Research Report: Investigating Cy5 Dye Emission Enhancement with Plasmonic Nanoantennas using Point Accumulation for Imaging in Nanoscale Topography (PAINT) Technique

I. Introduction

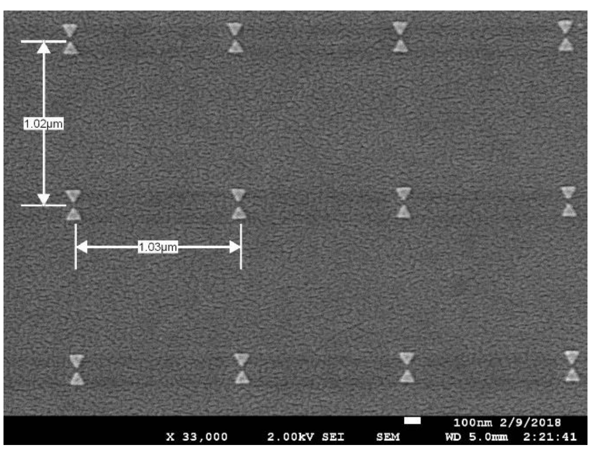
Super resolution microscopy has revolutionized imaging by surpassing the diffraction limit of conventional optical microscopy. Throughout this semester-long research endeavor, my primary focus was to investigate the enhancement of Cy5 dye molecule emission when combined with plasmonic nanoantennas using a modified version of the Point Accumulation for Imaging in Nanoscale Topography (PAINT) technique [1,2].

The main objective was to analyze the improvement in plasmonic nanoantenna electric field strength following computational optimization, contrasting it with conventional bowtie antennas. This heightened electric field strength would influence the radiative rate of the dye molecules used as probes in these experiments. Understanding this enhancement is critical for advancing more sensitive and higher resolution imaging techniques, providing insights into the geometric parameters that influence electric field enhancement, and enabling tailored antenna design.

II. Experimental Setup

To achieve this objective, I engaged in several experimental procedures:

Nanoantenna Characterization: Fabrication of the nanoantenna was followed by detailed characterization to confirm their optical properties. Specifically, SEM images we taken to confirm nanoantenna geometry and scattering spectra were collected to determine the antennas plasmon resonance as shown in Figures 1 and 2. Knowing the plasmon resonance of the structures allows us to design experiments that accurately compare enhancement due to coupling which is a function of spectral overlap between the emitter and the antenna.



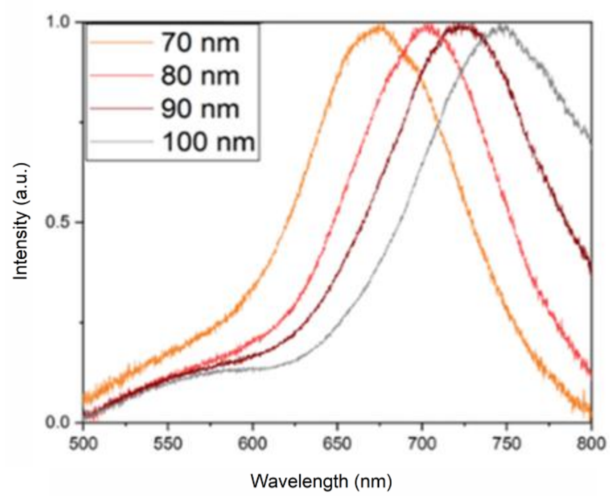


Figure 1. SEM image of Bowtie Nanoantenna arrays (LHS), Dark Field Scattering Spectra for representative bowtie side lengths (RHS).

