

Report: Ink Study of Two Ancient Fragments through Micro-Raman Spectroscopy.

James T. Yardley, Department of Electrical Engineering, Columbia University.

Alexis Hagadorn, Conservator and Head of the Conservation Program, Columbia University Libraries.

Date of study: March 11-12, 2013

Date of report: May 13, 2013

1. Executive Summary.

We have studied micro-Raman spectra associated with selected features from both sides of two manuscripts on papyrus: “Gospel of John” (GospJohn) and “Gospel of Jesus’ Wife” (GJW). These manuscripts were provided for purposes of this study through Prof. Karen King of Harvard. Our conclusions from this investigation are as follows.

“Gospel of John”.

- The ink used in this manuscript is primarily based on a carbon black pigment such as Lamp Black. The observed Raman spectra are very similar to the carbon-based inks studied for a wide variety of manuscripts including several dated from the early centuries of the Christian era.
- From the observed Raman spectra, we find no evidence for any constituents of ink or types of ink other than carbon black.
- We have found no significant difference in the Raman spectra obtained from different regions of either side of this manuscript.
- Since the Raman spectra for carbon-black based inks vary considerably based on the composition, preparation methods, and thermal history of the ink, the strong similarity of Raman spectra for Side 1 and for Side 2 of GospJohn suggests that the two sides of the manuscript are written in identical or similar inks.

“Gospel of Jesus Wife”.

- The ink or inks used in this manuscript are primarily based on carbon black pigments such as Lamp Black. The observed Raman spectra are very similar to the carbon-based inks studied for a wide variety of manuscripts including many dated from the early centuries of the Christian era.
- From the observed Raman spectra, we find no evidence for any constituents of ink or types of ink other than carbon black.
- The ink or inks used in GJW are similar to, but distinct from, the ink used for the GospJohn manuscript.
- Within the available accuracy of our measurements, our data are consistent with a single ink composition for each individual side of the GJW manuscript.
- The Raman spectra obtained from the recto side and from the verso side are very similar within experimental error, although the data admit the possibility that the recto and verso sides for this manuscript could be derived from different but similar batches of ink.

2. Introduction.

Columbia University is home to the Columbia Nanoscale Science and Engineering Center (NSEC) – a research center funded by the National Science Foundation to explore electron transport in molecular nanostructures. This research center houses a number of state-of-the-art materials characterization tools including a Micro-Raman Spectrometer. Through measurement of the spectrum of laser light scattered from selected microscopic regions of a document or manuscript, Raman spectroscopy provides a powerful non-destructive methodology for characterizing the chemical composition of inks and pigments. Columbia University's Rare Book and Manuscript Library houses one of the largest collections of ancient papyri in North America containing over 2150 papyri dating from the 3rd century BCE to the 7th century AD. In addition to this collection, the library houses many ostraca and numerous additional ancient documents and manuscripts. In 2012, we established a team for a collaborative venture to explore the inks of selected papyri and other materials from the Columbia collection. This initial team consists of (1) Alexis Hagadorn, Head of Conservation, Columbia University Libraries, (2) Dr. David Ratzan, Curator of the Papyri Collection, (3) Prof. James Yardley, Managing Director of the NSEC, along with (4) Prof. Roger Bagnall, Director of NYU's Institute for the Study of the Ancient World (ISAW).

At Harvard University, Prof. Karen King has been studying a papyrus fragment written in Egyptian Coptic which is especially interesting since although it appears to contain material similar to the Gospel of Thomas or other early Christian gospels, some of the text may possibly make reference to a wife of Jesus. For this reason this papyrus has been called the "Gospel of Jesus' Wife." Since this text is somewhat unprecedented in early Christian literature and questions have been raised about its authenticity, it therefore invites careful study and scrutiny. Thus it makes excellent sense to attempt to apply the expertise developed within the Columbia research team to this manuscript toward a characterization and understanding of the ink or inks present within this papyrus fragment, to see if the ink is consistent with its having been written in antiquity.

Accordingly on March 11 and 12 of 2013, Prof. King brought this manuscript and an additional papyrus fragment ("Gospel of John") to the Columbia laboratories where the entire team examined and studied the fragment. This report describes the experiments carried out, the results of the micro-Raman spectral studies, and presents the conclusions based on this initial analysis of the data.

3. Manuscripts and Handling.

This project was undertaken by the entire Columbia team in collaboration with Prof. Karen King of Harvard. Fig. 3-1 through 3-4 show this research team during the two-day investigation.

Gospel of Jesus Wife Fragment (GJW), 3/11/13.

The papyrus was received in a housing of transparent polymethylmethacrylate sheets [Plexiglas™] sealed around all edges with a paper or synthetic fiber web carrier tape. High resolution images provided by Karen King prior to testing had revealed some cracks in the papyrus surface, but overall it appeared stable and well-integrated.

The fragment was removed from the housing by slitting the tape seal with a scalpel. It released easily, though there was a static charge holding the recto to its corresponding Plexiglas™ surface. The mounting system for the fragment in the chamber consisted of a base board of “Eterno board” acid and lignin free 100% cellulose pulp board [from Tschudi of Switzerland, purchased at Talas]. The fragment was affixed to this by placing two sides under folded 10pt acid and lignin free board, fixed to the edge of the base board using a plastic binder clip [fig. 5-3]. Testing was carried out on the recto and verso, so at the mid-point of the tests, the fragment was inverted on the board.

Fig. 3-1. Alexis Hagadorn unsealing GJW manuscript using a scalpel.



While testing proceeded with the fragment in the chamber, the outer surfaces of the Plexiglas™ were

cleaned by mechanically removing the tape and wiping with a 25:75 isopropanol and water mixture applied on the tip of a cotton swab. The inner surfaces of the Plexiglas™ sheets were not cleaned, but two small particles, probably of papyrus, were removed using micro tweezers and placed in gelatin capsules.

Fig. 3-2. Prof. Karen King and James Yardley at the micro-Raman Spectrometer.



During the testing, the fragment was placed in different positions on the mount as necessary using a micro spatula to manipulate it because this allowed it to be

lifted most safely. This slight motion caused a loose fiber at the top edge of the fragment to become detached during the course of testing. In the photograph shown in Fig. 4-3, this fiber can be seen directly above the first epsilon in line 1 on the recto side. The fiber was collected using micro tweezers and placed in a gelatin capsule. All of the samples were placed in labeled bags and given to Karen King.

The fragment was replaced in the Plexiglas™ in the original orientation at the end of the day. The edges were partially sealed with Tyvek™ tape. [Pressure sensitive acrylic adhesive on a carrier of flashspun high-density polyethylene fibers, this adhesive residue is softened in ethanol for removal.] Both fragments were transferred to the Columbia Rare Book and Manuscript Library for overnight storage and retrieved the next morning.

Fig. 3-3. Curator David Ratzan examining papyrus fragment.



Gospel of John fragment and GJW fragment, 3/12/13.

The Gospel of John papyrus was removed from its housing by slicing around the taped edges with a scalpel. This papyrus consists of one larger fragment and a smaller fragment placed below it but not in direct contact within the housing. When the housing was opened, a static charge was holding the fragments to the

Plexiglas™ on the recto side, but they were loose and easy to release. It was seen that a third very small fragment, appearing to contain part of a letter of text, was sitting loosely on top of this smaller fragment. In the images provided by Karen King prior to testing, this very small item can be seen sitting on the lower edge of the smaller of the two sections of papyrus, slightly to the right of center on this fragment. The largest fragment was subjected to testing and was mounted using the same mounting system described for the GJW fragment. The smaller fragment and the very small fragment remained in the Plexiglas™ sandwich during the testing.

While testing proceeded, the Plexiglas™ edges were cleaned. The tape which had been used to seal this fragment was a synthetic carrier with some fibers in the support, and a thicker and stronger adhesive than that of the GJW housing tape. This adhesive was removed from the exterior of the housing using pure ethyl alcohol, and the interior of the housing was not cleaned.

During testing, a single fiber became detached from the Gospel of John fragment, while the fragment was repositioned as needed for the testing. The original position of the fragment could not be determined. This fragment

Fig. 3-4. Papyrologist Roger Bagnall examines manuscript in Raman lab.



was collected with micro-tweezers and placed in a gelatin capsule within a labeled bag. Two small particles, possibly fragments of ink, were collected from the inner surface of the housing, which was not otherwise cleaned, and these fragments were placed in gelatin capsules. The labeled fiber and particles were given to Karen King, along with labeled samples of the tape previously used to seal each housing.

The GJW fragment was then reopened for further testing. While this work proceeded, the Gospel of John was replaced in its housing, following the original orientation of the fragments within the Plexiglas™. Since the original location of the tiny loose element could not be determined, it was placed at Karen King's request at some distance from the other two items to indicate no known position relative to the other two. The housing was sealed around all four edges using Tyvek™ tape.

After the second round of testing on the GLW fragment, it was also rehoused in the original orientation within its Plexiglas housing and was sealed with Tyvek™ tape. Both fragments, now rehoused and sealed, were returned to Karen King in the plastic bags in which she had delivered them. Supplies: Eterno Board from Tshudi of Switzerland; Tyvek tape, #TAD097001, Talas, 330 Morgan Ave., Brooklyn, NY 11211 (<http://talasonline.com/>). Fig. 3-5 shows the GJW manuscript as rehoused and returned to Prof. Karen King.

Fig. 3-5. GJW manuscript as rehoused and returned to Prof. Karen King on March 12, 2013.



4. Photographs of Manuscripts.

For completeness in this report we will provide here photographs for both sides of the two manuscripts under investigation in this report. These photographs have superimposed a grid system that allows definition of specific regions by a letter/number combination.

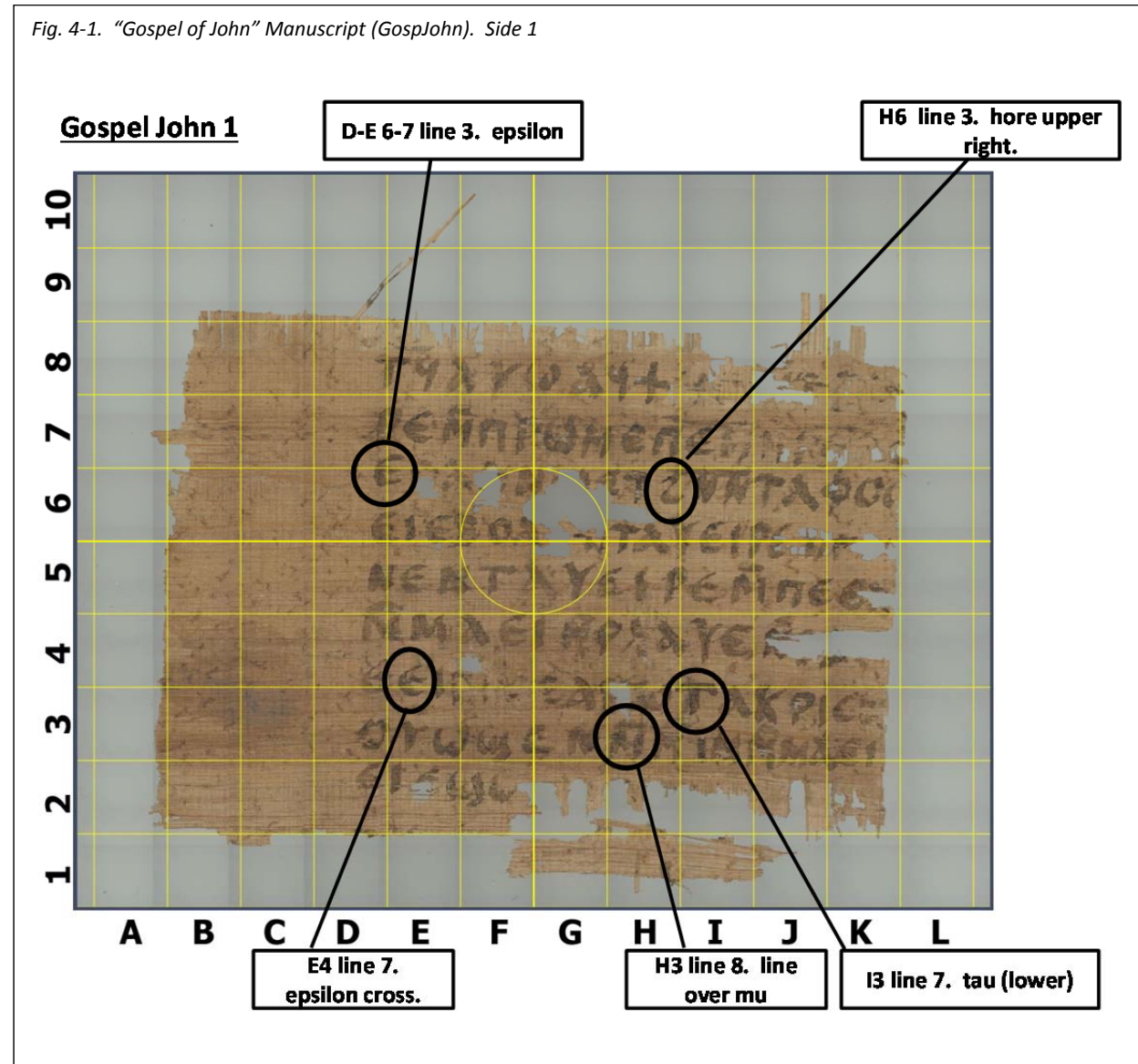


Fig. 4-2. "Gospel of John" Manuscript (GospJohn). Side 2.

