# Bio-Fluorescent Particle Counters and Industry Support for the Technology

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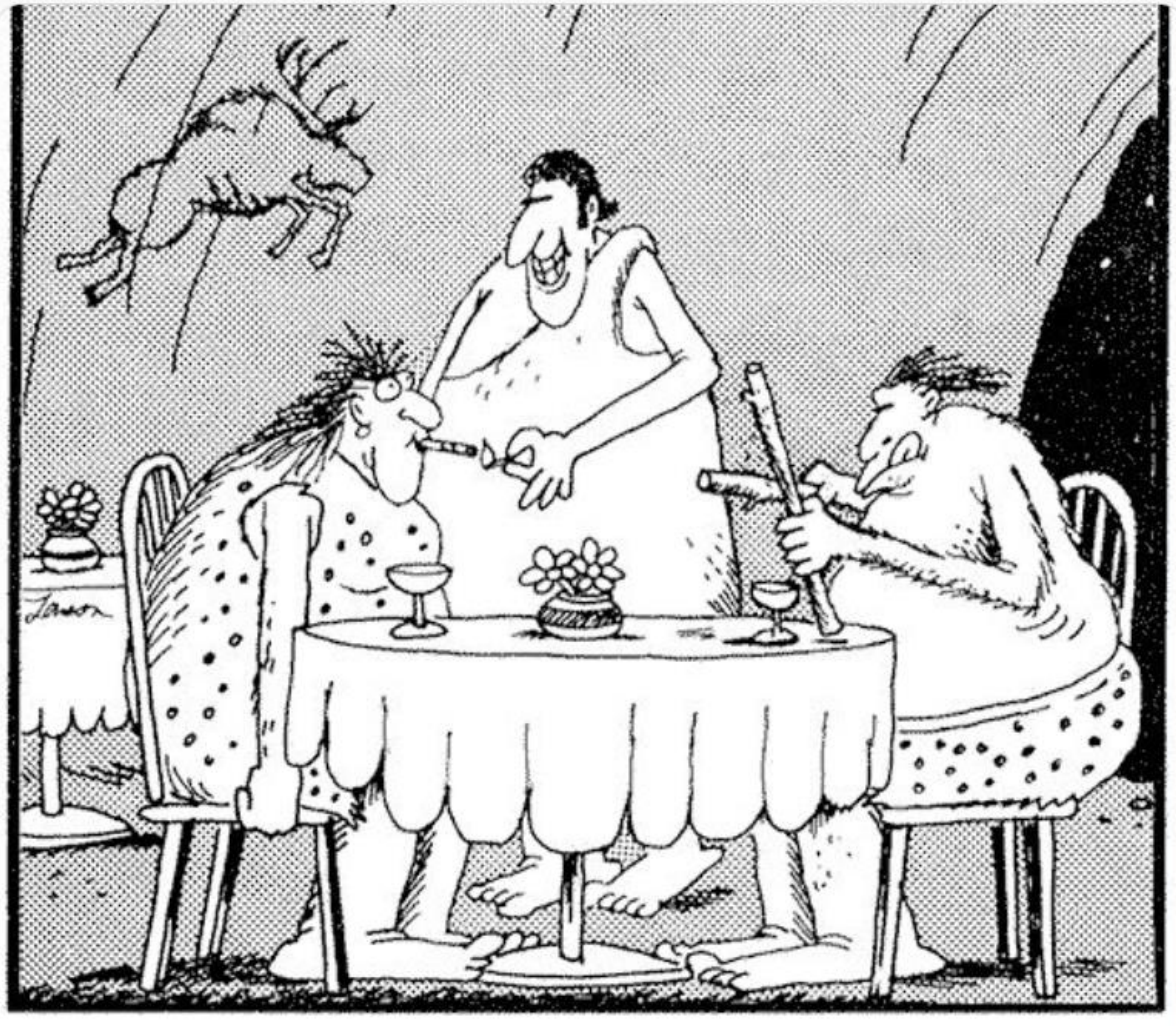
# Topics of discussion

