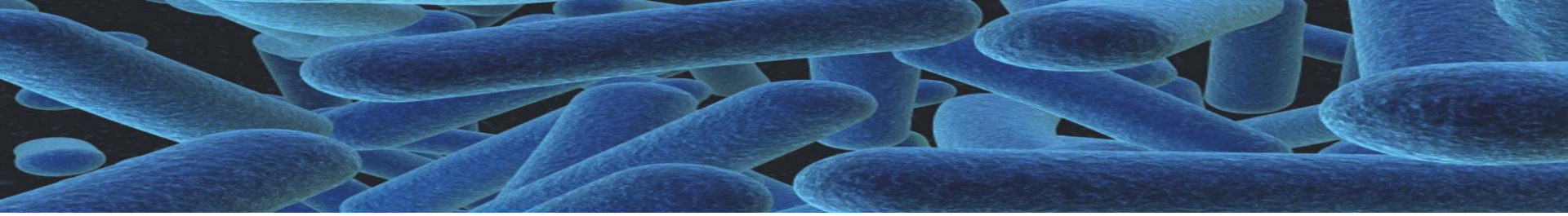


Evaluation, Validation and Implementation of Alternative and Rapid Microbiological Methods

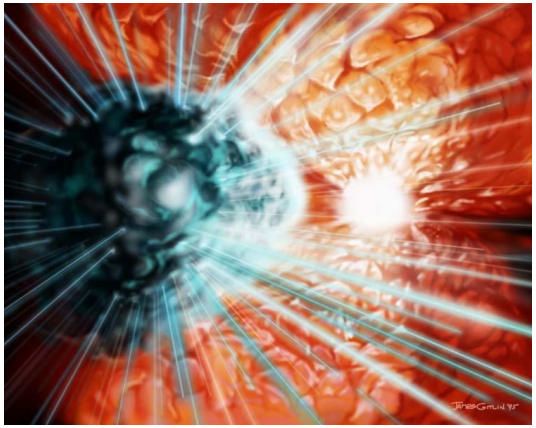
Spectroscopic-based Technologies

Michael J. Miller, Ph.D.

MICROBIOLOGY CONSULTANTS, LLC

Optical Spectroscopy

- Optical spectroscopy is an analytical tool that measures the interactions between light and the material being studied
- Light scattering is a phenomenon in which the propagation of light is disturbed by its interaction with particles



Rayleigh Scattering

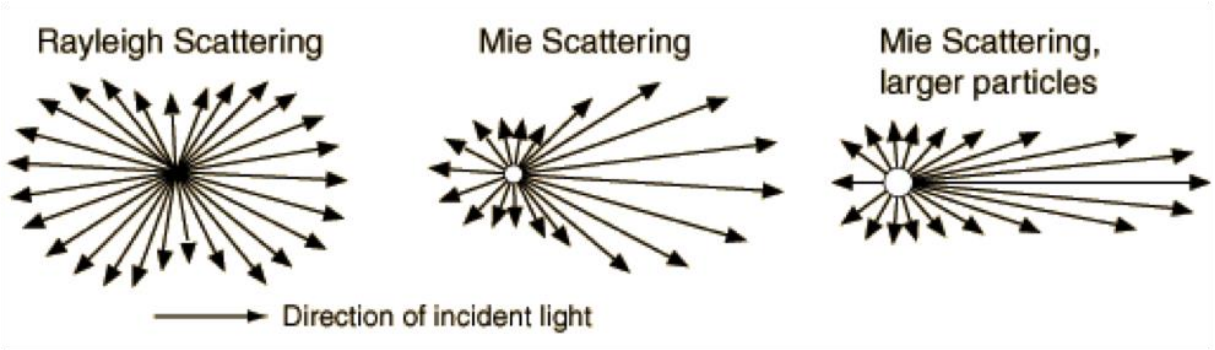
- When the particle size is much smaller than the wavelength of the light, the scattering is preferential to the shorter wavelength component of the incident light
- Blue skies are produced as shorter wavelengths of the incoming visible light (violet and blue) are selectively scattered by small molecules of oxygen and nitrogen

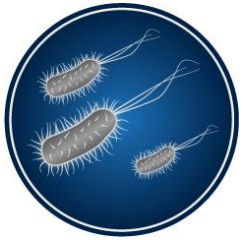
Mie Scattering

- When the particle size is much larger than the wavelength of the light, all visible wavelengths are scattered more or less equally
- Because cloud droplets are larger than the incoming visible light, almost all of the light that enters clouds will be scattered, producing a white color
- A similar effect occurs in mist or fog

Direction of Light Scattering

- In Rayleigh scattering, light is scattered in all directions and is not very sensitive to particle size
- In Mie scattering, the scattered light is concentrated in a forward direction, and the scattered portion of the light is proportional to the particle size
- This is why Mie scattering is used in many commercial particle detectors



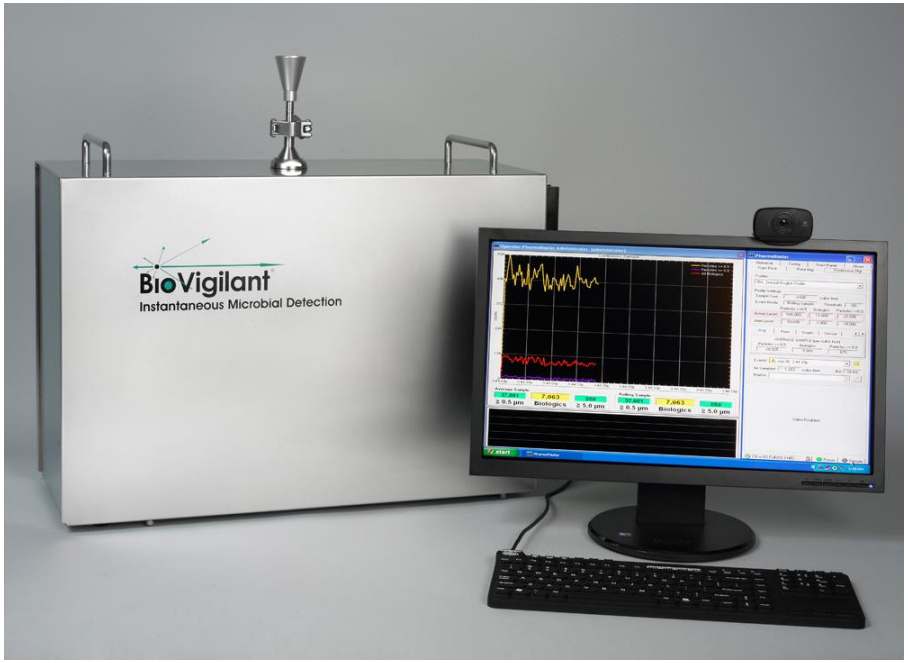


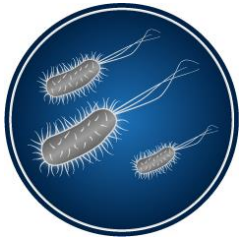
Scientific Principles

- Intrinsic fluorescence
- Raman spectroscopy

BioVigilant IMD-A

- A novel air monitoring RMM has been developed that is based on optical spectroscopy
- BioVigilant IMD-A (Instantaneous Microbial Detection in Air)

