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Effect of Measurement Parameters on UV-VIS Absorption Spectra —— 02

Applications Examples of Analysis Using Micro-Volume Measurement Cells —— 06

Q&A What is the difference between absorbance and optical density (O.D.)? —— 13

PQY-01 Photoreaction Evaluation System "Lightway" —— 14

Effect of Measurement Parameters on UV-VIS Absorption Spectra

Spectroscopy Business Unit, Analytical & Measuring Instruments Division
Hiroki Nishii

1. Introduction

Ultraviolet-visible spectrophotometers are used to measure the absorption spectra of target substances. However, changing measurement parameter settings, such as the slit width, scan speed, and data interval, can simultaneously change the width of peaks and noise level in resulting absorption spectra. Therefore, the optimal parameter settings for the given spectral measurement objectives must be specified.

This article describes how slit width, scan speed, and data interval parameter settings affect UV-VIS absorption spectra.

2. Measurement Parameters —Slit Width (Resolution)—

The slit width is a measurement parameter that specifies the width of the opening through which light enters and exits the monochromator used in a UV-VIS spectrophotometer. Given that light dispersed from the monochromator spreads out in the shape of a rainbow, the narrower the slit width, the more information can be acquired for specific wavelengths. For example, if 540 nm light is measured with a slit width setting of 1 nm, the information obtained includes a mixture of information from wavelengths ranging from 539.5 nm to 540.5 nm. That wavelength range is referred to as the resolution. A larger resolution value enables more accurate measurement of each wavelength. However, high resolution also has some disadvantages. Specifying a narrower slit width results in less light passing through the slit and less light reaching the detector.

That increases the amount of noise in acquired measurement data. With slit width and noise level having a mutually opposing relationship, the optimal slit width setting depends on the type of data desired.

The optimal resolution value will vary depending on the desired data to be acquired. Fig. 1 shows spectra of benzene in an ethanol solution measured at different resolutions.

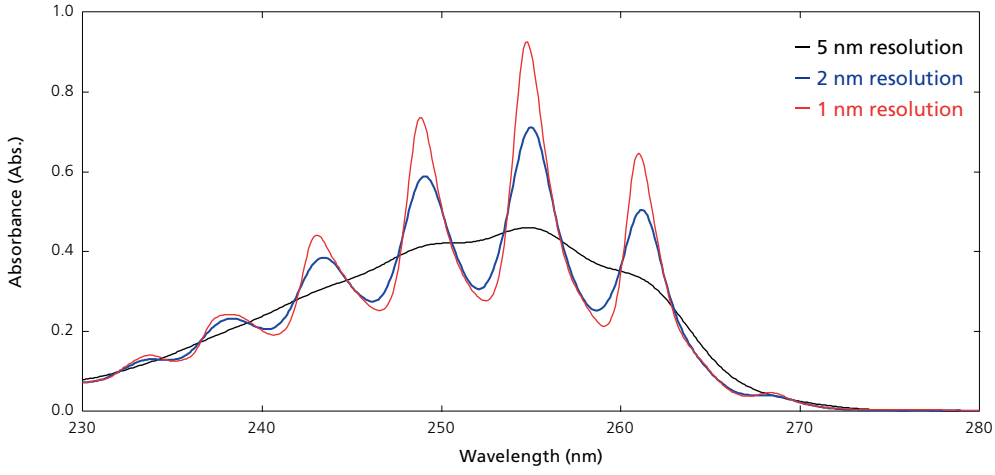


Fig. 1 Spectra of Benzene in Ethanol Solution

Benzene exhibits seven sharp peaks near the 230 to 270 nm area. The spectra show that decreasing the resolution successively smooths the peak until almost no peaks are detected with a resolution of 5 nm. As a general rule, it is known that peaks become difficult to detect at resolutions larger than the full width at half maximum (FWHM) of the peak being acquired. Optimize the slit width setting by specifying a wider slit width if the target peaks being measured are broad or noise is a concern or by specifying a narrower slit width for sharper peaks.

3. Measurement Parameters —Scan Speed—

Scan speed refers to how quickly wavelengths are scanned. During spectral measurements, the data integration time (length of time the detector detects light) is set automatically based on the specified scan speed. The faster the scan speed, the shorter the integration time. The slower the speed, the longer the time. Noise can be reduced with a longer integration time, but the slower scan speed will result in a longer measurement time.

Shimadzu UV-VIS spectrophotometers provide four scan speed levels (extra-slow, slow, medium, and fast); the UV-1900i model also includes an even faster survey mode (29,000 nm/min).

Fig. 2 shows baseline data measured with a UV-1900i system over the wavelength range from 400 to 900 nm at three scan speeds (survey, fast, and extra-slow). Table 1 shows the time required for the measurements.

