

# Application News

## No. A566

### Spectrophotometric Analysis

## Trace Measurement of Chlorophyll-a - Single-Drop Measurement Using TrayCell and the Utilization of Sub-Micro Cells -

Absorbance measurement is used in various fields as a means to quantitate substances. However, when the sample for measurement has a low concentration, measurement requires higher sensitivity and therefore fluorescence measurement may be employed instead. Particularly in the fields of chemistry and life science, there are cases where the amount of sample that can be obtained is insufficient for measurement, calling for techniques that enable measurement of even smaller trace amounts of sample.

This article introduces trace measurement results of chlorophyll-a using TrayCell™ (manufactured by Hellma Analytics), which enables fluorescence measurement of samples of only a few µL, and a sub-micro cell together with the RF-6000 spectrofluorophotometer.

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### TrayCell and a Sub-Micro Cell

Fig. 1 shows TrayCell and a sub-micro cell.

TrayCell is composed of a cell that contains a fibre-optic system and a cap. Measurement using TrayCell is possible by simply removing the cap, pipetting a sample droplet (min. sample volume: 2 µL) onto the cell window, capping, and setting the cell on the cell holder. The external dimensions of the sub-micro cell are the same as a regular 10-mm square cell and the minimum sample volume varies depending on the shape of the sample compartment of the cell.



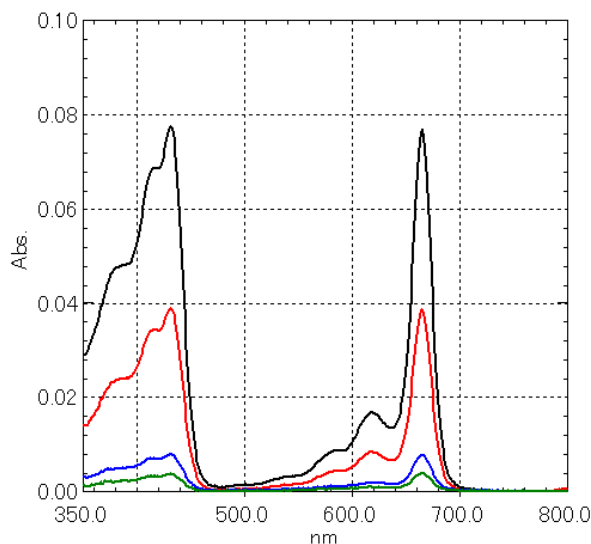
**Fig. 1 Appearance of Cells**  
Left: Sub-Micro Cell, Right: TrayCell

### Measuring Absorbance of Chlorophyll-a

The water quality of rivers and lakes is commonly inspected by measuring chlorophyll content. However, in the case of river water, high sensitivity is required for measurement since the chlorophyll concentration is low. Chlorophyll-a solutions (solvent: ethanol) of four differing concentrations between 1000 to 50 ng/mL were prepared and the absorbance was measured using the conditions listed in Table 1. The results of the measurements are shown in Fig. 2. The obtained absorbance spectra show that for the 50 ng/mL sample, absorbance is very weak at about 0.004 Abs and near the lower detection limit.

**Table 1 Measurement Conditions**

Instrument	: UV-1800
Wavelength Range	: 350 to 800 nm
Sampling Interval	: 1.0 nm
Scan Speed	: Low speed
Bandwidth	: 1.0 nm



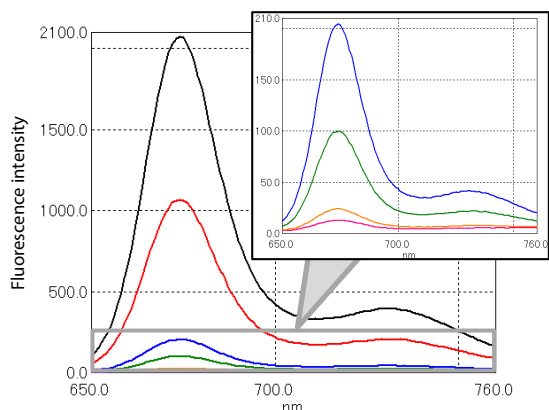
**Fig. 2 Absorbance Spectra of Chlorophyll-a Solutions**  
Black: 1000 ng/mL, Red: 500 ng/mL,  
Blue: 100 ng/mL, Green: 50 ng/mL

