LAbORATORY AND AIRBORNE TECHNIQUES FOR 
MEASURING FLUORESCENCE OF NATURAL SURFACES *

by

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**CONTENTS**

<table>
<thead>
<tr>
<th>Abstract</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>2</td>
</tr>
<tr>
<td>Current research in remote sensing of fluorescence</td>
<td>3</td>
</tr>
<tr>
<td>Airborne fluorometer</td>
<td>3</td>
</tr>
<tr>
<td>Airborne laser fluorometer</td>
<td>4</td>
</tr>
<tr>
<td>Laser fluorosensor</td>
<td>5</td>
</tr>
<tr>
<td>Other current research</td>
<td>5</td>
</tr>
<tr>
<td>Basic principles of measuring fluorescence</td>
<td>7</td>
</tr>
<tr>
<td>Assumptions in spectrofluorometry</td>
<td>8</td>
</tr>
<tr>
<td>Laboratory spectrofluorometer</td>
<td>10</td>
</tr>
<tr>
<td>Natural surfaces as viewed in a spectrofluorometer</td>
<td>12</td>
</tr>
<tr>
<td>The problem of reflected stray light</td>
<td>12</td>
</tr>
<tr>
<td>Components of light in fluorescence analysis of reflective samples</td>
<td>13</td>
</tr>
<tr>
<td>Effect of a filter on one side of the sample</td>
<td>15</td>
</tr>
<tr>
<td>Effect of a filter on both sides of the sample</td>
<td>16</td>
</tr>
<tr>
<td>Probable errors using three alternative techniques</td>
<td>17</td>
</tr>
<tr>
<td>Additional sources of error</td>
<td>19</td>
</tr>
<tr>
<td>Conclusions</td>
<td>20</td>
</tr>
<tr>
<td>References cited</td>
<td>21</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>22</td>
</tr>
</tbody>
</table>
Figure 1. Principal components of light in fluorescence analysis of reflective samples

Table 1. Comparison of three techniques for measuring fluorescence spectra of natural earth-surface samples

Table 2. Comparison of the three techniques for measuring fluorescence spectra under varying conditions of monochromator efficiency and reflectance of the sample
ABSTRACT

At least three research efforts were known to be in progress, as of November 1971, to develop techniques for remote sensing of fluorescence emitted by materials on the earth’s surface. Application of remote sensing of fluorescence to real earth-surface targets, and interpretation of the resulting data will require improved techniques for study of the fluorescence of similar samples in the laboratory.

These fluorescence-spectroscopy research projects are oriented toward remote sensing of substances as they are actually found on the earth's surface. The present report summarizes special laboratory techniques developed to provide ground-truth data in support of remote sensing of fluorescence. Techniques are described for obtaining fluorescence spectra from samples of natural surfaces that can be used to predict spectral regions in which these surfaces would emit solar-stimulated or laser-stimulated fluorescence detectable by remote sensor.

Scattered or reflected stray light causes large errors in spectrofluorometer analysis of natural sample surfaces. This and most other spurious light components can be eliminated by recording two successive fluorescence spectra for each sample, using identical instrument settings, first with an appropriate glass or gelatin filter on the excitation side of the sample, and subsequently with the same filter on the emission side of the sample. This technique appears more accurate than any alternative technique for testing the fluorescence of natural surfaces.
INTRODUCTION

Three research efforts were known to be in progress in 1971 to develop techniques for remote sensing of fluorescence emitted by natural surfaces such as some rock outcrops, surficial deposits of certain types, some soils, most vegetation, surficial films of certain pollutants on water, and a few substances dissolved or suspended in water.

Development and testing of a Fraunhofer line discriminator for remote sensing of solar-stimulated fluorescence has recently been under the auspices of NASA's Advanced Applications Flight Experiments Program, Langley Research Center. The sensor, basically an airborne fluorometer, has been successfully operated from a helicopter.

Two sensors for detection of laser-stimulated fluorescence are under development but had not yet been operated from the air as of late 1971. An airborne laser fluorometer has been constructed by the Canadian Department of Fisheries and Forestry, Ottawa, and a laser fluorosensor has been constructed by the Institute for Aerospace Studies at the University of Toronto.

