Abstract: Hong Kong is an area of complex topography, with mixtures of urban and greenbelt spaces. Local bioaerosol concentrations are multifaceted, depending on seasonal variations of meteorological conditions and emission sources. This study is the first known attempt at both quantitatively measuring and identifying airborne bioaerosol contributions, by utilising multiple single particle ultraviolet light-induced fluorescence spectrometers. Based in the Hong Kong University of Science and Technology’s super-site, a WIBS-NEO and PLAIR Rapid-E were operated from June to November, 2018. The purpose of this long-term campaign was to observe the shift in wind patterns and meteorological conditions as the seasons changed, and to investigate how, or if, this impacted on the dispersion and concentrations of bioaerosols in the area. Bioaerosol concentrations based on the particle auto-fluorescence spectra remained low through the summer and autumn months, averaging $4.2 \text{ L}^{-1}$ between June and October. Concentrations were greatest in October, peaking up to $23 \text{ L}^{-1}$. We argued that these concentrations were dominated by dry-weather fungal spores, as evidenced by their spectral profile and relationship with meteorological variables. We discuss potential bioaerosol source regions based on wind-sector cluster analysis and believe that this study paints a picture of bioaerosol emissions in an important region of the world.

Keywords: bioaerosol; UV-LIF spectrometry; fungal spores
1. Introduction

Hong Kong is a complex mixture of urban and greenbelt spaces. Although approximately 75% of the region is uninhabited rural land, Hong Kong is the 8th most densely packed city in the world, with a population of over 7 million people and an average of 6700 people per square kilometre (https://www.gov.hk/en/about/abouthk/factsheets/docs/population.pdf). Many studies focused on poor air quality, with asthma rates and respiratory allergies increasing each year [1,2]. Pollutants linked to anthropogenic activities dominated these studies, as the region becomes both increasingly urbanised and subject to pollutants released from the Chinese mainland [3]. However, other particle types such as bioaerosols were disregarded in their relative importance. These are defined as biological particles emitted from a variety of biogenic sources, including soil, oceans, plants and animals [4]. Although they encompass a large number of potential particles, dominant bioaerosols in outdoor environments are often bacteria, pollen and fungal spores [5].

Hong Kong has a monsoon-influenced climate with a clear annual cycle. During winter, there is a prevailing Northeasterly wind that blows from across mainland China [6]. This period is associated with high levels of pollution and aerosol loading [7], significantly raising the rates of daily mortality [8,9]. During summer, the summer monsoon prevails, bringing warmer and more humid winds from across the South China Sea. These winds are cleaner relative to the winds from the north, and so pollution levels lower accordingly. This cycle also has implications for bioaerosol concentrations. Woo et al. [10] used filter collection methods at four meteorological stations across Hong Kong to examine the microbial mixture. They observed seasonal differences in air-mass trajectories as an important factor, with greater quantities of marine-derived phylotypes present during summer and betaproteobacterial phylotypes—typically found in soil—present during winter. Interestingly, they found no significant effect of urbanisation and population growth on airborne microbial communities. This is in contrast to Yan et al. [11], who examined the composition of fungal spores in Beijing during haze and non-haze days. They found positive correlations between certain fungal groups, PM2.5 and PM10, as well as meteorological factors like relative humidity. These findings were previously supported elsewhere, for example by Liu et al. [12].

Bioaerosol exposure is important to understand because of the potential ramifications it has for both human and ecological health. Common fungal genera such as *Aspergillus* act as known carcinogens and allergens, and are implicated in various respiratory illnesses [13]. High concentrations exacerbate asthma attacks, with one study inferring that *Alternaria* spore counts of 1000/m³ more than doubled asthma deaths in Chicago [14]. Sensitivity to pollen is also a growing problem, with rates of physician-diagnosed hay fever amongst Chinese school children increasing from 2.9% to 4.1% in just seven years [15]. Whole pollen grains ranging in size from approximately 15–90 µm are the main driver of this condition, as they become deposited in the upper airways of the lungs. However, smaller pollen fragments are able to penetrate deeper, where they can act as a trigger for asthma. The fragmentation of pollen grains is driven in part by meteorological factors, with relative humidity being amongst the most influential. One study on Chinese elm by Miguel et al. [16] observed 70% of pollen grains rupturing after just 30 min of immersion in water. Pollen fragments are commonplace in the atmosphere, yet are not readily identifiable as pollen during microscopic analysis. Consequently, estimates of pollen concentrations and any subsequent respiratory issues are likely to be underestimates. Bioaerosols also affect the world’s flora. *Hymenoscyphus fraxineus* is a fungus originating from East Asia that is now responsible for Ash dieback disease in Europe and presents a significant threat to forest health [17]. Caribbean coral reefs are thought to be adversely affected by the spores of *Aspergillus sydowii* [18], and many major crop yields are reduced from low level yet persistent crop diseases [19].

Ultraviolet light-induced fluorescence (UV-LIF) spectrometry is an emerging technology that enables real-time online sampling of bioaerosols. It is founded on the principle that biological particles fluoresce when excited with ultraviolet light, resulting from the presence of biofluorophores. UV-LIF spectrometers are designed to detect emitted light at wavelengths associated with specific biofluorophores, thus, enabling particle identification. This technique was used to provide
measurements in a range of environments, including a high altitude Alpine site, rainforests, Antarctica, Beijing, and more [20–23]. These studies were predominantly conducted using various models of the Wideband Integrated Bioaerosol Sensor (WIBS), which has three fluorescent channels to aid in particle identification. However, as the technology develops, advancements were made in the number of channels available, with the PLAIR Rapid-E offering 32 channels. While such an instrument is capable of offering significant amounts of particle information, it is relatively untested in real-world conditions. A detailed review on the status of UV-LIF spectrometry and bioaerosol research was previously written by Huffman et al. [24]. Our study aimed to utilise both the well-characterised WIBS and the highly advanced PLAIR Rapid-E to better understand Hong Kong’s aerosol mixture and provide a comparison between their observations.

