# Instructions for Biowave S2100 UV/Vis Diode Array Spectrophotometer

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# 1 Introduction

The Biowave is a diode array UV/Vis spectrophotometer that has been specifically designed for the Life Sciences and biotechnology market. Popular methods are pre-programmed into the instrument for ease of use.

The Biowave spectrophotometer also offers: -

- Instantaneous scanning
- No moving parts
- Open sample compartment
- Space saving shape

We believe that the Biowave spectrophotometer will give many years of trouble free operation. We do however recommend that you read this manual prior to use.

We welcome any comments you may have regarding either the product or this manual. Please contact: -

Sales and Marketing Co-ordinator
Tel: +44 (0)1223 892688
Fax: +44 (0)1223 894118
E-mail: sales@wpaltd.co.uk

# 2 Conditions of Service

The Biowave spectrophotometer is intended for use under cover at a temperature of between 5 and 40°C.

If dangerous or aggressive chemicals are used, care should be taken to avoid spillage. Normal precautions should be taken against contact with all samples used, some of which may be harmful.

The following parts are user replaceable: -

| | **Cat No** |
|---|---|
| Tungsten Lamp | S2000T |
| Tungsten & Deuterium Lamp Assembly | S2000L |
| As for S2000L but accepting old unit in part exchange | S2000LX |
| Fan | FA074 |
| Cuvette Holder | CS003 |

For other problems please contact our sales office or local authorised dealer in your region.

**Health & Safety Notice**

**Instruments will only be accepted for repair or re-calibration when accompanied by a signed letter or certificate from the sender to the effect that there is no hazard to health due to biological, chemical or radioactive contamination. A copy of this certificate can be found on the base of the unit or at the end of this manual.**

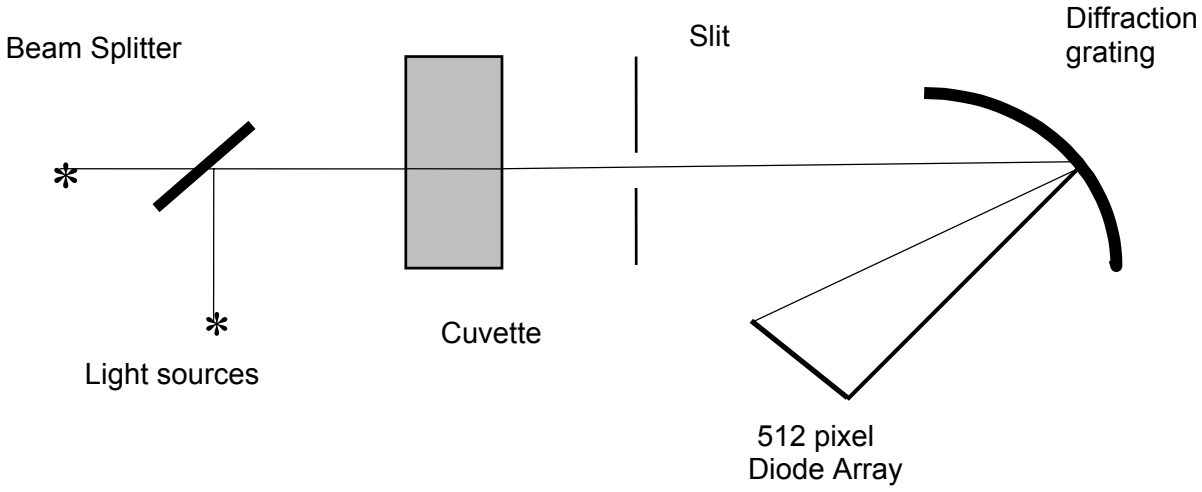
## 3 Principle of Operation

The optical layout comprises a concave grating and 512 pixel diode-array. On making a measurement both lamps (Deuterium and Tungsten) flash consecutively. A self-regulating circuit ensures flash to flash repeatability.

The data for each wavelength is recorded and stored in memory. Stray light is minimised by positioning the diffraction grating after the cuvette compartment, thereby eliminating the need for a light cover.

The use of a diode array detector allows simultaneous measurement across all wavelengths (scanning), and involves no moving optical parts, thereby minimising maintenance costs.

A simplified optical layout is presented below: -




***The added advantage of the above optical arrangement is that Stray light is minimised, thereby eliminating the need for a cover over the cuvette chamber.***

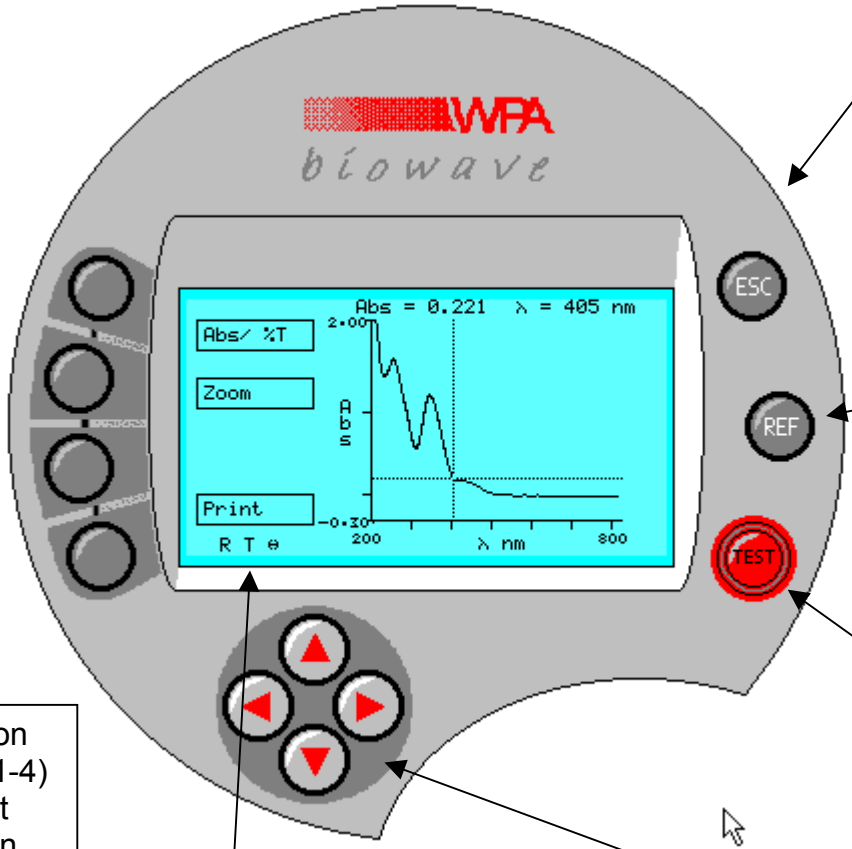
# 4 Specifications

| Specification | S2100 Biowave |
|---|---|
| Optical arrangement | Single beam, diode-array (512 pixel) using Rowland Circle optics with flat field corrected concave grating. |
| Wavelength range | 200 –820nm |
| Spectral bandpass | 5nm |
| Resolution/Bandwidth | 5nm |
| Stray Light | <0.1% at 220nm NAI and 340nm $NaNO_2$ |
| Wavelength Accuracy | ±1.5nm |
| Wavelength reproducibility | Better than ±0.6nm |
| Photometric modes | Abs, %T, $Conc^n$, ΔAbs/min, ΔAbs*factor/min |
| Photometric range | -0.3 - 1.999A<br>0 - 199.9%T |
| Photometric Reproducibility | ±0.002Abs (0 to 0.5Abs)<br>±0.007Abs (0.5-1.0Abs) |
| Photometric Accuracy | ±0.003Abs (0 to 0.5Abs)<br>±0.01Abs (0.5-1.0Abs) |
| Slew rate | Full spectrum measured simultaneously, measurement time <5 sec |
| Zero stability | ±0.003Abs/hour after 20min warm up |
| Noise | ±0.001Abs at 0A 500nm |

| Specification | S2100 Biowave |
|---|---|
| Preprogrammed methods | ds DNA<br>ss DNA<br>RNA<br>Oligonucleotides<br>Protein: Bradford, Lowry, BCA, Biuret & UV<br>Cell density measurement |
| Modes of operation | Scanning<br>Ratio ($A_{260}/A_{280}$ and $A_{260}/A_{230}$ )<br>Multi Wavelength<br>Absorbance<br>% Transmission<br>Concentration (linear least squares or Polynomial curved calibration)<br>Kinetics<br>Programmable - up to 99 methods stored in battery backed memory |
| Display | 240 x 128 pixel dot matrix LCD |
| Output | RS232 to WPA printer for text and graphics<br>RS232 ASCII for PC data logging |
| Light sources | Pulsed Deuterium and Tungsten halogen lamps giving life in excess of 15000 hours |
| Sample compartment | 40mm pathlength. Accepts 10mm and 40mm cuvettes, 15mm beam height, standard, semi-micro, micro and ultra micro cuvettes with volumes down to 70μl |
| Dimensions (l x w x d) | Approx. 380 x 140 x 275 mm |
| Weight | 4.5kg |
| Electrical Requirements | 90-250V 50/60Hz Max 100VA |

# 5 Controls

## 5.1




Escape key - takes the user back one step or aborts current operation

Reference key - zeros the instrument

Test key - makes a measurement across all wavelengths

Function keys (1-4) - select function identified in box.

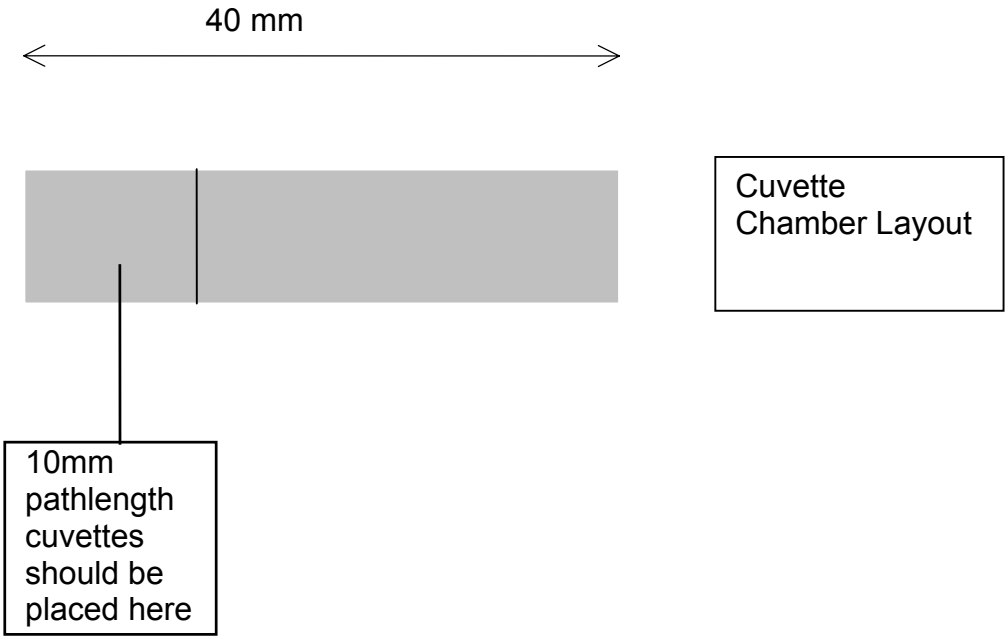
Symbols indicate which keys are operative

Cursor keys allow the user to navigate the screen

Note: The arrow keys and REF and TEST buttons are only operational in certain modes. When operational, symbols (R for Ref, T for Test, + arrows) will appear in the bottom left hand corner of the display. Similarly the Function keys on the left-hand side of the display are only operational when a box appears.