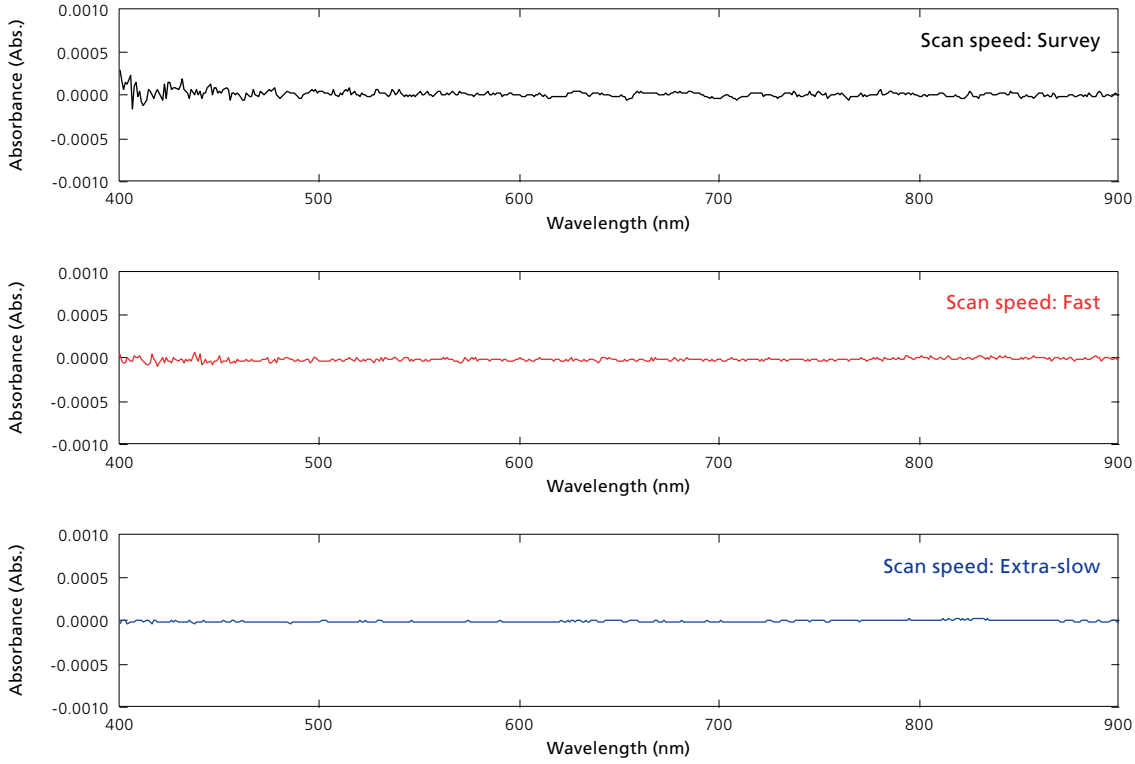


Fig. 2 Baseline Data Measured at Various Scan Speeds

Table 1 Measurement Time Required at Various Scan Speeds (at 1 nm data intervals)

Scan Speed	Measurement Time
Survey	About 15 sec
Fast	About 45 sec
Medium	About 107 sec
Slow	About 235 sec
Extra-Slow	About 870 sec

Fig. 2 shows how the slower the scan speed, the less baseline noise is included. Though not shown here, it is also necessary to be aware that increasing the scan speed will not only shorten the measurement time, but also may shift the position of peaks slightly in the short-wavelength direction.

If measuring a large wavelength range or only checking the approximate position of peaks, data can be acquired more efficiently by specifying a faster scan speed. On the other hand, if more accurate data acquisition is preferable, a slower scan speed is recommended.

4. Measurement Parameters —Data Interval—

The data intervals setting specifies the wavelength intervals between measuring each photometric value. Setting a shorter data interval value will result in acquiring more detailed photometric value information but will require a longer measurement time.

Fig. 3 illustrates the results that would be obtained from measuring a peak with different data intervals.