2. Materials and Methods

2.1. Site Description and Sampling Details

From June to November 2018, two UV-LIF instruments were continuously sampled inside the Hong Kong University of Science and Technology’s (HKUST)’ supersite, located on the eastern side of their campus (Latitude 22°19’56.8416” N, Longitude 114°16’1.1316” E). This site was built on a cliff facing Port Shelter and Silver Strand Bay, which is a suburban area with little residential or commercial development. The facility is composed of a weather-proof air-conditioned modular house of 80 m², which contains 10 sample inlets and an optical window for instrumentation, a 10 m high automatic weather station and an outdoor plinth area for 8 samplers. Sample inlets were approximately 1.5 m above the roof, with the inner diameters of pipes being approximately 2.8 cm. Humidity control systems including insulation tubing and a heating system were installed to the sampling lines, to avoid condensation. The air temperature inside the sampling lines was controlled within 34 to 36 °C through the heating system. The facility is used for a number of air quality monitoring projects, including those in collaboration with other institutions as well as the Hong Kong Environmental Protection Department (HKEPD).

The two bioaerosol instruments used in this study were the PLAIR Rapid-E and the WIBS-NEO, with each instrument connected to its own sample line. The PLAIR Rapid-E used a top hat type inlet with no upper particle size selection limit, while the WIBS-NEO sampled through a PM10 head. Details for each instrument can be found in Section 2.2. Both sample lines ran with additional flow to reduce the inlet transit time and to operate the PM10 head at the correct flow rate. Each instrument was calibrated approximately every two weeks, using NIST traceable glass beads and fluorescent glass particles of varying sizes (1, 2, and 4 µm) with the flow rates also monitored and maintained. The total flow of the sampling lines and flows of the two bioaerosol sensors were monitored and calibrated by the Brooks SHO-RATE Flowmeter and Gilibrator, respectively. Relative humidity and temperature were also recorded every minute at the same sampling site for the length of the campaign.

2.2. Instrumentation

The PLAIR Rapid-E could detect particles in a size range of 0.5–100 µm, allowing it to more effectively detect larger pollen grains and plant fragments than previous UV-LIF instrument designs [25]. Another advantage of the PLAIR Rapid-E was its high sample volume, sampling at ~3 L min⁻¹ to improve counting statistics for coarse particle sizes. It utilised a 337 nm gas laser to excite particles and offered 32 resolution channels that were able to detect emitted wavelengths from 350–800 nm. The PLAIR Rapid-E also measured the light scattered by a particle from 24 angles, in real time to approximate its shape. The rate of fluorescence decay was also recorded, further enabling identification of the protein-bearing particles. Particles between 0.5–5 µm were only detected during activation of the instrument’s ‘high-intensity mode’. Given the long-term nature of our monitoring campaign and a wish to preserve the strength of the laser, this mode was not employed. The results presented below were consequently only used for particles 5 µm and above.
The WIBS-NEO is a three channel UV-LIF spectrometer similar in design to various models of the WIBS-4 [24,26]. Each particle entering the instrument scattered light from a red 635 nm laser diode. The side-scattered light was collected by two high numerical-aperture chamber mirrors, passed through an aperture in one of the mirrors and onto a dichroic beam-splitter, before being detected by the FL2 (second fluorescence) channel photomultiplier tube (PMT). The scattered intensity was used to size the particles (in the range 0.5 to nominally 30 µm optical diameter) and subsequently trigger two filtered UV excitation flash lamps at 280 nm (Xe1) and 370 nm (Xe2), in succession. The scattering intensity was also recorded across a quadrature PMT and the standard deviation of the 4 scattering intensities were used to provide a measure of the particle asphericity (Asphericity Factor, AF). The detector system was timed to detect the 310–400 nm emission response (De1), followed by the 420–650 nm emission response (De2), following each respective excitation. In this study, we only focused on data from the Xe2/De1 bands, due to detector saturation.

2.3. Data Analysis

Some non-biological particles such as sea salt and mineral dust are capable of weakly fluorescing, potentially being recorded by UV-LIF instruments as false positives and skewing our observations. As such, a conservative 9 sigma threshold was used when setting the baseline for each channel of the WIBS-NEO. This was derived by the instrument recording the average background fluorescence in the absence of any particles, during what is known as the ‘Forced Trigger’ mode. A particle’s fluorescence must exceed 9 standard deviations of this value to be classified as fluorescent [27]. This was an effective way of removing interferents from our observations, while preserving the majority of ‘true’ bioaerosols [28]. The mechanisms of the WIBS-NEO was described in detail by Forde et al. [27]. For the PLAIR Rapid-E, a fluorescence baseline of 1500 arbitrary units (a.u.) was applied to each channel with the negative values capped at zero. This represented an arbitrary but conservative threshold. When applied, concentrations of fluorescent particles showed good agreement with those detected by the WIBS-NEO. As such, all fluorescent particles observed should contain biological material. De1 and De2 detector baseline shifts were carefully monitored and corrected for, but as such shifts might have adversely affected Fl1 and Fl3 data consistency, we focused on the Fl2 channel for this study. Single-channel instruments such as the UV-APS were successfully used in the past to detect fluorophores indicative of bacteria and other bioaerosols [29]. Deeper analysis was performed using data from the PLAIR Rapid-E, the results of which are described further below.

