

Fluorescence & Color



An overview of the fundamentals



of measuring fluorescent color



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Introduction

An overview of:

- *Measuring fluorescent color*
- *Chemistry & physics of fluorescent colors*
- *Measurement techniques*
- *Effects of different illuminants on fluorescent pigments*
- *Achieving accurate bispectral measurements for fluorescent colors*

Fluorescent pigments add brightness and color to our lives, but brightness and light to the consumer can spell difficulties in color matching and formulation for a manufacturer. While fluorescent pigments have been around for quite a few years, the proper formulation and quality control of colored materials containing them has been a challenge. This is primarily due to lack of proper instrumentation to make the necessary measurements. Another difficulty has been a general lack of understanding of how such materials function.

This technical note is intended to present an overview of the general principles associated with measuring fluorescent color. It will address the chemistry and physics of what causes fluorescent pigments to work and give a few examples of applications for the pigments. We'll also consider why standard spectrophotometers and colorimeters fail to give completely accurate results in measuring such materials. After an overview of the effects of different illuminants on fluorescent pigments, we'll discuss how geometry of measurement can affect results, and how several national laboratories are making such measurements. Finally, we'll take a look at one industrial solution that provides accurate bispectral measurements of fluorescent colored materials.

Fluorescence and Color

How we see colored objects is a well-studied phenomenon. The perceived color of an object is determined by the spectral composition of the light leaving it. The makeup of this light is a combination of the light that illuminates the object, and the way the object modifies that light. An object may appear quite different under daylight, fluorescent lights, or standard incandescent lamps, as is often observed when buying clothing. How many times has that brown pair of pants you liked in the store turned out to be olive green when seen in the sunlight?

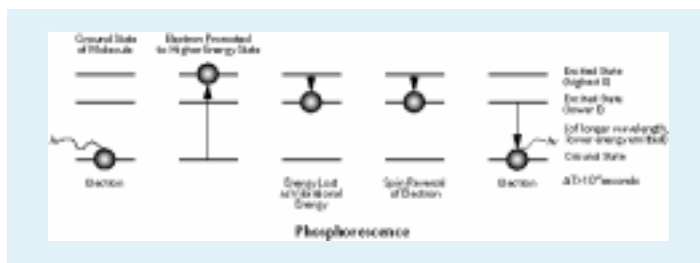
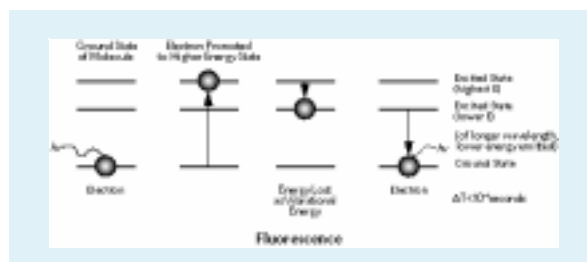
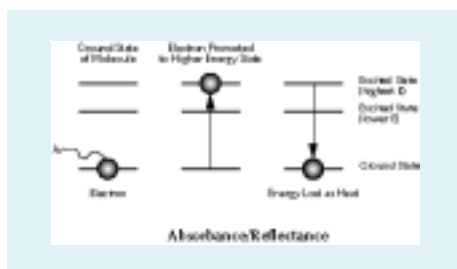
For non-fluorescent materials, the spectral reflectance can be determined using a spectrophotometer, and the color computed for specific illuminants. The determination of color becomes even more complex when one or more of the components of a pigment mixture exhibits fluorescence.

Fluorescence

Fluorescence is a phenomenon that occurs when a substance absorbs radiation of a certain wavelength, or group of wavelengths, and re-emits photons of different wavelength.

When certain compounds absorb light, an electron is excited to a higher vibrational energy state. The molecule then loses its excess of vibrational energy by collisions and falls to the lowest vibrational level of the energy state. In addition, almost all molecules occupying an energy state higher than the second undergo internal conversion and pass from the lowest vibrational level of the upper state to a higher vibrational level of a lower state which has the same energy. From there, the molecules again lose energy until the lowest energy level of the first excited state is reached. From this level, the molecule can return to any of the vibrational levels of the ground state, emitting its energy in the form of fluorescence. This phenomenon is illustrated in Figure 1.

