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Microcomputer-based Measurement of Algal Fluorescence as a Potential Indicator of Environmental Contamination

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The recent availability of inexpensive interfaces between microcomputers and video cameras have made possible a video imaging and analysis system which is able to measure the relative brightness of many individual points in a microscope image (Mayfield 1984). This paper describes one such system which has been designed to operate with a uv fluorescence microscope providing video images of the chlorophyll fluorescence of living algal cells. These images are then analyzed with the aid of an Apple II microcomputer. The effects of toxicants such as heavy metals and organic pollutants on chlorophyll fluorescence can be rapidly and easily measured with this system.

MATERIALS AND METHODS

An Apple II microcomputer with 64K total RAM memory (48K on the system board and 16K on an additional card) was equipped with a Digisector-65 video digitization interface card (Micro Works, Del Mar, CA). Briefly, this interface card accepts input from a black and white video camera or video cassette recorder and presents the image as a series of points on the Apple II high resolution graphics screen. There are different methods to digitize the image received; the simplest is to present only those points on the screen that are greater than a predetermined brightness value ranging from 0 to 63 (i.e., 64 brightness values). Controls on the interface card permit the brightness and contrast of the image to be varied. The resulting digitized image is a high contrast representation of the original video image. Programs supplied by the manufacturer of the interface card also allow these brightness limits to be adjusted under program control.

Alternatively, each point (called a pixel) in the video image can be individually addressed, and the current brightness value measured by the computer. With appropriate software a large number of points in a predetermined pattern can be addressed and evaluated. In this manner any video image can be converted to a series of brightness values and these values may be stored in a file on a disk drive attached to the

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microcomputer. The information contained in this file can then be further processed by an analysis program to extract and collate various types of information.

A Nikon Labophot microscope with a 100-watt mercury lamp providing epi-illumination through fluorescence objectives was equipped with a violet dichroic mirror and barrier filter system (Nikon Canada). The images of the chlorophyll fluorescence of the algal cells were relayed through a Panasonic WV-1050A high sensitivity black and white video camera to a video cassette recorder.

The video cassette recorder was interfaced through the Digisector-65 interface to the Apple II microcomputer for analysis of the images. A still frame image from the recorder was used as input for the digitization process. Still frame images are not essential for the digitization process but their use allows many more images to be stored on a tape because of the short taping duration required for each image. Normal taping and playback would require that each image be at least as long as the duration of the digitization process.

The program used for digitization was written partly in Applesoft BASIC and partly in assembly language. This assembly language programming was required because 10,000 individual points (pixels) on a square of 100 x 100 pixels were individually digitized and their brightness values measured. This process was too slow in BASIC (about 5 min) but was of acceptable duration (about 80 sec) in assembly language. As each point was addressed, its brightness was measured and the value stored in the memory of the computer. Upon completion of the scanning process, the entire 10,000 brightness values were transferred to a disk file. Since they were acquired in a known order, the image could be reconstructed using actual numbers for the brightness values (Fig. 1). In this case each brightness value was divided by 7 so that the values were between 0 and 9 on the printer. This was necessary because of limitation in the number of printing columns on the printers used.

Another program written in assembly language was used to analyze the information stored in the disk files. The program read in the 10,000 brightness values and categorized them into groups of 0-7, 8-15, 16-22, etc., up to 55-63. The average brightness of all points greater than 7 in brightness value was then calculated. Further analysis then calculated the average brightness of all points that were not black (i.e. all points greater than brightness level 0). The percentage of points with brightness levels greater than 0 was also calculated. These data were summarized and printed.

As a test of the video digitization program, a series of chlorophyll fluorescence intensity measurements were made of a pure culture of *Chlorella vulgaris*. The algal cells were exposed to $HgCl_2$ at a concentration of 50 mg l^{-1} in Bristols medium (Nichols and Bold 1963) and were examined with epifluorescent illumination after 1, 2, 4, and 22 h.

