Phytoplankton optics and flow cytometry in the marine environment.

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Two questions :

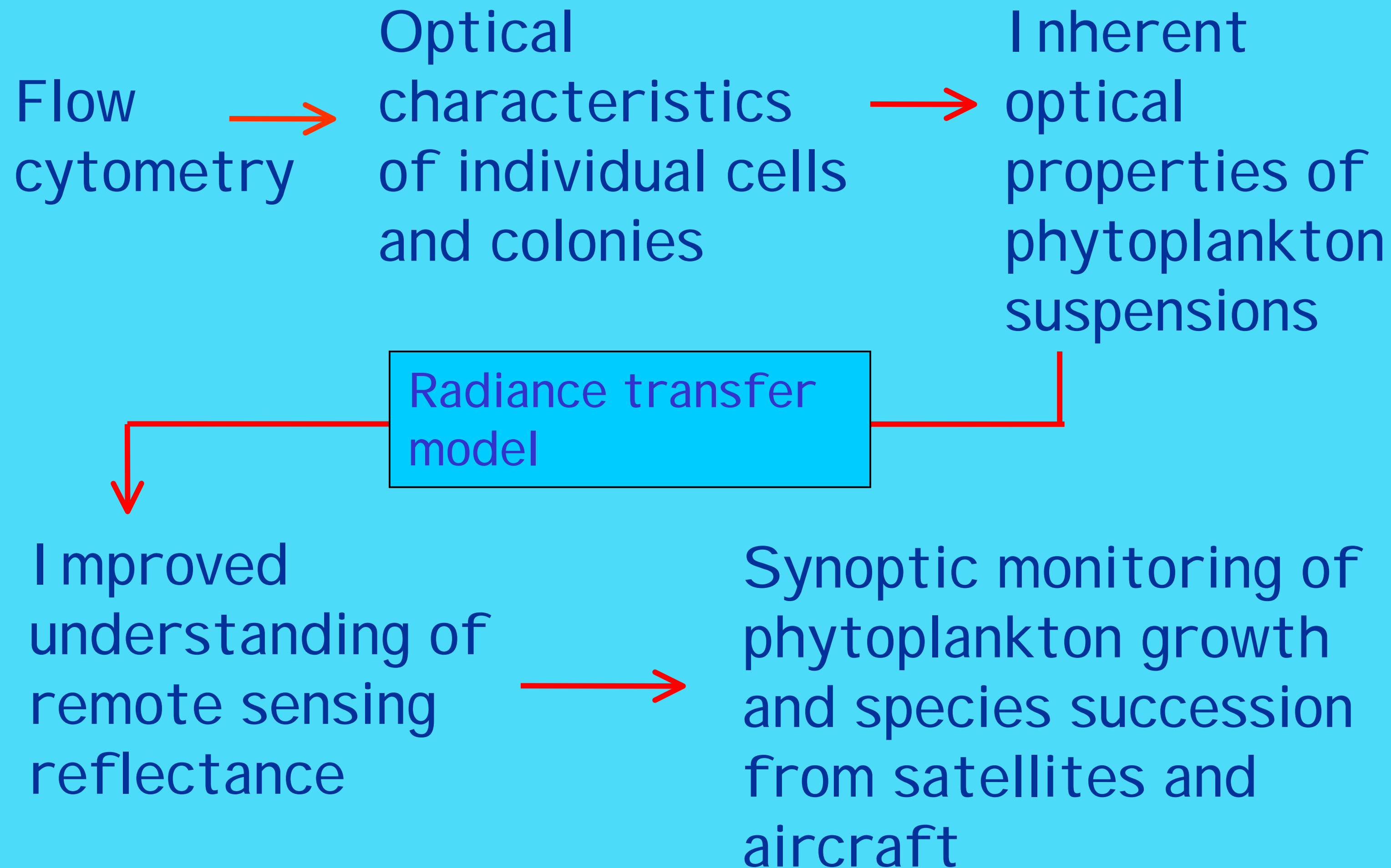
What can phytoplankton optics contribute to flow cytometry?

and:

What can flow cytometry contribute to phytoplankton optics?

An old idea: from cells to satellites

(see NATO ASI Proceedings G:27, 1990)



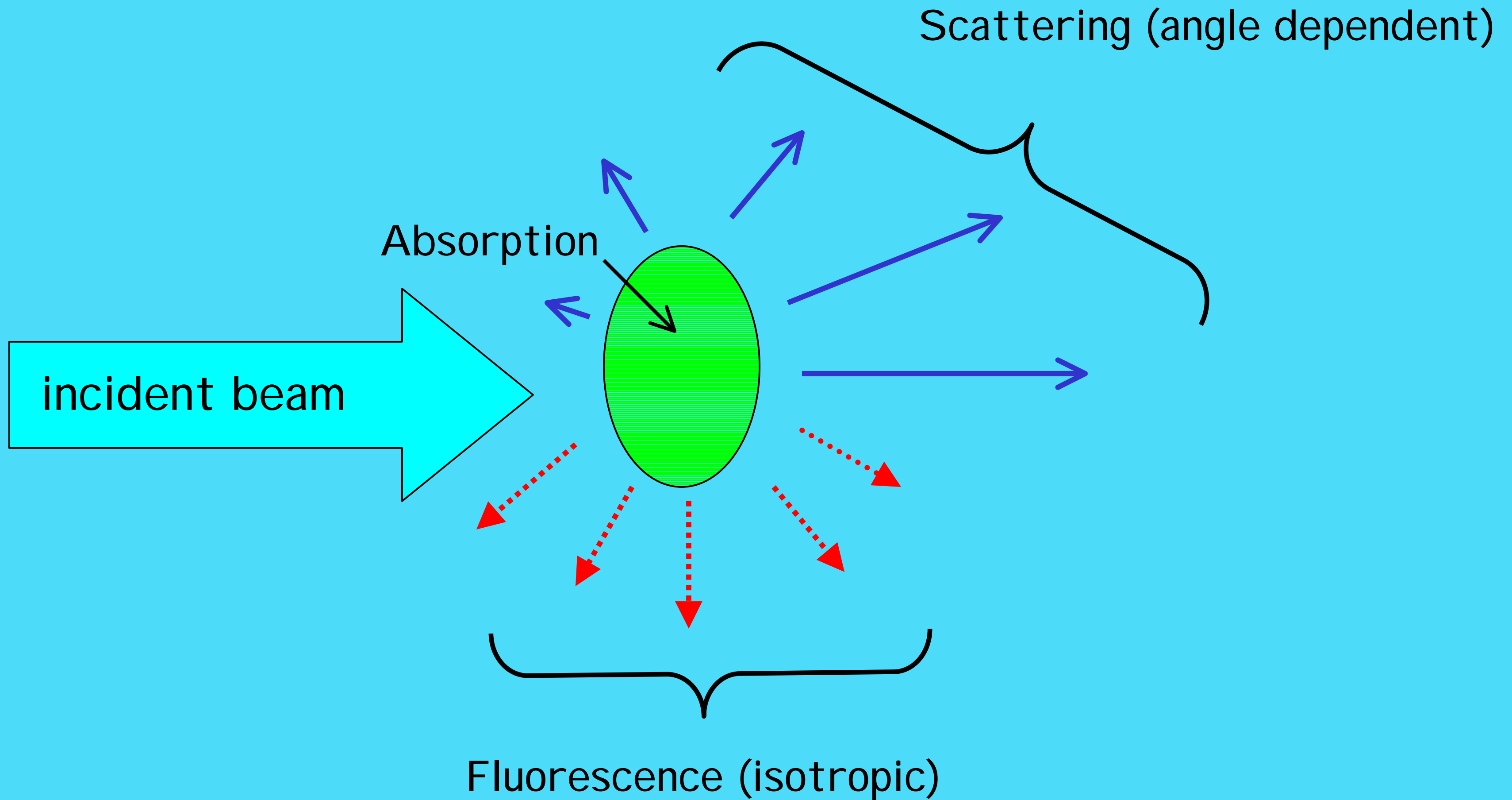
BUT:

There has been little progress in applying flow cytometry to oceanographic optics (as opposed to phytoplankton ecology and physiology) in the last 12 years.

