Assessing Accuracy of Surface Enhanced Raman Imaging

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Introduction

- Together with the diverse team of the Zavaleta Lab but more specifically with my PhD mentor, Alexander, we studied Surface Enhanced Raman Spectroscopy (SERS) particles
- Specifically studying the unique intensifying effects of gold nanoparticles on the Raman spectra of different molecules and objects
- We aim to use Raman spectroscopy in order to streamline the process of staining for many cancer biomarkers and eventually tailor a patients cancer treatment to their precise cancer configuration

Objective, Impact, and Goal of Mentor's Research

The Zavaleta Lab utilizes nanomedicine with the help of optical imaging to help surgeons better identify margins for tumor resection and even improve early cancer detection.

Raman Spectroscopy

- Type of optical imaging which targets a laser beam at molecules causing for new molecular vibrations in their excited state
- Spectra are collected when energized, inelastically scattered particles bounce back up into the lens creating distinctly unique spectra shared only by that molecule
- Can be used to show surgeons where healthy tissue ends and a malignancy begins as well as to help pathologists stain tissue samples with greater efficiency
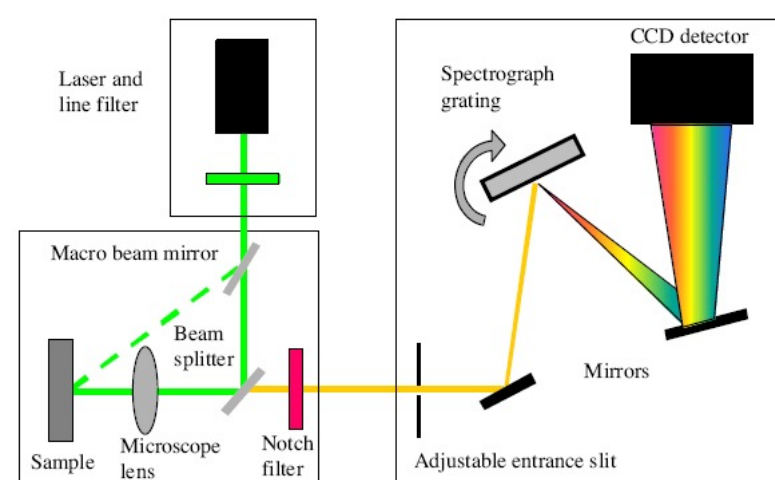
Figure 1: example of the function of a Raman Spectrometer Source: www.sas.upenn.edu

Methods and Techniques Used

SERS Nanoparticles Limit of Detection

- Performed a 2x dilution series across 13 tubes of BMMBP gold nanoparticles, until their spectra were no longer detected
- 200 microliters (μL) of pure Milli-Q water used as a control in each well-plate
- First well consisted of purely BMMBP gold nanoparticles, 100mL were then pipetted into the second well, this process was repeated 13 times
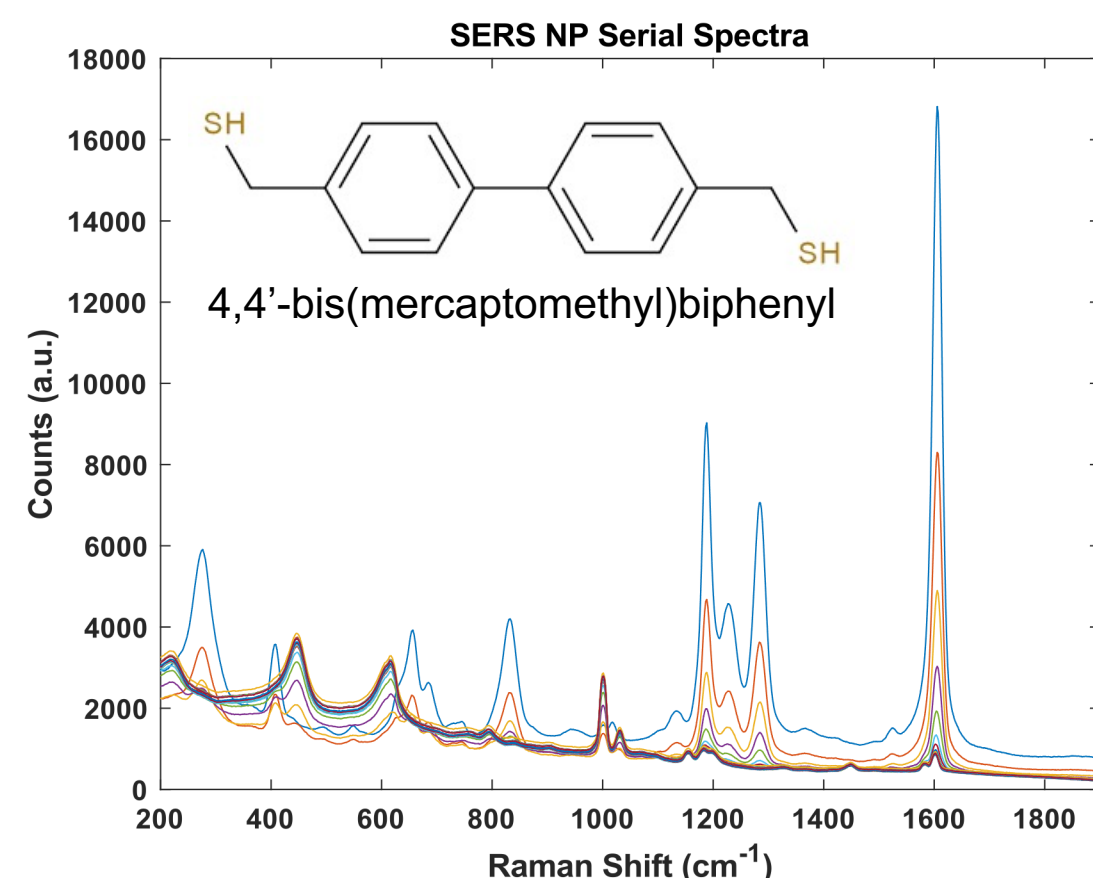
SERS NP Serial Spectra

