VWR® mySPEC spectrophotometer

INSTRUCTION MANUAL

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VWR International bvba

Researchpark Haasrode 2020

Geldenaaksebaan 464

B-3001 Leuven

+ 32 16 385011

http://be.vwr.com

Country of origin Germany

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1. Safety instructions

Before first use of the VWR mySPEC spectrometer please thoroughly read the instruction manual.

Special notice should be taken of the following:

Caution of dangerous voltage
Please ensure the voltage indicated on the AC adapter exactly matches your local AC power supply.
Only use the original AC adapter delivered with the VWR mySPEC spectrophotometer.

Caution liquids
Ensure that no liquids can enter the device. Do not fill the cuvette if it is already inserted in the cuvette slot.

Caution of dangerous explosive material
Explosive or reactive material must not be applied to the VWR mySPEC spectrophotometer.

Caution of damages
Please check the delivery for completeness and transport damage upon arrival. If any transport damage is noticed, contact your local distributor or the manufacturer immediately. Do not connect the VWR mySPEC spectrophotometer or one of its accessories to the AC wall outlet if you detect any damage.

Operating the instrument with the cover off exposes the operator to the instrument's sharp metal edges and delicate fibre optics. Removal of the cover may also void the warranty.

Intended use of the VWR mySPEC spectrometer:
Absorption spectroscopy of biological substances.
For research use only.

If the spectrophotometer is used in any manner not specified in these instructions, the intended protection of the user cannot be guaranteed.

The VWR mySPEC is designed only for indoor use under the following conditions:

- AC power: 100 – 240 VAC, 50 – 60 Hz, 12 W
- Temperature: 15 - 35 °C
- Humidity: 35 - 65%

The power supply can remain plugged into the VWR mySPEC while the instrument is not in use. When the instrument is plugged in but not in use, the power consumption is ~5 W and the flash lamp is not energised. The instrument does not use a power switch.

2. Installation

Content of delivery

- 1 VWR mySPEC spectrophotometer or VWR mySPEC Twin (with cuvette function)
- 1 AC power adapter
- 1 power cable
- 1 USB cable A-B
- Software CD including this manual
- Monitor with mounting foot

Unpacking

- Remove the instrument from its packaging and inspect it for signs of damage. If any are discovered, inform your supplier immediately

- The instrument must be placed on a stable, level surface and positioned such that air can circulate freely around the casing

- Ensure your proposed installation site conforms to the environmental conditions for safe operation

- The instrument is designed for indoor use only, temperature range of 15 to 35 °C and humidity of 35 to 65% are recommended

- If the instrument has just been unpacked or has been stored in a cold environment, it should be allowed to come to room temperature for 2 – 3 hours in the laboratory before switching on. This will prevent calibration failure as a result of internal condensation

- The instrument must be connected to the power supply with the power adaptor supplied. The adaptor can be used on 240 V, 50 – 60 Hz supplies. It will become warm once plugged in and should not be covered up

- It is recommended that users should thoroughly read this manual prior to use

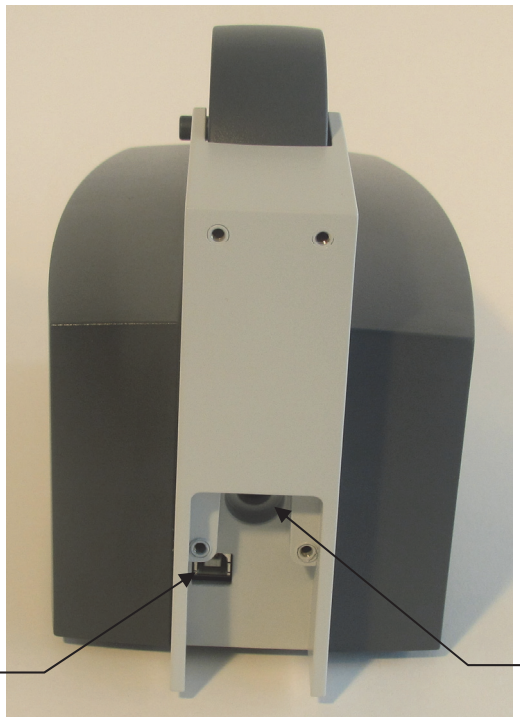
- Contact your supplier if you experience any difficulties with this instrument

Connections

AC power adapter

12 V power outlet connector for connection to the spectrophotometer

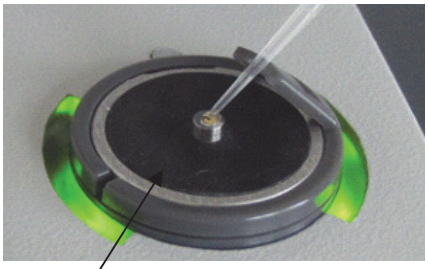
AC inlet cable connector



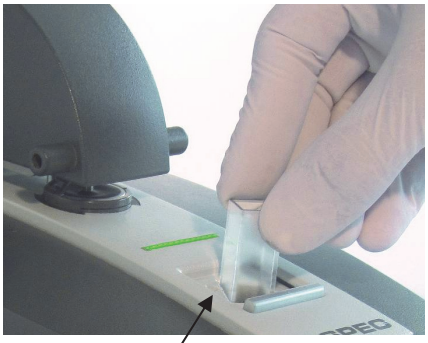
USB connector to PC

12 V power inlet for connection to the AC power adapter

Sample containers



Pedestal



Cuvette slot

Computer requirements

- Microsoft Windows Vista, Windows 7, 8 or 10 operating system
- 1,5 GHz or higher processor
- CD ROM drive
- 1 GB or more of RAM (2 GB if running Vista)
- 40 MB of free hard disk space
- 1 available USB port to connect the system to a computer

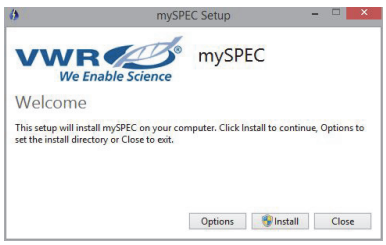
Software installation

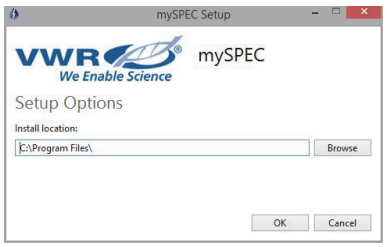
The system software must be loaded onto the PC before the USB cable is connected. Administrator access on the PC is required to install the software. To properly install the operating software, follow the steps below:

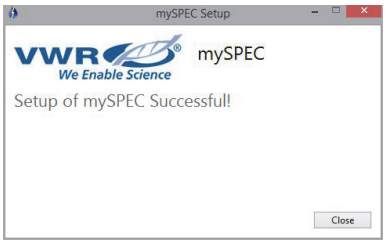
1. Close all programs and make sure the USB cable is unplugged.

2. Insert the CD-ROM in the CD-ROM drive. If the Auto run option is set on your computer, the installation automatically starts. If not, double click on the mySPEC.exe file on the CD to start the installation of the software.

3. The welcome screen is displayed, click on Install to continue







4. To select the Destination directory press Options (“C:\Program files\VWR\SPEC” by default). Then click on NEXT. Program files are installed in the specified directory.
5. After installation of the software connect the power supply and the USB cable to the VWR mySPEC system. If the Found New Hardware Wizard appears, the Windows XP, Vista, 7 and 8 (32 bit ans 64 bit) operating system will ask to allow it to search the internet for the proper software as shown. Select - **No, not this time**. Follow the prompts for automatic installation of the software.

The VWR mySPEC is now ready for operation. If the software does not start, verify if the power supply cable and the USB cable is connected and if the USB-drivers are installed correctly (found in the Device manager) If there are problems with the USB connections a yellow triangle at the USB controller will indicate the problem.

Cable Connections

To work with the system it is necessary to connect to following two cables to the instrument:

- The USB cable has to be connected to the instrument and the PC
- The power supply has to be connected with the plug and the back of the instrument.

3. Introduction

Instrument description

The VWR mySPEC is a simple to use UV/Vis spectrophotometer measuring 0,5 - 2 µl samples with high accuracy and reproducibility.
Typical sample volumes of 0,5 – 2 µl are pipetted onto an optical fibre and because of the surface tension of the sample a liquid bridge will be generated by hydrostatic tension which will be held between two optical fibres. During the procedure different path lengths will be created to enable the measurement of very low and even very high concentrated samples without the need for dilutions. Because of the use of a CCD array a full spectrum from 190 – 840 nm can be measured and displayed on the screen.

Sample size requirements

The sample size is not critical for measurement, the volume can be changed between 0,5 – 2 µl. For volume selection it is necessary that a liquid column forms after starting the measurement. Depending on the surface tension of the sample, different sample volumes are required. Additives like DNA, RNA buffer salt or proteins can reduce the surface tension of the sample. It is recommended to increase the sample volume to 1,3 – 2 µl to ensure liquid column formation if higher concentrations of additives are expected.

Aqueous solutions of nucleic acids: 1 µl
High concentration of nucleic acids or additives: 1,3 - 2 µl
Purified protein: 2 µl
Bradford, BCA, Lowry or Protein Pierce 660 nm assays: 2 µl
Microbial cell suspensions: 2 µl

Sample measurement

Before doing sample measurements in a VWR mySPEC spectrophotometer the system has to be blanked. A spectrum is taken of the reference solution (blank) and stored in the memory. When a sample is then measured, the intensity of light that was transmitted through the sample is recorded. Sample intensities, along with the blank intensities, are used to calculate the sample absorbance according to Beer-Lambert's law:

$A = \varepsilon * l * c$ $[c = A / (\varepsilon * l)]$

c: Concentration of the sample in moles/litre or molarity (M)
A: Absorbance represented in absorbance units (A)
ε: Extinction coefficient of the sample with units of litre/mol-cm
l: Optical path length in cm

The blank solution should have the same pH and similar ionic strength as the sample solution.

Instrument specifications

VWR mySPEC – pedestal mode

Minimum sample size:	0,5 µl
Path length:	1 mm (auto-ranging to 0,05 mm)
Light source:	Xenon flash lamp
Detector type:	2048 element linear silicon CCD array
Wavelength range:	190 - 840 nm
Wavelength accuracy:	±1 nm
Spectral resolution:	≤1,8 nm (FWHM @ Hg 253,7 nm)
Absorbance precision:	0,002 absorbance (1 mm path)
Absorbance accuracy:	±2% (at 0,76 absorbance at 257 nm)
Absorbance range:	0,02 - 300 (10 mm equivalent)
Detection limit:	2 ng/µl dsDNA
Maximum concentration:	15 000 ng/µl (dsDNA)
Measurement time:	<5 s
Footprint:	14x25 cm
Height:	19 cm
Weight:	2 kg
Sample pedestal construction material:	303 stainless steel and quartz fibre
Operating voltage:	12 VDC
Operating current:	0,8 A
Software compatibility:	Microsoft Vista, Windows 7, Windows 8 (32 and 64 bit) and Windows 10

VWR mySPEC Twin – cuvette mode

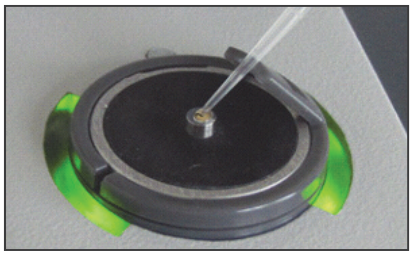
Beam height:	8,5 mm
Heating:	30 - 40 ±0,5 °C
Stirrer:	0, 50 or 1000 rpm
Path length:	10, 5, 2, 1 mm
Detection limit:	0,4 ng/µl dsDNA
Maximum concentration:	750 ng/µl (dsDNA)
Measurement time:	<3 s
Weight:	2,1 kg

All instruments are approved to CE and UL/CSA standards.

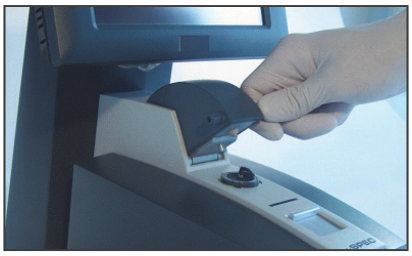
Measurements

Pedestal measurements

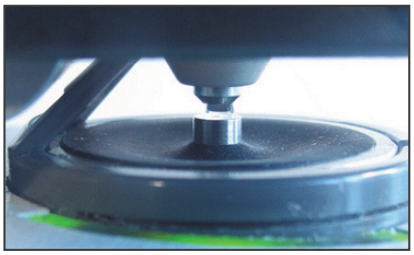
1. Open the arm and place 1 µl of sample directly onto the lower pedestal with the help of VWR mySPEC's pipetting aid.



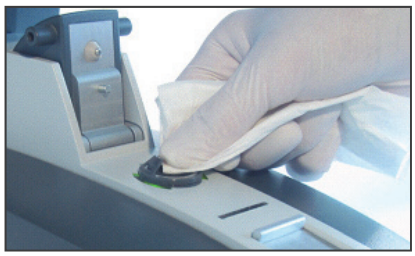
2. Close the arm.



3. A sample column is formed, the optimal path length is adjusted and the measurement starts. Measurement results are displayed within seconds and can easily be saved to file or printed.



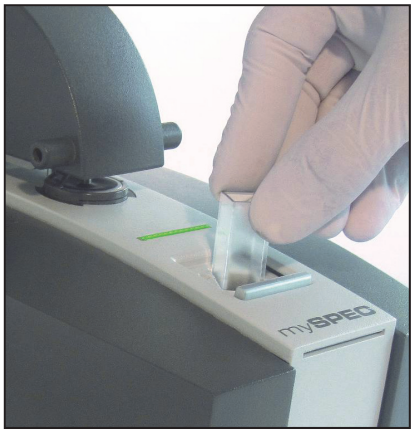
4. Simply wipe the pedestal surfaces clean and you are ready for the next measurement.



Cuvette measurements

The VWR mySPEC will accept 10, 5, 2 and 1 mm cuvettes. The optical beam (2 mm) is directed 8,5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.
When measuring samples with analytical wavelengths in the UV (<340 nm), use cuvettes made of quartz, as these pass UV wavelengths. It is essential to ensure that the sample volume in the cuvette is adequate to allow the light to pass through a representative portion of the sample when making a measurement.

1. Add the sample to the cuvette and ensure that the volume is sufficient to cover the light path.
2. Select the cuvette light path in the software and select the right path length of the cuvette.
3. Insert the cuvette into the instrument in the right position. The light beam will be indicated by an arrow.



4. Measure the sample by clicking on the '**Blank / Measure'** button in the software.
5. When the measurement is complete, remove the cuvette.

After cuvette measurements it is very important to clean and dry the cuvette carefully to avoid cross contamination during the next measurement.

4. Applications

Quick start

1. Start the software by double clicking on the **mySPEC** icon

2. Select the software application of interest.

3. Establish a blank using the right buffer.

   - Pedestal option: Pipette 1 - 2 µl of the blanking solution onto the bottom pedestal, lower the arm and click **Blank**.

   - Cuvette option (VWR mySPEC Twin only): Select **Use Cuvette**. Insert the cuvette noting the direction of the light path indicated by the etched arrow and click **Blank**.

4. Wipe away the blank from the upper and lower pedestals using a dry, lint-free laboratory wipe. Enter the sample ID in the field. Pipette 1 µl of sample and click **Measure**. The measurement will start automatically if the 'Autostart' function is activated.

5. Wipe away the blank from the upper and lower pedestals using a dry, lint-free laboratory wipe.

Measurement ranges

Sample type	**Lower detection limit**	**Approximate upper detection limit**	**Typical reproducibility** (5 replications)
Nucleic acids	2 ng/µl (pedestal)	≤15 000 ng/µl (dsDNA)	2 - 100 ng/µl: ±2 ng/µl
	0,4 ng/µl (cuvette)		>100 ng/µl: ±2%
Micro array	2 ng/µl (pedestal)	750 ng/µl (dsDNA)	2 - 100 ng/µl: ±2 ng/µl
	0,4 ng/µl (cuvette)		>100 ng/µl: ±2%
Protein A280	0,10 mg/ml (purified BSA pedestal)	400 mg/ml (purified BSA)	0,10 - 10 mg/ml: ±0,10 mg/ml >10 mg/ml: ±2%
	0,010 mg/ml (purified BSA cuvette)		
Proteins and labels	0,10 mg/ml	20 mg/ml (purified BSA)	0,10 – 10 mg/ml: ±0,10 mg/ml
	(purified BSA - pedestal)		
	0,010 mg/ml		
	(purified BSA cuvette)		
BCA	0,2 mg/ml	8,0 mg/ml	
	(20:1 reagent sample volume)		2% over the entire range
	0,01 mg/ml	0,20 mg/ml	0,01 mg/ml over the entire range
	(1:1 reagent sample volume)		
Modified Lowry	0,2 mg/ml	4,0 mg/ml	2% over the entire range
Bradford	100 µg/ml	8000 µg/ml	100 - 500 µg/ml: ±25 µg/ml
	(50:1 reagent sample volume)		500 - 8000 µg/ml: ±5%
Bradford	15 µg/ml	100 µg/µl	15 - 50 µg/ml: ±4 µg/ml
	(1:1 reagent sample volume)		50 - 125 µg/ml: ±5%
Pierce 660 nm	50 µg/ml (15:1 reagent sample volume)	50 - 125 µg/ml >125 µg/ml	50 - 125 µg/ml: ±3 µg/ml >125 µg/ml: 2%
	25 µg/ml (7,5:1 reagent sample volume)	25 - 125 µg/ml >125 µg/ml	25 - 125 µg/ml: ±3 µg/ml >125 µg/ml: 2%

Nucleic acid measurements

Overview

Nucleic acids can be quantified at 260 nm because at this wavelength there is a clearly defined peak maximum. A 50 µg/ml DNA solution, a 40 µg/ml RNA solution and 33 µg/ml solution of a typical synthetic Oligo nucleotide all have an optical density of 1,0 A in a 10 mm path length cuvette. These factors (50, 40 and 33) can be inserted into the formula (**c = (A * ε)/b**)

c = the nucleic acid concentration in ng/microlitre
A = the absorbance in A
ε = the wavelength-dependent extinction coefficient in ng-cm/microlitre
b = the path length in cm

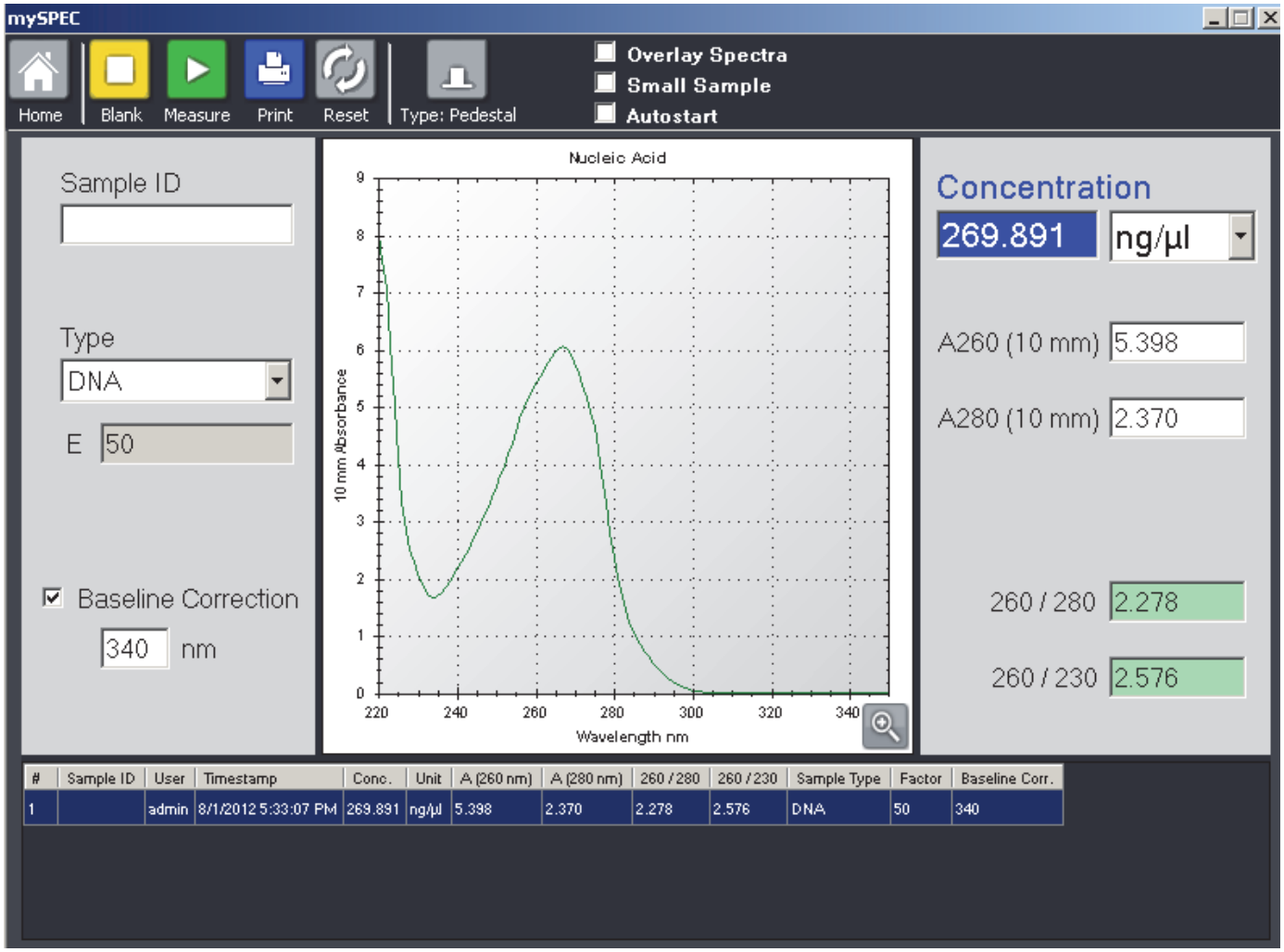
Measurement concentration ranges

The VWR mySPEC will accurately measure purified dsDNA samples ≤15 000 ng/µl without dilution.

A small sample volume option is available when samples have 10 mm equivalent absorbance values of 3,0 or higher (>150 ng/µl dsDNA).

Main screen

- After selecting the measurement application the lamp is automatically set to the correct intensity
- The following main screen will appear



The display shows results for a current sample normalised to a 10 mm path for all measurements.
The following features appear on the screen:

	Goes back to the main menu.
	Makes a blank measurement.
	Makes a sample measurement.
	Print results/screen.
	Resets blank value.
	Enlarges the spectrum to maximum size.

- **Sample ID:** Can be entered into this field

- **Type:** A drop down list from which the user can select the extinction coefficient of the nucleic acid

DNA = 50 for dsDNA
RNA = 40 for RNA
ssDNA = 33 for single-stranded DNA
Oligo DNA = the extinction coefficient will be calculated depending on user-defined base sequences
Oligo RNA = the extinction coefficient will be calculated depending on user-defined base sequences.
Custom = the user can enter an extinction coefficient.

- **Baseline Correction:** If selected, the samples “background noise" is normalised to 340 nm. The user can also manually enter a different wavelength for the normalisation of absorbance data.

