Pathogen ALERT

RAPID Reader

White Paper (NW_14)

Remote monitoring and rapid detection of *aspergillus fumigatus* using a self-contained and sterile cartridge loading system

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Abstract

Microbial contamination can result in serious disease outbreaks in healthcare environments and in mass food spoilage in the food & beverage and agricultural industries. This contamination can ultimately lead to increased mortality, illnesses, costs and reputational damage. Currently available bio-contamination detection systems are based primarily around selective placement of growth media plates in areas under investigation. These platforms must then be removed and transported to a laboratory, where they are typically incubated and studied to assess the growth of microbes. This process can take upwards of 42 hours, depending on the availability of an adequately equipped onsite laboratory. Impaction devices have been introduced to help test larger volumes of air in small areas; however, the incubation and visual examination of platforms still present a significant hindrance to rapid detection of contamination outbreaks. Furthermore, such investigations require dedicated trained personnel.

A technological overview and validation experimentation is presented herein, focussing on the rapid, automated detection of airborne *Aspergillus Fumigatus* spores present in the environment using the RAPID READER optical based sensor. *A. fumigatus* is a fungus shown to cause disease in immune-compromised individuals. *A. fumigatus* is a saprotroph (mycelium based growth) which has been found to be widespread in nature [1]. This study shows the ability of the RAPID READER system to detect, within a short timescale, the presence of *A. fumigatus* using the patented technology.

Technology

Microbial contaminants may be spread by various means e.g. direct transmission from contaminated surfaces or individuals by touch or through contaminated water supplies. However, in the aforementioned industries even with strict decontamination methods for surfaces, individuals and water supplies in place, microbial contamination still remains commonplace. An aspect of microbial contamination which is far more difficult to control is that of airborne contamination. Microbes may persist on hard to reach surfaces for up to several months or more. When disturbed, these microbes become airborne, enabling transmission to other areas of the particular facility in question. Furthermore, poor compliance with cleaning procedures and high footfall in other areas of a facility may result in regions of high levels of contamination. Again as this area is disturbed by the movement of individuals or machinery, microbial contamination from a ‘low risk’ (e.g. warehouse) to a ‘high risk’ (e.g. food production line) area, through airborne contaminants, is likely.

Current methods for assessing the levels of microbial contamination involve the use of (a) sampling and plating, (b) biochemical laboratory analysis and (c) optical methods. Assessments using methods (a) and (b) usually involve relatively long timescales (~ several days), highly trained staff and/or investment in costly chemicals and instrumentation. For example, samples must be prepared by an individual skilled in the field of microbiology before growing on nutrient media and enumeration or, in the case of polymerase chain reaction (PCR), samples must be isolated and one or a series of chemical preparations performed before the microbes are identified. Therefore, such methods are usually invoked in very specific laboratory analyses. Optical methods are most suited to remote monitoring of airborne microbial activity as technologies can be devised which may be deployed in the field and require minimal end user interaction.

The RAPID READER system operation is based on biophysical elastic light scattering induced by microbial colonies and the morphological changes they can induce on the surface on which they grow. It is known in the scientific literature that individual bacterial colonies act as biological spatial light modulators which produce unique scatter patterns. The RAPID READER system is based around the optical monitoring of laser light passing through a polymer and agar based sensor. Environmental air is impacted upon the sensor in short evenly spaced bursts, during which time airborne contaminants take hold and begin to grow. By selecting highly specific growth media with the appropriate optical characteristics, contaminants that require monitoring may be individually targeted. As the contaminant grows, the laser light passing through the medium begins to be affected. Scattering of the light occurs, accompanied by a change in the direct transmission intensity through the agar sample.

Originally *A. fumigatus* was thought to only reproduce asexually, however it was later discovered to also have a fully functioning sexual reproductive cycle [2]. This cycle is shown in Fig 1. During each cycle spores (ascospores and conidiospores depending on reproductive track) are created which may then become airborne and spread the mould beyond its immediate area. *A. fumigatus* causes roughly 90% of all invasive aspergillus infections in people, and has a high (50-95%) mortality rate [3]. The main route of infection is inhalation, and to a lesser extent open wounds, and infection is especially dangerous to immunocompromised patients.

