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Standard Guide for Microspectrophotometry in Forensic Paint Analysis¹

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^{ε1} NOTE—Corrections were made throughout editorially in April 2020.

INTRODUCTION

Color is one of the most important comparative characteristics of paints. The comparison of color is one of the first steps taken in a forensic paint comparison. Subjective terms such as “blue,” “violet,” or “purple” are descriptors of color but are inadequate for clear communication of color as terms ~~may~~ could suggest different colors to different people. It is essential to note that this guide does not propose the use of instrumental color comparison for objects that are distinguishable to the eye. Since the 1940s, analytical instruments have been able to discriminate colors that the average human eye cannot distinguish. Microspectrophotometers (MSPs), in particular, allow for an objective measurement of the color of small, millimetre or submillimetre samples and are generally more sensitive than the more subjective results of visual microscopical color comparisons.

Suitable instruments with appropriate optics, sensitivity, resolution, and dynamic range can measure spectral curves from small samples as that light is transmitted, absorbed, reflected, or emitted ~~(via~~ (by means of fluorescence) by the sample. The spectral limits of different instruments can vary and ~~may~~ can extend from the ultraviolet (UV) (~190 to 380 nm) through the visible spectral region (~380 to 780 nm) to the near infrared region (NIR) (~780 to 2500 nm). MSPs should not be confused with broadband or absorption filter-based tristimulus systems.

1. Scope

1.1 This guide is intended to assist forensic analysts who conduct UV, visible, NIR, or fluorescence emission spectral analyses on small fragments of paint or use Guide E1610, as this guide is to be used in conjunction with a broader analytical scheme.

1.2 This guide deals primarily with color measurements within the visible spectral range but will also include some details concerning measurements in the UV and NIR spectral ranges. The particular method(s) employed by each analyst depends upon available equipment, examiner training, sample suitability, and sample size.

1.3 This guide provides basic recommendations and information about microspectrophotometers.

1.4 This guide does not address other areas of color evaluation such as colorimetric values, paint surface texture or pigment particle size, shape, or dispersion within a paint film that are evaluated by other forms of microscopy.

1.5 This guide is directed at the color analysis of commercially prepared paints and coatings. It does not address the analysis or determination of provenance of artistic, historical, or restorative paints, but it ~~may~~ could be useful in those fields.

1.6 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.7 *This standard cannot replace knowledge, skill, or abilities acquired through education, training, and experience and is to be used in conjunction with professional judgment by individuals with such discipline-specific knowledge, skills, and abilities.*

1.8 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

¹ This guide is under the jurisdiction of ASTM Committee E30 on Forensic Sciences and is the direct responsibility of Subcommittee E30.01 on Criminalistics.

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1.9 This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.

2. Referenced Documents

2.1 *ASTM Standards:*²

D16 Terminology for Paint, Related Coatings, Materials, and Applications

E179 Guide for Selection of Geometric Conditions for Measurement of Reflection and Transmission Properties of Materials

E275 Practice for Describing and Measuring Performance of Ultraviolet and Visible Spectrophotometers

E284 Terminology of Appearance

E1610 Guide for Forensic Paint Analysis and Comparison

E1492 Practice for Receiving, Documenting, Storing, and Retrieving Evidence in a Forensic Science Laboratory

3. Terminology

3.1 *Definitions*—For definitions of paint-associated terminology used in this guide, see Terminologies **D16** and **E284**, and Guide **E1610**.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *charge-coupled device (CCD), n*—a silicon-based semiconductor chip consisting of a linear or two-dimensional array of photo sensors or pixels that transfers an electrical charge and converts it into a digital value.

3.2.2 *effect pigment, n*—any paint pigment that is designed to produce a significant change in color attribute(s) in a paint film when the film is viewed or illuminated from varied angles.

3.2.3 *grating, n*—parallel set of linear, regularly repeating grooves that, when illuminated, produces dispersion of light into its requisite wavelengths with maxima and minima of light intensity as a consequence of interference.

3.2.3.1 *Discussion*—

These maxima and minima vary in position with wavelength. This allows radiation of any given wavelength to be isolated from a mixture of wavelengths (for example, white light) and allows the grating to be used as part of a monochromator. The dispersion or ability to resolve separate wavelengths is expressed as the number of lines (or steps) in the grating per millimetre.

