

## Supporting Information

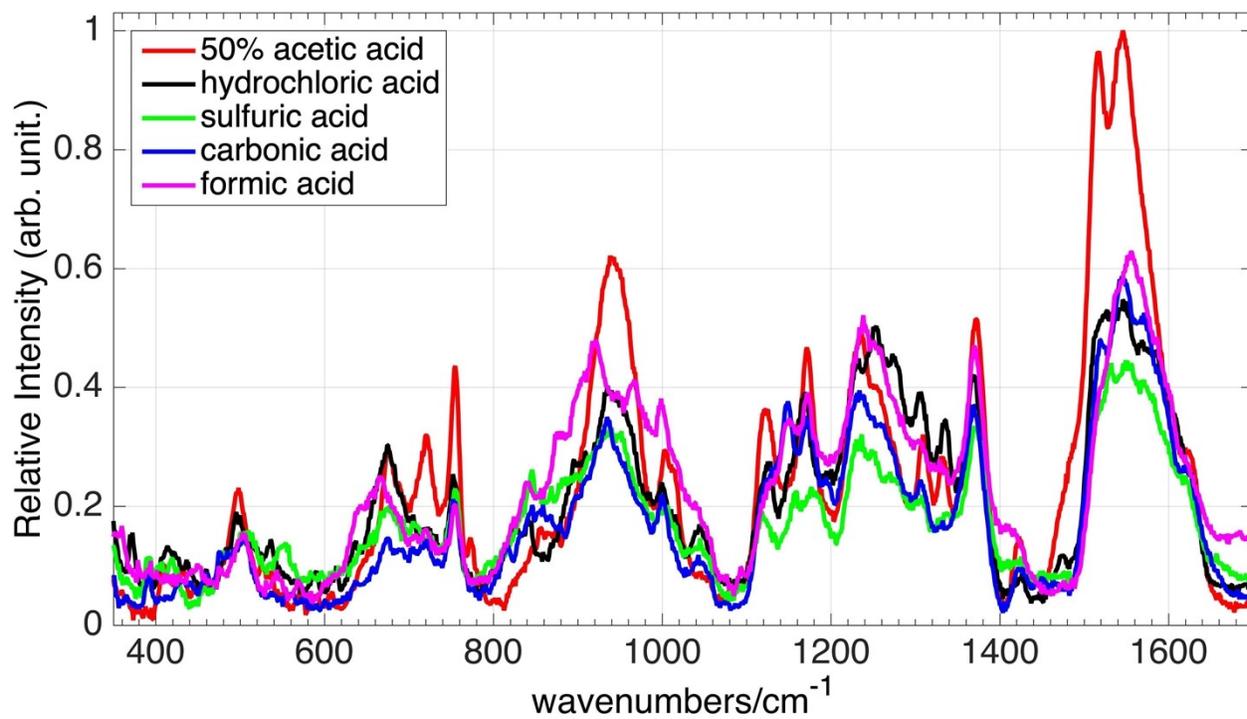
### A Surface Enhanced Raman Scattering Protocol for Robust Detection and Confirmatory Identification of Bloodstains

