In situ monitoring of ocean chlorophyll via laser-induced fluorescence backscattering through an optical fiber

Timothy J. Cowles, James N. Moum, Russell A. Desiderio, and Stanley M. Angel

The first seagoing test of a prototype laser/fiber-optic system for *in situ* detection of ocean chlorophyll fluorescence is described. Radiation at 488 nm originating from a shipboard argon laser was transmitted through 20 of 200-μm core optical fiber to the distal tip mounted on the microstructure profiler, the Rapid Sampling Vertical Profiler. The backscattered fluorescence emission signal was collected through the same fiber and processed on board ship. A series of measurements indicated that (1) successful isolation of ship-induced vibrations could be achieved using our optical bench framework to maintain optical alignments; (2) ambient chlorophyll concentrations could be detected *in situ*; (3) a Raman scattering signal from water could also be detected and should provide an internal standard against which chlorophyll fluorescence may be calibrated.

I. Introduction

An understanding of processes which control small-scale distributions of planktonic organisms has long been an objective of biological oceanographers.[1] Many biological and chemical interactions between organisms and their environment occur within spatial scales defined, at one extreme, by the centimeters or meters through which organisms swim, and at the other extreme, by the detection of predator or prey over a few millimeters to centimeters. Biological and chemical processes, and the observed distributions within the upper mixed layer, are constrained to a large degree by physical processes which operate over small spatial scales.[2]

The importance of the microscales of physical properties in the ocean has been recognized by physical oceanographers for quite some time. Scales of the order of 1 m and smaller are directly responsible for the vertical transport of mass (and hence all passive constituents such as nutrients and many phytoplankton) in the ocean. It is necessary to determine the nature of the small-scale physics to incorporate their effects (as subgrid scale processes) into models of the larger-scale circulation. To accomplish this, considerable effort has been spent on developing seagoing instrumentation to observe the smallest scales of fluctuations of temperature, conductivity, and velocity. As a result of the intensive effort put into instrument development, the behavior of the ocean (especially in response to surface forcing) has been revealed in new (and often unforeseen) ways. Recent reviews of the advances made in understanding the small-scale physics of the ocean are presented by Caldwell[3,4] and Gregg.[5]

Vertical distribution patterns of phytoplankton fluorescence have been examined extensively over the past 15 years using CTD-based *in situ* fluorometers.[6–8] (A CTD is a standard oceanographic instrument used to determine conductivity, temperature, and depth.) Because these measurements are limited by ship motion to 1–2 m vertical resolution, we have learned little about the spatial variability in phytoplankton abundance over scales of a few centimeters or less, which are relevant to the biological processes of food detection and consumption.[9–12] Because of the limited sampling capabilities to date, we have also learned very little of the biological response to rapid changes in the upper mixed layer induced by surface forcing. Recent data on zooplankton feeding behavior indicate that variations in phytoplankton cellular composition can markedly influence ingestion rates.[13] In addition, discrimination between individual algal cells by copepods may be mediated by chemical stimuli from the cells which are detectable over distances of a few millimeters or less.[13] We, therefore, require additional information about the distribution and physiological condition of algal cells over spatial scales of a few centimeters to understand the feeding responses of

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planktonic organisms as well as the response of algal cells to variations in the vertical light field, to variations in the nutrient gradient at the base of the thermocline, and to variations in the vertical mixing of mass and nutrients by turbulent processes. Only through directly coupling biological measurements with physical measurements can we begin to investigate the interactions between physical and biological processes on the relevant space and time scales.[14,15]

Considerable interest has developed during the past decade in applications of remote sensing of chemical signals using optical fiber detection systems.[16,17] These applications range from detection of contaminants in groundwater[18,19] and intravenous detection of physiological parameters[20] to detection of algal fluorescence in aquatic systems.[21,22] We have recently developed a prototype laser/fiber-optic system for determining ocean chlorophyll fluorescence, which is mated to a freely falling microstructure profiler [the Rapid Sampling Vertical Profiler (RSVP)[23]]. In this paper we describe the first field test of the protype laser/fiber-optic fluorometer. Our principal objectives in this development program are:

(1) to develop an oceanographic application for remote sensing of fluorescence using fiber-optic technology;

(2) to obtain coherence in measurement scales between physical processes and biological/chemical processes in the upper ocean;

(3) to define the smallest scales of variability of the chlorophyll fluorescence signal in the ocean.

