have logged on as an Administrator if you are running Windows NT.	Refer to the documentation supplied with NT.
You have installed the Cary software and the Help (2 <sup>nd</sup> CD).	Refer to 2.3.1
You have re-booted the PC.	Refer to your Windows documentation.



Note The Cary WinUV software will read Cary OS/2 and DOS data files, if you have any.

### 2.3.1 Installing the Cary WinUV software:

1. Turn on your PC.



Note If you are running Windows NT, logon as an Administrator or as a user with Administrator privileges.

2. Insert the Cary WinUV CD into the CD-ROM drive.
3. Select the "Install Cary Software" button in the window that appears.
4. Follow the instructions appearing on the screen. During the installation you will be asked to specify, amongst other things, the drive on which to install the software.



Note If you are installing a Solascreen package you will be asked if you want to copy videos for the Fabric and Sunglasses wizards to your hard disk. If you want to use either of these programs in their simplest mode the majority of the time then select 'Yes'. You will also be asked what type of interface and which Standards you want to install for the Fabric and Sunglasses applications. If you do not know what to select here then choose the Advanced interface and All Standards. You can change this later by referring to the online Help (search for fpchoice.exe if you are using the Fabric Protection application or sgchoice.exe for the Sunglass application).

When the installation is complete you should have a Cary Program group on the desktop containing a number of icons. The installation also places a Cary WinUV folder in the *Start/Programs* menu.



Note If you are running Windows NT and did not have Administrator privileges, the installation will fail. To successfully complete the installation, re-logon as an Administrator start the installation process again.

5. Remove the Cary WinUV CD from the CD-ROM drive and insert the Cary Help and Videos CD, as prompted.



Note You will need to insert the Cary Help and Videos CD into the CD-ROM drive each time you want to view videos included in the Help.

6. Follow the instructions appearing on the screen. You will be prompted to insert the Patches CDROM after the Help installation. You will need to restart Windows after the installation is finished. You can leave the Help & Videos CD in the drive if you like.



Note Patches are files designed to fix known software problems. When you insert the Patches CDROM it will autostart and you can then follow the instructions on the screen to install the patches.



Note If it has never been run before, the System Information application will appear maximized when Windows is restarted, prompting you to enter such information as the company details you would like to appear in reports and hint text properties. Otherwise, it will be minimized on the Windows taskbar.

### 2.3.2 Late breaking news

Before proceeding further you should read the Late breaking news documents supplied with the software. The documents contain the latest release information and important notes.

### 2.3.3 Changing the monitor resolution

For optimum viewing, your monitor resolution should be set to 800 x 600 pixels (or greater) and High colour (16 bit) (or greater).

To set the monitor resolution:

1. Click on the Windows "Start" button.
2. Point to *Settings* and select *Control Panel* from the flyout menu.
3. Double click on the *Display* icon.

4. From the Display Properties dialog, select the *Settings* tab.
5. In the *Desktop area* section, drag the sliding bar so that the pixel amount is set to "800 by 600 pixels".
6. In the Color palette section select High color (16 bit).
7. Select "OK". At the prompt, which informs you that your settings will be adjusted, select "OK" again. When asked if you wish to keep the setting, select "Yes".
8. You can then exit the Control Panel.

## 2.4 Starting the Cary software

To start the Cary WinUV software:

1. Click on the Windows "Start" button.
2. Point to *Programs* and select *Cary WinUV* from the flyout menu.
3. Select the desired application from the second flyout menu.

After the initial Cary flash screen appears, the selected application will open (refer to Chapter 3 for more information on the available applications).

## 2.5 Using the online Help

Your Cary software comes equipped with a comprehensive online Help system. The Help features explanations of each application including detailed step-by-step instructions on using the software. The Help also includes 'Tips and Tricks', 'Troubleshooting' and 'Contacts' sections in case you encounter difficulties using the software.

You can access the Help in a number of ways. You can:

- ❑ select the first item from the *Help* menu in an application or information about using that particular application.
- ❑ Select Help topics from the Help menu in an application to get to the Home page of the Help.
- ❑ Press the **F1** key for information about a dialog.
- ❑ Select Start, Programs, Cary WinUV, Cary Help to get to the Home page of the online Help.

## 2.5.1 Using Netscape Navigator instead of Internet Explorer

If, for some reason, you cannot load Internet Explorer, you can use Netscape Navigator as an alternative. The limitation with this is that Netscape does not support the unique online Help features outlined in section 2.1.2. Instead, you can only view the raw HTML files supplied on the Cary Help and Videos CD with little navigational ability.

If you install Internet Explorer to use the Help but would like Netscape to be your default browser, just select Default Browser On, when prompted.

In the later versions of Communicator, you will not be prompted to select Netscape as your default browser—you must re-enable this check. To do so, you must look in the directory:

C:\Program Files\Netscape\Users\XXX

at the prefs.js file for the user.

The relevant configuration is:

user\_pref("browser.wfe.ignore\_def\_check", VALUE);

Valid VALUES are: True (never check) and False (always check).

The configuration to determine if Internet Explorer checks to see if it should be the default browser is located in the Registry:

\HKEY\_CURRENT\_USER\SOFTWARE\Microsoft\Internet Explorer>Main\Check Associations

Valid values are "NO" (never check) and "Yes" (always check).

*This page is intentionally left blank*

# 3 Software overview

---

This chapter provides a brief introduction to the Cary WinUV software to help you familiarize yourself with its use. For more detailed information refer to the extensive online Help (see section 3.3 for instructions on using the Help).

## 3.1 Cary applications

The Cary WinUV software is made up of several different applications, depending on the Cary package ordered:

Package	Applications
SCAN	ADL Shell, Align, Simple Reads, GLP Administration, Validate, System Information, Scan with Maths and Advanced Reads
CONC	ADL Shell, Align, Simple Reads, GLP Administration, Validate, System Information, Scan with Maths plus Concentration and Advanced Reads
BIO	ADL Shell, Align, Simple Reads, GLP Administration, Validate, System Information, Scan with Maths, Concentration, Thermal*, Kinetics, Scanning Kinetics, Enzyme Kinetics, RNA/DNA and Advanced Reads
TABLET <sup>#</sup>	ADL Shell, Align, Simple Reads, GLP Administration, Validate, System Information, Scan with Maths, Concentration plus Dissolution and Advanced Reads
SOLASCREEN <sup>#</sup>	ADL Shell, Align, Simple Reads, GLP Administration, Validate, System Information, Scan, Maths, Advanced Reads, Fabric Protection and Sunglasses

*<sup>#</sup>Not available for the Cary 400 or 500*

*\* Not available for the Cary 50*

### **3.1.1 ADL Shell**

The Applications Development Language (ADL) is a built-in spectroscopy language supplied with the 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.

Refer to the online Help (see section 3.3) for a description of the settings in the ADL Shell application.

### **3.1.2 Advanced Reads**

The Advanced Reads application can be used to read multiple samples in a single run. You can use various accessories to take multiple sample solution and aliquot readings, in absorbance, percent transmittance, Abs<sup>\*</sup>F or percent reflectance mode, and find the mean.

Refer to the online Help (see section 3.3) for a description of the settings in the Advanced Reads application.

### **3.1.3 Align**

The Align application is used to align the lamp(s) in the instrument and various optical accessories such as the Fibre Optic Coupler. 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.

Starting the Align application brings up the Lamps tab of the Alignment window, which contains a Current Signal bar representing the energy of the lamp(s). To change the instrument parameters, select the Cary tab.

Refer to the online Help (see section 3.3) for a description of the settings in the Align application.

### **3.1.4 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 selected, 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.

Starting this application brings up the Concentration window. To set the instrument parameters, define the standards to be used and configure sampler accessories, select *Setup* from the menu. This will bring up the Setup window.

Refer to the online Help (see section 3.3) for a description of the settings in the Concentration application.

### **3.1.5 Dissolution (not available for Cary 400/500)**

The Dissolution application is used to monitor tablet dissolution, using up to 2 dissolution baths. The application enables you to collect data for up to 10 000 minutes (8 days) at defined time intervals.

The data can be displayed as absorbance, percent dissolved versus time or milligrams dissolved versus time. You can then calculate the time taken to reach a series of nominated percent dissolved values and/or calculate the percent dissolution of the tablets at given times.

Starting this application brings up the Dissolution window. To set the instrument and bath parameters, standards and test limits, click on the “Setup” button or select *Setup* from the menu.

Refer to the online Help (see section 3.3) for a description of the settings in the Dissolution application.

### **3.1.6 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, 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  $K_m$ .

Refer to the online Help (see section 3.3) for a description of the settings in the Enzyme Kinetics application.

### **3.1.7 Fabric Protection**

The Cary Fabric Protection Measurement system is designed to test the transmission of solar radiation (UVR) in the range of 280–400 nm (UVR) through unstretched dry textiles. The results obtained indicate the level of protection offered by fabric when worn in close proximity to the skin. Fabric that is not worn in close proximity to the skin may provide less UVR protection than indicated by the test due to UVR back scattering.

Refer to the online Help (see section 3.3) for a description of the settings in the Fabric Protection application.

### **3.1.8 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.

Refer to the online Help (see section 3.3) for a description of the settings in the GLP Administration application.

### **3.1.9 Kinetics**

The Kinetics application is used to obtain absorbance versus time data to enable you to determine the rate of reaction.

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.

Refer to the online Help (see section 3.3) for a description of the settings in the Kinetics application.

### 3.1.10 RNA/DNA

The RNA-DNA 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 Warbug and Christian coefficients; and,
- A(260) \* F.

Refer to the online Help (see section 3.3) for a description of the settings in the RNA/DNA application.

### 3.1.11 Scan

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

To configure the instrument and accessories, measurement mode, report settings and storage options, click on the “Setup” button or select *Setup* from the menu.

Refer to the online Help (see section 3.3) for a description of the settings in the Scan application.

### 3.1.12 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.

Refer to the online Help (see section 3.3) for a description of the settings in the Scanning Kinetics application.

### **3.1.13 Simple Reads**

The Simple Reads application is used to perform quick absorbance readings of samples at single wavelengths.

To configure the settings for the absorbance reading, click on the “Setup” button or select *Setup* from the Menu line. Then all you have to do is select the “Zero” button to zero the instrument, then select “Read” to take a reading.

Refer to the online Help (see section 3.3) for a description of the settings in the Simple Reads application.

### **3.1.14 Sunglasses**

The Cary Solascreen system is designed to test and determine the penetration of solar ultraviolet radiation in the range of 280 nm to 400 nm (UVR) through sunglasses and sunglare filters. It tests non-prescription general-use lenses not designed to attenuate solar radiation according to the Australian/New Zealand, British or American Fabric Standards.

Refer to the online Help (see section 3.3) for a description of the settings in the Sunglasses application.

### **3.1.15 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 (e.g. your company logo).

System Information 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.

Refer to the online Help (see section 3.3) for a description of the settings in the System Information application.

### 3.1.16 Thermal (not available for Cary 50)

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

Refer to the online Help (see section 3.3) for a description of the settings in the Thermal application.

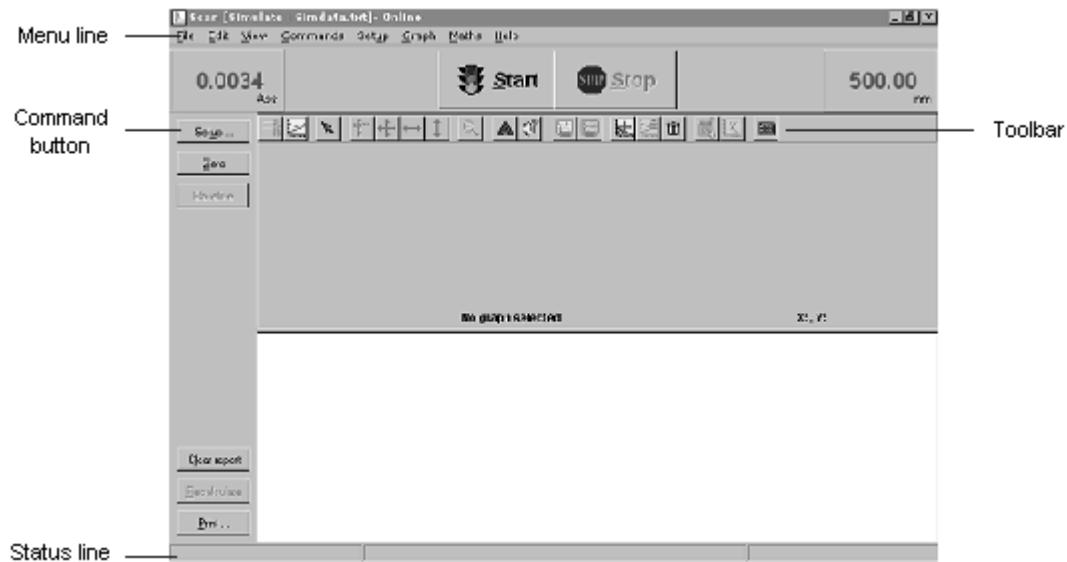
### 3.1.17 Validate

The Validate application enables you to carry out a number of performance tests to verify that the system is performing according to specification. Refer to the online Help (see section 3.3) for more information.

An optional Validation package is also available from Varian, 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.

## 3.2 Working with the Cary WinUV software

This section describes the various components of the software, and how to use them. A typical screen is as follows:



### 3.2.1 Menu line

Each application contains a Menu line directly beneath the window title bar. This bar displays a number of commands, e.g. *File*, *Edit*, *View* and *Help*.

Commands can be accessed by clicking on the desired item with the mouse or pressing **Alt** and the active (underlined) letter in the menu name (e.g. **Alt+E** to access the *Edit* menu). This will display a menu list. The options in the menu list can be accessed in the same manner. For a description of the various menu items and options refer to the online Help.



- Note When menu items or options appear grayed, they are unavailable for selection.

### 3.2.2 Toolbar

In some applications, such as Concentration and Scan, a Toolbar appears directly under the Menu line, containing a number of buttons which provide shortcuts to various functions in the software. To select a toolbar button, click on it with the mouse.



- Note The Toolbar may not be visible. To display the Toolbar, select *Toolbar* from the *View* menu. Conversely, if you wish to maximize your viewing area you can hide the Toolbar by selecting *Toolbar* from the *View* menu. (The options in the Toolbar are also available from the menu.)

To find out what a particular button does, hold the cursor over the button for a short time. After a brief delay, a Hint will appear showing what the button is used for.

### 3.2.3 Command buttons



Command buttons are used to carry out the action specified on the button or to access a related dialog.

Some applications, such as Concentration and Scan, have a group of buttons present on the left-hand side of the application window. These buttons provide shortcuts to various functions such as instrument setup and printing.



**Note** The Command buttons may not be visible. To display the buttons, select *Buttons* from the *View* menu. Conversely, if you wish to maximize your viewing area you can hide the buttons by selecting *Buttons* from the *View* menu. (The Command button options are also available from the menu.)

To select a command button, click on it with the mouse, or press the **Tab** key to move the focus to the button and press **Enter**.

### 3.2.4 Dialog boxes

In some cases, selecting a menu option or pressing a command button will activate a dialog box. This is a box that contains a number of different input fields relevant to that operation.

You can move the focus from field to field in a dialog box either by clicking on each entry item with the mouse or by pressing the **Tab** and **Arrow** keys to move the cursor from field to field. After entering/changing any values in a dialog box, press "OK" to close the dialog and save the changes.

### 3.2.5 Controls/settings

There are a number of different field types used in the WinUV software. This section describes the fields appearing throughout the software and how to enter values in these fields.



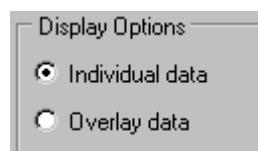
**Note** Fields that are unavailable for selection are said to be disabled and appear grayed.

#### Entry fields

SBW (nm)	1.500
Replicates	1

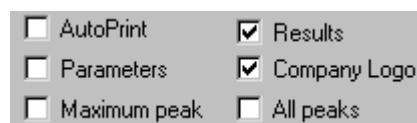
Entry fields accept text or numeric input. To make an entry, move the focus to the appropriate field, type in the required value, then move the focus elsewhere to accept the entry. You can modify numeric entry fields by pressing the **up arrow** or **down arrow** keys to increment or decrement the value by a predefined value.

### Radio buttons



Radio buttons are small, round buttons that are used to make a selection from a series of mutually exclusive items. You can select a radio button with the mouse by clicking on the button or the text beside it. Alternatively, you can move the focus to the group of buttons then select the desired option using the **arrow keys**. Selecting an option will deactivate all other options in the group as only one option may be selected at a time.

### Checkboxes



Checkboxes are small squares used to turn an option on or off. A checkbox item is selected when a tick (✓) appears in the checkbox. Within a group, any number of checkboxes may be selected.

To select or deselect a checkbox, simply click on it or the text beside it with the mouse. Alternatively, move the focus to the group of checkboxes, use the **arrow keys** to position the focus on the desired option and press the **spacebar** to select or deselect it.

### Drop down list



A drop down list is a selection field with an arrow icon to the right of it. Clicking on the arrow icon with the mouse, or pressing **F2** when the field has the focus, displays the list of options available for selection.

To make a selection from the list click on it once, or use the arrow keys to move the highlight bar to the desired option and press **F2** again to accept the selection.

### Table controls

	Rack	Tube
Standards	1	2
Samples	2	1
Blank	1	1
Rinse	0	0

Table controls (or multi-column controls) look like a table or spreadsheet. They contain cells that act as entry fields, distinguished by a white background. To edit the cell contents, double-click on the cell or move the focus to the field and press **F2**.

### 3.2.6 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 appear describing what the control or field does. If you hold the pointer over an entry field, the valid range for that field will appear.

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



- Note** You can alter the properties of the hints, such as the length of the delay before hints appear, from the Hints tab in the System Information application.

### 3.2.7 Status line

The Status line displays the current instrument status. Refer to the online Help for more information (see section 3.3).

### 3.2.8 File extensions

The various files, such as method files, report files and graphic files, are saved with a three letter extension that is representative of the type of file, and the application that created the file.

The following is used to represent the various file types:

File Type	Extension
Methods	.M**
Data	.D**
Report	.R**
Graph Template	.G**
Batch	.B**

The following is used to represent the various applications:

Application	Extension
Advanced Reads	.*AB
Concentration	.*CN
Dissolution <sup>#</sup>	.*TD
Enzyme Kinetics	.*EK
Fabric Protection	.*FP
Kinetics	.*KN
RNA/DNA	.*DN
Scan	.*SW
Scanning Kinetics	.*SK
Simple Reads	.*SR
Sunglasses	.*SG
Thermal <sup>†</sup>	.*TM
Validate	.*VO

<sup>#</sup>Not for Cary 400/500

<sup>†</sup>Not for Cary 50

For example, a method file from the Concentration application would have the file extension '.MCN'.



**Note** To allow you to identify the different file types in Windows Explorer, you need to disable the Windows option which hides filename extensions. To do this select *Options* from the *View* menu in Windows Explorer and deselect the 'Hide MS-DOS file extensions for file types that are registered' option on the *View* tab.

### 3.3 Online Help

The Cary WinUV software includes extensive online Help, which serves as your primary source of information on how to use your Cary system. It contains descriptions of the various windows, dialogs and fields that make up the software, as well as step-by-step instructions to help you perform various tasks.

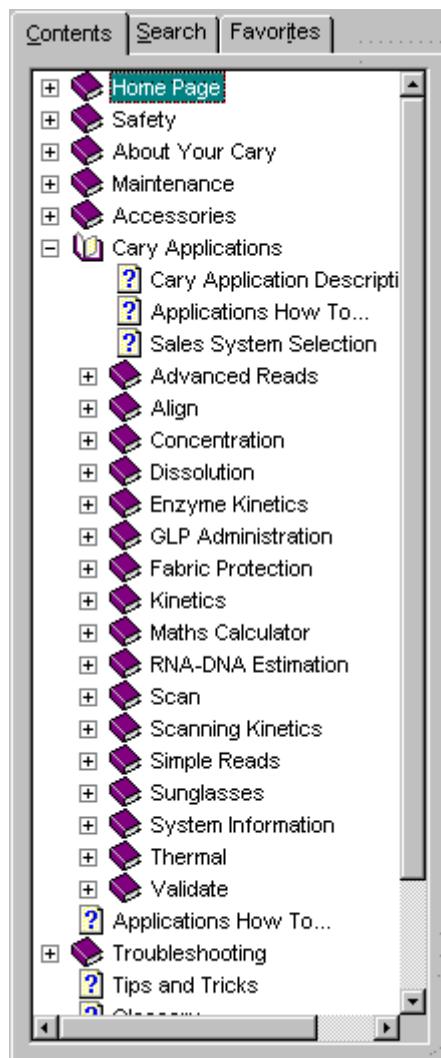
#### 3.3.1 Getting help

Since the help system is so extensive, it is advisable to familiarize yourself with the contents of the online Help by viewing the Cary Home page. To do this, select *Help Topics* from the *Help* menu in any application. The Cary Home page will appear in the browser.

