

Modulation and Study of Photoblinking Behavior in Dye Doped Silver-Silica Core-Shell Nanoparticles For Localization Super-Resolution Microscopy

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Introduction

The ability to control the photoblinking behavior of dye doped self-blinking fluorescent nanoparticles would make them an ideal label for localization based super resolution microscopy. The nanoparticles are composed of a silver core and a silica shell doped with Rhodamine 110. Because of the silver core the fluorescence intensity of the nanoparticles is enhanced through metal enhanced fluorescence (MEF), which is promising for increasing dye sensitivity. The nanoparticles are non-toxic, inert to the cellular environment and the conjugation of affinity ligands does not impact the optical properties, making them excellent labels for analysis of biological samples. Here it is shown that the photoblinking behavior can be controlled through changing the molecular oxygen content, the surrounding matrix, the core and shell size, and the dye concentration.

Methods

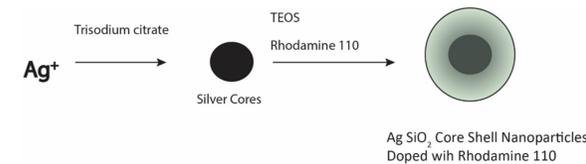


Figure 1: Synthesis of dye doped silver-silica core-shell nanoparticles.

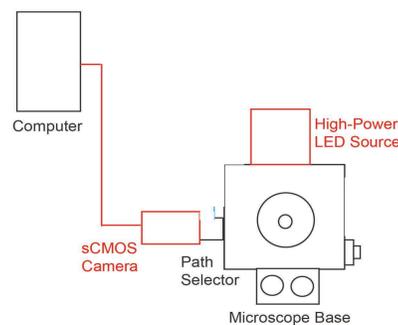


Figure 2: Schematic of the instrument set up.

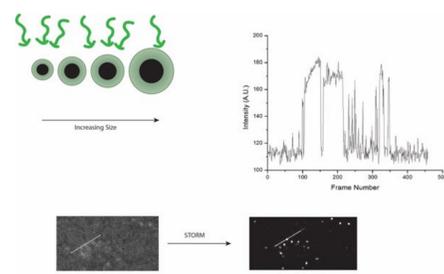


Figure 3: Schematic of the experimental process. Cores and shells of different sizes were synthesized and the duty cycle and intensity of the "on" states were measured for each set of synthesis conditions. This process was used to optimize particle size for STORM imaging.

Results

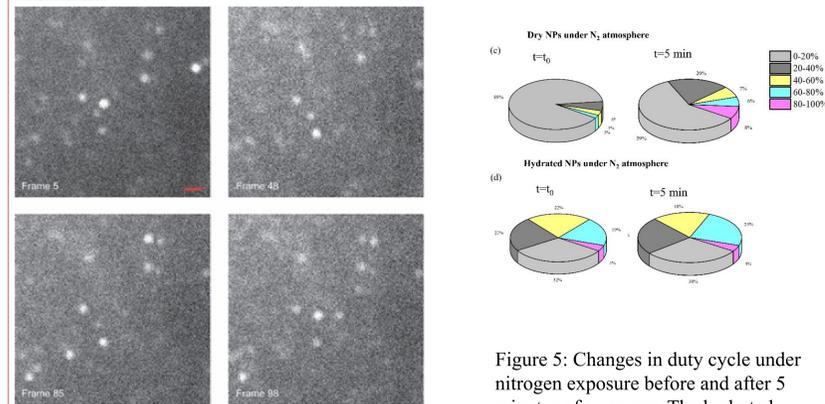


Figure 4: Time lapse of blinking nanoparticles. The rate and duty cycle are not uniform across a sample.

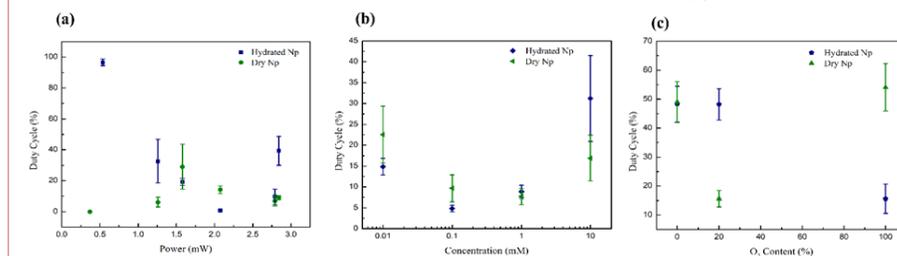


Figure 6: Distribution of duty cycle with varying (a) power of excitation source (b) concentration of dye solution, and (c) O₂ content in the nanoparticles for both dried and hydrated nanoparticles. T-tests were used to determine the statistical significance of the data. The dependence studies showed that blinking was independent of these three parameters for both dried and hydrated nanoparticles.

