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Mapping phytoplankton in situ using a laser-scattering sensor

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Abstract

A primary limitation of phytoplankton ecology research is the difficulty of describing patchiness and distributions of different phytoplankton groups. Chlorophyll fluorescence and optical backscatter are useful measurements that provide information about phytoplankton, but these measurements do not allow distinction of phytoplankton taxa. Traditional phytoplankton identification methods (such as microscopy, HPLC analysis, and flow cytometry) are labor intensive and therefore can provide only very limited coverage and resolution. Through lab experiments we show that the Laser In Situ Scattering and Transmissometer (LISST-100) instrument can accurately quantify phytoplankton cell dimensions for some cell shapes. Pseudo-spherical dinoflagellates are described with a single peak in the particle size distribution (PSD) at the cross-sectional dimension of the cells. Pennate diatoms are described with peaks in the PSD at the major and minor axis dimensions of the cells. Diatom cells with minor axis dimensions that vary along the major axis are described with one peak across the range of minor axis dimensions and a second peak at the major axis dimension. Through field experiments, we show that mapping the PSD in situ at high resolution permits description of patchiness and evolution of phytoplankton populations. We present two examples: (1) growing dominance of *Ceratium* species during a red tide bloom, and (2) concentration of *Pseudo-nitzschia australis*, a harmful algal bloom (HAB) species, at a water mass front. We conclude that synoptic mapping of the PSD can significantly advance phytoplankton ecology research in the coastal ocean.

Standard optical sensors such as backscattering instruments and chlorophyll fluorometers provide high spatial resolution with limited information about the particles within the sample. Methods involving the analysis of water samples, such as microscopic examination, flow cytometery, laboratory particle size analyzers, and HPLC analysis of pigments, provide low spatial resolution but produce high levels of information about the particles. Phytoplankton ecology studies would benefit from the ability to describe phytoplankton diversity at high spatial and temporal resolution. Of particular interest to detect and quantify are species that produce red tide and harmful algal bloom (HAB) events because of their significant impacts on human health, marine life, and fisheries (Hallegraeff 2003; Glibert et al. 2005; Kudela et al. 2005).

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Here we present methods for describing phytoplankton patchiness in situ by the application of a Laser In Situ Scattering and Transmissometry (LISST-100) instrument. The LISST-100 was originally designed and tested for sediment particles (Traykovski et al. 1999; Michelson and Pejrup 2001) and has been applied to studies of bacteria and phytoplankton populations in shallow (16 m) coastal waters and lakes (Serra 2001; Serra et al. 2002a, 2002b). In our application of the LISST to study coastal ocean phytoplankton populations, particle concentrations are lower than those of previous LISST applications. Therefore, we increased the sensitivity of our instrument by a factor of ten. We verified the LISST's ability to describe oceanic phytoplankton by comparing cell dimensions measured by the LISST with those measured using a microscope. These lab studies included cultures of dinoflagellates and diatoms, measured as monocultures and mixed cultures of different size classes.

The field program consisted of a 5-week time-series study of phytoplankton ecology in Monterey Bay, California (Fig. 1). Intensive in situ measurements during this study detailed development of two phytoplankton blooms: (1) a bloom of pennate diatoms of the genus *Pseudo-nitzschia*, some of which are HAB species (Scholin et al. 2000), and (2) a red tide bloom dominated by dinoflagellates of the genus *Ceratium*. By combining synoptic mapping of the PSD with multidisciplinary

