INTRODUCTION

Chlorophyll, the photosynthetic pigment in all plants, is a fluorescent molecule, thus it can be determined by fluorometry. Fluorometric techniques are now well established for both qualitative and quantitative measurement of the chlorophylls and pheophytins. For many applications, they have replaced the traditional spectrophotometric methods and have made analysis in the field practical.

ADVANTAGES

Fluorometric methods have many advantages over other methods. As one author stated, "Chlorophyll *a* was selected because... it is the only index of phytoplankton abundance presently available that can be measured by a continuous *in-situ* technique..."(1). According to another researcher, "The relative simplicity of these techniques enables much information to be rapidly gathered..." (2). A comparison study conducted by the U.S. Environmental Protection Agency has shown that fluorometric methods compare favorably with spectrophotometric results (3). Fluorometry has the following advantages over spectrophotometry:

- **Sensitivity:** Fluorometry is at least 1,000 times more sensitive than the spectrophotometric techniques (4 ,5). Up to 10 liters of water may be required for a single spectrophotometric chlorophyll determination (6, p. 186), but the fluorometer can obtain the same data from samples of 500 ml or less.

  Sometimes the large volumes required for spectrophotometric determination are nearly impossible to filter because of clogging problems.

  The spectrophotometric determination of chlorophyll involves filtration, disruption of the cells, and extraction of the chlorophyll, followed by absorbance measurements. The same extraction technique can be used to produce samples for fluorometric determination, with the advantage of greater sensitivity and thus smaller sample requirements.

- **Speed:** With a spectrophotometer, one must measure absorbance at several wavelengths (4, 6, p. 189); with the fluorometer, only one setting is needed.

- **Wavelength Settings:** Good results with the fluorometer do not depend on critical wavelength settings (4, 6, p. 191).

- **Cuvettes:** Fluorometric measurements are not critically dependent on cuvette handling and matching. Ordinary round borosilicate culture tubes normally are used as cuvettes for discrete samples.

- **On-the-Spot Results:** For many applications, the fluorometer can go on location and be used *in-vivo*, on a continuous-flow basis, eliminating delays for extraction, processing, and laboratory measurement. The field fluorometer can even operate on battery power in a small open boat. Information is continuously and immediately available. Thus the operating plan can be changed immediately according to interim results instead of having to wait for laboratory results and make repeated field trips.

IN-VIVO AND EXTRACTIVE METHODS: OVERVIEW

Where chlorophyll-containing organisms are small enough, as with phytoplankton, fluorescence may be measured directly, without extraction or chemical treatment. For many kinds of qualitative work, *in-vivo* measurement alone may answer the experimenter's questions. For quantitative determinations, the *in-vivo* data are calibrated by correlation with other measurements. (See CALIBRATION AND STANDARDS.)

In-vivo fluorescence measurements may be taken either: 1) On continuously flowing water with the **10-AU Field Fluorometer**; or 2) On discrete water samples ("grab samples") in the field with the **10-AU Field Fluorometer**, or in the laboratory with the **Trilogy Laboratory Fluorometer**. (This equipment is discussed further under SAMPLE SYSTEMS and EQUIPMENT SELECTION.)

IN-VIVO MEASUREMENTS

Direct fluorescence measurements of living cells have been put to many imaginative uses, and developments in this area are continuing at a rapid pace today.

Distribution and Life Pattern Studies

The first efforts at using fluorometry for chlorophyll determination in the field (7, 8, 9, p. 121) led experimenters to appreciate the variety of natural-distribution studies that the technique would permit. It was and remains the only way really to cover an area quickly for studies investigating the horizontal and vertical distribution of phytoplankton. Several broad types of studies have been done:

***In-vivo* Mapping** measurements have been used in both fresh and marine waters to develop population profiles (1, 2, 10, 11). Ocean mapping, on its own or as a verification tool for aerial and satellite surveys, has provided information for studies such as those on the location of upwelling of deep ocean water (fishing areas) (12, 13, 14, 15). Mixing patterns in lakes, estuaries, and ocean waters have been examined (11, 16, 17). The *in-vivo* method has been used to follow the progress of dinoflagellate blooms (18, 19), and the effect on productivity over an area affected by sewage discharges (20). A recent technique employed fluorometry for high-speed mapping of chlorophyll *a* in aquatic systems (21).