Preliminary field tests were conducted off the coast of Oregon from the R/V Wecoma on 24 Jan. 1988. Purposes of the cruise were to test the integrity of the optical system in shipboard conditions and to attempt to measure ambient phytoplankton pigment levels with the prototype system. We found that

(1) the fine optical alignment necessary for successful measurements could be maintained using our specially designed vibration-isolation optical bench frame;

(2) we could detect ambient chlorophyll concentrations *in situ* using a simple fiber optic arrangement on the RSVP;

(3) we could detect the Raman signal from water, which should provide an internal standard against which to calibrate chlorophyll fluorescence.

II. Experimental Arrangement

The prototype optical system was designed to monitor laser-induced fluorescence backscattering from seawater through an optical fiber (Fig. 1). An air-cooled argon laser (Continental Laser, Series 1000) was operated at a wavelength of 488 nm at 90-mW power; fluorescence excitation intensity was varied through the use of neutral density filters. An interference filter was used to block argon plasma lines. The laser beam was directed through a dichroic beam splitter which transmitted radiation of wavelengths shorter than 520 nm and reflected radiation of wavelengths >520 nm. A microscope objective (10 × 0.25 N.A.) was

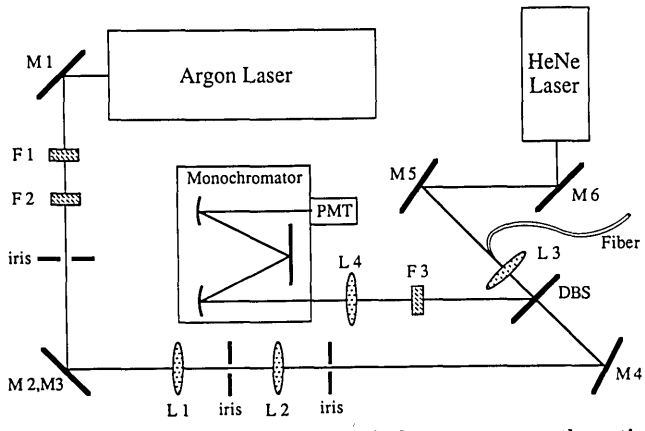

Fig. 1. Shipboard arrangement of optical components on the optical bench. *M*1–6, mirrors; *F*1, neutral density filters; *F*2, 488-nm bandpass interference filter; *M*2, *M*3, periscope; *L*1, *L*2, ×2 beam expanding telescope; DBS, dichroic beam splitter; *F*3, long-pass 530-nm interference filter; *L*4, achromatic lens; *L*3, 0.25-N.A. microscope objective. The He–Ne laser was used in the alignment procedure.

used to focus the laser beam onto the proximal tip of an optical fiber (Diaguide, 200-μm core, silica core and cladding, 0.2 N.A. 20 m long). Both tips of the fiber were polished using 0.3-μm lapping film. The laser light emanated from the distal end of the fiber in a cone determined by the numerical aperture of the fiber and the refractive index of water. Backscattered emission (fluorescence and Raman) from molecules in the sample volume defined by this cone was collected by the fiber and thereby guided back to the microscope objective, whereupon it was collimated. The dichroic beam splitter reflected the long-wavelength component of the collimated emission, which was focused into a 0.2-m monochromator (Photon Technology 01-001) using an achromatic lens. The slits were set at 400 μm to accommodate the magnification of the image of the fiber tip (the *f*/No. of the fiber was *f*/2.5, that of the monochromator effectively *f*/5.). It was necessary to use a long-pass interference filter to provide further discrimination against 488-nm radiation. The analog output of a photomultiplier tube with an extended red photocathode response (Hamamatsu R928) was digitized through a data acquisition interface in a microcomputer. The grating drive was software-controlled so that scan rates and lengths could be specified by the operator.

A He–Ne alignment laser was used to position coarsely the collection optics (microscope objective, dichroic beam splitter, and achromatic lens) and the laser beam steering optics. Final orientation of the collection optics was accomplished by launching the alignment laser beam into the distal tip of the fiber and monitoring the propagation of the red light out the proximal tip of the fiber through the optical train to the exit slit of the monochromator. Launching of the argon laser beam is noncritical because of the large core size of the fiber; it was checked by inserting the distal tip of the fiber into a power meter. Launch efficiency was estimated at 70% after consideration of reflection,

scattering, and absorption losses due to the elements of the optical train of the argon laser beam.