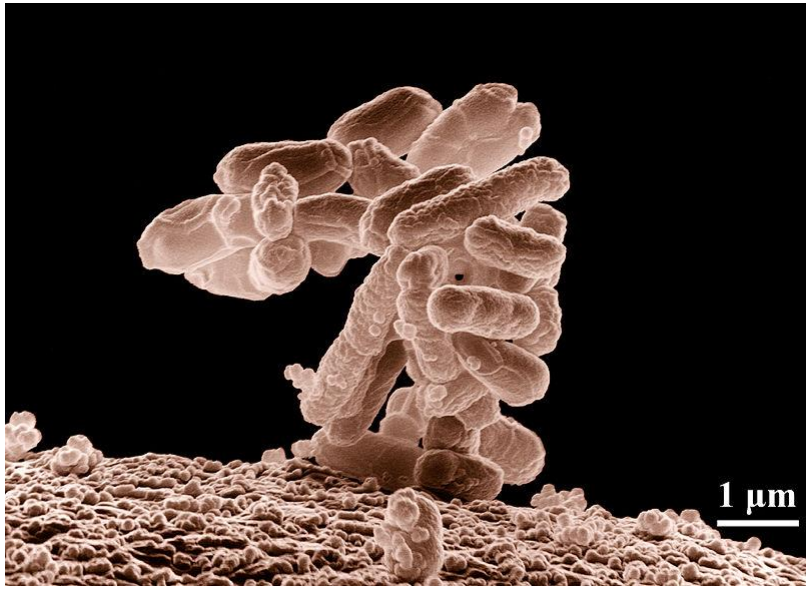
- Real time, bio-fluorescent particle counting (BPFC) technology
  - Principle of operation
  - Applications
- Industry support for BFPC
  - Working groups and collaborations
  - Publications




The Far Side by Gary Larson

# 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



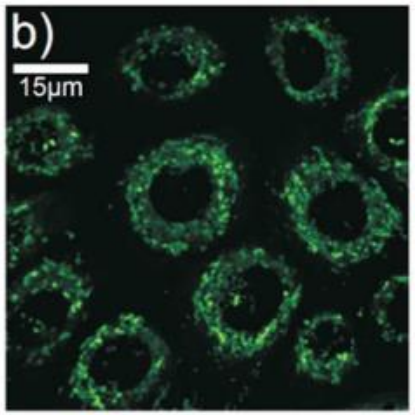

Electron micrograph of a cluster of *Escherichia coli* bacteria magnified 10,000 times - Wikipedia

# 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

| Fluorescent Biological Molecules | Approximate Fluorescent Emission Maximum (nm)* |
|---|---|
| Phenylalanine | 280 |
| Tyrosine | 300 |
| Tryptophan | 350 |
| NADH | 440, 460 |
| NADPH | 464 |
| Flavins | 535 |

A paruroctonus scorpion fluorescing under a blacklight (Wikipedia.org)

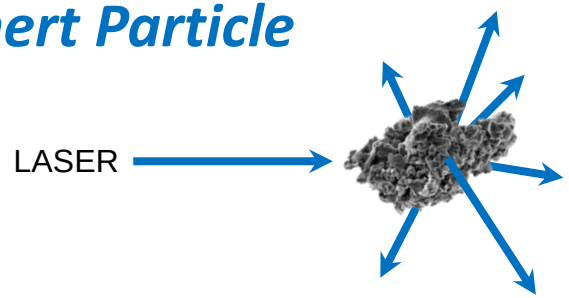



Autofluorescence of NADH in keratinocytes**

*Ammor, MS. (2007) Recent Advances in the Use of Intrinsic Fluorescence for Bacterial Identification and Characterization. Journal of Fluorescence. 17:455-459.
**Mellem D, et al. (2017) Fragmentation of the mitochondrial network in skin in vivo. PLoS ONE (2017) 12(6): e0174469. https://doi. org/10.1371/journal.pone.0174469

