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

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

Steven Vanni
ChemImage Corporation
7301 Penn Avenue
Pittsburgh, PA 15208
m: (412) 478-3504
f: (412) 241-7311
vanni@chemimage.com
www.chemimage.com

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Homeland Security
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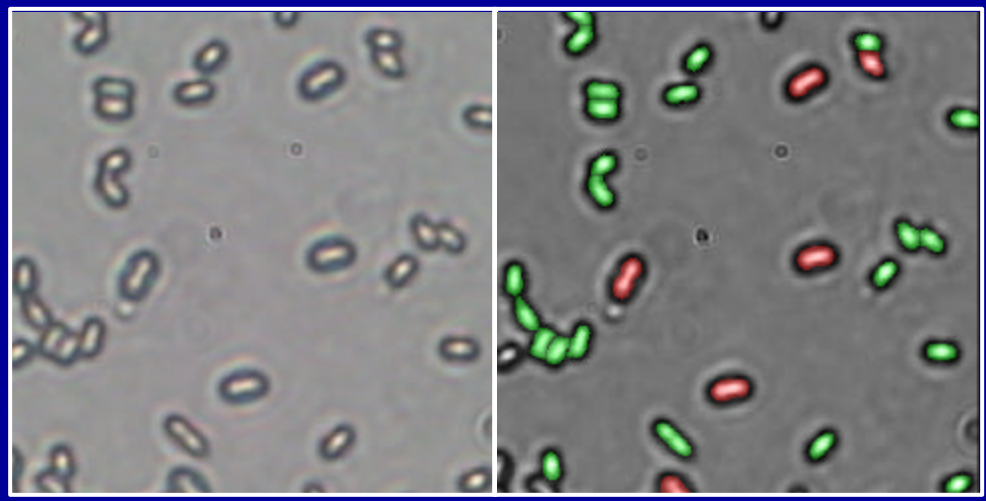
Security Division Symposium and Exhibition
18 June 2003
Reston, VA



In cooperation with:

Dr. Ted Hadfield
Dr. Kathy Kalasinsky
Dr. Vic Kalasinsky
Armed Forces Institute of Pathology

Dr. Steve Christesen
Dr. Alan Samuels
Janet Jensen
US Army, Edgewood Chemical & Biological Center

Dr. Jay Eversole
Naval Research Laboratory

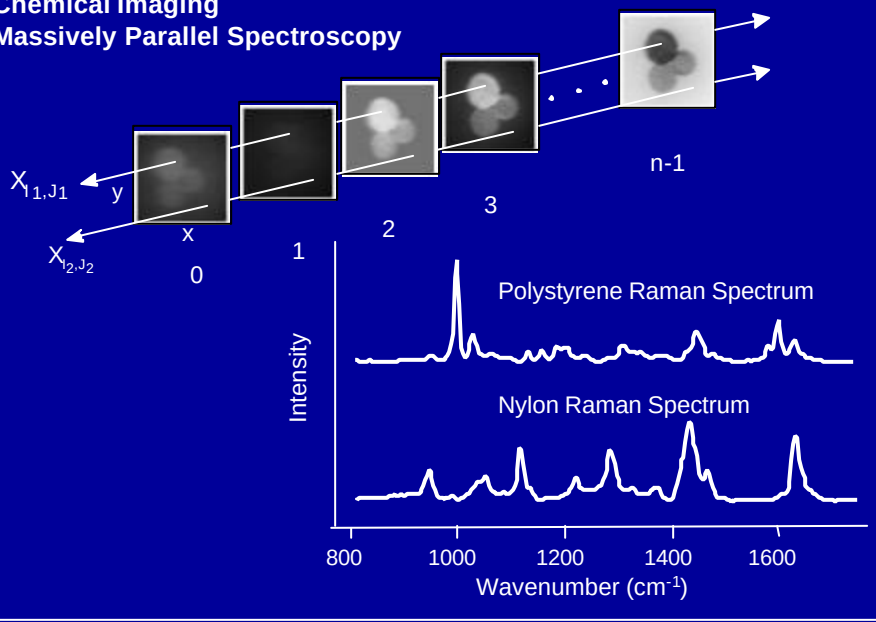
Dr. Patrick Treado
Dr. Matthew P. Nelson
Dr. Charles W. Gardner
Julianne Wolfe
Dr. Robert Schweitzer
Jason Neiss
ChemImage Corporation

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

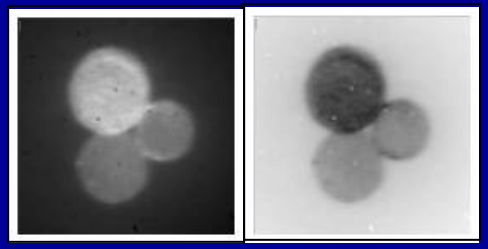
Conventional Imaging of Low Contrast Object

Components:
- Polystyrene
- Nylon

Chemical Imaging Massively Parallel Spectroscopy



Chemical Image Contrast based on Composition, Structure & Concentration



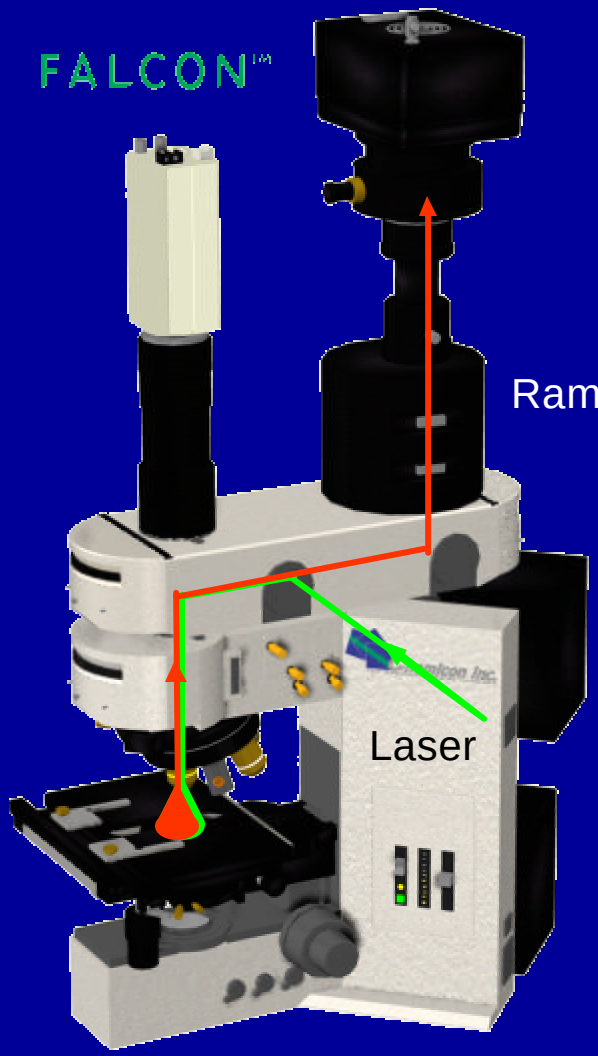
Raman image of Component A

Raman image of Component B

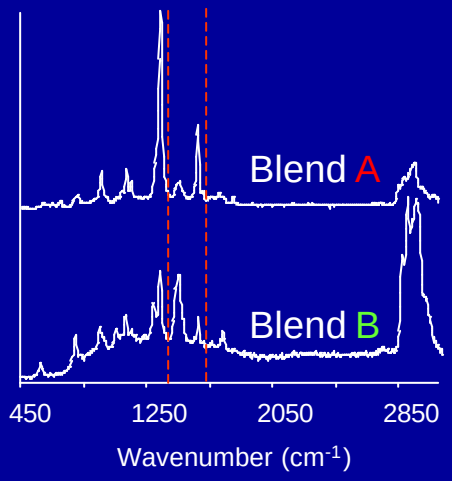
Why Chemical Imaging?

- Spectrum provides intrinsic contrast
- No need for dyes, stains or reagents
- No sample preparation
- Fast, noncontact & nondestructive
- Spectroscopy provides fingerprint for material

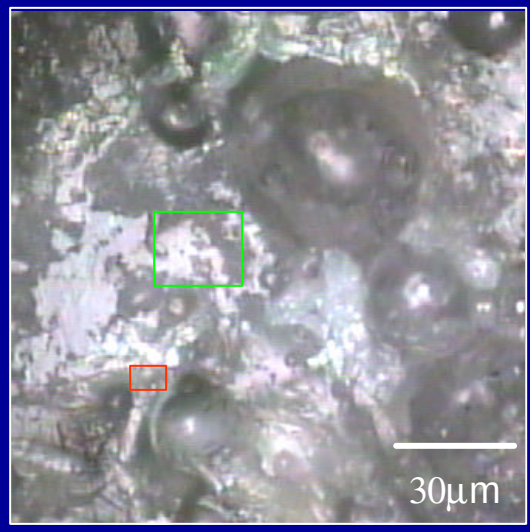
HOW DOES CHEMICAL IMAGING WORK?



