JASCO Applications Book

Spectrofluorometers

JASCO Corporation was founded in 1958 to provide the scientific community with optical spectroscopy products.

In the mid-1950's a group of researchers in the Institute of Optics of what is now Tsukuba University needed an Infrared Spectrophotometer for their research.
Since a commercially available instrument was not yet existing at the time, they undertook the challenge to develop their own.

The result was quite a success - a reliable instrument with excellent optical performance. As a second result, other research groups asked them to replicate the instrument for use within their laboratories.

Over the years the JASCO product line has grown to cover instruments used, not only in research but also for routine analysis applications in areas such as quality control, environmental analysis, and process control. The current spectroscopy product line encompasses instrumentation for the following methods:

- ***UV/Visible and NIR***
- ***Microscope Spectrophotomers***
- ***FT-IR, microscope FT-IR and FT-Raman***
- ***Dispersive RAMAN***
- ***Polarimeters***
- ***Spectrofluorometers***
- ***Portable Raman***
- ***Portable FT-IR***
- ***Fully Automated Dissolution Tester***

JASCO is also the world leader in the field of ***Circular Dichroism Spectropolarimeters*** and ***Vibrational Circular Dichroism Spectrometers.***

"serving the Science and Technology World by providing most advanced analytical instrumentation"

With the introduction of HPLC in the mid-1970's JASCO's experience in highly sensitive and accurate optical systems led to the development of a series of chromatographic detection systems. Fixed and variable wavelength UV/Visible and Fluorescence detectors were introduced featuring excellent sensitivity and reliability in compact modules. In order to offer complete HPLC systems JASCO developed a variety of novel solvent delivery systems as well as other accessories such as column ovens, autosamplers, and PC based control and analysis software.

Today JASCO offers a wide variety of ***HPLC modules***, accessories and analysis software. The new ***JASCO LC-4000 Liquid Chromatography*** series is designed to operate at pressures approaching 15,000 psi for either gradient or isocratic separations, providing researchers with a powerful tool when using the new generation of small particle columns. LC-4000 Series includes a versatile series of components offering unique flexibility to build systems for routine and specialized applications. LC-4000 features the widest choice of optical HPLC detector: UV, diode array, fluorescence, chemiluminescence, CD, chiral and refractive index detector.

Finally JASCO's modular ***Supercritical Fluid Chromatography*** and ***Supercritical Fluid Extraction*** platforms provide a low-cost, fast, green technology with reliable and worry-free performance for a wide variety of applications.

JASCO has a strong global presence, supplying customers in ***over 45 different countries.***

JASCO Europe *is in charge for marketing, sales, service and support for all Jasco products throughout* ***Europe, Middle East and Africa*****.**

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Make the most of your investment with

JASCO Service and Support

JASCO Service and Support agreement plans are designed for those laboratories pursuing superior productivity through the highest level of professional services.
The use of automated instrumentation is the right approach to meet today's laboratories productivity requirements, reducing analysis run times, enhancing sample throughput, and increasing analytical accuracy and precision. In this view, preventive maintenance is very important to maximize laboratory uptime and avoid unexpected expenses.

In addition to the analytical goal, proper installation and maintenance are required to achieve optimal performance. JASCO provides flexible service and support management solutions focused on your laboratory real objectives.

With its service network, JASCO is ready to maintain the perfect reliability of customer's instrumentation and minimize the laboratory down time.

· *Superior productivity*
· *Optimized analytical performance*
· *Lower cost of ownership*
· *Extended instrument life*

If your laboratory has specific Service and Support requirements, JASCO can help you with customized contract agreements. In addition, a full set of Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ) tests are available to verify the system proper installation, operation and performance, respectively.

Get the most from your investment with

JASCO Training Courses

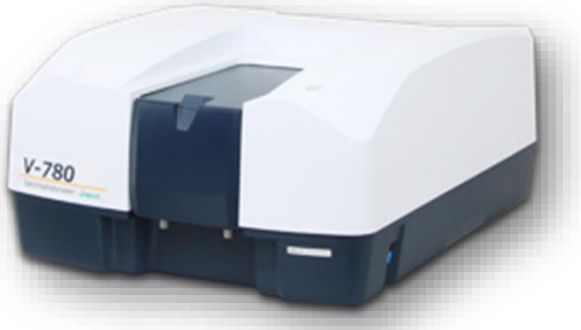
JASCO Training Courses ensure maximum skill development for the best value of your laboratory. Our team of highly-experienced specialists can help your staff to get the most from your instrument reducing your analysis run time and improve performance.

Build your knowledge with JASCO Training Courses:

· *Instrument and Software operation*
· *troubleshooting*
· *Maintenance*
· *Calibration*
· *Applications and Methods developments*
· *Operating Techniques*

V-730 – V-730bio – V-750 – V-760

UV-Vis Spectrophotometers



V-770 – V-780

UV-Vis/NIR Spectrophotometers

With more than fifty years of experience in the design of spectrophotometers, JASCO offers a complete range of UV-Vis/NIR instruments.
The ***V-700 series*** consists of six distinct models designed to meet a wide range of application requirements.

From an innovative optical layout to a simple comprehensive instrument control and data analysis software interface, the ***V-700 series*** does not compromise on accuracy, performance or reliability.

All spectrophotometers are controlled by ***Spectra Manager™***, JASCO's powerful cross-platform spectroscopy software package with USB communication.

FT/IR-4X

FT/IR Spectrometers

FT/IR-6X – FT/IR-8X

FT/IR Spectrometers

The ***FT/IR-4X, FT/IR-6X, FT/IR-8X*** models represent a broad range of instrumentation that redefine infrared spectroscopy as a powerful yet easy to use technique in a compact and reliable line of instruments with the highest signal-to-noise ratio.

All models are controlled by ***Spectra Manager™***, JASCO'S powerful cross-platform spectroscopy software package with USB communication.

All models feature an exclusive optics which maintains instrument optical alignment after beamsplitter changes or instrument movement.

IRT-5100 – IRT-5200

FT/IR Microscopes

IRT-7100 – IRT-7200

FT/IR Microscopes

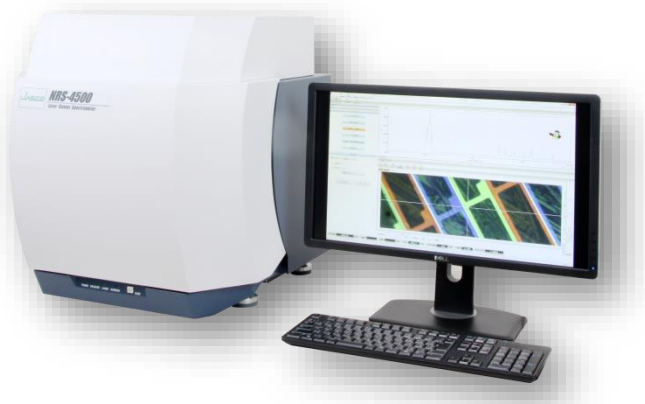
JASCO is proud to release four innovative FT-IR Microscope, the ***IRT-5000*** and ***IRT-7000***, providing several new functions that drastically improve infrared micro-spectroscopy analysis.

Both microscope systems can be easily interfaced with either the FT/IR-4X, FT/IR-6X and FT/IR-8x spectrometers, offering the most advanced microscopy and imaging systems available in the market today.

The microscope system automatically scans the specified points or area, rapidly collecting a full spectrum of each point without moving the sample stage.

NRS-4500

Laser Raman Spectrometer



The system offers space-saving, automated switching laser light source and alignment adjustment to assist the analysis, ***NRS-4500*** is easily used to quality control as well as research and development.

The micro-Raman ***NRS-4500*** is equipped with measurement assist function that can be easily setup operation and a user advice function that automatically analyzes the spectrum and obtain a high-quality data even at the first time.

QRI – Quick Raman Imaging function (available also on NRS-5000/7000 series) allows imaging speed x50 faster than traditional systems.
High Speed/High Accuracy Automatic Stage combined with High Speed EMCCD detector make it possible to shorten amount of imaging time dramatically.

NRS-5500 – NRS-5600

Laser Raman Spectrometers

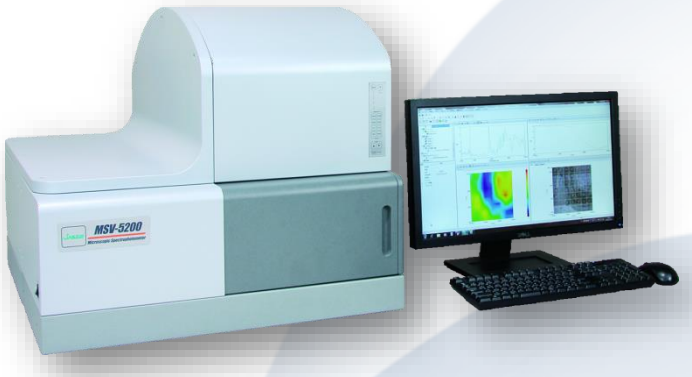
NRS-7500 – NRS-7600

Laser Raman Spectrometers

The performance expected on a micro-Raman spectrometer are fully provided with the JASCO ***NRS-5000/7000*** series Raman systems, assuring consistent performance for rapid acquisition of high quality data with automated system control and minimal optical adjustments.
For application expansion, an automated multi-grating turret, 2 internally mounted detectors and a maximum of 8 lasers ranging from the UV through the NIR are capable of integration with the instrument system.
Spectra Manager™ for the ***NRS-5000/7000*** offers revolutionary features to simplify previously difficult measurement and analysis tasks, while adding various user-support tools such as auto-fluorescence-correction, wavenumber correction, intensity correction, and a novel user-advice function.

MSV-5100 – MSV-5200 – MSV-5300

UV-Vis/NIR Microscopes



The MSV-5000 series is a microscopic spectrophotometer system providing transmittance/reflectance measurements of a microscopic sample area with a wide wavelength range from ultraviolet to near infrared.

MSV-5100 Spectrophotometer is a dedicated UV-Vis microscope with a wavelength range of (200-900 nm).

MSV-5200 Spectrophotometer includes a Peltier-cooled PbS detector and has a wavelength range of (200-2700 nm).

MSV-5300 Spectrophotometer incorporates an InGaAs detector to obtain optimized NIR measurements and has a wavelength range of (200-1600 nm).

J-1100 – J-1500 – J-1700

Circular Dichroism Spectropolarimeters

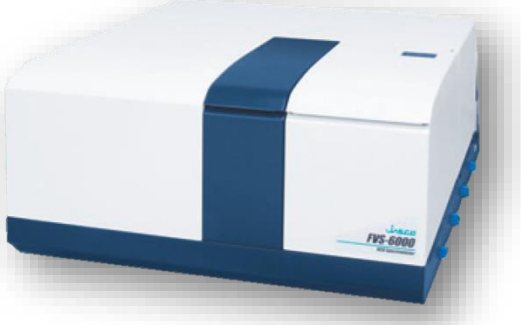
The latest effort in the JASCO commitment to lead the field of Circular Dichroism.

Unparalleled optical performance and optionally available measurement modes are combined in a manner to make the ***J-1000 Series Spectropolarimeter,*** a true "chiro-optical spectroscopy workbench", ***able to work up to 2,500 nm***.

Instrument control and data processing are handled effortlessly by our JASCO's user friendly and innovative cross-platform software, ***Spectra Manager™.***

FVS-6000

Vibrational Circular Dichroism



The ***FVS-6000*** not only allows you to easily obtain fingerprint VCD spectra, but also has several unique features such as a measurement range extension option of 4000-750 cm^{-1}.

Since the CD signals in the infrared region are one or more orders of magnitude lower than ECD signals in the UV-Vis region, high sensitivity and stability are required for a VCD spectrometer.

The **FVS-6000** is the VCD spectrometer of choice for highly sensitive VCD measurements.

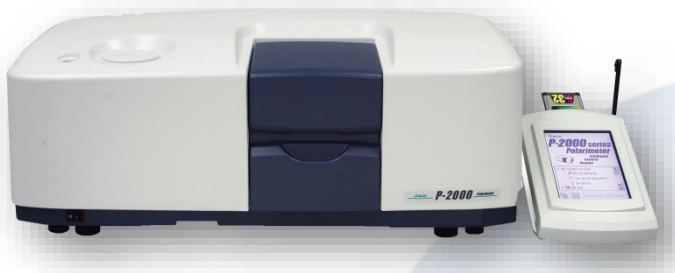
P-2000

Digital Polarimeter

The ***P-2000*** is designed as a customizable polarimeter with various options for a range of applications and budgetary requirements.

Options such as polarizers, wavelength filters, lamps and photomultiplier detectors provide a wide range of analytical wavelengths from UV-Vis to NIR.

A newly redesigned ***intelligent remote module (iRM)*** with a color LCD touch screen conveniently guides the operator through routines from data acquisition to data processing. The obtained data can be automatically printed to USB printers, or saved to a compact flash memory card for further processing on a PC.



FP-8250 – FP-8350
FP-8550 – FP-8650

Spectrofluorometers

Designed with the latest technology, the ***FP-8050 Series*** spectrofluorometers incorporate the highest sensitivity, fastest spectral scanning capability and excellent analysis-oriented functionality offering integrated solutions for NIR advanced materials research and biochemical analysis applications.

To meet the most stringent analysis demands, a variety of accessories are available for integration with a range of sophisticated control and analysis applications available in the user-friendly ***Spectra Manager™*** software to offer a flexible platform for any fluorescence and phosphorescence application.

RMP-510 – RMP-520 – RMP-530

Portable RAMAN Spectrometers

JASCO's new ***RMP-500*** Series has been developed to meet the needs of Material Science, Manufacturing and Biochemistry by combining the flexibility of a fiber optic probe with a portable Raman Microspectrometer.

The RMP-500 Series consists of three models, ***RMP-510, RMP-520, RMP-530*** ranging from small, portable units suitable for in-situ measurements to research-grade systems that will meet even the most difficult application requirements.

The **RMP-500** Series portable Raman spectrometer systems feature an integrated fiber optic probe with a small X-Y-Z stage, a compact laser, a high-throughput spectrograph and CCD detector.

VIR-100 – VIR-200 – VIR-300

Portable FT-IR Spectrometers

The ***VIR-100/200/300*** series are compact, lightweight, flexible FT-IR systems.

The collimated entrance and exit ports make it an ideal instrument for a wide range of applications.

The standard instrument includes a hermetically sealed interferometer, DLATGS detector, high intensity source, KRS-5 windows and automatic alignment. Options can be added for increased sensitivity, optional spectral ranges including NIR, and battery operation.

For even greater flexibility, external connection optics allows the user to install up to three different attachments in one system, selecting the most appropriate application accessory by simply switching the PC controlled optical configuration.

JASCO is the first manufacturer to develop a powerful, cross-platform software package, "***Spectra Manager***", for controlling a wide range of spectroscopic instrumentation. Spectra Manager program is a comprehensive package for capturing and processing data, eliminating the need to learn multiple software packages and offering the user a shallower learning curve.

Several types of measurement data files (UV-Vis/NIR, FT-IR, Fluorescence, etc.) can be viewed in a single window, and processed using a full range of data manipulation functions.

The latest version, Spectra Manager, includes four measurement programs, a spectra analysis program, an instrument validation program and the JASCO Canvas program as standard. It is possible to analyze data even during sample measurements.

Spectra Manager CFR provides features to support laboratories in compliance with 21 CFR Part 11.

LC-4000

High Performance Liquid Chromatography

The ***LC-4000*** Series is the latest in a long history of innovative HPLC systems developed by JASCO reaching all the way back to the start of commercial HPLC in the early 1970s.

The concept of the integrated ***LC-4000*** series HPLC provides key separation platforms at 50MPa, 70MPa and 130MPa which correspond to ***conventional HPLC***, the increasingly popular Rapid Analysis ***Fast HPLC*** and sub 2um ***U-HPLC*** respectively.

Each platform is supplied with a dedicated pump and autosampler matched to the operating pressure and share detectors optimized for high-speed 100Hz acquisition and the narrow peak shapes common to both Fast HPLC and U-HPLC.

In the LC-4000 series, SSQD technology (Slow Suction, Quick Delivery) has been re-developed, with a completely new solvent delivery mechanism offering the highest stability in solvent delivery across the entire analytical flow rate range used in the ***PU-4100*** Fast HPLC and ***PU-4200*** U-HPLC pump models.

JASCO has the largest range of optical detectors - from ***dual wavelength UV*** to ***diode array*** to unique ***chiral detectors***. All the detector are designed to meet U-HPLC requirements, data acquisition rate of 100Hz.

SFC-4000

Supercritical Fluid Chromatography

The JASCO ***SFC/SFE 4000*** integrated Analytical SFC system has been developed for all aspects of analytical SFC; including routine separation, method development and small scale preparation of samples at the mg scale.

With a simple intuitive software and robust engineering, the JASCO SFC system is a powerful tool for analytical separations.

Before separating and collecting the target chiral compound, finding the optimum separation condition is required (column, solvent, etc.).

JASCO offers a wide range of detectors with high pressure cells UV, Diode Array (real-time collection of 3D spectra and chromatogram) and the only CD detector available for SFC.
Especially, ***JASCO original CD detector*** measures optical isomers with circular dichromatic absorption, and can measure both CD and UV chromatograms as well as g-factor (CD/UV) chromatograms. Since g-factor in particular has a proportional relationship with the compositional ratio of optical isomer sample, the CD detector can perform compositional measurements and high purity fractionation for non-separated peaks.

LC-4500

Compact HPLC

The new ***LC-4500*** model has been developed taking into account laboratory space requirements.

The ***LC-4500*** compact HPLC provides outstanding performances in ***conventional HPLC***, making effective use of lab space.

PU-4580 enables to configure the various solvent delivery system, ***AS-4550*** can provide the various sample injection method and ***UV-4570*** UV-Vis detector offers you an enhanced sensitivity.

LC-4500 model has the key panel, which achieves simple operation.

Combination with existing LC-4000 modules can provides wider application.

Both HPLC and SFC/SFE systems are coupled with ***ChromNAV 2.0*** data system to offer both HPLC and spectral data handling for most of the detectors even with the dual wavelength UV detector.

A newly added feature of ***ChromNAV 2.0*** is the automatic e-mail notification on your smartphone/tablet, stay always updated on analysis status of your LC-4000. Full GLP compliance and 21 CFR part 11.

Table of Contents

Quantitative analysis of λDNA using SAF-850 One drop accessory

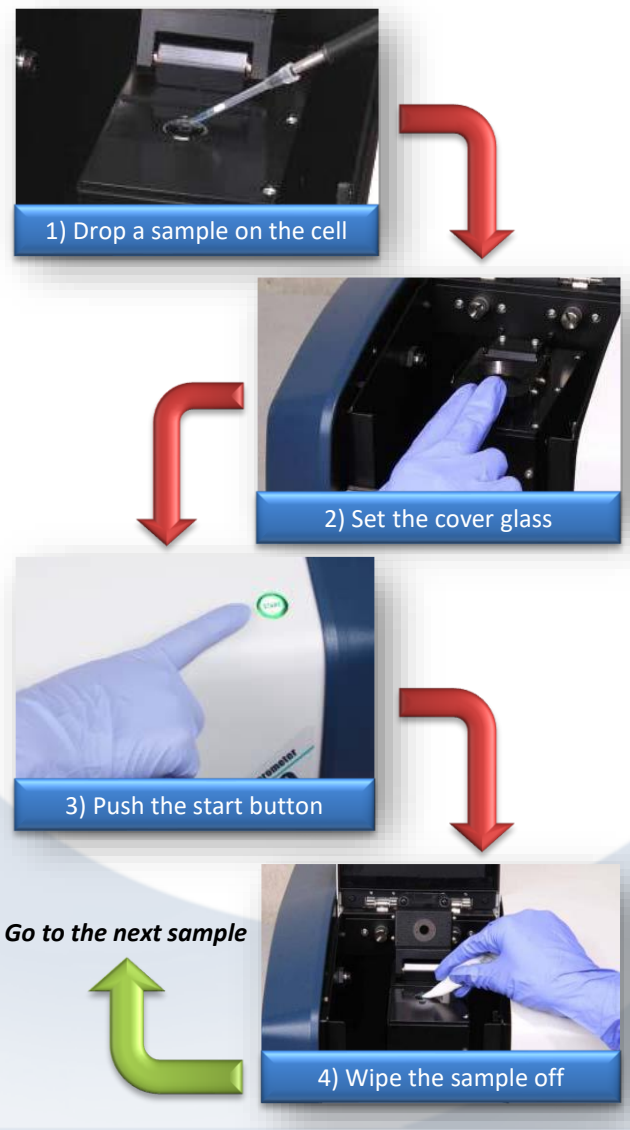
Introduction

SAF-850 One Drop accessory is a special accessory, enabling fluorescence measurement by just dropping 5 µL of the sample on a cell and then covering it with another glass. This simple way of measurement is the most suitable and effective for quantitative analysis of multiple samples such as fluorescently-labeled DNA and various kinds of fluorescent dye. In this application data, the measurement result of λDNA labeled with PicoGreen is shown.

