NDnano Summer Undergraduate Research
2016 Project Summary

1. Student name: Thomas Clarke

2. Faculty mentor name: Dr. Zachary Schultz

3. Project title: Identifying Nanostructure in Medieval Manuscripts

4. Briefly describe any new skills you acquired during your summer research:

During my 10 weeks, I have learned how to use different spectroscopic analytical techniques, including Raman spectroscopy, hyperspectral brightfield reflectance spectroscopy, and darkfield scattering spectroscopy. I also helped reconstruct a Raman microscope, which allowed me to better understand how that instrument works to successfully measure the intensity of Raman shifts.

5. Briefly share a practical application/end use of your research:

My research has shown that darkfield hyperspectral imaging and reflectance spectroscopy can be a good indicator of certain types of pigments that are present on manuscripts. Instead of taking spontaneous Raman spectra at random locations in search of signal, researchers with darkfield imaging capabilities can locate higher relative concentrations of certain pigments to examine. This will decrease the amount of time that the manuscripts need to be exposed to laser radiation.

Begin two-paragraph project summary here (~ one type-written page) to describe problem and project goal and your activities / results:

Medieval illuminated manuscripts are adorned with various dyes and pigments that contain information about the artifact's history and the techniques used in its creation. For example, identification of chemical species can often indicate the earliest possible date that a piece was painted.[1] Understanding the link between pigment nanostructure and their observable characteristics is also a valuable endeavor since it can suggest how to most accurately restore illuminated artifacts. There are currently several nondestructive methods being used to identify the various pigments and dyes present in an artifact. One method that is gaining popularity is Raman spectroscopy. Raman spectroscopy measures the amount of inelastic scattering resulting from a monochromatic beam that is directed at an analyte. Upon interaction with the analyte, the laser radiation often excites an electron from the ground state of the analyte molecule to a virtual state. A small amount of scattered light with a change in frequency is observed resulting from the electron's relaxation to a different vibrational state. Because each molecular species has a unique set of vibrational states, the Raman spectrum is unique to the species and can be used for

identification. However, finding areas with pigments that demonstrate good Raman scattering - and at sufficient concentration - can be difficult. Brightfield microscopy, imaging, or reflectance spectroscopy is often used in conjunction with Raman spectroscopy because it can show contrasts between many different colored pigments by detecting the amount of reflected and scattered light from an analyte; however, it has sometimes proven to be difficult to use these techniques to distinguish different pigments in mixtures. Since prolonged exposure to or high intensity of laser radiation can cause degradation[2], it would be useful to have a more effective means of identifying areas of high concentrations of Raman-active pigments in mixtures to limit the necessary radiation exposure in acquiring Raman spectra.