The shipboard optical system was mounted on an optical breadboard [Newport, 0.9 × 1.2m × 10 cm (3 × 4ft. × 4in.)] suspended from a rigid framework with a network of elastic shock cord; the framework was in turn mounted to the deck in the ship's dry lab with shock mounts. This vibration–damping system effectively eliminated high frequency ship vibrations. Although low frequency oscillations were passed by the system, accelerations were small enough so as not to affect optical alignments.

In the initial experiment described in this paper, the distal tip of the optical fiber was suspended in a 250-mliter beaker containing algal cells from a culture of the diatom *Thalassiosira weissflogii*. In subsequent shipboard tests, beakers were also used for the surface seawater and distilled water samples.

In situ measurements were recorded by mounting the distal tip of the optical fiber on the nose cone of the RSVP[23] and suspending it by hand at three depths within the upper mixed layer. The RSVP was designed to obtain rapid vertical profiles of temperature, conductivity, and velocity shear on scales of centimeters. Present capabilities allow up to 10 profiles/h to several hundred meters depth over time intervals of up to several weeks.[24] The instrument can be deployed from a moving ship as well as on station, permitting rapid areal surveys. Our ultimate objective is to deploy the RSVP in rapid profiling mode using the laser/fiber-optic fluorometer described here.

Surface samples of seawater were collected for testing. Calibration samples for chlorophyll and phaeophytin pigments were taken from surface water at the time of each experiment with the laser/fiber-optic system. Replicate samples (140 mliters) of seawater were filtered through GF/F filters and filters frozen for later pigment analysis by standard methods.[25]

III. Results

The initial shipboard tests were checks for vibration and ship motion on the output of the optical system. We maintained ~0.5 mW of laser power at the tip of the fiber and monitored the fluorescence spectrum of a stirred concentrated culture of the diatom *Thalassiosira weissflogii* by scanning the emission from 670 to 700 nm once a minute. We observed a gradual 15% decrease in fluorescence signal during 1.5 h of steaming at 12 knots in moderate seas. We attribute this decrease to sample degradation and/or light adaptation by the plankton. We observed no indication of signal dropouts which would have been the result of optical misalignment.

We recorded the emission spectrum of a sample of surface seawater on 488-nm excitation during shipboard laboratory tests. As a control, emission spectra of distilled water were obtained just prior to and immediately following the test with the seawater sample. This was done to eliminate the possibility that plankton were adhering to the fiber-optic tip. The emission spectrum from the seawater sample contained both a water Raman signal centered at 585 nm and a chlorophyll pigment peak centered at 685 nm (Fig. 2, *A*), while the distilled water spectrum displayed only the water Raman signal (Fig. 2, *B*). Subtraction of the distilled water spectrum from the sample spectrum provided the actual fluorescence spectrum from the pigments in the algae (Fig. 2, *C*).

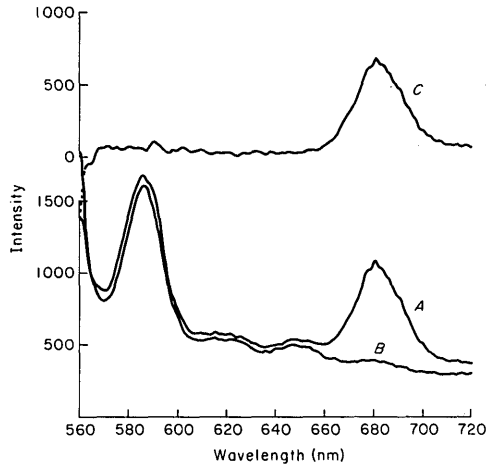

Fig. 2. Fluorescence spectra obtained with 488-nm Ar laser excitation through 20 m of 200-μm diam Diaguide fiber-optic cable of *A*, surface seawater; *B*, distilled water; and *C*, the difference spectrum of surface seawater minus distilled water. Note the water Raman signal centered at 585 nm and the strong chlorophyll fluorescence peak at 685nm. (Surface seawater chlorophyll pigment concentration was 0.98 μg liter^{-1}.)

