Application note

Forensic Blood Analysis

New Approach to Determine Time since Deposition of Blood at Crime Scenes

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Abstract

So far, there is no generally accepted procedure available that enables forensic scientists to determine the age of blood stains at a crime scene. Present methods are either limited in their predictive power or their analytical sensitivity.
In a recent study, by the National Center for Forensic Science at the University of Central Florida, a previously unidentified wavelength effect which shows a significant relationship to the lifetime of a blood stain has been discovered. The degree of this wavelength effect allows for more accurate determination of the time since the blood was deposited and it is now possible to differentiate between stains that were deposited minutes, hours, days and months ago. Using the NanoPhotometer® P-Class from Implen the scientists at the National Center for Forensic Science established that tiny bloodstains of only 1 µl could be used for this investigation. One of the benefits of the NanoPhotometer® P-Class is that it is portable and therefore may be taken to the crime scene to first of all confirm that the stain was blood and then to determine how long it had been there. The instrument is perfectly optimized for this forensic application as it may operate from a 12 V DC supply if required, has no moving parts for in-field reliability, does not require regular servicing or calibration and requires very little operator training plus of course it provides high performance on low sample volumes (Figure 1).

Figure 1: The NanoPhotometer® P-Class incorporates a simple menu driven user interface. The system is operational within 30 seconds and a typical analysis time is 5 seconds.

Methodology and technical requirements

After the blood samples have been obtained they were deposited on cloth and stored for different times and at different temperatures to simulate normal environmental conditions. The blood was then extracted with Tris-HCl, pH 8.0, spun and then stored at – 20 °C until required. The principle on which this work is based is that, as the blood ages, chemical reactions changing the spectral profiles of the blood samples still occur.
The spectral profiles of the representative blood samples were obtained with the NanoPhotometer® P-Class using 0.5 -3 µl of the extracted solution placed directly onto the LabelGuard™ analytical cell (Figure 2).

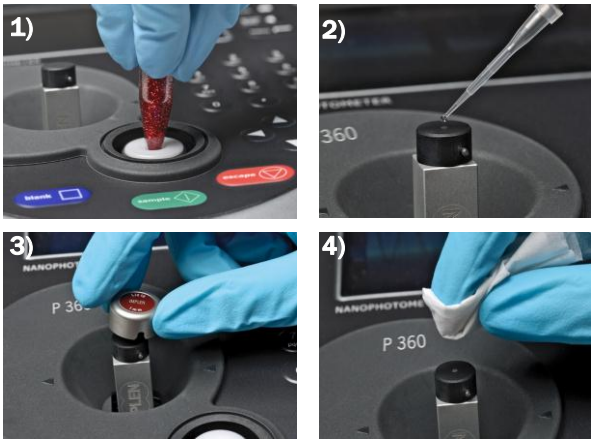

Figure 2: Analyzing the blood sample
1) Mix sample 2) Apply blood sample directly onto the centre of the measuring window. 3) Automatic sample dilution.
4) Quick and easy cleaning after the measurement.

The reproducibility of this technical design is excellent due to the fixed pathlength mechanism and the fully enclosed sample area to prevent evaporation or contamination. As the sample is compressed, between the cell and the lid, surface tension effects due to solvents are overcome and will not affect the results.
In addition to the blood analysis application the NanoPhotometer® P-Class is also an all-rounder for various spectrophotometrical applications in a modern laboratory. For example DNA/RNA specific applications are widely used in forensic laboratories when accurate quantification of concentration is required.

Based on the available methods for single/multiple wavelength and concentration measurements, full spectrum scans, standard curve determinations as well as ratio calculations and even kinetics, the user may generate customized applications for their individual needs. Predefined methods and functions are available for nucleic acid analysis, determination of dye labeling efficiency, protein quantification and cell density measurements.

Results and conclusion

Researchers at the National Center for Forensic Science at the University of Central Florida have investigated age related changes of the haemoglobin spectrum. Five different parameters were found to show age related changes: 1) changes in the maximum absorbance of the Soret band (ΔAbsSoret); 2) changes in the wavelength of the λmax for the Soret band (ΔλmaxSoret); 3) changes in the relative absorbance of the β band at 541 nm, compared to the λmin at 560 nm (ΔAbsβ(541–560)); 4) changes in the relative absorbance of the α band at 576 nm, compared to the λmin at 560 nm (ΔAbs α(576–560)); 5) the ratio of absorbance changes of the α and β bands (Figure 3).

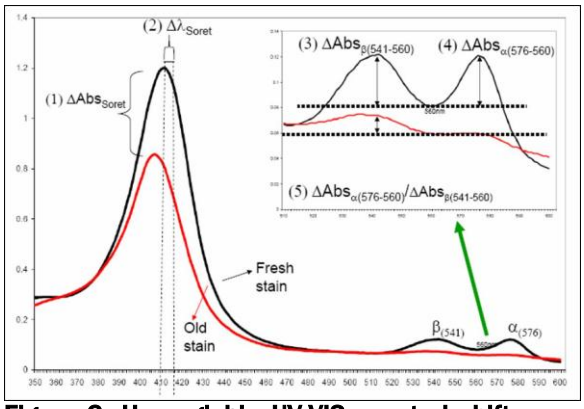

Figure 3: Hemoglobin UV-VIS spectral shift parameters. UV-VIS spectral profiles from bloodstains stored at room temperature for 15 minutes (black line) and 1 year (red line) were compared in order to identify potential differences between "fresh" and "old" stains. The putative time-dependent parameters (1–5) involving changes in the Soret band and α and β peaks are indicated (1–5).
(Source: Jack Ballantyne at University of Central Florida)

Graphing this Soret band wavelength shift as a function of stain age for bloodstains indicated a strong positive correlation so that it was possible to distinguish the deposition time of blood samples between months, days, hours and even minutes. When used in conjunction with the Implen NanoPhotometer® P-Class it may be possible to perform this analysis at the actual crime scene, first to confirm the presence of a bloodstain (through a characteristic UV-VIS spectral profile) and also to determine the time since deposition of that bloodstain using only microliter volumes of blood.

Reference:

Hanson EK, Ballantyne J (2010), A Blue Spectral Shift of the Hemoglobin Soret Band Correlates with the Age (Time Since Deposition) of Dried Bloodstains. PLoS One. 2010 Sep 20;5(9):e12830.
www.ncbi.nlm.nih.gov/pmc/articles/PMC2942901/?tool=pubmed

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