Measurement system

- FP-8200 Spectrofluorometer
- SAF-850 One Drop accessory
- λDNA labeled with PicoGreen®: 0, 1, 5, 10, 50, 100, 500, 1000 ng/mL

Measurement procedure



Results

1) Spectra

The emission spectra of sample with each concentration with Ex. Wavelength of 480 nm are shown in Figure 1. The result indicates that the peak wavelength is 523 nm.

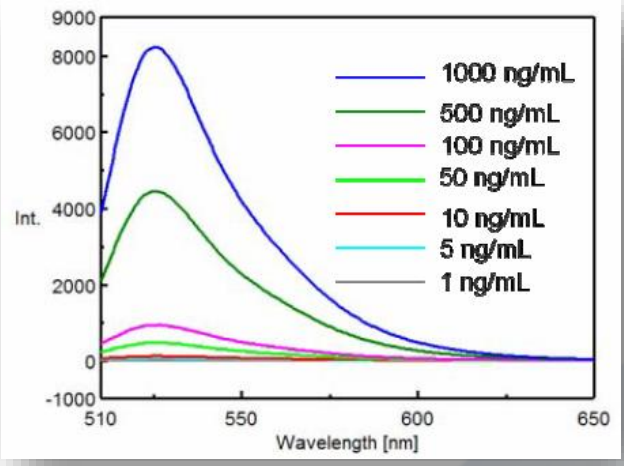


Figure 1 Emission spectra of λDNA labeled with PicoGreen®

Measurement conditions

Measurement mode: Emission
Ex wavelength: 485 nm
Measurement range: 510-650 nm
Ex bandwidth: 10 nm
Em bandwidth: 10 nm
Scanning speed: 100 nm/min
Data interval: 0.5 nm
Response: 2 sec
Sensitivity: 700V

Quantitative analysis of λDNA using SAF-850 One drop accessory

2) Measurement reproducibility

Table 1 shows the result of 5 times measurements of the sample with each concentration.

Measurement conditions

Measurement mode: Emission
Ex wavelength: 480 nm
Em wavelength: 523 nm
Ex bandwidth: 20 nm
Em bandwidth: 20 nm
Response: 1 sec
Sensitivity: 620 V

3) Linearity

The calibration curve was generated using the average value of the results in Table 1. Figure 2 shows the good linearity over a wide concentration range from 1ng/mL to 1000 ng/mL.

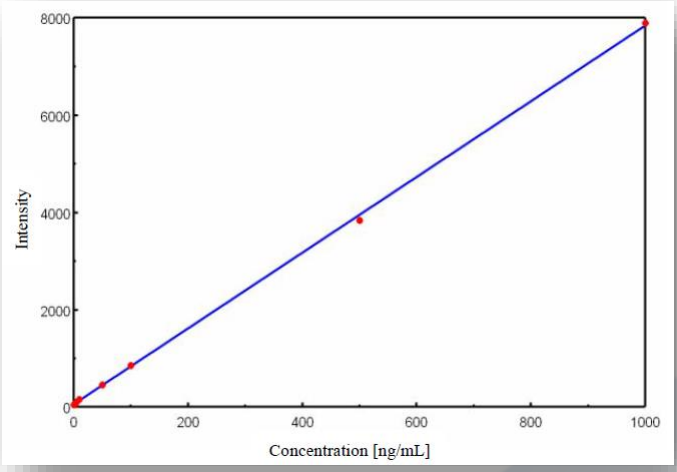


Figure 2 Calibration curve of λDNA labeled with PicoGreen®

Calibration curve equation : Int.= 7.780 x Conc.+ 57.5211
Correlation coefficient : 0.9999
Standard error : 6.819

Conc. [ng/mL]	1	2	3	4	5	Ave	SD	CV(%)
0	53.3	52.4	55.9	53.1	55.2	54.0	1.49	2.8
1	63.6	68.1	65.9	66.5	65.1	65.8	1.68	2.6
5	110.3	106.7	105.1	104.0	110.0	107.2	2.86	2.7
10	157.6	155.5	156.1	153.0	151.7	154.8	2.39	1.5
50	447.1	465.3	460.2	455.8	469.0	459.5	8.56	1.9
100	865.9	856.9	848.3	850.6	853.9	855.1	6.86	0.8
500	3842.7	3831.0	3858.0	3828.9	3811.0	3834.3	17.42	0.5
1000	7766.2	7992.1	7925.3	7972.8	7805.7	7892.4	101.15	1.3

Table 1 Measurement reproducibility

Activity measurement of trypsin using a fluorescence peptide substrate

(3) Enzyme activity measurement

Substrate solution 0.5 mL of each concentration was dropped at enzyme solution 2.5 mL, and time course measurement of the fluorescence intensity of the isolation AMC was performed to it. A result is shown in Figure 3.

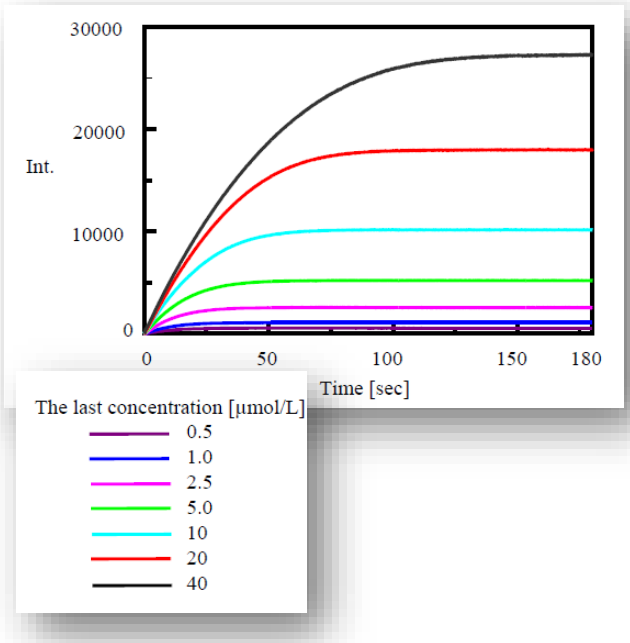


Figure 3 The pursuit result of an enzyme reaction process

Measurement parameters

Ex wavelength: 360 nm
Em wavelength:440 nm
Ex bandwidth: 5 nm
Ex bandwidth: 10 nm
Data interval: 0.1 sec
Response: 0.1 sec
Scan speed: 200 V

Analysis

[Kinetics Analysis] In quest of each initial velocity, the Lineweaver-Burk plot was performed using the program from inclination of the time variation data of each substrate concentration (Figure 4).

Km=5.99 and Vmax=35270 nmol/L-min-1 were obtained from this result.

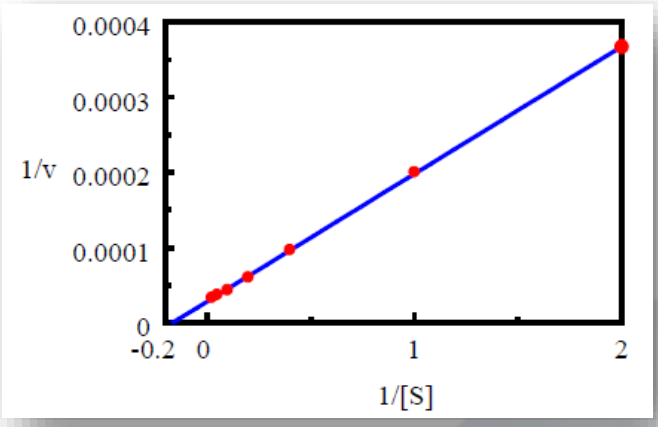


Figure 4 Lineweaver-Burk plot

Calculation result

Calculation method: Lineweaver-Burk Plot
Vmax: 35270
Km: 5.99
1/v = 0.000170 x 1/[S] + 0.0000284

Activity measurement of trypsin using a fluorescence peptide substrate

Introduction

Hydrolysis reaction is caused by making protease act on METHYLCOUMARIN-AMIDE (MCA) of peptide substrate, and 7-AMIDO-4-METHYLCOUMARIN (AMC) isolated.

This isolated AMC, fluorescence becomes the maximum by wavelength 440 nm. Protease activity can be measured using fluorescence spectrophotometer. We introduce the example which performed activity measurement of trypsin using a fluorescence peptide MCA substrate.

Measurement system

- FP-8300 Spectrofluorometer
- STR-812 Water thermostatted cell holder with stirrer
- CSP-829 Sample compartment lid with syringe port
- MCB-100 Mini Circulation Bath*1)
- VWKN-772 Kinetics Analysis Program

*1) The temperature of a circulation bath is set as 37 degrees by all the measurement.

Samples

Intensity standardization sample: 50 µmol/L AMC solution

Enzyme solution: 10 nmol/L Trypsin bovine pancreas typeVIII, 50 mmol/L Tris-HCl, 0.15 mol/L, NaCl, 1.0 mmol/L CaCl2, 0.1 mg/mL BSA

Substrate solution: Boc-Gln-Ala-Arg-MCA solution

(The concentration after mixture is adjusted to 0.5, 1, 2.5, 5, 10, 20, 40 µmol/L)

Concentration for adjustment [µmol/L]	240	120	60	30	15	6	3
The last concentration [µmol/L]	40	20	10	5	2.5	1	0.5

Measurements

(1) Fluorescence-spectrum measurement of AMC

Excitation and the Fluorescence spectrum of 50 µmol/L AMC were measured (Figure 2). It turns out that the fluorescence maximum wavelength from this result is 440 nm.

Measurement parameters

Ex wavelength: 360 nm
Em wavelength:440 nm
Ex bandwidth: 5 nm
Em bandwidth: 10 nm
Response: 0.5 sec
Sensitivity: 200 V
Data interval: 1 nm
Scan speed 500 nm/min

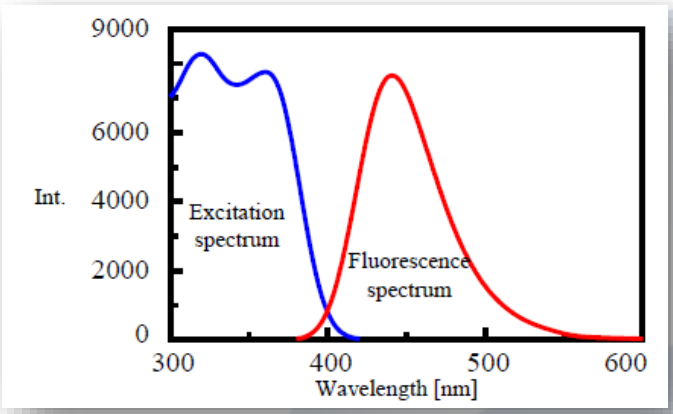


Figure 2 Fluorescence spectrum of AMC

(2) The vertical axis is changed into concentration from fluorescence intensity

Intensity standardization was performed in order to change the vertical axis into the numerical value equivalent to concentration.

50 µmol/L AMC solution of 0.5 mL is dropped at enzyme solution 2.5 mL. Fluorescence intensity of last concentration of 8.333 µmol/L AMC solution was set to 8333 µmol/L AMC solution.

(3) Enzyme activity measurement

Substrate solution 0.5 mL of each concentration was dropped at enzyme solution 2.5 mL, and time course measurement of the fluorescence intensity of the isolation AMC was performed to it. A result is shown in Figure 3.

Fluorescence Measurement of Heat-Denatured Lysozyme

Experiments for the denaturation of proteins are generally measured by Circular Dichroism. However, proteins contain aromatic amino acids (AAA) and will fluoresce when excited with UV light. During heat denaturation of proteins, the secondary structure of the protein will change and the aromatic amino acid residues will change slightly which can be detected by fluorescence.

Experimental

Emission (EM) spectra of lysozyme using an excitation (EX) of 280 nm are measured while controlling the temperature, to examine the relation between temperature and the fluorescence spectrum. The model FP-6500 spectrofluorometer and an ETC-272 Peltier thermostatted cell holder are used for the measurements in this experiment, using the instrument parameters outlined below.

Parameters

Temperature range	: 15 to 90°C
Temperature interval	: 5°C
EM bandwidth	: 5 nm
EX bandwidth	: 3 nm
EX wavelength	: 280.0 nm
Response	: 0.5 sec
Gain	: Medium
Wavelength range	: 290 to 450 nm

A buffered aqueous solution of 0.1mg/mL of lysozyme was used as the sample, the measurement performed by stirring the sample with a magnetic stirrer to ensure even sample temperatures in the cell. A temperature ramping rate of 1°C/min was controlled by the Peltier cell holder. Spectra are measured within 60 seconds after reaching the individual set temperature points.

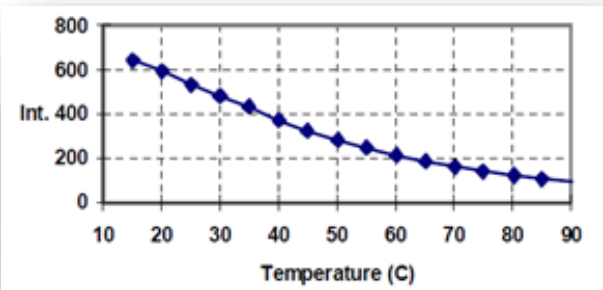


Figure 1 Temperature Dependence of Lysozyme aqueous solution at EX 280 nm, EM 340 nm

Results

The graph below (Figure 1) demonstrates the change of intensity vs. temperature at 340 nm. Lyzosyme is known to denature at a temperature of 70°C; however the graph of fluorescence vs. temperature shows only a decrease in intensity as the temperature increases. By contrast, the EM Spectra from 40 to 90 degrees plotted using a contour view demonstrates a transition of the spectra at 70 degrees (Figure 2).

The EM spectra at room temperature have an EM maximum at 340 nm with a corresponding maximum at 348 nm for 90 degrees. A plot of the intensity ratios for the two wavelengths versus temperature results in a graph that demonstrates a heat-denaturation at 70°C (Figure 3), in agreement with literature values.

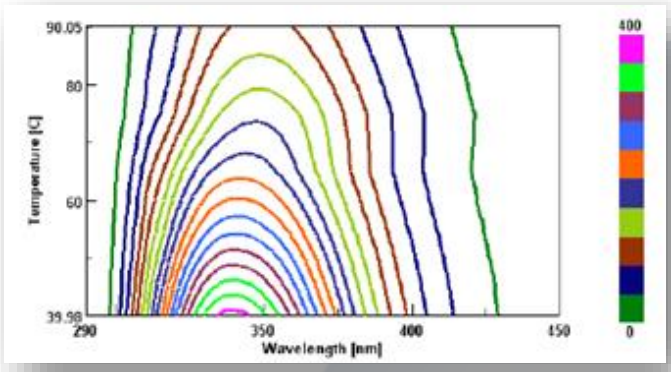


Figure 2 Contour view of EM spectra from 40 to 90 degrees

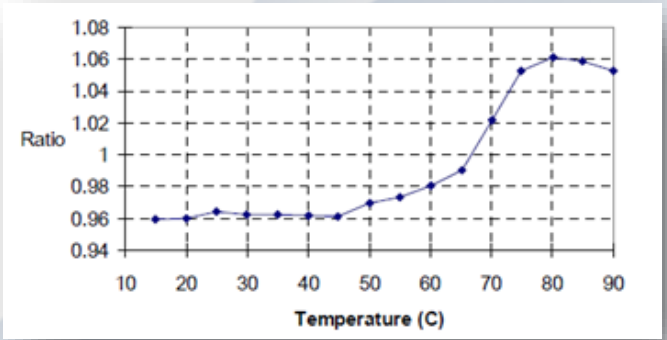


Figure 3 Intensity ratio of 340/348 nm vs. temperature

Acid Unfolding of Horse Cytochrome C Measured with a Fluorescence Stopped-Flow System

The fluorescence characteristics of tryptophan in proteins will vary depending on the structures surrounding the amino acid. The fluorescence of Cytochrome C is derived from the tryptophan in the residue position 59. The natural state of this tryptophan is so close to the Heme iron residue that the fluorescence is quenched by nonradiative energy transfer to the Heme iron. When Cytochrome C is denatured by an acid, the distance between the tryptophan and Heme iron changes and the fluorescence intensity grows. This application note demonstrates the measurement of the change in fluorescence intensity by the acid denaturation of Cytochrome C using the JASCO stopped-flow system.

Measurement/Analysis Systems
FP-6500 Spectrofluorometer
SFS-482 Stopped-Flow system (Cell length: 10 mm)
[Stopped-Flow Measurement] program
[Reaction Rate Calculation] program

Syringe configuration
S1: 10 mL, 0.5mg/mL Cytochrome C
S2: 10 mL, 0.1N sulfuric acid

Parameters:

Excitation bandwidth	: 5 nm
Emission bandwidth	: 5 nm
Response	: 2 seconds
Sensitivity	: Manual
Excitation wavelength	: 280 nm
Emission wavelength	: 340 nm
Measurement range	: 0-5000 milliseconds
Measurement interval	: 5 milliseconds
No. of accumulations	: 4
Flow time	: 35 milliseconds
Flow volume	: S1 = 100 µl; S2 = 100 µl
Mixing ratio	: S1:S2 = 1:1

Stopped-Flow System

Cell Holder and Cell

Experimental
Figure 1 illustrates the measured and calculated results of the Cytochrome C emission during the stopped-flow experiment.
The measured data shows an extreme change in the fluorescence intensity corresponding to the acid denaturation of Cytochrome C. The JASCO stopped-flow system enables data acquisition before the syringe movement is completed to ensure that the early stage of the reaction data before and after the flow time ends can be acquired.
The reaction rate was calculated with the [Reaction Rate Calculation] program. The calculated range was 35 to 5000 msec and a 2-step reaction mechanism was applied for the calculation. The calculated results show an excellent fit to the experimental data.

Calculation range: 35 to 5000 msec
Reaction rate formula: Y(t) = -142.667 * exp(-t / 432.854) + -47.7112 * exp(-t / 3611.11)
Step 1 time constant: 432.854 msec
Step 1 rate constant: 0.00231025 msec-1
Step 2 time constant: 3611.11 msec
Step 2 rate constant: 0.000276923 msec-1

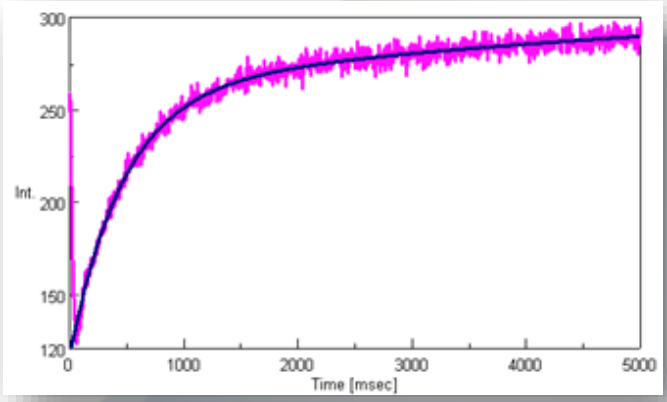


Figure 1 Measured and calculated results of Cytochrome C emission

Measuring fluorescence anisotropy spectrum of Rhodamine B

Measuring fluorescence anisotropy is performed using polarizers on both excitation(Ex) side and emission(Em) side. It is known that Rhodamine B has wavelength dispersion of fluorescence anisotropy depending on the excitation wavelength. Therefore the Rhodamine B was measured to obtain wavelength dispersion of fluorescence anisotropy and degree of polarization.

