



Evaluation of Organics Dissolved in Water by the Multivariate Analysis using 3D Fluorescence Spectra Measurement

3D fluorescence spectrum analysis (EEM: Excitation Emission Matrix, fluorescence fingerprint) is one of the methods used to evaluate the types and amounts of dissolved organic matters in water.

This time, the EEM of water samples collected at different river water purification processes in a purification plant were measured. By performing the PARAFAC¹⁾ analysis and main component analysis for the numerical data, the change in the organic matters dissolved in the water was evaluated.



F-7100 Fluorescence Spectrophotometer

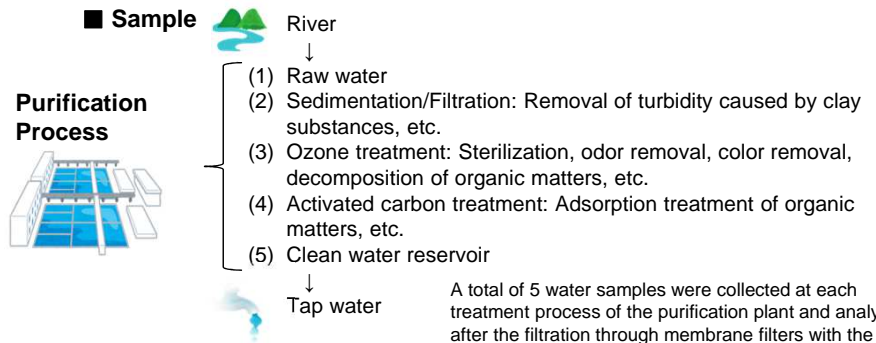
¹⁾ PARAFAC: Parallel Factor Analysis, Solo[®] 8.1.1 (Eigenvector Research, Inc., USA) was used.

Measurement of 3D Fluorescence Spectrum for Water Sample at Each Treatment Process

- ✓ By using F-7100 fluorescence spectrophotometer, the 3D fluorescence spectra of the water samples collected at different treatment processes in a purification plant were measured.
- ✓ The fluorescence intensity of the dissolved organic matters decreases as the water moves further in the treatment process and the fluorescence of the water from the clean water reservoir was very weak [Figure 1 (1) - (5)].

■ Analysis Conditions of F-7100 Fluorescence Spectrophotometer

Slit on excitation side: 5 nm
 Slit on fluorescence side: 5 nm
 Scan speed: 30,000 nm/min
 Response: Automatic
 Photomultiplier vol.: 500 V
 Spectral correction: ON