Figure 1
Energy level diagrams
for absorption/
phosphorescence/
fluorescence



Typically, molecules that display fluorescence are of two types: organic compounds with a high degree of conjugated unsaturation and extended p- cloud structure or inorganic compounds where it is relatively easy to promote an electron to a higher vibrational energy state (usually a d- or f- level). The spectrum of the emitted light is called the emission spectrum. Due to the conservation of energy, the wavelength of the emitted light is almost always at longer wavelength than the excitation radiation. This discussion will only address these cases. The difference between the excitation and the emission wavelength is called the Stokes Shift. Typically, the Stokes Shift for organic compounds is much smaller than for inorganic compounds and also usually occurs at longer wavelengths.

A similar phenomenon that can occur is phosphorescence, which can be observed in many pigments used in safety illumination of corridors or other places where directional markers are needed during power failures. While the mechanism on a molecular level is similar, phosphorescence will not be covered in this paper. Typically, the emission of phosphorescence takes place at least in $>10^{-4}$ seconds (it may last for minutes or even hours) while fluorescent emission takes place in $<10^{-4}$ seconds or less. One usually sees fluorescence in the 10^{-4} to 10^{-9} second range. Anything longer is considered phosphorescence. Perhaps the easiest definition is if you can see the emission after the illuminating source is removed, it is phosphorescence. If the emission stops when the illuminating source is removed, it is fluorescence.

What Does All This Have To Do With Color?

Organic fluors in nature are not all that rare. In fact, on a warm summer night, while enjoying a gin and tonic, you are consuming a very common fluorescent material– quinine! Those of us who work in fluorescence know that if we forget our standards when calibrating a spectrofluorimeter, relief is just a Schweppes away, as the quinine in tonic water is an excellent, reliable standard!

The reflectance of an object determines the color. In a non-fluorescent molecule, photons are still absorbed but they immediately drop back to the original energy state, dissipating the energy in the form of heat or simply emitting a photon of equal wavelength. The result is a typical absorption spectrum, since absorption is merely the log (1/R). If there are molecules in the pigment mixture that fluoresce, the resulting spectrum is a combination of a number of factors: (a) the reflectance of the non-fluorescing materials; (b) the absorbance of the fluorescent material due to the excitation of electrons to a higher energy state; (c) the emission spectrum of the fluorescing material. Truly a mess to interpret since both the absorption and fluorescence depend on the distribution of wavelengths in the illuminant used.

Fluorescence is also not uncommon in nature. Many organic and inorganic species fluoresce to some extent. Most soils containing humus fluoresce, as do many bacteria. Fluorescence is common in minerals as well. Many science museums will usually include a display of minerals illuminated by both tungsten lamps and black lights (UV source) to demonstrate this phenomenon. Almost all uranium compounds, commonly found in granite, fluoresce as do many zinc, cadmium, and copper ores.

Types of Fluorescent Pigments

Typical applications of inorganic fluors are in security documents, invisible markings on postage stamps and currency, and in fluorescent lamps, and CRT display tubes.

Commercial fluorescent pigments can be divided into three major groups – *inorganic fluors, optical whiteners, and daylight fluorescents*. All are significantly different in chemical structure and consequently all have their own particular niche applications due to their chemical and optical properties.

Inorganic fluorescent materials are perhaps the least utilized for industrial applications. These materials, generally based on zinc sulfide or cadmium sulfide doped with small amounts of another transition or rare earth element, are very stable. They do have a number of disadvantages that discourage their use in many applications. They are generally mildly toxic due to the heavy metals present in the compounds. Most all of the compounds are also only activated by UV to very Near-VIS radiation (300-420 nm), although some activate into the mid-visible range and a few even activate in the Near-IR. They also tend to have fairly low quantum efficiencies. Quantum efficiency is basically the number of photons out at the emission wavelength compared to number of photons in at excitation wavelengths. Many of these fluors tend to be somewhat phosphorescent, which may be an unwanted side effect.

Typical applications of inorganic fluors are in security documents (invisible markings on postage stamps and currency) and in fluorescent lamps, and CRT display tubes. A more recent application, based on their stability, is as fluorescence standards when combined with fluorocarbon resins at high temperature.