Particle concentrations were also considered as a function of back trajectory. One hundred and twenty hour-long back trajectories with a starting height of 10 m above ground level (approximately 20 m above sea level), using NOAA’s Hysplit merged with the Openair package [30] were calculated from the sampling site for the length of the monitoring campaign. These trajectories were run every 3 h across the whole campaign, using monthly meteorological data files downloaded from NOAA. The back trajectories were averaged into a number of distinct pathways known as ‘wind clusters’. A 6-cluster solution was used, revealing a range of sources with a mixture of terrestrial and marine influences.

3. Results

3.1. Campaign Observations

Particle concentrations across the length of the campaign are shown in Figure 1. They were lowest in August, began to increase in September and peaked at the start of October. This level was not sustained during the month, however, with concentrations falling before rising sharply again around the start of November. Concentrations were therefore highly variable, but generally highest from October onwards. From 5th July to 1st October, the mean concentration of particles greater than 5 µm recorded by the PLAIR Rapid-E was 4.2 ± 3.4 L⁻¹. The periods of enhancement observed during October peaked as high as 23 L⁻¹.
concentration from July 5th to 1st October was 20.6 ± 9.1 L\(^{-1}\). Two peaks were seen at the start of October and November, both exceeding 60 L\(^{-1}\).

Figure 1. Time-series of the concentration of biofluorescent particles (L\(^{-1}\)) from 5th July to 14th November, as measured by the PLAIR Rapid-E and WIBS-NEO, shown in panels (c,d) respectively. Particles ≥ 5 µm were included for the PLAIR Rapid-E, while the WIBS-NEO recorded particles of 0.5–30 µm. Temperature and relative humidity from 5th July to 1st November are also shown in panels (a,b) respectively, recorded at the same sampling site. Whiskers represent 5th and 95th percentile values and the width of each box represents one day. Median values are denoted by a horizontal line and mean values by the black dot. Grey dots represent hourly averages.

Similar trends were recorded by the WIBS-NEO. Peaks in biofluorescent concentrations at the beginning and end of October were observed again, although these periods of enhancement were less pronounced when compared to the PLAIR Rapid-E. It was a consequence of the WIBS-NEO recording down to a smaller size range that was responsible for the significantly higher concentrations of biofluorescent particles, when compared to the PLAIR Rapid-E. For the WIBS-NEO, the mean concentration from 5th July to 1st October was 20.6 ± 9.1 L\(^{-1}\). Two peaks were seen at the start of October and November, both exceeding 60 L\(^{-1}\).

The average relative humidity was lower from 1st October onwards than for the previous months, as shown in Figure 2. Two exceptionally low periods at the beginning and end of October were responsible for these averages. Both dry periods were sustained across multiple days with hourly
average values as low as 9%. For both temperature and relative humidity, there was also a greater
degree of diurnal variability from October onwards. From 5th July–1st October, diurnal temperatures
ranged from 26–29 °C, while for the period from 1st October, values ranged from 22–28 °C. The diurnal
pattern for relative humidity was similar across both periods, with the daily minimum occurred at
approximately 12:00. However, the minimum diurnal value from 5th July–1st October was higher than
the maximum diurnal value seen from 1st October onwards.

![Figure 2. Average diurnal patterns for relative humidity (a) and temperature (b) recorded at the
sampling site. Averages are taken for two periods, with 5th July–1st October shown in blue and 1st
October onwards shown in red.](image)

### 3.2. Biofluorescent Particle Properties

Differences appeared in the sizing of particles between instruments, with the PLAIR Rapid-E
skewing towards larger particles. This trend also appeared in the calibration data, suggesting it
is a consequence of differences in the method through which the size information was derived
by each instrument (see Supplementary Materials). The WIBS-NEO uses standard Mie scattering
approximations for sizing, and these were consistent with routine calibrations performed using NIST
traceable calibration particles. The PLAIR Rapid-E utilises geometrical optics for particles above 5 µm,
while using Mie scattering for particles 0.5–5 µm.

When comparing the size distribution of biofluorescent particles from 1st October (where significant
enhancements in concentrations were observed) to the preceding summer months, some differences
could be seen. Figure 3 shows that from 1st October onwards, the PLAIR Rapid-E detected a relative
increase in particles ~8 µm and up. The WIBS-NEO also captured this trend, but the inclusion of
sub-5 µm highlighted an interesting pattern. For the period beginning 1st October, the WIBS-NEO
observed a peak in the size distribution at a smaller size than in the previous months (~3 µm from 1st
October compared to 4 µm, previously). As such, the period associated with enhanced concentrations
displayed a greater range in the size distribution for biofluorescent particles.

The spectral profile of particles excited by the PLAIR Rapid-E was largely consistent across the
entire campaign. It can be seen in Figure 4 that fluorescent activity was predominantly recorded in
channels five and six, which covered the emission band 406–434 nm and reflected a narrow peak with
little to no shoulder. Fluorescence intensity in either channel was not correlated with particle size
($R^2 = 0.15$ and 0.16).
with cluster 1 typically showing higher concentrations during the day. For the WIBS-NEO in October, peak concentrations in cluster 1 appeared from 09:00 and maintained a high level throughout the day. At daytime, fluorescent particle concentrations, as shown in Figure 6. Both instruments broadly captured a similar mixture of terrestrial and oceanic influences. Differences could be seen. Figure 3 shows that from October 1st onwards, the PLAIR Rapid-E displayed significant enhancements in concentrations were observed). Some of these differences might be traceable to calibration particles. The PLAIR Rapid-E utilises geometrical optics for particles above 5 µm, with the width of each box reflecting the 14 nm range of the associated channel. Each red line represents the median value with the mean denoted by a dot. Whiskers represent 5th and 95th percentile values.