Vertical distribution studies. Dropping a probe and pumping water continuously or collecting grab samples below the level where pumping is practical has permitted vertical as well as horizontal mapping in studies aimed at understanding phytoplankton distribution (2, 13, 22-29).

Sinking-rate studies. Techniques for laboratory measurement of sinking rates of phytoplankton in both marine (30, 31) and fresh waters (32, 33) have been developed. These methods, although different, both involve determination of time required for cells to fall through the illumination area of a cuvette. Sinking-rate studies both in open ocean (34) and in lakes (35) have been done also.

Characterization of populations without resorting to microscopic counts has been another area of investigation (33, 36, 37). The effect of sewage discharges on species composition has been studied (38).

Biomass, standing crop, primary production. Much work has been done, with varying results, in attempts to relate *in-vivo* fluorometric measurements to other quantitative measures (1, 2, 9, p. 121, 10, 19, 20, 22, 26, 39, p. 57, 40, 41, 43 - 46). These studies have led to work on factors affecting fluorescence efficiency and the use of photosynthesis-inhibiting poisons.

Stress Effects

Variable results in the relationship of population level to *in-vivo* fluorescence, as well as environmental concerns, have inspired studies of the response of phytoplankton to many single-parameter stresses. The effects of metal ions, both as required, limiting nutrients and as growth-limiting toxins, have been examined, as have the influence of other toxins and of light levels (25, 33, 38, 39, 41, 43, 44, 45, 47 - 55). One might suspect that Kautsky effects would occur on entry of a dark-adapted sample into the lighted sample compartment of the fluorometer, but the illumination level (about 2 w/m$^{2)}$ is so low that this effect is not seen.

Water Quality

In related work, phytoplankton response to complex man-made interventions has been measured. Several studies have examined the effects on phytoplankton of power-plant cooling-system entrainment, including chlorination and the presence of other algicides and toxins as well as thermal stress. Heat has been found to have less effect than toxins. Some of these studies involved the use of extractive techniques: however, the results might have been more quickly and economically obtained through *in-vivo* measurement (44, 56 - 59).

Eutrophication of lakes, a matter of environmental interest, has been the subject of both historical and predictive studies (20, 60, 61). Related work has covered the effectiveness of sewage treatment processes (38, 48, 51).

Work with the Algal Assay Procedure bottle test (AAP:bt) of the U. S. Environmental Protection Agency has often involved fluorometry. Although there are difficulties stemming from the fact that this test attempts to relate laboratory cultures to natural populations, the test is widely used. (39, 40, 41, 44, 47; see also Bibliography.)

The effects of marine dumping of wastewater sludge have been studied in the Gulf of Mexico and in the New York Bight Apex (25, 28). Guidelines for such bioassays have been issued by the U.S. Environmental Protection Agency and the U. S. Army Corps of Engineers (44, 62).

The cultivation and nutrient balance of large-scale phytoplankton cultures for aquaculture/mariculture also has been examined with the use of the fluorometer (63).

Calibration, Standard, and Interferences: Overview

The readout produced by a fluorometer is relative and therefore must be related to the concentration of a standard during calibration. A standard is a known concentration or a known dilution of the substance to be measured. For studies on natural populations frequent calibration against a chlorophyll standard (64, 65) is important because:

1. The amount of organic substance associated with a given quantity of plant pigment varies widely, depending on the class and health of the organisms. For example, the conversion factor between chlorophyll *a* and total plant carbon can vary from 25 to 100 (6, p. 185) and, in special cases, even more (9, p. 121).

