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Chapter A12

FLUOROMETRIC PROCEDURES FOR DYE TRACING

By James F. Wilson, Jr., Ernest D. Cobb,
and Frederick A. Kilpatrick

BOOK 3

APPLICATIONS OF HYDRAULICS

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Preface

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This chapter is a complete update of the chapter by the same number published in 1968.

TECHNIQUES OF WATER-RESOURCES INVESTIGATIONS OF THE U.S. GEOLOGICAL SURVEY

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TWI 7-C1. Finite difference model for aquifer simulation in two dimensions with results of numerical experiments, by P. C. Trescott, G. F. Pinder, and S. P. Larson. 1976. 116 pages.

TWI 7-C2. Computer model of two-dimensional solute transport and dispersion in ground water, by L. F. Konikow and J. D. Bredehoeft. 1978. 90 pages.

TWI 7-C3. A model for simulation of flow in singular and interconnected channels, by R. W. Schaffranek, R. A. Baltzer, and D. E. Goldberg. 1981. 110 pages.

TWI 8-A1. Methods of measuring water levels in deep wells, by M. S. Garber and F. C. Koopman. 1968. 23 pages.

TWI 8-A2. Installation and service manual for U.S. Geological Survey monometers, by J. D. Craig. 1983. 57 pages.

TWI 8-B2 Calibration and maintenance of vertical-axis type current meters, by G. F. Smoot and C. E. Novak. 1968. 15 pages.

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SELECTED CONVERSION FACTORS

The following factors may be used to convert the International System of Units (SI) published herein to inch-pound units.

Multiply SI units	By	To obtain inch-pound units
	Length	
meter (m)	3.281	foot (ft)
	39.37	inch (in)
millimeter (mm)	0.03937	in
	Volume	
cubic centimeter (cm^3)	0.06101	cubic inch (in^3)
liter (L)	0.2642	gallon (gal)
	0.03531	cubic foot (ft^3)
milliliter (mL)	0.06101	in^3
	Mass	
gram (g)	0.002205	pound (lb)
kilogram (kg)	2.205	lb
	35.27	ounce (oz)
	Temperature	
degree Celsius (°C)	°F=9/5 °C+32	degree Fahrenheit (°F)
	°C=5/9 (°F−32)	

Other conversion relations that may be helpful to the user of this report are as follows:

angstrom (1 A)=10^{-10} meters (m)
kilometer (1 km)=10^3 m
1 m=10^3 millimeters (mm)
1 mm=10^3 micrometers (μm)
1 μm=10^3 nanometers (nm)

SYMBOLS AND UNITS

Symbol	*Explanation*	*Units*
C_f	Final concentration	µg/L
C_i	Initial concentration	µg/L
C_n	New concentration after a dilution	µg/L
C_s	Concentration of stock solution	µg/L
D_i	Dilution factor	--
D_T	Total dilution factor in a serial dilution	--
S_G	Specific gravity	--
V_d	Volume of the dye solution	L
V_w	Volume of added diluent	L
W_d	Weight of the dye	g

GLOSSARY

Absorption. The physical assimilation of dye molecules by organic or inorganic solids such as a bank, a bed, suspended material, or plantlife. Also, the assimilation and conversion to thermal energy of irradiating energy (light) by most substances, including all that are fluorescent.

Absorption spectrum. For a given substance, the characteristic relationship of the intensity of absorbed energy (light) to the wavelength of the incident energy. Related to, but not the same as, excitation spectrum.

Adsorption. The physical adhesion of dye molecules to the surfaces of solids such as a bank, a bed, suspended material, or plantlife. A primary cause of loss of dye in streams and aquifers.

Background. Fluorometer readings, other than those due to fluorescence of the tracer dye, that result from scattered light, from fluorescence of natural materials or pollutants, or from other causes.

Calibration. The relationship of fluorometer readings to dye concentration.

Concentration quenching. Not true quenching, but rather the reduction in the rate of increase of fluorometer readout with increasing dye concentration due to the increasing optical density of the dye itself. A problem only with very high concentrations. (*See* **quenching.**)

Converter (inverter). An electronic device used to change direct current from a storage battery into alternating current, as required by some fluorometers.

Cuvette (sample holder). A test tube or other device, usually made of Pyrex glass, for containing the water sample in the fluorometer.

Detectability. The extent to which a dye may be identified quantitatively in a water sample. Depends on the spectral characteristics of the dye, the potential interference by background materials, and the sensitivity of the fluorometer. (*See* **sensitivity.**)

Diluent. A diluting agent or solvent; distilled water is usually the diluent used in preparing standard dye solutions.

Dilution factor. The ratio of the volume of tracer solution to the total resultant solution. Used in computing concentrations of standard solutions.

Dummy cuvette. The opaque cuvette used to set the zero reading on the fluorometer dial before testing water samples.

Emission. The discharge of energy from an excited fluorescent substance. (*See* **fluorescence.**)

Emission (fluorescence) spectrum. For a given fluorescent substance, the characteristic relationship of the intensity of emitted energy (light) to the wavelength of the emitted energy. Except for magnitude, the relationship is independent of the wavelength of the absorbed light.

Excitation. For fluorescent substances, the state wherein certain electrons are raised temporarily to higher orbits owing to absorption of energy from an external source.

Excitation spectrum. For a given fluorescent substance, the characteristic relationship of the intensity of emitted light to the wavelength of the absorbed light. Related to, but not the same as, absorption spectrum.

Filter fluorometer; fluorimeter; fluorescence meter. An instrument containing a lamp or other means of exciting fluorescent radiation in a sample, with filters and a detector to measure relative fluorescent intensities caused by variations in concentration of the substance under examination.

Fluorescence. The emission of electromagnetic waves of characteristic energy when atoms or molecules decay from an excited state to a lower energy state. The excitation may be induced by subjecting the substance to radiation of slightly higher energy (shorter wavelength) than that of the characteristic emission, and ceases as soon as the external source is removed.

Fluorescence spectrum. *See* **emission spectrum.**

Fluorometer. *See* **filter fluorometer** *and* **spectrofluorometer.**

Loss of dye. Dye loss due to any or all of the following: absorption, photochemical decay, quenching, and chemical alteration.

Luminescence. Any emission of light not directly ascribable to heat. Fluorescence and phosphorescence are two examples.

Nanometer (nm). A unit of length equal to 10 angstroms, one-thousandth of a micrometer, and one-millionth of a millimeter. Used in expressing wavelength.

Neutral-density (ND) (range extension) filter. A filter used to reduce the amount of light reaching the photomultiplier tube in the fluorometer. Usually used in addition to other filters when dye concentrations are too high to obtain a reading, the ND filter reduces the intensity of the light, but does not change the spectral distribution of the light.

Photochemical decay (photodecomposition). Degradation of the fluorescence intensity of dye in a stream or a sample container by the action of light.

Quenching. The reduction of fluorescence due to any of a number of kinds of interaction of the dye molecules with other chemicals in the water. (*See* **concentration quenching.**)

Raman scatter. A spectrum produced when light is scattered as it passes through certain substances such as water. Some of the energy is absorbed; the scattered light has longer wavelengths than the incident light. (*See* **Rayleigh scatter.**)

Rayleigh scatter. A spectrum produced when light is scattered as it passes through certain substances such as water; the scattered light has the same wavelengths as the incident light. (*See* **Raman scatter** *and* **Tyndall scatter.**)

Selectivity. The capability to isolate a narrow spectral band of exciting or emitted light by selection of appropriate fluorometer filters. (*See* **sensitivity** *and* **specificity.**)

Sensitivity. The extent to which a given fluorometer can detect low concentrations of a given dye. Depends on the characteristics and interrelationships of the fluorometer components. (*See* **detectability, selectivity,** *and* **specificity.**)

Sorption. The process of taking up and holding either by absorption or adsorption. (*See* **absorption** *and* **adsorption.**)

Specificity. The capability to isolate fluorometrically the fluorescence of a particular dye from that of all other fluorescent substances present in a water sample. (*See* **selectivity** *and* **sensitivity.**)

Spectral-fluorescence characteristics. The excitation and emission spectra of a particular fluorescent substance, often identified by the wavelengths at which maximum excitation and maximum emission occur. (*See* **excitation spectrum** *and* **emission spectrum.**)

Spectral-transmittance characteristics. For a given filter or filter combination, the curve relating transmittance (percent transmission) to wavelength, sometimes identified by the wavelength of maximum transmission. (*See* **transmittance.**)

Spectrofluorometer (fluorescence spectrometer). A special type of fluorometer that may be used, among other things, to determine the spectral-fluorescence characteristics of fluorescent substances.

Standard solution. A sample containing a known concentration of dye in distilled water diluent. Used to calibrate a fluorometer.

