Spectroscopic Differentiation and Microscopic Mapping of Various Materials Using

Optical Pump-Probe Contrast

by

Jin Yu

Department of Chemistry
Duke University

Date:____________________

Approved:

___________________________
Warren S. Warren, Supervisor

___________________________
Martin C. Fischer

___________________________
Kevin D. Welsher

___________________________
Qiu Wang

Thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Chemistry in the Graduate School of Duke University

2018
ABSTRACT

Spectroscopic Differentiation and Microscopic Mapping of Various Materials Using Optical Pump-Probe Contrast

by

Jin Yu

Department of Chemistry
Duke University

Date:_______________________

Approved:

______________
Warren S. Warren, Supervisor

______________
Martin C. Fischer

______________
Kevin D. Welsher

______________
Qiu Wang

An abstract of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Chemistry in the Graduate School of Duke University

2018
Abstract

Understanding the photophysical properties of materials is important in order to identify them, predict structural and functional changes, and improve fabrication methods. Here I demonstrate the use of time-resolved nonlinear optical microscopy, pump-probe microscopy, on various materials (e.g., historic artwork pigments, modern automotive paints, melanin, and perovskite solar cells) to investigate their photophysical dynamics non-invasively with high molecular specificity and spatial resolution. In this dissertation, I discuss the challenges in the field of material science which are difficult to investigate using conventional analytical tools but which can be solved with the pump-probe technology by accessing intrinsic electronic and vibrational dynamics contrast microscopically in 3D.

In chapter 2, using Vermilion (HgS, notorious for losing its red color under light exposure) as an example, I demonstrate that pump-probe microscopy can map the distribution of α-, β-HgS, and liquid mercury on the microscopic scale. Then, I present studies investigating Vermilion’s degradation products under two light sources: an ultrafast near-infrared laser and an ultraviolet lamp. I even identify degradation products in discolored Vermilion of a 14th century painting.

Then, in chapter 3, I extend its application to a series of red organic pigments (ROPs) which exemplify the challenge in the field of conservation science due to the limitation of conventional nondestructive analysis tools. I highlight that intrinsic photov...
physical properties of ROPs provide molecularly specific contrast and utilize this contrast for high-resolution, three-dimensional imaging without the need for physical sample removal. Furthermore, I demonstrate the possibility of the initiation of the chemical breakdown of Carmine under NIR illumination.

In chapter 4, I highlight another potential application of pump-probe microscopy as an analytical tool for forensics of other types of organic colorants such as automotive paints. In addition, I present the importance of microscopic analysis using ultramarine blue pigments and discuss the non-radiative relaxation property of melanin in hair as an example.

Finally, in chapter 5, I present the progress on utilizing pump-probe microscopy for the mapping of charge carrier dynamics of organic-inorganic hybrid Perovskite (CH$_3$NH$_3$PbI$_3$-xCl$_x$) layers on the femto- to pico-second timescale and sub-micron spatial scale. The effects of single- and two-photon excitation, chemical composition, and aging on the granular level variations are discussed.
Dedication

For my parents, Kwang Kook and Ok Joo.
Contents
Abstract ........................................................................................................................................... iv
Dedication .......................................................................................................................................... vi
List of Tables ...................................................................................................................................... ix
List of Figures .................................................................................................................................... x
List of Abbreviation ........................................................................................................................ xv
1. Introduction .................................................................................................................................... 1
  1.1 Nonlinear optical mechanisms ................................................................................................. 2
  1.2 Pump-probe microscopy ........................................................................................................... 6
2. Visualization of degradation products of Vermilion (HgS).............................................................. 10
  2.1 Pump-probe signatures of α-, β-HgS and liquid Hg................................................................. 12
  2.2 Laser induced phase conversion .............................................................................................. 15
  2.3 UV and temperature assisted degradation ............................................................................ 23
  2.4 Degradation products in a 14th century painting .................................................................. 29
3. Spectroscopic Differentiation and Microscopic Mapping of Red Organic Pigments ................. 34
  3.1 Pump-probe spectroscopic database of red organic pigments .............................................. 36
  3.2 Non-invasive visualization of red organic pigments ............................................................... 39
  3.3 Visualization of spatial distributions of mixtures of laked-pigments in mock-up samples .......................................................................................................................................................................................................................................................... 47
  3.4 Initiation of chemical breakdown of Carmine under NIR light exposure .................. 50
4. Investigation of Other Types of Colorants .................................................................................... 56
  4.1 Modern automotive paints .................................................................................................... 57
4.2 Visualization of sulfur radicals in ultramarine blue pigments .......................... 62
4.3 Non-radiative relaxation dynamics of eumelanin in hairs............................... 72
5. Microscopic mapping of Perovskite charge carrier dynamics ............................ 77
  5.1 Comparison between conventional analytical methods and pump-probe microscopy .......................................................... 78
  5.2 Photo-physical dynamics of optical heterogeneous grains ............................. 81
  5.3 Effect of chloride doping and aging ......................................................... 86
6. Conclusion ..................................................................................................... 91
Appendix ......................................................................................................... 94
  1. Apparatus ............................................................................................... 94
  2. Degradation products of Vermilion ........................................................... 95
  3. Red organic pigments .............................................................................. 98
  4. Other types of colorants .......................................................................... 101
  5. Perovskite ............................................................................................ 102
References ....................................................................................................... 104
Biography ...................................................................................................... 114
List of Tables

Table 1 Expected bond dissociation energy of Carmine .................................................. 51
Table 2 Bi-exponential fitting of averaged pump-probe signals of three touch-up paints ........................................................................................................................................ 58
Table 3 Fluorescence lifetime of POI1 and POI2 in Figure 58b ...................................................... 85
List of Figures

Figure 1 Event in time after materials absorb light. ................................................................. 1

Figure 2 Fluorescence generated from single- (400 nm) and two-photon excitation (800 nm) in Rhodamine 6G. .................................................................................................................. 2

Figure 3 A range of conventional nonlinear optical mechanisms based on fluorescence detection. ................................................................................................................................. 4

Figure 4 A range of nonlinear interactions accessible with the pump-probe technique .... 5

Figure 5 Transient absorption spectroscopy set-up using single-color pump (blue) and supercontinuum probe ............................................................................................................. 6

Figure 6 Pump-probe microscopy set-up .................................................................................... 7

Figure 7 Noise spectrum of a laser source .................................................................................. 8

Figure 8 (a) Linear reflectance spectra of red and black HgS. (b) Pump-probe signals of panels c-e. Pump-probe images acquired at a pump / probe wavelength of 720 / 817 nm of (c) red HgS, (d) black HgS, and (e) metallic mercury ................................................................. 12

Figure 9 Power scaling of TPA and GSD signals in red HgS powder at 720 nm pump / 817 nm probe.............................................................................................................................. 13

Figure 10 (a-c) Pump-probe wavelength switching experiment. (d) A bright field image of a minor grain that shows GSD signals. .................................................................................. 14

Figure 11 The change on the surface of red HgS powder after excessive exposure to fs-laser power .............................................................................................................................. 16

Figure 12 Signal changes from TPA to ESA to GSD during laser-induced phase conversion ................................................................................................................................. 17

Figure 13 Pump-probe images from (a) intact HgO and (b) degraded HgO under fs-laser pulses .............................................................................................................................. 18

Figure 14 (a-b) Characterization of laser-induced degradation products using EDS and XRD. (c-d) Picture of red HgS powder after strong fs-laser exposure. ......................................... 19

Figure 15 Surface change of red HgS powders before/after exposure to fs-laser pulses of different pulse durations but identical average power. ............................................ 21
Figure 16 Prediction of energy diagram of α- and β-HgS................................. 22

Figure 17 Pump-probe imaging of UV-exposed red HgS powder on a glass slide at 720 / 817 nm................................................................. 24

Figure 18 Gradual appearance of ESA signals over time when red HgS powder is exposed to continuous UV light......................................................... 25

Figure 19 Regions that were exposed to UV radiation and that have developed ESA signals showed an increased susceptibility for phase conversion from α- to β-HgS under increased fs laser illumination................................................................. 26

Figure 20 Images of degradation products: collected discolored red HgS powder attached to double-sided tape (top row), color change of glass vials from transparent to silver (bottom row)........................................................................................................ 27

Figure 21 Characterization of discolored Vermilion powders under UV exposure at 22 and 57°C......................................................................................... 28

Figure 22 SEM images (a) bulk powder and (b) silver haze and corresponding (c) EDS signals. (d) Pump-probe analysis of silver haze on glass vials........................................ 29

Figure 23 A picture of the 14th century Italian painting (St. John the Evangelist, Ghissi, 1375, North Carolina Museum of Art). Inset is a magnified image showing the grey degradation on/in the Vermilion pigment layer.......................................................... 30

Figure 24 Pump-probe analysis on the cross-section sample acquired at 720 / 817 nm.. 31

Figure 25 (A) A SEM image of the cross-section sample in Figure 24. (B) EDS analysis results on the marked regions of the SEM image.................................................. 33

Figure 26 Structures of red organic dyes ........................................................................ 35

Figure 27 (a) Linear reflectance spectra of ROPs. (b-c) Pump-probe dynamics at two wavelength combinations: (b) 720 / 817 nm, (c) 600 / 817 nm.................................................. 37

Figure 28 (a) Cumulative phasor histogram of Carmine naccarat and Lac dye and (b) their false-color coded images................................................................. 40

Figure 29 Heterogeneous grains in Eosin Y powder observed at 720 / 817 nm........ 41

Figure 30 Pump-probe response of polycrystalline and crystalline Eosin Y................. 42
Figure 31 (a) Combined phasor plot of three pump-probe images; Purpurin, Alizarin, and Madder lake acquired at 720 / 817 nm. Phasor images of Purpurin (b), Alizarin (c), Madder lake (d). .................................................................43

Figure 32 pKa of Alizarin (a) and Purpurin (b). (c) Expected coordinating structure of 1 : 2 complex of Alizarin and Al^{3+} in the solid state. (d) Possible metal coordinating structures of Alizarin.................................................................45

Figure 33 Pump-probe microscopy of Alizarin and Purpurin lakes. .........................46

Figure 34 (a) A phasor distribution of Alizarin, Alizarin lake, Purpurin, Purpurin lake, and Madder lake. (b) Mapping of heterogeneous grains in Purpurin lake.........................47

Figure 35 (a-c) mock-up samples with different weight ratios of Madder lake and Carmine naccarat acquired; (a) 1:9, (b) 2:3, (c) 4:1. (d) in-situ depth sectioning images at variable depths.................................................................48

Figure 36 (a) Phasor histogram of a mock-up sample with 3:2 weight ratio of Madder lake and Carmine naccarat. (b) Delay traces corresponding to marked regions in panel a. (c) Phasor image.........................................................................................49

Figure 37 A structure of Carminic acid and its predicted laked form.............................50

Figure 38 IR spectra of Carminic acid and Carmine ..................................................52

Figure 39 (a) Absorption spectra of Carminic acid and Carmine in DMSO (b) pump-probe signatures of Carminic acid and Carmine powders at 720 / 817 nm ....................53

Figure 40 (a) Changes of absolute signal amplitude on Carmine powder mixed in linseed oil when scanned with increased laser illumination. (b) Pump-probe signal changes of Carmine powder when mixed with linseed oil. (c) Signal changes of Carmine under NIR illumination.........................................................................................54

Figure 41 (a) Linear reflectance spectra of three touch-up paints, (b) their pump-probe signals at 720 / 817 nm, and (c) the cumulated phasor plots of the paints. .......................57

Figure 42 Pictures showing scratched areas on Vehicle A (a) and B (b). BF images of paint flecks collected from scratched areas of Vehicle A (c) and Vehicle B (d).....................59

Figure 43 (a-b) Pump-probe images of reference paints of Vehicle A (a) and B (b). (c-d) Pump-probe image of scratched paints collected from Vehicle A (c) and B (d). (e) Pump-probe signatures extracted from marked regions in panel a-d ........................................61
Figure 44 Pump-probe image of (a) Lapis lazuli and (b) SUB and (c-d) their extracted pump-probe curves..................................................................................................................63

Figure 45 A switching experiment on Lapis lazuli tempered in gum...........................................65

Figure 46 A switching experiment on SUB tempered in gum.........................................................66

Figure 47 Characterization of red grains in Lapis lazuli .................................................................67

Figure 48 Pump-probe signatures of synthetic ultramarine red .....................................................68

Figure 49 Pump-probe signal dependence on SUB concentration..................................................69

Figure 50 Signal depth dependence of concentrated SUB .............................................................70

Figure 51 Polarization dependence of heterogeneous grains in SUB tempered in gum arabic.................................71

Figure 52 Pump-probe signature of eumelanin in a white hair ......................................................73

Figure 53 Pump-probe signals of end and root parts of black hairs.............................................74

Figure 54 The schematic explanation for the relationship between GSD process and the relaxation of excited electrons to the ground states via non-radiative relaxation.............75

Figure 55 (a) fs-TA spectrum of CH$_3$NH$_3$PbI$_3$ excited at 600 nm. (b) SEM image of CH$_3$NH$_3$PbI$_3$. (c) Pump-probe image acquired at 600 / 770 nm and (d) extracted TA delay traces.............................................................................................................................79

Figure 56 (a) Confocal, (b) pump-probe, and (c) FLIM images of CH$_3$NH$_3$PbI$_3$ having granular structures ........................................................................................................................................82

Figure 57 Power scaling results on CH$_3$NH$_3$PbI$_3$ having granular structures.........................83

Figure 58 (a) Pump-probe in Figure 56b, (b) FLIM, (c) fluorescence intensity image of CH$_3$NH$_3$PbI$_3$ having granular structures, and (d-e) their overlaying images..........................85

Figure 59 (a) SEM images of CH$_3$NH$_3$PbI$_{3-x}$Cl$_x$ (x = 0, 0.33, 0.5, 0.67). (b-e) fs-TA spectra of CH$_3$NH$_3$PbI$_{3-x}$Cl$_x$ (x=0, 0.33, 0.5, 0.67) at pump 802 nm.........................................................86

Figure 60 (a) Pump-probe images of CH$_3$NH$_3$PbI$_3$ (left) and CH$_3$NH$_3$PbI$_{2.67}$Cl$_{0.33}$ (right) at 817 / 742 nm and (b) extracted pump-probe responses............................................................87
Figure 61 Pump-probe responses of positive grains on Cl-doped PVSKs..............................88

Figure 62 Changes on pump-probe images and TA spectra of CH$_3$NH$_3$PbI$_{2.8}$Cl$_{0.2}$ with TiO$_2$ 5 days later .......................................................................................................................89

Figure 63 Fluorescence lifetime imaging microscopy set-up...............................................94
**List of Abbreviation**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-PF</td>
<td>Two-photon fluorescence</td>
</tr>
<tr>
<td>3-PF</td>
<td>Three-photon fluorescence</td>
</tr>
<tr>
<td>AOM</td>
<td>Acousto optic modulator</td>
</tr>
<tr>
<td>Arb. U.</td>
<td>Arbitrary unit</td>
</tr>
<tr>
<td>BF</td>
<td>Bright field</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge-coupled device</td>
</tr>
<tr>
<td>DSSC</td>
<td>Dye sensitized solar cell</td>
</tr>
<tr>
<td>EDS</td>
<td>Energy dispersive X-ray spectroscopy</td>
</tr>
<tr>
<td>ESA</td>
<td>Excited state absorption</td>
</tr>
<tr>
<td>FLIM</td>
<td>Fluorescence lifetime imaging microscopy</td>
</tr>
<tr>
<td>FOV</td>
<td>Field of view</td>
</tr>
<tr>
<td>GSD</td>
<td>Ground state depletion</td>
</tr>
<tr>
<td>HOMO</td>
<td>Highest occupied molecular orbital</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>LUMO</td>
<td>Lowest un-occupied molecular orbital</td>
</tr>
<tr>
<td>NIR</td>
<td>Near-infrared</td>
</tr>
<tr>
<td>OPO</td>
<td>Optical parametrical oscillator</td>
</tr>
<tr>
<td>PD</td>
<td>Photodiode</td>
</tr>
<tr>
<td>POI</td>
<td>Point of interest</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>PVSK</td>
<td>Perovskite</td>
</tr>
<tr>
<td>RIN</td>
<td>Relative intensity noise</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>ROPs</td>
<td>Red organic pigments</td>
</tr>
<tr>
<td>SE</td>
<td>Stimulated emission</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>SHG</td>
<td>Second harmonic generation</td>
</tr>
<tr>
<td>SRS</td>
<td>Stimulated Raman scattering</td>
</tr>
<tr>
<td>SUB</td>
<td>Synthetic ultramarine blue</td>
</tr>
<tr>
<td>TA</td>
<td>Transient absorption</td>
</tr>
<tr>
<td>TPA</td>
<td>Two photon absorption</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>XRD</td>
<td>X-ray powder diffraction</td>
</tr>
<tr>
<td>XRF</td>
<td>X-ray fluorescence</td>
</tr>
</tbody>
</table>
1. Introduction

When materials absorb light in the visible range, they show distinctive colors. This absorption property opens the capability to use materials in various ways (such as artwork pigments and automotive paints for decoration, melanin for protection of human beings under light exposure, and solar cells for generating charge carriers). Despite these disparate purposes, this similar origin of color allows us to understand materials’ interaction with light in a similar way.

\[ \text{Figure 1 Event in time after materials absorb light.} \]

It has been reported that time-resolved nonlinear optical spectroscopy is especially helpful to resolve electron transition dynamics of materials in the femto- to nano-second time scales (see Figure 1 for molecular event duration\(^1\)). The characterization of this photophysical dynamics helps identify materials and their possible chemical and functional changes, which ultimately help preserve materials’ properties and improve fabrication methods. However, conventional time-resolved optical spectroscopy usually averages
signals over substantially large areas, which obscures spatial variations. Unfortunately, chemical or functional changes of ensemble systems typically starts from small regions and even sometimes starts from minor components in the system, which are obviously difficult to trace using averaged spectra acquired from large regions. It brings out the need for microscopic analysis to understand photo-physical properties of individual components in ensemble systems. In this dissertation, I will demonstrate the use of time-resolved pump-probe optical microscopy on various materials to understand their photo-physical dynamics in 3D with the femto- to pico-second time scales and sub-microscopic spatial resolution.

