COVER SHEET

Performance assessment of UVAPS: Influence of fungal spore age and air exposure

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Abstract

This work focused on two main outcomes. The first was the assessment of the response of the Ultraviolet Aerodynamic Particle Sizer Spectrometer (UVAPS) for two different fungal spore species. The UVAPS response was investigated as a function of fungal age and the frequency of air current that their colonies exposure to. This outcome was achieved through the measurement of fungal spore fluorescent percentage and fluorescent intensity throughout a period of culturing time (three weeks), and the study of their fluorescent percentage as a function of exposure to air currents. The second objective was to investigate the change of fungal spore size during this period, which may be of use as a co-factor in this differentiation. Fungal spores were released by blowing the surface of the culture colonies with continuous filtered flow air. The UVAPS was used to detect and measure auto-fluorescing biomolecules such as riboflavin and nicotinamide adenine dinucleotide phosphate (NAD(P)H) present in the released fungal spores.

The study demonstrated an increase in aerodynamic diameter for fungal spores under investigation *(Aspergillus niger* and *Penicillium* species) over a period of time. The fluorescent percentage of spores was found to decrease for both fungal genera as they aged. It was also found that the fluorescent percentage for tested fungi decreased with frequency of air exposure. The results showed that, while the UVAPS could discriminate between *Aspergillus* and *Penicillium* species under well-controlled laboratory conditions, it is unlikely to be able to do so in the field.

Keywords: fungal spores, culturing time, fluorescent percentage, spore size

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1. Introduction

The presence of biological aerosols such as bacteria and fungi in the air is associated with risk to human health (Maus *et al.*, 2001; Murray *et al.*, 1990; Vijay *et al.*, 1999b). In particular, fungi are responsible for many allergic diseases such as asthma, allergic bronchopulmonary mycoses and hypersensitivity pneumonitis, to name just a few (Vijay et al., 1999). To assess and minimise risks to human health, it is important to develop a better understanding of the dynamics of these aerosols in the air, which can only be achieved with the application of fast and accurate real time detection methods.

Traditional methods used for detection of airborne biological aerosols are based on sample collection and subsequent laboratory analysis. As such, they only allow for snapshots of biological particle characteristics and are unable to yield high resolution time series of the characteristics. This is a major limitation, preventing progress on the understanding the dynamics of these particles. As such, further work aimed at extending the capabilities of real time detection techniques, is very important for developing a better understanding of the science of biological aerosols and without developing these techniques, it would be difficult to achieve any real progress.

During the last decade, different trials with instruments for measurement of fluorescence spectra as a method for real time detection of single viable airborne bioaerosols have been reported. These can be divided into three groups. The first group includes trials and studies to design and test an instrument capable of differentiating between biological and non biological aerosols such as a Fluorescence Spectrum Analyser and an Ultraviolet Aerodynamic Particle Sizer (UVAPS) (Brosseau *et al.*, 2000; Chen *et al.*, 1996; Hariston *et al.*, 1997; Hill *et al.*, 1995; Ho *et al.*, 1999; Kaye *et al.*, 2000; Nachman *et al.*, 1996; Pan *et al.*, 2003; Pinnick *et al.*, 1998; Pinnick *et al.*, 1995). The second group of studies aimed at designing and testing an instrument with the capability to characterise particle composition in order to discriminate between the bioaerosols themselves (Cheng *et al.*, 1999; Pan *et al.*, 1999; Seaver *et al.*, 1999; Sivaprakasam *et al.*, 2004; Weichert *et al.*, 2002). Some of these studies used multiple UV excitation wavelength to create more than one fluorescence spectra for each species under investigation (Cheng *et al.*, 1999; Sivaprakasam *et al.*, 2004). The third group coupled the UVAPS with other technologies, such as wet chemistry technology, so that if unusual aerosols were

detected by UVAPS, samples were collected for further analysis to identify the microorganisms using the wet chemistry technique (Ho, 2002). The key findings for the first group are most encouraging, however groups two and three still require further investigation.

