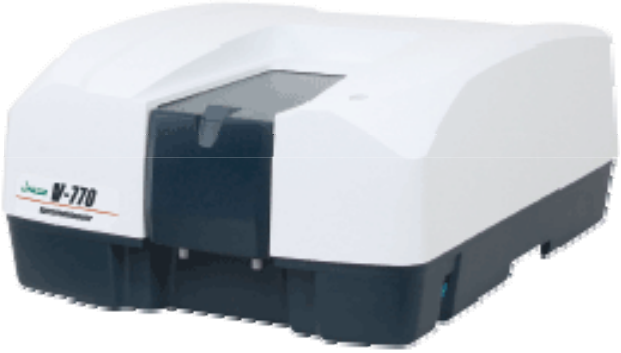
Application Note Booklet

UV Spectrometer



2710UV001

UV SPECTROMETER

CONTENTS

The Model SAH-769 One-Drop, A Dedicated Accessory for Extremely Small Amounts of Protein and Nucleic Acid Samples

JASCO introduces a new measurement method for an extremely small amount of DNA samples, or any other sample, by using the model V-730BIO spectrophotometer with the model SAH-769 One-Drop accessory.

The SAH-769 measures 5 or 0.6 µL of sample dropped on the disk cell with a 1- or 0.2- mm optical path, respectively. The precise optical path is secured by covering the liquid sample with the cover glass integrated with the unit. The cell and cover glass can be washed by simply wiping them clean with laboratory wipers. The simple method for the measurement of proteins and nucleic acids allows users to measure large numbers of samples promptly. The shorter optical path length configuration allows measurement of high concentration samples without further dilution.

The V-730BIO, the main unit of the system, utilizes a monochromator with a diffraction grating and a double-beam optical system to ensure high stability for extremely reliable measurements. The V-670BIO can be operated by the intelligent Remote Module (iRM) color touch panel control module or utilizing the cross-platform Spectra Manager software designed for the Windows operating system. In either case, the software includes standard programs for life science analyses. The [Protein/Nucleic Acid Measurement] program measures the sample absorbance at 260 and 280 nm to calculate the protein and nucleic acid ratio. The [Temperature Control Measurement] program with optional Peltier thermostatted cell holders can be utilized for the DNA melting analysis experiment.

System Configuration

V-730BIO UV/VIS Spectrometer for life science field

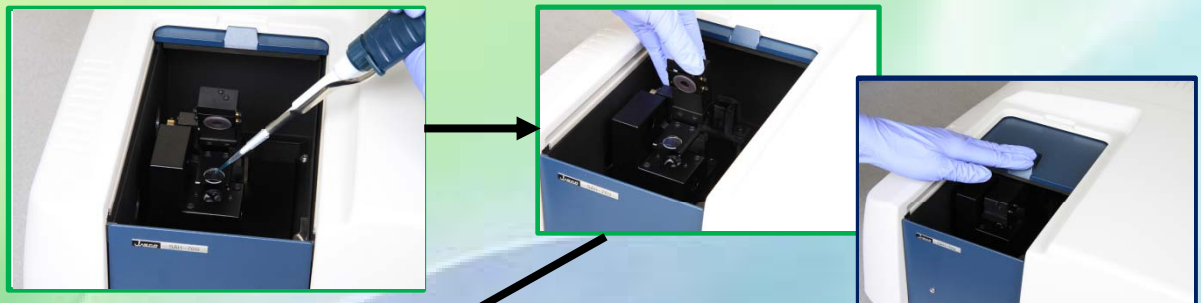
SAH-769 One drop accessory

Dedicated disk cell	1-mm optical path with 5 µL of sample volume
(Optional disk cell	0.2-mm optical path with 0.6 µL of sample volume)

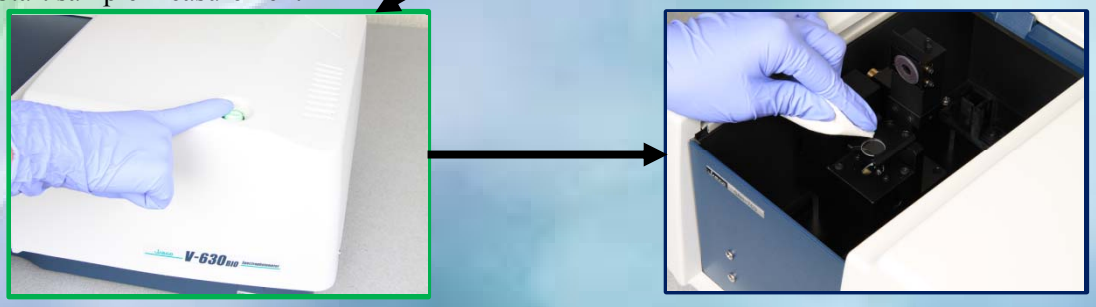
Measurement Procedure

1) Drop sample on the cell

2) Close the cover glass and the lid of sample compartment



3) Start sample measurement



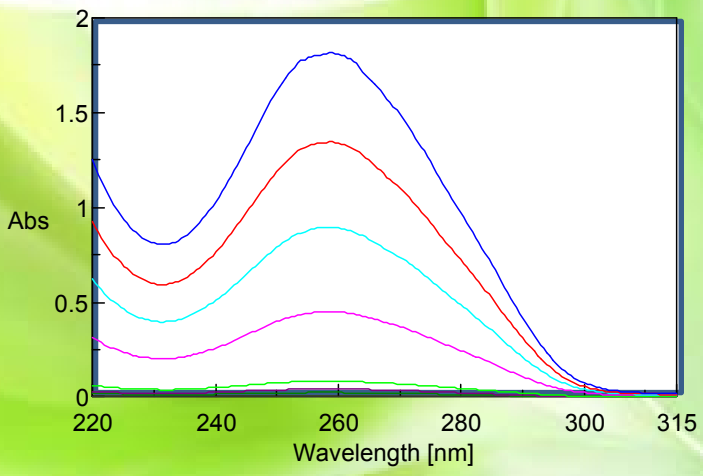
The measurement takes less than 20 seconds.

Measurement Results

Precision of Quantitative Analysis

Solutions of Calf Thymus DNA (KH_2PO_4 / NaOH buffer at pH7) at several concentrations were measured by using cells with 1-mm and 0.2-mm optical paths. The spectra (collected with identical instrument parameters) using each cell are illustrated in Figures 1 and 2. The graphs 1 and 2 illustrate the calibration curves created using the absorbance maxima at 260 nm. Both calibration graphs demonstrate good linearity.

Disk cell with a 1-mm optical path



Measurement Parameters
Data interval: 0.5 nm
Measurement range: 220 to 315 nm
Band width: 1 nm
Response: Medium
Scan Speed 200 nm/min

Figure 1. Absorbance spectra of DNA solution [optical path: 1 mm]

Graph 1 Calibration Curve [Optical path : 1 mm]

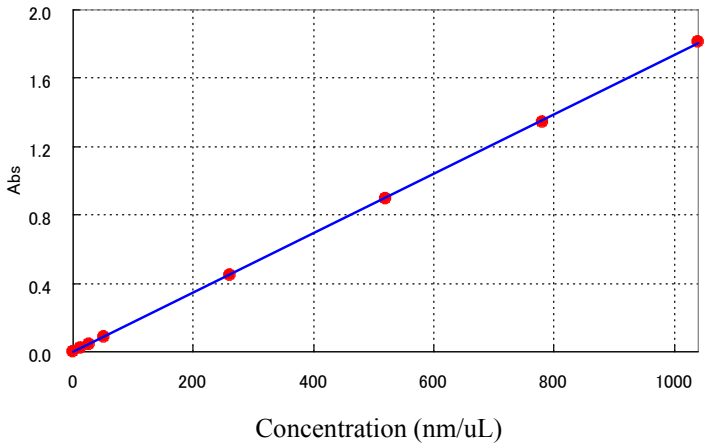


Table 1 Sample Conc. and Abs [OP: 1mm]

Legend	Conc. [ng/μL]	Abs
—	0	0.0005
—	13	0.0228
—	26	0.0417
—	52	0.0838
—	260	0.4500
—	520	0.8970
—	780	1.3443
—	1040	1.8137

y = 0.0017x - 0.0032	
Correlation coefficient	1.0000
Standard deviation	3.09

Disk cell with a 0.2-mm optical path

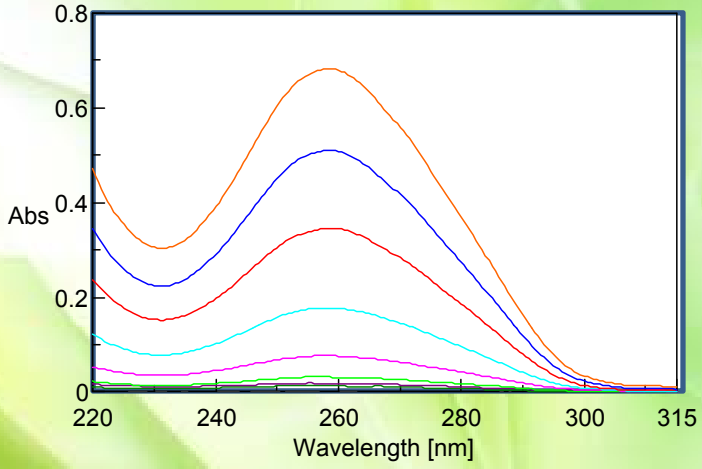


Figure 2 Absorbance spectra of DNA solution [optical path: 0.2 mm]

Graph 2 Calibration Curve [Optical Path: 0.2 mm]

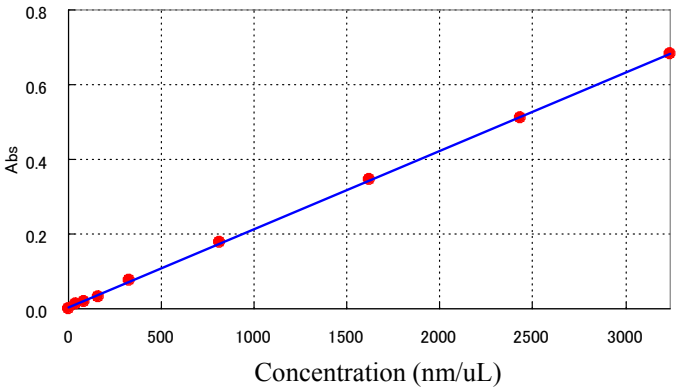


Table 2 Sample Conc. and Abs [OP: 0.2 mm]

Legend	Conc. [ng/µL]	Abs
—	0	-0.0009
—	40.5	0.0134
—	81	0.0180
—	162	0.0313
—	324	0.0766
—	810	0.1774
—	1620	0.3456
—	2430	0.5103
—	3240	0.6823

y = 0.0002x + 0.0029	
Correlation coefficient	0.999880002
Standard deviation	18.4930641

Contamination of Samples

The disk cell and cover glass were wiped clean after measuring the absorbance of a 5-µL high concentration DNA sample. Then, a 5 µL solvent was measured to evaluate sample cross-contamination of the disk cell. The results indicated in Table 3 indicate the wiping is enough to wash the sample from the cell.

Table 3: Carry over of DNA sample

	DNA sample		Solvent
Absorbance	1.8832	→	0.0002

Wiping sample

* Detection and quantitation limits are calculated as 3.3σ and 10σ, respectively.

Measurement reproducibility using SAH-769 one-drop measurement unit

Introduction

SAH-769 one-drop measurement unit is the premier accessory for multiple-sample quantitation of proteins and nucleic acids. Simply dispense one drop of sample (5 µL for 1 mm path length; 0.6 µL for 0.2 mm path length) on the cell, set the cover glass and start the measurement.

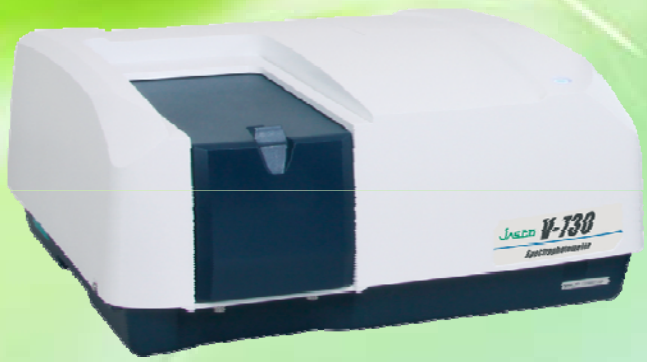
Here, we present the measurement reproducibility of calf thymus DNA using SAH-769 one-drop measurement unit with 1 and 0.2 mm path length cells.

Keywords

One-drop measurement, Protein, Nucleic acid, Reproducibility, Detection limit, Quantitation limit

Measurement system

V-730BIO UV/VIS Spectrometer for life science field
SAH-769 One drop accessory
Dedicated cells: 1 mm cell (minimum sample volume: 5 µL)
0.2 mm cell (minimum sample volume: 0.6 µL)



V-730 spectrometer

SAH-769 One drop accessory

Sample

Aqueous solution of calf thymus DNA

Procedure

A drop of sample was dispensed and measured, and then the measured sample was wiped from the cell. This procedure was repeated ten times.

