SEC2000 Spectra System

UV/Visible Spectrophotometer

Operating Manual

Operating Manual

Notice

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December 2010

Miniature Spectrophotometer

Operating Manual

- SEC2000 Detector
- SEC2000 light Source
- Visual Spectra 2.1
- Cuvette Holder
- Accessories

Thank you for purchasing *SEC2000* miniature spectrophotometer. The Palm sized compact design and diverse applications using optical sensing system will make *SEC2000* revolutionize analytical instrument markets. *SEC2000* interfaces PC via USB and the *Visual Spectra 2.1*, the software, is compatible with Windows 2000, Windows XP, 7 and 8. With the versatile spectrum display function and Time Serious Acquisition, the intensity change monitoring of the certain wavelength according to the time, *SEC2000* is suitable for not only optical and chemical analyses but also environmental and process monitoring. Also it can be applied to display and plasma research, color related industry, medical diagnosis, etc.

This manual contains general configuration, functions, and operating instructions of *SEC2000*. If you have any technical problems or question, please feel free to contact us.

We always make our effort to be the world's best company in the analysis system market with continuous commitment to make the world's best product.

Customer Service

BAS Inc.

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Caution

ALS provides one year guarantee for SEC2000 from the purchasing.
However, if the problem is caused as not paying attention to below, the cost can be charged to a user.

⚠ Precaution for Installation/Maintenance

- Do not expose the instrument to direct the sunlight.
- Avoid installing the instrument in wet locations and area with heat sources.
- Do not place the instrument in dusty place.
- Do not install the instrument close to magnets.
- Select a safe location for the instrument.
- Make sure the space of the instrument on a work desk and away from the areas where object could fall and damage the instrument.
- The optimal operating conditions are 5 ~ 35 °C and 30 ~ 70% RH.

⚠ Safety

To prolong the life of the system, please follow these precautions:

- Pack the system when it is not in use for extended periods of time.
- Do not clean the system with harsh solvents (benzene, thinner, alcohol, etc.).
- Do not spray the water to clean the instrument.
- Make sure the cables are not twisted or bent.
- Do not pull the cables with fierce force.
- Do not dismantle and modify the system.

Contents

1. Introduction

1-1. Standard Composition

Typical Miniature Spectrophotometer consists of four basic elements: SEC2000 (detector), Visual Spectra 2.1 (program software), SEC2000-TH (light source for Visible-Near infrared) or SEC2000-DH (light source for UV-Visible), and SEC2000-CUV (cuvette holder).

<Fig. 1-1-2> SEC2000 *UV-Visible Spectrometer*

<Table 1-1-1>

Item	Description	Item Code
Detector	SEC2000 Spectrometer	SEC2000-UV/VIS SEC2000-VIS/NIR
Software	Visual Spectra 2.1	Visual Spectra 2.1
Light Source	Tungsten-Halogen Lamp (for Visible)	SEC2000-TH
	Deuterium & Tungsten Halogen Lamp (for UV-Visible)	SEC2000-DH
Cuvette holder	3-Way Holder	SEC2000-CUV

1-2. Operating Principle

The light or excitation source sends light to the Cuvette holder containing sample. The light interacts with the sample. The light is collected by the spectrometer and the spectral information is sent to SEC2000. The energy is transmitted through Cuvette holder and is dispersed via a fixed grating across a linear charge-coupled device (CCD) array detector. A/D converter transforms the analog data collected from the spectrometer into digital information. This is passed to the software, providing the user with application-specific information.

1-3. System Overview

1-3-1. Features

- **Detector**

  CCD array detector provides the ability to acquire a full spectrum. In the same time, it takes a scanning unit to sample a single wavelength. The result is a flexible, low-cost spectrometer with no moving parts and a compact design with a familiar optical design - diffraction grating (symmetrical optical bench) – and reconfigures it for an optical bench small enough to fit into the palm of your hand.

- **Light Source**

  We offer a complete line of light sources for visible applications. This modular approach – components are easily mixed and matched – offers remarkably flexible applications.

1-3-2. Use

SEC2000 provides quantitative and qualitative analytical techniques for the absorbance and transmittance of visible light especially useful for chemiluminescence, electroluminescence and photoluminescence that are measured without light source.

1-3-3. Specifications

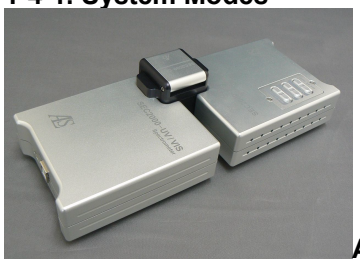
A miniature spectrometer, SEC2000 couples a reasonable cost and high-performance with 2,048-element linear CCD-array detector. Thanks to a modular approach, the components are easily mixed and matched, which offers remarkably flexible applications.

<Table 1-3-1>

Wavelength range	400 nm ~ 1000 nm (VIS/NIR) 200 nm ~ 900 nm (UV/VIS)
Resolution	~2.3 nm (50 μm slit, default) ~1.2 nm (25 μm slit, option)
Sensor	Silicon Charge Coupled Device (Si-CCD)-2,048 pixels
Grating	600 lines/mm
Physical dimension	35 x 98 x 250 mm
Integration time	12~13 scans/second

1-4. Application

1-4-1. System Modes



Absorbance/Transmittance Mode

Reflectance/Fluorescence Mode

Irradiance Mode

<Fig. 1-4-1>

1-4-2. Application Fields

<Table 1-4-1>

Type	Application Fields
Abs./Trans.	◆ Concentration of chemicals (solution) ◆ Polymer extrusion processes ◆ DNA quantification
Reflectance	◆ Freshness testing ◆ Film thickness/composition (Quality control) ◆ Activation energy of photo catalytic species ◆ Textile quality control
Emission (Irradiance)	◆ Astronomy (e.g., spectra of hale-Bopp, plasma monitoring) ◆ Metal *in situ* Monitoring ◆ Luminescence (PL, EL), LED & Laser wavelength
Scattering	◆ Oil concentrations of Oil/water System ◆ Raman Spectroscopy ◆ Physical transition phenomena (e.g., melting point, glass-transition crystallize temperature)
Fluorescence	◆ Marine organisms ◆ Biology (DNA, Protein, Cell proliferation assay, Histamine - analysis, Algae monitoring) ◆ Environmental fields (Waste water analysis, Ground water trace studies, Hydrocarbon detection, Dissolved oxygen) ◆ Plant efficiency (Plant physiology, Plant breeding, Horticulture, Agronomy, Agrochemicals, Pollution studies, Forestry, Ecology) ◆ Tissue diagnosis
Reflection Index	◆ Concentration in solution

2. SEC2000

2-1. Composition

- Product: SEC2000 spectra system
- Power source: 12 V adaptor/ input free volt
- Interface cable: USB cable
- Software: Visual Spectra 2.1

2-2. Specifications

<Table 2-2-1>

Platform	◆ Portable size (35 x 98 x 250 mm)
Optical design	◆ Diffraction grating (symmetrical optical bench) ◆ Compact design
Grating	◆ Standard 600 lines/mm ◆ Blazed at 500 nm (VIS/NIR), 400 nm (UV/VIS)
Detector	◆ 2,048 element linear silicon CCD array ◆ 14 μm x 200 μm per element ◆ Sensitivity: 86 photons/count ◆ Well depth: 16,000 photons ◆ Supply voltage: 5V DC (supply with USB port) ◆ Integration time: 1 msec ~ 10 sec ◆ Maximum clock frequency: 2 MHz ◆ Current consumption: 10 mA
Slit	◆ Standard 50 μm (in width)x1 mm
Resolution	◆ 0.8 ~ 8 nm FWHM (standard 2.3 nm)
Fiber optic connector	◆ SMA 905

3. Installation

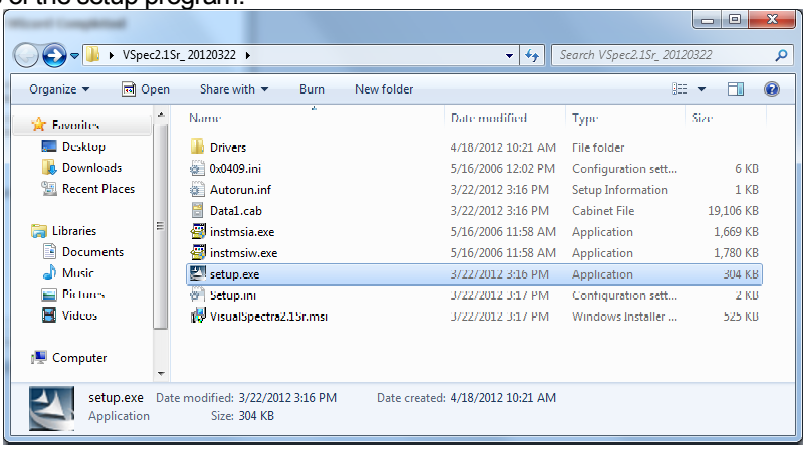
3-1. System Requirement

The minimum recommended PC configuration is:

1. CPU ：Intel Pentium 4 1.5Ghz / AMD XP 1500 ; Memory: 2GB（winXP）/3GB（win7 32bit）/8GB（win7 64bit）
2. Operating system (OS): windows XP(service pack 3)、windows 7 (service pack 1)

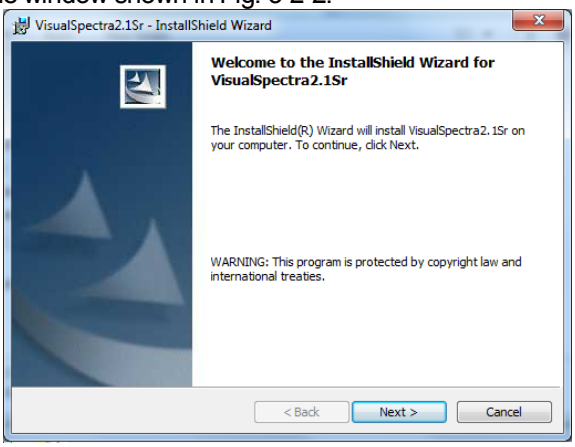
3-2. Software & Diver Installation

Open the **VSpec2.1Sr** software folder and click **setup.exe** as shown in Fig.3-2-1. Follow the instructions of the setup program.



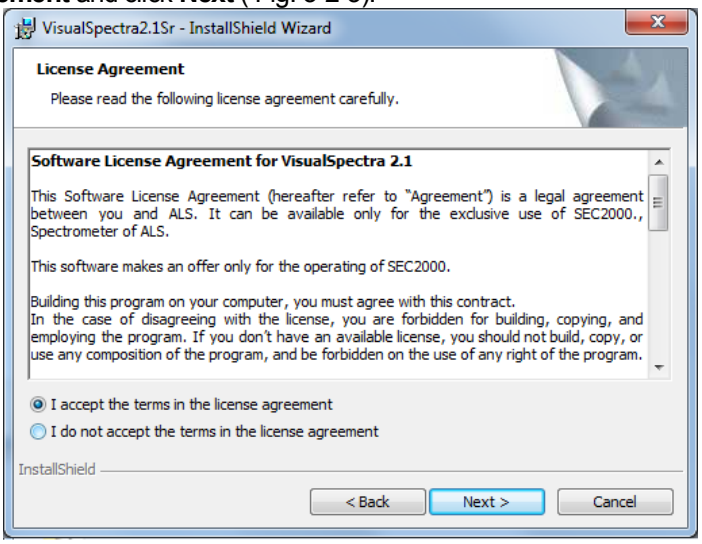
<Fig. 3-2-1>

First click **Next** in the window shown in Fig. 3-2-2.



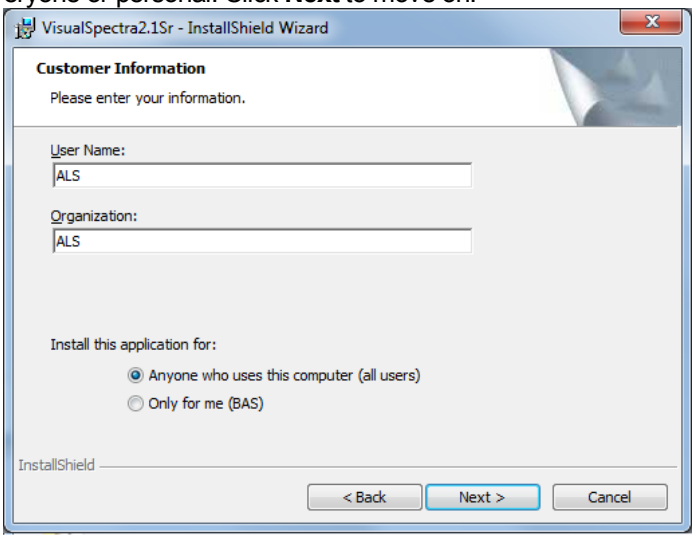
<Fig. 3-2-2>

When you have finished reading the **License Agreement**, select **I accept the terms in the license agreement** and click **Next** (Fig. 3-2-3).



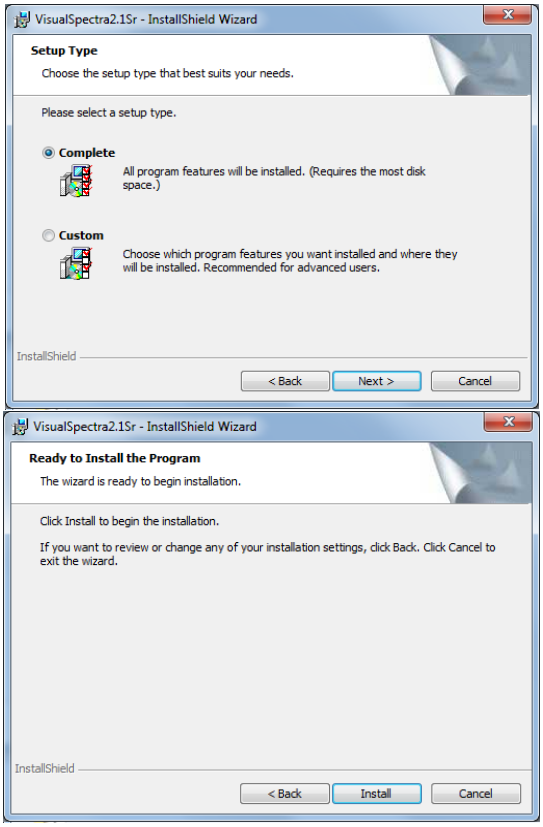
<Fig. 3-2-3>

Fill in the **User Name** and **Organization**, you can use the option to make this program available to everyone or personal. Click **Next** to move on.



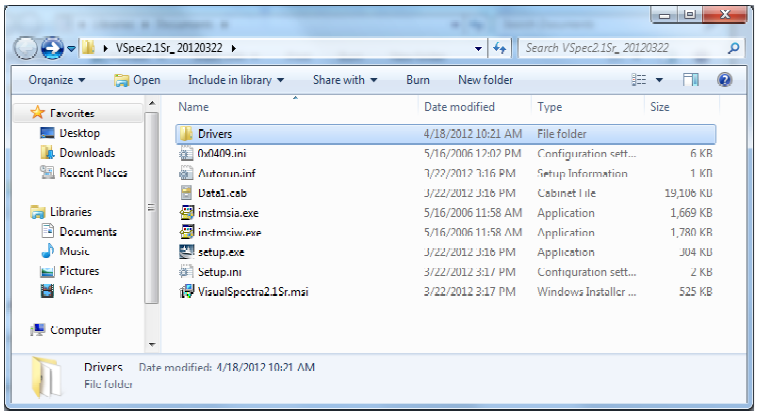
<Fig. 3-2-4>

Please select the **Complete** setup type**,** and click **Next.** If you are satisfied with your installation, select **Install**. The icon of *Visual Spectra 2.1* is created on your computer screen.



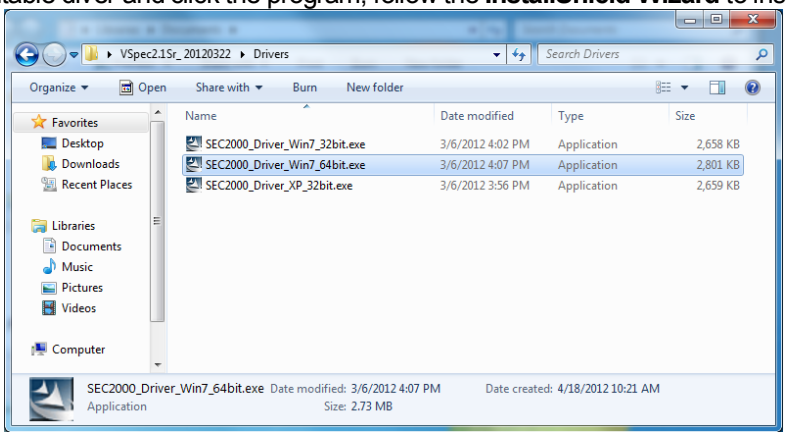
<Fig. 3-2-5>

Next, you should install the diver of the hardware. Open the **Divers** folder from the **VSpec2.1Sr** software source folder.

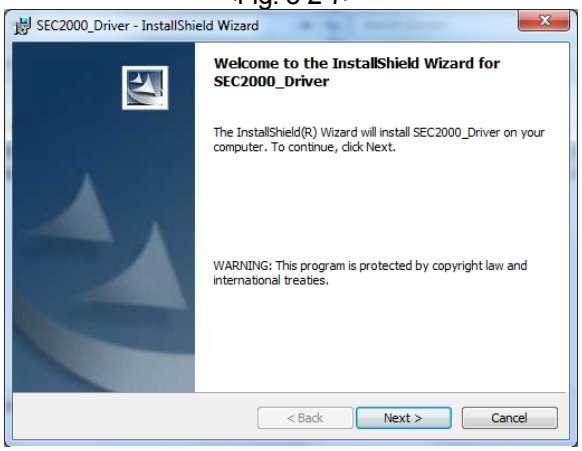


<Fig. 3-2-6>

Select the suitable diver and click the program, follow the **InstallShield Wizard** to install the diver.

