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USING ENVIRONMENT-SENSITIVE PROBES TO DETECT MYCOBACTERIUM TUBERCULOSIS WITH MACHINE LEARNING-BASED FLUORESCENCE MICROSCOPE OCTOPI

Presented By: Zachary Caterer

Primary Investigator: Mireille Kamariza, PhD

2023/08/30

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AGENDA

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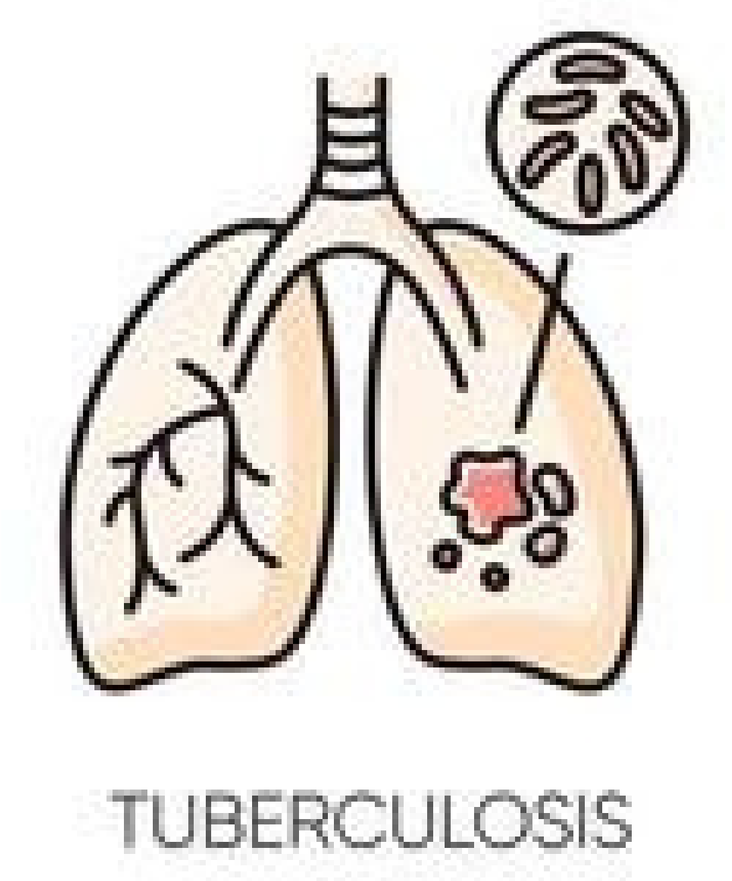
(1) Bagcchi S. WHO's Global Tuberculosis Report 2022. Lancet Microbe. 2023 Jan;4(1):e20. doi: 10.1016/S2666-5247(22)00359-7. Epub 2022 Dec 12. PMID: 36521512.



Background of the Study

Mycobacterium Tuberculosis (Mtb) is the causitive agent of Tuberculosis (TB) (1)

In 2021 10.6 Million people fell ill to TB and 1.6 Million people died (1)
Over 80% of TB cases and deaths are reported in low and middle income countries (1)