- **Concentration:** Based on absorbance at 260 nm and the default or user-defined extinction coefficient. Concentration units can be selected from the drop down box

- **A260:** Displays absorbance at 260 nm normalised to a 10 mm path length

- **A280:** Displays absorbance at 280 nm normalised to a 10 mm path length

- **260/280:** Ratio of absorbance at 260 and 280 nm. This ratio is used to assess the purity of DNA and RNA. A value of 1,8 – 2,1 indicates that the sample is pure. If the ratio is lower than the entered threshold value (see ‘Settings’) the coefficient will be marked in red. If the ratio is higher than the entered threshold value the coefficient will be marked in green. Low values may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm.

- **260/230:** Ratio of absorbance at 260 and 230 nm. This is a secondary measure of nucleic acid purity. The 260/230 values for a “pure” nucleic acid are often higher than the respective 260/280 values and are commonly in the range of 1,8 – 2,2. If the ratio is lower than the entered threshold value (see ‘Settings’) the coefficient will be marked in red. If the ratio is higher than the entered threshold value the coefficient will be marked in green. Low values may indicate the presence of co-purified contaminants.

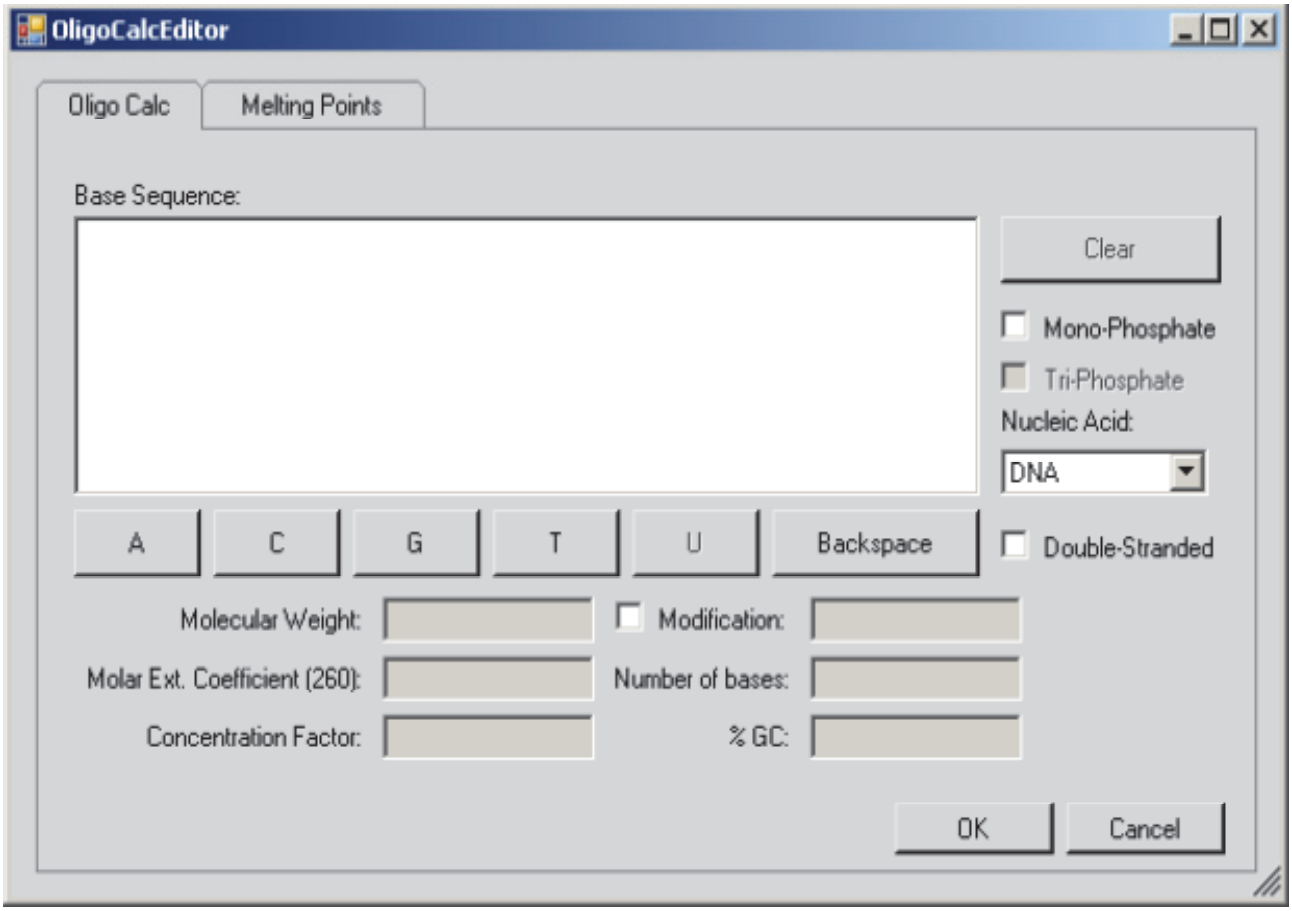
- **Overlay Spectra:** Select this function and the software will display multiple spectra at the same time

- **Small Sample:** Select this function and samples of 0,5 µl can be measured. The recommended sample concentration should be higher than 200 ng/µl.

- **Autostart:** Select this function and the sample measurement will start automatically if the sample arm is closed. The blank measurement must be done manually by using the ‘Blank’ button.

Oligo DNA/RNA

If the 'Oligo DNA' or 'Oligo RNA' function is selected from the drop down list from the 'Sample Type', the following screen will appear.



'Oligo Calc' is used to calculate molecular weights, extinction coefficients, concentration factors and melting points for specific nucleic acid sequences. Selecting this from the task bar will bring up two tabs:

- **Oligo Calc -** used to enter sequences of interest and select appropriate sample type variables
- **Melting Points** - displays the calculated melting points of a DNA strand. This tab is available only for DNA sequences

Using Oligo Calc

1. Enter a base sequence of interest into the field. Use the 'Base-Buttons' on the screen (A, C, G, T and U) or copy and paste the sequence into the display.
2. Select degree of phosphorylation.
3. Select **Double-Stranded**. The complementary base sequence will be included in the analysis.
4. From the drop down list, select the type of nucleic acid to be analysed. The default is DNA.
5. For additions to the base sequence select **Modification** and enter the molecular weight associated with the addition.
6. Click **OK**, the 'Molar ext. Coefficient' will be transferred automatically into the type field of the 'Nucleic Acid' module.

Oligo Calc analysis result fields include:

- **Molecular weight**
- **Extinction coefficient**

- **Concentration factor**
- **Number of bases**
- **% GC**- shows the percentage of the total number of bases made up by guanine and cytosine

To calculate the melting point of a DNA sequence

1. Enter a base sequence of interest into the field.
2. Enter the appropriate values in the each of the boxes:
   - **Oligo Molarity** - enters the Oligo molarity of the sample. A default value of 10 µM is displayed, but can be changed to a value more appropriate for the sample
   - **Cation Molarity** - enters the concentration of cations in the sample. A default value of 50 mM is displayed, but can be changed to a value more appropriate for the sample
   - **% Formamide** - enters the percentage concentration of formamide in the sample. A default value of 0,00% is displayed, but can be changed to a value more appropriate for the sample

Melting point analysis results:

- **Salt-Adjusted** - calculates the melting point of the base sequence without accounting for the effect of interaction between neighbouring bases
- **Nearest-Neighbor** - displays the melting point of the base sequence when the effect of interaction between neighbouring bases is taken into account

Making nucleic acid measurements

1. Select the **Nucleic Acid** application from the main menu - ensure the arm is closed.
2. Select the type of sample to be measured from the 'Type' drop down list - the default setting is DNA-50.
3. Choose the concentration units from the drop down list to the concentration box - the default units are ng/µl.
4. A default wavelength of 340 nm is automatically used for normalisation.
5. Establish a blank using the appropriate buffer.
6. Enter a sample ID in the appropriate field, load the first sample as described for the blank above and click **Measure.**
7. Click on  to enlarge the spectrum size. Click on  to reduce the spectrum size
8. Clean the upper and lower pedestal using a dry, lint-free laboratory wipe

After measuring:

- Simply wipe the upper and lower pedestals using a dry laboratory wipe so the instrument is ready to measure the next sample
- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples

The results will be displayed on the screen if no further measurement will be done. The next measurement will

overwrite the spectrum if ‘Overlay Spectra’ is not selected.
For a detailed view of the spectrum zoom use the left mouse button and draw a field of interested. The zoom will be done automatically. To minimise the zoom double click on the spectrum.

Micro Array

Overview

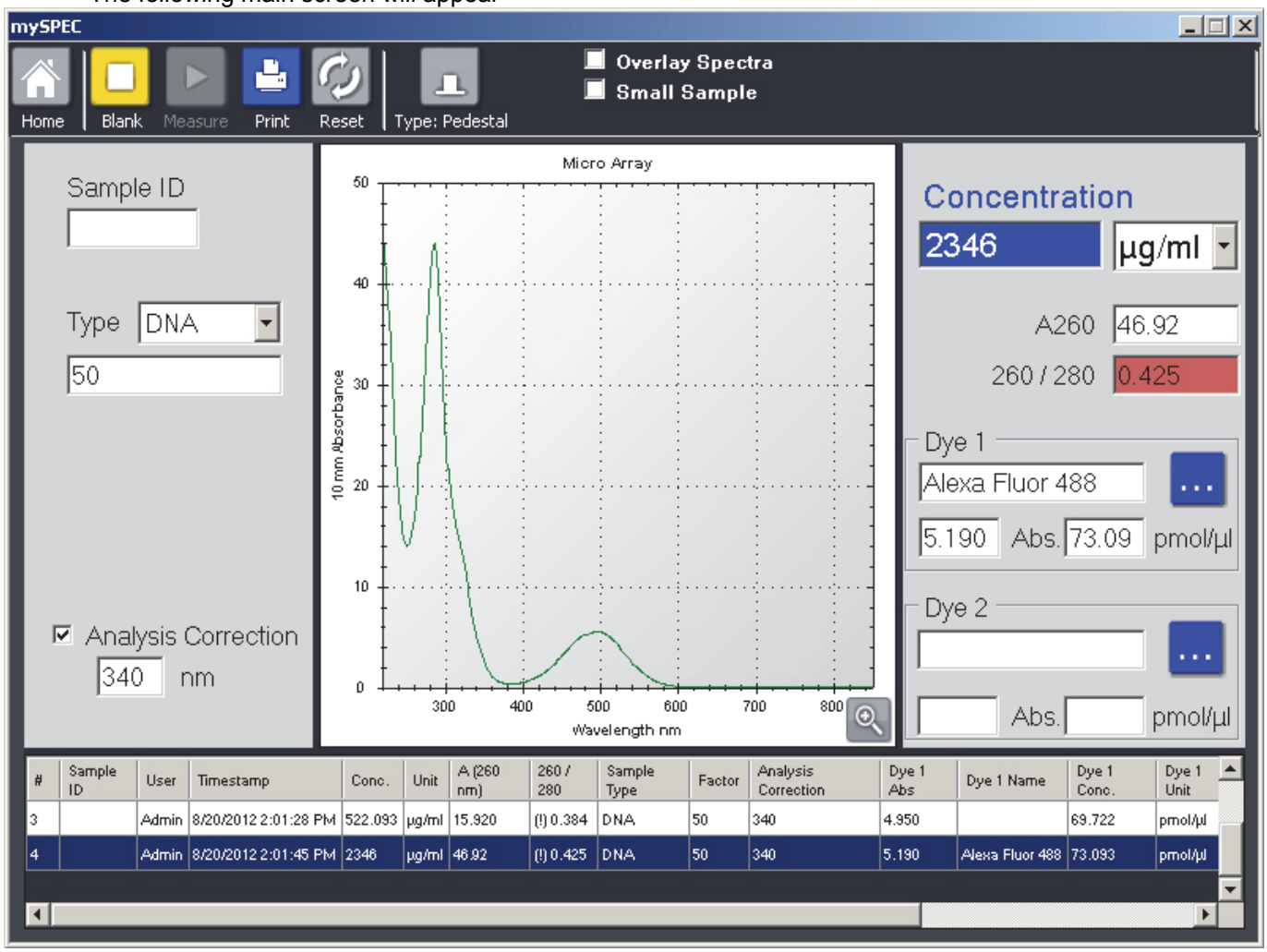
With this module you can measure pre-selected fluorescently-tagged hybridisation probes for use on micro arrays. The VWR mySPEC measures the absorbance of the fluorescent dye, as well as the concentration of the nucleic acid. Because of different the concentration ranges, the software automatically uses the optimal path length to measure the absorbance of each sample.

Measurement concentration ranges

The VWR mySPEC will accurately measure fluorescent dye and nucleic acid concentrations up to 100 pmol/µl (Cy3) and 750 ng/µl (DNA) respectively without dilution.

Main screen

- After selecting the measurement application the lamp is automatically set to the correct intensity
- The following main screen will appear



The display shows results for the current sample normalised to a 10 mm path for all measurements.
The following features appear on the screen.

Home	Goes back to the main menu.
Blank	Makes a blank measurement.

	Makes a sample measurement.
	Prints results/screen.
	Resets blank value.
	Enlarges the spectrum to maximum size

- **Sample ID:** Can be entered into this field

- **Type:** A drop down list from which the user can select the extinction coefficient of the nucleic acid

  DNA = 50 for dsDNA.
  RNA = 40 for RNA.
  ssDNA = 33 for single stranded DNA
  Oligo DNA = the extinction coefficient will be calculated depending on user-defined base sequences
  Oligo RNA = the extinction coefficient will be calculated depending on user-defined base sequences.
  Custom = the user can enter an extinction coefficient.

- **Analysis Correction:** If selected the "background noise" of the sample is normalised to 340 nm. The user can manually enter a different wavelength for the normalisation of the absorbance data.

- **Concentration:** Based on absorbance at 260 nm and the default or user-defined extinction coefficient. Concentration units can be selected from the drop down box.

- **A260:** Displays absorbance at 260 nm normalised to a 10 mm path length

- **260/280:** Ratio of absorbance at 260 and 280 nm. This ratio can be used to assess the purity of DNA and RNA. A value of 1,8 – 2,1 indicates that the sample is pure. If the ratio is lower than the entered threshold value (see ‘Settings’) the coefficient will be marked in red. If the ratio is higher than the entered threshold value the coefficient will be marked in green. Low values may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm.

- **Dye 1 (or 2)**: Select the dye(s) that are bound to the nucleic acid

  - **Abs** - the normalized absorbance of the respective dye at the 10 mm path length

  - **pmol/ µl -** is the concentration based on the respective dye’s extinction coefficient

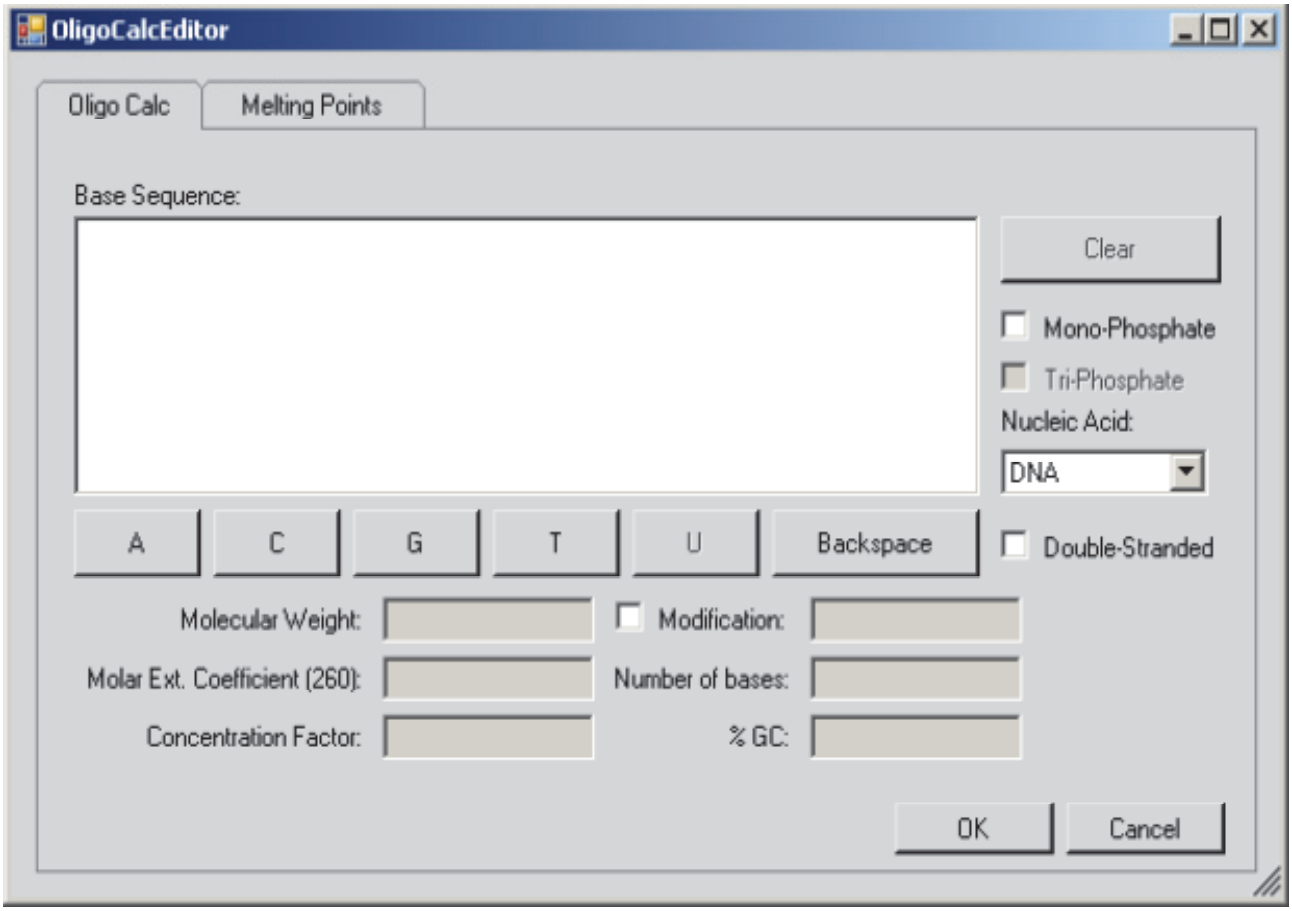
- **Overlay Spectra:** Select this function and the software will display multiple spectra at the same time

- **Small Sample:** Select this function and samples of 0,5 µl volume can be measured. The recommended sample concentration should be higher than 200 ng/µl’

- **Autostart:** Select this function and the sample measurement will start automatically if the sample arm is closed. The blank measurement must be done manually by using the ‘Blank’ button

Oligo DNA/RNA

If the 'Oligo DNA' or 'Oligo RNA' function is selected from the drop down list from the 'Sample Type', the following screen will appear.



'Oligo Calc' is used to calculate molecular weights, extinction coefficients, concentration factors and melting points for specific nucleic acid sequences. Selecting this from the task bar and it will bring up two tabs:

- **Oligo Calc** - used to enter sequences of interest and select appropriate sample type variables
- **Melting Points** - displays the calculated melting points of a DNA strand. This tab is available only for DNA sequences.

Using Oligo Calc

1. Enter a base sequence of interest into the field. Use the 'Base-Buttons' on the screen (A, C, G, T and U) or copy and paste the sequence into the display.
2. Select degree of phosphorylation.
3. Select '**Double-Stranded'**. The complementary base sequence will be included in the analysis.
4. From the drop down list, select the type of nucleic acid to be analysed. The default is DNA.
5. For additions to the base sequence select '**Modification'** and enter the molecular weight associated with the addition.
6. Click **OK**, the 'Molar ext. Coefficient' will be transferred automatically into the type field of the nucleic acid module.

Oligo Calc analysis result fields include:

- **Molecular weight**

- **Extinction coefficient**
- **Concentration factor**
- **Number of bases**
- **% GC** - shows the percentage of the total number of bases made up by guanine and cytosine.

To calculate the melting point of a DNA sequence:

- Enter a base sequence of interest into the field
- Enter the appropriate values in the each of the boxes:
  - **Oligo Molarity** - enters the Oligo molarity of the sample. A default value of 10 µM is displayed, but can be changed to a value more appropriate for the sample
  - **Cation Molarity** - enters the concentration of cations in the sample. A default value of 50 mM is displayed, but can be changed to a value more appropriate for the sample.
  - **% Formamide** - enters the percentage concentration of formamide in the sample. A default value of 0,00% is displayed, but can be changed to a value more appropriate for the sample

Melting point analysis results:

- **Salt-Adjusted** - calculates the melting point of the base sequence without accounting for the effect of interaction between neighbouring bases
- **Nearest-Neighbor** - displays the melting point of the base sequence when the effect of interaction between neighbouring bases is taken into account.

Making micro array measurements

1. Select the **Micro Array** application from the 'Nucleic Acid' menu - ensure the arm is closed.
2. Select the type of sample to be measured from the type drop-down list - the default setting is DNA-50.
3. Choose the concentration units from the drop down list of the concentration box - the default units are ng/µl.
4. A default wavelength of 340 nm is automatically used for normalisation.
5. Select the dye(s) to be measured from the 'Dye' drop down list.
6. Establish a blank using the appropriate buffer.
7. Enter a sample ID in the appropriate field, load the first sample as described for the blank above and click **Measure**.
8. Click on  to enlarge the spectrum size. Click on  to reduce the spectrum size.
9. Clean the upper and lower pedestal using a dry, lint free laboratory wipe.

After measuring:

- Simply wipe the upper and lower pedestals using a dry laboratory wipe so the instrument is ready to measure the next sample
- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples

The results will be displayed on the screen if no further measurement is done. The next measurement will overwrite the spectrum if ‘Overlay Spectra’ is not selected.
For a detailed view, zoom into the spectrum. Use the left mouse button and draw a field of interested. The zoom will be done automatically. To minimise the zoom double click into the spectrum.

Dye Editor

With the ‘Dye Editor’ additional dyes can be added into the list. To enter a new dye, click an empty row in the list. Enter all the dye information into the list. For detailed information of the dye ask the dye manufacturer. The 260 nm corrections will be automatically used for nucleic acid sample concentration calculations when the respective dye is selected. Once entered, the information is saved.

To delete a user-defined dye, highlight the row of interest by clicking in the grey box to the left and use the **Delete** button.

Protein A280

Overview

Protein determination at 280 nm
Protein can be determined in the near UV at 280 nm due to absorption by tyrosine, tryptophan and phenylalanine amino acids. Abs280 varies greatly for different proteins due to their amino acid content, and consequently the specific absorption value for a particular protein must be determined. The VWR mySPEC will accurately measure protein samples up to 400 mg/ml (BSA) without dilution using the pedestal mode. The software automatically uses the optimal path length to measure the absorbance of each sample.

The **Small Sample Volume** option is available when samples have 10 mm equivalent absorbance values of 3,0 or higher (>4,5 mg/ml BSA).