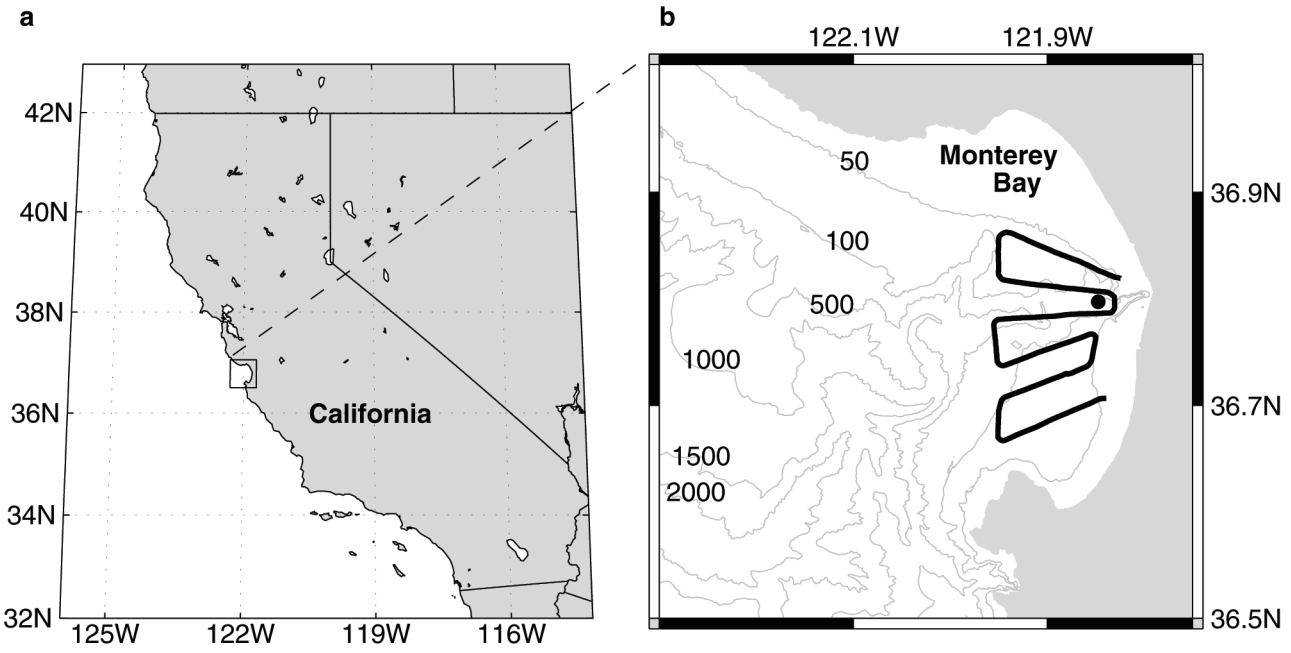

Fig. 1. Map of the study region. Locations of the survey line and phytoplankton sample station are shown in (b). Isobaths (m) are contoured in gray.

mapping from remote and in situ sensing, we were able to more fully describe the ecology of these blooms.

Materials and procedures

Instrument fundamentals—The principal of operation for the LISST-100 instrument is based on Mie theory (Gartner et al. 2001), which states that at small forward-scattering angles, laser light diffraction by spherical particles is essentially identical to diffraction by an aperture of equal size, i.e., the Fraunhoffer approximation (Gartner et al. 2001). This theory also states that a particle's composition and index of refraction have little or no effect on the distribution of light scattering at small angles in the forward direction. Modeling studies by Gordon and Du (2001) show that the particle size distribution (PSD) can be determined with forward scattering only, not backscattering, and that for forward scattering angles between 0° and 20°, the index of refraction of the particles has no effect on the scattering phase function. Thus, we are able to use the distribution of forward scattered laser light to measure particles with an index of refraction close to that of seawater (phytoplankton). Although nearly spherical particles are assumed in the operation of the LISST, MacCallum et al. (2004) obtained "reasonable agreement (between experimental results and Mie theory) within the first diffraction lobe for ellipsoidal particles."

The LISST-100 projects 670 nm laser light through a 20 cm path-length sample volume and records the forward scattering intensity over the range 0° to 19.5°. This scattering intensity is recorded as raw data. To produce an accurate PSD, background scatter is also required. This is determined by performing this same measurement on a sample of clear water, producing a "ZSCAT" background scatter file. The multi-angle intensity distribution of the raw data are converted to a particle size distribution (PSD) via a mathematical inversion that accounts for background scatter. For more on this inversion, see Hirleman (1987) or Agrawal and Pottsmith (2000). The PSD is computed as particle volume concentration in 32 logarithmically spaced size classes between 1.2 and 250 microns. For our experiments, the LISST-100 was modified by Sequoia Scientific in two simple and inexpensive ways. First, the gain was amplified by a factor of 10 to match the instrument's sensitivity with the particle concentrations found in the waters of Monterey Bay. The particle concentration range was defined from the examination of 3 years of light transmission and total suspended solids (TSS) data obtained from all seasons within Monterey Bay. Secondly, the batteries were removed and placed into a separate housing, allowing for a smaller package when powered from an external source.

Calibration—A fundamental issue of all electronic instruments is that they produce small amounts of noise in the data, and a fundamental issue with scattering instruments is that even pure water scatters light. Additionally, scratches on the windows and imperfections in the optics can contribute to scattered light. Gartner et al. (2001) found that even for high sediment concentrations, well-refined detector calibrations were essential for accurate size estimates. Because of these inherent issues and the low particle concentrations we typically measure, we have our LISST-100 calibrated and aligned at the factory annually, and collect background scattering data (ZSCAT) on each deployment. To determine the best possible ZSCAT for our conditions, we tested different methods including the use of factory ZSCAT values, laboratory DI (De-ionized

water) values, absolute lowest in situ values, and the median of lowest 1% of the in situ values.

For laboratory use, we create a ZSCAT using the purified media into which we are placing our sample, either filtered seawater (FSW) or clean growth media. Starting with a warmed up instrument, clean chamber and instrument lens, the sample chamber is filled, taking care to prevent bubbles from forming on the instrument's windows. This clean media sample is then measured and recorded in the same manner as a sample of phytoplankton. By comparing the results from this media blank to the factory ZSCAT, we can determine if there are any issues to remedy (such as bubbles, dirty windows, or internal electronics) before introducing the sample. If this procedure produces values close to or lower than factory ZSCAT values, the pure media sample is accepted, and data are collected for several tens of seconds. From this data set, the lowest values for each ring detector are extracted and used as ZSCAT values.

For field work, we use the full data set from each deployment to create an in situ ZSCAT. Even in the coastal zone environment where we do most of our work, particle concentrations at or below the detection limit of the instrument can often be found. In a typical deployment, we collect more than 6 h of continuous 1 Hz data from varying depths and locations, from which we select clear water samples. From the full data set, we extract the 1% of spectra having the highest transmission (clearest water). From this clearest water subset, we extract the minimum detector counts for each ring to produce the in-situ ZSCAT. Quality control of this process includes comparison of the noise (range) level in the clear water subset with the in situ and factory ZSCATs. If the noise range is acceptable, and the in situ ZSCAT is near or below factory calibration values and does not contain extraordinary single ring counts, the ZSCAT is accepted. In this way, we have been able to produce very clean data sets from oceanic water with low particle concentrations.