■ 3D Fluorescence Spectrum

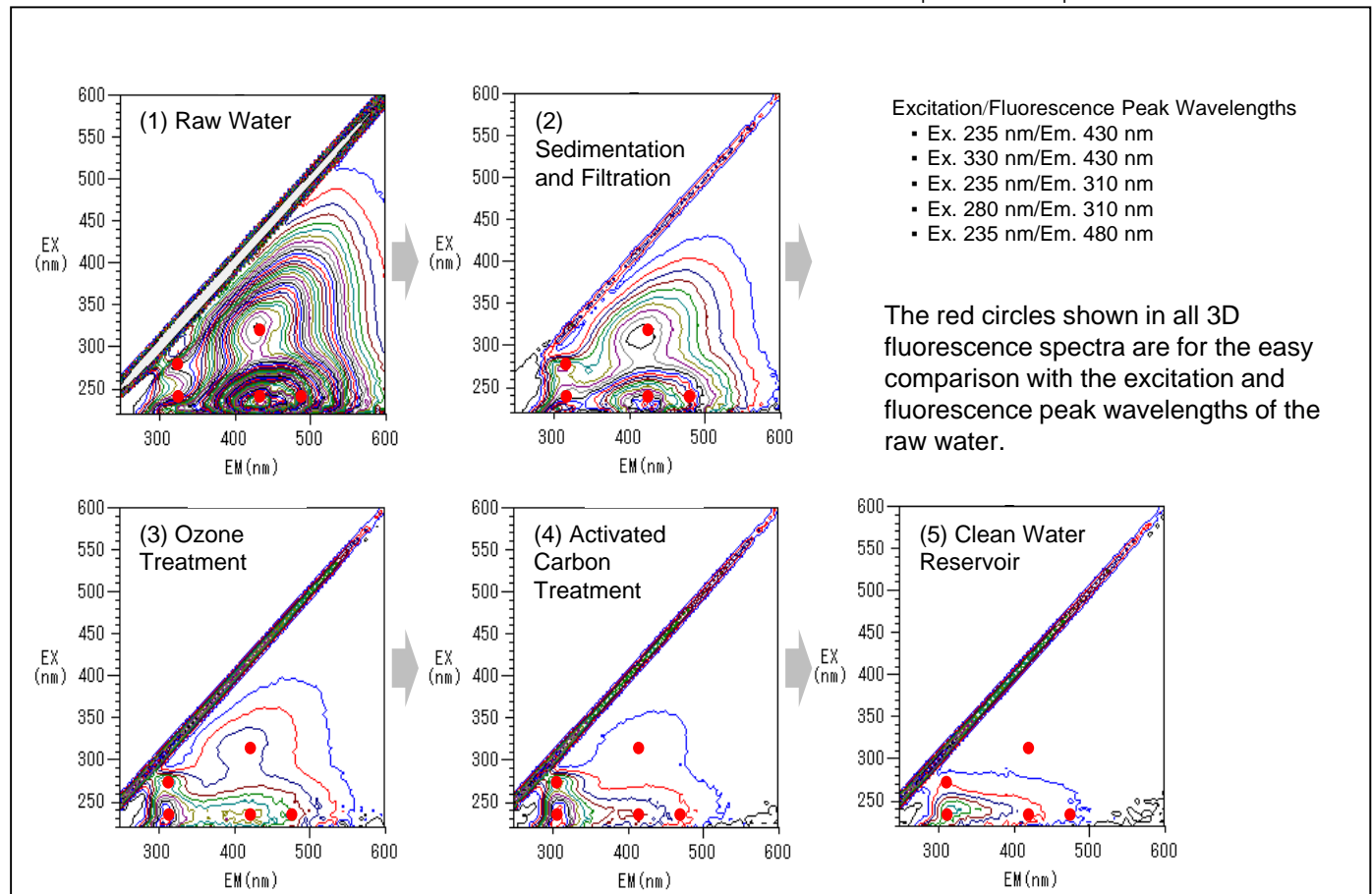


Figure 1 3D Fluorescence Spectra for Each Purification Process



Estimation of Substances Contained in Water at Each Purification Process by PARAFAC Analysis

- ✓ Quinine sulphate (QS) 1 µg/L was used to standardize the fluorescence intensity for each sample. (FL No.150002)
- ✓ From the sample spectrum standardized with quinine sulfate, the 3D fluorescence spectrum of the purified water, standardized in the same manner, was subtracted.
- ✓ In addition, the absorption spectra of 6 samples were measured by U-5100 UV-Visible spectrophotometer²⁾ and the corrections were made for the internal filter effects.

■ Analysis Conditions for U5100 Spectrophotometer

Optical path length : 10 nm
 Slit : 5 nm
 Scan speed : 400 nm/min
 Baseline correction : Purified water



2) The analysis can also be performed with the optional item for fluorescence spectrophotometer, absorbance cell holder (P/N: 650-0165).

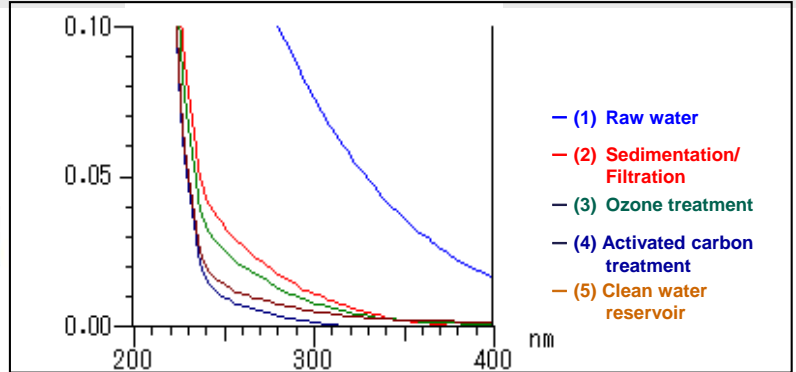


Figure 2 Absorption Spectra by U-5100

■ Formula for the correction of internal filter effect³⁾

$$I_{\text{corr}} = EEM_{\text{cor}} \exp(\text{Abs}_{\text{ex}} + \text{Abs}_{\text{em}}/2)$$

I_{corr} : Corrected fluorescence intensity
 EEM_{cor} : Measured fluorescence intensity (After background correction)
 Abs_{ex} : Absorbance at excitation wavelength
 Abs_{em} : Absorbance at fluorescence wavelength

A special order macroprogram was used for the correction of the internal filter effect.

