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Fluorescent Biological Aerosol Particles in Coastal Canada and the Link to Atmospheric Ice Nuclei

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Fluorescent Biological Aerosol Particles in Coastal Canada and the Link to Atmospheric Ice Nuclei

A Thesis

Presented to

The Faculty of Natural Sciences and Mathematics

University of Denver

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Jixiao Li

August 2015

Advisor: J. Alex Huffman
Abstract

Bioaerosols are a subgroup of atmospheric aerosols and are often linked to the spread of human, animal and plant diseases. Bioaerosols also may play an indirect effect on environmental processes, including the formation of precipitation and alteration of the global climate through their role as nuclei for cloud droplet formation. Several types of biological organisms (e.g., fungi and bacteria) have been shown to be effective ice nuclei (IN) and cloud condensation nuclei (CCN).

During 21 days in August 2013 we participated in a collaborative international campaign at a rural, coastal site near the village of Ucluelet on the west coast of Vancouver Island, British Columbia, Canada. The experiments were conducted as part of the NETCARE project (the NETwork on Climate and Aerosols: Addressing Key Uncertainties in Remote Canadian Environments), in part to examine cloud nuclei properties of marine aerosol. The study was conducted from a mobile trailer located approximately 100 m from the coast. A suite of aerosol instrumentation was operated for approximately one month. Key instruments utilized as a part of this thesis include the wideband integrated bioaerosol sensor (WIBS-4A) and the multiple orifice uniform deposition impactor (MOUDI) coupled with an off-line droplet freezing technique (DFT) for the measurement of ice nucleation activity of particles in immersion mode. The WIBS measures the concentration and properties of individual fluorescent particles suspended in
the air, which can serve as a proxy for airborne biological particle content. Particles shown to be fluorescent by the WIBS instrument were divided into seven categories based on the pattern of fluorescence each particle exhibited in the three fluorescent channels. Results of the WIBS analysis show that the fluorescent particle concentration in the region correlated well with IN number. The fluorescent particle concentration correlated well with the number of particles shown to be ice active as a function of both particle size and freezing temperature. Correlations involving marine aerosols and marine biological activity indicate that the majority of IN measured at the coastal site likely are not from have marine sources.
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Chinese student study in the US was something special, I believe breaking the ice between different cultures and passing through all the difficulties was a great lesson, which guided me through my MS journey. I am grateful to my family, especially to my father Li, Xingyan and my mother Ding, Yahong. Their support and their love is my strongest backup to finish my study.
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Chapter 1 Introduction

1.1. Overview: What are Primary Biological Aerosol Particles (PBAPs)

1.1.1 PBAPs definition

The atmosphere is a dynamic system vital to supporting life on Earth. The Earth’s atmosphere consists of 99.9% molecular dioxygen, dinitrogen, and noble gases with a small amount of trace gases. In the mix of atmospheric gases, solid and liquid aerosol particles are small pieces of condensed material that can be suspended in the atmosphere for up to weeks at a time\(^1\). Some of these particles are generated by biological activities\(^1,2\). Small particles that contain biological materials are called primary biological aerosol particles (PBAPs)\(^3,4\) and can be made up of bacteria, fungal spores, viruses, dust mites, fractions of biolysis materials, and excretions of microorganisms\(^5\). Small biological aerosols, such as bacteria and viruses, can agglomerate together or adsorb onto larger particles, whereas large particles like pollen are typically suspended in the air as individual grains. PBAPs have attracted increasing interest within atmospheric research communities in the last decades because of their potential influence on environmental processes such as cloud formation and for their potentially adverse effects on human\(^6\). The biological fraction of atmospheric aerosol is an intricate assortment of biologically derived material that can make up a significant percentage of both PM10 (particulate
matter with diameter less than 10 μm) and PM2.5 (particulate matter with diameter less than 2.5 μm) while often dominating the supermicron fraction of particulate matter\(^4\). Despres et al.’s study point out in clean tropical rainforest environment, fungal spores concentration could up to 45% of coarse particulate matter\(^4, 7\). Bioaerosols could also be an important factor that affect the PM especially in farming and agricultural activities\(^8\).