Optical whiteners are a particular class of organic compounds that have very specific fluorescent properties. These materials are used heavily in the textile, paper, and plastics industries to whiten materials. They have excitation maxima in the very near-UV (generally 340-400 nm) and emit in the 430-460 nm wavelength range, generally peaking around 440-450 nm. Optical whiteners act by adding a blue component to counteract the yellowness found in paper and textiles such as cotton and polyester, taking non-visible UV radiation and upconverting to blue. This effect is also used to brighten the appearance of pastel colors. The production and measurement of optical whiteners (a.k.a. brighteners) is a multi-million dollar industry throughout the world. Optical whiteners can take many forms. Typically the function groups on a highly unsaturated organic compound are modified to enhance solubility and uptake depending on the substrate upon which they are used. Two typical optical whiteners are shown in Figure 2.

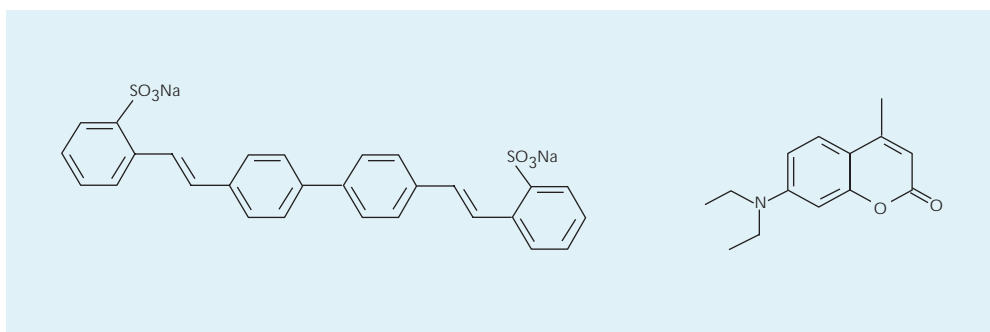


Figure 2
Chemical structures
of two optical whiteners

Daylight fluorescents are a fairly new and very heavily used class of organic compounds. These materials have both excitation and emission maxima in the visible region of the spectrum, so almost any illuminant can cause the excitation of the materials. These materials have many applications, from brightly colored papers to plastics products. An extremely important use is the formulation of these materials in safety applications such as protective garments and traffic safety signs. The latter two applications often combine the fluorescent component for daylight with retroreflective materials for night time usage to further enhance their night time conspicuity.

These materials and their formulations are effective because they greatly enhance the output of light at particular wavelengths far above what can be produced by reflectance alone. A red, yellow, or orange material of apparent reflectances far greater than 100% R can be produced by a combination of reflectance of the non-fluorescent component and emission of the fluorophor. Another advantage is that most of these materials are easily compounded into thermoplastics or incorporated into textile dyes. The problems in formulation are two-fold. First, too much of the fluorescent component adds expense and also may serve to be self-quenching, lowering the fluorescent component. Second is knowing exactly how much of the fluorescent component to add to achieve the desired color, since most color formulation software is not set up to accommodate the effects of fluorescent components.

Measurement of Fluorescence and Color with Standard Spectrophotometers

Spectrophotometers come in two types. First, there are those that scan over the spectrum, almost always from higher wavelength to lower using monochromatic illumination of the sample, stepping from wavelength to wavelength with a detector that indiscriminately collects the reflected or emitted photons. This type of instrument is typical of most analytical UV-VIS spectrophotometers.

The second type measures in the opposite way. The source illuminates the sample with all wavelengths at once, usually with a tungsten-halogen source then sorts out the reflected/absorbed wavelengths by use of an array of silicon photodiodes¹. This is a diode array spectrometer. For materials that do not fluoresce, the spectral data obtained from either of these instruments will, in theory, be identical. If a fluorescent material is part of the pigment mix, the results are greatly different, as shown in Figure 3.