3.2.4 *meaningful difference, n*—a feature or property of a sample that does not fall within the variation exhibited by the comparison sample, considering the limitations of the sample or technique, and therefore indicates the two samples do not share a common origin. The use of this term does not imply the formal application of statistics.

3.2.5 *measuring aperture, n*—element in the optical path of a MSP system that limits the area of illumination reaching the detector focal plane.

3.2.6 *metameric samples, n*—two or more samples that appear to have the same color under one type of illumination but may appear dissimilar under different lighting conditions, or two or more samples that appear to be the same color under all lighting conditions, yet their reflectance/transmittance spectral curves are different.

3.2.7 *microspectrophotometer (MSP), n*—a specialized spectrophotometer designed to measure the absorbance, reflectance and emission spectra of microscopic areas on samples.

3.2.8 *monochromator, n*—device designed to isolate narrow wavelength ranges of light from complex, broad-spectrum radiation.

3.2.9 *photomultiplier tube (PMT), n*—photosensitive vacuum tube device that quantitatively converts photons of light into electrical energy.

3.2.10 *pigment, n*—a finely ground, organic or inorganic, insoluble, and dispersed particle. Besides color, a pigment may provide many of the essential properties of paint, such as opacity, hardness, durability, and corrosion resistance (see also *effect pigment*).

3.2.11 *pixel binning, v*—the process of combining counts from adjacent pixels in a CCD detector during readout.

3.2.12 *spectral resolution, n*—measure of the ability to distinguish between adjacent peaks in a spectrum; it is usually determined by measuring peak width at half the maximum value of the peak height or full-width half-maximum (FWHM).

3.2.12.1 *Discussion*—

² For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

Spectral resolution is not to be confused with spatial resolution (the smallest features that may be resolved in the field of view of the MSP camera or eyepieces or may be used to refer to the smallest spectral sampling area of the MSP).

4. Summary of Guide

4.1 One of the most obvious properties of paint is its color. Pigments are used in paint to modify color or other properties. The interaction of pigments with light is very complex with light being scattered, absorbed, reflected, and emitted by a paint layer. A MSP can measure reflected, transmitted, or emitted radiation over a range of wavelengths.

4.2 MSP is an integrated instrument consisting of a microscope, a light source, a spectrophotometer, and a data-processing device. The microscope not only allows for analysis location selection but also reflects or transmits light energy efficiently, uniformly, and reproducibly and images light onto the spectrophotometer entrance aperture. The spectrophotometer contains a dispersive element, most commonly a diffraction grating, and a detector. The system is designed to measure the intensity of light energy with respect to its wavelength. All MSPs are single-beam instruments: a standard or a blank is measured, the result is stored, then a sample is measured and a ratio made to the standard to yield a transmittance or reflectance spectrum.

4.3 MSP analysis typically includes the visible spectral region (~380 to 780 nm), which captures information about the visual color of a sample. Most MSP systems are also sensitive to the NIR (~780 to 1100 nm). For UV-configured systems, the UV region (~190 to 380 nm) can provide additional information, for example, about UV absorbers in a clear coat layer. Furthermore, the spectrum of fluorescence emission (UV and visible excitation with UV to NIR emission) may also be captured.

4.4 MSP systems are generally used in forensic analysis because of the small sample sizes presented by paint film fragments. MSP is typically minimally destructive and microscopic samples can be analyzed. Instruments for color measurement from large samples can be used for color comparison, and can also be used for compiling color databases. Instruments for macroscopic color measurements produce results that are not necessarily directly comparable to MSP results.

4.5 Paint colors are usually measured in transmittance through thin sections. In transmittance measurements, a paint thin section is illuminated and the fraction of light transmitted or absorbed by the sample in the spectral range of interest (UV-Vis-NIR) is recorded relative to the light transmitted by a colorless background. Transmittance spectra may be plotted in either percent transmittance or absorbance.

4.6 In reflectance measurements of paint, a sample is illuminated and the fraction of light reflected from the sample in the spectral range of interest (UV-Vis-NIR) is recorded relative to the light reflected from a white reference standard. Reflectance spectral measurements are greatly affected by surface observation angle, surface texture, and the lack of microscopically reproducible diffuse reflectance standards. As a result, reflectance is seldom used for detailed color comparison, but it can be useful in exclusionary comparisons of bulk colors or for opaque samples. Reflectance spectra are plotted in percent reflectance.