SO:

Is the concept fundamentally flawed? What information can flow cytometry actually provide?

Basics: cell/beam interactions.



1. Absorption:

by chlorophylls (a,b,c), carotenoids and phycobiliproteins.

(not directly measurable by flow cytometry, but influences both fluorescence and scattering)

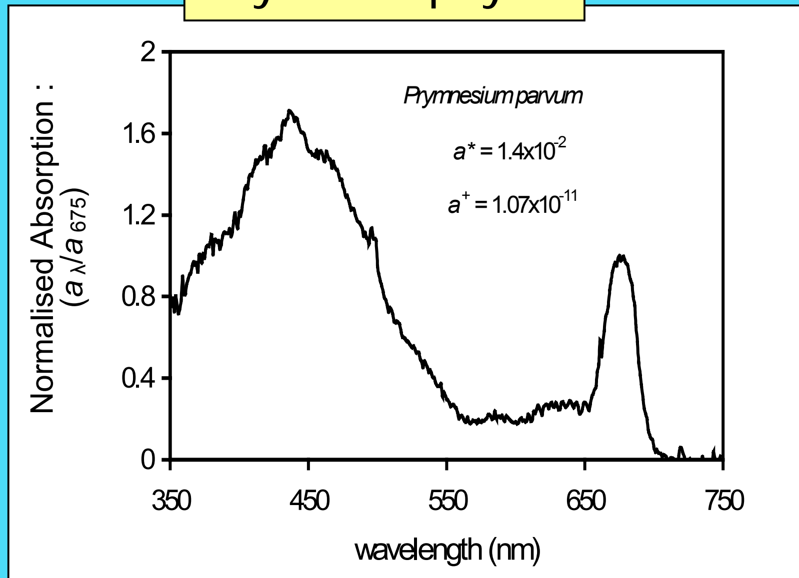
Group	Chl	Carotenoids	Phycobilins
Chlorophytes	a,b	+	-
Chrysophytes	a,c1+2	+	-
Cryptophytes	a,c2	+	+
Pyrrhophytes	a,c2	+	-
Cyanobacteria	a, (b)	+	+

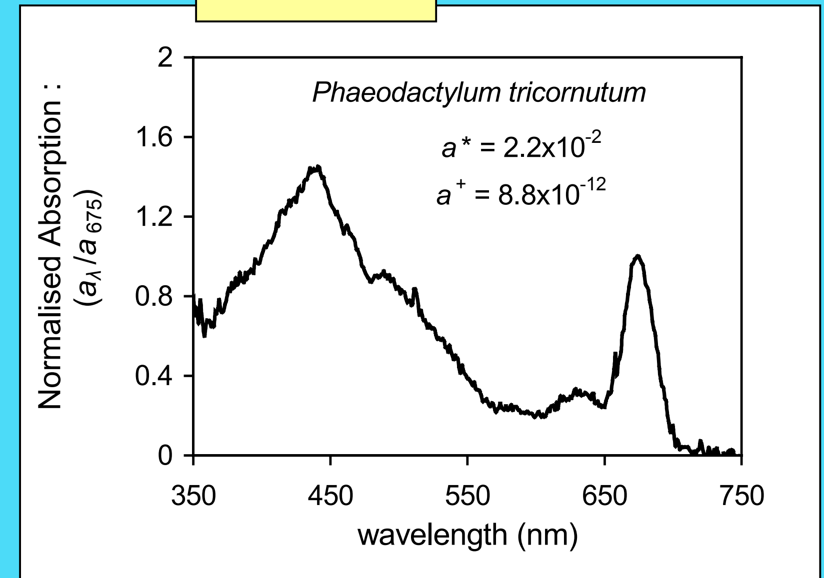
Note:

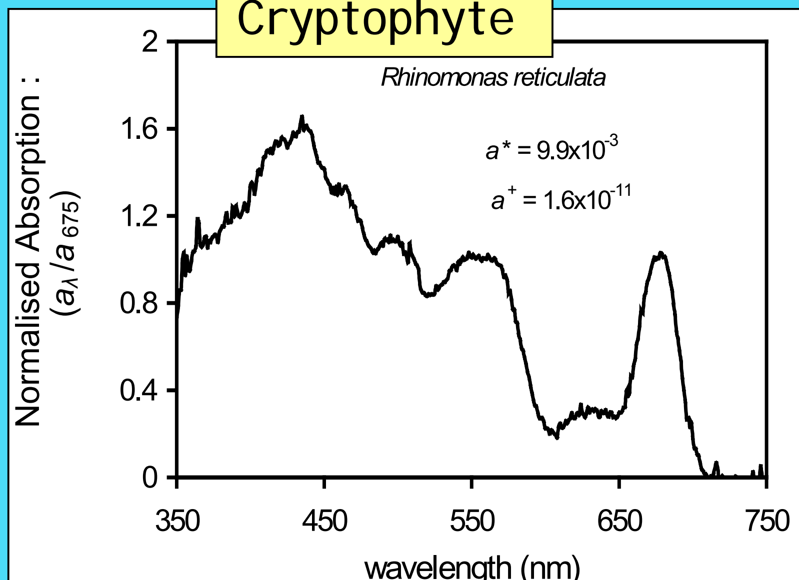
i. Absorption efficiency is subject to 'package effect', and is a function of cell size and intracellular pigment concentration.

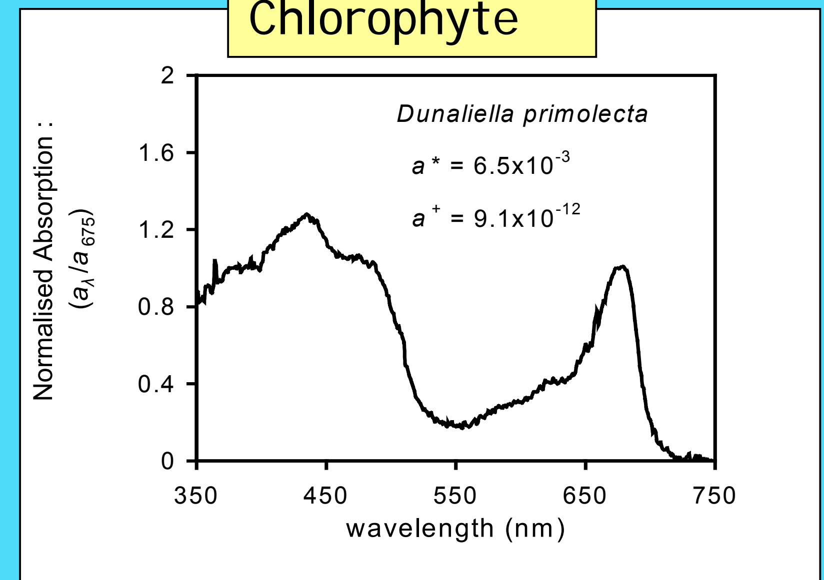
ii. Intracellular concentrations of all pigments vary in response to light exposure and nutrient status

