UV-VIS SPECTROSCOPY

# LAMBDA 465

# **UV Lab Software Users Guide**



#### **Release History**

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# I. Introduction

# I-1. Overview

This manual provides step-by-step instructions for the use of UV Lab software with a Lambda 465 Spectrophotometer.

UV Lab software must be installed onto a Microsoft<sup>®</sup> Windows 7, Windows 8 or windows 10 operating system to function properly.

The following icons are used throughout this manual to emphasize important information.

- General explanation about the main subject
- Detailed or expanded explanations about the main subject



Message contains important information about procedure or technique



Message contains helpful supplementary information

# I-2. Specifications of UV Lab

#### Support PDA UV-Vis Spectrophotometer

· Lambda 465 UV-Visible Spectrophotometer

#### **Control Accessories**

- · 8 Cell Water Jacketed Cell Changer
- Water Jacketed Automatic Referencing Stage
- · Auto Sipper System
- Single Peltier Temp ctrl Unit L465
- Multi Peltier Temp ctrl Unit L465
- · Reflectance Holder
- Autosampler
- DRA-100
- . Magnetic Stirrer Assembly (Auto Type)
- . Magnetic Stirrer Assembly (Manual Type)
- . Rapid Mixing Accessory

#### Minimum Computer Requirements

Processor : Intel<sup>®</sup> Core 1.5 GHz or faster RAM : At least 1GB Hard disk : 50GB with 1GB free Input devices : Mouse and keyboard Monitor : 1024x768 (minimum) Media : CD ROM Drive Port : USB port for the data acquisition

#### **Operating System**

- $\cdot$  Microsoft  $^{\circledast}$  Windows 7 or Windows 8 or Windows 10
- If using UV Lab Security Software : Microsoft<sup>®</sup> Windows 7 Professional, Ultimate, Enterprise, Windows 8, Windows 10

#### **Output Device**

Microsoft<sup>®</sup> Windows compatible printer

### UV Lab Software - Instrument Control, Data Acquisition and Standard Experiments

General Mode	
Wavelength Monitoring	$\cdot$ The full spectrum (190 ~ 1100 nm) of each sample is extracted at once
Equation Calculation	<ul> <li>Allows the user to enter support equations for the evaluation of the data</li> <li>Supports Functions: +, -, /, *, ABS, Exp, LN, LOG10, SQRT</li> </ul>

Find/Peak Valley · Find up to 30 of the peaks or valleys automatically or manually

Quantification Mode			
	Concentration Unit: All units user-specifiable		
Quantification	First, second and third order calibration curve fits		
Standard/Sample-	Supports zero offset of calibration curve		
•	Calculation of correlation coefficient		
Thickness Mode			
Thickness Measurement	Measure the thickness of thin film using the Reflectance Holder		
Kinetics Mode			
Time Based	· 3D Display: Time Display(X Axis), Zoom In/Zoom Out, Rotate Chart		
Kinetics	Full Spectrum		
	Time Unit: Min, Sec, Msec		
	· Zero Order, Initial Rate, First Order, Delta Au		
	• Data from single wavelength (using the multi-cell) or multiple wavelengths (using the single cell) can be extracted for the rate calculation		
Temperature	• 3D Display: Time Display(X Axis), Zoom In/Zoom Out, Rotate Chart		
Based	・Temperature Unit: °C		
Kinetics	<ul> <li>Temperature Limit: from -10 ℃ to 100 ℃</li> </ul>		
Ultra Kinetics	• Time Unit: Msec		
	Minimum Interval Time: 20 msec		

### UV Lab Bio Analysis (Optional) **Bio Mode** Nucleic Acid · General Ratio with two wavelengths for the calculation of user specified Analysis ratios. · Determine concentration of protein and nucleic acid using coefficients Baseline Correction **Protein Analysis** Predefined methods Bradford Protein Analysis at 595 nm ·Bicinchoninate (BCA) at 562 nm ·Biuret Protein Analysis at 540 nm ·Lowry Protein Analysis high sensitivity at 750 nm ·Lowry Protein Analysis low sensitivity at 500 nm ·Lowry Protein Analysis at 740 nm •Trinitrobenzene Sulfonate at 416 nm •Direct UV at 280 nm Direct UV at 205 nm Cell Density · Predefined methods · Cell Density calculated with absorbance of 600 nm Enzyme Activity · User Specifies One Activity Factor · Data from single wavelength (using the multi-cell) or multiple wavelengths (using the single cell) can be extracted for the rate calculation Baseline Correction Enzyme · Michaelis-Menten Mechanism · Lineweaver-Burk · Hanes-Woolf · Eadie-Hofstee · Calculate K<sub>m</sub>, V<sub>max</sub> from each plot • Temperature Unit: ℃ Thermal Denaturation • Temperature limit: from -10°C to 100°C $\cdot$ T<sub>m</sub> calculated with average method & 1st Derivative · Volume correction with user specified equation · Normalization with user specified factor • User defined equation allows calculation from T<sub>m</sub> value (ex: %G-C)

### Color Analysis Software (Optional)

Color Analysis Mode		
Color Analysis	. Color Difference Formula Function	
	. Measure the various Color Indices	
	. This can be added to any of UV Lab Software upon request.	

### Software (Optional)

Multi-Component Analysis (MCA) Mode

Multi-Component· Analyze complex compounds containing multiple components (up to 4Analysis (MCA)components)

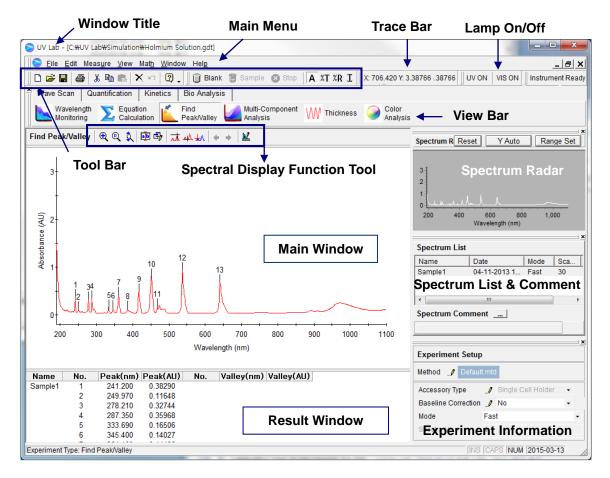
- $\cdot$  Define the concentration of each component
- .This can be added to any of UV Lab Software upon request.

Validation	
Validation	<ul> <li>Automatic Validation Wizard assists with the validation across the UV and Visible range</li> <li>Includes Photometric, Wavelength, Resolution, Stray Light, Stability and Dark Current tests</li> </ul>

#### UV Lab Security Software

Provides enhanced security with Windows 7 Professional, Enterprise, Ultimate, Windows 8, Windows 10 Operating Systems to assist with 21 CFR Part 11 compliance requirements for electronic signatures and electronic record traceability. This software package is supplied with the UV Lab Security Software Manual.

# I-3. UV Lab Software Interface



This chapter describes the unique display features of UV Lab Software.

### I-3-1. Window Title

Display the title of an active window or file. If data is saved as a specific file, its name will become the window title; otherwise, the title will be assigned automatically as [Untitled-1], [Untitled-2], etc.

### I-3-2. Main Menu

The Main Menu consists of a File Menu, Edit Menu, Measure Menu, View Menu, Math Menu, Window Menu and Help Menu.

📚 <u>F</u>ile <u>E</u>dit Meas<u>u</u>re <u>V</u>iew Mat<u>h</u> <u>W</u>indow Hel<u>p</u>

### I-3-3. Toolbar

The toolbar provides quick access to basic commands without opening a menu. Users can modify the configuration of the toolbars as desired.

Icon	Command	Hot Key	Icon	Command	Hot Key
	New	Ctrl + N	•	Contents	F1
<b>2</b>	Open	Ctrl + O		Blank	Alt + B
	Save	Ctrl + S		Sample	Alt + S
9	Print	Ctrl + P	8	Stop	
ж	Cut	Ctrl + X	А	Absorbance	Alt + A
	Сору	Ctrl + C	%T	Transmittance	Alt + T
<b>E</b>	Paste	Ctrl + V	۶R	Reflectance	Alt + R
×	Delete	Del	Ι	Energy	Alt + I
5	Undo	Ctrl + Z			

See II. File Menu, III. Edit Menu and IV. Measure Menu for more details.

### I-3-4. View Bar

There are four types of modes in the UV Lab software that can be selected by the user to analyze samples and manipulate collected data.

Mode	Functions
Wave Scan	Wavelength Monitoring Equation Calculation Find Peak/ Valley Thickness Measurement Color Analysis (Optional) Multi-Component Analysis (MCA) (Optional)
Quantification	Quantification Standard Quantification Sample
Kinetics	Time Based Kinetics Temperature Based Kinetics Ultra Kinetics (Optional)

Mode	Functions
Bio Analysis (Optional)	Nucleic Acid Analysis Protein Analysis Cell Density Enzyme Activity Enzyme Mechanism
	Thermal Denaturation

### I-3-5. Main Window & Spectral Display Function Tool

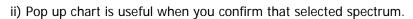
- Display the spectral data. If there are several spectra in the window, only an active red spectrum can be edited.
- Display the spectrum window and calibration curve in the quantification experiment, or the 3D spectrum and 2D spectrum in kinetics and bio experiments.
- Display a main window as required.

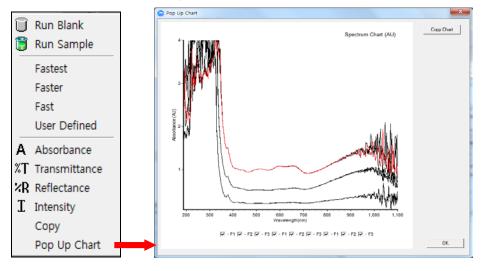
The following table provides a brief description of each Display Function Tool.

Toolbar	Command	Description
€ <b></b>	Zoom In	Zoom in the selected area
R	Zoom Reset	Reset the zoom area to the original size
Ş.	Y Axis Auto Scale	Allow the auto scale of Y-axis depending on the measurement result
*	Pick Peak	Look for peaks. Use in Peak/Valley Mode
Д	Pick Valley	Look for valleys. Use in Peak/Valley Mode
₩	Cursor	Display the cross lines for selecting data points
<b>\$</b>	To Left	Move the cursor to the left
•	To Right	Move the cursor to the right
View 3D	View 3D graphic mode	May be used in Kinetics & some Bio Modes
<u>a</u> b	Add Label	Add label on the spectrum.
d,	Edit Label	Edit label on the spectrum.
M	Properties	Display Interval, Change a chart (background & axis) color, legend display, grid, X-axis and Y-axis scale, and decimal point format, etc.

See VIII. Display Function Tools for more details.

- Display the optional function when you click right mouse in the main window.
  - i) Copy is useful when you paste into another program such as Microsoft Excel or other windows programs.





### I-3-6. Result window

- Display result values of performed measurements.
- Copy and paste into another program such as Microsoft Excel or other windows programs when you click right mouse in the result window.

Name	AU(440.000nm)	AU(465.000nm)	AU(546.000nm)	AU(590.000nm	n) AU(635.000nm)
Sample1	0.20616	0.29595	0.19140	0 10337	0.21510
				Сору	

### I-3-7. Trace bar

Display X-axis and Y-axis values of the mouse pointer on the spectrum.

```
X: 531.073 Y: -0.83019
```

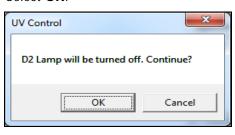
### I-3-8. Lamp ON/OFF

- Switch the lamp ON/OFF.
- Procedure

1. Followings are the status of the lamp.

UV ON VIS ON

- ► The left icon means the Deuterium lamp (UV lamp).
- The right icon means the Tungsten lamp (Visible lamp).
- 2. If you want to turn off the UV lamp, click **UV ON**. The following dialog box will be displayed. Select **OK**.



3. Then the UV lamp will be turned off and the icon will be changed as shown below.

UV OFF	VIS ON
--------	--------

4. If you want to turn on the UV lamp, click **UV OFF**. The following dialog box will be displayed. Select **OK**.

UV Co	ontrol
D2 I	Lamp will be turned on. It takes about 30 seconds. Continue?
	OK Cancel

- The UV lamp requires 30 seconds to be turned on completely after clicking **OK**.
- 5. Then the UV lamp will be turned on.

### I-3-9. Spectrum Radar

Display the specified range of spectrum. In the Time Based Kinetics, Enzyme Activity, Enzyme Mechanism modes, the overlay of all spectra is displayed in the Spectrum Radar window during the entire measurement.

S UV Lab - [C:#UV Lab#Simulation#Holmium Solution.qdt]	
Eile Edit Measure View Math Window Help	_   <del> </del>   ×
	JV ON VIS ON Instrument Ready
Wave Scan   Quantification   Kinetics   Bio Analysis	
Wavelength Z Equation Find Multi-Component W Thickness O Color Analysis	3
Find Peak/Valley ⊕ ℚ ℚ 및  ⊕ ↓ ↓ ← → 上	
	Spectrum R Reset Y Auto Range Set
Addr Spectrum Range Minimum Wavelength 130 nm Maximum Wavelength 1100 nm Minimum Y Value 0.2 Maximum Y Value 3.3 QK	3 2 200 400 600 800 1,000 Wavelength (nm) Spectrum List Name Date Mode Sca Sample1 04-11-2013 1 Fast 30 <
200 300 400 500 600 700 800 900 1000 1100	X
Wavelength (nm)	Experiment Setup
Name No. Peak(nm) Peak(AU) No. Valley(nm) Valley(AU)	Method 🥒 Default.mtd
Sample1 1 241.200 0.38290 2 249.970 0.11648	Accessory Type/ Single Cell Holder •
3 278.210 0.32744	Baseline Correction 🥒 No 🔹
4 287.350 0.35968	Mode Fast -
5 333.690 0.16506 6 345.400 0.14027	Spectra No. 1
Experiment Type: Find Peak/Valley	INS CAPS NUM 2015-03-13

Because of the limitation of memory, the Spectrum Radar window can display only up to 500 spectra.

- 1. By clicking the **Range Set Range Set** button, the X axis(wavelength) and Y axis (A, %T, %R and I) range are set.
- 2. Allow the auto scale of Y-axis (A, %T, %R and I) by clicking the Y Auto Y Auto button
- 3. By clicking the Reset Reset button, reset the Y-axis to the original size.

### I-3-10. Spectrum List & Comment

- Display the Name, Date, Mode, Scan No. and Integration No. of spectrum in the main window.
- Procedure
  - a. Spectrum List.
    - 1. Change a spectrum name by clicking it.

Spectrum List							
Name	Date	Mode	Sca	Inte			
Sample1	05-11-2015 1	Faster	3	1			
Sample2	05-11-2015 1	Faster	3	1			
4							
Spectrum Comment							

2. Enter a new name.

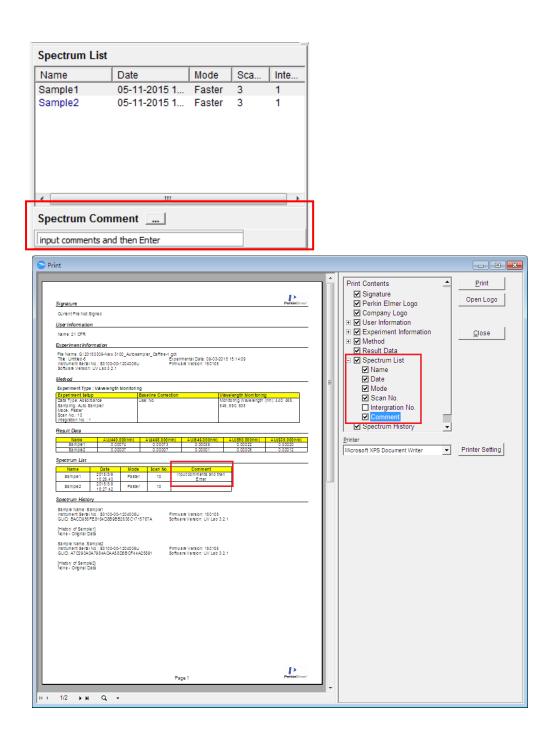
Spectrum List							
Name		Date	Mode	Sca	Inte		
test		05-11-2015 1	Faster	3	1		
Sampl	e2	05-11-2015 1	Faster	3	1		
r 🗆							
Spectrum Comment							

#### b. Spectrum Comment

1. Select the sample to comment in the spectrum list.

Spectrum List							
Name	Date	Mode	Sca	Inte			
Sample1	05-11-2015 1	Faster		1			
Sample2	05-11-2015 1	Faster	3	1			
•				•			
Spectrum Comment							

2. Enter a comment by selecting a spectrum, typing a comment and clicking **Enter**. You can check spectrum comment at the print.



### I-3-11. Experiment Setup

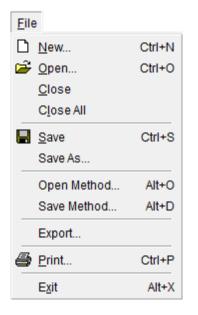
 Display and modify the experimental setup of Method, Accessory Type, Mode, Spectra No., Scan No. and Integration No.

Experiment Setup	Experiment Setup					
Method _/ Default.mtd	1					
Accessory Type	/	Single Cell Holder	•			
Baseline Correction		No	•			
Mode	Fas	ster		-		
Spectra No.	1					
Scan No.	10					
Integration No.						

Parameter	Function
Method	See V. Experiment Method for more details.
Accessory Type	Choose an accessory. Refer to each accessory manual for more details.
Baseline Correction	Choose Baseline Correction Yes or No. See V-1-1. Wavelength Monitoring for more details
Mode	Fast, Faster, Fastest and User defined Values may also be set for each mode. See <b>IV-4-1. Mode</b> for more details.
Spectra No.	See V-1-1. Wavelength Monitoring for more details.
Scan No.	See V-1-1. Wavelength Monitoring for more details.
Integration No.	See V-1-1. Wavelength Monitoring for more details.

# II. File Menu

The File menu includes commands to perform general file functions as shown in the following table.



Command	Function	
New	Open a new window	
Open	Open saved data	
Close	Close the current window	
Close All	II Close all windows	
Save	Save data	
Save As	Save data using a new file name	
Open Method	Open a saved method	
Save Method	Save a method	
Export	Export data to another program	
Print	Print results	
Exit	Exit UV Lab Software	

### II-1. New

■ Use the New command to open a new window.

#### Procedure

1. Select **New** to open a new window.

S New				×
Title		Untitled-2		<u>0</u> K
Comment				Cancel
Sample Nar	ne	Sample		
Experiment	Туре	Wavelength Monitoring	•	

2. Enter a Title and Comment (if desired), Sample Name (if desired) and select the

**Experiment Type**. If you do not enter a title, the title will be assigned automatically as [Untitled-1], [Untitled-2]....And if you do not enter a sample name, the sample name will be assigned automatically as [Sample 1], [Sample 2]....

3. Click OK.

# II-2. Open

- Use the Open command to open data in a file.
- Procedure
  - 1. Select a file to open, the title, date, comment, experiment type and spectrum is displayed.
  - 2. Select Open.
  - 3. The selected files can be displayed in a new window by clicking **Open in New Window** at the bottom of the box.

S Open	×
Look in: 🚺 Data	▼ ⇔ 🗈 💣 💷
1.qdt	
File name:     1.gdt       Files of type:     All Files (*.gdt)	Cancel
Title: EP Test Date: 4/10/2013 5:46:46 PM Experiment Type: Find Peak/Valley Comment:	3 2 1 0 200 400 600 800 1,000 Wavelength (nm)
Open in New Window	

The following file type options are available:

File Type	Description
All Files (*.*)	All kinds of files
UV Lab All Files	All kinds of UV Lab files (*.gdt, *.qdt, *.kdt,
	*.bdt, *.rdt, *.sdt, *.mgdt, *.akt)
UV Lab Wave Scan Files (*.gdt)	Wave Scan / Color Mode Sample/ MCA Mode
	Sample data
UV Lab Quantification Files (*.qdt)	Quantification Experiment data
UV Lab Kinetics data Files (*.kdt)	Time Based Kinetics & Temperature Based
	Kinetics data
UV Lab Bio Files (*.bdt)	Bio Mode Experiment data
UV Lab Color Target Files (*.sdt)	Color Mode Target data
UV Lab Color Standard Files (*.agdt)	Color Mode Standard data
UV Lab MCA Standard Files (*.mgdt)	MCA Mode Standard data
UV Lab Thickness Files (*.rdt)	Thickness Mode data
UV Lab Ultra Kinetics data Files (*.akt)	Ultra Kinetics data

### II-3. Close

- Use the Close command to close a window.
- Verify the data was saved before closing the window.

### II-4. Close All

Use the Close All command to close all windows.

### II-5. Save

- Use the Save command to save the data in the current window.
- Procedure
  - 1. To save data, select a folder to save data in, enter a file name, and select **Save**.

Save	×
Look in: 🕕 Data	▼ ← 🗈 💣 💷 ▼
1.gdt	
File <u>n</u> ame:	Save
Files of type: General Files (*.gdt)	✓ Cancel

Please refer to **II-2 Open** for file types.

# II-6. Save As

- Use the Save As command to save data using a new file name.
- Procedure
  - 1. To save data with a new file name, select a folder to save the file in, enter a file name and click **Save**.

Save As					X
Comp	outer 🕨 Local Disk (C:) 🕨 U	IV Lab ► Data 🗸	Search Data		Q
Organize 👻 New fo	older				(?)
Mesktop	<ul> <li>Name</li> </ul>	^	Date modified	Туре	
🗼 Downloads 🗐 Recent Places	1.gdt		4/11/2013 1:20 PM	GDT File	
<ul> <li>Libraries</li> <li>Documents</li> <li>Music</li> <li>Pictures</li> <li>Videos</li> <li>Computer</li> </ul>	E				
<b>•</b>					-
File <u>n</u> ame:					-
Save as type: Ge	eneral Files (*.gdt)				•
Hide Folders			<u>S</u> ave	Cano	el

Refer to **II-2. Open** for file types.

# II-7. Open Method

- Use the Open Method command to open a method stored in a file.
- Procedure
  - 1. To open a method, select a file to open and select **Open**.