2. The presence of humic materials, detritus, or competing dissolved fluorescing compounds may or may not interfere, depending on the nature of the study (36, 49, 52, 61, 64).

3. The *in-vivo* fluorescence efficiency of chlorophyll is species dependent (8, 10, 18, 19, 36, 45, 49, 50, 52, 53). It also depends on the age of a culture (43, 53).

4. The *in vivo* fluorescence efficiency of chlorophyll depends on the history of light exposure of the organisms. It is thus related to mixing history and diurnal cycles (23, 36, 43, 45, 49, 50, 52, 66).

5. Fluorescence efficiency *in-vivo* also depends on nutrient availability and the presence of toxins (36, 44, p. 11, 45, 50 - 53).

Anyone considering the use of *in-vivo* chlorophyll techniques should read the excellent articles by Kiefer (49, 50) and Loftus and Seliger (52). These papers indicate that stress effects may be greatly reduced and that much can be learned about the physical condition of the organisms under study.

EXTRACTIVE MEASUREMENTS

Fluorometric measurements on solvent extracts from disrupted cells usually are made to estimate the amount of chlorophyll and pheophytin (a degradation product of chlorophyll, which represents chlorophyll that has lost the central Mg ion). The extracted chlorophyll methods can be performed with a **10-AU Fluorometer** equipped with a discrete sample adaptor or with a **Trilogy Laboratory Fluorometer**.

The extractive method has been used in studies and is well established for quantitative use in the laboratory (27, 41, 56, 59). For most practical work, the pigment of interest is chlorophyll *a* and its degradation product, pheophytin *a*. Detailed procedures for estimating these fluorophores are available (4, 6, p. 201, 14). The U.S. Environmental Protection Agency Method 445.0 provides a step-by-step procedure for estimating chlorophyll *a* and pheophytin *a* (42). Basically, these methods involve six steps: filtration, extraction, measurement, acidification, remeasurement, and calculation.

Filtration

The sample is filtered under a vacuum through a glass fiber or Millipore filter. (For detailed procedure, see reference 42.)

Extraction

Refer to U.S.E.P.A. Method 445.0 for step-by-step extraction instructions (42). The central problem in extractive chlorophyll assay by any method always has been the quantitative removal of chlorophyll from the cells. Three types of solvent systems have been used in most work: 90% acetone, methanol, and DMSO. It should be noted that whatever procedure is used, chlorophyll is unstable in the presence of acid and of light. Trace amounts of acid will convert chlorophylls to the corresponding pheophytins; therefore, be sure your glassware is free of acid. Sunlight or fluorescent light will degrade chlorophylls very rapidly; incandescent light, in a matter of tens of minutes. Procedures should be carried out in subdued light, with dark storage between steps.

Grinding before extraction is recommended (4, 5, 39). A study of the efficiency of extraction of chlorophyll shows that grinding increases the release of chlorophyll by 5% to 60% (5). For marsh grass, a wrist-type shaker and stainless steel balls have been used (68). Some work with DMSO has been done without grinding (69, 70).

In both spectrophotometric and fluorescent work, 90% acetone is widely used for extraction. Some procedures recommend centrifuging this extract before measurement. This step can be omitted for fluorometry, since the technique is insensitive to turbidity at the usual levels. If high blanks occur or you are unable to zero the fluorometer with an acetone blank, you may find that your acetone is contaminated. Contamination of acetone with rolling oil from the manufacture of metal storage vessels is common.

Methanol is reported to be more efficient than acetone under some circumstances (70). Hot methanol also has been used (72). This extract is measured directly when high accuracy is not required and pheophytin levels are low. For greater accuracy, and where appreciable pheophytin is present, the methanol extract is dried and transferred to 90% acetone. The spectra of pheophytins *a* and *b* are pH sensitive in methanol, but not in 90% acetone. If this pH sensitivity were reversible, corrections could be made in the calculations, but it is not. The result is anomalous behavior in spectrophotometric determinations (70), and the same would be expected with fluorometric analysis.