Gospel John 2

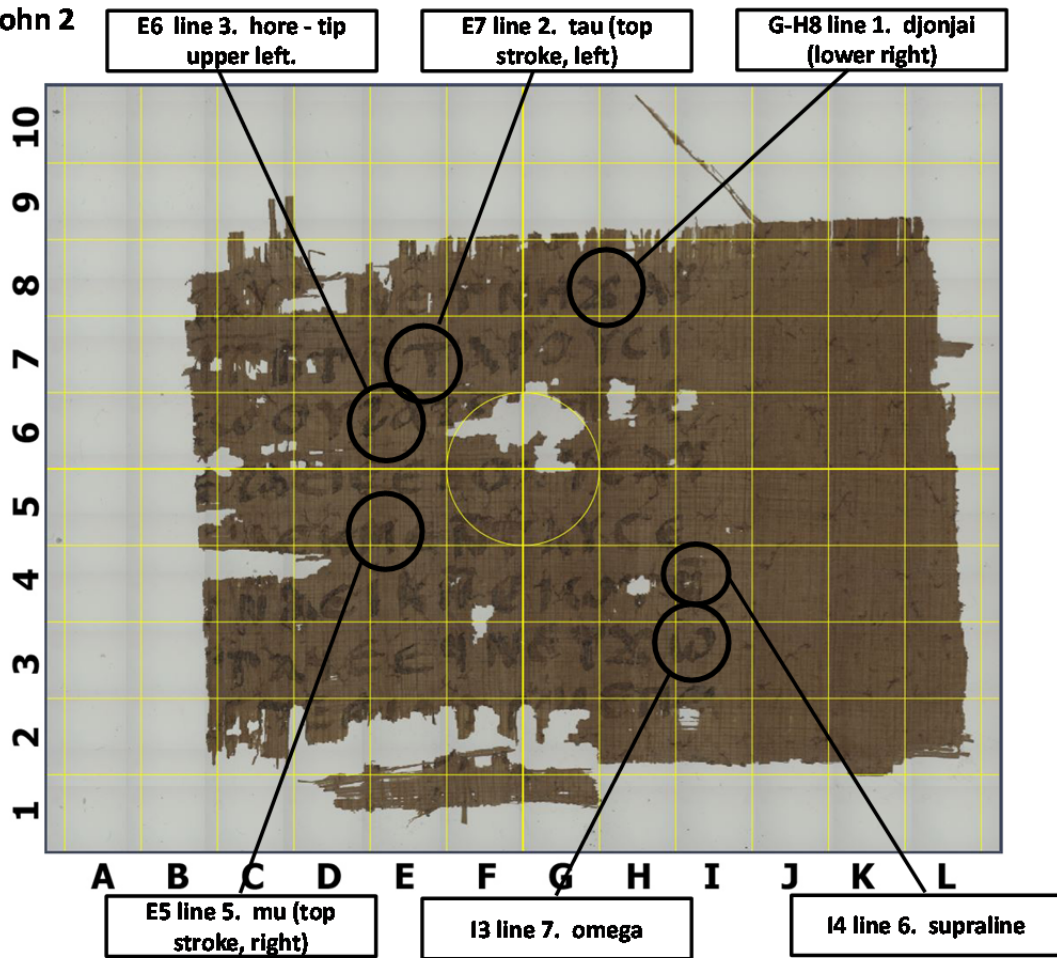


Fig. 4-3. "Gospel of Jesus Wife" or GJW, recto side.

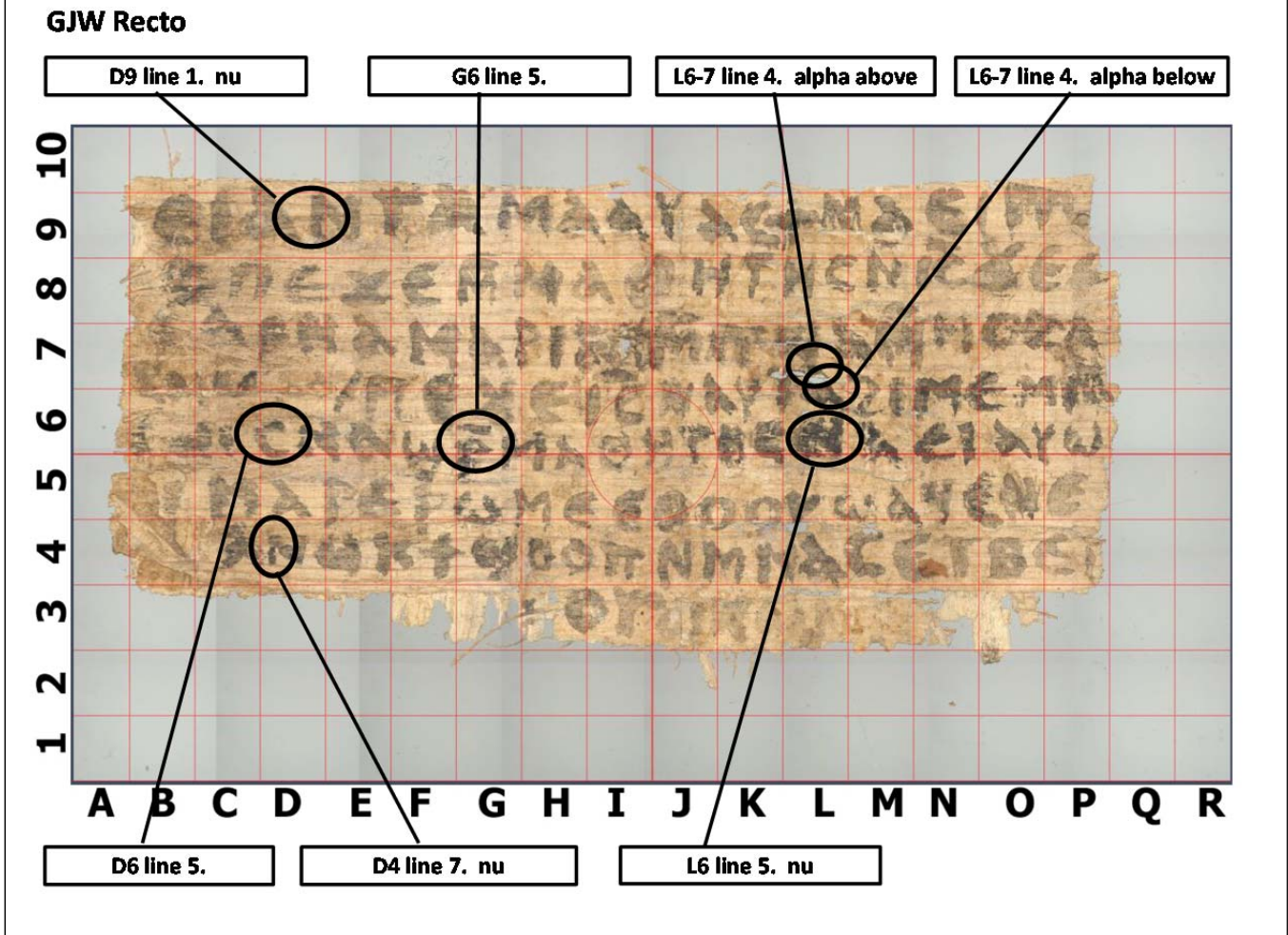
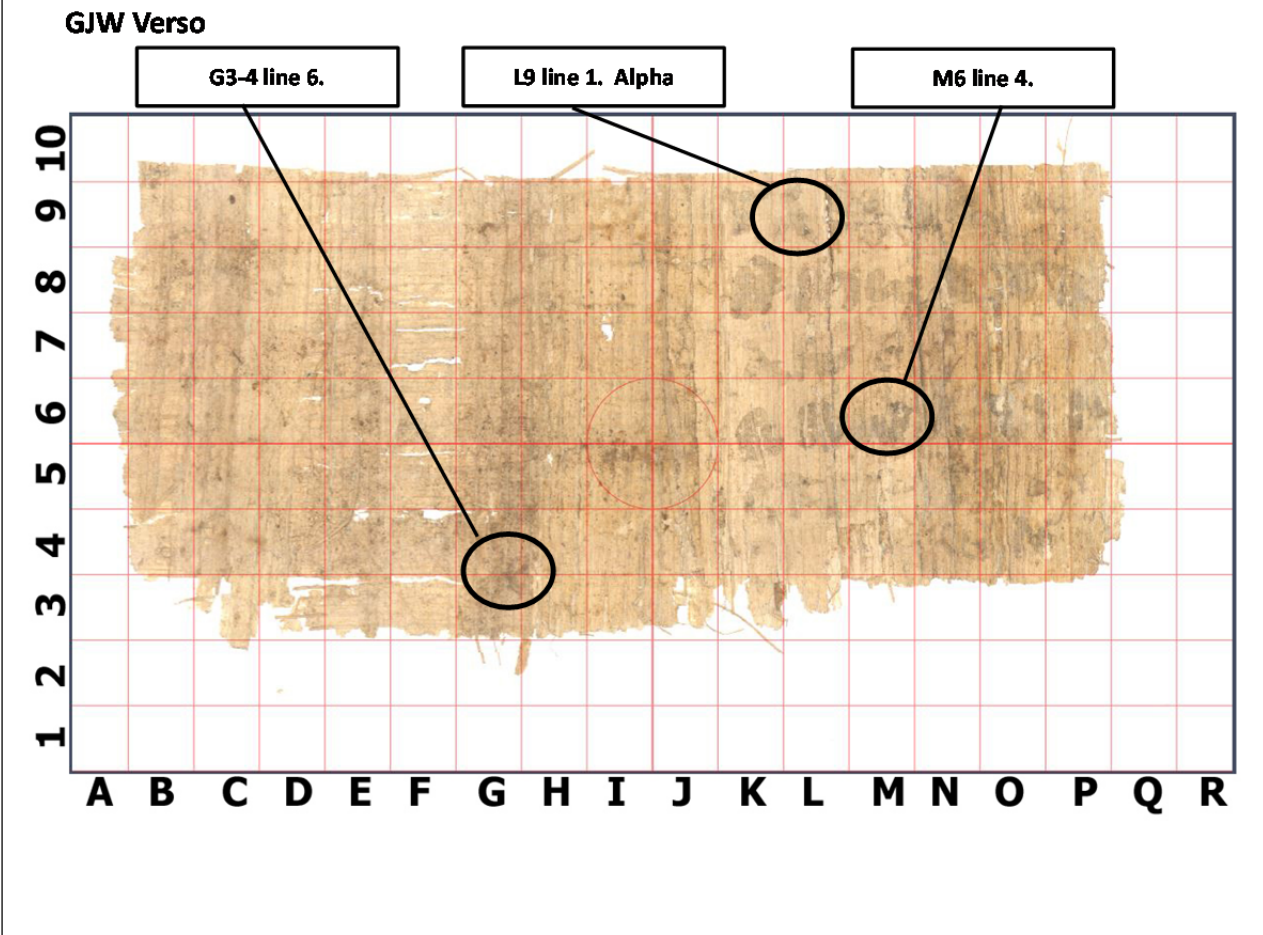


Fig. 4-4. "Gospel of Jesus Wife" or GJW, verso side.



