

HYPSOCHROMIC SPECTRAL SHIFTS OF THE HEMOGLOBIN SORET BAND CORRELATE WITH THE TIME SINCE DEPOSITION (TSD) OF DRIED BLOODSTAINS

Erin Hanson^{1,2}, M.S., and Jack Ballantyne^{1,2,3}, Ph.D.

¹*National Center for Forensic Science, Orlando, FL*

²*Graduate Program in Biomolecular Science, University of Central Florida, Orlando, FL*

³*University of Central Florida, Department of Chemistry, Orlando, FL*

The development of powerful and robust DNA typing strategies has made it possible to ascertain with a high degree of certainty whether a biological stain found at a crime scene originated from a particular individual. New methods are under development to allow for the determination of an individual's physical characteristics including eye color, hair color, skin pigmentation, height, weight, and relative age. Such a "genetic eyewitness," is not constrained by human recollection or subjective accounts and could provide an unbiased genetic estimator of the perpetrator's appearance, whether or not eyewitnesses are available. However, even with the wealth of new information being obtained from biological stains, the possibility of obtaining additional information from biological stains exists. For example, the ability to determine the relative time since deposition (TSD) of biological stains could provide law enforcement investigators with novel probative evidence by establishing an approximation of the time of commission of criminal offenses. However, no reliable TSD methods are available at present. The fundamental assumption of this project is that biochemical reactions still occur in the dried state and as dried stains age, damage and degradation to macromolecules such as DNA, RNA and protein occur. We have investigated whether macromolecular degradation intermediates can serve as molecular clocks for time since deposition (TSD) estimation.

We have developed a novel strategy for the determination of the time since deposition of dried bloodstains using spectrophotometric analysis of hemoglobin. Changes in the characteristic α and β bands (~540 and 576nm respectively) of the spectral profile of hemoglobin have previously been observed in aged bloodstains. However, the methodologies that utilize changes to these bands failed to gain widespread acceptance due to poor analytical sensitivity (large sample consumption) and inadequate resolution between stain ages. An examination of the Soret band in aged bloodstains has revealed a hypsochromic shift as the age of the stain increases which has not been previously identified. The extent of this shift permits a distinction to be made between stains that were deposited minutes, hours, days and months prior to analysis. This method therefore provides a higher resolution than any previously developed TSD methodology. The sensitivity of this method has been determined and as little as 20-40 nanoliters of blood can be used from bloodstains as small as 0.5 μ l. The effects of temperature and humidity have also been evaluated. Additionally, the ability to determine the time since deposition through analysis of the Soret band hypsochromic shift using a portable spectrophotometer has also been demonstrated.