A major objective of our laboratory research was to obtain fluorescence spectra from samples of natural surfaces that could be used to predict spectral regions in which these surfaces would emit solar-stimulated or laser-stimulated fluorescence. The objective of the present report is to describe special techniques of fluorescence analysis of sample surfaces, and efforts being made to develop an improved fluorescence technology applicable to earth-surface materials, both natural and artificially added.
The current status of research in remote sensing of fluorescence will be summarized prior to discussion of related laboratory techniques.

CURRENT RESEARCH IN REMOTE SENSING OF FLUORESCENCE

Airborne fluorometer

Development and testing of a Fraunhofer line discriminator for remote sensing of solar-stimulated fluorescence has been in progress since 1967, originally under the auspices of the National Aeronautics and Space Administration's (NASA's) Manned Spacecraft Center and recently under NASA's Advanced Applications Flight Experiments Program, Langley Research Center. The status of this research has been described (Hemphill, W.R. and Stoertz, G.E., 1971), recent laboratory studies having concerned the fluorescence of crude oil from the Santa Barbara spill of 1969, effluent from a phosphate processing plant in Florida, leaves from several tree species, and fresh water algae. Airborne tests of the sensor have been described, and other potential applications have been suggested (Hemphill, W.R., and others, 1969; Stoertz, G.E., and others, 1970). Basically the sensor could be viewed as an airborne fluorometer (Stoertz, G.E., Hemphill, W.R., and Markle, D.A., 1969).
Airborne laser fluorometer

No other fluorescence sensors are known to have been successfully operated from the air as yet, but two such sensors are evidently very close to this stage. An airborne laser fluorometer has been constructed by the Canadian Department of Fisheries and Forestry, Ottawa, Canada (Davis, Gross, and Kruus, 1971; also Gross, Harry, 1971, personal communication). The sensor was tested on the ground from a distance of 400 feet on petroleum and on a solution of Rhodamine 6G dye. Among the airborne applications foreseen by its developers are the mapping or recognition of oil films of fish or mineral origin, of fluorescent rocks and minerals such as scheelite (an ore of tungsten), of chlorophyll in plants, of rhodamine dyes used in hydrologic studies, of some industrial effluents such as lignin sulfonates, and of kelp beds in the waters off southern California. It is presumed that laboratory study of the fluorescence of these substances will be an important aid in interpreting the data that will eventually be obtained with the airborne laser fluorometer.
Laser fluorosensor

A laser fluorosensor has been constructed by the Institute for Aerospace Studies at the University of Toronto in Toronto, Canada (Measures, R.M., and Bristow, M., 1971; also Measures, R.M., 1971, personal communication). A prototype of the fluorosensor has been successfully tested in the laboratory, and in lieu of airborne tests reportedly will be tested from a van on a high cliff overlooking open water. Eventual airborne uses that are envisioned include most of the substances mentioned above, in addition to some pesticides in water and some pollutants in air. Tests in the laboratory were successful on such target materials as petroleum floating on water in films up to ten microns thick, calcium lignosulfonate in concentrations from 0.5 to 12 milligrams per liter (parts per million), Rhodamine BN dye in aqueous solutions, and chlorophyll in concentrations up to 600 micrograms per liter (parts per billion).

Other current research

In a recent review of available techniques for detecting oil in water (Klemas, Vytautas, 1971; also personal communication, 1971) it was concluded that the technique of fluorescence seems to offer more hope than other methods under consideration for sensing oil pollution. In laboratory tests of a sensing technique using an ultraviolet laser as a source of excitation it was found that the intensity of blue fluorescence could be correlated with oil-film thickness up to approximately 3.6 microns.
All of the research projects described above, and others currently in progress elsewhere, are oriented toward remote sensing of substances as they are actually found on the earth's surface, and in that sense they must use unconventional laboratory techniques. For example, the fluorescence of oil is studied in films floating on water, as it would be found in a real oil spill in nature, by contrast with conventional techniques that use solvents such as MIBK (methyl-iso-butyl-ketone). Investigations summarized in the present report were addressed toward that same problem -- to study the fluorescence of substances in their natural state, insofar as possible, in order to be able eventually to apply the results to interpretation of remote sensor data. As a result of pursuing this objective some findings may prove useful in more conventional laboratory studies of fluorescence, particularly of such materials as rocks, minerals, powders, leaves, glasses, filters, or the surfaces of liquids. Because, like remote sensing, the techniques are entirely non-destructive, they may have some application to study of gemstones or of living things. They may also have application to the study of turbid, colored, or opaque liquids, or to the sensing of fluorescence by any remote means including sensors of effluent streams or outflows from industrial plants. Sensors of the latter type might be situated only a few centimeters above the liquid surface.
BASIC PRINCIPLES OF MEASURING FLUORESCENCE