1.1 Nonlinear optical mechanisms

Imaging contrasts of pump-probe microscopy arise from nonlinear optical mechanisms. Nonlinear optical microscopy has several advantages over linear microscopy, especially in its virtual depth sectioning capability.  

![Fluorescence generated from single- (400 nm) and two-photon excitation (800 nm) in Rhodamine 6G (photograph courtesy of Dr. Fischer).](image)
Figure 2 illustrates the comparison of fluorescence signals generation under single- and two-photon excitation. When 400 nm (Figure 2a) or 800 nm (Figure 2b) of laser pulse trains focus on Rhodamine 6G, both excitations induces emission of light peaked around 550 nm. Single-photon excitation generates fluorescence from the entire volume while two-photon excitation generates emission only at the focal point. In the latter case, the shorter emission wavelength than excitation wavelength originates from nonlinear optical mechanism, two-photon fluorescence (2-PF, see energy diagram in Figure 3). Because this nonlinear optical interaction can only occur when two pulses are intensely focused, the signal is generated only at the focal point, therefore, this property leads to the depth resolution for virtual depth sectioning. In addition, the relatively longer excitation wavelength of two-photon excitation enables deeper penetration into the scattering media. The use of fluorescence signals generated from nonlinear optical processes as imaging contrast is obviously good to visualize spatial information, however, they limit the use on the object producing fluorescence. Pump-probe optical microscopy can overcome this limitation associated with these fluorescence-based nonlinear optical mechanisms but still taking the depth sectioning capability.³ Pump-probe microscopy uses intrinsic photo-physical dynamics of each material as an imaging agent, therefore, it requires no external labeling agent to produce decent fluorescence signal to visualize 3D information of the object.
A range of conventional nonlinear optical mechanisms based on fluorescence detection. Two-photon fluorescence (2-PF), three-photon fluorescence (3-PF), second harmonic generation (SHG). Red arrows represent incident light (excitation) and green arrows represent fluorescence emission.

For visualization and characterization, pump-probe microscopy resolves changes of electron population dynamics induced by interaction between matter and two laser pulses (i.e. pump and probe) at variable time delays (τ). Pump pulse excites electrons on ground states and the second pulse, probe, can trace transient electron transition dynamics on electronic and vibrational energy levels as a function of τ. These dynamics offers distinctive imaging contrasts to differentiate molecules: all molecules have distinctive electron transition and relaxation behaviors, therefore, tracing electron population can be employed to identify molecules. In contrast to conventional nonlinear optical contrast (based on fluorescence signals, e.g. 2-PF, 3-PF, SHG in Figure 3) which detects newly generated wavelength²⁴, pump-probe microscopy detects probe intensity changes (either gain or loss) when pump is on and off. Note that, by convention, probe loss (absorption) processes are defined as positive signals and probe gain processes are negative signals in pump-probe spectrum (i.e. transient absorption spectrum).
Figure 4 A range of nonlinear interactions accessible with the pump-probe technique: two-photon absorption (TPA), excited state absorption (ESA), stimulated emission (SE), ground state depletion (GSD), and stimulated Raman scattering (SRS).

Figure 4 displays a range of nonlinear optical mechanisms accessible with pump-probe spectroscopy and microscopy. Two-photon absorption (TPA) and stimulated Raman scattering (SRS) occur during the temporal overlap of two pulses due to involvement of virtual energy states. While the presence of pump leads to probe intensity loss in TPA, the presence of pump causes probe intensity gain in SRS when the pump wavelength is shorter than the probe. In excited state absorption (ESA), probe allows excited electron to access to higher excited energy levels. This process leads to probe absorption (positive sign) because the second transition is only allowed in the presence of pump. In stimulated emission (SE), probe can stimulate the radiative relaxation of excited electrons, that therefore leads to probe intensity gain in the presence of pump (negative sign). Ground state depletion (GSD) typically occurs when a sample absorbs both pump and probe, therefore, the greatest degree of probe absorption will occur on the sample in the absence of pump. In the presence of pump, the depletion of electrons on ground states leads to less probe absorption from the sample than in the absence of pump, therefore,
these differences produce probe gain signals. Because ESA, SE, and GSD processes are
associated with excited energy states, thermal relaxation (i.e. non-radiative relaxation,
typically on a timescale of few picoseconds, Figure 1) of the excited electrons produces
interesting contrast from the ranges of lifetimes. In ESA process, for example, thermal
relaxation of excited electron to the ground state produces a decrease in the population of
excited electron, therefore, this relaxation leads to a decrease in probe absorption over
time. Using these mechanisms, we can not only map out electron transition dynamics but
also identify individual components in the ensemble system.

1.2 Pump-probe microscopy

![Figure 5 Transient absorption spectroscopy set-up using single-color pump (blue) and supercontinuum probe (rainbow).]

Transient absorption spectroscopy (i.e. single-color pump and supercontinuum
probe, see Figure 5) has been employed in various fields for more than two decades, but
its weak signal-to-noise ratio had limited its spectroscopic mapping on the micrometer
scale. The use of a lock-in amplifier and pulse shaping have helped overcome this
challenge and lead to the spectroscopic mapping of transient absorption signals using pump-probe microscopy.\textsuperscript{5,8}

Figure 6 Pump-probe microscopy set-up. The pump beam is colored blue and the probe beam is colored red. The pump beam is amplitude modulated with an acousto-optic modulator (AOM) at 2 MHz. Nonlinearity causes modulation transfer to the probe pulse train and we measure AC signals using a photodiode (PD) and a lock-in-amplifier to detect the probe intensity change (inset shows TPA process as an example).

Figure 6 shows an experimental set-up for pump-probe microscopy. Output from a mode-locked Ti:Sapphire laser (Chameleon, Coherent, 80 MHz) splits into two pathways, and an optical parametrical oscillator (Mira-OPO, Coherent) tunes the wavelength of incoming laser pulse trains. With the help of an acousto optic modulator (AOM), the amplitude of either Chameleon or OPO pulse trains can be modulated to a high frequency (f>1 MHz) having a square function (note that Figure 6 shows the case that the amplitude of OPO pulse trains have modulated at 2 MHz). The pulse trains which is temporally modulated at 2 MHz is called the pump and the unmodulated pulse trains is
the probe. A translational stage can tune the traveling length for the pulse trains on Chameleon beam pathways, which control the inter-pulse time delay ($\tau$). Spatially overlapped pump and probe (with variable temporal time delays) pulses are focused on a sample using an objective lens (note that polarization angle between two pulse trains is parallel), and a scanning mirror controls these pulses to scan $x$-$y$ plane of the sample. Two scanning modes are typically used: one is for $x$-$y$ plane mapping at variable time delays ($\tau$) and a fixed focal point ($z$) and another is for virtual depth sectioning at a fixed time delay ($\tau$) and variable focal points ($z$).

![Noise spectrum of a laser source](image)

**Figure 7 Noise spectrum of a laser source**

The nonlinear interaction at the focus produces probe intensity changes through modulation transfer from pump. The inset in Figure 6 shows an example of modulation transfer to the probe pulse train in a TPA process. Although its intensity change is exaggerated to visualize the change, only $10^{-6}$ of the pump modulation might be transferred to the unmodulated probe pulse trains in a typical experiment. This intensity
change is far smaller than typical laser relative intensity noise (RIN), typical RIN of a mode-locked Ti:Sapphire laser is $1.5 \times 10^{-3}$ integrated over all time scales. Laser RIN is dependent on frequency ($f$) with a typical $1/f$ dependency, in addition for various discrete contributions below a few 100 kHz, therefore, this noise can be decreased by modulating pump pulse trains. As shown in Figure 7, $1/f$ noise is dominant in a low frequency range (i.e. below $f < 1$ MHz), and $1/f$ noise becomes negligible and the noise level approaches to the shot-noise level in a high frequency range ($f > 1$ MHz).\textsuperscript{3,9} Note that upper frequency limit is detector bandwidth. We can detect this probe intensity changes in either back-scattered light (when sample is optically opaque, e.g. artwork pigments on canvas) or transmitted light through samples. The former detection mode is called epi-mode and the latter one is called transmission-mode. In epi-mode, a polarizing beam splitter is used to collect depolarized beams in back-scattered lights (note that a non-polarizing beam splitter can be used for some polarization studies, e.g. polarization experiment in Figure 51). Probe intensity changes induced by nonlinear interaction are detected by a photodiode (Thorlab PDA55, bandwidth DC to 10 MHz) after rejecting pump pulse trains. The lock-in amplifier extracts the subtle amplitude and sign changes in the filtered probe light in the presence and the absence of pump.
2. Visualization of degradation products of Vermilion (HgS)

HgS has two major solid phase forms, each having a distinct color: $\alpha$-HgS (cinnabar, hexagonal crystal structure) is red and $\beta$-HgS (metacinnabar, cubic) is black\textsuperscript{10-15}. Vermilion (HgS) has been globally employed as a pigment in historical paintings. Due to its intense red color, it has been often assumed that Vermilion consists of $\alpha$-HgS. Interestingly, Vermilion tends to experience darkening over time under natural lighting\textsuperscript{16-17}, which differs with the original intent of the artist. This tendency to blacken over time has prompted studies of the underlying degradation mechanisms. However, the degradation products of Vermilion have been controversial; $\beta$-HgS\textsuperscript{18-20} and liquid Hg\textsuperscript{21-22} have been considered as discoloration products of $\alpha$-HgS.

A phase conversion from $\alpha$- to $\beta$-HgS has been reported to occur under intense pulsed laser exposure\textsuperscript{23-24}. In the absence of light, irreversible phase conversion can occur at temperature over 370°C\textsuperscript{12,15,25} (note that reversible phase conversion occurs under 370°C because $\alpha$-HgS is thermodynamically favored\textsuperscript{13}), and therefore researchers often assume that the phase conversion is an extremely rare event under normal conditions. Elemental microscopic analysis on some historical paintings has revealed a significant amount of chloride in the area of Vermilion discoloration, and a decrease in Hg contents as well\textsuperscript{26-27}. Therefore, chloride has been considered as a catalyst that helps the formation of liquid Hg in the presence of light\textsuperscript{10,17,19,21-22,28}, but the origin of chloride is speculative (see proposed chloride-assisted degradation processes in the presence of light (1, 2)\textsuperscript{21-22}).
\[
\text{HgS} + 4 \text{Cl}^- + 4 \text{H}_2\text{O} + 8 \text{H}^+ \rightleftharpoons \text{HgCl}_4^{2-}^{(\text{ads})} + \text{SO}_4^{2-} + 8 \text{H}^+ 
\]

(1)

\[
\text{HgCl}_4^{2-}^{(\text{ads})} + 2 \text{e}^- \rightleftharpoons \text{Hg}^{(\text{ads})} + 4 \text{Cl}^- 
\]

(2)

In microscopic studies, elemental analysis apparatuses (e.g. x-ray fluorescence (XRF), and scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS)) can trace changes in elemental ratios such as \( \alpha \)-HgS and liquid Hg\(^{27} \), but differentiation of \( \alpha \)- and \( \beta \)-HgS is challenging due to their identical molecular formulas. Some X-ray analysis techniques such as X-ray diffraction (XRD)\(^{12, 29} \) and x-ray photoelectron spectroscopy\(^{30} \) can differentiate two phase forms, but they provide little spatial resolution and grain-level resolution requires complex instrumentation such as synchrotron\(^{31} \).

Here we demonstrate the use of pump-probe optical microscopy as a new tool to study the degradation products and mechanisms of Vermilion in paint layers on the sub-micron spatial scale. For the study, we use red HgS (i.e. Vermilion), black HgS and liquid Hg (note that pure \( \alpha \)- and \( \beta \)-HgS are not commercially available). Firstly, we identify heterogeneous components in red HgS and investigate the role of heterogeneous grains during the laser-induced degradation. Secondly, we study the effect of pulsed near-infrared (NIR) light, continuous ultraviolet (UV) light and temperature on the degradation of red HgS powders containing negligible amounts of chloride. Lastly, we reveal degradation products in a 14th century painting containing discolored Vermilion layers.
2.1 Pump-probe signatures of α-, β-HgS and liquid Hg

I acquired a series of pump-probe images of red HgS, black HgS, and liquid Hg to obtain reference pump-probe signatures. Black HgS is obviously an absorptive powder, and consequently tempered into agarose gel to minimize sample damage due to absorption-induced heating. It is confirmed that all samples have distinctive nonlinear signatures at a pump / probe wavelength combination of 720 / 817 nm (Figure 8b). Red
HgS primarily shows TPA signals during temporal overlap of pulses because of the absence of energy states for one-photon transitions (see its linear reflectance curves in Figure 8a). Black HgS and liquid Hg produce negative signals originating from GSD process; liquid Hg contains additional long-lived ESA signals persisting for even longer time delays than those of GSD signals. Note that the negative signal of black HgS and liquid Hg at 720 / 817 nm persists at 817 / 720 nm, pointing to GSD as pump-probe mechanism (for such a wavelength switch, SRS would change sign and SE would be suppressed). While black HgS and liquid Hg show fairly uniform signals over entire fields of views, interestingly, red HgS shows the presence of minor components having negative signs at 0 fs, which is difficult to observe when averaging over the entire FOV.

Figure 9 Power scaling of TPA and GSD signals in red HgS powder at 720 nm pump / 817 nm probe. (a-b) Pump-probe signals at four power levels at ROI1 (a) and ROI2 (b). Inset is a pump-probe image acquired at 0.2 / 0.2 mW, \( \tau = 120 \) fs.

Several pump-probe microscopic and spectroscopic studies help confirm the presence of a few \( \beta \)-HgS in Vermillion. Vermillion’s extracted pump-probe signals from major and minor grains show instantaneous positive and negative signals, respectively.
(Figure 8). To understand the power dependence of positive and negative signals, a power study was conducted by acquiring a series of images at four power levels (0.1 / 0.1, 0.1 / 0.2, 0.2 / 0.1, 0.2 / 0.2 mW). Then, pump-probe delay traces at two ROIs (e.g. the regions marked in Figure 9a) were extracted. Figure 9 show that power dependence of both positive and negative signals is linear in pump and linear in probe.

![Figure 8](image)

Figure 8. To understand the power dependence of positive and negative signals, a power study was conducted by acquiring a series of images at four power levels (0.1 / 0.1, 0.1 / 0.2, 0.2 / 0.1, 0.2 / 0.2 mW). Then, pump-probe delay traces at two ROIs (e.g. the regions marked in Figure 9a) were extracted. Figure 9 show that power dependence of both positive and negative signals is linear in pump and linear in probe.

![Figure 9](image)

Figure 9. To understand the power dependence of positive and negative signals, a power study was conducted by acquiring a series of images at four power levels (0.1 / 0.1, 0.1 / 0.2, 0.2 / 0.1, 0.2 / 0.2 mW). Then, pump-probe delay traces at two ROIs (e.g. the regions marked in Figure 9a) were extracted. Figure 9 show that power dependence of both positive and negative signals is linear in pump and linear in probe.

