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SHIP AND SATELLITE BIO-OPTICAL RESEARCH IN THE CALIFORNIA BIGHT

Technical Report for NASA Grant NSG 1641

SHIP AND SATELLITE BIO-OPTICAL RESEARCH IN THE CALIFORNIA BIGHT

by

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Abstract

The objective of this research was to increase the understanding of meso-scale biological patterns and processes in productive coastal waters by: studying the physical and biological processes leading to chlorophyll variability; investigating the ecological and evolutionary significance of this variability, and its relation to the prediction of fish recruitment and marine mammal distributions; and assessing seasonal primary productivity (using chlorophyll as an indication of phytoplankton biomass) for the entire Southern California Bight (SCB) region.

Complementary and contemporaneous ship and satellite (Nimbus 7-CZCS) bio-optical data from the Southern California Bight and surrounding waters were obtained and analyzed for this research. These data were also utilized for the development of multi-platform sampling strategies and the optimization of algorithms for the estimation of phytoplankton biomass and primary production from satellite imagery.
Introduction and General Objectives

The spatial and temporal variability of phytoplankton communities has been widely documented and an understanding of this variability is important for the quantitative predictive modeling of phytoplankton dynamics. In addition, the variability of phytoplankton communities is thought to hold the key to understanding the relative importance of physical and biological factors structuring the marine food web. Also, this variability is a critical component in the design and implementation of multi-platform sampling strategies necessary for the accurate assessment of phytoplankton in highly productive coastal and upwelling regions of the world's oceans. To date, the accurate assessment of phytoplankton abundance, the successful modeling of phytoplankton dynamics, and the predictive linkage of phytoplankton production to higher trophic levels has been limited by a lack of synoptic data and adequate sampling strategies. Our research utilizes concurrent ship and satellite sampling strategies to obtain these synoptic data for the optimization of phytoplankton biomass estimates.

The Southern California Bight (SCB) is an eutrophic oceanographic region which is distinguished from oligotrophic regions by: higher average plant and animal biomass with a quantitatively different food web; presumed higher phytoplankton growth rates with high regional primary productivity; a lower ratio of gross to net primary productivity with a consequent lower uncertainty in discrete shipboard productivity 14C measurements; at least an order of magnitude more spatial and temporal variability, both of phytoplankton biomass and productivity, with a consequent greater sampling uncertainty regarding estimated averages of phytoplankton abundance and population growth rates. This greater variability plus the general importance of eutrophic regions provided a strong motive for the development of ship
and satellite sampling strategies to study phytoplankton dynamics in this representative and important oceanographic region.

Results

During the course of this research contemporaneous ship and satellite data were obtained, catalogued and analyzed. The results of this research are described in detail in several journal articles and an S.I.O. Report. The following presents a synopsis of these articles and the first page of each article is included in the appendix of this report.


This note considers how chlorophyll, as estimated from Coastal Zone Color Scanner (Nimbus 7 satellite) data, is related to the chlorophyll concentration in the ocean water column and describes the importance of this satellite information for phytoplankton ecology.


Concurrent ship and Nimbus-7 CZCS satellite data were utilized to optimize a general chlorophyll algorithm and to calculate the chlorophyll concentration in the Southern California Bight region. This optimization provides regional images of chlorophyll concentration that are consistent with independent shipboard chlorophyll determination within 40%. A time series of chlorophyll images (believed to be the first such time series of chlorophyll images), covering a three week period and concurrent with two oceanographic cruises, is presented.
These data reveal complex and changing chlorophyll patterns and significant shifts in the mean chlorophyll concentration, and provide a synoptic perspective of a complex oceanographic region that is impractical to obtain from ships alone.


Chlorophyll images from the Nimbus-7 CZCS were used to examine the statistical properties of chlorophyll distribution in the Southern California Bight and to answer the question "how synoptic are shipboard measurements of chlorophyll based on a standard grid of sampling stations?" Further, an algorithm was developed for estimating primary production from CZCS chlorophyll values. Areal integrated chlorophyll agreed well between satellite and ship data at the same time of sampling. In addition, a time sequence of satellite images revealed changes in chlorophyll related to meso-scale ocean warming and which was associated with decreasing primary production over a two week period. Prediction of primary production from CZCS chlorophyll data is currently limited by high standard error of estimates associated with present algorithms and sampling strategies, but this work indicates that concurrent ship and satellite data can be utilized (and have the potential) to provide a more accurate assessment of oceanic primary productivity on a regional basis.