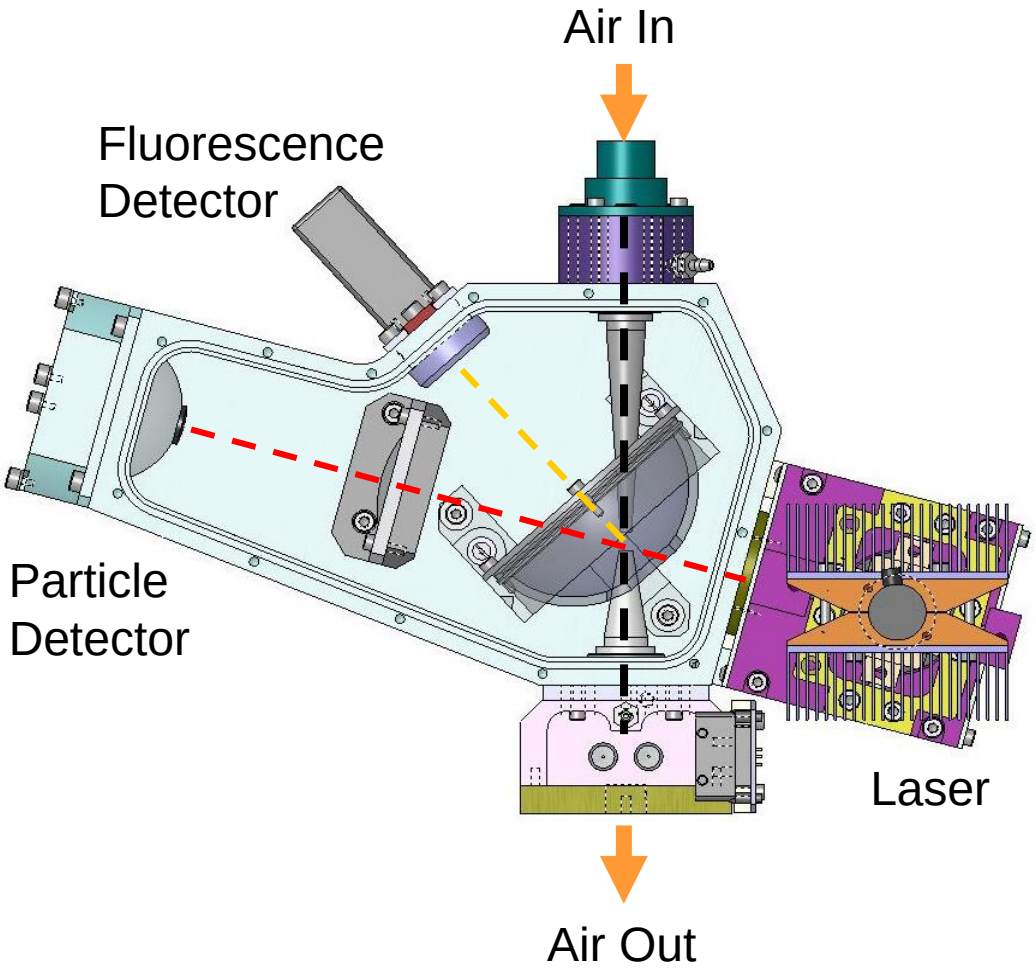




BioVigilant IMD-A

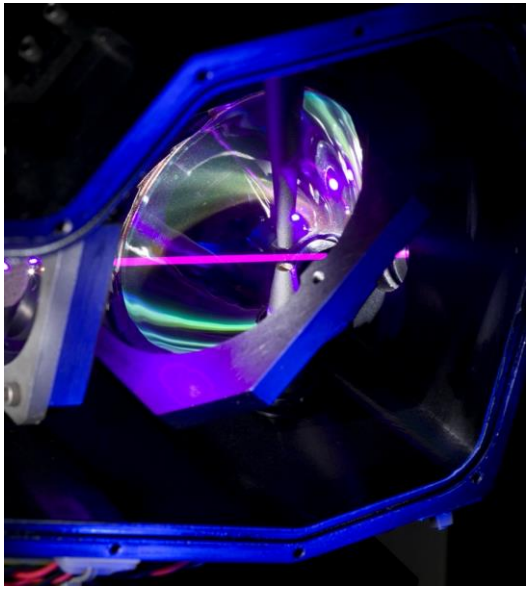
- Utilizes a Mie scattering particle detector to size and enumerate total particulates between 0.5 - >10 microns
- At the same time, particles that contain specific biological targets will auto-fluoresce as they pass through the laser
  - 405 nm excites NADH, riboflavin, dipicolinic acid
- Simultaneous detection of viable and non-viable particles
- Data is acquired and displayed in real time
- No reagents, media or consumables
- Monitor a single volume of air or operate continuously
  - 28 LPM model samples 1 cubic meter of air in ~35 minutes

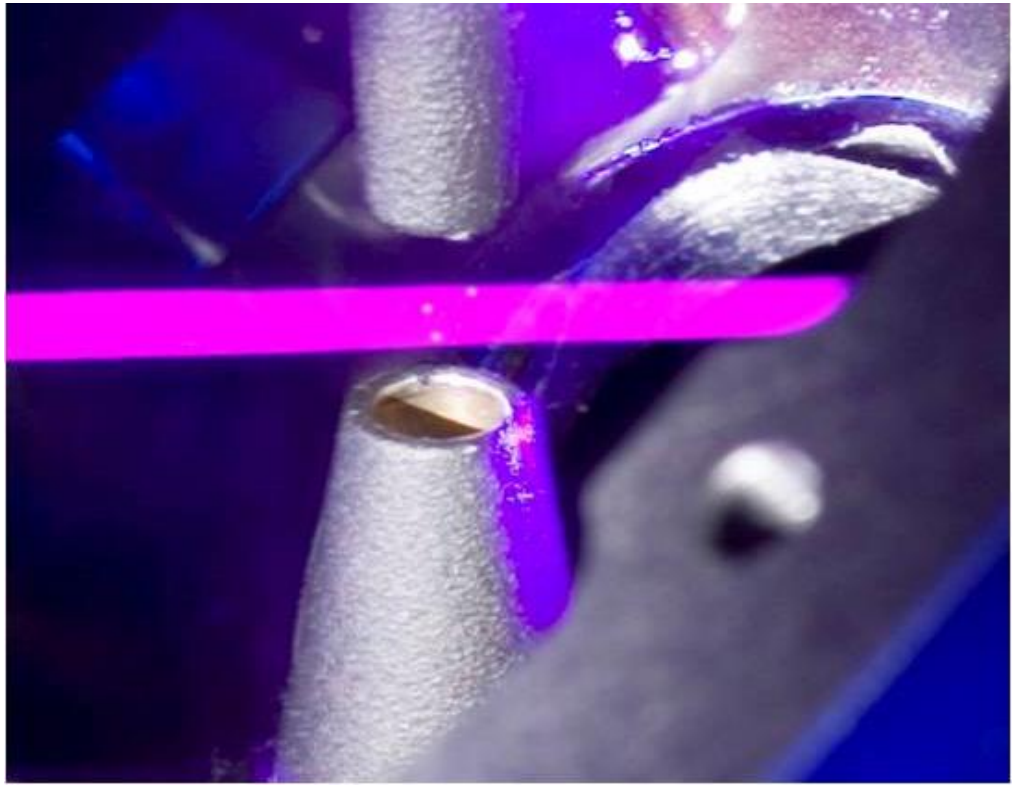
BioVigilant IMD-A



BioVigilant IMD-A

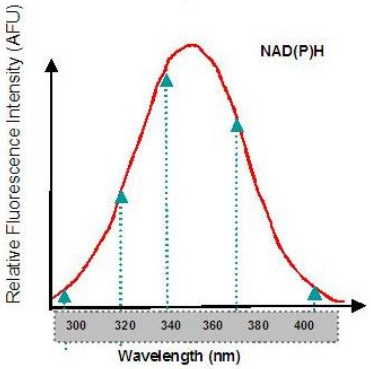
- Interrogation zone and laser excitation with single cell detection of airborne contaminants