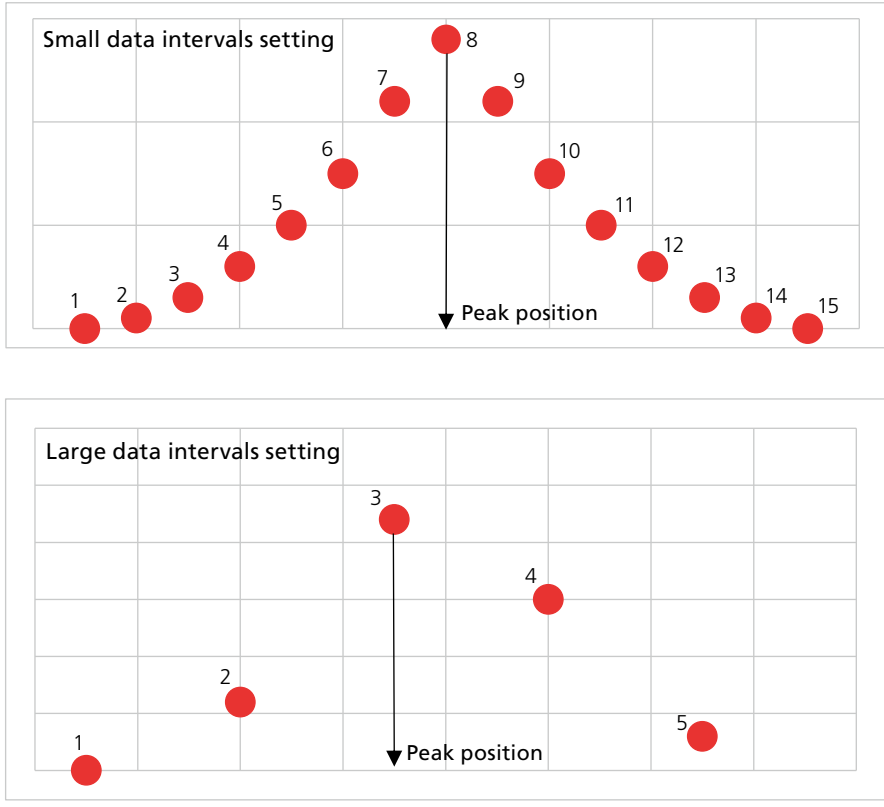


Fig. 3 Illustration of Measurements Obtained by Changing the Data Intervals Setting (Each Dot Indicates One Light Measurement Value)

Note that changing the data intervals setting can change the peak position. For the example in Fig. 3, if a small data intervals setting is specified, the peak position would be at the eighth red dot in the upper spectrum, whereas if a large data intervals value is specified, the peak position would be at the third red dot in the lower spectrum.

For Shimadzu UV-VIS spectrophotometers, the available data intervals settings are 0.05 nm, 0.1 nm, 0.2 nm, 0.5 nm, 1.0 nm, 2.0 nm, and 5.0 nm, depending on the model. (The UV-1900i model offers 1.0 nm and 2.0 nm settings for the ultra-fast survey scan mode.)

5. Measurement Parameters —Summary—

Table 2 summarizes the spectral effects of each parameter described above.

Table 2 Effects of Each Parameter on Spectra

Parameter	Setting	Resolution	Noise	Measurement Time
Slit Width	Wide	High	Low	—
	Narrow	Low	High	—
Scan Speed	Fast	—	High	Short
	Slow	—	Low	Long
Data Intervals	Large	—	—	Short
	Small	—	—	Long

6. Conclusions

This article described each parameter for spectral measurements and explained their effect on measurement data. More efficient and accurate measurement can be achieved by optimizing these parameter settings.

Hopefully, this article will be useful for customers struggling with measurements.

Applications

Examples of Analysis Using Micro-Volume Measurement Cells

Global Application Development Center, Analytical & Measuring Instruments Division
Yoshiyuki Tange

1. Introduction

A wide variety of measurement cells with different materials, volumes, and shapes are available for measuring the light absorbance of samples (solutions). The most commonly used cell is a quartz cell with a 10 mm optical path length, but in life science fields, where samples such as DNA/RNA and proteins are quantified, it is often difficult to obtain sufficient sample quantities, making it necessary to measure extremely small quantities.

This article describes the features of two types of cells (Nano Stick-S and TrayCell) that satisfy such needs and gives examples of using the cells for analysis.

One feature common to both cells is a short optical path length that enables not only measurement of very small sample quantities, but also measurement of high-absorbance samples without dilution, in some cases. Also, because small sample quantities are involved, after measurements the cell can be cleaned by simply wiping off the sample dripped onto the window plate, which makes the cleaning process extremely simple.

2. Nano Stick

Nano Stick cells are measurement cells made by SCINCO for extremely small quantities. The Nano Stick-S has one sampling port for inserting samples and the Nano Stick-D has two. (The Nano Stick-D has one port for a reference sample and one for the measurement sample, so that the same cell can be used to measure both reference and measurement samples by flipping the cell upside down.)

This article describes the Nano Stick-S. Fig. 1 shows Nano Stick-S cells and Table 1 shows their specifications.

Fig. 1 Nano Stick-S Cells

Table 1 Nano Stick-S Specifications

Optical Path Length	0.5 mm
Dimensions (W × D × H)	12.5×12.5×60.0 mm
Light Beam Height	15.0 mm
Minimum Sample Quantity	2 µL
Sampling Port Window Material	Quartz
Usable Wavelength Range	190 to 1,100 nm

The optical path length is 0.5 mm and the sample quantity required for measurements is an ultra-small 2 µL. Because the sampling port material is quartz, the cell can be used for a wide range of wavelengths, from ultraviolet to near-infrared.