The UVAPS operation is based on the excitation and emission of auto-fluorescent biomolecules, which exist in most bioaerosols. The main biomolecules present in fungal spores are reduced fluorescent coenzymes: nicotinamide-adenine dinucleotide (NADH), nicotinamide-adenine dinucleotide phosphate (NADPH), and riboflavin (Billinton and Knight, 2001; Brosseau *et al.*, 2000; Li *et al.*, 1991). However, the basis of the instrument's operation, the fluorescence of the excited biomolecules, has been found to be strongly affected by environmental and biomolecule-related factors. For example, Agranovski *et al.* (2003b) have shown that bacterial stress has an impact on these fluorescence properties. Huber *et al.* (2000) found that the fluorescence spectra for free NADH and those bounded to protein were different. Schmit and Brody (1976) found that *Neurospora crassa* spores had low level of reduced cofactors, NADH and NADPH, compared with a high level for its mycelia. It was also shown that many non biological aerosols such as peptone water, broth or other materials, had strong fluorescent signals (Agranovski *et al.* 2003a). Thus there is a need for a better understanding of these environmental factors and their effect on the instruments response to different bioaerosols.

It can be seen from the above summary that the UVAPS has been the main research tool for real time detection of viable bioaerosols to date. Whilst a significant amount of work has been published regarding the application of the UVAPS to the aerosols carrying bacteria (Agranovski *et al.* 2003a, Agranovski *et al.* 2003b), only a very limited amount of work has been done on the fluorescence spectra of fungal spores and application of the UVAPS to detection of fungal spores present in the air. Work by Kanaani *et al.*(2006), addressed the efficiency and the limits of the UVAPS in detecting fungal spores, showing the ability of the instrument in detecting and measuring fungal spores and that its upper detection-limit (the point beyond which the relationship between total and fluorescent particle concentration cease to be linear) of fluorescent particles was around 7×10^7 particles/m^3. However, there is still a need for further characterisation and validation of the instrument, and more research is necessary to develop a full understanding of the capabilities and limitations its application, for studies of airborne fungal spores.

The aim of this work was to characterise and quantify the effects of aging of fungal spores and of repeated air exposure, on the ability of the UVAPS to discriminate between different species. The fungi included in the study were *Aspergillus niger* and *Penicillium* species. The novel approach used in the study measured relative fluorescence intensities and, to some extent, spore size distribution, utilising the UVAPS for these two parameters for the first time, in order to investigate its potential to discriminate between fungal spores.

2. Materials and Methods

The work was conducted at the International Laboratory for Air Quality and Health (ILAQH) at Queensland University of Technology, inside a Class II, Type A, Biological Safety Cabinet (SG-400 SterilGARD, E-mail Westinghouse Pty Ltd., Australia).

Three sets of experiments were conducted using the UVAPS. The first experiment was to investigate the effects of fungal spore age on their size and fluorescent percentage, while the second experiment studied fungal spore fluorescent percentage as a function of the frequency of air exposure. The third experiment identified whether the particles released were spores or not. After the fungal species were cultured, they were released into a purpose-built box and their fluorescence signals and size distributions were measured using the UVAPS.

2.1. Aerosol preparation

Penicillium (ACM 4616) was inoculated onto three Sabouraud Dextrose Agar plates (SDA), and incubated at 25^{o}C for a total of twenty-one days. The incubated cultures were tested after two, four, seven, fourteen and twenty-one days. The same steps were followed in preparing *Aspergillus Niger* (ATCC 9142).

Another three plates of each species were incubated for seven days, released in the box and sampled by the UVAPS; then refrigerated for a further week, sampled again and later refrigerated for another week to be used a third time as discussed in section 2.2.3.

To minimize the impact of the variation in culture characteristics during fungal growth, which occurs commonly (Raper *et al.*, 1965), the following protocol in

culturing was applied. Firstly, a single culture divided into five segments was used instead of five separate cultures, and secondly, the replicate cultures were prepared using the same conditions as the original culture.