Tracer. In hydrologic tracing, any dissolved, suspended, or floating material used to determine the path and (or) rate of movement and dispersion of similar materials in the water. Tracers include natural materials and pollutants, such as sewage, as well as materials intentionally injected, such as floats, salts, radioisotopes, and fluorescent dyes.

Transmittance. The proportion of incident light that emerges from the opposite side of a fluorometer filter. (*See* **spectral-transmittance characteristics.**)

Tyndall scatter. A spectrum produced when light is scattered by suspended material in a water sample; the scattered light has about the same wavelengths as the incident light. (*See* **Rayleigh scatter.**)

Visible spectrum. The narrow band of the electromagnetic spectrum to which the human eye is sensitive; approximately the interval 380-800 nm, which includes the excitation and emission spectra of most of the tracer dyes.

Xanthene dyes. The group of brilliant fluorescent dyes characterized by the presence of the xanthene nucleus ($C_{13}H_{10}O$). Rhodamine WT and pontacyl pink are xanthene dyes used for water tracing.

FLUOROMETRIC PROCEDURES FOR DYE TRACING

By James F. Wilson, Jr., Ernest D. Cobb, and
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Abstract

This manual describes the current fluorometric procedures used by the U.S. Geological Survey in dye tracer studies such as time of travel, dispersion, reaeration, and dilution-type discharge measurements. The advantages of dye tracing are (1) low detection and measurement limits and (2) simplicity and accuracy in measuring dye tracer concentrations using fluorometric techniques.

The manual contains necessary background information about fluorescence, dyes, and fluorometers and a description of fluorometric operation and calibration procedures as a guide for laboratory and field use. The background information should be useful to anyone wishing to experiment with dyes, fluorometer components, or procedures different from those described. In addition, a brief section on aerial photography is included because of its possible use to supplement ground-level fluorometry.

Introduction

The extensive use of fluorescent dyes as water tracers began in the early to mid-1960' s. Prior to that time, floats, chemical salts, and actual contaminants had been used as tracers. After World War II, radioisotopes such as tritium (heavy hydrogen) gained favor as tracers, but their use was severely limited by handling problems, the special training required, and a general lack of understanding by the public. A search for a suitable substitute for radioisotopes led to the rediscovery of fluorescent dyes for tracing. Although fluorescein had been used occasionally for more than 50 years (Dole, 1906), Pritchard and Carpenter (1960) were the first to combine rhodamine B, a much better surface-water tracer than fluorescein, and greatly improved fluorometers for large-scale tracing studies.

Within the U.S. Geological Survey, feasibility test of dyes and fluorometers were made in 1961- 62 and were reported by Wright and Collings (1964). The initial application of fluorometry—and by far the most used application to date—was for the measurement of time of travel of solutes in streams (Buchanan, 1964; Hubbard and others, 1982). The procedures also were adapted to the measurement of stream discharge by dye-dilution methods (Cobb and Bailey, 1965; Kilpatrick, 1968). Fluorometry has also been applied to studies to determine reaeration rates of streams (Rathbun and others, 1977). Others in the Geological Survey who have contributed to the development of fluorometric procedures for dye tracing include E.L. Meyer, J.R. Kreider, and Bernard Dunn.

In addition to time-of-travel, dispersion, reaeration, and discharge measurements, hydrologic applications have included studies of waste buildup and flushing in estuaries (Bailey and others, 1966; Yotsukura and Kilpatrick, 1973) circulation and stratification of water in reservoirs; path tracing in cavernous limestone; measurement of ground-water time of travel (Freeze and Cherry, 1979, p. 426-430); determination of well-drilling fluid circulation time; studies of the uptake of irrigation waters by plants (Robinson and Donaldson, 1967); tagging of herbicide spray with dye in a canal to facilitate downstream sampling and testing for traces of the herbicide; and tagging of heated water discharges from power plants to facilitate downstream sampling.

The outstanding characteristics of dye tracing are (1) low detection and measurement

limits and (2) simplicity and accuracy in measuring dye tracer concentrations using fluorescent techniques. Nearly all applications of dye tracing include introduction of dye into a water body, subsequent collection of water samples over time and space to measure the response, and determination of the concentration of dye in the samples by means of a fluorometer. Dosing and sampling procedures and data analysis vary with each application; fluorometric procedures are generally the same for most applications and are the subject of this manual.

This manual is a revision of an earlier manual by Wilson (1968b) and is intended to be a companion to the manuals on individual applications such as discharge measurements (Kilpatrick and Cobb, in press), time-of-travel measurements (Hubbard and others, 1982), and others that may follow. This manual contains necessary background information about fluorescence, dyes, and fluorometers and a description of fluorometric procedures as a general guide in both laboratory and field applications of dye tracing. The background information also should be useful to anyone wishing to experiment with dyes, fluorometer components, or procedures different from those described. In addition, a brief section on aerial photography is included because of the possible use of aerial photography in dye tracing to supplement ground-level fluorometry.

The procedures described are subject to modification, as dyes, equipment, and techniques are continually being improved. Also, accuracy requirements for determining dye concentrations vary with individual applications and should be established in advance of an investigation in order to use the appropriate fluorometric procedures.

General Description of Fluorescence

Fluorometric analysis, or fluorometry, uses the physical phenomenon called fluorescence. Because fluorescence is the outstanding property of all tracer dyes, a general understanding of the phenomenon is necessary to assure its proper use in any application of dye tracing.

Basically, fluorescence is a form of luminescence, a broad term for any emission of light not directly ascribable to heat. Fluorescent substances emit radiation (light) immediately upon irradiation from an external source; emission ceases when the source is removed. A similar kind of luminescence is phosphorescence. However, phosphorescent substances store some of the irradiating energy, delaying emission, which continues after removal of the light source. For a detailed technical discussion of luminescence, see Bowen and Garlick (1966).

The almost instantaneous sequence of events in fluorescence is as follows: (1) absorption of energy from an outside source such as the Sun or an ultraviolet lamp, (2) excitation of some of the electrons of the fluorescent substance, resulting in enlarged electron orbits—the "excited state," and (3) emission of energy in the form of photons (light) as the excited electrons return to normal position—the "ground state." The emitted (fluoresced) energy nearly always has longer wavelengths and lower frequencies than the absorbed energy because some energy is lost in the process (Stokes' law). It is this property of dual spectra—a different specific combination of excitation and emission spectra for each fluorescent substance—that is used to make fluorometry an accurate and sensitive analytical tool. An excellent reference on the basic theory of fluorescence and fluorometry is Udenfriend (1962, p. 1–124).

In fluorometry, wavelengths are given in either nanometers or angstrom units. Nanometers are used in this manual.

Most substances are at least mildly fluorescent, and most fluorescence occurs in the 200- to 800-nanometer range of wavelengths—ultraviolet and visible light (fig. 1). Strongly fluorescent substances convert a high percentage of absorbed energy into emitted energy. Most strongly fluorescent substances fluoresce in the ultraviolet-to-green part of the spectrum. A few substances, including some of the preferred tracer dyes, fluoresce in the yellow-orange range. Dyes that are useful for tracer applications are strongly fluorescent and can be detected easily in small concentrations. The spectral properties of the tracer dyes and the corresponding fluorometer optics are discussed in subsequent sections of this manual.

Fluorescent materials likely to be found in some streams include algae and other naturally occurring organics, certain minerals, manmade

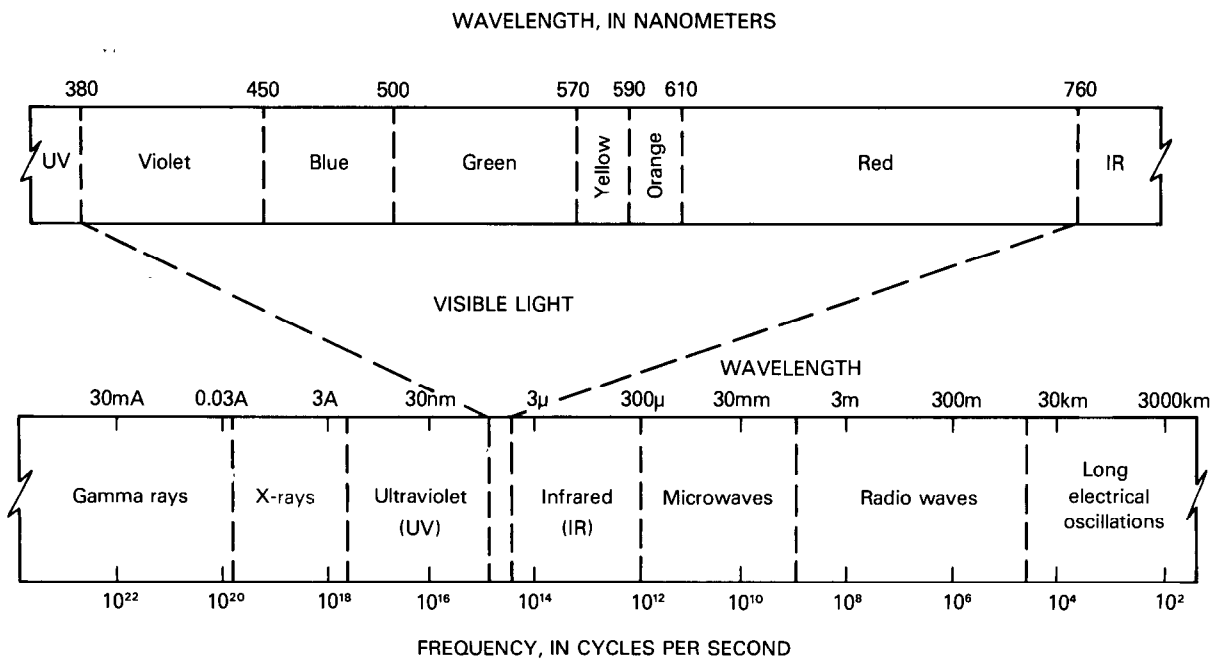

Figure 1.—The electromagnetic spectrum, with an enlargement of the visible spectrum. The fluorescent tracer dyes absorb and emit light at visible wavelengths.