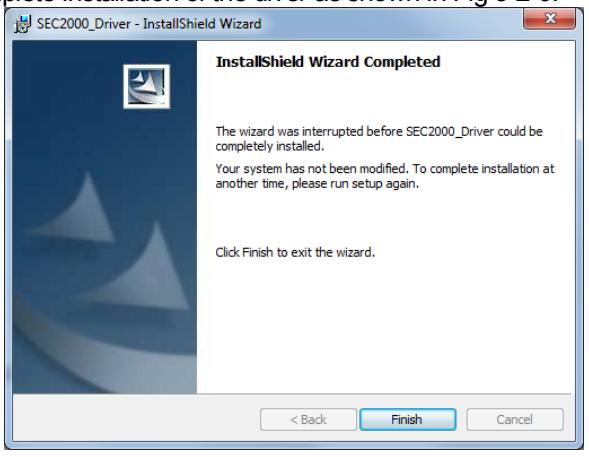


<Fig. 3-2-7>



<Fig. 3-2-8>

Clicking **Finish** to complete installation of the diver as shown in Fig 3-2-9.



<Fig. 3-2-9>

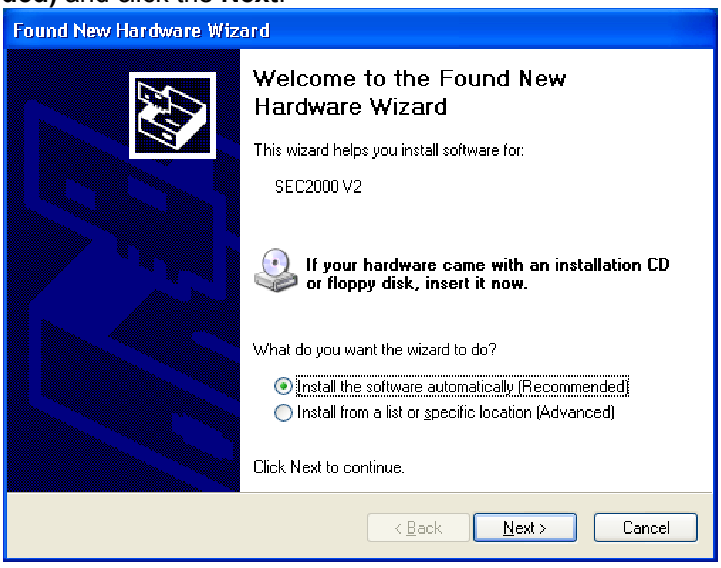
3-3. Connect SEC2000 to the PC

Using the enclosed USB cable, connect SEC2000 to the PC as shown in Fig.3-3-1.

<Fig. 3-3-1>

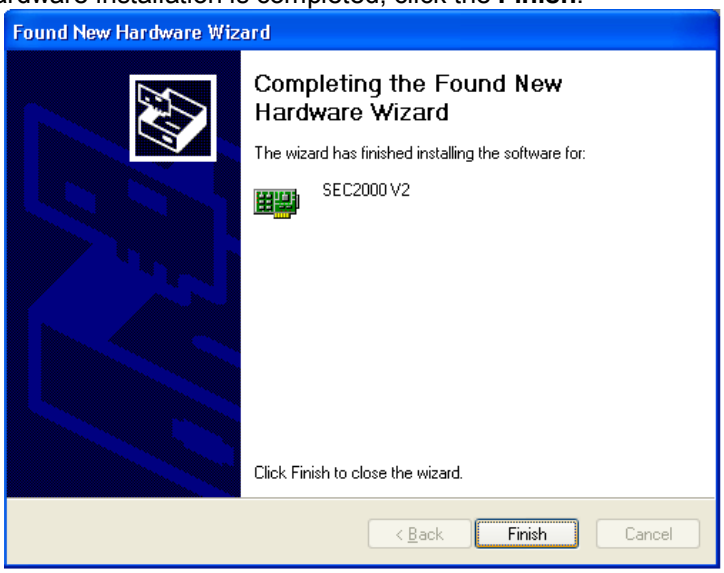
3-3-1. Windows XP

The computer automatically recognizes the USB interface. In the **Found New Hardware Wizard** screen, check **Install the software automatically (Recommended)** and click the **Next**.



<Fig. 3-3-2>

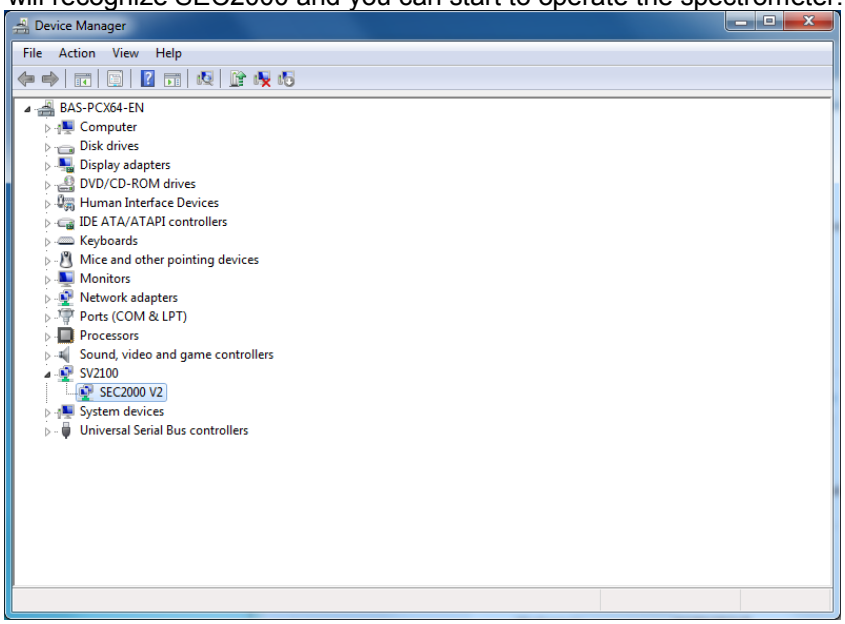
When the Hardware installation is completed, click the **Finish**.



<Fig. 3-3-3>

3-3-2. Windows 7

The PC will recognize SEC2000 and you can start to operate the spectrometer.



<Fig. 3-3-4>

4. Operation

4-1. Connection

SEC2000 consists of a light source, cuvette holder, detector and other accessories. The pre-set mode is for measuring absorbance or transmittance. It looks like a straight bar in the order of light source, cuvette holder, and detector, as shown in Fig. (a). To measure a reflectance spectrum, reconfigure it, as shown in Fig. (b). To measure an irradiance spectrum, remove light source, as shown in Fig. (c) of Fig. 4-1-1.

Note: When changing the combination of the device, the position of the light source and detector are also changed slightly. The spectral shape of the light source in Scope mode may be changed, due to changes of the intensity of the UV lamp and the VIS lamp. This will not affect the measurement.

(a) (b) (c)

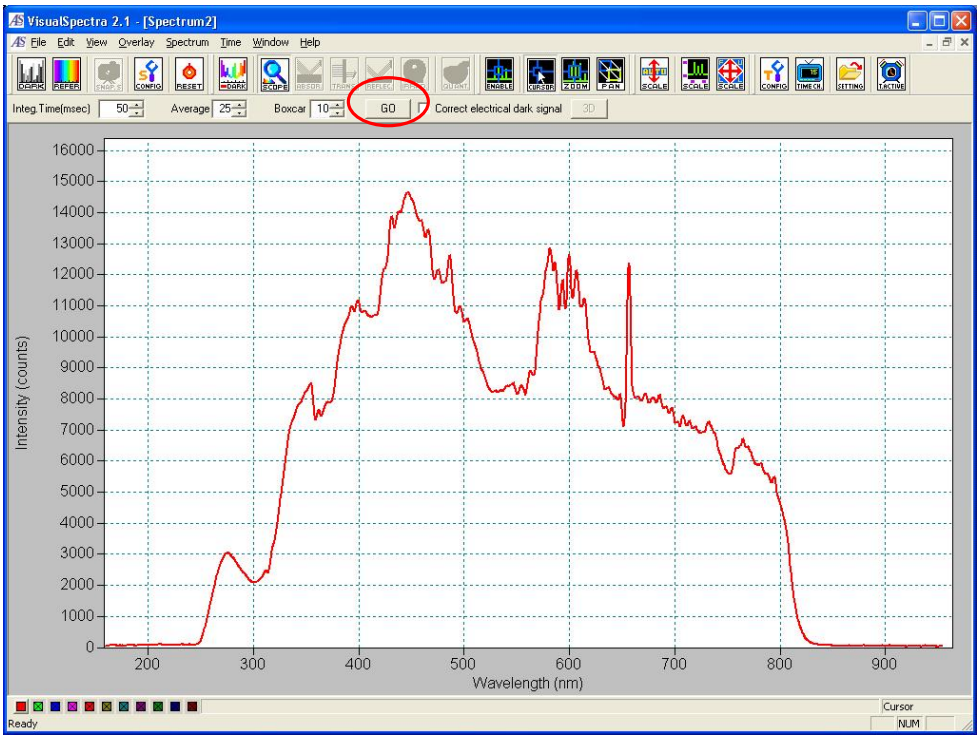
<Fig. 4-1-1>

Connect the *Spectra System* detector to a USB interface of the computer using the enclosed USB cable and plug in the enclosed electrical cord to Light Source. Then turn on the light.

<Fig. 4-1-2>

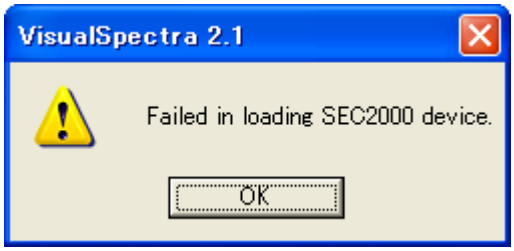
4-2. Program Start

Start *the Visual Spectra 2.1* program and click the **GO** icon. The trace line will appear, when the *Spectrometer* (*SEC2000*) has been connected properly with the computer.



<Fig. 4-2-1>

If the *Spectra System* driver is not installed properly or the USB connection fails between *Spectra System* and your computer, **Failed in loading SEC2000 device** message will appears. In this case, check the USB connection or reinstall the USB driver.



<Fig. 4-2-2>

4-3. Software Operation

4-3-1. Menu Descriptions

From the Main Menu bar, the following selections will produce the following drop-down options.

① **File**

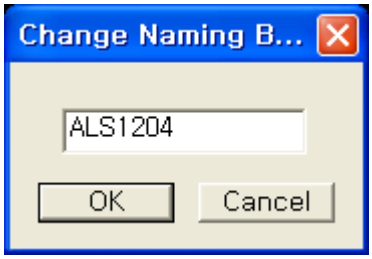
- **Open**

Dark (Ctrl+D)	A Dark spectrum (***. dark**) is read and displayed on Overlay1.
Sample Dark	A sample dark spectrum (***.spldark**) is read and displayed on Overlay1.
Reference (Ctrl+R)	A reference spectrum (***.refer**) is read and displayed on Overlay 2.
Sample (Ctrl+O)	A sample spectrum (***.sample**) is read and displayed on Master Trace.
Processed	Read the processed (absorbance/ transmittance/ irradiance) spectrum to Master Trace. It is activated when the dark and reference spectra are opened.
Settings	Set for a measuring spectrum and read the graphical view setting file (***.ini**).

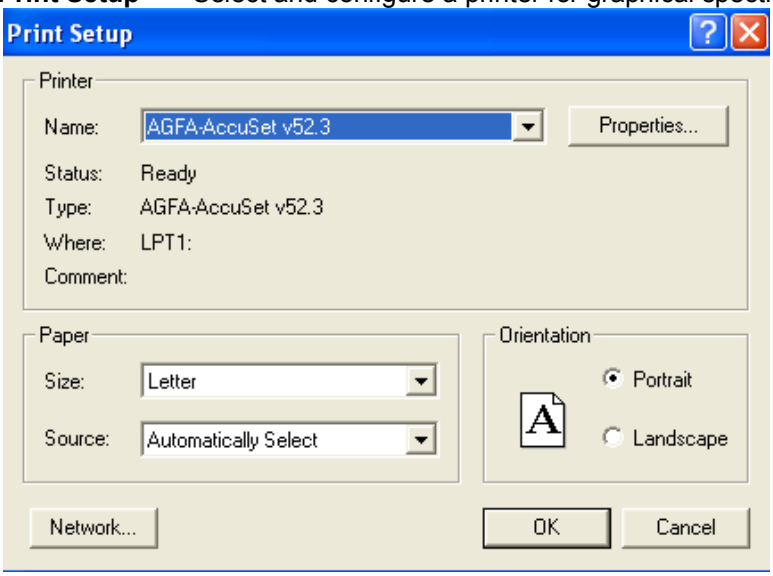
- **Save**

Dark	Save the current dark spectrum as a file (***.dark**).
Sample Dark	Save the current sample dark spectrum as a file (***.spldark**)
Reference	Save the current reference spectrum as a file (***. refer**).
Sample	Save the Master Trace's data as a file (***.sample**).
Processed	Save the Master Trace's data to file (processed spectrum, saved as ***.absorb/ *.trans/ *.irrad**.). It is activated when the dark and reference spectra are saved.
Establish environment	Establish environment for a measuring spectrum and save the graphical view setting file (***.ini**).

- **Auto Increment**

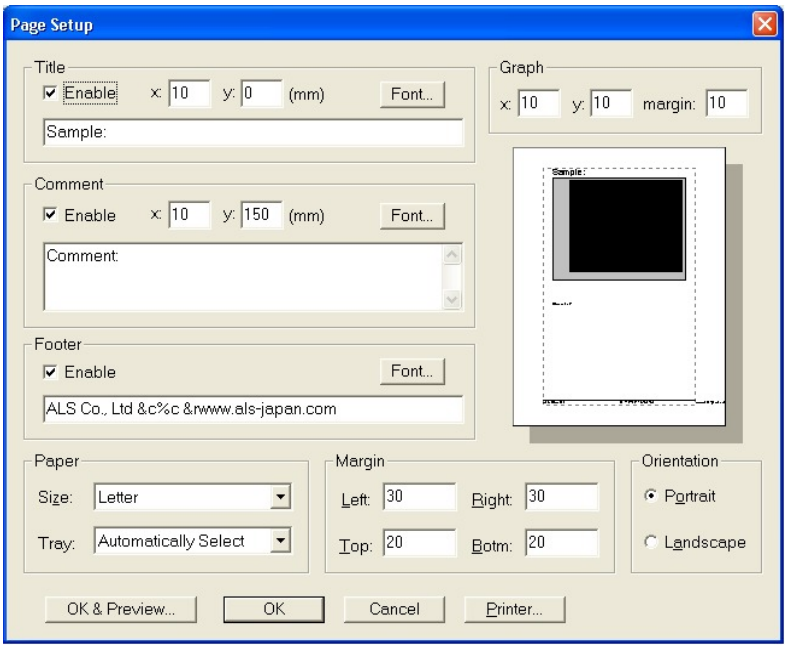
Enable	Generates a filename automatically for saved spectra.
Base Name	Set the base name for auto incremented files.  Ex. of file name: ALS1204,date→20110112, etc.
Starting Index	Set the starting index for auto incremented files.  Ex. of file name: ALS1204-1, ALS1204-2, etc.

- **Print Setup** Select and configure a printer for graphical spectrum printing



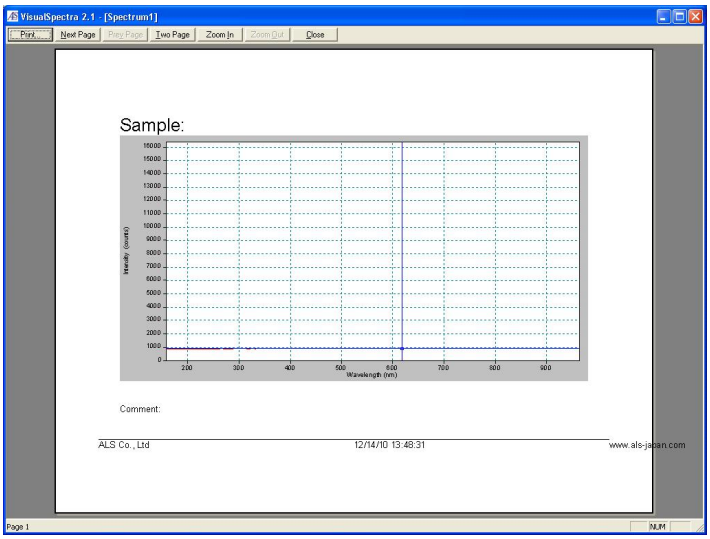
<Fig. 4-3-1>

- **Page Setup** Write Title, Comment and Footer for printing graphical spectrum.



<Fig. 4-3-2>

- **Print Preview** Preview the graphical spectra printing.

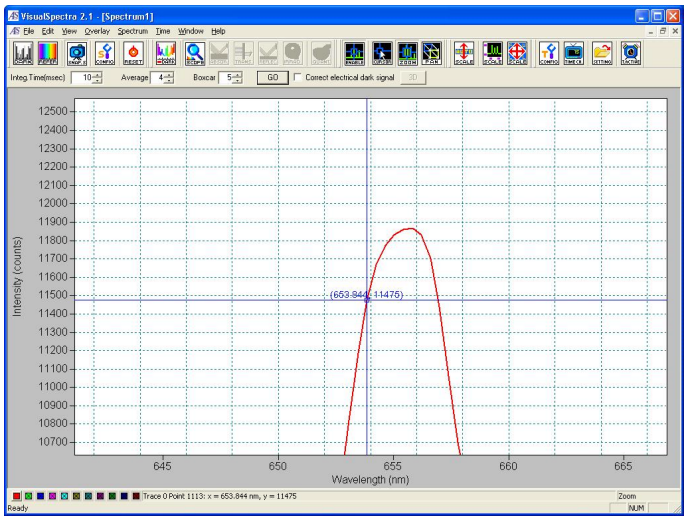


<Fig. 4-3-3>

- **Print (Ctrl+P)** Print current graphical spectra.
- **Exit** End program.

② **Edit**

- **Copy Graph (Ctrl+C)** Copy current graphical spectrum to a temporary site.
- **Copy Data** Copy current data to a temporary site.
- **Add Marker (Ctrl+A)** Make a marker for current graphical spectrum.