S Method Op	ben		×
Look in: 📗	Method	- + E (	* 📰 🕶
Default.n			
•			+
File <u>n</u> ame:			Open
Files of type:	Method Files(*.mtd)	▼	Cancel

## II-8. Save method

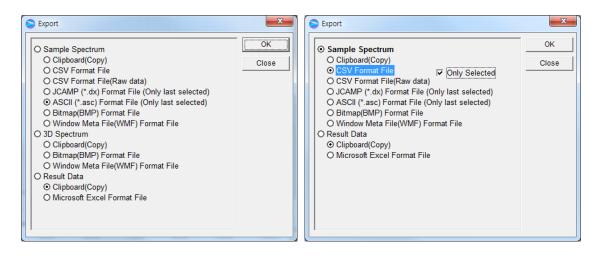
- Use the Save Method command to save the current setting for the data collection and processing methods.
- Procedure
  - 1. To save a method, enter a file name and select Save.

S Method Save	×
Look in: 🕕 UV Lab	▼ ← 🛍 💣 💷 ▼
AutoSave Calib Data Diag Help Image	Log Manual MultiStick Vucleic Acid Method Protein Method RawSave
< III	۱.
File <u>n</u> ame:	Save
Files of type: Method (*.mtd)	▼ Cancel

2. Method file extensions are automatically assigned as \*.mtd.

# II-9. Export

- Use the Export command to export the data to another program such as Microsoft Excel, or other Windows programs.
- Procedure
  - 1. Click **Export**. Choose the desired format type, as shown, and select **OK**.



File Type	Description
Export Sample Spectrum	
Clipboard(Copy)	Copy the spectrum as the picture
CSV Format File	Export a spectrum as a *.csv file. The interval
	between wavelengths is determined by the
	sampling interval. See VIII-4. Display
	Function Tools for more details.
Only Selected	Cehck: Export an only selected data.
CSV Format File (Raw data)	Export an original spectrum as *.csv file
JCAMP (*.dx) Format File (only last selected)	Export an original spectrum as *.dx file
ASCII (*.asc) Format File (only last selected)	Export an original spectrum as *.asc file
Bitmap(BMP) Format File	Export a spectrum as *. bmp file
Window Meta File(WMF) Format File	Export a spectrum as *.wmf file
Export 3D Spectrum	
Clipboard(Copy)	Copy the 3D spectrum as the picture

Clipboard(Copy) Bitmap Format File Window Meta File Format File

#### **Export Result Data**

Clipboard(Copy) Microsoft Excel Format File Copy the result data Export the result data as MS Excel format file

Export a 3D spectrum as \*.bmp file

Export a 3D spectrum as \*.wmf file

# II-10. Print

- Use the Print command to print or preview data in the current window.
- Procedure
  - 1. The following print-preview window will be displayed.
  - 2. Select the required print contents and select **Print**.

Print Contract Contra	
An	Print Contents         Print           Perkin Elmer Logo         Open Logo           © Company Logo         0           8 ⊡ User Information         10           19 ⊡ Werkind         ©           10 ⊡ Spectrum List         ©           10 ⊡ Sample Spectrum         0
Number     Number       With Mark Stream     With Mark Stream       With Mark Stream	Printer DocuCentre-IV C2263  Printer Setting
еца III р. н. О	•

Function	Description
Pre Page	Pre Page allows you to go back to the previous page
Next Page	Next Page allows you to go to the next page
Zoon In	Zoom In allow you to maximize the window
Zoom Out	Zoom Out allows you to revert the maximized the window to
	the standard size

- NOTE: User can change the company logo.
  - a. Click **Open Logo**.
  - b. Select the desired company logo. The selectable logo file is \*.bmp file and the recommended size is 110x50 pixels.
  - c. Check the company logo is changed.

## II-11. Exit

■ Use the Exit command to close UV Lab Software.



\_\_\_\_

-<>--

# III. Edit Menu

The Edit Menu includes commands to perform spectrum functions as shown in the following

ble.		
Edit		
🔊 Undo	Ctrl+Z	
X Cut Spectrum	Ctrl+X	
🗈 Copy Spectrum	Ctrl+C	
🔁 Paste Spectrum	Ctrl+V	
X Delete Spectrum	Delete	
Delete All	Ctrl+D	
SelectAll	Ctrl+A	
Command	Function	
Undo	Undo the previous edit operation	
Cut Spectrum	Remove the spectrum from a window	
Copy Spectrum	Copy the spectrum in a window	
Paste Spectrum	Paste the spectrum into a window	
Delete Spectrum	Delete the spectrum from a window	
Delete All	Delete all spectra from a window	
Select All	Select all the spectra in a window	

## III-1. Undo

- Use the Undo command to undo a previous edit operation.
- Procedure
  - 1. To undo an edit, click **Undo** in the Edit menu.

# III-2. Cut

- Use the Cut command to remove the selected spectra and place the cut items on the clipboard.
- Procedure
  - 1. Select a spectrum to cut.

- Select Cut in the Edit menu. If there are several spectra in the window, click Spectrum List to choose a particular spectrum to cut.
- 3. Place the cut spectrum in another location using the **Paste** command.

# III-3. Copy

- Use the Copy command to duplicate the selected items onto the clipboard.
- Procedure
  - 1. Select a spectrum to copy and click **Copy** in the Edit menu.
  - 2. Paste the copy into another location using the **Paste** command.

# III-4. Paste

- Use the Paste command to place a cut or copied item in the desired location.
- Procedure
  - 1. Cut or copy a spectrum to paste into a window.
  - 2. Select **Paste** in the selected window. Copies of the same item can continue to be pasted until another item is cut or copied.

# III-5. Delete

■ Use the Delete command to delete the selected spectrum from the active spectrum window.

- Procedure
  - 1. Select a spectrum to delete or click **Select All** to delete all spectra in the window.
  - 2. Click Delete.

# III-6. Select All

- Use the Select All command to select all spectra in the active spectral window.
- Procedure
  - 1. To select all, click Select All.

The Measure menu includes commands to perform measurement and diagnostic functions as shown in the following table.

Meas <u>u</u> re		Command	Function
🗍 Run Blank	Alt+B	Run Blank	Collect a blank spectrum
Run Sample	Alt+S	Run Sample	Collect a sample spectrum
Method Validation	Alt+M	Method	Set the mode and data collection parameters
Options		Validation	Verify instrument performs
Instrument	•	Options	Select measurement conditions [Mode, Instrument settings, etc.]
		Instrument	Check electronic and optical components

### IV-1. Run Blank

- Use the Blank command to collect a new blank spectrum.
- Procedure
  - 1. Place a blank in the sample holder.
  - 2. Select Blank.

Measure a new blank spectrum each time parameters for an experiment are changed.

## IV-2. Run Sample

- Use the Sample command to measure a sample.
- Procedure
  - 1. Place a sample in the sample holder.
  - 2. Select Sample.

This icon is activated after a blank is measured.

### IV-3. Method

- Use the Method command to set modes and parameters to control data collection.
- Procedure
  - 1. Select File menu.
  - 2. Select New. Select Experiment Type.

Quant	<u></u> K
	Cancel
Sample	
Quantification Standard	•)
	Sample

- 3. Click OK. See V. Experiment Method for more details.
- 4. The following method window is displayed. Set each parameter and click OK.

Method - C:#UV Lab#Default.mtd						
Experiment Type: Quantification Standard 🖨 Open 🖬 Save						
Experiment Setup »						
	line Correction tification Standard					» ×
	ysis Name		TEST			٦.
Con	centration Unit		ug/ml			
Use	Wavelength (nm)		880			
Stan	dard Replicate No.		1			
Sam	ple Replicate No.		1			
Curv	e Zero Offset		No			•
Curv	e Order		1			•
Deri	vative Order		0			•
<b>/</b> S	tandard Concentrat	ion				
		<u>S</u> ave as D	efault	<u>0</u> K	<u>C</u> ancel	
				~		_

5. Open or save a method using the and licons. The method will be saved as a \*.mtd file.

# IV-4. Options

Use the Options command to select the measurement and instrument settings, and for automatic interface setup.

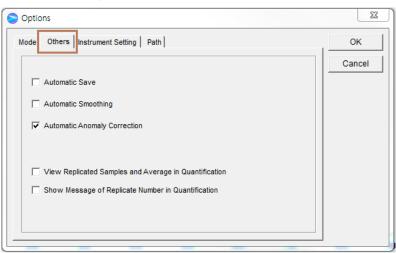
### IV-4-1. Mode

 Set customized parameters for each mode by entering a value in each box as shown and click OK. Default settings are shown below. Refer to V-1-1. Wavelength Monitoring for more details.

Node Others I	nstrument Setting Path	Faster	OK Cancel
Spectra No.	1	Spectra No. 1	
Scan No.	1	Scan No. 10	
Integration No.	1	Integration No.	
Fast Spectra No. Scan No. Integration No.	1 30 1		

### IV-4-2. Others

Use automatic function to save, smooth and correct data automatically. Select the functions to apply and select **OK**.



• If **Automatic Save** is checked:

- 1. An [AutoSave] folder is created under the UV Lab folder.
- 2. Acquired data is saved in the AutoSave > untitle-#(Date, Time) folder automatically as sample name .csv and date.time.csv.

- Acquired data is saved in AutoSave > PEData folder as \*.bak file. If the extension is changed from '\*.bak' to the extension of UV Lab files, e.g. \*.gdt etc., you can open this backup file from UV Lab software.
- Automatic Smoothing: Smoothes data automatically. In Automatic Smoothing, the Window Size and Polynomial Degree set is adjustable. Refer to VII-1. Smoothing for more details.
- Automatic Anomaly Correction: Corrects anomaly peaks automatically.
- View Replicated Samples and Average in Quantification: Refer to V-2.
   Quantification Mode for more details.
- Show Message of Replicate Number in Quantification: Refer to V-2.
   Quantification Mode for more details.

### IV-4-3. Instrument Setting

- Use instrument setting to setup the interface and peristaltic pump when necessary. Select **OK** when finished.
- As to the Peristaltic Pump Setup, refer to **Auto Sipper manual**.

S Options	23
Mode Other, Instrument Setting Path	ОК
Peristaltic Pump Setup	Cancel

### IV-4-3. Path

■ Use Path to designate the default data or method folder. Select **OK** when finished.

Options			63
Mode Others Instrument Setting Default data storage folder C: C: UV Lab	Path	Default method storage folder C: C: UV Lab	OK
Data		A Method	

## IV-5. Instrument

- The Self Diagnostics functions are important for ensuring optimum performance of the system.
- All electronic and optical parts of the system are automatically checked at startup, or on demand, to ensure the instrument is performing to specification.

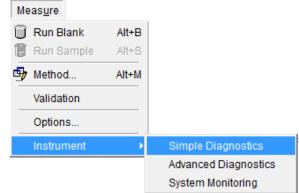
### **IV-5-1. Simple Diagnostics**

- Simple Diagnostics provides a limited set of diagnostics tests.
- Simple Diagnostics list

General	Shutter & Filter Speed measurement Check Dark Current Lamp Intensity evaluation Check Wavelength Check Noise level
---------	--

#### Procedure

1. To perform a Simple Diagnostics test, select **Simple Diagnostics** in the **Measure** menu.



2. Select Start.

Simple Diagnostics		×			
Current Test					
Status : Ready					
_ Total Test					
	[				
Open	Start	Close			

3. The following dialog box shows the status of the Simple Diagnostics.

Simple Diagnostics	×
Current Test Status : Ready	
Total Test	
Open	Start Close

4. Simple Diagnostics is finished.

UV Lab	×
End of Simple	e Diagnostic Test.
	OK.

5. Select View Result to view test result.

Simple Diag	nostics		×			
Current Test						
Result : 0.0001						
- Total Test -						
Open	View Result	Start	Close			

 Select Print or Save to print or save the results are required. The results of the Simple Diagnostics test are saved as month-day-year (hour-min)-S.dgs in the Diag folder of UV Lab folder.

	Simple Diagnostics R	esult	
I. Instrumental Inf	ormation		
Instrument Serial No.	465K5050501	DefIntNo.	1500
Firmware Version	130716	ScanNo.	10
Software Version	UV Lab 4.0.0	IntNo.	1
	<u> </u>	Partial Trans.	0
Deuterium Peak Char	1 486.0 nm 3119	Start Chan.	0
	656.1 nm 4962	End Chan.	0
		Dark Sub.	200
		Dark Cal.	0
		Rev. Data	0
		Save Raw	0
0.11	0.440505-0.20000		405 0007
Calib. parameters	-2.14653E6.32092	9E0.9836773	185.9937
Multicell Step		-	-
ARS Step		-	
2. Diagnostic Tes Test	t Results Permitted Value	Result	Status
Shutter Time	10 msec < Result < 80 msec	14.1 msec	PASS
Dark Current	1000 cnt < Result < 3000 cnt	2013 cnt	PASS
Dan Ourient	Avg.Intensity > 3000 cnt	3116 cnt	PASS
Intensity		485 96 nm	PASS
	+/-02 nm at 4860 nm	400.00 mm	
	+/- 0.2 nm at 486.0 nm +/- 0.2 nm at 656.1 nm	656 13 nm	PASS
Intensity Wavelength Noise	+/- 0.2 nm at 656.1 nm	656.13 nm	PASS
		656.13 nm 0.00002	PASS PASS

7. To open a saved result, select **Simple Diagnostics** in the **Measure** menu. Click **Open**.

Simple Diagno:	stics
Current Test	
Status : Read	У
Total Test —	
Open	[Start] Close

8. Select the desired file and click **OK**.

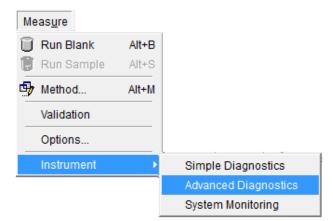
#### IV-5-2. Advanced Diagnostics

- Advanced Diagnostics provides a complete set of diagnostics tests.
- Advanced Diagnostics list

General	Shutter & Filter Speed measurement Check Cooling Fan Check Dark Current Lamp Intensity evaluation Check Wavelength Check Noise & Power
Lamp	Check Lamp On/Off and Power Indicating consumed life time of the Lamps and Time to Replace
Accessories	Check cell positions of the 8 Cell Water jacketed Cell Changer or ARS(Water Jacketed Automatic Referencing Stage)

#### Procedure

1. To perform an Advanced Diagnostics test, select **Advanced Diagnostics** in the **Measure** menu.



2. Select tests to run and click **Start**.

Division	Test	Permitted Value	Result	Status
General	Shutter Time	< 80 msec	-	N/A
	Fan Status	> 2000 rpm	-	N/A
	Dark Current	1000 cnt < Result < 3000 cnt	-	N/A
	Intensity	Avg. Intensity > 3000 cnt	-	N/A
	Wavelength	486.0 nm : +/- 0.2 nm	-	N/A
		656.1 nm : +/- 0.2 nm	-	N/A
	Noise	< 0.0001		N/A
	Power Status	12V : +/- 0.6 V	-	N/A
		-12V : +/- 0.6 V		N/A
		5V : +/- 0.25 V	-	N/A
✓ Lamp	On/Off Check	Deutrium Lamp On/Off (wait for 30sec)	-	N/A
		Tungsten Lamp On/Off		N/A
	Lamp Time	Deutrium Lamp : < 2000 hrs	-	N/A
		Tungsten Lamp : < 10000 hrs	-	N/A
	Lamp Power	Deutrium Lamp [0 V] : +/- 0.30 V		N/A
		Tungsten Lamp [5 V] : +/- 0.25 V	-	N/A
8 Multicell	Reset Multicell	Reset Multicell		N/A
	Position Check	Position Check	-	N/A
		Open	01/15/2015 1 Start	0:01:31 Close

 When tests are complete, click **Print** or **Save** to print or save the results as required. The results of the Advanced Diagnostics test are saved as **month-day-year** (hour-min)-A.dgs in the **Diag** folder.

Division	Test	Permitted Value	Result	Status		
General	Shutter Time	< 80 msec	13.705 msec	PASS		
	Fan Status	> 2000 rpm	2000 rpm	PASS		
	Dark Current	1000 cnt < Result < 3000 cnt	2095 cnt	PASS		
	Intensity	Avg. Intensity > 3000 cnt	14652 cnt	PASS		
	Wavelength	486.0 nm : +/- 0.2 nm	486.01 nm	PASS		
	656.1 nm : +/- 0.2 nm					
	Noise	< 0.0001	0.000080	PASS		
	Power Status	12V : +/- 0.6 V	12 V	PASS		
		-12V : +/- 0.6 V	-12 V	PASS		
		5V : +/- 0.25 V	5 V	PASS		
🔽 Lamp	On/Off Check	Deutrium Lamp On/Off (wait for 30sec)	ON	PASS		
		Tungsten Lamp On/Off	ON	PASS		
	🔽 Lamp Time	Deutrium Lamp : < 2000 hrs	935hours	PASS		
		Tungsten Lamp : < 10000 hrs	1946hours	PASS		
	Lamp Power	Deutrium Lamp [0 V] : +/- 0.30 V	0.09 V	PASS		
		Tungsten Lamp [5 V] : +/- 0.25 V	5 V	PASS		
8 Multicell   8	Reset Multicell	Reset Multicell	OK	PASS		
	Position Check	Position Check	OK	PASS		
01/15/2015 10:15:31						
		Open Save Print	Start	Close		

4. To open a saved result, select **Advanced Diagnostics** in the **Measure** menu and select **Open**.

Division	Test	Permitted Value	Result	Status
	Shutter Time	< 80 msec	-	N/A
	Fan Status	> 2000 rpm	-	N/A
	Dark Current	1000 cnt < Result < 3000 cnt	-	N/A
	Intensity	Avg. Intensity > 3000 cnt	-	N/A
	Wavelength	486.0 nm : +/- 0.2 nm	-	N/A
		656.1 nm : +/- 0.2 nm	-	N/A
	Voise	< 0.0001	-	N/A
	Power Status	12V : +/- 0.6 V	-	N/A
		-12V : +/- 0.6 V	-	N/A
		5V : +/- 0.25 V	-	N/A
✓ Lamp	On/Off Check	Deutrium Lamp On/Off (wait for 30sec)	-	N/A
		Tungsten Lamp On/Off	-	N/A
	Lamp Time	Deutrium Lamp : < 2000 hrs	-	N/A
		Tungsten Lamp : < 10000 hrs	-	N/A
	Lamp Power	Deutrium Lamp [0 V] : +/- 0.30 V	-	N/A
		Tungsten Lamp [5 V] : +/- 0.25 V	-	N/A
8 Multicell	Reset Multicell	Reset Multicell	-	N/A
	Position Check	Position Check	-	N/A
	0/12	Open	Start	Close

5. Select the desired file and click **OK**.

~>

## V. Experiment Method

## V-1. General Method

- This mode includes the following Experiment types:
  - Wavelength Monitoring
  - Equation Calculation
  - Find Peak/valley
  - Thickness
  - Color Analysis (Optional)
  - Multi-Component Analysis (MCA) (Optional)
- Method parameters in this mode can be modified after a measurement is complete. For example, users can modify the parameters of a Find Peak/Valley method after the measurement is complete and monitor their effect on the results.
- Results in this mode are interchangeable. For example, data measured using
   Equation Calculation method can be opened in a Wavelength Monitoring method.
- Perform a General Method measurement as follows:
  - 1. Select measurement mode.
  - 2. Set method parameters.
  - 3. Measure blank.
  - 4. Measure samples.
  - 5. Save or print results as required.

#### V-1-1. Wavelength Monitoring Mode

- Use Wavelength Monitoring to collect data over the full spectral range of the instrument, or a specified interval. Perform this type of experiment.
- Procedure
  - 1. Select New.
  - 2. Select **Wavelength Monitoring** in Experiment Type. The method dialog box will be displayed.

Experiment Setup		ž
Data Type	Absorbance	•
Sampling	Single Cell Holder	Ŧ
Mode	Faster	•
	1	
	10	
	1	
# Monitoring Wavele	ngth	

- 3. Setup the Experiment Setup parameters as follows:
  - a. Data Type: Select the units of the Y-axis. Absorbance, Transmittance or Reflectance.
  - b. **Sampling**: Select a sampling accessory.
  - c. **Mode**: Select test mode (Fast, Faster, Fastest) with fixed parameters for Spectra No., Scan No. and Integration No. or select User Defined to customize parameters.
  - d. Spectra No.: Determines how many times the sample is measured.
  - e. Scan No.: Determines how many scans are performed during a data collection.
     If the scan number is greater than one, the system averages all the collected data from all or the scans. This increases signal-to-noise ratio and total collection time. In general cases, we recommend setting this parameter to "30".
  - f. Integration No.: Determine the length of time a sample is exposed to the light source.A high integration number increases the signal-to-noise ratio.

## Integration No vs Integration Time

Integration time(ms) = 20.48 x Scan No x (integration NO + Default integration NO)

# The integration number needs to be chosen carefully so as not to saturate the spectrum.

#### P ightarrow What is Saturation?

When the integration number is too big, portions of the spectrum can be cut off because the detector reaches its maximum detection limit of light. This makes the difference between blank and sample data meaningless. The maximum value of Y axis should not exceed "60000" count.

4. Click **Baseline Correction** and set the baseline correction parameters.

Method - C:\UV Lab\Defau	lt.mtd		23
Experiment Type: Wavel	ength Monitoring	🗃 Open 📕 S	Save
Experiment Setup			» *
Baseline Correction Use	Yes		
Туре	Single Poin	t	-
Wavelength 1 (nm)	600		
	800		
<u>S</u> a	ve as Default	<u>0</u> K <u>(</u>	<u>ancel</u>

- ▶ Refer to the next page for more details.
- 5. Click **Monitoring Wavelength** and then the following dialog box will be displayed.

	Methoo	d - C:₩UV Lab₩Defau	t.mtd				23
	Experi	ment Type: Wavel	ength Monit	oring	🗃 Open	🔒 Sav	е
		ent Setup Correction					» »
	Waveler	ngth Monitoring					×
	/ Mor	itoring Wavelength					
(	🔵 Moni	toring Wavelength	×				
	<u>I</u> nsert	<u>D</u> elete					
	No.	Wavelength(	חה)				
١.	1		440				
ł.	2		465				
	3		546 590				
	5		635				
		<u>о</u> к	<u>C</u> ancel				

- 6. Enter the wavelengths to be monitored using **Insert** or **Delete** and click **OK**.
- 7. After completing parameter setup for Experiment Setup, Baseline Correction and Wavelength Monitoring, click **OK**.