Figure 10 (a-c) Pump-probe wavelength switching experiment. Images acquired at (a) 720 / 817 nm and (b) 817 / 720 nm (0.4 mW / 0.4 mW, $\tau = 200$ fs). (c) Extracted signals from ROIs marked in panel a-b. (d) A bright field image of a minor grain that shows GSD signals (top) and a corresponding pump-probe image acquired at 0 fs (bottom).

The next experiment was conducted using 720 / 817 nm and 817 / 720 nm to reveal the pump-probe mechanisms of both signals (Figure 10a-c). Two grains showing positive
and negative signals at 720 / 817 nm maintain their sign and decay dynamics at 817 / 720 nm, suggesting that the nonlinearity of the major grains is TPA and that of the minor grain is GSD. Power scaling and wavelength switching experiments indicate that minor grains have accessible electronic energy states for 720 and 817 nm, implying a visibly different color from major grains. A bright field (BF) image of red HgS confirms that minor grains are black, in contrast to major grains having red color (Figure 10d). In addition, similar decay dynamics of minor grains with black HgS indicate the presence of pre-existing β-HgS in Vermilion (Figure 8b).

### 2.2 Laser induced phase conversion

As known from laser ablation experiments, intense laser pulses can induce the conversion from α-HgS to β-HgS. In order to confirm this effect under femtosecond illumination and to determine damage thresholds, we deliberately increased powers of laser illumination. While we tested the limits, we observed that pre-existing β-HgS seeds a phase conversion above the damage threshold.

Figure 11 shows changes of pump-probe images near the surface of Vermilion when the sample was exposed to gradually increased power levels. Note that we induced degradation by acquiring a series of pump-probe image at gradually higher power levels, and monitored the degree of degradation using a “safe” power level (below the damage threshold) of 0.45 / 0.45 mW (a probe power of 0.45 mW corresponds to a pulse energy of 5.6 pJ, a temporal peak intensity of 7.5 GW/cm², an average fluence of 17 nJ/cm², see more
Figure 11 The change on the surface of red HgS powder after excessive exposure to fs-laser power. All images were taken at 720 / 817 nm, 0.45 / 0.45 mW, τ = 60 fs. (a) Intact Vermilion, after inducing degradation at powers of (b) 0.45 / 0.9 mW, and (c) 0.45 / 0.9 and 0.9 / 0.9 mW. (d) Occurrence of ESA signal in regions that turn from α- to β-HgS. Signals were extracted from the fixed ROI (marked in panels a-c) of a series of images acquired at different power levels (the number in parenthesis indicates the order of acquisition, followed by the power levels). (e) Normalized delay traces of black grains in non-degraded red HgS, black HgS, regions of red HgS that degraded during laser exposure, and deposits of the coverslip acquired at 720 / 817 nm. Powder tempered in agarose gel seem to exhibit slightly longer GSD.

details in Appendix). The region size of pre-existing β-HgS grains in Vermilion (Figure 11a) increased slowly when imaged with doubled probe power (0.45 / 0.9 mW, Figure 11b). After the sample was scanned with even higher power (0.9 / 0.45 and 0.9 / 0.9 mW, Figure 11c), the surface showed a more significant increase in β-HgS. Extracted pump-probe dynamics of degradation products are identical to pre-existing β-HgS (Figure 11e).
Along with the pump-probe changes from dominant nonlinearity of TPA to GSD, the surface of Vermilion powders shows visible changes in appearance from red to black.

![Image](image.png)

Figure 12 Signal changes from TPA to ESA to GSD during laser-induced phase conversion. Two successive pump-probe data sets acquired at a power level that induces conversion (720 / 817 nm, 0.75 mW each). (a,b) Surface changes from the first data set (a) and the second data set (b). $\tau = 400$ fs. (c) Occurrence of a positive ESA signal in various ROIs that turn from $\alpha$- to $\beta$-HgS. The switch from ESA to GSD occurred at varying time delay points during the first scan.

Interestingly, some areas having no pre-existing $\beta$-HgS also experience phase conversions; these areas tend to show occurrence of ESA signals during the phase
conversion. Pump-probe signals extracted from a fixed ROI in Figure 11 show changes in electron transition dynamics during phase conversion. This area initially showed TPA signals from $\alpha$-HgS at 0.45 / 0.45 mW. Before TPA turns to GSD processes, ESA signals lives until 1 ps at 0.9 / 0.9 mW. Note that the conversion from ESA to GSD randomly occurs at various time delays and powers during a series of data acquisition (see more details in Figure 12). After the pump-probe signal converted to negative signs, the area constantly showed GSD signals indicating the completion of a phase conversion. We attribute these ESA signals to an intermediate species generated from either chemical or physical changes.

![Figure 13 Pump-probe images from (a) intact HgO and (b) degraded HgO under fs-laser pulses. Imaging condition: 720 / 817 nm, 0.3 / 0.3 mW, $\tau$: 140 fs (left), 52 ps (right). Grains having long-lived positive signals are produced after illuminating HgO at 0.9 / 0.9 mW. (c) Pump-probe responses of marked region in panel a-b and liquid Hg.](image)

As a side note, when HgO powders are exposed to fs-laser pulses having high power, pump-probe signals change from TPA (intact HgO) to long-lived ESA signals that
extends to the time delays over 50 picoseconds, in addition to GSD signals (Figure 13, note that because HgO also has various solid phase forms and S and O are in the same group in the periodic table, I was curious whether HgO also will show similar phase conversion properties to HgS under fs-laser illumination or not). The signature of these degradation products closely resembles the signature of liquid Hg. This observation indicates HgO undergoes different chemical changes from red HgS under fs-laser exposure but the reason of producing different laser-induced degradation products is still under investigation.

Figure 14 (a-b) Characterization of laser-induced degradation products using EDS and XRD. (a) EDS spectra of degraded red HgS (signals were normalized by photon counts at 2.309 eV). All samples show similar Hg and S peak intensities at 2.195 (Hg), and 2.309 eV (S). (b) XRD spectra normalized by total intensity. (c-d) Picture of red HgS powder after strong fs-laser exposure. (c) the sample; (d) the coverslip removed from the sample after the experiment, showing black deposits.

EDS and XRD confirm the irreversible phase conversion form $\alpha$- to $\beta$-HgS under fs-laser pulses (Figure 14a, b). For EDS analysis, peaks of Hg (M$_\alpha$ 2.195 eV and M$_\beta$ 2.281 eV), S (K$_\alpha$ 2.309 eV), and Cl (K$_\alpha$ 2.622 eV and K$_\beta$ 2.812 eV) were monitored to study
elemental ratio changes on degraded Vermilion. Laser-induced degradation products still have a similar Hg and S ratio to that of intact red and black HgS, which indicates a negligible possibility of liquid Hg formation. No chlorine peak is observed, implying the presence of chloride or chlorine plays no essential role in fs-laser degradation. Because our XRD instrument does not offer microscopic resolution, we degraded a large area (size: 2 x 2 cm, see the image in Figure 14c) for XRD analysis. The diffraction pattern of red and black HgS correspond to reported patterns of α- and β-HgS, respectively. A peak at 30.5° shows most remarkable difference between red and black HgS; therefore, this peak is used for tracing phase conversion. Degraded red HgS shows an increase of peak intensity at 30.5°, indicating formation of β-HgS. This sample still shows a significant amount of α-HgS because the induced phase conversion only occurred partially on the surface but our X-ray diffractometry is unable to resolve layering information because it probes the entire volume.

α-HgS in red HgS exhibits only two-photon absorption at 720 / 817 nm, while black HgS shows linear absorption at both 720 and 817 nm. In order to investigate the role of linear and nonlinear absorption during fs-laser induced degradation, we conducted two imaging studies with different pulse durations and dwell times and monitored the degree of degradation.

First, we scanned three regions on Vermilion powders below a damage threshold (720 / 814 nm, 0.3 mW each) using fs-laser pulses with identical averaged power (1.2 mW)
Figure 15 Surface change of red HgS powders before/after exposure to fs-laser pulses of different pulse durations but identical average power (1.2 mW). The pulse width and wavelength used to induce degradation is (a) 1 ps, 814 nm, (b) 150 fs, 814 nm, and (c) 150 fs, 720 nm (modulated at 2 MHz). Pump-probe images on the top show the initial status of sample, and the bottom show the change of the surface after the sample was exposed to high power. (720 / 814 nm, 0.3 mW each, $\tau$: 80 fs).

but different pulse widths; (a) 814 nm, 1 ps (b) 814 nm, 150 fs, and (c) 720 nm, 150 fs in Figure 15. Then, the degree of degradation was monitored using initial monitoring condition (720 / 814 nm, 0.3 mW each). Note that we slightly shifted probe color from 817 to 814 nm for temporally broadening of the pulse using a band pass filter for 814 nm with bandwidth 1 nm). Negligible changes were observed on the sample exposed to 1 ps, 814 nm pulses while the phase conversion was observed under fs-laser illumination using 150 fs, 814 nm. The greatest degree of an increase in GSD signals was observed after Vermilion
was exposed to 720 nm, 150 fs (note that a modulated pulse train has roughly twice the peak intensity of unmodulated pulses at the same average power). We confirm that both 720 and 817 nm having shorter pulses cause a significant amount of phase conversion while the wider pulse induces negligible changes. It indicates that nonlinear optical absorption of α-HgS is a major factor of fs-laser induced degradation because higher peak power of a shorter pulse will induce higher degree of nonlinearity. Second, by changing the image scan speed we tuned the dwell time (10, 20 µs) and adjusted averaged power (1 / 1 mW, 0.5 / 0.5 mW, respectively) to keep the number of photons per individual pixel constant. For the two cases, thermal effects are almost identical but nonlinear optical absorption effect is stronger in the first case. We observed a considerable surface alteration in the case of having shorter dwell times, indicating nonlinear optical absorption as a key factor of discoloration.

![Energy Diagram of α- and β-HgS](image)

**Figure 16** Prediction of energy diagram of α- and β-HgS. TPA process in α-HgS (left) resulting from no accessible excited states for single photon excitation from 720 and 817 nm (indicated in middle), linear absorption of β-HgS leading to GSD process and local heating upon illumination (right).
A possible explanation for the seeding effect of β-HgS is that the rate of phase conversion of α-HgS is induced by nonlinear optical absorption and can be enhanced by a local heating from β-HgS (Figure 16).

Electron transition to excited energy states of α-HgS seems an essential step to initiate the fs-laser assisted phase conversion, because α-HgS has no accessible excited energy state for linear absorption of 720 and 817 nm—excited states can only be accessed by nonlinear optical absorption. Meanwhile, linear absorption of β-HgS can lead to an increase of temperature in its vicinity during exposure. This in turn enhances the rate of the phase conversion of α-HgS activated by nonlinear optical absorption. The observation of sputtered black grains in Figure 14d on the coverslip of the sample after exposure hints at a substantial temperature increase, but we were not equipped to quantify the temperature during the short laser exposure.

2.3 UV and temperature assisted degradation

Because nonlinear optical absorption is an extremely rare event in nature, its contribution to degradation is of little concern under normal environmental condition. To mimic natural system inducing light-assisted degradation, UV light was used as a light source.

For the initial experiment, UV light (intensity: 4.06 x 10^4 mW/cm^2, see details in Appendix) was used to expose red HgS powder placed on a glass slide, and then the samples were transferred to the microscope for imaging. Similar degradation products as
the ones obtained with fs-laser induced degradation were observed. Regions that received this much UV light underwent complete phase conversion, showing GSD signals from $\beta$-HgS (ROI2 in Figure 17). The appearance of a long-lived positive ESA signal was observed on the area that received only indirectly UV light, in addition to a TPA signal (ROI1).

![Pump-probe imaging of UV-exposed red HgS powder on a glass slide at 720 / 817 nm (0.2 mW each). (a) Pump-probe signatures of the sample’s surface at $\tau = 140$ fs. The upper part (ROI2) received more UV light than the lower part (ROI1). (b) Extracted pump-probe signals show apparent difference: TPA and ESA in ROI1 and GSD in ROI2.](image)

In order to monitor the gradual increase of ESA signal under UV exposure, a smaller amount of UV light (intensity: $1.14 \times 10^4$ mW/cm$^2$, details in Appendix) through the microscope was delivered onto a Vermilion powder and nonlinearity changes were directly monitored intermittently (note that heat-assisted effects were not simultaneously monitored for in this experiment because thermal expansion of Vermilion limits the monitoring of surface changes at a fixed focal point while Vermilion is heated). Figure 18 shows a gradual increase in ESA signals upon UV exposure, showing similar dynamics with an intermediate species observed in fs-laser induced degradation products. This
observation indicates that single photon absorption from UV light can induce the formation of intermediate species upon laser exposure. Because of limitations in the microscope optics, we could not deliver enough UV light to cause complete phase conversion.

Figure 18 Gradual appearance of ESA signals over time when red HgS powder is exposed to continuous UV light. UV light was introduced through the illumination objective in the microscope. Pump-probe signals were monitored using 720 / 817 nm, 0.3 / 0.3 mW.

Interestingly, regions that showed ESA signals seem to be more susceptible to phase conversion to β-HgS for increased fs-laser illumination powers. Figure 19 shows changes on a Vermilion surface containing two signatures (TPA and ESA) under high-power illumination. (TPA on the lower-left corner and ESA on the upper-right corner) While areas showing signatures of α-HgS constantly display TPA signals, regions having ESA signals turned to GSD signals under higher fs-laser illumination.
Figure 19 Regions that were exposed to UV radiation and that have developed ESA signals showed an increased susceptibility for phase conversion from $\alpha$- to $\beta$-HgS under increased fs laser illumination. Signature changes were induced and monitored simultaneously by using 720/817 nm with (a) 0.3/0.3 and (b) 0.9/0.9 mW ($\tau = 200$ fs). (c) Pump-probe signal changes on marked regions in panel a-b.

To study the effect of increased UV exposure and temperature, the experiment was conducted under higher UV illumination intensity and temperature assisting effect was simultaneously investigated. Note that the Vermilion powder was placed in a glass vial and UV light (intensity: $6.55 \times 10^4$ mW/cm²) illuminated into the vial for 5 minutes at two temperatures, 22 and 56°C. After illumination, we observed changes in two regions; discolored Vermilion powder and glass vials showing silver droplets. Formation of
degradation products is more pronounced for UV exposure at elevated temperatures, 56°C (Figure 20).

<table>
<thead>
<tr>
<th>Powder</th>
<th>Intact Vermilion</th>
<th>22°C</th>
<th>56°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass vial</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Figure 20 Images of degradation products: collected discolored red HgS powder attached to double-sided tape (top row), color change of glass vials from transparent to silver (bottom row). Degradation was induced at different temperature of the vial.*

We first characterized the visually discolored powder by transferring it onto a glass slide for pump-probe and XRD analysis. As the visual appearance indicates, the phase conversion to β-HgS is apparent and the degree of changes increases at higher temperature as shown in pump-probe image and spectra (Figure 21a-c): averaged pump-probe signals from entire FOV show a substantial nonlinearity change from intact Vermilion, an increase of GSD and suppression of TPA signals. Note that a long-lived ESA signal from intermediate was not observed probably due to high UV-illumination intensity nor was a liquid Hg signature observed. In contrast to pump-probe analysis, XRD spectra provided little information about the phase conversion, except for a slight peak broadening near 30.5° (Figure 21d).
Figure 21 Characterization of discolored Vermilion powders under UV exposure at 22 and 57°C. Pump-probe images of randomly selected regions of degraded powder produced at (a) 22 and (b) 57°C, and (c) their averaged pump-probe signals (FOV: 70 x 70 µm). (d) XRD spectra of discolored Vermilion samples shown in Figure 20.

Secondly, the haze of silver droplets, which had developed on a glass vial along with powder debris (see Figure 20), was analyzed using SEM-EDS and pump-probe microscopy. SEM-EDS spectra on the haze show an increase in Hg contents at 2.195 eV and 2.281 eV while bulk powder display a decrease of Hg peak compared to S at 2.309 eV, which indicates formation of vaporized Hg during light exposure (Figure 22a-c). In addition, both samples show no significant presence of chlorine peaks at 2.622 and 2.812
eV ($K_\alpha$ and $K_\beta$). Pump-probe analysis on the haze confirms liquid Hg had formed, in addition to $\beta$-HgS debris and the intermediate species (Figure 22c).

