Study of the diatom *Pinnularia sp.* using Raman spectroscopy

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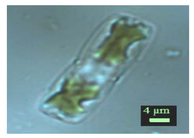
Abstract

Diatoms are essential producers in the marine ecosystem. They comprise a large amount of the biomass in our oceans and lakes. Benthic diatoms also show up in the sediment of lakes and oceans.

A spectroscopic study of diatoms, specifically the marine diatom *Pinnularia* sp., allows for better understanding of the structure and organization of the various components of these organisms. Diatoms are finding use in nanotechnology due to their unique nanostructured biosilica shells. Due to the importance of diatoms in the marine ecosystem and potential utility in nanotechnology advancements, a preliminary Raman spectroscopic study has been performed.

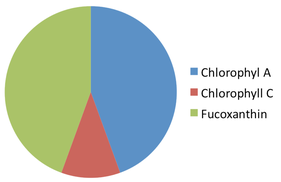
Diatoms



Living cell of *Pinnularia sp.*

Diatoms are single celled, photosynthetic marine organisms . Their shell, called the frustule, is composed of silica

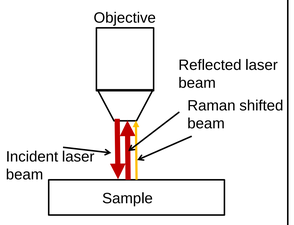
***Pinnularia sp.* Have 6 different pigments: chlorophyll a, chlorophyll c, fucoxanthin, diadinoxanthin, diatoxanthin, and beta-carotene. The latter 3 are, however, in sub-stoichiometric quantities.[1]**



Relative ratios of the 3 major pigments in the marine diatom *Pinnularia sp.*

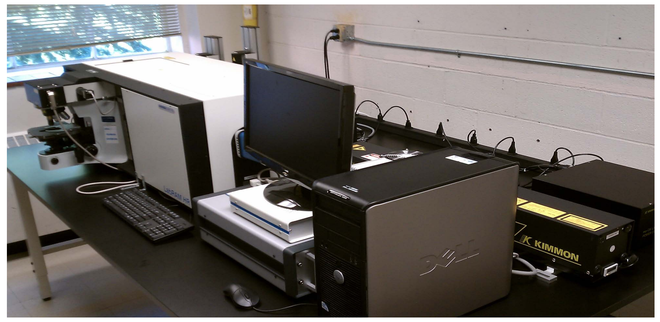
Raman Spectroscopy

Raman spectroscopy is a tool for determining the chemical composition of a sample. A laser of known frequency is shown onto a sample. This light interacts with the material through phonon interactions. These interactions cause a shift of a small amount of the reflected light, and this is the Raman shift.



Method

The Raman spectra were taken with a Horiba Jobin-Yvon LabRAM HR800 spectrometer with a Synapse CCD detector. Two different excitation lasers were used, a 532nm diode laser and a 785nm diode laser. All measurements were performed at room temperature and fluorescence has been subtracted from spectra. The setup is shown below.



Set up of Raman Spectrometer

The diatom spectrum is very complex due to its many components. In order to simplify the spectra, the pigments and silica shell were analyzed separately and then compared to the live cell spectrum.

Pigment analysis

The pigments were extracted with acetone from a concentrated diatom suspension. The diatoms were centrifuge at 170G for 5 minutes. The supernatant was removed and replaced with acetone. This was mixed and centrifuged again. The supernatant was removed and replaced with acetone. This solution was refrigerated and allowed to settle in the dark. The supernatant was extracted and Raman spectra were taken with the 785nm laser.

Chlorophyll a and chlorophyll c differ only by a single carbon chain, and chlorophyll A is much more abundant in the cell. Because of this, of these two pigments only chlorophyll a was assessed in this study.

Frustule analysis

The main component of both frustules and microscope slides is silica, thus it was necessary to examine the frustule spectrum on a different substrate. To this end, a 45 micron layer of titanium dioxide was deposited onto a glass substrate, a biofilm of *Pinnularia sp.* was grown on the titania, and the film was cleaned of organics with a mixture of ethanol and water. The Raman spectra were taken with a 532nm excitation wavelength.

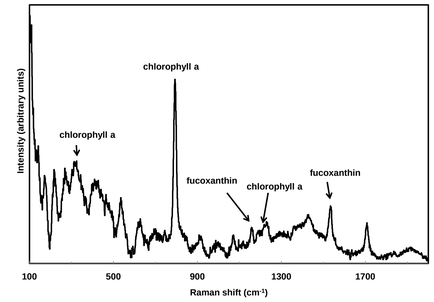
Diatom analysis

A microscope slide and cover slip were prepared with two layers of parafilm as a spacer between the two, holding the cover slip approximately 100 microns away from the slide. This allowed a thick sample of diatoms to be analyzed. The diatoms were concentrated by centrifuging a cell suspension. The soft pellet was then transferred to the microscope slide and the cover slip was placed on top of the parafilm spacers. The spectra were taken with the 785nm excitation laser.

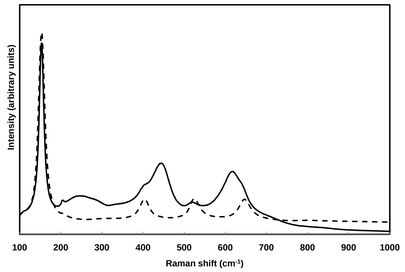
Results

Pigments

The pigment spectra shown to the right shows chlorophyll a peaks at 319.6 cm^{-1} caused by the magnesium center, at 793.0 cm^{-1}, and at 1188.9 cm^{-1} due to methyl rocking modes. This spectra also shows peaks for fucoxanthin at 1157.1 cm^{-1} caused by C-C stretching modes and 1552.0 cm^{-1} caused by C=C stretching modes.



Raman spectra of *Pinnularia sp.* pigments in acetone , 785nm excitation



Dashed line: titania substrate, 532nm excitation
Solid line: cleaned frustules on titania substrate, 532nm excitation
Normalized to peak intensity at 152.3 cm^{-1}

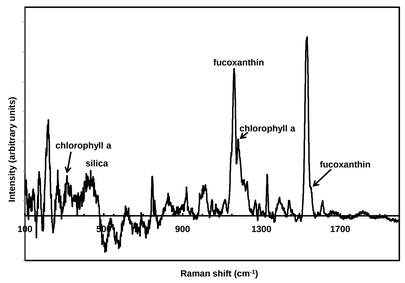
Frustules

In the dashed line spectrum to the left, the four distinctive peaks for titania in the anatase phase can be seen at 152.3 cm^{-1}, 403.3 cm^{-1}, 524.7 cm^{-1}, and 646.1 cm^{-1}.

The solid line spectrum is of cleaned frustles on the same titania substrate, thus the only addition is silica. In this spectrum, the anatase titania peaks are still present, but silica peaks have now emerged at 442.7 cm^{-1} and 617.4 cm^{-1}.

Diatoms

To left, the spectra of live cells is shown. The peaks of both of the pigments and silica can be seen. Fucoxanthin peaks can be seen at 1160.3 cm^{-1} and 1545.9 cm^{-1}, chlorophyll A peaks at 314.2 cm^{-1} and 1186.0 cm^{-1}, and a silica peak at 445.2 cm^{-1}.



Raman spectra of live *Pinnularia sp. cells*, 785nm excitation

References

1. **Gildenhoff, Nina. "The excitation energy transfer in the trimeric fucoxanthin-chlorophyll protein from *Cyclotella meneghiniana* analyzed by polarized transient absorbtion spectroscopy." *Chemical Physics*. 373. (2010): 104-109.**