Any bubbles, dust, or other contaminants in the cell can cause scattering reflection that can affect data. Therefore, the Nano Stick-S includes a bubble checker (Fig. 2) that can be used to check the sample for bubbles, dust, or other contaminants before starting measurements.

Fig. 3 shows how the Nano Stick-S cell is used.

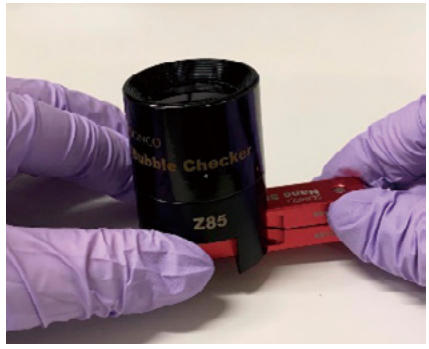


Fig. 2　Checking for Contaminants with the Bubble Checker

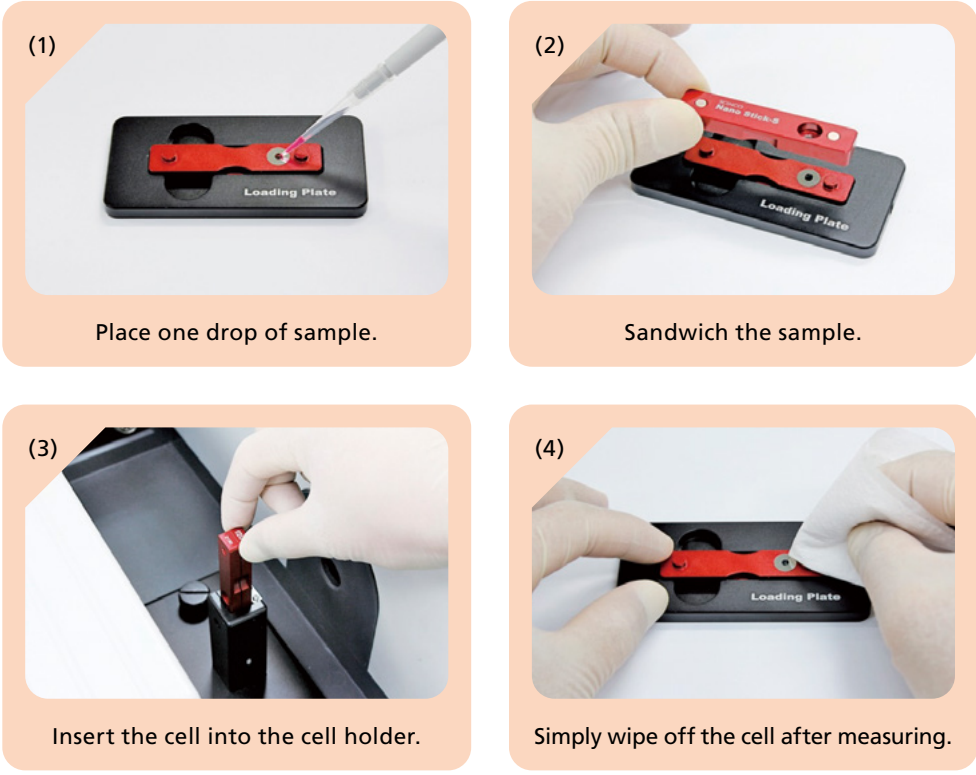


Fig. 3　How the Nano Stick-S Cell is Used

The following describes how the cell is used.
(1) Separate the cell into two parts and place one drop of sample on the sampling port.
(2) Place the upper portion on top to hold the sample sandwiched between the two sections.
(3) Insert the Nano Stick-S cell into the cell holder and measure the sample.
(4) After measuring, separate the cell into its two parts and wipe off any remaining sample from the sampling port.

3. TrayCell

The TrayCell is a measurement cell made by Hellma for extremely small quantities. Fig. 4 shows TrayCell cells and Fig. 5 illustrates their internal optics.

Fig. 4 TrayCell Cells

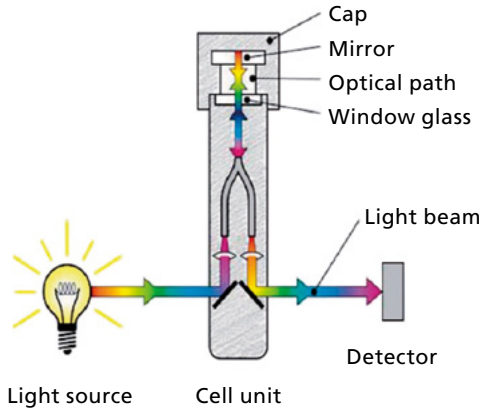


Fig. 5 Optics inside TrayCell

The TrayCell consists of a cell unit and cap, with the optical path length determined by the distance between the window glass and mirror inside the cap.

