Polarization Discrimination Technique to Separate Overlapping Fluorescence and Elastic Scattering Applied to Algae in Seawater

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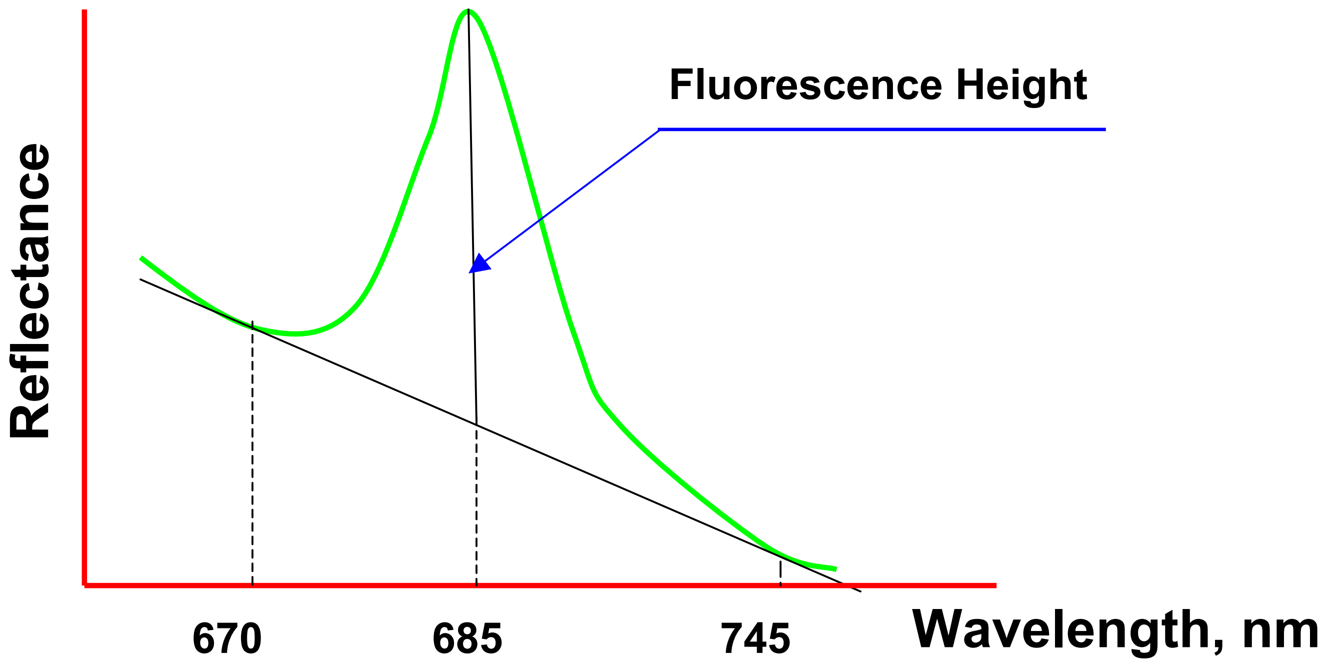
Motivation