2.2. Apparatus description

The Ultraviolet Aerodynamic Particle Sizer, [UVAPS, model 3312, TSI, St. Paul., MN,] is the instrument which was designed to monitor and detect bioaerosols. It provides accurate particle count size distributions, as well as a real-time fluorescence for particles with aerodynamic diameters of 0.5-15 μm. Fluorescence measurements are produced by exciting particles with an UV laser beam at a wavelength of 355 nm and then detecting the fluorescence emission from 420 to 575 nm.

The fluorescence spectra detected by the UVAPS are very sensitive to change in the UV laser pulse energy and photomultiplier tube (PMT) gain, i.e. doubling the value of each of the two parameters would lead to doubling of the measured fluorescence intensity (Agranovski et al., 2003a; TSI-Incorporated, 2000). The detected threshold baseline was controlled and checked during the course of this study. The UV laser pulse energy was set to 50 ± 1% of the lasers full power and 482V were applied to the PMT to produce a measurable gain.

Using the UVAPS, aerosols with no fluorescent compounds appear in channel 1 and in a very small fraction at channel 2; while the bioaerosols (with endogenous metabolites) appear in the channels from 3 to 64. The particles with higher fluorescent intensity will be found at higher channels (TSI-Incorporated, 2000).

The mixing chamber in this study, used to provide homogenous particle distribution before sampling to the UVAPS, was made of aluminium with one side made of Perspex used as a door. Its dimensions were (100cm×39cm×39cm).

2.3. Experimental Methodology

The instrument calibration and background measurements were monitored before and after each experiment conducted in this study. The average background was subtracted from each single reading before data interpretation. However, in most cases it was found to be negligible compared to the tested sample. In all these experiments

the UVAPS sample time was 20 seconds. Each data point presented in this paper is a mean value of at least three replicate measurements.

The experiments were conducted at temperatures inside the box ranging from 22 to 26^{o}C and relative humidity of 50 to 54%. This is a moderate humidity range, in which the spores remain as singlets and do not aggregate (Reponen *et al.*, 1996). In contrast to the humidity, temperature plays an insignificant role in the hygroscopic growth of the particles (Li and Hopke 1993), therefore room temperature was used for convenience and to represent typical indoor environment

2.3.1. UVAPS calibration.

The UVAPS was calibrated using 0.993 µm particle diameter monodisperse Polystyrene Latex (PSL) Particles (Duke Scientific Corporation, Palo Alto, CA) and 0.91 µm particle diameter Blue Fluorescent (BF) microspheres (Duke Scientific Corporation, Palo Alto, CA). Both were used at a density of 1.05 g/cm^3 and their aerodynamic diameters (diameter of unit-density sphere) were 1.02 and 0.93 µm, respectively (Willeke and Baron, 1993). Suspension of each was made by diluting one drop from stock in sterilized distilled water to a concentration of the order of 10^7 particle/m^3.

PSL aerosols were generated using a 6-jet Collison nebulizer (BGI Inc., Waltham, MA). The nebulizer was operated at a flow rate of 7 L/min and the supply compressed air was filtered with a HEPA filter. Droplets carrying aerosols were dried before entering the mixing chamber by a silica gel dryer, and in addition, when entering the chamber, by compressed-HEPA-filtered-air. A schematic representation of the experimental set-up is presented in Figure 1.

Monodispersed polymer standard particle suspensions, from DYNO PARTICLES AS, of 0.1% solids (SS-2-PXG 0.1%), (SS-5-PXG 0.1%) and (SS-7-PXG 0.1%) and of the density of 1.05 g/cm^3, corresponding to particle aerodynamic diameters of 2.05, 5.12 and 7.17 µm respectively, were also used for calibration of the UVAPS. Procedure as recommended by the UVAPS manufacturer (TSI) was followed in the instrument calibration, and it was conducted before and after each experiment.

The mean aerodynamic diameters of standards obtained were within the diameter accuracy provided by the supplier (± 3%). The size range of standard particles that were used for calibration covers the size of fungal spores under investigation.