References 4, 5, and 6 (funded under other grants) discuss the biological characteristics of coral reefs and the differences in photoadaptation strategies between benthic and pelagic phytoplankton. This is an important step towards understanding how light is utilized by these two groupings of algae. Further, the research described in these references lays the groundwork for the remote sensing of coral reefs utilizing existing Landsat and CZCS data.


A bio-optical technique is presented which allows both the classification and optical modeling of natural waters. The spectral diffuse attenuation coefficient for irradiance (300 nm to 700 nm) has been related to two biological quantities; the total chlorophyll-like pigment concentration and the dissolved organic material (DOM). This model is a component model which augments our previous work in that it includes new data in the analysis, utilizes an improved analytic fit, extends into the UV region of the spectrum, and adds a DOM component. This model, which permits quantitative calculation of spectral irradiance at any point in the water column in a variety of biogeneric water types, facilitates predictive modeling and is directly useful for sensitivity analyses of both aircraft and satellite remote sensing applications.

This document presents fundamental terms describing the transfer of radiative energy in natural waters and relevant optical properties of this medium. The aim of this work is to recommend a standard terminology for underwater optics and optical remote sensing of the oceans.


Satellite imagery is now being used to estimate the near-surface chlorophyll concentration for large ocean areas. To assess the accuracy and precision of these remote sensing techniques, contemporaneous ship and satellite data for the determination of chlorophyll at sea, our analyses have led us to review the literature of fluorometry in order to re-examine the assumptions and approximations made when using this field technique. This report gives a summary of the general concepts of fluorometry, examines important assumptions, presents established fluorometer calibration techniques, and estimates the accuracy and precision of this methodology.

In addition to the above publications which have already been accepted, several other publications are in progress as a result of this research and some of these have been given as talks at American Geophysical Union and/or the American Society of Limnology and Oceanography meetings. However, the above articles (with complete abstracts given in the appendix) represent the work completed as of 31 January 1982.
Appendix

Publications supported wholly or in part by NASA grant NSG 1641 as of 31 January 1982. Please see attached pages.
Remote Sensing and Depth Distribution of Ocean Chlorophyll

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ABSTRACT: This note considers how chlorophyll, as estimated from Coastal Zone Color Scanner (Nimbus 7 satellite) data, is related to the chlorophyll concentration in the oceanic water column. In spite of some limitations, the remotely sensed chlorophyll concentration can be used as an index of the mean water column chlorophyll. Chlorophyll imagery provides synoptic measurements impractical to obtain from ships alone, and hence may be of considerable importance in phytoplankton ecology.

It has been demonstrated that data from the Coastal Zone Color Scanner (CZCS) on the Nimbus 7 satellite can be processed to provide quantitative chlorophyll maps of an oceanographic region within an accuracy of 0.5 log C (Morel and Prieur, 1977; Gordon et al., 1980; Hovis et al., 1980; Smith and Baker, submitted, 1981). This note discusses the issue of how chlorophyll, as estimated from CZCS imagery, is related to the water column chlorophyll concentration.

The problem is best explored in terms of optical attenuation lengths as distinct from geometrical depths. Gordon and McCluney (1975) defined, $Z_{90}$, as the depth of penetration of light above which 90% of the diffusely reflected irradiance (excluding specular reflectance) originates. They showed that for a homogeneous ocean

$$Z_{90} = K^{-1}$$  \hspace{1cm} (1)

where $K$ = diffuse attenuation coefficient for downwelling irradiance. More recently, it has been shown (Gordon and Clark, 1980), that for remote sensing purposes, the concentration of the constituent under consideration should be weighted by a factor

$$g(Z) = \exp \left\{ -2K \cdot Z \right\}$$  \hspace{1cm} (2)

Heuristically, this weighting factor can be viewed as being derived from the irradiance arriving at the surface having been attenuated by $\exp \left\{ -2K \cdot Z \right\}$ from the surface to the depth $Z$ and by the same factor on the return to the surface. Gordon and Clark (1980) conclude that the remotely sensed concentration of chlorophyll is given by

$$C_{\text{SAT}} = \frac{\int_0^{Z_a} C(Z) \cdot g(Z) \cdot dZ}{\int_0^{Z_a} g(Z) \cdot dZ}$$  \hspace{1cm} (3)

where $C(Z)$ is the concentration of chlorophyll as a function of depth.

