A DETAILED CRITICAL REVIEW OF THE CHEMICAL STUDIES ON THE TURIN SHROUD: FACTS AND INTERPRETATIONS

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INTRODUCTION:

In 1978, a team of scientific researchers (STURP: Shroud of Turin Research Project) was allowed for the first time to carry out a scientific comprehensive study of the Turin Shroud. Visual examination, macro and microphotographies, X-Ray radiographies; IR, visible and UV reflectance spectroscopy and photographs and UV-Vis fluorescence studies were conducted *in situ*.
32 surface samples (5 cm2 each) were obtained from specific locations using inert, non-reactive pure hydrocarbon sticky tapes for later examination.
The results of the studies were published in different peer-reviewed scientific journals in the following years.

In 1981, STURP officially concluded that: «*No pigments, paints, dyes or stains have been found on the fibrils. X-ray, fluorescence and microchemistry on the fibrils preclude the possibility of paint being used as a method for creating the image. Ultra Violet and infrared evaluation confirm these studies.(…) The scientific consensus is that the image was produced by something which resulted in oxidation, dehydration and conjugation of the polysaccharide structure of the microfibrils of the linen itself.(…) Thus, the answer to the question of how the image was produced or what produced the image remains, now, as it has in the past, a mystery. We can conclude for now that the Shroud image is that of a real human form of a scourged, crucified man. It is not the product of an artist. The blood stains are composed of hemoglobin and also give a positive test for serum albumin. The image is an ongoing mystery and until further chemical studies are made, perhaps by this group of scientists, or perhaps by some scientists in the future, the problem remains unsolved*".

In sharp contrast, Walter McCrone who also examined the samples, mainly by the mean of Polarized Light Microscopy, concluded that the Shroud was in fact a "beautiful painting" by a mediaeval artist who used red ochre as pigment in a collagen binder for the image and a larger amount of the same kind of pigment with vermilion (HgS) added to paint the "blood".

In 1988, the results of the radiocarbon dating of a single small piece of the Turin Shroud (age: 1260-1390 with 95% confidence) apparently demonstrated that McCrone was right.

In 2005, the late Raymond Rogers published in the peer-reviewed journal *Thermochimica Acta*[1] his personal studies on the chemistry of some linen threads obtained from the radiocarbon area compared with that of the main part of the Shroud and concluded: "*the radiocarbon sample was not part of the original cloth of the Shroud of Turin. The radiocarbon date was thus not valid for determining the true age of the Shroud*".

If Rogers is right (which will not be discussed here), the question to know if the Shroud is or not a medieval painting becomes again of highest interest.

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Too often, contradictory or vague statements are found in the Shroud's literature and it is difficult to gather and compare the original articles of the researchers.

The goal of this paper is to present and discuss as completely and objectively as possible the results of these chemical studies, mainly on the basis of almost all the original peer-reviewed articles and of a personal research in the scientific literature available on the Internet. Comments are presented as such.

I) MICROPHOTOGRAPHY:

1) Image areas

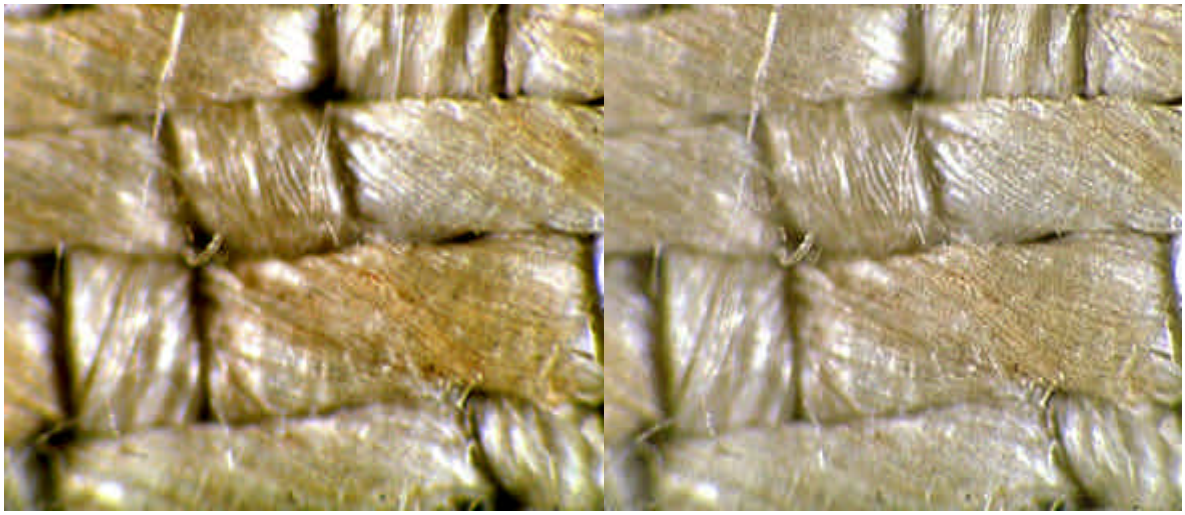


Image areas (Left: contrast enhanced). ME-29. 64× © 1978 Mark Evans

The **yellow** color forming the image is extremely **superficial without any sign of capillarity**: the colored fibers appear only on the topmost segments of the threads, the coloration extends only 2 or 3 fibers deep into the thread structure and does not appear under the crossing threads[2]. It is **discontinuous**: on a single exposed thread, one can find bundles of colored fibers adjacent to bundles of uncoloured fibers. The intensity of the color appears to be roughly the same at small scale: the macroscopic differences in color intensity seem to be mainly the result of a larger or smaller number of colored fibers per unit area (the "half-tone effect"). The colored fibers are **not cemented** together[3]. No or few particles appear under the microscope at 50 × magnification.

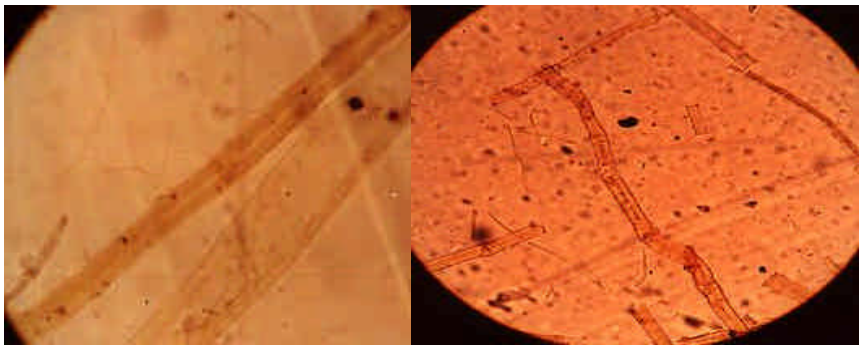
2) <u>Blood stains</u>

Dense blood stains (Scourge mark on back). ME-06. 32× © Mark Evans

The properties of the blood areas are the exact **opposite** of those of the image areas: the red to red-brown material has soaked into the entire thickness of the cloth as a liquid material, reaching the opposite side. Many fibers are cemented together and red spots are obvious. A large number of different particles are seen under the microscope.

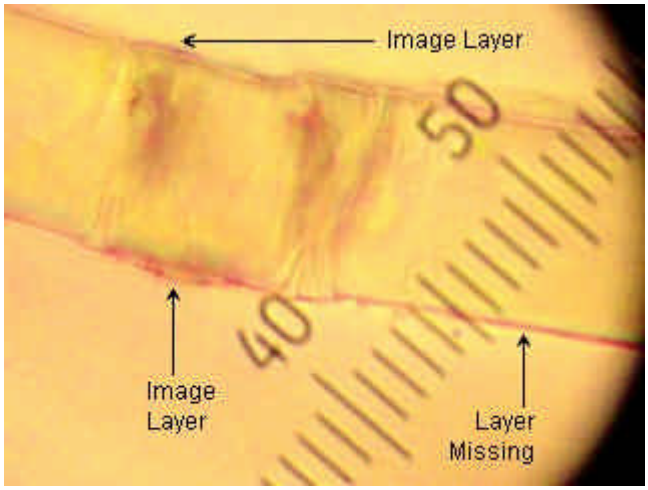
II) <u>MICROSCOPY OF IMAGE FIBERS:</u>

- The medulla of the image fibers is clear, colorless (on the left). The medulla of scorched fibers is colored (on the right). (Photos © Ray Rogers)



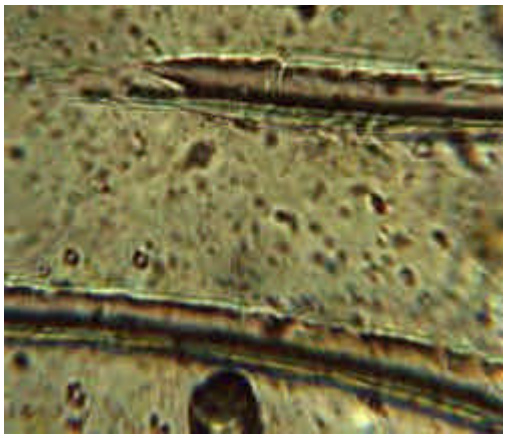
- A large number of fibers were examined by Ray Rogers between the crossed polarizing filters of a petrographic microscope. Under appropriate conditions (at “extinction”), the crystallite structure of cellulose of fresh flax fibers is demonstrated by the fact that the segments between the normal bright nodes are perfectly black. Everything that changes this structure (aging, heating, radiations…) can be detected.
Rogers found no difference in the crystallite structure of image fibers in comparison with non-image fibers[4].

- High resolution contrast-phase microscopy of some image fibers shows what appears to be a yellow “thin layer” (thickness: 0.2 to 0.6 microns) at the surface. This layer is brittle (yellow flakes are missing) and has a shrunken appearance.



Surface yellow “thin layer” on image fiber © Ray Rogers

- Some structures (the so-called “ghosts”) that look like “molds” of fibers (including the typical dense nodes of flax fibers) have been occasionally found in the tapes used in 1978 to remove surface fibers from the Shroud. According to Ray Rogers, they do not contain fiber (no cellulosic birefringent structure) but have the same chemical characteristics as those of the image fiber surface (reaction with diimide). They are probably pure samples of the image surface thin layer[5].



“Ghosts” of image fibers © Ray Rogers

III) EFFECTS OF THE 1532 FIRE AND OF THE WATER USED FOR EXTINCTION:

Some burned regions intersecting the image have been carefully examined on the Shroud. The image color does not show any change in color or density in these areas where the temperature must have reached at least 250°C to 300° C. (the temperature needed for the beginning of the discoloration of cellulose fibers within a few minutes): no color gradient in

the adjacent image area is seen. The combustion of cellulose also produces many different highly reactive water-soluble products which must have been in contact with the image: no effect is seen[6].

In view of the 1978 STURP examination, many experiments were carried out on linen samples coated with most of the known pigments, binders and dyes used in the Middle-Ages. They were burned and scorched in the same way as seen on the Turin Shroud. All of them show visible color changes near the burned or scorched area by direct effect of heating or reactions with pyrolysis products[7].