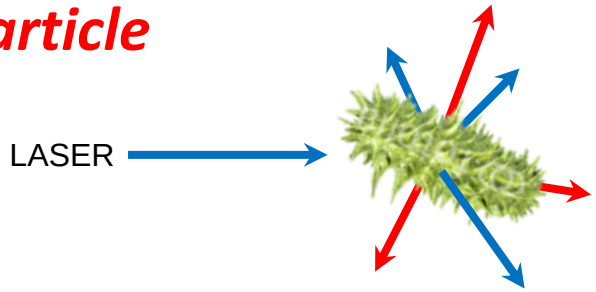
# BFPC Technology

***Inert Particle***




**Scattered Light**

***Bio-fluorescent Particle***




**Scattered Light** & **Characteristic Intrinsic Fluorescence**

System Algorithms

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

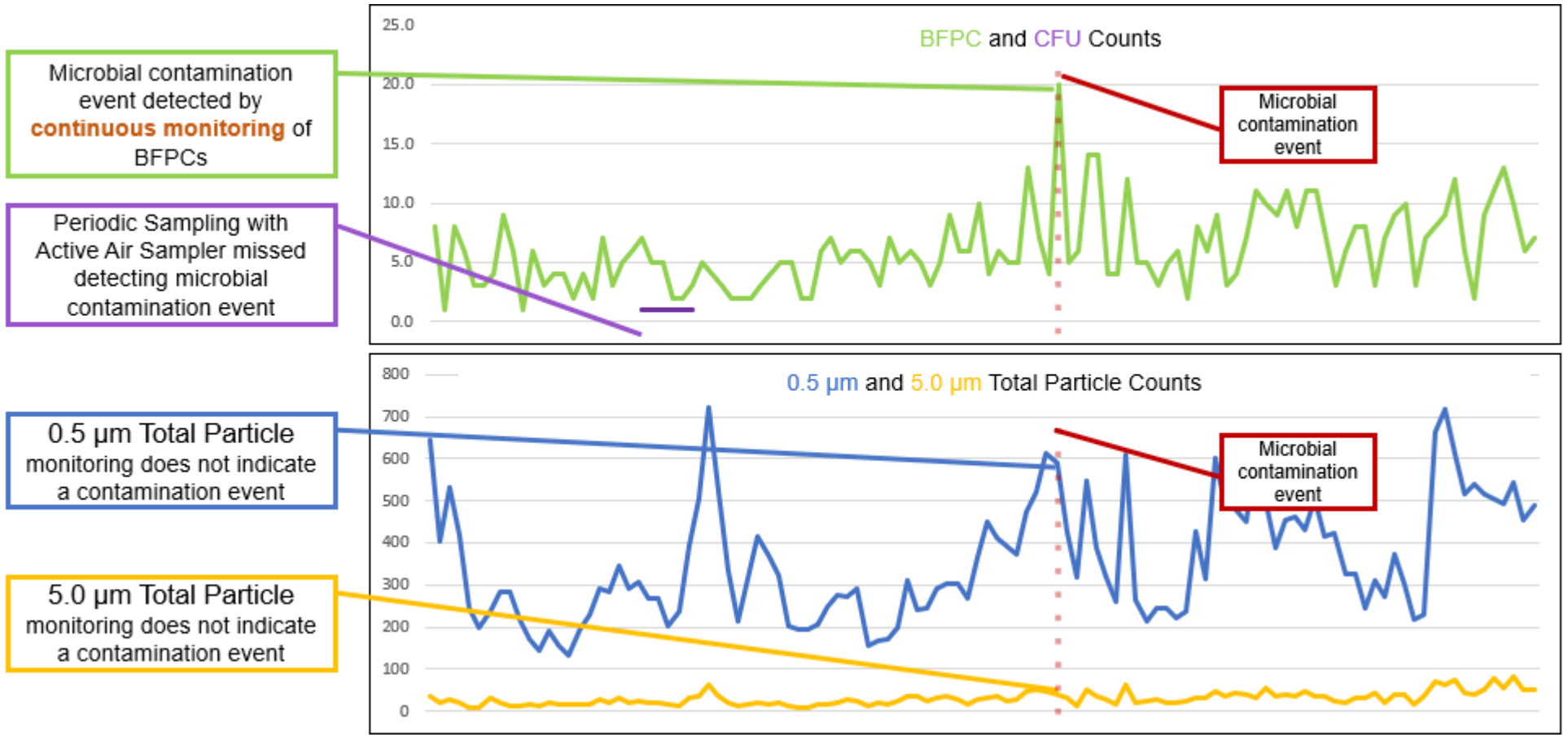
# BFPC Detection

- 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



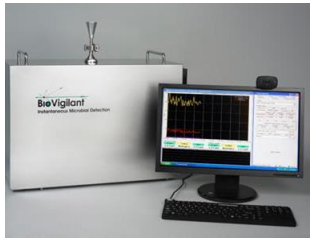

* Diagram from Process and Environmental Monitoring Methods working group BFPC Overview presentation

# 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 ($M^3$) 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 – $M^3$ Collaboration

| | |
