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SPECTROFLUOROMETER TECHNIQUES APPLICABLE TO SAMPLE SURFACES *

by

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ABSTRACT

Fluorescence analysis of the surfaces of samples, both solid and liquid, is required to provide ground-truth data in support of remote sensing of the fluorescence of earth surfaces. To facilitate this type of test and to increase reproducibility of fluorescence spectra, modified spectrofluorometer designs are suggested and improved laboratory techniques are described.

A horizontal sample orientation would be advantageous for testing such surfaces as liquids, floating films, natural soils, unconsolidated sediments, and moist samples. Integration of fluorescence from a large surface would be an advantage, either by spinning the sample or an optical component in order to sweep the field of view over a circular area about 1 cm. in diameter while maintaining high spectral resolution by means of narrow slits.

Severe limitations in testing of sample surfaces include the behavior of many samples as "quantum counters", to some degree, and the incorporation of scattered or reflected stray light into their fluorescence spectra. Procedures for evaluating or correcting for these sources of error are discussed.

Methods for correcting fluorescence spectra to remove instrumental artifacts are described in detail, including computational and graphical procedures for rapid manual correction by nomographs and

similar techniques. Expedient methods for the selection and use of optical filters, the evaluation of filter effectiveness, and the selection of optimum wavelength settings in spectrofluorometry are outlined.

SPECIAL SPECTROFLUOROMETER TECHNIQUES FOR SAMPLE SURFACES

Study of the fluorescence of sample surfaces, whether of solids or liquids, may impose special requirements and limitations in the areas of: 1) sample holders and compartments; 2) positioning of samples; 3) concentration; 4) choice of excitation versus emission spectra; and 5) stray light.

Sample holders and compartments

To permit efficient study of a wide range of sample surfaces, including rocks, minerals, leaves, unconsolidated sediments, and liquids, it is advantageous for the sample compartment in the spectrofluorometer to be large enough to accommodate samples of a reasonably large size and to be versatile. Most sample compartments that we have seen could be greatly improved for this purpose. Generally the sample compartments are easily removed and there is space for an improved compartment having most of the desired versatility. Several desirable characteristics are enumerated below, of which some might require changes in basic optical design that should be feasible as soon as there is greater demand for instruments to study fluorescence of sample surfaces.

1) Large size and versatility. It is desirable that the compartment accommodate rock and mineral samples measuring approximately 3 x 3 x 1-1/2 inches, and the minimum useful size would accept samples measuring approximately 3 x 2 x 1/2 inch. Provision is needed for testing leaves and for holding them in a reasonably flat position without crushing. It would be advantageous to be able to test leaves in vivo, requiring access for a stem and space immediately adjacent for a pot or beaker to contain a living plant. Provision is needed for testing small quantities of powder, preferably the amount needed to cover a circle no more than 1/2 inch in diameter and no thicker than about 0.2 mm. It is desirable that the sample be capable of being rotated through small angles while the compartment is closed so that the view angle can be made optimum. It would also be advantageous to be able to adjust the location of the sample with the lid closed in order to test various spots on the surface. It should be possible to easily change from solid to liquid samples.

2) Provision for filters. Testing of sample surfaces, including liquid surfaces, requires the use of filters on both the excitation and emission sides of the sample. There should be two spaces large enough for filters measuring 2 in. x 2 in., and having a thickness of at least 7 mm. and preferably 1/2 inch. Filters that are built into some instruments are permanently mounted on one side of the sample, consequently there is still a requirement for filters that can be moved alternately

from one side to the other as required for work with sample surfaces. There is a need for inexpensive non-fluorescent filters with bandwidths of about 10 to 20 nm or narrower, peak transmittances over 50%, and peak wavelengths spaced every 10 to 20 nanometers throughout the visible spectrum. Available inexpensive glass and gelatin filters would fill this need except that their coloring may be provided by fluorescent dyes.

3) Provision for angular alignment of beam or sample. Because the incident and viewed beams in spectrofluorometers are basically at right angles, it is necessary to position a sample surface at some angle so that the major part of the incident beam will be reflected at an angle away from the viewed beam. Figure 1 illustrates two basic acceptable methods for accomplishing this. In Figure 1A the incident beam is normal to the sample surface, the sample is in a fixed angular position, and a small rotating mirror provides the means for making small adjustments in the angle of view to accommodate irregularities in the surface of the sample. In Figure 1B and 1C the incident beam has an angle of incidence of about 30° with the normal to the sample surface, while the angle of view is 60° from this normal. Consequently the bulk of reflected light will be at angles midway between these two beams.

These two alternatives are nearly equal in terms of optical efficiency. The design using a rotating mirror appears preferable because the mirror can be rotated more conveniently than the sample in attaining optimum settings. This design minimizes the

chance of damage to friable samples or to optical or other components in the sample compartment. A design superior to either of these might be similar to Figure 1A, except that the front surface of the sample would be totated by about 10^{o} in a clockwise direction in order to further decrease the possibility of incident light being reflected at the angle of view. However considerable change in design might be required to accommodate the various components. The unit might need to be as large as that shown in Figure 1C.

4) Other desirable provisions. For testing of liquid surfaces, floating films on liquids, natural soils, unconsolidated sediments, and moist samples of any kind, it would be advantageous to place the sample in its natural horizontal position. Horizontal testing would require a deeper sample compartment and the use of two mirrors, one of which might need to rotate through a small angle. Further, to minimize heterogeneity and preferred orientation, it would be desirable to scan a larger area of the sample surface. Although there are several ways to accomplish this, the most practical would be a configuration in which the incident beam was normal to the sample surface, and the sample was rotated rapidly on a small turntable; alternatively a mirror or some other optical component might rotate.

Positioning of samples for surface testing

In testing the surfaces of samples for fluorescence, whether they are solids or liquids, there is a complex geometric relation between the widths of the two beams as defined by the slits, the angular orientation of the sample surface, the exact location of the sample surface, and if a cuvette or powder cell is used, the thickness of the cell wall or window. These relations are illustrated in Figure 2, drawn approximately to scale and enlarged 10 times. Because of the large number of variables, the degree of reproducibility of fluorescence intensities is fair for powdered samples or for liquid surfaces, fair to poor for flat surfaces such as sawed rocks and smooth leaves, and very poor for the rough natural surfaces of solid samples. For the solid samples it is most important to improve reproducibility so that laboratory and remote sensor data can be better correlated.

Although instrument parameters and sample position are usually adjusted to give maximum intensity, there are at least two difficulties in finding the optimum position for testing a sample surface: 1) confusion of fluorescence and reflectance; and 2) variations in concentration of the fluorescent substance. These are discussed below and the geometric relations illustrated on Figure 2.

1) Confusion of fluorescence and reflectance. As a rough sample surface is maneuvered in the incident beam to attain maximum fluorescence the angle of the sample with respect to that beam will continually change as a result of small irregularities in the surface. This will cause varying amounts of reflected light to follow the path of the viewed beam, and if the incident beam contains a significant percentage of stray light there generally will be sample positions in which the reflected stray light will exceed the fluorescence emission. It was described previously (Stoertz, G.E. and Hemphill, W.R., 1972) how these components can be separated by the use of filters. In order to do this while maneuvering a sample for optimum position, it is necessary to use a trial-and-error procedure in which the sample is adjusted, intensity is recorded with filter first on excitation side and then on emission side, the sample is re-adjusted, and the process is repeated. This procedure becomes prohibitively time-consuming because of the following related problem.

2) Variations in concentration of the fluorescent substance. Unlike liquids, fluorescence of natural solids is irregularly distributed across their surfaces. This is true even of leaves that have a completely uniform green appearance. The only exception is in the case of finely-ground and well-mixed powdered samples that are pressed uniformly against a quartz cell window. Consequently in adjusting sample position

to attain maximum fluorescence, the effects due to pure geometry are confused with those due to irregular distribution of the fluorescent substance across the sample surface. Thus "hot spots" are usually tested, rather than spots representative of the whole surface. Because of this and related problems there is a real need for better techniques and/or instrumentation, some of which have been mentioned above. These may include normal incidence of the excitation beam, use of a rotating sample turntable, and use of a rotating mirror or other optical component.

Present instrumentation and techniques for measuring fluorescence intensity of sample surfaces do not give sufficiently reproducible results, particularly from rough surfaces. The comparison of fluorescence intensities for two or more surfaces, for example, may be subject to large errors. Comparison of the fluorescence of two or more liquid surfaces may be more reliable, provided the following precautions are taken:

1) The cell wall thicknesses must be as nearly identical as possible for all samples. It may be desirable to use the same side of a single cuvette for each measurement.

2) The square prism formed by the intersection of the incident beam and the viewed beam must be so situated that it falls largely within the liquid rather than in the quartz cell wall.

3) In order to achieve maximum fluorescence, which indicates the optimum position, the square prism mentioned above must generally

intersect a small area of the liquid surface.

4) In the case of highly colored or absorptive liquids, the area of intersection must be as large as possible, whereas in clear transmissive liquids it must be as small as possible; the optimum position will therefore differ with every change in these optical properties.