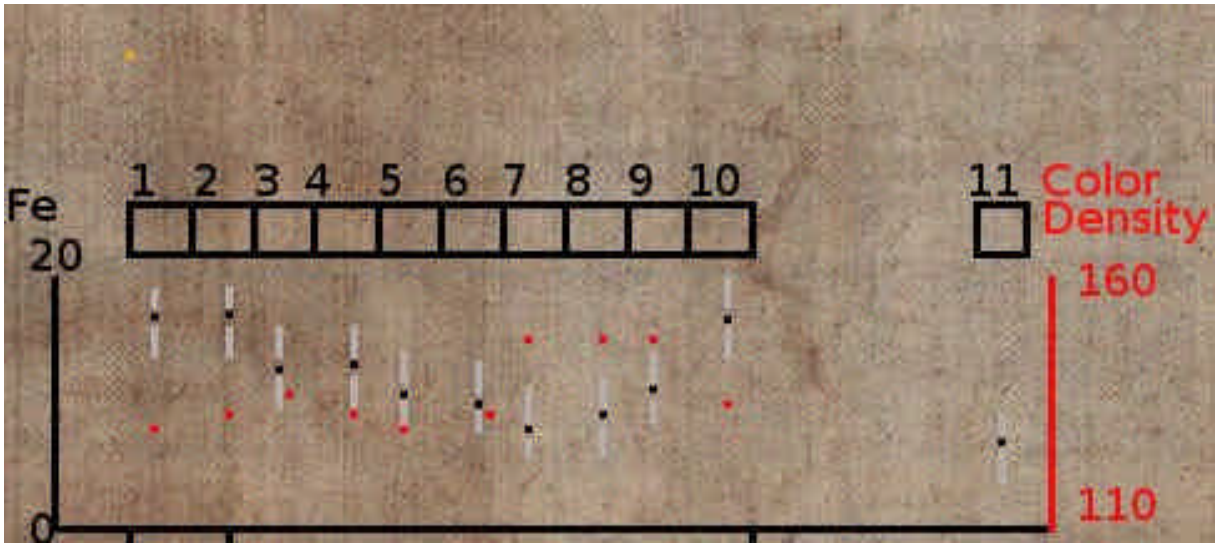
IV) PHYSICAL METHODS

1) Photography in transmitted visible light: the blood stains and the water stains margins are well marked but the image is not visible confirming its superficial nature.

2) X-Ray low energy radiography[8]: this method is used to detect differences in densities. The wave structure is clearly seen as expected, since all the threads contain high levels of Ca covalently bound to the cellulose as demonstrated by X-Ray fluorescence and microchemical studies. The water stains margins are also visible, demonstrating a high concentration of dense elements through a chromatographic mechanism.
The dense blood stains are *not* seen. Since the blood stains penetrate the cloth and are therefore concentrated, this observation suggests that high concentrations of iron and heavy metals like Hg (vermillion) are not present in the dense blood stains. To the contrary, the mineral content of a blood exudate is comparatively low[9].
However, the reported sensitivity to Fe2O3 (iron oxide) is not sufficient to eliminate the possibility of iron oxide pigments (or vermilion) being present in the blood stains.

3) X-Ray fluorescence[10] is a very powerful method to determine the concentration of inorganic elements having an atomic number above 16 with accuracy depending on the element.
27 spectra of the Shroud were collected, each from a 1.3 cm2 illuminated spot on the Shroud. Iron, Calcium and Strontium were found on all the spectra.
Calcium (116-253 microgr/cm2) and Strontium (0.6-2.9 microgr/cm2) appear as a relatively uniform background distribution while Iron local concentrations vary.
Measured Iron concentrations (rounded) were about 50-60 microgr/cm2 in blood areas (3 samples), 24-35 microgr/cm2 in the water stain near the dorsal-foot image (5 samples), 8-18 microgr/cm2 in the facial image areas (see below), and 6.8 (+/- 2.1) microgr/cm2 in the unique background area tested (area 11 on the figure below).

11 spectra were obtained from the facial region where the highest density differences can be seen.



XRF facial scans (areas 1 to 11): measurements of the Iron content (microgr. /cm2): black dots; I have added the mean reflected light density of the same areas (on greyscale): red dots (only indicative because of the great scattering of the values).

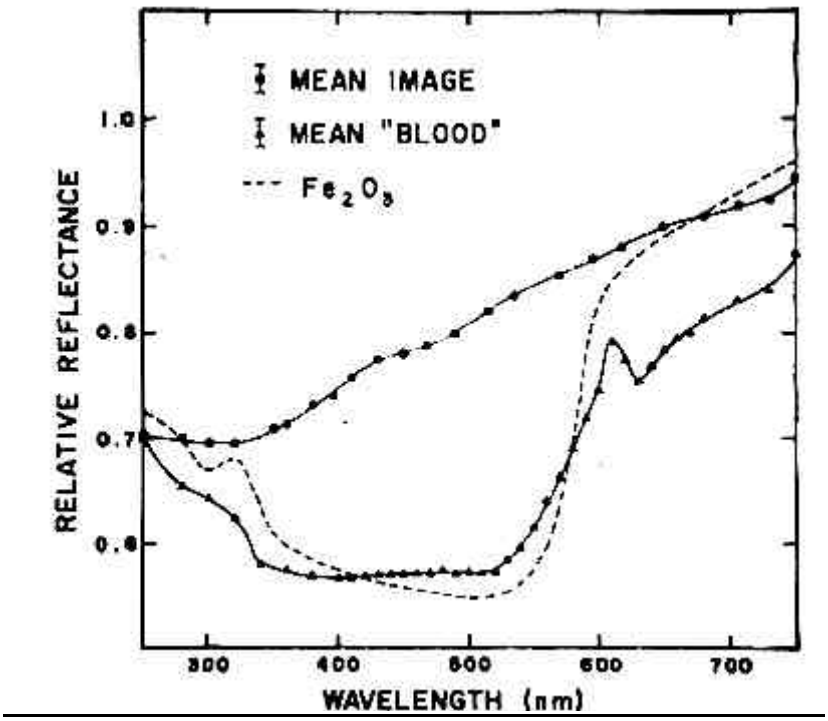
[***Comments***: One can see that there is no statistical difference in iron content from area 3 to 9, although there is no obvious image in areas 7 to 9 and a (relatively) "strong" image in areas 4 to 6. It must be noticed that the area 10 is considered as a partially "blood" area by the authors.]

On the basis of experiments with various concentrations of hematite (Fe2O3), the authors estimated that the sensitivity of their measurements on the Shroud was not sufficient to reject the hypothesis of painting with hematite.

In addition, measuring the Iron content of whole blood on cellulose paper, the authors concluded that the iron content of the blood stains on the Shroud is consistent with blood. However, they were not able to detect Potassium (K) on the Shroud (blood contains Potassium), but "*poor signal-to-noise ratios may preclude definite conclusions on this point*". No other element was detected on the Shroud within the limits of the method; mercury (Hg) was not found but the sensitivity of the method with regard to Hg under these conditions was not measured or quoted in this publication.

4) Visible, infra-red and UV-Vis reflectance spectroscopy[11], [12] have been used to compare spectra of the different regions on the Shroud as well as to compare them with spectra of different kinds of controls (linen with hematite, blood coated linen etc...)

In general the different areas show different spectra: particularly, the spectral reflectance characteristics of the blood stains are clearly different from those of the image under visible, IR or UV illumination.
For example (see below), the spectrum in visible range of pure image is a monotone curve without any particular feature. To the contrary, linen coated with hematite shows a low reflectance (strong absorption) region, relative to background, between 350-550 nm., and an abrupt increase of reflectance between 550-650 nm. Such pattern is typical of any kind of pigments which absorb light only in a specific region of the spectrum. This is absolutely not the case for the image spectrum on the Shroud.



Reflectance spectra in visible range (relative to background): mean of different image and blood areas (with statistical variance) compared with typical spectrum of hematite.

The second important fact is that the spectra of the image are very similar to the spectra of the slightly scorched areas under visible, IR and (with some differences) UV light. This fact suggests that the image chromophores involve some kind of dehydrated polysaccharides since the first step of thermal degradation of cellulose is dehydration.

The IR spectra of the Shroud blood stains show marked differences with the spectra of whole recent blood on cotton but it is not known if these differences are chemical or surface effects. [***Comments***: this does not at all preclude the possibility for the "blood" stains on the Shroud to be real blood as discussed below: see V-5)]

5) UV-Vis Fluorescence spectra and photographic studies:
The UV fluorescence studies are important to determine the chemical differences. They complete the reflectance spectroscopic studies because the physical mechanism of the emission of fluorescence is different from that of reflectance spectroscopy which is based on the absorption of light.

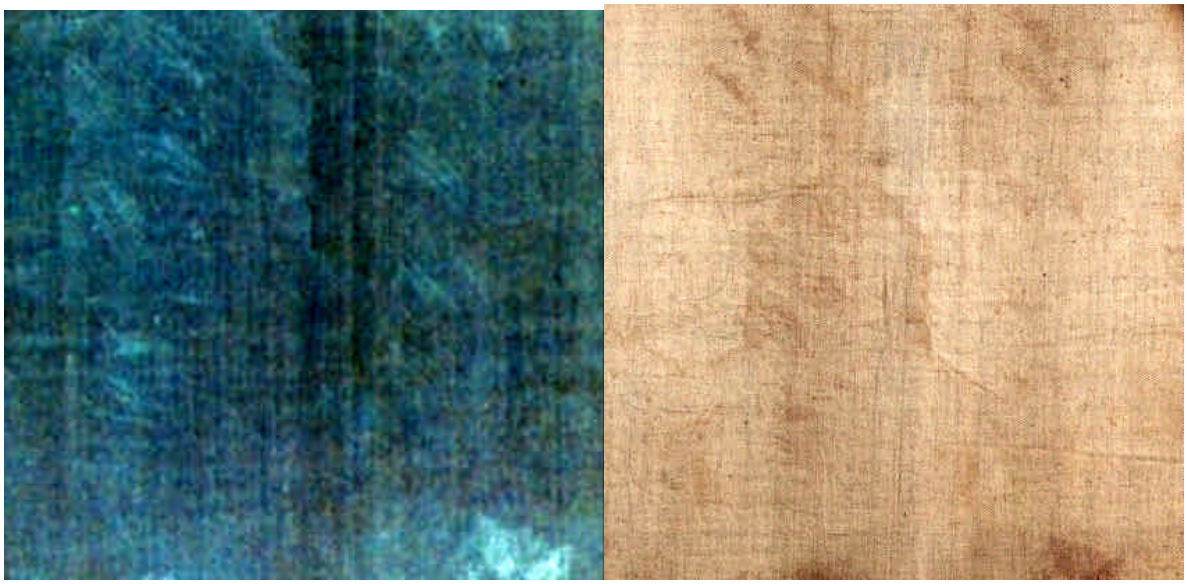
The compared reflectance vs. fluorescence spectra of different image areas show that the higher the absorbance, i.e. the higher the concentration of chromophores, the lower the fluorescence. This is also true for the scorched areas and the blood stains. This implies that the main effect of all these stains, including the image, seems to be the quenching of the background fluorescence[13].

This is confirmed by the photos of the Shroud in the visible under pure UV illumination[14]. They show yellow-greenish background fluorescence, no fluorescence emission of the image (brown) and of the blood stains (dark brown to black spots) and the characteristic reddish orange fluorescence in the slight scorches.