Raman Spectra

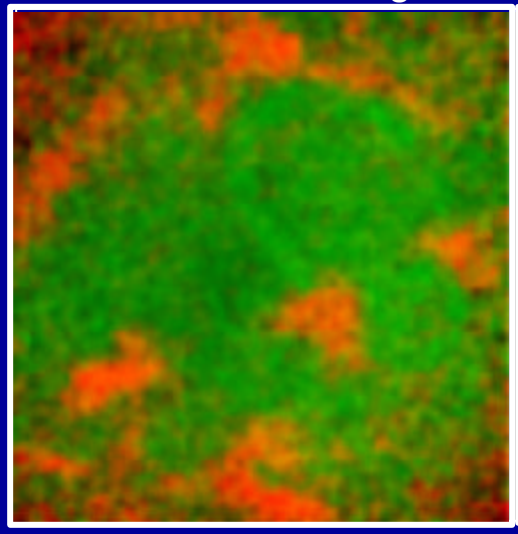


Polymer Blend Microscope Image

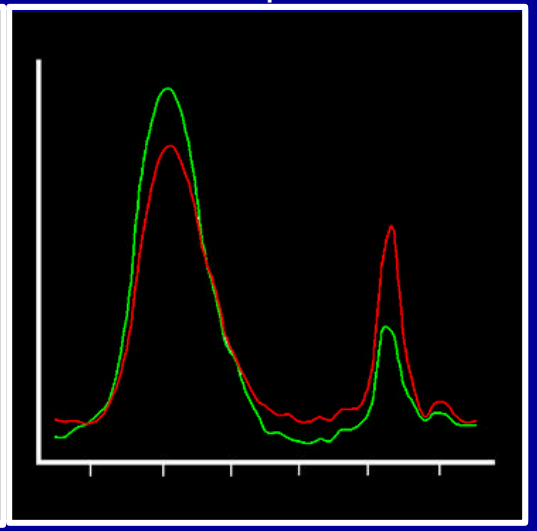


- High performance plastics (ex. car bumpers) are blends of polymers
- Chemical imaging improves cost performance

Chemical Image

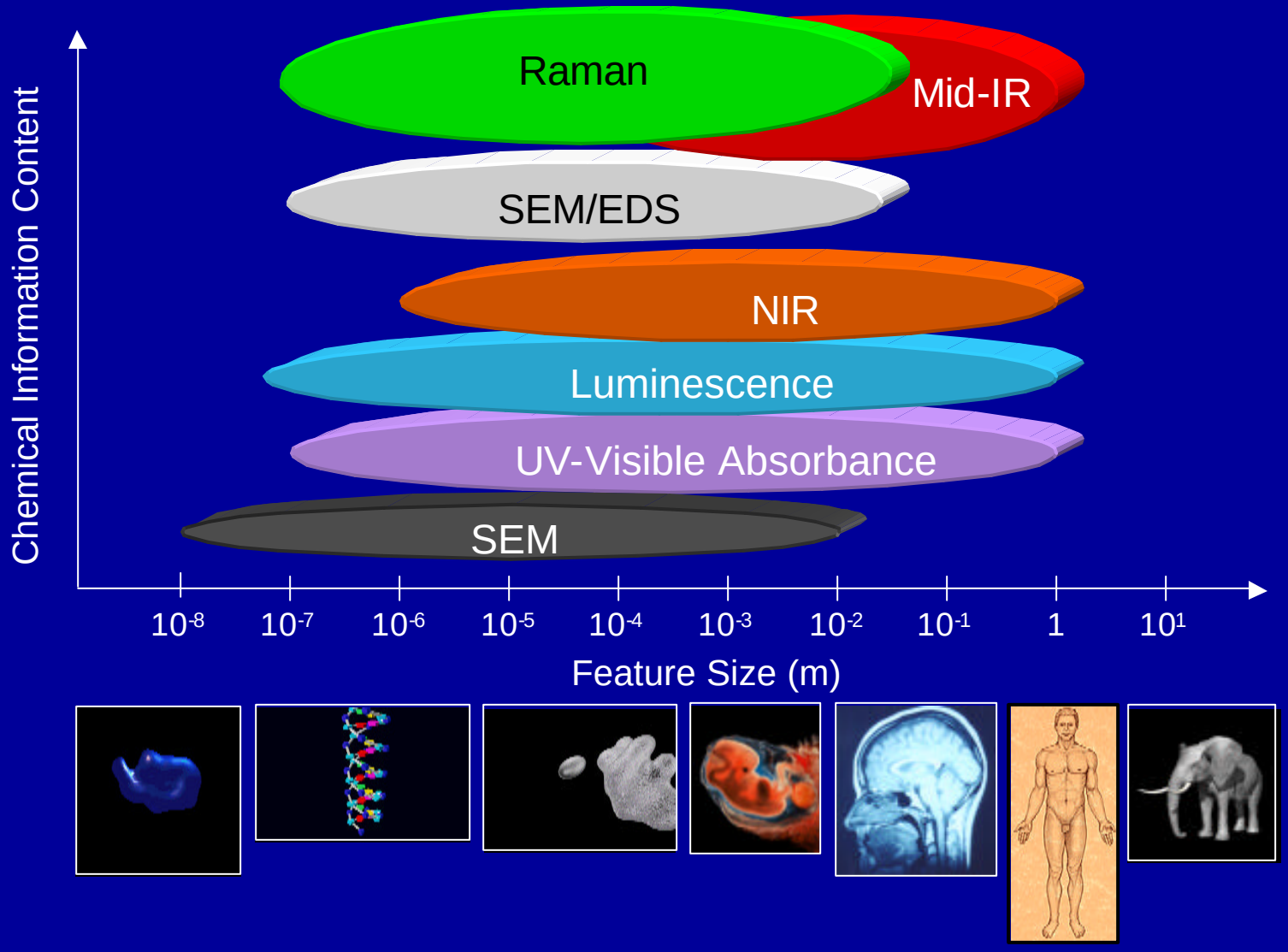


Raman Spectra



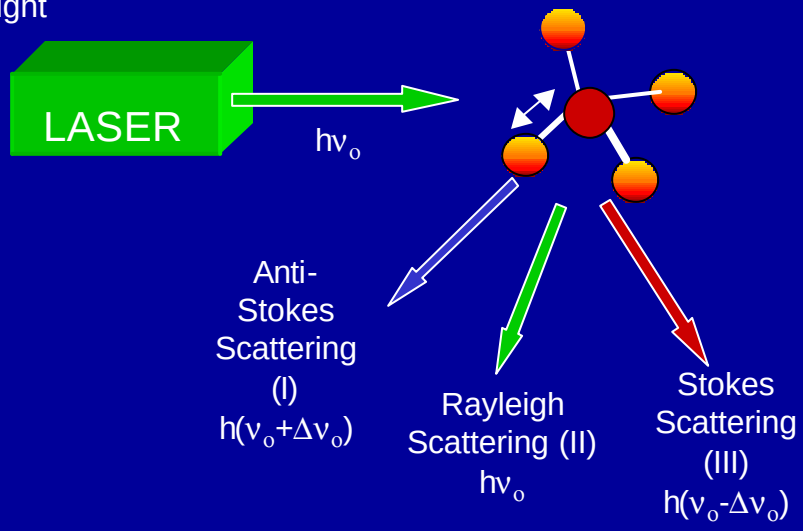
Chemical Imaging Techniques

- Chemical Imaging integrates multiple, orthogonal detection strategies



Raman Spectroscopy

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



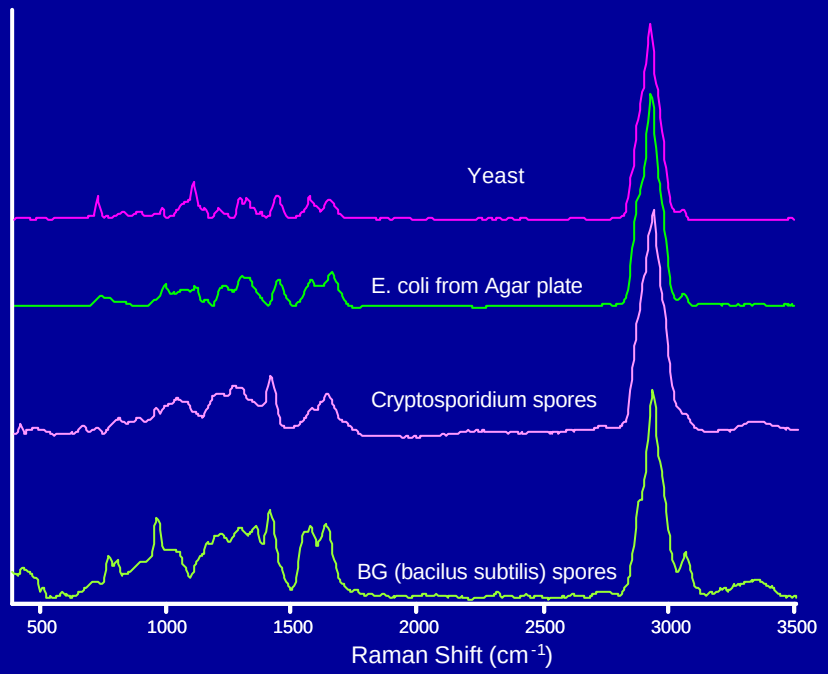
Advantages

- H_2O 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 10^6 photons a Raman photon
- Moderate sensitivity (0.1-1 wt%)
- Fluorescence interference (can be minimized)
- Not quantitative unless internal standard is used

