Ultrafast Pump-Probe Microscopy in Cultural Heritage Research

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Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Philosophy in the Department of
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ABSTRACT

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Abstract

The materials and working method of a painting can reveal important information about our cultural history, as well as lend the conservator the necessary knowledge for treatment options. The removal of a cross-section sample reveals the three-dimensional (3d) structure of the painting and can be used to identify materials. However, cross-section samples are destructive and provide only local information. Nonlinear optical ultrafast pump-probe microscopy, originally developed for biomedical imaging, can provide high resolution 3d images with chemical contrast. In this dissertation, I adapt pump-probe microscopy to multiple materials and applications in cultural heritage research. Pump-probe dynamics were found to be sensitive to the ratio of the two chromophores present in the precious blue pigment lapis lazuli and its synthetic analogs, ultramarines blue and violet. Virtual pump-probe cross-sections were combined with nonlinear fluorescence contrast to study differences between the interactions of paper supports with inorganic crystalline pigments and organic dyes. Multiple early Italian paintings (The Crucifixion by Puccio Capanna, The Martyrdom of St. Alexander and The Body of Christ Supported by Angels attributed to Lorenzo Lotto) were imaged in-situ, in conjunction with traditional conservation science methods, as a part of a technical case study. Thus, pump-probe microscopy offers an important new tool for gaining fundamental insights into our cultural heritage.
Dedication

I dedicate this dissertation to my truly wonderful and amazing husband. Victor, I absolutely could not have done this without your unconditional love and support.

This dissertation is also dedicated to my three beautiful children. Charlie, Oscar, and Estelle, you each brought a joy to my life that continues to make everyday special and my deepest desire is that in completing this difficult task I can give you the lives you deserve.
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1. The Scientific Research of our Cultural Heritage

This chapter will discuss the research methods frequently applied to the study of cultural heritage within the context of the Italian renaissance pigment palette and painting technique. Though the focus of this work will revolve around pigment research, we will briefly discuss the construction of panel paintings. It serves as a prelude for the major focus of this dissertation, applications of pump-probe microscopy to cultural heritage research.

*I dim the lights in the photography suite of the North Carolina Museum of Art’s conservation lab. The halogen lamp is humming and our infrared camera is in position; an early Italian painting from the Pavian school is ready for its close-up. The lamp’s IR light passes through the pigments on the surface (which are transparent to this wavelength), is absorbed by any carbon containing underdrawing materials and reflected off of the white ground of the painting back to the camera. Click. The IR image reveals a hidden surprise; the man painted on the surface of the painting has an entirely different man underneath him.*
Figure 1: Digital and infrared photograph of Madonna and Child with St. John the Evangelist, a Donor, and St. Anthony Abbot. Originally painted in the Pavian Workshop between 1400-1450, IR-reflectography reveals the donor’s face was repainted.

The donor in this painting is the man kneeling and praying to the Virgin Mary and the baby Jesus. The donor is most likely the man who originally commissioned the
painting and had himself painted in as a character in reverence. However, the IR reflectogram of the painting shows a completely different donor face (more hair, lower set eyes, and smaller jawline) indicating that the painting was originally made for someone else and that someone painted the face we see in the bright field image at a later time. This type of practice was common when a painting changed owners.

The investigation of the Pavian school painting described above is a good example of applying modern scientific techniques to study cultural heritage. The conservator can directly apply knowledge of the materials and methods of the artist to a proper treatment and conservation plan for the historic artwork, as well as use such information to better understand past cultures. For example, replacing faces within artwork is a common practice dating back to the sculptures of ancient Rome (1). Various techniques have discovered completely different paintings underneath the paintings we see; synchrotron radiation has shown that Rembrandt van Rijn (2) and Vincent Van Gogh (3, 4) commonly re-used their canvases.

1.1 The Italian Painting Technique

Cennino Cennini’s book, *Il Libra dell ‘Arte (The Craftsman’s Handbook)* (5), explains how to create a panel painting from the very beginning. Cennini’s treatise on Italian painting begins with the initial steps for preparing the wood panel and ends with varnishing the finished painting, including important instructions on how to prepare colors and other necessary skills. Although no paintings survive that can be directly
attributed to Cennini, he comes from a master-pupil line dating back to Giotto, who many believe to be the father of Italian Renaissance painting.

Italian Renaissance paintings are well known by their religious subject matter. However, the artist did not arbitrarily pick the subject matter; rather a client would commission a painting or altarpiece and choose the subject matter. In this view, the painter was very much a professional craftsman (hence the title of Cennini’s book). The master painter often had his own workshop, which he filled with apprentices who each had their own job. A carpenter was in charge of preparing the panels from raw wood; another apprentice would prepare the panel ground for painting; this apprentice would pass the prepared panel to the gilder, then painter, and so on (6). This important consistency of tasks is very useful for the conservator and curator who are responsible for attributing paintings to their rightful painter. For example, it is highly unlikely that two different panels were from the same workshop if the panels have different ground preparations. We will now discuss each step in the Italian painting technique with reference to the analytical methods often used in their study.

1.1.1 Panel Construction

In general, Italian panel and subsequent altarpiece construction was quite laborious and began with the preparation of the wood. The workshop carpenters tended to utilize the entire log, often cutting it into planks of varying sizes that were stored until seasoned (dried) to avoid warping. Due to the limited choice of wood in
Italy panels were typically constructed on poplar, which is weak and soft with large open cells. Poplar shrinks and swells in climate changes and is particularly prone to bug infestation (like wood-worm and death-watch beetles) (6). For this reason, panel transfer is a common conservation practice (currently being addressed at the Getty Conservation Institute (7)) for many Italian panels. The original wood is stripped away; the back of the painting is then supported with a canvas that is ultimately attached to another panel. To the conservator, the state of the panel is of great importance and must be investigated before any treatment can take place, especially as stabilizing the panel may be the first step in conservation. Dendrologists and botanists can identify the age and type of wood, based on simple visual observation and polarized light microscopy (8). In addition, an x-ray of the painting will immediately reveal any major flaws or cracks in the wood and can also highlight the construction of the panel, if it involved multiple pieces jointed together. Sometimes canvas impressions in the paint layer can also reveal methodology (such as if the ground were sized prior to painting) or transfer processes during previous restorations. Figure 2 shows the x-ray of the Pavian school painting (seen in Figure 1), and reveals the cradle system that was used for conserving the support as well as original areas of loss and damage.
Figure 2: X-radiography of Madonna and Child with St. John the Evangelist, a Donor, and St. Anthony Abbot.

Note that some details can be seen at the bottom of the x-ray where the painting has a leather strip covered in typeset. Conservators speculate this typeset was added to the painting at the time of ownership transfer, when the original face was re-painted.

1.1.2 Gesso Ground

Paint (pigments in binder) cannot be applied to bare untreated wood. Wood is very porous; if left untreated the raw wood absorbs the binding medium leaving the
pigments with nothing to adhere them to the panel. Further, gilding (the application of gold leaf) must be carried out on a very smooth surface. Panels that were ready for use were prepared with a ground (a coating on the wooden support that makes it suitable as a painting surface). The ground is important because long-term preservation will depend on good adhesion of the ground to the panel and of the paints to the ground.

The first stage of ground preparation involves sizing the wood with several coats of animal glue and then applying strips of glue-soaked canvas. This seals and flattens the wood, as well as provides a surface for the first layers of gesso. Gesso is the term traditionally used to describe a mixture of animal glue with gypsum (calcium sulfate, CaSO₄·2H₂O). As Cennini describes these steps (5), the application of gesso to the prepared wood is an extremely tedious process. The preparation of gesso from raw gypsum is a complicated procedure that takes at least a month and yields two types of gesso, grosso (a course gesso) and sottile (a fine gesso). The prepared panels are painted first with several layers of gesso grosso and then with several layers of gesso solittle. The overall flatness of the ground can be ensured by dusting the dried gesso layers with charcoal and scraping the surface with the flat edge of an iron spatula until charcoal can no longer be seen.

While a gesso ground is common in Italian paintings, it is certainly not the only methodology for preparing a panel. Lead white and chalk, or a mixture of the two (termed loot wit), are also frequently used materials in grounds. There are many
examples of these materials discovered in the ground of paintings from various regions during the Renaissance time period. Non-destructive spectroscopic techniques, such as x-ray fluorescence intensity spectroscopy (XRF) and reflectance spectroscopy (FORS), can distinguish between gesso, chalk, and lead white in some cases. While XRF is more sensitive to heavy elements and can easily identify lead white by the lead M-line (9), the lead line overlaps with the weak sulfur K-line. It is not possible to use XRF alone to identify gesso or distinguish it from chalk (calcium carbonate) if lead white is present. FORS is able to identify the presence of gypsum, chalk, and lead, as well as to distinguish between them (10), based on the molecular vibrations of each compound. Unfortunately, these techniques do not provide any quantitate depth information. If lead white was present in a pigment layer (a very common occurrence) neither of the two techniques could distinguish between surface lead or ground lead.

1.1.3 Preliminary Drawing

Once the panel is prepared, Cennini also instructs on how to make the preliminary drawing, often using charcoal, black inks, or sinopie (a reddish earth pigment), which is typically studied using IR reflectography. Generally the painting is illuminated with a halogen lamp (all IR wavelengths) and the reflected IR light is captured with an IR camera and appropriate optical filter. Most colored pigments are transparent to IR light, while carbon-containing pigments, such as charcoals or black inks, will absorb the IR light (8). The captured image will appear dark anywhere the IR
was absorbed, i.e., anywhere there are carbon-containing pigments. Interpretation of the results can be difficult because any carbon-based pigments in the surface paint layers will absorb IR and appear dark. Again the lack of depth resolution can make it difficult to distinguish between, for example, a carbon black underdrawing or the use of a carbon black for shading in the pigment layer.

1.1.4 Gilding

The gilder typically applied the gold leaf before any paint was applied to the panel. He would create divisions between areas to be gilded and to be painted by lightly scoring the panel with a stylus into the gesso. The area to be gilded was then prepared with a bole (a soft, greasy-textured red-brown clay). Bole serves two purposes; it provides a smooth cushioned sticky surface for the gold leaf to be burnished and imparts a warm rich color to the gold. Gold leaf is thin enough to appear green and cold in color when placed directly on a white background. The sheets of gold leaf have been estimated to be roughly 250 nm thick, by assuming that one would make 100 sheets from a single gold florin (6)! Bole is prepared from earth pigments, which have a variety of compositions depending on the region or origin, and have been heavily used through history (11).

1.1.5 The Paint Film

Pigments were typically produced from natural sources such as minerals, insects, or plants. Pigments that are central to this dissertation include the precious blue mineral
pigment lapis lazuli and the organic blue dye indigo. These pigments will be discussed in greater detail later. Tables 1 through 5 list the main pigments (organized by color) in the Italian palette, including composition and relevant notes. The following tables were summarized from material in a series of books, *Artists’ Pigments: A Handbook of Their History Characteristics* (12-15).

Table 1: Red Pigments

<table>
<thead>
<tr>
<th>Name</th>
<th>Composition</th>
<th>Origin and Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vermillion</td>
<td>Mercury(II) sulfide, α-HgS</td>
<td>• Synthetic (8th C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Naturally occurring as the mineral cinnabar</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Light induced transformation to black α’-HgS</td>
</tr>
<tr>
<td>Red Lead</td>
<td>Dilead(II) lead(IV) oxide, Pb₃O₄</td>
<td>• Synthetic (5th C BCE)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Naturally occurring as mineral minimum</td>
</tr>
<tr>
<td>Realgar</td>
<td>Arsenic(II) sulfide, α-As₄S₄</td>
<td>• Mineral (antiquity)</td>
</tr>
<tr>
<td>Hematite</td>
<td>Iron(III) oxide, α-Fe₂O₃</td>
<td>• Mineral (antiquity)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Refers to pure mineral, more common is a red ocher (Fe₂O₃ + clay + silica).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• A variety of earth pigments exist with ferric oxide as the coloring agent (ranging in color)</td>
</tr>
<tr>
<td>Carmine</td>
<td>Carminic Acid, C₂₂H₂₀O₁₃</td>
<td>• Natural (antiquity)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• From female cochineal scale insect (termed cochineal carmine if source is known)</td>
</tr>
<tr>
<td></td>
<td>Kermesic Acid, C₁₈H₁₂O₉</td>
<td>• Natural (antiquity)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• From female kermes scale insect (termed kermes)</td>
</tr>
<tr>
<td>Name</td>
<td>Composition</td>
<td>Origin</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Lac</td>
<td>Laccaic Acid A, C_{26}H_{19}NO_{12}</td>
<td>• Natural (antiquity)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• From laccifer lacca kerr scale insect</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Also source for shellec resin</td>
</tr>
</tbody>
</table>

Table 2: Green Pigments

<table>
<thead>
<tr>
<th>Name</th>
<th>Composition</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verdigis</td>
<td>Copper(II) ethanoate, Cu(CH_3COO)_2</td>
<td>• Synthetic (antiquity), prepared from acetic acid on copper plates</td>
</tr>
<tr>
<td>Copper Resinate</td>
<td>Copper salts of resin acids</td>
<td>• Synthetic glaze, created from verdigris and turpentine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Dates of use uncertain, but most common in 15\textsuperscript{th} C Italian oil paintings</td>
</tr>
<tr>
<td>Terre Verde</td>
<td>Variations on green silicates, glauconite and celadonite</td>
<td>• Mineral (antiquity)</td>
</tr>
<tr>
<td>Malachite</td>
<td>Basic copper(II) carbonate, CuCO_3\cdotCu(OH)_2</td>
<td>• Mineral (antiquity), but infrequent use in European paintings</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Associated in nature with blue mineral, azurite (although malachite is more abundant)</td>
</tr>
</tbody>
</table>

Table 3: Yellow Pigments

<table>
<thead>
<tr>
<th>Name</th>
<th>Composition</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead Tin Yellow</td>
<td>Lead tin oxide, Pb_2SnO_4</td>
<td>• Synthetic (~ 13\textsuperscript{th} C)</td>
</tr>
<tr>
<td>Type I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orpiment</td>
<td>Arsenic(III) sulfide, As_2S_3</td>
<td>• Mineral, in use since antiquity</td>
</tr>
</tbody>
</table>
### Table 4: Blue Pigments

<table>
<thead>
<tr>
<th>Name</th>
<th>Composition</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lapis Lazuli</strong></td>
<td>Sulfur containing sodium aluminum silicate, (Na,Ca)[Al₆Si₆O₂₄] (SO₄,S,Cl)₂</td>
<td>• Mineral (lazurite in purest form)</td>
</tr>
<tr>
<td></td>
<td>Denotes repeating crystal framework unit.</td>
<td>• Used as a stone since antiquity, as a pigment only since ~ 6th C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Synthetic (ultramarine blue) developed in 1828</td>
</tr>
<tr>
<td><strong>Azurite</strong></td>
<td>Basic copper(II) carbonate, 2CuCO₃•Cu(OH)₂</td>
<td>• Mineral, associated in nature with green mineral, malachite.</td>
</tr>
<tr>
<td><strong>Egyptian Blue</strong></td>
<td>Calcium copper(II) silicate, CaCuSi₄O₁₀</td>
<td>• Synthetic (~ 3100 BCE)</td>
</tr>
<tr>
<td><strong>Indigo</strong></td>
<td>C₁₆H₁₀N₂O₂</td>
<td>• Natural (antiquity)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• True indigo source is the Indigofera tinctoria plant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Dyer’s woad, (Isatis tinctoria) used as dark blue dye, but eventually replaced by true indigo</td>
</tr>
</tbody>
</table>

### Table 5: White and Black Pigments

<table>
<thead>
<tr>
<th>Name</th>
<th>Composition</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gypsum</strong></td>
<td>Calcium sulfate dehydrate, CaSO₄•2H₂O</td>
<td>• Mineral</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Gesso is traditional term applied to animal glue and gypsum mixture for preparing panels.</td>
</tr>
<tr>
<td><strong>Chalk</strong></td>
<td>Calcium carbonate, CaCO₃</td>
<td>• Natural (calcite), present in mineral, animal, and vegetable</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Synthetic chalk (precipitated chalk), created ~ 1850</td>
</tr>
<tr>
<td>Pigment Type</td>
<td>Description</td>
<td>Notes</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| **Lead White**               | Basic lead(II) carbonate, $4\text{PbCO}_3\cdot2\text{Pb(OH)}_2$            | • Synthetic (although exists in pure form as the rare mineral hydrocerussite)  
  • In use since antiquity    |
| Carbon Blacks Based on elemental carbon | Lampblack (Composition varies based on starting material)                    | • Collected from a sooting flame, for example burning linseed oil in a lamp  
  • In use since antiquity    |
|                              | Bone/Ivory Black (Most likely containing calcium and phosphates from bone)  | • Prepared by charring animal bones.  
  • In use since antiquity    |
|                              | Plant Blacks (Composition varies based on plant material)                    | • Prepared by charring plants, for example charcoal is typically from wood.  
  • In use since antiquity (wood charcoal probably the earliest)            |

In the workshop, painters were responsible for prepping and refining the pigment into a suitable material for painting. Different pigments required different amounts of preparation, though some could be occasionally bought as a powder from apothecaries. The painter himself would dig up colored earth pigments, which can be prepared with a simple washing and grinding process. Raw lapis lazuli, on the other hand, is a stone that must be ground and cleaned in a complex process to extract the blue coloring. Cennini himself believes the preparation to a lengthy and arduous task, thus he advises

“… that making it is an occupation for pretty girls rather than for men; for they are always at home, and reliable…” (5)
The pigments were typically ground on a porphyry stone with water, to remove lumps and reduce particle size, but each pigment requires a different amount of grinding. Ground pigment is stored topped with the water to keep the pigment from drying out. In the early 14th century, the medium of choice for panel painting was egg yolk. Egg yolk dries and sets very quickly, demanding a very organized and systematic form of painting and discourages thick textured layers that will crack as the water dries (6). Around the 1500’s, Italian painters moved from egg yolk to oils as a binding media, although the methodology of this period is poorly understood. Currently, the gold standard technique for identifying binding materials is taking a paint sample and preparing it for different mass spectrometry techniques (16). Unfortunately these chemical analysis methods are completely destructive. Raman spectroscopy (17) and FORS (10, 18) can use the molecular vibrations specific to binding molecules to identify binders in theory, but the methods have had some difficulty in practice. Raman signals are inherently weak and easily overwhelmed by fluorescence that typically accompanies organic materials. FORS has shown some promise but is still lacking in specificity.