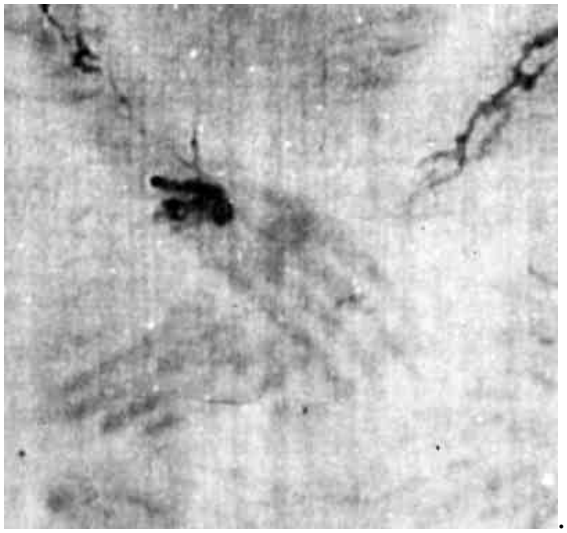
The non-fluorescence of the blood areas is consistent with blood since the porphyrin structure of haemoglobin is a strong near-ultraviolet absorber[15].
The non-fluorescence of the image compared with the reddish fluorescence of the scorches suggests that the image is not simply a scorch. However, the fact that the scorches of the 1532 fire were produced under special conditions (in a casket under low oxygen level) does not allow definite conclusions.
Finally, no protein by-product like a collagen binder seems to be present in the image area: such kind of material, even aged, is usually strongly fluorescent under UV light.

The UV photography also provides 2 kinds of features which are not seen under visible light: many groups of 3-4 narrow lines near the marks of scourging (consistent with a Roman flagrum) and a small fluorescent halo around the main blood stains (serum?)

See photos below.



Negative of the UV-Vis photography on the left and visible photography on the right of the same region (back of the legs) showing the narrow lines of the scourge marks under UV light. Most of them are not seen in visible.



UV photography showing a fluorescent (white) halo around the blood of the wrist wound, not seen under visible light. (© Judica)

6) Pyrolysis Mass Spectrometry[16]:
Samples of different areas of the Shroud have been tested by this highly sensitive but destructive method. The goal was to detect small amounts of non-carbohydrate organic materials that could have been used as painting material. The method was sufficiently sensitive to detect traces of the low-molecular-weight fractions (oligomers) of the polyethylene bag that was used to wrap one of the samples.
Seven samples were used: one modern linen, one pure image, one "background" (from the atypical "Raës sample" adjacent to the radiocarbon dated sample), one image/ water stain and three blood samples.

No contaminant was found on the image samples: neither pigment, nor nitrogen-containing material like proteins or proteinous binder.
The Raës sample showed a significant content of pentosans (plant gum) which was absent from all the other samples.
At least one of the blood samples (the "Zina thread" from the image heel) showed a strong peak for hydroxyproline at low temperature. This amino-acid is present in animal proteins including blood proteins or collagen.
In addition no lignin was discovered in the shroud samples, confirming the microchemical tests (see below).

7) FT-IR and UV-Vis Microspectroscopy[17] experiments were later performed on fibers from different areas of the TS: five non-image, four water stain, four scorch, two serum, two image, two from the "Holland backing cloth", and two blood "globs". In addition fibers coming from the radiocarbon area were also obtained for these analyses but this question is out of the scope of this paper. The spectra were also compared with the spectra of different dried "blood" simulacra (one as "paint": iron oxide and cinnabar in a 5% gelatine solution, and the other as a mixture of whole blood with bilirubin/human albumin). Dried whole blood, bilirubin and hemoglobin samples were used as control.

FT-IR spectra of the Shroud fibers on the pure image samples showed none of the characteristic amide bands of proteinous material. These bands were easily seen in the blood globs and serum fibers, again confirming the microchemical studies (see below).

A progressive "oxidation pattern" based on analysis of carboxylic acid salt and conjugated ketone regions was found: the image fibers are more oxidized than the background but less than the scorched fibers, in agreement with the spectroscopy and microchemical data.

Both FT-IR and UV-Vis spectra of Shroud blood and serum samples are in good agreement with the spectra of blood controls, but the presence of relatively high levels of bilirubin is clearly required as expected for "traumatic" blood exudates.
The mineral blood simulacra showed, to the contrary, a complete mismatch with the Shroud blood data (except for protein peaks).

V) MICROSCOPY AND MICROCHEMICAL TESTS:

Walter McCrone (deceased), an expert in microscopy, was first allowed by STURP to study the 32 surface samples. He used mainly Polarized Light Microscopy (PLM) investigations and some classical forensic tests for blood on the Shroud samples. His method was as follows: without a prior knowledge of the location of the samples, he looked at the samples through the microscope. He found yellow fibers and many sub-micron small red particles that he identified as iron oxide pigments on the basis of their optical properties. These red particles were found on two thirds of the image samples (or all image samples, depending on the source), in greater amount on all blood samples and none on background fibers.
From this spatial correlation, he finally concluded (after some changes in his claims) that the image was painted with iron oxide in a collagen binder and that the blood was the same material in greater amount. Later he found that the "blood" also contains vermilion (HgS). He published his discoveries in the journal of his own Institute: *Microscope*, and later in 2 peer-reviewed journals. Some other investigations were also performed by the McCrone Associates electron optics Group.

Heller (MD, medical Physics and Chemistry, deceased) and Adler (Chemist, specialized in blood and porphyrin chemistry[*], deceased) performed microscopy and many exhaustive microchemical tests on the same samples. The main differences with McCrone reside in their systematic approach, the best representativeness of the samples and "objects" studied, the many microchemical tests performed and their use of appropriate controls. They concluded that the "blood" on the Shroud was real old blood exudates and that the image was the result of some kind of dehydration-oxidation of the cellulose. They published their discoveries in peer-reviewed journals.

One must realize that there are many difficulties in the **interpretation** of the observed features on the samples: 1) the representativeness of the sample and of the different "objects" seen in the sample, 2) the validity of some tests which were not necessarily developed on this kind of material, 3) the history of the Shroud.
About this last point for example, the Shroud was rolled and unrolled, folded and unfolded, so that particles originating from an area can be found in another area. This is particularly true for the "blood" particles because the surface of the red material in "blood" areas appears often abraded. The water has also transported some materials. The effect of the 1532 fire must also be considered. Finally, it is known from historical documents that a great number of painted

[*] This fact can easily be verified on « Google scholar »: Adler wrote many papers in peer-reviewed journals about porphyrins. This is important because the heme group of blood is a porphyrin.

reproductions have been pressed against the Shroud for "sanctification" purpose, allowing some contamination with pigment particles.

Heller and Adler, in their main published work[18], worked on 22 of the 32 sticky tape samples received from McCrone and removed from the Shroud surface in 1978: 6 blood samples, 2 pure body image samples, 1 non-image, 2 body image adjacent to blood marks, 4 pure water stain, 3 water stain/body image, 2 scorch, the other coming from a patch and the Holland backing cloth. In addition they distinguished "inside water stain" from "water stain margin" and also "blood areas" from "adjacent to the blood areas".
For the microscopic survey, they worked with different high magnification[19] (1000 ×) microscopes (light, polarization and phase contrast) similar to McCrone's.
For the reasons developed above (representativeness), they decided to gather the "objects" in different classes on the basis of their shape, color and optical properties. For a class to be assigned to a specific area, it was decided that a minimum threshold of 15 objects of the same class had to be present on the sample. Then, at least 5 objects of each class were tested against appropriate controls.
In order to test the sensitivity and specificity of the chemical tests, they used as control: a 300 years old Spanish linen cloth partially covered with two-years old blood, samples of a Coptic (350 A.D) and of Pharaonic (1,500 B.C.) burial linen and many laboratory controls.

1) Light Microscopy and optical properties of the fibers and particles

Walter McCrone summarized in 1990[20] his previous observations published in Microscope[21] as follows:
There are 19% (10-26%) yellow fibers in non-image areas and 46% (29-72%) yellow fibers in image areas.
Microscopically, the image consists of yellow fibers and red particles. The red particles, seen only under high-magnification light microscopy (600-1000×) are more abundant in the red blood images, and the yellow fibers are the major colored substance in the body image. There is no red particle in the non-image samples.
The red particles are often visible as encrustations or agglomerates in the blood areas, while they are more widely dispersed in the body-images areas.
The red particles have varying degrees of hydratation, color, and refractive index (from about 2.5 to 3.01). These properties are characteristic of the artist's earth pigment, red ochre, used for at last 30 000 years. A significant proportion of the Shroud red ochre is hematite (anhydrous Fe2O3), accounting for the observed birefringence of many of the individual particles.
When a tape is applied on linen painted with highly diluted iron-earth pigment in gelatine and examined under the microscope, the red coated fibers and particles are indistinguishable from those of the Turin Shroud. Diluted blood after drying was examined in the same way and showed a brownish gelatinous coating (refractive index about 1.55) and no particle on the fibers.

As described above, Heller and Adler used another method to characterize the different objects under the microscope. The following table summarizes the results of this classification[22].

DESIGNATION	PREDOMINANT LOCATIONS	COMMENTS
Very pale fibrils	Backing “Holland Cloth”	Very smooth surfaces
Pale yellow fibrils	Non-image areas	Slightly corroded surfaces
Yellow fibrils	Pure body image areas	Corroded surfaces
Dark fibrils	Scorch areas	Light to dark brown color; very corroded surfaces
Red coated fibrils	Blood areas	- Color: orange to red - Surfaces: smooth to fractured to particulate - Coating not birefringent
Golden yellow coated fibrils	Margins of blood areas	Except for color, the same general characteristics as above
Red particulate coated fibrils	Water stain margins and blood scorch areas.	- Color: deep red to brown - birefringent, pleochroic - some particles are found in the lumen of the fibril
“Shards”	Blood areas and adjacent areas	Fractured replica of the coating of the red coated fibrils; orange to red and not birefringent or pleochroic
Orange globs	Blood areas and adjacent areas	Large “globs”; red-orange to orange-yellow; not birefringent or pleochroic
Brown globs	Blood-scorch margins	Brown version of the orange globs with small dark particulates embedded therein
Black particulates	Scorch areas	Irregular, opaque particles.

They also compared these objects with those obtained on the Spanish linen control under various conditions: untreated, saturated with 0.1% gelatine solution, saturated with 0.1% gelatine and 0.1% Fe2O3 (iron oxide), saturated with 2-years old human whole blood. All these controls were then heated at 100°C for 1 hour (to mimic aging) and 250°C. for ½ hour. Iron oxide in gelatin resembles birefringent red particulate coated Shroud fibrils and is frequently clumped. Heating tends to break apart the clumps, leaving separate fine particles. Whole blood closely resembles Shroud red coated fibrils, except redder color. By heating, the control “aged” real blood coated fibers resembles Shroud red fibrils more closely in color and polarization properties.

[***Comments***:

The main optical properties[23] of a microscopic object are:
1) Refractive index: the Refractive Index or Index of Refraction is the ratio of the velocity of light in a vacuum relative to the velocity of light in a medium (solid, liquid, gas); it is expressed as n, and varies with wavelength (?) and temperature (°C). A common standard temperature is 25°C (another is 20°C); a common standard wavelength is 589 nm (Fraunhofer D); a common precision used in microscopy is ±0.001. Example: $n_D^{25} = 1.528$

2) Isotropy/Anisotropy: Refers to substances that have one (isotropy) or more than one (anisotropy) refractive index;
3) Birefringence: the numerical difference between the maximum and minimum refractive indices of anisotropic substances. Birefringence may be qualitatively expressed as low (0 - 0.010), moderate (0.010 – 0.050), or high (>0.050); often birefringence >0.2 is termed "extreme".

An isotropic material, like blood, is not birefringent, while an anisotropic material (like crystals) is birefringent. For example, the birefringence of water ice is moderate (0.014), the birefringence of calcite is high (-0.172) and that of hematite is "extreme" (-0.21 to -0.23).

