Biochrom Libra S50, S60, S70 & S80

UV Visible Spectrophotometers

User Manual

DECLARATION OF CONFORMITY

This is to certify that the following Biochrom manufactured products conform to the requirements of the following Directives -:

Biochrom Libra S50 (80-7000-00, 80-7000-01, 80-7000-02, 80-7000-03, 80-7000-04)
Biochrom Libra S60 (80-7000-10, 80-7000-11, 80-7000-12, 80-7000-13, 80-7000-14)
Biochrom Libra S70 (80-7000-20, 80-7000-21, 80-7000-22, 80-7000-23, 80-7000-24)
Biochrom Libra S80 (80-7000-30, 80-7000-31, 80-7000-32, 80-7000-33, 80-7000-34)

2006/95/EC	Low voltage equipment safety directive
98/79/EC	In Vitro Diagnostic Medical Devices Directive
2004/108/EC	EMC directive
2002/96/EC	EC Directive on Waste Electrical and Electronic Equipment (WEEE) 2003/108/EC & 2008/34/EC
2002/95/EC	Restrictions of the use of certain Hazardous Substances in Electrical and Electronic Equipment (ROHS)
2006/42/EC	Machinery directive
1999/5/EC	Radio and Telecommunications Terminal Equipment Directive (instruments fitted with Bluetooth accessory only)

Standards to which conformity is declared, where relevant, are as follows

EN61010-1:2001	Safety requirements for electrical equipment for measurement, control and laboratory use. General requirements
EN61010-2-101:2002	Particular requirements for IVD medical equipment
EN61326-1:2006	Electromagnetic compatibility - generic emission standard electrical equipment for measurement, control & laboratory use.
EN12100-1,2:2003(+A1:2009)	Safety of machinery–Basic concepts, general principles for design
EN14121-1:2007	Safety of machinery, Risk assessment

For further information, including unpacking, positioning and installation of the products please refer to the user manual.

Signed:

Brian Clarkstone
Technical Director
Biochrom Ltd

CONTENTS:

HEALTH & SAFETY

General safety

This equipment has been designed to conform to the following directives

2006/95/EC	Low Voltage Equipment Safety Directive
98/79/EC	In Vitro Diagnostic Medical Devices Directive
2004/108/EC	EMC Directive
2002/96/EC	EC Directive on Waste Electrical and Electronic Equipment (WEEE) 2003/108/EC, 2008/34/EC
2002/95/EC	Restrictions of the use of certain Hazardous Substances in electrical and electronic equipment (ROHS)
2006/42/EC	Machinery directive
1999/5/EC	Radio and Telecommunications terminal equipment directive (instruments fitted with Bluetooth accessory only)

Standards to which conformity is declared include

- EN61010-1:2001 Safety requirements for electrical equipment for measurement, control and laboratory use.
- EN 61010-2-101:2002 Particular requirements for IVD medical equipment
- EN 61326-1:2006 Electromagnetic compatibility - generic emission standard Electrical equipment for measurement, control and laboratory use. Classified as basic immunity criterion A and Class B for conducted and radiated emissions
- EN 12100-1,2:2003 (+A1:2009) Safety of machinery – Basic concepts, general principles for design
- EN 14121-1:2007 Safety of machinery, Risk assessment

General Hazards

There a number of warning labels and symbols on your instrument. These are there to inform you where a potential danger exists or particular caution is required. Before commencing installation, please take time to familiarise yourself with these symbols and their meaning.

This instrument is intended for use by individuals trained in and familiar with the use of spectrophotometers and their associated hazards. In the event of a malfunction or hazard occurring, the user responsible shall disconnect the unit from power and isolate for decontamination and /or repair.

This instrument is subject to the following hazards:

High voltages exist inside the unit. Repair and maintenance should only be carried out by individuals trained specifically to work on these instruments

The UV source contained within the unit generates a light beam that traverses the sample chamber and is accessible in the lamp chamber. Under normal use the lamp beam is confined within the instrument. The unit should not be operated with the sample chamber lid open or the lamp housing lid removed. Prolonged exposure to the beam may cause permanent eye damage.

There are no bio-hazardous materials within the unit; however this unit could be used with bio-hazardous samples. Before using the instrument the customer should have in place decontamination procedures designed to protect laboratory workers from occupationally acquired infections. The sample chamber cell holders are removable and may be decontaminated using the appropriate disinfectant for the bio hazard in question, rinsed with distilled water and then allowed to dry. The sample chamber and exterior may be wiped with a suitable disinfectant cleaning wipe.

- Decontamination. Equipment returned for repair should include an appropriate decontamination certificate
- It is the responsibility of the customer to ensure that the user of the equipment is provided with a safe working environment.
- Any chemicals used in Analyses should be used, stored and disposed of in accordance with manufacturer's guidelines and local safety regulations
- Toxic Fumes. Efficient laboratory ventilation must be provided when working with volatile solvents or toxic substances
- Waste disposal. Disposal of some solvents and chemicals may be classed as hazardous waste and must be dealt with in accordance with local regulatory practice.
- Personal protective equipment. This is not required to operate the unit but the samples measured may require PPE. A local risk assessment should be carried out.

This equipment may be connected and controlled from a PC. To preserve the integrity of the measuring equipment it is essential that the attached PC itself conforms to basic safety and EMC standards and is set up in accordance with the manufacturers' instructions. If in doubt consult the information that came with your PC. In common with all computer operation the following safety precautions are advised.

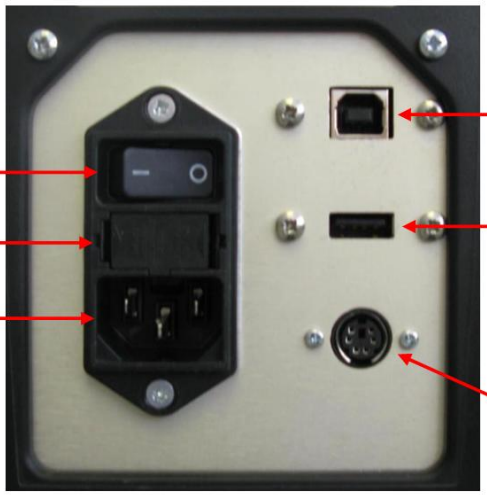
- To reduce the chance of eye strain, set up the PC display with the correct viewing position, free from glare and with appropriate brightness and contrast settings
- To reduce the chance of cross contamination from biological samples, use appropriate personnel protection measures and disinfectant wipes on keyboard and mouse.

Care must be taken when handling all heated accessories

Unpacking and Installation

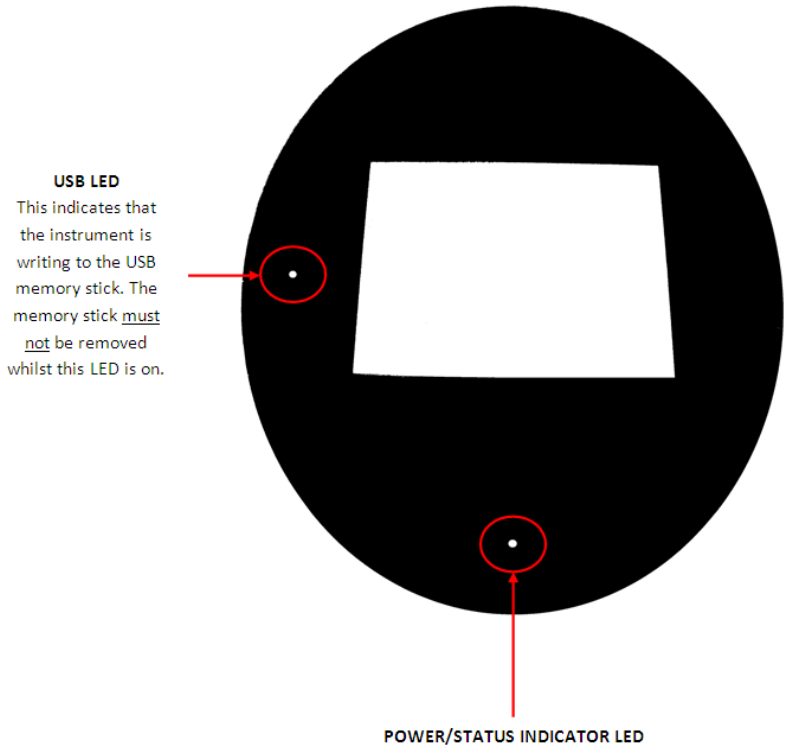
- These instruments weigh approximately 18.5kg. Follow you local regulations for safe handling and lifting of this equipment.
- Inspect the instrument for any signs of damage caused during transit. If any damage is discovered. Do not use the instrument and report the problem to your supplier.
- The instrument must be placed on a stable, level bench or table capable of taking its weight with sufficient space around the instrument for ventilation to circulate freely.
- Ensure your proposed installation site conforms to the environment conditions for safe operation
    - Indoor use
    - 5 to 40 °C
    - Maximum relative humidity 90% up to 31 °C decreasing linearly to 50% at 40 °C
- Extremes of temperature may require recalibration of the unit for optimum performance
- If the instrument has been stored in a cold environment then it should be allowed to come to thermal equilibrium for 2 to 3 hours before operation so that start up calibration is not compromised by condensation.
- The equipment must be connected to the local supply outlet using the provided power cables. It can be operated from 85 to 264 V~ 50 or 60 Hz.
- Replace power inlet fuses only with the same type and rating as follows
    - For Deuterium/Tungsten units T 1.6 A H 250 V AC (Anti-Surge, High breaking capacity)
    - For Xenon units T 1.6A H 250 V AC (Anti-Surge, High breaking capacity)
- Power rating is
    - 100 VA for Xenon units
    - 150 VA for Deuterium/Tungsten units
- The instrument should be positioned so that the power cable may be readily removed in the event of a hazard or malfunction occurring.
- Site the instrument in an atmosphere free from dust and corrosive fumes.
- Use the on/off switch on the left hand side of the instrument. The instrument will automatically perform some start up self diagnostic checks on switch on. Please wait for these to finish before attempting to use the equipment.

Instrument Connections



Equipment Operation

Controls and Indicators



INTENDED USERS

The instrument is intended to be used by scientists and technicians who possess basic laboratory and technical skills and have the knowledge and understanding of the hazards involved, with the unit and the samples used, to operate it in a safe manner.

Instrument Preparation

- Switch on the unit and allow it to finish its start up calibration
- Best performance is obtained if the instrument is allowed to warm up and stabilise for at least 30 minutes
- If applicable connect the unit to a PC using a USB cable and refer to the online help and user manual
- Select the appropriate application or method
- Where relevant, set up the application parameters for the sample
- For Deuterium/ Tungsten units a precision mode is available. To use this mode enter the desired application and switch to precision mode
- Select cuvette cells to use. It is important to use cells of the correct type. Most samples are measured using a standard 10mm path length cell. Special cells and accessories are available for larger or smaller path lengths and sample volumes. It is important to use cells of the

correct type. Some cells absorb in the UV and are not suitable for UV sample measurement. Cells used for samples should be free from dust, residue or scratches.

- Before preparing samples and sample reference blanks, you must be familiar with hazards arising from handling the sample materials and where necessary observe local regulatory practice, personnel protection equipment and measures designed to ensure your safety.
- Prepare the sample blanks (references). A reference is typically the solution that the sample is dissolved in. For Split beam models a reference is required. For Dual beam units the reference may be placed in the reference path or a separate sample reference may be made by first placing the reference in the sample path and performing a reference measurement.
- Prepare the sample solutions. The sample solution would normally comprise the sample under test dissolved in the reference solution
- When placing the cells in the equipment ensure the cell is orientated so that the light energy will pass through the cell
- For further information on running applications and methods refer to the user manual

Post Run procedures

- Empty cuvette cells of sample and rinse with deionised water
- Clean cuvettes periodically with commercially available cleaning solution or dilute detergent solution followed by a thorough rinse in deionised water.
- Note that some samples and solvents may be classified as hazardous or bio hazardous waste. The disposal of such substances must be carried out in accordance with local regulatory practice

Performance Validation

Good laboratory practice recommends that the unit is periodically checked for optical performance.

- Switch on validation checks. When the unit is powered up it performs wavelength accuracy and lamp energy checks. Once complete the unit will beep and show a green on light.
- Periodically wavelength, stray light and absorbance should be checked to ensure the unit is performing to specification. Deterioration in performance may indicate that the instrument needs servicing or that a poor baseline has been saved. Performance validation can b carried using traceable reference materials.

If the equipment is operated in a manner not specified, then the protection provided by the equipment may be impaired and the instrument warranty withdrawn.

User Maintenance

- Other than the Tungsten and Deuterium lamps in certain instruments there are no user serviceable parts in this equipment.
- To prevent contamination
  - Cell holders and accessories should be removed and cleaned with commercially available cleaning solution or dilute detergent followed by a through rinse in deionised water. Allow to dry thoroughly before use
  - Casework and the sample compartment may be wiped down with commercially available disinfectant wipes
- The lamps used in the unit age over time and less energy will be available to pass through the sample. Where energy has fallen to around 50% of the installation energy you are

advised to have the lamps changed. Deuterium and Tungsten lamps may be replaced by the user. Xenon lamp replacement can only be carried out by a qualified service engineer.

- To replace a Deuterium or Tungsten lamp ;
  - Switch the instrument off, disconnect the power supply cord and allow lamps to cool.
  - Locate the lamp access cover at the rear of unit and remove the two top screws and slide top cover back and lift off. Never operate the unit with the lamp housing cover removed.

  - Remove the lamp and dispose of in accordance with local regulatory practice.
  - Follow the handling instructions supplied with the lamp. Do not touch the glass envelopes of the replacement bulbs directly.
  - Replace the lamp cover and screw securely.
  - Attach power cord, switch on and wait at least 30 minutes for the unit to warm up.
  - Perform a new instrument baseline (on all bandwidth settings if variable bandwidth unit) and save this as the permanent baseline (see the online help or user manual).
  - Reset the lamp hours after replacing the lamp (see the online help or user manual).

TROUBLESHOOTING

PROBLEM	POSSIBLE CAUSE
Start up calibration fails	Check sample (and reference) beam is clear before switch on Possible optical failure, contact service support
Negative absorbance reading	Check that sample and reference cells have not been swapped Check sufficient height of sample solution (beam height is nominally 15 mm above the cell base for normal measurements and it is recommended that it be filled to 20 mm above the base)
Unexpected results	Check for bubbles in solution
Absorbance values higher than expected	Check for use of incorrect cell type Check cells are free from finger prints Check cells for contamination Check cell orientation Check reference used Possible optical alignment problem, contact service support
Absorbance value lower than expected	Check sufficient height of sample solution Check sample and reference are not the same Check sample compartment lid is properly closed Possible stray light issue, contact service support
Instrument will not reference	Check orientation of cuvette Check for use of incorrect cell type
Poor reproducibility with nucleic acid analysis	Check for particles, try using background correction Try increase in concentration

Customer Support Contacts

Note: If you experience any problems with your instrument, please refer to the troubleshooting guide on page 8. If you require further assistance, please contact customer support at

http://www.biochrom.co.uk/content/1/11/support.html

Customer Support ROW	+44 (0)1223 427890	support@biochrom.co.uk
Customer Support US		support@biochrom.co.uk

Service, Repair or Return

Good laboratory practice recommends that the unit be periodically serviced to ensure the unit is suitable for purpose. It is recommended that the instrument be serviced annually. You can arrange this through your distributor. Prior to Inspection, Servicing, Repair or Return of Medical and Laboratory Equipment the unit must be decontaminated.

A returns policy operates on this equipment. Before returning the equipment to the distributor or manufacturer

- Fill in a returns request form. Available from the web site or your local distributor
- Return the unit together with a completed declaration of contamination status form. Available from the web site or your local distributor
- Please note that instrumentation will not be accepted for servicing or return until a completed declaration has been received
- Instrumentation that has not been cleaned sufficiently or decontaminated may be subject to additional charges and return delay

Disposal

Decontamination

In use this product may have been in contact with bio hazardous materials. Before disposal all accessories should be removed and thoroughly cleaned in disinfectant and then rinsed with distilled water. All outside surfaces and sample chamber walls must be wiped down with disinfectant wipes suitable for purpose

WEEE

A label with a crossed-out wheeled bin symbol indicates that the product is covered by the Waste Electrical and Electronic Equipment (WEEE) Directive and is not to be disposed of as unsorted municipal waste. Any products marked with this symbol must be collected separately and in accordance with local regulatory practice.

INTRODUCTION TO THE BIOCHROM LIBRA SPECTROPHOTOMETER

The Biochrom Libra S50, S60, S70 & S80 are a range of standalone, easy to use, split beam and double beam UV/visible spectrophotometers with high resolution colour touch screens. All Biochrom Libra spectrophotometers offer a comprehensive range of spectrophotometric and life science applications.

A spectrophotometer is an optical arrangement that is designed to pass light energy through a sample. The reduction of transmittance of the light energy and corresponding absorbance peaks, caused by the sample under test, can be used in a qualitative way to determine sample composition or in a quantitative way, by comparing with known concentration standards, to determine the concentration of a sample. In other types of studies the tracking of absorbance over time can be useful to study chemical reactions and biological processes.

The Biochrom Libra range of spectrophotometers are designed to produce light energy from the far ultraviolet through the visible light in the range 190 to 1100 nm. Many materials, and in particular solutions of materials will absorb light energy within this region. This makes the Biochrom Libra spectrophotometers applicable to a wide range of market sector needs including applications in Life sciences, clinical, pharmaceutical, cosmetics, food & drink, agricultural, industrial, environmental, toxicology, water treatment and teaching. There are a large number of published methods and assays available for these.

The icons described throughout this manual use the names quoted in the Table of Icons on page 80, if you are unsure of any of their functions please refer to this section.

For detailed descriptions of the functions of parameter boxes please refer to the Glossary of Boxes section.

Note: Throughout this manual all screen shots are shown on a white background, this is purely for illustrative purposes.

USE WITH RESOLUTION PC SOFTWARE

When connected to a PC the Biochrom Libra S50, S60, S70 & S80 spectrophotometers can be controlled using the Resolution PC software packages. Operation using Resolution PC software is described in the Resolution user manual or Resolution help file.

FREQUENTLY USED ICONS

BUTTON	NAME	FUNCTION
	Forward arrow	Advances to the next screen in a sequence
	Back arrow	Returns to the previous screen in a sequence
	Tick	Confirms selection/entry. Saves and exits
	Cross/exit	Exit without saving
BUTTONS ON THE SAMPLE MEASUREMENT SCREEN		
	Take reference	Performs a reference measurement
	Take measurement	Performs a sample measurement
	Options arrow	Opens the options menu on the sample measurement screen
BUTTONS ON THE OPTIONS MENU		
	Method parameters	Takes the user from the sample measurement screen to the first method parameter screen
	Save data	Allows the user to manually save sample data to a specified location.
	Save method	Allows the user to save the current method parameters to the internal memory or a USB stick
	Print	Prints the sample data from the specified printer
	Auto print	Toggles auto print on and off
	Sample Manager	Accesses Sample Manager
	Trace Manager	Accesses Trace Manager (wavescan and kinetics only).

PERFORMING A MEASUREMENT

BIOCHROM LIBRA S50

The Biochrom Libra S50 is a split beam UV visible spectrophotometer that contains a single cell holder for both reference and sample measurements. Therefore before performing a sample measurement it is necessary to perform a reference measurement to correct for solvent and/or cuvette effects. This is done as follows:

1. Insert a cuvette containing the solvent in the cell holder and close the sample chamber lid (it is possible to reference against air by leaving the cell holder empty during a reference measurement).
2. Press take reference.
3. When the reference is complete, remove the cuvette containing solvent from the cell holder, insert a cuvette containing the sample solution and close the sample chamber lid.
4. Press take measurement.
5. Repeat steps 3 & 4 until all sample data has been collected. See the section Saving and Printing for post measurement options.

Note: A single reference will suffice for subsequent analyses in the same series.

BIOCHROM LIBRA S60, S70 & S80

The Biochrom Libra S60, S70 & S80 are double beam UV visible spectrophotometers that contain cell holders for both samples (situated at the front of the cell chamber) and references (situated at the back of the cell chamber). As double beam spectrophotometers continually reference against the reference cell holder it is possible to perform measurements in one of three ways.

To reference against air:

1. Insert a cuvette containing the sample solution in the sample cell holder, ensure the reference cell holder is empty and close the sample chamber lid.
2. Press take measurement.
3. Repeat until all sample data has been collected. See the section Saving and Printing for post measurement options.

To reference against a solvent:

1. Insert a cuvette containing the sample solution in the sample cell holder, insert a cuvette containing the solvent in the reference cell holder and close the sample chamber lid.
2. Press take measurement.
3. Repeat until all sample data has been collected. See the section Saving and Printing for post measurement options.

Note: This methodology is used throughout the user manual.

To correct for both solvent and cell effects (simulate matched cuvettes):

1. Insert cuvettes containing the solvent in both the sample and reference cell holders and close the sample chamber lid.
2. Press take reference.
3. Remove the cuvette containing solvent from the sample cell holder, empty out the solvent and replace with the sample solution.
4. Insert the cuvette containing the sample solution in the sample cell holder and close the sample chamber lid.
5. Press take measurement.
6. Repeat steps 4 and 5 until all sample data has been collected. See the section Saving and Printing for post measurement options.

Note: This methodology ensures the most accurate measurements.

LAMP MODE

When creating a method using a Biochrom Libra S70 or S80 spectrophotometer the user will have the option of setting the Lamp Mode to Precision or Pulse. These modes are described below.

PRECISION

Precision Mode is used for applications requiring the most accurate measurements. Both the tungsten and deuterium lamps will remain permanently switched on.

PULSE

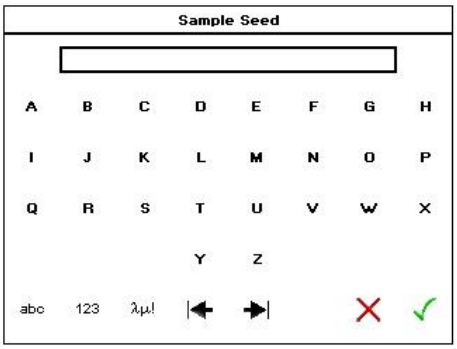
Pulse mode is used to help preserve lamp life and reduce running costs as the tungsten and deuterium lamps will be switched off after 15 minutes of inactivity.

Note: As the Biochrom Libra S50 & S60 use press to read xenon lamps, measurements can only be made in pulse mode.

TYPES OF BOXES

The Biochrom Libra use different kinds of boxes for parameter selection and entry, these include.

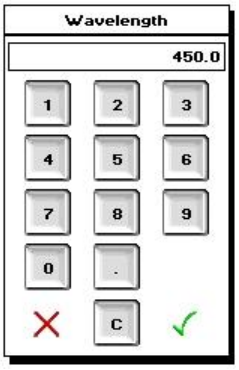
Alphanumeric Text Entry



The alphanumeric text entry box allows the user to enter letters, numbers and symbols by pressing the abc, 123 and λμ! buttons, respectively. It is possible to toggle between upper and lower case letters and through a list of symbols by pressing the abc and λμ! buttons twice.

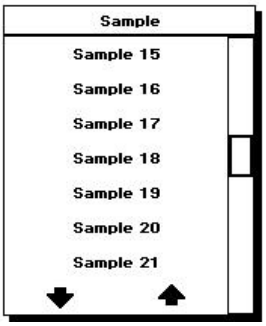
Note: The layout of the screen is dependent on the text entry mode set under User Interface in Settings.

Numeric Entry



The numeric entry box allows the user to include numbers in the method parameters. Depending on the numeric box selected it may be possible to add both positive and negative numbers.

Combo Box

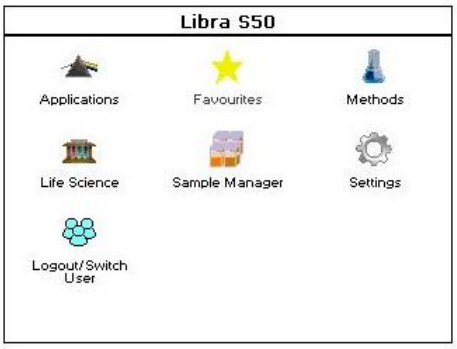


Where there are greater than two options, the user will be presented with a combo box listing all of these. If there are greater than 8 options the user can scroll through these using either the page up and page down arrows or the scroll bar.

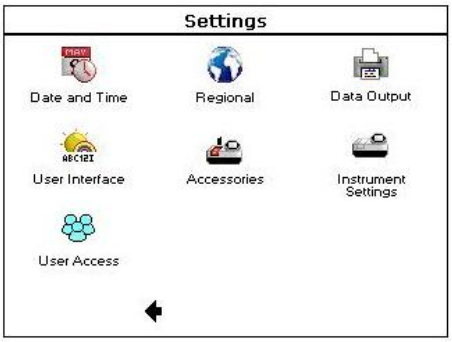
Note: If a box only contains two options *i.e.* On or Off, pressing this box will toggle between the options and not produce a combo box.

SETTINGS

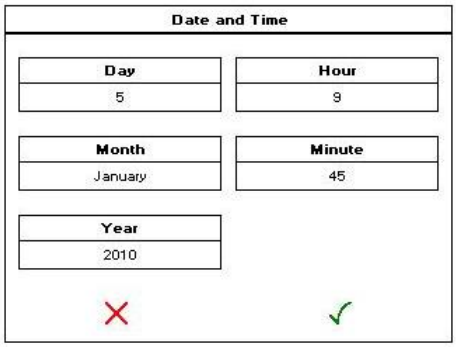
Settings are accessed via the Settings button on the main screen (see below)



Note: When User Access Is turned on the Settings option will only appear on the main screen for users with Administrator or Supervisor privileges.



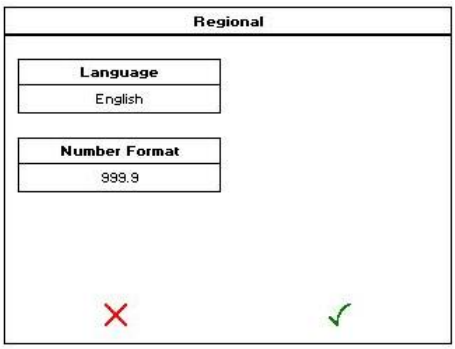
DATE & TIME



The Biochrom Libra will arrive with the UK time and date set. This can be changed by pressing the Date and Time button.

After the desired date and time have been entered, select the tick to save and exit or the cross to exit without saving.