Absorption spectra vary between taxa



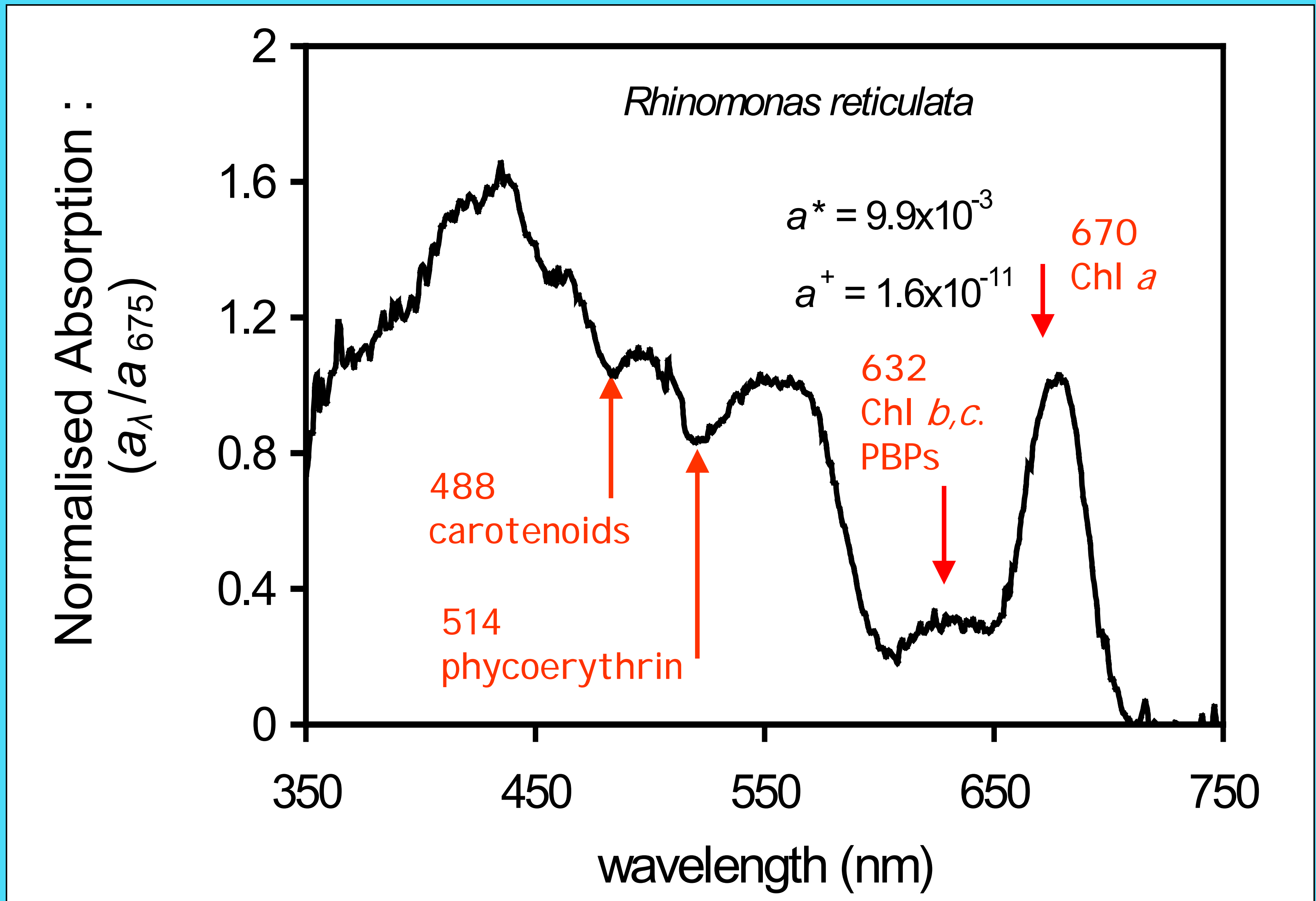






Laser wavelengths and pigment absorption bands.

(most chlorophyll fluorescence is not directly excited)



2. Fluorescence:

only from chlorophyll *a* associated with PSII antenna (peak at 685 nm) and phycoerythrin (peak at 580 nm)

Group	PSII antenna	Phycoerythrin
Chlorophytes	Chl a	-
Chrysophytes	Chl a	-
Cryptophytes	Chl a	+ (if N replete)
Pyrrhophytes	Chl a	-
Cyanobacteria	Chl a ? (phycobilisomes)	+ (in dim light)

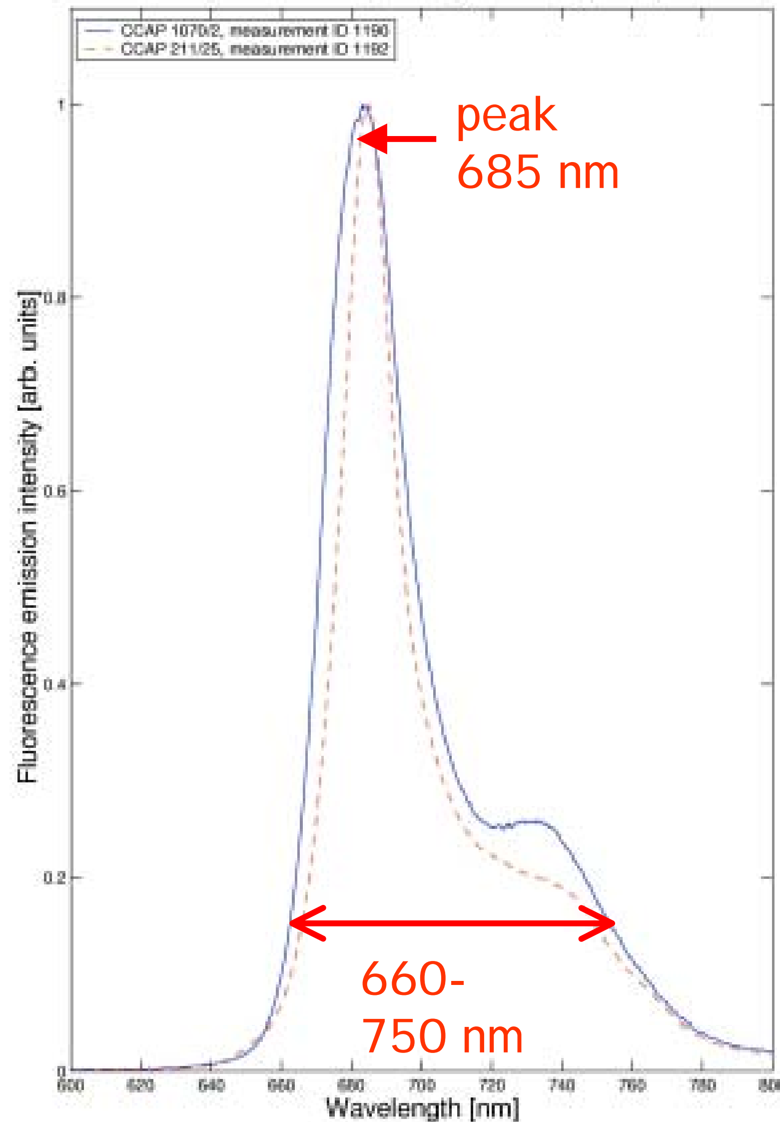
1. Phycoerythrin content of cryptophytes is strongly influenced by nitrogen status.

2. Electron transfer from RC to PQ pool takes 1-2 ms: no time for photochemistry. Yield varies from F_o to F_{max} depending on photon flux.

3. Chlorophyll fluorescence yield can be strongly reduced by non-photochemical quenching.

Spectral characteristics of Chl a fluorescence

(Room-temperature single cell measurements for a diatom and a chlorophyte.)



3. Scattering:

depends on size, shape and refractive index.

1. Mie theory gives exact solutions for spheres of uniform refractive index, and acceptable approximations in some other cases.