The painters in the workshop used a thin and delicate layering technique, utilizing their pigments to create bright and vibrant colors. However the limited palette of the renaissance meant that multiple pigments were often used together to create the desired effect. For example, kermes or cochineal would be glazed over or mixed with lapis lazuli to create a purple hue. This use of lapis lazuli is of particular interest
because during this time period there was only one source for the precious blue pigment (the mountains of Afghanistan), making it more expensive than gold. To save cost, lapis lazuli was typically adulterated with a cheaper blue pigment such as azurite or indigo. Although there were a few green pigments available, green was more typically created by the mixing (or layering) of a blue and yellow pigment for more vibrant coloring. Pure greens were used more often as glazes.

1.2 Noninvasive and Invasive Analysis Techniques

From this discussion, it should be clear that Italian panels were created from the ground up; the layering structure of the painting contains a great deal of information regarding the methodology. As previously suggested, many techniques that can identify materials do not contain quantitative depth information about the stratigraphy of the painting, and as such, the three-dimensional structure of a painting is typically studied by the removal of a cross-section sample. A small paint sample is removed and encased within a hard resin that is polished down to the surface of the paint sample. As an example, we will discuss both noninvasive and invasive analysis of a pre-14th century Italian painting, The Crucifixion.

1.2.1 Noninvasive Analysis of The Crucifixion

*The Crucifixion* (figure 3) has been attributed to Italian artist Puccio Capanna and was painted in roughly 1330 CE. According to the examination of the painting by the Chief Conservator of the North Carolina Museum of Art (NCMA) (19), the painting
materials and panel construction are believed to follow the style and technique as described by Cennino Cennini.

Figure 3: Digital photograph of The Crucifixion, from the collection of the North Carolina Museum of Art as gifted by Samuel H. Kress Foundation. The painting is roughly 5 x 7 inches.

X-radiography, infrared reflectance imaging, and ultraviolet visible fluorescence photography are excellent nondestructive tools for providing information about a painting’s support, compositional paint changes, under-drawings, paint and varnish
applications and restorations (8) over the entire painting. Macro-photographs of The Crucifixion are seen in figure 4.

![Figure 4: X-ray, IR, and UV/VIS photographs of The Crucifixion. The x-ray can be difficult to interpret due to the cradle added to the panel. A photograph of the reverse of the panel is inset in the x-ray, to show the pattern.](image)

It is believed The Crucifixion may have undergone a panel transfer process, although this cannot be confirmed from the conservation file. The x-ray photograph has indications of cloth incorporated into the ground, but it is uncertain if this is the original cloth or part of a transfer process. Conservators at the Kress Foundation cradled the panel in 1939 using mahogany and maple (seen in the in-set of the x-ray), although wood analysis in 1987 by Elizabeth Wheeler indicates the presence of fir and European walnut. However, how much of the original wood remains is uncertain. In this case, the IR photograph does not contain much information in regards to any original underdrawing and the UV/VIS photograph is uniformly glowing across the surface,
consistent with the report that the Kress Foundation covered the surface with a layer of damar varnish in 1939. These types of images can contain a wealth of information important to the conservator, but they contain no chemical information in regards to material identification and no quantitative depth information.

There are many commercially available systems that are portable and can be taken directly to the painting, museum, or even used in the field to identify materials (mainly pigments) in historic artworks including XRF, FORS, and Raman. These are generally single spot (or micro) techniques, performed on areas of interest and not an entire painting. These spectroscopic techniques do not contain depth information and put information from all the layers into one spectrum. This limitation is highlighted in the XRF spectra in figure 5.

![XRF spectra of Mary's blue robe](image)

**Figure 5**: XRF spectra taken from three different areas of Mary’s blue robe.
In regards to the painting’s 3d structure, for example, all the spectra contain a very strong contribution from lead (lead white), but it is impossible to tell if this is due to a mixture with a pigment or in the ground. Similarly, the presence of titanium could be attributed to the pigment titanium white, which would have been used in a panel transfer process or in restorations to paint layers. The XRF spectra are also lacking in pigment specificity. Many of the lighter elements could be attributed to minerals in earthy pigments used in the gilding, to lapis lazuli, or to a mineral based ground, such as chalk or gypsum. However, due to the strong lead peak overlapping the sulfur peak, it is not possible to identify lapis lazuli or distinguish between chalk (calcium carbonate) and gypsum (calcium sulfate).

To attain better material specificity, elemental analysis is generally used in conjunction with a molecular technique for greater specificity. Raman spectroscopy is based on a scattering phenomenon that occurs due to molecular vibration when light interacts with a sample. (20). The difference in energy between the incident and scattered photon is equivalent to the vibrational energy of the molecule in question, which gives the technique its high molecular specificity. In the field of cultural heritage research, Raman spectroscopy has frequently been used for in-situ identification of pigments (21). Unfortunately, Raman suffers from an intrinsically weak signal (only 1 in roughly a billion photons will scatter inelastically) and is easily overwhelmed by fluorescence in the presence of organics. Raman was performed in-situ on The Crucifixion
in conjunction with the XRF analysis to confirm the various pigments. Table 6 summarizes the results of XRF and Raman.

**Table 6: Pigment Identification from XRF and Raman Analysis**

<table>
<thead>
<tr>
<th>Area</th>
<th>Pigment Assignment</th>
<th>Raman</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue Mantel</td>
<td>Lead White (Pb), trace Azurite (Cu),</td>
<td>Lapis Lazuli</td>
</tr>
<tr>
<td></td>
<td>Lapis Lazuli (trace minerals)</td>
<td></td>
</tr>
<tr>
<td>Red Robe, Mary</td>
<td>Vermillion (Hg), Calcium Ground (Ca),</td>
<td></td>
</tr>
<tr>
<td>Magdalene</td>
<td>Red Ocher (Fe, trace minerals)</td>
<td></td>
</tr>
<tr>
<td>Blue Robe, Saint</td>
<td>Lead White (Pb), trace Azurite (Cu),</td>
<td>Lapis Lazuli, Azurite,</td>
</tr>
<tr>
<td></td>
<td>Lapis Lazuli (trace minerals)</td>
<td>Gypsum, Calcite</td>
</tr>
<tr>
<td>Red Robe, Soldier</td>
<td>Vermillion (Hg), Lead White (Pb)</td>
<td></td>
</tr>
<tr>
<td>Angel, Left</td>
<td>Lead white (Pb), Azurite (Cu), Gold (Au),</td>
<td>Lapis Lazuli, Azurite,</td>
</tr>
<tr>
<td></td>
<td>Ocher or Lapis Lazuli (Fe and trace</td>
<td>Lead White</td>
</tr>
<tr>
<td></td>
<td>minerals)</td>
<td></td>
</tr>
<tr>
<td>Blue Cross</td>
<td>Azurite (Cu), Lead White (Pb)</td>
<td>Lapis Lazuli, Azurite</td>
</tr>
</tbody>
</table>

From this table it is clear that multiple complementary techniques are often required to completely understand the use of materials. Many of the techniques above can be used as imaging spectroscopy methods, in which each pixel of an image contains chemical information. Other examples include hyper-spectral imaging and various synchrotron radiation based x-ray techniques. Hyper-spectral imaging spectroscopy has mapped egg yolk and animal skin glue in early Renaissance paintings using molecular contrast (18). This technique has been applied on a macro-scale; mapping the use of a particular pigment or binder over an entire painting (10), but does not give stratigraphic
information. The elemental information provided by x-ray techniques has been extremely useful in studying pigment degradation (9, 22, 23). For example, synchrotron radiation sources have proven useful in examining cadmium yellow degradation in paintings by Van Gogh (24) and Matisse (25, 26). Synchrotron based methods have also successfully revealed hidden paintings under the surface of paintings by the masters, Rembrandt (2) and Van Gogh (3, 4). Recent technological advances could allow for mobile scanning macro-XRF systems (27, 28), which has had some promise in studying underpaintings (29).

Unfortunately, the removal of a cross-section sample is typically required to discern the 3d structure of the painting because these spectroscopic techniques can only provide *non-depth* specific material information.

### 1.2.2 Invasive Analysis of The Crucifixion

In *The Crucifixion*, cross-sectional analysis with bright field microscopy (figure 6), revealed that Mary’s robe is painted with a thick and pure layer of lapis lazuli.
Figure 6: Bright field image of cross-section sample from the edge of Mary’s mantel in *The Crucifixion*.

This excessive use of lapis lazuli is of particular interest because of the great cost of the pigment. *The Crucifixion* is thought to originally be the central compartment of one panel of a diptych altarpiece (*Madonna and Child Enthroned with Angels, The Annunciation, and Female Saints*), but without further study of that altarpiece, such a connection cannot be made. A good indication would be the use of lapis lazuli in the Virgin’s blue mantel. If Mary’s robe in *Madonna and Child Enthroned with Angels, The Annunciation, and Female Saints* had a similar thickness, it would be a good indicator that the panels originally went together.

Cross-sections need to be small but also as representative of the painting as possible. However with typical sizes of up to 0.5 mm, cross-sections really only contain
local layering information, which is seen in figure 7, where the cross-section from the robe of a floating angel reveals a completely different layering technique.

![Cross-section image](image)

**Figure 7:** Bright field image of a cross-section from a floating angel’s robe on *The Crucifixion*. This cross-section sample highlights how conservators can study the 3d structure of a painting to study methodology.

The methodology (paint layering techniques) can be easily visualized with bright field imaging. For example, gold was applied to the panel by water gilding onto a red mordant (as seen in figure 7) and gold embroidered decoration was applied by mordant gilding (as seen in figure 6). Fluorescence microscopy provides complementary information about organics (such as binders, varnishes, or glues) that may be absent in the bright field image. Figure 8 shows the fluorescence image of the same cross-section above, but in this case there is a clearly visible organic layer on the top.
Figure 8: Fluorescent image of the same cross-section seen in figure 7. Fluorescence images can provide additional information; here a layer of organic material on top glows brightly, which is dark in the case of the bright field image.

Cross-sections can also be analyzed with various analytical techniques to identify the artists’ materials. SEM-EDS is a common technique for cross-section analysis, allowing the materials in the cross-section to be imaged with high spatial resolution and elemental contrast. For certain pigments, it is straightforward, such as finding lead signals in the white pigment in the ground (lead white) or mapping mercury and sulfur to the red grains in the pigment layer (vermilion). Figure 9 shows the SEM-EDS analysis of the angel’s robe cross-section from The Crucifixion, seen in figure 7.
Figure 9: SEM-EDS analysis of the same cross-section from figures 7 and 8. SEM-EDS of a cross-section provides elemental information while retaining spatial reference. Here, we identified a gypsum and lead white ground, hematite or earth pigment based bole and mordant, gold leaf, and a mixture of lead white and lapis lazuli. The organic layers present (as seen in the fluorescent image) cannot be identified with this technique.

Sodium, calcium, aluminum, silicon, and sulfur all map to the blue particles (lapis lazuli). Calcium, sulfur, and lead also map to the white ground (gypsum and lead white). Iron (with silicon and some aluminum) map to the bole and mordant (earth pigment/hematite). The gold mapping is clearly attributed to the gilding. Pigment identification using elemental analysis was straightforward (aided by the bright field
image) for this cross-section; however, this is not always the case. For example, the elements in lapis lazuli are common among mineral pigments, and while the sulfur is a good indicator, it also maps frequently in the cross-section. Raman spectroscopy is also used to analyze cross-section samples to provide the complementary molecular information if necessary.

There is also an array of micro-chemical tests that can be applied to cross-sections for material (both organic and inorganic) identification; an entire chapter in Steward’s book is devoted to such tests (8). However, each micro-chemical test necessitates the surface of the cross-section to be re-polished and, eventually, the cross-section will be completely destroyed.

Due to the destructive nature and limited (local only) information provided by cross-section samples, there is clear motivation to develop an in-situ spectroscopic imaging technique that can provide both chemical and spatially resolved depth information. While it is clearly desirous to limit damage to historic artworks, there is a wide range of heritage objects, such as historic paper documents or illuminated manuscripts, which cannot be sampled. Further, even on paintings, there are areas of interest that a conservator would never sample, such as icons’ faces.

1.3 Experimental Details for Techniques Used Throughout Dissertation.

The experimental techniques listed below were also applied to the study of the *The Crucifixion*, and used throughout the remainder of this dissertation, where noted.
1.3.1 FORS Analysis

A fiber-optic spectroradiometer, FS3 (ASD. Inc. Boulder, CO) was used to obtain FORS spectra from the mock paintings. The spectrometer operates from 350 nm to 2500 nm with a spectral sampling of 1.4 nm from 350 to 1000 nm. The spectral resolution at 700 nm is 3 nm. The light source of a leaf probe head (ASD. Inc.) was used at a distance of 20 cm to illuminate the samples (~400 lux) and the fiber was placed ~1 cm from the object, giving a ~3 mm spot size at the painting. We averaged 2 spectra with a total acquisition time of <5 s per point.

1.3.2 Bright Field Microscopy

Examinations were carried out using a Leica DMRX polarizing light microscope in reflected mode. Brightfield illumination was achieved using a tungsten-halogen lamp. Leica Fluortar x20 and x50 objectives were used. Fluorescence observations were made using a high-pressure mercury lamp and a Leica D cube (Excitation UV / Violet; Excitation filter BP 355-425nm; Dichromatic mirror 455nm; Suppression filter LP 470nm). Images were captured using a Canon EOS 2D Mark ii digital SLR body affixed to the photo port on the DMRX. Canon image capture software was operated using a MacBook Pro.

1.3.3 X-Ray Fluorescence Intensity Spectroscopy

The elemental compositions of major color areas across the paintings were studied using a Bruker Tracer III-SD handheld x-ray fluorescence spectrometer (Rh-tube,
40 keV, 11 µA, 180 s spectral accumulations). Spectra were collected in a non-contact configuration under a vacuum that ranged from 10-25 torr.

1.3.4 SEM-EDS

SEM-EDS analyses were carried out using a Hitachi S3-400N variable pressure scanning electron microscopy (VP-SEM) fitted with an Oxford Instruments X-Max detector and Oxford Instruments INCA X-ray spectrometer. Samples were placed onto conductive carbon stubs using carbon tape. A tungsten filament served as the electron beam source. Samples were examined in an uncoated state at chamber pressures ranging from 35 – 40 Pa at a working distance of 10 mm. The accelerating voltage used was 20 kV. EDS regions of interest (ROI) images were generated using a Hitachi backscattered electron detector and were captured through the Oxford INCA spectrometer analysis software. Electron density differences (inferred to be elemental differences) among constituents within the sample were visually presented as varying gray level values in the backscattered image. Characteristic X-ray energies were collected over a 0 (zero strobe used for calibration reference) - 20 keV, processed and displayed as both x-y plots and element maps (i.e., element detection in areas within a given ROI visually expressed as a distribution map). Characteristic X-rays detected provided a basis from which to draw sample constituent inferences, while the element maps revealed distributions of the constituents within the ROI.
1.3.5 Raman Microscopy

Raman spectra were collected with a Renishaw inVia Raman microscope using the 532 nm line for excitation and calibrated with the 520.5 cm⁻¹ silicon Raman band. Spectra were collected with a 20x objective, 1800 l/mm grating, with laser power and collection times varying for each spot to optimize signal and avoid sample damage.

1.3.6 Macrophotography

IR and UV/VIS images were captured with a Nikon D200 during exposure of the paintings to a tungsten lamp and black light, respectively. X-radiography was performed using a Picker Hotshot unit with varying operating voltages, tube currents, and exposure times for the best image contrast. Paintings were exposed with underlying Kodak film, developed in-house and photographed with a Nikon D200 for digitization.
2. Three-dimensional Imaging Spectroscopy in Cultural Heritage Studies

The imaging methods discussed in chapter one can be characterized as linear optical microscopy techniques. Unfortunately, linear microscopies tend to trade off between spatial resolution, depth penetration, and chemical specificity. High depth resolution is particularly difficult to achieve in samples that are highly absorbing and scattering. Multi-photon microscopy techniques are nonlinear and have been a mainstay for 3d imaging in scattering media since the 1990’s. This chapter will briefly discuss the current linear 3d imaging techniques and then focus on nonlinear optical microscopy and the application of nonlinear microscopy to cultural heritage research.

2.1 Linear Imaging Microscopy

An important consideration in the discussion of optical techniques is that many methods have developed for the purposes of imaging deeply into biological samples. Skin is not that different from a painting. For example a sample of skin will have several layers that contain biological pigments, such as eumelanin, pheomelanin, and hemoglobin, encased in ‘binding’ material (skin tissue). The difficulties of 3d imaging into the skin would cause a doctor to take a biopsy (just as a conservator must take a cross-section). Marvin Minksy developed confocal microscopy from a desire to visualize brain cells in 3d, problematic because of the densely packed tissue of the central nervous system (30).
Confocal techniques use a pinhole in front of the detector to reject out-of-focus light, cleanly separating signal at different depths and allowing for optical sectioning (31). Many linear techniques can adopt the confocal geometry and, though pioneered for biological applications, confocal x-ray (32-34), Raman (35, 36), and fluorescent (37) microscopies have proven very useful in the field of cultural heritage. Unfortunately, the addition of a pinhole greatly reduces the efficiency of signal collection because of the rejection of any out of focus photons. However, multiple scattering events in the sample can cause out of focus photons to get through the pinhole, reducing resolution. In highly scattering samples, such as tissue, imaging depth is limited to less than 100 µm with confocal techniques.