In basic principle, measurement of fluorescence offers advantages over such other optical techniques as absorption spectrophotometry, because in fluorescence the purpose is to measure a feeble light against a nearly black background, while in absorption the purpose is to measure a small difference between two relatively bright light sources. Because of this basic difference, fluorescence is far more sensitive at very low concentrations. In addition, the configuration of the laboratory apparatus is basically different in the two techniques, fluorescence using an angle of 90° or less between the incident beam and the viewed beam, in order to minimize interference by the source light, whereas in absorption spectrophotometry the angle is effectively 180°.

The approach of this report will concern spectral and optical considerations as they relate to instrument configuration and laboratory techniques. Application of the results to remote sensing will be discussed in a separate report. Basic techniques of spectrofluorometer analysis have been described previously in numerous publications (e.g., Udenfriend, Sidney, 1962 and 1969), as have theoretical and analytical aspects.
Basically, fluorescence has been defined as the emission of electromagnetic radiation from a photon-excited state of a molecule when it returns to the ground state, provided there is no intersystem crossing (American Instrument Company, Inc., unpublished data). With intersystem crossing (i.e., triplet-to-singlet or singlet-to-triplet transitions, involving the net reversal of spin of one electron in an atom or molecule) the emission is termed phosphorescence, a phenomenon that is relatively slow by comparison with fluorescence. In fluorescence the mean lifetime of the excited state (singlet) is on the order of $10^{-8}$ seconds, while in phosphorescence the lifetime of the excited state (triplet) is longer than $10^{-8}$ seconds (Udenfriend, Sidney, 1962, p. 11).

Typical time intervals required for these electronic transitions are $10^{-4}$ seconds for phosphorescence, $10^{-9}$ seconds for fluorescence, and $10^{-15}$ seconds for absorption and reflectance.

**Assumptions in spectrofluorometry**

Our measurements would most correctly be termed luminescence, a term that encompasses both fluorescence and phosphorescence. Luminescence might be distinguished from reflected or scattered light by means of time intervals. However, we distinguished these phenomena by means of spectral character of the incident and the viewed beams of light. This
The method assumes that when an incident beam of nearly monochromatic light strikes a sample any viewed light of significantly longer wavelength will be fluorescence or phosphorescence emitted by the sample.

The basic techniques used in spectrofluorometry by most laboratories still depend on this assumption, and generally it involves little error. In the case of crystalline materials, a significant portion of the emitted light may be phosphorescence, particularly from phosphate rock, certain evaporite minerals, and certain calcareous samples.

In discussing the results, we have used the term fluorescence as a general term for emitted light, a usage that is consistent with the terms spectrofluorometer, fluorescence spectrophotometer, airborne fluorometer, airborne laser fluorometer, and laser fluorosensor. Generally these instruments sense total emitted light, comprising both fluorescence and phosphorescence.

One additional assumption that is commonly made in most spectrofluorometry is that the incident beam is, indeed, monochromatic, or practically so. In the case of solid samples, and particularly of natural earth-surface materials having rough or highly reflective surfaces, and when off-the-shelf spectrofluorometers are used, this assumption is quickly found to be invalid. We found that new techniques were needed in order to obtain data that would have value, particularly when the samples were only weakly fluorescent.
Laboratory spectrofluorometer

The basic instrument used in this work is a spectrofluorometer (or spectrophotofluorometer or fluorescence spectrophotometer), consisting of a light source such as a 150-watt xenon arc lamp, an excitation monochromator, a sample compartment, an emission monochromator, a photomultiplier, and a recorder that can display either an excitation or emission spectrum. An excitation spectrum is a measure of the intensity of fluorescence at specific wavelengths when a sample is sequentially illuminated by various wavelengths in the spectrum of incident light. An emission spectrum is a measure of both intensity and wavelength of fluorescence when a sample is illuminated by monochromatic incident light.

