Bio-Fluorescent Particle Counters and Industry Support for the Technology

Allison Scott
Facilitator, M³ Collaboration
Principal Scientist, MicronView LLC
Topics of discussion

• Real time, bio-fluorescent particle counting (BPFC) technology
  • Principle of operation
  • Applications

• Industry support for BFPC
  • Working groups and collaborations
  • Publications
As Thak worked frantically to start a fire, a Cro-Magnon man, walking erect, approached the table and simply gave Theena a light.
Bio-Fluorescent Particle Counters (BFPC)

- BFPCs are a form of enhanced particle counter
- BFPCs use the detection of:
  - Scattered light for particle enumeration
  - Intrinsic fluorescence for classification of particles as bio-fluorescent particles (BFP) or inert

Electron micrograph of a cluster of *Escherichia coli* bacteria magnified 10,000 times - Wikipedia
Intrinsic Fluorescence

• Some particles naturally fluoresce through absorption of energy from light and release of this energy as light at a longer wavelength.

• All cells contain many such fluorescent molecules with NAD(P)H and Riboflavin being examples that fluoresce under 405nm light.

<table>
<thead>
<tr>
<th>Fluorescent Biological Molecules</th>
<th>Approximate Fluorescent Emission Maximum (nm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine</td>
<td>280</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>300</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>350</td>
</tr>
<tr>
<td>NADH</td>
<td>440, 460</td>
</tr>
<tr>
<td>NADPH</td>
<td>464</td>
</tr>
<tr>
<td>Flavins</td>
<td>535</td>
</tr>
</tbody>
</table>


BFPC Technology

Continuous and real-time BFPC outputs:
- Total particle counts
- Auto-fluorescent counts (AFU)

* Diagram from Process and Environmental Monitoring Methods working group BFPC Overview presentation
BFPC Detection

- BFPCs are a non-growth-based method
  - The BFPC Auto-Fluorescent Unit (AFU) is a unit that reflects both size and fluorescence of a particle and can detect viable but non-culturable cells in a sample
  - The Colony-forming unit (CFU) is a unit used to estimate the number of viable and culturable bacteria or fungal cells in a sample

- Different method of detection than the traditional growth-based method
  - CFU ≠ AFU

- Similar to a classical particle counter, but enhanced
  - Like a particle counter, BFPC detect scattered light and report total particle counts
  - Unlike a particle counter, BFPC also detect fluorescence and report biologic counts
BFPC Detection

• **Advantages**
  • Continuous monitoring – supports data trending and increased process understanding
  • Real-time results – support
    • Timely indication of adverse trends, and
    • Faster root cause identification.
  • Minimization of operator presence in critical environments (air monitoring)
  • Support personnel training due to real-time total particle and biologic count feedback

• **Potential Limitations**
  • Do not identify
    • Mitigation – use the traditional method if an over action event is identified on BFPC
  • Some interferent materials can be classified as an AFU
    • Mitigation – understand potential interferents in environment, minimize or replace materials if possible
Process Understanding Comparison

- Microbial contamination event detected by continuous monitoring of BFPCs
- Periodic Sampling with Active Air Sampler missed detecting microbial contamination event
- 0.5 μm Total Particle monitoring does not indicate a contamination event
- 5.0 μm Total Particle monitoring does not indicate a contamination event

* Diagram from Process and Environmental Monitoring Methods working group BFPC Overview presentation
BFPCs for Environmental Monitoring

- BFPC technology has been available since the early 1990’s
  - Initially targeted government applications including bio-terrorism
  - Systems such as the UV-APS, FL-APS3, BioScout, and WIBS
- Around 2009 the first air based BFPC, and in approximately 2015 the first water based BFPC were introduced to the pharmaceutical industry

**Air Based BFPC**
- PMS BioLaz
- Azbil Biovigilant IMD-A
- TSI BioTrak
- MicronView BAMS

**Water Based BFPC**
- Mettler Toledo 7000 RMS
- Azbil Biovigilant IMD-W
BFPC Applications - Air Monitoring

Monitoring of controlled areas
- Aseptic suites
- Fill lines
- RABS/ Isolator systems
- Compressed gasses
- Media/ water fills
- Gowning Rooms
- Biosafety cabinets/Flow hoods

Monitoring to Return to Production
- Routine maintenance/calibration
- New construction or equipment
- Accelerate return from shutdown

Energy reduction – green initiatives
- HVAC flow reduction studies

Risk assessment
- Sample Site Selection
- Dynamic Modeling
- FMEA

Investigations
- EM excursions
- Root cause investigation/troubleshooting
- Verify CAPA effectiveness

Operator training
- Gowning training/qualification
- Aseptic technique
BFPC Applications – Water Monitoring

**Production Monitoring**
- Purified water
- Water for injection
- Predictive monitoring
- Sanitization

**Monitoring to Return to Production**
- Routine maintenance/calibration
- New construction or equipment
- Accelerate return from shutdown
- Real-time cleaning verification