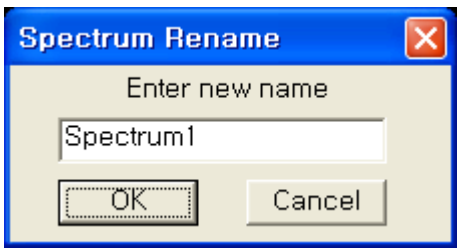


<Fig. 4-3-4>

- **Erase Marker (Ctrl+E)** Erase a maker that is located near a current cursor.
- **Erase All Markers** Erase all markers.

③ **View**

- **Rename Window** Rename a graphical spectrum window.

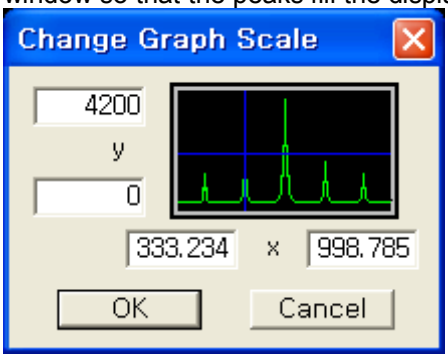


<Fig. 4-3-5>

- **Mouse Tracking**

Cursor	Change mouse status to cursor.
Zoom	Change mouse status to Expand/Reduce.
Pan	Change mouse status to drawing.

- **Spectrum Scale**

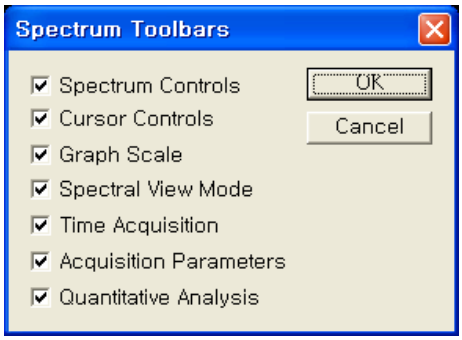
Auto Scale	Adjusts the scale of the spectrum in the current spectral window so that the peaks fill the display vertically. 
Change Scale	Adjusts the scale of the spectrum optionally.
Full Scale	Adjusts the scale for all data to be displayed.

- **Cursor Display** Enable or disable the display of cursor for the spectral window.
- **Grid Display** Enable or disable the display of grid for the spectral window.
- **Marker Display** Enable or disable the display of maker for the spectral window.
- **Color**

Trace	Master: Choose a master color for the spectral windows. Overlay1~10: Choose each overlay for the spectral windows.
Background	Choose a background color for the spectral window.
Frame	Choose a frame color for the spectral window.
Cursor	Choose a cursor color for the spectral window.
Grid	Choose a grid color for the spectral window.

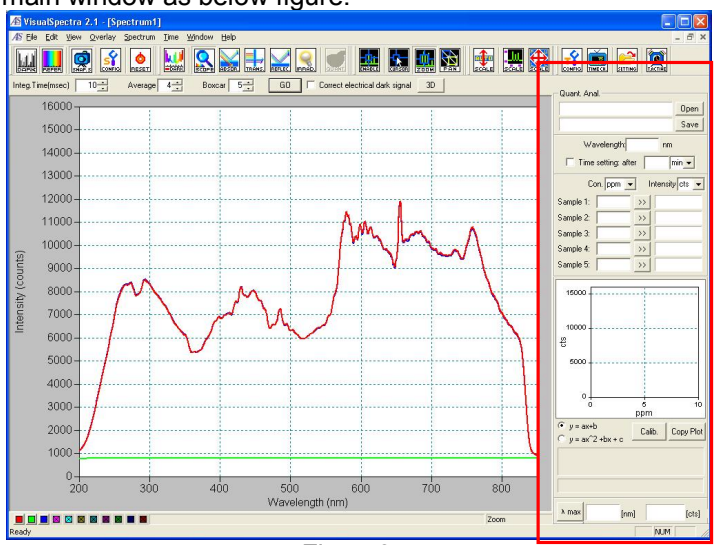
Text	Choose a text color for the spectral window.
Tick	Choose a tick color for the spectral window.
Marker	Choose a marker color for the spectral window.
Default	Return all colors to the original.

- **Main Status bar** Toggle the status bar display in the main visual spectral window.
- **Spectrum Tool bar** Toggle the tool bar display in the main visual spectral window.



<Fig. 4-3-6>

If the"**Quantitative Analysis**" is checked, the window will be displayed on the right side of the main window as below figure.



<Fig. 4-3-7>

④ **Overlay**

- **Spectrum**
- **Time Series**

- **Add Overlay 1 ~ 10** Display the data for each spectral window (1 ~ 10).
- **Clear** Clear selected overlay data from the spectral window.
- **Add Overlays (Multiple Selection)** Display several data at the same time.
- **Clear All** Clear all overlay data from the spectral window.

⑤ **Spectrum**

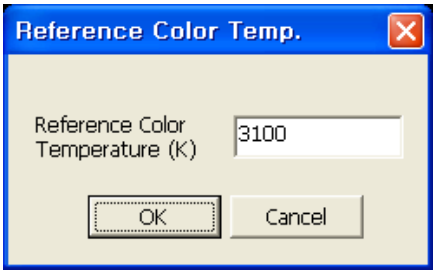
- **Config. Spectrometer** Confirm the parameters of spectrometers for wavelength calibration.

<Fig. 4-3-8>

- **Reset Spectrometer** This function cannot be used for user.
- **Calib. Spectrometer** This function cannot be used for user.
- **Store Dark** Save the spectrum measurement result as dark spectrum to memory and display on overlay 1.
- **Store Reference** Save the spectrum measurement result as reference spectrum to memory and display on overlay 2.
- **Snapshot** Halt data acquisition and take a snapshot of activity in the spectral window.
- **Scope Mode** Present the data in scope mode.
- **Scope Mode Minus Dark** Switch the current spectral window into scope mode and subtract the stored dark spectrum.
- **Absorbance Mode** Display data in absorbance mode. Please save the Dark and Reference spectra before using this function.
- **Transmittance Mode** Display data in transmittance mode. Please save the

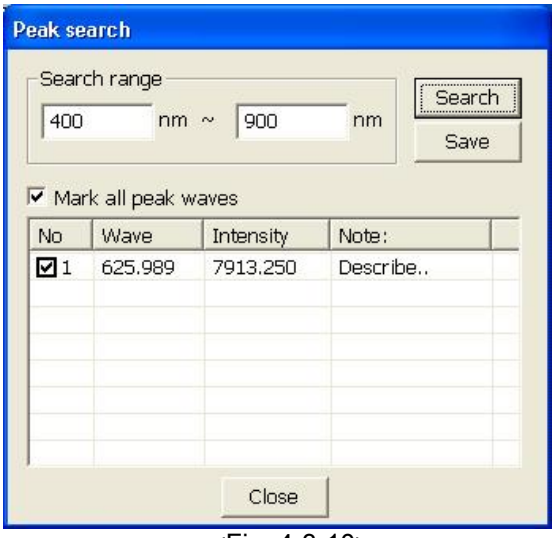
Dark and Reference spectra before using this function.

- **Reflectance Mode** Display data in reflectance mode. Please save the Dark and Reference spectra before using this function.
- **Relative Irradiance Mode** Display data in relative irradiance mode. Please save the Dark and Reference spectra before using this function.
- **Reference Color Temp.** Input the color temperature (K) of your blackbody reference lamp used for relative irradiance measurements.



<Fig. 4-3-9>

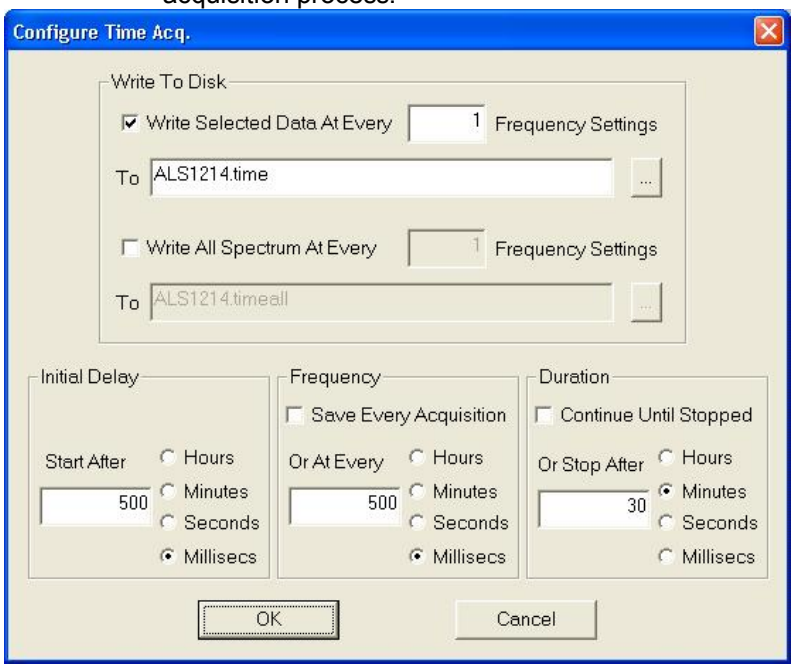
- **Quantitative Analysis** It is effective only on absorbance mode.
- **Peak Search** Search peaks in the specified range. Click **Save**, the peak data will be saved as ***.csv** file.



<Fig. 4-3-10>

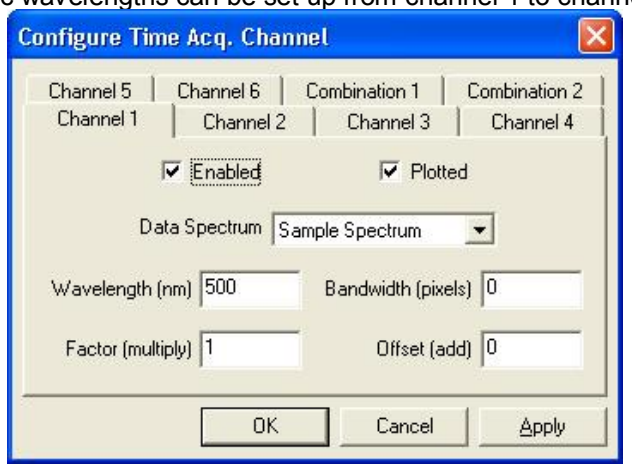
⑥ **Time** Time course measurements of the specific wavelength or full spectrum change is performed.

- **Configure Time Acquisition** Configure and establish the parameters for time acquisition process.



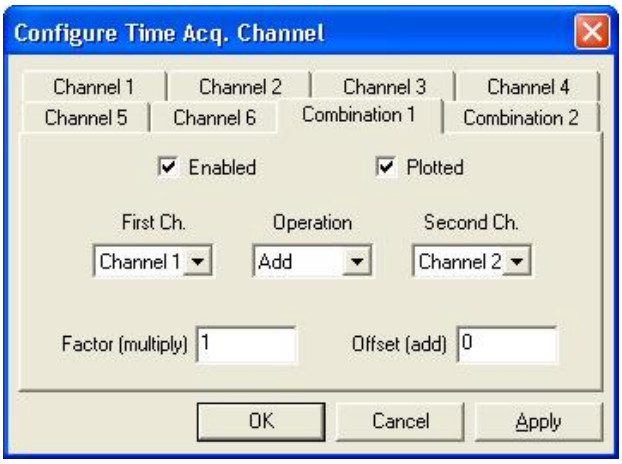
<Fig. 4-3-11>

- **Configure Time Acq. Channel** Set up the channels for time acquisition process. Six specific wavelengths can be set up from channel 1 to channel 6.



<Fig. 4-3-12>

Combination 1 and 2 can perform operation among channel 1 ~ channel 6.



<Fig. 4-3-13>

- **Restore Parameters**

  Select and open a file with the complete set of time acquisition parameters, including the configuration settings for **Time Acq. Channel**.

- **Save Parameters**

  Save a complete set of time acquisition parameters, including the configuration settings for **Time Acq. Channel**.

- **Activate** Activate the time acquisition mode.

⑦ **Window**

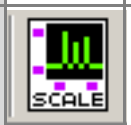
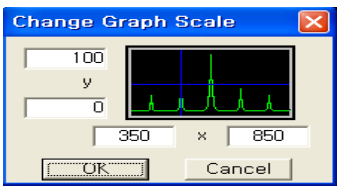
- **Cascade** Arrange all spectral windows in a horizontally overlapping design.
- **Tile** Arrange all spectral windows side by side.

⑧ **Help**

- **Help Topics** Present the user manual for the system.
- **About *Visual Spectra 2.1*** Present the version information of Visual Spectra 2.1.

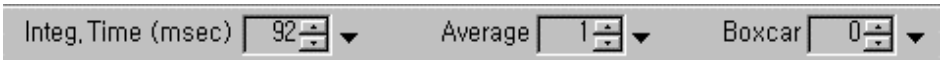
4-3-2. Functions

① Icons

Minus Dark		Select this icon if the baseline of the resulting graph is unstable based on the present Y coordinate, or to adjust the Y coordinate based on the dark level. If this icon is clicked, the baseline will be forced to be Y (Intensity) = 0 which will be reflected in the graph. Though this function is used for adjusting an unstable distribution of a spectrum, you must pay attention to the fact that it is based on the original data when saved as a file.
Auto Scale		For a detailed view, select this button to enlarge the Y-coordinate into full screen. The X-coordinates may be enlarged beyond the edge of the screen in this mode.
Full Scale		This icon displays the X-axis and Y-axis in a full screen.
Scale		For a detailed view, this mode allows the user to examine the graph with the X-axis and Y-axis scaled to the user's preference. Simply input the upper range of each axis accordingly. 
Enable		This function provides the coordinate of a particular peak or point. After choosing **"ENABLE"** icon, select the target point. Then the result will be recorded on the point that the mouse indicates.
Cursor		With this function selected, clicking on a point will provide the coordinate information (Wavelength, Intensity) shown on the status bar at the bottom of the screen. In accordance with the movement of the mouse, new coordinate information will be shown.

Zoom		This shows an enlarged image of the desired region. You can observe the graph by zooming in and out of spectrum with the method mentioned in the **"Full scale".** After choosing the **"Zoom"** button, click and drag the cursor over the desired region with the left button of the mouse pressed, and then you can see the detailed graph in the scoped area.
Pan		To see a broader spectrum, choose the **"PAN"** button. Click and drag the graph, then X-axis and Y-axis also change following the graph movement.
Snap Shot		Snap Shot makes real-time values halt. When the **"SNAP.S"** button is clicked, the window will be stopped and all other icons will become inactivated. If you click **"SNAP.S"** button again, the icons become active.

② **Integration Time, Average, & Boxcar**



<Fig. 4-3-14>

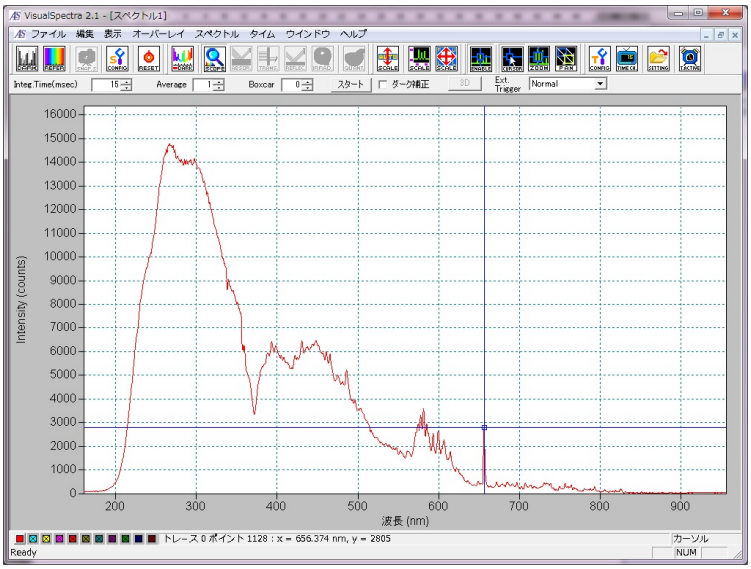
Integration Time	Function of spectral signal amplifying. The larger the number, the larger the signal (increase in detection sensitivity). If the signal intensity exceeds 16,000 counts, the signal will be saturated and not be detected. Therefore, the signal should be adjusted below 16,000 counts. In case that the concentrations between samples have large difference, make the integration time as low as possible.
Average	Function of spectral signal stabilizing. The higher the figure, the more the signal is stabilized. When the figure is too high, it is hard to get real-time data. Decide the least figure so the spectral signal of sample does not move up and down too much. If the real time values of the sample concentration continue to change very frequently, lower this function to meet your experiment.
Boxcar	Function of spectral signal smoothing. The higher the figure, the more the signal smoothes. If the figure is too high, the limit of resolution lowers. Decide the lowest figure so the spectral signal does not vary much. When you want to separate intensities of more than two nearby wavelengths, lower this function to meet your experiment.

5. Measurement

5-1. Calibration

All spectrometers are delivered to the user after an initial calibration but some can drift slightly over a period of time and depending on the environment (If the mirror and diffraction grating are not aligned, please contact us.).

It can be confirmed by the following way whether wavelength calibration is need. Open the software and turn on the UV lamp. Confirm the following peaks in scope mode. As shown in Fig. 5-1-1, sharp peaks at 656 nm labeled D_α, are the feature peaks of the deuterium arc lamp. The wavelength calibration is required when the feature peaks is gap.



<Fig. 5-1-1> Emission spectrum of the ultraviolet deuterium arc lamp showing characteristic hydrogen Balmer lines.

5-2. Dark and Reference Measurements

When Dark and Reference are measured, continue with the experiment applying the same Integration Time value. To block the light towards the detector *SEC2000*, close the shutter of the light source.

① The power supply is switched on and turned ON the UV lamp and halogen lamp. Wait for 20 to 30 minutes until UV lamp is stabilized.

② Put the reference sample into the cuvette holder, and open the light source shutter

fully.

③ Adjust the Integration Time to control the maximum value of the Y axis of the graph (less than 16000).