2.3.2. The fungal spore size and fluorescent percentage as a function of fungal age

The experiments were conducted using the set-up presented in Figure 2. The dry generation method, which was described in detail by Kanaani *et al* (2006), was used for fungal spore release. In this method, the compressed air was filtered by a HEPA filter and introduced into the mixing chamber at a flow rate of 10 L/min in all the cases, except for the two-day-old cultures for which a flow rate of 20 L/min was used. Spore release was induced by directing a narrow jet of air at the surface containing *Aspergillus* or *Penicillium* mycelia at an angle of 60^{o} from a distance of 1.5 cm. This short distance above the spores was used so it would not affect the adjacent sections. The culture dish was divided into approximately five equal sections by marking the dish bottom using a narrow paper tape and pen. Each marked section was used for experiments applying a specific culture time, i.e. two, four, seven, fourteen or twenty-one days. The exhaust airflow of the UVAPS was HEPA- filtered and the airflow was returned to the biological cabinet to prevent any contamination (Figure 2).

Sampling by the UVAPS was conducted from the same sampling point within the chamber during all the experiments to measure spore concentration, fluorescent particle counts and total particle counts.

2.3.3. Fungal spore fluorescent percentage as a function of the frequency of exposure

The same method of spore release was used as described above. The culture dish was divided into three, approximately equal, spore covered sections. After the first seven days of culturing time, measurements were conducted for one section only. The airflow was directed towards each individual section via specific exposure points and the air speed decreased as the distance from the exposure point increased. As such, it was at a minimum when it reached the adjacent section, so the effect on the adjacent section was negligible. This section was exposed to the air current at a flow rate of 10 L/min for 20 minutes, during which the sampling from the chamber was conducted. The culture was then refrigerated for seven days and the measurements were repeated for the previously exposed section, as well as conducted for the second section. After

the culture had been left in the refrigerator for another seven days, measurements of fungal spores from all the three sections were conducted. Hence the last (third) section was exposed to compressed air once, the second section was exposed twice and the first section underwent three exposures. The experiment was repeated three times with three cultures exposed to same conditions.

2.3.4. Fungal particle identification

In parallel to culture testing, the released fungal particles were identified, using an optical microscope, to determine whether they were in fact spores or not. Pieces of transparent adhesive tape and uncoated microscope slides were placed inside the box, so released particles attach to or fall on them. This was followed by microscopy testing to identify the releasing particles, as described in detail by Kanaani *et al.* (2006). A light microscopy (Model CX31RTSF, Olympus Corporation, Tokyo, Japan) was used to identify the particles.

3. Results

3.1. Fungal particle identification

Using the optical microscope and based upon particle characteristics such as shape, size and appearance, the particles were identified as spores. However, fungal hypha was not recognized on these slides. Using the UVAPS, the mean aerodynamic diameter for *Aspergillus* and *Penicillium* fungal particles at age one and two weeks were 2.40± 0.12 and 3.55± 0.14, respectively. The mean aerodynamic diameters results that obtained by UVAPS for *Aspergillus* and *Penicillium* were in good agreement with literature data for the spores under investigation (Ramirez, 1982; Raper *et al.*, 1965). The above results support each other and indicate that the sampled particles were fungal spores, for detail see Kanaani *et al.*(2006) and the following section in this study.

3.2. Fungal spore size

It can be seen from Figure 3a and Table 1a that the mode aerodynamic diameter of *Aspergillus* spores was 3.05μm for two-, four- and seven-day-old spores. By the fourteenth and twenty first-day of culturing time, the mode increased to reach 3.28 and 3.52 μm, respectively. The percentage of spores in the large size range (beyond the mode, i.e. 3.52-6.24 μm) also increased (the spectrum shifted to the right), whilst spores in the small size range (lower than 3.28 μm) decreased (Table 1a).

Figure 3b and Table 1b show that the spores of *Penicillium* follow the same trend as that of the *Aspergillus.* While the spore mode was 2.29 μm for two-, four- and seven-day-old spores, it increased to 2.46 μm and 2.64 μm for the fourteen- and the twenty-one-day-old spores, respectively. The percentage of spores in the large size range (beyond 2.46 μm) increased with age, while spore size lower than 2.46 μm decreased (Table 1b).