## 6 Cuvette Chamber

The cuvette holder has been designed to accommodate a variety of cuvette sizes in an accurate and repeatable manner.




**Note: It is important to align the square cuvettes to the left-hand side of the chamber. The alignment of the cuvette is important to ensure re-producible readings**.

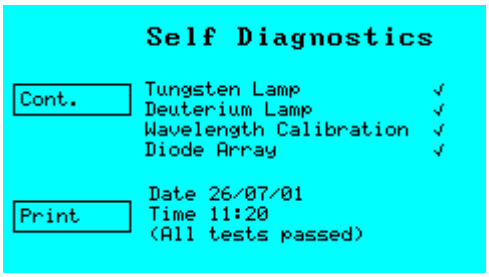
# 7 Installation & Start-up

Unpack the Biowave spectrophotometer and ensure that you have received the following in good condition: -

S2100 Biowave Spectrophotometer
Power Lead
Starter pack of 10 disposable UV cuvettes
Dust cover
Instruction manual
Warranty Card
3.5 inch floppy disc with MS Excel™ Add-in / Analysis spreadsheet

Place the unit on a level surface, insert the mains cable in the rear socket and then connect to the electricity supply (90-250V 50/60Hz).

Switch the instrument on using the switch at the rear. The Biowave logo should appear followed by a self-diagnostic screen as below:




Check that all tests are passed or contact our sales office/authorised dealer.

**Warm Up Time**

To allow the optical and electronic components to stabilise it is recommended to allow a minimum of 10 minutes for the unit to warm up. For minimum drift, leave the instrument for 45 minutes to 1 hour.

It is best practice to always take a reference measurement prior to making a TEST, however the Biowave will remember the last reference until the unit is switched off or re-referenced.

**Lamp Failure**

Failure of the lamps indicates either that there was a cuvette in the compartment when the instrument was switched on (simply remove the cuvette and Re-test) or that the lamp has failed, or has output too low for good performance.

Failure of one lamp will not affect the other lamp - so if the Deuterium lamp fails you can still make good visible measurements (380 to 825 nm).

See section on Servicing & Maintenance

**Failure in Wavelength Calibration**

The wavelength calibration is performed by observing the key spectral data from the Deuterium lamp and ensuring that the peak remains in the correct position. A failure in wavelength calibration infers some movement of the fixed optical system or exposure to extreme temperatures.

Try leaving the unit on for a few minutes and then re-start.

If the failure re-occurs please consult your local dealer/service agent or our service department.

**Note: The unit will continue to operate despite being out of wavelength calibration.**

**Failure of Diode Array**

On start up the unit checks that all pixels (512) are operating. A failure of any pixel will give the above error.

The unit can still be used as operation at other wavelengths will not be affected. The faulty pixel will show itself as a line on the wavelength scan.

If the failure occurs please consult your local dealer/service agent or our service department.

# 8 General Operations

## 8.1 *Referencing*

When R is displayed in the bottom left hand corner of the display, it is possible to Reference the instrument by pressing REF.

The reference *across all wavelengths* will be held in the memory until the unit is switched off, or re-referenced. However for best results, reference the unit before each measurement is taken.

The reference material will normally be a low absorbing material, often the solvent used with the sample, which is mostly water.

**If the reference Absorbance is too high the instrument will display "----" If this appears, first check the type of cuvette being used (Quartz for UV measurements). If this is satisfactory consider an alternative reference material.**

## 8.2 *Measurement at Low Levels (<0.050A)*

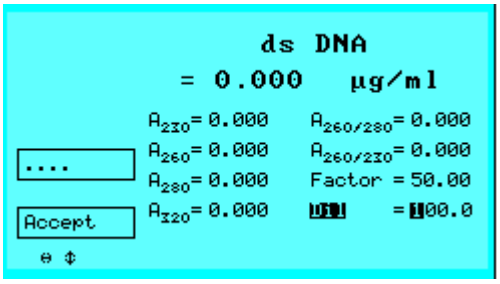
Due to the narrow beam size needed for use with small volume cuvettes, the Biowave is sensitive to variations in cuvettes and to scratches or dirt on the inside or outside of the cuvette.

We therefore recommend using the same cuvette as both the reference to measure the sample.

## 8.3 *Dilution*

Some methods contain a dilution factor.

This is to be used where samples are diluted prior to measurement. All the screens work in a similar manner – with the up/down cursors being used to change the value and the left/right cursors to select the digit to be changed. A separate function button allows the decimal point to be changed.



The factor will be used as a multiplier for the value obtained.
E.g. If the user has diluted a sample 1:9 sample/diluent, the factor used will 10.000.

Concentration = 10.000 * Abs * Factor

Where the factor relates to the specific method.

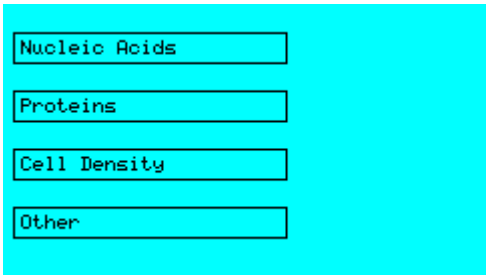
### 8.4 *Background Absorbance Correction*

Some samples can be turbid and this can affect the readings obtained. The Biowave contains the facility to correct for this by subtracting the Absorbance at 320nm from the values taken at 260 and 280nm.

This correction facility has the symbol $A_{corr}$ which will appear in the bottom left hand corner of the screen when active. The correction facility can be applied to all Nucleic Acid measurements and also to the direct UV Protein method.

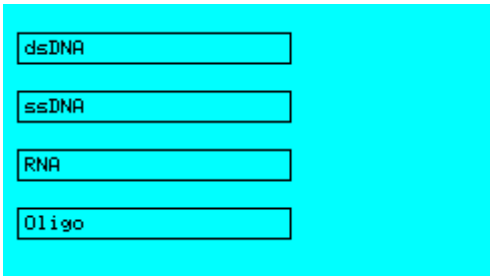
# 9 Software Operation

After the spectrophotometer has performed the self diagnostics press the Cont. Function Key to proceed to the first menu screen.




## 9.1 *Nucleic Acids*

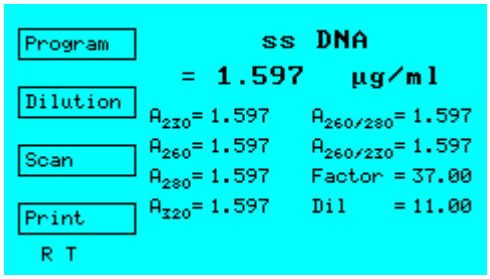
This Function key displays the choice of Nucleic Acid methods pre-programmed into the instrument.




Press the Function key for the desired sample type to bring up a further menu which allows the user to enter the assay conditions.

### 9.1.1 dsDNA, ssDNA, RNA

All three methods work in a similar fashion, so for ease of representation only the dsDNA is represented here.

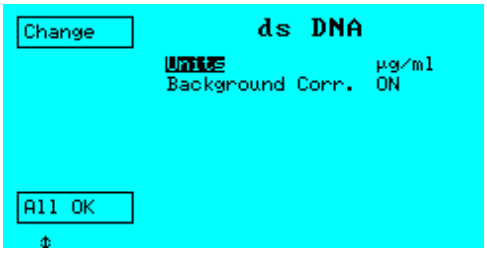


The ratio readings give information about the purity of the sample. The A260/230 reading give a measure of phenol contamination following extraction .

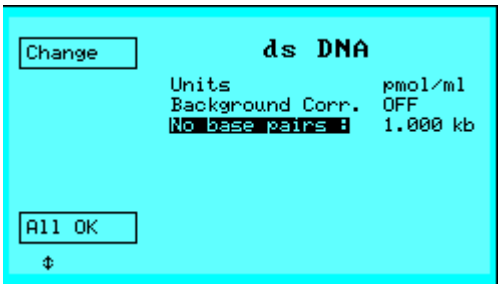
The A260/280 ratio measures protein contamination.

#### 9.1.1.1 Programming

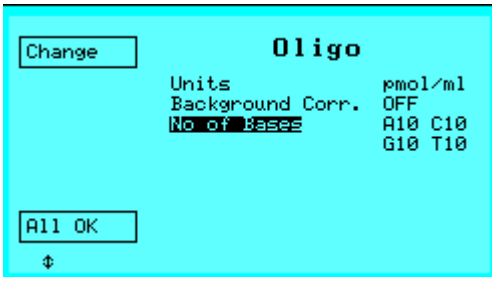
The program button allows the units of concentration to be changed between µg/µl, µg/ml and pmol/ml and the background correction to be turned on and off using the appropriate arrow keys. Press Function Key 4 “All OK” to proceed to the next screen.




If pmol/ml is selected then an additional line will appear for the No of Base Pairs to be entered




For the concentration of Oligo’s in pmol/ml, instead of the calculation using the number of bases, it uses the sequence of bases (see Appendix II for details of calculation).




Pressing Change allows the No.of Bases to be entered using the cursor keys in the normal manner (similar to Dilution Factor).

The Factor used in the calculations defaults to the values below:-

| | |
|---|---|
| dsDNA | 50.00 μg/ml |
| ssDNA | 37.00 μg/ml |
| RNA | 40.00 μg/ml |
| Oligo | 33.00 μg/ml |

These can be changed – see section 10.1

## 9.2 *Protein Methods*

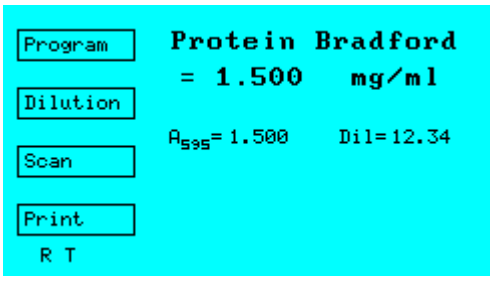
This gives the user the option of the pre-programmed methods of:

- Protein BCA
- Protein Bradford
- Protein Lowry
- Protein Biuret
- Protein UV

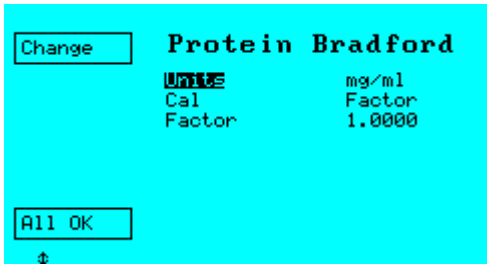
A choice of 4 colorimetric protein methods are available + one direct UV Absorbance method.

### 9.2.1 Colorimetric Protein methods

We have described the Bradford Assay, but the other assays work in an identical fashion, with the wavelength and name changing.



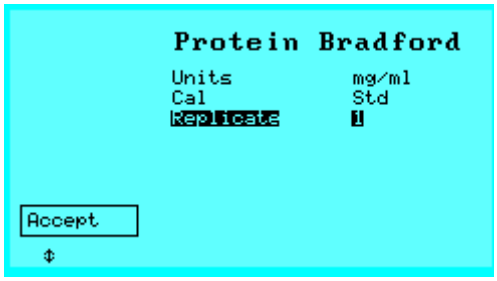

The program button allows the units of concentration to be changed , and the calibration between standard and factor to be selected .