There are non-confocal 3d imaging techniques; two examples are optical coherence tomography (OCT) and terahertz imaging. OCT has had a very large impact in the field of ophthalmology (38) and terahertz imaging has also been applied to a wide range of biomedical applications (39). There have been successful extensions of these technologies to cultural heritage. OCT imaging of varnished wood (as applied to a violin) allowed for the 3d visualization of fibers and cell wall distribution as well as the surface state of the wood (40). Terahertz imaging has been applied to study hidden paintings (41), though quantitative depth information was difficult to extract due to image artifacts caused by an uneven surface. One disadvantage of both techniques is that image contrast is largely based on refractive index mismatches and therefore only
provides structural contrast, which is not material specific and limits the application to cultural heritage.

There are several interesting 3d x-ray methods that have been applied to paintings (42, 43), and as mentioned in chapter one, x-ray techniques have been successful in revealing hidden paintings and studying the degradation of heavier metal pigments. Unfortunately, this is also a limitation of the technique; light element mineral pigments (lapis lazuli) and organic dyes (cochineal) are not easily detected and the presence of lead white (or other heavy metals) can cause unwanted absorption effects, such as shielding other elements. In many x-ray techniques, the 3d data must be mathematically reconstructed and many, though not all, require the use of a synchrotron for high intensity and stable monochromatic x-ray light.

2.2 Nonlinear Imaging Microscopy

Nonlinear optical processes can combine high depth and spatial resolution for imaging in highly scattering media. In 1990 Watt Webb developed a nonlinear microscopy technique utilizing two-photon excited fluorescence (2PEF), a technique frequently applied today in biomedical applications (44-46).

Nonlinear techniques generate signal only at the focal position and optical sectioning is possible without the need for a pinhole (allowing all signal photons to be collected for greater signal to noise). Typical nonlinear regimes use longer (IR) wavelengths and ultrafast (< 0.2 ps) pulses. Longer wavelengths allow for greater
penetration depth and tend to reduce linear absorption and scattering in the sample, while short laser pulses provide high peak intensities at low average power.

Figure 10 compares linear fluorescence to nonlinear fluorescence.

**Figure 10:** Linear (400 nm excitation, left) and nonlinear (800 nm excitation, right) fluorescence in rhodamine 6G. Note that the nonlinear absorption is localized to occur at the focal point.

The dye, Rhodamine 6G, is shown in one case absorbing one 400 nm photon and, in the other, simultaneously absorbing two 800 nm photons. In each case, the dye is excited and fluoresces. However, with one photon absorption the dye also fluoresces outside of the focal point and signal, in the absence of a detector pinhole, is collected from all the sections in the dye that interact with the laser. In contrast, nonlinear fluorescence from the dye can only occur at the focal point, where the laser’s photon density is highest, and optical sectioning is possible.

Traditional nonlinear imaging techniques, such as 2PEF, second harmonic generation (SHG), and third harmonic generation (THG) rely on processes that generate light of a different color (or wavelength) than the excitation light. Fluorescence or
harmonic generation can then be easily collected and used for image contrast. SHG has been frequently utilized as a method for imaging collagen in a range of tissues (47) and cellulose (48), but these materials occur frequently in historic artworks. Collagen is present in parchments made from animal skins and in animal glue binders. Cellulose is in many wood derived papers and also in plant based binders, such as gum arabic. These traditional nonlinear imaging methods have found a few applications to cultural heritage; recent research includes the 3d imaging of varnish and a red organic dye in mock-up systems, as well as wood in-situ on a violin using a combination of second harmonic generation and two-photon excited fluorescence (49). Another example includes mapping oil and varnish interfaces with third harmonic generation and 3PEF (50). However, these fluorescent and harmonic processes generally lack chemical specificity. In the case of the oil and varnish interfaces, confocal Raman was previously used to aid in the depth resolved identification of materials, although quantification of the real thickness of the samples was not possible.

In biomedical applications, specificity is often addressed by the addition of chromophores to the sample, but this is not possible in the study of historic artworks. Further, most inorganic pigments neither fluoresce nor generate appreciable harmonic light. It would be advantageous to expand the range of detectable molecular signatures to processes that don’t generate light of a different color. This is the important advantage of pump-probe microscopy.
2.2.1 Femtosecond Optical Pump-Probe Microscopy

Near-infrared femtosecond pump-probe optical microscopy can detect a range of transient absorption processes (51), including signals from excited state absorption, ground state depletion, and stimulated emission (52). Two ultrafast laser pulse trains (typically 0.2 ps pulse duration) are coupled into a laser-scanning microscope. A time delay is set between the pump and probe pulses such that the sample is first electronically excited and then its response is probed at a later time (up to 100 ps). Different molecular processes have different effects on the probe pulse as a function of pump intensity and pump-probe delay (see figure 11).

![Energy level diagrams](image)

**Figure 11**: Energy level diagrams representing transient absorption mechanisms detectable with pump-probe microscopy.
In sequential two-photon absorption, the probe is absorbed only by molecules in the excited state, hence the presence of the pump increases the probe absorption (the absorption then diminishes for longer delays). In contrast, for ground state depletion the probe is absorbed by molecules remaining in the ground state, which has been partially depleted by the pump, hence the presence of the pump decreases the probe absorption (probe absorption increases back to the equilibrium value for long delays).

In the case of these absorptive processes, the generated signal is not separable from the excitation beams because it is merely causing a loss or gain of the probe pulse, a change that is very small compared to the intensity and noise of the laser beams. However, by intensity modulating the pump beam at a frequency higher than the laser noise, modulation is forced onto the probe during nonlinear interactions in the focal volume within the sample, illustrated in figure 12. A photodiode and lock-in amplifier can then sensitively detect the signal (51).

![Intensity Modulation Transfer Diagram]

**Figure 12:** Intensity modulation transfer from pump to probe during a nonlinear interaction in the sample.
The modulation frequency is typically several MHz, chosen to overcome the noise spectrum of laser fluctuations. Pump-probe microscopy, like other nonlinear imaging methods, is much less affected by light scattering than conventional microscopy; the signal is proportional to the product of the intensities of the two lasers, causing scattered light to produce much less signal, giving the method its power in 3d imaging. A schematic of our experimental setup is shown in figure 13 (53, 54).

![Pump-Probe Microscopy Experimental Set-Up](image)

**Figure 13: Experimental Set-Up**
Our experimental set-up includes a Ti:Sapphire modelocked laser (repetition rate of 80 MHz, 810 nm pulse duration of roughly 150 fs), which pumps an optical parametric oscillator (OPO) to generate an output pulse in the visible to the near-IR of a similar pulse duration. The pump pulse train is intensity-modulated at 2 MHz using an acousto-optic modulator (AOM). The probe pulse is unmodulated and the interpulse delay is controlled via an adjustable optical path length in the probe arm. The two beams are overlapped on a dichroic mirror and sent collinearly into a laser-scanning microscope. The pulses are focused onto the sample with a 20x 0.7 NA air objective or a 60x 0.9 NA air objective. Back-scattered light is collected using a photodiode and lock-in amplifier to create pump-probe images or directed via a dichroic onto a photomultiplier tube for nonlinear fluorescence images.

In general, a series of images are taken at different pump-probe time delays, where each pixel will contain the intensity of the probe. This pump-probe image stack allows us to observe the change in the probe intensity over time. Pump-probe delay curves are chemically specific (although an instantaneous two-photon absorption signal is not always specific). We can create 3d images by selecting an appropriate pump-probe time delay and taking a series of en-face images (xy images perpendicular to the beam axis) at different depths (z-direction).
2.3 Extension of Pump-Probe Microscopy to Artist Pigments

Pump-probe microscopy, developed mainly for biomedical applications, has imaged a variety of biological pigments with unique contrast. One example is distinguishing between oxy- and deoxy-hemoglobin to image blood vessels in a live mouse ear (53, 54). Pump-probe microscopy has also differentiated between eumelanin and pheomelanin (55, 56) in the imaging of pigmented skin lesions (57) and ocular cancer (58). Pump-probe microscopy imaged eu-and pheo-melanin with subcellular resolution (59) to visualize in 3d melanin caps on basal cells. Further, the unique pump-probe dynamics of these two biological pigments were found to vary in the presence of iron (60), indicating the technique can provide unique contrast for 3d imaging as well as be a useful tool in studying photochemistry and function. The technique has also been applied to fossilized eumelanin (61). This dissertation focuses on extending our technique to the artist’s pigment palette.

At the same pump-probe wavelength combination used to image melanins (720 and 810 nm, respectively), we generated unique pigment contrast for lapis lazuli and its synthetic equivalent, ultramarine blue, as well as another blue pigment, indigo, and the red pigments caput mortuum and vermillion (62). Caput mortuum is an iron oxide based earth pigment; iron oxides are discussed briefly in chapters 5 and 6. These results, along with images of the bulk pigments, are shown in figure 14.
Figure 14: Pump-probe dynamics of several blue and red pigments generated from 720-810 nm, pump-probe, wavelength combination.

While these initial results are promising, achieving pump-probe contrast in fine art objects has a unique set of challenges different from skin imaging. Artist colorants range from organic dyes to inorganic minerals with colors spanning the entire visible spectrum, in contrast to the limited biological chromophores; for example, hemoglobin, eumelanin, and pheomelanin all provide unique image contrast with the 720-810 nm pump-probe wavelength combination. Several historically important pigments remain invisible to this wavelength combination, such as azurite, malachite, and several organic dyes. More recently (63) we have shown that it is possible to address the complexity introduced by the large range of possible pigments in the paint layers by increasing the
spectral range of the pump and probe beams, which we will discuss in the next section, as well as how to apply this specificity to 3d imaging.

2.4 Pump-Probe Virtual Cross-Sections

In a typical painting, the 3d structure could consist of multiple colorants in layers, mixtures, or a combination of layers and mixtures. As briefly discussed in chapter one, the palette was limited during the Italian renaissance and purples were often made using combinations of red pigments such as kermes or red madder (both substituted anthraquinones) mixed or layered with blue mineral pigments (lapis lazuli or azurite). The combination of kermes and lapis lazuli gives a rich purple that would be suitable for iconic figures in a painting, an example of which would be the lavender robe of Pilate in the painting Christ Before Pilate, in the permanent collection of the North Carolina Museum of Art. A combination with the cheaper azurite can give a darker muted purple useful for less prominent figures.

We created two purple mock-up paintings; one a blue pigment (synthetic ultramarine) covered with a thin glaze of red pigment (quinacridone red, a modern transparent light-stable replacement for the natural substituted anthraquinone) and in the other, the two pigments are mixed. It should be noted in the mixed painting that we used natural lapis lazuli and not the synthetic version of the blue pigment. Figure 15 shows bright field images of the mock-ups and highlights how current methods (fiber optics reflectance spectroscopy) cannot provide depth information. The reflectance of
the two paintings identifies the materials but gives little clue as to which painting is layered and which is mixed.

Figure 15: Purple mock-up paintings and resulting reflectance spectra. Quinacridone red is indicated by the reflectance peak at 600 nm, while lapis lazuli has a reflectance peak around 400 nm and increased reflectance at 700 nm (due to increasing transparency of lapis in the IR and reflectance from the white ground of the sample). Figure adapted from reference (63).
As previously discussed, we can create a virtual cross-section by taking a series of en-face images at different depths with an appropriate pump-probe time delay and wavelength combination, i.e., imaging parameters that fully separate ultramarine blue from quinacridone red. Such information can be characterized from pump-probe image stacks taken at different wavelength combinations of the two pigments in question. In this case we were able to image a physical cross-section from the mock-ups, to characterize the two pigments. The spectroscopic results, as well as the bright field and pump-probe images, are presented in figure 16.

**Figure 16:** Pump-probe delay traces for ultramarine blue and quinacridone red at a variety of pump/probe wavelengths (indicated in the legend in nm). The two pigments show vastly different time responses dependent on pump and probe wavelength. Bright field images of the physical cross-sections are shown with their respective pump-probe image. The pump-probe images were taken at an interpulse delay of 0.1 ps and a wavelength combination of 615/810 nm. Quinacridone red is false-colored red and ultramarine blue cyan. The pump-probe images are 365 µm x 90 µm in size. The total power for this data collection was 5 mW. Figure adapted from reference (63).
At a pump-probe wavelength combination of 615-810 nm, the signal in quinacridone red is positive and decays in time. In ultramarine blue the signal is negative, also decaying in time. The combination of positive and negative pigment-specific transient absorption signals provides an ideal case for creating a virtual cross-section. Interestingly, at pump/probe wavelengths of 655-810 nm, the transient absorption amplitudes for these pigments are reversed (although much weaker in magnitude for quinacridone red). The temporal decay characteristics of the pigments also vary with pump/probe wavelengths, providing yet another method of pigment separation. There is no signal from the acrylic binder. The bright field and pump-probe microscopic images of the physical cross-sections taken from the two mock-up paintings give similar results in terms of both the distribution of pigments and the layer thickness. In the layered case, the red glaze is ~5 μm and the synthetic ultramarine ~25 μm thick, while the mixed sample has one layer with a varying thickness of 25 to 90 μm. The pigments in the pump-probe images are assigned false-colors according to their pump-probe response; the red glaze (colored red) has a positive response and the ultramarine blue (colored cyan) has a negative response. It is important to note for future work we can build a pump-probe library with cross-section samples from a variety of historical artworks that have already been characterized with currently accepted analytical techniques.
Pump-probe virtual cross-sections (generated at a wavelength combination of 615/810 with an interpulse delay of 0.1 ps) immediately reveal the stratigraphy of the two mock-up paintings, as seen in figure 17. Further, each pigment presents with unique contrast and we can see variations in the artist’s brushwork.

Figure 17: Pump-probe virtual cross-sections of the mixed and layered mock-ups. A volume set of pump-probe images of the intact mock-up paintings was taken at a wavelength combination of 615/810 nm, fixed interpulse delay of 0.1 ps, and total power of 3 mW, with a 20x 0.7 NA objective. One image of each set is shown, false-colored red for quinacridone red and cyan for ultramarine blue. Each *en-face* (xy) image is 365 µm x 365 µm and the virtual cross-sections are 365 µm x 90 µm. Figure adapted from reference (63). Virtual cross-sections distinguish the mixed from the layered methodology.
At this particular pump wavelength (615 nm), our imaging depth in lapis lazuli was limited to roughly 10 μm due to absorption by ultramarine, but tuning the pump wavelength to 710 nm should increase the penetration through this pigment six-fold, at the expense of a negligible signal from red glaze. Although this difference in depth penetration is not extremely evident in our mock-up sample due to the layer thickness being only roughly 25 μm thick, figure 18, highlights the use of a higher NA objective (60x 0.9 NA) to cleanly resolve the 5 μm thick layer of red glaze and our ability to pick different wavelength combinations to obtain different information in virtual cross-sections.

![High Resolution Pump-Probe Virtual Cross-Sections](image)

Figure 18: High-resolution virtual cross-sections at two different wavelength combinations. These virtual cross-sections were created using a 60x 0.9 NA objective with wavelength combinations of 615/810 and 710/810 at a fixed interpulse delay of 0.1 ps and total power of 3 mW. The virtual cross-sections are 155 μm x 40 μm (scale bar represents 20 μm) and false-colored red for quinacridone red and cyan for ultramarine blue.

This technique has several advantages over the removal of a physical cross-section, besides its nondestructive nature. Because we map out an entire volume, we can
create virtual slices from the entire field of view in any direction. This allows us to visualize differences in brushwork or abrupt changes in layering that may not be evident in a physical cross-section, in which accessible information is dependent on the sampling orientation. In addition, we can sample from many areas anywhere in the painting, which is not possible when acquiring physical cross-sections (generally conservators do not remove samples from pristine areas of the paintings).

2.5 In-Situ Pump-Probe Cross-Section of The Crucifixion

As an in-situ proof of principle experiment, we were able to image an intact painting, *The Crucifixion* by Italian painter Puccio Capanna painted in roughly 1330 CE. Prior analysis of the painting, discussed in chapter one, indicated thick layers of lapis lazuli in Mary’s mantel, which permitted us to test our depth penetration, while the purplish robe of a floating angel presented an opportunity to image multiple layers in a historic painting, similar to our mock-up paintings.

Prior cross-sectional analysis of the Virgin Mary’s robe indicates that the robe had been painted with a thick (up to 60 µm) layer of lapis lazuli, which is quite unusual given the high cost of the pigment. Pump-probe imaging in the center of the robe gave virtual cross-sections consistent with this lapis lazuli thickness, and figure 19 highlights the ability of this method to noninvasively image through a thick pigment layer.
Figure 19: Investigation of the Virgin Mary’s mantel in Puccio Cappana’s, *The Crucifixion*. The bright field image of the physical cross-section is 200 µm x 365 µm, shown below a photograph of the painting under our pump-probe microscope. To create the virtual cross-section the painting was imaged in an area containing only a single layer of lapis lazuli (in contrast to the physical cross-section) with a wavelength combination of 720/810 nm, an interpulse delay of 0.2 ps and a total power of 2.7 mW. The *en-face* image (365 µm x 365 µm) is roughly 30 µm under the surface of the robe and is shown with a virtual cross-section (60 µm x 365 µm). Lapis lazuli is false-colored blue and mineral impurities that naturally occur with the pigment are colored magenta. Figure adapted from reference (63).

The physical cross-section was removed from an area at the edge of Mary’s robe, which was embroidered with gold leaf, which explains in this case the presence of gold leaf and mordant above the layer of lapis. This was in contrast to the pump-probe image, which we took in the middle of the robe, expecting to image only lapis and no gold leaf or other gilding artifacts. We then compared pump-probe virtual cross-sections...
to the physical cross-section taken from the purplish area of the floating angel (as seen in chapter one) in order to image through multiple layers in a historic panting.

In this case, the bright field and pump-probe microscopic images of the physical cross-section give similar results in terms of both the distribution of pigments and the layer thickness, with a few exceptions. Bright field and SEM-EDS analysis indicates a very delicate and thin layering of pigments containing, from top to bottom, a faded red glaze, a mixture of lead white and lapis lazuli, iron oxide, an organic coating, gold leaf, an iron rich mordant (a mixture of pigments and oil used to adhere the gold leaf (64)), and a gypsum ground. Pump-probe analysis identifies only three distinct decay behaviors (at a 710/810 wavelength combination) in lapis lazuli, iron oxide, and gold. Here, we classified the temporal dynamics in the pump-probe signals using phasor analysis, a method that is commonly used to visualize decay times in fluorescence lifetime measurements (65) and that was recently adapted to pump-probe work (66).

