LIGHT-SCATTER ANALYSIS OF MICROALGAE

CORRELATION OF SCATTER PATTERNS FROM PURE AND MIXED ASYNCHRONOUS CULTURES

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ABSTRACT A method is described for the first time for rapid and accurate discrimination among several algal types by their light-scattering properties alone. Using a multiangle light-scattering flow system, we obtained light-scatter patterns for individual cells in asynchronous cultures of *Chlorella*, *Chlamydomonas*, and *Anacystis*. The patterns are consistent and distinct for each species. By these signatures, each algal type can be recognized within mixtures.

INTRODUCTION

We present here a flow-system method in which several individual algal species can be rapidly and accurately identified on a cell-by-cell basis by their light-scattering properties alone. This is of interest to environmental scientists and water resource planners who, wishing to have more detailed knowledge of the phytoplankton community in aquatic ecosystems, must rely on present water analysis techniques (microscopic, colorimetric, and turbidimetric), which tend to be slow and tedious. In this method, the cells are introduced into a flow chamber, where they pass individually through a focused laser beam (6,328 Å) at rates up to 1,000 cells/s. The intensity of light scattered from the cell is measured simultaneously at 32 angles between 0° and 21° with respect to the laser beam axis. The scatter patterns are transferred to a computer for analysis by a mathematical clustering algorithm. We have obtained these patterns for several algal types; the patterns for three microalgal species, *Anacystis nidulans*, *Chlamydomonas reinhardii*, and *Chlorella pyrenoidosa*, are described herein. The patterns for each species are sufficiently distinct so that discrimination among algal types in mixtures can be readily achieved. This technique may be particularly important in water pollution studies, where the composition, abundance, and distribution of algae in a water sample can be related directly to the level of inorganic and organic pollution in the sample (1).

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MATERIALS AND METHODS

Algal Culture

Asynchronous populations of the green algae *C. pyrenoidosa* (strain 71105) and *C. reinhardii* (Harvard strain minus mating type) and the blue-green alga *A. nidulans* (ICC No. 625) were grown by previously described mass culture techniques (2). The algae were grown at 37, 25, and 39°C, respectively, in the described media (3–5). In these experiments, the algae were grown from agar slants in 500-ml and 2-liter Bellco spinner flasks (Bellco Glass, Inc., Vineland, N.J.) with subsequent inoculation in a 60-liter mass culture apparatus (2).

Samples for light-scatter measurements were taken during exponential growth, determined by absorbance at 550 nm by a Gilford Model 240 spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). The algae were removed from the growth apparatus, separated from the growth medium by centrifugation, and washed twice with sterile isotonic saline.

Cells of *C. reinhardii* are motile and are usually oval with two flagella arising from the anterior end. The cell size ranges from 10 to 30 μm, depending on the phase of the cell cycle (5). *Chlamydomonas reinhardii* is a member of the class of algae known as the *Chlorophyceae* and belongs to the subclass *Volvocales* (6). Cells of *C. pyrenoidosa* are usually round, lack flagella, and are nonmotile. The cell size is 5–15 μm, depending on the cell cycle phase. They are part of the *Chlorophyceae* class and the subclass *Chlorococcales* (7). The blue-green alga *A. nidulans* is mostly unicellular and is similar in size and shape to rodlike bacteria. The cells are 2–3 μm in length, and multiplication is by binary fission (8). The longest multiple cell filaments consist of four cells. Most of the cells in an exponentially growing culture are single.