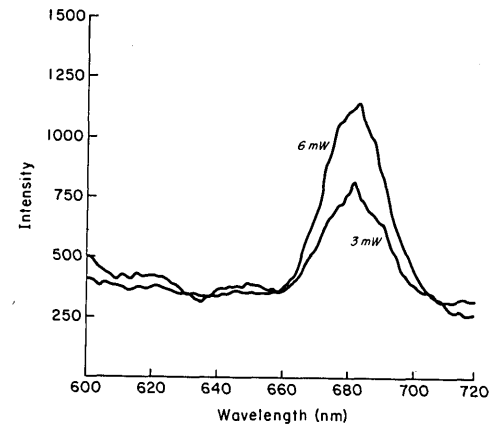

Fig. 3. Comparison of fluorescence spectra at 6- and 3-mW laser power at the tip. Integrated values between 670 and 705 nm were 63,085 and 33,725, respectively.

We adjusted the output power of the Ar laser through the optical fiber to optimize the fluorescence detection of algae in the surface seawater sample. We found that doubling laser power out the tip of the fiber from 3 to 6 mW resulted in a doubling of fluorescence output from the illuminated algae (Fig. 3), indicating that our detector was not saturating. We maintained this power level for the remaining experiments.

The optical fiber was then attached to the RSVP with the distal tip oriented vertically downward (Fig. 4) and the instrument deployed over the side of the

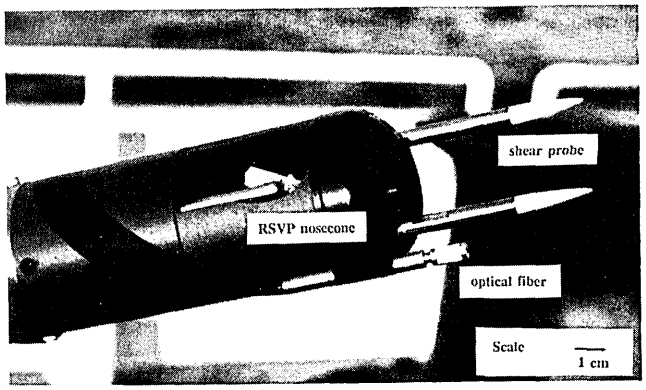

Fig. 4. Placement of the fiber-optic tip mounted on the outer diameter of the RSVP sensor nose cone. The two white-tipped shear probes are 0.64 cm in diameter. A thermistor and white conductivity cell are located in a flowthrough passageway ~6.4 cm to the left of the fiber tip.

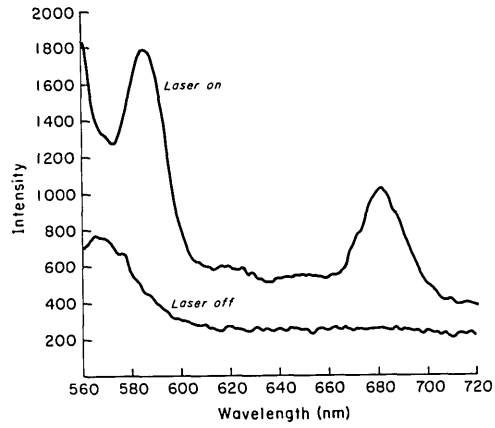

Fig. 5. Fluorescence spectrum from 9-m depth obtained with the laser ON compared with the upward radiance spectrum collected with the laser OFF.

vessel at depths of 1, 5, and 9 ms. At each depth, a fluorescence spectrum with 488-nm excitation was obtained (Fig. 5), followed by an upwelling radiance spectrum taken in the absence of laser radiation. The upwelling radiance spectrum was subtracted from the fluorescence spectrum at each depth, so that the spectra in Fig. 6 have the influence of ambient light removed. Concurrent measurements of temperature, conductivity, and pressure from sensors on the RSVP were made. Table I provides the mean physical properties at each depth. Chlorophyll calibration samples were obtained from the surface water, and the concentration is listed in Table I. Time series (80 s) of physical measurements at each depth revealed considerable variability, as illustrated for the 9-m series in Fig. 7. A maximum depth variation of 30–40 cm occurred due to ship motion, but the temperature and salinity variations observed near the 20-, 40-, and 70-s time points are uncorrelated with depth variations. Each series was separated by 15–30 min, and hence these do not represent a vertical profile. Rather, the apparently denser surface water is due to horizontal variability, as illustrated in Fig. 7.

