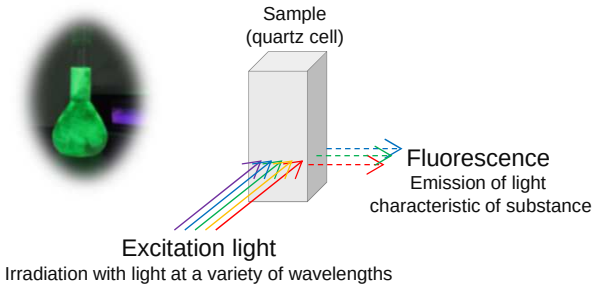
# Description of Fluorescence Fingerprint Analytical Method

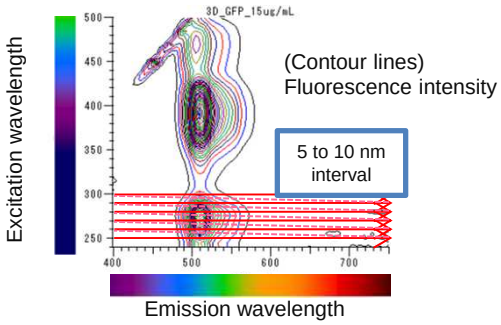
This report presents a summary of fluorescence fingerprinting (3-D fluorescence spectra), which has been a topic of discussion in recent years, and its relationship to fluorescence phenomenon, measurement methods, fundamental principles, and applications.

## Fluorescence Phenomenon and 3-D Fluorescence Spectra (Fluorescence Fingerprint)

- ✓ A sample is irradiated with light at a variety of wavelengths, and the fluorescence emitted from the compound is observed.
- ✓ The excitation wavelength, fluorescence wavelength, and fluorescence intensity vary with the type and concentration of the compound.
- ✓ The fluorescence properties of the sample (fluorescence fingerprint) can be acquired using the 3-D fluorescence spectrum mode of the fluorophotometer.

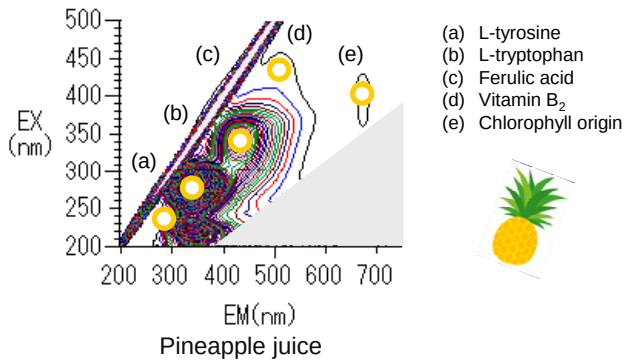



With a fluorophotometer, a sample is irradiated with light from the UV to the visible range (200 to 900 nm), and the fluorescence emitted from the sample (200 to 900 nm) is observed.

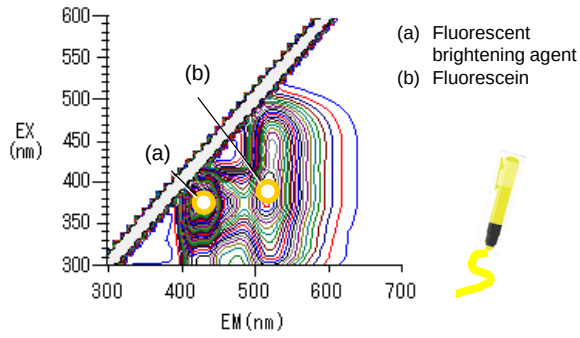



With a fluorophotometer, after fixing the excitation wavelength and scanning for emission wavelengths, the operation of scanning for fluorescence wavelengths at the next excitation wavelength is repeated to construct 3-D fluorescence spectra.

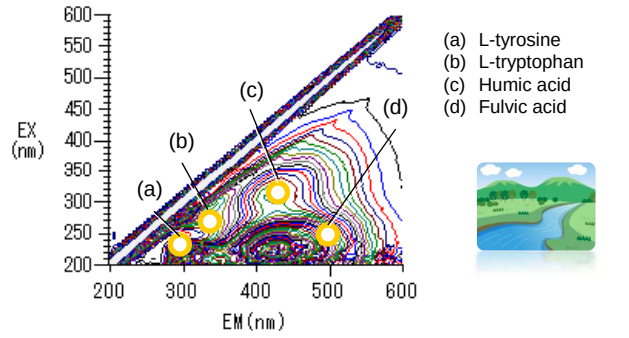
Figure 1 – Description of fluorescence phenomenon and measurement principles for 3-D fluorescence spectra