2. T-matrix algorithm gives scattering solutions for non-spheres of simple shape and structure.

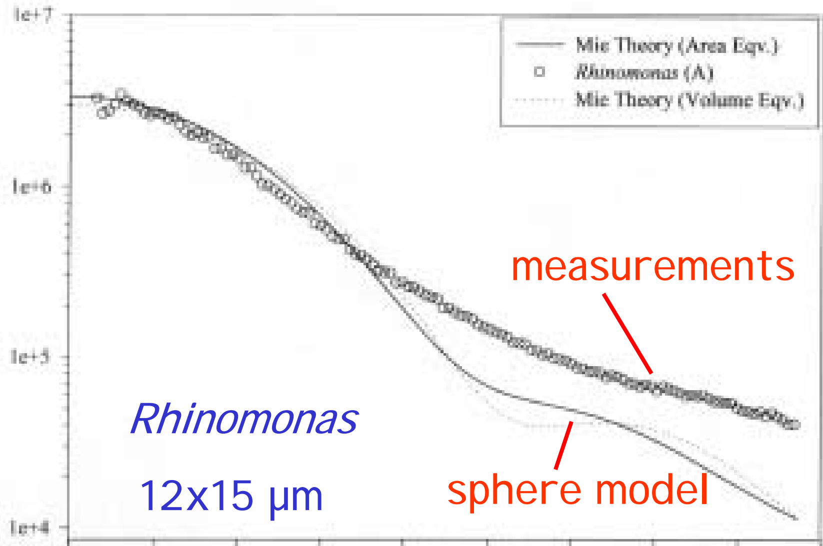
But:

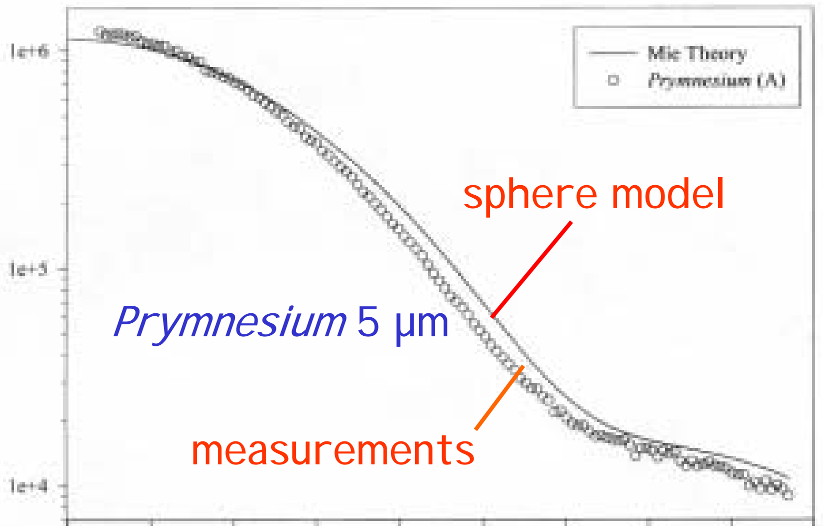
1. Real cell shapes can be difficult to describe mathematically.

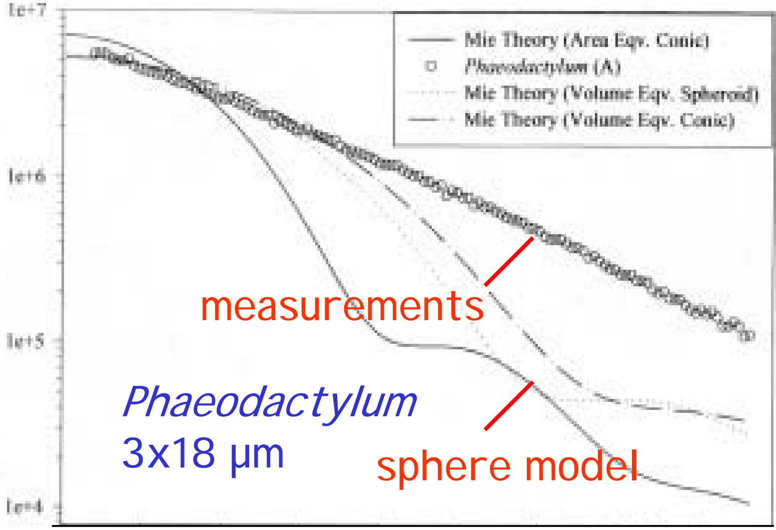
2. For cells of complicated structure, refractive index becomes a curve fitting parameter rather than a physical constant.

3. Signal magnitude is very sensitive to collection geometry, especially beam obscuration bars.

How well does Mie theory predict scattering from phytoplankton cells?





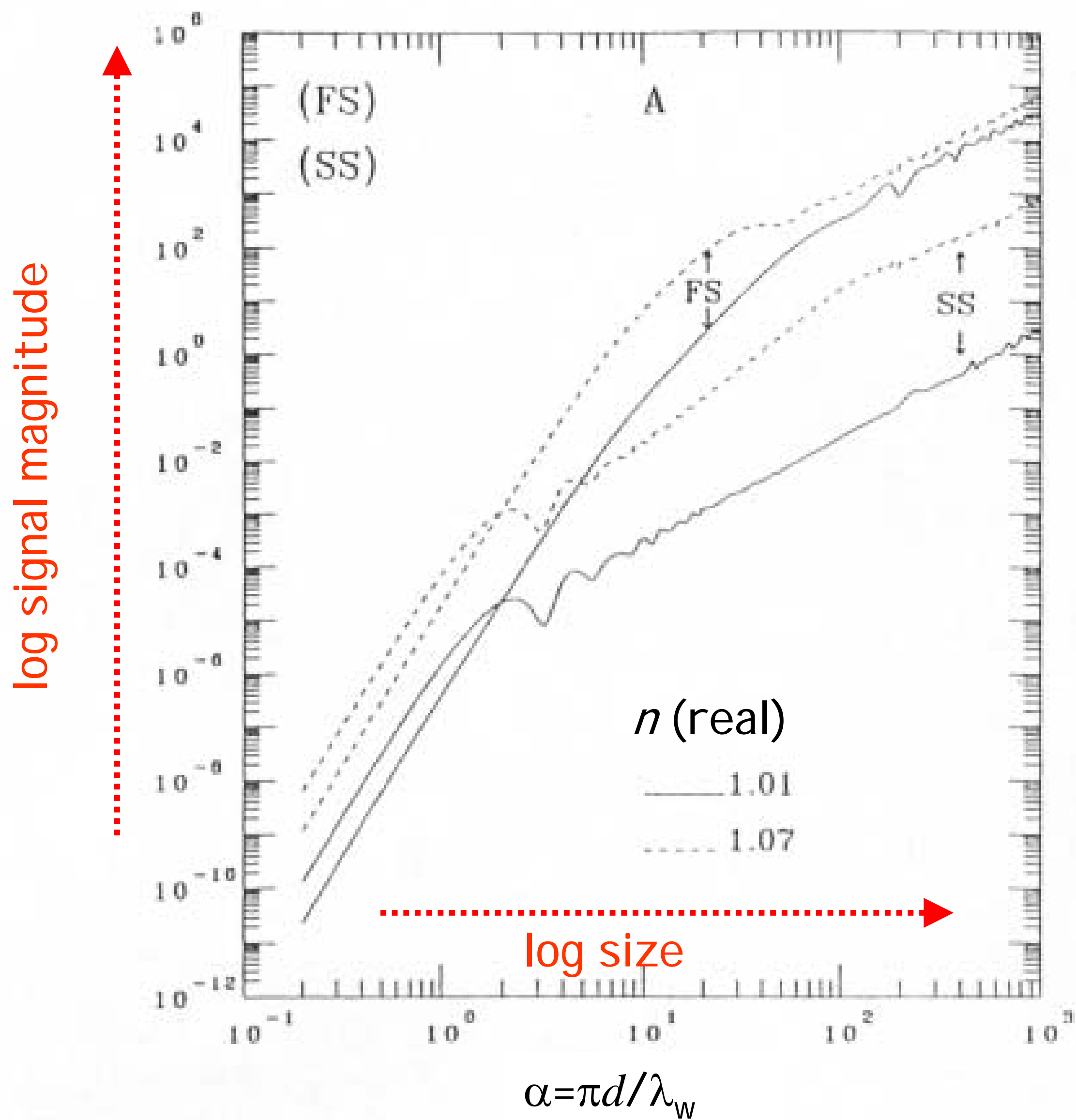


log intensity (arb. u.)