To access the online Help for a particular application, start the application and select application *Help* (e.g. *Scan Help*) from the *Help* menu (or press the **F1** button). This will open the Help specific to the selected application in the browser.

### 3.3.2 Navigating the Help

You can move around the Help using the Contents window (pictured below).



Note To display the Contents window, press the "Show" button in the Browser menu.

The Contents list is manipulated by clicking on the icons and the associated text:

- ❑ To expand/contract a section in the Contents list, double-click on the ‘bookcase’ or ‘folder’ icon for that section, or click on the +/- sign next to the icon.

- ❑ To display a topic, click on the text associated with that topic.

The topics in the Help are often cross-referenced with other related topics. A cross-reference appears as text with a solid underline (blue by default). To jump to the related topic, click on the underlined text with the mouse. To return to the previous topic, click on the “Back” button in the Browser toolbar.

### **3.3.3 Searching for Help**

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

To search for information on a particular subject:

1. Select the Search tab.
2. Enter the word(s) you want to search for and press the “List Topics” button or click **Return** on the keyboard to list all the relevant documents containing the search word.  
The number of topics found will be displayed. You can select to 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 clicking on it with the mouse then pressing the “Display” button. Alternatively, double click on the topic. All occurrences of the key word will appear highlighted.
4. If this does not provide the information you require, enter a more specific word or additional words in the keywords field and try again. You can enter the words AND, OR, NEAR and NOT inbetween your key words. These are accessible by clicking  to the right of the keywords field.

Another way to narrow your search is to use the checkboxes 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.

### 3.3.4 Finding key words within a Help topic

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 topic:

1. Click anywhere on the Help page.
2. On the keyboard, hold down the **Ctrl** key and press **F**. The Find dialog will appear.
3. Enter the word you wish to look for in the *Find what:* field.
4. If you like, limit the Find using the checkboxes. eg. Select 'Match case' to ignore key words with upper and lower cases different to that entered.
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.
6. Press the "Find Next" button or press the **Enter** key to begin the Find. The first occurrence of the designated word will be highlighted.
7. Press the "Find Next" button or **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.

### 3.3.5 Tracking 'favorite' Help 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, follow these steps:

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

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

If a topic is no longer required in the Favorites list, highlight it then press the “Remove” button.

### **3.3.6 Printing the Help**

You can print the current Help topic for a particular application.

To obtain a printed copy of the current Help topic accessed from the ‘Search’ or ‘Favorites’ facility:

1. Press the “Print” button at the top of the Help window or click anywhere in the Help window, hold down the **Ctrl** key on your keyboard and press **P**. This will open the Print dialog.
2. Press the “OK” button.

The topic will be printed on the default printer.

If you have accessed the topic you would like to print by clicking on it in the ‘Contents’ listing, you may still access the Print dialog by clicking anywhere in the Help window, holding down the **Ctrl** key on your keyboard and pressing **P**.

However, pressing the “Print” button will bring up the Print Topics dialog. This gives you the option of printing the selected topic only or the selected heading and all subtopics. Once you have made your selection and pressed the “OK” button, then the Print dialog will appear. Press “OK” here to print your selection on the default printer.

To change the default printer:

1. Select the appropriate printer from the Name drop down list in the Printer group of the Print dialog.
2. Click “Properties” to display the printer Properties dialog. Change any settings, if required, then click “OK” to exit the Properties dialog.
3. Click “OK” to close the Print dialog.

For more information on changing the printer options, hold the cursor over the area of interest in the Print dialog and press the **F1** key.

*This page is intentionally left blank*

# 4 How to...

---

This chapter provides step-by-step instructions on how to perform operating procedures using your Cary instrument and various software applications.

For more detailed information on the software applications or your Cary instrument, refer to the extensive online Help (see section 3.3 for instructions on using the Help).

Step-by-step instructions for common measurements are provided for the following Cary software applications:

- |  |  |
|--|--|
| <input type="checkbox"/> Advanced Reads    | <input type="checkbox"/> RNA/DNA           |
| <input type="checkbox"/> Concentration     | <input type="checkbox"/> Scan              |
| <input type="checkbox"/> Dissolution       | <input type="checkbox"/> Scanning Kinetics |
| <input type="checkbox"/> Enzyme Kinetics   | <input type="checkbox"/> Simple Reads      |
| <input type="checkbox"/> Fabric Protection | <input type="checkbox"/> Sunglasses        |
| <input type="checkbox"/> Kinetics          | <input type="checkbox"/> Thermal           |

## 4.1 Advanced Reads

### 4.1.1 How to manually read samples

This demonstrates how to read samples using no accessories.

#### Start the Advanced Reads application

1. On the Windows Taskbar, click “Start” to display the *Start* menu.
2. Move the cursor over the *Programs* menu item to display the *Programs* menu.
3. Select *Advanced Reads* in the *Cary WinUV* menu.
4. Select your instrument type if necessary and press “OK” to open the Advanced Reads application.  
(Alternatively, you can select the Advanced Reads icon in the Cary WinUV folder on the desktop.)



Note If you are running a GLP system, you may be prompted to enter a password before accessing the Advanced Reads application.

#### Set up data collection parameters

##### 5. Setup dialog

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

To do this:

#### Set up instrument parameters such as wavelength, SBW, Y mode etc.

##### 6. Setup dialog / Cary tab

- (a) In the *Wavelength* field enter the wavelength that you want monitored.
- (b) In the *Ave Time* field enter the required value. A good starting point is 0.1 sec.
- (c) If you are not using a Cary 50, enter the required spectral bandwidth in the *SBW* field. Use the maximum setting unless your method specifies another value.



Note If using a microcell a smaller SBW should be selected.

- (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 same solution or '3' for triplicate aliquots.
- (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*.

#### Set up lamp options

##### 7. Setup dialog / Options tab

If you are using a Cary 50, proceed to step 8.

- (a) Select the Options tab.
- (b) Check the 'Auto lamps off' checkbox if you want to automatically turn off the lamps at the end of the collect. This option enables you to preserve the lifetime of the lamps. It is especially useful for when performing reads overnight or unattended for long periods of time.
- (c) Click on the UV/Vis radio button if you want both the lamps on during sample reading.

- (d) Enter the wavelength at which you would like the source lamp to change from the UV to the Vis/NIR lamp. The recommended changeover is 350 nm for lamps with a UV cutoff. If you are using a Cary 500, enter the detector changeover wavelength as well.
- (e) In the Beam Mode group, select the beam mode that you require. In most cases this should be 'Double Beam' and 'Normal'.

#### Set up accessories

##### 8. *Setup dialog / Accessories tabs*

Select the Accessories 1 and Accessories 2 tabs and make sure that no accessories are selected.

#### Set up reporting and printing requirements

##### 9. *Setup dialog / Reports tab*

- (a) Select the Reports tab and specify your reporting requirements for this method.
- (b) Enter your name in the *Name* entry field.
- (c) Enter a comment relating to your experiment in the *Comment* entry field.
- (d) Set up your report style by selecting the appropriate checkboxes in the Options group.

For example, select the AutoPrint checkbox to obtain a printout of your report automatically.

Select the Parameters checkbox to include your experimental parameters in the report.



**Note** If AutoPrint is selected, then 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.

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

#### Set up your samples and increment names

##### 10. *Setup dialog / Samples tab*

- (a) Select the Samples tab. This dialog box allows you to enter a list of sample names that will be used during your analysis.

- (b) Enter the number of samples that you are going to use in the *Number of Samples* field. The table below this field will expand or contract to match your choice.
- (c) In the Samples table (in the Sample Names group), enter the name of each sample. You can enter up to 20 characters for each name.
- (d) If the samples have the same name with a different numeric extension, enter the name in the first sample position and then press the “Increment” button.

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

11. *Setup dialog / Auto Store tab*
  - (a) Select the Auto Store tab.
  - (b) 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.

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

12. Select the Show Status Display checkbox on any of the tabs to display various information fields on your current reaction.

#### **Finish setup**

13. Once you are satisfied with your method setup click “OK” to confirm any changes you have made and close the Setup dialog.

#### **Zero the instrument**

14. To zero the instrument:
  - (a) Click the “Zero” button. Alternatively, select *Zero* in the *Commands* menu to perform a zero. The Zero dialog box will appear.
  - (b) Place a blank in the sample compartment and press “OK”.

#### **Read samples**

15. To read samples:
  - (a) Click the “Start” button. The Sample Selection dialog box will appear.
  - (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. Press “OK” to read the sample. (If replicates have been nominated, then the result is reported after the final sample replicate is read.)
  - (d) Repeat for the remaining samples.

**Save your data**

16. To save your data:
  - (a) Click on the *File* menu item and select *Save Data As*.
  - (b) Enter the file name for this read in the *File name* field.
  - (c) Click “Save”. The data will be stored as a Batch file.

## 4.2 Concentration

### 4.2.1 How to perform a calibration and manually measure concentrations

This demonstrates how to perform a multi-standard calibration and measure sample concentrations using no accessories.

#### Start the Concentration application

1. On the Windows Taskbar, click “Start” to display the *Start* menu.
2. Move the cursor over the *Programs* menu item to display the *Programs* menu.
3. Select *Concentration* in the *Cary WinUV* menu.
4. Select your instrument type if necessary and press “OK” to open the Concentration application.  
(Alternatively, you can select the Concentration icon in the Cary WinUV folder on the desktop.)



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

#### Set up data collection parameters

5. *Setup dialog*  
Click the “Setup...” button or select *Setup* from the Menu line to display the Setup dialog and specify the method parameters for a new method.

To do this:

#### Set up instrument parameters such as wavelength, spectral bandwidth, Y mode etc.

6. *Setup dialog / Cary tab*
  - (a) In the *Wavelength* field enter the wavelength that you want monitored.
  - (b) In the *Ave Time* field enter the required value. A good starting point is 0.1 sec.

- (c) Unless you are using a Cary 50, enter the required spectral bandwidth in the *SBW* field. Use the maximum setting unless your method specifies another value.



Note If using a microcell a smaller SBW should be selected.

- (d) Select 'Replicates' (if you require them) and set the value to the number of replicates that you are using.
- (e) Unless you are using a Cary 50, select the Y mode you require. Click "Abs" to specify the 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* entry fields to specify the displayed ordinate range. Note that these are starting values only. The WinUV software will automatically rescale the calibration graph as the standards are measured.

### Set up the calibration

#### 7. *Setup dialog / Standards tab*

- (a) Select 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 pressed.
- (c) Set the appropriate Units for your standards for reporting purposes.



Note If you are performing a calibration for use with the Dissolution application, then the Units specified must be mg/mL or g/L.

- (d) Set the *Standards* field to the number of standards that you are using in your calibration. The table below this field will expand or contract to match your choice.
- (e) In the Standards table, enter the concentration of each standard into the 'Conc' column.
- (f) Select the type of curve fitting required for your calibration in the 'Fit Type' group.
- (g) Enter the required  $R^2$  value or correlation coefficient into the *Min R<sup>2</sup>* field. The closer the number is to 1.000 the better the fit. Typically, 0.95 is used.

### Set up lamp options

#### 8. *Setup dialog / Options tab*

If you are using a Cary 50, proceed to step 9.

- (a) Select the Options tab.

- (b) Check the ‘Auto Lamps Off’ checkbox if you want to automatically turn off the lamps at the end of the collect. This option enables you to preserve the lifetime of the lamps. It is especially useful for when performing Concentration runs overnight or unattended for long periods of time.
- (c) Click on the “UV/Vis” button if you want both the lamps on during the run.
- (d) Enter the wavelength at which you would like the source lamp to change from the UV to the Vis/NIR lamp. The recommended changeover is 350 nm for lamps with a UV cutoff. If you are using a Cary 500, enter the detector changeover wavelength as well.
- (e) In the Beam Mode group, select the beam mode that you require. In most cases this should be ‘Double Beam’ and “Normal”.

#### Set up accessories

##### 9. *Setup dialog / Accessories tab*

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

#### Set up reporting and printing requirements

##### 10. *Setup dialog / Reports tab*

- (a) Select the Reports tab and specify your reporting requirements for this method.
- (b) Enter your name in the *Name* entry field.
- (c) Enter a comment relating to your experiment in the *Comment* entry field.
- (d) Set up your report style by selecting the appropriate checkboxes in the Options group.

For example, select the AutoPrint checkbox to obtain a printout of your report automatically.

Select the Parameters checkbox to include your experimental parameters in the report.

Select the Graph checkbox to include a calibration graph in the generated report.



**Note** If AutoPrint is selected, then 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 selecting the *Report* option in the *View* menu.

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

#### **Set up your samples and increment names**

##### **11. Setup dialog / Samples tab**

- (a) Select the Samples tab. This dialog box allows you to enter a list of sample names that will be used during your analysis.
- (b) Enter the number of samples that you are going to use in the *Number of Samples* field. The table below this field will expand or contract to match your choice.
- (c) In the Samples table, enter the name of each sample. You can enter up to 20 characters for each name.
- (d) If the samples have the same name with a different numeric extension, enter the name in the first sample position and then press the "Increment" button.

#### **Set up your samples for weight and volume correction**

##### **12. Setup dialog / Samples tab**

- (a) Select 'Corrections' in the Weight/Volume Corrections group 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.

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

##### **13. Setup dialog / Auto Store tab**

- (a) Select the Auto Store tab.
- (b) Select 'Storage off'. The method (with calibration), collected data and report will not be automatically saved. However, you can manually save it all at the end of the collection.

**Set up visual system monitoring**

14. Select the Show Status Display checkbox on any of the tabs to display various information fields on your current reaction.

**Finish setup**

15. Once you are satisfied with your method setup click "OK" to confirm any changes you have made and close the Setup dialog.

**Zero the instrument**

16. To Zero the instrument:

- (a) Click the "Zero" button to zero the system. Alternatively, select *Zero* in the *Commands* menu to perform a zero.
- (b) Place a blank in the sample compartment and press "OK".

**Perform the calibration**

17. To perform the calibration:

- (a) Press the application "Start" button or select *Start* in the *Commands* menu. The Standard/Sample Selection dialog box will appear.
- (b) Select the standards and samples to be used in the analysis. By default all standards and samples are selected.
- (c) Click "OK" to exit the Standard/Sample Selection dialog box.
- (d) The Present Standard dialog box will prompt you to place the appropriate standard in the sample compartment. Press "OK" to measure the standard.
- (e) Repeat until you have measured all 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 press "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.

### Measure the sample

18. To 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. Press "OK" to measure the sample and calculate its concentration. (If replicates have been nominated, then the concentration is calculated after the final sample replicate is read.)
  - (b) Repeat for the remaining samples.

### Save the data

19. To save your data:
  - (a) Click on the *File* menu item and select *Save Data As*.
  - (b) Enter the file name for this Concentration run in the *File name* field.

## 4.3 Dissolution

The following Dissolution How to... procedures are described.

- How to perform a Dissolution run (Cary 100/300)
- How to perform a Dissolution run (Cary 50)

### 4.3.1 How to perform a Dissolution run (Cary 100/300)

#### Open the Dissolution application

1. On the Windows Taskbar click "Start" to display the *Start* menu.
2. Move the cursor over the *Programs* menu item to display the *Programs* menu.
3. Select *Dissolution* in the *Cary WinUV* menu to open the Dissolution application.
4. Select your instrument type (if necessary) and press "OK" to open the Dissolution application.  
(Alternatively, you can double click on the Dissolution icon in the Cary WinUV folder on the desktop.)



**Note** If you are running a GLP system you may be prompted to enter a password before accessing the Dissolution application.

### Set up method parameters

#### 5. *Setup dialog*

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.

To do this:

### Set up instrument control and run timing parameters

#### 6. *Setup dialog | Cary tab*

On the Cary tab, specify the Instrument parameters for your analysis as follows:

- (a) Enter the *Wavelength*, *Ave* (averaging) *Time*, and *SBW* you require in the corresponding entry fields.
- (b) Select the ordinate mode you require (“Abs”, “%Dissolved” or “mg Dissolved”).
- (c) Enter an upper range and lower range value in the *Y Min* and *Y Max* entry fields to specify the displayed ordinate range.

#### 7. Specify the Collect Timing parameters:

- (a) Select the units for the collect. Click “Hours” to specify the time in hours or “Minutes” to specify the time in minutes.
- (b) Enter the number of stages you require in the *Number of Stages* entry field. The number you set here will be reflected in the table below.



**Note** Using more than one stage enables you to collect data more frequently during the stages with a fast tablet dissolution rate and to collect data less frequently where you know there will be a slower rate of dissolution.

- (c) Enter the Cycle and End time for each stage.

### Set up the bath controls including temperature, stir and pump times

#### 8. *Setup dialog | Bath tab*

- (a) Select the Bath tab to specify the Bath settings and controls for your analysis.
- (b) Select the Apparatus method type, the number of baths to be used and the vessels to be analyzed.

- (c) If Dual Pathlength is required, select the Use Dual Pathlength checkbox and specify whether you wish the data to be collected in the rear or front cells.



**Note** The Dual Pathlength option is not valid for a two bath system or when analyzing vessels Samples + Std + Blank on a one bath system.

- (d) Enter the VanKel Bath temperature, Method temperature and Temperature Tolerance.



**Note** Select the Warm up Bath checkbox if you would like to warm up the bath at a preset time. Click on the Bath tab to specify a date and time to begin the bath warmup.

- (e) Enter the various pump times in the corresponding entry fields.
- (f) Enter the Stir Rate in revolutions per minute.
- (g) Select the Infinity Stir Time checkbox if you want to perform an infinity measurement at the end of the last collect cycle.
- (h) Select the Initial Fast Stir checkbox if you want to perform a fast stir after adding the medium solution to the vessels to remove any trapped air bubbles around the paddles/baskets.

#### **Set up the sample information for tablet information and potency correction**

##### **9. Setup dialog | Samples tab**

- (a) Select the Samples tab and enter the sample information for your analysis.
- (b) Enter the Tablet Potency, Vessel Volume and the type of Sample Medium used.
- (c) Check the Weight Correct checkbox to apply the weight corrections.
- (d) Enter the Method Weight for each bath unit.
- (e) Enter the Actual tablet weights for each vessel.

#### **Set up the standard options for your collection**

##### **10. Setup dialog | Stds tab**

- (a) Select the Stds tab to specify the Standards Values.
- (b) Enter the *100% Std Abs*, *Standard Weight* and *Standard Volume* in the corresponding entry fields.



**Note** The 100% Std Abs entry field will be disabled if the specified Vessels type is Sample + Std or Sample + Std + Blank; or if the Use Multi-Std Calibration checkbox has been checked.

- (c) Specify the Std Medium and Std Reference material used for the analysis.
- (d) If required, check the Use Multi-Std Calibration checkbox to turn on the Multi-Std Calibration facility.



**Note** The Multi-Std Calibration facility requires a stored calibration to be selected by clicking on . The stored calibration must be calibrated and generated using the Concentration application with the same method parameters used in the Dissolution method.

- (e) If measuring a standard online, check the Use Limits checkbox to apply the Standard Control Limits during the analysis.
- (f) Enter the Standard Limits values when using the Standard Control Limits facility.
- (g) Specify the If Outside Limits action when using the Standard Control Limits.