**1.1.2 Size and distribution of PBAPs**

The range of sizes at which PBAPs can exist is very broad and individual particles can occur at very different sizes based on their type. The largest PBAPs are generally pollen grains, with an average aerodynamic diameter of approximately larger than 5 μm\(^9\) and which can be as large as 100 μm\(^10\). So in general intact pollen grains are not counted as a part of the PM10 measurement. Ambient atmospheric number concentrations of pollen have been measured to be up to 1000-8000 grains m\(^-3\) during pollination season\(^11\). Fungal and algae spores are commonly monitored in greater quantities than pollen, but at smaller sizes. Number concentrations have been cited between 10\(^3\) to 10\(^4\) m\(^-3\) over vegetated regions and they exhibit aerodynamic diameters most frequently it is about 0.5 μm\(^4, 9\). Bacteria generally represent an even smaller class of biological particles with average aerodynamic diameter of 0.2 μm\(^9\). Viruses exhibit sizes between 0.0051 μm and 0.25 μm\(^9, 12\). This allows them to travel deep into respiratory pathways and hence can cause potentially lethal infections, though most airborne viruses are not suspended individually, but are agglomerated inside or on the surface of much larger particles or droplets\(^4\).
1.1.3 Release mechanisms

PBAPs can be released or transferred into the atmosphere in a number of different ways. Passive release of PBAPs relies on external factors for dispersal such as meteorological conditions of high wind, humidity, and rain, with a particular emphasis on air flow\(^\text{10}\). Wind is an effective separation method for many pollen and fungal spore types\(^\text{10}\). Humidity and temperature are usually interconnected with wind release, as the bonds holding pollen and spores to the host surfaces tend to be dehydrated by the air flow\(^\text{13}\). Rainfall is commonly linked with the wash-out of ambient particles; however it has been shown to cause the release of certain fungal spores, as well\(^\text{7}\). This process is achieved by raindrops imparting mechanical energy to fungal spores and bacteria on terrestrial surfaces (i.e. plant leaves). In the ocean PBAP can be passively produced by impacting waves, tides, and bubble bursting\(^\text{14}\). Marine bioaerosol could be an important media for sea-air transportation of bacteria and viruses. A study by Aller et al. suggested that bacteria and viruses were enriched near the sea-surface microlayer (SML) and a large number of marine microorganisms could be transmitted into the air as marine aerosols\(^\text{15}\). PBAPs released from marine environment may be transported up to thousands of kilometers and could influence the global distribution of bioaerosols\(^\text{15}\).

PBAPs can also be released actively. This means that the host organism dynamically releases particles. Certain fungal spore types are known to absorb water during rainfall or periods of high humidity, which causes a gradual pressure build up within the cell holding the spores\(^\text{16}\). Once the osmotic pressure reaches a certain threshold
the fungal spores are forcibly shot into the surroundings\textsuperscript{17}. There are various mechanisms of active PBAP release, but a review of these topics is beyond the scope of this text.

\subsection*{1.1.4 Factors affecting PBAPs concentrations}

Many factors can affect the atmospheric concentration of PBAPs. Meteorological effects such as wind, rainfall, solar radiation and humidity level can influence PBAPs concentration by influencing release mechanisms and deposition rates. Many types of PBAPs, such as pollen and spores, are ultimately linked to the life cycles of their host organisms, their production, and biodegradation materials\textsuperscript{4}. Consequently higher amounts of some biological aerosols (e.g. pollen) are often seen in the spring and summer seasons, because the high temperature enhances biological activities, whereas airborne fungal spores are often highest in the fall\textsuperscript{3,18}. Seasonal and diurnal variations affect the PBAPs concentration as a function of factors such as temperature, snow cover, and bioactivity\textsuperscript{3}. A study by Liao et al. showed the growth of certain fungal spores is related to the relative humidity of the environment\textsuperscript{19}. The study showed that increasing the relative humidity also led to a higher concentration of spores. Wind is also a key factor for PBAP transportation, which causes the concentration change among different regions\textsuperscript{20}. Rainfall and other forms of precipitation usually reduce the concentration of PBAPs, because the rain drops “wash out” most of the bioaerosol in ambient. However, in some cases wind and rainfall can also impart physical force that causes the plant to move, which may also release the PBAPs. There have been numerous studies from a variety of world-wide locations indicating the concentration of PBAPs link to climate, such as cloud formation.
and percepitation\textsuperscript{21, 22}. Many PBAP types have been shown to exhibit a diurnal pattern\textsuperscript{4, 23}. Certain fungal spore that prefer high humidity environments are released during the nighttime, whereas others that prefer high temperature and dry conditions are often released during the day\textsuperscript{24, 25}.