When shipboard chlorophyll has been determined at only a few discrete depths in the water column, then $C_{\text{SAT}}$ may be approximated by the mean chlorophyll concentration to a depth of one attenuation length, $C_x$ (Smith and Baker, 1978a). The mean chlorophyll to the euphotic depth, $C_T$, is obtained from an integral of $C(Z)$ over 4.6 attenuation lengths (i.e. the 1% level). If, as is generally the case, $C(Z)$ is not too complex then $C_{\text{SAT}}$, $C_x$ and $C_T$ are highly correlated, as has been previously shown using data from 140 stations (Smith and Baker, 1978a). Thus $C_{\text{SAT}}$ can be used as an index of the mean chlorophyll concentration in the water column.

In order to illustrate this for a variety of water types, Fig. 1 and Table 1 show 3 chlorophyll profiles (Cullen and Eppley, 1961) ranging from productive coastal water to oligotrophic waters. It is useful to renormalize these profiles as shown in Fig. 2 where chlorophyll has been plotted as a function of attenuation lengths of photosynthetic available radiant energy (PAR). When plotted in this manner, a given percentage PAR level (the 1% euphotic depth for example) and/or the
Oceanic Chlorophyll Concentrations as Determined by Satellite (Nimbus-7 Coastal Zone Color Scanner)

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Abstract

Data gathered concurrently by ships and satellite (Nimbus-7 Coastal Zone Color Scanner) have been compared to optimize a general chlorophyll algorithm and to calculate chlorophyll concentrations in the Southern California Bight. This optimization provides regional images of chlorophyll concentration that are consistent with independent shipboard chlorophyll determinations within ±40%. Chlorophyll images from satellite passes on 27 February, 6 March and 11 March 1979 are presented along with shipboard measurements to reveal complex and changing chlorophyll patterns including a significant decline in the mean chlorophyll concentration during the 2 wk study. These data provide a synoptic view of a complex oceanographic region which is impractical to obtain from ships alone. It is shown how concurrent ship and satellite data can be used for the quantitative definition and statistical analysis of an oceanic habitat descriptor (chlorophyll) for the modeling of the marine environment.

Introduction

The Coastal Zone Color Scanner (CZCS) on the Nimbus-7 satellite is the only satellite sensor now in orbit for the purpose of assessing living marine resources (Gordon et al., 1980; Hovis et al., 1980). The CZCS has provided us with quantitative chlorophyll images of the entire Southern California Bight region. These images, confirmed by concurrent surface data, revealed complex changes in chlorophyll concentrations during a 2 wk period in late winter of 1979.

The Southern California Bight is a relatively productive coastal region, bounded on the north and east by the North American coastline from Point Conception, California to Cabo Colnett, Baja California, Mexico; and on the west by the California Current (Southern California Coastal Water Research Project [SCCWRP], 1973). The southeasterly sweep of the coastline below Point Conception and the island and bank system shoreward of the continental shelf break provide a topography that contains, and perhaps spawns, the Southern California Eddy. This consistent but not constant, large (>200 km Diam), cyclonic eddy (Osen, 1984) is the dominant pattern in a region characterized by admixture of several water types with considerable horizontal variability (Jones, 1971) and with episodic upwelling (Reid et al., 1958).

The biological implications of the variability and the upwelling in this region have been extensively studied for many years: California Cooperative Oceanic Fisheries Investigations (CalCOFI) (1963-1981), Cushing (1971), Southern California Coastal Water Research Project (SCCWRP) (1973), Eppley et al. (1975 and references therein). The upwelling and general circulation variability (Skogsberg, 1936; Bolin and Abbott, 1963; Smith, 1973) within the California Current System have a significant effect on the biota in the Southern California Bight. Further understanding of the biota in this complex and variable region may benefit significantly from synoptic data that is impractical to obtain from ships alone.

