Instantaneous Microbial Detector for Pat Application

J.P. Jiang, BioVigilant, Tucson, Arizona

For the PAT application of microbial monitoring instruments in pharmaceutical field, certain performance features of a detector technology are highly desirable, among them are: (1) real time microbial detection; (2) capability of continuous monitoring of microbial level in the environment; (3) broad detection range of microbes; (4) freedom from sample preparation and (5) ease of operation. To provide these features for pharmaceutical PAT applications, BioVigilant has designed and manufactured an optical instrument for real time detection of microbes, its Instantaneous Microbial Detector (Model IMD). We will describe the functionality of the IMD instrument.

In contrast with the conventional testing methods, which use culturing or chemical reagents, Instantaneous Microbial Detection uses strictly optical methods. As a result, it does not grow anything and it does not use reagents or stains. Since the light interacts with aerosol material instantly, the detection of microbes is affected in real time and the environmental monitoring by this method can be continuous. The principle of operation of the IMD is simultaneous measurements of individual particle's size and UV-induced intrinsic fluorescence signal. The particle sizing is done utilizing the widely known and used principle of Mie Scattering, but in a novel way in order to simultaneously measure for the presence or absence of the intrinsic fluorescence of certain metabolites necessary for life. Microbes contain metabolites such as NADH and riboflavin, which, when excited at a certain wavelength, emit intrinsic fluorescence signal which can be captured by BioVigilant's IMD devices. (This intrinsic fluorescence emanates from the microbe itself and is different from the fluorescence that is used in the antibody dye labeling methods, which comes from the reagents that attach to the microbe.) This fluorescence signal detection serves as a marker for biological activity of the aerosol. This marker is used to differentiate bio-aerosols from inert dust particles. The particle size information provides a qualitative classification of aerosols for differentiating viable microbes from other environmental particulate. Figure 1 shows the size range of various relevant particulate in the environment.

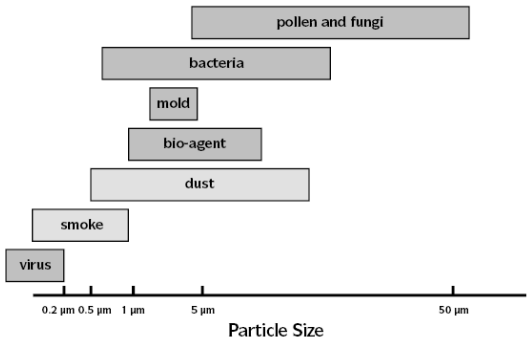


Fig. 1. Size range of various environmental particulates

The physical apparatus of the IMD fluorescence microbial sensor and the optical layout of the instrument are depicted in Fig. 2.