5) If comparison is to be made between a highly colored liquid and a relatively clear liquid the most valid comparison would be based on positions which were optimum for each liquid; this would require that the cuvette be adjusted differently for each test.

6) Adsorption of fluorescent solutes on the walls of the cell will be a significant source of error in measuring fluorescence of liquid surfaces, and will probably cause variation from place to place, resulting in poor reproducibility; this will probably be a source of error at all concentrations, although most serious at low concentrations.

7) Better reproducibility would be expected when slits are wider, because an increase in the cross-sectional area of the square prism will allow greater tolerance in positioning of the cell, better reproducibility of exact slit widths, better integration of small variations due to surface adsorption, and greater tolerance of small differences in cell wall thickness. However, the use of wider slits to attain better reproducibility would sacrifice spectral resolution, hence some intermediate slit width will give optimum results.

The concentration factor in testing sample surfaces

Problems related to concentration that have already been mentioned, and that are unique to testing of sample surfaces are: 1) adsorption of fluorescent solutes on the inner surfaces of cell walls; and 2) great variations in concentration of the fluorescent substance across the surface of a solid sample. Another factor related to concentration is that many natural solids display far higher concentrations of fluorescent components than do natural liquids, and the more concentrated liquids would normally be diluted for spectrofluorometer tests. Fluorescence measurements depend for their high sensitivity and accuracy on the use of extremely dilute solutions, commonly less than 100 micrograms per liter (100 parts per billion).

At higher concentrations the phenomenon of concentration quenching causes serious departures from linearity of concentration versus fluorescence, with the result that an inordinate number of standard samples would be needed in order to attain high accuracy in measuring concentration of unknown samples. If concentration of a solute is increased beyond some point there is an actual decrease in measurable fluorescence with further increase in concentration because the useful wavelengths in the incident beam are largely absorbed before the light reaches the critical square prism that is being viewed, or because the fluorescence emitted within this prism is largely re-absorbed before it reaches the outer wall of the cell.

A similar phenomenon occurs in solid samples, but with additional complications. In an extreme case, the concentration of the fluorescent solid may be so great that a large portion of the incident light energy actually serves to excite fluorescence in the sample. In this case, the amount of fluorescence emitted would depend largely on the available light instead of on the substance. Consequently the excitation spectrum would be an image of the source-light spectrum rather than an image of the fluorescence excitation properties of the substance.

The best example of this phenomenon is a "quantum counter", which is simply a concentrated fluorescent solution that absorbs practically all the incident light energy within the thin surface layer of the solution, converting a large portion of this energy into fluorescence that is emitted within the field of view of the emission optics. A solution often used as a quantum counter is rhodamine B in ethylene glycol, at a concentration of 3 to 5 grams per liter. The excitation spectrum of this solution, monitored at 610 to 630 nm, will be an image of the spectral distribution of incident light, in quanta per wavelength interval.

We observed this phenomenon to some degree in many solid and liquid samples, and it appears to be this phenomenon more than any other that explains the marginal results often obtained in fluorescence studies of solid samples. Any sample exhibits this effect if the amount of fluorescence emitted within the field of view is directly proportional to the amount of incident light available throughout a broad spectral band.

Many solids and highly colored liquids (e.g., oil films) display this effect, behaving as "quantum counters" to some degree. If efforts are made to correct the excitation spectrum of a quantum counter for spectral variations in source intensity, the corrected spectrum will be nearly a straight line. In effect, the uncorrected excitation spectrum is largely artifact with little or no spectral fluorescence excitation data.

Methods used to correct fluorescence spectra are discussed in a later section. It is noteworthy that the foregoing effect is likely to be present to some extent in nearly all excitation spectra from solid samples. Efforts are still being made to find a reliable means to evaluate or correct for this. Powdering the sample and diluting might be beneficial, but this would destroy the original surface which is of interest in remote sensing applications. Fortunately, reliable emission spectra can evidently be obtained from sample surfaces, even when the fluorescent component is highly concentrated.

Evaluation of stray light in a spectrofluorometer

It was concluded above that stray light is present in all spectrofluorometers, but that its presence is critical only in the testing of sample surfaces or other highly reflective targets. Because the adverse effects of stray light can be largely removed by use of filters placed alternately on both sides of a sample, the remaining problems posed by stray light are that: 1) it represents a loss of potential efficiency and sensitivity, because the stray light effectively reduces the useful light

in the incident beam and the useful portion of the graph plotted by the recorder; 2) a portion of the reflected stray light is combined in the spectrum along with the pure fluorescence, and may be expected to constitute from 15% to 50% of the intensity of the fluorescence from a sample surface; and 3) the presence of a large percentage of stray light may make it difficult to recognize another spurious element such as the fluorescence of the filter. A method for evaluating the amount of stray light in a spectrofluorometer is summarized immediately below.

Figure 3 shows two emission spectra actually recorded by a spectrofluorometer for moderately reflective samples of phosphate rock and of ulexite. Each consists of a pair of spectra recorded as described previously, with a filter alternately before and after the sample in the optical path. If the filter itself had contributed appreciable fluorescence, this component would be present in both the upper and the lower curves, because the movement of the filter with respect to the sample should not materially effect the fluorescence of the filter. The fluorescence of the sample would be included only in the upper curve. Therefore the ratios between the two curves at intervals along the spectrum should remain fairly constant unless both of these curves contained a second fluorescent component (e.g., a filter fluorescence component). In that case the second fluorescence component would be likely to reveal itself in the form of a peak or some other consistent change in the ratios from one end of the spectrum to the other.

These relations are shown in Table 1. The ratios do not reveal any significant or consistent change, or any concealed peak that might suggest incorporation of a second fluorescence component within either set of spectra. In both cases the lowest values are at or near the peak fluorescence of the sample, and higher values occur at both longer and shorter wavelengths. This is the kind of result that suggests a small error in the zero level. Practically all discrepancies between the upper and lower curves can be ascribed to stray light rather than to filter fluorescence.

The conclusion from these two samples is that stray light comprised at least 38% of the total peak height recorded for phosphate rock and at least 66% for ulexite. Therefore stray light reflected from the phosphate rock was at least 61% as intense as the fluorescence from that sample, while in the case of the ulexite sample the stray light was at least 194% as intense as the fluorescence. In these and similar applications it could be concluded that the presence of stray light might reduce the effectiveness of this spectrofluorometer to as little as one-third the effectiveness of a perfectly efficient instrument.

CALIBRATION OF A SPECTROFLUOROMETER AND CORRECTION OF FLUORESCENCE SPECTRA

A primary purpose of spectrofluorometry is to determine the true excitation and emission peak wavelengths for fluorescent substances. It should be possible to extract this type of information from the spectrum of any sample short of a perfect quantum counter. However, the more closely a sample approaches the characteristics of a quantum counter, the more difficult it becomes to correct its excitation spectrum.

The object of our efforts was to remove instrumental artifacts from the spectra of sample surfaces, including solids. However absorptive, reflective, fluorescent, or concentrated such a sample might be, it should be possible to extract from its spectra the true excitation and emission peak wavelengths. These peaks are useful in predicting wavelengths that would be most effective in remote sensing of fluorescence from similar targets on the earth's surface.

Instrumental artifacts that are inevitably incorporated into fluorescence spectra include: 1) spectral variations in intensity of the source lamp; 2) spectral variations in sensitivity of the photomultiplier tube; 3) transmittance of the filter at the wavelength setting of the monochromator; 4) sensitivity setting of the instrument at time of test; 5) slit-width setting at time of test; 6) actual sensitivity of the instru-

ment due to all other factors at time of test (e.g., line voltage, warm-up time, etc.). The first two are spectrally-related factors, and these need to be corrected to extract peak wavelengths of excitation and emission from the spectra. The other four factors are related only to overall intensity and are of secondary importance because fluorescence intensity of sample surfaces is also closely related to the positioning of the sample in the instrument. Methods for correction of the six instrumental artifacts will be described below, with emphasis on the two spectrally-related factors. Later the factors will all be incorporated into a single formula for correction of spectra.

Correction for source-lamp spectrum

In order to relate fluorescence spectra obtained with a laboratory spectrofluorometer to fluorescence that would result from stimulation by sunlight or by laser as in remote sensing applications, it is necessary to eliminate artifacts resulting from spectral variations in the intensity of the source lamp used in the spectrofluorometer. This is effectively accomplished by viewing a quantum counter consisting of rhodamine B in ethylene glycol, as described above. Such a solution maintains a constant ratio of quanta absorbed at 280 to 600 nanometers

to quanta emitted at 630 nanometers (Perkin-Elmer Instrument News, 1970, p. 12). The excitation spectrum is obtained through a red filter (e.g., Kodak Wratten gelatin filter no. 29) with excitation slits equivalent to about 4 nanometers of the spectrum and emission slits equivalent to about 36 nanometers. Because of the broad emission slits the emission monochromator may be set anywhere from 610 to 630 nanometers. However, Wratten gelatin filter no. 26 would be preferable with a setting of 620 nanometers, and no. 25 with a setting of 610 nanometers.