Dimethyl sulfoxide (DMSO) has been effective for the extraction of macrophytic brown algae, which are resistant to extraction by other means (69). It also has been used successfully with diatoms and blue-green algae but has worked less well with coccoid green algae unless grinding is done (72). In both cases, extraction appeared complete, with no pigment left in the residue. DMSO apparently disrupts the internal plastid membrane structure of the cell in brown and blue-green algae.

Seely, et al. (69) followed DMSO extraction of brown algae with acetone extraction and a separation procedure, but Shoaf and Lium (72) went on to compare absorption spectra of acetone dilutions of DMSO extracts of diatoms and blue-green algae with those of 90% acetone extracts. The absorption spectra were almost identical, and acid ratios (see below) were the same in both.

Some workers have proceeded to separate extracted pigments by paper chromatography before measurement, rather than continue with the measurement and calculation sequence to be outlined below (73).

Measurement

The extract is poured into a cuvette (normally a borosilicate culture tube) and measured using the fluorometer. Where pheophytin or other interfering pigments are not present, this reading is directly proportional to chlorophyll concentration. Where pheophytin is suspected, acidification and remeasurement follow (unless the Welschmeyer Non-acidification Method is being used; see section below).

Acidification and Remeasurement

Note: The following section applies only to traditional chlorophyll measurements which require a measurement after acidification. If you are using the Welschmeyer Non-acidification Method, you will skip this step and the calculation step that follows (see next section).

Addition of acid converts chlorophyll to pheophytin. In early work, acetone saturated with oxalic acid was used (5). Now HCl is used almost exclusively, because the reaction is more rapid and complete (4). Conversion is nearly instantaneous for chlorophyll *a*, complete in about two minutes for chlorophyll *b* and three minutes for chlorophyll *c* (74).

Those using the methanol extraction technique should review the work of Marker (70), since anomalous effects do occur on acidification.

After acidification, the fluorescence of the sample is again measured. The ratio of the fluorescence before acidification (R_b) to that after (R_a), or R_b/R_a, is called the acid ratio (6, p. 203 Refer to U.S.E.P.A. Method 445.0 for step-by-step acidification instructions (42)).

The Acid Ratio

Saijo (34, 75) has shown that values from unity to 11.5 may be obtained if the excitation wavelength is varied from 410 to 440 nm in a spectrofluorometer. The primary reason for this shift is that the excitation wavelength of chlorophyll *a* is at about 440 nm, while that of pheophytin *a* is at about 420 nm.

Chlorophylls *a*, *b*, and *c* and their pheophytins are quite different in both their excitation and their emission wavelengths. Each pair has its own acid ratio. A careful study of the acid ratios of various mixtures of pure chlorophylls *a* and *c* shows this effect (4). Use of an emission filter that sharply rejects the fluorescence of chlorophyll *c* and pheophytin *c* makes the acid ratio independent of the amount of chlorophyll *c* present.

The acid ratio also is affected by photomultiplier, lamp, and optical filter characteristics. Thus it must be determined individually for each instrument and must be checked if components are changed.

The Welschmeyer Non-Acidification Method

This method (71) is very useful for determining chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. It does not require the acidification step. It employs narrow band optical filters to measure primarily chlorophyll *a*, excluding most chlorophyll *b* and the pheopigments. Under the most extreme ratio of chlorophyll *a/b* likely to occur in nature, the conventional acidification technique may result in an approximately 60% underestimation of chlorophyll *a*. The non-acidication method yields only a 10% overestimation of true chlorophyll *a*. The U.S.E.P.A. has shown that the technique compares favorably with conventional fluorescence acidification and spectrophotometric methods (3).

Calculations

The most general case for calculation of the amounts of various pigments present is given, with a scheme of analysis, in reference 77. For most work, however, such detail is not needed. Formulas for the calculation of chlorophyll *a* and pheophytin *a* are given in the literature, without derivation (4, 5, 6, p. 203, 7). Refer to U.S.E.P.A. Method 445.0 for step-by-step calculation instructions (42).