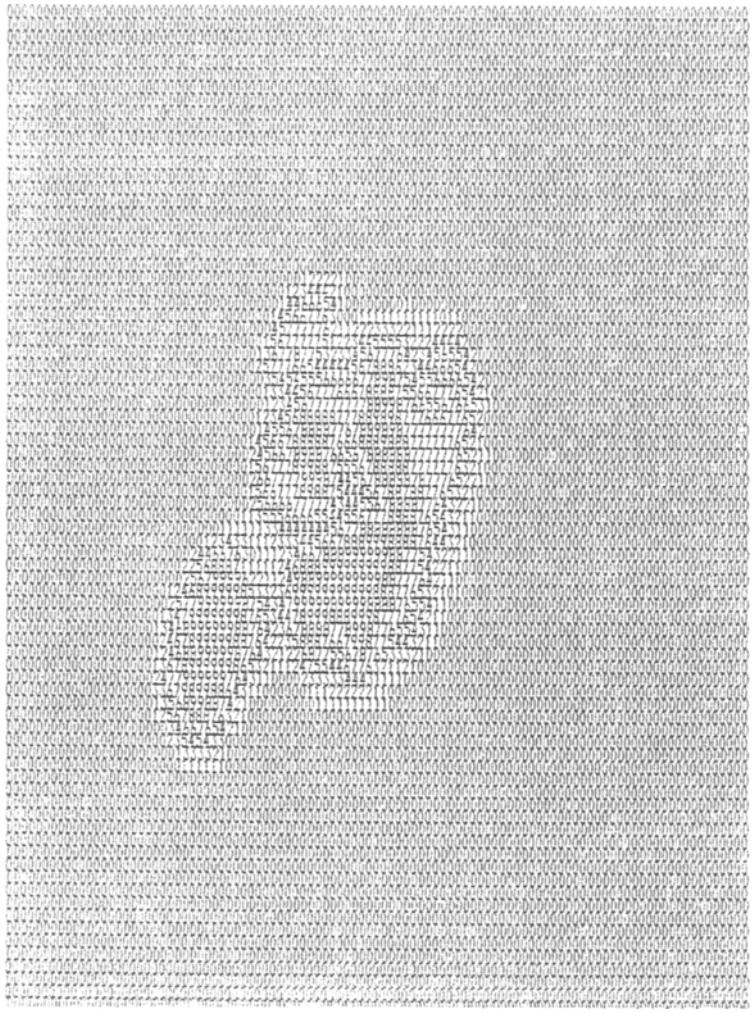

Figure 1. A representation of the brightness levels in a microscope image of *Chlorella vulgaris* cells. The brightness levels of 0 to 63 have been divided by 7 to allow the printer to format the image. Some vertical elongation of the image occured because of printer formatting limitations.

A total of 10 images for each time were digitized, and the data were stored onto a disk file.

In another test aboard the research vessel C.S.S. Bayfield, a natural phytoplankton assemblage was collected from an offshore station of Lake Ontario in the vicinity of Toronto from the 10-meter depth that showed a phytoplankton maximum. A water sample was collected, a portion was preserved with Lugol's solution for taxonomic identification and enumeration (Munawar and Munawar 1978) while a 4-l portion was concentrated by filtering through a 20 μm Nitex screen and then through a 0.45 μm Millipore membrane filter. The <20 μm size fraction of the algae was therefore collected on the 0.45 μm filter and the >20 μm

fraction, which was retained by the Nitex screen, was washed back and filtered onto a 0.45 µm filter. Both of these concentrated size fractions were washed back from wet membranes with a fast jet of water to a 4-ml total volume. A microscopic examination indicated that the >20 µm size fraction contained abundant organisms (Table 1) and it was therefore used as a test assemblage for the toxicity testing.

Table 1. Species composition of the >20 µm size assemblage of the phytoplankton used in the bioassay from Lake Ontario determined by the inverted microscope method.

Species	Abundance
Oscillatoria limetica Lemm.	+++
O. tenuis Agardh	+++
Chlamydomonas sp.	++
Ankistrodesmus falcatus var. spirilliformis G.S. West	+++
Scenedesmus bijuga (Turp.) Lagerheim	++
S. quadricauda (Turp.) de Brebisson	++
Coelastrum sphaericum Naegeli	++
Ochromonas sp.	+
Dinobryon sociale Ehr.	+
Kephyrion ovum Pascher	+
Nitzschia sp.	+++
Synedra acus Kutz.	+++
Gymnodinium varians Maskell	++
Peridinium sp.	+

+++ dominant
++ common
+ present

The selected >20 µm size fraction was examined immediately with the Nikon Labophot uv microscope, and representative fields were recorded with a Sony 2000 portable video recorder. Portions of the samples were then treated with two dosages of a mixture of metals (1X and 10X the recommended Water Quality Objectives for the protection of the Great Lakes biota, see Wong *et al.* 1978 and Munawar 1982). The control and the treated samples were then kept in the dark and transported back to the laboratory. After 20 h these samples were once again examined with the fluorescence microscope. Twenty representative fields for each treatment were prepared, recorded, digitized and analyzed.

RESULTS AND DISCUSSION

The effect of mercury on the average chlorophyll fluorescence intensity of *Chlorella vulgaris* cells is presented in Fig. 2. Progressively longer exposure to mercury decreased the average chlorophyll fluorescence of the cells. All were significantly different at the P=0.01 level.