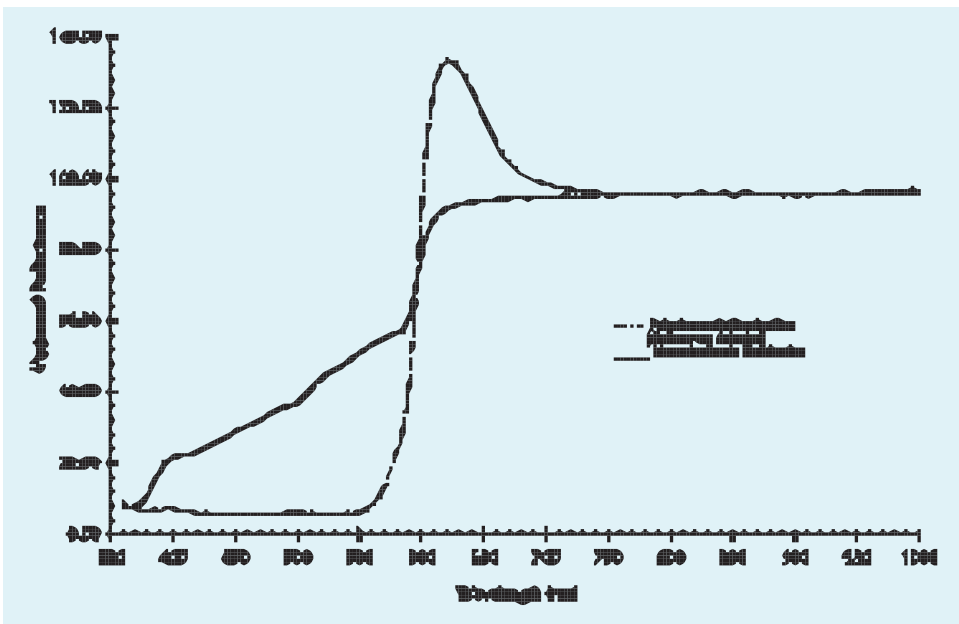


Figure 3
Comparison of red/orange daylight fluorescent film measured on scanning (monochromatic illumination) and diode array-based (polychromatic illumination) instruments.

Figure 3 shows a red/orange daylight fluorescent type material measured on a scanning spectrophotometer. Notice that the absorbance due to excitation shows up as a combination of absorbance and emission, since the detector is unable to differentiate between photons of different wavelengths. The second curve shows the same red/orange daylight fluorescent type material measured on a diode array spectrophotometer with polychromatic illumination. Notice that the absorbance due to excitation shows up as an absorbance and the emission appears as an enhancement to the reflectance.

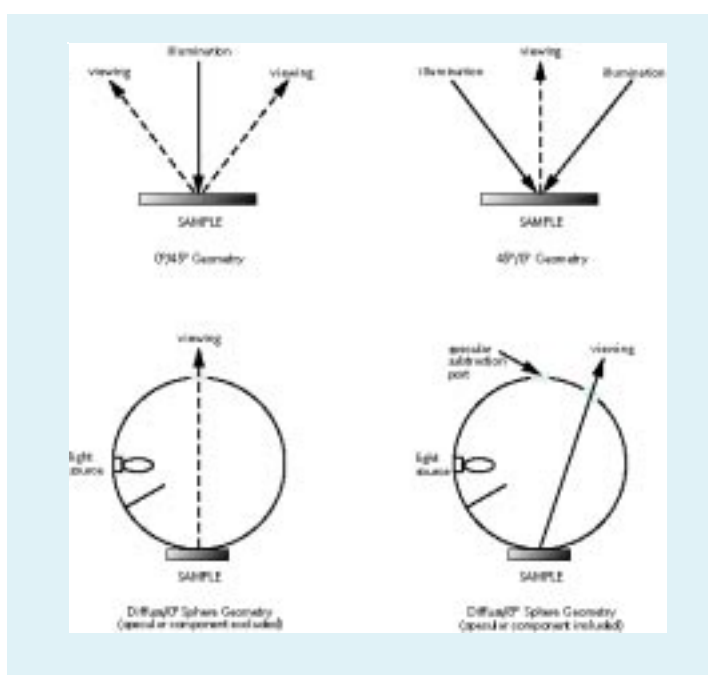
¹ Many instruments either use a xenon arc source or a combination of tungsten-halogen and deuterium lamps to increase the range further into the ultraviolet wavelength region.

Geometric Considerations for Colorimetry

The geometries we'll discuss are those of *illumination angle* – the angle at which the illuminating beam strikes the object to be measured, and *collection angle* – the angle at which the detector views the measured object.