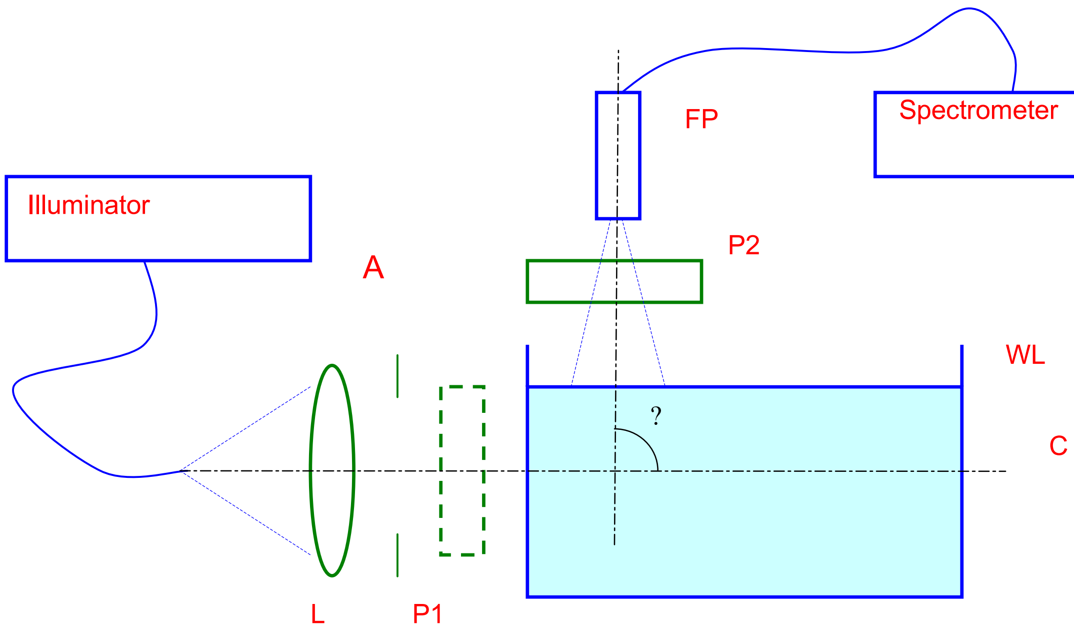
- Chlorophyll concentration and photosynthetic activity can be measured from fluorescence spectra
- Determination of the Chlorophyll Spectra is difficult in turbid waters due to the scattering signatures of suspended solids
- Separation of chlorophyll fluorescence from the background scattering using the polarized nature of scattering and the unpolarized nature of fluorescence can work in coastal waters using simple illumination sources including sun light

Fluorescence Height



Traditional method of the fluorescence height calculation over baseline

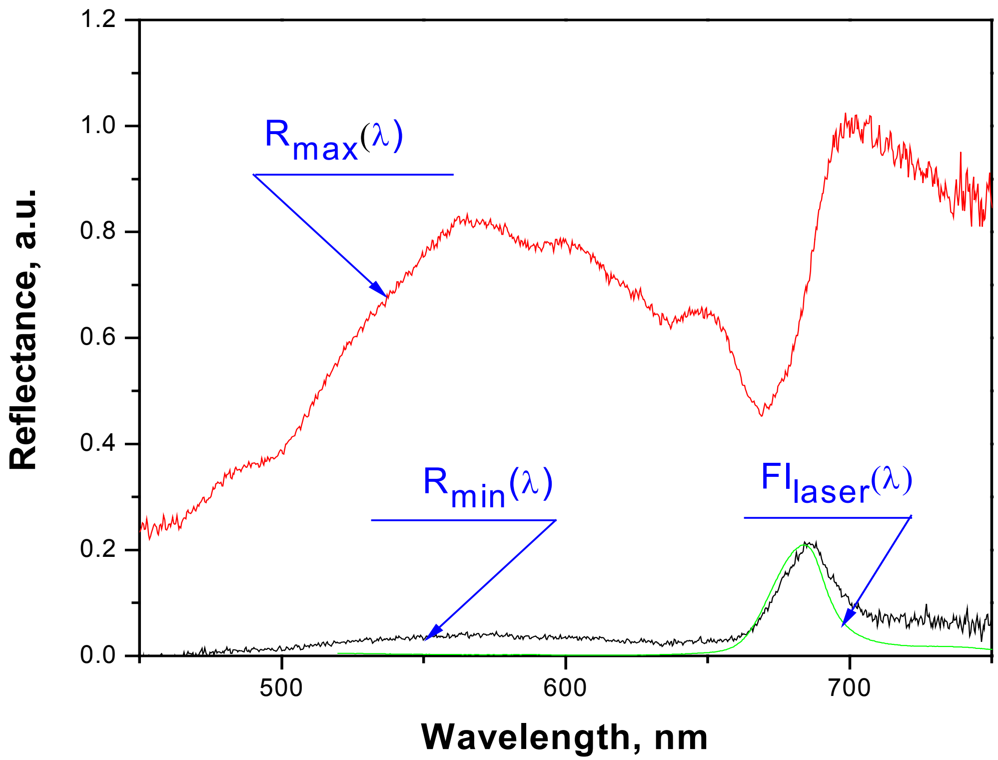
Experimental Setup



L – lens, FP – fiber probe, A – aperture, P1, P2 – polarizers,
C – cuvette with algae, WL – water level.