pollutants such as paper and textile dyes, certain petroleum products, and laundry-detergent brighteners. Wright and Collings (1964, p. 749–750) and Williams and Bridges (1964, p. 372–376, 387–393) discuss the spectral-fluorescent properties of some common organic substances.

Fluorescence intensity is affected in varying degrees by certain physical and chemical factors such as solvent, concentration, temperature, pH, photochemical decay, and fluorescence quenching. Udenfriend (1962, p. 96-114) and Williams and Bridges (1964, p. 377-385) present excellent general discussions of these and other factors. Studies limited to an evaluation of the properties of fluorescent dyes were reported by Pritchard and Carpenter (1960), Feuerstein and Selleck (1963b), Wright and Collings (1964), and Smart and Laidlaw (1977). These properties are discussed in more detail in the next section.

Fluorescent Dyes

Types recommended for tracing

Hundreds of commercial dyes are available in a variety of colors. A great number are strongly fluorescent, but only a few exhibit the combination of properties essential for water tracing. Two dyes, variations of the same basic organic structure (xanthene), are preferred for use as water tracers: rhodamine WT and pontacyl pink (also known as intracid rhodamine B, pontacyl brilliant pink B, and acid red 52). These dyes are generally good tracers because they are (1) water soluble, (2) highly detectable—strongly fluorescent, (3) fluorescent in a part of the spectrum not common to materials generally found in water, thereby reducing the problem of background fluorescence, (4) harmless in low concentrations, (5) inexpensive, and (6) reasonably stable in a normal water environment.

A third dye, acid yellow 7 (also known as lissamine FF), lends itself to water tracing in certain environments. Only a small amount of field testing has been performed with this dye. Smart and Laidlaw (1977) reported on the dye's characteristics. It appears to have many good qualities for water tracing. Brian G. Katz (hydrologist, U.S. Geological Survey, written commun., 1982) reported, however, that background fluorescence was observed to increase with increasing concentrations of total organic

carbon for the optical system used with acid yellow 7 dyes. This seems to be one of the principal drawbacks to this dye, because there often is a high natural background in streams, and during unsteady flow the background may vary with time.

Dennis E. Ford and Kent W. Thornton (Ford, Thornton, Norton, and Associates, written commun., 1982) found in laboratory tests that acid yellow 7 has a solubility of 2.5 g/100 mL. Brian Katz (U.S. Geological Survey, oral commun., 1982) reported that the practical upper limits of solubility in his tests with the dye were about 1 g/100 mL.

Characteristics of the preferred tracer dyes are presented in table 1. It should be noted that for some dyes there are several manufacturers, but only a few are mentioned in table 1.

Rhodamine WT is preferred for most water-tracing uses. It is easy to use and has many other characteristics that are desirable for water tracing. The other dyes may be used to advantage under special conditions.

Although acid yellow 7 has not been used extensively in field tests, it is believed to be a good dye for use in waters containing small or constant concentrations of organic matter.

All of the dyes listed in table 1 are believed to have some use for ground-water tracing, although acid yellow 7 has not been used for that purpose. There may be problems with the use of any of these dyes in tracing ground water through clay and silt soils because of sorption on the large surface areas associated with these soils.

In the past, rhodamine B and BA have been widely used for water tracing, but because of their high adsorption tendencies they are not recommended for this purpose and are not referred to further in this manual.

Table 1.—Characteristics of preferred tracer dyes

Dye color, formula, and common name	Other names	Cost per pound as specified	Remarks
Rhodamine WT	Intracid rhodamine WT	$9.50[a]. Available in 20-percent solution (specific gravity, 1.19, 9.92 pounds per gallon at 62° F.	High detectability, low sorptive tendency, good diffusity, low acidity. Fluorescence variable with temperature.
Acid red 52 ($C_{27}H_{29}N_2O_4S_2Na$) Often referred to as pontacyl pink	Intracid rhodamine B Pontacyl brilliant pink B	$15.14[a]. Available as powder.	Fairly high detectability, low sorptive tendency, good diffusity, low decay rate, fairly stable at pH extremes. Fluorescence variable with temperature.
Acid yellow 7	Lissamine FF Lissamine yellow FP Brilliant acid yellow 8G Overacid brilliant sulpho flavine FF	$17.85[b]. Available as a powder.	Fairly high detectability, low sorptive tendency, good diffusity, fairly stable at pH extremes, little affected by temperature. Subject to background interference.

[a]Costs provided by Crompton and Knowles Corporation, P.O. Box 68, Skokie, Illinois 60076, phone 312/675-5510, on March 29, 1984, for 25-pound orders.

[b]Costs provided by Organic Chemical Corporation, P.O. Box 4258, East Providence, Rhode Island 02914, phone 401/434-3300, on March 29, 1984, for 25-pound orders.

Note: Costs of dye vary with the amount ordered. Additional charges may be made for handling and shipping.

Properties of dyes

The outstanding property of the dyes, discussed previously, is their strong fluorescence. Additional properties that affect the use of the dyes as tracers are discussed below. Good references on the subject are Feuerstein and Selleck (1963a, 1963b) and Smart and Laidlaw (1977).

Factors that affect fluorescence

Fluorescence may vary with the solvent used, but in hydrologic tracing the effect is constant because the solvent always is basically water; other chemicals present in the water may affect fluorescence in other ways.

In dilute solutions (solutions in which less than 5 percent of the exciting light is absorbed) and for a given fluorometer setup, fluorescence varies directly with dye concentration; this important fact is discussed in the section "Fluorometer Calibration."

Aside from concentration, the most significant factor affecting fluorescence of dilute solutions is sample temperature. Fluorescence activity increases (resulting in higher readings) as sample temperature decreases; lower readings are obtained as temperature increases. Temperature effects must be accounted for in data analysis. Temperature-correction curves for rhodamine WT, pontacyl pink, and acid yellow 7 are given in figure 2. It can be seen from figure 2 that acid yellow 7 is relatively insensitive to temperature changes.

If calibration standards and field samples are brought to a common temperature before the samples are analyzed, temperature effects can usually be ignored—unless the samples are allowed to warm up while being tested in the fluorometer.

The pH of the sample also may affect fluorescence intensity. Fluorescence of rhodamine WT is stable in the pH range 5-10 and decreases outside those limits (see curves prepared by Feuerstein and Selleck, 1963a, p. 13). Fluorescence of pontacyl pink and acid yellow 7 is stable in the pH range 4-10 and decreases outside those limits (Smart and Laidlaw, 1977, p. 19, 20).

Decreased fluorescence due to pH variation is not usually a serious problem, except possibly in highly acidic streams, where apparent recovery of dye could be very small. Feuerstein and Selleck (1963a, p. 12; 1963b, p. 24) indicated that by adjusting the pH of a solution, one can restore fluorescence to full strength. This, however, would not rectify any nonreversible chemical effects due to reaction of dye with the acidic compound itself.

For tracing purposes, fluorescence also can be adversely affected by quenching, which results from the action of other chemicals in the solution. The quenching agent may do any or all of the following (Williams and Bridges, 1964, p. 383-385): (1) absorb exciting light, (2) absorb light emitted by the dye, and (3) degrade the excited-state energy. A fourth possibility is that the quenching agent may chemically change the fluorescent compound; this change is nonreversible. Chlorine, for example, is known to quench the fluorescence of rhodamine dyes. For this reason chlorinated tapwater generally should not be used to prepare standard solutions. When tapwater is the only water available, small concentrations of chlorine can usually be removed by allowing containers of the water to sit open to the atmosphere for about 12 hours. An effect similar to true quenching, concentration quenching, occurs when relatively high concentrations of dye produce a screening effect on both the exciting light and the emitted light. This problem can be overcome simply by diluting a measured amount of sample with a measured amount of water.