Table 2 lists the TrayCell specifications.

Table 2 TrayCell Specifications

Optical Path Length	0.1, 0.2, 1.0, 2.0 mm
Dimensions (W × D × H)	12.5×12.5×59.5 mm
Light Beam Height	15.0 mm
Minimum Sample Quantity	0.7 to 3.0 µL (0.1 mm optical path length) 0.7 to 4.0 µL (0.2 mm optical path length) 3.0 to 5.0 µL (1.0 mm optical path length) 6.0 to 10.0 µL (2.0 mm optical path length)
Window Material	Quartz
Usable Wavelength Range	190 to 1,100 nm

With the standard cap, the optical path length can be set to 0.2 mm or 1.0 mm. With the optional cap, the optical path length can also be set to 0.1 mm or 2.0 mm. The sample quantity required for measurements varies depending on the optical path length, but the minimum volume is an extremely small 0.7 µL. The quartz window plate material enables a wide wavelength measurement range, from ultraviolet to near-infrared.

Fig. 6 shows how TrayCell cells are used.

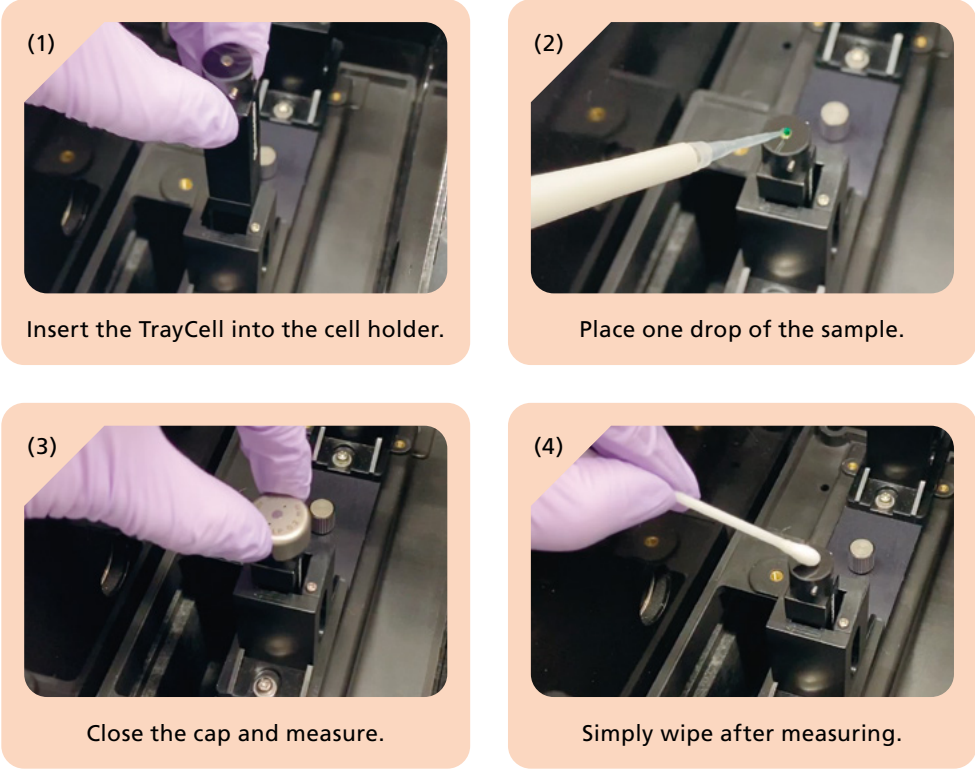


Fig. 6 How TrayCell Cells are Used

The following describes how the cell is used.
(1) Insert the TrayCell into the cell holder.
(2) Remove the cap and drip the necessary sample volume onto the cell.
(3) Attach the cap and measure the sample.
(4) After measuring, remove the cap and wipe off the sample with a cotton swab or by other means.

4. Evaluating the Quantitative Analysis Capabilities of Two Types of Micro-Volume Measurement Cells

The calibration curve linearity and repeatability of the Nano Stick-S and TrayCell cells were evaluated. Lambda-DNA is a type of double-stranded DNA. Five standard samples of Lambda-DNA with different concentrations ranging from 27.5 to 440 ng/μL were used as the measurement samples. Table 3 lists measurement conditions, Fig. 7 shows overlays of spectra measured from each concentration using the respective micro-volume measurement cells, and Fig. 8 shows the resulting calibration curves.