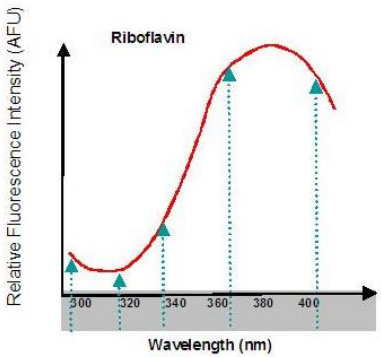


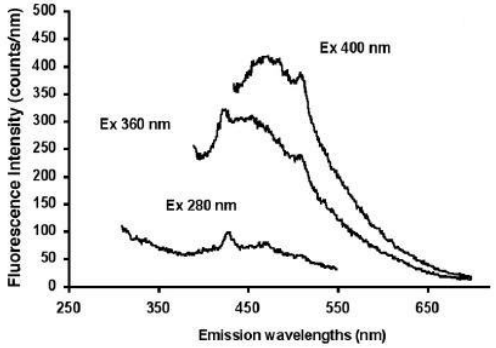


BioVigilant IMD-A

- NADH, riboflavin, and dipicolinic acid are primary markers

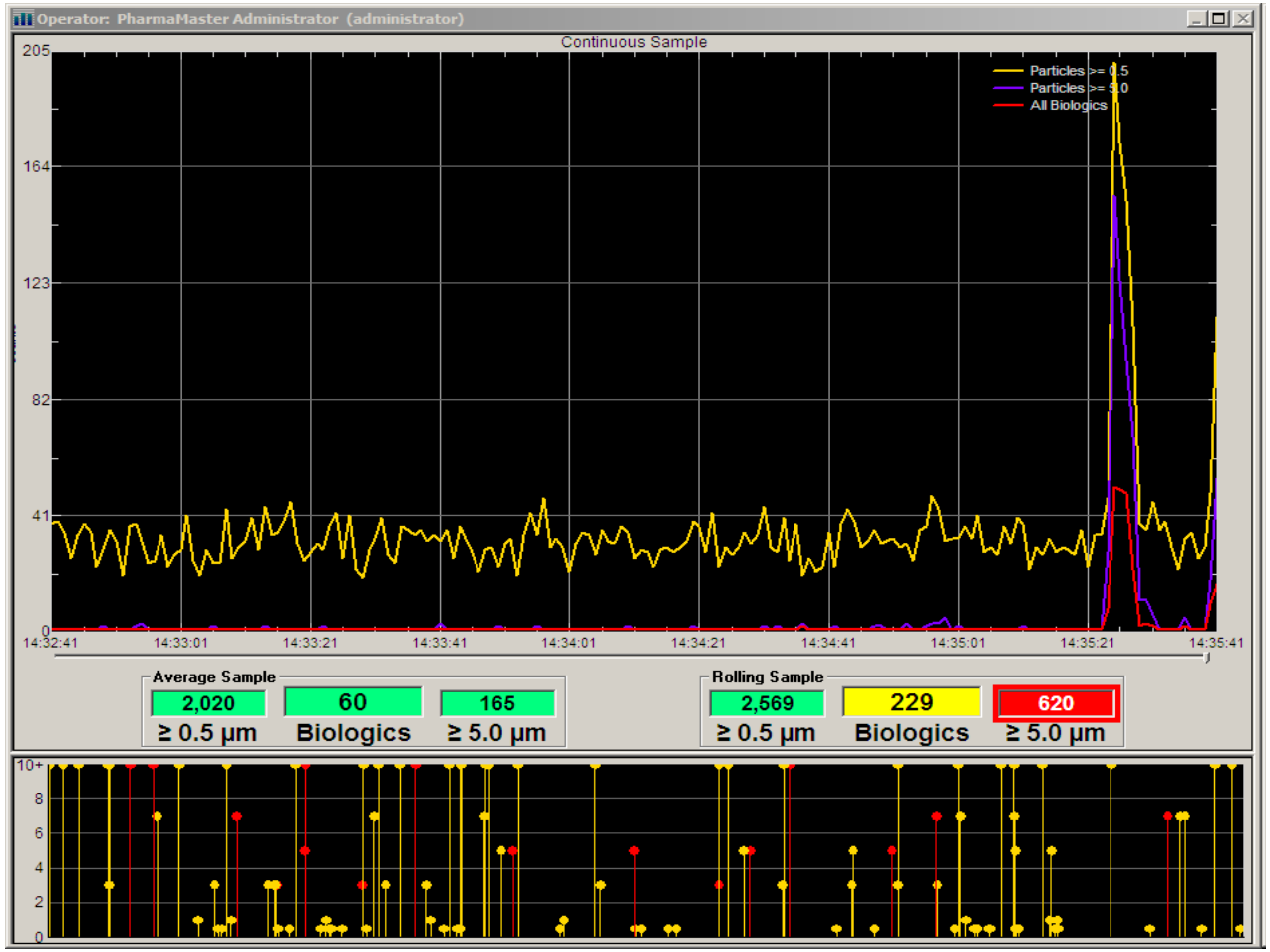






- A light source in the wavelength range of 340-405 nm is a suitable choice of exciting these compounds
- Smaller 405 nm diode lasers became available when the IMD-A was being developed

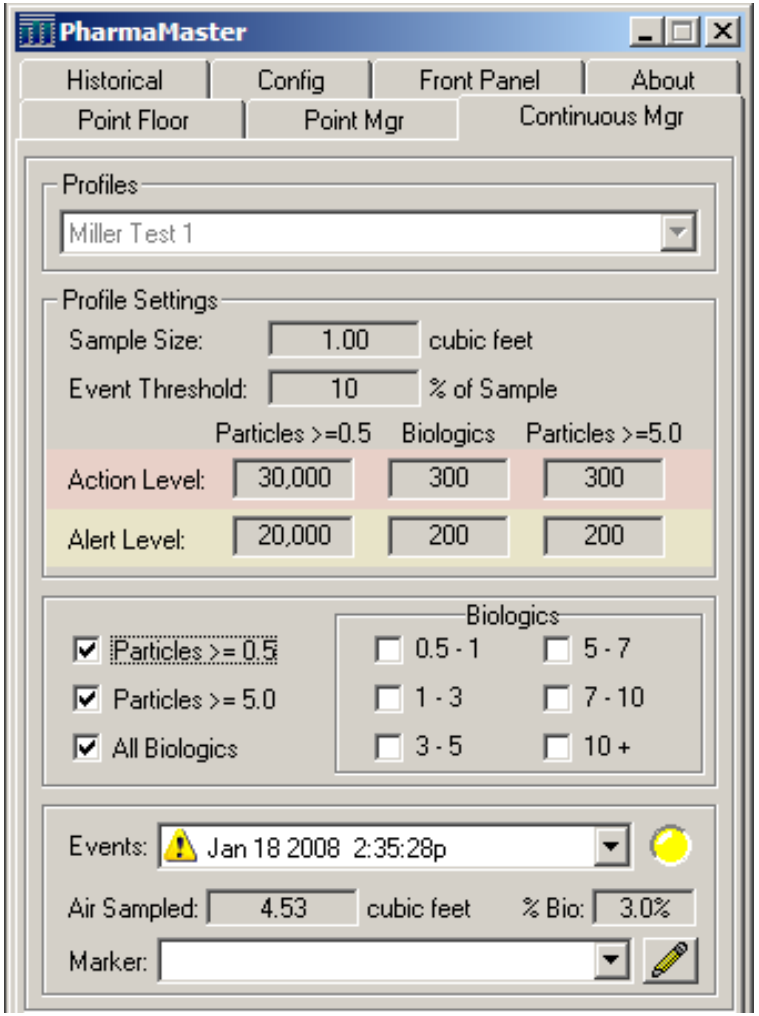
BioVigilant IMD-A

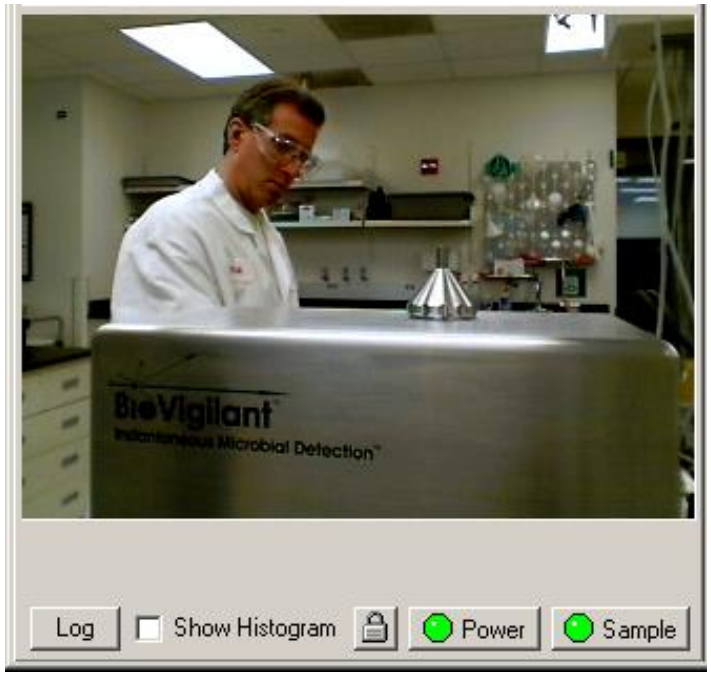


- Continuous monitoring refreshed every second. Note the spike in counts at the right.
  - 0.5 µ (yellow)
  - 5.0 µ (blue)
  - Viable (red)

- Data is acquired every second
  - Non-viable (yellow)
  - Viable (red)