Instrumentation

A comprehensive description of the flow system, data acquisition, and data processing techniques appears elsewhere (9). The cells were suspended in isotonic saline and introduced into a flow chamber (10), where they passed individually through a 5-mW helium-neon laser beam focused to a spot size of 50 μm. The width of the sample stream could be controlled by varying the pressure difference between the sample stream and the sheath which surrounds it. As a cell traversed the laser beam, light scattered in all directions. A portion of the forward scattered light was sampled by a detector array consisting of 32 concentric rings of photodiode material (Recognition Systems, Inc., Van Nuys, Calif. model WRD-6420A). With the exception of the innermost ring, each ring intercepted a polar angle $\theta_i \pm \triangle\theta_i$, where $\triangle\theta_i$ ranged from 0.06° to 0.9°. Both θ_i and $\triangle\theta_i$ increased monotonically with polar angle. Each ring spanned an azimuthal angle of 180°. The mean polar angle of the innermost ring was 0.0°, and this ring subtended a polar angle of 0.14° and had an azimuthal angular range of 360°. The negative signal on this innermost ring was first inverted and then included in the scatter pattern. A schematic drawing of the instrument is given in ref. 9.

The current pulse generated by the flash of light on each ring was converted to a voltage signal, which was logarithmically amplified. The voltage peak was sensed and held, then digitized by a fast eight-bit analog-to-digital converter, and subsequently stored on the disk memory of a PDP 11/45 computer (Digital Equipment Corp., Maynard, Mass.). Therefore, each cell has 32 measured light-scatter intensities, which can be considered its "signature." This signature can be thought of as a point in 32-dimensional space. A mathematical clustering algorithm has been developed[1] which groups together similar scatter patterns with no a priori information about the cells that produce the patterns. The density of each point was determined by calculating the Euclidean distance to the 15 nearest neighbors of each point. The

[1]Goad, C. A. 1978. A clustering algorithm for mixtures of monotone densities. In preparation.

array of densities forms a surface above the 32-dimensional hyperplane. The hills on this surface are regions of high density and represent the clusters into which the scatter patterns are grouped. Every scatter pattern is assigned to some cluster. This algorithm is described in more detail elsewhere (9) and is similar to one used for classifying reaction mechanisms in high-energy physics (11). In the results presented below, each cluster is represented by a band two standard deviations wide centered about a mean scatter pattern for the cluster. Cluster analysis is performed on 400 points for each sample.

RESULTS AND DISCUSSION

Fig. 1 *a* shows a collection of 100 randomly selected scatter patterns from *A. nidulans*. In this and all succeeding figures, the ordinate contains approximately three decades of log light-scatter intensity, while the detector element angle is given on the abscissa. Two clusters are identified by the clustering algorithm, as shown in Fig. 1 *b*. Each cluster is hatched differently and is enclosed by a pair of lines, each indicating an excursion of one standard deviation from the mean of the scattered intensity for that cluster. The percent of cells in each cluster is marked. In every sample, 400 randomly selected cells were used for cluster analysis. The presence of the second cluster (7%) at higher scattering intensity is due to a small fraction (< 10% as verified by microscopic count) of the asynchronous culture nearly at the point of fission. Since this rod-shaped organism reproduces in a longitudinal manner, it would be expected that, due