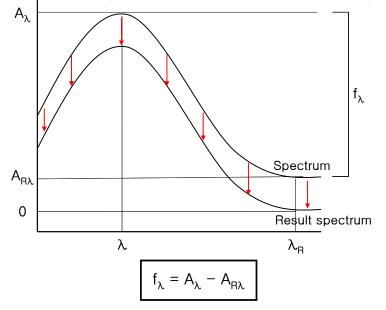
- 8. Measure the blank.
- 9. Measure the samples.
- 10. Save or print spectrum and results as desired.
  - Wavelength Monitoring | 🏵 🍳 🎗 師 🖶 🛻 🔶 M 3 23 🝃 Edit Sample Absorbance (AU) ок Old Name: Sample New Name: Sample Cancel 0 400 500 700 800 200 300 600 900 1000 1100 Wavelength (nm) Name AU(440.000nm) AU(465.000nm) AU(546.000nm) AU(590.000nm) AU(635.000nm) -0.00403 -0.00313 -0.00093 Sample 0.08837 0.06396
- ▶ To Edit a sample name, double click the sample line to be changed in the list.



Baseline Correction is a technique that can be used to improve the precision of results by minimizing the effects of any changes that cause a linear baseline shift, for example, a drift in lamp intensity. This technique is particularly useful for samples with low absorbance. The value is calculated by method suited to each condition. Result spectrum is presented that eliminates baseline values from the absorbance. There are three methods of calculating the baseline values.

#### Single Point

Use when the baseline shift is the same at all wavelengths. A reference wavelength on the baseline is selected. Baseline value is eliminated by subtracting the absorbance at the reference wavelength from the absorbance of the full wavelength.



 $f_{\lambda}$  is the function result at wavelength  $\lambda$ 

 $A_{\lambda}$  is the absorbance at wavelength  $\lambda$ 

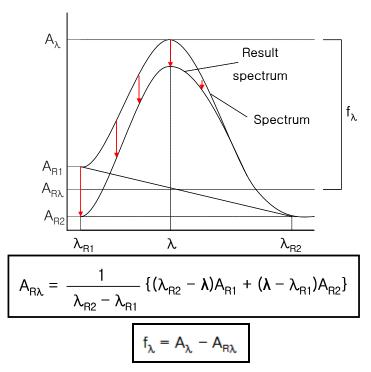
 $A_{R\lambda}$  is the absorbance at reference wavelength  $\lambda_R$ 

#### Range Average

An extension of the single point method, it is used when it is difficult to select a reference wavelength as a point. The reference wavelength replaces the single wavelength absorbance value with the average absorbance value over a wavelength range.

#### Three Points

Use to correct a slant baseline. The absorbance values from the two reference wavelengths,  $A_{R1}$  and  $A_{R2}$ , define a straight line, which is used to calculate the reference absorbance( $A_{R\lambda}$ ) at the wavelength( $\lambda$ ). Resulting spectrum are calculated using the following equation.



$$\begin{split} f_\lambda \text{ is the function result at wavelength } \lambda \\ A_\lambda \text{ is the absorbance at wavelength } \lambda \\ A_{R\lambda} \text{ is the reference absorbance at wavelength } R_\lambda \end{split}$$

#### V-1-2. Equation Calculation Mode

- Use Equation Calculation to collect data for a calculated result using a specified equation.
- Procedure
  - 1. Select New.
  - 2. Select Equation Calculation in Experiment Type. The method dialog box is displayed.

S Method			×
Experiment Type: Equation	Calculation	🚔 Open 📕 Sav	/e
Experiment Setup Baseline Correction			» »
Equation Calculation			*
Equation Name	Ratio		
Equation Unit	Au		
"/ Equation Expression			
Save	as Default	OK Car	ncel

- 3. Setup Experiment Setup and Baseline Correction parameters. See V-1-1. Wavelength Monitoring for more details.
- 4. Click **Equation Calculation** and set parameters as follows.
  - a. Equation Name: Enter the equation name.
  - b. **Equation Unit**: Enter the units of the Y-axis. Absorbance, transmittance or reflectance.
- 5. Click **Equation Expression** The following dialog box will be displayed.

Insert	Delete					
No. 1	Wavelength(nm) 300				Wav	ve1+Wave2
2	400	Wave1				
		Wave2	Back	space	CI	ear
		Wave3	7	8	9	1
		Wave4	4	5	6	*
		Wave5	1	2	3	-
		Wave6	0		۸	+
		Wave7		(		)
		Wave8	A	BS	E	XP
		Wave9	LN	LOG10	SC	RT

6. Enter the wavelengths, which will be monitored using Insert and Delete.

on absorbance result		
e absorbance result		
	at the selected v	vavelength.
on can contain up t	o nine.	
ct		
У		
te the absolute val	le	
te e(exp)		
te the Natural loga	rithm	
te the common log	arithm	
te the square root		
	on can contain up to ct y ate the absolute valu ate e(exp) ate the Natural loga	y ate the absolute value ate e(exp) ate the Natural logarithm ate the common logarithm

7. Enter the equation to apply to the data using the calculator keys and click **OK**.

- 8. After setting parameters for Experiment Setup, Baseline Correction and Equation Calculation, select **OK** in the method setup window.
- 9. Measure the blank.
- 10. Measure the sample.
- 11. Save or print the data and spectra as required.

#### V-1-3. Find Peak/Valley Mode

- Use Find Peak/Valley to determine the maxima and minima of Y-values in the defined wavelength range of the spectrum.
- Procedure
  - 1. Select New.

2. Select **Find Peak/Valley** in the Experiment Type. The method dialog box is displayed.

S Method	×						
Experiment Type: Find Peak/Valley							
Experiment Setup »							
Baseline Correction Find Peak/Valley	<u>*</u>						
Automatic Find	Yes 🔹						
Absorbance Threshold (AU)	0.02						
Transmittance Threshold (%)	2						
Intensity Threshold (cnt.)	10						
Find Peak	Yes 👻						
Peak No.	7 🔹						
Find Valley	Yes 👻						
Valley No.	7 🔹						
Start Range (nm)	190						
End Range (nm)	1100						
<u>S</u> ave as E	Default OK Cancel						

- Setup experiment and baseline correction parameters. See V-1-1. Wavelength Monitoring for more details.
- 4. Setup peak/valley parameters.
  - a. Automatic Find: Select Yes or No for the automatic location of peaks and valleys.
  - b. **Absorbance Threshold (AU)**: Enter an absorbance value for the threshold. Peaks about this threshold are included in the result window.
  - c. **Transmittance Threshold (%)**: Enter a transmittance value for the threshold. Peaks about this threshold are included in the result window.
  - d. **Intensity Threshold (cnt.)**: Enter an intensity value for the threshold. Peaks about this threshold are included in the result window.
  - e. Find Peak: Select Yes or No for finding peaks.
  - f. Peak No.: Select the number of peaks to find.
  - g. Find Valley: Select Yes or No for finding valleys.
  - h. Valley No.: Select the number of valleys to find.
  - i. Start Range (nm): Enter the start wavelength for the desired range to search.
  - j. End Range (nm): Enter the end wavelength for the desired range to search.
- 5. After setting parameters for Experiment Setup, Baseline Correction and Find Peak/Valley, click **OK**.
- 6. Measure the blank.

- 7. Measure samples. Peaks and valleys are found automatically.
- For manual peak finding, use the following icons to pick peaks and valleys or seek data.
   Select Pick Peak/Valley icon. See VIII-3. Pick Peak/Valley for more details.

채 Pick Peak 🛛 🚧 Pick Valley

These icons are not activated if Automatic Find is specified.

9. Save or print data and spectra as required.

#### V-1-4. Thickness Mode

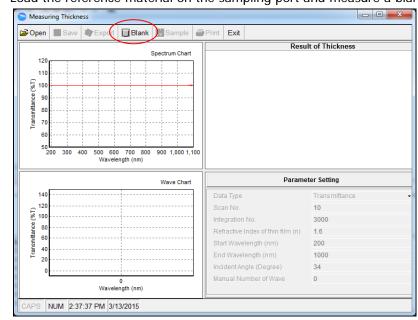
- Use Thickness Mode to measure the thickness of a film.
- The **Reflectance accessory** is required to perform reflectance measurements in this mode.
- Procedure
  - 1. Select New.
  - 2. Select **Thickness** in the Experiment Type. The following window is displayed.

S Measu	ring Thickne	ess										×
🗳 Open	Save	Export	🗍 Blank	I. Sample	6	Print	Exit					
140			.1	Spectrum Chart						Resul	t of Thickness	
(120 (1%) 100 2000 2000 2000 2000 2000 2000 2000		Wavele	0 ngth (nm)									
				Wave Chart					F	Paramet	er Setting	
140 (120 (120) 300 300 80 60 10 10 20 0		Wavele	0 ength (nm)			Sca Inte Ref Sta End	rt Wave d Wavel ident An	Inde eleng lengt ngle (	x of thin fill th (nm) th (nm) (Degree) er of Wave	m (n)	Transmittance 10 3000 1.6 200 1000 34 0	
CAPS	NUM 2:37	7:37 PM 3/1										 

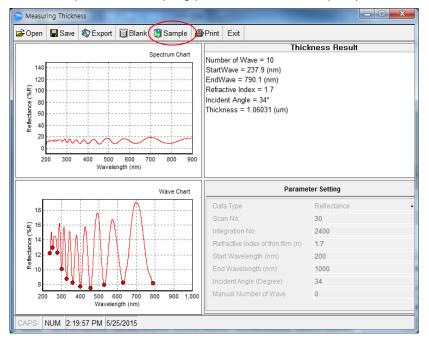
- 3. Click **Parameter Setting** as follows.
  - a. Data Type: Choose Reflectance or Transmittance.
  - b. **Scan No.:** This parameter determines how many scans are performed during a data collection. If a number of greater than one is specified, the collected data is averaged.

This increases the signal-to-noise ratio and also the total collection time.

- c. Integration No.: This parameter determines the length of time a sample is exposed toLight source. A high integration number increases the signal-to-noise ratio. However,you must control the integration time carefully so as not to saturate the spectrum.
- d. **Reflective index of thin film (n):** Enter the known value for the coating material of the thin film.
- e. Start Wavelength (nm): Enter the start wavelength for the measurement.
- f. End Wavelength (nm): Enter the end wavelength for the measurement.
- g. Incident Angle (Degree): 34 ° (fixed for Reflectance Accessory)
- h. **Manual Number of Wave (M.N.W)**: The required fringe (wave) numbers are used to calculate the thickness between start wavelength and end wavelength. M.N.W. can be modified after the measurement is complete.
  - i . 0: Automatic fringes (wave) are detected and counted automatically. The software identifies the valleys closest to the user entered Start Wavelength and End Wavelength values, counts the number of fringes (wave) between them, and calculates the thickness.
  - ii. If the software can not locate the appropriate fringe (wave) shapes and locations, set a value more than 2 in the Manual Number of Wave box. See point 9, below.
- 4. Click OK.



5. Load the reference material on the sampling port and measure a blank spectrum.



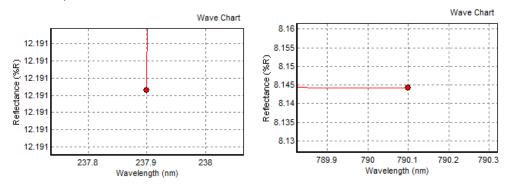
6. Load a sample on the sampling port and measure sample spectrum.

7. Film thickness calculations are performed automatically using the following equation.

$$d = \frac{w}{2\sqrt{n^2 - \sin^2\theta}} \cdot \frac{\lambda_1 \cdot \lambda_2}{\lambda_2 - \lambda_1} \cdot \frac{1}{1000} \,(\mu m\,)$$

Where: d = film thickness

- w = number of fringes (waves) between  $\lambda_1$  and  $\lambda_2$
- n = reflective index of thin film
- $\theta$  = Angle of incidence
- $\lambda_1 \& \lambda_2 = \text{peak wavelengths (nm)}$
- 8. **Zoom in** this extracted spectrum to see start and end wave positions precisely. In this example, the start wave is 237.9 nm and the end wave is 790.1 nm.



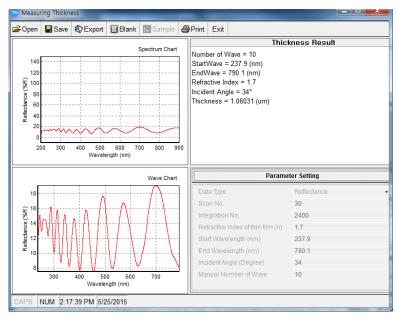
 In some cases, the software may not find the peak because the wave of the spectrum is too small. In this case, thickness can be calculated manually.

Click **Experimental Setup** and zoom in on the area of the wave in the spectrum.

- a. Count the number of waves and define the start and end wavelength.
- To compare the manual result with the result of an automatic calculation of thickness, enter the value of the start wavelength (237.9 nm) and end wavelength (790.1 nm) and click OK.

ОК							
Data Type	Reflectance						
Scan No.	30						
Integration No.	2400						
Refractive Index of thin film (n)	1.7						
Start Wavelength (nm)	237.9						
End Wavelength (nm)	790.1						
IncidentAngle (Degree)	34						
Manual Number of Wave	10						

c. The result of the manual thickness calculation is shown. In this example, the thickness calculated is 1.06031 um and is the same as the automatic thickness calculation.



#### V-1-5. Color Analysis Mode (Optional)

- Use Color Analysis to measure Color Scale values including CIE color (L\*, a\*, b\*) and Hunter
  - (Lab). The test can be used for:
  - Quality Control of the color difference
  - Variable Color Indices
  - Confirming a Yxy chromaticity diagram.

#### Procedure

- 1. Select New.
- 2. Select **Color Analysis** in the Experiment Type. The method dialog box is displayed.

S Color Analysis											
<b>≌Open -</b> Sa	ave • 🔷 Export •	🗍 Blank	🗂 Standard	E Target	I↓ Sam	nple 🥔 Print •	Exit				
Parameter Setti	ng OK		Result Spe	ectrum		Result (	hromaticity I	Diagram		Result Concentra	tion Chart
Instrument Soup		120 110 90 (L2) 90 100 90 10 50 20 10 20 20		10 800 ngth (nm)	1,000	0.8 0.7 0.6 0.5 0.4 0.3 0.2 0.1 0 0	2 0.4 x	0.6 0.8	∀H&	0 Yelowi	ness
Result D	ata View					Co	lor Result Va	lues			
1) Color Quality F	Result	No 1	Name	d∗L	d*a	d∗b C	IE dE∗ab	L+ a	3*	b*	
2) Color Indices F	Result			1							
Whiteness	•										
Target Color	Sample Color										
		• D \\ (	Color Samp	le Data /	APHA	. STD 🖌 Gard	ner STD	7			<u> </u>

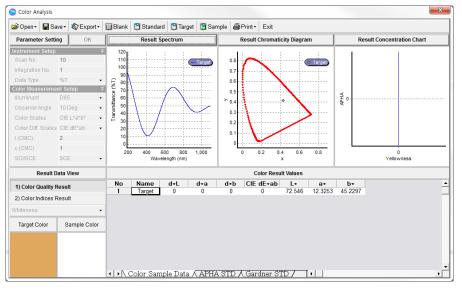
- 3. Click **Parameter Setting** and setup instrument parameters as follows:
  - a. **Scan No.:** This parameter determines how many scans are performed during a data collection. If a number of greater than one is specified, the collected data is averaged. This increases the signal-to-noise ratio and also the total collection time.
  - b. **Integration No.**: This parameter determines the length of time a sample is exposed to the light source. A high integration number increases the signal-to-noise ratio.
  - c. Data Type: Select Transmittance (%T) or Reflectance (%R).

# A Diffuse Reflector is required to measure the color of a sample in reflectance mode (%R).

- 4. Setup color measurement parameters as follows:
  - a. **Illuminance**: Select the appropriate illuminant. For example, if the illuminant is natural light, select D65. Options include: A, C, D50, D55, D65, F1~F12 (total 17).
  - b. **Observer Angle**: Select the Observer Angle, 2° or 10°.

- c. Color Scales: Select the Color Scales. Common choices are 'CIE Yxy' or 'CIE L\*a\*b'.
- d. Color Difference Scales: For color difference measurements, select the Color Difference Scale. CIE dE\*ab is a common choice as it returns the widely used "Delta E" value.
- e. **CMC(I:c)**: Value "I" and "c" are "2" and "1" respectively. For example, CMC(2:1) is generally used in textile and dye industry. The I, c value can only be set for CMC and BFD scales.
- f. **SCI/SCE**: The Diffuse Reflector Accessory Integrating Sphere uses a 0/d (normal illumination/diffuse viewing) geometry. All measurements with this accessory exclude the specular component of the reflection and are therefore SCE.
  - SCI: Specular Component Included (spin) Specular reflectance is included
  - SCE: Specular Component Excluded (spex) Specular reflectance is excluded USE: Comparison of instrument & visual color difference
- g. **Decimal Place**: Enter the desired number of decimal places to display in the result data.
- 5. Choose color index parameters as follows:
  - a. Whiteness: Select the Whiteness.
  - b. Yellowness: Select the Yellowness.
  - c. **Decimal Place**: Enter the desired number of decimal places to display in the result data.
  - d. **APHA Concentration**: If APHA measurement is used, enter the values of the APHA stock solution.
  - e. **Gardner Concentration**: If Gardner measurement is used, enter the values of the Garner stock solution.
- 6. When parameter setup is complete, select **OK**.
- 7. Measure the Blank Blank. This is typically a clear, colorless solution for transmission work, or the include Spectralon<sup>®</sup> disk if using the integrating sphere.

8. Measure the Target Target and check the color scale result. Only one Target can be measured in each window. All samples are compared to the Target for the purpose of calculating Delta E (dE) values in color comparison measurements.



9. Measure Samples Sample and check the color difference values.

Parameter Sett	ave• 🖏 Export•	1		rd 🛅 Targ Spectrum			Print - Exit esult Chromati	icity Diagra	n	De	sult Concentration Chart
Instrument Setur			Neount	spectrum			esur cironad	icity Diagra		110	
Scan No.	10	120 110				0.8	~				
Integration No.	1	100			Target	0.7			Target		
Data Type	%T •	90			- 2						
Color Measurem		F 80		~	- 4	0.6					
Illuminant	D65 •	8 60	1	$\langle \rangle$	- 5	0.5	1			4H 0	
Observer Angle	10 Deg -	tie 50	· • • • • • • • • • • • • • • • • • • •	/N		> 0.4				8	
Color Scales	CIE L*a*b*	(1%) 80 70 60 50 40	1		$\sim$	0.3	1				
Color Diff. Scale	s CIE dE*ab	. 50	1 /			0.2					
I (CMC)	2	10				0.1	$\sim$				
c (CMC)	1	0				0	<u>¥</u>				
SCI/SCE	SCE	20		600 800 relength (nm)	1,000	0		.4 0.6 ×	0.8		0 Yellowness
CONCOL	002			cicity (iiii)				^			T CHOWING 30
Result	Data View						Color Resu	ult Values			
1) Color Quality	Result	No	Name	d+L	d∗a	d∗b	CIE dE*ab	L*	a*	b∗	
2) Color Indices	Pocult	2	Target 1	0 -0.0002	0 -0.0008	0 0.0007	0 0.0011	72.546 72.5458	12.3253 12.3245	45.2297 45.2304	
2) Color maices	rtesuit	3	2	-0.3333	0.0896	0.5941	0.6871	72.2127	12.3245	45.8238	
	•	4	3	-0.6688	0.1794	1.2098	1.3939	71.8772	12.5047	46.4395	
Whiteness		5	4	-1.0067	0.272	1.8501	2.1237 2.8769	71.5393	12.5973 12.6908	47.0798	
Whiteness Target Color	Sample Color	6	5	-1.3476	0.3655						

- 10. Print, save and export data as required.
  - a. **Open**: Open Target, Standard or Sample data files.
  - b. Save: Save the Target, Standard or Sample data separately.
  - c. **Export**: any of the following:
    - i . Result Data from the Target/Sample to an Excel readable file (\*.csv).
    - ii. The Result Spectrum

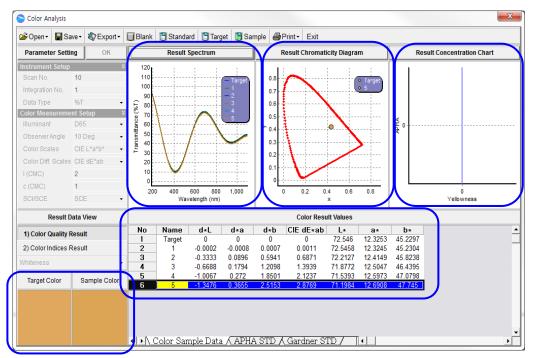
- iii. The Chromaticity Diagram
- iv. The Concentration Chart
- d. **Name**: To change the name of each data, click the name column in the color result value window.

No	Name	d*L	d∗a	d∗b	CIE dE*ab	L*	a*	b*
1	Target	0	0	0	0	72.546	12.3253	45.2297
2	1	-0.0002	-0.0008	0.0007	0.0011	72.5458	12.3245	45.2304

Change name and enter.

No	Name	d*L	d∗a	d∗b	CIE dE∗ab	L*	a*	b*
1	Target	0	0	0	0	72.546	12.3253	45.2297
2	Test	-0.0002	-0.0008	0.0007	0.0011	72.5458	12.3245	45.2304

11. Analyze and interpret your data using the information in the fields in the window described below.

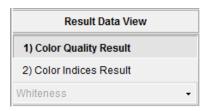


- a. Target / Sample Color: Display the color of Target and Sample.
- B. Result Spectrum: Display the transmittance or reflectance spectra of Standard / Sample. Click Result Spectrum to display these spectra in the full window.
- c. Result Chromaticity Diagram: Display the xy chromaticity diagram and position of Standard and Sample is indicated as point. Select Result Chromaticity Diagram to display this diagram in the full window.
- d. Result Concentration Chart: Display the relation between color index

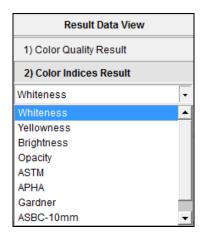
(concentration) of APHA / Gardner and their absorbance. Select

**Result Concentration Chart** to display this chart in the full window.

- e. **Color Result Values**: Display results of **Target**, **Standard**, **and Sample** in accordance with the preset parameters.
- f. **Result Data View**: Select **Color Quality Result** or **Color Indices Result** to see various color difference values.
  - i . Color Quality Result: Used to see the general color difference value.



ii. **Color Indices Result**: Used to see the result for Whiteness, Yellowness, Brightness, Opacity, ASTM, APHA, Gardner, ASBC-10, EBC-10.