Blood is isotropic (not birefringent) and, according to McCrone: "*blood in any form or any organic derivative of blood has refractive indices less than 1.60*"[24].
Red ochre is a mixture of iron oxide particles ranging from pure hematite (anhydrous Fe2O3, extreme birefringence, very high R.I: 2.8-3) to variously hydrated iron oxide (Fe2O3×H2O, birefringent or not, with various high refractive indices, usually above 2.5).
It is important to realize that a given iron oxide pigment particle (except hematite) *can be isotropic (birefringent) or not*, depending on its state of hydration and cristallinity.
This is the case for the earth iron pigments like red and yellow ochre, although they also do contain variable amounts of highly birefringent particles: hematite[25].
The true test which distinguishes iron oxide particles (birefringent or not) from organic particles like blood is the measure of the refractive index.

There is obviously a problem with the opposite observations of McCrone and Heller and Adler on the optical properties of the particles.

It is not clear how McCrone performed his observations to obtain the optical properties cited. In his first paper[26] (1980), he wrote: "*This material, **when examined on the tapes** with higher magnification and transmitted polarized light, is identical in appearance and properties (color, pleochroism, shape, size, cristallinity, refractive indices and birefringence) to the particles of hydrous and anhydrous iron oxide particles, collectively known as iron earth pigment, used since the days of the caverman*". This was confirmed later (1998) by McCrone's statement: "*On examining thousands of red image particles **on the Shroud tapes**, I saw no low refractive red particles except rose madder particles…*"[27]

Heller noticed that the Mylar tape was optically active so that any red particle looks birefringent when the light has to pass through the tape and particle[28]. Certainly, the tape also changes the measured refractive indices of the particles.
What is sure is the fact that, contrary to STURP instructions, McCrone affixed all the tape samples to microscope cover glasses. From the above McCrone's citation ("...*When examined on the tapes*...") and from the fact that Heller and Adler received their 22 samples in this form[29], it is highly probable that most (if not all) of McCrone's optical observations were performed on the Mylar tape. Consequently, one must be sceptical about the optical properties (birefringence and refractive indices) of the material in McCrone's claims and one must consider that he identified the red ochre mainly on the basis of the "*size, shape and color*".

In sharp contrast, Heller and Adler removed all the specimens from the tapes before their studies. Then, they removed the adhesive with toluene and verified the final removal of the adhesive under UV irradiation (the adhesive gives a bluish-white fluorescence).

Concerning the particles they wrote: "*Most of the red particulates ranged from submicron to about 3 microns, and the birefringent red particulates from 0.7 to about 1 micron in diameter. These particulates were therefore too small for accurate quantitative determination of their optical parameters by the standard methods. Highly variable results were obtained even for refractive index measurements by immersion methods. However, when a red non-birefringent red particulate coated fibril and a birefringent red particulate coated fibril were compared side by side immersed in benzene (index of refraction=1.50), the Becke line movements were in opposite directions. Therefore, the refractive index of the non-birefringent red particles is less than 1.5, while it is greater than 1.5 for the birefringent red particles, indicating that they are quite different materials.*"[30]
The Becke method is the standard method to determine the refractive index of a microscopic object. McCrone confirmed this fact: "*There is a very simple optical test that differentiates blood from red ochre or vermilion - the Becke line test for refractive index*"[31].
That is exactly what Heller and Adler did *but* on the objects removed from the tapes, contrary to McCrone.

To summarize, Heller and Adler found birefringent and non-birefringent particles with highly variable refractive indices. While the birefringent particles can not be blood (they identified them later as iron oxide), the non-birefringent particles can be blood as well as earth iron pigments (except hematite). However, much more important are the red coated fibrils tested *side by side* (i.e. exactly in the same conditions at the same time) which show very different *connected* properties: birefringence and refractive index above 1.5 on one side versus no birefringence and refractive index below 1.5 on the other side.
The refractive index below 1.5 of the non-birefringent red coated fibrils definitely excludes iron oxide pigments as origin of their red color.

There are clearly two very different kinds of red "objects" (particles, "globs" or agglomerates, shards, incrustations and red coated fibrils) on the Shroud, something that McCrone failed to recognize.]

2) Elemental analysis:

The following elements were tested by Heller and Adler[32], generally with 2 different microchemical tests: Fe, Ca, Cu, Mn, Ni, Co, Al, Zn, Ag, Hg, As, Sb, Pb, Sn, Cr.
Only Fe and Ca were found above trace level.

Iron could be found *without* acidic predigestion on all the non-blood samples: it is iron bound on the surface of the fibrils during the retting of the flax fibers (like Ca).
The shards and the globs in the blood areas gave the test for iron in the blood areas but *only* after digestion with aqua regia which is able to remove the iron from the heme of hemoglobin.
To the contrary, strongly positive iron tests could be obtained on the birefringent red particles with concentrated hydrochloric acid (HCl) *alone*, in which they slowly dissolve, as expected for iron oxide.

[***Comments:***
Aqua Regia is a mixture of concentrated hydrochloric acid and nitric acid which is used to dissolve some noble metals like gold or platinum that can not be dissolved in concentrated acids like HCl alone.

Here, the nitric acid (a strong oxidant) is required to liberate the iron from the heme of hemoglobin. The iron then passes into solution and dissolves in HCl where it can be tested by the reagents.
Iron oxide is soluble in concentrated HCl alone without the need of aqua regia.

These microchemical tests prove again that there are two different kinds of red particles and coatings on the Shroud: birefringent iron containing "objects" that could be dissolved in HCl alone and liberate high amounts of iron: iron oxide *and* non birefringent objects that need aqua regia (not only HCl) to liberate lower amounts of iron, as expected for hemoglobin which contains relatively little iron.]

The origin of the Iron oxide found on the Shroud:

Many hypotheses can be found to explain the presence of the iron oxide on the Shroud:

1) Earth pigment in the McCrone's paint hypothesis
2) Iron oxide formed in burned blood through a well-known reaction
3) Iron contained in the water used to extinguish the 1532 fire and/or cellulose bound iron, both "chromatographing" to the water stains margins where it could precipitate as Fe(OH)3 and finally produce iron oxide in the form of the birefringent red particles observed[33].

The predominant location of the birefringent red particles and coatings (water stains margins and blood scorch areas) found by Heller and Adler seems to be in agreement with both the hypotheses 2) and 3).
These authors performed experiments on the old "Spanish linen" control in the conditions of the extinguishing of the 1532 fire and observed iron oxide particles of the same appearance and properties as those found in the water stain margins on the Shroud[34].

Another possibility to find the origin of the iron oxide particles is to check for impurities. According to Heller and Adler, the earth iron oxide pigments used in the paint are almost always contaminated with Mn and/or Co and/or Ni and/or Al above the 1% level. More than 50 birefringent (i.e. iron oxide) red coated fibers were tested (after digestion with aqua regia) together for these species specifically and none of them was found above 1% level. Prof. Riggi also examined Shroud particles with electron microprobe and found none of the expected contaminants but when he examined old and new "Venetian" red (another kind of red ochre), he found the contaminants.
Heller and Adler concluded that "*the geochemistry of iron is such that to find an iron earth pigment as pure as this would be most unusual unless pure hematite were used*".

[***Comments:***
One must be very careful here. The 50 fibers tested by Heller and Adler were certainly from the water stains margins and *not* from blood areas. This is consistent with the fact that they did not find Al, contrary to what was found in the blood areas by them as well as by McCrone (see below).
Therefore, this experiment confirms that the iron oxide found in the water stains margins is pure and certainly not iron oxide pigment, *but it says nothing about the iron oxide (if any) found in the "blood"*.]

The McCrone Associates electron optics Group performed Energy dispersive X-ray Analysis (EDXRA) on 11 "Blood-Image-Area Shards"[35] from tape 3CB. One was different from the others (no color, only Ca).

The characteristics of the 10 others are as follows:

- Diameter: 5-23 microns (flat particles)
- Color: orange to red-orange to red
- Major elements (peak heights above background): Ca: 36-65 (7/10), Fe: 22-66 (7/10), Cl: 20-60 (6/10), Si: 27-57 (8/10), Al: 8-28 (8/10).
- Minor elements: Na: 1-9, Mg: 1-8, P: 2-16, K: 1-9.
- They also found Hg/S which is the basis of the claim of vermilion on Shroud blood image: this point will be studied later.

Heller and Adler also performed EDXRA[36] on 16 different globs and fibers from blood image, body image and non-image tape samples. The fibers all showed strong Ca and Fe signals and the globs all show Na, Mg, Al, Si, P, S, Cl, K, Ca and Fe but no quantitative data or peak counts are given. They also could detect Hg from vermilion but only in the "track" sample (see below vermilion) and no Co, Mn or Ni.

Heller and Adler stated that all the elements (except Hg) found in this study are in fact also found in whole blood.
McCrone stated that the contaminants found were consistent with earth iron oxide that contains quartz, limestone or feldspar impurities.

In addition, X-ray Diffraction (XRD) analyses were performed by McCrone Associates on 10 other "shards" from tape 3 CB (blood area). The refractive indices ranged from 1.46 to 3.36 (above 2.22 for 7/10). In the same article, McCrone concluded from this analysis that: "*the XRD data were obtained on single red particles aggregates (blood-image shards) (…) amounting to <1 ng. of which possibly 0.2-0.3 ng. is pigment. Less than one-half of the pigment is crystalline (hematite or vermilion), hence the lines in the XRD pattern are very spotty and difficult to measure. The agreement with known hematite data is, nonetheless, convincing.*"

[***Comments***:
McCrone used the term "red ochre" to define the alleged pigment in the painting theory. More precisely, he explained: "*the iron earth pigments are hydrous iron oxides ranging in color and refractive index from yellow ochre to red ochre depending on their history*".

Ochres are defined as clays used to make the earth colors[37], in other words tinted clays. Their color, varying from dull yellow to red or brown, is given by the presence of different iron oxyhydroxides and oxides, mainly goethite (FeO(OH)) and hematite. They were used since the prehistoric times but "*the mineralogy of earthy pigments found in historical paintings is not satisfactorily described*"[38]. If the red ochre of the alleged painting is an earth pigment it is therefore probably contaminated with some minerals found in clays. However there is a high mineralogical variability in the natural earths.
In the group of the natural earth pigments, the color of the red earths is mainly given by hematite and red clays are very common in nature. Relatively pure massive hematite of iron-ore grade free of clay minerals can be found in nature, and pure hematite was also artificially produced at least from Middle Ages by calcination of ferrous sulphate.
There are four main families of clays: kaolinite, montmorillonite-smectite, illite and chlorite. Clays always contain Al and Si. In addition smectite contains Ca, Mg and Na, illite contains K, Mg and Fe and chlorite can contain Mg, Fe, Ni or Mn. Other contaminants like gypsum (containing Ca and S) can be found.

Because of the high mineralogical variety of earth iron pigments it seems difficult to conclude with certainty that the lack of Ni, Co and Mn is a definitive proof of the fact that the iron oxide does not come from earth. However, unless earth iron oxide is pure hematite (which is not the case according to McCrone), Al and Si seem to be always associated with Fe in earth iron pigments.