Objects tested: algae ***Isochrysis sp., Tetraselmis striata***, ***Thalassiosira weissflogii***, concentrations up to 10^6 cells/mL, algae with clays.

Polarized Illumination



$$R_{\max}(\lambda) = R_{\perp}(\lambda) + 0.5Fl(\lambda),$$

$$R_{\min}(\lambda) = R_{\|}(\lambda) + 0.5Fl(\lambda),$$

Near zero if no depolarization valid for spherical particles

$$Fl(\lambda) \approx 2R_{\min}(\lambda)$$

Generally validated using laser induced fluorescence but significant error results due to scattering component

Improved Removal of scattering

Strong correlation between sum and difference signals exists for scattering contribution

$$R_D(\lambda) = R_{\max}(\lambda) - R_{\min}(\lambda) = R_{\perp}(\lambda) - R_{\parallel}(\lambda)$$

$$R_S(\lambda) = R_{\perp}(\lambda) + R_{\parallel}(\lambda).$$

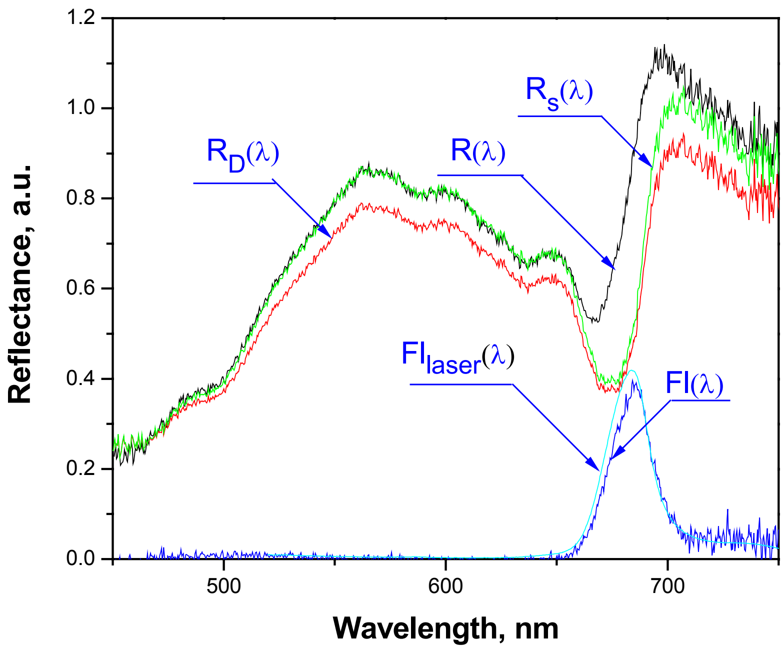
$$R_S = AR_D + B$$

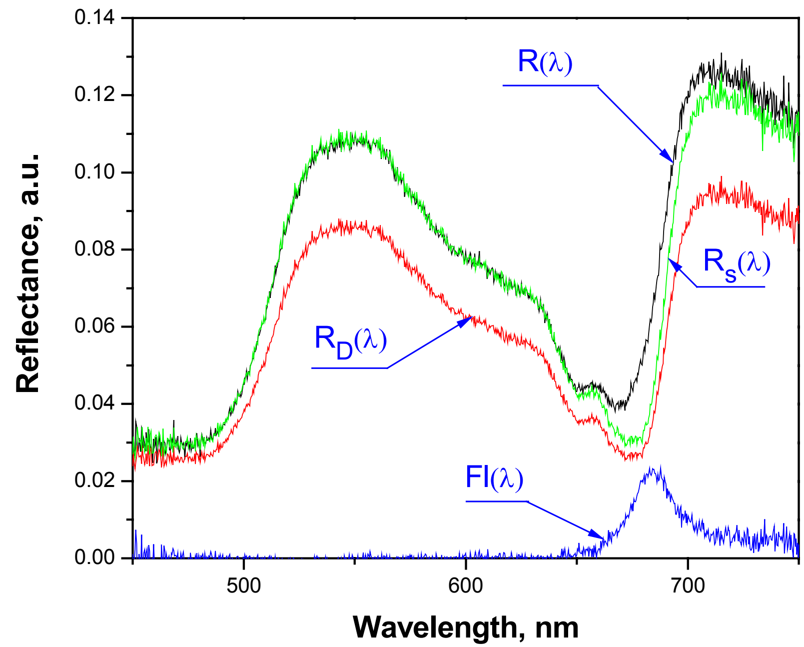
In fluorescence band $$R(\lambda) = R_{\max}(\lambda) + R_{\min}(\lambda) = R_{\perp}(\lambda) + R_{\parallel}(\lambda) + Fl(\lambda),$$