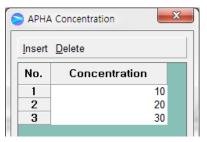
#### APHA, Gardner Measurement Procedure

To perform APHA/Gardner Measurements, follow these additional steps.

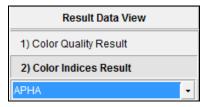
1. Check **APHA/Gardner Use** in the [Parameter Setting]→[Color Index Setup]. Click the **edit** button to setup the measurement.

Color Index Setup	×				
Whiteness	CIE				
Yellowness	ASTM D1925-7 👻				
Decimal Place	4				
APHA/Gardner	Use				
"/ APHA Concentration					
	entration				

2. Enter the concentration for Gardner or APHA standard solutions.



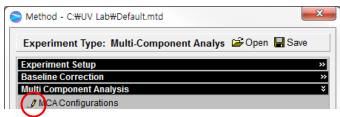
3. Click Color Indices Result and select APHA or Gardner.



- 4. Measure the Blank Blank, and Standard samples Standard.
- 5. Save Standards. (for later use)
- 6. If necessary, measure the Blank again.
- 7. If the difference value between target and each sample is needed, measure the Target.
- 8. Measure the Sample.

#### V-1-6. Multi-Component Analysis (MCA) Mode (Optional)

- Use Multi-Component Analysis (MCA) to analyse complex compounds containing several compounds. Up to 4 components in the mixture can be analysed. It is used to define the concentration of each component. Preform this test as follows:
- Procedure
  - 1. Click New.
  - 2. Select **Multi-Component Analysis** in the Experiment Type. The method dialog box will be displayed. Click **MCA Configurations.**



- 3. The standard measurement window will be displayed. Click **New** icon.
- Click Experimental Setup in the Multi-Component Analysis window. If you do not want to measure the new standards, click Open Standard and choose the saved standards data.

ık 🖪 Standard 🖨 Print 🏶 Exp	ort 📳 Go To Measure Sample
Spe	ctrum Chart
Aksortiance (4U)	overlangth (mr.)
Experiment	al Setup OK
Data Type	Absorbance -
Scan No.	10
Integration No.	1
Apply Derivative	
	1
	*/ *
	Spe (n) augusty Experiment Data Type Scan No. Integration No.

5. Setup parameters as follows.

Experimental Set	up C	OK
Instrument Setting		*
Data Type	Absorbance	•
Scan No.	10	
Integration No.	1	
Apply Derivative		
Derivative Order	1	
Multi-Component Analysis Settin	g	×
Wavelength Method	Selected Wavelength	-
"/ Wavelength Method Setting.		
Number of Standard (1~4)	4	
	•	

#### Instrument Setting

Enter the instrumental parameters. (Data Type, Scan No., Derivative)

- a. Data Type: Select Absorbance or Transmittance.
- b. Scan No.: This parameter determines how many scans are performed during a data collection. If a number of greater than one is specified, the collected data is averaged. This increases the signal-to-noise ratio and also the total collection time.
- c. **Integration No**.: This parameter determines the length of time a sample is exposed to Light source. A high integration number increases the signal-to-noise ratio.

d. **Apply Derivate:** Check ( $\sqrt{}$ ) **Apply Derivative** to obtain the data after applying derivative and enter the derivative order number (1~4).

#### Multi-Component Analysis Setting

- a. Wavelength Method: Select a calculation method for MCA.
  - i . Selected Wavelength: Click Wavelength Method Setting and enter wavelengths to use for the test and click OK. Insert and Delete can be used to

change the number of wavelengths used for the test.

Selected Wavelength Meth							
Insert Delete							
No.	Wavelength(nm)	)					
1	4	150					
2	5	550					
3	6	650					
4	1	750					
5	8	350					
	<u>O</u> K <u>C</u> a	ncel					

ii . Range Wavelength: Enter the wavelength range to use for the test and click OK.

SMCA Wavelength Method								
Ranged Wavelength Method								
From	200	nm						
То	1000	nm						
	ок	Cancel						

- b. Number of Standard (1~4): Enter the number of standards to be measured.
- c. Concentration Unit: Enter units for the standards.

d. **Standard Parameter Setting**: The following text box is displayed. Enter the concentration of each standard in the text box and select **OK**. **Insert** and **Delete** can be used to change the number of standards for the test.

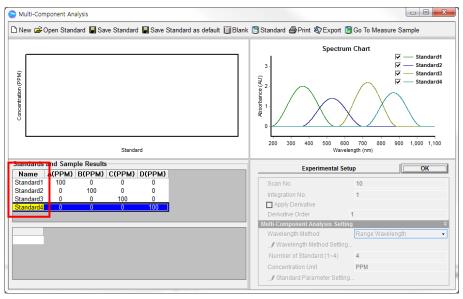
	S MCA	Standard Concentration					×	J
	<u>I</u> nsert	<u>D</u> elete						
	No.	Concentration (A)	Conentration (B) (	Concentration (C)	Concentration (D)	No.	Name	
	1	100	0	0	0	1	A	I
	2	0	100	0	0	2	В	l
	3	0	0	100	0	3	C	
1	4	0	0	0	100	4	D	
								I
								I
								l
								1
								1
					-			1
							0	ſ
							<u>OK</u> ancel	
L								1

6. After setting all experimental parameters, click **OK**.

Experimental Setu	ip	ОК
Instrument Setting		×
Data Type	Absorbance	•
Scan No.	10	
Integration No.	1	
Apply Derivative		
Derivative Order	1	
Multi-Component Analysis Setting	)	*
Wavelength Method	Selected Wavelength	•
"/ Wavelength Method Setting		
Number of Standard (1~4)	4	
	•	

7. Measure the blank Click **Blank** icon.

 Measure the standards. Click Standard Standard icon. Enter the name of the Standard and click the Enter key.



9. Save the measured standard data.

If the standard measurement value is not saved, the sample concentration is calculated with the standard values saved as Default after measuring an unknown sample.

- 10. To measure the unknown sample, save standards data and click **Go To Measure Sample**.
- 11. The sample measurement window is displayed.

ΡE	lle Edit Measure View Math Window Help	
	🖇 🖬 🥔 🕺 🛍 🛍 🗡 🕫 🕲 🔒 🗑 🕲 🗛 XT XR I 🛛 X: 740.707 Y: 3.20378	UV ON VIS ON Instrument Ready
Way	e Scan Quantification Kinetics Bio Analysis	
-	Wavelength Sequation Find Calculation Find Peak/Valley Analysis W Thickness OC Cold Analysis	or Iysis
ulti (	Component Analysis 奥风久国际小 + + 國	Spectrum R Reset Y Auto Range Se
0	3+	3]
8		2
	1	1
5		200 400 600 800 1.000
e (A		Wavelength (nm)
pane	-	Spectrum List
Absorbance (AU)		Name Date Mode Sca.
-		
	+	
		Spectrum Comment
3	<sup>1</sup>	spectrum comment
	200 300 400 500 600 700 800 900 1000 1100	
	Wavelength (nm)	Experiment Setup
lam	e A(PPM) B(PPM) C(PPM) D(PPM) Chi Square	Method / Default.mtd
anna	MITTMI DIFTMI CITTMI DIFTMI CIII SQUARE	Accessory Type / Single Cell Holder •
		Baseline Correction / No ·
		Mode Fastest
		Scan No. 1

- 12. Measure the blank.
- 13. Measure samples. Contents of each standard component in the unknown sample and its Chi Square value are displayed.
- 14. Change the name of the sample. Double-click the sample. Change the sample name and select **OK**.

Name	A(PPM)	B(PPM)	C(PPM)	D(PPM)	Chi Square
Sample1	0.0000	0.4711	0.0000	37.6810	0.0904
Sample2	0.0000	0.4069	0.0000	32.5434	0.0674
Edit Name Old Name New Name	Sample1				
			ОК	Cancel	

15. Save the data. Print or export data and spectrum as required.

Chi-square,  $\chi^2$ 

The Chi-square distribution is used in the following cases;

- a. to examine the discrepancy between an observed frequency and an expected frequency when more two results are acquired
- b. to examine whether the sample distribution corresponds to a binomial distribution or a normal distribution
- c. to examine whether two variables are independent each of other or not.

The following statistic can be used as a tool to measure the discrepancy between observed frequency and expected frequency:

$$\chi^{2} = \frac{(o_{1} - e_{1})^{2}}{e_{1}} + \frac{(o_{2} - e_{2})^{2}}{e_{2}} + \dots + \frac{(o_{k} - e_{k})^{2}}{e_{k}} = \sum_{i=1}^{k} \frac{(o_{i} - e_{i})^{2}}{e_{i}} \dots \dots \dots (1)$$

If the total frequency is "N", the following equation is formulated:

$$\sum o_i = \sum e_i = N \quad \dots \quad (2)$$

The former equation can be also expressed as:

$$\boldsymbol{x}^2 = \sum \frac{\boldsymbol{o}_i^2}{\boldsymbol{e}_i} - N \quad \dots \quad (3)$$

if  $\chi^2 = 0$ , the observed frequency corresponds to the expected frequency. exactly,

if  $\chi^2 > 0$ , they do not correspond exactly. That is, the larger the value of  $\chi^2$ , the larger discrepancy between the observed frequency and the expected frequency.

## V-2. Quantification Mode

- Use Quantification to calculate calibration coefficients using the measured data from a set of standards.
- Method parameters in this mode can be modified after a measurement is complete. For example, users can modify the wavelength at which the test is performed after the measurement is complete and monitor its effect on the linearity of the calibration curve used to quantify the sample.
- Perform a Quantification Method measurement as follows:
  - 1. Select measurement mode.
  - 2. Set method parameters.
  - 3. Measure blank.
  - 4. Measure standards.
  - 5. Measure samples.
  - 6. Save or print results as required.

#### V-2-1. Quantification Standard / Sample Mode

- Use Quantification Standard/Sample to quantify a sample at a single wavelength using a reference standard.
- Procedure
  - 1. Click New.

2. Select **Quantification standard** in Experiment Type. The Method dialog box is displayed.

S Method - C:#UV Lab#DefaultMS.mtd								
Experiment Type: Quantification Standard 🗳 Open 🕁 Save								
Experiment Setup »								
Baseline Correction Quantification Standard	» *							
Analysis Name	test							
Concentration Unit	%							
Use Wavelength (nm)	500							
Standard Replicate No.	1							
Sample Replicate No.	1							
Curve Zero Offset	Yes 🔹							
Curve Order	1 -							
Derivative Order	0 -							
"I Standard Concentration								
Save as	Default <u>O</u> K <u>C</u> ancel							

- 3. Setup Experiment Setup and Baseline Correction parameters. See IV-1-1. Wavelength Monitoring for more details.
- 4. Click **Quantification Standard** and set parameters as follows:
  - a. Analysis Name: Enter the analysis name.
  - b. **Concentration Unit**: Enter the units for the standards.
  - c. Use Wavelength (nm): Enter the wavelength to use for the test.
  - d. **Standard Replicate No**.: Enter the number of repeated standard measurements. The average value of each measurement is displayed after measuring the times entered before.

e. Sample Replicate No.: Enter the number of repeated sample measurements.

There are two ways to check the sample measurement result. Select **Measure**  $\rightarrow$  **Options** in the Main menu.

		Options	
Meas <u>u</u> re		Mode Others Instrument Setting	OK
🗍 Run Blank	Alt+B	C Automatic Save	
🔋 Run Sample	Alt+S	C Automatic Smoothing	
🔄 Method	Alt+M	Automatic Anomaly Correction	
Validation		₩ View Replicated Samples and Average in Quantification	
Options		Show Message of Replicate Number in Quantification	
Instrument	+		

i ) In case of checking (  $\lor$  ) View Replicated Samples and Average in

**Quantification** in the **Others** tab, each repeated measurement result and the averaged value are displayed.

R^2: 0.99908 Function: Y = 1.12E-02X + -3.57E-02 Remain Standard Measurement No. : 0							
Name	Concentration(mg/L)	AU(440.00nm)	<b>Dilution Factor</b>	Original Conc.(mg/L)			
Sample1	24.15	0.2337	1.0	24.15			
Sample2	24.15	0.2337	1.0	24.15			
Sample3	24.14	0.2336	1.0	24.14			
Average	24.15	0.2337	1.0	24.15			
Sample4	51.39	0.5376	1.0	51.39			
Sample5	51.35	0.5372	1.0	51.35			
Sample6	51.39	0.5376	1.0	51.39			
Average	51.38	0.5375	1.0	51.38			

ii) In case of checking ( $\lor$ ) View Replicated Samples and Average in

Quantification and Show Message of Replicate Number in Quantification

in the **Others** tab, below message are displayed when each repeated measurement.

ſ	UV Lab	ĺ	UV Lab
	Now, measuring sample number 1 of 3		Now, measuring sample number 3 of 3
	ОК		ОК

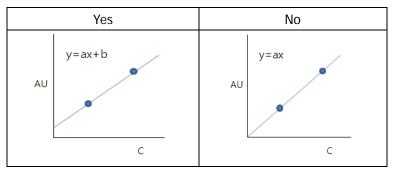
iii) In case of unchecking View Replicated Samples and Average in

#### Quantification and Show Message of Replicate Number in Quantification

in the **Others** tab, the only averaged value of each repeated measurement is displayed.

R <sup>2</sup> : 0.99919 Function: Y = 1.12E-02X + -3.78E-02 Remain Standard Measurement No. : 0						
Name Concentration(mg/L) AU(440.00nm) Dilution Factor Original Conc.(mg						
Sample1	24.18	0.2335	1.0	24.18		
Sample2	51.19	0.5364	1.0	51.19		
Sample3	99.36	1.0767	1.0	99.36		

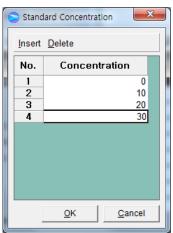
f. Curve Zero Offset: Select Yes or No to use.



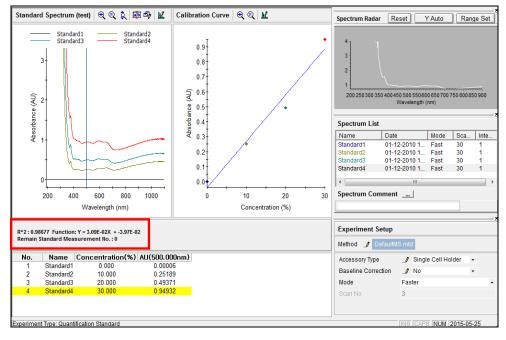
- g. **Curve Order**: Select a 1<sup>st</sup>, 2<sup>nd</sup> or 3<sup>rd</sup> order fit for the calibration curve.
- h. **Derivative Order**: Choose the Derivative Order among 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup>. The Y-axis of spectrum and calibration curve is changed as selected.

#### 5. Select Standard Concentration.

Enter the concentration for each standard in the test box, as shown below and select OK.
 Insert and Delete can be used to change the number of standards for the test.

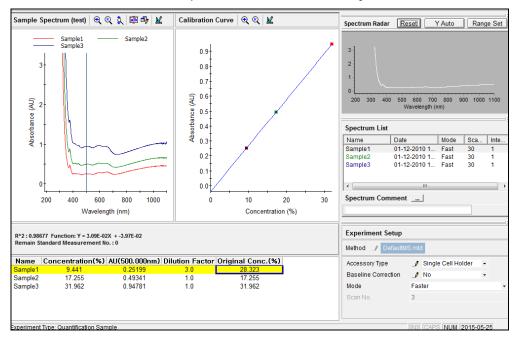


- After setting parameters for Experiment Setup, Baseline Correction and Quantification Standards is complete, click OK in the method setup window.
- 8. Measure the blank.
- Measure the standards according to their concentrations. The spectra and resulting calibration curve are displayed as follows. The equation and correlation coefficient for the curve are displayed below the Standard Spectrum window.





- · · · · ·
- 11. Measure the sample (unknown).



12. The concentration of each sample is calculated automatically.

13. If samples were diluted prior to measurement, the original concentration may be calculated using a dilution factor as shown below:

R^2:0.98677 Function: Y = 3.09E-02X + -3.97E-02 Remain Standard Measurement No. : 0					
Name	Concentration(%)	AU(500.000nm)	<b>Dilution Factor</b>	Original Conc.(%)	
Sample1	9.441	0.25199	3.0	28.323	
Sample2	17.255	0.49341	1.0	17.255	
Sample3	31.962	0.94781	1.0	31.962	

- 14. Save or print data and spectra as required.
- To edit a sample name or a dilution factor, double click the sample line to be changed in the list.

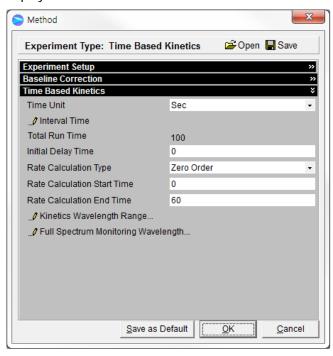
Sedit Sample		X
Old Name:	Sample3	ОК
New Name:	Sample 1	Cancel
Old Dilution Factor:	1	
New Dilution Factor:	1	

## V-3. Kinetics Mode

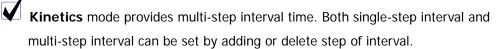
- Use Kinetics to test reaction rate. This mode includes the following Experiment Types:
  - Time Based Kinetics
  - Temperature Based Kinetics
  - Ultra Kinetics
- Perform a Kinetics Method measurement as follows:
  - 1. Select measurement mode.
  - 2. Set method parameters.
  - 3. Measure blank.
  - 4. Measure samples.
  - 5. Save or print results as required.

#### V-3-1. Time Based Kinetics Mode

- Use Time Based Kinetics to test the reaction rate against time
- Procedure
  - 1. Click New.
  - 2. Select **Time Based Kinetics** in the Experiment Type. The method dialog box is displayed.



- Setup Experiment Setup and Baseline Correction parameters. See V-1-1. Wavelength Monitoring for more details.
- 4. Select **Time Based Kinetics** and setup test parameters as follows:
  - a. Time Unit: Choose a time unit (min, sec, msec).
  - b. Interval Time: Set the interval time during Start and End Time. Select Insert and Delete to add interval time.
    - i . Time Unit Min: Recommended Interval is over 1min
    - ii . Time Unit sec: Recommended Interval between 5  $\sim$  60 sec
    - iii . Time Unit msec: Recommended Interval between 650  $\sim$  2000 msec



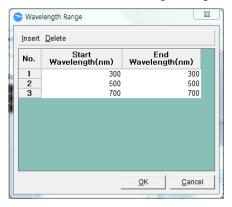
Interval Time					
No.	Start Time	End Time	Interval		
1	0	30	5		
2	30	50	1		
3	50	100	10		

- c. Total Run Time: Show the total run time for testing samples.
- d. **Initial Delay Time**: Set the holding time before the first sample measurement. If "0" is entered for the initial delay time, the measurement is taken immediately.
- e. Rate Calculation Type: Select the order for the rate calculation. Select
   Form: Zero Order, Initial Rate, First Order and Delta Au. See the end of this section for more information.
- f. Rate Calculation Start Time: Enter the time to start calculating the rate.
- g. Rate Calculation End Time: Enter the time to stop calculating the rate.

The Start and End Time must be within the total run time set for the test.

5. Click Kinetics Wavelength Range.

6. Enter the desired wavelength range for the measurement and click **OK**.



The number of **wavelength Range** should be matched the number of Using Cell. If the Wavelength Range is set as the picture below, multi cell number should be selected three cells and in numerical order on **using cells** in the **8 Multi-Cell Holder Setup** window. For example, Cell No.2 for wavelength 300 nm, Cell No.3 for wavelength 500 nm, Cell No.5 for wavelength 700 nm.

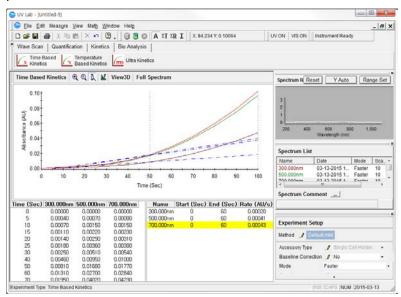
🔵 Wave	length Range	X	n	S Multi-Cell Holder Setup	
Insert	<u>D</u> elete				
No.	Start Wavelength(nm)	End Wavelength(nm)		Available Cells Using Cells Cell No. 1 Cell No. 2 Cell No. 3 Delete	1
1 2 3	300 500 700	300 500 700		Cell No. 5         Cell No. 5         Up           Cell No. 5         >         Cell No. 7         Down	
				Blank Position 1 Auto Measure Blank	
		<u>O</u> K			
				Reset OK Cancel	

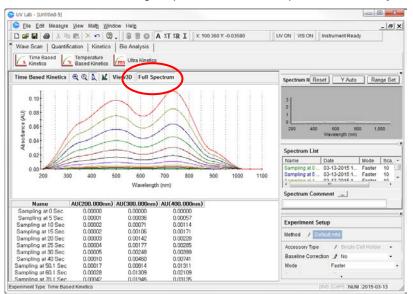
7. Select Full Spectrum Monitoring Wavelength.

8. Enter the wavelength for the measurement and select **OK**.

S Monitoring Wavelength							
Insert Delete							
No.	Wavelength(	nm)					
1		200					
2		300					
3		400					
	<u>0</u> K	<u>C</u> ancel					

- 9. After setting parameters for Experiment Setup, Baseline Correction and Time Based Kinetics is complete, select **OK**.
- 10. Measure the blank.
- 11. Measure samples. The overlay of all the full spectra is displayed in the Spectrum Radar window during the entire measurement.
- 12. After the experiment is complete, the Regression Curve is generated as shown in the picture below.