④ By clicking the "**Reference**" icon in the program, the spectrum is obtained at the maximum light intensity. Save the reference spectrum by "**File**"/"**Save**"/"**Reference**".

⑤ Close the light source shutter, and click the "**Dark**" icon in the program to obtain a spectrum under the minimum conditions without light. Save the dark spectrum by "**File**"/"**Save**"/"**Dark**".

⑥ Scope mode is the basic measurement mode. After measuring the spectrum in Dark and Reference, the **"Absorbance"**, **"Transmittance"**, **"Reflectance"** and **"Irradiance"** measurement mode icons become active.

5-3. Sample Measurement

Apply the same condition with which the Reference was measured (the shutter open & the same Integration Time). Put the sample into the cuvette holder, and close the cover. Save the sample spectrum by "**File**"/ "**Save**"/ "**Sample**".

5-4. Absorbance/Transmittance Measurement

Absorbance/Transmittance spectra are used to measure how much light is absorbed by a sample. For most samples, absorbance is linearly related to concentration of the substance. The software calculates absorbance (*A*) using Beer's law and shows the relationship between absorbance and concentration as below equation:

$$A=\varepsilon bc$$

Where *A* is the absorbance of the solution, ε is the molar absorptivity $(mol^{-1} \cdot cm^{-1})$, *b* is the pathlength of radiation through the absorbing medium (cm) and *c* is the concentration (mol/L) of the solution.

The absorbance is measured with the following procedure.

<Fig 5-4-1>

① First, the light source and detector of SEC2000 are set as shown in Fig. 5-4-1.

② The power supply is switched on and turned ON the UV lamp and halogen lamp. Wait for 20 to 30 minutes until the lamps are stabilized.

③ Open the software and click "**Go**" icon, the spectrum signal will appear. On Scope Mode, put the reference sample into the cuvette holder, and open the light source shutter fully.

④ Adjust the Integration Time to control the maximum value of the Y axis of the graph (less than 16,000).

⑤ By clicking the "**Reference**" icon in the program, the spectrum is obtained at the maximum light intensity. Save the reference spectrum by "**File**"/"**Save**"/ "**Reference**".

⑥ Close the light source shutter (Do not close the power switch), and click the "**Dark**" icon in the program to obtain a spectrum under the minimum conditions without light. Save the dark spectrum by "**File**"/"**Save**"/"**Dark**".

⑦ Put the sample into the cuvette holder, and open the light source shutter fully. Click the "**Absorbance Mode**"/"**Transmittance Mode**" icon to change the Mode. Store the spectrum by "**File**"/"**Save**"/"**Processed**" or "**Sample**" to save an absorbance or transmittance spectrum.

Notes

When absorbance/transmittance is saved by "**File**"/"**Save**"/"**Processed**", the data of absorbance/transmittance is displayed only absorbance/transmittance mode. However, if absorbance/transmittance is saved "**File**"/"**Save**"/"**Sample**", the saved data is convertible for other mode.

5-5. Reflectance Measurement

Reflection is the change in direction of a wave front at an interface between two dissimilar media so that the wave front returns into the medium from which it originated. The reflection of light may be *Specular* (that is, mirror-like) or *Diffuse* (that is, not retaining the image, only the energy) depending on the nature of the interface. The glossier the surface is, the closer to specular reflection it is.

Reflection is expressed as a percentage (%r) of reflective light quantity from a standard reference substance:

$$\%r = \{ (S-D) / (R-D) \} \times 100\ (\%)$$

Where *r* is the reflectance of the sample, *S* is the reflect light amount which means applied light to the sample surface; *R* is the reflect light amount which applied light to the standard reflective material surface; *D* is the amounts of dark light when light intercepts.

5-5-1. Reflectance Measurement 1

In this measurement, the shape of the sample should be board like and it can be set into the cuvette holder at 45 degrees.

The reflectance is measured with the following procedure.

<Fig. 5-5-1> Configuration example of reflectance measurement.

① First, the light source and detector of SEC2000 are set as shown in Fig. 5-5-1.

② The power supply is switched on and turned ON the UV lamp and halogen lamp.

Wait for 20 to 30 minutes until the lamps are stabilized.

③ Open the software and click "**Go**" icon, the spectrum signal will appeared. On Scope Mode, put the standard reflective material into the cuvette holder, and open the light source shutter fully.

④ Adjust the Integration Time to control the maximum value of the Y axis of the graph (less than 16,000).

⑤ By clicking the "**Reference**" icon in the program, the spectrum is obtained at the maximum light intensity. Save the reference spectrum by "**File**"/"**Save**"/ "**Reference**".

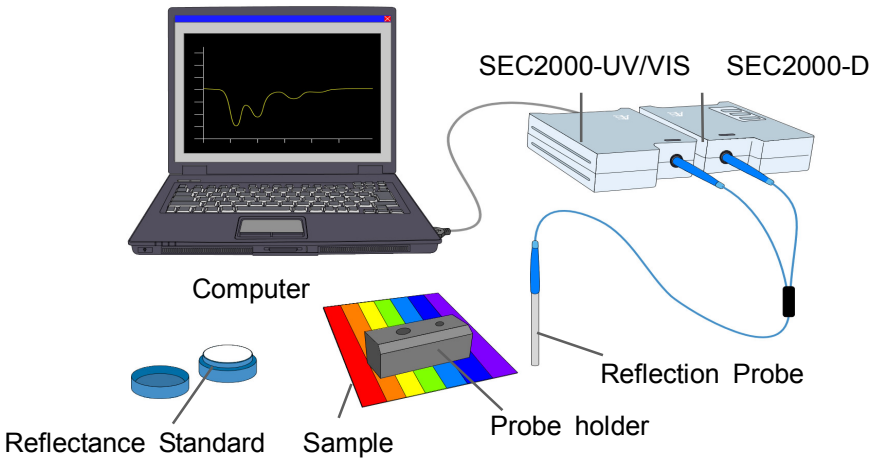
⑥ Close the light source shutter (Do not close the power switch), and click the "**Dark**" icon in the program to obtain a spectrum under the minimum conditions without light. Save the dark spectrum by "**File**"/"**Save**"/"**Dark**".

⑦ Put the sample into the cuvette holder, and open the light source shutter fully. Click the "**Reflectance Mode**" icon to change the Mode. Save the spectrum by "**File**"/"**Save**"/"**Processed**" or "**Sample**".

5-5-2. Reflectance Measurement 2

In this measurement, the Reflection probe is used.

The reflectance is measured with the following procedure.



<Fig. 5-5-2> Configuration example of reflectance measurement using reflection probe.

① First, the light source and detector of SEC2000 are set as shown in Fig. 5-5-2.

② The power supply is switched on and turned ON the UV lamp and halogen lamp. Wait for 20 to 30 minutes until the lamps are stabilized.

③ Open the software and click "**Go**" icon, the spectrum signal will appeared. On Scope Mode, open the light source shutter. Put the reflection probe to the standard reflective material. Move the probe tip to adjust the reflection light intensity to maximum. Fix the probe tip to holder by screw, and then the distance from the tip to measurement surface is fixed.

④ Adjust the Integration Time to control the maximum value of the Y axis of the graph (less than 16,000).

⑤ By clicking the "**Reference**" icon in the program, the spectrum is obtained at the maximum light intensity. Save the reference spectrum by "**File**"/"**Save**"/ "**Reference**".

⑥ Close the light source shutter (Do not close the power switch), and click the "**Dark**" icon in the program to obtain a spectrum under the minimum conditions without light. Save the dark spectrum by "**File**"/"**Save**"/"**Dark**".

⑦ Put the reflection probe to sample surface, and open the light source shutter fully. Click the "**Reflectance Mode**" icon to change the Mode. Save the spectrum by "**File**"/"**Save**"/"**Processed**" or "**Sample**".

5-6. Irradiance Measurement

Have you ever seen the colors of light emitted by the sun, fluorescent lights, white lights and lamps? These colors look white to our eyes. Red, yellow and green traffic signal colors are radiation of light. The material emitting light is called the light source. The emission of light by the light source is called Luminescence.

How is the color of the light source generated? Shall we perform experiments for the Luminescence Spectrum of the light?

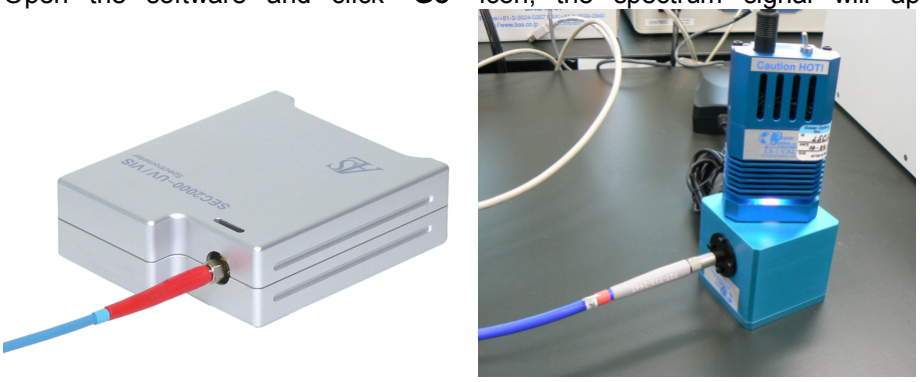
5-6-1. Relative irradiance measurement

① First, connect the detector of SEC2000 and integrating sphere by optical fiber. Set the standard light source to integrating sphere as shown in Fig. 5-6-1.

② Turn on the power of the standard light source. Wait for 20 to 30 minutes until the

lamps are stabilized.

③ Open the software and click "**Go**" icon, the spectrum signal will appear.



<Fig. 5-6-1> Configuration example of relative irradiance measurement.

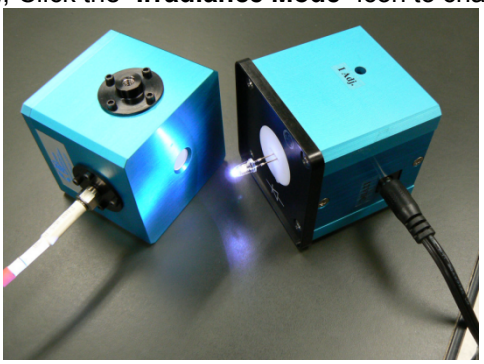
④ "**Spectrum**"/" **Reference Color Temp.**" Input the color temperature (K) of the standard light source used for relative irradiance measurements.

⑤ On Scope Mode, Adjust the Integration Time to control the maximum value of the Y axis of the graph (less than 16,000).

⑥ By clicking the "**Reference**" icon in the program, the spectrum is obtained at the maximum light intensity. Save the reference spectrum by "**File**"/ "**Save**"/ "**Reference**".

⑦ Close the light source shutter (Do not close the power switch), and click the "**Dark**" icon in the program to obtain a spectrum under the minimum conditions without light. Save the dark spectrum by "**File**"/"**Save**"/"**Dark**".

⑧ Connect a luminescence sample to integrating sphere (set a LED bulb to integrating sphere), Click the "**Irradiance Mode**" icon to change the Mode.



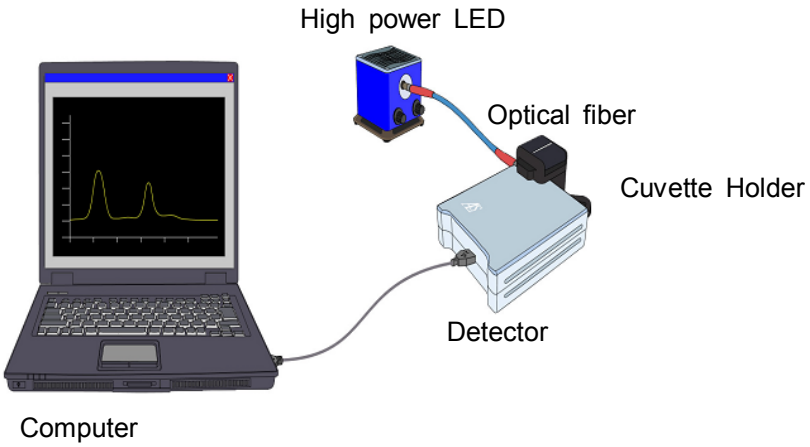
<Fig. 5-6-2> Relative irradiance measurement of LED bulb.

⑨ Save the spectrum by “**File**”/”**Save**”/”**Processed**” or “**Sample**”.

5-6-2. Fluorescence / luminescence-spectrum measurement

In this measurement, the detector of SEC2000 and cuvette holder are used.

① First, connect the detector of SEC2000, cuvette holder and light source by optical fiber as shown in Fig. 5-6-3.



<Fig. 5-6-3> Configuration example of fluorescence measurement.

② Put the sample into the cuvette holder and close the cover of cuvette holder.

③ Open the software and click “**Go**” icon, the spectrum signal will appear.

④ Turn on the power of the excitation light source. On Scope Mode, it shows the fluorescence of the sample. Adjust the Integration Time to control the maximum value of the Y axis of the graph (less than 16,000).

⑤ Save the spectrum by “**File**”/”**Save**”/ “**Sample**”.

⑥ When you measure a luminescence spectrum, please set a luminescence sample to detector (intercepting a surrounding light), and continue the procedure①～④, Save the luminescence spectrum by “**File**”/”**Save**”/ “**Sample**”.

5-7. Time Series Acquisition (TSA)

Time Series Acquisition is monitoring of the intensity change of absorbance, transmittance and radiation illumination. Time course of chemical and physical changes of the sample is measured. This function is applicable to a chemical substance,

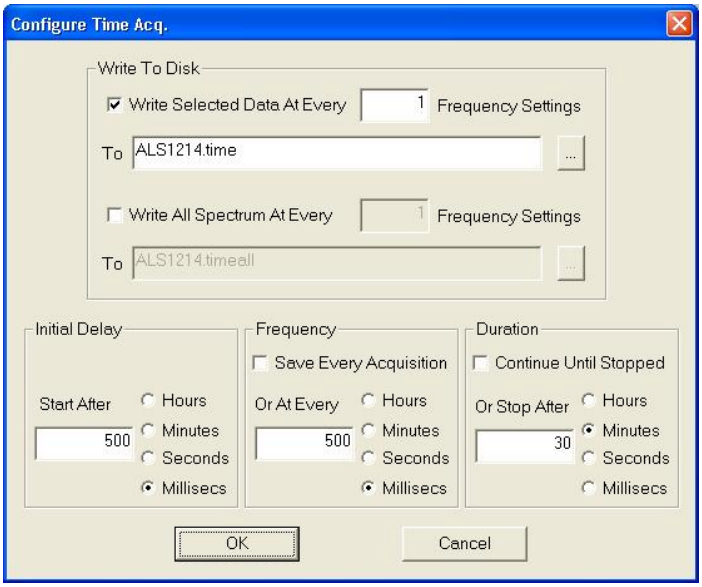
environment and process management.

The measurement can be carried out in Scope, Absorbance, Transmittance and Reflectance modes. Doing time series acquisition measurement, please follow the steps:

① First, open "**Menu**"/"**Time**"/"**Configure Time Acquisition**", or click the 

icon.

② The following window will appear. Configure and establish the parameters for time acquisition process.



<Fig. 5-7-1>

Write to Disk	-Check the "**Write Selected Data At Every 1 Frequency Settings**", and configure the file name and folder to store the specific wavelength intensity by fixed time interval. You need to complete "**Configure Time Acq. Channel**" also. Please refer the step ⑦ to configure the channels. -Check the "**Write All Spectrum At every 1 Frequency Settings**", and configure the file name and folder to store the full spectrum by fixed time interval. If you only want to store the full spectrum, there is no need to set up the "**Configure Time Acq. Channel**".
Initial Delay	Set the start time of the measurement (After pushing the play button). The measurement will start after "**Initial Delay**" time.
Frequency	Set the time interval. Larger than 100 ms.
Duration	Set the period of measurement.

③ Write the folder and file name for the time series acquisition process.

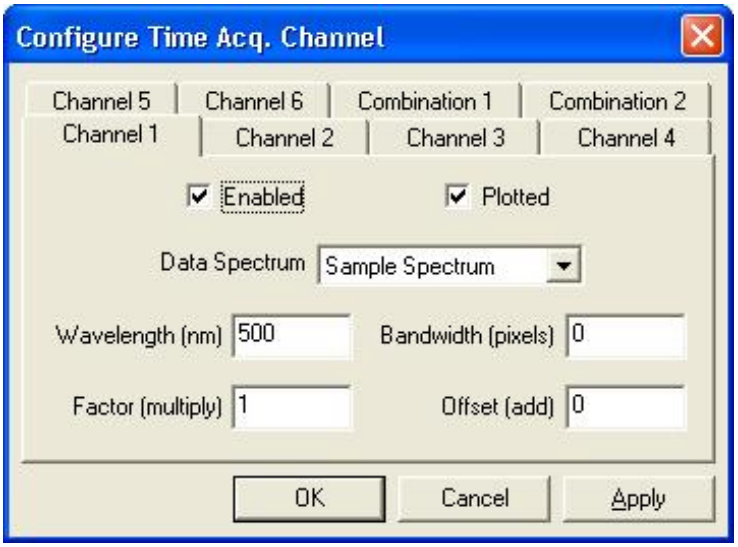
④ Enter the "**Initial Delay**" to set starting time.

⑤ Enter the "**Frequency**" to set the data collection frequency.

⑥ Enter the "**Duration**" to set the data collection period.

⑦ Open "**Menu**"/"**Time**"/"**Configure Time Acquisition Channel**", or click the 

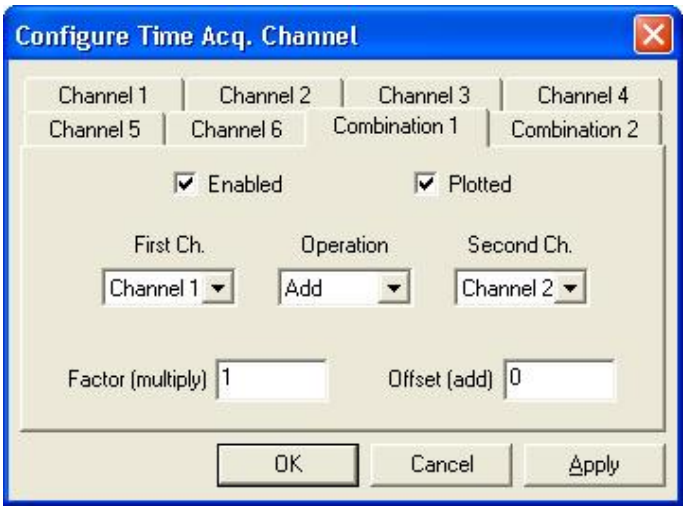
icon. In this window you can configure the wavelength channels. Six specific wavelengths can be set up from channel 1 to channel 6.