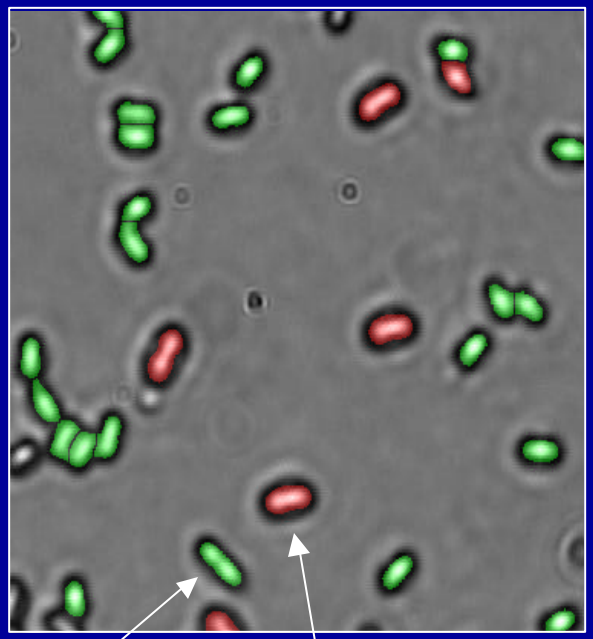
Raman Spectra are Specific



Chemical Imaging vs. Competitive Chemical Imaging Technology

ChemImage Technology

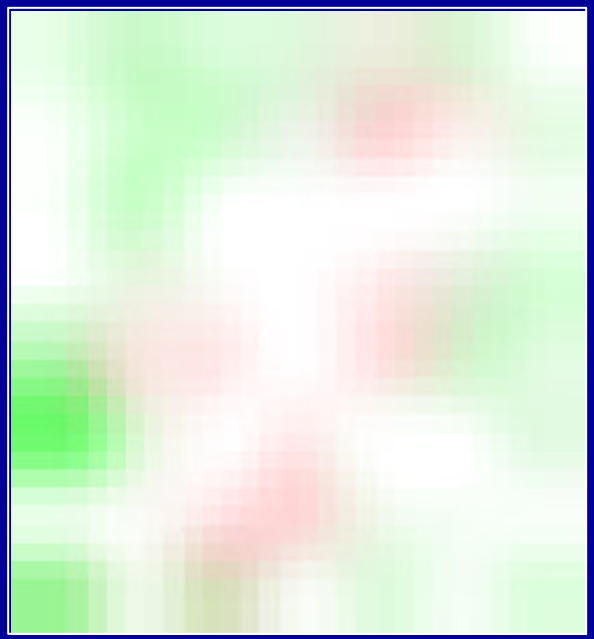
Acquisition Time: 10 Seconds
Information Content: 256,000 Pixels



Conventional Microscopy

Conventional Technology

Acquisition Time: 2.8 Hours
Information Content: 1024 Pixels

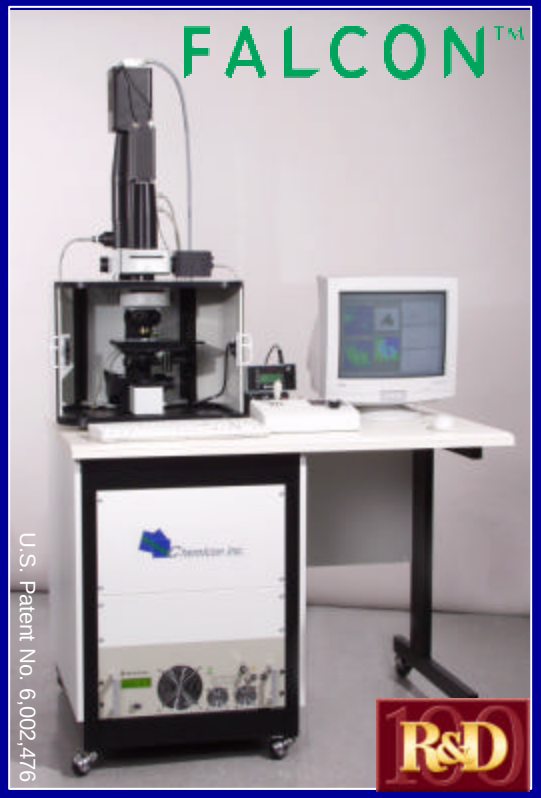


Why is ChemImage's Technology Unique?

- 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

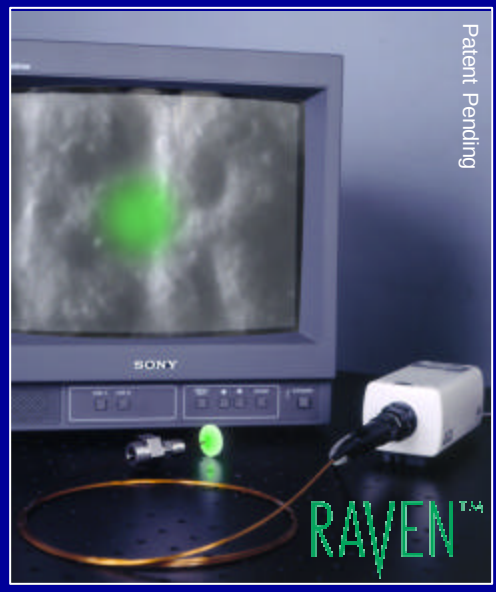


- Dispersive Raman platform
- High definition imaging
- 250nm spatial resolution
- Entry level systems
- Volumetric imaging capable
- Raman, PL, Fluorescence, NIR, Color

Macro

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

Remote



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

Software

ChemImage Application Examples

Forensics: fingerprint detection

Light Background Extract

Dark Background Extract

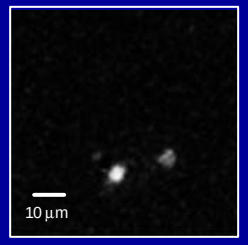
- Latent fingerprint not previously detectable with existing technology
- Technique is non-destructive

Counterfeit $10 Bill

Pharmaceuticals: drug particle size

Conventional Microscopy

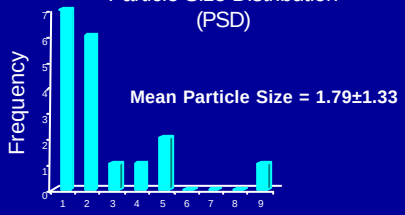
Raman Chemical Image



Drug Particles

- Drug particle size not detectable with any other existing technology

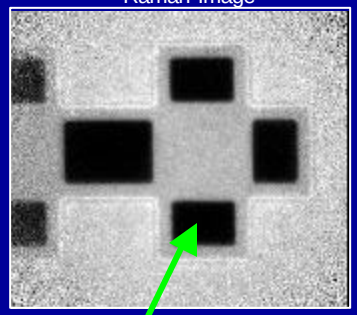
Particle Size Distribution (PSD)



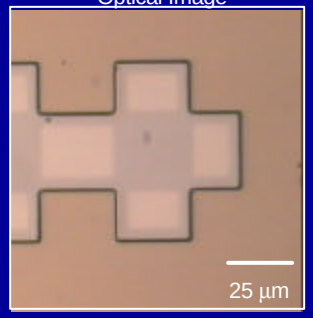
Particle Size (Max. Chord, µm)

Semiconductors: ion implant imaging

Raman Image



Optical Image



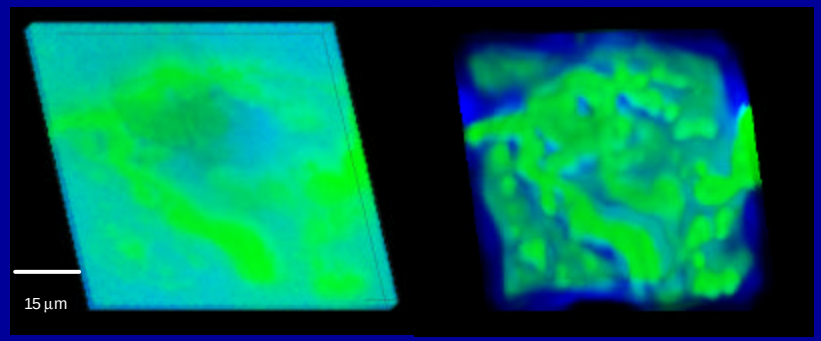
Implant: 2.0 x 10^{15} As cm^2

- Raman imaging has unparalleled sensitivity for ion implantation

Polymers: 3D blend imaging

3D Raman Chemical Image (Normalized Data)

3D Raman Chemical Image (Deconvolved Data)



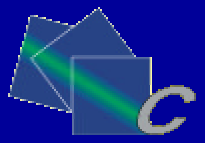
- Volumetric Raman Chemical Imaging provides non-destructive whole object molecular imaging

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)



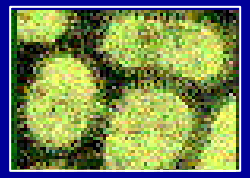
Chemical-Biological Warfare Threat Detection and Identification Methods

Biological → Chemical

Bacteria	Rickettsiae	Viruses
Anthrax Plague Rabbit Fever Diptheria	Typhus Spotted Fever Q-Fever	Yellow Fever Dengue Fever Influenza

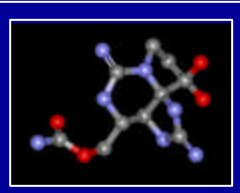
1 x 4 μm

0.25 x 1 μm



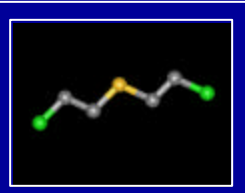
0. 1μm
(100 nm)

Toxins
Botulinum Toxins Mycotoxins Staphylococcus Saxitoxin



0.0001μm
(1nm)

Chemicals
Sarin Soman Mustard

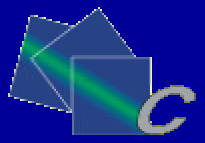


0.0001μm
(1nm)

- 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

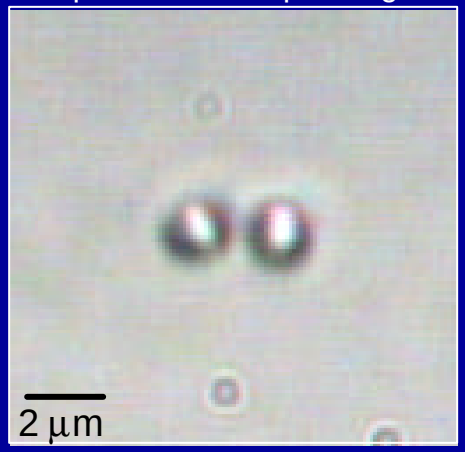


Matthias-Maser, S; "Primary Biological Aerosol Particles," in Atmospheric Particles, ed. By R. M. Harrison and R. Van Grieken (Wiley, New York, 1998), p. 349.