Figure 3. Biofluorescent particle size distribution (dN/dlogDp (L⁻¹)) for the WIBS-NEO (a) and PLAIR Rapid-E (b) for two periods (see text). Blue line—average for the period of 4th July–1st October; red line—average for the period 1st October–14th November. x-axis values represent the average size of the bins used. Shaded areas represent ± 1 standard deviation.

3.3. Back Trajectory Analysis

As discussed in Section 2.3, a 6 cluster solution was used to calculate back trajectories that dominated during the length of our campaign. These different clusters can be seen in Figure 5, with a mixture of terrestrial and oceanic influences.

Wind cluster 1 had the most influence from the mainland and was also associated with the highest fluorescent particle concentrations, as shown in Figure 6. Both instruments broadly captured similar diurnal patterns (as shown in Figure 7), although this was more pronounced for the PLAIR Rapid-E, with cluster 1 typically showing higher concentrations during the day. For the WIBS-NEO in October, peak concentrations in cluster 1 appeared from 09:00 and maintained a high level throughout the day,
falling slightly in the evening, before rising again at 21:00. It could be seen that not every wind cluster was present each month, with cluster 1 only appearing from September and cluster 2 also being absent during August. Clusters 3–6 regularly appeared from July-September (with the exception of cluster 5 in July), but all were absent during October and November. The effective diameter (also known as the weighted mean diameter) of biofluorescent particles detected by both UV-LIF instruments also changed as a function of wind cluster. As such, wind cluster 1 was not only associated with higher bioaerosol concentrations, but also with larger particles. This change was particularly pronounced for the WIBS-NEO.

Figure 5. A 6 cluster solution when performing cluster analysis on all back trajectories from July 3rd to November 14th, using NOAA’s Hysplit, merged with the Openair package. Each trajectory represents 120 h and start from a height of 10 m above ground level.

Figure 6. Average daily concentrations (L^{-1}) and effective diameter (µm) of particles detected by both the PLAIR Rapid-E and WIBS-NEO, as a function of wind cluster. Panels (a) and (b) represent PLAIR Rapid-E and WIBS-NEO concentrations, respectively. Panels (c) and (d) represent PLAIR Rapid-E and WIBS-NEO Effective Diameter, respectively. Concentrations recorded by the WIBS-NEO are for particles across the instrument’s entire size range (0.5–30 µm).
Figure 7. Mean concentrations (L⁻¹) of biofluorescent particles as a function of wind cluster, split by month and hour of day. Concentrations are for particles ≥5 µm for the PLAIR Rapid-E and ≥0.5 µm for the WIBS-NEO. Concentrations as a function of cluster are calculated in three-hour intervals. Panel (a) represents the PLAIR Rapid-E and panel (b) represents the WIBS-NEO.

It can be seen in Figure 8 that periods of exceptionally low relative humidity were almost exclusively associated with wind cluster 1 and typically contained much higher particle concentrations. For biofluorescent particles recorded by the PLAIR Rapid-E, we noted an $R^2$ value of 0.40 ($p \leq 0.001$) with relative humidity from July to October. This negative relationship was strengthened to 0.60 from 1st October onwards, with both peaks coinciding with the lowest relative humidity observed (see Supplementary Materials).
Figure 8. PLAIR Rapid-E particle concentrations (L$^{-1}$) as a function of relative humidity. The colourbar represents the wind cluster that was dominant at the time of recording. Data points represent three-hour averages across the entire campaign.

4. Discussion

The biofluorescent particle concentrations we observed appeared to be low, when compared to previous studies for other major cities. Yue et al. [23], used a WIBS-4 in Beijing and observed more than an order of magnitude increase in biofluorescent particles greater than 0.8 µm, with an average concentration across their sampling period of 642 ± 297 L$^{-1}$. The lower level at HKUST could be explained by dominant marine air masses at this coastal suburban site.

The specificity of a fluorophore’s excitation and emission bands helped to inform particle identification. Our model of the PLAIR Rapid-E used a gas laser that excited at 337 nm, and we observed the greatest fluorescent response in channels five and six (406–434 nm). Channel 2 of the WIBS-Neo excited particles with 370 nm light and used a detection plate sensitive to 310–400 nm. Although there was no perfect overlap between these instruments, they were both close to the excitation/emission bands commonly associated with NADH [31]. Other possible fluorophores at these wavelengths included sporopollenin (a biopolymer found in the outer coating of pollen grains and plant spores), DNA, phenols, and terpenoids [32,33].

Previous literature attempted to characterise bioaerosols in Hong Kong, but there were some instrumental limitations in our ability to replicate their findings. For example, Woo et al. [10] found many marine-derived phylotypes during summer, with Chlorophyta dominating their samples. These are a taxon of green algae, for which previous literature suggests Chlorophyll-α has a much higher excitation wavelength of 400–485 nm [35]. Although Xe2 of the WIBS-Neo was close to the lower end of this excitation band, the emitted wavelengths were higher than the instrument’s detection range, at 685 nm. As such, our instruments would register any airborne concentrations of Chlorophyta as non-fluorescent particles and would overlook them during analysis.