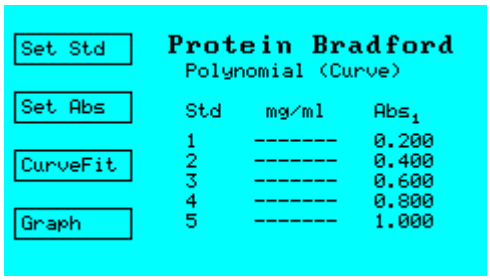
### 9.2.1.1 Standard Calibration

If the factor of the calibration graph is not known, the calibration graph can be constructed. “Standard” is chosen by using the ←→ keys and pressing Function Key 4 “Accept”

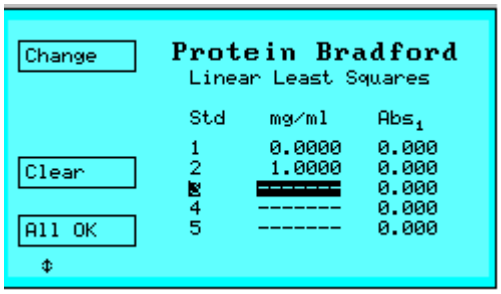
On selecting set Standard, the number of replicates will then appear beneath.



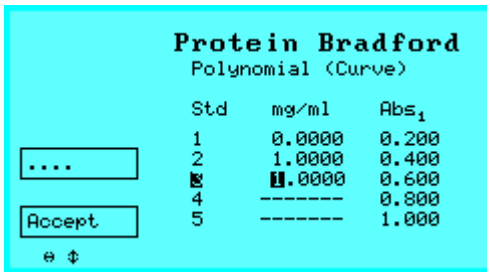

The calibration values can then be input.




Pressing Set Std will allow the concentration of the standards to be input.




Press change to modify the standard value. The cursor keys allow the digits to be changed - move to the next digit using ←→. Function Key 3 “……” moves the decimal point by one space when pressed.




Press Accept to confirm. Once all the standard values are correct press Function Key 4 “All OK”.

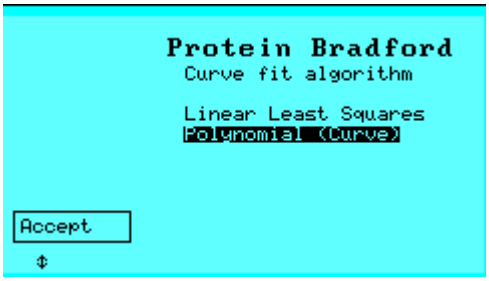
The cursor then moves to the first Absorbance entry for Std 1.

Absorbance values can be entered manually by pressing the Change button and using the cursor keys, as for the standards, or directly, by placing the appropriate standard in the cuvette chamber and pressing TEST (having first taken a valid reference).

Upon entering the Abs value for the first standard ($Abs_1$) , use the arrow keys to move to the next replicate ($Abs_2$) or to the next standard if only 1 replicate is required. Ensure that for all standards selected there are Absorbance values entered. The left arrow can be used to scroll across the screen revealing the values entered for $Abs_1$, $Abs_2$ and $Abs_3$ .

### 9.2.1.1.1 Curve fitting

On completion the choice of Curve fit can be made.




If the relationship between Abs and concentration is anticipated to be a linear one, then select Linear Least Squares. This will create a best fit straight line based on all the data points entered.

If the relationship is anticipated to be non linear, select Polynomial (curve). This will attempt to fit a polynomial expression *exactly* to the points given.

The order of the polynomial depends upon the number of points chosen. For 2 points a straight line equation is selected eg:-

2 point : y = a + bx
3 point : y = a + bx + $cx^2$

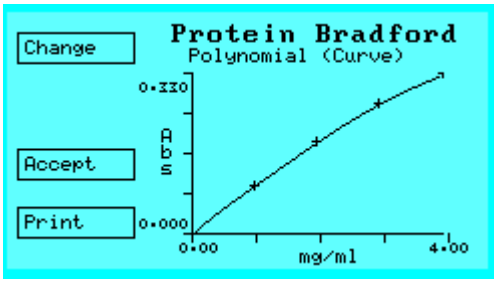
**INVALID CURVES**

Because the equation fits a curve exactly to the points, there is a chance that the resultant curve is not valid. If the curve selected contains points of inflection (where the slope changes sign eg maxima and minima) it would give rise to potentially more than one Concentration for a chosen Abs value. This is clearly unacceptable and therefore the program displays “Invalid Curve”.

To overcome this we recommend that the number of points is reduced. If this still fails it may be sensible to select Linear Least Squares.

Where replicates are being used, the unit takes an average value for each standard.

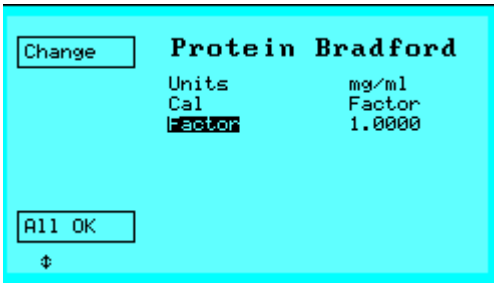
To display the graph press Graph .



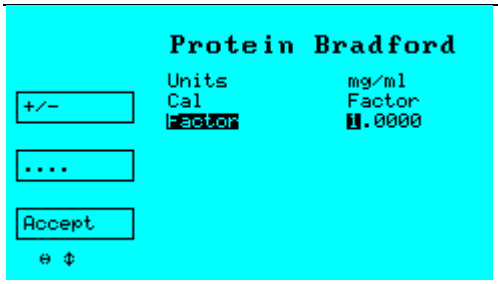

### 9.2.1.2 Factor Calibration

If the calibration for the method is already known, and represents a straight line, then select Cal = Factor.

In this case a Factor will be displayed.

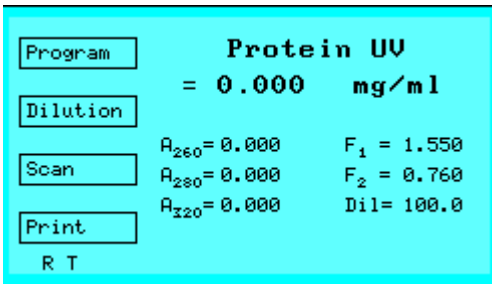



To change the factor, press Change and proceed as normal.

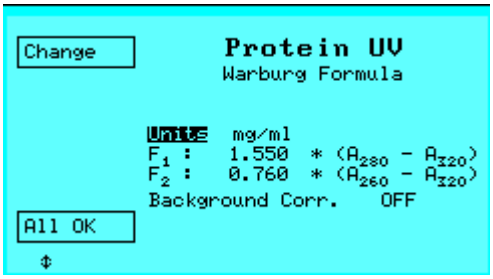



### 9.2.2 Direct UV Absorbance determination of Proteins

For a rapid, rough estimate of Protein Concentration the Direct UV method is ideal. The method is based upon the Christian Warburg Expression (see Appendix II) .



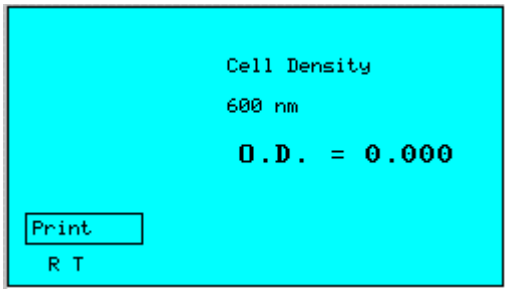

Pressing Program allows units, factors and background absorbance ($A_{320}$) status to be changed.



## 9.3 *Cell Density Measurement*

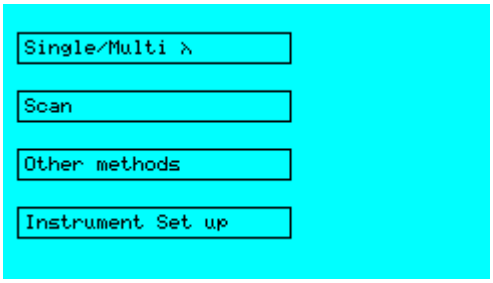
The cell density function provided a convenient short cut to cell density measurements. As this is effectively a turbidimetric method, different instruments will tend to give different Absorbance values due to the scattering effect of particles in solution.

This cell density function both sets the wavelength of measurement and also provides an auto-correction to bring readings closer to an experimentally established norm.




**Note :** The correction function operates by measuring the Absorbance at two different wavelengths and applying an algorithm based on experimental data obtained using a variety of instruments and different organisms. For further information please consult our sales office.

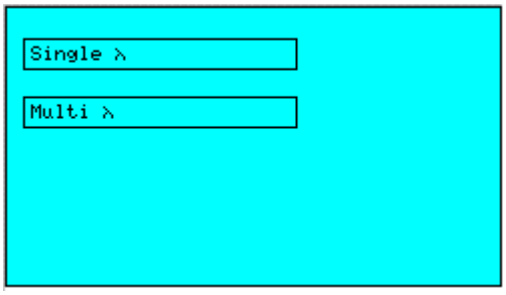
## 9.4 *Other*



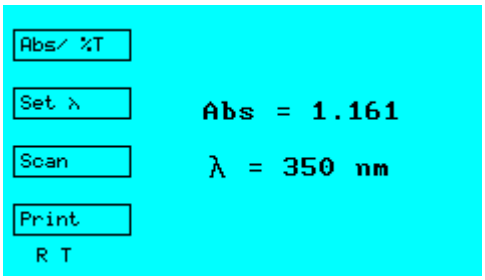

The “Other” Function Key takes the user to a screen to perform standard spectrophotometric functions.

## 9.5 *Single/Multi* λ

This function key takes the user to a further menu screen allowing the option of single or multi wavelength measurements.




### 9.5.1 Single measurement

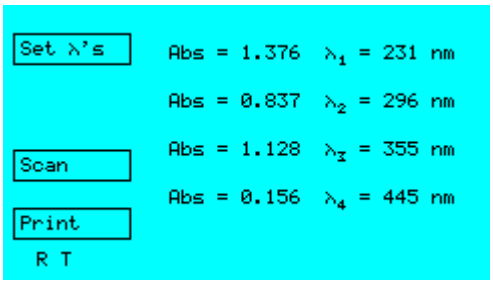



This mode should be used when readout in Absorbance or %Transmission is required for a single wavelength.

You can toggle between Abs and %T by pressing Function Key 1.

**To avoid accidental changes to the wavelength setting the arrow keys only become functional when "Set λ" is pressed. Use the ↑↓ keys to change wavelength then press "Accept λ" to accept.**

### 9.5.2 Multi Wavelength



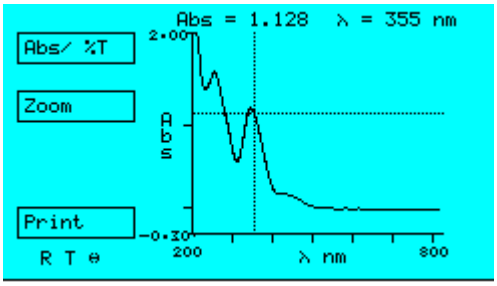

The wavelength settings can be changed in a similar manner to the single measurement mode and are memorised until re-set.

Pressing Function Key 1 highlights the first wavelength setting, others can be accessed by pressing Function Key 1 again. Pressing the ↑↓ arrows allows the wavelength value to be changed. Pressing Function Key 1 once more confirms the choice and moves to the next wavelength.

Once the user is satisfied with the settings press Function Key 4 "All OK". Reference and Test are then active so you can make a measurement.

## 9.6 *Scanning*

The 512-pixel diode array captures readings from the complete wavelength range each time a measurement is made.