5. Spectroscopic Measurements and Techniques.

Micro-Raman spectroscopy provides the basis for a non-destructive technique to characterize the chemical composition of inks and pigments. Fig. 5-1 gives a basic schematic diagram for a Raman spectrometer. In this system a laser provides exciting light that is focused onto a sample through an optical system including a microscope. The optical system directs light scattered from the sample in response to the laser excitation into a spectrometer consisting of a slit, a diffraction grating, and some additional optics. This spectrometer spreads out the scattered light by wavelength to create a spectrum of the scattered light spread across an array of photodetectors (CCD array). In the Raman scattering process a small fraction of molecules within the exciting beam absorb one quantum of energy corresponding to creation of vibrational motion within and simultaneously emit a quantum of light with energy reduced (and therefore wavelength increased or shifted toward the red part of the spectrum) by the amount of the vibrational quantum absorbed in the molecule. Therefore the measurement of scattered light intensity as a function of wavelength provides direct information about the nature of the molecules under investigation through observation of the characteristic vibrational resonances as peaks in the display of scattered light as a function of wavelength. Since dyes and pigments tend to have vibrational motions that are strongly coupled to light, Raman spectroscopy is particularly effective for examination of dye molecules and pigments within the sample. In *micro-Raman* spectroscopy, the exciting laser radiation is focused to a very small spot with a high-power microscope objective. This allows study of dyes and pigments in selected microscopic regions of the sample.

In this investigation, we obtained micro-Raman spectra using a conventional commercial micro-Raman instrument (Renishaw inVia Model). We used 633 nm (red) laser excitation (10 mWatt maximum power) for all spectra using 0.5%-10% of the laser power focused onto the sample through a conventional X100 microscope objective. We have found that this power level generally does not

Fig. 5-1. Basic schematic of the Renishaw Raman spectrometer system.

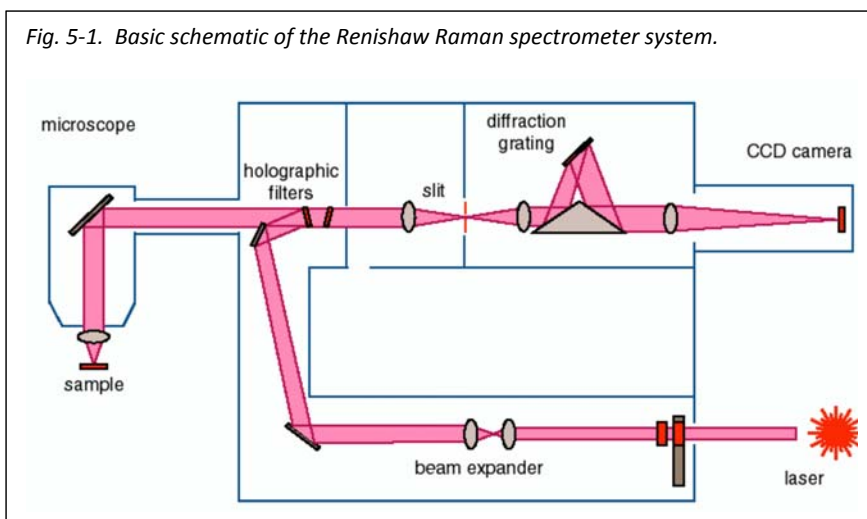
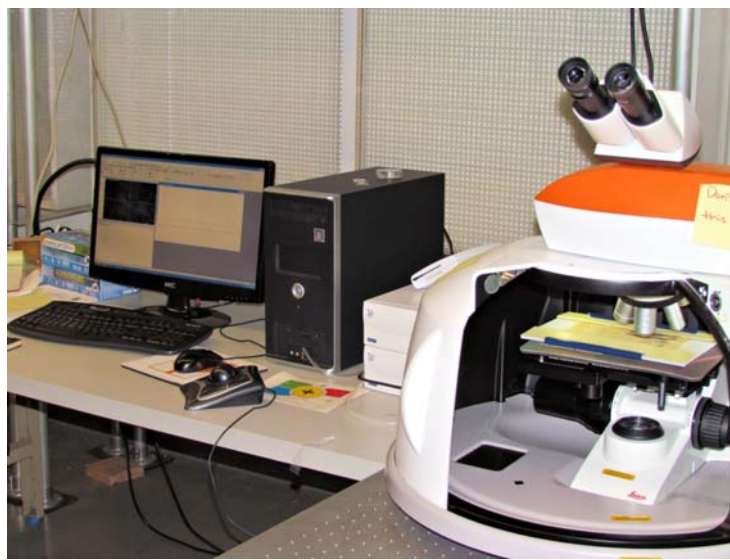
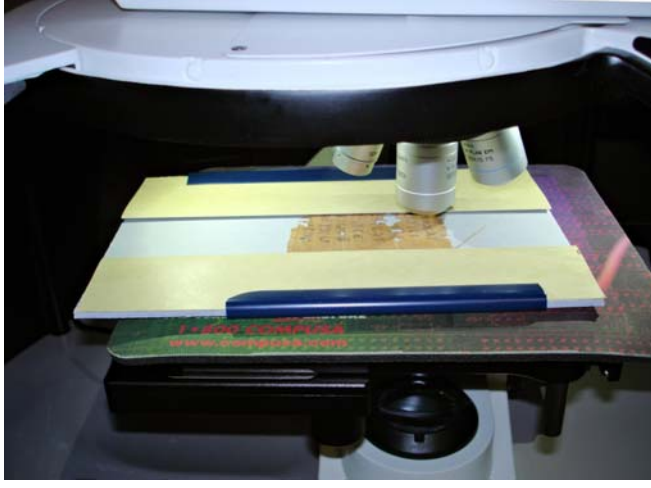


Fig. 5-2. Basic photograph of Renishaw Raman Apparatus showing sample chamber, display, and control interface.



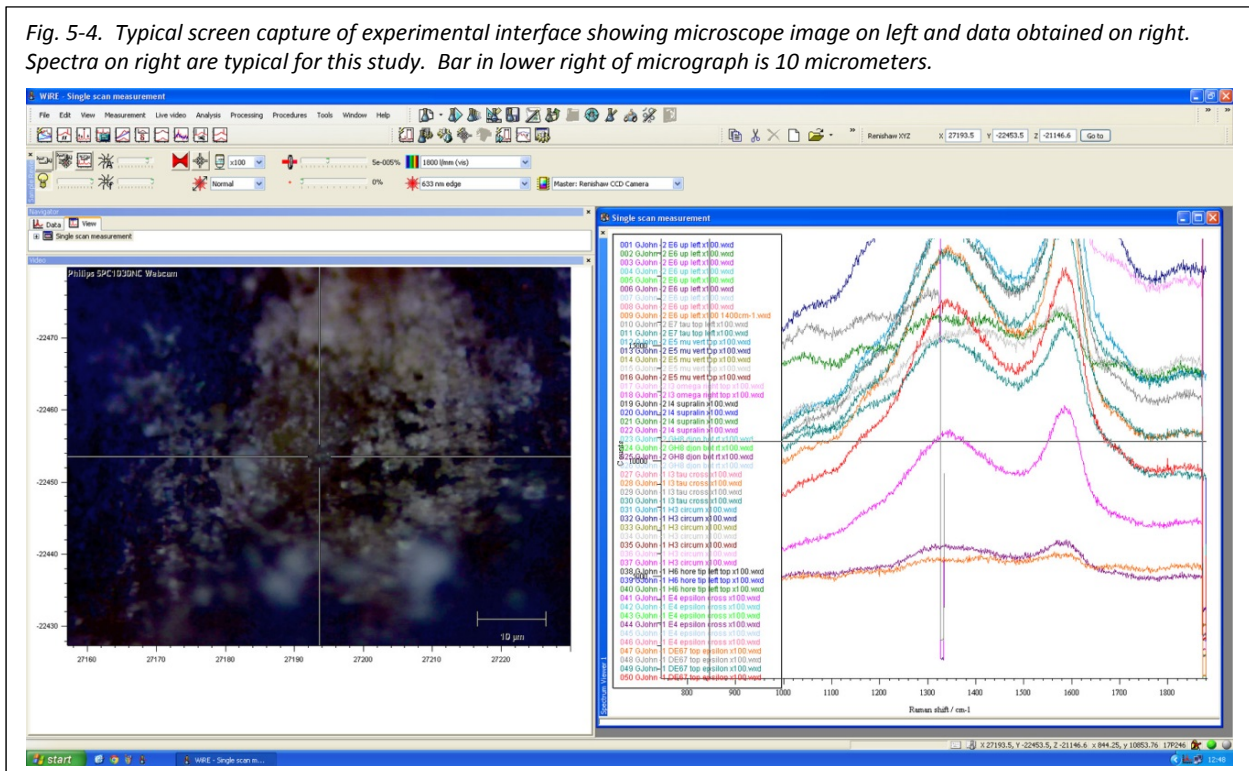
generate sample damage under normal spectroscopic examination. We manually placed the manuscript or fragment under investigation within its mounting system, as described in Section 3, under the microscope objective using initially an X20 objective. This allows visual confirmation of focus onto the chosen general region for study. We then changed to the X100 objective for careful focus, sample translation, and data collection. The instrument provides a visual display of the microscope image. In addition, the instrument provides the capability for direct control of focus and position of the stage under the fixed microscope objective using a track-ball interface. This allows the user to precisely locate and define a specific region for study. Fig. 5-2 shows a photograph of the experimental apparatus. Fig. 5-3 shows the manuscript and its mounting system in place within the Raman sample chamber underneath the X100 microscope objective.

Fig. 5-3. Photograph showing the GospJohn manuscript, Side 2, and its holder within the Raman sample chamber.



We used the following methodology to select a specific microscopic region of the manuscript for spectral examination. The microscope image display gives direct real-time presentation of the general region of the document under investigation. Fig. 5-4 shows an experimental screen shot from this study

Fig. 5-4. Typical screen capture of experimental interface showing microscope image on left and data obtained on right. Spectra on right are typical for this study. Bar in lower right of micrograph is 10 micrometers.



in which the microscope image may be seen on the left hand side. The spatial resolution of the microscope using the X100 objective is approximately 1 μ (micrometer or “micron”); the corresponding

depth of field (depth of the region in focus) is only about 3-4 μ . Since the overall topography of the manuscript varies through a depth of perhaps 20 microns or more, the microscope examines most carefully and collects light most efficiently from a region approximately 1 micron in diameter through a depth of approximately 3-4 microns. Thus we choose a specific region for investigation through direct translation of the stage and through change in the microscope focus, both of which can be directly controlled through the track-ball interface. In a typical microscope image we can see individual grains or domains of ink as well as papyrus fibers and other miscellaneous microscopic objects, depending upon magnification chosen. In order to minimize reflection and scattered light from the papyrus background, we selected regions or individual grains that showed the most intense black color (for example the region in the crosshairs of the image in Fig. 5-4). We also note that, because of the complex microscopic topography of the manuscript, the incident laser radiation may be reflected or refracted from the papyrus under investigation in complex ways. For this reason the light may be more effectively coupled into some regions than others. The Raman spectrometer provides the capability to superimpose a very weak (typically 10^{-5} per cent) exciting laser image upon the sample, reflection from which is visible also on the system monitor (not visible in Fig. 5-4). By monitoring the reflected laser light from the sample we are able to choose a region which gives maximum reflection from individual ink grains. We have found that this process provides the maximum micro-Raman signal. We believe that this is because strong reflection corresponds to optimal coupling of the laser beam both into and out of the chosen region for study.