3) Yuki Ito. Groundwater Flow in Cenozoic Sedimentary Aquifer Characterized by Excitation-emission Matrix and Multivariate Analysis The Hitachi Scientific Instrument News., Vol.59 No.2 (2016)

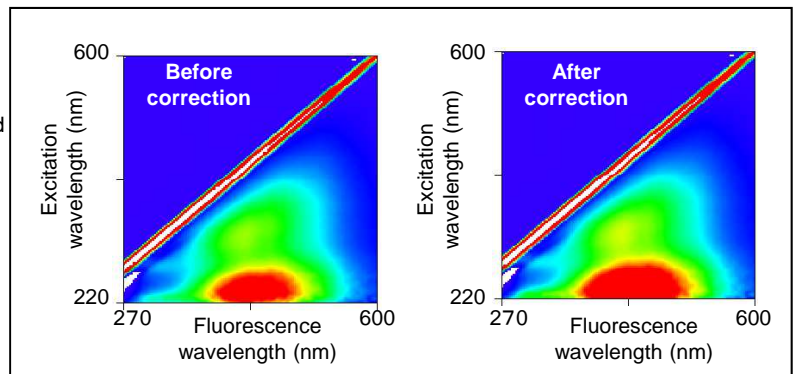
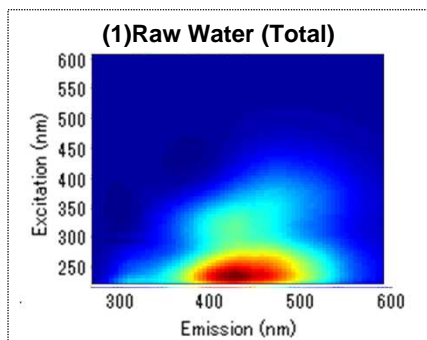


Figure 3 Fluorescence Intensity Corrected for Internal Filter Effect

■ 3D Fluorescence Spectra of Water from Purification Processes and PARAFAC Analysis Results



By performing PARAFAC analysis, the peaks could be separated into at least 3 substances. The substances were estimated to be the ones described below based on the historically reported examples of the excitation and fluorescence wavelengths for the substances³⁾.

- (a) Fulvic acid derivative
- (b) Humic acid derivative
- (c) Protein derivative