|---|---|
| Steering Committee | • Facilitates Sub-Team meetings, publications, group communication<br>• Organizes annual summit meetings |
| Sub-Team #1 | • Challenges associated with BFPC implementation<br>• AFU ≠ CFU<br>• BFPC Validation |
| Sub-Team #2 | • Establishing baseline<br>• Setting Alert and Action levels |
| Sub-Team #3 | • Communications toolbox, User Requirements Specification Template<br>• Modern Microbial Method Initial Evaluation Roadmap<br>• Water BFPC Data Analytics |

# $M^3$ 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*

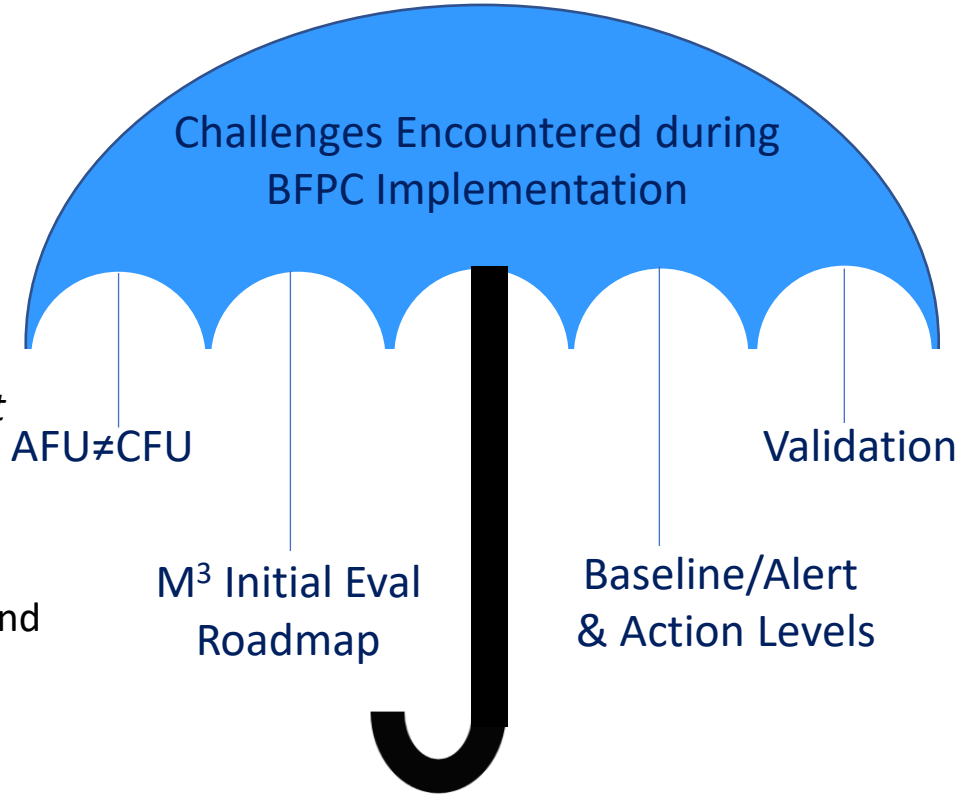
- 23 February 2023:

  Speaker: Caroline Dreyer, Novo Nordisk

  *Novo Nordisk's Path to BFPC Implementation: Navigating the Maze of Regulatory Expectations*

# $M^3$ Collaboration – Publication Strategy

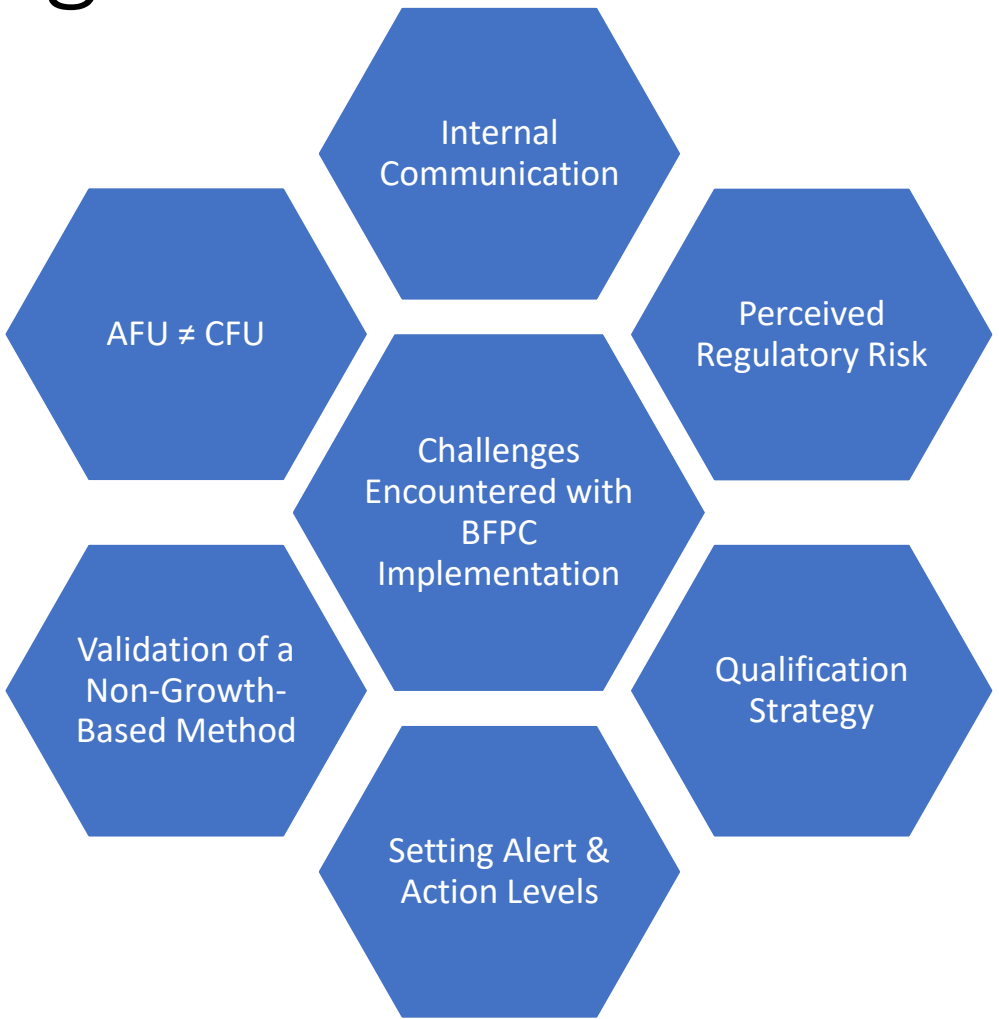
- **Teaser article**
  - *Initial Evaluation Roadmap for Modern Microbial Methods*
- **Umbrella publication strategy**
  - Overarching article – *Challenges Encountered in the Implementation of BFPC Systems as a Routine Microbial Monitoring Tool*
- **Sub-articles**
  - *Understanding the Non-equivalency 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…