Parameters

Wavelength: 260 nm
Bandwidth: 1 nm
Response: Medium

Results

1 mm path length cell

Table 1 Reproducibility of absorbance

Conc. [ng/µL]	0	2.4	4.8	9.6	19.3	38.6	77.2	154.4	308.8	617.5
1	-0.0008	0.0047	0.0084	0.0179	0.0321	0.0676	0.133	0.259	0.516	0.998
2	0.0003	0.0059	0.0093	0.0146	0.0332	0.0677	0.131	0.260	0.518	1.003
3	0.0012	0.0056	0.0083	0.0162	0.0334	0.0705	0.130	0.260	0.519	1.003
4	0.0015	0.0063	0.0072	0.0181	0.0345	0.0679	0.130	0.262	0.512	1.024
5	-0.0002	0.0067	0.0071	0.0166	0.0329	0.0676	0.132	0.264	0.517	0.993
6	-0.0013	0.0053	0.0088	0.0204	0.0331	0.0672	0.130	0.259	0.522	0.996
7	0.0013	0.0049	0.0082	0.0170	0.0336	0.0695	0.129	0.259	0.511	0.996
8	0.0002	0.0036	0.0089	0.0168	0.0326	0.0680	0.133	0.260	0.509	1.006
9	0.0027	0.0058	0.0089	0.0177	0.0315	0.0676	0.134	0.260	0.509	1.000
10	-0.0004	0.0043	0.0069	0.0153	0.0353	0.0692	0.132	0.267	0.509	0.995
A.V. [Abs]	0.0004	0.0053	0.0082	0.0171	0.0332	0.0683	0.131	0.261	0.514	1.001
S.D.	0.0012	0.0010	0.0008	0.0016	0.0011	0.0011	0.0015	0.0026	0.0047	0.0089
C.V. [%]	N/A	17.9	10.3	9.6	3.3	1.6	1.2	1.0	0.9	0.9

Calibration equation: Abs = 0.00163 x Conc. + 0.00366

Correlation coefficient: 0.9998

Table 2 Reproducibility of concentration

Conc. [ng/µL]	0	2.4	4.8	9.6	19.3	38.6	77.2	154.4	308.8	617.5
1	-2.8	0.6	2.9	8.8	17.5	39.3	79.8	157.3	315.0	611.5
2	-2.1	1.4	3.4	6.7	18.2	39.4	78.0	157.7	316.3	614.6
3	-1.5	1.2	2.9	7.7	18.3	41.1	78.0	157.9	316.9	614.7
4	-1.3	1.7	2.2	8.9	18.9	39.5	77.7	158.8	312.4	627.6
5	-2.4	1.9	2.1	8.0	18.0	39.3	78.7	160.0	316.0	608.8
6	-3.1	1.0	3.1	10.3	18.1	39.1	77.5	157.0	318.7	610.4
7	-1.4	0.7	2.8	8.2	18.4	40.5	77.4	157.1	312.0	610.3
8	-2.1	0.0	3.2	8.1	17.8	39.6	79.3	157.8	311.1	616.5
9	-0.6	1.3	3.2	8.6	17.1	39.3	80.0	157.4	310.9	612.8
10	-2.5	0.4	2.0	7.1	19.5	40.3	78.9	162.2	311.0	609.9
A.V. [ng/µL]	-2.0	1.0	2.8	8.2	18.2	39.8	78.5	158.3	314.0	613.7
S.D.	0.75	0.59	0.52	1.00	0.68	0.66	0.95	1.63	2.88	5.48
C.V. [%]	N/A	57.4	18.7	12.2	3.7	1.7	1.2	1.0	0.9	0.9

Detection limit*: 5 ng/µL

Quantitation limit*: 10 ng/µL

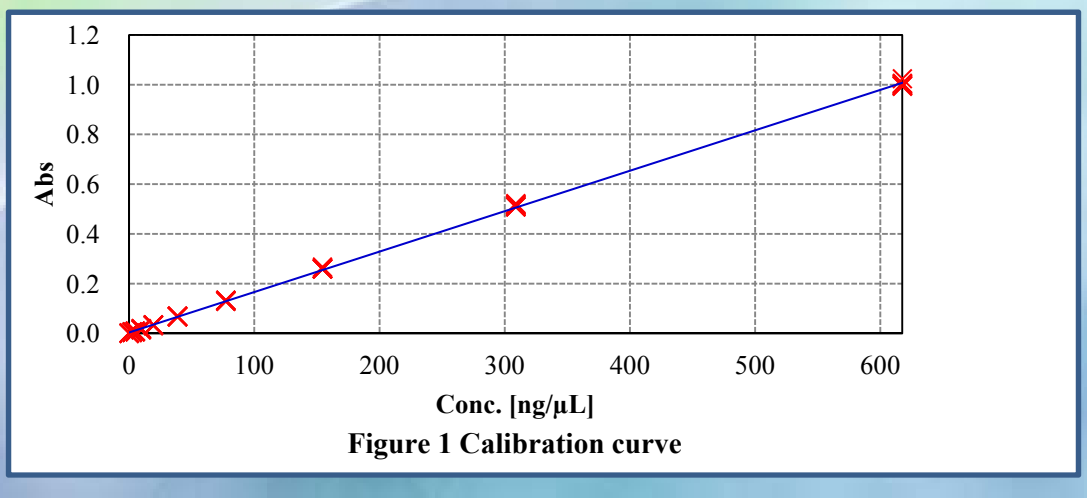


Figure 1 Calibration curve

0.2 mm path length cell

Table 3 Reproducibility of absorbance

Conc. [ng/µL]	0	38.6	77.2	154.4	308.8	617.5	1235	2470	4940
1	0.0008	0.0122	0.0196	0.0460	0.0842	0.166	0.326	0.646	1.297
2	-0.0011	0.0165	0.0223	0.0430	0.0847	0.166	0.330	0.645	1.319
3	-0.0001	0.0160	0.0195	0.0427	0.0873	0.170	0.328	0.653	1.320
4	-0.0004	0.0112	0.0176	0.0430	0.0863	0.165	0.331	0.654	1.284
5	0.0069	0.0152	0.0180	0.0410	0.0860	0.168	0.327	0.647	1.307
6	-0.0005	0.0139	0.0184	0.0424	0.0845	0.170	0.326	0.660	1.303
7	0.0006	0.0137	0.0206	0.0450	0.0839	0.170	0.331	0.659	1.312
8	0.0019	0.0127	0.0200	0.0440	0.0875	0.168	0.330	0.658	1.301
9	0.0011	0.0130	0.0212	0.0435	0.0851	0.169	0.338	0.663	1.296
10	0.0007	0.0134	0.0194	0.0426	0.0879	0.171	0.326	0.664	1.308
A.V.	0.0010	0.0138	0.0197	0.0433	0.0857	0.168	0.329	0.655	1.305
S.D.	0.0023	0.0017	0.0015	0.0014	0.0015	0.0020	0.0037	0.0071	0.0110
C.V.	N/A	12.2	7.4	3.3	1.7	1.2	1.1	1.1	0.8

Calibration equation: Abs = 0.000264 x Conc. + 0.00281
Correlation coefficient: 0.9999

Table 4 Reproducibility of concentration

Conc. [ng/µL]	0	38.6	77.2	154.4	308.8	617.5	1235	2470	4940
1	-7.8	35.5	63.7	163.8	308.5	617.6	1224	2438	4909
2	-15.0	51.9	74.0	152.4	310.6	620.2	1242	2434	4991
3	-11.1	50.0	63.5	151.2	320.4	635.6	1233	2467	4993
4	-12.0	31.9	56.3	152.3	316.8	616.2	1246	2468	4856
5	15.7	46.8	57.5	145.0	315.3	625.2	1228	2441	4946
6	-12.4	41.9	59.3	150.3	309.7	635.3	1227	2493	4932
7	-8.4	41.2	67.4	160.1	307.6	632.5	1245	2489	4963
8	-3.4	37.5	65.0	156.1	321.3	625.1	1241	2484	4924
9	-6.4	38.6	69.9	154.4	312.0	629.5	1271	2503	4904
10	-8.1	40.1	63.0	150.9	322.6	637.3	1227	2505	4950
A.V.	-6.9	41.5	63.9	153.7	314.5	627.5	1238	2472	4937
S.D.	8.60	6.36	5.51	5.34	5.58	7.77	14.19	27.04	41.61
C.V.	N/A	15.3	8.6	3.5	1.8	1.2	1.1	1.1	0.8

Detection limit*: 50 ng/µL
Quantitation limit*: 100 ng/µL

*The detection limit is calculated using 3.3σ. The quantitation limit is calculated using 10σ.
σ is the standard deviation in 0 ng/µL of a sample concentration.

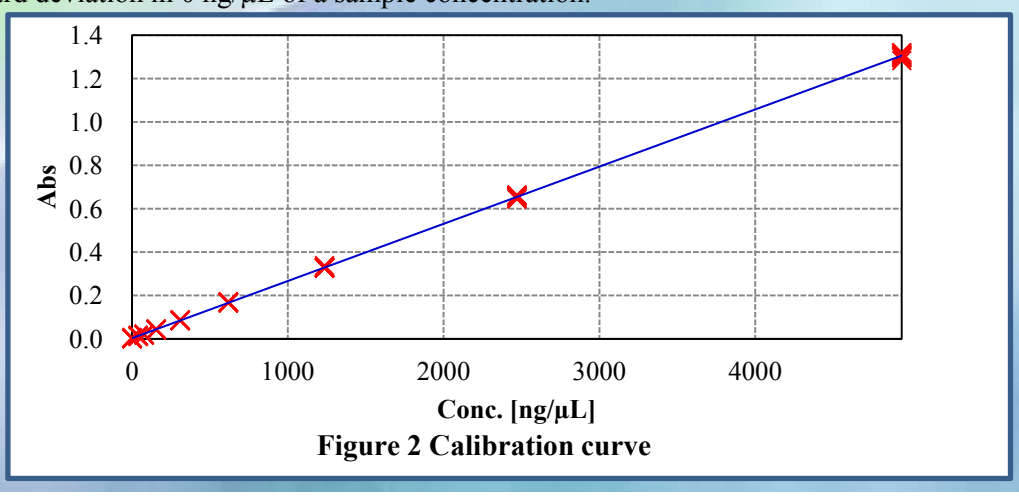


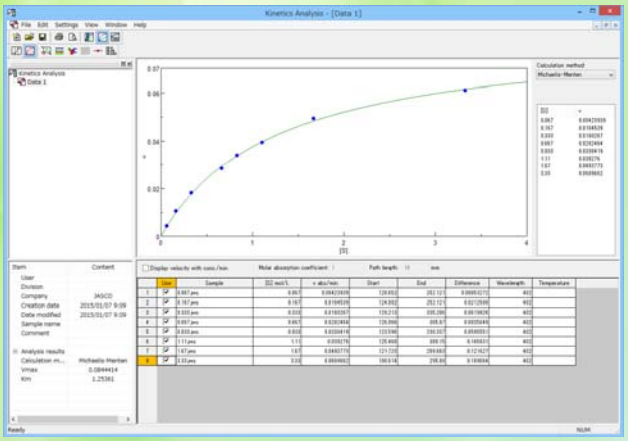
Figure 2 Calibration curve

Measurement of ALP Activity using the Kinetics Analysis System

Introduction

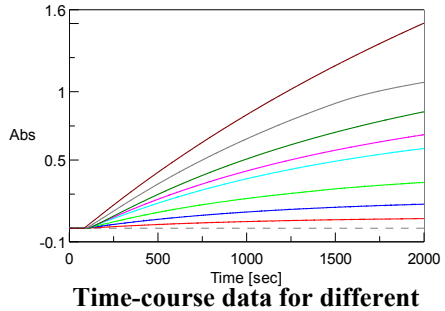
The kinetics analysis system can evaluate enzyme activity, the Michaelis constant (K_m), the maximum velocity (V_{max}) and the enzyme inhibition constant, and create a kallidinogenase activity table based on the Japanese Pharmacopoeia in this application

V-730 UV/Vis spectrophotometer
STR-773 Water thermostatted cell holder with stirrer
CSP-909 Sample holder cover with syringe port

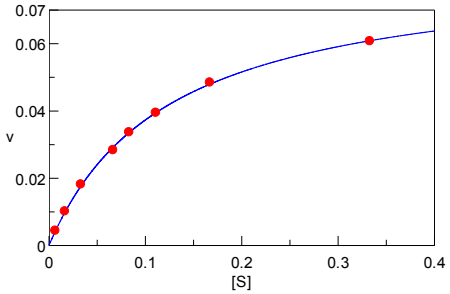


[Kinetics Analysis] program

The enzyme alkali phosphatase (ALP) catalyzes the hydrolysis of *p*-nitrophenylphosphate to an inorganic phosphate and *p*-nitrophenol. Since the *p*-nitrophenol exhibits a stable yellow in an alkali solution, a time-course measurement was performed at the wavelength where the maximum absorption occurred. The ALP activity was calculated by plotting the initial velocity v (slope) against the substrate concentration [S].



Time-course data for different substrate concentrations



Michaelis-Menten plot

Analysis results

V_{max}	0.0835
K_m	0.1238

System	Model No.	Product name		Remarks
Instrument	V-730ST	UV/Vis spectrophotometer		Model V-730DS is shipped with a PC.
Accessory	STR-773	Water thermostatted cell holder with stirrer		
Optional program	CSP-909	Sample holder cover with syringe port		
Optional program	VWKN-772	Kinetics analysis program		
Description	A syringe is separately required. Applicable syringe: Micro syringe with 2 inch (approx. 50 mm) needle length Program functions: Enzyme activity calculation, Km/Vmax calculation, enzyme inhibition constant calculation, and activity table determination			

DNA Melting Measurement with the PAC-743/743R Water-cooled Peltier Cell Changer Measurement and temperature sensor in Cell

For the melting measurement of DNA samples, a temperature sensor can be inserted into the sample cells and the actual temperatures of samples plotted on the horizontal axis in order to increase the accuracy of the temperature readings for the melting experiment. This measurement technique is easy to be applied for 10-mm rectangular cells with a larger sample volume. However, for cells with a small sample volume such as the 8-position micro cell (100µl), a temperature sensor blocks the instrument optical path. It is then difficult to obtain both absorbance and temperature of a sample simultaneously.
Here, a DNA measurement example using the 8-position micro cell with a temperature sensor is outlined. By using one of the 8-position micro cells as a temperature monitor (Figure 1), the horizontal axis of the temperature course data can be plotted with actual temperatures obtained by the sensor. This increases the temperature accuracy of measurements with the 8-position micro cell.

Measurement System
V-730 UV/VIS Spectrometer
PAC-743 water-cooled Peltier cell changer
8-position micro cell block
8-position micro cell
Silicon cap
Cap pressure fixture
Sensor in cell

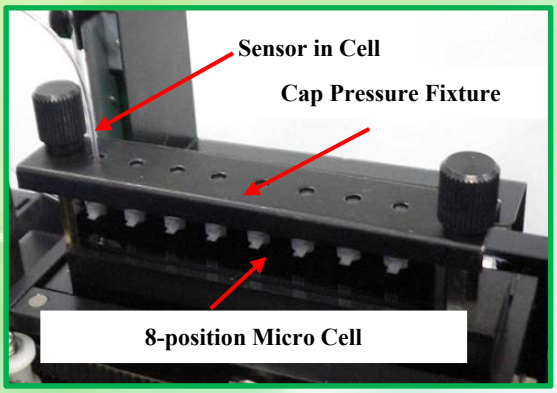


Figure 1: Sample Setting

Measurement Program
VWTP-959 Temperature Gradient Measurement Program

Sample
Poly (dA-dT)-Poly(dA-dT) pH7 KH_2PO_4-NaOH buffer solution (20 µg/mL)


Figure 2: Silicon Cap

Measurement Parameters
Number of cells: 7 *1)
Temperature control sensor: holder
Temperature monitor sensor: cell 8 *2)
Start condition: Keep within +/- 0.01-C of the target temperature for 3 seconds
Data interval: 1 ºC (20-50 ºC), 0.1-C (50-70 ºC), 1-C (70-100 ºC)
Ramp rate: 2 ºC/min
Response: 0.24sec
Measurement wavelength: 260 nm
Reverse temperature measurement: ON

*1) Cell 8 was used only for temperature monitor. Absorbance was not measured.
*2) A silicon cap with a hole illustrated in Figure 2 was used for the temperature monitor cell. Silicon caps without holes were used for cells 1 to 7.

Results

Measurement results are illustrated in Figure 3. The horizontal axis of the graph was plotted versus the values of the temperature monitor sensor in cell 8. The sample measurement required a total of 5 hours; 2.5 hours for the temperature increase data and 2.5 hours for the reverse temperature course. Table 1 indicates the melting temperature calculated from the temperature course data outlined in Figure 3. These results demonstrate melting temperatures for the various cells from 61.8 to 62.2ºC (Ave. of 6.20ºC), with a standard deviation of 0.13 ºC, and a coefficient of variance of 0.20%.

The same measurement was performed while obtaining sample temperatures using the standard temperature sensor (the holder sensor) within the PAC-743/743R accessory. Table 2 records the melting temperatures calculated from the temperature data using the holder sensor. Measurement results from this experiment provide a melting point varying from 63.0 to 63.3˚C that is around one degree higher than the temperature obtained when using the sensor in the sample cell. These results indicate that the temperature of the holder was around one degree higher than the actual temperature of the sample. On the other hand, the standard deviation and coefficient of variance are the same for both measurements. Considering these results, the holder sensor offers sufficient capability to measure a reproducible melting temperature for all sample cells. However, to obtain the absolute value for sample melts, a cell temperature sensor is highly recommended.

