BIOTRAK® REAL-TIME VIABLE PARTICLE COUNTER: DISCRIMINATION CAPABILITY

Introduction

The BIOTRAK Particle Counter is a full-featured instrument that detects the total number of particles in the air as well as determines which of those particles are viable in nature. Additionally, the BIOTRAK Particle Counter incorporates a particle collection filter so the optically analyzed particles are collected and available for subsequent speciation analysis. The BIOTRAK Particle Counter incorporates proven technologies leveraging TSI's experience in particle measurement theory, instrument development, and calibration.

The BIOTRAK Particle Counter falls into a class of instruments known as Rapid Microbiology Methods (RMM) that have garnered significant interest over the past several years. RMM techniques offer significant potential benefits to manufacturing spaces concerned with environmental microbiological contamination. The key differentiation that the BIOTRAK Particle Counters offer is the ability to detect the presence of viable particles in **real time**. When compared to the traditional active air sampling and compendial culture based count methods that require 2 to 4 days of incubation and analysis, the BIOTRAK Particle Counter provides:

- High resolution trending of microbial particle levels in the manufacturing environment
- Instantaneous notification of microbial contamination events, which enables:
  - Segregation of potentially exposed product
  - Environmental alert and action level alarms
  - Rapid initiation of root cause investigation
- Process Analytical Technology (PAT) based process control input
- Potential for real-time product release
- Real-time feedback to support personnel training on proper clean room practices

One of the key operating characteristics of a real-time viable particle detector is its ability to discriminate viable particles from non-viable particles. For more information on The Theory of Operation, please see Application Note CC-101.

Discrimination

The BIOTRAK Particle Counter incorporates patented[*] technology based on Laser Induced Fluorescence (LIF). LIF utilizes the intrinsic fluorescence of microbial constituents to determine whether a particle is viable. Certain cell metabolites associated with cell viability fluoresce when excited by ultra-violet light. The metabolites most commonly associated with cell viability are tryptophan, NADH, and the flavin's (riboflavin). Figure 1 shows the wavelength dependant excitation and emission bands for riboflavin.

The metabolites fluoresce when excited by any wavelength within the excitation band. The emission spectrum is independent of excitation wavelength although it will only emit at wavelengths above the excitation wavelength. The emission magnitude does change, depending upon the excitation factor shown in the Figure 1a. Each viability metabolite has its own distinctive LIF excitation and emission curves. A viable particle (microorganisms) contains a complex mixture of metabolites and fluoropheres and the emission spectrum is complex. Due to this variability, it is not possible to obtain species information.

Figure 2 (Jeyes et al 2007, Advanced Trigger Development. *Lincoln Laboratory Journal* Vol. 17 No. 1 pp 29-62) illustrates complex excitation-emission spectral contours for the primary viability markers, a common bacteria (*Bacillus globigii*), and some fluorescing non-viable particulates.
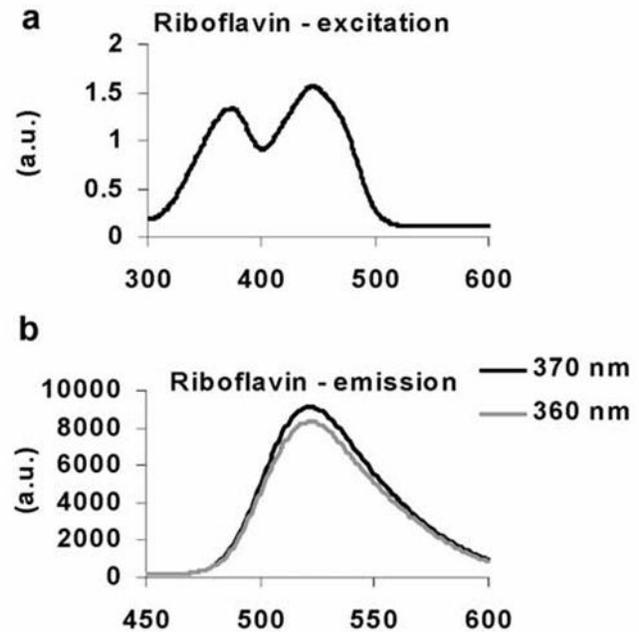

