UV-VIS SPECTROSCOPY

LAMBDA 465

UV Lab Software Users Guide

PerkinElmer

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® 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® 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

· Microsoft® Windows 7 or Windows 8 or Windows 10
· If using UV Lab Security Software : Microsoft® Windows 7 Professional, Ultimate, Enterprise, Windows 8, Windows 10

Output Device

· Microsoft® Windows compatible printer

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

General Mode	
Wavelength Monitoring	· The full spectrum (190 ~ 1100 nm) of each sample is extracted at once
Equation Calculation	· Allows the user to enter support equations for the evaluation of the data · Supports Functions: +, - , /, *, ABS, Exp, LN, LOG10, SQRT
Find/ Peak Valley	· Find up to 30 of the peaks or valleys automatically or manually
Quantification Mode	
Quantification Standard/ Sample	· Concentration Unit: All units user-specifiable · First, second and third order calibration curve fits · 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 Kinetics	· 3D Display: Time Display(X Axis), Zoom In/Zoom Out, Rotate Chart · 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 Based Kinetics	· 3D Display: Time Display(X Axis), Zoom In/Zoom Out, Rotate Chart · Temperature Unit: ℃ · Temperature Limit: from -10℃ to 100℃
Ultra Kinetics	· Time Unit: Msec · Minimum Interval Time: 20 msec

UV Lab Bio Analysis (Optional)

Bio Mode	
Nucleic Acid Analysis	· General Ratio with two wavelengths for the calculation of user specified 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 Mechanism	· Michaelis-Menten · Lineweaver-Burk · Hanes-Woolf · Eadie-Hofstee · Calculate K_m, V_{max} from each plot
Thermal Denaturation	· Temperature Unit: ℃ · Temperature limit: from -10°C to 100°C · T_m calculated with average method & 1st Derivative · Volume correction with user specified equation · Normalization with user specified factor · User defined equation allows calculation from T_m 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 Analysis (MCA)	· Analyze complex compounds containing multiple components (up to 4 components) · Define the concentration of each component .This can be added to any of UV Lab Software upon request.

Validation

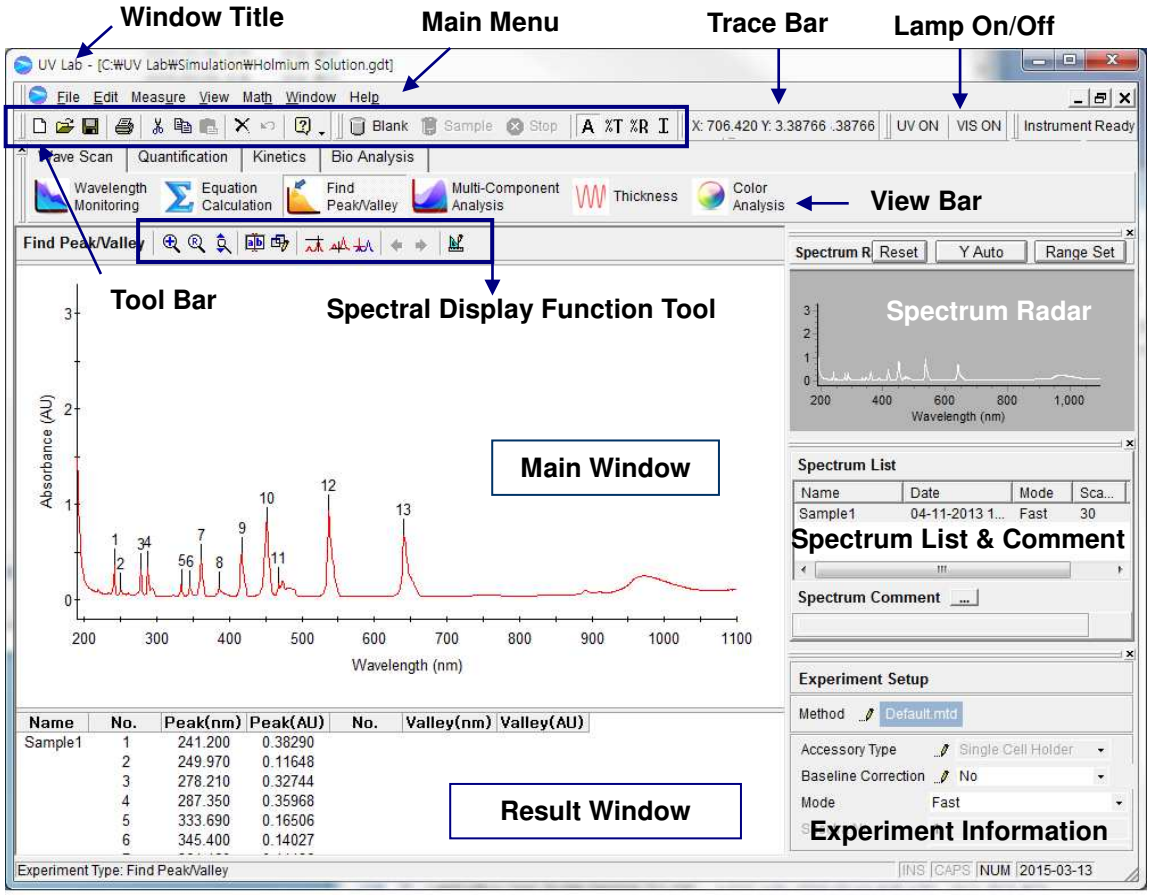
Validation	· Automatic Validation Wizard assists with the validation across the UV and Visible range · Includes Photometric, Wavelength, Resolution, Stray Light, Stability and Dark Current tests

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.

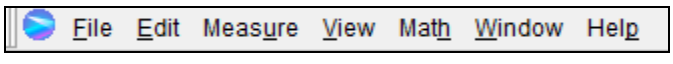


I-3-1. Window Title

▣ 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.



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
	Open	Ctrl + O		Blank	Alt + B
	Save	Ctrl + S		Sample	Alt + S
	Print	Ctrl + P		Stop	
	Cut	Ctrl + X		Absorbance	Alt + A
	Copy	Ctrl + C		%T Transmittance	Alt + T
	Paste	Ctrl + V		%R Reflectance	Alt + R
	Delete	Del		Energy	Alt + I
	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.

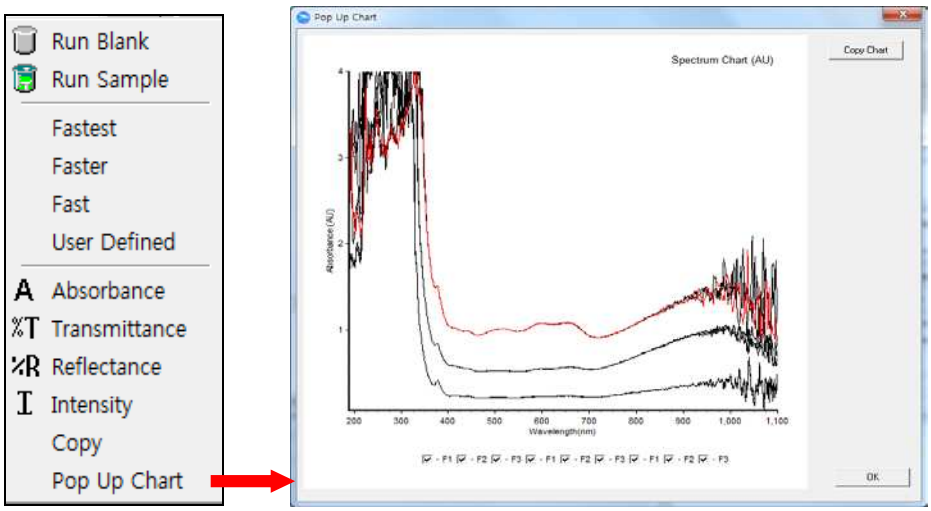
Toolbar	Command	Description
	Zoom In	Zoom in the selected area
	Zoom Reset	Reset the zoom area to the original size
	Y Axis Auto Scale	Allow the auto scale of Y-axis depending on the measurement result
	Pick Peak	Look for peaks. Use in Peak/Valley Mode
	Pick Valley	Look for valleys. Use in Peak/Valley Mode
	Cursor	Display the cross lines for selecting data points
	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
	Add Label	Add label on the spectrum.
	Edit Label	Edit label on the spectrum.
	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.

ii) Pop up chart is useful when you confirm that selected spectrum.



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)	AU(635.000nm)
Sample1	0.20616	0.29595	0.19140	0.10337	0.21510

Copy

I-3-7. Trace bar

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

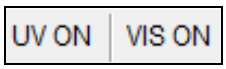
X: 531.073 Y: -0.83019

I-3-8. Lamp ON/ OFF

▣ Switch the lamp ON/OFF.

▣ Procedure

1. Followings are the status of the lamp.



▶ 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.

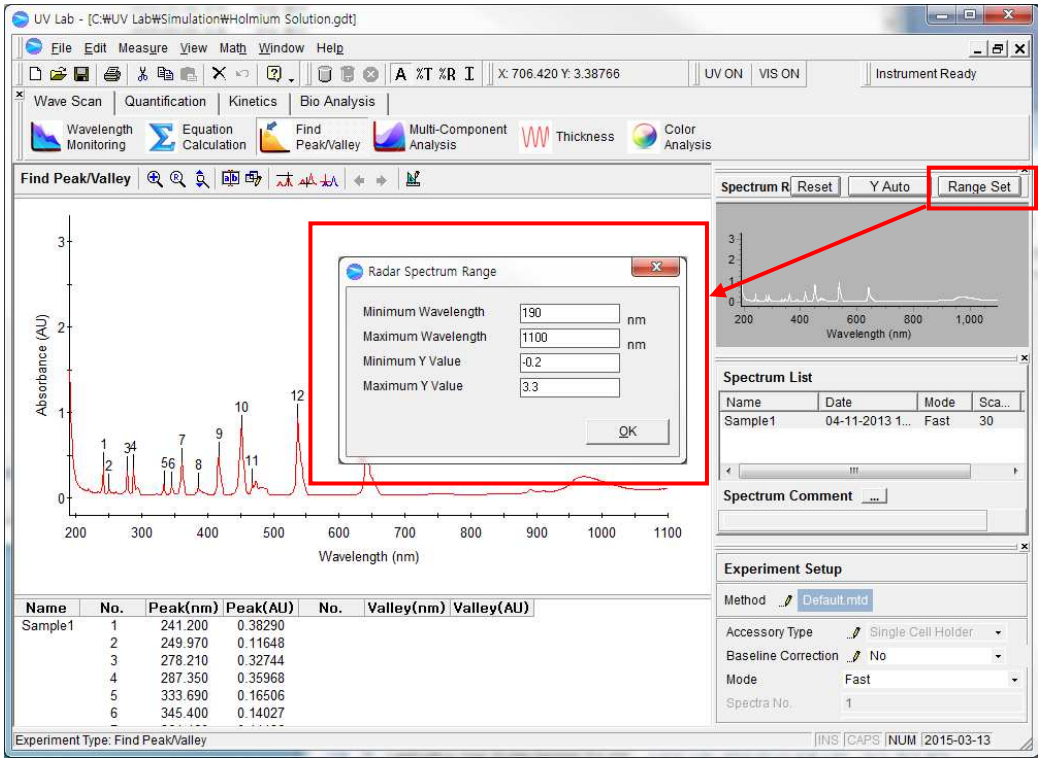
4. If you want to turn on the UV lamp, click UV OFF. The following dialog box will be displayed. Select OK.

▶ 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.



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

1. By clicking the 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 button
3. By clicking the Reset button, reset the Y-axis to the original size.

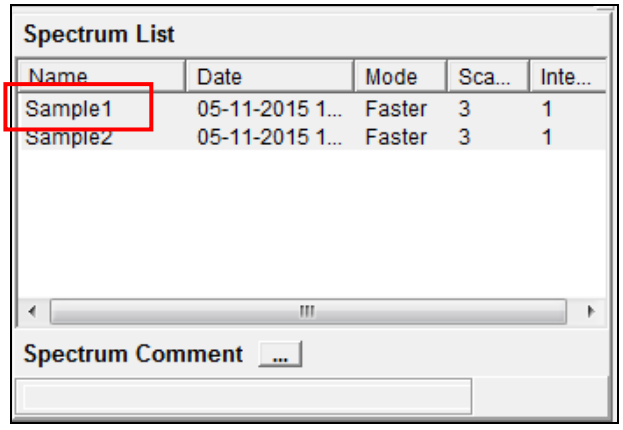
I-3-10. Spectrum List & Comment

▣ Display the Name, Date, Mode, Scan No. and Integration No. of spectrum in the main window.

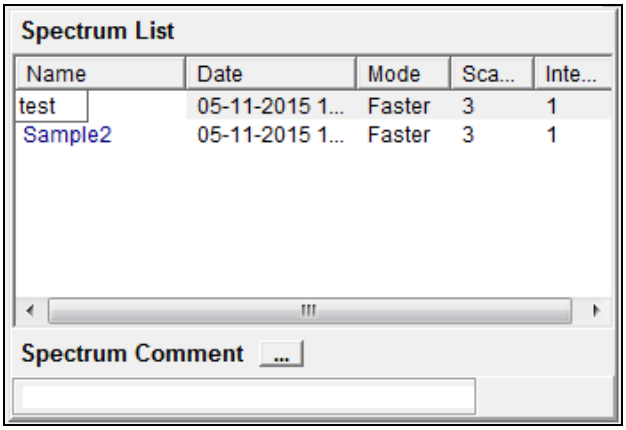
▣ Procedure

a. Spectrum List.

1. Change a spectrum name by clicking it.

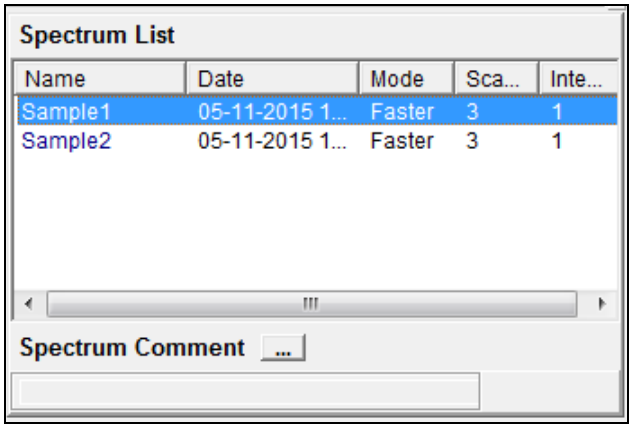


2. Enter a new name.

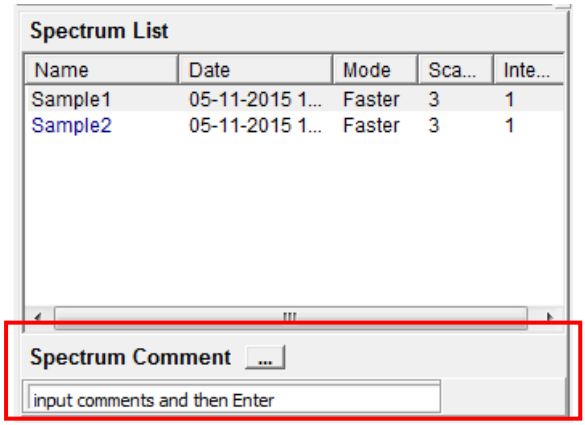


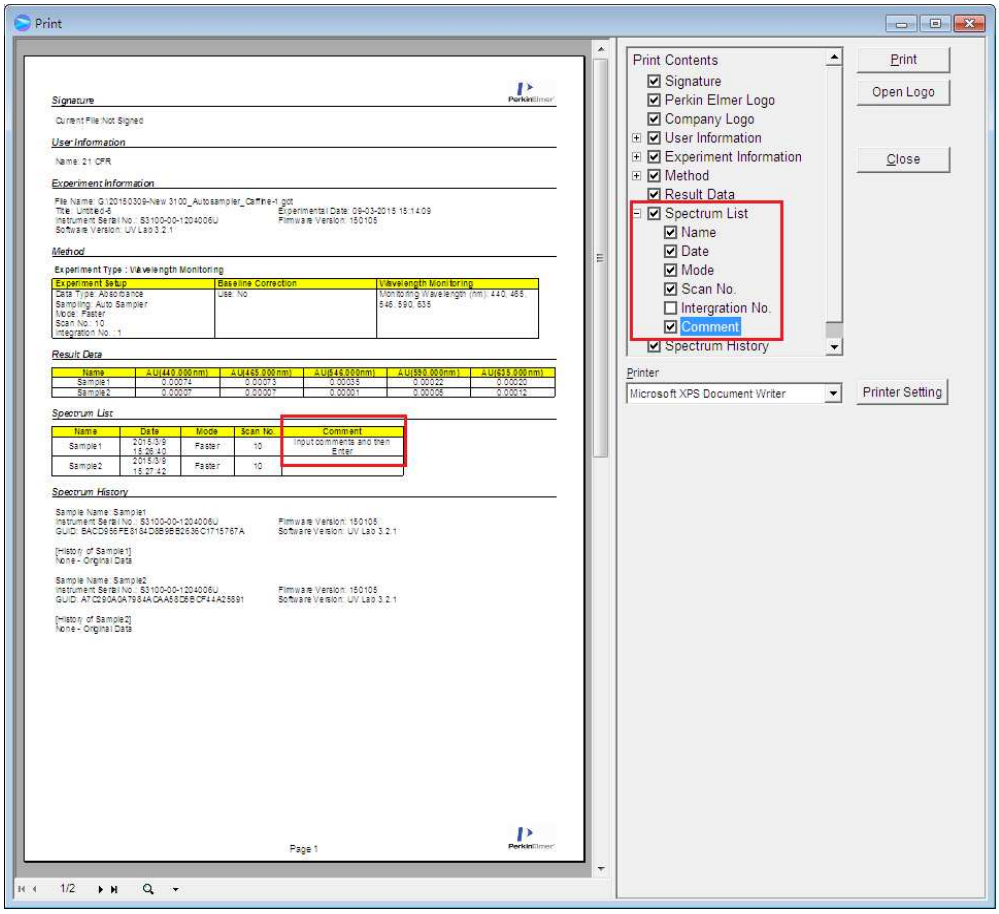
b. Spectrum Comment

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



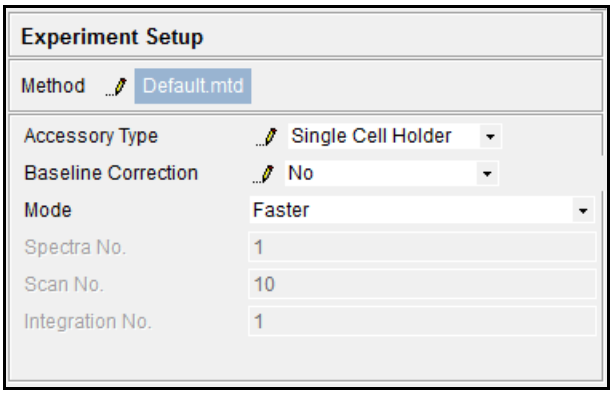
2. Enter a comment by selecting a spectrum, typing a comment and clicking Enter. You can check spectrum comment at the print.





I-3-11. Experiment Setup

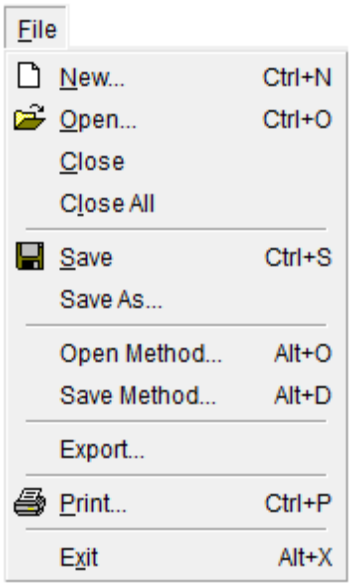
▣ Display and modify the experimental setup of Method, Accessory Type, Mode, Spectra No., Scan No. and Integration No.



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 IV-4-1. Mode 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.



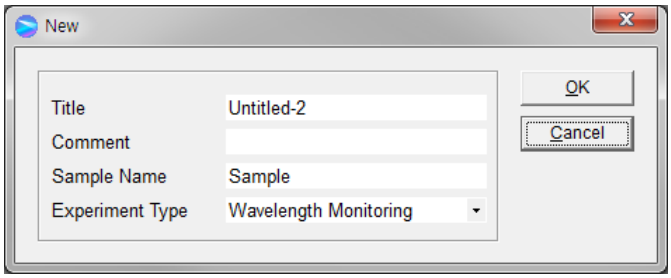
Command	Function
New	Open a new window
Open	Open saved data
Close	Close the current window
Close All	Close all windows
Save	Save data
Save As	Save data using a new file name
Open Method	Open a saved method
Save Method	Save a method
Export	Export data to another program
Print	Print results
Exit	Exit UV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

II-1. New

▣ Use the New command to open a new window.

▣ Procedure

1. Select New to open a new window.



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

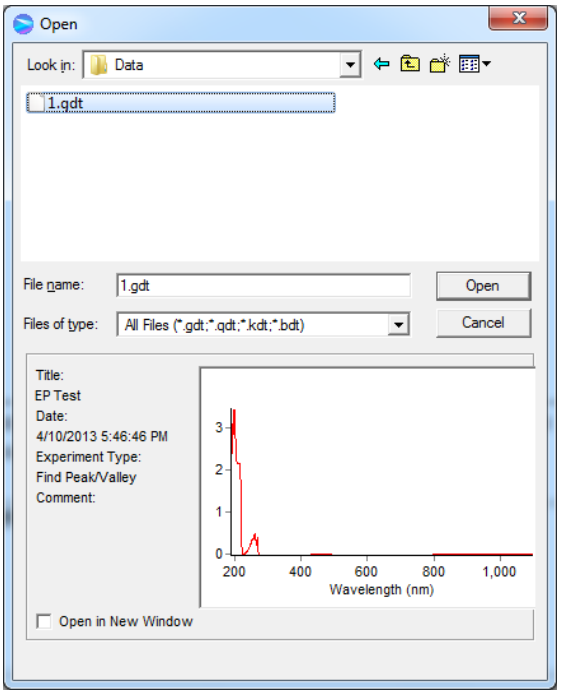
▣ Use the Open command to open data in a file.

▣ 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.



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

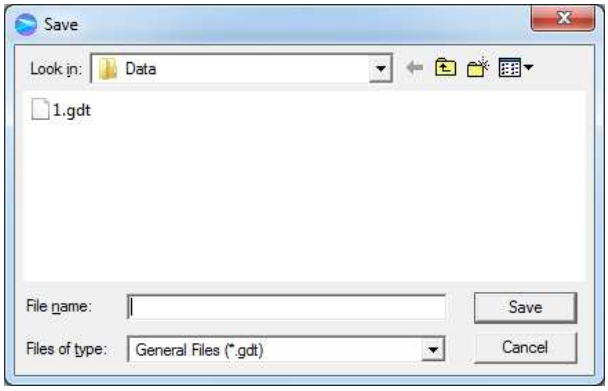
- Use the Close command to close a window.
- Verify the data was saved before closing the window.

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.



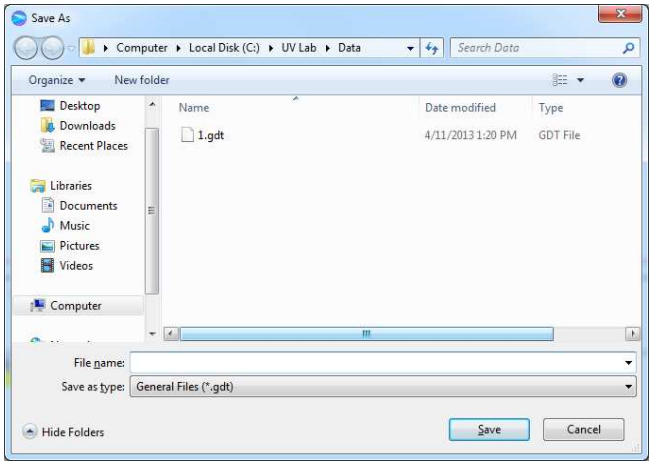
Please refer to II-2 Open for file types.

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.



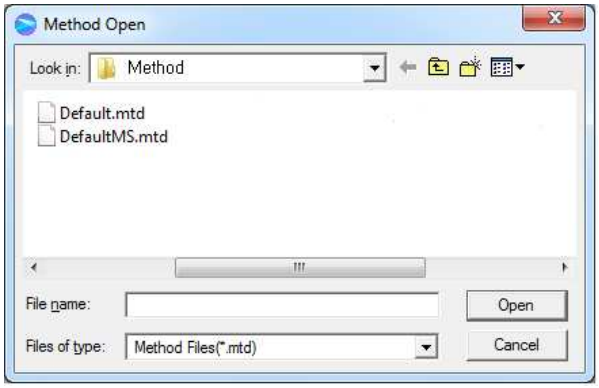
Refer to II-2. Open for file types.

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.

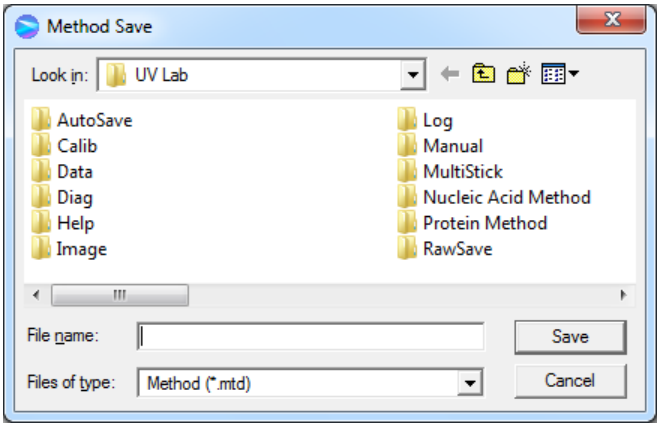


II-8. Save method

▣ 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.



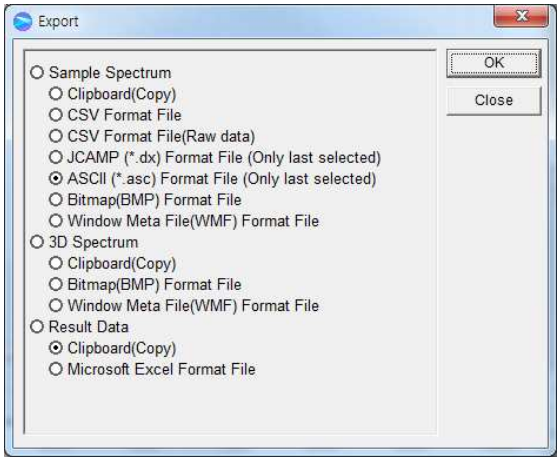
2. Method file extensions are automatically assigned as *.mtd.

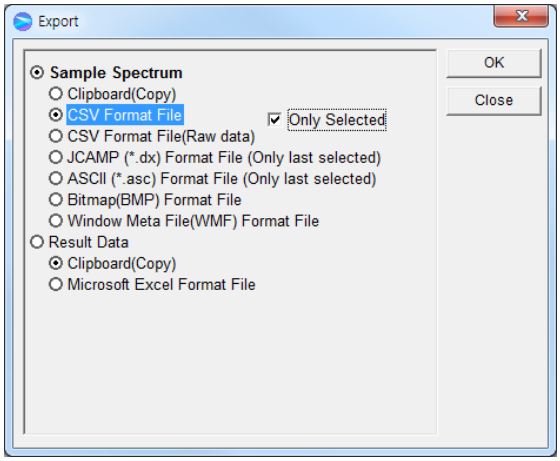
II-9. Export

▣ 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
Bitmap Format File	Export a 3D spectrum as *.bmp file
Window Meta File Format File	Export a 3D spectrum as *.wmf file
Export Result Data	
Clipboard(Copy)	Copy the result data
Microsoft Excel Format File	Export the result data as MS Excel format file

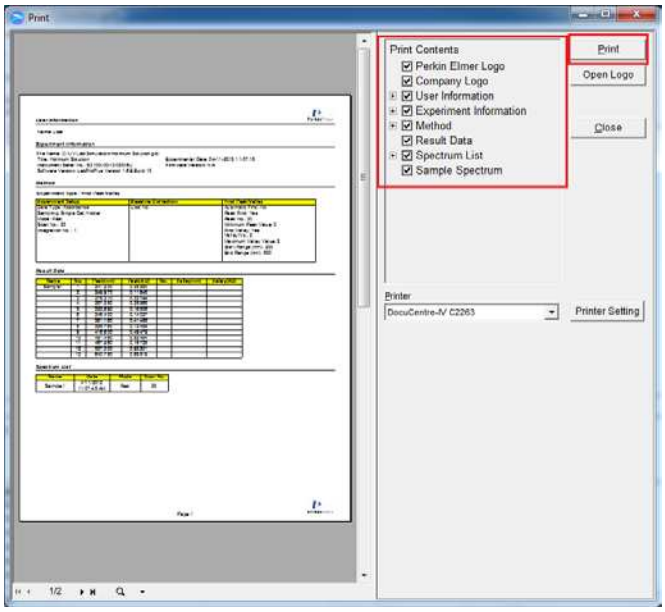
II-10. Print

▣ Use the Print command to print or preview data in the current window.

▣ Procedure

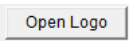
1. The following print-preview window will be displayed.

2. Select the required print contents and select Print.



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.*

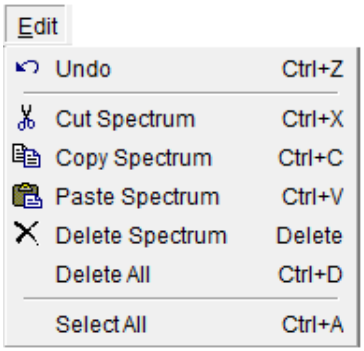
c. *Check the company logo is changed.*

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 table.



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.

2. 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

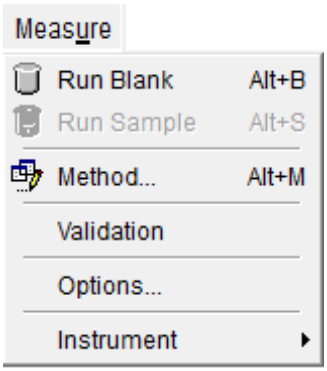
▣ Use the Select All command to select all spectra in the active spectral window.

▣ Procedure

1. To select all, click Select All.

IV. Measure Menu

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



Command	Function
Run Blank	Collect a blank spectrum
Run Sample	Collect a sample spectrum
Method	Set the mode and data collection parameters
Validation	Verify instrument performs
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.