Spore sizes of *Aspergillus* and *Penicillium* are cited in literatures as falling in the ranges from 2.5 to 5.0 μm (Raper *et al.*, 1965) and 2 to 4 μm (Ramirez, 1982), respectively. The results of this study are in agreement with these figures.

3.3. Fungal spore fluorescence as a function of culturing time

Total particle number is the number of particles counted by the UVAPS in the channels 1-64, while fluorescent particle number is the number of particles counted in the channels 2-64. Fluorescent percentage is the number of fluorescent particles as a percentage of total particle number.

Figure 4 shows that total fluorescent percentage decreases with increasing age of the spores for both *Aspergillus* and *Penicillium*. The total fluorescent percentage of *Penicillium* is higher than that of *Aspergillus* for each age of the spores.

The results also show similar trends for spore size and its fluorescent percentage for both *Aspergillus* and *Penicillium* species (Table 2), i.e. fluorescent percentage of spores increase with increasing aerodynamic diameter. As presented in Table 2, the fluorescent percentage for *Aspergillus* spores with diameters of 2.64μm and 4.70μm, were 48.1 ± 13.7 and 93.3 ± 5.0, respectively. Alternatively, *Penicillium* spores showed a fluorescent percentage of 58.9 ± 14.0 and 97.8 ± 2.0, for diameters of

1.84µm and 3.05µm, respectively. These measurements were conducted using spore samples obtained after seven days of culturing. A similar trend was found for the other ages.

3.4. Fluorescent Intensity

The intensity of spore fluorescence was investigated in this work by two different methods. Firstly, investigation was conducted in terms of the spore concentration percentage (for all diameters in a single channel) versus fluorescent intensity, which is proportional to the channel number. Spore concentration percentage is the ratio of the sum of spore concentration of all diameters with same fluorescent intensity, and therefore the same fluorescent channel (single channel) to the sum of spore concentration of all diameters in all 64 channels expressed in percentage. The fluorescent intensity distributions are shown in Figure 5. The fluorescence intensity distribution of 0.993 µm monodispersed Polystyrene Latex (PSL) Particles was markedly different to that of 0.91 µm Blue fluorescent (BF) microspheres. The non fluorescent aerosol PSL showed 99.9% ± 0.1 in the first channel and 0.1% in the second channel, while 2.9% ± 0.1 of BF was found in the first channel and 96.5% ± 0.2 in the last channel (the highest intensity channel [64]). However for *Penicillium* and *Aspergillus* species, fluorescent intensity distribution was different, as expected, to the distribution for PSL and BF particles; but also different between the two species. This can be seen from the results presented in Figure 5a for *Aspergillus* and *Penicillium* species cultured for seven days, where *Aspergillus* fungal spore percentage in the first four channels is higher than that for *Penicillium* spores. For channels above the fourth, the inverse situation was obtained and in many of the channels the *Penicillium* spore percentage was double that of *Aspergillus*.

However, when comparing spectra of the two species cultured for different periods of times the situation changes. Figure 5b shows that the spectrum of seven-days-old *Aspergillus* is almost identical to the spectrum of fourteen-day-old *Penicillium*. This indicates the change of spectra with age of species and thus the importance of taking culture age into account when comparing fungal species.

The second method of investigating spore fluorescent intensity utilized the spore concentration percentage (for specific diameters in a single channel) versus channel number. Spore concentration percentage, in this case, is the ratio of the spore

concentration of a specific diameter in one channel to the sum of spores of that specific diameter in all 64 channels expressed in percentage. The results of application of this method are presented in Figure 6 (including only every second value, to make the diagram clear).

The channel numbers on the UVAPS reflect the degree of fluorescence, with increasing numbers reflecting an increase in the fluorescent intensity. It can be seen from Figures 6a and b that high concentrations were recorded for small spores in the lower intensity channels (1-5), and low concentrations in the higher intensity channels (6-64); while the inverse was found for the larger spores. The same trend was found between spore size and their fluorescent intensity. Figure 6a illustrates, for example, that *Aspergillus* spores of size 2.84 µm were present only up to channel 29, while the 3.79 and 4.37 µm spores were present up to 51, and 64 channels, respectively. There was, with very limited exception, a proportional relationship between the spore size and their concentration in the channels from 6 to 64. On the other hand the results in channel 1, the non fluorescent channel, showed an inverse relationship between spore concentration and its size, with 2.46 and 2.84 µm spores showing the highest concentration (of 50.3% and 44.3% respectively) and 4.37 µm spores, the lowest (at 5.8%).