Table 3 Measurement Conditions

Instrument	UV-1900i UV-VIS spectrophotometer
Measurement Wavelength Range	220 to 330 nm
Data Intervals	1.0 nm
Scan Speed	Slow
Slit Width	1.0 nm
Cell Optical Path Length	0.5 mm (Nano Stick-S) 1.0 mm (TrayCell)

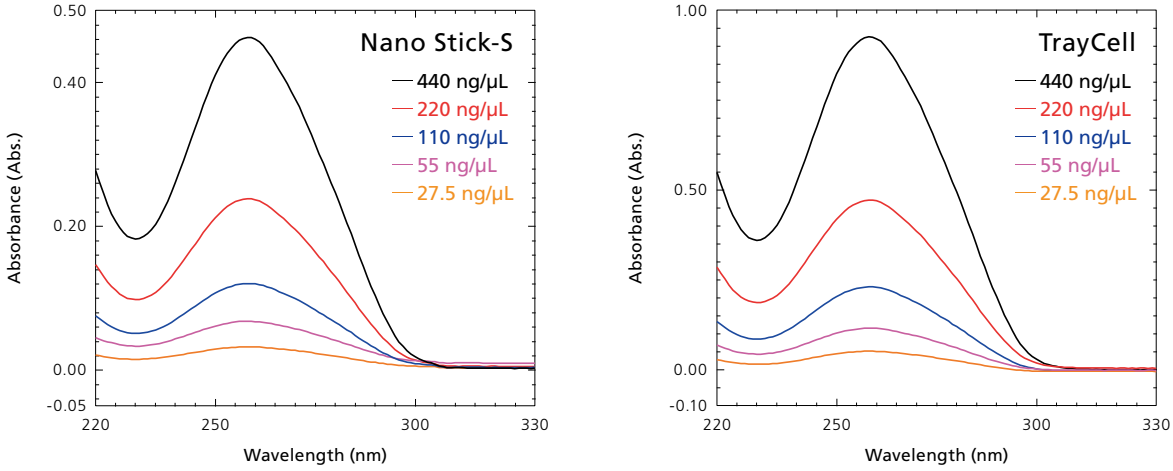


Fig. 7 Overlays of Lambda-DNA Absorption Spectra for Each Concentration

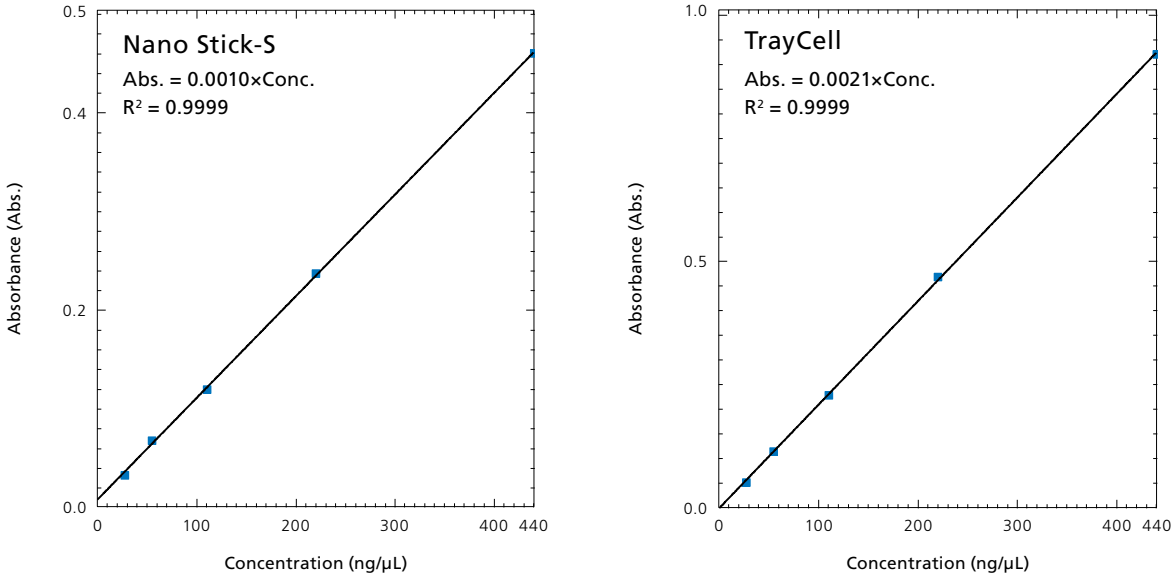


Fig. 8 Calibration Curves

Both cell types achieved a 0.9999 coefficient of determination and excellent linearity.

Table 4 shows results from 10 repeated measurements of the 440 ng/µL concentration sample. Given that the two cell types have different optical path lengths, they were evaluated based on the coefficient of variation (CV) value (standard deviation divided by the mean value), rather than the standard deviation value. Absorbance was determined by subtracting the absorbance at the baseline position (320 nm) from the absorbance value at the peak position (260 nm) (A_{260} - A_{320}).