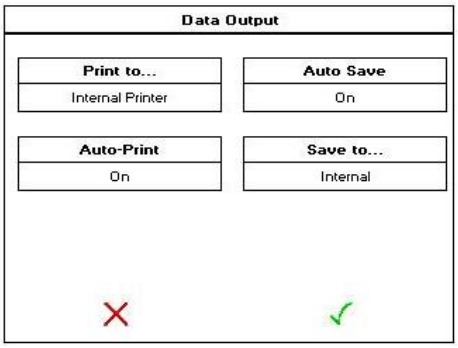
REGIONAL



The Biochrom Libra will arrive with the language set to English. This can be changed by pressing the Language button; the options are English, French, German and Spanish.

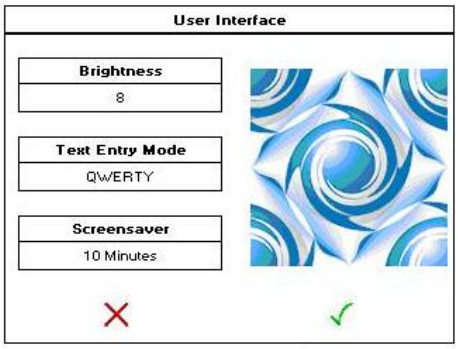
To save any alterations press the tick, to exit without saving press the cross.

Data Output



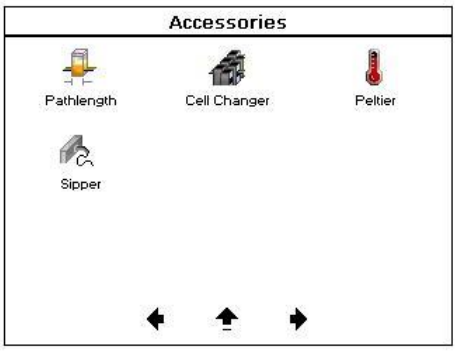
This is the default saving and printing settings that will be used in all application method parameters.

User Interface



Allows the user to set the desired brightness level of the screen, the alphanumeric text entry mode and the duration after which the screensaver will be displayed (if required).

Accessories

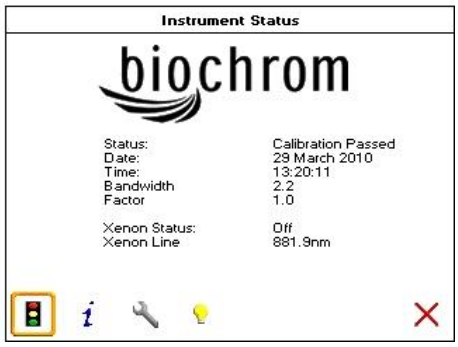


Displays accessories that are fitted to the instrument and allows the user to set the desired accessory parameters. For specific details see the Accessories section.

Instrument Settings

The following options are included under instrument settings:

Instrument Status

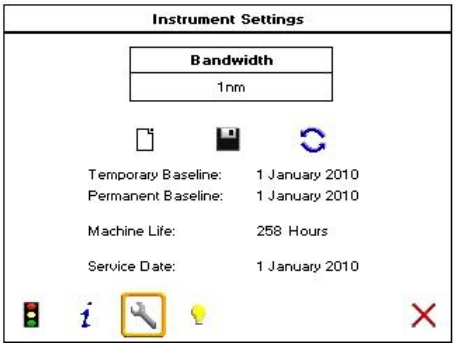


This displays the current status of the instrument.

Instrument Information

Displays the serial number, user-interface (UI) version, build and release dates and instrument control (IC) version.

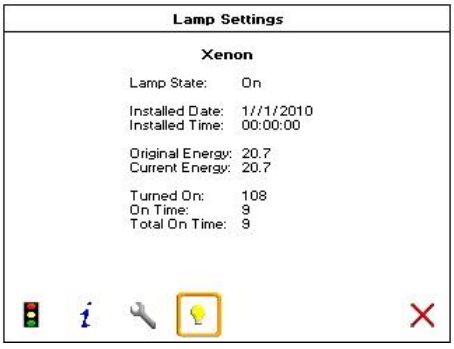
Instrument Settings



Instrument Settings allows the user to:
1. Collect a new, temporary baseline. This will be stored until the instrument is switched off.
2. Save the temporary baseline. This will become the permanent baseline and be stored until overwritten.
3. Restore the original baseline. If measurements show the temporary baseline to be poor quality the permanent baseline can be restored.

Note: Using the Biochrom Libra S80 the bandwidth at which the baseline is measured is selected by pressing the *Bandwidth* box

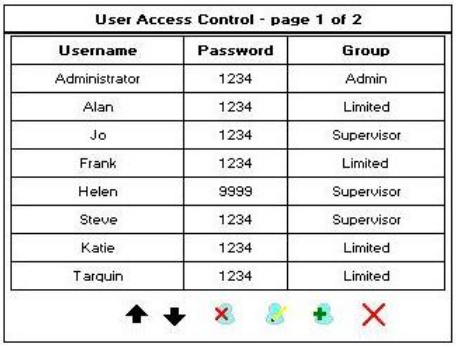
Lamp Settings



Displays the current lamp status and allows the user to reset lamp life (tungsten and deuterium lamps only).

To exit at any time press the exit button located in the bottom right hand corner.

USER ACCESS

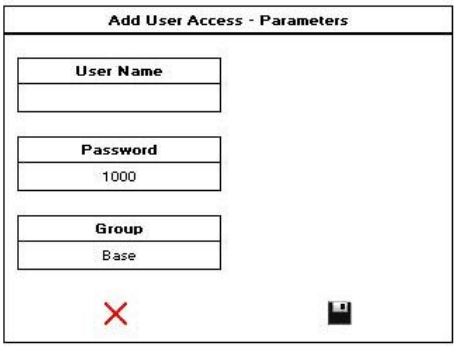


The Biochrom Libra have the option to assign users different access rights. These are set via the user access button.

Note: User access is only available to users who have administrator privileges.

Adding a user

To add a new user to the instrument, press the 'Add user' button. The Biochrom Libra can store up to 16 individual users.



Each user is given a user name (using alphanumeric entry), a 4-digit password and assigned to one of three user groups depending on the access level they require. The table below outlines the features each user group can access.

User Group	Run Applications & Saved Methods	Save Sample Data	Delete Sample Data from the instrument's memory	Save Methods	Access Settings Menu	Access User Settings
Limited	✓	✓	✗	✗	✗	✗
Supervisor	✓	✓	✓	✓	✗	✗
Admin	✓	✓	✓	✓	✓	✓

Editing a user

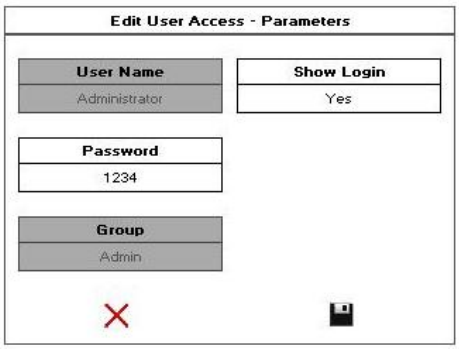
To edit a user's details, highlight the desired user and press the 'Edit user' icon. This allows the username, password and user group to be edited/updated as above.

Deleting a user

To delete a user from the instrument, highlight the desired user and press the 'Add user' icon. Any methods or data created by this user will not be deleted.

Note: It is not possible to delete the default administrator account.

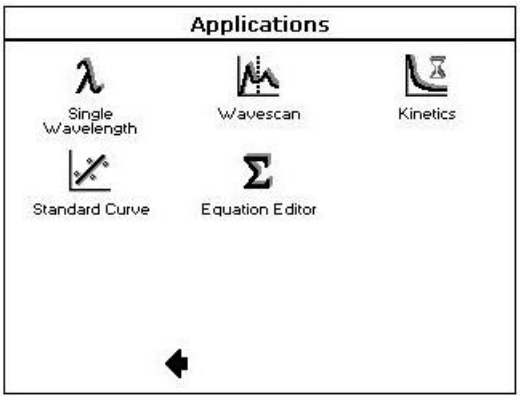
Editing User Access



To disable user logins and user access, highlight the default administrator account and press the 'Edit User' icon to display the screen left.

Note: With *Show Login* set to No the instrument will not display the 'Switch User' button on the main screen and the instrument will always be in Administrator mode.

APPLICATIONS

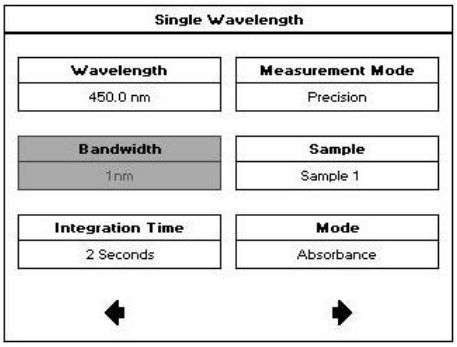


Single Wavelength	Absorbance, % transmission or concentration measurements at a single, specified wavelength.
Wavescan	Wavelength scan between two, user defined wavelengths in the range 190 to 1100 nm. The Biochrom Libra allows data overlay, post-scan data manipulation and user configurable peak and valley functions.
Kinetics	Serial and parallel measurements of absorbance *versus* time to determine rate or end points. The Biochrom Libra allows data overlay, post-scan data manipulation and user defined sectors.
Standard Curve	Concentration measurement at a single wavelength determined by the generation of a calibration curve of known standards.
Equation Editor	Allows users to create their own unique methods that include calculations and thresholds.

SINGLE WAVELENGTH

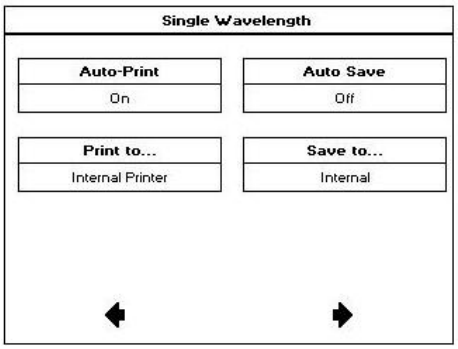
The Single Wavelength application performs simple absorbance (A) and % transmission (%T) measurements on samples, measuring the amount of light that has passed through a sample relative to a reference (this can be air).

MEASUREMENT PARAMETERS



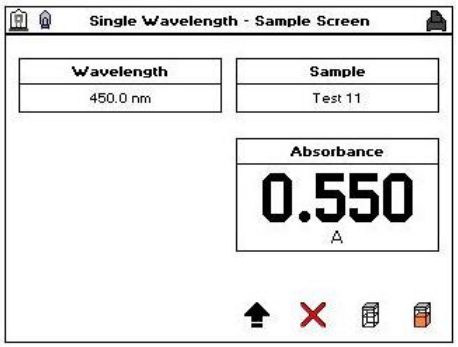
Set *Mode* to Absorbance or %T. *Wavelength*, *Bandwidth* (Libra S80 only), *Integration Time* and *Lamp Mode* can be set as required. The Sample Seed entered under *Sample* will be the filename used for any data file saved automatically.

You may advance to the next screen at any time by pressing the forward arrow and return to the previous screen by pressing the back arrow.



Set the outputs required in your method. For more information see the section Saving and Printing.

TAKING A MEASUREMENT



To perform a measurement using split beam instruments (Libra S50). Insert a cuvette containing the reference solution in the cell holder and press the reference button. Remove and replace with a cuvette containing the sample and press the take measurement button. A single reference suffices for subsequent analyses in the same series.
To take a measurement using double beam instruments (Libra S60, S70 & S80) insert a cuvette containing the reference solution in the reference cell holder and a cuvette containing the sample in the sample cell holder and press the take measurement button. The reference measurement will be automatically subtracted from the sample measurement.

SAVING & PRINTING

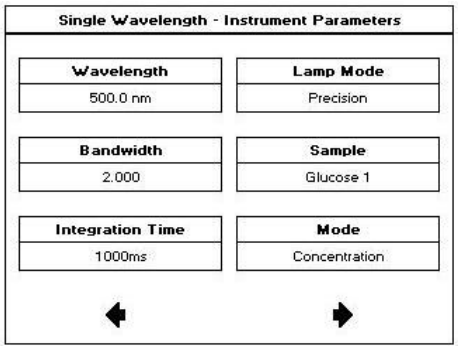
For details of manual saving and printing see the Saving and Printing section.

CONCENTRATION VIA FACTOR

This mode within the Single Wavelength application makes simple concentration measurements on samples. Concentration is obtained by multiplying the measured absorbance at a specific wavelength by a factor. The factor may be known in advance or may be calculated by the instrument by measuring a standard of known concentration. Examples of concentration measurements include cholesterol, glucose and urea.

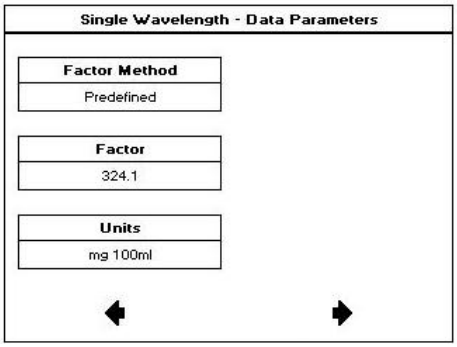
MEASUREMENT PARAMETERS

From the main screen of the Biochrom Libra select Applications followed by Single Wavelength to display the screen below.

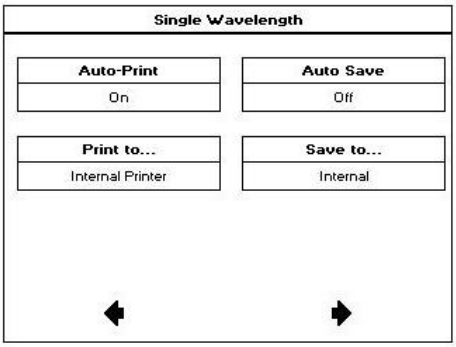


Set *Mode* to Concentration. *Wavelength*, *Bandwidth* (Libra S80 only), *Integration Time* and *Lamp Mode* can be set as required.

You may advance to the next screen at any time by pressing the forward arrow and return to the previous screen by pressing the back arrow.

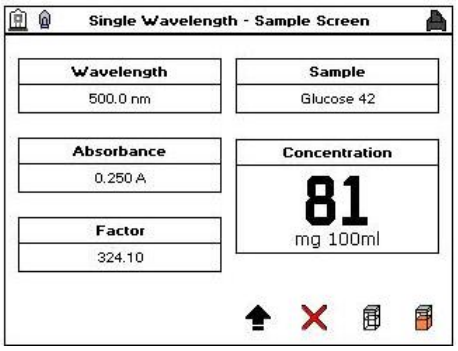


Set *Factor Method* as required, enter *Factor* using numeric entry and *Units* using alphanumeric entry.



Set the outputs required in your method. For more information see the section Saving and Printing.

TAKING A MEASUREMENT



To perform a measurement using split beam instruments (Libra S50). Insert a cuvette containing the reference solution in the cell holder and press the reference button. Remove and replace with a cuvette containing the sample and press the take measurement button. A single reference suffices for subsequent analyses in the same series.
To take a measurement using double beam instruments (Libra S60, S70 & S80) insert a cuvette containing the reference solution in the reference cell holder and a cuvette containing the sample in the sample cell holder and press the take measurement button. The reference measurement will be automatically subtracted from the sample measurement.

Note: For assays where there is no established concentration factor, calibration should be carried out using prepared standards (see Standard Curve for details of how to perform this).

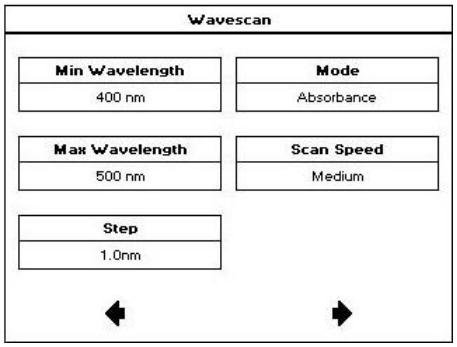
SAVING & PRINTING

For details of manual saving and printing see the Saving and Printing section.

WAVESCAN

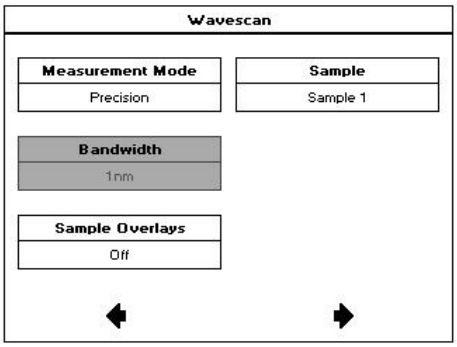
A measurement of the absorbance or %transmission of a sample over a specified wavelength range is one of the most useful physical characteristics of a compound, both as means of identification (qualitative analysis) and of estimation (quantitative analysis). The observed features arise due to the various electronic transitions that are possible within a molecule. The Biochrom Libra offer a range of post-scan data manipulation options including: 1st order derivative, enabling identification of multiple, unresolved peaks; 2nd order derivative, enabling identification of peak shoulders (inflections); 4th order derivative, which identifies both multiple peaks and inflections at the same time; Smoothing, utilises the Savitzky-Golay algorithm to smooth data and increase the signal to noise ratio; Enhanced, which enhances features, sharpening peaks and valleys.

MEASUREMENT PARAMETERS



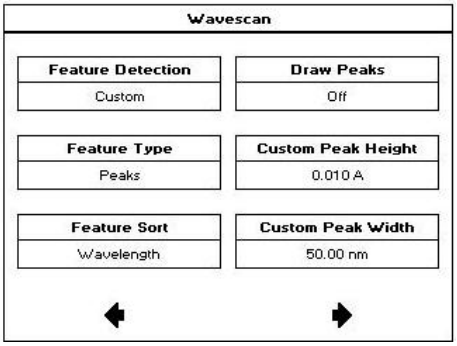
Use *Max Wavelength* and *Min Wavelength* to set the required wavelength range (the Biochrom Libra scan from high to low wavelengths). Set *Step*, *Mode* and *Scan Speed* as required. If *Scan Speed* is set to Integration the user will also be required to set the *Integration Time*.

You may advance to the next screen at any time by pressing the forward arrow and return to the previous screen by pressing the back arrow.



Set *Lamp Mode*, *Bandwidth* (Libra S80 only) and *Sample Overlays* as required. The *Sample Seed* entered under *Sample* will be the filename of any automatically saved file.

Note: With *Sample Overlays* set to ≥2 the data all wavelength scans will be automatically saved to the instrument's internal memory and will be displayed in Trace Manager.



This measurement parameters screen allows the user to set the following parameters:

Feature Detection: Determines the number of peaks or valleys that will be automatically detected. Options are Coarse, Sensitive or Custom.

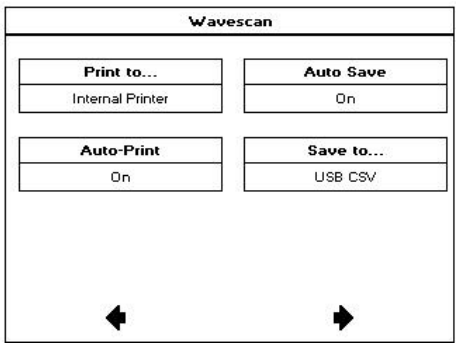
Feature Type: The feature types that will be detected by the software. Options are Peaks or Valleys.

Feature Sort: Determines the how the features will be displayed in the data table. Options are Wavelength or Magnitude.

Draw Peaks: Switches the display of peak cursors on and off. The cursors show vertical dashed lines displaying the measured peak height and horizontal dashed lines showing the peak width.

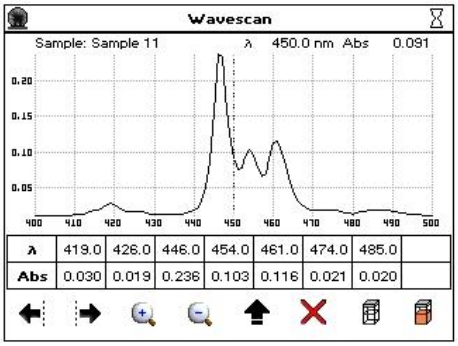
Custom Peak Height: Only displayed if *Feature Detection* is set to Custom. This is the minimum height the peak has to be above the higher of the two adjacent minima for the peak to be detected.

Custom Peak Width: Only displayed if *Feature Detection* is set to Custom. This is the minimum width of the peak as determined by the difference in wavelength between the higher of the two adjacent minima and the opposing intersection of that higher minimum level and the peak profile.



Set the outputs required in your method. For more information see the section Saving and Printing.

TAKING A MEASUREMENT



To perform a measurement using split beam instruments (Libra S50). Insert a cuvette containing the reference solution in the cell holder and press the reference button. Remove and replace with a cuvette containing the sample and press the take measurement button. A single reference suffices for subsequent analyses in the same series.
To take a measurement using double beam instruments (Libra S60, S70 & S80) insert a cuvette containing the reference solution in the reference cell holder and a cuvette containing the sample in the sample cell holder and press the take measurement button. The reference measurement will be automatically subtracted from the sample measurement.

With *Feature Detection* set to Coarse, Sensitive or Custom the sample measurement screen will display a table below the scan. This table will display the *Feature Type* selected in the method parameters. To manually add a peak or valley to the table, position the cursor over the desired feature by either touching the feature or using the left and right cursors and press an empty cell in the table.

Note: Details of how to perform overlays, data manipulation and selecting saved files can be found in the Trace Manager section.

KINETICS

Kinetics measurements made using a UV/visible spectrophotometer measure the change in absorbance at a single, fixed wavelength over a specified period. This can be used to provide useful information when an appropriate factor, defined in a reagent kit protocol, is applied. Reagent test kits are routinely used for the enzymatic determination of compounds in food, beverage and clinical laboratories.

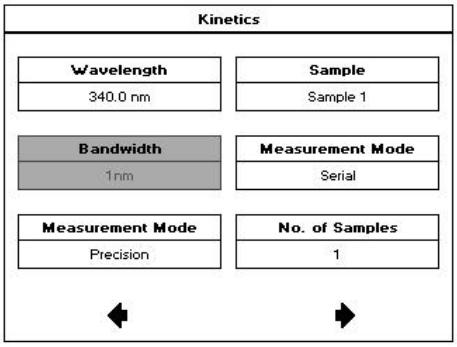
UV/visible spectrophotometric kinetic assays are considered one of the most convenient measurements for enzymatic assays since they allow the rate of the reaction to be measured continuously.

SERIAL KINETICS MEASUREMENTS

Serial kinetics is the measurement of the absorbance of a single sample over a specified duration at a specified interval. As the Biochrom Libra are capable of taking up to 1 reading per second serial kinetics measurements can be used for rapid rate reactions.

Note: Without a cell changer connected, the instrument is only capable of performing serial kinetics measurements.

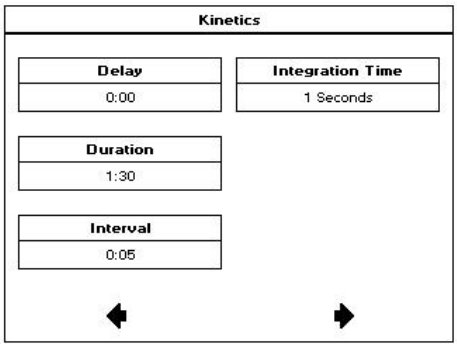
MEASUREMENT PARAMETERS



Wavelength, Bandwidth (Libra S80 only), *Integration Time* and *Lamp Mode* can be set as you require. The *Sample Seed* entered under *Sample* will be the filename of any automatically saved file. *No. of Samples* is described below.

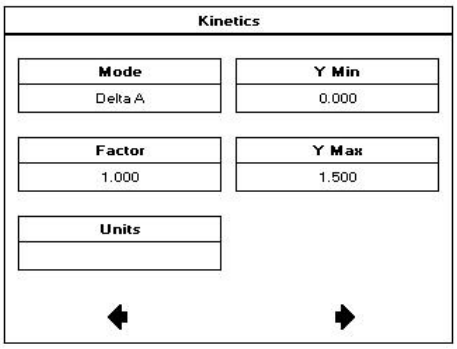
Note: *Measurement Mode* only appears if a cell changer is connected. If this option is not displayed the instrument will perform a serial kinetics measurement.

Note: When performing a serial kinetics measurement with >1 sample the measurement proceeds as follows: Sample 1 will be measured for the full duration at the specified interval, after this is complete sample 2 will be measured for the full duration at the specified interval. The measurement will continue in this manner until all samples have been recorded. When measuring >1 sample, all data will be overlaid at the end of the measurement and automatically saved to the instrument's internal memory.

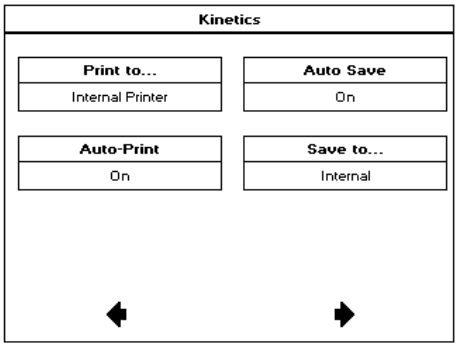


Set the *Delay* (time before first measurement), *Duration* (total measurement time, up to 180 minutes), *Interval* (duration between readings, from 1 second to duration) and *Integration Time* that you require.

Note: The integration time is determined by the interval (i.e. the maximum integration time is half the interval time)

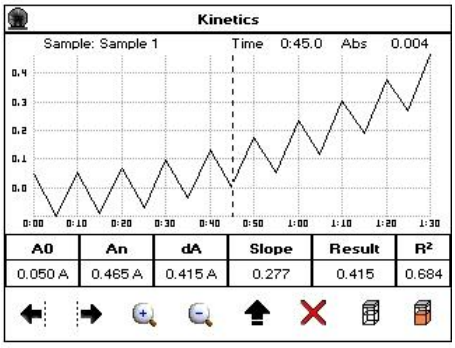


Mode has options for Delta A, Final A and Slope and is the value that will be multiplied by the *Factor* to give the *Result* on the sample measurement screen. *Units* are entered using alphanumeric text entry and will appear on any printed or exported data. Y min and Y max are what is displayed during the measurement, the y axis auto-scales upon completion.