Phasor analysis generates a histogram based on the single frequency sine and cosine transforms for every pixel in the pump-probe image. The histogram, called a phasor plot, will separate the pixels according to lifetimes, according to the following equations:

\[
g(\omega) = \frac{\int I(t) \cos(\omega t) dt}{\int |I(t)| dt}
\]

\[
s(\omega) = \frac{\int I(t) \sin(\omega t) dt}{\int |I(t)| dt}
\]
where $\omega$ is the chosen frequency, $I(t)$ is the time dependent probe intensity, and $g(\omega)$ and $s(\omega)$ are the phasor plot axes. False-colored images based on specific lifetimes are generated by color-coding specific areas on the phasor plot (generally those that cluster together indicating very similar lifetimes). The false colored pump-probe image correlates well with the bright field image, despite the lack of signal in some regions. The decay behaviors and cross-section images can be seen in figure 20.

![Physical Cross-Section and Pump-Probe Delay Behavior](image)

**Figure 20:** Cross-section analysis of the angel’s purple robe in Puccio Cappana’s, *The Crucifixion*. A bright field image of the physical cross-section taken from the angel’s robe is shown with its respective pump-probe image that was taken at a wavelength combination of 710/810 nm with a total power of 1.5 mW. Each image is 55 $\mu$m x 545 $\mu$m. The pump-probe image (presented here at 0.2 ps) has been false-colored cyan for lapis lazuli and red for the two iron rich pigments above and below the gold layer. Figure adapted from reference (63).

At the chosen wavelength combination, we do not see a signal in the faded red glaze, lead white, organic coating, or gypsum, save for a few mineral impurities that may be present in those layers. We obtain signal from gold; however, the gilding could
not be spatially resolved and at this wavelength combination, iron oxide and mordant showed signals with identical decay behaviors. We imaged an area adjacent to the sample site and acquired volume data with a fixed pump-probe delay of 0.2 ps, which yields positive pump-probe signals from iron oxide, gold, and mordant and negative signals from lapis lazuli. The pump-probe dynamics of iron oxide/mordant and gold could be cleanly separated by acquiring pump-probe delays at each depth, but this is not practical with our current setup. Hence in these pump-probe images we color-coded the positive signal orange, encompassing any of the three materials, and negative signals cyan (lapis lazuli). These images and their resulting virtual cross-sections, including a maximum intensity projection of the volume along the y direction, are seen in figure 21.

Figure 21: Pump-probe analysis of the angel’s purple robe in Puccio Cappana’s, The Crucifixion. A series of en-face (185 µm x 185 µm) images were taken
with a 0.2 ps delay at different depths with a wavelength combination of 710/810 nm at a total power of 1.5 mW. The images have been false-colored cyan for lapis lazuli and orange for components that have a positive pump-probe signal. Virtual cross-sections are 50 µm x 185 µm. Figure adapted from reference (63).

At the probed location we found a composition that is slightly different from the physical cross-section, but this is not entirely surprising because cross-sections only contain local information. The en-face images show positive signal on the surface, most likely from iron oxide, negative signal in the center from lapis lazuli, and positive signal again underneath the lapis lazuli, which is most likely gold with possible contributions from mordant (the gilding in this region is heavily cracked, exposing the mordant underneath). This view is supported by virtual cross-sections extracted from this dataset. The virtual xz slice, and even more so the maximum intensity projection, suggest either a mixture or very thin layers of iron oxide with lapis lazuli, and gold leaf with mordant underneath.

In this case, we did not learn new historically relevant information in regards to the painting because The Crucifixion has been studied invasively with cross-sectional analysis. However, we were able to sample areas from which a conservator would not take a cross-section sample (the middle of Mary’s robe and undamaged areas of the angel’s robe). We did find different pump-probe dynamics in the aged lapis lazuli on Mary’s robe in comparison to modern lapis lazuli samples, which we will discuss in the next chapter. In chapter 5 we will address a technical case study and show how pump-
probe microscopy can complement traditional methods of study to aid in the attribution of two Italian roundels to Lorenzo Lotto.

2.6 Conclusion

We have shown that pump-probe microscopy is capable of providing pigment specific dynamics that can be utilized to create noninvasive virtual cross-sections in historic artwork. Although there are many considerations for the future development of pump-probe for use in cultural heritage applications, the remainder of this dissertation focuses on using our current microscope set-up to study several materials in depth, including the blue mineral pigment, lapis lazuli, and the organic blue dye, indigo. We will investigate the molecular origin of our pump-probe signal in lapis lazuli and combine pump-probe pigment contrast of indigo with nonlinear fluorescence from paper fibers to examine multi-modal imaging in studying paper samples. As previously stated, this dissertation culminates in a technical study in which pump-probe microscopy is used in lieu of physical cross-section samples.
3. Connections between pump-probe signal and molecular signatures in lapis lazuli

In the previous chapter we discussed the ability of pump-probe microscopy to provide high-resolution 3d pigment specific images. As demonstrated in pump-probe virtual cross-sections, the unique contrast afforded by selection of particular pump-probe wavelength combinations allows us to address the complexity of multiple pigments encountered in works of art. The image contrast provided by pump-probe microscopy is related to the excited and ground state dynamics of the pigments. However, unlike some linear spectroscopic techniques like FT-IR or Raman, pump-probe signatures do not have an obvious interpretation in terms of molecular structure. Molecular specificity in pump-probe can be further complicated by the fact that pump-probe delay dynamics in a pigment can change depending on the wavelength combination and power, although we exploit this complication for image contrast.

In an effort to further our understanding of the molecular basis of the pump-probe signal, we studied the ultramarine pigments (including the precious blue pigment, lapis lazuli) in depth. We chose lapis lazuli in part because it has a strong pump-probe response at our standard pump-probe imaging wavelength combination of 720-810 nm, and also because of the pigment’s cultural importance throughout 6,000 years of history. Although we have discussed (in chapter two) that synthetic lapis lazuli (ultramarine blue) has unique responses to different wavelength combinations, in this chapter we will thoroughly investigate the pigment’s response to the 720-810 nm
wavelength combination and correlate this to the well-established molecular signatures of the pigment.

As our samples, we will investigate natural lapis lazuli from Afghanistan and Chile and, as comparisons, synthetic ultramarines blue, violet, and red. We also examine sodalite, a typically colorless mineral crystal in the same family as lapis that lacks sulfur. First, we will first discuss the history, chemistry, and photochemistry of lapis lazuli and its relation to synthetic ultramarine pigments. Second, we will examine the pump-probe response of each pigment and how our choice of pump-probe wavelengths interacts with the pigments’ color center. Lastly, we co-register pump-probe and Raman images of the same crystals, leading us to conclude that pump-probe microscopy is sensitive to the ratio of the two chromophores present in lapis lazuli and ultramarines blue and violet. We find that our pump-probe interaction selectively excites the main blue chromophore, $S_3$, and causes stimulated emission in the associated yellow chromophore, $S_2$.

3.1. The History of Lapis Lazuli

Lapis lazuli is a very important material in cultural heritage. The blue stone has appeared in our cultural history for over 7,000 years; examples of its use stretch back to the centuries before the Common Era. King Tutankhamen’s death mask is constructed of gold and lapis lazuli stone, the Royal Tombs of Ur (67) contained many ceremonial objects carved from lapis lazuli, and many other examples are littered throughout
history. As a pigment, lapis lazuli is often seen in iconic figures; for example, in Italian paintings only the robe of Mary, very important saints, or the cross of Jesus would be painted with lapis lazuli. Even in these cases lapis lazuli was typically extended with a cheaper blue pigment (5) because it was more expensive than gold. It is the rarity of the material that imparted a seemingly ritualistic value to its use.

The most common ancient source of lapis lazuli was the Sar-i-Sang mines in Badakhshan, Afghanistan, but other ancient sources are thought to be the Pamir Mountains in Tajikistan and Lake Baikal in Siberia Russia (68, 69). The Andes Mountains in Chile is a modern day source (70), discovered in the 18th century, close to the time that J.B. Guimet performed the first industrial synthesis of ultramarine blue (71). The distance from these ancient sources to Italy (or the ancient cities of Egypt and Mesopotamia) is thousands of miles, accounting for the difficulty in obtaining the stone and its subsequent iconic use. In addition to the distance hindering its use, the sources were also very difficult to mine. The Sar-i-Sang mines, located 7,500 feet above sea level, are accessible only by ragged, narrow footpaths that are destroyed by harsh rains and snowstorms every winter, leaving only three months of the year to work the mines. Lapis from the other ancient sources were even more difficult to access; the deposits in the Pamir mountains are roughly 16,500 feet above sea level and Lake Baikal is even further from cities of trade (3,000 miles from ancient Mesopotamia and nearly twice that distance to reach Italy) (68, 72).
The formation of lapis, discussed in the next section, only occurs within the rock wall. Hence the cave wall had to be cracked open before the raw lapis lazuli could be removed. This was typically done by thermo-shocking the rock face; in ancient times this was accomplished by heating the wall with fire and then quickly cooling with water (72). The Sar-i-Sang mines are still worked for the stone today, and though little else has changed in its accessibility, dynamite is used to ease the burdens of mining.

3.1.2 The Formation of Lapis Lazuli

The stones that are mined and later worked into usable gems or ground into pigments are called lapis lazuli. The mineral responsible for the blue color is lazurite. Lazurite is a derivative of the aluminosilicate sodalite with a crystal frame of the formula \( \text{Na}_8[\text{Al}_6\text{Si}_6\text{O}_{24}]\text{Cl}_2 \), brackets indicating the repeating unit of the crystal structure. In the sodalite group, alternating \( \text{AlO}_4 \) and \( \text{SiO}_4 \) tetrahedra are corner-linked, giving the crystal a cubo-octahedral cavity that entraps sulfur anions, see lapis rock and lazurite crystal structure in figure 22 (73).
Figure 22: Raw lapis lazuli and crystal structure of blue mineral, lazurite. The rock has gold pyrite veins and includes other mineral impurities. The blue coloring, due to the mineral lazurite, has an alumino-silicate that entraps radical sulfur anions. Photograph of raw lapis rock adapted from reference (72). Crystal structure adapted from reference (74).

However, lazurite formation can only occur under certain geological conditions through a process termed contact metamorphism. In brief, lazurite formation involves magma from the Earth’s core leaking onto the surface and crystallizing into granite against other rocks, dolomite or limestone. Lazurite forms at this interface, where high pressures, temperatures, and magmatic gases cause chemical changes in the rocks (75, 76). Contact metamorphism is a highly complex process, the mechanism of which is still not entirely known for forming lapis lazuli. Different regions will have different magma and rock compositions; hence many accessory minerals occur naturally with the
formation of lazurite. Geo-sourcing attempts are often done in relation to these accessory minerals and not on lazurite itself.

Ion beam analysis and x-ray techniques (77) have been particularly useful in studying the trace elements associated with lapis lazuli and applied to geo-sourcing. Woolastonite has been found to be associated only with natural lapis lazuli from Chile (78). Diopside is common to all sources of lapis lazuli but has an associated fluorescence pattern that has been found in Afghan sources (79). Lapis lazuli rocks of a Siberian origin have an exceedingly high concentration of strontium (80).

Lapis lazuli as a pigment undergoes a preparation process that removes many of the accessory minerals and it can be difficult to geo-source using lazurite alone. However, recent research has postulated that geo-sourcing based on single lazurite crystals is possible. Specifically, Raman spectroscopy revealed CO\textsubscript{2} trapped within the lazurite cage in Afghan samples (81), while soft x-ray signatures (82) and sulfur K-edge x-ray absorption near edge structure spectroscopy (XANES) (83) have shown differences in the chromophore of lapis lazuli dependent on source.

3.1.3 Photochemistry in Ultramarines

The nature of the chromophores in ultramarine pigments has been under study for over 40 years with Raman spectroscopy as the key investigation technique (84-86). The deep blue color in lazurite originates from a charge transfer process due to molecular orbital transitions in S\textsuperscript{\textfrak{v}} (87), which has a very strong and broad absorption
with $\lambda_{\text{max}} \sim 590-625$ nm. Raman spectroscopy has been particularly useful in studying the $\text{S}^-$ (bent $C_{2v}$ symmetry) because the anion has three distinct Raman active vibrational modes (the fundamental symmetric S-S stretch at 540 cm$^{-1}$, a symmetric S-S-S bend at 240 cm$^{-1}$, and an anti-symmetric S-S stretch at 580 cm$^{-1}$). These modes are also IR active.

$\text{S}^-$ commonly occurs alongside small amounts of the yellow chromophore $\text{S}_2^-$, characterized by an absorption band with $\lambda_{\text{max}} \sim 380-400$ nm. Raman spectroscopy can also identify $\text{S}_2^-$, but it has only one Raman active mode, a S-S stretching mode at $\sim 590$ cm$^{-1}$. Typically, the 580 cm$^{-1}$ peaks of $\text{S}_3^-$ can only be clearly detected in Raman spectroscopy if the sample is excited by 647.1 nm (88), which is far enough from the absorption of $\text{S}_2^-$ that its weak stretching mode is not detected. As a result the Raman shift of $\text{S}_2^-$ is often seen moving between 582-590 cm$^{-1}$ because of a superposition of the $\text{S}_2^-$ and $\text{S}_3^-$ (580 cm$^{-1}$) peaks. Full details on bond angels, charge distribution, spectroscopic constants, and molecular orbital calculations of the $\text{S}_3^-$ and $\text{S}_2^-$ anions can be found in refs (84-86, 88, 89).

Ultramarine red and violet have similar spectroscopic properties but show absorptions around 520 nm and Raman shifts around 675 cm$^{-1}$, which has been attributed to a red chromophore, $\text{S}_4$ (84). This red chromophore is not present in the natural lapis lazuli or the synthetic ultramarine blue.

Alongside the extensive spectroscopic study of the chromophores in lazurite, there is also a body of research in regards to the synthesis of ultramarine pigments to
investigate the causes of variations in the blue intensity. Ultramarine blue synthesis involves heating china clay, soda ash, sulfur, and a reducing agent to 800°C and then allowing the product to cool and admitting oxygen (71). Lazurite naturally has particles featuring a range of blue hues, and in the ultramarine pigments, manipulating the ratio of $S^-$ and $S^2-$ can completely change the coloring from blue to green and yellow.

### 3.2 Molecular Analysis of Bulk Natural and Synthetic Ultramarine Pigments

To confirm the spectroscopic properties of our samples, purchased from Kremer Pigments, we performed FORS (figure 23) and Raman spectroscopy (figure 24) on bulk pigments placed on glass microscope slides in no binding media.
Reflectance spectroscopy results are consistent with established results. Natural (Chilean and Afghan) lapis lazuli and synthetic ultramarines blue and violet show reflectance dips centered around 400 and 625 nm, due to electronic absorption in $S_2$ and $S_3$, respectively. The main absorption feature in ultramarine red is shifted to 520 nm due to the red molecular chromophore, $S_4$, and a small absorption around 520 nm can also be seen in ultramarine violet. The colorless sodalite has no discernable features.
Figure 24: Raman spectroscopy on bulk natural and synthetic pigments. Five spectra each from random areas within the bulk pigments were averaged together to generate each spectrum in the figure (technical details described in chapter one).

Raman spectroscopy of these bulk pigments shows nothing unexpected; see table nine below for a full listing of peak assignments. The most distinctive features in the Raman spectra are the $S_2$ and $S_3$ fundamental peaks (585 and 545 cm$^{-1}$, respectively), in natural (Chilean and Afghan) lapis lazuli and synthetic ultramarines blue and violet. Ultramarine red has a drastically different spectrum because the main chromophore is the $S_4$ molecule; although there is still a weak peak indicating the presence of $S_2$ and a very weak $S_2$ peak. Sodalite shares no spectral features with the ultramarines because it is lacking the sulfur color center.
### Table 7: Raman Peak Assignments

<table>
<thead>
<tr>
<th>Band Wavenumber (cm(^{-1}))</th>
<th>Band Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>258 (m)</td>
<td>(V_2) (bending vibration of (S_3))</td>
</tr>
<tr>
<td>287 (w)</td>
<td>(? V_2) (stretching vibration of (S_2))</td>
</tr>
<tr>
<td>356 (vs)</td>
<td>(S_4)</td>
</tr>
<tr>
<td>548 (s)</td>
<td>(V_1) (symmetric stretching of (S_3))</td>
</tr>
<tr>
<td>585 (m)</td>
<td>(V_1) (stretching vibration of (S_2))</td>
</tr>
<tr>
<td>653 (s)</td>
<td>(S_4)</td>
</tr>
<tr>
<td>673 (vs)</td>
<td>(S_4) (?(V_1))</td>
</tr>
<tr>
<td>805 (w)</td>
<td>(V_1+V_2) ((S_3))</td>
</tr>
<tr>
<td>837 (w,sh)</td>
<td>(? V_1+V_2) (5(\nu))</td>
</tr>
<tr>
<td>1028 (m)</td>
<td>(S_4) (356+673)</td>
</tr>
<tr>
<td>1095 (m)</td>
<td>(2V_1) ((S_3))</td>
</tr>
<tr>
<td>1126 (m,sh)</td>
<td>(~ 2V_1) ((S_2))</td>
</tr>
<tr>
<td>1345 (w)</td>
<td>(S_4) (2\times673)</td>
</tr>
<tr>
<td>1352 (w)</td>
<td>(2V_1+V_2) ((S_3))</td>
</tr>
<tr>
<td>1641 (m)</td>
<td>(3V_1) ((S_3))</td>
</tr>
</tbody>
</table>

In the table, \(w, m, s, vs,\) and \(sh\) indicate peak intensity for weak, medium, strong, very strong, and shoulder, respectively. Question marks in the band assignment are due to unverified assignments. The table was reproduced from Clark’s assignments in references (84-86).