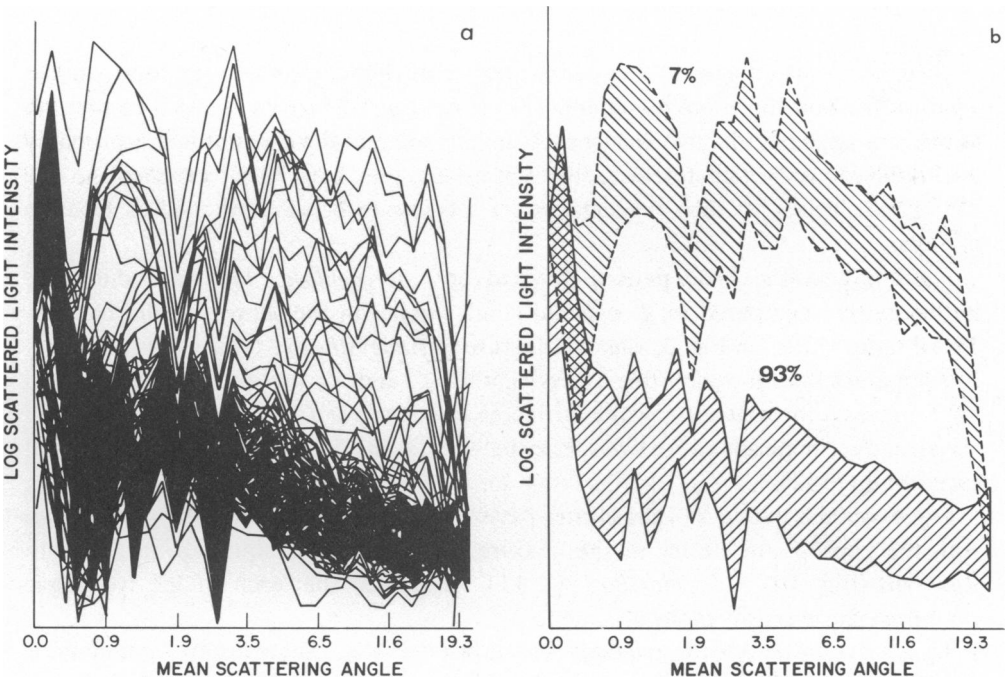

FIGURE 1 (*a*) Light-scatter patterns from 100 randomly selected cells of *Anacystis nidulans*. (*b*) Two clusters are identified by the clustering algorithm for *Anacystis*. The percent of cells in each cluster is indicated.

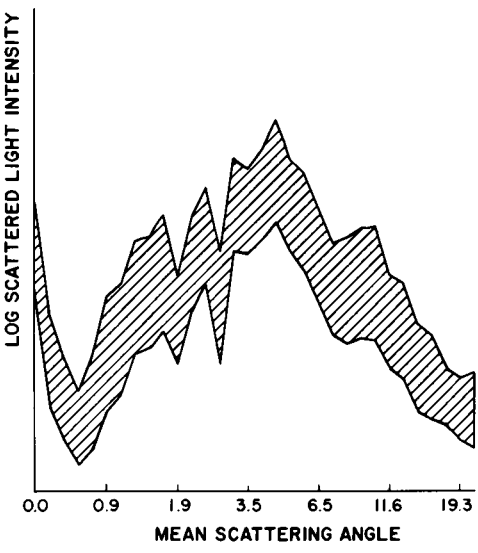

FIGURE 2

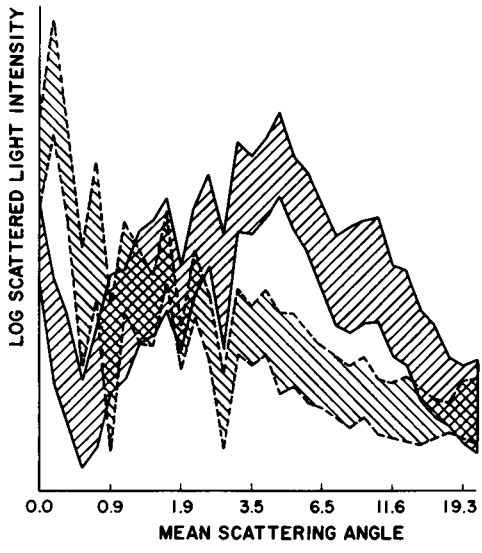

FIGURE 3

FIGURE 2 Cluster pattern for *Chlorella pyrenoidosa*.

FIGURE 3 Cluster patterns for a mixture of *Anacystis nidulans* (hatching with positive slope) and *Chlorella pyrenoidosa* (hatching with negative slope).

to their larger size, these cells would scatter more light, especially at lower angles where diffraction effects are dominant (12). Inspection of Fig. 1 *a* shows a continuum of patterns spanning the two clusters. Although some of these patterns are probably attributable to cells in intermediate stages of fission, they have been "assigned" to one of the two clusters, as their percentages are too low to be discriminated as separate clusters.