Recently, satellite data for the Southern California Bight have been used to complement surface measurements. Bernstein et al. (1977) utilized the very high resolution radiometer surface temperature data from the NOAA-3 satellite to investigate the formation of eddies in the California Current. Lasker et al. (in press) studied thermal imagery provided by the advanced, very high resolution radiometer aboard the NOAA-6 satellite to describe ocean processes related to the spawning of the Northern anchovy.
Correlation of Primary Production as Measured Aboard Ship in Southern California Coastal Waters and as Estimated from Satellite Chlorophyll Images

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Abstract

Chlorophyll images suitable for the regional analysis of the Southern California Bight were obtained by satellite (Nimbus-7 Coastal Zone Color Scanner) on 27 February, 6 March and 11 March 1979. An algorithm to convert satellite chlorophyll data to primary production has been developed. In addition, primary production was measured along with other biological and physical factors from ships cruising in the Southern California Bight during late February and early March 1979. There was fair agreement between shipboard measurements and satellite estimations; for example, primary production (mg carbon m⁻² d⁻¹) averaged 554 mg from shipboard measurement and 403 mg from satellite estimation when averaged for the entire Bight for the course of the study. The high standard error associated with the variance in productivity index used in our algorithm prevented more accurate conversion of chlorophyll concentrations into primary productivity. However, the synoptic data provided by concurrent ship and satellite measurements can be used to quantify, and perhaps reduce, sampling errors associated with ship sampling alone and to provide estimates of the accuracy of primary productivity on a regional basis.

Introduction

The California Current System in general and the Southern California Bight region in particular have been surveyed by research vessels for many years in the California Cooperative Oceanic Fisheries Investigations (CalCOFI) and more recently in the Southern California Bight Studies (SCBS). The region is characterized by admixtures of several water types (Reid et al., 1958; Emery, 1960; Cushing, 1971; Jones, 1971; Eppler et al., 1978; Smith and Baker, 1981). For studies of such complex regions, we have developed techniques which will provide synoptic data at higher frequencies than can be provided by ships alone.

Recently, satellite data have been used to complement surface measurements (Bernstein et al., 1977; Gordon et al., 1980; Esteller et al., in press) in the Southern California Bight. Three chlorophyll images of the Bight were taken by the Coastal Zone Color Scanner (CZCS: Hovis et al., 1980) on the Nimbus-7 satellite (Smith and Baker, 1981). These images are taken at a point of departure for the work presented here. The present work first quantitatively compares data from a grid of ship stations with synoptic satellite chlorophyll images. The chlorophyll data from these images is then utilized to estimate primary production for comparison with shipboard measurements and other empirical predictions of primary production.

Materials and Methods

Shipboard Measurements

Biological and optical data from the Southern California Bight were obtained from the R.V. "New Horizon" between 24 February and 12 March 1979 (stations shown in Fig. 1 of Smith and Baker, 1981) and from the R.V. "E.B. Scripps" (Cruise SCBS14) between 3 March and 8 March 1979 (present Fig. 1). The SCBS cruise was the 14th of a series that began in fall of 1974. The shipwork at 15 of these 16 stations included measurement of a depth profile of quantum scalar irradiance (i.e., photosynthetically available radiant energy, PAR) with the instrument described by Booth (1976) and a cast of 5-liter PVC Niskin bottles 6 in the euphotic zone (at depths corresponding to the 90, 37, 29, 12, 4 and 17 levels of PAR) and 2 selected greater
The reefs of the Florida Keys are widely known and have drawn the attention of scientists since the early 1800's. The Keys themselves are the fossil remains of Pleistocene reefs from North Key Largo south to Key West, (Hoffmeister and Multer, 1964). Their species composition can be seen in nearly every canal cut and rock quarry. Receiving less attention however, are the reefs that make up the present living chain of reef from Fowey Rocks south to the Dry Tortugas. These reefs are distributed in and along the outer edge of the shallow lagoon on the seaward side of the Keys. In all, there are probably over one hundred individual reefs, however there are less than 25 that could be considered to be more than patch reefs. The largest, most well-developed outer reefs presently are, in north-south order, Carysfort, Molasses, Looe Key, the Sambos, Long Key, Loggerhead Reef. Of these Looe Key, Carysfort, Molasses and Long Key are similar in that they have a rich coral community which exhibit species zonation patterns similar to other caribbean reefs (Goreau, 1959), and exhibit a morphology of considerable magnitude that appears to be the result of active coral growth. The two that appear most similar in morphology are Long Key, Dry Tortugas and Carysfort, Key Largo. Each is exposed to prevailing seas and has approximately the same depth range and
species composition. As a first comparison they appeared to be the two reefs that might reveal the most about the Florida Keys reefs as they exist at the northern and southern ends of the Keys.