For *Penicillium*, large spores (2.64 and 3.05 µm) can be found in all sixty-four channels, with the spore concentration increasing in the higher channels with increasing fungal spore size (Figure 6b). Spores of smaller diameters (1.98 and 2.29 µm), were found mainly in the lower channels, with very low concentrations (less than 0.1%) present in the higher intensity channels. For example spore with 1.98 µm diameters were present up to channel 21, while the 2.29 µm diameter spores were present up to channels 40. In general, from channel 15 to channel 64, the spore percentages were proportional to their sizes, i.e. when the spore size increases, its concentration percentage in the higher channels increase. The same trend between spore size and concentration as that observed for Aspergillus was found in channel 1, with the exception of 3.05 µm diameter spores, which showed a minor deviation from this relation.

It was observed that twenty-one-day-old fungal spores for both species were found at lower fluorescent intensity channels than those that were seven-day-old (graphs not included). The spore concentration decreased in the higher channels and increased in the lower channels (1-5). For example, the concentration of the large *Penicillium*

spores (3.05 µm) in the last channel was 2.3% for seven days old spores, while it decreased to about 0.2% for twenty-one-day-old spores.

3.5. Fungal spore fluorescent percentage as a function of frequency of the exposure

The investigation of the effect of the frequency of the exposure to air on the fungal spore fluorescent percentage showed that the percentage decreases with the increasing number of exposure times (Figure 7). The section that was measured for the third time (exposed three times to air current) showed the lowest fluorescent percentage (67.5% ± 5.4). The section exposed twice showed a fluorescent percentage of 75.3 % ± 3.8, while the section exposed only once (after fourteen days refrigeration) demonstrated the highest fluorescent percentage (79.2% ± 3.9). Using the same procedure, *Penicillium* showed a similar relationship (Figure 7).

4. Discussion

In this work fungal spore size and fluorescence of *Aspergillus* and *Penicillium* species were investigated using the UVAPS. The spore sizes were studied as a function of fungal spore age, while the fluorescence was measured as a function of age and number of air exposures.

The concentration levels of the generated spores were not high enough to result in rapid coagulation, which would interfere with the interpretation of the results of the study. The ranges of the total spore concentrations, in all these studies except for two days culturing time, were from 3.05 to 10.46 and from 3.56 to 14.4 particle/cm^3 for *Aspergillus* and *Penicillium*, respectively. The concentrations for two days culturing time were very small (of the order of 0.2 #/cm3 after blank subtraction). A linear relationship between total particle concentration and fluorescent particle concentration was identified for all of the concentrations obtained in this study, as was shown in previous work (Kanaani et al., 2006).

4.1. Fungal spore size as a function of age

The change of spore size distribution for fungal species as a function of time is a parameter that may help in the discrimination between the species. In this study, the mode of the spore size distribution (Table 1) was investigated as a function of time under well controlled conditions as a factor aiding in discrimination between *Aspergillus* and *Penicillium*. However, analysis of the entire spore size distribution proved to be more helpful in discriminating between the two species (Figure 3c).

While the location of the modes of *Aspergillus* and *Penicillium* spores of the same age, i.e. twenty-one-day-old (3.52 and 2.64 μm respectively), were quite different, the modes of different ages, i.e. 4-day-old *Aspergillus* and twenty-one-day old *Penicillium* spores, were less different (3.05 and 2.64 μm). Thus, as the difference between their ages increased the ability to discriminate between them decreased (Figure 3c).

4.2. The effect of age on fungal spore fluorescent percentage

Figure 4 shows that the fluorescent percentage decreases for both fungal spores during the culturing time, and that at all stages the fluorescent percentage of *Penicillium* is more than that of *Aspergillus*. This may be interpreted as follows.