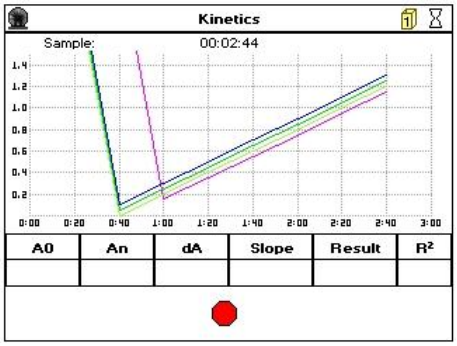
Set the outputs required in your method. For more information see the section Saving and Printing.

TAKING A MEASUREMENT

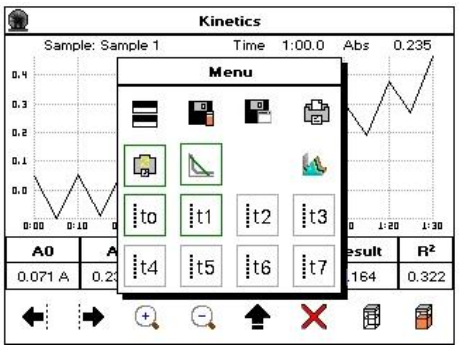


To perform a measurement using split beam instruments (Libra S50). Insert a cuvette containing the reference solution in the cell holder and press the reference button. Remove and replace with a cuvette containing the sample and press the take measurement button. A single reference suffices for subsequent analyses in the same series.
To take a measurement using double beam instruments (Libra S60, S70 & S80) insert a cuvette containing the reference solution in the reference cell holder and a cuvette containing the sample in the sample cell holder and press the take measurement button. The reference measurement will be automatically subtracted from the sample measurement.

Note: Measurements will commence after the specified *Delay* period (if applicable).

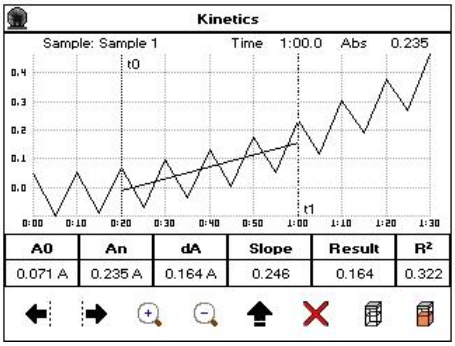


A measurement can be stopped at any time by pressing the Stop button at the bottom of the screen. All data collected to this point will be displayed on screen and can be saved.



The data displayed in the table below the scan refers to the full measurement range. To obtain data for a specific section it is necessary to add sections, this is done as follows: Set the cursor to the desired start position by either pressing on the scan or using the cursors, select t0 from the options menu, set the cursor to the desired end position and select t1 from the options menu. This can be repeated to add up to 4 discrete sections.

Note: Sections must be added in numerical order *i.e.* t1 must be added after t0, t2 after t1 etc.



With sections defined, the data displayed in the table below the scan is determined by the position of the cursor. Only when the cursor is positioned in the section of interest will this data be displayed.

A line of best fit can be added to any section by positioning the cursor in the desired section and selecting the add line of best fit button from the options menu

Note: Details of how to perform overlays, data manipulation and selecting saved files can be found in the Trace Manager section.

SAVING AND PRINTING

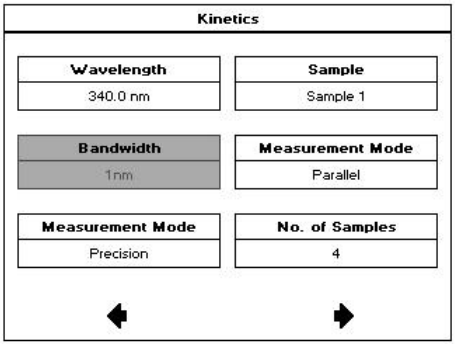
For details of manual saving and printing see the Saving and Printing section.

PARALLEL KINETICS MEASUREMENTS

Parallel kinetics is the measurement of the absorbance of >1 sample (up to 8) at a set interval over a specified duration. *E.g.* for 4 samples a measurement would proceed as follows: The instrument will measure samples 1 to 4, pause for the appropriate interval, measure samples 1 to 4, pause and continue in this manner until the full duration has been reached.

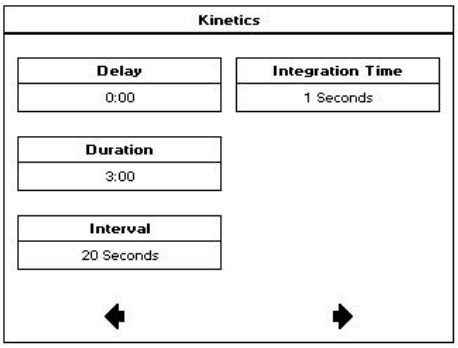
Note: As parallel kinetics requires a cell changer the option of performing a parallel kinetics measurement is only available when a cell changer is connected.

MEASUREMENT PARAMETERS

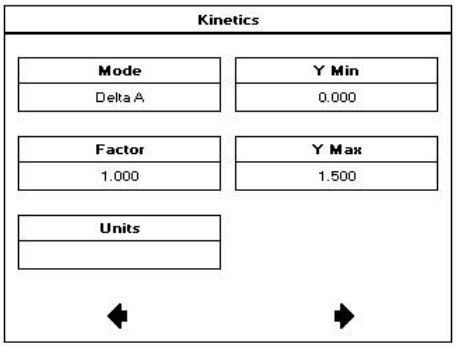


Wavelength, Bandwidth (Libra S80 only), *Integration Time* and *Lamp Mode* can be set as you require. The *Sample Seed* entered under *Sample* will be the filename of any saved file. *No. of Samples* is described above. *Measurement Mode* should be set to Parallel

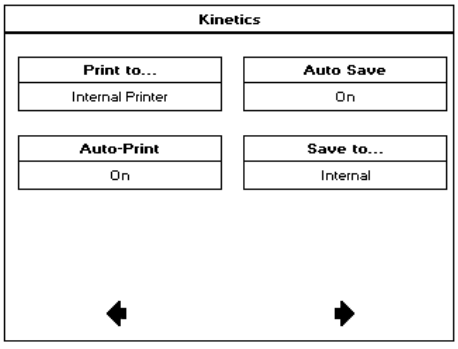
Note: For parallel kinetics measurements the minimum number of samples is 2



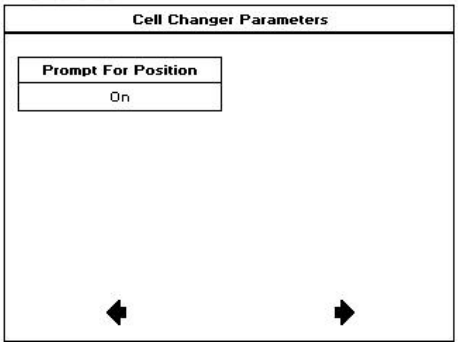
Delay and *Duration* (up to 180 minutes) can be set as required. The options available under *Interval* are determined by the number of samples set on the previous page.



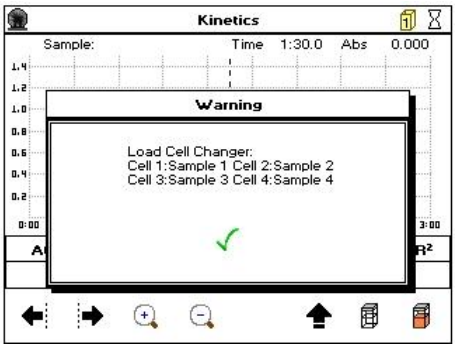
Mode has options for Delta A, Final A and Slope and is the value that will be multiplied by the *Factor* to give the *Result* on the sample measurement screen. *Units* are entered using alphanumeric text entry and will appear on any printed or exported data. Y min and Y max are what is displayed during the measurement, the y axis auto-scales upon completion.



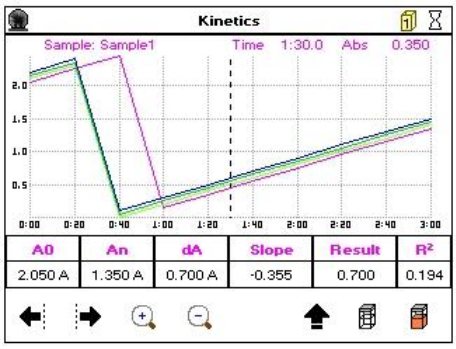
Set the outputs required in your method. For more information see the section Saving and Printing.



As parallel kinetics requires a cell changer, you will be given the option of viewing the prompt for sample position before collecting data. All other cell changer parameters are determined by the method parameters. This prompt is shown below.

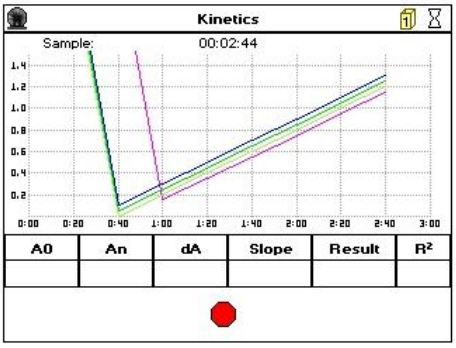


TAKING A MEASUREMENT

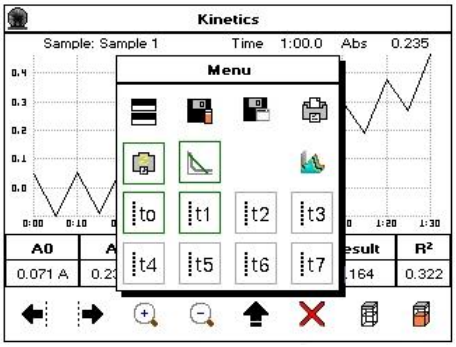


To perform a measurement using split beam instruments (Libra S50). Insert a cuvette containing the reference solution in the position 1 of the cell holder, insert cuvettes containing samples in positions 2 through 8 and press the take measurement button.

To take a measurement using double beam instruments (Libra S60, S70 & S80), insert a cuvette containing the reference solution in the reference cell holder and a cuvettes containing samples in positions 1 through 8 and press the take measurement button. The reference measurement will be automatically subtracted from the sample measurement.

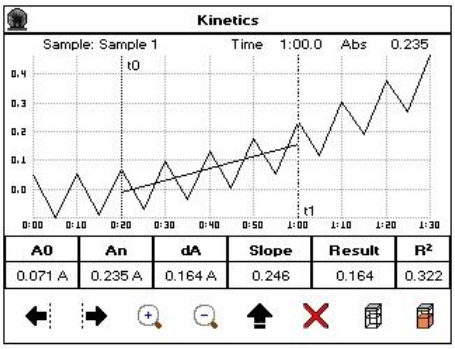


A measurement can be stopped at any time by pressing the Stop button at the bottom of the screen. All data collected to this point will be displayed on screen and can be saved.



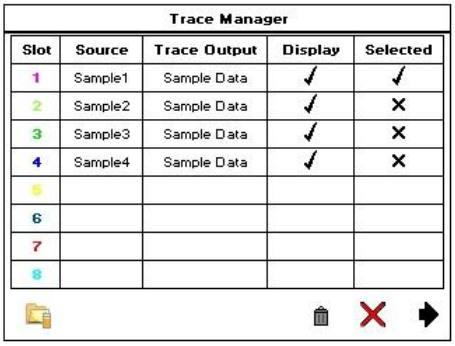
The data displayed in the table below the scan refers to the full measurement range of the selected trace (see below for selecting a trace). To obtain data for a specific section it is necessary to add sections, this done as follows: Set the cursor to the desired start position by either pressing on the scan or using the left and right cursors, select t0 from the options menu, set the cursor to the desired end position and select t1 from the options menu. It is possible to add up to 4 discrete sections.

Note: Sections must be added in numerical order *i.e.* t1 must be added after t0, t2 after t1 etc.



With sections defined, the data displayed in the table below the scan is determined by the position of the cursor. Only when the cursor is positioned in the section of interest will this data be displayed. This refers to the selected scan only and is indicated by the colour of the column headers in the table.

A line of best fit can be added to any section by positioning the cursor in the desired section and selecting the add line of best fit button from the options menu



To allow post scan data manipulation all samples measured are automatically included in Trace Manager (see below for full details of Trace Manager).

Note: The data displayed in the table below the scan refers to the Selected sample only.

SAVING AND PRINTING

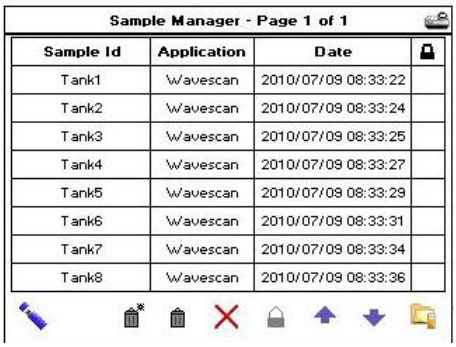
For details of manual saving and printing see the Saving and Printing section.

TRACE MANAGER – OVERLAYING & MANIPULATING WAVESCAN & KINETICS FILES

Trace Manager is the application used by the Biochrom Libra to overlay and manipulate wavescan and kinetics files. Samples are loaded into Trace Manager as described below:

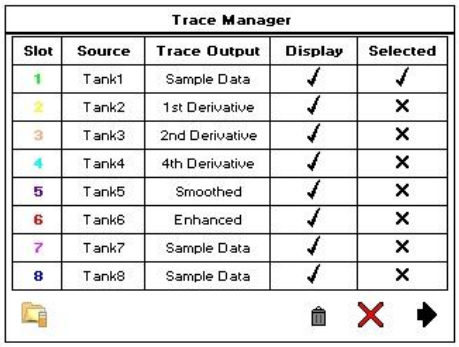
USING SAMPLE MANAGER

Wavescan and kinetics files can be loaded directly into Trace Manager by selecting the required files from Sample Manager on the main screen. This procedure is outlined below:



Highlight the required files by pressing on the appropriate row and load these into Trace Manager by pressing the Sample Manager icon (bottom right corner). If the files you require are not displayed on the screen you can scroll through the pages using the up and down arrows. Pressing on the Sample ID and Date column headers will sort the files alphabetically and chronologically, respectively.

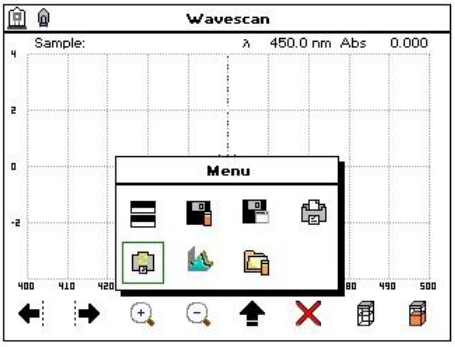
Note: If a USB memory stick is inserted, it is possible to toggle between files stored on the internal and USB memories using the icon in the left hand corner. The icon in the top right corner of the screen will display the memory that is currently in use.



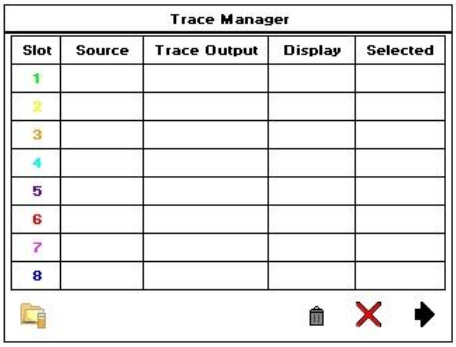
All recalled files will be displayed on the Trace Manager screen (up to 8). The colour of the *Slot* number will be the colour of the trace when it is displayed on screen. To toggle which files are displayed on the measurement screen press in the appropriate *Display* box (up to 8 files can be displayed). To select which files data will be displayed on the measurement screen press in the appropriate *Selected* box (only one file may be selected). Press the forward arrow to view the overlaid data

FROM WITHIN AN APPLICATION

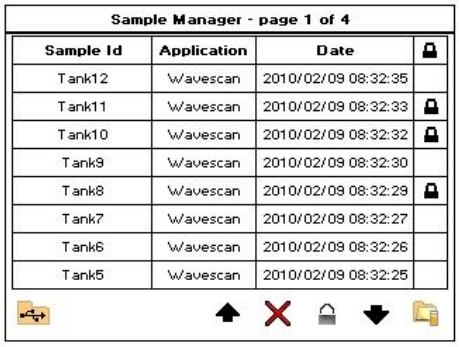
To overlay saved files with a live trace displayed, Trace Manager can be accessed from within the application. This procedure is required for Limited users as they do not have the ability to access Sample Manager on the main screen.



Trace Manager can be accessed from the Wavescan and Kinetics measurement screens using the Trace Manager icon on the options menu.

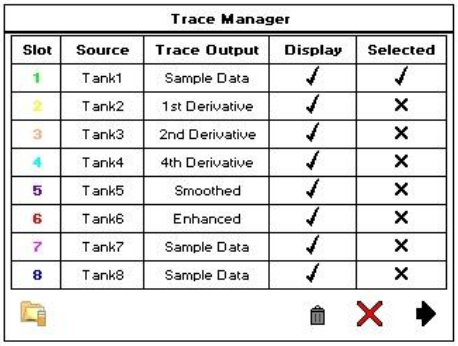


When accessed with no overlays displayed, Trace Manager will be empty. Files are added to this screen by selecting the Sample Manager icon in the left hand corner of the screen and loading saved files as described below.



Highlight the required files by pressing on the appropriate row and load these into Trace Manager by pressing the Sample Manager icon. If the files you require are not displayed on the screen you can scroll through the pages using the up and down arrows. Pressing on the Sample ID and Date column headers will sort the files alphabetically and chronologically, respectively.

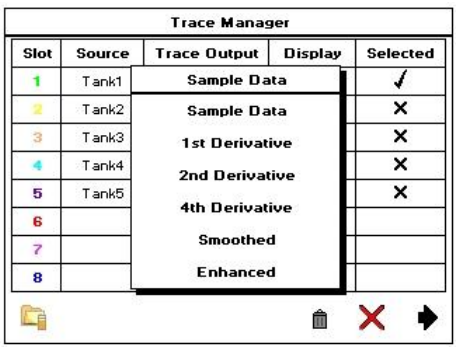
Note: If a USB memory stick is inserted, it is possible to toggle between files stored on the internal and USB memories using the icon in the left hand corner. The icon in the top right corner will display the memory that is currently in use.



All recalled files will be displayed on the Trace Manager screen (up to 8). The colour of the *Slot* number will be the colour of the trace when it is displayed on screen. To toggle which files are displayed on the measurement screen press in the appropriate *Display* box (up to 8 files can be displayed). To select which files data will be displayed on the measurement screen press in the appropriate *Selected* box (only one file may be selected). Press the forward arrow to view the overlaid data.

POST SCAN MANIPULATION

Trace Manager allows the user to manipulate recalled wavescan and kinetics data using the procedure outlined below.



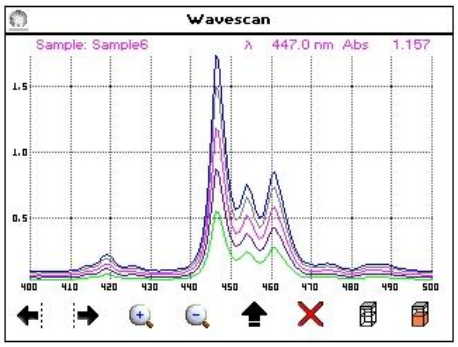
With the required files loaded into Trace Manager press the appropriate *Trace Output* box to display the manipulation options.

Wavescan post scan manipulations are:

Sample Data	Displays the raw wavescan data (this is the default option).
1st – 4th Derivative	Displays the derivative data to the desired order.
Smoothed	Uses the Savitzky-Golay algorithm to reduce noise and smooth the data.
Enhanced	Enhances features, sharpening peaks and valleys.

Kinetics post scan manipulations are:

Sample Data	Displays the raw kinetics data (this is the default option).
Low	Applies a low level of smoothing to the data
Medium	Applies a medium level of smoothing to the data
High	Applies a high level of smoothing to the data



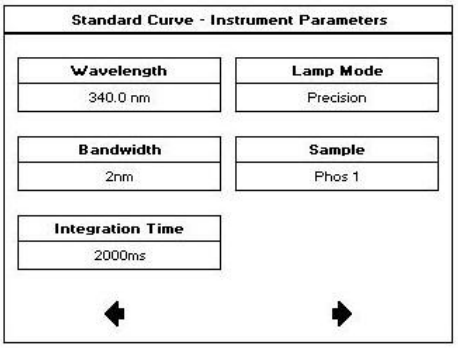
After the manipulations and display options have been set as required press the forward arrow in the right corner to display the recalled data on the sample measurement screen.

Note: For wavescan and serial kinetic measurements with overlays set to >1 and parallel kinetic measurements, data will be automatically saved into the instrument's internal memory and displayed in Trace Manager. To ensure the optimum performance of the instrument it is recommended that unwanted files are deleted from the internal memory at regular intervals (see Sample Manager – deleting files from the internal memory).

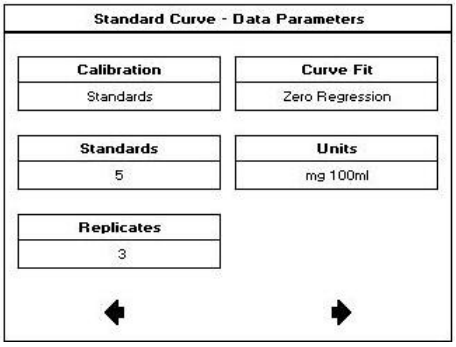
STANDARD CURVE

The construction of a multi-point calibration curve from standards of known concentration to quantify unknown samples is a fundamental use of a spectrophotometer. The Biochrom Libra range of instruments has the advantage of being able to store calibration curves with a method. Each calibration curve can be created using up to 9 standards, with each standard measurement being made of up to 3 replicates.

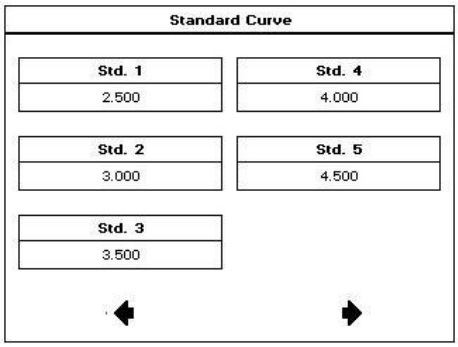
CREATING A STANDARD CURVE



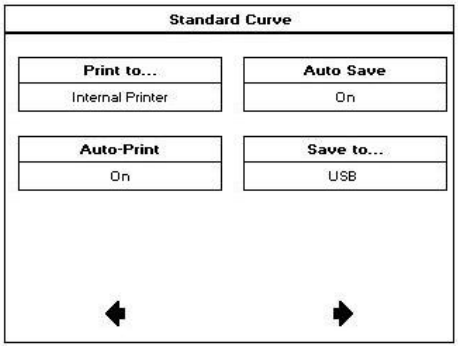
Set *Wavelength*, *Bandwidth* (Libra S80 only), *Integration Time* and *Lamp Mode as* required. The Sample Seed entered under *Sample* will be the filename of any file saved automatically.



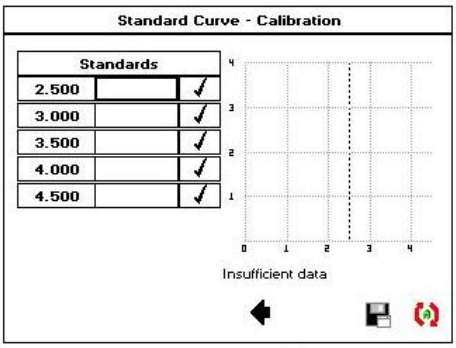
Calibration can be set to *Standards* (the user is required to prepare and measure standards) or *Manual* (the user inputs both the standard concentrations and standard absorbances). Set *Standards, Replicates, Curve Fit* and *Units* as required in your application.



Set the concentration values for each of the standards using the numeric entry box.

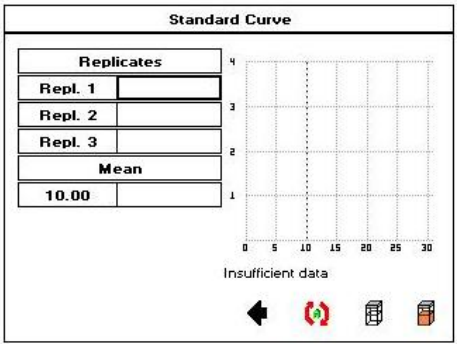


Set the outputs required in your method. For more information see the section Saving and Printing.



To create the standard curve when using replicates press the replicates button in the bottom right corner to take you to the screen shown below. With *Replicates* off standards can be measured directly as described below.

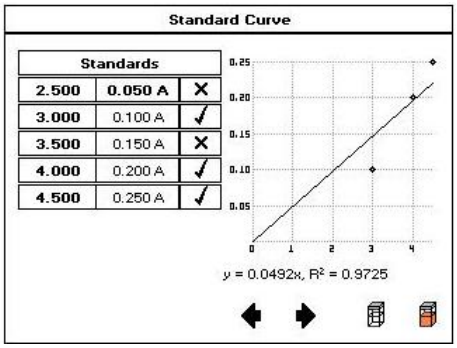
Note: Pressing the save method icon before any standards have been measured will save the method parameters only. Recalling a method containing method parameters only requires the user to construct a standard curve before measuring samples.



To create a standard curve using the Biochrom Libra S50, insert the cuvette containing the reference solution in the cell holder and press the take reference button. Remove and replace with a cuvette containing the first standard/replicate in the series and press the take measurement button. A single reference suffices for standard curve creation. To create a standard curve using double beam instruments (Biochrom Libra S60, S70 & S80) insert the cuvette containing the reference solution in the reference cell holder and the cuvette containing the first standard/replicate in the sample cell holder and press the take measurement button. The reference measurement will be automatically subtracted from the standard measurement.

Continue recording all standards/replicates until the standard curve has been completed. To repeat any standard measurements simply press the desired result, insert the correct standard and press take measurement.

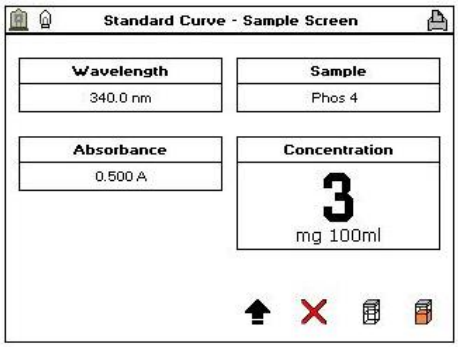
Note: After all replicates have been taken for a particular standard pressing the replicates icon takes the user to the next standard that was specified in the method.