In either type of spectrum it is necessary to define the wavelength of the fixed monochromator if the spectrum is to have meaning. Since diffraction gratings are generally used in each monochromator the slit widths that delimit the two light beams will also define the widths of the spectral bands, and therefore the amount and the wavelength range of light in each beam. Accurate measurement of slit widths is essential if spectra are to be compared. As mentioned above, a condition generally overlooked is the effectiveness of the monochromator in transmitting only monochromatic light to the exclusion of all other wavelengths.
Any monochromator will transmit some stray light, or light at wavelengths outside the band defined by the slits, because the rulings on the grating contain irregularities, the beams are not perfectly collimated, the optical surfaces may be dusty, and because of many other factors. Difficulty of eliminating this stray light is one reason why fluorescence has been applied predominantly to liquid samples or to finely ground powders pressed against a perfectly flat window.

One objective of our research has been to obtain fluorescence spectra from samples of natural surfaces that could be used to predict spectral regions in which these surfaces would fluoresce if they were illuminated by sunlight. In order for the results to be of use in predicting detectivity by a remote sensor it is also desirable to eliminate from the spectra any components resulting from spectral variations either in the light source, in the efficiency of the monochromators, or in the sensitivity of the photomultiplier. This objective is in accord with proposals that have been made for standardization of methods of reporting fluorescence spectra. (Udenfriend, Sidney, 1969, p. 592-593).
The problem of reflected stray light

It was mentioned above that monochromators transmit varying amounts of stray light in addition to monochromatic light in the spectral band defined by the angle of the diffraction grating and the slit widths. In fluorescence analysis of a clear liquid, most stray light in the instrument is excluded from the light paths by means of baffles, light sinks, and the fact that the two beams are at right angles. When a light-colored rough-textured crystalline sample is viewed, however, a significant amount of light from the incident beam may be included in the viewed beam. The monochromatic component in this beam can be excluded merely by setting the emission monochromator at a longer wavelength than the excitation monochromator. However if the incident beam contains a significant percentage of stray light of this longer wavelength it will be inseparable from any fluorescence at the same wavelength.

In testing samples of white crystalline material (e.g., the borate mineral ulexite from salt flats in the Atacama Desert, a mineral that commonly displays moderately strong, yellowish fluorescence) we found that reflected stray light might comprise between one-third and two-thirds of the recorded intensity of the viewed beam after it passed through the emission monochromator. The magnitude of the problem is illustrated by Figure 1, which serves to illustrate the principal components of light that need to be considered in both the incident beam and the viewed beam.
Components of light in fluorescence analysis of reflective samples

Figure 1 is based on light intensities that correspond roughly with those that might be expected in a "worst-case" laboratory situation with representative natural crystalline materials and typical commercial spectrofluorometers. The relative intensities are shown graphically, and are derived from two assumptions based on our laboratory experience: 1) that the desired monochromatic component comprises one-third of the total intensity of the incident beam; and 2) that the fluorescence component comprises one-half (50%) of the intensity of the viewed beam as it leaves the sample. It is assumed, for convenience, that each component of the incident beam is equally effective in stimulating fluorescence of the sample, is equally reflected or scattered by the sample, and that the efficiency of both monochromators is equal under the experimental conditions.

The basic components of the two beams can therefore be summarized as follows (percentages based on assumptions enumerated above):

Basic components of the incident (excitation) beam striking the sample:

1) Monochromatic source light (e.g., 33%)
2) Stray source light (e.g., 67%)

General components of the viewed beam leaving the emission monochromator:

A) Monochromatic fluorescence (e.g., 17%)
B) Stray fluorescence (e.g., 33%)
C) Monochromatic reflectance (e.g., 17%)
D) Stray reflectance (e.g., 33%)
Specific components of the viewed beam leaving the emission monochromator:

A1) Monochromatic fluorescence stimulated by monochromatic source light (e.g., 6%)

A2) Monochromatic fluorescence stimulated by stray source light (e.g., 11%)

B1) Stray fluorescence stimulated by monochromatic source light (e.g., 11%)

B2) Stray fluorescence stimulated by stray source light (e.g., 22%)

C1) Monochromatic reflectance from monochromatic source light cannot occur because the two monochromators are assumed to be set at different wavelengths, and the bands are assumed not to overlap (i.e., 0%)

C2) Monochromatic reflectance from stray source light (e.g., 17%)

D1) Stray reflectance from monochromatic source light (e.g., 11%)

D2) Stray reflectance from stray source light (e.g., 22%)

