

Optimizing Inter-Well Illumination Uniformity in Backscattering UTI Diagnostic Device

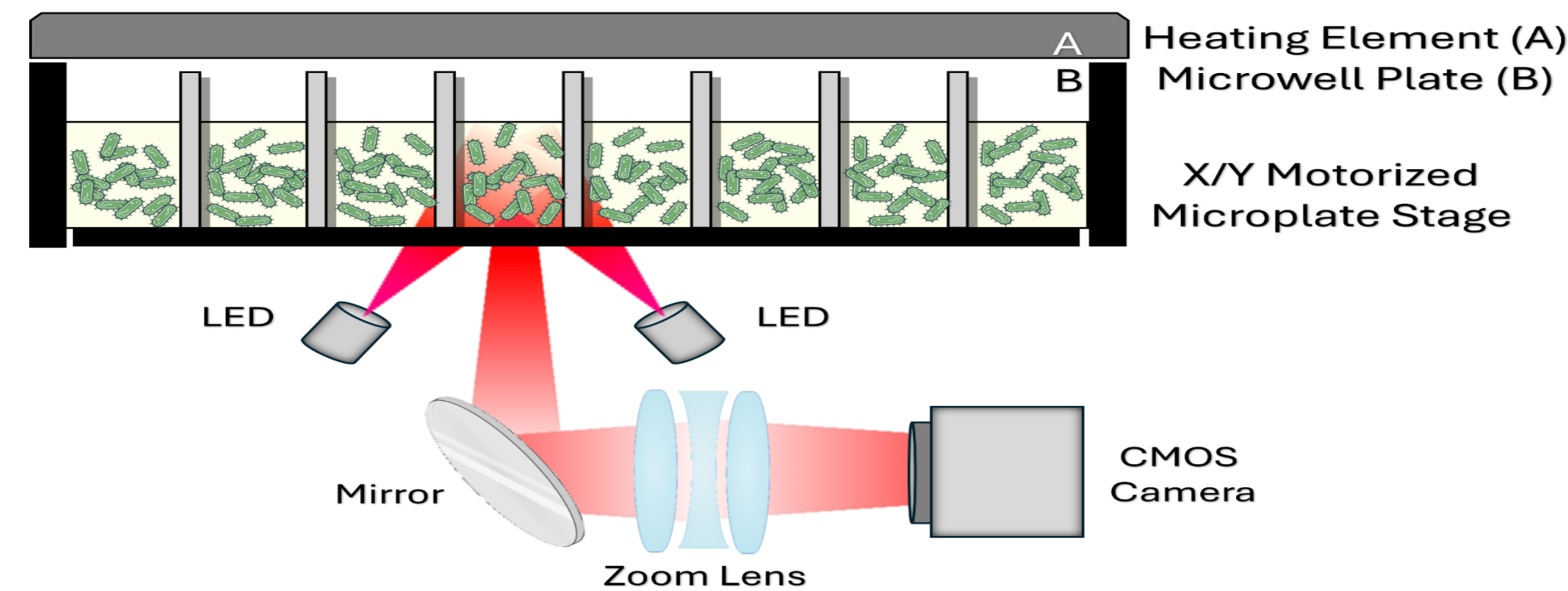
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Significance