If correction of short ultraviolet wavelengths were needed, a quantum counter of "BPEA" in a concentration of about 1 mg/liter has been recommended, using a yellow filter, and for excitation wavelengths from 200 to 280 nanometers (Anakrion, R.E., 1971, personal communication). Because of their strong coloring, quantum counters must be tested on their front surfaces rather than by transmitted light. The resulting excitation spectrum then should have a vertical scale that is very nearly equivalent to units of quanta per wavelength interval (Porro, T.J., 1971, personal communication). The spectrum should be recorded after the source lamp is thoroughly warmed-up and stabilized, and the spectrum will vary with age of the lamp, particularly in the ultraviolet. It is most profitable to perform this calibration after the lamp has been used for at least 10 or 15 hours.

Correction for photomultiplier tube sensitivity

Artifacts resulting from spectral variations in sensitivity of the photomultiplier tube can be effectively corrected by use of a standard calibrated lamp whose spectral irradiance is known when it is operated at a specified voltage, amperage, or color temperature. An emission spectrum is recorded while light from the lamp is reflected into the emission side of the spectrofluorometer by means of a surface that is nearly neutral. Additional factors should be applied to correct for the spectral reflectance of that surface, if necessary. We used a 1000-watt standard lamp, operated at 120.2 volts, and reflected specularly from the side of a standard quartz cuvette backed by black felt. Other suitable materials are magnesium oxide, magnesium carbonate, and barium sulfate, but some of these discolor with use, must be freshly scraped, and may require an additional correction for spectral reflectance. It is desirable for slits to be sufficiently wide to be nearly equivalent to a 4-nanometer spectral band, and attenuation of the intense light to an acceptably low level should be accomplished by means of the shutter or slits closest to the photomultiplier tube. If the lamp has been calibrated in units of irradiance it is necessary to multiply irradiance by the corresponding wavelengths in order to convert to a factor proportional to quanta per wavelength interval (Schneider, W., 1971, pers. commun.). Further aspects of this method of calibration have been described (Chen, 1967, p.342-352; Udenfriend, 1969, p.609-11)

Correction for filter transmittance

When filters are used as described in a previous report (Stoertz, G.W., 1972), attenuation of light can be simply corrected by means of a single attenuation factor calculated for the wavelength setting of the fixed monochromator. This is facilitated by selecting in advance a limited number of wavelength settings (e.g., multiples of 10 nm.) to be used, and by determining the most effective filter for use with each such setting. However two important factors limit the accuracy of this correction: 1) the slit widths used during the recording of a spectrum to be corrected should be nearly equal to the slit widths used when the filter correction factor was calculated; and 2) the filter factor must be determined when the filter is on the side closest to the fixed monochromator.

Different narrow-band filters may be more effective for use on the emission side as opposed to the excitation side of the sample, even at the same wavelength settings. This results from the fact that a filter used on the emission side, with a fixed emission monochromator setting of 486 nm (for use during recording of excitation spectra), will be most effective if it has a steep transmittance profile on the low-wavelength side of 486 nm. However the same filter, if used on the excitation side, with a fixed excitation monochromator setting of 486 nm (for use during recording of emission spectra), will be most effective if it has a steep profile at wavelengths higher than 486 nm. Selection of optimum filters for given wavelengths will be discussed in a following section of this report.

Correction for sensitivity

Correction for sensitivity of the spectrofluorometer is facilitated by pre-selecting a limited number of settings to be used. These are already limited by the design of some instruments. Whatever the design, it is advisable to calibrate instrumental settings stated on instrument dials or control panels.

There is a possibility of error due to non-linearity of the recorder, such that pen deflection produced by a given increment of light may vary from the lower part of the chart to the upper part. In calibrating for this error it is important that the zero level of the recorder be accurately adjusted, otherwise a small zero error might be interpreted as non-linearity in pen response. Although this possible source of error was investigated for the recorder used in our research, we found non-linearity to be negligible in that recorder, so no such correction factor was necessary in our calculations.

Correction for slit widths

This correction is also facilitated by pre-selecting a limited number of slit widths to be used, this being particularly advantageous because so many other factors are related to slit width (i.e., light source calibration, photomultiplier tube sensitivity, and filter transmittance). All spectra can then be adjusted for equivalence to arbitrary slit widths by application of a few correction factors such as tabulated below:

Multiply reading at slit width:	By this factor:	To obtain reading equivalent to slit width:
12	0.065	6
10	0.13	6
9	0.18	6
5	1.94	6
4.5	3.01	6
4.4	3.34	6

Correction for temporal changes in light intensity

Light output of the instrument may vary as a result of such things as arc instability, warm-up time, and changes in line voltage. It is advantageous to monitor such changes in intensity by maintaining a standard sealed cuvette of distilled water, and testing it immediately after each spectrum is recorded. A suitable method that has been suggested (Porro, T.J., 1971, pers. commun.) is to excite the water at an arbitrary wavelength such as 350 nm, and record the Raman scatter peak near 400 nm by scanning emission from approximately 380 to 420 nm.

Temperature

Temperature is a significant factor in fluorescence. In quantitatively analyzing liquids for fluorescent solutes the temperature must be controlled by a water bath (Kilpatrick, F.A., 1969, pers. commun.) Our initial experiments with solids were largely qualitative, hence the control of temperature was omitted. In remote sensing by airborne fluorometer over water or land targets the factor of temperature may need to be considered, as moderate temperature changes result in an appreciable change in fluorescence intensity. Apparent temperature of the water surface could be measured from an aircraft with an infrared radiometer (8-14 micrometers). The relation between temperature and fluorescence of Rhodamine WT dye has been analyzed (Stoertz, G.E., 1969, p. 34; Wilson, J.F., Jr., 1967, written commun); for that compound, if temperature increases from $0^{o}C$ to $15^{o}C$ fluorescence decreases more than one-third, and comparable changes would be expected in solids.

Application of the correction factors

Correction of each spectrum requires application of three or more fixed correction factors and one wavelength-dependent factor, the latter being most conveniently applied at uniform intervals across the spectrum (e.g., every 10 nanometers). These factors are applied to the "uncorrected spectrum", which is the measured difference between the two spectra recorded with a selected filter on the excitation and emission sides of the sample, as follows:

$$\text{corrected spectrum} = \frac{\text{uncorrected spectrum} \times \text{sensitivity factor}}{\text{wavelength-dependent factor} \times \text{fixed wavelength factor} \times \text{filter factor}}$$

A typical data sheet for correction of the excitation spectrum of a solution of Rhodamine WT is shown in Table 2. It may be advantageous to use a limited number of instrumental settings and filters, in which case a pre-computed table of adjustment factors can be prepared. Table 3 illustrates a typical section of such a table for correction of emission spectra. The only computation then required is to multiply a single factor from the body of the table by the uncorrected emission spectrum in order to obtain the corrected emission spectrum.

Verifying validity of a corrected spectrum

In the case of pure dilute solutions of fluorescent compounds the peak wavelengths and general configuration of the corrected spectrum can be verified by comparison with published data. However in the case of natural samples, particularly of solids, the fluorescent substance is commonly impure, and may display fluorescence from more than one component. To verify validity of spectra from such samples it is necessary to find some method other than reference to published data, unless the substance is relatively pure, fairly concentrated, and readily identified (e.g., samples of the mineral willemite).

An expedient method to estimate validity of corrected fluorescence spectra from natural sample surfaces is to tabulate peak wavelengths for a number of samples, both in the corrected and the uncorrected spectra. An increase in the number of peaks in the corrected spectrum would suggest either that instrumental artifacts had been over-corrected, resulting in spurious troughs in the spectrum, or that new artifacts had been introduced by the correction process.

Peaks are generally more numerous in the uncorrected excitation spectrum of a sample than in its uncorrected emission spectrum. This is the result of multiple peaks in the spectral output of the source lamp, which are uincorporated as artifacts in the excitation spectrum, whereas

the emission spectrum contains artifacts from the spectral sensitivity of the photomultiplier tube, which is commonly a fairly smooth curve having a single peak.

The multiple peaks seen in excitation spectra for solid sample surfaces may still remain even after application of all calibration curves and correction factors. In these cases it is probable that the true excitation data are obscured by a combination of two or more problems: 1) instrumental artifacts; 2) differential spectral reflectance of stray light; 3) partial behavior of the sample as a quantum counter; and 4) concentration quenching or self-absorption of fluorescence.

The foregoing expedient method for verifying validity should also be applicable to spectra recorded by a corrected-spectrum spectrofluorometer in which instrumental artifacts are designed to be automatically removed. These instruments are subject to the same limitations and sources of error that are encountered during manual calibration and correction procedures.