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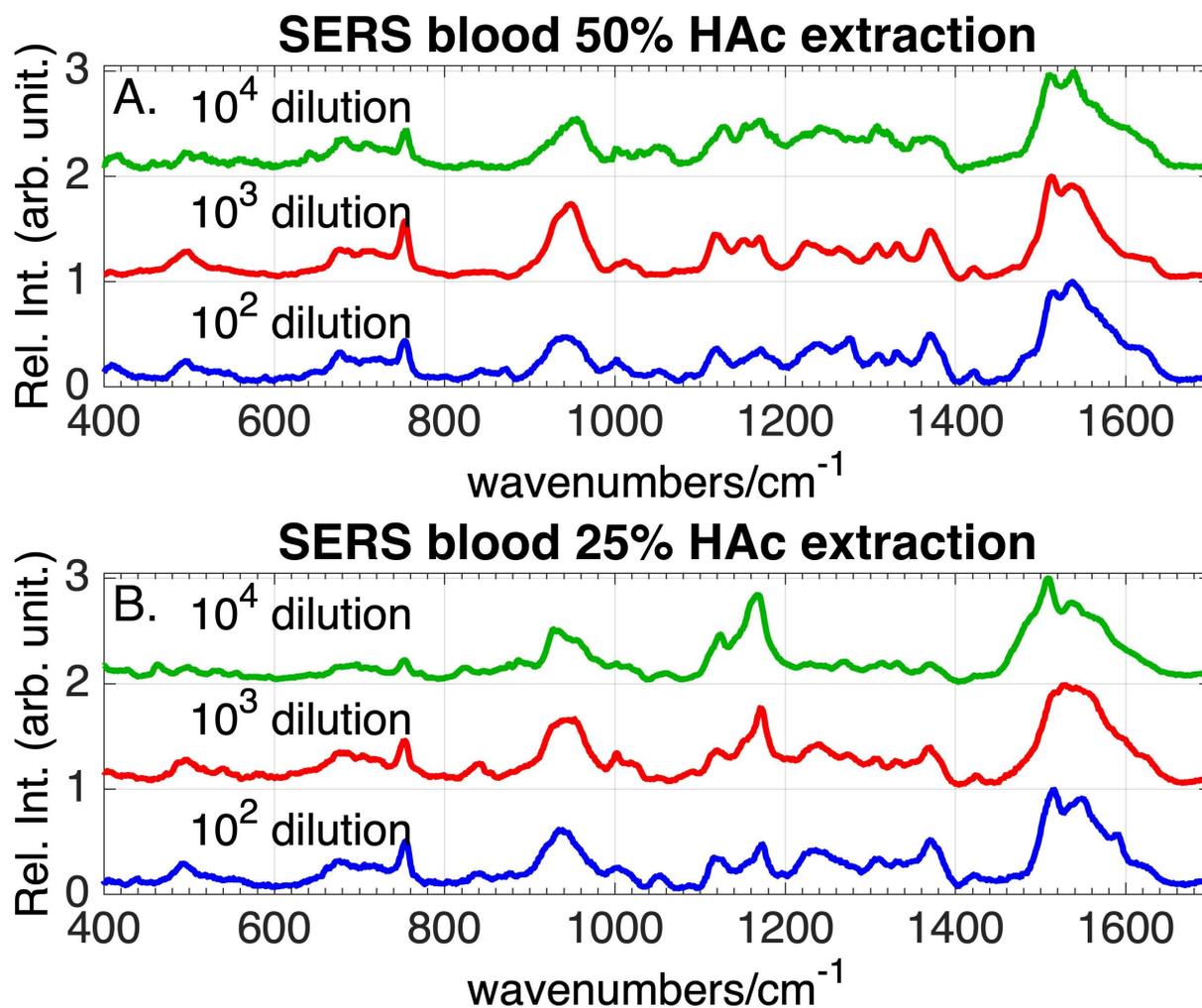
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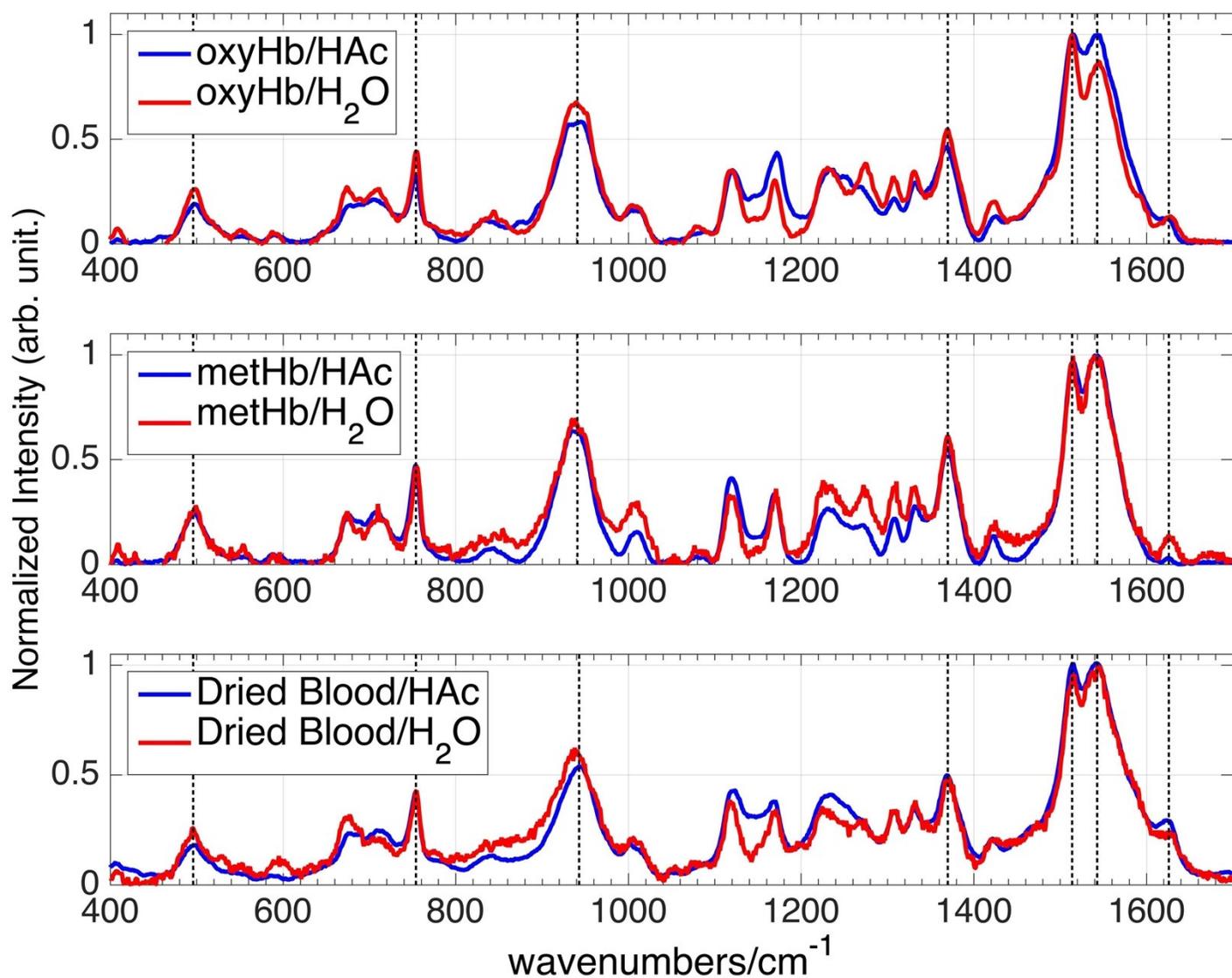


