Raman Chemical Imaging Provides Rapid, Non-Invasive and Reagentless Biothreat Detection

Session VIII: Technology Forum Focus Groups
Group I: Chemical/Biological/Explosive Detection & Security

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Raman Chemical Imaging Provides Rapid, Non-Invasive and Reagentless Biothreat Detection

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Chemical Imaging - Molecular spectroscopy and digital imaging for chemical analysis of materials

Conventional Imaging of Low Contrast Object

Components:
- Polystyrene
- Nylon

Chemical Image Contrast based on Composition, Structure & Concentration

Why Chemical Imaging?
- Spectrum provides intrinsic contrast
- No need for dyes, stains or reagents
- No sample preparation
- Fast, noncontact & nondestructive
- Spectroscopy provides fingerprint for material
• High performance plastics (ex. car bumpers) are blends of polymers
• Chemical imaging improves cost performance
Chemical Imaging integrates multiple, orthogonal detection strategies.
Raman Spectroscopy

- Inelastic scattering phenomenon
- Laser based technique
- Probes energy of molecular vibrations - provides a “fingerprint”

Advantages

- H₂O not an interference (compatible with aqueous systems)
- Little to no sample preparation
- Small amount of sample required
- Operates in ‘reflectance’ mode
- Diverse experimental conditions (fiber optics & microprobes)
- Suitable for *in situ* monitoring (no vacuum required)
- 50 – 4,000 cm⁻¹ coverage in single instrument
- Glass optics including fiber optics and fiberscopes
- Usually nondestructive and noninvasive
- Suitable for aqueous, gaseous and solid samples
- Inorganic and organic material analysis
- High spatial resolution (250 nm) imaging

Disadvantages:

- Weak phenomena – 1 out of 10⁶ photons a Raman photon
- Moderate sensitivity (0.1-1 wt%)
- Fluorescence interference (can be minimized)
- Not quantitative unless internal standard is used
ChemImage Technology
Acquisition Time: 10 Seconds
Information Content: 256,000 Pixels

Conventional Technology
Acquisition Time: 2.8 Hours
Information Content: 1024 Pixels

Why is ChemImage’s Technology Unique?
• Requires No Sample Pre-Treatment
• Rapid Analysis Time: Typically 5 Minutes or Less
• Valid Results: No Need for Additional Tests
• Not Limited to a Specific Biothreat Agent
• Has the Ability to Detect Multiple Agents
• Readily Adaptable to New Biothreat Agents
ChemImage Instrumentation Platforms

**Micro**
- FALCON™
- Large surface area analysis
- Macro/Micro zoom optics
- NIR, Raman, PL, Fluorescence, Color

**Macro**
- CONDOR
- Real-time video imaging
- Laser Raman spectroscopy
- NIR, Fluorescence & Raman Chemical Imaging

**Remote**
- RAVEN™
- Chemical imaging

**Software**
- ChemAnalyser™
- Image analysis, measurement, and visualization

- Dispersive Raman platform
- High definition imaging
- 250nm spatial resolution
- Entry level systems
- Volumetric imaging capable
- Raman, PL, Fluorescence, NIR, Color

U.S. Patent No. 6,002,476
Patent Pending
ChemImage Copyright

ChemImage Application Examples

Forensics: fingerprint detection
- Latent fingerprint not previously detectable with existing technology
- Technique is non-destructive

Pharmaceuticals: drug particle size
- Drug particle size not detectable with any other existing technology

Semiconductors: ion implant imaging
- Raman imaging has unparalleled sensitivity for ion implantation

Polymers: 3D blend imaging
- Volumetric Raman Chemical Imaging provides non-destructive whole object molecular imaging
Biothreat Detection
• Molecular analysis complicated and difficult for mixtures of really small things
• Bioagents are the hardest: small, complex organisms, in cluttered backgrounds
• Bioagents are usually invisible, odorless, taste-free; human senses can not recognize when exposure has occurred

How are We Addressing the Problem?
• Molecular chemical imaging technology has demonstrated great promise in addressing this problem
• Works even for single bacteria … and… orders of magnitude faster than conventional techniques
• Chemical molecular identification possible – now being validated with Government Labs (AFIP, ECBC, NRL)
Chemical-Biological Warfare Threat Detection and Identification Methods

**Bacteria**
- Anthrax
- Plague
- Rabbit Fever
- Diptheria

**Rickettsiae**
- Typhus
- Spotted Fever
- Q-Fever

**Viruses**
- Yellow Fever
- Dengue Fever
- Influenza

**Toxins**
- Botulinum Toxins
- Mycotoxins
- Staphylococcus
- Saxitoxin

**Chemicals**
- Sarin
- Soman
- Mustard

- Chemical Imaging & Spectroscopy
- MALDI-TOF Mass Spectrometry
- Flow cytometry
- Polymerase chain reaction
- Whole-cell immunosensors
  - Colorimetric
  - DNA-based
  - Particle tag-based
  - Gravimetric
  - Electrochemical