Use of nomographs for rapid correction of peak wavelengths

In some applications the main objective of spectrofluorometer analysis may be to determine wavelength of the true excitation or emission peaks, or to determine the true height of such peaks. To do this by manual computation of a fully corrected spectrum would be time-consuming, but the same result can sometimes be accomplished rapidly by means of a nomograph. Figure 4 illustrates such a nomograph that was

constructed to permit the measurement of excitation peak wavelengths and heights from uncorrected excitation spectra recorded directly by a particular spectrofluorometer with a specific lamp and phototube. The nomograph is simply overlain on the recorded spectrum, provided that certain conditions have been met, and the peak wavelength and height are read directly at the point where the highest curve intersects the recorded spectrum. The conditions required to permit application of this nomograph were: 1) recording at a specified wavelength scale (20 nm per cm); 2) use of a specified emission setting (486 nm); 3) use of specified slit widths (12 nm); 4) use of specified sensitivity (approx. 30X); and 5) use of a specified yellow filter ("HCE #2").

However, such a nomograph should have a broader applicability for tests made with a given instrument, and could serve for calculation of peak wavelengths alone, or of comparison of the peak heights for two or more samples, provided the following minimum conditions were met: 1) wavelength scale same as nomograph; 2) slit widths same as specified on nomograph; and 3) all other factors equal for both samples. This means that for practical purposes only one nomograph would be required for each type of spectrum recorded by a given instrument, provided slit widths were kept constant and as long as lamp and phototube adjustment was maintained. This method of correction may be the most satisfactory for many spectrofluorometer applications that require only peak wavelengths and heights.

OPTICAL FILTERS AND WAVELENGTH SETTINGS FOR TESTING SAMPLE SURFACES

It has been concluded (Stoertz, G.E. and Hemphill, W.R., 1972) that successful testing of the fluorescence of sample surfaces, particularly of solids, requires the use of filters. It was also concluded that different characteristics are required for maximum effectiveness of a filter when used for emission spectra as opposed to excitation spectra. The purpose of this section is to describe methods used for selecting the optimum filter for each purpose and for selecting optimum wavelength settings for each filter that is available. It should be noted that well-matched sets of narrow-band filters are available at uniform wavelength intervals throughout the visible spectrum. The use of such filters would simplify the problem of filter and wavelength selection, but measurements would still be required in order to correct for filter transmission. However in our spectrofluorometer tests we used inexpensive filters having a wide range of characteristics and with a little extra effort these can prove as useful as filters costing more than twenty times as much.

Selection of optimum filters

The most important characteristic for filters to be used for excitation spectra is a steeply sloping transmission curve at wavelengths slightly shorter than the wavelength setting to be used. For emission spectra a steeply sloping curve at slightly longer wavelengths is desired. Narrow-band filters can serve both purposes, but sharp cut-on filters are equally suitable for recording of excitation spectra, and sharp cut-off filters for recording of emission spectra. A wavelength other than that of peak transmission is frequently the most effective for use with a given filter. The critical factor is the steepness of the slope of the transmission curve at or near some wavelength setting of the monochromator with which the filter is proposed for use.

An expedient method was used to estimate the effectiveness of all available filters. It was reasoned that high transmittance in the vicinity of the proposed wavelength would be an asset as would low transmittance at longer wavelengths, for use with emission spectra (or low transmittance at shorter wavelengths, for use with excitation spectra). It was reasoned that if average slit widths used during the recording of spectra were adjusted so that an effective spectral band about 10 nm wide were used, a high transmittance in that band would be advantageous. Because of the presence of some stray light at all wavelengths it is evident that any transmittance at wavelengths outside the central 10-nm band would allow leakage of some stray light. However, it appears that the stray

light generally falls off gradually with spectral distance from the central wavelength. To evaluate this would require detailed data on the spectral distribution of stray light in each monochromator.

As an expedient method, it was estimated that in our test conditions a spectral band 40 nm wide adjacent to the central band was most crtical in determining filter effectiveness. The average transmittance per wavelength interval (roughly equivalent to the integrated area under the transmittance curve) in this band was subtracted from the transmittance of the central band in order to obtain an expedient index of filter effectiveness. A different index of filter effectiveness would need to be calculated for every wavelength setting proposed for use with each filter, and a different index for its use with fixed excitation or emission monochromator settings. These calculations were completed for 46 selected filters, but wavelengths considered were limited to 10-nm intervals for convenience. Filters that were evaluated include 14 Corning-glass filters, 29 Wratten gelatin filters, and 3 narrow-band interference filters.

The results of these evaluations are summarized in Table 4. Only the most effective filters for this application are included in the table. Measured transmittances of the filters are also shown on the table for the wavelengths at which they proved most effective, as these transmittances would need to be applied as correction factors in spectrofluorometer applications. It should be noted that these transmittances and effectiveness ratings were measured in relation to use of specific filters in a

specific manner and in a certain position in one spectrofluorometer. These figures may vary in different batches of glass or sheets of gelatin. The data are presented to illustrate procedures that can be used to significantly improve the accuracy of spectrofluorometer analyses, and that may be necessary in testing of sample surfaces.

Selection of optimum wavelength settings

The data shown on Table 4 can be used to advantage in selecting optimum wavelength settings for the fixed monochromators in spectrofluorometry. Commonly there will be a considerable latitude in selecting these settings, because many points on the short-wavelength side of the excitation peak of a sample will be almost equally suitable for use in recording the emission spectrum, and conversely, many points on the long-wavelength side of the emission peak will be almost equally suitable for use in recording the excitation spectrum. If the fluorescence characteristics of the sample allow this latitude, which they commonly do, the choice of instrumental settings may as well be made on the basis of instrumental factors, which include light-source intensity, phototube sensitivity, and effectiveness of the available filters. An example of this is tabulated below, relative to recording of an emission spectrum:

Alternative wavelength settings of excitation monochromator	Best available filter for the alternative settings	Filter transmittance	Filter "effectiveness"
410 nm.	No. 36	40.8%	21.2%
420 nm.	No. 36	38.4%	30.1%
430 nm.	No. 36	26.6%	25.9%

If none of the three settings (410, 420, and 430 nanometers) were preferable from the standpoint of the sample, then it might at first glance be concluded from filter transmittances that the setting of 410 nanometers would be preferable. However the first choice should instead be 420 nanometers, because the filter is considerably more effective at that setting. The second choice would appear to be 430 nanometers on this basis. However, the intensity of the lamp source should also be considered, since greater availability of light might conceivably offset the greater filter effectiveness at 420 nanometers. This type of data can be obtained by reference to Table 5, a summary of all calibration data for a spectrofluorometer. That table shows that light intensity would be about 68% at settings of 410 nm or 420 nm, but only about 62% at a setting of 430 nm. Therefore the setting of 420 nm. would clearly be preferable from all standpoints.

Fluorescence of optical filters

It was mentioned previously that most inexpensive optical filters fluoresce, since they derive their coloring from fluorescent dyes. Normally, to use such filters in fluorescence analysis would seem potentially disastrous, particularly for highly sensitive work, yet they are commonly used for this purpose. The filter fluorescence would definitely be a potential source of serious error in spectrofluorometry of solid samples, of front surfaces of liquids, or other reflective samples. However the technique of alternately placing the filter on both sides of the sample appears to eliminate this problem, because the filter fluorescence is present in both spectra, and subtraction of the spectra should largely eliminate the error.

In the course of our work the fluorescence of at least 46 filters was evaluated and the detailed data will be included in a subsequent report. The same problemsmare encountered in measuring the fluorescence of a filter as are encountered in any other solid sample. Any attempt to measure the fluorescence spectrum directly will include spurious factors due to reflected stray light, stray fluorescent light, etc. Therefore it is necessary to use a second filter as a standard of reference, and to record two spectra, with the second filter on both sides of the first filter, alternately. It is effective to place the first filter in the same position as an opaque sample, and to test its front-surface for fluorescence. However this result will commonly give a different spectrum than would be obtained by testing the fluorescence in transmitted light -- the way in which the filter would actually be used in a spectrofluorometer. Because of these complications a description of these test results will be deferred to a subsequent report.

Some unpublished data on the fluorescence of optical filters has been made available and is briefly summarized below. This data was obtained from the following sources: 1) Corning Glass Works; 2) Baird-Atomic, Inc.; and 3) Eastman Kodak Company.

The Corning Glass Works advised that practically all of their colored glass filters fluoresce at certain wavelengths (Saxton, R.C., 1971, written communication). They also have manufactured a non-fluorescing sharp-cut yellow filter (CS-3-144) for use in fluorometric analysis.

Baird-Atomic, Inc. has made a detailed study of the fluorescence of the following glass filters manufactured by the Corning Glass Works: 0-54, 0-52, 3-73, 3-72, 3-71, 3-70, 3-69, 3-68, 3-67, 3-66, 2-73, 2-63, 2-62, and 2-61 (Hornig, A.W., and Karandanis, D., 1966, unpublished report). They found that the 2- filters fluoresce weakly near 600 nm, that filters 3-73 and 3-72 fluoresce in the blue but have tails into the yellow, and that filters 0-52 and 0-54 apparently fluoresce in the violet.

The Eastman Kodak Company has made studies of the relative fluorescence of Wratten gelatin filters. Among the filters that were found to be slightly fluorescent were filter numbers 1A, 2A, 2E, 3, 4, 8, 12, 15, 16, 21, 22, and 92, while filter no. 29 was rated "fluorescent" (Nadin, H.R., 1971, written communication).

