Laser Induced Fluorescence
LIF Fundamentals and Applications
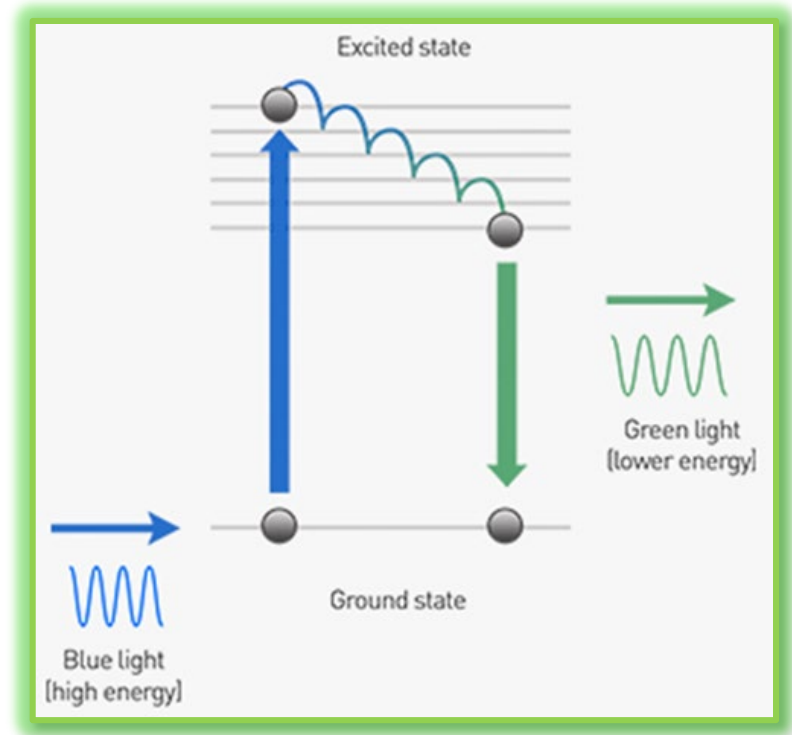
TSI Inc. Model 9510-BD
BioTrak® Real-time viable particle counter

What is fluorescence?

+ Molecule absorbs energy from light source
+ Some energy is lost to molecular vibrations
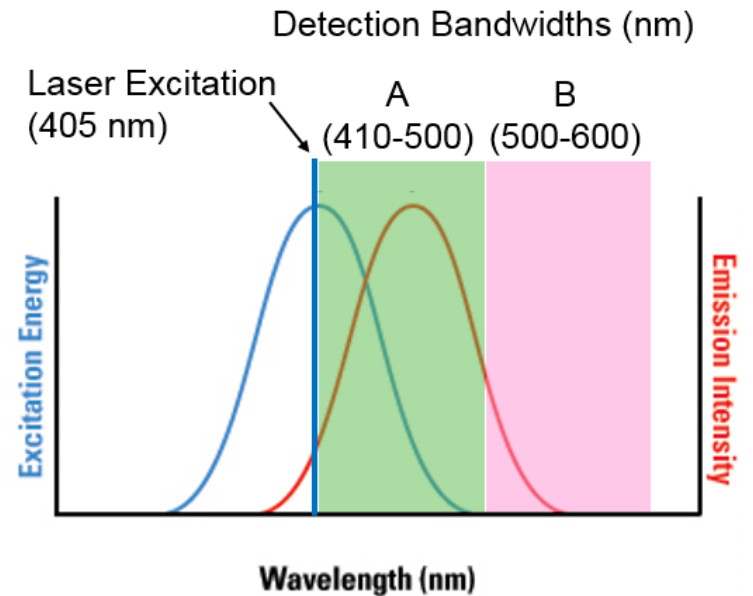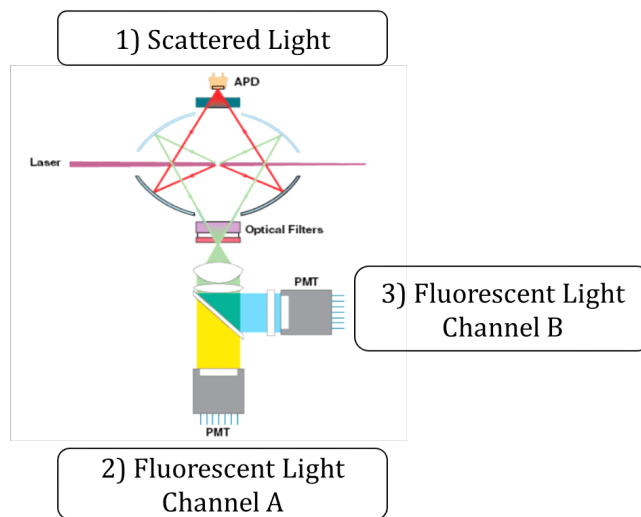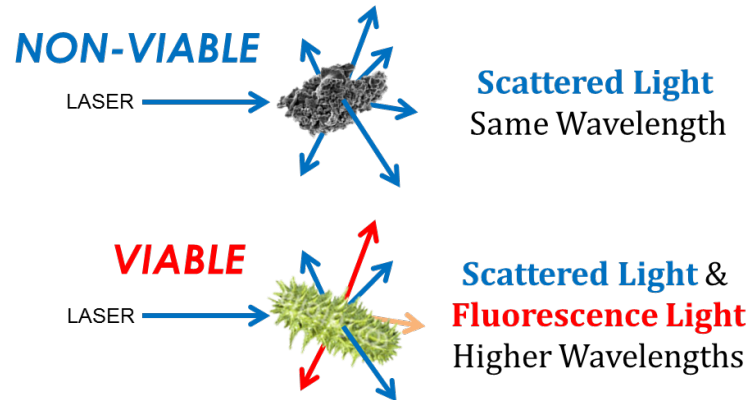+ Remaining energy released as light at a higher wavelength