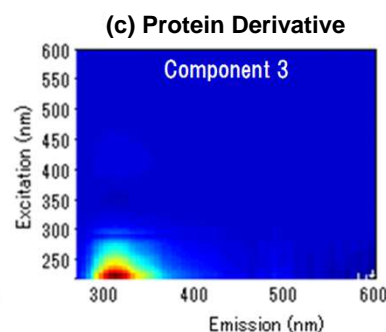
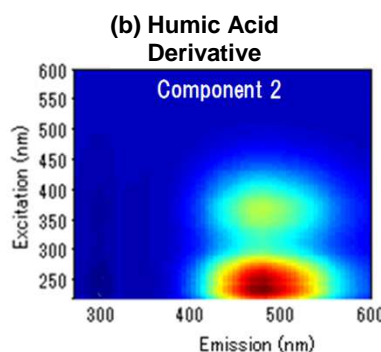
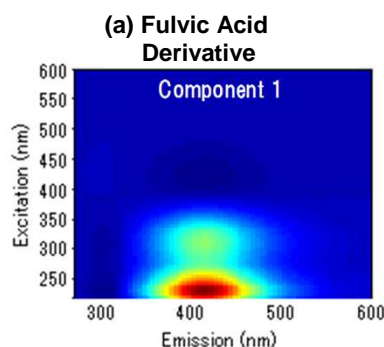
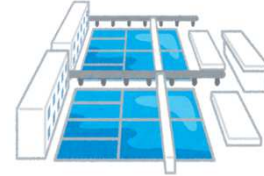
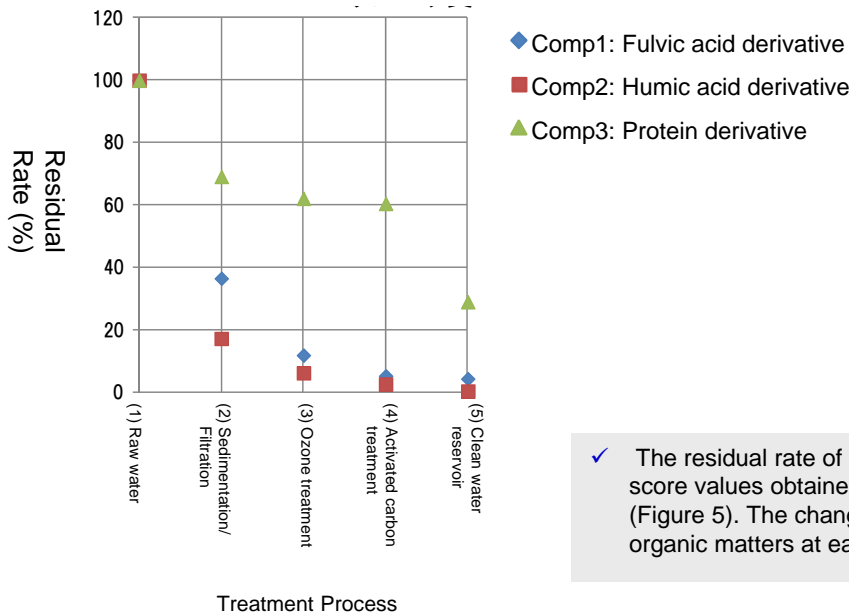


Figure 4 Analysis Data (3D Fluorescence Spectra Separated for Each Substance)



Residual Rates for Humic Substances and Protein in Water from Treatment Processes

Residual Rate (%)

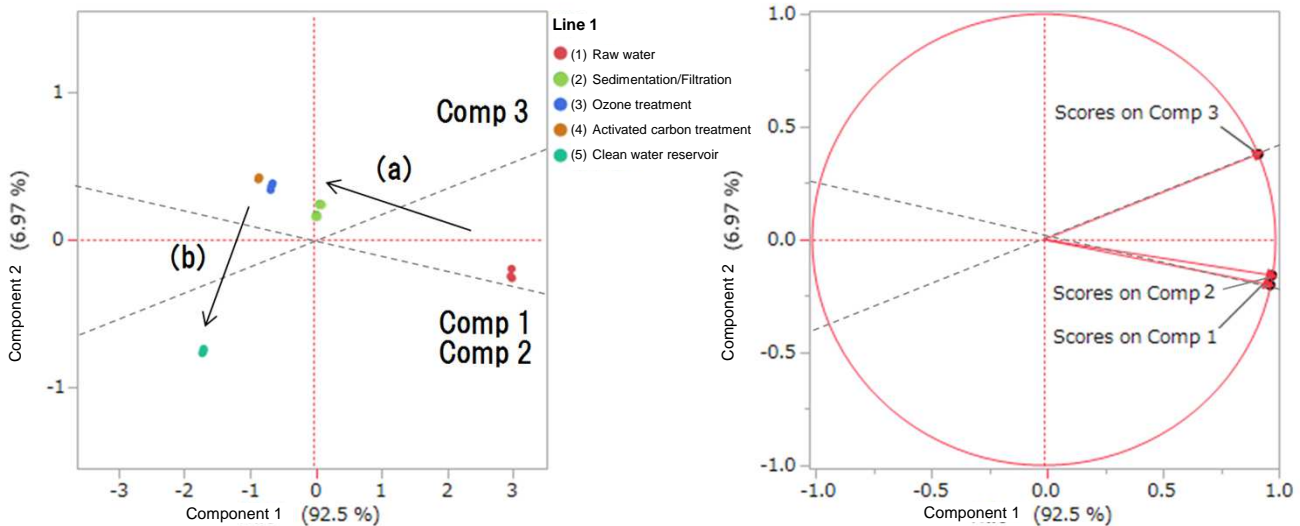


✓ The residual rate of each substance was calculated based on the score values obtained by PARAFAC analysis described above (Figure 5). The changes (effect for the removal) for dissolved organic matters at each process can be confirmed.