Thanks to the Snite Art Museum of the University of Notre Dame, non-destructive spectroscopic techniques were allowed to be taken on a 15th century manuscript. Using Raman spectroscopy, several different pigments were characterized, including: azurite, lead tin yellow type I, and vermillion. The Raman spectra of these three pigments from the manuscript are shown in Figure 1. However, much of my work this summer seems to suggest that darkfield microscopy, imaging, and scattering spectroscopy can often be a better indicator of pigments and dyes on a manuscript, especially in mixtures. Whereas brightfield microscopy observes the reflected and scattered light together from an analyte, darkfield microscopy only detects the scattered light. A stark contrast between these two techniques was seen in a region containing lead tin yellow type I. Normalized hyperspectral brightfield reflectance spectra and darkfield scattering spectra were taken in this region under the same white light intensity, as seen in Figure 2. Twenty-six individual locations were then analyzed for their brightfield and darkfield intensities at wavelengths in the visible region. Raman spectra were then obtained from these same 26 locations in this region, each using a 633 nm laser at 0.053 mW. It became evident that the Raman spectra in the more yellow regions of the darkfield image have more intense peaks corresponding to lead tin yellow type I. To show this, the area under the brightfield, darkfield, and (brightfield – darkfield)/(brightfield + darkfield) spectra was obtained between 570 and 590 nm. This estimates the intensity of the detected reflected + scattered, pure scattered, and pure reflected yellow light respectively. These intensities of detected yellow light at each location were then plotted against the peak area of the corresponding Raman spectra