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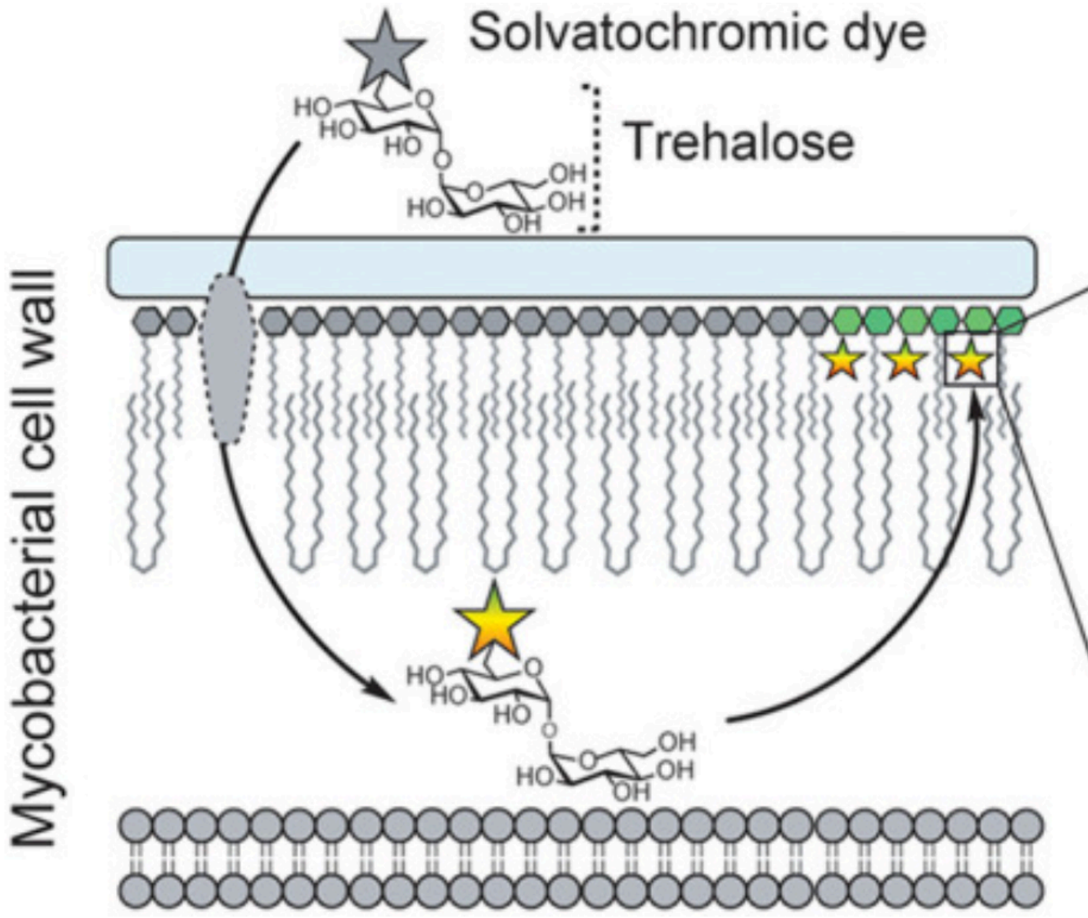
(1)Kamariza M, Keyser SGL, Utz A, Knapp BD, Ealand C, Ahn G, Cambier CJ, Chen T, Kana B, Huang KC, Bertozzi CR. Toward Point-of-Care Detection of Mycobacterium tuberculosis: A Brighter Solvatochromic Probe Detects Mycobacteria within Minutes. JACS Au. 2021 Jul 26;1(9):1368-1379. doi: 10.1021/jacsau.1c00173. PMID: 34604847; PMCID: PMC8479770.

Solvatochromic Probes

Trehalose probes can be metabolized by Ag85 forming Trehalose Monomycolate (TMM) (1)

Dyes are designed to change their fluorescence intensity when transitioning from an aquous to hydrophobic environment (1)



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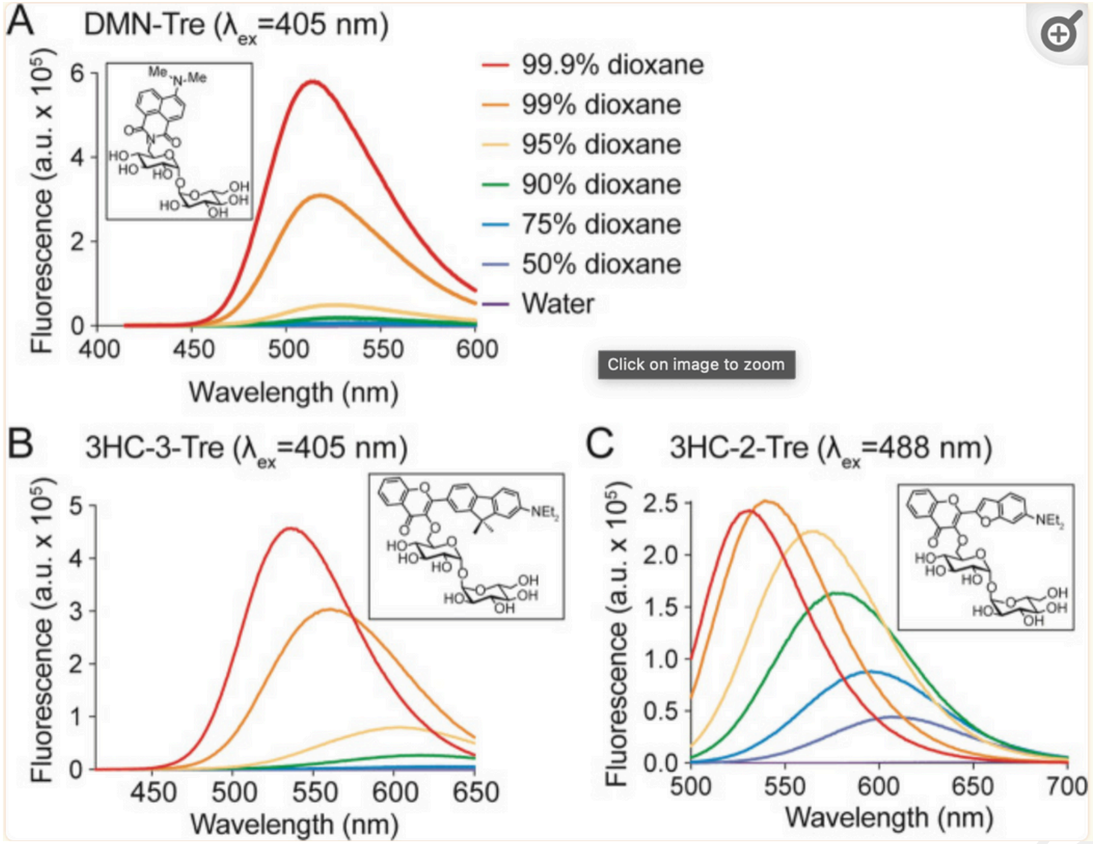
(1) Kamariza M, Keyser SGL, Utz A, Knapp BD, Ealand C, Ahn G, Cambier CJ, Chen T, Kana B, Huang KC, Bertozzi CR. Toward Point-of-Care Detection of Mycobacterium tuberculosis: A Brighter Solvatochromic Probe Detects Mycobacteria within Minutes. JACS Au. 2021 Jul 26;1(9):1368-1379. doi: 10.1021/jacsau.1c00173. PMID: 34604847; PMCID: PMC8479770.