13. To see the full wavelength spectrum of the samples, click Full Spectrum.

14. Print and save spectra and data as required.

## ? Rate Calculation Type

Four Rate Calculation types are available. These include:

#### Zero order

Uses a linear fit to calculate the rate, k, by linear regression using the equation:

 $\mathbf{A}_{t}=\mathbf{A}_{0}-\mathbf{\textit{k}}t$ 

 $\mathbf{A}_t$  is the absorbance at time t.

 $\mathbf{A}_{\mathbf{o}}$  is the absorbance at the start of the calculation time range.

**k** is the zero order rate constant [Units: AU/s].

#### Initial Rate

Uses a quadratic fit to calculate the rate, k, by linear regression using the equation:

#### $\mathbf{A}_{\mathrm{t}} = \mathbf{A}_{\mathrm{0}} + \mathbf{k}\mathbf{t} + \mathbf{t}^{2}$

 $\mathbf{A}_t$  is the absorbance at time t.

 $A_0$  is the initial absorbance.

k is the initial rate [Units: AU/s].

#### First order

Uses an exponential fit to calculate the rate, k, using the rate law:

### $\mathbf{A}_{t} = \mathbf{A}_{\infty} + (\mathbf{A}_{o} - \mathbf{A}_{\infty}) e^{-kt}$

This rate law can be rearranged to

### $\ln(A_t - A_{\infty}) = \ln(A_o - A_{\infty}) - kt$

 $\mathbf{A}_t$  is the absorbance at time  $\mathbf{t}$ .

 $\mathbf{A}_{\mathbf{0}}$  is the initial absorbance.

**k** is the first order rate constant [Unit: 1/s].

#### Delta AU

Uses the difference between the absorbance at the start of the calculation time range and the absorbance at the end. This calculation is very simple and can be expressed as:

#### $Delta AU = A_t - A_0$

 $\mathbf{A}_{\mathbf{t}}$  is the absorbance at time  $\mathbf{t}$ .

 $\mathbf{A}_{\mathbf{0}}$  is the initial absorbance.

Delta AU [Units: AU].

### V-3-2. Temperature Based Kinetics Mode

■ Use Temperature Based Kinetics to test the reaction rate against temperature.

#### Procedure

1. Select New.

 Select Temperature Based Kinetics in Experiment Type. The method dialog box is displayed.

S Method - C:\UV Lab\Default.mtd	22
Experiment Type: Temperatur	e Based Kine 🗃 Open 🔚 Save
Experiment Setup Baseline Correction	»
Temperature Based Kinetics	*
	min)
Start Temperature(°C)	30
End Temperature(°C)	100
Holding Time (min.)	0
Rate Calculation Type	Zero Order 🔹
Calculation Start Temperature(°C)	30
Calculation End Temperature(°C)	80
Save as I	Default <u>O</u> K <u>C</u> ancel

 Setup Experiment Setup and Baseline Correction parameters. See V-1-1. Wavelength Monitoring for more details. In Sampling Type of Experiment Setup, select Single Cell Peltier or Muti-Cell Peltier.

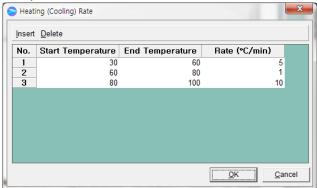
For more details of the Peltier Accessory temperature setting, refer to the **Single Peltier Temp Ctrl Unit L465** or **Multi Peltier Temp Ctrl Unit L465** manual.

- 4. Select **Temperature Based Kinetics** and setup test parameters as follows:
  - a. Edit Heating(Cooling) Rate (°C/min): Set the temperature interval for one minute.

Temperature based kinetic mode provides multi-step ramping. Both single-step ramping and multi-step ramping can be set by adding or delete step of interval.



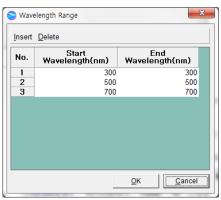
# Sample is measured as interval of rate.



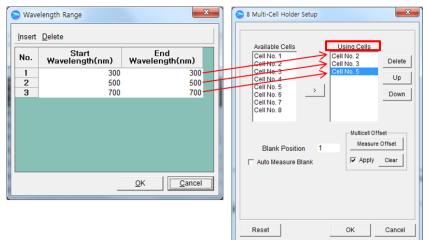
- b. Start Temperature: Enter the start temperature for the measurement.
- End Temperature: Enter the end temperature for the measurement. C.
- Holding Time: Select the holding time at each temperature. d.
- e. Rate calculation Type: Select the rate calculation type. See V-3-1. Time Based Kinetics for more information.
- Calculation Start Temperature: Enter the start temperature for the calculation. f.
- g. Calculation End Temperature: Enter the end temperature for the calculation.

V The Calculation Start and Calculation End Temperature must be within the Start and End temperatures for the measurement.

- 5. Select Wavelength Range.
- 6. Enter the wavelength range for the measurement and click **OK**.



The number of wavelength Range should be matched the number of Using Cell. If the Wavelength Range is set as the picture below, peltier multi cell number should be selected three cells and in numerical order on using cells in the 8 Multi-Cell Holder Setup window. For example, Cell No.2 for wavelength 300 nm, Cell No.3 for wavelength 500 nm, Cell No.5 for wavelength 700 nm.



- 7. Measure the Blank.
- Measure samples. 8.
- 9. Print and save spectra and data as required.

### V-3-3. Ultra Kinetics Mode

- Use Ultra kinetics to measure a very fast kinetic reaction.
- Measurement can be performed with an interval time as low as 20 ms in this mode. Perform this type of experiment as follows:
- Procedure
  - 1. Click New.

CUltra Kinetics			
🗅 New 🚔 Open 🔳 Save 🌚 Exp	ort• 🔟 Blank 🔝 Sample 🙁 Stop 🖨 Print Exit		
(N)	Kinetic	Data sheet	Spectrum Chart
Academice (AL)	0 Time (s)		OK           Experiment Setup           Interval Time (s) (0.02s ~)           0.02           Total Data Number (~ 50000)           Fill Baseline Correction           Base Wave (nm) 400           Fill Baset Mark (nm) 200           One Spectrum Monitoring           Display Start Wave (nm) 1000           Grad Baset Mark (nm) 1000
Kinetics Rate 1 = Equation 2 : Kinetics Rate2 = Equation 2 :	Title : Untitle Comment : Experiment Date :	<u>+7</u>	Display End Wave (nm)     1000     If All Spectra Monitoring       Edited Science     Monitoring       Kinetics Wave 1 (nm)     500     Calculation       Kinetics Wave 2 (nm)     550     Zero Order       Start Time (s)     8     End Time (s)     11.5       Factor     0.01     Start Time (s)     11.5

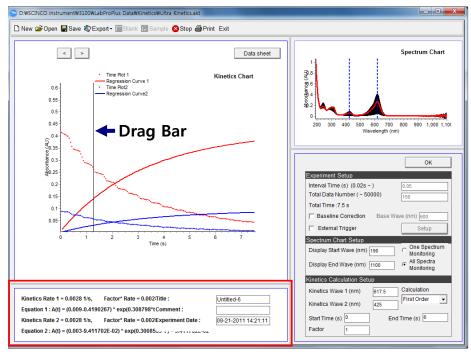
2. Select **Ultra Kinetics** in the Experiment Type. The method dialog box is displayed.

3. Enter each parameter as follows. The interval time and total time are calculated automatically in the Test Result window.

	ОК
Experiment Setup	
Interval Time (s) (0.02s ~ )	0.02
Total Data Number ( ~ 50000)	1000
Total Time :	
Baseline Correction Base Way	re (nm) 400
External Trigger	Setup
Spectrum Chart Setup	
Display Start Wave (nm) 200	C One Spectrum Monitoring
Display End Wave (nm) 1000	<ul> <li>All Spectra Monitoring</li> </ul>
Kinetics Calculation Setup	
Kinetics Wave 1 (nm) 500	Calculation
Kinetics Wave 2 (nm) 550	Zero Order 👻
Start Time (s) 8 End	Time (s) 11.5
Factor 0.01	

- a. **Interval Time (s)**: Enter the interval time between measurements. This parameter is related to Integration No. The minimum interval time is 0.02 sec.
- b. **Total Data Number**: Enter the number of data points desired. The max. Sampling No. is 50000.
- c. **Total Time (s)**: Will be set automatically according to both the Interval time and the Total Data Number.
- d. **Baseline Correction**: Check ( $\sqrt{}$ ) Baseline Correction box to apply for the Baseline correction and enter the wavelength in the Base Wave(nm).

- e. Display Start Wave (nm): Enter Start Wave for the display in the Spectrum chart.
- f. **Display End Wave (nm)**: Enter End Wave for the display in the Spectrum chart.
- g. **One Spectrum Monitoring**: Show the last spectrum obtained.
- h. All Spectra Monitoring: Show the whole spectrum obtained.
- i. **Kinetics Wave 1 / 2 (nm)**: Enter the specific wavelength 1 / 2 to calculate the reaction rate.
- j. **Calculation**: Select the order for the rate calculation among Zero Order, First Order and Delta Au. (Refer to *Time Based Kinetics* chapter for the details)
- k. Start Time (s): Enter the time to start calculating the rate.
- I. End Time (s): Enter the time to stop calculating the rate.
- Both the start time and the end time shouldn't be a bigger than the total run time.
- m. Factor: Enter the factor for the calculation.
- 4. After setup is complete, click **OK**.
- 5. Measure the Blank.
- 6. Measure the sample. Each reaction rate of selected wavelength 1 and wavelength 2 will be calculated in the kinetics chart.



**Drag Bar**: Confirm the spectrum data at specific time which is pointed by drag bar at the Kinetics Chart

- 7. Save the data. [\*.akt]
- The set-up parameters of 'Base Wave', 'Display Start Wave', 'Display End Wave', 'Kinetics Wave', 'Start Time', 'End Time' and 'Factor' can be changed after the test and then the new results will be shown accordingly.
- 8. Print, save or export data and spectrum as required.

## V-4. Bio Mode

■ Use Bio to perform pre-programmed biological test. This mode includes the following

Experiment Types:

- Nucleic Acid Analysis
- Protein Analysis
- Cell Density
- Enzyme Activity
- Enzyme Mechanism
- Thermal Denaturation
- Perform a Bio Method measurement as follows:
  - 1. Select measurement mode.
  - 2. Set method parameters.
  - 3. Measure blank.
  - 4. Measure samples.
  - 5. Save or print results as required.

### V-4-1. Nucleic Acid Analysis Mode

- Use Nucleic Acid Analysis Methods to perform ratio and concentration measurements on samples containing proteins and nucleic acids.
- Procedure
  - 1. Select New.
  - 2. Select **Nucleic Acid Analysis** in the Experiment Type. The method dialog box is displayed. Select the cell pathlength in the Nucleic Acid method and click **Open**.

S Method O	)pen
Look in: 🚺	Nucleic Acid Method 💽 🔶 📸 🔻
	ck 0.2mm.mtd
🔄 🔄 Nano Stie	ck 0.5mm.mtd
Pathleng	ith 1mm.mtd
Pathleng	yth 10mm.mtd
File <u>n</u> ame:	Pathlength 1mm Open
Files of type:	Method Files(*.mtd)  Cancel

3. Setup Experiment Setup and Baseline Correction parameters. See V-1-1. Wavelength Monitoring for more details.

S Method - C:#UV Lab#N	Jucleic Acid Method₩Pa	athlength 1mm.mtd
Experiment Type: Nu	cleic Acid Analysis	🚔 Open 🔚 Save
Experiment Setup		»
Baseline Correction Nucleic Acid Analysis		<u>*</u>
Analytical Name	1mm	
Calculation Type	dsDNA	•
Concentration Unit	ng/ul	
Cell Pathlength (mm)	1	
Dilution Factor	1	
Nucleic Acid Factor	50	
	Dama an Dafault	
	Save as Default	<u>OK</u> <u>Cancel</u>

- 4. Setup test parameters in the Nucleic Acid Analysis tab as follows:
  - a. Analytical Name: Enter the analytical name.
  - b. Calculation Type: Select the calculation type.

Calculation Type	dsDNA 🗸
	dsDNA
	ssDNA
	RNA
	Oligo DNA
	Warburg-Christian
	Kalb and Bernlohr

## ?> Calculation Type

The Nucleic Acid application is used for determining the concentration and purity of nucleic acid samples. The reading at 260 nm allows to calculate the concentration.

#### dsDNA, ssDNA, RNA, Oligo DNA

[Nucleic Acid Concentration]  $ug/ml = A_{260} x$  extinction coefficient x D.F

Where,

 $A_{260}$  is Absorbance at 260 nm.

Extinction coefficient :

The generally accepted extinction coefficients for nucleic acids are:

Coefficient	Value
dsDNA	50 ug/ml
ssDNA	37 ug/ml
RNA	40 ug/ml
Oligo DNA	33 ug/ml

D.F is dilution factor.

#### Warburg-Christian

The Warburg-Christian assay with pre-selected parameters calculates protein and nucleic acid concentrations (in micrograms per  $m\ell$ ) using the following equations with absorptivities calculated by Warburg and Christian.

 $[Protein] = (1552 * A_{280}) - (757.3 * A_{260}),$ 

 $[\text{Nucleic Acid}] = (62.9 * A_{260}) - (36.0 * A_{280})$ 

where, the absorbance at 260 nm and 280 nm are corrected for the baseline at 320 nm.

#### Kalb and Bernlohr

The Kalb and Bernlohr assay with pre-selected parameters calculates protein and nucleic acid concentrations (in micrograms per  $m\ell$ ) using the following equations. [Protein] = (183.0 \* A<sub>230</sub>) - (75.8 \* A<sub>260</sub>),

[Nucleic Acid] =  $(49.1 * A_{260}) - (3.48 * A_{230})$ 

where, the absorbance at 260 nm and 230 nm are corrected for the baseline at 320 nm.

- c. Concentration Unit: Enter the concentration unit for the samples.
- d. Cell Pathlength: Enter the cell (beam) pathlength.
- e. Dilution Factor: Enter the dilution factor.
- f. Nucleic Acid Factor: Will be set automatically according to calculation type.

UV Lab uses factors 50, 37, 40 and 33 as default settings for dsDNA, ssDNA, RNA and Oligonucleotides.

- 5. After setting parameters for Experiment Setup, Baseline Correction and Nucleic Acid Analysis is complete, click **OK**.
- 6. Measure the blank.
- 7. Measure samples.
- 8. Save and print spectra and data as required.

### V-4-2. Protein Analysis Mode

- Use Protein Analysis to quantify the amount of protein in a sample. Select from pre-programmed frequently used methods for quantifying proteins.
- Procedure
  - 1. Select New.
  - 2. Select Protein Analysis and select OK.
  - 3. Method Open dialog box is displayed. Choose the method and select OK.

S Method Open	x
Look in: 🕕 Protein Method	- ← 🗈 👉 🖬 -
BCA.mtdBSA-5mm.mtdBiuret.mtdBSA-10mm.mtdBradford.mtdLowry.mtdBSA-0.2mm.mtdLowryHigh.mtdBSA-0.5mm.mtdLowryLow.mtdBSA-2mm.mtdProtein205.mtd	Protein280.mtd
File <u>n</u> ame:	Open
Files of type: Method Files(*.mtd)	Cancel

- 4. The method dialog box is displayed. Setup test parameters and click **OK**.
- 5. Measure the Blank.
- 6. Measure samples.
- 7. Save and print spectra and data as required.

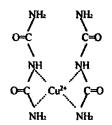
## ? Protein Analysis Method

The study of many biochemical processes depends upon an accurate measurement of the amount of protein in solution. This has led to the development of several protein quantification methods, the most common of which are described below.

		[nm]		
Biuret	Dilute copper sulfate in strong alkali	540	200–2000	Biuret.mtd
Lowry (high sensitivity)	Dilute copper sulfate in strong alkali and Folin-Ciocalteu reagent	750	4–200	Lowryhigh.mtd
Lowry (low sensitivity)	Dilute copper sulfate in strong alkali, Folin-Ciocalteu reagent	500	60–400	Lowrylow.mtd
Lowry (modified)	Dilute copper sulfate in strong alkali and Folin-Ciocalteu reagent and dithiothreitol	740	3–200	Lowry.mtd
Bradford	Goomassie Brilliant Blue G250 in dilute acid	595	2–50	Bradford.mtd
Bicinchoninic acid (BCA)	Bicinchoninic acid	562	4–400	BCA.mtd
Trinitrobenzene Sulfonate (TNBS)	Hydrochloric acid and trinitrobenzene reagent	416	0.5–100	TNBS.mtd

#### 1. Biuret Method

Biuret (NH<sub>2</sub>-CO-NH-CO-NH<sub>2</sub>) produces a violet chelate compound when reacting with alkali  $CuSO_4$ .



Using the theory that a compound which has more than two peptide bonds also produces a chelate compound, by the same procedure as in the Biuret case, one can determine the protein concentration. Approximately,  $1 \sim 10 \text{ mg}$  of protein can be quantified by the Biuret method. Using a microassay one can measure as  $0.25 \text{ mg} \sim 2.0 \text{ mg}$  of protein. A chelate compound is usually stable for  $1 \sim 2$  hours but its chromaticity gradually increases with time. The absorbance of the sample in the test tube is measured at 540 nm.

#### 2. Lowry Method

The Lowry procedure is one of the most venerable and widely used protein assays, being first described in 1951 [Lowry et al., J. Biol. Chem. 193: 265-275 (1951)]. Under alkaline conditions, copper complexes with protein. When folin phenol reagent (phospho-molybdic-phosphotungstic reagent) is added, the folin-phenol reagent binds to the protein. Bound reagent is slowly reduced and changes color from yellow to blue. The Lowry method is more sensitive than the Biuret method and can measure  $10 \sim 200 \ \mu g$  of protein. The absorbance of the sample in the test tube is measured at **750 nm**.

#### 3. Bradford Method

One of the most widely used methods was developed by Bradford; it is based upon a shift in the absorption spectrum of Coomassie Brilliant Blue G-250 when the dye binds to protein in an acidic solution. The method is fast, convenient, and produces an equivalent absorbance change for many different proteins. The absorbance of the resulting solution is measured spectrophotometrically at **595 nm** and compared to a standard curve generated using known quantities of a control protein in the dye solution. In this method, color reaction is completed very quickly (in 2 min.) and it is stable for 1 hour. The Bradford method is more sensitive than the Lowry method and can measure  $1 \sim 20$  $\mu$ g of protein using micro assay. The Bradford method is faster and is seldom affected by non-protein components.

#### 4. BCA Protein Assay

BCA Protein Assay uses a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantification of total protein. This method combines the well-known reduction of  $Cu^{+2}$  to  $Cu^{+1}$  by protein in an alkaline medium (the Biuret reaction) with the highly sensitive and selective colorimetric detection of the cuprous cation ( $Cu^{+1}$ ) using a unique reagent containing BCA. The purple-colored reaction product in this assay is formed by the chelation of two molecules of BCA with one cuprous ion. This water-soluble complex exhibits a strong absorbance at 562 nm that is nearly linear with increasing protein concentrations over a broad working range (20-2,000 µg/ml). The BCA method is not a true end-point method; i.e., the final color continues to develop. However, following incubation, the rate of continued color development is sufficiently slow to allow large number of samples to be assayed together.

### V-4-3. Cell Density

- Use Cell Density to determinate the absorbance at 600 nm.
- Procedure
  - 1. Click New.
  - 2. Select Cell Density and click OK.

3. **Method** dialog box is displayed.

Method	X
Experiment Type: Ce	ell Density 🗃 Open 📕 Save
Experiment Setup Baseline Correction	»
Cell Density	*
Factor	1
Multiplier	1
Units	OD

- Setup Experiment Setup and Baseline Correction parameters. See V-1-1. Wavelength Monitoring for more details. Setup test parameters and click OK.
  - a. Factor: Set a desired factor value. [used when entering cell/ml in Units]
  - b. Multiplier: Set a desired Multiplier value. [used when entering cells/ml in Units]
    - Factor and Multiplier define the conversion of the measured OD to the number of cells per milliliter (e.g.: Factor 5, Multiplier: 100,000,000)
       1 OD 600 = 5 x 10<sup>8</sup> cells/ml
  - $\checkmark$  When entering OD in Units, set the Factor and Multiplier as 1.
  - c. **Units**: Enter OD or cells/ml.
- 5. Measure the Blank.
- 6. Measure samples.
- 7. Save and print spectra and data as required.

### V-4-4. Enzyme Activity Mode

- Use Enzyme Activity Methods to calculate enzyme activity from a set of kinetic samples.
- Procedure
  - 1. Click New.
  - 2. Select **Enzyme Activity** in the Experiment Type. The method dialog box is displayed.

Experiment Setup Baseline Correction	>
Enzyme Activity	
Time Unit	Sec -
Total Run Time	60
Initial Delay Time	0
Interval Time	10
Rate Calculation Type	Zero Order 🗸
Start Time	0
End Time	60
Enzyme Activity Unit	mol/sec
Enzyme Activity Factor	0.4

- 3. Setup Experiment Setup and Baseline Correction parameters. See V-1-1. Wavelength Monitoring for more details.
- 4. Select **Enzyme Activity** and setup test parameters as follows:
  - a. Time Unit: Choose a time unit (min, sec, msec).
    - i . min unit: Recommended Interval Time: over 1min
    - ii. sec unit: Recommended Interval Time: 5 ~ 60 sec
    - iii. msec unit: Recommended Interval Time: 650 ~ 2000 msec
  - b. Total Run Time: Enter the total time for measuring samples.
  - c. **Initial Delay Time**: Set the holding time before the first measurement. If "0" is entered for the initial delay time, the measurement is taken immediately.
  - d. Interval Time: Set the interval time between the measurements.
  - e. Rate Calculation Type: Select the order for the rate calculation. Select form: Zero Order, Initial Rate, First Order and Delta Au. See V-3-1. Time Based Kinetics for more information.
  - f. Start Time: Enter the time to start calculating enzyme activity.
  - g. End Time: Enter the time to stop calculating enzyme activity.
  - h. Enzyme Activity Unit: Enter the enzyme activity unit.
  - i. **Enzyme Activity Factor**: Enter the enzyme activity factor to calculate the enzyme activity. This value can be calculated using the equation in the box below.

