A New Opportunity Using Elemental Microbiological Multi-variate Analysis for the In Situ Identification of Astrobiological Materials

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Introduction

It is known that LIBS is a sensitive spectrochemical technique that can rapidly determine a target's elemental composition with excellent spatial resolution and minimal sample preparation. It has only recently begun to be used for characterizing biological samples such as microorganisms (i.e. bacteria) and tissues. On the basis of a bacterium's unique elemental (mostly inorganic) composition, LIBS can be utilized to provide a potentially faster, more portable, and more robust technology than many other methods to perform rapid measurements which are useful for the detection and identification of harmful pathogens in real-time at the point-of-care.

We have already demonstrated that bacteria do possess unique atomic signatures that are robust through time and environment that can be measured via LIBS and that these signatures can be used to rapidly identify an unknown bacterial specimen – Elemental Multi-variate Microbiological Analysis. Extensive studies are now underway to determine the biological variability of these signatures, the effects of sample contamination and mixing, the limits of detection, the ultimate specificity, and to answer numerous other important questions to transition from a laboratory technique to a fieldable, clinical technology.

Chemometric Analysis

Numerous multi-variate chemometric routines have been applied to classify LIBS spectra:

- discriminant function analysis (DFA) / linear discriminant analysis (LDA)
- principal least squares–discriminant analysis (PLS-DA)
- principal component analysis (PCA)
- artificial neural networks (ANN)

Specificity of LIBS Spectra

RESEARCH question: Can we identify bacteria using LIBS spectra?

Hypothesis: Bacteria possess unique elemental (mostly inorganic) composition that can be classified using multi-variate chemometric analysis.

Testing Hypothesis

- Bacteria are cultured on a solid nutrient agar plate. Individual colonies are picked to create test samples.
- LIBS spectra are obtained from these test samples and used to train a multivariate discriminant analysis.
- The trained model is then used to test the identification accuracy of bacteria.

Results

- LIBS spectra obtained from bacteria prepared identically over the span of months/days/years. This suggests that inherent repeatability is not a limiting factor.
- The DFA grouped LIBS spectra by genus (e.g. Staph or Strep), or by species (e.g. E.coli or M. smegmatis) on the basis of real, reproducible differences between these groups and similarities among members within the group.

State of Growth / Nutrient Media

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Presence of Biochemicals

Along with other bacteria, biological samples may contain other biochemicals and/or inorganic salts which may obscure the bacterial signal.

- Aim: To investigate the effect that salts and proteins in sterile urine have on the identification of Staphylococcus epidermidis.
- Sample S. epidermidis were prepared in identical fashion, then suspended in de-ionized water (Goren norm) and sterile urine (3-red triangles).
- Spectra were mounted without washing or any preparation and analyzed with EMMA.
- All specimens possess identical LIBS spectra and were 100% identified correctly and discriminated from E.coli and Strep. 

Dilution Experiment / Linearity

Some microbiological tests are cell number-dependent. The LIBS total spectral power should be linearly dependent on cell-number, but specificity should not depend on cell number.

- Aim: To demonstrate that EMMA specificity is not dependent on cell number.
- Serial dilutions of M. smegmatis were prepared and mounted identically.
- Bacterial specimens at all titers (concentrations) were identified correctly and discriminated from a mutant strain of M. smegmatis and S. viridans.

Mixing Experiment

- Non-sterile samples may contain other types of bacteria which may confuse the LIBS signature.
- Aim: To examine the discrimination ability in mixed cultures.
- Mixtures of known mixing fraction were prepared from suspensions of E. coli and Strep.
- Six mixtures were prepared with a ratio of E.coli to Strep of 0.2, 0.5, and 0.8, each with 1050-10-15-20 E. coli/mL.
- Multiplicate 5 mL tubes of these mixtures were prepared, thoroughly sampled six times, and then centrifuged for 2 minutes at 3000 rpm.
- Specimens were mounted and analyzed via EMMA.
- Down to 60-90% mixing fraction, the LIBS discrimination was identified with 95% specificity.
- Specifically decreased to 30% for 30% mixing fraction, as expected.
- Anecdotally any sample classified as anything other than one of the two mixing species.

Alive / Dead / Inactivated

In the environment or in vivo the bacteria may be in various stages of growth: actively reproducing (log phase), in stasis (lag phase), or may have been inactivated due to exposure to antimicrobials, UV light, starvation, etc.

- Aim: To examine the effect that the bacterial stage of growth has on the LIBS spectral fingerprint.
- Specimens of non-pathogenic E. coli were prepared identically and harvested in log-phase (reproducing exponentially).
- After harvesting, one specimen was killed via autoclaving, one specimen was mounted on agar and exposed to 248 nm ultraviolet light for 5 minutes.
- All bacteria possessed identical LIBS spectra and were classified as E. coli.