<Fig. 5-7-2>

Enabled	Check the "**Enabled**" to active the "**Channel 1**".
Plotted	Check the "**Plotted**" to plot the data on window.
Data Spectrum	Choose the spectrum type. **Sample/Dark/Reference spectrum**.
Wavelength	Please input the wavelength (nm) to measure by channel 1.
Bandwidth (pixels)	Decide the wavelength bandwidth to calculate peak area from the measured values. In this case, note that the unit is not **nm** but **pixels** (3 pixels≈1 nm). Since the value of peak area is bigger than the value of peak height, the plot range is beyond the Y-coordinate limit on the graph. It is better to make the bandwidth value **0** (zero) except in case that the measured values should be shown with peak area.
Factor	The measured value is multiplied by the number in "**Factor**".
Offset	When the plots are overlapped with one another as a result of setting up several Channels at the same time, fill one figure in "**Offset**" and increase the plot value. Fill it with real value only when the plots are overlapped.

⑧ Combinations 1 and 2 can perform operation among channel 1 ~ channel 6.



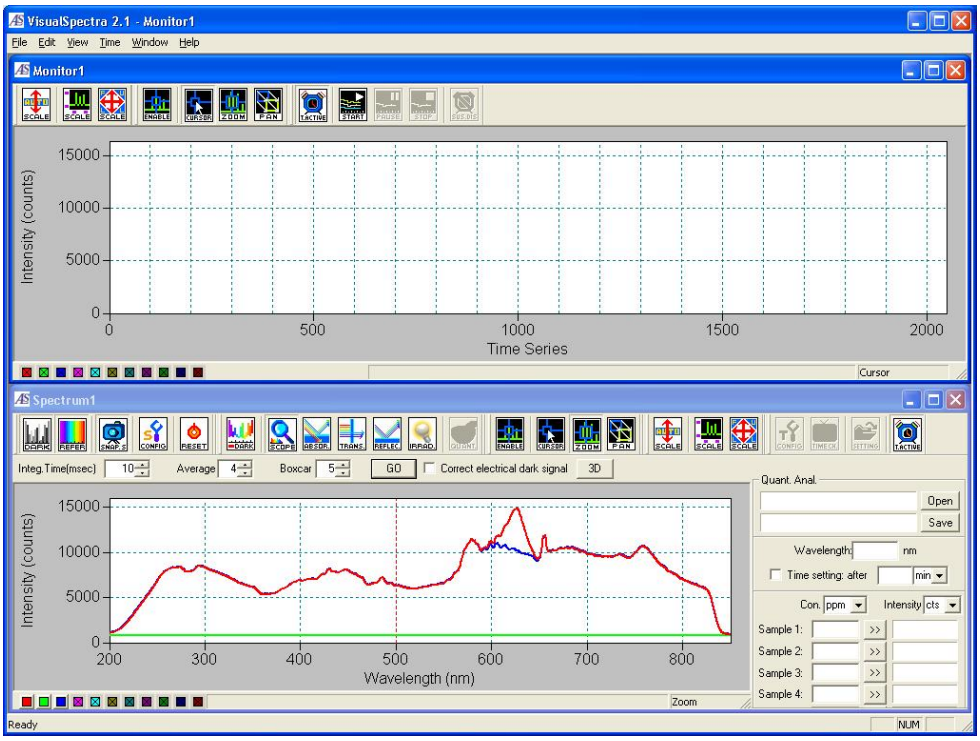
<Fig. 5-7-3>

⑨ The parameters of step ②-⑧can be saved by “**Manu**”/”**Time**”/”**Save Parameter**”. Next time, when you want to collect the data at the same condition, you should open the parameters by “**Manu**”/”**Time**”/”**Save Re-memory Parameter**” or click 

icon to.

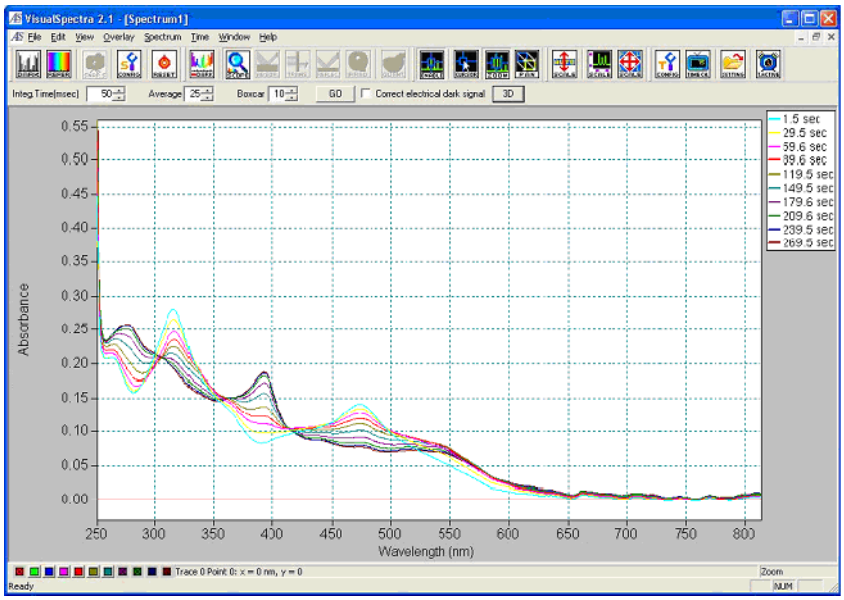
⑩ After configuring the channel parameters, open “**Time**”/” **Activate Time Acquisition**” or click 

icon to active the following window. The upper window ”Monitor 1” is for channel monitoring. The lower window ”Spectrum 1” is for original spectrum. Click the start “►” icon of “**Monitor 1**”, then the data collection will be carried out on the set-up conditions.



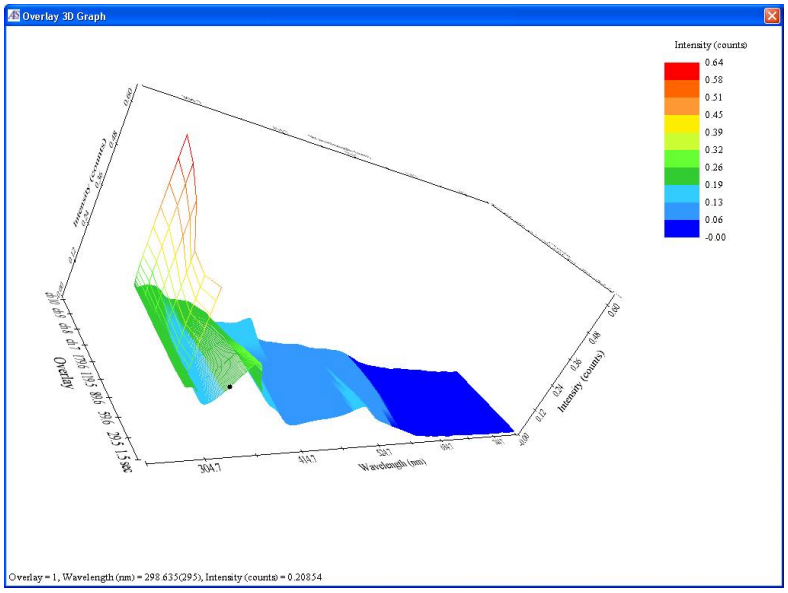
<Fig. 5-7-4>

⑪ The saved full spectrum files can be displayed using "**Overlay**"/"**Time series**"/"**Add overlay (Multiple Selection)**"



<Fig. 5-7-5>

⑫ The data can be displayed by 3D figure after clicking the  icon.



<Fig. 5-7-6>

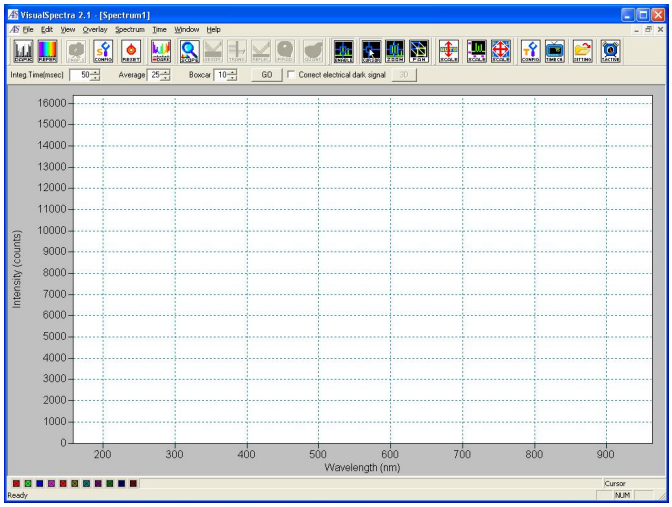
6. Analysis

6-1. Overlay

Data can be overlaid on software for analysis. The overlay method is different according to the type of the data. Please proceed to the following steps to concrete your data.

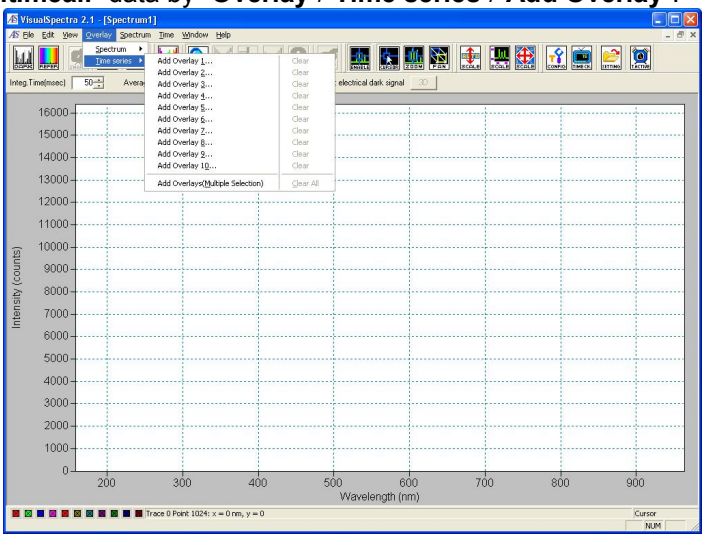
6-1-1. Overlay of Time Series data

① Open the software.



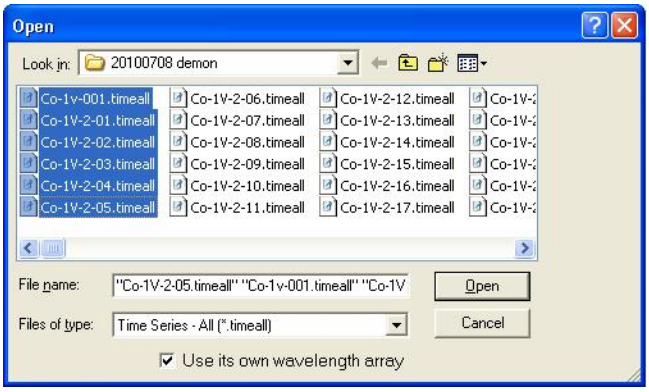
<Fig. 6-1-1>

② Open a "***.timeall**" data by "**Overlay**"/"**Time series**"/"**Add Overlay**".



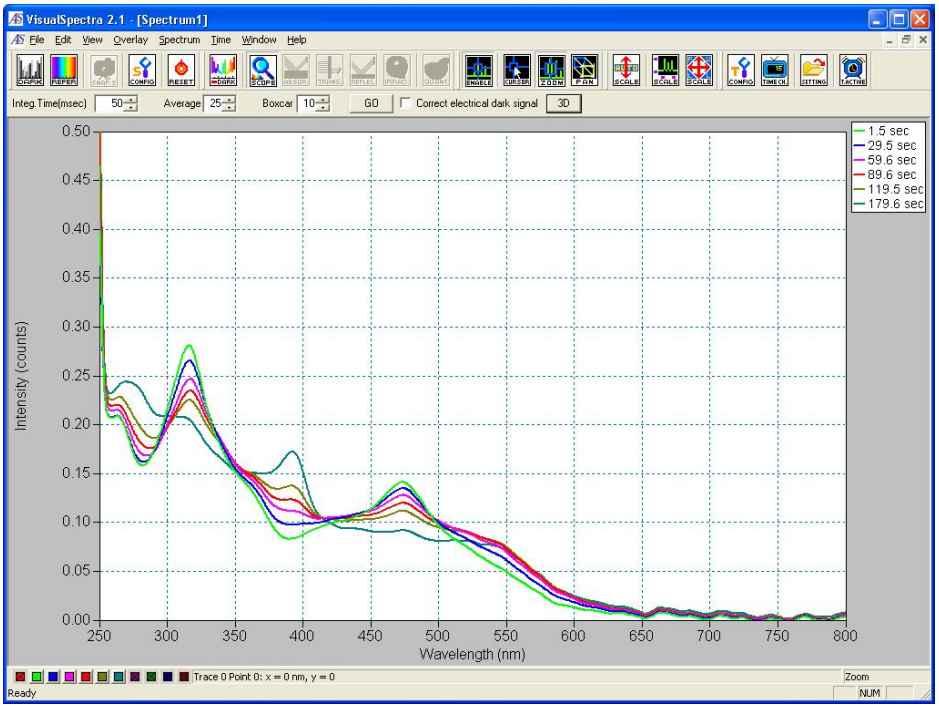
<Fig. 6-1-2>

③ Fig.6-1-3 shows opening data by "**Add Overlay** (**Multiple Selection**)". You may overlay 10 files at the same time.



<Fig. 6-1-3>

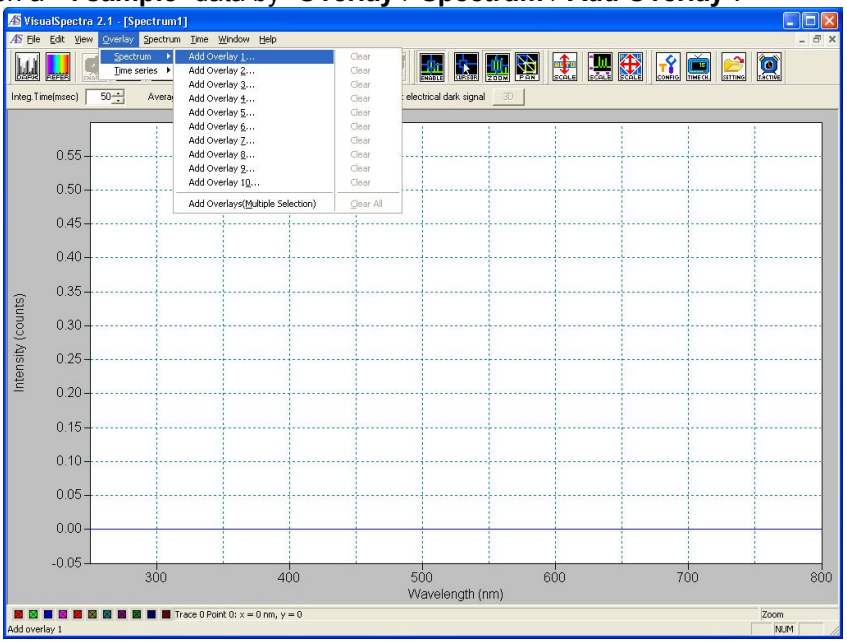
④ Adjust the scale, specify the optimal range, and then the data are displayed. The legend is shown in the right side of the graph. The data name is shown at the time of the measurement of the time course.



<Fig. 6-1-4>

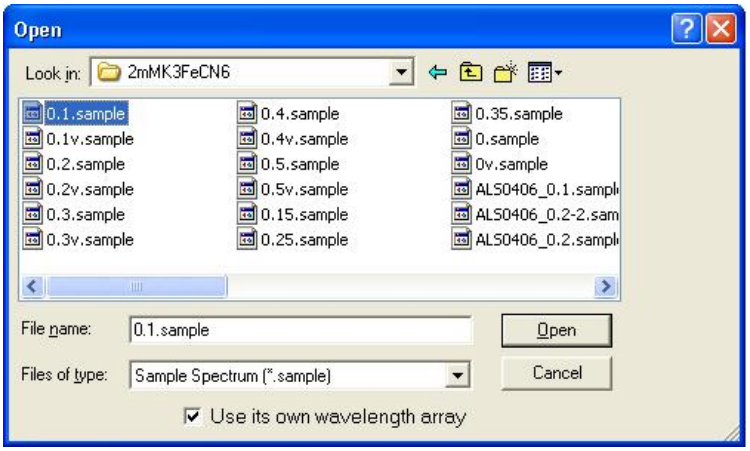
6-1-2. Overlay of Sample data

⑥ Open a "***. sample**" data by "**Overlay**"/"**Spectrum**"/"**Add Overlay**".