CALIBRATION AND STANDARDS

When, Why, and How?

As mentioned, the reading of a fluorometer is relative and must be related to a standard during calibration. In addition, *in-vivo* work is affected by the variability discussed in previous sections, caused by species, environmental factors, and history. The usual calibration procedure for field *in-vivo* measurements involves periodic collection of a sample for extraction whenever it appears that conditions affecting the *in-vivo* fluorescence may have changed. The appearance of the water may be a good enough indicator of a changed system. Flemer (19) found good correlation between *in-vivo* and extractive spectrophotometric techniques as long as samples were grouped according to the visual color of the water. In areas where considerable background variation occurs, however, Esaias found it necessary to take calibration samples on a regular schedule (15). These samples may be taken at the exhaust outlet of the fluorometer during continuous-flow work. They should be stored in dark bottles and kept cold while in transit to the laboratory. Be sure to note the time, the location, and the readings of the samples that are taken so they can be cross-referenced to logged data.

Many procedures depend on the well known trichromatic spectrophotometric procedure (6, p. 185). This technique requires an accurately calibrated spectrophotometer. To illustrate this point, we checked the calibration of a Cary model 14 at the hydrogen line at 656.3 nm and found it was within 0.2 nm. A fresh extract of lawn grass in 90% acetone was scanned. The extract was found to contain 3.31 micrograms per ml of chlorophyll *a* and 0.89 micrograms per ml of chlorophyll *b* by the Parsons and Strickland formula (6). Calculations then were made, based on this scan, in which the spectrophotometer was set 2.5 nm higher, then 2.5 nm lower than the hydrogen line. Chlorophyll *a* values were 2.95 and 3.43 micrograms per ml, respectively, while chlorophyll *b* values were 1.38 and 0.52 micrograms per ml, respectively.

Use of a spectrophotometer with a wide bandwidth also can cause serious errors. A bandwidth of 3 nm or less is recommended (6, p. 195). Weber (76) has shown that chlorophyll *a* recovery falls drastically as bandwidth is increased. With a 0.1 nm bandwidth considered to yield 100% recovery, he shows 98.8% recovery at 2 nm, 78.6% at 10 nm and 48.5% at 20 nm.

Yardsticks: Primary Standards

The standard chosen depends on the nature of the problem being studied. The trend is to report chlorophyll *a* concentration directly, rather than to relate it to biomass or cell count. A detailed procedure that relates *in-vivo* chlorophyll to chlorophyll *a* standard is available (77). It has been found useful where background fluorescence is relatively regular and constant. A description of steps to be taken where background is variable has been provided to us by Dr. Wayne Esaias (15).

Pure chlorophyll *a* prepared in 90% acetone/10% distrilled water is now available from Turner Designs (P/N 10-850) The set of two ready-to-use fluorometric standards consists of one high-level and one low-level concentration solution. The pure chlorophyll *a* standard may be converted quantitatively to pheophytin *a* by the addition of HCl in an acetone solution (4). One also may prepare pure standards of chlorophylls *a*, *b*, and *c* and of their pheophytins by extractive methods (4, 70, 79). The use of paper chromatography may be considered here (73).

Where the desired result is biomass or cell count, and a single species is being studied, there is no need to use an intermediate chlorophyll standard. Direct standardization against biomass or cell count is best, where *in-vivo* measurements or simple extractive techniques are used (39, 47).

Secondary Standards

Unfortunately, chlorophyll is unstable in solution, therefore making repeated calibrations with chlorophyll may be expensive or time consuming. Fortunately, once a fluorometer has been calibrated, a secondary standard having reasonably similar spectral characteristics may be used. The secondary standard is read at the time of initial calibration against chlorophyll. Its reading (which may even be on a different scale) is noted. Thereafter, the fluorometer may be checked and adjusted with this secondary standard.

