Computer aided fluorescence imaging of photosynthetic systems: Application of video imaging to the study of fluorescence induction in green plants and photosynthetic bacteria

James M. Fenton & Antony R. Crofts
Department of Physiology and Biophysics, University of Illinois, 407 S. Goodwin Ave., Urbana, IL 61801, USA

Received 7 September 1989; accepted in revised form 1 May 1990

Key words: Video imaging, fluorescence induction kinetics, photosynthesis

Abstract

A fluorescence video imaging system utilizing relatively inexpensive commercial components is described. The instrument utilizes a black and white CCD video camera detector, a commercial video imaging board and an IBM-AT compatible computer. The color output of the imaging board greatly aids in the user's ability to visually discriminate areas of interest in the video field. Software development that enables the user to capture kinetic traces in real time from the video images is also described. The system is used to monitor fluorescence from photosynthetic systems. The usefulness of the system in screening for photosynthetic mutants is also demonstrated. The cost of the system can be kept below $12,000.

Abbreviations: CCD – charge-coupled device; DCMU – diuron, 3-[3,4-Dichlorophenyl]l,l-dimethylurea; AGC – automatic gain control; LUT – look-up table; AOI – area of interest; CPU – central processing unit; RAM – random access memory; ADC – analog-to-digital converter; FVIPS – fluorescence video image processing software; I/O – input/output; F_0 – dark-level fluorescence; OIDPSMT – characteristic transient components, where O is dark level, I is intermediary peak, D is dip, P is peak of fast transient, S is quasi-steady state level, M is second maximum, T is terminal level

Introduction

The fluorescence emission from photosynthetic systems provides a simple and non-invasive means of studying the photochemically initiated electron transfer reactions that occur in the photosynthetic membranes. Since fluorescence from photosynthetic systems is inversely related to the probability of photochemical trapping, the fluorescence induction caused by a dark-light transition provides useful information on the overall rate of flow of electrons through the electron transport chain (Papageorgiou 1975). Although fluorescence methods have been used to provide information about the physiological state of intact plant systems, or to screen for mutant strains of algae and bacteria, most experimental systems have used a single element detector, and have therefore been restricted to sampling an average from a region of interest (Schreiber 1983, Bennoun et al. 1977). It would often be convenient to be able to measure fluorescence from several regions of interest, or to be able to observe an image of the fluorescence intensity from a field of view. Fluorescence imaging has been used by Youvan et al. (1983) through infra-red photography, to screen for high-fluorescence mutants of photosynthetic bac-
teria, and more recently Youvan has explored the use of reflectance spectroscopy imaging (Yang and Youvan 1988) as a screening method. Daley et al. (1989) have also used video imaging techniques to visualize the non-uniformity of non-photochemical quenching in leaves after the addition of abscissic acid. By combining the measurement of the induction curves with video imaging techniques, Omasa et al. (1987), have shown that it is possible to observe a variety of conditions which affect the electron transport chain, such as the incorporation of herbicides into leaves, and inhibition of photosynthesis by industrial pollutants.

In the case of algae, small plants and photosynthetic bacteria, the antagonism between fluorescence and photochemistry provides a useful technique for the rapid screening and characterization of photochemically and photophysically interesting mutations. In order to provide more than a cursory screening, the method requires that induction curves should be measured from areas of interest of a few square millimeters, corresponding to the size of typical bacterial colonies on plates. This has provided the main impetus for the design and construction of the instrument described in this paper, which was developed to follow the rapid induction curves (<1 s) exhibited by photosynthetic bacteria, is sensitive to infrared light out to 1100 nm, and provides a simple, and relatively inexpensive, method of collecting fluorescence induction data in real time, and of rapidly presenting this information to the user through image analysis and processing.

Materials and methods

Apparatus

The fluorescence video imaging apparatus consisted of an optical box containing illumination facilities, sample chamber, filters, shutter, and a miniature CCD-device television camera. This was linked to a video processing board in a desk-top computer equipped with suitable displays to present video and graphic data to the user. An outline of the apparatus is shown in Fig. 1.

In the system presented here the processor used to digitize and store video image data was a Matrox MVP-AT board (Matrox Electronic Systems Ltd., Dorval, Quebec). The board plugs directly into 2 slots inside an IBM PC-AT or compatible computer. In the present apparatus, a PC’s Limited 286 computer (now marketed by Dell Computer Corp., Austin, Texas) was used, and operated at 6 MHz in order to maintain compatibility with the MVP-AT board. The MVP-AT provides 1 megabyte of memory in which up to four $512 \times 512 \times 8$ bit video images can be stored. The video memory is dual-ported RAM that can be directly accessed in 64 kilobyte blocks by the CPU, thus allowing rapid and independent computer access to the video information. This ability was used to develop software that allows kinetic information at specific locations to be stored in real time. Additionally, the board provides a powerful math processor.