Excited state

Green light
[lower energy]

Ground state

Blue light
[high energy]

What is Autofluorescence?

+ Cells contain many molecules that fluoresce when exposed to UV light
+ Metabolites of microorganisms
+ Look for autofluorescence to detect viable particles

Molecule	Approximate Peak Fluorescence (nm)
NAD(P)H	450
Retinol	500
Riboflavin	550
Folic acid	450
Pyridoxine	400
Tyrosine	305
Tryptophan	325
Flavin	540

Detecting Autofluorescence

NON-VIABLE

LASER →

Scattered Light
Same Wavelength

VIABLE

LASER →

Scattered Light &
Fluorescence Light
Higher Wavelengths

1) Scattered Light

APD

Laser

Optical Filters

PMT

PMT

3) Fluorescent Light
Channel B

2) Fluorescent Light
Channel A

Detection Bandwidths (nm)

Laser Excitation
(405 nm)
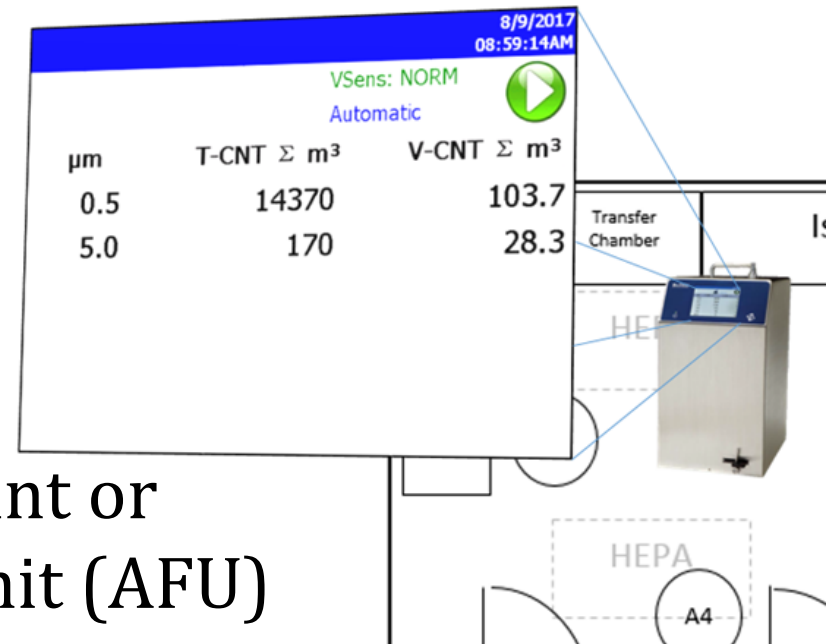
A
(410-500)

B
(500-600)

Excitation Energy

Emission Intensity

Wavelength (nm)