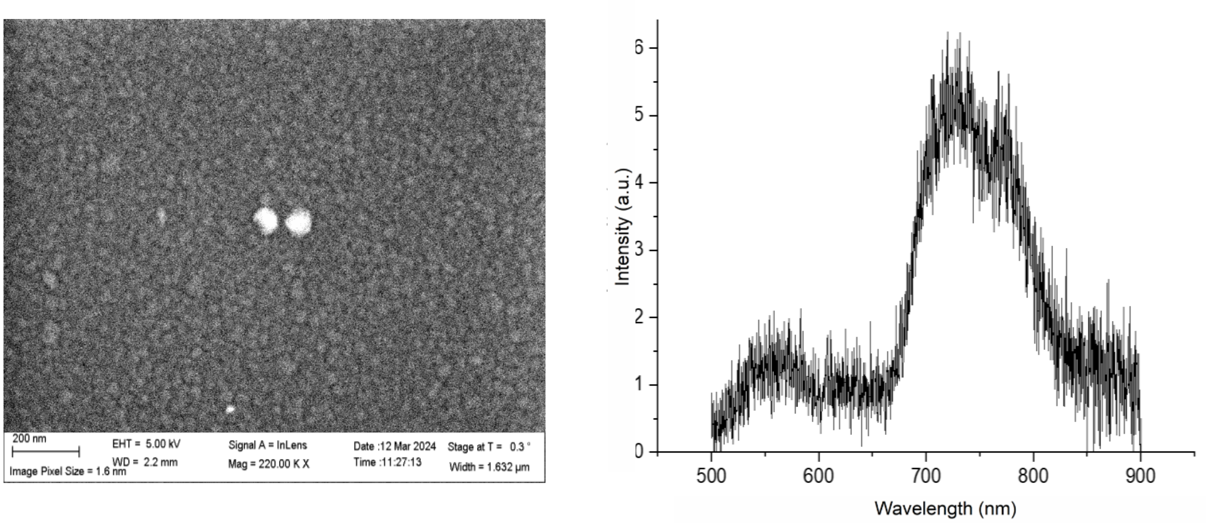


Figure 2. SEM image of Optimized Structure Nanoantenna arrays (LHS), Dark Field Scattering Spectra of Optimized Structures (RHS).

Using Super Resolution Microscopy to Study Cy5 Dye Emission: Incorporation of the PAINT technique into the microscopy setup enabled nanoscale imaging of the coupled dye molecules.

In our experiments we looked at fluorescence emission for organic molecules in a solution. The molecules diffuse freely until they absorb to the surface of our sample. At this point their diffusion is slow enough to image their emission on our EMCCD camera. We collect emission from many molecules over 1000s of frames to sufficiently sample and extract useful information. By studying the emission from these dye molecules, we learn about how their local environment is influencing their decay pathways which we understand from Figure 3. If we see an increase in radiative rate, we verify field enhancement of the antenna.

