Investigating the color of the blood stains on archaeological cloths: the case of the Shroud of Turin

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The unique reddish blood stains on the archaeological cloth known as the Shroud of Turin caught the attention of several scholars, who proposed different hypotheses to explain the unusual blood color. To date, just few hypotheses have been tested experimentally, and the results are debatable. In this paper we test the strength of two hypotheses (namely, the presence of carboxyhemoglobin and the long term influence of ultraviolet light on high-bilirubin blood) by the spectral reflectance of the blood stain regions on the Shroud and by color analyses of ultraviolet irradiated high-bilirubin blood stains on linen. The relevance of these simple methods to the study of stained textiles is discussed.

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The Shroud of Turin is an ancient cloth of linen 4.4 m long and 1.1 m wide. The linen bears faint front and dorsal images of a full-sized bearded man apparently laid out in death as if the image had been formed while the cloth was longitudinally folded over a human body.

On the Shroud there are also several reddish stains giving the appearance of blood stains. They are placed at various locations over the body image including the wrist, the feet, around the head and on the back.

The Shroud is considered one of the most studied archaeological objects in history [1, 2, 3] and independent forensic analyses confirmed that blood stains on it are indeed real blood as they contain specific blood components, including hemoglobin, immunoglobulin, bilirubin, and serum albumin [4, 5, 6]. A recent review of the main scientific data available on the Shroud can be found in [7].

1. *ODD COLOR OF THE BLOOD STAINS ON THE SHROUD OF TURIN*

The reddish color of blood stains is an amazing feature of the Shroud, as dried blood turns dark brown due to oxidation of oxy-hemoglobin to met-hemoglobin and hemichrome. Various hypotheses were proposed to explain the odd color of the Shroud blood stains. Table 1 summarizes the main proposals and existing data.

Table 1. Summary of the main proposals and attempts to explain the reddish color of blood marks on the Shroud.

POSSIBLE CAUSE OF THE REDDISH COLOR OF THE BLOOD STAINS ON THE SHROUD	*PROPOSED BY*	*EXPERIMENTAL DATA*
Pigments (red ochre, vermillions, alizarin)	Mc Crone [8]	Mc Crone (1980) [8]. Analysis of sub-micron particles from adhesive-tapes. Data available.
Saponaria Officinalis	Rogers, Arnoldi [9]	Soran (1977). Blood stains+saponaria claimed still reddish after 25 years. Data and pics not available.
Free bilirubin bound with albumin	Adler [10]	Goldoni et al. (2000) [11] Svensson (2010) [12]. Negative results with both free and non-free bilirubin. Data and pics available.
Bilirubin + UV	Goldoni et al. [11]	Goldoni et al. (2000) [11] Positive results. Pic available. Data not available.
Carboxyhemoglobin	Baima Bollone et al. [13]	Kearse (2017) [14]. Negative results on bloodstains 12 days old. Data and pics available
Pigments/dyes added to blood	Van der Hoeven [15]	Fanti, Zagotto (2017) [16]. Analysis of sub-micrometer particles from encrusted linen fibers and dust. Data available.

Let us comment on each proposal in Table 1.

Mc Crone in [8] reported few iron oxide particles, micrometer-size cinnabar and small traces of vermilion (HgS) observed in adhesive-tape samples taken from the surface of the Shroud by the team of the Shroud of Turin Research Project (STuRP) in 1978.
Mc Crone deduced that blood stains and body images were painted. However, there is a large body of scientific evidence [2–4, 7, 9, 10, 17–20] that the materials reported in [8] cannot be construed as evidence that the Shroud image and blood stains are painted. In particular, the presence of these microscopic debris particles was attributed to the historically recorded practice of sanctifying copies by pressing them on the Shroud [21], as it happened on the 4th May of almost every year across the XVII century [22, 23], thus providing unwitting transfer of debris from the painted copies to the Shroud.

Concerning Saponaria officinalis, Rogers and Arnoldi in [9] wrote: "The warp of ancient linen was protected with starch during weaving and the finished cloth was washed in Saponaria officinalis suds. Saponaria is hemolytic, which could explain why the old blood stains on the cloth are still red. Diane Soran (deceased) of Los Alamos, tested hemolysis on Saponaria-washed cloth before we went to Turin. The blood is still red on those 25-year-old samples. Controls are black." Unfortunately, experimental data and photographs are not available and the claimed redness of the old blood stains due to Saponaria officinalis cannot be confirmed.