Figure 22 SEM images (a) bulk powder and (b) silver haze and corresponding (c) EDS signals. (d) Pump-probe analysis of silver haze on glass vials. The region of a silver droplet, clearly exhibiting the pump-probe signatures of liquid Hg (720 / 817 nm, 1.5 mW each, 160 fs).

### 2.4 Degradation products in a 14th century painting

Lastly, pump-probe imaging and microscopic analysis were conducted on a 14th century Italian painting (Figure 23, John the Evangelist Reproving the Philosopher Crato by Francesuccio di Cecco Ghissi, 1375, egg tempera on panel; North Carolina Museum
of Art) having darkened Vermilion layers. The North Carolina Museum of Art provided a cross-section sample (size: 1 x 0.3 mm), which contains areas that were identified by a conservator as discolored Vermilion.

Confocal reflectance and pump-probe images were simultaneously acquired at 720 / 817 nm. The confocal reflectance image highlights a layering structure of the cross-sectioned area as its BF image indicates (insect in Figure 24a): varnish, Vermilion, and ground supports. Some areas on the top of Vermilion layer show little reflection, however, confirmation of chemical or physical changes of this area is challenging because weak reflected signal can result from either higher absorption of blackened products or less scattering from empty areas such as physical cracks. In contrast, the simultaneously
Figure 24 (a) A pump-probe image of the cross-section sample acquired at 720 / 817 nm, 0.3 / 0.3 mW, $\tau = 260$ fs (top: the surface of the painting). Squares indicate ROIs (magenta, red, green, cyan, and blue: ROI1 to 5, respectively), with their pump-probe signals shown in (b). Inset on left and right is a BF image and a confocal reflectance image of the same FOV. (c) Regions that were containing ESA signals near the surface of a cross-section sample (top) showed an increased susceptibility for phase conversion $\beta$-HgS under increased fs-laser illumination (bottom). White squares indicate the identical areas. Imaging condition: 0.3 / 0.3 mW, 720 / 817 nm, $\tau = 90$ fs.
acquired pump-probe images display obvious difference in molecular information of darkened areas and even slightly degraded Vermilion layers showing invisible color changes. Note that varnish and ground layers generate a negligible amount of pump-probe signal. GSD signals from β-HgS are detected on the top of the Vermilion layer showing visible discoloration (e.g. ROI1 in Figure 24a), while the inner layer shows dominant TPA signal resembling intact α-HgS (ROI5, note that there is a small additional ESA signals, which could indicate a slight degradation in inner layer as well). Even when Vermilion is visibly non-degraded, gradual signal variations are observed from deeper to the top layers (see signal changes from ROI5 to ROI2 in Figure 24a-b). Contribution of ESA signals for the time delays in the picosecond range increases from the bottom to upper layers, similar to the signals we observed in fs-laser and UV exposure experiment.

Figure 24c shows susceptibility to phase conversion of the regions showing long-lived ESA signals in the Vermilion layers. Another region showing long-lived ESA signals towards the surface was selected and pump-probe signature changes were monitored at 0.3 / 0.3 mW (an image on top of Figure 24c). This area was imaged twice with higher power, 0.9 / 0.9 mW, and then changes were monitored at 0.3 / 0.3 mW (bottom). GSD signals increase mostly near the surface under higher fs-laser illumination power, indicating the surface containing ESA signals is more susceptible to phase shift (similar observation as Figure 19). This result implies higher contents of the intermediate species
in Vermilion layers towards the surface, and its nonlinearity could indicate incipient degradation of Vermilion layers.

Figure 25 (A) A SEM image of the cross-section sample in Figure 24. (B) EDS analysis results on the marked regions of the SEM image. Note that we observe a substantial amount of S in the all areas, including resin, varnish and support. Because of this background, we were unable to quantitatively determine the Hg to S ratio in different areas containing Vermilion.

SEM-EDS analysis reveals changes in elemental ratios in the cross section (note that the white square in Figure 25 corresponds to the region in Figure 24). The area showing β-HgS and gradual signal changes displays no clear changes of Hg and S ratios. Furthermore, a noticeable amount of chlorine is not observed in any area. To reveal phase and chemical changes on these areas a follow-up μ-XRD experiment is in preparation.
3. Spectroscopic Differentiation and Microscopic Mapping of Red Organic Pigments

In this chapter, the application of pump-probe microscopy will be extended to a series of red organic pigments (ROPs) which exemplify the challenge in the field of conservation science. Conjugated π-systems in organic ROPs result in the dye's red colors, which are generated from electronic transitions in the visible (VIS) range (Figure 26). Because the organic structures (i.e. dye) are unstable and change their absorption properties depending on environment such as solvent and pH (e.g. Eosin Y and Eosin Y free acid, see structures in Figure 26), precipitated dyes with metallic cations, called lake, have been employed to increase stability and make them less dependent on environment.

The use of ROP lakes is prevalent in historical artwork, but they are notorious for fading under light exposure over time as Vermilion undergoes discoloration. Unfortunately, non-invasive characterization of their degradation processes is challenging primarily due to the lack of atomic specificity. While x-ray contrast is powerful for inorganic pigments, its use for ROPs is obviously challenging, except for the bromine-based detection of Eosin Y<sup>32-33</sup>. The most chemical specific techniques such as chromatography, nuclear magnetic resonance, and mass spectroscopy require a physical removal of samples<sup>34-37</sup>, which is obviously destructive and limited in scope and sampling location. Several micro- and non-destructive approaches have been developed with
Figure 26 Carmine naccarat is the lake pigment based on (a) Carminic acid. Madder lake is based on (b) Alizarin and (c) Purpurin. Lac dye consists of (d) Laccaic acid D and (e) Laccaic acid A,B,C, and E. (f) Eosin Y and its closed ring form (g) Eosin Y free acid.

optical contrast based reflection (fiber-optic reflection spectroscopy\textsuperscript{41-43}), absorption (Fourier transform infrared spectroscopy\textsuperscript{44,45}), fluorescence\textsuperscript{46-47} (in some cases even time-resolved\textsuperscript{48}), and Raman scattering\textsuperscript{44,49} (Surface-enhanced Raman scattering microscopy, note that the requirement for micro-sampling or close contact to a nanoparticle-covered probe tip poses a significant challenge)\textsuperscript{49-52}. These contrasts can be used for spectroscopic mapping, but direct projection of these signals onto an imager does not explore the depth dimension. Combined optical coherence tomography\textsuperscript{53} (a scattering-based contrast method) and two-photon fluorescence\textsuperscript{54} have intrinsic depth sectioning.
capability and can measure structure, but little detailed chemical information can be obtained.

Here we demonstrate the use of pump-probe microscopy to analyze and visualize a series of ROPs. Distinctive pump-probe signatures of insect-based (Carmeine naccarat and Lac dye), plant-based (Madder lake), and synthetic (Alizarin, Purpurin, Alizarin lake, Purpurin lake, Eosin Y, Eosin Y free acid, Carminic acid, and Carmine) pigments will be highlighted. Using the pump-probe signatures, spatial variation resulting from chemical changes or pigment mixture will be mapped with micrometer scale resolution, and capability to visualize the depth dimension will be discussed. Lastly, I will discuss the effect of NIR light exposure on electronic and vibrational energy levels of Carmine.

3.1 Pump-probe spectroscopic database of red organic pigments

Some knowledge of absorption spectra of the pigments is essential to choose appropriate wavelengths for pump-probe analysis. All ROPs exhibit similar linear reflectance spectra (Figure 27a), and the edge of an absorption feature was selected as pump, 600 and 720 nm. This is because absorption that is too high reduces the possible penetration depth and too little absorption leads to weak observable signals (note that the pump initializes all pump-probe mechanisms, therefore, strong signals require for the pump to excite enough electrons from the ground state). 817 nm is also close to the end of an absorption edge and was selected as the probe, mainly due to restriction of the experimental set-up, but offered low enough absorption for high penetration.
Figure 27 (a) Linear reflectance spectra of ROPs. (b-c) Pump-probe dynamics at two wavelength combinations. Pump/probe wavelength and power are: (b) 720 nm, 1.5 mW / 817 nm, 1.5 mW; (c) 600 nm, 0.25 mW / 817 nm, 0.5 mW. The traces are averaged signals from the x-y FOV of (b) 365 x 354 µm and (c) 182 x 177 µm.

To minimize possible signal variations from pigment-binder interaction, unmodified powder was investigated except for Purpurin. Purpurin shows a gradual signal decrease during pump-probe data acquisition, and we attribute this reduction to local heating caused by the laser (note that it has been reported that the thermal stability of Purpurin is lower than other red organic dyes and the formation of metal complexes...
substantially increases its thermal stability\textsuperscript{55}). To minimize degradation during data acquisition, Purpurin was tempered into an agarose gel.

Figure 27b and c show pump-probe signatures of various ROPs at 720 / 817 nm and 600 / 817 nm, respectively. In this study, we fixed the z-position at slightly below the surface and scanned pump-probe signals of x-y planes at variable time delays. Note that these spectra show averaged pump-probe signals from the entire x-y field of view (FOV). Although the linear reflectance spectra of the pigments are very similar and mostly featureless, their nonlinear optical pump-probe signatures vary significantly.

In two insect-based pigments, Carmine naccarat and Lac dye, 720 / 817 nm pulses produce an instantaneous positive signal and a long-lived positive signal resulting from TPA and ESA, respectively. Pumping at 600 nm leads to electron population increases in excited states, thus ESA signals are dominant at 600 / 817 nm.

Madder root produces Purpurin and Alizarin dyes in nature and adding metal cations into these dyes helps form stable metal-dye complexes called Madder lake\textsuperscript{56-57}. Despite the similarities in Purpurin and Alizarin structures, they show dramatic differences at 720 / 817 nm: Purpurin shows dominant negative signals resulting from combination of GSD or SE (major) and TPA (minor) while Alizarin displays TPA and long-lived signals from ESA. 600 / 817 nm enables Purpurin to access next excited states resulting in ESA signals but no qualitative dynamic change is observed in Alizarin. A possible explanation of this behavior is the observation that both dyes exhibit fluorescence...
extending into the 800 nm range, with Purpurin having the much higher quantum yield and smaller Stokes shift (indicating SE as a major contribution for the negative signal at 720 pump)\textsuperscript{57}.

The averaged pump-probe response of Eosin Y, a brominated derivative of fluorescein, displays the opposite sign at two wavelength combinations: dominant negative signals resulting from SE (major), TPA, ESA and GSD at 720 / 817 nm and positive signals from ESA at 600 / 817 nm.

**3.2 Non-invasive visualization of red organic pigments**

The pump-probe spectroscopic signatures in Figure 27 show important pigment characteristics but do not take advantage of the high spatial resolution afforded by pump-probe imaging. First, a visualization study was conducted on two insect-based pigments (Carmine and Lac dyes) which are challenging to differentiate non-invasively due to the similarities in their chemical structures. However, their pump-probe responses resulting from intrinsic photo-physical properties help identify and differentiate them. To highlight the difference of Carmine and Lac dye, pump-probe signals of individual pixels on their images are plotted into a phasor histogram (see Appendix for explanation of Phasor analysis\textsuperscript{58-59}). The histograms in Figure 28 illustrate the importance of choosing appropriate colors – for a 600 nm pump the two pigments are barely separated, while the 720 nm pump yields a clear separation due to the qualitatively different shapes of the
pump-probe traces. As narrow distributions of phasor coordinates in the plot indicate, spatial variation of signal is not apparent in both Carmine and Lac dye.

Figure 28 (a) Cumulative phasor histogram of four pump-probe images. Carmine naccarat and Lac dye are imaged using two wavelength combinations (600 / 817 nm and 720 / 817 nm). (b) False-color coded images of Carmine naccarat (600 / 817 nm – magenta, 720 / 817 nm – green) and Lac dye (600 / 817 nm – orange, 720 / 817 nm – cyan). Scale bar: 50 µm.

The spatial resolution of pump-probe microscopy proves especially useful for pigments that are heterogeneous such as Eosin Y and Madder-related pigments. Despite the synthetic origin of Eosin Y, its pump-probe image in Figure 29 is heterogeneous, which may result from variations in chemical structures or crystallinity on the grain level\textsuperscript{38}. This image highlights that a small subset of crystals showed markedly different pump-probe dynamics, TPA and ESA, from dominant crystals producing SE signals.

To trace the origin of variation in Eosin Y, pump-probe characteristic changes from Eosin Y free acid to Eosin Y was investigated. Note that a closed 5-membered ring in Eosin
Y free acid reduces the length of the conjugated system leading to its pale orange appearance (see their structures in Figure 26). Eosin Y free acid is insoluble in water and shows TPA and weak ESA signals at 720 / 817 nm (Figure 30b). Its appearance changes to red immediately after adding NaOH solution, because ring opening extends the length of the conjugated system. Pump-probe signatures of this solution show an increase in negative signals resembling those of Eosin Y. To check the influence of crystallinity and polycrystallinity on pump-probe dynamics, the solution (i.e. Eosin Y free acid in NaOH solution) was placed on the glass slide and crystallization was induced after covering with a cover slip. The vicinity of the edge of the solution underwent fast drying while the center of solution covered with a glass underwent crystallization (see BF images in Figure 30a). Pump-probe images in Figure 30c-d display the structures near the edge and center of the sample: the edge of a sample seems to have polycrystalline structures while the center part shows needle-like crystal structures.
Figure 30 Pump-probe response of polycrystalline and crystalline Eosin Y. (a) Surface image of polycrystalline (underwent fast drying, top, left) and crystalline structures (bottom, left), and a picture of Eosin Y free acid after adding NaOH solution and covering with a cover glass (right). (b) Signal changes of Eosin Y free acid after adding NaOH solution and removing water. The number in legend from 1 to 5 indicates investigating areas from close to an edge of sample (corresponding to pump-probe image: c) to the center showing crystals (see pump-probe image in d).

Imaging condition: 720 / 817 nm, 1.5 mW each, $\tau = 380$ fs.

Both two areas show the presence of heterogeneous grains having pure SE and SE and TPA, but formation of a long-lived ESA signal is not observed. Another interesting feature associated with crystallinity is the variation of decay behaviors of negative signals from the edge to the center (see signature change from 1 to 5 in Figure 30b): polycrystalline
structures generate negative signals decaying slowly while well-crystalline structures show faster relaxation behaviors. The signature of crystalline structures is similar to the negative signal in unmodified Eosin Y powder, indicating the origin of negative signals in Eosin Y.

Figure 31 (a) Combined phasor plot of three pump-probe images; Purpurin, Alizarin, and Madder lake acquired at 720 / 817 nm. Phasor images of Purpurin (b), Alizarin (c), Madder lake (d). In Madder lake image (d), the color is varied from blue to green along the indicated trajectory in a. (x-y FOV: 365 x 354 µm).
The next imaging study was focused on visualization of Madder-related pigments (Alizarin, Purpurin, and a commercially available Madder lake) using phasor analysis (Figure 31). The two pure chemicals, Alizarin and Purpurin, have no spatial variation of signals, leading to a narrow distribution on a phasor plot. Their signatures in each pixel are color-coded in yellow and magenta, respectively, and pump-probe images show fairly uniform color distribution due to their homogeneity. Expectedly, Madder lake shows heterogeneity in the pump-probe dynamics. Its negative components in blue closely resemble those of Purpurin in magenta. In addition, positive components of Madder lake in green show separated distribution on phasor plots from those of Alizarin in yellow. Note that sub-pixel mixtures would show phasor coordinates along the colored path indicated in the phasor plot. A small third region (grey) is distinct from the others and is likely caused by other inclusions in the natural sample.

The phasor analysis in Figure 31 indicates that the photo-physical origin of heterogeneity associated with Madder lake mainly result from the formation of dye—metal complex, in addition to partial contribution from natural impurities. To understand the possible influence of the laking process, Alizarin and Purpurin were precipitated with \( \text{Al}^{3+} \) after deprotonating them in \( \text{K}_2\text{CO}_3 \) solution\(^{56} \) (see pKa of Alizarin and Purpurin\(^{46,60} \) in Figure 32). In the cultural heritage science field, it has been predicted that bidentate coordination of Alizarin with \( \text{Al}^{3+} \) produces a 2:1 complex of Alizarin and \( \text{Al}^{3+} \) in the solid
state\textsuperscript{61} (Figure 32c). It is also reported that Alizarin has three potential coordinating sites with metal based on characterization on Sn—Alizarin complexes\textsuperscript{62} (Figure 32d)).