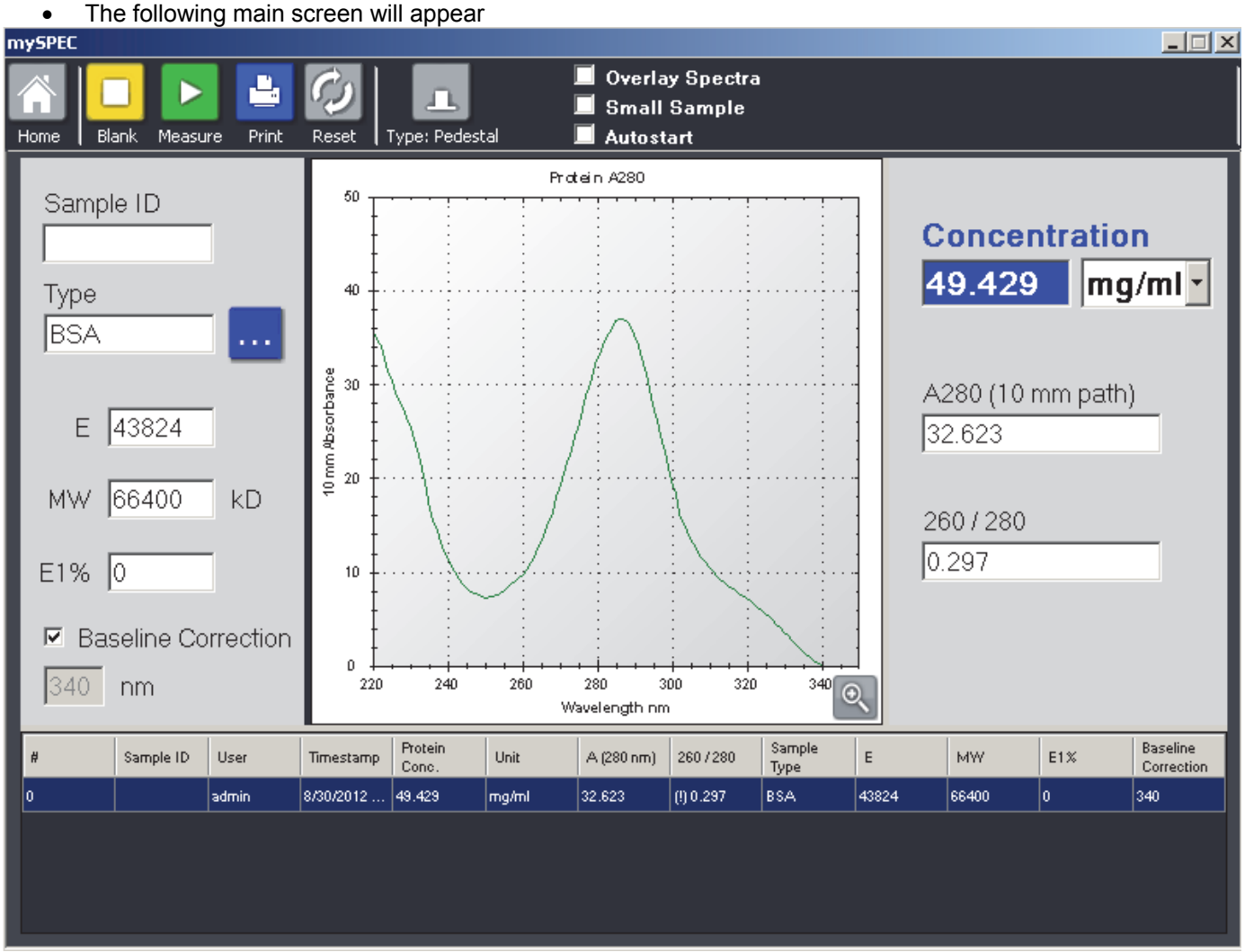
Sample volume requirements

The sample size is not critical for the measurement, the volume can be changed between 0,5 – 2 µl. For the volume selection it is necessary that a liquid column forms after starting a measurement. Depending on the surface tension of the sample, different sample volumes are required. Additives like proteins can reduce the surface tension of a sample. It is recommended that you increase the sample volume to 2 µl to ensure liquid column formation if proteins are measured.

When using the cuvette option, it is essential that sufficient sample volume be used to ensure that the light path is passing through a representative portion of the sample. The optical beam (2 mm) is directed 8,5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

Main screen

- After selecting the measurement application the lamp is automatically set to the correct intensity
- The following main screen will appear



The display shows results for the current sample normalised to a 10 mm path for all measurements.
The following features are on the screen:

	Goes back to the main menu.
	Makes a blank measurement.
	Makes a sample measurement.
	Prints results/screen.
	Resets blank value.
	Enlarges the spectrum to maximum size.

- **Sample ID:** A sample ID can be entered into this field

- **Type:** A drop down list from which the user can select or enter the extinction coefficient of a protein. This list can be extended by frequently used proteins.

Abs 1 = 1 mg/ml	A general reference setting based on a 0,1% (1 mg/ml) protein solution producing an absorbance at 280 nm of 1,0 A.
BSA	Bovine Serum Albumin reference. Unknown (sample) protein concentrations are calculated using the extinction coefficient of 43824 at 280 nm for a 1% BSA solution (MW = 66400 Da).
IgG	IgG reference. Unknown (sample) protein concentrations are calculated using the extinction coefficient of 210000 at 280 IgG solution (MW = 150000 Da).
Lysozyme	Lysozyme reference. Unknown (sample) protein concentrations are calculated using the extinction coefficient of 38940 at 280 nm Lysozyme solution (MW = 14300 Da).
Other proteins	For other reference proteins the user can enter; the molar extinction coefficient and the molecular weight of the protein, the mass extinction coefficient for a 1% solution, or the E1% value.

E = Mass extinction

MW = molecular weight of the protein

E1% = extinction coefficient E1% (g/100 ml)-1 cm (equivalent to the inverse of the 10 mm absorbance of a 1% solution of the protein under test).

- **Baseline Correction**: If selected, the default wavelength for the bichromatic normalisation is 340 nm. The user can manually enter a different wavelength for bichromatic normalisation of the absorbance data. In either case, the baseline is automatically set to the absorbance value of the sample at the selected wavelength. All wavelength data will be referenced from this value. If the baseline correction is not used, the spectra can be offset from the baseline and the calculated protein concentration may be higher than the true value.

- **Concentration:** Concentration based on absorbance at 280 nm and the selected extinction coefficient. Concentration units are chosen from the adjacent drop down list. The default selection is mg/ml.

- **A280 (10 mm path): Absorbance** at 280 nm for the protein sample being measured. The displayed absorbance value is normalised to a 10 mm path.

- **260/280:** Ratio of the absorbance at 260 and 280 nm

Making protein A280 measurements

1. Select the **Protein A280** application from the main menu **Proteins -** ensure the arm is down**.**

2. Select the type of sample to be measured from the 'Type**'** drop down list.

3. Choose the concentration units from the drop down list adjacent to the colour coded box. Default is mg/ml.

4. A default wavelength of 340 nm is automatically used for normalisation. Select an alternative reference wavelength or choose not to have the spectrum normalised by deselecting the **Baseline Correction** box.

5. Select '**Overlay Spectra'** to display multiple spectra at the same time.

6. Establish a blank using the appropriate buffer. The blank solution generally is a buffer that the molecule of interest is suspended or dissolved in. This solution should be the same pH and of a similar ionic strength as the sample solution.

   - Pedestal option: Pipette 2 µl of the appropriate blanking solution onto the bottom pedestal, lower the arm and click the **Blank** button

   - Cuvette option (VWR mySPEC Twin only): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8,5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations

7. Enter a sample ID in the appropriate field, load the first sample as described for the blank above and click **Measure.**

After measuring:

- Simply wipe the upper and lower pedestals using a dry laboratory wipe so the instrument is ready to measure the next sample

- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples

Proteins and labels

Overview

Protein determination at 280 nm
Protein can be determined in the near UV at 280 nm due to absorption by tyrosine, tryptophan and phenylalanine amino acids. The Abs 280 varies greatly for different proteins due to their amino acid content and consequently the specific absorption value for a particular protein must be determined. The VWR mySPEC will accurately measure protein samples up to 100 pmol/µl (Cy3) and 20 mg/ml (BSA) respectively without dilution. The software automatically uses the optimal path length to measure the absorbance of each sample.

The **small sample volume** option is available when samples have 10 mm equivalent absorbance values of 3,0 or higher (>4,5 mg/ml BSA).

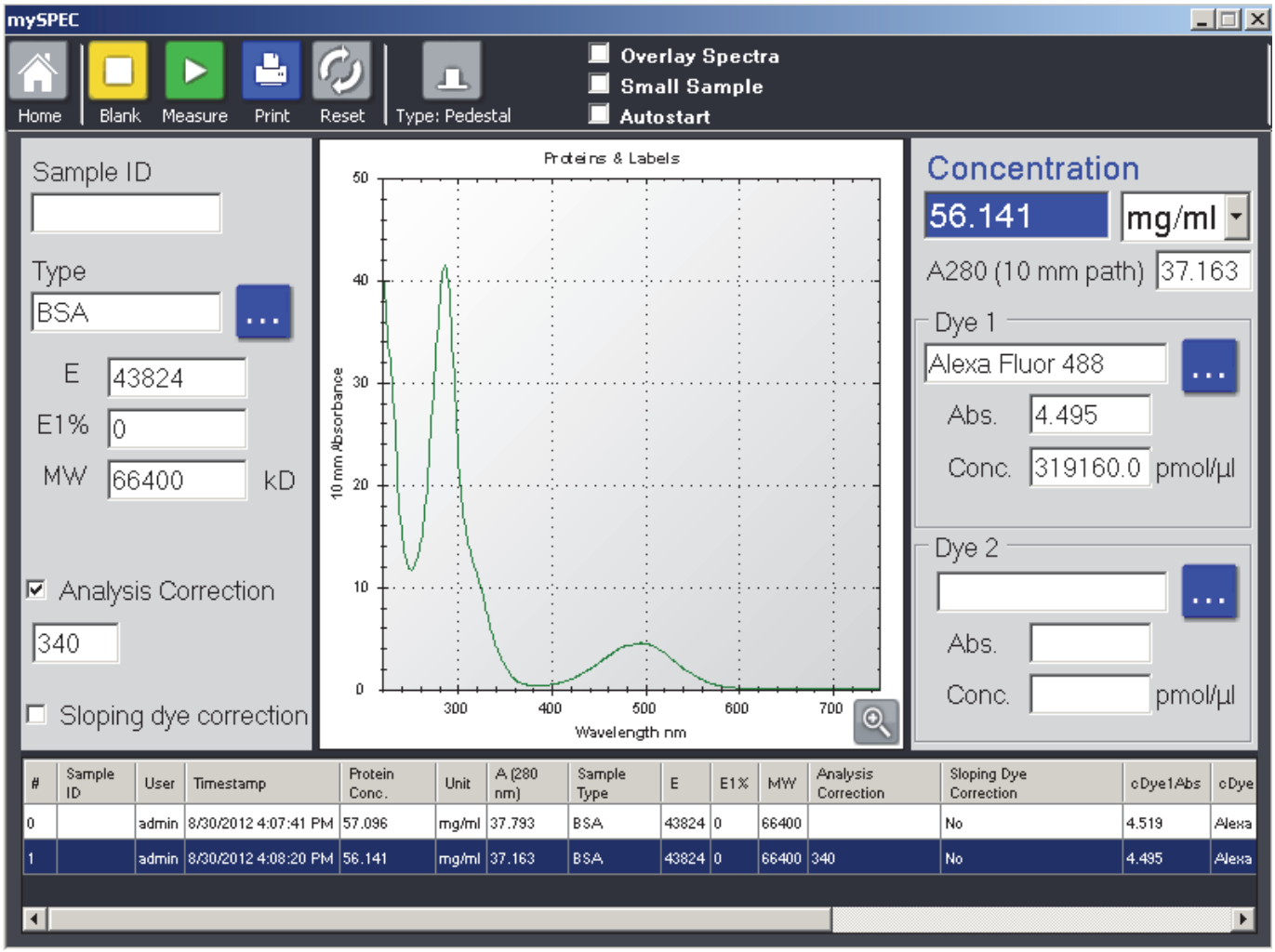
Sample volume requirements

The sample size is not critical for measurement, the volume can be changed to between 0,5 – 2 µl. For volume selection it is necessary that a liquid column forms after starting a measurement. Depending on the surface tension of the sample, different sample volumes are required. Additives like proteins can reduce the surface tension of the sample. It is recommended that you increase the sample volume to 2 µl to ensure liquid column formation if proteins are to be measured.

When using the cuvette option, it is essential that sufficient sample volume be used to ensure that the light path is passing through a representative portion of the sample. The optical beam (2 mm) is directed 8,5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

Main screen

- After selecting the measurement application, the lamp is automatically set to the correct intensity
- The following main screen will appear



The display shows results for the current sample normalised to a 10 mm path for all measurements.
The following features are on the screen:

Home	Goes back to the main menu.
Blank	Makes a blank measurement.
Measure	Makes a sample measurement.
Print	Prints results/screen.
Reset	Resets blank value.
	Enlarges the spectrum to maximum size

- **Sample ID:** Can be entered into this field

- **Type:** A drop down list from which the user can select or enter the extinction coefficient of the protein. This list can be extended by frequently used proteins.

Abs 1 = 1 mg/ml	A general reference setting based on a 0,1% (1 mg/ml) protein solution producing an absorbance at 280 nm of 1,0 A.
BSA	Bovine Serum Albumin reference. Unknown (sample) protein concentrations are calculated using the extinction coefficient of 43824 at 280 nm for a 1% BSA solution (MW = 66400 Da).
IgG	IgG reference. Unknown (sample) protein concentrations are calculated using the extinction coefficient of 210000 at 280 IgG solution (MW = 150000 Da).
Lysozyme	Lysozyme reference. Unknown (sample) protein concentrations are calculated using the extinction coefficient of 38940 at 280 nm Lysozyme solution (MW = 14300 Da).
Other proteins	For other reference proteins the user can enter; the molar extinction coefficient and the molecular weight of the protein, the mass extinction coefficient for a 1% solution or E1% value.

E = mass extinction

MW = molecular weight of the protein

E1% = extinction coefficient E1% (g/100 ml)-1 cm (equivalent to the inverse of the 10 mm absorbance of a 1% solution of the protein under test).

- **Analysis Correction:** If selected the "background noise" of the sample is normalised to 340 nm. The user can manually enter a different wavelength for the normalisation of the absorbance data.

- **Sloping Dye Correction:** When selected the software will subtract the value of a sloping baseline from 400 to 750 nm from the absorbance at the dye wavelength. This only affects the reported absorbance of the dye peak(s) and dye concentration(s).

- **Concentration:** Concentration based on absorbance at 280 nm and the selected extinction coefficient. Concentration units are chosen from the adjacent drop down list. The default selection is mg/ml.

- **A280 (10 mm path):** Absorbance at 280 nm for the protein sample being measured. The displayed absorbance value is normalised to a 10 mm path.

- **Dye 1 (or 2)**: Select the dye(s) that are bound to the proteins

  - **Abs** - the normalised absorbance of the respective dye at the 10 mm path length

  - **pmol/ µl** - is the concentration based upon the respective dye's extinction coefficient

Making proteins and labels measurements

1. Select the **Proteins & Label's** application from the main menu **Proteins** - ensure the arm is down**.**

2. Select the type of sample to be measured from the 'Type**'** drop down list.

3. Choose the concentration units from the drop down list adjacent to the colour coded box - default is mg/ml.

4. Use the drop down lists in the 'Dye 1' or' Dye 2' box to select the appropriate dye (or dyes). If the protein has been labelled with only one dye choose **None** as the dye type for 'Dye 2'.

5. A default wavelength of 340 nm is automatically used for bichromatic normalisation for the protein component of the sample. Select an alternative reference wavelength or choose not to have the spectrum normalised by deselecting **Analysis Correction**.

6. Select **Overlay Spectra** to display multiple spectra at the same time.

7. Select **Sloping Dye Correction 400-750 nm** if desired.

8. Establish a blank using an appropriate buffer. The reference or blank solution generally is the buffer that the molecule of interest is suspended or dissolved in. This solution should be the same pH and of a similar ionic strength as the sample solution.

9. Pedestal option: Pipette 2 µl of dH_2O onto the bottom pedestal, lower the arm and click the **Blank** button.

10. Cuvette option (VWR mySPEC Twin only): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8,5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

11. Enter a sample ID in the appropriate field, load the first sample as described for the blank above and click **Measure**.

After measuring:

- Simply wipe the upper and lower pedestals using a dry laboratory wipe so the instrument is ready to measure the next sample

- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples

Protein BCA

Overview

The BCA method is a colorimetric method for determining the total protein concentration in unpurified protein samples. The method depends on a reaction between cupric ions and amino acid residues. This assay combines the reaction with the enhancement of cuprous ion detection using bicinchoninic acid (BCA) as a ligand, giving an absorbance maximum at 562 nm.
The Protein BCA application requires a standard curve be generated before sample protein concentrations can be measured.

BCA assay measurement ranges

Commercial BCA protein kit manufacturers typically outline procedures for different protein concentration ranges:

- A regular assay uses a 20:1 reagent/sample volume ratio to detect a concentration range between ~0,20 to 8,0 mg/ml (BSA). When setting up an assay for pedestal measurements, a minimum sample volume of 4 µl in 80 µl of BCA reagent is recommended

- A mini assay uses a 1:1 reagent/sample volume ratio to detect a concentration range between 0,01 to 0,20 mg/ml (BSA). To prepare sufficient volume of these 1:1 mixtures for pedestal measurements, a minimum sample volume of 10 µl and 10 µl of BCA reagent (in a PCR tube) is recommended

Follow the assay kit manufacturer's recommendations for all standards and samples (unknown). Ensure each is subjected to the same timing and temperature throughout the assay.

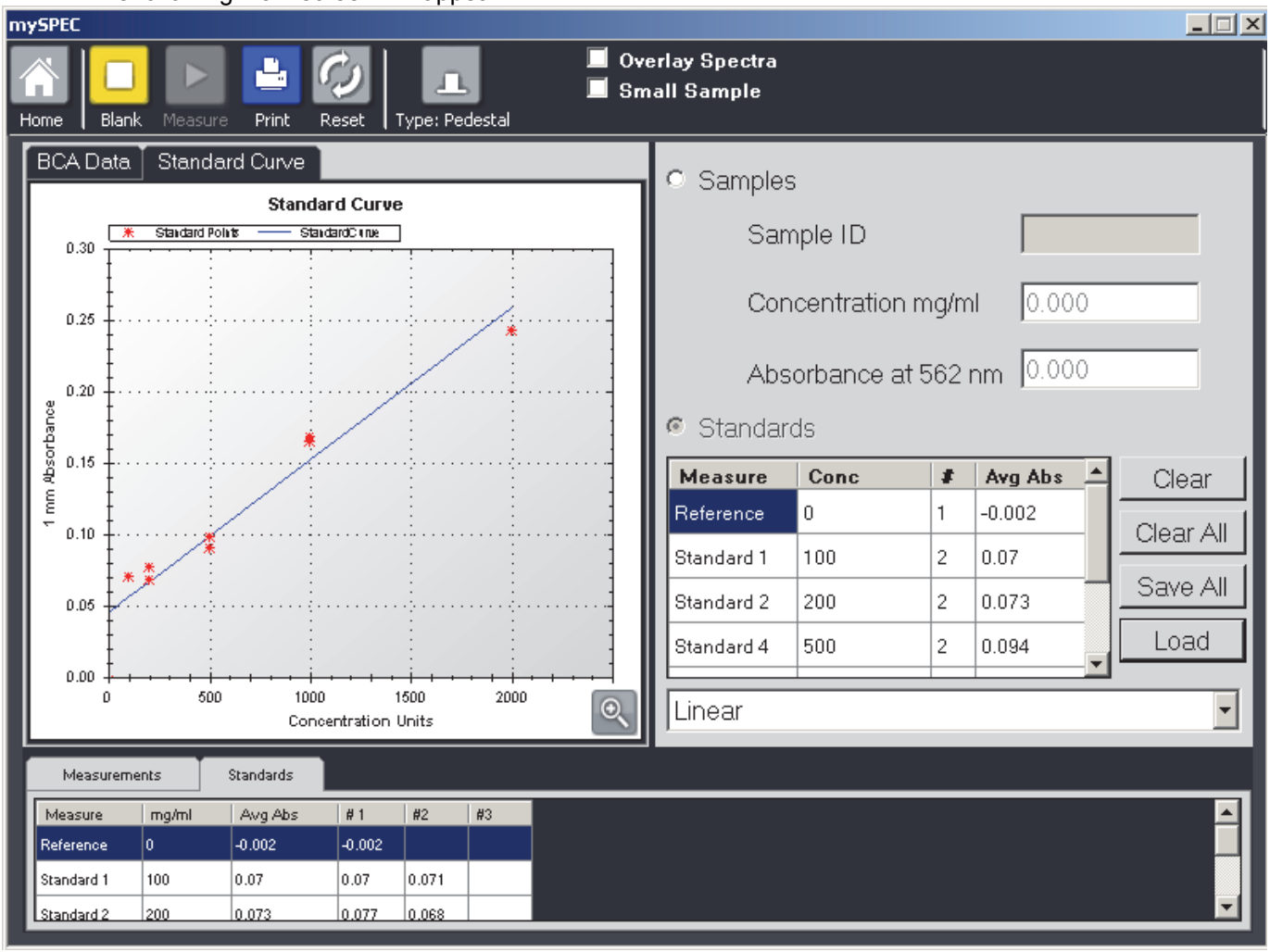
Sample volume requirements

The sample size is not critical for the measurement, the volume can be changed between 0,5 – 2 µl. For volume selection it is necessary that a liquid column forms after starting a measurement. Depending on the surface tension of the sample, different sample volumes are required. Additives like proteins can reduce the surface tension of a sample. It is recommended that you increase the sample volume to 2 µl to ensure liquid column formation if proteins are to be measured.

When using the cuvette option, it is essential that sufficient sample volume be used to ensure that the light path is passing through a representative portion of the sample. The optical beam (2 mm) is directed 8,5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

Main screen

- After selecting the measurement application, the lamp is automatically set to the correct intensity
- The following main screen will appear



The spectral display shows data for the current sample normalised to a 1 mm path if measured using the pedestal mode. The path length information is indicated on the Y axis.

	Goes back to the main menu.
	Makes a blank measurement.
	Makes a sample measurement.
	Prints results/screen.
	Resets blank value.
	Enlarges the spectrum to maximum size.

- **BCA Data tab** - displays current measurement data

- **Standard Curve tab -** displays the plot of the standard curve, the type of curve being displayed. Curve types include interpolation, linear and polynomials. The default selection is “linear”.

- **Samples button** - once a valid standard curve is measured the sample button will activate. Enter the sample ID, the concentration and absorbance at 562 nm will be displayed in the field.

- **Standards radio button** – enter the names and concentrations of standards when selected

BCA standard curves

A standard curve is required for BCA assays.

- A standard curve can be generated using a minimum of two points which may include two standards or a reference (BCA reagent/assay buffer only – no protein) and one standard

- The feature allows for multiple replicates for up to seven standards. Enter the standard concentration into the field and measure the absorbance. It is recommended you measure three replicates of each concentration.