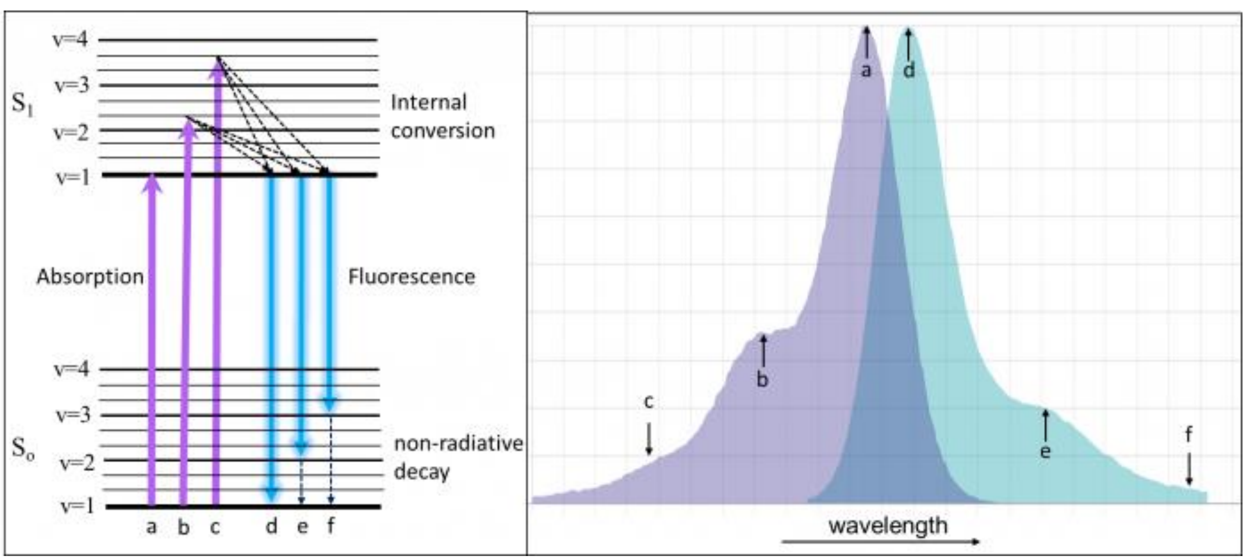


Figure 3. Typical Jablonski diagram for an organic molecule. A molecule is excited from the ground state S_0 to the vibronic ground state of the first excited state S_1. It can either decay radiatively (fluorescence) or non-radiatively. *Image reproduced without permission from [3]*

III. Results

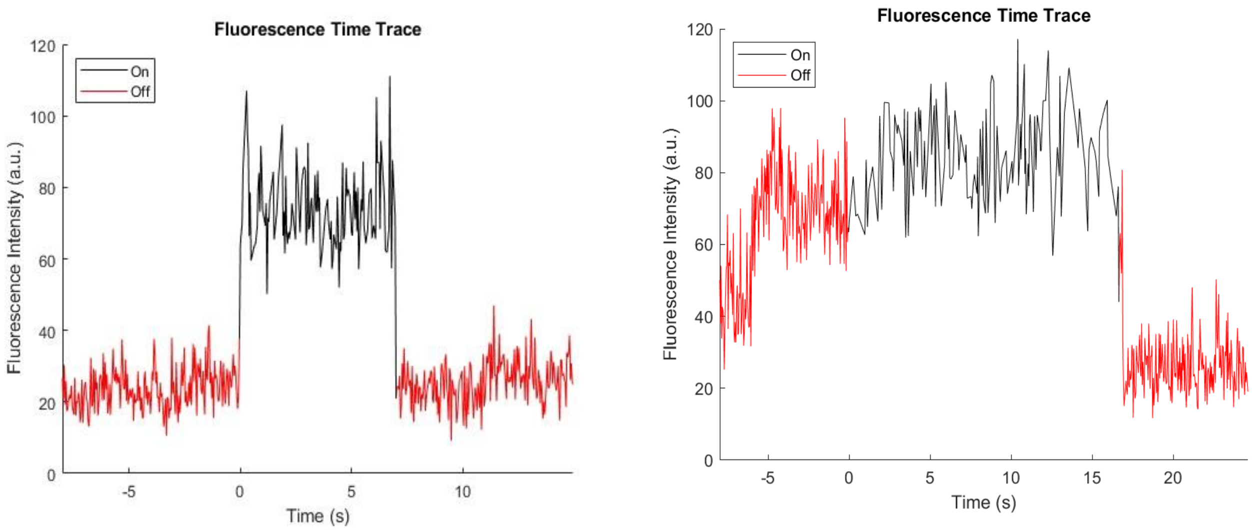


Figure 4. Single molecule time traces for optimized structures (LHS) and bowties (RHS). Molecules show similar track lengths, but lower intensity fluctuations associated with the strong gradient of the EM field in the gap indicating weaker EM field in the gap region for the optimized structure.

The experimental outcomes demonstrated significant emission enhancement of Cy5 dye molecules when coupled with plasmonic nanoantenna. This enhancement was observed through increased brightness and spatial resolution in the PAINT images compared to the control samples without nanoantenna. The nanoantenna geometry and spacing played a critical role in the emission enhancement, as evidenced by the obtained results shown in Figures 4 and 5. From the data we see that the optimized structures did not perform better than the representative 100 nm side length bowtie nanoantenna. We attribute this deviation to the limited data collected. In future work, by increasing sample size, we expect to see the trend of greater spectral overlap resulting in larger track durations of the emitter.