In tests conducted in Baltimore storm sewers using chlorinated hydrant water, Katz (U.S. Geological Survey, written commun., 1982) found no effect of the chlorine on the fluorescence of acid yellow 7, but he did find significant effects on the fluorescence of rhodamine WT. On the other hand, Ford and Thornton (Ford, Thornton, Norton, and Associates, Ltd., written commun., 1982) found sizable decreases in fluorescence of acid yellow 7 when solutions containing 100 and 500 μg/L (micrograms per liter) of acid yellow 7 and 1.0 and 5.25 mg/L (milligrams per liter) of residual chlorine were tested in a laboratory. It may be concluded that chlorinated water should be avoided, if possible, both in field tests and in the preparation of standards.

High levels of oxygen, such as in highly aerated streams, appear to have the same effect as chlorine. This will not ordinarily be a problem,

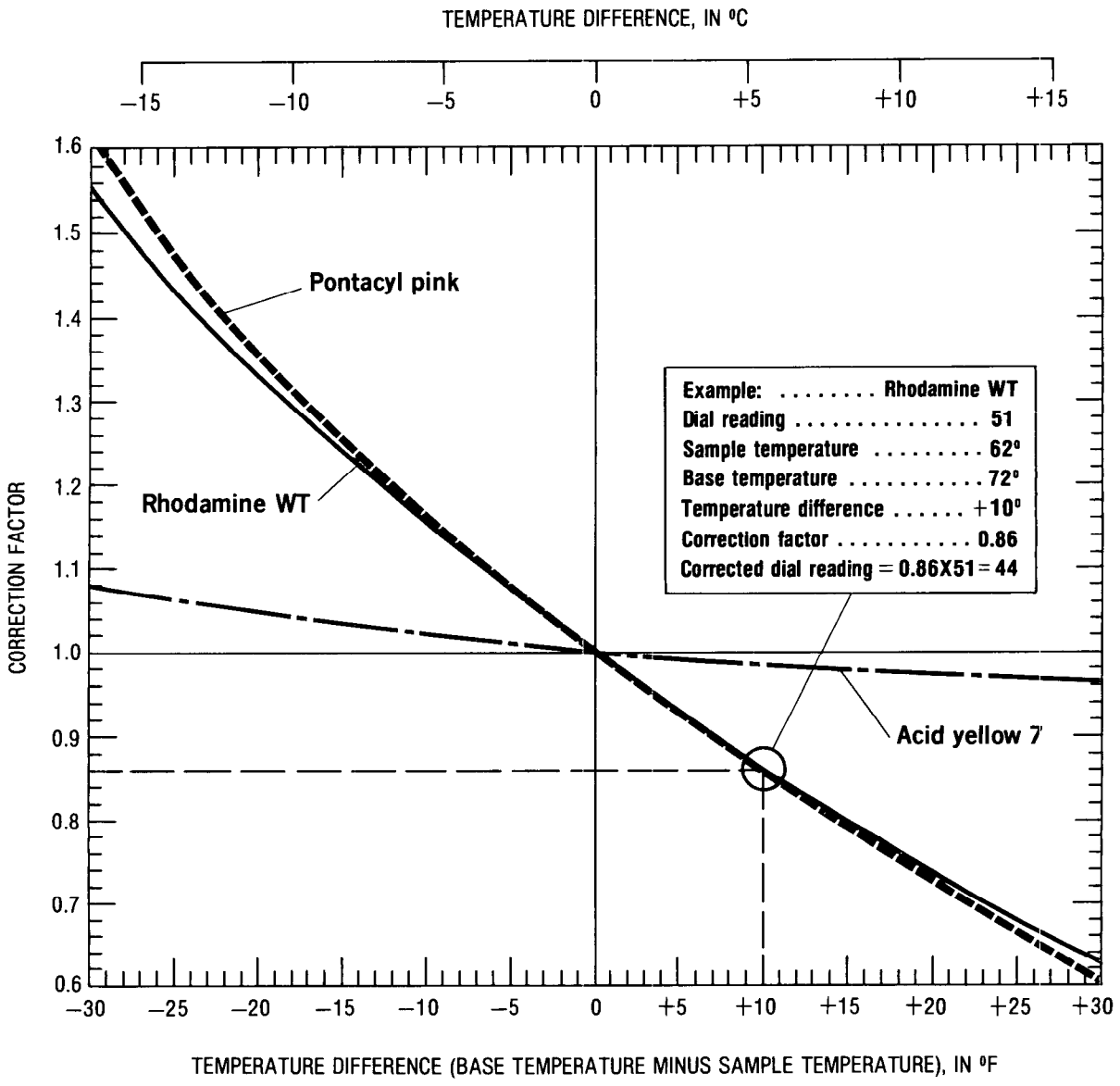

Figure 2.—Temperature-correction curves for rhodamine WT, pontacyl pink, and acid yellow 7 dyes. Curve for acid yellow 7 modified from Smart and Laidlaw (1977, fig. 2).

unless the stream being tested is virtually all "white water." Under these infrequent conditions, unusally large losses of rhodamine WT have been measured.

A permanent reduction in fluorescence can be caused by photochemical decay, or photodecomposition. Bright sunlight has this effect on the rhodamine dyes, and the effect increases gradually with time. Stream depth, turbidity, and cloudy skies tend to minimize photochemical decay, so the effect usually is significant only for applications requiring recovery of a large percentage of the dye or where the dye is exposed to sunlight for several days. Smart and Laidlaw (1977, p. 24) indicate that the photochemical decay rate of acid yellow 7 appears to be an order of magnitude less than that of pontacyl pink.

Sorption

One of the most important characteristics of dyes used for water tracing is the tendency to adhere to suspended and bed materials, aquatic plants and the like (adsorption), or to be absorbed by such materials. It is important that the sorption tendency of a dye used as a water tracer be as low as possible. Rhodamine WT, pontacyl pink, and acid yellow 7 are only slightly susceptible to adsorption in most situations. Generally, organic sediments adsorb more dye than inorganic sediments. In laboratory tests reported by Smart and Laidlaw (1977, p. 25-29), acid yellow 7 generally seemed to be more resistant to adsorption on mineral sediments than either rhodamine WT or pontacyl pink. Acid yellow 7 also seems to be more resistant to adsorption by organic matter than does rhodamine WT. There is some evidence in the report by Smart and Laidlaw (1977, p. 25-29) that pontacyl pink is superior or equal to acid yellow 7 in water containing significant amounts of organic matter.

Although sorption (adsorption and (or) absorption) is not a factor that affects fluorescence, the results are similar to those from photochemical decay or chemical quenching. Like the losses due to other causes, sorptive loss contributes to decreased recovery of dye, but it is impossible to separate quantitatively these three causes of dye loss in a stream. Dye losses have a direct bearing on the accuracy of discharge measurements but are rarely serious enough to threaten the results of time-of- travel or dispersion measurements. Sorption, of course, is a critical factor in ground-water tracer studies.

Biological effects

A number of tests have been conducted concerning the effects of rhodamine WT and pontacyl pink on aquatic life and on laboratory test animals. Parker (1973) reported on a test in which eggs and 12-day-old larvae of the Pacific oyster (*Crassostrea gigas*) were exposed for 48 hours in water at 24° C with rhodamine WT dye concentrations ranging from 1 to 10,000 µg/L. All of the tested eggs developed to larvae without abnormalities and all of the 12-day-old larvae survived with no abnormalities. An additional test was made by Parker (1973) on silver salmon and Donaldson trout. No mortalities or other problems were observed when the fish were exposed to water containing 10,000 µg/L of rhodamine WT for 17.5 hours and then to water containing 375,000 µg/L of the dye for an additional 3.2 hours.

J.S. Worttley and T.C. Atkinson (reported as personal commun., 1975, in Smart and Laidlaw, 1977) exposed a number of freshwater and brackish water invertebrates, including water flea (*Daphnia magna*), shrimp (*Gammarus zaddachi*), log louse (*Asellus aquaticus*), may fly (*Cloeon dipterum*), and pea mussel (species *pisidium*), to water containing up to 2,000,000 µg/L of rhodamine WT for periods of up to 1 week. No significant differences in mortality between the test and control animals were observed.

D.E. Donaldson (U.S. Geological Survey, written commun. cited by Wilson, 1968b, p. 6, and by Smart and Laidlaw, 1977, p. 30) prepared a drinking-water solution of 10 µg/L of rhodamine WT that was given to rats. He observed a slight loss of body weight and some effect on certain body organs—especially the liver—when the rats were given this solution for a prolonged time, compared with rats in a control group.