4,4'-bis(mercaptomethyl)biphenyl

Figure 2: SERS Nanoparticles spectra of BMMBP during dilutions PC: Alex

- As seen in figure 1, the same unique Raman spectra of BMMBP is seen throughout the experiment, getting continuously less defined

Linearity of 1600 cm-1 Raman Band

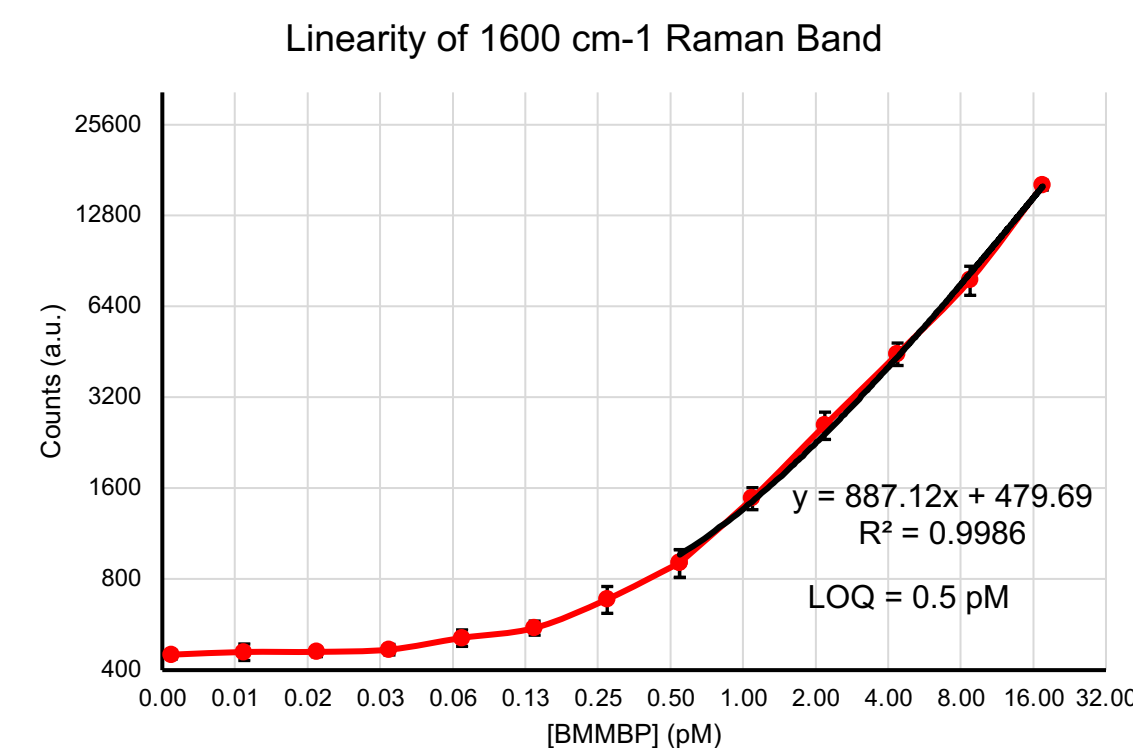$y = 887.12x + 479.69$
$R^2 = 0.9986$
LOQ = 0.5 pM