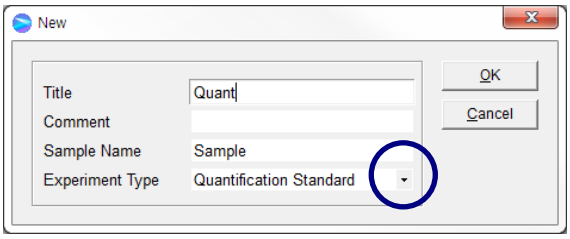
IV-3. Method

▣ Use the Method command to set modes and parameters to control data collection.

▣ Procedure

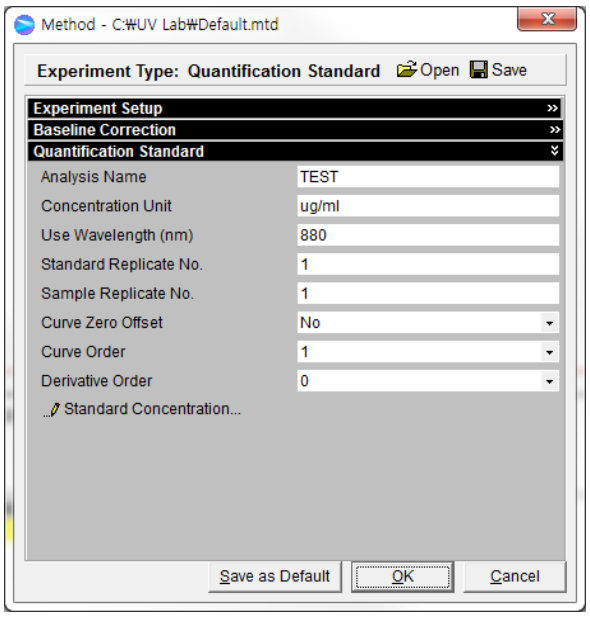
1. Select File menu.

2. Select New. Select Experiment Type.



3. Click OK. See V. Experiment Method for more details.

4. The following method window is displayed. Set each parameter and click OK.



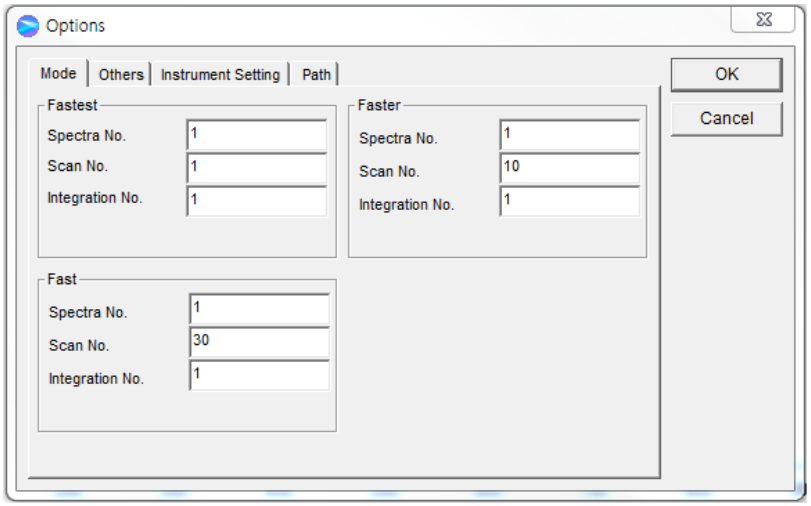
5. Open or save a method using the and icons. The method will be saved as a *.mtd file.

IV-4. Options

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

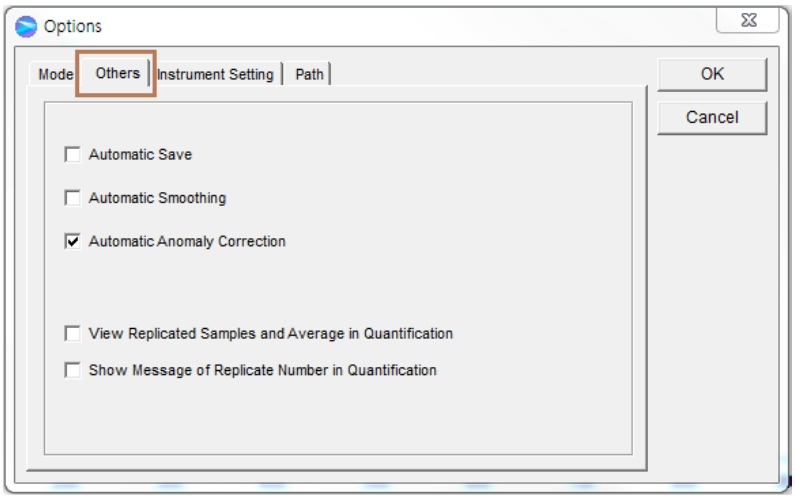
IV-4-1. Mode

1. 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.



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.

3. 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.

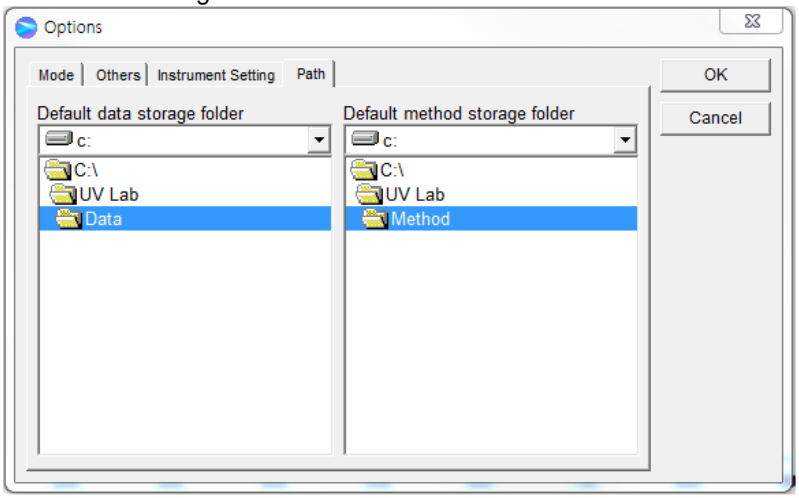
IV-4-3. Instrument Setting

▣ 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.

IV-4-3. Path

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



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

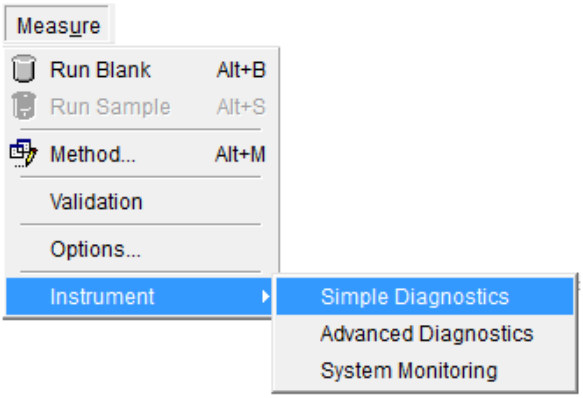
▣ Simple Diagnostics provides a limited set of diagnostics tests.

▣ Simple Diagnostics list

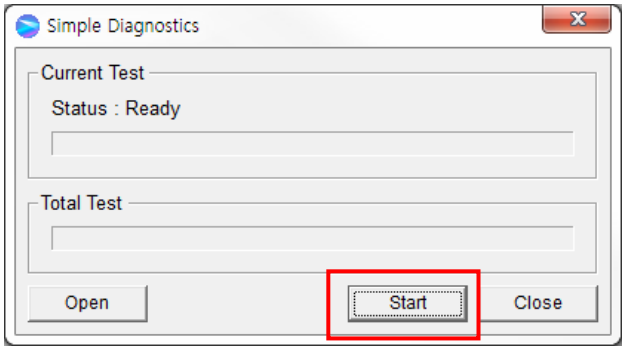
General	Shutter & Filter Speed measurement Check Dark Current Lamp Intensity evaluation Check Wavelength Check Noise level

▣ Procedure

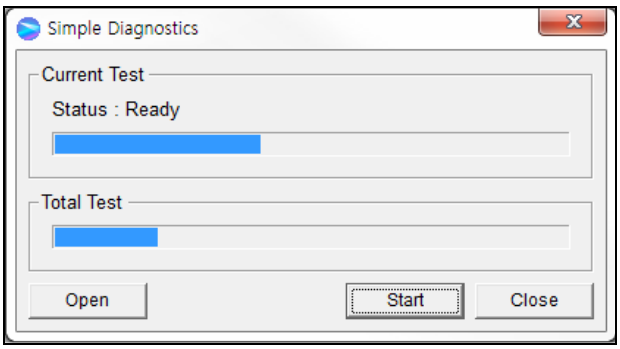
1. To perform a Simple Diagnostics test, select Simple Diagnostics in the Measure menu.



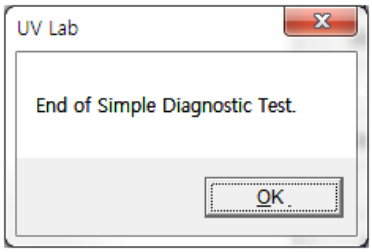
2. Select Start.



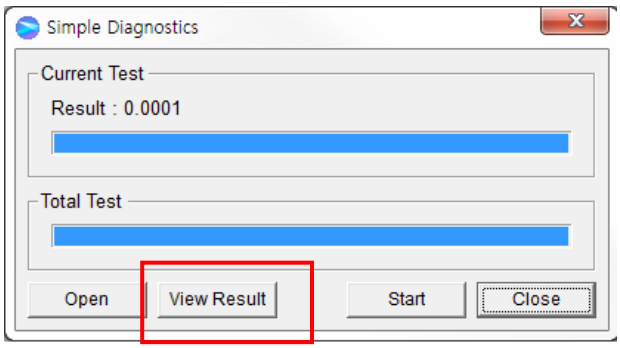
3. The following dialog box shows the status of the Simple Diagnostics.



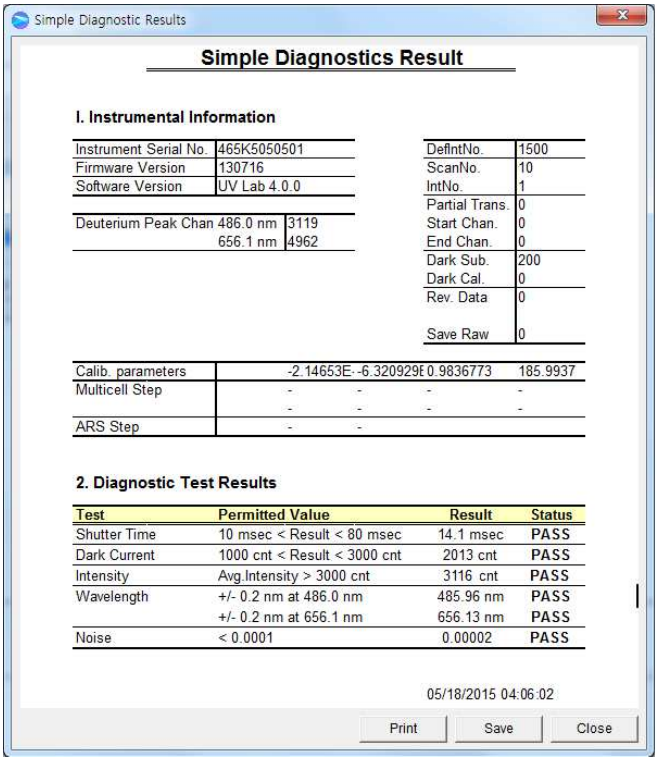
4. Simple Diagnostics is finished.



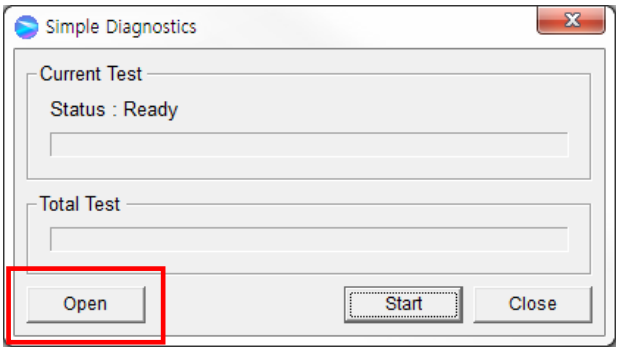
5. Select View Result to view test result.



6. 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.



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



8. Select the desired file and click OK.

IV-5-2. Advanced Diagnostics

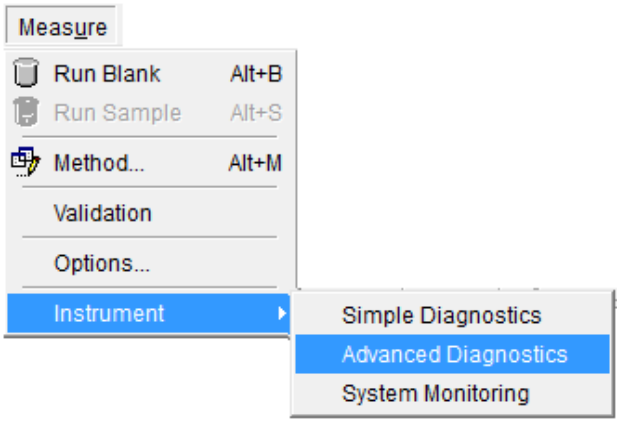
▣ Advanced Diagnostics provides a complete set of diagnostics tests.

▣ Advanced Diagnostics list

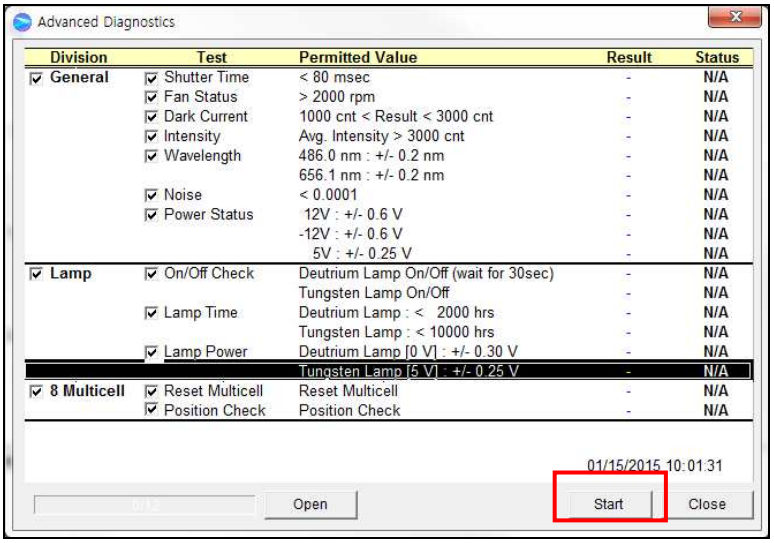
General	Shutter & Filter Speed measurement Check Cooling Fan Check Dark Current Lamp Intensity evaluation Check Wavelength Check Noise & Power
Lamp	Check Lamp On/Off and Power Indicating consumed life time of the Lamps and Time to Replace
Accessories	Check cell positions of the 8 Cell Water jacketed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

▣ Procedure

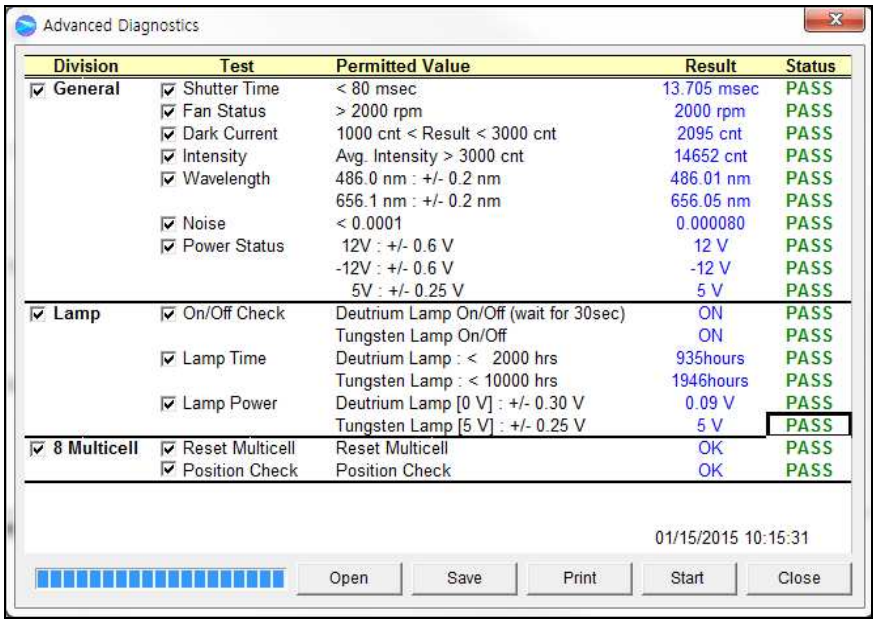
1. To perform an Advanced Diagnostics test, select Advanced Diagnostics in the Measure menu.



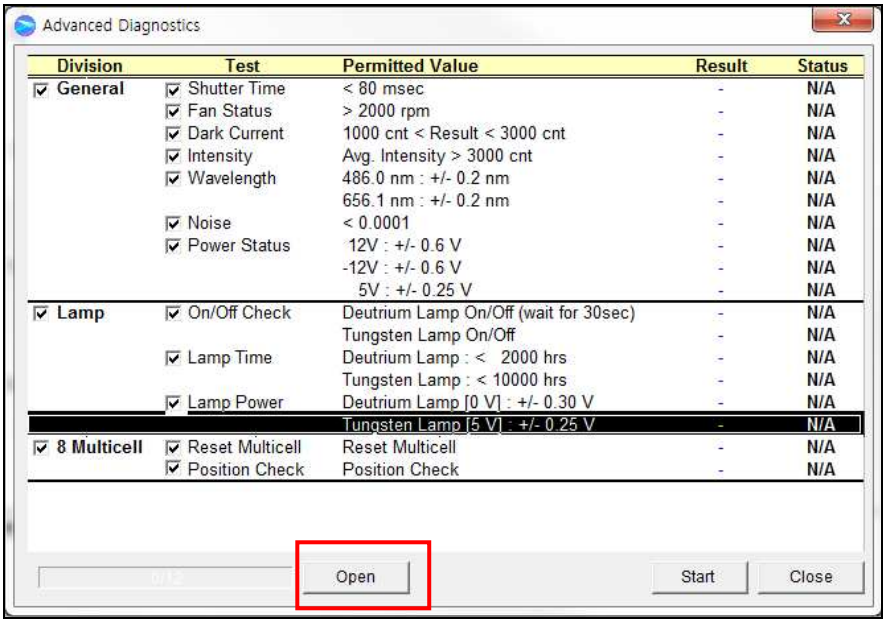
2. Select tests to run and click Start.



3. 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.



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



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:

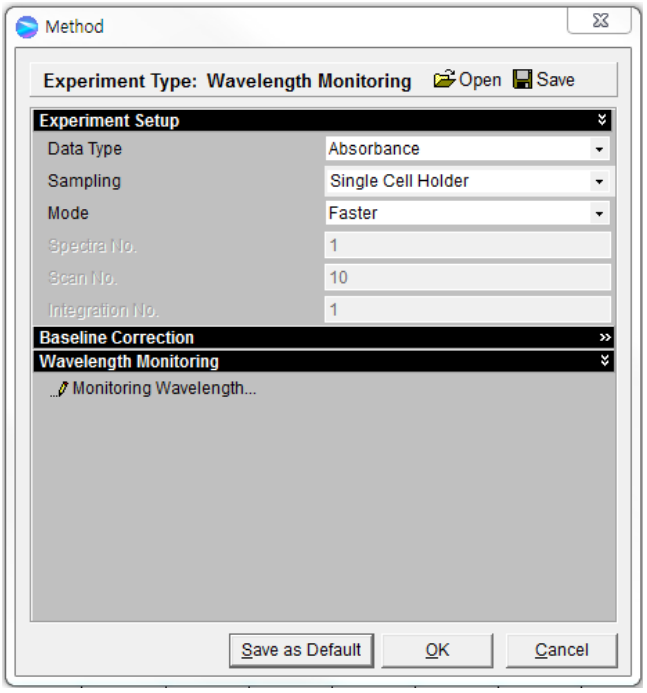
1. Select measurement mode.
2. Set method parameters.
3. Measure blank.
4. Measure samples.
5. Save or print results as required.

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.



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

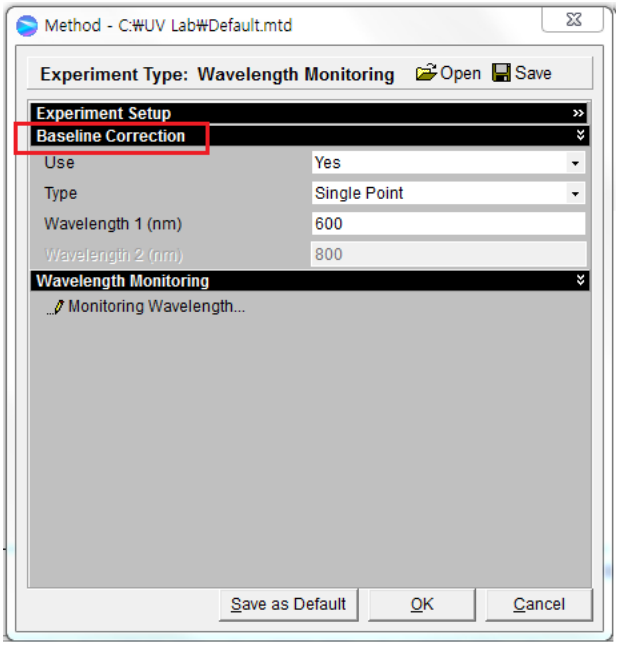
Integration time(ms) = 20.48 x Scan No x (integration NO + Default integration NO)

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

? ***What is Saturation?***

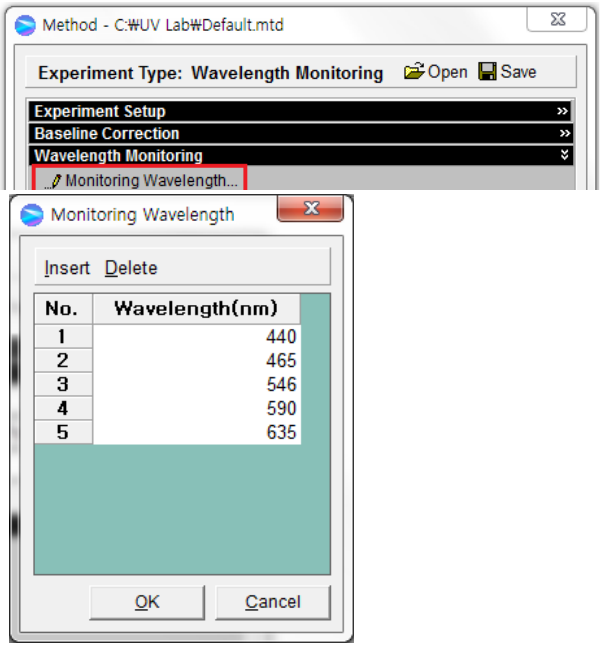
When the integration number is too big, portions of the spectrum can be cut off because the detector reaches its maximum detection limit of light. This makes the difference between blank and sample data meaningless. The maximum value of Y axis should not exceed “60000” count.

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



▶ Refer to the next page for more details.

5. Click Monitoring Wavelength and then the following dialog box will be displayed.



6. Enter the wavelengths to be monitored using Insert or Delete and click OK.

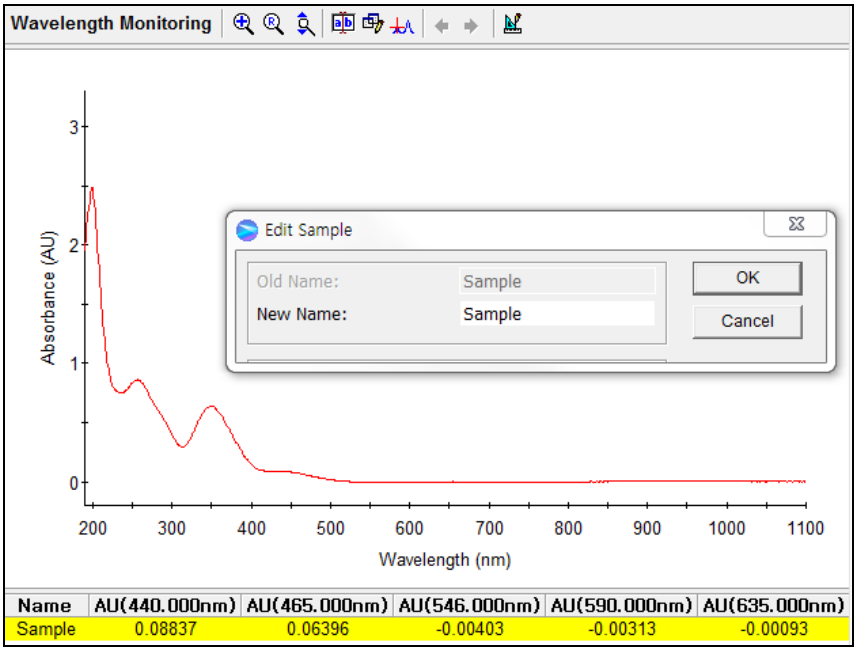
7. After completing parameter setup for Experiment Setup, Baseline Correction and Wavelength Monitoring, click OK.

8. Measure the blank.

9. Measure the samples.

10. Save or print spectrum and results as desired.

▶ To Edit a sample name, double click the sample line to be changed in the list.

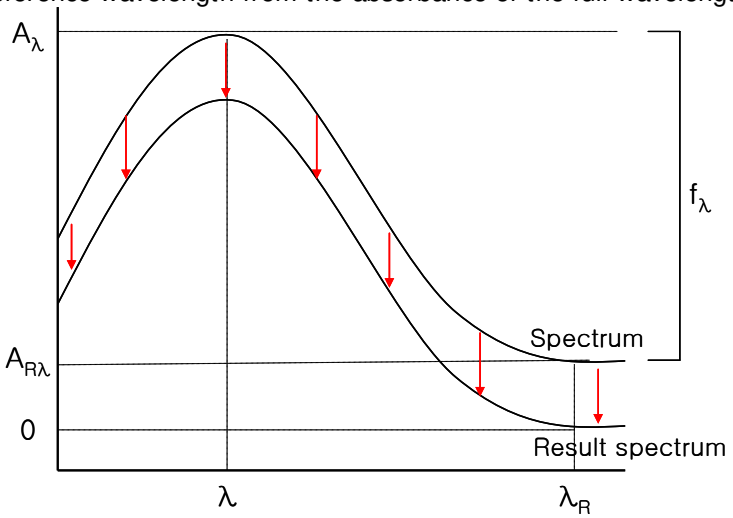


Baseline Correction

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.



$$f_\lambda = A_\lambda - A_{R\lambda}$$

f_λ is the function result at wavelength λ

A_λ is the absorbance at wavelength λ

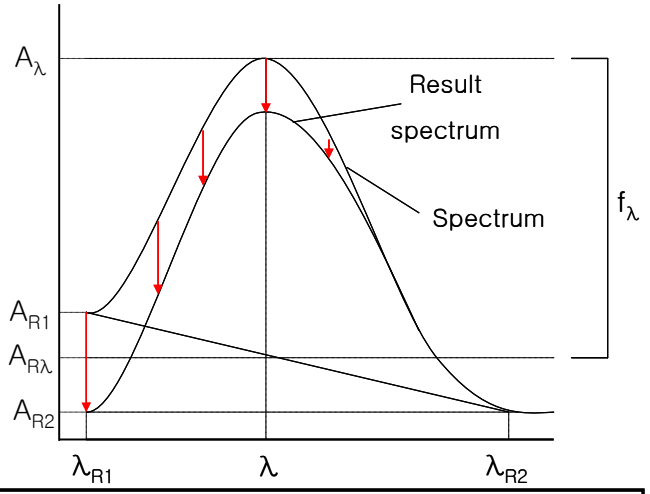
$A_{R\lambda}$ is the absorbance at reference wavelength λ_R

▶ 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(λ). Resulting spectrum are calculated using the following equation.



$$A_{R\lambda} = \frac{1}{\lambda_{R2} - \lambda_{R1}} \{(\lambda_{R2} - \lambda)A_{R1} + (\lambda - \lambda_{R1})A_{R2}\}$$

$$f_\lambda = A_\lambda - A_{R\lambda}$$

f_λ is the function result at wavelength λ
A_λ is the absorbance at wavelength λ
$A_{R\lambda}$ is the reference absorbance at wavelength R_λ

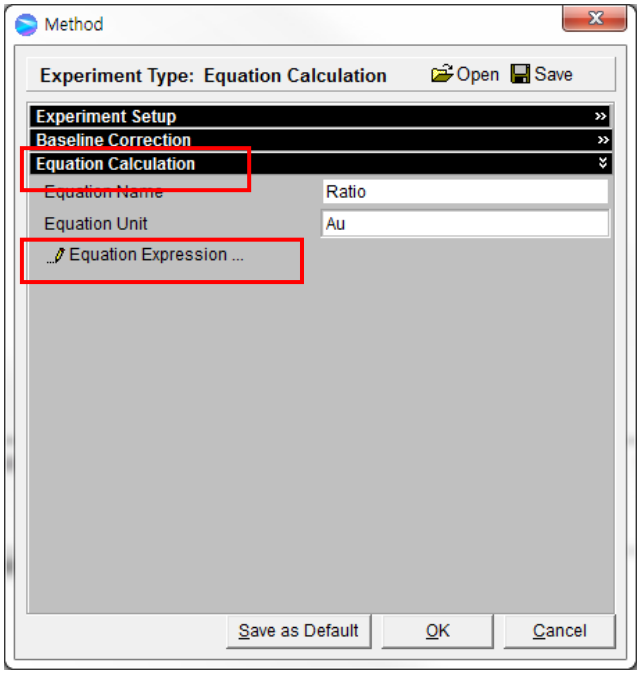
V-1-2. Equation Calculation Mode

▣ Use Equation Calculation to collect data for a calculated result using a specified equation.