Due to the nature of the *A. fumigatus* growth, which generates a web-like mesh called a mycelium (shown in Fig 2), the scattering of light is enhanced through diffraction as the colonies thicken. Moreover, changes caused on the surface by the growths (chemical and topographical), exacerbate the scatter effect. Colonies can form from as little as a single airborne spore. Given that the transmitted and scattered light paths are modified by the growth of microbes on the agar, both the transmission and scatter intensities are constantly monitored for changes during operation, in order to evaluate the presence and growth of *A. Fumigatus* on the sample.

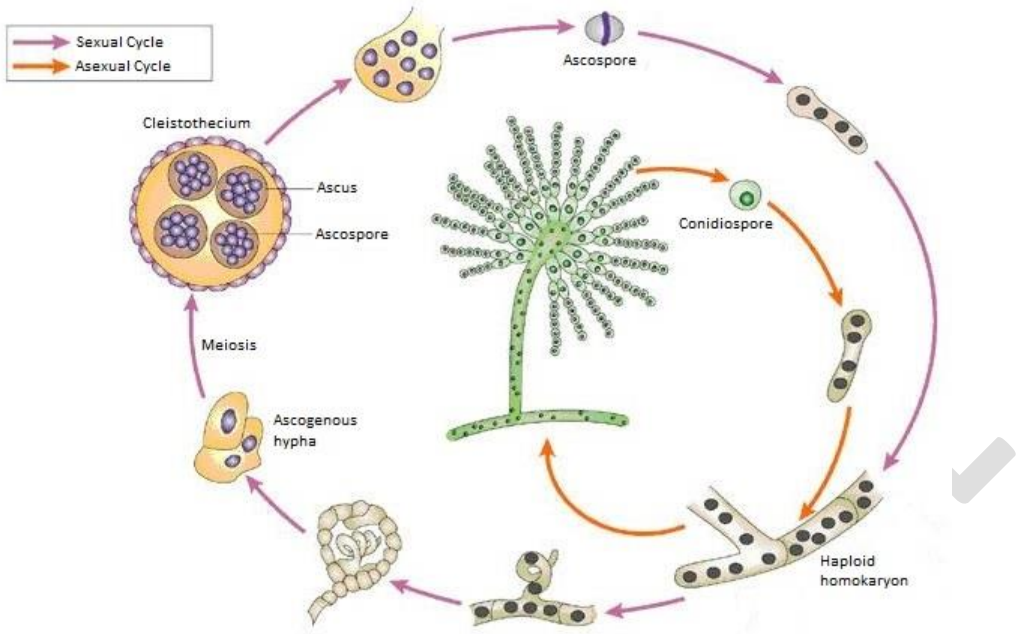

Figure 1 → Asexual and Sexual life cycle of A. fumigatus mould

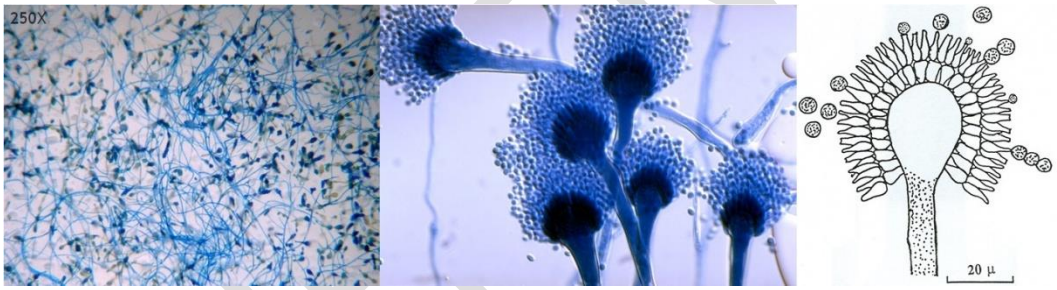

Figure 2 → Mycelium growth typical of A. fumigatus

Using this laser/optical method colonisation events are detected before any obvious visual growth has occurred, allowing for early detection of contamination in at risk areas. The data is continuously uploaded onto a cloud server, where algorithms for extracting patterns in the data can detect the presence of the contaminant and for example generate alerts to warn personnel about potential outbreaks. This allows for a valuable head start on contamination events, giving time to engage counter measures before the contaminant has taken hold or become widespread.