Of the foregoing seven components of light that can occur (excluding C1) only the first (monochromatic fluorescence stimulated by monochromatic source light, component #A1) is desired as a constituent of an excitation or emission spectrum. The percentages shown in the foregoing tabulation are again based on the assumptions enumerated above (p. 13). They illustrate that the desired fluorescence component might comprise as little as 6% of the total intensity of the emission beam as it is sensed at the photomultiplier. The remaining 94% would then consist of spurious values. The object of our work was to eliminate these spurious values from the fluorescence spectra of natural earth-surface samples insofar as possible.
Effect of a filter on one side of the sample

Inexpensive optical filters are available that will transmit monochromatic light in relatively narrow bands. Moreover, filters that transmit very low levels of stray light are readily available, either of colored glass or colored gelatin. If such a filter were placed on the emission side of a sample, to transmit a spectral band corresponding approximately to the band defined by the emission monochromator setting and slit widths, the components of light leaving the emission monochromator would then be:

A1) Monochromatic fluorescence stimulated by monochromatic source light (e.g., 6%)

A2) Monochromatic fluorescence stimulated by stray source light (e.g., 11%)

C2) Monochromatic reflectance from stray source light (e.g., 17%)

This would give greatly improved results over those obtainable with the use of monochromators alone, because two-thirds of the spurious values would have been eliminated, and the desired fluorescence component (#A1) would have been effectively increased by a factor of three. This use of optical filters is commonplace in spectrofluorometry, the filter being placed on the emission side for recording of excitation spectra (i.e., where excitation monochromator must be scanned) and on the excitation side for recording of emission spectra (i.e., where emission monochromator must be scanned). In testing a clear liquid by examining the fluorescence transmitted from the center of a cuvette, as opposed to testing the fluorescence from the liquid surface, this method of using filters is completely adequate, because the reflected component is negligibly small.

Commonly it is not even necessary to use a filter when testing clear liquids in cuvettes.
Effect of a filter on both sides of the sample

For the recording of an excitation spectrum from any type of sample (liquid or solid), the filter would be placed on the emission side of the sample, as described above. If it were sufficient to use a single excitation wavelength to stimulate the fluorescence, a suitable filter also could be placed on the excitation side. This is the technique used in a filter fluorometer. It requires no monochromators, but does not produce spectra. In a spectrofluorometer, if a filter that matched the emission monochromator setting were to be used instead on the excitation side, the relevant components of the beam from the light source would be determined by the following conditions:

1) Monochromatic source light would not strike the sample because it would ideally be completely excluded by the filter, whose transmittance corresponded to that of the emission monochromator setting (i.e., this component would be 0%, or close to it).

2a) Stray source light of wavelengths transmitted by the filter would strike the sample.

2b) All other stray source light would be excluded by the filter (i.e., this component would be 0%, or close to it).

In other words, the only component of source light that would actually be incident on the sample would be component #2a. Assuming that component #2a strikes the sample and stimulates some fluorescence, the resulting components that would leave the emission monochromator and be sensed by the photomultiplier (while the filter remained on the excitation side) would be:

A2a) Monochromatic fluorescence stimulated by stray source light transmitted by the filter.

B2a) Stray fluorescence stimulated by stray source light transmitted by the filter.

C2) Monochromatic reflectance from stray source light transmitted by the filter (full amount is recorded with filter on either side).

D2a) Stray reflectance from stray source light transmitted by the filter.
If the spectrum recorded with the filter on the excitation side were subtracted from that recorded with the filter on the emission side, the difference between the two could be represented by:

\[
(A_1 + A_2 + C_2) - (A_{2a} + B_{2a} + D_{2a} + C_2)
\]

\(\text{(filter on emission side)} - \text{(filter on excitation side)}\)

Since component \(C_2\) occurs in both spectra, and since component \(A_1\) is the desired fluorescence component, it can be seen that the difference between the two curves would be approximately equal to the desired fluorescence component if it could be shown that \(A_2\) is approximately equal to \((A_{2a} + B_{2a} + D_{2a})\).

**Probable errors using three alternative techniques**

When reasonable values are substituted for these components, it is found that the difference between the two spectra will always be somewhat larger than the desired fluorescence component, which is the pure monochromatic fluorescence stimulated by pure monochromatic source light. In conditions estimated to be typical during spectrofluorometer tests of rough-surfaced reflective earth-surface samples, the recorded intensities are estimated to commonly range from 15% to 50% higher than the pure fluorescence component as defined above. The derivation of these values is shown on Tables 1 and 2, including a comparison of probable errors that typically would be encountered using the three alternative techniques.
Table 2 shows that whereas the range of probable error is 15% to 50% when a filter is alternately placed on both sides of the sample, the error would probably range from about 50% to 500% if the filter were used on only one side of the sample, as in conventional techniques. If no filter were used at all under these conditions (i.e., moderate to high sample reflectance, moderate to low monochromator efficiency, and moderate fluorescence intensity) errors from about 100% to over 1000% might be anticipated.