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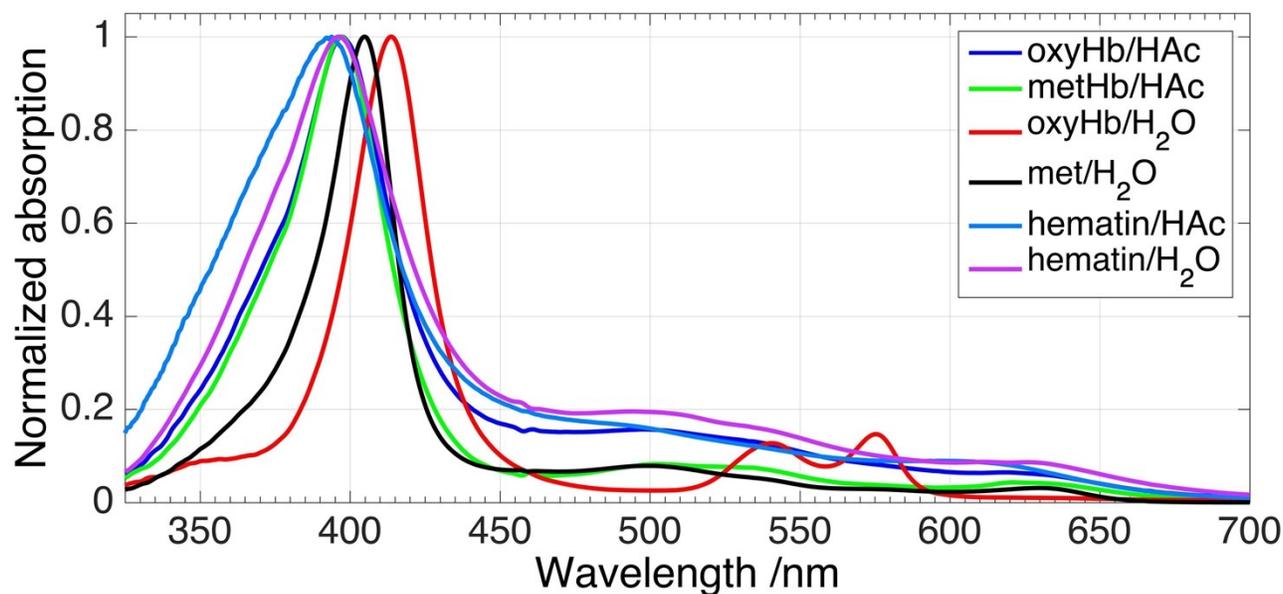
**Figure S1.** SERS spectra of dried blood on Au as a function of the acid used for the 1  $\mu\text{L}$  extraction procedure. Acetic acid (HAc) results in the most intense SERS feature and a SERS spectrum that matches that of RBCs and hemoglobin in water.