![Diagram of Alizarin and Purpurin pKa and coordination structures](image)

**Figure 32** pKa of Alizarin (a) and Purpurin (b). (c) Expected coordinating structure of 1 : 2 complex of Alizarin and Al\textsuperscript{3+} in the solid state. (d) Possible metal coordinating structures of Alizarin

Interestingly, synthesized Alizarin and Purpurin lakes show different degree of heterogeneity and signal changes under pump-probe microscope (Figure 33). Alizarin lake shows fairly uniform distribution of ESA signals (but different from Alizarin) on its image. In contrast to Alizarin lake, averaged pump-probe signals of Purpurin lake result from superposition of ESA and negative signals (see ROI1 and 2 in Figure 33b). Extracted pump-probe signals highlight the influence of laking process in pump-probe dynamics: metal—Alizarin and Purpurin coordination generate additional accessible excited states for ESA signals, and Purpurin lake also induces the relaxation behavior changes in negative signal of Purpurin.
Figure 33 Pump-probe microscopy of Alizarin and Purpurin lakes. (a) Alizarin lake ($\tau = 0$ fs), (b) Purpurin lake ($\tau = 200$ fs), and (c) pump-probe signatures of dye and lake of Purpurin and Alizarin. Imaging condition: 720 / 817 nm (1.5 mW each). All samples were tempered in agarose gel.

Phasor analysis$^{58, 63}$ in Figure 34 highlights variations of pump-probe dynamics caused by lake formation. Homogeneity of Alizarin lake samples generates its narrow distribution on a phasor plot and it is closer to the endpoint of Madder lake (1) than Alizarin. Expectedly, Purpurin lake shows broad distribution of phasor coordinates between two endpoints (i.e. purple and red bulbs in Figure 34a). One endpoint in purple closely resembles the distribution of Purpurin and Madder lake (2) and another in red
shows a remarkable shift from Purpurin to Madder lake (1). This distribution implies the possible influence of Purpurin lake on the heterogeneity in Madder lake. Figure 34b exhibits the spatial distribution of heterogeneous components having distinct signatures in Purpurin lake.

Figure 34 (a) A phasor distribution of Alizarin, Alizarin lake, Purpurin, Purpurin lake, and Madder lake. (b) Mapping of heterogeneous grains in Purpurin lake. Two endpoints were colored in purple and red, and sub-pixel mixtures between endpoints are colored path.

Although both Alizarin and Purpurin lakes display closer distribution to the end point of Madder lake (1), resulting from additional ESA signal, neither lakes show a perfect overlap with Madder lake. Further influences from laking processes in Madder lake (1) is under investigation.

3.3 Visualization of spatial distributions of mixtures of laked-pigments in mock-up samples
Figure 35 (a-c) mock-up samples with different weight ratios of Madder lake and Carmine naccarat acquired; (a) 1:9, (b) 2:3, (c) 4:1. (d) in-situ depth sectioning images at variable depths (720 nm / 817 nm, 1.5 mW each, $\tau = 0$ fs).

To illustrate the ability of pump-probe microscopy to visualize spatial distributions of mixtures of ROPs in 3D, mock-up samples were prepared on canvas with mixtures of two lake pigments, Carmine naccarat and Madder lake, having different weight ratios (1:9, 2:3, and 4:1). Because of the similarity in visible color, these samples appear featureless under a conventional bright field microscope. According to Figure 27, Carmine and Madder predominantly show pump-probe signals of opposite sign for a 720 nm / 817 nm pump / probe wavelength combination at $\tau = 0$ fs, making this condition ideal for mapping these pigments in heterogeneous mixtures. Figure 35a-c shows pump-probe images of mock-up samples at time delay ($\tau$) = 0 fs, clearly showing the different mixture ratios of the three samples. In Figure 35d we show a sequence of images of mock-
up sample (1:9) as a function of depth, imaged under the same conditions. Note that these images are acquired in situ, i.e., without the need for sample removal from the canvas.

![Phasor histogram and delay traces](image)

**Figure 36** (a) Phasor histogram of a mock-up sample with 3:2 weight ratio of Madder lake and Carmine naccarat. (b) Delay traces corresponding to marked regions in panel a. (c) Phasor image. Imaging condition: 720 nm / 817 nm, 1.5mW each; image dimension is (730 x 708) µm.

From above studies we know that Madder lake contains two signs and its ESA signal has the positive sign at time delay 0 fs like Carmine. To further differentiate the components in the mixtures, phasor analysis is used. Figure 36a and b show the resulting...
phasor histogram and the delay traces corresponding to the regions indicated in the histogram. Although Carmine naccarat also has long-lived positive signals from ESA, it is possible to distinguish them based on the difference in their pump-probe dynamics.

Figure 36c shows an image of sample B (corresponding to a depth of 45 µm) using the color scheme as indicated in the phasor histograms. The image clearly shows the map of Carmine and both components of Madder lake.

### 3.4 Initiation of chemical breakdown of Carmine under NIR light exposure

Carmine is a lake pigment made by precipitating Carminic acid with metallic cations such as Al$^{3+}$ and Sn$^{2+}$. It has been reported that carbonyl and hydroxyl group in Carminic acid coordinate with metal cations (Figure 37) that therefore increases its stability$^{64-66}$. The coordination enhances the stability of Carminic acid, unfortunately, Carmine still experiences fading upon light exposure$^{66}$. Its susceptibility for degradation motivates me to initiate an investigation on degradation processes of Carmine.

![Carminic acid](image1.png)

![Carminic lake](image2.png)

**Figure 37** A structure of Carminic acid and its predicted laked form.
A possible explanation for fading would be found in the particle in a box model (3): a decrease in the length of a conjugated system (L) induces the blue shift of an absorption band of Carminic acid due to an increase in HOMO—LUMO gap, ΔE. Consequently, less absorption of visible lights leads to a loss of its color under light exposure\textsuperscript{67-68}.

\[ \Delta E_n = \frac{\hbar^2}{8mL^2}((n + 1)^2 - n^2) \quad (3) \]

In advance of breakage of conjugation bonds, I expect Carmine experiences additional process: a bond breakage between metal and carbonyl group of Carminic acid. A breakage of conjugated systems requires higher energy for bond dissociation of C=O, C=C, C—C than C=O···Al\textsuperscript{3+} (Table 1)\textsuperscript{69-70}. Therefore, from purely energetic considerations, an external energy from light exposure may induce the breakage of this weakest bond and it may offer the possibility for laked forms to turn to unlaked forms (i.e. dye) as an initial degradation product.

**Table 1 Expected bond dissociation energy of Carmine**

<table>
<thead>
<tr>
<th>Bond</th>
<th>Bond dissociation energy (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C=O···Al\textsuperscript{3+}</td>
<td>40</td>
</tr>
<tr>
<td>C=C</td>
<td>150</td>
</tr>
<tr>
<td>C=O</td>
<td>179</td>
</tr>
<tr>
<td>C—C</td>
<td>80 ~ 100</td>
</tr>
<tr>
<td>C—H</td>
<td>100</td>
</tr>
<tr>
<td>C—O</td>
<td>85</td>
</tr>
<tr>
<td>C—O—Al\textsuperscript{3+}</td>
<td>122</td>
</tr>
</tbody>
</table>
For the study, Carmine and Carminic acid were purchased from Sigma Aldrich and TCI, respectively. Note that Sigma Aldrich specifies Carmine contains around 40% of unreacted Carminic acid and Al$^{3+}$ is used to form Carminic lake. IR spectra of Carmine and Carminic acid powder confirms the influence of Al$^{3+}$—Carminic acid complex on carbonyl stretch (Figure 38); Carminic acid shows strong carbonyl peak near 1703 cm$^{-1}$ and interaction with metal cation leads to this peak shift to 1637 cm$^{-1}$.

![Image of IR spectra](image)

**Figure 38 IR spectra of Carminic acid and Carmine**

Linear optical absorption spectra of Carminic acid and Carmine in DMSO show that the coordination between Carminic acid and Al$^{3+}$ induces red shift of an absorption band of Carminic acid (Figure 39a). It also illustrates that Carmine possesses a potential to be excited to electronic energy states under light exposure above 700 nm while Carminic acid shows weak absorption of the wavelength above 650 nm. A pump-probe wavelength combination of 720 / 817 nm (both at NIR ranges) reveals further electron
transition properties of Carminic acid and Carmine (Figure 39b). Both Carminic acid and Carmine show instantaneous positive signals during temporal overlap of pulses; Carminic acid shows slight contribution of GSD signals and Carmine contains additional long-lived positive signals. In Carmine, it seems that pre-existing Carminic acid generates TPA signals and the lake formation leads to ESA signals which highlight the presence of long-lived electrons for the time delays in the picosecond range under 720 nm light.

![Figure 39](image.png)

**Figure 39** (a) Absorption spectra of Carminic acid and Carmine in DMSO (b) pump-probe signatures of Carminic acid and Carmine powders at 720 / 817 nm

Interestingly, a series of pump-probe spectra of Carmine acquired at 720 / 817 nm highlights that destabilization of Carmine under 720 nm may induce chemical changes on lake forms (Figure 40a). These changes seem to cause the opposite trends on two pump-probe signatures: TPA increases while ESA decreases. Note that for this study, Carmine was mixed with linseed oil to minimize the possible effect of laser-induced thermal degradation (note that linseed oil has SRS signals at 720 / 817 nm due to the vibrational stretching of C—O at 1747 cm$^{-1}$ in carbonyl group$^{71}$ as shown in Figure 40d, and it weakens
the amplitude of TPA signals in Carmine. See changes in Figure 40b). It seems that a bond breakage on carbonyl—\( \text{Al}^{3+} \) in Carmine leads to the formation of Carminic acid, resulting in an increase in TPA and a decrease in ESA. This observation raises possibility of laser-induced bond breakage of metal—Carminic acid at NIR ranges.

Figure 40 (a) Changes of absolute signal amplitude on Carmine powder mixed in linseed oil when scanned with increased laser illumination. A series of pump-probe spectra was acquired at fixed pump powder (0.59 mW) and variable probe powers; (1) 0.59, (2) 0.89, (3) 1.18, (4) 0.59, (5) 2.36, (6) 0.59, (7) 0.59 mW. Numbers in parenthesis indicate a sequence of data acquisition. Signals observed at identical power (0.59 / 0.59 mW) were plotted in a. (b) Pump-probe signal changes of Carmine powder when mixed with linseed oil. All regions show variations on instantaneous signals. Signals were normalized by the data point at 7 ps. (c) Signal changes of Carmine under NIR illumination.
In order to check the possible degradation effect of NIR light exposure, filtered NIR light from a halogen lamp was delivered through the microscope onto Carmine powder and signature changes were directly monitored intermittently (Figure 40c, note that light longer than 715 nm was used for the experiment). Both TPA and ESA decrease after NIR illumination; the degree of amplitude decrease is higher in ESA signals. The decrease of TPA signal indicates a possibility that pre-existing Carminic acid also underwent degradation. Unfortunately, it seems that monitoring the change only on Carminic lake is difficult with the Carmine due to pre-existing dye, therefore, degradation monitoring on dye-free Carmine is in preparation.

The observations raise another possibility of NIR light induced degradation of Carmine although UV range of light has been often assumed as a reason of fading of Carmine. It is possible to expect that an increase of Carminic acid upon light exposure enhances the rate of further chemical breakdown because of its instability and its capability to produce radicals upon light exposure\textsuperscript{72-73}. To confirm the formation of Carminic acid as an early-stage degradation product of Carmine, imaging study using stimulated Raman microscopy\textsuperscript{50} is in preparation, which will offer us better insights on changes of carbonyl—metal bond upon light exposure.
4. Investigation of Other Types of Colorants

Beyond imaging red inorganic and organic pigments in cultural heritage objects, pump-probe contrast could also prove helpful in studying other types of colorants such as modern automotive paints, ultramarine blue pigments in historical artwork, and melanin in hair. For example, when investigating vehicle accidents, small automotive paint samples are often collected and analyzed (e.g. in hit-and-run incidents). Although automotive paints and artwork pigments have different purposes in terms of their uses, we expect investigation of automotive paints would be in the same manner as ROPs because interaction between light and organic colorants would produce distinctive markers to identify and characterize them. In chapter 4.1, I will demonstrate that the use of pump-probe microscopy as a tool to analyze a series of red organic automotive paints and will describe identification processes used on automotive paint flecks generated from a hit-and-run car accident. In 4.2, I will highlight the need for microscopic analysis to understand photo-physical properties of ultramarine blue pigments by taking various phenomena (e.g. concentration and depth dependence of pump-probe signals and micron-size grains having distinctive depolarizability) as examples. In addition, I will discuss the capability of pump-probe microscopy as a detection tool for sulfur radical anions in ultramarine blue pigments. In 4.3, I will describe the changes of GSD signatures as a function of varying oxidation states of eumelanin in human hair.
4.1 Modern automotive paints

The concern about the toxicity of inorganic paints containing heavy metals has induced a shift towards from the use of inorganic pigments to organic pigments in modern automotive paints\textsuperscript{74}. From the above study, we know pump-probe microscopy can differentiate a series of ROPs, therefore, it is possible to expect that pump-probe microscopy can also differentiate other types of ROPs used for automotive paints.

![Graphs showing linear reflectance spectra and pump-probe signals of three touch-up paints.](image)

**Figure 41** (a) Linear reflectance spectra of three touch-up paints, (b) their pump-probe signals at 720 / 817 nm, and (c) the cumulated phasor plots of the paints.
To demonstrate the capability of pump-probe microscopy, touch-up paints made for matching the color with one automotive were purchased from three companies. Not surprisingly, their reflectance spectra show similar linear absorption features because they were originally generated to have identical colors. On the other hand, their pump-probe spectra display noticeable differences between their nonlinear mechanisms at 720 / 817 nm. Multi-exponential fitting on the averaged signal from the entire FOV shows apparent differences in the decay times and relative amplitudes (Table 2). Distribution of the three paints in a phasor plot shows partial overlap though they are still distinguishable, indicating that pump-probe microscopy can also be used as an identification technique for automotive paints.

Table 2 Bi-exponential fitting of averaged pump-probe signals of three touch-up paints

<table>
<thead>
<tr>
<th>Paint</th>
<th>( T_1/\text{ps} ) ( \text{A}_1 )</th>
<th>( T_2/\text{ps} ) ( \text{A}_2 )</th>
<th>Instantaneous amplitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paint 1</td>
<td>1.47±0.23 \ (0.011±0.001)</td>
<td>30.7±4.1 \ (0.013±0.001)</td>
<td>0.0270±0.0002</td>
</tr>
<tr>
<td>Paint 2</td>
<td>1.11±0.12 \ (0.028±0.001)</td>
<td>25.4±3.9 \ (0.014±0.001)</td>
<td>0.0350±0.0004</td>
</tr>
<tr>
<td>Paint 3</td>
<td>0.87±0.09 \ (0.066±0.003)</td>
<td>5.3±0.4 \ (0.023±0.003)</td>
<td>0.2100±0.0012</td>
</tr>
</tbody>
</table>

Identification of automotive paints using pump-probe microscopy is especially useful for a hit-and-run accident, which requires the find of a suspect, because pump-probe signatures of the paints remaining on a victim’s vehicle can be used as the evidence.
The next study was focused on identification of automotive paint flecks collected from two vehicles, which seemed to have been involved in the same hit-and-run accident.

Vehicle A is a victim’s vehicle which originally had silver paint on its entire body. However, the accident produced structural deformation and blue scratches on the side of Vehicle A, which is an obviously different from its original color (Figure 42a). Macroscopic information indicated that Vehicle B had probably glazed the side of Vehicle A: Vehicle B, which had blue paint, had scratches containing silver paint at the same height as the scratches on Vehicle A (Figure 42b).
For further identification, optical microscopic analysis was conducted on two paint flecks collected from each vehicle; one contains the original paint for a reference and another contains scratched areas showing the color of the foreign paint. BF images of the scratched samples of the two vehicles show similar paint colors, silver and blue, but distinct structural shapes (Figure 42c-d). For example, the victim’s vehicle (Vehicle A) shows stripes, indicating that an external force from another vehicle generated the trajectory of blue paint. In contrast, Vehicle B shows pressed paint piles which seem that its physical pressure on another object induced cumulation of paint piles on Vehicle B. Although visible color, the height of paint scratches, and their microscopic structures explain some causality about the accident, their molecular information is obscured.

To trace molecular signatures in the paint fragments, the initial pump-probe analysis was conducted on reference samples containing the two vehicles’ original paints. Vehicle A and B have distinct and distinguishable pump-probe signatures: Vehicle A has heterogeneous components (negative and positive signals) and Vehicle B has dominant positive signals showing different relaxation behaviors compared to those in Vehicle A (see Figure 43e).