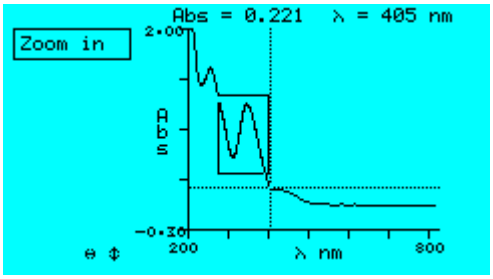


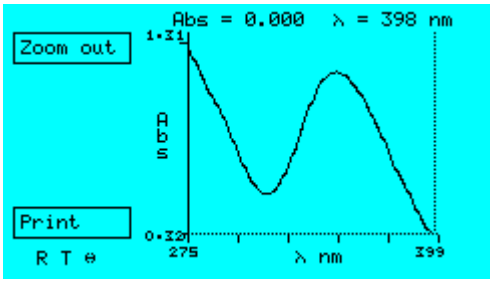

The scan displaying this information can be viewed on the graphics display.

Two cursors can be moved across the screen (using ←→) to identify the wavelength and reading at a particular point in the spectrum.

### "Zoom" Function

To magnify a portion of the spectrum press Function Key 2. A box will appear (at the inter-section of the cursors); this can be re-positioned using the arrow keys. Once the position is OK press the "Zoom In" key.

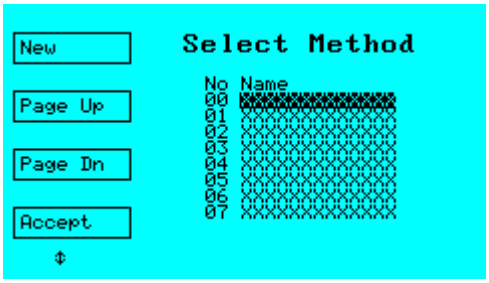



The spectrum is automatically re-sized to fill the screen. The cursors can be used once again to identify the reading at a particular wavelength.

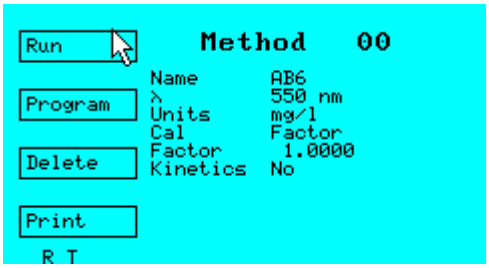
## 9.7 *Other methods*

Up to 99 methods can be user programmed. These enable readout in concentration units, using linear or non-linear calibration curves. The facility for kinetic operation is also included.



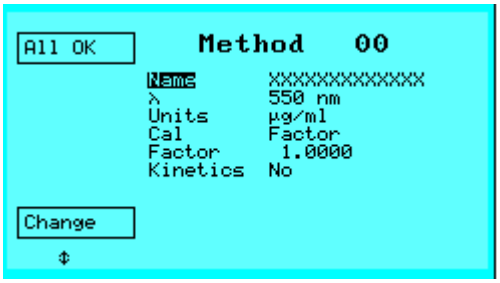

To select a method, move the cursor to the desired number using the Page up or Page down functions and the arrow keys and then click on “Accept”. Alternatively select a new method to bring up the next free location.

Once selected the following screen will be displayed:




### 9.7.1 To Program a New Method (or re-program an existing method)

The following screen is displayed if a new method is selected, or when re-programming an existing method.



Programming a method is simple.

To change each selection you need to do the following:-

- Position the cursor over the relevant line
- Press CHANGE
- Use arrow keys to amend the entry
- Press ACCEPT to confirm

The table below is a summary of the information that can be programmed into each method.

| | |
|---|---|
| **Name** | This can be an alpha-numeric name of up to 13 characters - including spaces |
| λ | Wavelength setting. |
| **Cal** | Calibration. Can be based on a factor or standard (Std) |
| **Units** | A choice of units are available, or none |
| **Factor** | This is only relevant for the Factor calibration – it will not be displayed if Std Cal chosen |
| **Kinetics** | Yes/No/Fixed time<br><br>This provides the option of measurement at pre-set time intervals (single wavelength only) for Rate of Reaction studies and enzyme assays. The fixed time option allows only one measurement to be taken after a user determined time. |

To assign a name (of up to 13 letters) position the cursor on the **Name** using the ↑↓ keys and then press Function Key 4 to “Change”.

Using the arrow keys assign the name (the ←→ arrows move between digits and the ↑↓ arrows select letters or numbers) and then press Function Key 4 once again to confirm.

**Note: Not all the letters need to be used, however to save a method a name must be entered or otherwise the new values will revert to default values.**

Position the cursor against the next option and repeat the procedure to select the desired option.

### 9.7.1.1 Changing Wavelength

Use up/down arrows to change the wavelength.

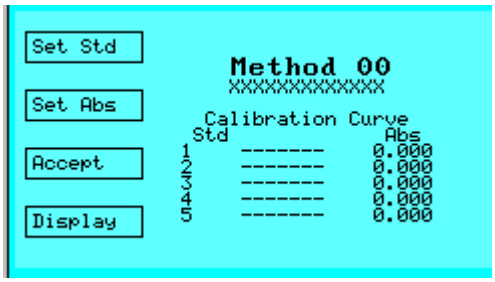
**Note: The wavelength changes slowly at first and then more quickly dependent upon how long the button is pressed.**

### 9.7.1.2 Calibration

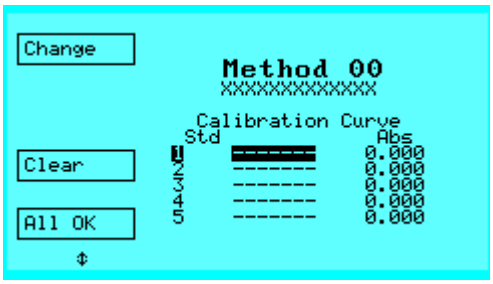
#### 9.7.1.2.1 Standard Calibration

If the factor of the calibration graph is not known, the calibration graph can be constructed. "Standard" is chosen by using the ←→ keys and pressing Function Key 4 "Accept"

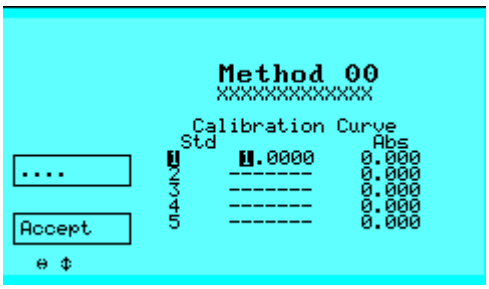
On selecting Std the following screen will be displayed allowing Std values to be entered.




Pressing Set Std will allow the concentration of the standards to be input.




Press change to modify the standard value. The cursor keys allow the digits to be changed - move to the next digit using ←→. Function Key 3 "……" moves the decimal point by one space when pressed.



Press Accept to confirm. Once all the standard values are correct, press Function Key 4 “All OK”.

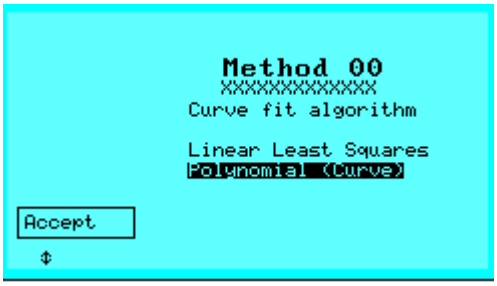
The cursor then moves to the first Absorbance entry for Std 1.

Absorbance values can be entered manually by pressing the Change button and using the cursor keys as for the standards, or directly, by placing the appropriate standard in the cuvette chamber and pressing TEST (having first taken a valid reference).

On entering Abs for the first standard the cursor will move to the next standard. Ensure that for all standard values entered there are equivalent Absorbances. The user can choose between 2 to 5 Standards.

**Curve fitting**

On completion the choice of Curve fit can be made.




If the relationship between Abs and concentration is anticipated to be a linear one, then select Linear Least Squares. This will create a best fit straight line based on all the data points entered.

If the relationship is anticipated to be non linear, select Polynomial (curve). This will attempt to fit a polynomial expression *exactly* to the points given.

The order of the polynomial depends upon the number of points chosen. For 2 points a straight line equation is selected eg:-

2 points : $y = a + bx$
3 points : $y = a + bx + cx^2$

**INVALID CURVES**

Because the equation fits a curve exactly to the points, there is a chance that the resultant curve is not valid. If the curve selected contains points of inflection (where the slope changes sign, e.g. maxima and minima) it would give rise to potentially more than one Concentration for a chosen Abs value. This is clearly unacceptable and therefore the program display “Invalid Curve”.

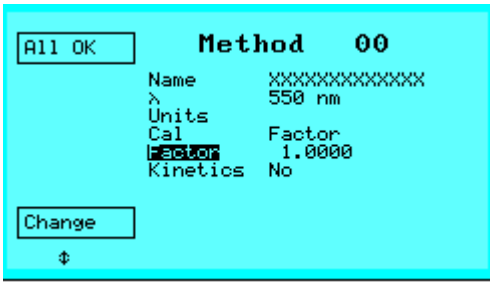
To overcome this we recommend that the number of points are reduced. If this still fails it may be sensible to select Linear Least Squares.

To display the graph press Graph .

### 9.7.1.2.2 Factor Calibration

If the calibration for the method is already known, and represents a straight line, then select Cal = Factor.

In this case a Factor will be displayed.




To change the factor, press Change and proceed as normal.

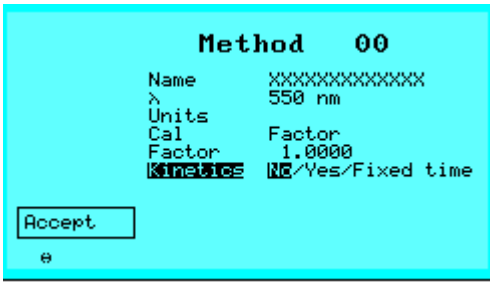
### 9.7.1.3 Units

A choice of units can be made for concentration measurements. These will appear on the final result screen when the Method is run.

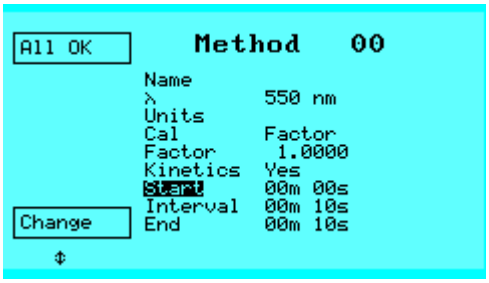
### 9.7.1.4 Kinetics

The kinetics facility allows the user to run a series of measurements at programmed intervals.

To program a method to include multiple measurements select:-




Selecting “Yes” presents the user with some additional options:-




**Start Time**

This is the delay before the first measurement is made.

**Interval**

This is the interval between measurements. There is a minimum interval of 10 seconds.

**End Time**

This increases in increments of the interval time.

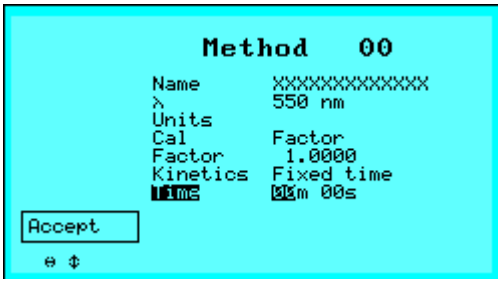
The software automatically calculates the change in Absorbance per minute (ΔA/min) using a least squares correction and multiplies this by the chosen factor.
The correlation coefficient is displayed (1.000 is a perfect fit, 0.000 is no fit), along with ΔA/min and the concentration (ΔA/min. Factor).