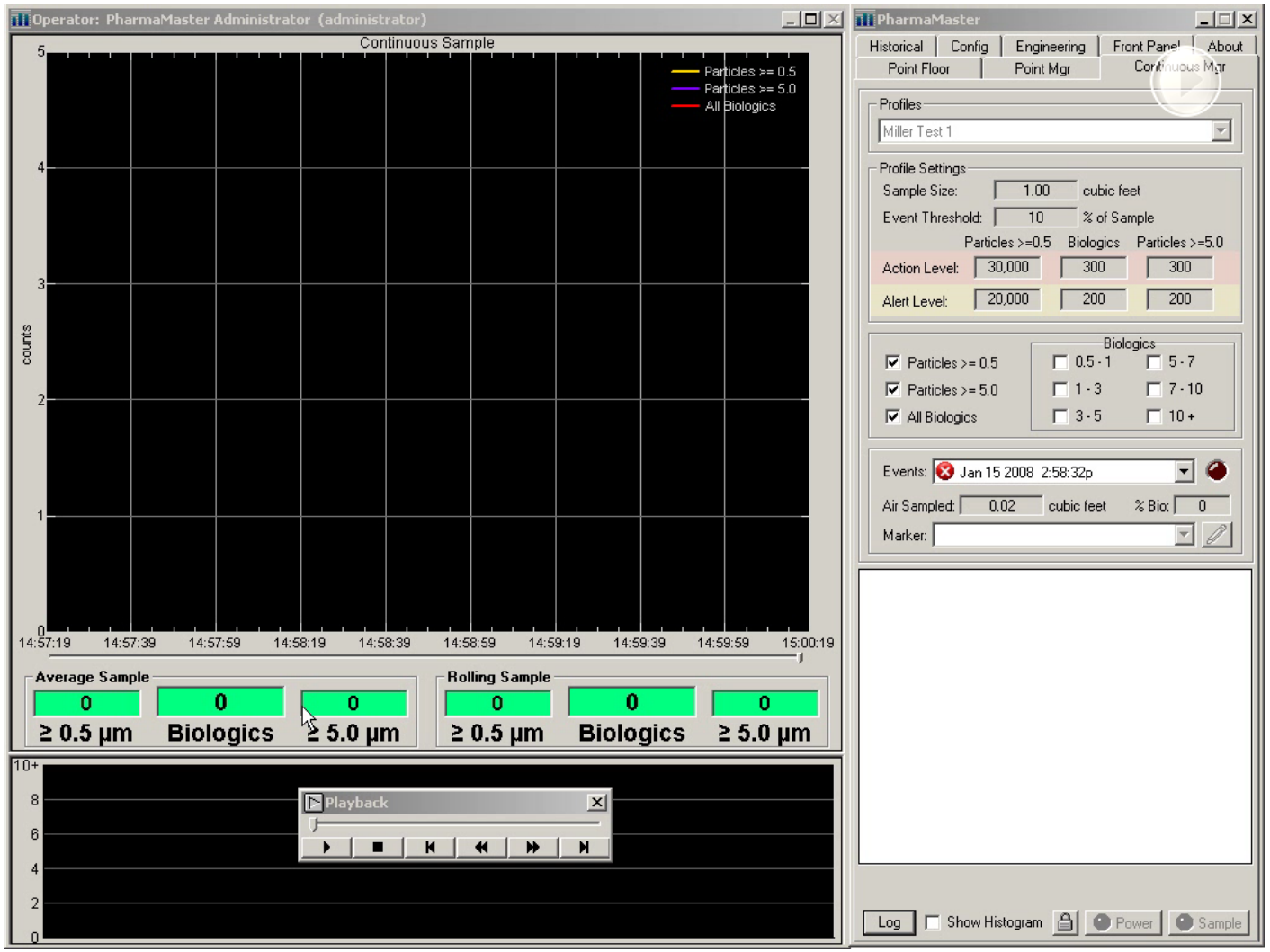
BioVigilant IMD-A

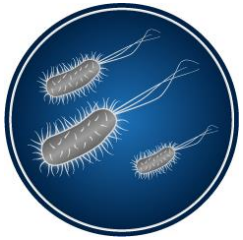




Video is synced with monitoring data and can be used to support a root cause analysis of a contamination event

Archived Data and Video





Case Study

- Static monitoring in filling and transfer isolators
- Transfer of components into filling isolator
- Dynamic monitoring during aseptic fill
- Interventions
- Simulated mouse-hole
- Glove integrity testing

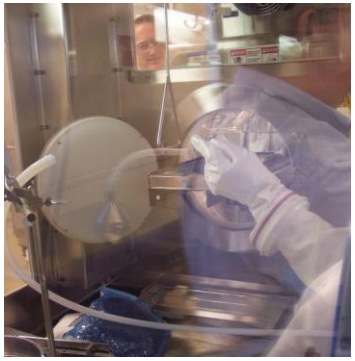
The following study was published in PDA Journal of Pharmaceutical Science and Technology; 2009, 63 (3) 258-282.

Monitoring Isolators

- Studies were conducted in a manufacturing isolator pilot facility
- *The surrounding room was not classified or controlled*

Transfer of Sterile Components

Activity	Total Volume Sampled	Sampling Time	Total Particles ≥ 0.5 µm	Total Particles ≥ 5.0 µm	Total Viable Particles
Transfer of autoclaved components from the 8-glove isolator into the filling isolator	1.75 m^3	1 hr 1 min	1	0	0
Transfer of dry heat oven sterilized components from the 3-glove isolator into the filling isolator	0.33 m^3	11 min 29 sec	0	0	0

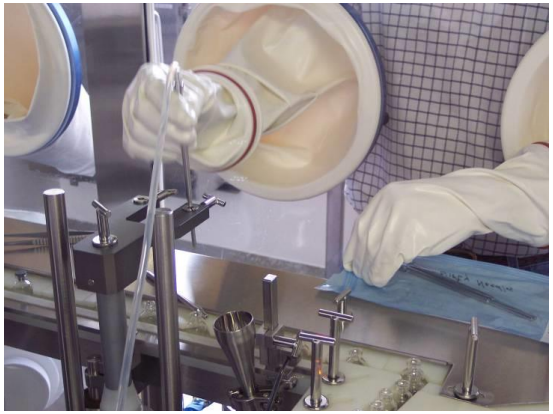


Dynamic Monitoring of Sterile Fill

Activity	Total Volume Sampled	Sampling Time	Total Particles ≥ 0.5 µm	Total Particles ≥ 5.0 µm	Total Viable Particles
Monitoring next to filling needle	1.01 m^3	35 min 23 sec	21	3	0
Monitoring transfer of vials during fill	0.10 m^3	3 min 23 sec	0	0	0

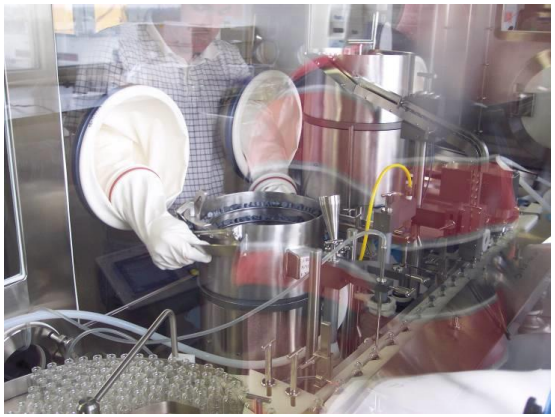
Interventions

Activity	Total Volume Sampled	Sampling Time	Total Particles ≥ 0.5 µm	Total Particles ≥ 5.0 µm	Total Viable Particles
Sterile filling needle replacement	0.05 m^3	1 min 47 sec	0	0	0



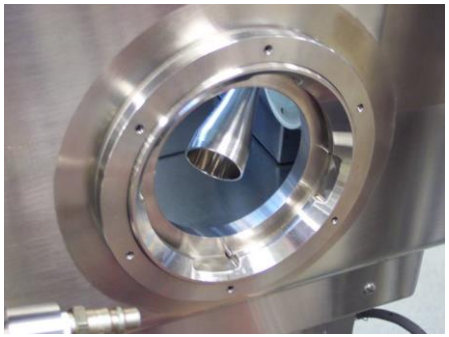
Interventions

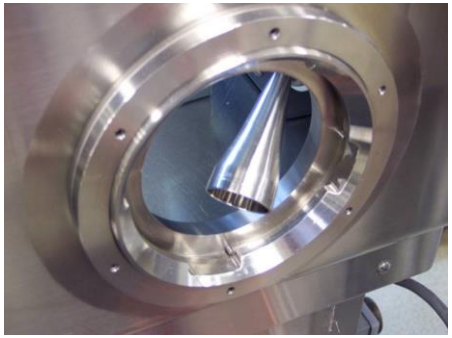
Activity	Total Volume Sampled	Sampling Time	Total Particles ≥ 0.5 µm	Total Particles ≥ 5.0 µm	Total Viable Particles
Detaching stopper bowl, moving bowl to opposite side of isolator, returning bowl to original position and reattaching	0.07 m^3	2 min 29 sec	1	0	0