▣ Procedure

1. Select New.

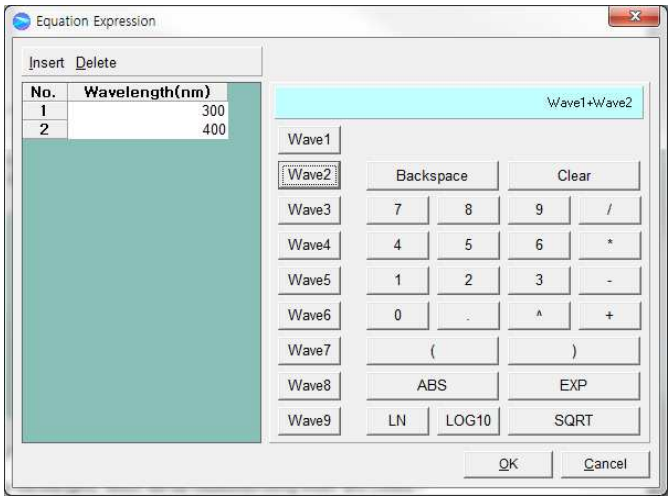
2. Select Equation Calculation in Experiment Type. The method dialog box is displayed.



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.



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

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

Key	Function
Wavelength	Use the absorbance result at the selected wavelength. Equation can contain up to nine.
+	Add
-	Subtract
/	Divide
*	Multiply
ABS	Calculate the absolute value
EXP	Calculate e(exp)
LN	Calculate the Natural logarithm
LOG10	Calculate the common logarithm
SQRT	Calculate the square root

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.

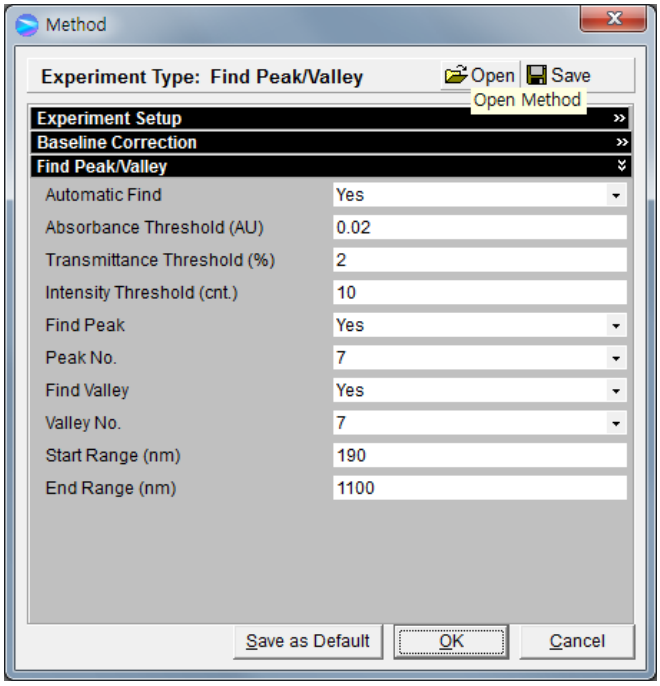
V-1-3. Find Peak/ Valley Mode

▣ 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.



3. 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.

8. 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.

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

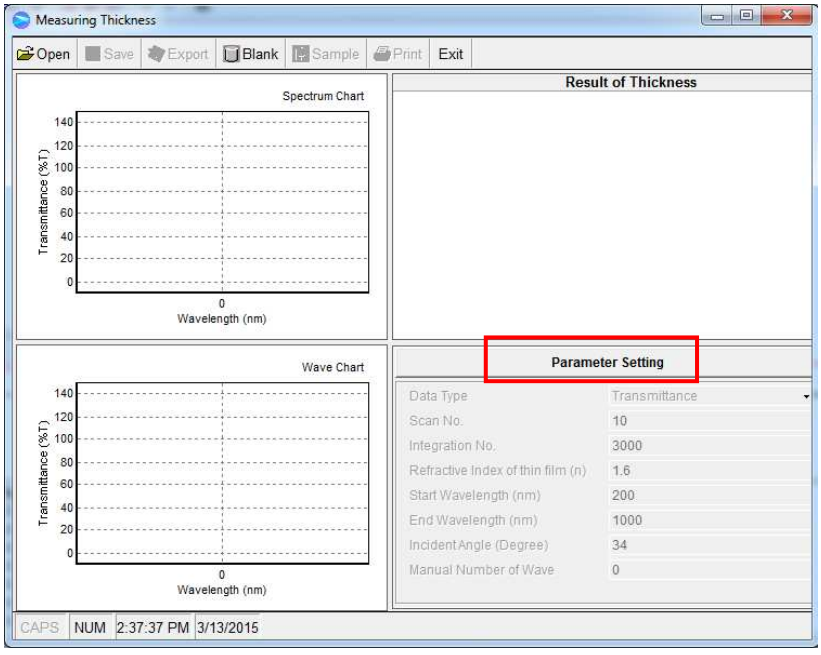
▣ Use Thickness Mode to measure the thickness of a film.

▣ 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.



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 to Light 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)

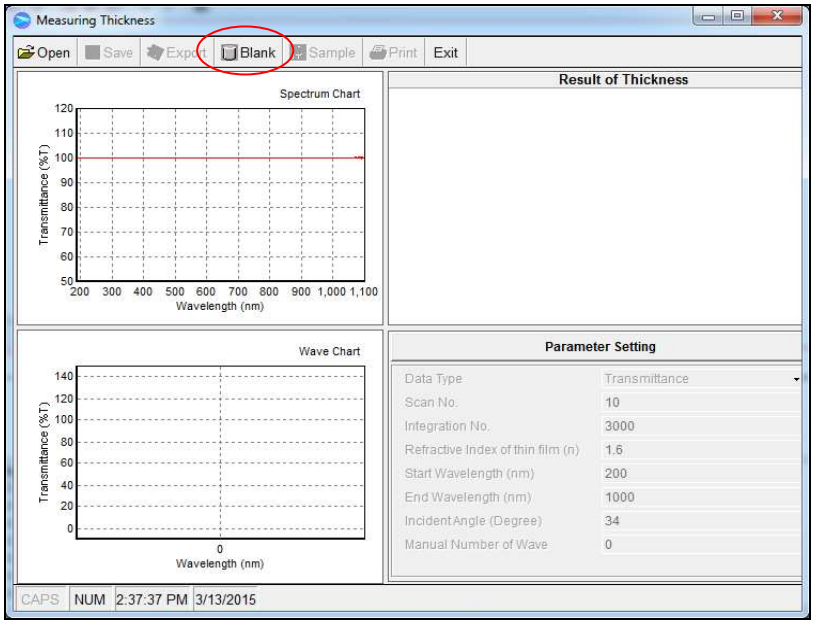
h. Manual Number of Wave (M.N.W): The required fringe (wave) numbers are used to calculate the thickness between start wavelength and end wavelength. M.N.W. can be modified after the measurement is complete.

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.

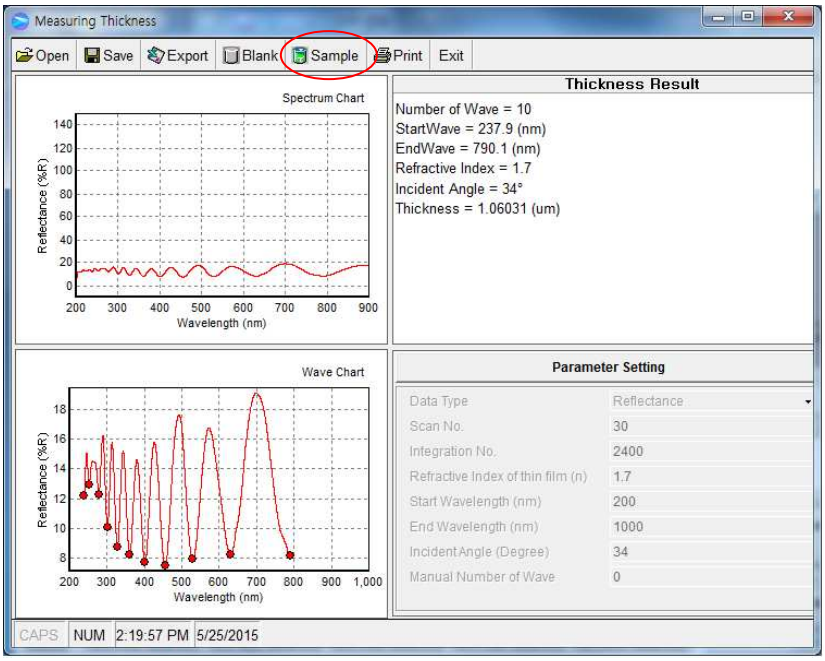
ⅱ. If the software can not locate the appropriate fringe (wave) shapes and locations, set a value more than 2 in the Manual Number of Wave box. See point 9, below.

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

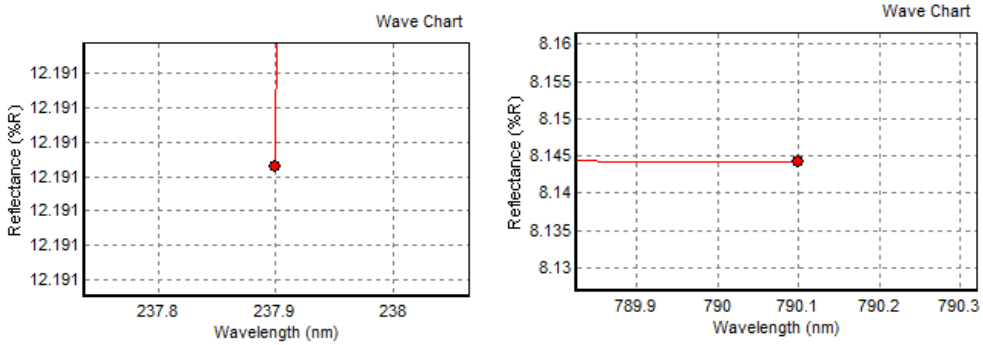
w = number of fringes (waves) between λ_1 and λ_2

n = reflective index of thin film

θ = Angle of incidence

λ_1 & λ_2 = 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.

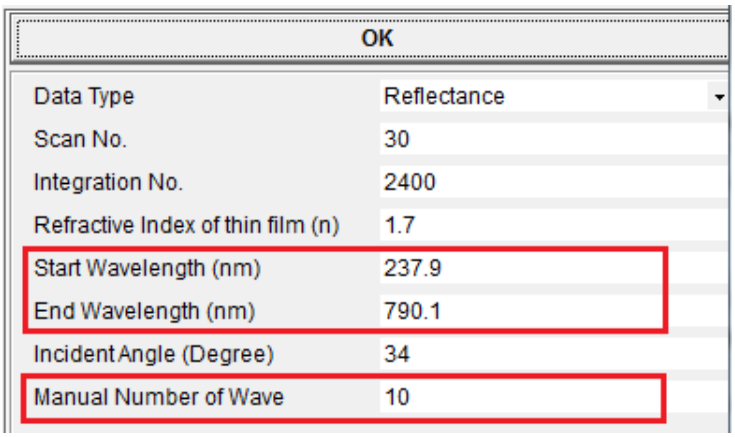


9. In some cases, the software may not find the peak because the wave of the spectrum is too small. In this case, thickness can be calculated manually.

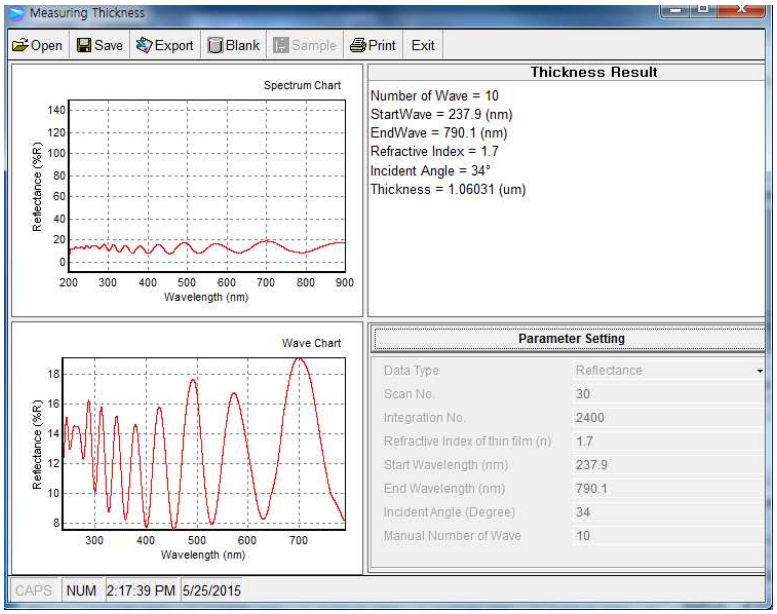
   Click Experimental Setup and zoom in on the area of the wave in the spectrum.

   a. Count the number of waves and define the start and end wavelength.

   b. 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.



   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)

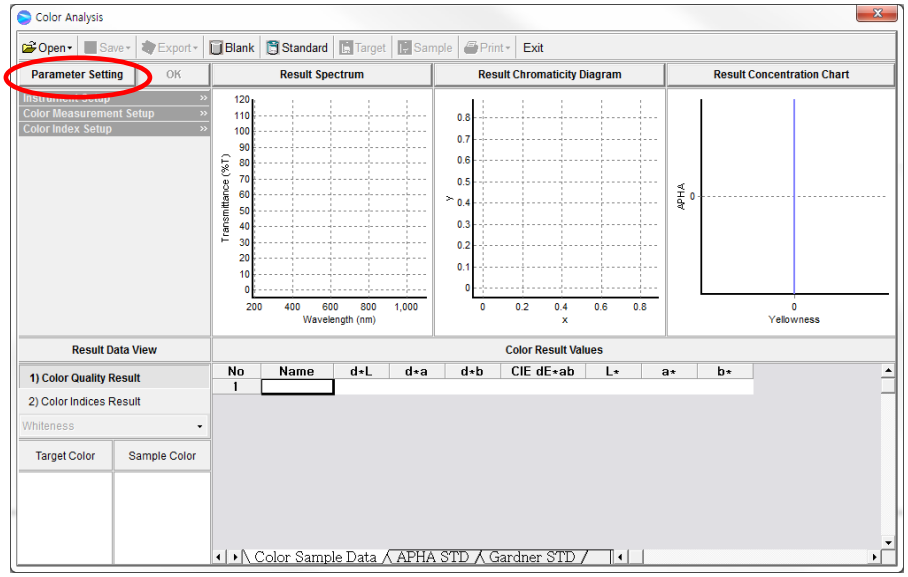
▣ Use Color Analysis to measure Color Scale values including CIE color (L*, a*, b*) and Hunter (Lab). The test can be used for:

- 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.



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(l:c): Value "l" and "c" are "2" and "1" respectively. For example, CMC(2:1) is generally used in textile and dye industry. The l, 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.

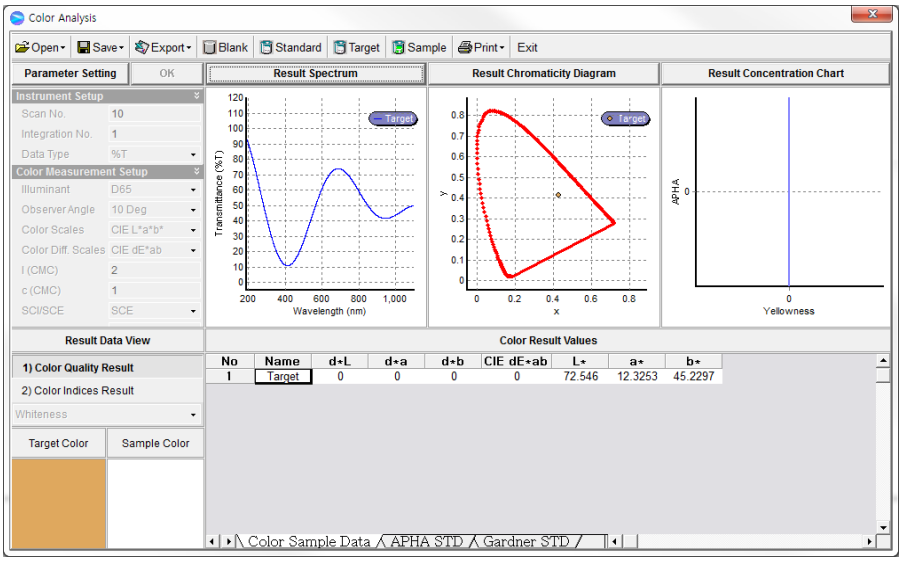
6. When parameter setup is complete, select OK.

7. Measure the Blank

. This is typically a clear, colorless solution for transmission work, or the include Spectralon® disk if using the integrating sphere.

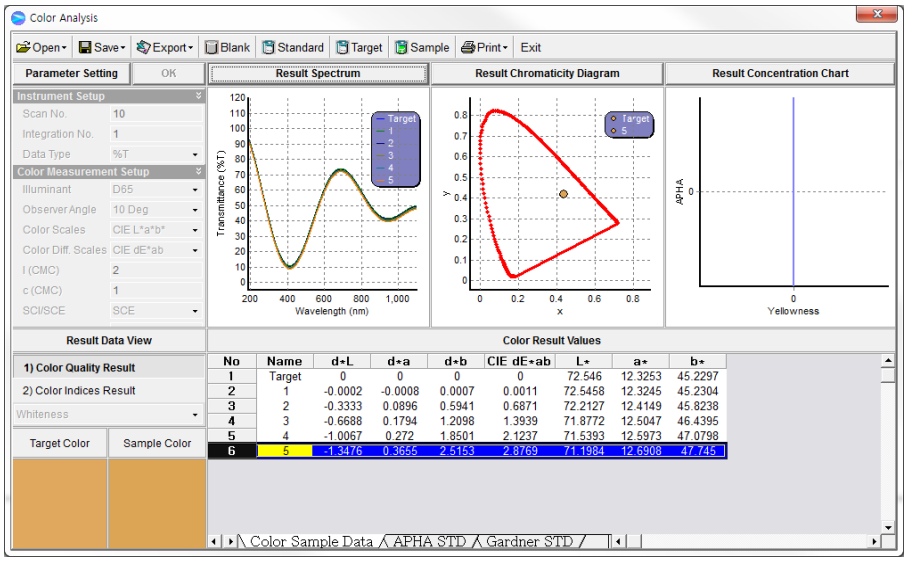
8. Measure the Target

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

and check the color difference values.



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

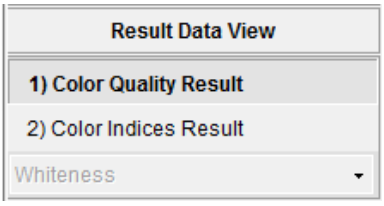
(concentration) of APHA / Gardner and their absorbance. Select

to display this chart in the full window.

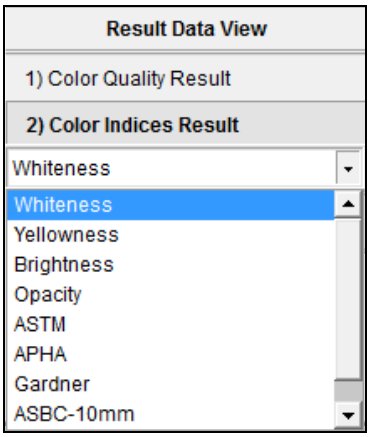
e. Color Result Values: Display results of Target, Standard, and Sample in accordance with the preset parameters.

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.



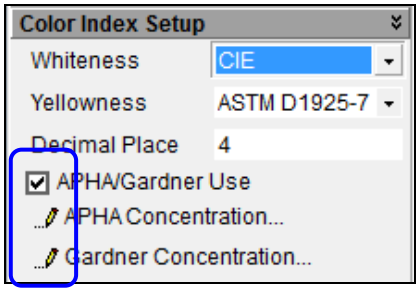
ⅱ. Color Indices Result: Used to see the result for Whiteness, Yellowness, Brightness, Opacity, ASTM, APHA, Gardner, ASBC-10, EBC-10.



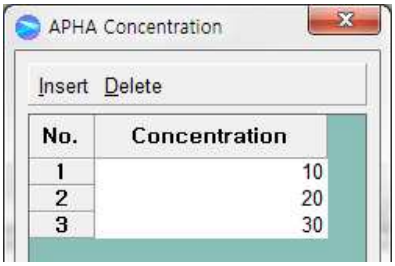
▣ APHA, Gardner Measurement Procedure

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

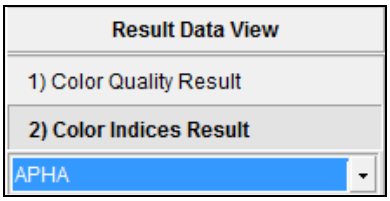
1. Check APHA/ Gardner Use in the [Parameter Setting]→[Color Index Setup]. Click the edit button to setup the measurement.



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



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



4. Measure the Blank

, and Standard samples

.

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.

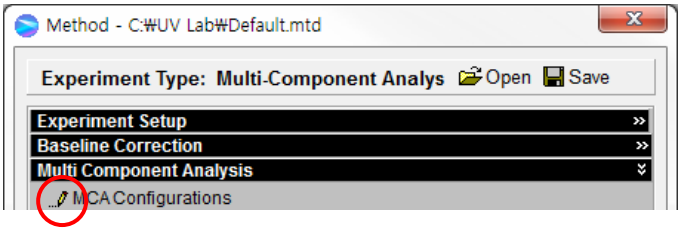
V-1-6. Multi-Component Analysis (MCA) Mode (Optional)

▣ 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:

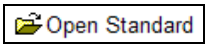
▣ Procedure

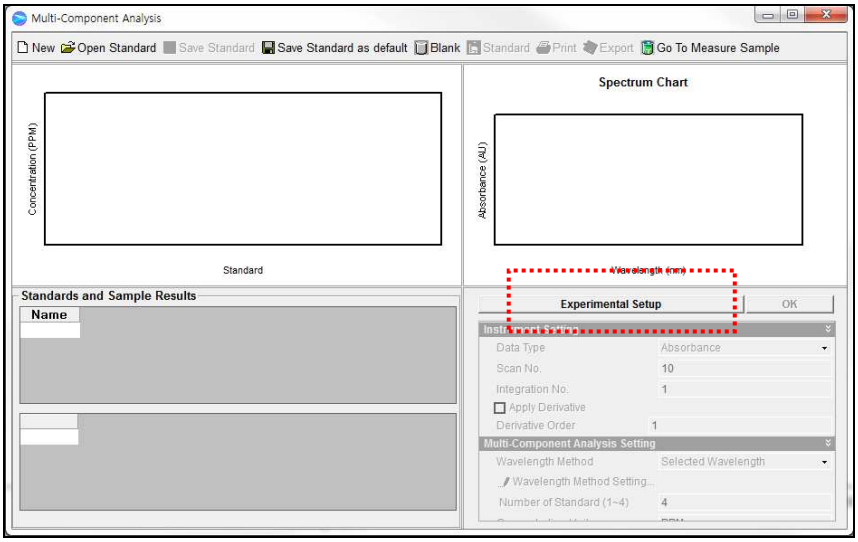
1. Click New.

2. Select Multi-Component Analysis in the Experiment Type. The method dialog box will be displayed. Click MCA Configurations.

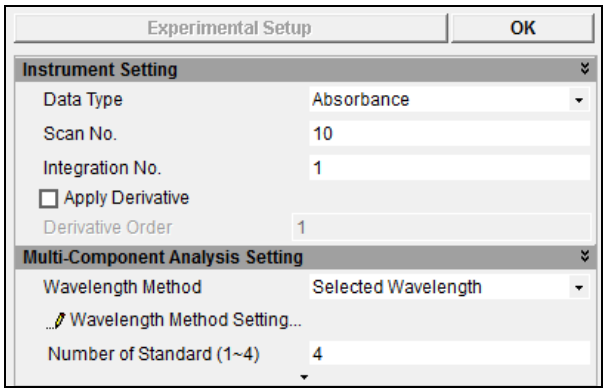


3. The standard measurement window will be displayed. Click New icon.

4. 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.



5. Setup parameters as follows.



▶ 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.

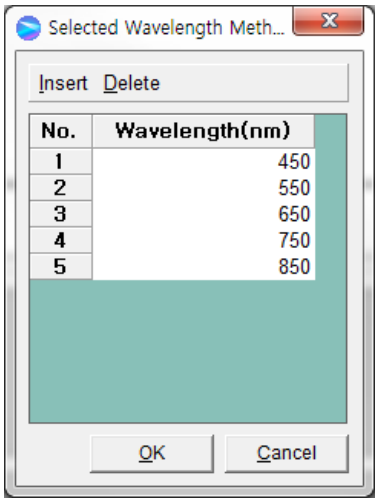
c. Integration No.: This parameter determines the length of time a sample is exposed to Light source. A high integration number increases the signal-to-noise ratio.

d. Apply Derivate: Check (√) Apply Derivative to obtain the data after applying derivative and enter the derivative order number (1~4).

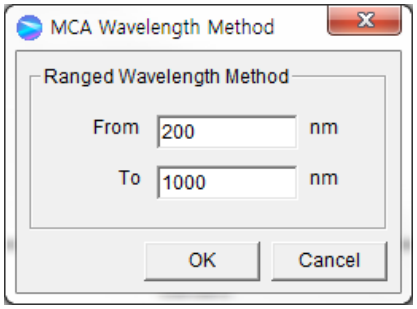
▶ Multi-Component Analysis Setting

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.



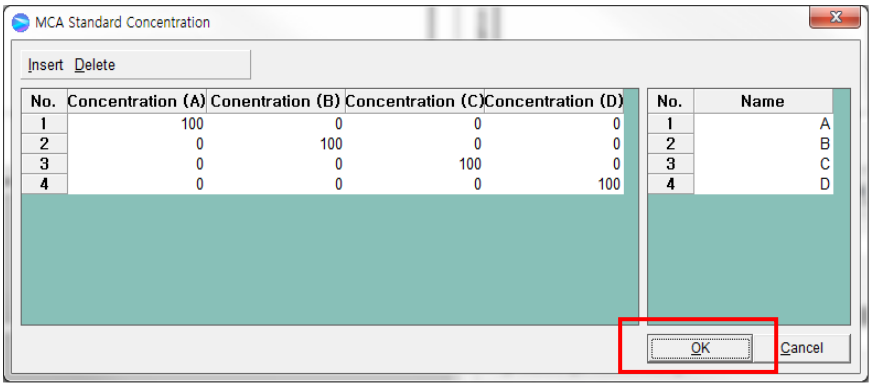
ⅱ. Range Wavelength: Enter the wavelength range to use for the test and click OK.



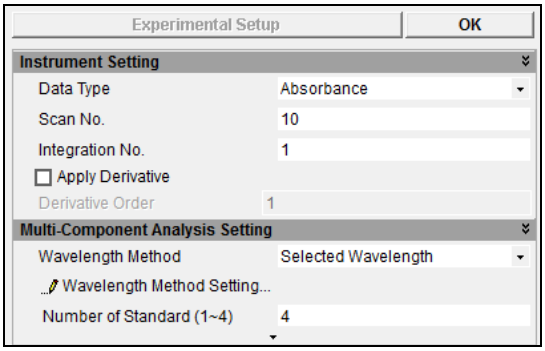
b. Number of Standard (1~4): Enter the number of standards to be measured.

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.



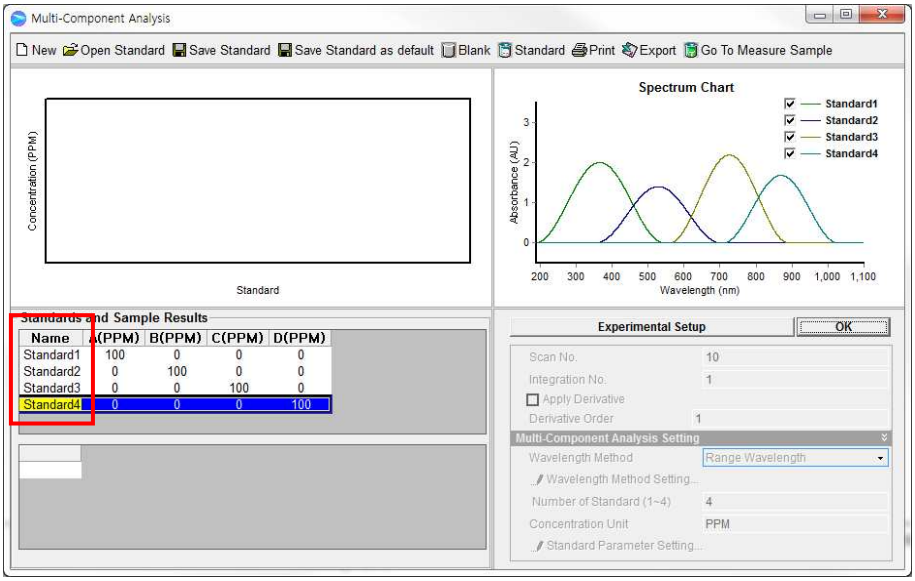
6. After setting all experimental parameters, click OK.



7. Measure the blank Click Blank icon.

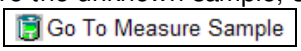
8. Measure the standards. Click Standard

icon. Enter the name of the Standard and click the Enter key.

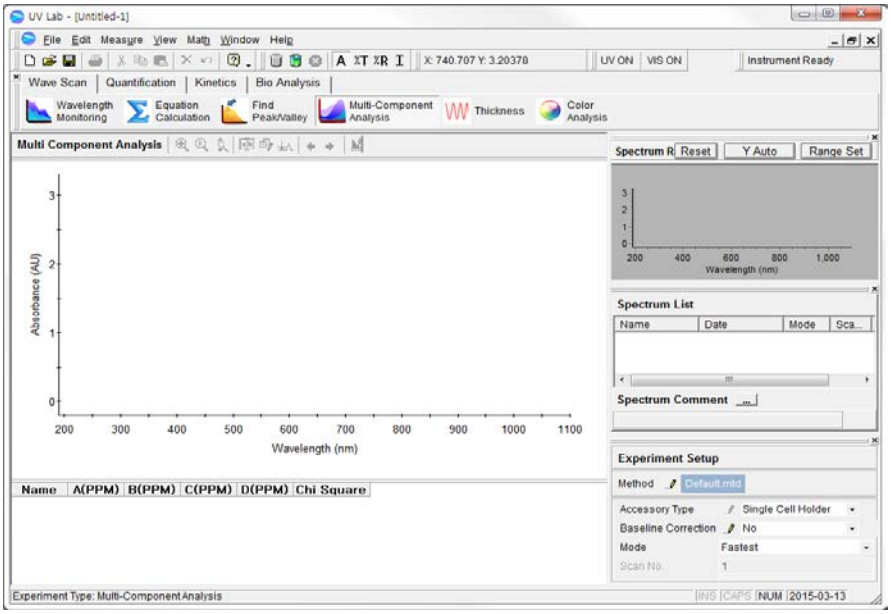


9. Save the measured standard data.

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.