An error between 15% and 50% should be viewed in relation to the ideal goal of pure monochromatic fluorescence stimulated by pure monochromatic source light. From this viewpoint errors of that magnitude are tolerable, if not actually favorable. To approach more closely to this ideal goal would probably require use of a phosphorescence accessory, imposing limitations of a different type. It is concluded that the use of filters in the manner described above is inexpensive, quick, easy, and more accurate than any alternative technique that we know of for testing natural earth-surface samples.
Additional sources of error

We have already considered seven components of light recorded by a spectrofluorometer. If the filter or other optical parts are weakly fluorescent, the number of light components will be greatly increased and analysis of sources of error would be more difficult. Fortunately errors arising from this source tend to compensate each other.

Some problems resulting from fluorescence of filters and other optical parts are nearly unavoidable, because fluorescence has been found in the quartz windows of powder cells for a spectrofluorometer, in the glass or cement of one or more lenses in a spectrofluorometer, and in the majority of filters that have been tested. The filter problem will be more fully discussed in a subsequent report. Fluorescence of quartz cells is commonly a weak violet or blue-violet that is most strongly excited near 250 nm (Price, J.M., and others, 1962, p. 530-532). For this reason it is advisable to: 1) use the best grade of quartz cells; 2) to check cells, cell windows, lenses, sample holders, and filters for fluorescence with long- and short-wavelength ultraviolet light; and 3) to avoid excitation near 250 nm unless it is certain that all transparent components are non-fluorescent. These precautions would be much less important in conventional spectrofluorometer research with liquids in standard cuvettes, because the critical area of intersection of the incident beam and the viewed beam is in the center of the cell, well away from the cell walls or other optical parts.
CONCLUSIONS

1) At least three advanced research efforts are in progress to develop techniques for remote sensing of the fluorescence of natural surfaces. These will require improved techniques for measuring the fluorescence of similar samples in the laboratory.

2) When a light-colored, rough-textured crystalline sample is viewed in a typical commercial spectrofluorometer, the viewed beam leaving the emission monochromator will commonly contain seven significant components.

3) Of the seven components of the viewed beam, only monochromatic fluorescence stimulated by monochromatic source light is a desired constituent of a fluorescence spectrum, and in some applications this may be the smallest component of the seven. The remaining six components are all spurious values.

4) Most of the six spurious components can be eliminated by recording two successive spectra for one sample, using identical instrument settings, first with an appropriate glass or gelatin filter on the excitation side of the sample, and then with the same filter on the emission side of the sample. This technique is inexpensive, quick, easy, appears universally applicable to all types of samples, and appears more accurate than any alternative technique that we have seen for testing samples of natural surfaces.
REFERENCES CITED


ACKNOWLEDGMENTS

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**Figure 1.** PRINCIPAL COMPONENTS OF LIGHT IN FLUORESCENCE ANALYSIS OF REFLECTIVE SAMPLES (components identified below)

A1: Monochromatic fluorescence stimulated by monochromatic source light
A2: Monochromatic fluorescence stimulated by stray source light
B1: Stray fluorescence stimulated by monochromatic source light
B2: Stray fluorescence stimulated by stray light
C2: Monochromatic reflectance from stray light
D1: Stray reflectance from monochromatic source light
D2: Stray reflectance from stray source light

(all components are identified above)

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<tr>
<th>*</th>
<th>A1:</th>
<th>6%</th>
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<tr>
<td>*</td>
<td>A2:</td>
<td>11%</td>
</tr>
<tr>
<td>*</td>
<td>B1:</td>
<td>11%</td>
</tr>
<tr>
<td>*</td>
<td>B2:</td>
<td>22%</td>
</tr>
<tr>
<td>*</td>
<td>C2:</td>
<td>17%</td>
</tr>
<tr>
<td>*</td>
<td>D1:</td>
<td>11%</td>
</tr>
<tr>
<td>*</td>
<td>D2:</td>
<td>22%</td>
</tr>
</tbody>
</table>