DMN and 3HC Trehalose

4,4-N,N-dimethylaminonapthalimide (DMN-Tre)
3-Hydroxychromone (3HC-Tre)

Probes enable no wash visualization in 30 minutes(1)
Advantages of these probes: speed affordability, operationally simple etc
Synthetically convenient (Bioorthogonal or Click chemistry) (1)
Chemically stable



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(1) Octopi: Open configurable high-throughput imaging platform for infectious disease diagnosis in the field Hongquan Li, Hazel Soto-Montoya, Maxime Voisin, Lucas Fuentes Valenzuela, Manu Prakash bioRxiv 684423; doi: https://doi.org/10.1101/684423

Octopi

Introduction of Octopi: a low-cost ($250-$500), reconfigurable autonomous microscopy platform.

Machine Learning Pipeline (MLP) and automated slide scanning system

Successfully applied to automated malaria parasite detection in blood smears.

Utilized spectral shift for detecting DAPI-stained Plasmodium falciparum parasites.

Screens over 1.5 million red blood cells per minute, achieving high sensitivity and specificity.

Potential for large-scale robotic microscope network to enhance disease diagnosis. (1)

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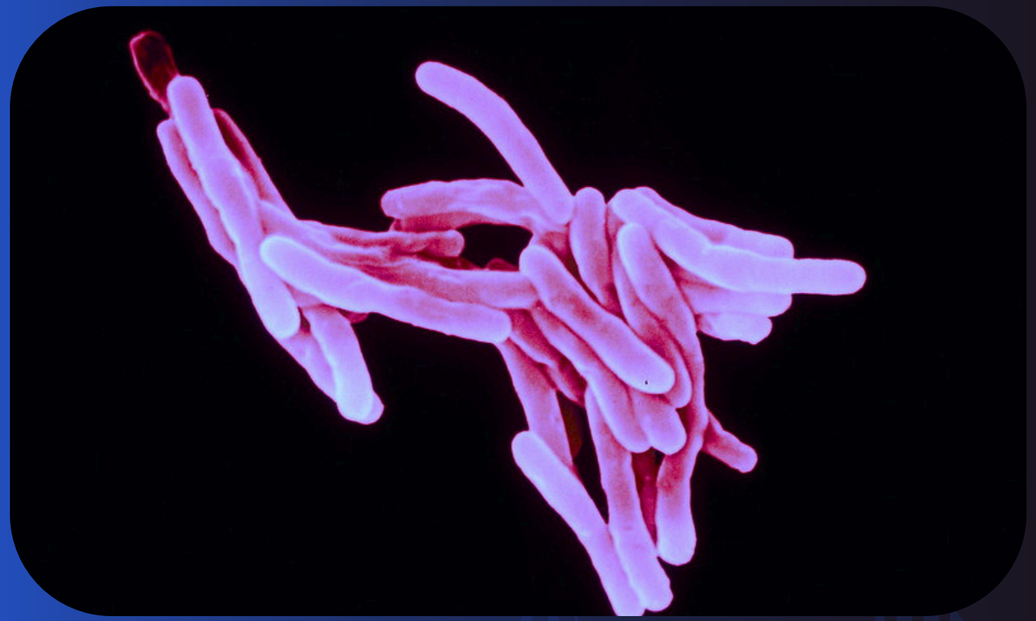
Research Objectives

SCOPE OF THE STUDY

Integrate a novel MLP that integrates with Octopi's automaticated slide scanning system using the solvatochromic probes