To ignore any outlying standard measurements, press the tick in the appropriate row to toggle it to a cross. Any ignored measurement will be automatically removed from the standard curve. These can be reinstated by pressing the cross.

TAKING A MEASUREMENT

After the standard curve has been collected press the forward arrow to proceed to the sample measurement screen.



To perform a measurement using the Biochrom Libra S50, insert the cuvette containing the reference solution in the cell holder and press the take reference button. Remove and replace with a cuvette containing the sample and press the take measurement button. A single reference suffices for subsequent analyses in the same series.
To take a measurement using double beam instruments (Biochrom Libra S60, S70 & S80) insert the cuvette containing the reference solution in the reference cell holder and the cuvette containing the sample in the sample cell holder and press the take measurement button. The reference measurement will be automatically subtracted from the sample measurement.

To view the standard curve whilst on the sample measurement screen, simply press the 'View Curve' icon that appears under the options menu.

Note: Saving the method using the Save Method icon that appears on the options menu will save both the method parameters and the standard curve. Recalling a method containing method parameters and standard curve allows the user to measure samples directly.

SAVING & PRINTING

For details of manual saving and printing see the Saving and Printing section.

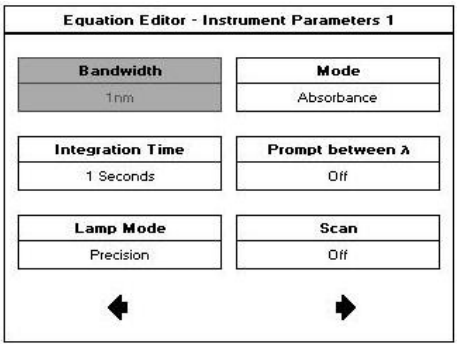
Equation Editor

The Equation Editor application allows users to create their own unique methods that include calculations and thresholds. Examples of methods that can be created using Equation Editor include percentage strength calculations and olive oil and chlorophyll analyses.

GETTING STARTED

The use of Equation Editor is outlined below.

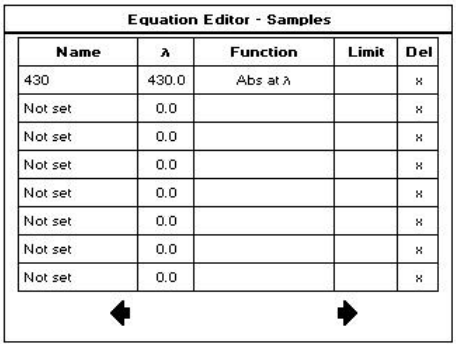
MEASUREMENT PARAMETERS



The first screen of Equation Editor allows the user to set the measurement parameters that will be used for all subsequent measurements. *Bandwidth* (Libra S80 only), *Integration Time*, *Lamp Mode* and *Mode* are used as in all other applications. The use of *Prompt between λ* and *Scan* are described below.

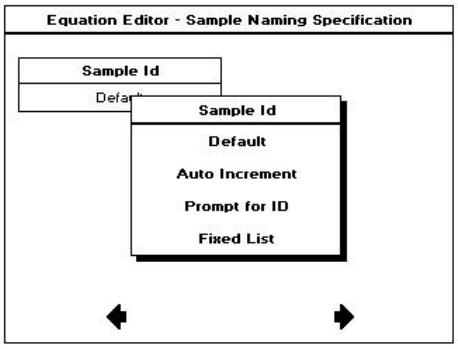
Prompt between λ off	The instrument will measure wavelength 1, measure wavelength 2, measure wavelength 3 etc and then perform any calculations.
Prompt between λ on	The instrument will measure wavelength 1, prompt, measure wavelength 2, prompt, measure wavelength 3 etc and then perform any calculations. This is used for equations that require wavelength measurements of different samples *e.g.* chlorophyll analysis.
Scan off	The instrument will only measure the wavelengths specified in the sample specification table (see below). Having scan off speeds up analyses as only the required wavelengths are measured.
Scan on	The instrument will perform a full wavelength scan over a range set under the sample measurement parameters screen. With scan on the user can set both the scan speed and the step size.

You can advance to the next screen at any time by pressing the forward arrow and return to the previous screen by pressing the back arrow.



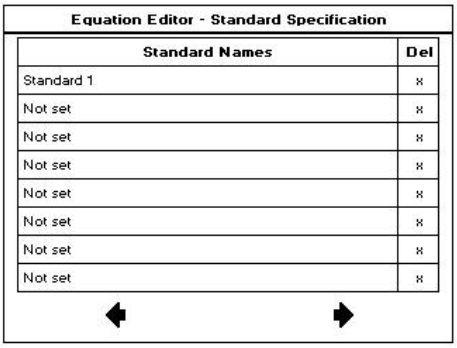
Using the Sample Measurement table the user inputs all of the measurements that are required in the method *i.e.* the screen left shows a fixed wavelength measurement at 430.0 nm that is named 430. Data is inputted as described below. Before any data is inputted Name will read 'Not set' and will not appear in the Sample Data list on the Equation Builder.

Name	Pressing this allows the user to input an alphanumeric name for the measurement. The name inputted here will be used as the Sample Data in the Equation Builder (inputted data only appears in the Equation Builder if the user has defined a name).
Wavelength (λ)	Pressing this produces the number entry box that allows the user to input the wavelength for measurement.
Function	Pressing this displays a combo box with the following options. Abs / %T at λ – measurement will be at the wavelength inputted by the user only. Peak closest to λ – the instrument automatically finds the peak closest to the inputted wavelength. Valley closest to λ – the instrument automatically finds the valley closest to the inputted wavelength.
+/-	Used with Peak closest to λ and Valley closest to λ only. This is the range over which the instrument will scan for a peak or valley from the inputted wavelength.
Del	Pressing this deletes the row. If the data is used in an equation this will also be deleted.



Equation Editor has four options for sample naming conventions. These are shown below:

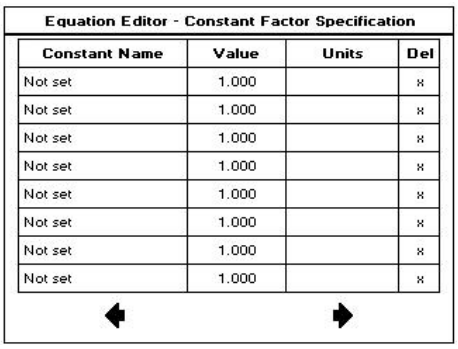
Default	The sample name consists of Sample and an incrementing number.
Auto Increment	The sample name is a combination of sample seed and an incrementing sample number. The user will be prompted to enter the sample seed for each new batch of samples.
Prompt for ID	The user is prompted to enter the sample name before running each sample.
Fixed List	The user will be prompted to enter the number of samples required. Sample names for each sample are then entered on the subsequent screens. The inputted sample names are saved for each method.



The Standard Specification screen is used to declare a list of all of the standard solutions which will be referenced when creating an equation.

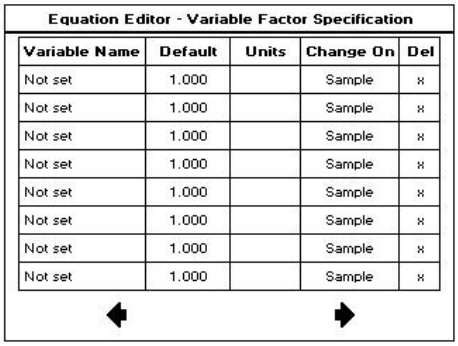
E.g. the equation for percentage strength at 500 nm compares the absorbance of a sample to the absorbance of a control standard. This is where the control standard would be defined.

Standard names are entered by pressing on the desired row and entering the standard name using alphanumeric text entry. Standard measurements can be made for any measurements specified in the Samples table.



The Constant Factor Specification screen is used to declare any constants used in the equation. Data is inputted as described below. Before any data is inputted *Constant Name* will read 'Not set' and will not appear in the Constants list on the Equation Builder.

Constant Name	Pressing this allows the user to input an alphanumeric name for the constant.
Value	Pressing this produces the number entry box that allows the user to input the value of the constant.
Units	Pressing this allows the user to enter units for the constant using alphanumeric text entry. If this column is left blank, no units will be displayed on exported or printed data.
Del	Pressing this deletes the row. If the constant is used in an equation this will also be deleted.



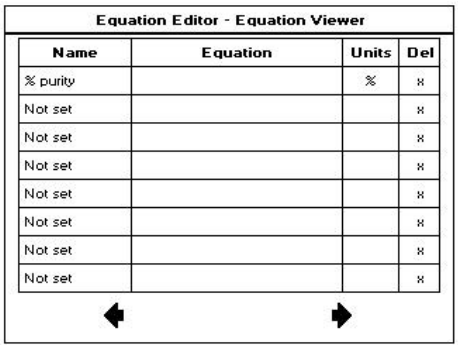
The Variable Factor Specification screen is used to declare any variables used in the equation. Data is inputted as described below. Before any data is inputted, *Variable Name* will read 'Not set' and will not appear in the Variables list on the Equation Builder.

Variable Name — Pressing this allows the user to input an alphanumeric name for the variable factor.

Default — Pressing this produces the number entry box that allows the user to input the default variable factor. Default values can be edited during a measurement.

Units — Pressing this allows the user to enter units for the variable factor using alphanumeric text entry. If this column is left blank, no units will be displayed on exported or printed data.

Change Each — Pressing this toggles between sample and batch. When set to sample the method will prompt for a variable to be entered before each sample measurement. When set to batch, the method will prompt for a variable to be entered at the start of each sample batch.



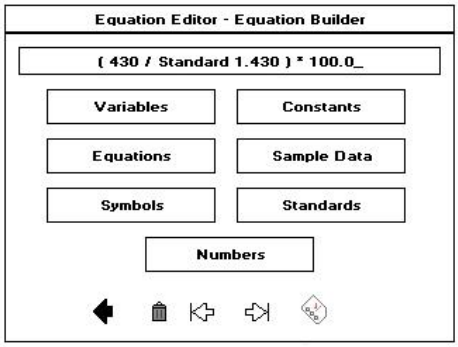
The Equation Viewer screen provides an overview of any equations created, as well as allowing the user to create new or edit existing equations. Data is inputted as described below. Before any data is inputted Name will read ‘Not set’ and will not appear on the results screen.

Name — Pressing this allows the user to input a unique, alphanumeric name for the equation. The names entered here will be displayed in the ‘Equations’ combo box on the Equation Builder screen as well the results screens. Therefore any equation can be easily identified when using it in other equations.

Equation — Pressing this takes the user to the ‘Equation Builder’ (see below). Any equation constructed in the Equation Builder will be displayed in this box.

Units — Pressing this allows the user to enter units for the result of the equation using alphanumeric text entry. If this column is left blank no units will be displayed alongside the result.

Del — Pressing this deletes the row and removes the equation.

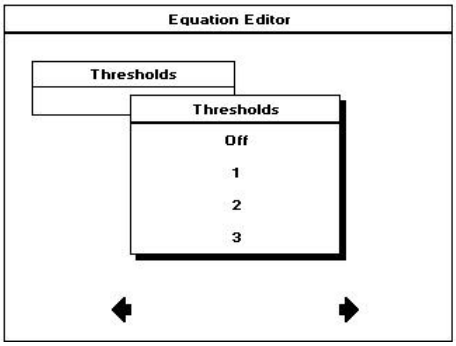


The Equation Builder allows the user to create any equations required in the method. Data is inputted as described below. To allow data to be inserted or deleted the cursor can be moved left and right using the left and right arrows. Data can be deleted using the delete icon and thresholds added using the thresholds icon (see below).

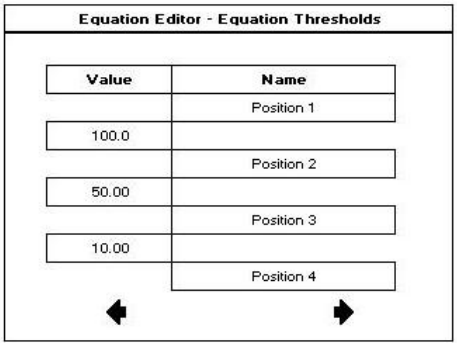
Variables	Pressing on this displays a list that contains any variables added to the 'Variables' table by the user. Selecting the desired variable enters it into the equation.
Constants	Pressing on this displays a list that contains any constants added to the 'Constants' table by the user. Selecting the desired constant enters it into the equation.
Equations	Pressing on this displays a list that contains any equations that have been created in this method. Selecting the desired equation enters it into the equation.
Sample Data	Pressing on this displays a list that contains all of the readings specified by the user in the Sample Measurement table. Selecting the desired sample data enters it into the equation.
Symbols	Pressing on this displays a list containing mathematical symbols and the logic gates AND and OR. Selecting the desired symbol enters it into the equation.
Numbers	Pressing this produces the number entry box that allows the user to directly input numbers into the equation.

Note: All of the data above appears in the lists as it was inputted in the appropriate table.

After the equation has been inputted the user has two options. If the result is to be viewed as a number, press the back arrow to return to the Equation Viewer screen. If the result is to be viewed with a user specified pass/fail limit press the 'Thresholds' button to set appropriate thresholds for the measurement.



The first thresholds screen allows the user to input how many thresholds are required for a result.



With Thresholds set to 3 the screen will display the table layout shown left. Setting Thresholds to 2 and 1 will reduce the number of values you can input to 2 and 1, respectively.

Value — Pressing this produces the number entry box that allows the user to directly input numbers for threshold values.

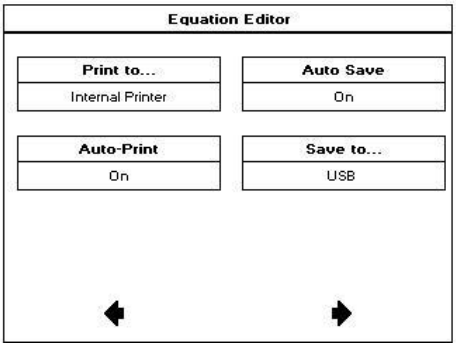
Name — Pressing this allows the user to enter the text that will be displayed for each result using alphanumeric entry.

Using the example above: A result of ≥100 will return the answer Position 1, a result of <100 and ≥50 will return the answer Position 2, a result of <50 and ≥10 will return the answer Position 3 and a result of <10 will return the answer Position 4.

Note: When using AND or OR logic in an equation the result will be returned as a binary (1 = true and 0 = false). These can be incorporated into the thresholds by setting the *Thresholds* to 1, *Value* to 1 and setting the appropriate names (as the name above value corresponds to ≥1 this is the response that will be displayed for true results).

After all of the thresholds have been set, pressing the forward arrow will return the user to the Equation Builder screen. The Equation Viewer screen can be accessed using the back arrow.

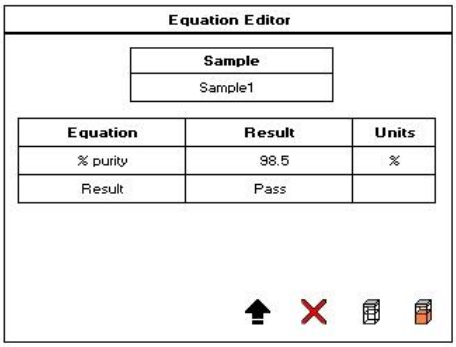
Continue as described above until all equations have been created. Once complete pressing the forward arrow will take the user to the output options screen below.



Set the outputs required in your method. For more information see the section Saving and Printing.

Note: Automatically saving sample data with sample naming set to ‘Prompt for ID’ or ‘Fixed list’ will save the data using the first inputted sample name as the filename.

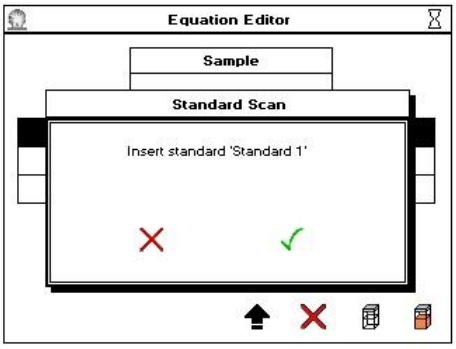
PERFORMING A MEASUREMENT – NO STANDARDS



To perform a measurement using split beam instruments (Libra S50). Insert a cuvette containing the reference solution in the cell holder and press the reference button. Remove and replace with a cuvette containing the sample and press the take measurement button. A single reference suffices for subsequent analyses in the same series.
To take a measurement using double beam instruments (Libra S60, S70 & S80) insert a cuvette containing the reference solution in the reference cell holder and a cuvette containing the sample in the sample cell holder and press the take measurement button. The reference measurement will be automatically subtracted from the sample measurement.

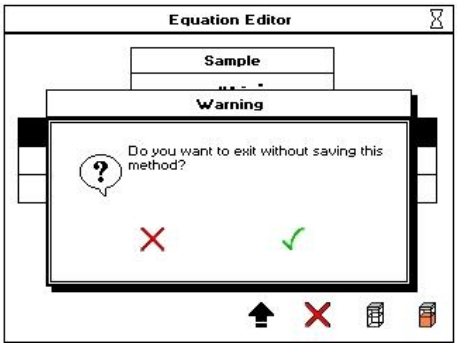
Note: Pressing on the equation name allows the user to view the equation and the individual measurement results.

USING STANDARDS



When performing a measurement using a method that includes standards, the first press of the take measurement button will produce a message box that prompts the user to insert a specific standard. After all standards have been measured, subsequent presses of the take measurement button will perform sample measurements.

SAVING METHODS AND EXITING

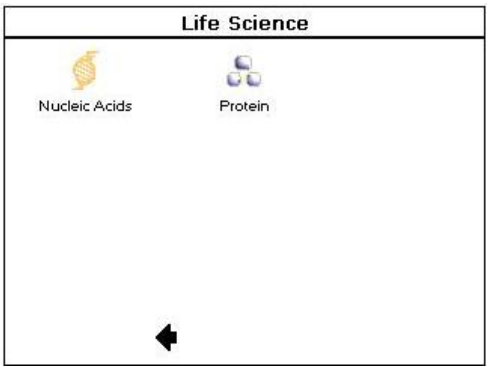


As methods developed using Equation Editor may have taken time to input, the software will prompt the user to save the method before exiting. Pressing the cross will return to the user to the results screen, where they can save the method, pressing the tick will exit without saving. Details of method saving can be found below in the Saving Methods section.

SAVING AND PRINTING

For details of manual saving and printing see the Saving and Printing section.

LIFE SCIENCE APPLICATIONS



This contains two sub folders; Nucleic Acids and Protein. The contents of these sub folders are detailed below:

NUCLEIC ACIDS

DNA	Utilises the absorbance measurements at 230, 260 & 280 nm with optional background correction to perform a concentration and purity check for DNA samples.
RNA	Utilises the absorbance measurements at 230, 260 & 280 nm with optional background correction to perform a concentration and purity check for RNA samples.
Oligo	Utilises the absorbance measurements at 230, 260 & 280 nm with optional background correction to perform a concentration and purity check for oligo samples.
Cydye DNA	Measures the labelling efficiency of fluorescently labelled DNA probes to ensure that there is sufficient amount of each probe to give satisfactory signals. The DNA yield is measured at 260 nm whilst the incorporation of the dyes is measured at the absorption maxima. This method is also useful for measuring the yields and brightness of fluorescently labelled *in-situ* hybridization probes.
TM Calc	The Tm Calculation application calculates the theoretical melting point from the base sequence of a primer. It is done using nearest neighbour thermodynamic data for each base in the nucleotide chain in relation to its neighbour

PROTEIN

BCA	Quantitative determination of protein concentration utilising the absorbance measurement at 562 nm
Bradford	Quantitative determination of protein concentration utilising the absorbance measurement at 595 nm
Lowry	Quantitative determination of protein concentration utilising the absorbance measurement at 750 nm

Biuret	Quantitative determination of protein concentration utilising the absorbance measurement at 546 nm
Protein UV	Direct UV determination of protein concentration at 280 nm using the Christian Warburg calculation
Protein A280	Direct UV determination of protein concentration using BSA, IgG, Lysozyme, Molar Extinction. Mass Extinction or E1% calculations

NUCLEIC ACID APPLICATIONS

DNA, RNA & Oligo

Nucleic acids can be quantified at 260 nm because it is well established that solutions of DNA and RNA in 10 mm pathlength cells with an optical density (absorbance) of 1.0 have concentrations of 50 µg/ml and 40 µg/ml, respectively. Oligonucleotides typically have a factor of 33 µg/ml, although this does vary with base composition and can be calculated if the base sequence is known.

$$\text{Concentration} = A260 \times \text{Factor}$$

Biochrom Libra use the default factors 50, 40 and 33 for DNA, RNA and oligonucleotides, respectively. Compensation for dilution and pathlength can also be entered.

NUCLEIC ACID PURITY CHECKS

- Nucleic acids extracted from cells are accompanied by proteins and extensive purification is required to separate these protein impurities. The ratio of A260/A280 gives an indication of a sample's purity, with pure DNA and RNA preparations typically having ratios of ≥1.8 and ≥2.0, respectively. Deviations from these values indicate the presence of impurities, but care must be taken when interpreting results.
- Concentration also affects both the A260 and A280 readings. If a solution is too dilute, the readings may be at the instrument's detection limit and results may vary as there is less distinction of the A260 peak and A280 slope from the background absorbance. For accurate measurements A260 should always be greater than 0.1.
- Elevated A230 values can also indicate the presence of impurities (230 nm is near the absorbance maximum of peptide bonds and also indicates buffer contamination since EDTA and other buffer salts absorb at this wavelength). When measuring RNA samples, the A260/A230 ratio should be >2.0. Ratios lower than 2.0 generally indicate contamination with guanidinium thiocyanate, a reagent commonly used in RNA purification and which absorbs over the 230 - 260 nm range. A wavelength scan of the nucleic acid is particularly useful for RNA samples.

BACKGROUND CORRECTION

- To compensate for the effects of background absorbance caused by turbidity, high absorbance buffer solutions and the use of reduced aperture cells the Biochrom Libra have the option of background correction at a 320 nm.

- When used, A320 is subtracted from A260 and A280 prior to use so that:

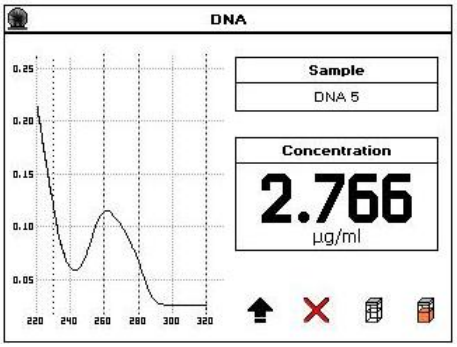
$$\text{Concentration} = (A260 - A320) \times \text{Factor}$$

$$\text{Abs ratio} = (A260 - A320) / (A280 - A320)$$

$$\text{Abs ratio} = (A260 - A320) / (A230 - A320)$$

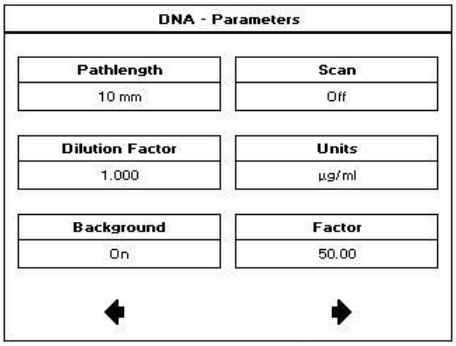
- The use of background correction can remove variability due to handling effects of low volume disposable cells.

Spectral scan of nucleic acid



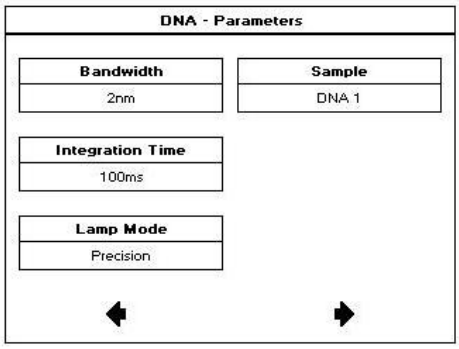
Note: An absorbance maximum near 260 nm and absorbance minimum near 230 nm, a flat peak near 260 nm and steep slope at 280 nm and very little absorbance at 320 nm

MEASUREMENT PARAMETERS

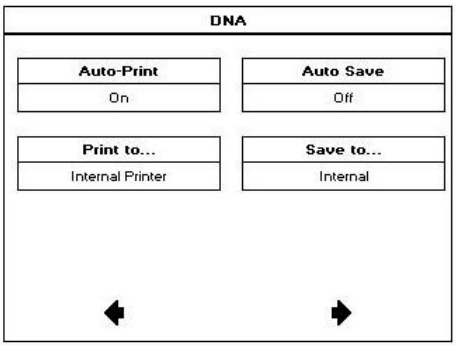


Set *Pathlength* and *Dilution Factor* to the required values. Check above to see if Background correction is required. To perform a scan over the range 220 – 330 nm for each measurement set *Scan* to *On.* Set *Units* to encompass the expected concentration of your samples, the default *Factor* will update automatically depending on the units selected *i.e.* for units of µg/ml the default factor will be 50.00. If the *Factor* required differs from the default value this can be edited using numeric entry.

You may advance to the next screen at any time by pressing the forward arrow and return to the previous screen by pressing the back arrow.

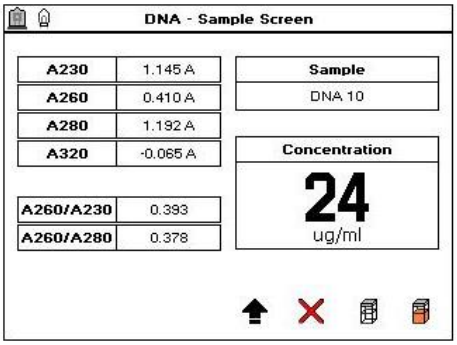


Bandwidth (Libra S80 only), *Integration Time* and *Lamp Mode* can be set as you require. The *Sample Seed* entered under *Sample* will the filename of any saved file.



Set the outputs required in your method. For more information see the section Saving and Printing.