1.1.5 Atmospheric transport

Like non-biological aerosols, PBAPs suspended in the atmosphere are affected by gravity, atmospheric dynamics, and deposition pathways. PBAPs distributed in the atmosphere may be transmitted on the surface of a dust particles, which can be transported up to thousands of kilometers, such as from the Sahara Desert across the Atlantic Ocean\textsuperscript{26, 27, 28}. Dust is generally understood to constitute up to approximately 50\% of tropospheric aerosol mass, or up to 1000 ~ 3000 Mt yr\textsuperscript{-1}\textsuperscript{29}.

Given that long-distance transport of biological aerosol can result in deposition of invasive species to new settlement regions\textsuperscript{4}, it is important to accurately predict the source of bioaerosol transmission using atmospheric models\textsuperscript{22}. This requires not only a detailed understanding of aerodynamic transmission, but also an understanding of the biogeography of the source and the microbial environment in route.

1.2 Why do we care about PBAPs

1.2.1 Air quality and health effects

PBAPs can influence human health in both indoor and outdoor environments\textsuperscript{30}. Kalogerakis et al. monitored bioaerosol activities in several indoor area in Athens, Greece
and showed that they play an important role of indoor air quality\textsuperscript{31}. Indoor PBAPs can come from both inside or outside. Resident, furniture, and construction material can each host and emit microorganism\textsuperscript{30}. For water-soaked building materials, as occurs during flood situations, growth of bacterial and fungal is a potential hazard to human health, because they can release high counts of bacteria and mold spores. Pathogenic and toxic bioaerosol can also be spread maliciously, as demonstrated by the cases of anthrax spores distributed in the form of white powder placed in postal envelopes and sent to government workers in the United States in 2001\textsuperscript{32}.

Many types of PBAPs can cause serious health effects, because they can be inhaled to the mouth and nasal passages and can be small enough to be inhaled deep into the lungs\textsuperscript{33}. The largest pollen PBAPs with 10-100 µm can get trapped in nasopharynx and trachea\textsuperscript{34}. The smallest of PBAPs, such as viruses, can travel much deeper and can even pass directly from lung alveoli into the bloodstream\textsuperscript{35}.

\textbf{1.2.2 Environment effects}

Atmospheric aerosol particles can absorb and scatter incoming solar radiation and outgoing long-wave radiation, affecting the ground-air radiation balance\textsuperscript{36}. Aerosol particles which can generate ice crystals by freezing supercooled water droplets or water vapor deposited on their surface are called ice nuclei (IN). Aerosols that act as the site for condensation of water vapor into liquid cloud droplets are called cloud condensation nuclei (CCN). IN and CCN are important factors that influence the optical properties, mass, and lifetime of a cloud. Clouds can exert both direct and indirect influence on the
energy balance of a parcel of air, and ultimately on the globe. Possible atmospheric ice nuclei include mineral dust, soot, crystalline salts, and biological aerosol particles. Mineral dust has been well-studied as an important factor that affect atmospheric ice nuclei, it theoretically can be 100% activated to ice crystals. PBAPs can also act as IN and CCN, hence affecting the mass of the cloud. Other than these effects, bioaerosols have a potentially important role in atmospheric chemistry and physics processes. Bioaerosols can 1) Effect the chemical processes by microbial degradation, and 2) influence heterogeneous chemistry, like at the air/snow interface. Aerosol particles form the nucleus for the condensation of cloud droplets, and their number and properties influence cloud microphysical properties. How active particles act as IN and CCN depends on their size and hygroscopicity. The figure shows a simple mechanism that how bioaerosol be generated and effect the climate matter.

