A91 UV-Visible Microspectrometer Parameters That Affect Spectral Quality, Reliability, and Power of Discrimination

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After attending this presentation, attendees will benefit by gaining a deeper understanding of how UV-visible microspectrophotometry (MSP) spectral data quality, reliability, and discrimination power are improved through testing and controlling critical instrumental parameters, specifically, attention to sample preparation and using polarized light when analyzing optically birefringent samples.

This presentation will impact the forensic science community by recommending guidelines for collection of high-quality spectral data and interpretations in ultraviolet-visable MSP analysis.

Microanalysis of transfer (trace) evidence involves the application of a microscope and microscopical techniques for the observation, collection, documentation, and analysis of micrometer-size particles. Microspectroscopy is the union of microscopy and spectroscopy for microanalysis. Microscopy is the art and science of creating, observing, recording, and interpretation of magnified images. Analytical spectroscopy is the science of studying the absorption, reflection, and emission of electromagnetic radiation to determine the structure and chemical composition of materials. Both microscopy and spectroscopy contribute specific scientific information about a material's structure and composition. Both scientific methodologies originate with optics and the interaction of radiant energy with matter. Microspectrophotometry is perhaps one of the oldest microanalytical techniques with roots traced to the microspectrograph in the mid-1800s when used by Henry Clifton Sorby in order to compare the transmission spectrum of light traversing different directions in crystals. Microspectrophotometry has various contrast methods. These techniques continue to be used today in forensic science laboratories for the characterization and comparison of naturally colored and dyed or pigmented transfer evidence such as architectural, automotive, and artistic paints, natural and synthetic fibers, color-dyed human and animal hairs, thin polymer films, and minerals.

This research reports the critically important role of factors such as: (1) using a research PLM with a circular rotating stage to precisely orient the sample; (2) controlling the orientation of polarizer in the condenser; (3) the quality of the spectrometer used to record transmittance; (4) importance of slide and cover slip; (5) using a refractive index oil that closely matches the refractive index of the specimen to minimize scattering and lensing effects; and, (6) the proper treatment of data, including spectral normalization, in order to determine real variance in the data. This research reports how wavelength accuracy was determined using a combination of mercury-argon and krypton line sources. Photometric accuracy was determined using a series of neutral density filters. The system's noise was determined by measuring root mean square (RMS) noise of the transmittance. The results of this research will be presented in a two-prong approach: instrumental parameters and sample-dependent factors.

Frequently, inherent instrument-induced polarization effects are not realized by the untrained user. Spectral data collected on fibers and films demonstrate that instrument-induced polarization may result in erroneous interpretations, false exclusions, and false inclusions based on changes to spectral features such as bathochromic or hypsochromic peak shifts, and hyperchromic or hypochromic intensity shifts. The results will also demonstrate that sample preparation and specimen orientation are critical to obtaining high-quality (high signal-to-noise) data. Specimens are generally not flat or parallel materials so physical characteristics such as cross-section morphology, varying levels of pigmentation, variations in dye uptake, and degree of delustrant will all effect the quantitative transmittance level, the quality of the spectral data, and the degree of spectral consistency. The results will demonstrate that proper data processing (normalization) will minimize and control sample variations due to sample thickness (pathlength) and sample concentration variations in the specimen.

In conclusion, the proper use of the microscope is critical to obtaining reproducible, high-quality, high-resolution data which is essential for optimum spectral differentiation. When the operator systematically controls as many instrument parameters as possible, the quality of the spectral data is improved, which results in a higher signal-to-noise and better spectral discrimination. The goal of this presentationis that the guidelines that will be presented will be considered for adoption by SWGMAT, ENFSI, and other forensic science technical groups.

Microspectroscopy, Trace Evidence, Criminalistics