- Standards must be entered before unknown samples are measured. Once the first sample is measured, no additional standards or replicates should be added

- A standard can be deleted at any time before a sample has been measured. Click into the results table and click on **Clear** to delete this standard. If you click on **Clear all** the whole table will be deleted.

- Click on **Save all** to save the standard curve

- Click on **Load** to load a previous standard curve (mss-File).

- It is recommended that users follow the protein assay manufacturer's guidelines for generating standard curves and create new standard curves for every measurement

Making BCA assay measurements

- Refer to the manufacturers' guidelines and recommendations for sample preparation

- A Zero Reference standard is a solution of the same buffer/dye reagent composition as the other standards and samples but without any protein added

- Prepare both standards and unknowns in the same manner. Use diluents of the same pH and ionic strength for all blanks, standards and unknown samples

- Standards diluted from a stock must cover the expected range of the unknown samples. Sample protein concentrations are not extrapolated beyond measured standard concentrations.

Procedure

1. Select the **Protein BCA** application from the **Protein** menu - ensure the arm is down.

2. Enter the values for each standard concentration in the right pane table. The software allows for the reference and up to 7 additional standards. The reference and/or standards can be measured in replicates.

3. Select **Overlay Spectra** to display multiple spectra at the same time.

4. Establish a blank using the appropriate buffer - the blank for colorimetric assays is generally dH_2O.

5. Pedestal option: Pipette 2 µl of dH_2O onto the bottom pedestal, lower the arm and click **Blank.**

6. Cuvette option (VWR mySPEC Twin only): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8,5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

7. Under the 'Standards' tab, highlight a standard and load as described for the blank above. Click **Measure** to initiate the measurement. Be sure that you measure all standards prior to measuring samples.

8. After all the standards have been measured, click on the **Samples** button. Enter a sample ID. Load 2 µl of sample when using the pedestal or insert a cuvette. Click **Measure** to initiate the measurement. It is not necessary to blank the instrument between the standard and the unknown sample measurements.

After measuring:

- Simply wipe the upper and lower pedestals using a dry laboratory wipe so the instrument is ready to measure the next sample

- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples

Protein Bradford

Overview

The Bradford method depends on quantifying the binding of a dye, Coomassie Brilliant Blue, to an unknown protein and comparing this binding to that of different, known concentrations of a standard protein at 595 nm and normalised at 750 nm, this is usually BSA (bovine serum albumin)
Like the other colorimetric assays, the Bradford assay requires a standard curve be generated before sample proteins can be measured.

Bradford assay measurement ranges

Commercial Bradford protein kit manufacturers typically outline procedures for different protein concentration ranges:

- A regular assay uses a 50:1 reagent/sample volume ratio to detect a concentration range between ~0,1 mg/ml to 8,0 mg/ml (BSA). The best linearity is in the 0,01 - 1 mg/ml range. When setting up an assay for pedestal measurements, a minimum sample volume of 4 µl in 200 µl of Bradford reagent is recommended

- A mini assay uses a 1:1 reagent/sample volume ratio to detect a concentration range between 15 – 125 µg/ml (BSA). To prepare sufficient volume of these 1:1 mixtures for pedestal measurements, a minimum sample volume of 10 µl and 10 µl of BCA reagent (in a PCR tube) is recommended.

Follow the assay kit manufacturer's recommendations for all standards and samples (unknown), ensuring they are subjected to the same timing and temperature throughout the assay.

Protein standards (BSA) for generating a standard curve can also be provided by the manufacturer for the Bradford assay.

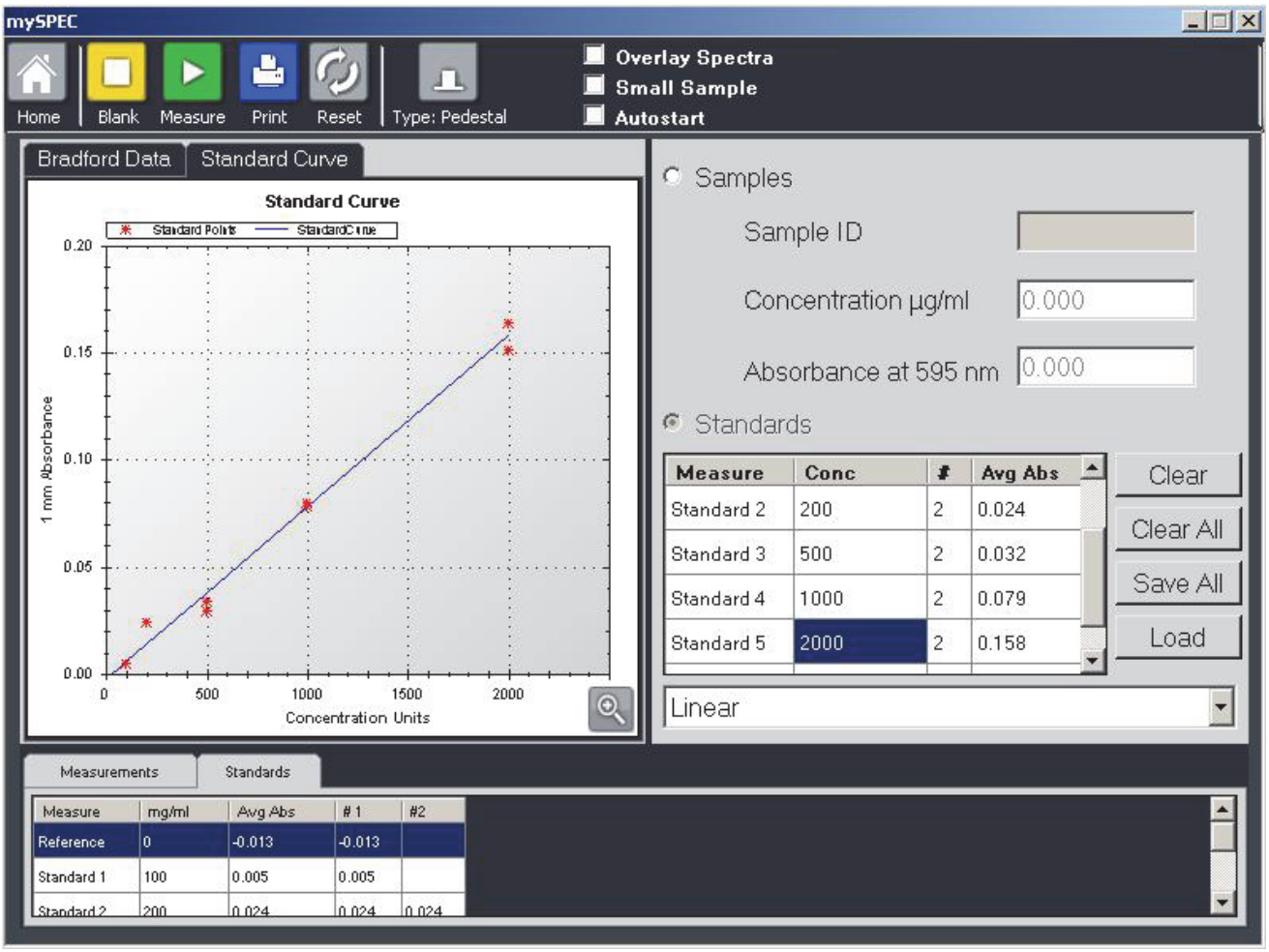
Sample volume requirements

The sample size is not critical for the measurement, the volume can be changed between 0,5 – 2 µl. For the volume selection it is necessary that a liquid column forms after starting a measurement. Depending on the surface tension of the sample, different sample volumes are required. Additives like proteins can reduce the surface tension of the sample. It is recommended that you increase the sample volume to 2 µl to ensure liquid column formation if proteins are to be measured.

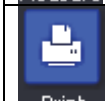
When using the cuvette option, it is essential that sufficient sample volume be used to ensure that the light path is passing through a representative portion of the sample. The optical beam (2 mm) is directed 8,5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

Main screen

- After selecting the measurement application, the lamp is automatically set to the correct intensity
- The following main screen will appear



The spectral display shows data for the current sample normalised to a 1 mm path if measured using the pedestal mode or the path length selected if using the cuvette option (VWR mySPEC Twin).

	Goes back to the main menu.
	Makes a blank measurement.
	Makes a sample measurement.
	Prints results/screen.
	Resets blank value.
	Enlarges the spectrum to maximum size

- **Bradford Data tab** - displays current measurement data

- **Standard Curve tab -** displays the plot of the standard curve, the type of curve being displayed. Curve types include interpolation, linear and polynomials. The default selection is “linear”.

- **Samples button** - once a valid standard curve is measured the sample button will be activated. Enter the sample ID, the concentration and absorbance at 595 nm will be displayed in the field.

- **Standards button** - enter the names and concentrations of standards when selected

Bradford standard curves

A standard curve is required for a Bradford assays.

- A standard curve can be generated using a minimum of two points which may include two standards or a reference (BCA reagent/assay buffer only – no protein) and one standard

- The feature allows for multiple replicates for up to seven standards. Enter the standard concentration into the field and measure the absorbance. It is recommended that you measure three replicates of each concentration.

- Standards must be entered before unknown samples are measured. Once the first sample is measured, no additional standards or replicates should be added.

- A standard can be deleted at any time before a sample has been measured. Click into the results table and click on **Clear** to delete this standard. If you click on **Clear all**, the whole table is deleted.

- Click on **Save all** to save the standard curve

- Click on **Load** to load a previous standard curve (mss-File)

- It is recommended that the user follow the protein assay manufacturer's guidelines for generating standard curves and creates new standard curves for every measurement

Making Bradford assay measurements

- Refer to the manufacturers' guidelines and recommendations for sample preparation

- The Zero Reference standard is a solution of the same buffer/dye reagent composition as the other standards and samples but without any protein added

- Prepare both standards and unknowns in the same manner. Use a diluent of the same pH and ionic strength for all blanks, standards and unknown samples.

- Standards diluted from a stock must cover the expected range of the unknown samples. Sample protein concentrations are not extrapolated beyond measured standard concentrations.

Procedure

1. Select the **Protein Bradford** application from the **Protein** menu - ensure the arm is down.

2. Enter the values for each standard concentration in the right pane table. The software allows for the reference and up to 7 additional standards. The reference and/or standards can be measured in replicates.

3. Select **Overlay Spectra** to display multiple spectra at the same time.

4. Establish a blank using the appropriate buffer - the blank for colorimetric assays is generally dH_2O.

   - Pedestal option: Pipette 2 µl of dH_2O onto the bottom pedestal, lower the arm and click **Blank**

   - Cuvette option (VWR mySPEC Twin only): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8,5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

5. Under the 'Standards' tab, highlight a standard and load as described for the blank above. Click **Measure** to initiate the measurement. Be sure that you measure all standards prior to measuring samples.

6. After all of the standards have been measured, click on the **Samples** button. Enter a sample ID. Load 2 µl of sample when using the pedestal or insert a cuvette. Click **Measure** to initiate the measurement. It is not necessary to blank the instrument between the standard and the unknown sample measurements.

After measuring:

- Simply wipe the upper and lower pedestals using a dry laboratory wipe so the instrument is ready to measure the next sample
- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples

Protein Lowry

Overview

The 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 650 nm and normalised at 405 nm; this is usually BSA.
The Lowry assay is an alternative method for determining protein concentration based on the widely used and cited Lowry procedure for protein quantification. Like the other colorimetric assays, the Lowry assay requires a standard curve be generated before sample proteins can be measured.

Lowry assay measurement range

To accurately prepare standards, it is recommended that a 20 µl minimum sample volume and 100 µl of Lowry reagent be used for each reaction. On the VWR mySPEC the Lowry assay can be used to measure samples from ~0,20 to 4 mg/ml.

Follow the assay kit manufacturer's recommendations for all standards and samples (unknown). Ensure each is subjected to the same timing and temperature throughout the assay.

Protein standards (BSA) for generating a standard curve can also be provided by the manufacturer for the Lowry assay.

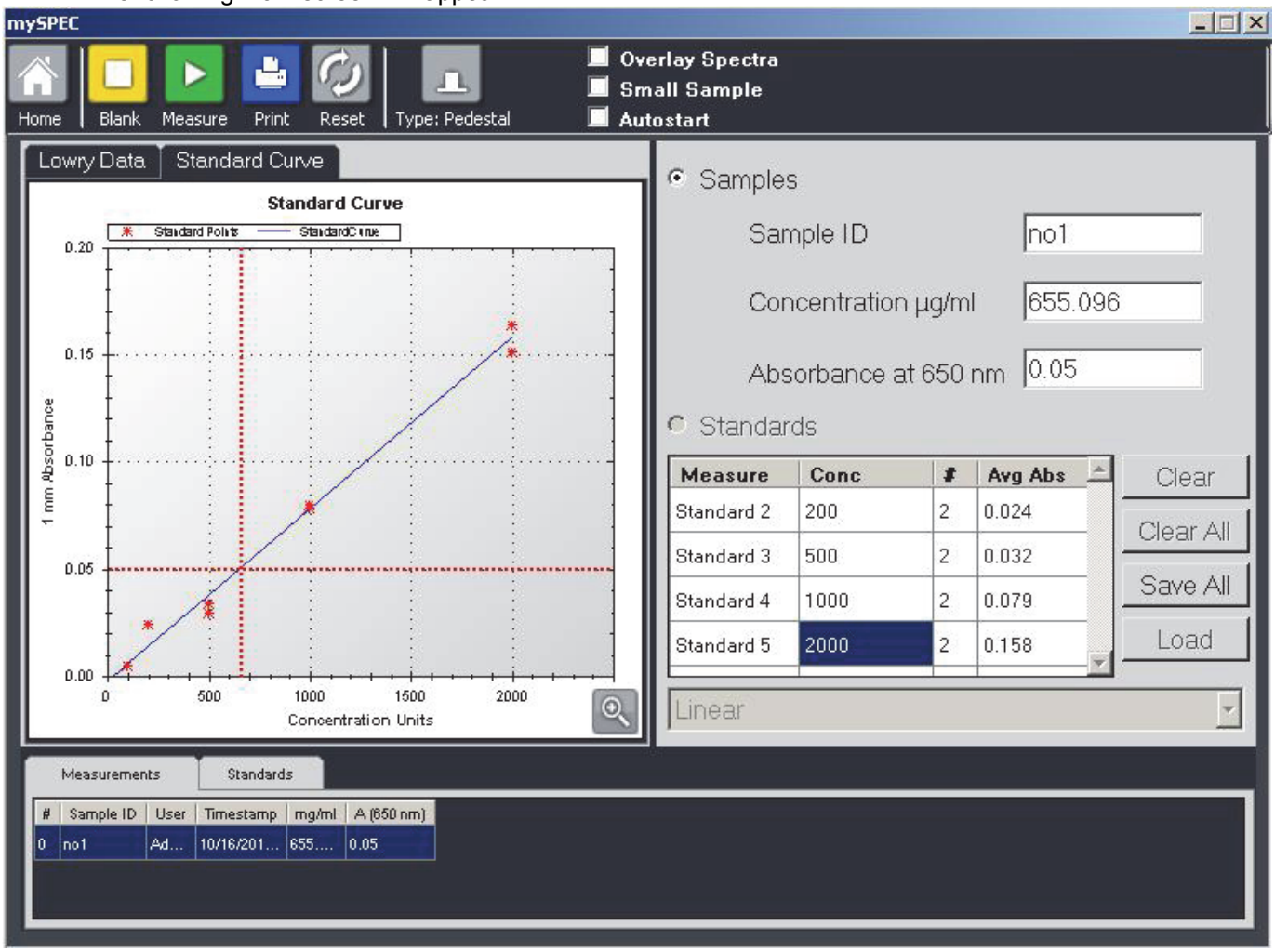
Sample volume requirements

Sample size is not critical for measurement, the volume can be changed between 0,5 – 2 µl. For the volume selection it is necessary that a liquid column forms after starting a measurement. Depending on the surface tension of the sample, different sample volumes are required. Additives like proteins can reduce the surface tension of the sample. It is recommended that you increase the sample volume to 2 µl to ensure liquid column formation if proteins are to be measured.

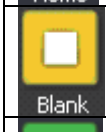
When using the cuvette option, it is essential that sufficient sample volume be used to ensure that the light path is passing through a representative portion of the sample. The optical beam (2 mm) is directed 8,5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

Main screen

- After selecting the measurement application, the lamp is automatically set to the correct intensity
- The following main screen will appear



The spectral display shows data for the current sample normalised to a 1 mm path if measured using the pedestal mode or the path length selected if using the cuvette option (VWR mySPEC Twin only).

	Goes back to the main menu.
	Makes a blank measurement.
	Makes a sample measurement.
	Prints results/screen.
	Resets blank value.
	Enlarges the spectrum to maximum size.

- **Lowry Data tab** - displays current measurement data

- **Standard Curve tab -** displays the plot of the standard curve, the type of curve being displayed. Curve types include interpolation, linear and polynomials. The default selection is “linear”.

- **Samples button** - once a valid standard curve is measured the sample button will activate. Enter the sample ID and the concentration and absorbance at 650 nm will be displayed in the field.

- **Standards Radio button** – enter the names and concentrations of standards when selected

Lowry standard curves

A standard curve is required for Lowry assays.

- A standard curve can be generated using a minimum of two points which may include two standards or a reference (BCA reagent/assay buffer only – no protein) and one standard

- The feature allows for multiple replicates for up to seven standards. Enter the standard concentration into the field and measure the absorbance. It is recommended that you measure three replicates of each concentration.

- Standards must be entered before unknown samples are measured. Once the first sample is measured, no additional standards or replicates should be added.

- A standard can be deleted at any time before a sample has been measured. Click into the results table and click on **Clear** to delete this standard. If you click on **Clear all**, the whole table is deleted.

- Click on **Save all** to save the standard curve

- Click on **Load** to load a previous standard curve (mss-File)

- It is recommended that users follow the protein assay manufacturer's guidelines for generating standard curves and creates new standard curves for every measurement

Making Lowry assay measurements

- Refer to the manufacturers' guidelines and recommendations for sample preparation

- The Zero Reference standard is a solution of the same buffer/dye reagent composition as the other standards and samples but without any protein added

- Prepare both standards and unknowns in the same manner. Use a diluent of the same pH and ionic strength for all blanks, standards and unknown samples.

- Standards diluted from a stock must cover the expected range of the unknown samples. Sample protein concentrations are not extrapolated beyond measured standard concentrations.

Procedure

1. Select the **Protein Lowry** application from the **Protein** menu - ensure the arm is down.

2. Enter the values for each standard concentration in the right pane table. The software allows for the reference and up to 7 additional standards. The reference and/or standards can be measured in replicates.

3. Select **Overlay Spectra** to display multiple spectra at the same time.

4. Establish a blank using the appropriate buffer - the blank for colorimetric assays is generally dH_2O.

5. Pedestal option: Pipette 2 µl of dH_2O onto the bottom pedestal, lower the arm and click **Blank.**

6. Cuvette option (VWR mySPEC Twin only): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8,5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

7. Under the 'Standards' tab, highlight a standard and load as described for the blank above. Click **Measure** to initiate the measurement. Be sure that you measure all standards prior to measuring samples.

8. After all of the standards have been measured, click on the **Samples** button. Enter a sample ID. Load 2 µl of sample when using the pedestal or insert a cuvette. Click **Measure** to initiate the measurement. It is not necessary to blank the instrument between the standard and the unknown sample measurements.

After measuring:

- Simply wipe the upper and lower pedestals using a dry laboratory wipe so the instrument is ready to measure the next sample
- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples

Protein Pierce 660 nm

Overview

The Protein Pierce 660 nm assay reagent is a ready to use formulation that offers rapid, accurate and reproducible colorimetric detection of minute amounts of protein in solution. The reagent is ideal for measuring total protein concentration in samples containing reducing agents and/or detergents.

Protein Pierce 660 nm reagent and protocols

This protocol uses a 15:1 reagent/sample volume ratio. When setting up an assay for pedestal measurements, a minimum sample volume of 4 µl in 60 µl of reagent is recommended.

Follow the assay kit manufacturer's recommendations for all standards and samples (unknowns), ensuring they are subjected to the same timing and temperature throughout the assay.

Protein standards (BSA) for generating a standard curve can also be provided by the manufacturer for the Pierce 660 nm assay.

Pierce 660 nm assay measurement range

When using a dilution of 4 µl of sample in 60 µl of reagent, the Protein Pierce 660 nm assay concentration range of detection is 50 to 125 µg/ml.

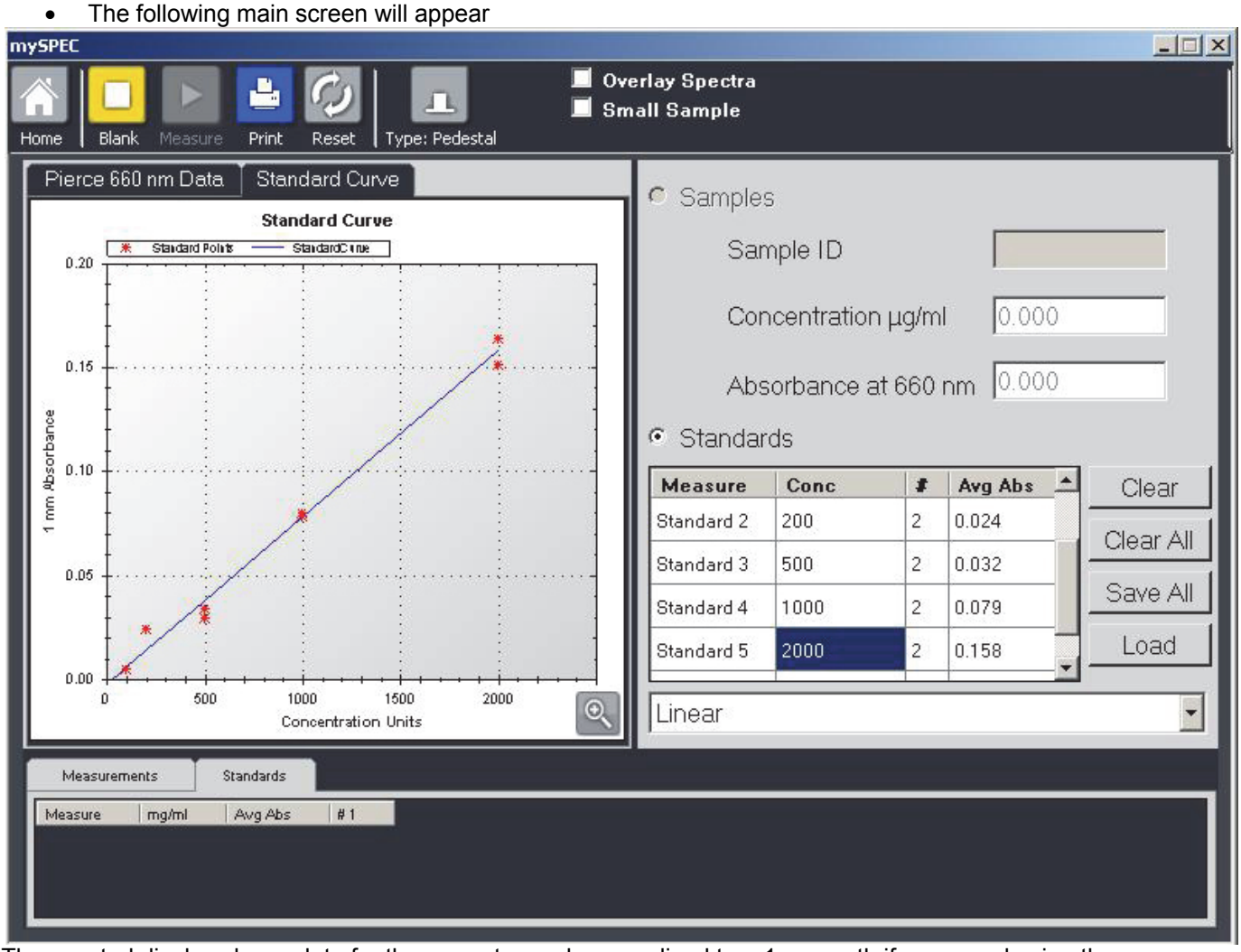
Sample volume requirements

The sample size is not critical for the measurement, the volume can be changed between 0,5 – 2 µl. For the volume selection it is necessary that a liquid column forms after starting a measurement. Depending on the surface tension of the sample, different sample volumes are required. Additives like proteins can reduce the surface tension of the sample. It is recommended that you increase the sample volume to 2 µl to ensure liquid column formation if proteins are to be measured.