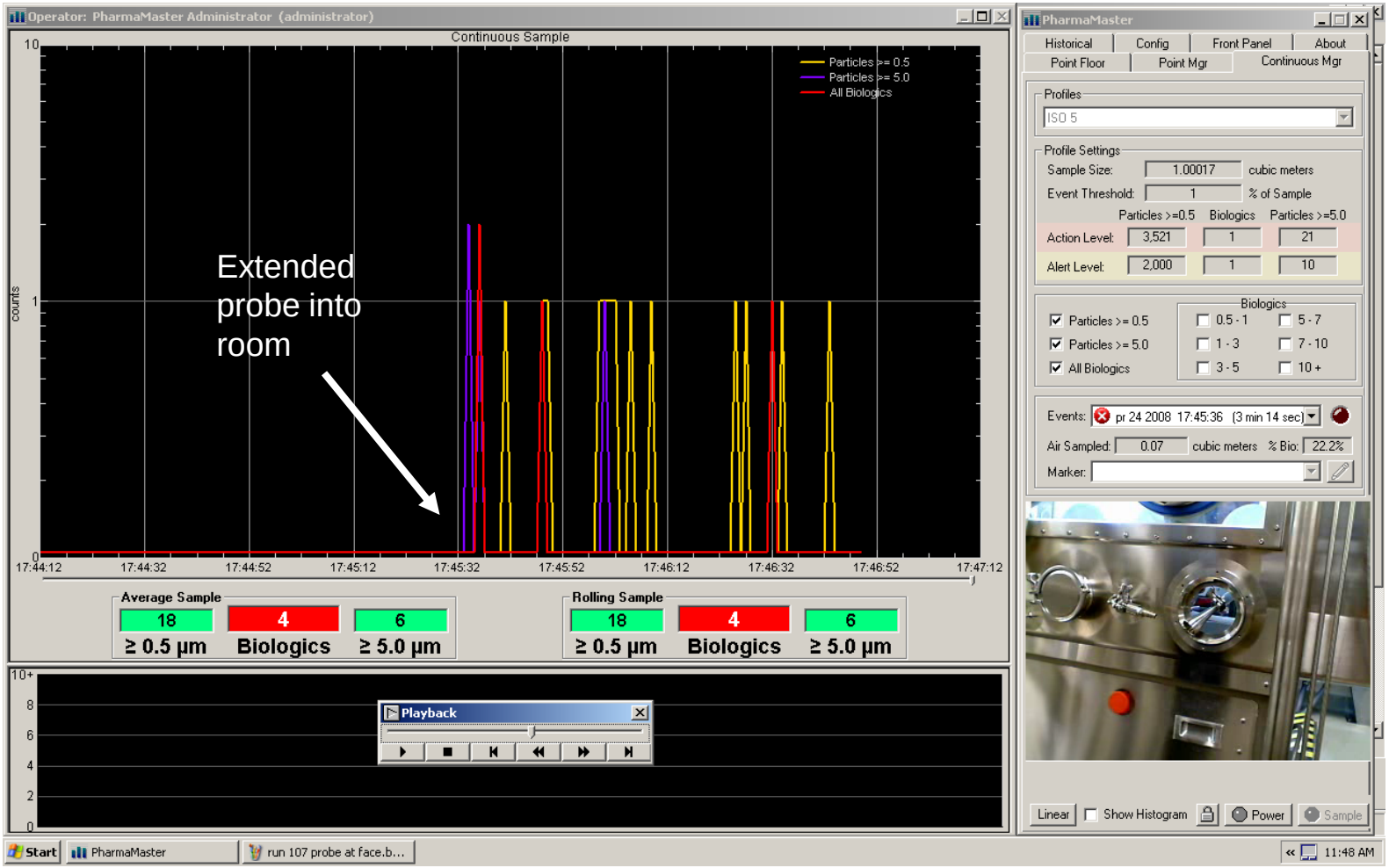
Simulated Mousehole

Activity	Total Volume Sampled	Sampling Time	Total Particles ≥ 0.5 µm	Total Particles ≥ 5.0 µm	Total Viable Particles
Monitoring mousehole with probe inside isolator	0.10 m^3	3 min 24 sec	1	0	0
Monitoring mousehole with probe at isolator-room interface	0.03 m^3	1 min 01 sec	0	0	0
Monitoring mousehole after probe is pushed into room	0.10 m^3	3 min 37 sec	38	11	12





Simulated Mousehole



Glove Integrity Testing

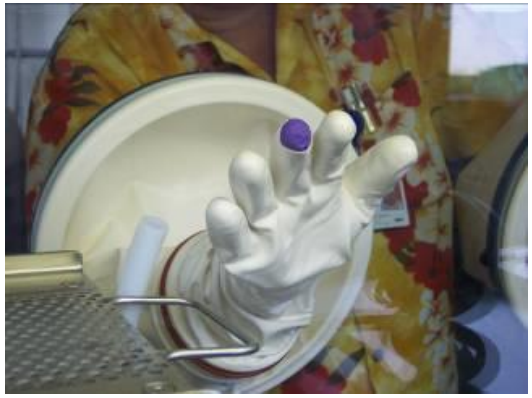
- It has been suggested that pinholes in isolator gloves represent an increased risk of contamination
- Engineered pinholes in Hypalon gloves
  - 75-100 µm and 200-250 µm
- Monitored with and without clamped finger, with inserted hand wearing a sanitized inner glove, bare hand
- Tested under positive and negative pressure