Figure 1. Riboflavin excitation and emission curve
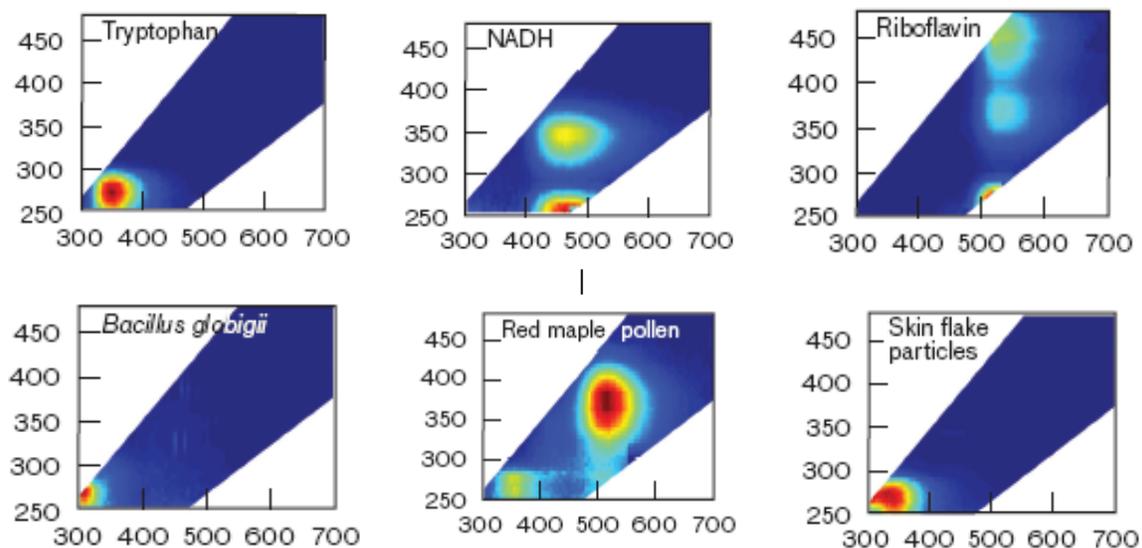(Plytycs et al *Folia Histochemica et Cytobiologica* 2006)

Figure 2. Excitation emission spectra of viability markers tryptophan, NADH, and riboflavin; the common microorganism Bacillus globigii; and common nonviable fluorescent particles red maple pollen and skin flakes

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[*]The BIOTRAK incorporates one of more of the following patents: 6,167,107; 5,701,012; 5,895,922; 6,831,279; 7,261,007.

In these plots, the Y-axis is the excitation wavelength while the X-axis is the fluorescence emission wavelength. These are full spectral response curves where the particles where excited at many wavelengths and the fluorescence response measured at multiple wavelengths. The ideal instrument would be able to duplicate this functionality. In reality, each additional excitation wavelength requires an additional laser and a spectrometer is required to separate the fluorescence emission into its constituent wavelengths. This type of measurement requires expensive laboratory equipment and significant sample preparation.

Although not a full spectrometer, the BIOTRAK Particle Counter separates the broad fluorescence emission into two bands in order to improve discrimination and utilize the spectral information present. The BIOTRAK Particle Counter utilizes a third generation optics engine resulting from over 20 years of technology and product development for military and homeland defense bio-threat detection products. Figure 3 shows the optics engine and its principle components. Three optical signals utilized to determine particle viability:

- APD—Particle Size
- PMTA—Short Wavelength Fluorescence Channel
- PMTB—Long Wavelength Fluorescence Channel