When using the cuvette option, it is essential that sufficient sample volume be used to ensure that the light path is passing through a representative portion of the sample. The optical beam (2 mm) is directed 8,5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

Main screen

- After selecting the measurement application the lamp is automatically set to the correct intensity
- The following main screen will appear



The spectral display shows data for the current sample normalised to a 1 mm path if measured using the pedestal mode or the path length selected if using the cuvette option (VWR mySPEC Twin only).

	Goes back to the main menu.
	Makes a blank measurement.
	Makes a sample measurement.
	Prints results/screen.
	Resets blank value.
	Enlarges the spectrum to maximum size.

- **Pierce 660 nm Data tab** - displays current measurement data

- **Standard Curve tab** - displays the plot of the standard curve, the type of curve being displayed. Curve types include interpolation, linear and polynomials. The default selection is “linear”.

- **Samples button** - once a valid standard curve is measured the sample button is activated. Enter the sample ID, the concentration and absorbance at 650 nm will be displayed in the field.

- **Standards button** – enter the names and concentrations of standards when selected

Pierce 660 nm standard curves

A standard curve is required for Pierce 660 nm assays.

- A standard curve can be generated using a minimum of two points which may include two standards or a reference (BCA reagent/assay buffer only – no protein) and one standard

- This feature allows for multiple replicates for up to seven standards. Enter the standard concentration into the field and measure the absorbance. It is recommended that you measure three replicates of each concentration.

- Standards must be entered before unknown samples are measured. Once the first sample is measured, no additional standards or replicates should be added.

- A standard can be deleted at any time before a sample has been measured. Click into the results table and click on **Clear** to delete this standard. If you click on **Clear all**, the whole table is deleted.

- Click on **Save all** to save the standard curve

- Click on **Load** to load a previous standard curve (mss-File)

- It is recommended that users follow the protein assay manufacturer's guidelines for generating standard curves and create new standard curves for every measurement

Making Pierce 660 nm assay measurements

- Refer to the manufacturers' guidelines and recommendations for sample preparation

- The Zero Reference standard is a solution of the same buffer/dye reagent composition as the other standards and samples but without any protein added

- Prepare both standards and unknowns in the same manner. Use diluents of the same pH and ionic strength for all blanks, standards and unknown samples.

- Standards diluted from a stock must cover the expected range of the unknown samples. Sample protein concentrations are not extrapolated beyond measured standard concentrations.

Procedure

1. Select the **Protein Pierce 660 nm** application from the **Protein** menu - ensure the arm is down.

2. Enter the values for each standard concentration in the right pane table. The software allows for the reference and up to 7 additional standards. The reference and/or standards can be measured in replicates.

3. Select '**Overlay Spectra'** to display multiple spectra at the same time

4. Establish a blank using the appropriate buffer - the blank for colorimetric assays is generally dH_2O

   - Pedestal option: Pipette 2 µl of dH_2O onto the bottom pedestal, lower the arm and click **Blank**

   - Cuvette Option (VWR mySPEC Twin only): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8,5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations

5. Under the 'Standards' tab, highlight a standard and load as described for the blank above. Click '**Measure'** to initiate the measurement. Be sure that you measure all standards prior to measuring samples

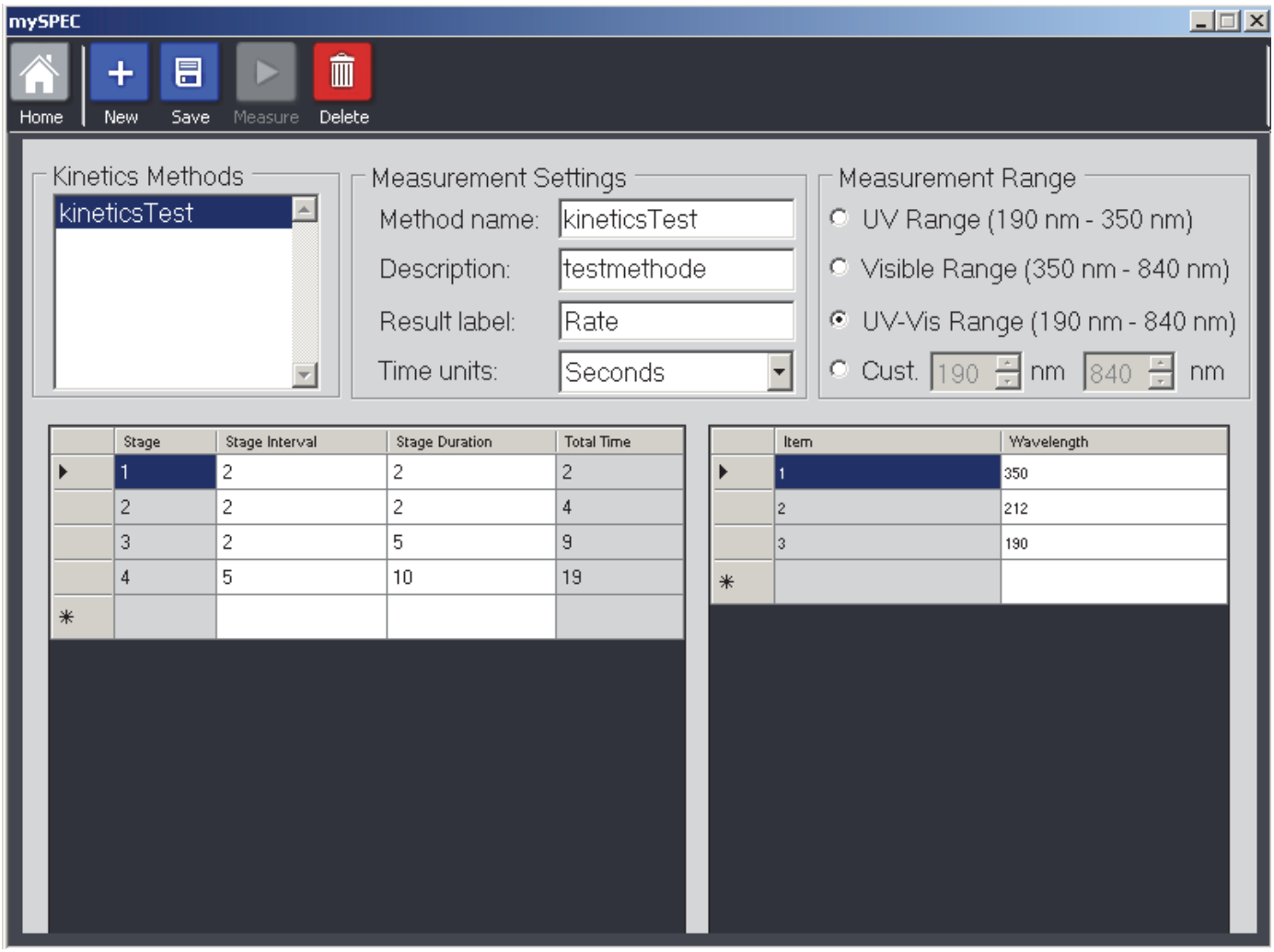
6. After all of the standards have been measured, click on the '**Samples'** button. Enter a sample ID. Load 2 µl of sample when using the pedestal or insert a cuvette. Click' **Measure'** to initiate the measurement. It is not necessary to blank the instrument between the standard and unknown sample measurements

After measuring:

- Simply wipe the upper and lower pedestals using a dry laboratory wipe so the instrument is ready to measure the next sample
- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples

Kinetics Editor

The VWR mySPEC can be used to make time-based kinetic measurements. The ‘Kinetics Editor’ can be used to create, edit, save and view custom kinetic methods. The ‘Kinetics Editor’ is located at Main Menu/Editors/Kinetic Editor Measurements which can also be made without saving a method. The software does not support kinetic measurements using the pedestal mode.



Screen features

When the **Kinetics Editor** is selected, the following icons are displayed

	Creates a new custom method.
	Saves the current method definition.
	Starts a kinetics method measurement. The kinetics method does not need to be saved prior to running. This icon will be greyed out until the minimum setting requirements for a new method have been entered.
	Deletes a kinetics method. This icon will be greyed out until the minimum setting requirements for a new method have been entered

Kinetic methods

Select displayed method to change or edit functions in this method. The **Measurement Settings** and **Measurement Range** will be shown on the screen.

Creating a new kinetic method

- Click on **New** to create a new method
- Insert the measurement settings and measurement range (spectral display wavelength range: UV, Visible, UV/Vis or custom) onto the screen

Two screen panels are used to define a method:

- **Measure Stage** – defines measurement stage intervals and the duration of each stage. To add the first stage, enter a value in the highlighted **Stage Interval** field. Next, click on and enter a value in the highlighted **Stage Duration** field. Click **Enter** and cumulative time will auto-populate. The next row will now be available for additional stage entries. The minimum stage interval allowed is 2,0 seconds.

- **Wavelengths to Monito**r - monitors multiple wavelengths during the reaction. To add the first wavelength, highlight the empty field and enter a value. The next row will become available for additional wavelength selections.

  - A cursor (coloured vertical line during the measurement) will be positioned at each of the monitored wavelengths. These cursors can be moved and the associated changes reflected in the rate display.

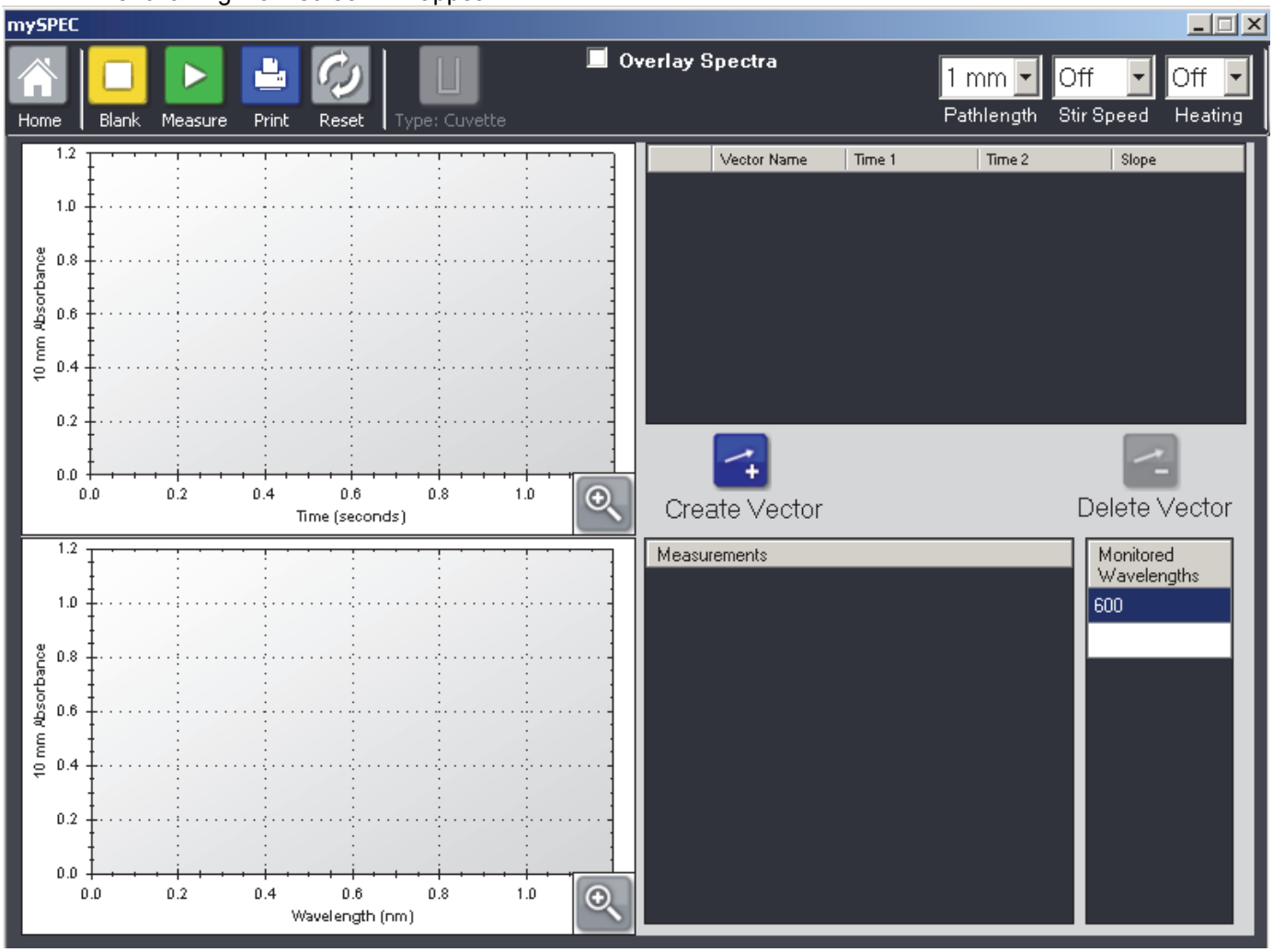
  - Wavelengths can be removed by selecting the wavelength and delete the value

A kinetics method can either be saved or immediately run without saving. To save a kinetics method, click on the **Save-Button**.

To start the kinetics measurement, click on the **Measure-Button** or open the ‘Kinetic Method’ from the ‘Main Menu’ (Further Method/Kinetics).

Measure kinetic measurements

- After selecting the kinetic application the lamp is automatically set to the correct intensity
- The following main screen will appear



There are two graphical displays in the right pane of the kinetics screen:

- The wavelength table on the top of the display shows the absorbance values at each stage of the measurement for all of the wavelengths being monitored

  - The spectrum plot at the bottom of the display shows a spectra for each measurement taken at each stage. The data is displayed with absorbance as the vertical axis and wavelength as the horizontal axis. Each spectrum in the display is colour coded. The time and date each spectrum was collected is shown to the right of the spectrum plot area using the same colour as the associated spectrum. The coloured vertical line crosshairs correspond to the wavelengths the method was initially set to monitor.

- The right display allows rate vectors to be calculated for selected portions of associated data curves. A data curve is the series of absorbance values at the wavelength selected in the spectrum plot area at each stage of the measurement.

Calculating a slope

1. Click on **Create Vector**.
2. Add the wavelength of interest onto the screen.
3. Enter the initial and end time point of interest.
4. Click on the **Create-Button** to calculate vector and slope - the vector will be displayed in the upper screen.

Making kinetic measurements

1. Select a kinetics method from the main menu (Further Method/Kinetics). Alternatively, choose **Measure** from the **'**Kinetics Editor' application.

2. Select the cuvette path length - the default selection is 10 mm.

3. Select the temperature by clicking on **Heating** and select a temperature between 30 – 40 °C. Allow the cuvette holder to reach temperature before proceeding.

   - It may take some minutes for the cuvette holder to reach temperature

4. Select **Stir Speed** if stirring is required. There are two speed values available (slow and fast).

5. Establish a blank using the appropriate buffer. Insert a cuvette with the blank solution into the instrument, noting the direction of the light path indicated by the etched arrow and choose **Blank**.

6. Insert a cuvette with the sample and choose **Measure.**

   - The optical beam (2 mm) is directed 8,5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

7. After the measuring the software will ask for a file name and storage location.

8. Remove the cuvette, and follow the recommendations of each specific cuvette manufacturer for cleaning and maintenance.

Cell Cultures

Overview

With the ‘Cell Cultures’ method, absorbance turbidity can be measured.

It is important to know that for exact measurements a long path length (1 cm) is required and the cell type is in suspension. It is recommended to do cell measurements with cuvettes and not with the pedestal mode.

The ‘Cell Cultures’ application displays the sample spectrum from 250 to 700 nm.

Sample size requirements

The sample size is not critical for the measurement, the volume can be changed between 0,5 – 2 µl. For the volume selection it is necessary that a liquid column forms after starting a measurement. Depending on the surface tension of the sample, different sample volumes are required. Additives like proteins can reduce the surface tension of the sample. It is recommended that you increase the sample volume to 2 µl to ensure liquid column formation if proteins are to be measured.

When using the cuvette option, it is essential that sufficient sample volume be used to ensure that the light path is passing through a representative portion of the sample. The optical beam (2 mm) is directed 8,5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

Sample homogeneity

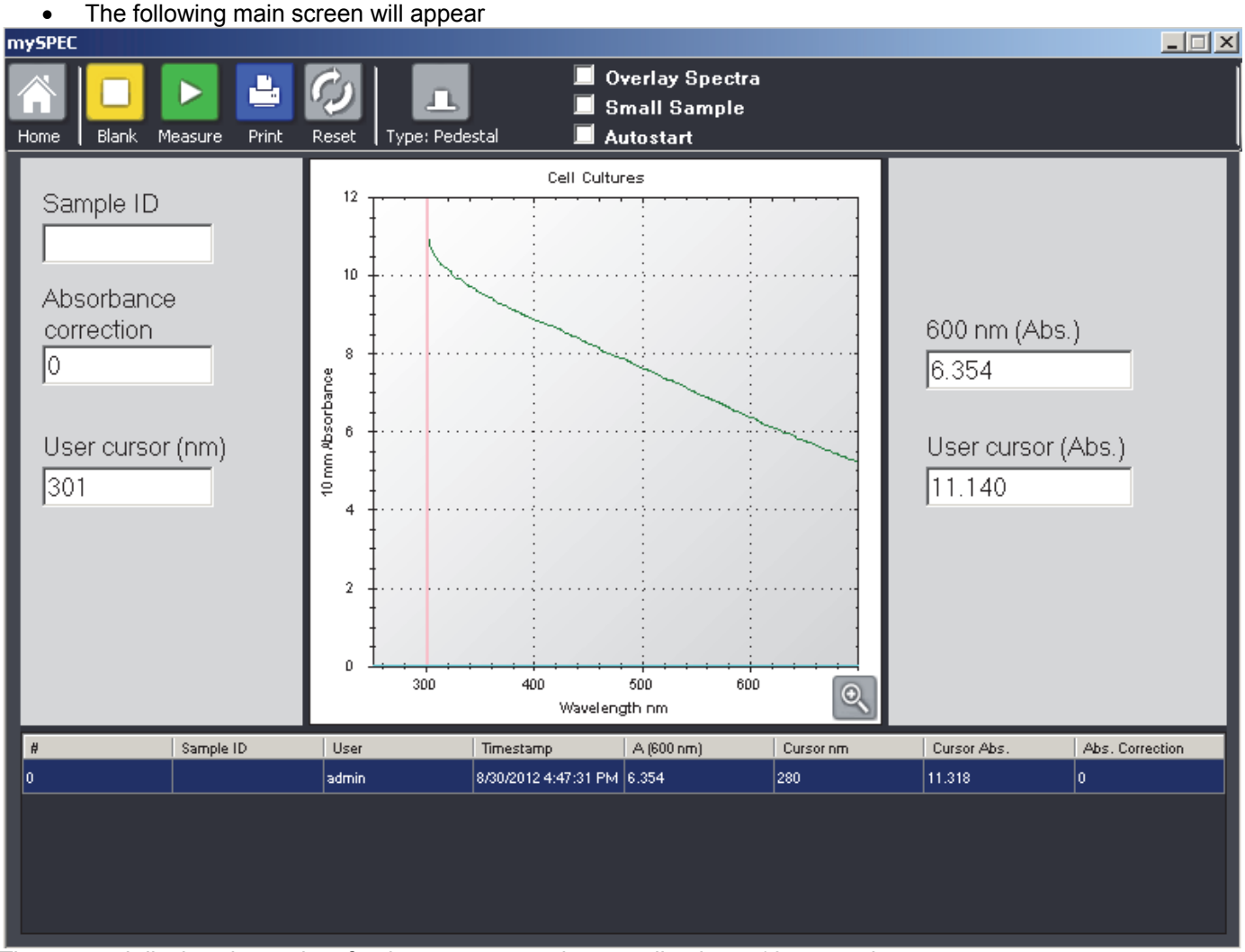
Ensure that the cell suspension is homogeneous before removing an aliquot for its “absorbance” measurement. Vigorous mixing may be required before measuring aliquots of concentrated samples on the measurement pedestals. Use the stirring capability when making microbial cell culture cuvette measurements.

Measurement range

Due to its shorter path lengths, the VWR mySPEC can directly monitor concentrated cell suspensions.
Dilute microbial cell cultures if using the cuvette mode.

Main screen

- After selecting the 'Cell Cultures' method ('Further Method') the lamp is automatically set to the correct intensity
- The following main screen will appear



The spectral display shows data for the current sample normalised to a 10 mm path.

	Goes back to the main menu.
	Makes a blank measurement.
	Makes a sample measurement.
	Prints results/screen.
	Resets blank value.
	Enlarges the spectrum to maximum size.

- **Sample ID** – Enter the name of the sample
- **Absorbance Correction –** user-defined baseline cursor value

- **User Cursor (nm)** – user-defined cursor position. The cursor **can** be moved by selecting it and horizontally dragging to a different position or by entering the desired wavelength.

- **600 nm (Abs)** – 600 nm absorbance value, as measured from the user-defined baseline

- **User cursor (Abs)** – absorbance value at the user-defined wavelength, as measured from the user-defined baseline

Making cell culture measurements

1. Select the '**Cell Cultures'** application from the main menu - ensure the arm is down.

2. Select '**Overlay Spectra'** to display multiple spectra at the same time.

3. Establish a blank using the appropriate buffer. The reference or blank solution generally is the buffer that the molecule of interest is suspended or dissolved in. This solution should be the same pH and of a similar ionic strength as the sample solution.