CONCLUSIONS

1) Improvements are needed in spectrofluorometer versatility and techniques in order to facilitate testing of the fluorescence of sample surfaces, particularly rough surfaces, and to improve reproducibility of spectra from such samples.

2) A horizontal sample orientation would be advantageous for testing of liquid surfaces, floating films on liquids, natural soils, unconsolidated sediments, and moist samples. For some applications, integration of fluorescence from a large area would be an advantage, by spinning either the sample or an optical component.

3) Surfaces of natural samples commonly display effects similar to concentration quenching. Many behave, to some extent, as quantum counters, in that their excitation spectra may depend to a greater degree on the quanta of incident light received than on the character of the sample. Their emission spectra do not have that limitation.

4) Stray light in a spectrofluorometer is a severe limitation in testing of sample surfaces, but the amount of stray light can generally be evaluated with little difficulty.

5) Determination of correct peak excitation and emission wavelengths from fluorescence spectra requires correction of only the wavelength-dependent variables, specifically source-lamp intensity and photomultiplier sensitivity. This type of correction can be accomplished rapidly by means of simple nomographs.

6) Spectrofluorometer test results can be significantly improved by careful selection of optical filters and wavelength settings. For best results filters should be quantitatively evaluated for their effectiveness in selectively transmitting light of given wavelengths. Expedient methods for selecting optimum filters and wavelengths can be devised to match actual experimental conditions.

7) Optical filters commonly available are liable to be fluorescent, and if used in testing fluorescence of sample surfaces could cause errors. These errors apparently can be removed by use of a technique described in a previous report.

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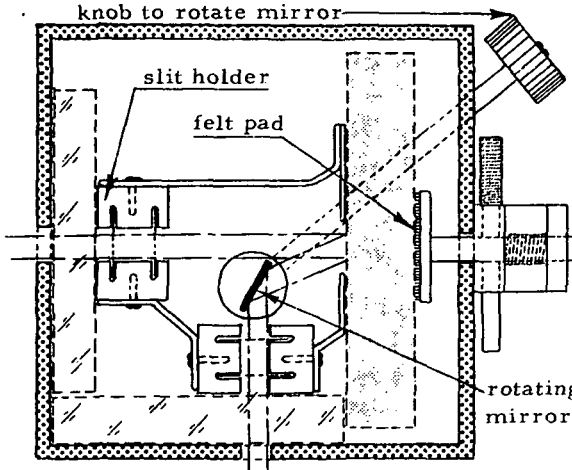


Fig. 1A. Possible design using normal incidence, a rotating mirror, and a fixed sample (the sample might also be rotated 10° clockwise)

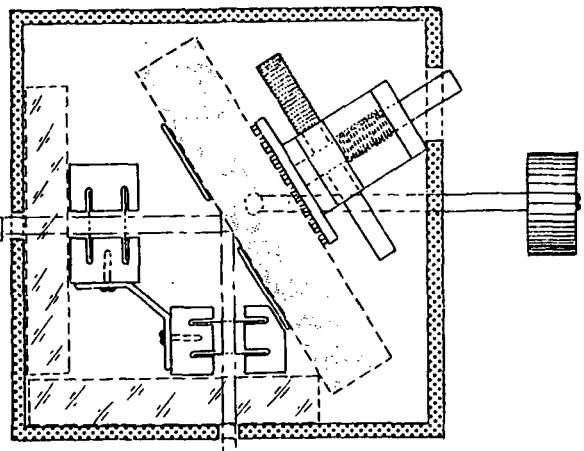


Fig. 1B. Possible design using 30° incidence and a sample holder like that of the MPF

Fig. 1C. Design similar to Fig. 1B but with an enlarged sample compartment utilizing all available space in the SPF spectrofluorometer

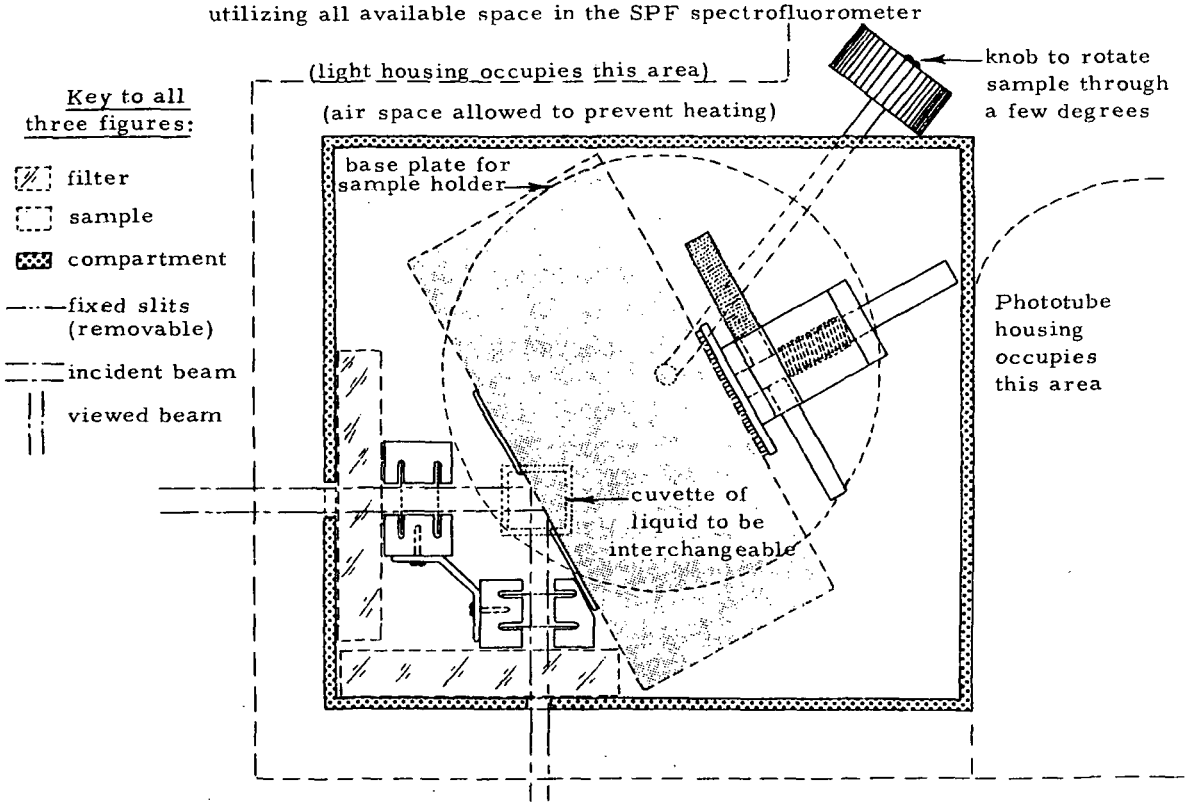


Figure 1. Alternate designs to provide for angular alignment of beam or sample in a spectrofluorometer sample compartment for testing the surfaces of samples (configuration of compartment based on SPF; holder based on MPF solid sample accessory)

Figure 2. Geometry relative to testing of sample surfaces in a spectrofluorometer (enlarged 10X; 1 cm=1 mm)

Key: opaque solids; powder; liquids; glass cell wall; effective target; viewed beam; incident beam; alignment of front surface of solid sample holder

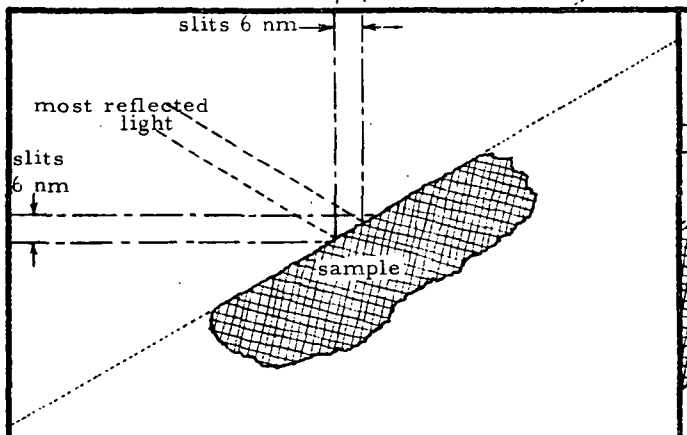


Figure 2A. Solid sample holder in optimum adjustment for a flat opaque sample.

