Using Real-time Multiband Fluorescence Signatures to Discriminate between Bioaerosol Classes

Darrel Baumgardner, Kevin McCabe, Greg Kok, Gary Granger, Mark Hernandez

Droplet Measurement Technologies
Columbia Gorge College
University of Colorado at Boulder
Civil, Environmental & Architectural Engineering
Primary Biological Aerosol (PBA) Discrimination: WHY?

PBA from aquatic & terrestrial sources are present in the atmosphere:

Potential for effects on atmospheric processes by acting as nuclei

Nutrient transport implications

Potential for environmental health impacts:

(-) microbes, allergens, toxins, hypersensitivity drivers, weapons

(+) aerosolized vaccines
PBA “Discrimination”: what does this actually mean?
PBA “Discrimination”: what does this actually mean?

- DNA
- DNA?
- COUNT!
- “ACTIVITY”
- “VIABILITY”
- “TOXICOLOGY”
- “ACTIVITY”
- PM vs PM\textsubscript{BIO}
- Optical
- “SIGNATURE(s)” (Laser Induced Fluorescence)
Evolution of the Wideband Integrated Bioaerosol Sensor - WIBS

<table>
<thead>
<tr>
<th>Detector</th>
<th>Channel FL 1</th>
<th>Channel FL2</th>
<th>Channel FL3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Excitation</td>
<td>Detection</td>
<td>Excitation</td>
</tr>
<tr>
<td>WIBS-3 (λ)</td>
<td>280 nm</td>
<td>320-600 nm</td>
<td>280 nm</td>
</tr>
<tr>
<td>WIBS-4 (λ)</td>
<td>280 nm</td>
<td>310-400 nm</td>
<td>280 nm</td>
</tr>
</tbody>
</table>

ONLINE SINGLE PARTICLE MEASUREMENTS:

Optical size, three fluorescence channels, asymmetry factor $A_f$

$0.5 < D_p < 20 \, \mu m$

Maximum Fluorescent Processing Concentration: $2 \times 10^4 \, \#/L$
across all 3 channels: (c.a. $10^4 / \text{minute}$)

Maximum PM Processing Concentration: $1 \times 10^5 \, \#/L$

Flow Rate c.a. 1 L/min

Modified from, M. Gallagher, U. of Manchester

Systematically Challenge Each Discrimination Sphere

- **Identification**
  - DNA

- **Abundance**
  - DNA
  - COUNT !

- "Viability"
- "Toxicology"
- "Activity"

PM vs PM\(_{BIO}\)
- Optical
- "Signature(s)"
  - (Laser Induced Fluorescence)
Bioaerosol Chamber Studies

Physical Factors

Defined Environment $= f (\text{RH}, T, \lambda v)$

Pure Culture & Environmental Samples

Pressurized Nebulizer

(many types)
Bioaerosol Chamber Studies

Physical Factors

Defined Environment = f (RH, T, λν)
Particle Size Distribution should be Considered as a Critical Test Parameter

Pure Culture & Environmental Samples

Pressurized Nebulizer

Many types

Aerodynamic Particle Sizer

GMD = 1.23 \mu m
GSD = 1.49 \mu m
Chamber Studies should be at Physical Equilibrium

Pure Culture & Environmental Samples

Pressurized Nebulizer

~ 10 min*

RH

T

CHALLENGE PROCEDURE

Pressurized Nebulizer → Pure Culture & Environmental Samples → Sample Collection → Microscopic Analysis → # / m³ vs. Time → Samples Collected
COMPARING WIBS to DIRECT COUNT of FUNGAL SPORES from Air-O-Cell CASSETTES

RH = 30%  T = 22 C

n = 3
Examples of Environmental Aerosol Samples
(flood impacted areas)

Indoors

Outdoors

Aerosolized Flood Water Sample Challenges: A National Reconnaissance
Aerosolized Flood Water Sample Challenges:

Don’t Assume Cytometry is Going to “cut it”

Optimized Flow Cytometer Counts

Direct Microscopic Count (# cells / cm³)
PBA “Discrimination”: what does this actually mean?

Lab and Field Verify the Ability of 3 channel Fluorescence to “recognize” distinct cell types

Optical Signatures (UV LIF)
Do Phenotypic Groupings Emerge that Can be Useful for “Discrimination”? 

**Diagram:**
- **Pressurized Nebulizer** 
  - [Diagram of Pressurized Nebulizer]
- **Pure Culture** 
  - [Diagram of Pure Culture]
Do Phenotypic Groupings Emerge that Can be Useful for “Discrimination”?