Environmental Monitoring using LIF

+ Biofluorescent Particle Counters (BFPC)
+ Sampling method identical to particle counting
 - Fully automated
  - Real-time results

+ Viable Particle Count or Autofluorescing Unit (AFU)

8/9/2017
08:59:14AM

VSens: NORM
Automatic

µm	T-CNT Σ m³	V-CNT Σ m³
0.5	14370	103.7
5.0	170	28.3

Transfer Chamber

HE

HEPA

A4

Environmental Monitoring using Traditional Microbiology

+ Collect an air sample onto growth media passively or actively

AIR SAMPLE
AIRBORNE PARTICLES
GEOMETRIC CHARACTERISTICS
PETRI DISH
AIR FLOW
AIR SAMPLER
VACUUM PUMP
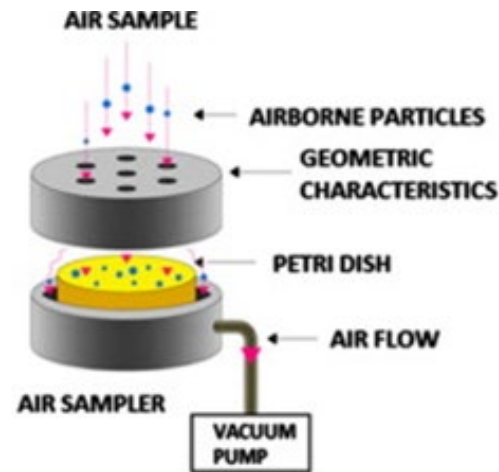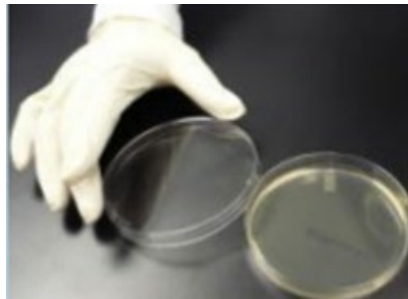
+ Incubate plates to allow for the formation of visible colonies (≥ 2 days)
+ Count the colony forming units (CFU)

Method Comparison

No method detects everything

+ Each provides an estimate of airborne viable contamination based on detection capabilities

+ Traditional methods estimate based on growth under the chosen incubation conditions

+ LIF methods estimate based on fluorescence consistent with what is expected for viable microorgansims

Method Comparison

Timeliness of results can be the difference between preventing an adverse event or being able to react to one

