Biological and Chemical Microstructure in Coastal Areas

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LONG-TERM GOALS

We seek to gain a quantitative understanding of the physical, chemical, biological, and optical dynamics that structure the planktonic ecosystem *on the scales of the organisms.* By quantifying the variability of the organisms and their environment at the scales that the organisms live their lives, we will gain insight into the dynamics that create structure and influence the function of the planktonic ecosystem.

OBJECTIVES

Our objectives in this phase of the proposed work were to develop image-processing algorithms to allow enhanced and automated analysis of the images obtained with our free-falling two-dimensional imaging fluorometer system (FIDO-ϕ). These algorithms would allow us to perform continuous counts of fluorescent objects throughout the water column, measure their sizes, and perform image pattern-recognition procedures in order to distinguish among object types.

APPROACH

FIDO-ϕ is a free-falling vehicle carrying a laser, sensitive CCD camera, computer, depth/tilt/roll sensors, and a CTD/fluorometer/transmissometer package. The laser is formed into a 6.5 mm thick sheet that extends at a 45° angle below the vehicle. Chlorophyll *a* fluorescence stimulated by the laser is imaged by the camera, oriented at 90° to the laser sheet. The imaging area is 32x32 cm, with 312x312 μm resolution per pixel. Previous work has shown that the system can image individual cells at least as small as 5 μm; the fluorescent organisms appear as individual bright points in the images.

The vehicle falls at a pre-determined rate (usually 3-10 cm/s), dropping a weight at 100 m, and returning to the surface. Fluorescence images are acquired every 2 seconds, resulting in 150-800 images per profile, depending on drop speed. Experiments with scale models in a flume, and observation of the images themselves indicate that the vehicle does not disturb the water it is imaging.

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In a series of profiles recorded ~10 km west of San Diego, CA, we gathered ~10,000 images. Comparisons of the average fluorescence of an image with bulk fluorescence measured by a "regular" fluorometer are consistent. However, the two-dimensional nature of the images allows unprecedented observation of the numbers, sizes, and spatial arrangement of individual cells throughout the water column. Our work in this phase consisted to two tasks:

Task 1: Due to light spreading and attenuation, the incident irradiance in the imaging plane varies as a function of range from the light source. Since observed fluorescence is proportional to the incident irradiance, the same particles appear brighter or dimmer depending where they are in the image. The effects of the beam pattern can be normalized by multiplying the observed intensities by a number that is inversely proportional to the incident irradiance at each point. For low densities of absorptive and scattering matter, this procedure is simple; however, in the presence of strong absorbers and scatterers, the incident intensity changes in an image dependent matter. Removing these systematic effects is the goal of Task 1.

Task 2: One of the great advantages of our system is that we produce *images* of the stimulated fluorescence. These images can provide a great deal more information than that provided by traditional fluorometers when analyzed by image-processing techniques that characterize the shapes and densities of the fluorescent objects. Since our profiles consist of many thousands of images, automated image-processing methods for characterizing the images are necessary. The goal of Task 2 is to explore the development of image-processing algorithms to characterize and estimate the abundances of different shapes observed in the images.

WORK COMPLETED

Task 1: Correcting the incident beam pattern. We tested 3 different methods for correcting the images for the spreading of the incident laser beam.

Method 1: Averaging of all images followed by smoothing. This is the technique outlined in Franks and Jaffe (2001). All images in a profile are averaged and then smoothed. We have used 5^{th} order polynomial smoothing, opening-closing image dilation methods, smoothing filters and convolution, and median filters. The fastest method is the smoothing filter. Each individual image is then divided by the averaged/smoothed beam pattern to "flatten" the image. This method works best in relatively clear waters (<0.7 μg chl *a* / liter) with little scattering of the laser sheet. In more chlorophyll-rich waters, the phytoplankton cells cause a great deal of scattering, significantly altering the shape of the beam pattern which causes depth-dependent (chlorophyll-dependent) variations in the accuracy of correction among images.

Method 2: Averaging images of similar chlorophyll concentration. In this method, images are binned by their average fluorescence, and then averaged and smoothed as in method 1. This technique reduces the noise of relatively clear images, and reduces the problem of chlorophyll-dependent changes in the beam pattern. The main problem with this technique is re-scaling the images after normalization by the smoothed beam pattern so that all images in a profile can be compared to each other. We explored normalization among images by scaling to the maximum fluorescence in the beam pattern, scaling to the median or mean, and normalizing by the background "noise" in an image.

Method 3: Filtering of individual images. In waters of high chlorophyll concentration, variations in the incident beam are strong enough that each image must be corrected individually. All the smoothing techniques were tested on the images, and the median filter was found to work well (though it was relatively slow). The median filter allowed accurate visualization of the large individual cells throughout much of the image, but suffers from the same inter-image normalization problem as method 2.