Table 4 Repeatability Evaluation Results

No.	Nano Stick-S	TrayCell
	Absorbance Value (A_{260} - A_{320})/Abs.	
1	0.461	0.932
2	0.457	0.929
3	0.464	0.931
4	0.458	0.929
5	0.458	0.934
6	0.459	0.933
7	0.459	0.930
8	0.459	0.933
9	0.459	0.927
10	0.460	0.939
Mean Value	0.459	0.932
Standard Deviation	0.0020	0.0034
Coefficient of Variation (CV)	**0.0043**	**0.0036**

The CV values in Table 4 indicate that the TrayCell provided better repeatability than the Nano Stick-S.

5. Measuring High-Absorbance Samples

Paints, stains, printer inks, and other such samples contain large amounts of pigments that result in high absorbance values. When measuring such samples using a typical quartz cell with a 10 mm optical path length, samples must be successively diluted in multiple steps, because absorbance cannot be lowered to within the available measurement range with a single dilution. Stepwise dilution not only requires additional time and trouble, but can also introduce errors. In contrast, using a micro-volume measurement cell with a shorter optical path length, as described in this article, will result in lower peak intensity, so that even samples with high absorbance levels can be measured without multiple dilutions.

The following describes using a 10 mm quartz cell and a TrayCell (0.2 mm optical path length) to measure high-absorbance water-soluble red stain samples. Ultrapure water (Milli-Q) was used to dilute samples by 5000 times (in two steps) for the 10 mm quartz cell and by 100 times for the TrayCell before sample measurement. Table 5 lists measurement conditions and Fig. 9 shows the resulting spectra.

Table 5 Measurement Conditions

Instrument	UV-1900i UV-VIS spectrophotometer
Measurement Wavelength Range	300 to 800 nm
Data Intervals	1.0 nm
Scan Speed	Medium
Slit Width	1.0 nm
Cell Optical Path Length	10 mm (quartz cell) 0.2 mm (TrayCell)

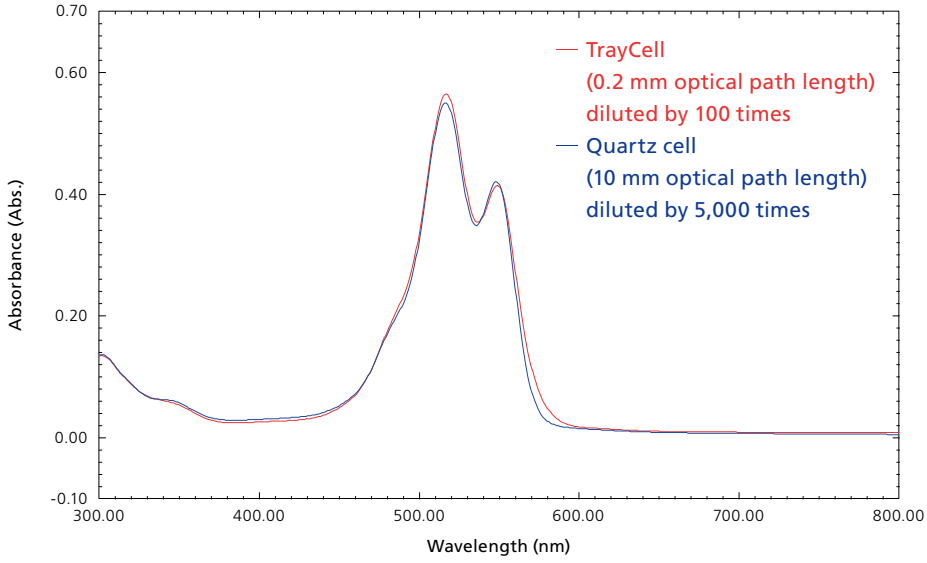


Fig. 9 Absorption Spectra of Water-Soluble Red Stain

A comparison of the spectra shows that the different dilution rates and cells have some effect on spectra, but the spectra are roughly the same.

The results show that the red stain sample measured in this example had to be diluted in two steps for the 10 mm quartz cell, whereas equivalent data could be obtained using the TrayCell with only one dilution step. However, samples containing components that are colloidally dispersed during the sample dilution process require particular caution because they can be affected by light scattering, which can elevate the baseline.

6. Summary

Table 6 summarizes the advantages of using a Nano Stick-S or TrayCell micro-volume measurement cell described in this article.

Table 6 Advantage of Nano Stick-S and TrayCell Cells

Cell	Advantages
Nano Stick-S	• Enables measurement of micro-volumes. • Measures high-absorbance samples easily. • Bubble checker enables checking for bubbles, dust, or other contaminants in cells.
TrayCell	• Enables measurement of micro-volumes. • Measures high-absorbance samples easily. • Selectable optical path length • Superior repeatability

The results showed that a Nano Stick-S or TrayCell cell can be used to achieve accurate quantitative analysis of DNA/RNA, protein, and other samples measured in life science fields and to easily measure high-absorbance samples.