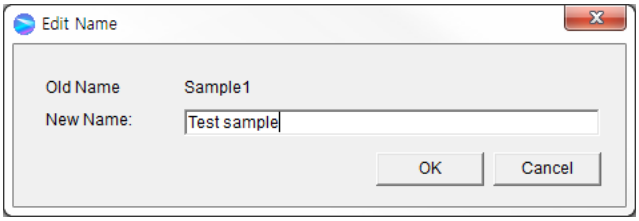


12. Measure the blank.

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



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

Chi-square, χ^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:

$$\chi^2=\frac{(o_1-e_1)^2}{e_1}+\frac{(o_2-e_2)^2}{e_2}+\ldots+\frac{(o_k-e_k)^2}{e_k}=\sum_{i=1}^{k}\frac{(o_i-e_i)^2}{e_i} \quad \ldots\ldots\ldots(1)$$

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

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

The former equation can be also expressed as:

$$\chi^2=\sum\frac{o_i^2}{e_i}-N \quad \ldots\ldots(3)$$

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 χ^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.

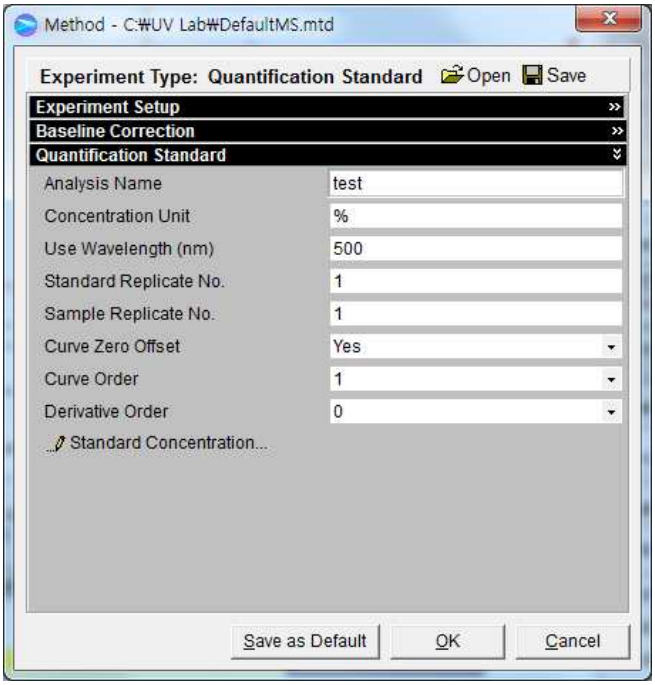
V-2-1. Quantification Standard / Sample Mode

▣ 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.

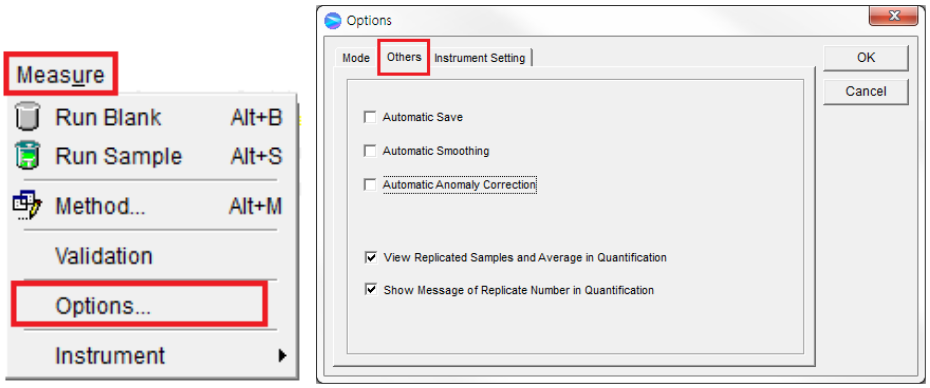


3. Setup Experiment Setup and Baseline Correction parameters. See IV-1-1. Wavelength Monitoring for more details.

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 → Options in the Main menu.

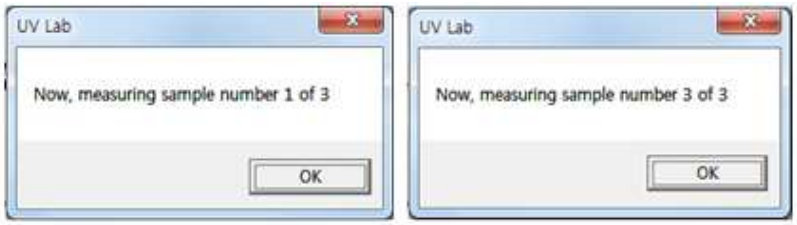


i) In case of checking (∨) View Replicated Samples and Average in Quantification in the Others tab, each repeated measurement result and the averaged value are displayed.

R^2 : 0.99908 Function: Y = 1.12E-02X + -3.57E-02
Remain Standard Measurement No. : 0

Name	Concentration(mg/L)	AU(440.00nm)	Dilution Factor	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 (∨) 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.

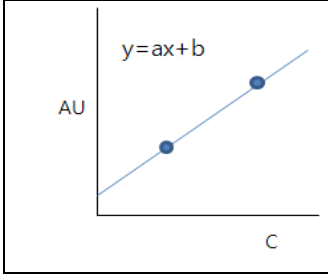
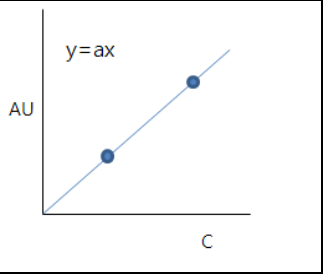


iii) In case of unchecking View Replicated Samples and Average in Quantification and Show Message of Replicate Number in Quantification in the Others tab, the only averaged value of each repeated measurement is displayed.

R^2 : 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/L)
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.

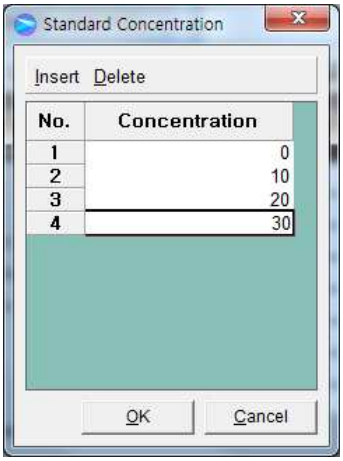
Yes	No
	

g. Curve Order: Select a 1st, 2nd or 3rd order fit for the calibration curve.

h. Derivative Order: Choose the Derivative Order among 1st, 2nd and 3rd. The Y-axis of spectrum and calibration curve is changed as selected.

5. Select Standard Concentration.

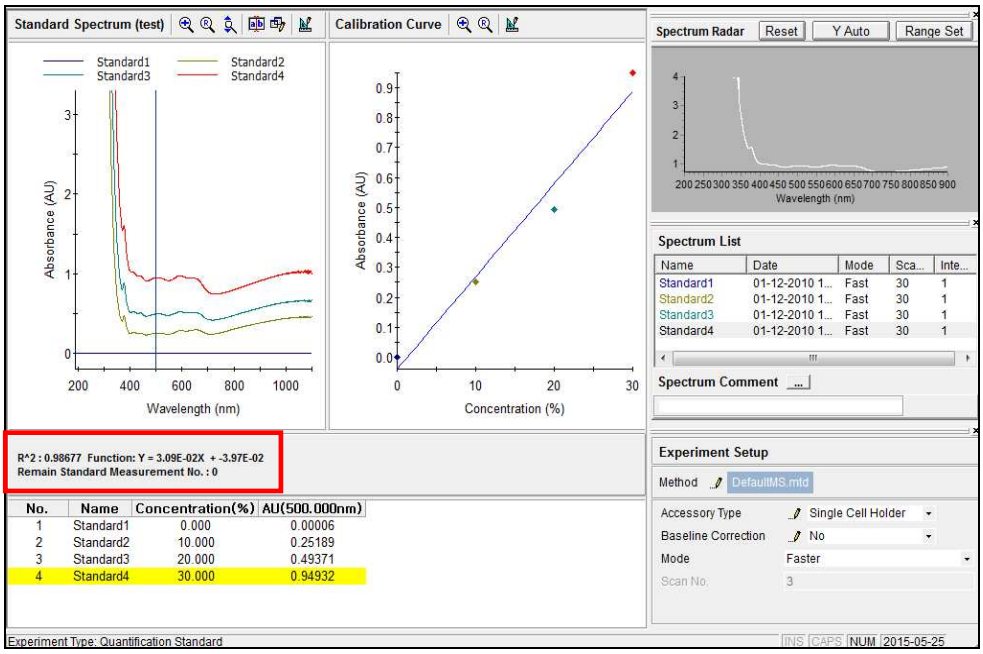
6. Enter the concentration for each standard in the test box, as shown below and select OK. Insert and Delete can be used to change the number of standards for the test.

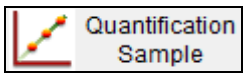


7. After setting parameters for Experiment Setup, Baseline Correction and Quantification Standards is complete, click OK in the method setup window.

8. Measure the blank.

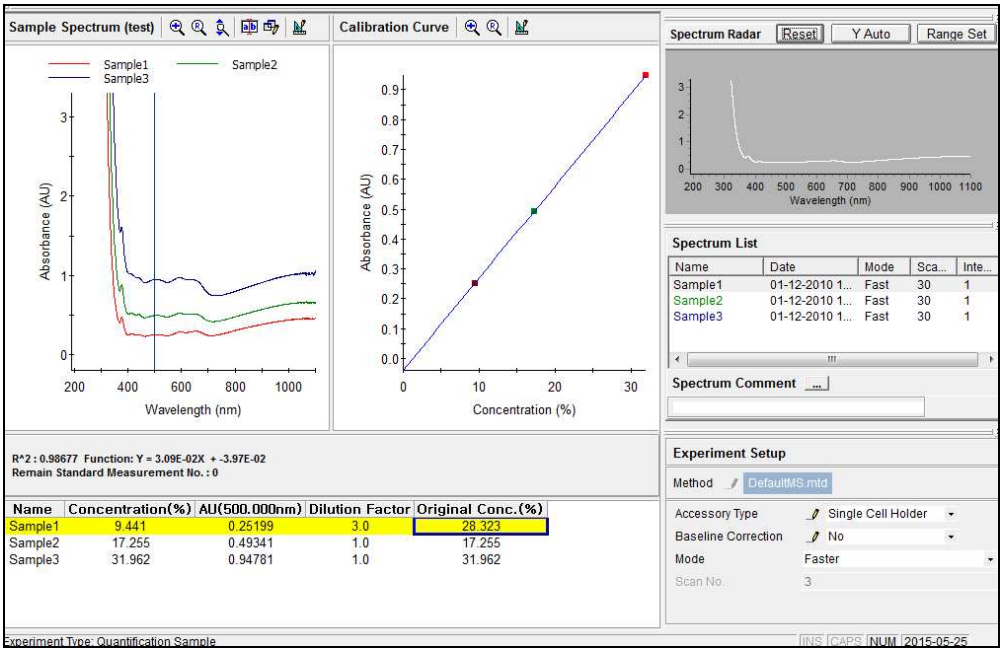
9. Measure the standards according to their concentrations. The spectra and resulting calibration curve are displayed as follows. The equation and correlation coefficient for the curve are displayed below the Standard Spectrum window.



10. Select Quantification Sample. 

11. Measure the sample (unknown).

12. The concentration of each sample is calculated automatically.



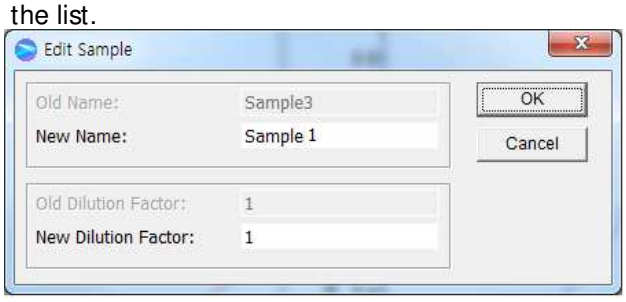
13. If samples were diluted prior to measurement, the original concentration may be calculated using a dilution factor as shown below:

R^2 : 0.98677 Function: Y = 3.09E-02X + -3.97E-02
Remain Standard Measurement No. : 0

Name	Concentration(%)	AU(500.000nm)	Dilution Factor	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.



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.

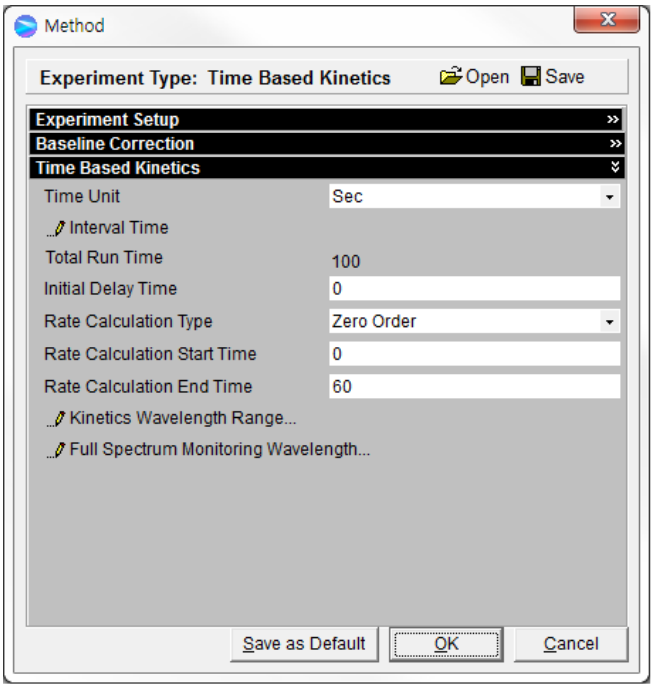
V-3-1. Time Based Kinetics Mode

▣ Use Time Based Kinetics to test the reaction rate against time

▣ Procedure

1. Click New.

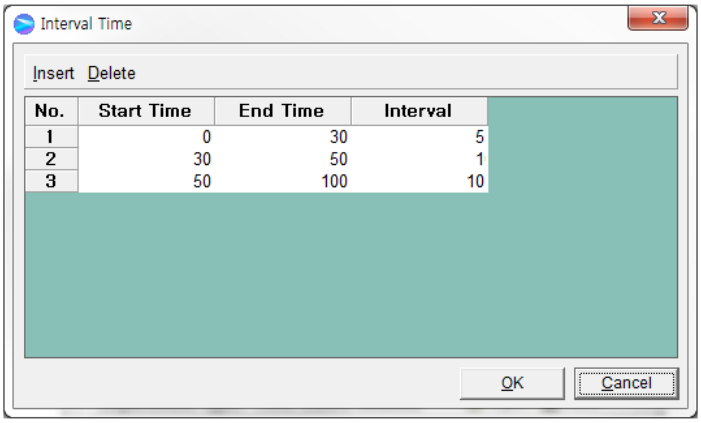
2. Select Time Based Kinetics in the Experiment Type. The method dialog box is displayed.



3. 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 ~ 60 sec
      iii. Time Unit - msec: Recommended Interval between 650 ~ 2000 msec

   Kinetics mode provides multi-step interval time. Both single-step interval and multi-step interval can be set by adding or delete step of interval.

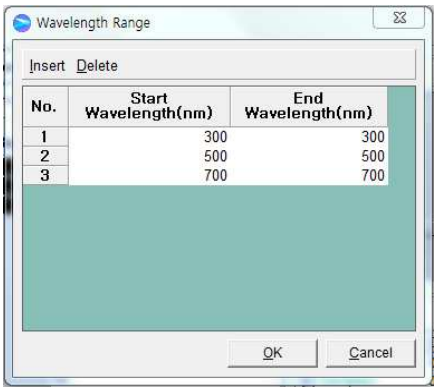


   c. Total Run Time: Show the total run time for testing samples.
   d. Initial Delay Time: Set the holding time before the first sample measurement. If "0" is entered for the initial delay time, the measurement is taken immediately.
   e. Rate Calculation Type: Select the order for the rate calculation. 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.

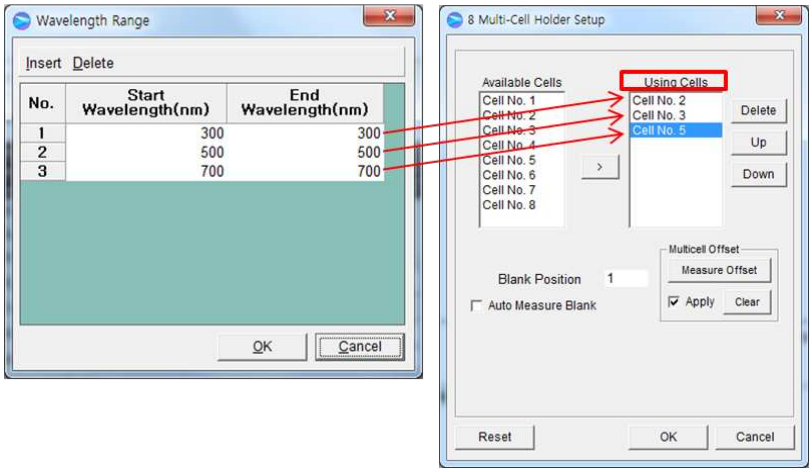
   The Start and End Time must be within the total run time set for the test.

5. Click Kinetics Wavelength Range.

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

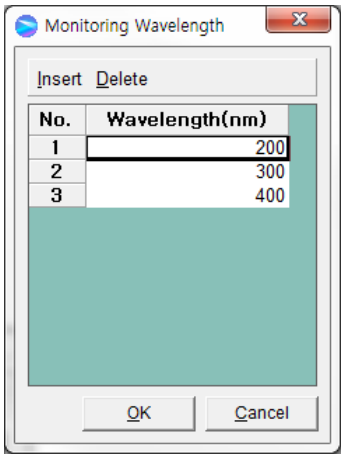


The number of wavelength Range should be matched the number of Using Cell. If the Wavelength Range is set as the picture below, multi cell number should be selected three cells and in numerical order on using cells in the 8 Multi-Cell Holder Setup window. For example, Cell No.2 for wavelength 300 nm,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



7. Select Full Spectrum Monitoring Wavelength.

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

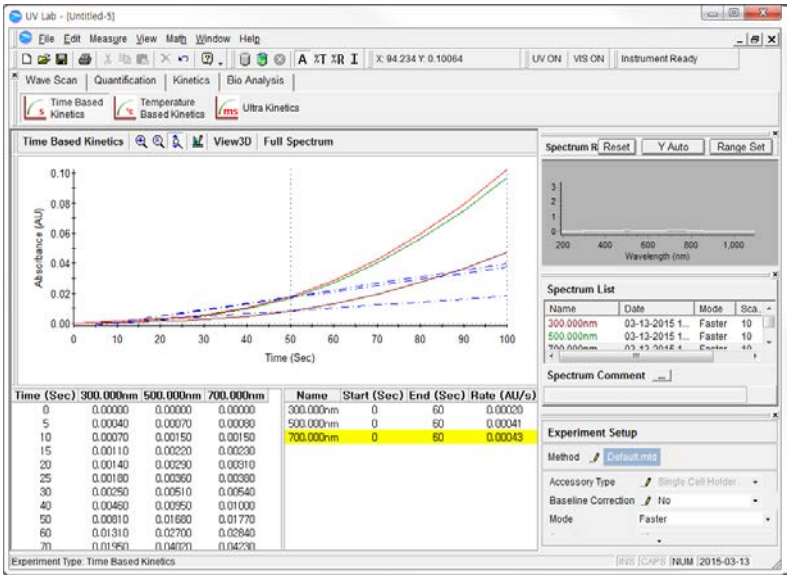


9. After setting parameters for Experiment Setup, Baseline Correction and Time Based Kinetics is complete, select OK.

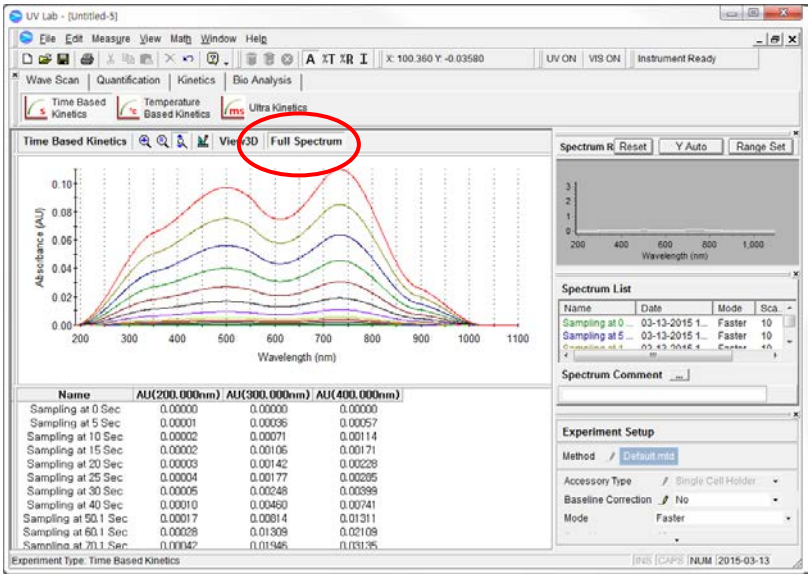
10. Measure the blank.

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:

  $A_t = A_0 - kt$

  A_t is the absorbance at time t.

  A_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:

  $A_t = A_0 + kt + t^2$

  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:

$$A_t = A_\infty + (A_o - A_\infty)\, e^{-kt}$$

This rate law can be rearranged to

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

A_t is the absorbance at time t.

A_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:

  $$\text{Delta AU} = A_t - A_0$$

  A_t is the absorbance at time t.

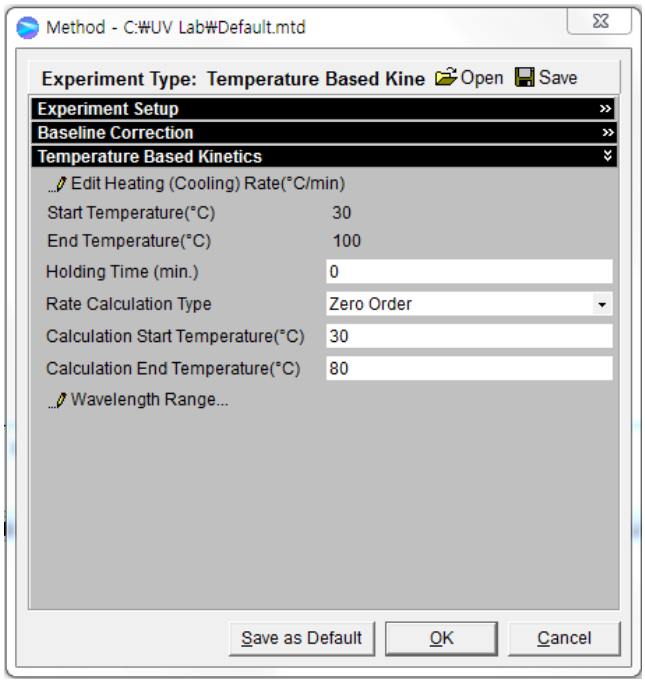
  A_0 is the initial absorbance.

  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.

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



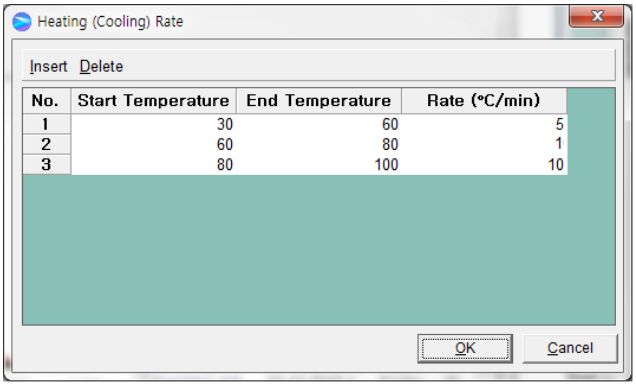
3. Setup Experiment Setup and Baseline Correction parameters. See V-1-1. Wavelength Monitoring for more details. In Sampling Type of Experiment Setup, select Single Cell Peltier or Muti-Cell Peltier.

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 (℃/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.

c. End Temperature: Enter the end temperature for the measurement.

d. Holding Time: Select the holding time at each temperature.

e. Rate calculation Type: Select the rate calculation type. See V-3-1. Time Based Kinetics for more information.

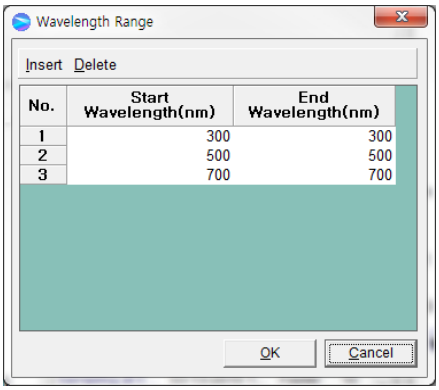
f. Calculation Start Temperature: Enter the start temperature for the calculation.

g. Calculation End Temperature: Enter the end temperature for the calculation.

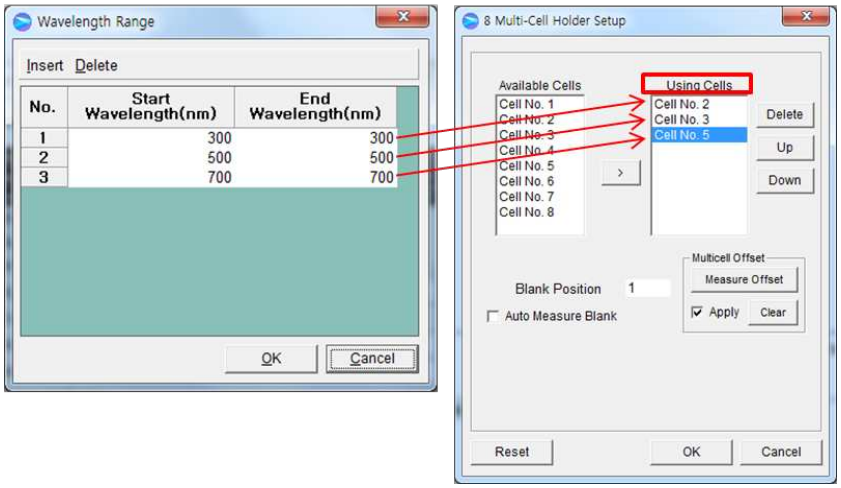
The Calculation Start and Calculation End Temperature must be within the Start and End temperatures for the measurement.

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.

8. Measure samples.

9. Print and save spectra and data as required.

V-3-3. Ultra Kinetics Mode

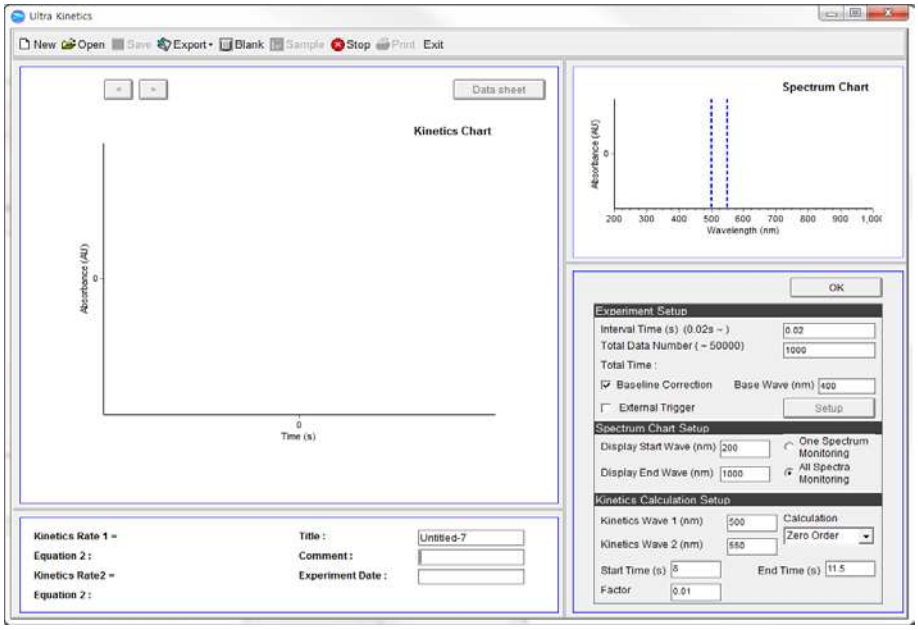
▣ Use Ultra kinetics to measure a very fast kinetic reaction.

▣ Measurement can be performed with an interval time as low as 20 ms in this mode. Perform this type of experiment as follows:

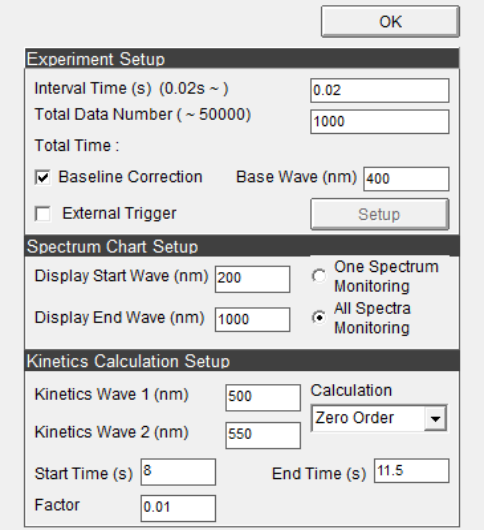
▣ Procedure

1. Click New.

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.



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 (√) 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.

l. End Time (s): Enter the time to stop calculating the rate.