<Fig. 6-1-5>

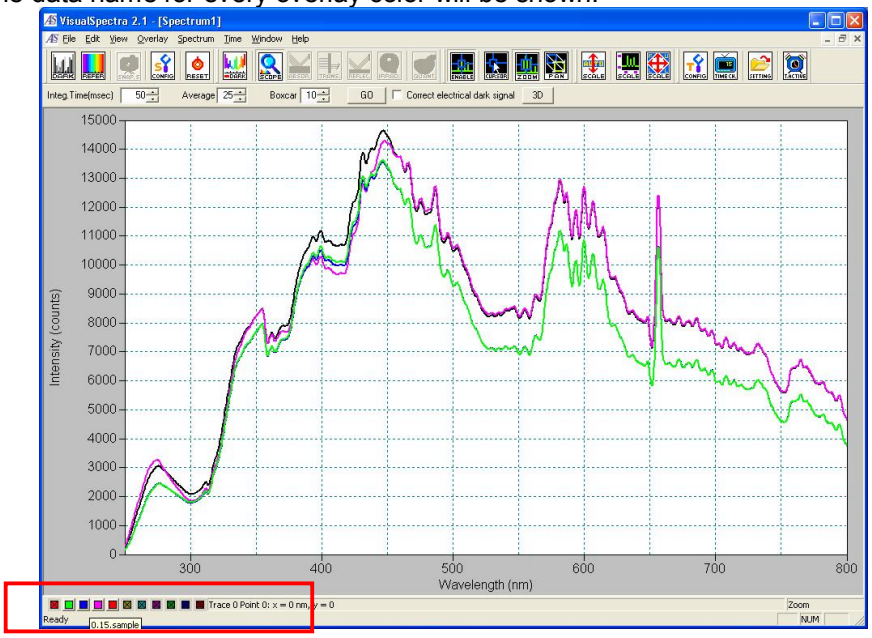
⑦ Fig. 6-1-6 shows opening data by "**Add Overlay** (**Multiple Selection**)". You may overlay 10 files at the same time.



<Fig. 6-1-6>

⑧ Adjust the scale, specify the optimal range, and then the data are displayed. There is no legend. Place your mouse on the lower left color icon (11 pieces), and then

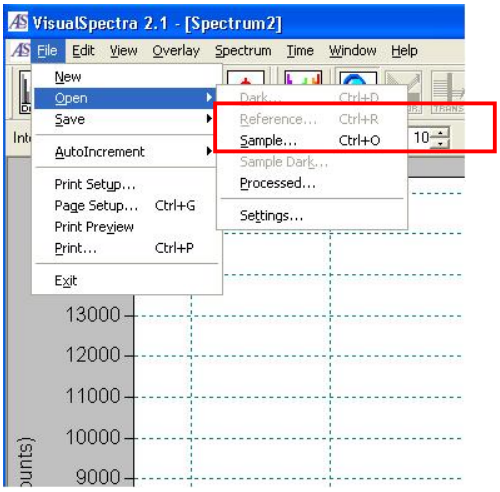
the data name for every overlay color will be shown.



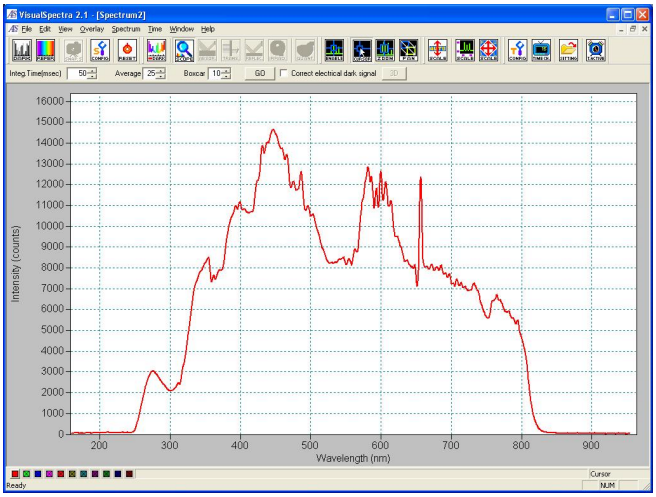
<Fig. 6-1-7>

6-1-3. Overlay of Processed data

① To overlay a processed data (***.absorb/*.trans/*.refl/*.irrad**), you must install the **Dark** and **Reference** data first. Open any sample data by "**File**"/"**Open**/ "**Sample**".

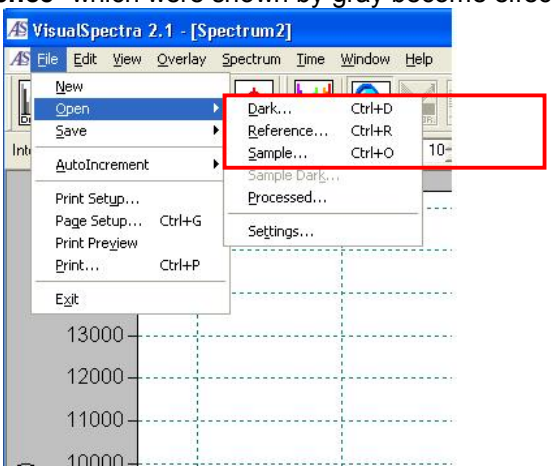


<Fig. 6-1-8>



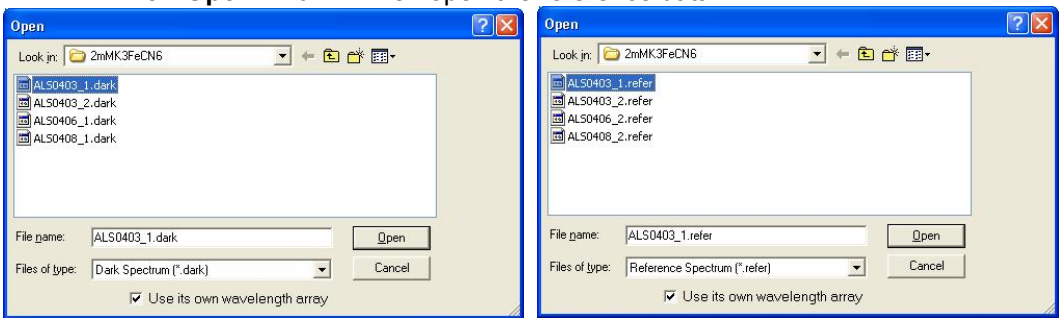
<Fig. 6-1-9>

② "**Dark**" and "**Reference**" which were shown by gray become effective.



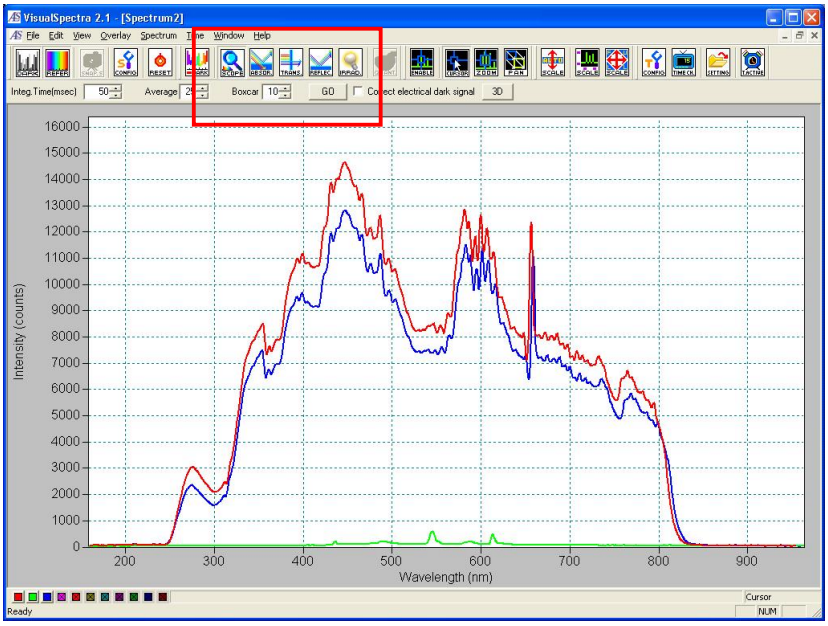
<Fig. 6-1-10>

③ Open the dark data saved in the same condition as saving processed data, by "**File**"/"**Open**"/"**Dark**". Then open the reference data.



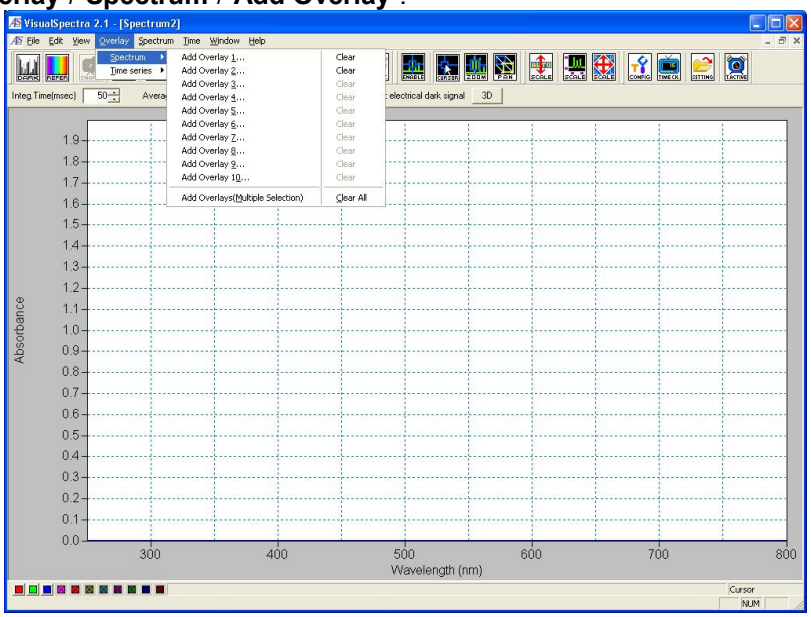
<Fig. 6-1-11>

④ Dark and reference spectra are automatically overlaid to Overlay1 and 2. The icons of **Absorbance/ Transmittance/ Reflectance/ Irradiance** become effective.



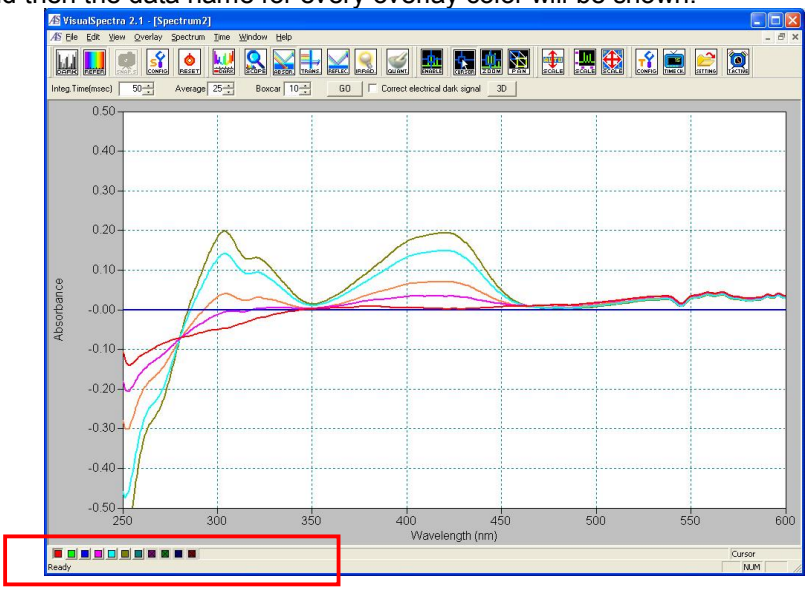
<Fig. 6-1-12>

⑤ Change the mode to Absorbance Mode. Then overlay a "***.absorb**" data by "**Overlay**"/"**Spectrum**"/"**Add Overlay**".



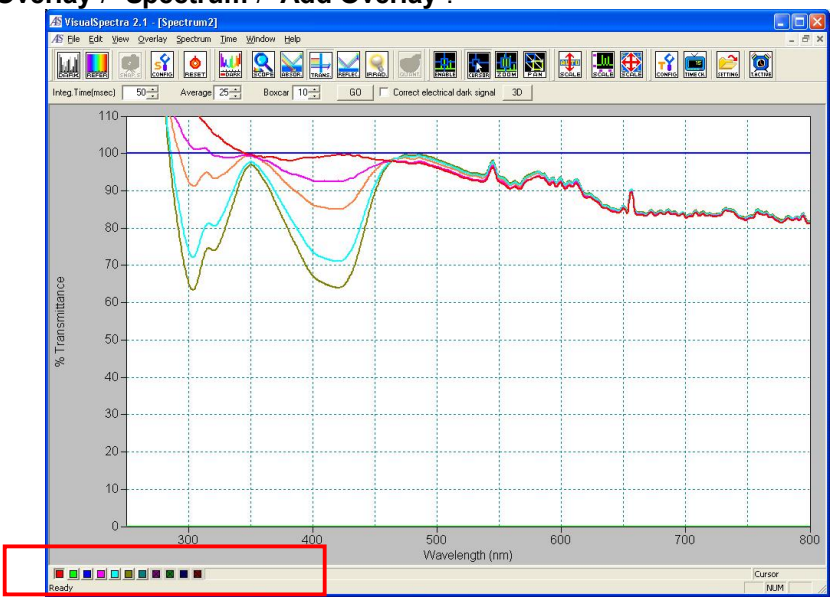
<Fig. 6-1-13>

⑥ Adjust the scale, specify the optimal range, and then the data are displayed. There is no legend. Place your mouse on the lower left color icon (11 pieces), and then the data name for every overlay color will be shown.



<Fig. 6-1-14>

⑦ To overlay a ***.trans/*.refl/*.irrad** data, same to the step ⑤, change the mode to Transmittance/Reflectance/Irridiance Mode, and then overlay the data by "**Overlay**"/ "**Spectrum**"/ "**Add Overlay**".



<Fig. 6-1-15>

6-2. Quantitative Analysis with Calibration Method

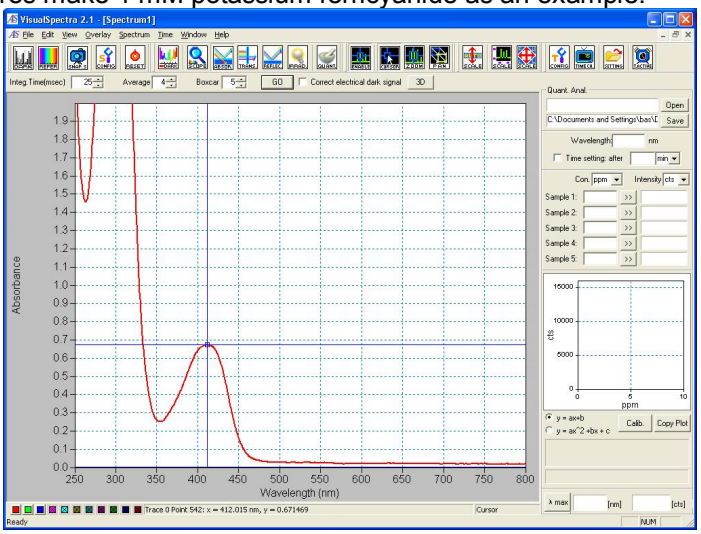
6-2-1. Calibration method by calculating the absorbance area

The measuring results are quantitatively analyzed by using the absorbance area of the selected wavelength range after calibrating the baseline. It is possible only in absorbance mode.

① Set the spectrometer system as shown in Fig. 6-2-1.

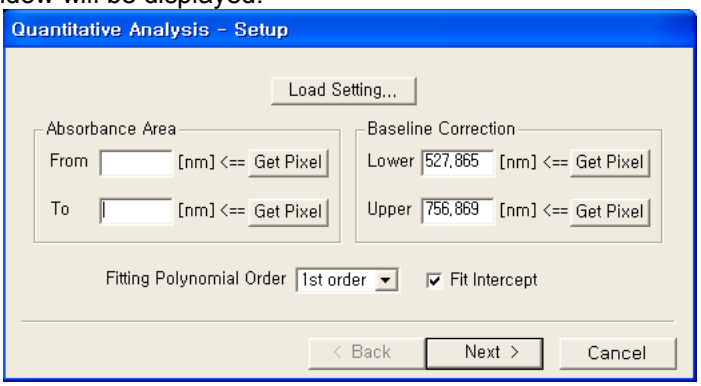
<Fig. 6-2-1> Configuration for an absorbance experiment.

② A reference spectrum and a dark spectrum are measured in the same procedure as absorbance measurement. Put a standard sample into the cuvette holder in absorbance mode, then the screen will become as Fig. 6-2-2. The following procedures make 1 mM potassium ferricyanide as an example.



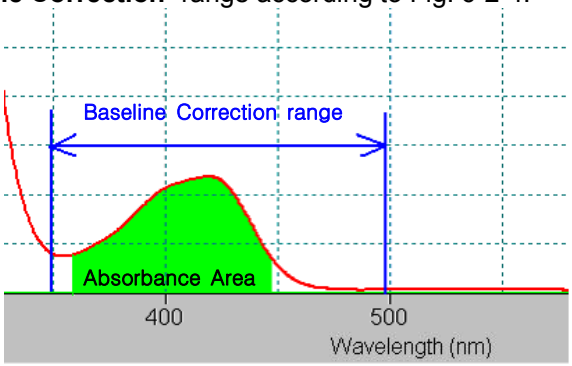
<Fig. 6-2-2> Absorbance of 1 mM $K_3Fe(CN)_6$ solution.

③ Click "**Spectrum**" / "**Quantitative Analysis**", then the "**Quantitative Analysis-Setup**" window will be displayed.



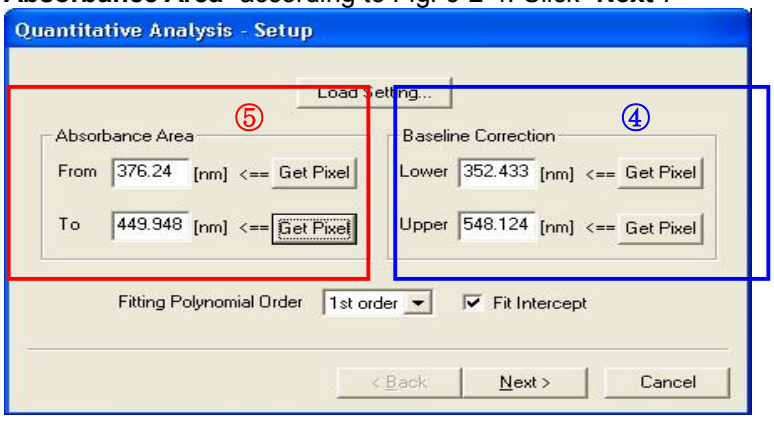
<Fig. 6-2-3>

④ Set the "**Baseline Correction**" range according to Fig. 6-2-4.