5. Significance and Use

5.1 This guide is designed to assist an analyst in the selection of appropriate sample preparation methods and instrumental parameters for the analysis and comparison of paint pigments and colors. When used for comparison purposes, the goal is to determine whether any meaningful differences exist between the samples.

5.2 Paint sample spectra can be measured by reflectance or transmittance spectroscopy for comparison purposes. Transmittance measurements are generally preferred and are especially necessary for the analysis of UV absorbers in clear coats and the detailed analysis of effect pigments that are not opaque. Emission comparison via by means of fluorescence is also measurable.

5.3 It is not the intention of this guide to present comprehensive theories and methods of MSP. It is necessary that the analyst have an understanding of UV-Vis-NIR MSP and general concepts of specimen preparation before using this guide. This information is available from manufacturers' reference materials, training courses, and references such as Eyring (1),³ Stoecklein (2), and Purcell (3).

6. Sample Preparation

6.1 The general collection, handling, and tracking of samples should meet or exceed the requirements of Practice E1492.

6.2 ~~The~~ Verify that the work area and tools used for the preparation of samples ~~shall be~~ are free of all extraneous materials that could transfer to the sample.

6.3 *Transmittance Measurements:*

6.3.1 ~~Thin sections shall be prepared and examined~~ Prepare hand-cut or microtome-cut thin sections for transmittance measurements. ~~These may be prepared as hand-cut or microtome-cut sections.~~ Although embedding followed by microtomy may be considered labor-intensive, this method (when questioned and known samples are mounted together) produces samples with an equivalent path length. Samples with equivalent path lengths are preferred for all transmission measurements and required for analysis of UV absorbers when the comparison relies upon differences in relative concentrations (4).

³ The boldface numbers in parentheses refer to a list of references at the end of this standard.

6.3.2 Paint sections of approximately ~~3- μm~~ 3 μm thick are generally appropriate for measurements of pigmented layers. ~~Thicker sections may be necessary for lightly~~ Lightly colored samples or clear coats could require thicker sections (for example, up to ~~20- μm~~ 20 μm thick) (4, 5) for increased sensitivity of the instrument to low concentrations of UV absorbers or pigments.

6.3.3 Photometric reproducibility ~~may~~could be difficult to achieve with thin peels of paint layers or hand-cut cross sections due to uncontrolled path lengths within and between samples. This ~~may not be problematic when spectral curves exhibit exclusionary features, but it can lead to difficulties in the interpretation of shade (that is, intensity) differences.~~ differences when spectral curves do not exhibit exclusionary features.

6.4 *Reflectance Measurements:*

6.4.1 ~~Specular~~ Conduct specular reflection measurements ~~shall be conducted~~ on clean and undamaged sample areas. Clean the surface of the sample carefully with deionized water, or alcohol if necessary. Reflectance measurements are usually conducted without further preparation of the sample surface so that surface features (for example, weathering) that might distinguish between samples are not altered. In some cases, it ~~is~~ will be necessary to remove surface features. Surface weathering, for example, can be removed by polishing the paint using a diamond paste or another polishing medium so the measured properties of the surface are comparable to data for new, undamaged paint surfaces. However, caution is recommended before removal of features as the potential for other evidential loss increases. ~~Effort should be made to retain~~ Retain a portion of the original unaltered ~~sample~~ sample whenever possible.

6.4.2 To obtain optimal results during spectral measurements, prepare the surface ~~shall be so it is~~ free of scratches, dirt, and blemishes and mounted perpendicular to the optical axis of the microscope. ~~The paint sample surface shall be in sharp focus.~~ Obtain a sharp focus on the paint sample surface.

6.4.3 ~~Cross~~ Conduct cross section reflectance measurements ~~shall be conducted~~ on polished or microtome cut surfaces. Prepare samples for comparison in the same manner. Simplify and enhance reproducibility of the surface finish by mounting and polishing edge-mounted known and questioned samples side by side.

6.4.4 When the signal-to-noise ratio of a reflectance measurement is poor due to small sample size, ~~preparation~~ prepare and ~~analysis of~~ analyze a thin cross section by ~~transmittance is necessary.~~ transmittance.

6.5 For paint smears, different portions of the smear can be separated and mounted for analysis. These results can provide conclusive exclusionary data but ~~may not provide~~ could lack meaningful associative information. Smear paints are difficult for reflectance measurements; analyzing smears by transmittance methods is recommended.