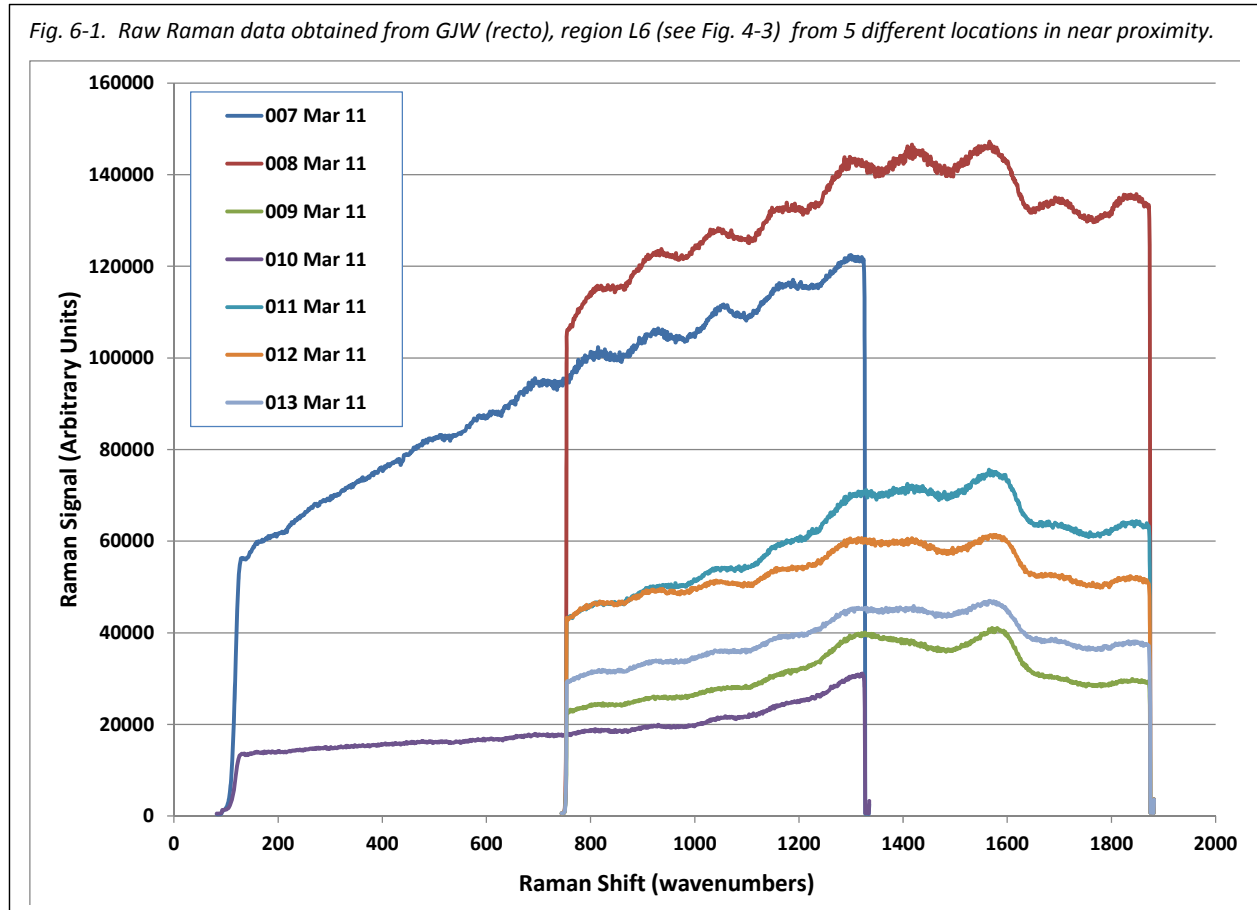
As shown in Fig. 5-1, the instrument collects the radiation from the microscope assembly and disperses it according to wavelength using a conventional 1800 line/mm grating onto a photodetector array (or "CCD array"). The resulting spectrum of collected light is then presented by the commercial instrument, through a computer interface, as intensity or "signal" as a function of energy difference between the photon energy corresponding to the wavelength of the exciting light and the photon energy corresponding to the wavelength of the collected light. The normal units for this energy difference (or "Raman shift") in Raman spectroscopy are wavenumbers (or cm^{-1}). These units may be converted into proper energy units of erg/molecule through multiplication by the factor hc where h is the Planck constant and c is the speed of light. Since a finite range of wavelengths can be monitored by a given diffraction grating/CCD array combination, the central Raman shift under examination must be defined by the user, determining the range of Raman shifts that are explored in a single scan of the instrument. Fig. 5-4 shows, on the right hand side, the visual display of several Raman spectra as signal intensity plotted as a function of Raman shift (i.e. vibrational energy loss within the sample). Each spectrum with its experimental settings may also be saved as a computer file for subsequent analysis. As mentioned above, the Raman spectrum is indicative of those specific molecular species, within the optical collecting region of the spectrometer, which have strong Raman response, such as dyes and pigments.

Experimentally, then, we chose each particular microscopic region for study using the trackball system to move the table holding the sample and also to change the focus and thereby the focal region. We monitored the reflected laser reflection to choose regions where the optical coupling seems optimal. We then set the experimental parameters for the spectrometer including laser power, central Raman shift examined (which determines the range of Raman shifts studied in an individual scan), duration of each individual scan (typically 5-10 seconds), and number of scans to be included in the reported output (typically 3-12). We chose the latter two parameters to provide for sufficient signal, without saturating the very sensitive detection system. In general we expect to obtain better signal-to-noise for longer scans or for increased numbers of individual scans collected. However there is one important complication for examination of practical manuscripts: The dimensions of the manuscript may drift as thermal changes, changes in humidity, and other environmental factors cause physical movement in the

manuscript. To the extent that this happens, the actual region in focus that is under investigation will drift as well. Thus the actual region being examined could change during the course of the measurement. In addition the coupling efficiency of light into the region of investigation may change, possibly causing the overall signal level may drift. Thus we cannot continue to collect information on a timescale long compared to these drift times. For the specific manuscripts studied here, the system maintained focus reasonably well during the data collection period of 10-60 seconds. We monitored this by examining the state of the focus when the each data set is completed since the microscope monitor is disabled during the actual scan. However in a small number of spectra we did observe clear signs of baseline drift. These spectra were not included in the data analysis.

6. General Observations and Data Analysis.

Over a two day period, we collected approximately 140 Raman spectra from specific regions of the two sides of the two manuscripts, examining in detail the Raman shift range from 150 cm^{-1} to 1900 cm^{-1} . The



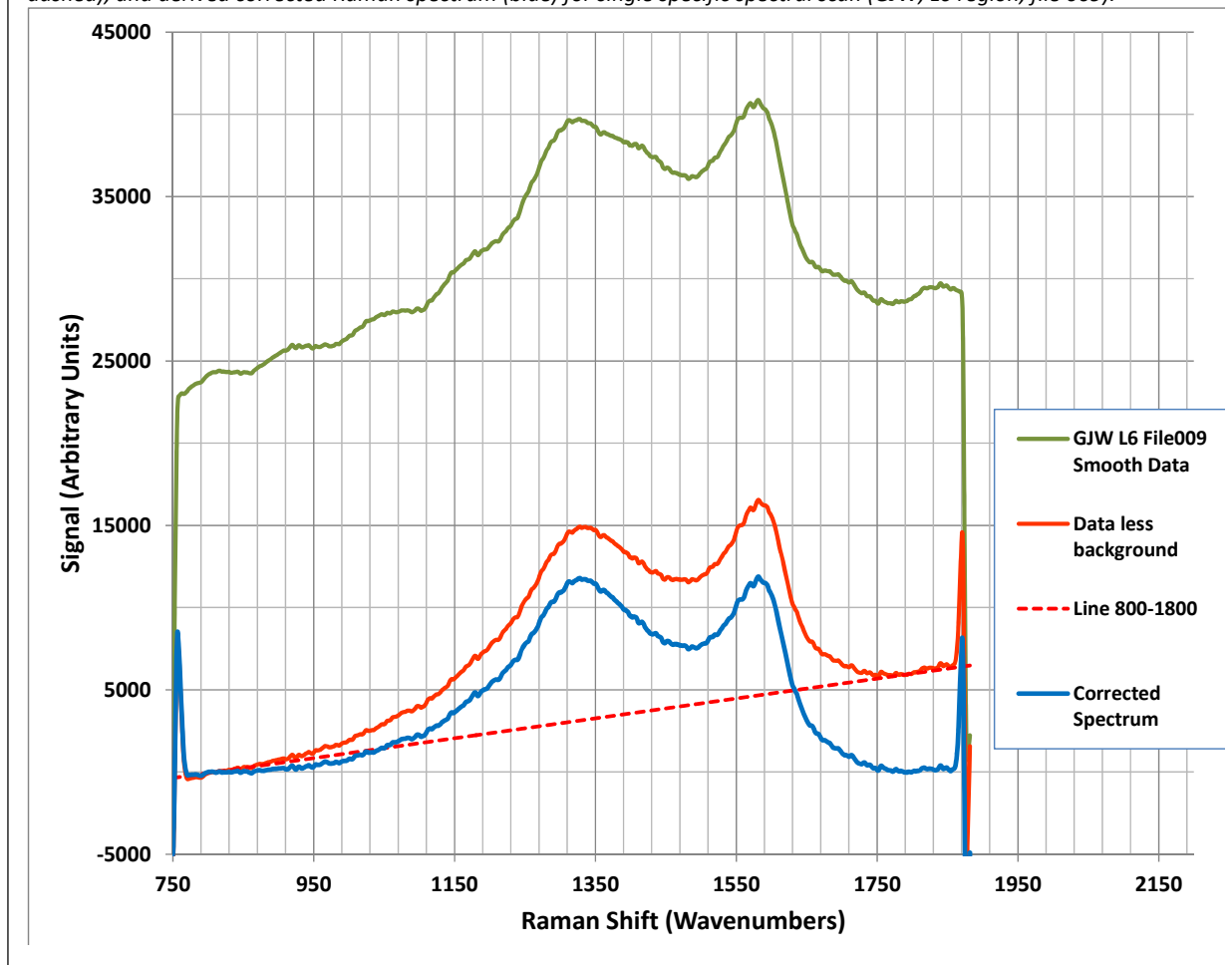
individual regions investigated are designated in Fig. 4-1 through Fig. 4-4. In addition we obtained several scans in the spectral range from 1900 cm^{-1} to 2750 cm^{-1} . Note: the instrument cannot provide Raman signals at Raman shifts of less than 120 cm^{-1} because of special filters used to prevent laser radiation at the exciting wavelength from entering the spectrometer system and saturating the detectors.

Fig. 6-1 shows, for example, the raw Raman scattering data of signal level (in arbitrary units – actual value depends on the duration of each individual spectral scan and the number of acquisitions used to obtain each spectrum) as a function of Raman shift. These data represent seven different acquisitions obtained from GJW, recto side, position L6 (see Fig. 4-3), all taken from spatial regions in near proximity. These data illustrate a number of key factors for the obtained Raman spectra. Firstly the overall signal varies considerably depending on the precise region examined and upon the coupling efficiency for light into and out of the region being studied. For example the data of file 007 and file 008 (as indicated in the legend for Fig. 6-1) were obtained sequentially, but do not show the same signal level at 800 cm^{-1} shift. Secondly on many spectral scans, there is a somewhat periodic or oscillating signal with a period of approximately 180 cm^{-1} with relative amplitude that varies from scan to scan. We have previously investigated this phenomenon in some detail. The oscillating signal results from radiation coming from the underlying material that is subject to optical interference in the detector array. We have found that

the net observed signal can be represented as the sum of a Raman scattering signal and a “background signal”, the latter of which varies with Raman shift. We have tested this in many independent experiments. Thirdly in the range of Raman shift shown in Fig. 6-1, from the instrumental lower limit of 120 cm^{-1} to 1900 cm^{-1} , except for the background signal mentioned above, there are no spectral features apparent other than two peaks which can be seen in all scans – a spectrally-narrower peak near 1600 cm^{-1} and a broader one peaking near 1350 cm^{-1} . We observed the same general qualitative pattern shown in Fig. 6-1 for all Raman spectra obtained for both sides of the two manuscripts under investigation. In no case did we observe any other distinct spectral features.

For analysis of these data then we carried out the following procedure. First we smoothed the data using a Loess smoothing scheme that provides a weighted average of 9 points near each individual data point. This effectively averages the signal over a spectral width of about 3 cm^{-1} . The upper trace of Fig. 6-2 for example shows such a smoothed signal for one particular Raman scan (file 009 from GJW, position L6 – see green line of Fig. 6-1). We then subtracted a baseline obtained from the same series of

Fig. 6-2. Smoothed raw spectrum (green), data less base background (red), linear fit between 800 cm^{-1} and 1800 cm^{-1} (red, dashed), and derived corrected Raman spectrum (blue) for single specific spectral scan (GJW, L6 region, file 009).



