## Comparative analysis of hyperspectral and near-infrared

## spectroscopy for bloodstain deposition time estimation

## **Supplementary information**

In this study, cement board, gypsum board, and iron plate were used as substrates, as they are commonly found at crime scenes. Samples with permeable (cement board, gypsum board) and non-permeable (iron plate) substrates were used. Permeable materials cause bloodstains to dry faster, influencing the spectral characteristics and accelerating the aging process of bloodstains.Non-permeable materials do not absorb blood, causing the bloodstains to remain more on the surface, leading to slower evaporation and a more gradual aging and degradation process.The use of these two types of materials allows for a comprehensive analysis of bloodstain deposition characteristics on different substrates and their effects on bloodstain deposition time.Blood samples were collected from two volunteers at the affiliated hospital of Yunnan Police College at 10:30 am on July 12, 2023. The volunteers included a healthy 35-year-old male and a healthy 35-year-old female, both without diabetes, hypertension, or other blood diseases. During the collection, 10 ml of venous blood was collected from each volunteer using 2 ml vacuum tubes.

At 10:30 on the same day, blood was evenly applied to each substrate, with six bloodstains applied per substrate, labeled as Sample 1 to Sample 6, and each bloodstain with a thickness of 0.5 mm. According to the scale shown in the figure, each bloodstain had a diameter of 3.0 cm and an area of 4.71 cm<sup>2</sup>. The prepared samples were placed in an environment with a temperature of 24°C and humidity of 34% (both maintained until the end of the experiment). Spectral data collection was conducted at 11:18 am.

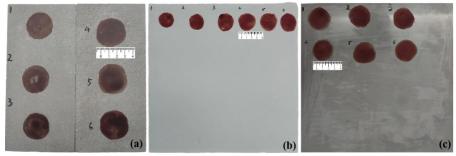


Fig S1. Bloodstains samples on different substrates, where (a) is a cement board, (b) is a gypsum board, and (c) is an iron board.

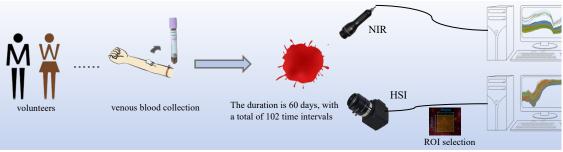


Fig S2. Diagram of the main experimental steps.

The spectra data collected by the NIR were exported using the MicroNIR\_1\_5\_7 software, while the HSI data were exported using ENVI software. The regions of interest (ROI) in the HSI data were manually set around the target area using the ROI function in ENVI, and the average spectra within the ROI were calculated. The ROI size was  $240 \times 240$  pixels, and the ROI was selected from the central area of the spectral image. Fig 2 shows the selection of the ROI in the HSI bloodstains samples: a is an example on cement board, b is an example on gypsum board, c is an example on iron board, and d shows the pixel size of the ROI.

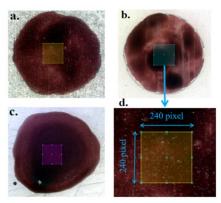


Fig S3. Selection of regions of interest (ROI) in HSI bloodstains samples, where a. is cement board, b is gypsum board, c is iron board, d is the example of ROI selection area.

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Data	Material	preprocessing	Latent variable	RMSEC	RMSEP	<b>R</b> <sup>2</sup>	RMSECV	Bias
HSI	permeation		13	7.54	8.95	0.76	0.19	0.08
	impermeable	SNV	10	7.13	8.46	0.78	0.15	0.09
NIR	permeation		13	7.05	8.01	0.79	0.17	0.07
	impermeable		10	6.94	7.72	0.81	0.14	0.06

Table S1. Summary of the figures of merit for the PLS regression model.