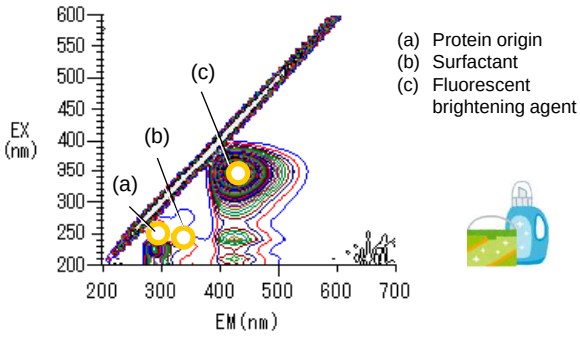

Pineapple juice




Paper marked with highlighter pen




River water




Laundry detergent

The fluorescence from familiar samples can also be confirmed. As can be seen above, we measured autofluorescence from various components found in pineapple juice, river water, highlighter ink, and laundry detergent.
(Substance identifications were deduced from literature and other information.)

Figure 2 – Fluorescence fingerprint of various samples

# Fluorescence Fingerprint Measurement Procedures

■ Fluorescence Fingerprint Measurement Procedures

(1) Sampling
↓
(2) Preparation
↓
(3) Sample mounting
↓
(4) Measuring operation
↓
(5) Data processing

## (1) Sampling

Fluorescence fingerprints can be obtained from not only liquid samples but solid samples as well. In addition, various sample forms such as pastes, powders, granular, and plate-like can be accommodated.

### Liquid samples

- Liquid (low viscosity): Raw water, etc.
- Liquid (high viscosity): Oil, eggs, milk, etc.
- Paste: Mayonnaise, cream, etc.

### Solid samples

- Powder: Grain powder, spices, etc.
- Granular: Rice, beans, supplements, etc.
- Plate-like (cut): Meat, fruit, glass, plastic, etc.

## (2) Preparation

Fluorescence fingerprint analysis measures the autofluorescence that is emitted from the sample itself with minimum preparation. A bare minimum number of preparation steps such as filtration, centrifugation, dilution, pulverization and homogenization are necessary.

### Liquid sample

- Filtration
- Centrifugation
- Dilution

➔ Results in a uniform sample condition

### Solid sample

- Pulverization, homogenization: pulverizer → sieve
- Pelletization: pulverizer → tableting machine
- Cutting

➔ Produces a uniform sample surface

## (3) Sample mounting

For liquid samples, place the sample into an ordinary quartz 10 mm square cell, and measure the fluorescence from the side. For highly concentrated samples or solid samples, set the sample at an incline, and measure the fluorescence from the surface. For samples having a non-uniform shape, reproducibility of sample mounting is improved by using an integrating sphere.

### Liquid sample

- Liquid (low viscosity): 10 mm square cell
- Liquid (high viscosity): wide cell + solid sample holder
- Paste: wide cell + solid sample holder

### Solid sample

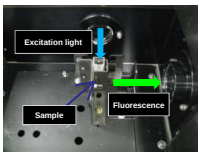
- Powder: powder cell + solid sample holder
- Granular: wide cell + integrating sphere
- Plate-like (cut): quartz plate + solid sample holder


10 mm square cell

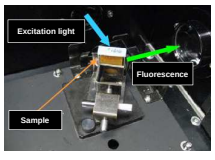

Wide cell


Powder cell

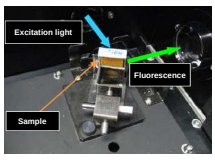

Wide cell



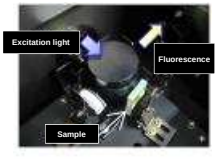
Side photometric system



Surface photometric system



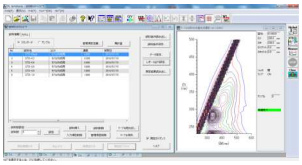
Surface photometric system

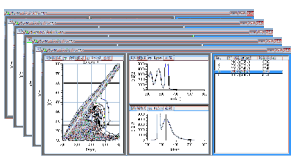


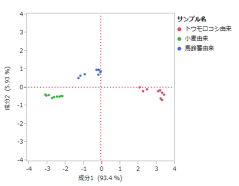
Integrating sphere photometric system

## (4) Measuring operation
## (5) Data analysis

Set the measurement conditions, and record the sample name and the like in the sample information table. Subsequently, make measurements in accordance with the table. Analyze measurement data with multivariate analysis software.