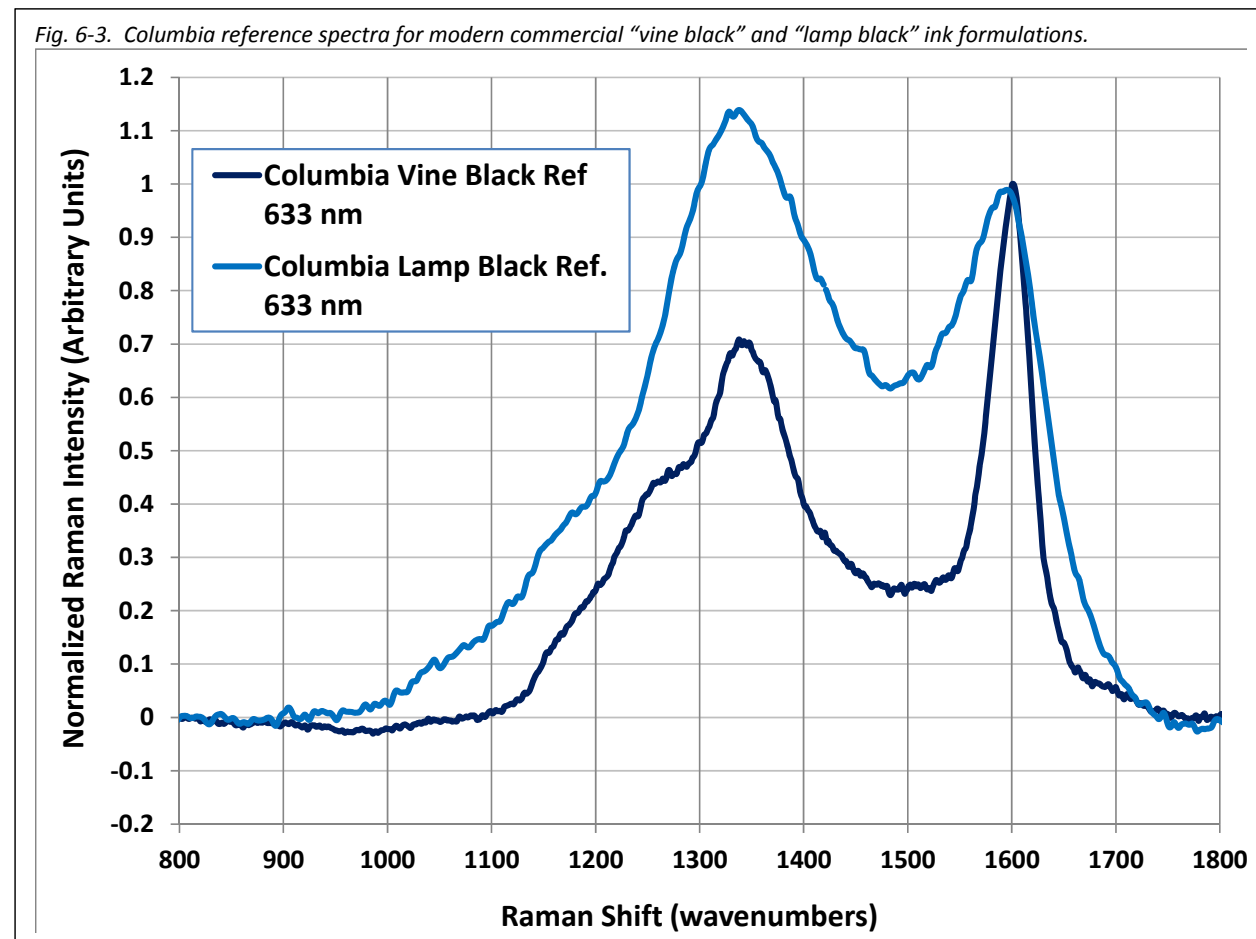
experiments for a region away from dark pigment grains (i.e., we subtracted an experimental background). During the course of the experimentation we obtained a number of these background spectra. For the data analysis here we have chosen to use a single background spectrum which exhibits little variation with Raman shift over the range $750\text{--}1900\text{ cm}^{-1}$ other than the oscillatory behavior. Thus

the second step in the data analysis is to subtract this specific background spectrum multiplied by a mathematical factor chosen for each measured spectrum to eliminate the oscillations of signal with Raman shift from the raw spectrum. This process provides a spectrum with the background removed. The second (red) trace for Fig. 6-2 shows the corrected data obtained for this particular spectrum. In general specific Raman features in these corrected spectra appear upon an additional background varying slowly with Raman shift due to a variety of sources. For purposes of the data analysis here, we have chosen to fit a straight line between the spectral points at 800 cm^{-1} and 1800 cm^{-1} which are well out of the Raman shift range for the spectral features of interest (as seen in the dashed red line of Fig. 6-2). We then subtracted this additional base level to derive a final corrected spectrum (shown in the blue line of Fig. 6-2). Such baseline subtraction is very common in Raman spectroscopy where broad background signals are almost always present. We have experimented extensively with variations in this procedure including changing the linear baseline choice, changing the choice of background used for subtraction, and other variations in procedure. These variations have no substantial change in the resulting corrected spectrum. We note particularly that the resulting corrected spectrum is very insensitive to the precise value of the multiplicative factor used to remove the background since in all data chosen for study, this background is small. For comparison purposes, as a final step, we normalized the amplitude for each spectrum to the value of 1.0 at its peak value between 1550 and 1650 cm^{-1} (i.e., the peak amplitude near 1600 cm^{-1} was set to a value of 1.0). This enables the spectral features derived from each spectrum to be quantitatively compared. The reason for this choice of normalization derives from our understanding of the origins of these spectra features as discussed below.

Using the above well-defined procedure we analyzed each of the 140 individual spectra that were taken during the two-day experimental period. We discarded those spectra that did not provide sufficiently-high signal-to-noise ratios to be useful, excessively high background contributions, or that exhibited anomalous baseline shifts (possibly due to instability in focus). However in all cases the basic observed spectra exhibited the same overall qualitative spectral behavior as seen in the spectra chosen for detailed analysis. Otherwise all data were retained even though the signal-to-noise was higher in some cases than others. The spectra shown in Fig. 6-1 and 6-2 above are quite typical of the greatest part of the spectral data.

Most dyes or pigments exhibit characteristic Raman spectra. The published literature includes many detailed descriptions of the use of Raman spectroscopy to characterize pigments and ink materials¹⁻⁴. Furthermore, several catalogs of observed Raman spectra derived from manuscripts based on papyrus, vellum, parchment and other substrates have been published in libraries and other review papers^{5, 6}. Black ink pigments generally fall into the categories carbon black (many variations)⁷, iron gall⁸⁻¹¹, hematite¹², magnetite¹³, various iron oxides¹⁴, lead oxide, lead sulfate, and lead sulfide. Modern black inks are derived from complex formulations, typically exhibiting numerous sharp spectral features at Raman shifts less than 1000 cm^{-1} ¹⁵⁻¹⁸. Each of these general types exhibits well-characterized and unique general spectral characteristics. However, details of Raman spectra for black pigments are in many cases dependent upon specific chemistry which can vary within each of these classes of pigments. Some black pigments are based on various forms of “carbon black” which we will use here as a generic term for carbonaceous particulate material containing both amorphous carbon and partially graphitic or crystalline carbon. Carbon Black is normally produced by incomplete combustion of oils, tars, and other natural products. Furthermore the detailed shapes for some Raman spectra (especially carbon black spectra) are also dependent on specific pigment preparation conditions and can depend somewhat upon characteristics of the type of Raman spectrometer, including for example the excitation wavelength. For this reason at Columbia we have developed a set of reference spectra based on known formulations of modern pigments, obtained with our own particular instrument¹⁹. Fig. 6-3 shows two

such reference spectra for carbon black pigments: one based on a “vine black” pigment prepared from soot derived from burned vegetable matter, and one based on a “lamp black” pigment prepared from



soot from burning oil, both placed on a modern papyrus substrate. These spectra were derived using precisely the same instrumentation and data analysis schemes outlined above and used for this study.

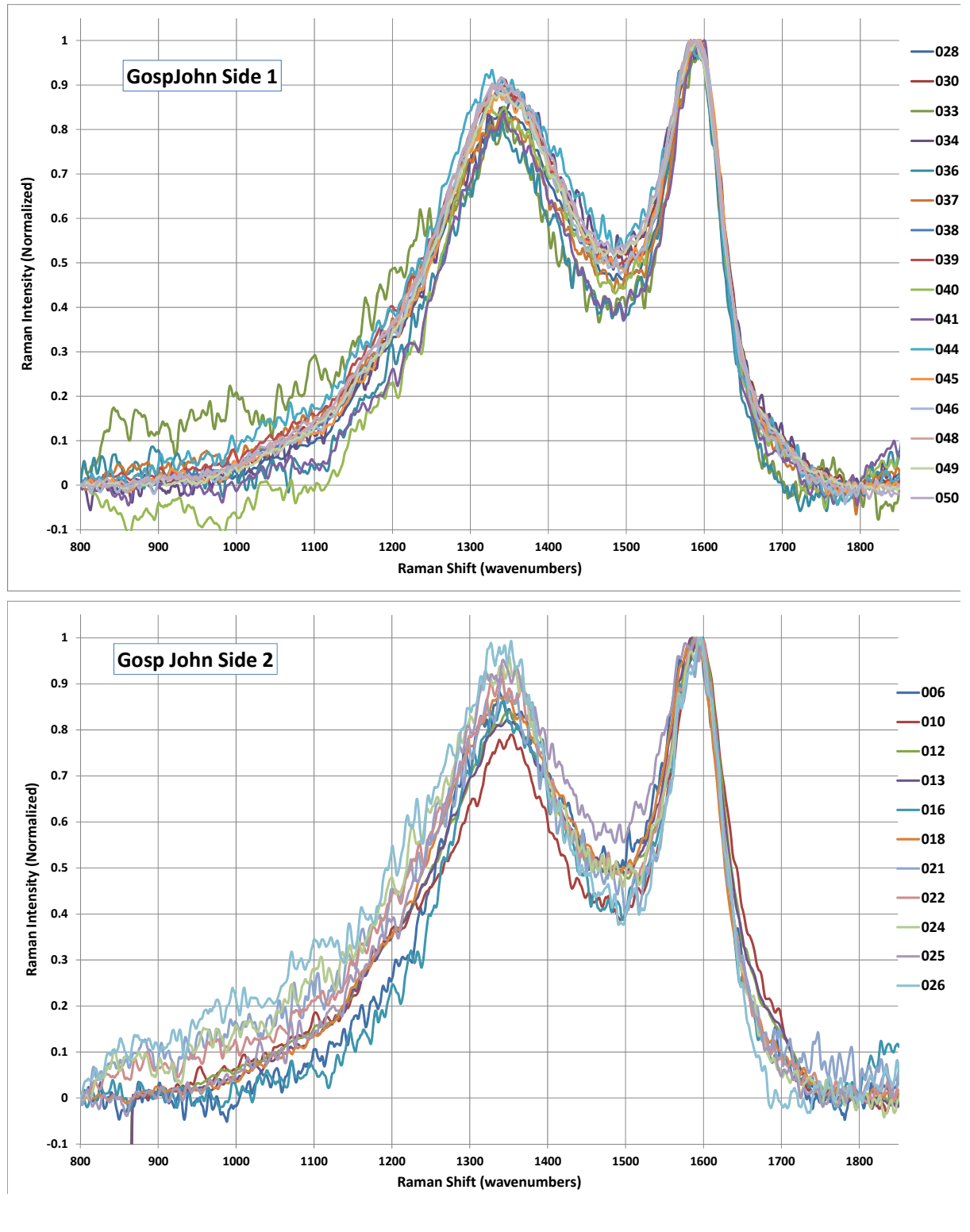
For the manuscripts of this investigation, we did not find spectral features characteristic of any of these well-known black pigments other than those which clearly belong to carbon black. The spectra illustrated in Fig. 6-2 are clearly similar to our reference spectra for lamp black and vine black shown in Fig. 6-3. These spectra are also quite similar to many reported Raman spectra of black features for papyrus and other manuscripts both ancient and modern^{20, 21}. We can thus state with a high degree of confidence that carbon black is present to a major extent as a black pigment in both of the manuscripts under study. Based on the absence of other Raman features, it is very unlikely that there are any other significant colorants or pigments present, at least in the specific regions investigated.

The origin of the spectral features for the Raman spectra various forms of carbon black has puzzled spectroscopists for many decades²². Because of the importance of carbon black and related forms of carbon this has been the subject of extensive investigation²³. Graphite, a crystalline form of carbon, shows only a sharp peak near 1580 cm^{-1} in this spectral range. Early studies of the Raman spectra of carbon black materials showed that the features near 1580 cm^{-1} were fairly consistent while those at other wavelengths, especially the prominent band near 1350 cm^{-1} varied in relative intensity and spectral distribution with exciting wavelength and sample preparation²⁴⁻²⁹. Modern interpretations are

that the higher energy feature relates to the proper Raman spectrum of ordered carbon in large crystalline graphitic structures while the lower energy features near 1350 cm^{-1} arise from nanoscale particulates with structural symmetry reduced from that of graphite³⁰⁻³². Thus the shape of the lower energy bands and their intensity relative to the higher energy band is highly characteristic of the preparation methodology and thermal history of the material. For this reason the Raman spectrum in the 1350 cm^{-1} band for inks based on carbon black vary considerably in shape and in signal intensity relative to the 1600 cm^{-1} band.