### 3.3 Pump-Probe Analysis of Natural and Synthetic Ultramarine Pigments

Previous pump-probe spectroscopy of lapis lazuli (from Afghanistan, Chile, and synthetic ultramarine blue) indicated that there are differences in pump-probe response
among these pigments (62). The FORS and Raman spectroscopic results lead us to believe that these lifetime differences could be due to the variation in the ratio of $S_2$ and $S_3$, which is the main molecular difference between the pigments. In order to test this hypothesis, we created dispersed pigment samples on microscope slides that were suitable for imaging with pump-probe microscopy in conjunction with bright field and Raman microscopy. In five different spots from each sample (marked with silver for spatial reference), we took a pump-probe delay stack at a wavelength combination of 720-810 nm and analyzed the lifetime variations using phasor analysis. The spectroscopic results are seen in figure 25.

Figure 25: Cumulative phasor analysis and resulting pump-probe spectra of ultramarine samples. On each prepared sample, we took a pump-probe delay stack at a wavelength combination of 720-810 nm, pump-probe, with a total power at the sample of 3 mW and 20x 0.7 NA air objective from five random areas that had been
marked with silver for spatial reference. These 20 delay stacks were analyzed with phasor analysis at 0.12 THz (excluding sodalite and ultramarine red that have no pump-probe signal).

Cumulative phasor analysis (65, 66) of all the pump-probe images from each sample show a range of lifetime responses with the exception of sodalite and ultramarine red, which do not have a pump-probe signal at this particular wavelength combination. In cases with two distinct and easily separated signals (for example, a positive and negative signal), we can use phasor information to color our pump-probe images and spatially identify where a particular pump-probe decay originates in the image. In this case, although the lifetimes of natural pigments tended to cluster together as did the synthetic pigments, the lifetimes were not easily separated and there was no clear distinction between the natural and synthetic pigments on the phasor plot. Thus, we chose the pump-probe extreme responses (circled and plotted in figure 25 as blue and yellow) to serve as a basis set for linear spectral unmixing.

To perform linear unmixing, we assume that each pixel in our pump-probe image is a linear combination of the two spectral responses seen in fig 25. We can then calculate the coefficients necessary for each pixel to equal such a linear combination and use these coefficients to generate an image with our desired coloring. The linear decomposed images will then show the distribution of the slow and fast pump-probe decays across the pigment crystals. Linear unmixing is similar to principle component analysis (PCA) described in reference (57), but in this case the spectral responses of our
samples is known and PCA is generally performed with no \textit{a-priori} knowledge. Bright field and pump-probe linear decomposition images can be seen in figure 26.

<table>
<thead>
<tr>
<th>Linear Decomposition Analysis of Ultramarine Pigments</th>
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</thead>
<tbody>
<tr>
<td><strong>Natural Ultramarine Blue</strong></td>
</tr>
<tr>
<td>Chile Lapis Lazuli</td>
</tr>
<tr>
<td>Afghan Lapis Lazuli</td>
</tr>
</tbody>
</table>

Figure 26: Bright field images and pump-probe linear decomposition images of ultramarine pigments. The images show correlation between the pump-probe signal and the blue coloring of the crystal with sub-crystal resolution. Pump-probe images were taken with a wavelength combination of 720-810 nm, pump-probe, total power of 3 mW at the sample, 20x 0.7 NA air objective. Each image is 547 x 467 \( \mu \)m. Images were false-colored according the basis set seen in figure 25.

As seen most clearly in the Chilean lapis lazuli sample, our pump-probe signal correlates to the color center of the crystal (with sub-crystal resolution) and not with the entire pigment crystal itself. Since we know this blue coloring is due to the ratio between \( S_2 \) and \( S_3 \) and that a heterogeneous mixture of the chromophores within lazurite crystals (90) is common in natural samples, we believe the sub-crystal changes in our pump-probe dynamics are due to the chromophore ratio. Further, in cases where there is no \( S_2 \) and very little to no \( S_3 \) (sodalite and ultramarine red), we do not get any
pump-probe signal. The red molecular chromophore, S₄, does not absorb our pump-probe wavelengths and would not interfere with our signal. From these images it is clear that the natural ultramarine blue samples are dominated by a long negative decay and the synthetic samples by a fast negative decay with excited state absorption.

A power study of natural (figure 27) and synthetic ultramarine (figure 29) allowed us to elucidate the nonlinear interactions in the samples.

---

**Figure 27:** Fixed pump and fixed probe power study on natural Afghan lapis lazuli. A power study on natural Afghan lapis lazuli was performed by holding the probe or pump power constant while changing the power the in other beam. At each power combination, we took a pump-probe delay stack with 720-810 nm pump-probe wavelength combination and 20x 0.7 NA air objective. Each pump-probe spectrum was taken from the same spot on the sample (a prepared canvas painted with lapis lazuli in egg yolk binder).

Power studies on natural Afghan lapis lazuli indicate that the negative component is due to a process that absorbs one photon from the pump and one from the
probe (as the power of either the pump or probe doubles, the signal also doubles). The pump is most likely selecting $S_3$ because it falls within the range of the charge transfer process $\sim 625$ nm. It is possible that the 810 nm probe is then causing ground state bleach (GSB) or stimulated emission (SE) in $S_3^{-}$. However if that were the case, we would expect to see the same signal in ultramarine blue and violet, which are also dominated by the $S_3^{-}$ peaks. In our molecular analysis, the only difference between these pigments is the shoulder peak that indicates $S_2^{+}$, which is weaker in ultramarine blue and nearly absent in ultramarine violet. This seems to indicate that the change in our pump-probe spectra is due to the presence of the $S_2^{+}$ radical. One possibility is that the excitation of the $S_3^{-}$ charge transfer process later transfers its energy to the local $S_2^{+}$ chromophore, which then interacts with the probe in GSB or SE. SE seems more likely because the 810 nm probe is far from the peak absorption of $S_2^{+}$ ($\sim 400$ nm). Furthermore, the Stokes shift of $S_2^{+}$ is quite large ($\sim 10,000$ cm$^{-1}$), and luminescence data show broad emission from $S_2^{+}$ between 516 and 1033 nm (88), which is within the energetic range of our probe. Transient white light absorption (figure 28) is useful in studying the dynamics in natural lapis lazuli at different wavelengths simultaneously. For example, in a stimulated emission process the further the probe moves away from the pump, the stronger the negative signal becomes, which would not be the case in a spectral hole burning (ground state bleach) situation (60, 91).
Figure 28: Transient white light absorption spectra of natural Afghan lapis lazuli pumped at 720 nm with a pump power of 6 mW (power per wavelength in white light was not calibrated). Natural Afghan lapis lazuli was diluted with linseed oil and cycled through a flow cell during spectroscopy.

Transient white light absorption is the same as pump-probe spectroscopy, but the probe is white light. Spectra are taken at different time-delays between the pump (720 nm) and the white light, but the probe now contains absorption information from all wavelengths. Full technical experiments details for this method can be seen in reference (92). The signal is saturated around the 720 nm pump and a negative signal can be seen on either side of the pump. It is possible the negative signal between 800 and 1100 nm is due to a stimulated emission process. However, there is also a negative
signal between 550 and 675 nm that cannot be explained unless the sample is undergoing multiple nonlinear photon processes, processes involving multiple photons from the pump and the probe. Transient white light experiments are typically conducted at much higher peak powers than what we used in our pump-probe microscope (further discussed in the 6th chapter), making the likelihood of higher order nonlinear processes possible. Unfortunately, due to these higher order nonlinear processes, we could not connect the photodynamics of the transient white light spectrum to our power study.

Power studies on synthetic ultramarine blue (figure 29) allowed us to investigate the origin of the excited state signal that is absent in natural lapis samples.

Figure 29: Fixed pump and fixed probe power studies on synthetic ultramarine blue. A power study on synthetic ultramarine blue was performed by holding the probe or pump power constant and changing the power the in other arm.
and taking a pump-probe delay stack with 720-810 nm pump-probe wavelength combination and 20x 0.7 NA air objective. Each pump-probe spectrum was taken from the same spot on the sample (a prepared canvas painted with ultramarine blue in egg yolk binder).

In synthetic ultramarine blue, the $S_2^-$ concentration is very low and excited state absorption begins to compete with the strong probe-selected $S_3^-$ charge transfer interaction. The excited state absorption is nonlinear in pump and probe, requiring multiple photons from the pump and probe to occur. From these pump-probe curves, the excited state absorption responds more strongly to the pump, which is most likely because the pump is closer to $S_3^-$ absorption. Because the pump and probe are at the very edge of the charge transfer band, it seems likely that we would see excited state absorption in $S_3^-$ given enough power/photon absorption. The results of pump-probe imaging and this power study lead us to believe that at this wavelength combination, we are sensitive to the concentration of the $S_2^-$ chromophore but the sample must initially be excited via the charge transfer in $S_3^-$. It may be the case that pumping closer to the absorption of $S_2^-$ would give completely different dynamics and new information about the lesser-studied yellow chromophore.

3.4 Co-registration of Pump-Probe and Raman Images

For a closer comparison, we took Raman maps in the same pump-probe sampling area. In the Raman maps, a Raman spectrum was collected pixel by pixel in a selected area. To analyze the Raman maps in a similar manner as the pump-probe images, we took the average bulk spectrum of natural (Afghan) and synthetic
ultramarine blue to generate a basis set for linear unmixing. As seen in figure 29, the only difference between the two normalized spectra is the ratio of the $S_2$ and $S_3$ peak. The Raman maps presented in the figure below represent distribution maps of the chromophore ratio. Since we believe that the longer negative decay was due to the crystal having more $S_2$, we used the same coloring scheme as in the pump-probe images; stronger $S_2$ shoulder in blue and weaker $S_2$ shoulder in green. The results are seen below (figure 30) with corresponding pump-probe images.

![Raman Basis Set](image)

**Figure 30:** Comparison of Raman and pump-probe response in single crystals from natural and synthetic ultramarine blue. Pump-probe images were taken with a wavelength combination of 720-810 nm, pump-probe, total power of 3 mW at the
sample, 20x 0.7 NA air objective. Raman images were collected with a 532 nm excitation line at varying powers and collection times to maximize signal and avoid damage. Each scale bar represents 10 μm.

It is difficult to complete a one-to-one comparison on the two images because they were each taken on a different system, with slightly different resolutions and at slightly different depths. Due to time constraints, the Raman images have a resolution of roughly half compared to the pump-probe images. Nevertheless, the pump-probe signal seems to correlate to the change in the $S_2$ and $S_3$. This is perhaps most evident when looking at the synthetic versus the natural samples as a whole. However, even among the natural crystals, the Raman images show more heterogeneity whenever the crystals feature more of the fast decaying behavior. A stronger argument could be made if we were able to correlate two main properties together from each analysis, for example pump-probe lifetime decay with Raman peak intensity ratios. Unfortunately such a correlation involves a rigorous fitting routine. Fitting the Raman curves was particularly challenging in this case, because we were examining a peak that essentially changes from a strong to weak shoulder and fitting typically failed in the event of a weak shoulder. In linear decomposition, we were able to use all of the raw data without having to make any fitting or model assumptions.
3.5 Application of Pump-Probe Dynamics to Historic Artwork

In some cases modern pigments do not give us the same pump-probe response as aged pigments in historic artworks, as was found to be the case in *The Crucifixion* (figure 31).

Figure 31: Pump-probe decay dynamics of aged and modern lapis lazuli. All curves were produced at a wavelength combination of 720-810 nm, but modern samples were imaged with a total power of 3 mW at the sample, while historic
samples were imaged with a total power of 1 mW. Curves from the aged sample were produced by averaging pump-probe dynamics in all crystals from a cross-section sample taken from Mary’s robe in *The Crucifixion*.

As discussed, we believe the pump-probe signal at 720-810 nm in natural and synthetic ultramarine blue is sensitive to the ratio of the blue and yellow chromophores, $S^2$ and $S^3$, respectively. The decay response of the lapis lazuli crystals from *The Crucifixion* shows heterogeneity, as expected. However, the slower-decaying pump-probe time response in the aged pigments decays on a faster timescale than observed in modern Afghan lapis lazuli. The overall faster negative decay observed from lapis lazuli in *The Crucifixion* is very similar to the time response we expect when pigment crystals have a lower concentration of $S^2$ and we were most likely not imaging the painting with enough power to see the excited state absorption response. This change in the slower-decaying pump-probe time response of the aged pigments (in comparison to modern natural lapis lazuli) could simply be due to initial chemical differences between the modern replicates and the rock that was originally mined 600 years ago. Another possibility is that we are imaging a chemical change that has occurred in the pigment over the last 600 years. For example, research on the faded blues of the Sistine chapel (93) suggests that lapis lazuli degrades because the aluminum silicate framework breaks down, releasing the chromophores. It is possible we are imaging another aging process in which $S^2$ is depleted relative to $S^3$. 
Such a correlation is difficult to make without first quantifying the chromophore ratio with the pump-probe signal. However, this could be done by co-localizing pump-probe images with a range of techniques, such as XANES, EPR, NMR, and Colorimetry, which have all shown promise in characterizing chromophore concentrations and their hue (83, 93-95). Further, we would need to characterize a much wider range of samples, from modern lapis lazuli mined from various sources to aged lapis lazuli available through pre-existing cross-sectional analysis. Additional information from pump-probe spectroscopy could then lead to new breakthroughs concerning degradation pathways, which are still not fully understood, and characterization of blue hues among the pigments.

3.6 Conclusion

As discussed in section 3.1, a great deal of research, past and on going, has dealt with the photochemistry of lapis lazuli and its implications. Our results indicate that pump-probe microscopy can add to this extensive field. It could be possible to quantify the concentration of $S_2$ in each pigment crystal and such knowledge could be applied to geo-sourcing or knowledge of the causes of the deep blue coloring.

The intense blue coloring in lazurite and synthetic ultramarines increases upon heating (thought to convert $S_2$ to $S_3$), which has been applied in the area of geo-sourcing (90). Similar heating experiments have been used in conjunction with XANES to determine the concentration of the $S_3$ in pigment crystals and concluded that extensive
annealing in natural samples allows for a strong blue color with relatively little sulfur (83, 94). Pump-probe correlation between concentration and distribution of $S_2$ (along with reflectance spectroscopy) could reveal that the yellow chromophore plays more of a role in the intensity of the coloring than originally thought. Because chromophore heterogeneity could be applied to geo-sourcing, pump-probe microscopy may be a complementary technique that allows for geo-sourcing on lazurite alone in the absence of trace minerals.

A deeper understanding of the relationship between our pump-probe signal and pigment chemistry could be applied to many different areas in cultural heritage. As noted in chapter two, this type of understanding has led to interesting discoveries in melanin chemistry which could be applied to earlier diagnosis of melanoma (60). In this field, we use such knowledge to understand material degradation, pigment/media/support interactions, object manufacture and many other such issues of interest to conservators. In the next chapter, we will use unique pump-probe dynamics to study differences in the interaction of inorganic pigments versus organic dyes on paper supports in conjunction with nonlinear fluorescent contrast.
4. Multi-Modal Nonlinear Imaging in Paper and Textiles

Paper and other textiles are exceedingly important materials in the history of mankind. Papermaking was thought to originate in China in 200 BCE, though the craft of making paper disseminated from China into Europe over many centuries (96, 97). Paper was originally manufactured from waste fibers; discarded cloth (typically cotton, linen, or hemp) was soaked in water, drained through a sieve, and then pressed and dried. The history of paper should also hint at the importance of textiles; paper is a cheaper alternative for writing than is silk or other woven materials, which were the original media for recording text or other documents. In some cases, typically in medieval Europe, parchment or vellum (made from animal skin) was deemed more appropriate for religious writings. However after the first printing of the Gutenberg Bibles, paper again became the preferred medium for printed books (96).

Unfortunately paper, historic textiles, and illuminated (painted) or un-painted manuscripts are very fragile, nearly impossible to sample, and difficult to examine. Simple mechanical stress from holding open a manuscript or unrolling a scroll could cause extensive damage. However, the proper treatment (conservation and restoration) of such samples is important; fibrous supports are very susceptible to deterioration because they are composed of natural bio-materials (cellulose, collagen, or lignin) and frequently treated with dyes that may not be light stable. Although there is a large body
of research (98-101) related to material identification and degradation quantification, most techniques require sampling.

In this chapter we will discuss current research in characterizing fibrous artworks and the complementary nature of nonlinear microscopy to these analytical techniques. We extend our pump-probe contrast to include nonlinear fluorescence and second harmonic generation to pigments on paper and cloth supports. First, we will examine the interaction of the inorganic crystalline pigment ultramarine blue with different types of paper and compare this with the organic dye indigo. Second, we generate virtual cross-sections from paper samples painted with both indigo and ultramarine blue in layers versus mixtures. Last, we examine indigo dyed cotton cloth. In each case we find that we can combine pump-probe pigment contrast with nonlinear fluorescent fiber contrast to obtain 3d information that is otherwise unavailable to the conservator.

4.1 Current Research in Historic Paper, Manuscripts, and Textiles

Paper (and various textiles) are made of natural cellulose fibers, which are produced by cotton plants in a very pure form or combined with lignin in woody plants (101). Paper composition and quality has varied through out history; European paper in the middles ages was nearly pure cellulose and obtained from cotton, linen, or hemp rags and treated with animal glue for sizing. However, as paper demand became higher over time, it was more common to use raw wood pulp for paper, which contains more...
impurities than pure cellulose like lignin. Other additives can be included in the paper making process to improve the quality or appearance of the paper. Unfortunately cellulose degrades over time, due to many factors including bacteria and fungi, chemical changes from oxidation or air pollution, causing the paper to deteriorate (100). Identifying these degradation pathways is essential to the proper treatment of these precious historic materials, and a great deal of research has been devoted to the study of molecular degradation in paper. There are several good reviews of the spectroscopic techniques used for characterizing degradation in paper documents (100, 101). The Dead Sea scrolls are an example of important historic documents that have been characterized and studied with multi-spectral imaging (102), polarized Raman spectroscopy (99), confocal XRF (103), and other analytical techniques (104).