Turner Designs now offers solid secondary chlorophyll *a* standards (P/N 8000-952 for the Trilogy and P/N 10-AU-904 for the 10-AU-005-CE). The secondary standards require no special handling or storage conditions and the stability is guaranteed for two years.

Several other secondary standards have been suggested. Coproporphyrin has been used and is readily available. It is well known in the clinical laboratory, and its stability is documented.

Caution: Because the acid solution of coproporphyrin is corrosive, we do not recommend it for direct calibration of continuous flow systems.

Temperature Effects

The fluorescence of chlorophyll varies with temperature. In vivo chlorophyll fluorescence has a temperature coefficient of -1.4%/°C, while that of extracted chlorophyll in 90% acetone is lower, -0.3%/°C (7). The fluorescence of secondary standards such as coproporphyrin also varies with temperature, and each secondary standard has its own temperature coefficient. Whenever the temperature coefficient of the measured substance and the standard differ, the difference in temperature coefficients must be accounted for. Where discrete samples are being measured, the best technique is to maintain both samples and standards at the same temperature. Where you are performing continuous-flow *in-vivo* work, the temperature of the sample is usually quite stable and is difficult to adjust. For most work, calibrating at the anticipated temperature of the *in-vivo* sample will be sufficient. If not, the temperature coefficients of both sample and standard must be determined, and corrections must be applied manually, (or use the 10-AU-500 temperature compensation package to automatically correct for temperature changes; contact Turner Designs for details). If extracted chlorophyll is being measured against a chlorophyll standard, the two must be at the same temperature, but this temperature need not be fixed. A simple water bath and a brief wait for temperature equilibrium to be reached will suffice.

For measurement of extracted chlorophyll, the Turner Designs **10-AU** or **Trilogy Fluorometers** will normally be equipped with cuvette adaptors for borosilicate (glass) culture or test tubes. Samples at or near room temperature

may be left in the instrument for about one half minute before a drift in reading caused by sample warming will be noted. This period is at least three times the amount of time required to take an accurate reading. A sample put into the fluorometer, even briefly, should not be reread until it has reached equilibrium in the water bath. Some warming of the tube will have occurred and will have been transmitted to the sample by the time of reinsertion.

SAMPLE SYSTEMS

Continuous Flow Sampling

The **10-AU Fluorometer** is normally supplied with a 10-AU-020 High-Volume Continuous-Flow Cuvette. Sample is supplied directly to the flow cell using a pump.

The most satisfactory system to use is a submersible pump. This approach virtually eliminates problems of gas-bubble formation caused by leaks. For shallow sampling, a battery-operated bilge pump (Turner Designs part# 10-590) is commonly used. The typical output of about 400 gallons per hour is adequate for shallow sampling. No depth rating normally is given, but this pump probably should not be considered for depths greater than about 2 meters.

Caution: If you are using a deck-mounted centrifugal pump in a small boat where wave action causes pitching and rolling, be sure the intake hose does not leave the water. If air enters the system even for a short time, flow will stop and the pump will have to be primed again.

Large pieces of debris (which may lodge in elbows and constrictions and create problems with pumps) may be removed by intake filtration. A simple and effective intake system consists of a pipe perforated with many holes and wrapped with plastic screen (80). The tubing used may be polyethylene or plastic garden hose. The use of rubber tubing is not recommended. The section of tubing closest to the fluorometer must be opaque for a space of one to two feet to prevent outside light from reaching the photomultiplier tube. Light penetration to the photomultiplier is easy to check: with the instrument set on a high sensitivity range, simply shade the tubing. Direct sunlight and shade should give the same reading. Opaque tubing also may be needed in experiments where *in-vivo* measurements are thought to be affected by light history.

Sweet and Guinasso (82) have shown that if a long opaque hose is used, high pumping rates may give an increased fluorescence. This effect disappears at pumping rates below 600 ml/minute. If the organisms are given the opportunity to dark adapt, keep the flow below 600 ml/minute or at a constant rate throughout a series.