# $M^3$ Collaboration – Challenges Article

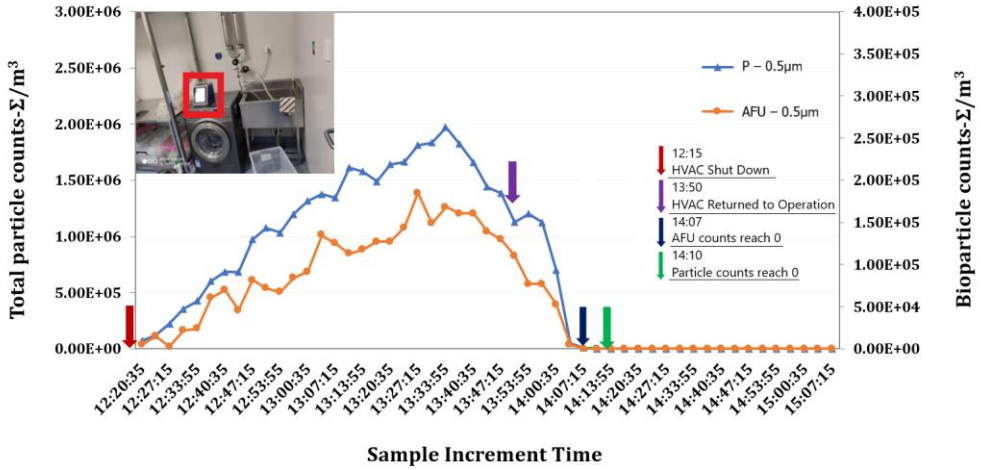
- *Challenges Encountered in the Implementation of BFPC Systems as a Routine Microbial Monitoring Tool*
  - Published in PDA JPST July 2022
  - https://journal.pda.org/content/early/2022/07/15/pdajpst.2021.012726
- Discusses potential challenges and ways of mitigating



# PEMM – BFPC EM & Troubleshooting Article

## Air BFPC

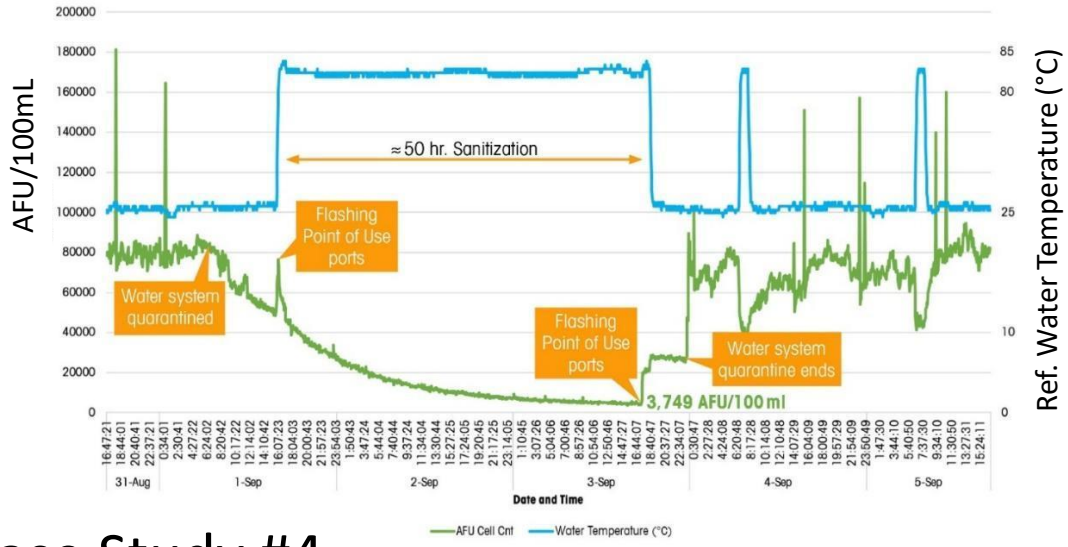
- Case Study #1
  - Cleanroom Shutdown & Recovery