#### **Set up the collection limits to ensure samples are within laboratory specifications**

##### **11. Setup dialog | Limits tab**

- (a) Select the Limits tab to specify the Sample Control Limits.
- (b) Check the Use Limits checkbox if you want to apply Sample Control Limits to the analysis.
- (c) Enter the number of limits you require in the *Number of Limits* entry field. The number you set here will be reflected in the table below.
- (d) Enter the Limit Information you require to be applied to the Sample analysis.



**Note** The time points entered will default to the nearest time points at which the measurement was made. No extrapolation of data is allowed.

- (e) Specify the If Outside Limits action when using the Sample Control Limits.

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

##### **12. Setup dialog | Reports tab**

- (a) Select the Reports tab to specify your reporting requirements for this method.

- (b) Enter your name in the *Name* entry field.
- (c) Enter a comment relating to your experiment in the *Comment* entry field.
- (d) Setup your report style by selecting the appropriate radio buttons in the Options group.

For example, select the Auto Print checkbox to obtain a print out of your report automatically.

Select the Parameters checkbox to include your experimental parameters in the report.

Select the Graph checkbox to include a graph in the generated report.



- Note** If Auto Print is selected, then the system will send the report information to the specified printer as well as the Report area. However if Auto Print is not selected, the report will only be sent to the Report area and can be viewed by selecting the *Report* option in the *View* menu.

- (e) Specify which time points are to be used in the report. For example, select either All time points, Sample Control Limit time points or User defined time points to appear in the report.
- (f) Enter the % Dissolved time points you require in the report.



- Note** To edit the Time and % Dissolved point tables click the right mouse button over the respective tables for the menu options.

- (g) Select the AutoConvert option you require.

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

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

13. Select the Show Status Display checkbox on any of the tabs to display various information fields on your current reaction.

#### **Finish setup**

14. Once you are satisfied with your method Setup click "OK" to close the Setup dialog.

**Check the placement of the Dissolution flowcells**

15. Click the “Loading Guide” button to check that the dissolution flowcells have been positioned correctly in the 8 x 6 Multicell Holder Accessory. This identifies the correct dissolution flowcell positions for the type of method setup specified for the run, e.g. Samples + Std dual path rear cells etc.



- Note Make sure you insert the flowcells into the Multicell Holder with the flow arrow in the right direction.

Once you are satisfied with your positioning click “OK” to exit the Cell Loading Guide.

16. Ensure that the tubing from the bath(s) and pump is securely connected to the dissolution flowcells.



- Note Each dissolution flowcell will have a downward arrow indicating the inlet flow. Care must be taken when handling the dissolution flowcells to ensure that the cell windows are not scratched, chipped or marked with fingerprints. The tubing to and from the cells, pump and bath must be checked for leaks to ensure analytical integrity.

**Check system communication and ensure correct mechanical responses**

17. To check communication and mechanical responses:
  - (a) Click the “Diagnostics” button or select *Diagnostics* from the *Commands* menu to check the bath and Multicell Holder communication.
  - (b) Select the cells in the Cary Apparatus group and check that the correct movement to each cell occurs.
  - (c) Select the bath you would like to test.
  - (d) Select the “Communication Setup” button and enter the correct settings.
  - (e) Click on the “Bath Communication Tests” button to start the test sequence. Follow the various prompts that appear.

**Zero the instrument**

18. To zero the instrument:
  - (a) Click the “Zero” button to display the Zero All Cells dialog.
  - (b) Click “OK” to zero each cell.

### Start the Dissolution run

19. To start the run:
  - (a) Press the "Start" button to start a data collection. Alternatively you can select *Start* in the *Commands* menu.
  - (b) The Sample ID dialog will appear. Enter your sample name here and press "OK".
  - (c) The 'Save As...' dialog box will appear prompting you to save the method, and collected data in a Dissolution Batch file (\*.BTD).
  - (d) Select the folder in which you want to save your Dissolution Batch file.
  - (e) Enter the name of the file in the *File name* entry field then click "Save". The method, collected data and generated report will be saved.
20. A number of prompt messages will appear during the analysis. Read the message carefully and select the appropriate response. The type of prompt messages that will appear will depend on the method parameters used for the data collect.



**Note** During a run, the software will ask you if you want to use the automatic temperature sensing in all vessels. If you are using a 7000 bath, then you must choose "No" and the software will use the temperature probe(s) in the bath(s) as the control temperature.

#### 4.3.2 How to perform a Dissolution run (Cary 50)

##### Open the Dissolution application

1. On the Windows Taskbar click "Start" to display the *Start* menu.
2. Move the cursor over the *Programs* menu item to display the *Programs* menu.
3. Select *Dissolution* in the *Cary WinUV* menu to open the Dissolution application.
4. Select your instrument type (if necessary) and press "OK" to open the Dissolution application.  
(Alternatively, you can double click on the Dissolution icon in the Cary WinUV folder on the desktop.)



**Note** If you are running a GLP system you may be prompted to enter a password before accessing the Dissolution application.

### Set up method parameters

5. 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.

To do this:

### Set up instrument control and run timing parameters

6. *Setup dialog | Cary tab*

On the Cary tab, specify the Instrument parameters for your analysis as follows:

- (a) Enter the *Wavelength* and *Ave (averaging) time* you require in the corresponding entry fields.
  - (b) Select the ordinate mode you require (“Abs”, “%Dissolved” or “mg Dissolved”).
  - (c) Enter an upper range and lower range value in the *Y Min* and *Y Max* entry fields to specify the displayed ordinate range.
7. Specify the Collect Timing parameters:
    - (a) Choose the units for the collect. Select “Hours” to specify the time in hours or “Minutes” to specify the time in minutes.
    - (b) Enter the Number of Stages you require in the *Number of Stages* entry field. The number you set here will be reflected in the table below.



- Note** Using more than one stage enables you to collect data more frequently during the stages with a fast tablet dissolution rate and to collect data less frequently where you know there will a slower rate of dissolution.

- (c) Enter the Cycle and End time for each stage.

### Set up the bath controls including temperature, stir and pump times

8. *Setup dialog | Bath tab*

- (a) Select the Bath tab to specify the Bath settings and controls for your analysis.
- (b) Select the Apparatus method type, the number of baths to be used and the vessels to be analyzed.
- (c) If Dual Pathlength is required, select the Use Dual Pathlength checkbox and specify whether you wish the data to be collected in the rear or front cells.



- Note** The Dual Pathlength option is not valid for a two bath system.

- (d) Enter the VanKel Bath temperature, Method temperature and Temperature Tolerance.



**Note** Select the Warm up Bath checkbox if you would like to warm up the bath at a preset time. Click on the Bath tab to specify a date and time to begin the bath warmup.

- (e) Enter the various pump times in the corresponding entry fields.
- (f) Enter the Stir Rate in revolutions per minute.
- (g) Select the Infinity Stir Time checkbox if you want to perform an infinity measurement at the end of the last collect cycle.
- (h) Select the Initial Fast Stir checkbox if you want to perform a fast stir after adding the medium solution to the vessels to remove any trapped air bubbles around the paddles/baskets.

#### **Set up the sample information for tablet information and potency correction**

##### **9. Setup dialog | Samples tab**

- (a) Select the Samples tab to enter the sample information for your analysis.
- (b) Enter the Tablet Potency, Vessel Volume and the type of Sample Medium used.
- (c) Check the Weight Correct checkbox to apply the weight corrections.
- (d) Enter the Method Weight for each bath unit.
- (e) Enter the Actual tablet weights for each vessel.

#### **Set up the standard options for your collection**

##### **10. Setup dialog | Stds tab**

- (a) Select the Stds tab to specify the Standards Values.
- (b) Enter the *100% Std Abs*, *Standard Weight* and *Standard Volume* in the corresponding entry fields.



**Note** The *100% Std Abs* entry field will be disabled if the specified Vessels type is Sample + Std or Sample + Std + Blank; or the Use Multi-Std Calibration checkbox has been checked.

- (c) Specify the Std Medium and Std Reference material used for the analysis.
- (d) If required, check the Use Multi-Std Calibration checkbox to turn on the Multi-Std Calibration facility.



**Note** The Multi-Std Calibration facility requires a stored calibration to be selected by clicking on . The stored calibration must be calibrated and generated using the Concentration application with the same method parameters used in the Dissolution method.

- (e) If measuring a standard online, check the Use Limits checkbox to apply the Standard Control Limits during the analysis.
- (f) Enter the Standard Limits values when using the Standard Control Limits facility.
- (g) Specify the If Outside Limits action when using the Standard Control Limits.

#### **Set up the collection limits to ensure samples are within laboratory specifications**

##### **11. Setup dialog | Limits tab**

- (a) Select the Limits tab to specify the Sample Control Limits.
- (b) Select the Use Limits checkbox if you want to apply Sample Control Limits to the analysis.
- (c) Enter the number of limits you require in the *Number of Limits* entry field. The number you set here will be reflected in the table below.
- (d) Enter the Limit Information you require to be applied to the Sample analysis.



**Note** The time points entered will default to the nearest time points at which the measurement was made. No extrapolation of data is allowed.

- (e) Specify the If Outside Limits action when using the Sample Control Limits.

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

##### **12. Setup dialog | Reports tab**

- (a) Select the Reports tab to specify your reporting requirements for this method.
- (b) Enter your name in the *Name* entry field.
- (c) Enter a comment relating to your experiment in the *Comment* entry field.
- (d) Setup your report style by selecting the appropriate radio buttons in the Options group.

For example, select the Auto Print checkbox to obtain a print out of your report automatically.  
Select the Parameters checkbox to include your experimental parameters in the report.  
Select the Graph checkbox to include a graph in the generated report.



**Note** If Auto Print is selected, then the system will send the report information to the specified printer as well as the Report area. However if Auto Print is not selected, the report will only be sent to the Report area and can be viewed by selecting the *Report* option in the *View* menu.

- (e) Specify which time points are to be used in the report.

For example, select either All time points, Sample Control Limit time points or User defined time points to appear in the report.

- (f) Enter the % Dissolved time points you require in the report.



**Note** To edit the Time and % Dissolved point tables click the right mouse button over the respective tables for the menu options.

- (g) Select the AutoConvert option you require.

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

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

13. Select the Show Status Display checkbox on any of the tabs to display various information fields on your current reaction.

#### **Finish setup**

14. Once you are satisfied with your method Setup click "OK" to close the Setup dialog.

#### **Check the placement of the Dissolution Flow Cells**

15. Click the "Loading Guide" button or select *Loading Guide* from the *Commands* menu to check that the dissolution flowcells have been positioned correctly in the 18 Cell Holder Accessory. This identifies the correct dissolution flowcell positions for the type of method setup specified for the run, e.g. Samples + Std dual path rear cells etc.



Note Make sure you insert the flowcells into the Multicell Holder with the flow arrow in the right direction.

Once you are satisfied with your positioning click "OK" to exit the Cell Loading Guide.

16. Ensure that the tubing from the bath(s) and pump is securely connected to the dissolution flowcells.



Note Each dissolution flowcell will have a downward arrow indicating the inlet flow. Care must be taken when handling the dissolution flowcells to ensure that the cell windows are not scratched, chipped or marked with fingerprints. The tubing to and from the cells, pump and bath must be checked for leaks to ensure analytical integrity.

#### **Check system communication and ensure correct mechanical responses**

17. To check communication and mechanical responses:
  - (a) Click the "Diagnostics" button or select *Diagnostics* from the *Commands* menu to check the bath and Multicell Holder communication.
  - (b) Select the cells in the Cary Apparatus group and check that the correct movement to each cell occurs.
  - (c) Select the bath you would like to test.
  - (d) Select the "Communication Setup" button and enter the correct settings.
  - (e) Click on the "Bath Communication Tests" button to start the test sequence. Follow the various prompts that appear.

#### **Zero the instrument**

18. To zero the instrument:
  - (a) Click the "Zero" button to display the Zero All Cells dialog.
  - (b) Click "OK" to zero each cell.

#### **Start the Dissolution run**

19. To start the run
  - (a) Press the "Start" button to start a data collection. Alternatively you can select *Start* in the *Commands* menu.
  - (b) The Sample ID dialog will appear. Enter your sample name here and press "OK".

- (c) The Save As... dialog box will appear prompting you to save the method, and collected data in a Dissolution Batch file (\*.BTD).
  - (d) Select the folder in which you want to save your Dissolution Batch file.
  - (e) Enter the name of the file in the *File name* entry field then click "Save". The method, collected data and generated report will be saved.
20. A number of prompt messages will appear during the analysis. Read the message carefully and select the appropriate response. The type of prompt messages that will appear will depend on the method parameters used for the data collect.



**Note** During a run, the software will ask you if you want to use the automatic temperature sensing in all vessels. If you are using a 7000 bath, then you must choose "No" and the software will use the temperature probe(s) in the bath(s) as the control temperature.

## 4.4 Enzyme Kinetics

The following Enzyme Kinetics How to... procedures are described:

- How to perform a temperature controlled run using the Multicell Holder (Cary 100–500)
- How to perform a temperature controlled run (Cary 50)

### 4.4.1 How to perform a temperature controlled run using the Multicell Holder (Cary 100–500)

This demonstrates 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, 400 and 500).

#### Start the Enzyme Kinetics application

1. On the Windows Taskbar, click "Start" to display the *Start* menu.
2. Move the cursor over the *Programs* menu item to display the *Programs* menu.
3. Select *Enzyme Kinetics* in the *Cary WinUV* menu.

4. Select your instrument type (if necessary) and press "OK" to open the Enzyme Kinetics application.  
(Alternatively, you can double click on the Enzyme Kinetics icon in the Cary WinUV folder on the desktop.)



Note If you are running a GLP system, you may be prompted to enter a password before accessing the Enzyme Kinetics application.

#### **Set up data collection parameters**

5. 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.

To do this:

#### **Set up Multicell Holder accessory with multi zero, no blank correction**

6. *Setup dialog | Accessories tab*

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

- (a) Select the Accessories tab.
- (b) Select the Use Cell Changer checkbox to enable the accessory.
- (c) For Cary 100/300 instruments, choose the type of Multicell Holder you are using (6 x 6 or 8 x 6).
- (d) Ensure that you have this accessory installed prior to commencing the run.
- (e) Choose the Select Cells radio button and select the cells you require from the available cells in the Use Cells group.



Note For Front Beam analysis, select the checkboxes 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.

- (f) Check the Multi Zero checkbox to turn on the Multi Zero facility.
- (g) Ensure that the Blank Correction checkbox is not checked.

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

## 7. To set up accessories:

- (a) If you are not using a Peltier controlled accessory (e.g. the water thermostatted 8 x 6) ensure that you have the Temperature Controller accessory installed prior to commencing the run, and,
  - (i) Tick Automatic Temperature Setting and select the 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 8 part (d)).
- (b) In the Temperature Display group, select Block and Probe 1 to view the temperature of the Multicell Holder block and one temperature Probe in the Status Display window.

**Set up instrument parameters such as wavelength, SBW, Y mode etc.**8. *Setup dialog | Cary tab*

Select the Cary tab and specify the Instrument parameters for your analysis.

- (a) Enter the *Wavelength*, *SBW* and *Ave* (averaging) *Time* you require in the corresponding entry fields.
- (b) Select the ordinate mode you require. Click “Abs” to specify the 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 Fibre Optic Probe.
- (c) Enter an upper range and lower range value in the *Y min* and *Y max* entry fields to specify the displayed ordinate range.
  - (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 in the Accessories tab. (Step 7 part (a)(ii)).

**Set up rate parameters such as the number of different rates in the reaction, the speed and duration of the data collection**

9. Select the Advanced Collect radio button in the Collect Timing group. 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 not be much activity.

- (a) Enter the number of different reaction rates that you require in the *Number of Stages* entry field. The number you set here will be reflected in the table below.
- (b) Vary the number of data points collected per cell per run by setting the Dwell time for each rate Stage.
- (c) 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.
- (d) Specify the duration of the measurement run by setting the Stop time for each rate Stage.

**Set up lamp and graphics options**

10. *Setup dialog | Options tab*

- (a) Select the Options tab.
- (b) Check the Auto Lamps Off checkbox if you want to automatically turn off the lamps at the end of a collect. This option enables you to preserve the lifetime of the lamps. It is especially useful for when performing data collections overnight or unattended for long periods of time.
- (c) Click on the UV/Vis radio button if you want both the lamps on during the run.
- (d) Enter your required *Source Changeover and Detector Changeover* (Cary 500 only) wavelengths in the corresponding entry fields.
- (e) Set the monochromator exit slit height to full or reduced (Cary 400 and 500 only).
- (f) In the Display Options group, select the way in which you want the data displayed as it is collected. Choose the Individual Data radio button to display the collected data of each sample in individual graph boxes. Choose the Overlay Data radio button to superimpose the collected data of each sample in the Enzyme Kinetics run in one graph box.

**Set up V<sub>0</sub> calculation**

11. *Setup dialog | Analyze tab*
  - (a) Select the Analyze tab.
  - (b) Set up the Start and Stop times for your V<sub>0</sub> Calculation.
  - (c) Enter the correct product absorptivity for your reaction.
  - (d) Enter the correct cell pathlength for your reaction.

**Set up calculations for the maximum rate (V<sub>max</sub>) and substrate concentration that gives half the maximum rate (K<sub>m</sub>)**

12. To set up V<sub>max</sub> and K<sub>m</sub> calculations:
  - (a) Select the method by which the data obtained from your selection in the Plot/Fit group will be calculated. Choose Linear Least Square or Marquardt by selecting the appropriate radio button.
  - (b) Choose the inhibitor model for your analysis. Select either the Non Competitive, Competitive or Uncompetitive radio button.
  - (c) Check the box/es beside the Plot/Fit type/s that will be used to determine V<sub>max</sub> and K<sub>m</sub> values.
  - (d) Select the Auto Calculate checkbox to automatically perform enzyme kinetics calculations on collected data at the end of each run. These results will be displayed in the Report area.

**Set up reporting and printing requirements**

13. *Setup dialog | Reports tab*
  - (a) Select the Reports tab.
  - (b) Enter your name in the *Name* entry field.
  - (c) Enter a comment relating to your experiment in the *Comment* entry field.
  - (d) Set up your report style by selecting the appropriate checkboxes in the Options group.

For example, select the Auto Print checkbox to obtain a printout of your report automatically.  
Select the Parameters checkbox to include your experimental parameters in the report.  
Select the Graph checkbox to include a graph in the generated report.



**Note** If Auto Print is selected, then the system will send the report information to the specified printer as well as to the Report area. However if Auto Print is not selected, the report will only be sent to the Report area and can be viewed by selecting the *Report* option in the *View* menu.

- (e) Select the Autoconvert option you require.

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

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

14. *Setup dialog | Auto Store tab*
  - (a) Select the Auto Store tab to set up whether the collected data is to be saved, and if so, when the Cary should store the information.
  - (b) Select Storage On (Prompt at Start), to set up the Cary to prompt you for file name before the start of an Enzyme Kinetics reaction.

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

15. Select the Show Status Display checkbox on any of the tabs to display various information fields on your current reaction.

#### **Finish setup**

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

#### **Zero the instrument**

17. To zero the instrument
  - (a) Click the "Zero" button to zero the system. Alternatively, select *Zero* in the *Commands* menu to perform a zero. A Cell Loading Guide dialog box will be displayed.
  - (b) If you want, 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 6, 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 press "OK", the system will perform a zero on the cell position in the light path, i.e. the system will not reset the Multicell Holder to position 1.

### Start the run

18. To start the run:

- (a) Press the "Start" button to start a data collection. Alternatively you can select *Start* in the *Commands* menu. *Do not add your active reagent at this time*. The system will display a Cell Loading Guide dialog box.
- (b) If you want, 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 then display the Save File dialog box.
- (d) Enter the file name for this run in the *File name* field and press "Save". The Sync Start dialog box will appear.
- (e) Reset the Multicell Holder to position to cell 1 using the "Reset Slide" button. Add your active reagent just before the Count Down reaches 0:00 or commence the data collection by pressing the "OK" button.

### Enter substrate and inhibitor concentrations

19. Once the run has started, enter the substrate and inhibitor concentrations.



Note If no information has been entered in the User Data Form during the collect, no calculation is performed.