Unit = 
$$\frac{\mu \text{mol produced}}{\min} = \frac{\triangle A}{\triangle t} * \left[ \frac{1}{\epsilon (M^{-1} \text{cm}^{-1})} b * \frac{10^6 \mu M}{M} * V_f (L) \right]$$

 $\boldsymbol{\varepsilon}$  = molar absorption coefficient (M<sup>-1</sup>cm<sup>-1</sup>)

**b** = cell pathlength (cm)

 $V_f$  = final volume in the cuvette (I)

**A** = absorbance

t = time (min)

- 5. Click Wavelength Range.
- 6. Enter the wavelength range for each measurement and click **OK**. See **V-3-1**. Time

Based Kinetics for more information.

S Wavelength Range		
<u>I</u> nsert	<u>D</u> elete	
No.	Start Wavelength(nm)	End Wavelength(nm)
1	260	260
2	280	280
		OK <u>C</u> ancel

- 7. After setting parameters for Experiment Setup, Baseline Correction and Enzyme Activity is complete, click **OK**.
- 8. Measure the Blank.
- 9. Measure Sample. The overlay of all the spectra is displayed in the Spectrum Radar window during the entire measurement.
- 10. After the experiment complete, the Regression Curve is generated.
- 11. Save and print spectra and data as required.

### V-4-5. Enzyme Mechanism Mode

■ Use Enzyme Mechanism Methods to collect the plots, K<sub>m</sub> and V<sub>max</sub> from a set of kinetic samples.

#### Procedure

- 1. Click New.
- 2. Select Enzyme Mechanism in the Experiment Type. The method dialog box is displayed.

Baseline Correction Enzyme Mechanism		
Time Unit	Sec	
Total Run Time	60	
Initial Delay Time	0	
Interval Time	10	
Rate Calculation Type	Zero Order	
Start Time	0	
End Time	60	
Enzyme Activity Unit	mg	
Enzyme Activity Factor	0.9	
"Ø Substrate Concentration		

- 3. Setup Experiment Setup and Baseline Correction parameters. See V-1-1. Wavelength Monitoring for more details.
- 4. Click Enzyme Mechanism and setup test parameters as follows:
  - a. Time Unit: Choose a time unit (min, sec, msec).
    - i . min unit: Recommended Interval Time: over 1min
    - ii. sec unit: Recommended Interval Time: 5 ~60 sec
    - iii. msec unit: Recommended Interval Time: 650 ~ 2000 msec
  - b. Total Run Time: Enter the total run time for measuring samples.
  - c. **Initial Delay Time:** Set the holding time before the first measurement. If "0" is entered for the initial delay time, the measurement is taken immediately.
  - d. Interval Time: Set the interval time between the measurements.
  - e. Rate Calculation Type: Select the order for the rate calculation. Select form: Zero Order, Initial Rate, First Order and Delta Au, See V-3-1. Time Based Kinetics for more information.

- f. Start Time: Enter the time to start calculating enzyme activity.
- g. End Time: Enter the time to stop calculating enzyme activity.
- h. Enzyme Activity Unit: Enter the enzyme activity unit.
- i. **Enzyme Activity Factor**: Enter the enzyme activity factor to calculate the enzyme activity.
- 5. Select Substrate Concentration.
- 6. Enter the wavelength range and substrate concentration for each test and select **OK**.

Substrate Concentration				
Insert Delete				
Sample No.	Start Wavelength(nm)	End Wavelength(nm)	Substrate Concentration	
1	300		10	
2	300	300	20	
3	300	300	30	
Use con	centration as sample name		<u>O</u> K <u>C</u> ancel	

- 7. After setting parameters for Experiment Setup, Baseline Correction and Enzyme Mechanism is complete, select **OK**.
- 8. Measure the blank.
- 9. Measure samples in the order of the Substrate Concentration. The overlay of all the full spectra is displayed in the Spectrum Radar window during the entire measurement.
- 10. To see enzyme plot, click ViewEMResult.



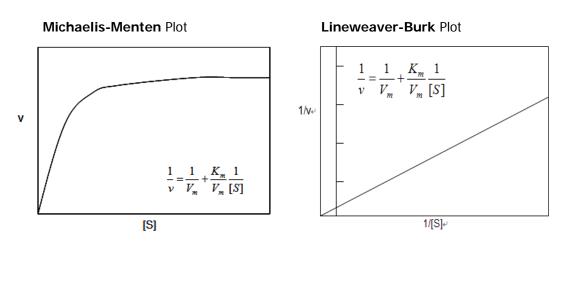
11. Click ▼ and select a plot type to open. (Michaelis-Menten, Lineweaver-Burk, Hanes-Woolf, Eadie-Hofstee Plot)

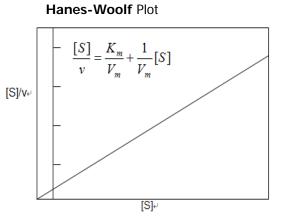


- 12. The graph can be viewed if a single plot is selected.
- 13. Save and print spectra and data as required.

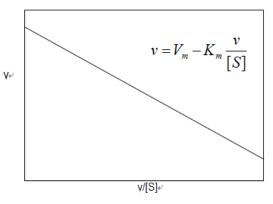
### ?> Enzyme Mechanism Plots

The effect of the substrate concentration on the rate of an enzyme-catalyzed reaction is shown graphically by the Michaelis-Menten plot. The Michaelis- Menten plot is constructed from the analysis of a set of samples with varying substrate concentration. The concentrations for each set are entered and stored with the rates of each sample. Three other plots are derived from the Michaelis-Menten equation, namely, **Lineweaver-Burk** plot, **Hanes-Woolf** plot, **Eadie-Hofstee** plot.





Eadie-Hofstee Plot



### V-4-6. Thermal Denaturation Mode

- Use Thermal Denaturation modes to collect the temperature based data and perform a classical DNA melting experiment.
- Procedure
  - 1. Select New.
  - 2. Select Thermal Denaturation in Experiment Type. The method dialog box is displayed.

S Method	22		
Experiment Type: Thermal Denaturation 🖉 Open 📕 Save			
Experiment Setup Baseline Correction	»		
Thermal Denaturation	*		
	nin)		
Start Temperature(°C)	30		
End Temperature(°C)	100		
Holding Time (min.)	0		
Volume Correction	No 👻		
	Volume (T)=0.99829+104.5*10^-6		
Tm_Method	1st Derivative -		
DNA Pair Length(K)	10		
Equation Name	%		
Equation Expression	2.44 * (Tm - 81.5 - 16.66 * log (M)+50		
Calculation Start Temperature(°C)	30		
Calculation End Temperature(°C)	80		
Molarity (mol/L)	8		
<u>S</u> ave as D	efault <u>O</u> K <u>C</u> ancel		

- Setup Experiment Setup and Baseline Correction parameters. See V-1-1. Wavelength Monitoring for more details. Select the Single Cell Peltier or Multi-Cell Peltier in Sampling Type of Experiment Setup.
- 4. Click **Thermal Denaturation** and setup test parameters as follows:
  - a. Edit Heating(Cooling) Rate (°C/min): Set the temperature interval for one minute.

Thermal mode provides multi-step ramping. Both single-step ramping and multi-step ramping can be set by adding or deleting step of ramping. Measure the sample as rate interval.

Sample is measured as interval of rate.

	ng (Cooling) Rate			
No.	Start Temperature	End Temperature	Rate (°C/min)	
1	30	60		5
2	60	80		6
3	80	100		10
			<u></u>	<u>C</u> ancel

- b. Start Temperature: Enter the start temperature for the measurement.
- c. End Temperature: Enter the end temperature for the measurement.
- d. Holding Time: Enter the holding time at each temperature.
- e. **Volume Correction**: If volume correction is selected, the baseline corrected absorbance value is corrected for the thermal expansion of an aqueous buffer. The default equation for volume correction is:

Volume (T) =  $0.99829 + 104.5 \times 10^{-6}T + 3.5 \times 10^{-6}T^2$ 

- f. **Tm method**: Select a method for determining Tm (DNA melting temperature). Options include: 1st derivative and Average.
- g. **DNA Pair Length (K):** Enter the DNA pair length. If a DNA pair length is above 5000, enter as "0".
- Equation Name and Expression: The melting range is calculated within the specified calculation range by defining the low temperature where the slope begins to increase steadily, and the high temperature where the slope approaches zero again. The default equation for the calculation of %G-C (Guanine-Cytosine) base pairs is:

Where M is the molarity in mol/I, K is the DNA base pair length.

If a DNA base pair length (K) is entered as '0', then the equation becomes:

%G-C= 2.44 \* ( Tm - 81.5- 16.66 \* log(M) )

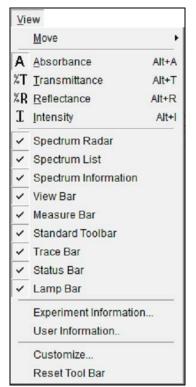
- i. **Calculation Start Temperature**: Enter the start temperature for calculating the Tm value using the selected method.
- j. **Calculation End Temperature**: Enter the end temperature for calculating the Tm value using the selected method.

- 5. After setting parameters for Experiment Setup, Baseline Correction and Thermal Denaturation are complete, select **OK**.
- 6. Measure the blank.
- 7. Measure samples.
- 8. Save and print spectra and data as required.

# VI. View Menu

■ The View menu includes commands to change and customize software windows as show

below.



Move	Select another experiment mode	
Absorbance	Display the unit of Y-axis by absorbance	
Transmittance	Display the unit of Y-axis by transmittance	
ReflectanceDisplay the unit of Y-axis by reflectance		
Intensity	Display the unit of Y-axis by intensity	
Spectrum Radar         Display spectrum radar on the screen		
Spectrum List Display spectrum list on the screen		
<b>Spectrum Information</b> Display spectrum information on the screen		
View Bar	Display view bar on the screen	
Measure Bar Display standard toolbar on the screen		
Standard Toolbar	Display standard toolbar on the screen	
Trace Bar	Display trace bar on the screen	

Status Bar	Display status bar on the screen	
Lamp Bar	Display lamp bar on the screen	
Experiment Information	ormation Display experiment information	
User Information	Display user information	
Customize	Change toolbar style	
Reset Tool Bar	Reset toolbar on the screen	

### VI-1. Move

Use the Move command to select the experiment method. A new window and method will be displayed.

View		
<u>M</u> ove ►	~	Wavelength Monitoring
		Equation Calculation
		Eind Peak/Valley
		Thickness
		Color Analysis
		Multi Component Analysis
		Quantification Standard
		Quantification Sample
		Time Based Kinetics
		Temperature Based Kinetics
		Ultra Kinetics
		Nucleic Acid Analysis
		Enzyme Activity
		Enzyme <u>M</u> echanism
		Thermal Denaturation
		Protein Analysis
		Cell Density

## VI-2. Absorbance

- Use the Absorbance command to convert the unit of the Y-axis to absorbance mode.
- Procedure
  - 1. Select **Absorbance**, and the unit of the Y-axis changes to absorbance mode.

### VI-3. Transmittance

■ Use the Transmittance command to convert the unit of the Y-axis to transmittance mode.

Procedure

1. Select **Transmittance** and the unit of the Y-axis changes to transmittance mode.

## VI-4. Reflectance

- Use the Reflectance command to convert the unit of the Y-axis to reflectance mode.
- Procedure
  - 1. Select Reflectance and the unit of the Y-axis changes to reflectance mode.

## VI-5. Intensity

Use the Intensity command to convert the unit of the Y-axis to intensity mode.

Procedure

1. Select Intensity and the unit of the Y-axis changes to intensity mode.

## VI-6. Experimental Information

- Use the Experiment Information command to exchange the experiment information.
- Procedure
  - 1. Select Experiment Information.

Experimental Informat	ion	X
Title	Untitled-1	<u>0</u> K
System Name	Undefined	Cancel
Experiment Date	5/25/2015 4:49:28 PM	
Comment	TEST	
Sample Name	Sample	

2. Enter Title, System Name and Comment information.

- Print 
   Print Contents

   ✓ Perkin Elmer Logo

   ✓ Company Logo

   ● User Information

   ✓ Title

   ✓ Comment

   ✓ Comment

   ✓ System Name

   ✓ Instrument Serial No.

   ✓ Instrument Serial No.
   Print P Open Logo User Inf vame: s Experiment information Plie Name: C VUV LabiData 123.gdt Thie: Untitled-1 Comment: TEST Instrument Serial No.: 465K505050 Software Version: UV Lab 4.0.0 <u>C</u>lose Experimental Date: 05-25-2015 16:49:28 System Name: Undefined Firmware Version: 130716 Method ientType : Viävelength Mi Instrument Serial N
   Firmware Version
   Software Version
   Software Version
   Result Data
   Spectrum List
   Sample Spectrum Experiment Setup Data Type: Absorbance Sampling: Single Cell Holder Mole: Factor Viave length Monitoring Monitoring Wavelength (hm): 440, 465, 546, 590, 635 Result Data AU(440.000 nm) AU(455.000 nm) AU(515.000 nm) AU(555.000 nm) AU(555.000 nm) 0.05617 0.06680 0.192.22 0.034.06 0.1075.3 Name Sample1 Spectrum List Printer 
   Name
   Date
   Mode
   Scan No.

   Sample1
   5/25/2015
   Faster
   10

   4:49:57 PM
   Faster
   10
   ▼ Printer Setting Microsoft XPS Document Writer Sample Spectrum al. Mat Perkelle Page 1 1/1 Q -H 4 ► 11
- 3. Select OK. You can check Experiment Information at the print.

## VI-7. User Information

- Use the User Information command to exchange the user information.
- Procedure
  - 1. Select User Information.

User Informa	tion	×
Name	admin	<u>OK</u>
Company	AAA	<u>C</u> ancel

2. Enter Name and Company information and click **OK**. You can check Company information at the print. You can check **User Information** at the print.

Print	
User Information         Concentry AAA           Superineer Information         Concentry AAA           Superineer Information         Experimental Date (50-4-001 51:0.0.4)           The user of Use Data (50-201 52:0.0.4)         Filtermental Date (50-4-001 52:0.0.4)           Scheart Home Over Use 0.20         Filtermental Date (50-4-001 52:0.0.4)           Detains Home Over Use 0.20         Filtermental Date (50-4-001 52:0.0.4)           Detains Home Over Use 0.20         Filtermental Date (50-4-001 52:0.0.4)           Detains Home Over Use 0.20         Experiment Table           Department Table         Use Not Restrict (Intro)           Date (Streame Care Loss)         Use Not Restrict (Intro)	Print Contents         Print           Ø Company Logo         Open Logo           Ø Longoavy Logo         Open Logo           Ø Mame         Øcompany Logo           Ø Longoavy Logo         Open Logo           Ø Longoavy Logo         Øcompany Logo           Ø Mame         Øcompany           Ø Method         Øcompany           Ø Result Data         Øcopertum List
Result Data           Name         AU(#40.000nm)         AU(#55.000nm)         AU(555.000nm)         AU(555.000nm)	Brinter Printer Setting Microsoft XPS Document Writer  Printer Setting
Fage 1	Parente la

## VI-8. Customize

- Use the customize command to hide and create the toolbars and disable all of the user's editing options.
- Procedure
  - 1. Click **Toolbars** tab.

2. Select toolbars desired.

<ul> <li>Standard Toolbar</li> <li>Main Menu</li> <li>View Bar</li> <li>Spectrum Radar</li> <li>Spectrum List</li> <li>Experiment Setup</li> <li>Trace Bar</li> <li>Status Bar</li> <li>Measure Bar</li> <li>Lamp Bar</li> </ul>	New Rename Delete Reset
--	----------------------------------

3. Select New. Enter the new toolbar name and select OK.

Customize		 • 4		9	23
Experies -	oolbar	 Cance	23	aw hame elete set	
		Keyboard		Clo	se

4. Select the **Commands** tab.

Customize	? ×
Toolbars Commands Options	
Categories:	Commands:
File Edit	New
Measure View	Open 🗏
Math	Close
Window Help	Close All
	Save _
Description	
	Modify Selection
	Keyboard Close

5. You can create a tab. Select **Keyboard**. The following dialog box will be displayed.

Categories:	C <u>o</u> mmands:		
File	New	•	Close
Edit Measure		=	
View	Open		
Math	Close		Assign
Window	Close All		Remove
Help	Ciose All		
	Cave	Ŧ	Reset All
Press <u>n</u> ew shortcut key:	C <u>u</u> rrent Keys:		
Description:			

- 6. Select **Options** tab.
- 7. Choose the options required, and then select **Close**.

Customize	? ×
Toolbars Commands Options	
Personalized Menus and Toolbars           Menu show recently used tools first           Show full menus after a short delay	
Reset Menu Usage Data Other	
Large Icons	
Show ScreenTips on Toolbars	
Show shortcut Keys in ScreenTips	
Menu animation: (None)	
Keyboard	Close



# VII. Math Menu

The Math menu includes commands to analyze the collected data.

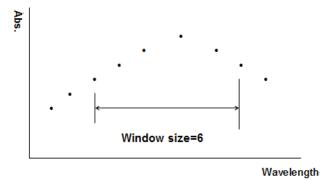
Mat <u>h</u>		
Sm <u>o</u> othing Derivative	Smoothing Derivative	Smooth the spectrum Obtain the data after applying a derivative
Scalar <u>A</u> dd Scalar <u>M</u> ultiply Scalar <u>D</u> ivide	Scalar Add	Add a constant value to the y-value in the spectrum
Log A <u>d</u> d	Scalar Multiply	Multiply the y-value in the spectrum by a constant value
<u>S</u> ubtract A <u>v</u> erage	Scalar Divide	Divide the y-value in the spectrum by a constant value
	Log	Compute the common logarithm of the y-value in the spectrum
	Add Subtract Average	Obtain the added data of selected spectra Obtain the subtracted data of two spectra Obtain the average data of the selected spectra
	Area	Perform to calculate the area

## VII-1. Smoothing

- Use the Smoothing command to smooth the spectrum.
- Procedure
  - 1. Select **Smoothing**. The following dialog box is displayed.

Smoothing		<b>—</b> ×	
Smoothing Window Size Polynomial Degree	Yes 19 5		
Processing Spectrum	Add new spectrum	4	

- 2. Set the function parameters.
  - a. Smoothing: Select Yes or No.
  - b. Window Size: Select the data point to use to smooth the spectrum.



- c. Polynomial Degree: Select the dimension of curve fitting.
- d. Processing Spectrum: Select Add new spectrum or Change original spectrum.
- 3. After setting parameters is complete, click **OK**. The result is displayed in the main window.



UV Lab uses the Savitsky-Golay method for the data smoothing. Using the Savitsky-Golay method results in the elimination of (window size)/2 points on each end of the smoothed value in the middle of the window. It is the preferred method for noise reduction and is also recommended for smoothing because no truncation of the data occurs.

### VII-2. Derivative

■ Use the Derivative command to obtain the derivative data of the spectrum.

#### Procedure

1. Select **Derivative**. The following dialog box will be displayed.

Derivative Order	1	•	<u>O</u> K
Processing Spectrum	Add new spectrum	•	<u>C</u> ancel

 Click OK after entering the Derivative Order number and selecting the Processing Spectrum. The result will be displayed in the main window.

### VII-3. Scalar Add

- Use the Scalar Add command to add a value to the Y-axis of a spectrum
- Procedure
  - 1. Select Scalar Add. The following dialog box will be displayed.

Scalar Add	x
0.2	<u>0</u> K
Add new spectrum	<u>C</u> ancel
Change original spectrum	

- 2. Enter the value to add to the spectrum.
- 3. Select Add new spectrum or Change original spectrum.
- 4. Click **OK**. The result will be displayed in the main window.

## VII-4. Scalar Multiply

- Use the Scalar Multiply command to multiply the Y-axis of a spectrum by a value
- Procedure
  - 1. Select **Scalar Multiply**. The following dialog box will be displayed.

Scalar Multiply	×
2	<u>о</u> к
<ul> <li>Add new spectrum</li> <li>Change original spectrum</li> </ul>	Cancel

- 2. Enter the value to multiply the spectrum.
- 3. Select Add new spectrum or Change original spectrum.
- 4. Select **OK**. The result will be displayed in the main window.

## VII-5. Scalar Divide

- Use the Scalar Divide command to divide the Y-axis of a spectrum by a value
- Procedure
  - 1. Select **Scalar Divide**. The following dialog box will be displayed.

<u>0</u> K
<u>C</u> ancel

- 2. Enter the value to divide the spectrum.
- 3. Select Add new spectrum or Change original spectrum.
- 4. Select **OK**. The result is displayed in the main window.

## VII-6. Log

Use the Log command to compute the log of a spectrum.

#### Procedure

1. Click Log. The following dialog box will be displayed.

×
<u>0</u> K
ancel

- 2. Select Add new spectrum or Change original spectrum.
- 3. Select **OK**. The result is displayed in the main window.

## VII-7. Add

- Use the Add command to add the Y-axis values of selected spectra.
- Procedure
  - Select desired spectra to add together by clicking the spectra while holding down the Ctrl key.
  - 2. Click **OK**. The result is displayed in the main window.

## VII-8. Subtract

- Use the Subtract command to subtract the Y-axis values of two spectra.
- Procedure
  - 1. Select two spectra to subtract by clicking the spectra while holding down the **Ctrl** key. The following dialog box is displayed.

Subtract	×
Sample1 - Sample2	<u>O</u> K
C Sample2 - Sample1	<u>C</u> ancel

2. Select the appropriate equation.

3. Click **OK**. The subtracted result is displayed in the main window.

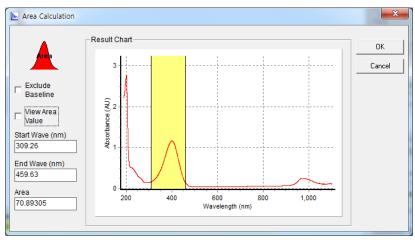
## VII-9. Average

- Use the Average command to calculate the average of selected spectra.
- Procedure
  - 1. Select the spectra to average together by clicking the spectra while holding down the **Ctrl** key or using the **Select All** command.
  - 2. Select **OK**. The average result is displayed in the main window.

