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Investigation Of Bacteriophage-host Interactions Using Single Molecule Localization Microscopy

Andrew Kick United States Military Academy **Katherine Hebert** United States Military Academy **Aidan Tran** United States Military Academy **Lance Richardson** United States Military Academy **Patrick Armstrong** United States Military Academy **Jackson Pophal** United States Military Academy

Multi-drug resistant pathogenic bacteria have developed through mutation and selection to overcome our suite of antibiotics or alternatively could be developed through bioengineering by state or non-state actors: in both cases, these pathogenic bacteria pose a grave threat to public health and particularly wounded Servicemembers. Providing new therapeutic solutions against multi-drug resistant bacteria to physicians and military health protection agencies is critically important. Bacteriophages (bacteria-specific viruses) are an emerging therapeutic with great potential for physicians to regain the initiative on pathogenic bacteria by unilateral treatment or in combination with traditional antibiotics. Characterization of the bacteriophage / bacteria interaction is incompletely understood: Single Molecule Localization Microscopy (SMLM / super-resolution microscopy) will facilitate a more complete visualization and analysis of the bacteriophage's infection, replication, and killing of bacteria. Constructing a SMLM will bring a high-end SMLM capability to West Point at a fraction of the cost for a new instrument, train research Cadets on constructing a super-resolution microscope, and better characterize bacteriophage / host interactions to assist DoD-research in developing bacteriophage as effective anti-bacterial therapeutics. SMLM has opened the door to new exploration of the microscopic world below the Abbé diffraction limit of optical microscopy (approximately 200 nm) and has the theoretical possibility of improving image resolution to 20 nm or better. Super-resolution microscopes are generally restricted to premier imaging facilities due to their cost.

Escherichia coli (E. coli) and the T4 phage were selected for the first bacteriophage / host interaction to investigate because E. coli is a biosafety level 1 bacteria and there are extensive research publications on this interaction. During Academic Year 2022, the Cadet research team purchased the microscope components and constructed a working SMLM ((λ=638 nm), as well as, established the T4 phage infection procedures. The team developed a novel fluorescent staining model for T4 phage, not previously published in the literature enabling images to be produced illustrating phage infection of E. coli. The Cadet research team is improving image quality / resolution and adding a second laser (λ=488 nm) to illuminate more details of the bacterial structure using a different fluorescent marker. After overcoming these final technical challenges, the Cadets will shift their focus to characterizing the E. coli / T4 phage interactions, specifically T4 phage's capacity to penetrate an E. coli biofilm. Upon establishment of this infection and evaluation system at West Point, Cadets will apply it to characterize the interactions of novel phage with BSL-2 pathogenic bacteria and develop new methods for evaluation of bacteriophage penetration into biofilms and the efficacy of cotreatment of bacteriophage and antibiotics against multi-drug resistant pathogenic bacteria.

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