- (a) Open the User Data Form, by right clicking in a graph box and selecting User Data Form from the list or by selecting it from the *Graph* menu.
- (b) The table that appears has Data Names and may have *V<sub>0</sub>* values already entered in the first two columns. In the third and fourth columns, enter your values for [S] and [I] in  $\mu\text{molar/L}$ .
- (c) Press "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.

#### 4.4.2 How to perform a temperature controlled run (Cary 50)

This demonstrates how to perform a single cell, multi-rate Enzyme Kinetics run at 37 °C using the single cell Peltier accessory (Cary 50).

##### Start the Enzyme Kinetics application

1. On the Windows Taskbar, click “Start” to display the *Start* menu.
2. Move the cursor over the *Programs* menu item to display the *Programs* menu.
3. Select *Enzyme Kinetics* in the *Cary WinUV* menu.
4. Select your instrument type (if necessary) and press “OK” to open the Enzyme Kinetics application.  
(Alternatively, you can double click on the Enzyme Kinetics icon in the Cary WinUV folder on the desktop.)



**Note** If you are running a GLP system, you may be prompted to enter a password before accessing the Enzyme Kinetics application.

##### Set up data collection parameters

5. 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.

To do this:

##### Ensure Multicell Holder accessory is not selected

6. *Setup dialog | Accessories tab*

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

- (a) Select the Accessories tab.
- (b) Ensure the Use Cell Changer checkbox is not selected.

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

7. To set up accessories:

- (a) In the Temperature group, check ‘Automatic Temperature Setting’ to enable the single cell Peltier accessory.
- (b) Set the monitoring temperature by entering the Block temperature as 37 °C.

- (c) In the Temperature Display group, select Probes 1 and 2 to view the temperature of two temperature Probes in the Status Display window.

**Set up instrument parameters such as wavelength, Y mode etc.**

8. *Setup dialog | Cary tab*

Select the Cary tab and specify the Instrument parameters for your analysis.

- (a) Enter the *Wavelength* and Ave (averaging) *Time* you require in the corresponding entry fields.
- (b) Enter an upper range and lower range value in the *Y min* and *Y max* entry fields to specify the displayed ordinate range.

**Set up rate parameters such as the number of different rates in the reaction, the speed and duration of the data collection**

9. To set up different data collection procedures the multiple rates in your reaction:

- (a) Select the Advanced Collect radio button in the Collect Timing group.



**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.

- (b) Enter the number of different reaction rates that you require in the *Number of Stages* entry field. The number you set here will be reflected in the table below.
- (c) 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.
- (d) Specify the duration of the measurement run by setting the Stop time for each rate Stage.

**Set up collection display options**

10. *Setup dialog | Options tab:*

- (a) Select the Options tab to select the way in which you want the data displayed as it is collected.
- (b) Choose the Individual Data radio button to display the collected data of each sample in individual graph boxes. Choose the Overlay Data radio button to superimpose the collected data of each sample in the run in one graph box.

**Set up V<sub>0</sub> calculation**

11. *Setup dialog | Analyze tab*
  - (a) Select the Analyze tab.
  - (b) Set up the Start and Stop times for your V<sub>0</sub> Calculation.
  - (c) Enter the correct product absorptivity for your reaction.
  - (d) Enter the correct cell pathlength for your reaction..

**Set up calculations for the maximum rate (V<sub>max</sub>) and substrate concentration that gives half the maximum rate (K<sub>m</sub>)**

12. To set up V<sub>max</sub> and K<sub>m</sub> calculations:
  - (a) Select the method by which the data obtained from your selection in the Plot/Fit group will be calculated. Choose Linear Least Square or Marquardt by selecting the appropriate radio button.
  - (b) Choose the inhibitor model for your analysis. Select either the Non Competitive, Competitive or Uncompetitive radio button.
  - (c) Check the box/es beside the Plot/Fit type/s that will be used to determine V<sub>max</sub> and K<sub>m</sub> values.
  - (d) Select the Auto Calculate checkbox to automatically perform enzyme kinetics calculations on collected data at the end of each run. These results will be displayed in the Report area.

**Set up reporting and printing requirements**

13. *Setup dialog | Reports tab*
  - (a) Select the Reports tab.
  - (b) Enter your name in the *Name* entry field.
  - (c) Enter a comment relating to your experiment in the *Comment* entry field.
  - (d) Set up your report style by selecting the appropriate checkboxes in the Options group.  
For example, select the Auto Print checkbox to obtain a printout of your report automatically.  
Select the Parameters checkbox to include your experimental parameters in the report.  
Select the Graph checkbox to include a graph in the generated report.



**Note** If Auto Print is selected, then the system will send the report information to the specified printer as well as to the Report area. However if Auto Print is not selected, the report will only be sent to the Report area and can be viewed by selecting the Report option in the *View* menu.

- (e) Select the Autoconvert option you require.

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

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

##### **14. Setup dialog | Auto Store tab**

- (a) Select the Auto Store tab to set up whether the collected data is to be saved, and if so, when the Cary should store the information.
- (b) Select Storage On (Prompt at Start), to set up the Cary to prompt you for file name before the start of an Enzyme Kinetics reaction.

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

##### **15. Select the Show Status Display checkbox on any of the tabs to display various information fields on your current reaction.**

#### **Finish setup**

##### **16. Click "OK" to save any changes you have made and close the Setup dialog.**

#### **Zero the instrument**

##### **17. To zero the instrument:**

- (a) Click the "Zero" button to zero the system. Alternatively, select *Zero* in the *Commands* menu to perform a zero. A Loading Guide dialog box will be displayed.
  - (b) If you want, change the names of the blank sample.
  - (c) Place the blank solution in the correct cell position and click "OK".
- The system will perform an instrument zero on the blank solution.

### Start the run

18. To start the run:
  - (a) Press the “Start” button to start a data collection. Alternatively you can select *Start* in the *Commands* menu. *Do not add your active reagent at this time.* The system will display a Loading Guide dialog box.
  - (b) If you want, change the names of the sample.
  - (c) Place the sample solution in the correct cell position and click “OK”. The system will set up the Graphics area and then display the Save As dialog box.
  - (d) Enter the file name for this run in the *File name* field and press “Save”. The Sync Start dialog box will appear.
  - (e) Add your active reagent just before the Count Down reaches 0:00 or commence the data collection by pressing the “OK” button.

### Enter substrate and inhibitor concentrations

19. Once the run has started, enter the substrate and inhibitor concentrations.



**Note** If no information has been entered in the User Data Form during the collect, no calculation is performed.

- (a) Open the User Data Form, by right clicking in a graph box and selecting User Data Form from the list or by selecting it 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  $\mu\text{molar/L}$ .
- (c) Press “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.

## 4.5 Fabric Protection

The following Fabric Protection How to... procedure is described:

- How to perform a scan using the Cary 100/300/400/500 (Australian/New Zealand Standard)

#### **4.5.1 How to perform a scan using the Cary 100/300/400/500 (Australian/New Zealand Standard)**

1. Under the *Options* menu, select *Australian* and the number of samples to be tested.
2. Click on the "Sample Info." button. Enter all relevant information in the Sample Info. dialog and click "OK".
3. Select the *Setup* menu to display the Setup dialog.  
Select 'Report Graph' if you want the trace(s) to be displayed in the report.  
Select 'Report XY Table' for XY pairs data to be displayed in the report.  
Select 'Storage on (prompt at start)' to save the data.
4. Click on the "Baseline" button and follow the message prompts to collect 100%T and 0%T baseline scans.

##### **Scanning Sample 1**

5. First measurement (Position 1, 0°):
  - (a) Click the "Scan" button. You will be prompted to select your data saving options.
  - (b) Select a file name and file type and click "Save". A dialog will appear indicating the International Standard as well as the sample and measurement number. You will be prompted to place your sample at 0° (Position 1). You also have the option of changing the name of the scan.
  - (c) To test the fabric sample, slide the Fabric holder out of the accessory and place the sample into the Fabric holder.
  - (d) Slide the holder back into position, replace the covers and click "OK" to perform the test.
6. Second measurement (Position 2, 90°):
  - (a) A dialog like the previous will appear indicating the new measurement number for Sample 1. You will be prompted to place your sample at 90° (Position 2). You also have the option of changing the name of the scan.
  - (b) To test the sample in position 2, slide the Fabric holder out of the accessory and turn the sample 90° from the original measurement position.
  - (c) Slide the fabric holder back into position, replace the covers and click "OK" to perform the test.
7. This process will continue until the number of samples selected from the Options menu in Step 1 have been scanned.

## 4.6 Kinetics

The following Kinetics How to... procedures are described:

- How to perform a multi-wavelength, single cell, single rate Kinetics run (Cary 100–500).
- How to perform a temperature controlled Kinetics run using the Multicell Holder (Cary 100–500)
- How to perform a temperature controlled Kinetics run (Cary 50)

### 4.6.1 How to perform a multi-wavelength, single cell, single rate Kinetics run (Cary 100–500)

This demonstrates how to perform a multi-wavelength, single cell Kinetics run at ambient temperature (Cary 100, 300, 400 and 500).

#### Start the Kinetics application

1. On the Windows Taskbar, click “Start” to display the *Start* menu.
2. Move the cursor over the *Programs* menu item to display the *Programs* menu.
3. Select *Kinetics* in the *Cary WinUV* menu.
4. Select your instrument type (if necessary) and press “OK” to open the Kinetics application.  
(Alternatively, you can double click on the Kinetics icon in the Cary WinUV folder on the desktop.)



**Note** If you are running a GLP system, you may be prompted to enter a password before accessing the Kinetics application.

#### Set up data collection parameters

5. *Setup dialog*

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.

To do this:

**Set up instrument parameters such as wavelength, spectral bandwidth, Y mode etc.**

6. *Setup dialog / Cary tab*
  - (a) Select the Cary tab.
  - (b) In the *Wavelength* field enter the wavelengths that you want monitored.
  - (c) In the *SBW* field enter the required spectral bandwidth.
  - (d) In the *Ave Time* field enter the required value.
  - (e) Select the ordinate mode you require. Click “Abs” to specify the Absorbance mode or “%T” to specify percent Transmittance.
  - (f) Enter an upper range and lower range value in the *Y min* and *Y max* entry fields to specify the displayed ordinate range.
  - (g) Select the abscissa (X) mode you require. Click “Min” to time in minutes or “Sec” to time in seconds.

**Set up rate parameters such as the number of different rates in the reaction, the speed, and the duration of the data collection**

7. *Setup dialog / Cary tab*
  - (a) Select the ‘Simple Collect’ radio button in the Collect Timing group. This sets 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.

**Set up lamp and graphics options**

8. *Setup dialog / Options tab*
  - (a) Select the Options tab.
  - (b) Check the ‘Auto Lamps Off’ checkbox if you want to automatically turn off the lamps at the end of a collect. This option enables you to preserve the lifetime of the lamps. It is especially useful for when performing Kinetics data collections overnight or unattended for long periods of time.
  - (c) Click on the ‘UV/Vis’ radio button if you want both the lamps on during the run.
  - (d) Enter your required *Source Changeover* and *Detector Changeover* (Cary 500 only) wavelengths in the corresponding entry fields.

- (e) Set the monochromator exit slit height to full or reduced (Cary 400 and 500 only).
- (f) In the Display Options group, select the way in which you want the data displayed as it is collected. Choose the 'Individual Data' radio button to display the collected data of each sample in individual graph boxes. Choose the 'Overlay Data' radio button to superimpose the collected data of each sample in the Kinetics run in one graph box.

#### Set up accessories

##### 9. *Setup dialog / Accessories tab*

Select the Accessories tab and ensure that no Accessories are selected.

#### Set up rate calculation, rate order and other rate parameters

##### 10. *Setup dialog / Analyze tab*

- (a) Select the Analyze tab.
- (b) Select the 'Auto Calculate' checkbox to automatically perform a rate calculation on collected data at the end of each run.
- (c) Select 'Simple Calculate' to setup one rate calculation for the entire Kinetics run.
- (d) Set up the reaction Start and Stop times and select the reaction order.
- (e) If you select a 'first order' or 'second order' Simple Calculate rate calculation you can use the Manual Guess group to manually enter the parameters: A0, Alnf and Rate (k). It is presumed that you have a reasonable idea of the values for these fit parameters, as they will be used as a first guess for the Marquardt non-linear regression analysis.



**Note** If you do not choose 'Manual Guess' then the Cary system will automatically calculate the values: A0, Alnf and Rate when the "Start" button is pressed.

- (f) Enter a value in the *Factor* field to calculate enzyme activity. The numerical multiplication factor is applied to the absorbance.

- (g) If you are performing a second order reaction, enter the initial concentration of substrate before reaction.
- (h) Select 'Display Fit' to automatically overlay the calculated lines of best fit onto the plotted data.

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

##### **11. Setup dialog / Reports tab**

Select the Reports tab and specify your reporting requirements for this method.

- (a) Enter your name in the *Name* entry field.
- (b) Enter a comment relating to your experiment in the *Comment* entry field.
- (c) Set up your report style by selecting the appropriate checkboxes in the Options group.

For example, select the 'Auto Print' checkbox to obtain a printout of your report automatically.

Select the 'Parameters' checkbox to include your experimental parameters in the report. Select the 'Graph' checkbox to include a graph in the generated report.



**Note** If 'Auto Print' is selected, then the system will send the report information to the specified printer as well as the Report area. However, if 'Auto Print' is not selected, the report will only be sent to the Report area and can be viewed by selecting *Report* in the *View* menu.

- (d) Check *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', then at the end of the data collection the system will automatically generate a report and store the data both in the Cary format as well as ASCII XY pairs format in the current folder.

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

##### **12. Setup dialog / Auto Store tab**

- (a) Select the 'Auto Store' tab to set up whether the collected data is to be saved, and if so, when the Cary should store the information.
- (b) Select 'Storage Off'. The method, collected data and report will not be saved.

**Set up visual system monitoring**

13. Select the ‘Show Status Display’ checkbox to display various information fields on your current reaction.

**Finish setup**

14. Once you are satisfied with your method setup click “OK” to confirm any changes you have made and close the Setup dialog.

**Zero the instrument**

15. To zero the instrument:
  - (a) Click the “Zero” button to zero the system. Alternatively, select *Zero* in the *Commands* menu to perform a zero. A Loading Guide dialog box will be displayed.
  - (b) If you want, 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).

**Start the Kinetics run**

16. To start the Kinetics run:
  - (a) Press the application “Start” button to start a data collection. Alternatively, you can select *Start* in the *Commands* menu. *Do not add your active reagent at this time*. The system will display a Loading Guide dialog box.
  - (b) If you want, 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 Sync Start dialog box.
  - (d) Add your active reagent just before the Count Down reaches 0:00 or commence the data collection by pressing the “OK” button.

The Cary will start the data collection.

**Save your data**

17. To save your data:
  - (a) Click on the *File* menu and select *Save Data As*.
  - (b) Enter the file name for this Kinetics run in the *File name* field.
  - (c) Press "Save".

The data will be stored as a Batch file.

### **4.6.2 How to perform a temperature controlled Kinetics run using the Multicell Holder (Cary 100–500)**

This demonstrates 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, 400 and 500).

**Start the Kinetics application**

1. On the Windows Taskbar, click "Start" to display the *Start* menu.
2. Move the cursor over the *Programs* menu item to display the *Programs* menu.
3. Select *Kinetics* in the *Cary WinUV* menu.
4. Select your instrument type (if necessary) and press "OK" to open the Kinetics application.  
(Alternatively, you can double click on the Kinetics icon in the Cary WinUV folder on the desktop.)



**Note** If you are running a GLP system, you may be prompted to enter a password before accessing the Kinetics application.

**Set up data collection parameters**

5. *Setup dialog*  
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.

To do this:

**Set up Multicell Holder accessory with multi zero**

6. *Setup dialog / Accessories tab*  
As various options do not become available until the appropriate accessories are selected, you need to select these first.

- (a) Select the Accessories tab.
- (b) Select the 'Use Cell Changer' checkbox to enable the accessory.
- (c) For Cary 100/300 instruments, choose the type of Multicell Holder you are using (6 x 6 or 8 x 6).
- (d) Ensure that you have this accessory installed prior to commencing the run.
- (e) Choose the 'Select Cells' radio button and select the cells you require from the available cells in the Use Cells group.  
For Front Beam analysis, select the checkboxes 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.
- (f) Check the 'Multi Zero' checkbox to turn on the Multi Zero facility.
- (g) Ensure that the Blank Correction checkbox is not checked.

**Set up accessories for reaction temperature control and temperature display****7. Setup dialog / Accessories tab**

- (a) If you are not using a Peltier controlled accessory (e.g. the water thermostatted 8 x 6) ensure that you have the Temperature Controller accessory installed prior to commencing the run, and,
  - (i) Select 'Automatic Temperature Setting' and select the Temperature Controller to enable the accessory.
  - (ii) Set the monitoring temperature by entering the Block temperature as '36 °C'. (The monitoring device is selected in step 8 part (e)).
- (b) In the Temperature Display group, select 'Block' and 'Probe 1' to turn on monitoring of the Multicell Holder block and one temperature Probe.

**Set up instrument parameters such as wavelength, spectral bandwidth, Y mode etc.****8. Setup dialog / Cary tab**

Select the Cary tab and specify the instrument parameters for your analysis.

- (a) Enter the *Wavelength*, *SBW* and *Ave (averaging) Time* you require in the corresponding entry fields.

- (b) Select the ordinate mode you require. Click "Abs" to specify the Absorbance mode or "%T" to specify percent Transmittance.
- (c) Enter an upper range and lower range value in the *Y min* and *Y max* entry fields to specify the displayed ordinate range.
- (d) Select the abscissa (X) mode you require. Click "Min" to time in minutes or "Sec" to time in seconds.
- (e) 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 in the Accessories tab.  
(Step 7 part (a)(ii)).

**Set up instrument parameters such as wavelength, spectral bandwidth, Y mode etc.**

9. *Setup dialog / Cary tab*

Select the 'Advanced Collect' radio button in the Collect Timing group. 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 kinetics 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* entry field. The number you set here will be reflected in the table below this field.
- (b) Vary the number of data points collected per cell, per run by setting the Dwell time for each rate Stage.
- (c) 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.
- (d) Specify the duration of the Kinetics run by setting the Stop time for each rate Stage.

**Set up lamp and graphics options**10. *Setup dialog / Options tab*

- (a) Select the Options tab.
- (b) Check the 'Auto Lamps Off' checkbox if you want to automatically turn off the lamps at the end of a collect. This option enables you to preserve the lifetime of the lamps. It is especially useful for when performing Kinetics data collections overnight or unattended for long periods of time.
- (c) Click on the 'UV/Vis' radio button if you want both the lamps on during the run.
- (d) Enter your required Source Changeover and Detector Changeover (Cary 500 only) wavelengths in the corresponding entry fields.
- (e) Set the monochromator exit slit height to full or reduced (Cary 400 and 500 only).
- (f) In the Display Options group, select the way in which you want the data displayed as it is collected. Choose the 'Individual Data' radio button to display the collected data of each sample in individual graph boxes. Choose the 'Overlay Data' radio button to superimpose the collected data of each sample in the Kinetics run in one graph box.

**Set up rate calculation, rate order and other rate parameters**11. *Setup dialog / Analyze tab*

- (a) Select the Analyze tab.
- (b) Select the 'Auto Calculate' checkbox to automatically perform a rate calculation on collected data at the end of each run.
- (c) Select 'Advanced Calculate' to set up multiple rate calculations for the Kinetics run.
- (d) Enter the number of different rate calculations that you require in the *Number of Stages* entry field. The number you set here will be reflected in the table below.
- (e) 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 Manual Guess group to manually enter the parameters: A<sub>0</sub>, A<sub>inf</sub> and Rate (k). It is presumed that you have a reasonable idea of the values for these fit parameters, as they will be used as a first guess for the Marquardt non-linear regression analysis.

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

#### Set up reporting and printing requirements

##### 12. *Setup dialog / Reports tab*

- (a) Select the Reports tab.
- (b) Enter your name in the *Name* entry field.
- (c) Enter a comment relating to your experiment in the *Comment* entry field.
- (d) Set up your report style by selecting the appropriate checkboxes in the Options group. For example, select the 'Auto Print' checkbox to obtain a printout of your report automatically.  
Select the 'Parameters' checkbox to include your experimental parameters in the report. Select the 'Graph' checkbox to include a graph in the generated report.