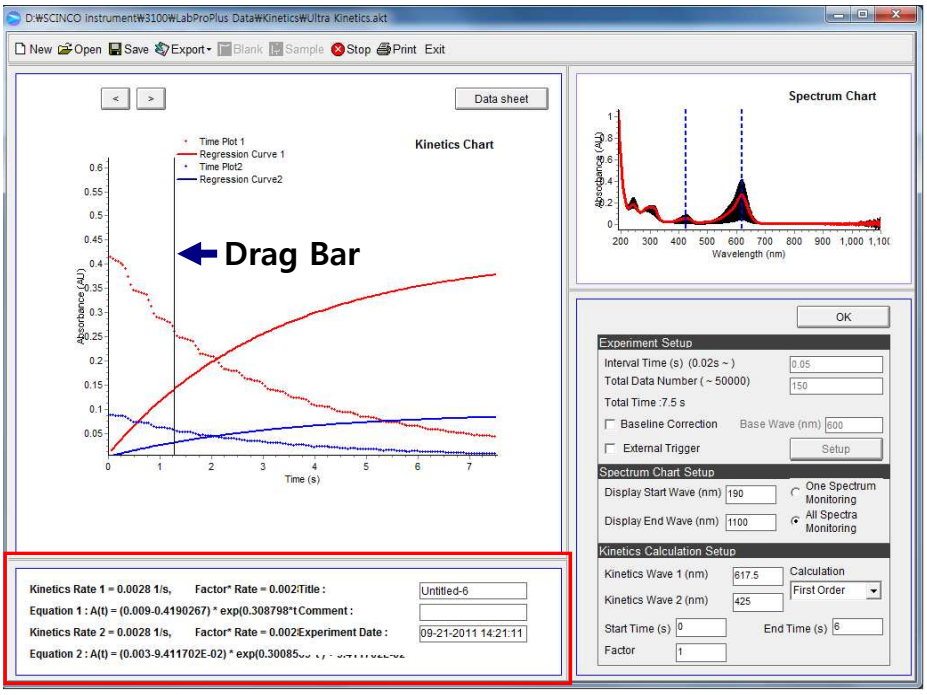
☑ Both the start time and the end time shouldn't be a bigger than the total run time.

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.

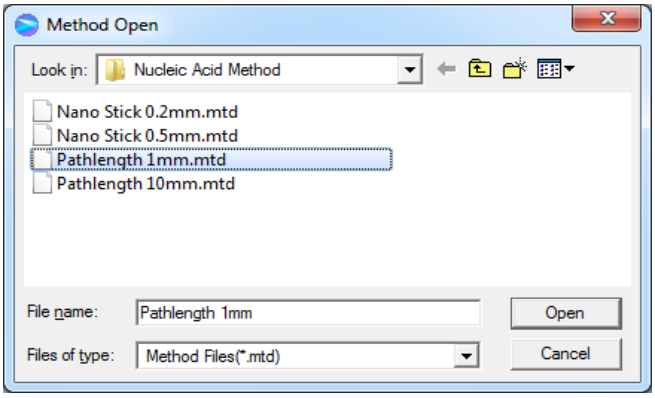
V-4-1. Nucleic Acid Analysis Mode

▣ Use Nucleic Acid Analysis Methods to perform ratio and concentration measurements on samples containing proteins and nucleic acids.

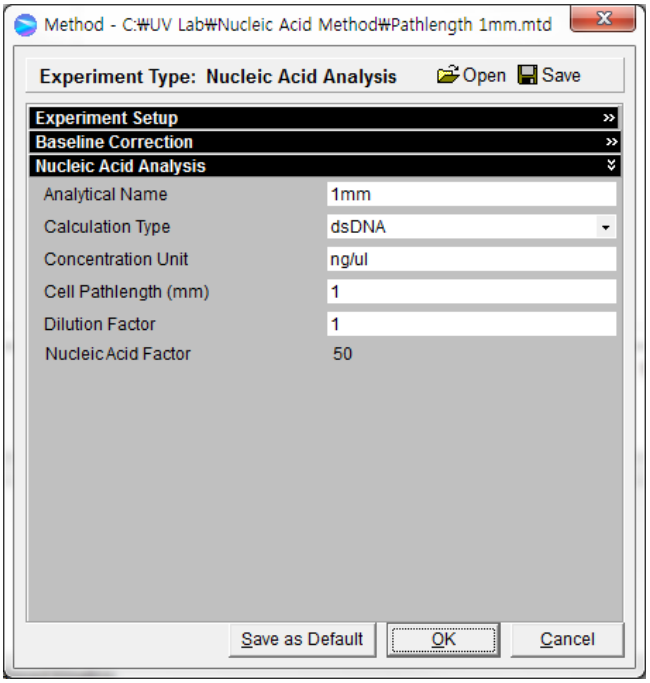
▣ Procedure

1. Select New.

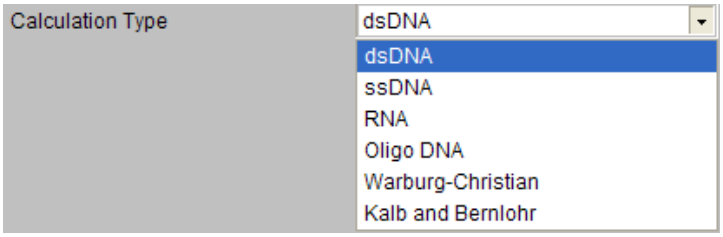
2. Select Nucleic Acid Analysis in the Experiment Type. The method dialog box is displayed. Select the cell pathlength in the Nucleic Acid method and click Open.



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



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

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 mℓ) using the following equations with absorptivities calculated by Warburg and Christian.

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

[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 mℓ) using the following equations.
[Protein] = (183.0 * A_{230}) - (75.8 * A_{260}),

[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.

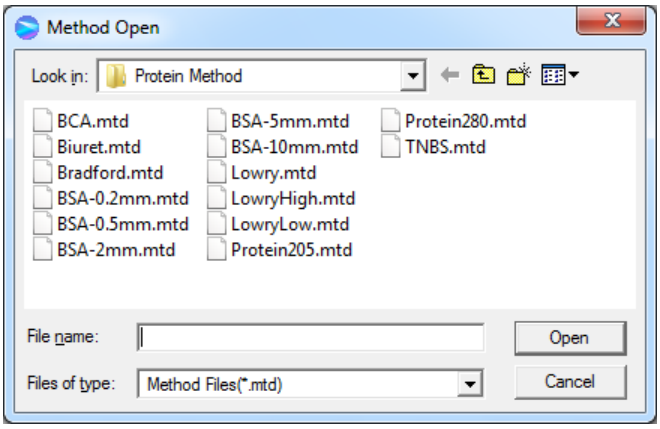
5. After setting parameters for Experiment Setup, Baseline Correction and Nucleic Acid Analysis is complete, click OK.
6. Measure the blank.
7. Measure samples.
8. Save and print spectra and data as required.

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.



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.

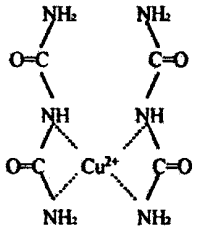
Protein Analysis Method

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_2-CO-NH-CO-NH_2) 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, 1~10 mg of protein can be quantified by the Biuret method. Using a microassay one can measure as 0.25 mg~2.0 mg of protein. A chelate compound is usually stable for 1~2 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 10~200 μg 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 1~20 μg 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

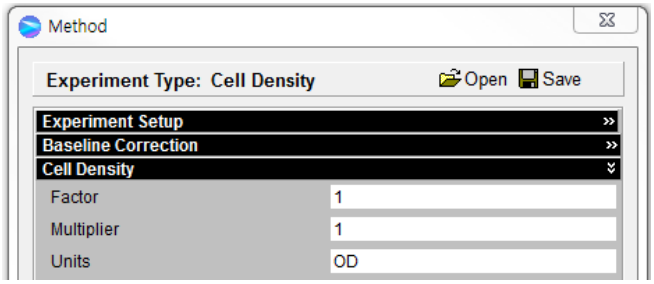
▣ Use Cell Density to determinate the absorbance at 600 nm.

▣ Procedure

1. Click New.

2. Select Cell Density and click OK.

3. Method dialog box is displayed.



4. 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 10^8 cells/ml

   ☑ 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.

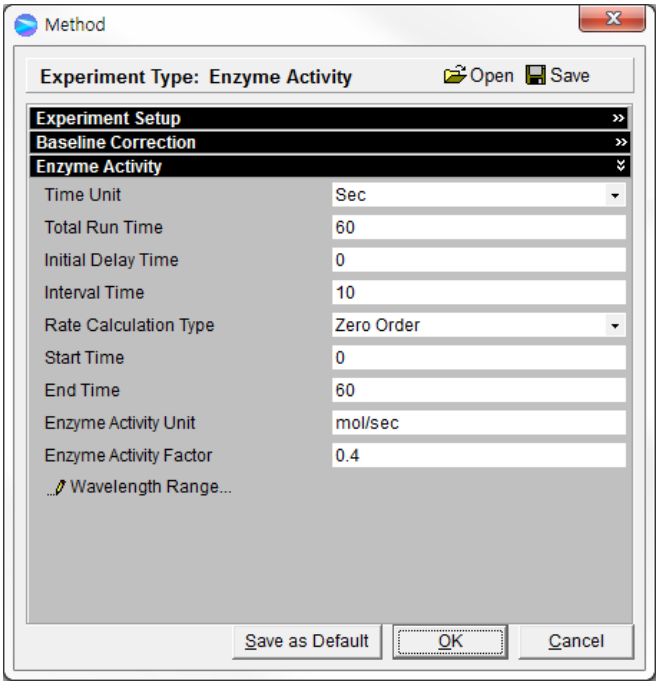
V-4-4. Enzyme Activity Mode

▣ 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.



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.

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

ε = molar absorption coefficient ($M^{-1}cm^{-1}$)

b = cell pathlength (cm)

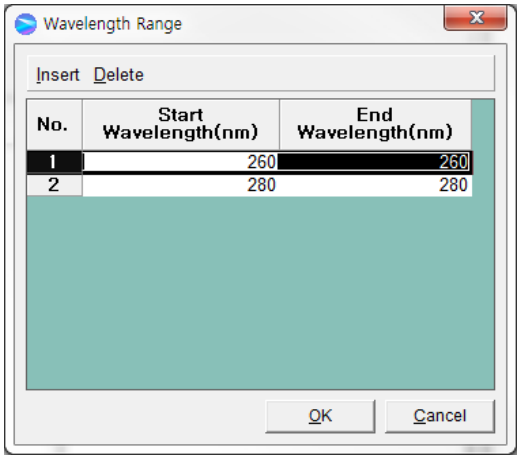
V_f = final volume in the cuvette (l)

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.



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.

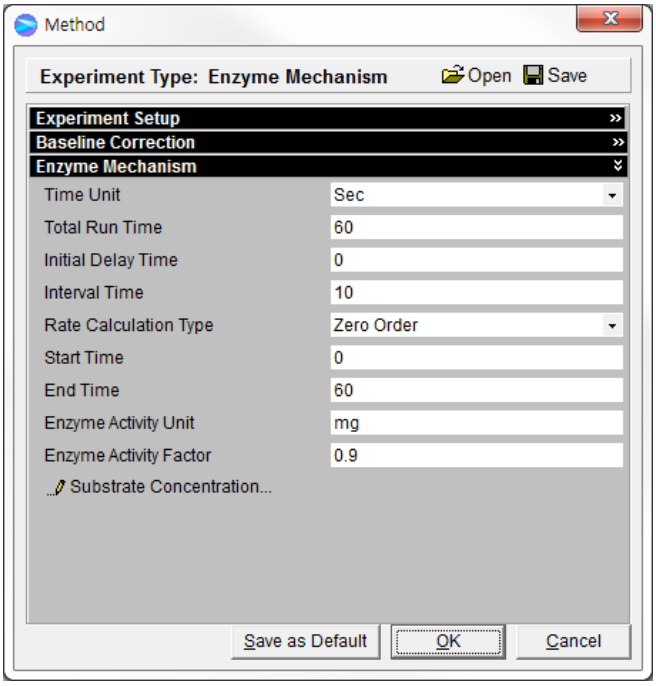
V-4-5. Enzyme Mechanism Mode

▣ Use Enzyme Mechanism Methods to collect the plots, K_m and V_{max} from a set of kinetic samples.

▣ Procedure

1. Click New.

2. Select Enzyme Mechanism in the Experiment Type. The method dialog box is displayed.



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.

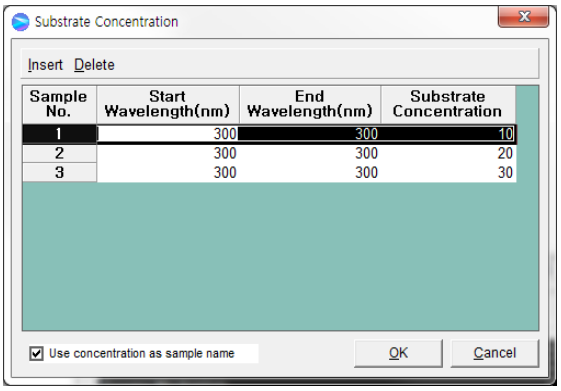
g. End Time: Enter the time to stop calculating enzyme activity.

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.

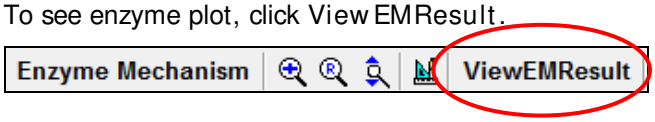


7. After setting parameters for Experiment Setup, Baseline Correction and Enzyme Mechanism is complete, select OK.

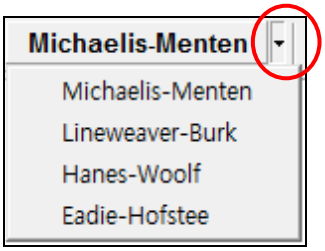
8. Measure the blank.

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 View EM Result.



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



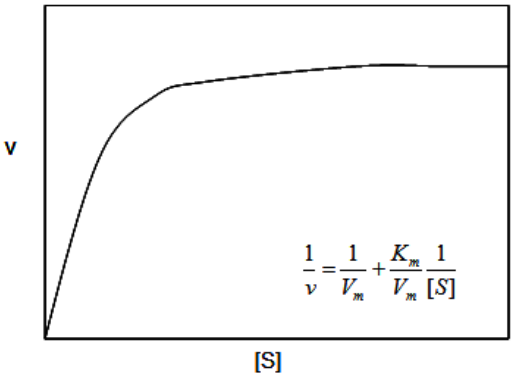
12. The graph can be viewed if a single plot is selected.

13. Save and print spectra and data as required.

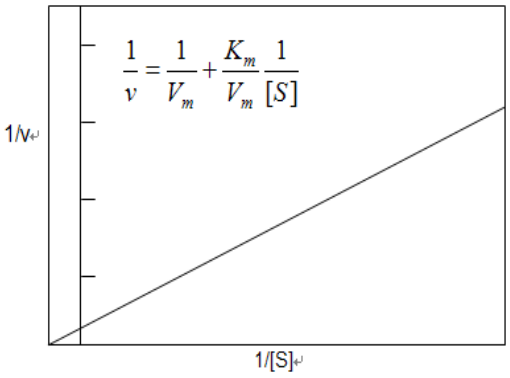
Enzyme Mechanism Plots

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.

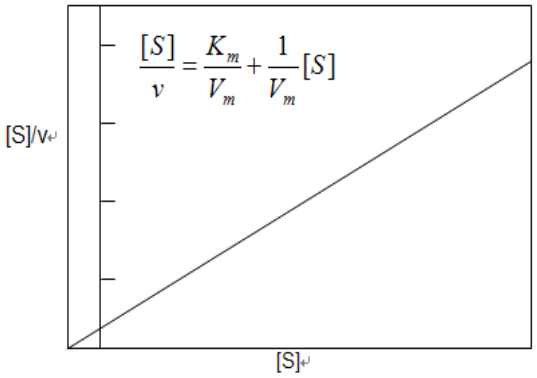
Michaelis-Menten Plot



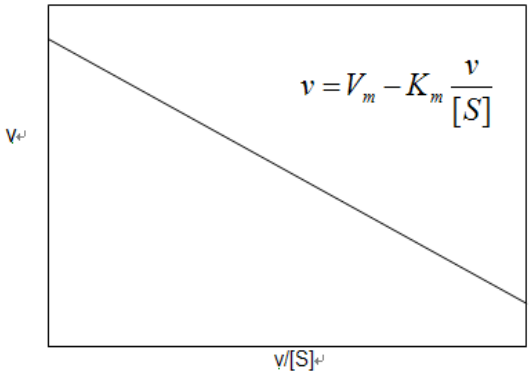
Lineweaver-Burk Plot



Hanes-Woolf Plot



Eadie-Hofstee Plot



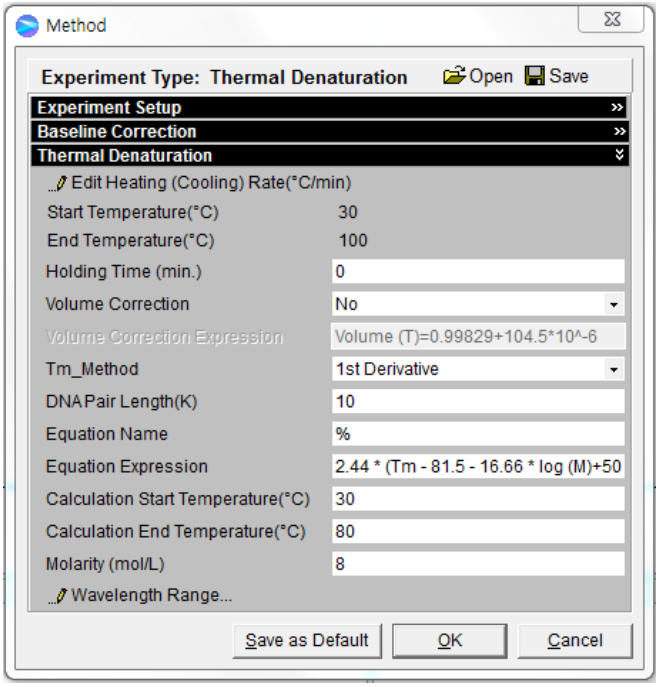
V-4-6. Thermal Denaturation Mode

▣ 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.

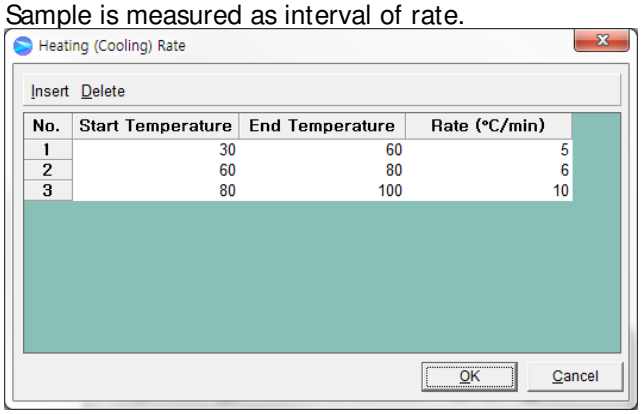


3. Setup Experiment Setup and Baseline Correction parameters. See V-1-1. Wavelength Monitoring for more details. Select the Single Cell Peltier or Multi-Cell Peltier in Sampling Type of Experiment Setup.

4. Click Thermal Denaturation and setup test parameters as follows:
   a. Edit Heating(Cooling) Rate (℃/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.



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:

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

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".

h. 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:

$$\%\ G\text{-}C = 2.44 * (Tm - 81.5 - 16.66 * \log(M) + 500/K)$$

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

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

$$\%\ G\text{-}C = 2.44 * (Tm - 81.5 - 16.66 * \log(M))$$

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.

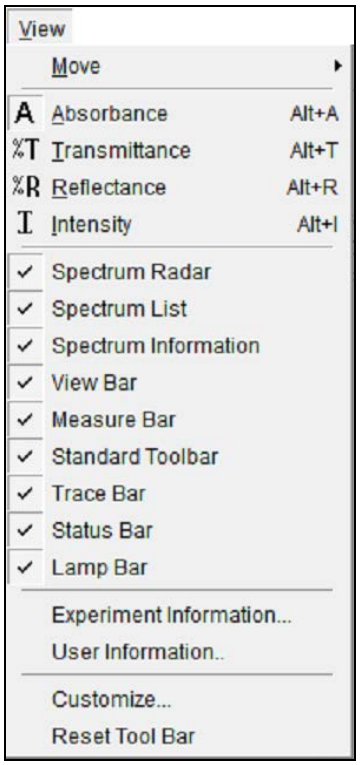
6. Measure the blank.

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.

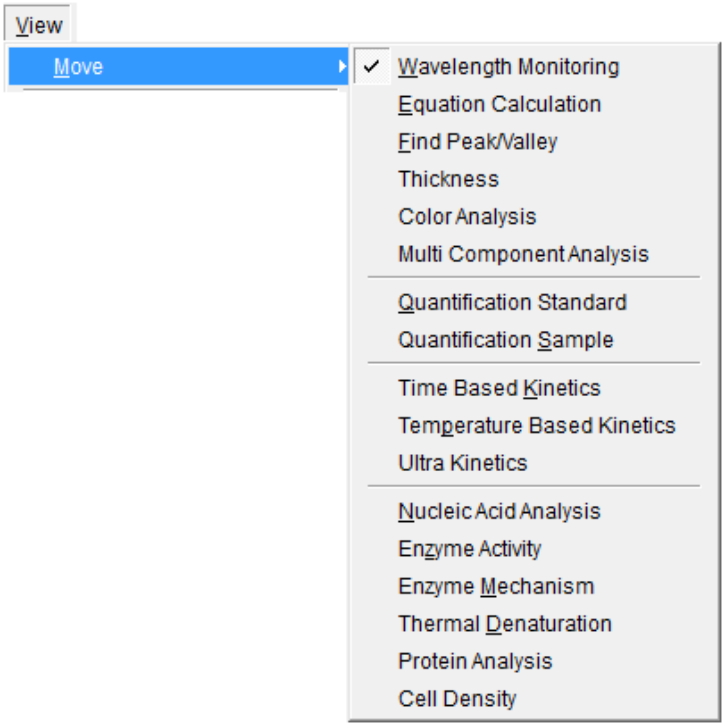


Move	Select another experiment mode
Absorbance	Display the unit of Y-axis by absorbance
Transmittance	Display the unit of Y-axis by transmittance
Reflectance	Display the unit of Y-axis by reflectance
Intensity	Display the unit of Y-axis by intensity
Spectrum Radar	Display spectrum radar on the screen
Spectrum List	Display spectrum list on the screen
Spectrum Information	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	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.



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

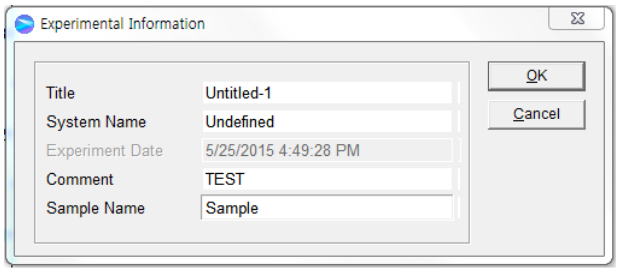
1. Select Intensity and the unit of the Y-axis changes to intensity mode.

VI-6. Experimental Information

▣ Use the Experiment Information command to exchange the experiment information.

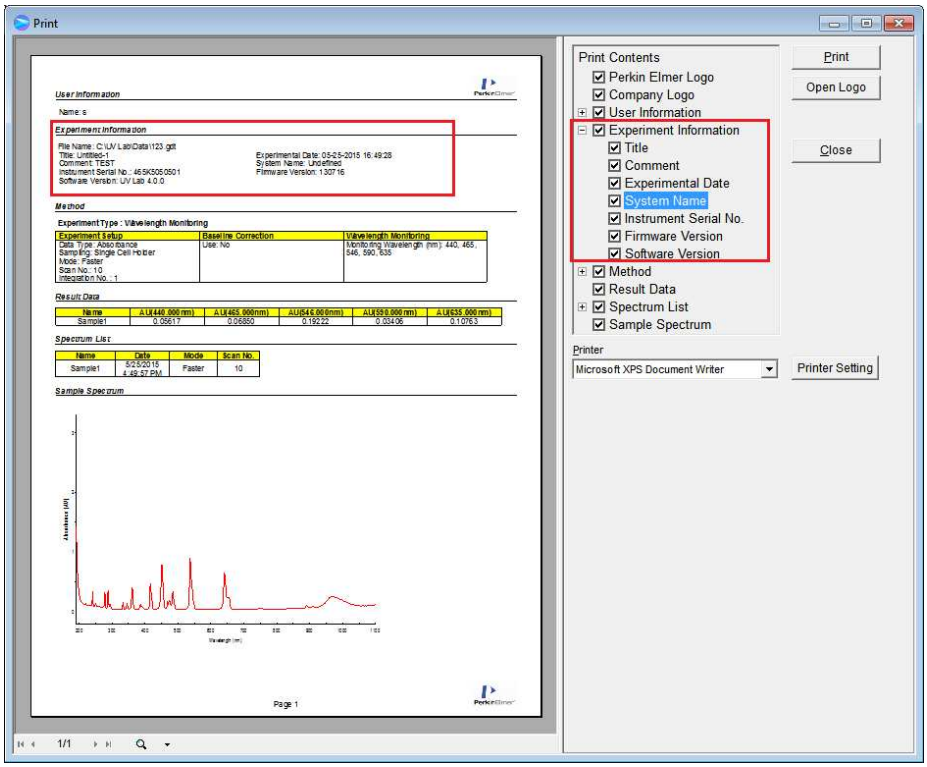
▣ Procedure

1. Select Experiment Information.



2. Enter Title, System Name and Comment information.

3. Select OK. You can check Experiment Information at the print.

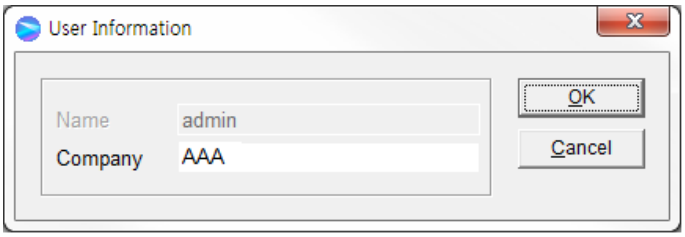


VI-7. User Information

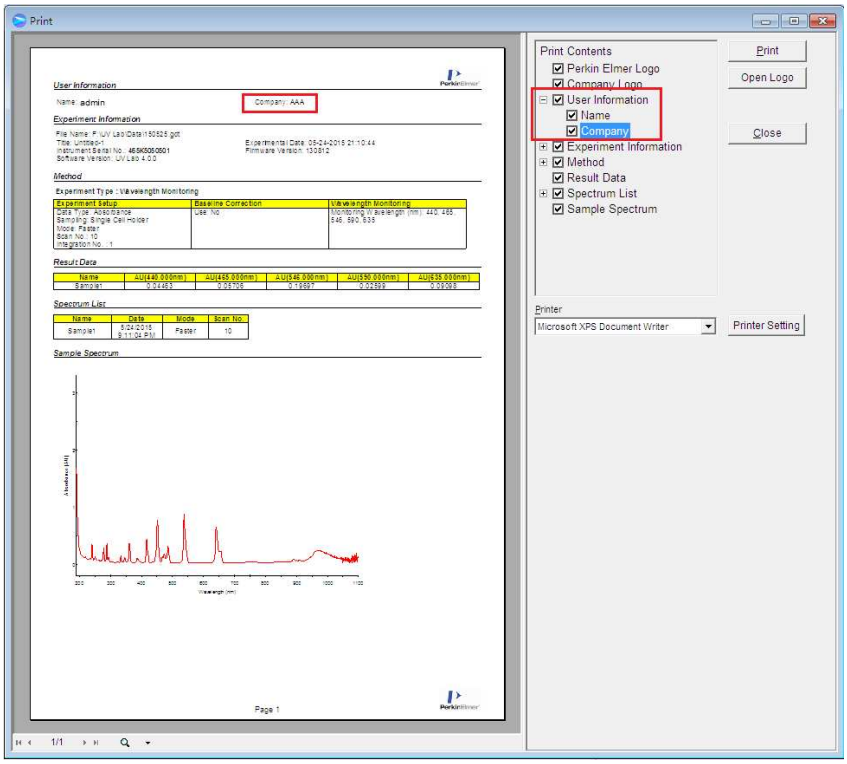
▣ Use the User Information command to exchange the user information.

▣ Procedure

1. Select User Information.



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.



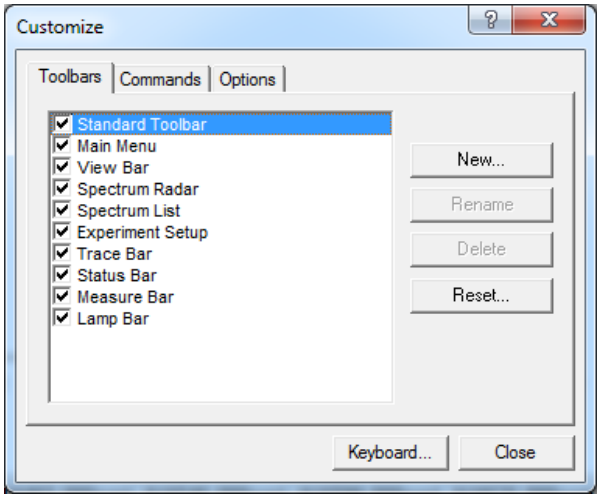
VI-8. Customize

▣ Use the customize command to hide and create the toolbars and disable all of the user's editing options.

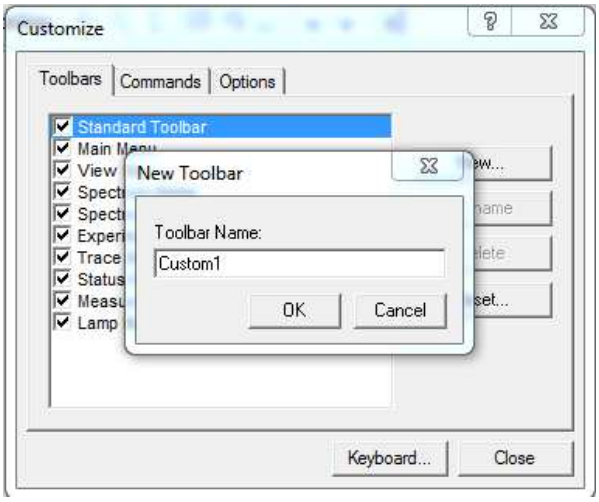
▣ Procedure

1. Click Toolbars tab.

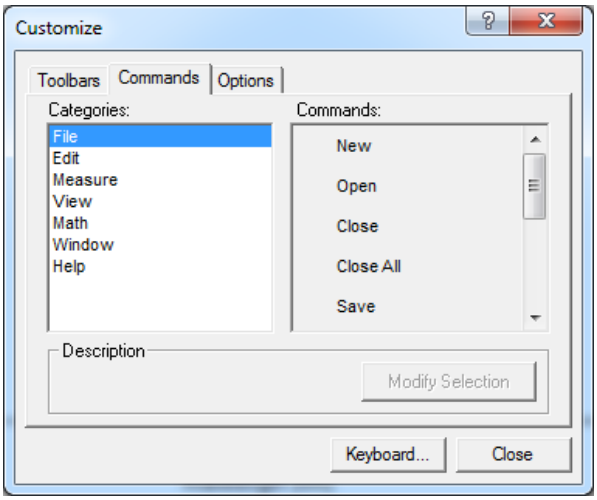
2. Select toolbars desired.