Fig. 2 contains the cluster pattern obtained for *C. pyrenoidosa*. The major difference in this pattern compared to *A. nidulans* (Fig. 1 *b*) lies in the shapes of the clusters. This is more evident in Fig. 3, where a mixture of *Anacystis* and *Chlorella* is analyzed. It is apparent that, for scattering angles between 1° and 5°, the intensity of scattered light decreases for *Anacystis*, while an increase is seen for *Chlorella*. This results in excellent discrimination of the two species between 3° and 5°. In fact, resolution is still excellent up to 12°.

Fig. 4 contains overlays of the cluster patterns obtained for each of the pure species with the appropriate cluster in the mixture. The overlap is quite good for both *Anacystis* (Fig. 4 *a*) and *Chlorella* (Fig. 4 *b*). It is clear that each of the two algae can be recognized easily in the mixture.

The cluster patterns for *C. reinhardii* are shown in Fig. 5. There are two populations virtually indistinguishable in size but differing in light-scatter intensity over the angular range between 3° and 5°. Cell replication studies (13) during the vegetative cycle have shown that there is a biosynthetic rate-limiting step that could effectively

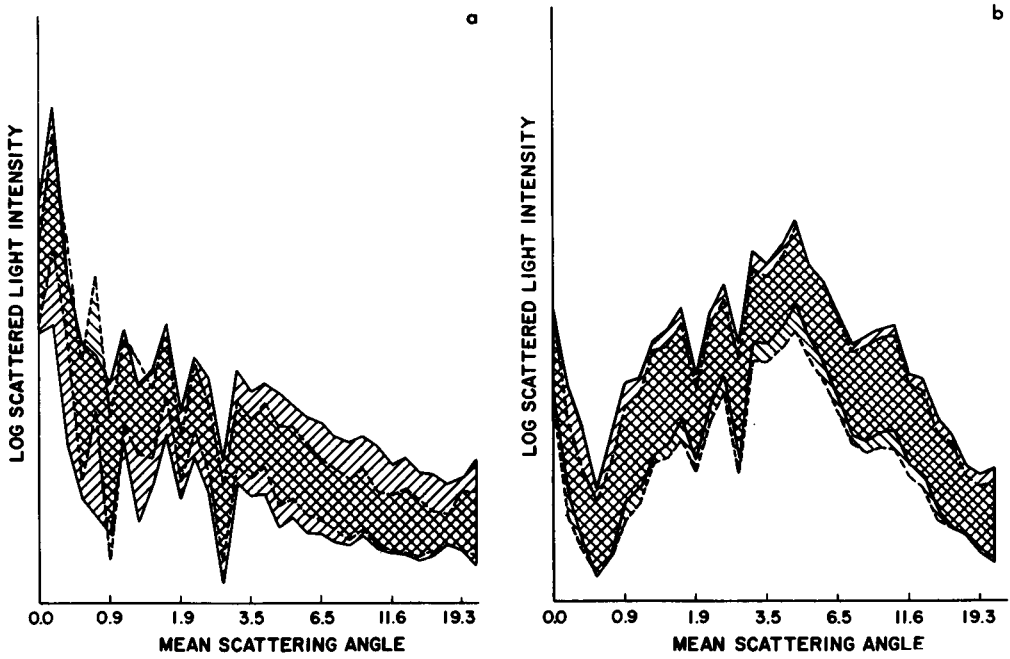

FIGURE 4 (*a*) Overlay of cluster patterns from 93% cluster of *Anacystis* alone (hatching with positive slope) with the *Anacystis* cluster in the *Anacystis-Chlorella* mixture (hatching with negative slope). (*b*) Overlay of cluster patterns from *Chlorella* alone (hatching with positive slope) with *Chlorella* from the *Anacystis-Chlorella* mixture (hatching with negative slope).