Lab monoculture evaluation—Monoculture stock was obtained from Provasoli-Guillard National Center for Culture of Phytoplankton (CCMP) and from a lab that cultures phytoplankton at MBARI (C. A. Scholin). These cultures were grown and maintained in a climate-controlled incubator. The dinoflagellates were of the genera A*lexandrium*, *Ceratocorys,* and *Pyrocystis*. The diatoms were of the genera *Pseudo-nitzschia* and *Asterionella*. During the growth phase and prior to measurement in the LISST, a 2 mL sub-sample of each culture was filtered onto a Poretics 0.2 μm polycarbonate filter, and cell abundance and size measurements were assayed with epifluorescence microscopy (Buck et al. 1996). Measurement in the LISST was then performed in the following way. After the instrument was warmed up and allowed to stabilize, the LISST sample chamber was prefilled with clean growth media (usually F/1) and visually inspected for bubbles. The LISST SOP software was then started, and a ZSCAT was recorded as described above. A known volume of culture was then injected into the sample chamber. A smaller volume from the same pipette was placed on a slide for measurement and enumeration under a microscope as described above. During the measurement period, the sample chamber was periodically mixed with a bulb pipette to keep the suspension well mixed.

Field deployment of LISST—We deployed the LISST-100 on a Sea Sciences Acrobat Towfish along with the following sensors: a Falmouth Scientific conductivity-temperature-depth (CTD) sensor, a WetLabs chlorophyll fluorometer, and a WetLabs optical backscatter sensor (OBS). By using the towfish as a deployment platform, we were able to acquire real time data and provide continuous power to the LISST. This allowed real-time quality control for sensor fouling by jellyfish or kelp, and it removed the limitation of on-board data storage and battery power.

This suite of optical measurements allows us to distinguish between waters dominated by phytoplankton scatterers and waters dominated by nonphytoplankton scatterers. Waters dominated by non-phytoplankton particles are evident by the combination of high optical backscatter and low chlorophyll florescence. This combination is commonly seen in intermediate nepheloid layers extending from the bottom nepheloid layer. However, all of the LISST data presented in this paper coincided with the indications of phytoplankton-dominated waters. Further, all mapping was conducted with sufficient distance from possible sediment sources, and during the late dry season when input of terrestrial particulate matter from coastal waterways is at a minimum.

Reduction of field data for phytoplankton studies—Scalar measurements such as temperature, salinity, chlorophyll fluorescence, and optical backscatter made from the towed undulating vehicle provide a single measurement at each point, while the LISST provides a spectrum of 32 values at each point. Interpreting this dense data requires data reduction techniques. Serra (2001) reduced LISST data into single scalars and found that particle volume concentration produced the best correlation with microscope counts. We applied different techniques depending on the nature of the PSD within each bloom and took advantage of the spectral nature of the data. For the red tide bloom dominated by dinoflagellates within a narrow size range, we used the fact that the spectra were characterized by a single narrow peak. For the bloom of the HAB species of pennate diatom, we used the fact that the spectra were characterized by two distinct peaks, at the major and minor axis dimensions of the cells.

To describe patchiness of a bloom of the pennate diatom *Pseudo-nitzschia australis* (a HAB species), we used empirical orthogonal function (EOF) decomposition (Preisendorfer 1988; Kelly 1988; Bretherton et al. 1992). This method, equivalent to principal component analysis, is effective for determining the dominant modes of variability in a temporal-spatial series. In the case of PSD data, the spatial elements are the 32 size-class bins of the sensor, and the temporal elements are the points at which the PSD is measured during the course of a survey. We used the most efficient method of EOF decomposition, singular value decomposition (Kelly 1988). The dominant mode resulting from

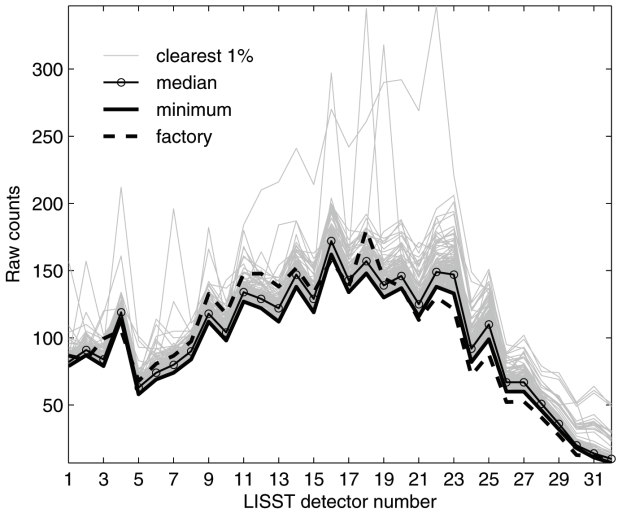

Fig. 2. Example of a successful in situ calibration plot showing scans from the clearest 1% of all samples taken during the deployment, the median and minimum values for each detector ring, and the factory calibration values.

this analysis exhibited a spectral shape matching the PSD of *Pseudo-nitzschia* cultures measured in the lab. Therefore, mapping the amplitude of the dominant mode found in the in situ data permitted mapping the intensity of the *Pseudo-nitzschia* bloom. Validation of the bloom was aided by phytoplankton enumeration data from time-series stations in Monterey Bay.