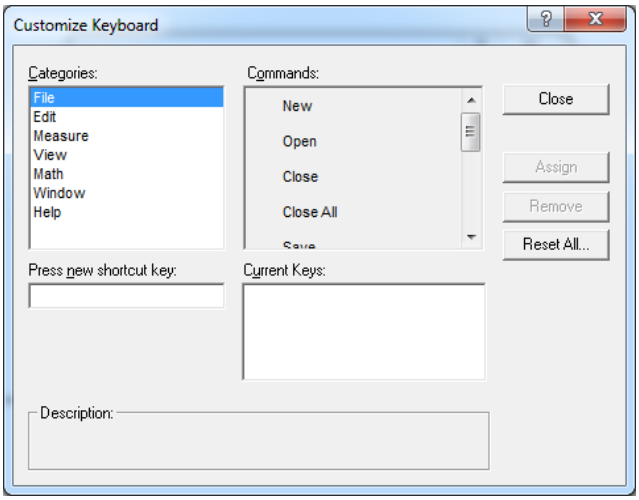
3. Select New. Enter the new toolbar name and select OK.



4. Select the Commands tab.

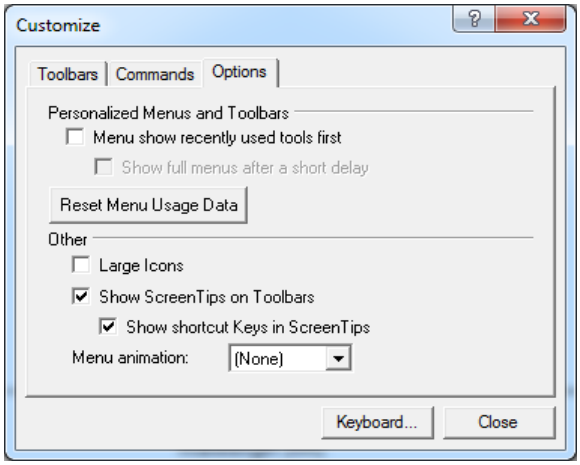


5. You can create a tab. Select Keyboard. The following dialog box will be displayed.



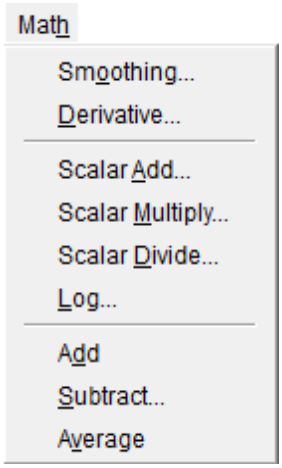
6. Select Options tab.

7. Choose the options required, and then select Close.



VII. Math Menu

▣ The Math menu includes commands to analyze the collected data.



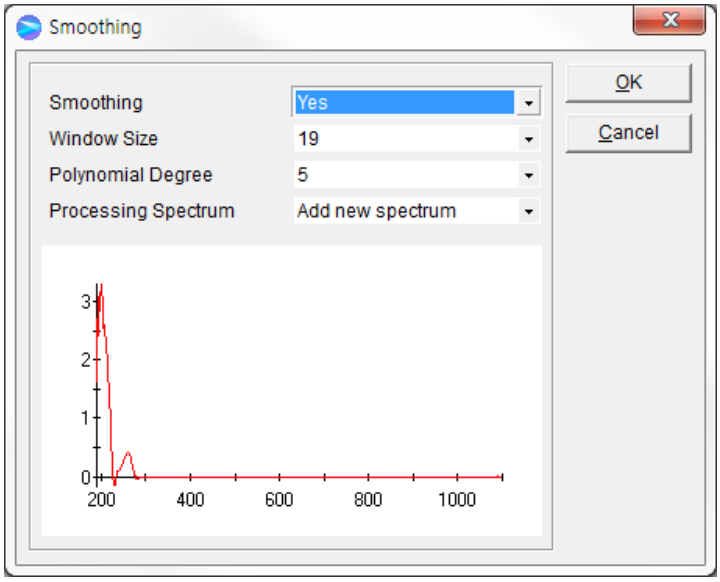
Smoothing	Smooth the spectrum
Derivative	Obtain the data after applying a derivative
Scalar Add	Add a constant value to the y-value in the spectrum
Scalar Multiply	Multiply the y-value in the spectrum by a constant value
Scalar Divide	Divide the y-value in the spectrum by a constant value
Log	Compute the common logarithm of the y-value in the spectrum
Add	Obtain the added data of selected spectra
Subtract	Obtain the subtracted data of two spectra
Average	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

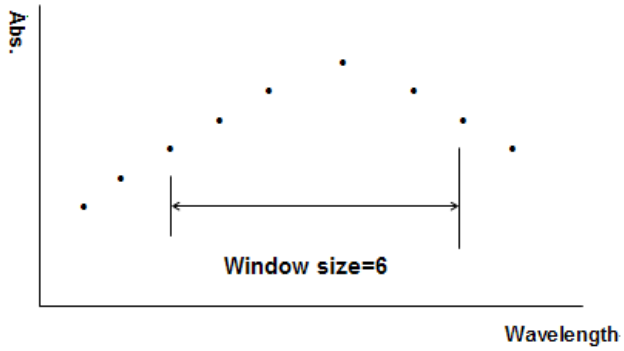
1. Select Smoothing. The following dialog box is displayed.



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.

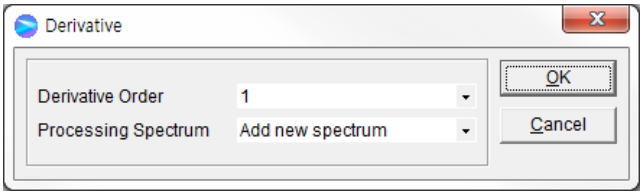
Savitsky-Golay Smoothing

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.

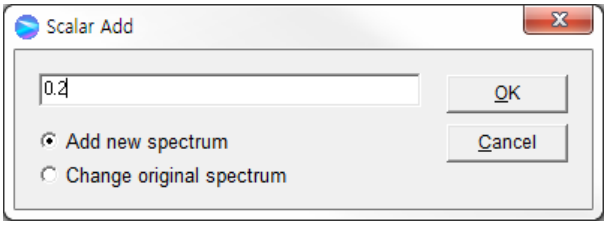


2. Click OK after entering the Derivative Order number and selecting the Processing Spectrum. The result will be displayed in the main window.

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.



2. Enter the value to add to the spectrum.

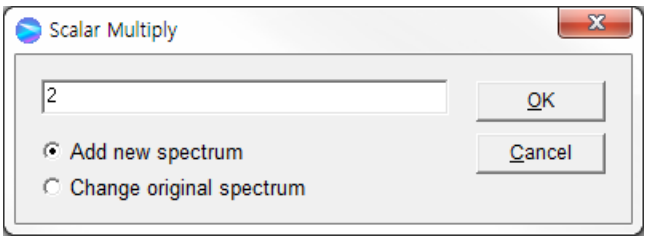
3. Select Add new spectrum or Change original spectrum.

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.

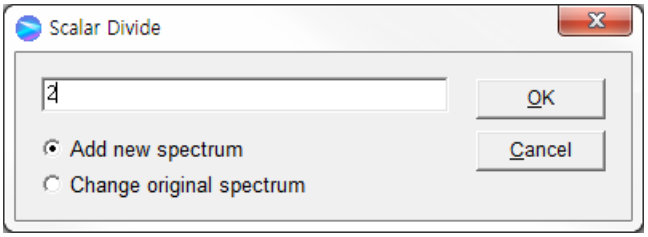


2. Enter the value to multiply the spectrum.
3. Select Add new spectrum or Change original spectrum.
4. Select OK. The result will be displayed in the main window.

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.

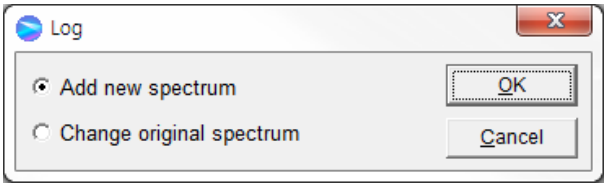


2. Enter the value to divide the spectrum.
3. Select Add new spectrum or Change original spectrum.
4. Select OK. The result is displayed in the main window.

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.



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

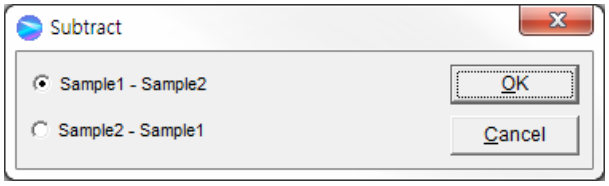
1. Select desired spectra to add together by clicking the spectra while holding down the Ctrl key.

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.



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

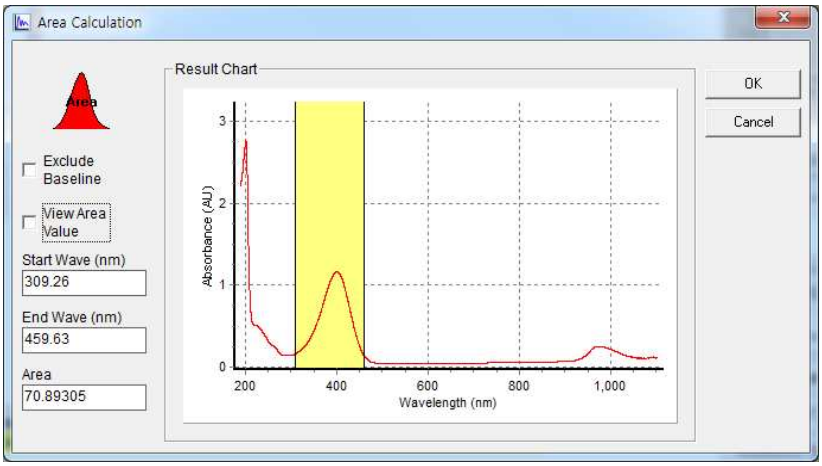
▣ Use the Average command to calculate the average of selected spectra.

▣ 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.

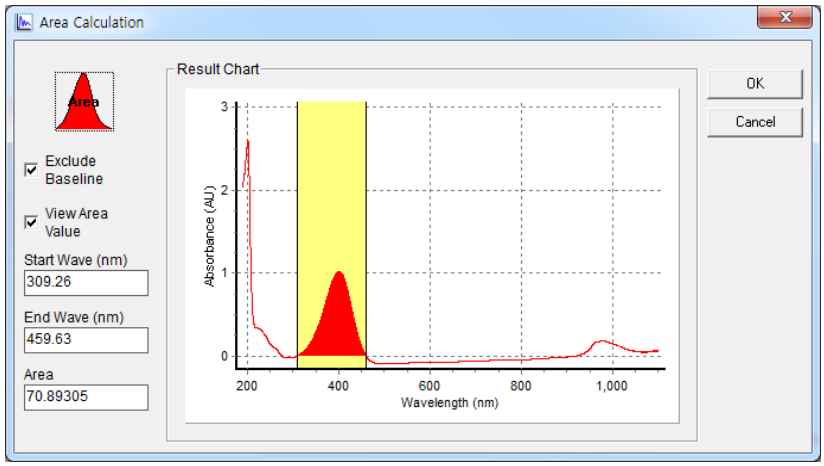
c. **Start Wave (nm)**: Enter the start wavelength for the area calculation.

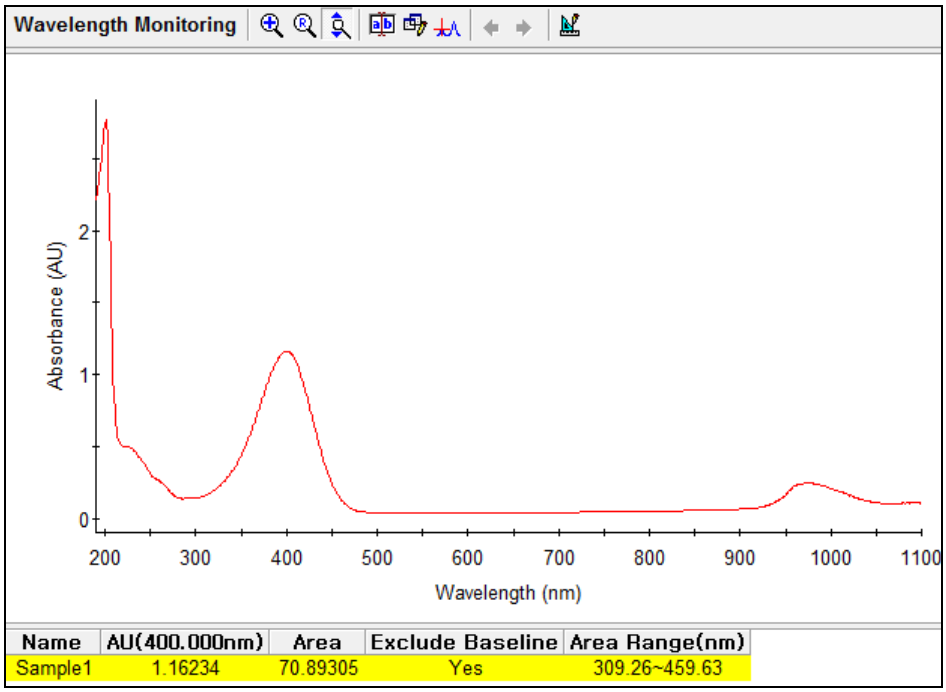
d. **End Wave (nm)**: Enter the end wavelength for the area calculation.

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.

	Zoom In		Pick Peak
	Zoom Reset		Pick Valley
	Y Axis Auto Scale		Cursor
	Add Label		To Left
	Edit Label		To Right
	Properties	View 3D	View 3D for kinetics mode

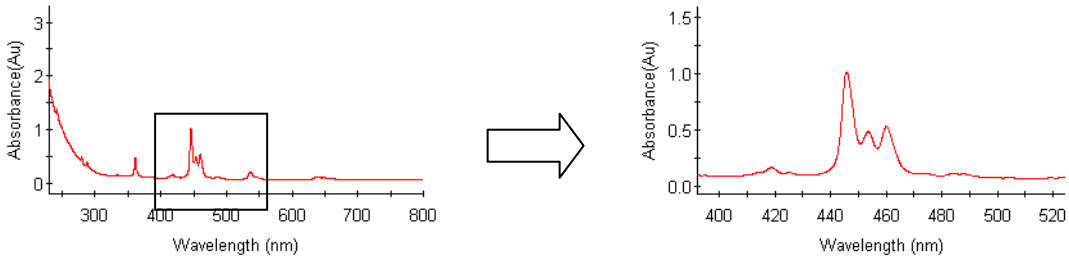
VIII-1. Zoom In / Reset / Auto scale

▣ 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.

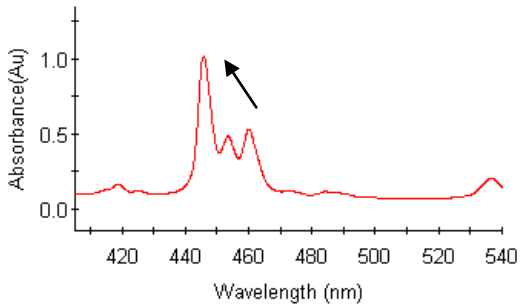
VIII-2. Add / Edit / Delete Label

▣ Use these icons to add, edit and delete labels in the main window.

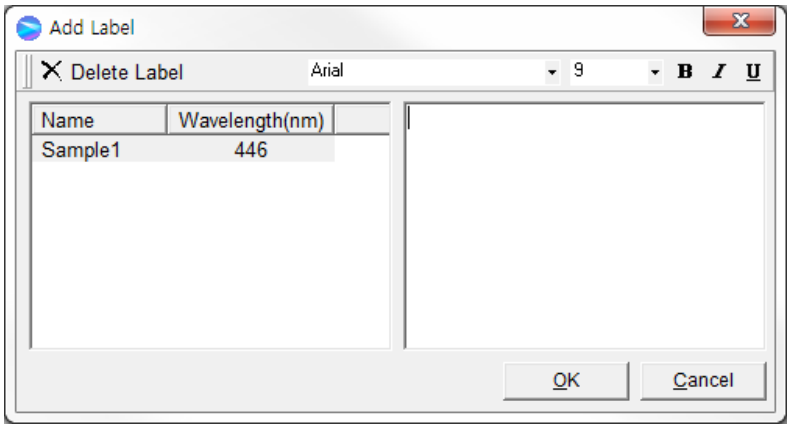
▣ Procedure

1. Select Add Label.

2. Set the label at the required position using the mouse, as shown below.

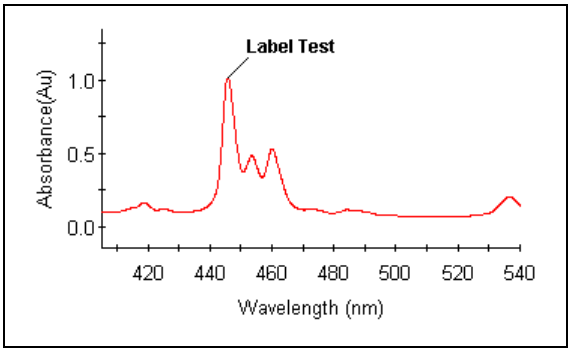


3. The following dialog box will be displayed.



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.

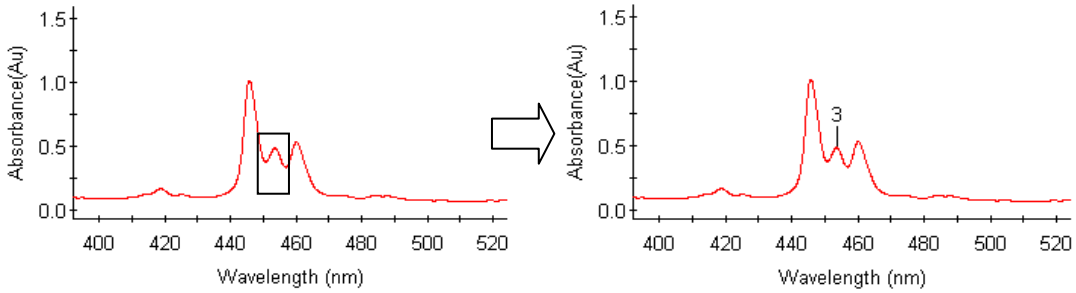
VIII-3. Pick Peak / Valley

▣ 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.

4. Select delete or delete all as shown.

Name	No.	Peak(nm)	Peak(AU)
Sample1	1	241.200	0.3829
	2	249.970	0.1165
	3	278.210	0.3274
	4	287.350	0.3597
	5	333.690	0.1651
	6	345.400	0.1403

Delete
DeleteAll
Copy

VIII-4. Properties and Display Interval

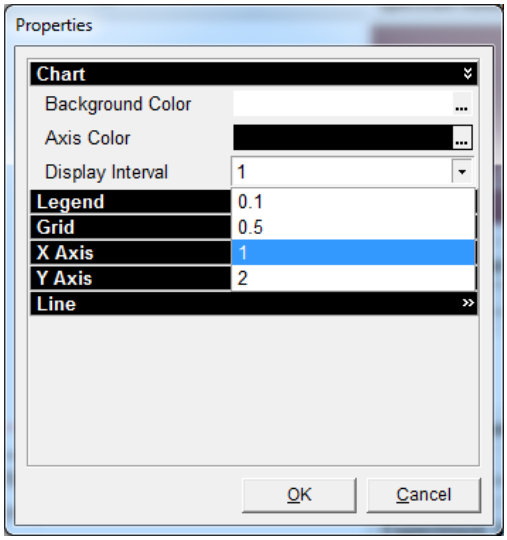
▣ Use these icons to change chart properties in the main window, such as the chart color, display interval, grid, X Axis, Y Axis and line properties.

▣ Procedure

1. Select Properties.

2. Change properties as follows.

3. Select OK when finished.



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.

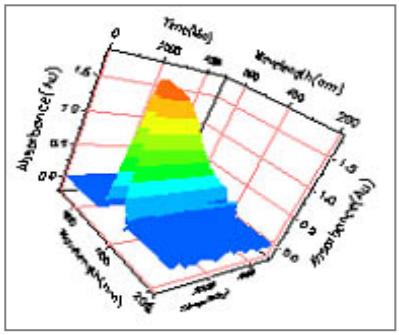
d. Grid: Select to display X and Y grids.

e. X, Y Axis: Set the range of X and Y Axis.

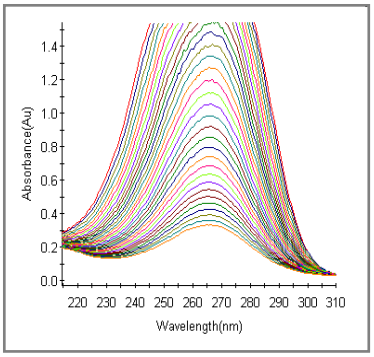
f. Line: Select the color and pattern of the spectrum lines.

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.



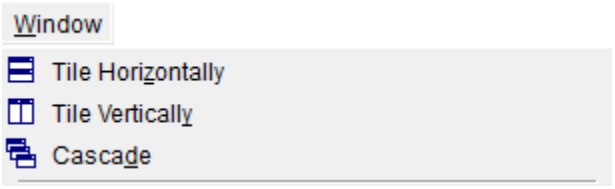
3D Graphic Spectra Mode



Time Based Kinetics

IX. Window Menu

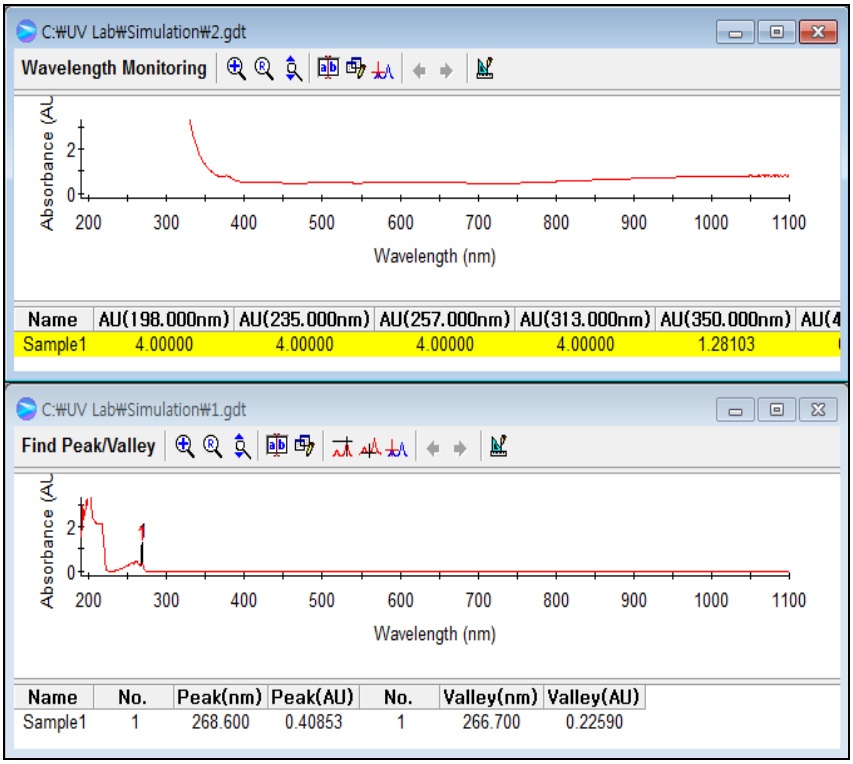
▣ The Window Menu to arrange the windows and show the current windows.



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

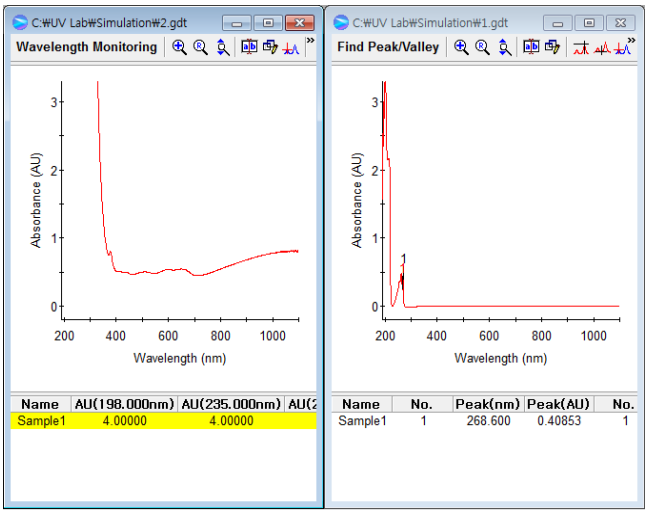
IX-1. Tile Horizontally

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



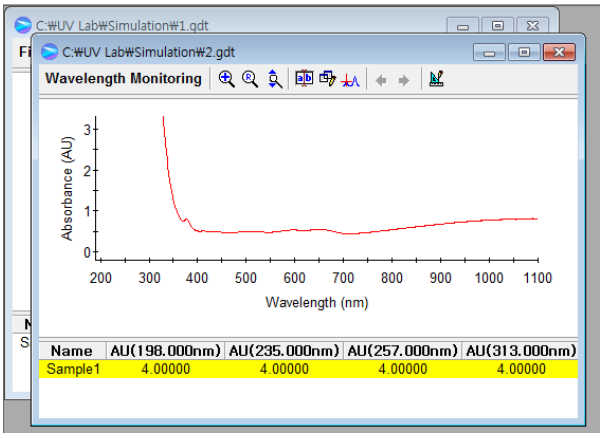
IX-2. Tile Vertically

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

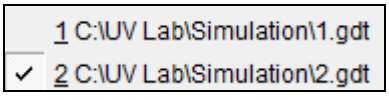


IX-3. Cascade

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



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



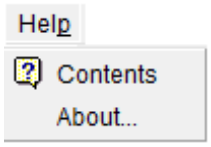
▣ Procedure

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.



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

X-1. Contents

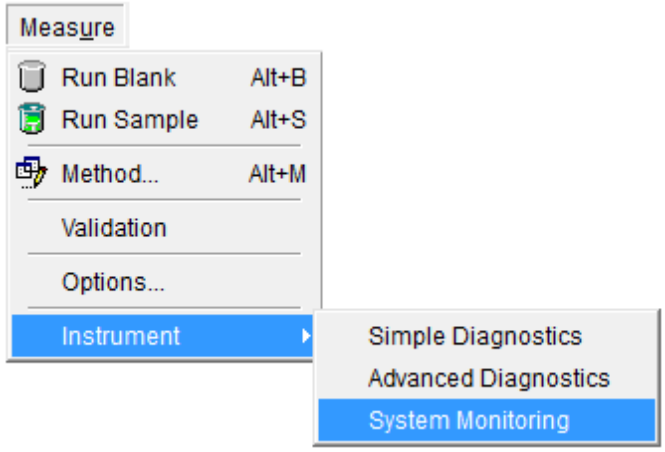
▣ Use the help section for suggestions on using UV Lab software more effectively.

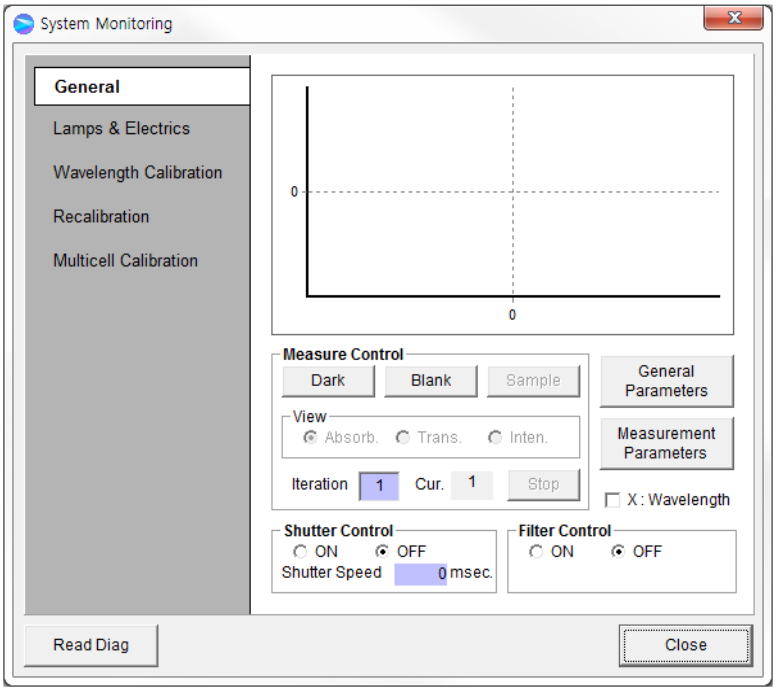
X-2. About...

▣ 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.





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.

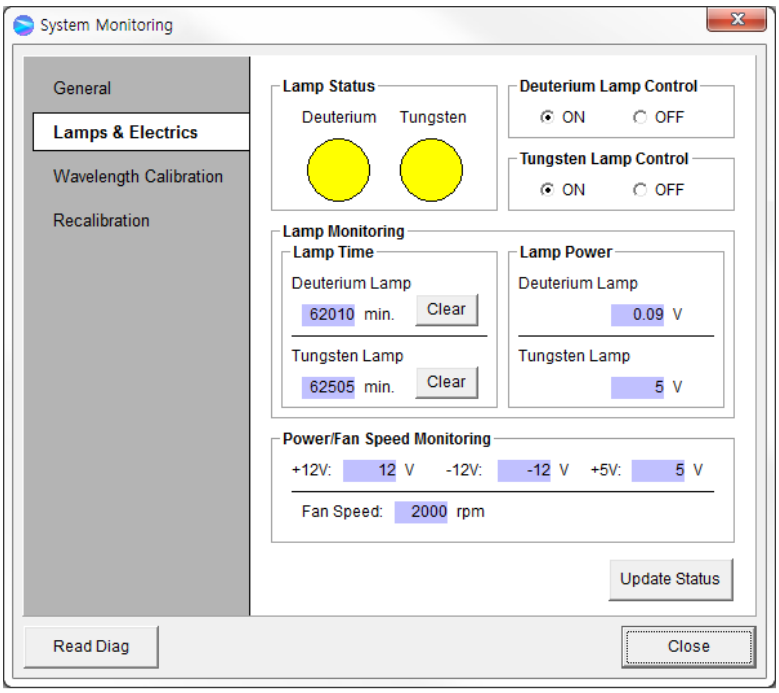
XI-1. General

▣ 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.



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.