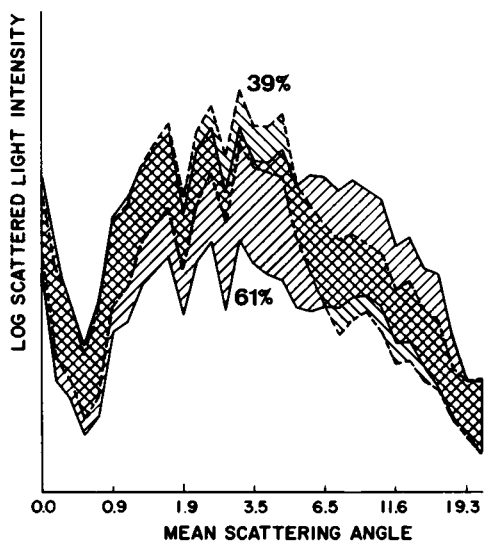

FIGURE 5 Cluster patterns for *Chlamydomonas reinhardii*.

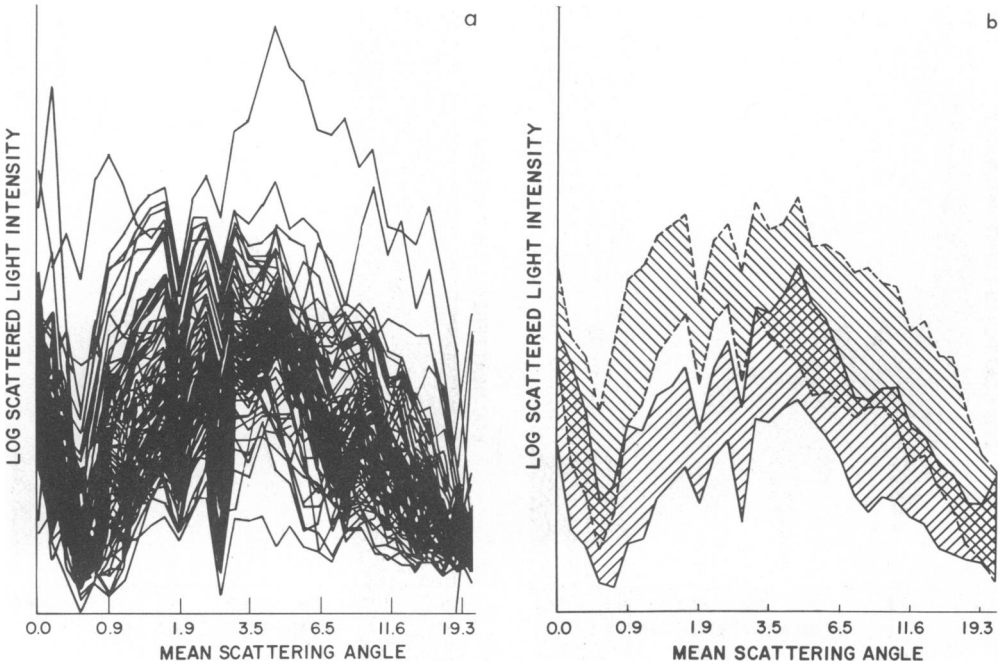

FIGURE 6 (*a*) Light-scatter patterns for 100 randomly selected cells from a mixture of *Chlorella pyrenoidosa* and *Chlamydomonas reinhardii*. (*b*) Cluster patterns for a mixture of *Chlorella pyrenoidosa* (hatching with positive slope) and *Chlamydomonas reinhardii* (hatching with negative slope).

divide the population into two subpopulations. Using a modification of the original Meselson and Stahl experiment (14), which demonstrated semiconservative replication of *Escherichia coli* DNA, Chiang (15) has demonstrated the biphasic nature of chloroplast DNA synthesis during synchronous vegetative growth of *C. reinhardii*. Their results suggest that there are two populations inherent within an asynchronous culture, consistent with what is seen in Fig. 5.

