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MEASURING THE DELAYED FLUORESCENCE AND CHEMILUMINESCENCE
OF CHLOROPHYLL IN VIVO

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This article will describe simple ways to measure delayed light emission and chemically induced luminescence of chlorophyll (Chl) in green plants and algae, using relatively inexpensive equipment (total cost less than \$2000 in 1970). The basic needs are: (1) A detector (photomultiplier) that is sensitive enough in the red, from about 650 to 750 nm, operated in conjunction with an amplifier and a strip chart recorder or an oscilloscope. (2) A means of first subjecting the plant material to a program of illumination and/or chemical change, and then quickly presenting it to the detector. The provision for illumination is essential, even when chemiluminescence is to be measured, because the chemically induced luminescence of Chl in vivo generally require that the system first be activated by light¹.

In the ensuing description some specific commercial components are mentioned. These are given for the convenience of the reader, but are only representative of a great variety of comparable and competitively priced components.

A very simple and useful arrangement is shown in Fig. 1. It is only necessary to purchase a Photomultiplier Microphotometer from American Instrument Co., Silver Springs, Maryland, plus a Bausch and Lomb VOM-5 Recorder from any major scientific supplier, and a small spotlight such as is used as a microscope illuminator. The Microphotometer should be chosen with a Type 1P22 photomultiplier, for sensitivity in the red. The photometer head has a filter housing in which a sample cell (Cuvette or small test tube) can be supported to receive material from an external syringe. A red filter, such as a Corning No. CS2-58, can be placed between the sample cell and the photomultiplier in order to reduced the response to "false" light.

In operation the plant material, a suspension of chloroplasts or algae, is held in the syringe and a reagent can be placed, if desired, in the sample cell. After a suitable illumination program the material is injected into the sample cell and its luminescence is recorded. A second syringe could be fitted so as to deliver a second chemical perturbation after the plant material has been injected into the sample cell.

The foregoing instrument is limited because of its marginal sensitivity in the red and its time constant of about 0.1 sec. With a little effort one can make an apparatus designed more specifically for the purpose at hand, at a cost of less than \$1200 including shop charges but excluding the recorder or oscilloscope.

As shown in Fig. 2, the optical arrangement can be extended to allow illumination of the material in the sample cell as well as in the syringe. This permits measurements with leaf sections and other structures too large to pass through a syringe. It also allows measurement of the prompt fluorescence, using a combination of blue exciting light and a red filter in the photomultiplier window to intercept scattered exciting light. A suitable combination of filters is Corning Nos. 4-96 (blue) and 2-64 (red).

The best choice of photomultiplier is one having the S-20 spectral response, which affords high sensitivity in the red together with a reasonably low dark current. While an improvement can be had by cooling such a photomultiplier to dry ice temperature, this is not necessary in most application. Suitable photomultipliers are: E. M. I. Type 9558B or 9698B (Whittaker Corp., Gencom Div., 80 Express St., Plainview, New York), and RCA Type 7265 or 7326 (Radio Corp. of America, Electron Tube Division, Harrison, New Jersey).

A satisfactory way to apply high voltage to the photomultiplier is shown in Fig. 3 a. There are many regulated DC high voltage supplies on

the market, designed specifically for use with photomultipliers. One such is the Model 245 made by Keithley Instruments, Inc., 28775 Aurora Road, Cleveland, Ohio.

The photocurrent can be delivered to a commercial "electrometer amplifier" or "picoammeter", costing in the neighborhood of \$600 to \$1000; for example the Model 602 Electrometer made by Keithley Instruments. However, a simple and completely suitable electrometer amplifier can be made at much less cost, using a high quality operational amplifier such as the Type PP25C from Philbrick Researches, Inc., Allied Drive at Route 128, Dedham, Massachusetts. This little feedback amplifying module is powered by about 5 ma at ± 15 v DC, obtained from batteries or from Philbrick's Model PR-30 power supply. The amplifying circuit is shown in Fig. 3 b. With a resistance of 10^9 ohm in the feedback loop as shown, an input of 10^{-9} amperes is transformed into an output of 1 volt. A "precision" 10^9 ohm resistor can be obtained from Keithley Instruments or from Victoreen Instrument Co., 5806 Hough Ave., Cleveland, Ohio.

The output of the amplifier can be fed into any suitable recorder, such as the Bausch and Lomb VOM-5 mentioned earlier, or into an oscilloscope. The circuit of Fig. 3 b includes a variable resistance-capacitance smoothing network by which faster components of noise can be eliminated (at the cost, of course, of a slower response). The values of R and C in this network were chosen to be compatible with a recorder or oscilloscope of high input impedance (about one megohm or more).

The foregoing instruments have been chosen for their simplicity. The investigator who wishes to construct a Becquerel Phosphoscope², or a stop-flow apparatus fitted for optical work³, or a pumped circulating

or "continuous squirt-mixing" arrangement will enjoy certain advantages, but the simple method described here is useful for a great variety of experiments.

REFERENCES

1. B. C. Mayne, Photochem. Photobiol. 8, 107 (1968).
2. R. K. Clayton, Biophys. J. 9, 60 (1969).
3. Q. H. Gibson, and L. Milnes, Biochem. J. 91, 161 (1964).

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Fig. 1. A simple arrangement for measuring the luminescence of chlorophyll in chloroplasts and algae, using a Photomultiplier Microphotometer from American Instrument Co. (see the text).

Fig. 2. A more elaborate arrangement for measuring chlorophyll luminescence and fluorescence in vivo, using components as described in the text and in Fig. 3.

Fig. 3. (a) A generally satisfactory way to connect a photomultiplier. The cathode receives negative high voltage up to about -1000 v. (b) An electrometer circuit using an operation amplifier (see the text) to convert a small photocurrent to a voltage signal compatible with a recorder or oscilloscope. An adjustable smoothing filter is included.

(a)

(b)

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Fig. 3