GOALS

1. Determine the detection protocol
2. Access the limits of detection
3. Implement clinical samples



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METHODOLOGY

QUALITATIVE METHODS

Bacterial Culture Inoculation and Metabolic Labeling

Confocal Fluorescence Microscopy (Leica)

Octopi Microscope

QUANTITATIVE METHODS

Leica Software

Fiji

Python

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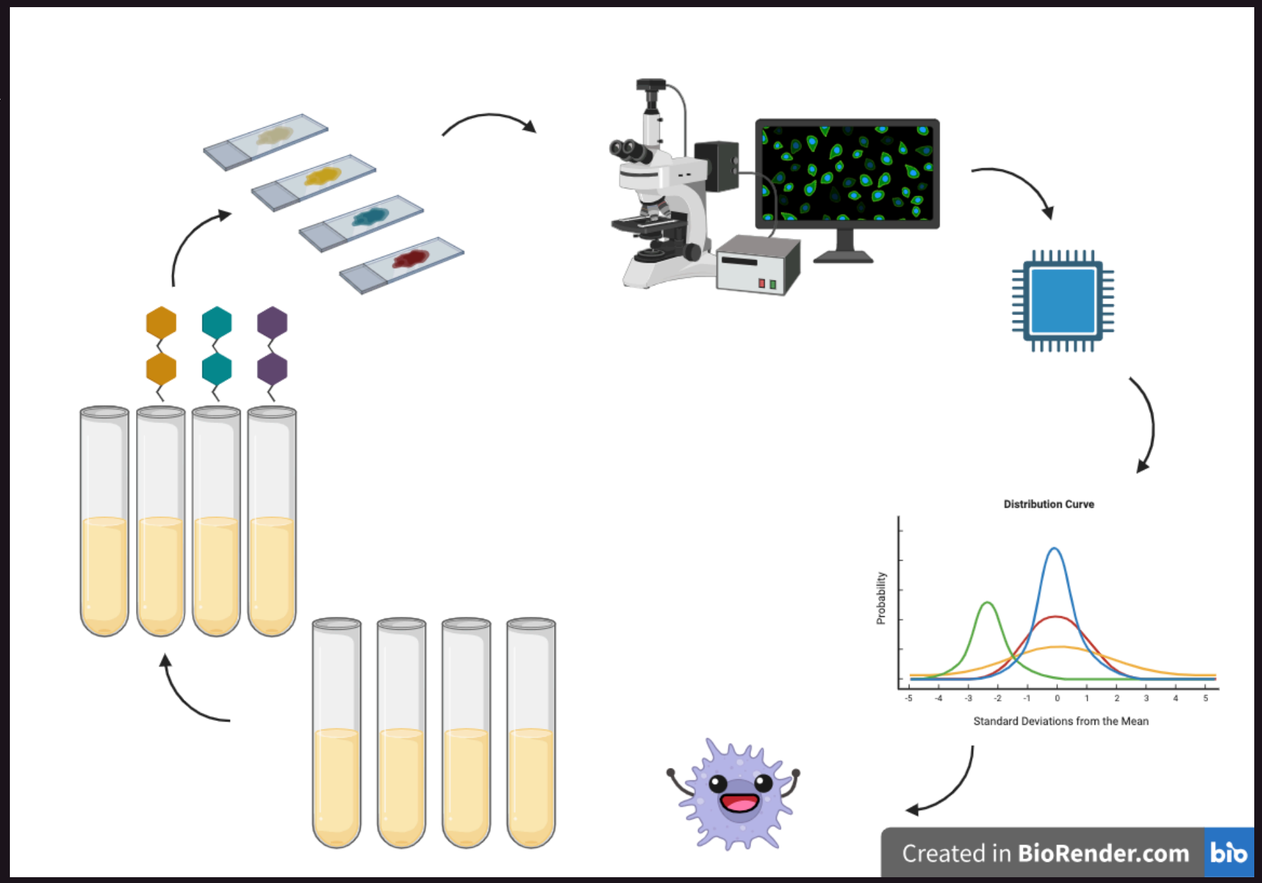
METHODOLOGY