A detection algorithm analyzes the signals and determines if the measured particle is viable in nature. The use of thee parameters in the viability detection algorithm allows for good discrimination of viable particles from benign environmental particles.
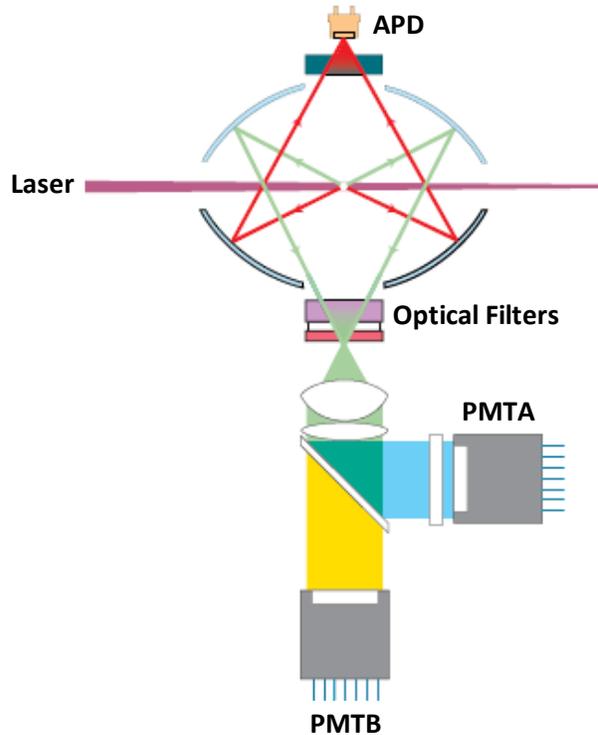
Figure 3. BIOTRAK Single Particle LIF Optics

The use of multiple parameters in the discrimination algorithm allows for better discrimination. Discrimination is the ability to differentiate viable from non-viable benign particulates.

Figure 4 illustrates the discrimination improvement offered by incorporating two fluorescence channels into the discrimination algorithm. Bermuda grass pollen is a non-viable particle that fluoresces while *Ralstonia picketti* is a microorganism. The same data is plotted in both figures. It is not possible to differentiate the viable particle from the non-viable particles in Figure 4A. The data is intermixed without any noticeable grouping. Figure 4B illustrates the power of adding an additional discrimination parameter. Two clear data groupings emerge when a second fluorescence channel is added to the analysis.