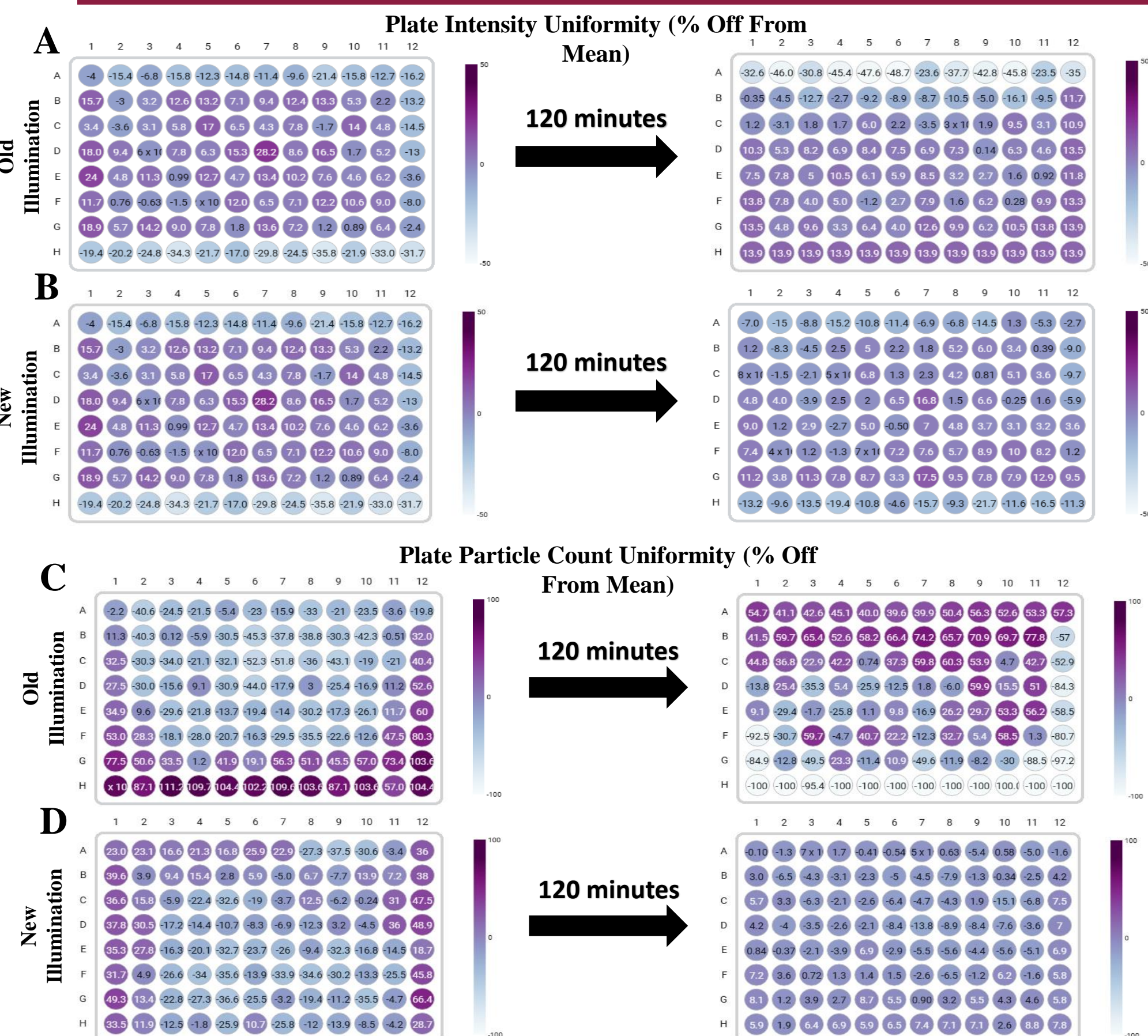
- **Large Volume Scattering Imaging (LVSIm) Device:** Developed to enable rapid bacterial detection through video-based imaging methods [1].
- **Challenge - Uneven Illumination:** The original illumination setup caused pronounced variability in well intensities and particle counts, particularly at the edges and bottom of the plate.
- **Impact on Data Quality:** Non-uniform lighting introduced bias and reduced reproducibility in measurements between samples.
- **Project:** Implement a new illumination approach to achieve uniform lighting across the 96-well plate, minimizing variability and creating more robust and reliable data for rapid bacterial detection workflows using the LVSIm.