Glove Integrity Testing

- Monitored while moving and flexing fingers

Glove Integrity Testing

- Monitored with cut finger

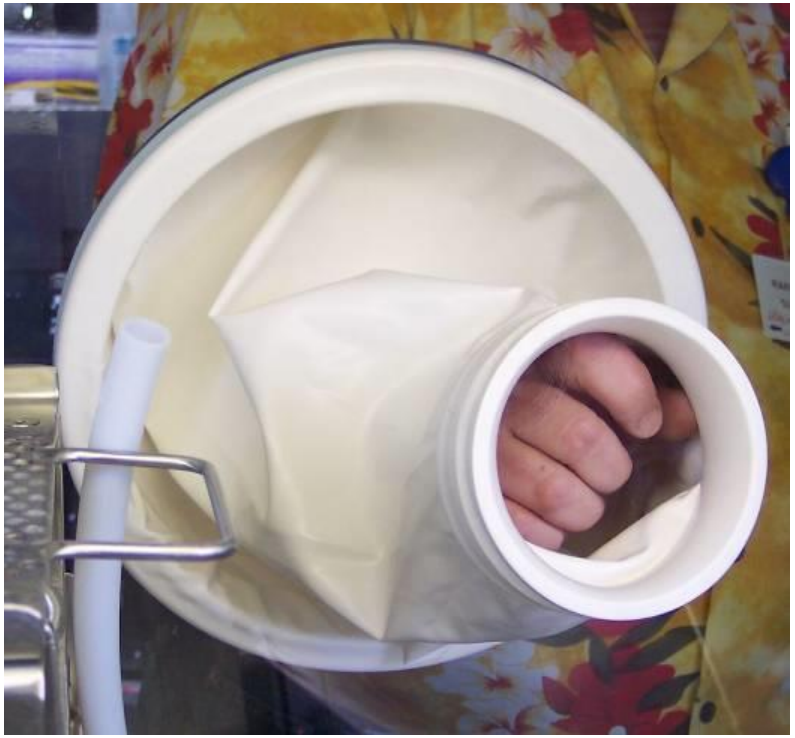


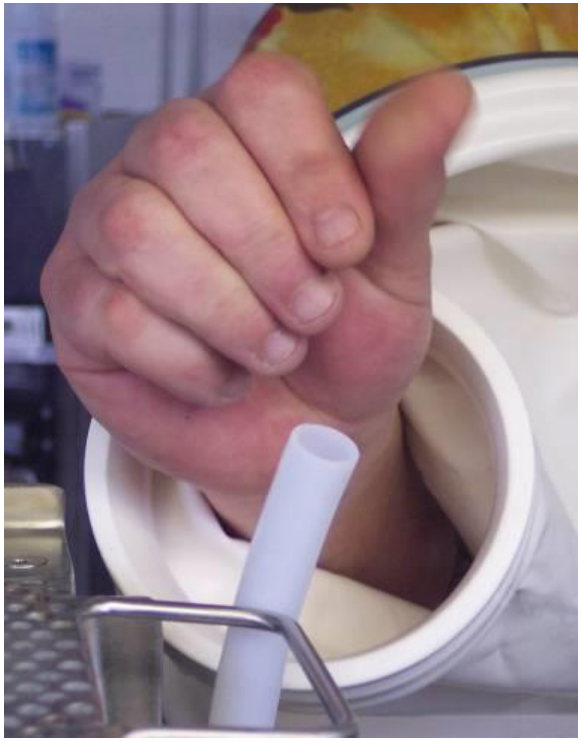
Glove Integrity Testing

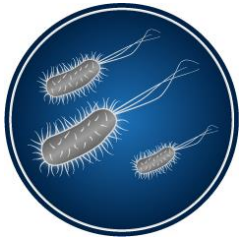
- Monitored with all fingers cut off

Glove Integrity Testing

- Monitored with glove completely removed



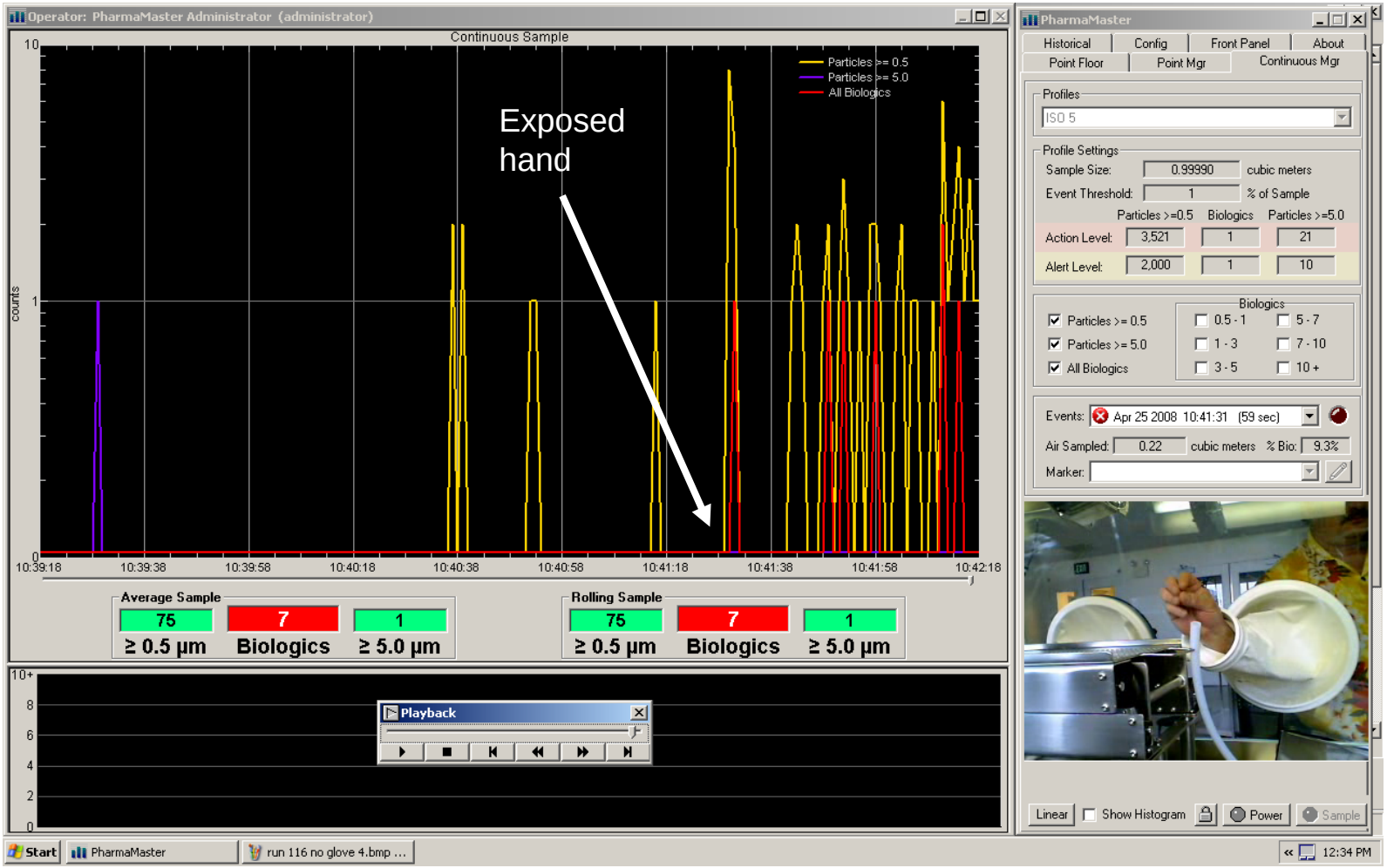




Glove Integrity Testing

Activity	Total Volume Sampled	Sampling Time	Total Particles ≥ 0.5 µm	Total Particles ≥ 5.0 µm	Total Viable Particles
Monitoring a 75-100 µm pinhole	0.45 m^3	15 min 35 sec	7	0	0
Monitoring a 200-250 µm pinhole	0.22 m^3	7 min 51 sec	0	0	0
Monitoring a glove with a cut fingertip	0.30 m^3	10 min 5 sec	12	2	1
Monitoring a glove with all fingers cut off and when the glove was removed and particles introduced	0.23 m^3	8 min 19 sec	75	1	7

Glove Integrity Testing

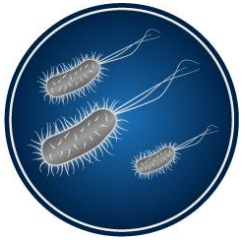


Particle Measuring Systems BioLaz

- Real-time, continuous monitoring of air
- Simultaneous detection of viable and total particles
- Fluorescence of NADH and riboflavin
- Quantification of individual airborne microorganisms
- Intended to be integrated with your current, traditional monitoring program
- Supplements existing viable and nonviable monitors for batch release

TSI BioTrak

- Real-time, continuous monitoring of air
- Simultaneous detection of viable and total particles
- Auto-fluorescence of cellular targets
- 28 LPM samples 1 cubic meter of air in ~35 minutes
- Compliant to ISO-21501-4 and JIS
- Gelatin filter to capture organisms for subsequent growth and testing, such as microbial ID

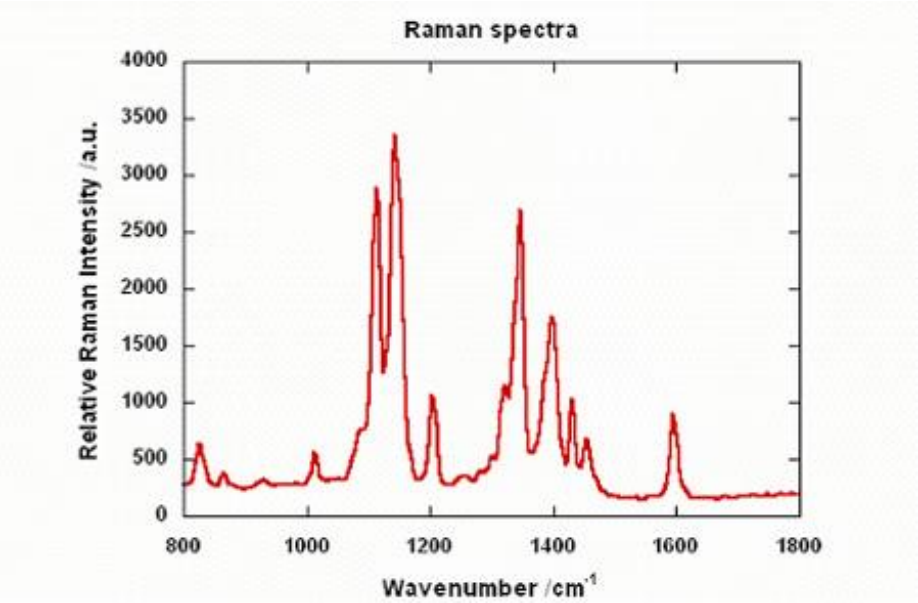


Real-Time Microbiology

- These three real-time RMM systems represent a significant paradigm shift in the way which we conduct microbiology in support of pharmaceutical manufacturing
- Real-time, in-process environmental monitoring is aligned with PAT and QbD initiatives
- May support the parametric release of aseptically filled product
  - But we will still need real-time in-process monitoring of the product stream and filling
  - Detection, enumeration and identification
  - Technologies are currently available and in development that will meet these needs

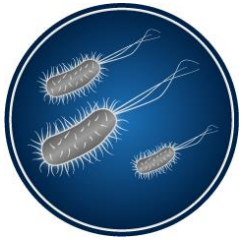
Raman Spectroscopy

- Scattered light can also provide information about molecular vibrations and rotations of molecules (Raman)
- Each microorganism has its own unique Raman spectrum which can be used as a fingerprint for identification



Battelle REBS

- Resource Effective Bio-Identification System
- Based on Raman spectroscopy
- Analysis of liquids and aerosols (military application)
- Effective for bacteria, viruses, and toxins

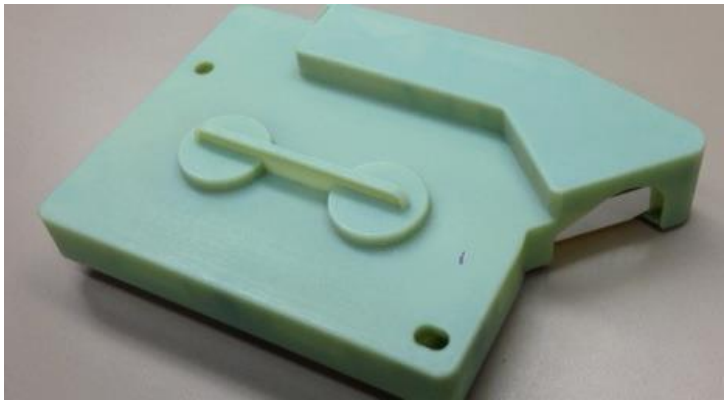


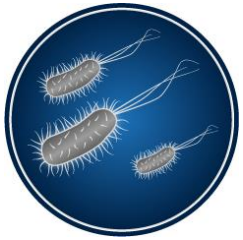
Battelle REBS

- Primary pharmaceutical use is in-line testing of purified water and other aqueous formulations
- Detection, identification, and enumeration (1 cfu/mL)
- Capable of identifying over 100 pathogens in less than 30 minutes with continuous sample collection
- Sampling and time to result: 2 hours using Raman spectroscopy
- However, the system will not differentiate viable from dead cells

Battelle REBS

- Samples are collected on a non-destructive, collection device (cassette tape). Raman is run on the sample section. A count and ID is provided.
- When a positive is detected, you can cut out the sample section, place into a conical tube containing PBS and 1% triton and vortex for further analysis (culturing or PCR)