Figure 43c and d highlight apparent structural differences (stripe- and pile-like structures) of paint flecks from the scratched areas with characteristic pump-probe contrast. This pump-probe investigation reveals that blue scratches on Vehicle A have the identical pump-probe signature of Vehicle B’s original paints. Cross-checking on a
Figure 43 (a-b) Pump-probe images of reference paints of Vehicle A (a) and B (b) acquired at 720 / 810 nm, t = 80 fs. (c-d) Pump-probe image of scratched paints collected from Vehicle A (c) and B (d) at τ = 320 fs. (e) Pump-probe signatures extracted from marked regions in panel a-d. Identical pump-probe signature of Vehicle B was found in scratched regions in Vehicle A.
scratched sample collected from Vehicle B also contains apparently same pump-probe signatures as Vehicle A. This result highlights another capability of pump-probe microscopy as an identification tool for pigments in the forensic science field.

4.2 Visualization of sulfur radicals in ultramarine blue pigments

Lapis lazuli (natural ultramarine blue) is an important historical pigment and its limited source in nature makes it expensive more than gold. To substitute it, an alternative pigment called synthetic ultramarine blue (SUB) has been synthesized\textsuperscript{75}. \textit{Na}_6\textit{Ca}_2\textit{Al}_6\textit{Si}_6\textit{O}_{24}(\textit{S}_n\textit{SO}_4)_2 and \textit{Na}_7\textit{Al}_6\textit{Si}_6\textit{O}_{24}\textit{S}_3 are characterized molecular formula for natural and synthetic ultramarine blue pigments\textsuperscript{76}, respectively, and they consist of two key components, a trisulfur radical anion (\texttildelow S_3) and a sodalite-cage structure\textsuperscript{75-78}. The charge transfer of \texttildelow S_3 produces blue colors but its unpaired electron makes this species reactive and unstable\textsuperscript{79-81}. This unstable radical anion becomes stable in a sodalite-cage structure due to chemical protection. Therefore, a breakage of sodalite framework causes the appearance change of ultramarine blue pigments (typically under acidic conditions)\textsuperscript{82-84}. Other types of sulfur species have also been reported as chromophores producing distinct colors in a sodalite framework: \texttildelow S_2 is yellow-green and \textit{S} and \texttildelow S_4 are red color\textsuperscript{80,85}, and synthetic pigments containing these radicals are called synthetic ultramarine green and synthetic ultramarine red, respectively\textsuperscript{84}. Electron paramagnetic resonance and resonance Raman spectroscopy reveal that ultramarine blue pigments contain \texttildelow S_3, in addition to \texttildelow S_3\textsuperscript{77-78,86-87}. We expect that pump-probe microscopy can characterize and
visualize various sulfur radical anions in natural and synthetic ultramarine blue pigments using their distinct electron transition dynamics with the sub-micrometer resolution. To reveal the presence of minor components, I initiated the microscopic analysis on ultramarine blue pigments.

Figure 44 Pump-probe image of (a) Lapis lazuli and (b) SUB acquired at 720 / 817 nm (Pump / Probe, 0.63 / 0.65 mW) \( \tau = 160 \) fs. Marked regions in pump-probe images with red, yellow, green, blue squares correspond to ROI 1 to 4, respectively. Their extracted pump-probe curves of (c) Lapis lazuli and (d) SUB. Signals were detected using transmission mode.

Several microscopic analyses on natural and synthetic ultramarine blue pigments reveal interesting photo-physical properties of micro-grains showing characteristic
pump-probe contrast. Figure 44 shows that Lapis lazuli and SUB have different grain sizes but their heterogeneous grains show similar nonlinearities to each other at 720 / 817 nm. Both Lapis lazuli and SUB show dominant negative signals resulting from $\cdot S^-$ (see their pump-probe responses in ROI4 of Figure 44), and the negative signals of SUB decay faster than those of Lapis. Meanwhile, positive signals in ROI1 of Lapis lazuli show similar signatures to those of SUB (red curves), in addition to negative signals in ROI2 (yellow curves). Some signals of grains in SUB or Lapis are not found in other pigments (e.g. ROI3). If impurities from external sources generate pump-probe signatures of minor grains in Lapis lazuli, these signals should not be observed on synthetic pigments. However, similar pump-probe signals of minor grains in two pigments imply that the grains may originate from identical starting materials for sulfur-sodalite cage structures.

To understand the origin of these pump-probe signatures, a switching experiment was conducted on both pigments at a fixed imaging location. When switching the wavelength combination from 720 / 817 nm to 817 / 720 nm, dominant negative signals from Lapis (see ROI2 in Figure 45) decrease significantly (but still remain a small contribution), in addition to an increase of ESA signals. It indicates that the dominant negative signals of Lapis lazuli at 720 / 817 nm is superposition of SE (dominant), GSD, and ESA signals. Signals of some grains, such as ROI1, change the sign completely (indicating a presence of ESA and GSD signatures), while some grains retain their signs at switched cases, such as ROI3 (GSD) and 4 (indicating ESA and TPA signals).
Figure 45 A switching experiment on Lapis lazuli tempered in gum. Pump/probe images acquired at a wavelength combination; (a) 720 / 817 nm, (b) 817 / 720 nm. Pump-probe signals were extracted from marked regions in panel a-b, and their pump-probe responses are plotted with identical colors with those of squares. Changes of pump-probe signals on identical locations when wavelength combination is (c) 720 / 817 nm and (d) 817 / 720 nm. (transmission mode).

At 817 / 720 nm, not surprisingly, dominant negative signals (e.g. ROI2-4) from SUB decrease significantly as Lapis lazuli shows a remarkable decrease in the contribution of negative signals. This observation indicates that SE is a dominant nonlinearity of SUB as Lapis Lazuli. Some minor grains such as ROI1 show significant sign changes from
positive to negative at the switched wavelength, implying that positive signals in ROI1
are a superposition of ESA and GSD signals.

![Figure 46](image)

Figure 46 A switching experiment on SUB tempered in gum. Pump-probe images acquired at a pump / probe wavelength combination; (a) 720 / 817 nm, (b) 817 / 720 nm. Pump-probe signals were extracted from marked regions in panel a-b, and their pump-probe responses are plotted with identical colors with those of squares in (c) 720 / 817 nm and (d) 817 / 720 nm. (transmission mode)

Interestingly, one distinct signal (superposition of TPA and ESA signals in Figure 45) is only observed on red grains in Lapis lazuli (see a BF image in Figure 47a). Because this signature is only observed in Lapis lazuli, it seems to originate from external impurity during natural mining processes of Lapis lazuli. SEM-EDS analysis reveals different
elemental ratios between red grains and blue grains. A blue grain in Figure 47b shows a substantial amount of sulfur elements, in addition to various atoms originating from the sodalite cages structure. On the other hand, red grains show a negligible amount of elements observed in the blue grain and display strong iron and oxygen peaks, which indicates the presence of iron oxide in natural ultramarine blue pigments.

Figure 47 Characterization of red grains in Lapis lazuli. (a) A BF image (top) and its SEM image (bottom). (b) Corresponding EDS spectra to marked region in panel a. (c-d) Pump-probe image of blue and red grains (white square corresponds to the area in SEM image in a) acquired at 720 / 817 nm, 0.6 mW each, τ = 80 fs, transmission mode (c) and their extracted pump-probe signatures (d).
Other than iron oxide, the similar nonlinear contrast of natural and synthetic ultramarine blue implies that diverse components may not be created by external impurities but may naturally originate during their formation processes, which might lead to the formation of various sulfur radical species. This hypothesis can be supported by imaging study results on synthetic ultramarine red, which show the presence of GSD and ESA components observed on Lapis lazuli and SUB as their minor components. Therefore, my hypothesis is that various nonlinear contrasts in ultramarine blue pigments originate from various sulfur radical anions. To prove this hypothesis, follow-up experiment (co-registering pump-probe and Raman microscopic image) is in preparation.

Figure 48 Pump-probe signatures of synthetic ultramarine red acquired at 720 / 817 nm, 0.8 mW each, $\tau = 160$ fs (transmission mode)

Interestingly, the microscopic analyses on ultramarine blue pigments give us a clue not only about heterogeneous grains and impurities, but also about changes on averaged pump-probe curves resulting from the different degree of signal contribution of heterogeneous components.
Figure 49 Pump-probe signal dependence on SUB concentration. Picture of (a) concentrated and (b) diluted SUB. (c) Comparison between a center and an edge of concentrated SUB at 1.5 / 3.0 mW. Inset corresponding to the red curve ($\tau = 160$ fs).

Pump-probe signal changes at four power levels on (d) concentrated and (e) diluted SUB. (Pump / probe : 720 / 810 nm, epi detection). x-y FOV: 525 x 525 $\mu$m.

For example, averaged pump-probe signals of SUB (typically FOV is bigger than 100 x 100 $\mu$m) show concentration and depth dependence, but these features of ensemble system can be understood by microscopic analysis. Figure 49a and b display pictures of two SUB powder samples tempered in gum arabic having different degrees of concentrated SUB grains. Pump-probe signals of concentrated and diluted SUB samples vary significantly when the center of the samples is investigated (Figure 49d-e): the
contribution of ESA signals is more pronounced in the concentrated SUB than in those of diluted one. Not surprisingly, the edge of the concentrated sample shows a decrease in ESA signals and its averaged spectrum is mostly similar to one of the diluted SUB sample (Figure 49c).

Figure 50 highlights changes in averaged signals of SUB depending on z direction: the surface of SUB powder shows dominant negative signals while middle and bottom parts show additional contribution from ESA signal around τ = 2 ps.

![Graph](image)

**Figure 50 Signal depth dependence of concentrated SUB. Pump / Probe wavelength: 720 / 810 nm (1.5 / 3.0 mW). x-y FOV: 525 x 525 µm. Epi detection mode, signals were normalized by negative minima.**

The next microscopic imaging study on heterogeneous components reveals that different degrees of depolarizability of individual micro-grains leads to the interesting behaviors (i.e. concentration and depth dependence) of the ensemble system. To understand depolarizability associated with individual grains, identical regions in SUB showing heterogeneous grains were monitored using parallel and perpendicular
polarization between pump and probe (inset in Figure 51a shows a BF image of imaged areas showing blue and red grains in SUB). When polarization between pump and probe pulses changes from parallel to perpendicular, the signal amplitude of SE (e.g. ROI3) and ESA (ROI2) decreases dramatically while another ESA signal in ROI1 shows a negligible amplitude change (Figure 51).

Figure 51 Polarization dependence of heterogeneous grains in SUB tempered in gum arabic. Images acquired at 720 / 817 nm (0.3 mW each), τ = 160 fs with (a) parallel and (b) perpendicular polarization between pump and probe. Inset is a corresponding BF image to pump-probe images. (c) Extracted pump-probe signals from three ROIs (transmission mode).
With this different degree of heterogeneous grains’ polarization dependence, we can explain signal variations depending on imaging depth. Incident beams are depolarized in the middle or bottom layers more than top layers primarily due to scattering of micro grains. Therefore, a higher degree of depolarization of the incident beam in the middle or bottom layer causes a decrease in signal amplitude of SE while ESA signals are less affected, which results in the increase of positive signals in middle and bottom layers of SUB. The concentration dependence can also be explained in the same manner. Stronger depolarization of incident beams in concentrated SUB grains causes a weaker contribution of SE signal. Therefore, the contribution of ESA signals increases in the averaged pump-probe curves, in contrast to diluted SUB grains. We have observed these behaviors mainly on SUB and not on lapis lazuli, which implies that smaller grains of SUB increase the degree of depolarization of incident pulses leading to the interesting phenomena in SUB (note that the size of SUB is around 2 µm while the size of Lapis is around 30 µm).

4.3 Non-radiative relaxation dynamics of eumelanin in hairs

Melanin is organic pigments, which have broad optical absorption and fast non-radiative relaxation behaviors of excited electron. These properties allow melanin to act as a photo-protecting layer in human skin and hair. In this subchapter, changes of non-radiative relaxation behaviors of eumelanin (brown or black melanin) in white hairs and
oxidized black hairs will be discussed using relaxation behaviors of GSD signals (note that eumelanin is brown or black melanin).

Stress from my life as a graduate student induces an exponential growth in the distribution of white hairs among my black hairs. My initial interest was focused on understanding possible nonlinear optical property changes on eumelanin in white hair. For pump-probe analysis, a pump-probe wavelength combination of 770 / 730 nm was employed (note that one former Warren group member, Kukyoun Ju, has demonstrated that 770 / 730 nm is useful to understand excited-state population dynamics of eumelanin using dominant GSD signatures). A pump-probe image of white hair in Figure 52 displays discontinuous distributions of negative signals generated by black pigments in white hairs. Extracted pump-probe responses of eumelanin in white hairs are identical to those in black hairs, which indicates that a negligible change in chemical composition of
eumelanin is associated with white hairs and melanocytes merely produce less eumelanin in white hairs.

The next study was focused on understanding the oxidation effect on non-radiative relaxation behaviors of eumelanin in hair. The use of surfactant and natural aging induce the oxidation of eumelanin in human hairs over time. For the comparison, pump-probe signals of hair root and end (i.e. tip) parts of black hairs were acquired (hair length: ~30 cm). Absolute signal amplitude is typically weaker in the hair end parts, and normalized pump-probe signals with negative minima show that GSD signals in hair end parts have longer lived GSD signals than those in the root part.

![Figure 53 Pump-probe signals of end and root parts of black hairs. 770 / 730 nm, 2 mW each.](image)

The relationship between the GSD signals and non-radiative relaxation behaviors can be explained by schematic explanation in Figure 54. In GSD process, pump-probe signals (i.e. Δtransient absorption, ΔTA) will be maximized at 0 ps because electrons excited by the pump do not relax back to ground states, which, therefore, leads to weaker
Figure 54 The schematic explanation for the relationship between GSD process and the relaxation of excited electrons to the ground states via non-radiative relaxation. (a) The electron population on excited states in the presence (left) and absence (right) of pump at $t = 0$ ps. (b) Detected probe intensity. Pump modulation transfers to probe while the nonlinear interaction occurs between two pulses. Due to the competition between pump and probe, detected probe intensity is larger in the presence of pump. This competition process maximizes $|\Delta T_A|$ at $t = 0$ ps. (c) Population changes at $t>0$ ps. Pulse duration allows pump to excite electrons without competition with probe. Process (i) and (ii) show fast and slow non-radiative relaxation of electrons, respectively. Fast non-radiative relaxation allows probe to excite more electrons on the ground state, and it induces smaller $|\Delta T_A|$ (left) than...
slow relaxation (right) as shown in (d). (e) Expected pump-probe responses of process (i) and (ii) when we plot $\Delta TA$ as a function of $\tau$.

transition from probe. This is because that pump-probe signal is proportional to the probe intensity change between two cases (i.e. when pump is on and off), the probe intensity difference will be maximized when the pump excites a larger number of electrons than the probe. At $\tau > 0$ ps, faster thermal relaxation of electrons leads to a decrease in electron population in the excited state (see process (i) in Figure 54c). It increases the number of electrons in the ground state, and the next pulse (probe) can excite more electrons than slower relaxation (ii), which results in smaller $\Delta TA$. Therefore, when we plot $\Delta TA$ as a function of $\tau$, faster non-radiative relaxation will show faster decaying curve.

Consequently, faster signal reduction in GSD of hair root parts represents faster non-radiative relaxations and slower signal decrease in GSD of hair end parts represents slower non-radiative relaxation. Slower non-radiative relaxation on the oxidized part indicates a reduction in the capability of melanin layers in hair roots to act as photoprotecting layers. In addition, the longer-lived GSD signals in hair roots imply that it has a higher potential to react with other species.
5. Microscopic mapping of Perovskite charge carrier dynamics

Surprisingly, many historical artwork pigments have been employed as core components in photovoltaic devices. One yellow inorganic pigment, CdS, has a strong preference to form n-type semiconductors\(^88-89\) and its coupling with CuS has been reported as the firstly developed thin film solar cell\(^90\). β-HgS nanoparticles (Vermilion’s degradation product in chapter 2) has also been highlighted as a promising material for a hybrid solar cell to produce charge carriers\(^91-92\). The use of red organic dyes, for example, has been reported as core materials producing photoelectrons in dye sensitized solar cells (DSSCs)\(^88, 93-96\). Therefore, understanding photo-physical properties (especially electron transition dynamics and photo-induced degradation processes) of artwork specimen is not only useful to preserve cultural heritages but also important to improve the device performance of photovoltaic materials. As we saw the instability of red organic dyes (e.g. degradation properties of Purpurin and Carminic acid) in cultural heritage objects, the instability of organic dyes induces the shifts from DSSCs to another paradigm, hybrid organic-inorganic metal halide perovskite (PVSKs), CH\(_3\)NH\(_3\)PbX\(_3\) (X = Cl, Br, and I)\(^97-98\).