   - Pedestal option: Pipette 2 µl of the appropriate blanking solution onto the bottom pedestal and click **Blank**

   - Cuvette option: Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8,5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

4. Enter a sample ID in the appropriate field, load the first sample as described for the blank above and click **Measure.**

After measuring:

- Simply wipe the upper and lower pedestals using a dry laboratory wipe so the instrument is ready to measure the next sample

- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples

UV/Vis

Overview

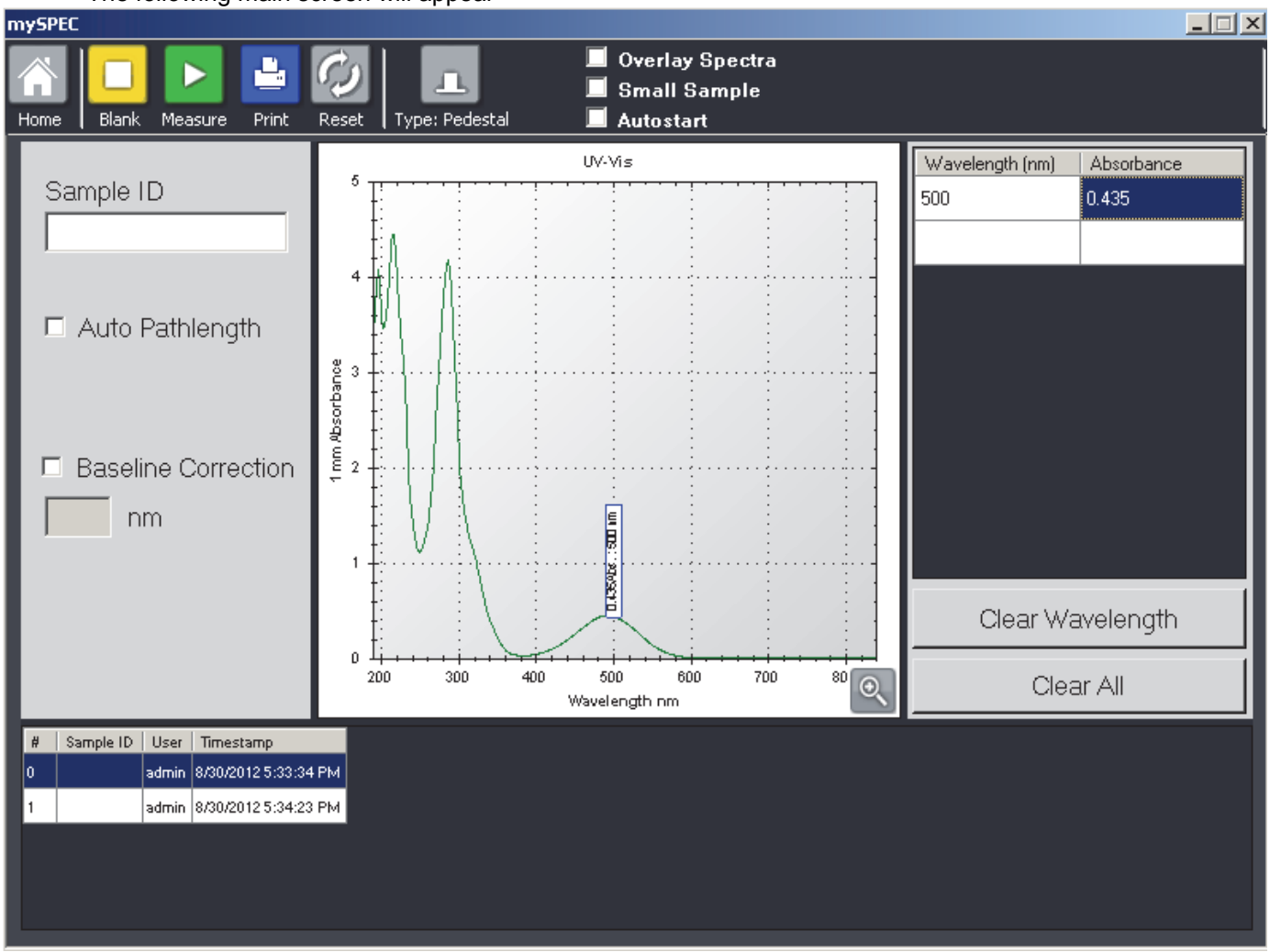
The UV/Vis application allows measurements known from conventional spectrophotometer. Sample absorbance is displayed on the screen from 190 to 840 nm.

Measurement concentration ranges

The VWR mySPEC can measure samples with 10 mm path length absorbance equivalent of 300 A when using the auto path length feature.

Main screen

- After selecting the 'UV/Vis' method (Further Method) the lamp is automatically set to the correct intensity
- The following main screen will appear



The spectral display shows data for the current sample normalized to a 10 mm path length.

Home	Goes back to the main menu.
Blank	Makes a blank measurement.
Measure	Makes a sample measurement.

	Prints results/screen.
	Resets blank value.
	Enlarges the spectrum to maximum size.

The following features are to the right of the spectral display:

- **Sample ID -** enter the name of the sample
- **Auto Path Length** – automatically allows the software to use the optimal pedestal path length for high concentration samples. If selected, the shorter path length will be used when any wavelength between 220 to 840 nm has 1 mm equivalent absorbance value of 1,25 or higher.
- **Baseline Correction -** allows for specification of a wavelength for the bichromatic normalisation of absorbance data for the entire spectrum. If the baseline correction is not used, the spectra can be offset from the baseline.
- **Wavelength -** adds cursor wavelength and cursor absorbance to the table
- **Clear wavelength –** clears one entry from the wavelength table
- **Clear all –** clears all entries from the wavelength table

Making UV/Vis measurements

1. Select the 'UV/Vis' application from the main menu - ensure the arm is down.
2. Select a reference wavelength or choose not to have the spectrum normalised by deselecting the **Baseline Correction** box.
3. Enter the wavelengths to monitor in the absorbance **nm**. Select the first row, enter the wavelength. The next row will now be highlighted and additional wavelengths can be added as above.
4. Select **Overlay Spectra** to display multiple spectra at the same time.
5. Establish a blank using the appropriate buffer. The blank solution generally is the buffer that the molecule of interest is suspended or dissolved in. This solution should be the same pH and of a similar ionic strength as the sample solution.
   - Pedestal option: Pipette 1 - 2 µl of the appropriate blanking solution onto the bottom pedestal, lower the arm and click **Blank**
   - Cuvette option (Model 2000c only): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8,5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations
6. Enter a sample ID in the appropriate field, load the first sample as described for the blank above and click **Measure**.

After measuring:

- Simply wipe the upper and lower pedestals using a dry laboratory wipe so the instrument is ready to measure the next sample
- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples.

User Defined

Overview

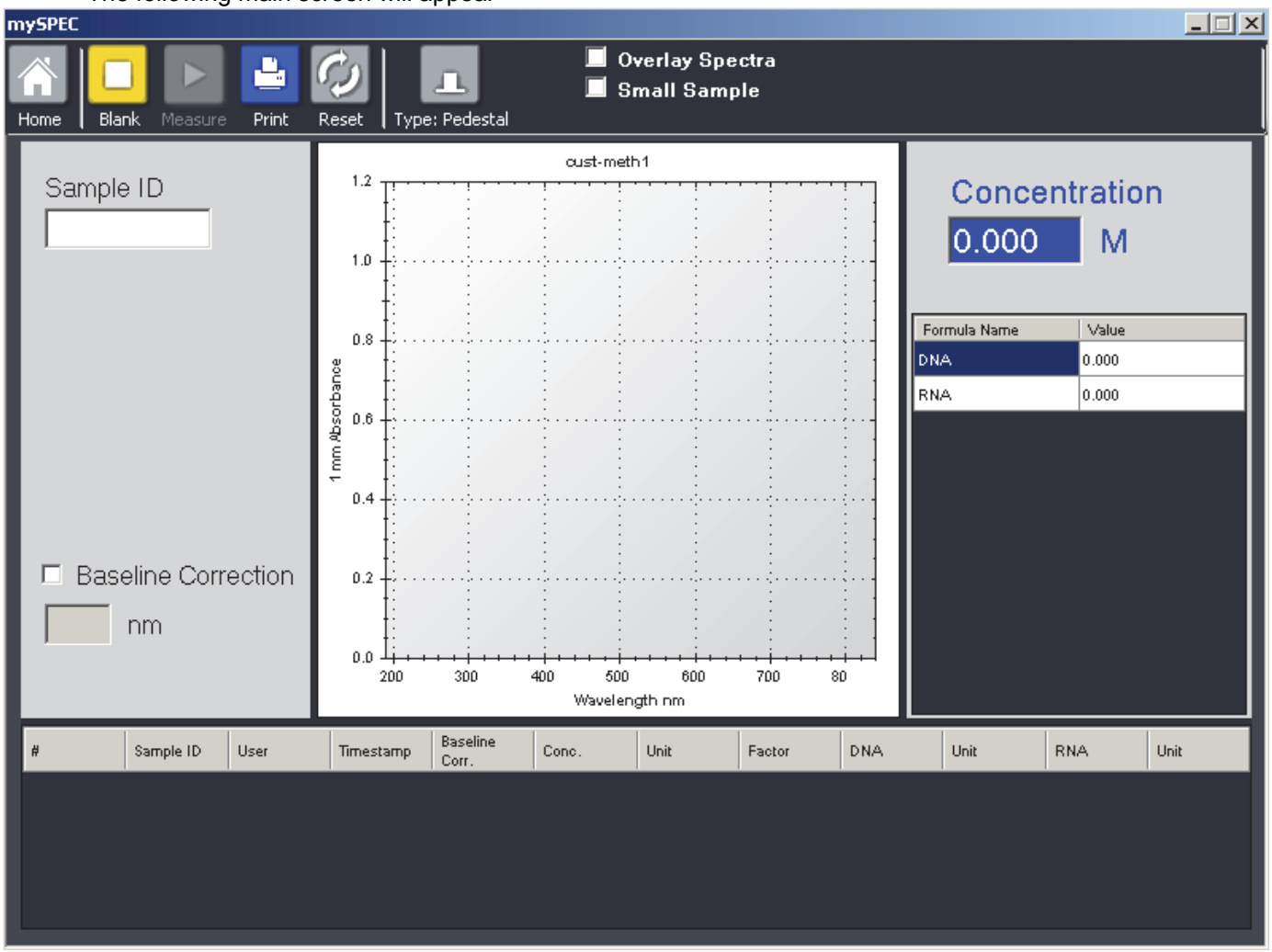
The ‘User Defined Method’ allows measurements known from a conventional spectrophotometer, or used to create, edit, save and view custom methods generated in the ‘Method Editor’ (see Method Editor).

Measurement concentration ranges

The VWR mySPEC can measure samples with 10 mm path length absorbance equivalent of 300 A.

Main screen

- After selecting the ‘User Defined Method’ the lamp is automatically set to the correct intensity – then select the ‘Module’ you want to use from list
- The following main screen will appear



Home	Goes back to the main menu.
Blank	Makes a blank measurement.
Measure	Makes a sample measurement.
Print	Prints results/screen.

	Resets blank value.
	Enlarges the spectrum to maximum size.

The following features are on the display:

- **Sample ID -** enter the name of the sample
- **Baseline Correction -** allows for specification of a wavelength for the bichromatic normalisation of absorbance data for the entire spectrum. If the baseline correction is not used, the spectra can be offset from the baseline
- **Concentration -** indicates calculated concentration (depends on programmed setting, see 'Method Editor' for further information).
- **Formula Name / Value** – optional calculation of different concentrations depending on the settings set in the 'Method Editor'

Making user-defined measurements

1. Select the 'User Defined' application from the main menu - ensure the arm is down.
2. Select **Baseline Correction** box if correction is needed then enter the wavelength used for normalisation of absorbance data for the entire spectrum.
3. Select **Overlay Spectra** to display multiple spectra at the same time.
4. Establish a blank using the appropriate buffer. The blank solution generally is the buffer that the molecule of interest is suspended or dissolved in. This solution should be the same pH and of a similar ionic strength as the sample solution.
5. Pedestal option: Pipette 1 - 2 µl of the appropriate blanking solution onto the bottom pedestal, lower the arm and click **Blank**.
6. Cuvette option (VWR mySPEC Twin): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8,5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.
7. Enter a sample ID in the appropriate field, load the first sample as described for the blank above and click '**Measure'**.

After measuring:

- Simply wipe the upper and lower pedestals using a dry laboratory wipe so the instrument is ready to measure the next sample
- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples

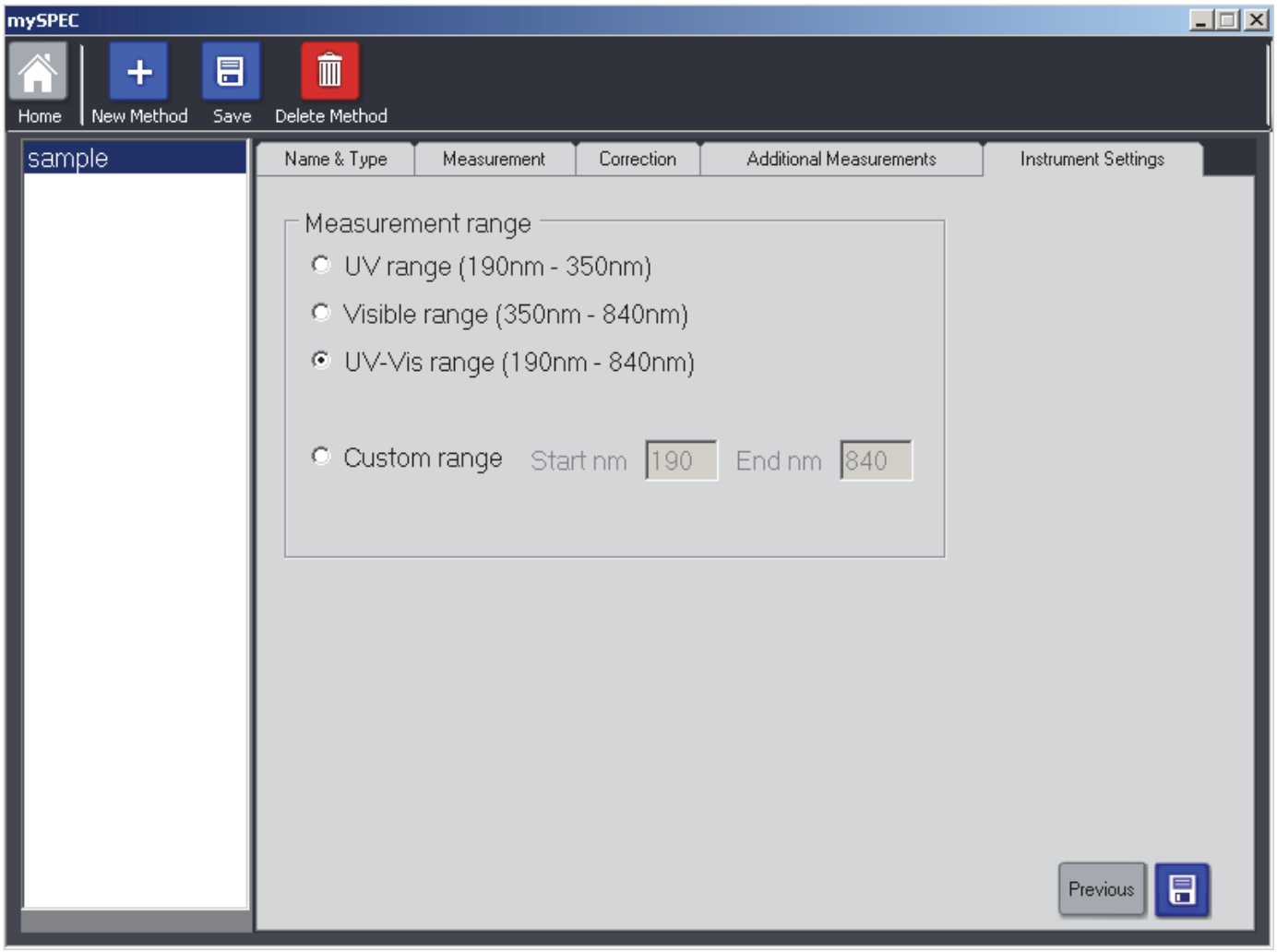
5. Editors

The ‘Editor’ application allows you to create, edit, save and view custom methods or customise kinetic measurements. All the other pop-up lists like ‘Dye-‘, ‘Units-‘, or ‘Protein-Editor’ can be used to add further information into the lists.

Method Editor

This feature includes 6 tabs to define the limits for settings available within the ‘Method Editor’.

Method Editor features



When **Method Editor** is started, the following icons will appear at the top of the screen.

 New Method	Creates a new custom method.
 Save	Saves a new custom method.
 Delete Method	Deletes the highlighted method in the method list box.

Main features

The 'Method Editor' includes 5 or 6 tabs to define settings for a new method

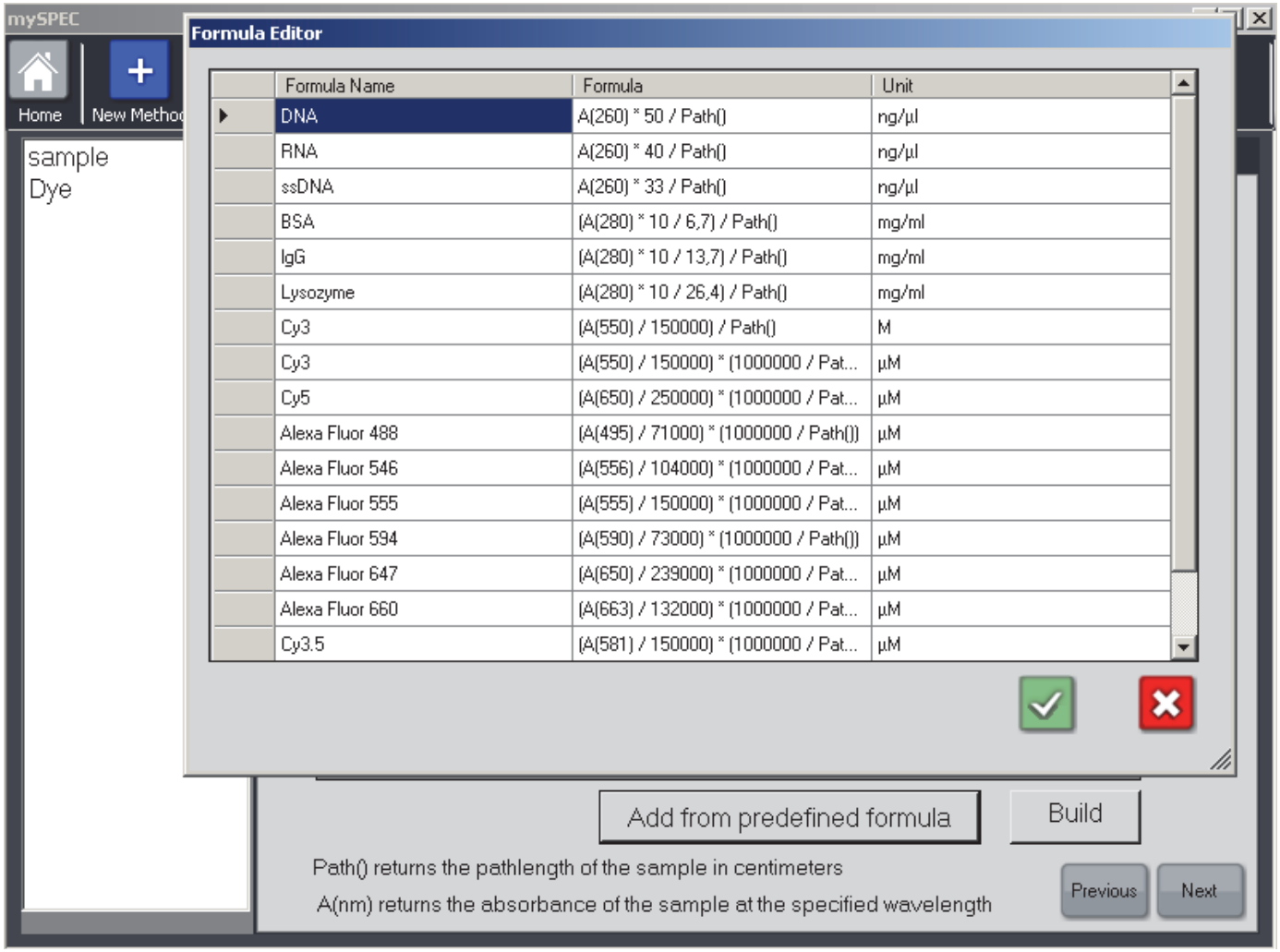
- **Name & Type** - defines the custom method name and type. The type refers to the method of calculating the sample concentration and offers a choice of multiplying absorbance values by factors/extinction coefficients or using standard curves. There are three different types:
  - **Manually entered factor/extinction coefficient** - a molecular weight is required if the results units and the user-defined factor/extinction coefficient units are not both weight- and molarity-based
  - **Standard curve** - uses a simple standard curve based on the absorbance of a set of standards at the specified analysis wavelength to determine a sample concentration
  - **Standard curve with two wavelengths** - calculates the sample concentration by taking the average of values determined by each of two standard curves associated with the user-defined wavelengths
- **Measurement -** defines the analysis wavelength and the preferred result units. The measurement results or concentration will be calculated using the absorbance value at the analysis wavelength and by applying the selected method type (factor or standard curve). The result name should be added into the field 'Result Name' like 'Concentration'. Select the amount of decimal digits from 0 – 6 by clicking into the field.
- **Correction** - selects the spectrum baseline correction - the baseline correction offsets the spectrum such that the absorbance at the correction wavelength is subtracted from the entire spectrum
- **Standard** - an additional tab that appears when a standard curve option is selected as the method type. This tab is used to define the initial standard names and concentration values that appear in the custom method and is used to define the curve fit used in the method. Curve fit choices include: linear, linear through zero, interpolation, 2^{nd} and 3^{rd} order polynomials.
  - A standard curve can be generated using a minimum of two points which may include two standards or a reference (assay buffer only – no protein) and one standard
  - The multipoint curve capability allows for multiple replicates for up to seven standards
  - Additional standards can only be added before unknown samples are measured. Once the first sample is measured, no additional standards or replicates can be added
  - A standard can be deleted at any time before a sample has been measured
- **Additional Measurements** - adds user-selected or user-defined formulas for additional processing of data. Refer to "Creating new formulas" below for additional details regarding how to build a custom formula
- **Instrument Settings** - selects the spectral display wavelength range for the method

Creating new formulas

The ‘Additional Measurements’ tab is used to define measurements that are included in the newly created method.

New measurement values are defined by entering the desired name, the formula used to calculate the value and the unit of measure associated with the value. A formula can be added manually, selected from a list of pre-defined formulas, or constructed from a set of equation templates using the ‘Build Formula’ button.

A list of pre-defined formula will appear after pressing the button ‘Add from predefined formula’. Clicking on the formula and pressing OK will add the formula into the ‘Method’.



Making custom method measurements

1. Select the ‘User Defined’ application from the main menu - ensure the arm is down.

2. Select the **Baseline Correction** box if a correction is needed. Enter the wavelength used for normalisation of absorbance data for the entire spectrum.