Smart and Laidlaw (1977, p. 30) reported that subcutaneous injections of 50 µg of pontacyl pink caused inflamed sores at the injection sites and a pronounced loss of body weight, whereas injections of rhodamine WT caused no traumatic ill effects even after 56 days of this treatment.

No known studies have been made of the effects of acid yellow 7 on test animals. According to Smart and Laidlaw (1977, p. 30), the manufacturers have indicated that acid yellow 7 is unlikely to cause any unusual toxic hazards.

A letter by the director of the Criteria and Standards Division of the Office of Drinking Water, U.S. Environmental Protection Agency (EPA), dated April 10, 1980, states that "[EPA] would not object to [rhodamine WT's] use as a tracer in lieu of additional information on human toxicology or a change in the position of the Food and Drug Administration."

S.L. Abidi (1982) reported on laboratory tests showing that when rhodamine WT is mixed with streamwater containing nitrites,

diethylnitrosamine (DENA), a carcinogen, may be formed. Johnson and Steinheimer (1984) conducted a number of tests relative to DENA formation and persistence. They found that DENA in a simulated stream environment has a half life of less than 3 hours. They also analyzed water samples from four streams taken during rhodamine WT tracer studies and could not detect DENA in any of the samples. Nitrite concentrations in the four streams varied from 2 to 46 μg/L.

Users of rhodamine WT should take special precautions to avoid direct contact with the dye. Rubber or plastic gloves should be worn when handling concentrated dye solutions. When the dye does come in contact with the skin, it should be washed off immediately. Pipetting of dye solutions should be done with a squeeze bulb or by using a long piece of flexible tubing to prevent accidental ingestion of the dye.

The Geological Survey policy for the use of rhodamine dyes by its employees is that the maximum permissible concentration of the dye is 10 μg/L at any water intake that ultimately results in direct or indirect human consumption. Concentrations at water intakes should be kept well below this level; many dye studies can be designed for maximum concentrations of 1 μg/L at such critical points as water intakes.

Spectral characteristics

It is common practice to define fluorescent substances as those that absorb light at one wavelength and emit light at a longer wavelength. Actually, each fluorescent substance is characterized by a specific excitation spectrum and a specific emission spectrum. The excitation spectrum is the variation in intensity of emitted light with the wavelength of the absorbed light. (The excitation spectrum is equivalent to the absorption spectrum, which relates absorbed light to wavelength.) The emission spectrum is the variation in intensity of emitted light with the wavelength of the emitted light. The characteristic spectra for several tracer dyes are shown in figure 3. Usually the wavelengths corresponding to maximum excitation and emission intensity are given to represent the spectral-fluorescence characteristics of a substance.

Spectral-fluorescence characteristics of rhodamine WT and pontacyl pink are very similar, as can be seen in figure 3. Therefore, it is impossible to differentiate between these dyes in a common solution. Fortunately, however, few other materials exhibit characteristics similar to those of the two dyes. The use of highly selective color filters in a fluorometer permits easy isolation of the fluorescence of the dyes from that of most other materials found in streams.

The spectral-fluorescence characteristics of acid yellow 7 are considerably different from those of the other dyes. The excitation spectrum peaks in the violet color range and the emission spectrum peaks in the green part of the spectrum. It is possible to differentiate quantitatively between acid yellow 7 and the other two dyes shown in figure 3; however, the emission characteristics of acid yellow 7 are somewhat similar to the emission characteristics of other materials that may be found in streams. This can cause interference of background fluorescence with fluorescence of the dye. Therefore, before a test, samples of the water in which acid yellow 7 is to be used as a tracer should be checked on a fluorometer for magnitude and variability of the background readings. The fluorometer filters used to detect acid yellow 7 are different from those used for the other dyes. Filter selection is discussed in the next section.

Fluorometers

General description

The two fundamental types of fluorometers are (1) fluorescence spectrometers or spectrofluorometers, used for spectral analyses of fluorescent substances (Udenfriend, 1962, p. 62-86), and (2) filter fluorometers, discussed in this section.

A filter fluorometer, or fluorimeter, is an instrument that gives a relative measure of the intensity of light emitted by a sample containing a fluorescent substance; the intensity of fluorescent light is proportional to the amount of fluorescent substance present. However, a fluorometer reading by itself is a number having little meaning until it is compared with readings for samples of known concentrations

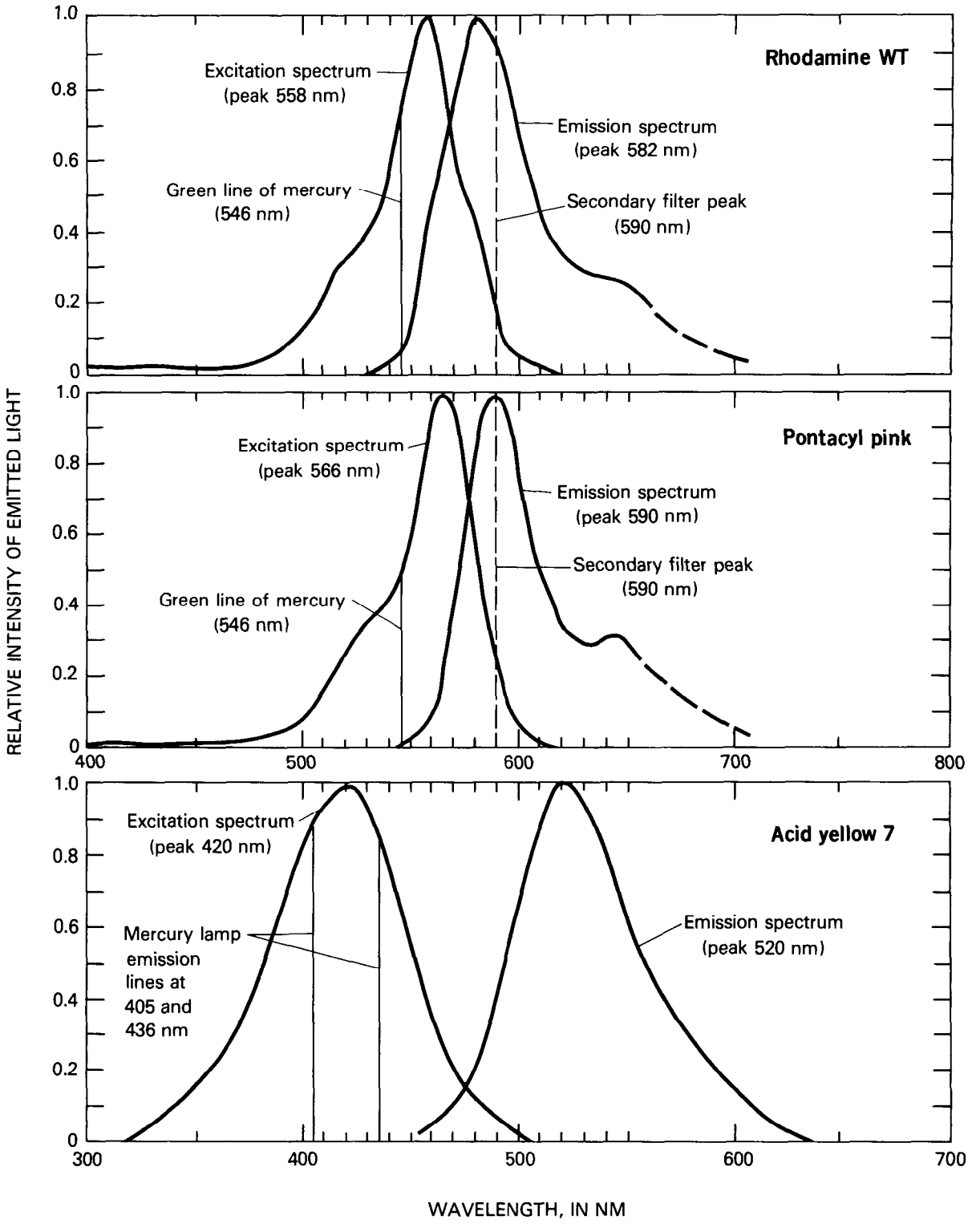

Figure 3.—Excitation and emission spectra of rhodamine WT, pontacyl pink, and acid yellow 7 dyes. Spectrofluorometric analysis for rhodamine WT and pontacyl pink courtesy of G.K. Turner Associates. Spectra for acid yellow 7 adapted from Smart and Laidlaw (1977, fig. 1).

(standards) on the same fluorometer under the same instrument and temperature conditions. Generally, a reading for a given sample on one fluorometer cannot be compared directly with a reading for the same sample on a different fluorometer. Every fluorometer is different and must be individually calibrated.

A filter fluorometer consists of six basic components, shown in figure 4. This basic structure is found in all commercial fluorometers (Udenfriend, 1962, p. 62–78).