This communication is the result of two parallel studies on the distribution of reef-building corals on Carysfort Reef, Key Largo and Long Key Reef, Dry Tortugas. The aim of the projects was to characterize the species composition of reef-building corals from both localities, establish base line data for future studies and through comparison, attempt to identify the impact of man on the reefs of the Key Largo area. Participants in the project include K. Lukas, J. Thompson, D. Girardin, K. Gordon, J. Halas, C. Richardson. Other contributing investigators include J. W. Japp and Jo Wheaton-Smith from the Department of National Resources, State of Florida, and G. Davis of the National Park Service. All assisted in phases of the field work and all are due grateful thanks. This research was supported by the Smithsonian Institution and Harbor Branch Foundation with logistical support in the Dry Tortugas provided by National Park Service, Everglades, Florida.
Depth-dependant photosadaptation

by Zooxanthellae of the reef coral Montastrea annularis

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Running Head: Photoadaptations by reef coral Zooxanthellae
Abstract

Zooxanthellae living in colonies of a Carribbean reef coral, Montastrea annularis, photoadapt to depth-dependent attenuation of submarine light. In shallow-living coral colonies, the zooxanthellae appear photoadapted to function at high light intensities and do poorly if transplanted to low light intensities; in contrast, zooxanthellae in deeper-living coral colonies can be damaged by high light intensities. The adaptation to decreasing light intensity and changing spectral quality appears to be accomplished by increasing the size of the photosynthetic unit (PSU), as opposed to increasing the number of PSU's/cell. Whole cell absorption increases with depth partially offsetting the loss of light energy due to depth dependent attenuation. Calculations of photosynthetically usable radiation, the light an alga is capable of absorbing in its own submarine habitat, suggest that the algae at different depths are optimizing rather than maximizing their ability to harvest submarine light energy.
Photoecology of pelagic and benthic marine algae

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ABSTRACT

The bio-optical state of ocean waters is dependent on the concentration of phytoplankton pigments. Changes in ocean color affect the chromatic adaptation of phytoplankton suggesting a feedback loop between phytoplankton abundance and photoadaptation. Such density dependent changes are not affected by benthic communities pointing to a fundamental difference in the photoecology of benthic and pelagic marine communities.

Measurements of underwater spectral irradiance and algal spectral absorbance suggest that the spectral signature of photosynthetically absorbed radiation (PUR) may be a community specific parameter which may be used to investigate the dynamics of solar radiation absorption by marine ecosystems.
Bio-optical classification and model of natural waters II

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This study is also a contribution to the research encouraged by the IAPSO Working Group on Optical Oceanography.
A bio-optical technique is presented which allows both the classification and optical modeling of natural waters. The spectral diffuse attenuation coefficient for irradiance \((700 \text{ nm})\) has been related to two biological quantities: the total chlorophyll-like pigment concentration and the dissolved organic material (DOM). This model is a component model which augments our previous work in that it includes new data in the analysis, utilizes an improved analytic fit, extends into the UV region of the spectrum, and adds a DOM component. This model permits quantitative calculation of spectral irradiance at any point in the water column in a variety of non-homogeneous water types, facilitating predictive modeling.
Terminology and Units in Optical Oceanography

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INTRODUCTION

This document presents fundamental terms describing the transfer of radiative energy in natural (sea and lake) waters and relevant optical properties of this medium. The table is divided into four parts:

1. Fundamental quantities
2. Radiant energy in natural waters
3. Material characteristics
4. Inherent optical properties of natural water.

The first aim of this document is to recommend a standard terminology for underwater optics which fully respects the rules of the "International Systems of Units". Consequently, quantities, symbols, and units in Part 1 are transcribed from the table ISO 31/VI (International Organization for Standardization, 1973) dealing with light and related electromagnetic radiations. Quantities in Part 3 are partly based on the same ISO Table and also on the more detailed nomenclature developed by the Commission Internationale de l'Eclairage (1957).