Measuring operation


Measurement data

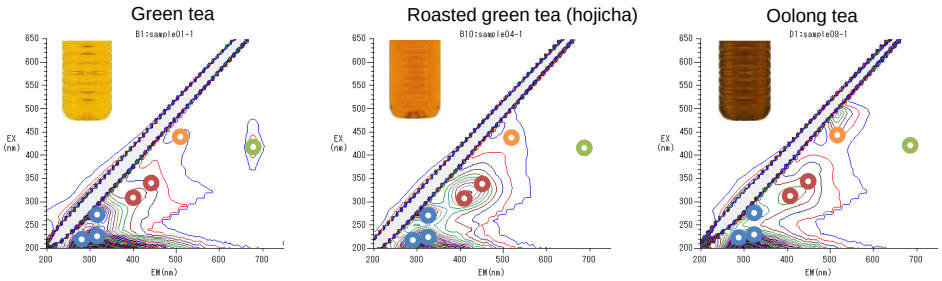

Data analysis

# Multivariate analysis application example using fluorescence fingerprinting

- ✓ Measurements of multiple specimens are performed based on the sample information table. This is useful for quality control and process validation.
- ✓ Fluorescence fingerprint measurement data can be batch output by the multivariate analysis software.
- ✓ Sample classification and accept/reject determination is carried out using multi-specimen fluorescence fingerprint data.
- ✓ By reading in the model coefficients, multivariate analyses can be calculated at the time of sample measurement, and an accept or reject determination can be made immediately after measurement.

## ■ Classification of tea by fluorescence fingerprint analysis (principal component analysis)

300 µL of each tea is added dropwise into a microplate, and the fluorescence fingerprint is automatically measured by the EEM Multi system. The fluorescence of each sample is ascertained.




EX/EM
- 210/270
- 220/315
- 280/315
- 310/400
- 360/450
- 410/675
- 470/515

- Amino acid origin
- Ferulic acid origin
- Vitamin $B_{12}$ origin
- Chlorophyll origin

Figure 3 – Fluorescence fingerprint of each tea

Comparison of differences in tea making processes

| Process | Green tea | Roasted green tea (hojicha) | Oolong tea |
|---|---|---|---|
| Fermentation | – | – | △ (half-fermented) |
| Steaming | ○ | ○ | ○ |
| Kneading | ○ | ○ | ○ |
| Drying | ○ | ○ | ○ |
| Roasting | – | ○ | – |

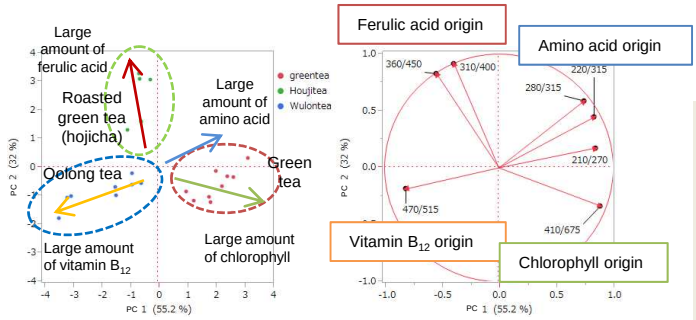



Figure 4 – Multivariate analysis (principal component analysis) results

### Points

Fluorescence fingerprints were detected from the autofluorescent components of tea. Principal component analysis was applied to the fluorescence fingerprints. The change at each tea production stage can be captured visually by retaining principal components (component 1, component 2, etc.) limited to those with large changes in wavelength. Here, the X-axis is correlated with amino acids and chlorophyll, and the Y-axis is correlated with ferulic acid.

It can be seen that in green tea, components originating from amino acids and chlorophyll have strong fluorescence intensity, that in roasted green tea (hojicha), components originating from ferulic acid have strong fluorescence intensity, and that in Oolong tea, vitamin $B_{12}$ components have strong fluorescence intensity.

This technique is useful when controlling processes, discriminating between similar products, and interpreting measurement data.

## ■ Olive oil grade discrimination (discriminant analysis)

There are cases in which ordinary olive oil is falsely labeled and sold as highest-grade extra virgin oil. From the olive oil, the fluorescence fingerprints stemming from peroxide (red box) and stemming from chlorophyll (blue box) were ascertained.
The olive oils can be distinguished by multivariate analysis of the intensity distributions.