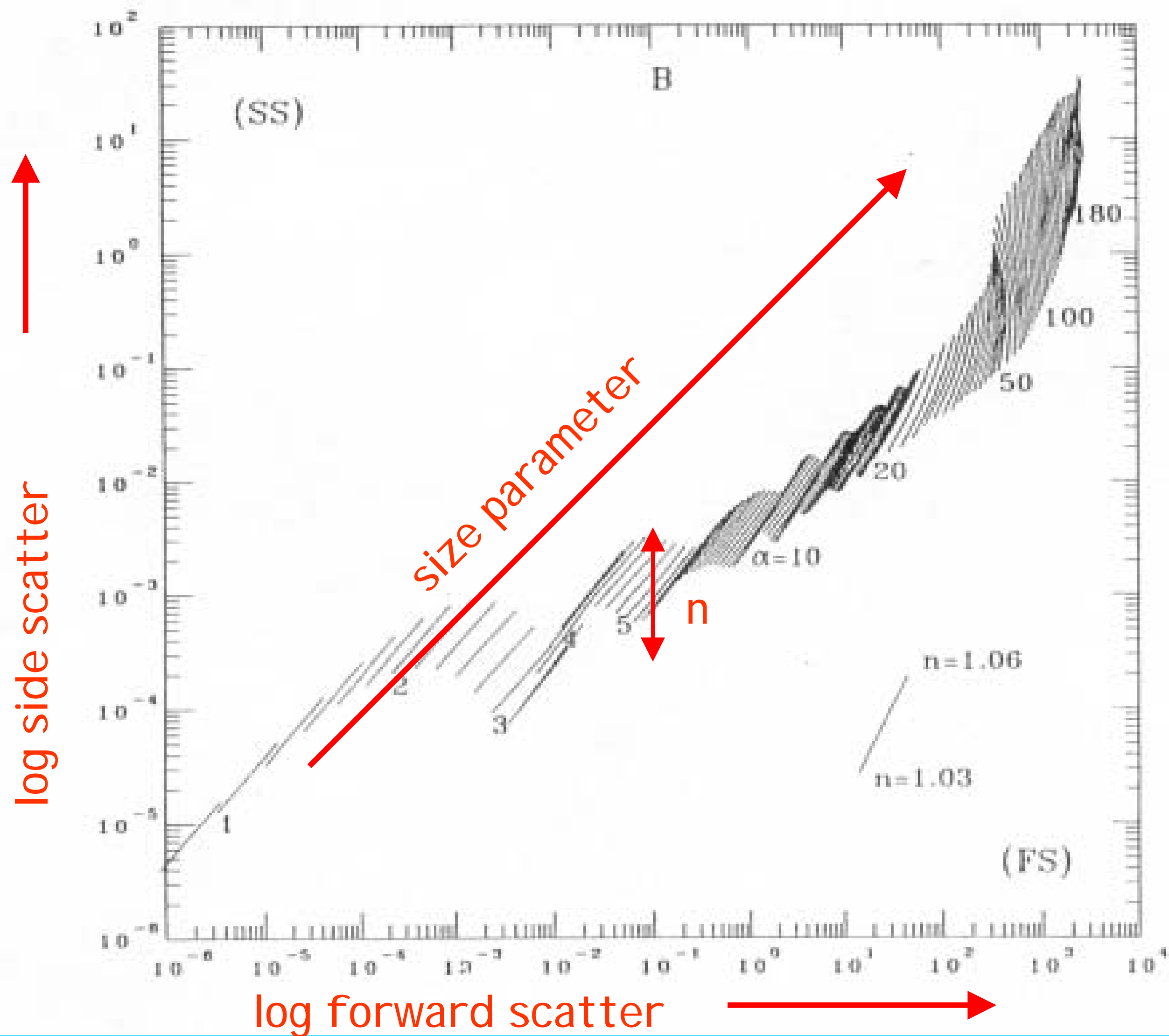
0 9

Angle (degrees)

Nevertheless, Mie theory can provide insights into the complexity of the problem of interpreting light scattering signals from a flow cytometer.



Mie calculation by Morel (1990) for forward scatter (2-18°) and side scatter (72-108°)



Mie calculation by Morel (1990) for forward scatter (2-18°) and side scatter (72-108°).

Crude summary of Morel's results

(note these apply strictly to a simple sphere illuminated by a 488 nm plane wave)

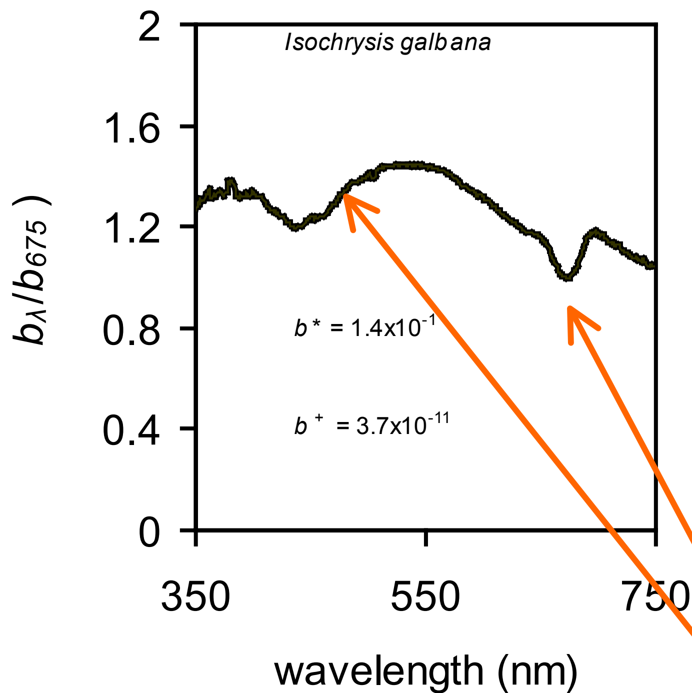
diameter (d, μm)	FS α	SS α	Notes
<2	d^6	d^6	FS and SS very sensitive to size
2-50	d^6->d^4	d^2	Details complex, d vs n ambiguity
>50	d^2	d^2	FS -> cross section FS/SS -> n

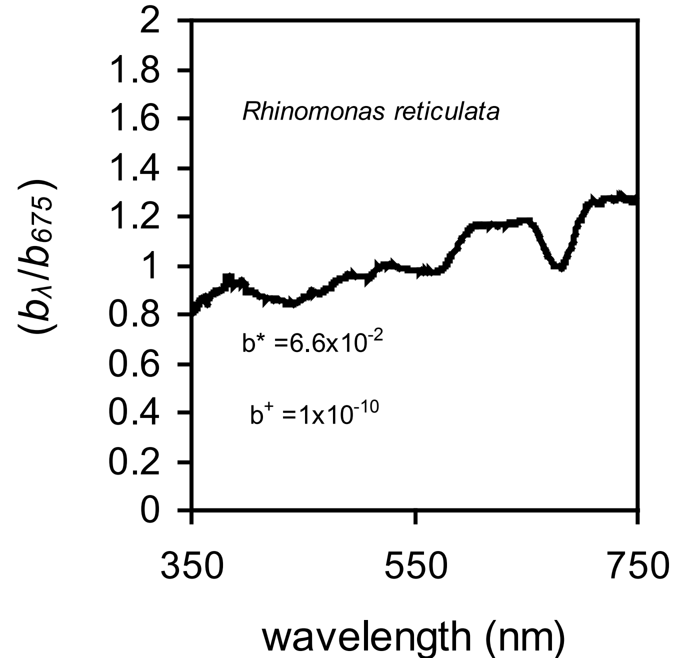
pico-plankton

nano-plankton

large cells and colonies

What about wavelength dependence of scattering?