To describe the rapid increase in *Ceratium* species during a red tide bloom, we used the size class information from the LISST. Specifically, we computed average vertical profiles of the percent of PSDs having the maximum concentration in the size range of the red tide species (40 to 80 μm). Each survey consisted of approximately 170 vertical profiles made by the towed vehicle along the track line shown in Fig. 1b. The change in the average vertical profiles between surveys illustrated average depth-dependent growth in the dominance of the red tide species. To further characterize the bloom, we computed average vertical profiles of total particle concentrations for each survey. The change in the average vertical profile of total concentration between individual surveys illustrated the average depth-dependent increase in phytoplankton abundance.

Remote sensing—Interpretation of LISST data from the red tide bloom were greatly augmented by remote sensing. We present near-concurrent remote sensing from satellite and aircraft. The satellite image is from the Sea-viewing Wide Field-of-view Sensor (SeaWiFS). Details of the SeaWiFS data processing are found in Ryan et al. (2005). The airborne remote sensing is from the portable hyperspectral imager for low light spectroscopy (PHILLS) II sensor. Details of the PHILLS data processing are found in Kohler et al. (2004) and Bissett et al. (2005). The PHILLS data were corrected for atmospheric and illumination effects to produce remote sensing reflectance, Rrs (Montes et al. 2001). From the Rrs data, we derived a red tide indicator image, based on the ability to detect extreme blooms by their high reflectance in the near-infrared (Gower et al. 2005). This indicator uses bands centered at 708, 540, and 420 nm for the rgb composite image.

Assessment

Calibration—The first requirement was to determine the optimal ZSCAT method. The importance of optimal ZSCAT increases as the concentration of particles decreases. Our analyses of the available methods show that the best results are obtained by using the media being sampled under the conditions during which the sample is being taken, i.e., phytoplankton growth media in the lab, and the clearest seawater encountered during a deployment. In ideal conditions for an in situ ZSCAT, a subset of detector counts from the clearest waters (having the highest transmission) are tightly grouped around the factory calibration, and the minimum values within the subset fall near or below the factory values. If the lens is dirty, the in-situ values fall above the factory calibration by more than a few percent, and there is a significant spread in the values from the clearest water subset. Bubbles in the sample chamber are easily identified by large variations in the raw detector counts. An example in situ calibration plot with a clean lens and well-functioning instrument is shown in Fig. 2.

Lab experiments—The second requirement was to determine if optimally calibrated LISST measurements in the lab using monocultures of phytoplankton matched measurements of phytoplankton size made using a microscope. Using the laboratory procedure described above, we found that the LISST produces distinct PSD peaks at the expected diameter of nearly spherical phytoplankton (Fig. 3a). Combining two pseudo-spherical species in the sample chamber also results in distinct PSD peaks at the cross-sectional dimensions of each species (Fig. 3b). In the case of a monoculture of pennate diatoms such as *Pseudo-nitzschia,* the PSD shows two peaks (Fig. 3c), with one peak corresponding to the major (length) axis and one to the minor (width) axis. In the case of a species with a range of minor axis dimensions, *Asterionella*, we found that the LISST accurately quantifies the single major axis dimension and the range of minor axis dimensions. From these experiments, we conclude that the LISST can accurately describe a diverse range of phytoplankton shapes and sizes.

Field results—With an optimized calibration method, and laboratory results supporting the conclusion that the LISST can accurately describe phytoplankton dimensions, we then evaluated the application of the LISST in field studies of phytoplankton ecology. The phytoplankton enumeration time series of MBARI showed that the pennate diatom *Pseudo-nitzschia* was abundant during our study period (Fig. 4). LISST PSDs measured from this bloom were similar to LISST PSDs measured from *Pseudo-nitzschia* lab cultures (Fig. 5).

A towfish survey during the bloom encountered patchy distributions of chlorophyll and optical backscatter (Fig. 6). Within the first 35 km of the 13 September 2002 survey, two