- Pure Culture
- Pressurized Nebulizer
- Specific Fluorescent Spectra
  + Size (OD)
  + (Shape Factor)

Public Enemies
- Aspergillus spp
- Penicillium spp
- Stachybotrys spp
- Ragweed spp
- Pine spp
- Alder spp

Environmental Microbiology Laboratory
EMPAT NO: 102795
APPROACH FOR COMPILING PHENOTYPIC GROUPINGS FROM FLUORESCENCE PATTERNS

FLUORESCENCE CHANNEL RESPONSE

Fluorescence Channel Patterns:
- FL 1
- FL 2
- FL 3
- FL 1 + 2
- FL 1 + 3
- FL 2 + 3
- FL 1 + 2 + 3

Species Coverage:
- Alternaria sp
- Aspergillus sp
- Cladosporium sp
- Chaetomium
- Botrytis
- Fusarium sp
- Gibberella
- Gliocladium
- Penicillium
- Phoma sp
- Sclerotinia
- Trichoderma
- Ustilaginoidea
- Aster sp
- Ambrosia artemisia
- Artemisia absinthium
- Cupressus similis
- Eucalyptus globulus
- Funaria sp
- Fagus
- Juniperus sp
- Picea omorika

Color Legend:
- 100% Group Frequency
- 80%
- 60%
- 40%
- 20%
ADDING DIMENSIONS TO COMPILE DISTINCT GROUPING PATTERNS:
ADDING DIMENSIONS TO COMPILE DISTINCT GROUPING PATTERNS:
ADDING DIMENSIONS TO COMPILE DISTINCT GROUPING PATTERNS:
ADDING DIMENSIONS TO COMPILE DISTINCT GROUPING PATTERNS:
EMERGING PATTERN RECOGNITION:

All of the fungal species in this group fluoresce in channels 1 & 2 (only).

Within this group, average optical diameters range from 2 $\mu$m to 7 $\mu$m.

And fluorescence intensity from 200 to 1200 “relative” counts.

Hence, some degree of phenotypic grouping by “genera” is emerging.
FLOW CHART FOR
PHENOTYPIC ALGORITHM
DETERMINATIONS

Decision Level I
Fluorescence Pattern
F1,F2,F3,F12,...,F123

Group A
Decision Level II
Optical Diameter

Group N
Decision Level II
Optical Diameter

Group A-1
Decision Level III
F1,F2,F3 intensities

Group A-n
Decision Level III
F1,F2,F3 intensities

Group N-1
Decision Level III
F1,F2,F3 intensities

Group N-n
Decision Level III
F1,F2,F3 intensities

Group A-1-i
Decision Level IV
Shape Factor

Group A-n-x
Decision Level IV
Shape Factor

Group N-1-i
Decision Level IV
Shape Factor

Group N-1-x
Decision Level IV
Shape Factor

Mold Group= A-1-i-a

Mold Group= N-1-i-a
SUMMARY OF TESTING TO DATE:

18 fungal isolates divided into 7 distinct groups

13 pollens isolates divided into 3 distinct groups

Within each group, spores and grains can have significantly different:

- flouresing patterns,
- optical diameters,
- different fluorescing intensities

(shape factors)
AIHA EMPAT PROGRAM:
Environmental Microbiology Proficiency Analytical Testing

ERMI (Environmental Relative Moldiness Index)
Hypothesis: even after remediation, aerosol bioburden in water damaged homes is higher indoors than immediately outdoors

• Bioaerosol Concentrations indoors < outdoors

• Ecology indoors = outdoors

(DeKoster, 1995; Yang, 1993; Robertson, 1997; Schillinger et al, 1999)
## Percentage of Spore Type as Analyzed by EMSL

<table>
<thead>
<tr>
<th>Room</th>
<th>Alternaria</th>
<th>Ascospores</th>
<th>Aspergillus</th>
<th>Basidiospores</th>
<th>Chaetomium</th>
<th>Cladosporium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exterior</td>
<td>1</td>
<td>36</td>
<td>7</td>
<td>31</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Basement</td>
<td>4</td>
<td>23</td>
<td>45</td>
<td>15</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>Bedroom</td>
<td>1</td>
<td>8</td>
<td>65</td>
<td>14</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Den</td>
<td>1</td>
<td>15</td>
<td>11</td>
<td>14</td>
<td>0</td>
<td>53</td>
</tr>
<tr>
<td>Hall</td>
<td>0</td>
<td>18</td>
<td>13</td>
<td>12</td>
<td>0</td>
<td>47</td>
</tr>
<tr>
<td>“Inside”</td>
<td>0</td>
<td>41</td>
<td>8</td>
<td>29</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Kitchen</td>
<td>1</td>
<td>28</td>
<td>19</td>
<td>13</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Livingroom</td>
<td>0</td>
<td>43</td>
<td>11</td>
<td>40</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>“Upstairs”</td>
<td>1</td>
<td>11</td>
<td>50</td>
<td>32</td>
<td>0</td>
<td>16</td>
</tr>
</tbody>
</table>
Threshold for Size and F1 intensity patterns

Threshold for concentration patterns