To program the time, place the cursor over the desired selection and press Function Key 4 to change.

Use the arrow keys to move between digits and to increase or decrease the value. The interval time is restricted to values above 10 seconds. The maximum period is 59m 59s after completion of the START time.

Note: If running a long kinetics method, it is advisable to leave the unit on for at least 30 minutes to allow the lamps to fully stabilise, otherwise a slight long term drift will be apparent.

#### 9.7.1.4.1 Fixed Time Kinetics

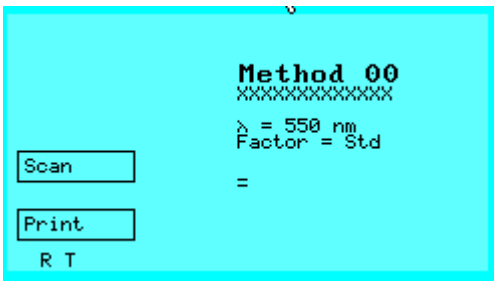



This allows the user to set a timed delay before a measurement is made. This is suited to tests where the final result depends upon allowing the sample to react for a certain length of time and is especially useful where the Absorbance is likely to attain a certain value and then decay.

See Appendix I for calculation.

### 9.7.2 Run a Method

The following screen should be displayed:




Pressing REF will zero the instrument and clear the screen.
Pressing TEST will take a measurement (or if kinetics have been selected, start the run) and display the result in selected units.

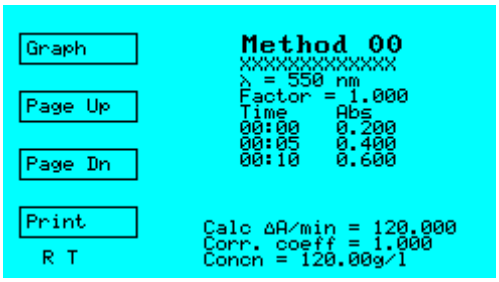
**Note:** Kinetics cannot be accepted if Factor = Std

### 9.7.2.1 Kinetics Run

During the kinetics run the screen will initially show the Start Time less time elapsed.

Once this reached zero, the first measurement will be taken, followed by more measurements at each selected interval until the End time is reached.

Each measurement is displayed on the screen in turn. Once all the readings have been taken, the calculated ΔA/min, correlation coefficient and concentration will be displayed.




The ΔA/min is calculated using a Least Squares Fit. You can view the graph by pressing Function Key 1, or scroll through the readings using the page up or page down keys.

To repeat the test, re-press TEST.

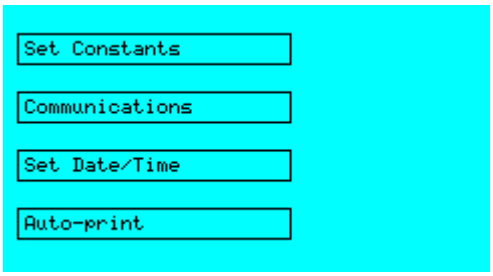
To abort a run, press ESC.

Values may be printed to a printer or to a PC.
A maximum of 20 readings can be accommodated in the memory.

### 9.7.2.2 Fixed time Kinetics

The screen will initially show the delay time – reducing down to zero. Once the time has elapsed a reading will be taken and the result displayed on the screen.

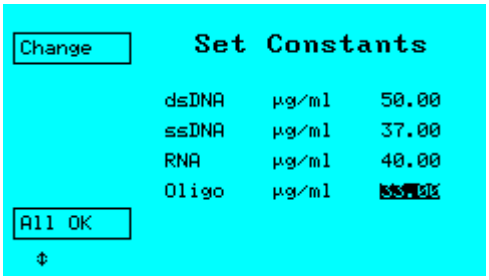
# 10 Instrument Set-up




## 10.1 *Set Constants*

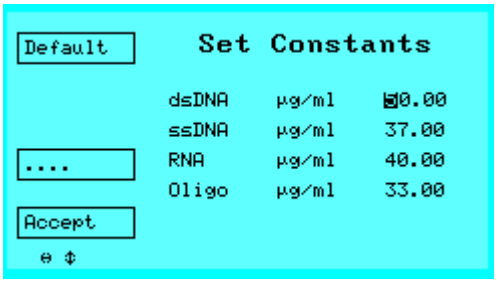
This function changes the factors that are used in the Nucleic Acid calculations.

The default factors are initially displayed:




Choose the factor to be changed using the ↑↓ keys.

Press change. The numeric value is changed using the ↑↓ keys. To move between the figures use the ←→ keys. Function Key 3 "**.....**" alters the position of the decimal place.

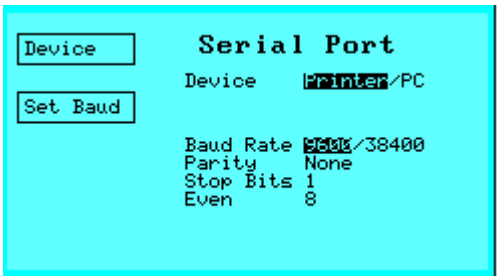



The Default key returns the factor to the factory setting.

When the factor is correct press Function Key 4 "Accept".

Once all factors are correct press Function Key 4 "All OK". The factors will then be used in all Nucleic acid calculations.

## 10.2 *Communications*




The Biowave has a bi-directional RS232 port that can be used to connect to a PC or 40-column printer.

The communication settings are the same for PC and Printer, but have a different format. They are accessed from the “Set up instrument” then “Communications” buttons from the entry menu.

RS232 settings are as follows. The Baud rate is user adjustable by clicking on function key 2 “Set Baud” to toggle between the two available values : 9600 or 38400.

**IMPORTANT NOTE : For connection to the printer S2000P only the 9600 Baud setting may be used.**

| Setting | Printer/PC |
|---|---|
| Baud Rate | 9600 or 38400 |
| Parity | None |
| Stop Bits | 1 |
| Data Bits | 8 |

If connecting to a PC at 38400 Baud the length of the cable between instrument and PC should be no longer than 3m.

The device required is selected by pressing the Device button, which toggles the unit between PC and Printer mode. The PC output is in comma separated, ASCII format. The printer format is detailed in section 11.

### 10.3 *Set Time/Date*


The time and date are available in the following formats: -

Euro Format
Time hh:mm (24 hour clock)
Date dd/mm/yy

OR

US Format
Time hh:mm (24 hour clock)
Date mm/dd/yy

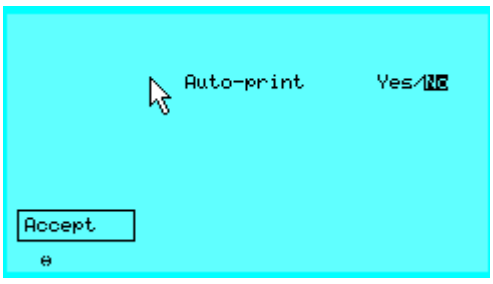
Click on “Format” to toggle between the two formats. These are retained even if the unit is switched off. The embedded processor is year 2000 compliant.

To set the Time/Date enter the “Set up Instrument” screen and select “Set Time/Date”.

Use the ↑↓ to change the setting or use the ←→ arrows to move between time and date settings.

**Press “Accept” to confirm**

### 10.4 *Auto-Print*



When the Auto-print is ON, the instrument automatically increments the Sample Number and outputs the data in the set mode (PC or Printer) each time TEST is pressed (but not REF). The Sample Number screen is not displayed.

When Auto-print is OFF, printing is via the Print button.

NOTE: the Print button remains active even when Auto-print is ON in case duplicates are required.

# 11 Printing

The unit is designed to print to a 40 column serial printer.

**Printer Specifications**

| | |
|---|---|
| Type | Serial RS232 |
| Protocol: Text | EPSON ESC/POS format |
| Protocol:Graphics | Sends graphics command 1B(hex),01(hex); then 40 bytes of data, the lower 6bits of each containing the graphics dots comprising that line. Repeated for each graphics line of dots. |
| Width | 40 column |
| Handshaking | RTS/CTS |
| or | Wire in DTR line to the CTS feed from Biowave |

The WPA S2000P printer from WPA meets all the above specifications.

To avoid any possible damage, ensure that both printer and spectrophotometer are switched off prior to connection. There are 2 different print formats dependent upon which mode of operation has been selected.

In the majority of cases the user will be asked to confirm the **Sample Number**, or revise. This will then appear on the printout.

# 12 Connection to PC

The Biowave S2100 can be used in conjunction with a PC in one of two ways:-

1. By installing the WPA Biowave Excel Add-in and controlling the operation from Excel
2. By downloading data as a text (csv) text file using “Hyperterminal” (Windows 95/98 TM) – with the option of then importing this into Excel.

Before either can be used, the correct connection must be established.

## 12.1 *Setting Up the Biowave to a PC Link*

First connect the Biowave and the PC using a suitable cable, such as WPA part number S2000PC. Connections to the most common PC COM ports are shown in the Table below, together with the necessary connectors.

| **FROM:** | **TO:** | |
|---|---|---|
| **Biowave 9 way D type Female** | **COMM1 9 way D type Female** | **COMM2 25 way D type Female** |
| Pin 2 | Pin 3 | Pin 2 |
| Pin 3 | Pin 2 | Pin 3 |
| Pin 7 | Pin 8 | Pin 5 |
| Pin 8 | Pin 7 | Pin 4 |
| Pin 5 (screen) | Pin 5 (screen) | Pin 7 (screen) |

## 12.2 *Using the Biowave Excel Add-In*

This Excel Add-in enables remote operation of the Biowave S2100 from a PC and also automatically imports the data into MS Excel without having to go via a HyperTerminal programme. It greatly simplifies the transfer of data to the PC and provides a simple route toward overlaying spectra on the same graph using Excel graphing facilities.

**NOTE: When using the Excel Add-In it is very important that the data is saved on a very regular basis to avoid accidental loss. Use of the “auto-save” add-in can assist with this but care needs to be taken to change the file name to avoid overwriting previous stored data.**

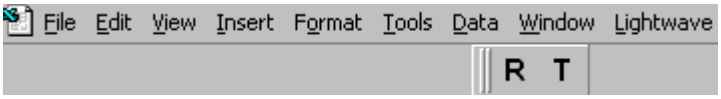
### 12.2.1 To install

The following files are provided on Floppy disc:

| | |
|---|---|
| letimer.ocx | LightwaveNT.bat |
| Mscomm32.ocx | Regsvr32.exe |
| Lightwave.xla | Lightwave.hlp |
| Lightwave.bat | Scrrun.dll |

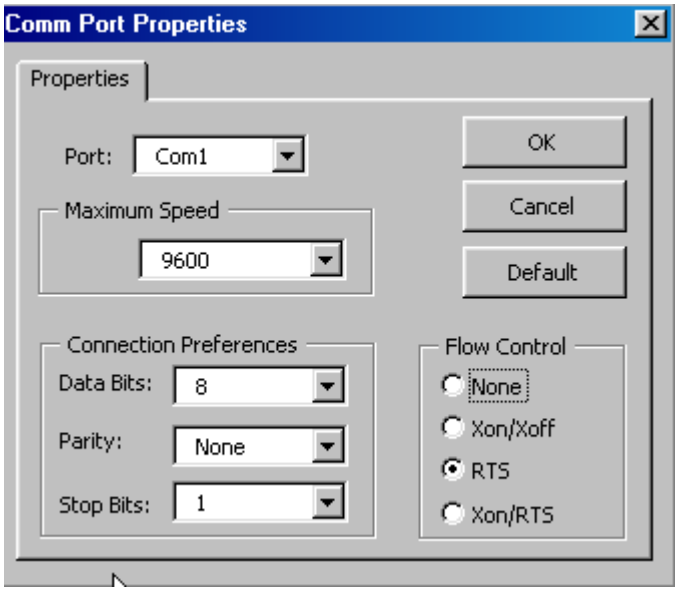
1. Insert disk into the A: drive
2. Select START, RUN, A:\Lightwave.BAT( If using Windows 95,98,ME or 2000 ) or if using WindowsNT run A:\ LightwaveNT.bat. This will install the .ocx files into the c:\windows\system directory, register the files and transfer the help file to c:\windows\help
3. Open Excel
4. Select Tools, Add-ins ,Browse. Select the a:\ drive and highlight Lightwave.xla and click OK

The Lightwave Add-In should activate, and a Lightwave menu item appear between the Window and Help items. Two icons, one marked R (reference) and the other T (for test) should appear.