Task 2: Currently, we are exploring the hypotheses that the images reveal depth-dependent changes in not only abundance by also shape (=species). Our initial efforts are to "hand process" the images by selecting several distinctive shapes that appear in the images. First an object "primitive" was prepared, containing the object being sought, and with reduced background noise. This object was then correlated with others in an image, and the object rotated through 360° by 5° increments to explore all two-dimensional orientations of the object. Objects showing >0.9 correlations were considered to be the same. This primitive pattern is robust to the effects of incident beam spreading.

We also developed image-processing software that enumerates all fluorescent objects in a beam-corrected image. Objects above a predetermined fluorescence threshold were counted, and their areas and centroids calculated. Algorithms were developed to determine nearest-neighbor distances among objects in an image.

RESULTS

We were successful in developing several algorithms for correcting images for spreading of the incident beam. These corrections allowed us to enumerate individual cells within each image, and throughout the water column. An example profile is shown in Figure 1, where the cell counts are plotted beside the bulk fluorescence. (We recognize that many of the fluorescent objects we image are not "cells", but may also be detrital material, herbivore guts, fecal pellets, etc.) Most of these "cells" are likely >5 μm, and represent 10-20% of the total fluorescence, but may represent a disproportionate fraction of food available to crustacean zooplankton. A striking feature of the vertical distribution of these cells is that the vertical gradients are much higher than the gradients of the bulk fluorescence. Often we observe changes in cell concentration of >5x over a vertical distance of 10-30 cm. Furthermore, the peaks in cell concentration do not always correspond with peaks in the bulk fluorescence. This is particularly evident in the distribution of the "very large cells" – objects that were >50 pixels in area. These large objects appeared in only one isolated layer, centered at about 43.5 m, near the bottom of the chlorophyll maximum layer. The objects were intensely fluorescent elongate objects 5-10 mm long, and 0.6-1 mm wide. This further supports our hypothesis that the planktonic ecosystem is highly structured on scales of 10's of cm, and that this ecologically important structure has not been adequately measured by previous instruments.

With our image analysis software we were able to calculate the two-dimensional locations of the objects in an image, and derive nearest-neighbor statistics (Figure 1). In the example shown, the maximal bulk chlorophyll concentration was 0.65 μg/liter, and the nearest-neighbor distances were about 2-4 mm. Considering that most of the phytoplankton are much smaller than the pixel resolution of the camera (312x312 μm), this is a relatively large distance between cells (hundreds of body lengths). The variance of nearest-neighbor distances suggests that the cells were distributed according to a two-dimensional Poisson distribution throughout much of the water column. In several regions the variance was higher than predicted by a Poisson distribution, implying that the organisms were more

clumped (more cells closer together than predicted, and more isolated cells than predicted by Poisson). This may show aggregation of cells, or may be related to binary fission of daughter cells, or even grazing-induced patchiness of cells.