6.6 *Mounting Samples:*

6.6.1 ~~Questioned and known samples shall be mounted and prepared~~ Mount and prepare questioned and known samples in the same ~~manner whenever possible.~~ manner.

6.6.2 For reflectance measurements, record the angle of illumination and ~~collection shall be recorded.~~ collection.

6.6.3 For transmittance measurements, mount the thin section or smear particles ~~are mounted~~ on a microscope slide under a coverslip in an appropriate refractive index medium (typically one that is close to that of the sample).

6.6.3.1 ~~The~~ Use a microscope slide ~~shall have with~~ transmittance characteristics appropriate for the region of the spectrum being analyzed. Glass (borosilicate) slides and coverslips are suitable for measurements in the visible and NIR portions of the spectrum. ~~Samples~~ Mount samples to be analyzed in the UV region of the spectrum ~~shall be mounted~~ on quartz or fused silica; however, not all slides have the same UV transmittance characteristics.

6.6.3.2 ~~A~~ Add a mounting medium ~~shall be added~~ between the slide and coverslip for transmittance measurements. ~~The~~ Select a mounting medium ~~to be selected shall be~~ that is compatible with the sample (that is, will not dissolve the sample) and with the spectral range being investigated (that is, glycerol is transparent to the UV region of the spectrum). ~~Possible mounting~~ Mounting media include, but are not limited to, water, xylene, xylene substitutes, glycerol, refractive index oils ($n = 1.52$ or 1.66 are common).

7. Performance Checks

7.1 Prior to use of the instrument, turn on the microscope, illumination sources, and spectrometer ~~shall be turned on and permitted to stabilize.~~ The lamp shall be allowed and allow them to stabilize. Allow the lamp to warm up and stabilize according to manufacturers' instructions or laboratory experience, whichever yields consistent results.

7.2 Checking instrument performance ensures/verifies that an instrument is operating within required standards and any errors that might affect data or analytical conclusions are known, limited, accounted for, or corrected. It is essential to demonstrate wavelength and absorbance/photometric accuracy through a performance check, such as that described in Practice E275. ~~Performance check procedures shall be undertaken prior to analyses. It is also recommended that a performance check be implemented after any maintenance. To ensure that historical performance check data are comparable, the system shall be set to a similar configuration each time the system is checked.~~

7.2.1 Conduct a performance check daily, prior to analyses.

7.2.2 A performance check should be conducted prior to use after any maintenance.

7.2.3 Use a similar configuration each time a performance check is conducted on the system to ensure that historical performance check data are comparable.

7.3 ~~A Maintain a record of the performance checks shall be maintained.~~ all performance checks. A historical record of this data provides a mechanism for monitoring system performance and provides an operator with an early warning of system trends and possible deterioration.

7.4 Performance check parameters include:

7.4.1 ~~Wavelength Accuracy—Wavelength~~ Routinely check wavelength accuracy over the measured range ~~shall be routinely checked~~ with the aid of National Institute of Standards and Technology (NIST)-traceable holmium, erbium, or didymium oxide filters. The resolution used during the wavelength accuracy checks should be the same or higher than that used in casework and consistent for each wavelength accuracy check. ~~These measurements shall be carried out in transmittance.~~ Transmittance is used for these measurements.

7.4.2 ~~Photometric Accuracy—~~Neutral density filters are used to demonstrate the photometric response of the system is linear. A typical set of neutral density calibration filters ~~might~~ could include some or all of the following filters: 0.1, 0.5, 1.0, 2.0, 2.5, and 3.0 absorbance units.

8. Instrument and Scanning Parameters

8.1 Microscope parameters include:

8.1.1 ~~Illuminator—An~~ Select an illuminator appropriate to the analysis being ~~conducted shall be selected.~~ The conducted. Select the emission spectrum of the illuminator that is selected shall be of has sufficient intensity across the entire wavelength range of interest so as to provide a spectrum with an acceptable signal-to-noise ratio. Tungsten, halogen, and xenon are commonly used for visible and NIR analysis. Commonly, xenon lamps are used for UV analysis and mercury lamps are used for fluorescence excitation. While LED illuminators are available over much of the spectrum, they are of little utility for MSP due to their lower intensity and limited spectral range (3).

