Transmission measurement of Volvox by using MSV-5000 series

Introduction

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

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

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

System configuration

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

Sample

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

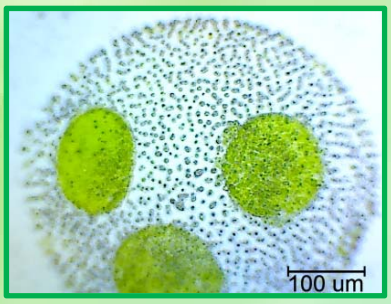

Fig. 1. Observation Image of dried Volvox

Measurement conditions

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

Spectrum measurement

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

Results

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

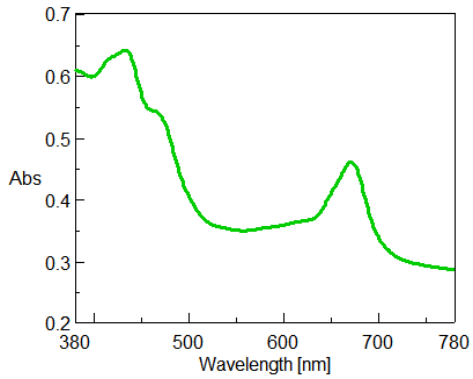

Fig. 2. Absorption spectrum of Volvox

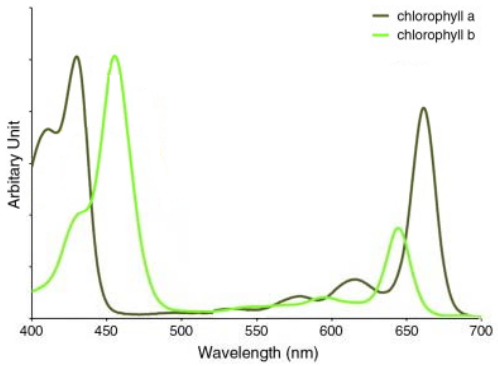

Fig. 3. Absorption spectra of chlorophylls[2)] (with Acetone solvent)

Fixed Wavelength Mapping Measurement

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

Measurement conditions

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

Results

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

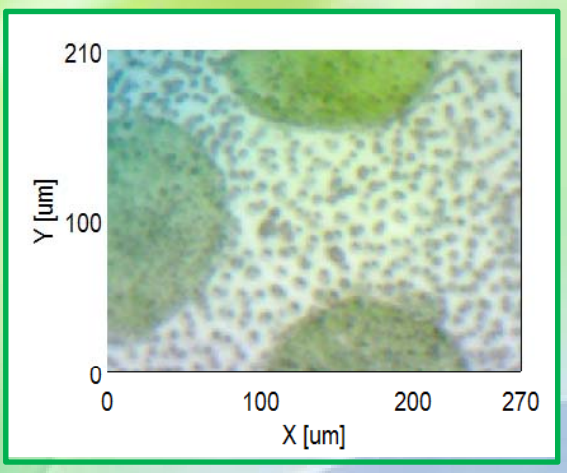

Fig. 4. Observation Image

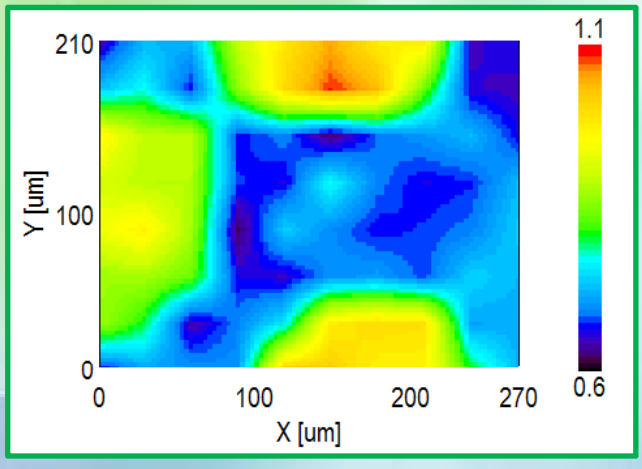

Fig. 5. Color-coded diagram of mapping measurement

Reference Literatures

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