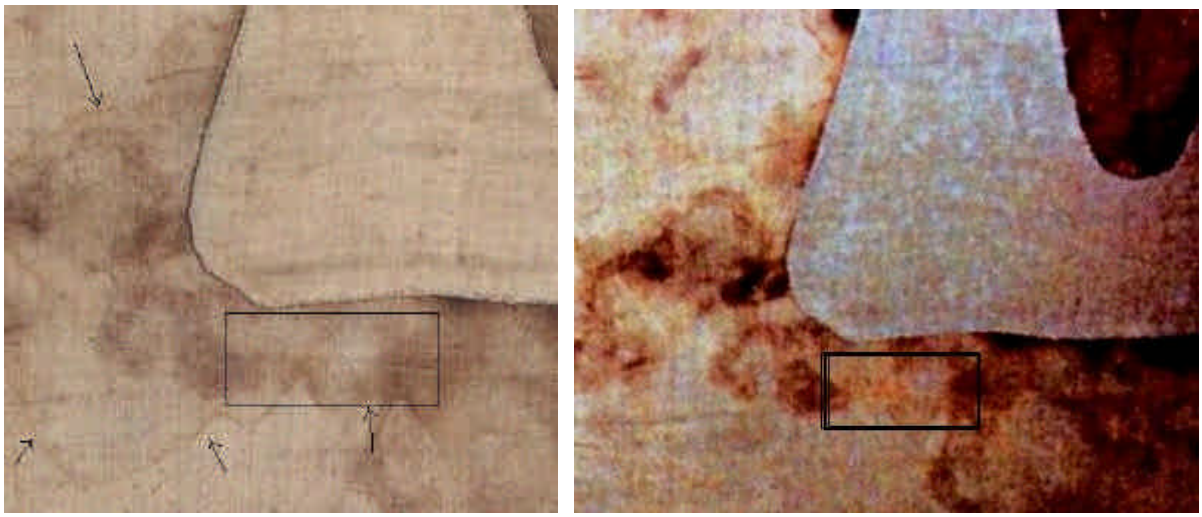
Now the question is: are the McCrone Associates EDXRA data of the 10 shards coming from the 3CB blood sample consistent with blood?

SEM/EDX data from dried blood residues were studied by Dixon and al[39].
In particular, samples of blood smears, crusts and dried blood on cloth (up to 7 years old) and graphite were analyzed after subtraction of the spectra generated by the substrate.
We must remember that the peak heights in the qualitative EDXRA spectra can not be directly related to the actual amounts of the measured species.
All the blood spectra showed a characteristic pattern: Chlorine (Cl), Sulphur (S) and Potassium (K) peaks predominate in nearly the same ratio.
Blood on cloth gave the following results, in % (rounded): Cl: 22-27, S: 12-22, K: 11-23, Si: 7-17, Ca: 5-14, P: 0.4-13, Al: 1-9, Na: 0-5, Mg: 1-3, Fe: 0-2.

Although the data are presented differently (% vs. peaks heights), the comparison with the EDXRA data of the 10 "blood" shards on the Shroud is interesting:
- For the 3 major peaks of real blood: Cl on Shroud is consistent with blood, S is present but the actual peak count on the Shroud is unknown (see vermilion Hg/S chapter below) and the very small peak of K seems not compatible with K in whole blood. However, Adler gives a possible explanation for this fact (see below).
- The counts for Si and Al relative to Cl in the Shroud "blood" shards are not inconsistent with the relative percentages of the same elements in real blood. Therefore, one can not eliminate blood on the basis of their presence in the "blood" shards on the Shroud as it has been claimed.
- Ca is also found in blood at a level roughly consistent with what is found in the Shroud "blood", and the small excess could come from the calcium bound to the cellulose.
- The iron peak height of whole blood in EDXRA spectra is surprisingly low: there is much more Fe in the Shroud red shards than expected for whole blood.

On the other hand, are the EDXRA data consistent with yellow or red ochre (Fe2O3.H2O)?
According to the McCrone particle atlas, these iron earth pigments show: O and Fe as major elements (>5%), C, Al and Si as minor elements (1-5%) and Cl, K and Ti as trace elements (<1%). Minor and trace elements may vary, depending on impurities and source.
Obviously, all the elements are consistent with EDXRA data of the 10 Shroud shards except Cl which is found on the Shroud as a *major* element and not at all as a *trace* element.
However, contamination with Cl containing particles is possible or, alternatively Cl can be associated with vermilion because Hg in vermilion particles often contains Cl in addition to S[40] .

Let us now examine the 3CB sample from which the EDXRA and XRD data were obtained by McCrone's Associates.



The photo on the left is in visible light and that on the right is the UV-Vis fluorescence slightly enhanced photo. The rectangle shows roughly the location of the sample 3CB.
We can see clearly that this sample is one of the worst "blood" samples possible for the analysis. There is very little "blood" (only on the edges), the area is in a water stain and near the water stain margins (arrows) and it is a light scorch as demonstrated by the reddish characteristic fluorescence and its proximity with a patch and a burned area.

Therefore the analysed particles can be anything: pigments, heated blood strongly contaminated with iron oxide from the water stain and/or from burned blood (explaining perhaps the high iron level) or anything else.
The representativeness of the sample is so bad that absolutely no conclusion from EDXRA can be drawn.]

<u>Vermilion</u>:

Heller and Adler[41] found a large (150×50 microns) prismatic hexagonal object on sample 6BF (blood, lance wound, front). They tested this object for metallic species and found only Hg: it was cinnabar, the natural form of vermilion. Fragments of this particle were also found in a "track" on the same sample. They decided to test with their microchemical reagents for Hg a number of red particles on *all* the samples and found that *none* of the red particles, even from the same tape away from the "track" gave the test for Hg. Control tests established that a negative test for Hg means that Hg can not be present above 1% (weight).
In addition, we have seen above that they also examined 16 different globs and fibrils from blood, body image and non-image tape samples using an electron microprobe. They found Hg only in the same "track" sample.
From these studies, they concluded that vermilion (or cinnabar) is only "incidental".

In the same 10 shards from the blood area 3-CB described above, McCrone Associates also found characteristic Hg/S peaks[42]. The peak heights are as follows: = 10 units: 4/10, 11-35 units: 2/10, 36-55 units: 4/10.
In addition, a back-scattered electron image of a 3-CB blood-image-area-shard, providing a mapping of the spatial locations of the elements, shows that mercury and sulphur are spatially associated in one part of the shard, while iron is in another part of the same shard. From this observation and the fact that no vermilion was observed on body-image tape fibers, McCrone

concluded: "*It seems reasonable that the Shroud was first painted with a red ochre paint and then the blood images were enhanced with a vermilion paint*".
Finally, McCrone also used a PLM microchemical test for Hg (mirror formation by precipitation from HgS solution by metallic copper) on a single blood-image shard (again from 3CB sample) compared with many tiny simulated known vermilion "shards". The photo shows a very weak color (at the limit of visibility) compared with the color of most of the simulacra.

[***Comments***:

The presence of vermilion on the Shroud is of paramount importance because, contrary to iron oxide, it can be only a paint pigment.
However, the question is: if there is vermilion on the Shroud, is it present in sufficient amount in the right location to account for a true painting of the blood stains or, alternatively, is it an incidental artefact (contamination)?

In EDXRA spectra, the peaks of Hg and S overlap. It means that from the peak height alone, it is impossible to determine the relative quantities of Hg (which can only come from vermilion) and S (which is not necessarily associated with vermilion but also with blood for example).
In this method, Hg can be identified by the peaks on the high energy lines at 9.99 and 11.82 keV. and S on the basis of the relative intensities of the Hg 2.20 line and these higher energy lines[43]. Although the Shroud spectra show some high energy peaks characteristic of Hg, McCrone did not calculate the amount of vermilion. Therefore it is sure, from the presence of both Hg/S peak and Hg peaks, that there is HgS (vermilion) in these agglomerates but the amount is unknown: the quantitative data given above (Hg/S peak heights) do not represent necessarily the true HgS content but perhaps a mixture of HgS (vermilion) and S from another origin.
It must also be recalled that the spectra were obtained from 10 shards coming from only a single (3CB) blood sample, which is in addition a very "bad" blood sample as we have seen above.

With the same EDXRA method, Heller and Adler found Hg *only* in globs coming from another (6BF) blood sample, the so-called "track" where they found the cinnabar particle. None of the other fibers and globs they tested, including blood samples, showed Hg with this method.
Their microchemical testing for Hg of many red particles from *all* the other samples showed that Hg is not present above 1% level, while the same microchemical test showed a strong reaction on the great cinnabar particle and also on the tiny particles all around in the "track".
In sharp contrast with McCrone, at least two of the blood samples tested by Heller and Adler were areas with high red material density: 6BF (blood, front, lance area) where they found Hg only in the "track" and 3EF (wrist blood image area, front) were they apparently did not find Hg.
From these 2 experiments taken together, it is clear that there is very likely no vermilion at all on most of the blood stains.

McCrone did not detect vermilion particles in his first extensive PLM survey of the Shroud samples, although he particularly focused on the blood samples. He was "*embarrassed by the finding of vermilion by the McCrone Associates electron optics group*" and began to see vermilion particles only after this finding[44].

He then wrote[45]: "*Many loose particle aggregates, picked from the blood-image tapes, show red particles different in shape and color from red ochre, but characteristic of the artist's pigment, vermilion (HgS)*" and added that it is not ground mineral cinnabar but "*a dry-process form first prepared by alchemists about 800 A.D*".
In fact, it is difficult, even for an experienced PLM expert like McCrone to differentiate iron oxide pigments and vermilion without using EDXRA, as he recognized himself: "*SEM/EDS is most useful for differentiating between pigment look-alikes (e.g., ultramarine and smalt, **hematite and vermilion**), and **an experienced PLM analyst** is often relieved to have confirmation by SEM/EDS of such look-alikes in a given sample*"[46].

In short, Heller and Adler found vermilion (or cinnabar) only in a specific location (the "track") of a single blood area sample (6BF: front, lance blood) and none on the many red particles and fibers from *all* the other samples (including strongly colored blood area samples) with both microchemical tests (sensitivity: 1%) and EDXRA.
McCrone did not see vermilion particles in his first extensive study but only after the McCrone Associates experiments and probably only in the 3CB sample* (back, lance blood flow). The McCrone associates EDXRA studies showed the presence of vermilion in unknown amount in ten particle agglomerates of the same 3CB sample.

Therefore one can conclude from the PLM, EDXRA and microchemical studies that vermilion is proved to be present only in one blood sample (3CB: lance blood, back) and in a very specific region of another blood sample ("track" in 6BF: lance blood, front), that the amount of vermilion particles in these areas is certainly low and that there is very likely no vermilion in most of the other blood samples.]

2) <u>Tests for the organic dyes</u>:

These tests were performed by Heller and Adler[47] on image fibers.
The color of all the organic dyes can be modified by acids and/or bases or can be extracted by some kind of solvent.
21 solvents were used on image fibers, covering a large range of pH and solubility.
Only diimide (a powerful reducing agent) and, much more slowly, hydrazine were able to remove the color of the image, living a colorless intact fiber.
This result not only rules out the hypothesis of an organic dye but also suggests that the chromophores are oxidized molecules at the surface of the colored fibers.

3) <u>Tests for the organic functional groups</u>:

These tests were performed by Heller and Adler[48]
Sixteen functional groups of interest were tested by 1 or 2 tests on non-blood fibers (image, non-image, scorches).
Only the carbonyl and aldehyde groups tested positive: weakly on the non-image, moderately on the image and strongly on the scorches, confirming again the FT-IR and other spectroscopy data suggesting some kind of oxidation-dehydratation of the image fibers.
No lignin was found by the standard phloroglucinol/HCl test on all the samples.

* At least in the peer-reviewed article (Ref.20), all that is shown (spectra, figures, photos) concerning vermilion refers to material of the 3 CB sample only.