The system is designed to ensure no cross contamination is possible. The RAPID READER design incorporates a sterilised, environmentally controlled cartridge system which contains the sensor and other components, which can be loaded and unloaded as needed. The design is optimised to exclude external light sources while directing the air down into the centre of the test area; in a similar manner to that of an air impactor device.

This cartridge is completely self-contained, and once replaced, all traces of the previous growth are removed. Obviation of cross contamination is aided by the fan system 'pulling' the air through the cartridge, ensuring no possibility of contamination from the fan itself.

Specificity of Growth media

As an example, Malt Extract Agar (MEA) is used as the growth media for *A. fumigatus*. MEA is recommended for the growth of fungi, particularly yeast and moulds. Agar, compared to jelly, allows for rapid growth of the contaminant without removal of media. Mould will typically live off the nutrient rich agar substance without destroying it, whereas jelly is typically eaten away causing drastic changes in the optical properties, potentially leading to false results.

Moulds and yeast can tolerate lower pH levels than most bacteria. To aid in the specificity of the MEA, and inhibit potential bacterial colonisation, lowering of the growth mediums pH can be performed during the agar production through the addition of lactic acid. This will make the medium inhospitable for bacterial growth, allowing the mould more time to settle without producing a false positive result due to omnipresent airborne bacteria.

Validation Data

The RAPID READER provides a digital output of the scattering and transmission intensities of the laser source passing through the growth media. Upon the event of a colonisation, the intensities will begin to shift long before the visible presence is apparent to the naked eye. A typical data set is shown in Fig 3 below.

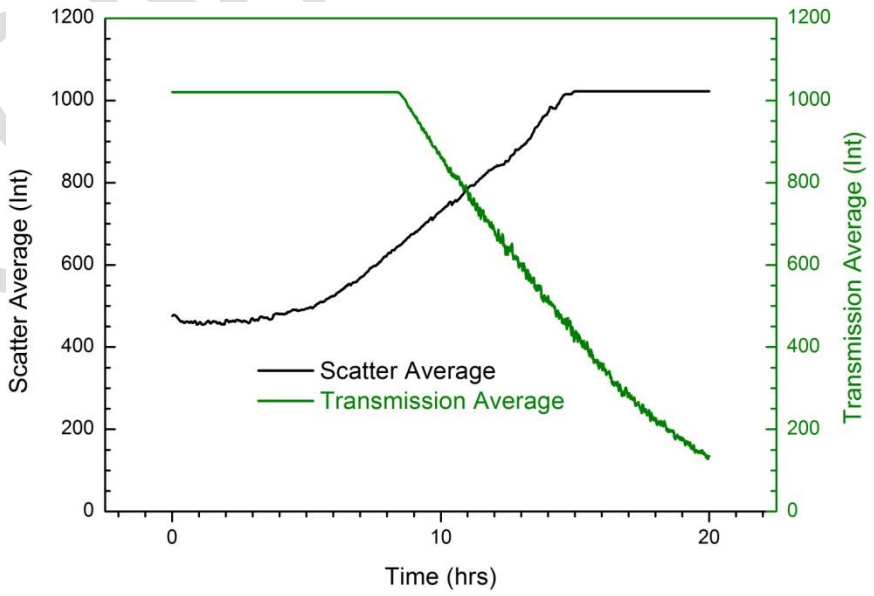

Figure 3 → Scatter and Transmission Intensities versus time after the growth media has been contaminated. Note in this case the scattered signal begins to change strongly at approx.. ~6 hours

The mould associated scattering of the light is the first phenomenon to be observed, as even low amounts of mycelial growth will cause significant diffraction of the laser beam. Furthermore, as the colonies grow to a certain density, they attenuate the beam along the transmission axis. This is observed in the sharp fall in the intensity of the trnsmitted beam which takes place after c. 9 hours (Fig 3).

Summary

The method of detection shown herein allows for remote rapid detection of airborne contaminants, and enables continuous, near-real-time monitoring of specific areas. The detection times are well below those using current laboratory based methods. By means of incorporating suitable selective or differential agars into the sensor system, the device is well suited to the rapid and automated detection of specific airborne contaminants.

References

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