LVSIm Principle

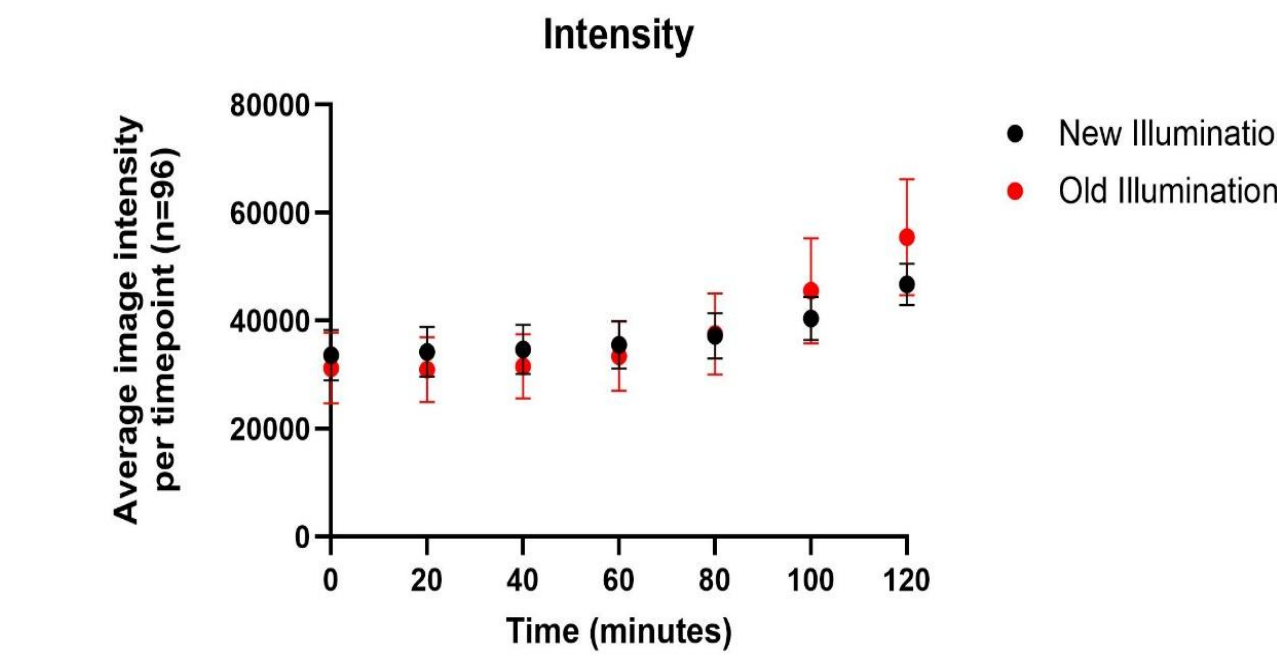


- **LVSIm Design:** Combines dual LEDs and low magnification zoom lens to capture backscattered light.
- **High-Throughput Format:** Uses a 96-well plate to support parallel measurement of multiple samples.
- **Illumination Issues:** Current LED positioning causes reflections from the plate's outer skirt, resulting in increased intensity at the plate edges.
- **Focus:** Optimize LED angle, height, and location to minimize scatter variability across wells, creating consistent illumination among wells and providing a reliable reading.

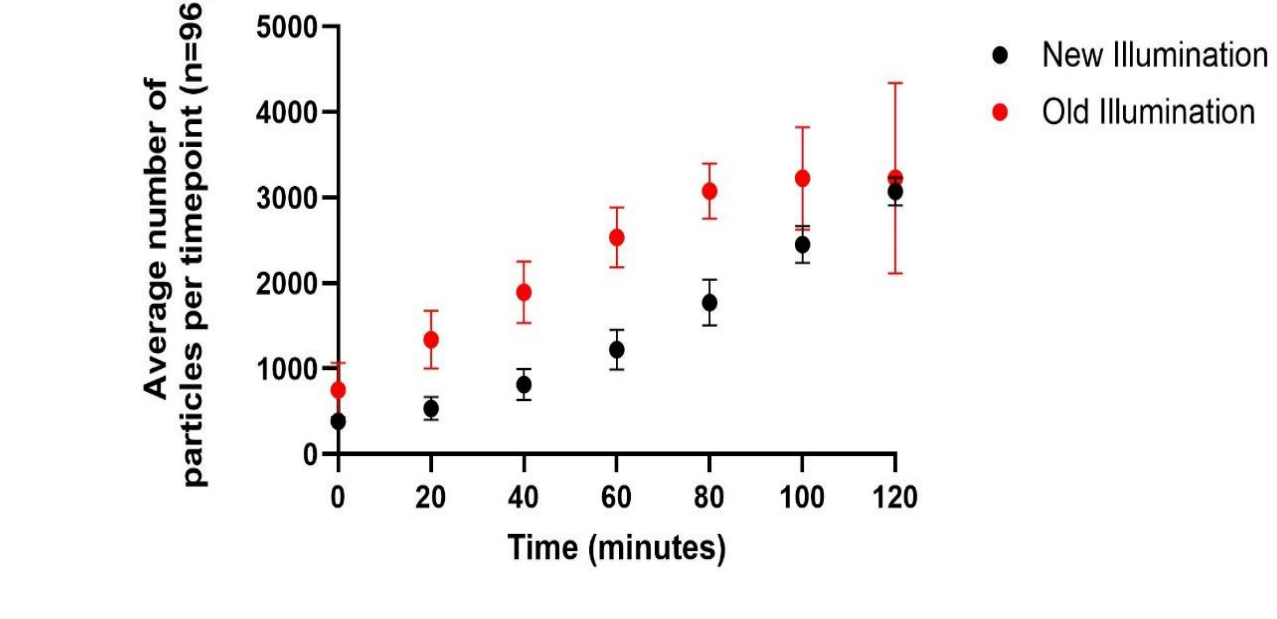
Results



Variability Comparison Between Illumination Setups



Variability Comparison Between Illumination Setups - Particle Count



- Heatmaps (A–D) compare the uniformity of *E. coli* growth in a 96-well plate under the old versus new illumination setups. Panels (A–B) display each well's intensity deviation from the plate mean over a two-hour period, while (C–D) show the corresponding particle count deviation. Figures (E–F) illustrate inter-well variability at 20-minute intervals throughout the two-hour span, showing the differences between the two setups.
- Repositioning the light sources diagonally along the plate's X-axis reduces interactions with the plate skirt, thereby minimizing edge effects. This configuration provides more uniform illumination across the wells and decreases variability in both intensity and particle counts.

Future Improvements

- **Expanded Illumination Coverage:** Adding two additional light sources illuminates each well from multiple angles. This would in turn evenly illuminate all corners of the image for better lighting coverage in the image.

Acknowledgements

I would like to thank Dr. Shaopeng Wang and the Optical Biosensing Lab for the opportunity to contribute to this project. Special thanks to my graduate student mentor Brandyn Braswell.

References

1. Zhang, F., Jiang, J., McBride, M., Zhou, X., Yang, Y., Mo, M., Peterman, J., Grys, T., Haydel, S. E., Tao, N., & Wang, S. (2021). Rapid antimicrobial susceptibility testing on clinical urine samples by video-based object scattering intensity detection. *Analytical Chemistry*, 93(18), 7011–7021. <https://doi.org/10.1021/acs.analchem.1c00019>