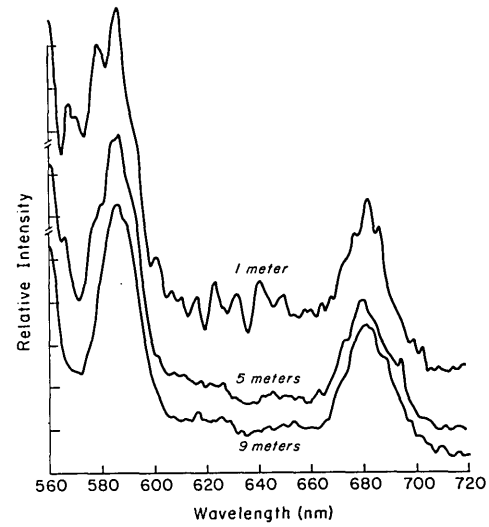

Fig. 6. Fluorescence spectra from 1, 5, and 9 m with background radiance spectra subtracted. Spectra are offset on ordinate to illustrate the similarity between the spectra.

IV. Discussion

We were able to detect ambient pigment concentrations of 0.9–1.1 μg liter^{-1} in the upper 10 m of the water column using the prototype instrumentation. Oscillations in the 1-m spectrum, which are correlated with ship motion through the pressure record from the RSVP, are presumably due to deviations from the vertical in angular orientation of the fiber-optic tip. These periodic deviations from vertical would result in a periodic increase in the ambient background light level in the emission spectrum, which cannot be can-

Table I. Summary of Physical and Optical Properties at the Three Depths Sampled with the RSVP and Fiber-Optic Fluorometer

Depth (m)	Temp. (°C)	Salin. (psu)	Density (kg m^{-3})	Int. Raman peak	Int. chlor. peak	Ratio of chl:Raman	Xtrct Chl	Xtrct Phaeo
1.06	10.03	31.55	1024.26	53749	29080	0.541	0.96	0.29
4.50	10.06	31.49	1024.23	56701	25431	0.449		
8.57	10.15	31.68	1024.37	50316	24302	0.483		

Depth, temperature, salinity, and density values are the means from an 80-s times series. Integrated Raman (570–600-nm) and chloropohyll (670–705-nm) fluorescence values were obtained from the spectral scans shown in Fig. 6 after offset and baseline corrections. Extracted (Xtrct) pigment values were obtained from surface seawater samples collected at the time of profiling.

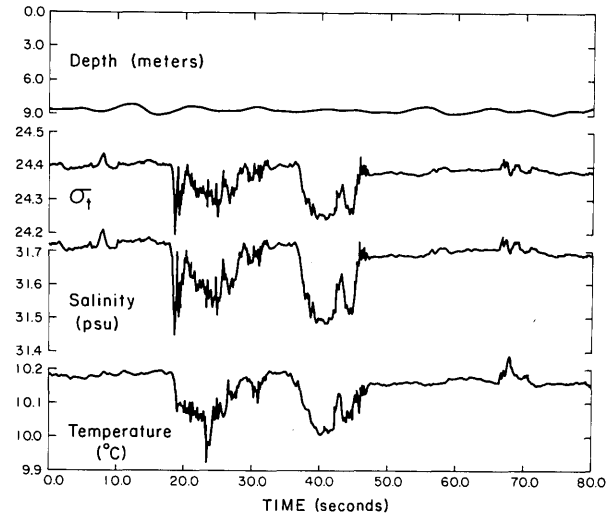

Fig. 7. Time series (80 s) of pressure, σ_t, salinity, and temperature from 9-m depth [σ_t = (density, −1000) * 1000]. Salinity and σ_t were computed using standard algorithms from measurements of pressure, temperature, and electrical conductivity.

celed by subtraction of the upwelling radiance recorded at a different time. This effect will decrease with depth as the background light is attenuated, and the effect is negligible in spectra from 5 and 9 m.

The ratio of pigment fluorescence signal to water Raman signal has been used by several investigators in airborne remote sensing applications as a means to determine effective viewing volumes due to variations in aqueous optical attenuation coefficients.[26-28] We also used the integrated Raman signal as an internal calibration standard. This technique provides for compensation of variations in laser power at the fiber-optic tip as well as variations in optical attenuation characteristics within the sampling volume during a profile.

A difficulty encountered in our implementation of this method of data reduction lies in the accurate determination of baselines used to determine the integrated peak areas. On 488-nm irradiation the Raman signal from water occurs at ~ 585 nm. As previously noted, upwelling radiance intensities in this region of the spectrum are significant near the surface. A more serious problem lies in the presence of Raman scattering and fluorescence from the core and cladding of the optical fiber,[29] which is the source of the high signal levels on the blue side of the water Raman bands in the spectra presented in Fig. 6. This fiber emission also limits the sensitivity of our single-fiber delivery and collection system in two ways. The longer the optical fiber, the more fiber emission, so that sensitivity is a function of fiber length. The fiber emission is linear in laser intensity, whereas the chloropyll signal from plankton eventually saturates due to light adaptation and biodegradation. Planned improvements to our fluorometer include a means for subtracting out the interfering effects of the fiber emission.