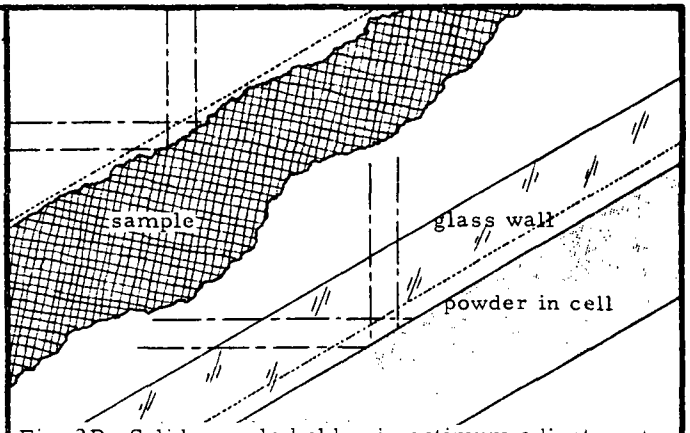


Fig. 2B. Solid sample holder in optimum adjustment for rough sample and for testing powder in powder cell

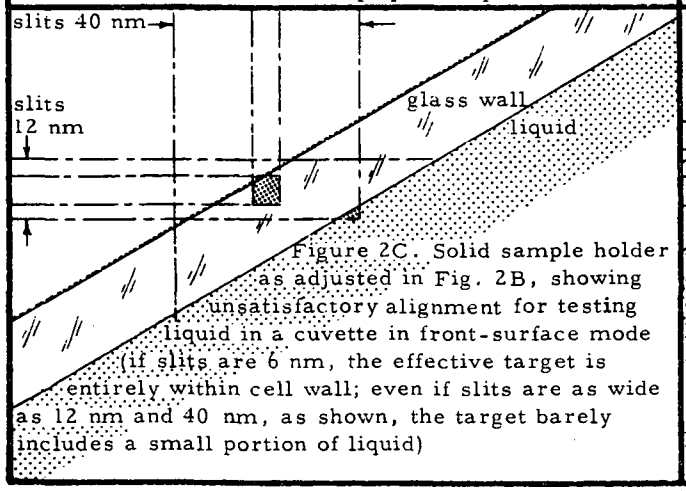


Figure 2C. Solid sample holder as adjusted in Fig. 2B, showing unsatisfactory alignment for testing liquid in a cuvette in front-surface mode (if slits are 6 nm, the effective target is entirely within cell wall; even if slits are as wide as 12 nm and 40 nm, as shown, the target barely includes a small portion of liquid)

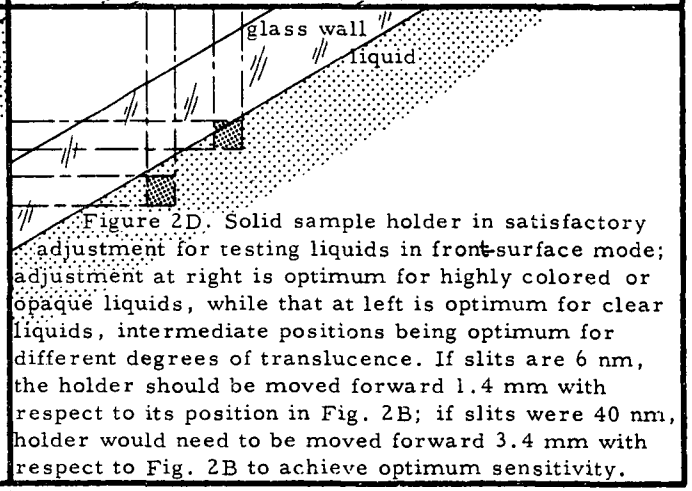
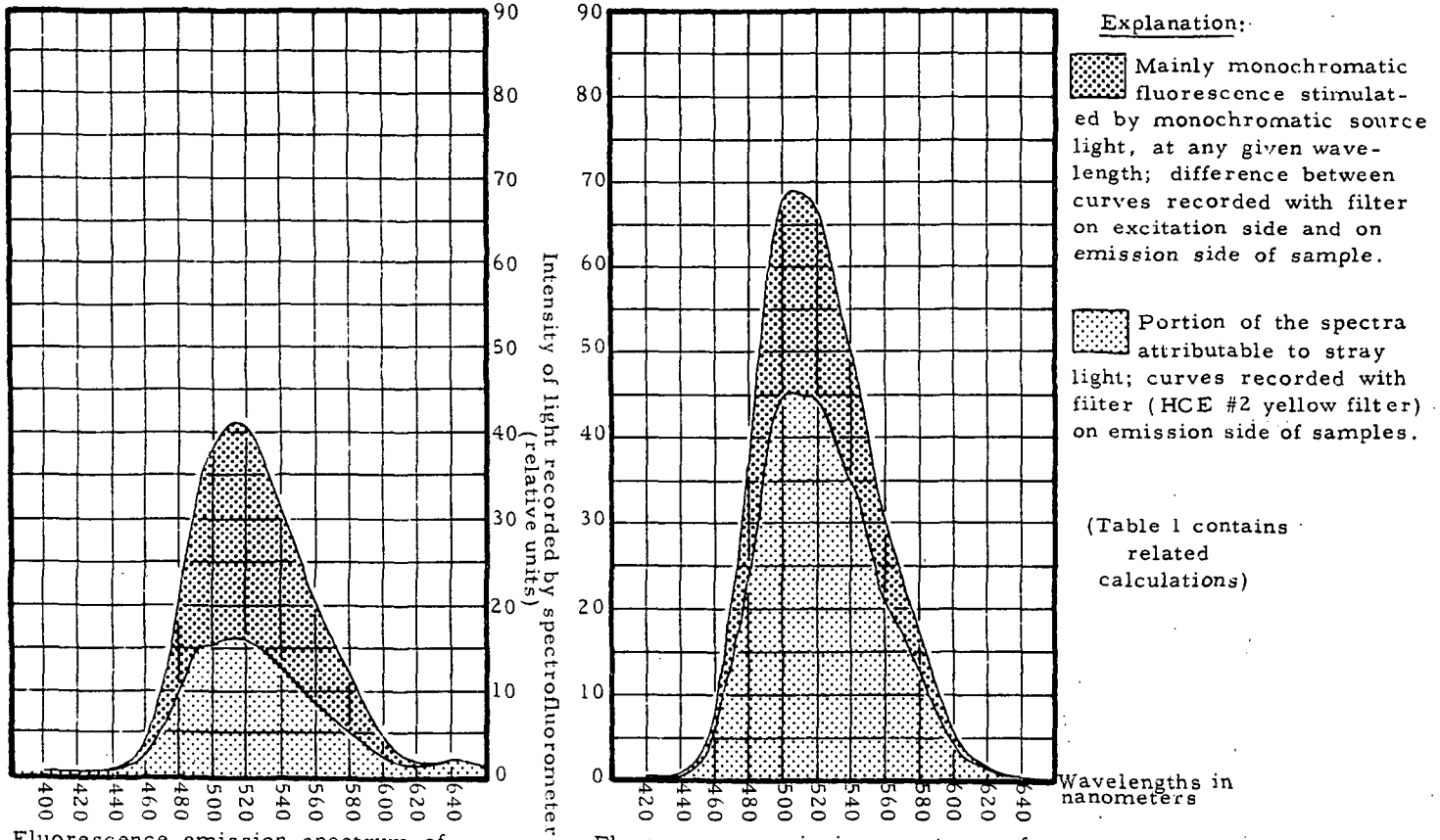


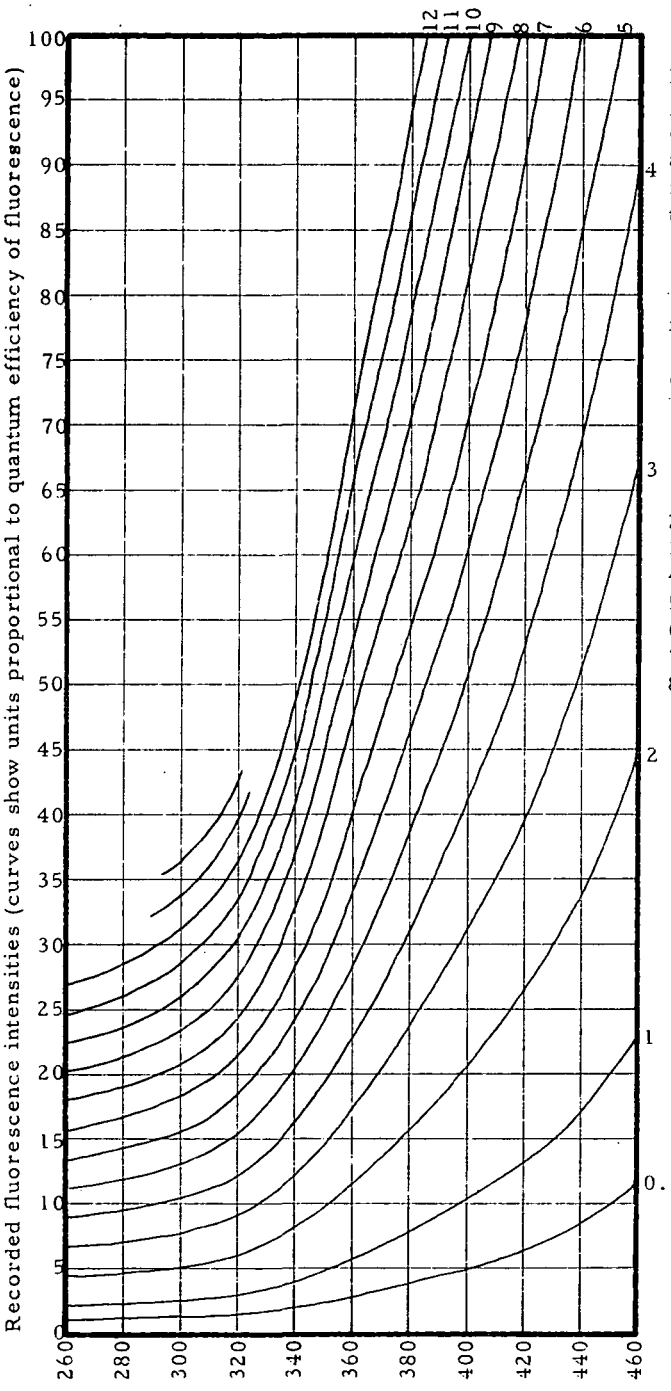
Figure 2D. Solid sample holder in satisfactory adjustment for testing liquids in front-surface mode; adjustment at right is optimum for highly colored or opaque liquids, while that at left is optimum for clear liquids, intermediate positions being optimum for different degrees of translucence. If slits are 6 nm, the holder should be moved forward 1.4 mm with respect to its position in Fig. 2B; if slits were 40 nm, holder would need to be moved forward 3.4 mm with respect to Fig. 2B to achieve optimum sensitivity.