Concerning free bilirubin bound with albumin, Adler noted in [10] that "As a consequence of traumatic wounds, as in case of flagellation and of crucifixion, red blood cells break and the released hemoglobin forms aggregates with haptoglobulin (…) and is also degraded by hepatic enzymes that convert them in bilirubin. Therefore, the bilirubin binds the proteic complexes, mainly with albumin, taking a yellow-orange color." Goldoni et al. [11] tested the hypothesis of Adler using artificial bilirubin, with negative results. Independently, Svensson [12] obtained negative results using high-bilirubin blood from a patient with jaundice. However, the high level of bilirubin due to hepatitis is mainly composed of conjugated bilirubin, while in the Adler' hypothesis the main part of bilirubin might be free bilirubin.

After testing Adler's hypothesis, Goldoni et al. argued something else could be involved. They proposed a photochemical modification of bilirubin by ultraviolet (UV) radiation and tested this hypothesis by UV lamp irradiation of several bilirubin titers artificially created by chemical reactions. They obtained 'positive by eye' results, showing a single picture without any numerical/spectral/colorimetric data [11]. As a consequence, the hypothesis of Goldoni needs to be validated by more accurate and quantitative data.

Baima Bollone et al. [13] detailed how endogenous carbon monoxide (CO) generated during the breakdown of red blood cells, might bind to hemoglobin creating a stable complex carboxyhemoglobin which could be responsible for the reddish appearance of the blood stains. Independently of our experiments described in the following, recently Kearse evaluated the effect of CO exposure on the color of fresh blood stains [14], showing that CO treatment enhanced the redness of fresh blood stain but the effect does not persist over 12 days when samples are exposed to air.

Van der Hoeven in [15] presented data elaborations suggesting that the Shroud blood stains, originally made by blood, mixed with dyes and/or pigments applied to the linen. Recently, this hypothesis was partially supported by the analyses of small crusts from Shroud fibers on adhesive-tapes taken in 1978 [16] and authors suggested that the blood stains may have been touched up at some point during the Shroud's history. Due the microscopic size of material analyzed, however, the result in [16] cannot be extrapolated to the sum of all of the blood stains present on the Shroud. This result is indeed compatible with the hypothesis of particle transfer from the copies sanctified by contact [21–23]. It is worth mentioning that both dye and blood-related materials were found by spectroscopic analyses of the artistic blood stains on the "shroud of Arquata", a copy of the Shroud (1653 AD) whose body figures were made without any drawings or dyes [24].

Clearly, the hypotheses listed in Table 1 are not compatible with each other, and it could be helpful to test experimentally their strength. In our laboratories there are instruments suitable to test the validity of the proposals by Goldoni [11] and by Baima Bollone [13], as described in the following.

2. MATERIAL AND METHODS

In order to test the validity of the Goldoni's proposal [11] we designed the following experiments:

a) UV irradiation of both normal- and high-bilirubin blood stains on linen.

b) Use of three UV doses, namely 24 J/cm^2, 16 J/cm^2 and 11.1 J/cm^2 respectively equal to those allowing 1) a coloration of linen, 2) a latent coloration of linen, and 3) below threshold for linen coloration.

c) Two control blood samples, one with normal bilirubin irradiated, the other with high-bilirubin, not irradiated.

d) Perform colorimetric analysis immediately after irradiations and 4 years later, seeking for long term effects, if any.

The high-bilirubin blood was from a Caucasian male, 45 years old, diagnosed jaundice by hepatitis HBV+. Blood analyses gave the following results: Total Bilirubin: 8.8 mg/dl (normal range (n.r.) 0.3–1.1 mg/dl); Conjugated Bilirubin: 7.7 mg/dl (n.r. 0.1–0.3 mg/dl); Hemoglobin: 15.2 g/dl (n.r. 13.5–18 g/dl); Albumin: 3.4 g/dl (n.r. 3.5–5 g/dl).

We put 17 high bilirubin blood stains and 2 normal blood stains on several pieces of linen cut from a never used and never washed cloth woven in 1950. We have previously shown [25] the absolute reflectance of this linen cloth matches that of the Shroud in the UV range.

We have shown in [25, 26] that pulsed UV and VUV laser irradiation generates a linen coloration only when the radiation dose is above a given value. That is, the photo-chemical process of linen coloration is a threshold effect. Namely, we obtained a Shroud-like, superficial linen coloration when the total laser fluence was above 22 J/cm^2 and the laser pulsewidth shorter than 50 ns. Here the total fluence is defined as

$$F_T = (N/A) \times \iint_s F(x,y)\, dxdy, \qquad (1)$$

where N = number of laser shots, A = area of the linen irradiated by the laser and F (x, y) = fluence at point x, y of the transverse area s of the laser beam.