A worksheet named “sample” will also be created. This is required to enable the data to be imported.

The following screen will appear (or can be selected by highlighting Comms under the Lightwave toolbar)



Select which COM port you want to use and set the Baud rate – the other values should be the defaults (these should be the defaults):-

| | |
|---|---|
| Maximum speed | 9600 Baud |
| Data Bits | 8 |
| Parity | None |
| Stop Bits | 1 |
| Flow control | RTS |

Press OK

Once this is done turn the Biowave on and connect it to the correct COM port (selected above) on the PC. Once the Biowave is past the Diagnostics screen select “Instrument Setup” and “Communications”. Ensure that the Device is set to PC and select the Baud rate **to match the value set above**.

**Note : It is imperative that the Baud rates match – any mismatch will prevent communication and will require both S2100 and Excel to be re-started.**

Running at 38400 enables data to be downloaded at a faster rate, resulting in a total measurement to display time of around 15 seconds, compared to 21s for the slower Baud rate.

### 12.2.2 Data Format

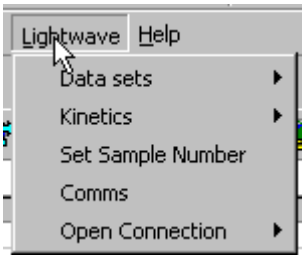
Data is downloaded in the following format:-

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Date: | 23/8/2001 | 23/8/2001 | 23/8/2001 |
| 2 | Time: | 11:34:31 | 11:47:10 | 11:47:31 |
| 3 | Sample no | 2 | 3 | 4 |
| 4 | | | | |
| 5 | Wavelengtl | Abs | Abs | Abs |
| 6 | | | | |
| 7 | 200 | 0.538 | 0.001 | 1.229 |
| 8 | 201 | 0.538 | 0.001 | 1.229 |
| 9 | 202 | 0.535 | 0 | 1.223 |
| 10 | 203 | 0.532 | 0 | 1.221 |
| 11 | 204 | 0.53 | 0.001 | 1.224 |
| 12 | 205 | 0.53 | 0.001 | 1.224 |
| 13 | 206 | 0.529 | 0.001 | 1.227 |
| 14 | 207 | 0.531 | 0 | 1.224 |
| 15 | 208 | 0.533 | 0.001 | 1.229 |
| 16 | 209 | 0.534 | 0.001 | 1.232 |

The format is the same – it makes no difference which Biowave screen you are in.
Any subsequent data analysis must be performed with this base data.

### 12.2.3 Options

The Lightwave add-in gives you the following options:-




**Date sets** – allows the choice of overwrite or increment. If overwrite is selected any new data will overwrite existing data (which will be lost unless saved).
If increment is selected the data will be added alongside existing data.

**Kinetics –** this option allows measurements to be taken over time. It is possible to set data intervals of between 15s (@38400 Baud) and 10 minutes, with a maximum of 255 measurements. Data is automatically incremented.

**Set Sample Number** – The unit automatically increments the sample number (unless in Kinetics mode). The sample number can be re-set using this function.

**Comms** – this allows the Baud rate to be changed.

**NOTE : Opening this option automatically resets other options to their default values. Running at 38400 enables data to be downloaded at a faster rate, resulting in a total measurement to display time of around 15 seconds, compared to 21s for the slower Baud rate.**

**Open Connection** – Before data can be downloaded the Lightwave connection must be opened.

### 12.2.4 To operate

1. Once this is done turn the Biowave on and connect it to the correct COM port (selected above) on the PC using the cable Cat No TU2030. Once the Biowave is past the Diagnostics screen select “Instrument Setup” and “Communications”. Ensure that the Device is set to PC and select the Baud rate.
2. Check that the Baud rate on the Biowave and on the PC are consistent.
3. Open the connection using the Open Connection option on the Lightwave menu
4. Select Overwrite or Increment
5. Set sample number as required
6. Place reference cuvette in the Biowave
7. Click on

   R

   A box should appear after approx. 7s indicating that a successful Reference has been performed.
8. Place sample cuvette in the Biowave
9. Click on

   T
10. After between 15 and 21 seconds data should appear in the sample spreadsheet

**IMPORTANT: Please ensure that Sample Data is saved very regularly, ideally using autosave. With any communications program there is a risk that the program will hang and in this circumstance, data may be lost.**

### 12.2.5 Kinetics

It is possible to perform Tests at time intervals and download the data into a new column for each Test. To do this select Lightwave, then Kinetics, then Kinetics ON.

This will automatically select the increment data option. You will then have to enter the time interval – the minimum is 15s (9600 Baud) and 21s (38400 Baud) and a maximum of 10 minutes. It is possible to record up to 255 measurements in one worksheet giving a maximum total time of 42.5 hours.

To start the kinetics run, click on T (having first referenced if required).
To stop click the STOP KINETICS button.

### 12.2.6 Problem Solving

During Installation

Please inform WPA of any problems encountered when installing or running the Lightwave Excel Add-in.

1. If the .BAT file does not run properly it is possible to do these operation manually as follows:-

    1) Copy both .ocx files to the c:\windows\system directory
    2) Copy the Lightwave.hlp to the c:\windows\help directory
    3) Select START RUN , in the open text box type
       Regsvr c:\windows\system\mscomm32.ocx, then repeat
       Regsvr c:\windows\system\ietimer.ocx

    4) Open EXCEL and proceed as before

2. If the Add-in option on Excel is not available please install this option from original setup disk or speak to your systems administrator.

3. If the Visual Basic Editor starts this may indicate that a necessary file, scrrun.dll, is missing from the \windows\system directory. You can copy this file from the A:\ drive to the C:\windows\system directory – if a previous version is already installed do not overwrite. Please inform WPA and/or consult your systems administrator if the problem does not resolve itself.

4. Some problems may be caused if the PC lead is connected to the wrong COM port or using the wrong cable. Please check this if any problems are being encountered.

### 12.2.7 Error messages during operation

**Status Port Open** – this indicates that connection has been lost with the Lightwave. Try re-opening the connection. If this fails then switch the Lightwave OFF then ON and once through the diagnostics screen select OPEN CONNECTION.

**Overrun Error** - this indicates that values are being overwritten in the hardware buffer and is related to baud rate, computer speed, and how many other activities the PC is trying to perform. Data will not be downloaded in this condition.

If this error occurs you can minimise the likelihood by

1. Using a Baud rate of 9600

2. Closing down other non essential programs and background functions

**Any Visual Basic Editor message –** the Excel add-in relies on visual basic programming. If the editor starts please ensure that you close it down immediately and do not alter any programme script. If this re-occurs please report to WPA.

**Other problems**

The most likely cause of problems is that the incorrect Baud rate is selected. This can result in *Status Port Open* messages – see above.

The Biowave is configured to be used with commonly available software for RS232 communications such as “terminal” (Windows 3.11™) or “Hyperterminal” (Windows 95/98 ™). These are usually located under Program Manager “Accessories" with your PC. After you have the data displayed on your VDU you can easily save the information and import it to other applications to store or analyse.

Note that the Windows 3.11 'Terminal' facility is very dependant on the speed of the base machine. A slow 386 or 486 machine may miss data. In such cases, it is advisable to use a DOS based package such as 'Odyssey' to collect data.

Before disconnecting or connecting the instrument turn the PC and Biowave off to avoid damage to any part.

### 12.2.8 Using the Biowave Analysis Program

Along with the Excel Add-in, WPA have also provided an Excel spreadsheet which helps display downloaded data in a more useable format. Please see instruction provided on the disc in pdf format.

### 12.3 *Connecting to Hyperterminal*

In addition to being able to use the direct import into Excel it is also possible to store data as a text file . This procedure is outlined in Appendix 2

## 12.4 *Using Bi-directional communications*

1. Connect the Biowave to a PC using “HyperTerminal” (see Appendix 2).

2. Establish communication between the Biowave and the PC by pressing Control and the letter H together (*Control H)*. The PC should respond with ‘HELLO’ on the PC screen.

3. To make a reference reading press *Control R*. A series of R’s will run across the bottom of the Biowave screen and the PC will respond with ‘REF DONE’.

4. To read a sample press *Control T*. The Biowave will respond with a row of T’s running across the bottom of the Biowave screen. The data obtained will be transmitted to the PC in comma delimited ASCII [Wavelength, Value (Abs)]. A message of ‘TEST DONE’ will be received when all the data has been transmitted.

NOTE: Inputting any other characters will give the prompt ‘COMMAND NOT RECOGNISED’.

# 13 Servicing and Maintenance

The Biowave has been designed to be extremely robust and require minimal servicing.

The unit performs a series of self-diagnostic checks each time it is switched on to check the:

- **Lamp performance**
- **Wavelength calibration**
- **Diode Array**

The only user serviceable parts are the lamps, fan and cuvette chamber.

## 13.1 *Replacing the Lamps*

**The Biowave uses novel technology that will ensure that the lamp replacement is a very infrequent event (the lamps should last >15,000 hours).**

**The innovative method of operation requires a particular type of lamp. The lamp alignment is also crucial to the correct operation and we cannot guarantee performance to the published specification if lamps other than those from WPA are used.**

The part numbers for replacement lamps are as follows: -

| | |
|---|---|
| Tungsten Lamp | S2000T |
| Tungsten & Deuterium Lamp Assembly | S2000L |
| As for S2000L but accepting only unit in part exchange | S2000LX |

The lamps come complete with comprehensive fitting instructions.

**CARE: The lamps may be hot, and fingermarks may burn to the surface rendering the bulb useless. DO NOT TOUCH THE GLASS ENVELOPE OF THE LAMP WITH YOUR HANDS.**

## 13.2 *Routine Maintenance*

Although very little attention will be required, we nonetheless recommend the following: -

1 Keep the instrument clean. Immediately wipe off any spilt liquids. Clean with a slightly damp cloth. Non-abrasive water based soap or detergent may be used.
3 Remove the cuvettes from the instrument when not in use.
4 At regular intervals check the mains cable for wear and tear and replace if damaged.
5 Store in a cool place away from corrosive chemicals or fumes.

The cuvette compartment has a drain hole to ensure that any spillage within the cuvette compartment will not damage the instrument. The cuvette holder can be pulled out to clear any spillage and to access the cuvette compartment. Check that the cuvette tray has not accumulated any liquid or deposits from the storage of wet cuvettes.