There is strong evidence to suggest that the bioaerosol concentrations we observe are predominantly fungal spores. Turner et al. [36] previously made detailed observations of the fungal genera in Hong Kong and how they vary by season, with manual off-line microscopical analysis. Dominant genera
include *Cladosporium*, *Aspergillus*, *Penicillium*, and *Aureobasidium*, which together accounted for 65.7% of isolates seen across an entire year. Among these, *Cladosporium* was the most prevalent. Although a hugely variable and broad genus, many *Cladosporium* spp. are ‘dry-weather spores’, noted for their negative correlation with relative humidity [37,38] and therefore demonstrate a good agreement with our observations. The observed diurnal pattern in concentrations also support this conclusion, with previous studies finding peaks in *Cladosporium* counts, at similar times [39], although this appeared to be highly variable, possibly depending on the region [40,41]. *Cladosporium* spores were previously shown to fluoresce at similar wavelengths to the channels in which we observed activity. O’Connor et al. [42] excited two *Cladosporium* spp. with 370 nm light and observed clear peaks at ~415 nm. Furthermore, exciting the spores with 350 nm light produced almost identical results, suggesting that the 337 nm laser used in the PLAIR Rapid-E might be sufficient. They observed similar spectral profiles in other fungal spores, including *Alternaria*, *Penicillium*, and *Aspergillus*.

Pollen was considered, but did not sufficiently explain our observations. This was despite our instruments detecting fluorescent activity that is potentially indicative of sporopollenin. Hong Kong has two pollen seasons occurring in spring and autumn, neither of which coincide with the enhancements we observed. Furthermore, the spectral signal of pollen is expected to be broader as more channels becoming activated. O’Connor et al. [42] observed three peaks at 420 nm, 465 nm, and 560 nm in *Betulaceae* samples, while grass pollen had an additional peak at 675 nm. It was surprising that we did not see evidence of pollen’s presence during our campaign. Concentrations of particles greater than 5 µm remained very low during the summer months, averaging ~3 L⁻¹. It was possible our sampling site was not suitable for capturing pollen, given the close proximity to the sea. As the back trajectories all cross the South China Sea during the months when the pollen levels are expected to be greatest, there is little opportunity for the collection and transport of pollen grains before reaching our sampling site. Fragmentation of pollen grains also remains an issue for detection, with previous studies suggesting an association between biofluorescent fragment concentrations and rain or thunderstorms [16,43].

Bacteria are not thought to be the dominant bioaerosol, despite the fluorophore we likely observed—NADH—being prevalent in bacteria (hence, it is usually used as an indicator of bacterial metabolic activity) [44]. NADH is ubiquitous amongst other bioaerosols, however, its presence alone is not enough for the identification of bacteria. Our conclusions were also in contrast to the observations made by Woo et al. [10], who highlight increased concentrations of soil bacteria during winter, with northerly trajectories carrying soil particles through Aeolian processes. However, the northerly wind clusters associated with higher particle concentrations only show this trend when other conditions are met. When relative humidity is moderate or high, particle concentrations from wind clusters one and two are no different from the concentrations seen in clusters 3–6. As our observations clearly capture the importance of dry periods in driving particle enhancements, and as previous studies found no correlation between bacterial concentrations and relative humidity [45], it is likely that we are observing something else. As previously discussed, past studies confirmed the presence of fungal spores in the region, for which many species are ‘dry-weather’. We would expect these fungal spores to also fluoresce in the channels we see activity in. We also argue that because the dry periods are associated with northerly trajectories, this artificially inflates the importance of wind trajectory. We find little evidence that northerly trajectories are carrying particles from new source regions, and it is instead possible that associated meteorological conditions drive bioaerosol release from more local sources. It should be noted here that the two driest periods we observed were exceptional, even for northerly winds. Hong Kong’s dry period typically began in November and extended through to February. As such, the first dry period we observed at the beginning of October was unusually early. Furthermore, values for relative humidity did not normally fall as low as we recorded. From 1981–2010, Hong Kong’s mean relative humidity during October was 73% ([https://www.hko.gov.hk/en/cis/normal/1981_2010/normals.htm](https://www.hko.gov.hk/en/cis/normal/1981_2010/normals.htm)), while in 2018 it was 51%. As such, although these conditions clearly enhanced the concentrations of certain bioaerosols, we believe enhancement events such as these would be relatively infrequent.
We acknowledge that the average size of particles detected by the PLAIR Rapid-E was large for fungal spores. Previous studies suggest that *Cladosporium herbarum* spores average around 3 \(\mu m\) [27]. However, these studies were done using different UV-LIF spectrometers to the PLAIR Rapid-E, for which our calibration data suggest that there is a difference in its particle sizing. As such, it is possible many of the fungal spores recorded by the PLAIR Rapid-E would register as smaller if sampled by a different instrument. When looking at particle concentrations across the entire size range of the WIBS-NEO, a peak could be seen for sizes similar to previous studies on fungal spores [27]. Furthermore, *Cladosporium* spp. were highly variable and data on the exact species present in Hong Kong is limited. Consequently, it is also possible that the spores in this region were larger than those used in previous laboratory experiments.

5. Conclusions

This was the first study to quantify bioaerosol concentrations in Hong Kong using the UV-LIF technology. The long-term nature of this campaign enabled us to observe how these concentrations might also change, depending on the season. From 5th July to 1st October, the PLAIR Rapid-E recorded average concentrations of particles greater than 5 \(\mu m\) equal to \(4.2 \pm 3.4\) L\(^{-1}\), while the WIBS-NEO detected \(20.6 \pm 9.1\) L\(^{-1}\) for particles greater than 0.5 \(\mu m\). Two significant peaks were seen during October by both instruments, exceeding 23 L\(^{-1}\) in the PLAIR Rapid-E and 60 L\(^{-1}\) in the WIBS-NEO. Previous literature suggest that wind trajectories are the dominant influence on bioaerosols in Hong Kong. Southerly winds were attributed with enhanced levels of algae, while northerly trajectories were argued to transport soil bacteria from the mainland. By contrast, we observed relative humidity as the most influential factor, with low levels likely initiating spore release from dry-weather fungi, such as *Cladosporium*.