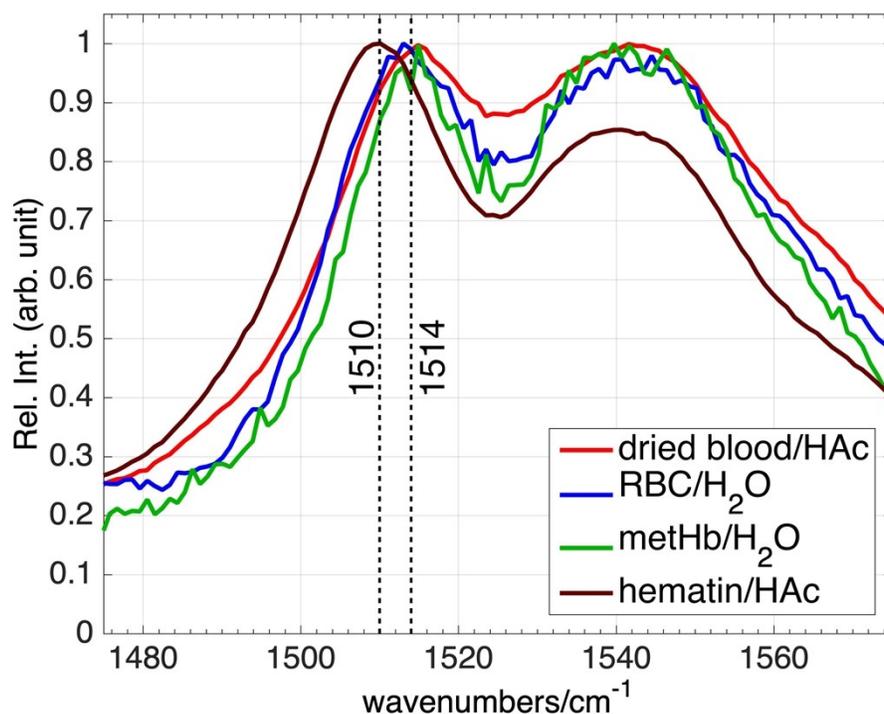
**Figure S2.** SERS spectra of dried diluted blood demonstrated superior performance, in terms of reproducible vibrational spectra due to (A.) 50% HAc (RHS), and (B.) 25% HAc, are compared. As seen above, a more consistently reproducible SERS spectrum for the dried stains of diluted blood is observed for the 50% HAc extraction procedure.



