NEXT-GENERATION BIOAEROSOL DETECTION & IDENTIFICATION

Multi-dimensional Portable Mass Spectrometry For Biological Detection

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Recently, there has been an explosion of new rapid detection and diagnostic assays which can be used in the field or at the bedside. Modern genomic technologies have addressed specificity but are costly to develop and validate, require a significant amount of sample preparation, are generally performed in laboratory space, and can result in false negatives via genetic engineering or naturally acquired mutations. Mass spectrometry (MS) technologies have best-in-class sensitivity and specificity for detecting pathogens in clinical settings utilizing an approach known as mass fingerprinting. Mass fingerprinting is achieved by extracting biomolecules from samples and acquiring a precursor mass scan (MS1) on a mass spectrometer to yield a low fidelity 'fingerprint'.

Currently developed portable instrumentation has been targeting these unique features for the purpose of biological detection or peptide sequencing. To overcome the limitations of the current state-of-the-art in mass spectrometry to achieve bacterial identification, we have developed a multi-dimensional mass fingerprinting technique that enables biological identification down to the strain-level on a low-resolution mass spectrometer capable of tandem MS. For this approach, rather than interrogating a sample that is processed into proteins/peptides using a single MS scan, we propose to analyze an unprocessed raw sample (containing all biochemical species; lipids, proteins, peptides, and metabolites) by combining Matrix-Assisted Laser Dissociation/Ionization with low resolution-tandem mass spectrometry. We obtain and record a mass scan (MS1) of the sample as introduced into the mass spectrometer via MALDI. We then obtain and record a series of fragment mass scans (MS2) from mass windows (~m/z 100-200) of the MS1 scan. MS1/2 spectral data is combined, generating a unique high-fidelity fingerprint before classification using a supervised classifier. Once technical and biological replicates have been collected and successfully quality controlled, we will develop a comprehensive set of identifying fingerprints from across all targeted biological agents. With this peak list, we will use well-known and interpretable machine learning algorithms such as support vector machine (SVM), random forest (RF), and linear discriminant analysis (LDA).

To demonstrate the feasibility of this technique, we generated crude lysates from three different microbial species in biological triplicate, two unique strains for each, using a combination of organic solvents. These extracts were mixed with MALDI matrix and allowed to dry immediately before analysis. The spectral data were subjected to an unsupervised principal component analysis (PCA) to compare our ability to distinguish each sample from one another based upon multivariate separation within the data (MS1 only or MS1+MS2). Collectively, this data demonstrates important proof-of-principle for this mass fingerprinting approach and justifies future work focused on deployment using a portable instrument with tandem MS capabilities. Also, by integrating supervised machine learning with this new dimension of data, we hope to make an identification based upon our library and make a high confidence presumptive identification based upon spectral commonalities seen at the various phylogenetic levels. This approach is superior to MS1-only capable instrumentation developed a priori, and will provide a robust, fieldable biological detection modality for the Warfighter.