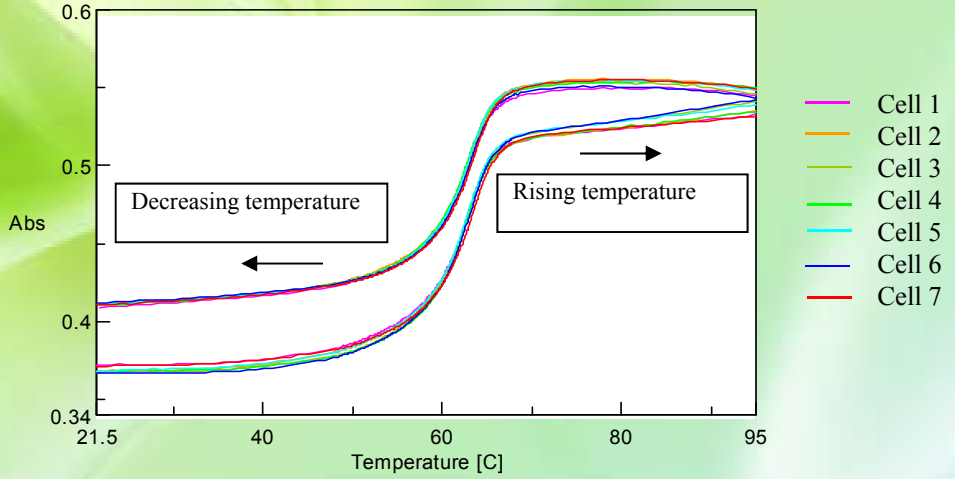


Figure 3: Temperature Course Data

Table 1: Melting Temperature (sensor in cell)

	Temperature (°C)	
	Rising	Falling
Cell 1	62.0	61.9
Cell 2	62.0	62.1
Cell 3	61.9	62.1
Cell 4	61.9	62.0
Cell 5	61.8	61.9
Cell 6	62.0	61.9
Cell 7	62.2	62.3
Ave.	62.0	62.0
Std. Dev.	0.13	0.13
C. V.	0.20	0.20

Table 2: Melting Temperature (holder sensor)

	Temperature (°C)
Cell 1	63.3
Cell 2	63.0
Cell 3	63.2
Cell 4	63.0
Cell 5	63.1
Cell 6	63.1
Cell 7	63.1
Cell 8	63.3
Ave.	63.1
Std. Dev.	0.13
C. V.	0.20

DNA Melting Measurements

with the PAC-743/ 743R Water-cooled Peltier Cell Changer "1 mm 10 µL 8-Position Micro Cell Block"

When performing a DNA Melting measurement, most of the samples are only available in extremely small amounts. Due to a limited amount of sample, it is essential that the amount used for measurement/analysis purposes be as little as possible. However, when sampling a small amount in a high temperature range, volatilization of the sample occurs, frequently complicating the analysis process.

Additionally, in order to increase the accuracy of the temperature readings of the samples, one of the 8-Position Micro Cell Block cells is utilized for the dedicated temperature monitoring so that the temperature in a cell can be used in the melting data.

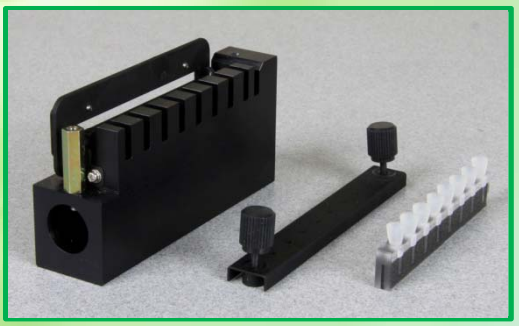


Figure 1. 8-position automatic cell changer (Left) / Lid retainer (Center) / cell and silicon lid (Right)

Measurement Systems
V-730 UV/VIS Spectrometer
PAC-743 Water-cooled Peltier Cell Changer
1 mm 8-position micro cell block
1 mm 8-position micro cell
Silicon Cap (attached to 1 mm 8-position micro cell block)
Cap fixture (attached to 1 mm 8-position micro cell block)

Measurement program
VWTP-959 [Temperature Ramping Measurement /Melting Analysis] Program

Sample
Poly (dA-dT)
Poly (dA-dT) pH7KH2PO4-NaOH buffer solution (200 µg/mL)

Measurement Parameters

Start condition:	Keep within ±0.10 ºC of the temperature setting for 3 seconds	
Data interval:	1 ºC (20 - 50 ºC), 0.1 ºC (50 - 70 ºC), 1 ºC (70 - 100 ºC)	
Temperature gradient:	2 ºC / min	
Response:	Fast	
Wavelength for measurement:	260 nm	
	Holder sensor:	Internal Cell Sensor:
Number of cells:	8	7
Temperature control sensor:	holder	holder
Temperature monitor sensor:	holder	cell (8)

Results

The melting curves from the results of sample measurements with all eight micro cell using the holder sensor are plotted as shown in Figure 2. The time required for the measurement was totally 2.5 hours and the changes of samples volume during the measurement process are shown in Figure 3. During the measurement, Nujol was placed on top of the sample cells to prevent the sample from adhering to silicon caps.

After completion of the measurement of samples, the solution levels for each of the 8 cells were higher than the upper limit of cells (indicated using a red dotted line), and the decrease in sample volume were almost not observed by the human eye. In short, by using the silicon cap and cap fixture, volatilization of samples can be prevented.

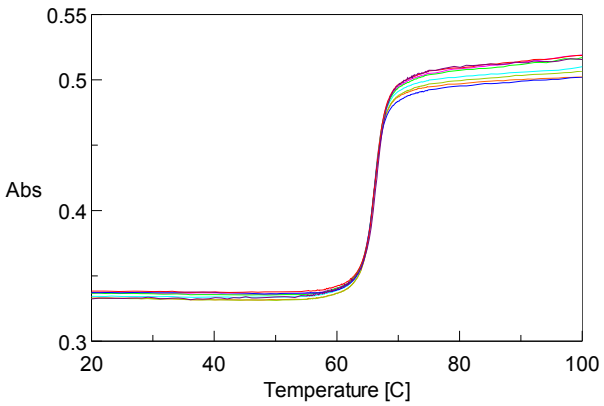


Figure 2. Melting curve data
[Plotted by using Holder Sensor]

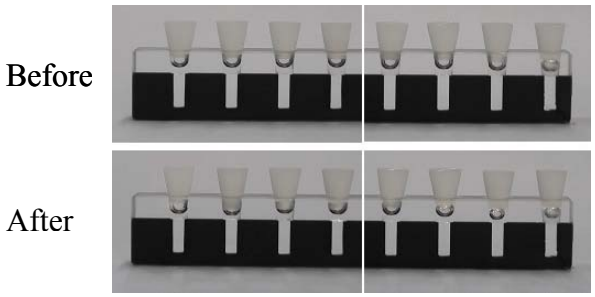


Figure 3. Change in sample volume before and after measurement
[Cell 1 from left]: Upper limit of cell

In order to enhance the accuracy of the temperature, one of the eight cells (referred to as "cell 8") was used exclusively to monitor the sample temperature. Figure 4 shows a result of melting curves using the temperature readings from internal cell sensor. These temperature values were plotted in the horizontal axis in Figure 4 using data collected from internal cell sensor in cell 8. No evaporation was observed in this case.

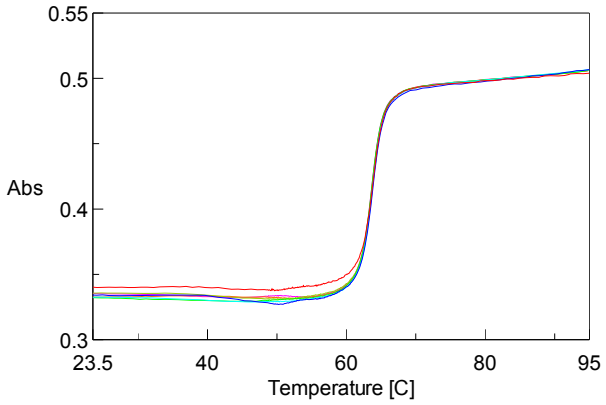


Figure 4. Melting curve data
[Plotted by using Internal Cell Sensor]

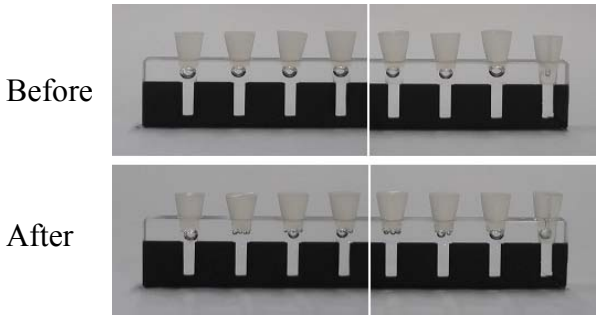


Figure 5. Change in sample volume before and after measurement
[Cell 1 from left]: Upper limit of cell

The results of the melting points, calculated from the melting curves data in Figure 2 and 4, are shown in Table 1 and 2 below. The results using the holder sensor are shown in Table 1 ranging melting temperature between 66.0 ºC ~66.2 ºC (average 66.1 ºC) with standard deviation of 0.08 ºC and coefficient of variance of 0.13%. On the other hands, the results using internal cell sensor are shown in Table 2 ranging melting temperatures between 63.6 ºC ~ 63.8 ºC (average 63.7 ºC) with standard deviation of 0.08 ºC and coefficient of variance of 0.12%. This indicates that the temperature using the holder sensor was approximately 3.5 ºC higher than using the internal cell sensor. From these data, it can be concluded that the actual temperature of the holder was 3.5 ºC higher than the temperature of the original sample in the cell, while both the standard deviation and coefficient of variance had no major differences in their result.

In conclusion, to compare the melting temperatures relatively between each sample, the holder sensor is believed to be sufficient, while the internal cell sensor in the sample cell is ideal for measuring the absolute value of melting temperatures.

Table 1. Melting temperature [Holder Sensor]

	Temp [ºC]
Cell 1	66.1
Cell 2	66.0
Cell 3	66.0
Cell 4	66.1
Cell 5	66.1
Cell 6	66.0
Cell 7	66.2
Cell 8	66.2
Ave.	66.1
S.D.	0.08
C.V.	0.13

Table 2. Melting temperature [Internal cell Sensor]

	Temp [ºC]
Cell 1	63.6
Cell 2	63.6
Cell 3	63.6
Cell 4	63.6
Cell 5	63.7
Cell 6	63.7
Cell 7	63.8
Ave.	63.7
S.D.	0.08
C.V.	0.12

DNA melting by One Drop measurement using capillary jacket

Intruduction

Melting measurement is one of remarkable measurement methods in Biotechnology field such as DNA melting and thermal denaturation of Proteins. For those measurements, 10mm rectangular type cell has been generally used, which requires large volume of sample for each measurement.

By using JASCO's water-cooled Peltier thermostatted cell holder PAC-743/743R with micro 8-position cell it is possible to measure sample with volume as small as 10 µL *, however, for measurement of sample with much lower volume, it has been wished for a long time to have an accessory enabling such requirement.

*Refer to DNA melting measurement #3 in using of PAC-743/743R No.090911-012.

As one of solutions, JASCO introduces an application "DNA melting by One Drop measurement method" using JASCO V-730 BIO Spectrophotometer with capillary jacket for melting measurement, capillary and adaptor.

Capillary jacket for melting measurement

Capillary is disposal type quartz glass cell, whose optical pathlength is 0.5 mm and minimum sample volume is 3 µL. After sampling by a capillary phenomenon, both edges of capillary are lapped by seal, which helps to avoid volatilization of sample. Such capillary is inserted into capillary jacket for melting measurement to be mounted to 6 channel cell block of Peltier cell holder or to PAC-743/ PAC 743R Water-cooled Peltier thermostatted cell holder. This capillary jacket has temperature sensor insertion port and temperature measurement in this port helps to measure accurate actual temperature of sample.

Fig.1 Capillary

Fig.2 Capillary jacket (L) and Adaptor (R) for melting measurement.

Measurement System

V-730 BIO	UV/VIS Spectrophotometer
ETCS-761	Water-cooled Peltier thermostatted cell holder with stirrer
OPS-515	Temperature sensor assy.
MCB-100	Mini water circulation bath

Capillary jacket for melting measurement, adaptor, capillary, seal material.

Measurement Program

Temperature ramping / DNA melting program (Standard software of V-730 Bio)

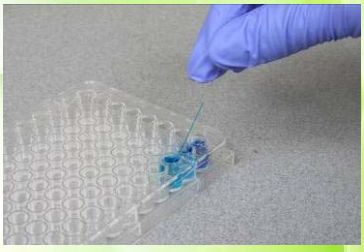
Sample

Poly (dA-dT)-Poly(dA-dT) pH7 phosphoric acid buffer

Measurement Condition

Start setting:	3 seconds in the set temperature +/- 0.10 degrees C
Data acquisition interval:	0.1 degrees C
Temperature gradient:	1 degrees C / min
Response:	0.24sec
Measurement wavelength:	260 nm

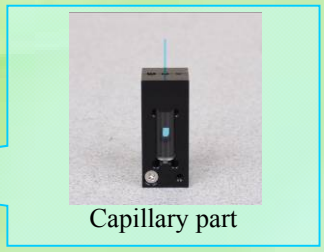
Measurement Steps



Sampling by using capillary

Sealing both edges

Setting capillary jacket into cell holder



Capillary part

Setting temp. sensor and capillary into jacket

Result

Fig. 3 shows the result of measurement of Poly (dA-dT)-Poly(dA-dT).

The green spectrum is the measurement result data by using 10 mm rectangle type cell and blue one is the result data by using capillary cell. Temperature is measured by the temperature sensor inside of capillary jacket.

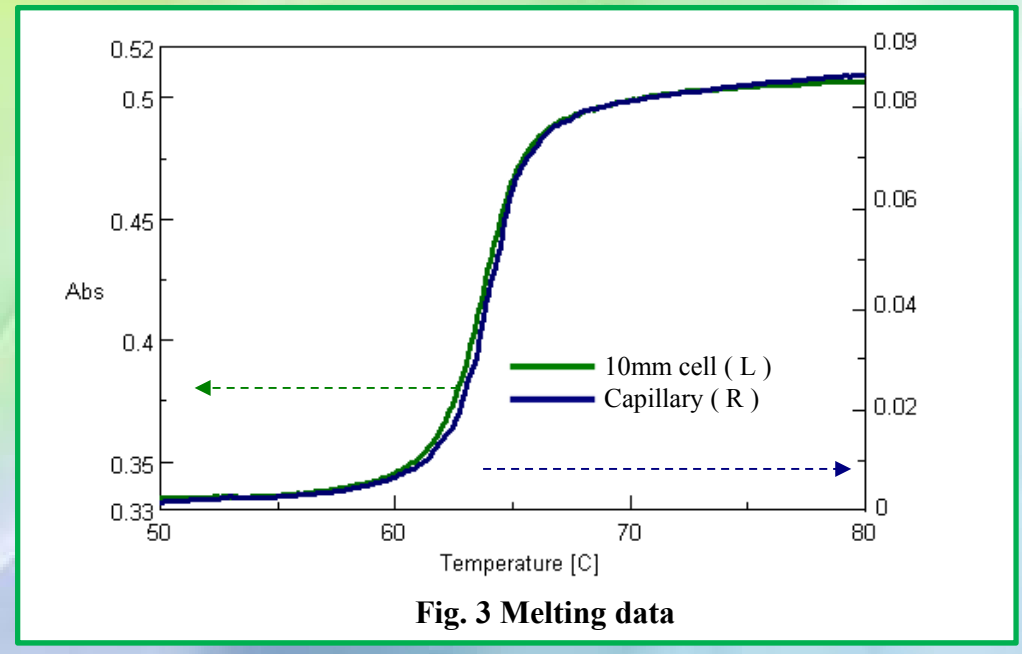


Fig. 3 Melting data

As the result of calculation based on above data for melting temperature, the similar results have been obtained for both measurement, such as 63.8 degrees C in 10 mm rectangular type cell and 63.9 degrees C in capillary cell respectively. It obviously proves that the measurement using small volume capillary is as reliable as measurement by general method using 10 mm cell.

