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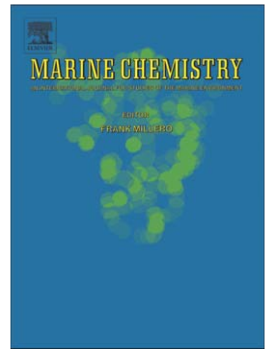

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The direct measurement of intracellular pigments in phytoplankton using resonance Raman spectroscopy

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ABSTRACT

Most current methods for analysis of phytoplankton lack the sensitivity to understand processes at the scale of individual cells. Here we describe the use of resonance Raman spectroscopy to provide detailed pigment information on individual phytoplankton cells in a non-destructive manner. To validate this technique we examined pigment spectra from cultured algae and field diatoms. Carotenoid standards for β-carotene, fucoxanthin and lutein measured with our system were highly linear, with the sensitivity based on the proximity of the excitation wavelength to that of the pigment's electronic transition. When analyzing individual cells, the position of the main C=C stretching frequency was conserved in both laboratory-cultured and field-collected diatoms, occurring at slightly higher wavenumbers for cultured *Phaeocystis* - especially when cells were grown without UV radiation. Total fucoxanthin per cell measured for *Phaeocystis* was within the range of literature values based on bulk-pigment methods (e.g. HPLC). Additionally, due to the method's non-destructive nature, rapid photopigment reorganization by a diatom was visualized and a six-fold reduction in local-area fucoxanthin concentration was quantified in less than two minutes. Our results provide methodology proof of concept and suggest it may be a candidate technology for addition to existing particle characterization platforms (e.g. flow cytometers) to provide new information previously missed with current configurations using similar monochromatic excitation sources.

Keywords: carotenoids; *P. antarctica*; pigments; resonance Raman

1 Introduction:

Aquatic phytoplankton (single-cell microalgae) produce annually the equivalent amount of organic matter as all terrestrial plants, despite having a 500x lower biomass. To capture light and subsequently harness the energy for cellular metabolism and growth, most phytoplankton use the ubiquitous pigment Chlorophyll a (Chl a). Chl a absorption is limited to red and blue regions; therefore, phytoplankton have evolved a suite of accessory pigments called carotenoids, which provide a dual service of expanding the available wavelengths of light available for

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