Figure 1 shows a high-bilirubin blood stain before and after the irradiation by Lambda Physik LPX-305 excimer laser pulses (308 nm, 0.4 J/pulse, 30 ns) focused by a lens to deliver a total fluence F_T = 24 J/cm^2, which is above threshold for linen coloration. The irradiated area shows vaporization and ablation of the blood stain. All 3 irradiated blood stains were damaged.

Fig. 1. Left: close-up view of a high-bilirubin blood stain before irradiation. Right: the same blood stain after laser irradiation of its upper half with a UV dose above threshold for Shroud-like linen coloration. The irradiated area of the blood was ablated by UV radiation.

As detailed in [25, 26] we generated a latent coloration of the linen, which becomes visible after artificial or natural aging of linen, following laser irradiations that at first did not generate any visible effect. Here we irradiated 4 high-bilirubin blood stains and one normal blood stain by LPX-305 excimer laser pulses focused to have a F_T = 16 J/cm^2 which is below threshold for linen coloration but sufficient to generate a latent coloration. All blood stains appear not damaged after irradiation. Figure 2 shows a typical undamaged blood stain after the irradiation.

Finally, we have irradiated 5 high-bilirubin blood stains and one normal blood stain by a blacklamp (Sylvania bulb, spectrum of emission 340 nm - 390 nm peaked at 364 nm, 17.1 nm FWHM measured by the spectrometer Hamamatsu mod. C10082CAH). At 6.5 cm distance from the lamp, the UV intensity on blood stains measured by an absolute UV photodiode Newport 818-UV/DB connected to a power meter 843-R was 1.54 mW/cm^2. After two hours the F_T (see eq. (1)) was 11.1 J/cm^2, that is, 69% the threshold value for latent coloration of linen [25, 26]. Figure 3 shows one of the lamp-irradiated blood stain.

The remaining 5 high-bilirubin blood stains and one normal blood stain were not irradiated.

Using a camera Fujifilm FinePix S1600 equipped with a lens Fujinon 28-420 mm we photographed all blood stains at the same time and under the same lighting conditions. We performed a simplified red, green, blue (RGB) color analysis by three steps:

1) Calibration of RGB values on a reference white.

2) Selection of the same elliptic surface inside each blood stain, major axis 151 pixels, minor axis 124 pixels, for a total of 14,700 pixels, paying attention to avoid zones which are non homogeneous in color.

3) Use of the histogram function available in the software Corel PaintShop Pro to obtain the average values for R, G, and B of the elliptic area inside each blood stain.

Fig. 2. High-bilirubin blood stain after laser irradiation with a UV dose below threshold for linen coloration but sufficient to generate a latent coloration. The blood stain appears undamaged.

Fig. 3. High bilirubin blood stain just after lamp irradiation with a UV dose below threshold for both linen coloration and latent coloration. The blood stain appears undamaged.

In addition, the histogram function provides hue (H), saturation (S) and lightness (L) values in the range (0 – 255). HSL is a representation of the RGB color model, designed to more closely align with the way human vision perceives color-making attributes. Table 2 shows HSL average values, referred to the linen, just after UV irradiations and 4 years later.

Table 2. Hue, saturation and lightness of linens photographed just after irradiations and 4 years later. Values are averaged over 5 linen sampling. Each error is the data dispersion within one standard deviation of the mean.

	2013	2017
Hue	166.6 ± 3.1	165.2 ± 3.2
Saturation	8.1 ± 0.9	8.7 ± 0.5
Lightness	167.6 ± 2.6	169.6± 1.7