PVSKs have attracted interest as next generation solar cell materials due to their potential for high power conversion efficiency and simple fabrication methods\(^97-98\). Despite the rapid development of PVSKs in the material science field, the characterization of photo-physical properties and dynamics is far from exhaustive, leaving open questions, such as the role of trap states, influence of defects, and the origin of luminescence\(^99\). It has
been reported that PVSK layers show grain-level spatial variations\textsuperscript{86}, but the role of heterogeneous grains has been controversial\textsuperscript{89}. Conventional spectroscopic analysis tools (such as transient optical absorption spectroscopy\textsuperscript{97, 100}) offer information on charge carrier dynamics but often cannot resolve the heterogeneous grains. Nano imaging tools, such as scanning electron microscopy (SEM), can resolve the spatial structure but provide little information on dynamic optical and electronic properties.

In this chapter, the use of time-resolved nonlinear optical microscopy, pump-probe microscopy, will be demonstrated to measure optical and electron dynamics in PVSKs on the femto- to pico-second timescale and sub-micron spatial scale. We investigate crystalline CH$_3$NH$_3$PbI$_3$ and CH$_3$NH$_3$PbI$_{3-x}$Cl$_x$ perovskite thin layers deposited on glass substrates and map charge carrier dynamics that result in various nonlinear contrasts such as TPA, GSD, SE, and ESA. Based on these contrasts, we can visualize the heterogeneous grains and investigate the influence of different fabrication methods on the electronics properties. I will present progress in utilizing pump-probe microscopy for mapping of charge carrier dynamics in order to explore the effects of PVSK composition, manufacturing, and aging.

5.1 Comparison between conventional analytical methods and pump-probe microscopy

For pump-probe imaging studies, we have a choice of pump and probe wavelength (with some restrictions imposed by the laser system). In order to survey appropriate wavelength combinations we performed transient absorption spectroscopy,
employing a fixed wavelength pump and a white light continuum probe. For the pump wavelength we chose 600 nm, well above the band gap of these perovskite (PVSK) samples.

Figure 55 (a) fs-TA spectrum of CH$_3$NH$_3$PbI$_3$ excited at 600 nm. Each color represents one inter-pulse time delay ($\tau$). (b) SEM image of CH$_3$NH$_3$PbI$_3$ with an inset of the pump-probe image (c) to compare structure between the two images. The pump-probe image in (c) was acquired at 600 / 770 nm (Pump / Probe, 0.1 / 0.84 mW) at $\tau = 0$ fs. (d) Extracted TA delay traces from areas indicated in image (c).

Figure 55a shows a transient absorption spectrum with probe wavelengths around the band edge, where we can observe transient gain processes from ground state depletion and stimulated emission (samples exhibit luminescence near the band edge).
has been noted that the band edge location is influenced by the perovskite’s manufacturing and environmental conditions. In order to be sensitive to spectral shifts we chose a wavelength of 770 nm, which is close to, but not centered at, the transient gain peak at the band edge for our imaging studies.

With pump / probe wavelengths of 600 nm / 770 nm we performed fs-transient absorption (TA) imaging (i.e. pump-probe imaging) in CH$_3$NH$_3$PbI$_3$. Figure 55c shows a transient absorption image at pulse overlap (inter-pulse delay of τ = 0 fs). In Figure 55b the TA image is inset on a conventional SEM image (note that all SEM images in this chapter were taken by our collaborator in Case Western Reserve University). The images do not show the same region of interest, but the structural features match well. In the TA image, we observe spatial heterogeneity: the majority of the fiber-like structures exhibit a positive TA response (transient absorption) signals at pulse overlap, while some boundaries show a negative (transient gain) response. More dynamical information can be obtained by acquiring a series of images at different inter-pulse delays (delay traces). Figure 55d shows transient absorption dynamics of the two distinct regions of interest (ROI, marked with squares in Figure 55c), along with the delay traces averaged over the entire imaging area. The positive signal in the fiber structure quickly turns into a negative (transient gain) signal with a lifetime of several hundred ps (ROI1). The minor component with the negative signal at τ = 0 fs decays with an initial decay time of about 15 ps after which a long decay behavior is recovered (ROI2). The averaged delay trace reflects the
trace one obtains from the spectroscopy setup, yet it is dominated by the major components, while the components from areas with the short decay behaviors are essentially lost in the averaging process. This microscopic TA mapping thus reveals the presence of heterogeneous grains in the PVSK layer itself, mostly invisible with a spectroscopic apparatus.

5.2 Photo-physical dynamics of optical heterogeneous grains

The inter-pulse delay accessible with our microscope is limited by the mechanical delay stage to sub-nanoseconds. In order to access longer time scales it is possible to compare TA dynamics with data obtained from fluorescence lifetime imaging microscopy (FLIM). Our FLIM system operates with an excitation wavelength of 817 nm and emission wavelengths adjustable by optical filters. In order to compare TA and FLIM data in the same region of the sample we performed TA imaging with 817 nm excitation (this forced us to use a slightly lower probe wavelength of 742 nm, which is still near the band edge). The pump energy at 817 nm is below the band edge; hence, we now probe two-photon excited charge carrier dynamics. A new generation of microscope will be able to measure both contrast types within a wider wavelength range.

Figure 56 shows images of CH₃NH₃PbI₃ layers with several contrast types of the same sample area. Note that the prominent circular feature was only included in the images to serve as a landmark for image registration and was not included during analysis. Pump and probe wavelengths were 817 nm and 742 nm, respectively. Figure 56a
Figure 56 (a) Confocal, (b) pump-probe, and (c) FLIM images of CH$_3$NH$_3$PbI$_3$ having granular structures. Imaging conditions: (a) 817 / 742 nm, (b) 817 / 742 nm at $\tau = 400$ fs, and (c) two-photon excitation at 817 nm. (d) Extracted TA spectra (from data in (c)). Scale bar for all figures: 10 µm.

shows a reflectance confocal image, in which areas of reduced backscattered light (e.g. due to higher absorption or less scattering) are colored darker. Figure 56b displays results from transient absorption microscopy, while Figure 56d shows extracted TA delay traces for two selected ROIs, along with an averaged TA delay curve. Based on the comparison of confocal and TA images, ROI 1 and 2 show planar and granular structures, respectively. Interestingly, both ROI 1 and ROI 2 contain dominant multi-photon absorption and long-
Figure 57 Power scaling results on CH$_3$NH$_3$PbI$_3$ having granular structures. (a-b) Pump-probe images acquired by using 817 / 742 nm and various power levels on the fixed FOV ($\tau = 230$ fs). Pump / probe power levels are (a) 0.3 / 0.3 mW and (b) 0.3 / 0.15 mW. (c-d) Power dependence of two ROIs marked in panel a and b: (c) ROI1' and (d) ROI2'. ROI1' and ROI2' have similar responses to ROI1 and ROI2 in Figure 56, respectively.

Lived negative signals (GSD or SE); however, only ROI 2 contains additional short-lived negative components decaying within the time scale of about 1 ps. This short-lived state might indicate the presence of an additional energy state in regions like ROI 2, and the short lifetime points towards fast non-radiate relaxation of this excited state back to the ground state (radiative relaxation typically occurs on the nanosecond, non-radiative on
the picosecond timescale (see Figure 1), more details about the nonradiative relaxation in Figure 54). TA images acquired at different power levels (Figure 57) revealed that the positive TA signals scaled linear in pump and quadratic in probe (consistent with a three-photon absorption) and the negative signals scaled quadratic in pump and linear in probe (consistent with two-photon-induced ground state bleach for short times and two-photon-excited/linearly induced stimulated emission for long times). The sign of transient absorption signals cannot differentiate between SE and GSD as the photophysical origin of the short-lived (or long-lived) negative signals. As in the directly pumped case of Figure 55, the spatially resolved TA maps reveal heterogeneous dynamics obscured in conventional TA spectroscopic studies.

Since the charge carrier dynamics measured by TA (reflecting both radiative and non-radiative dynamics) varies within the PSVK sample we also expect variations in radiative emission. A FLIM image in Figure 56b illustrates the heterogeneous distribution of fluorescence lifetime. We observe two major decay components ($\tau_1$) of different amplitudes ($A_1$) as shown in Table 3. The emission signal is fairly uniform over the entire FOV at early times; however, the long-decay component ($\tau_{1.2}$) varies significantly within the PVSK layer. Regions exhibiting a strong long-lived FLIM component also exhibit a stronger long-lived negative TA signal (as exemplified by ROI2 in Figure 56). Figure 58 shows an overlay of these two contrasts. These multimodal imaging studies illustrate that both pump-probe microscopy and FLIM can spatially map and correlate heterogeneous
Figure 58 (a) Pump-probe in Figure 56b, (b) FLIM, and (c) fluorescence intensity image of CH$_3$NH$_3$PbI$_3$ having granular structures. Imaging acquisition condition: (a) 817 / 742 nm (pump / probe, 0.6 mW each) at $\tau = 400$ fs, and (b, c) 817 nm (0.6 mW). (d) Overlaying image between panel a and b. (e) Overlaying image between panel b and c.

Table 3 Fluorescence lifetime of POI1 and POI2 in Figure 58b

<table>
<thead>
<tr>
<th></th>
<th>(\tau_{r,1}) (ps)</th>
<th>(A_{r,1}) (%)</th>
<th>(\tau_{r,2}) (ps)</th>
<th>(A_{r,2}) (%)</th>
<th>Offset</th>
</tr>
</thead>
<tbody>
<tr>
<td>POI1</td>
<td>376.1</td>
<td>90.5</td>
<td>2673</td>
<td>9.5</td>
<td>167.6</td>
</tr>
<tr>
<td>POI2</td>
<td>376.2</td>
<td>83.7</td>
<td>3129.2</td>
<td>16.3</td>
<td>273.1</td>
</tr>
</tbody>
</table>

components in PVSKs having distinctive charge-carrier relaxation behaviors. Small areas with additional energy states are apparent which exhibit fast (picoseconds) non-radiative and slow (nanosecond) radiative relaxation decays.
5.3 Effect of chloride doping and aging

Figure 59 (a) SEM images of CH$_3$NH$_3$PbI$_3$-$_x$Cl$_x$ ($x = 0, 0.33, 0.5, 0.67$, left to right). (b-e) fs-TA spectra of CH$_3$NH$_3$PbI$_3$-$_x$Cl$_x$ ($x=0, 0.33, 0.5, 0.67$) at pump 802 nm (0.5 mW). $x$ is (b) 0, (c) 0.33, (d) 0.5 and (e) 0.67, respectively.

We also utilized transient absorption microscopy to study the effects of chloride addition on the physical structure and optical properties of PVSK layers. For these studies we prepared PVSK layers having various chloride contents, i.e. CH$_3$NH$_3$PbI$_3$-$_x$Cl$_x$ ($x=0, 0.33, 0.5, 0.67$). Their SEM images (Figure 59a) indicate that the addition of chloride influences their spatial structure such as fiber thickness and density. Conventional (spatially averaged) fs-TA spectroscopy (Figure 59b) offer more insights to electronic structure and charge carrier dynamics, but do not explore the spatial heterogeneity.
Figure 60 (a) Pump-probe images of CH$_3$NH$_3$PbI$_3$ (left) and CH$_3$NH$_3$PbI$_{2.67}$Cl$_{0.33}$ (right) at 817 / 742 nm (Pump/probe, 0.15 mW each), $\tau$: 100 fs (top), 5 ps (bottom). At $\tau = 5$ ps, long-lived ESA signals (color-coded in red) can be observed in the chloride-doped PVSK. (b) Extracted pump-probe responses from panel a. ROI1 and 3 show similar dynamics to ROI2, and ROI4, respectively. ROI5 shows a distinct TA response only observed in CH$_3$NH$_3$PbI$_{2.67}$Cl$_{0.33}$.

Figure 60 shows TA results from CH$_3$NH$_3$PbI$_3$ and CH$_3$NH$_3$PbI$_{2.67}$Cl$_{0.33}$. In both samples, we see heterogeneity not only in the structure, but also in the transient absorption dynamics. The data indicates that the addition of chloride induces the formation of regions exhibiting long-lived ESA signals. In Figure 60a, pump-probe images at $\tau = 5$ ps highlight the presence of grains having positive signs in the Cl-doped samples while pump-probe images at $\tau = 100$ fs mostly offer structural images (i.e. fiber-like structure with positive TA signals and small regions with negative signals). In Figure 60b, extracted TA spectra from selected ROIs in Figure 60a represent the influence of chloride addition on charge carrier dynamics. While ROI1 and 2 in CH$_3$NH$_3$PbI$_3$ have similar decay behaviors to ROI3 and 4 in CH$_3$NH$_3$PbI$_{2.67}$Cl$_{0.33}$ respectively, ROI5 shows
unique ESA signals of CH$_3$NH$_3$PbI$_{2.67}$Cl$_{0.33}$. These decay curves and mapping results indicate that the addition of chloride leads to the generation of regions with distinct TA behaviors rather than modifying the overall electron dynamics of CH$_3$NH$_3$PbI$_3$. The gradual increase of these regions’ ESA signals over about 5 ps may indicate that excited electron states relax to another electronic excited state via non-radiative relaxation during a few picoseconds, after which the resulting population seems to slowly (hundreds of picoseconds) decay to lower energy states. Moreover, the different chloride ratio in PVSks does not seem to affect the decay behavior of these ESA signals (Figure 61). However, it might be possible that the electronic excited state producing ESA signals in chloride-containing PVSK solar cells affect their efficiency because this state enables to trap the electron over an extended period.

Figure 61 Pump-probe responses of positive grains on Cl-doped PVSks.
Figure 62 (a-b) Pump-probe images of CH$_3$NH$_3$PbI$_{2.8}$Cl$_{0.2}$ with TiO$_2$. (c) Averaged pump-probe responses of entire FOVs of two images. Panel b was acquired 5 days later than panel a and different FOVs were imaged. Imaging acquisition condition: 817 / 742 nm (0.3 mW each), $\tau = 5$ ps.

Another interesting feature of the regions exhibiting long-lived ESA signals is that they seem to undergo degradation over time scales of days. This effect is essentially obscured in conventional TA spectroscopy because in the spatially averaged decay data the small regions do not contribute substantially. The pump-probe image of CH$_3$NH$_3$PbI$_{2.8}$Cl$_{0.2}$ (this sample was deposited on a TiO$_2$ layer) in Figure 62 visualizes the distribution of grains having long-lived positive signals among dominant granular structures having negative signal. While positive regions significantly reduce after 5 days
as shown in Figure 62b, the averaged TA spectrum in Figure 62c shows only negligible changes. This observation indicates that ESA components in chloride containing PVSks are degradable within a week and pump-probe microscopy may help understand photo-
physics of these components.
6. Conclusion

In chapter 2, I have showed that pump-probe microscopy can nondestructively visualize the distribution of α-, β-HgS, and liquid Hg on the microscopic scale. Nonlinear absorption generated from TPA induces the irreversible phase shift of α- to β-HgS under fs-laser illumination. The rate of the phase conversion is enhanced by a local heating from the pre-existing β-HgS due to its linear absorption. Furthermore, UV exposure produces both β-HgS and metallic Hg, and that elevated temperatures accelerate this process. The formation of long-lived ESA signals was observed during the degradation process under fs-laser and UV exposure. We attribute these ESA signals to an intermediate species generated from either chemical or physical changes. While Cl-assisted Hg production has clearly been established as a degradation pathway in Vermilion, our studies point to HgS phase conversion as an alternate degradation pathway in the absence of Cl. Lastly, analysis of a 14th century painting shows the increase of β-HgS in the discolored Vermilion. We expect the detection of ESA signals in visibly non-degraded vermilion layers could indicate the presence of early-stage degradation of Vermilion.

In chapter 3, I have demonstrated that time-resolved pump-probe spectroscopy can be used to differentiate various red organic pigments (ROPs, e.g. Carminic acid, Carmine, Lad dye, Alizarin, Purpurin, Madder lake, Eosin Y). To differentiate ROPs based on their intrinsic photo-physical dynamics, the ability to control multiple parameters (a wavelength combination of pump and probe, and time delay) is important. Pump-probe
3D mapping analyses show its ability to visualize spatial distribution of mixtures of ROPs in 3D without the need of sample removal. Furthermore, I have demonstrated its capability to resolve heterogeneous components in pigments resulting from variations in crystallinity on the grain level or the formation of metal—dye complex. Finally, I have addressed another potential application of pump-probe microscopy as a detection tool to characterize the initiation of the chemical breakdown of Carmine under NIR illumination.