According to ASTM and CIE definitions, the illumination angle is always the first number (or letter) you will see with the collection angle following separated by a slash (/) or colon (:). Thus, if one illuminates a sample with 45° incident light and has a detector that views the samples from above (normal to the sample or 0°) the geometry is properly described as 45°/0°. This geometry is quite common in inexpensive color analyzers and compact reflectance measurement instruments. If one reverses the detector and light source, the geometry now becomes 0°/45°. Pretty simple, right? Now, suppose you illuminate at 0° but use an integrating sphere as a collector, which catches all forward scatter over 180°. This is called diffuse collection, abbreviated as 'd'. The geometry is called 0°/d.² The reverse geometry, using the sphere as the illuminant and viewing the sample at normal incidence, is d/0°. The four geometries shown in Figure 4 describe most of the applications you'll see. There is the odd 30°/d geometry designated in one ASTM specification and multi-angle geometries as well, but if you remember incidence angle first, collection angle second, you'll have no trouble deciphering geometric nomenclature.

Figure 4
Measurement Geometries



Helmholtz Reciprocity

Helmholtz Reciprocity states, in simple terms, that light doesn't care what direction it's coming from or going to, so that 0°/45° and 45°/0° geometries are equivalent. For directional/directional geometry (a number followed by another number), that is true. The definitive experimental work was done by Frank Clarke³ at the National Physical

Laboratory in the UK about 10 years ago. This principal also holds in most cases for directional/hemispherical or hemispherical/directional geometry (#/d or d/#). It does not hold true if materials are luminescent and the illuminating radiation causes the material being measured to luminesce.

That last sentence is the rub. There is not a consensus in the fluorescence community as to what is the proper geometry of measurement. The paper and textile industries, under the auspices of TAPPI and AATCC, have traditionally written their measurement specifications around d/0° or d/8°. Paint and plastics researchers within CIE and ASTM don't yet have final standards, but many of the draft standards are leaning towards 0°/45° or 45°/0°. The real problem is that there are good technical arguments to be made for each geometry.

2. You will occasionally (especially in European circles) see 8°/t and 8°/d. The 't' is for total reflectance (specular component included) and the 'd' is for diffuse only (specular component excluded).

3. F.J.J. Clarke and D.J. Parry, *Helmholtz Reciprocity: its validity and application to reflectometry*, *Lighting Research and Technology* 17 (1) 1985.

Integrating Sphere Spectrophotometers

Integrating sphere-based spectrophotometers are generally used in industry for color matching. They are much better at eliminating effects due to sample non-homogeneity or variation in scattering effects of surfaces. When designed with diffuse polychromatic or monochromatic illumination and a diode array detector, the fluorescent component can be discriminated from the reflected component by subtraction. The problem lies in that once a sample fluoresces, its fluorescent emission becomes part of the diffuse illumination. Fluorescent emission is lambertian in its character. In other words, the emitted component is non-directional. The problem now lies with the secondary illumination – the combination of the original illuminant and the fluorescent component. If the fluorescing species has a small Stokes Shift, and the excitation and emission bands overlap at all, there is some uncertainty as to what illumination is responsible for what. Granted, the amount of radiation emitted is very small compared to the main illumination, there are concerns about the overall effect of this secondary illumination. If the excitation and emission bands don't overlap, this problem doesn't exist. One shortcoming of sphere-based measurements is the problem of gloss. Gloss adds a lightness component (the L in $L^*a^*b^*$ or $L^*c^*h^*$) that is totally dependent on illumination and viewing angle.

Generally, one wants to do formulations in a specular excluded mode. This can be done with an integrating sphere – you can illuminate or collect at 0° , so the specular component goes out the port (or the sample isn't illuminated at that angle). Another method is to use a light trap. But, how big should the light trap be? Or, how big should the input/collection port be? Both of these issues cause problems with sphere efficiency and inter-instrument comparisons.

When one uses a $0^\circ/45^\circ$ or $45^\circ/0^\circ$ geometry, sample re-emission is no longer a problem, since there is no "re-radiation" by the sphere. The only illuminant the sample sees is that of the original illumination source. While this sounds like a true solution to the problem of the sphere geometry, it's not quite so simple since directional/directional geometries have difficulty with samples that have different scattering characteristics. So, while a $45^\circ/0^\circ$ instrument may be fine for determining the color of a matte paper sample or uniform plastic part, it doesn't work as well for a textured cloth sample or samples with effect pigments included (e.g. those found in metallic or pearlescent paints). Another advantage of directional/directional geometry is that it is by geometry specular excluded, so sample gloss is not a complicating factor.