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RESULTS

(A) 20X
(B) 60X
(C) 160X

8. Figures

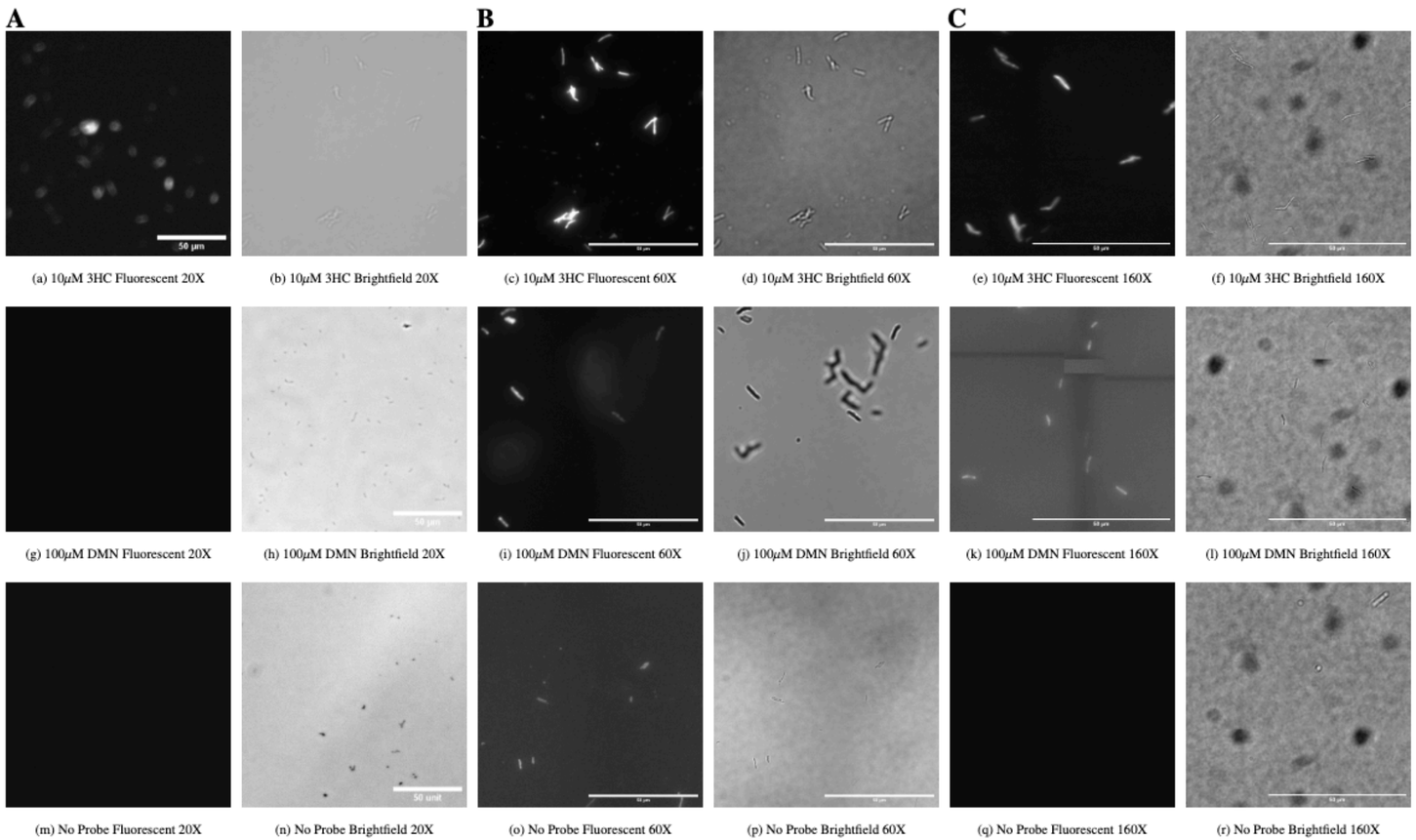

Figure 1: Probe Performance and Imaging at Different Leica Microscope Objectives. Each subfigure presents a comparative analysis of probe performance. This figure depicts 3 groups (A), (B), (C). (A) Images depict Leica 20X objective. (B) Images depict the Leica 60X objective. (C) Images depict the Leica 160X objective. Subfigures (a)-(f) showcase the results of imaging experiments conducted for 10μM 3HC-Tre. Subfigures (g)-(l) showcase the results of imaging experiments conducted for 100μM DMN-Tre. Subfigures (m)-(r) showcase the results of imaging experiments conducted with no probe (unlabeled) Msmeg. All incubations were performed for 3 hours, and probed for 30 minutes. Subfigures (a, c, e, g, i, k, m, o, q) showcase fluorescent images. Subfigures (b, d, f, h, j, l, n, p, r) showcase brightfield images. Scale bar is 50 μm for all figures.