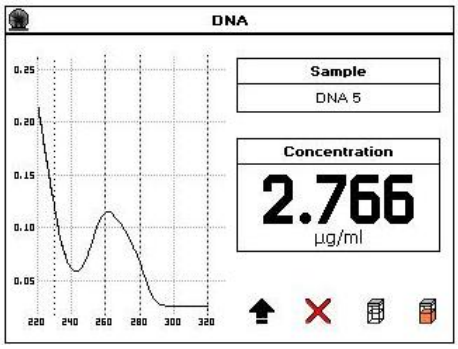
TAKING A MEASUREMENT



To perform a measurement using split beam instruments (Libra S50). Insert a cuvette containing the reference solution in the cell holder and press the reference button. Remove and replace with a cuvette containing the sample and press the take measurement button. A single reference suffices for subsequent analyses in the same series.

To take a measurement using double beam instruments (Libra S60, S70 & S80) insert a cuvette containing the reference solution in the reference cell holder and a cuvette containing the sample in the sample cell holder and press the take measurement button. The reference measurement will be automatically subtracted from the sample measurement.

If *Background* is set to *On* the A320 result will be included in the left hand column and automatically subtracted from the displayed A230, A260, A280, A260/A230, A260/A280 results.



If *Scan* is set to *On* in method parameters a scan of the most recently run sample can be viewed by pressing the *View Scan* icon in the options menu.

Note: When saving sample data, scan files will not be saved. The *Wavescan* application should be used to save scans of nucleic acid samples.

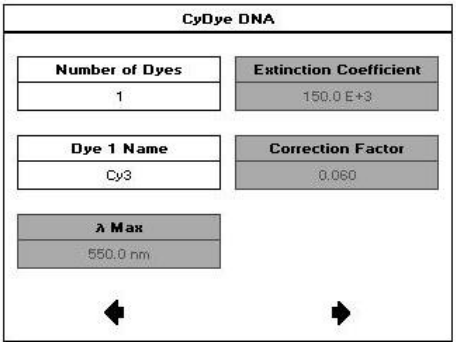
SAVING & PRINTING

For details of manual saving and printing see the Saving and Printing section.

CYDYE DNA

The measurement of the labelling efficiency of fluorescently labelled DNA probes before 2-colour micro-array hybridization ensures that there is sufficient amount of each probe to give satisfactory signals. The data also provides an opportunity to balance the relative intensities of each fluorescent dye by adjusting the concentration of each probe before hybridization. The DNA yield is measured at 260 nm whilst the incorporation of the dyes is measured at the absorption maxima. This method is also useful for measuring the yields and brightness of fluorescently labelled *in-situ* hybridization probes.

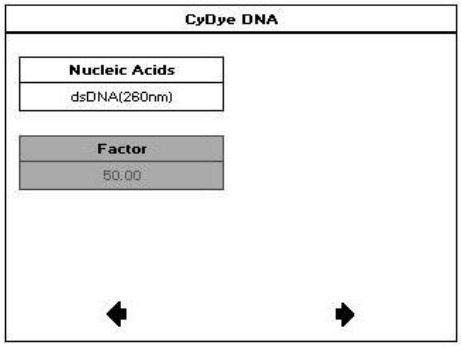
MEASUREMENT PARAMETERS



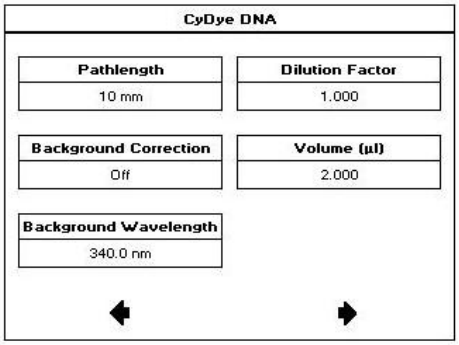
Number of Dyes, this can be set to 1 or 2. *Dye 1 Name* allows the user to select the dye used in the measurement, the Biochrom Libra have 19 dyes pre-programmed and the option for user entry using the Custom Dye option. *λ Max*, *Extinction Coefficient* and *Correction Factor* are only editable when using the Custom Dye option.

You may advance to the next screen at any time by pressing the forward arrow and return to the previous screen by pressing the back arrow.

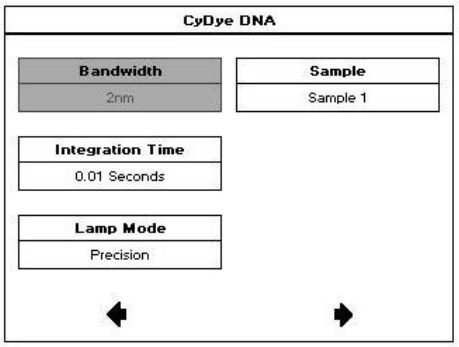
With *Number of Dyes* set to 2, the next method parameter screen allows the user to specify the second dye used in the measurement.



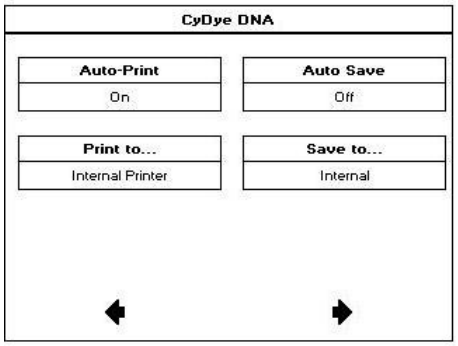
If the measurement requires the calculation of DNA Concentration and/or DNA Quantity the relevant nucleic acid can be inputted. A custom factor can be entered by selecting Custom in *Nucleic Acids*.



This parameters screen allows the user to set the pathlength of the cuvette being used, whether background correction is required and at what wavelength, the dilution factor and volume of the sample in µl. All of these will be used in the calculations.

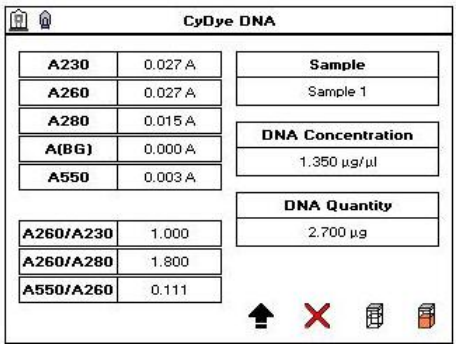


Bandwidth (Libra S80 only), *Integration Time* and *Lamp Mode* can be set as you wish. The *Sample Seed* entered under *Sample* will the filename of any saved file.

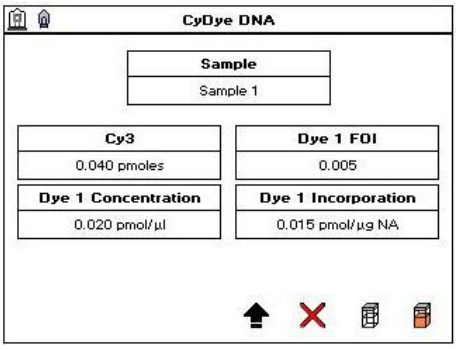


Set the outputs required in your method. For more information see the section Saving and Printing.

TAKING A MEASUREMENT

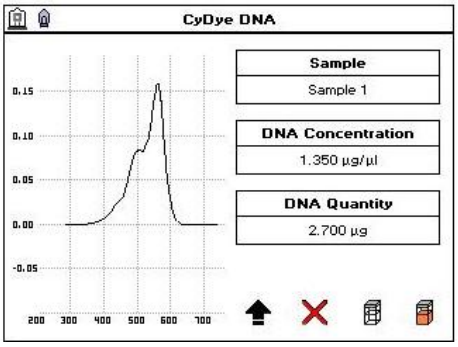


To perform a measurement using split beam instruments (Libra S50). Insert a cuvette containing the reference solution in the cell holder and press the reference button. Remove and replace with a cuvette containing the sample and press the take measurement button. A single reference suffices for subsequent analyses in the same series.



To take a measurement using double beam instruments (Libra S60, S70 & S80) insert a cuvette containing the reference solution in the reference cell holder and a cuvette containing the sample in the sample cell holder and press the take measurement button. The reference measurement will be automatically subtracted from the sample measurement.

If *Background Correction* is set to *On* the *Background Wavelength* set in the method will be included in the left hand column and automatically subtracted from all results.



To toggle between DNA & dye parameters press the toggle icon on the options menu. Cydye DNA measurements are wavelength scanning measurements, to view the scan for a particular measurement press the scan button in the options menu.

SAVING & PRINTING

For details of manual saving and printing see the Saving and Printing section.

TM CALCULATION

The Tm Calculation application calculates the theoretical melting point from the base sequence of a primer. It is done using nearest neighbour thermodynamic data for each base in the nucleotide chain in relation to its neighbour (Breslauer *et al*, *Proc.Natl. Acad. Sci. USA,* **1986**, *83*, 3746). The data obtained are useful in both the characterisation of oligonucleotides and in calculating Tm for primers used in PCR experiments.

The ACGT/U sequence entered in the method parameters is used to calculate the theoretical Tm, the theoretical absorbance (Absorbance units/mmol) and the conversion factor (mg/ml). This is possible as the stability of a bent and twisted sequence of bases such as an oligonucleotide is dependent on the actual base sequence. These calculated thermodynamic interactions between adjacent base pairs have been shown to correlate well with experimental observations.

The Tm Calculation application uses matrices of known, published thermodynamic values and extinction coefficients to calculate Tm and the theoretical absorbance/factor of an entered base sequence.

Tm is calculated using the equation:-

$$Tm = \frac{\Delta H \times 100}{\Delta S + (1.987 \times \log_e(\mathbf{c}/4 + 53.0822)} - 273.15 + \log \mathbf{[salt]}$$

where ΔH and ΔS are the enthalpy and entropy values, respectively summed from respective 2 × 4 × 4 nearest neighbour matrices

c is the Primer concentration of oligonucleotide (pmoles/ml) in the calculated Tm or the measured concentration in measured Tm. In the latter case, concentration is obtained from the equation:

$$c = \frac{\text{Abs(260 nm)} \times \text{Calculated factor} \times \text{pathlength multiplier} \times 10\,000}{\mathbf{MW}}$$

Calculated factor and MW are defined below

[salt] is the buffer molarity plus total molarity of salts in the hybridization solution (moles/l)

Weights for ΔS are indexed by adjacent paired bases. A similar equation applies to weights for ΔH, again indexed by adjacent bases.

Note: Bivalent salts may need normalising using a multiplying factor of 100 because of their greater binding power.

THEORETICAL ABSORBANCE

The Theoretical Absorbance is based on a calculation as follows:

For each adjacent pair of bases (nearest neighbours) an extinction coefficient weight is accumulated using a 4 × 4 table (one for either DNA or RNA). This total weight is doubled and then for each

internal base a counterweight is subtracted using another 1 × 4 table. The end bases are excluded from the latter summation.

Total Extinction Coefficient E = Σ (2 × aTable[base_type][base(n)][base(n+1)])-
Σ(tTable[base_type][base(n]])

CONVERSION FACTOR

The Conversion Factor is given by = $\frac{\text{Molecular weight}_{ABCDE}}{\Sigma_{EABCDE}}$

where

E_{ABCDE} = [2 × (EAB +EBC +ECD +EDE) –EB –EC –ED]

• The molecular weight (MW) of a DNA oligonucleotide is calculated from:

MW (g/mole) = [(dA × 312.2) + (dC × 288.2) + (dG × 328.2) + (dT × 303.2.)] + [(MWcounter-ion) × (length of oligo in bases)]

(for RNA oligonucleotide, (dT × 303.2) is replaced by (dU × 298.2)

The MW calculated using this equation must be adjusted for the contribution of the atoms at the 5' and 3' ends of the oligo.

For phosphorylated oligos: Add [17 + (2 × MW of the counter-ion)]
For non-phosphorylated oligos: Subtract [61 + (MW of the counter-ion)]

The MW (g/mole) of the most common oligo counter ions are:

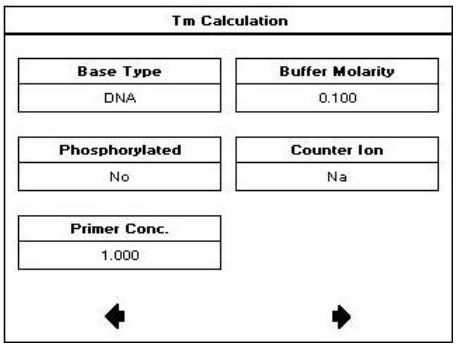
Na (sodium)	23.0
K (potassium)	39.1
TEA (triethylammonium)	102.2
Other	Defaults to 1.0 (variable 0.1–999.9)

Calculated molecular weight: a weight is added for each base looked up from a table. The weight of the counter ion is added for every base from a small table for the known ions. If phosphorylated, then the system adds 17.0 plus two counter ions otherwise it subtracts 61.0 and one ion.

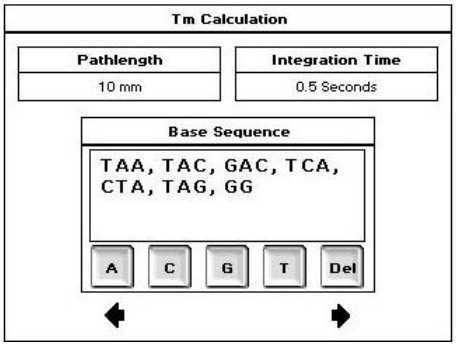
Theoretical Absorbance: for each adjacent pair of bases (nearest neighbours) a weight is accumulated using a table. For each internal base a weight is subtracted using another table. Separate tables are used for DNA and RNA.

Calculated factor: this is the calculated molecular weight divided by the theoretical absorbance.

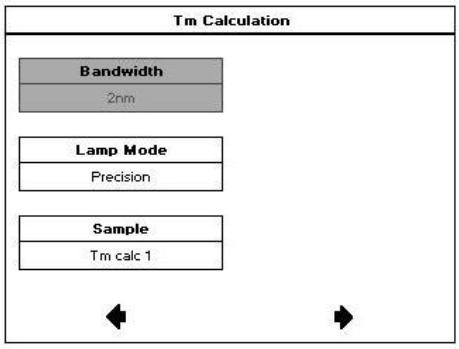
MEASUREMENT PARAMETERS



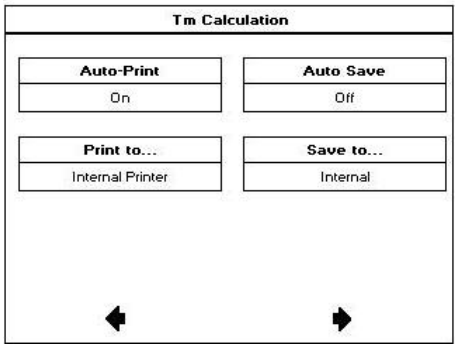
Set the required *Base Type* (DNA/RNA), whether the base is *Phosphorylated*, the *Primer Concentration* (pmoles/ml), *Buffer Molarity* and *Counter Ion*. *Counter Ion* has options for Na (sodium), K (potassium), TEA (triethylammonium) and Other, allowing the user to set the required molecular weight (MW) of the counter ion.



Set the *Pathlength* and *Integration Time* you require. *Base Sequence* allows the user to enter the known base sequence triplets using the buttons A, C, G and T/U. To improve readability a comma is added after each triplet.

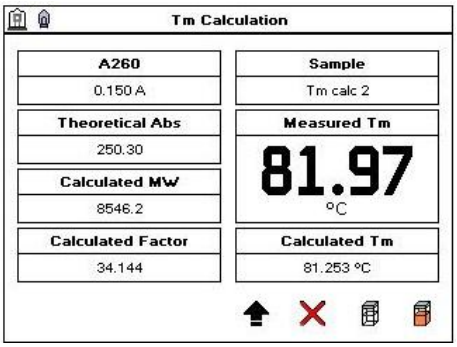


Bandwidth (Libra S80 only) and *Lamp Mode* can be set as you wish. The *Sample Seed* entered under *Sample* will the filename of any automatically saved file.



Set the outputs required in your method. For more information see the section Saving and Printing.

TAKING A MEASUREMENT



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To take a measurement using double beam instruments (Libra S60, S70 & S80) insert a cuvette containing the reference solution in the reference cell holder and a cuvette containing the sample in the sample cell holder and press the take measurement button. The reference measurement will be automatically subtracted from the sample measurement.

SAVING & PRINTING

For details of manual saving and printing see the Saving and Printing section.

PROTEIN APPLICATIONS

The Biochrom Libra contains dedicated methods for both colorimetric protein assays and direct UV measurements.

BCA, BRADFORD, LOWRY & BIURET PROTEIN ASSAYS

The BCA, Bradford, Lowry and Biuret protein assays are well established spectrophotometric methods for determining the amount of protein in a sample. The exact choice of the assay depends upon the concentration of protein being measured and the detergents/reducing agents used in purification. Detailed protocols are supplied with all assay kits and should be followed closely to ensure accurate results are obtained. An outline of the protein assays offered by the Biochrom Libra is provided below:

Bradford method:	Quantifies the binding of the dye Coomassie Brilliant Blue to an unknown protein and compares this binding to that of different, known concentrations of a standard protein at 595 nm. The standard protein is usually bovine serum albumin (BSA).
Biuret method:	Depends on reaction between Cu^{2+} ions and amino acid residues in an alkali solution. The resulting copper complex absorbs light at 546 nm.
BCA method:	Depends on reaction between Cu^{2+} ions and amino acid residues. In addition, this method combines this reaction with the enhancement of Cu^{+} ion detection using bicinchoninic acid (BCA) as a ligand, giving an absorbance maximum at 562 nm. The BCA process is less sensitive to the presence of detergents used to break down cell walls.

Lowry method: Depends on quantifying the colour obtained from the reaction of Folin-Ciocalteu phenol reagent with the Tyrosyl residues of an unknown protein and comparing with those derived from a standard curve of a standard protein at 750 nm (usually BSA).

DETERMINATION OF PROTEIN CONCENTRATION USING THE BICINCHONINIC ACID (BCA) PROTEIN ASSAY

The principle of the bicinchoninic acid (BCA) protein assay relies on the formation of a Cu^{2+}-protein complex under alkaline conditions, followed by reduction of the Cu^{2+} to Cu^{+}. The amount of reduction is proportional to the amount of protein present. BCA forms a purple-blue complex with Cu^{+} in alkaline environments, thus providing a basis to monitor the reduction of alkaline Cu^{2+} by proteins. The BCA assay can be used to quantify proteins in the concentration range 0.2 to 1.0 mg/ml. It is compatible with many detergents but not compatible with reducing agents such as dithiothreitol above 1 mM.

GETTING STARTED

It is always advisable to prepare the standard in the same buffer as the sample to minimise any interference effects. BCA assays are routinely performed at 37 °C. Colour development begins immediately and can be accelerated by incubation at higher temperatures. Higher temperatures and/or longer incubation times can be used for increased sensitivity.

MATERIALS REQUIRED

Bicinchoninic Acid Kit for Protein Determination
Suitable tubes with caps to hold and mix 2.1 ml samples and to heat at up to 60 °C
Plastic disposable cuvettes
Standard protein solution of known concentration (1 mg/ml)
Incubator or block heater to heat sample tubes

PREPARATION OF THE BCA WORKING REAGENT

BCA reagents A and B are available commercially from a number of different sources. Instructions given here are for the kit supplied by Sigma Aldrich, other methods will be similar. Always refer to the manufacturer's instructions.

1. Mix 50 parts of Reagent A (a solution containing bicinchoninic acid, sodium carbonate, sodium tartrate and sodium bicarbonate in 0.1N NaOH, pH 11.25) with 1 part of Reagent B (4% (w/v) $CuSO_4.5H_2O$), preparing sufficient reagent for all the standards and samples. 2ml of working reagent is required for each sample.

2. Mix until the solution is a uniform light green colour. The solution is stable for 1 day.

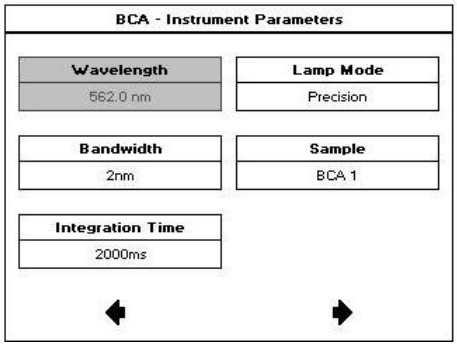
STANDARD PREPARATION

1. Prepare a series of protein standards ranging in concentration from 0.2 to 1.0 mg/ml such that the final volume for the assay is 0.1 ml. The Biochrom Libra can measure up to 9 standards and up to 3 replicates.
2. Add 2.0 ml of the BCA working reagent to each standard, vortex gently and incubate using one of the following parameters: 60 °C for 15 minutes, 37 °C for 30 minutes or room temperature from 2 hours to overnight.
3. If required, allow the tubes to cool to room temperature.

SAMPLE PREPARATION

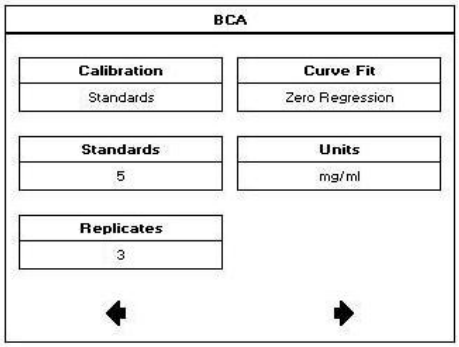
1. Prepare the unknown samples as described above ensuring that the final volume is 0.1 ml.
2. Add 2.0 ml of the BCA working reagent to each sample, vortex gently and incubate using one of the following parameters: 60 °C for 15 minutes, 37 °C for 30 minutes or room temperature from 2 hours to overnight.
3. If required, allow the tubes to cool to room temperature.

CREATING A STANDARD CURVE



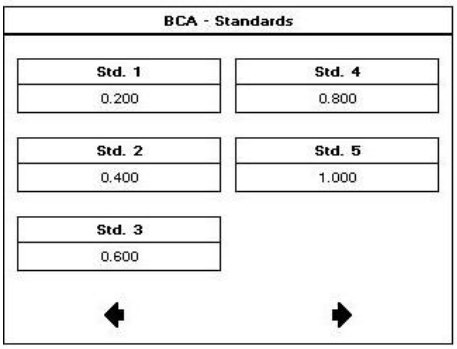
For BCA measurements *Wavelength* is set to 562.0 nm. *Bandwidth* (Libra S80 only), *Integration Time* and *Lamp Mode* and *Sample* can be set as you wish.

You may advance to the next screen at any time by pressing the forward arrow and return to the previous screen by pressing the back arrow.

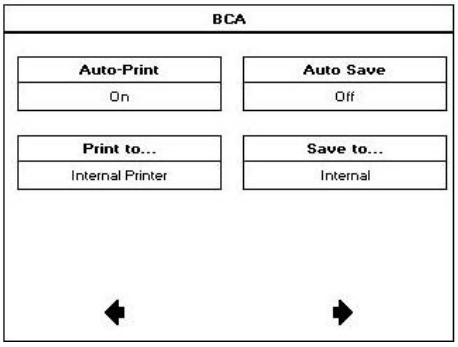


Set *Calibration* to Standards, *Curve Fit* to Zero Regression and enter *Units* of mg/ml. The number of standards and replicates can be set as you wish (for optimum accuracy it is recommended that the number of standards is ≥4 and replicates is >1).

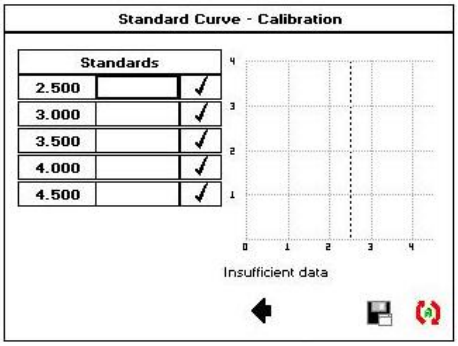
Note: With *Calibration* set to Standards the user is required to prepare and measure standards with *Calibration* set to Manual the user inputs both the standard concentrations and absorbances.



Enter the concentration of prepared standards using the numeric keypad.

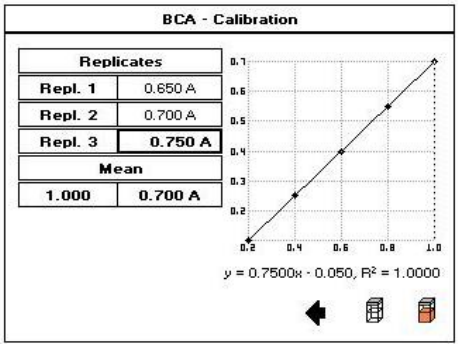


Set the outputs required in your method. For more information see the section Saving and Printing.

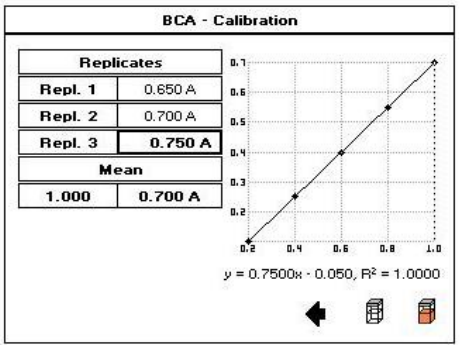


To create the standard curve when using replicates press the replicates button in the bottom right corner to take you to the screen shown below. With *Replicates* off standards can be measured directly as described below.

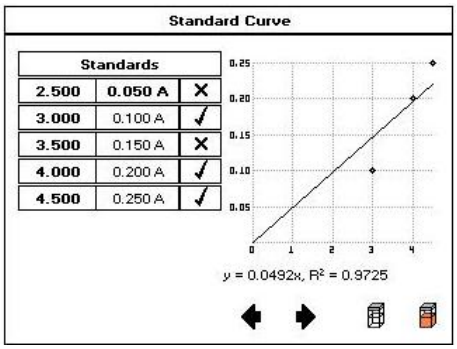
Note: After all replicates have been taken for a standard pressing the replicates icon takes the user to the next standard that was specified in the method.



To create a standard curve using the Biochrom Libra S50, insert the cuvette containing the reference solution in the cell holder and press the take reference button. Remove and replace with a cuvette containing the first standard/replicate in the series and press the take measurement button. A single reference suffices for standard curve creation. To create a standard curve using double beam instruments (Biochrom Libra S60, S70 & S80) insert the cuvette containing the reference solution in the reference cell holder and the cuvette containing the first standard/replicate in the sample cell holder and press the take measurement button. The reference measurement will be automatically subtracted from the standard measurement.