+ Traditional methods take days to obtain results – can only try to determine the possible impact to product of the viable contamination detected
+ LIF methods obtain results in real-time – can be proactive and immediately work to mitigate the issue to prevent an impact to your product
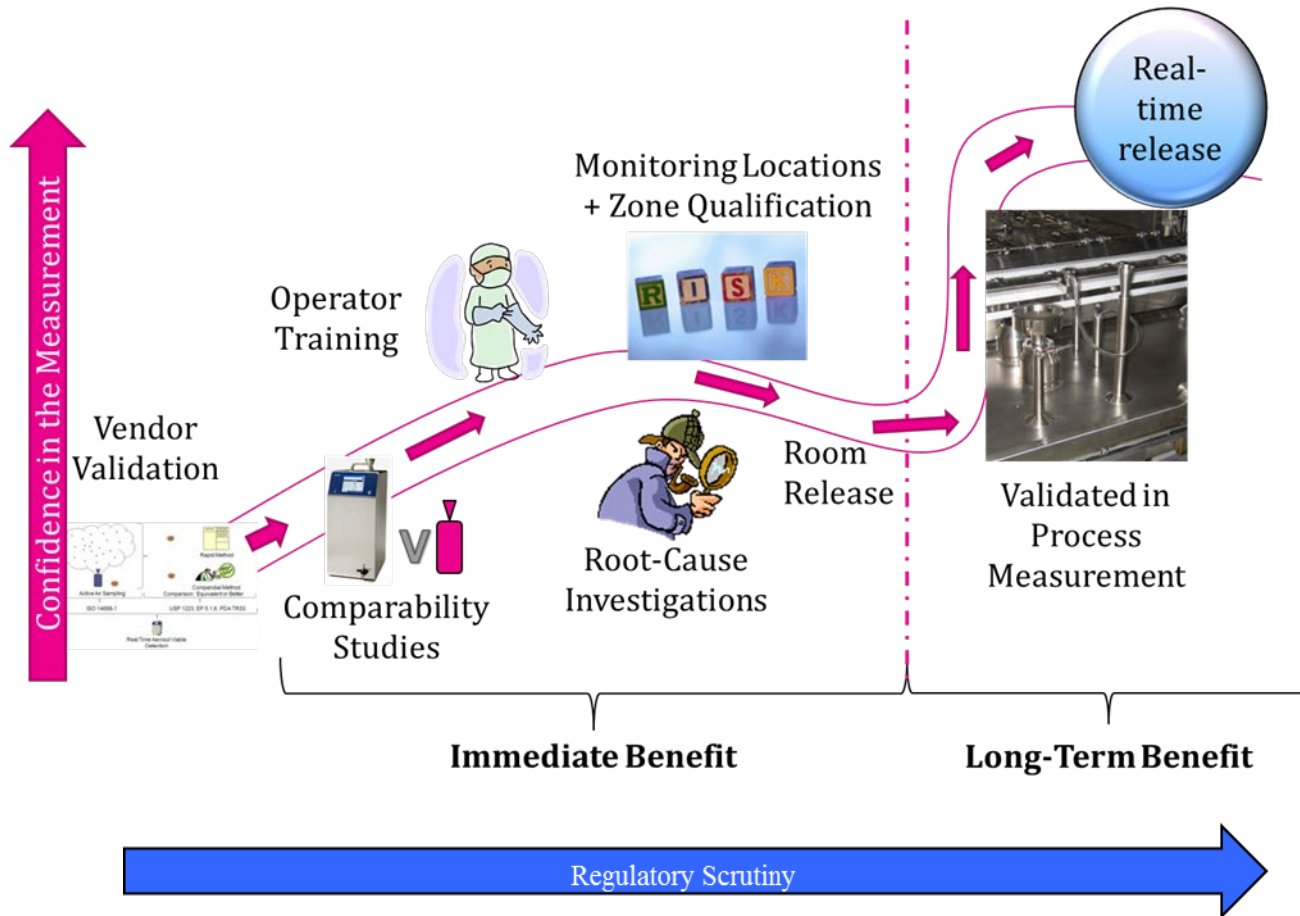
Method Comparison

Manual methods can have data integrity concerns

+ Traditional methods rely on manual plate counts
   – susceptible to human error
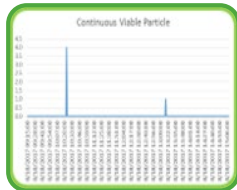
+ LIF methods are fully automated

LIF Applications

LIF Applications

Root Cause Analysis

Real-Time Monitoring

Continuous Monitoring

Scanning

LIF Applications

Monitoring in Grade C/D Areas:

+ Continuous monitoring supplements current EM

+ Reduce risk by being proactive instead of
    reactive

+ Reduce sampling

LIF Applications

Monitoring Aseptic Processes:

+ Continuous monitoring system

+ Only requires an isokinetic probe in Grade A area

+ Immediate actions can be taken in the event of
   an excursion

In-process Environmental Monitoring

1. No interventions •No: product waste, nozzle clogging, downtime, extra sterility testing…
2. Increase process knowledge• real-time, continuous data
3. Real-time corrective action
4. Readily integrated & automated • Samples like total particle counter
5. Automated operation, alarms, analysis, etc… (FMS)

Benefits:
- **Increase Throughput**
- **Reduce Risk**

Implementation

Challenges

+   Understanding the results

+   Regulatory scrutiny

- Will depend on application
- Agencies encourage the use of new technologies
- How will inspectors react?
- BioPhorum Operations Group (BPOG)
- Process and Environmental Monitoring Method (PEMM)

Implementation

BPOG Article in the PDA Journal April 2019:
Continuous Microbiological Environmental
Monitoring for Process Understanding and
Reduced Interventions in Aseptic Manufacturing
Jeffrey Weber, James Hauschild, Pieta Ijzerman-Boon, et al.
PDA J Pharm Sci and Tech 2019, 73 121-134
Access the most recent version at
doi:10.5731/pdajpst.2018.008722

Implementation

+ Process and Environmental Monitoring Methods (PEMM) group shares information about the technology and applications for real-time biofluorescent particle detection methods and how they help to address major industry concerns. To find the recent article go to: https://www.tsi.com/products/cleanroom-particle-counters/real-time-viable-particle-counter/biotrak-real-time-viable-particle-counter/

Questions?

11/12/2019