Such fungi were shown to share similar spectral profiles to the particles we observed and dominated Hong Kong spore counts in previous studies. Spore release for such genera was shown to occur during the day, showing further agreement with our observations. Two prolonged periods of low relative humidity were associated with exceptionally high bioaerosol concentrations, which were possibly indicative of spore showers. Both dry periods were also associated with northerly trajectories, potentially inflating the importance of wind trajectory. We did not find strong evidence that northerly trajectories carry fungal spores from new source regions, but rather the associated meteorological conditions might drive spore release from more local sources. Future work should attempt to use UV-LIF spectrometers of higher excitation wavelengths, with the aim of capturing a different subsection of the bioaerosols present in the region. Since the current measurement site has a close proximity to the South China Sea, the observations in our study during summer months when marine air masses dominate, probably reflect a relatively lower range of bioaerosol concentrations in Hong Kong. It would also be interesting to investigate the long-term variations of bioaerosol concentration and composition in different terrain and land-use situations, to further elucidate the spatio-temporal characteristics of fluorescent bioaerosols in Hong Kong.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4433/11/9/944/s1, Figure S1: Time series of the thresholding applied to the three channels of the WIBS-NEO over the course of the campaign. Due to drift in channels 1 and 3, only particles that fluoresced in channel 2 were included in the analysis. Figure S2: Size distribution of NIST traceable calibration particles as recorded by the PLAIR Rapid-E. As 4 \(\mu m\) particles were predominantly used it is clear the instrument is oversizing when compared to the WIBS-NEO. Figure S3: Size distribution of NIST traceable calibration particles as recorded by the WIBS-NEO. As 4 \(\mu m\) particles were predominantly used it is clear the instrument is accurately sizing the calibration particles. Figure S4: WIBS-NEO particle concentrations (L\(^{-1}\)) as a function of relative humidity. The colourbar represents which wind cluster was dominant at the time of recording. Data points represent three-hour averages across the entire campaign.

Author Contributions: D.M. is the primary author and responsible for most of the analysis across both instruments. J.L. monitored both instruments for the length of the campaign and was responsible for regularly calibrating them. I.C. pre-processed all data and provided guidance on both analysis and written components. M.F. was responsible for setting up the instruments at the sampling site with the appropriate experimental design. M.N.C. assisted in
organising and cross-project funding. D.T. and M.G. acted as supervisors to D.M., having secured funding for the project and forming the collaboration with HKUST. W.C. is the co-investigator of this research project, responsible for instrument operation and data acquisition. A.K.H.L., J.C.H.F. and J.Y. supervised the research project in design and discussion. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by a collaborative project between HKUST and the University of Manchester. The project focussed on building a collaborative framework for improved understanding of airborne biological particles (UOM173). D.M. is a PhD student who has received funding from NERC and is a member of the Doctoral Training Partnership (DTP).

**Acknowledgments:** We would like to thank K.S. and N.S. at Droplet Measurement Technologies (DMT) for their loan of the WIBS-NEO during this campaign.

**Conflicts of Interest:** The authors declare no conflict of interest and the funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