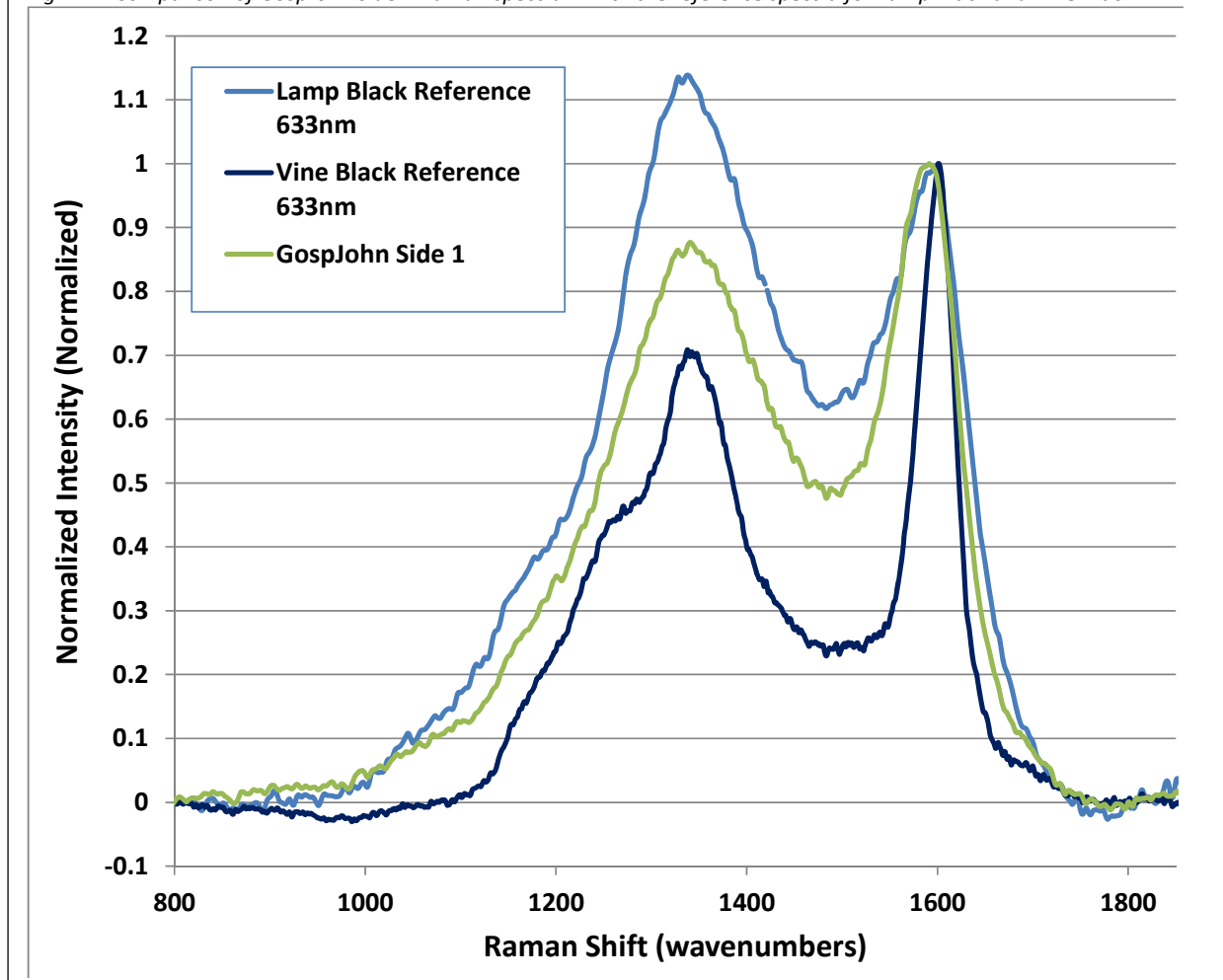
7. Gospel of John (GospJohn): Observations and Results.

Fig. 7-1. Raman spectra for the manuscript Gospel of John, Side 1 and Side 2 obtained at various regions within the manuscript. The numbers at the right represent individual experiment designations.



Since the Gospel of John document proved to be simpler to interpret, we will discuss it first. Fig. 7-1 shows all of the retained observed Raman spectra from the Gospel of John, side 1 and side 2. From these figures it is clear that the Raman spectra for each side are essentially identical within the overall experimental error. The spectra show one relatively-narrow peak near 1597 cm^{-1} and a broader peak

Fig. 7-2. Comparison of GospJohn Side 1 Raman spectrum with the reference spectra for Lamp Black and Vine Black.

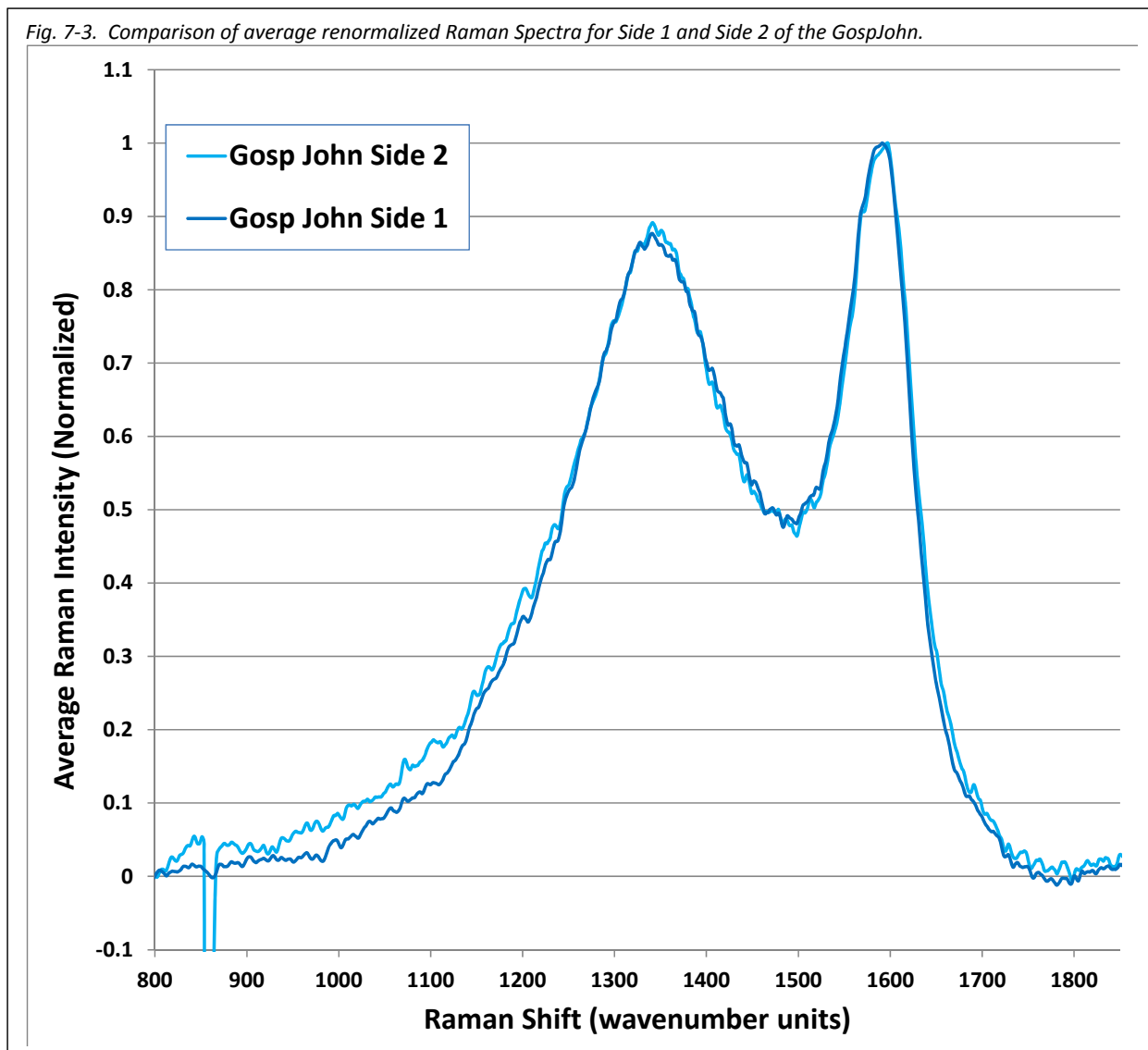


near 1341 cm^{-1} . These features are characteristic of the Raman Spectrum of many forms of carbon black as discussed above. The origin of these features in the Raman spectrum of carbon black, which has been the subject of intense research over the past three decades, is described in Section 6. For the purpose of this report however we can state clearly that these spectra match closely those reported in the literature for numerous variations of carbon based ink, especially lamp black inks. This point is made clear in Fig. 7-2 which compares directly the averaged and renormalized spectra for Side 1 of GospJohn with our reference lamp black and vine black spectra. The observed GospJohn Side 1 spectrum falls between these two reference spectra and conforms to the same general shape.

An examination of Fig. 7-1 shows that the spectra of Side 1 and Side 2 of this manuscript are also quite similar. We can test this hypothesis by computing an average spectrum for side 1 and one for side 2 based on all data retained for analysis. The resulting average spectra are demonstrated in Fig. 7-3. These spectra are certainly identical within the experimental error. Since it has been quite well established that the specific shape of the broader band near 1340 cm^{-1} is very sensitive to the precise nature of the ink preparation, these data strongly imply that the ink used in the production of side 1 of

this manuscript is the same as the ink used to produce side 2. The similarity of the observed Raman spectra shown in Fig. 7-1 taken at several different regions within the manuscripts as designated in Fig.

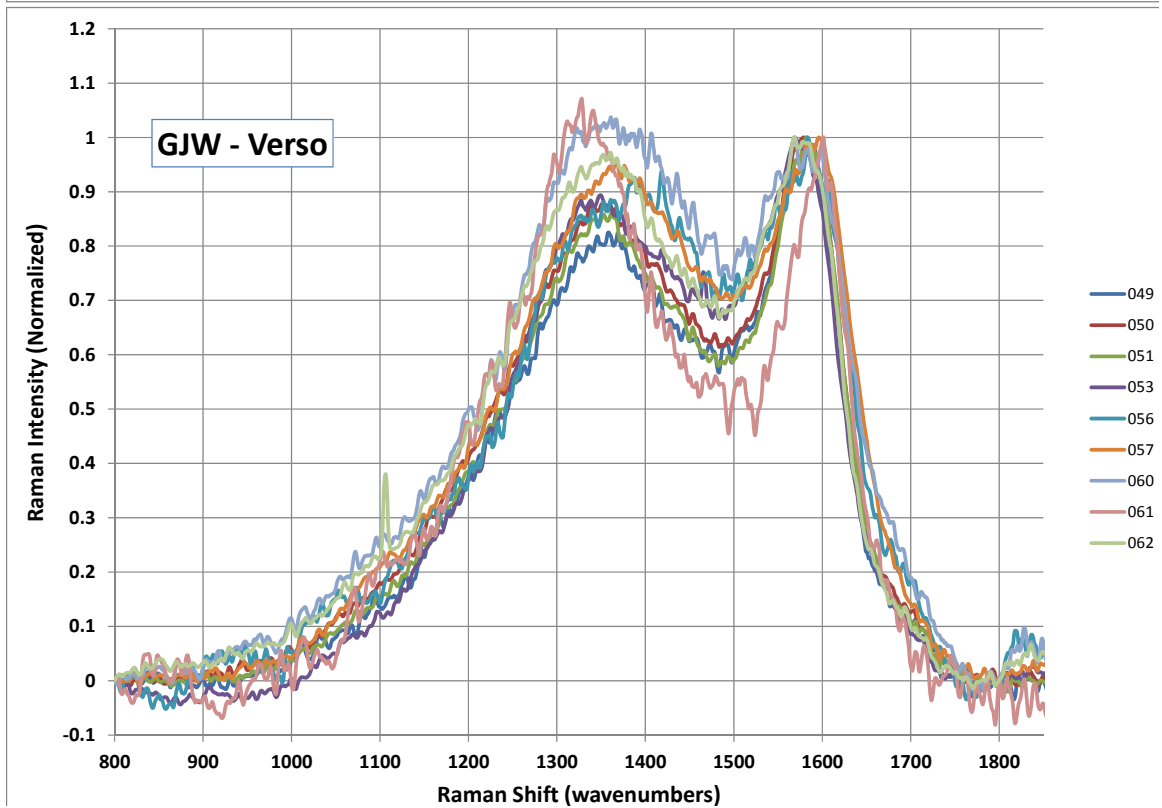
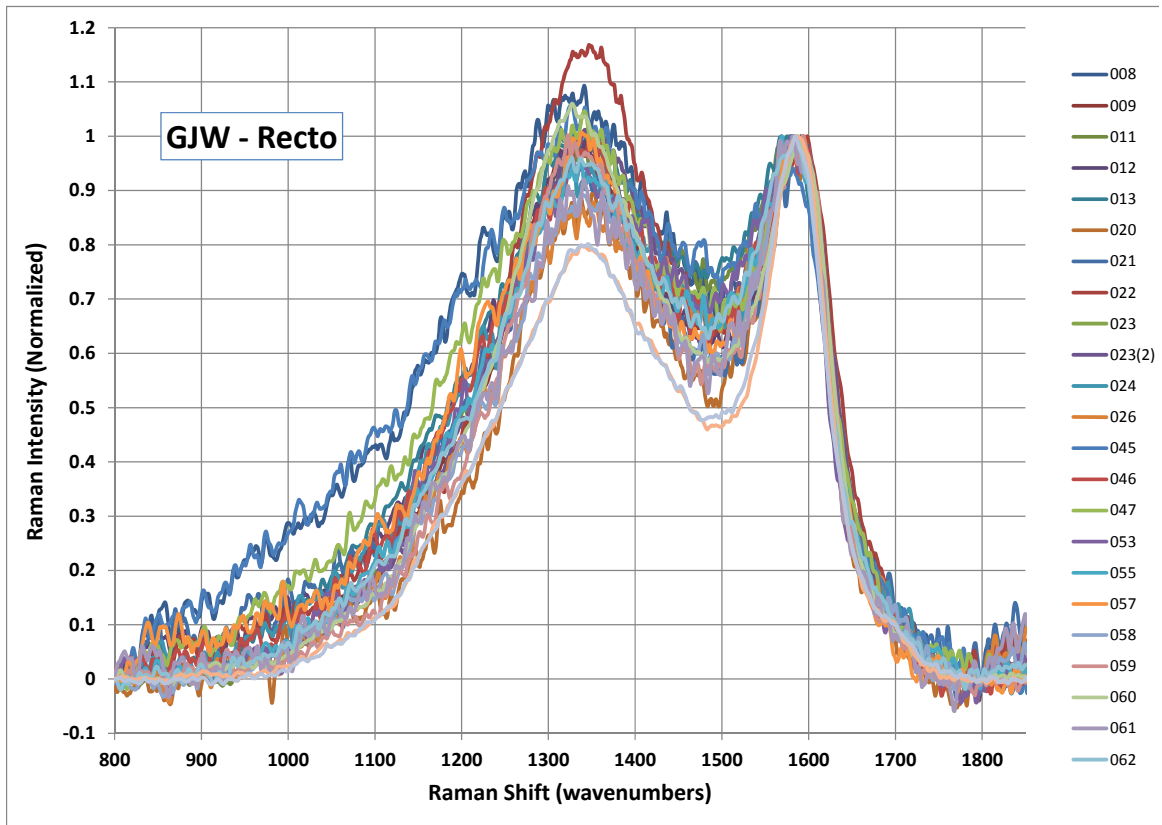
Fig. 7-3. Comparison of average renormalized Raman Spectra for Side 1 and Side 2 of the GospJohn.