Parts 2 and 4 deal more specifically with quantities and parameters belonging to optical oceanography. Basically, this vocabulary derives from and complements a previous terminology recommended by the Committee on Radiant Energy in the Sea, set up by IAPO (International Association of Physical Oceanography) and published by its chairman, Pr. N.G. Jerlov, in 1964 (and thereafter in 1968 and 1976; as introductory material in his books, "Optical Oceanography" and "Marine Optics"). This IAPO standard terminology on under-water optics has introduced some specific new definitions which the recommendations contained in a preliminary work prepared by R.W. Freiser (1960) have proved very useful. The sym-
FLUOROMETRIC TECHNIQUES
FOR THE MEASUREMENT OF OCEANIC CHLOROPHYLL
IN THE SUPPORT OF REMOTE SENSING

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FLUOROMETRIC TECHNIQUES
FOR THE MEASUREMENT OF OCEANIC CHLOROPHYLL
IN THE SUPPORT OF REMOTE SENSING

1. INTRODUCTION
Satellite imagery is now being used to estimate the near-surface chlorophyll concentration for large ocean areas. To assess the accuracy and precision of these remote sensing techniques, contemporaneous ship and satellite data for the determination of oceanic chlorophyll concentrations have been collected. Since chlorophyll fluorometry is a widely used technique for the determination of chlorophyll at sea, our analyses have led us to review the literature of fluorometry in order to re-examine the assumptions and approximations made when using this field technique.

This report gives a summary of the general concepts of fluorometry, examines important assumptions, presents an outline of an established fluorometer calibration technique, and estimates the accuracy and precision of this methodology. Included are field observations of the discrete chlorophyll fluorescence ratio, of the variability of discrete chlorophyll determinations using various particle collecting filters, and of diurnal fluorescence ratio comparisons.

The launch of the Nimbus-7 satellite in October 1978 carrying the Coastal Zone Color Scanner (CZCS) has made possible optical measurements of the oceans from space. Algorithms are presently being developed to relate these optical spectral measurements to the chlorophyll present in the water. Our purpose is to outline our own consistent methodology of along track shipboard fluorescence for the concurrent comparison with satellite derived values of chlorophyll. This report is not intended as a definitive statement on fluorometric techniques, but rather a working document describing our field techniques. Hence, although the theory is general, the specifics apply to the Turner and Turner Designs fluorometers.

2. FLUOROMETRY
Fluorometry, the measurement and use of fluorescence, is a technique of quantitative chemical analysis ideally suited to field use. This technique of optical measurement is inherently sensitive, offers specificity, and is versatile, simple and relatively inexpensive. As a result, fluorometry has found a wide range of applications in the study of natural waters including: chlorophyll and phaeopigment analysis, nephelometry, detection of fluorescent pollutants as well as a host of analyses which make use of fluorescent dyes as tracers.

Fluorometric techniques have the further advantage that several field proven instruments are commercially available to the potential user. These instruments are distinguished not only by their reliability but also by the thoroughness of the accompanying operational instructions and supporting scientific reference material. Consequently, it is a relatively efficient process, starting with little or no experience, to put fluorometric techniques into use. On the other hand, there are a number of approximations and possible variations in methodology which can reduce the potential accuracy and precision of fluorometric techniques. Our objective is to choose a methodology that optimizes the accuracy and precision of determining oceanic chlorophyll concentrations from shipboard, especially for the purpose of comparison with concurrent satellite imagery.

CONCEPTS
Absorption and "instantaneous" re-emission of radiant energy from a molecule or atom accompanied by a change in wavelength as well as direction, is known as fluorescence. When a quantum of light is absorbed by a molecule, the molecule is raised to an excited state. There are a variety of ways the excited molecule can manifest or dissipate this energy. First, if the energy of the absorbed quanta exceeds the energy of the chemical bonds that hold the molecule together, the molecule may be torn apart. This is called photolysis. Second, if an excited state is produced, the molecule is in an unstable condition and can achieve a more stable state by converting this excitation to vibrational energy, which in turn is dissipated as heat to the surroundings. Third, an excited molecule can cause chemical transformation to occur. An important example of this is photosynthesis.

In addition to these "radiationless" processes there are a number of light-scattering processes in which an incident photon at one frequency is absorbed resulting in re-emission at a different (lower) frequency. When the absorption and re-emission occurs as a single transition, the phenomenon is called the Raman effect (Jenkins and White, 1957). The intensity of light scattered in the Raman effect is much lower than that of fluorescence.