for the lead tin yellow type I peak at 118 cm^{-1}. As seen in Figure 2, the results show a relatively strong correlation between the pure scattered yellow intensity and the Raman intensity, a weaker correlation for the scattered + reflected yellow intensity and Raman intensity, and little correlation for the pure reflected yellow intensity and Raman intensity. This potentially shows that darkfield imaging and scattering spectra can identify areas of higher concentration better than corresponding brightfield techniques. Further investigation is being conducted to examine other pigments on the manuscript as well as determine what aspects of these pigments' nanostructures cause the observed differences in Raman intensity.
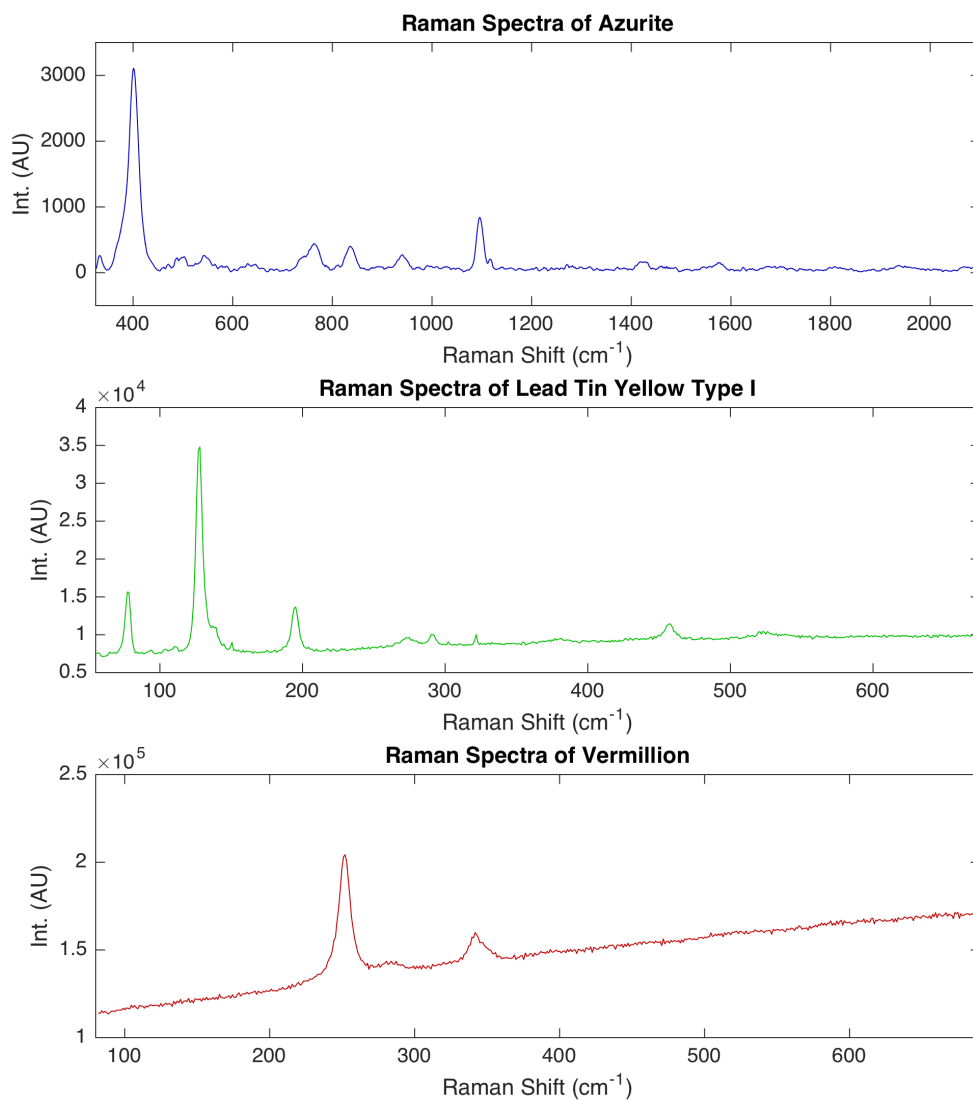
Figure 1: The Raman Spectra of Azurite (using 532 nm laser, 0.3 mW), Lead Tin Yellow Type I (using 633 nm laser, 0.053 mW), and Vermillion (using 633 nm laser, 0.053 mW).
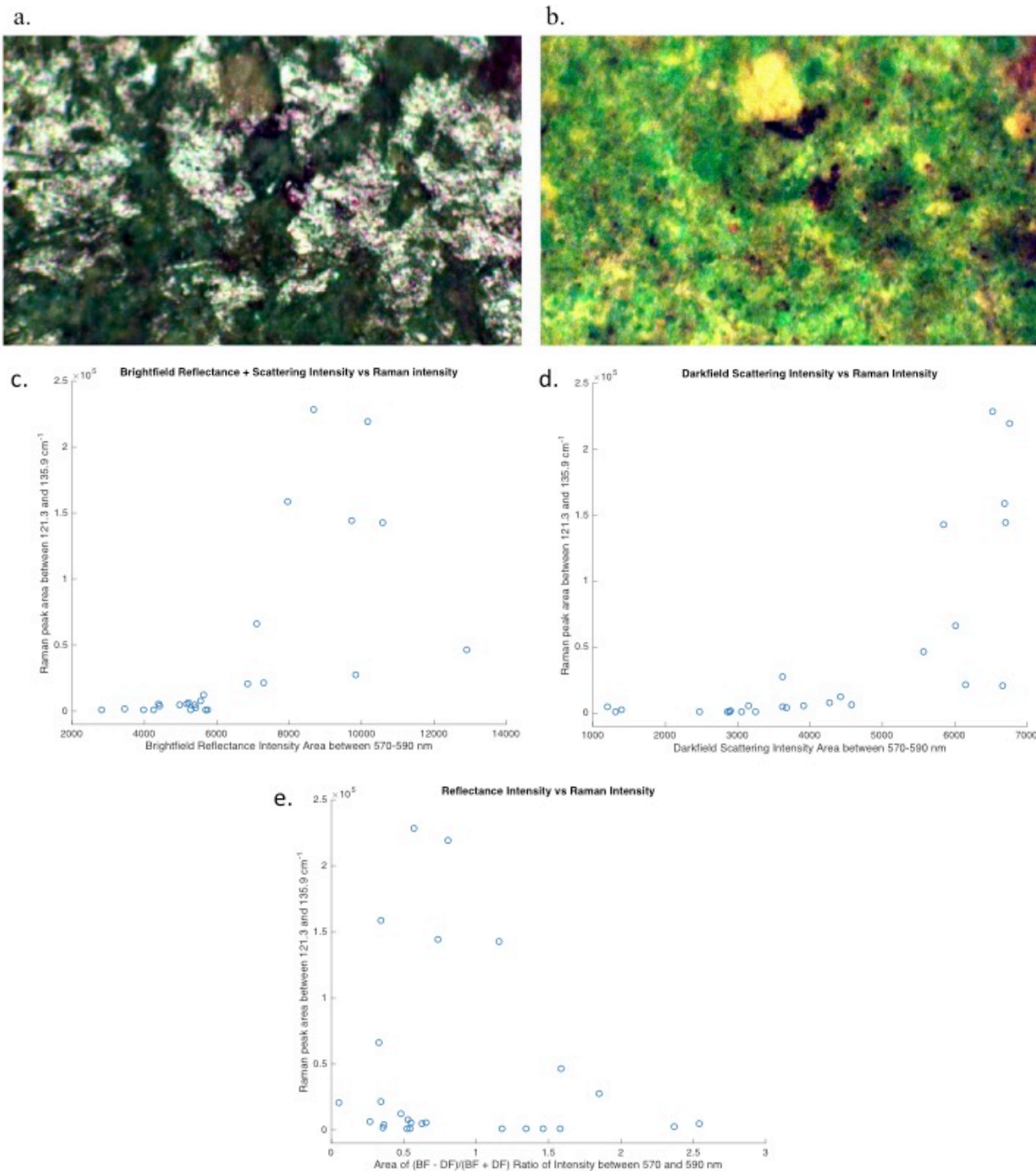
Figure 2: a. Brightfield image of region containing lead tin yellow type I.   b. Darkfield image of region containing lead tin yellow type I.   c. Scatter plot of brightfield intensity vs. Raman intensity.   d. Scatter plot of darkfield scattering intensity vs. Raman intensity.   e. Scatter plot of pure reflectance intensity vs. Raman intensity

References:

[1] Burgio, L.; Ciomartan, D. A.; Clark, R. J. H. Spectroscopy of inorganic materials Pigment identification on medieval manuscripts, paintings and other artefacts by Raman microscopy: applications to the study of three German manuscripts. *J. Mol. Struct.* **1997***, 405*, 1-11.

[2] Bertrand, L.; Schoeeder, S.; Anglos, D.; Breese, M. B. H.; Janssens, K.; Moini, M.; Simon, A. Mitigation strategies for radiation damage in the analysis of ancient materials. *Trac-Trends Anal. Chem.* **2015***, 66*, 128-145.