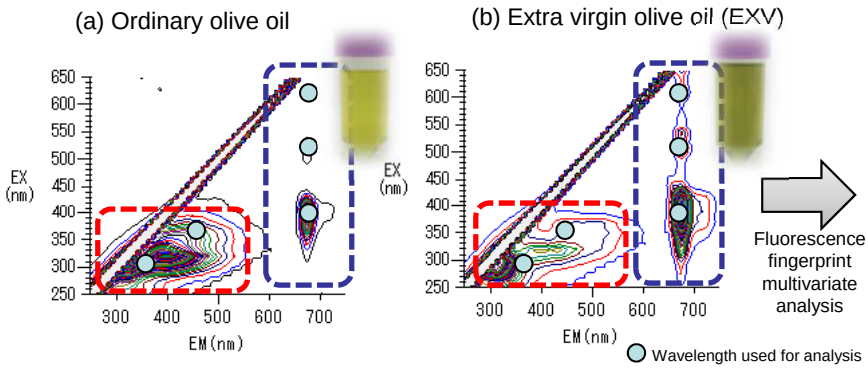
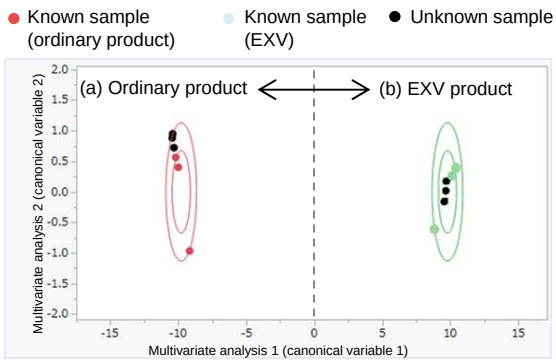



Figure 5 – Olive oil fluorescence fingerprint




Canonical variable 1 and canonical variable 2 are derived by discriminant analysis from the fluorescence intensity of each sample (height of contour lines)

Figure 6 – Multivariate analysis (discriminant analysis) results

### Points

Measurement results for the known samples are read in, and model coefficients are calculated by the multivariate analysis software.

# Technical Report

FL No. 170002 Fluorescence Spectrophotometer

HITACHI
Inspire the Next

## EEM Basic ~Measurement system for raw material discrimination and process confirmation~

- ✓ The model F-7100 fluorescence spectrophotometer is a basic system equipped with an automatic filtering attachment.
- ✓ Fluorescence fingerprints can be acquired at about 2 to 3 minutes per sample.

### ■ Equipment exterior

Automatic filtering attachment

Model F-7100 Fluorescence Spectrophotometer

### ■ System configuration example

<Main unit>
Model F-7100 Fluorescence Spectrophotometer
<Optional items>
· Automatic filtering attachment (P/N: 5J0-0158)
· Solid sample holder (P/N: 5J0-0152)
<Optional programs>
· Dedicated analysis software for multivariate analysis

## EEM Multi ~Automatic Multi-Sample Discrimination System with Microplate~

- ✓ Handles continuous multi-sample measurement of fluorescence fingerprints using a microplate attachment.
- ✓ 96 liquid samples can be continuously measured with the microplate, and 18 powder samples can be continuously measured with the powder sample guide.

### ■ Equipment external appearance

96-well microplate

Powder sample guide

Model F-7100 Fluorescence Spectrophotometer
(Microplate attachment installed)

### ■ System configuration example

<Main unit>
Model F-7100 Fluorescence Spectrophotometer
(Optional items)
· Automatic filtering attachment (P/N: 5J0-0158)
· Microplate attachment (P/N: 5J0-0139)
· Powder sample guide (special order item)
*Powder samples are loaded into the sample cup (0.25 mL) for automatic analysis use.
<Optional programs>
· Dedicated analysis software for multivariate analysis

## EEM Direct ~Discrimination System with Inline Optical Fiber~

- ✓ Samples with irregular shapes or large samples can be directly measured without placing the sample into a sample chamber by using an optical fiber.
- ✓ The spot irradiated by excitation light from the optical fiber is approximately 5 mm, so micro regions can be measured.

### ■ Equipment external appearance

Sample installation part

Model F-7100 Fluorescence Spectrophotometer
(Optical fiber attachment installed)

### ■ System configuration example

<Main unit>
Model F-7100 Fluorescence Spectrophotometer
(Optional items)
· Optical fiber attachment (special order item)
Optical fiber: One 2-branched optical fiber (2 m) attached
Measurement wavelength range: 250 to 700 nm
(250 to 800 nm when R928F used)
Intensity specification: Solid sample holder fluorescence ratio 1/100 or greater
<Optional programs>
· Dedicated analysis software for multivariate analysis

**[KEY WORDS]**

fluorophotometer, F-7000, F-7100, F-2700, detergent, olive oil, fluorescent paint, edible oil, multivariate analysis, principal component analysis, discriminant analysis, PARAFAC, fluorescence fingerprint, EEM, Excitation Emission Matrix

*"EEM" is a registered trademark in Japan of Hitachi High-Tech Science Corporation

Note: The data published in this document are to present examples of measurements and are not a guarantee of performance.

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