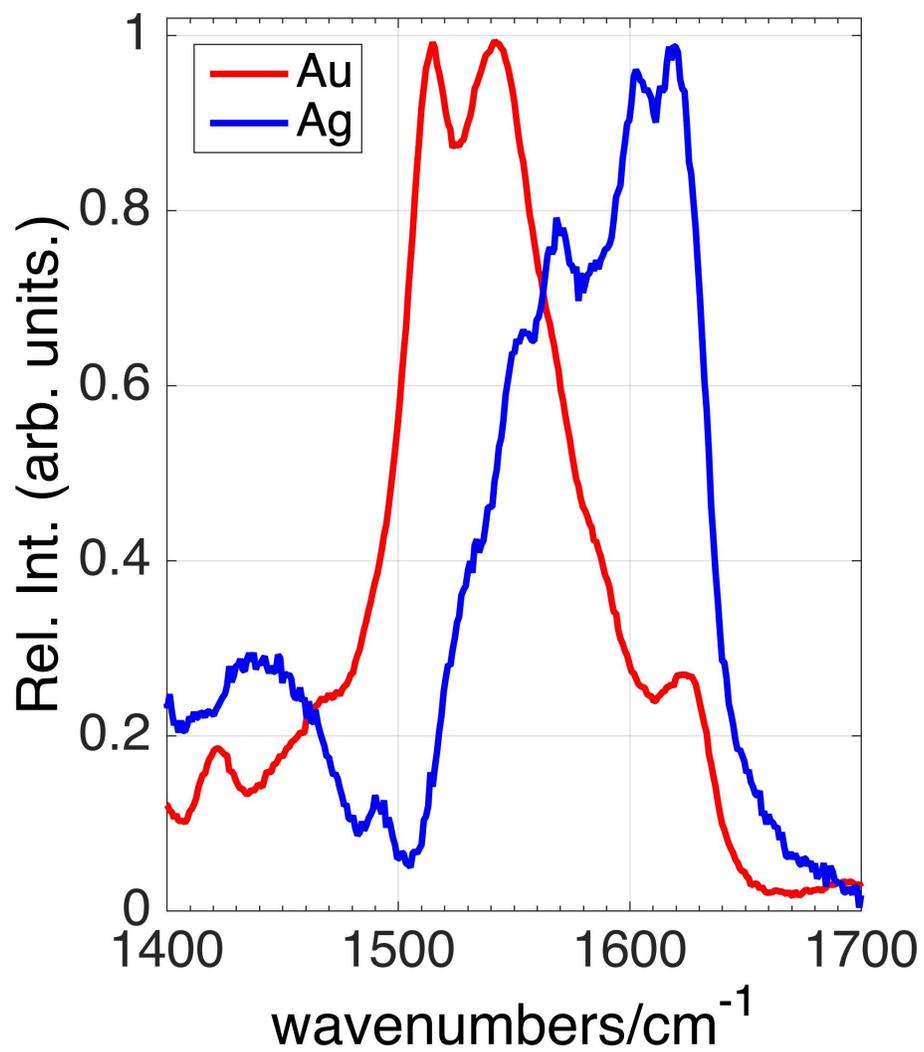
**Figure S3.** SERS spectra of dried blood, metHb and oxyHb in H<sub>2</sub>O and 50% acetic acid (HAc). The dried blood in water sample was prepared by taking a small piece of dried blood (> 24 hours) and sonicated to break up and help dissolve in H<sub>2</sub>O before being deposited on the Au SERS substrate.