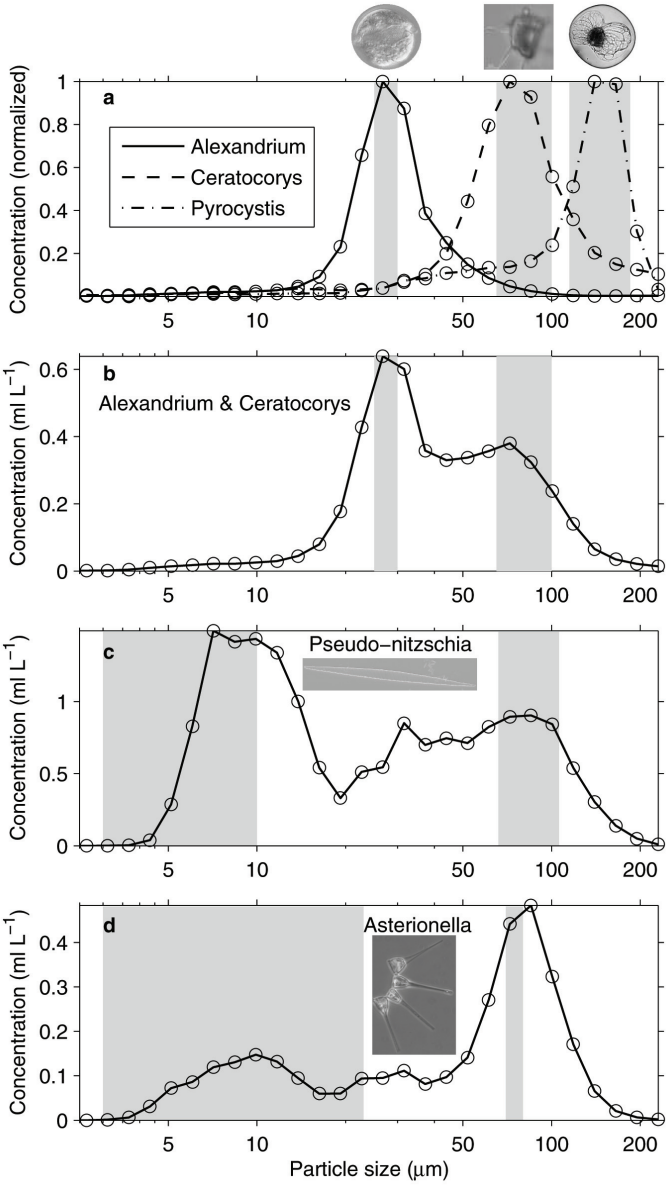

Fig. 3. Laboratory results from LISST experiments with phytoplankton cultures. (a) A composite overlay of results from three separate experiments with different species. The PSDs are normalized to their maxima. (b) Results of a single experiment containing two species. (c) PSD for a monoculture of a *Pseudo-nitzschia*. (d) PSD of a monoculture of *Asterionella*. Gray bars represent the range of cell dimensions measured via microscope for the nearly spherical species (a,b), and the minor (short) and major (long) axes of non-spherical species (c,d).

patches of high chlorophyll fluorescence were encountered (Fig. 6b, centered at ~8 km and 27 km along transect). Temperature measurements showed that the second patch was in a frontal zone (Fig. 6a). Whereas the high chlorophyll fluorescence in this patch at the front coincided with high optical backscatter, the patch centered at ~8 km did not (Fig. 6a-c). We tested whether the LISST could discriminate between these patches, and if the LISST could identify and map the distribution of *Pseudo-nitzschia*. The EOF decomposition of the PSD data showed that the dominant (first) mode had a spectral shape very similar to our lab PSD measurements for *Pseudo-nitzschia australis* monocultures (Fig. 6d). Further, we found that the magnitude of the first mode was very similar to the magnitude of the laboratory signal, when laboratory sample concentrations were at similar levels as the field concentration (Fig. 5,6d). By mapping the amplitude of this EOF, we found that particles with a shape signature similar to *Pseudo-nitzschia* were found primarily at the high-chlorophyll patch in the frontal zone that coincided with high optical backscatter (Fig. 6d, patch centered at 27 km along the transect). Analysis of LISST and ancillary oceanographic data thus suggests a concentrated patch of *Pseudo-nitzschia* in a frontal zone.

Later in our survey period, conditions favoring a red tide bloom of dinoflagellates developed in the bay (Ryan et al 2005). Microscopic examination of water samples identified the dominance of the species *Ceratium dens* and *Ceratium furca* (Fig. 7a). The cross-sectional dimensions of *C. furca* and *C. dens* sampled in this bloom ranged between ~40 and 80 μm. Particle size spectra showed that the *Ceratium* size class was abundant in patches throughout the monitored volume. A spectrum representative of these patches that showed a concentration peak centered at 60 μm is shown in Fig. 7b (spectrum A).

We applied the extensive PSD measurements from the LISST, together with multidisciplinary mapping of properties by in situ and remote sensing, to identify the probable source of the seed population for this red tide bloom. The bloom occurred immediately following the intrusion of offshore waters into Monterey Bay. Two possibilities were examined, seeding from intruding waters, and seeding from a preexisting population in the resident bay waters. Particle concentrations within these waters were very low, making them an unlikely source. Further, the sampled intrusion waters showed a local minimum in concentrations in the *Ceratium* size class (Fig. 7b, spectrum B). In contrast, the *Ceratium* size class signature was present in patches of resident bay waters (Fig. 7b, Spectrum C). Following this in situ mapping with the LISST, the front propagated northward, and remote sensing imagery indicated strong development of the red tide in nearshore waters, on the bay water side of the front (Fig. 8). These multi-platform observations support the conclusion that the seed population for the red tide bloom was within resident bay waters, not in the intruding waters. Following these observations of bloom initiation, the red tide spread rapidly throughout the bay (Ryan et al. 2005).

The extensive particle size spectrum measurements may also be applied to characterize bloom growth through the water column (Fig. 7c). Between 2 October and 9-11 October, average particle concentrations within the volume increased only above 25 m depth, with the greatest increase in the shallowest waters. Also during this time the particle size spectrum shifted dramatically toward the *Ceratium* size class (40 to 80 μm). On 2 October, less than 15% of size spectra at all depths had