A number of companies market fluorometers that can be used for water tracing. Reference to a specific fluorometer and components is for the purpose of illustration and should not be regarded as an endorsement of a particular brand of equipment. In this report, three different brands of fluorometers are discussed. There may be other fluorometers that are as useful for dye tracing as those mentioned. The brands discussed are known to be used in the Geological Survey.

Some representative fluorometers of the types that are presently used in the Geological Survey are shown in figures 5, 6, and 7. The Turner model 111 fluorometer (fig. 5) has been extensively used by the Geological Survey. Figure 8 is a functional diagram of this fluorometer. Other fluorometers generally are based on similar principles. One basic difference is that the FLM/AMINCO fluorocolorimeter (fig. 7) and the Turner Designs model 10 fluorometer rely on solid-state electronic amplification of the photomultiplier signal, whereas the Turner model 111 fluorometer relies on a mechanical servomotor arrangement (fig. 8).

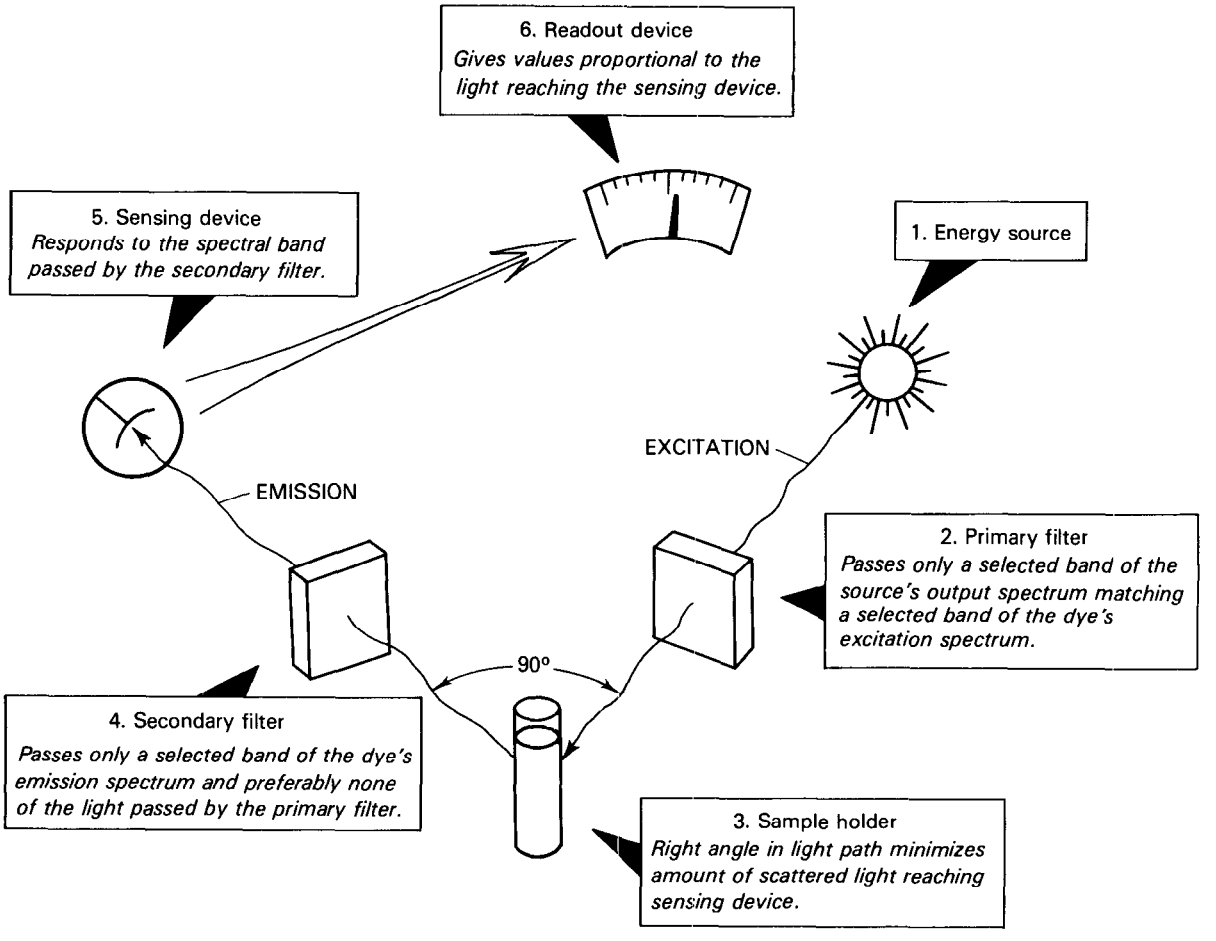

Figure 4.—Basic structure of most filter fluorometers.

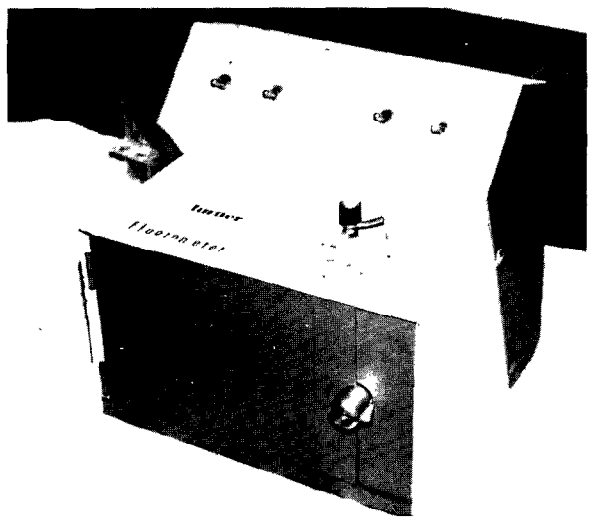

Figure 5.—The Turner model 111 fluorometer.

Selection of optical components

The sensitivity of a fluorometer determines the lower limit of detectability of a dye. For a given fluorometer and dye, instrument sensitivity, and hence dye detectability, depend on the characteristics and interrelationships of the optical components of the instrument. For maximum sensitivity, all components of the fluorometer's optical system must be properly matched to the dye and physically aligned. However, when sensitivity of the instrument is increased, undesirable effects such as background interference also may be increased. If the change is to be useful, the net effect of a contemplated change in optical components must be favorable to dye detectability.

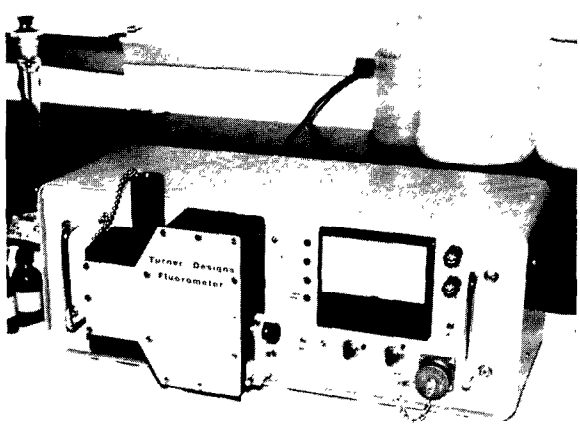

Figure 6.—The Turner Designs model 10 fluorometer.

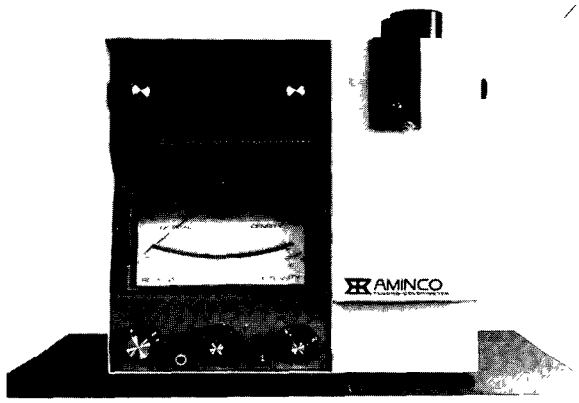

Figure 7.—The FLM/AMINCO fluorocolorimeter (photograph by Marvin D. Duerk, U.S. Geological Survey).

Lamps

The objective in both lamp and filter selection is to obtain as much sensitivity to the dye as possible without sacrificing selectivity. Selectivity is the capability of isolating a part of the fluorescence spectrum of the dye from potentially interfering background fluorescence.

There are three lamps that may be used with rhodamine WT and pontacyl pink dyes. Two of the three, the general-purpose ultraviolet (UV) lamp and the far-UV lamp, are low-pressure mercury-vapor lamps that emit discontinuous spectra of high-intensity monochromatic lines easily isolated by the proper filters. Most of the mercury lines are in the UV or violet part of the spectrum, but the "green line" at 546 nm is close to the peak excitation wavelengths of rhodamine WT and pontacyl pink (fig. 3). The 546-nanometer line emitted by the far-UV lamp, which is clear glass, is more than twice as strong as that emitted by the general-purpose UV lamp, which has a white phosphor coating.