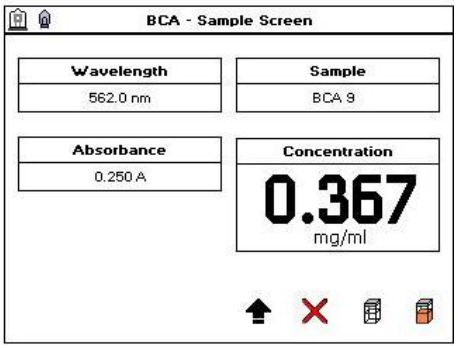
Continue recording all standards/replicates until the standard curve has been completed. After the standard curve has been collected press the forward arrow to proceed to the sample measurement screen.



To ignore any outlying standard measurements press the tick next in the appropriate row to toggle it to a cross. Any ignored measurement will be automatically removed from the standard curve. These can be reinstated by pressing the cross.

Note: Pressing the save method icon before any standards have been measured will save the method parameters only. Recalling a method containing method parameters only will require the user to construct a standard curve before measuring samples.

TAKING A MEASUREMENT



To perform a measurement using the Biochrom Libra S50, insert the cuvette containing the reference solution in the cell holder and press the take reference button. Remove and replace with a cuvette containing the sample and press the take measurement button. A single reference suffices for subsequent analyses in the same series.

To take a measurement using double beam instruments (Biochrom Libra S60, S70 & S80) insert the cuvette containing the reference solution in the reference cell holder and the cuvette containing the sample in the sample cell holder and press the take measurement button. The reference measurement will be automatically subtracted from the sample measurement.

To view the standard curve whilst on the sample measurement screen, simply press the ‘View Curve’ icon that appears under the options menu.

Note: Saving the method using the Save Method icon that appears on the options menu will save both the method parameters and the standard curve. Recalling a method containing method parameters and standard curve allows the user to measure samples directly.

SAVING & PRINTING

For details of manual saving and printing see the Saving and Printing section.

DETERMINATION OF PROTEIN CONCENTRATION USING DIRECT UV METHODS

The direct UV method of protein determination has a number of advantages over traditional colorimetric assays in that it does not rely on an external protein standard and the sample is not consumed in the assay. However the presence of nucleic acid in the protein solution can have a significant effect due to strong nucleotide absorbance at 280 nm. This can be compensated by measuring A260 and applying the equation of Warburg and Chrsitian for the protein crystalline yeast enolase (Equation 1).

$$\text{Protein concentration (mg/ml)} = (1.55 \times \text{Abs280}) - (0.76 \times \text{Abs260}) \quad \textbf{1}$$

$$\text{Protein concentration} = (\text{Factor 1} \times \text{Abs280}) - (\text{Factor 2} \times \text{Abs260}) \quad \textbf{2}$$

Biochrom Libra use default A260 and A280 factors of 0.76 and 1.55, respectively. These factors can be edited so that the equation can be applied to other proteins (Equation 2). Compensation for background, dilution and pathlength can also be entered.

To customise Equation 2 for a particular protein, the A260 and A280 values should be determined at known protein concentrations to generate simple simultaneous equations, which, when solved provides the two coefficients. In cases where Factor 2 is found to be negative, it should be set to zero since it means there is no contribution to the protein concentration due to absorbance at 260 nm.

The A260/A280 ratio also gives an indication of protein purity; a ratio of 0.57 can be expected for pure protein samples.

BACKGROUND CORRECTION

- To compensate for the effects of background absorbance caused by turbidity, high absorbance buffer solutions and the use of reduced aperture cells the Biochrom Libra can use background correction at a 320 nm.
- When used A320 is subtracted from A260 and A280 prior to use so that:

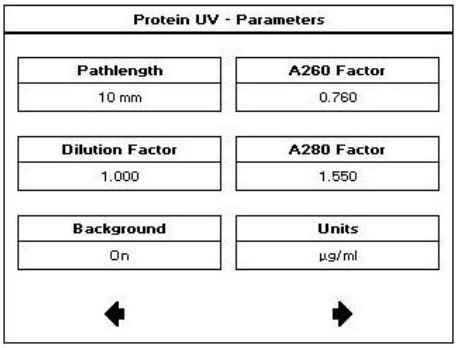
$$\text{Protein concentration} = [\text{Factor 1} \times(\text{Abs 280} - \text{Abs 320})] - [\text{Factor 2} \times(\text{Abs 260} - \text{Abs 320})]$$

$$\text{Ratio} = (\text{Abs 260} - \text{Abs 320}) / (\text{Abs 280} - \text{Abs 320})$$

- The use of background correction can remove variability due to handling effects of low volume disposable cells.

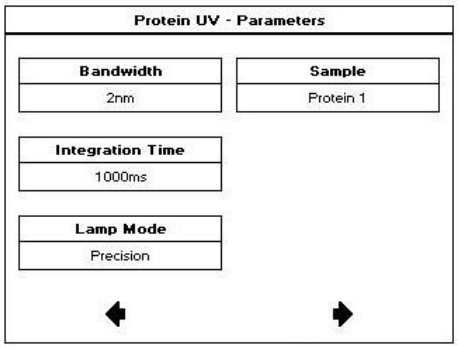
PROTEIN UV

MEASUREMENT PARAMETERS

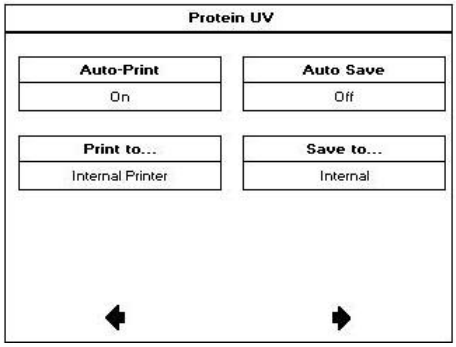


Set *Pathlength* and *Dilution Factor* to the required values. Check above to see if background correction is required. The default values for *A260 Factor* and *A280 Factor* are 0.76 and 1.55, respectively; these can be edited by pressing the appropriate box. Set *Units* to encompass the expected concentration of your samples

You may advance to the next screen at any time by pressing the forward arrow and return to the previous screen by pressing the back arrow.

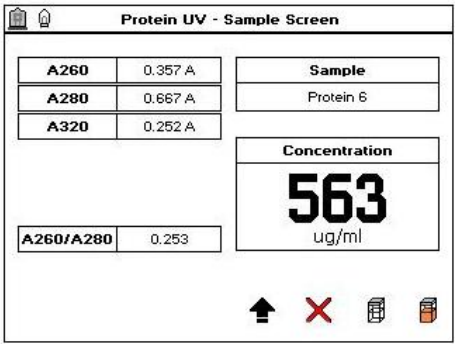


Bandwidth (Libra S80 only), *Integration Time* and *Lamp Mode* can be set as you wish. The *Sample Seed* entered under *Sample* will the filename of any saved file.



Set the outputs required in your method. For more information see the section Saving and Printing.

TAKING A MEASUREMENT



To perform a measurement using the Biochrom Libra S50, insert the cuvette containing the reference solution in the cell holder and press the take reference button. Remove and replace with a cuvette containing the sample and press the take measurement button. A single reference suffices for subsequent analyses in the same series.

To take a measurement using double beam instruments (Biochrom Libra S60, S70 & S80) insert the cuvette

containing the reference solution in the reference cell holder and the cuvette containing the sample in the sample cell holder and press the take measurement button. The reference measurement will be automatically subtracted from the sample measurement.

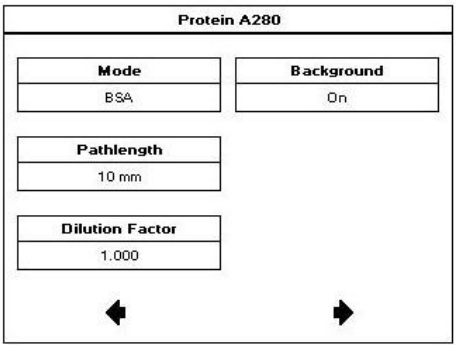
If *Background* is set to *On* the A320 result will be included in the left hand column and automatically subtracted from the displayed A260, A280 and A260/A280 results.

SAVING & PRINTING

For details of manual saving and printing see the Saving and Printing section.

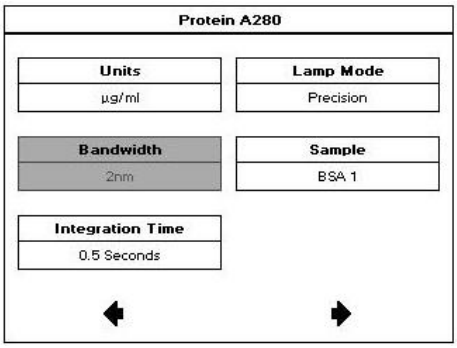
PROTEIN A280

MEASUREMENT PARAMETERS

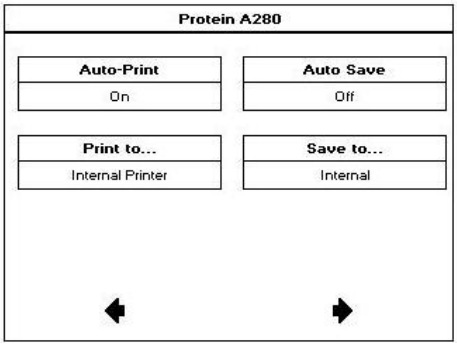


Select the *Mode* you require, Biochrom Libra have options for Christian Warburg, BSA, IgG, Lysozyme, Molar Extinction, Mass Extinction and E1% (for Molar Extinction the user will also be required to enter the molar extinction coefficient, and molecular weight and atomic units for Mass Extinction).

Set *Pathlength* and *Dilution Factor* to the required values. Check above to see if background correction is required.

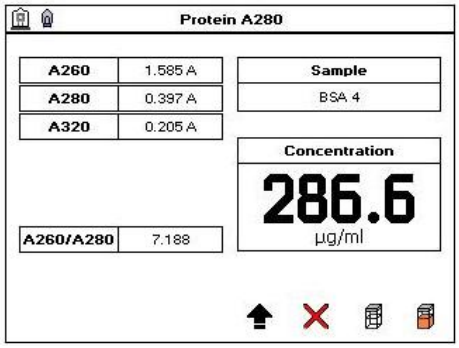


Set *Units* to encompass the expected concentration of your samples. *Bandwidth* (Libra S80 only), *Integration Time* and *Lamp Mode* can be set as you wish. The *Sample Seed* entered under *Sample* will the filename of any saved file.



Set the outputs required in your method. For more information see the section Saving and Printing.

TAKING A MEASUREMENT



To perform a measurement using the Biochrom Libra S50, insert the cuvette containing the reference solution in the cell holder and press the take reference button. Remove and replace with a cuvette containing the sample and press the take measurement button. A single reference suffices for subsequent analyses in the same series.

To take a measurement using double beam instruments (Biochrom Libra S60, S70 & S80) insert the cuvette containing the reference solution in the reference cell holder and the cuvette containing the sample in the sample cell holder and press the take measurement button. The reference measurement will be automatically subtracted from the sample measurement.

If *Background* is set to *On* the A320 result will be included in the left hand column and automatically subtracted from the displayed A260, A280 and A260/A280 results.

SAVING & PRINTING

For details of manual saving and printing see the Saving and Printing section.

SAVING & PRINTING

Biochrom Libra UV/visible spectrophotometers allow users to save and print sample data. This can either be included automatically as a method parameter or performed manually from the sample measurement screen. This section details the saving and printing options offered by all Biochrom Libra spectrophotometers.

SAVING SAMPLE DATA

The Biochrom Libra allows users to save sample data in three different formats:

INTERNAL

The sample data is saved to the instrument's internal memory format. See the Sample Manager section for details on saving and recalling data from the internal memory.

Note: To ensure the optimum performance of the Biochrom Libra it is recommended that unwanted data be deleted from the instrument's internal memory at regular intervals.

USB

The sample data is saved to a USB memory stick in format that can be read by Biochrom Libra spectrophotometers only. Files in this format cannot be opened by Microsoft Excel or other spreadsheet programmes.

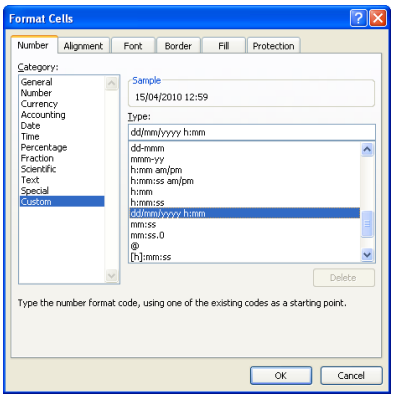
Note: Sample data will be saved to the Biochrom Libra Samples directory on the USB memory stick; if this directory is not present it will be created.

USB CSV

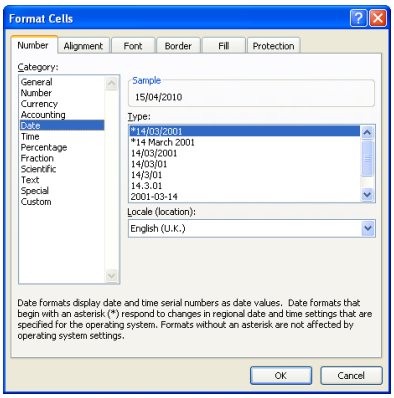
The data is saved to a USB memory stick in comma separated variable (CSV) format allowing it to be opened directly using Microsoft Excel or other spreadsheet programmes. Files in this format cannot be opened using the instrument.

Note: To view the data displayed in the File Created, Date and Time cells in a recognised format this will need formatting as described below:

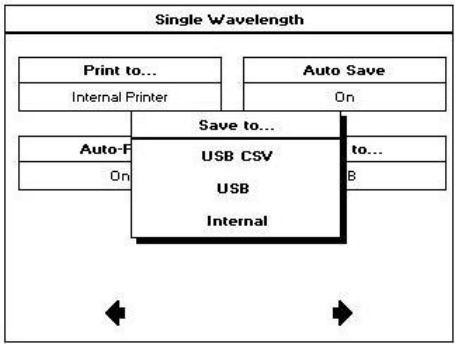
File Created: Right click in the appropriate cell and select Format Cells from the list, select Custom dd/mm/yyyy h:mm from the list on the right hand side (see below) and select ok.



Date / Time: Right click in the appropriate cell and select Format Cells from the list, under Category select Date or Time and the desired format from the list on the right hand side (see below) and select ok.



AUTOMATIC SAVING



The option to save sample data automatically is set under method parameters. With Auto Save set to on, the save location can be set to Internal, USB or USB CSV (the USB options are only available if a USB memory stick is inserted).

The filename given to an automatically saved file will be either the Sample Seed entered in the method parameters or, if the user chooses not to enter a Sample Seed, Default. The Biochrom Libra will only save one 'Default' file per application; subsequent saves of files without a sample seed will overwrite the previous default file.

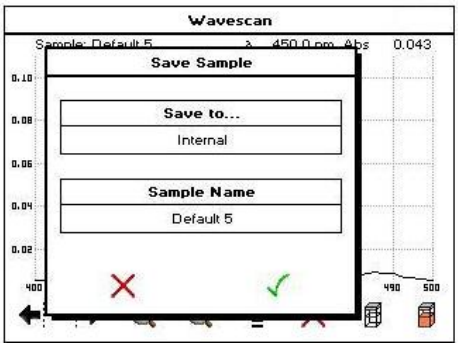
As sample data is saved when the user exits the application removing a USB memory stick before exiting will result in loss of data.

Note: All files are appended with a unique time and date stamp it is therefore possible to create two or more files sharing the same name.

Note: With Overlays ≥2 in Wavescan and Number of Samples ≥2 in Kinetics the overlaid data will always be saved automatically to the instrument's internal memory.

MANUAL SAVING

If a method does not require sample data to be saved each time a measurement is taken it is possible to manually save sample data in one of the formats outlined above. This procedure is described below:



After collecting all required sample measurements select save sample data from the options menu on the sample measurement screen to display the dialogue box shown left. The save location and filename are set by pressing the Save to and Sample Name boxes, respectively. If no Sample Name is entered the file will be titled Default

Note: Any sample data saved manually will override the auto save function.

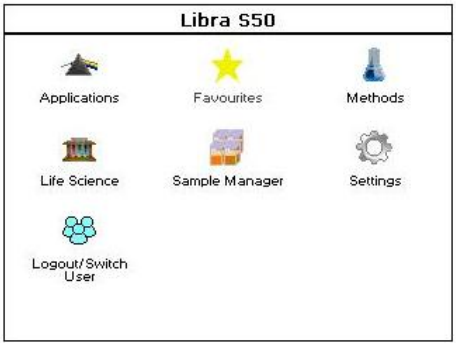
EXPORTING DATA

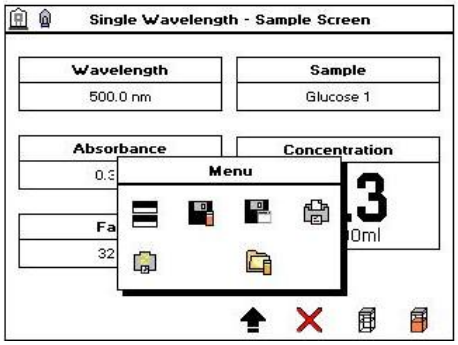
The Biochrom Libra allow users to recall saved sample data from the internal memory or a USB memory stick and save this in another format. This is done as follows:

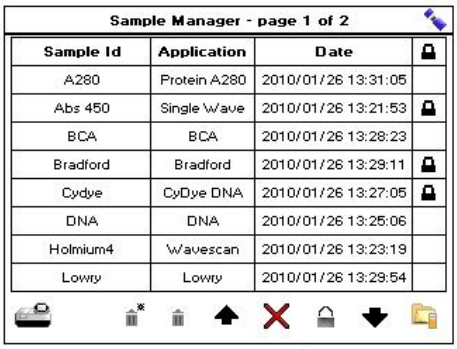
Recall saved data using Sample Manager and press the save sample button on the options menu to display the save sample dialogue box. Set the desired save location and filename using the Save to and Sample Name boxes, respectively and press the tick to confirm the data export.

SAMPLE MANAGER

Sample Manager is the application used by the Biochrom Libra for saving and recalling data from both the instrument's internal memory and the instrument's USB format. Sample Manager can be accessed from either the main screen using the Sample Manager button (below left) or from within an application using the Sample Manager icon on the options menu (below right).







To recall a saved file, highlight the desired file and press the Sample Manager icon in the right hand corner of the screen. Data will be displayed on the Sample Measurement screen (see Recalled Files below).

With a USB memory stick inserted it is possible to toggle between the internal and USB memories using the icon in the left hand corner of the screen. The location of the displayed data will be indicated by the icon in the top right hand corner.

Sample Manager has been designed to make finding saved files as simple as possible. Therefore it is possible to arrange files alphabetically, by application or by date/time saved by pressing the column headers Sample ID, Application and Date, respectively.

If there are too many saved data files to fit on a single screen this is signified at the top of the screen, *e.g.* Page 1 of 2. Scrolling through the screens is achieved using the up and down arrows at the bottom of the screen.

Note: Sample Manager can only display the first 100 sample data files; if the internal memory or USB stick contains >100 files these can be viewed be deleting or moving (USB only) unwanted data.

DELETING DATA FROM THE INTERNAL MEMORY

To ensure that the internal memory of the instrument does not contain too many unwanted data files, Sample Manager allows you to delete files. This can be done in one of three ways:

Deleting a single file: Highlight the file for deletion and press the delete button

Deleting multiple files: Highlight multiple files and press the delete button

Deleting all files: Press the 'Delete all' icon at the bottom of the screen.

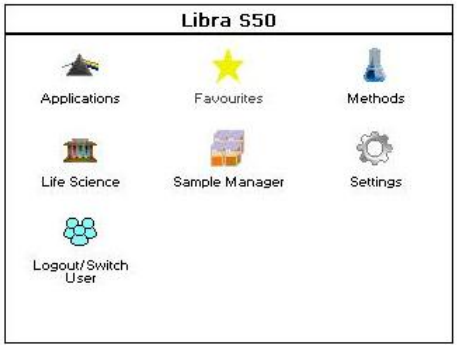
Note: It is only possible to delete data using Sample Manager accessed from the main screen. Sample Manager does not allow a user to delete sample data files from a USB memory stick; this must be done using a PC.

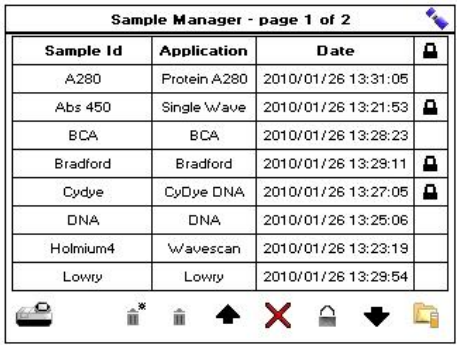
Sample Manager allows the user to lock files saved to the internal memory to prevent the accidental deletion of files containing precious data. To lock files, highlight the required data and press the lock icon at the bottom of the screen. Locked files are signified by the lock icon in the right hand column. Once a file is locked selecting 'Delete' or 'Delete All' does not clear this data from the instruments' internal memory. To unlock a file, highlight the appropriate locked data and press the lock icon at the bottom of the screen.

Note: As sample data files saved to a USB memory stick must be deleted using a PC it is not possible to lock USB sample data using Sample Manager.

To exit from Sample Manager press the 'Exit' icon.

ACCESSING SAMPLE MANAGER FROM THE MAIN SCREEN



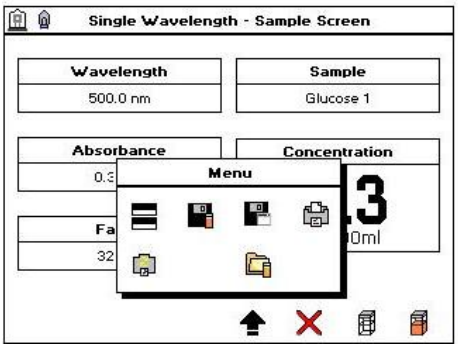


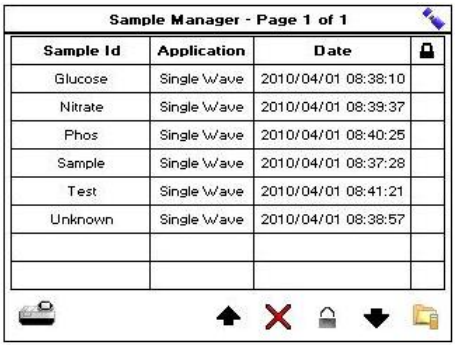
When accessed from the main screen Sample Manager will display all files held on the internal memory or USB memory stick and allows the user to lock and delete files saved to the internal memory. As Sample Manager accessed from the main screen allows users to delete files from the instrument's internal memory this option is disabled for Limited users.

To allow post scan manipulation of saved wavescan and kinetics data, files loaded from Sample Manager are loaded directly into Trace Manager (see Trace Manager section for details).

Note: With large numbers of files held on the internal memory there may be a short delay before Sample Manager opens.

ACCESSING SAMPLE MANAGER FROM WITHIN AN APPLICATION

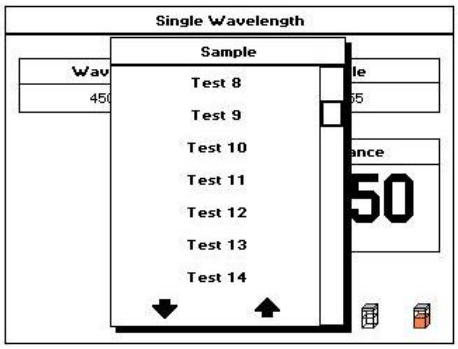




When accessed from within an application Sample Manager will only display files belonging to that specific application and only allows users to recall saved data and lock files.

RECALLED FILES

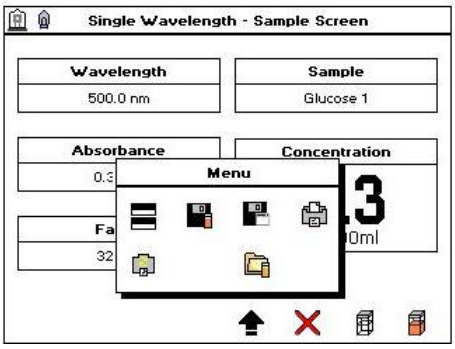
When recalled, sample data files will display the first sample recorded in a measurement. To access all sample data within a recalled file press the Sample box to display a list of all samples contained within the file. Pressing on the desired file will populate the boxes on the sample measurement screen with the saved data. Choosing to measure another sample with an old sample's data displayed simply updates the sample measurement screen and the appropriate boxes.



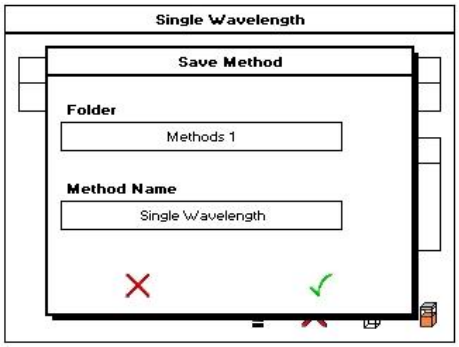
Note: Wavescan and kinetics sample data is recalled using Trace Manager. For details see the Trace Manager section for details.

SAVING METHODS

The Biochrom Libra allow users to store methods to the both the instruments internal memory and to USB memory sticks. The procedure for saving methods is described below:



After selecting the desired application and setting the required method parameters select the Save Method icon from the options menu on the sample measurement screen. Set the desired file name and save location using the dialogue box shown below.

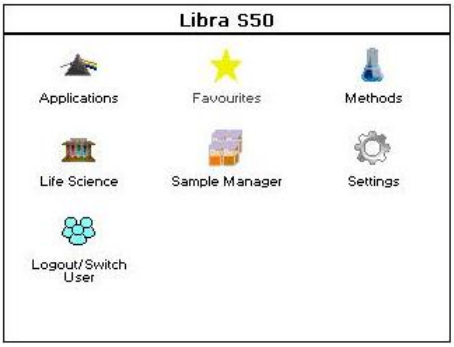


Pressing the Folder box produces a list of available save locations. USB will only appear on the list if a USB memory stick is inserted.

Pressing the Method Name box allows the user to set the desired method name using alpha numeric text entry.

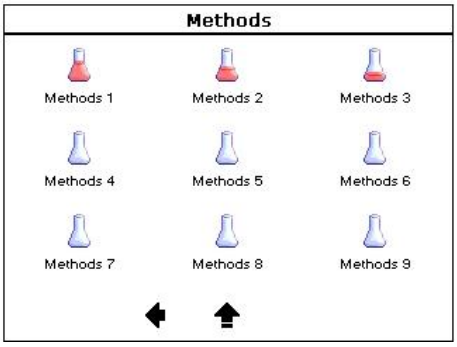
Press the tick to save and exit or the cross to exit without saving.