3. Select ‘**Overlay Spectra’** to display multiple spectra at the same time.

4. If running a method that uses a standard curve, enter the values for each standard concentration in the right pane table. The software allows for the reference and up to 7 additional standards. The reference and/or standards can be measured in replicate.

5. Establish a blank using the appropriate buffer. The blank solution generally is the buffer that the molecule of interest is suspended or dissolved in. This solution should be the same pH and of a similar ionic strength as the sample solution.

   - Pedestal option: Pipette 1 - 2 µl of the appropriate blanking solution onto the bottom pedestal - lower the arm and click **Blank**

   - Cuvette option (VWR mySPEC Twin): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8,5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

6. Enter a sample ID in the appropriate field, load the first sample as described for the blank above and click **Measure**.

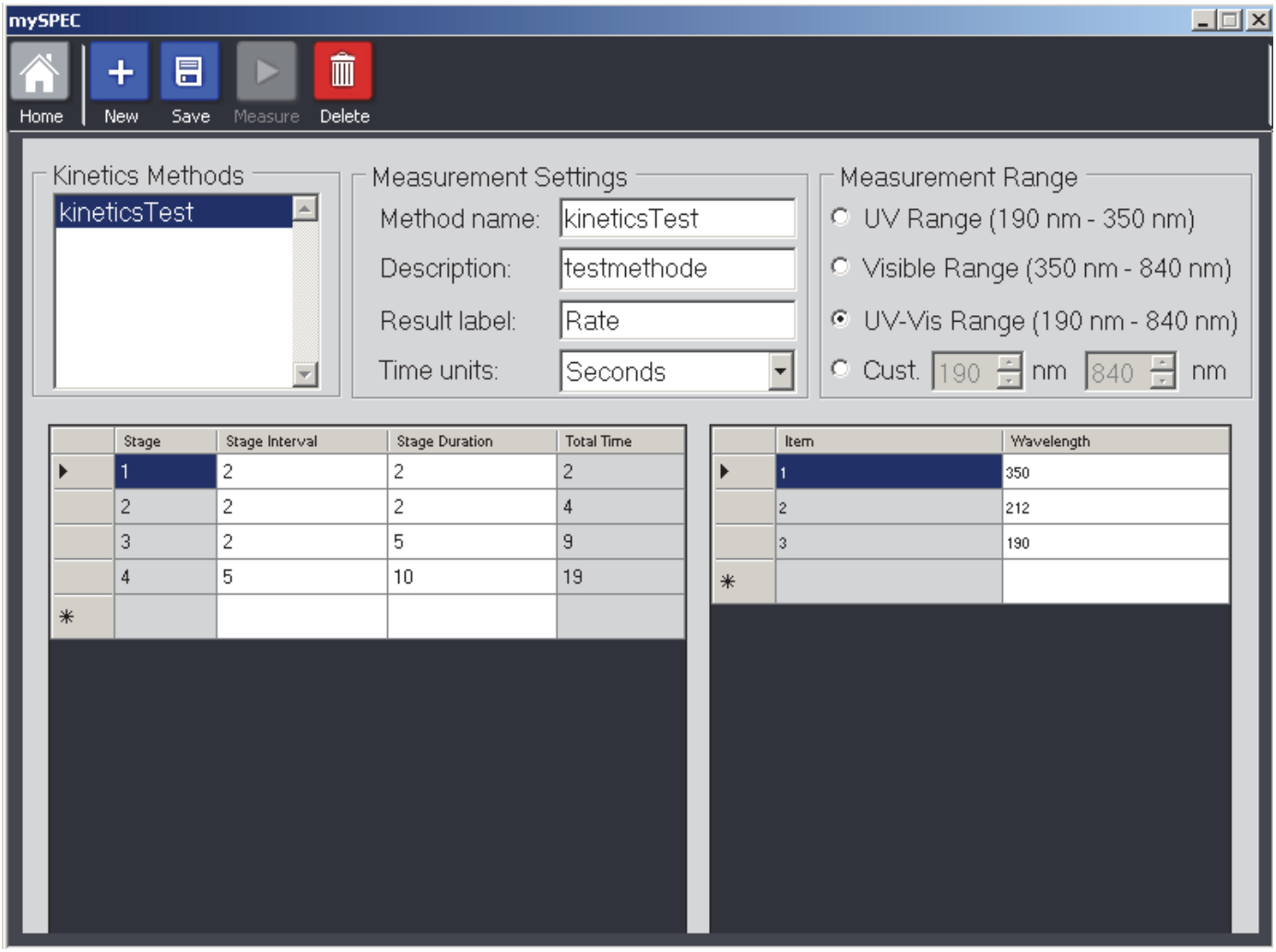
After measuring:

- Simply wipe the upper and lower pedestals using a dry laboratory wipe so the instrument is ready to measure the next sample

- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples

Kinetics Editor

The VWR mySPEC can be used to make time-based kinetic measurements. The ‘Kinetics Editor’ is used to create, edit, save and view custom kinetic methods. The ‘Kinetic Editor’ is located at Main Menu/Editors/Kinetic Editor. The software does not support kinetics measurements using the pedestal mode.



Screen features

When the ‘**Kinetics Editor’** is selected, the following icons will be displayed.

	Creates a new custom method.
	Saves the current method definition.
	Starts a kinetics method measurement. The Kinetics Method does not need to be saved prior to running. This icon will be greyed out until the minimum setting requirements for a new method have been entered.
	Deletes a Kinetics Method. This icon will be greyed out until the minimum setting requirements for a new method have been entered.

Kinetic Methods

Select displayed method to change or edit functions in this method. The **Measurement Settings** and **Measurement Range** will be shown on the screen.

Creating new Kinetic Method

- Click on **New** to create a new method
- Insert the ‘Measurement Settings’ and ‘Measurement Range’ (spectral display wavelength range: UV, Visible, UV/Vis or custom) into the screen

Two screen panels are used to define a method:

- **Measure Stage** – defines measurement stage intervals and the duration of each stage. To add the first stage, enter a value in the highlighted **Stage Interval** field. Next, click on and enter a value in the highlighted **Stage Duration** field. Click **Enter** and cumulative time will auto-populate. The next row will now be available for additional stage entries. The minimum stage interval allowed is 2,0 seconds.

- **Wavelengths to Monito**r - monitors multiple wavelengths during the reaction. To add the first wavelength, highlight the empty field and enter a value. The next row will become available for additional wavelength selections.

  - A cursor (coloured vertical line during the measurement) will be positioned at each of the monitored wavelengths. These cursors can be moved and the associated changes will be reflected in the rate display.

  - Wavelengths can be removed by selecting the wavelength and delete the value

A kinetics method can either be saved or immediately run without saving. To save a kinetics method, click on the **Save-Button**.

To start a kinetics measurement, click on the **Measure-Button** or open ‘Kinetic Method’ from the ‘Main Menu’ (Further Method / Kinetics).

Dye Editor

This software uses the general form of the Beer-Lambert equation to calculate fluorescent dye concentrations in the 'Micro Array and Proteins & Labels' applications. The user can choose to enter new dye types in the 'Dye/Chrom. Editor'. A table of extinction coefficients for the pre-defined dyes is shown below.

Dye list for the 'Dye and Chromophore Editor'					
Dye	**Unit**	**Coeff. (l/mol-cm)**	**Analysis Wavelength (nm)**	**260 nm correction**	**280 nm correction**
Cy3	µM	1,50E+05	550	0,04	0,05
Cy3.5	µM	1,50E+05	581	0,08	0,24
Cy5	µM	2,50E+05	650	0,00	0,05
Cy5.5	µM	2,50E+05	675	0,05	0,18
Alexa Fluor 350	µM	1,90E+04	346	0,25	0,19
Alexa Fluor 405	µM	3,45E+04	401	0,23	0,70
Alexa Fluor 488	µM	7,10E+04	495	0,30	0,11
Alexa Fluor 532	µM	8,23E+04	525	0,24	0,09
Alexa Fluor 546	µM	1,04E+05	556	0,21	0,12
Alexa Fluor 555	µM	1,50E+05	555	0,04	0,08
Alexa Fluor 568	µM	9,31E+04	578	0,45	0,46
Alexa Fluor 594	µM	7,30E+04	590	0,43	0,56
Alexa Fluor 647	µM	2,39E+05	650	0,00	0,03
Alexa Fluor 660	µM	1,32E+05	663	0,00	0,10
Alexa Fluor 680	µM	1,84E+05	679	0,00	0,05
DyLight 488	µM	7,00E+04	493	0,02	0,15
DyLight 649	µM	2,50E+05	654	0,00	0,04
FITC	µM	6,80E+04	495	0,20	0,32
Oyster-500	µM	7,80E+04	503	0,29	0,21
Oyster-550	µM	1,50E+05	553	0,04	0,05
Oyster-556	µM	1,55E+05	560	0,03	0,04
Oyster-645	µM	2,20E+05	649	0,05	0,06
Oyster-650	µM	2,00E+05	653	0,04	0,06
Oyster-656	µM	2,00E+05	660	0,03	0,04
Pacific Blue	µM	4,60E+04	416	0,15	0,20
r-PE	µM	2,00E+05	566	0,00	0,18
Texas Red	µM	8,00E+04	595	0,23	0,18

Setting up a new dye

1. Select the **Dye Editor** application from the main menu **Editors**.
2. A list of dyes will appear.
3. Select the last row and enter the dye specific coefficient and values. Ask the dye supplier for the specific values.
4. Click on the **Save** button to save the dye into the list.
5. The new dye will be included in the drop down list in the 'Micro Array and Proteins & Labels' applications if you click on Dye1 or Dye2.

Unit Editor

The user can choose to enter new units in the ‘Unit Editor’.

Setting up a new unit

1. Select the **Unit Editor** application from the main menu **Editors**.
2. A list of units will appear.
3. Select the last row and enter the unit specific values.
4. Click on the **Save** button to save the new unit into the list.

Protein Editor

For purified protein analysis and concentration measurements 4 pre-defined protein settings are available. The options can be viewed by selecting the drop down list in the **Protein A280 Method** and **Proteins and Labels Method**.

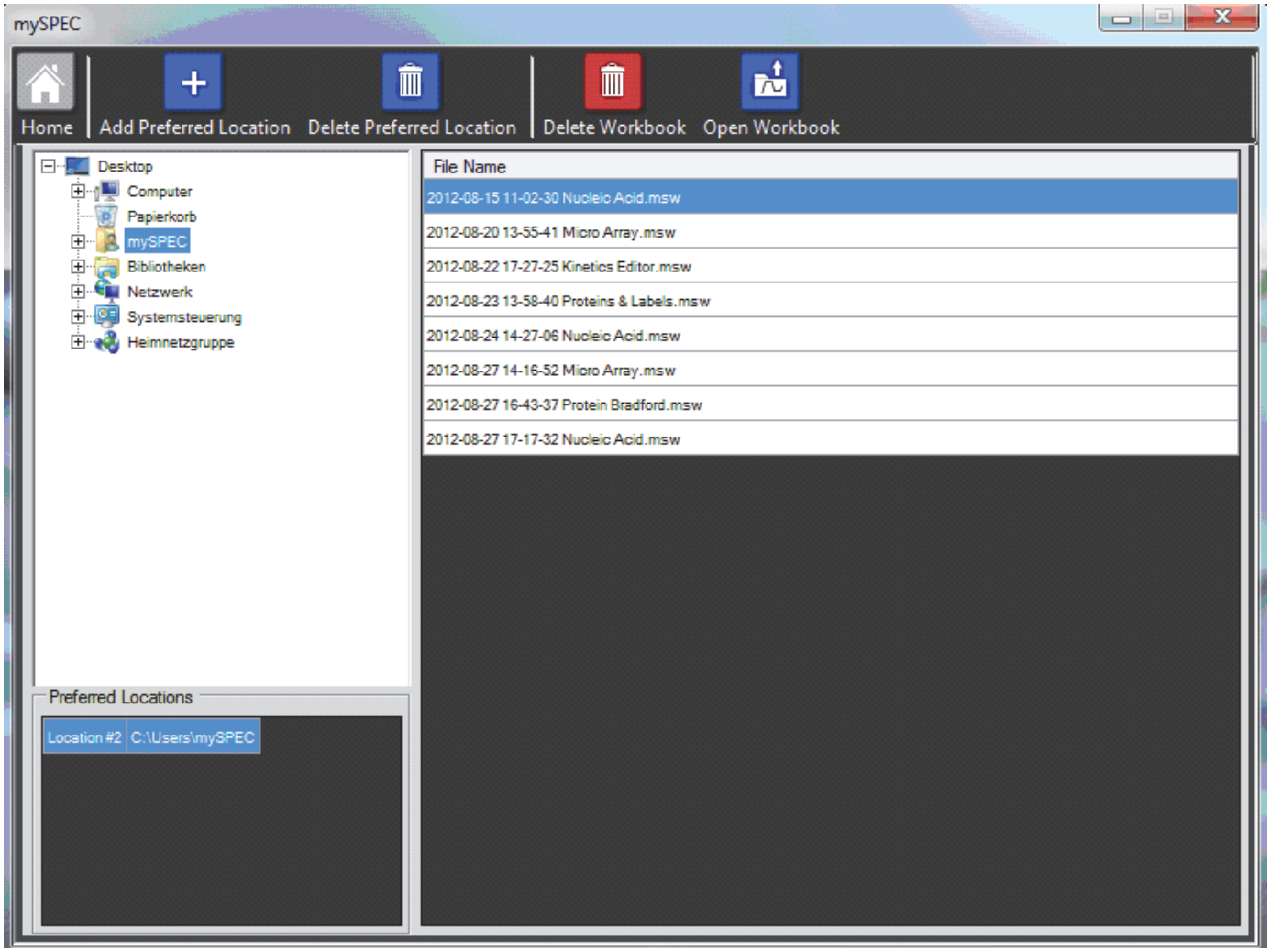
Setting up a new protein

1. Select the **Protein Editor** application from the main menu **Editors**.
2. A list of pre-installed proteins will appear.
3. Select the last row and enter the protein specific values.
4. Click on the **Save** button to save the new protein into the list.

6. Data, reports and settings

Data

Sample measurement data is recorded in files that can be filed in a user-specified location. The left pane task bar is used to access saved files and to pre-define preferred locations, where the data is saved.



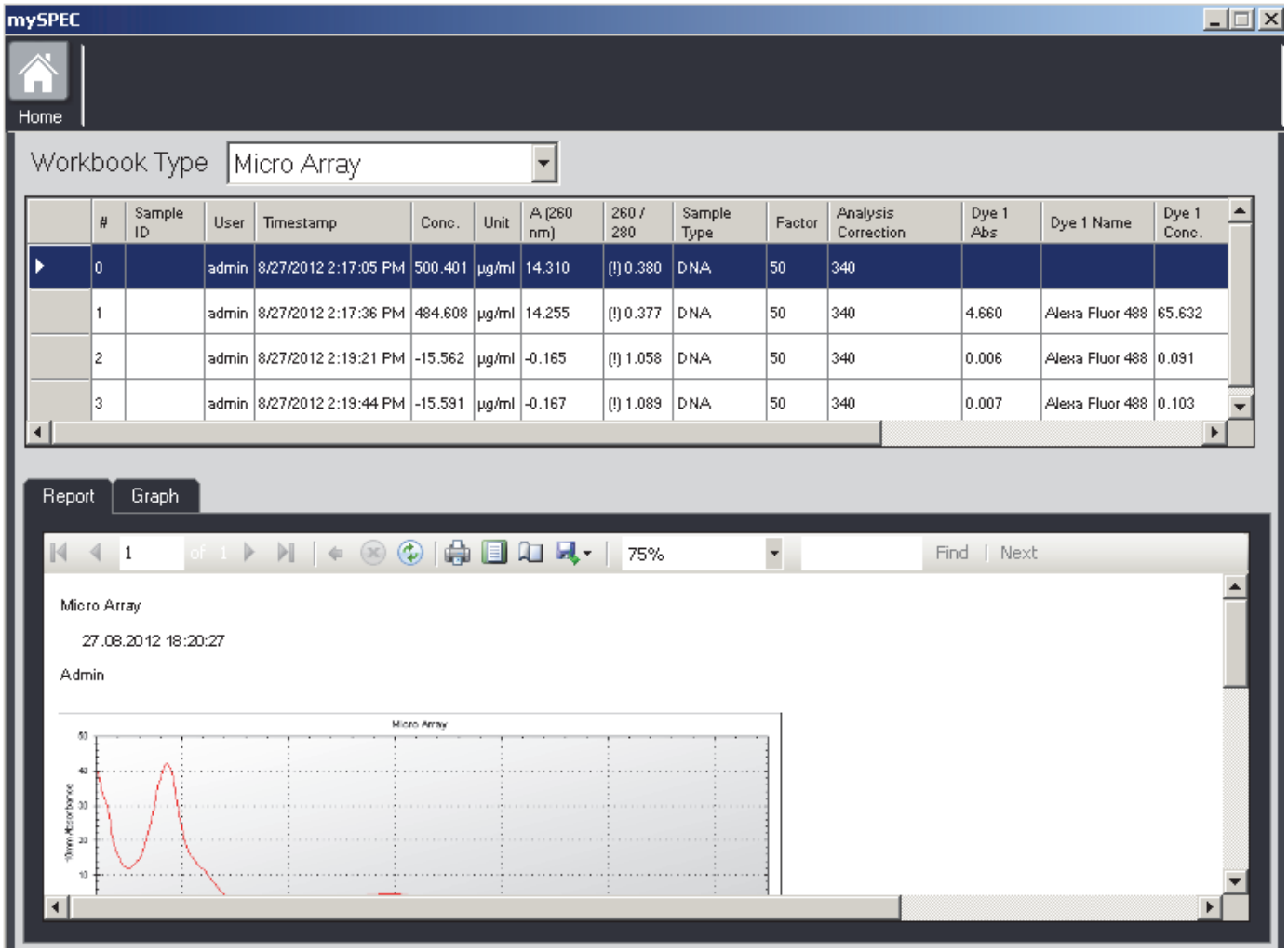
Screen features

When the '**Data Module'** is selected, the following icons will be displayed.

 Add Preferred Location	Add a preferred location of data storage into the software for fast and easy access.
 Delete Preferred Location	Delete a preferred location from the list after pre-selection.
 Delete Workbook	Delete mySPEC Data file.
 Open Workbook	Open selected mySPEC Data file in the application module.

Reports

Reports are user-configurable tables that display sample data. A report can be accessed by clicking the 'Reports' task bar, which appears once an application or a custom method is opened.



Two tabs appear in the right pane when the ‘Reports’ task bar is selected:

- **Report** - displays the sample data archived in the opened workbook. A graph displaying the spectrum of the highlighted sample in the report table is shown at the bottom of the page. Multiple spectra can be displayed by highlighting the sample data in the report table.
- **Graph** - A graph displaying the spectrum of the highlighted sample in the report table is shown

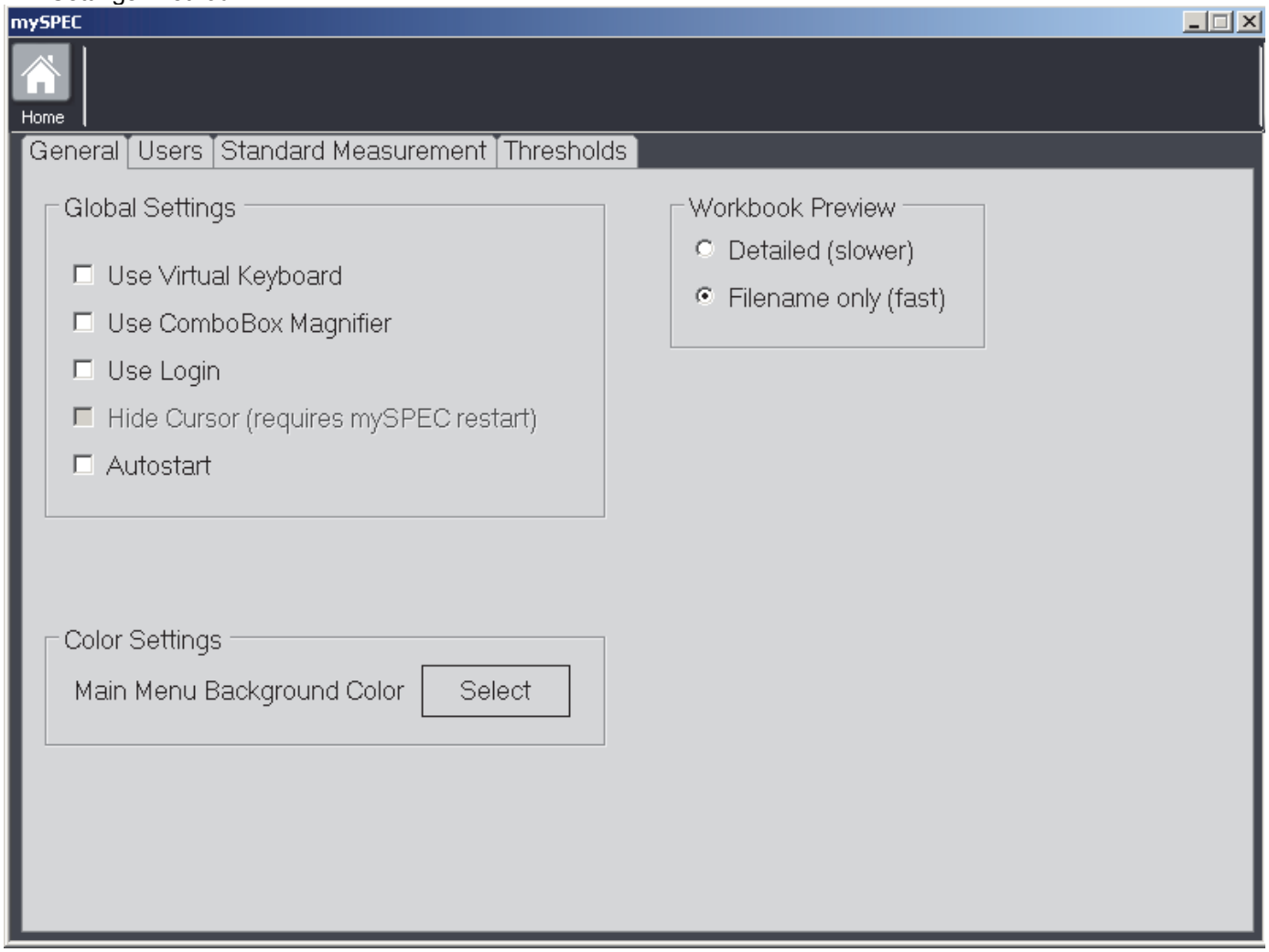
In the ‘Report’ tab the following tools can be used:

- **Print (**  **)** - sends a report to the default system printer and changes the settings of the printer ()

Export ( **)** - saves a report either as an Excel, PDF or Word file.

Settings

The settings can be accessed by clicking the ‘Settings’ button in the main menu. Three tabs appear in the ‘Settings’ method.