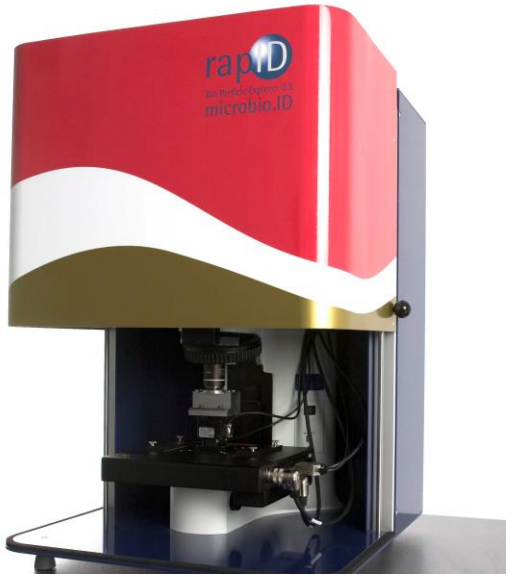


Rap.ID Particle Systems

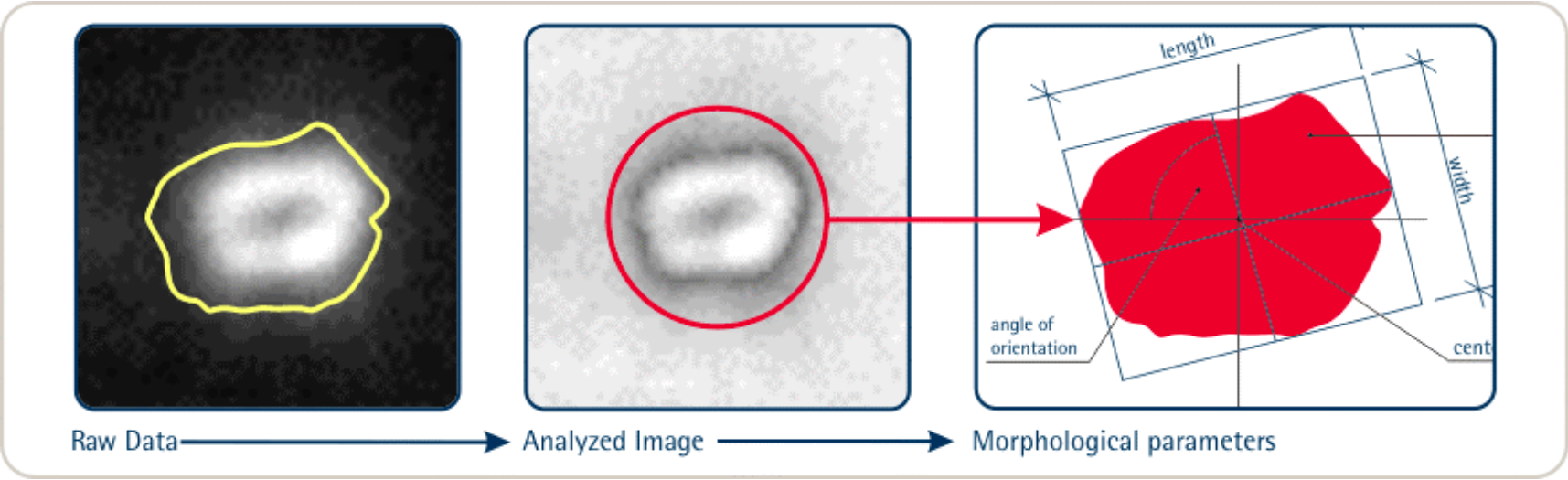
- Viability staining and automated image analysis using dark field illumination
  - Detects viable particle quantity, shape, and size for particles ranging from 0.5 µm and larger
- Confocal Raman laser beam (532 nm) is then automatically aligned with the viable particle locations
- A spectral signature generated and is compared with a library of known microorganisms (database is still being developed)

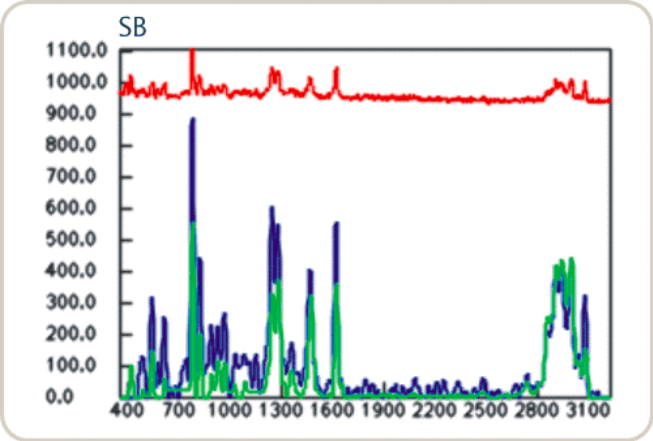
Rap.ID Particle Systems

- Particles are collected on metal foil using impaction or filtration methods for airborne or liquid samples
- Viability staining and particle enumeration in 4 minutes
- Followed by microbial identification of a single viable cell is 1-5 seconds
- 150 bacteria and spore entries in database, customizable
- 300-600 individual ID's per hour
- >150 samples per 8 hours
- Non-destructive for further analysis



Rap.ID Particle Systems



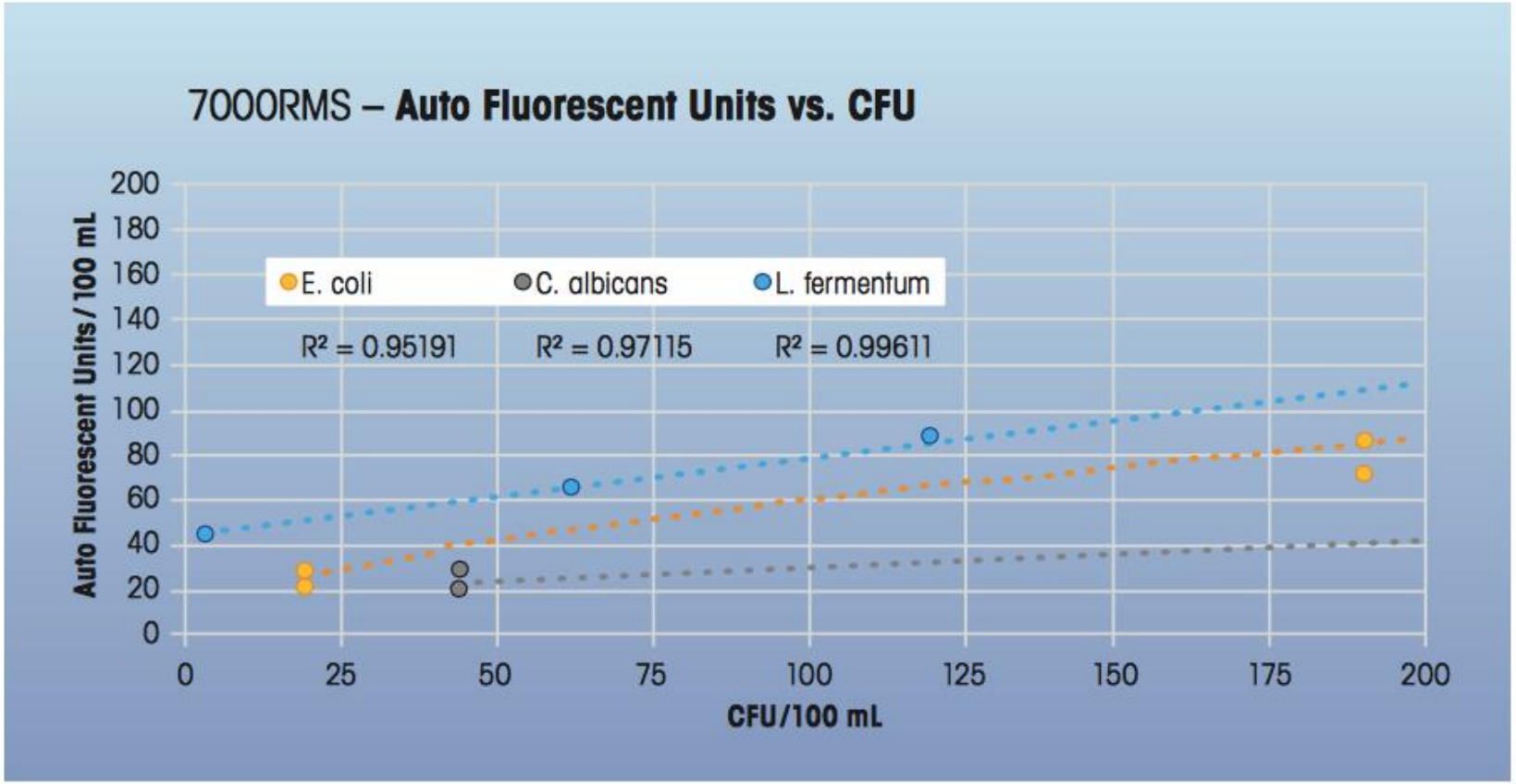


Mettler-Toledo 7000RMS Bioburden Analyzer

- Mie scattering and intrinsic fluorescence of microorganisms in liquids
- 30 mL/minute flow rate
- Continuous monitoring or point sampling
- On-line
- 5 - 90° C water
- Linear range: 1-700 counts/mL