Figure 5 Residual Rate for Each Substance at Each Treatment Process

Evaluation of Water at Each Treatment Process by Main Component Analysis

Main Component Analysis



- ✓ The main component analysis was performed by using JMP⁴) multivariate analysis software with the score values obtained in PARAFAC analysis. The main component analysis is a method allows the easy distinction between each of the samples by consolidating a large amount of data into main component scores for the representation as lower-dimensional models. In each plot, the 3D fluorescence spectrum data is shown as one point in the coordinate system.
- ✓ Figure 6 is the scattered diagram for the main component analysis. The x-axis is for the magnitude of the fluorescence intensity while the y-axis corresponds to the ratio of protein derivatives to humic acid/fulvic acid derivatives. By using the vectors in Figure 7, the axis corresponds to the increase or decrease of each organic matter is shown as the dotted line in Figure 6.
- ✓ By studying the residual rates of the substances at each treatment process shown in Figure 5 and the vector direction of the factor loading shown in Figure 7, it can be confirmed that the fluorescence intensity decreases and humic acid and fulvic acid derivatives are removed as the process moves from (1) raw water to (4) activated carbon treatment [Figure 6 (a)].
- ✓ Similarly, Figure 6 confirms that protein, etc. are also removed from (4) activated carbon treatment to (5) clean water reservoir [Figure 6 (b)].
- ✓ By using the multivariate analysis, the fluorescence fingerprint data can be consolidated to lower-dimensional models, allowing the easy distinction between samples.



Introduction of Automatic Fluorescence Fingerprint Analysis System for Water Samples

By using the autosampler and the system with the high sensitivity flow cell, the fluorescence fingerprint analysis for water samples can be automated with the sensitivity equivalent to that for the analysis with a rectangular cell.



■ Autosampler

- Liquid handling robot: Auto Liquid Handling System Model 223: Gilson Company, Inc.
- Peristaltic pump: Peristaltic Pump MINIPULS Evolution: Gilson Company, Inc.

■ Fluorescence spectrophotometer

- F-7100 Fluorescence Spectrophotometer

(Example of Optional Item Configuration)

- Automatic filter accessory
- High sensitivity flow cell unit (custom-made)
- Absorbance flow cell holder (custom-made)
- Optional program EEM Assist Program
- Analysis software Solo® 8.1.1 (Eigenvector Research, Inc., USA)
 JMP 12.2 (SAS Institute Inc., Cary, NC, USA)

- ✓ The system is connected with an autosampler and thus, the automatic fluorescence fingerprint analysis for multiple samples becomes easy.
 - Measurement time : 5 min/sample (200-600 nm, 5 nm interval)
 - Sample volume : 20 mL/sample (with high sensitivity flow cell)
 - Maximum sample load : 56 samples
- ✓ F-7100 model has the sensitivity 1.5 times higher compared to the conventional model. The standard installation also includes the Xe lamp having the lifetime 5 times longer than the conventional model.
- ✓ By using the automatic filter accessory, it is possible to measure the fluorescence fingerprint excluding unnecessary multi-dimensional light.
- ✓ By using the absorbance flow cell holder (special order), the absorption spectra can be measured automatically.
- ✓ By importing the output data file into Solo analysis software, PARAFAC analysis can be performed.

* "EEM Assist" is a trademark of Hitachi High-Tech Science Corporation.
EEM Assist: Excitation-Emission Matrix Assist

* "JMP" is a registered trademark of SAS Institute Inc. of USA and it is used in Japan and some other countries.

[KEY WORDS]

Note: These data are an example of measurement; the individual values cannot be guaranteed.

Environmental Analysis Related, Tap Water, Fluorescence Spectrophotometer, F-7000, F-7100, F-2700, Quinine Sulfate, FL Intensity Standardization, PARAFAC, Multivariate Analysis, Fluorescence Fingerprint, Quinine Sulfate Dihydrate, EEM