$\rightarrow$ $Fl(\lambda) = R(\lambda) - R_S(\lambda),$ where $R_S = AR_D + B$

Usefulness is due to fact that R_D can be calculated even in fluorescence band

Extracted Fluorescence





Algae ***Isochrysis sp.***

(brown algae spherical d ˜ 5 μm)

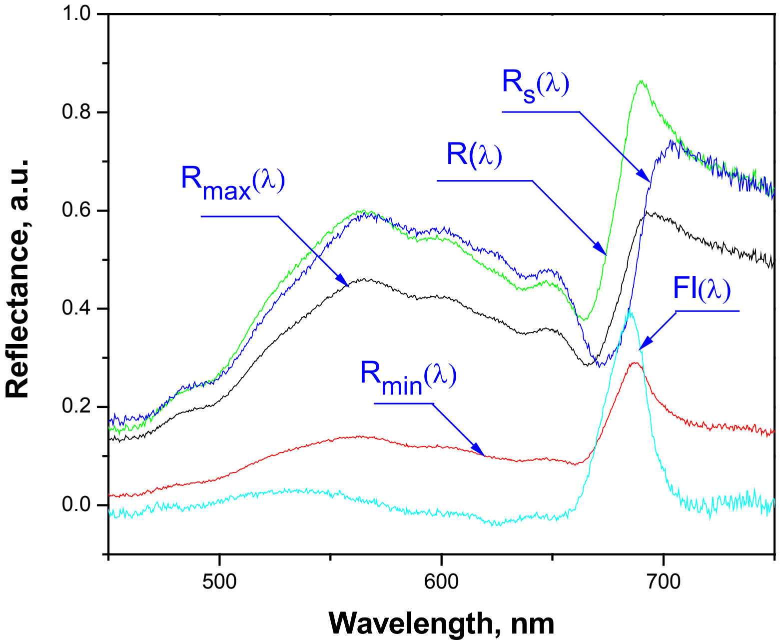
Algae ***Tetraselmis striata***

(green algae slightly ellipsoidical d ¡ 12 μm)