4-1 and Fig. 4-2 suggests strongly that the ink from all regions on both sides is very similar if not identical (at least as characterized by Raman spectroscopy).

8. Gospel of Jesus' Wife (GJW) – Results and Interpretations.

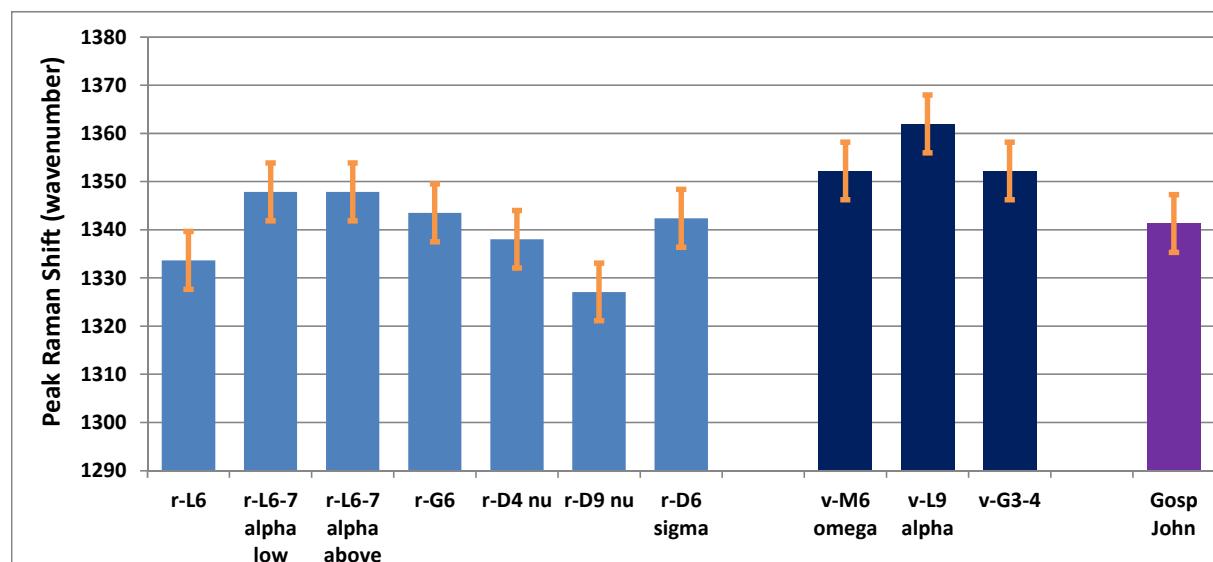
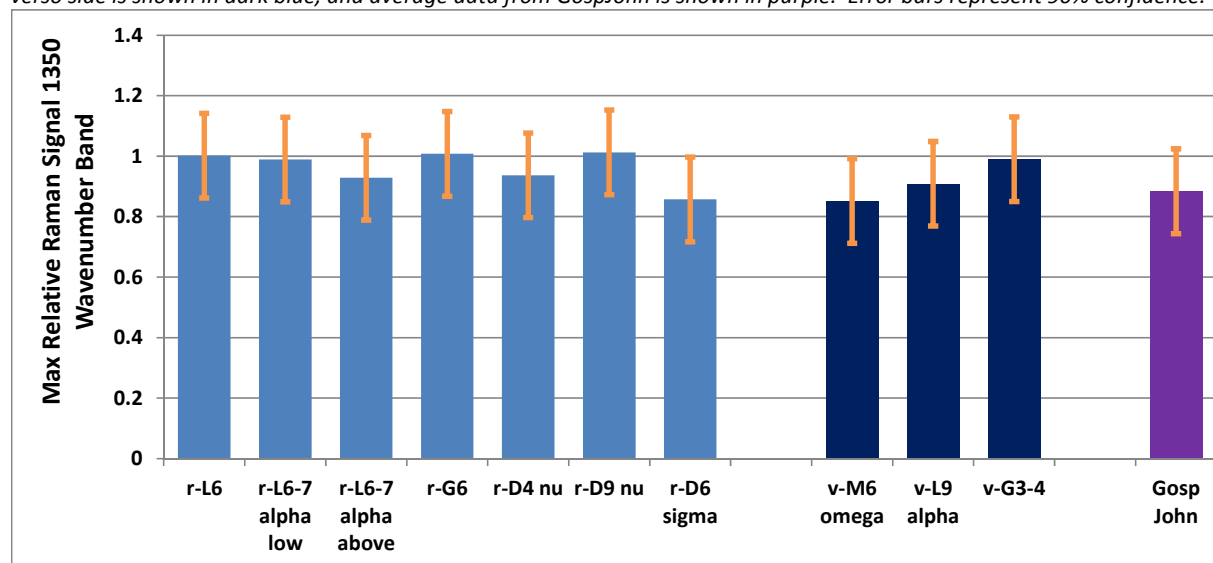
Fig. 8-1. Raman spectra for recto and verso sides of GJW. Numbers on right are experimental designations.



General Observations. For the GJW manuscript we explored Raman spectra at multiple selected locations on both recto and verso sides (see Fig. 4-3 and Fig. 4-4). Since our analysis is based on a total of 25 spectra from the recto side taken from 7 different locations and a total of 9 different spectra obtained from 3 different locations, we have a limited data set to examine. Fig. 8-1 shows a composite of all spectra retained for both recto and verso sides. These spectra are clearly similar to those found for the GospJohn manuscript discussed in Section 7. Thus it is clear that the ink examined through these Raman spectra are based primarily on carbon black pigments. In Fig. 8-1 the spectrum-to-spectrum variations appear to be greater than for those shown in Fig. 7-1. This may be due to the intrinsic signal to noise for the experiments or it may be due to differences in inks for the recto versus the verso sides, or it may be due to possible difference in inks in different regions within a single side.

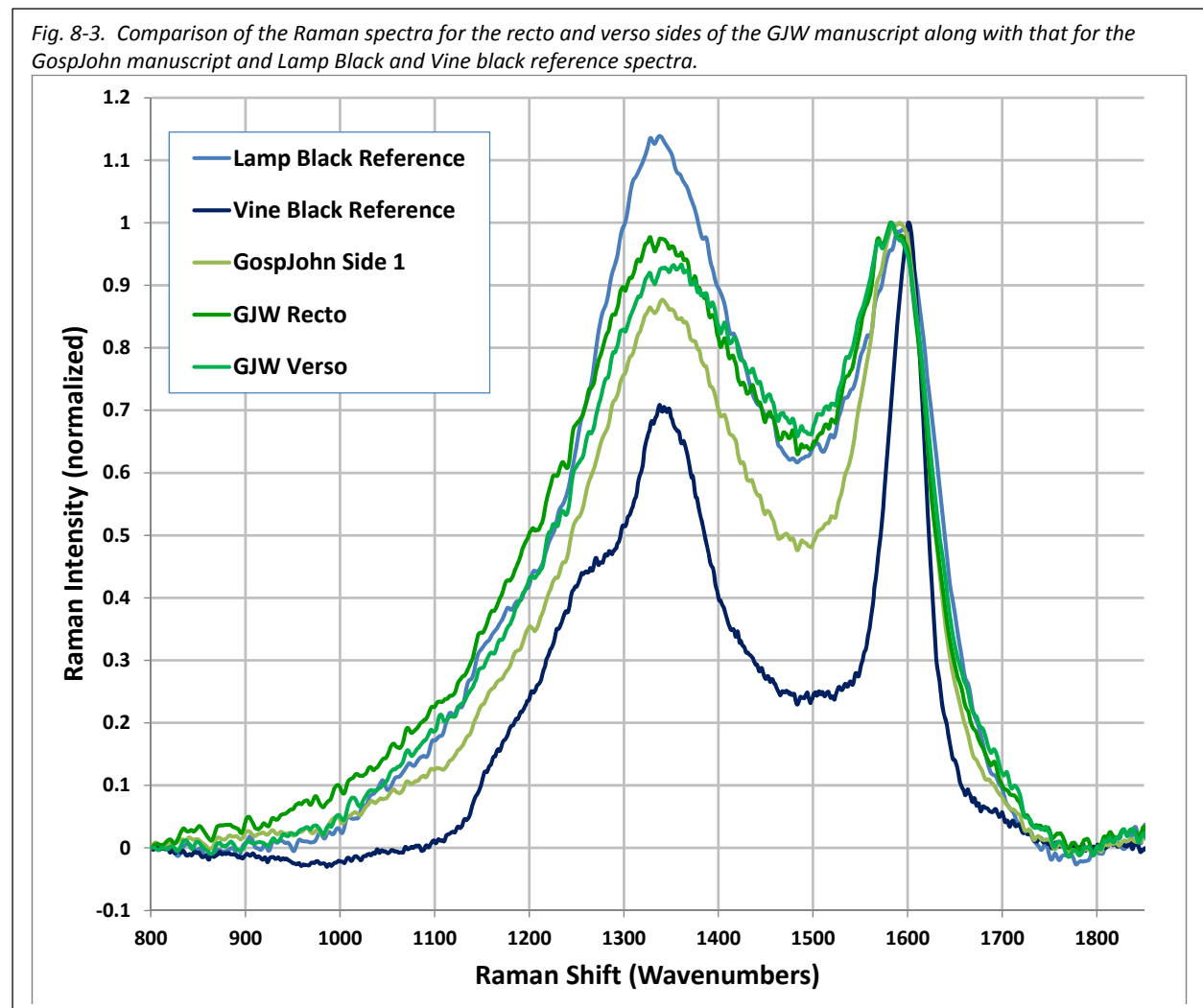
Examination of Different Features in Text. In order to examine the possibility that the ink composition may vary for different specific features or regions of the GJW manuscript, we have examined the

Figure 8-2. Comparison of the average peak position and peak amplitude of the 1350 cm^{-1} band in Raman spectra obtained for ink in specific regions of the GJW document (designated as in Fig. 4-3 and 4-4) where recto side is shown in light blue, verso side is shown in dark blue, and average data from GospJohn is shown in purple. Error bars represent 90% confidence.



average normalized Raman spectra taken from different individual locations on the GJW manuscript. Here, we must emphasize that there is a limited number of observations for each location and thus the data are less precisely determined. For quantitative comparison of the individual spectra we have chosen to examine the peak amplitude of the 1350 cm^{-1} band relative to the sharp 1600 cm^{-1} band and we have chosen to examine the peak Raman shift of the 1350 cm^{-1} band. The charts in Fig. 8-2 show actual Raman shift positions and amplitude (relative to the 1600 cm^{-1} peak) for spectra averaged from specific regions of the recto and verso sides of the GJW document. The charts also show the values of these parameters for the average spectra from GospJohn document. The specific regions are designated in the charts for correlation to the photographs shown in Fig. 4-3 and 4-4. Because of the limited data, the uncertainties for each of the chart elements are difficult to quantify but are certainly relatively large. The error bars in Fig. 8-2 represent the 90% confidence range ($\pm 1.68 \sigma$ where σ is the standard deviation, determined from a typical data set). Our conclusion is that there is no clear or convincing indication of any specific differences in ink composition for specific regions of the document. These charts do suggest some difference between recto and verso sides of the document, especially clear in the measurement of peak Raman shift. We will examine this possibility later in this report.