4) <u>Tests for proteins</u>:

McCrone used the standard Amido-black test for detection of proteins. The positive result he found is the main basis of his claim of a proteinous medium as paint binder.
At micro level, he showed that the amido-black stained a thin layer around some fibers and also the agglomerates, some containing visible submicron red particles[49].
However, from his articles or even from his book (see Ref.67), the amido-black testing was performed *only* on fibers from blood area samples (1AB, 3CB and 6 AF).

In the same paper, he wrote:"*Amido black is a stain for any proteinaceous substance; hence I could only conclude that the paint medium was* ***blood****, or tempera produced during the Middle Ages from egg, cheese, or collagen (…). However, if the Shroud paint medium is collagen, it would show one unique difference from egg or cheese tempera, and from blood. Collagen lacks cystine and cysteine, two sulphur-containing amino acids present in other proteins (including blood)"*.
He used the standard PLM test for sulphur-containing substances: "*the catalytic decomposition of NaN3 in solution to yield a froth of N2 bubbles*", the iodine/azide test.
He tested a linen fiber from the Turin Shroud, two real-blood-coated linen fibers and blood particles. The N2 bubbles appeared around the real-blood fibers and particles but none around the Turin Shroud fiber.
He concluded that the proteinaceous medium used in the "blood" stains on the Shroud was collagen and not blood or other sulphur-containing proteins.

Heller and Adler[50] found that Amido-black also stained weakly pure cellulosic material and strongly old control linen or artificially aged linen. Moreover, the resulting stain can not be removed by proteases, demonstrating that the color was not bound to proteins but more likely to carbonyl cellulosic groups. Therefore this classical test for the detection of proteins is not valid (at least at macro level) on old cellulosic material like the Shroud.

They tested eight different reactants for protein detection on many controls. Some were not specific enough for proteins in the conditions of the Shroud or not sensitive enough.
Finally, only fluorescamine was found both specific for proteins and highly sensitive *to the nano to picogram level* in the conditions of the Shroud.
On the Shroud, all the tested objects (red coated fibers, shards, globs) found mainly in the blood areas tested positive *while the pure image yellow fibers and the red birefringent fibers found mainly in the water stain margins tested negative*.

The classical Bromcresol Green test specific for the albumin was also used, although of moderate sensitivity (0.1 microgr.).
The test was positive on the golden yellow fibers of the blood stains margins and also on some globs in the blood areas.

Finally, proteases were found to be able to dissolve completely and quickly *without any visible residue* all the kinds of colored material found in the blood areas and blood margins (red or olive yellow coating of fibers, shards and globs). The same proteases showed "*absolutely no effect*" on the background non-image fibers *and on the yellow coating of pure image fibers* or the deep red particulate coating of the fibers in the water stains margins away from the blood areas.

[***Comments***:

McCrone found a proteinaceous medium (coating and agglomerates) on blood samples fibers with amido black and Heller and Adler also found proteins with their highly sensitive and specific fluorescamine test: blood areas do contain proteins, either from blood or collagen.

McCrone also performed the standard test for sulphur-containing amino acids and tested a single fiber from a blood sample. His controls were 2 real-blood particles and two fibers covered with dried recent blood.
If one looks closely at the figure 21 in McCrone's paper where the experiment is shown, it appears that the Shroud fiber has only two tiny red-brown dots covering less than 10% of the fiber length while 90% of the fiber is nearly colorless. To the contrary, the control fibers are entirely colored, i.e.100% of their surfaces is covered with a thick recent blood layer. Even there, N2 bubbles are seen only on roughly half of their surfaces. In contrast the 2 real blood control particles are rounded by a very large amount of bubbles. Therefore, it seems that this test is much more suitable for particles than for fibers as McCrone himself wrote ("*A standard PLM test for sulphur-containing substances* ***in particle form***..."). It is amazing that McCrone did not apply this test to the many particle agglomerates that he found.
Perhaps, most important is the fact that, according to Ray Rogers (personal communication), the fiber tested came from the 6AF area which is close to a scorch, which explains why he did not get the test.
Therefore, this negative test applied to tiny amounts (sensitivity not tested) of this centuries-old material on a single fiber taken in a previously heated area with bad controls (large amounts of recent blood) is, without any doubt, meaningless.

McCrone did not test the pure image yellow fibers for proteins.
Heller and Adler tested these fibers specifically, not only with their highly sensitive and specific fluorescamine test (as determined by their well-suited controls), but also with proteases solutions, which are able to dissolve only proteinaceous material.
Even if one alleges that the fluorescamine test is not sensitive enough to detect proteins in the thin yellow coating of the pure image fibers[51] (which is doubtful), the test with proteases is the absolute proof that the pure image fibers do not contain proteins, while the blood fibers do: proteases work even on these centuries-old partly denaturated proteins.

Moreover, the fact that the proteases quickly and completely dissolve all the types of blood objects (fiber coating, shards and globs) without any visible residue also shows that the iron in these objects is *not* iron oxide. If blood objects were iron oxide (or vermilion) particles embedded in a proteinaceous medium, the proteases would have dissolved the medium living at the same location a number of submicron red colored particles easily seen. This is demonstrated by the fact that the proteases also dissolved the brown globs of the burned blood, leaving small visible dark particle residues (probably carbonized material).]

5) Tests for blood components:

McCrone performed the usual forensic tests for blood (benzidine, sulphuric acid, phenolphthalein, Takayama and Teichman tests): the blood samples tested negative and he simply claimed that "there is no blood on the Shroud".

Before that, in 1973, an Italian scientific group of researchers was allowed, for the first time, to test a few blood samples on the Shroud and the same negative results were obtained with the same kind of tests. However, their report stated that "*the pigmented encrustations did not pass into solution in the solvents, acids and the alkalies we used*"[52], so that they cautiously concluded that no definitive statement could be drawn.

Knowing that, Heller and Adler assumed that false negative results would be obtained if the material can not be adequately solubilised, as can occur with a very aged strongly denaturated sample[*]. In fact, they were concerned by the poor specificity of these usual forensic tests. Therefore, they developed another method[53] on the 300 years old "Spanish linen" control impregnated with 12 month-old blood. This method is based on the detection of porphyrin. Hemoglobin contains proteins (the "globin") and heme, which is a chemical structure containing a single Fe ion (Fe2+ in hemoglobin, Fe3+ in methemoglobin). However, porphyrins are widely found in nature, particularly in chlorophyll where the central atom is magnesium instead of iron.
It is known that porphyrin can be detected with a high sensitivity and a high specificity by a characteristic red visible fluorescence under longwave UV illumination in a dark room. In order to use this test on the "blood" fibers of the Shroud, Heller and Adler had to find a method to 1) solubilise the material and 2) displace the Fe from the heme to obtain the porphyrin to be tested.

After many tests on the Spanish fibers, they found that concentrated hydrazine (a strong reductant) was able to solubilise the one-year blood material. Then the concentrated formic acid displaced the iron, so that acid porphyrin could be obtained and tested.
As expected, positive tests were obtained on the Spanish linen blood and the sensitivity of the method in these conditions was about 1 nanogram.
On the Shroud, positive tests for porphyrin were obtained on the non-birefringent red coated fibrils of the blood areas, on the shards and the large orange globs[54].

To further confirm the presence of hemoglobin, they carried out the hemochromagen (a standard test for hemoglobin) and cyanmethemoglobin microspotting colorimetric tests on the shards and red coated fibers with positive results.

Microspectrophotometry in the visible range of the Spanish stained fibrils showed clearly the characteristic spectrum of a fully oxidized denatured met-hemoglobin, while because of the high degree of scattering, the same method on the blood Shroud fibers was only "indicative" of the presence of this kind of old denatured hemoglobin.
However, all the fibers (i.e. blood Spanish linen control fibers and Shroud blood fibers) showed the intense Soret (400-450 nm.) band which is characteristic and specific for a regular porphyrinic material like hemoglobin.
Later, Adler[55] wrote that the spectra of the non-birefringent red-coated blood fibrils from the Shroud did not match the spectrum of methemoglobin in blood solution as it is given in the literature. However, he found "*a spectrum that was characteristic of only one known group of compounds- the so-called high-spin, high-iron porphyrins*" which are found in a special form of methemoglobin: methemoglobin in its para-hemic form. Now, this kind of para-hemic species formed spontaneously in living persons when high quantities of hemoglobin are released in the serum from broken red cells, i.e. under hemolysis conditions.

[*] However, Adler wrote (in Ref.54): "We did get positive Benzedine tests by increasing sharply the peroxide content".

Finally, they tested for bile pigments with the Erlich's reagent with the same microspotting method. The characteristic azobilirubin color could be detected on the olive colored shards found mainly around the blood areas, on the orange globs and weakly on the more orange colored fibers. Further, the test color was sensitive to acid, turning a paler purple and was discharged by 10 minutes of short wave UV light as is characteristic of this color test.
These 2 tests clearly show that unusual high amounts of bilirubin or bile pigments are present in the blood and serum areas. This fact should also explain the unusual red color of the blood on the Shroud by the mixture of brown met-hemoglobin with yellow-orange bilirubin.
Normal blood contains only small amounts of bilirubin. Hemolysis (the breakdown of the red cells) in living bodies can result from prolonged hard traumatic shocks and produce large amounts of bilirubin as found in the blood of the Shroud.

[***Comments***:

Adler, as a recognized expert in blood and porphyrins, presents here an impressive set of proofs that hemoglobin (methemoglobin) is present on the Shroud.
- There is porphyrin, the characteristic part of hemoglobin, and this porphyrin is not from chlorophyll because, according to Adler, "*its fluorescence does not have to be generated*"[56], i.e. the fluorescence appears spontaneously under UV, which is not the case here, as demonstrated by the UV fluorescence studies on the whole Shroud.
- There is hemoglobin as demonstrated by the microchemical spot tests (hemochromagen and cyanmethemoglobin tests) and the characteristic Soret band. While the absorption spectra of haem derivatives differ from derivative to derivative, all have in common this strong absorption at about 400-425 nm[57].
- This hemoglobin (methemoglobin) is in a very special form (para-hemic) which develops spontaneously at the surface of hemoglobin films *and* in living bodies that undergo hemolysis, as in severe traumatic conditions. Hemolysis in the living also produces bilirubin which was found in the blood at extraordinary high levels, particularly in the olive colored shards found in the serum areas around the blood stains as expected. In addition, this discovery explains the unusual red color of this old blood as the result of a mixture of brown-orange methemoglobin in the para-hemic form and yellow-orange bilirubin.
As Adler wrote: "*No one would have ever dreamed, when we first started doing the analysis, that the chemistry would provide corroborating evidence to what the pathologists concluded long ago about the Shroud figure*."

Now the question is: why are the usual forensic tests for blood negative, according to McCrone?
These tests all involve the catalytic peroxidative action of the heme group in producing either a colored or fluorescent oxidized form of some dye. These tests are widely used in forensics to detect blood with a good sensitivity but a rather poor specificity: they are called "presumptive tests" (benzidine, phenolphtaleine…) and must be confirmed by more specific tests (Takayama). However, the question here is that of the sensitivity.
My researches have shown that in forensic sciences these tests are well suited for relatively recent blood stains and I was not able to find studies on material older than a few months in the forensic literature.

It is well-known that the solubility of a blood stain in various solvents decreases with its age[58], confirming what was observed on the Shroud.