While characterizing and monitoring the state of preservation or degradation of these samples is important, most works on paper or manuscripts cannot be sampled. Raman spectroscopy has proven to be a useful non-invasive method for investigating pigment palettes in illuminated manuscripts (21, 35, 105, 106), but it is very limited in its ability to study organic materials such as dyes or lakes. FORS has recently been used to identify dyes and shows promise in identifying binding media in ancient manuscripts (18, 107). There are several other spectroscopic tools useful in studying colorants in historic paper and many are similar to those used in paintings (as discussed in chapter one). However, special considerations must be taken to account for the delicacy of
paper. Nonetheless, x-ray techniques (108) and FT-IR (109) are both frequently applied to study pigments on paper, as well as the paper support (98). The difficulty of identifying dyes is an important consideration, especially when dealing with textiles that are typically treated with organic dyes.

The dyes on historic textiles are typically studied by chromatographic techniques performed on large removed samples (16, 110, 111). Another Raman technique, surfaced-enhanced Raman spectroscopy (SERS) has shown promised in identifying red dyes (112). In SERS, a small amount of dye is adsorbed onto a metal (typically silver or gold) substrate leading to greatly enhanced Raman scattering signal and a quenching of fluorescence. This technique has been applied to watercolors, organic dyes, and historic textiles (113-115). Although considered to be micro-destructive because of the small sample sizes required (down to 25 μm in diameter), it would ultimately be beneficial to investigate such works completely non-invasively.

Nonlinear fluorescence and second harmonic generation are common tools in bio-medical imaging (44-46) for studying natural materials. In particular, collagen is know for its SHG signal (47), although this contrast has not been utilized in paper samples. Nonlinear fluorescent microscopy has recently been utilized to examine wood fibers in a violin (49) and analyze oil-varnish interfaces (50), but most inorganic pigments do not fluoresce or generate harmonic light and as such cannot be studied in conjunction with their support.
4.2 Virtual Nonlinear Multi-Modal Cross-Sections in Paper Samples

We have shown the ability of pump-probe microscopy to create virtual cross-sections in paintings (63) and here we extend our 3d sampling to paper artworks. We created several mock-up samples on different types of paper, including wood pulp, whatman, linen, and cotton papers. The most common way to make paper involves a process called pulping: the cellulose source (raw wood, cotton or linen cloths) is first soaked and ground in water to release the cellulose fibers, drained through a wire-mesh tray, and then pressed or hammered into a thin sheet. This type of paper is called laid paper and tends to be uneven with an impression left by the wire mold. For raw wood pulp, this technique tends to break the cellulose into shorter fibers and leave a high content of lignin (causing the paper to be brittle and acidic (116, 117)). Cotton and linen are more flexible by comparison because they yield longer cellulose fibers with no lignin (96). Whatman paper is also made from pure cellulose sources, such as cotton, but is filtered through a very fine woven wire mold that is then dipped into animal size (glue), creating a very flat and smooth surface without any mold impression. Whatman paper is frequently called wove paper and has been popular amongst artists, especially watercolorists, since its invention in the 17th century (118).

Our current pump-probe microscope is set up to collect nonlinear fluorescence simultaneously with pump-probe signal, giving us a way to collect signal both from the pigments and their supports in separate channels. Simultaneous signal collection in
separate channels gives us the flexibility to study each material individually as well as enables straightforward co-registration of both channels. Each paper was painted with ultramarine blue in various binders. The choice of binders or support had no effect on the pump-probe signal and each type of paper had nonlinear fluorescence. Results from the depth imaging of ultramarine blue on wood paper is presented in figure 32.

<table>
<thead>
<tr>
<th>Representative Bright Field Image</th>
<th>Pump-Probe, Ultramarine Blue</th>
<th>Nonlinear Fluorescence</th>
<th>NLF + PP</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
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</table>

**Figure 32:** Pump-probe and nonlinear fluorescent images of ultramarine blue on wood pulp paper at the surface of the paper and at different depths. The representative bright field image was taken at an arbitrary depth in the sample with a 20x air objective. It is 703 x 467 µm. Pump-probe signal was collected simultaneously with fluorescence signal in separate channels by taking a series of en-face (xy) images at different depths with a fixed interpulse delay of 0 fs, wavelength combination of 715-810 nm, pump-probe, and total power at the sample of 2 mW using a 60x 0.9NA air objective. Pump-probe contrast in ultramarine blue has been false-colored magenta, nonlinear fiber fluorescence green, and the two channels are added together for co-registration. Each nonlinear image is 185 x 185 µm.
A representative bright field image of the sample shows that ultramarine blue pigment particles cling to the paper’s fibers (wood pulp in this case), which are a combination of cellulose and lignin. The bright field image is not completely in-focus because bright field microscopy lacks high depth resolution and the paper consists of multiple layers of fibers. We collected pump-probe signal from the ultramarine pigment, false-colored magenta, simultaneously with nonlinear fluorescence signal from the wood pulp fibers. At these wavelength combinations (720-810 nm pump-probe) cellulose is known to have second harmonic generation (SHG) and lignin two photon excited fluorescence (2PEF). 2PEF typically refers to two degenerate photons. However, for the remainder of this discussion, we will use 2PEF to describe the sample fluorescing after absorbing one photon from the pump and one from the probe. Although 2PEF and SHG can be spectrally separated using appropriate optical filters, our current set up detects both without separation. Thus, the fluorescence images have been false-colored green to indicate a combination of both SHF and 2PEF. The channels can be viewed separately or together as an addition image, which highlights similarly to the bright field image how the ultramarine particles cling around the fibers. Pump-probe can extend the depth resolution that is available from bright field microscopy, as seen in the addition images taken from different depths of the paper. Even with a layer of ultramarine blue painted on the paper, we were able to collect fluorescence from the fibers and to image completely through some fibers to see others below.
Addition images (pump-probe pigment contrast with nonlinear fiber fluorescence) from whatman, linen, and cotton paper along with their representative bright field images can be seen in figure 33.

![Pump-Probe + Nonlinear Fluorescence in Various Papers with Bright Field Images](image)

**Figure 33:** Nonlinear multi-modal images of Whatman, linen, and cotton paper with bright field images. Nonlinear multi-modal images were created by collecting pump-probe signal simultaneously with fluorescence signal in separate channels in a series of en-face (xy) images at taken at different depths with a fixed interpulse delay of 0 fs, wavelength combination of 715-810 nm, pump-probe, and total power at the sample of 2 mW using a 60x 0.9NA air objective. Co-registered images have been false-colored to show the pump-probe contrast in ultramarine blue as magenta and nonlinear fiber fluorescence green. Each nonlinear image is 185 x 185 µm. Representative bright field images were taken at an arbitrary depth in the sample with a 20x air objective. Each image is 703 x 467 µm.
In each case we are able to collect a fluorescence signal from the fibers painted with ultramarine blue, which we see in all types of paper. Cotton is nearly pure cellulose, which unlike the other materials mainly generates SHG. Whatman, linen, and wood pulp have a strong fluorescent signal when both pulses are overlapped due to 2PEF in the material. Hence taking a z-stack at an overlap of 0 fs utilizes a strong pump-probe pigment response and nonlinear fluorescence fiber response. In the event of SHG only, there is no time dependence because the sample interacts with two photons from the same pulse.

**4.2.1 Nonlinear Multi-Modal Analysis of Indigo in Paper Samples**

Ultramarine blue is a crystalline inorganic pigment and was shown to cluster around the fibers in the different types of paper. To determine if organic dyes interacted differently with fibers and if we could image such an interaction using multi-modalities, we painted indigo from a watercolor pan and indigo pigment tempered in gum on wood and cotton paper. From each area we took a series of pump-probe delay stacks at different depths, as well as z-stacks at fixed inter-pulse delays to determine if there was visual or spectroscopic evidence of indigo-fiber interaction. Spectroscopic results from the two indigo samples on wood are presented in figure 34.
Figure 34: Spectroscopic differences between pan watercolor indigo and indigo pigment tempered in gum. Pump-probe spectra were generated with a wavelength combination of 715-810 nm, pump-probe, and total power at the sample of 2 mW using a 60x 0.9NA air objective at an arbitrary depth of high signal in the samples. FORS spectra were collected from the surface of each indigo painted sample from a similar area as to that sampled with pump-probe.

From previous work with indigo we expected a long-lived excited state signal, as seen in the pump-probe delay spectrum of indigo tempered in gum. However, indigo from a watercolor pan has a completely different pump-probe time response. At an inter-pulse delay of 0 fs (the time at which the images were taken), both have a positive response, but after roughly 200 fs, indigo from a watercolor pan shows a long negative decay. Initially we believed this was due to the binder because we found no spectroscopic differences in the pump-probe dynamics due to depth (which could be caused by interactions with the fiber), only differences originating from the indigo being tempered in gum or from a watercolor pan. Watercolors are typically made by creating
a very thick paste of pigment with little gum, which is dried and then extended with water when the artist is ready to paint. This led us to believe that our pump-probe differences could be due to pigment-binder interactions in an over- versus an under-bound environment. However, a systematic study of varying amounts of gum added to indigo failed to reproduce the pump-probe dynamics seen above. FORS investigation of the two samples found that the indigo tempered in gum consisted of true indigo, while the indigo from the watercolor pan did not actually consist of indigo. Confirmation of this came from the Winsor and Newton watercolor catalogue; what they call indigo is actually a mixture of carbon black, quinacridone, and copper phthalocyanine. In a spectroscopic study of the pump-probe dynamics we can distinguish between pan watercolor and gum tempered indigo. However, in creating virtual cross-sections the pump-probe interpulse delay was set to 0 fs and, at this delay, both types of indigo have a positive signal and are not distinguishable. Hence, both types will be referred to as indigo for the remaining discussion.

Similar analysis was done to ultramarine blue samples for comparison and the dynamics were found to have no dependence on depth in the paper or, as previously stated, on the binding medium. Indigo did not show any time response differences depending on whether or not the dye was on the surface or inside of a fiber, however it is possible to see indigo embedded within a fiber while ultramarine clings to the outside, as will be discussed below.
4.2.2 Determining Layering Methodology in Paper Samples with Nonlinear Multi-Modal Imaging

We were able to collect pump-probe image contrast and fluorescent fiber contrast for both indigo and ultramarine painted individually on paper, however most historic artworks on paper or ancient manuscripts, like paintings, contain multiple pigments in layers or mixtures. We painted cotton paper with ultramarine blue layered thinly over indigo, a common technique to extend the expensive lapis lazuli pigment. Results in figure 35, consistent with the previously discussed color scheme, indigo is false-colored blue, ultramarine magenta, and nonlinear fluorescence green. The addition image is inset with a representative bright field image and features a virtual cross-section.
Figure 35: Nonlinear multi-modal image of ultramarine blue layered on indigo on cotton paper. This nonlinear multi-modal image was created by collecting pump-probe signal simultaneously with fluorescence signal in separate channels in a series of en-face (xy) images at taken at different depths with a fixed interpulse delay of 0 fs, wavelength combination of 715-810 nm, pump-probe, and total power at the sample of 2 mW using a 60x 0.9NA air objective. Co-registered images have been false-colored to show the pump-probe contrast in indigo as blue, ultramarine as magenta, and nonlinear fiber fluorescence green. The en-face image is 185 x 185 μm and inset with a representative bright field image (703 x 467 μm). The virtual cross-section was generated by removing a z-slice from the xy volume cube and is 70 x 185 μm.

The cotton paper was painted with a thin wash of each pigment and it is possible that the indigo had not yet dried when the ultramarine was applied or that the indigo was ‘re-wetted’ by the application of the ultramarine. In either case, the resulting cross-section shows that the two pigments are mixed rather than layered. The virtual cross-section indicates that the indigo may be embedded within the fiber as opposed to the ultramarine that appears to be only on the surface. The indigo signal persists further in depth than the ultramarine and adds to the fiber signal more so than the ultramarine blue.

It may be the case that thin washes of any two layers simply do not make distinct layers, be it on paper or other supports. To test our virtual cross-section capabilities with the fluorescence modality, we created a similar mock-up on wood but with an excess of gum Arabic to create layers (and compared this to a mixture). A representative bright field image, an en-face pump-probe image, fluorescence fiber image, and virtual cross-sections are seen in figure 36.
Figure 36: Nonlinear multi-modal virtual cross-section of thick layers of ultramarine blue over indigo on wood pulp paper. The representative bright field image (703 x 467 µm) shows the highly reflective and glossy surface of the heavily gummed ultramarine blue. We took a series of en-face pump-probe and nonlinear fluorescent images at different depths with a fixed interpulse delay of 400 fs, wavelength combination of 715-810 nm, pump-probe, and total power at the sample of 16 mW using a 60x 0.9NA air objective. Co-registered images have been false-colored to show the pump-probe contrast in indigo as blue, ultramarine as magenta, and nonlinear fiber fluorescence green. The en-face images are 185 x 185 µm. The
virtual cross-section was generated by removing a z-slice from the xy volume cube (indicated by the dashed white line in the pump-probe image) and each image is 90 x 185 µm.

The bright field image of the paper sample highlights the extra gum that has been used in creating the two layers; the surface is very shiny and reflective and the pigment layer is dense. Pump-probe images confirm the high pigment density, regardless of the additional gum, and despite the thick gum and pigment layers, we were able to collect fiber fluorescence through the entire layer (a fluorescence image from 30 µm below the painted surface is shown in the above figure). The virtual cross-sections also show the indigo embedded in the fiber; the heavy indigo and gum layer has completely blocked the ultramarine from interacting within the paper fibers but the heavy gum use did not keep the indigo from embedding within the fibers.

In the case of heavy gum use, we see a clear difference between the layered and mixed samples. The two pigments mixed together can be seen in figure 37.
Figure 37: Nonlinear multi-modal virtual cross-section of thin washes of ultramarine blue over indigo on wood pulp paper. This nonlinear multi-modal image was created by collecting pump-probe signal simultaneously with fluorescence signal in separate channels in a series of en-face (xy) images at taken at different depths with a fixed interpulse delay of 200 fs, wavelength combination of 715-810 nm, pump-probe, and total power at the sample of 8 mW using a 60x 0.9NA air objective. Co-registered images have been false-colored to show the pump-probe contrast in indigo as blue, ultramarine as magenta, and nonlinear fiber fluorescence green. The en-face image is 185 x 185 µm and inset with a representative bright field image (703 x 467 µm). The virtual cross-section was generated by removing a z-slice from the xy volume cube and is 50 x 185 µm.

These results indicate that pump-probe microscopy is an extremely promising tool for the noninvasive 3d investigation of works on paper. Although we have previously shown our ability to distinguish between pigments in a mixture versus a
layer, here we have extended this to include information about the support using an additional nonlinear imaging modality. Even in thick paint layers featuring multiple pigments and heavy binder use, we were able to extract a fluorescent signal from the support at depths up to 90 µm in paper.

4.3 Nonlinear Multi-Modal Microscopy in Textile Investigation

As previously discussed, SERS is a promising technique to study organic dyes and has been applied to paper samples and historic textiles. Unfortunately, sampling is required, and because the technique is spectroscopic, it does not retain important spatial information. As a non-invasive imaging tool, multi-modal nonlinear microscopy could easily complement standard SERS and other analytical tools for textile and paper artworks. Not only can we study fibers using nonlinear fluorescence, but we can also image organic dyes using pump-probe contrast. Although we have worked more with indigo, preliminary results from cochineal and kermes carmine and madder lake show that pump-probe could be a useful tool in identifying these dyes. When extending our technique to a piece of cotton cloth dyed with indigo, we found we could image up to 300 µm in depth, results shown in figure 38.
Figure 38: Pump-probe and nonlinear fluorescent images of indigo dyed cotton cloth at the surface of the cloth and at different depths. The representative bright field image was taken at an arbitrary depth in the sample with a 10x air objective. It is 1.5 x 0.9 mm. Pump-probe signal was collected simultaneously with fluorescence signal in separate channels by taking a series of en-face (xy) images at different depths with a fixed interpulse delay of 200 fs, wavelength combination of 715-810 nm, pump-probe, and total power at the sample of 16 mW using a 60x 0.9NA air objective. Pump-probe contrast in indigo has been false-colored magenta, nonlinear fiber fluorescence green, and the two channels are added together for co-registration. Each nonlinear image is 185 x 185 µm.

Our penetration depth and the dye’s interaction with the cotton fiber are more evident when visualizing the virtual cross-section through the cloth. We can see that the dye has completely penetrated each fiber and we have imaged this effect through multiple fibers 300 µm in depth (figure 39).
Figure 39: Nonlinear multi-modal virtual cross-section of indigo dyed cotton cloth. This virtual cross-section was generated by removing a z-slice from the xy volume cube featured in figure 36, using a fixed interpulse delay of 200 fs, wavelength combination of 715-810 nm, pump-probe, and total power at the sample of 16 mW using a 60x 0.9NA air objective. Each image is 300 x 185 µm. The top panel is pump-probe contrast in indigo, middle is nonlinear fluorescence in cotton fibers, and the bottom is the co-registration of pump-probe and fluorescence.
4.4 Discussion and Conclusion

In each mock-up (ultramarine and indigo on paper, both individually and together) we were able to image pigments and their support through depths of up to 300 μm, while retaining important spatial information for each material. This high-resolution 3d imaging with such chemical information is not currently possible with standard techniques. Although artists have long utilized the interaction of paint washes in fibrous supports in creating watercolors, tapestries, or other work, this research represents the first 3d in-situ view of paint or dye and their fabric interactions. The implications of these results could have a large impact on the conservation and study of paper and textiles. For example, thin washes of pigment on paper as seen in figure 35, show how the medium is drawn into the fiber leading the pigment clinging to the fiber (in the case of ultramarine) and the indigo embedded within. We found no evidence of pigments remaining in the voids of the paper fibers. This is in contrast to the case where heavy gum was used to create layers and the paper appears to behave much more like a canvas. The gum fills the voids of the paper and the upper layer (in our mock-up, ultramarine blue) is completely unavailable to penetrate into the fibers. However, indigo appears to have been drawn into the fiber despite the heavy gum use. These preliminary results indicate pump-probe could be useful in studying the movement of pigment and binder through paper samples, which may be useful in understanding degradation, particularly if paper samples are found to deteriorate from the surface...
inwards and if binder use provides any protection to inner fibers. It is also possible with multiple pump-probe virtual cross-sections to re-create the weaving pattern in textiles, which would aid in understanding the manufacturing process of ancient clothing, tapestries, or other textiles.
5. Technical Case Study with Pump-Probe Microscopy

Conservators, art historians, and curators investigate objects in the museum every day for a variety of reasons, the most immediate being to ensure a proper treatment of historic artworks. However, there are many implications for cultural history, ancient economies, and mores of long lost societies to be discovered during these investigations. In general, technical case studies represent the close collaboration of art historians, conservators, curators, and scientists. Further, within the scientific investigation of historic artworks, there is no ‘one size fits all’ because objects in the museum are highly complex systems and many techniques must be employed in a complementary manner for material and methodology studies. One example of this highly collaborative work is the case study on the 17th Flemish oil painting, The Armorer’s Shop, attributed to David Teniers The Younger (119). In this study, art conservators, scientists, and art historians worked together to discover that the lower panel, featuring a heap of armor, was painted first and incorporated into the larger structure later.