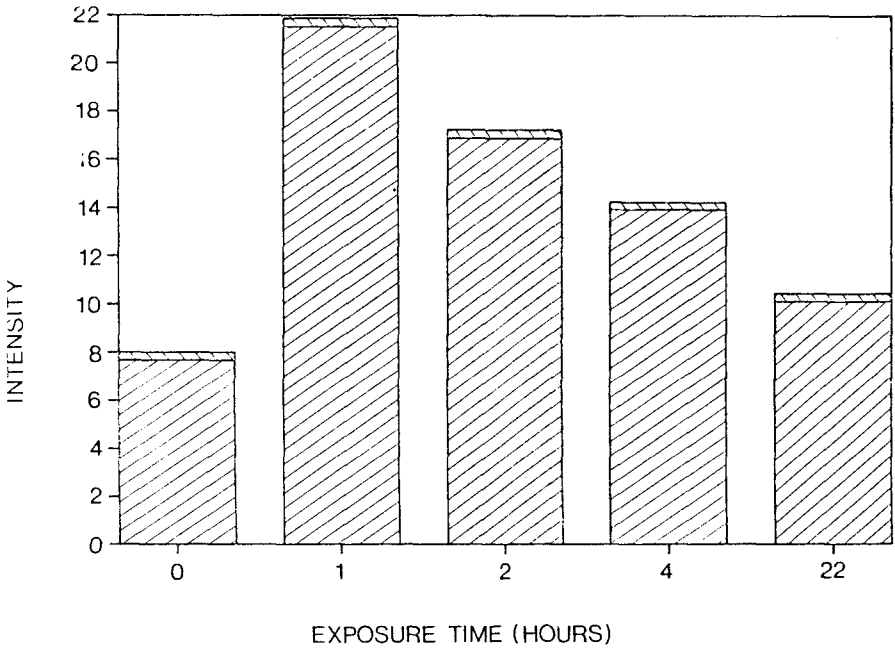

Figure 2. Effects of 50 μg ml^{-1} $HgCl_2$ on chlorophyll fluorescence intensity of *Chlorella vulgaris* cells under uv illumination.

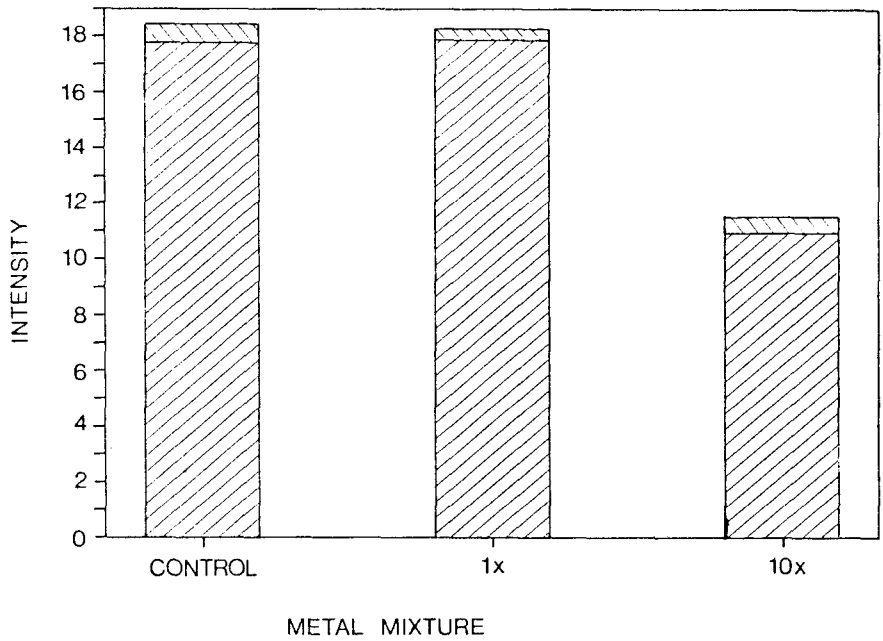

Figure 3. Effects of 1X and 10X metal mixtures on chlorophyll fluorescence intensity of a natural phytoplankton assemblage from Lake Ontario.

Treatment of the natural population from Lake Ontario with 1X and 10X the recommended levels of metals resulted (Fig. 3) in a significant decrease in chlorophyll fluorescence at the 10x concentration. There was no significant decrease after the 20 h exposure at the 1X concentration.

The two tests of the method showed that it was able to distinguish changes in chlorophyll fluorescence intensities due to treatment with mercury and mixtures of metals. More comparative testing is required to validate this toxicity measurement approach, but it seems that the responses of the chlorophyll to uv light during testing differs according to the prior treatment with metals.

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