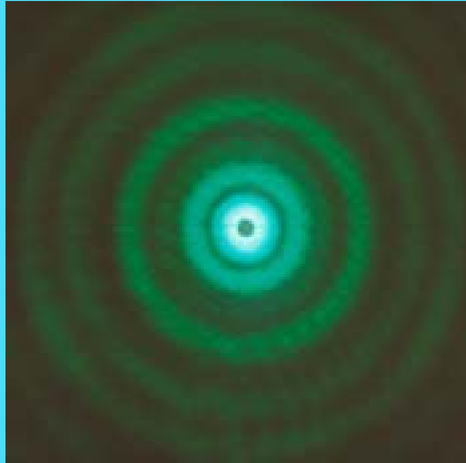




Note Kettler-Helmhotz influence of absorption bands on scattering efficiency.

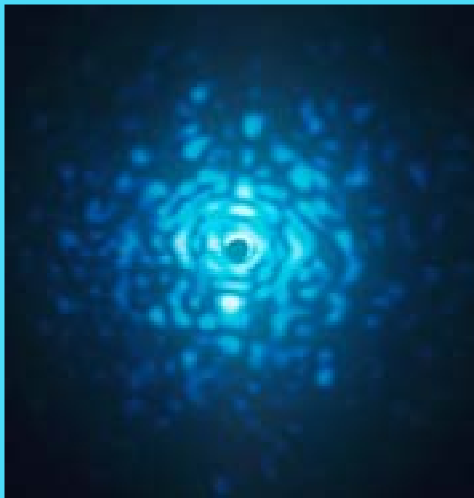
Is single-particle scattering really so complicated?

Single particle forward scattering patterns



latex bead

Anabaena



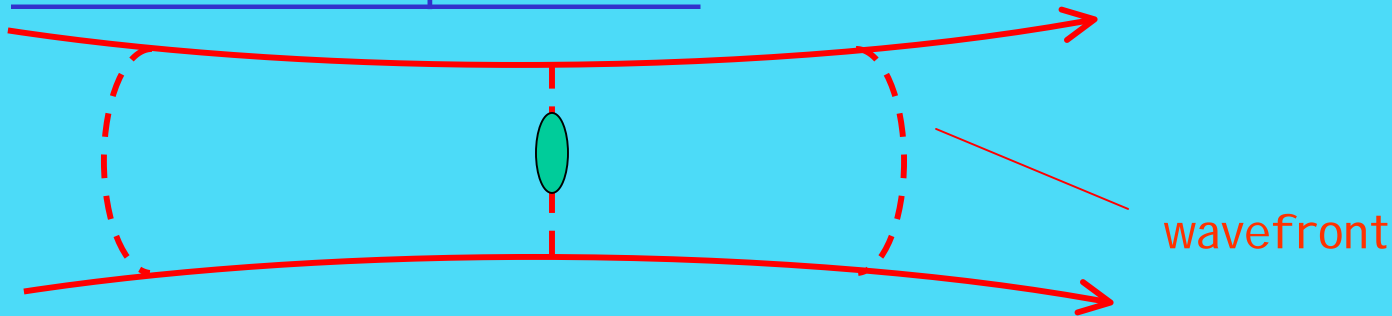
Pediastrum

Ceratium

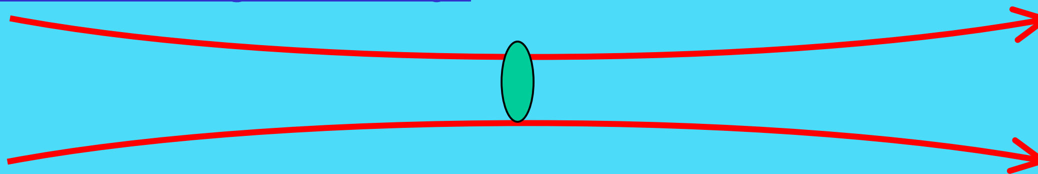
The importance of
instrument geometry:

flow cytometry signals
are strongly dependent on
the relative size of the
cell and the beam waist

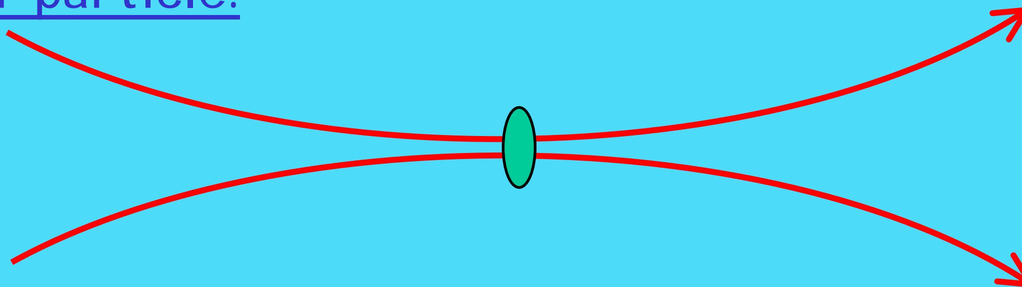
Case I: particle much smaller than beam. 'Classical' optical interaction with plane wave.



Case II: particle and beam of similar size. Result depends on exact geometry.

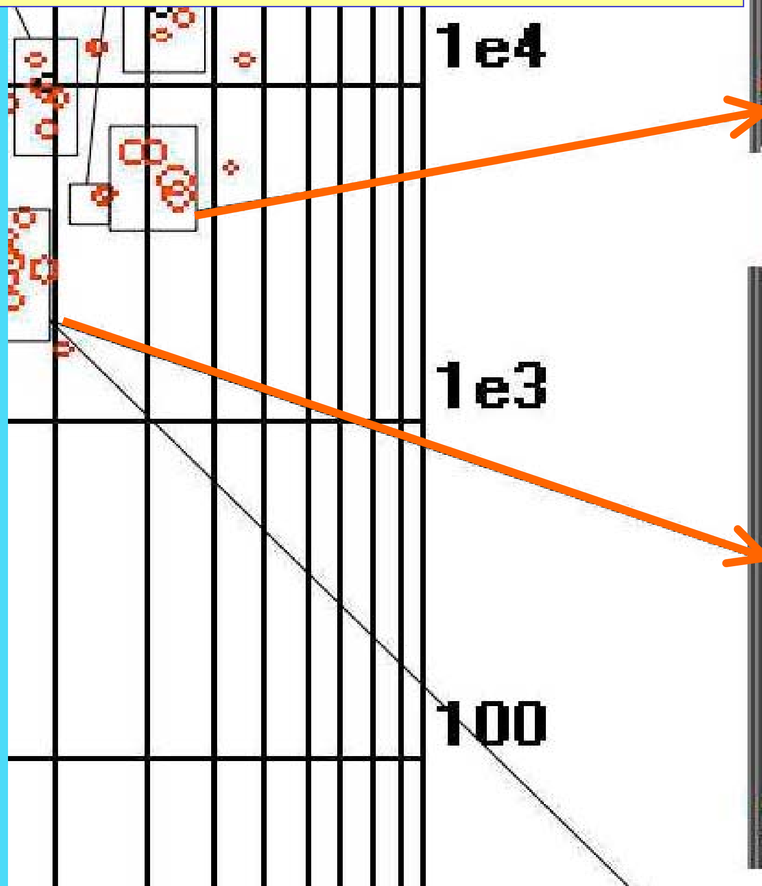


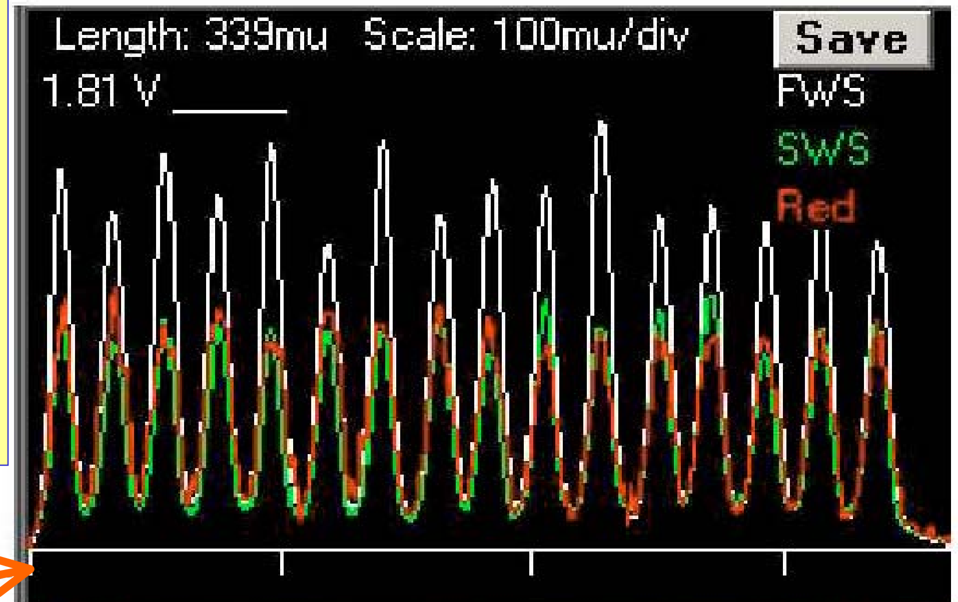
Case III: particle larger than beam waist. Optical scanning of particle.