Illuminants and Their Effect on Fluorescence

The color of objects is dependent upon both the pigments used and the illumination under which we observe them. This becomes even more of a problem when one or more of the pigments involve luminescence, since fluorescent emission is dependent on both the wavelength distribution and intensity of the illuminating source.

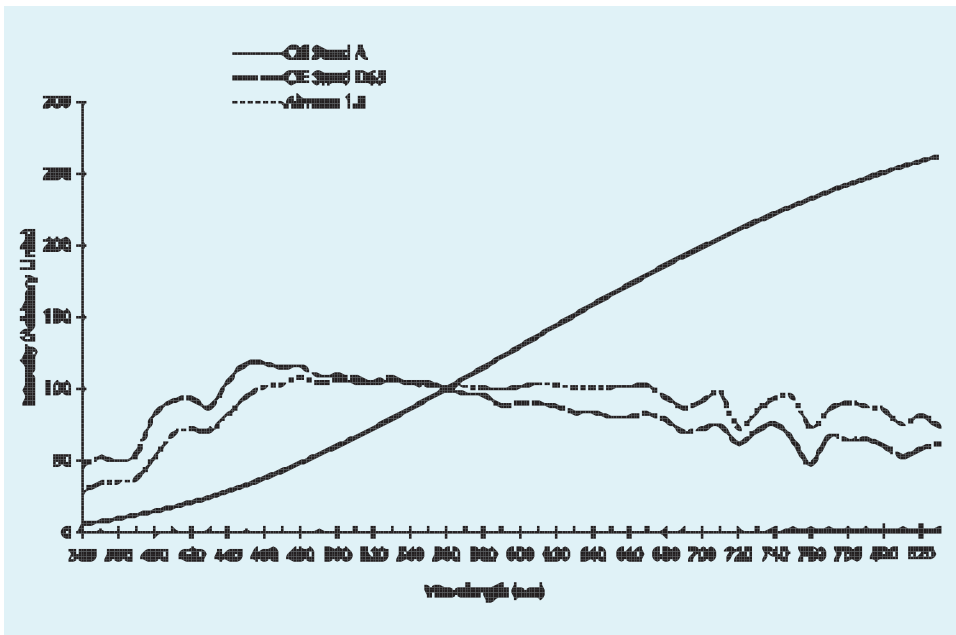


Figure 5
Comparison of
Illuminants A,
D65, and
Solar Airmass 1.5

If there were only one illuminant, say sunlight, color matching would be simplified to a great extent. It's quite difficult to use sunlight as a source for a spectrophotometer, as you might imagine, but artificial concepts of daylight simulation exist. Perhaps the closest is D65, which simulates daylight with a correlated color temperature of 6500 K. While a close approximation of D65 has been realized at B.A.M. the Federal Institute for Materials Research and Testing Germany, by use of a complex series of filters, it is usually thought of as a construct upon which calculations are based.

The use of sunlight or one of its simulants would be fine if materials were only used outdoors. Unfortunately, for formulators, mankind spends a great deal of time indoors under a wide variety of illumination conditions. Some of these illuminants have very little structure, such as tungsten filament lamps, and are quite predictable. Others, such as fluorescent lamps or high intensity discharge lamps have a great deal of spectral structure. Incidentally, the only “official” CIE illuminants are A (incandescent lighting) and D65 (daylight). Industrial color matching, however, uses many other illuminants to more closely match real life situations.