The third lamp, the green T-5 envelope lamp, emits a continuous spectral band from less than 520 nm to more than 560 nm, peaking at 546 nm. Because the lamp output is a band instead of a monochromatic line, more light passes the primary filter. The advantage of this form of

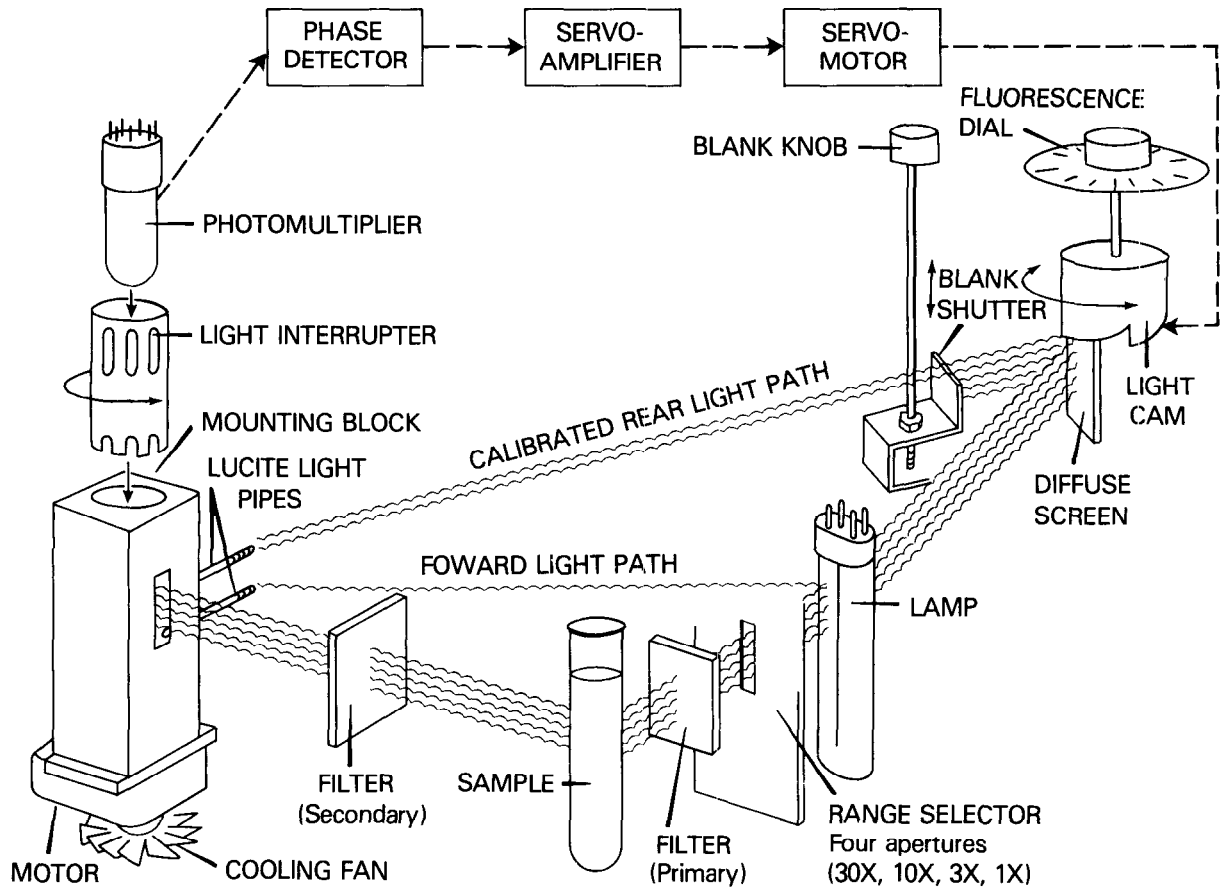

Figure 8.—Functional diagram of the Turner model 111 fluorometer. Modified from drawing furnished by G.K. Turner Associates.

output is increased sensitivity to the rhodamine WT and pontacyl pink dyes, on the order of tenfold, compared with the far-UV lamp. A disadvantage is a possible increase in background interference for two reasons: (1) a small part of the light in the 560- to 570-nanometer range passes straight through the filter system to the photomultiplier, and (2) the fluorometer is more sensitive to scattered light and to background materials that are not strongly excited by the 546-nanometer line alone. The first source of interference is constant and presents no great problem. The second source may not be present at all, or it may be great enough to preclude use of this lamp. In general, this second source has not been found to be a problem. Both the far-UV and the green T-5 lamps have been used extensively with the rhodamine dyes and are recommended for use with the Turner model 111 fluorometer. The green T-5 envelope lamp is not needed with the FLM/AMINCO fluorocolorimeter because the range of sensitivity is adequate with the far-UV lamp.

The far-UV lamp or equivalent is recommended for use with acid yellow 7 dye. This lamp has useful outputs at 254, 297, 313, 405, 436, and 546 nm. The outputs at 405 and 436 nm are both near the peak of the excitation spectrum of acid yellow 7 (see fig. 3).

Filters

The purpose of using color filters in a fluorometer is to limit, as much as possible, the light reaching the photomultiplier to the light fluoresced by the dye. Filter selection must be based on (1) the useful output spectrum of the lamp, (2) the spectral-fluorescence characteristics of the dye, (3) potential interference from fluorescence of materials present in the stream,

and (4) potential interference from light scattered by the sample.

A preferred filter system for use with rhodamine WT and pontacyl pink dyes consists of a primary filter combination peaking at 546 nm and a secondary filter combination peaking at 590 nm. The primary filter combination can be obtained by using two Corning 1-60 and one Wratten 61 filters. The secondary filter combination can be obtained by combining a Corning 4-97 and a Corning 3-66 filter. The spectral-transmittance characteristics of the two filter combinations and of the components of the secondary filter are shown in figure 9.

The loss in transmittance resulting from combining filters is very apparent. It also may be seen in figure 9 that the green line of mercury is completely screened out by the secondary filter, even though there is a slight overlap of the spectra of the primary and secondary filters. This overlap is significant only if the green T-5 lamp is used. The orange filter (Corning 3-66) is always placed closest to the photomultiplier and the blue filter (4-97) closest to the sample to eliminate any fluorescence of the filters themselves.

Although rhodamine WT and pontacyl pink, as shown in figure 3, have slightly different spectral-fluorescence characteristics, the filter combinations shown in figure 9 are good for both dyes. It can be seen in figure 3 that the green line emitted by the far-UV lamp and passed by the primary filter is more efficient in exciting rhodamine WT than it is for pontacyl pink. To examine the effect of the secondary filter, one should compare the entire filter transmission spectrum with the entire fluorescence spectrum of the dye.

The 546/590 filter combination is especially preferred when turbidity is present, as the 590-nanometer secondary filter eliminates most of the scattered light. In the absence of high background levels, sensitivity may be increased by replacing the primary filter with a combination of one Corning 1-60 and one Wratten 58. (This substitution is not recommended if the green T-5 lamp is used.) The secondary filter may be replaced by a Wratten 23A, which has a transmission spectrum very similar to that of the Corning 3-66 shown in figure 9. Background interference (from other fluorescent substances or from scattered light) is a potential source of trouble with the 23A because of the open end of its transmission spectrum. Usually, substitutions in the 546/590 combination are unnecessary. However, when turbidity and background fluorescence are almost totally absent and the experimenter is willing to perform the extra