The variability observed in time series at a fixed depth reinforces the importance of high resolution sampling of biological features within the upper mixed layer. In these experiments, ~ 60 s were required to obtain a full fluorescence spectrum; however, water properties changed significantly over much shorter time periods (Fig. 7). Hence the fluorescence spectrum has smeared this variability over the time required for a spectral scan. In fact, the water Raman peak obtained may not be representative of the water from which the chlorophyll fluorescence peak was obtained. Our next step in the development process is to obtain faster spectral scans to match the detection of temperature and conductivity on the RSVP and to attempt to resolve the smallest scales of variability of the chlorophyll fluorescence signal in the ocean.

The second version of the laser/fiber-optic fluorometer will be tested as a profiling instrument during the summer of 1988. Incorporation of an intensified diode array as the detector in place of the single photomultiplier tube used in this study will permit spectral scans at the rate of 30 Hz. Results of these tests will be reported elsewhere.

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Meetings *continued from page 587*

1990
February

5–9 **Laser Applications to Chemical Analysis Top. Mtg.,** Incline Village, NV *OSA Mtgs. Dept., 1816 Jefferson Pl., NW, Wash., DC 20036*

11–16 Advanced Technology Optical Telescopes IV & Instrumentation in Astronomy VII, Tucson *SPIE, P.O. Box 10, Bellingham, WA 98227*

11–17 Medical Imaging IV Mtg., Newport Beach *SPIE, P.O. Box 10, Bellingham, WA 98227*

13–15 **Optical Remote Sensing of the Atmosphere Top. Mtg.,** Incline Village, NV *OSA Mtgs. Dept., 1816 Jefferson Pl., NW, Wash., DC 20036*

14–19 O-E/LASE '90 Optoelectronics & Laser Applications in Science & Engineering Mtg., exhibit & course, Los Angeles *SPIE, P.O. Box 10, Bellingham, WA 98227*

March

4–9 1990 Santa Clara Symp. on Microlithography, Santa Clara *SPIE, P.O. Box 10, Bellingham, WA 98227*

18–23 Advances in Semiconductors: Physics & Device Applications course, Newport Beach *SPIE, P.O. Box 10, Bellingham, WA 98227*

April

1–6 Advances in Semiconductors & Semiconductor Devices, Physics & Device Applications, Newport Beach *SPIE, P.O. Box 10, Bellingham, WA 98227*

16–20 1990 Tech. Symp. Southeast on Optics, Electrooptics, & Sensors, *SPIE, P.O. Box 10, Bellingham, WA 98227*

May

21–25 Lasers & Electo-Optics Conf., Anaheim *OSA Mtgs. Dept., 1816 Jefferson Pl., NW, Wash., DC 20036*

21–25 Int. Quantum Electronic Conf., Anaheim *OSA Mtgs. Dept., 1816 Jefferson Pl., NW, Wash., DC 20036*

June

17–20 Image Preservation Symp., Rochester *SPSE, 7003 Kilworth La., Springfield, VA 22151*

July

29–3 Aug. O-E/Fibers '90, San Diego *SPIE, P.O. Box 10, Bellingham, WA 98227*

August

19–24 34th Ann. Int. Tech. Symp. on Optical & Optoelectronic Applied Sci. & Eng., San Francisco *SPIE, P.O. Box 10, Bellingham, WA 98227*

26–31 Euroanalysis VII Conf., Vienna *M. Grasserbauer, c/o Interconvention, Austria Center Vienna, A-1450 Vienna, Austria*

September

4–7 Advanced Processing Technologies for Optical & Electronic Devices Mtg., Boston *SPIE, P.O. Box 10, Bellingham, WA 98227*

4–7 O-E/Lase East, Boston *SPIE, P.O. Box 10, Bellingham, WA 98227*

November

4–9 **OSA Annual Mtg.,** Boston *OSA Mtgs. Dept., 1816 Jefferson Pl., NW, Wash., DC 20036*

4–9 **OPTCON'90,** Boston *OSA Mtgs. Dept., 1816 Jefferson Pl., NW, Wash., DC 20036*