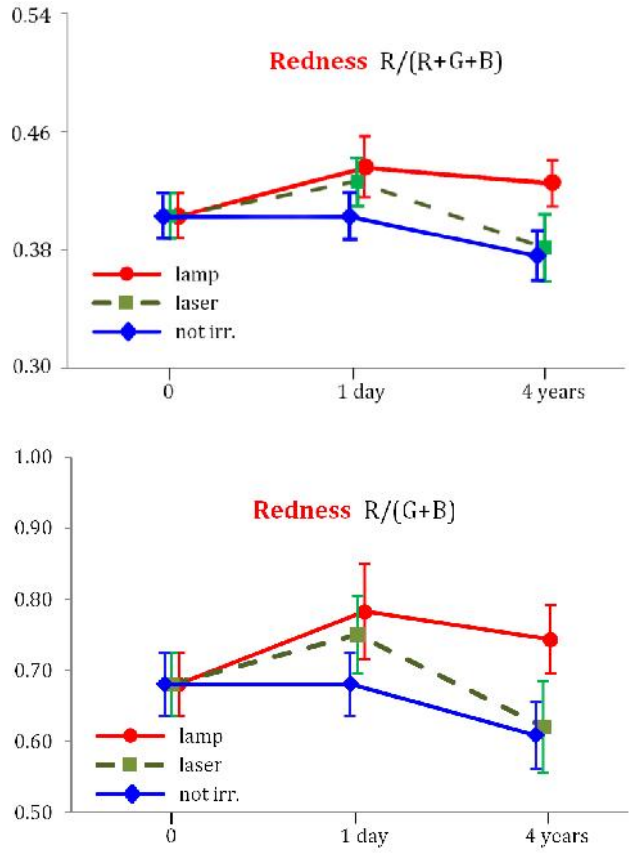
The data in Table 2 show that the average HSL values did not change considerably in 4 years, confirming that all linen samples were illuminated and photographed in the same setting and lighting conditions.

After calculating the average values of R, G and B in each irradiation condition we have considered two parameters for estimating the reddish color of the blood stains, namely R/(R+G+B) and R/(G+B) and two parameters for estimating the yellowish color of the blood stains, (R+G)/(R+G+B) and (R+G)/B. Figure 4 shows the plots of the four parameters at three times: before irradiation, one day after irradiation and four years after irradiation. Due to the ablative effect visible in fig. 1, we did not perform color analyses of the 3 blood stains irradiated with a laser dose above threshold for linen coloration. Then, three groups of blood stains are reported in fig. 4: a) not irradiated, b) irradiated by laser pulses with a dose below threshold for linen coloration but sufficient to generate a latent coloration and c) irradiated by the lamp with a dose below threshold for both coloration and latent coloration.

Figure 4 does not show the data of the 2 blood stains with normal bilirubin (one of them irradiated) because their redness and yellowish parameters match the corresponding values of the high-bilirubin blood stains which were not irradiated.

The two redness parameters in fig. 4 provide the same trend. As the R/(G+B) parameter gives a larger relative variation compared to R/(R+G+B), we have chosen R/(G+B) to evaluate the quantitative redness variation of the blood stains in Table 3. The same holds for the yellowing parameter (R+G)/B.

Figure 4 and Table 3 show that 30-ns UV laser pulses irradiation produces a reddish shift of the color of high-bilirubin blood stains one day after the irradiation, but the redness enhancement does not persist after 4 years. On the contrary, continuous wave (cw) UV irradiation of high-bilirubin blood stains generates a color shift toward the red-yellow that persists over time as it is still visible and measurable after 4 years.



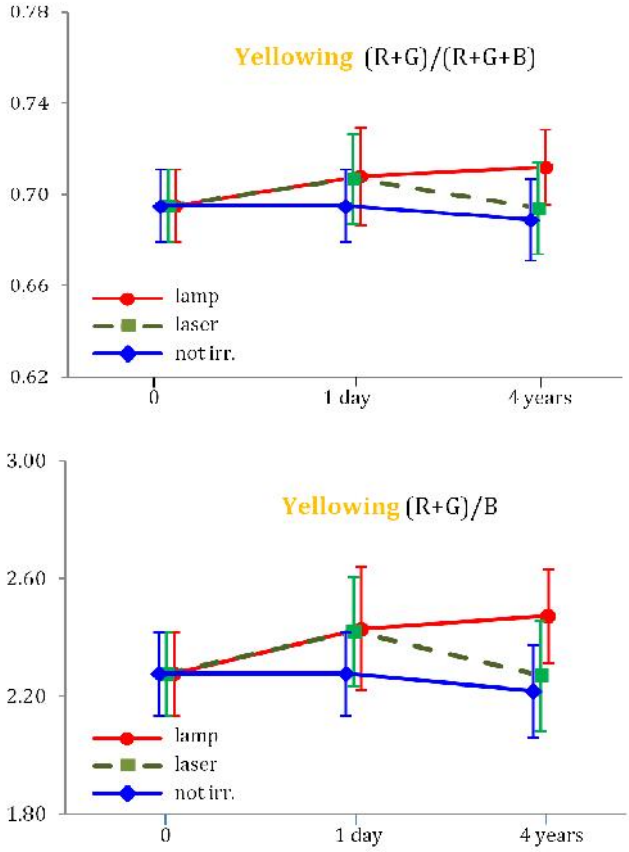


Fig. 4. Blood stains redness and yellowing –each calculated according with two distinct parameters– before irradiation, one day after irradiation and four years after irradiation. Diamond blue, circle red and square green respectively refer to high-bilirubin blood stains not irradiated, irradiated by lamp, irradiated by laser. Bars represent the data dispersion within one standard deviation of the mean. Redness and yellowing values of control blood stains are the same than those of high bilirubin not irradiated.