Figure 3: Linear trend seen in intensity of gold nanoparticles until they plateau and the signatures become unreadable PC: Gabriella and Alex

Results and Discussion

Demultiplexing Raman Spectra

- Demultiplexing: a process of trial and error can be used to work backwards in distinguishing different dyes using the Direct Classical Least Squares Algorithm
- Dilutions allow for one to find the range of linearity needed for demultiplexing
- This relies on the idea that the spectra of each dye used will be unique and linear
- By using the ideal concentration of Milli-Q water to nanoparticles, one can then stain a singular tissue sample with many dyes to identify biomarkers and demultiplex the image in order to detect each stain
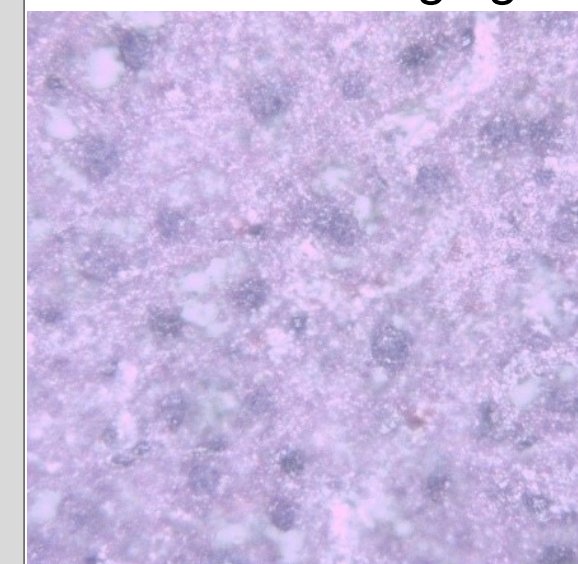- Future studies may include using differently designed Si phantoms to measure imaging resolution

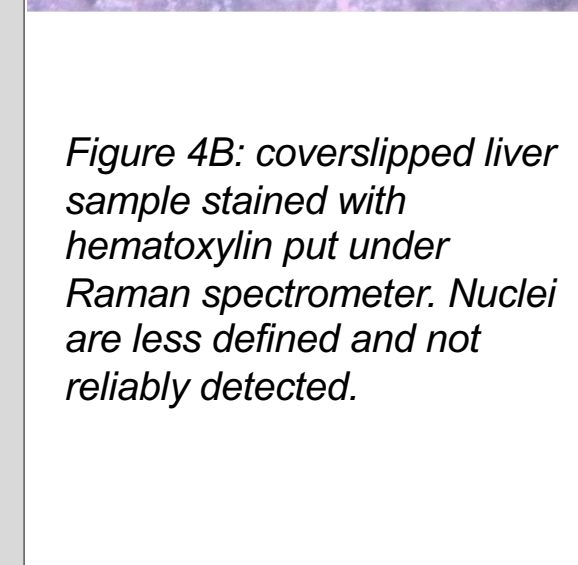*Figure 4A: Hematoxylin stained liver sample under bright light microscope*