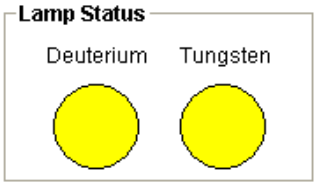
d. Power / Fan Speed Monitoring: Use to check the power status and fan speed.

e. Update Status: Use to update the status of the displayed window after modifying the current status of the instrument.

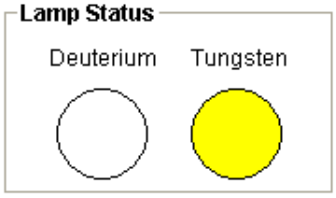
XI-2-1. Lamp Status

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.

1. 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.

XI-2-3. Lamp Monitoring

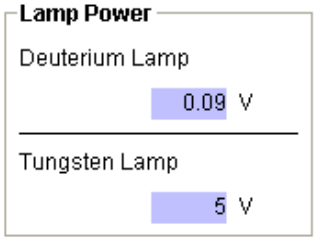
Use to check the lamp power and time used.

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

Lamp Life Time

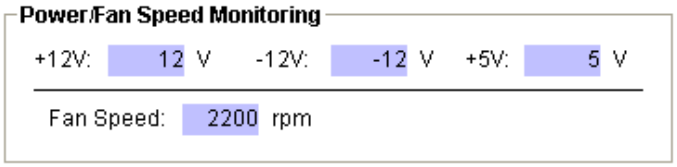
- Deuterium lamp: 2,000 hours
- Tungsten lamp: 10,000 hours

2. Lamp Power: View the power status of each lamp.



XI-2-4. Power / Fan Speed Monitoring

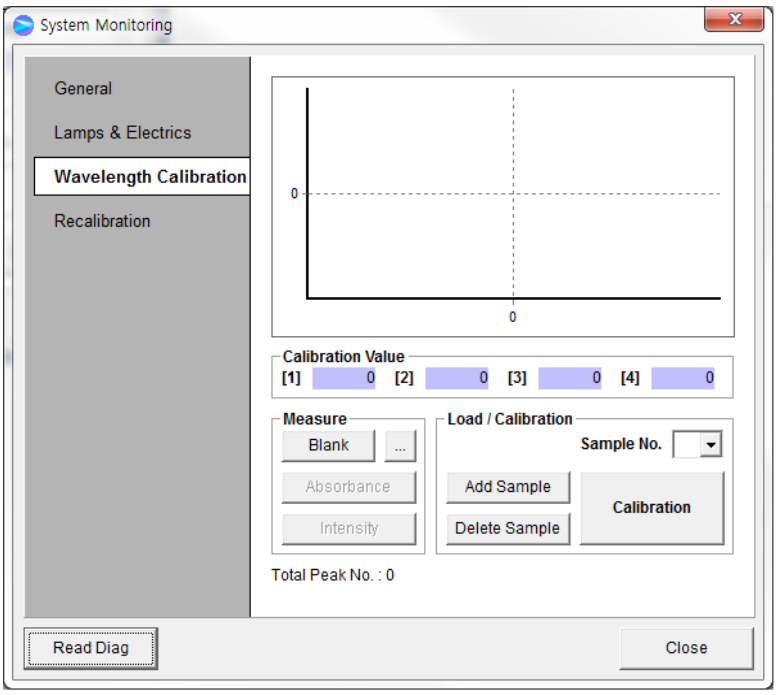
View status for the power and the fan speed of the instrument.



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.



a. Calibration Value: Indicate 4 factors for calibration curve.

b. Measure: Use to measure the blank, absorbance and intensity.

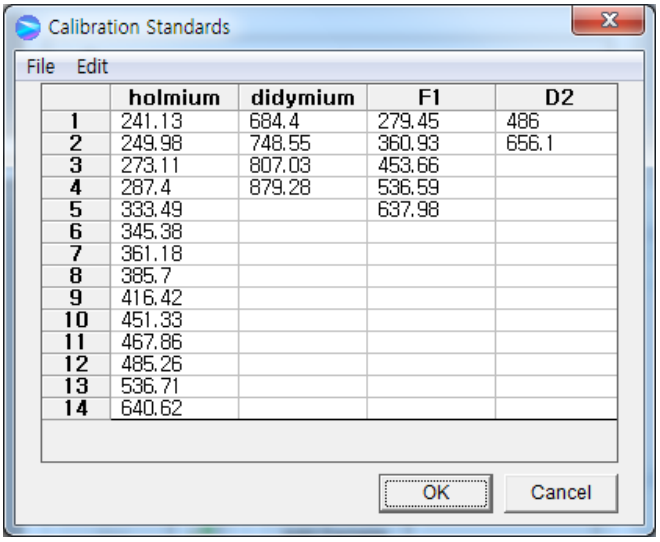
c. Load/ Calibration: Use to retrieve spectra of standard samples stored in a current window and to perform calibration progress.

d. Total Peak No.: Show the total peak numbers of entered values of standard materials.

XI-3-1. Wavelength Calibration Procedure

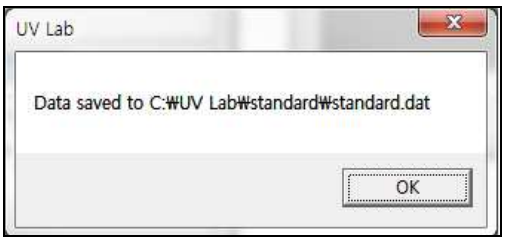
1. Select Wavelength Calibration. The Calibration Standards dialog box is displayed.

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.

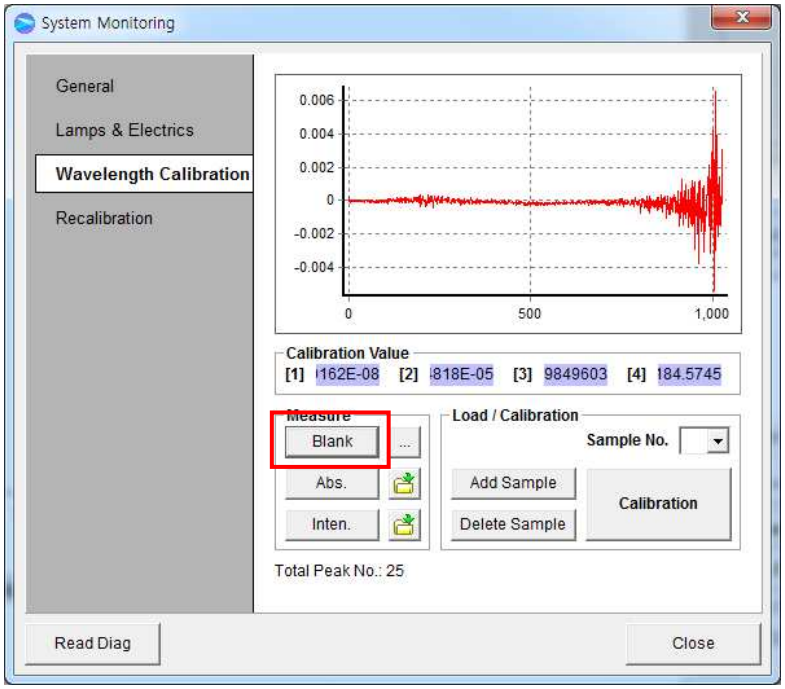


- Holmium: Refers to the Holmium Oxide Solution Standard. Enter the 14 values from the certificate of calibration closest to those shown in the default table above based on a SBW of 1.5 nm.
- Didymium: Refers to the Didymium Filter Standard. Enter the 4 values from the certificate of calibration closest 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.

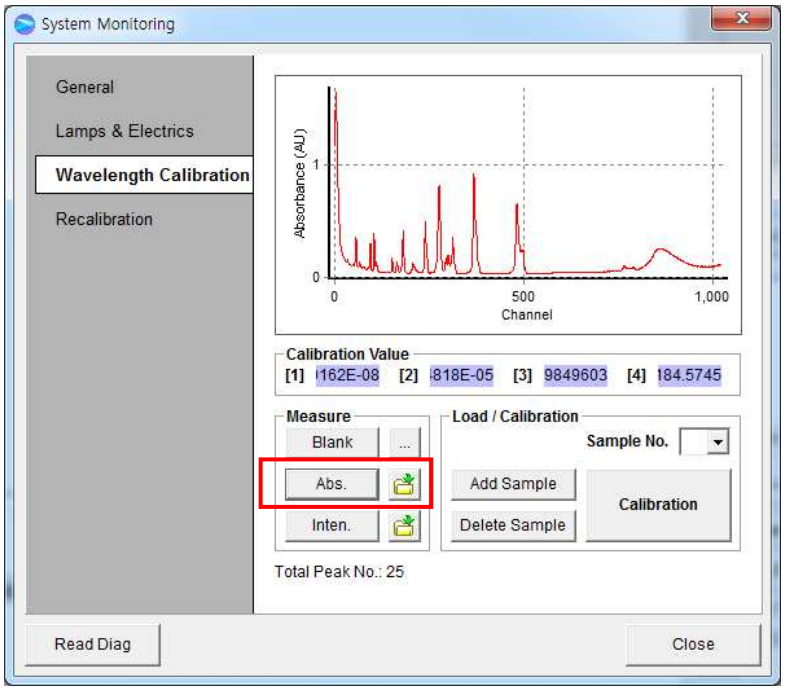
3. The following dialog box is displayed. Select OK.



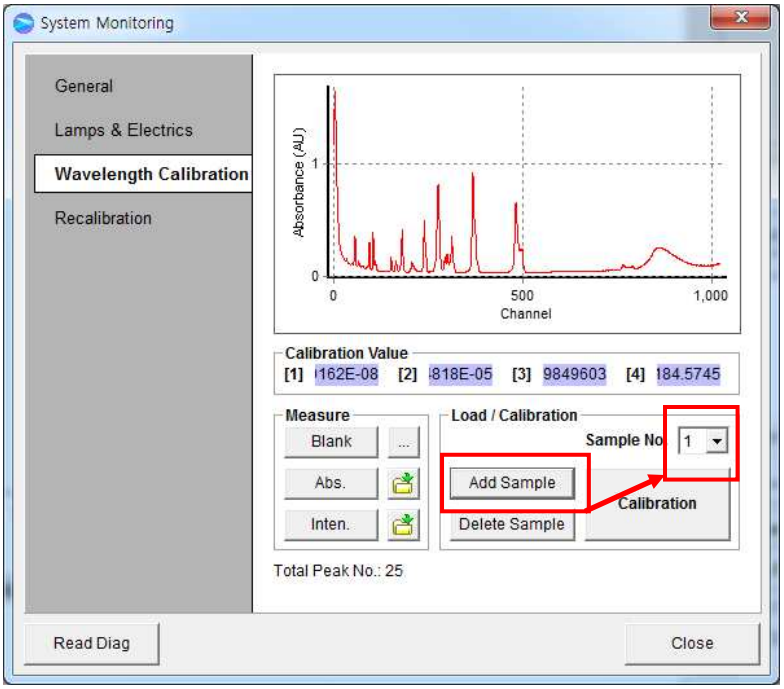
4. Remove the sample from the cell holder and select Blank.



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

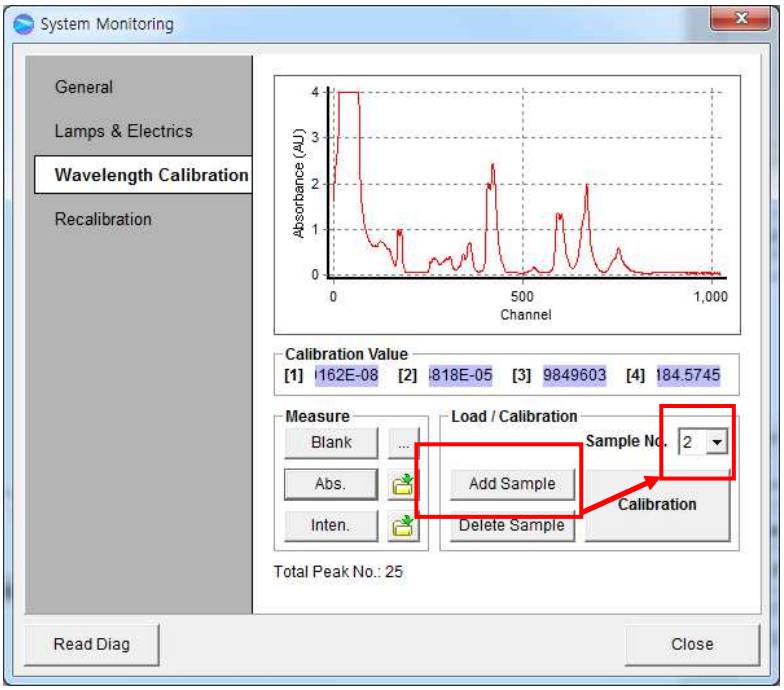


6. Select Add Sample to save the data from Sample 1 and select 1 for the Sample No.



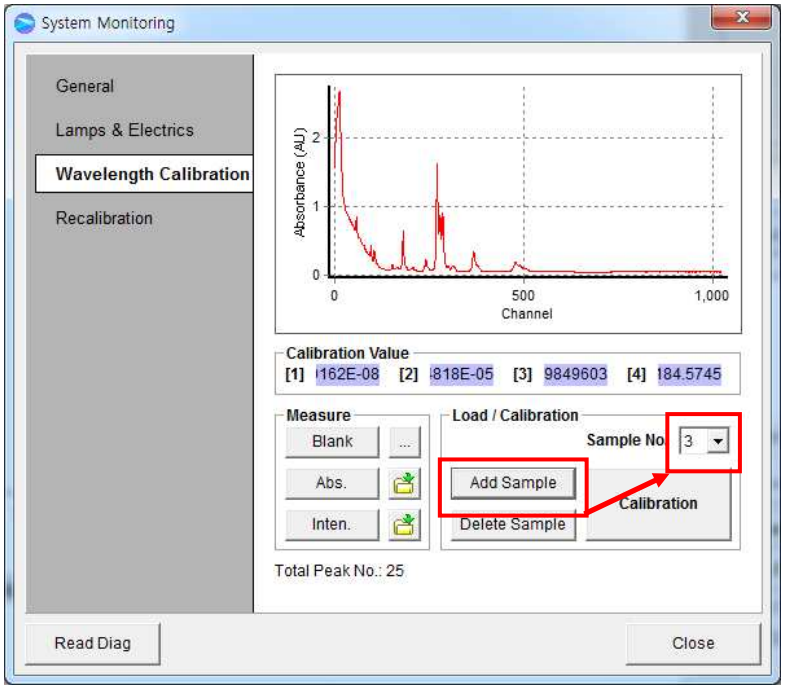
7. Place the Standard Sample 2 (Didymium filter) in the cell holder and click Absorbance.

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

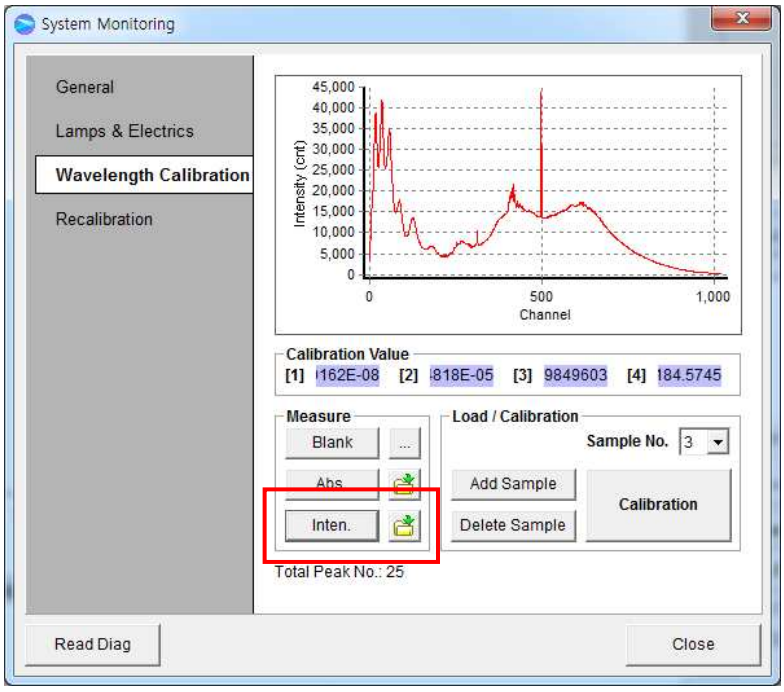


9. Place the Standard Sample 3, F1 (Holmium Oxide) filter in the cell holder and select Absorbance.

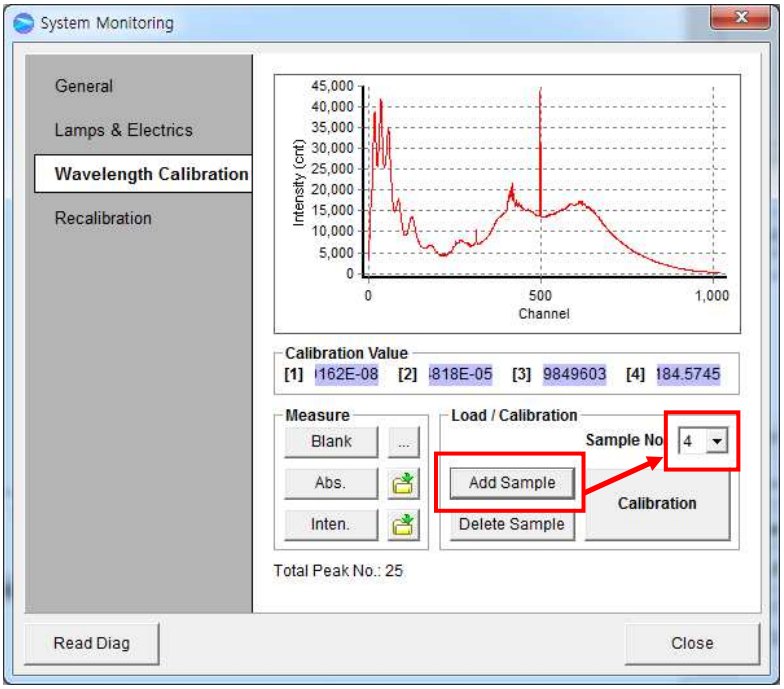
10. Select Add Sample to save the data for Standard Sample 3 and select 3 for the Sample No.



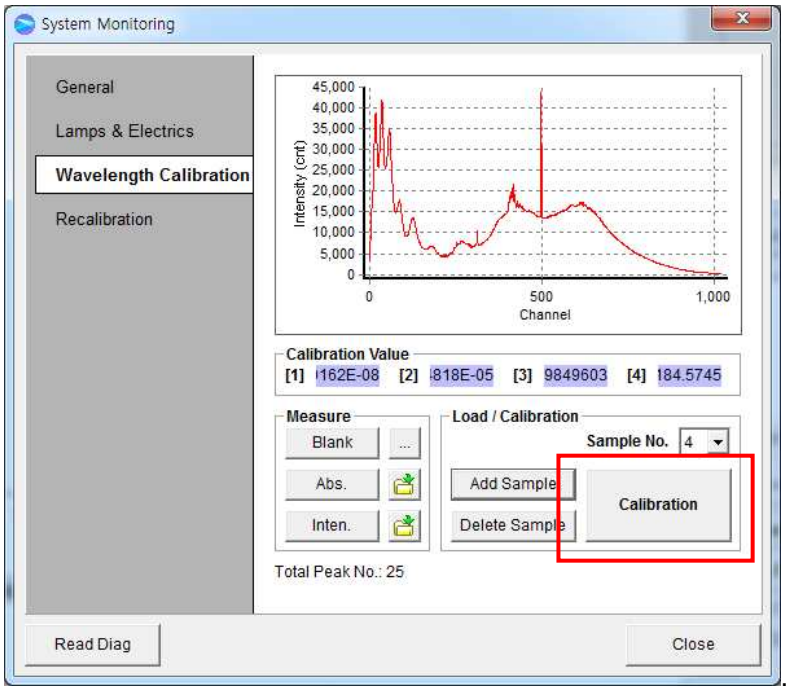
11. Remove all samples from the cell holder and click Intensity to measure Standard Sample 4 (D_2 peak).



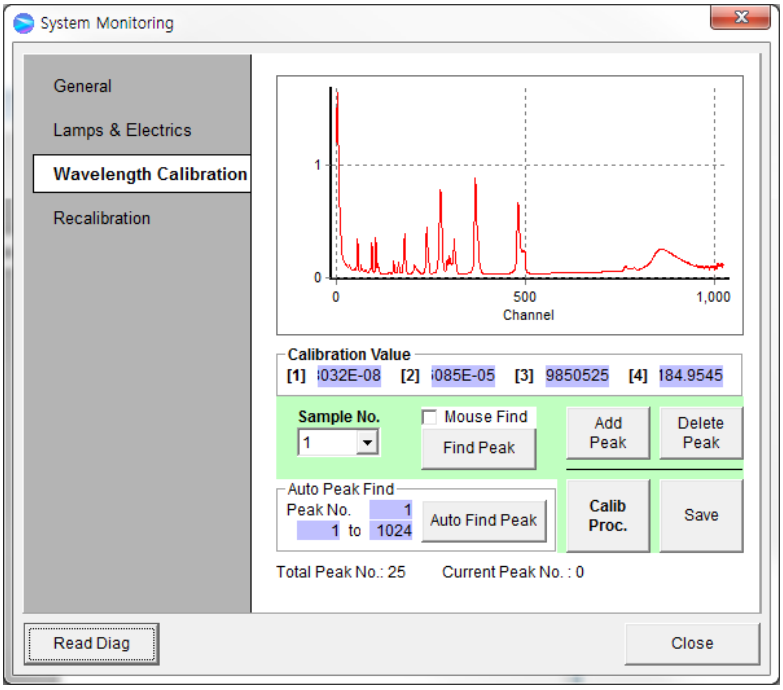
12. Select Add Sample to save the data of Standard Sample 4 and select 4 for the Sample No.



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

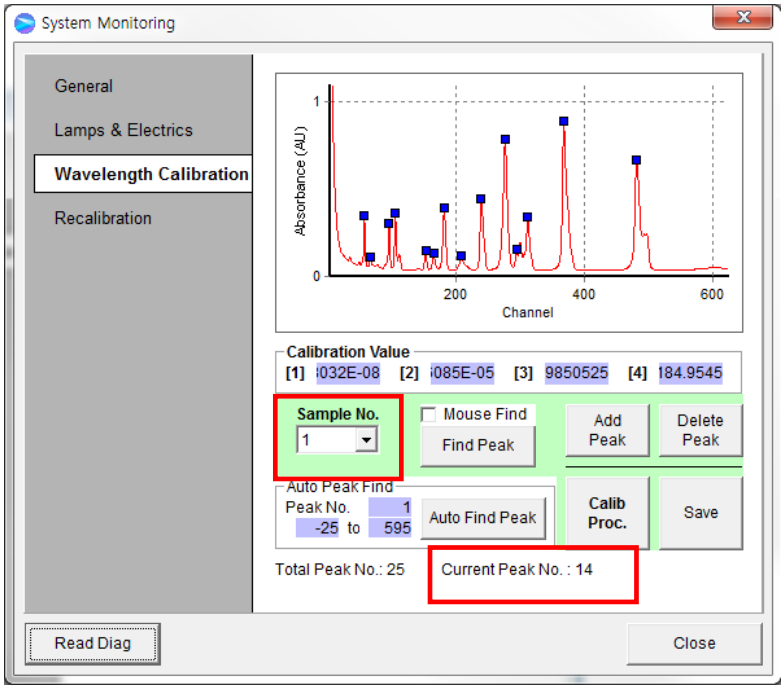


14. The following dialog box is displayed. Find the peaks for each standard sample and add them in this window. See XI-3-3. Peak finding for more information.



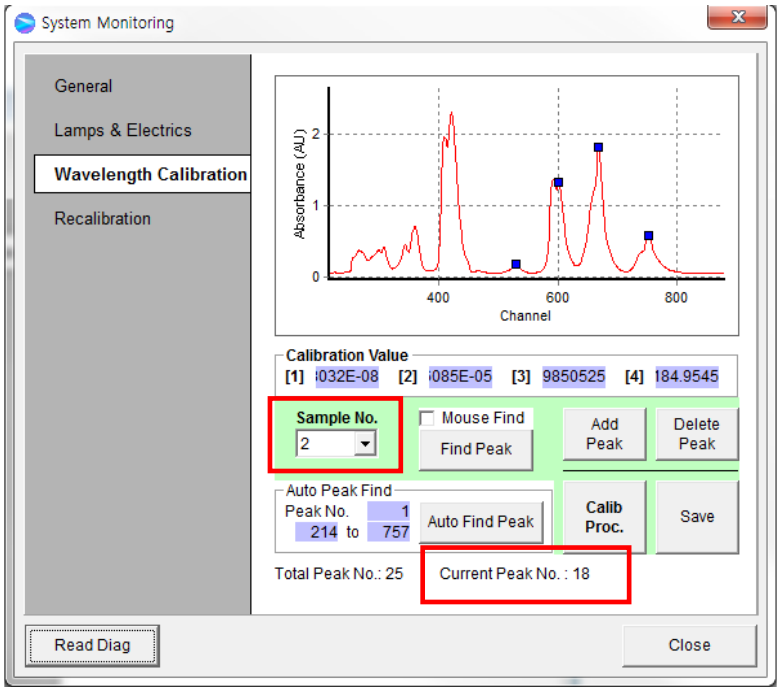
15. Select '1' in the [Sample No.].

16. Find 14 peaks for Standard Sample 1 (Holmium oxide solution) and add them as shown below.



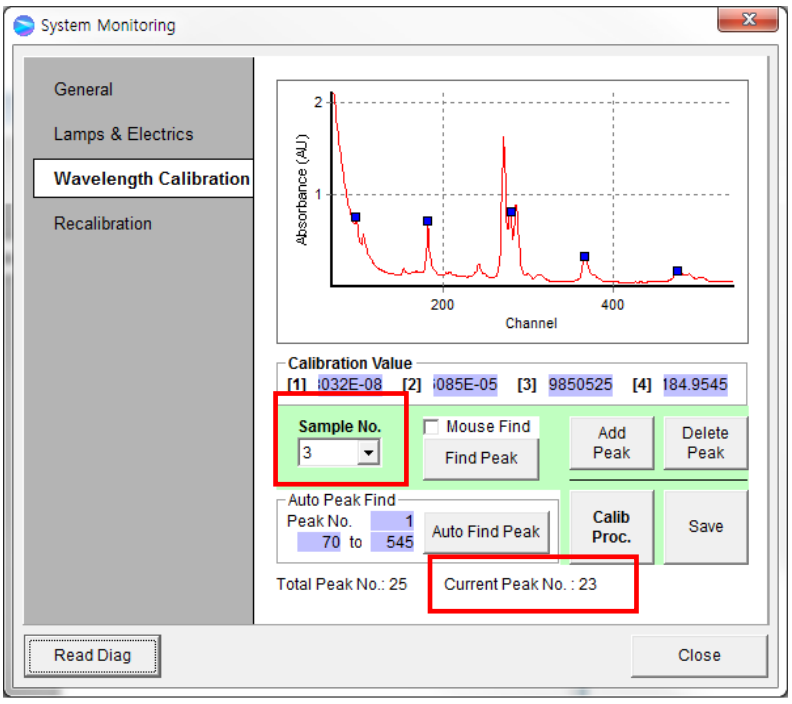
17. Select ‘2’ in the [Sample No.].

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



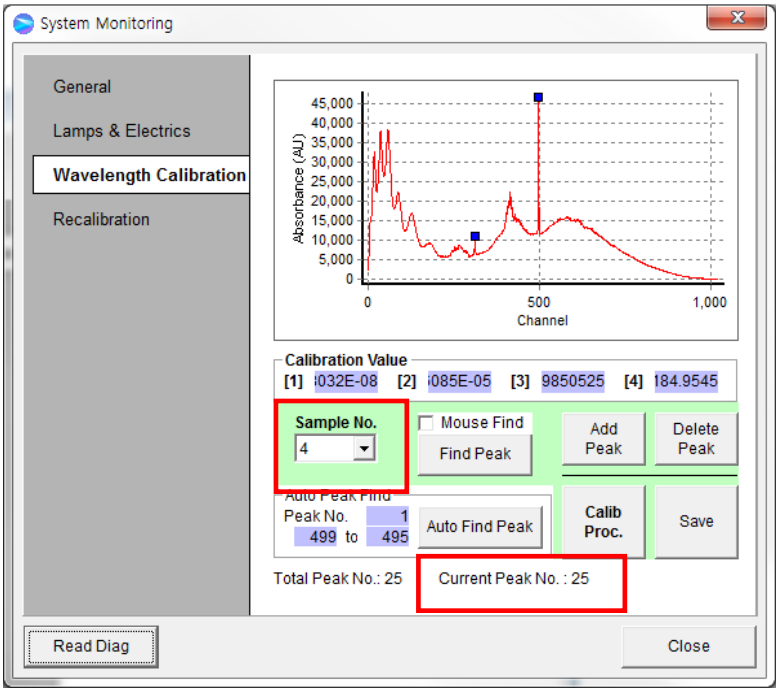
19. Select ‘3’ in the [Sample No.].

20. Find 5 peaks for Standard Sample 3 (F1 Filter) and add then as shown below.



21. Select ‘4’ in the [Sample No.].

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.

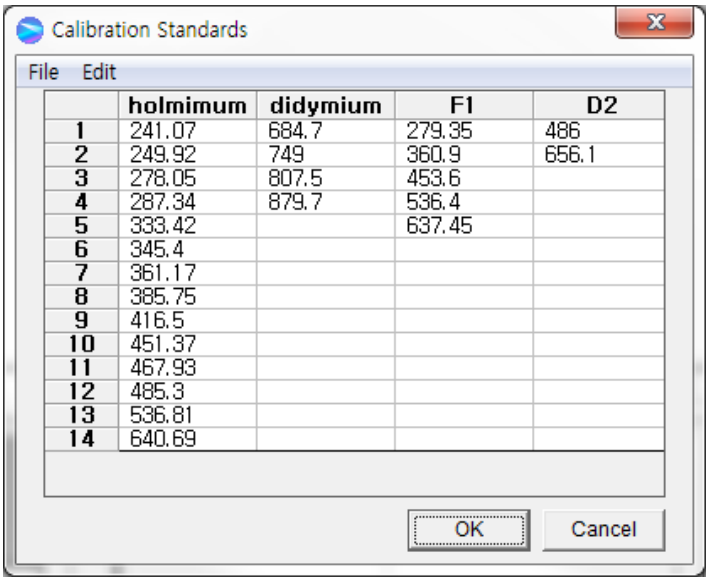
. Check the result of the calibration curve and verify that all of the data fits in the following Wavelength Error Trend window. If the calibration result is acceptable, select OK. If the result is not acceptable (R^2 > 0.9999 Error Max. and Min. < ±1), repeat the calibration procedure.