Measurement of the reduction reaction of 2,6-dichloroindophenol (DCIP) using the absorption stopped-flow system

Introduction

Using the absorption stopped-flow measurement system, consisting of FS-110 fast scan spectrophotometer and SFS-852 stopped-flow system, two to four kinds of liquid sample can be mixed quickly and the changes in absorption spectra can be measured at intervals of 5 msec. This system allows measurement of rapid enzymatic, catalytic and oxidation-reduction reactions.

This application note illustrates an example of determining the reaction rate by using the absorption stopped-flow system for the reduction of DCIP, whose color in aqueous solution is changed from blue to colorless as a result of reaction with *L*-ascorbic acid.

Keywords Stopped-flow, Reaction rate, Fast scan spectrophotometer

Measurement and analysis system

Absorption stopped-flow measurement system
- FS-110 fast scan spectrophotometer
- SFS-852 stopped-flow system
- Stopped-flow measurement program
- Reaction rate calculation program

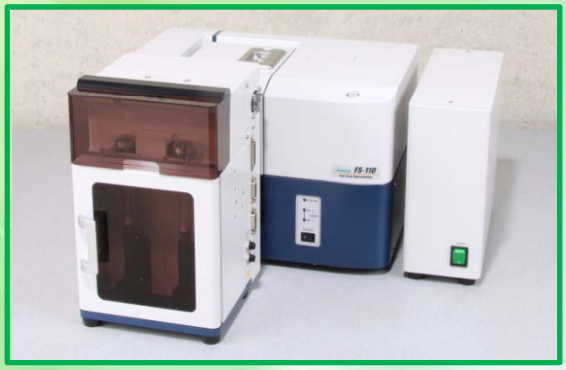


Figure 1 Absorption stopped-flow system

Samples

- 20 mmol/L *L*-ascorbic acid aqueous solution (Dissolve the *L*-ascorbic acid with NaOH/Na_2HPO_4 aqueous solution and make it to a constant volume. Then, adjust the pH to 7.6.)
- 1 mmol/L DCIP aqueous solution

Measurement conditions

Spectrophotometer		Stopped-flow system	
Optical pathlength:	2 mm	Time of solution sending:	10 msec
Wavelength range:	300 to 800 nm	Mixing ratio:	1:1
Data interval:	1 nm	Volume of solution sending:	50 µL
Measurement interval:	0.010 sec	The measurement is started when the syringe is stopped.	
Measurement time:	0 to 3 sec		

Results

Figure 2 shows the 3D spectra of the sample. When the reaction is started, the spectrum indicates an absorption maximum at approximately 600 nm and the sample exhibits a blue color. Then, the absorbance in the visible wavelength range changes to approximately zero within 1 sec after starting the measurement, and the sample turns colorless.

Figure 3 shows the time course data for absorbance at the absorption maximum (604 nm) and the curve fitted to the data between 0.03 and 2.00 sec for the reaction range, assuming the reaction to be a primary reaction. The fitted curve is in excellent agreement with the measurement results. A reaction rate of 4.3 sec^{-1} was calculated.

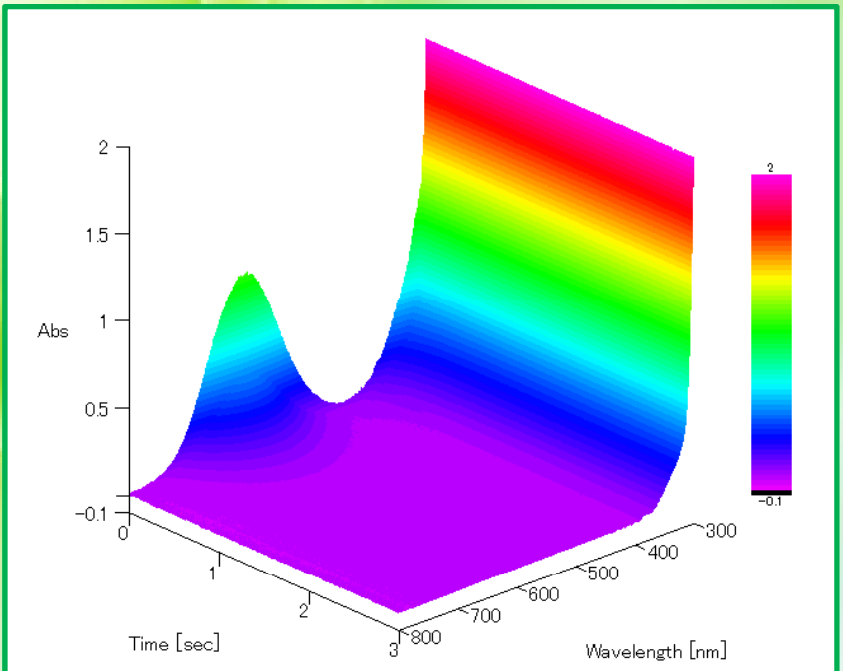


Figure 2 3D spectra of reduction of DCIP

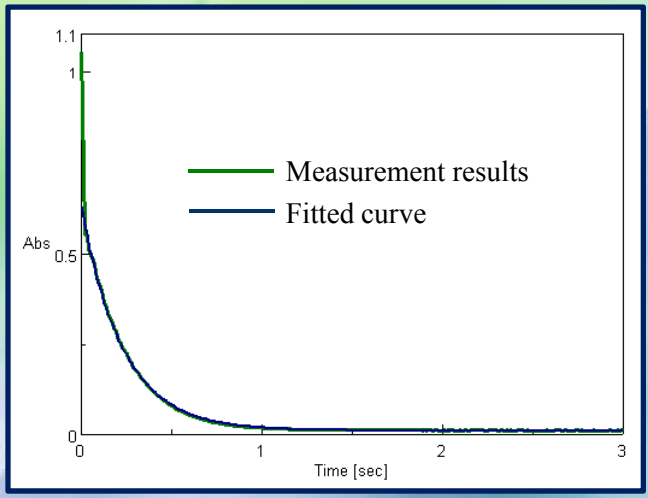


Figure 3 Time-course measurement results and fitted curve for sample absorbance at 604 nm

Curve fitting analysis parameters

Number of reaction steps:	1
Reaction range:	0.03 to 2.00 sec

Curve fitting analysis result

Reaction rate calculation equation:
Y(t) = 0.6150x exp(-t/0.2305)

Baseline equation:	Y(t) = 0.0105
Time constant:	0.2309 sec
Rate constant:	4.3296 sec^{-1}
Half-life period:	0.1600 sec

Transmission measurement of Volvox by using MSV-5000 series

Introduction

The MSV-5000 series microscopic spectrophotometer is capable to analyze micro sample/area by both transmission/reflection measurement in the region from UV to Near-IR, which can be applied to characterization of micro sized sample/area and also impurity analysis.

Recently, this type of technology is getting very popular in bioscience field such as the analysis of localized constituents in living cells. Volvox which has localized cellular density due to its internal daughter colonies, was measured to obtain absorption spectra and fixed-wavelength mapping.

Keywords : microscopic measurement, biochemical, spectrum imaging (mapping)

System configuration

MSV-5100 UV/Vis/NIR Microscopic Spectrophotometer
MAXY-501 Automatic XYZ Stage

Sample

Water containing Volvox was dropped onto a microscope slide glass and dried. (Fig.1)

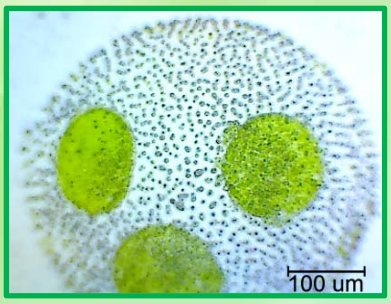


Fig. 1. Observation Image of dried Volvox

Measurement conditions

Spectral bandwidth (UV/Vis):	5.0 nm	Scanning speed:	1000 nm/min
Response:	Quick	Data pitch:	1 nm
Cassegrain objective:	16 times	Aperture:	50 mmϕ

Spectrum measurement

One of the daughter colonies inside of mother colony is measured to obtain absorption spectrum.

Results

Measured absorption spectrum is shown in Fig. 2. Chlorophyll a and chlorophyll b are well known as major chlorophylls included inland plants and green alga [1),2)] and those absorption spectra are shown in Fig. 3.[2)] This published data on the literature is measured under acetone solvent condition, and the peak positions of those chlorophylls are slightly different depending on the solvent used, but it's only approx. 2-7 nm difference in wavelength. Comparing the spectra of chlorophylls with Volvox spectrum, it is assumed that chlorophyll a and b are included in Volvox.

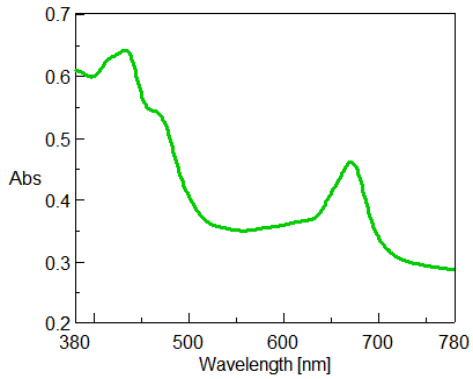


Fig. 2. Absorption spectrum of Volvox

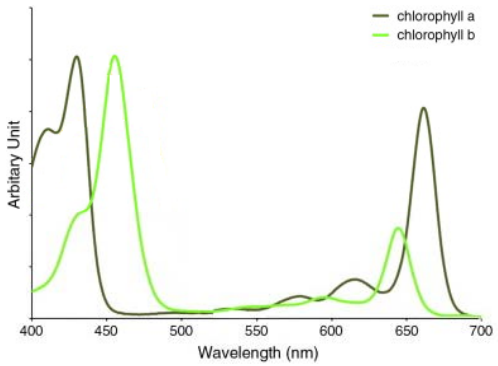


Fig. 3. Absorption spectra of chlorophylls[2)] (with Acetone solvent)

Fixed Wavelength Mapping Measurement

Fixed wavelength mapping measurement was executed at 672 nm since the peak was observed at this 672 nm by the absorption spectrum measurement. Mapping measurement at specific fixed wavelength makes it possible to generate high speed mapping data.

Measurement conditions

Measurement Mode:	Lattice measurement
Measurement wavelength:	672 nm
Response:	Fast
Spectral Bandwidth:	2 nm
Cassegrain objective:	16 times
Aperture:	30 μmϕ
Measurement interval:	30 μm

Results

Observation image and its color-coded diagram by mapping measurement are shown as below and it is confirmed that the area with higher cellular density in observation image is exactly in good agreement with the area of higher absorbance in color-coded diagram.

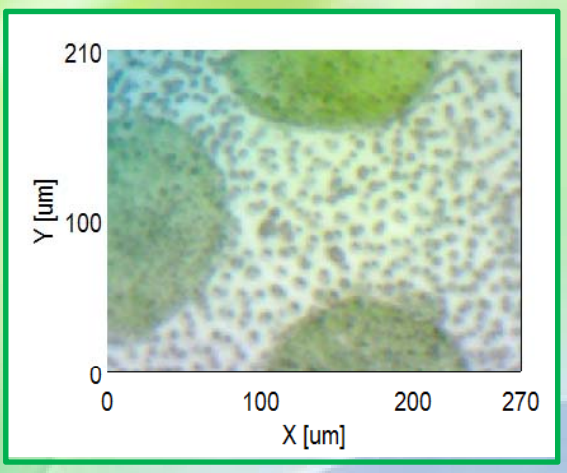


Fig. 4. Observation Image

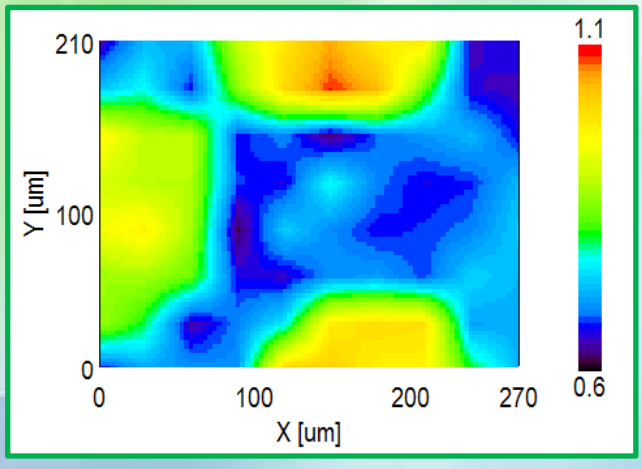


Fig. 5. Color-coded diagram of mapping measurement

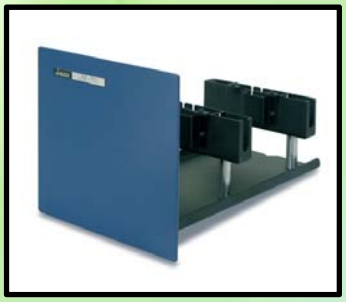
Reference Literatures

1) Hiroshi Terayama ed., *Kisoseikagaku (Revised Edition)*. Shoukabou, **1970**, p.130-131.
2) *Biochimica et Biophysica Acta (BBA) - Bioenergetics*. **2011**, *1807*, 968-976.

Chromaticity and turbidity quantitative measurement

<Chromaticity>

Chromaticity measurement is to check the coloration degree of clean water and wastewater using humic acid. This test is applied by observing the absorption at 390 nm which shows the yellow color of humic acid by using UV/Vis spectrophotometer.



Measurement/analysis system

- V-730/750/760/770/780 UV/Vis spectrophotometer
- LSE-701 Long path cell holder
- VWWQ-953 Chromaticity/turbidity measurement program
- Rectangular cell, 50 mm or 100 mm

Standard sample

Cobalt chloroplatinate which has a color similar to yellow-brown of humin is used as standard sample for chromaticity. 2.49 g of Potassium chloroplatinate and 2.02 g of cobalt chloride are dissolved in 200 mL of hydrochloric acid, and then purified water is added to make the solution of total volume 1 L. This solution is neat standard sample with chromaticity 1000 degree.