8.1.1.1 Instrument design with regard to lighting can affect the ease of collecting reflectance measurements. Epi-illumination results in specular reflectance of the illuminating light, causing a loss of detail in the viewed image, making layer distinction difficult to impossible at times. The illumination/measuring geometry in reflectance analysis of 45°/0° is preferred such that the specularly reflected portion is not gathered by the microscope. This geometry can be best achieved with the help of a darkfield/brightfield illuminator fitted with darkfield objectives. However, such illumination excludes UV measurements as UV transmitting darkfield objectives are not available. A general discussion of geometric considerations for reflectance and transmittance measurements is found in Guide E179.

8.1.1.2 Background/system/reference transmittance spectra can be used to monitor illuminator performance and warn of unsuitable system alignment.

8.1.1.3 ~~Illumination Centration—~~Slight adjustments to the position of the bulb ~~may~~ can serve to increase or decrease the emission over specific regions of the spectrum. For example, it is possible to maximize UV illumination, often at the expense of some light in the visible wavelengths. Generally, the slight loss of intensity in the visible region is not problematic due to the high intensity of modern bulbs.

8.1.1.4 ~~Illumination Intensity—~~For some illuminators, this ~~may~~ can be a fixed parameter. When the voltage of an illuminator is adjustable, it should be held fixed following the photometric intensity performance check (7.4.2).

8.1.2 ~~Field Diaphragm—~~To minimize stray light, with the specimen in focus, the edges of the field diaphragm should be brought into view and sharply focused with adjustment of the substage condenser. The field diaphragm is then opened so that its edges are either just outside the collection aperture or just beyond the field of view. ~~The~~ Readjust the focus and size of the aperture ~~shall be adjusted if when the objective is changed.~~

8.1.3 ~~Substage Aperture (that is, Condenser Iris)—~~The substage aperture should be opened until the desired image contrast is obtained. As adjustment of this aperture impacts the amount of light reaching the detector, this aperture setting ~~may~~ could be different from that used to produce an ideal image (6). In some instances, it is desirable to further increase the opening of the substage aperture ~~in order~~ to allow more signal to reach the detector. Aperture adjustment should occur prior to the collection of the background spectrum, and ~~the aperture shall~~ should remain in a fixed position between the background and sample spectra collection and between samples when they are being compared. The appropriate aperture level is typically that which produces an emission intensity for the most intense emission peak of no more than approximately 80 % of the detector saturation value. It is critical that the detector is not saturated anywhere over the region being measured.

8.1.4 ~~Objective—An objective shall be selected~~ Select an objective that permits imaging of the paint layer or inclusion to be analyzed. A balance between the objective magnification and size of the measuring aperture is selected by the analyst. Typically, measurements are made using objectives between 10× and 50×. Quartz optics are required for measurements made in the UV region. Once the appropriate objective is selected, measure all samples being compared ~~shall be measured~~ at a fixed magnification.

8.1.5 ~~Measuring Aperture—~~If the MSP system is equipped with variable or multiple collection apertures, select and use the same aperture ~~shall be selected and used~~ for the measurement of all samples being compared. In general, select the largest aperture that will remain within the boundaries of the sample area to be ~~measured is recommended.~~ measured. This is particularly important for paints with effect pigments, which show variation over larger areas. The edge of a layer or sample should be avoided due to edge effects that ~~may~~ could impact the spectrum. For smears and thin peels, an oversized aperture (one that extends beyond the boundary of the sample) is undesirable in transmittance measurements as it increases the noise in the spectrum. In fluorescence

emission, the spectrum is not the result of a ratio to a reference scan; the strength of the signal is determined by absolute counts. Therefore, an oversized aperture when analyzing smears or thin peels for fluorescence emission ~~may~~can be used to increase the signal reaching the detector. As the background is black, there is no significant increase in noise to detract from the quality of the collected data.

8.1.6 *Filter Cube*—Some systems have a filter cube turret. Filter cubes ~~may~~can include a blank cube for transmission measurements, a mirror for reflection, and combinations of excitation and barrier filters for fluorescence measurements. ~~The analyst shall ensure that~~ Use a filter cube appropriate to the measurement being made ~~is in place. All~~made. Measure all samples being compared under a given configuration ~~shall be measured~~ with the same filter cube in place.