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RESULTS

(A) 3HC-Tre

(B) DMN-Tre

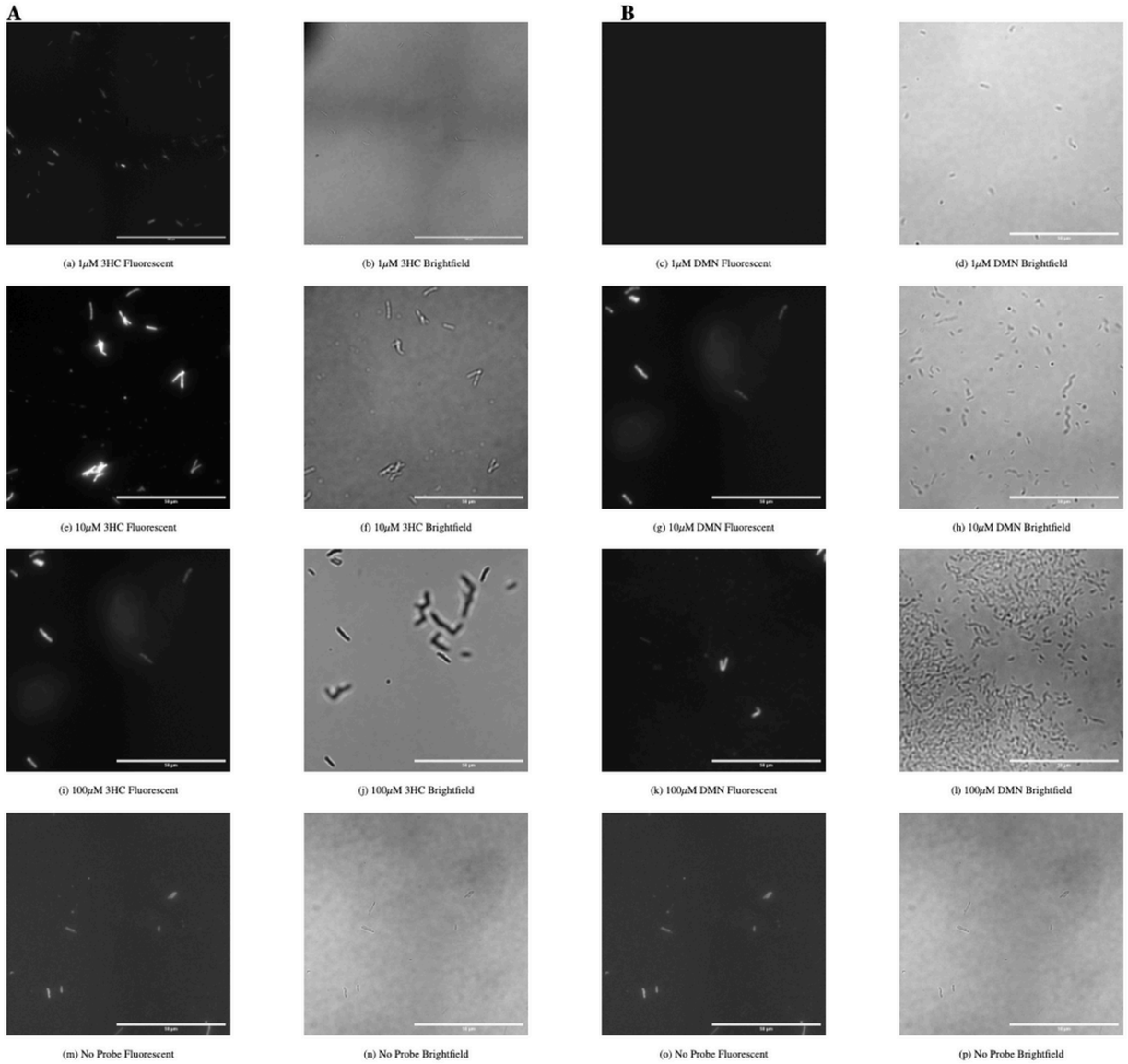

Figure 2: Dose-Dependent Effects of 3HC-Tre and DMN-Tre Probes on Cellular Imaging. This figure depicts 2 groups, (A), (B). (A) depicts images using 3HC-Tre probes, while the rightmost column is the fluorescent, and the leftmost column shows the brightfield images. (B) depicts images using DMN-Tre probes, with a similar format to (A). Subfigures (a)-(d) demonstrate the 1μM. Subfigures (e)-(h) demonstrate the 10μM 3HC-Tre and DMN-Tre probes. Subfigures (i)-(l) demonstrate the 100μM 3HC-Tre and DMN-Tre probes. Subfigures (m)-(p) demonstrate the 0μM 3HC-Tre and DMN-Tre probes. The images provide insights into the effects of varying probe concentrations on cell behavior and imaging outcomes. All images used Leica's 60X objective. Scale bar is 50μm for all images.