Figure 3. Graphical method for evaluating the amount of stray light in a spectrofluorometer



Fluorescence emission spectrum of phosphate rock from Saudi Arabia (#21272); excitation at 320 nanometers

Fluorescence emission spectrum of ulexite from Salar de Ascotan (#S30), northern Chile; excitation at 370 nm.



Explanation:

Nomograph is for illustrative purposes only, since curves change with changes in lamp and phototube use. Curves show fluorescence intensities in excitation spectra under conditions shown below; for solid samples, stray light effects should be corrected, insofar as possible, by measuring the difference between spectra recorded with the filter on excitation and emission sides.

Applicable conditions:

1) MPF-2A spectrofluorometer (as adjusted in December 1970)
2) Chart speed 1 cm. per 20 nm.
3) Emission setting 4861 A
4) Slit widths all 12 nm.
5) HCE #2 yellow filter
6) Xenon lamp, 150 watts
7) Phototube R 106
8) Sensitivity setting #4 (30 x)

Figure 4. Example of a nomograph for rapid correction of excitation peak wavelengths and heights recorded by a particular spectrofluorometer under certain fixed conditions.

Table 1. Ratios calculated for the spectra shown on Figure 3, supporting the conclusion that the lower curves represent stray light rather than filter fluorescence.

Wavelength	Phosphate rock			Ulexite		
Nanometers	Upper curve	Lower curve	Ratio (%)	Upper curve	Lower curve	Ratio (%)
460	3.1	1.4	45.2	7.7	5.2	67.5
470	9.5	4.6	48.4	21.2	14.5	68.4
480	19.5	8.9	45.6	37.0	24.4	66.0
490	30.7	13.4	43.6	56.5	37.7	66.7
500	36.8	14.3	38.9	67.3	44.2	65.7
510	39.7	14.9	37.5	68.2	44.4	65.1
520	39.3	14.8	37.7	66.3	43.9	66.2
530	35.4	13.3	37.6	57.8	38.8	67.1
540	30.3	11.5	38.0	49.4	34.0	68.8
550	24.4	9.3	38.1	39.2	27.2	69.4
560	19.3	7.5	38.9	29.2	20.3	69.5
570	14.6	5.7	39.0	23.0	16.4	71.3
580	10.8	4.1	38.0	16.8	12.2	72.6
590	6.8	2.6	38.2	10.3	7.5	72.8
	Averages		40.3%*	. . . . .		68.4%*

* (presumed to be equivalent to the average percentages of stray light in the signals recorded)

Table 2. Typical data sheet showing correction of the excitation spectrum for a solution of Rhodamine WT (100 micrograms per liter; 100 parts per billion)

Sample type: Rhodamine WT	Date of test: Nov. 4, 1971	Spectrofluorometer: MPF-2A	Sensitivity: 4
Sample no.: Concentration 100 ppb	Mode of test: Front surface	Phototube: R 136	Excitation slit width: 6 nm
Collector or source: Vance Kennedy	Curve no.: 448 D	Light source: Xenon 150-watt	Emission slit width: 6 nm
Type of spectrum: Excitation	A: Fixed wavelength factor: 5.2	D: Fixed adjustment factor (D=C/AB): 319.0	Uncorrected peak: 555 nm.
Fixed monochromator wavelength: 5890A	B: Filter factor: 71.1	Summation from (240) to (580): 877	Corrected peak: 555 nm
Filter no.: Corning 2-73	Sensitivity factor (C): 1.00	for equivalence to sensitivity: (4)	Remarks: Note front surface mode used

Wavelength (nm)	Uncorrected spectrum (#1)	Variable wavelength factor (#2)	Adjustment factors (D/#2)(#3)	Corrected spectrum (#1x#3)	Wavelength (nm)	Uncorrected spectrum (#1)	Variable wavelength factor (#2)	Adjustment factors (D/#2) (#3)	Corrected spectrum (#1x#3)
230					470	1.4	88.4	3.61	5.05
240	0	2.9	110.	0	480	2.0	81.0	3.94	7.88
250	0.2	6.3	50.6	10.1	490	3.0	76.7	4.16	12.5
260	0.8	10.8	29.5	23.6	500	4.7	70.6	4.52	21.2
270	0.8	16.1	19.8	15.8	510	7.8	68.1	4.68	36.5
280	0.9	22.5	14.2	12.8	520	10.8	69.3	4.60	49.7
290	1.5	30.1	10.6	15.9	530	12.2	66.7	4.78	58.3
300	1.7	37.2	8.58	14.6	540	17.4	63.7	5.01	87.2
310	2.2	44.6	7.15	15.7	550	25.2	60.5	5.27	132.8
320	1.8	49.7	6.42	11.6	560	26.9	58.1	5.49	147.7
330	1.2	51.4	6.21	7.45	570	16.0	56.6	5.64	90.2
340	1.2	58.3	5.47	6.56	580	4.3	56.0	5.70	24.5
350	2.0	66.4	4.80	9.60	590				
360	2.3	68.5	4.66	10.72	600				
370	1.4	58.9	5.42	7.59	610				
380	0.9	56.3	5.67	5.10	620				
390	0.9	62.2	5.13	4.62	630				
400	1.1	70.8	4.51	4.96	640				
410	1.1	68.1	4.68	5.15	650				
420	1.1	68.2	4.68	5.15	660				
430	1.0	62.2	5.13	5.13	670				
440	0.7	52.8	6.04	4.23	680				
450	0.6	51.3	6.22	3.73	690				
460	0.7	62.5	5.10	3.57	700				

Table 3. Section of a pre-computed table for rapid correction of emission spectra, requiring application of a single factor to obtain the corrected spectrum.

Explanation: The table is for illustrative purposes only; it is applicable only to a specific spectrofluorometer (MPF-2A) as it was adjusted from April to June, 1971, and when used with slits 6 nm, PMT R-106. Table shows "adjustment factors, col.# 3" for each setting.

Excitation setting (wavelength, nm)		320	320	320	320	320	320	330	330	330	330	340	340	340	340	380	380
Filter number (Corning glass #)		7-54	7-54	7-54	7-54	7-54	7-54	7-54	7-54	7-54	7-54	7-54	7-54	7-54	7-54	7-51	7-51
Sensitivity setting (dial readings)		1	2	3	4	5	6	2	3	4	5	1	2	3	5	1	2
A: Fixed wavelength factor		.497	.497	.497	.497	.497	.497	.514	.514	.514	.514	.583	.583	.583	.583	.563	.563
B: Filter factor		.528	.528	.528	.528	.528	.528	.561	.561	.561	.561	.596	.596	.596	.596	.549	.549
C: Sensitivity factor (to adjust to sensitivity 4		30.89	10.09	2.90	1.00	0.29	.109	10.09	2.90	1.00	0.29	30.89	10.09	2.90	0.29	30.89	10.09
D: Fixed adjustment factor (C/AB		117.7	38.45	11.05	3.81	1.105	.415	34.99	10.06	3.468	1.006	88.9	29.04	8.345	2.834	100	32.6
'Col. # 2'	Wavelength																
0.211	330	558	182	52.4	18.1	5.2											
0.200	340	589	192	55.3	19.1	5.5	2.08	175	50.3	17.3	5.03						
0.196	350	601	196	56.4	19.4	5.6	2.12	179	51.3	17.7	5.13	454	148	42.6	4.26		
0.196	360	601	196	56.4	19.4	5.6	2.12	179	51.3	17.7	5.13	454	148	42.6	4.26		
0.196	370	601	196	56.4	19.4	5.6	2.12	179	51.3	17.7	5.13	454	148	42.6	4.26		
0.191	380	616	201	57.9	19.9	5.8	2.17	183	52.7	18.2	5.27	465	152	43.7	4.37		
0.194	390	607	198	57.0	19.6	5.7	2.14	180	51.9	17.9	5.19	458	150	43.0	4.30	515	168
0.200	400	589	192	55.3	19.1	5.5	2.08	175	50.3	17.3	5.03	445	145	41.7	4.17	500	163
0.202	410	583	190	54.7	18.9	5.5	2.05	173	49.8	17.2	4.98	445	144	41.3	4.13	495	161
0.194	420	607	198	57.0	19.6	5.7	2.14	180	51.9	17.9	5.19	458	150	43.0	4.30	515	168
0.185	430	636	208	59.7	20.6	6.0	2.24	189	54.4	18.7	5.44	481	157	45.1	4.51	541	176
0.176	440	669	218	62.8	21.6	6 3	2.36	199	57.2	19.7	5.72	505	165	47.4	4.74	568	185
0.169	450	696	228	65.4	22.5	6.5	2.46	207	59.5	20.5	5.95	526	172	49.4	4.94	592	193
0.161	460	731	239	68.6	23.7	6.9	2.58	217	62.5	21.5	6.25	552	180	51.8	5.18	621	202
0.150	470	785	256	73.7	25.4	7.4	2.77	233	67.1	23.1	6.71	593	194	55.6	5.56	667	217
0.139	480	847	277	79.5	27.4	7.9	2.99	252	72.4	24.9	7.24	640	209	60.0	6.00	719	235
0.132	490	892	291	83.7	28.9	8.4	3.14	265	76.2	26.3	7.62	673	220	63.2	6.32	758	247