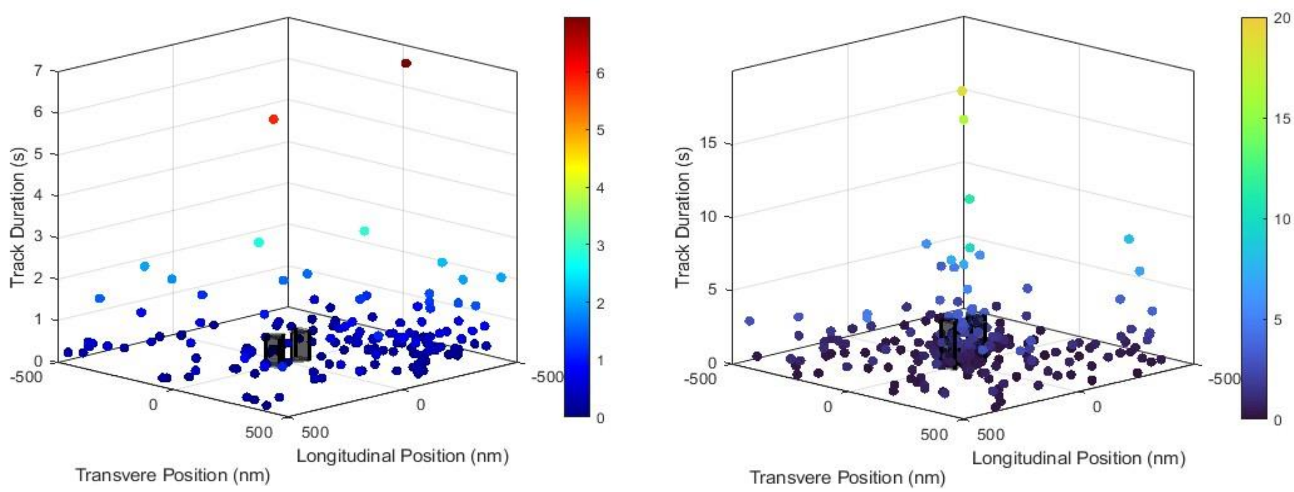


Figure 5. Single molecule tracks for optimized structures (LHS) and bowties (RHS). Shorter track durations for the optimized structures were observed. There is also decreased confinement when compared to bowtie reference.

IV. Discussion

The results of this study are consistent with previous research on plasmonic nanoantennas and their influence on fluorescence enhancement. However, the current computational design approach has not yet effectively predicted optimal nanoantenna configurations for electric field enhancement. Nonetheless, we aim to achieve more accurate antenna designs through further investigation. By continuing to refine our optimizations, we anticipate determining the optimal geometric configuration that maximizes Cy5 dye emission. These optimized designs will be fabricated and evaluated using the experimental techniques outlined in this report.

The observed enhancement from both optimized nanoantennas and bowtie structures highlights the potential of such nanoantennas in advancing the capabilities of super resolution microscopy through plasmonics. This potential includes mapping of hotspots, reconstruction of nanoparticle shapes, and mapping of reactions on nanoparticle surfaces.

V. Conclusion

In conclusion, this semester-long research project successfully investigated the emission enhancement of Cy5 dye molecules using plasmonic nanoantenna optimized via computational methods. The results contribute to the broader understanding of how plasmonic nanoantenna can improve the sensitivity and resolution of fluorescence imaging techniques. Moving forward, further exploration and refinement of nanoantenna designs could lead to even more impactful advancements in super resolution microscopy.

VI. References

[1] A. Sharonov and R. M. Hochstrasser, "Wide-field subdiffraction imaging by accumulated binding of diffusing probes," Proceedings of the National Academy of Sciences, vol. 103, no. 50, pp. 18911-18916, 2006.

[2] Smith, A. et al. “Plasmonic Nanoantennas for Enhanced Fluorescence Microscopy” Journal of Nanotechnology, 12(3), 45-58, 2018

[3] Omega Filters. "Fluorescence Overview." Omega Filters. Accessed April 18, 2024.