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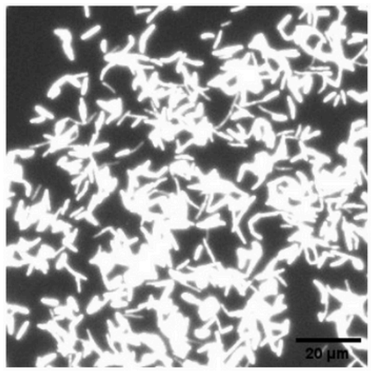
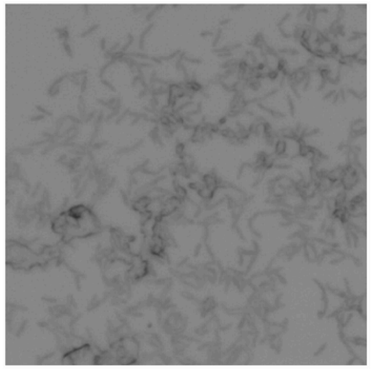
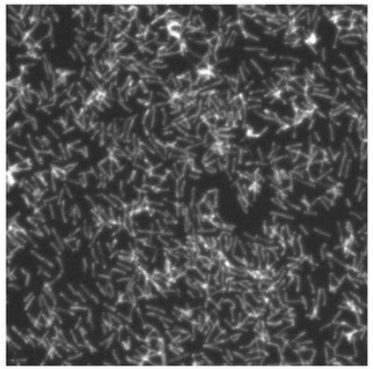
RESULTS



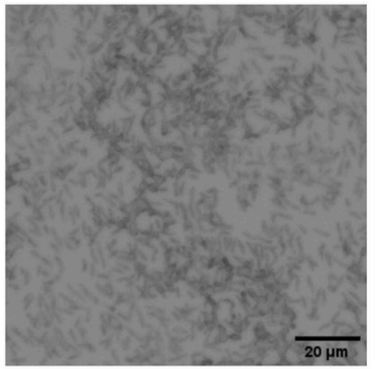
(a) 10µM 3HC Fluorescent



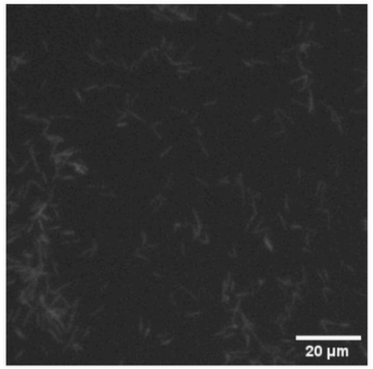
(b) 10µM 3HC Brightfield



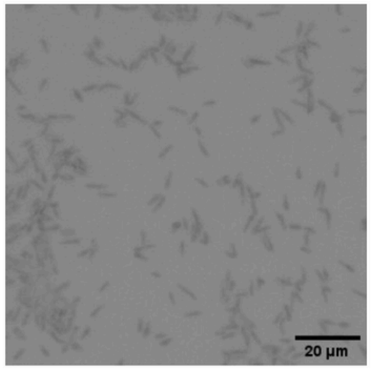
(c) 100µM DMN Fluorescent



(d) 100µM DMN Brightfield



(e) No Probe Fluorescent



(f) No Probe Brightfield

Figure 3: Comparative Cellular Imaging Using the Octopi Microscope. This figure presents a comparative analysis of cellular imaging outcomes facilitated by the Octopi microscope. Subfigures (a, c, e) showcases fluorescent images, while subfigures (b, d, f) displays brightfield images. Subfigures (a, b) illustrates cells incubated with a 10μM 3HC-Tre probe for 30 minutes. Subfigures (c, d) depicts cells incubated with a 100μM DMN-Tre probe for 30 minutes. Subfigures (e, f) features control cells incubated without any probe. All samples were incubated for 3 hours. The scale bar is set at 20μm for all images.