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

26. Select OK to finish the wavelength calibration.

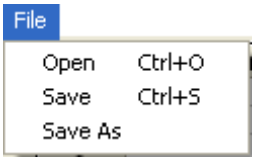
XI-3-2. File & Edit Menu

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.

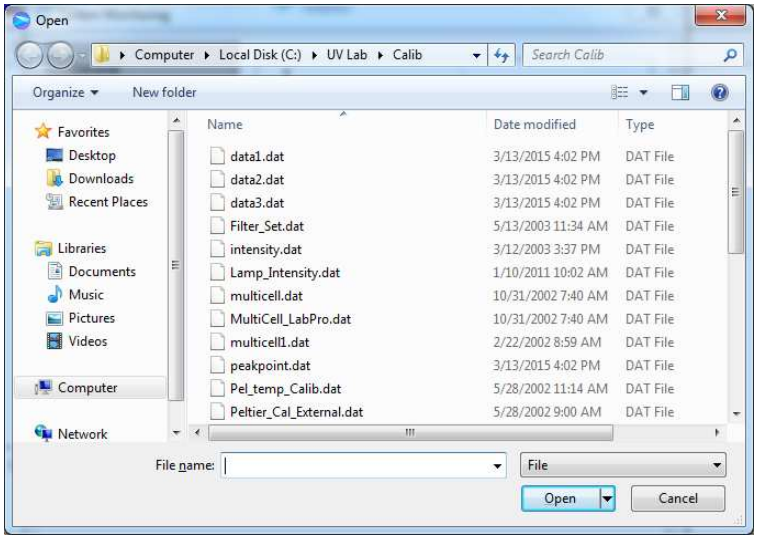


	holmimum	didymium	F1	D2
1	241.07	684.7	279.35	486
2	249.92	749	360.9	656.1
3	278.05	807.5	453.6	
4	287.34	879.7	536.4	
5	333.42		637.45	
6	345.4			
7	361.17			
8	385.75			
9	416.5			
10	451.37			
11	467.93			
12	485.3			
13	536.81			
14	640.69			

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

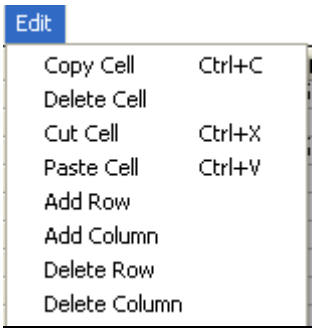


- Open: Use the Open command to retrieve data for a standard sample stored in a current windows.



- 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.

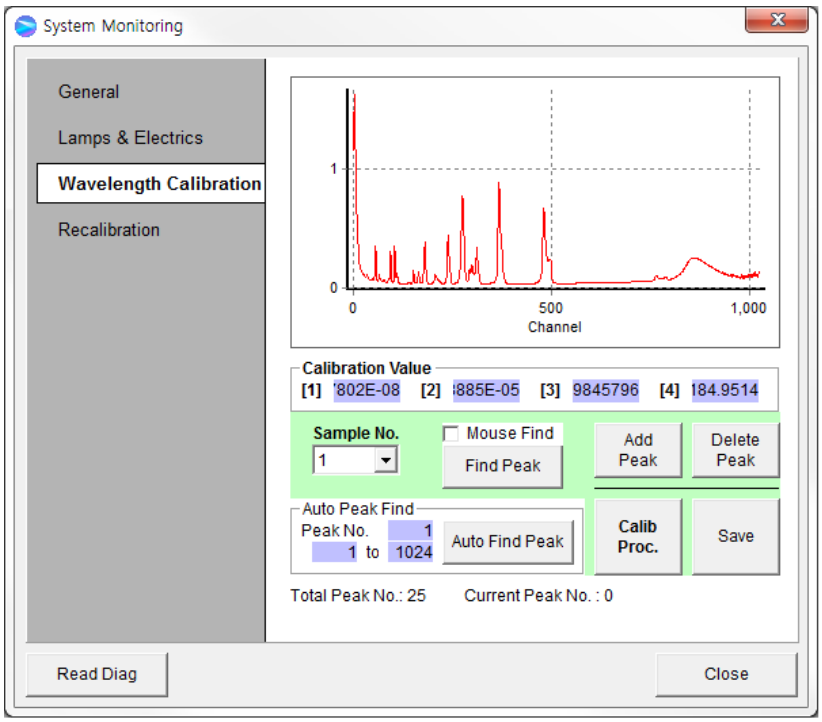
2. Available commands and its functions in Edit menu are as follows.



- 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.

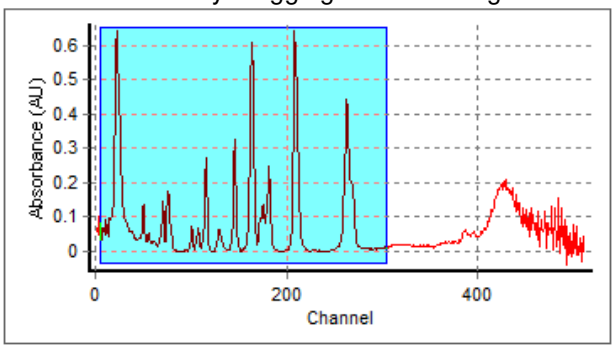
▶ Mouse Find: Use to find and add peak using mouse dragging. Check Mouse Find and designate peak finding area. One peak is found and added automatically in the designated area without selecting Find Peak or Add Peak.

Peak Finding Procedure

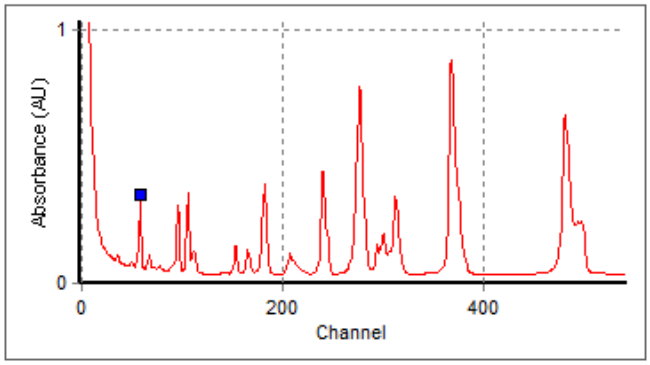
1. Select the peak finding area.

   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.



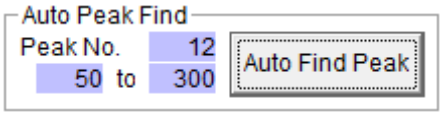
2. Select Mouse Find. Click and drag the mouse from the upper-left to the lower-right over the tip of a peak. A small blue square appears at the peak position identified.

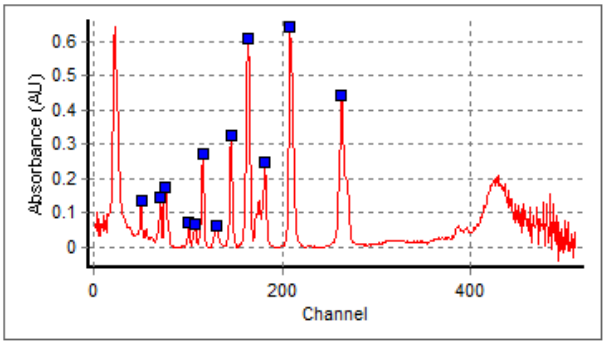


If it is difficult to find the correct peak, unclick Mouse Find, repeat step 1 designating a smaller area of the spectrum and try again.

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.

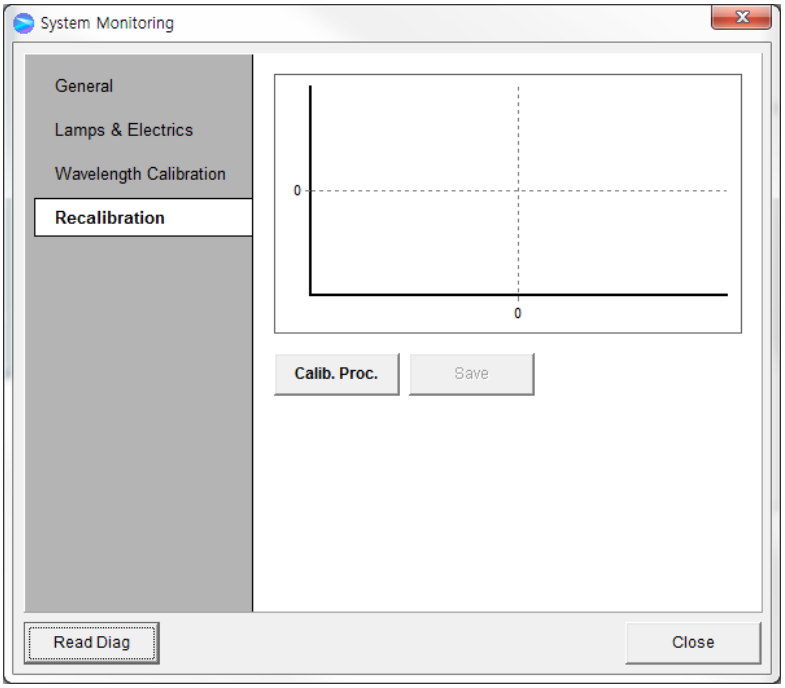




XI-4. Recalibration

▣ Use to recalibrate the wavelength using D_2 peaks.

1. Select Recalibration in the System Monitoring. The below dialog box appears.



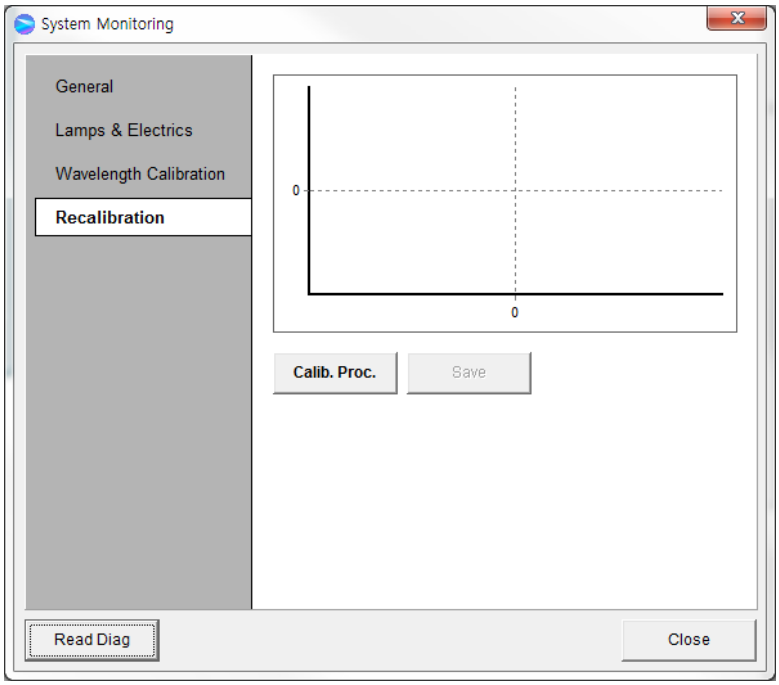
▶ Calib. Proc.: Use to progress recalibration.

▶ Save: Use to save recalibrated result.

XI-4-1. Recalibration Procedure

Use to recalibrate the wavelength using D_2 Peak.

Select Recalibration in the System Monitoring. The below dialog box appears.



1. Measure D_2 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.

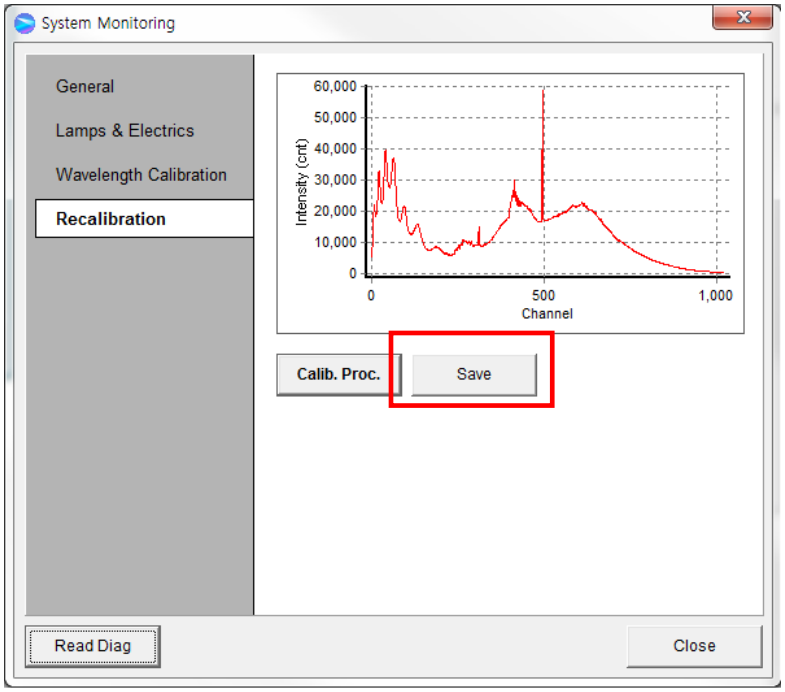
b. If the value of 'Average Dev. Peak' is less than 0.2 nm:
Recalibration is not needed because the difference between the D_2 peak and currently measured wavelength is less than 0.2.

XI-4-2. In case Recalibration needs

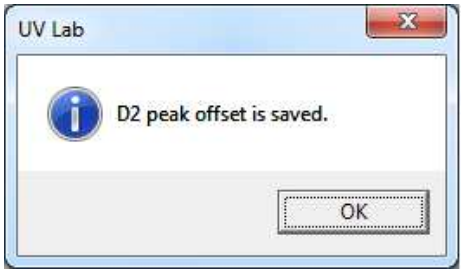
1. To recalibrate, select Yes.

2. After recalibration is completed, select OK.

3. Select Save to save the data.

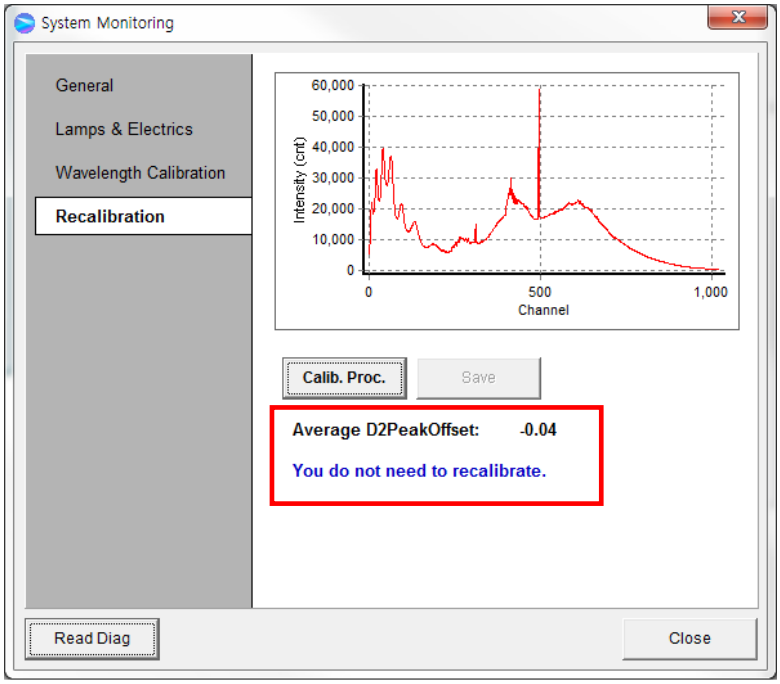


4. The following dialog box appears. Select OK.



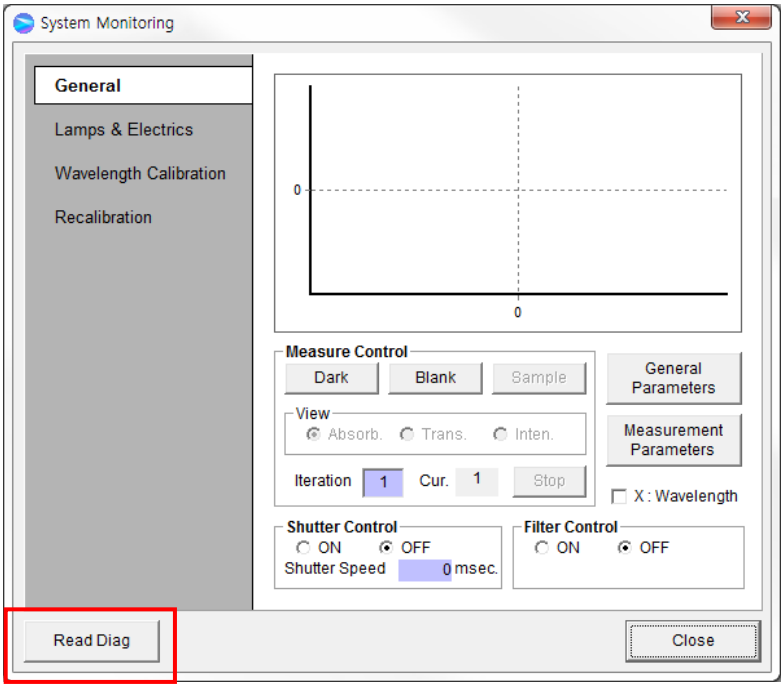
XI-4-3. In case Recalibration is not needed

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



XI-5. Read Diag

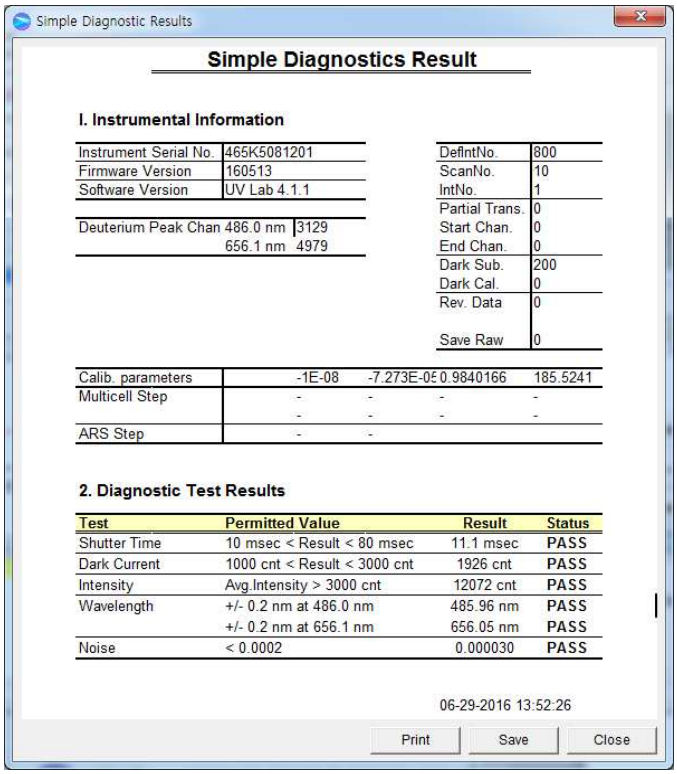
▣ Use Read Diag to open result files for Simple or Advanced Diagnostic.



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

1. Select Read Diag. The Open Diagnostic Result window is displayed.

2. Select the desired file in the Diag folder and select Open. The selected Simple Diagnostics Result window is displayed as shown.
   (Result file measured in the simple diagnostic mode are "*-S.dgs")

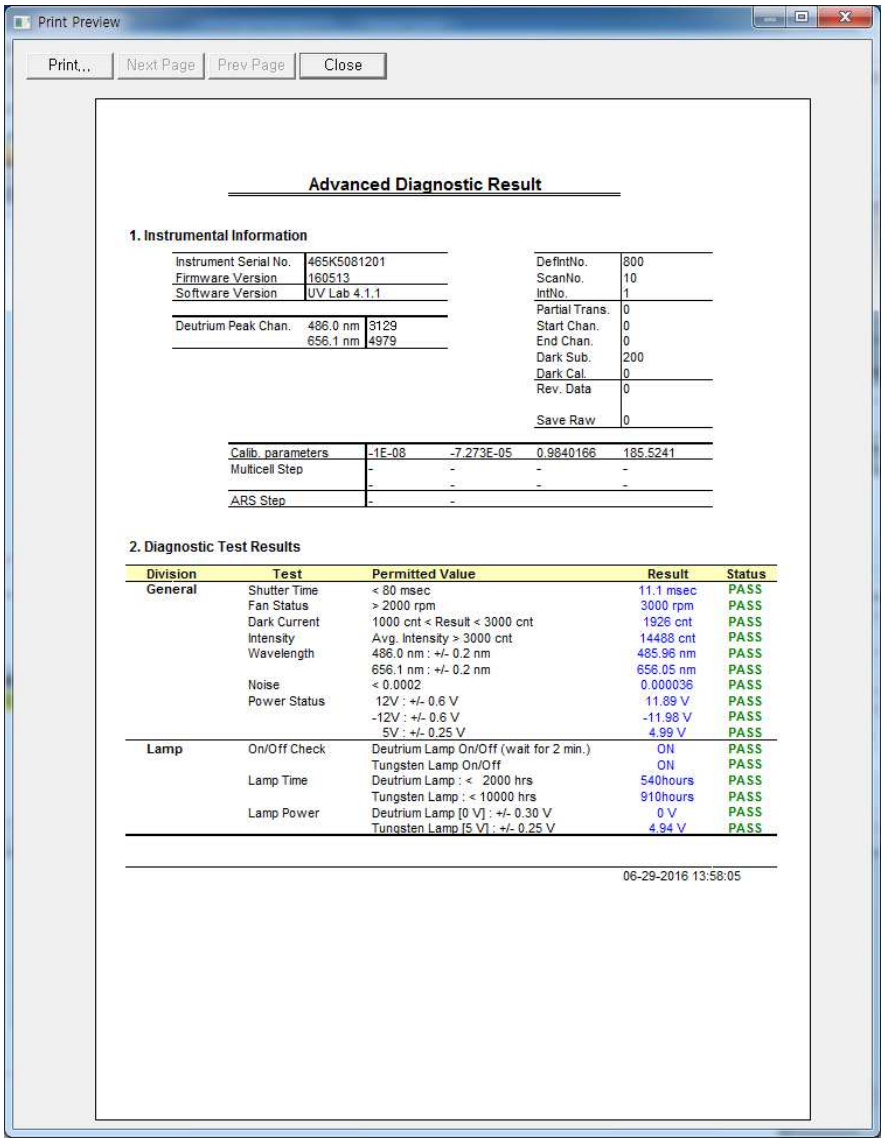


XI-5-2. Open the Result of Advanced Diagnostics

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.

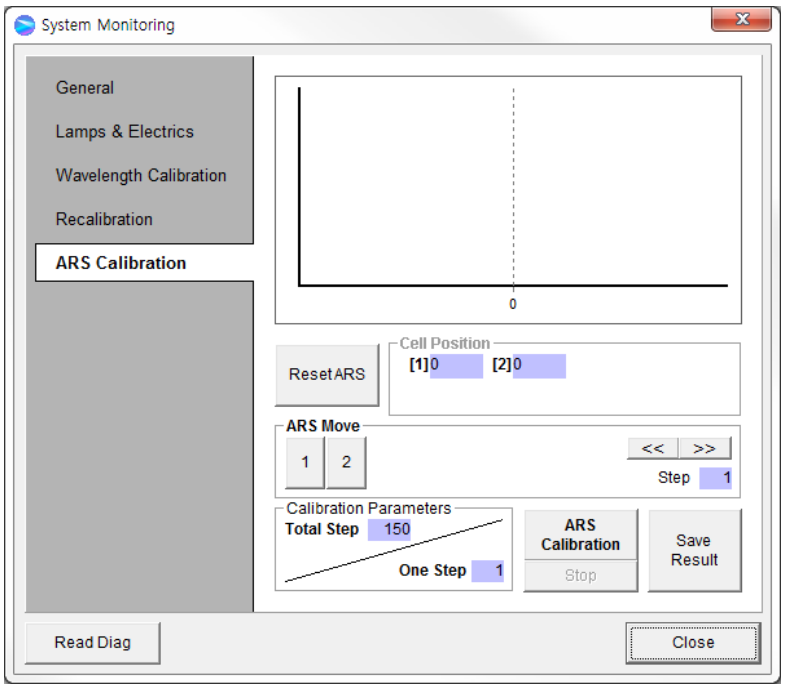
(Result file measured in the advanced diagnostic mode are "*-A.dgs")



XI-6. Automatic Referencing Stage Calibration

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

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.

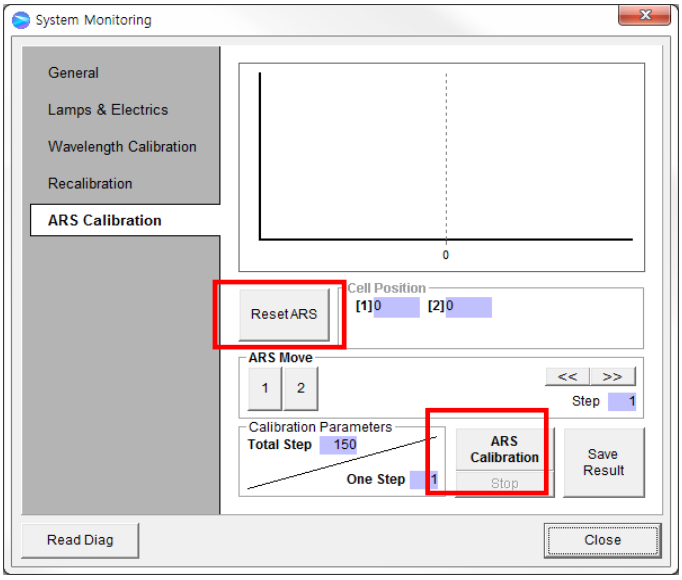
▶ Stop: Use to stop ARS Calibration.

▶ Save Result: Use to save calibrated result.

XI-6-1. ARS Calibration Procedure

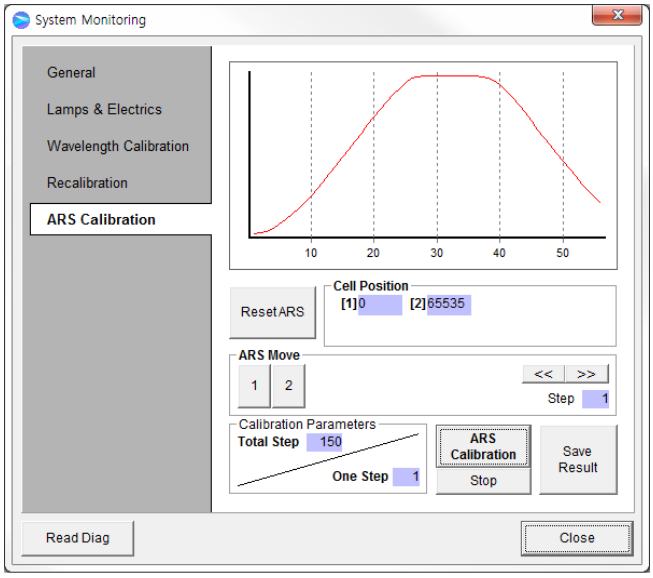
1. Select Reset ARS to format the ARS.

2. Select ARS Calibration as shown below. Default Value: 150 (Total Step), 1 (One Step)



3. Remove all samples from ARS and select OK.

4. Then ARS calibration will start. The current process of calibration is shown.



5. When calibration is finished, select OK.

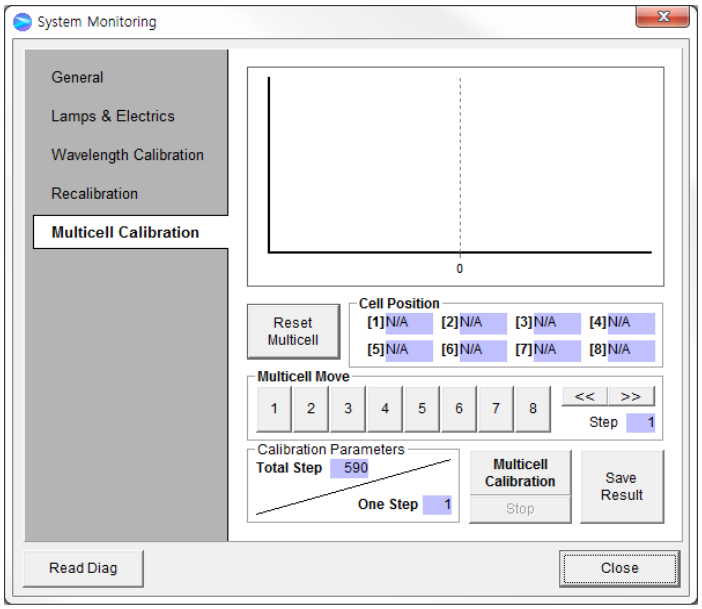
6. Select Save Result

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.



▶ 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 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.

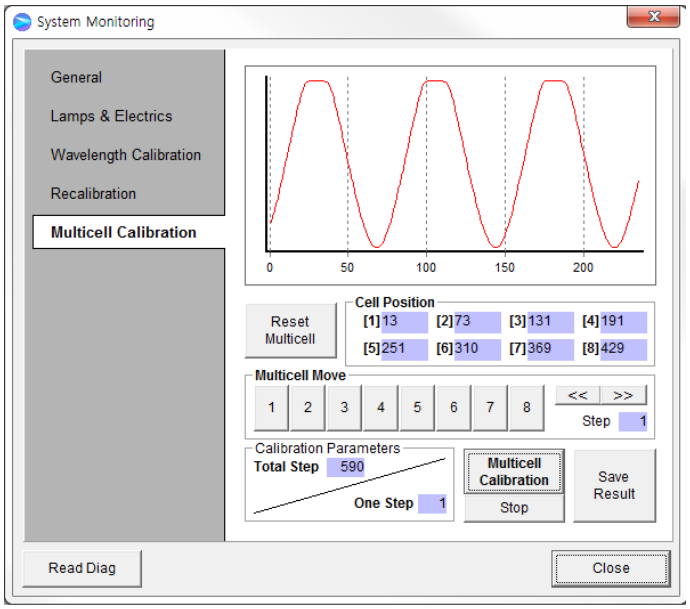
XI-7-1. Multicell Calibration Procedure

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.



5. When calibration is finished, click OK.

6. Select Save Result

to save data.