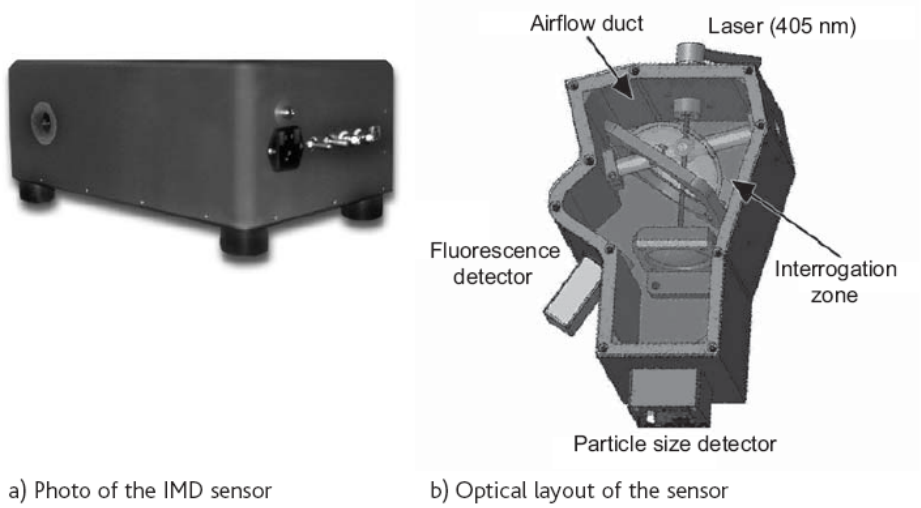
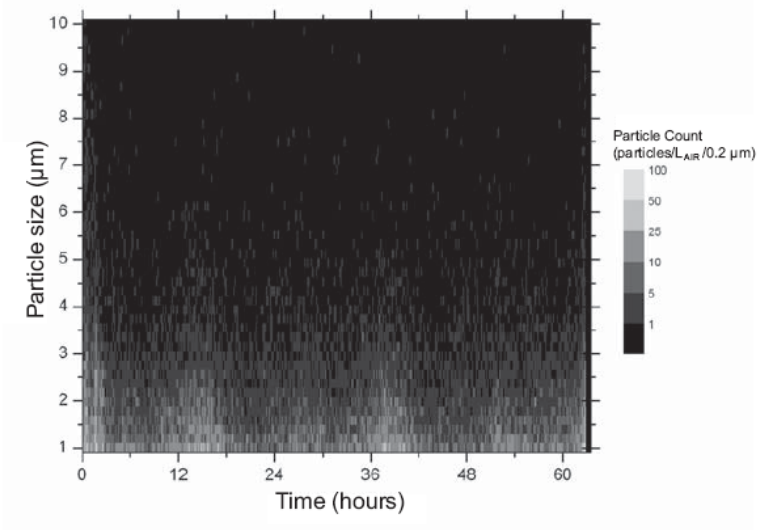
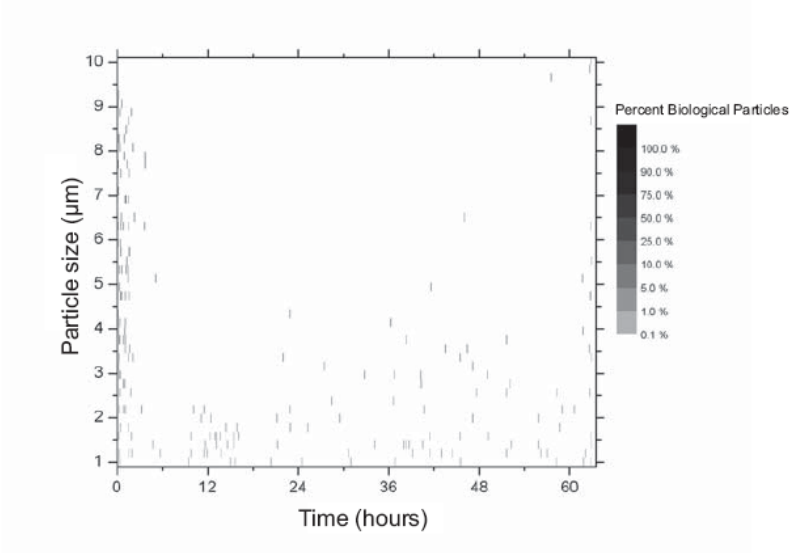


Fig. 2 Model IMD-A airborne microbial sensor

In PAT application, an instrument capable of instantaneous and continuous microbial detection will be useful. IMD-A microbial detector has these attributes for applications in pharmaceutical environmental monitoring. Fig. 3 shows the data display of IMD sensor's measurement of total particle counts and bio-aerosol counts in an office building. The monitoring time was over 60 hours continuously. The measurement data are shown in a format of time-resolved histogram of particle size. Several interesting data trending features can be discerned from these graphs: the diurnal variation of the particle counts, the influence of the human presence in microbial counts (the peak of bio-aerosol counts shown at the beginning of the IMD measurement run which started on a Friday afternoon, Fig. 3 (b)). These features show that IMD sensor can be a useful tool in implementing PAT initiative. It can be used for the purpose of promptly identifying potential route of contamination or the root cause of a microbial excursion in a pharmaceutical facility.



a) Time-resolved size histogram of total airborne particles in office room air



b) Time resolved histogram of bio-aerosol in the same office space

Fig. 3 IMD-A sensor data displays of a) total indoor particle counts and b) bio-aerosols in an indoor office space

In summary, IMD sensor is designed to offer real-time microbial detection on a continuous basis. The IMD sensor is based on optical detection of intrinsic fluorescence from certain metabolites inside microbes, so it has a broad microbial detection range and provides monitoring results instantaneously. It can be useful in implementing PAT initiative in pharmaceutical fields.