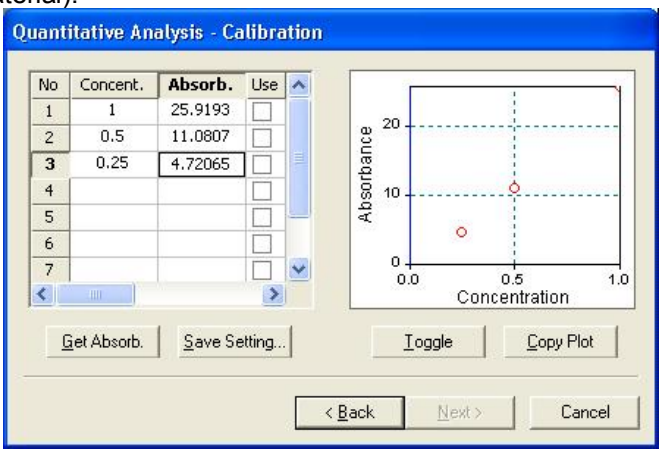
<Fig. 6-2-4>

⑤ Set the "**Absorbance Area**" according to Fig. 6-2-4. Click "**Next**".



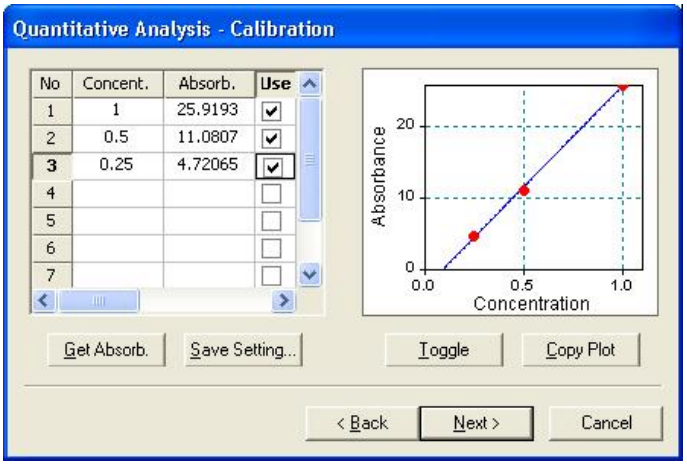
<Fig. 6-2-5> Selection of wavelength range for the absorbance area and Baseline Correction.

⑥ On the step of "**Quantitative Analysis- Calibration**", to measure the absorbance of the standard material with known concentration, put a standard sample into the cuvette holder, and input data in "**Concent.**" Column. Move your mouse to "**Absorb.**" column, and click "**Get Absorb.**" icon to calculate the area of the standard sample (get a calibration line with at least three different concentrations of standard material).



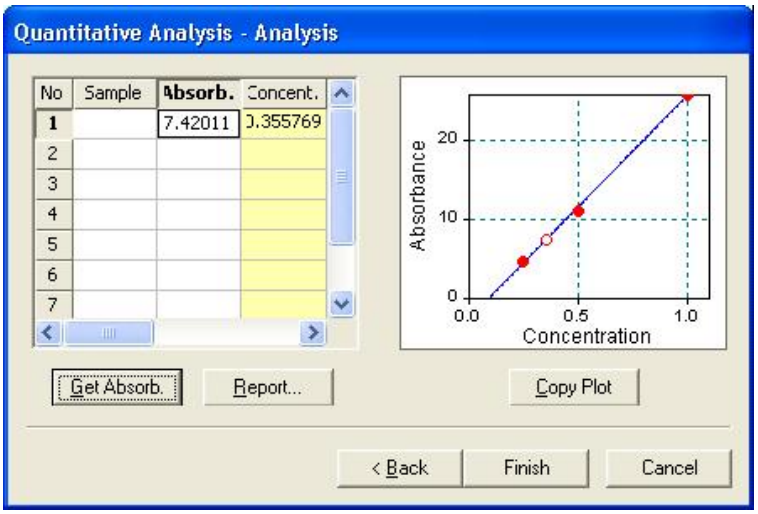
<Fig. 6-2-6> Absorbance of $K_3Fe(CN)_6$ solutions (Concentration: 1, 0.5 and 0.25 mM).

⑦ Click "**Use**", then calibration line is obtained as shown in Fig. 6-2-7.



<Fig. 6-2-7> Calibration line with three different concentrations.
The standard samples are shown in closed circle.

⑧ Click "**Next**", on the step of "**Quantitative Analysis-Analysis**", to measure the absorbance with unknown concentration of sample, put a sample into the cuvette holder, move your mouse to "**Absorb.**" column, and click "**Get Absorb.**" Icon. Then the concentration of the unknown sample will be calculated and shown in "**Concent.**" column.



<Fig. 6-2-7> Quantitative analysis with calibration method.

The unknown concentration of the sample is shown in open circle.

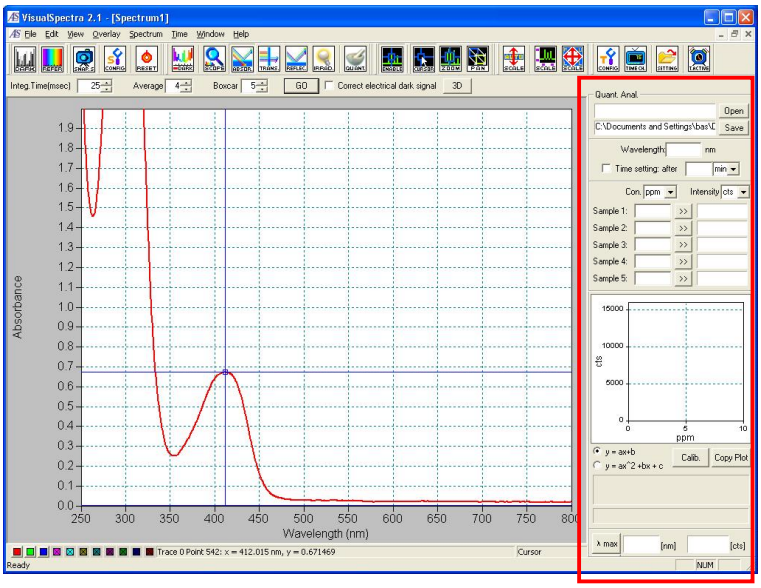
6-2-2. Calibration method by calculating the peak absorbance.

Analyze the concentration of a sample by making calibration line with the peak absorbance of standard solutions. It is possible only in absorbance mode.

① Set the spectrometer system as shown in Fig. 6-2-8.

<Fig. 6-2-8> Configuration for an absorbance experiment

② A reference spectrum and a dark spectrum are measured in the same procedure as absorbance measurement. Put a standard sample into the cuvette holder in absorbance mode, the screen will become as Fig. 6-2-9. The following procedures make 1 mM potassium ferricyanide as an example.

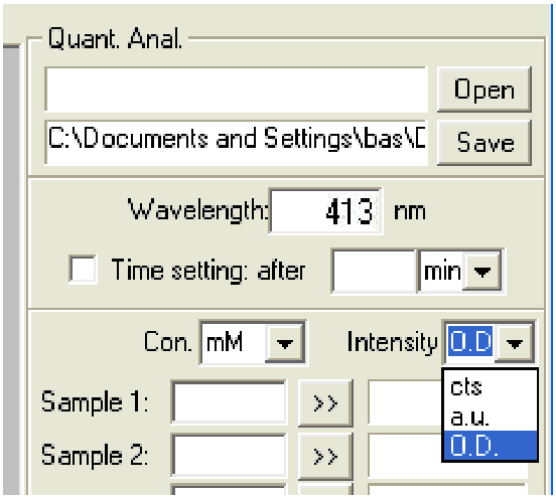


<Fig. 6-2-9> Absorbance of 1 mM $K_3Fe(CN)_6$ solution.

③ Open the "**Quantitative Analysis**" window by "**View**"/"**Spectrum Tool Bar**"/ check the "**Quantitative Analysis**". The window of "Quantitative Analysis" is shown on the right side of the main screen (Fig. 6-2-9).

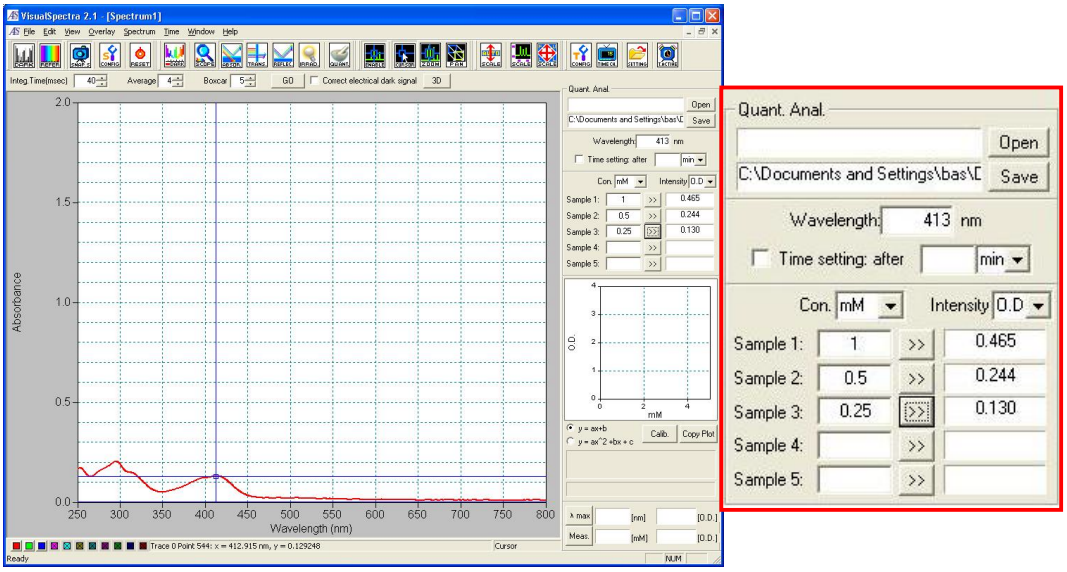
④ Set parameters (Fig. 6-2-10)

- Set the file name and folder of saving calibration curve by "**Save**".
- Set the peak wavelength of the sample by "**Wavelength_ nm**", we set 413 nm for the ferricyanide.
- Set the unit of solution concentration by "**Con.**", we choose "**mM**".
- Set the unit of absorbance by "**Intensity**", we choose "**O.D.** ".



<Fig. 6-2-10>

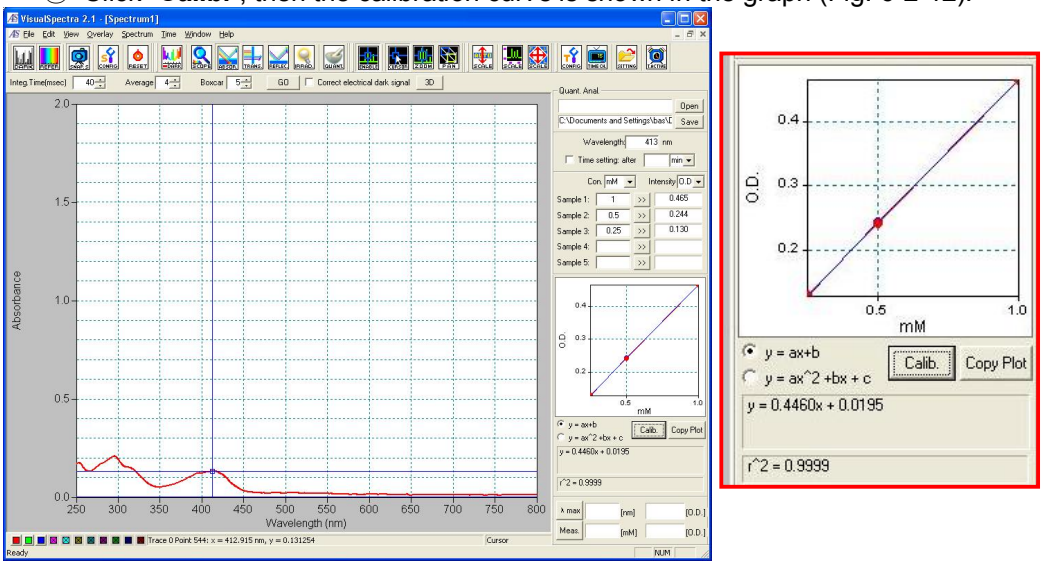
⑤ Input the standard concentration of “Sample 1” to “Sample 5”, and click “>>” The vertical axis of the peak is read.



<Fig. 6-2-11>

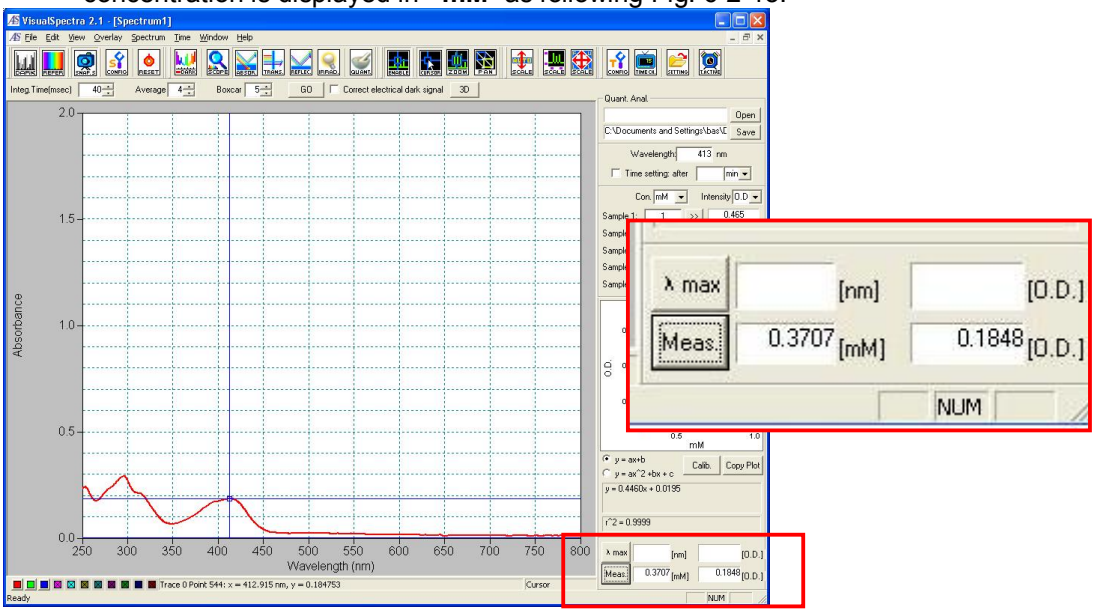
⑥ Change the standard, and get the peak absorbance of each concentration.

⑦ Click “**Calib.**”, then the calibration curve is shown in the graph (Fig. 6-2-12).



<Fig. 6-2-12> Calibration line with three different concentrations.

⑧ Put an unknown sample into the cuvette holder, and click “**Meas.**” icon. The peak absorbance of the sample is measured and displayed in “**O.D.**”, and the concentration is displayed in “ **mM**” as following Fig. 6-2-13.



<Fig. 6-2-13>

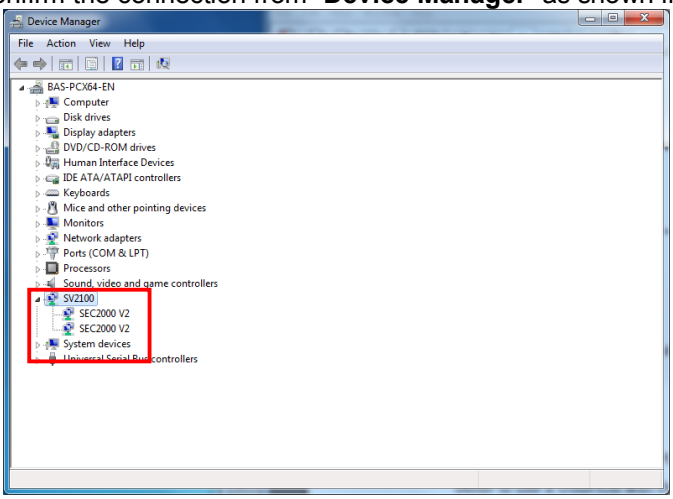
7. Multi-Channel

7-1. Purpose of Multi-Channel

This function is designed for you to use several spectrometers on one PC. You can use five spectrometers at the same time.

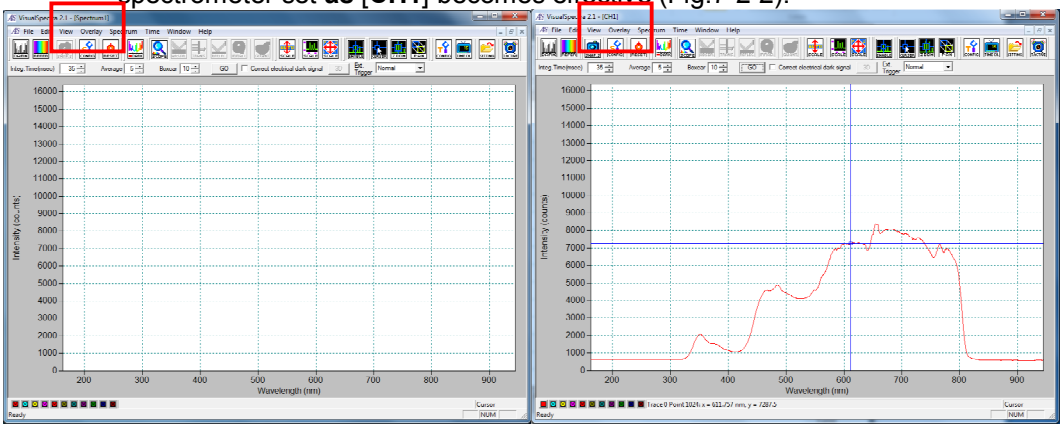
7-2. Configure the software

① Connect the spectrometers to PC with USB cables. Using a notebook PC, you had better to use a USBHUB with the power supply to connect the spectrometers. And you can confirm the connection from "**Device Manager**" as shown in Fig.7-2-1.