The question of the detection of blood residues on archaeological artifacts is controversial and always related to blood residues on stones or potteries often buried in soil in archaeological context.
From a laboratory study[59] and references included herein, the following conclusions can be drawn: the heme component of blood is relatively stable but the protein undergoes some degradation and the solubility of hemoglobin solutions allowed to dry on soil and clay particles is low.
More interesting are the results of heme detection on solid samples. A thick blood smear was applied on a glass slide and allowed to dry at room temperature for 14 days. Then, it was covered with soil or clay (in order to simulate burial conditions) for 6 weeks or buried in soil for 1 year. Then the reagents (here: TMB, derived from Benzidine, and phenolphthalein) were directly applied on the resulting solid samples. TMB did not detect heme either after 6 weeks or 1 year, while phenolphthalein detected heme easily after 6 weeks but only weakly on 2 of the 3 samples after 1 year, showing a decreasing sensitivity with time.

Although the conditions of these experiments are not those of the Shroud[1], it seems that the usual forensic tests for detection of blood are not well suited for the detection of the very small amounts (the tests on the Shroud were performed on single fibers, each certainly containing much less than a drop of blood) of a very old blood. The sensitivity of the reagents to hemoglobin in solution was tested and found to be 0.1 to 1 microg. /10 microl. Since a drop of liquid has a volume of 50 microl., and assuming that there is 5 microl. of blood on a single fiber (1/10 of a drop), the tests would detect 1 to 10 ng. of recent hemoglobin (no substantial difference was observed in the sensitivity of phenolphthalein for hemoglobin in solution and hemoglobin dried on soil or clay in this experiment). Therefore the sensitivity of these forensic tests is probably at the limit of detection for recent blood on the Shroud fibers. If we add the effect of aging and the poor solubility, it is not astonishing that McCrone tests were negative.
Regarding this question, McCrone simply wrote: "*One might argue that first century blood, or even 14TH century blood, would have to behave differently, yet similar tests on Persian burial silks and Egyptian mummy wrappings show no difference from my year-old blood spots*"[60] without giving any reference.]

6) <u>Immunological tests</u>:

In these experiments, specific antibodies are obtained and purified when foreign antigens are injected into the blood of a rabbit.
Human blood contains 2 main types of antigens: polypeptide type antigens (albumin for example) and oligosaccharide type antigens where the antigenic fraction is a small sugar molecule (ABO antigens on red cells for example).

If one injects these molecules into the blood of a rabbit, the animal produces antibodies that are highly specific for the antigen. These antibodies can then be used to recognize the antigens by different methods. The antibodies against human albumin react only with human albumin and not, for example, with albumin of dogs. However, if the antigens come from animal species which are closely related (human and baboons for example) false positive reactions can be observed (cross serology). In addition, antibodies against oligosaccharide type antigens can also react with other types of molecules like bacterial cell wall antigens.

[1] For textiles, I have found the following quote : "A suitable microscale Takayama crystalline test has not yet been developed for aged textile bloodstains" in http://aic.stanford.edu/jaic/articles/jaic43-03-002.html

Positive immunochemical tests were independently obtained by Heller and Adler and two teams of researchers[61] with antibodies for human whole serum and human albumin (polypeptide type antigens) as well as for blood type antigens and human globulins (oligosaccharide type antigens) on samples of the blood areas.
Many controls were used (baboon, dogs, rabbits etc.) in order to avoid problems of cross-serology. As expected, antibodies against proteins from primate species also reacted, but weakly, with the blood on the Shroud.
This pattern of tests allows one to identify the blood as human or at least of primate origin with a high degree of certainty.

VI) DISCUSSION :

1) The body-image:

- For McCrone, the image is comprised of a number of earth pigment red ochre particles well dispersed in a highly diluted yellowed collagen binder applied as a liquid. The area density of the pigment particles is relatively low, and the yellow color of the image fibers is mainly given by the collagen binder, which yellowed with aging.
As mentioned above, McCrone[62] applied an increasing number of tempera "paint" drops (from 5 up to 80) on a modern linen cloth. Each drop was from the same solution of 10 ppm red ochre in a 1% aqueous gelatin solution[2] . Therefore, he obtained spots of different known iron concentrations on roughly the same surface. On the photo shown in this paper, the concentrations vary from 0.2 microg. /spot up to 3.2 microg. /spot. The color resembles that of the image on the Shroud and becomes visible above 2.2 microg. In addition, there is clearly visible shading from 2.2 to 3.2 microg.
[*Comments*: unfortunately, to my knowledge, McCrone did not provide microphotographs of these colored threads, nor studies of the fluorescent and spectral properties of the spots. However, an interesting conclusion is that 2-3 microgr. of such iron oxide paint is able to give a visible discoloration and that the variations in iron oxide concentrations which give an appropriate shading are much lower than the sensitivity of the STURP's XRF instruments.]

- For STURP/Heller and Adler, the yellow color of the pure image fibers is not a painting and does not contain any proteinous binder. The chromophores are the result of some kind of dehydration, oxidation and conjugation by-products of the polysaccharide polymers of the cellulose of the fibers. They did not try to explain this fact. If there are very little iron-containing particles on the body-image fibers (except for the water stains), they have nothing to do with the image color[63].

Comparing the two statements, one understands that both agree that the image is mainly the thin yellow colored layer at the surface of the fibers and not a question of particles.

If the yellow color of the body-image comes from a dried highly diluted liquid collagen solution, it is difficult or even impossible to explain the extreme superficiality, the lack of capillarity and the discontinuous distribution. To the contrary, if an artist had applied a concentrated gel-like solution, which would be more consistent with those properties, the fibers would have been cemented together, which is not the case. Incidentally, McCrone

[2] In another paper, McCrone wrote « the paint on the shroud was dilute (0.01% in a **0.01% gelatin solution**) ». See: http://www.shroud.com/bar.htm

observed among all the samples, only 2 fibers cemented together with a yellow residue and in addition in a blood tape sample: 1AB[64].

Collagen (or gelatin and other animal glues) is a paint binder which is characterized by its solubility in hot water, its lack of solubility in organic solvents and the fact that it softens in cold water[65]. Water was tested by Heller and Adler on image yellow fibers and no reaction was observed. However, I do not know if it was hot or cold water. During the 1532 fire, the burned and scorched areas were submitted to very high temperatures. A collagen binder near these areas would have certainly been destroyed and the hot water (not very high temperature is required) would have dissolved the binder. Nothing like that is observed in the corresponding areas. To the contrary, STURP hypothesis is consistent with these observations.

Contrary to what is often claimed, none of the spectral properties of the whole Shroud can eliminate McCrone's hypothesis with certainty. Iron concentration variations by XRF measurements within the image are not accurate enough. The monotone curves on reflectance spectra (if the amount of pigment is very low), the similarity of UV and IR spectra with the scorches and even the lack of fluorescence of the image would theoretically *perhaps* be related to the aged collagen (although this is not proved at all).
Nonetheless, the STURP hypothesis is more easily consistent with all these observations.

Finally, the only absolute proof will be found in the search for proteins with the best tests available, both for sensitivity and specificity: no amide band with FTIR microspectrometry, no nitrogen-containing product with Pyrolysis-Mass spectrometry (PMS), negative fluorescamine test and no removal of the color with proteases (while the same test removed the red coating of blood fibers).
McCrone's retort was that: "…*STURP's protein tests were not sensitive enough to detect protein in the thinner body-image areas*…"[66] and he did not comment on the protease testing.
We have seen that PMS testing were designed to increase the sensitivity and traces of the low-molecular-weight fractions (oligomers) of the polyethylene bag that was used to wrap one of the samples were detected. PMS was performed not on a single fiber but on a sample, i.e. thousands of fibers: obviously, proteins would have been detected.
Heller and Adler demonstrated that fluorescamine is able to detect nano to picograms of proteins on old linen. They tested many image fibers from the different samples: it is highly doubtful that they would not be able to find proteins on at least some of the fibers.
Finally, the protease testing is perhaps the most important for our purpose because of the opposite behaviour observed for the image and blood fibers. Actually, these blood fibers can be considered as the "absolute" control. The dissolution of the red coating on blood fibers shows that the proteases dissolve the old denaturated proteins (either blood or collagen): if the yellow color of the image fibers is old aged collagen, the color must disappear in the protease solution, whatever its amount.

On the other hand, McCrone simply did not test for proteins (and did not test at all) the body-image fibers[67]. His claim of a collagen binder on the image fibers is *only* based on visual examination.

Finally, chemical analysis of the functional groups, FTIR Microspectroscopy of the image fibers and the lack of effect of all the solvents except diimide (and hydrazine) strongly support the STURP hypothesis of the image color being a dehydration-oxidation of the cellulose.

2) <u>The Blood-image</u>:

- <u>McCrone</u>: "blood" image consists of greater amounts of red ochre pigments particles and collagen binder than in the body-image. The pigments particles are not "widely dispersed" but often embedded in agglomerates by the binder. The particles are often visible in the agglomerates. In addition, vermilion particles are visible in blood areas but not in image areas.

- <u>STURP/Heller and Adler</u>: blood image consists of real blood. This blood is not whole blood but blood clot exudates. The hemoglobin is an old methemoglobin in its para-hemic form and the blood contains extraordinary high levels of bilirubin. In addition, there are haloes of serum around the blood marks. Careful examination of the blood marks shows that they are depressed in the centres, raised on the edges and that small serum haloes can be seen around all of them[68].
A consistent explanation for all these facts follows: the man whose image is seen on the Shroud suffered very strong traumatic conditions (scourging …) and many red cells hemolysed (red cells walls were broken) so that free hemoglobin appeared in the plasma. Under these conditions and within a few minutes, this hemoglobin is destroyed in the spleen and the liver to produce high levels of bilirubin. In addition, if the turn-over is not sufficient, the excess of hemoglobin spontaneously goes to the para-hemic form. In the wounds, the blood then began to clot: most of the red cells were retained in the clots, explaining why they are not found on the Shroud. Later, the material that was in contact with the shroud was therefore composed of serum (including its proteins, mainly albumin), hemoglobin (and quickly methemoglobin) in its para-hemic form and high amounts of bilirubin. Finally, on the cloth itself, the clotting process continued, so that most of the para-hemic methemoglobin (with the iron) was retained in the centre of the clot, while a serum halo appeared around the clots by clot retraction. The final clotting of the blood exudates on the Shroud explains the clotted appearance of the blood marks. Incidentally, it must be known that in blood, the red color does not come from the relatively low content of iron but from the heme of hemoglobin.

McCrone often claimed that he was an "*expert in using microscopical methods to identify the pigments, media and supports for each paint used in a painting*".
There is no doubt about that. But it is clear that he was not an expert in differentiating old blood particles from pigment particles only by looking through a microscope for the simple reason that he surely never saw such old blood. Neither McCrone nor Heller and Adler were "experts" in this field (and certainly nobody is): in this regard, the Shroud was a new challenge for science.

Heller and Adler, as well as McCrone, looked at real blood on linen and obtained opposite results. For Heller and Adler, blood on the Shroud closely resembles real blood while for McCrone it is the contrary.
Here the details are very important.
McCrone's real blood was in fact *highly diluted* (a 3% aqueous blood solution), recent, and painted on modern linen. It is not astonishing, in these conditions, that "*the diluted-blood-painted images, when taped, are very different from Turin shroud image tapes*" and that "*the brownish gelatinous blood coating on the linen fibers …shows no red particles…*"[69].
Up to now, I have avoided questioning the intellectual honesty of the researchers. But here, the "test" is so bad and so far away from the question that it is unfortunately obvious that McCrone's "observation" is simply nonsense.