Table 3. Summary of the average values of redness R/(G+B) and yellowing (R+G)/B of high-bilirubin blood stains, and their variation with time and with respect to the non irradiated blood stains. Before irradiation, both redness and yellowish values were the same of those reported for not irradiated blood stains. Errors represent the data dispersion within one standard deviation of the mean.

REDNESS AND YELLOWING OF BLOOD STAINS	1 DAY AFTER IRRADIATION	4 YEARS AFTER IRRADIATION	VARIATION vs. time	VARIATION vs. not irradiated
Redness of not irradiated blood stains	0.68±0.04	0.61±0.05	- 10.3%	_
Redness of laser irradiated blood stains	0.76±0.05	0.62±0.06	-18.4%	+1.6%
Redness of lamp irradiated blood stains	0.78±0.07	0.74±0.05	-1.3%	+21.3%
Yellowish of not irradiated blood stains	2.28±0.14	2.22±0.16	-2.6%	_
Yellowish of laser irradiated blood stains	2.42±0.19	2.28±0.19	-5.8%	+2.7%
Yellowish of lamp irradiated blood stains	2.42±0.21	2.46±0.16	+1.7%	+10.8%

3. SPECTROSCOPIC EVIDENCE

On occasion of the last exhibition in 2015, we measured the spectrum of the reflectance R(λ) of the mean of four blood stained areas on the Shroud of Turin. This spectrum was used to design the illumination set-up based on a dedicated algorithm able to calculate the optimum lighting spectrum to perceive the low-contrast body images on the Shroud, while minimizing the potential photo-induced damage of light [27].

The spectral reflectance of the most relevant points on the Shroud in the configuration 45/0 (illuminating light incident at 45° with respect to the Shroud normal, and 0° observation along the normal of the Shroud) was measured using a PR650 Photoresearch spectrophotometer (range 380-780 nm, with 4 nm step) and a stabilized lighting source (CIE standard A). The measurements were performed with the Shroud in the vertical position like during the public exhibition. Being thick blood stains opaque in the spectral region between near UV and near infrared (transmittance T = 0), the spectral absorbance is given by A(λ) = 1 - R(λ), as shown in fig. 5.

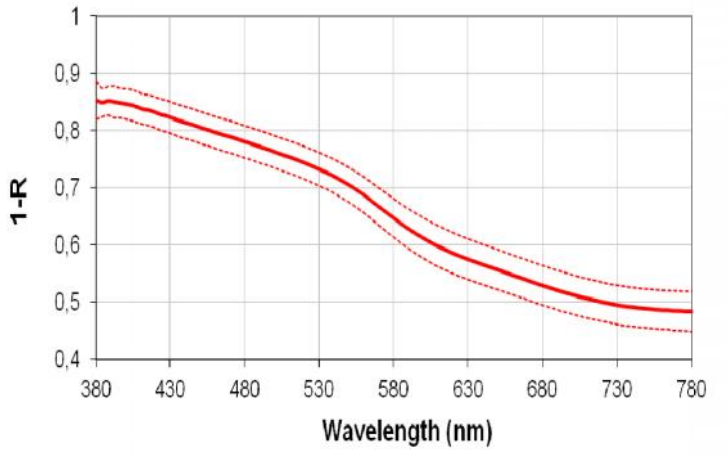


Fig. 5. Spectrum between the near UV and near infrared of the absorbance A(λ) = 1 - R(λ) of the mean of four blood stains on the Shroud of Turin. Dotted lines delimit the variance of the mean. Instrument bandpass is 4 nm.