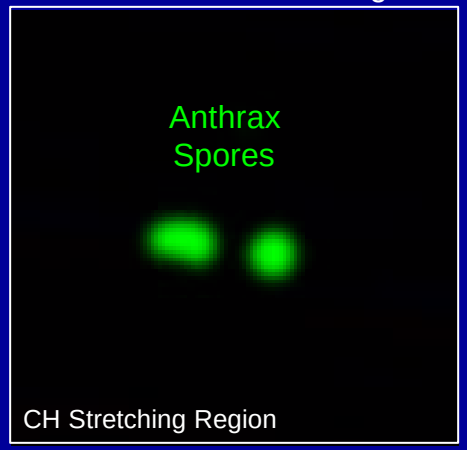
Raman Spectra of Anthrax Spores

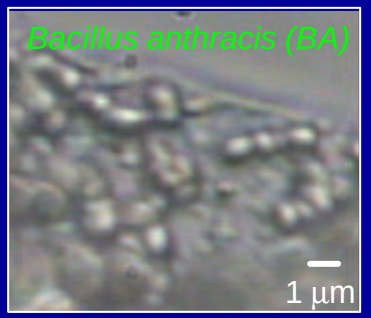
Using a ChemImage FALCONÔ Raman Chemical Imaging Microscope

Optical Microscope Image

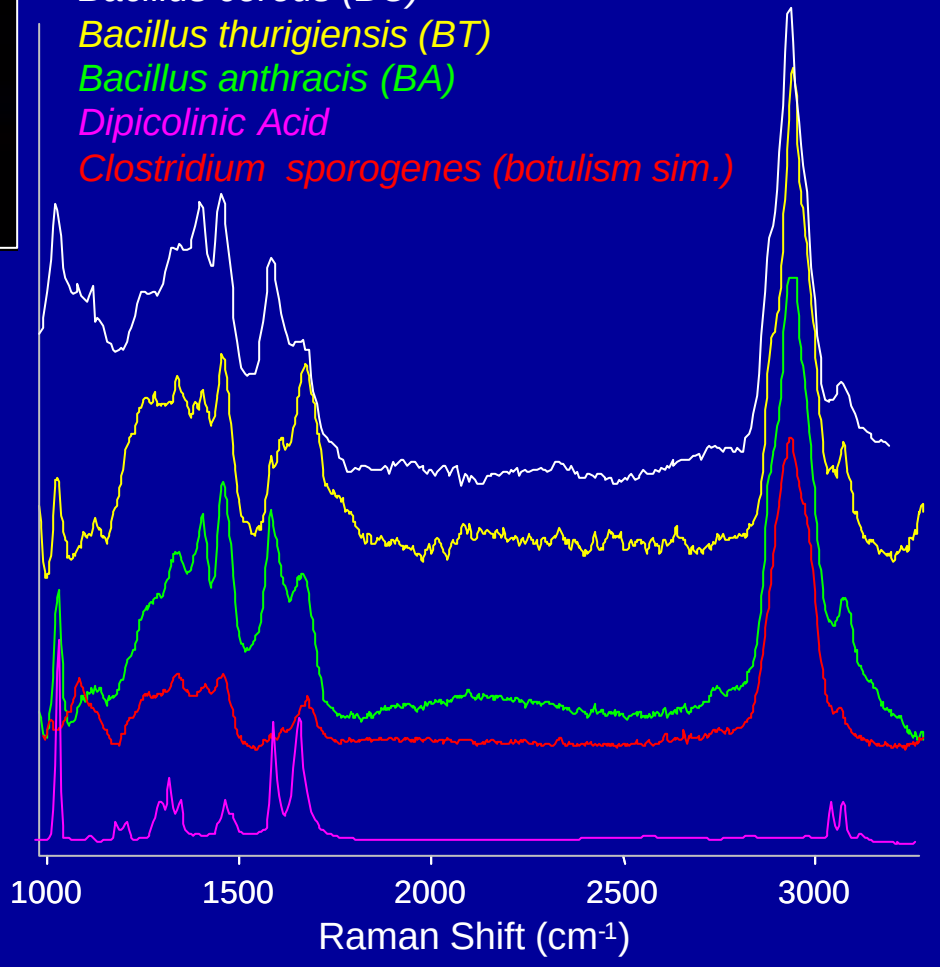


Raman Chemical Image





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



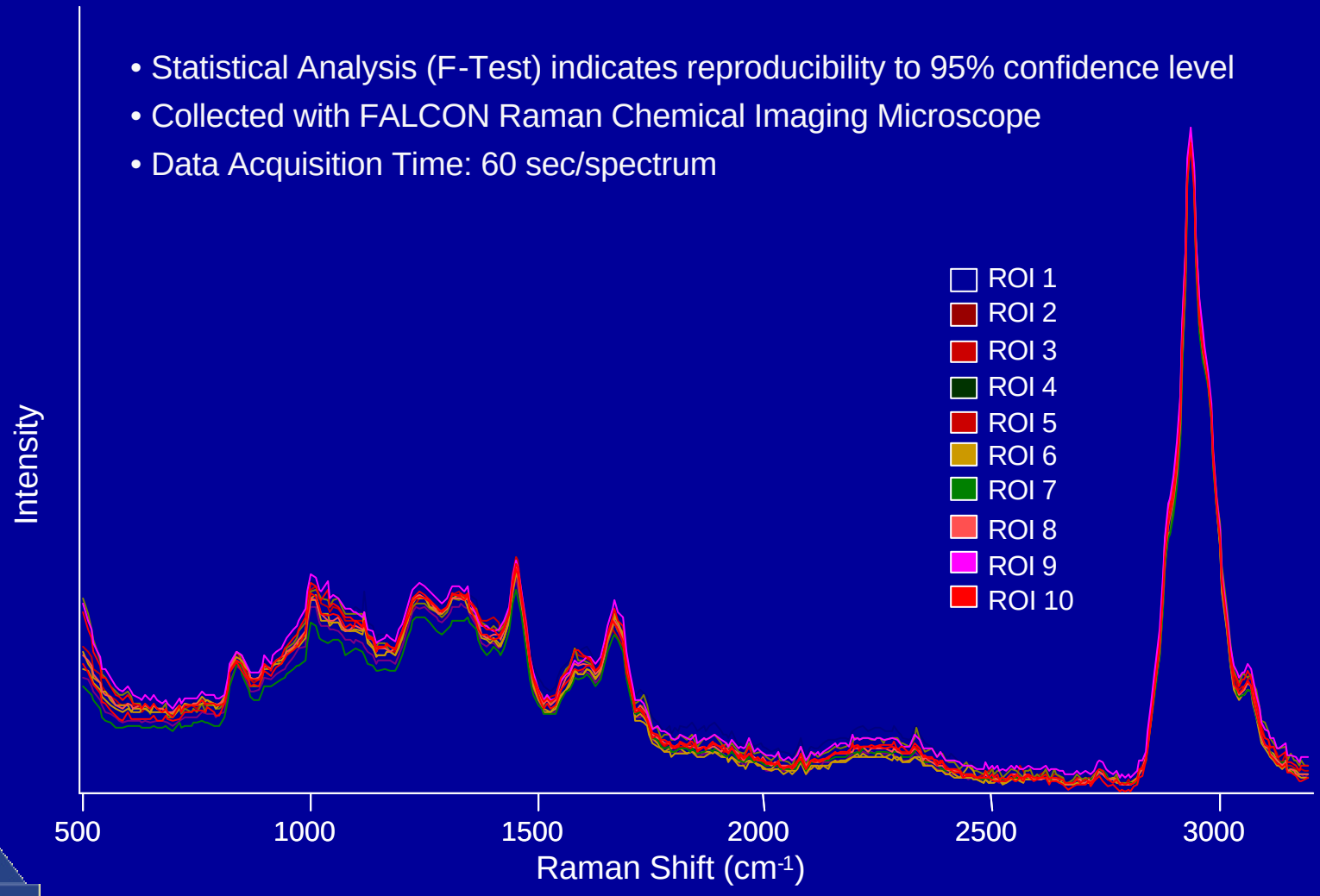
In cooperation with:
Dr. Ted Hadfield
Armed Forces Institute of Pathology

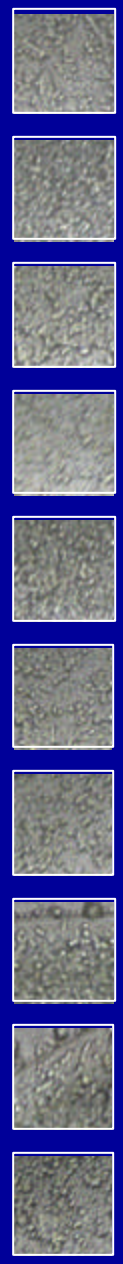
Raman Spectra are reproducible

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



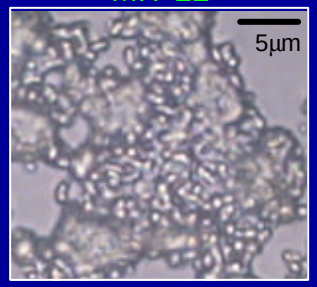


Raman Spectra of RAAD Program BG Spores

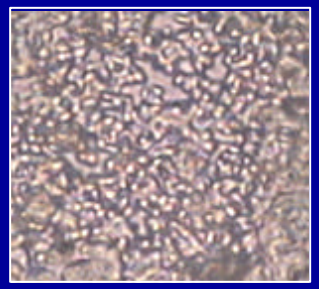
Using a ChemImage FALCONÔ Raman Chemical Imaging Microscope