Figure 4B: coverslipped liver sample stained with hematoxylin put under Raman spectrometer. Nuclei are less defined and not reliably detected.
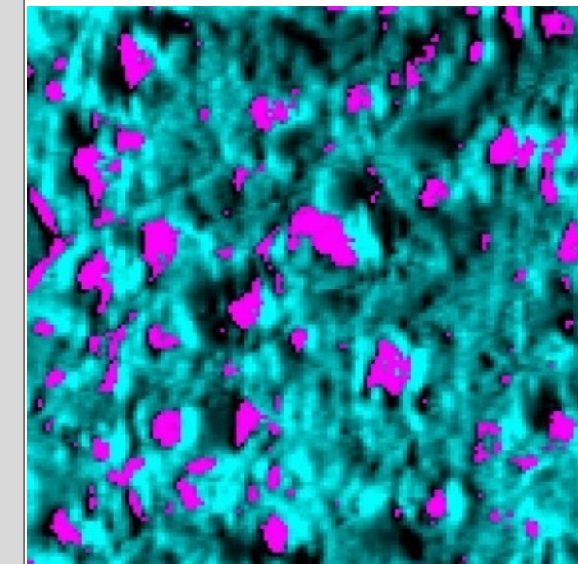
Figure 4C: uncoverslipped liver sample stained with hematoxylin put under Raman spectrometer. Nuclei are well defined and correlated with bright light image. PC: Alex
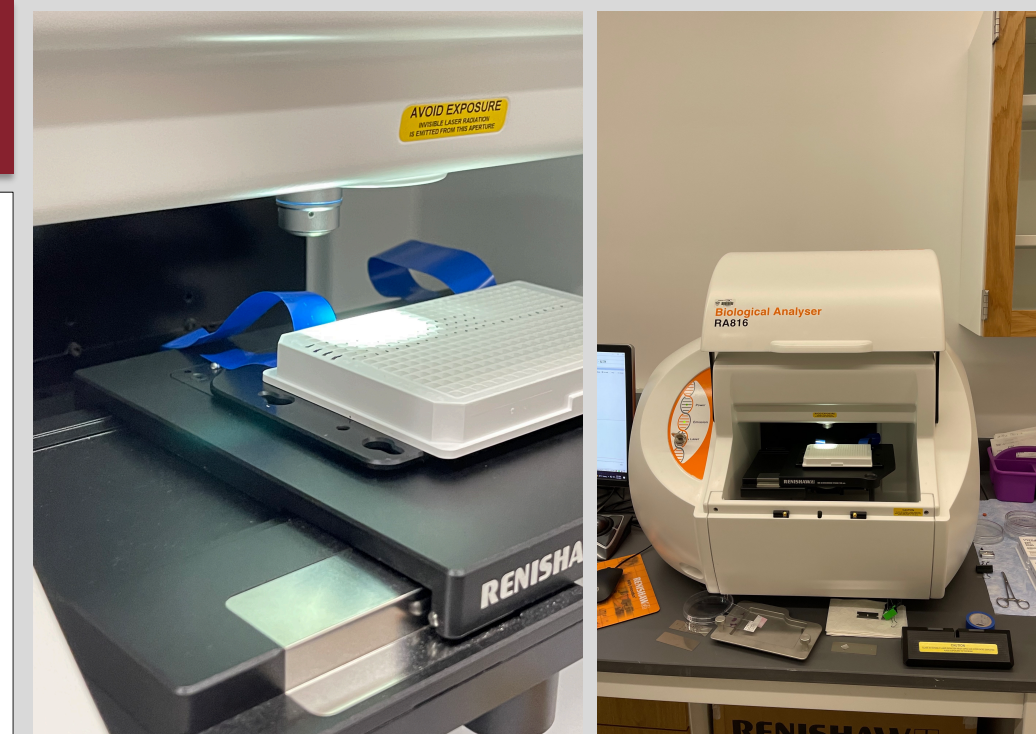
Figure 5: Raman Spectrometer PC: Gabriella

References

1. Zavaleta, C. L.; Kircher, M. F.; Gambhir, S. S., Raman's "effect" on molecular imaging. J Nucl Med 2011, 52 (12), 1839-44.
2. Davis, R. M.; Campbell, J. L.; Burkitt, S.; Qiu, Z.; Kang, S.; Mehraein, M.; Miyasato, D.; Salinas, H.; Liu, J. T. C.; Zavaleta, C., A Raman Imaging Approach Using CD47 Antibody-Labeled SERS Nanoparticles for Identifying Breast Cancer and Its Potential to Guide Surgical Resection. Nanomaterials (Basel) 2018, 8 (11).
3. Zavaleta, C. L.; Smith, B. R.; Walton, I.; Doering, W.; Davis, G.; Shojaei, B.; Natan, M. J.; Gambhir, S. S., Multiplexed imaging of surface enhanced Raman scattering nanotags in living mice using noninvasive Raman spectroscopy. Proc Natl Acad Sci U S A 2009, 106 (32), 13511-6.

Acknowledgements