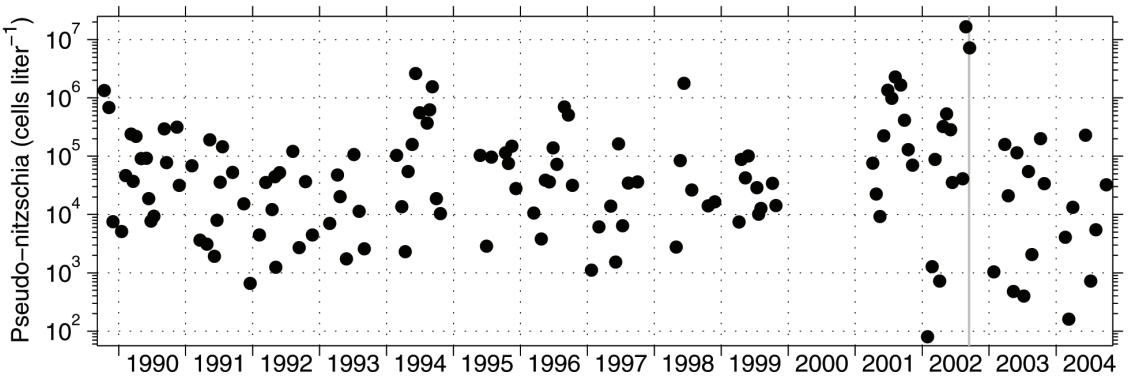

Fig. 4. Counts of *Pseudo-nitzschia* in Monterey Bay from 1989 to 2004, highlighting the very high counts that occurred in the fall of 2002. The location of the monitoring station is shown in Fig. 1b.

maximum concentrations within the *Ceratium* size class. By 9-11 October, more than 75% of all size spectra within the upper 5 m had maximum concentrations within the *Ceratium* size class. The surface-intensified dominance of this size class is consistent with the nature of red tide blooms, which form dense aggregations near the surface.

Discussion

Our laboratory results show that the LISST-100 is able to accurately describe phytoplankton cell dimensions for cells having diverse shapes and sizes. This includes pseudo-spherical cells, pennate cells having a large aspect ratio, and irregularly shaped cells having a consistent major axis dimension but a minor axis dimension that varies significantly along the major axis. This alone is a surprising and significant result, considering that the instrument was designed for studies of sedimentary processes involving higher concentrations of particles, and that the mathematical inversion method of deriving a PSD from multi-angle forward scattering assumes nearly spherical particles.

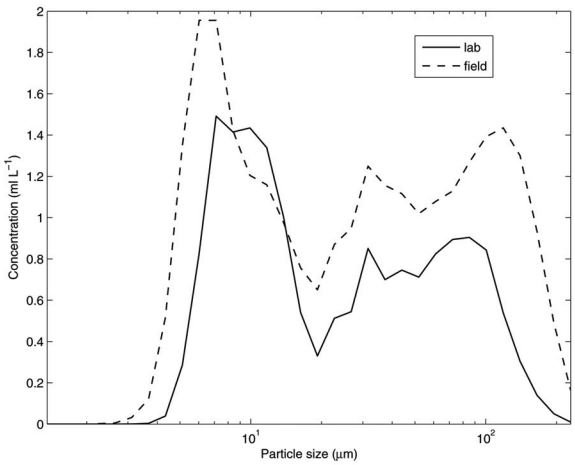

Fig. 5. Comparison of laboratory PSD for a monoculture of *Pseudo-nitzschia* at ~2.5×10^6 cells/L and a representative PSD acquired in the field from within a bloom of *Pseudo-nitzschia*

The laboratory results, in turn, provide a foundation for applying the LISST-100 to in situ mapping of phytoplankton. The goal is to resolve PSD variability in phytoplankton-dominated waters at the scales. We currently resolve water properties measured by other sensors. Because the PSD is a 32-

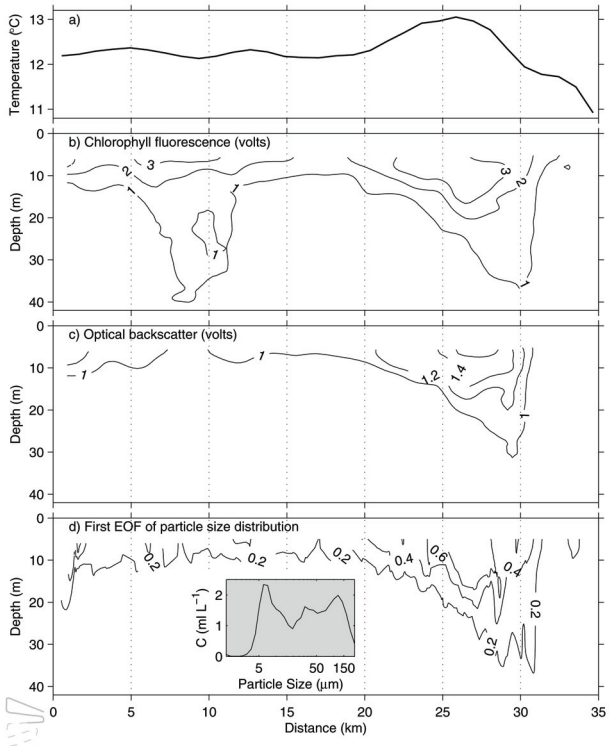

Fig. 6. Optical distinction of a *Pseudo-nitzschia* patch at a front, mapped along the survey track shown in Fig. 1b. (a) Average temperature at 10 m depth. (b) Chlorophyll fluorescence. (c) Optical backscatter. (d) First EOF of the PSD series. The EOF 1 amplitude is mapped, and the spectral shape of the first mode is shown in the inset. Note the similarity to the PSD of *Pseudo-nitzschia* shown in Fig. 5. Spatial reference for distance is represented as the survey track next to the 0-point on the distance axis. The survey started at the NE corner, and the portion of the full transect shown in the sections is represented by the solid line portion of the full survey track.