**Risk assessment**
- Grab sample timing & frequency
- Loop component replacement
- Loop health

**Investigations**
- Excursions
- Root cause investigation/troubleshooting
- Verify CAPA effectiveness

**Green initiatives**
- Energy savings through sanitization or heating temperature reduction studies
- Maximizing water usage/minimizing down time

**Training**
- Maintenance/Engineering, component replacement.
BFPC Industry Support
Industry Support

**BioPhorum Fill Finish Alternative and Rapid Micro Methods (ARMM) BFPC Team**
- Air based BFPC focus
- Started in 2017

**Process & Environmental Monitoring Methods (PEMM) Working Group**
- Air and water BFPC focus
- Started in 2014

**Modern Microbial Methods (M³) Collaboration**
(Est. 2021)

**Online Water Bioburden Analyzer (OWBA) Working Group**
- Water rapid method focus
- Started in 2013

**Kilmer Community Rapid Microbiology Methods Group**
- Rapid Micro Method focus
- Started in 2019
## Industry Support – M³ Collaboration

### Steering Committee
- Facilitates Sub-Team meetings, publications, group communication
- Organizes annual summit meetings

### Sub-Team #1
- Challenges associated with BFPC implementation
- AFU ≠ CFU
- BFPC Validation

### Sub-Team #2
- Establishing baseline
- Setting Alert and Action levels

### Sub-Team #3
- Communications toolbox, User Requirements Specification Template
- Modern Microbial Method Initial Evaluation Roadmap
- Water BFPC Data Analytics
M³ Collaboration – Virtual Summits

• 4 March 2021:
  Speaker: Dr Anthony Cundell, Microbiological Consulting LLC and USP Microbiology Expert Committee
  *Application of USP<1223> to the Validation of Biofluorescent Particle Monitoring (BFPM)*

• 24 February 2022:
  Speaker: Dr Anthony Bevilacqua - Mettler-Toledo Thornton and USP Chemical Analysis Expert Committee
  *Water Based BFPC Test Case Overview and USP Pharm. Waters Expert Panel Current Work*

• 23 February 2023:
  Speaker: Caroline Dreyer, Novo Nordisk
  *Novo Nordisk’s Path to BFPC Implementation: Navigating the Maze of Regulatory Expectations*
M³ Collaboration – Publication Strategy

- **Teaser article**
  - Initial Evaluation Roadmap for Modern Microbial Methods

- **Umbrella publication strategy**
  - Overarching article – Challenges Encountered in the Implementation of BFPC Systems as a Routine Microbial Monitoring Tool

- **Sub-articles**
  - Understanding the Non-equivalency of BFPCs versus the Colony-Forming Unit (submitted to PDA JPST)
  - Validation of BFPC for Use in GMP Manufacturing Environments (working on draft)
  - Article focusing on what baseline counts include and how to establish alert and action levels (working on final draft)
  - Article on water-based BFPC Data Analytics

- **Toolbox Documents**
  - Modern Microbial Method User Requirements Specification Template

More articles to be added soon...

Email: ModernMicrobialMethods@gmail.com • LinkedIn: www.linkedin.com/company/modern-microbial-methods • Website: www.ModernMicrobialMethods.com
M³ Collaboration – Challenges Article

- Challenges Encountered in the Implementation of BFPC Systems as a Routine Microbial Monitoring Tool
  - Published in PDA JPST July 2022
  - https://journal.pda.org/content/early/2022/07/15/pda.jpst.2021.012726
- Discusses potential challenges and ways of mitigating
PEMM – BFPC EM & Troubleshooting Article

Air BFPC
- Case Study #1
  - Cleanroom Shutdown & Recovery
- Case Study #2
  - Verification of Low Particulate Wall Refurbishment Technology

Water BFPC
- Case Study #3
  - Troubleshooting a contaminated WFI system
- Case Study #4
  - Diagnosis of Non-Biological Water Loop Particulates

2018-2022 Industry Publications & Webinars


- Montenegro-Alvarado JM. **Pfizer** Leveraging rapid microbiological methodology in forensic evaluation to identify elusive root cause. *Amer Pharm Rev. [Internet]*. 2018 Sep

- **Online Water Bioburden Analyzer Workgroup**. A better approach to pharmaceutical water testing – user requirements for an online water bioburden analyzer. *Pharm Online*. 2018 Nov


2018-2022 Industry Publications & Webinars


Conclusions

• BFPCs are a modern microbial method that have been used in the Pharmaceutical industry for over a decade

• BFPCs offer:
  • Continuous sampling - better process understanding and trending
  • Real-time results - proactive instead of reactive response
  • Automated testing - improved data integrity and reduced risk to process

• Industry working groups continue to collaborate on the implementation, testing and validation of BFPC systems to support broader adoption and awareness
Thank you!

Allison Scott, Principal Scientist
a.scott@micronview.com