**Note** If 'Auto Print' is selected, then the system will send the report information to the specified printer as well as to the Report area. However if 'Auto Print' is not selected, the report will only be sent to the Report area and can be viewed by selecting *Report* in the *View* menu.

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

**Set up storage of collected data**13. *Setup dialog / Auto Store tab*

- (a) Select the Auto Store tab to set up whether the collected data is to be saved, and if so, when the Cary should store the information.
- (b) Select ‘Storage On (Prompt at Start)’, to set up the Cary to prompt you for a file name before the start of a Kinetics reaction.

**Set up visual system monitoring**

## 14. Select the ‘Show Status Display’ checkbox to display various information fields on your current reaction.

**Finish setup**

## 15. Click “OK” to save any changes you have made and close the Setup dialog.

**Zero the instrument**

## 16. To Zero the instrument:

- (a) Click the “Zero” button to zero the system. Alternatively, select *Zero* in the *Commands* menu to perform a zero. A Cell Loading Guide dialog box will be displayed.
- (b) If you want, 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).

**Start the Kinetics run**

## 17. To start the Kinetics run:

- (a) Press the application “Start” button to start a data collection. Alternatively, you can select *Start* in the *Commands* menu. *Do not add your active reagent at this time*. The system will display the Save As dialog box.
- (b) Enter the file name for this Kinetics run in the *File name* field and press “Save”. The system will display a Cell Loading Guide box.
- (c) If you want, 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 appear.

- (e) Reset the Multicell Holder to position to cell 1 using the “Reset Slide” button.
- (f) Add your active reagent just before the Count Down reaches 0:00, or commence the data collection by pressing the “OK” button.

#### **4.6.3 How to perform a temperature controlled Kinetics run (Cary 50)**

This demonstrates how to perform a single cell, multi-rate Kinetics run at 37 °C using the single cell Peltier accessory (Cary 50).

##### **Start the Kinetics application**

1. On the Windows Taskbar, click “Start” to display the *Start* menu.
2. Move the cursor over the *Programs* menu item to display the *Programs* menu.
3. Select *Kinetics* in the *Cary WinUV* menu.
4. Select your instrument type (if necessary) and press “OK” to open the Kinetics application.  
(Alternatively, you can double click on the Kinetics icon in the Cary WinUV folder on the desktop.)



**Note** If you are running a GLP system, you may be prompted to enter a password before accessing the Kinetics application.

##### **Set up data collection parameters**

5. *Setup dialog*  
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.

To do this:

##### **Ensure Multicell Holder accessory is not selected**

6. *Setup dialog / Accessories tab*  
As various options do not become available until the appropriate accessories are selected, you need to select these first.
  - (a) Select the Accessories tab.
  - (b) Ensure the Use Cell Changer checkbox is not selected.

**Set up accessories for reaction temperature control and temperature display****7. Setup dialog / Accessories tab**

- (a) In the Temperature group, check Automatic Temperature setting to enable the single cell Peltier accessory.
- (b) Set the monitoring temperature by entering the Block temperature as 37 °C.
- (c) In the Temperature Display group, select Probe 1 to view the temperature of one temperature probe in the Status Display window.

**Set up instrument parameters such as wavelength, average time, X mode etc.****8. Setup dialog / Cary tab**

- (a) Select the Cary tab
- (b) In the *Wavelength* field enter the wavelengths that you want monitored.
- (c) Enter an upper range and lower range value in the *Y min* and *Y max* entry fields to specify the displayed ordinate range.
- (d) Select the abscissa (X) mode you require. Click "Min" to time in minutes or "Sec" to time in seconds.

**Set up rate parameters such as the number of different rates in the reaction, the speed, and the duration of the data collection****9. Setup dialog / Cary tab**

- (a) Select the 'Advanced Collect' radio button in the Collect Timing group. 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 kinetics reaction and to collect data less frequently where you know there will not be much activity.
- (b) Enter the number of different reaction rates that you require in the Number of Stages entry field. The number you set here will be reflected in the table below.
  - (c) 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.

- (d) Specify the duration of the measurement run by setting the Stop time for each rate Stage.

#### **Set up collection display options**

- 10. *Setup dialog / Options tab*
  - (a) Select the Options tab.
  - (b) Select the Individual Data radio button to display a separate graph for each cell, or Overlay Data to display all results in the one graph.

#### **Set up rate calculation, rate order and other rate parameters**

- 11. *Setup dialog / Analyze tab*
  - (a) Select the Analyze tab.
  - (b) Select the 'Auto Calculate' checkbox to automatically perform a rate calculation on collected data at the end of each run.
  - (c) Select 'Advanced Calculate' to set up multiple reaction rate calculations for the Kinetics run.
  - (d) Enter the number of different rate calculations that you require in the Number of Stages entry field. The number you set here will be reflected in the table below.
  - (e) 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 Manual Guess group to manually enter the parameters: A0, Ainf and Rate (k). It is presumed that you have a reasonable idea of the values for these fit parameters, as they will be used as a first guess for the Marquardt non-linear regression analysis.

If you do not choose Manual Guess then the Cary system will automatically calculate the A0, AInf and Rate values when the "Start" button is pressed.

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

### Set up reporting and printing requirements

#### 12. Setup dialog / Reports tab

- (a) Select the Reports tab.
- (b) Enter your name in the *Name* entry field.
- (c) Enter a comment relating to your experiment in the *Comment* entry field.
- (d) Set up your report style by selecting the appropriate checkboxes in the Options group.

For example, select the 'Auto Print' checkbox to obtain a printout of your report automatically. Select the 'Parameters' checkbox to include your experimental parameters in the report. Select the 'Graph' checkbox to include a graph in the generated report.



**Note** If 'Auto Print' is selected, then the system will send the report information to the specified printer as well as the Report area. However, if 'Auto Print' is not selected, the report will only be sent to the Report area and can be viewed by selecting the *Report* option in the *View* menu.

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

### Set up storage of collected data

#### 13. Setup dialog / Auto Store tab

- (a) Select the Auto Store tab to set up whether the collected data is to be saved, and if so, when the Cary should store the information.
- (b) Select 'Storage On (Prompt at Start)' to set up the Cary to prompt you for a file name before the start of a Kinetics run.

### Set up visual system monitoring

- 14. Select the 'Show Status Display' checkbox to display various information fields on your current reaction.

**Finish setup**

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

**Zero the instrument**

16. To Zero the instrument:
  - (a) Click the "Zero" button to zero the system. Alternatively, select *Zero* in the *Commands* menu to perform a zero. A Loading Guide dialog box will be displayed.
  - (b) If you want, 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.

**Start the Kinetics run**

17. To start the Kinetics run:
  - (a) Press the application "Start" button to start a data collection. Alternatively, you can select *Start* in the *Commands* menu. *Do not add your active reagent at this time*. The system will display the Save As dialog box.
  - (b) Enter the file name for this Kinetics run in the *File name* field and press "Save". The system will display a Loading Guide dialog box.
  - (c) If you want, change the name of the sample.
  - (d) Place the sample solution in the correct position and click "OK". The system will set up the Graphics area and then display Sync Start dialog box.
  - (e) Add your active reagent just before the Count Down reaches 0:00 or commence the data collection by pressing the "OK" button.

## 4.7 RNA/DNA

The following RNA/DNA How to... procedures are described:

- How to perform a temperature controlled run using the Multicell Holder (Cary 100–500)
- How to perform a run using the Multicell Holder (Cary 50)

#### 4.7.1 How to perform a temperature controlled run using the Multicell Holder (Cary 100–500)

This demonstrates 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, 400 and 500).

##### Start the RNA-DNA application

1. On the Windows Taskbar, click “Start” to display the *Start* menu.
2. Move the cursor over the *Programs* menu item to display the *Programs* menu.
3. Select *RNA - DNA* in the *Cary WinUV* menu.
4. Select your instrument type (if necessary) and press “OK” to open the RNA-DNA application.

(Alternatively, you can double click on the RNA-DNA icon in the Cary WinUV folder on the desktop.)



- Note** If you are running a GLP system, you may be prompted to enter a password before accessing the RNA-DNA application.

##### Set up data collection parameters

5. *Setup dialog*

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.

To do this:

##### Set up Multicell Holder accessory with multi zero

6. *Setup dialog / Accessories 1 tab*

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

- (a) Select the Accessories 1 tab.
- (b) Select the Use Cell Changer checkbox to enable the accessory.
- (c) For Cary 100/300 instruments, choose the type of Multicell Holder you are using (6 x 6 or 8 x 6).
- (d) Ensure that you have this accessory installed prior to commencing the run.

- (e) Choose the Select Cells radio button and select the cells you require from the available cells in the Use Cells group.  
For double beam analysis, select the checkboxes Cell 1 through to the Cell 6 (6 x 6) or Cell 1 through 8 (8 x 6).
- (f) Check the Multi Zero checkbox to turn on the Multi Zero facility.  
The Multi Baseline facility will not be accessible until completing Step 8.

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

7. *Setup dialog / Accessories 1 tab*

- (a) If you are not using a Peltier controlled accessory (e.g. the water thermostatted 8 x 6) ensure that you have the Temperature Controller accessory installed prior to commencing the run, and,
  - (i) Tick Automatic Temperature Setting and select the 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 8 part (d)).
- (b) In the Temperature Display group, select Block and Probe 1 to view the temperature of the Multicell Holder block and one temperature Probe in the Status Display window.

**Set up instrument parameters such as wavelength, etc.**

8. *Setup dialog / Cary and Baseline tabs*

- (a) Select the Cary tab.
- (b) Enter the first and second wavelengths at which you would like to measure your sample/s.
- (c) If you require background correction, select the Background Correction checkbox and enter a background wavelength in the corresponding field.
- (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 in the Accessories 1 tab (Step 7 part (a)(ii)).

- (e) To perform a wavelength scan, check ‘Scan Samples’. The Baseline tab will appear. Make your Wavelength Scan selections on the Cary tab, then select the Baseline tab.
- (f) Select ‘Baseline correction’. To use a previously stored baseline (\*.CDN), click on 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 tab and check the Multi Baseline checkbox which will now be activated.

#### **Set up source, SBW and display options**

- 9. *Setup dialog / Options tab*
  - (a) Select the Options tab.
  - (b) Check the Auto Lamps Off checkbox if you want to automatically turn off the lamps at the end of a collect. This option enables you to preserve the lifetime of the lamps. It is especially useful for when performing data collections overnight or unattended for long periods of time.
  - (c) Click on the UV/Vis radio button if you want both the lamps on during the run.
  - (d) Enter your required *Source Changeover and Detector Changeover* (Cary 500 only) wavelengths in the corresponding entry fields.
  - (e) Enter the spectral bandwidth of your instrument in the *SBW* field.
  - (f) Set the appropriate Slit Height (Cary 400/500 only).
  - (g) In the Display Options group, select the way in which you want the data displayed as it is collected. Choose the Individual Data radio button to display the collected data of each sample in individual graph boxes. Choose the Overlay Data radio button to superimpose the collected data of each sample in the run in one graph box.

#### **Set up samples and increment names**

- 10. *Setup dialog / Samples tab*
  - (a) Select the Samples tab. This tab allows you to enter a list of sample names that will be used during your analysis.

- (b) 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.
- (c) In the Sample Names list, enter the name of each sample. You can enter up to 20 characters for each name.
- (d) De-select the samples that you do not wish to analyze by clicking beside the desired sample in the small first column to remove the red tick.
- (e) If the samples have the same name with a different numeric extension, enter the name in the first sample position and then press the “Increment” button.
- (f) If you would like multiple readings of the same aliquot, select the Replicates checkbox and enter the number of replicates required in the data entry field that appears.

#### **Set up analysis parameters**

##### **11. Setup dialog / Analyze tab**

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

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

##### **12. Setup dialog / Reports tab**

- (a) Select the Reports tab.
- (b) Enter your name in the *Name* entry field.
- (c) Enter a comment relating to your experiment in the *Comment* entry field.
- (d) Set up your report style by selecting the appropriate checkboxes in the Options group.  
For example, select the Auto Print checkbox to obtain a printout of your report automatically.  
Select the Parameters checkbox to include your experimental parameters in the report.  
If you have selected ‘Scan Samples’ on the Cary tab, select the Graph checkbox to include a graph in the generated report.



**Note** If Auto Print is selected, then the system will send the report information to the specified printer as well as to the Report area. However if Auto Print is not selected, the report will only be sent to the Report area and can be viewed by selecting the *Report* option in the *View* menu.

- (e) Select the Autoconvert option you require.

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

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

13. *Setup dialog / Auto Store tab*
  - (a) Select the Auto Store tab to set up whether the collected data is to be saved, and if so, when the Cary should store the information.
  - (b) Select Storage On (Prompt at Start), to set up the Cary to prompt you for a file name before the start of an RNA-DNA run.

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

14. Select the Show Status Display checkbox on any of the tabs to display various information fields on your current reaction.

#### **Finish setup**

15. Click "OK" to save any changes you have made and close the Setup dialog.

#### **Zero the instrument**

16. To zero the instrument:
  - (a) Click the "Zero" button to zero the system. Alternatively, select Zero in the *Commands* menu to perform a zero. A Cell Loading Guide dialog box 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 7 part (e), 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 pressing "OK", the system will perform a zero on the cell position in the light path, i.e. the system will not reset the Multicell Holder to position 1.

**Perform a baseline correction (if not using a stored baseline)**

17. If you have selected to perform a baseline correction (Step 8(f)) and are not using a stored baseline, take a baseline reading now by following the steps below. Otherwise, proceed to Step 18.
  - (a) Click the "Baseline" button. A Cell Loading Guide dialog box will appear.
  - (b) Load the blank/s as depicted.
  - (c) Click "OK". The system will collect a baseline for each cell in use and the word 'baseline' will appear above the ordinate instrument status reading.

**Start the RNA-DNA run**

18. To start the run:
  - (a) Press the "Start" button to start a data collection. Alternatively you can select *Start* in the *Commands* menu. The system will display the Save As dialog box.
  - (b) Enter the file name for this RNA-DNA run in the *File name* field and press "Save". The system will display a Cell Loading Guide dialog box.
  - (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.

**4.7.2 How to perform a run using the Multicell Holder (Cary 50)**

This demonstrates how to perform a multicell wavelength scan with baseline correction, multi zero and multi baseline (Cary 50).

**Start the RNA-DNA application**

1. On the Windows Taskbar, click "Start" to display the *Start* menu.
2. Move the cursor over the *Programs* menu item to display the *Programs* menu.
3. Select *RNA - DNA* in the *Cary WinUV* menu.
4. Select your instrument type (if necessary) and press "OK" to open the RNA-DNA application.  
(Alternatively, you can double click on the RNA-DNA icon in the Cary WinUV folder on the desktop.)



**Note** If you are running a GLP system, you may be prompted to enter a password before accessing the RNA-DNA application.

**Set up data collection parameters****5. Setup dialog**

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.

To do this:

**Set up instrument parameters such as wavelength, etc.****6. Setup dialog / Cary and Baseline tabs**

- (a) Select the Cary tab.
- (b) Enter the first and second wavelengths at which you would like to measure your sample.
- (c) If you require background correction, select the Background Correction checkbox and enter a background wavelength in the corresponding field.
- (d) If you are performing a wavelength scan, check ‘Scan Samples’. The Display Options group will be activated and the Baseline tab will appear.
- (e) Enter a Start and Stop wavelength value and select a Scan Rate.
- (f) In the Display Options group, select the way in which you want the data displayed as it is collected.  
Choose the Individual Data radio button to display the collected data of each sample in individual graph boxes. Choose the Overlay Data radio button to superimpose the collected data of each sample in the run in one graph box.
- (g) Select the Baseline tab and check ‘Baseline correction’.
- (h) To use a previously stored baseline (\*.CDN), click on the “Baseline...” button and browse for the appropriate file. Otherwise, you can perform your baseline correction at the beginning of the run.

**Set up Multicell Holder accessory with multi zero****7. Setup dialog / Accessories 1 tab**

- (a) Select the Accessories 1 tab.
- (b) Select the Use Cell Changer checkbox to enable the accessory.
- (c) Ensure that you have this accessory installed prior to commencing the run.
- (d) Choose the Select Cells radio button and select the cells you require from the available cells in the Use Cells group.

- (e) Check the Multi Zero checkbox to turn on the Multi Zero facility.
- (f) Check Multi Baseline to turn on the Multi Baseline facility.

#### **Set up samples and increment names**

- 8. *Setup dialog / Samples tab*
  - (a) Select the Samples tab. This tab allows you to enter a list of sample names that will be used during your analysis.
  - (b) 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.
  - (c) In the Sample Names list, enter the name of each sample. You can enter up to 20 characters for each name.
  - (d) De-select any samples that you do not wish to analyze by clicking beside the desired sample in the small first column to remove the red tick.
  - (e) If the samples have the same name with a different numeric extension, enter the name in the first sample position and then press the “Increment” button.
  - (f) If you would like multiple readings of the same aliquot, select the Replicates checkbox and enter the number of replicates required in the data entry field that appears.

#### **Set up analysis parameters**

- 9. *Setup dialog / Analyze tab*

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

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

- 10. *Setup dialog / Reports tab*
  - (a) Select the Reports tab.
  - (b) Enter your name in the *Name* entry field.
  - (c) Enter a comment relating to your experiment in the *Comment* entry field.
  - (d) Set up your report style by selecting the appropriate checkboxes in the Options group.

For example, select the Auto Print checkbox to obtain a printout of your report automatically.  
 Select the Parameters checkbox to include your experimental parameters in the report.  
 If you have selected 'Scan Samples' on the Cary tab, select the Graph checkbox to include a graph in the generated report.



**Note** If Auto Print is selected, then the system will send the report information to the specified printer as well as to the Report area. However if Auto Print is not selected, the report will only be sent to the Report area and can be viewed by selecting the *Report* option in the *View* menu.

- (e) Select the Autoconvert option you require.

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

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

##### 11. *Setup dialog / Auto Store tab*

- (a) Select the Auto Store tab to set up whether the collected data is to be saved, and if so, when the Cary should store the information.
- (b) Select Storage On (Prompt at Start), to set up the Cary to prompt you for a file name before the start of a RNA-DNA run.

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

##### 12. Select the Show Status Display checkbox on any of the tabs to display various information fields on your current reaction.

#### **Finish setup**

##### 13. Click "OK" to save any changes you have made and close the Setup dialog.

#### **Zero the instrument**

##### 14. To zero the instrument:

- (a) Click the "Zero" button to zero the system. Alternatively, select *Zero* in the *Commands* menu to perform a zero. A Cell Loading Guide dialog box 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 7 part (e), 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 pressing OK, the system will perform a zero on the cell position in the light path, i.e. the system will not reset the Multicell Holder to position 1.

#### **Collect baselines (if not using a stored baseline)**

15. If you have selected to perform a baseline correction (Step 6(g)) and are not using a stored baseline, take a baseline reading now by following the steps below. Otherwise, proceed to Step 16.
  - (a) Press the "Baseline" button to collect a baseline for each cell. A Cell Loading Guide dialog box will appear.
  - (b) Load the blank/s as depicted.
  - (c) Click "OK". On completion of the baseline collections, the word 'baseline' will appear in red above the ordinate instrument status reading.

#### **Start the RNA-DNA run**

16. To start the run:
  - (a) Press the "Start" button to start a data collection. Alternatively you can select *Start* in the *Commands* menu. The system will display the Save As dialog box.
  - (b) Enter the file name for this RNA-DNA run in the *File name* field and press "Save". The system will display a Cell Loading Guide dialog box.
  - (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.

## **4.8 Scan**

The following Scan How to... procedures are described.

- How to perform a scan with baseline correction (Cary 100–500)
- How to perform a scan in Signal-to-Noise mode (Cary 100–500)
- How to perform a scan in Independent mode (Cary 500)
- How to perform a scan with baseline correction (Cary 50)

#### 4.8.1 How to perform a scan with baseline correction (Cary 100–500)

This demonstrates how to perform a wavelength scan from 500 nm to 400 nm with baseline correction (Cary 100, 300, 400, 500).