In sharp contrast, Heller and Adler looked at the 2 year-old real blood on old linen and found that it "*closely resembles Shroud red coated fibrils, except redder color*", and that the resemblance increased after heating at 100°C. for one hour (aging). Although, they did not mention particles, it is not difficult to understand that with aging and mechanical stress, the coating will loose many tiny red particles all around, as well as the observed "shards" that are obvious fractured replicas of the red coatings.

Regarding the spectral characteristics of the blood areas on the whole Shroud, none appear to be inconsistent with McCrone's claims. This is the same for the STURP hypothesis and in addition, the iron content of the blood areas were shown to be consistent with real blood.
Only one fact seems to exclude the possibility for the blood stains to be real blood: the low level of potassium (K) as demonstrated by XRF at macro level as well as EDXRA at micro level (if the particles tested are really blood particles).
However, as Adler wrote, we must remember that the blood on the Shroud is not whole blood but blood exudates. Potassium amount is (in gr. /cm3): $1.6\text{-}2.4 \times 10^{-3}$ in whole blood and $1.4\text{-}2.2 \times 10^{-4}$ in serum[70]. Almost all the potassium is in the red blood cells. This is true even if the blood was partially hemolysed. When the blood began to clot in the wounds, most, if not all, the intact red cells were retained in the clots that were not transferred on the Shroud.
In the Heller hypothesis, the unusual low level of K is therefore readily explained, although not definitely proved by experiments.

Everybody can see the fluorescent halo around the main bloodstains in the UV-Vis photographs. In fact, small fluorescent haloes were also observed around all the blood marks, even around the scourges.
This simple observation is and remains probably the most definite proof that the blood is real blood.
There is no way to observe such a spontaneous behaviour for a painting. Therefore, to obtain these fluorescent haloes, the artist would have to spend hundred hours to deliberately paint them with collagen (which, incidentally, would be not fluorescent in body-image areas)…so that they would be invisible with the naked eyes.
I let the reader decide the absolute nonsense of such a possibility.
Could the blood marks have been painted with real human (or primate) blood?
Here is the very convincing answer of Adler[71]: "*We have shown …that [the blood] must have been taken from the exudate of a clot at a certain point in the clotting process. An artist would therefore have needed the exudate from the wounds of a severely tortured man, or baboon, and he would need to take the substance within a 20-minute period after the clotting had begun, and paint it on the cloth with the serum edges and all the other forensic precision that we see there*."

We turn now to the optical properties of the different red objects seen in the blood areas.

We have seen that they are the main basis of McCrone paint hypothesis.
From McCrone himself, it is clear that most of his observations were performed on the anisotropic Mylar tapes that change the optical properties.
It is important to notice that McCrone's claims about the red particles have changed at various times[72] : the paint was composed of 1) simply iron oxide particles, then 2) jeweller's rouge, an iron oxide available only after about 1800, identical to hematite, then 3) earth iron oxide pigment: the red ochre in a proteinous binder and finally, after the discovering of Vermilion by McCrone associates, 4) his final claim of liquid earthy iron-oxide paint with liquid vermilion paint to enhance the red color of the "blood".

Concerning the color and shapes of the particles, we see that McCrone, at least in his first careful observations, was not able to visually distinguish jeweller's rouge from red ochre, although jeweller's rouge smallest particles (about one micron) have uniform red color and uniform "equant" shapes under high magnification microscopy, contrary to red ochre particles that are yellow to red and have irregular shapes[73].
In addition, McCrone did see at first neither the proteinous binder nor the vermilion particle aggregates.

In short, McCrone's claims about the optical properties (birefringence, refractive index...) of the particles are meaningless in the conditions of his observations and even his statements on their color and shape properties are highly questionable.

The only microchemical tests performed by McCrone were the usual forensic tests for blood that are in fact very likely not suitable for the detection of blood on the Shroud for the reasons developed above.

Finally, the only tests that seem to be in good agreement with McCrone hypothesis are found in the elemental composition as determined by EDXRA and XRD of a few red agglomerates found in a single blood sample. However, the sample from which the particles analysed came from was so bad (very little red material, scorch near a burned area and in a water stain) that nothing important can be drawn: it can be blood as well as pigment or anything else. This area is clearly not representative of the true "blood" areas.
Vermilion is in fact the only absolute proof that there are paint particles in blood areas. But we have demonstrated by careful examination of all the data that it is only found in two blood areas and absent from all the other blood areas tested for Hg, even from the strongest stained. Therefore, it is very likely a contamination and can not account for the color of the blood stains.

We have shown that, with an exhaustive comparative study of a number of fibers from all kind of fibers, Heller and Adler were able to demonstrate clearly that the large majority of the red objects found specifically in the blood samples were not birefringent, not pleochroic and have a refractive index below 1.5, which definitely eliminate red ochre pigments.
They would have been able to conclude from these properties and visual resemblance with blood that the blood on the Shroud was real. However, they probably performed all the tests available to completely characterize the material, step by step, with the help of many controls. In addition, these studies were in the field of expertise of these authors.
They were worried not only by the sensitivity but also the specificity of their tests.

To summarize, they found on blood fibers:

- Proteins: fluorescamine testing, FTIR microspectrometry and proteases testing.
- Albumin of primate origin: microchemical testing, immunological testing.
- Porphyrin: hydrazine/formic acid fluorescence specific test. This porphyrin is not from vegetal origin.
- Hemoglobin/methemoglobin: specific Soret bands in spectra, indicative reflection spectra[*], positive hemochromagen tets, positive cyanmethemoglobin tests.

[*] This statement was confirmed by Dr.Bruce Cameron, experimenter in hemoglobin, who found that the spectra were those of and old acid methemoglobin. Cited in http://www.shroud.com/pdfs/ford1.pdf

- Specifically, methemoglobin in its para-hemic form found particularly in hemolysed blood (spectra).
- High levels of bilirubin (microchemical testing), consistent with the para-hemic methemoglobin, both consistent with the partial hemolysis of the blood of a tortured living man.
- Relatively low amounts of iron liberated *only* after digestion with Aqua Regia as expected for heme bound iron, contrary to the iron of iron oxide pigments.
- Iron content (XRF) consistent with blood.
- Absence of any visible iron oxide residue after dissolution with both proteases and hydrazine.
- Positive highly specific immunological tests with antibodies against whole human serum, human albumin, blood type antigens and human globulins.
- Laboratories experiments with blood+bilirubin matching the microspectrometry spectra observed on the Shroud fibers and showing a complete mismatch with iron oxide+vermilion in gelatine paint simulacra.

Even if one claims, as it as been done in non convincing "studies" (not published in any peer-review)[74], that some of these tests are not specific enough, it is simply impossible for all these tests to be wrong together.

The only possible conclusion is that the "blood" on the Shroud is real old denaturated human (or at least primate) blood.

VII) CONCLUSION:

I examined carefully, objectively and in detail almost *all* the published articles about this important question. All the arguments presented in these studies have been discussed: *nothing was omitted* (at least to my knowledge), contrary to what is too often observed on both sides of the controversy. In addition, I have tried to find in scientific papers on the Internet the answers to some of the most important questions.

I can conclude what follows:

- STURP, Heller and Adler conducted their studies following the true scientific method, using the largest set of methods and instruments available, carefully comparing the properties of all the types of objects, testing all the samples for a good representativeness, developing appropriate methods and using a number of controls. On the opposite McCrone's conclusions were in fact based on questionable visual examinations, a few poor tests on bad samples, using no or bad controls and without distinguishing between blood and image samples etc.

- STURP conclusions are compatible with all the observed facts, while McCrone's are not compatible with many of them. The STURP findings are self-consistent, and, some of them, could not have been imagined at the beginning (the bilirubin and the particular nature of the blood). They are also perfectly consistent with the forensic studies.

- STURP's interpretation is able to explain most of McCrone's findings[*] on the basis of his biased conditions of observations, his use of bad tests and finally wrong interpretations, while

[*] As another example, it was demonstrated by Jackson that the distribution of the particles found by McCrone can be easily reproduced by translocation of the particulate material of the blood stains in folding and unfolding the cloth several times. Cited in Ref.2

McCrone's hypothesis can not explain most of the facts observed by STURP or even by everybody else (fluorescent serum halo for example).

At the end of this paper, I would like to add some remarks not directly related with chemistry:

McCrone wrote at the end of his peer-reviewed article: "*These results suggest that a talented artist carefully studied the New Testament …and other artists' painting of Christ*[**]*. He then thought about a shroud image in terms of a dark tomb. Instead of the usual portrait with normal light and shadow, he assumed that the image could only be produced by body contact with the cloth. He painted directly on the cloth to image the body-contact points (forehead, bridge of the nose, cheekbones, mustache, beard, etc., over the entire body, front, and back). This automatically creates a negative image*".

Let's look again at the well-known photographs:

Now the question is: can anyone imagine that a "talented" artist would have painted the image (on the left) and would have obtained by an extraordinary chance the wonderful highly realistic image (which is in fact related to the unique three-dimensional characteristic of the Shroud image) seen on the right (the negative photograph) *without having in mind even the possibility to imagine such effect*? In addition, why would he avoid painting the two "bands" on the left and the right of the face. This effect is also seen in many other locations on the Shroud, strongly suggesting a relationship between the image and the slightly different chemical properties of the different batches of linen threads used to weave the cloth?

For this reason, many efforts have been made to reconcile McCrone's alleged findings of paint pigments as the cause of the image with these extraordinary properties. The most

[*] * Christ was never represented with a wound in the wrist, as on the Shroud, but in the palm.

interesting seemed to be Joe Nickell's transfer-rubbing over a bas relief. Unfortunately, McCrone examined the colored fibers obtained with this method and found that there were very different from the shroud's fibers….

Definitely, the Shroud is not a painting and the blood is real blood.
Whatever its true age, it is and remains a unique object in History and an enigma despite many years of intensive scientific efforts.
In recent years, some non-destructive methods have been developed that could be easily applied to the Shroud.
Will the researchers again have access to the relic?

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[58] In Ref.57

[59] D.M. Gurfinkel and U.M. Franklin. A Study of the Feasibility of Detecting Blood Residue on Artifacts. *Journal of Archaeological Science* 1988, 15, 83-97.

[60] In Ref.20

[61] P.Baima Bollone, *Sindone o No ?*, Societa Editrice Internazionale, Turin (1990) and E.Jumper, A.Adler and al., *ACS Adv.in Chem.*, 205 (1984):447. Both are cited in Ref.3.

[62] In Ref.20

[63] **Note**: the density of red particles in the body-image areas is certainly very low. Heller and Adler, as it seems, did not find many particles and Ray Rogers wrote me: "*My visible-light petrographic microscope has a resolution of about **300 nanometers**. I can easily resolve the pigment particles that are and were used for painting. **They did not appear on any of my tapes**. I have spent at least hundreds of hours (probably thousands over 26 years) studying my tapes, and I have taken hundreds of photomicrographs of different samples. Joe Kohlbeck, a professional microscopist at Hercules Corp., also looked at my samples*". Ray Rogers. *Personal communication (e-mail), 5 March 2005*.

[64] In Ref.20

[65] http://www.naturalpigments.com/education/article.asp?ArticleID=111

[66] In Ref.44

[67] McCrone finally admitted this fact when he wrote : « Obviously, I could not have conducted staining tests for collagen tempera on the body-image fibers … » in Ref.44

[68] In Ref.55

[69] In Ref.20

[70] http://en.wikipedia.org/wiki/List_of_human_blood_components

[71] In Ref.55

[72] See http://www.shroud.com/pdfs/ford1.pdf

[73] http://www.mccroneatlas.com

[74] See in Ref.72 for a complete comment of these « studies ».