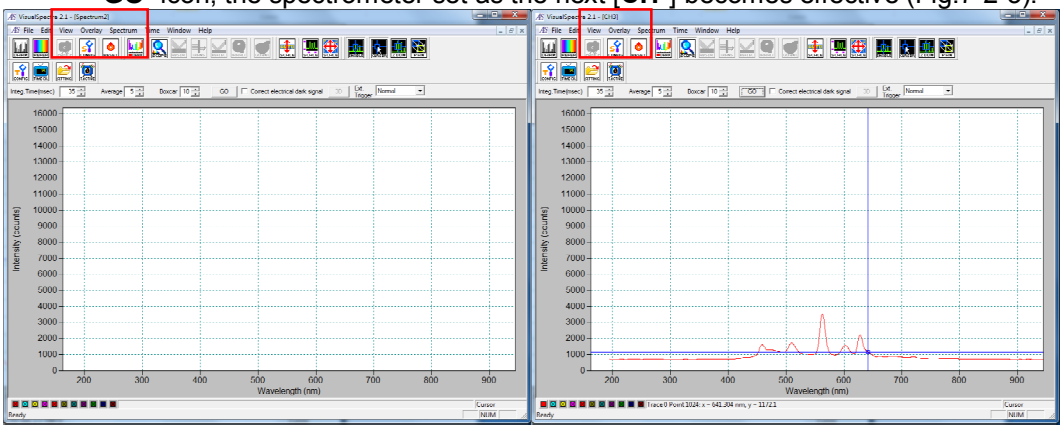
<Fig.7-2-1>

② Open the software, it shows as [**Spectrum1**]. Click the "**GO**" icon, the spectrometer set **as** [**CH1**] becomes effective (Fig.7-2-2).



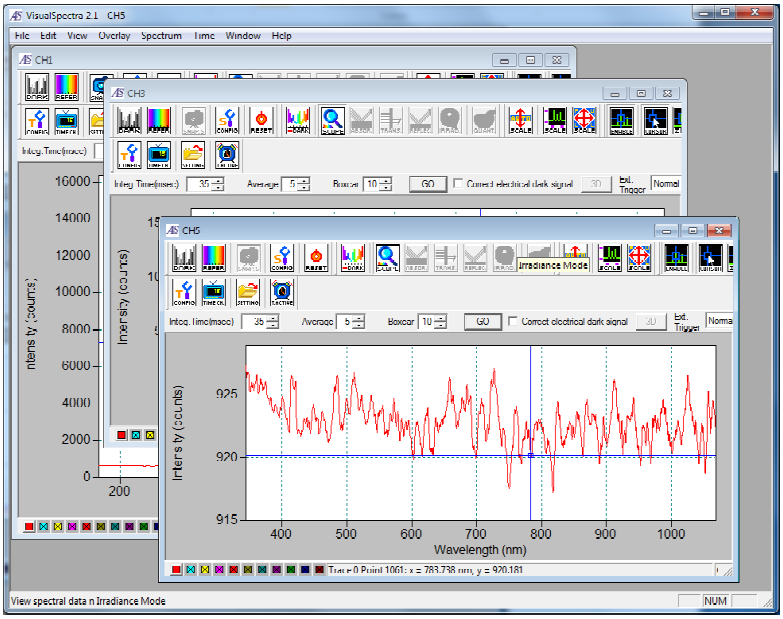
<Fig.7-2-2>

③ Click “**File**”/ ”**New**”, you can open a new window shown as [**Spectrum2**]. Click the “**GO**” icon, the spectrometer set as the next [**CH***] becomes effective (Fig.7-2-3).



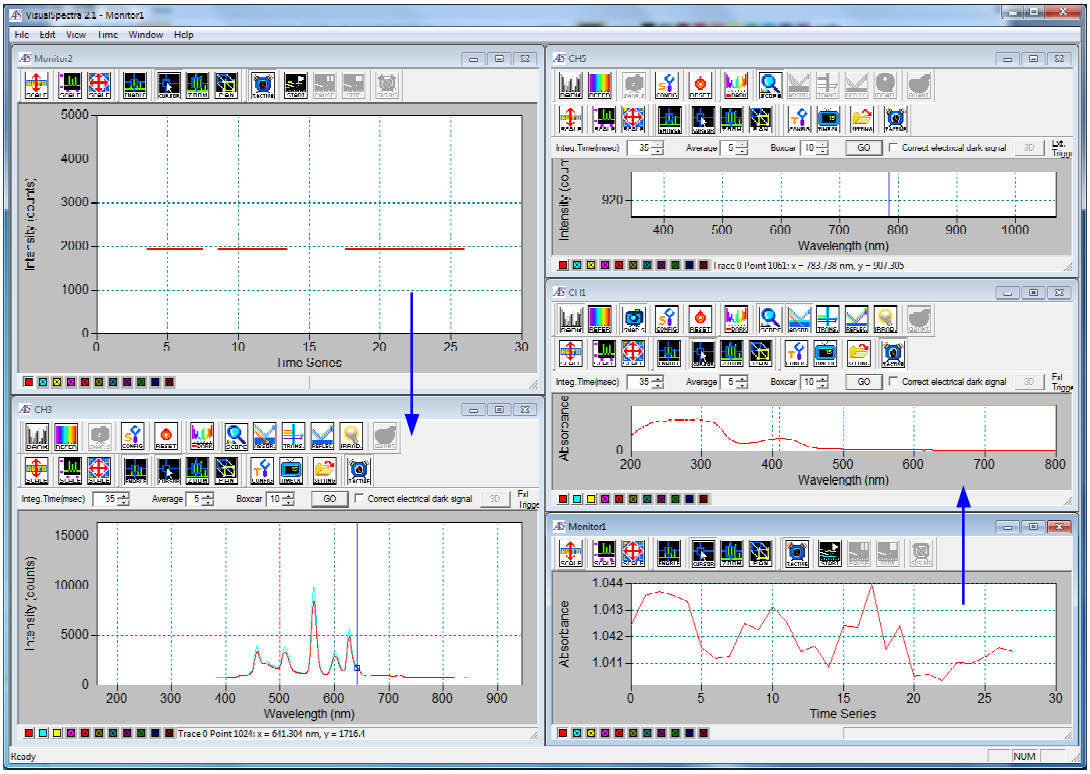
<Fig.7-2-3>

④ Regulate each window to suitable size.



<Fig.7-2-4>

⑤ The time course measurement for each channel is displayed as [**Monitor1**] and [**Monitor2**]. It indicates opening number in turn, but not corresponds to Channel number. As shown, for example, in Fig. 7-2-5, Monitor1 corresponds to CH1, and Monitor2 corresponds to CH3.

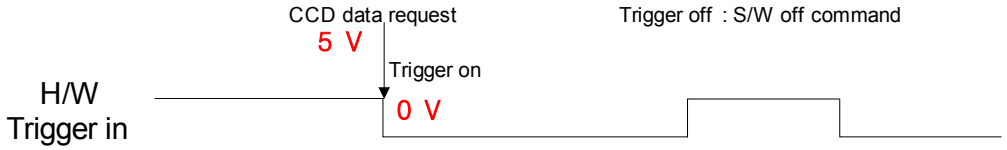

Monitor2
Intensity (counts)
Time Series
CH5
Wavelength (nm)
CH3
Wavelength (nm)
Monitor1
Absorbance
Time Series
Ready

<Fig.7-2-5>

8. Trigger

8-1. External trigger

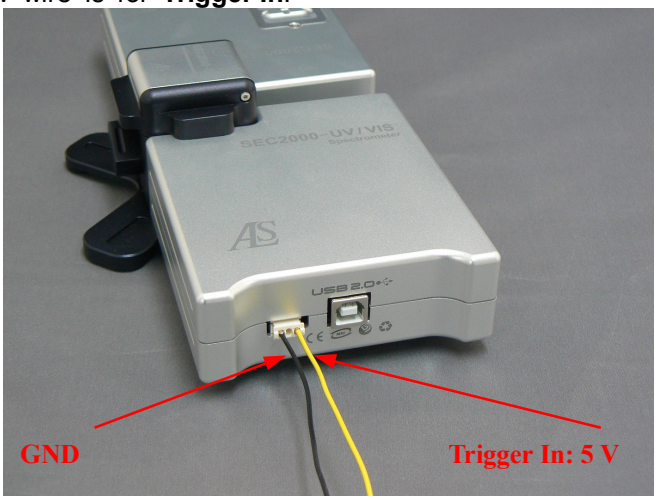
It is a new developed function for external trigger to synchronize spectrum measurement with other measurements (for example, electrochemistry measurement). Start the spectrum measurement by an external TTL signal. Fig.8-1-1 shows the signal chart of **Low Level** mode. When the external signal (Trigger In) changes from 5 V to 0 V, the spectrum measurement starts. There are **High Level** mode also. It's signal chart is reversed to the Low Level mode. The follow procedure describes the operation on the Low Level mode.



<Fig. 8-1-1 External Trigger Signal: Low level>

8-2. External trigger

Fig.8-2-1 shows the Trigger In connector. The black wire is for **Ground** (GND) and the yellow wire is for **Trigger In**.

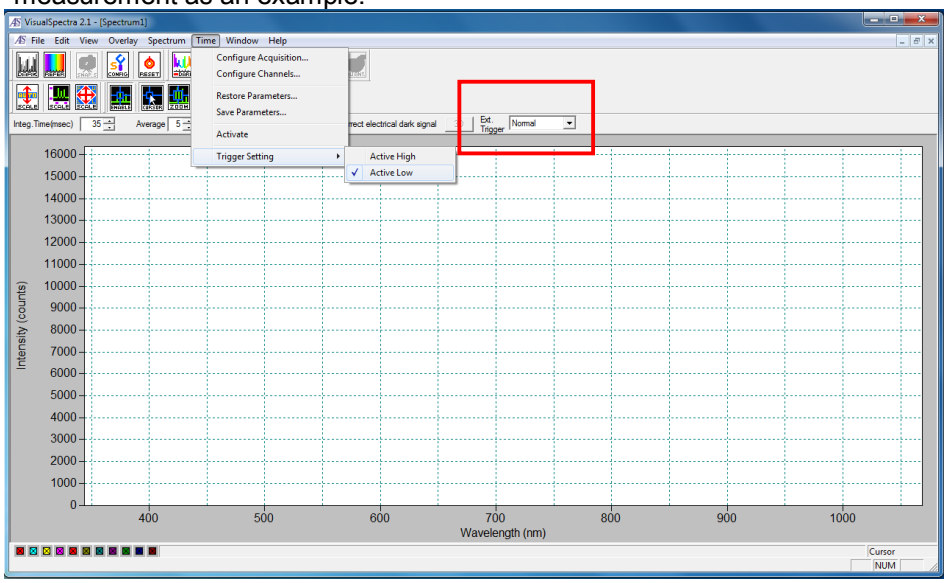


<Fig. 8-2-1 External Trigger connector>

8-3. Procedure of the software setting

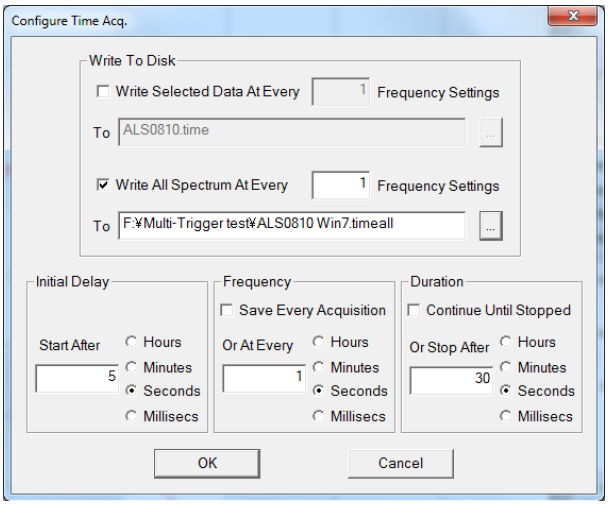
① Open the software, and save the "**Reference**" and "**dark**" spectra as common.

② Choose the trigger mode from "**Time**" / "**Trigger Setting**" / "**Active high**" or "**Active low**". We introduce you the Setting of Trigger with the absorbance measurement as an example.



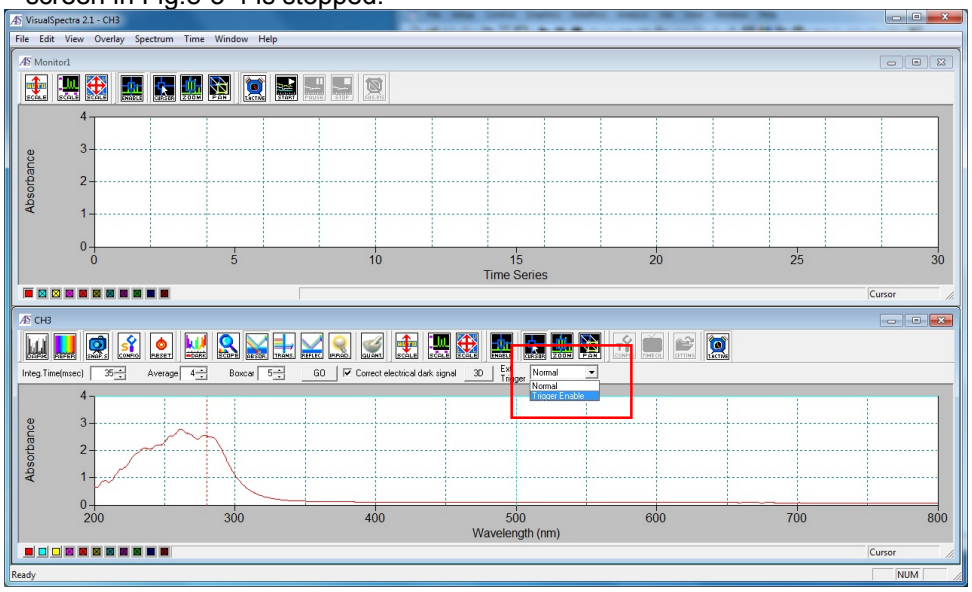
<Fig. 8-3-2 >

③ Set the "**Configure Time Acquisition**" (Fig. 8-3-3) at "**Ext. Trigger**" "**Normal**" condition(Fig.8-3-2). The detail of Time acquisition setting is described in **5-7.** of the Chapter 5.



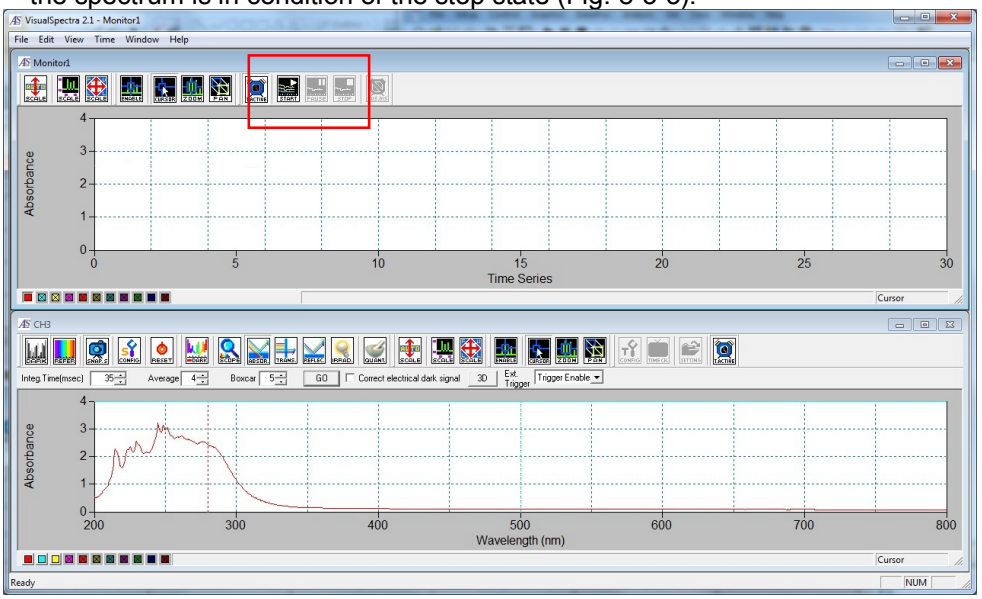
<Fig. 8-3-3 >

④ Change the "**Ext. Trigger**" to "**Trigger Enable**". The signal of the lower spectrum screen in Fig.8-3-4 is stopped.



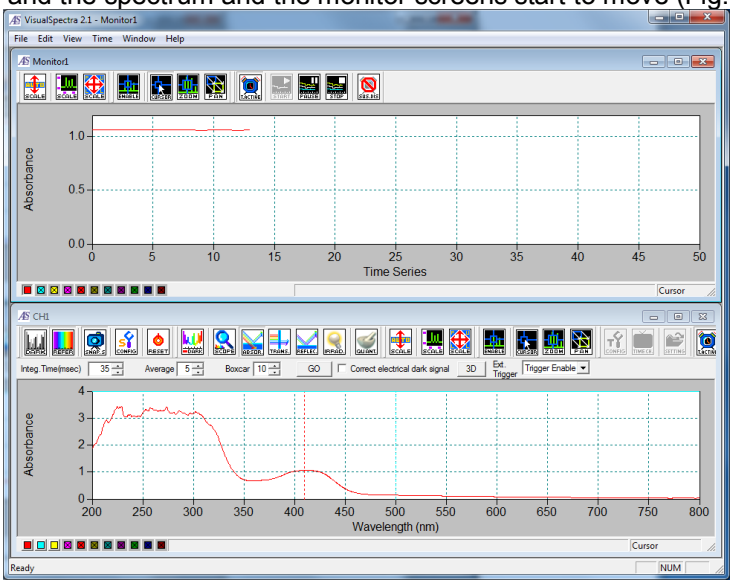
<Fig. 8-3-4 >

⑤ Click the "**START**" icon in the Monitor1 screen. During this time the external TTL is 5 V. Waiting for the change of the external TTL, all screens are on standby, and the spectrum is in condition of the stop state (Fig. 8-3-5).



<Fig. 8-3-5 >

⑥ When the external TTL changes from 5 V to 0 V, the spectrum measurement is begun, and the spectrum and the monitor screens start to move (Fig. 8-3-6).



<Fig. 8-3-6 >

⑦ To stop the monitor, you can configure the "**Duration**" or click the "**STOP**" icon in the Mornotor1 screen.