METHODS SAVED TO THE INTERNAL MEMORY



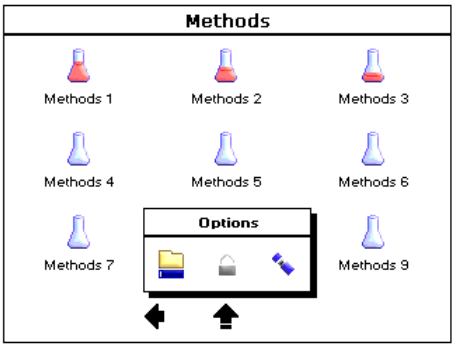
Methods saved to the instrument's internal memory are stored in either the Methods or Favourites folders, both of which are accessed via the main screen. The Biochrom Libra are capable of storing up to 90 methods on the instrument's internal memory.

METHODS FOLDER



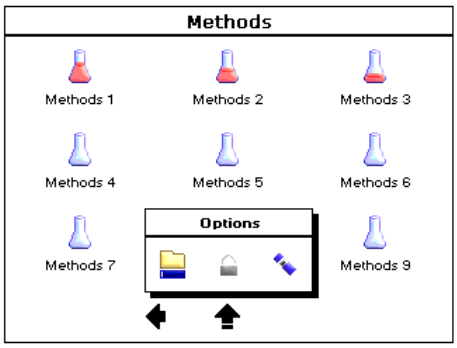
The Methods folder is made up of 9 folders each capable of storing up to 9 methods. The method folder icons have been designed to give the user an indication of the number of methods that are stored in that folder.

RENAMING METHOD FOLDERS

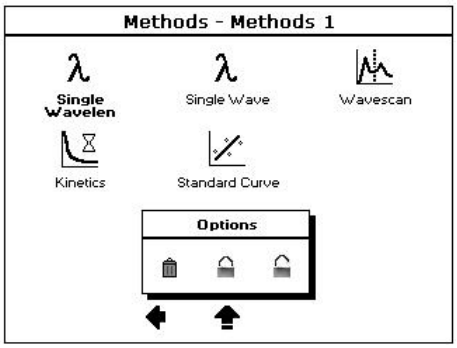


Using the Rename Folder icon on the options menu (the left hand icon) it is possible to rename any of the 9 method folders using alphanumeric text entry.

LOCKING SAVED METHODS

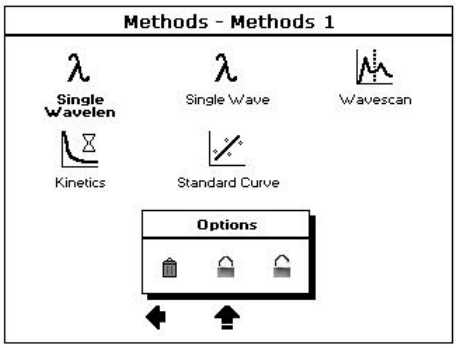


Using the lock folder icon on the options menu (the centre icon) it is possible to add pass code protection to any of the method folders. Locked folders cannot be renamed and are indicated by a padlock.



Within a methods folder it is possible to add pass codes to individual methods and to lock them from deletion using the lock icon on the options menu (the centre icon). Locked methods can be unlocked by using the unlock icon on the options menu (the right hand icon), selecting the desired method and entering the correct pass code.

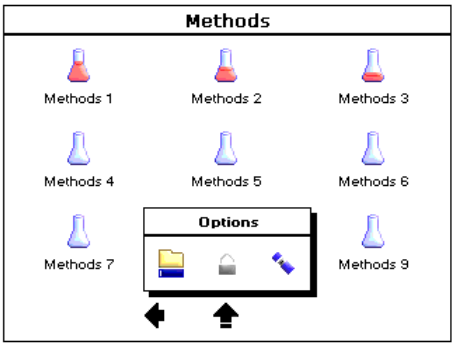
DELETING SAVED METHODS



Within a methods folder it is possible to delete saved methods using the delete icon on the options menu (the left hand icon) and selecting the desired file.

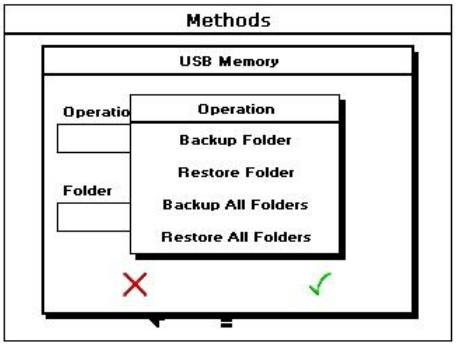
Note: Locked methods must be unlocked to allow deletion.

BACKING UP METHOD FOLDERS TO USB



With a USB stick inserted it is possible to use the USB icon on the options menu (the right hand icon) to:

Backup Folder: Copies all methods from a specified folder to a USB memory stick

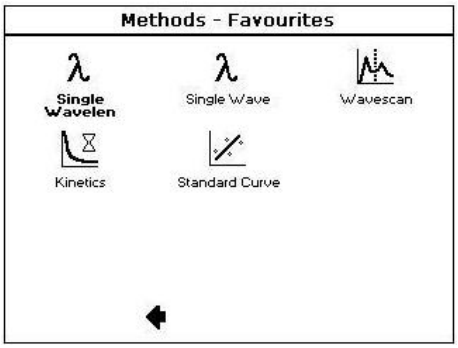


Restore Folder: Copies a backed up method folder from the USB memory stick to the internal memory

Backup All Folders: Copies all method folders from the internal memory to a USB memory stick

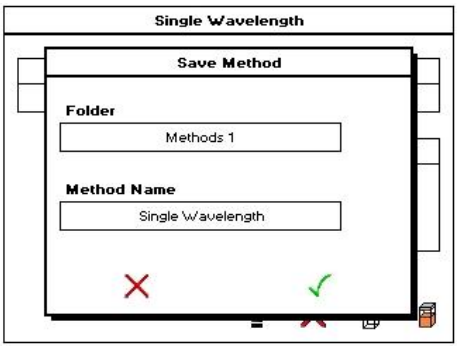
Restore All Folders: Copies all backed up method folders from the USB memory stick to the internal memory

FAVOURITES FOLDER



The Favourites folder is capable of storing up to 9 user defined methods. Methods stored in the Favourites folder can be locked and deleted as described above.

SAVING METHODS TO USB



To save files to a USB memory stick follow the procedure described above and select USB in the Folder box. The USB option will only appear in the list if a USB memory stick is inserted.

Methods saved to a USB memory stick will appear in the Biochrom Libra Methods folder in the root directory.

Note: Although it is possible to save an unlimited number of method files to a USB memory stick only 9 can be displayed on the instrument at any one time. As these will only be read from the Biochrom Libra Methods folder, additional files can be stored in other locations.

PRINTING

PRINTING SAMPLE DATA

The Biochrom Libra allows users to print sample data in one of three ways:

Note: Only available printers will be shown in the *Print to…* options box.

INTERNAL PRINTER

Fitting of the internal printer is described in the Biochrom Libra Accessories Manual.

PRINT VIA COMPUTER (PVC)

Print *via* Computer (PVC) is an application running under Windows™ to enable the Biochrom Libra to transfer data into a PC environment. From there the data can be printed or saved in a variety of formats, including graphics and text formats or as an Excel™ file. PVC can store data either to a common directory or be configured to save to independent directories by both file format and connection.

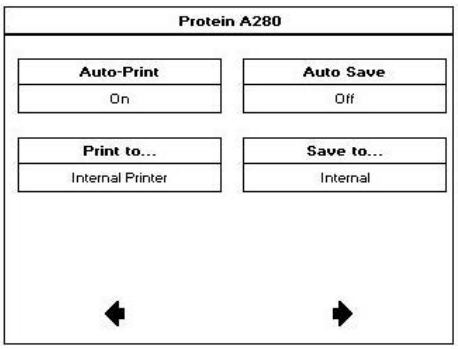
PVC is capable of supporting several instruments simultaneously, limited only by hardware and the speed of the host system and is able to operate via USB and Bluetooth simultaneously.

Installation and operating instructions for PVC can be found on the PVC CDROM.

BLUETOOTH®

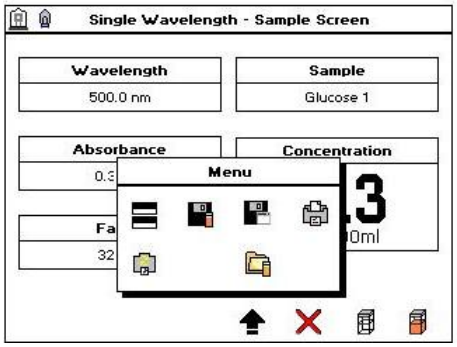
The Bluetooth accessory is a factory fitted option.

AUTOMATIC PRINTING



The option to print sample data automatically is set under method parameters. With *Auto Print* set to on, the print location can be set to one of the available options.

MANUAL PRINTING



If a method does not require sample data to be printed each time a measurement is taken it is possible to manually print sample data. This procedure is described below:

Set the desired print location in *Print to…* After collecting all required sample measurements select the Print icon from the options menu on the sample measurement screen.

ACCESSORIES

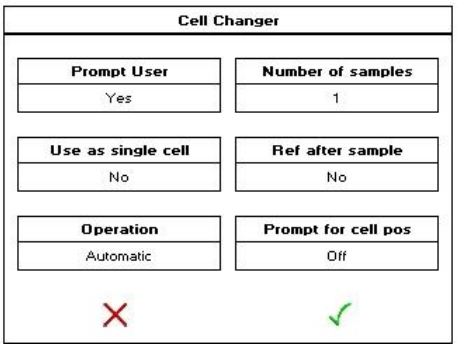
FITTING

Fitting of accessories is described in the Biochrom Libra Accessories Manual. The instrument must be switched off when fitting and removing accessories.

CELL CHANGER

OPERATION

With a cell changer connected, the Accessories folder under Settings will contain the Cell Changer icon. This provides the user with the means to set the default configuration for the cell changer accessory.



PROMPT USER

Yes: The Cell Changer parameter screen is displayed as part of the application parameters, allowing the user to set cell changer parameters each time an application is run. The default configuration set here pre-selected.

No: The Cell Changer parameter screen is not displayed as part of the application parameters. The default settings configured in the Accessories folder are used.

The Prompt feature is designed to save the user time and effort configuring each application for the cell changer and will also allow accessory parameter screens to be skipped on loading applications.

USE AS SINGLE CELL

Yes: Stops the cell changer accessory moving, meaning that the cell position 1 will remain in the sample beam for each measurement. When enabled, the options related to cell changer are removed from the screen. As this option allows the user to use the cell changer as a standard single cell holder this removes the need to keep swapping accessories.

No: The cell changer accessory will be used.

OPERATION

Automatic: By pressing the Start Measurement button the unit will collect the number of samples specified automatically.

Manual: By pressing the Start Measurement button the unit will measure the first sample, display a prompt to start next measurement, measure the second sample, prompt display a prompt to start next measurement etc.

NUMBER OF SAMPLES

Allows the user to set the number of samples to be measured using the numeric entry box. In Fixed Wavelength applications, if the number of samples is >8 the user will be prompted to load the cell changer after the first 8 samples. In Wavescan and Kinetics the maximum number of samples is 8.

Note: Number of Samples refers to the number of samples only and does not include standard or reference measurements.

REF AFTER CYCLE (BIOCHROM LIBRA S50 ONLY)

Yes: With >7 samples the measurement will proceed as follows: take a reference from cell position 1 measure samples 1 to 7, prompt user to insert next samples, take a reference from position 1 measure samples etc

No: With >7 samples the measurement will proceed as follows; take a reference from cell position 1 measure samples 1 to 7, prompt user to insert next samples, measure samples etc (i.e. a reference measurement is taken at the start of the measurement only).

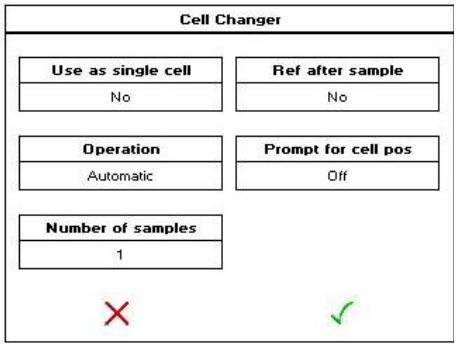
Note: With ≤7 samples Ref After Cycle on or off is recorded in the same manner.

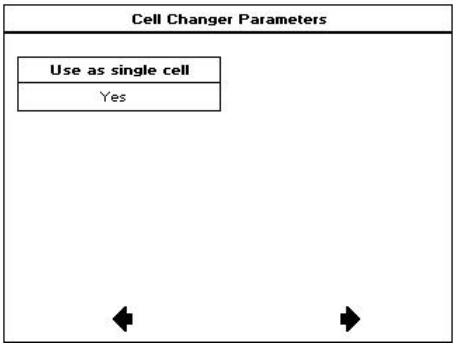
PROMPT FOR CELL POS

On: After pressing the Start Measurement button the software will display the positions each sample and reference (Libra S50 only) should be placed.

Off: The unit will perform the measurement without displaying this message.

The Cell Changer parameter screen that is displayed in the application method parameter screens is shown below (with use as single cell on or off). All options are as described above.



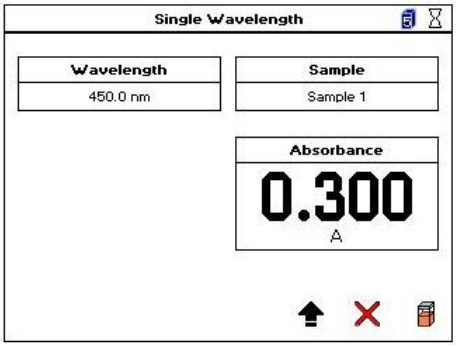


TAKING A MEASUREMENT WITH A CELL CHANGER

When using the cell changer the software will not display the Take Reference button.

Libra S50 — The reference measurement will always be taken from position 1 of the cell holder (if Ref after cycle is set to on a reference measurement will be taken from position 1 on each occasion)

Libra S60, S70 & S80 — As these instruments continually reference against the reference cell holder there is no requirement to perform a reference measurement before collecting sample data.



The icon in the top right corner of the sample measurement screen indicates the cell position that is in the sample measurement beam.

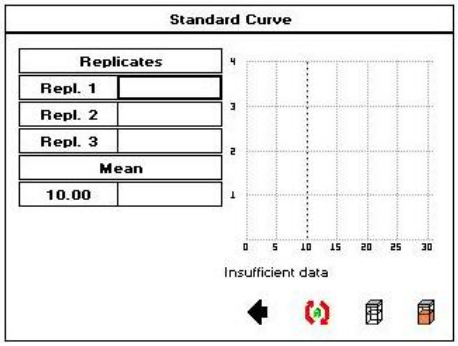
SAVING METHODS & RESULTS

Any method saved with the cell changer connected will save the inputted accessory parameters. If a user attempts to use a cell changer method without this accessory connected the software will display the warning, 'The following accessories are missing: Cell Changer The cell changer must be fitted before this method can be loaded.'

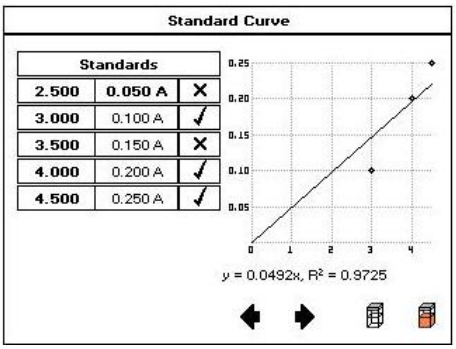
INCORRECT FITTING

If the cell changer is fitted incorrectly it will not be recognised during the instruments' start up calibration tests. If this occurs the instrument should be switched off, disconnected from the mains and the accessory refitted, paying close attention to the internal accessory connector.

USING THE CELL CHANGER TO CREATE A STANDARD CURVE



Pressing the take measurement button when on the Replicates screen will re-measure the highlighted replicate only. This measurement will always be made from cell position 1 of the cell changer.



Pressing the take measurement button on the construct standard curve screen will re-measure all standards whether measuring replicates or not.

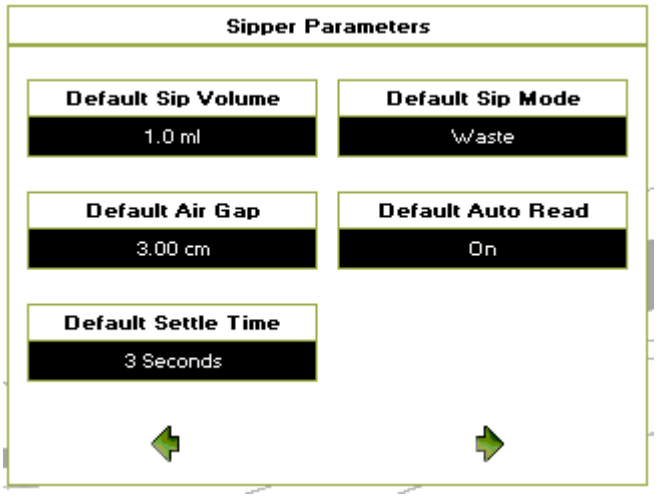
Sipper

The sipper and flowcell accessories for the Libra range of instruments are designed as an aid to speed up sample handling. The system comprises a peristaltic pump head that is controlled by the software to sip a known, calibrated volume of sample and move this into a flowcell which is in the sample position of the spectrophotometer.

The sipper can be set up and used within all applications and sipper parameter are stored as part of an application method

Setting sipper defaults

It is simple to customise the default parameters of the sipper for a particular installation by setting all of the default values. Every application will then load these defaults each time the sipper page is opened however they can be changed within the application. These default values set within the instrument under the Settings, Accessories, Sipper Icons and are stored even when the instrument is switched off.



Sip Volume

- Amount of sample to be sipped up. Range of values = 0.3ml to 50ml.

Note: working with volumes less than 1ml will require careful setting of the air gap to minimise cross contamination between samples.

Air-gap

- Gap between successive samples which is set by pumping air after the tube has been removed from the sample liquid – set in cm of tubing. Range of values = 0 to 200cm

The sipper has the ability to program an air gap between samples to help minimise cross contamination and to allow users flexibility to work with different volumes and lengths of sipper inlet tubing. When the start button is pressed the sipper pumps up the volume of sample that is set within the method and the user is then prompted to remove the tubing from the sample. After a short delay the pump starts again and the sample is moved along the tubing and air is sucked into the end of the tubing. The default air gap set in the software is 40cm which has been set to work with a 65cm length of tubing on the flowcell inlet. If a different length of tubing is used then a different default air gap will need to be set. To calculate this default air gap:

- Measure the length of tubing connected to the flowcell tubing inlet and take 20cm from this value
- From the home page go into a single wavelength method and enter through until you get to the sipper parameter page. Set the volume to 1ml and the air gap to the value measured above. Set the settle time to 1 second and Auto read to off.
- Sip a sample of water, remove the tube from the sample when prompted and note the position of the end of the sample slug – you need this to be stopping above the inlet position to the flowcell. For a 1ml sample volume you should have this stopping 15-25cm above the flowcell inlet. To move the position of the sample press the menu icon, parameters icon, step through the method until you reach the sipper parameters and adjust the air gap value. Increasing the value will move the end of the sample closer to the flowcell inlet, lowering the value will move it further away.
- Once the value has been determined exit the Single wavelength application, go to Settings, accessories, sipper and enter this value in the default air gap box.

Settle time

- Amount of time before the system is ready to measure after the sample has entered the flowcell. Value entered in seconds. Range of values = 1 to 30 seconds

Sip Mode

- Choice of Waste or Return. Waste mode turns the pump head in one direction and the sample goes to waste after measurement. If return is set then pressing the start button a second time will reverse the motor and the sample will be pumped back out of the inlet tube into the sample vessel. Cross contamination between samples will be worse if return mode is used.

Auto Read

- Auto read on will automatically start the measurement in the current application after the settle time has passed. If Auto read is off the user will manually have to press the reference or run icon for each measurement.

If you press the Settings, accessories, sipper icons as well as the defaults that have been set above there are other icons at the bottom of the screen for Calibration of the sipper volume and washing of the sipper.

Calibration

The sipper software contains a routine that allows users to calibrate the sipper to ensure that the volume of liquid set within the method matches the actual volume being sipped. Before starting the calibration routine check that it is actually necessary:

- Sample volume – set up a single wavelength method which includes the sipper and set the sip volume to 1ml. Fill a 10ml measuring cylinder, sip 5 times from the measuring cylinder taking notice of the messages regrdaing removing the tube for the air gap. After 5 sips divide the volume of sample sipped by 5 – the value should be approximately 1ml. If the vaklue varies by more than 0.2mls then recalibrate the sipper as below:
- Fill a 10ml graduated measuring cylinder with water up to the 10ml line. Press the calibrate icon, put the end of the sample tube into the measuring cylinder, press the start button and the sipper will start pumping. Once this has finished repeat 4 more times. Read off from the measuring cylinder the actual amount that has been sipped and enter it into the box on the screen and then press the Tick icon to exit.

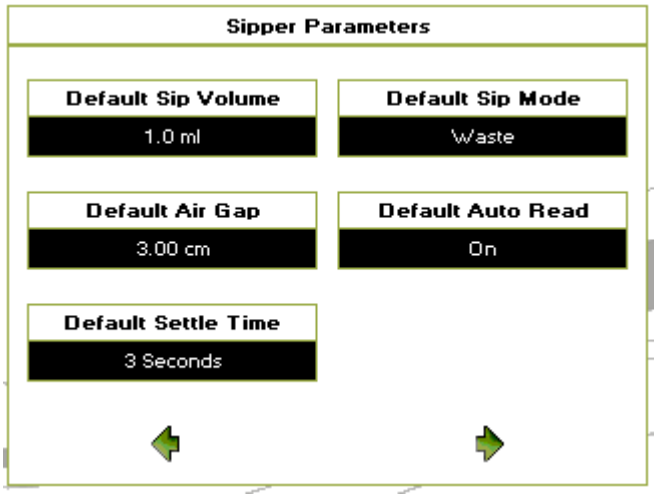
If you need to rest the calibrations to the default value then sip once and enter the value of 1ml.

Wash mode

This enables you to wash the sipper at the end of a batch of samples. Press the wash icon, dip the sample tube into water and allow it to flush the system.

Using the sipper within an application

All sipper parameters can be set as part of a method, the following screen will appear as part of the application method when the sipper is connected. The screen will open using the default sipper values stored under the Settings, Accessories, Sipper Icons.



The process of using the sipper is as follows:

1. Choose the application that you want to run by pressing the relevant icon
2. Set up the instrument parameters, data handling parameters and then sipper parameters. The sipper parameters shown will be the ones that are set on the sipper default page. These sipper parameters can be changed as part of this application method and the new values saved as part of the method
3. Put the sample tubing into the reference solution and press the silver button on the front of the sipper. System will sip the set volume and then prompt you to remove the sample tubing
4. System will then pump the set air gap and then wait the set settle time. If auto read is on the system will automatically reference, if it is off then press the clear cuvette icon to reference
5. Put the sample tube into the sample, press the silver button on the front of the sipper. System will sip the set volume and then prompt you to remove the sample tubing
6. System will then pump the set air gap and then wait the set settle time. If auto read is on the system will automatically run the sample, if it is off then press the orange cuvette icon to run the sample. Repeat for subsequent samples

Cleaning/Maintenance/Troubleshooting

To ensure good performance from your sipper system it is essential that routine maintenance is carried out.

After batch of samples

- Run the sipper Wash mode pumping deionised water, for 1 minute then remove sample tubing and pump air until all liquid is pumped into the waste vessel

At end of day

- Open up pump head to relax pressure on pump head tubing

Weekly

- Check pump head tubing for signs of wear – replace if necessary
- Check all connections for leaks
- Depending upon types of sample being run it may be advantageous to clean the flowcell properly (e.g. if “sticky” protein solutions are being run). Unscrew the 2 connectors, take the flowcell out of the instrument, fill with a detergent solution and leave to soak overnight. Empty and then flush with deionised water for 1 minute using the wash mode

Problems with sipper systems are generally caused by wrongly set parameters or poor maintenance. Typical faults and their cures include:

Sample not being sipped

- Check pump head closed
- Check pump head tubing for damage
- Check flowcell is connected and connectors are tightened
- Check PTFE tubing is pushed firmly into pump head tubing
- Check PTFE tubing is not crushed or damaged
- Check flowcell for blockages
- Check waste pipe for blockages

Sample being pumped right through flowcell

- Check air gap and reset to correct value

Bubbles in flowcell

- Check flowcell connectors are tightened
- Clean flowcell with detergent solution

Inconsistent readings

- Check volume being sipped – try increasing the volume
- Adjust air gap so the end of the sample is closer to the flowcell inlet - more of the sample flushes the previous sample out of the flowcell

Wrong volume being sipped

- Check sipper calibration

TECHNICAL SPECIFICATIONS

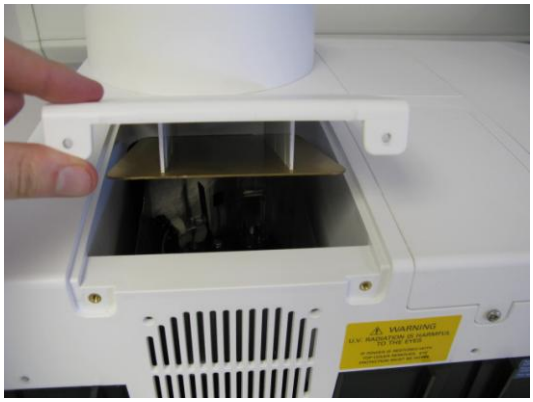
Parameter	Libra S50 Specification	Libra S60 Specification	Libra S70 Specification	Libra S80 Specification
Configuration	Split Beam	Double Beam	Double Beam	Double Beam
Lamp	Xenon	Xenon	Tungsten/Deuterium	Tungsten/Deuterium
Wavelength Range	190nm to 1100nm	190nm to 1100nm	190nm to 1100nm	190nm to 1100nm
Wavelength Accuracy	+/- 0.5nm across wavelength range	+/- 0.5nm across wavelength range	+/- 0.3nm across wavelength range	+/- 0.3nm across wavelength range
Wavelength Reproducibility	+/- 0.1nm	+/- 0.1nm	+/- 0.1nm	+/- 0.1nm
Bandwidth	2nm	2nm	1nm	Variable (0.5nm, 1nm, 2nm, 4nm)
Toluene in Hexane EP Resolution			>2.0	>2.0 at 1nm bandwidth
Stray Light	<0.050%T at 220nm using NaI and 340nm using $NaNO_3$	<0.050%T at 220nm using NaI and 340nm using $NaNO_3$	<0.025%T at 220nm using NaI and 340nm using $NaNO_3$ <1%T at 198nm using KCl	<0.025%T at 220nm using NaI and 340nm using $NaNO_3$ <1%T at 198nm using KCl
Photometric Range	-4A to 4A	-4A to 4A	-4A to 4A	-4A to 4A
Photometric Accuracy	+/-0.002A at 0.5A +/-0.004A at 1A +/-0.006A at 2A	+/-0.002A at 0.5A +/-0.004A at 1A +/-0.006A at 2A	+/-0.002A at 0.5A +/-0.004A at 1A +/-0.006A at 2A	+/-0.002A at 0.5A +/-0.004A at 1A +/-0.006A at 2A
Photometric Reproducibility	+/-0.002A at 1A	+/-0.002A at 1A	+/-0.002A at 1A	+/-0.002A at 1A
Scan Speed	>2400 nm/min	>2400 nm/min	>2400 nm/min	>2400 nm/min
Zero Stability	+/-0.001A/hr	+/-0.001A/hr	+/-0.0003A/hr in precision mode	+/-0.0003A/hr in precision mode
Noise	< 0.00005A RMS @ 0A at 700nm and 500nm over 20 measurements	< 0.00005A RMS @ 0A at 700nm and 500nm over 20 measurements	< 0.00005A RMS @ 0A at 700nm and 500nm over 20 measurements	< 0.00005A RMS @ 0A at 700nm and 500nm over 20 measurements

CHANGING TUNGSTEN AND DEUTERIUM LAMPS

Lamps should only be removed after they have cooled completely, attempting to remove lamps before they have cooled may result in injury.