**References**

1. Leung, R.; Wong, G.; Lau, J.; Ho, A.; Chan, J.; Choy, D.; Douglass, C.; Lai, C. Prevalence of asthma and allergy in Hong Kong schoolchildren: An ISAAC study. *Eur. Respir. J.* 1997, 10, 354–360. [CrossRef] [PubMed]
2. Yang, Y.; Tang, R.; Qiu, H.; Lai, P.C.; Wong, P.; Thach, T.Q.; Allen, R.; Brauer, M.; Tian, L.; Barratt, B. Long term exposure to air pollution and mortality in an elderly cohort in Hong Kong. *Environ. Int.* 2018, 117, 99–106. [CrossRef] [PubMed]
3. Cao, J.J.; Shen, Z.X.; Chow, J.C.; Watson, J.G.; Lee, S.C.; Tie, X.X.; Ho, K.F.; Wang, G.H.; Han, Y.M. Winter and summer PM2.5 chemical compositions in fourteen Chinese cities. *J. Air Waste Manag. Assoc.* 2012, 62, 1214–1226. [CrossRef] [PubMed]
4. Després, V.; Huffman, J.A.; Burrows, S.M.; Hoose, C.; Safatov, A.; Buryak, G.; Fröhlich-Nowoisky, J.; Elbert, W.; Andreae, M.; Pöschl, U.; et al. Primary biological aerosol particles in the atmosphere: A review. *Tellus B Chem. Phys. Meteorol.* 2012, 64, 15598. [CrossRef]
5. Jones, A.M.; Harrison, R.M. The effects of meteorological factors on atmospheric bioaerosol concentrations—A review. *Sci. Total Environ.* 2004, 326, 151–180. [CrossRef]
6. Yan, Y.Y. Surface wind characteristics and variability in Hong Kong. *Weather* 2007, 62, 312–316. [CrossRef]
7. Shi, W.; Wong, M.S.; Wang, J.; Zhao, Y. Analysis of airborne particulate matter (PM2.5) over Hong Kong using remote sensing and GIS. *Sensors* 2012, 12, 6825–6836. [CrossRef]
8. Wong, C.M.; Ma, S.; Hedley, A.J.; Lam, T.H. Effect of air pollution on daily mortality in Hong Kong. *Environ. Health Perspect.* 2001, 109, 335–340. [CrossRef]
9. Qian, Z.; Lin, H.M.; Stewart, W.F.; Kong, L.; Xu, F.; Zhou, D.; Zhu, Z.; Liang, S.; Chen, W.; Shah, N.; et al. Seasonal pattern of the acute mortality effects of air pollution. *J. Air Waste Manag. Assoc.* 2010, 60, 481–488. [CrossRef]
10. Woo, A.C.; Brar, M.S.; Chan, Y.; Lau, M.C.; Leung, F.C.; Scott, J.A.; Vrijmoed, L.L.; Jawar-Reza, P.; Pointing, S.B. Spatial variation in airborne microbial populations and microbially-derived allergens in a tropical urban landscape. *Atmos. Environ.* 2013, 74, 291–300. [CrossRef]
11. Yan, D.; Zhang, T.; Su, J.; Zhao, L.L.; Wang, H.; Fang, X.M.; Zhang, Y.Q.; Liu, H.Y.; Yu, L.Y. Diversity and composition of airborne fungal community associated with particulate matters in Beijing during haze and non-haze days. *Front. Microbiol.* 2016, 7, 487. [CrossRef] [PubMed]
12. Liu, Z.; Li, A.; Hu, Z.; Sun, H. Study on the potential relationships between indoor cultivable fungi, particle load and children respiratory health in Xi’an, China. *Build. Environ.* 2014, 80, 105–114. [CrossRef]
13. Richardson, M.; Bowyer, P.; Sabino, R. The human lung and Aspergillus: You are what you breathe in? *Med. Mycol.* 2019, 57, S145–S154. [CrossRef] [PubMed]
14. Targonski, P.V.; Persky, V.W.; Ramekrishnan, V. Effect of environmental molds on risk of death from asthma during the pollen season. *J. Allergy Clin. Immunol.* 1995, 95, 955–961. [CrossRef]
15. Wang, H.; Zheng, J.; Zhong, N. Time trends in the prevalence of asthma and allergic diseases over 7 years among adolescents in Guangzhou city. *Zhonghua Yi Xue Za Zhi* 2006, 86, 1014–1020.
16. Miguel, A.G.; Taylor, P.E.; House, J.; Glovsky, M.M.; Flagan, R.C. Meteorological influences on respirable fragment release from Chinese elm pollen. *Aerosol Sci. Technol.* 2006, 40, 690–696. [CrossRef]
17. Stocks, J.J.; Buggs, R.J.; Lee, S.J. A first assessment of Fraxinus excelsior (common ash) susceptibility to *Hymenoscyphus fraxineus* (ash dieback) throughout the British Isles. *Sci. Rep.* 2017, 7, 1–7. [CrossRef]

18. Rypien, K.L. African dust is an unlikely source of *Aspergillus sydowii*, the causative agent of sea fan disease. *Mar. Ecol. Prog. Ser.* 2008, 367, 125–131. [CrossRef]

19. Fisher, M.C.; Henk, D.A.; Briggs, C.J.; Brownstein, J.S.; Madoff, L.C.; McCraw, S.L.; Gurr, S.J. Emerging fungal threats to animal, plant and ecosystem health. *Nature* 2012, 484, 186–194. [CrossRef]

20. Crawford, I.; Lloyd, G.; Herrmann, E.; Hoyle, C.; Bower, K.; Connolly, P.; Flynn, M.; Kaye, P.; Choularton, T.; Gallagher, M. Observations of fluorescent aerosol-cloud interactions in the free troposphere at the High-Altitude Research Station Jungfraujoch. *Atmos. Chem. Phys.* 2016, 16, 2273–2284. [CrossRef]

21. Gabey, A.; Stanley, W.; Gallagher, M.; Kaye, P.H. The fluorescence properties of aerosol larger than 0.8 μm in urban and tropical rainforest locations. *Atmos. Chem. Phys.* 2011, 11, 5491–5504. [CrossRef]

22. Crawford, I.; Gallagher, M.W.; Bower, K.N.; Choularton, T.W.; Flynn, M.J.; Ruske, S.; Listowski, C.; Brough, N.; Lachlan-Cope, T.; Fleming, Z.L.; et al. Real time detection of airborne fluorescent bioparticles in Antarctica. *Atmos. Chem. Phys.* 2017, 17, 14291–14307. [CrossRef]

23. Yue, S.; Ren, H.; Fan, S.; Wei, L.; Zhao, J.; Bao, M.; Hou, S.; Zhan, J.; Zhao, W.; Ren, L.; et al. High abundance of fluorescent biological aerosol particles in winter in Beijing, China. *ACS Earth Space Chem.* 2017, 1, 493–502. [CrossRef]

24. Huffman, J.A.; Perrin, A.E.; Savage, N.J.; Clot, B.; Crouzy, B.; Tummon, F.; Shoshanim, O.; Damit, B.; Schneider, J.; Sivaprasakam, V.; et al. Real-time sensing of bioaerosols: Review and current perspectives. *Aerosol Sci. Technol.* 2019, 54, 1–31. [CrossRef]

25. Crouzy, B.; Stella, M.; Konzelmann, T.; Calpin, B.; Clot, B. All-optical automatic pollen identification: Towards an operational system. *Atmos. Environ.* 2016, 140, 202–212. [CrossRef]

26. Perrin, A.; Schwarz, J.; Baumgardner, D.; Hernandez, M.; Spracklen, D.; Heald, C.; Gao, R.; Kok, G.; McMeeking, G.; McQuaid, J.; et al. Airborne observations of regional variation in fluorescent aerosol across the United States. *J. Geophys. Res. Atmos.* 2015, 120, 1153–1170. [CrossRef]