Mettler-Toledo
7000RMS Bioburden Analyzer

Mettler-Toledo 7000RMS Bioburden Analyzer

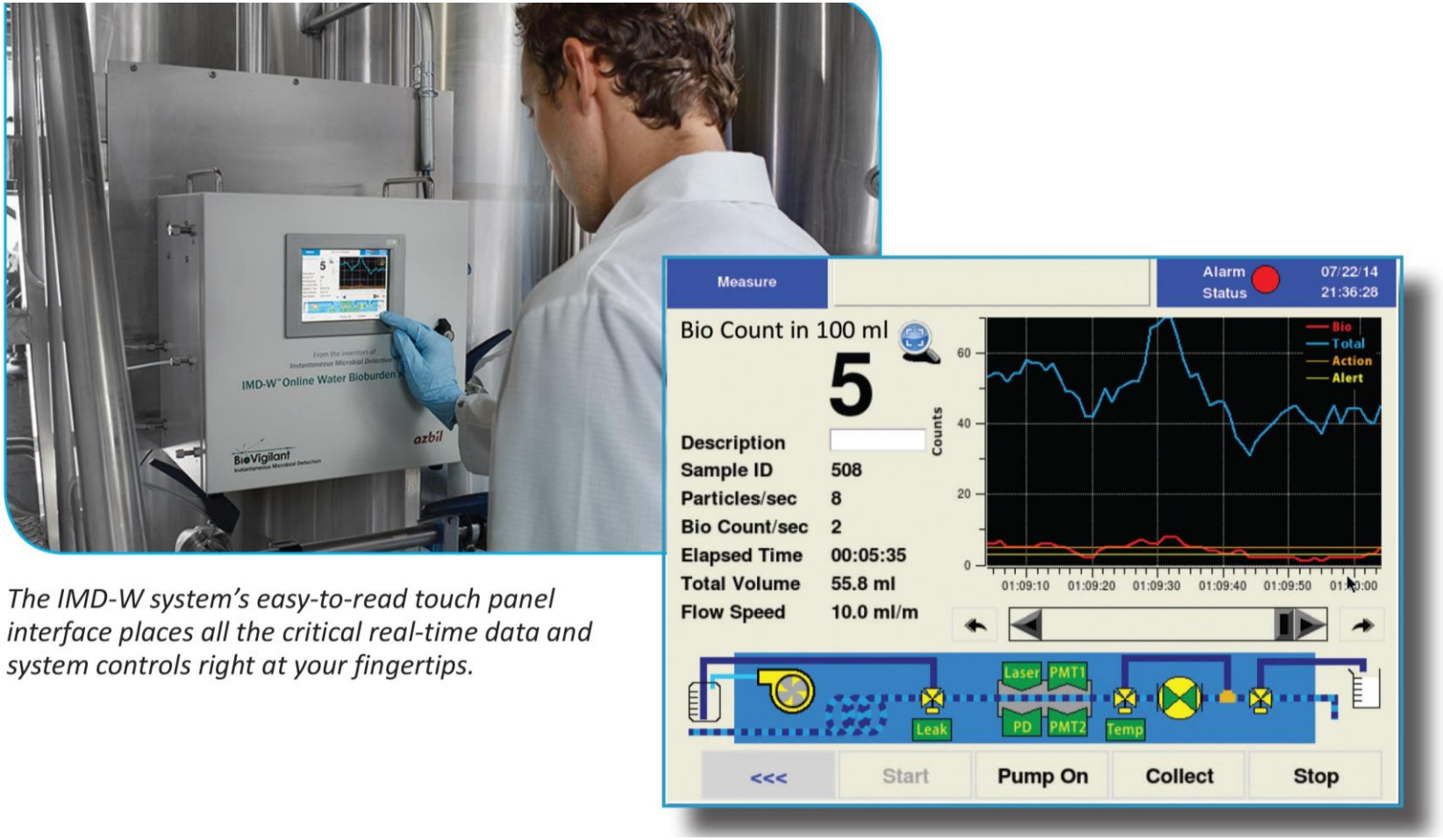


Correlation graph of plate count (CFUs) and 7000RMS (Auto Fluorescent Units)

BioVigilant IMD-W

- Mie scattering and intrinsic fluorescence of microorganisms in liquids
- 10 mL/minute flow rate
- Continuous monitoring or point sampling
- On-line
- 0 - 90° C water
- Detection limit: 1 biocount

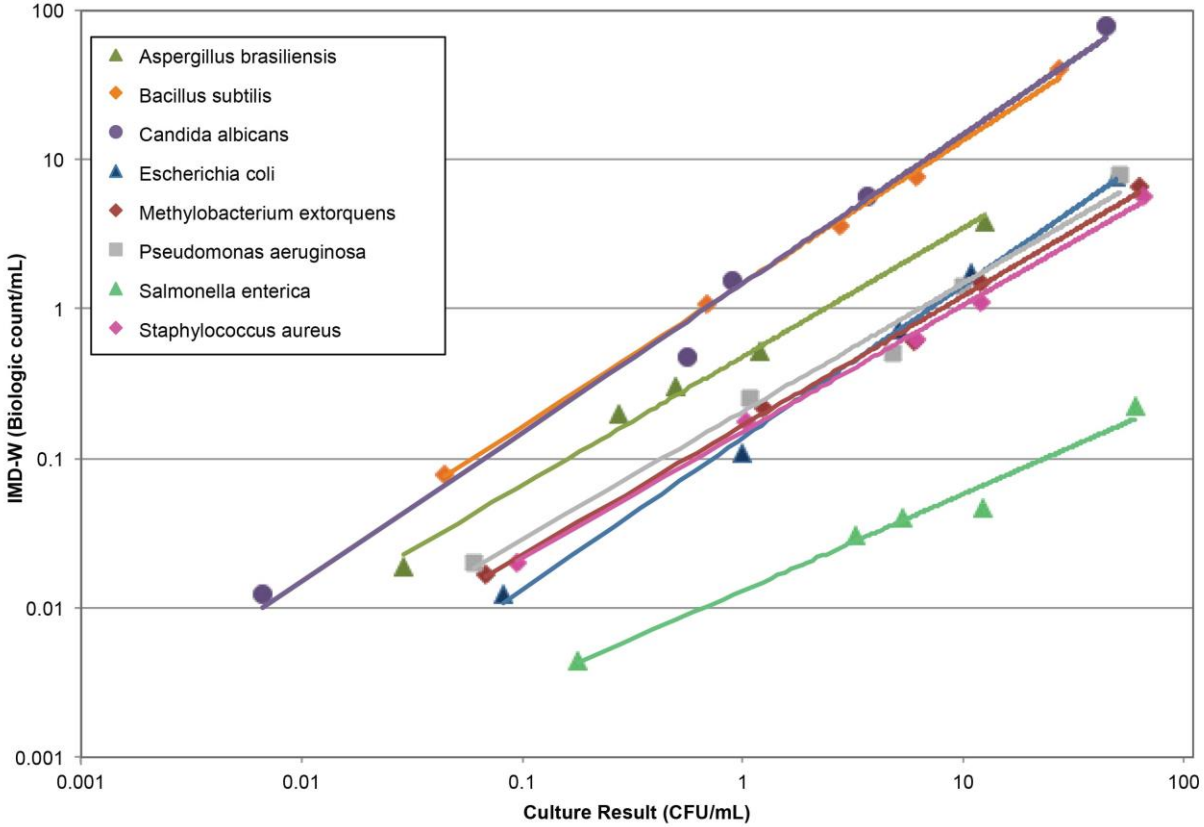
BioVigilant IMD-W



The IMD-W system's easy-to-read touch panel interface places all the critical real-time data and system controls right at your fingertips.

BioVigilant IMD-W

Correlation to Culture Counting Method



The IMD-W system's fluorescing particle counts show a high level of correlation to conventional CFU cultured counts across a wide dynamic range.

R^2 Values

Microorganism Tested	Coefficient of Determination (R^2)
A. brasiliensis	0.992
B. diminuta	0.677
B. subtilis	0.998
C. albicans	0.991
E. coli	0.997
M. extorquens	0.996
P. aeruginosa	0.985
P. putida	0.712
S. enterica	0.980
S. aureus	0.997

Coefficient of determination (R^2) values are shown for the relationship between IMD-W biologic counts and culture CFU results. A value close to one shows a high level of correlation in the results from both methods.