Beyond imaging red inorganic and organic pigments in cultural heritage objects, in chapter 4, I have highlighted that pump-probe contrast could also prove helpful in studying other types of colorants such as modern automotive paints, ultramarine blue pigments, and melanin in hair. In chapter 4.1, pump-probe imaging studies on a series of red organic automotive paints show the capability of pump-probe to differentiate other types of pigments having similar linear reflectance spectra in the non-destructive analysis. Imaging study results on automotive paints collected from vehicles involved in a hit-and-run accident demonstrate the capability of pump-probe microscopy in the forensic analysis of pigmented samples as well. In chapter 4.2, pump-probe analyses reveal that Lapis lazuli and synthetic ultramarine blue have heterogeneous grains which may originate from the presence of other sulfur radical anions and natural impurities. Furthermore, pump-probe study results on individual micrograins emphasize the importance of microscopic analyses to understand various phenomena such as depth and concentration dependence of the ensemble systems. In chapter 4.3, pump-probe analyses
show that eumelanin in white and black hairs have identical pump-probe signals. Lastly, slower non-radiative relaxations of melanin in hair end parts indicates a reduction in the capability of melanin layers to act as photo-protecting layers.

In chapter 5, I have demonstrated that time-resolved nonlinear optical microscopy, pump-probe microscopy, can measure optical and electron dynamics in organic-inorganic hybrid perovskite (PVSK) solar cells on the femto- to pico-second timescale and sub-micron spatial scale. The relaxation of charge carriers via radiative and non-radiative decay can be monitored using both pump-probe microscopy and FLIM with the observable time window from femto- to nano-second. I have confirmed the presence of heterogeneous components which optically behave differently using pump-probe microscopy and FLIM. Furthermore, I have demonstrated that these variations in grain levels can result from different fabrication methods, chloride doping, and aging, and that pump-probe microscopy can observe these behaviors non-destructively.
Appendix

1. Apparatus

Fluorescence lifetime imaging microscopy (Figure 63): A Ti:sapphire laser (Chameleon, coherent) was used for two-photon excitation and photons were detected using a time correlated single photon counting module (PMC-100-4, Becker & Hickl GmbH).

![Fluorescence lifetime imaging microscopy set-up](image)

**Figure 63 Fluorescence lifetime imaging microscopy set-up**

Linear reflectance and absorption spectra: A Cary 5000 spectrometer was used for the characterization.

X-ray Diffraction Analysis: X-ray diffractograms were acquired with a Panalytical X'Pert Pro MRD HR XRD system with a 1/2 degree slit, a step size of 0.05°, an acquisition time per step of 1 s, and Cu-Ka radiation (generator voltage: 45 kV, tube current: 40 mA).
Scanning electron microscopy-electron dispersive X-ray spectroscopy: A FEI XL30 ESEM with Bruker XFlash 4010 EDS detector was used for the characterization (primary voltage: 20 keV, working distance: 15 mm).

UV light source: A X-Cite 120 (Hg Arc lamp, 120 W) fluorescence illuminator was used as UV source.

2. Degradation products of Vermilion

Chemical sources: Mercury (II) sulfide red (Sigma Aldrich), mercury sulfide black (Alfa Aesar), liquid mercury (Sigma Aldrich). All chemicals were used without further purification steps.

Detection mode for pump-probe signals: All pump-probe signals in chapter 2 were collected by using epi-mode.

2.1 Preparation of red HgS, black HgS, and liquid Hg

For pump-probe imaging studies in Figure 8: In order to reduce the chance for laser-induced degradation on red and black HgS, the powder was embedded in agarose gel (the gel enhances the heat diffusion). Firstly, powder was placed on the glass slide. 1 g of agarose powder was mixed with 10 mL of water and heated at mild temperate until the mixture turned transparent. Then, the transparent solution was applied onto the powder and after about 30 seconds, the solution turned to gel. Then, the sample was covered with a cover slip.
For imaging studies in Figure 8: Liquid Hg was transferred on a glass slide and then covered with a coverslip. The edge of the coverslip was sealed with glue.

For XRD and SEM-EDS analysis in Figure 14: Double-side tape was placed on a glass slide (size: 2 x 2 cm), then red or black HgS powder was placed on the tape.

### 2.2 fs-laser induced degradation products of red HgS

For pump-probe imaging studies in Figure 11: Red HgS powder was placed between a glass slide and a coverslip and then placed in the pump-probe microscope. The imaging conditions were: pump/probe 720 / 817 nm, 256 x 256 pixels, dwell time: 20 μs, number of τ points: 56 (from -8 to 52 ps). Figure 11a was acquired at 0.45 / 0.45 mW, then one set of pump-probe images was acquired at 0.45 / 0.9 mW. After slight phase conversion was observed, the pump/probe power was readjusted to the original power levels (0.45 / 0.45 mW) in order to monitor the surface changes (Figure 11b). Then, two sets of pump-probe images were acquired at 0.9 / 0.45 mW and 0.9 / 0.9 mW to induce more severe degradation. Figure 11c was subsequently acquired at 0.45 / 0.45 mW.

For XRD and SEM-EDS analysis in Figure 14: Red HgS powder was placed between a glass slide and a cover slip; the edges were sealed with glue. The glass slide was placed in the pump-probe microscope, and the surface of red HgS was scanned at a very high power to induce damage: pump / probe (720 / 817 nm, 2.3 / 5 mW, τ = 0 fs), image FOV: 350 x 350 μm, 128 x 128 pixels, pixel dwell time: 20 μs. Once the conversion
(negative pump-probe signal) was observed, the sample was translated in the x- or y-direction by one FOV (350 \( \mu \)m).

### 2.3 UV induced degradation of red HgS

UV light delivered through a fiber bundle to red HgS powders on a microscope slide in Figure 17: Red HgS powder (50 mg) was placed on a glass slide and the surface was flattened. UV light from a Hg lamp was exposed on the powder (UV exposure intensity: \( 4.06 \times 10^4 \) mW/cm\(^2\)). After exposure, the sample was transferred to the microscope.

UV light delivered through the optical system in the microscope in Figure 18: Red HgS powder (50 mg) was placed on the glass slide and the surface was flattened. UV light was exposed onto the surface (UV exposure intensity: \( 1.14 \times 10^4 \) mW/cm\(^2\)) and pump-probe signal changes were recorded over time.

UV light delivered through a fiber bundle to red HgS powders in a glass vial in Figure 20, Figure 21, and Figure 22: Red HgS powder (0.5 g) was placed in a 20 mL glass vial and the vial was covered with white paper. UV light from a Hg lamp was used to expose the powder for 5 min (UV exposure intensity: \( 6.55 \times 10^4 \) mW/cm\(^2\)).

### 2.4 Damage threshold fluence

The damage threshold for illumination in Figure 11 was experimentally determined to be 0.45 mW for the pump and probe beam when repeatedly scanning over the sample (the pulse delays were scanned). For the probe beam, an average power of 0.45
mW corresponds to a pulse energy of 5.6 pJ. Focusing the 150 fs pulse to a 400 nm radius beam in the microscope results in a temporal peak intensity of 7.5 GW/cm² and an average intensity of 90 kW/cm². For a stationary beam this would correspond to a fluence of 1.1 mJ/cm², but because of the beam scanning the average fluence is 17 nJ/cm². For the modulated pump beam, the average fluence is the same while the peak fluence is roughly doubled.

3. Red organic pigments

Pigment sources: Carmine naccarat (42100), Lac dye (36020), and Madder lake (372141) were purchased from Kremer Pigments. Purpurin, Alizarin, and Eosin Y were purchased from Acros. Carmine and Carminic acid were purchased from Sigma Aldrich and TCI, respectively. All pigments were used without further purification steps.

Detection mode for pump-probe signals: All pump-probe signals in chapter 3.1 to 3.3 were collected by using epi-mode and the signals in chapter 3.4 were collected using transmission mode.

3.1 Preparation of reference samples

Preparation of powder samples in Figure 27: Pigment powder was placed between a glass slide and a cover slip.

Preparation of Purpurin samples in Figure 27: Agarose (0.50 g) was added into DI water (15 mL) in a 20 mL glass vial, then the mixture was heated at 50°C for 10 min. Purpurin powder (40 mg) was tempered into 1 mL agarose solution, then the mixture was
applied onto the glass slide and cooled at room temperature. After a gel was formed, a cover slip was placed on top to prevent dehydration.

### 3.2 Synthesis of Alizarin and Purpurin laked pigments

Preparation of Alizarin lake in Figure 33: K$_2$CO$_3$ (1.51 g, 10.95 mmol) and DI water (50 mL) were added into a 50mL conical tube. In another a 50 mL conical tube, alum (AlK(SO$_4$)$_2$·12H$_2$O, 3.04 g, 6.40 mmol) and DI water (50 mL) were added. Purpurin (0.30 g, 1.25 mmol) and a stir bar were added into a 125 mL Erlenmeyer flask and 20 mL of the alkaline solution (2.19 x 10$^{-4}$ M of K$_2$CO$_3$) was added. When the alkaline solution was added into alizarin powder, reaction mixture immediately turned to purple color. The reaction mixture was mixed for 10 min at 22°C. Then, 20 mL of alum solution (AlK(SO$_4$)$_2$·12H$_2$O, 2.56 mmol) was added into the reaction mixture for 1 hour at 22°C. Note that when the alum solution was added into the reaction mixture, the solution immediately turned to red color from purple. The reaction mixture was filtrated using a Buchner funnel and feed was washed with DI water until it showed clear filtrate. Red powders were obtained.

Preparation of Purpurin lake in Figure 33, Figure 34: K$_2$CO$_3$ (1.51 g, 10.95 mmol) and DI water (50 mL) were added into a 50mL conical tube. In another a 50 mL conical tube, alum (AlK(SO$_4$)$_2$·12H$_2$O, 3.04 g, 6.40 mmol) and DI water (50 mL) were added. Purpurin (0.30 g, 1.17 mmol) and a stir bar were added into a 125 mL Erlenmeyer flask and 20 mL of the alkaline solution (2.19 x 10$^{-4}$ M of K$_2$CO$_3$) was added. The reaction
mixture was mixed for 13 min at 22°C. Then, 30 mL of alum solution (AlK(SO₄)₂·12H₂O, 3.84 mmol) was added into the reaction mixture for 25 min at 22°C. The reaction mixture was filtrated using a Buchner funnel and feed was washed with DI water until it showed clear filtrate. Red powders were obtained.

3.3 Mock-up samples

Pump-probe imaging studies in Figure 35 and Figure 36: Carmine naccarat and Madder lake powder were mixed with a Carmine weight fraction of 8% (sample A), 38% (sample B), and 77% (sample C). Binder was prepared using Gum arabic (3 g) and DI water (45 ml). Power samples (0.5 g) and gum arabic solution (1 ml) were then mixed using a glass mortar and pestle. The mixed samples were applied to the canvas with a brush.

3.4 Phasor analysis

Phasor analysis is a computationally simple technique, which maps the qualitative behavior of the pump-probe dynamics onto a two-dimensional plot by calculating the two quadrature components, g(ω) and s(ω), of a single-frequency Fourier transform (4, 5).\textsuperscript{58-59} Note that ω is a given frequency (0.25 THz is used for pump-probe signals decaying within subhundred picoseconds for effective Fourier transformation) and I(τ) is a pump-probe signal at a given inter-pulse time delay (τ). g(ω) and s(ω) are real and imaginary parts of single-Fourier transformation of signals, respectively, and they are normalized by the absolute value of I(τ).
\[ g(\omega) = \frac{\int I(\tau) \cos(\omega \tau) d\tau}{\int |I(\tau)| d\tau} \]  \hspace{1cm} (4)
\[ s(\omega) = \frac{\int I(\tau) \sin(\omega \tau) d\tau}{\int |I(\tau)| d\tau} \]  \hspace{1cm} (5)

An example of such analysis is shown in Figure 28 for Carmine naccarat and Lac dye. The left panel shows phasor histograms for images acquired at two wavelength combinations. Note that dashed lines in phasor plots indicate pump-probe signals having a single-exponential decay. For the phasor histogram every pixel above a set threshold is accumulated at the location of the phasor coordinates corresponding to the pixel's pump-probe behavior. The phasor images depict color coded images corresponding to specific regions in the phasor plot (the colors match the regions in the phasor plots).

4. Other types of colorants

Detection mode for pump-probe signals: All pump-probe signals in chapter 4.1 and 4.3 were collected by using epi-mode and the signals in chapter 4.2 were collected using epi or transmission mode (see captions of individual figures).

4.1 Auto paints

For pump-probe studies in Figure 41: Three touch-up paints, matched to color R-513 Ralley Red of a 2009 Honda Civic, were purchased from different suppliers: Dupli color (paint 1), Auto Paint Depot (paint 2), and ERA paints (paint 3). These paints were applied onto a glass slide then completely dried before conducting imaging studies.
For pump-probe studies in Figure 42 and Figure 43: Paint flecks were collected from two vehicles involved in a hit-and-run car accident. A Honda Accord (Vehicle A) and a Ford escape (Vehicle B).

4.2 Ultramarine blue pigments

Chemical sources: Synthetic ultramarine blue, dark (45010), synthetic ultramarine red (42601), and Lapis lazuli (10530) were purchased from Kremer pigments.

Preparation of ultramarine pigments: All ultramarine pigments were prepared by tempering them in gum Arabic solution (Gum arabic (1 g) and DI water (10 ml)), then, completely dried mixtures were used for the studies.

4.3 Melanin

Sample sources: Hair samples were collected from Jin Yu in 2017.

5. Perovskite

Sample sources: All PVSK samples were fabricated by a collaborator (Charles Kolodziej) in Case Western Reserve University, OH. After the fabrication, the samples were sealed using epoxy glue and covered with a cover slip and shipped to Duke University, NC.

Detection mode for pump-probe signals: All pump-probe signals were collected by using transmission mode.
References


contribution of chemical analyses and imaging to the assessment of color changes in the red lake pigments. *Heritage Science* 2017, 5 (1).


43. Cucci, C.; Delaney, J. K.; Picollo, M., Reflectance hyperspectral imaging for investigation of works of art: old master paintings and illuminated manuscripts. *Accounts of chemical research* 2016, 49 (10), 2070-2079.


46. Miliani, C.; Romani, A.; Favaro, G. J. J. o. P. O. C., Acidichromic effects in 1, 2-di- and 1, 2, 4-tri-hydroxyanthraquinones. A spectrophotometric and fluorimetric study. 2000, 13 (3), 141-150.


71. Gomez, M.; Reggio, D.; Lazzari, M., Linseed oil as a model system for surface enhanced Raman spectroscopy detection of degradation products in artworks. 0 (0).


Biography

Jin Yu received her B.S. degree in 2009 and her M.S. degree in 2011, both in Chemistry from Ewha Womans University, South Korea, with a research focus on organic synthesis. Pursuing a doctorate in Chemistry, she began her graduate studies at Duke University in 2013. She initially worked with Prof. Warren Warren on the synthesis of long-lived $^{13}$C-labeled magnetic resonance imaging agents. She is currently working with Prof. Warren Warren and Prof. Martin Fischer on applying nonlinear optical microscopy to the investigation of heterogeneous components in various materials such as artwork pigments and semiconducting materials. Jin’s research interest focuses on molecular imaging and molecular spectroscopy using the intrinsic photo-physical property of molecule itself. She was recognized for her graduate work at Duke, receiving the Kathleen-Zielek fellowships. She also presented her researches on numerous occasions, including at the Gordon Research Conference, Material Research Society meeting, and Optical Society of America incubator meeting. During her doctoral degree, she published three manuscripts; Yu, Jin, Warren S. Warren, and Martin C. Fischer. “Spectroscopic Differentiation and Microscopic Imaging of Red Organic Pigments Using Optical Pump–Probe Contrast.” *Analytical Chemistry* 90, no. 21 (2018): 12686-12691. Zhou, Zijian, Jin Yu, Johannes FP Colell, Raul Laasner, Angus Logan, Danila A. Barskiy, Roman V. Shchepin et al. “Long-lived $^{13}$C$_2$ nuclear spin states hyperpolarized by parahydrogen in reversible exchange at microtesla fields.” *The Journal of Physical Chemistry Letters* 8, no. 13 (2017): 3008-