Particular care should however be taken to avoid spillage when handling aggressive or organic liquids as these can damage the plastic mouldings.

## 13.3 *Decontamination*

If the S2100 is sprayed to decontaminate it, please ensure that the optics are protected by temporarily sealing the optic opening in the cuvette chamber with tape.

## 13.4 *Calibration*

WPA can provide a service and calibration for your instrument including the provision of a certificate traceable to international standards.

Please contact our sales office for further details.

# 14 CE Marking

European Directives for Low Voltage and Electro Magnetic Compatibility

**ALL WPA INSTRUMENTS COMPLY WITH EUROPEAN DIRECTIVES FOR EMC AND LOW VOLTAGE SAFETY AND ARE ACCORDINGLY CE MARKED**

It should be noted that external equipment connected to inputs or outputs could increase the susceptibility of measurements to interference if placed in the maximum electric fields prescribed by the Standard. Although the situation can be alleviated by the use of screened connections, WPA instruments should not be used under such extreme conditions, which would themselves constitute a danger to the operators.
The unit has been tested using a 2m RS232 cable connected; however a longer lead may affect the level of EMC emission.

# 15 Guarantee

The Biowave is guaranteed against faulty workmanship and components for two years. In the unlikely event of service requirements (other than Lamp replacement) during, or after, the guarantee period, do not open the instrument to attempt repairs.

Please return the instrument either to WPA or to your local distributor. Whenever an instrument is returned, please ensure that a label indicating your name, address, telephone and extension number is securely attached to the instrument.

# 16 Health & Safety Certificate and Declaration of Decontamination Status

Before an instrument can be accepted for repair, service or return to stock this form must be completed and returned to WPA showing that no hazard to health exists to WPA due to Physical, Chemical, Biological or Radioactive contamination of the unit. Where the unit has been used in potentially hazardous environments evidence of decontamination conducted should be given. **WPA reserve the right to destroy the said instrumentation (at the cost of the user) after a period of 3 months has expired between the instrument being received and the satisfactory completion of this certificate**.

Instrument Model Number …….
Instrument Serial Number ……..

Circle A if applicable, otherwise circle and complete all parts of section B

A This equipment (including any accessories) has not been in contact with blood or other body fluids, respired gases or pathological samples. It represents no hazard to health due to Physical, Chemical, Biological or Radioactive contamination of the unit.

B 1. This equipment been exposed internally or externally to hazardous materials as indicated below:-

( ) Yes ( ) No Body fluids, respired gases, pathological samples
( ) Yes ( ) No Other biohazards
( ) Yes ( ) No Chemicals or substances hazardous to health
( ) Yes ( ) No Radioactive hazards
( ) Yes ( ) No Other hazards

Please attached further details.

2. Has this equipment been cleaned and decontaminated. ( ) Yes ( ) No

How was this contamination removed?……

.....

I declare that I have taken all reasonable steps to ensure the accuracy of the above information.

Signed ..... Name (print) .....

Company/Establishment…..……Position.....

Date .....

# Appendix 1 – Calculations

### *Nucleic Acids*

#### dsDNA

Results for dsDNA μg/mL = Factor * $A_{260}$ * Dil Factor
Results for dsDNA μg/μL = Factor * $A_{260}$ * Dil Factor
Results for dsDNA pmol/mL = (Factor*1000/No base pairs * 2 * 330) * $A_{260}$* Dil Factor

The default value for the Factor is 50 for μg/ml and 0.050 for μg/μL
The size is measured in No of Base Pairs (kB)
The relative molar mass is taken to be 330 kDa.

#### ssDNA

Results for ssDNA μg/mL = Factor * $A_{260}$* Dil Factor
Results for ssDNA μg/μL = Factor * $A_{260}$* Dil Factor
Results for ssDNA pmol/mL = (Factor*1000/No bases * 330) * $A_{260}$* Dil Factor

The default value for the Factor is 37 for μg/ml and 0.037 for μg/μL
The size is measured in No of Bases (kB)
The relative molar mass is taken to be 330 kDa

#### RNA

Results for RNA μg/mL = 40 * $A_{260}$* Dil Factor
Results for RNA μg/μL = 0.04 * $A_{260}$* Dil Factor
Results for RNA pmol/mL = (Factor*1000/No bases * 330) * $A_{260}$* Dil Factor

The default value for the Factor is 40 for μg/ml and 0.040 for μg/μL
The size is measured in No of Bases (kB)
The relative molar mass is taken to be 330 kDa

#### Oligonucleotides

Results for Oligo μg/mL = Factor * $A_{260}$* Dil Factor
Results for Oligo μg/μL = Factor * $A_{260}$* Dil Factor
Results for Oligo pmol/mL = ($A_{260}$* Dil Factor * 100,000) / (1.5 * Adenine + 0.71*Cytosine + 1.2*Guanine + 0.84*Thymine)

The default value for the Factor is 33 for μg/ml and 0.033 for μg/μL

The concentration of Oligonucleotides has been based upon an estimate of the Molar Extinction Coefficient according to the following formula:-

$\varepsilon \cong 10000 * (1.5A+0.71C+1.2G+0.84G)$

Where the values for A,C,G,T are number of bases.

Concentration in pmol/ml = $(A_{260} * 10^9 ) / \varepsilon$

***Protein UV Absorbance method***

Results for mg/ml = $1.55 * A_{280} - 0.76 * A_{260}$
Results for μg/ml = $(1.55 * A_{280} - 0.76 * A_{260} ) * 1000$

***Background Absorbance***

For all the above methods if Background Correction is set to ON, the Absorbance results for $A_{260}$ and $A_{280}$ used in the above calculations are as follows

$A_{c260} = A_{260} – A_{320}$
$A_{c280} = A_{280} – A_{320}$

**16.1** ***Other methods***

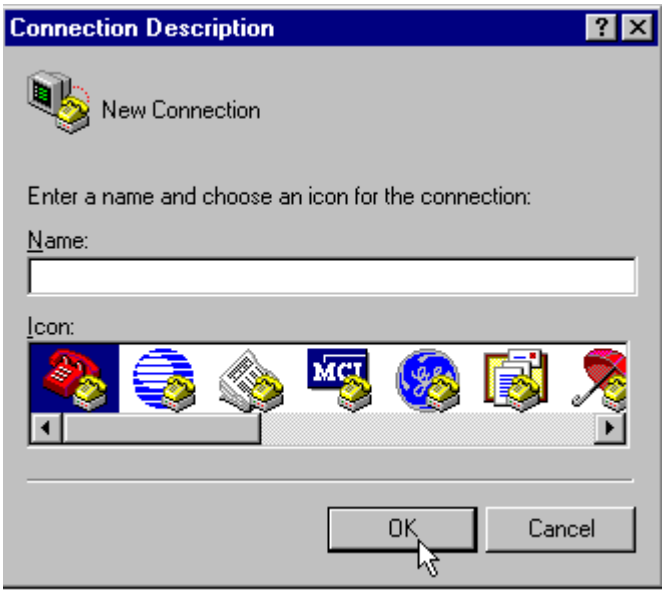
| **Type of method** | **Calculation** |
|---|---|
| Non kinetics | Result = Factor (calc from std cal or factor)* Abs |
| Kinetics = Fixed Time | Result = Factor * Final Abs |
| Kinetics = YES | Result = Factor * ΔA/min<br>Where delta A/min is calculated using a least squares linear fit. |

# Appendix 2 -Connecting to HyperTerminal and downloading results

### Windows 95 or 98 (or NT users)

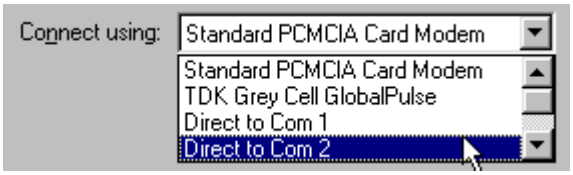
Open HyperTerminal program under Program Manager “Accessories”.

Double Click on icon “Hypertrm”



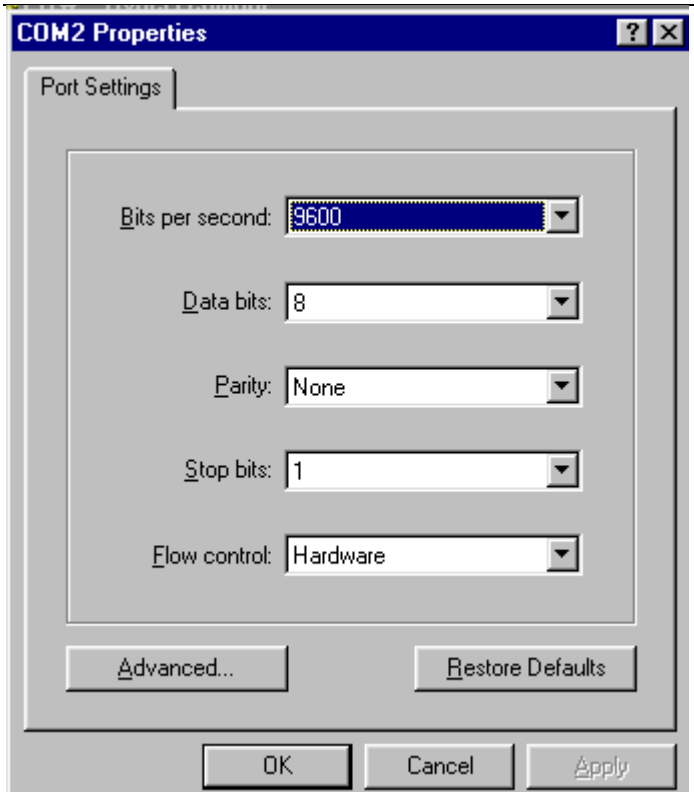

You will be asked for a suitable name - e.g Biowave and an icon. Once this is set up you can click on this icon and the settings will be loaded correctly.

You will then be asked to configure the phone number. Ignore the first options and move the "Connect using" option.

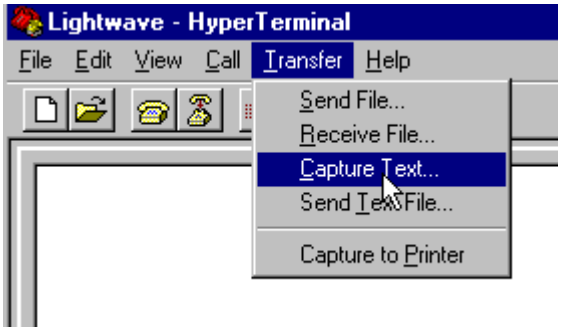

Select the correct COM port (which will be used to receive the Biowave data) under “Connect Using” and then OK.

Now set up the COM port: -




Set the settings as above then press OK.

To capture data, select Transfer - Capture Text.




You will be asked for a file name - e.g. Biowave.txt. Then Press START.