### Measuring Fluorescence of Trace Chlorophyll-a

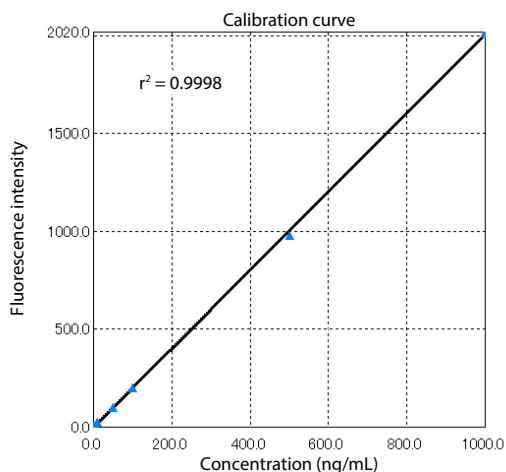
Chlorophyll-a solutions of six differing concentrations between 1000 and 5 ng/mL were prepared and measured using the RF-6000 spectrofluorophotometer and a trace measurement cell. Calibration curves were created based on the measurements. Measurement was done using TrayCell and a sub-micro cell (100 µL) respectively using the conditions listed in Table 2. Figs. 3 and 4 show the spectra and calibration curve from the measurements using TrayCell and Figs. 5 and 6 show the spectra and calibration curve from the measurements using the sub-micro cell.

**Table 2 Measurement Conditions**

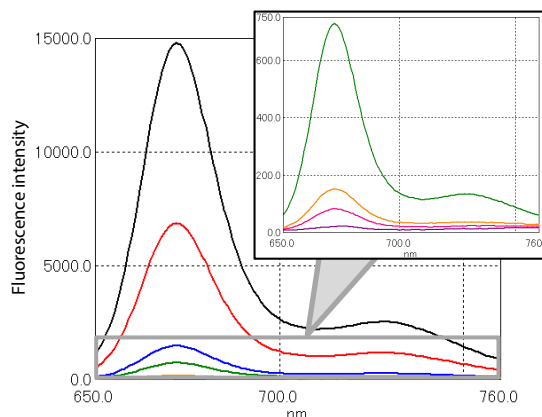
Instrument	: RF-6000
Excitation Wavelength	: 618 nm
Fluorescence Wavelength /	: 675 nm/650 to 760 nm
Fluorescence Wavelength Range	
Data Interval	: 1.0 nm
Scan Speed	: 60 nm/min
Integration Time	: 1 sec
Bandwidth	: Ex/Em 10.0/10.0 nm (TrayCell) Ex/Em 5.0/5.0 nm (Sub-micro cell)
Sensitivity	: High (TrayCell) Low (Sub-micro cell)
Repetition	: 3 times



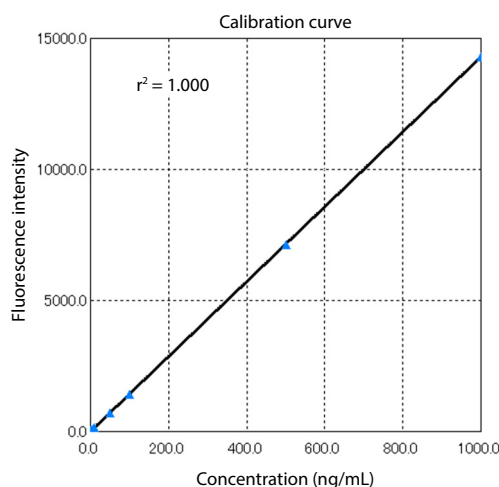
**Fig. 3 Fluorescence Spectra of Chlorophyll-a Solutions Obtained Using TrayCell**  
Black: 1000 ng/mL, Red: 500 ng/mL, Blue: 100 ng/mL, Green: 50 ng/mL, Orange: 10 ng/mL, Pink: 5 ng/mL



**Fig. 4 Calibration Curve of Chlorophyll-a Solutions Using TrayCell**



**Fig. 5 Fluorescence Spectra of Chlorophyll-a Solutions Obtained Using a Sub-Micro Cell**  
Black: 1000 ng/mL, Red: 500 ng/mL, Blue: 100 ng/mL, Green: 50 ng/mL, Orange: 10 ng/mL, Pink: 5 ng/mL, Purple: 1 ng/mL



**Fig. 6 Calibration Curve of Chlorophyll-a Solutions Using a Sub-Micro Cell**

Although detection of low-concentration samples by absorption spectroscopy is considered to be difficult, fluorescence measurements using both trace measurement cells resulted in spectra with little noise.

As for the quantitation precision, the calibration curve obtained with TrayCell shows linearity down to 5 ng/mL despite the extremely small sample amount that was used. With the sub-micro cell, since the sample amount was larger than that used with TrayCell, signals were stronger and the obtained calibration curve shows linearity down to 1 ng/mL.

### Conclusion

Using either TrayCell or a sub-micro cell together with the RF-6000 spectrofluorophotometer not only enables the measurement of trace samples of only a few µL up to a few hundred µL, but also the measurement of low-concentration samples with good precision.

TrayCell is a trademark of Hellma GmbH.

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