- Interlaboratory results are reproducible
- BG traceable to Dugway

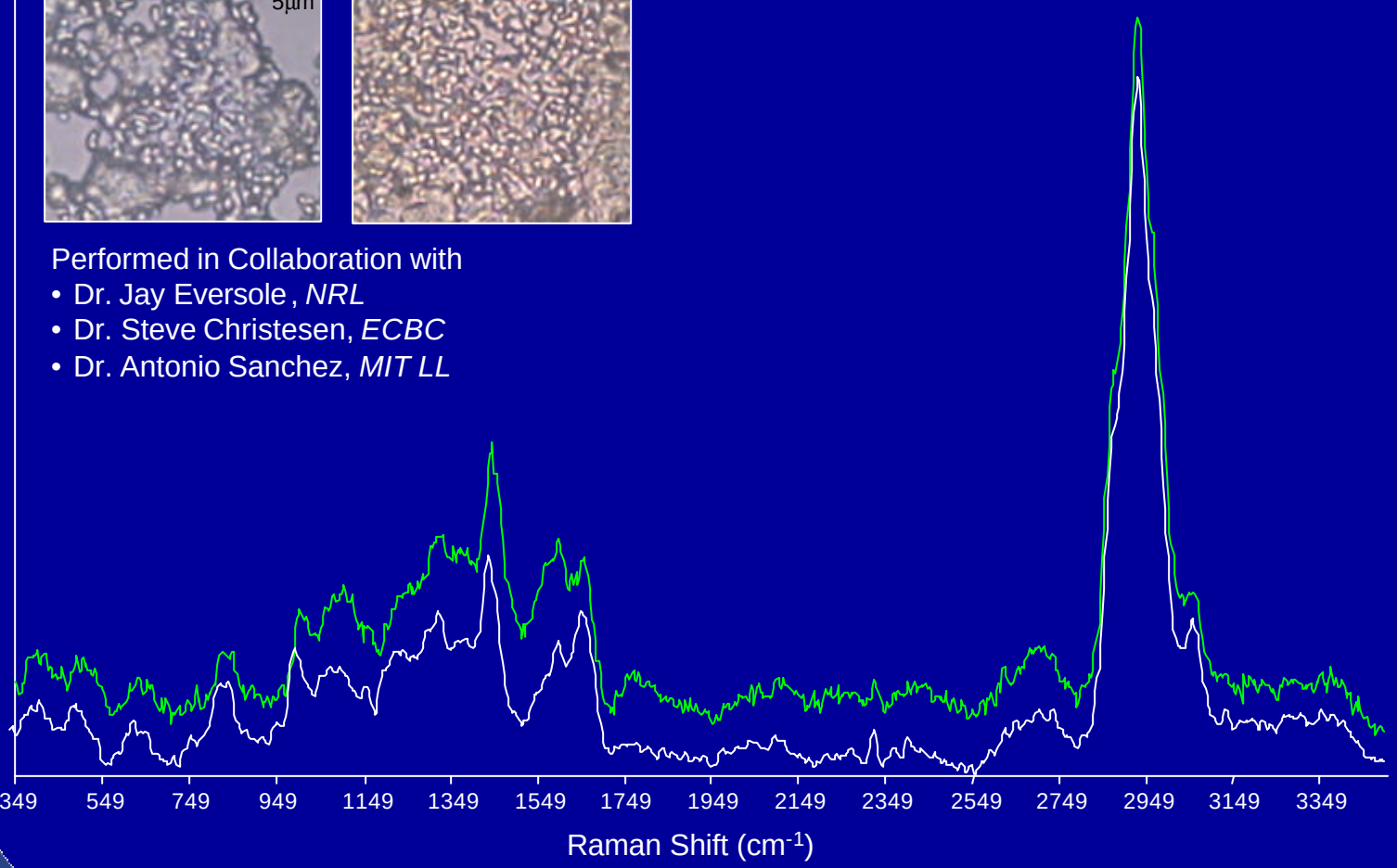
MIT LL



NRL



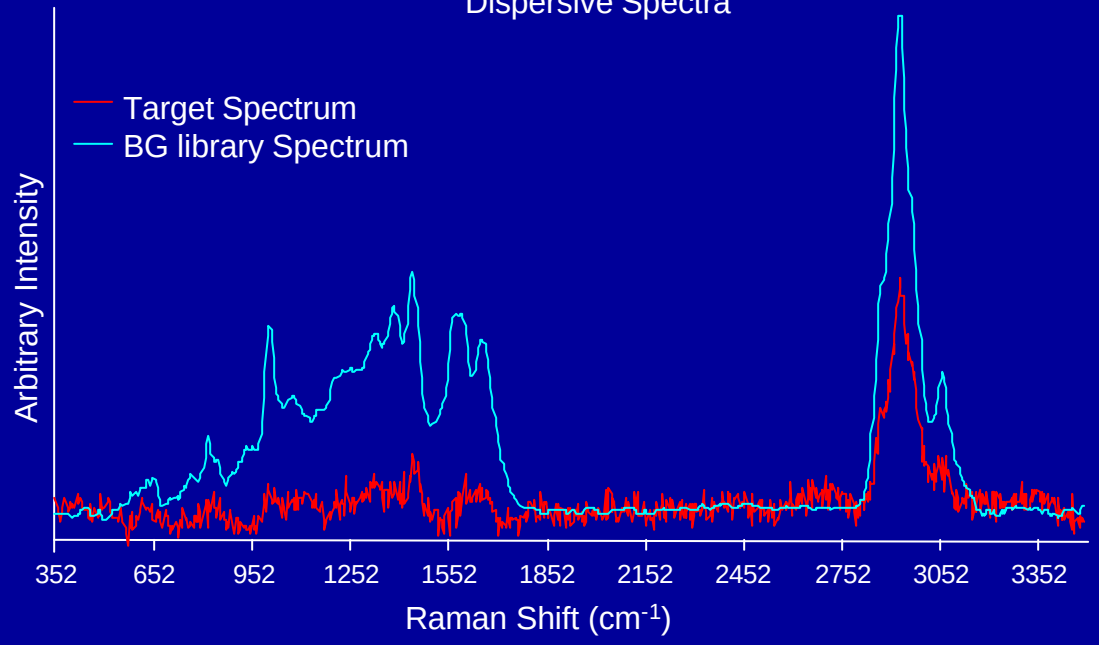
Performed in Collaboration with
- Dr. Jay Eversole , *NRL*
- Dr. Steve Christesen, *ECBC*
- Dr. Antonio Sanchez, *MIT LL*



