

## B127 Infrared Spectroscopy for Characterization of Bloodstain Age Using the Amide Spectral Regions From Blood Proteins

Anthony R. Trimboli, BS\*, and Stephen L. Morgan, PhD, University of South Carolina, 631 Sumter Street, Department of Chemistry and Biochemistry, 631 Sumter Street, Columbia, SC 29208

After attending this presentation, attendees will better understand methods for the characterization of age of blood stains using attenuated total reflectance infrared spectroscopy (ATR/FTIR) will be discussed. In particular, the value of monitoring of the changes in the Amide III region spectra of blood for determining the age of a bloodstain will be demonstrated. Attendees will also learn how multivariate calibration methods can be used to model variations in spectral intensity as a function of aging.

The research described in this presentation will impact the forensic science community by establishing the scientific basis for a direct spectroscopic method of dating bloodstains discovered at crime scenes.

The research described in this presentation was designed to establish the scientific basis for a direct spectroscopic method of dating bloodstains discovered at crime scenes. The estimation of age of blood stains may provide leads for the future direction of forensic investigations. Rapid tools for estimation of blood stain age might also provide investigators the ability to determine the relevance of an item of trace evidence involving blood.

Methods to replace the currently used enhancement reagents and presumptive tests for blood (such as the use of luminol, phenolphthalein, and leucomalachite green) have been sought continuously. Likewise, methods for dating blood stains have been widely proposed as well. Visible light techniques for aging of blood stains by ratioing intensities from different spectral regions (Kind et al. 1972) laid the groundwork for future spectroscopic studies. Other methods such as immunoelectrophoresis (Rajamannar, 1977) and high performance liquid chromatography (Andrasko, 1997) have also been applied to the problem of dating blood stains. Most recently, electron spin resonance spectroscopy (Fujita, et al. 2005) and DNA analysis (Anderson, et al. 2005) have also been used to assign a date to a bloodstain.

The research concerns the use of bands in the IR region from around 1650 cm<sup>-1</sup> to 1200 cm<sup>-1</sup> that are relevant to the secondary structure of blood proteins. IR spectroscopy is fast, reliable and ATR sampling does not require extensive sample preparation. Varying concentrations of blood were subjected to ultraviolet and visible light for controlled time intervals in an artificial light box. FTIR absorbance spectra (128 scans, 4000 to 800 cm<sup>-1</sup> spectral range, 4 cm<sup>-1</sup> resolution) using the ATR diamond crystal in ambient atmosphere as a background. IR spectral data sets, including replicate spectra, were modeled for a training set of data as a function of the various aging intervals using multivariate calibration techniques (partial least squares). Finally, the spectral regions most relevant to the systematic changes occurring during the aging of blood stains were identified. The predictive accuracy of the calibration was evaluated by predicted the aging intervals for a separate test set of data of equal size to the training set.

Bloodstain Aging, FT-IR, Multivariate Calibration