Test method

Measuring the absorbance of sample in cell of 50 mm or 100 mm pathlength at the wavelength of 390 nm

Procedure

1. Standard solution is prepared from neat standard sample diluted by purified water. Blank sample is purified water filtrated using 0.2 µm membrane filter.
2. Chromaticity calibration curve is created from the measurement results of blank sample and standard solution prepared in 1.
3. Sample water is filtrated using membrane filter or centrifuged and the supernatant is used as sample.
4. The absorption of sample prepared in 3 at 390 nm is measured, and chromaticity is calculated from the results and calibration curve.

Calibration curve

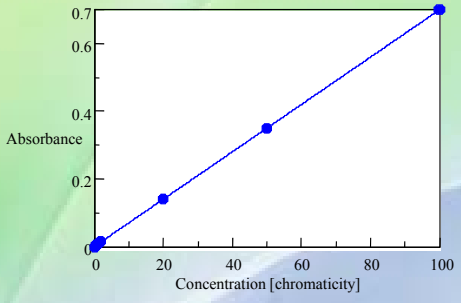


Figure 1. Chromaticity calibration curve

Table 1. Chromaticity calibration curve

Concentration [chromaticity]	Absorbance	Quantitative value [chromaticity]
0	0.000	-0.06
0.5	0.004	0.51
1	0.007	0.97
2	0.015	2.04
20	0.141	20.07
50	0.350	49.99
100	0.699	99.99

Standard solutions with chromaticity at 0, 0.5, 1, 2, 20, 50, 100 degree were measured using 50 mm light pathlength cell and these results are shown in the center column of Table 1. Measured absorbance values are input to the calibration curve and the calculated quantitative values of chromaticity are shown in the right column of Table 1. From the above results, the standard deviation (s) between the obtained quantitative value and actual chromaticity is 0.04(6) degree, detection limit, 0.15 and quantitation limit, 0.46 degree. *1)

*1) Detection limit is calculated from 3.3σ and quantitative limit is calculated from 10σ.

Calibration curve information: $y = 0.0070x + 0.0004(5)$ $R^2 = 1.0000$

<Turbidity>

In turbidity measurement, the turbidity degree due to insoluble particles, microbe and organic substance in clean water and wastewater is tested. Scattering light at 660 nm is measured using UV/Vis spectrophotometer in transmission measurement method or integrating sphere photoelectric spectrophotometry.

Standard sample

Immixture polystyrene suspension is used as standard sample of turbidity. Mixture of 5 kinds of polystyrene particles shown in the Table 2 is stated as neat solution of turbidity at 100 degree, which is commercially available.

Table 2. Polystyrene standard particle of turbidity at 100

Category	Normal diameter (μm)	Mixture ratio (%)
No. 6	0.5	6
No. 7	1	17
No. 8	2	36
No. 9	5	29
No. 10	10	12

[Transmission measurement method]

Measurement/analysis system

- V-730/ / 750 / 760 / 770 / 780 UV/Vis spectrophotometer
- LSE-701 Long path cell holder
- Quantitative measurement program
- Rectangular cell, 20 mm 50 mm and 100 mm

Procedure

1. Neat sample solution is diluted by purified water and prepared as standard solution. Purified water filtrated using 0.2 μm membrane filter is used as blank sample.
2. Turbidity calibration curve is created from the measurement results of blank sample and standard solution prepared in 1.
3. The absorbance of sample water at 660 nm is measured, and turbidity is calculated from the results and calibration curve.

Calibration curve

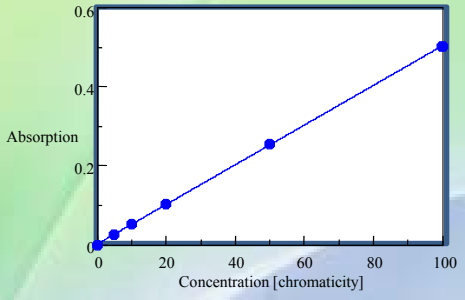


Figure 2. Turbidity calibration curve (Transmission measurement method)

Table 3. Turbidity calibration curve (transmission measurement method)

Concentration [turbidity]	Absorbance	Quantitative value [turbidity]
0	0.000	-0.27
5	0.026	4.81
10	0.052	10.07
20	0.102	20.09
50	0.256	50.64
100	0.502	99.67

Standard solutions with turbidity at 0, 5, 10, 50, 100 degree were measured using 20 mm light pathlength cell and the results are shown in Table 3. By the same method as chromaticity, the standard deviation is calculated as 0.36 degree, detection limit as 1.18 and quantitation limit as 3.6 degree.

Calibration curve information: $y = 0.0050x + 0.0014$ $R^2 = 1.0000$

Evaluation of sun protection fabrics by using a UPF evaluation system

Introduction

UPF (UltraViolet Protection Factor) is used to indicate the UV shielding performance of sun protection for fabric products. The 'UPF value' represents the ratio of time for sunburn by UV with and without the protection of the fabric material or product. For example, in the case of skin irradiated by ultraviolet light in 10 minutes with a UPF 50 cloth, it takes 500 min (50 (UPF) x 10 min) to obtain the same amount of sunburn to the skin without using the cloth product. The test method of for UPF calculations when using a UV-Vis spectrophotometer is defined in AS/NZS 4399:1996, BS EN 13758-1:2002, the AATCC Test Method 183:2010, and GBT18830:2009.

In this application data, the evaluation of the UPF, UPF rating, UVA transmittance, and UVB transmittance of sun protection fiber products defined in AS/NZS 4399:1996 by using the UPF calculation system of a UV/Vis spectrophotometer is explained. Also, the fluorescence properties are explained by using a spectrofluorometer because fabric products sometimes emit fluorescence by UV light irradiation.

Keywords: UPF, Sun protection fiber, AS/NZS 4399:1996, BS EN 13758-1:2002, AATCC Test Method 183:2010, GBT18830:2009

Calculation Method

·UPF

UPF is calculated by equation (1).

$$UPF = \frac{\sum_{290}^{400} E(\lambda)\cdot S(\lambda)}{\sum_{290}^{400} E(\lambda)\cdot S(\lambda)\cdot T(\lambda)} \times 100 \qquad (1)$$

E(λ): CIE reference erythema dose spectrum
S(λ): radiation intensity distribution of sunlight
T(λ): diffuse transmittance spectra (%T)

·UPF rating

To calculate the UPF rating, measure the transmittance spectrum at more than four different points for the same sample and round down the value, calculated by equation (2), by equation (5).

$$\text{UPF rating} = UPF_{AVE} - E \qquad (2)$$

$$UPF_{AVE} = \frac{UPF_1 + UPF_2 + \cdots + UPF_N}{N}$$

$$E = \frac{t_{k,\alpha}}{\sqrt{N}} \times SD$$

$$SD = \sqrt{\frac{\sum_{i=1}^{N} (UPF_i - UPF_{AVE})^2}{N-1}}$$

UPF_i: UPF of i_{th} point on a sample
$t_{k,a}$: The value which provides 0.5 % as the border value of probability of one side in the t distribution
a: Probability of one side (0.005)
k: Degree of freedom (N-1)

If the UPF rating is smaller than the minimum of each UPF, the value which is calculated by equation (3) are rounded down by 5.

$$\text{UPF rating} = \text{lowest UPF} \qquad (3)$$

if UPF rating is more than 50, UPF rating is defined as 50+

・UVA transmittance, UVB transmittance

UVA transmittance is calculated by equation (4) by using the average of the transmittance in the range from 315 nm to 410 nm.

UVB transmittance is calculated by equation (5) by using the average of the transmittance in the range from 290 nm to 315 nm.

$$UVA = \frac{T_{315} + T_{320} + T_{325} + \cdots + T_{400}}{18} \qquad (4)$$

$$UVB = \frac{T_{290} + T_{295} + T_{300} + \cdots + T_{315}}{6} \qquad (5)$$

Sample

T shirt (black)	polyester 100%
Sports shirt (black)	polyester 100%
Arm cover (black)	rayon 55% polyester 45%

Measurement

・3D fluorescence measurement

3D fluorescence measurements of the samples were conducted using a spectrofluorometer to identify the fluorescence property using excitation wavelengths in the range from 290 nm to 400 nm.

・Transmittance spectra measurement

(1) Baseline measurements were performed using a Spectralon reference tile.

(2) Transmittance spectra measurements were performed at 4 different points in the same sample.

* If fluorescence was observed in the 3D fluorescence measurement, a Fluorescence Cut Filter Block and a Fluorescence Cut Filter (U-330) are required to measure samples which emit fluorescence in the wavelength range from 450 to 650 nm.

Measurement parameters

・Spectrofluorometer

Excitation bandwidth:	5 nm	Emission bandwidth:	5 nm
Scan speed:	5000 nm/min	Response:	10 msec
Data interval:	0.5 nm	Spectra correction:	ON

・Spectrophotometer

UV/Vis bandwidth:	5.0 nm	Scan speed:	100 nm/min
Response:	0.96 sec	Data interval:	1 nm

Results

·Results of 3D fluorescence measurements

Figure 1 illustrates the 3D fluorescence measurement of the T shirt.

No fluorescence was observed for the excitation wavelengths from 290 to 400 nm.

No fluorescence was observed for the sports shirt and arm cover samples.

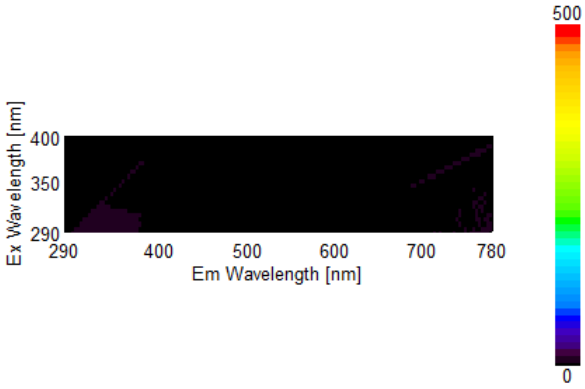


Fig. 1 3D fluorescence spectra of T shirt

·Results of transmittance measurements

Figure 2 shows the transmittance spectrum of each sample.

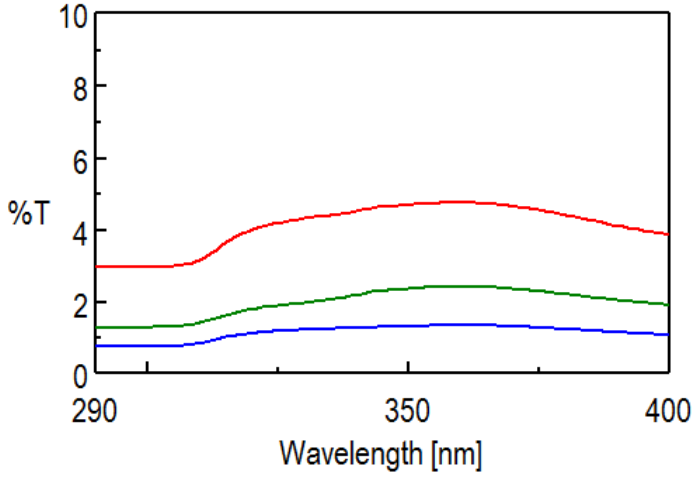


Fig. 2 Transmittance spectrum of each sample

Red line: T shirt

Blue line: Sports shirt

Green line: Arm cover

Figure 3 illustrates the UPF measurement software display and Table 1 provides the analysis results for the samples. As shown in Figure 3, the [UPF measurement] program can objectively compare the performance of the ultraviolet shielding as a result of the numerical calculation of the UV shielding performance of fabric products. Moreover, the program corresponds to various standards including BS EN 13758-1:2002, AATCC Test Method 183:2010, and GBT18830:2009.

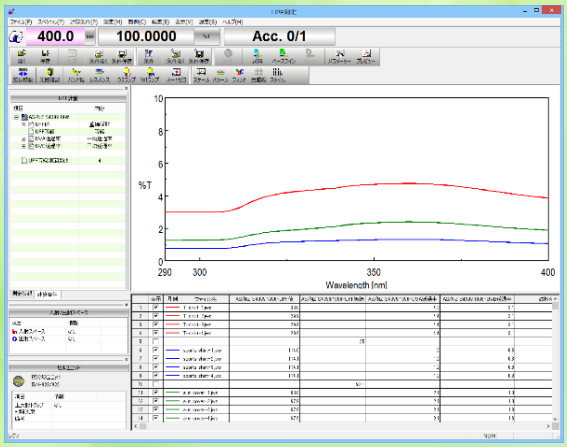


Fig. 3 UPF Measurement Program

Table 1 Analysis result based on AS/NZS 4399:1996

Sample	No.	UPF	UPF rating	UVA Transmittance (%)	UVB Transmittance (%)
T shirt	1	30.0	25	4.4	3.1
	2	30.0		4.4	3.1
	3	30.0		4.3	3.1
	4	30.0		4.4	3.1
Sports shirt	1	114.3	50+	1.2	0.8
	2	114.2		1.2	0.8
	3	114.1		1.2	0.8
	4	114.4		1.2	0.8
Arm cover	1	68.2	50+	2.1	1.3
	2	68.0		2.1	1.3
	3	68.1		2.1	1.3
	4	68.0		2.1	1.3

Evaluation of the privacy film using an automated absolute reflectance measurement accessory

Introduction

A privacy film which is used for smartphone displays has a characteristic structure in which clear layers and light shielding layers are interlaminated. This structure prevents a smartphone display from bystanders 'peeking' at the screen while the viewing angle depends on the height and pitch of the light shielding layers in the louver layers.

To evaluate the viewing angle or the transmittance of the louver layer, an absolute reflectance measurement accessory is an effective tool. The accessory can be used to set samples at a specified angle by rotating the sample to the source incidence and/or detector angles. In this application, the angle dependence of the transmittance spectra of the privacy film for a smartphone is explored.

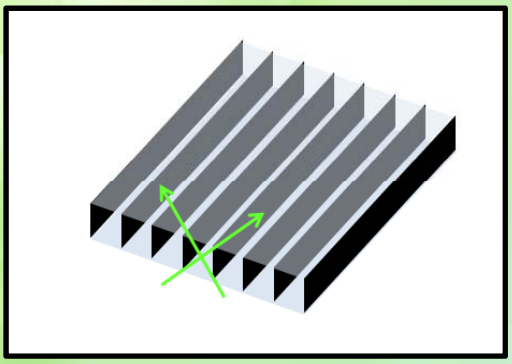


Fig. 1 Structure of louver layer

Keywords: Angle scanning measurement, Absolute reflectance measurement unit, Privacy film

Sample

Privacy film for smartphones (Figure 2)
Specification: View angle 65°
Anti-glare processing

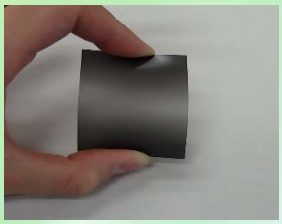


Fig. 2 Privacy film sample

Measurement condition

Position:	transmittance, asynchronous
Detection angle:	0°
Incidence angle:	−60° to 60°
Measurement interval:	2°
Measurement mode:	%T
Wavelength range:	380 to 780 nm
Bandwidth:	5 nm
Scan speed:	400 nm/min
Response:	0.96 sec

System configuration

P/N	Description
7067-J051A	V-750ST UV/VIS Spectrometer
7082-J019A	ARMV-919 Automated absolute reflectance measurement accessory
	Incidence and Detection Angle Limitation Mask (Custom-ordered)
4880-6532A	VWAM-968 Absolute reflectance spectra measurement program

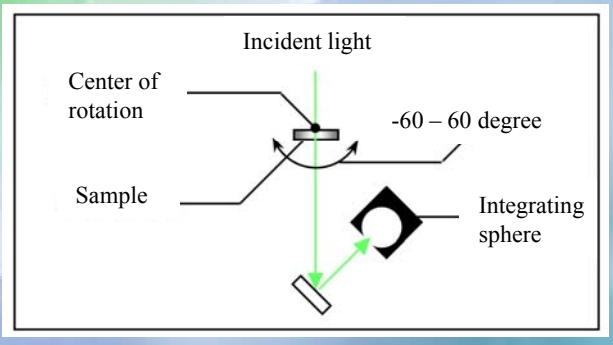


Fig. 3 Measurement image

Fig. 4 Absolute reflectance measurement system

Result

Figure 5 illustrates the interval data in which spectra were acquired every 2º from -60º to 60º.