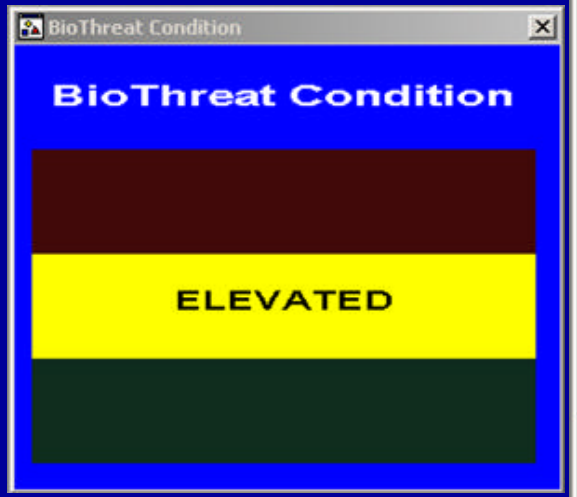
Rapid Automated Detection of BG Spores

1 second Acquisition Time

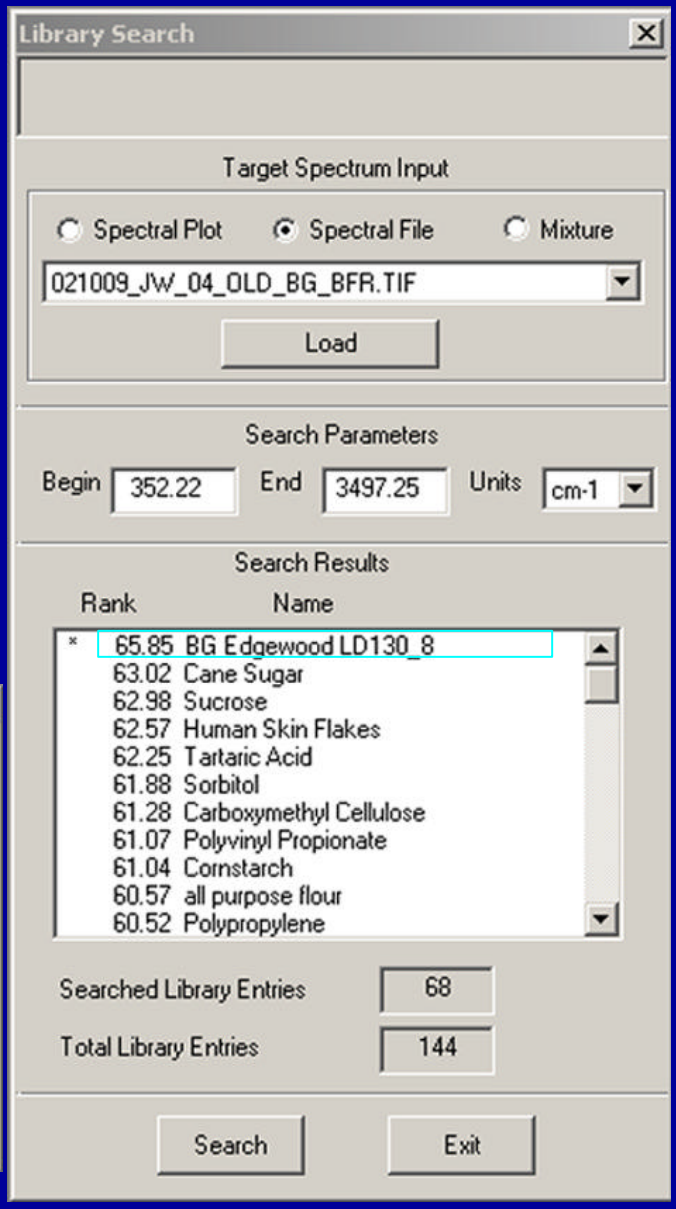
Dispersive Spectra



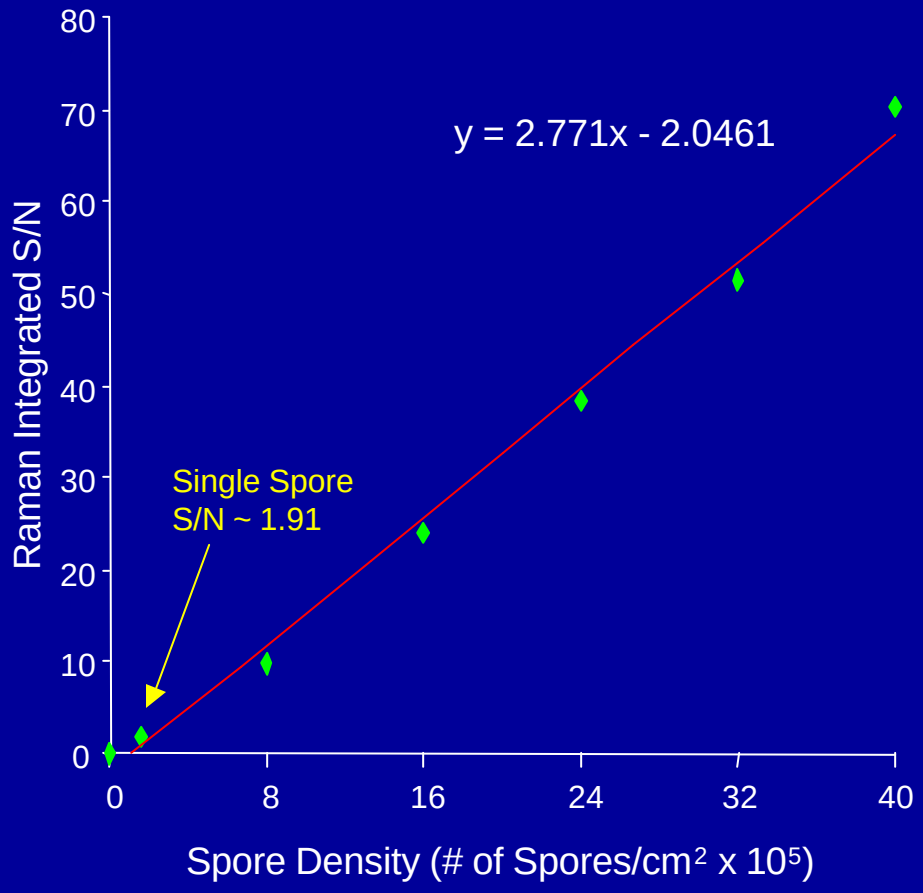
- True positive identified within 1 sec, using automated approach