##### Start the Scan application

1. On the Windows Taskbar, click “Start” to display the *Start* menu.
2. Move the cursor over the *Programs* menu item to display the *Programs* menu.
3. Select *Scan* in the *Cary WinUV* menu.
4. Select your instrument type (if necessary) and press “OK” to open the Scan application.  
(Alternatively, you can double click on the Scan icon in the Cary WinUV folder on the desktop.)



**Note** If you are running a GLP system you may be prompted to enter a password before accessing the Scan application.

##### Set up data collection parameters

5. *Setup dialog*

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.

To do this:

##### Set up instrument parameters such as X Mode, Y Mode, wavelength range etc.

6. *Setup dialog / Cary tab*

- (a) Set the appropriate abscissa mode for the scan in the *X Mode* field, for example ‘nanometers’.
- (b) Set the wavelength range for the scan by entering the values you require in the *Start/Stop* fields, for example ‘500/400’.
- (c) In the *Y Mode* field, select the ordinate mode in which you want the collect data to be displayed, for example ‘Abs’.
- (d) Enter an upper range and lower range value in the *Y min* and *Y max* entry fields to specify the displayed ordinate range.

- (e) Set the speed of the data collection. With the Cary instruments you do this by setting the Ave Time and Data Interval. In the *Ave Time* field enter the required value. A good starting value is 0.1 second.
- (f) In the Data Interval field, enter the wavelength increment you require between data points. A good starting point is 0.5. The Cary will automatically update the Scan Rate field when you select it.
- (g) Make sure that Cycle Mode is not selected.

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

- 7. *Setup dialog / Options tab*
  - (a) Select the Options tab.
  - (b) Set the SBW for the run. If you are using a Cary 400, select 'Fixed SBW' for the run.
  - (c) Set the Beam Mode for the run. This is commonly set to 'Double'.
  - (d) For Cary 500 do not set the Energy. The Cary will use a fixed SBW for the run and will alter the Energy level automatically to maintain a constant signal level.
  - (e) For Cary 400/500 set the Slit Height to 'Full'.
  - (f) Click on the "UV/Vis" button if you want both lamps on during the run.
  - (g) Check the 'Auto Lamps Off' checkbox if you want to automatically turn off the lamps at the end of a collect. This option enables you to preserve the lifetime of the lamps. It is especially useful when performing Scan runs overnight or unattended for long periods of time.
  - (h) Do not select the 'Signal-to-Noise Mode' checkbox.
  - (i) In the Display Options group, select the way in which you want the data displayed as it is collected. Choose the 'Individual Data' radio button to display the collected data of each sample in individual graph boxes. Choose the 'Overlay Data' radio button to superimpose the collected data of each sample in the Scan run in one graph box.
- 8. *Setup dialog / Independent tab*  
If you are using a Cary 500 do not change any options on this tab.

**Set up the baseline correction**

9. *Setup dialog / Baseline tab*
  - (a) Select the Baseline tab.
  - (b) Select ‘Baseline Correction’. This will force the Cary 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 at this stage. To do so, press the “Baseline” button and open the saved CSW baseline file.

**Make sure that no accessories are selected**

10. *Setup dialog / Accessories 1 tab*

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

11. *Setup dialog / Accessories 2 tab*

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

12. *Setup dialog / Accessories 3 tab*

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

**Set up reporting and printing requirements**

13. *Setup dialog / Reports tab*

- (a) Select the Reports tab and specify your reporting requirements for this method.
- (b) Enter your name in the *Name* entry field.
- (c) Enter a comment relating to your sample in the *Comment* entry field.
- (d) Set up your report style by selecting the appropriate checkboxes in the Options group.

For example, select the ‘AutoPrint’ checkbox to obtain a printout of your report automatically.

Select the ‘Parameters’ checkbox to include your experimental parameters in the report.

Select the ‘Graph’ checkbox to include a graph in the printed report.



**Note** If ‘AutoPrint’ is selected, then 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 selecting *Report* in the *View* menu.

- (e) Set up the Peak Table reporting.
  - (i) Select Peak Labels.
  - (ii) Press the “Peak Information” button and choose the type of Peak Labels, the Peak Style and set the Peak Threshold. Press “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.
- (f) 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.
- (g) Select the ‘Autoconvert’ option you require. If you select ‘Select for ASCII (csv)’ or ‘Select for ASCII (csv) with Log’, then at the end of the data collection the system will automatically generate a report and store the data both in the Cary format as well as ASCII XY pairs format in the current folder.

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

##### **14. Setup dialog / Auto Store tab**

- (a) Select the Auto Store tab to set up whether the collected data is to be saved, and if so, when the Cary should store the information.
- (b) Select ‘Storage On (Prompt at Start)’.

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

##### **15. Select the ‘Show Status Display’ checkbox to display various information fields on your current reaction.**

#### **Finish setup**

##### **16. Once you are satisfied with your method setup click “OK” to confirm any changes you have made and close the Setup dialog.**

#### **Zero the instrument**

##### **17. Click the “Zero” button to zero the system. Alternatively, select Zero in the *Commands* menu to perform a zero.**

#### **Measure a baseline**

##### **18. To measure a baseline:**

- (a) Click the “Baseline” button to set up the baseline collection the system. Alternatively, select Baseline in the *Commands* menu.

- (b) When prompted, insert the blank sample into the sample compartment front beam and press "OK". The Cary will collect the baseline scan. After the collection, the word 'baseline' will appear 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.



**Note** If the word 'baseline' is gray and italicized, the baseline file is still valid. The gray and italics indicates that the Cary is idling outside the abscissa range of the baseline file.

#### Start the Scan run

19. Click the application "Start" button to start a data collection. Alternatively, select *Start* in the *Commands* menu.

#### Set up file name for the data and sample names

20. To set up a file name:
  - (a) Once you press "Start", the Windows Save As dialog will appear. Enter the appropriate file name for data and press "Save".
  - (b) The Sample Name dialog will now appear. Enter the appropriate name for your sample and press "OK". This is the name that the trace will be displayed and printed with.

The Scan run will commence and the corrected trace will appear 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 in the Reports tab of the Setup dialog.

### 4.8.2 How to perform a scan in Signal-to-Noise mode (Cary 100-500)

This demonstrates how to perform a wavelength scan from 500 nm to 400 nm in signal-to-noise mode (Cary 100, 300, 400, 500).

#### Start the Scan application

1. On the Windows Taskbar, click "Start" to display the *Start* menu.
2. Move the cursor over the *Programs* menu item to display the *Programs* menu.
3. Select *Scan* in the *Cary WinUV* menu.
4. Select your instrument type (if necessary) and press "OK" to open the Scan application.  
(Alternatively, you can double click on the Scan icon in the Cary WinUV folder on the desktop.)



Note If you are running a GLP system you may be prompted to enter a password before accessing the Scan application.

#### Set up data collection parameters

##### 5. Setup dialog

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.

To do this:

#### Set up instrument parameters such as X Mode, Y Mode, wavelength range etc.

##### 6. Setup dialog / Cary tab

- (a) Set the appropriate abscissa mode for the scan in the *X Mode* field, for example ‘nanometers’.
- (b) Set the wavelength range for the scan by entering the values you require in the *Start/Stop* fields, for example ‘500/400’.
- (c) In the *Y Mode* field select the ordinate mode in which you want the collect data to be displayed, for example ‘Abs’.
- (d) Enter an upper range and lower range value in the *Y min* and *Y max* entry fields to specify the displayed ordinate range. If the data goes outside the display region you can use the “Autoscale” button during the data collection to automatically scale the display.
- (e) You now need to set the speed of the data collection. With the Cary instruments you do this by setting the Ave Time and Data Interval. In the *Ave Time* field enter the required value. 0.1 sec is a good starting value.
- (f) In the *Data Interval* field, enter the wavelength increment you require between data points. A good starting point is 0.5. The Cary will automatically update the *Scan Rate* field when you select it.
- (g) Make sure that Cycle Mode is not selected.

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

##### 7. Setup dialog / Options tab

- (a) Select the Options tab.
- (b) If you are using a Cary 400, select ‘Fixed SBW’ for the run.
- (c) If you are using a Cary 100, 300 or 500, set the SBW for the run. A good starting point is 2 nm if your method does not specify a SBW.

- (d) Set the Beam Mode for the run. This is commonly set to 'Double'.
- (e) If you are using a Cary 500, do not set the Energy. The Cary will use a fixed SBW for the run and will alter the Energy level automatically to maintain a constant signal level.
- (f) If you are using a Cary 400/500, set the Slit Height to 'Full'.
- (g) Click on the "UV/Vis" button if you want both the lamps on during the run.
- (h) Check the 'Auto Lamps Off' checkbox if you want to automatically turn off the lamps at the end of a collect. This option enables you to preserve the lifetime of the lamps. It is especially useful when performing Scan runs overnight or unattended for long periods of time.
- (i) Tick the 'Signal-to-Noise Mode' checkbox, and set the required values for the Acceptable S/N and the S/N Timeout, for example '1000 and 1 s'.
- (j) In the Display Options group, select the way in which you want the data displayed as it is collected. Choose the 'Individual Data' radio button to display the collected data of each sample in individual graph boxes. Choose the 'Overlay Data' radio button to superimpose the collected data of each sample in the Scan run in one graph box.

8. *Setup dialog / Independent tab*

- (a) Make sure that Independent Control is not checked.
- (b) Set measurement Mode to Auto.

**Set up the baseline correction**

9. *Setup dialog / Baseline tab*

- (a) Select the Baseline tab.
- (b) Select 'Baseline Correction'. This will force the Cary to perform a baseline correction on the sample data. The correction will be performed on each point before it is displayed.



Note A stored baseline can be used at this stage. To do so, press the "Baseline" button and open the saved CSW baseline file.

**Make sure that no accessories are selected**

10. *Setup dialog / Accessories 1 tab*

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

11. *Setup dialog / Accessories 2 tab*  
Make sure that no options are selected on this tab and no accessories are installed.
12. *Setup dialog / Accessories 3 tab*  
Make sure that no options are selected on this tab and no accessories are installed.

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

13. *Setup dialog / Reports tab*
  - (a) Select the Reports tab and specify your reporting requirements for this method.
  - (b) Enter your name in the *Name* entry field.
  - (c) Enter a comment relating to your experiment in the *Comment* entry field.
  - (d) Set up your report style by selecting the appropriate checkboxes in the Options group.  
For example, select the ‘AutoPrint’ checkbox to obtain a printout of your report automatically.  
Select the ‘Parameters’ checkbox to include your experimental parameters in the report.  
Select the ‘Graph’ checkbox to include a graph in the generated report.



**Note** If ‘AutoPrint’ is selected, then 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 selecting the *Report* option in the *View* menu.

- (e) Set up the Peak Table reporting.
  - (i) Select Peak Labels.
  - (ii) Press the “Peak Information” button and choose the type of Peak Labels, the Peak Style and set the Peak Threshold. Press “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.
- (f) 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.

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

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

##### **14. Setup dialog / Auto Store tab**

- (a) Select the Auto Store tab to set up whether the collected data is to be saved, and if so, when the Cary should store the information.
- (b) Select ‘Storage On (Prompt at End)’.

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

##### **15. Select the ‘Show Status Display’ checkbox to display various information fields on your current reaction.**

#### **Finish setup**

##### **16. Once you are satisfied with your method setup click “OK” to confirm any changes you have made and close the Setup dialog.**

#### **Zero the instrument**

##### **17. Click the “Zero” button to zero the system. Alternatively, select Zero in the Commands menu to perform a zero.**

#### **Measure a baseline**

##### **18. To measure a baseline:**

- (a) Click the “Baseline” button to set up the baseline collection the system. Alternatively, select Baseline in the Commands menu.
- (b) When prompted, insert the blank sample into the sample compartment front beam and press “OK”. The Cary will collect the baseline scan. After the collection, the word ‘baseline’ will appear 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.



**Note** If the word ‘baseline’ is gray and italicized, the baseline file is still valid. The gray and italics indicates that the Cary is idling outside the abscissa range of the baseline file.

#### **Start the Scan run**

##### **19. Click the application “Start” button to start a data collection. Alternatively, you can select Start in the *Commands* menu.**

**Set up sample names**

20. Once you press “Start”, the Sample Name dialog will appear. Enter the appropriate name for your sample and press “OK”. The Scan run will commence and the corrected trace will appear in the Graphics area.  
If for a particular point, the set Acceptable S/N cannot be met in the set S/N Timeout time, then 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.

**Save the collected data**

21. When the Cary has measured the sample, the Save As dialog box will appear. Enter the appropriate name for your sample press “Save”. The Cary will then create the report and also print it, if ‘AutoPrint’ has been selected in the Reports tab of the Setup dialog.

**4.8.3 How to perform a scan in Independent mode (Cary 500)**

This demonstrates how to perform a wavelength scan from 2200 nm to 400 nm in Independent mode (Cary 500 only).

**Start the Scan application**

1. On the Windows Taskbar, click “Start” to display the *Start* menu.
2. Move the cursor over the *Programs* menu item to display the *Programs* menu.
3. Select *Scan* in the *Cary WinUV* menu.
4. Select your instrument type (if necessary) and press “OK” to open the Scan application.  
(Alternatively, you can double click on the Scan icon in the Cary WinUV folder on the desktop.)



**Note** If you are running a GLP system you may be prompted to enter a password before accessing the Scan application.

**Set up data collection parameters**

5. 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.

To do this:

**Set up instrument parameters such as X Mode, Y Mode, wavelength range etc.**

6. *Setup dialog / Cary tab*
  - (a) Set the appropriate abscissa mode for the scan in the *X Mode* field, for example ‘nanometers’.
  - (b) Set the wavelength range for the scan by entering the values you require in the *Start/Stop* fields, for example ‘2200/400’.
  - (c) In the *Y Mode* field select the ordinate mode in which you want the collect data to be displayed, for example ‘Abs’.
  - (d) Enter an upper range and lower range value in the *Y min* and *Y max* entry fields to specify the displayed ordinate range. If the data goes outside the display range during the collection you can use the “Autoscale” to automatically scale the data.
  - (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.

**Set up SBW, lamp and graphics options**

7. *Setup dialog / Options tab*
  - (a) Select the Options tab.
  - (b) Do not set the SBW or Energy as the settings in Independent mode will override these.
  - (c) Set the Beam Mode for the run. This is commonly set to ‘Double’.
  - (d) Set the Slit Height to ‘Full’.
  - (e) Click on the “UV/Vis” button if you want both the lamps on during the run.
  - (f) Check the ‘Auto Lamps Off’ checkbox if you want to automatically turn off the lamps at the end of a collect. This option enables you to preserve the lifetime of the lamps. It is especially useful when performing Scan runs overnight or unattended for long periods of time.

- (g) In the Display Options group, select the way in which you want the data displayed as it is collected. Choose the 'Individual Data' radio button to display the collected data of each sample in individual graph boxes. Choose the 'Overlay Data' radio button to superimpose the collected data of each sample in the Scan run in one graph box.

#### **Set up the signal level controls (SBW/Energy) for the scan**

8. *Setup dialog / Independent tab*
  - (a) Select the Independent tab.
  - (b) Tick the 'Independent Control' checkbox.
  - (c) Select the Measurement Mode as 'Auto'. This will mean that the Cary will use a Fixed SBW in the UV-Vis region and a fixed Energy level in the NIR region.
  - (d) In the UV-Vis group, 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.
  - (e) In the NIR group, set the parameters you require in the NIR region. Good starting values are: Ave Time = 0.1, Data Interval = 4, Scan Rate = 2400, Energy=1.

#### **Set up baseline correction**

9. *Setup dialog / Baseline tab*
  - (a) Select the Baseline tab.
  - (b) Select 'Baseline Correction'. This will force the Cary 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 at this stage. To do so, press the "Baseline" button and open the saved CSW baseline file.

#### **Make sure that no accessories are selected**

10. *Setup dialog / Accessories 1 tab*  
Make sure that no options are selected on this tab and no accessories are installed.
11. *Setup dialog / Accessories 2 tab*  
Make sure that no options are selected on this tab and no accessories are installed.
12. *Setup dialog / Accessories 3 tab*  
Make sure that no options are selected on this tab and no accessories are installed.

## Set up reporting and printing requirements

### 13. Setup dialog / Reports tab

- (a) Select the Reports tab and specify your reporting requirements for this method.
- (b) Enter your name in the *Name* entry field.
- (c) Enter a comment relating to your experiment in the *Comment* entry field.
- (d) Set up your report style by selecting the appropriate checkboxes in the Options group.

For example, select the ‘AutoPrint’ checkbox to obtain a printout of your report automatically. Select the ‘Parameters’ checkbox to include your experimental parameters in the report. Select the ‘Graph’ checkbox to include a graph in the generated report.



**Note** If ‘AutoPrint’ is selected, then 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 selecting the *Report* option in the *View* menu.

- (e) Set up the Peak Table reporting.
  - (i) Select Peak Labels.
  - (ii) Press the “Peak Information” button and choose the type of Peak Labels, the Peak Style and set the Peak Threshold. Press “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.
- (f) 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.
- (g) Select the ‘Autoconvert’ option you require. If you select ‘Select for ASCII (csv)’ or ‘Select for ASCII (csv) with Log’, then at the end of the data collection the system will automatically generate a report and store the data both in the Cary format as well as ASCII XY pairs format in the current folder.

**Set up storage of collected data**

14. *Setup dialog / Auto Store tab*
  - (a) Select the Auto Store tab to set up whether the collected data is to be saved, and if so, when the Cary should store the information.
  - (b) Select 'Storage On (Prompt at End)'.

**Set up visual system monitoring**

15. Select the 'Show Status Display' checkbox to display various information fields on your current reaction.

**Finish setup**

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

**Zero the instrument**

17. Click the "Zero" button to zero the system. Alternatively, select *Zero* in the *Commands* menu to perform a zero.

**Measure a baseline**

18. To measure a baseline:
  - (a) Click the "Baseline" button to set up the baseline collection. Alternatively, select *Baseline* in the *Commands* menu.
  - (b) When prompted, insert the blank sample into the sample compartment front beam and press "OK".

The Cary will collect the baseline scan. After the collection, the word 'baseline' will appear 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.



**Note** If the word 'baseline' is gray and italicized, the baseline file is still valid. The gray and italics indicates that the Cary is idling outside the abscissa range of the baseline file.

**Start the Scan run**

19. Click the application "Start" button to start a data collection. Alternatively, you can select *Start* in the *Commands* menu.

**Set up sample names**

20. Once you press "Start", the Sample Name dialog will appear. Enter the appropriate name for your sample and press "OK". The Scan run will commence and the corrected trace will appear in the Graphics area.

**Save the collected data**

21. When the Cary has measured the sample, the Save As dialog box will appear. Enter the appropriate name for your sample press "Save". The Cary will then create the report and also print it, if 'AutoPrint' has been selected in the Reports tab of the Setup dialog.

**4.8.4 How to perform a scan with baseline correction (Cary 50)**

This demonstrates how to perform a wavelength scan from 500 nm to 400 nm with baseline correction (Cary 50).

**Start the Scan application**

1. On the Windows Taskbar, click "Start" to display the *Start* menu.
2. Move the cursor over the *Programs* menu item to display the *Programs* menu.
3. Select *Scan* in the *Cary WinUV* menu.
4. Select your instrument type (if necessary) and press "OK" to open the Scan application.  
(Alternatively, you can double click on the Scan icon in the Cary WinUV folder on the desktop.)



- Note If you are running a GLP system you may be prompted to enter a password before accessing the Scan application.

**Set up data collection parameters**

5. *Setup dialog*

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.

To do this:

**Set up instrument parameters such as wavelength range, Y Mode, scan speed etc.**

6. *Setup dialog / Cary tab*

- (a) Set the wavelength range for the scan by entering the values you require in the *Start/Stop* fields, for example '500/400'.

- (b) In the *Y Mode* field select the ordinate mode in which you want the collect data to be displayed, for example 'Abs'.
- (c) Enter an upper range and lower range value in the *Y min* and *Y max* entry fields to specify the displayed ordinate range.
- (d) Make sure that 'Cycle Mode' is not selected.
- (e) Set the Beam Mode for the run. This is should be set to 'Dual Beam'.
- (f) In the Scan Controls group, select 'Simple' and click a scan speed button. Alternatively, you can select 'Advanced' and enter an Ave Time and Data Interval (the Cary will then select the Scan Speed).
- (g) In the Display Options group, select the way in which you want the data displayed as it is collected. Choose the 'Individual Data' radio button to display the collected data of each sample in individual graph boxes. Choose the 'Overlay Data' radio button to superimpose the collected data of each sample in the Scan run in one graph box.