General Features of Inks for GJW. The data shown above suggest some systematic difference between the ink for the recto and verso sides of the GJW manuscript. Fig. 8-3 shows averages of spectra



obtained for the recto and verso sides of GJW. This figure also presents the normalized spectrum determined for the GospJohn manuscript and, for comparison purposes, the Columbia reference spectra for modern lamp black and vine black inks on modern papyrus. These spectra make clear that the inks used for the GospJohn manuscript are quite distinct from the inks used in the GJW manuscript, as characterized by Raman spectroscopy. The basic features for the GJW spectra lie somewhere between those for the lamp black reference and the vine black reference. However they are well within the general reported Raman spectra for carbon-based inks used in ancient papyrus and other ancient manuscripts.

Comparison of Recto and Verso Inks. The Raman spectra for the recto side could differ from the Raman spectra for the verso side if, for example, the recto and verso sides were created at different times with different ink compositions. The spectra on average could also be different if there were modifications to the overall ink composition on one side or the other. They could also differ if the two sides experienced different chemical or physical environments throughout their lifetimes. Because these data represent the average of many spectra, the accuracy for the average spectra from GJW shown in Fig. 8-3 is higher than for the data shown in Fig. 8-2. The differences in average spectra for recto and verso sides are thus summarized in the chart below which shows approximate 90% confidence levels (1.68 times the standard deviation).

<u>Manuscript</u>	<u>Peak Position for 1350cm⁻¹ Band</u>	<u>Relative Amplitude for 1350cm⁻¹ Band</u>
GJW, recto	1334.8 +/- 13 cm ⁻¹	0.986 +/- 0.13
GJW, verso	1352.2 +/- 13 cm ⁻¹	0.927 +/- 0.13

These data and the data of Fig. 8-2 indicate only very small measured average differences between the ink compositions for the two sides of the GJW document, both in terms of the peak position for the 1350 cm⁻¹ band and in terms of its intensity relative to the primary 1600 cm⁻¹ peak (at least with respect to the individual regions sampled). The observed spectra for recto and verso sides, in both peak position and relative amplitude, are similar within the 90% confidence limit uncertainties for these measurements. In addition we find no significant measured differences between the Raman spectra obtained at different locations on an individual side of the document. Although the Raman spectra obtained from the recto side and from the verso side are similar within experimental error, the data as shown in Fig. 8-2 admit the possibility that the recto and verso sides for this manuscript could be derived from different but similar batches of ink.

9. Conclusions.

We have studied micro-Raman spectra associated with selected features from both sides of two manuscripts on papyrus: “Gospel of John” (GospJohn) and “Gospel of Jesus Wife” (GJW). Our conclusions are as follows:

“Gospel of John”.

- The ink used in this manuscript is primarily based on a carbon black pigment such as Lamp Black. The observed Raman spectra are very similar to the carbon-based inks studied for a wide variety of manuscripts including several dated from the early centuries of the Christian era.
- From the observed Raman spectra, we find no evidence for any constituents of ink or types of ink other than carbon black.
- We have found no significant difference in the Raman spectra obtained from different regions of either side of this manuscript.
- Since the Raman spectra for carbon-black based inks vary considerably based on the composition, preparation methods, and thermal history of the ink, the strong similarity of Raman spectra for Side 1 and for Side 2 of GospJohn suggests that the two sides of the manuscript are written in identical or similar inks.

“Gospel of Jesus Wife”.

- The ink or inks used in this manuscript are primarily based on carbon black pigments such as Lamp Black. The observed Raman spectra are very similar to the carbon-based inks studied for a wide variety of manuscripts including many dated from the early centuries of the Christian era.
- From the observed Raman spectra, we find no evidence for any constituents of ink or types of ink other than carbon black.
- The ink or inks used in GSW are similar to, but distinct from, the ink used for the GospJohn manuscript.
- Within the available accuracy of our measurements, our data are consistent with a single ink composition for each individual side of the GJW manuscript.
- The Raman spectra obtained from the recto side and from the verso side are very similar within experimental error, although the data admit the possibility that the recto and verso sides for this manuscript could be derived from different but similar batches of ink.

References and Footnotes

1. Robin JH Clark. "Pigment identification on medieval manuscripts by Raman microscopy". *Journal of molecular structure* 347, 417-427 (1995)
2. Robin JH Clark. "Pigment identification by spectroscopic means: an arts/science interface". *Comptes Rendus Chimie* 5, 7-20 (2002)
3. Gregory D Smith and Robin JH Clark. "Raman microscopy in archaeological science". *Journal of Archaeological Science* 31, 1137-1160 (2004)
4. Lucia Burgio, Robin JH Clark and Richard R Hark. "Raman microscopy and x-ray fluorescence analysis of pigments on medieval and Renaissance Italian manuscript cuttings". *Proceedings of the National Academy of Sciences* 107, 5726-5731 (2010)
5. Ian M Bell, Robin JH Clark and Peter J Gibbs. "Raman spectroscopic library of natural and synthetic pigments (pre- \approx 1850 AD)". *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 53, 2159-2179 (1997)
6. Lucia Burgio and Robin JH Clark. "Library of FT-Raman spectra of pigments, minerals, pigment media and varnishes, and supplement to existing library of Raman spectra of pigments with visible excitation". *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 57, 1491-1521 (2001)
7. Eugenia P Tomasini, Emilia B Halac, María Reinoso, Emiliano J Di Liscia and Marta S Maier. "Micro-Raman spectroscopy of carbon-based black pigments". *Journal of Raman Spectroscopy* 43, 1671-1675 (2012)
8. Alana S Lee, Peter J Mahon and Dudley C Creagh. "Raman analysis of iron gall inks on parchment". *Vibrational spectroscopy* 41, 170-175 (2006)
9. Alana S Lee, Vincent Otieno-Alego and Dudley C Creagh. "Identification of iron-gall inks with near-infrared Raman microspectroscopy". *Journal of Raman Spectroscopy* 39, 1079-1084 (2008)
10. Marina Bicchieri, Michela Monti, Giovanna Piantanida and Armida Sodo. "All that is iron-ink is not always iron-gall!". *Journal of Raman Spectroscopy* 39, 1074-1078 (2008)
11. Marina Bicchieri, Michela Monti, Giovanna Piantanida and Armida Sodo. "Non-destructive spectroscopic investigation on historic Yemenite scriptorial fragments: evidence of different degradation and recipes for iron tannic inks". *Analytical and bioanalytical chemistry*, 1-9 (2013)
12. Rosalie David, HGM Edwards, DW Farwell and DLA De Faria. "Raman spectroscopic analysis of ancient Egyptian pigments". *Archaeometry* 43, 461-473 (2001)
13. L Slavov, MV Abrashev, T Merodiiska, Ch Gelev, RE Vandenberghe, I Markova-Deneva and I Nedkov. "Raman spectroscopy investigation of magnetite nanoparticles in ferrofluids". *Journal of Magnetism and Magnetic Materials* 322, 1904-1911 (2010)
14. D Bikiaris, Sister Daniilia, S Sotiropoulou, O Katsimbiri, E Pavlidou, AP Moutsatsou and Y Chrysoulakis. "Ochre-differentiation through micro-Raman and micro-FTIR spectroscopies: application on wall paintings at Meteora and Mount Athos, Greece". *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 56, 3-18 (2000)
15. Laurence C Abbott, Stephen N Batchelor, John R Lindsay Smith and John N Moore. "Resonance Raman and UV-visible spectroscopy of black dyes on textiles". *Forensic science international* 202, 54-63 (2010)

16. Irina Geiman, Marco Leona and John R Lombardi. "Application of Raman Spectroscopy and Surface-Enhanced Raman Scattering to the Analysis of Synthetic Dyes Found in Ballpoint Pen Inks". *Journal of forensic sciences* 54, 947-952 (2009)
17. RE Littleford, MP Hughes, G Dent, D Tackley and WE Smith. "Surface-Enhanced Resonance Raman Scattering of Black Inkjet Dyes in Solution and *in Situ* Printed onto Paper". *Applied spectroscopy* 57, 977-983 (2003)
18. James D Womack, Thomas J Vickers and Charles K Mann. "Determination of azo dyes by resonance-enhanced Raman spectroscopy". *Applied spectroscopy* 41, 117-119 (1987)
19. Kremer Pigments (247 West 29th Street, NY 10001) supplied the two carbon black pigments: Furnace black (denoted here as "Lamp Black", item #47250 and German vine black, item #47000. Papyrus sheets were purchased from Dick Blick Art Materials (P.O. Box 1267, Galesburg, IL 61402-1267) item #11239-1001. Gum arabic from acacia tree, item #51198-250G was purchased from Aldrich. The gum arabic was dissolved in deionized water to achieve the desired working consistency. Either lamp black or vine black was mixed into this solution. A pen tip was fabricated by cutting the tip of a cotton swab with a knife and a new "pen" was used for each type of ink. Ink was applied direct to new papyrus sheets using the pen tip.
20. Lucia Burgio and Robin JH Clark. "Comparative pigment analysis of six modern Egyptian papyri and an authentic one of the 13th century BC by Raman microscopy and other techniques". *Journal of Raman Spectroscopy* 31, 395-401 (2000)
21. J Ambers. "Raman analysis of pigments from the Egyptian Old Kingdom". *Journal of Raman Spectroscopy* 35, 768-773 (2004)
22. F Tuinstra and J L_ Koenig. "Raman spectrum of graphite". *The Journal of Chemical Physics* 53, 1126 (1970)
23. MA Tamor and WC Vassell. "Raman "fingerprinting" of amorphous carbon films". *Journal of Applied Physics* 76, 3823-3830 (1994)
24. M Nakamizo, R Kammereck and P Letal Walker. "Laser Raman studies on carbons". *Carbon* 12, 259-267 (1974)
25. Yan Wang, Daniel C Alsmeyer and Richard L McCreery. "Raman spectroscopy of carbon materials: structural basis of observed spectra". *Chemistry of Materials* 2, 557-563 (1990)
26. RO Dillon, John A Woollam and V Katkanant. "Use of Raman scattering to investigate disorder and crystallite formation in as-deposited and annealed carbon films". *Physical Review B* 29, 3482 (1984)
27. A Cuesta, P Dhamelincourt, J Laureyns, A Martinez-Alonso and JM Diaz Tascón. "Raman microprobe studies on carbon materials". *Carbon* 32, 1523-1532 (1994)
28. A Sadezky, H Muckenhuber, H Grothe, R Niessner and U Pöschl. "Raman microspectroscopy of soot and related carbonaceous materials: Spectral analysis and structural information". *Carbon* 43, 1731-1742 (2005)
29. M Ramsteiner and J Wagner. "Resonant Raman scattering of hydrogenated amorphous carbon: Evidence for π -bonded carbon clusters". *Applied physics letters* 51, 1355-1357 (1987)

30. AC Ferrari, JC Meyer, V Scardaci, C Casiraghi, Michele Lazzeri, Francesco Mauri, S Piscanec, Da Jiang, KS Novoselov and S Roth. "Raman spectrum of graphene and graphene layers". *Physical review letters* 97, 187401 (2006)
31. AC Ferrari and J Robertson. "Interpretation of Raman spectra of disordered and amorphous carbon". *Physical Review B* 61, 14095 (2000)
32. Andrea Carlo Ferrari and John Robertson. "Raman spectroscopy of amorphous, nanostructured, diamond-like carbon, and nanodiamond". *Philosophical Transactions of the Royal Society of London. Series A: Mathematical, Physical and Engineering Sciences* 362, 2477-2512 (2004)