**Incident (excitation) beam striking the sample**

<table>
<thead>
<tr>
<th>1: Monochromatic source light (light of desired wavelengths)</th>
<th>2: Stray source light (light of undesired wavelengths)</th>
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<td>33%**</td>
<td>67%**</td>
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**Percentages are based on assumptions enumerated in the text (p. 13); they correspond to a probable "worst-case" likely to be encountered when a commercial spectrofluorometer is applied to fluorescence analysis of a white crystalline solid sample.**
Table 1. Comparison of three techniques for measuring fluorescence spectra of natural earth-surface samples

<table>
<thead>
<tr>
<th>SPECTROFLUOROMETER TECHNIQUE</th>
<th>Components of light recorded, on spectra (using symbols defined in text)</th>
<th>PROBABLE &quot;WORST-CASE&quot;</th>
<th>ESTIMATED COMMON CASE</th>
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<tr>
<td></td>
<td>Recorded intensity based on assumptions enumerated in text (p. 13)</td>
<td>Assumed intensity of pure fluorescence stimulated by pure source light</td>
<td>Percent error (&quot;worst case&quot;)</td>
</tr>
<tr>
<td>Standard technique using no filter</td>
<td>A + B + C + D [17 + 33 + 17 + 33 = 100%]</td>
<td>5.6%</td>
<td>1690%</td>
</tr>
<tr>
<td>Narrow-band filter used on side of fixed monochromator (filter assumed 100% effective)</td>
<td>A₁ + A₂ + C₂ [6 + 11 + 17 = 33%]</td>
<td>5.6%</td>
<td>496%</td>
</tr>
<tr>
<td>Narrow-band filter used on both sides of sample, alternately (one spectrum is subtracted from other)</td>
<td>((A₁ + A₂ + C₂) - (A₂a + B₂a + D₂a + C₂) \Rightarrow A₁ + A₂ - A₂a - B₂a = D₂a) ((6 + 11 + 17) - (1.7 + 3.3 + 3.3 + 17) = 8.4%)</td>
<td>5.6%</td>
<td>50%</td>
</tr>
<tr>
<td>Ratio of stray light to monochromatic light in the spectrofluorometer</td>
<td>Spectrofluorometer technique (described in the accompanying text)</td>
<td>R/F* = 100%</td>
<td>R/F* = 50%</td>
</tr>
<tr>
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<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>200%</td>
<td>Standard technique using no filter</td>
<td>100 1690%</td>
<td>100 1250%</td>
</tr>
<tr>
<td></td>
<td>Narrow-band filter on side of fixed monochromator (filter assumed 100% effective)</td>
<td>33.3 496%</td>
<td>33.3 350%</td>
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<tr>
<td></td>
<td>Narrow-band filter on both sides of sample, alternately (one spectrum subtracted from other)</td>
<td>8.4/ 50 13.3/ 80 18.3/ 97 20.6/ 104</td>
<td></td>
</tr>
<tr>
<td>50%</td>
<td>Standard technique using no filter</td>
<td>100 350%</td>
<td>100 237%</td>
</tr>
<tr>
<td></td>
<td>Narrow-band filter on side of fixed monochromator (filter assumed 100% effective)</td>
<td>66.7 200%</td>
<td>66.7 125%</td>
</tr>
<tr>
<td></td>
<td>Narrow-band filter on both sides of sample, alternately (one spectrum subtracted from other)</td>
<td>30.0 35% 40.6/ 37 51.1/ 38 56.0 39</td>
<td></td>
</tr>
<tr>
<td>20%</td>
<td>Standard technique using no filter</td>
<td>100 188%</td>
<td>100 116%</td>
</tr>
<tr>
<td></td>
<td>Narrow-band filter on side of fixed monochromator (filter assumed 100% effective)</td>
<td>83.3 140%</td>
<td>83.3 80%</td>
</tr>
<tr>
<td></td>
<td>Narrow-band filter on both sides of sample, alternately (one spectrum subtracted from other)</td>
<td>40.2 16% 53.7/ 16 67.3/ 16 73.4/ 16</td>
<td></td>
</tr>
</tbody>
</table>

(* R/F represents the ratio of reflectance/fluorescence)

Table 2. Comparison of three techniques for measuring fluorescence spectra under varying conditions of monochromator efficiency and reflectance of the sample