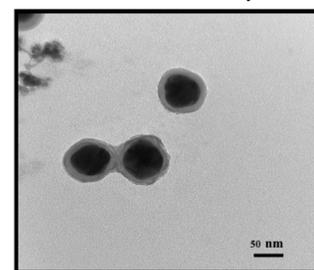


Figure 7: Transmission electron microscopy (TEM) image of the nanoparticles studied. The silver core and the silica sol gel shell are clearly visible and easily distinguishable from one another. The nanoparticles have a symmetrical core-shell structure with the core diameter of approximately 85 nm. The total diameter of the nanoparticle is 100±20 nm, confirmed by Dynamic Light Scattering (DLS). Due to the elapsed time between synthesis and analysis (approximately 12 – 14 hours) there are particles shown clumped together; the more time passes the more they clump.

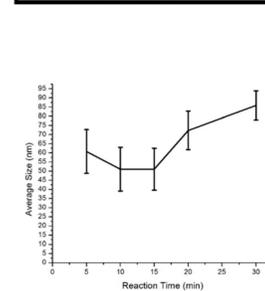


Figure 8: The core size, measured by DLS, is shown to change with the reaction time.

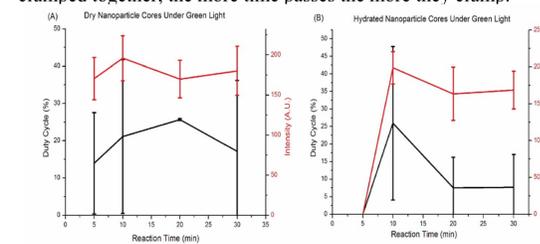


Figure 9: The duty cycle and average intensity of the nanoparticle cores under dry and hydrated conditions. There was determined to be no statistical difference in duty cycle and intensity as the core size varies.

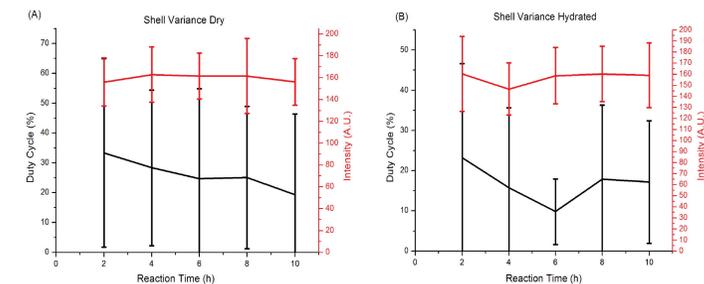


Figure 10: Duty cycle and intensity of the core-shell nanoparticle without dye. The cores were the 10-minute reaction time cores and the shell reaction time was varied in order to control the thickness.

Super Resolution

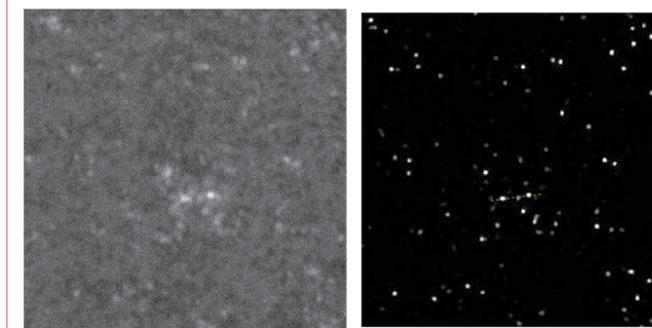


Figure 11: An image of nanoparticles before STORM localization and the same nanoparticles after STORM analysis, confirming that they are single nanoparticles and not clusters, shown as bright white spots on a black background.

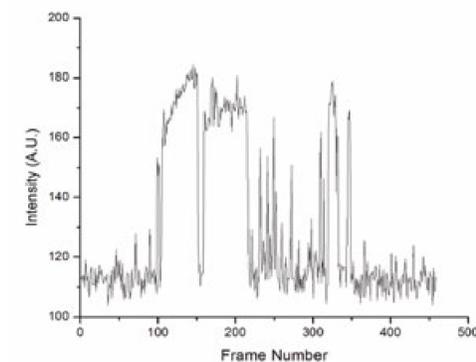


Figure 12: A graphical depiction of the photoblinking behavior of the final nanoparticle built for optimal photoblinking behavior through the manipulation of the core and shell sizes.

Acknowledgements

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References:

Chakraborty, C., S. B. Thompson, V. J. Lyons, C. Snoeyink and D. Pappas (2019). "Modulation and study of photoblinking behavior in dye doped silver-silica core-shell nanoparticles for localization super-resolution microscopy." *Nanotechnology*.
S. Thompson, D. Pappas (2020). "Core Size does not Affect Blinking Behavior of Dye-Doped Ag@SiO₂ Core-Shell Nanoparticles for Super-Resolution Microscopy." *RSC Advances*.

Future Work

- Conjugation to affinity ligands and adherence to cells
- Super resolution imaging of cells using the nanoparticles

Conclusions

- Blinking independent of excitation source power and dye concentration
- Higher duty cycles under no oxygen atmospheric conditions
- High oxygen atmospheric conditions did not significantly impact duty cycle
- More dramatic change for dry nanoparticles than for hydrated nanoparticles
- Photoblinking behavior is random
- Photoblinking behavior can be controlled through modification of the core and shell structures
- Duty cycle for completed nanoparticle under hydrated conditions ranges from 2% to 70% and exhibits rapid photoblinking