- Chemical Imaging & Spectroscopy
  - Mass spectrometry
  - 2D gel electrophoresis
  - Immunosensors
    - DNA-based
    - Colorimetric (ELISA)
    - Nanoparticle tag
      - Quantum dot
      - Upconverting phosphor
    - Gravimetric
    - Electrochemical

- Chemical Imaging & Spectroscopy
  - Ion mobility spectrometer
  - Mass spectrometry
  - SAW sensor with sorption coating
  - FPW sensor with sorption coating
  - FTIR

• Raman analysis rapidly provides molecular fingerprint (<5 min)
• Confirmatory test
• Single spore detection limit
• Diagnostic for:
  • Species
  • Strain
  • Viable vs Nonviable

In cooperation with:
Dr. Ted Hadfield
Armed Forces Institute of Pathology
AFIP Samples – *B. Anthracis* in Sporulation Broth

Dispersive Raman Spectroscopy – 10 Different Regions of Interest

- Statistical Analysis (F-Test) indicates reproducibility to 95% confidence level
- Collected with FALCON Raman Chemical Imaging Microscope
- Data Acquisition Time: 60 sec/spectrum
Raman Spectra of RAAD Program BG Spores
Using a ChemImage FALCON™ Raman Chemical Imaging Microscope

Perform in Collaboration with
- Dr. Jay Eversole, NRL
- Dr. Steve Christesen, ECBC
- Dr. Antonio Sanchez, MIT LL

- Interlaboratory results are reproducible
- BG traceable to Dugway
**Rapid Automated Detection of BG Spores**

1 second Acquisition Time

**Dispersive Spectra**

- Target Spectrum
- BG library Spectrum

- True positive identified within 1 sec, using automated approach
Bacillus Anthracis Limit of Detection Using Raman Chemical Imaging

Single Spore Detection in 25 sec

\[ y = 2.771x - 2.0461 \]

Fused Raman/Optical Image

Single Spore S/N ~ 1.91

Spore Density (\# of Spores/cm\(^2\) x \(10^5\))

Raman Integrated S/N
Raman Analysis of BG/Aspergillus terreus Mixture
Using a ChemImage FALCON™ Raman Chemical Imaging Microscope
• Using un-optimized discrimination approach, at 90% probability of detection, 5X improvement in false alarm rate demonstrated
Chemical Imaging Analysis of Mixtures

- Mixtures are not homogeneous on the microscopic scale
- Spectra obtained from different sample locations are different
- Chemical Imaging (i.e. spatially-resolved spectroscopy) rapidly provide a set of spectra incorporating these variations
- Data analysis tests set of mixture spectra against all of the compounds in its library and determines the compounds in its library likely to be present
- A ranking system selects and reports the compounds present

Specimen is white powder containing (Talc, Tylenol, BG spores)

Spatially Resolved Spectra

Reflectance images with sampling grid

Results of Automated Identification
Raman Chemical Imaging of Anthrax on Food & Bodily Fluids

Using a ChemImage FALCON™ Raman Chemical Imaging Microscope

Mucin, BG, and Onion Raman Spectra

Threats to Food
BG and Onion
Optical Image

Threats to Humans
BG and Mucin
Optical Image

Raman Chemical Image

3 µm
**Blind Study of BG/Mucin Samples**

**Sensitivity** = 100%
**Selectivity** = 100%

*Sensitivity (true positive rate): %Sensitivity = (TP/(TP+FN))*100, where TP = true positives and FN = false negatives.

*Selectivity (true negative rate): %Selectivity = (TN/(FP+TN))*100, where TN = true negatives and FP = false positives.
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EAGLE Transportable Microscope System

- ChemImage has successfully demonstrated its EAGLE Transportable Microscope System
- Anthrax (simulant) detected in seconds – comparable to FALCON
- EAGLE can automatically identify presence of biothreat by using Biothreat Database
- Features
  - Fluorescence, Colorimetric Chemical Imaging (targeting)
  - Dispersive Raman Chemical Imaging (identification)
  - Wireless and Remotely Controlled
  - Live Digital Video

Fluorescence Chemical Imaging
BG/Diesel Soot/Road Dust Mixture

Fluorescence Chemical Image

Fluorescence Spectra

Bacillus globigi (BG)
Diesel Soot
Road Dust (non fluorescing)
Conclusions

- Chemical Imaging detection is non-invasive, non-contact and does not require significant sample preparation or reagents
- ChemImage technology can allow users (physicians, law enforcement, soldiers, researchers) see and identify materials (cancer, biothreats, evidence) that you can’t detect now
- Chemical Imaging is inherently orthogonal, integrating multiple detection strategies into the same system
- Normal Raman spectroscopy is highly selective and sensitive (single spore detection demonstrated) when targeted
- Optical imaging and fluorescence imaging sensitive means for targeting
- Chemical Imaging provides excellent sampling statistics, which compensates for spore to spore variability and enables morphometric assessment
- Widefield illumination important for Chemical Imaging
- Near term deployment of Chemical Imaging technology conceivable, based on mature, commercially available Raman technology
- Technology scalable
  - Field transportable technology demonstrated
  - Fully portable technology feasible
  - Basis for a hand held point Chemical Imaging sensor