- 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

Hooper S, et al. Advanced Environmental Monitoring and Troubleshooting with Bio-Fluorescent Particle Counters: Four Case Studies from the Process and Environmental Monitoring Methods Working Group. *Eur Pharm Rev*, Oct 2022

# 2018-2022 Industry Publications & Webinars

- Montenegro-Alvarado JM, et al. **Pfizer** case study: rapid microbial methods for manufacturing recovery after Hurricane María. *Pharm Online*. 2018 July
- 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
- Weber J, et al. **BPOG** Continuous microbiological environmental monitoring for process understanding and reduced interventions in aseptic manufacturing. *PDA J Pharm Sci Technol.* 2019 Mar/Apr;73(2):121-134
- Russ M. **Genentech** Webinar – Changing a Paradigm: Implementing a Real Time Microbial Detection Analyzer in Pharmaceutical Water. *Amer Pharm Rev.* 2019 Mar 14
- Ayers F, et al. **PEMM** Bio-Fluorescent Particle Counter-Based Real-Time Feedback and Control of Processing Conditions, Eur Pharm Rev, Aug 2019 ed.
- Benkstein K, et al. Evaluating changes to *Ralstonia pickettii* in high-purity water to guide selection of potential calibration materials for bioburden analyzers. *J Ind Microbiol Biotechnol.* 2019 Jul; 46: 1469-1478.

# 2018-2022 Industry Publications & Webinars

- Bar R. Charting and Evaluation of Real-Time Continuous Monitoring Water Bioburden. *PDA J Pharm Sci Technol.* 2019 Sep; 73 (5) 496-509
- Prasad A, et al. **BPOG** Practical applications of bio-fluorescent particle counting in Environmental Monitoring Investigations. *PDA J Pharm Sci Technol.* 2020 Jan/Feb;74
- Hjorth J, et al. GMP Implementation of Online Water Bioburden Analyzers. *Pharmaceutical Engineering*. 2021 Jan/Feb
- Scott A, et al. **PEMM** A Discussion on Bio-Fluorescent Particle Counters: Summary of the Process and Environmental Monitoring Methods Working Group Meeting with the FDA Emerging Technology Team. *Pda J Pharm Sci Technol.* 2021
- Briglia C, et al. **M³** Initial Evaluation Roadmap for Modern Microbial Methods. *PDA Letter.* 2022 Apr
- Scott A, et al. **M³** Challenges Encountered in the Implementation of Bio-Fluorescent Particle Counting Systems as a Routine Microbial Monitoring Tool. *Pda J Pharm Sci Technol.* 2022
- Hooper S, et al. **PEMM** Advanced Environmental Monitoring and Troubleshooting with Bio-Fluorescent Particle Counters: Four Case Studies from the Process and Environmental Monitoring Methods Working Group. *Eur Pharm Rev.* 2022 Oct
- Behrens D, et al. Application of Biofluorescent Particle Counters for Real-Time Bioburden Control in Aseptic Cleanroom Manufacturing. *Appl Sci.* 2022 Aug.

# 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!

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