Measurement principle

With Ex side polarizer in the vertical(V) position, each excitation spectrum is measured under Em side polarizer in the vertical(V) and horizontal(H) positions. These fluorescence intensities of the excitation spectra are defined as IV and IH. In order to correct the difference of sensitivity for polarization on Em side detector, the spectra are measured with Ex side polarizer in the H and Em side polarizer in the V and H positions, and the ratio is multiplied by IH to obtain IH(corrected). Then calculated fluorescence anisotropy(r) is obtained using the following equation.

r = { IV- IH(corrected)} / {IV +2 IH(corrected)}

Measurement condition

Instrument: FP-6500
Polarizer: FDP-203 polarizer
Measurement mode: Ex spectrum
Ex side bandwidth: 3 nm
Em side bandwidth: 10 nm
Response: 0.5 sec
Sensitivity: Medium
Measurement wavelength range: 350-580 nm
Data acquisition interval: 0.2 nm
Em wavelength: 625.0 nm
Wavelength scan speed: 200 nm/min

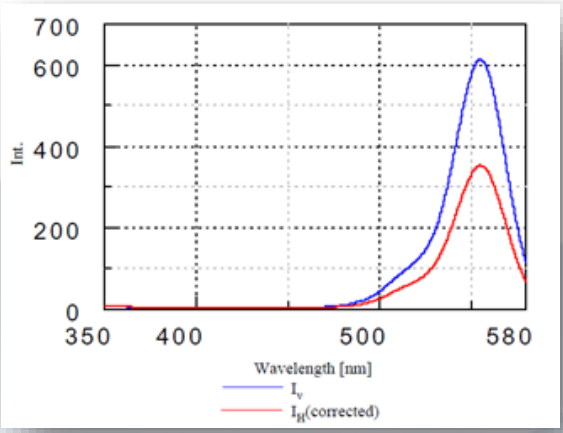


Figure 1 Ex spectra of IV and IH(corrected)

Measurement procedure

1. Set Ex side polarizer in the V position and set Em side polarizer also in the V position. Using FP-6500 spectrofluorometer, measure Ex spectrum of ethylene glycol solution (0.588 mg/mL). Then the fluorescence intensity of the spectrum is defined as IV.
2. Set Ex side polarizer in the H position and set Em side polarizer in the V position, and measure spectrum in the same way like item 1.
3. Set Ex side polarizer in the H position and set Em side polarizer in the H position, and measure spectrum in the same way like item 1.
4. Set Ex side polarizer in the V position and set Em side polarizer in the H position, and measure spectrum in the same way like item 1.
5. Calculate the ratio of the above item 2.and 3. results, and then approximate average value is determined to be ratioavg = 3.0.
6. Multiply the spectrum of the above item 4. with the ratioavg = 3.0, to determine this fluorescence intensity as IH(corrected).
7. Calculate the fluorescence anisotropy(r) using IV and IH(corrected).

Measurement result

The absorption band that has peak wavelength around 560 nm shows large constant fluorescence anisotropy(r). On the other hand, r around 430nm, 360 nm show negative peaks, and shows small positive peak around 380 nm. These fluorescence anisotropy variation suggests that different electronic transition bands are overlapped.

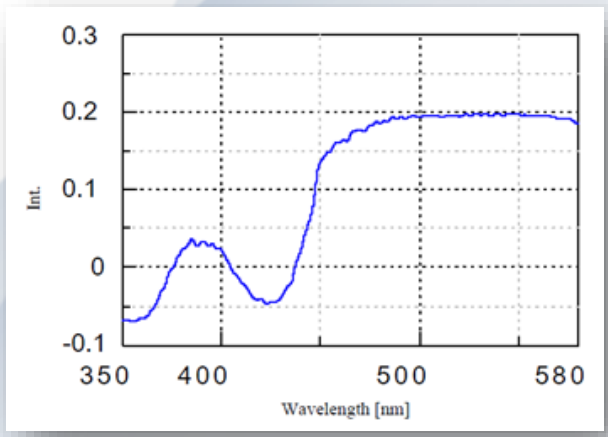


Figure 2 Ex spectrum of fluorescence anisotropy

Fluorescence depolarization measurement for Liposome using FP-6500

1. Fluorescence polarization and depolarization

The molecule is excited (S0 --> S1) by the absorption of light from ground state to transition state and stabilized in the most stable vibrational state by its molecular relaxation process, and then it returns again (S1 --> S0) to the ground state by radiating fluorescence light.In the process of S0 --> S1 or, S1 --> S0, there is a directionality (transition moment), determined by molecular structure, that is closely related to polarization direction of excitation and fluorescence light.

(1)Absorption process: With exciting by linearly polarized light, probability of excitation of each molecule is expressed as below.
P(α) = P0cos2α (α: Angle between polarization direction and transition moment, P0: proportional constant).
When vector of electric field of excitation light and transition moment are in parallel condition, the probability of excitation will become maximum.

(2) Radiation process: Fluorescence in the process of S1 --> S0 has polarizing direction in conformity with the direction of transition moment. Fluorescence light from the molecule excited by polarized light has polarization property depending on the polarizing direction of excitation light. Moreover, such polarization property of the fluorescence light will be affected also by the rotation of the molecule due to Brownian motion, etc. until the fluorescence is radiated. In other words, when fluorescence is radiated before the rotation of its molecule, fluorescence light will be strongly polarized towards direction of the polarization of excitation light, but on the other hand, when fluorescence is radiated after rotation of its molecule in completely random direction, the fluorescence light will be no longer polarized. This is called “depolarization”.

2. Measurement method and measured example for fluorescence depolarization

In order to measure the fluorescence depolarization by FP-6500 spectrofluorometer, automatic polarization measurement unit and its software are needed.

By setting the Excitation (Ex) side polarizer in the vertical position(V), both fluorescence intensities(IVV and IVH) are measured by setting the fluorescence(Em) side polarizer in the horizontal position(H) and V. In order to correct the sensitivity difference of the Em side detector against polarization, the spectrum is measured by setting the Ex side polarizer in H position and setting the Em side polarizer in V and H position to obtain instrumental function, G(=IHV/IHH), and the correction is applied multiplying IVH by G factor. Accordingly, each parameter is expressed by the following formula.

Total fluorescence intensity (F):	$I_{VV} + 2G \cdot I_{VH}$
Fluorescence anisotropy (r):	$\frac{I_{VV} - G \cdot I_{VH}}{I_{VV} + 2G \cdot I_{VH}}$
Polarization degree (P):	$\frac{I_{VV} - G \cdot I_{VH}}{I_{VV} + G \cdot I_{VH}}$

As an example, measurement was made using 0.22 mg/L rhodamine B in glycerin solution.

Measurement conditions
Automatic polarization measurement unit: ADP-303
Water-cooled Peltier thermostatted automatic polarization measurement unit.
Measurement mode: Fluorescence
Excitation wavelength: 550 nm,
Emission Wavelength: 600 nm
Excitation bandwidth: 5 nm
Emission bandwidth: 5 nm
Response: 0.1 sec
Measurement temperature: 25 degrees C

No.	IVV	IVH	G	F	P	R
1	244.1098	30.737	2.833	418.2683	0.4741	0.3754
2	244.3634	30.7426	2.833	418.5530	0.4745	0.3757
3	243.7140	30.6777	2.833	417.5363	0.4743	0.3755
4	243.3629	30.6519	2.833	417.0391	0.4740	0.3753
5	242.9584	30.5799	2.833	416.2262	0.4743	0.3756
Ave.	243.7017	30.6778	2.833	417.5246	0.4742	0.3755
S.D.	0.5639	0.067	0	0.9398	0.0002	0.0002
C.V.	0.2314	0.2184	0	0.2251	0.0353	0.0419

Chart 1 Fluorescence polarization of 0.22 mg/L rhodamine B in glycerin solution

Fluorescence depolarization measurement for Liposome using FP-6500

3. Fluorescence polarization measurement of Liposome

Fluorescence polarization depends on the time from excitation of the molecule to its radiation and on how long polarization property of the initially excited molecule is kept without some movement of the molecule.

Therefore, in other words, by measuring the degree of fluorescence polarization, it can be known how difficult the molecule can move. The factors related to molecule movement are as follows.

(1) Molecular size (The larger molecule is harder to move.)
(2) Viscosity of environment where molecule exists (In higher viscous condition, molecule is harder to move.)
(3) Strength and degrees of freedom of molecule in bondage.

The above factors are taken into consideration mainly for measurement of biological samples, and especially are utilized for analysis of the size and conformation change for biological polymer and the local conformation. Here is a result of polarization degree measured under the condition that diphenyl hexatriene (DPH) is added to liposome (lipid bilayer) that is generated by dispersion of the phosphatide in water and the temperature is changed from 25 degrees C to 55 degrees C.

DPH is held in oriented form between lipid bilayer and is restricted to move at a low temperature, but when membrane drastically flickers by the phase transition of lipid bilayer at 40 degrees C, the movement of DPH is activated, and depolarization is clearly observed.

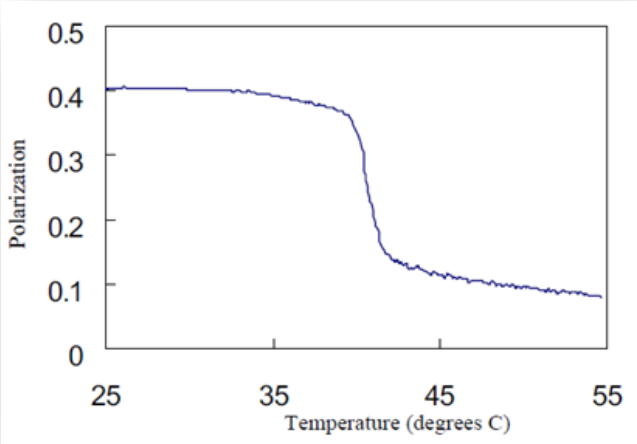


Figure 1 Temperature gradient measurement for fluorescence polarization of DPH added to liposome

DPH is held in oriented form between lipid bilayer and is restricted to move at a low temperature, but when membrane drastically flickers by the phase transition of lipid bilayer at 40 degrees C, the movement of DPH is activated, and depolarization is clearly observed.

Diffuse Reflectance Measurement of Fluorescent Powder

Fluorescent powder has been evaluated by its fluorescence spectrum, excitation spectrum, external/internal quantum efficiency and luminescent color. In addition, diffuse reflectance spectrum is now required for obtaining the findings related to absorption spectrum. However, in the diffuse reflectance measurement by using of spectrophotometer with integrating sphere, since the scattered light and fluorescence from sample cannot be separated, only sum of scattered light and fluorescence light will be obtained as the result of reflectance measurement. Therefore, such artifact must be corrected to obtain the true reflectance.

In this application, by using sodium salicylate, a typical fluorescence powder as a sample, the procedure to eliminate the fluorescence component from such reflectance artifact will be demonstrated.

In order to reduce the reflectance artifact by fluorescence, firstly, the L42 cut filter was placed in front of the detector so that scattered light caused from irradiation light can be transmitted through the filter and, the fluorescence can be cut by the filter. Secondly, in the single beam mode, spectra of standard white plate and sample were measured without using of L42 filter. Thirdly, the same single beam spectra were measured with using the L42 filter.

The transmittance of L42 will be the ratio between two spectra of standard white plate measured with filter and without filter (a) of Fig. 1). The single beam spectra of sodium salicylate measured with filter and without filter are shown in b) of Fig. 1. The L42 cut filter can transmit the 50% of light at 420 nm, and can cut the light of wavelength shorter than 420 nm and so it is evident that the transmittance in the range shorter than 420 nm was significantly reduced when measured with filter. Also, the spectrum of sodium salicylate measured without filter indicates that most of the signal intensity shorter than 370 nm was from fluorescence.

The fluorescence spectra of sodium salicylate measured with L42 filter and without filter are shown in Figure 2.

The peak measured with filter (light blue in color) was observed as fluorescence and the peak without filter (pink in color), as total fluorescence and then the ratio of peak area was calculated, which is a factor to obtain the total fluorescence from fluorescence. The calculated ratio was 1.0556. By multiplying the factor 1.0556 with the single beam spectrum of sodium salicylate measured with filter (light blue in color; Figure 1b), the total fluorescence was obtained. Then, the true diffuse reflectance component was obtained by the subtracting the total fluorescence from single beam spectrum of sodium salicylate measured without filter (pink in color; Figure 1b).

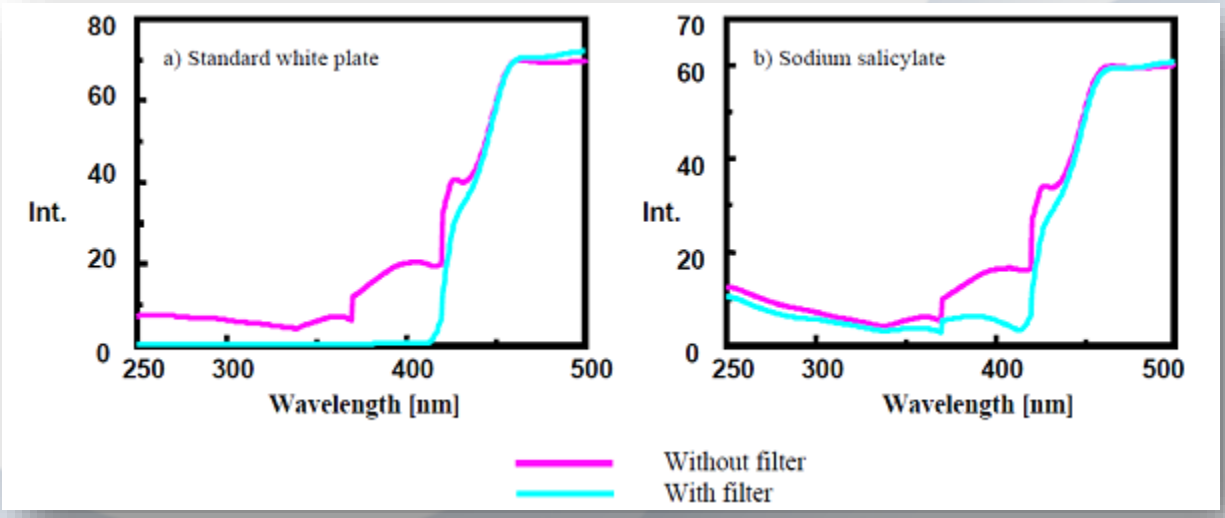


Figure 2. Fluorescence spectra of sodium salicylate.

Diffuse Reflectance Measurement of Fluorescent Powder

The true diffuse reflectance component of sodium salicylate is shown in Figure 3 together with the diffusive reflectance component of the standard white plate obtained in the same procedure. Since such component of standard white plate can be considered as 100% Line, the diffuse reflectance spectrum of sodium salicylate (Figure 4) was obtained by the ratio of two spectra in Figure 3.

NOTE:
Cut-off wavelength of L42 filter is 410 nm. Therefore, this correction procedure can be applied only to the wavelength range shorter than 410 nm.

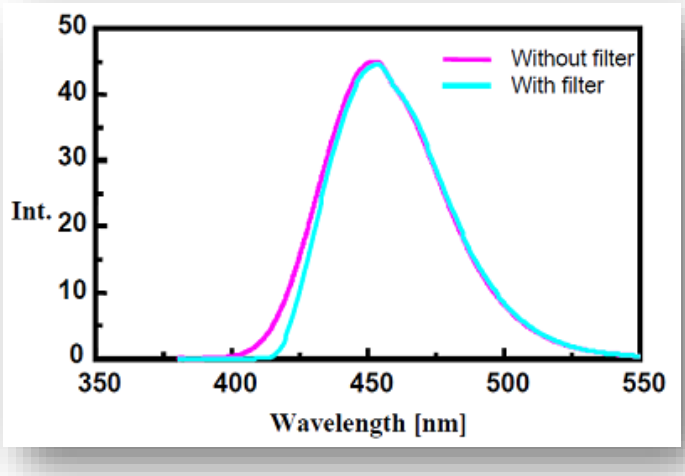


Figure 2. Fluorescence spectra of sodium salicylate.

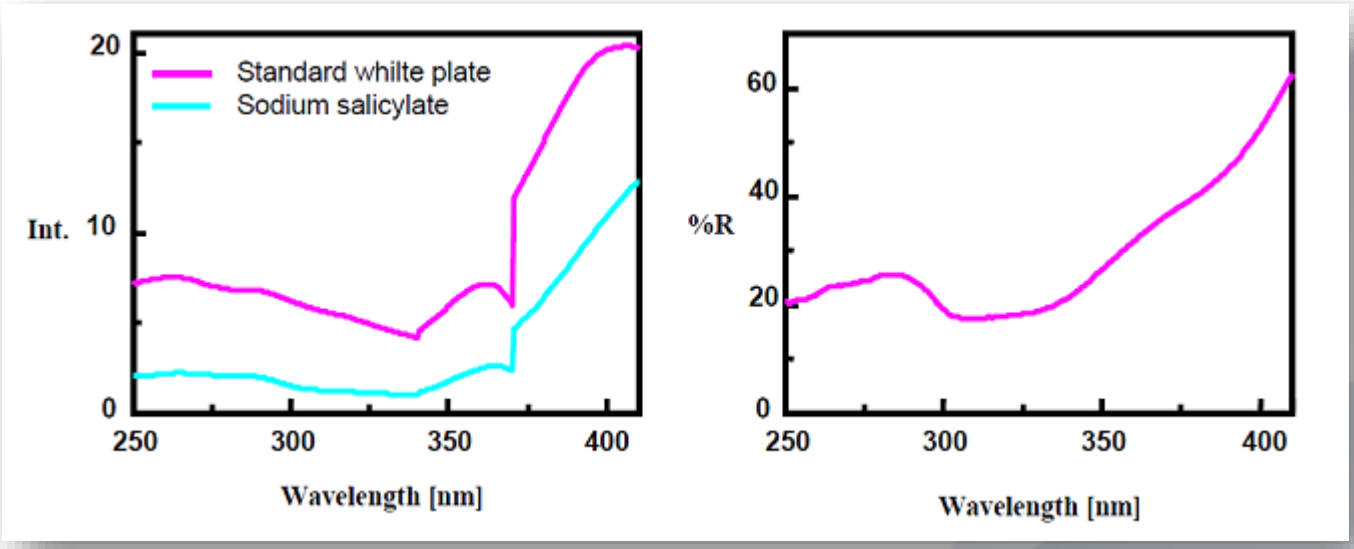


Figure 3. Diffuse reflectance component.

Figure 4. Diffuse reflectance spectrum of sodium salicylate

Phosphorescence Spectrum Measurement for Quantum Efficiency

Introduction

Phosphorescence substances have attracted attention as luminescent material for organic EL device.

Quantum efficiency of phosphorescence substances is required for developing such materials. Although the conventional integrating sphere measures sample spectrum at room temperature, phosphorescence is observed by cooling the sample to the temperature of liquefied nitrogen at 77K. JASCO developed a new dedicated system for calculating quantum efficiency from the measured phosphorescence spectra at 77K.

Phosphorescence Quantum Efficiency Measurement System

FP-6500 Spectrofluorometer
100-mm Cooling Integrating Sphere
FWSQ-6017 Quantum Efficiency Calculation Program
Sample: solid (6 to 7 mm sq x 1.0 to 1.5 mm tick)
powder
liquid

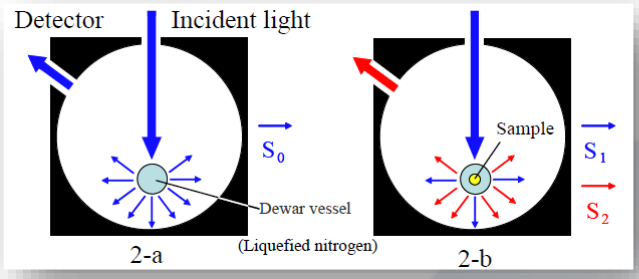


Figure 2 Sample position in the integrating sphere

Measurement Procedure to Calculate Quantum Efficiency

Quantum efficiency is obtained by the ratio between "photon number absorbed by sample" and "photon number emitted by sample". To measure phosphorescence spectra with an integrating sphere for the calculation, first, place a Dewar vessel with coolant such as liquefied nitrogen in the sphere and measure a spectrum of incident light as illustrated in Fig. 2-a. The peak area appears in the Ex wavelength range of the spectrum, illustrated with blue in Fig. 3, indicates incident photon number S0. Then, place a sample inside the vessel and measure a spectrum including scattering light of incident light and sample emission as illustrated in Fig. 2-b. The peak area appears in the Ex or Em wavelength range of the spectrum, illustrated with red in Fig. 3, indicates photon number unabsorbed by sample S1 or emitted by sample S2, respectively.