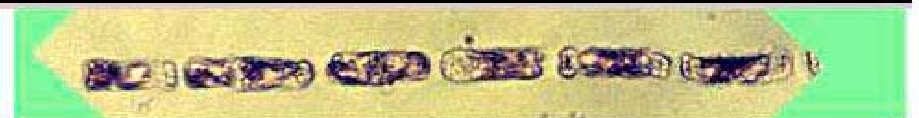


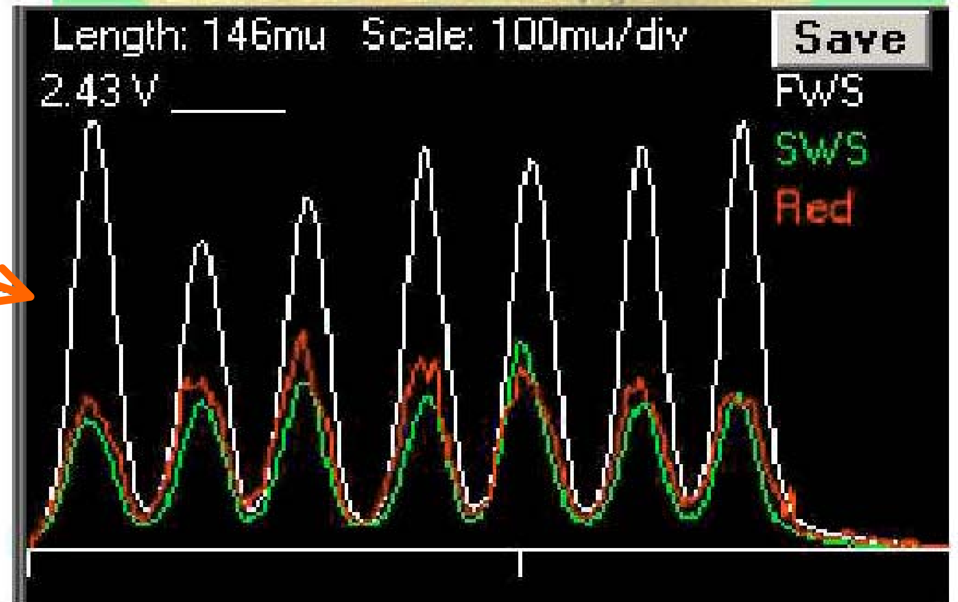
Example: pulse profiles measured with a 3 μm high beam waist:

Skeletonema costatum









From theory to practice.....

a low power submersible flow cytometer for use in an Autonomous Submersible Vehicle.

Design outline.

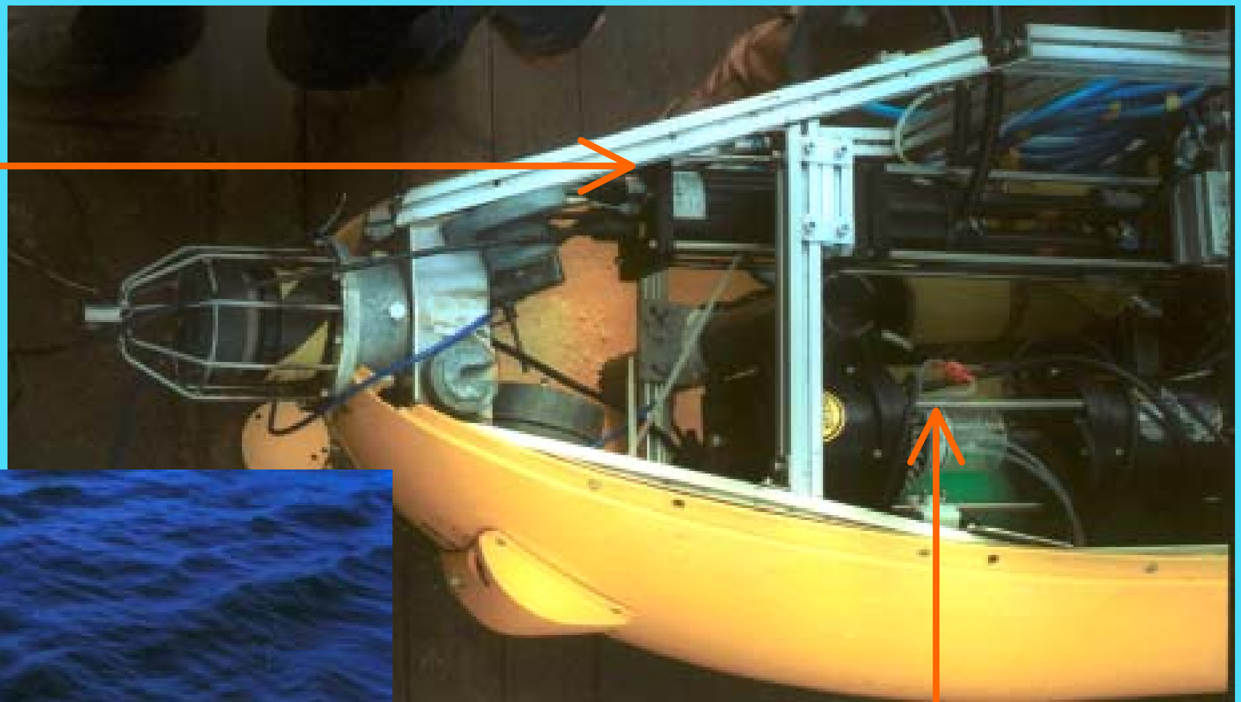
1. Use single red solid state laser operating at 670 nm (low power, good beam quality)