Hopefully, this article will be helpful for selecting an appropriate measurement cell.

Q&A

What is the difference between absorbance and optical density (O.D.)?

The calculation method for absorbance and optical density is identical, but they are based on different concepts.

Absorbance

Absorbance is an index that indicates the amount of light a substance absorbs. When light is shone onto a substance, some of the light energy is absorbed and the rest of the light passes through the substance (Fig. 1). UV-VIS spectrophotometers calculate absorbance based on the amount of incident light (I_0) and the amount of light transmitted through the sample (I) (Eq. 1).

Absorbance = $\log_{10}(I_0/I) = \log_{10}10^{\varepsilon cL} = \varepsilon cL$ ··· Eq. 1

where ε: Molar absorption coefficient, c: Concentration (mol/L), and L: Optical path length (cm)

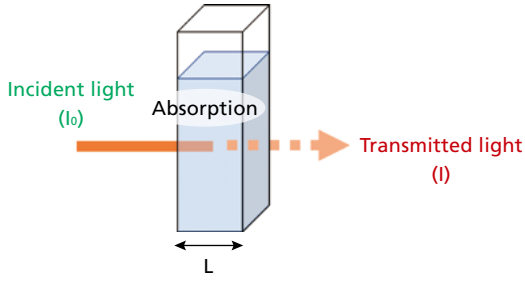


Fig. 1 Illustration of Absorbance Measurement

The absorbance values determined by equation 1 are proportional to concentration c and optical path length L (Lambert-Beer law). If a transparent sample with minimal light scattering is measured, the attenuation of incident light as it passes through the sample is assumed to be due to light absorption, but if a sample with turbidity is measured, apparent values are determined by both light absorption and light scattering.

Optical Density (O.D.)

Optical density (O.D.) is an index that indicates the amount of light scattering (turbidity) in substances. For colloidal solutions and other samples with turbidity, the attenuation of light transmitted through the sample is determined by both the light absorbed and scattered by the substance.

In other words, the O.D. value is derived from absorbance based on an optical path length of 10 mm (Eq. 2).

O.D. = Absorbance × 10/L ··· Eq. 2

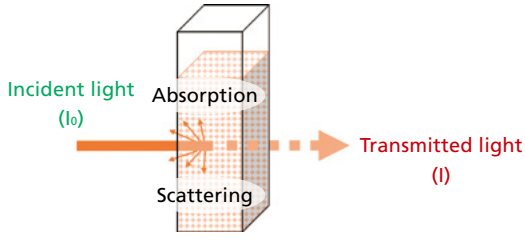


Fig. 2 Illustration of Measuring Optical Density

The index is commonly used for quantitative analysis of nucleic acids and proteins without the calibration curve method or to evaluate (compare) the properties of multiple glass materials.

PQY-01 Photoreaction Evaluation System

Lightway

Fully Automatic Measurement of Photoreaction Quantum Yield

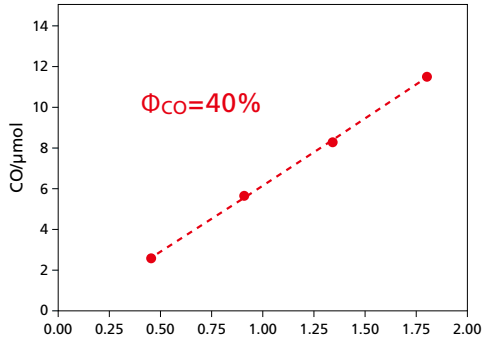
Shortens Experimental Process

The Lightway system significantly shortens time compared to conventional methods that use a chemical actinometer.

Measures Photon Count Accurately

- Calculates number of photons absorbed without using a chemical actinometer.
- Eliminates human error because no adjustments are necessary.
- Uses LED light source that provides stable output for long periods.

Using a Ru-Re Supramolecular Complex Photocatalyst to Measure Photoreaction Quantum Yield of CO_2 Reduction Reaction



Absorbed Photon Number/10^{19}

Quantity of Carbon Monoxide Generated vs. Number of Photons Absorbed

Carbon monoxide gas generated from the reduction reaction was measured using a gas chromatograph (vertical axis).
The number of absorbed photons measured using the Lightway system was plotted on the horizontal axis. The resulting slope was used to calculate the photoreaction quantum yield.
The quantum yield of CO generated from the photocatalytic reaction was 40 %.

Note: If generated products are liquid, they can be measured using a liquid chromatograph.

Source: Professor Osamu Ishitani and Assistant Professor Yusuke Tamaki of the Department of Chemistry, School of Science, Tokyo Institute of Technology

Relevant Instruments

Nexis GC-2030 Gas Chromatograph

Nexera Series Liquid Chromatograph

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