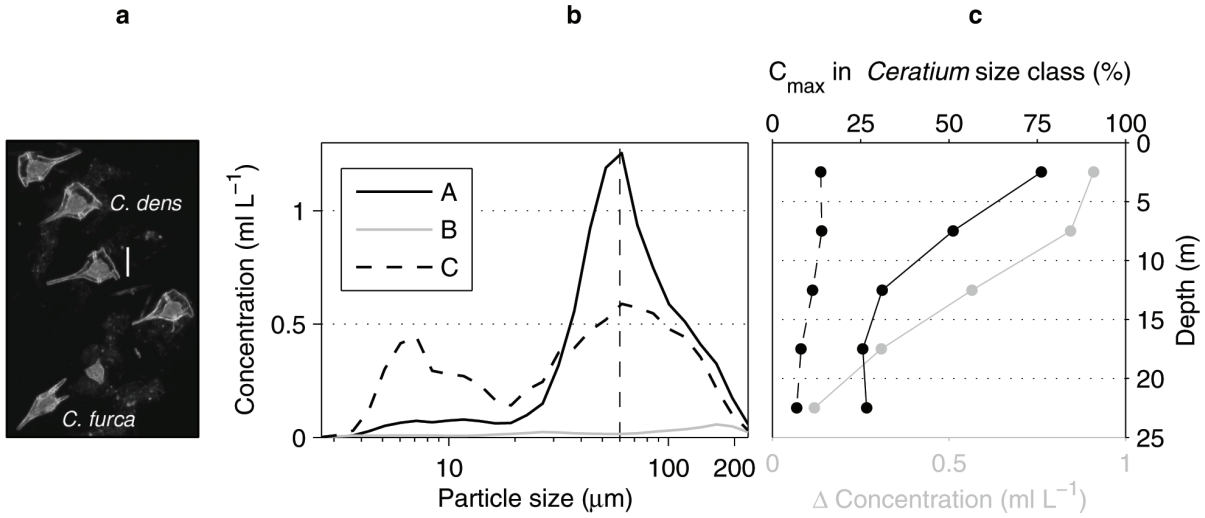

Fig. 7. Image (a) showing the red tide species from a sample within the monitored volume on 8 October 2002. Scale bar is 60 µm. (b) Representative particle size spectra measured by LISST-100 on the towed instrument system. PSD A is from within the red tide bloom showing a peak at 60 µm. PSD B is from the clean offshore waters that flushed the bay. PSD C is from the resident bay waters, also with a 60 µm peak. Panel (c) shows changes in time in the PSD. The dashed black line shows the percentage of PSD having maximum concentrations within the Ceratium size class on 2 October, and the solid black line shows this percentage for survey data from 9-11 October. The gray profile shows the change in total particle concentrations within the range 1-250 µm between 2 October and 9-11 October.

element spectrum measured at each point in a survey, data reduction techniques are critical to understanding the dense data provided by the LISST-100. We demonstrated that the PSD of a HAB diatom species measured in situ closely matched that measured in the lab, and we applied statistical analysis of the PSD series measured in situ to map bloom intensity and relate its spatial variation to environmental conditions. Specifically, we showed that the HAB species was concentrated in a frontal zone, and that this bloom patch was distinct in multiple optical measurements. In the case of a major red tide bloom, analysis of the PSD data permitted description of the increasing concentration of particles, particularly in the size class of the red tide species observed in situ.

The in situ case studies presented represent an important area of phytoplankton ecology research relevant to marine ecology and human interactions with the coastal ocean.

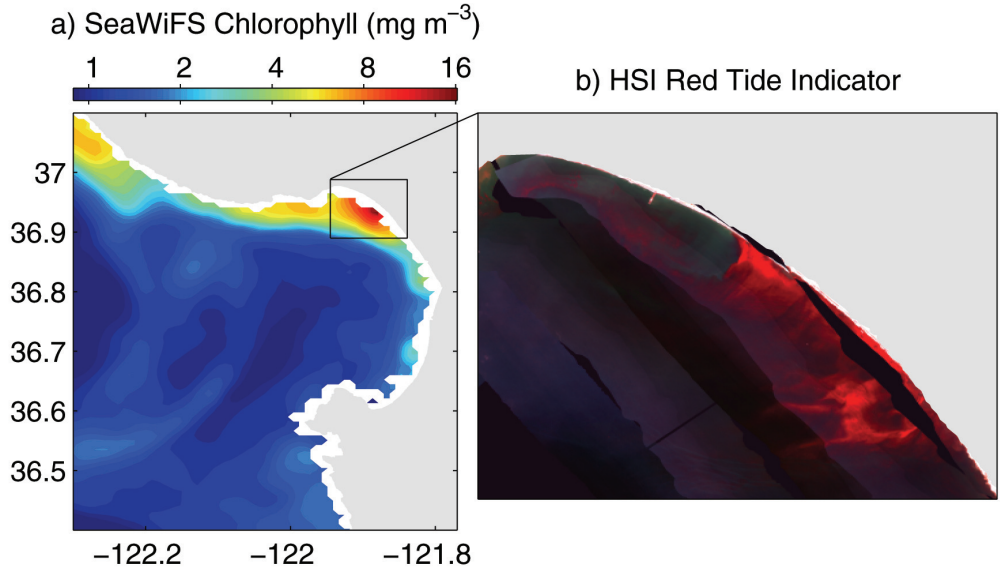

Fig. 8. Observation of the red tide seed population in resident bay waters, 1 October, 2002. (a) SeaWiFS chlorophyll, 1 km resolution. (b) HyperSpectral Imaging (HSI) Red Tide Indicator derived from the Portable Hyperspectral Imager for Low Light Spectroscopy (PHILLS) II sensor, 2 m resolution. Red regions of the HSI Indicator image indicate high reflectance in the near infrared due to the red tide bloom.