Fig. 6*a* shows the raw data obtained from a mixture of *Chlorella* and *Chlamydomonas*. It is not obvious from this figure that there is more than a single population. Even though the two species have very similar cluster shapes (cf. Figs. 2 and 5), the clustering algorithm effectively separates the two species, as demonstrated in Fig. 6*b*. Comparison of the two clusters shows that most effective discrimination is achieved between 1° and 2° (that is, on the basis of size).[2] Fig. 7*a* shows the overlay of the 61% cluster from pure *Chlamydomonas* (Fig. 5) with the cluster identified as *Chlamydomonas* in the mixture (Fig. 6*b*). The same is shown for *Chlorella* in Fig. 7*b*. The good overlap indicates that even in mixtures unambiguous discrimination of morphologically similar algal species can be obtained.

[2]It has been determined experimentally (12) that particle size is directly proportional to the intensity of light scattered in the forward lobe of the diffraction pattern. We have calculated (16) the first minimum in the diffraction pattern to be 2.2° for *Chlamydomonas* and 4.4° for *Chlorella*.

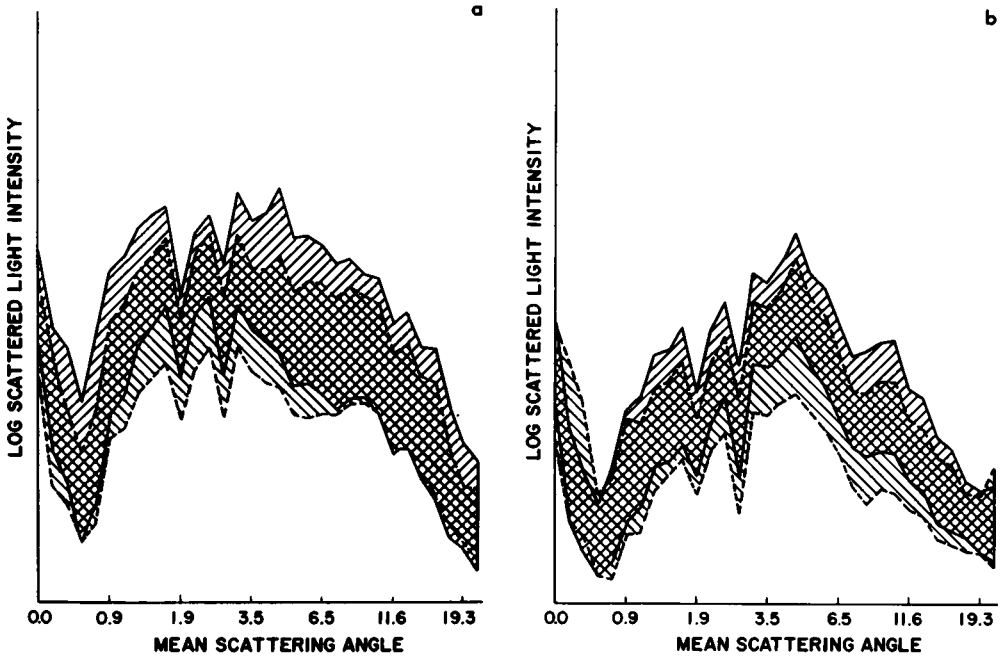

FIGURE 7 (*a*) Overlay of the 61% cluster from *Chlamydomonas* alone (hatching with negative slope) and *Chlamydomonas* from the *Chlorella-Chlamydomonas* mixture (hatching with positive slope). (*b*) Overlay of cluster pattern from *Chlorella* alone (hatching with positive slope) and *Chlorella* from the *Chlorella-Chlamydomonas* mixture (hatching with negative slope).

The data presented here demonstrate the ability to characterize asynchronous populations of pure and mixed populations of microalgae by light-scattering techniques. We have obtained scatter patterns characteristic for specific algal types using these methods. This technique should be a valuable tool for rapid acquisition of information concerning the condition of aquatic ecosystems using algae and other zooplankton as bio-indicators of freshwater and marine environments.

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