Name	AU(350.000nm)	AU(440.000nm)	AU(546.000nm)	AU(590.000nm)	AU(635.000nm)
Sample4	2.67510	0.99745	0.96795	1.03574	1.00894
Sample5	2.67174	0.99715	0.96829	1.03403	1.00831
Average of Sample4 & Sample5	2.67342	0.99730	0.96812	1.03488	1.00863

## VII-10. Area

- Use the Average command to calculate the average of selected spectra.
- Procedure
  - > Use the Area command to calculate the are in the specified range
  - Procedure
  - 1. Select Area. The following dialog box is displayed.



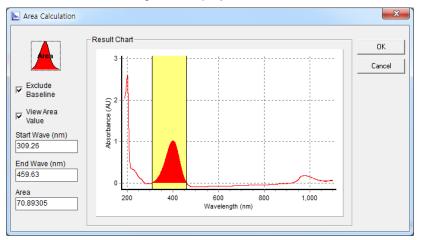
2. Set the parameters.

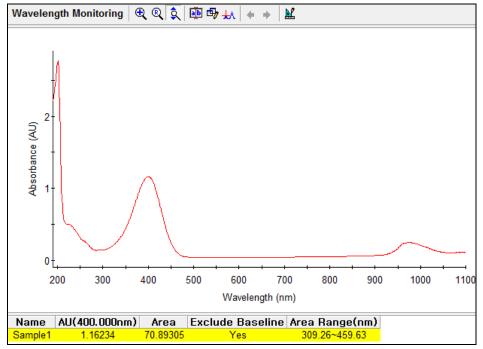
a. Exclude Baseline: Exclude the baseline from the calculated area value automatically.

- b. View Area Value: Display the area value in the result window.
- c. Start Wave (nm): Enter the start wavelength for the area calculation.
- d. End Wave (nm): Enter the end wavelength for the area calculation.
- e. Area: Display the calculated value.
- 3. After entering the parameters, click Area icon.



4. The calculated area and range are displayed in the Area tab. If you check View Area value, the area and range are displayed in the result window.

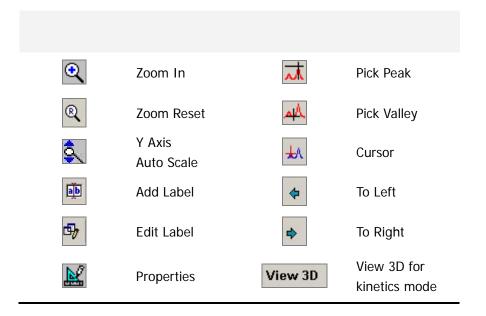




-<>

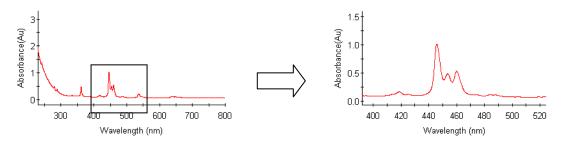
# **VIII**. Display Function Tools

■ Use the display function tools to modify the display of the main window as desired.



## VIII-1. Zoom In / Reset / Auto scale

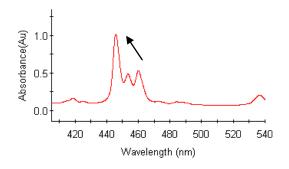
- Use these icons to zoom , reset and auto scale the selected area in the main window.
- Procedure
  - 1. Select Zoom In.
  - 2. Select the zoom area using the mouse, as shown.



- 3. To restore the original range, click **Zoom Reset**.
- 4. To Auto scale the Y axis, click the Y Axis Auto Scale.

## VIII-2. Add / Edit / Delete Label

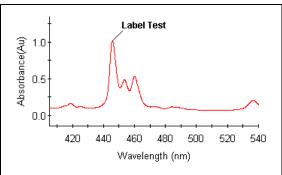
- Use these icons to add, edit and delete labels in the main window.
- Procedure
  - 1. Select Add Label.
  - 2. Set the label at the required position using the mouse, as shown below.



3. The following dialog box will be displayed.

S Add Label			×
Delete Label	Arial	<b>-</b> 9	• B <i>I</i> <u>U</u>
	ngth(nm) 46		
		<u>0</u> K	<u>C</u> ancel

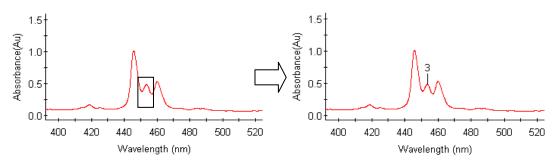
- 4. Enter comments, and select a font style and size.
- 5. Select OK.



6. To edit or delete the labels, click Edit Label and edit or delete the labels as desired.

## VIII-3. Pick Peak / Valley

- Use these icons to pick peaks/ valleys or seek the data.
- Procedure
- 1. Select Pick Peak/ Valley.
- 2. Select the spectral range using the mouse. The labels of the peaks or valleys are displayed as shown below.



3. To delete a peak/valley, select the peak/valley in the result window and click **the right mouse**.

Name	No.	Peak(nm)	Peak(AU)	)
Sample1	1	241.200	0.3829	
	2	249.970	0.1165	
	3	278.210	0.3274	Delete
	4	287.350	0.3597	_
	5	333.690	0.1651	Delete <u>A</u> ll
	6	345.400	0.1403	Сору

4. Select delete or delete all as shown.

## VIII-4. Properties and Display Interval

Use these icons to change chart properties in the main window, such as the chart color,

display interval, grid, X Axis, Y Axis and line properties.

Procedure

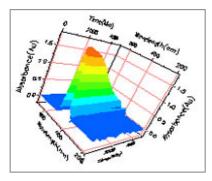
- 1. Select Properties.
- 2. Change properties as follows.
- 3. Select **OK** when finished.

Properties	_
Chart	*
Background Color	
Axis Color	
Display Interval	1
Legend	0.1
Grid	0.5
X Axis	1
Y Axis	2
Line	»
	<u>Q</u> K <u>C</u> ancel

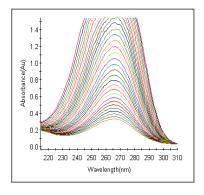
- a. Chart: Choose the colors of the background and Axis color.
- b. Display Interval: Select the wavelength display interval: 0.1 nm, 0.5 nm, 1 nm, 2 nm.
- c. Legend: Select to display the legend on the chart and where to position it.
- d. Grid: Select to display X and Y grids.
- e. X, Y Axis: Set the range of X and Y Axis.
- f. Line: Select the color and pattern of the spectrum lines.

## VIII-5. 3D Graphic mode

 It is possible to use the 3D the graphic mode in the kinetics modes: Time Based Kinetics and Temperature Based Kinetics, and the Bio modes: Enzyme and Thermal Denaturation. Click
 Veiw 3D to use this feature.



3D Graphic Spectra Mode



**Time Based Kinetics** 

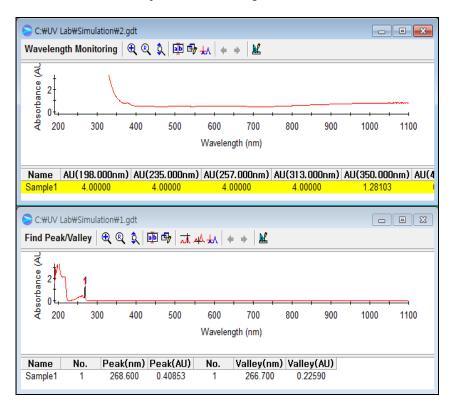
# IX. Window Menu

■ The Window Menu to arrange the windows and show the current windows.

Window         Tile Horizontally         Tile Vertically         Cascade	
Command	Function
Tile Horizontally Tile Vertically Cascade	Display the windows in the horizontal tile mode Display the windows in the vertical tile mode Display the windows in the cascade mode

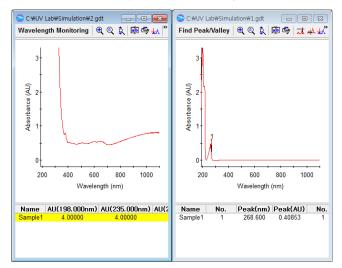
## IX-1. Tile Horizontally

■ Use the Tile Horizontally command to align the windows in the horizontal tile modes as below.



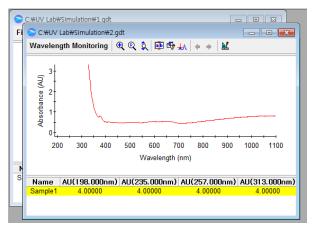
## IX-2. Tile Vertically

Use the Tile vertically command to align the windows in the vertical tile mode as below.



## IX-3. Cascade

Use the Cascade command to align the windows in the cascade mode as below.



■ The titles of the current windows are displayed as below.

<u>1</u> C:\UV Lab\Simulation\1.gdt
 <u>2</u> C:\UV Lab\Simulation\2.gdt

- Procedure
  - 1. To view a different window, click the window you want to view and the selected window is displayed.
  - 2. When the data in the window is saved, the file titles are listed.

# X. Help Menu

■ The Help Menu contains the Help contents for UV Lab software.

Help	Command	Function
Contents		
About	Contents	Display UV Lab Software Users guide as PDF file
	About	Display the version of UV Lab Software

## X-1. Contents

■ Use the help section for suggestions on using UV Lab software more effectively.

## X-2. About...

**About UV Lab** contains information on the version of the software as shown below.



<>

# XI. System Monitoring

Use System Monitoring to check the overall condition of the instrument. It provides a step-by-step explanation with figures, for the wavelength calibration of the spectrophotometer and the position calibration of the ARS (Water Jacketed Automatic Referencing Stage) and 8 Cell Water Jacketed Cell Changer.

Meas <u>u</u> re				
🗍 Run Blank	Alt+B			
🔋 Run Sample	Alt+S			
📴 Method	Alt+M			
Validation				
Options				
Instrument	Þ	Simple Dia	gnostics	
		Advanced D	iagnostic	s
		System Mor	nitoring	
System Monitoring				X
General	1			
Lamps & Electrics				
Wavelength Calibration				
Recalibration	0			
Multicell Calibration				
	L		0	
	Meas	sure Control	-	
		Dark Blank	Sample	General Parameters
	Viev		Inten.	Measurement Parameters
	Itera	ation 1 Cur. 1	Stop	X: Wavelength
	0	ter Control ON © OFF ter Speed <mark>0</mark> msec.	C ON	OFF
Read Diag				Close

Command	Function
General	Use to test sample simply for checking the instrument, to set the parameters for the measurement and to check the shutter and the filter. This mode for Service Engineer.
Lamp & Electrics	Use to check the status of the Lamp and the Electrics (Power, Fan) and to control the Lamp ON/OFF.
Wavelength Calibration	Use to perform a wavelength calibration of the instrument using standard samples.
Recalibration	Use to perform a wavelength calibration of the instrument using D2 Peaks.
ARS Calibration	Use to calibrate the cell position of the Water Jacketed Automatic Referencing Stage. This option only appears, if an ARS accessory is recognized by the instrument.
Multicell Calibration	Used to calibrate the cell position of the 8 Cell Water Jacketed Cell Changer. This option only appears if an 8 Multi-Cell accessory is recognized by the instrument mode appears.
Read Diag	Use to open the result file measured using Simple or Advanced Diagnostic.

## XI-1. General

• Use this mode for service engineer.

## XI-2. Lamp & Electronics

- Use this mode to check and control the status of the lamps and electrics, power and fan.
- 1. Select **Lamps & Electronics** in the System Monitoring. Then following dialog box is displayed.

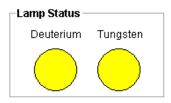
System Monitoring General Lamps & Electrics Wavelength Calibration Recalibration	Lamp Status Deuterium Tungsten Deuterium Tungsten Lamp Monitoring Deuterium Lamp 62010 min. Clear Tungsten Lamp 62505 min. Clear Power/Fan Speed Monitoring +12V: 12 V -12V: Fan Speed: 2000 rpm	-Deuterium Lamp Control ON OFF Tungsten Lamp Control OFF Lamp Power Deuterium Lamp 0.09 V Tungsten Lamp 5 V -12 V +5V: 5 V
Read Diag		Update Status Close

- a. Lamp Status: Use to verify the ON/OFF status of the lamps.
- b. Lamp Control: Use to control ON/OFF status of the lamps.
- c. Lamp Monitoring:
  - Lamp Time: Use to check the time used.
  - Lamp Power: Use to check status of lamp's power.
- d. Power / Fan Speed Monitoring: Use to check the power status and fan speed.
- e. **Update Status**: Use to update the status of the displayed window after modifying the current status of the instrument.

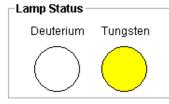
#### XI-2-1. Lamp Status

Use to check the ON/OFF status of lamps. When lamp is turned on, the circle appears yellow. When it is turned off the circle appears white.

Example 1: Both lamps are turned on.



Example 2: Only the VIS (tungsten) lamp is turned on.



### XI-2-2. Lamp Control

Use to control the ON/OFF status of the lamps.

- Select **On** in the Deuterium Lamp Control to turn on the Deuterium(UV) lamp. The user must wait until the process of updating the status is completed and disappears.
- 2. Select **OFF** in the Deuterium Lamp Control to turn it off.



- 3. Select **ON** in the Tungsten Lamp Control to turn on the Tungsten (VIS) lamp.
- 4. Select **OFF** in Tungsten Lamp Control to turn it off.

Deuterium La	mp Control ——
C ON	OFF

#### XI-2-3. Lamp Monitoring

Use to check the lamp power and time used.

1. **Lamp Time**: View the exhausted time of lamp. Select **Clear** to reset the time count only when installing a new lamp.

Lamp Time ——	
Deuterium Lamp	
6455 min.	Clear
Tungsten Lamp	
6245 min.	Clear

#### Lamp Life Time

- Deuterium lamp: 2,000 hours
- Tungsten lamp: 10,000 hours
- 2. Lamp Power: View the power status of each lamp.

-Lamp Power	·
Deuterium La	amp
	0.09 V
Tungsten Lar	mp
	5 V

### XI-2-4. Power / Fan Speed Monitoring

View status for the power and the fan speed of the instrument.

Power/Fan Speed Monitoring							
FOWGIA	an speeu	WOING	л шу				
+12V:	12 \	′ -1	2V:	-12	V +5V	ή ε	i V
Fan S	peed:	2200	rpm				

## XI-3. Wavelength Calibration

- Use this mode to calibrate the wavelength using a standard sample.
- 1. Select **Wavelength Calibration** in the System Monitoring. Then following dialog box will be displayed.

System Monitoring	×
General Lamps & Electrics Wavelength Calibration Recalibration	Calibration Value [1] 0 [2] 0 [3] 0 [4] 0 Measure Blank Absorbance Intensity Delete Sample Calibration
Read Diag	Total Peak No. : 0 Close

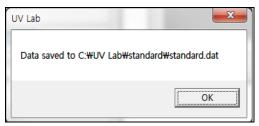
- a. Calibration Value: Indicate 4 factors for calibration curve.
- b. Measure: Use to measure the blank, absorbance and intensity.
- c. Load/Calibration: Use to retrieve spectra of standard samples stored in a current window and to perform calibration progress.
- d. Total Peak No.: Show the total peak numbers of entered values of standard materials.

#### XI-3-1. Wavelength Calibration Procedure

- 1. Select Wavelength Calibration. The Calibration Standards dialog box is displayed.
- 2. The "Standard.dat" file, which is stored in the Standard folder as the default, is opened. These values correspond to the wavelength positions of the absorbance peaks of the standard samples. Check these values for accuracy. Change the values to match those in the calibration certificate of the standard samples used, if necessary, and select OK.

	holmium	didymium	F1	D2
1	241.13	684.4	279.45	486
2	249.98	748.55	360.93	656.1
3	273.11	807.03	453.66	
4	287.4	879.28	536.59	
5	333.49		637.98	
6	345.38			
7	361.18			
8	385.7			
9	416.42			
10	451.33			
11	467.86			
12	485.26			
13	536.71			
14	640.62			

- ▶ Holmium: Refers to the Holmium Oxide Solution Standard. Enter the 14 values from the certificate of calibration closest to those shown in the default table above based on a SBW of 1.5 nm.
- Didymium: Refers to the Didymium Filter Standard. Enter the 4 values from the certificate of calibration closest to those shown in the default table above based on a SBW of 1.5 nm.
- F1: Refers to the Holmium Oxide Filter Standard. Enter the 5 values from the certificate of calibration closest to those shown in the default table above based on a SBW of 1.5 nm.
- D2: Refers to the Instrument's deuterium lamp and is not an external standard. Use the default values provided in the table.
- 3. The following dialog box is displayed. Select **OK.**



System Monitoring	×
General	0.006 -
Lamps & Electrics	0.004
Wavelength Calibration	0.002
Recalibration	0
	-0.004
	0 500 1,000
	Calibration Value [1] 162E-08 [2] 818E-05 [3] 9849603 [4] 184.5745
	Heasure         Load / Calibration           Blank    Sample No.
	Abs. Add Sample Calibration
	Total Peak No.: 25
Read Diag	Close

4. Remove the sample from the cell holder and select **Blank**.

5. Place standard sample 1 (Holmium oxide solution) in the cell holder and click **Absorbance**.

System Monitoring	×
General Lamps & Electrics Wavelength Calibration Recalibration	Image: Calibration Value         Calibration Value         [1] 1162E-08       [2] 818E-05       [3] 9849603       [4] 184.5745         Measure       Image: Calibration       Sample No.       Image: Calibration         Blank       Image: Calibration       Sample No.       Image: Calibration         Inten.       Image: Calibration       Calibration         Total Peak No.: 25       25
Read Diag	Close

System Monitoring	×
General Lamps & Electrics Wavelength Calibration Recalibration	Calibration Value [1] 162E-08 [2] 818E-05 [3] 9849603 [4] 184.5745 Measure Blank Abs. Inten. Total Peak No: 25
Read Diag	Close

6. Select **Add Sample** to save the data from Sample 1 and select **1** for the Sample No.

7. Place the Standard Sample 2 (Didymium filter) in the cell holder and click Absorbance.

System Monitoring	×
General Lamps & Electrics Wavelength Calibration Recalibration	4 (n) aue 2 aue 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
	Calibration Value [1] 162E-08 [2] 818E-05 [3] 9849603 [4] 184.5745 Measure Blank Add Sample Calibration Inten. Delete Sample Calibration Total Peak No.: 25
Read Diag	Close

8. Select Add Sample to save the data for Standard Sample 2 and select 2 for the Sample No.

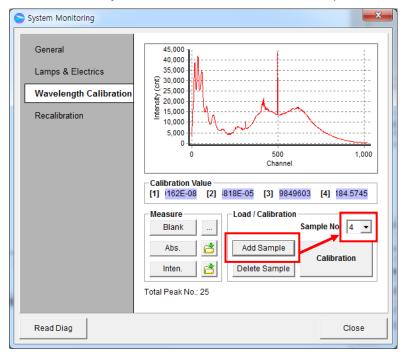
9. Place the Standard Sample 3, F1 (Holmium Oxide) filter in the cell holder and select **Absorbance**.

System Monitoring	×
General Lamps & Electrics Wavelength Calibration Recalibration	Calibration Value [1] [162E-08 [2] 818E-05 [3] 9849603 [4] [84.5745]
	Measure       Load / Calibration         Blank          Abs.          Inten.          Delete Sample       Calibration         Total Peak No.: 25
Read Diag	Close

10. Select Add Sample to save the data for Standard Sample 3 and select 3 for the Sample No.

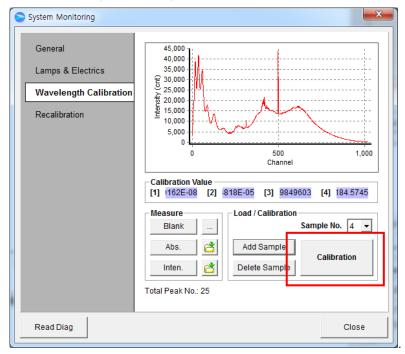
11. Remove all samples from the cell holder and click **Intensity** to measure Standard Sample 4 (D<sub>2</sub> peak).

System Monitoring	×
General Lamps & Electrics Wavelength Calibration Recalibration	45,000 40,000 35,000 25,000 10,000 5,000 0 0 5,000 0 0 5,000 0 0 5,000 0 0 5,000 0 0 5,000 0 0 5,000 1,000 5,000 0 0 5,000 0 0 0 0 0 0 0 0 0 0
	Calibration Value [1] 162E-08 [2] 818E-05 [3] 9849603 [4] 184.5745 Measure Blank Add Sample Calibration Inten. Total Peak No.: 25
Read Diag	Close

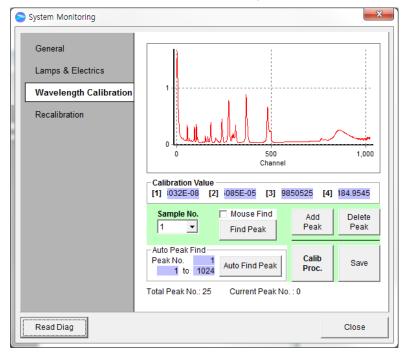


12. Select Add Sample to save the data of Standard Sample 4 and select 4 for the Sample No.

13. After measuring and adding all standard samples, click Calibration.



14. The following dialog box is displayed. Find the peaks for each standard sample and add them in this window. See **XI-3-3**. **Peak finding** for more information.



- 15. Select '1' in the [Sample No.].
- 16. Find **14 peaks** for Standard Sample 1 (Holmium oxide solution) and add them as shown below.

System Monitoring
General         Lamps & Electrics         Wavelength Calibration         Recalibration         Calibration         Calibration         Calibration         Image: Calibration Value         Value Peak Find         Peak No.         Peak No.         Calibration         Auto Peak Find         Peak No.         Calib         Peak No.         Calibration         Save         Total Peak No.: 25         Current Peak No.: 14
Read Diag         Close

17. Select '2' in the [Sample No.].

System Monitoring		×
General Lamps & Electrics Wavelength Calibration Recalibration	Calibration Value	800
	[1]         1032E-08         [2]         1085E-05         [3]         9850525         [4]           Sample No.          Mouse Find         Add         Add         Peak	4] 184.9545 Delete Peak
	Auto Peak Find Peak No. 1 214 to 757 Auto Find Peak Calib Proc.	Save
	Total Peak No.: 25 Current Peak No. : 18	
Read Diag		Close

18. Find **4 peaks** for Standard Sample 2 (Didymium filter) and add them as shown below.

- 19. Select '3' in the [Sample No.].
- 20. Find **5 peaks** for Standard Sample 3 (F1 Filter) and add then as shown below.