Figure 6 outlines the transmittance spectra at 0º, 10º, 20º, 30º, 40º, 50º, and 60º.

Figure 7 provides the angle scanning transmittance data from −60º to 60º at 550 nm.

Figures 5 and 6 indicate that the film absorbs blue light at less than 400 nm and keeps the transmissivity constant at more than 400 nm, which means that the film displays the light to eyes without a large color change. As illustrated in Figure 7, the transmittance is approximately 5% near the nominal view angle of ±32.5º. This characteristic is quite suitable for user privacy.

As indicated in this report, the absolute reflectance measurement system is best suited to evaluate the incident angle dependence properties of various transmittance spectra.

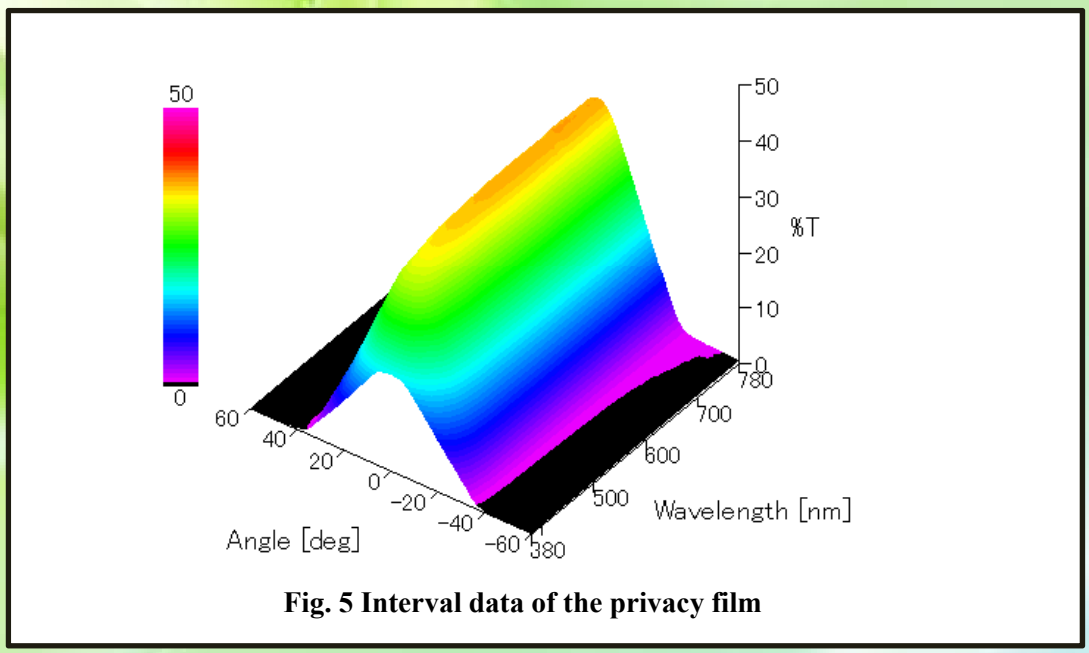


Fig. 5 Interval data of the privacy film

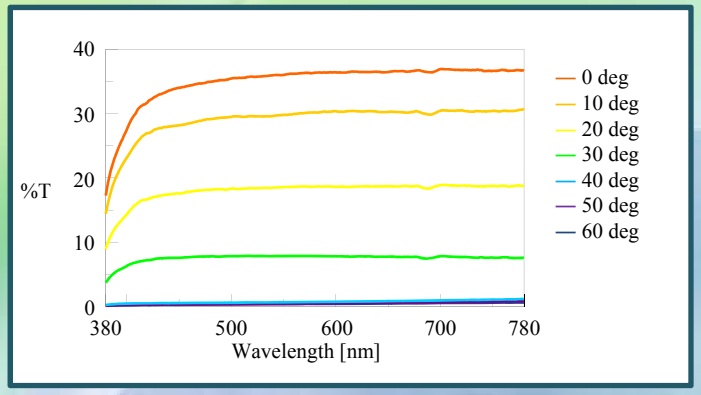


Fig. 6 Transmittance spectrum at every 10º from 0º to 60º

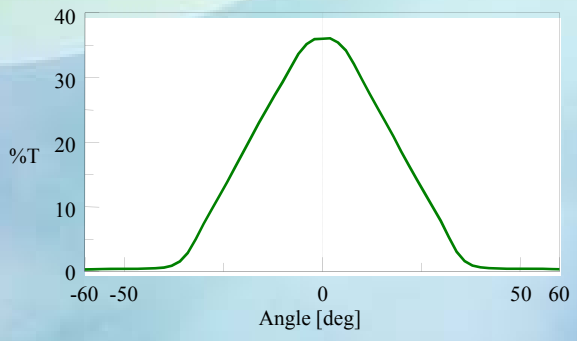


Fig. 7 Angle dependence of transmittance spectra at 550 nm

Horizontal Integrating Sphere / PIV-756/PIN-757

Fig1. The horizontal Integrating sphere

The model PIV-756/PIN-757 Horizontal Integrating sphere (Figure 1) is an accessory for the V-600 series spectrophotometers (V-750/760/770) for measurement of the diffuse transmittance and reflectance of samples. Using a simple horizontal positioning of samples, placement of uneven or irregular samples can be visually determined. The horizontal integrating sphere accessory offers precise measurement and increases the operating efficiency even for amorphous samples and samples that can not be placed vertically.

To demonstrate the capabilities of this accessory, a sample that cannot be placed vertically, such as a contact lens, is examined using the horizontal integrating sphere.

Measurement with a Conventional Integrating Sphere

To measure a contact lens with a horizontal integrating sphere, one of the possible measurement methods is to hold the sample in a saline solution by placing it between two quartz plates (Figure 2).
With this measurement method, air bubbles in the cell and deformation of the lens may prevent precise measurements of the sample. Also, the vertical placement of the sample makes the determination of the desired measurement area very difficult. Moreover, hard contact lenses may crack or be damaged when placing the lens between the quartz plates.

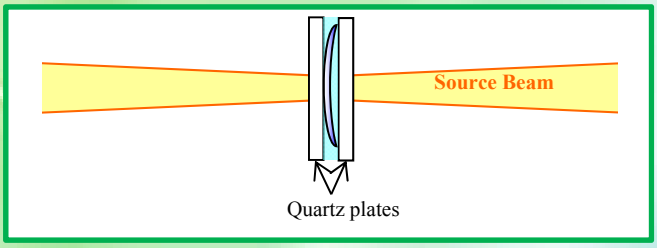


Fig2. Contact lens between quartz plates

Measurement with the Horizontal Integrating Sphere

With the horizontal integrating sphere, samples are horizontally placed on the integrating sphere. It is not necessary to contain the contact lens within quartz plates. A dedicated sample holder was prepared by using a quartz cell. With its circular design, a contact lens can be held in the appropriate solution during analysis.

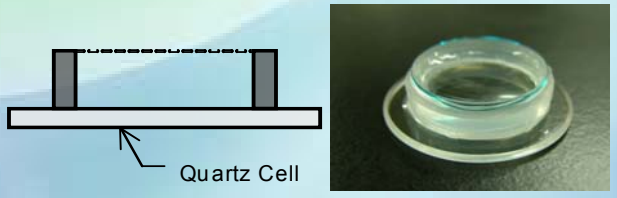


Fig 3. Dedicated holder for contact lens

The net transmittance of the sample can be obtained by measuring a blank with the lens holder filled with the preservative solution. This blank measurement cancels any stray light reflected by the surfaces of the sample holder and the lens solution.

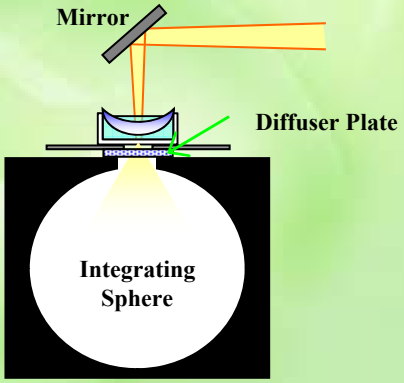


Fig4. Sample placed on the horizontal integrating sphere

As shown in Figure 4, the sample holder with the sample solution was placed on the integrating sphere. With the horizontal integrating sphere, the sample can be easily observed such that a specific measurement area can be selected. Hard contact lenses can also be measured with no physical stress on the lens sample.

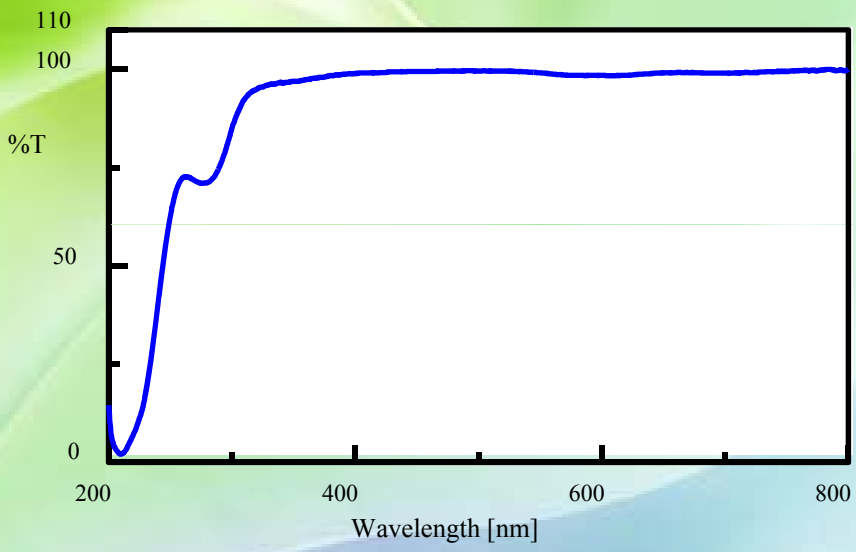


Fig 5. Spectrum of hard contact lens sample

Applications of the Horizontal Integrating Sphere

As the example demonstrates, the horizontal integrating sphere is the best accessory for samples that can not be measured with vertical placement. This accessory offers easy measurements for small or amorphous samples as well as samples that can not be set vertically. Since the sample is placed on the top of the accessory, it can be measured while avoiding sample damage and also provide precise sample measurement of sample areas by simple visual examination of the specific area to be measured.

Thickness Analysis of natural oxide film on microscopic Si pattern

Introduction

The MSV-5000 series microscopic spectrophotometer is for transmission/reflection measurement in a wide wavelength range from ultraviolet to near-infrared. It allows the measurement of the area of as small as 10 μm diameter and the built-in high-resolution camera enables to observe the samples precisely to determine the area to be measured. This instrument is most suitable to measure the minute samples or samples having microstructure. This time, the sample on which Si patterns of 35 μm widths are lined up on Ti substrate with 14 μm intervals was measured as a microstructure sample. Actually, the thickness of SiO_2 formed upon Si was analyzed from the obtained reflectance spectrum, because Si is easily oxidized in the air to form thin oxide film of SiO_2.

Keywords microscope, silicon, absolute reflectance

Measurement System

MSV-5200 Microscopic spectrophotometer
VWML-791 [Multi-Layer Analysis] program

Sample: Si and Si oxide film on the Ti substrate

Measurement condition

UV/Vis spectral bandwidth:	5.0 nm
NIR spectral bandwidth:	20.0 nm
Scan speed:	100 nm/min
Response:	Slow
Data interval:	0.5 nm
Cassegrain objective mirror:	16x
Incidence angle:	23°
IN aperture:	10 μmϕ
OUT aperture	10 μmϕ

Measurement

1. Baseline: Al vapor-deposited mirror as a reference is used for baseline measurement.
2. Measurement area: The sample is observed by the high-resolution camera to determine the measurement area (Fig. 1). The red spot in Fig. 1 shows the size and position of detected light.
3. Sample measurement: The reflectance spectrum is measured.
4. Transforming into absolute reflectance: The absolute reflectance spectrum of the sample is calculated by multiplying the obtained relative reflectance by the absolute reflectance of Al vapor-deposited mirror.

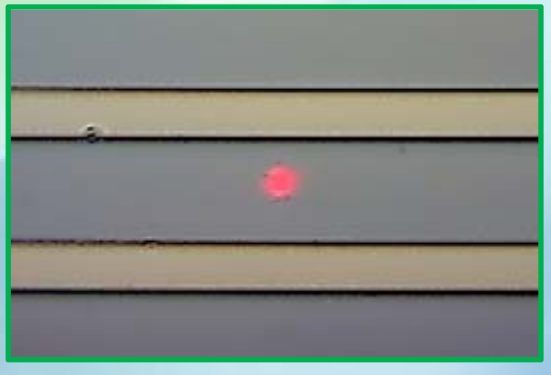


Fig. 1. Observation figure of measurement position

Analysis

Reflectance(R) is expressed by the equation of refractive index of the film (n_i), extinction coefficient (k_i), the angle of incidence (q_i), wavelength (λ) and the film thickness (d_i). This time, optical constants of Si and SiO_2 are used from the literature value. and then the film thickness of SiO_2 is estimated by using [Multi-Layer Analysis] program by fitting the calculated reflectance spectrum to the measured one to make the thickness value reasonable.

Measurement Results

Measured absolute reflectance spectrum is shown in Fig. 2. MSV-5000 series adopts the confocal optical system, which enables the measurement eliminating the influence of back side reflection. In the range over 1100 nm where the light passes through Si, the spectrum would not be influenced by the back side reflection.

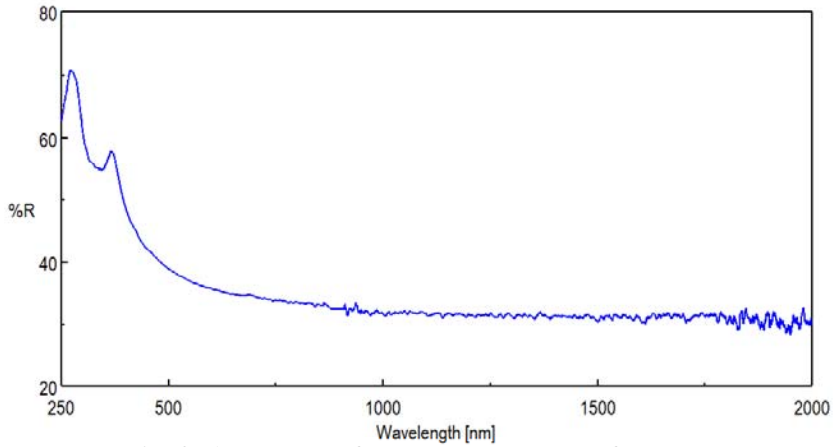


Fig. 2. Absolute reflectance spectrum of sample

Analysis Results

The result of fitting the reflectance spectra using [Multi-Layer Analysis] program is shown in Fig. 3. The error between measured spectrum and calculated one was within 2% (Fig. 3) and the film thickness of SiO_2 was calculated to be 7.6 nm.