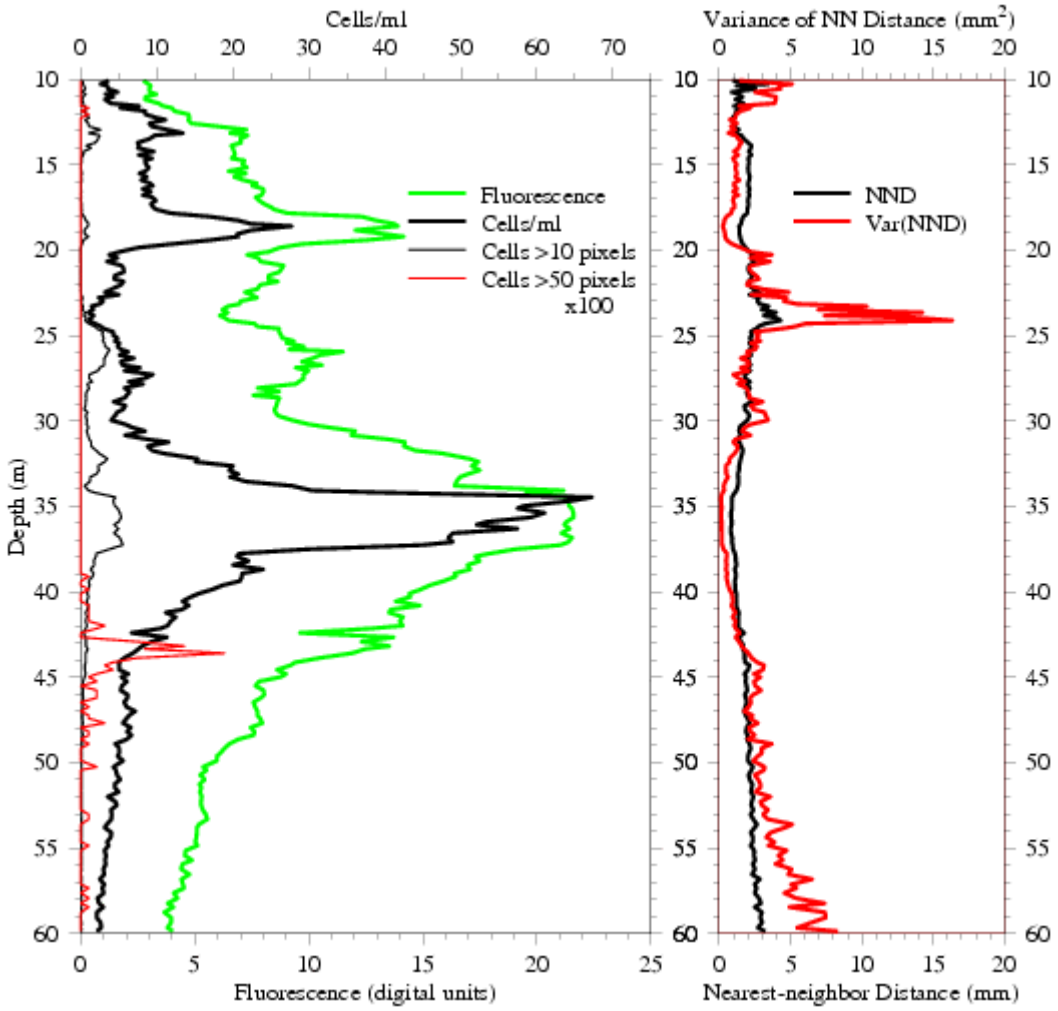

Figure 1. Left panel: vertical profiles of chlorophyll a fluorescence (green line), cell concentration (thick black line), concentration of cells larger than 10 pixels (thin black line), and concentration of cells (objects) larger than 50 pixels (thin red line). Note that the cell concentration profiles tend to have shaper vertical gradients than the fluorescence profiles. Two thin layers of all cells are apparent (19 m and 35 m). The largest objects were found only in a thin layer at 44 m depth. Right panel: Vertical profiles of nearest-neighbor distance (black line) and the variance of the nearest-neighbor distance (red line). When the mean=variance, the distribution of cells is consistent with a Poisson distribution.

Careful examination of peaks of cell concentration showed that individual peaks often contained object shapes that were unique to the layer. This suggests that the layers – often <1 m thick – contain different species and species assemblages than layers only a few meters above or below.

IMPACT/APPLICATIONS

Our results show a high degree of microscale spatial structure in the vertical phytoplankton distributions. Layers <1 m thick were common occurrences, and image analysis showed that unique cell types were often associated with the layers. Thin layers separated by only a few meters in depth could have completely different phytoplankton communities.

One interesting result of our analyses was the observation of "cryptic layers" – layers of enhanced concentrations of certain phytoplankton types that were not associated with layers of bulk fluorescence. Thus bulk fluorescence was not a good indicator for the presence of layers. The occurrence of these cryptic layers suggests that the planktonic ecosystem is highly structured in space, with potentially unique, relatively isolated communities layered vertically. This observation has implications for the structure and function of the planktonic ecosystem, as well as the evolution of planktonic species.

RELATED PROJECTS

In our NSF-OTIC sponsored project with Steven Monismith (Stanford University) we are using the FIDO-ϕ as a framework for constructing a free-falling stereo PIV (particle image velocimetry) system. In this system the laser sheet will be oriented vertically, and imaged using two cameras – one on each side of the sheet. By taking a rapid series of images of scattered light, followed by an image of fluoresced light, we will be able to construct three-dimensional velocity vectors with mm-cm spatial resolution over the imaging plane. If this program is successful, we will be able to construct two-dimensional maps of turbulent water motions, calculate maps of the dissipation of turbulent kinetic energy, and explore the correlations of the phytoplankton with these microscale physical structures. When combined with the image-processing algorithms developed during this ONR work, this program will allow an unprecedented view into the physical-chemical-biological interactions structuring the planktonic ecosystem at micro scales.

REFERENCES

Franks, P.J.S. and J.S. Jaffe. 2001. Microscale distributions of phytoplankton: initial results from a two-dimensional imaging fluorometer. Mar. Ecol. Prog. Ser. 220:59-72.

HONORS/AWARDS/PRIZES

P.J.S. Franks. 2002. H. Burr Steinbach Visiting Scholar, WHOI.

J.S. Jaffe. 2002. Made Fellow of the American Acoustical Society.

J.S. Jaffe. 2003. H. Burr Steinbach Visiting Scholar, WHOI.