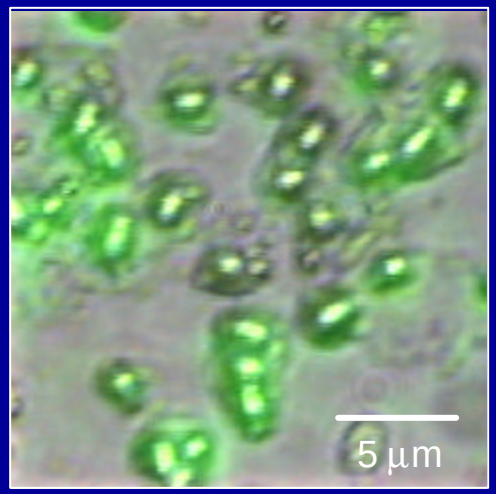
Spectral Library Search Dialog Box



Bacillus Anthracis Limit of Detection Using Raman Chemical Imaging



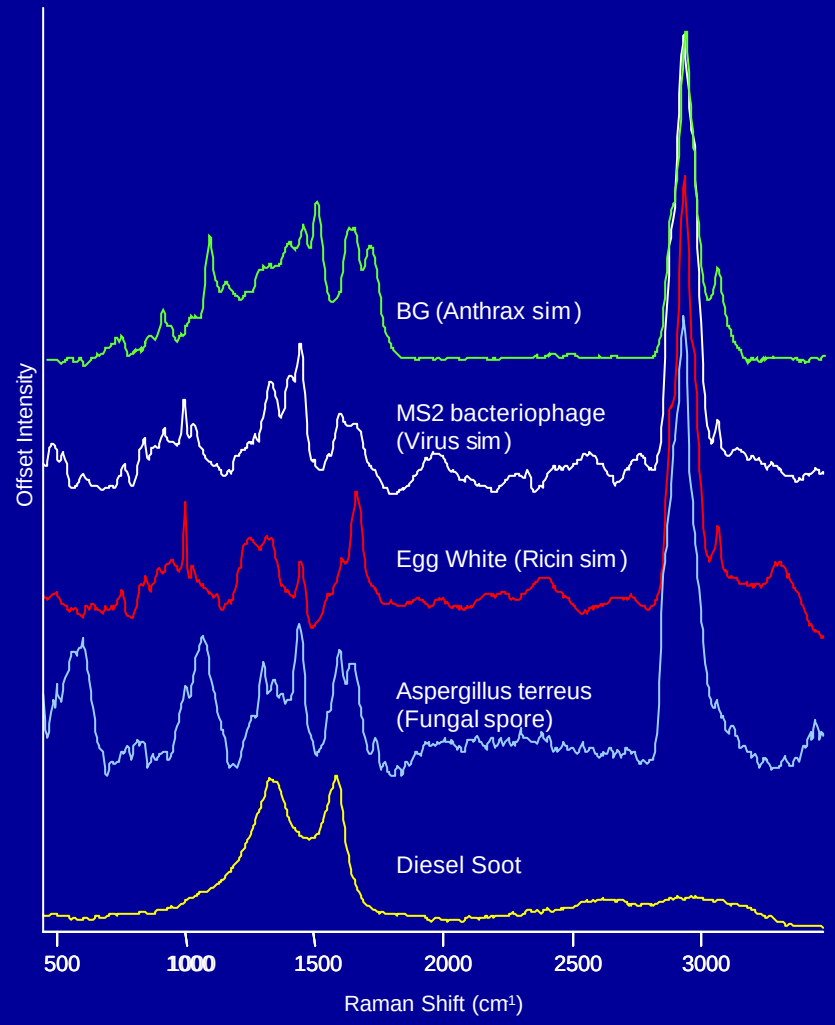
Fused Raman/Optical Image



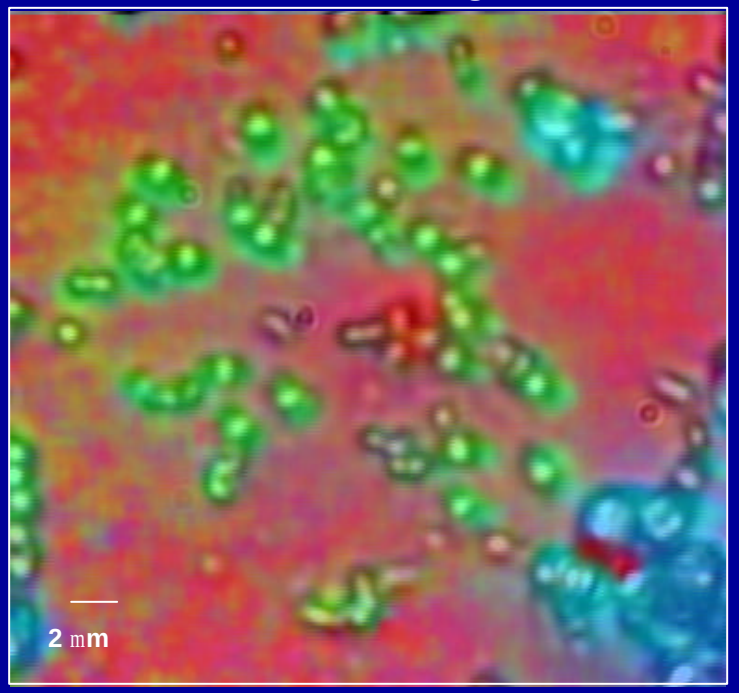
Single Spore Detection in 25 sec

Raman Analysis of *BG*/*Aspergillus terreus* Mixture

Using a ChemImage FALCONÔ Raman Chemical Imaging Microscope



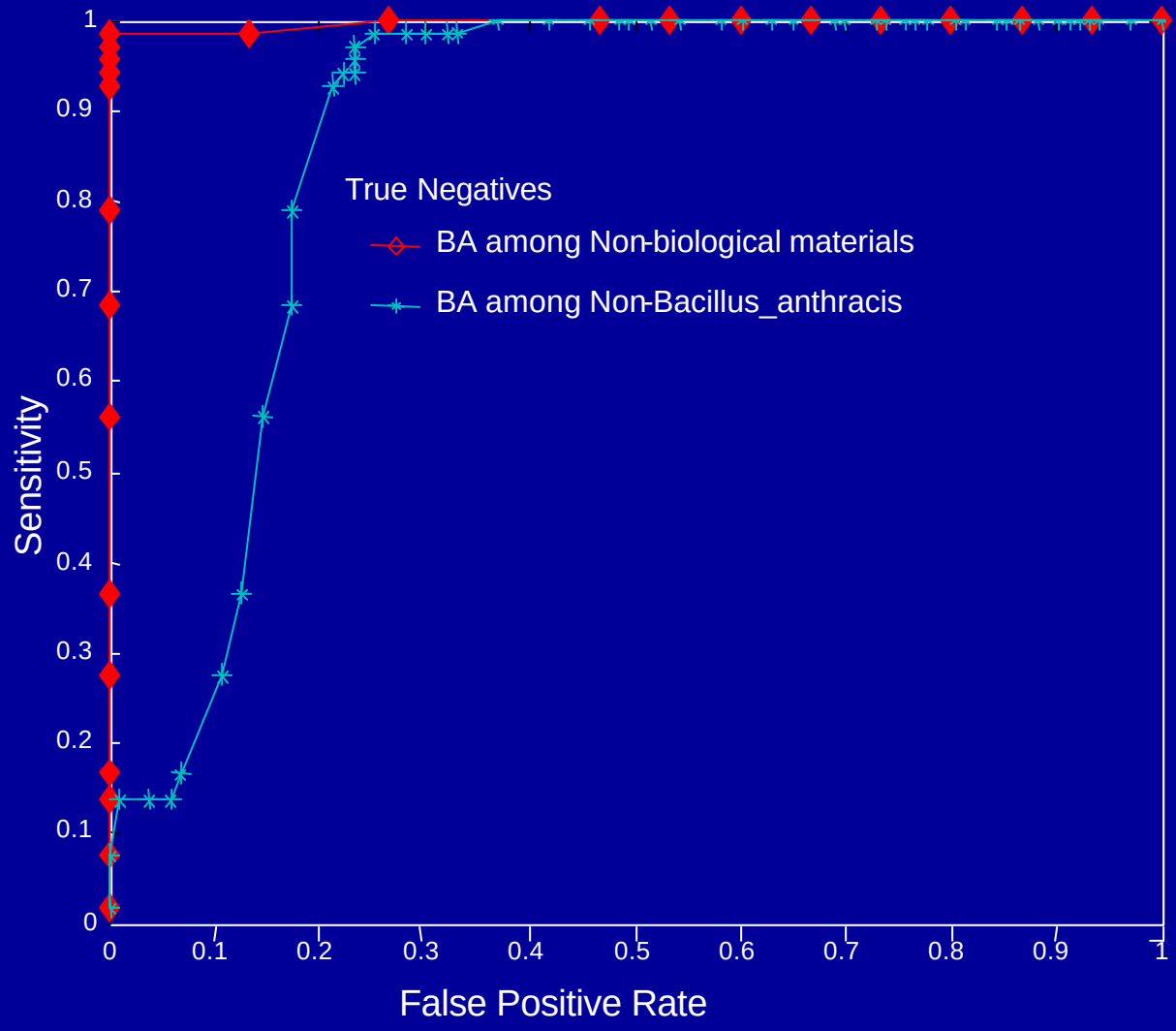
Raman Image



BG
conidia of A. terreus

Preliminary Receiver Operator Characteristic (ROC) Curve
Bacillus anthracis Discrimination Assessment

- Using un-optimized discrimination approach, at 90% probability of detection, 5X improvement in false alarm rate demonstrated

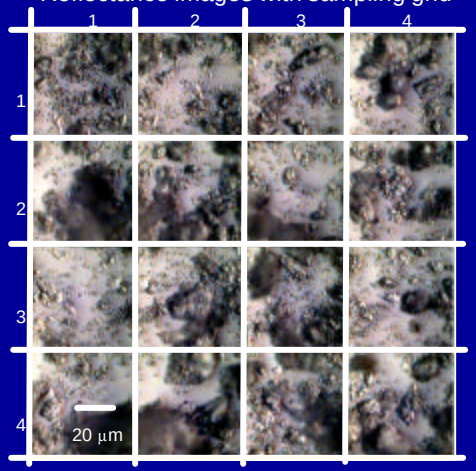


Chemical Imaging Analysis of Mixtures

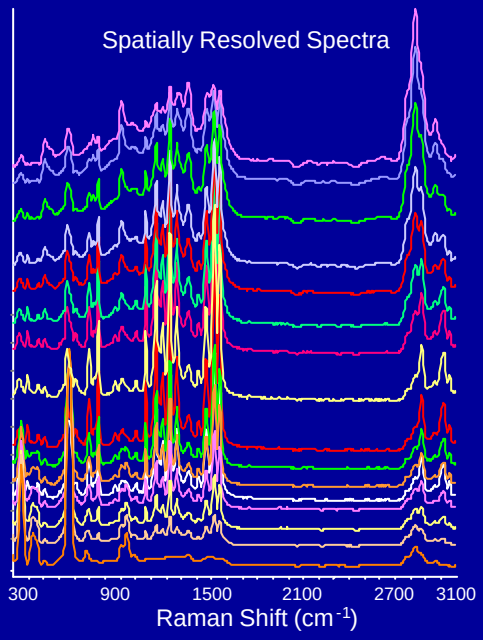
- Mixtures are not homogeneous on the microscopic scale
- Spectra obtained from different sample locations are different
- Chemical Imaging (i.e. spatially-resolved spectroscopy) rapidly provide a set of spectra incorporating these variations
- Data analysis tests set of mixture spectra against all of 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)

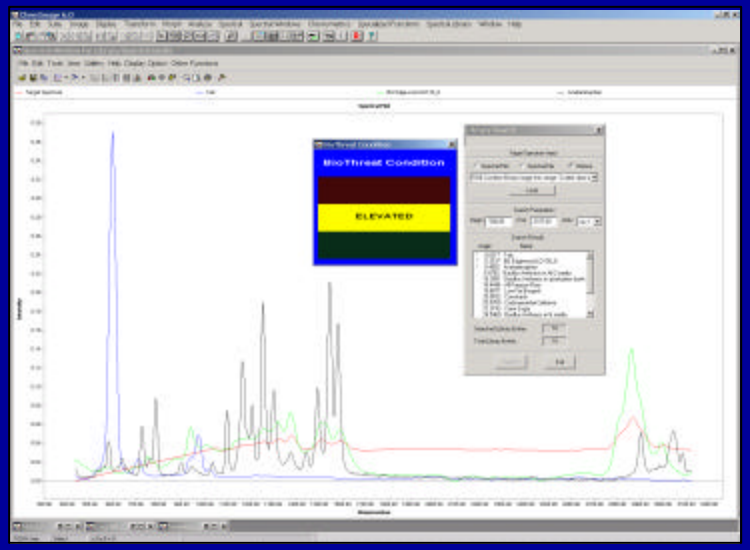
Reflectance images with sampling grid



Spatially Resolved Spectra

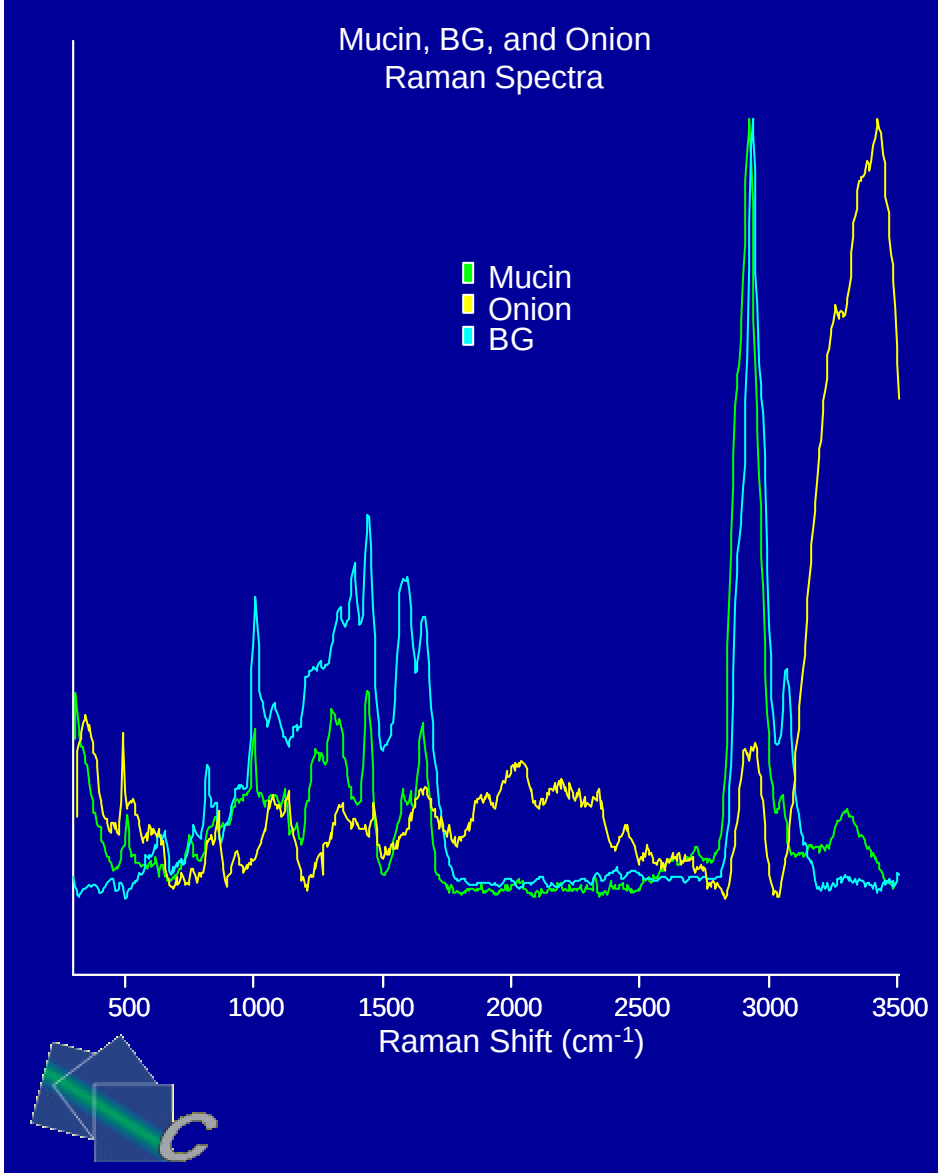


Results of Automated Identification



Raman Chemical Imaging of Anthrax on Food & Bodily Fluids

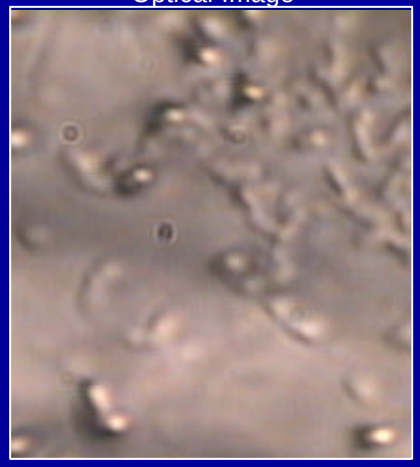
Using a ChemImage FALCON® Raman Chemical Imaging Microscope