- **General** – the main difference in the mySPEC workaround is, if you work with a normal PC or with a Tablet (Touch). If a Touch is used it is recommended that you activate the virtual keyboard function for adding letters and numbers without a keyboard. It is easier to work with the mySPEC Touch if the ‘Combo Box’ magnifier is activated. If a ‘Login’ is not required de-activate this field and the software will start automatically without different permissions. If the auto start function is activated, sample measurement will start automatically if the sample arm is closed. A blank measurement must be started by clicking on the blank icon. ‘Hide Curser’ can be selected if the mySPEC Touch is used.

- **User -** manages which users have permission to change or configure new users and to separate what measurement is done from which user. Add a user name and a password to create a new user. “Admins” are allowed to set-up and delete new users and delete measurement data (workbooks). New parameters can be added into the ‘Dye Editor’, ‘Protein Editor’ and ‘Unit Editor’ if an ‘Admin User’ is logged in. Password of the user Admin is: admin

- **Thresholds -** A260 and A280 are the optical spectrometer measurement of absorbance at the wavelengths of 260 and 280 nm respectively. A260 is frequently used to measure DNA/RNA concentration and A280 is used to measure protein concentration. A ratio of A260/A280 >1,8 suggests little protein contamination in a DNA/RNA sample. Absorption at 230 nm can be caused by contamination by phenolate ion, thiocyanates, and other organic compounds. For a pure RNA sample an A260/A230 ratio >1,8 is recommended. Set your specific A260/A280 and A260/230 ratio value and the mySPEC software will indicate after a Nucleic Acid or Micro Array measurement if the A260/A280 and A260/A230 value is fine (green) or not so good (red).

7. Maintenance

Safety

Operating the instrument with the cover off exposes the operator to the instrument's sharp metal edges and delicate fibre optics. Removal of the cover may also void the warranty.

The VWR mySPEC is designed only for indoor use under the following conditions:

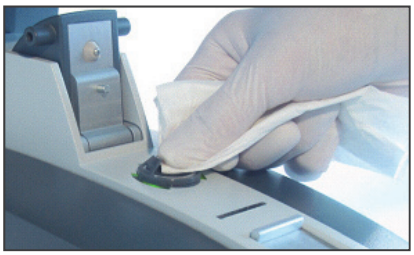
- Temperature: 15 - 35 °C
- Humidity: 35 - 65%

The power supply can remain plugged into the VWR mySPEC while the instrument is not in use. When the instrument is plugged in but not in use, the power consumption is ~5 W and the flash lamp is not energised. The instrument does not use a power switch.

Maintenance of the sample retention system

Cleaning

The primary maintenance requirement for the VWR mySPEC is keeping the measurement pedestal surfaces clean. Upon completion of each sample measurement, wipe the sample from the upper and lower pedestals with a clean, lint-free lab wipe to prevent sample carryover and avoid residue build up.



Use 2 µl water aliquots to clean the measurement surfaces after high concentration samples to ensure no residual sample is retained on either measurement surface.

1. Apply 3 - 5 µl of dH_20 onto the bottom pedestal.

2. Lower the upper pedestal arm to form a liquid column; let it sit for approximately 2 - 3 minutes.

3. Wipe away the water from both the upper and lower pedestal with a clean lab wipe.

- **Additional cleaning**
  When a more rigorous cleaning protocol is required (i.e. dried proteins), substitute 0,5 M HCl for the dH_2O in the procedure above. Follow with 3 - 5 µl of dH_2O.

- **Decontamination**
  A sanitising solution, such as a 0,5% solution of sodium hypochlorite (1:10 dilution of common commercial bleach solutions – freshly prepared), can be used to ensure that no biologically active material is present on the measurement pedestals. Follow with 3 - 5 µl of dH_2O.

The use of detergents or isopropyl alcohol is not recommended as they may uncondition the pedestal measurement surfaces. If a solution containing either is used, it is important to follow with 3 - 5 µl of dH_2O.

Solvent compatibility

VWR mySPEC spectrophotometer pedestals are compatible with most solvents typically used in life science laboratories. These include: Methanol, ethanol, n-propanol, isopropanol, butanol, acetone, ether, chloroform, carbon tetrachloride, DMSO, DMF, Acetonitrile, toluene, hexane, benzene, sodium hydroxide, sodium hypochlorite (bleach), dilute HCl, dilute HNO_3, dilute acetic acid.

All forms of Hydrofluoric Acid (HF) and Tetrahydrofuran (THF) are incompatible, as the fluoride ion will dissolve the quartz fibre optic cable.

Do not allow alcohol, bleach, acetone or other solvents to remain on the diaphragm surrounding the pedestal for more than a minute as the adhesive keeping the seal in place may be adversely affected.

Decontamination of measurement pedestals

If decontamination is necessary, a sanitising solution, such as a 0,5% solution of sodium hypochlorite (1:10 dilution of common commercial bleach solutions, freshly prepared), can be used to ensure that no biologically active material is present on the measurement pedestals.

Maintenance of the cuvette system

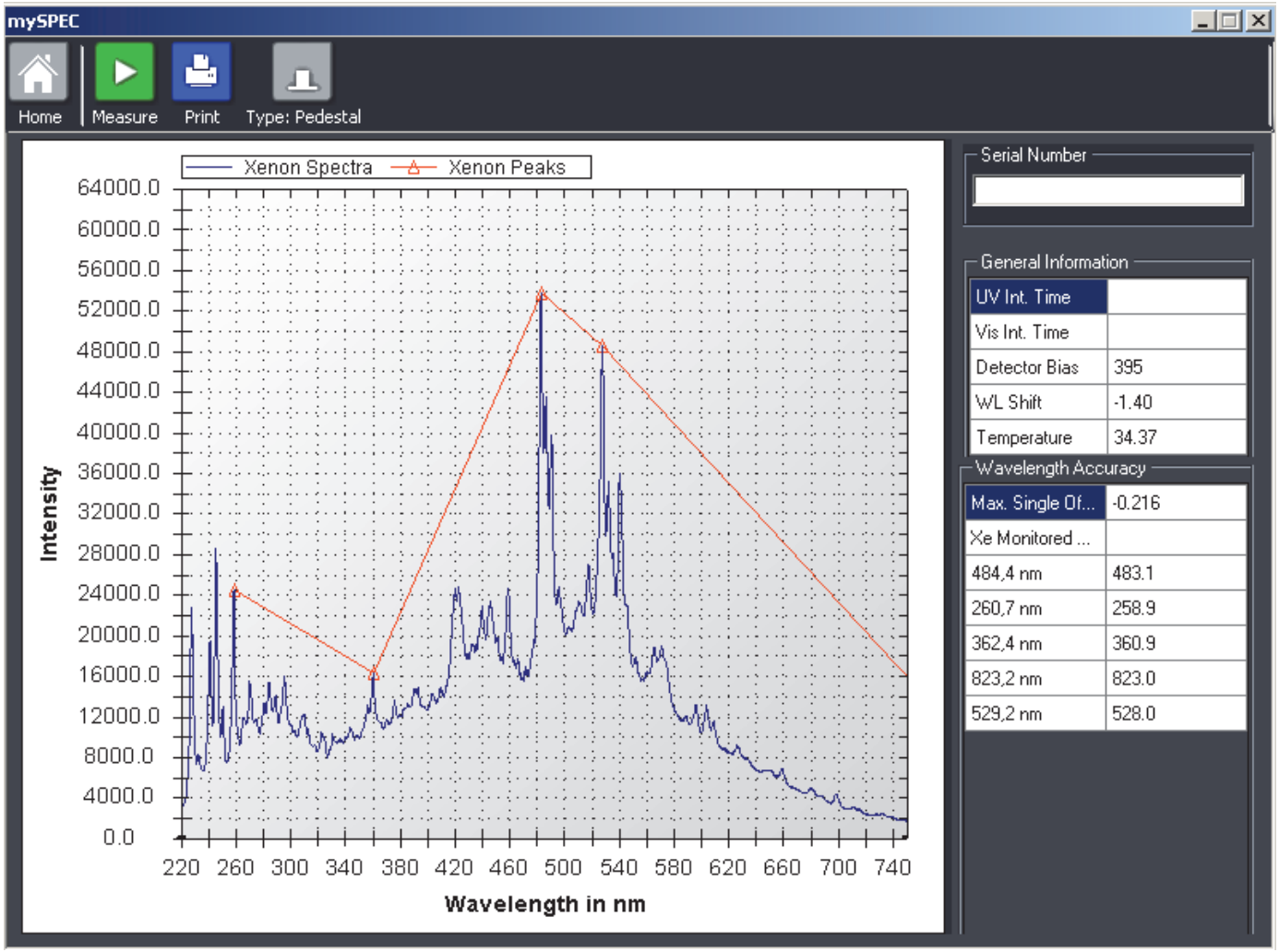
The cuvette holder assembly block can be cleaned of excess dust using canned air. If any liquid gets into the block, it is best to use a lab wipe or a lab cotton swab to absorb the liquid.

8. Diagnostics and troubleshooting

The ‘**Diagnostics’** task bar accesses both the ‘Intensity Check’ and the ‘Calibration Check’. The ‘Intensity Check’ confirms that the internal spectrometer is functioning as expected and the ‘Calibration Check’ confirms that the instrument is performing within the pedestal path length calibration specifications.

Intensity Check

With the sampling arm down, click **Measure** to perform an intensity check.



Clean the pedestal as described below, before starting the ‘Intensity Check’.

1. Apply 3 - 5 µl of dH_2O onto the bottom pedestal.
2. Lower the upper pedestal arm to form a liquid column; let it sit for approximately 2 - 3 minutes.
3. Wipe away the water from both the upper and lower pedestal with a clean, dry lab wipe.

If the bias indicator is too high, please ensure that the instrument is not located in a cold environment. Allow the unit to warm up to room temperature to repeat the intensity check.

Contact VWR International if the spectrum does not look like the upper one.

Calibration Check

It is recommended that a pedestal calibration check be performed every six months to verify that the instrument is performing within specifications.

A vial of mySPEC calibration fluid is required to run the calibration check procedure (Cat. No. 732-2537). The calibration fluid is an aqueous potassium dichromate ($K_2Cr_2O_7$) solution used to confirm the path length accuracy of VWR mySPEC spectrophotometers.

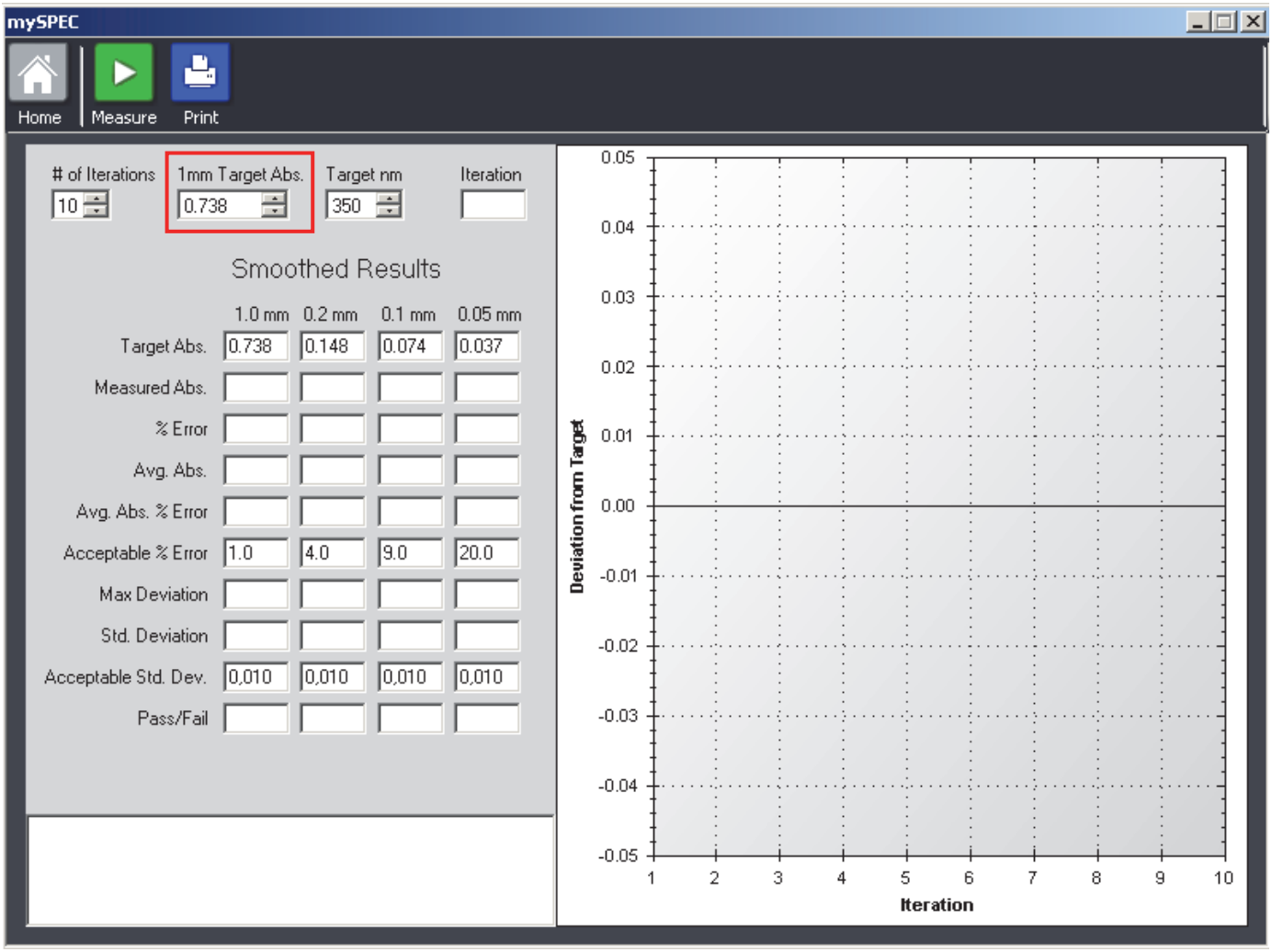
Note: The calibration fluid is supplied in a single use vial. Calibration fluid must be used within one hour of opening the vial. Exposure to the environment or transferring of the fluid to another container may result in a significant change in concentration.

Pedestal calibration check procedure

1. Ensure the measurement pedestals are clean and that a 1 µl water sample “beads” up on the lower pedestal.

2. Open the mySPEC software, and using the **Diagnostics** button, and choose **Calibration Check.**

3. Enter the actual Target Absorbance stated on the calibration fluid vial in the appropriate field as shown in the image below.



4. Add 1 µl of dH_2O to the pedestal, lower the arm and click **Measure.**

5. Wipe the upper and lower pedestals using a dry laboratory wipe.

6. Vigorously shake the calibration fluid vial to thoroughly mix the solution.

7. Follow the on-screen prompts in the ‘Customer Guidance’ text box. Using individual 2 µl aliquots of the calibration fluid, measures 10 replicates. After the 10th measurement, the calibration check results will be displayed on-screen in the ‘Customer Guidance’ text box.

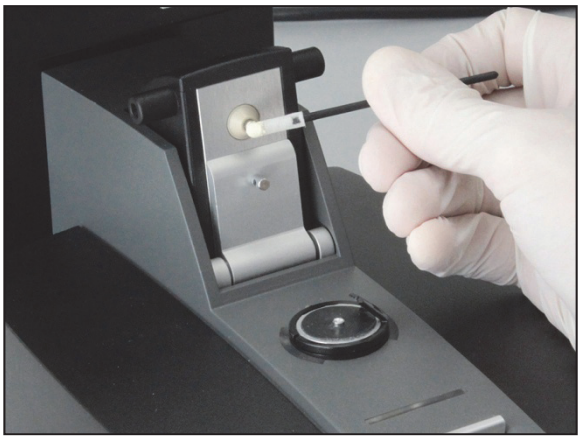
If the instrument still does not pass the calibration check using 2 µl samples recalibration is required. Contact support.

Print a screen shot of the ‘Calibration Check’ to send it to the support team.

mySPEC Reconditioning

Please use the mySPEC Reconditioning Kit as a rapid method of reconditioning the pedestals when the surface properties have been compromised and liquid columns breaks during measurement.

1. Open the vial and use the applicator to apply a very thin layer of the Reconditioning solution on the upper and lower surface of the pedestals.
2. Wait for 30 seconds to dry
3. Remove the Reconditioning solution with a clean and dry laboratory wipe, by intensive rubbing the surface of the upper and lower pedestal.
4. Repeat step 1 – 3 till an 1 µl aliquot of water creates a well-defined drop.



Technical service

Web Resources

Visit the VWR website at **www.vwr.com** for:

• Complete technical service contact information

• Access to the VWR Online Catalogue, and information about accessories and related products

• Additional product information and special offers

Contact us For information or technical assistance contact your local VWR representative or visit. www.vwr.com.

Warranty

VWR International warrants that this product will be free from defects in material and workmanship for a period of two (2) years from date of delivery. If a defect is present, VWR will, at its option and cost, repair, replace, or refund the purchase price of this product to the customer, provided it is returned during the warranty period. This warranty does not apply if the product has been damaged by accident, abuse, misuse, or misapplication, or from ordinary wear and tear. If the required maintenance and inspection services are not performed according to the manuals and any local regulations, such warranty turns invalid, except to the extent, the defect of the product is not due to such non-performance.

Items being returned must be insured by the customer against possible damage or loss. This warranty shall be limited to the aforementioned remedies. IT IS EXPRESSLY AGREED THAT THIS WARRANTY WILL BE IN LIEU OF ALL WARRANTIES OF FITNESS AND IN LIEU OF THE WARRANTY OF MERCHANTABILITY.

Compliance with local laws and regulations

The customer is responsible for applying for and obtaining the necessary regulatory approvals or other authorisations necessary to run or use the Product in its local environment. VWR will not be held liable for any related omission or for not obtaining the required approval or authorisation, unless any refusal is due to a defect of the product.

Equipment disposal

This equipment is marked with the crossed out wheeled bin symbol to indicate that this equipment must not be disposed of with unsorted waste.
Instead it's your responsibility to correctly dispose of your equipment at lifecycle -end by handling it over to an authorized facility for separate collection and recycling. It's also your responsibility to decontaminate the equipment in case of biological, chemical and/or radiological contamination, so as to protect from health hazards the persons involved in the disposal and recycling of the equipment.
For more information about where you can drop off your waste of equipment, please contact your local dealer from whom you originally purchased this equipment.

By doing so, you will help to conserve natural and environmental resources and you will ensure that your equipment is recycled in a manner that protects human health.

Thank you

Local VWR offices in Europe and Asia Pacific

Australia
VWR International Pty.LTD
Unit 1/31 Archimedes Place
Murarrie, Queensland 4172
Tel.: 1300 727 696
Fax: 1300 135 123
Email: sales@au.vwr.com

Belgium
VWR International bvba
Researchpark Haasrode 2020
Geldenaaksebaan 464
3001 Leuven
Tel.: 016 385 011
Fax: 016 385 385
E-mail: customerservice@be.vwr.com

China
VWR (Shanghai) Co., Ltd
Rm.219
No. 2100, Dongming Road
Pudong New District
Shanghai, 200126
Tel.: +86-21 589 868 88
Fax: +86-21 585 588 01
E-mail: info_china@vwr.com

Czech Republic
VWR International s. r. o.
Veetee Business Park
Pražská 442
CZ - 281 67 Stříbrná Skalice
Tel.: +420 321 570 321
Fax: +420 321 570 320
E-mail: info@vitrum.cz

Denmark
VWR - Bie & Berntsen
Transformervej 8
2730 Herlev
Tel.: 43 86 87 88
Fax: 43 86 87 90
E-mail: info@dk.vwr.com

Finland
VWR International Oy
Valimotie 9
00380 Helsinki
Tel.: +358 9 80 45 51
Fax: +358 9 80 45 52 00
E-mail: info@fi.vwr.com

France
VWR International S.A.S.
Le Périgares – Bâtiment B
201, rue Carnot
94126 Fontenay-sous-Bois cedex
Tel.: 0 825 02 30 30 (0,18 EUR TTC/min)
Fax: 0 825 02 30 35 (0,18 EUR TTC/min)
E-mail: info@fr.vwr.com

Hungary
VWR International Kft.
Simon László u. 4.
4034 Debrecen
Tel.: (52) 521-130
Fax: (52) 470-069
E-mail: info@hu.vwr.com

India
VWR Lab Products Private Limited
No.139. BDA Industrial Suburb,
6th Main, Tumkur Road, Peenya Post,
Bangalore, India – 560058
Tel.: +91-80-28078400
Fax: +91-80-28078410
E-mail: vwr_india@vwr.com

Ireland / Northern Ireland
VWR International Ltd / VWR International (Northern Ireland) Ltd
Orion Business Campus
Northwest Business Park
Ballycoolin
Dublin 15
Tel.: 01 88 22 222
Fax: 01 88 22 333
E-mail sales@ie.vwr.com

Italy
VWR International S.r.l.
Via San Giusto 85
20153 Milano (MI)
Tel.: 02-3320311/02-487791
Fax: 02-332031307/02-40090010
E-mail: info@it.vwr.com

The Netherlands
VWR International B.V.
Postbus 8198
1005 AD Amsterdam
Tel.: 020 4808 400
Fax: 020 4808 480
E-mail: info@nl.vwr.com

New Zealand
VWR International LP
241 Bush Road
Albany
0632 New Zealand
Email: sales@globalscience.co.nz

Norway
VWR International AS
Haavard Martinsens vei 30
0978 Oslo
Tel.: 02290
Fax: 815 00 940
E-mail: info@no.vwr.com

Poland
VWR International Sp. z o.o.
Limbowa 5
80-175 Gdansk
Tel.: 058 32 38 210
Fax. 058 32 38 205
E-mail: labart@pl.vwr.com

Portugal
VWR International - Material de Laboratório, Lda
Edifício Neopark
Av. Tomás Ribeiro, 43- 3 D
2790-221 Carnaxide
Tel.: 21 3600 770
Fax: 21 3600 798/9
E-mail: info@pt.vwr.com

Singapore
VWR Singapore Pte Ltd
18 Gul Drive
Singapore 629468
Tel: +65 6505 0760
Fax: +65 6264 3780
E-mail: sales@sg.vwr.com

Spain
VWR International Eurolab S.L.
C/ Tecnología 5-17
A-7 Llinars Park
08450 - Llinars del Vallès
Barcelona
Tel.: 902 222 897
Fax: 902 430 657
E-mail: info@es.vwr.com

Sweden
VWR International AB
Fagerstagatan 18a
163 94 Stockholm
Tel.: 08 621 34 00
Fax: 08 621 34 66
E-mail: info@se.vwr.com

Switzerland
VWR International GmbH
Lerzenstrasse 16/18
8953 Dietikon
Tel.: 044 745 13 13
Fax: 044 745 13 10
E-mail: info@ch.vwr.com

Turkey
VWR International Laboratuar Teknolojileri Ltd.Şti .
Orta Mah. Cemal Gürsel Caddesi
Ördekcioglu Işmerkezi No.32/1
34896 Pendik - Istanbul
Tel.: +90216 598 2900
Fax: +90216 598 2907
Email: info@tr.vwr.com

UK
VWR International Ltd
Customer Service Centre
Hunter Boulevard
Magna Park
Lutterworth
Leicestershire
LE17 4XN
Tel.: 0800 22 33 44
Fax: 01455 55 85 86
E-mail: uksales@uk.vwr.com