The quantum efficiency is calculated from "photon number absorbed by sample = S0 - S" and "photon number emitted by sample S2":

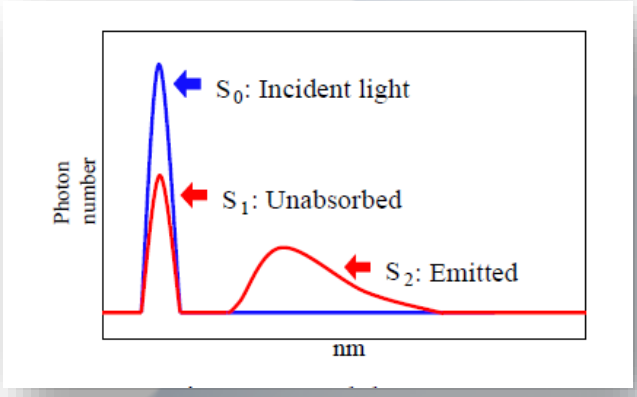


Figure 3 Model spectra

$$\textbf{Quantum Efficiency} = \frac{\text{Photon number emiitted by sample}}{\text{Photon number absorbed by sample}} = \frac{S_2}{S_0 - S_1}$$

Phosphorescence Spectrum Measurement for Quantum Efficiency

Fluorescence Quantum Efficiency of Quinine Sulfate

To confirm whether placing the Dewar vessel and coolant*1) in the sphere has any effect on the measurement results, quinine sulfate with well-known fluorescence quantum efficiency was measured by using this system. Fig. 4 illustrates the measured spectra and Table 1 shows the calculation results of quantum efficiency (ϕ). The calculated fluorescence quantum efficiency was 0.56 that corresponds well with the literature-based value of 0.546*2). From this calculation results, the effect of placing vessel inside the sphere can not be confirmed.

Measurement Parameters

- Ex bandwidth 5 nm
- Em bandwidth 5 nm
- Response 0.5 sec
- Ex wavelength 350.0 nm
- Data interval 1 nm
- Scan speed 1000 nm/min

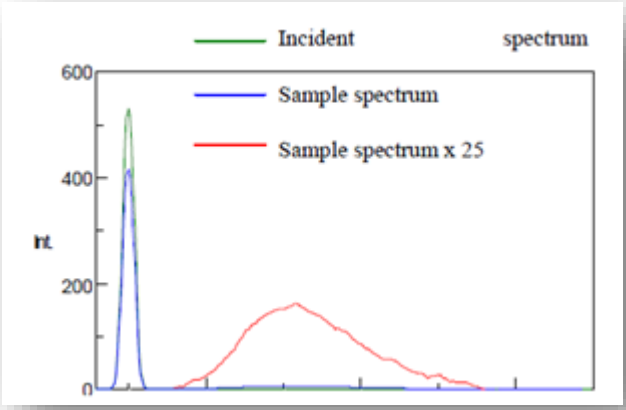


Figure 4 Spectra of Quinine Sulfate

	Int.
S_0	5377.3
S_1	4092
S_2	715.4
Φ [Measured]	0.56
Φ [Literature]*2)	0.546

Table 1 Fluorescence quantum efficiency of Quinine sulfate

Phosphorescence Quantum Efficiency of Benzophenone

Benzophenone was measured as a representative phosphorescence substance. The sample was cooled by liquefied nitrogen. Fig. 5 illustrates the measured spectra and Table 2 shows the calculation results of quantum efficiency (ϕ). The calculated phosphorescence quantum efficiency was 0.93 that corresponds well with the literature-based value of 0.9*3).

Measurement Parameters

- Ex bandwidth 5 nm
- Em bandwidth 5 nm
- Response 0.5 sec
- Ex wavelength 335.0 nm
- Data interval 1 nm
- Scan speed 1000 nm/min

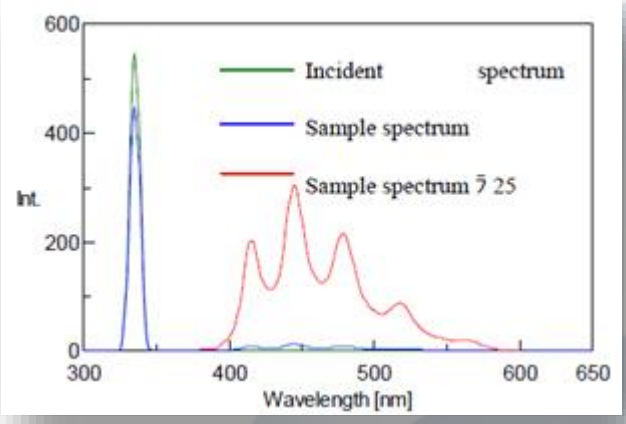


Figure 5 Spectra of Benzophenone

	Int.
S_0	4954.3
S_1	4074.0
S_2	819.3
Φ [Measured]	0.93
Φ [Literature]*3)	0.9

Table 2 Phosphorescence quantum efficiency of Benzophenone

*1) In this measurement, the Dewar vessel was filled with water instead of liquid nitrogen

*2) Melhuish, W.H., J.Phys.Chem. **65**, 229, 1961

*3) The chemical society of Japan, Courses in Experimental Chemistry 3 basic physical chemistry, Maruzen ISBN: 4-621-07303-6

Relative quantum yield measurement using FP-8000 series

Introduction

Fluorescence quantum yield is defined as the ratio of the number of photons emitted from sample as fluorescence to the number of photons in the excited light absorbed. Absolute method and relative method are known measuring methods. Regarding the absolute method, it is necessary to detect all the fluorescence from the sample, requiring the integrating sphere, while relative method can calculate quantum yield of unknown sample by comparing the intensity of standard fluorescence with unknown sample, and accordingly the relative method is easier to get the results of quantum yield. In this experiment, an example will be shown for the calculation of quantum yield of Rhodamine B when fluorescein is used as the standard sample.

Calculation method for relative quantum yield

In order to calculate relative quantum yield,

1) Quantum yield of standard sample is required and in addition
2) Absorbance at excitation wavelength and
3) Area of emission spectrum with spectral correction are also required for the standard sample and the unknown sample respectively. Moreover, when the solvent of the standard sample is different from that of unknown sample,
4) Average refractive index value in the wavelength range to calculate the area of emission spectrum is required. When the standard or unknown samples for emission spectrum measurement are diluted
5) Dilution ratio is required.

	Item	Samples	Expression
1)	Quantum yield	Standard	f_{st}
2)	Absorbance at Ex Wavelength	Standard Unknown	A_{st} A_x
3)	Area of emission spectrum	Standard Unknown	F_{st} F_x
4)	Average refractive index value of solvent	Standard Unknown	n_{st} n_x
5)	Dilution ratio	Standard Unknown	D_{st} D_x

Table 1 Required parameters to calculate relative quantum yield

By using the parameters in the Table 1, relative quantum yield of unknown sample, f_x is shown by the following equation (1).

$$\Phi_x = \Phi_{st} \bullet \left(\frac{A_{st}}{A_x}\right) \bullet \left(\frac{F_x}{F_{st}}\right) \bullet \left(\frac{n_x^2}{n_{st}^2}\right) \bullet \left(\frac{D_x}{D_{st}}\right) \qquad (1)$$

In addition to [Absorbance measurement] program to measure 2)Absorbance at excitation wavelength and [Spectra measurement] program to get 3)Emission spectrum area, [Relative quantum yield calculation] program to calculate relative quantum yield of unknown sample based on equation (1) is installed as standard to the FP-8000 series. So everything from measuring to analyzing relative quantum yield can be performed only by using standard programs.

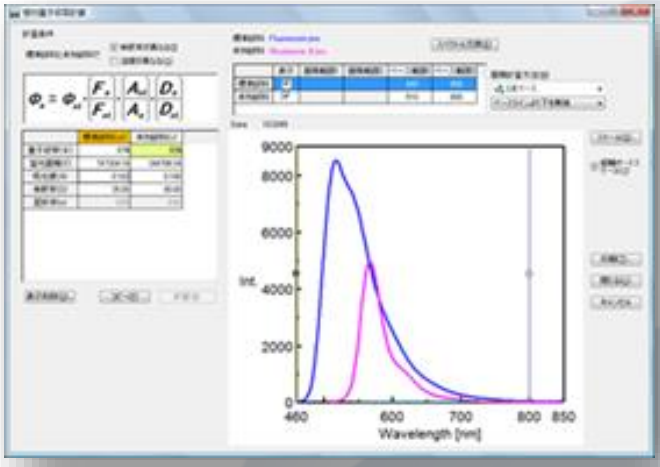


Figure 1 [Relative quantum yield calculation] program screen

Measuring System

FP-8500 - Spectrofluorometer*1)
FUV-803 - Absorbance measurement cell block

**1) Emission spectrum with spectral correction is required for relative quantum yield calculation. The spectral correction was performed using Rhodamine B on Ex side, and ESC-842Calibrated WI light source on Em side for this measurement.*

Relative quantum yield measurement using FP-8000 series

Samples

	Name	Concentration	Solvent
Standard	Fluorescein	For Absorbance:5400 µg/L For Emission:72 µg/L	Ethanol
Unknown	Rhodamine B	For Absorbance:7200 µg/L For Emission:36 µg/L	Ethanol

Calculation procedure of parameters required to calculate relative quantum yield

1) Quantum yield of standard sample
Published value of 0.97 from literature[*2)] was used for quantum yield of fluorescein.

**2) Literature: Kazuhiko Kinoshita and Koshin Mihashi, Fluorescence measurements - Applications for Biochemical Sciences. (The Spectroscopic Society of Japan, Measurement Method Series 3) Japan Scientific Societies Press, 1983.*

2) Absorbance at Ex wavelength
Absorption spectra was measured using [Absorbance measurement] program and FUV-803 Absorbance measurement cell block. Results are shown in Fig. 2. From the results, it was confirmed that absorbance of the fluorescein at Ex wavelength, 450 nm was 0.490 and the absorbance of the Rhodamine B at Ex wavelength 500 nm was 0.225.

Measurement Conditions

Ex bandwidth	2.5 nm
Em bandwidth	10 nm
Scan speed	200 nm/min
Data interval	1 nm
Response	0.5 sec
PMT voltage	230 V
Filter	Used

Attenuator B was used.[*3)]

**3) The attenuator B is available as a standard of spectrofluorometer.*

Area of emission spectrum

Emission spectra were measured using [Spectra measurement] program. In order to prevent from reabsorption of fluorescence, sample solution was diluted so that the absorbance of the sample solution becomes less than 0.02 for this measurement.(Details of dilution ratio will be described in the item 5).) Obtained emission spectra were shown in Figure 3.

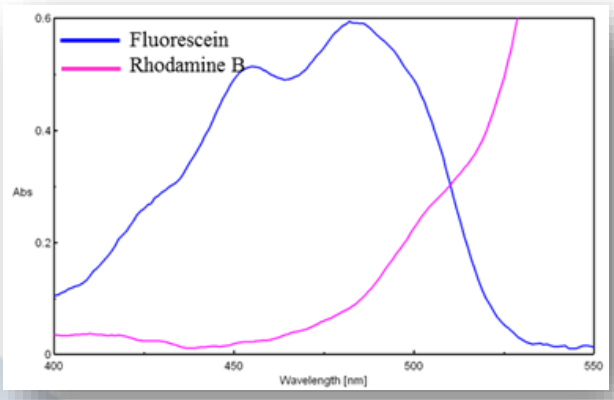


Figure 2 Absorption spectra

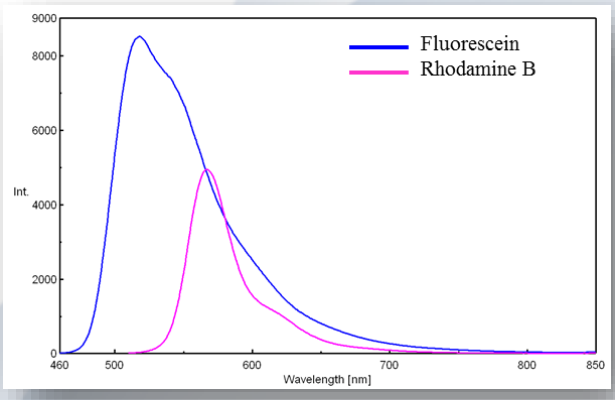


Figure 3 Emission spectra

Standard (Fluorescein)	Unknown (Rhodamine B)
0.490	0.225

Table 2 Absorbance at Ex wavelength

Standard (Fluorescein)	Unknown (Rhodamine B)
747204	243513

Table 3 Area of Emission spectrum

Relative quantum yield measurement using FP-8000 series

Measurement Conditions

Ex bandwidth	5 nm
Em bandwidth	5 nm
Scan speed	200 nm/min
Data interval	0.5 nm
Response	0.5 sec
PMT voltage	430 V
Filter	Used
Spectral correction	ON

Average refractive index of solvent

As ethanol is used on both standard and unknown samples for this measurement, average refractive index of the solvent is not required. When the solvents used are different from each other, published value is used.

Dilution Ratio

Comparing with absorbance measurement in the item 2), standard sample solution was diluted by 75 times and unknown sample, by 100 times in the item 3) emission spectra measurement.

Analysis Results

Quantum yield of Rhodamine B was calculated by applying parameters obtained by "calculation methods 1)~5) to get parameters which are needed to calculate relative quantum yield" to the equation (1). As a result, quantum yield of 92 % which is within the range of published value, [*3)] 69~97% was obtained.

Standard (Fluorescein)	Unknown (Rhodamine B)
75	100

Table 4 Dilution Ratio

Published value	Measured value
69~97%	92%

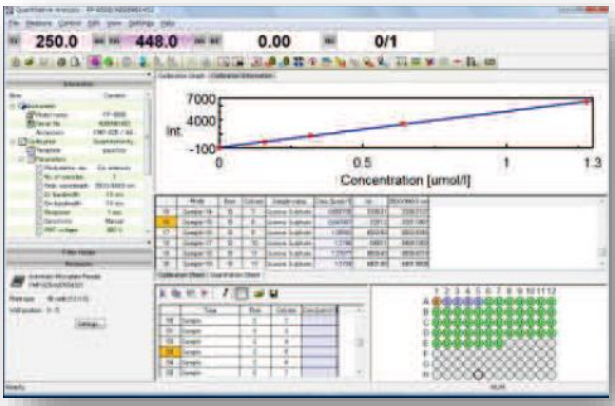
Table 5 Quantum yield of Rhodamine B

Fluorescence Measurements for Quinine Sulfate using Microplate Reader

Introduction

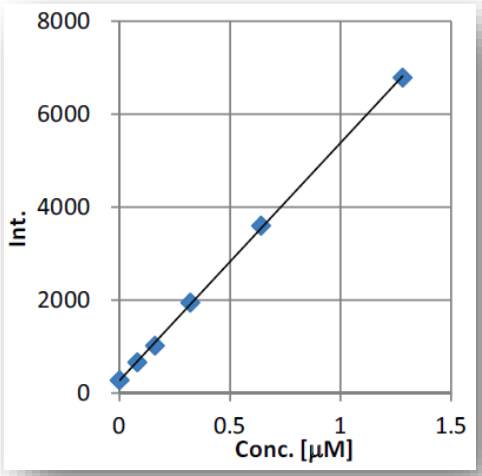
The FP-8000 series spectrofluorometer with the FMP-825 microplate reader allows multiple liquid samples to be automatically measured by moving the wells. Using this system, quantitative analysis, in addition to spectrum and fixed-wavelength measurements can be carried out.

FP-8500 Spectrofluorometer
FMP-825 Microplate reader



[Quantitative Measurement] program

A calibration curve for quinine sulfate was created using a 96-well microplate reader. Based on the reproducibility for blank samples, the quantitation and detection limits were calculated.



Calibration curve for quinine sulfate

Calibration: Int=5117 x Conc.+270
Corr. Coeff.: 0.9998
Quantitation limit: 5 x 10^{-8}mol/

Measurement parameters

- Sample volume: 300 mL
- Ex wavelength: 250 nm
- Em wavelength: 448 nm
- Ex bandwidth: 10 nm
- Em bandwidth: 10 nm
- Speed: 30 mm/sec
- Response: 50 msec
- Gain: 400 V

Near-Infrared Phosphor Measurement using FP-8700 spectrofluorometer

Introduction

Near-infrared fluorescence label is getting paid attentions in several markets recently due to it's high stability and safety issue against human body. Especially, these labels can be applied to biochemistry, Medical science and Life science. I hi li In this application note, the excitation and fluorescence of samples as $YVO_4:Nd^{3+}$, $YVO_4:Nd^{3+},Yb^{3+}$ are measured in near-infrared region and reported.

Sample

$YVO_4:Nd^{3+}$, $YVO_4:Nd^{3+},Yb^{3+}$

Measurement Condition

[Excitation spectra]

- Ex wavelength range 350-850 nm
- Em wavelength 883 nm
- Ex bandwidth 10 nm
- Em bandwidth 10 nm
- Scan speed 500 nm/min
- Response 0.5 sec
- Data interval 0.2 nm
- Sensitivity 600 V
- Spectral correction ON

[Fluorescent spectra]

- Ex wavelength 600 nm
- Em wavelength range 850-1500 nm
- Ex bandwidth 10 nm
- Em bandwidth 10 nm
- Scan speed 500 nm/min
- Response 0.5 sec
- Data interval 0.2 nm
- Sensitivity 600 V
- Spectral correction ON

System configuration

- FP-8700 Spectrofluorometer [1)]
- FPA-810 Powder sample cell block
- PSH-101 Powder sample cell
- ESC-842 Calibrated light source (WI)
- ILF-890 100mm dia. Integrating Sphere
- KBr plate holder
- IR microscope KBr plate (5 x 5)
- FWQE-880 Quantum yield calculation program

1) FP-8700 is prepared as two models, high sensitivity model (wavelength range from 300 nm to 1400 nm), wide range model (wavelength range from 300 nm to 1700 nm), due to the difference of fluorescent side detector. The measurements in this application note is performed using wide range model.

Result

Excitation spectra of $YVO_4:Nd^{3+}$ and $YVO_4:Nd^{3+},Yb^{3+}$ are shown in figure 2 and the excitation states from ground state of Nd^{3+} ($^4I_{9/2}$) are shown in here. Fluorescence spectra of $YVO_4:Nd^{3+}$ and $YVO_4:Nd^{3+},Yb^{3+}$ are shown in figure 3 and the energy deactivation from excitation states of Nd^{3+} ($^4F_{3/2}$) and Yb^{3+} ($^2F_{5/2}$) are also shown in here. The calculation result of Quantum yield are shown in table 1.

As a result, this near-infrared fluorescence measurements is an effective tool to develop the phosphor based on multicolor or transmittivity in physiological tissue.

