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UCRL-PROC-224796

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September 27, 2006

Chemical and Biological Countermeasure Technologies for Homeland Security
Boulder, CO, United States
October 10, 2006 through October 13, 2006

Characterization and Detection of Biological Weapons with Atomic Force Microscopy

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Critical gaps exist in our capabilities to rapidly characterize threat agents which could be used in attacks on facilities and military forces. DNA-based PCR and immunoassay-based techniques provide unique identification of species, strains and protein signatures of pathogens. However, differentiation between naturally occurring and weaponized bioagents and the identification of formulation signatures are beyond current technologies. One of the most effective and often the only definitive means to identify a threat agent is by its direct visualization. Atomic force microscopy (AFM) is a rapid imaging technique that covers the size range of most biothreat agents (several nanometers to tens of microns), is capable of resolving pathogen morphology and structure, and could be developed into a portable device for biological weapons (BW) field characterization. AFM can detect pathogens in aerosol, liquid, surface and soil samples while concomitantly acquiring their weaponization and threat agent digital signatures.

BW morphological and structural signatures, including modifications to pathogen microstructural architecture and topology that occur during formulation and weaponization, provide the means for their differentiation from crude or purified unformulated agent, processing signatures, as well as assessment of their potential for dispersion, inhalation and environmental persistence. AFM visualization of pathogen morphology and architecture often provides valuable digital signatures and allows direct detection and identification of threat agents.

We have demonstrated that pathogens, spanning the size range from several nanometers for small agricultural satellite viruses to almost half micron for pox viruses[1,2], and to several microns for bacteria and bacterial spores[3-5], can be visualized by AFM under physiological conditions to a resolution of ~20-30 Å. We have also demonstrated[2] that viruses from closely related families could be differentiated by AFM on the basis of their structural attributes. Similarly, we have shown[3-5] that bacterial spore coat structures are phylogenetically and growth medium determined. These findings validate that AFM can identify species/formulation-specific signatures that could used to reconstruct production conditions. In addition, we showed that internal structures of pathogens could be revealed by chemical and enzymatic dissection, thus providing additional AFM threat agent signatures. We have developed AFM-based immunochemical labeling procedures for threat-specific epitope visualization, which extend the specificity of structural information that AFM can provide.

AFM enables sensitive detection/identification/detection of threat agents (e.g Sterne. *B. anthracis* spore sample having a concentration of ~10^6 spores/ml) and allows their identification in environmental cluttered samples.

AFM analysis has the capacity for accessing forensically important data and rapid BW identification and weaponization characterization. This work was performed under the auspices of the U.S.DOE at UC, LLNL under contract number W-7405-ENG-48.

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