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RESULTS

(A) Octopi
(B) Leica 60X

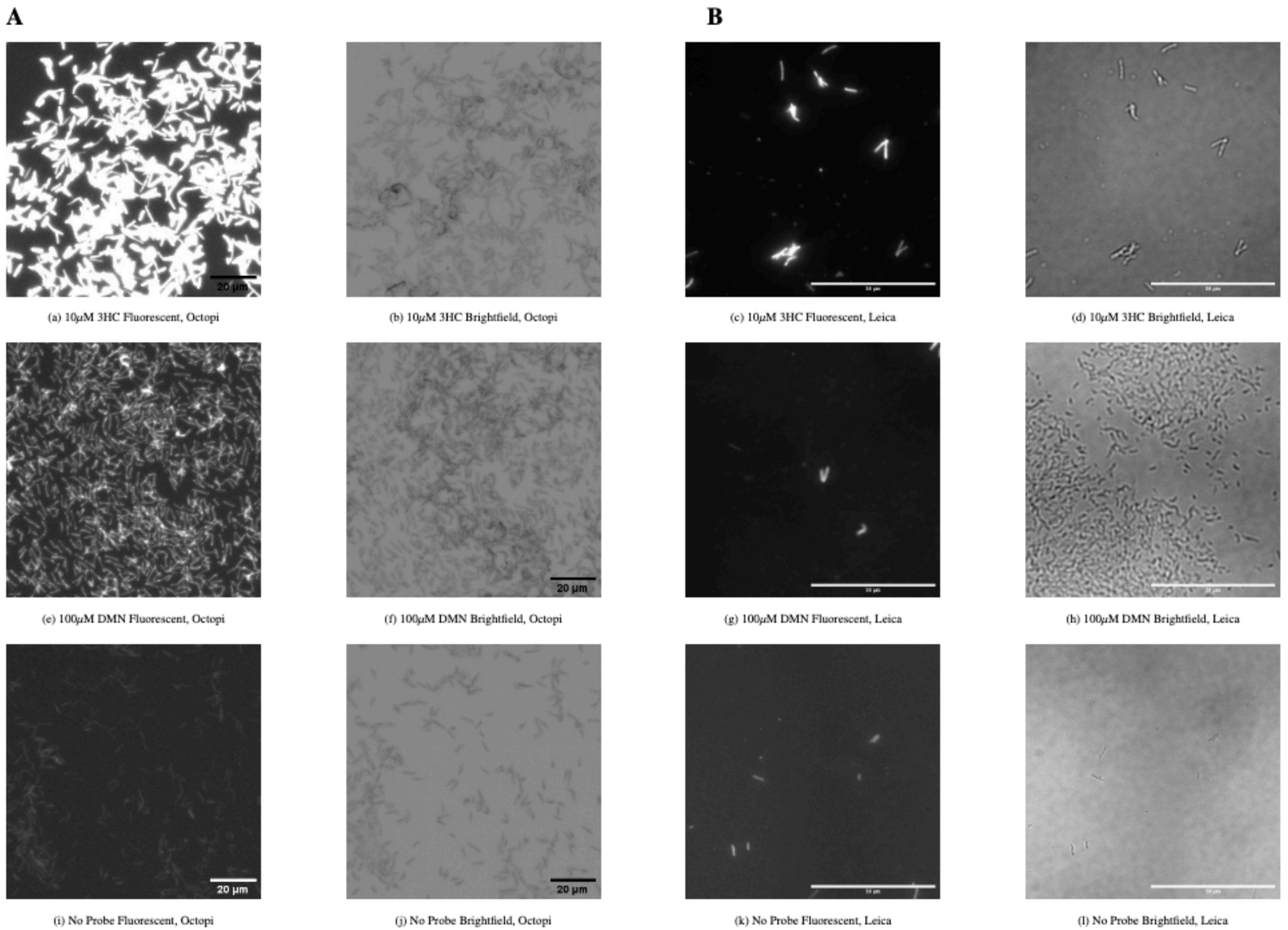

Figure 4: Comparative Analysis of Cellular Imaging Between Octopi and Leica (60X Objective). This image depicts 2 groups (A), (B). (A) images acquired using Octopi microscope. (B) images acquired Leica microscope with 60X objective. Each pair of subfigures represents the same cellular sample imaged by both systems. Subfigures (a - d) represent the same sample incubated with a 10μM 3HC-Tre probe, (e - h) with a 100μM DMN-Tre probe, and (i - l) without any probe. Subfigures (a, c, e, g, i, k) correspond to the fluorescent images while subfigures (b, d, f, h, j, l) represent the brightfield images. All samples were incubated for 3 hours, and probed for 30 minutes. The scale bar is set at 20μm for Octopi images and 50μm for Leica images.

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Conclusions and Future Direcions

CONCLUSIONS

- Successful demonstration of environment-sensitive probes (DMN-Tre and 3HC-Tre) in labeling Msmeg bacteria using Octopi and Leica fluorescence microscopes.
- Combined use of brightfield and fluorescence microscopy for visualizing labeled bacteria across magnifications, forming a foundation for future high-throughput image analysis with machine learning.

FUTURE DIRECTIONS

- Focus on finalizing machine learning-based diagnostic pipeline, integrating brightfield and fluorescence images, and optimizing algorithms for quantitative insights.
- Transition from Msmeg to Mycobacterium tuberculosis (Mtb) to validate approach on disease-causing bacteria.
- Conduct experiments to define sensitivity and specificity thresholds of environment-sensitive probes and Octopi microscopy for robust detection limits.
- Optimize Octopi microscope for TB diagnosis, including autofocus system refinement.

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Nicolas Wheeler PhD (UWEC)

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Questions??

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