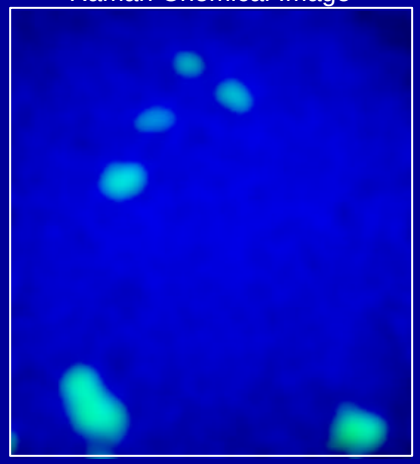
Threats to Food

BG and Onion

Optical Image



Raman Chemical Image

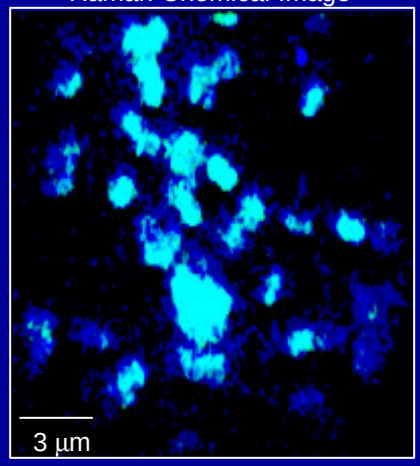


Threats to Humans

BG and Mucin

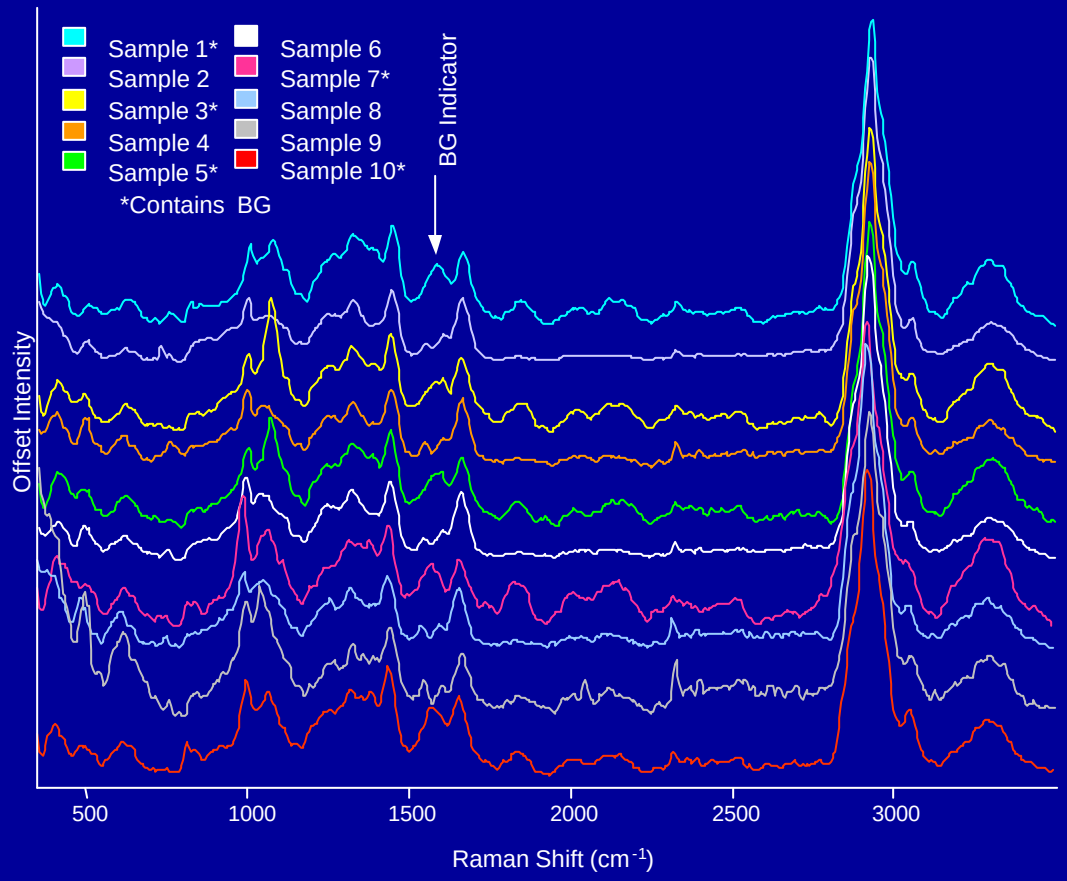
Optical Image

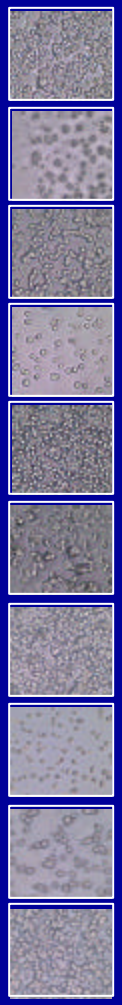
Raman Chemical Image



Blind Study of BG/Mucin Samples

Sensitivity* = 100%
Selectivity* = 100%





Sample	Actual	Predicted
1	+	+
2	-	-
3	+	+
4	-	-
5	+	+
6	-	-
7	+	+
8	-	-
9	-	-
10	+	+

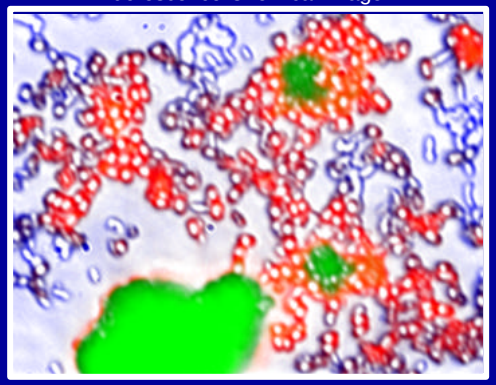
***Sensitivity** *(true positive rate)*: %Sensitivity = (TP/(TP+FN))*100, where TP = true positives and FN = false negatives.
***Selectivity** (*true negative rate)*: %Selectivity = (TN/(FP+TN))*100, where TN = true negatives and FP = false positives.

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

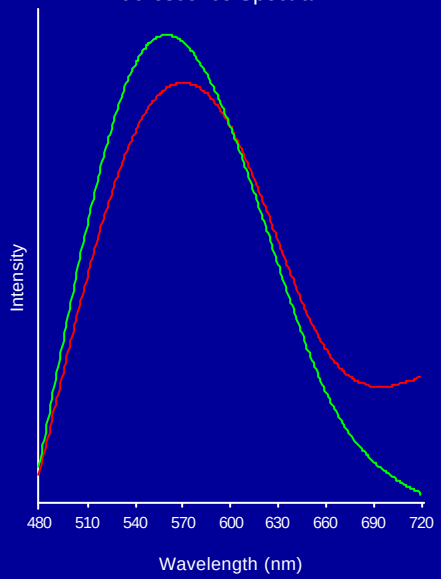
Fluorescence Chemical Imaging
BG/Diesel Soot/Road Dust Mixture

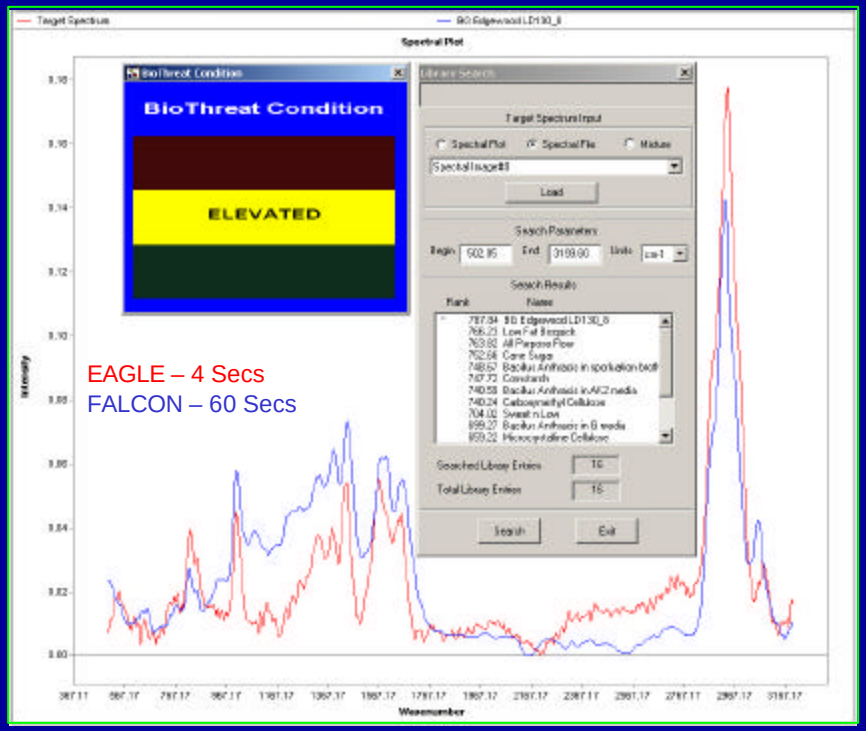
Fluorescence Chemical Image



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

Fluorescence Spectra





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