Figure 1.1 Cycling and effects of primary biological aerosol particles in the atmosphere and biosphere. Figure from the open source of: https://astrowriter.files.wordpress.com/2011/04/aerosol1.png?w=544
Ice nucleation on the surface of a particle requires the supersaturation (i.e. greater than RH=100%) of water vapor with respect to ice$^{43}$. The supersaturation is determined by the size and soluble mass content of particles as defined by Köhler theory, which describes the combined effects of the elevation of RH by the curved surface of particles and lowering of RH by dissolved solute$^{6,44}$. The greater the supersaturation required for a particle to initiate ice formation, the lower the required temperature must be. It has been suggested that the large (i.e. > 2 µm) PBAPs may act as IN at lower supersaturation values than other particle types, such as mineral dust$^{45}$. Cloud water droplets do not freeze directly at 0 °C but can remain in a super-cooled liquid state down to temperatures of approximately -37.5°C$^{46}$. At higher temperatures, aerosol particles are required initiate ice formation via a process called heterogeneous freezing. This ability is not exclusive to biological matter; however, it has been shown that some microorganisms can initiate freezing of water at much warmer temperatures than any non-biological materials. This is thought to happen because of the chemical nature of proteins on the surface of the organisms$^{47}$. The nucleating ability of PBAP may affect the growth and formation of clouds, in part, by initiating cloud growth at lower altitudes than when seeded exclusively by mineral dust. The additional cloud cover can affect the radiative balance of the Earth because it trap or reflect radiation, leading to both an increase and decrease in warming of a certain region, depending on the altitude and properties of the clouds. Clouds can also affect the precipitation patterns of a region. PBAPs as IN and giant CCN are considered to play a potentially important role in precipitation from “mixed phase” clouds (mixed with solid and liquid water phases).
1.3 Fluorescent aerosol in marine environment

Understanding of the physical and chemical properties of marine aerosol particles is important because these particles play an important role in atmospheric processes. Marine aerosols affect radiative transfer and climate directly by scattering and absorbing radiation and indirectly by influencing the droplet size distribution and albedo of marine boundary layer clouds. There are various sources that generate aerosols in marine environment, including: bubble bursting, impact of wave, wind, and precipitation. These activities make significant contribution to creation of marine aerosols. Because oceans cover approximately 70% of the globe, it is important to understand the marine sources of aerosols, including IN and CCN.

It is well known that biological aerosols affect the IN and CCN formation in continental region. Burrow et al showed two distinct lines of evidence for the existence of marine biogenic atmospheric ice nuclei, evidence from IN counts in the atmosphere and from laboratory testing of source material. There is evidence from in situ observations reported in a number of studies scattered over four decades suggesting that in remote, biologically active areas of the ocean, the background atmospheric IN concentrations measured on ships are highly influenced by local marine biological activity and sea spray production. Information on the concentrations and activity of INs is needed to predict the frequency and properties of mixed-phase and ice clouds in the atmosphere and hence the effect of aerosol particles on climate and precipitation. Later research found that atmospheric IN concentrations was higher in ocean upwelling regions, or were associated with high concentrations of biogenic materials in samples of ocean water. However, the
paper pointed out that these historical measurements suffer from the limitations of the filter membrane method used at the time, which had significant systematic errors. Laboratory study of marine aerosols based on sea ice and generated sea spray aerosol using ocean water has bacteria and phytoplankton found in these samples. It is possible that the release of biological particles from the oceans may be a source of INs in the atmosphere.

For this study, we hypothesize that biological particles play an important role in cloud formation by acting as IN in marine regions. To measure the biological aerosols and the major source of INs, we set up a remote measurement site on the coast in western Canada; we investigated correlations between INs number concentrations and different types of ambient biological aerosols. Measurements were conducted in August, 2013 as part of the Network on Climate and Aerosols: Addressing Key Uncertainties in Remote Canadian Environments (NETCARE) project. A primary goal of the study was to investigate whether primary biological particles are a major source of INs at the marine environment.