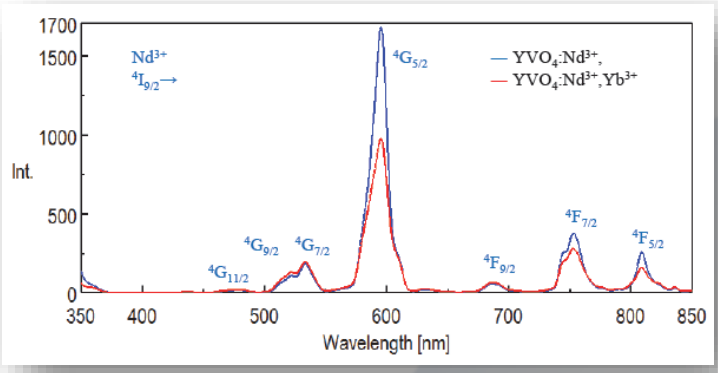


Figure 2 Excitation spectra of near-infrared phosphor

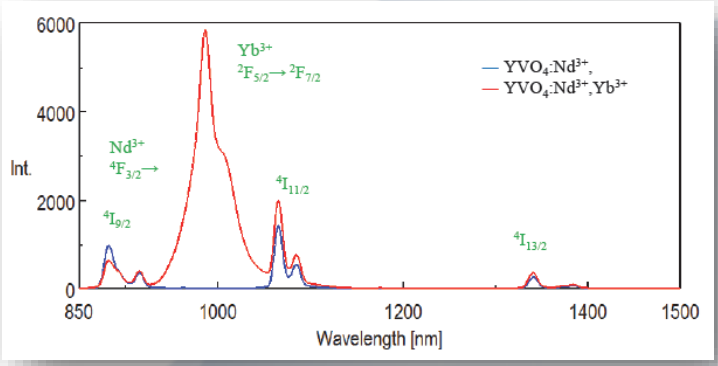


Figure 3 Fluorescence spectra of near-infrared phosphor

	Absorptance [%]	Outer quantum efficiency [%]	Internal quantum efficiency [%]
YVO4:Nd3+	9.0	2.5	28.5
YVO4:Nd3+,Yb3+	21.0	12.4	59.1

Table 1 Quantum yield calculation

Sensitivity evaluation of biosensor by using FRET

Introduction

FRET is the mechanism that the energy is transferred non-radiatively from an excited state donor to a neighbor acceptor (Figure 1). FRET is an abbreviation for Forester (or fluorescence) resonance energy transfer.

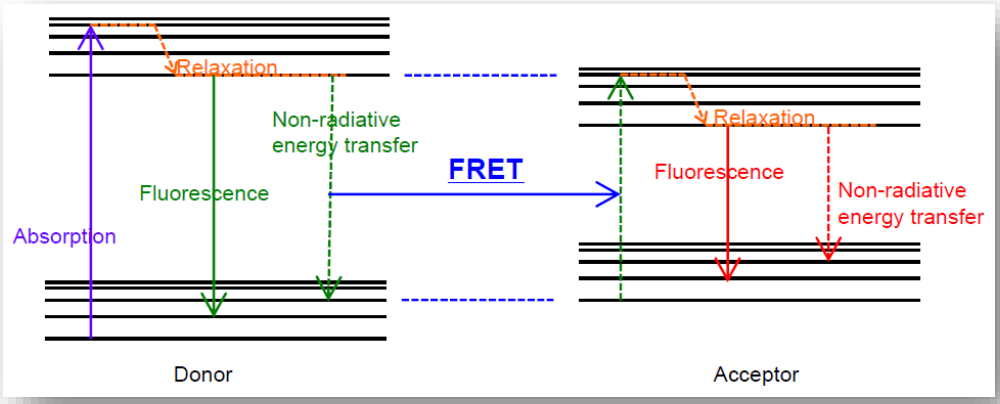


Figure 1 - Energy transition in a FRET pair

FRET is monitored by the spectrofluorometer, which measures the fluorescence/quenching of acceptor or excited donor. FRET efficiency depends on the following factors (Figure 2).

- Spectral overlap between the donor and acceptor Donor. As the overlap area of the donor fluorescence spectrum and the acceptor absorption spectrum is larger, FRET efficiency is higher
- Distance between donor and acceptor FRET efficiency is inversely proportional to the six power of distance between the donor and acceptor
- Orientation of donor and acceptor

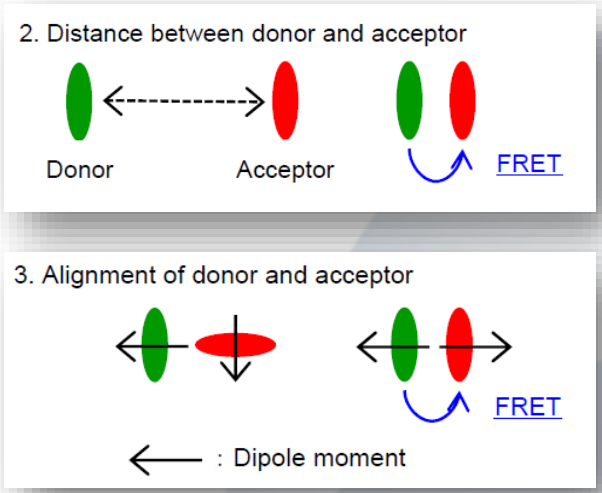
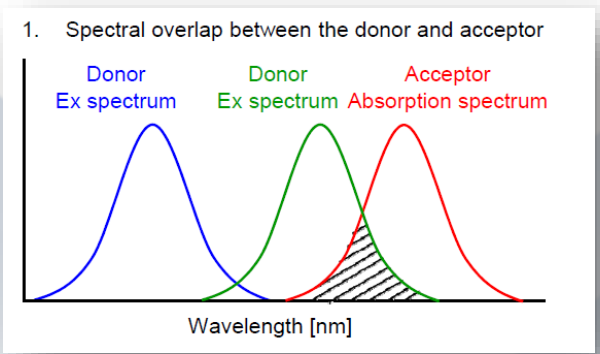


Figure 2 - Factors that influence FRET

FRET efficiency is a maximum when the two dipole moments are parallel or anti-parallel to each other, and no energy transfer occurs when the dipole moments are perpendicular to each other.



Typically, when the distance between the donor and the acceptor is 1 -10 nm, FRET is occurred. This mechanism is used for the following applications.

- Monitoring the protein conformational change by fusing the donor and acceptor to two different sites in the same protein (intramolecular FRET [1])
- Monitoring the protein-protein interactions by fusing the donor and acceptor to two different host proteins (intermolecular FRET [1])

Sensitivity evaluation of biosensor by using FRET

One of FRET application examples is the murine anti-TNT monoclonal antibody (anti-TNT mAb), which can form a complex with trinitrotoluene (TNT). Anti-TNT mAb absorbs the excitation light of 280 nm, and produces the fluorescence light of 340 nm. When anti-TNT mAb forms a complex with TNT, FRET is occurred between the anti-TNT mAb (donor) and TNT (acceptor). The energy is transferred from the anti-TNT mAb excited at 280 nm to the TNT, and the fluorescence at 340 nm from the anti-TNT mAb is quenched (Figure 3). By utilizing this interaction as biosensor, this application note shows the evaluation of the sensitivity between the aromatic nitro compounds and anti-TNT mAb [2].

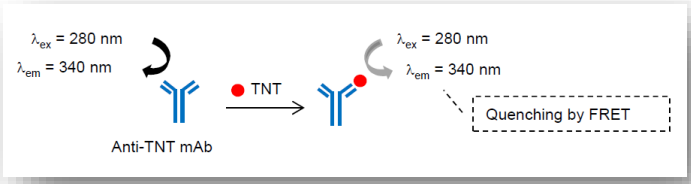


Figure 3
Schematic illustration of fluorescence quenching by FRET

Measurement

10 uL of 4.4x10-8 mol/L TNT solution was added to 800 uL of 1.6x10-7 mol/L anti-TNT mAb, and the fluoresence measurement was performed. After measurement, the final concentration of TNT was increased step-by-step by adding each 10 uL of TNT solution (4.4x10-7 mol/L, 4.4x10-6 mol/L, 4.4x10-5.5 mol/L and 4.4x10-5 mol/L), and fluorescence measurement of each different TNT concentration solution was performed.

Regarding the other aromatic nitro compounds, similar measurements were performed.

Reagents and Samples

- murine anti-TNT monoclonal antibody (anti-TNT mAb): 1.6x10-7 mol/L [1]

Aromatic nitro compounds

- Trinitrotoluene (TNT) : 4.4x10-8, 4.4x10-7, 4.4x10-6.5, 4.4x10-6, 4.4x10-5.5, 4.4x10-5 mol/L
- Trinitrobenzene (TNB) : 4.7x10-8, 4.7x10-7, 4.7x10-6.5, 4.7x10-6 mol/L
- 2,6-dinitrotoluene (2,6-DNT) : 5.5x10-8, 5.5x10-7, 5.5x10-6, 5.5x10-5, 5.5x10-4 mol/L
- 2-Nitrotoluene (NT) : 7.3x10-8, 7.3x10-7, 7.3x10-6, 7.3x10-5, 7.3x10-4 mol/L
- Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX): 4.5x10-8, 4.5x10-7, 4.5x10-6, 4.5x10-5, 4.5x10-4 mol/L
- Solvent
- pH 7.4 phosphate buffer

Measurement condition

- Ex wavelength: 280 nm
- Measurement range: 300 - 500 nm
- Excitation bandwidth: 1 nm
- Emission bandwidth: 20 nm
- Scan speed: 200 nm/min
- Data acquisition interval: 0.5 nm
- Response: 1 sec Sensitivity: 500 V
- Spectral correction: ON

Measurement results

The fluorescence spectra of mixture solution of anti-TNT mAb and TNT were shown in Figure 4. As the TNT concentration increases, the fluorescence intensity of anti-TNT mAb becomes weak. Similar measurements were performed using other aromatic nitro compounds as samples. The degree of progress of complex formation reaction was determined by the decrease of the fluorescence intensity at 340 nm.

Sensitivity evaluation of biosensor by using FRET

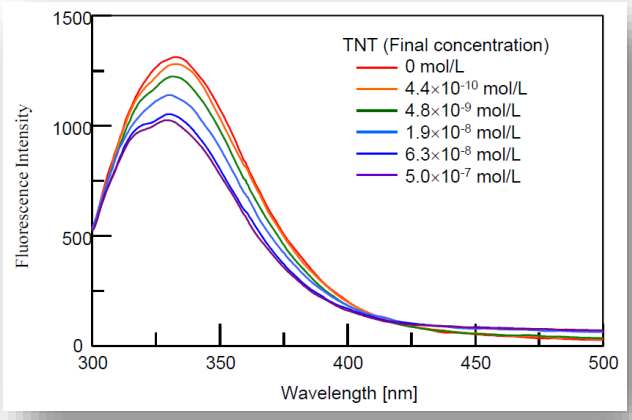


Figure 4 - Fluorescence spectra of solutions containing anti-TNT mAb and various concentration of TNT

Figure 5 shows the relations between the dose of aromatic nitro compounds and the reaction ratio.
From this curve, the half maximal inhibitory concentration (IC50) was estimated (Table 1).
This result shows that anti-TNT mAb have sensitivity to TNT, TNB, NT and 2,6-DNT, especially have highly sensitivity to TNT and TNB whereas can't detect RDX.
From these results, anti-TNT mAb is expected as high sensitive nitro compounds biosensor without using fluorescent label.

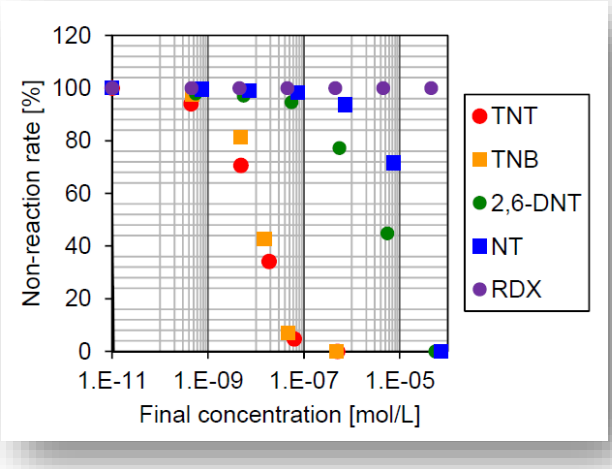


Figure 5
Dose-response curve of Anti-TNT mAb and aromatic nitro compounds

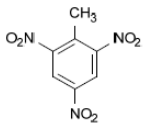
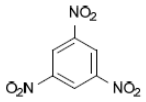
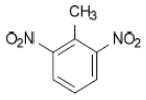
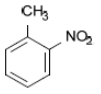
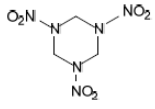
Name	Structure	IC_{50} [M]
TNT		4.0E-08
TNB		3.8E-08
2,6-DNT		7.9E-06
NT		3.6E-04
RDX		-

Table 1 - Analysis of binding of different explosive compounds to anti TNT

System configuration

- FP-8500ST Spectrofluorometer
- STR-812 Water thermostatted cell holder with stirrer
- CGP-859 Sample compartment lid with pipette port (for Gilson P-200)
- FMH-802 5mm Micro cell jacket
- ESC-842 Calibrated light source (WI)
- J/3-5.45/Q/5 Fluorimeter rectangular quartz micro cell, 5mm x 5mm x L45mm
- Micro stirrer bar, F-4036-02-NEW

Acknowledgement
Special thanks to Hiroshi Nakayama of Panasonic corporation who cooperated with the experiment.

[1] Kevin Truong and Mitsuhiko Ikura, Current Opinion in Structural Biology 2001, 11:573–578
[2] Satoko Suzuki, Toshifumi Uchiyama, Ken-ichi Akao, Koushi Nagamori, Hiroshi Nakayama and Yuji Ito TNT sensing using non labeling FRET Japan Taiwan Medical Spectroscopy International anti-Ito, non-FRET, Japan-Symposium/14th Annual Meeting of the Japan Association of Medical Spectroscopy, AWAJI, JAPAN 2016

Upgrade of simple coumarin analysis system to high sensitivity one

In order to prevent from producing illegal light diesel oil which contains kerosene or heavy oil, 1 ppm of coumarin is added in the related oils of the diesel (kerosene or A heavy oil) as discrimination label. The analysis procedures to determine mixing with the discrimination label and its mixing concentration are standardized by Advisory body in National Petroleum Dealers Association. Simple analysis using test tube and quantitative analysis using separating funnel are described in the instruction manual of the procedure. We have already introduced simple quantitative analysis system incorporating easy-to use simple analysis and accuracy of quantitative analysis with the preparation performed using test tube, detecting fluorescence intensity and judging concentration using spectrofluorometer. Usual coumarin determination purpose is to analyze quantitatively more than a couple of percent of the related oils, while there is another analysis case which needs to analyze quantitatively to less than 1 percent. We would like to introduce a system to upgrade the above simple analysis system to high sensitivity system with improved detection limit and quantitation limit drastically.

1. Measurement principle

Coumarin is hydrolyzed in alkaline solution and becomes Cis-O-hydroxycinnamic acid. In addition, the Cis-O-hydroxycinnamic acid is isomerized by ultraviolet radiation and becomes Trans-O-hydroxycinnamicacid.The Trans-O- hydroxycinnamic acid radiates green fluorescence(Ex 360 nm, Em 500 nm). In this quantitative analysis procedure, this green fluorescence is detected.

2. Measurement system

Filters on both EX and EM sides are used to reduce scattering light, enabling the analyzing system to assure high sensitivity measurement.

- FP-6300 Spectrofluorometer
- Test tube holder for coumarin measurement
- U330 filter (EX side), WG305 filter (EM side) *1)

3. Tools to be used

- Round-bottom screw cutting test tube (18 mm outer diameter x 160 mm length)
- Stirrer bar (3 mm diameter x 10 mm length)
- Shaker

4. Preparation of reagents

1) Alkaline solution reagents

Dissolve 10 g of sodium hydroxide and 20 g of sodium nitrate into Millipore water, and prepare 100 mL solution. The alkaline solution is kept in polyethylene vessel.

2) Alcohol solution

Mix 40 mL of 1-butanol and 30 mL of ethanol in this proportions.

3) Undiluted coumarin solution [1000 ppm]

Dissolve 100 mg of the coumarin into aromatic solvent (such as n-propyl benzene).

4) Standard coumarin solution [0.1 ppm]

Dilute 100 µL of the undiluted coumarin solution using n-dodecane (1 ppm). Take 100 µL of the 1 ppm coumarin solution, and dilute it using the n-dodecane and prepare 100 mL solution.

5) Standard sample

Mix each of solutions in accordance with the following ratio.

Concentration of additive [%]	Standard coumarin solution [0.1 ppm] (mL)	n-Dodecane (mL)	Alkaline solution (mL)	Alcohol solution (mL)
0%	0	4.2	3	4.8
1%	0.06	4.14	3	4.8
2%	0.12	4.08	3	4.8
4%	0.24	3.96	3	4.8
6%	0.36	3.84	3	4.8
8%	0.48	3.72	3	4.8
10%	0.96	3.24	3	4.8

Table 1
Mixing ratio of standard solution

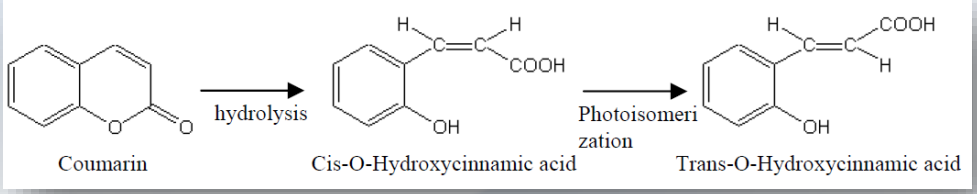


Figure 1
Hydrolysis and photoisomerization of coumarin

Upgrade of simple coumarin analysis system to high sensitivity one

5. Measurement procedure

Prepare test tubes containing standard samples which were prepared in the [4. Preparation of reagents 5) Standard sample]. Shake these test tubes to hydrolyzes coumarin in the test samples, and the coumarin is extracted in the alkaline solution. Then, perform photoisomerization reaction by radiating excitation light (360 nm) of spectrofluorometer on the alkaline solution, and detect fluorescence intensity at 500 nm and generate calibration curve.

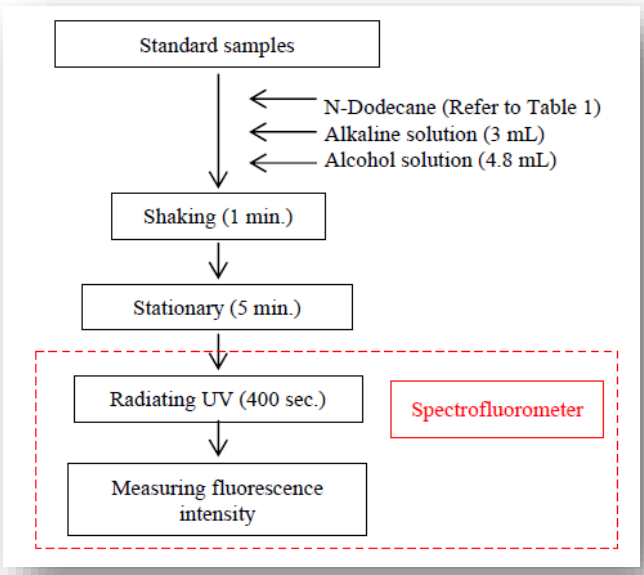


Figure 2 Flow chart of analysis procedure

1) Put each of standard samples into test tubes *1) .
2) Add n-dodecane (refer to Table 1), 3 mL of alkaline solution and 4.8 mL of alcohol solution.
3) Put stoppers on the test tubes, and shake 1 minute using shaker to hydrolyze coumarin and extract to alkaline solution.
4) Keep stationary for 5 minutes after the shaking. By keeping stationary, the above extracted solutions are separated as lower layer of alkaline solution, middle layer of alcohol solution and upper layer of diesel oil.
5) After keeping stationary for 5 minutes, put a stirrer into test tube and set it to test tube holder for spectrofluorometer. Radiate UV light (360 nm) on the alkaline solution layer for 400 seconds with rotating the stirrer, for photoisomerization reaction. Stop rotating the stirrer and read fluorescence intensity with EX 360 nm, EM 500 nm and generate calibration curve.

*1) Regarding low concentration coumarin measurement, it is necessary to wash thoroughly those test tool such as test tube.

6. Measurement condition

After monitoring the process of photoisimerization by using [Time course measurement] program, the spectra were measured using [Spectrum measurement] program and fluorescence intensity was detected at Em wavelenght = 500 nm.

