Computer-based image analysis for the automated counting and morphological description of microalgae in culture

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Abstract

A largely unexplored area is the application of digital image processing to counting and sizing of microalgal cells from culture. Commercial systems are available, but have not been tested, nor necessarily optimized for high speed counting and sizing of phytoplankton. The present work describes the design, construction, specifications and comparative performance of an inexpensive system optimized for counting and sizing microalgal cells. This system has been tested with cells of the picoplankton to nanoplanckton size ranges (1–20 μm). The hardware was a widely available standard microcomputer, an inexpensive video camera and monitor, and a video digitization board (frame grabber). A modifiable menu-driven program (PHYCOUNT) was written and provisions made to make this program available to other workers. The program is constructed such that it can be adapted to a variety of hardware setups (Video digitization boards). Comparison of growth curves for microalgae revealed there were no significant differences in division rate and cell yield as assessed by the image analysis method compared to manual counts with a hemacytometer. Several hundred cells were counted routinely within 10–15 s, far exceeding the counting rate achieved by hand tally. A variable transect feature allowed sampling every nth pixel and provided a substantial increase in execution speed. More than 1000 counts can be done per day. A protocol for the use of 96-well plates of polyvinyl chloride as counting chambers contributed to the processing of large numbers of samples rapidly. Other routines developed provided subtended area, defined the coordinates of cell perimeter, and derived cell length and width. The calculation of the latter two parameters was usually done off-line as data output is in standard numerical form accessible by other programs. Experience with daily use of the PHYCOUNT program and imaging hardware reveal that the system is reliable for cell counting and sizing. The presence of bacteria in the algal cultures does not affect cell counting or sizing.
Introduction

Standard methods for the evaluation of growth in microalgae include manual cell counting (Guillard, 1973), pigment analysis (Hanssmann, 1973) and turbidity (Sorokin, 1973). Automated methods include resistive-type counters (Parsons, 1973) or laser-based zone counting (Boyd, 1978).

One approach which has not been as widely explored is the use of digital image processing to count cells. We chose to examine this for a variety of reasons. The similarity to manual counting by microscopy is a particular advantage, as this is considered to be an accurate method by many workers and is widely employed. Another advantage is that the image analysis hardware contains no moving parts, thereby decreasing potential downtime. This method is also unaffected by the presence of bacteria in cultures which might affect other methods such as turbidity measurement.

There are commercial units which remain untested in comparison to standard manual methods for assessment of cell division rate in microalgal cultures. Another problem with commercial systems is their high cost which puts them out of the reach of many laboratories interested in using them as dedicated systems for the assessment of cell growth (Cynar et al., 1985). The system described is inexpensive (see Discussion). Commercial programs are often embedded in hardware or source programs are not available to the user, preventing modification or customization. Such software is often proprietary (Sieracki et al., 1985).

The aim of the present work was to determine whether a completely automated image analysis systems devoted to microalgal work could be constructed from readily available components and demonstrated to function effectively in comparison to standard methods. This has not been done for either a commercial or non-commercial system. Particular attention was paid to constructing the system from widely available and inexpensive parts to ensure the widest possible potential applicability for workers in other laboratories. Requirements for the program included high speed execution as counting functions are ordinarily applied to large numbers of experimental samples, and data output in a form easily usable in other programs.

Materials and methods

Organisms and culture conditions

The origin, taxonomic status and cultivation of chlorophyte Nannochloris bacillaris Naumann (isolate UWASH 20-2-2) has been described previously (Brown & Elfman, 1983). This organism is a picoplankter with cell diameter of approximately 1.4 μm. Also, two species of the nanoplankton size range were studied with this system (both approximately 10 μm in diameter). The first was a freshwater member of the Prymnesiophyceae, Chrysochromulina breviturrita Nich. Its origin, and methods for culture have also been described (Wehr et al., 1985). The second nanoplankter, Euglena gracilis (University of Western Ontario strain #40) was grown in an inorganic medium supplemented with 50 mL/L soil extract (Smith & Wiedeman, 1964) at 20 °C under 60 μmol photon m⁻² s⁻¹ illuminance from cool-white fluorescent lamps. All cultures were axenic with the except for E. gracilis which was bacterized.

Microscopy

A Leitz Ortholux research microscope was used with brightfield optics. Kohler illumination was essential for this work with a carefully centered light source and bright, even illumination to allow good gray level discrimination by the computer. Evenness of illumination was usually tested by increasing the contrast control on the image monitor (see below) and decreasing brightness to a low level to emphasize bright areas and shadows. Illumination was adjusted so that these areas are eliminated. Depth of field was adequate and presented no problems if care was taken to allow cells settle to the bottom of the chamber.