Because red tide dinoflagellates include many HAB species, and because *Pseudo-nitzschia australis* is a HAB species, as are other species of this genus, better methods to study intense blooms dominated by these species will contribute to understanding of HAB ecology. Monterey Bay hosts an extremely diverse phytoplankton community, however bloom patches dominated by one or a few species do not appear to be uncommon. We have studied more red tide blooms, and we have repeatedly found intense blooms dominated by single species or a few species. Red tide blooms during the fall of 2004 and 2005 were dominated by the same three dinoflagellates of the genera *Cochlodinium*, *Akashiwo*, and *Ceratium* (Kudela et al. 2007). We have also found red tide patches in nearshore waters of the bay frequently dominated by a single species of *Ceratium*. Synoptic mapping of the PSD to characterize this patchiness will add an important dimension to phytoplankton ecology studies, and improved methods for studying these events will be beneficial for understanding and managing coastal waters. The methods described here for in situ mapping of phytoplankton patchiness and variability used enumeration of plankton samples to support interpretation of the dense PSD data sets. Plankton enumeration will always be essential in studies of phytoplankton ecology, however the labor intensive nature of this method severely constrains the spatial and temporal resolution that can be achieved from it. The primary strength of the methods introduced here is the ability to map at high-resolution the PSD for distinguishing biological particle populations of high ecological interest, and to do so at scales resolved by other sensors describing the environmental properties.

A primary requirement for applying these methods in situ is that relatively high concentrations of particles are needed to provide sufficient signal for the LISST. From extensive deployment of the LISST-100 on towed and autonomous platforms, we find that the increased sensitivity of our instrument provides strong signal for phytoplankton concentrations typically observed in the bay. Another requirement of applying these methods is that acquiring PSDs usable for identifying phytoplankton depends on encounter of patches dominated by one to a few species that have a unique spectral signature. The case studies we presented may appear to be unique or rare, however, we argue that they are not at all the exception. First, these blooms, dominated by one to a few species, happened in a short period during a 5-week study; to the extent that this time period is a representative random sample, such blooms would not be considered rare. Second, we are finding in subsequent studies that within the diverse phytoplankton assemblage of the bay, patches dominated by one to a few species are regularly encountered. The efficacy of EOF decomposition of PSD series also rests on this requirement; it will be most effective where clear peaks exist in the PSD series. A third primary requirement for in situ application of these methods is that in order to determine whether the observed variability in the PSD is caused by variability in the phytoplankton populations, ground truth from plankton enumeration is essential. As demonstrated in the case studies presented, this combination can be very effective. A key requirement in this is to match enumeration samples as closely as possible to patches of interest mapped by the LISST. This is difficult to do with random sampling. An active and important area of development in this regard is automated acquisition of water samples based on sensor readings, such that dense phytoplankton patches encountered by the LISST can be detected and a water sample taken. We are now routinely deploying the LISST on an autonomous underwater vehicle (AUV) carrying physical, nutrient, and bio-optical sensors. This AUV is now returning water samples from prescribed locations within a survey grid. Thus a single platform is hosting not only the ability to more thoroughly describe phytoplankton variability, but also to validate the LISST-based description from water samples, and to detail the environmental variability underlying the observed phytoplankton variability. For effective feature detection and response (acquire sample), advances in artificial intelligence (AI) on-board the AUV are required. For another category of features, intermediate nepheloid layers, detection, and response AI software has already been developed and tested on our AUV (Fox et al. 2007), and we are proceeding with other categories of feature detection and response, including dense phytoplankton patches.

This research indicates a few important directions for future work. Whereas the clearly demonstrated ability of the LISST to quantify phytoplankton dimensions is an important result that stands alone, the ability to accurately quantify concentrations and derive estimates of cell counts would be very useful. We have found that the instrument's volume concentration estimate increases proportionally with increases in particle concentration. However, we have not found close agreement between microscopic counts and count estimates based on computations assuming spherical cells, the simplest test case using pseudo-spherical cells. This deserves further experimentation. Another important area of future work is to understand how to characterize chain-forming species in situ. This depends partly on the sampling method. During the in situ studies presented here, the LISST was deployed with an open flow path. We now deploy the LISST on an AUV, with a sample chamber fit around the sample volume and a rapid flow of seawater pumped through the chamber. This method of sampling would be more likely to break up chains and permit clearer detection of individual cell dimensions.

In summary, the methods introduced here demonstrate the ability to describe phytoplankton patchiness in situ at high resolution, at least for phytoplankton patches that have sufficient particle density and distinction in the particle size distribution. For blooms of many red tide and harmful algal species, these requirements are often met, and these methods will be effective. The data density resulting from high resolution mapping of the PSD requires effective methods of data reduction to derive ecologically meaningful results. Further

developments of this approach should include improved interpretation of particle volume concentrations relative to cell counts, additional methods of data reduction, and application of artificial intelligence on autonomous vehicles to acquire ground-truth water samples at critical locations identified by on-board sensors.

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