Time course measurement	
Ex bandwidth *2)	20 nm
Em bandwidth	10 nm
Response	2 sec
Sensitivity	High
Measurement range	0 - 400 sec
Data acquisition interval	2 sec
Ex wavelength	360 nm
Em wavelength	500 nm

Spectrum measurement	
Ex bandwidth	10 nm
Em bandwidth	10 nm
Response	Fast
Sensitivity	High
Measurement range	380 - 650 sec
Data acquisition interval	1 nm
Ex wavelength	360 nm
Scan speed	1000 nm/min

*2) Ex bandwidth was set at 20 nm to perform photoisomerization effectively for the Time course measurement. Ex bandwidth was set at 10 nm for the Spectrum measurement in order to suppress reduction of fluorescence intensity due to photolysis.

Upgrade of simple coumarin analysis system to high sensitivity one

7. Calibration curve

Time course measurement data and spectral measurement data of standard samples with additive materials concentration of 0 ~ 10 % are shown in the Figure 3 and 4. From the Figure 3, it is observed that photoisomerization finished in 150 seconds from starting UV light radiation.

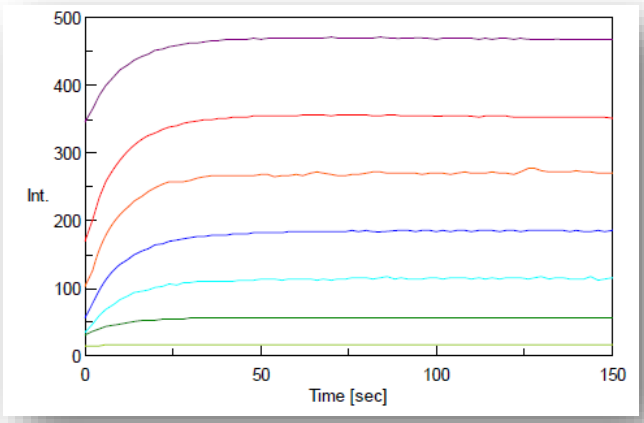


Figure 3 Photoisomerization situation

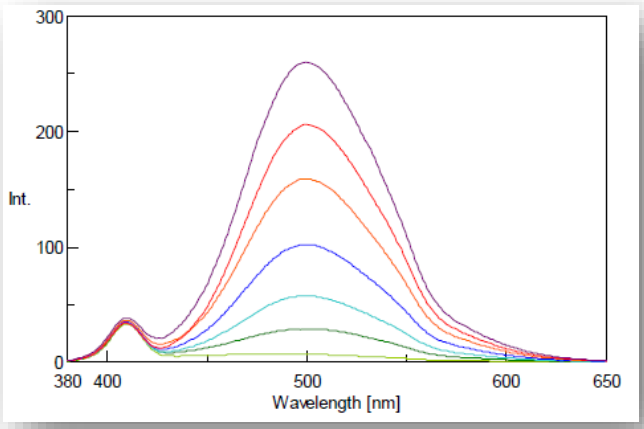


Figure 4 Spectra after finishing photoisomerization

Calibration curve plotting fluorescence intensity at spectrum peak wavelength of 500 nm with additive material concentration is shown in Figure 5.
0.9993 of correlation coefficient for the calibration curve was obtained, showing good linearity.

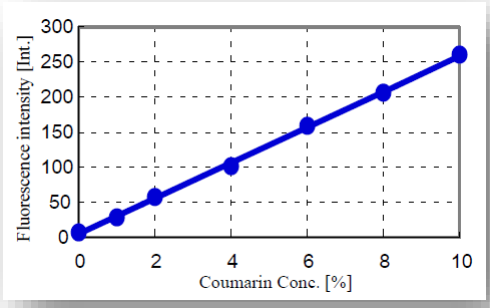


Figure 5 Calibration curve

Additive conc. [%]	Fluorescence intensity
0	6.7077
1	28.7548
2	57.3873
4	101.829
6	158.903
8	205.882
10	260.236

Calibration curve information
y = 25.367x + 4.7604
R2 = 0.9993

The measurement using 0% and 1 % concentration standard solution was repeated 5 times, and standard deviation for fluorescence intensity was 0.4357 and standard deviation for coumarin concentration was 0.0172. Considering such results, it is possible to perform analysis with 0.06% detection limit and 0.2 % quantitation limit. *3)

*3) Detection limit was calculated by 3 sigma and quantitation limit was calculated by 10 sigma.

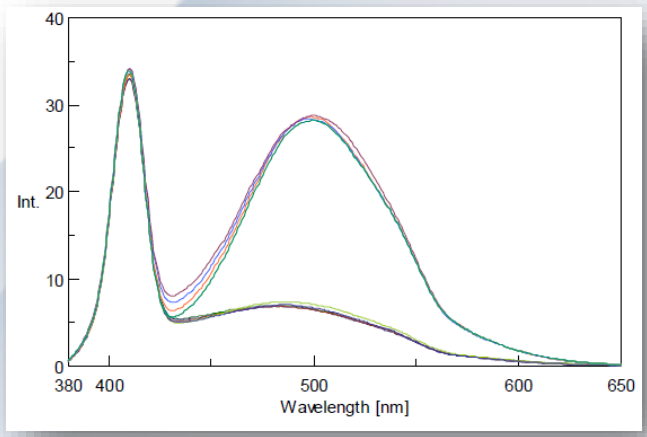


Figure 6 Spectra of conc. 0 and 1 % coumarin solution (5 spectra each)

Fluorescence Detection of Counterfeit US Currency

Each year, significantly more money is stolen with a pen than with a gun. Much of the fraud is due to counterfeiting since the current technology available for personal computers, scanners, and color copiers have attained the capability to accurately reproduce paper currency. Counterfeit currency costs governments and businesses billions of dollars a year. During fiscal year 1997 alone, a total of $136,205,241 in counterfeit U.S. currency appeared worldwide. This application demonstrates how a simple fluorescence instrument can be used to verify the identity of currency through the presence of the security thread and the background fluorescence of the bill. This fluorescence intensity can also be used to study the aging and manufacturing reproducibility of currency.

Introduction

United States currency is the most counterfeited currency in the world with Canadian money a close second, these currencies popular due to their status as a 'hard' currency, similar to gold. Large numbers of bills in all denominations are counterfeited and passed (given as payment) to unsuspecting merchants and banking institutions. In the past, only large denominations like the $100 and $50 bill were counterfeited. Currently, however, numerous businesses report the receipt of counterfeit $5, $10, and $20 notes with the $20 note being passed with the highest frequency. One of the most common types of counterfeit detection is provided by ultraviolet (UV) light. The operating principle is simple; if the bill being checked has fluorescence then it is genuine. The idea behind this is that the paper used in the printing of real currency has a high starch content making it appear dull under UV light. Counterfeiters generally use bond paper that has virtually no starch and is bleached. The bleached bond paper fluoresces under UV light. The characteristic background fluorescence of all the US currency studied is shown in Figure 1. UV detection of counterfeit currency has been in use since approximately 1976, and has been fairly effective. Recently, a new security measure, the security thread, has been implemented to prevent counterfeiters from passing off a bill as one of a higher denomination.

A security thread is a thin thread or ribbon running through a bank note substrate. The thread in U.S. currency has printing and on the new $50 note, micro-printing and graphics. The thread in the new notes glows when held under an ultraviolet light. In the $100 note will glow pink, and in the $50 note it glows yellow. In addition, it is visible in transmitted light, but not in reflected light.

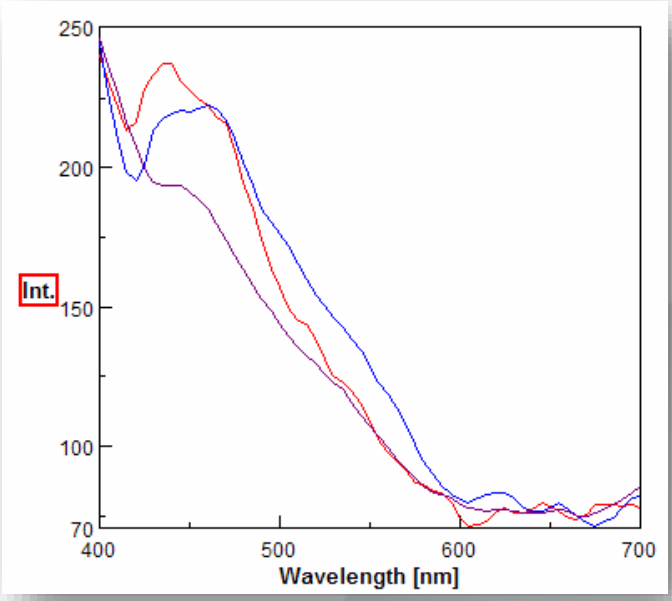


Figure 1 Scan of the background fluorescence of US, Japanese, and Canadian currency. All these monies have a similar background.

These security threads make it difficult to copy currency with a commercial color copier. Using a unique thread position for each denomination starting with the $100 note prevents certain counterfeit techniques, such as bleaching ink off a lower denomination and using the paper to "reprint" the bill as a higher value note. Table 1 designates the color of the emitted fluorescence of the security threads.

Denomination	Fluorescence of Security Thread
$100	Pink/Orange
$50	Yellow
$20	Green
$10	Red
$5	Blue

Table 1 Security threads in US Currency

Fluorescence Detection of Counterfeit US Currency

Experimental

A Jasco FP6200 fluorescence spectrophotometer equipped with a solid sample holder accessory was used for all analyses. The solid sample holder is kinematically mounted in the instrument sample compartment to provide the proper orientation of the sample to the excitation and emission ports. Bills of denomination $5, $10, $20 and $100 were placed in the sample holder such that the security thread was exposed to the excitation beam. Emission scans of both sides of each bill were collected and several locations on the security thread for each bill were also examined. The background fluorescence of the bill was subtracted from each scan of the security thread. Figure 2 illustrates the JASCO solid sample holder.

Figure 2 Jasco solid sample holder with $20.00 bill.

Spectra were collected from 400-565nm. Instrument parameters were as follows:

- Band width (Ex) 5 nm (Em) 5 nm
- Measurement range 400 - 600 nm
- Data pitch 1nm
- Excitation Wavelength 365.0 nm
- Scanning speed 125 nm/min.

Representative scans of the security threads are shown in Figure 3. The emission bands are wide and distinctly identify each bill. The fluorescence intensity of the security threads appeared to decrease with apparent use and handling of the bills, as newer bills displayed a stronger fluorescence intensity. The fluorescent threads examined in this study did not have auniform intensity along the length of the thread. This suggests that fluorescence could be used for quality control of the production of these security devices.

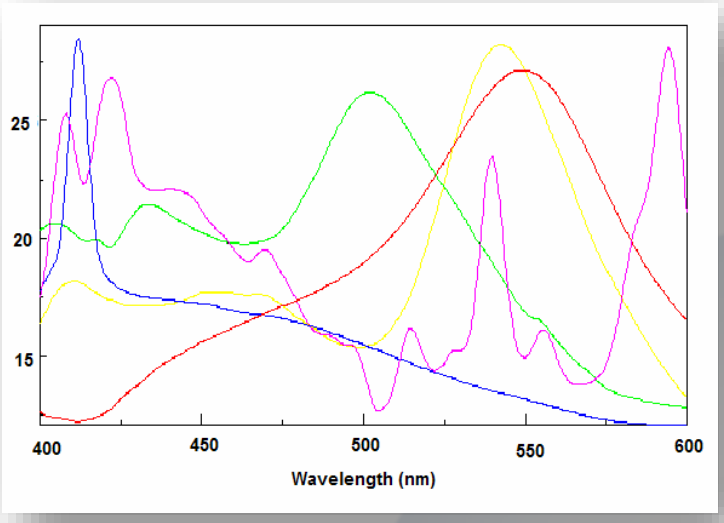


Figure 3 Spectral scans of the fluorescent strands in a 5, 10, 20, and 100 dollar bill

Conclusions

Simple fluorescence instrumentation can be used to evaluate the authenticity of U.S. currency. Using a fluorescence instrument as shown, the signal from the bill and its inherent security thread can be monitored to ensure currency validity and possibly provide a method for the examination of aging of a bill.

Absolute quantum yield measurement of solution using FP-8000 series

Introduction

Fluorescence quantum yield is defined as the ratio of the number of photons emitted from sample as fluorescence to the number of photons in the excited light absorbed. Absolute method and relative method are known as measuring methods. Relative method is comparing the intensity of standard fluorescence with unknown sample to calculate quantum yield of the unknown sample. Therefore obtained results depend on the accuracy of standard sample's quantum yield value. On the other hand, quantum yield can be obtained directly by the absolute method, because the absolute method allows to detect all the fluorescence from the sample and integrates using integrating sphere, enabling more accurate quantum yield measurement. In this experiment, some examples will be shown for the calculation of solution sample's quantum yield of which published values from literature are known by the absolute method.

Measuring system

- FP-8500 Spectrofluorometer*1)
- ILF-835 100mmF Integrating sphere unit
- 1 mm pathlength solution cell
- FWQE-880 Quantum yield calculation program

*1) Emission spectrum to which spectral correction is performed is required for quantum yield calculation. The spectra correction was performed using Rhodamine B on EX side ,and was also performed on EM side using standard white plate for synchronous spectrum (250-450 nm) and ESC-842 (450-700 nm).

Samples

- 200 ppm Quinine sulfate (Solution: 1.0 N H2SO4)
- 15 ppm Fluorescein (Solvent: 0.1 N NaOHaq)
- 200 mg/mL tryptophan (Solvent: Ultra pure water)

Measuring method for absolute quantum yield

1) Measuring incident light

Confirm nothing is set on the sample cell holder in the integrating sphere, and measure spectrum of the incident light. Obtained peak area is defined as area from incident light, S0 (equivalent number of photons in the incident light).

2) Measuring sample

Set the sample on the sample holder, and measure scattering and emission spectra of the sample. Obtained excitation wavelength peak area is defined as area scattered from sample, S1(equivalent number of photons which were not absorbed), and peak area in the emission wavelength range is defined as area emitted from sample, S2.

3) Calculating quantum yield

Calculate in accordance with the following.

Sample absorption[%]=(S0-S1)/S0 x 100

External quantum yield[%]=S2/S0 x 100

Internal quantum yield[%]=S2/(S0-S1) x 100

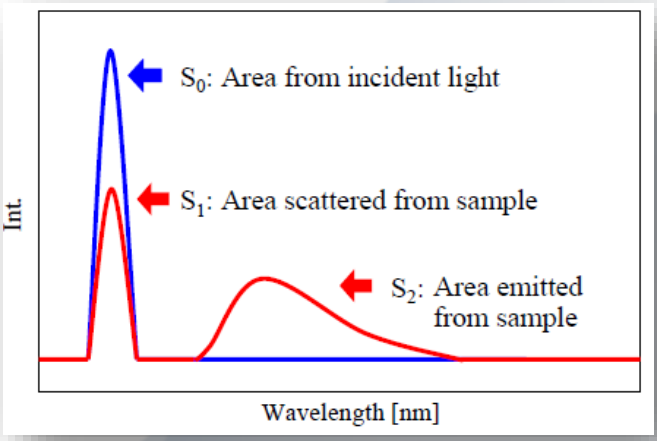


Figure 3 Image of S0, S1, S2

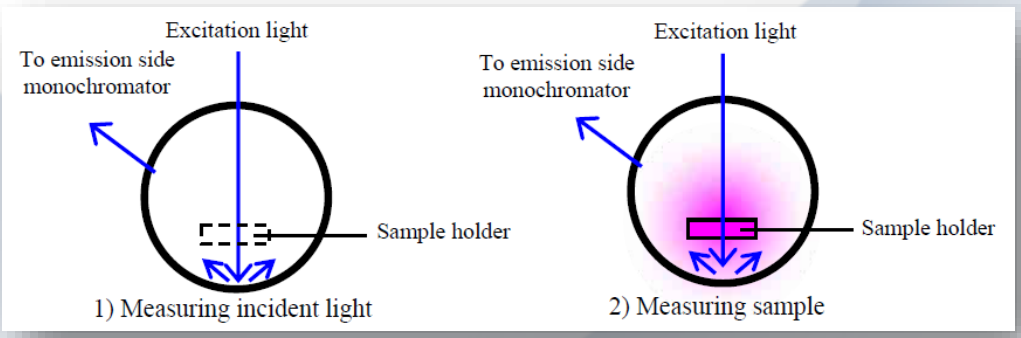


Figure 2 Diagram of integrating sphere for measuring incident light and measuring sample

Absolute quantum yield measurement of solution using FP-8000 series

Measurement results

Sample spectra measurement results are shown in the Figure 4 ~ 6.

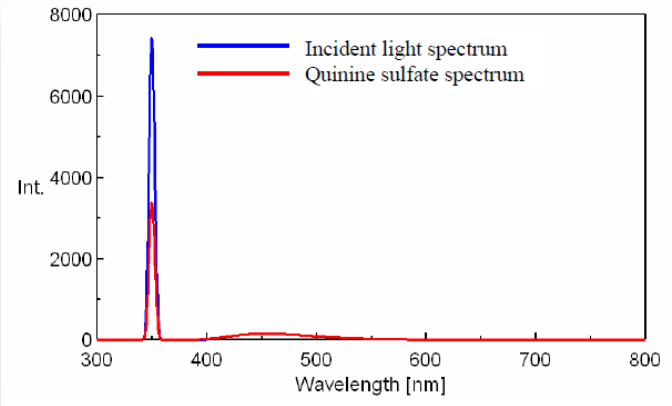


Figure 4 Emission spectrum of quinine sulfate

[Measurement condition]

- Mode: Emission
- Ex bandwidth: 5 nm
- Em bandwidth: 5 nm
- Ex wavelength: 350.0 nm
- Measurement range: 300 - 800 nm
- Scan speed: 200 nm/min
- Data interval: 0.1 nm
- Response: 0.5 sec
- PMT voltage: 350 V

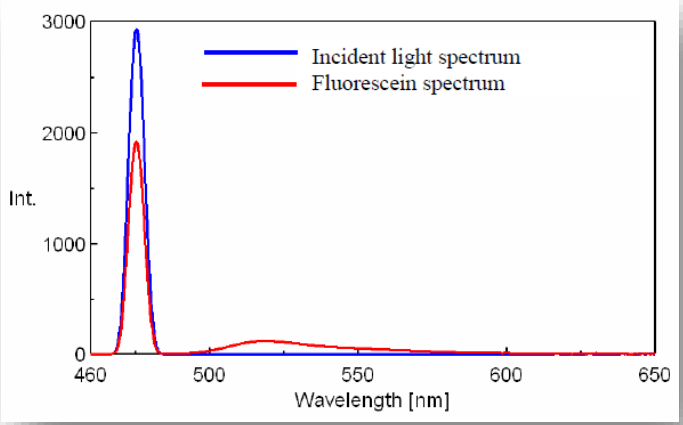


Figure 5 Emission spectrum of fluorescein

[Measurement condition]

- Mode: Emission
- Ex bandwidth: 5 nm
- Em bandwidth: 5 nm
- Ex wavelength: 475.0 nm
- Measurement range: 460 - 650 nm
- Scan speed: 200 nm/min
- Data interval: 0.1 nm
- Response: 0.5 sec
- PMT voltage: 250 V

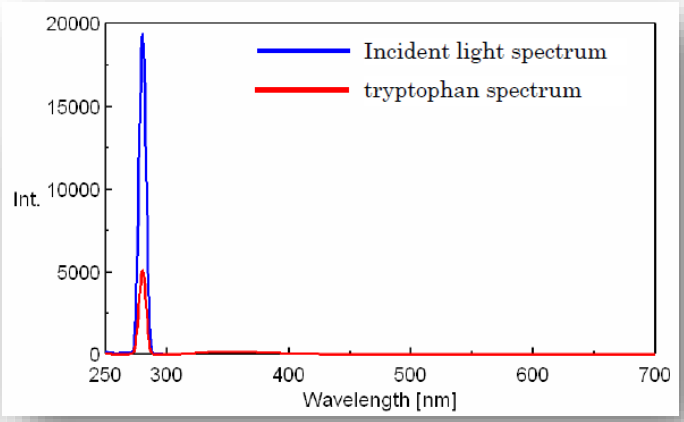


Figure 6 Emission spectrum of tryptophan

[Measurement condition]

- Mode: Emission
- Ex bandwidth: 5 nm
- Em bandwidth: 5 nm
- Ex wavelength: 280.0 nm
- Measurement range: 250 - 700 nm
- Scan speed: 200 nm/min
- Data interval: 0.1 nm
- Response: 0.5 sec
- PMT voltage: 400 V

Analysis results

Table 1 shows area from incident light (S0), area scattered from sample (S1), area emitted from sample (S2) calculated by each of sample's spectra and wavelength range. Calculation results of quantum yield using the values on the Table 1 and equations on the 3) are shown in the Table 2. Obtained results are within the range of published values from literatures for any samples.