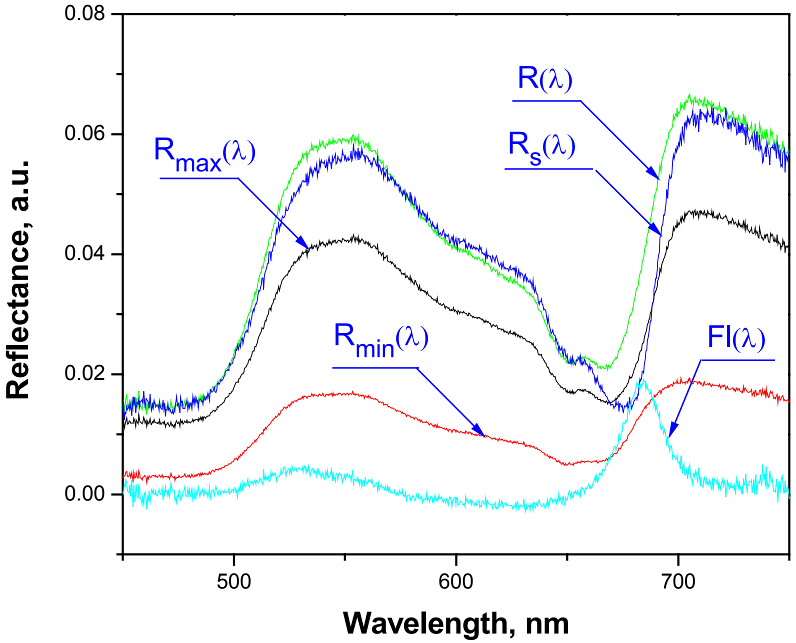
Technique with 2 polarizers

Unpolarized source

Light scattered by the algae illuminated by unpolarized light has some degree of polarization and can be also analyzed using polarization discrimination with the same linear regression approach



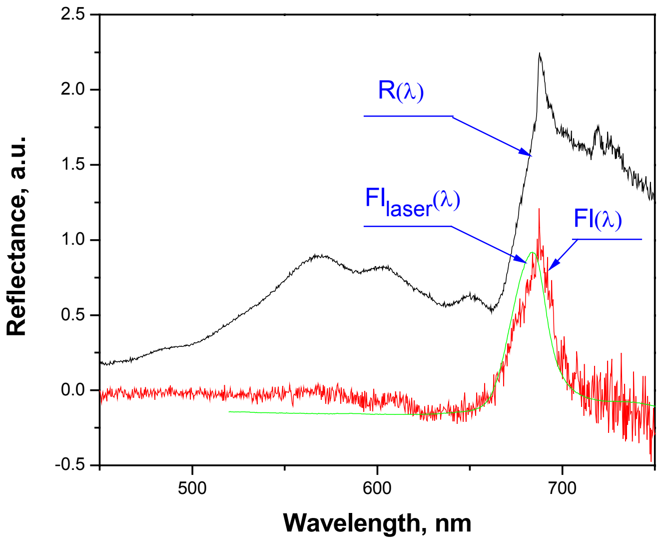
Algae ***Isochrysis sp.*** (brown algae)



Algae ***Tetraselmis striata*** (green algae)

Sunlight source

$$\theta_{sol} = 50^\circ$$



Algae ***Isochrysis sp.***

(brown algae)