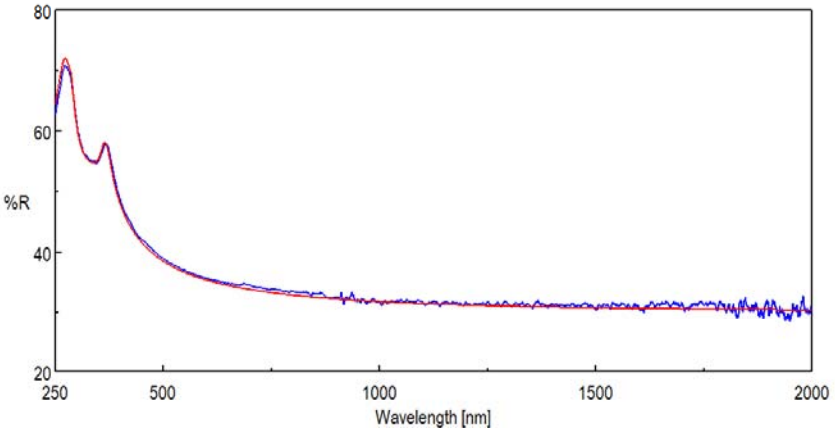


Fig. 3. Fitting result of reflectance spectra

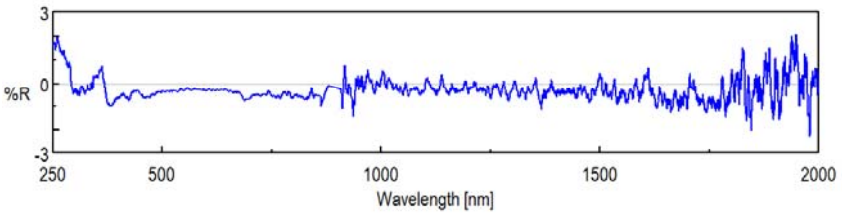


Fig. 4. The error between measured spectrum and calculated one

[Integrating sphere photoelectric spectroscopy]

Measurement/analysis system

V-750/760/770/780	UV/Vis spectrophotometer
ISV-922/ISN-923/ISN-901i	Integrating sphere unit
VWWQ-789	Chromaticity/turbidity measurement program

Rectangular cell, 10 mm, 20 mm, 30 mm and 50 mm

Procedure

1. Standard solution is prepared from neat standard sample diluted by purified water. Blank sample is purified water filtrated using 0.2 µm membrane filter.
2. Turbidity calibration curve is created from the measurement results of blank sample and standard solution prepared in 1. Firstly, standard white plate is mounted in integrating sphere and total light transmittance (T_t) is measured, and then the plate is removed and diffuse transmittance (T_d) is measured.
3. T_t and T_d of sample water at 660 nm is measured, and turbidity is calculated from the results and calibration curve.

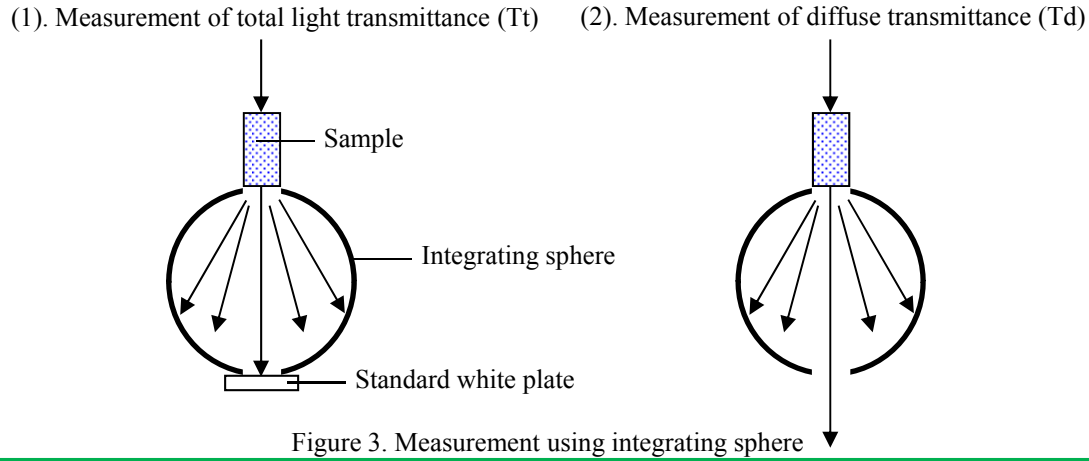


Figure 3. Measurement using integrating sphere

Calibration curve

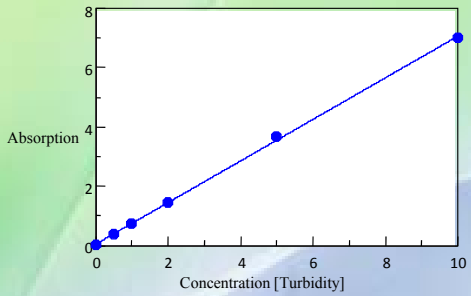


Figure 4. Turbidity calibration curve (integrating sphere photoelectric spectroscopy)

Table 4. Turbidity calibration curve (Integrating sphere photoelectric spectroscopy)

Concentration [turbidity]	Td/Tt x 100	Quantitative value [turbidity]
0	0.004	-0.05
0.5	0.389	0.50
1	0.726	0.98
2	1.435	1.99
5	3.666	5.17
10	7.003	9.92

Standard solutions with turbidity at 0, 0.5, 1, 2, 5, 10 degree were measured using 20 mm light pathlength cell and the results are shown in Table 4. From the calibration curve, the standard deviation is calculated as 0.08(6) degree, detection limit as 0.28 and quantitation limit as 0.86 degree. *2)

50 mm light pathlength rectangular cell is recommend to be used for the analysis of low turbidity sample with concentration close to or less than quantitation limit .

Calibration curve information: $y = 0.7018x + 0.0397$ $R^2 = 0.9997$

Transmittance and Reflectance Measurement System for Minute Lenses

Introduction

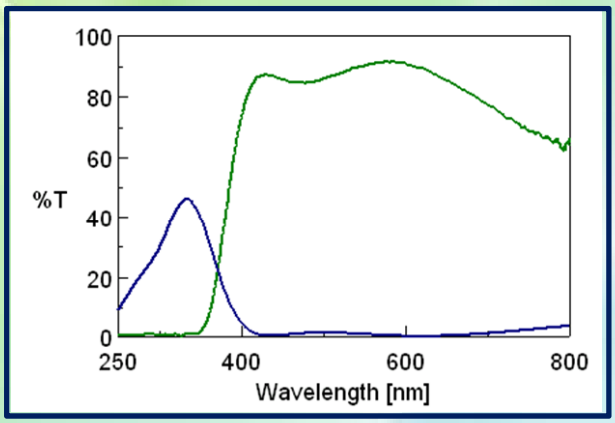
Small type lens is widely used in various products as smart phone , tablet PC etc,,. This note show the transmittance and reflectance measurement for 1-mm-diameter lenses used in mobile-phone cameras.

MSV-5200 UV/Vis/NIR Microspectrophotometer

Integrating sphere for MSV-5200

For samples that refract light such as lenses, the transmittance can be measured using an integrating sphere. The reflectance can also be measured if the aperture size is sufficiently reduced so that the area being analyzed can be considered to be flat.

1-mm-diameter mobile-phone camera lens



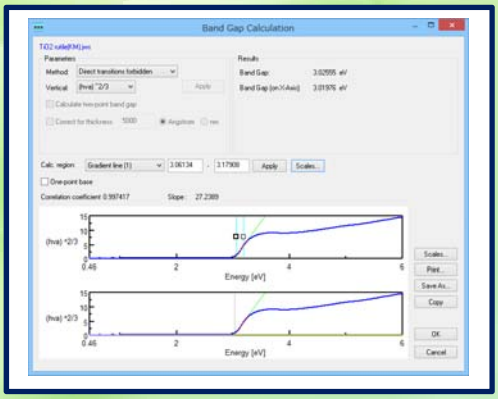
System	Model No.	Product name	Code	Remarks
Instrument	MSV-5200-16-ST	UV/Vis/NIR Microspectrophotometer	6973-J008A	Wavelength range: 200 to 2700 nm Supplied with 16x Cassegrain objective, converging mirrors, and manual stage.
Optional accessories	MISP-552	Integrating sphere for MSV-5200		Wavelength range: 250 to 2000 nm Only for the dedicated manual stage for the integrating sphere. Not applicable for diffuse transmittance or reflectance measurements. Normal (non-diffuse) reflectance measurements can be performed using the reflectance optical path.
Description	Available models are the MSV-5100 (200 to 900 nm) and MSV-5300 (200 to 1600 nm). The type of integrating sphere varies depending on the instrument model, and determines the wavelength range that can be used.			

Bandgap Measurements for Titanium Dioxide Powder

Introduction

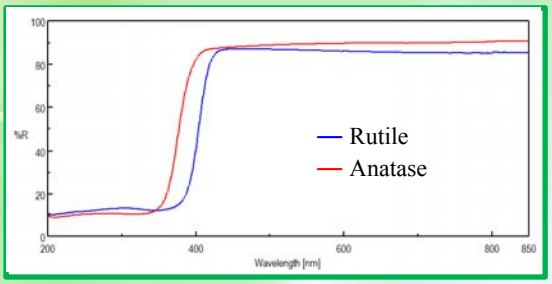
The bandgap energy for semiconductor materials can be determined from the transmittance or reflectance spectrum using a JASCO spectrophotometer.

V-770 UV/Vis/NIR spectrophotometer
ISN-923 Integrating sphere unit



VWBG-773 [Band Gap Calculation] program

Diffuse transmittance spectra for rutile and anatase titanium dioxide powder were measured. Since for titanium dioxide, absorption occurs due to forbidden direct transitions, plotting hν against $(h\nu\alpha)^{2/3}$ allows the bandgap energy to be determined.



Diffuse transmittance spectrum for TiO2

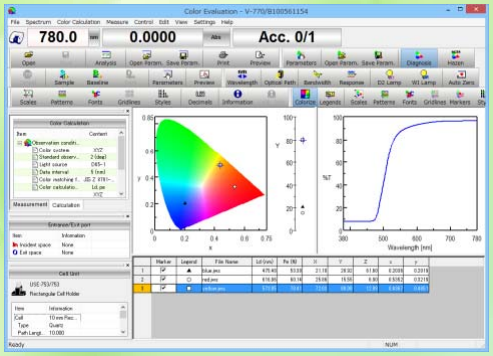
System	Model No.	Product Name		Remarks
Instrument	V-770ST	UV/Vis/NIR spectrophotometer		Model V-770DS is shipped with a PC.
Optional accessory	ISN-923	Integrating sphere unit		
Optional accessory	PSH-002	Powder cell		
Optional program	VWBG-773	[Band Gap Calculation] program		
Description				
Notes for ordering	Although, in principle, the bandgap should be calculated using the absorption coefficient, when only bandgap absorption occurs, the bandgap energy can be determined directly from the transmittance or reflectance spectrum. If the sample is a thin film and an interference pattern is present in the transmittance or reflectance spectrum, use an absolute reflectance measurement unit to measure the spectrum. The interference effect can be eliminated by calculating 100-%T-%R.			

Reflection Object Color Measurements using Color Diagnosis System

Introduction

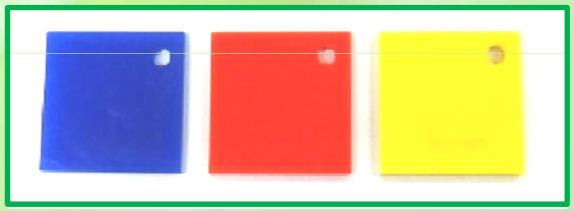
The color diagnosis system for the V-700 series can evaluate the reflection or transmission object color determined based on the CIE (Commission Internationale de l'Éclairage) standard.

V-750 UV/Vis spectrophotometer
ISV-922 Integrating sphere unit

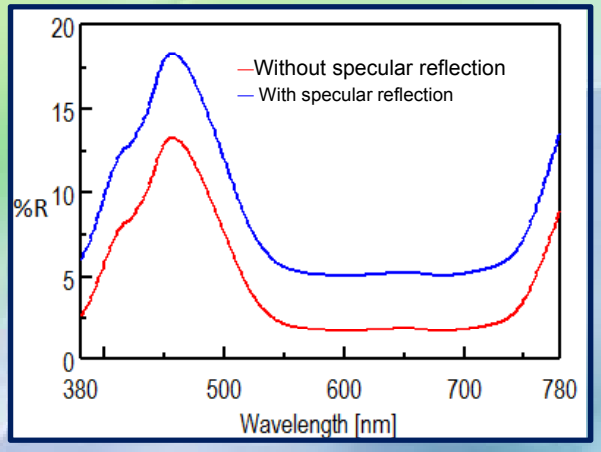


VWCD-960
[Color Evaluation (Color Diagnosis)] program

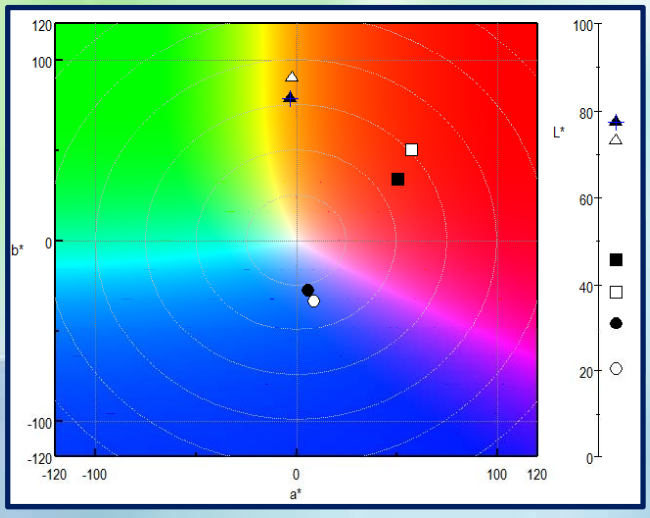
Reflectance spectra for colored plastic reflective plates were measured with or without the specular component using an integrating sphere unit. If the spectrum includes the specular component, it is found that the lightness is exaggerated and the chroma is diminished. Accordingly, to evaluate the color of a mirror or a mirror-like sample surface, it is necessary to remove the specular component during measurement.