2. Measure chlorophyll fluorescence above 680 nm, forward scatter and side scatter.

3. Wide bore fluidics, efficient particle alignment, 3 μm x 1000μm beam

4. Full pulse digitisation (lengths and internal structure with ~ 3 μm sampling).

ac9+ attenuation and absorption spectrophotometer



Aquamonitor

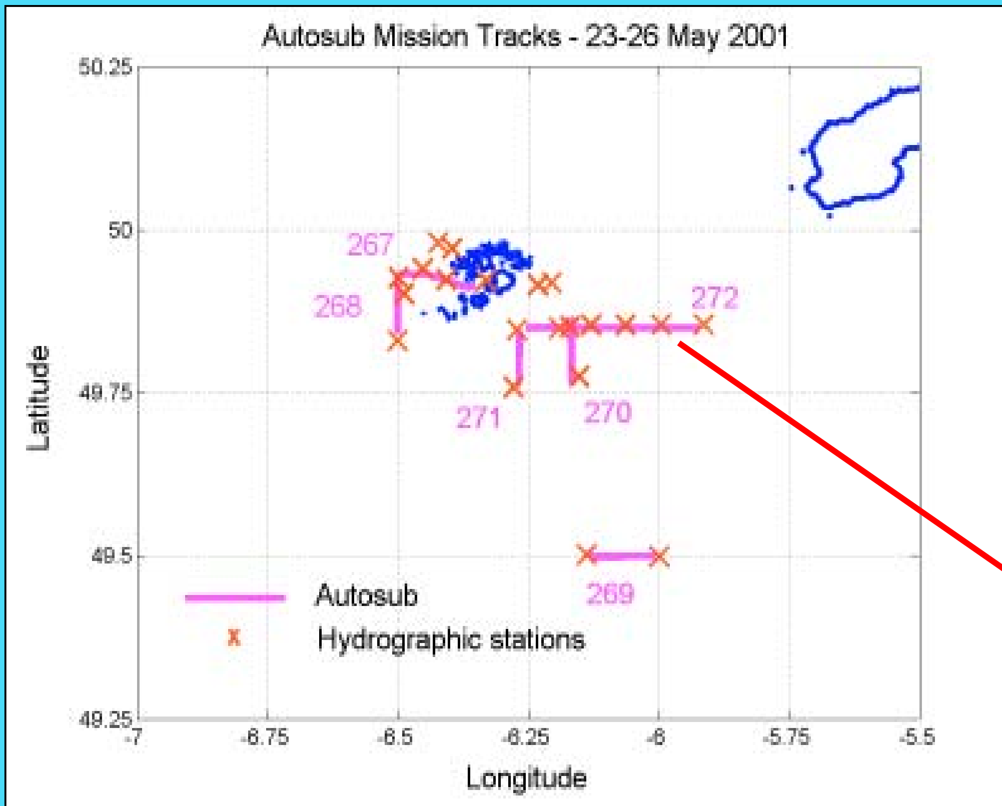
water sampler

in situ

flow cytometer

Optical instruments in Autosub

Transect of edge of bloom SE of Isles of Scilly, dominated by coccolithophores.



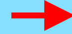
Autosub tracks and hydrographic stations

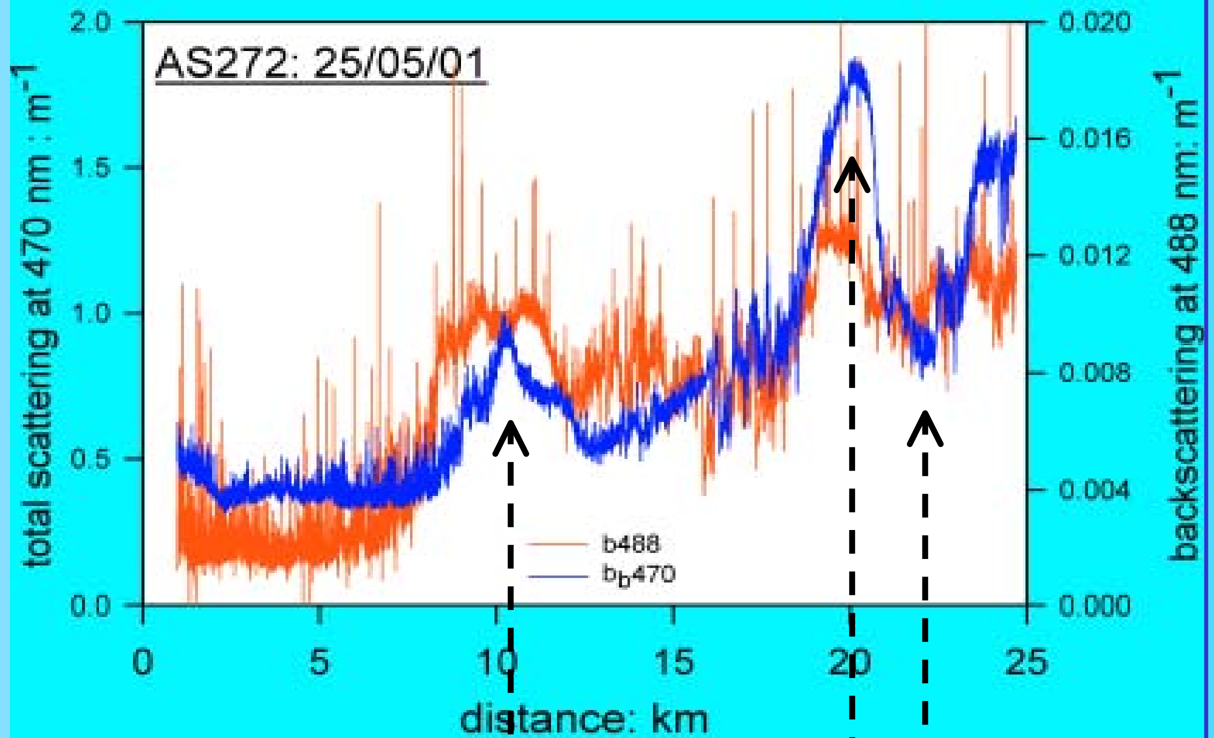
SeaWiFS RGB image

24 May 2001

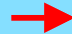
(Remote Sensing Unit, PML)

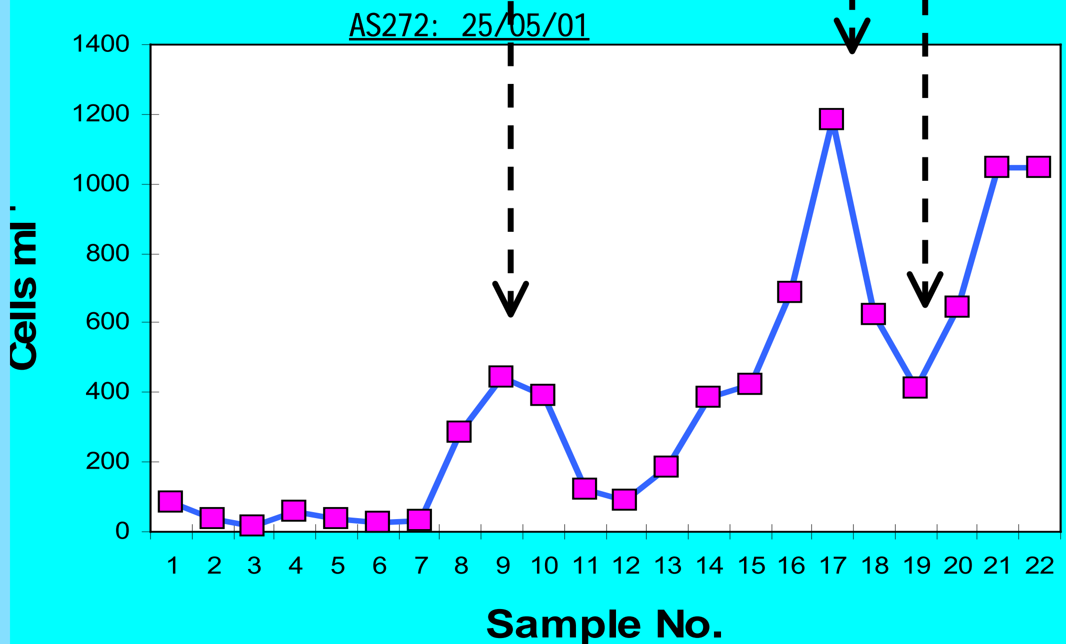
Ac9 scattering and Hydroscat backscattering transects from Autosub





Emiliana huxlei cell density measured under way by in situ flow cytometry correlates well with backscattering, less well with total scattering





Points for discussion:

1. Signals from flow cytometers depend on the interaction of the measurement geometry with individual cells and colonies *Measurements are instrument-specific.*

2. Absolute optical measurements require detailed knowledge of both instrument configuration and particle characteristics. *This is rarely feasible.*

3. The current strength of flow cytometry lies in particle discrimination and enumeration. *Supplements other measurements of phytoplankton optical properties, but is unlikely to replace them.*

4. *In situ* flow cytometry is now possible (Dubelaar et al., Olson et al.). *Will provide a valuable extension to optical capabilities of AUV's, moorings and sea bed installations.*

The End