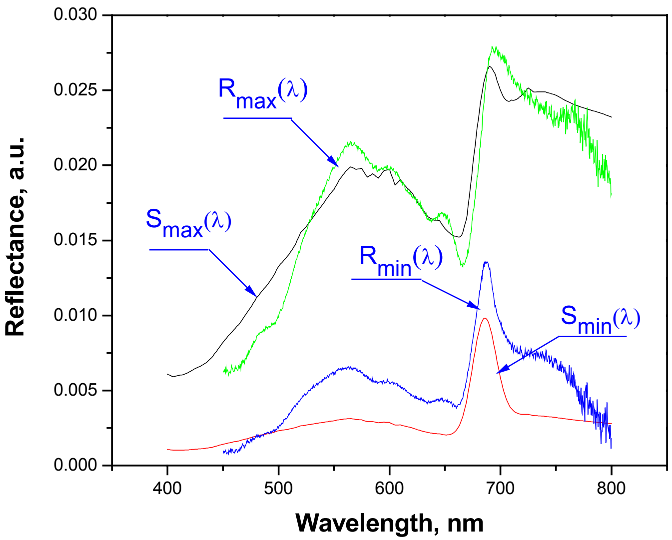
Technique with 1 polarizer

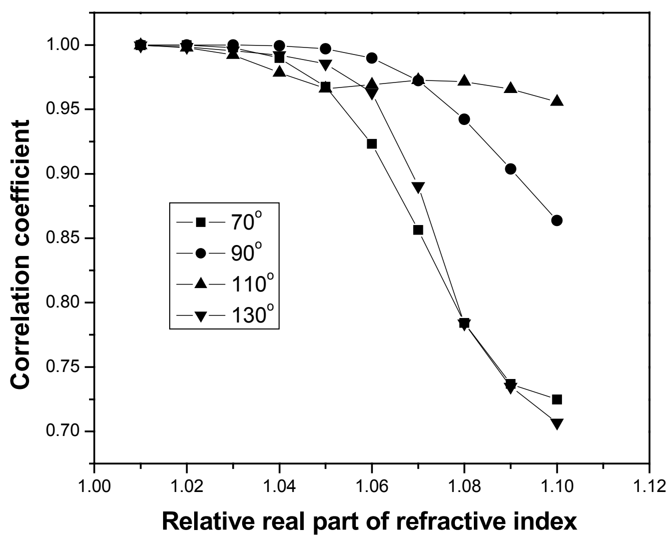
Very good agreement with laser induced fluorescence

Mie Scattering Simulation Model Results

Mean 2.5 μm, σ = 0.5 μm from measurement.

Correlation between $(S_{max} - S_{min})$ and $(S_{max} + S_{min})$



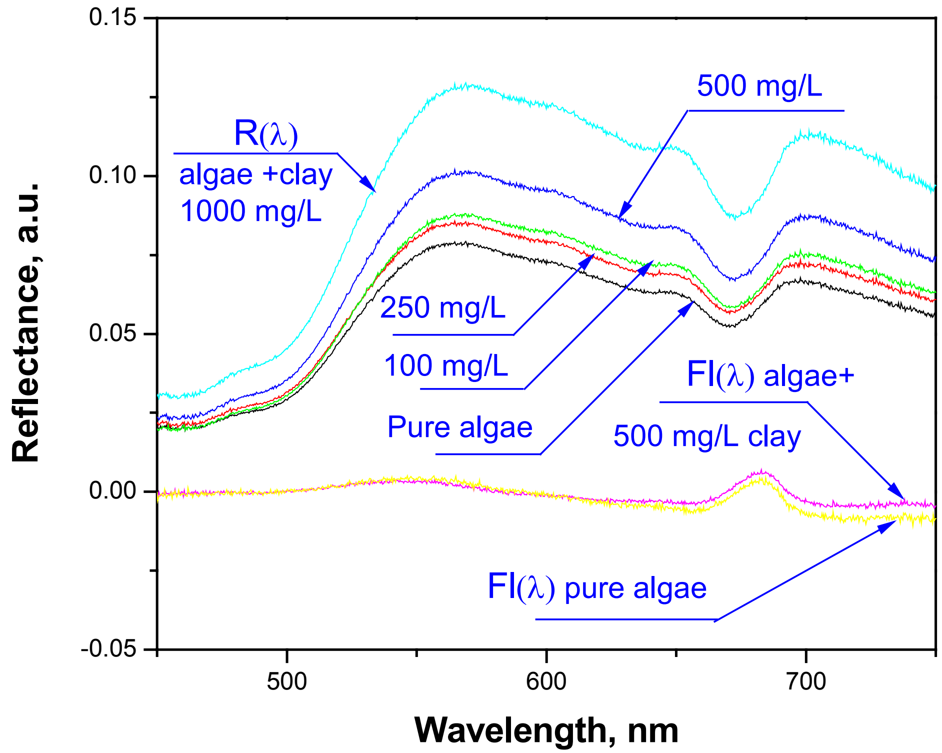


Complex scattering index obtained from *Isochrysis sp* spectral attenuation data and Kramers-Kronig relations using a two oscillator model

Good agreement

Good correlations exist as long as real part of refractive index is sufficiently low (i.e. highly absorbing)

Algae with clays



Clay – Na-Montmorillonite, particle size 1-6μm

Fluorescence curves retrieved from reflectance of pure algae and algae with clay (500 mg/L) are very close

Conclusions/Future Work

- Separation of Chlorophyll Fluorescence from scattering using polarization discrimination has been demonstrated
- Implementation of the technique using both white light and sun light sources has proven successful
- Fluorescence extraction has been obtained even with the presence of high concentration of scattering medium

Conclusions/Future Work

- Validation with laser induced fluorescence has been performed
- Mie scattering calculations are used to demonstrate the validity and limitations of the method
- Future simulations and experiments should determine the range of the technique's applicability.