8.1.7 *Phototube Diverter*—Some systems have an adjustable phototube that permits light to be diverted to the ocular, spectrometer/camera, or split. Spectral artifacts (for example, interference fringes on the baseline) ~~may~~could be visible if the diverter is in the split position. ~~It is recommended the diverter~~ The diverter should be set such that all light is directed to the spectrometer during measurements to ensure the highest possible signal to noise ratio.

8.2 Spectrometer parameters include:

8.2.1 *Detector*—While the detector is not an adjustable variable post-purchase, it is a critical component during data collection. Two main detector types are available for use with MSP systems:

8.2.1.1 Photomultiplier tube (PMT) detectors consist of a photocathode, held at a positive potential, and a series of dynodes with successively lower potential to amplify the signal and convert photons of light into electrical energy. PMTs are sensitive, provide a high signal-to-noise ratio, and have good spectral resolution. PMTs are typically single channel detectors and are generally used in scanning spectrophotometers in conjunction with a grating, where the sample is scanned by stepping through wavelengths to create a spectrum point by point.

8.2.1.2 Semiconductor detectors are composed of a monochromator fitted with a diffraction grating and an array detector (for example, charged couple devices [CCD]) that acts as the photosensitive device. A CCD detector generally has a lower signal-to-noise ratio as compared to a PMT detector, but measurement time is drastically reduced because of simultaneous detection of the full spectral range. For this reason, CCD detectors are far more common than PMT detectors. The semiconductor detector's resolution will depend on the number of pixels in the array, the dispersion and line spacing of the grating, and the distance between the grating and the array.

8.2.2 *Wavelength Range*—~~A~~ Select a wavelength range ~~shall be selected~~ to reflect the spectral range to be measured and one that is appropriate to the instrument capabilities, sample, and sample preparation conditions as discussed in Section 6. This range typically falls between 190 and 1100 nm (transmittance UV-Vis-NIR).

8.2.3 *Resolution*—The resolution is predominantly defined by the grating and slit size. For most commercial MSP instruments, the grating and slit size are fixed, which results in a fixed maximum resolution. For CCD detectors, pixel size and spacing also have an effect on resolution due to pixel binning. Pixel binning (that is, resolution factor) reduces the resolution and increases the signal to noise ratio. The resolution factor is selected as a balance between signal to noise ratio and spectral resolution.

8.2.4 *Integration Time*—Most MSP systems allow the user to define the integration time. Some software packages have a built-in functionality, which automatically adjusts the integration time. The integration time is determined from a background spectrum. When automatic integration time is not available, set the integration time ~~shall be set~~ such that the detector electronics are not saturated.

8.2.5 *Number of Scans*—Most MSP systems allow the user to define the number of scans to average for a single spectrum. ~~The~~ Select the optimum number of scans ~~should be selected to yield that yields~~ the desired signal to noise ratio. Generally, the signal to noise ratio is increased by the square root of the number of scans.

9. Sample Analysis

9.1 Prior to sample analysis, allow the instrument ~~shall be allowed~~ to stabilize and ~~its performance checked~~ then conduct a performance check in accordance with Section 7.

9.2 ~~Illumination~~ Optimize the illumination field, measuring aperture, and magnification ~~shall be optimized~~ for the sample under investigation. Experimental conditions and instrument settings (that is, objective, aperture size, lamp voltage, scan/spectrum averaging, spectral resolution, or sample focal plane) should be identical for known and questioned samples ~~in order~~ to minimize experimental errors and sampling variations.

9.2.1 ~~These experimental~~ Experimental conditions ~~shall~~ include the length of time that a sample resides in the illumination beam. Sample temperature can increase with examination time as the sample absorbs heat, which can cause irreversible degradation in some dyes and pigments (for example, bleaching). Minimizing the time the sample is illuminated with UV light helps reduce specimen heating and UV degradation during analysis.

9.2.2 Consistent sample conditions are especially critical when collecting fluorescence data because the amplitude of the resulting spectrum is directly correlated with the collection conditions (that is, there is no reference scan to ratio against).

9.2.3 Inherent to any grating based spectrophotometer, energy diffracted from grating rulings can be polarized (predominantly polarized perpendicular to the rulings) and can result in spectral artifacts. This effect (Wood's anomaly) can be observed as a distinct transmittance maximum within the spectrum. Therefore, consistent or similar sample orientations are preferred when comparing samples.