It has been reported that the viability of fungal spores decline from the moment that they are released (Flannigan and Miller, 1994). *Aspergillus* and *Penicillium* spores have been found to survive in dry air for decades (Flannigan and Miller, 1994), however in humid environment, fungal spores' viability declines with time. For example, *Penicillium chrysogenum* spores subjected to moving air at 75% RH showed a reduction in culturability (Muilenberg and Burge, 1994), and dehydrated *N. crassa* conidia have remained viable for several years but they lost viability after nine days when stored under conditions of 100% relative humidity (Griffin, 1994). In this work the relative humidity during incubation, which was 60% ± 9, could have been the parameter resulting in the decrease of fungal spore viability with age. The decrease in the levels of cofactors such as nicotinamide-adenine dinucleotide (NADH), nicotinamide-adenine dinucleotide phosphate (NADPH), and riboflavin with age is responsible for the decrease of fluorescence signals.

The different fluorescent percentage between *Aspergillus* and *Penicillium* fungal spores pointed out the possibility of using this parameter to differentiate between the two species. However, when conducting the experiments under conditions similar to those expected for a real home environment, and thus by varying culture age and air exposure, the results were not so encouraging. Figure 4 shows that *Aspergillus* after four days had the same fluorescent percentage as *Penicillium* after fourteen days culturing time, and also that fourteen-day-old *Aspergillus* had the same fluorescent percentage as twenty one days old *Penicillium*. This indicates that it is not possible to differentiate between mixed populations of species of mixed ages, as would be the case in a typical home environment.

For both *Aspergillus* and *Penicillium*, the increase of fluorescent percentage with the increase in spore sizes, (Table 2), is likely to be a consequence of the increase of total fluorescence amount with spore size. Hence the proportion of spores with fluorescence exceeding the threshold detection baseline increased (as a results of spore growing), and was detected by UVAPS as fluorescent particles, and subsequently located within 2-64 channels. This was the case for size channels with average or high number of spore counts; but not for size channels with low spore counts (Table 2). This could be explained as resulting from the influence of particle coincidence (phantom) (Heitbrink and Baron, 1991; Holm et al., 1997) and the slower recovery of the UV laser compared to the recovery of the APS laser of the UVAPS (Agranovski *et al.*, 2003a), which leads to lower estimates of the real fluorescent percentage. The effect of the last phenomenon was obvious when the fluorescent particle number was low i.e. nine particles in the 4.70µm and eight particles in the 3.52µm channels for *Aspergillus* and *Penicillum*, respectively (Table 2).

4.3. The effect of age on the fungal spore fluorescent intensity

All fungal spores contain the same sources of autofluorescence such as the reducing fluorescent coenzymes nicotinamide-adenine dinucleotide (NADH) and nicotinamide-adenine dinucleotide phosphate (NADPH), as well as the metabolic function riboflavin (vitamin B_2) (Billinton and Knight, 2001; Brosseau *et al.*, 2000; Li *et al.*, 1991). However, fluorescent intensity of these biomolecules may vary according to the environmental conditions under which the fungal colonies are placed, and due to their concentration.

Under well-controlled conditions fluorescent intensity constitutes another parameter, which helps to differentiate between fungal species under investigation. In order to extract more information from spore fluorescence signals, spore fluorescence intensity was considered according to two methods (Figures 5 & 6). The spectra, as shown in Figure 5, aid in discriminating between the two species of the same age; but they are not of much help for spores of mixed ages as would normally be the case in home environment. In particular, for spores of the same age, spore size distributions (the second method, see section 3.4.) were different for the two species with two major differences, as can be concluded from inspection of Figure 6. Firstly, the spore concentrations of *Penicillium* in higher intensity channels were more than that for *Aspergillus.* Secondly, in contrast to *Aspergillus*, *Penicillium* was detected in all of the higher intensity channels. The entire size distributions could serve as finger prints (or signatures) for each of the species (Figure 6a &b). However, it was found that each genus changed its fingerprint dramatically with age, which makes it difficult to discriminate between mixed ages on the basis of their fluorescent intensity. In spite of both spore species containing the same biomolecules (NAD(P)H)and riboflavin, they were found to be distributed in different channels, which implies that the amount of these biomolecules and their location within the spore are different for the same and different species (i.e. same species but different age and different species of the same or different ages). Under-well controlled laboratory condition the spore size distribution, together with spore fluorescent percentage and intensity proved to be useful parameter in differentiating between the species under investigation. However in ambient air, where large populations of fungal species of different ages are present (Dix and Webster, 1995; Vijay et al., 1999a) as well as other biological and non biological airborne particles, the task of differentiating between fungal spores, using the UVAPS, appears to be very difficult, or even impossible.