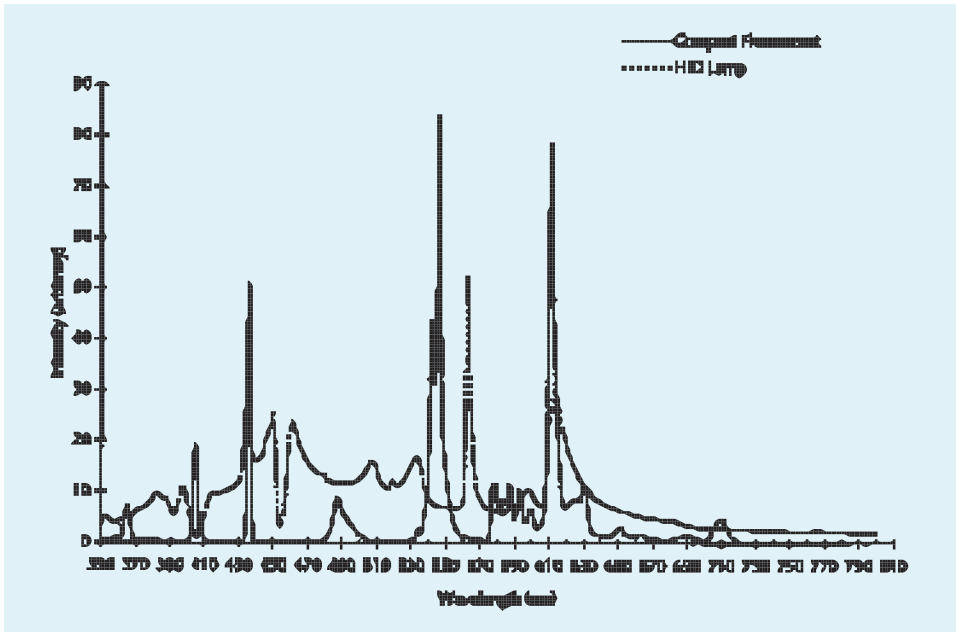


Figure 6
Compact Fluorescent/
HID Lamp Illuminants

While each of these sources require consideration in formulation of color, they may wreck havoc on formulations in which fluorescent pigments are included. Consider a compact fluorescent source as shown in Figure 6. If the excitation band for the fluor is narrow and occurs in a region of the spectrum where the output of the source is zero, there will be no fluorescent contribution to the color. Move the excitation band or shift the lamp output a few nanometers and the fluorescent component may dominate the color. As there are dozens of fluorescent illuminants and quite a few other sources used in commercial lighting, color matching becomes more and more challenging.

Bispectral Fluorescent Color Measurement

To properly match fluorescent color, quite a bit more information is needed than is measured by standard spectrophotometers or colorimeters. One needs to separate the reflected component from the fluorescent component from a theoretically spectrally flat source, then apply the corrections for an illuminant. This can be accomplished by a number of methods. Perhaps the most universally accepted is using a system with two monochromators, one on the illumination side, and one on the collection side.

The illumination (or excitation) monochromator is set to a wavelength with the collection (or emission) side scanned over the wavelength range of interest. The excitation monochromator is then stepped to the next wavelength, and the process repeated. The result is a data table that is not simply data pairs as in color (wavelength/reflectance), but a three dimensional matrix (as shown in Figure 7) where the reflectance is on the diagonal and the fluorescent emission is below the diagonal. This is known as a bispectral color measurement as both a fluorescence and a reflectance spectrum are being generated, with both the source side and the output side being monitored.

