RB.1: Detection of Bacterial Endospores using QCL Spectroscopy Coupled to Unmanned Vehicles and Artificial Intelligence (AI)/Machine Learning (ML)

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Soft Target Engineering to Neutralize the Threat Reality

SENTRY Challenge

Bacterial endospores are a type of defensive mechanism that contain tough structures that keep themselves inactive until a germination opportunity arises. Its diminutive size, which can range from 0.95 ± 0.11 µm in width to 1.31 ± 0.17 µm in length, might cause a delay in identification of several hours or even days. They can be immediately utilized for terrorism and are comparatively undetectable. After the anthrax assaults in 2001, which were caused by exposure to endospores that can cause illness and, in the worst cases, death, there was an upsurge in research to find a way to stop this from happening again.

This research is based on the detection of endospores from *Bacillus cereus* (***Bc***) and *Bacillus thuringiensis* (***Bt***), biological simulants to *Bacillus anthracis* (***Ba***) due to their genetic resemblance. This detection will be achieved through spectroscopic techniques without compromising personnel safety, with the use of UV (Unmanned vehicles).

Accomplishments

Spectral acquisitions of *Bt* endospores with Quantum Cascade Laser (QCL) spectroscopy in transmission after various purification treatments (**Figure 1**).

The optimal purification procedure was the chemical method using ethanol, as shown in **Figure 5**. The purification was done in order to remove vegetative cells from the samples.

The endospores can be distinguished using Quantum Cascade Laser (QCL) spectroscopy in transmission mode (**Figure 2**).

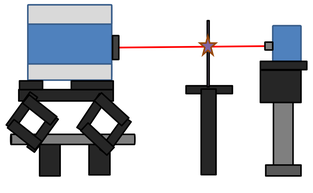

Figure 1. QCL in transmission mode for sample acquisition.

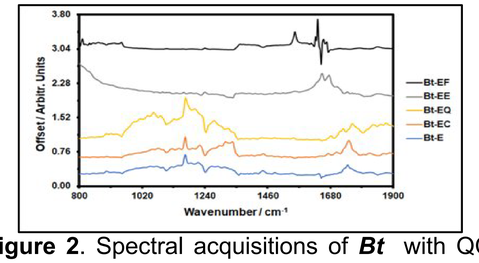

Figure 2. Spectral acquisitions of ***Bt*** with QCL transmission.

Addressing the Challenge

It is important to correctly and quickly detect, without endangering lives, biological threats such as endospores that are dangerous to humans. In the anthrax assaults of 2001, envelopes containing *Bacillus anthracis* (***Ba***) endospores were discovered. Five individuals died from illness, 31 people tested positive for spore exposure, and 10,000 people were labeled "at-risk" of exposure. Thirty five postal facilities and commercial mailrooms were poisoned as a result of this incident, and the United States Postal Service (USPS) shut down two processing and distribution centers (P&DC). This instance demonstrates the reach, stealth, and danger that biological threats can possess. Rapid detection of biological risks is required, while minimizing the cognitive strain on employees.

To track changes and/or development of endospores at various stages, their production and purification was actively optimized. This was done with the intention of differentiating between endospore-producing bacteria that have genetic similarity to *Ba* using spectroscopic techniques. Quantum Cascade Laser (QCL), a recently developed technique with energy source brightness > synchrotrons, was the spectroscopic method employed. These powerful spectroscopic sources can be wavenumber (micros) tuned from ~ 2000 to about 790 cm^{-1}. This range matches the fundamental vibrational absorption bands of many biological species (Carrión-Roca *et.al*, 2023). After discerning and characterizing the spectra, the team will assemble the QCL in an UV, such as a Husky A200™ Rover (Clearpath Robotics, Inc.) in combination with an in-house developed hexacopter. This combination of technologies provides the benefits of a safer solution through remote/autonomous operation and broad area coverage. These characteristics will aid first-responders in identifying, analyzing, and prioritizing their work even through dangerous or usually inaccessible areas to personnel.

Endospore production, identification and future QCL-Rovers (Drones)

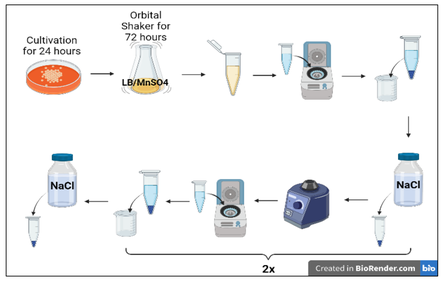

Figure 3. Procedure of cell expansion of bacterial samples to obtain endospores.

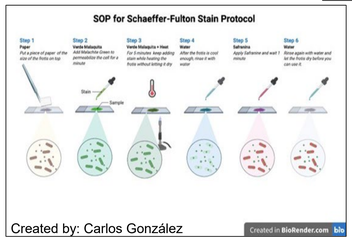

Figure 4. Schaeffer-Fulton Staining protocol.

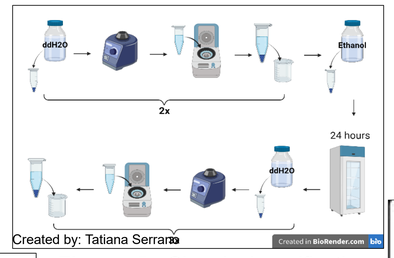

Figure 5. Chemical purification procedure using ethanol.

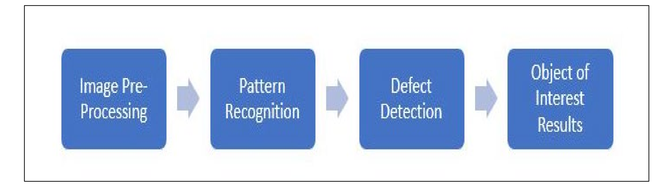

Figure 7. Pattern Recognition Sequence to be applied (using Principal Components Analysis, PCA).

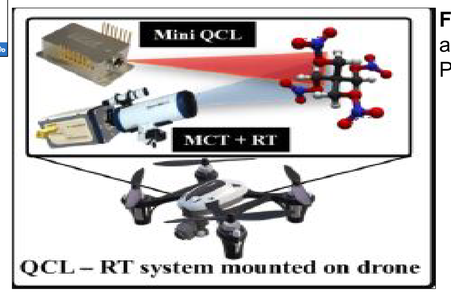

Figure 6. Model of QCL-UAV (Rovers & Drones) Assemblies

Next Steps

- Prototype: Mounting of QCL onto UV, and stress-testing for faults
- Simulating autonomous operation of system
- Begin interaction with local first responders to receive usability feedback
- Continue spectral acquisitions of other biothreat simulants
- Begin bioaerosols detection experiments at a distance
- Begin programming in AI & ML

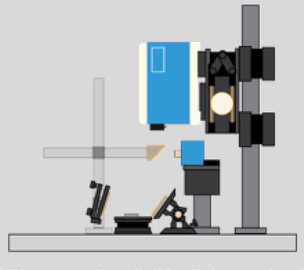

Figure 8. QCL-GA optical setup for sample acquisition

- Examining samples of the bacteria at various phases of growth to see the behavior of the endospores
- Further biological spectral acquisition characterization
- For the acquisition of spectra, utilize the optical grazing angle (GA) mount for the QCL (QCL-GA)

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