

FUNDAMENTALS OF REAL-TIME VIABLE PARTICLE MONITORING: HOW DOES IT WORK?

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Real-time viable particle monitoring instruments have been available for over five years. These instruments can overcome many of the obstacles presented by traditional growth-based viable monitoring (i.e., poor recovery of some organisms, long incubation times, and risk of contaminating product during interventions). However, due in part to a lack of understanding in the technology used by these instruments, implementation thus far has been limited. This white paper is intended to be a general description of this technology and how it is used to detect viable particles in real-time.

Laser Induced Fluorescence

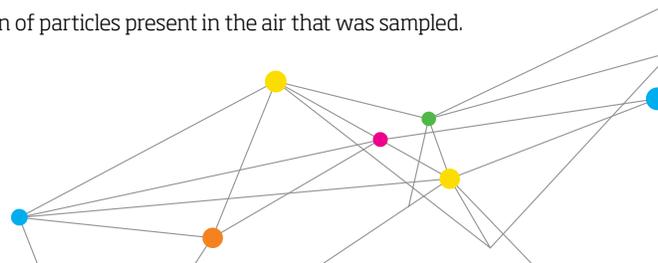
The technology behind real-time viable particle monitoring is laser induced fluorescence (LIF). It may also be referred to as enhanced active air particle counting or auto-fluorescent active air particle counting. The core technology involves detecting the effects a particle has on a laser beam as the particle passes through it. Counting viable particles by this method more closely resembles optical particle counting than it does counting colonies using traditional microbiological methods.

Optical Particle Counting Basics

Optical particle counting has been widely accepted for decades for determining the concentration and size distribution of total airborne particles in cleanrooms. Particles are sampled and directed through an optics chamber where they pass through a laser beam, causing the light to scatter. Light scattering events are counted with respect to the volume of air sampled, and the intensity of each event is measured to determine the size of each particle. The optical particle counter uses this data to calculate and report the concentration and size distribution of particles present in the air that was sampled.



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Viable Particle Counting with Laser Induced Fluorescence

Much like an optical particle counter, an instrument using LIF passes a particle through a laser beam and detects the light that was scattered. However, unlike an optical particle counter, the LIF instrument also detects if the particle fluoresced. A particle fluoresces if it absorbs some of the light and reemits it at a higher wavelength as shown in

Figure 1. Fluorescence is a good indicator for determining if a particle is viable or non-viable because living microorganisms contain relatively high concentrations of molecules that fluoresce. Because this method relies on optical characteristics instead of culture conditions (i.e., media type, incubation temperature, incubation time, etc.), it generally detects more viable particles than growth-based methods. It produces a more sensitive measurement for viable contamination.

Reliability of Results

Unfortunately, microorganisms are not the only particles that fluoresce. Early LIF instruments had very high rates of false positives because they had a difficult time discriminating between microorganism carrying particles and other fluorescing particles. These instruments based viability determination on only two optical parameters, light scattering and fluorescence in a single range of wavelengths.

Figure 2 Panel A demonstrates how a non-viable fluorescing particle, in this case pollen, would be hard to differentiate from a viable particle using these instruments. In order to reliably discriminate between the two, more information is needed. This led to the development of instruments that detected fluorescence in a second range of wavelengths. For example, the TSI BioTrak® Real-Time Viable Particle Counter detects fluorescence in the ranges of 430-500 nm and 500-650 nm. **Figure 2 Panel B** shows how this greatly improves the ability to discriminate between viable and non-viable fluorescing particles. This improved capability made LIF suitable to use in aseptic areas where the detection of a single viable contaminant may require an investigation and possibly even the loss of product.

Airborne particles having optical characteristics consistent with a microorganism are counted as viable.

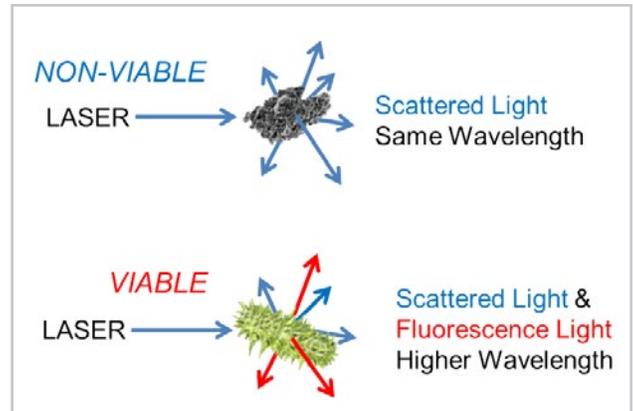


Figure 1. Simplified representation of the optical characteristics of viable and non-viable particles.

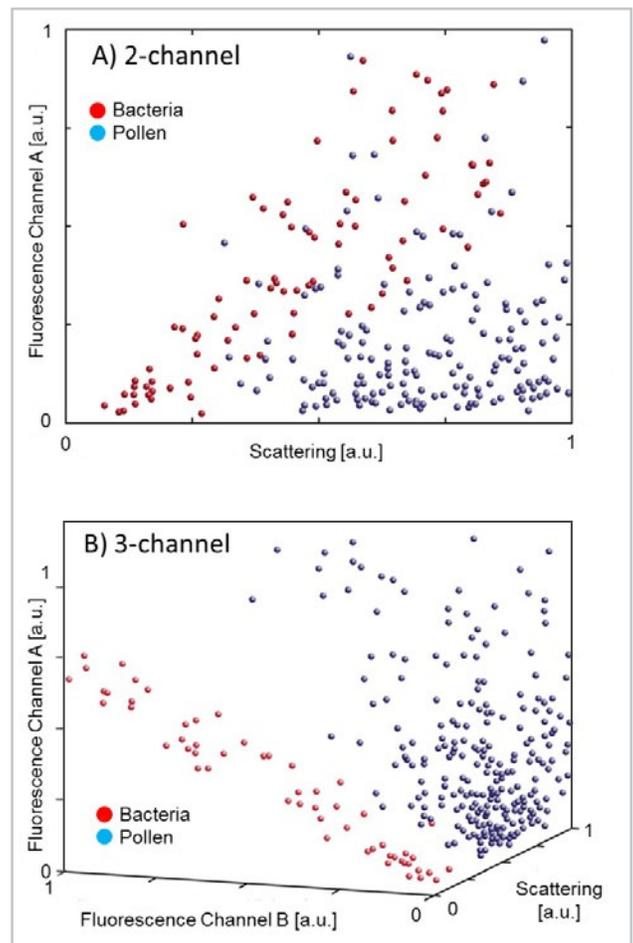
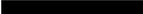


Figure 2. Improved viable discrimination using three optical parameters.



Summary

LIF technology uses the fluorescent properties of microorganisms to determine the concentration of viable particles present in an air sample. The results are reported in real-time in much the same fashion as an optical particle counter reports total particle counts. Improvements in technology over the years have resulted in instruments that are suitable for use in cleanrooms of any class. This includes aseptic areas where the benefits of higher sensitivity and real-time results can better assure the quality of the product being manufactured.



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