Table 1 Detail of quantum yield calculation

	Area from incident light [S0]	Area scattered from [S1]	Area emitted from [S2]	Scattered WL Range [nm]	Emitted WL Range [nm]
Quinine sulfate	48267	22538	14304	320-365	365-750
Fluorescein	19174	12515	6116	465-485	485-630
Tryptophan	136135	35842	12101	270-290	290-550

Table 2 Calculation results of quantum yield

	Sample Absorbance	External quantum yield	Internal quantum yield	Internal quantum yield (published values)
Quinine sulfate	53.3%	29.6%	55.6%	50-57%[*2)]
Fluorescein	34.7%	31.9%	91.8%	85-92%[*2)]
Tryptophan	73.7%	8.9%	12.1%	12-14%[*3)]

*2) Literature: The Spectroscopical Society of Japan, (Japan Scientific Societies Press)
*3) Literature: Principles of fluorescence spectroscopy, Joseph R. Lakowicz, Springer

System evaluation of spectrofluorometer FP-8500 with optical fiber

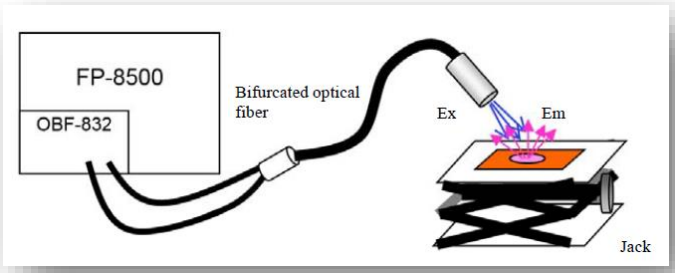
Introduction

By using OBF-832 optical fiber unit, it becomes easy to use an optical fiber as a fluorescence detection unit of the analyzer. With the use of the optical fiber, the measurement can be performed by just approaching a probe to a sample. It is possible to measure a sample which is larger than sample chamber, to trace in vivo reaction, to measure under the severe environment where people can not approach such as high/low temperature and high pressure.

In this application note, part of fluorescent samples were measured by using FP-8500 spectrofluorometer and OBF-832 optical fiber unit.

Measurement System and condition

- FP-8500 Spectrofluorometer
- OBF-832 Optical fiber unit
- Calibration Calibration light source / standard white plate
- Samples Red and Yellow referee cards



Sample measurement

Calibrated light source measurement under dark room condition

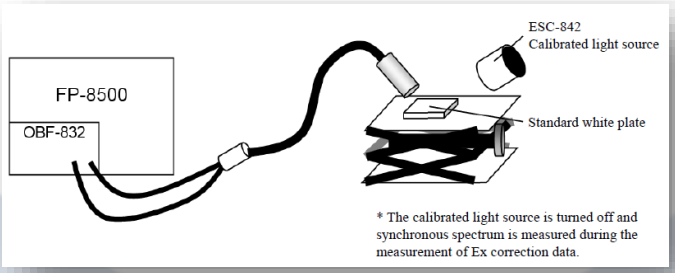


Figure 1 Measurement system

Measurement Condition

- Measurement mode: fluorescence
- Excitation bandwidth: 10 nm
- Emission bandwidth: 10 nm
- Excitation wavelength: 310 nm
- Measurement range: 400 - 800 nm
- Scan speed: 100 nm/min
- Data interval: 0.5 nm
- Response: 1 sec
- PMT voltage: 350 V (yellow card), 550 V (red card)

Measurement Results

Figure 2 and 3 show the measurement results. The profiles of each spectra measured with the optical fiber and with FLH-809 film holding block were consistent with each other. From the result, this system is effective in the case such as the measurement of a sample which is larger than sample chamber and the measurement under the severe environment.

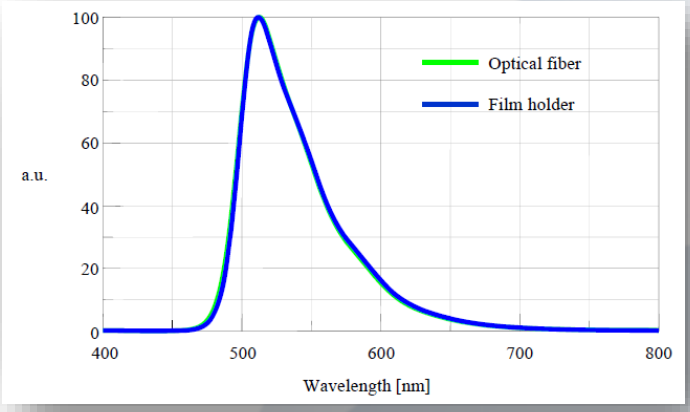


Figure 2 Peak normalized fluorescence spectrum of the yellow card

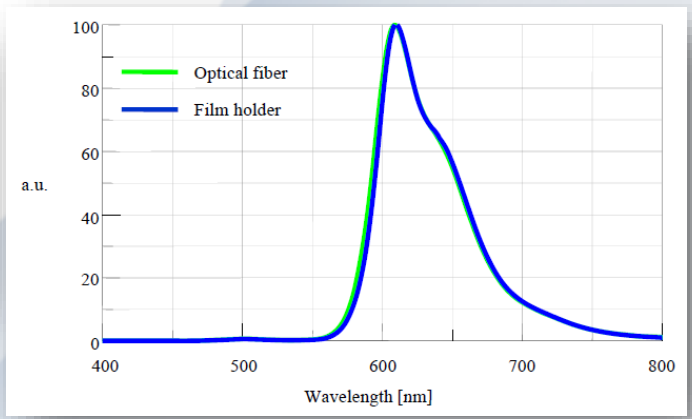


Figure 3 Peak normalized fluorescence spectrum of the red card

Component Analysis of an Excitation-Emission Matrix of Water Samples using PARAFAC Analysis

Introduction

Excitation-Emission Matrix (EEM) can be used in a wide variety of applications, especially in the analysis of environmental water. EEM provides the following information; (1) determining of the origin of environmental water and chromophoric dissolved organic matter (CDOM), (2) monitoring of the component variation from influences such as climate patterns or local weather.

Environmental water typically contains a variety of fluorescent materials (humic acid, fulvic acid, protein, amino acid, chlorophyll, synthetic compounds etc.) and analysis using EEM includes several fluorescence peaks, resulting in very complicated data which may be difficult to interpret.

Parallel Factor Analysis (PARAFAC) is a type of multivariable analysis, which can be used to extract the components from mixed 3-D fluorescence data. JASCO has developed an advanced solution using PARAFAC, which can help to interpret the complicated data found in the EEM.

As an example of component analysis by PARAFAC, this application note shows the 3-D fluorescence measurement and component analysis results of a mixed sample (Tryptophan, humic acid and folic acid).

Experimental

Measurement condition

- Excitation bandwidth: 5 nm
- Fluorescence bandwidth: 10 nm
- Scan speed: 5000 nm/min
- Response: 50 msec
- Data interval: 1 nm
- Sensitivity: High
- Filter: Used

Solutions of tryptophan (0.0175 mg/L), humic acid (0.5 mg/L) and folic acid (1 mg/L) were prepared in the following mixture ratios (tryptophan: humic acid: folic acid): 6:2:2, 5:5:0, 5:0:5, 4:4:2, 4:2:4, 2:6:2, 2:4:4, 2:2:6, 0:5:5.

The excitation and emission spectra were obtained and corrected for using calibrated light sources (WI and D2).

Instrument

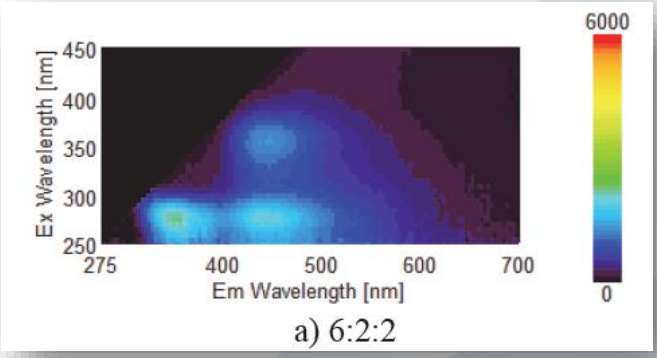
- FP-8300ST Spectrofluorometer
- ESC-842 Calibrated light source (WI)
- ESC-843 Calibrated light source (D2)
- FUV-803 Absorbance measurement cell block
- PLS Toolbox (Solo) Chemometrics Software*1)
- [IFE Correction] program*2)

*1) Made by Eigenvector Research Inc.
*2) Custom-ordered

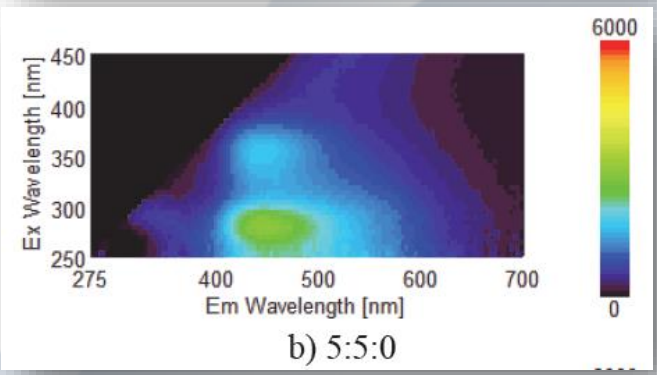
Result

The 3-D fluorescence spectra of ultra pure water and sample were obtained and corrected for using the calibrated excitation emission spectra. The ultra pure water spectra were then subtracted from the sample spectra to remove the water Raman peaks.

The subtracted sample spectra are shown in Figure 1.

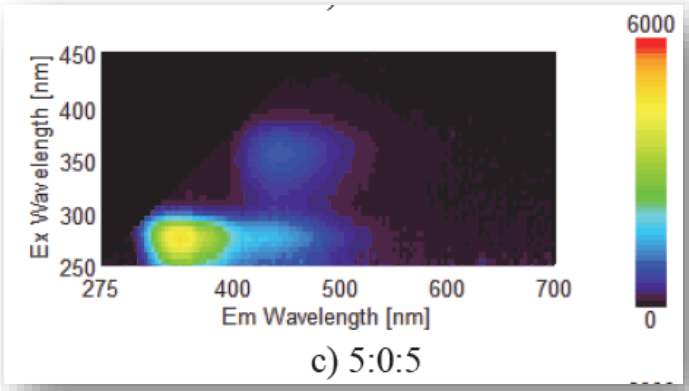


a) 6:2:2

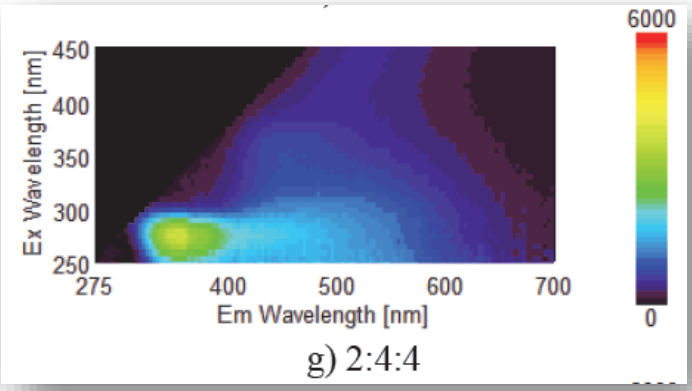


b) 5:5:0

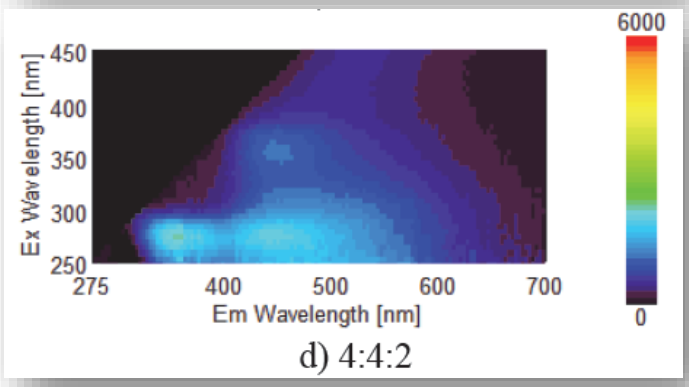
Component Analysis of an Excitation-Emission Matrix of Water Samples using PARAFAC Analysis



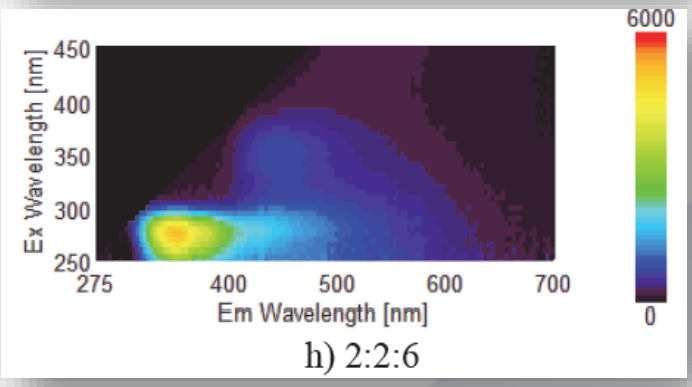
c) 5:0:5



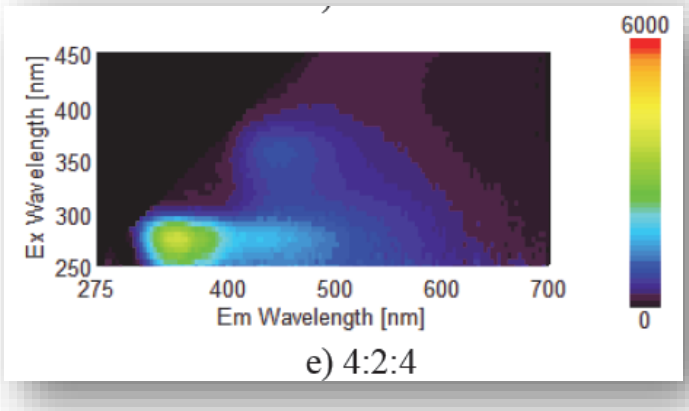
g) 2:4:4



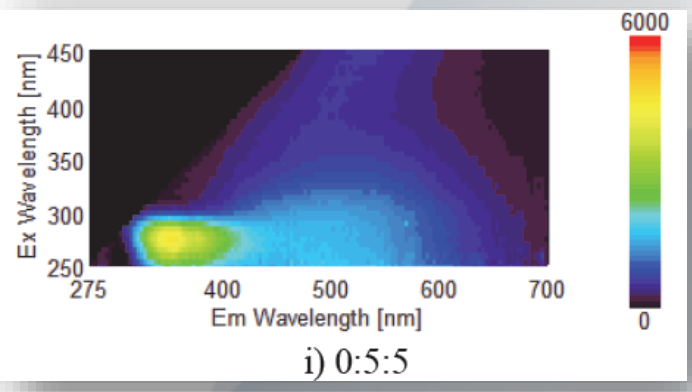
d) 4:4:2



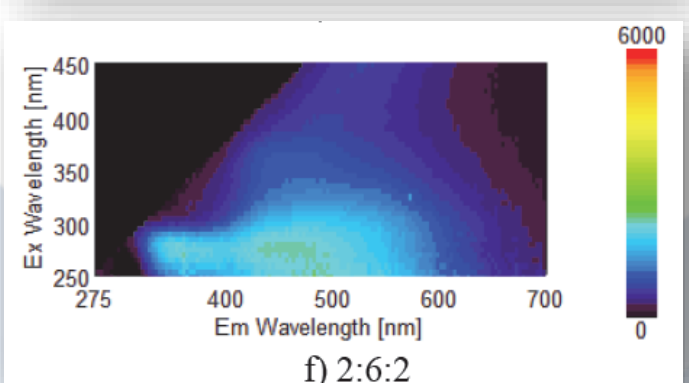
h) 2:2:6



e) 4:2:4



i) 0:5:5



f) 2:6:2

Figure 1
3-D fluorescence spectra of the different mixture ratios (tryptophan: humic acid: folic acid)

Component Analysis of an Excitation-Emission Matrix of Water Samples using PARAFAC Analysis

Component analysis using PARAFAC analysis was performed on the 3-D fluorescence data and the number of component spectra was set as 3. Figure 2, left shows the component spectra calculated by PARAFAC, and Figure 2, right shows the 3-D fluorescence data of the pure sample (tryptophan, humic acid and folic acid). As shown in Figure 2, the component spectra are closely similar to the 3-D fluorescence data of the pure sample.

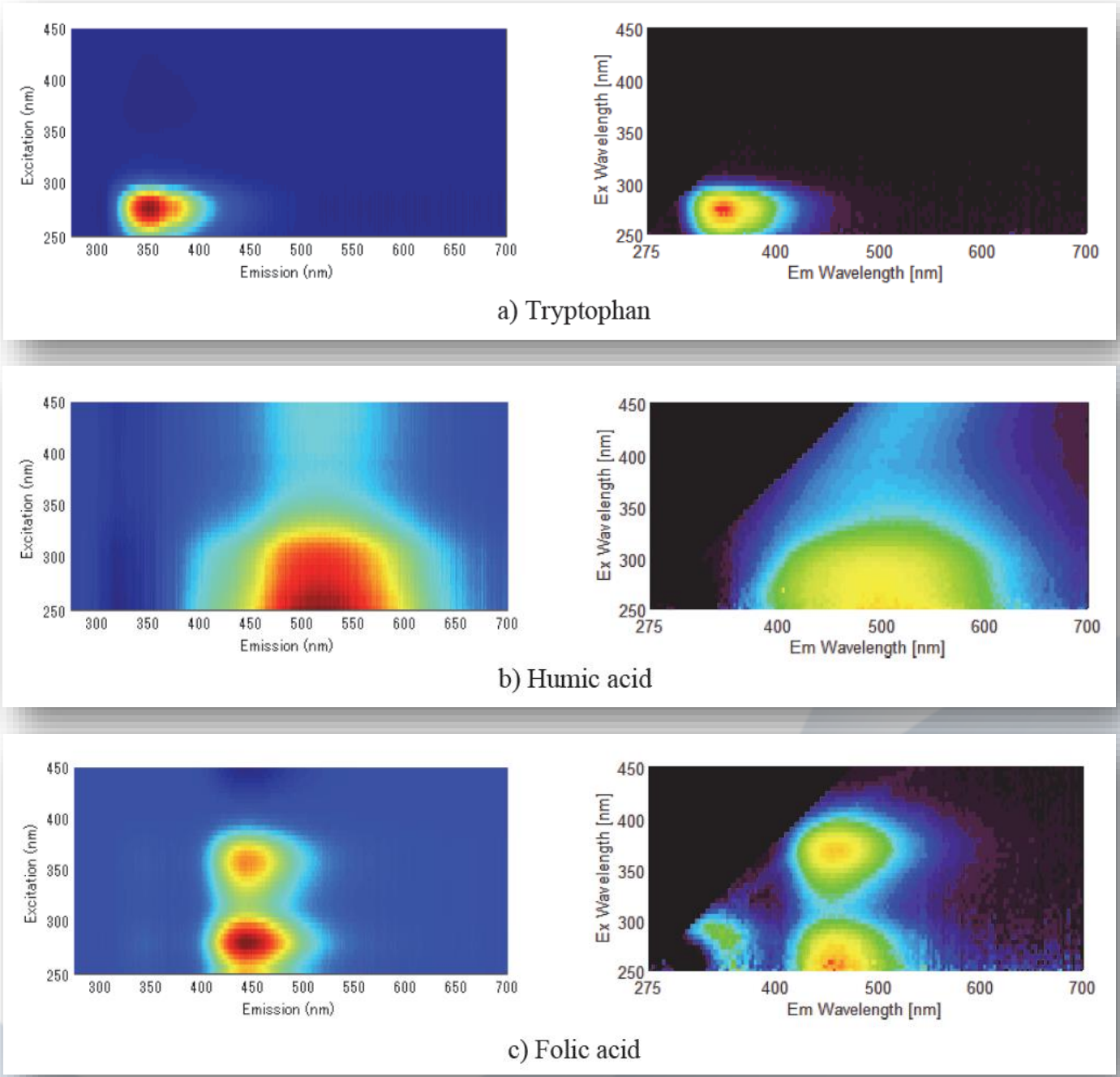


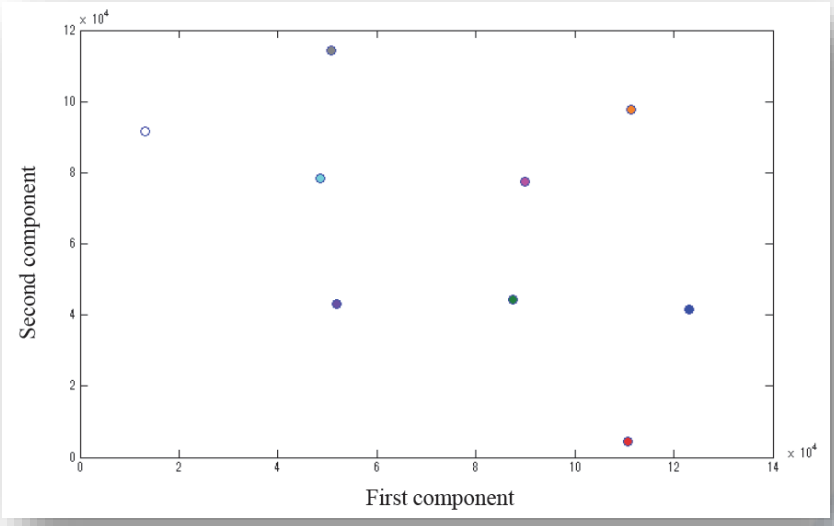
Figure 2
3-D fluorescence spectra of the pure samples (right) and component spectra by PARAFAC (left).