The BIOTRAK Particle Counter's discrimination algorithm incorporates three parameters to determine if a particle is viable in a similar manner as this visualization exercise shown in Figure 4. The data illustrated in Figure 4 was generated in a laboratory, but is indicative of the signatures obtained during operation in a manufacturing environment.
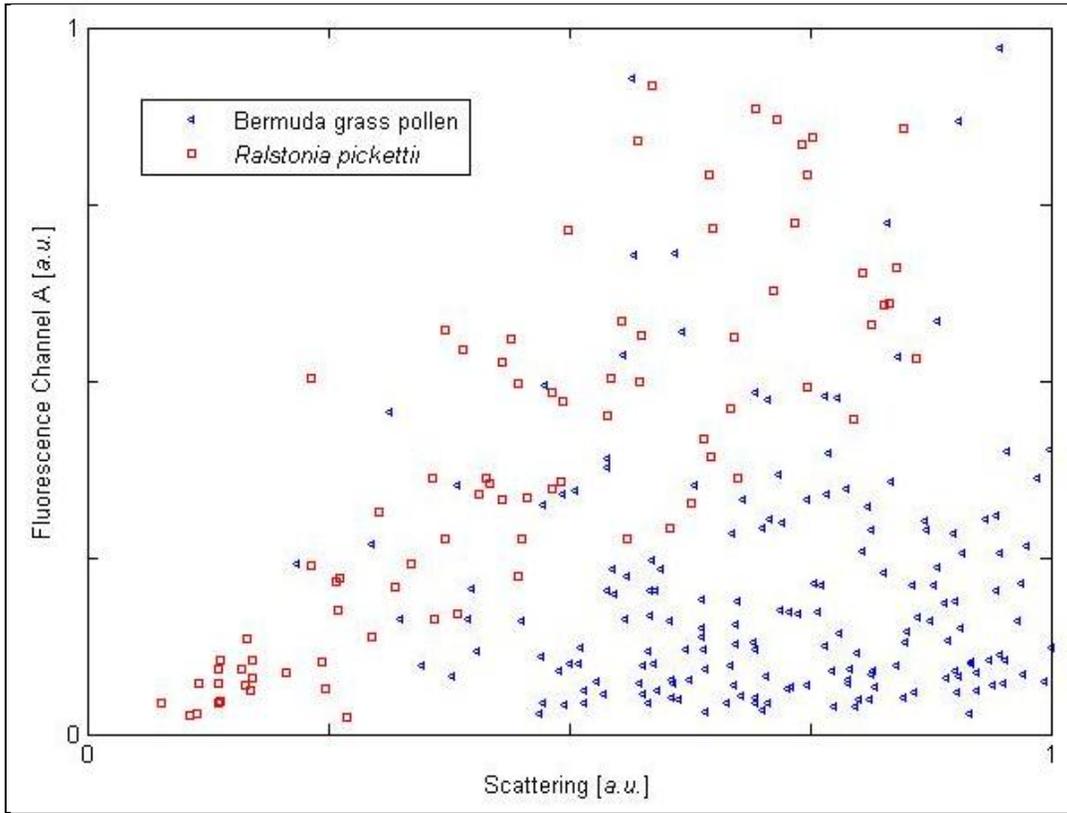
Figure 4A. Raw Data with Two Discrimination Parameters: Particle Size and Fluorescence
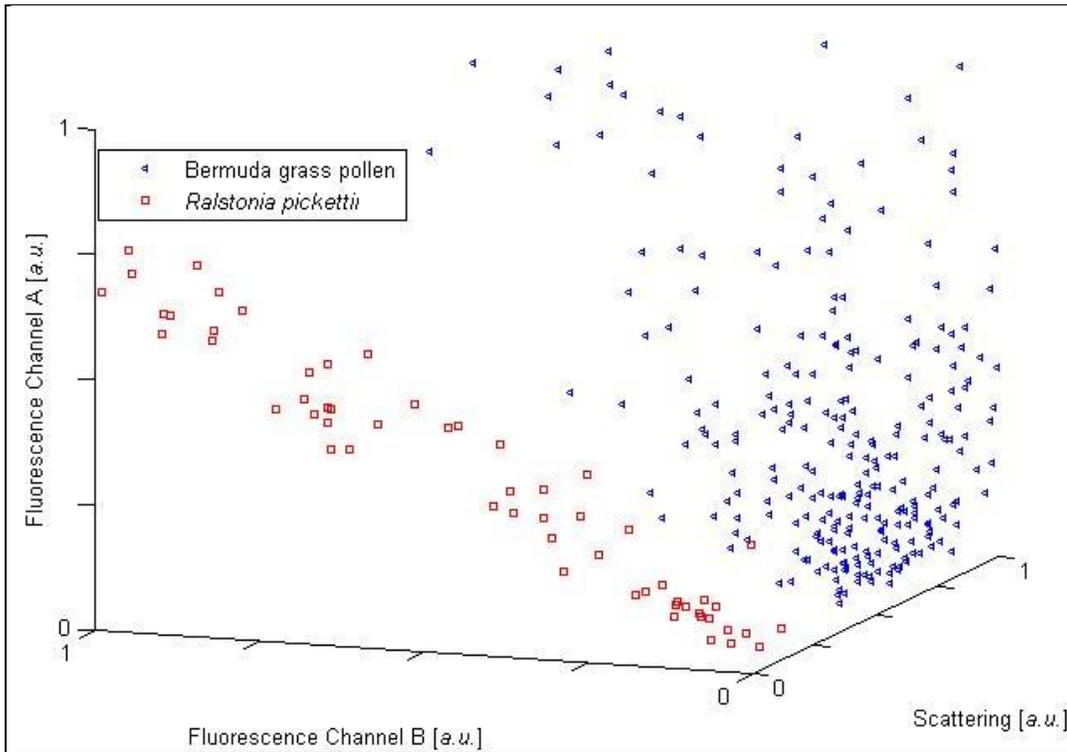

Figure 4B. Raw Data with Three Discrimination Parameters:
Particle Size, Fluorescence A, and Fluorescence B

Conclusion

The laser induced fluorescence signatures generated from environmental particles are complex. It is a challenging task to discriminate viable from non-viable particles that have fluorescence characteristics. The BIOTRAK Particle Counter incorporates three discrimination parameters into its viability detection algorithms greatly enhancing its ability to correctly classify viable versus non-viable particles.

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