1. Switch off the Biochrom Libra and ensure that the instrument is disconnected from the mains.

2. From the back of instrument remove the screws securing the lamp chamber (this is located above the fan grating).



3. Remove the lamp chamber lid to reveal both the tungsten and deuterium lamps (see below).

LAMP CHAMBER OF THE BIOCHROM LIBRA S70 & S80

REMOVING & REPLACING THE TUNGSTEN LAMP

To remove the tungsten lamp, pull the metal clip away from the tip of the lamp and carefully pull the lamp to remove.

To insert the new lamp, pull the metal clip away from the housing to provide space for the new lamp to be inserted and carefully insert the pins of the lamp into the lamp housing. When inserted in the housing carefully place the metal clip on the tip of the lamp.

Note: When replacing a tungsten lamp, the lamp envelope must not be touched. If the envelope is touched during installation the lamp must be thoroughly cleaned with a light cloth.

REMOVING & REPLACING THE DEUTERIUM LAMP

The deuterium lamp is removed by carefully pulling the lamp upwards. To insert the new lamp, line up the lamp pins with the holes in the lamp housing and push into place. The deuterium lamp can only be inserted in the correct position

Note: After installation the deuterium lamp must be thoroughly cleaned with a light cloth to remove any marks.

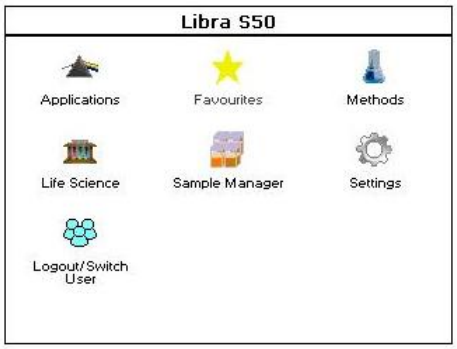
REPLACING THE LAMP CHAMBER LID

After the new lamp has been installed the lamp chamber lid should be replaced and screwed in place. The instrument will now be ready to use.

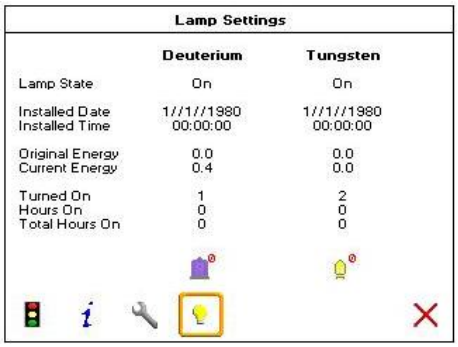
Note: A new baseline should always be measured after installing a lamp. This procedure is described in Instrument Settings (p. 16). The baseline should only be collected after the lamp has warmed up sufficiently (45 minutes).

RESETTING LAMP LIFE

With new lamps installed it is necessary to reset the lamp life on the software, this is done as follows:



From the main page, select Settings followed by Instrument Settings.

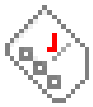


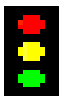
Select the lamp status icon at the bottom of the screen and press the appropriate Reset Lamp Life icon to reset the lamp life information for the desired lamp. The icons are detailed in the Table of Icons section.

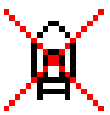
Note: Only users with Administrator privileges are capable of resetting lamp life information.

TABLE OF ICONS

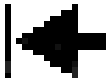
ICON	TITLE	FUNCTION
	Back arrow	Returns the user to the previous screen.
	Forward arrow	Advances the next screen in a sequence.
	Open options menu	Displays the relevant options menu, the exact content of the menu will depend upon the location.
	Page down	Allows the user to navigate to the next page
	Page up	Allows the user to navigate to the previous page
	Cursor left	Used in the wavescan and kinetics applications to move the cursor left. The position of the cursor and the corresponding x and y values are displayed above the scan.
	Cursor right	Used in the wavescan and kinetics applications to move cursor right. The position of the cursor and the corresponding x and y values are displayed above the scan.
	Toggle data viewed / reset	When used in the Cydye DNA application this toggles the data displayed between DNA and dye data. Under Instrument Settings this will resets a value.
	Delete all	Deletes all saved data/methods. Delete all is a two stage process.
	Delete	Deletes the highlighted data/method. Delete is a two stage process

	Thresholds	Used in Equation Editor. This allows users to add thresholds (pass/fail) limits to their results.
	Ok / accept	Used to confirm / accept any changes.
	Exit	Exits from an application or screen. If exit is selected without saving, any changes will be lost.
	Lock	Locks sample data file/method folder against accidental deletion.
	Unlock	Unlocks a locked method folder.
	Instrument	Used in Sample Manager to access sample data files stored on the instrument's internal memory or to indicate that the data being displayed is from the internal memory.
	Add linear section	Used in the kinetics applications to view a line of best fit on the scan.
	Section	Used in kinetics applications to view data in a specific section. It is possible to add t0 through to t7.
	Method folder	Methods saved internally can be stored in one of nine method folders (each capable of storing up to nine individual methods).
	Rename method folder	Accessed via the options menu on the methods screen, this allows the method folder to be renamed.

	Toggle view scan on or off	Used in the nucleic acid applications DNA, RNA and Oligo this allows the user to view a survey scan of the last sample run (in the region 220 - 320 nm).
	Toggle auto print on or off	Accessed via the options button on the sample screen. Pressing this button toggles auto print on and off.
	View method parameters	Accessed via the options button on the sample measurement screen. Pressing this button takes the user back to the method parameters.
	Print data	Accessed via the options button on the sample measurement screen. Pressing this button prints the sample data.
	Save method parameters	Accessed via the options button on the sample measurement screen. This allows a method to be saved to a location specified by the user.
	Save sample data	Accessed via the options button on the sample measurement screen. This allows sample data to be saved to a location specified by the user.
	Sample Manager	Accessed via the options button on the sample measurement screen. Displays the sample data held in the either the internal memory or on a USB memory stick.
	Instrument Information	Accessed via instrument settings. This displays instrument information such as product name, serial number etc.
	Instrument Settings	Accessed via instrument settings. This allows the user to set the default bandwidth, save new baselines and view the date of the last service.
	Instrument Status	Accessed via instrument settings. This displays the current status of the instrument.

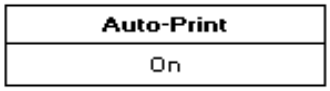
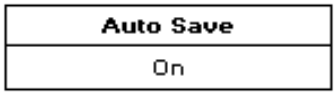
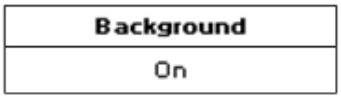
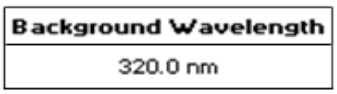
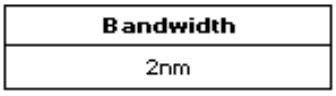
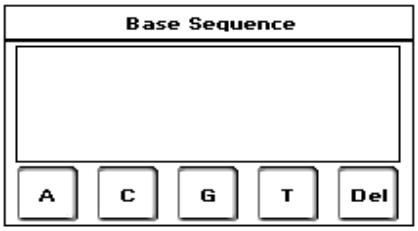
	Lamp Settings	Accessed via instrument settings. This displays the lamp status and allows the user reset the lamp life (S70 & S80 only).
	Save instrument options	Accessed via instrument settings. This saves the instrument options.
	Cell changer number	Displayed on the status bar. This indicates that the cell changer is connected and the cell position that is in the sample beam.
	Auto print - Bluetooth	Displayed on the status bar. This indicates that the instrument will automatically print all sample data to the Bluetooth device.
	Auto print - internal printer	Displayed on the status bar. This indicates that the instrument will automatically print all sample data to the internal printer
	Auto print - USB	Displayed on the status bar. This indicates that the instrument will automatically print all sample data to the USB printer
	Deuterium lamp off	Displayed on the status bar. This indicates that the deuterium lamp is off.
	Deuterium lamp fail	Displayed on the status bar. This indicates that the deuterium lamp has failed.
	Deuterium lamp on	Displayed on the status bar. This indicates that the deuterium lamp is on.
	Deuterium lamp warming	Displayed on the status bar. This indicates that the deuterium lamp is warming up.
	Tungsten lamp fail	Displayed on the status bar. This indicates that the tungsten lamp has failed.

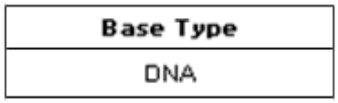
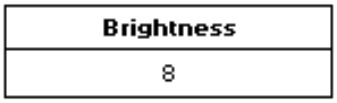
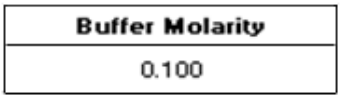
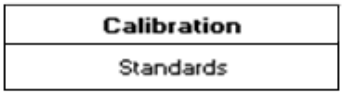
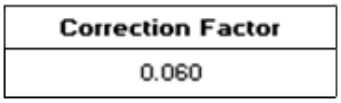
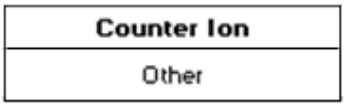
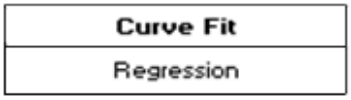
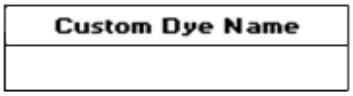
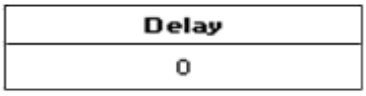
	Tungsten lamp off	Displayed on the status bar. This indicates that the tungsten lamp is off.
	Tungsten lamp on	Displayed on the status bar. This indicates that the tungsten lamp is on.
	Tungsten lamp warming	Displayed on the status bar. This indicates that the tungsten lamp is warming up.
	Xenon lamp failed	Displayed on the status bar. This indicates that the xenon lamp has failed.
	Instrument busy	Displayed on the status bar. This indicates that the instrument is performing a measurement.
	Printing to Bluetooth device	Displayed on the status bar. This indicates that the instrument is printing to a Bluetooth device.
	Printing to internal printer	Displayed on the status bar. This indicates that the instrument is printing to the internal printer.
	Printing to USB	Displayed on the status bar. This indicates that the instrument is printing to a USB printer.
	Toggle view standard curve	When used in standard curve applications *i.e.* Lowry protein assay, this toggles the sample measurement screen to display or hide the standard curve.
	Replicates	Used in standard curve applications to collect data from a group of replicates.
	Take sample measurement	Commences a sample measurement. When using a Libra S50 a reference measurement must be taken prior to a sample measurement

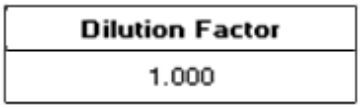
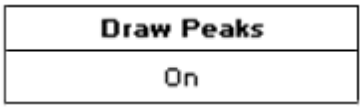
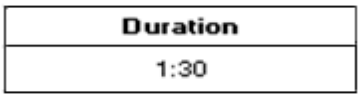
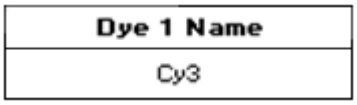
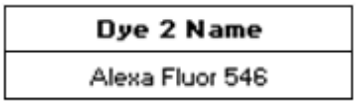
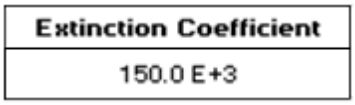
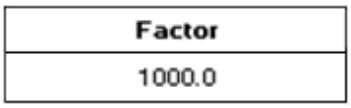
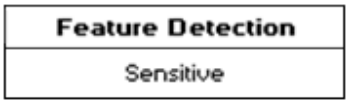
	Take reference measurement	Commences a reference measurement.
	Backspace / delete	Used in text entry mode to move the cursor backwards (left) and delete any unwanted characters.
	Forward space	Used in text entry mode to move the cursor forwards (right).
	Lower case	Selects lower case letters when in text entry mode.
	Symbols	Selects symbols when in text entry mode.
	Upper case	Selects upper case letters when in text entry mode.
	Numeric	Selects numeric entry when in text entry mode.
	Trace Manager	Used in the wavescan and kinetics applications. This allows the user to overlay up to 8 samples data files and to choose what type of data is displayed *i.e.* raw data, smoothed data, 1st derivative.
	USB	Used on the Sample Manager screen to access data stored on a USB memory stick. Used in the options menu in the methods folder to allow the user to backup methods to a USB memory stick.
	Add user	Displayed under User Access, this allows anyone with Administrator privileges to add another user to the instrument.
	Delete user	Displayed under User Access, this allows anyone with Administrator privileges to delete users from the instrument.
	Edit user	Displayed under User Access, this allows anyone with Administrator privileges to edit currently users' parameters.

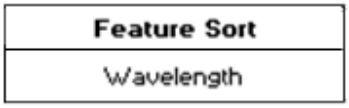
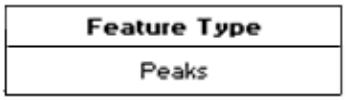
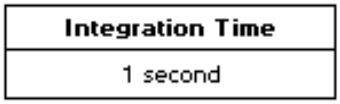
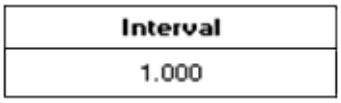
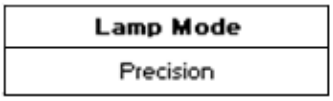
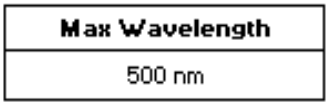
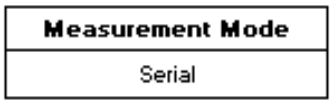
	Zoom in	Used in the wavescan and kinetics applications. This allows the user to zoom into a specific region of a scan.
	Zoom out	Used in the wavescan and kinetics applications. This allows the user to zoom out and return to the original scan.

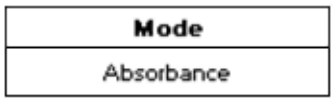
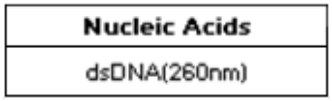
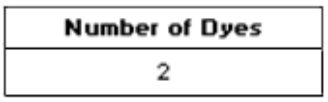
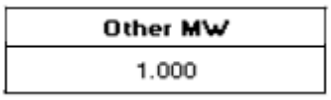
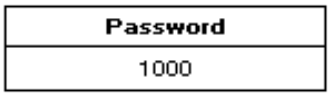
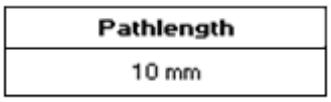
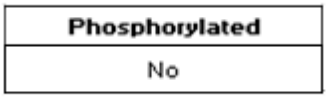
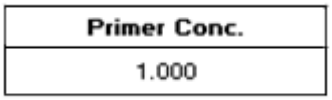
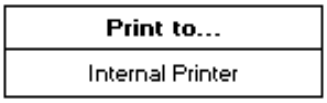
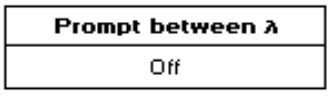
GLOSSARY OF BOXES

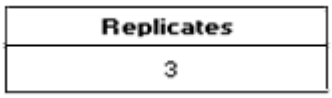
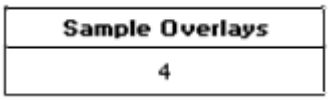
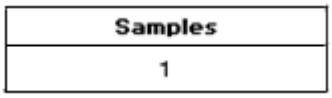
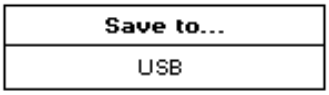
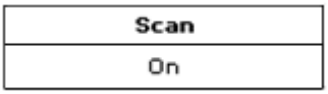
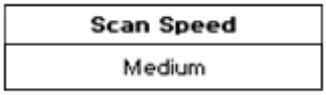
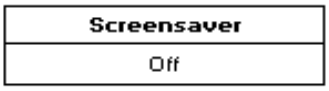
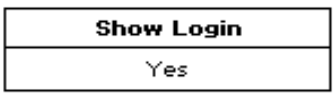
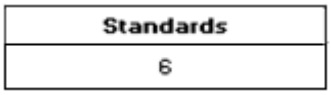
BOX	FUNCTION
	Toggles between on and off. Used in all applications to set whether sample data is printed automatically or not
	Toggles between on and off. Used in all applications to set whether sample data is saved automatically or not
	Can be toggled on and off. Used in nucleic acid and protein measurements to subtract the absorbance value at 320 nm. This is done to allow for the effects of turbidity, high absorbance buffer solutions and the use of reduced aperture cells.
	Set by numeric entry. Used in Cydye DNA measurements only and enables the user to specify the wavelength of background correction.
	For variable bandwidth instruments (Libra S80) this gives the option to set the bandwidth to 4, 2, 1 or 0.5 nm for a measurement. For fixed bandwidths instruments (Libra S50, S60 and S70) this box will be greyed out.
	Used in Tm calculation to set the sequence of bases. The bases A, C, G, T can be added in DNA mode and the bases A, C, G, U can be added in RNA mode. Bases are grouped in threes to improve readability.

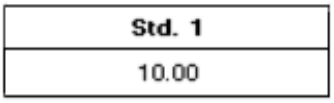
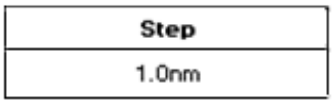
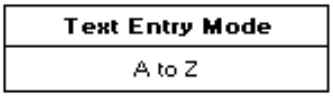
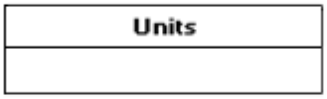
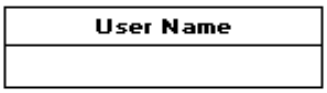
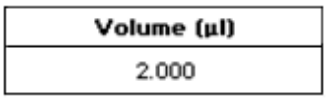
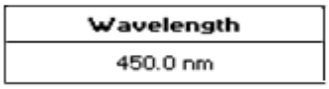
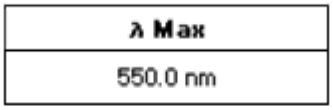
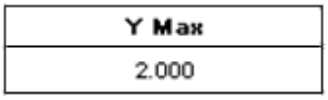
	Used in Tm calculation to toggle between DNA and RNA base types.
	Used in User Interface in Settings to set the brightness of the screen.
	Numeric entry, used in Tm calculation only. Buffer molarity = buffer molarity + total molarity of salt (moles / L).
	Used in all standard curve applications to set the method used to collect the standard curve. Options are for Standards or Manual.
	Set by numeric entry, used in the Cydye DNA application only. This is the correction factor applied to the absorbance of the dye at a specified wavelength.
	Used in Tm calculation only this allows the user to add the type of counter ion used. The options are sodium (Na), potassium (K), triethylammonium (TEA) or other. If other is selected the molecular weight of this counter ion must be added using Other MW.
	Used in all standard curve applications this is the curve fit that will be applied to the standards' absorbance values. Options are Zero Regression, Regression, Interpolation and Cubic Spline.
	Alphanumeric entry for custom dye name, used in Cydye DNA only.
	Numeric entry. This is the delay required before a kinetic measurement commences.

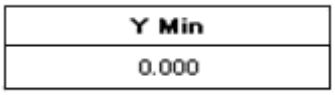
	Numeric entry. This is used in nucleic acid and protein applications to compensate for the absorbance of highly concentrated samples.
	Used in wavescan only, this switches display of peak cursors on and off. Peak cursors show vertical dashed lines displaying the measured peak height and horizontal dashed lines showing the peak width
	Numeric entry. This is the duration over which a kinetic measurement is performed.
	Used in Cydye DNA application only. This is the first dye type used in the measurement.
	Used in Cydye DNA application only. This is the second dye type used in the measurement (where applicable).
	Numeric entry for Cydye DNA application only. This is the extinction coefficient of the specified dye. Note for dyes included in the Libra software these values are not editable and the box will be greyed out.
	Numeric entry to 3 decimal places. In concentration and nucleic acid measurements multiplying absorbance readings by this factor gives the concentration value. In kinetic measurements the result is calculated by multiplying this factor by absorbance, delta absorbance or the slope.
	Used in wavescan measurements only. This determines the sensitivity of the peak or valley detection *i.e.* sensitive will detect more peaks or valleys than course. Options are; Off, Coarse, Sensitive or Custom (when custom is selected the minimum peak height and width must be entered).

	Toggles between wavelength and absorbance, used in wavescan measurements only. This determines how features will be ordered in the peak/valley table below the scan.
	Toggles between peaks and valleys, used in wavescan measurements only. This determines what feature type will be detected.
	Used in User Access to set the group a user will belong to and what access they will be granted.
	Used in all applications. This is the duration the instrument will take a reading at an individual wavelength. The longer the integration time, the greater the signal to noise ratio and the greater the accuracy.
	Numeric entry. This is the interval at which serial kinetics readings will be taken.
	Used by the S70 & S80 in all applications to toggle between Precision and Pulse mode.
	Numeric entry. This is the upper limit of a wavescan measurement. Note that when using the Libra S50, S60, S70 or S80 the max wavelength must always be greater than the min wavelength by at least the step value.
	Toggles between the kinetics measurement modes, serial and parallel.
	Numeric entry. This is the lower limit of a wavescan measurement. Note that when using the Biochrom Libra the max wavelength must always be greater than the min wavelength by at least the step value.

	Used in the Single Wavelength, Kinetics and Protein A280 applications to set the required measurement mode.
	Used in Cydye DNA application only, this sets the nucleic acid used in the measurement.
	Toggles between 1 and 2. Used in Cydye DNA application only to set the number of dyes used in the measurement.
	Numeric entry. This option is only used if the counter ion type in the Tm calculation is set to Other.
	Used in User Access to set a password for new users
	Used in the nucleic acid applications, Protein A280 and Protein UV. This is the pathlength of the cuvette used in the measurement. Options are for 10, 5, 2 and 1 mm.
	Toggles between yes and no. Used in Tm calculation to set if the sample to be measured is phosphorylated or not.
	Numeric entry to 3 decimal places. Used in Tm calculation to sets the primer concentration in pmole/mL.
	Used in all applications to set the desired print location. Only available printers are displayed.
	Toggles between On and Off, used in Equation Editor only. With Prompt on the measurement will proceed as follows: measure wavelength 1, prompt for sample, measure wavelength 2, prompt for sample etc.

	Used in all standard curve applications. This is the number of times a standard measurement is repeated before the mean of these values is plotted on the standard curve. Options are off (1 measurement), 2 or 3.
	Used in wavescan measurements, this determines how many samples will be overlaid on the graph. Options are off, 2 to 8.
	Used in kinetic measurements, this sets the number of samples that will be measured during the method. Options are 1, 2 or 3.
	Used in all applications to set the desired save location. Only available locations are displayed.
	Toggles between on and off. Used in DNA/RNA/Oligo measurements this sets whether, as a further analytical tool, the instrument will also perform a scan over the wavelength range 220 - 320 nm.
	Used in wavescan measurements, this determines the speed at which the scan is performed. Options are Slow, Medium, Fast or Integration. Note that when integration is selected the user will be prompted to set the integration time (see box above).
	Used in User Interface in Settings to set the time before the Biochrom screensaver will be displayed
	Used by the Default Administrator in User Access to set if user login will be displayed or not.
	Used in all standard curve applications. This is the number of standards that will be used to create the standard curve; options are from 1 to 9.

	Numeric entry to 2 decimal places. Used in all standard curve applications this is the concentration of the standard.
	Used in wavescan measurements, this determines the interval at which wavescan measurements are made (in nm). Options are for 0.1, 0.2, 0.5 or 1 nm. Smaller intervals give greater detail but lead to longer scan times.
	Used in User Interface in Settings to set the text entry mode used for alphanumeric text entry.
	Used in applications that give a concentration result. Units are entered via either alphanumeric entry or from a list of options.
	Used in User Access when creating a new user.
	Numeric entry to 3 decimal places, this is used in the Cydye DNA application and is the volume of the probe in µL.
	Numeric entry to 1 decimal place and is used in all fixed wavelength applications to determine the wavelength at which the measurement will be performed.
	Numeric entry to 1 decimal place. Used in the Cydye DNA application, this is the wavelength at which the absorbance of the dye will be measured. Note for dyes included in the Libra software these values are not editable and this box will be greyed out.
	Numeric entry to 3 decimal places. This is the maximum value of the Y axis shown during a kinetics measurement. Note the graph will automatically rescale at the end of the measurement to give the optimum Y max.

	Numeric entry to 3 decimal places. This is the minimum value of the Y axis shown during a kinetics measurement. Note the graph will automatically rescale at the end of the measurement to give the optimum Y min.