4.4. The effect of air current on spore fluorescent percentage

The results presented in Figure 7 demonstrate the effect of air current on fungal spore fluorescence signals. The decrease in fluorescent percentage with the increase in the number of exposures to air, for both of fungal spore types, was in agreement with a previously reported study that investigated the effect of sampling time on fungal culturability (Wang *et al.*, 2001). Wang *et al.*(2001) showed that the relative

culturability of *P. melinii* and *A. versicolor,* using two personal filter samplers, was much higher when sampled for 10 minutes than when sampled for 10 hours for both samplers. Stanevich and Petersen (1990), also found that the viability of five-minute samples taken with an Andersen N6 sampler was six times lower than that of one-minute samples. The decrease in both culturability and fluorescent percentage is likely to be due to the impact of exposure to air current and desiccation stress.

Fungal spores grow in different places in the indoor environment, and consequently are subject to air exposure for different periods of time and under different air speeds. According to the findings from this study, the variation in the number of exposure times to the air, air speed and the duration of exposure periods will complicate the process of differentiating between different fungal genera.

There are many other variables affecting fungal differentiation using the UVAPS such as UV exposure, surfaces on which they are growing (for example timber, tile or gypsum) and relative humidity, which will be the topics of future studies.

5. Conclusions

This study showed that the fungal spore size of the genera under investigation (*Aspergillus and Penicillium*) increased with culturing time. Spore size distribution helped as additional parameter in differentiating between the genera of the same age; but not for genera of different ages. The fluorescent spore percentage decreased with increasing fungal spore age and also with the number of times the fungal spores were exposed to air currents. Based on fungal spore size distributions, together with fluorescent percentages and intensities, the study demonstrated the ability of UVAPS to discriminate between two fungal spore species under controlled laboratory conditions. In the field, however, it would not be possible to use the UVAPS to differentiate between different fungal spores due to the presence of different micro-organisms of varying ages and subjected to different environmental conditions. In addition, the environment may contain non biological aerosols which, when illuminated with the same wavelength as the spores, will fluoresce, making the task of differentiation more difficult.

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The captions of all tables and figures are:

1. Figure 1. Experimental set-up for the UVAPS calibration.
2. Figure 2. Experimental set-up (direct method generation).
3. Table 1. Average percentage of different sizes of fungal spores at different ages; (a) *Penicillium*; (b) *Aspergillus*
4. Figure 3. Average spore size distribution spectra as a function of culturing time: (a) *Aspergillus;* (b) *Penicillium*; (c) Comparison of *Aspergillus* and *Penicillium*.
5. Figure 4. Fluorescent percentage as a function of culturing time of *Aspergillus* and *Penicillium*.
6. Table 2. Fluorescent percentage of fungal spores (*Aspergillus* and *Penicillium*) after seven days culturing time.
7. Figure 5. Spore concentration percentage (for all diameters in a single channel) as distributed between UVAPS channels (channel numbers reflect degree of fluorescence): (a) *Aspergillus* and *Penicillium* species after seven days culturing time; (b) *Aspergillus* after seven days and *Penicillium* after fourteen days culturing time.
8. Figure 6. Average spore concentration percentage (for specific diameter in a single channel) of seven day old spores as distributed between UVAPS channels (channel numbers reflect degree of fluorescence): (a) *Aspergillus* (b) *Penicillium*.
9. Figure 7. Fungal spore fluorescent percentage as a function to number of times exposed to air flow rate of 10 L/min.