1.4 How PBAPs are detected

There are many methods to detect the PBAPs in the atmosphere. The measurement techniques use some form of sampling device to physically measure or count the concentration or number of particles in the atmosphere.

Impactors are among the most common devices by which bioaerosols are sampled in ambient air, especially for pollen. Cascade impactors are sampling devices that
simultaneously collect numerous particle size ranges from ambient air. They perform this function by allowing the differential deposition of particles as a function of aerodynamic diameter. Ambient air enters through the upper section of the sampler and an appropriate flow rate is maintained by the application of a vacuum generated by a pump. Particle matter in different size ranges is impacted and collected onto a substrate in this way.\textsuperscript{49} The micro-orifice uniform deposit impactor (MOUDI) is a cascade impactor with cut-sizes down to 0.056 μm and which provides size resolved aerosol particles for the various stages.\textsuperscript{50} After air samples are collected by impactors, it usually relates to apply cultivation or microscopy techniques to characterize the PBAPs. Cultivation methods are only capable of detecting certain types of bacteria, fungi and algae. Cultivation is particularly useful for measuring individual species or groups of microorganisms and even characterizing certain bacterial strains. In addition to cultivation, microscopic techniques were important to study PBAPs. Light microscopy and fluorescence microscopy are two commonly used techniques which have been applied to study the structural/shape information of PBAPs. These techniques can identify biological particles via direct counting and visualization. However, microscopy application can only be used to distinguish some broad groups of microorganisms.

Single particle analysis with online methods requires shorter time and fewer samples than some culture-based sampling techniques, but can also yield detailed information about individual particles in a sample. Several decades ago military communities endeavored to develop an effective online measurement of ambient bioaerosols used as bio-warfare materials\textsuperscript{51, 52}. Since that point, the use of real-time
spectroscopic techniques has flowed over into research communities who utilize the ability to characterize individual aerosols by differences in their fluorescent properties.

Fluorescence spectroscopy is one of the real-time techniques that are commonly used for the analysis of bioaerosols. The systems can operate continuously, and possibly remotely. Several instrumental systems have been developed over the last few years to distinguish biological from non-biological particles, mainly based on UV–Vis fluorescence spectroscopy techniques of individual particles. Fluorescence of microorganisms occurs due to intrinsic fluorophors such as: amino acids (e.g. tryptophan), cellular metabolites including nicotinamide adenine dinucleotide (NADH) or flavins including riboflavin. Several instruments have been developed to detect biological aerosols in real-time. The Ultraviolet Aerodynamic Particle Sizer (UV-APS, TSI, Shoreview, MN, USA) and the Wideband Integrated Bioaerosol Sensor (WIBS, Droplet Measurement Technologies, Boulder, CO, USA) are two commercially available instruments that have been deployed in a number of recent studies of ambient bioaerosol. More details of the fluorescence spectroscopy technique will be introduced in section 1.5.

1.5 Fluorescence spectroscopy PBAPs Instruments: WIBS and UV-APS

1.5.1 Fluorescence spectroscopy technique

Materials exposed to incident light radiation can either absorb or scatter incoming photons. During this absorption or scattering process, energy is imparted to a molecule that can be dissipated either through heat, radiation, or luminescence. Molecular
fluorescence can be regarded as a light scattering process with three steps: 1) Ground state of the molecule become to a higher vibrational state of an excited electronic state but absorbs photons. 2) The excited state molecule energy through non-radiative decay (heat) and transition of the electron to the lowest vibrational level. 3) Return to the electronic ground state by radiative (fluorescence) or non-radiative (quenching) emission.\textsuperscript{55} Fluorescence spectroscopy has become an efficient technique for many analytical sciences, especially within biological research. The techniques can be divided into the general classes of extrinsic and intrinsic fluorescence, and the fluorescence properties of a molecule can be characterized by parameters of steady state fluorescence spectroscopy, such as excitation wavelength ($\lambda_{\text{ex}}$) and emission wavelength ($\lambda_{\text{em}}$).\textsuperscript{55} Based on many studies the combined fluorescent channel of biological aerosol particles was an effective method to analyze the biological particles.\textsuperscript{56}