27. Forde, E.; Gallagher, M.; Walker, M.; Foot, V.; Attwood, A.; Granger, G.; Sarda-Esteve, R.; Stanley, W.; Kaye, P.; Topping, D. Intercomparison of Multiple UV-LIF Spectrometers Using the Aerosol Challenge Simulator. *Atmosphere* 2019, 10, 797. [CrossRef]

28. Savage, N.J.; Krentz, C.E.; Könenmann, T.; Han, T.T.; Mainelis, G.; Pühler, C.; Huffman, J.A. Systematic characterization and fluorescence threshold strategies for the wideband integrated bioaerosol sensor (WIBS) using size-resolved biological and interfering particles. *Atmos. Meas. Tech.* 2017, 10, 4279–4302. [CrossRef]

29. Agranovski, V.; Ristovski, Z.D.; Ayoko, G.A.; Morawaska, L. Performance evaluation of the UVAPS in measuring biological aerosols: Fluorescence spectra from NAD (P)H coenzymes and riboflavin. *Aerosol Sci. Technol.* 2004, 38, 354–364. [CrossRef]

30. Carslaw, D.C.; Ropkins, K. Openair—An R package for air quality data analysis. *Environ. Model. Softw.* 2012, 27, 52–61. [CrossRef]

31. Rehman, A.U.; Anwer, A.G.; Gosnell, M.E.; Mahbub, S.B.; Liu, G.; Goldys, E.M. Fluorescence quenching of free and bound NADH in HeLa cells determined by hyperspectral imaging and unmixing of cell autofluorescence. *Biomed. Opt. Express* 2017, 8, 1488–1498. [CrossRef] [PubMed]

32. Lakowicz, J.R.; Shen, B.; Gryczynski, Z.; D’Auria, S.; Gryczynski, I. Intrinsic fluorescence from DNA can be enhanced by metallic particles. *Biochim. Biophys. Res. Commun.* 2001, 286, 875–879. [CrossRef] [PubMed]

33. Roshchina, V.V. Autofluorescence of plant secreting cells as a biosensor and bioindicator reaction. *J. Fluoresc.* 2003, 13, 403–420. [CrossRef]

34. Wang, H.; Zhu, R.; Zhang, J.; Ni, L.; Shen, H.; Xie, P. A novel and convenient method for early warning of algal cell density by chlorophyll fluorescence parameters and its application in a highland lake. *Front. Plant Sci.* 2018, 9, 869. [CrossRef] [PubMed]

35. Lamb, J.J.; Rekke, G.; Hohmann-Marriott, M.F. Chlorophyll fluorescence emission spectroscopy of oxygenic organisms at 77 K. *Photosynthetica* 2018, 56, 105–124. [CrossRef]

36. Turner, P. The fungal air spora of Hong Kong as determined by the agar plate method. *Trans. Br. Mycol. Soc.* 1966, 49, 255–267. [CrossRef]

37. Grinn-Gofron’, A.; Strzelczak, A. Changes in concentration of Alternaria and Cladosporium spores during summer storms. *Int. J. Biometeorol.* 2013, 57, 759–768. [CrossRef]
38. Kasprzyk, I.; Kaszewski, B.M.; Weryszko-Chmielewska, E.; Nowak, M.; Sulborska, A.; Kaczmarek, J.; Szymanska, A.; Haratym, W.; Jedryczka, M. Warm and dry weather accelerates and elongates Cladosporium spore seasons in Poland. *Aerobiologia* 2016, 32, 109–126. [CrossRef]

39. Hameed, A.A.; Khoder, M.; Yuosra, S.; Osman, A.; Ghanem, S. Diurnal distribution of airborne bacteria and fungi in the atmosphere of Helwan area, Egypt. *Sci. Total Environ.* 2009, 407, 6217–6222. [CrossRef]

40. Bardei, F.; Bouziane, H.; del Mar Trigo, M.; Ajouray, N.; El Haskouri, F.; Kadi, M. Atmospheric concentrations and intradiurnal pattern of Alternaria and Cladosporium conidia in Tétouan (NW of Morocco). *Aerobiologia* 2017, 33, 221–228. [CrossRef]

41. Pady, S.; Kramer, C.; Clary, R. Diurnal periodicity in airborne fungi in an orchard. *J. Allergy* 1967, 39, 302–310. [CrossRef]

42. O’Connor, D.J.; Iacopino, D.; Healy, D.A.; O’Sullivan, D.; Sodeau, J.R. The intrinsic fluorescence spectra of selected pollen and fungal spores. *Atmos. Environ.* 2011, 45, 6451–6458. [CrossRef]

43. Schäppi, G.F.; Suphioglu, C.; Taylor, P.E.; Knox, R.B. Concentrations of the major birch tree allergen Bet v 1 in pollen and respirable fine particles in the atmosphere. *J. Allergy Clin. Immunol.* 1997, 100, 656–661. [CrossRef]

44. Wos, M.; Pollard, P. Cellular nicotinamide adenine dinucleotide (NADH) as an indicator of bacterial metabolic activity dynamics in activated sludge. *Water Sci. Technol.* 2009, 60, 783–791. [CrossRef]

45. Islam, M.; Ikeguchi, A.; Naide, T. Concentrations of Aerosol Numbers and Airborne Bacteria, and Temperature and Relative Humidity, and Their Interrelationships in a Tie-Stall Dairy Barn. *Animals* 2019, 9, 1023. [CrossRef]