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

7. *Setup dialog / Baseline tab*
  - (a) Select the Baseline tab.
  - (b) Select 'Baseline Correction'. This will force the Cary 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 at this stage. To do so, press the "Baseline" button and open the saved CSW baseline file.

#### **Make sure that no accessories are selected**

8. *Setup dialog / Accessories 1 tab*  
Make sure that no options are selected on this tab and no accessories are installed.
9. *Setup dialog / Accessories 2 tab*  
Make sure that no options are selected on this tab and no accessories are installed.

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

10. *Setup dialog / Reports tab*
  - (a) Select the Reports tab and specify your reporting requirements for this method.

- (b) Enter your name in the *Name* entry field.
- (c) Enter a comment relating to your experiment in the *Comment* entry field.
- (d) Set up your report style by selecting the appropriate checkboxes in the Options group.  
For example, select the ‘AutoPrint’ checkbox to obtain a printout of your report automatically. Select the ‘Parameters’ checkbox to include your method parameters in the report. Select the ‘Graph’ checkbox to include a graph in the generated report.



**Note** If ‘AutoPrint’ is selected, then 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 selecting *Report* in the *View* menu.

- (e) Set up the Peak Table reporting.
  - (i) Select Peak Labels.
  - (ii) Press the “Peak Information” button and choose the type of Peak Labels, the Peak Style and set the Peak Threshold. Press “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.
- (f) 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.
- (g) Select the Autoconvert option you require. If you select ‘Select for ASCII (csv)’ or ‘Select for ASCII (csv) with Log’, then at the end of the data collection the system will automatically generate a report and store the data both in the Cary format as well as ASCII XY pairs format in the current folder.

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

##### **11. Setup dialog / Auto Store tab**

- (a) Select the Auto Store tab to set up whether the collected data is to be saved, and if so, when the Cary should store the information.
- (b) Select ‘Storage On (Prompt at Start)’.

**Set up visual system monitoring**

12. Select the 'Show Status Display' checkbox to display various information fields on your current reaction.

**Finish setup**

13. Once you are satisfied with your method setup click "OK" to confirm any changes you have made and close the Setup dialog.

**Zero the instrument**

14. Click the "Zero" button to zero the system.

**Measure a baseline**

15. To measure a baseline:
  - (a) Click the "Baseline" button to set up the baseline collection the system. Alternatively, select *Baseline* in the *Commands* menu.
  - (b) When prompted, insert the blank sample into the sample compartment front beam and press "OK".  
The Cary will collect the baseline scan. After the collection, the word 'baseline' will appear 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.



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

**Start the Scan run**

16. Click the application "Start" button to start a data collection. Alternatively, you can select *Start* in the *Commands* menu.

**Set up file name for the data and sample names**

17. To set up a file name:
  - (a) Once you press "Start", the Windows Save As dialog will appear. Enter the appropriate name for your Scan run and press "Save".
  - (b) The Sample Name dialog will now appear. Enter the appropriate name for your sample and press "OK". The Scan run will commence and the corrected trace will appear 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 in the Reports tab of the Setup dialog.

## 4.9 Scanning Kinetics

The following Scanning Kinetics How to... procedures are described.

- How to collect data using the Multicell Holder with temperature control (Cary 100–500)
- How to collect data (Cary 50)

### 4.9.1 How to collect data using the Multicell Holder with temperature control (Cary 100–500)

This demonstrates how to perform a multicell, multi-stage wavelength scan with baseline correction from 600 to 500 nanometers at 37 °C using the Temperature Controller accessory with the Multicell Holder accessory (Cary 100, 300, 400 and 500).

You can then use the data to create kinetics continuums in order to calculate reaction rates.

#### Start the Scanning Kinetics application

1. On the Windows Taskbar, click "Start" to display the *Start* menu.
2. Move the cursor over the *Programs* menu item to display the *Programs* menu.
3. Select *Scanning Kinetics* in the *Cary WinUV* menu.
4. Select your instrument type (if necessary) and press "OK" to open the Scanning Kinetics application.  
(Alternatively, you can double click on the Scanning Kinetics icon in the Cary WinUV folder on the desktop.)



Note If you are running a GLP system, you may be prompted to enter a password before accessing the Scanning Kinetics application.

#### Set up data collection parameters

5. *Setup dialog*  
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.

To do this:

**Select baseline correction type**

6. *Setup dialog / Baseline tab*
  - (a) Select the Baseline tab.
  - (b) Select the Baseline correction radio button. 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 at this stage. To do so, press the "Retrieve Baseline file" button and open the saved \*.CSK baseline file.

**Set up Multicell Holder accessory with multi zero**

7. *Setup dialog / Accessories tab*
  - (a) Select the Accessories tab.
  - (b) Ensure that you have the appropriate accessories installed prior to commencing the run.
  - (c) Select the Use Cell Changer checkbox to enable the Multicell Holder accessory.
  - (d) If the option is available, choose the type of Multicell Holder you are using (6 x 6 or 8 x 6).
  - (e) Choose the Select Cells radio button and select the cells you require from the available cells in the Use Cells group.  
For Front Beam analysis, select the checkboxes 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.
  - (f) Check the Multi Zero checkbox to turn on the Multi Zero facility.

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

8. *Setup dialog / Accessories tab*
  - (a) If you are not using a Peltier controlled accessory (e.g. the water thermostatted 8 x 6) ensure that you have the Temperature Controller accessory installed prior to commencing the run.
  - (b) Tick Automatic Temperature Setting and select the 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 10 part (j).)

- (d) In the Temperature Display group, select Block and Probe 1 to view the temperature of the Multicell Holder block and one temperature Probe in the Status Display window.

**Set up instrument parameters such as X mode, Y mode, wavelength range etc.**

**9. Setup dialog / Cary tab**

Select the Cary tab and specify the Instrument parameters for your analysis.

- (a) Set the appropriate abscissa mode for the scan in the *X Mode* field, for example nanometers.
- (b) Set the wavelength range for the scan by entering the values you require in the *Start/Stop* fields, for example 500/400.
- (c) In the *Y Mode* field, select the ordinate mode you require. Click Abs to specify the Absorbance mode or %T to specify percent Transmittance.
- (d) Enter an upper range and lower range value in the *Y min* and *Y max* entry fields to specify the displayed ordinate range.
- (e) You now need to set the speed of the data collection. With the Cary instruments you do this by setting the Ave Time and Data Interval. In the *Ave Time* field enter the required value. 0.1 sec is a good starting value.
- (f) In the *Data Interval* field, enter the wavelength increment you require between data points. 0.5 nm is a good starting point. The Cary will automatically update the *Scan Rate* field when you select it.

**Set up rate parameters such as the number of different rates in the reaction, the speed and duration of the data collection**

**10. Setup dialog / Cary tab**

Select the Advanced Collect radio button in the Collect Timing group. 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 scanning kinetics reaction and to collect data less frequently where you know there will not be much activity.

- (g) Enter the number of different reaction rates that you require in the *Number of Stages* entry field. The number you set here will be reflected in the table below.

- (h) 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.
- (i) Specify the duration of the Scanning Kinetics run by setting the Stop time for each rate Stage.
- (j) 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 in the Accessories tab (Step 8 part (c)).

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

##### **11. Setup dialog / Options tab**

- (a) Select the Options tab.
- (b) Set the SBW for the run.
- (c) Set the Slit Height to Full.
- (d) Check the Auto Lamps Off checkbox if you want to automatically turn off the lamps at the end of a collect. This option enables you to preserve the lifetime of the lamps. It is especially useful for when performing data collections overnight or unattended for long periods of time.
- (e) Click on the “UV/Vis” button if you want both the lamps on during the run.
- (f) Enter your required *Source Changeover* and *Detector Changeover* (Cary 500 only) wavelengths in the corresponding entry fields.

#### **Set up analysis parameters**

##### **12. Setup dialog / Analyze tab**

This tab is used for post-run analysis.

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

##### **13. Setup dialog / Reports tab**

- (a) Select the Reports tab.
- (b) Enter your name in the *Name* entry field.
- (c) Enter a comment relating to your experiment in the *Comment* entry field.
- (d) Set up your report style by selecting the appropriate checkboxes in the Options group.

For example, select the Auto Print checkbox to obtain a printout of your report automatically.  
 Select the Parameters checkbox to include your experimental parameters in the report.  
 Select the Graph checkbox to include a graph in the generated report.



- Note**
- If Auto Print is selected, then the system will send the report information to the specified printer as well as to the Report area. However if Auto Print is not selected, the report will only be sent to the Report area and can be viewed by selecting the *Report* option in the *View* menu.
  - (e) Check Include X-Y Pairs Table to view a list of abscissa values and their corresponding ordinate values.
  - (f) Select the Autoconvert option you require.
  - (g) If you choose Select for ASCII (csv) or Select for ASCII (csv) with Log, then at the end of the data collection the system will automatically generate a report and store the data both in the Cary format as well as ASCII XY pairs format in the current folder.

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

##### **14. Setup dialog / Auto Store tab**

- (a) Select the Auto Store tab to set up whether the collected data is to be saved, and if so, when the Cary should store the information.
- (b) Select Storage On (Prompt at Start), to set up the Cary to prompt you for a file name before the start of a Scanning Kinetics run.

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

##### **15. Select the Show Status Display checkbox on any of the tabs to display various information fields on your current reaction.**

#### **Finish setup**

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

#### **Measure a baseline**

##### **17. To measure a baseline:**

- (a) If you do not have a valid baseline file, the Cary will prompt you to click the "Baseline" button. Click the "Baseline" button to set up the baseline collection.

- (b) The system will display a Cell Loading Guide dialog box. If you want, 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 appear 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 File | Save Method As. 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.

#### **Zero the instrument**

18. To zero the instrument:

- (a) Click the "Zero" button to zero the system. Alternatively, select *Zero* in the *Commands* menu to perform a zero.
- (b) If you want, change the name of the blank/s.
- (c) Insert blank samples into the appropriate positions of the Multicell Holder and click "OK".

#### **Start the Scanning Kinetics run**

19. To start the run:

- (a) Click the "Start" button to start a data collection. Alternatively you can select *Start* in 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 tab of the Setup dialog. If so, enter the file name for this Scanning Kinetics run in the *File name* field and press "Save".
- (b) The system will display a Cell Loading Guide dialog box. If you want, 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 appear.

- (d) Add your active reagent just before the Count Down reaches 0:00 or commence the data collection by pressing the “OK” button.
- 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.

#### 4.9.2 How to collect data (Cary 50)

This demonstrates how to perform a multicell, multi-stage wavelength scan with baseline correction from 600 to 500 nanometers at ambient temperature (Cary 50).

You can then use the data to create kinetics continuums in order to calculate reaction rates.

##### Start the Scanning Kinetics application

1. On the Windows Taskbar, click “Start” to display the Start menu.
2. Move the cursor over the *Programs* menu item to display the *Programs* menu.
3. Select *Scanning Kinetics* in the *Cary WinUV* menu.
4. Select your instrument type (if necessary) and press “OK” to open the Scanning Kinetics application.  
(Alternatively, you can double click on the Scanning Kinetics icon in the Cary WinUV folder on the desktop.)



Note If you are running a GLP system, you may be prompted to enter a password before accessing the Scanning Kinetics application.

##### Set up data collection parameters

5. *Setup dialog*  
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.

To do this:

##### Set up instrument parameters such as wavelength range, Y Min/Max etc.

6. *Setup dialog / Cary tab*  
Select the Cary tab and specify the Instrument parameters for your analysis.

- (a) Set the wavelength range for the scan by entering the values you require in the *Start/Stop* fields, for example 500/400.
- (b) Enter an upper range and lower range value in the *Y min* and *Y max* entry fields to specify the displayed ordinate range.
- (c) You now need to set the speed of the data collection. With the Cary instruments you do this by setting the Ave Time and Data Interval. In the *Ave Time* field enter the required value. 0.1 sec is a good starting value.
- (d) In the *Data Interval* field, enter the wavelength increment you require between data points. 0.5 nm is a good starting point. The Cary will automatically update the *Scan Rate* field when you select it.

**Set up rate parameters such as the number of different rates in the reaction, the speed and duration of the data collection**

7. *Setup dialog / Cary tab*

- (a) Select the Advanced Collect radio button in the Collect Timing group. 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 scanning kinetics reaction and to collect data less frequently where you know there will not be much activity.
- (b) Enter the number of different reaction rates that you require in the Number of Stages entry field. The number you set here will be reflected in the table below.
  - (c) 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.
  - (d) Specify the duration of the Scanning Kinetics run by setting the Stop time for each rate Stage.

**Select baseline correction type**

8. *Setup dialog / Baseline tab*

- (a) Select the Baseline tab.
- (b) Select the Baseline correction radio button. 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 at this stage. To do so, press the "Retrieve Baseline file" button and open the saved \*.CSK baseline file.

#### **Set up Multicell Holder accessory with multi zero**

9. *Setup dialog / Accessories tab*
  - (a) Select the Accessories tab.
  - (b) Ensure that you have the appropriate accessories installed prior to commencing the run.
  - (c) Select the Use Cell Changer checkbox to enable the Multicell Holder accessory.
  - (d) Choose the Select Cells radio button and select the cells you require from the available cells in the Use Cells group.
  - (e) Check the Multi Zero checkbox to turn on the Multi Zero facility.

#### **Set up analysis parameters**

10. *Setup dialog / Analyze tab*

This tab is used for post-run analysis.

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

11. *Setup dialog / Reports tab*

- (a) Select the Reports tab.
- (b) Enter your name in the *Name* entry field.
- (c) Enter a comment relating to your experiment in the *Comment* entry field.
- (d) Set up your report style by selecting the appropriate checkboxes in the Options group.

For example, select the Auto Print checkbox to obtain a printout of your report automatically. Select the Parameters checkbox to include your experimental parameters in the report. Select the Graph checkbox to include a graph in the generated report.



**Note** If Auto Print is selected, then the system will send the report information to the specified printer as well as to the Report area. However if Auto Print is not selected, the report will only be sent to the Report area and can be viewed by selecting the *Report* option in the *View* menu.

- (e) Check Include X-Y Pairs Table to view a list of abscissa values and their corresponding ordinate values.

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

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

##### **12. Setup dialog / Auto Store tab**

- (a) Select the Auto Store tab to set up whether the collected data is to be saved, and if so, when the Cary should store the information.
- (b) Select Storage On (Prompt at Start), to set up the Cary to prompt you for a file name before the start of a Scanning Kinetics run.

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

##### **13. Select the Show Status Display checkbox on any of the tabs to display various information fields on your current reaction.**

#### **Finish setup**

##### **14. Click “OK” to save any changes you have made and close the Setup dialog.**

#### **Measure a baseline**

##### **15. If you do not have a valid baseline file, the Cary will prompt you to click the “Baseline” button.**

- (a) Click the “Baseline” button to set up the baseline collection.
- (b) If you want, 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 appear 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.

#### **Zero the instrument**

##### **16. To zero the instrument:**

- (a) Click the “Zero” button to zero the system. Alternatively, select *Zero* in the *Commands* menu to perform a zero.
- (b) If you want, change the name of the blank/s.
- (c) Insert blank samples into the appropriate positions of the Multicell Holder and click “OK”.

### Start the Scanning Kinetics run

17. To start the run:

- (a) Click the “Start” button to start a data collection.  
Alternatively you can select *Start* in 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 tab of the Setup dialog. If so, enter the file name for this Scanning Kinetics run in the *File name* field and press “Save”.

- (b) The system will display a Cell Loading Guide dialog box. If you want, 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 appear.
- (d) Add your active reagent just before the Count Down reaches 0:00 or commence the data collection by pressing the “OK” button.

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

## 4.10 Simple Reads

The following Simple Reads How to... procedure is described:

- How to perform a measurement at a single wavelength

### 4.10.1 How to perform a measurement at a single wavelength

#### Start the application

1. On the Windows Taskbar click “Start” to display the *Start* menu.
2. Move the cursor over the *Programs* menu item to display the *Programs* menu.
3. Select *Simple Reads* in the *Cary WinUV* menu to open the Simple Reads application.

4. Select your instrument type (if necessary) and press "OK" to open the Simple Reads application.  
(Alternatively, you can double click on the Simple Reads icon in the Cary WinUV folder on the desktop.)



**Note** If you are running a GLP system you may be prompted to enter a password before accessing the Simple Reads application.

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

5. Click the "Setup..." button or select *Setup* from the Menu line to display the Setup dialog box. This displays the Cary tab where you can specify the parameters for a new read.
6. Select 'Read at Wavelength' and enter the required wavelength at which you want to perform the read.
7. In the Y Mode group select the button corresponding to the ordinate mode you require. The ordinate mode determines the way in which the photometric value is measured and displayed in your report.
8. Click the "OK" button. The instrument will change to the new wavelength.

#### **Zero the instrument**

9. Make sure that the sample compartment is clear (or you have a blank in position) and zero the instrument by pressing the "Zero" button. (Wait for the ordinate reading to reach '0').

#### **Read the sample**

10. Insert the sample into the sample compartment.
11. Wait while the instrument changes to the specified wavelength. The current wavelength is displayed in the Abscissa status display at the top and right of the application window.
12. Click the "Read" button to perform photometric measurements on the sample at the specified wavelength.

The result of the sample read appears in the Report area and includes the ordinate reading obtained and the wavelength at which the reading was measured.

#### **Print the results**

13. You can print the contents of the Report area by pressing the "Print" button.

## 4.11 Sunglasses

The following Sunglasses How to... procedure is described:



**Note** This example assumes that you are using either the Simple or Advanced interface for the Sunglasses application. If you are using the Wizard (black screen with photo of the Sun) you will need to change to another interface to use this example. To change the interface refer to the online Help by clicking on the Windows Start button, select Programs, Cary WinUV, Cary Help, Software help, Sunglasses, Click on the General information folder and select the How to change the Sunglass application interface link.

- How to perform a scan

### 4.11.1 How to perform a scan

1. Under the *Options* menu, select the desired [\*sg\\_options.htm\*](#) International Standard(s) from the *Options* menu.
2. Click on the Sample Info." button.
3. Enter all relevant information in the Sample Info. dialog and click "OK".
4. Select the *Setup* menu to display the Setup dialog.  
Select Report Graph' if you want the trace(s) to be displayed.  
Select 'Report XY Table' for XY pairs data.  
Select 'Storage on (prompt at start)' to store the data.

#### Collect a Baseline

5. Click the Baseline" button to collect the baseline. You will be prompted to insert a blank sample into the sample compartment and click "OK" to collect the Baseline.  
Once collected, the word 'Baseline' will appear in the Status line on the main application window.



**Note** The "Baseline" button reverts to a "Stop" button while the Baseline is being collected. You can stop the collection by clicking this button.

#### Position the sunglasses

6. The sunglasses need to be placed in the correct position in the Cary to obtain accurate results. To do this:

- (a) Hold the sunglasses so that the lens is centered on the cushion located on the sample compartment wall.
- (b) With the sunglasses held in one hand, slide the barrel towards the sunglasses until the barrel cushion is pressed firmly against the lens. Apply sufficient pressure so that when you let go of the sunglasses they are supported between the compartment wall cushion and the barrel cushion. Tighten the barrel screw to hold the sunglasses in position. If they slip out of position, or fall to the bottom of the sample compartment, move the barrel away from the glasses and try again.

**Performing a scan**

7. When the sunglasses are securely in place, click the "Scan" button to perform the test.

**Collect results**

8. Once the test is complete the results will be displayed as set in the Setup dialog in Step 4.

**Print**

9. Click the "Print" button if you would like to print out the results.

## 4.12 Thermal

The following Thermal How to... procedures are described.

- ❑ How to perform a run and determine Tm using Derivative calculations
- ❑ How to perform a run and determine Tm using Hyperchromicity calculations

### 4.12.1 How to perform a run and determine Tm using Derivative calculations

This demonstrates how to perform a Thermal run using a single temperature rate and automatically calculate Tm from the first derivative.