The absorbance spectrum in fig. 5 shows an inflection at 560 nm ± 10 nm. Comparing fig. 5 with the spectral absorbance of human carboxyhemoglobin (HbCO) and met-hemoglobin (MetHb) reported in [28] we observe the inflection is compatible with the presence of MetHb. Let us remind MetHb is the result of the drying process of blood in air, as hemoglobin becomes deoxygenated and converts to MetHb. On the contrary, the spectral absorbance of HbCO, showing five inflections between 450 nm and 600 nm with the typical "double horn" shape peaked at 538 nm and 570 nm, see fig. 1 in [28], is entirely different from the monotonic "reversed S" absorption shape in fig. 5. As a consequence, the spectrum of the Shroud blood stains in fig. 5 is not compatible with the presence of HbCO proposed by Baima Bollone [13] as one of the main causes of their reddish color.

4. SUMMARY AND REMARKS

The reddish stains on the archaeological cloth known as the Shroud of Turin give the appearance of blood stains and independent forensic analyses confirmed that they contain specific blood components, including immunoglobulin, hemoglobin, bilirubin, and albumin, which are consistent with the presence of real blood [3–6].

Table 1 summarizes the main proposals and attempts to explain the unique reddish color of blood stains on the Shroud. Only a few of these proposals have been tested experimentally to date, and several results are debatable because data are not sufficient, or were obtained in vitro, or cannot be retrieved. We have tested the strength of two proposals, namely, the presence of HbCO [13] and the long term influence of UV light on high-bilirubin blood [11], by the study of the spectral reflectance of the blood stain regions on the Shroud and by RGB color analyses of UV irradiated high-bilirubin blood.

Concerning the long term influence of UV light on high-bilirubin blood, the results in figure 4 and in Table 3 show that irradiation of high-bilirubin blood stains by nanosecond UV laser pulses makes the blood stains slightly redder at the moment, but after 4 years the effect does not persist. On the contrary, cw UV irradiation of high-bilirubin blood stains generates a color shift toward the red-yellow still visible and measurable after 4 years. We observed that the average redness and yellowish parameters of control blood stains with normal bilirubin (one of them irradiated) match the corresponding values of high-bilirubin blood stains which were not irradiated. As a consequence, we may deduce that the redness enhancement is mainly due to the interaction between UV radiation and bilirubin, as postulated in [11].

The colorimetric results in Figure 4 and Table 3 give quantitative confirmation of the "positive by eye" picture framed by Goldoni after UV irradiation of a high-bilirubin stain on a linen cloth treated with aloe, myrrh and sweat, reported without any colorimetric/spectral data in [11]. By the way, our linen was not added by any material, and therefore aloe, myrrh and sweat do not seem to play any role in the reddish color shift of UV-irradiated high-bilirubin blood stains.

It may be interesting to check how long a sunlight exposure should last to attain the UV dose delivered by our lamp, assuming the UV spectrum of sunlight at sea level (bandwidth 300 nm–400 nm) has the same effect on high-bilirubin blood stains of the lamp we used (bandwidth 340 nm–390 nm) and neglecting the effects of visible and infrared sunlight. According to the absolute spectrum of the sunlight at the sea level in [29], in a clear day the Sun at the azimuth delivers a maximum UV intensity of 3.6 mW/cm^2. Then, a straightforward calculation

leads to 51 minutes of direct sunlight irradiation at normal incidence as the minimum time necessary to reach the UV dose of 11.1 J/cm^2 delivered by our lamp on blood stains.

Concerning the presence of HbCO, the reverse S shape of the spectral absorbance of the Shroud blood stains in fig. 5 is entirely different from the "double horn" shape of the spectral absorbance of HbCO (see e.g., fig. 1 in [28]). As a consequence, our results argue against the hypothesis that HbCO may account for the reddish color of the Shroud blood stains, as proposed in [13].

We have observed an inflection at 560 nm ± 10 nm in the spectral absorbance of the blood stains on the Shroud of Turin, see fig. 5. This inflection is partially visible in the 1978 spectral measurements made by STuRP [30], yet our spectrum shows a better sigmoid shape and is far more accurate than that by STuRP, due to intrinsic limits of the technology available in 1978. The observed spectral inflection is consistent with the presence of MetHb, therefore our spectroscopy results provide additional evidence that the blood stains on the Shroud contain aged hemoglobin degraded in MetHb as suggested by previous studies using different tools [4, 5, 17, 18, 31].

In our opinion, the clue of spectral inflection around 560 nm is promising in the studies of stains on archaeological textiles like, e.g., the Sudarium of Oviedo [32] as a non invasive, fast and simple check of the presence of aged blood. Moreover, specific models for the propagation of the radiation in inhomogeneous materials [24, 33–35] could provide information on the concentration of the compounds and blood related materials responsible for the visual appearance of stained archaeological textiles.

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