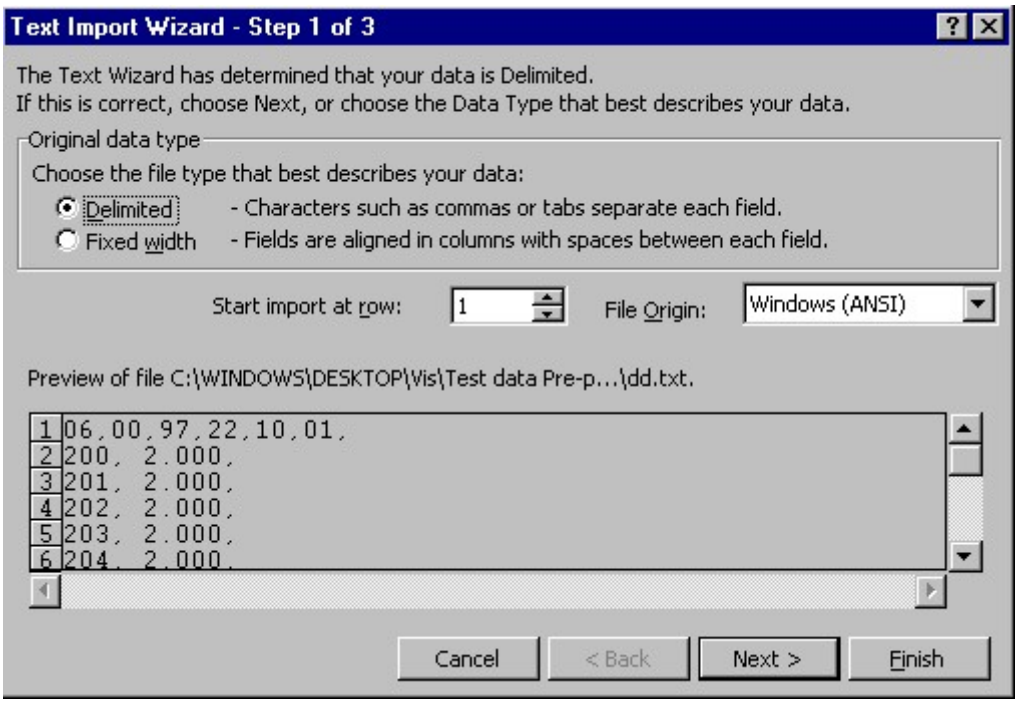
Pressing PRINT on the S2100 will send data to the PC (ensure that PC is selected under Communication).

To End data transfer Select Transfer - Capture Text- Stop.

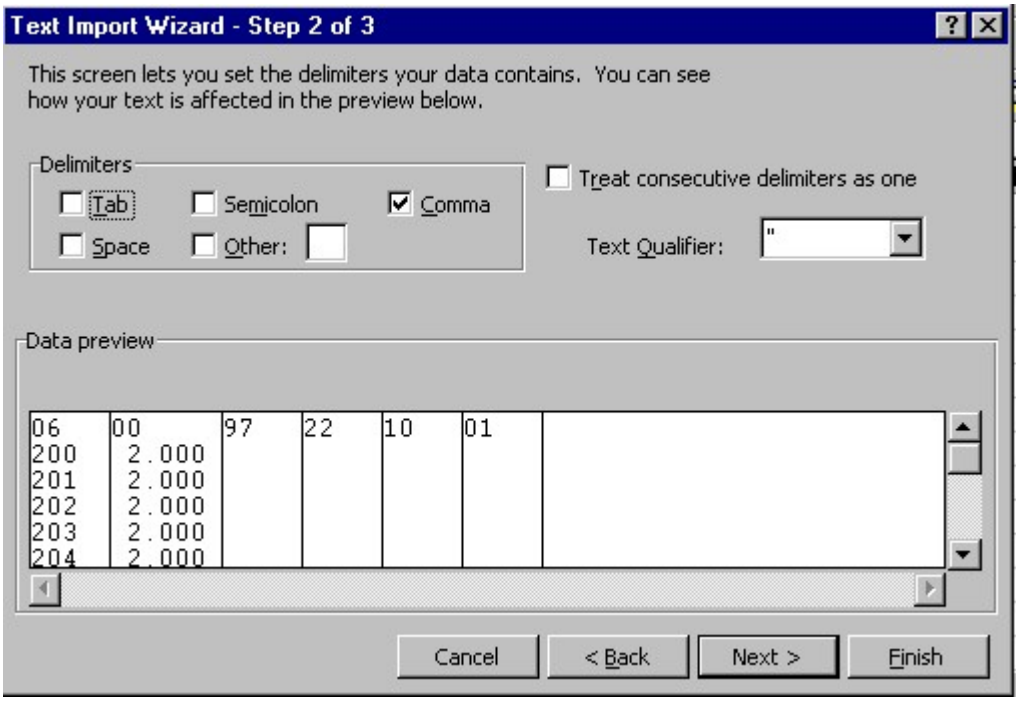
The file can then be imported into EXCEL™ directly using the Import Wizard within MS Excel ™. The import wizard will detect the format automatically.

**Importing Into Excel (or similar spreadsheet program)**

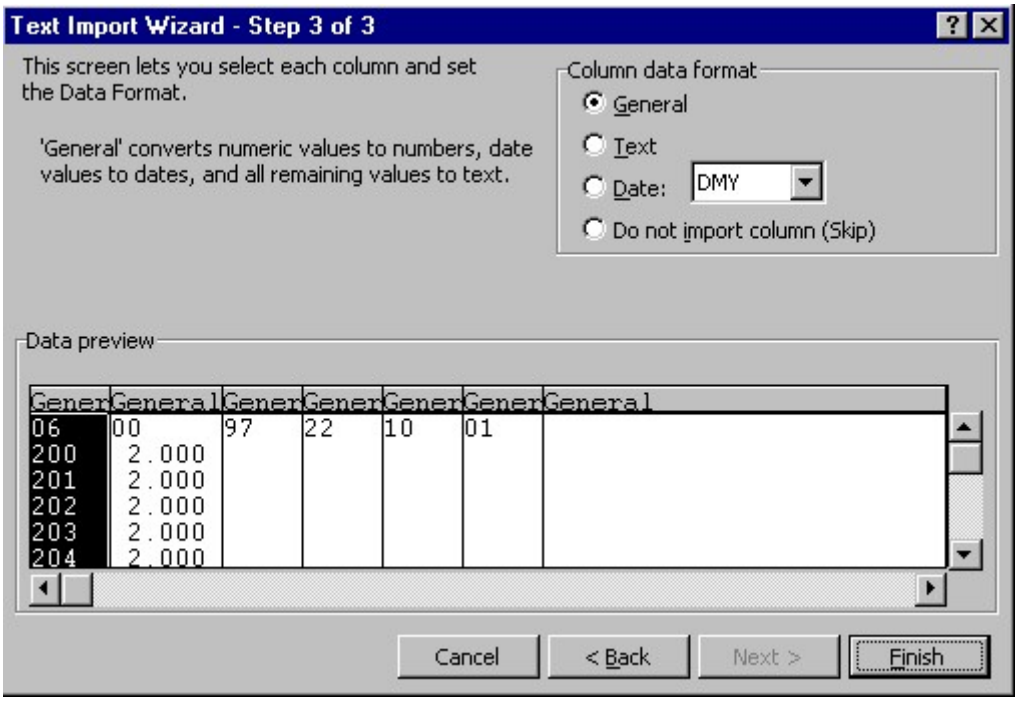
Open Excel™ and open the data file (*.txt); the following screen will be displayed:-




Choose Delimited (if not already chosen) and press NEXT.



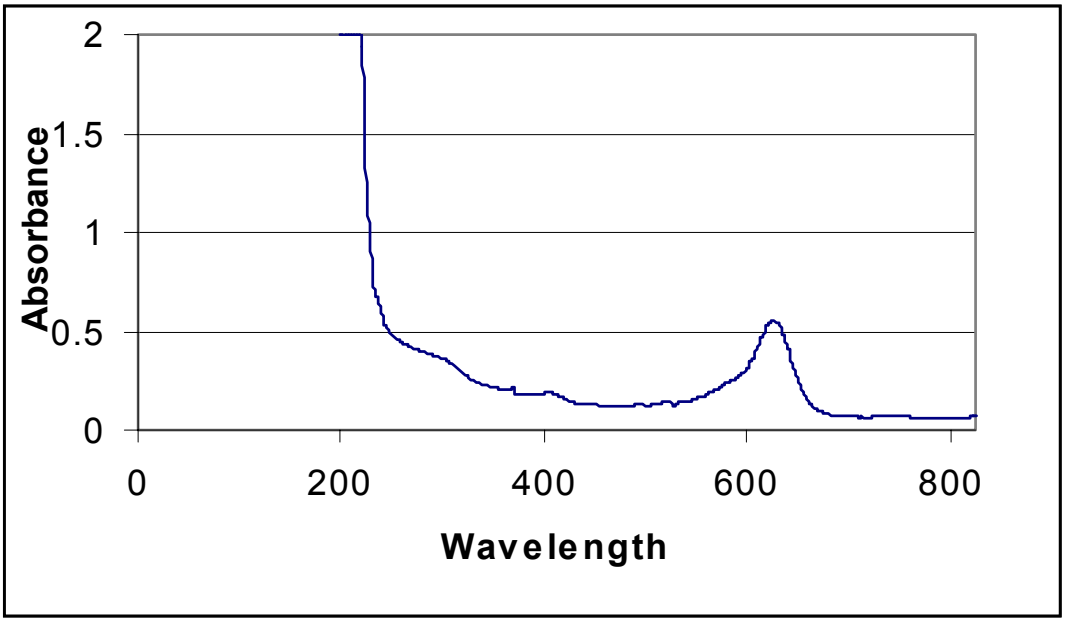

Select comma, then NEXT




Select General, then FINISH. The data will then appear as indicated in the excel spreadsheet. To create a graph of the data (if scan data) use the graph facility in Excel. You will need to re-arrange the columns (wavelength first, Absorbance (or %T) second).

**WPA has set up an Excel™ file (sample.xls) to import the data and display Spectra. This can be obtained from our sales office and e-mailed to you or put onto a disc. Alternatively the data can be imported into you own Excel file.**

A typical printout would look like that below:-



The PC format is designed to be compatible with most Windows™ spreadsheet software including LOTUS 123™ and MS EXCEL™.

**Windows 3.11™**

Open the Terminal emulator and under the “Settings” menu, run down to “Communications” and set the following parameters.

| | | |
|---|---|---|
| **COM1/COM2** | : | Depending on which port you are using. (Usually the mouse is in COM1 and COM2 is available) |
| **BAUD RATE** | : | 9600 |
| **PARITY** | : | None |
| **STOP BITS** | : | 1 |
| **DATA BITS** | : | 8 |
| **FLOW CONTROL** | : | “Hardware” with the parity check and carrier Detect boxes inactive. |

Once you have set these values click OK. You can save these settings using the file menu.

Whenever you use the programme again you can open the saved file to retrieve the settings.

**Data output format** is as follows:

ASCII strings, comma de-limited.

**Format 1**

Dd,mm,yy,hh,min,sample number or ref,value,units, wavelength

**Format 2 Spectral data**

Dd,mm,yy,hh,min, sample number or ref, carriage return, value1, wavelength 1, carriage return ,value 2, , wavelength 2, carriage return ……., value 512, wavelength 512, carriage return, checksum

**[1]Format 3**

[1] “Windows" and "Windows 95/98" are acknowledged to be trademarks of Microsoft Group

Dd,mm,yy,hh,min, method no, name, sample number or ref, elapsed time in mm/ss, value, *repeat for other values* , delta A/min, correlation coefficient, display, units

**Format 4**

dd,mm,yy,hh,mm,sample no or ref, value, units, wavelength, value, units, wavelength, value, units, wavelength , value, units, wavelength

**Format 5**

dd,mm,yy,hh,mm,method no, name, Std,value,Abs, Std,value,Abs, Std,value,Abs, etc

**Format 6**

dd,mm,yy,hh,mm,method no, name,sample no or ref, value, units, wavelength, Factor or curve