**Start the Thermal application**

1. On the Windows Taskbar click "Start" to display the *Start* menu.

2. Move the cursor over the *Programs* menu item to display the *Programs* menu.
3. Select *Thermal* in the *Cary WinUV* menu.
4. Select your instrument type (if necessary) and press “OK” to open the Thermal application.  
(Alternatively, you can double click on the Thermal icon in the Cary WinUV folder on the desktop.)



**Note** If you are running a GLP system, you may be prompted to enter a password before accessing the Thermal application.

#### **Set up data collection parameters**

5. *Setup dialog*

Select the “Setup...” button or select *Setup* from the menu to display the Setup dialog and specify the method parameters for a new method.

To do this:

#### **Set up instrument parameters such as wavelength, spectral bandwidth, etc.**

6. *Setup dialog / Cary tab*

- (a) Select the Cary tab and specify the instrument parameters for your analysis.
- (b) In the *Wavelength* field enter the wavelength that you want monitored.
- (c) In the *SBW* field enter the required spectral bandwidth.
- (d) In the *Ave Time* field enter the required value.



**Note** For slow changes in temperature (i.e. slow ramp rates) it is recommended that a long Ave Time (e.g. 2–3 seconds) be set.

- (e) Enter the minimum and maximum graph ordinate values in the *Y Min* and *Y Max* fields respectively.

#### **Set up rate parameters such as the number of different rates in the reaction, the speed and the duration of the data collection**

7. *Setup dialog / Cary tab*

- (a) In the Cary tab specify the Collect Temperatures parameters:
- (b) In the *Start °C* entry field, enter the temperature at which you want to commence the data collection.



**Note** Once you press “OK” to dismiss the Setup dialog, the Cary will drive the temperature to the specified Start Temperature.

- (c) In the *Return °C entry* field, enter the temperature that you want the Temperature Controller to drive to at the end of the data collection.
- (d) Select the Monitor that the Cary will use to record the temperature for each data point during the run.
- (e) Select Simple Collect to set a single rate of temperature change for the entire Thermal run.
- (f) Enter the Data Interval, ramp Rate, and End temperature and Hold time you require in the corresponding cell in the single row table. Select the stages where you want to collect data in the Collect Data column.

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

- 8. *Setup dialog / Options tab*
  - (a) Select the Options tab and specify the source conditions and data display options for your analysis.
  - (b) Tick the ‘Auto Lamps Off’ checkbox if you want to automatically turn off the lamps at the end of a collect. This option enables a user to preserve the lifetime of the lamps. It is especially useful for when performing Thermal data collections overnight or unattended for long periods of time.
  - (c) Click on the ‘UV/Vis’ radio button if you want both the lamps on during the run.
  - (d) In the Display Options group, select the way in which you want the data displayed as it is collected. Choose the ‘Individual Data’ radio button to display the collected data of each sample in individual graph boxes. Choose the ‘Overlay Data’ radio button to superimpose the collected data of each sample in the Thermal run in one graph box.

#### **Set up accessories**

- 9. *Setup dialog / Accessories tab*

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

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

- 10. *Setup dialog / Analyze tab*
  - (a) Select the Analyze tab.
  - (b) Select smoothing and nominate the smoothing interval and filter size to control the number of measurement points to be averaged.

## Set up a derivative method of analysis

### 11. Setup dialog / Analyze tab

- (a) Check the 'Derivative' option to choose the derivative method to calculate the temperature at which 50% of the DNA strands have separated (Tm).
- (b) Select the 'Autocalculate' checkbox to automatically perform a derivative calculation on collected data at the end of each run.



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

Now set the parameters to define the range over which to calculate and plot the first derivative of the selected scan. To do this:

- (c) 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.
- (d) 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.

- (e) In the *Low Calculation Limit* entry field, enter the temperature where you want to start the calculation of the derivative for the plot.
- (f) In the *High Calculation Limit* entry field, enter the temperature where you want to end the calculation of the derivative for the plot.

**Set up other recalculations and normalisation**

12. *Setup dialog / Reports tab*
  - (a) Select the Calculations tab to enter the calculation parameters.
  - (b) Check Calculation and enter or select an equation in the field to perform a calculation.
  - (c) Check Correction and enter the Correction Temperature to perform a Thermal Expansion Correction.
  - (d) Check Normalize and enter the temperature at which you would like the data to be normalized in the field.

**Set up reporting and printing requirements**

13. *Setup dialog / Reports tab*
  - (a) Select the Reports tab.
  - (b) Enter your name in the *Name* entry field.
  - (c) Enter a comment relating to your experiment in the *Comment* entry field.
  - (d) Set up your report style by selecting the appropriate radio buttons in the Options group.  
For example, select the 'Auto Print' checkbox to obtain a printout of your report automatically.  
Select the 'Parameters' checkbox to include your method parameters in the report.  
Select the 'Graph' checkbox to include graphics in the generated report.



- Note If 'Auto Print' is selected, then the system will send the report information to the specified printer as well as the Report area. However, if 'Auto Print' is not selected, the report will only be sent to the Report area and can be viewed by selecting *Report* in the *View* menu.
- (e) Select the Autoconvert option you require. If you select 'ASCII' or 'ASCII with Log', then at the end of the data collection the system will automatically generate a report and store the data both in the Cary format as well as ASCII XY pairs format in the current folder.

**Set up storage of collected data**

14. *Setup dialog / Auto Store tab*
  - (a) Select the Auto Store tab.
  - (b) Select 'Storage Off'.

**Set up visual system monitoring**

15. Select the 'Show Status Display' checkbox to display various information fields on your current reaction.

**Finish setup**

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

**Zero the instrument**

17. To Zero the instrument:
  - (a) Press the "Zero" button to zero the system. Alternatively, select *Zero* in the *Commands* menu to perform a zero. A Loading Guide dialog box is 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.

**Equilibrate the sample**

18. Make sure that the Cary has reached the Start temperature and has been at this temperature for 2–3 minutes. If you enter a hold time in the *Stage 1 Hold* field, 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 press the "Start" button.

**Start the Thermal run**

19. To start the Thermal run:
  - (a) Push the application "Start" button to start a data collection. Alternatively, you can select *Start* in the *Commands* menu. The system will display a Loading Guide dialog box.
  - (b) Load your sample into the front cell holder.
  - (c) Enter the name of your sample in the text entry field then press "OK". The Cary will wait for the specified Hold time and then perform the Thermal run.

**Cary actions after the run**

20. At the end of the run, depending on what you have set in previous steps, the User Data Form may appear. If it does, then fill-in the appropriate entries in the columns provided for the sample and press "OK". The calculation equation will appear in the report.

Also, as Autocalculate (Derivative group) was selected on the Analyze tab of the Setup dialog box, a derivative calculation will be performed at the end of the data collection.

The sample under investigation, with corresponding Tm values, will appear in the Report area as well as parameters used to generate the selected data file, Temperature profile, and the method used to contain the Tm value.

A plot will appear in the Graphics area indicating the original temperature data as well as a plot of the first derivative of the selected scan. The point at which the derivative plot peaks (i.e. maximum or minimum gradient on the original plot) is reported as the Tm value and the original plot is marked with an arrow to indicate this point.

To view a plot of the collected data, from the View menu select the Graph option.

#### **Save your data**

21. To save your data:

- (a) Click on the *File* menu item and select *Save Data As...*
- (b) Click in the *Files of Type* field and select Batch.
- (c) Enter the file name for this Thermal run in the *File name* field.
- (d) Click "Save".

The data will be stored as a Batch file.

#### **4.12.2 How to perform a run and determine Tm using Hyperchromicity calculations**

This demonstrates how to perform a Thermal run with multiple temperature ramps and determine Tm using a hyperchromicity calculation.

##### **Start the Thermal application**

1. On the Windows Taskbar click "Start" to display the *Start* menu.
2. Move the cursor over the *Programs* menu item to display the *Programs* menu.
3. Select *Thermal* in the *Cary WinUV* menu.
4. Select your instrument type (if necessary) and press "OK" to open the Thermal application.  
(Alternatively, you can double click on the Thermal icon in the Cary WinUV folder on the desktop.)



**Note** If you are running a GLP system, you may be prompted to enter a password before accessing the Thermal application.

### Set up the data collection parameters

#### 5. Setup dialog

Click the “Setup...” button or select *Setup* from the Menu line to display the Setup dialog and specify the method parameters for a new method.

To do this:

### Set up instrument parameters such as wavelength, spectral bandwidth etc.

#### 6. Setup dialog / Cary tab

- (a) Select the Cary tab and specify the instrument parameters for your analysis.
- (b) In the *Wavelength* field enter the wavelength that you want monitored.
- (c) In the *SBW* field enter the required spectral bandwidth.
- (d) In the *Ave Time* field, enter the required value.



**Note** For slow changes in temperature (i.e. slow ramp rates) it is recommended that a long Ave Time (e.g. 2–3 seconds) be set.

- (e) Enter the minimum and maximum graph ordinate values in the *Y Min* and *Y Max* fields respectively.

### Set up the collect temperature parameters

#### 7. Setup dialog / Cary tab

- (a) In the *Start °C* entry field, enter the temperature at which you want to commence the data collection.



**Note** Once you press “OK” to dismiss the Setup dialog, the Cary will drive the temperature to the specified Start Temperature.

- (b) In the *Return °C* entry field, enter the temperature that you want the Temperature Controller to drive to at the end of the data collection.
- (c) Select the Monitor that the Cary will use to record the temperature for each data point during the run.
- (d) Select the ‘Advanced Collect’ radio button.
- (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 temperature you require for each stage in the ramp table. Select the stages where you want to collect data in the Collect Data column.

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

8. *Setup dialog / Options tab*
  - (a) Select the Options tab to specify the source conditions and data display options for your analysis.
  - (b) Tick the 'Auto Lamps Off' checkbox if you want to automatically turn off the lamps at the end of a collect. This option enables a user to preserve the lifetime of the lamps. It is especially useful for when performing Thermal data collections overnight or unattended for long periods of time.
  - (c) Click on the 'UV/Vis' radio button if you want both the lamps on during the run.
  - (d) In the Display Options group, select the way in which you want the data displayed as it is collected. Choose the 'Individual Data' radio button to display the collected data of each sample in individual graph boxes. Choose the 'Overlay Data' radio button to superimpose the collected data of each sample in the Thermal run in one graph box.

#### **Set up the Multicell Holder accessory**

9. *Setup dialog / Accessories tab*
  - (a) Select the Accessories tab.
  - (b) Ensure that you have the Thermostattable 6 x 6 Multicell Holder accessory installed prior to commencing the run.
  - (c) Select the 'Use Cell Changer' checkbox to enable the accessory.
  - (d) Choose the 'Select Cells' radio button and select the cells you require from the available cells in the Use Cells group.
  - (e) Check the 'Multi Zero' checkbox to turn on the Multi Zero facility.
  - (f) In this example, deselect Blank Correction.

#### **Set up temperature display**

10. *Setup dialog / Accessories tab*

In the Temperature Display group, select 'Block' and 'Probe 1' to turn on monitoring of the Multicell Holder block and one temperature probe in the Status Display window.

### Set up the RBA accessory

11. *Setup dialog / Accessories tab*

- (a) In the RBA group, tick the 'Use RBA' checkbox.
- (b) Select either Attenuation and enter the Abs value or select Position and enter the angle position.

### Set up the analysis to reduce measurement interference

12. *Setup dialog / Analyze tab*

Select the Analyze tab and select 'Smoothing'. Nominate the smoothing Interval and Filter Size to control the number of measurement points to be averaged.

### Set up your method of analysis

13. *Setup dialog / Analyze tab*

- (a) Select the Analyze tab.
- (b) Check the 'Hyperchromicity' option to calculate the temperature at which 50% of the DNA strands have separated (Tm).



**Note** The hyperchromicity calculation is performed once the run is completed.

- (a) Check 'Van't Hoff Calculation' to calculate a Van't Hoff plot ( $\ln(K(T))$  vs  $1000/T(\text{deg K})$ ) and activate the Hyperchromicity group.
- (b) Select the 'Self Complementary' radio button if the DNA molecule being analyzed consists of identical strands. Otherwise select 'Non Self Complementary'.
- (c) Enter the concentration in the *Total Conc of Strands* entry field.
- (d) Enter the Moleularity (i.e. the number of strands) of the hybrid. For example, enter '2' in this field if the DNA molecule being analyzed consists of two strands.
- (e) Check the 'Delta G' checkbox to perform Delta G (free energy) and K (equilibrium constant) calculations.
- (f) In the *Number of Temperatures* field that is now accessible, 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.
- (g) Enter the required temperature in each cell in the Temperature table.

**Set up other recalculations and normalizations**

14. *Setup dialog / Calculations tab*
  - (a) Select the Calculations tab.
  - (b) Check Calculation and enter or select an equation in the field to perform a calculation.
  - (c) Check Correction and enter the required Correction Temperature to perform a Thermal Expansion Correction.
  - (d) Check Normalize and enter the temperature at which you would like the data to be normalized in the field.

**Set up your reporting and printing requirements**

15. Select the Reports tab and specify your reporting requirements.
  - (a) Enter your name in the *Name* entry field.
  - (b) Enter a comment relating to your experiment in the *Comment* entry field.
  - (c) Set up your report style by selecting the appropriate radio buttons in the Options group.  
For example, select the 'Auto Print' checkbox to obtain a printout of your report automatically.  
Select the 'Parameters' checkbox to include your method parameters in the report.  
Select the 'Graph' checkbox to include graphics in the generated report.



**Note** If 'Auto Print' is selected, then the system will send the report information to the specified printer as well as the Report area. However, if 'Auto Print' is not selected, the report will only be sent to the Report area and can be viewed by selecting *Report* in the *View* menu.

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

**Set up storage of collected data**

16. *Setup dialog / Auto Store tab*
  - (a) Select the Auto Store tab to set up whether the collected data is to be saved, and if so, when the Cary should store the information.
  - (b) Select 'Storage On (Prompt at End)'.

**Set up visual system monitoring**

17. Select the 'Show Status Display' checkbox to display various information fields on your current reaction.

**Finish setup**

18. Once you are satisfied with your method setup click "OK" to save any changes made and close the Setup dialog.

**Zero the instrument**

19. To Zero the instrument:

- (a) Press the "Zero" button to zero the system. Alternatively, select *Zero* in the *Commands* menu to perform a zero. The system will display a Cell Loading Guide dialog box.
- (b) Place the blank solutions in the correct positions in the holder then press the "OK" button to zero the specified cells.

**Equilibrate the Samples**

20. Make sure that the Cary has reached the Start temperature and has been at this temperature for 2–3 minutes. If you enter a hold time in the *Stage 1 Hold* field, 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 press the "Start" button.

**Start the Thermal run**

21. To start the Thermal run, click the "Start" button or select *Start* in the *Commands* menu.

The Save As dialog will appear.

**Set up file name and trace names for the data**

22. To set up file and trace names:

- (a) Enter the appropriate name for your Thermal run and click "Save". The system will now display a Cell Loading Guide dialog box.
- (b) Place the solutions in the correct cell positions in the holder.
- (c) Enter the name of each sample in the corresponding text entry field then click "OK".

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

**Cary prompts after the run**

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

**Hyperchromicity calculations**

24. The Cary will now prompt you to use the Thermal ruler to define various limits for the hyperchromicity calculations.
  - (a) A prompt will appear asking you to select the Associated DNA limits using the Ruler. Press the "OK" button on this prompt. The cursor changes to ruler mode.
  - (b) Place your mouse on the Thermal curve at one end of the linear region of the Associated DNA part of the curve (prior to transition). Click and hold down the left mouse button, then drag the mouse along the curve and release the left mouse button 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.
  - (c) An Associated DNA Limits dialog will appear displaying the upper and lower limits that you selected with the Thermal Ruler. Press the "OK" button to accept the calculated line.
  - (d) A prompt will then appear asking you to select the Deassociated DNA limits using the Ruler. Press the "OK" button on this prompt. The cursor changes to ruler mode.
  - (e) Place your mouse on the Thermal curve at one end of the linear region of the Deassociated DNA part of the curve (after the transition). Click and hold down the left mouse button, then drag the mouse along the curve and release the left mouse button 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 will appear displaying the upper and lower limits that you selected with the ruler. Press the "OK" button to accept the calculated line.



**Note** The Associated and Deassociated DNA Limits dialogs 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 the "Retry" button on either dialog 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 appear. 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 appear asking you to select the range on the Alpha curve that will be used for the Tm calculation. Press the "OK" button to make your selection.
- (h) Place your mouse on the alpha curve at one end of the linear region. Click and hold down the left mouse button, then drag the mouse along the curve and release the left mouse button at the other end of the linear region.
- (i) An Alpha Tm Limits dialog box will appear displaying the selected limits. Click the "Retry" button to re-select the limits if required. Press the "OK" button to accept the selected ranges. A linear least squares line is drawn between the selected points and the value of temperature where  $\alpha = 0.5$  is calculated to give the Tm value. (The Tm value is automatically reported in °C and K.)

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

- (a) 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 appear asking you to select the required range on the Van't Hoff plot to calculate Delta G and K. Press the "OK" button on this dialog.
- (b) Place your mouse on the Van't Hoff curve at one end of the linear region. Click and hold down the left mouse button, then drag the mouse along the curve and release the left mouse button at the other end of the linear region.

- (c) A Van't Hoff Limits dialog box will also appear displaying the upper and lower limits that you selected with the ruler. Press the "OK" button to accept the calculated line. Alternatively, you can select the "Retry" button to re-select the limits using the ruler.



Note If there is an error in the Van't Hoff calculation you will be prompted to re-select 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° and Delta S° respectively).

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

- K(Tm)experimental
  - K(Tm)from first principles
  - K(Tm)experimental/K(Tm)from first principles
25. Step 24 will be repeated for each sample trace measured.

# 5 Troubleshooting

---

If you are having problems with your Cary system, check the following information to see if there is a solution to your problem:

## **Installation and startup problems**

1. Check the troubleshooting section in the Hardware manual supplied with the instrument.
2. Check the information provided below.

## **Software problems**

1. First check the information below.
2. Check the troubleshooting information provided in the on-line Help. To do this, select the Windows “Start” button, then *Programs, Cary WinUV, Cary Help*. Select Troubleshooting and follow the links from there.
3. Check the Late Breaking news sheets that were shipped with the Cary WinUV CDROMs.
4. If you still have not found the solution to your problem then you can email a question to: [uv@osi.varian.com](mailto:uv@osi.varian.com). Alternatively, you can contact your local Varian office or representative..

## **5.1 Instrument offline**

### **Problem**

When I start the Cary WinUV software the application reports that the instrument is “Offline”.

### **Solution**

Check the connection of the main instrument cable attaching the PC to the instrument.

Ensure that the instrument has completed its initialization tests before you start the Cary software.

Refer to chapter 4 in the Hardware manual provided with the instrument for more information.

## 5.2 Connect button instead of Start

### Problem

When I start the WinUV application I want to use the Start button has changed to a Connect button, why?

### Solution

When you first start the 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. Just wait until the Connect button changes to Start. If it does not change then check to see if you have any other Cary applications running. If they are not collecting data you can just press the Connect button to get control of the instrument.

## 5.3 Not enough memory

### Problem

After installing version 2 of the Cary WinUV software I get an error saying that there is not enough memory and the Cary WinUV software will not run.

### Answer

Check that you have version 4.0 or later of Microsoft's Internet Explorer (IE4) installed (to check the version start Internet Explorer and select About Internet Explorer from the Help menu. The version number will be displayed).

## 5.4 Poor display of videos and photographs

### Problem

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

### Solution

Increase your display settings to a higher resolution as follows:

1. Select "Start", *Settings, Control Panel, Display*.
2. Select the Settings tab and adjust the Color palette to a higher screen resolution (i.e. 16 bit or 32 bit color) providing your monitor and display adapter allow it.

3. Press "OK".



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

## 5.5 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?

### Answer

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

## 5.6 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?

### Answer

If you are running under Windows NT you need to be logged on as an Administrator to make the changes in System Information.

*This page is intentionally left blank*