Formation of Bubbles in the Sample Line

Bubbles result from any of four factors:

1. Leaks in the sample system, especially in pump seals.
2. The wave action mentioned above.
3. Evolution from water that is supersaturated with oxygen.
4. Evolution from water that is supersaturated with oxygen at some reduced pressure point in your sample system.

An occasional bubble passing through the system will cause the reading to jump, but it will not cause serious error. A steady stream of bubbles, however, will cause serious error.

A submerged centrifugal pump has an advantage in that air cannot leak into the pump seals. Leaks in deck-mounted pump seals must be identified and repaired. Supersaturated water is seldom a problem in chlorophyll determination: oxygen evolution from water under reduced pressure is the major cause of bubble formation in sampling systems.

Oxygen evolution from unsaturated water may occur either when a submerged pump is run so fast that supersaturated conditions are reached in the low-pressure region behind its impeller blades or when a deck-mounted pump draws down the pressure at the top of a long column of water in the intake hose. In either case, the time required for these microbubbles to be redissolved is much greater than the time of transit to the fluorometer.

The solution for deck-mounted pumps is to mount the pump as low as possible, keep the hose low, and maintain a low flow rate. For submersible pumps, keep the pump speed down. If these tactics do not eliminate bubbles, mount a

bubble trap at a peak in the hose path before it enters the fluorometer. In severe cases, it may be necessary to use an ultrasonic bath to agglomerate the microbubbles into large bubbles, followed by a tangential bubble trap.

Use of a Bubble Trap: Many scientific research vessels have constructed PVC bubble traps to avoid many of the troubles mentioned above. This is something for the user to consider if the instrument is intended to be used on a boat in open water.

Grab Sampling

While work with the continuous-flow system has great advantages in ease and speed, certain situations require the use of discrete ("grab") samples. Where water below the depth of the reach of a pump is to be analyzed, grab samples must be taken. Work in shallow estuaries also requires grab samples to be taken because mud (not simple turbidity) interferes with continuous-flow systems.

The 25 x 150 mm Cuvette is recommended for the best sensitivity for analyzing grab samples. Where grab sample volume is limited, however, the Cuvette Holder for 13 x 100 mm cuvettes may be used; the minimum sample volume is only 4 ml.

EQUIPMENT SELECTION

Fluorometer

Since Turner Designs offers several fluorometers, the first thing the user should consider is where the measurement will be taken. In the laboratory environment, consider the **Trilogy Laboratory Fluorometer** or the **10-AU-005 Fluorometer** equipped with borosilicate cuvette adaptor. If the measurement will be done in the field, a **10-AU-005 Field Fluorometer** should be used. When using the 10-AU fluorometer, the red-sensitive photomultiplier is strongly recommended.

Note: The field fluorometer, designed for rugged service, is splash resistant and can withstand temporary immersion (carrying case cover on or off), as long as the Continuous-Flow Cuvette is installed.

Photomultipliers

Our experience is that the enhanced red-sensitivity photomultiplier is required for *in-vivo* chlorophyll determinations in extremely clear lake or deep ocean water. For estuary work, or for algal growth determinations, the standard photomultiplier in the Turner Designs 1**0-AU** may be adequate.

Although all evidence to date indicates that the only difference between the use of the standard photomultiplier and the enhanced red-sensitivity surface is one of ultimate detectability rather than accuracy, practically all published articles on *in-vivo* chlorophyll have specifically referenced use of the red-sensitive photomultiplier. For this reason, if legal or regulatory proceedings hinge on the data, the red-sensitive photomultiplier is advised.

For further information on photomultipliers and for curves representing typical spectral response characteristics, see reference 81.

Cuvettes Adaptor

The **10-AU Field Fluorometer** can be supplied with a Cuvette Adaptor for 25 x 150 mm tubes or for 13 x 100 mm tubes. Refer to the Turner Designs 10-AU Manual for correct installation and removal of cuvette adaptors, and connection to hoses and auxiliary equipment. The Trilogy Laboratory Fluorometer comes with an adaptor that will allow 12 x 75 mm round bottom borosilicate tubes or 12 x 35 mm screw top vials to be used.