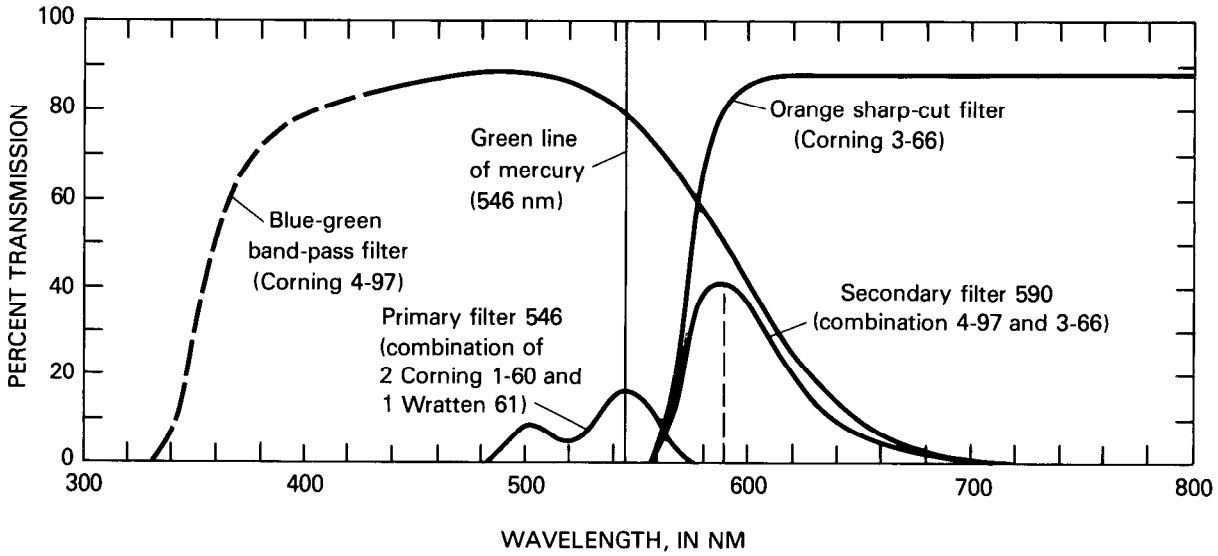

Figure 9.—Spectral-transmittance characteristics of preferred filters for rhodamine WT and pontacyl pink dyes. Sources: Corning Filter Catalog for curves for filters 4-97 and 3-66; Feuerstein and Selleck, 1963b, figure 3, for curve for primary filter combination 546. Separate curves for Corning 1-60 and Wratten 61 not shown. (See Corning Glass Works, 1962, and Eastman Kodak Co., 1965.)

work needed to be assured that there is no interference, the substitute mentioned above may be used to advantage.

For acid yellow 7 dye, the primary filter recommended by Sequoia Turner Corporation (written commun., 1981) is a combination filter consisting of a filter with a color specification 2A and filter 47B. The 47B filter transmits wavelengths between about 390 and 500 nm, with a peak transmittance at about 436 nm. The 2A filter is placed nearest to the lamp. A preferred secondary filter for acid yellow 7 dye is filter 2A–12, which passes wavelengths greater than 510 nm.

The filters must be replaced periodically, as they become clouded with use. Occasional comparison of fluorometer calibration curves will indicate their deterioration and need for replacement.

Occasionally, very high dye concentrations will necessitate the use of a neutral-density (ND) filter. Although ND filters are available in a wide range of transmission percentages, only one or two are necessary to have on hand. A 10-percent ND filter, which passes only 10 percent of the light reaching it, is recommended. In extreme cases, a 1-percent ND filter may be used, but dilution of the sample can be used as a means of analyzing samples of such high concentration in the rare instances they are obtained. The ND filter should be placed over the secondary color filter in the fluorometer. Because the FLM/AMINCO fluorocolorimeter has such a wide range of scales, it is seldom necessary to use an ND filter with this instrument.

Sample holders

The standard cuvette for testing a single sample in the Turner model 111 fluorometer is glass, 3.5 cm^3 in volume, 12 mm in diameter, and 75 mm long. The Turner Designs model 10 fluorometer uses a glass cuvette 13 mm in diameter by 100 mm long which fits inside a temperature-controlled holder. The FLM/AMINCO fluorocolorimeter uses a 10- by 75-millimeter cuvette.

The amount of light fluoresced by a given concentration varies directly with the size of the sample holder. Some fluorometers provide for the use of cuvettes of different sizes. For example, the flow-through door on the Turner Model 111 fluorometer has provision for a variety of cuvette sizes ranging from 1 to 20 cm^3.

A square cuvette 4.5 cm^3 in volume and 10 mm square by 75 mm long can be used in the standard door of the Turner model 111 without the high-sensitivity kit (the high-sensitivity kit requires the use of a round cuvette). This quartz glass cuvette is designed to increase sensitivity to turbidity in samples but is also useful in producing lower and more consistent background readings when turbidity is low. In this instance, the green T–5 lamp may be used to acquire the desired sensitivity. The FLM/AMINCO fluorocolorimeter does not require modification to increase sensitivity, since it has high-sensitivity capability built into it.

Two methods may be used to improve the sensitivity of the Turner model 111 fluorometer. Most commonly, a high-sensitivity conversion kit is installed in the standard door when the far-UV lamp is used. The kit uses mirrors and a glass prism to direct fluorescent light more efficiently toward the photomultiplier tube, thus increasing the sensitivity of the single-sample cuvette about tenfold. The high-sensitivity kit is a built-in component of the constant-temperature door. These doors are described in the section on "Accessory Equipment." It should be noted that slight misalignment of the high-sensitivity kit can cause calibration difficulties. Most nonlinear calibrations can be traced to this source.

A second method of improving the sensitivity of the Turner model 111 fluorometer is use of the green T-5 lamp with the standard door and without the high-sensitivity kit. The high-sensitivity kit and the T-5 lamp should not be used in combination. The use of this lamp eliminates the possibility of misalignment of the high-sensitivity kit, since it is not used, and thus eliminates this cause of nonlinear calibration. Either the more accurate square cuvette or the round cuvette can be used with this combination.

A cuvette itself may be fluorescent or may cause some undesirable scattering of light. The filters used in dye tracing seem to prevent most of this kind of interference. For precision work, the same cuvette or matched cuvettes should be used to avoid any small differences in readings that might be due to light scattered by different cuvettes.

Photomultiplier

A photomultiplier is a special vacuum tube that detects incident radiation and amplifies the resulting electronic signal. The photomultiplier tube used in most fluorometers is sensitive primarily to the blue and UV end of the spectrum, while the rhodamine WT and pontacyl pink dyes fluoresce primarily in the orange range. However, some of the standard tubes are very sensitive to the red wavelengths, which accounts in part for a wide variation in sensitivity among instruments. Red-sensitive photomultiplier tubes are available for some fluorometers. The fluorometer manufacturer should be contacted concerning the availability and installation of such tubes if they are desired. The sensitivity of the red-sensitive photomultiplier tube can be as much as three to five times that of the standard tube.

Range control

Most fluorometers have a means of manually controlling available sensitivity. In the Turner model 111, this control is accomplished by a range selector between the lamp and the primary filter (see fig. 8). The four positions are called 30×, 10×, 3×, and 1×, indicating their approximate relative sensitivity. For example, the 30× scale gives a reading approximately three times as high as the reading on the 10× scale for the same fluorescent sample. However, the true relationship between the scales varies among instruments. Average values for a group of four instruments were found to be 19×, 7.6×, 2.8×, and 1× when the far-UV lamp was used. There is evidence that these ratios vary with intensity of lamp output.

Sensitivity, or range control, is electronically adjusted in the FLM/AMINCO fluorocolorimeter by use of multiplier and fine-adjust controls. The multiplier control is used to select one of seven ranges of sensitivity available in the measuring circuit. These scales of relative intensity are as follows: ×100, ×30, ×10, ×3, ×1, ×.3, and ×.1. All scales are exact multiples. For example, when the 0 to 0.1 (×.1) scale is selected, 0.1 is full scale. When the multiplier switch is changed to the 0 to 1.0 (×1) scale, 0.1 is one-tenth of full scale and indicates the same relative intensity. This allows background to be suppressed on the most sensitive scale and to remain suppressed on all scales. This, along with the fact that scales are exact multiples, is very useful during calibration when performing precision analysis.

Other fluorometers have similar manual controls for sensitivity. Some fluorometers, such as the Turner Designs model 10 fluorometer, provide for optional automatic selection of sensitivity scale—a useful feature for continuous sampling.

Preferred systems

For general use with rhodamine WT or pontacyl pink dyes, a preferred system for use with the Turner model 111 fluorometer consists of either the standard door, high-sensitivity kit, and far-UV lamp or the standard door without the high-sensitivity kit but with the green T-5 lamp. In either system, the 546- and 590-nanometer filters should be used. Equivalent lamps and filters should be used with other fluorometers. The Turner Designs "rhodamine accessory kit" contains the proper lamp and filters for the Turner Designs model 10 fluorometer. The FLM/AMINCO also has a "rhodamine kit" for use with its fluorocolorimeter. Other fluorometers may not have high-sensitivity kits or may have other means of increasing sensitivities. For maximum sensitivity with the flow-through door, the green T-5 lamp is recommended, as the high-sensitivity kit is not part of this door. It is not necessary to modify the FLM/AMINCO's fluorocolorimeter or the Turner Designs model 10 with a high-sensitivity kit as these instruments have the needed sensitivity built into them.

The high-sensitivity kit should not be used when using acid yellow 7 because the fluorometer is quite sensitive to this dye and the high-sensitivity kit may enhance background fluorescence too much. For use with acid yellow 7, the far-UV lamp, the 2A and 47B filters (used as a primary filter), and the 2A-12 filter (used as a secondary filter) are preferred.

Accessory equipment

Temperature-control apparatus

Because fluorescence depends on temperature, all fluorometer readings must be either