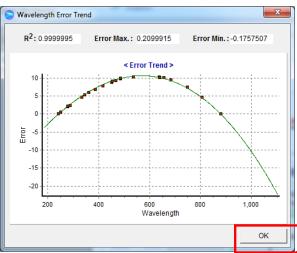


21. Select '4' in the [Sample No.].

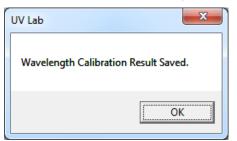
System Monitoring General Lamps & Electrics Wavelength Calibration Recalibration	45,000 40,000 (m) 35,000 925,000 40,0000 40,00000000	
		1,000 [4] 184.9545
	Sample No. 4 Auto Peak Find Peak No. 499 to 495 Auto Find Peak Calib Proc.	Delete Peak Save
Read Diag	Total Peak No.: 25 Current Peak No.: 25	Close

22. Find **2** peaks for Standard Sample 4  $(D_2)$  and add them as shown below.

- 23. Check the numbers of found peaks. If all peaks were founded, verify that the current Peak No. is equal to the Total Peak No.
- 24. Click **Calib Proc.** Check the result of the calibration curve and verify that all of the data fits in the following Wavelength Error Trend window. If the calibration result is acceptable, select **OK**. If the result is not acceptable ( $R^2 > 0.9999$  Error Max. and Min.  $<\pm 1$ ), repeat the calibration procedure.



25. Select **Save**. The below dialog box is displayed.



26. Select **OK** to finish the wavelength calibration.

#### XI-3-2. File & Edit Menu

After measuring and adding all standard samples, click **Calibration**. The following dialog box is displayed. Peak points can be found and added for each standard sample here.

File	Edit				
		holmimum	didymium	F1	D2
	1	241.07	684.7	279.35	486
	2	249.92	749	360.9	656.1
	3	278.05	807.5	453.6	
	4	287.34	879.7	536.4	
	5	333.42		637.45	
	6	345.4			
	7	361.17			
	8	385.75			
	9	416.5			
	10	451.37			
	11	467.93			
	12	485.3			
	13	536.81			
	14	640.69			

1. Available commands in File menu and their function include:

File	
Open	Ctrl+O
Save	Ctrl+S
Save A	s

Open: Use the Open command to retrieve data for a standard sample stored in a current windows.

Com	nputer	Local Disk (C:)      UV Lab     Calib	✓ 4 Search Calib	
Organize 🔻 New	folder		8	= - 1 (
🔆 Favorites	^	Name	Date modified	Туре
🧮 Desktop		data1.dat	3/13/2015 4:02 PM	DAT File
鷆 Downloads		data2.dat	3/13/2015 4:02 PM	DAT File
📃 Recent Places		data3.dat	3/13/2015 4:02 PM	DAT File
		Filter_Set.dat	5/13/2003 11:34 AM	DAT File
门 Libraries		intensity.dat	3/12/2003 3:37 PM	DAT File
Documents	=	Lamp_Intensity.dat	1/10/2011 10:02 AM	DAT File
🎝 Music		multicell.dat	10/31/2002 7:40 AM	DAT File
Pictures		MultiCell_LabPro.dat	10/31/2002 7:40 AM	DAT File
😸 Videos		multicell1.dat	2/22/2002 8:59 AM	DAT File
		peakpoint.dat	3/13/2015 4:02 PM	DAT File
🖳 Computer		Pel_temp_Calib.dat	5/28/2002 11:14 AM	DAT File
		Peltier_Cal_External.dat	5/28/2002 9:00 AM	DAT File
🗣 Network	Ψ.			,
F	ile nar	ne:	▼ File	

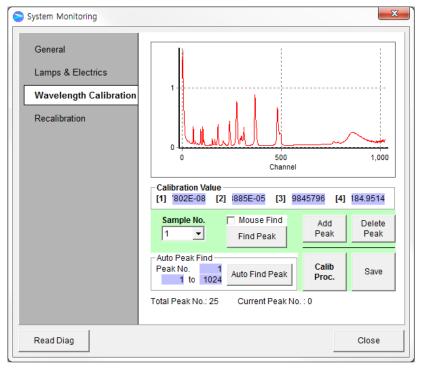
- Save: Use the Save command to save data in the current window. The save window showing the storage position will appear when Save is clicked.
- Save As: Use the Save as command to save data using a new file name. Select the folder to save file in. Input the file name, and click Save.
- 2. Available commands and its functions in Edit menu are as follows.

Edit		
Co	py Cell	Ctrl+C
De	elete Cell	
C	ut Cell	Ctrl+X
Pa	iste Cell	Ctrl+V
Ac	ld Row	
Ac	ld Column	
De	elete Row	
De	elete Column	

- Copy Cell: Use the Copy Cell command to copy cell.
- **Delete Cell**: Use the Delete Cell command to delete cell.
- Cut Cell: Use the Cut Cell command to cut cell.
- ▶ Paste Cell: Use the Paste Cell command to paste cell.
- Add Row: Use the Add Row command to add row.
- Add Column: Use the Add Column command to add column.
- **Delete Row:** Use the Delete Row command to delete row.
- **Delete Column:** Use the Delete Column command to delete column.

### XI-3-3. Peak Finding

Peak finding is used to manually identify the correct peaks in the reference samples to use for wavelength calibration as shown.



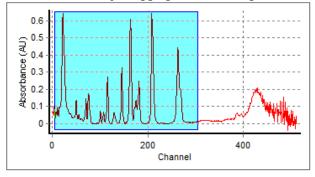
- Sample No. : The number of samples which was added, and find peak points by designating each sample spectrum.
- Find Peak, Add Peak, Delete Peak: Use to find peaks in the spectrum manually. Zoon the peak point to find in the spectrum and click Find Peak. The highest peak in the area zoomed is found automatically. Select Add Peak to save the peak. Select Delete Peak to delete it.
- Calib Proc.: Select Calib Proc. after finding a satisfactory peak point for all spectra.
   Wavelength Calibration will be performed and an Error Trend window will appear.
- Save: Click Save to save satisfactory results.
- Mouse Find: Use to find and add peak using mouse dragging. Check Mouse Find and designate peak finding area. One peak is found and added automatically in the designated area without selecting Find Peak or Add Peak.

# Peak Finding Procedure

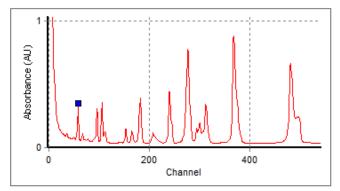
1. Select the peak finding area.

Drag the mouse left to right on the main screen as shown below. The original condition

can be restored by dragging the mouse right to left while pushing the left mouse button.



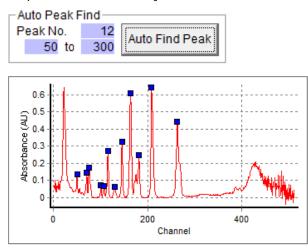
2. Select **Mouse Find**. Click and drag the mouse from the upper-left to the lower-right over the tip of a peak. A small blue square appears at the peak position identified.



If it is difficult to find the correct peak, unclick **Mouse Find**, repeat step 1 designating a smaller area of the spectrum and try again.

If the wrong peak position is identified, unclick **Mouse Find** and click **Delete Peak**. The last peak position to be identified is deleted.

Auto Find Peak / Peak No.: Use to find peaks with Auto Find Peak. Designate a peak finding area and enter the number of peaks to find. Click the Auto Find Peak. The amount of peaks can be found by the order of their value.



## XI-4. Recalibration

- $\blacksquare Use to recalibrate the wavelength using D_2 peaks.$
- 1. Select **Recalibration** in the System Monitoring. The below dialog box appears.

System Monitoring		x
General Lamps & Electrics Wavelength Calibration Recalibration	Calib. Proc. Save	
Read Diag	Close	

- Calib. Proc.: Use to progress recalibration.
- **Save:** Use to save recalibrated result.

#### XI-4-1. Recalibration Procedure

Use to recalibrate the wavelength using  $D_2$  Peak.

Select Recalib	ration in the	System	Monitorina	The below	dialog box	annears
Juliu Recallo		System	wormoning.		ulaiby box	appears.

System Monitoring		×
General Lamps & Electrics Wavelength Calibration Recalibration	00 Calib. Proc.	
Read Diag	Clo	DSe

- 1. Measure D<sub>2</sub> by selecting **Calb. Proc**...
- 2. Remove sample from cell holder and select **OK**.
- 3. After measuring  $D_2$ , the result is displayed in two ways according to average dev. peak value.

#### a. If the value of 'Average Dev. Peak' is more than 0.2 nm:

Recalibration is needed because the difference between  $D_2$  peak and currently measured wavelength is more than 0.2.

#### b. If the value of 'Average Dev. Peak' is less than 0.2 nm:

Recalibration is not needed because the difference between the  $D_2$  peak and currently measured wavelength is less than 0.2.

### XI-4-2. In case Recalibration needs

1. To recalibrate, select Yes.

UV Lab	×
Do you want to Recalibrate?	
Yes	No

- 2. After recalibration is completed, select **OK**.
- 3. Select **Save** to save the data.

System Monitoring	×
General Lamps & Electrics Wavelength Calibration Recalibration	60,000 50,000 40,000 30,000 10,000 0 500 10,000 Channel Calib. Proc. Save
Read Diag	Close

4. The following dialog box appears. Select **OK**.



### XI-4-3. In case Recalibration is not needed

System Monitoring General Lamps & Electrics Wavelength Calibration Recalibration	60,000       50,000       40,000       20,000       10,000       0       500       Channel	X
Read Diag		Close

1. If recalibration is not needed, the following dialog box appears.

## XI-5. Read Diag

■ Use **Read Diag** to open result files for Simple or Advanced Diagnostic.

System Monitoring	×
System Monitoring General Lamps & Electrics Wavelength Calibration Recalibration	0 Measure Control Dark Blank Sample General Parameters
	View © Absorb. © Trans. © Inten. Iteration 1 Cur. 1 Stop X: Wavelength
	Shutter Control     Filter Control       C ON
Read Diag	Close

### XI-5-1. Open the Result of Simple Diagnostics

- 1. Select Read Diag. The Open Diagnostic Result window is displayed.
- Select the desired file in the Diag folder and select Open. The selected Simple Diagnostics Result window is displayed as shown.

		-41 0		
	Simple Diagno	SUCS R	esuit	-
I. Instrumental In	formation			
Instrument Serial No	. 465K5081201	-	DefIntNo.	800
Firmware Version	160513	-	ScanNo.	10
Software Version	UV Lab 4.1.1	-	IntNo.	1
		-	Partial Trans.	0
Deuterium Peak Cha	an 486.0 nm 3129	-	Start Chan.	0
	656.1 nm 4979	_	End Chan.	0
			Dark Sub.	200
			Dark Cal.	0
			Rev. Data	0
			Save Raw	0
Calib. parameters	-1E-08	-7.273E-	050.9840166	185.5241
Multicell Step	-	-	-	-
	-	-	-	-
ARS Step	-	- C		
2. Diagnostic Tes	Permitted Value		Result	Status
Shutter Time	10 msec < Result <	80 msec	11.1 msec	PASS
Dark Current	1000 cnt < Result <	3000 cnt	1926 cnt	PASS
Intensity	Avg.Intensity > 3000		12072 cnt	PASS
	+/- 0.2 nm at 486.0 n	im	485.96 nm	PASS
Wavelength			656.05 nm	PASS
Wavelength	+/- 0.2 nm at 656.1 n	1111		
Wavelength Noise	+/- 0.2 nm at 656.1 n < 0.0002	IIII	0.000030	PASS

(Result file measured in the simple diagnostic mode are "\*-S.dgs")

#### XI-5-2. Open the Result of Advanced Diagnostics

1. Select **Read Diag.** Then the **Open Diagnostic Result window** will be displayed.

2. Select the desired file in **Diag** folder and select **Open**. The selected **Diagnostics Result** window is displayed as shown.

(Result file measured in the advanced diagnostic mode are "\*-A.dgs")

Next Page	Prev Page Clos	se					
	Adva	nced Diagnostic R	esult	-			
1. Instrument	al Information						
			DefintNo.	800	-		
			IntNo.	1	-		
Deutriu			Start Chan.	0			
	030.11	4010	Dark Sub.	200			
			Rev. Data	0	-		
			Save Raw	0	-		
	Calib. parameters	-1E-08 -7.273E-	0.9840166	185.5241	-		
		<u> </u>	-	-	-		
	<u>,</u>	•			-		
Division General	Test Shutter Time	Permitted Value < 80 msec		Result 11.1 msec	Status PASS		
	Fan Status	> 2000 rpm		3000 rpm	PASS		
					PASS PASS		
	Wavelength	486.0 nm : +/- 0.2 nm		485.96 nm	PASS		
		656.1 nm : +/- 0.2 nm		656.05 nm	PASS		
					PASS PASS		
	Power Status				PASS		
		5V : +/- 0.25 V		4.99 V	PASS		
Lamp	On/Off Check			ON	PASS		
	Lamp Time				PASS PASS		
	camp time			910hours	PASS		
	Lamp Power		-/- 0.30 V	0 V 4.94 V	PASS PASS		
		rangoton camp to vy.		4.04 0	1400		
	2. Diagnostic Division General	1. Instrumental Information         Instrument Serial No.       465K50         Firmware Version       100 Lab         Software Version       0V Lab         Deutrium Peak Chan.       486.0 n         656.1 m       656.1 m         Calib. parameters         Multicell Step         ARS Step         2. Diagnostic Test Results         Division       Test         General       Shutter Time         Power Status       Dark Current         Intensity       Wavelength         Noise       Power Status         Lamp       On/Off Check         Lamp Time       Calip. parameters	Instrumental Information           Instrument Serial No. 160513           Software Version           UV Lab 4.1.1           Deutrium Peak Chan. 480 0 nm 3129           656.1 nm 3129           656.1 nm 3129           Muticel Step           -           ARS Step           Division           Test           Division           Test           Operating Software Version           Data Status           ARS Step           -           ARS Step           Division           Test           Data Current           Data Current <td colspan="2" d<="" td=""><td>Instrument Serial No.         465K5081201         DefintNo.           Firmware Version         U/V Lab 4.1.1         ScanNo.         ScanNo.           Deutrium Peak Chan.         486.0 nm         3129         Start Chan.           Deutrium Peak Chan.         486.0 nm         3129         Start Chan.           Deutrium Peak Chan.         486.0 nm         3129         Start Chan.           Deutrium Peak Chan.         486.0 nm         4979         Start Chan.           Dark Sub.         Dark Sub.         Dark Sub.         Dark Sub.           Dark Sub.         Dark Sub.         Dark Sub.         Dark Sub.           Muticell Step         -         -         -           ARS Step         -         -         -           ARS Step         -         -         -           Division         Test         Permitted Value         General           General         Shutter Time         &lt; 80 msec</td>         -           Fan Status         &gt; 2000 rpm         Dark Current         1000 cnt           Dark Current         1000 cnt         -         -           Noise         &lt; 0.0002</td> rd         -           Power Status         12V: +/- 0.6 V         -	<td>Instrument Serial No.         465K5081201         DefintNo.           Firmware Version         U/V Lab 4.1.1         ScanNo.         ScanNo.           Deutrium Peak Chan.         486.0 nm         3129         Start Chan.           Deutrium Peak Chan.         486.0 nm         3129         Start Chan.           Deutrium Peak Chan.         486.0 nm         3129         Start Chan.           Deutrium Peak Chan.         486.0 nm         4979         Start Chan.           Dark Sub.         Dark Sub.         Dark Sub.         Dark Sub.           Dark Sub.         Dark Sub.         Dark Sub.         Dark Sub.           Muticell Step         -         -         -           ARS Step         -         -         -           ARS Step         -         -         -           Division         Test         Permitted Value         General           General         Shutter Time         &lt; 80 msec</td> -           Fan Status         > 2000 rpm         Dark Current         1000 cnt           Dark Current         1000 cnt         -         -           Noise         < 0.0002		Instrument Serial No.         465K5081201         DefintNo.           Firmware Version         U/V Lab 4.1.1         ScanNo.         ScanNo.           Deutrium Peak Chan.         486.0 nm         3129         Start Chan.           Deutrium Peak Chan.         486.0 nm         3129         Start Chan.           Deutrium Peak Chan.         486.0 nm         3129         Start Chan.           Deutrium Peak Chan.         486.0 nm         4979         Start Chan.           Dark Sub.         Dark Sub.         Dark Sub.         Dark Sub.           Dark Sub.         Dark Sub.         Dark Sub.         Dark Sub.           Muticell Step         -         -         -           ARS Step         -         -         -           ARS Step         -         -         -           Division         Test         Permitted Value         General           General         Shutter Time         < 80 msec	I. Instrumental Information           Instrument Serial No. 1465K5061201           Software Version         Utab 4.1.1           Deutrium Peak Chan. 1465.0 nm 3129           Deutrium Peak Chan. 100           Optimized Section 100           Deutrium Peak Chan. 100           Deutrium Peak Chan. 1010           Deutrium Peak Chan. 1010           Optimized Section 100           Deutrium Peak Chan. 1020           Deutrium Peak Chan. 1010           Deutrium Peak Chan. 1010           Optimized Section 1010           Calib. parameters           -1E-08         -7.273E-05         0.98401166         185.5241           Muticell Step         -           -         -           ARS Step         -           -           Dark Current 10000 cnt         1825 cnt           Dark Current 10000 cnt         1928 cnt           Dark Current 10000 cnt         1928 cnt           Dark Current 10000 cnt         1928 cnt

## XI-6. Automatic Referencing Stage Calibration

In this mode, you can calibrate the cell position of the ARS (Water Jacketed Automatic Referencing Stage).

System Monitoring	×
General Lamps & Electrics Wavelength Calibration Recalibration ARS Calibration	Cell Position       [1]0       [2]0       ARS Move       1     2       Calibration Parameters       Total Step       150       One Step       Stop         Save       Result
Read Diag	Close

#### 1. Select ARS Calibration in the System Monitoring.

- **Reset ARS:** Use to move to '0' step of ARS position.
- Cell Position: Show the saved steps for each cell position of the ARS.
- ARS Move:
- Use to move ARS position by clicking \_\_\_\_\_ buttons.
- Use to move ARS position using \_\_\_\_\_ buttons by entered step.
- Calibration Parameters:
- Total Step: Show the total steps(150) of the operating ARS.
- One Step: Show the unit of the operation of the ARS pulse (usually used as 1 value).
- **ARS Calibration:** Use to perform ARS Calibration.
- **Stop:** Use to stop ARS Calibration.
- Save Result: Use to save calibrated result.

#### XI-6-1. ARS Calibration Procedure

- 1. Select **Reset ARS** to format the ARS.
- 2. Select ARS Calibration as shown below. Default Value: 150 (Total Step), 1 (One Step)

System Monitoring	X
General Lamps & Electrics Wavelength Calibration Recalibration ARS Calibration	Cell Position
l	ARS Move     <<<>>>       1     2       Calibration Parameters     Step       Total Step     150       One Step     1       Stop     Save       Result     Stop
Read Diag	Close

- 3. Remove all samples from ARS and select **OK**.
- 4. Then ARS calibration will start. The current process of calibration is shown.

System Monitoring	×
General Lamps & Electrics Wavelength Calibration Recalibration ARS Calibration	
	Reset ARS     Cell Position [1]0     [2]65535       ARS Move     << >>       1     2     Step       Calibration Parameters     ARS       Total Step     150       One Step     1       Stop     Save       Result     Stop
Read Diag	Close

5. When calibration is finished, select **OK**.

Save Result

6. Select Save Result

to save data. Then select OK.

## XI-7. Multi-Cell Holder Calibration

- Use to calibrate the cell position of the 8 Cell Water Jacketed Cell Changer.
- 1. Select Multicell Calibration in the System Monitoring. The following dialog box is displayed.

System Monitoring	×
General	
Lamps & Electrics	
Wavelength Calibration	
Recalibration	
Multicell Calibration	
	0
	Cell Position
	Reset [1]N/A [2]N/A [3]N/A [4]N/A Multicell
	[5]N/A [6]N/A [7]N/A [8]N/A
	1 2 3 4 5 6 7 8 <<<>>> Step 1
	Calibration Parameters Total Step 590 One Step 1 Stop Stop
Read Diag	Close

- **Reset Multicell:** Use for formatting the Cell holder.
- Cell Position: Show the saved data for each cell position of the Cell holder.
- Multicell Move:
- Use to move Cell position by clicking 1 2 3 4 5 6 7 8 buttons.
- Use to move Cell position using \_\_\_\_\_ buttons by the entered step.
- Calibration Parameters:
  - Total Step: Show the limitation of operation of the Cell holder pulse. Usually you can use the set value as a default (590).
  - One Step: Show the units of the operation of the Cell holder pulse. (usually used as 1 value).
- Multicell Calibration: Use to find each position of the Cell holder.
- **Stop:** Use to stop Cell holder Calibration.
- Save Result: Use to save the data after Cell holder Calibration.

#### XI-7-1. Multicell Calibration Procedure

- 1. Select Reset Multicell to format the 8 Cell Water Jacketed Cell Changer.
- 2. Enter the value in Calibration Parameters.
- 3. Select Multicell Calibration. Remove all samples from Cell holder and select OK.
- 4. The Cell holder Calibration will start. The current process of calibration is shown.

Lamps & Electrics Wavelength Calibration Recalibration Multicell Calibration	
	0         50         100         150         200           Cell Position           Multicell         [1]13         [2]73         [3]131         [4]191           [5]251         [6]310         [7]369         [8]429           Multicell         Move          <         >>>           1         2         3         4         5         6         7         8         <<>>>
	Calibration Parameters Total Step 590 One Step 1 Stop Stop Stop

5. When calibration is finished, click **OK**.