Table 4. Transmittance and effectiveness of some optical filters when used at fixed monochromator settings in a spectrofluorometer.

Monochromator setting (nm)	Emission spectra			Excitation spectra			Monochromator setting (nm)	Emission spectra			Excitation spectra		
	Most effective filter	Filter transmittance	Filter effectiveness	Most effective filter	Filter transmittance	Filter effectiveness		Most effective filter	Filter transmittance	Filter effectiveness	Most effective filter	Filter transmittance	Filter effectiveness
220	n a	n a	n a	n a	n a	n a	480	CS-5-60	5.9	13.5	Wr.#4	74.8	65.0
230	n a	n a	n a	n a	n a	n a	4861	Int.4862	8.7	20.6	Wr.#4	81.7	±60
240	CS-7-54	13.4	n a	CS-7-54	10.2	3.0	490	Wr.#75	15.7	10.5	Wr.#4	85.3	53.9
250	CS-7-54	29.4	n a	CS-7-54	16.5	26.3	500	Wr.#75	11.6	8.9	Wr.#8	63.6	43.9
260	CS-7-54	34.1	n a	CS-7-54	19.1	38.5	510	Wr.#75	6.7	5.6	Wr.#8	79.0	45.7
270	CS-7-54	42.5	n a	CS-7-54	23.3	41.0	520	CS-4-105	7.4	7.5	Wr.#12	57.8	46.1
280	CS-7-54	42.2	n a	CS-7-54	29.5	36.0	530	Wr.#74	15.8	8.3	Wr.#12	79.2	59.6
290	CS-7-54	46.1	n a	CS-7-54	34.4	26.3	540	Wr.#74	12.9	10.0	CS-3-68	69.1	64.1
300	CS-7-54	49.7	n a	CS-7-54	37.6	18.8	550	CS-4-102	7.2	8.2	CS-3-68	83.0	69.4
310	CS-7-54	50.2	n a	CS-7-54	40.7	11.8	560	CS-4-102	4.7	6.6	Wr.#21	68.9	54.8
320	CS-7-54	52.8	3.3	CS-7-51	14.6	9.8	570	Wr.#73	8.1	4.0	Wr.#21	83.2	57.9
330	CS-7-54	56.1	4.5	CS-7-51	25.6	28.3	580	Wr.#73	5.7	4.8	Wr.#22	87.9	63.6
340	CS-7-54	59.6	9.2	CS-7-51	31.6	47.6	5890	Int.5890	4.6	15.1	CS-2-73	71.1	±59
350	CS-7-54	63.7	18.9	CS-7-51	38.2	48.1	590	Int.5890	2.6	12.7	CS-2-73	71.2	59.0
360	CS-7-54	61.4	33.9	CS-7-51	43.5	36.0	600	Wr.#72E	5.6	2.3	Wr.#24	72.2	61.5
370	CS-7-51	51.4	52.0	CS-5-56	36.3	33.8	610	Wr.#72B	5.1	4.3	Wr.#25	70.8	61.3
380	CS-7-51	54.9	62.2	CS-5-56	46.0	35.9	620	Wr.#72B	2.2	2.6	Wr.#26	75.3	49.8
390	CS-7-51	44.5	49.0	CS-5-56	52.7	33.3	630	Wr.#72B	0.8	1.4	Wr.#29	66.8	53.1
400	CS-7-51	20.8	22.4	Wr.#1A	64.4	49.7	640	Wr.#72B	0.3	0.5	Wr.#92	48.1	42.0
410	Wr.#36	40.8	21.2	Wr.#1A	76.1	52.5	650	Int.6583	32.8	4.5	Wr.#92	48.9	38.6
420	Wr.#36	38.4	30.1	Wr.#2A	63.3	46.2	6563	Int.6583	47.8	19.5	Int.6583	20.9	32.8
430	Wr.#36	26.6	25.9	CS-3-73	60.0	70.0	660	Int.6583	18.2	17.0	Int.6583	22.0	33.4
440	Wr.#98	39.1	23.3	CS-3-73	70.5	62.6	670	Int.6583	2.9	1.3	CS-2-64	52.5	24.6
450	Wr.#98	31.4	24.6	Wr.#2E	85.0	49.5	680	n a	n a	n a	Wr.#70	7.6	11.7
460	CS-5-60	11.7	23.8	Wr.#3	48.3	36.2	690	n a	n a	n a	Wr.#70	5.8	6.8
470	CS-5-60	5.9	22.8	Wr.#4	74.8	60.0	700	n a	n a	n a	n a	n a	n a

Symbols: CS-Corning glass; Wr.-Wratten gelatin; Int.-Interference; n a - not available.

Table 5. Summary of calibration data for a spectrofluorometer, for use in selecting optimum wavelength settings. (See Table 4 for filter factors)

Wavelength factors (A)			Wavelength factors (A)			Sensitivity (C)			Slit width factors		
Wavelength (nm)	Photo-tube factors	Xenon lamp factors	Wavelength (nm)	Photo-tube factors	Xenon lamp factors	Instrument setting:	Adjustment factor (C) multiplied by setting (col. to left)	to obtain equivalent sensitivity:	Slit width: (nm)	Adjustment factor multiplied by slit width (col. to left)	to obtain equivalent slit width:
220	n a	n a	480	13.9	81.0	1	282.5	6	12	0.065	6
230	n a	n a	4861	13.3	79.0	2	92.3	6	10	0.13	6
240	n a	n a	490	13.2	76.7	3	26.52	6	9	0.18	6
250	106.9	6.3	500	12.3	70.6	4	9.15	6	5	1.94	6
260	72.6	10.8	510	11.4	68.1	5	2.69	6	4.5	3.01	6
270	55.7	16.1	520	10.6	69.3	1	105.03	5	4.4	3.34	6
280	42.7	22.5	530	9.7	66.7	2	34.31	5			
290	33.7	30.1	540	9.0	63.7	3	9.86	5			
300	27.3	37.2	550	8.6	60.5	4	3.40	5			
310	24.2	44.6	560	7.3	58.1	1	30.89	4			
320	22.2	49.7	570	6.6	56.6	2	10.09	4			
330	21.1	51.4	580	5.7	56.0	3	2.90	4			
340	20.0	58.3	5890	5.2	56.5	1	10.65	3			
350	19.6	66.4	590	5.1	55.9	2	3.48	3			
360	19.6	68.5	600	4.4	50.9	1	3.06	2			
370	19.6	58.9	610	3.7	51.4	6	0.37	5			
380	19.1	56.3	620	3.1	62.3	6	0.109	4			
390	19.4	62.2	630	2.6	54.2	6	0.038	3			
400	20.0	70.8	640	2.2	43.6	6	0.0108	2			
410	20.2	68.1	650	1.8	43.9	6	0.0035	1			
420	19.4	68.2	6563	1.5	50.7	5	0.29	4			
430	18.5	62.2	660	1.3	52.3	5	0.101	3			
440	17.6	52.8	670	1.1	51.8	5	0.029	2			
450	16.9	51.3	680	0.9	42.2	5	0.0095	1			
460	16.1	62.5	690	0.7	51.4	4	0.34	3			
470	15.0	88.4	700	0.4	n a	4	0.099	2			

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16. Abstracts: Fluorescence analysis of the surfaces of samples, both solid and liquid, is required to provide ground-truth data in support of remote sensing of the fluorescence of earth surfaces. To facilitate this type of test and to increase reproducibility of fluorescence spectra, modified spectrofluorometer designs are suggested and improved laboratory techniques are described.

Methods for correcting fluorescence spectra to remove instrumental artifacts are described in detail, including computational and graphical procedures for rapid manual correction by nomographs and similar techniques. Expedient methods for the selection and use of optical filters, the evaluation of filter effectiveness, and the selection of optimum wavelength settings in spectrofluorometry are outlined.

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