Component Analysis of an Excitation-Emission Matrix of Water Samples using PARAFAC Analysis

Figure 3 shows the score plot of the first component (tryptophan) and the second component (humic acid) as calculated by PARAFAC.
The ratio of the components calculated by PARAFAC is similar to the mixture ratio of tryptophan and humic acid.

Conclusion

These results demonstrate that PARAFAC analysis is a useful method to identify a component spectrum in a mixed spectrum, and can be used to determine the components in a mixed solution from the peak information of each component spectrum. PARAFAC can also be used to provide quantitative analysis for each component in the mixture.



Marker	Tryptophan	Humic acid	Folic acid
●	6	2	2
●	5	5	0
●	5	0	5
●	4	4	2
●	4	2	4
●	2	6	2
●	2	4	4
●	2	2	6
○	0	5	5

Figure 3 - Score plot of tryptophan and humic acid

Component Analysis of an Excitation-Emission Matrix of Water Samples using PARAFAC Analysis - APPENDIX

<Appendix>

General measurement and data processing procedures (for analysis of environmental water)

This appendix shows the general measurement and data processing procedures for analyzing the environmental water.

[Measurement]

1. Acquires the excitation correction data and emission correction data by using calibrated light source unit (WI and D2) *1)
2. Measures the fluorescence intensity of 10 µg/L quinine sulfate in 0.1 N sulfuric acid solution at 350 nm excitation wavelength and 455 nm emission wavelength.
3. Performs the 3D fluorescence measurement with ultra pure water.
4. Performs the 3D fluorescence measurement with sample.
5. Performs the absorbance measurement with sample at the wavelength range which fluorescence measurement has been performed in, if it is necessary to correct for inner filter effect *2).

*1) The normalization wavelength of excitation correction data is specified as 350 nm and the normalization wavelength of emission correction data is specified as 455 nm.

*2) The correction of inner filter effect is required when analyzing the environmental water including high concentration fluorescent materials (soil, lake, and lower reach of rivers).

[Data-processing]

1. Corrects the 3D fluorescence data of ultra pure water and sample by using the excitation and emission correction data in order to correct for spectral characteristic of the spectrofluorometer *3).
2. Calculates the subtraction spectrum between the 3D fluorescence data of sample and the one of ultra pure water to remove the water Raman peaks *4).

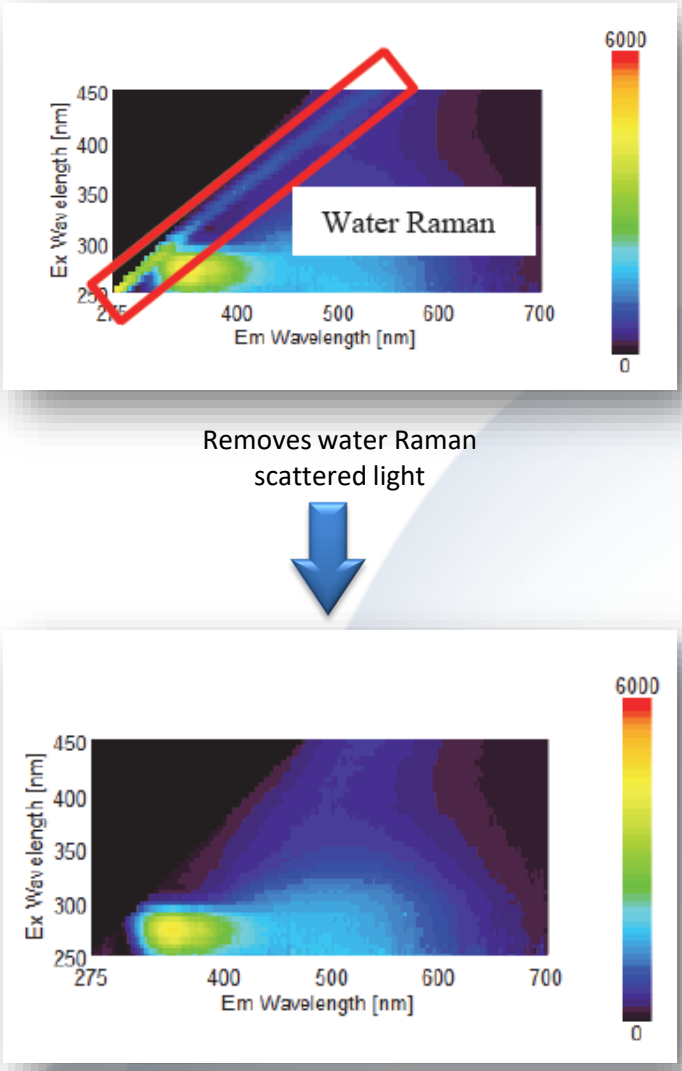


Figure 1
Data processing of 3D fluorescence data

Component Analysis of an Excitation-Emission Matrix of Water Samples using PARAFAC Analysis - APPENDIX

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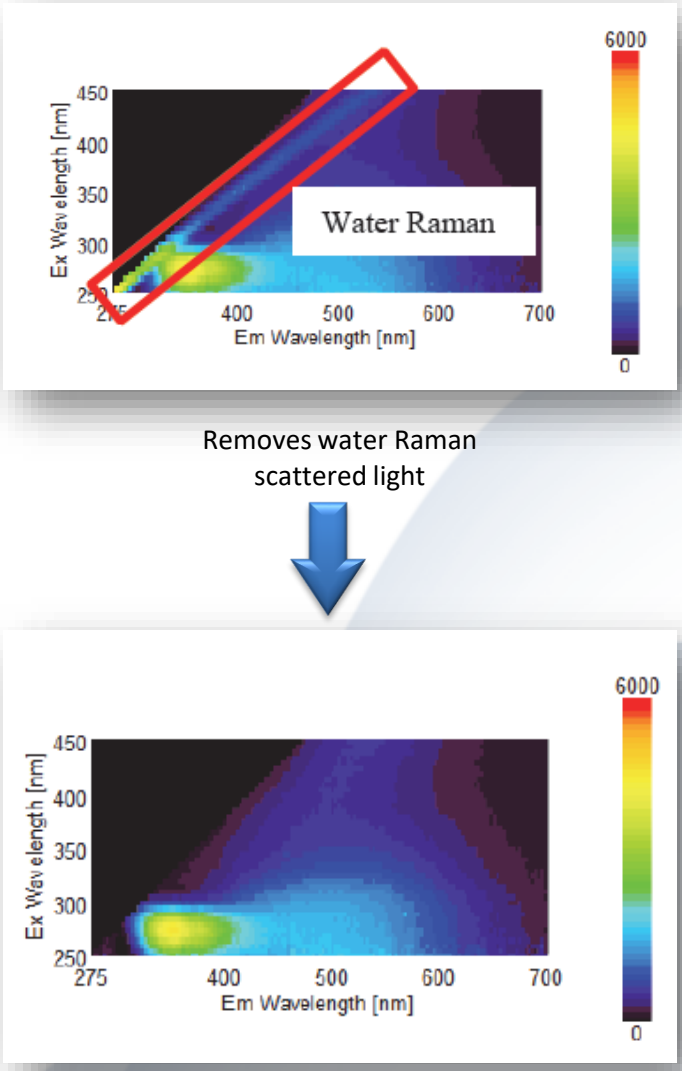


Figure 1
Data processing of 3D fluorescence data

High-Speed Measurement and EEM Interpretation for Olive Oil Analysis

Introduction

Excitation-Emission Matrix (EEM) is used with fluorescence spectroscopy for identification and characterization in mixture analysis. Recently, EEM has started to become an accepted method for determining the type, quality and geographical origin of food products. To firmly establish EEM as a reliable method for this type of analysis, more references are required, and therefore many more samples should be measured (from hundreds to thousands).

In order to increase sample throughput, JASCO has developed a system for high-speed 3-D fluorescence measurement using the FP-8500 Spectrofluorometer coupled with an MV-3500 multi-channel spectral detector (Figure 1).

To demonstrate the ease-of-use of this high speed measurement system, this application note reports the results for the measurement of a variety of olive oil samples. In addition, this note shows the use of EEM interpretation for characterizing the olive oil samples.

Figure 1
3-D fluorescence high-speed measurement system

System configuration

- FP-8500ST Spectrofluorometer
- MV-3500 Portable spectrophotometer *1)
- FMD-860 cell holder for high-speed scanning *1)
- Straight bundle optical fiber *1)
- ESC-842 Calibrated light source (WI)
- ESC-843 Calibrated light source (D2)
- PLS Toolbox (Solo) Chemometrics Software *2)

*1) Custom-ordered
*2) Made by Eigenvector Research Inc.

Sample

- Olive oil, diluted in hexane 1:350 to a low absorbance (<0.02 AU)
- Extra virgin olive oil: Six samples (A, B, C, D, E, F)
- Pure olive oil: Two samples (G, H)

Measurement and Analysis Procedure

Measurement was performed using an FP-8500 spectrofluorometer with MV-3500 multi-channel spectrophotometer. The measurement and data processing procedures are as follows.

[Measurement]

1. Acquire the excitation and emission correction data using calibrated light sources (WI and D2)
2. Perform 3-D fluorescence measurement with hexane.
3. Perform 3-D fluorescence measurement with the samples. Three data sets were collected for each sample, which was removed and replaced between scans.

[Data processing]

- Correct the 3-D fluorescence data of hexane and sample using the excitation and emission correction data.
- Calculate the subtraction spectrum between the 3-D fluorescence data of the sample and hexane to remove the hexane Raman peaks.

Measurement condition

- Excitation bandwidth: 10 nm
- Emission bandwidth: 5 nm
- Excitation data interval: 5 nm
- Emission data interval: 1 nm
- Response: 20 sec
- Filter: Used

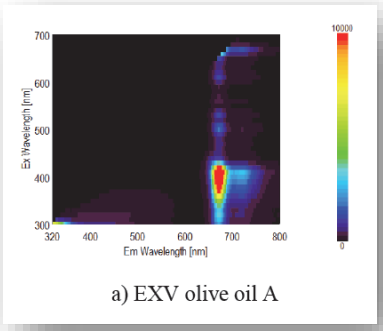
Measurement Results

Figure 2 shows 3-D fluorescence data of each sample after data processing.

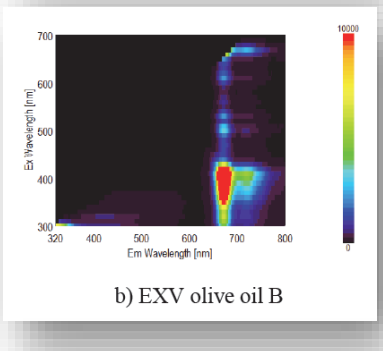
This system can acquire a complete 3-D fluorescence spectrum within 40 sec for high-speed measurement.

High-Speed Measurement and EEM Interpretation for Olive Oil Analysis

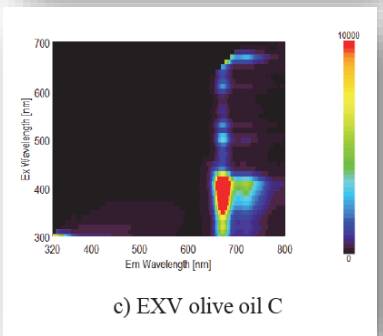
Figure 2, Peak locations, and estimate of the components from a reference paper 1, oxidation product (Ex: 300 to 400 nm, Em: 320 to 500 nm), vitamin E (Ex: 300 to 400 nm, Em: 500 to 600 nm) and chlorophyll (Ex: 300 to 700 nm, Em: 650 to 800 nm).



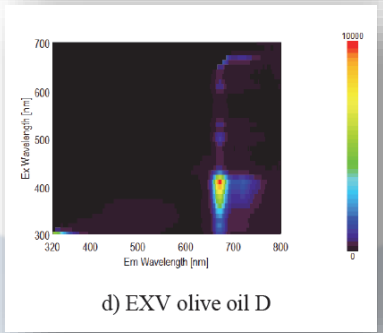
a) EXV olive oil A



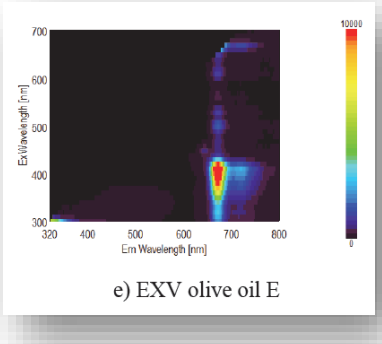
b) EXV olive oil B



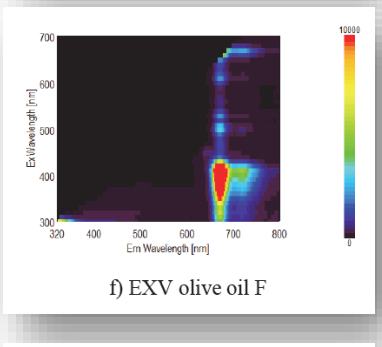
c) EXV olive oil C



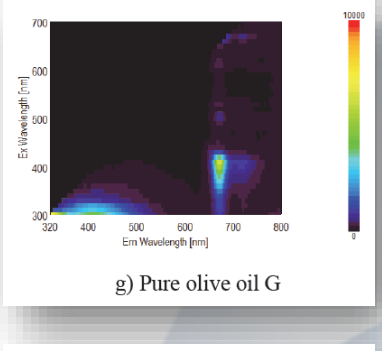
d) EXV olive oil D



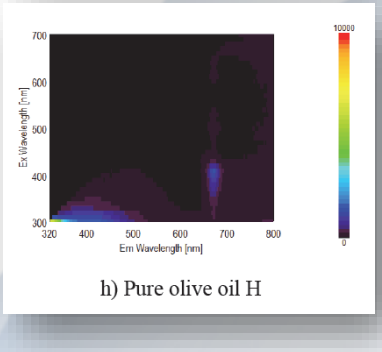
e) EXV olive oil E



f) EXV olive oil F



g) Pure olive oil G



h) Pure olive oil H

Figure 2
3D fluorescence data of each sample

High-Speed Measurement and EEM Interpretation for Olive Oil Analysis

Analysis results

Next, parallel factor analysis (PARAFAC) was performed using the 3-D fluorescence data. The number of component spectra was set to two.

Figure 3 shows the component spectra calculated by PARAFAC. The spectra show that the first component spectrum is chlorophyll and the second is an oxidation product and vitamin E.

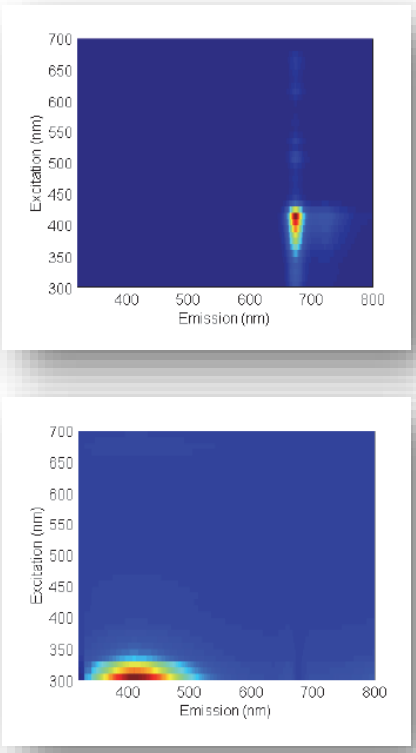


Figure 3

Component spectra calculated by PARAFAC
(Up: first component, Down: second component)

Figure 4 shows the score plot for the first component (chlorophyll) and the second component (an oxidation product and vitamin E). Regarding the distribution in the extra virgin olive oil, the score for the first component is large and the second is small, by comparison the distribution in the pure olive oil, shows that the score for the first component is small and the second is large. This trend is related to the production process of the olive oil.

Extra virgin olive oil is produced by squeezing and filtering the fruit of the olive without any chemical treatment process, and the acidity of the olive oil is less than 0.8%. On the other hand, pure olive oil is a blend of virgin olive oil and refined olive oil, the acidity of pure olive oil is typically less than 1 %. In addition, the chlorophyll content of pure olive oil is small because it is reduced during the production process for refining the olive oil.

These results provide good information about the characteristics of olive oils.

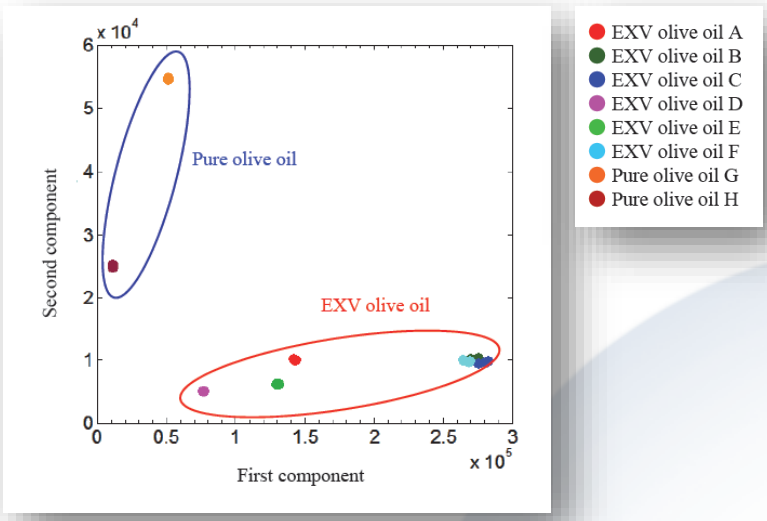


Figure 4

Score plot of the first and second components

Conclusion

JASCO has developed a high-speed fluorescence measurement system, which can be used for highthroughput sample measurement.

In addition, we found that PARAFAC is a powerful tool for the interpretation of EEM. PARAFAC can be used to extract the component spectra from 3-D fluorescence data, for use in estimating the compounds from their component spectra. The score plot calculated by PARAFAC provides information about the characteristics of a sample, and is useful for determining the type and quality of the samples.

Reference

1) Kongbonga YGM, Ghalila H, Onana MB, Majdi Y, Lakhdar ZB, Mezlini H and Ghalila SS, Food and Nutrition Sciences, 2, 692-699, (2011)

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