Further, this study, using a combination of cross-section analysis with FT-IR, Raman, and SEM-EDS, dendrochronology, and x-ray techniques (including traditional non-invasive XRF, synchrotron based confocal XRF, and x-radiography), led to the conclusion that the smaller panel was not painted by Teniers but most likely Jan Breughel the Younger. In this example, x-radiography allowed for visualization of the unusual panel construction and a dendrologist confirmed that the armor plank was
painted roughly 20 years before the remainder of the panel. Material analysis (with XRF and cross-section analysis) highlighted similar palettes, but confocal XRF discovered the use of an imprimatura on the armor plank absent from the larger panel and indications that the additional ground was applied to the larger panel to smooth gaps from height differences when the armor plank was incorporated into the larger panel. Art historical evidence was able to link the armor plank to Breughel.

This chapter will focus on the technical case study of two roundels, *The Body of Christ Supported by Angels* and *The Martyrdom of St. Alexander*, gifts from Kress Foundation that are in the permanent collection of the North Carolina Museum of Art. Although this dissertation has focused solely on pump-probe microscopy, here we will show pump-probe microscopy providing complementary information to traditional conservation science techniques. First, we will discuss a brief history of the two roundels and the difficulty of attributing both to the Italian artist, Lorenzo Lotto. Second, we will investigate the paintings using x-radiography, IR reflectography, and UV/VIS photography for style and construction comparison on the macro-scale. Third, we will perform in-situ material analysis on areas of interest with XRF and FORS. Lastly, in lieu of physical cross-section removal and analysis, we will apply pump-probe microscopy to create virtual cross-sections (63).
5.1 A Brief History of The Body of Christ Supported by Angels and The Martyrdom of St. Alexander

In 1512 nobleman Alessandro Martinengo commissioned Lorenzo Lotto to create an elaborate altarpiece for the church of Santo Stefano at Fortino in Bergamo. The Body of Christ Supported by Angels and The Martyrdom of St. Alexander roundels were decorative inserts in the frame of the altarpiece. However, when the church was demolished in 1561, the altarpiece moved from place to place and many of the components disappeared (120). The Kress Foundation acquired these two paintings in 1950 from Contini Bonacossi of Florence after they had spent time in a private collection in Milan. Scenes from the life of St. Alexander were rarely depicted in art, yet The Martyrdom of St. Alexander roundel is appropriate to the Santo Stefano altarpiece. He the patron saint of the city of Bergamo and he is also depicted in the main panel gathered around the throne of the Madonna. The Body of Christ Supported by Angels is also reflective of the style of angels near the top of the Santo Stefano’s altarpiece main panel. Unfortunately, the paint application and compositional sophistication of The Martyrdom of St. Alexander is of inferior quality to The Body of Christ Supported by Angels, leading art historians, curators, and conservators to believe that Lorenzo Lotto did not paint this roundel. There is speculation that sometime between the dismantling of the frame and the acquisition of the pieces by Kress, someone created a copy of The Martyrdom of St. Alexander. Although it would be difficult to prove, one possibility is that the friars in the church wanted to keep the original roundel of The Martyrdom of St. Alexander, as he was
their patron saint. Another simpler explanation is that a copy was created because the
original was lost or destroyed. While it is possible that an apprentice in Lorenzo Lotto’s
workshop painted the St. Alexander roundel, it is unlikely that Lotto would not have an
a hand in painting St. Alexander, who is a central saint in the Bergamo altarpiece.

Examination of the two roundels (121, 122) estimates both to be composed of
pine; the edge of each panel is concealed with a non-original veneer strip. Both
paintings contain an interlayer of canvas without a weave impression in the paint layer,
indicative of a transfer process. It is unknown if any original panel wood remains for
either painting. Although both paintings appear to have been painted in an oil medium,
neither have any brushwork evidence that indicates the use of egg tempera. The
composition of The Martyrdom of St. Alexander extends to the edge of the panel, unlike
The Body of Christ Supported by Angels in which the design is circumscribed by a 3/16
neutral border. However, it does not appear that this margin on The Martyrdom of St.
Alexander is an original paint layer. The two paintings differ greatly in subject
development and composition. The Body of Christ Supported by Angels models the figures
with tightness and precision and features a complex use of glazing to impart vibrant
color and depth to the overall composition. The Martyrdom of St. Alexander features no
such complex glaze use and renders the figures with inferior structure in fractured
brush strokes. The main goal of researching these paintings is to discover if The
Martyrdom of St. Alexander can be attributed to Lorenzo Lotto in its current state.

Photographs of the two paintings can be seen in the next section.

5.2 Macro-photography

Support, compositional paint changes, under-paintings, brush strokes and varnishes or restorations were examined with x-radiography, infrared reflectography, and ultraviolet visible fluorescence photography (figure 40).
Figure 40: Non-invasive macrophotography (digital photograph, x-radiograph, infrared reflectogram, and ultraviolet visible photograph) of *The Body of Christ Supported by Angels*.

The digital photograph of *The Body of Christ Supported by Angels* represents an extensive restoration effort. The Chief Conservator of the North Carolina Museum of
Art in-painted the loss (clearly seen in the x-ray, IR, and UV/VIS photographs), corrected Christ’s leg position from a previous restoration, and coated the painting in a new layer of varnish. There are other important details in the above photographs: the x-ray highlights very uniform brushwork and the IR image shows that in the initial drawing the position of Christ’s eyes were slightly different, both of which indicates very deliberate and thoughtful work. A much different methodology is reveal in macrophotographs of The Martyrdom of St. Alexander (figure 41).
Figure 41: Non-invasive macrophotography (digital photograph, x-radiograph, infrared reflectogram, and ultraviolet visible photograph) of *The Body of Christ Supported by Angels*.

*The Martyrdom of St. Alexander* has yet to undergo any restoration, although the roundel has been cleaned and had its old varnish removed. Treatment is a very delicate
and lengthy process; the NCMA’s work on *The Body of Christ* took nearly a year and if *The Martyrdom of St. Alexander* cannot be attributed to Lorenzo Lotto than its restoration is low priority in relation to other objects in the collection. There are striking differences between the two paintings, evident in the above photographs. The final coloring of the St. Alexander roundel is nowhere near as bright or vibrant as the Body of Christ roundel. The x-ray of St. Alexander also reveals a much sloppier brushwork that appears uneven and not uniform, making the final appearance less skillful and more hurried. The IR photograph of the St. Alexander roundel does not contain any information on the nature of the under-drawing; either there is no under-drawing or we simply cannot see it because there are no differences in the final painting. There is very little information to glean from the UV photograph, except that this painting appears to have little loss.

The differences in brushwork alone in these two paintings hint at the idea that these roundels were painted by different artists. However, we also wanted to investigate the painting materials and layering techniques.

### 5.3 Non-invasive material analysis of The Body of Christ Supported by Angels and The Martyrdom of St. Alexander

Painting materials were analyzed non-destructively using XRF and FORS, as described in chapter one. Figure 42 shows the XRF and FORS spectra from an area on the angel’s green dress that highlights the complementary nature of the two techniques in pigment identification.
Figure 42: Photograph and x-ray of the sampled area on *The Body of Christ Supported by Angels* with the resulting XRF and FORS spectra.

The elemental information necessary to interpret the XRF spectra and make pigment assignments was taken from the book series *Artists’ Pigments: A Handbook of their History and Characteristics* (12-15). The XRF spectrum from the green dress is dominated by a calcium and copper peak, which could indicate the use of a copper green pigment (malachite, copper resinate, or verdigris) and either chalk (calcium
carbonate) or gypsum (calcium sulfate). Carbon and sulfur are both fairly light and would have relatively weak x-ray peaks, but because the lead lines overlap with sulfur lines, it would not be possible to distinguish to sulfur in this case.

A FORS spectrum of the same spot complements the elemental data to allow for the identification of gypsum and verdigris (10). The three peaks at 1447, 1490, and 1537 nm are hydroxyl stretches typically seen in gypsum (along with the peaks at 1943 and 2219 nm). The peak at 2219 nm could also be associated with a CO stretch that would be associated with copper carbonate pigments (like azurite, malachite, verdigris, or copper resinate). Azurite spectra typically have strong features at 2286 and 2351 nm that are missing from this spectrum. The absorption over the visible range with increased reflection around 1000 nm allowed us to identify verdigris as the green pigment in the dress. Recent case studies of other Lotto altarpieces also confirms his preference for verdigris (123).

Another detail is that the skirt of the dress appears to be painted with a different technique than the sleeve. There is very little indication of lead in both spectra, and the x-ray of the dress is dark as compared to the sleeve, suggesting that after the panel was prepared with the gypsum ground the artist reserved this area for the dress and simply glazed the verdigris on top of the ground. This is in contrast with most other areas of the panel in which it appears that lead white was used as a ground under the figures. Other features in the FORS spectrum of interest include the lipid peaks at 1733, 1750,
2305, and 2348 nm, which have been associated most likely with an oil medium (18).

The overall results are summarized in tables 8 and 9.

**Table 8: Summary of Material Findings in The Body of Christ Supported by Angels.**

<table>
<thead>
<tr>
<th>Sample Spot</th>
<th>Material Inference</th>
<th>FORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angel’s Green Dress</td>
<td>Calcium ground and copper green</td>
<td>Gypsum and verdigris</td>
</tr>
<tr>
<td>Sleeve of Green Dress</td>
<td></td>
<td>Gypsum, lead white, azurite, and verdigris</td>
</tr>
<tr>
<td>Angel Face in Green Dress</td>
<td></td>
<td>Gypsum and vermillion</td>
</tr>
<tr>
<td>Angel’s Red Wings</td>
<td>Calcium ground, lead white, iron oxide, and vermilion</td>
<td>Gypsum, vermillion, red dye, and lead white</td>
</tr>
<tr>
<td>Angel’s Red Dress</td>
<td>Calcium ground, lead white, iron oxides</td>
<td>Gypsum and organic red dye</td>
</tr>
<tr>
<td>Sleeve of Red Dress</td>
<td></td>
<td>Gypsum, lead white, and possible azurite</td>
</tr>
<tr>
<td>Angel Face in Red Dress</td>
<td>Lead white, vermilion, iron oxides</td>
<td>Gypsum, lead white, vermilion</td>
</tr>
<tr>
<td>Angel’s Green Wing</td>
<td>Lead white and copper green</td>
<td>Gypsum and azurite</td>
</tr>
<tr>
<td>Face of Christ</td>
<td></td>
<td>Gypsum, lead white, with possible red lead and iron oxide</td>
</tr>
<tr>
<td>Body of Christ</td>
<td></td>
<td>Gypsum, lead white, with possible red lead, azurite, and iron oxide</td>
</tr>
</tbody>
</table>

The palette from *The Body of Christ Supported by Angels* is consistent with pigments often used in the early 1500’s, including gypsum, lead white, azurite, verdigris, vermilion, red dyes, and iron oxides. In addition to the pigments listed above, each XRF spectrum from the panel also contained peaks from aluminum, silicon,
potassium, titanium, iron, nickel, and strontium. Aluminum and silicon frequently occur as impurities from mineral-based pigments and could be associated with the ground (calcium sulfate), earth pigments (iron oxide derivatives), or azurite. Strontium is also typically found in XRF spectra when the ground of the painting is calcium-based (either chalk or gypsum) (119). The titanium and potassium could be explained by a possible canvas transfer and restoration treatment. Titanium white and glue would have been used to fix the paint layers to a new panel, accounting for the presence of these elements in each spectrum, although it is also possible that animal glue was used in the original preparation of the ground because the use of an animal glue as a binder for gypsum is a common technique.

Table 9: Summary of Material Findings from *The Martyrdom of St. Alexander*.  

<table>
<thead>
<tr>
<th>Sample Spot</th>
<th>Material Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Alex’s Hair</td>
<td>Iron oxide</td>
</tr>
<tr>
<td>St. Alex’s Head</td>
<td>Vermillion, iron oxides</td>
</tr>
<tr>
<td></td>
<td>Gypsum, lead white, and iron oxide</td>
</tr>
<tr>
<td>St. Alex’s Armor</td>
<td>Lead white and possible copper pigment</td>
</tr>
<tr>
<td></td>
<td>Lead white, malachite, and indigo</td>
</tr>
<tr>
<td>St. Alex’s Clothes</td>
<td>Lead white and possible vermilion and copper pigment</td>
</tr>
<tr>
<td></td>
<td>Gypsum, organic red dye, and iron oxide</td>
</tr>
<tr>
<td>Executioner’s Head</td>
<td>Iron Oxide</td>
</tr>
<tr>
<td>Executioner’s Back</td>
<td>Some vermilion and iron oxide</td>
</tr>
<tr>
<td>Executioner’s Sash</td>
<td>Gypsum, lead white, possible malachite and indigo</td>
</tr>
<tr>
<td>Executioner’s Pants</td>
<td>Gypsum, vermilion, and an organic red dye</td>
</tr>
<tr>
<td>Ground Under Executioner</td>
<td>Gypsum, indigo, and iron oxide</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Ground Under St. Alex</td>
<td>Calcium ground with possible vermilion and copper pigment</td>
</tr>
<tr>
<td>Blue Sky</td>
<td>Lead white and azurite</td>
</tr>
<tr>
<td>Blue Village</td>
<td>Gypsum and indigo</td>
</tr>
</tbody>
</table>

The palette findings for *The Martyrdom of St. Alexander* were similar, although this panel contained indigo in addition to azurite. This additional blue pigment is not very telling as far as the provenance of the two paintings, but there were some differences between the panels in the material analysis. Again, aluminum, silicon, titanium, iron, nickel, and strontium are common to each XRF spectrum but, in this roundel, there is very little potassium. This could indicate that no glue was used during a panel transfer process, which would not help with attributing the roundel to an artist, or more significantly, that the two panels were originally prepared differently. For example, oil based grounds, typically mixtures of lead white with oil, are much more common in the 17th century. The XRF spectra from the St. Alexander roundel is dominated by strong lead peaks, which could corroborate the idea of an oil based ground.

However, there are some instances in which FORS could identify lipid vibrational features consistent with egg yolk or oil and gave no indications of animal glue (which could indicate the presence or lack of potassium was due to restoration and not to elements in the actual painting). Many of the lipid peaks in the FORS spectra
from *The Body of Christ Supported by Angels* are those typical of oil, which is in contrast with *The Martyrdom of St. Alexander* that has peaks more consistent with egg yolk. There are also a few areas in *The Body of Christ Supported by Angels* that could indicate the use of both egg yolk and oil (Lotto was known for using mixed media in his paintings). Unfortunately it is not possible to conclusively assign the peaks to oil or egg yolk using FORS spectra alone. Despite the possibility of a different ground preparation material, the ground application and subsequent texture is very different between the two roundels. In the St. Alexander roundel the ground was brushed first in a diagonal direction and then in a circular fashion. It is unlikely the ground application would be so different if both panels were from the same workshop because panel preparation was typically the job of one person.

5.4 Virtual Pump-Probe Cross-Sections from *The Body of Christ Supported by Angels* and *The Martyrdom of St. Alexander*

Based on our material analysis we chose three areas on each painting for comparison with pump-probe microscopy (figure 43).
Figure 43: Sampling areas chosen for pump-probe microscopy. Spots indicated with a star were sampled with pump-probe microscopy and information obtained from same color stars was compared.

According to the FORS and XRF material analysis, each area of interest is painted with similar pigments (vermillion, organic red dye, and iron oxides). Although we have not yet worked extensively with red dyes, from previous work (62), we know that vermillion has an instantaneous positive signal and iron oxides have a summation of an instantaneous signal with a very long lived excited state at this wavelength combination. Spectroscopic pump-probe analysis was performed on each area of interest by taking a delay stack at different depths and the subsequent spectra were analyzed using phasor analysis (results in figure 44).
Figure 44: The phasor plot was generated by batch processing pump-probe delay stacks taken from different depths at each sampled area of the paintings. The phasor frequency was 0.15 THz. The pump-probe delay dynamics are the averaged curves taken from the corresponding colored region of interest on the phasor plot. These same colors are used in the subsequent pump-probe images to indicate delay behavior.

With the exception of one negative component (from a material found in the angel’s red wing), a majority of the pigments have a positive signal as expected from vermilion and iron oxides. Vermillion and iron oxide both have a strong instantaneous signal. However, iron oxides also have a long-lived excited state signal as well as lifetime variations occurring due to natural heterogeneity. These positive signals are difficult to cleanly separate without using multiple wavelength combinations. As such, each image is false-colored magenta for positive signal (vermilion, iron oxide, or a mixture) and yellow for negative signal. In order to generate virtual cross-sections, we took a series of xy images at different depths with one-micron step size (up to 90 µm)
with a fixed inter-pulse delay of 0 fs. Results from the red wing in *The Body of Christ Supported by Angels* and the executioner’s red shorts in *The Martyrdom of St. Alexander* can be seen in figure 45.