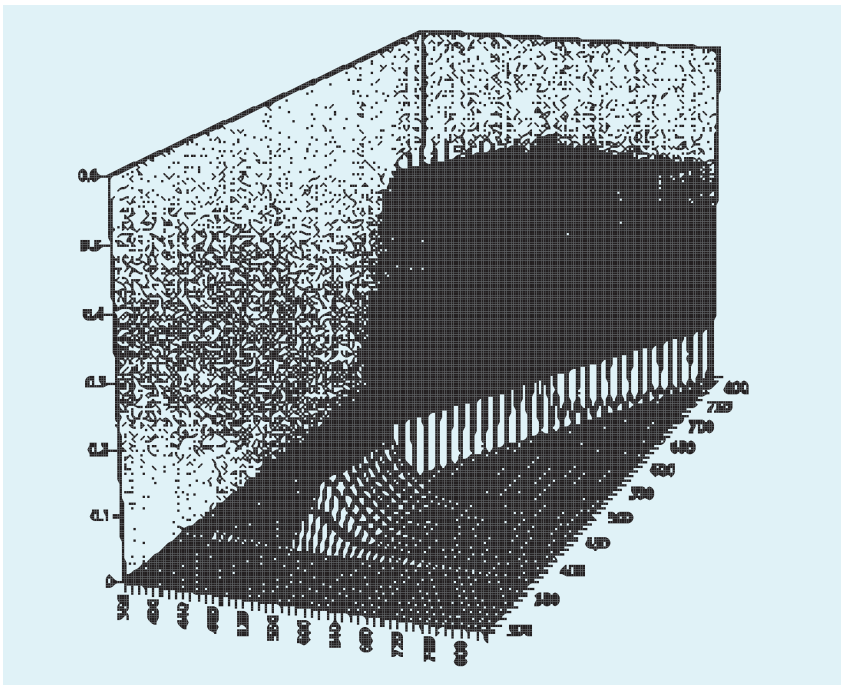


Figure 7
Bi-spectral Color
Measurement

The method described above is that used by two of the national laboratories that perform such measurements – NRC in Canada and the B.A.M. in Germany. This method is highly accurate but also highly tedious with measurements literally taking hours to perform. A slightly different tactic has been taken by the National Physical Laboratory (NPL) in the UK. This is also the path that we at Labsphere chose to follow in our development of a novel bispectral fluorescent color analyzer. This instrument uses a source side monochromator identical to that used in the method described above, but the collection side is quite different.

**National Laboratories
with Fluorescence
Measurement Capabilities**

*National Physical Laboratory,
Quantum Metrology Division,
Queen's Road, Middlesex
TW11 0LW United Kingdom*

*National Research Council, Canada,
Institute for Measurement Standards
Building M-36, Montreal Road,
Ottawa, Ontario K1A 0R6*

*Bundesanstalt für Materialforschung
und Prüfung,
Optical Properties of Materials Division,
Unter den Eichen 87, W-1000
Berlin 45, Germany*

On the collection side, the light which reflects/emits from the sample is imaged onto a polychromator which splits the radiation into its spectral components. This spectrum is then projected onto a silicon photodiode array, which collects all the wavelengths at once, eliminating the need for a scanning monochromator on the collection side. The resolution of the source side illumination is dependent on the monochromator, while the resolution on the collection side is due to both the emission monochromator and the diode array used. This method greatly speeds up the measurement process.

This concept can be designed into either the sphere or directional/directional geometries. The current design for the above mentioned instruments is either 45°/0° or 0°/45°.

From the data generated by a bispectral instrument, accurate quality control and formulation of colored materials containing fluorescent pigments can be realized. The method of monochromatic illumination and collection allows other illuminants to be applied to the data to accurately predict how a color will appear under a variety of illumination and viewing conditions. In addition, the separation of fluorescent component from the reflected component allows color formulation software to aid in the color matching of complex pigment mixtures that cannot be easily done by other means.

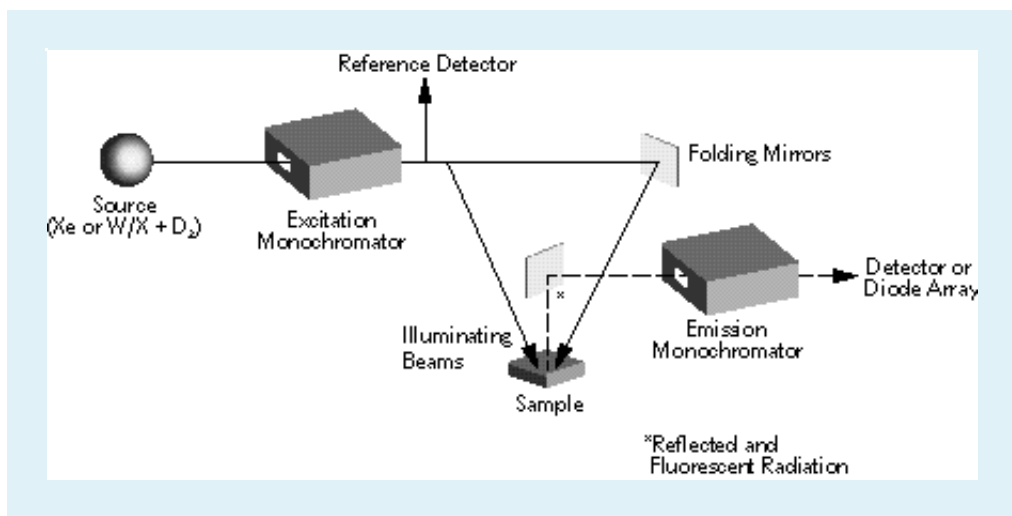


Figure 8
Schematic diagram
of a bispectral
fluorescence
instrument



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