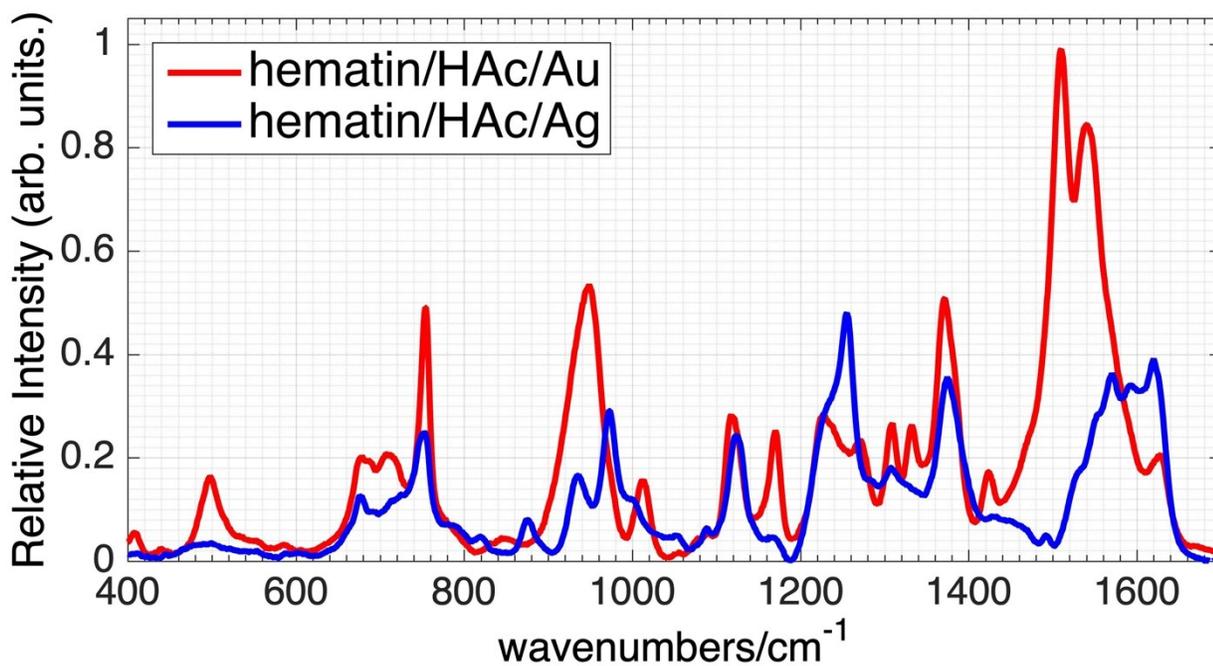
**Figure S4.** Normalized uv-vis absorption spectra of oxyHb, metHb and hematin in water and 50% acetic acid (HAc) solutions.



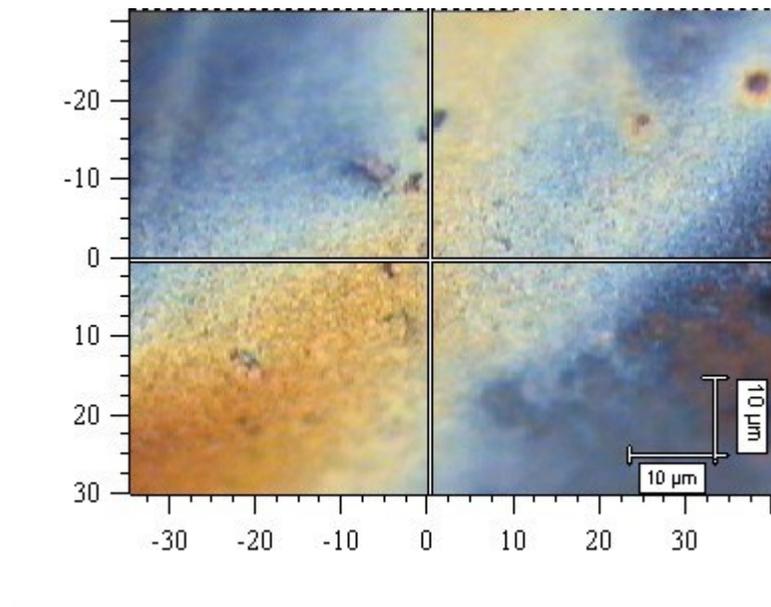
**Figure S5.** 785 nm excited SERS spectra of dried blood extracted by 50% HAc, RBC and metHb in water, and hematin (Fe(III) PPIX OH) in 50% acetic acid. Only the hematin spectrum shows a small but consistent ( $\sim 4$   $\text{cm}^{-1}$ ) red shift of the  $\nu_{38}$  band relative to the RBC, metHb and dried blood spectra. This difference is attributed to the globin complexation which is lacking in the hematin.



**Figure S6.** Comparison of 785 nm SERS spectra of 24 hour dried blood extracted by the 50% acetic acid procedure in the 1400 – 1700 cm<sup>-1</sup> region on Au and Ag substrates. Spectra are normalized by the maximum intensity in this region.



**Figure S7.** Comparison of 785 nm SERS spectra of hematin in 50% HAc on Au and Ag substrates. The differences observed in these Au and Ag spectra are nearly identical to the differences observed for the SERS spectra of dried blood extracted by 50% HAc procedure on Au and Ag substrates. (Figure 3 in the text.)



**Figure S8.** Image of dried bloodstain extracted by 50% acetic acid and placed on SERS substrate. No RBCs are in the region of the laser focus centered at the origin.