**Figure 45**: Pump-probe depth images of the Angel’s red wing and the executioner’s red pants. Pump-probe analysis was performed by taking a series of xy images at different depths using a 60x 0.9 NA air objective, with a pump-probe wavelength combination of 715-810 nm, fixed interpulse delay of 0 fs, and total power at the sample of 2 mW. Any components with a positive signal were false-colored magenta and negative components yellow. Each image is 185 x 185 μm.

The red wing from *The Body of Christ Supported by Angels* appears to have a negative component that is completely missing from the red shorts in *The Martyrdom of St. Alexander*. The negative signal is thought to be coming from a glaze, which is
corroborated by the virtual cross-sections generated from these volume cubes (figure 46). Unfortunately, we have not yet done an extensive survey of organic red dyes for material comparison in pump-probe dynamics.

![Virtual Cross-Section from *The Body of Christ*](image1)

![Virtual Cross-Section from *The Martyrdom of St. Alexander*](image2)

**Figure 46:** Pump-probe virtual cross-sections from the Angel’s red wing and the executioner’s red pants. Virtual cross-sections were generated by pulling out a random z slice from the volume cube of xy images taken at different depths. Virtual cross-sections are 60 x 185 µm.

The virtual cross-section highlights the very different methodology between the red wing and the executioner’s red pants. The red wing was most likely a mixture of vermillion and earth pigments with a thin glazing on top, whereas the red pants show only one layer that is structurally different. Although the materials in each case give a positive signal, which could indicate that they are the same material, it’s possible that the pigments were prepared in a different way on the St. Alexander roundel that led to a more amorphous structure.
A similar analysis was done on the faces of the angel and St. Alexander. Figure 47 shows an en-face image from the angel’s eyelid, pump-probe dynamics of two pigment crystals, and a virtual cross-section.

Figure 47: Virtual cross-section and pump-probe dynamics from the Angel’s eyelid. A pump-probe delay stack was taken roughly 10 µm below the surface at the sampling area of the angel’s eyelid. The en-face xy images show time response differences between materials in an image at 0 fs versus 380 fs. The pump-probe delay behavior is shown for two areas of interest, highlighted by white boxes (the dashed boxed is indicated by the dashed curve on the plot). A virtual cross-section generated from the same area is shown at 0fs. All images were taken with a 60x 0.9 NA air objective, a pump-probe wavelength combination of 715-810 nm, and total power at the sample of 2 mW. En-face images are 185x 185 µm and the virtual cross-section is 60 x 185 µm.

Although phasor analysis was not able to cleanly separate the multiple positive signals in this layer, we can view images at different inter-pulse delays to see a mixture of different pigments. At a delay of 0 fs, most of the pigments give a bright positive signal. However, once the two pulses are delayed by 380 fs, the signal for many of the pigments disappears. The pump-probe dynamics indicate an instantaneous signal (consistent with vermillion) and another longer-lived excited state absorption signal.
This signal is different than expected for an iron oxide, which typically has a strong instantaneous signal with a weaker long-lived signal. Unfortunately, the XRF and FORS material analysis do not indicate the use of a red dye or another material to which we can attribute this excited state absorption signal, but it appears to be coming from a material other than vermillion and that these two pigments are mixed together in this layer. We took a series of images at different depths with a fixed delay of 0 fs, and from this volume cube, the virtual cross-section (a z-slice) shows no indication of layering, but rather one layer of materials with the positive signal. This cross-section in conjunction with the en-face images lead us to believe the angel’s eyelid was not painted with a glazing technique, as in the red wing, but by mixing together vermillion with other pigment (possibly iron oxide) and painting a single layer over the face.

The results for St. Alexander’s eyelid (figure 48) are similar in appearing to be one layer with a mixture of pigments, except here we see vermillion and the pump-probe behavior expected of iron oxides.
Figure 48: Virtual cross-section and pump-probe dynamics from St. Alexander’s eyelid. A pump-probe delay stack was taken roughly 10 µm below the surface at the sampling area of the St. Alexander’s eyelid. The en-face xy images show time response differences between materials in an image at 0 fs versus 380 fs. The pump-probe delay behavior is shown for two areas of interest, highlighted by white boxes (the dashed boxed is indicated by the dashed curve on the plot). A virtual cross-section generated from the same area is shown at 0 fs. All images were taken with a 60x 0.9 NA air objective, a pump-probe wavelength combination of 715-810 nm, and total power at the sample of 2 mW. En-face images are 185x 185 µm and the virtual cross-section is 60x 185 µm.

Unfortunately in the case of the angel’s red dress, the particles are too sparse to conclusively observe layering as in figure 45 and analysis of the red shorts on St. Alexander reveals a single layer very similar to that seen in the figures above.

5.5 Attribution of The Body of Christ Supported by Angels and The Martyrdom of St. Alexander

Evidence from the macrophotography, in particular the x-ray and IR photographs, highlight vast differences in brushwork and underpainting. The Body of Christ Supported by Angels was constructed with much more attention to detail in contrast to the rushed and sloppy brushwork of the St. Alexander roundel. The material
analysis with XRF and FORS show a very similar pigment palette. However, this is a common pigment palette in the 16th and 17th century, meaning the roundels could be contemporaries or could have been created a century apart. There are indications of different ground preparation and binder use, but these are unfortunately inconclusive.

Pump-probe investigation of the glazing technique on the Angel’s red wing versus the executioner’s red pants show that the Angel’s wing was painted with multiple layers of different pigments whereas the red shorts seem to have only one layer. Not only is the layering structure between the two paintings different, but the structural contrast in the images further seem to indicate that the paint preparation was also different. In the case of the faces of the Angel versus St. Alexander, the methodology seems similar (one layer with multiple pigments), but the materials are different. It is also worth noting that the pump-probe analysis of the faces provided information that would have remained completely unavailable to the conservator because these are areas from which a conservator would never consider taking a sample.

Recent analytical case studies performed on several other Lotto altarpieces confirm our analysis; Lotto frequently used mixtures of vermillion with red lakes (carmine and madder) and hematite. The grounds in those studies were found to be prepared with gesso and animal glue and covered with a tinted primer (123). These results are similar to our findings in The Body of Christ Supported by Angels. Based on our investigation, we believe that Lorenzo Lotto did not paint The Martyrdom of St. Alexander.
When the altarpiece was dismantled many of the panels were dispersed among private collectors and one possibility is that the St. Alexander roundel was damaged during dismantling or traveling and a private owner attempted to repair it before selling it to someone else. Another possibility is that the monastery of Bergamo (home to the altarpiece before dismantling) wished to keep the St. Alexander roundel because he was the patron saint of the town and they made a copy. It would be very difficult to prove either of these possibilities for certain, but it does appear that *The Martyrdom of St. Alexander* was not from the workshop of Lorenzo Lotto.

### 5.6 Pump-Probe Microscopy in Cultural Heritage Research

We previously demonstrated that pump-probe microscopy could provide nondestructive 3d imaging of paintings and here we have extended this to work in conjunction with traditional analytical methods to provide complementary information in a technical case study. These results are very promising for the use of pump-probe microscopy as an alternative to physical cross-section removal. In the case of these two paintings, we found that at a 720-810 nm pump-probe wavelength combination that the pigments did not separate easily. However, pigment separation can be solved in several ways. As seen in figures 47 and 48, we can utilize the pigment’s individual delay behavior and take pump-probe delay traces at different depths. Here we only took up to three delay stacks at different depths, which was not enough to generate a high-resolution virtual cross-section with pigment separation. Such a process, taking delay
stacks at multiple depths, is quite time consuming. An alternative is to take multiple
depth stacks at a variety of inter-pulse delays, which still utilizes the pigments different
time responses (for example the instantaneous signal in vermillion versus the longer
lived signal in iron oxide).

Pigments have signals that are wavelength dependent, and as such, imaging the
two paintings with multiple wavelength combinations could have also aided in
separating pigments cleanly. It is possible that pigments were present which were
invisible to the 720-810 nm wavelength combination. However this is where pump-
probe works well with other methods that can identify pigments and materials. When
pump-probe wavelengths are in the visible range, organic glazes, binders, and varnishes
are typically invisible. These materials could have greatly aided in attribution,
especially when FORS indicated, though inconclusively, that different biding media
were used in the two paintings. Frequently, these materials can cause interference due
to their fluorescence in traditional spectroscopic techniques, but they leave our pump-
probe signal completely unaffected. However, in this case, it would have been
advantageous to study the binders. Chapter four discussed extending our technique to
multi-modal nonlinear microscopy for nonlinear fluorescence or harmonic generation,
and in the future, this could be applied to organic media in paintings, which has been
shown in some recent 3D imaging work (49, 50). Overall, pump-probe microscopy
successfully investigated the methodology in these two paintings and provided unique
information, otherwise unavailable without invasive and destructive sampling, to help
determine that Lorenzo Lotto most likely did not paint The Martyrdom of St. Alexander.
6. Conclusion and Future Work

This dissertation studied a variety of materials and successfully took part in a technical case study on two historic paintings using the wavelength combination of 720-810 nm. Here we present initial results from the pump-probe imaging of earth pigments that indicate we could study pottery manufacture in the future. We will also discuss expanding our attainable pump-probe pigment contrast using transient white light spectroscopy to create a pigment spectral database.

6.1 Pump-Probe Dynamics of Iron Oxide Based Earth Pigments

In addition to the materials discussed throughout this dissertation, we have also imaged a large set of natural iron oxide and iron hydroxide earth pigments that range in color from browns to yellows and reds to purples. These prehistoric pigments are as ubiquitous in our cultural heritage as they are in the Earth (11). Yet, their chemical similarity and natural heterogeneity makes these pigments difficult to characterize with current spectroscopic techniques (124, 125). Here we show clear distinction between green, red, and yellow earth pigments, even at the same pump-probe wavelengths we used to study the blue pigment lapis lazuli. In figure 49, we see qualitatively different pump-probe delays for natural green umber, a manganese substituted iron oxide, hematite, pure red iron oxide, and limonite, pure yellow iron hydroxide. We have also tested groups of similarly colored red, yellow, and brownish earth pigments, and found pump-probe delay behavior unique to each pigment.
Figure 49: Pump-probe investigation of iron oxide pigments. All the pump-probe delays were obtain at wavelengths of 710 nm and 810 nm respectively, with a total power on the sample of 4.2 mW. (A) Pump-probe delays for hematite, limonite, and natural green umber. The New World potshard (B) has a pump-probe delay characteristic to earth pigments, with a strong instantaneous response and a long-lived signal persisting after 120ps. (C) Pump-probe delays of heat-treated hematite. Dry hematite powder was heated in a tube furnace between 400° -1100 C, removing
samples after heating for 45 minutes in 100 increments, and painted onto a canvas in a tempera binder (only every other temperature is included here).

The thermal characteristics of the earth pigments have long been utilized to achieve color changes; for example, yellow earths can be heated to give a red earth, which in turn can be heated to a black earth. The famous red and black look of Attic wares came from exploiting these thermal properties in the iron-rich Attic clays (126, 127). Identifying the firing conditions of various potshards could not only elucidate their manufacture for important archeological information, but also indicate whether or not various shards belong together for restoration purposes. We find the pump-probe delay of a New World potshard (figure 49) has the characteristic decay behavior of an earth pigment, which is not surprising. Unfortunately, we do not know the composition or firing conditions of the potshard, but it is safe to assume it would behave similar to hematite, the pure mineral iron oxide that creates the red color. Although rudimentary, we heated hematite at temperatures similar to that of a kiln, and the pump-probe delay at each sampled temperature show qualitative differences. Figure 49 features the pump-probe delays for hematite at a few of the sampled temperatures and an inset showing the characteristic color change of the pigment from red to black. Our treatment of the earth pigments has thus far been qualitative, but it is clear that we can further characterize these pigments for broad applications in cultural heritage.

Earth pigments were frequently used in ancient Greek polychromy (128), as well as in many Early Renaissance paintings as the ground for gilding, called bole(64) (this
technique was used in *The Crucifixion*, and in many ancient funerary relics (129). In addition, Greek statuary was not white, as believed for centuries; it was brightly colored (130-132). Paint fragments are detected today by grazing incidence ultraviolet light, but the ability of pump-probe microscopy to penetrate a scattering surface could have revolutionary impact.

### 6.2 Spectral Database for Pump-Probe Pigment Response

Although we have shown that we are able to observe a variety of unique contrast from different pigments at the 720-810 nm pump-probe wavelength combination, our current experimental set up is slightly limited to fully investigate historic artworks. Several important pigments (azurite, malachite, and madder lake to name a few) are invisible to this wavelength combination and we also fail to see signals in binders or varnishes. Our experimental set up is tunable to a point; however picking random wavelength combinations in an attempt to find signal in the wide range of materials in the artist’s palette is completely impractical. A simple solution to this problem is to create a pump-probe spectral database of materials using transient white light absorption. Initial results are promising. Figures 50 - 52 show transient white light absorption in vermilion, verdigris, and malachite.
Figure 50: White light transient absorption of vermillion, pumped at 720 nm with a power of 5 mW.

At our typical pump-probe imaging wavelength of 720-810 nm, we have only seen an instantaneous positive signal in vermillion. However, these results indicate that by tuning the probe further towards the IR we could obtain a long-lived negative signal. Had we used such a pump-probe wavelength combination in our study of the two Lotto paintings, we could separated vermillion from iron oxides much more easily as iron oxides were found to have no signal in the IR region during transient absorption experiments. Unfortunately, due to time constraints, we have not yet had time to
investigate the Lotto paintings under multiple wavelength combinations. White light transient absorption also revealed signals in two pigments that are invisible to the 720-810 nm wavelength combination, verdigris and malachite.

Figure 51: Transient white light absorption spectrum of verdigris pumped at 720 nm with a power of 12.5 mW.

Although verdigris was pumped at 720 nm, there is no signal with an 810 nm probe. However, these transient absorption spectra indicate we could tune the probe further into the IR or into the visible range to achieve negative or positive contrast.
Figure 52: Transient white light absorption spectra of malachite pumped at 820 nm with a power of 10 mW.

Malachite shows no signal when pumped with 720 nm, but we able to find transient absorption signals by tuning the pump to 820 nm. Again, time constraints have not yet allowed us to image the Lotto paintings with multiple wavelengths, but it is clear from these results we could gain further information about the two roundels. For example, we could investigate the glazing technique on the green dress of the angel, identified as verdigris. We could also study the angel’s green wing, which contains either layers or mixtures of azurite and malachite.
While these transient absorption spectra contain a wealth of spectroscopic information, it is important to note that such a technique could not be applied to historic artwork, but rather provide the information needed to determine appropriate pump-probe wavelength combinations for given materials. White light transient absorption spectroscopy is often performed with low repetition rate laser systems (1 kHz, in this case), due to their high peak power. This is in part because generating white light from a sapphire plate requires high power. Furthermore, the white light continuum is noisy and observing transient absorption signals over this noise (~ 0.2 mOD) can be difficult. Pump-probe microscopy, as discussed in this dissertation, extracts signals that are roughly 100 times smaller than the observed signals from transient white light absorption. This is an important point for investigating historic artwork because we are imaging at much lower peak powers (~ 1/100) than compared with transient white light absorption, but still providing the same spectroscopic information. Using spectroscopic information from a transient white light database, in conjunction with other material identification techniques, would allow us to quickly select appropriate imaging parameters for historic artwork. Adapting transient absorption directly to the lower powers needed for imaging artwork will require some technological innovations; specifically, the white light continuum fluctuates from shot to shot by an amount much larger than the expected signal, and this will have to be improved.
6.3 Conclusion

The results of this dissertation have shown that pump-probe microscopy is a promising tool for gaining new insights into cultural heritage without the need for invasive sampling. There are also vast implications for future work. In our efforts of building a pump-probe database, we hope to find wavelength combinations that allow was to image organic materials. For example, organic red dyes are notorious for fading, frequently leaving behind nothing but an aluminum substrate. Red dyes are also difficult to identify (when not faded), using current noninvasive techniques. In our study of indigo, pump-probe was shown to be sensitive to the blue dye and found spectroscopic differences between true indigo and the mesh of organic dyes utilized to create an indigo watercolor pan. Identification and examination of degradation in dye materials could have vast implications for conservators. Furthermore, discovering pump-probe signal in binders would have many applications, including applications to degradation pathways, pigment/binder interactions, and technical case studies of various artworks. Pump-probe has also been shown to work in conjunction with traditional analytical methods and to provide complementary information that would otherwise be unavailable. Thus, this dissertation has proven the applicability of pump-probe microscopy to a wide range of issues in the scientific research of cultural heritage.
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Biography

Tana Elizabeth Villafana was born in Winston-Salem, North Carolina on April 12th, 1984. She received her bachelor of science from the University of Illinois at Chicago in December 2008 and worked closely under the supervision of Dr. Robert J. Gordon during undergraduate research. During her graduate work at Duke University she published the following papers; Samineni, P.; deCruz, A.; Villafana, T. E.; Warren, W. S.; Fischer, M. C., Pump-probe imaging of historical pigments used in paintings. Optics Letters 2012, 37 (8), 1310-1312. and Villafana, T. E.; Brown, W. P.; Delaney, J. K.; Palmer, M.; Warren, W. S.; Fischer, M. C., Femtosecond pump-probe microscopy generates virtual cross-sections in historic artwork, Proceedings of the National Academy of Sciences 2014, 111 (5), 1708-1713. She won a number of awards during her graduate career including the NCR RAP Postdoctoral Research Fellowship (2015-2017), John T. Chambers Scholarship (2013-2014), William Krigbraum and Charles Bradsher Fellowship (Spring 2014), SPIE Student Travel Grant Award for SPIE O3A (2013), Charles Bradsher and Joe Taylor Adams Fellowship (Spring 2013), and APS/DLS Travel Grant Award for FIO/LS 2012 Conference (Fall 2012). Her research also generated public interest among several interviews; [Duke U. discovers new use for laser in art world](#) by Associated Press, [Art Under a New Wavelength](#) aired by UNC-TV, and [Lasers ID Ancient Artists’ Intent](#) shared in the Duke Community.