Lamps and Filters for the 10-AU Fluorometer

Three kits are available that contain lamps and filters for different applications. Each kit comes with complete instructions as to which lamps and filters to use for individual types of work. A summary of where each optical kit might be used is given below. (For more information, ask for the Optical Configuration Guide, available from Turner Designs.) The kits listed below have different part #'s for each instrument, please consult the Ordering Information Guide for the appropriate instrument to find the part number of the kits described below.

Kit #1 **Chlorophyll Optical Kit (*In-vivo* & Extractive Measurement)**
For *in-vivo* and in-vitro chlorophyll measurement. This kit should be used for traditional *in-vivo* chlorophyll (Lorenzen) and *in-vitro*/extractive acidification methods, including Strickland & Parsons, Standard Methods for Water and Wastewater, and EPA 445.0.
The 10-AU-600 Red-Sensitive Photomultiplier Tube is required.

Daylight White Lamp (F4T5D)
Excitation Filter (340-500 nm) (CS-5-60)
Emission Filter (>665 nm) (CS-2-64)
Reference Filter (1 N.D.) - 10-AU optical kits only
Attenuator Plate (1:5) - 10-AU optical kits only

Kit #2 **Chlorophyll *a* Optical Kit (Extractive Measurement)**
For in-vitro/extractive chlorophyll measurement only. This kit is designed for use with the Welschmeyer (1993) Non-Acidification Method. This optical kit is selective for chlorophyll *a* and discriminates against accessory chlorophylls and pheopigments. The 10-AU-600 Red-Sensitive Photomultiplier Tube is required.

10-089 Blue Lamp (F4T4.5B2 equiv.)
10-113 Excitation Filter (436 nm)
10-115 Emission Filter (680 nm)
Reference Filter (1 N.D.) - 10-AU optical kits only

Kit #3 **Chlorophyll *a* Optical Kit (*In-vivo* Measurement)**
For *in-vivo* chlorophyll *a* measurements of fresh water samples and samples high in blue-green algae (phycocyanin). This optical kit is selective for chlorophyll *a* and discriminates against emission interference. The 10-AU-600 Red-Sensitive Photomultiplier Tube is required.

10-089 Blue Lamp (F4T4.5B2 equiv.)
Excitation Filter (340-500 nm) (CS-5-60)
10-115 Round Emission Filter (680 nm)
Reference Filter (1 N.D.) - 10-AU optical kits only
Attenuator Plate (1:5) - 10-AU optical kits only

Modules for the Trilogy Fluorometer

Three modules are available for the chlorophyll analyses for the Trilogy Fluorometer:

Chlorophyll a acidification module:	(P/N 7200-040)
Chlorophyll a non-acidification module:	(P/N 7200-046)
Chlorophyll a in vivo module:	(P/N 7200-043)

For information about other available modules please visit http://www.turnerdesigns.com/t2/instruments/trilogy.html.

Pump

A pump is necessary to pump sample through the fluorometer's flow cell. The 10-590 bilge pump is available from Turner Designs. Contact us for details.

Data Collection

The **10-AU Field Fluorometer** has three methods for data collection, which can be used simultaneously:

1. The analog voltage output can be used with a logger or chart recorder;

2. The RS-232 serial data output can be used with a computer or other serial device;

3. The optional Internal Data Logger (10-AU-450), where the 10-AU will log data directly into the instrument for later downloading and analysis (converted to ASCII format). The addition of Electronic Chart Recording to the

Internal Data Logger permits examination of trends in the field without downloading by displaying 240 data points at a time on the digital display. The Internal Data Logger is particularly useful for *in-vivo* studies where additional data collection equipment is unavailable and when many data points are to be recorded.

The **Trilogy Laboratory Fluorometer** has a standard RS-232 serial output for use with a computer or serial printer. A compact serial printer is available as an option. (Ask Turner Designs for details.)

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