Colored plastic reflective plates



Reflectance spectrum for blue reflective plate



● Blue (with specular reflection)	○ Blue (without include specular reflection)
■ Red (with specular reflection)	□ Red (without include specular reflection)
▲ Yellow (with specular reflection)	△ Yellow (without include specular reflection)

L*a*b* chromaticity diagram

Corresponding standards

Standard	Standard Name	Remarks
JIS Z 8722:2009	Methods of colour measurement -- Reflecting and transmitting objects.	Reflection object color: the φ60-mm or φ150-mm integrating sphere unit corresponds to geometric condition d. Transmission object color: the standard cell holder or film holder corresponds to geometric condition e. The φ60-mm or φ150-mm integrating sphere unit corresponds to geometric condition f.
CIE No.15:2004	COLORIMETRY, THIRD EDITION	

Light source	Standard
D65, A	JIS Z 8781-2: 2012, CIE 15: 2004, ISO 11664-2:2007 / JIS Z 8701: 1999, JIS Z 8720: 2000, ASTM E308: 2008
B	JIS Z 8701: 1982
C	JIS Z 8701: 1999, JIS Z 8720: 2000, CIE 15: 2004, ASTM E308: 2008
D50, D55, D75	CIE 15: 2004 / JIS Z 8720: 2000, ASTM E308: 2008
F1, F3, F4, F5, F6, F8, F9, F10	CIE 15: 2004
F2, F7, F11, F12	CIE 15: 2004, ASTM E308: 2008

Color System	Standard
XYZ	JIS Z 8701: 1999, CIE No.15: 2004, ISO 11664-1: 2007, ASTM E308: 2008
L*a*b*	JIS Z 8781-4: 2013, CIE No.15:2004, ISO 11664-5: 2009, ASTM E308: 2008
Lab	JIS Z 8730: 2002, ASTM D2244:2011
L*u*v*	JIS Z 8781-5: 2013, CIE No.15: 2004, ISO 11664-5: 2009, ASTM E308: 2008
Munsell	JIS Z 8721: 1993

Color-matching Function
JIS Z 8781-1: 2012, ISO 11664-1: 2007 / JIS Z 8701: 1999, ASTM E308: 2008 / JIS Z 8701: 1982, CIE 15: 2004

Color Difference	Standard
$\triangle E^*_{ab}$, $\triangle L^*$, $\triangle a^*$, $\triangle b^*$	JIS Z 8730: 2009, CIE 15: 2004, ISO 7724/3: 1984, ASTM D2244: 2011
$\triangle E^*_{ab}$, $\triangle L^*$, $\triangle C^*_{ab}$, $\triangle H^*_{ab}$	JIS Z 8730: 2009, CIE 15: 2004, ISO 7724/3: 1984, ASTM D2244: 2011
$\triangle E_{00}$, $\triangle E_{00}$, $\triangle L'$, $\triangle C'$, $\triangle H'$	JIS Z 8730: 2009, CIE 15: 2004, ASTM D2244: 2011
$\triangle E_{94}$, $\triangle L^*$, $\triangle C^*_{ab}$, $\triangle H^*_{ab}$	JIS Z 8730: 2009, CIE 15: 2004 / ASTM D2244: 2011
$\triangle E_{CMC(l:C)}$, $\triangle L^*$, $\triangle C^*_{ab}$, $\triangle H^*_{ab}$	JIS Z 8730: 2009, ISO 105-J03: 2009, ASTM D2244: 2011
$\triangle E^*_H$, $\triangle L$, $\triangle a$, $\triangle b$	JIS Z 8730: 1980, ASTM D2244: 2011
$\triangle E^*_{uv}$, $\triangle L^*$, $\triangle u^*$, $\triangle v^*$, $\triangle C^*_{uv}$, $\triangle H^*_{uv}$	JIS Z 8730: 2009, CIE 15: 2004, ASTM D2244: 2011

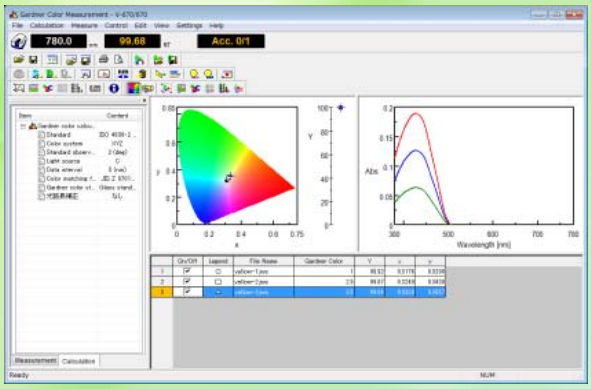
Calculation Items	Standard
Tri-stimulus values: XYZ	JIS Z 8701: 1999, CIE 15:2004, ISO 11664-1: 2007, ASTM E308: 2008
Chromaticity coordinates: x, y	JIS Z 8701: 1999, CIE 15: 2004, ASTM E308: 2008
Dominant wavelength: l_d, excitation purity: p_e	JIS Z 8701: 1999, CIE 15: 2004
Lightness index: L*, Chromaticity coordinates: a*, b*	JIS Z 8781-4: 2013, CIE 15: 2004, ISO 11664-4: 2009, ASTM E308: 2008
ab Chroma: C^*_{ab}, ab Hue angle: h_{ab}	JIS Z 8781-4: 2013, CIE No.15: 2004, ISO 11664-4: 2008, ASTM E308: 2008
Lightness index: L, chromaticity coordinates: a, b	JIS Z 8730: 2002, ASTM D2244: 2011
Lightness index: L*, chromaticity coordinates: u*, v*	JIS Z 8781-5: 2013, CIE 15: 2004, ISO 11664-5: 2009, ASTM E308: 2008
Hue: H, lightness: V, chroma: C	JIS Z 8721: 1993
Whiteness index: WI	JIS Z 8715: 1999 / CIE 15: 2004 / ASTM E313: 2010 / ASTM E313: 1996 / ASTM E313: 1973
Tint: T_w	JIS Z 8715: 1999 / CIE 15: 2004 / ASTM E313: 2010 / ASTM E313: 1996
Yellowness: YI	JIS K 7105: 1981 / JIS K 7373: 2006, ASTM E313: 2010

Gardner Color Measurement System

Introduction

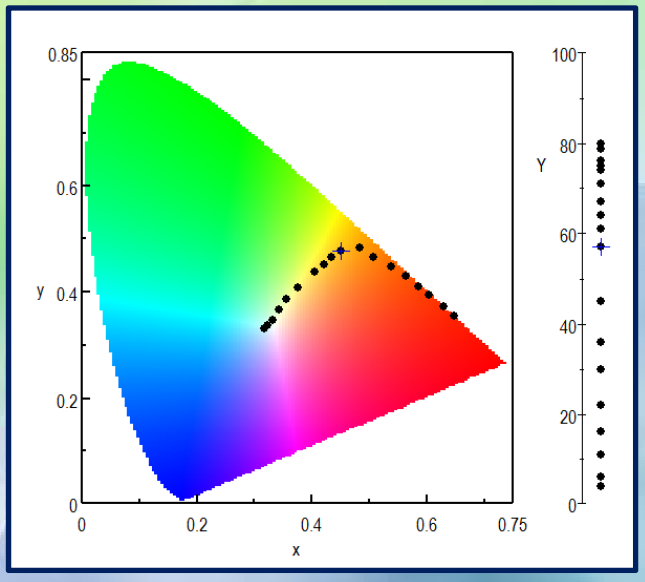
The Gardner color measurement system can evaluate the Gardner color as a measure of the degree of coloration of samples such as boiled oil, varnish, petroleum, liquids used in the production of chemicals, and substances that become molten when heated.

V-750 UV/Vis spectrophotometer



[Color Evaluation-Gardner Color] program (example)

The Gardner scale is defined based on the chromaticity of glass or liquid standards numbered from 1 for the lightest (light yellow) to 18 for the darkest (brownish red). By identifying the standard solution that is closest in color to the sample solution, the standard color number for the sample can be evaluated. In this program, the chromaticity coordinates x and y, and the tristimulus value Y for the standard samples can be registered by entering them directly or by calculating them from the spectrum for the standard sample. The sample spectrum is then measured and its Gardner color is calculated either from the color difference between the sample and the standard, or by comparing their x and y values.



XYZ color system with standard solution coordinates plotted

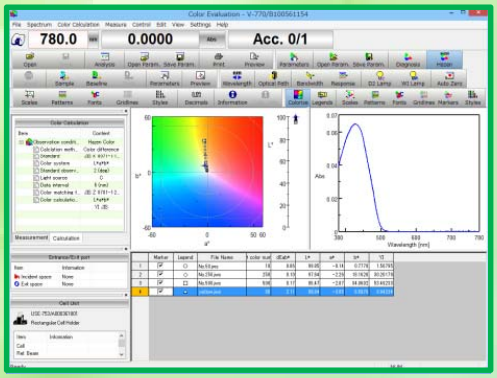
This system is compliant with ISO 4630-2:2004 Clear liquids - Estimation of colour by the Gardner colour scale - Part 2: Spectrophotometric method.

Hazen Color Measurement System

Introduction

The Hazen color measurement system can evaluate the Hazen color of samples such as drying oil, varnish, petroleum, liquids used in the production of chemicals, and substances that become molten when heated.

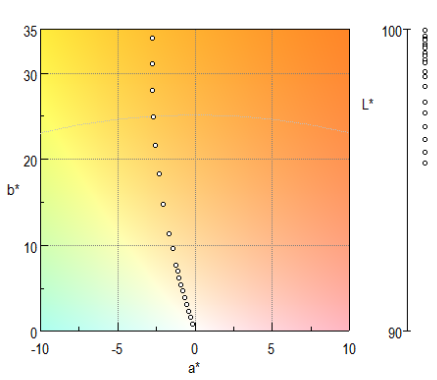
V-750 UV/Vis spectrophotometer
LSE-701 Long path cell holder
50-mm rectangular cell



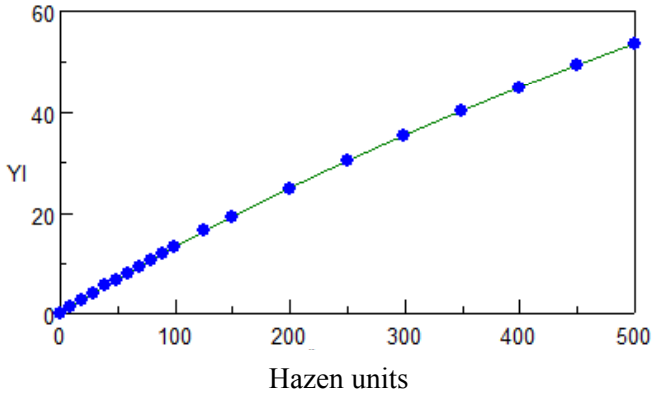
VWHC-977
[Color Evaluation–Hazen Color] program

The Hazen scale is defined based on the chromaticity of numbered standard solutions with different concentrations and yellow hues. By identifying the standard solution that is closest in color to the sample solution, the standard color number for the sample can be evaluated. In the [Color Evaluation-Hazen Color] program, any of the following methods can be used to determine the color in Hazen units.

1. **The color difference between standard and sample solutions**
2. **A calibration curve for the relation between the yellowness index YI and Hazen units for standard solutions**
3. **A calibration curve for the relation between the chromaticity coordinates b* and Hazen units**
4. **A calibration curve for the relation between the absorbance at a specified wavelength and Hazen units for standard solutions**



L*a*b* color system with standard solution coordinates plotted



Calibration curve for YI and Hazen units

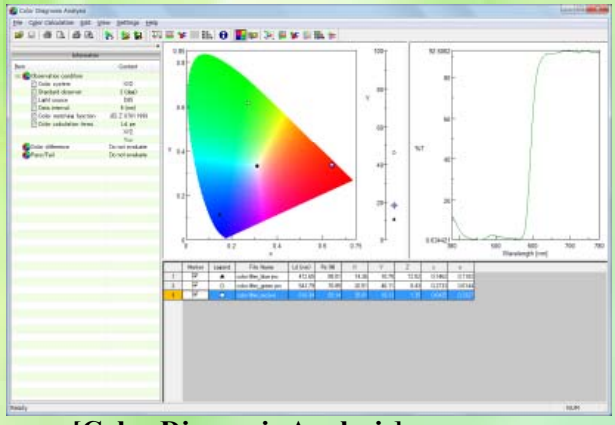
This system is compliant with ISO 6271-2:2004 Clear liquids - Estimation of colour by the platinum-cobalt scale- Part 2: spectrophotometric method. In this program, the visual test defined in JIS K 0071-1:1998, ISO 6271-1:2004 or ASTM D 1209-05 is applied to the UV/Vis spectrophotometer.

Color Analysis System for RGB Color Filters

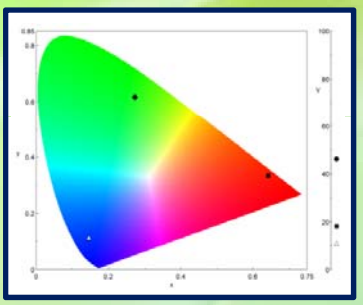
Introduction

This system measures the transmittance for each pixel in RGB color filters used for flat panel displays, and analyzes the color. The transmittance spectrum for the three colors in the filter was measured, and the calculation results were plotted as a chromaticity diagram using the [Color Diagnosis Analysis] program

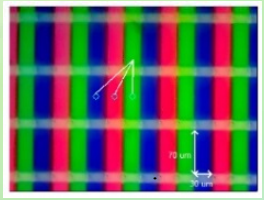
MSV-5200 UV/Vis/NIR Microspectrophotometer



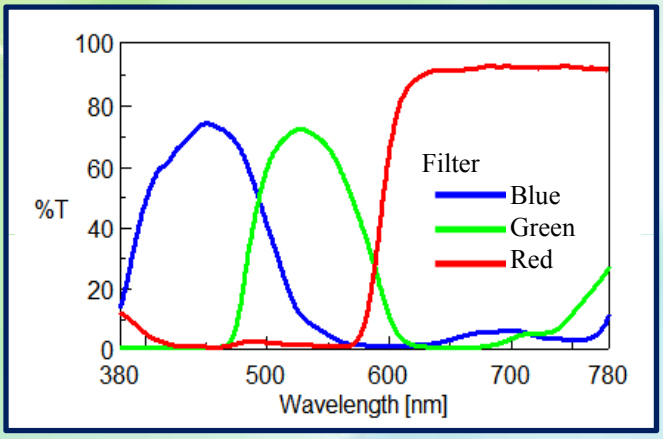
[Color Diagnosis Analysis] program



Chromaticity diagram (XYZ color system)



Observed image



Transmittance spectrum

System	Model No.	Product name	Code	Remarks
Instrument	MSV-5200-16-ST	UV/Vis/NIR Microspectrophotometer	6973-J008A	Wavelength range: 200 to 2700 nm , Supplied with 16x Cassegrain objective mirror, convergence mirror and manual stage.
Optional program	MCAN-581	[Color Diagnosis Analysis] program	G478	Dedicated analysis program for Spectra Manager Ver.2 Data in multiple file formats (*.jwa, *.jwb, *.jwd) can be analyzed.
Special accessory	MAXY-501-F	Automatic XYZ stage	6973-J201A	Optionally installed in factory. Movable distance*: X: 76 mm, Y: 52 mm, Z: 25 mm. * Varies depending on the magnification of the objective or convergence mirror, or the type of sample.
Description	Available models are the MSV-5100 (200 to 900 nm) and MSV-5300 (200 to 1600 nm). Using the automatic XYZ stage, mapping, line and multipoint measurements can be performed, in addition to automatic focusing and multiple-image acquisition. The joystick is an optional extra.			