The fluorescence signals presented here are often associated with the presence of biological particles that contain fluorophores such amino acids.\textsuperscript{4, 25} For example, tryptophan (Trp) constitutes nearly 5\% of the dry weight of \textit{Bacillus subtilis} spores.\textsuperscript{58} Coenzymes are also commonly seen fluorophores. Two pyridine nucleotides, nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH), are well known fluorescent coenzymes.\textsuperscript{55}

One possible source of non-biological interference is polycyclic aromatic hydrocarbons (PAH). PAH occur in the atmosphere primarily in a gas state as products of incomplete combustion. PAH can adsorb to other particles like organic aerosols, and they are known to be highly fluorescent, especially in the spectral band that corresponds to
Another source of non-biological fluorescence interference could be humic-like substances (HULIS). HULIS is a mixture of heterogeneous compounds formed by the natural decay and oxidation of biological material. Fluorescence of HULIS substances has been observed in several commonly used spectral positions like 310-400nm emission at 280nm excitation. There is not an efficient way to differentiate between biological particles and HULIS particles suspended in the air. PAH particle usually have a diameter less than 0.5 µm; and HULIS usually can be found in both aquatic and terrestrial regions. Different fluorophores could have an overlapping fluorescence emission, as well. Examples of other biological fluorophores that have relatively high absorption coefficients for 280 nm excitation with a non-zero quantum yield are riboflavin and flavin adenine dinucleotide (FAD). These molecules may have fluorescence in a large wavelength range and they are hard to characterize individually. Mineral dust is also a major factor that doing contribution of the global atmospheric aerosol, and it’s well known that some minerals have photoluminescence properties. Different of naturally occurring minerals such as silicate, carbonate, phosphate and sulfate minerals have been found to emit the fluorescence at ~500 and ~700 nm when excited at different wavelengths.

However, a number of advantages of the fluorescence spectroscopy have been reported, including: (i) the fluorescence particle counter for measuring the elastic scattering and undispersed fluorescence of individual particles; (ii) the use of a conditional sampling strategy for measuring single-particle fluorescence spectra of individual bioaerosol particles excited at certain wavelength; and (iii) a conditional
laser-firing strategy, wherein particles are excited by a pulsed UV laser that is triggered to fire when the red laser is scattered\textsuperscript{62}. Further updates of the UV-laser technique, crossed-beam triggering system, and reflective optics for detection, will result in a superior capability for effectively measuring fluorescence aerosols in the atmosphere.

1.5.2 WIBS

Wideband Integrated Bioaerosol Sensor mark 4A (WIBS-4A) is an analysis instrument that can precisely detect single mold and fungal spores, pollen grains, and other bioaerosols\textsuperscript{64}. It detects a single particle’s light scattering, fluorescence emission (with 3 channels), size, and asymmetry factors for particles between 0.5 microns to 20 µm in size. It can calculate the particle concentration and size distributions. This instrument uses the ultraviolet xenon flash lamp as a light source to excite the aerosols and emitted the fluorescence. During the operation, aerosol is drawn from the ambient atmosphere via a laminar-flow delivery system. This system renders suspended particles in essentially single file as they traverse the focused laser beam. The total aerosol flow is typically approximately 2.3 l/min, of which approximately 1.7 l/min is filtered before being re-introduced to form a sheath flow. The 0.63 l/min sheath flow confines the remaining sample flow as well as a small bleed flow that continually purges the optical chamber of any fugitive particles. Each particle entering the laser scatters light in all directions. The side-scattered light is collected by the two high numerical-aperture chamber mirrors and passes through an aperture in one of the mirrors, then through a dichroic beam-splitter, and onto the FL2 channel quadrant photomultiplier tube (PMT).
This produces an electrical scatter pulse, as shown in the value of asymmetry factor (AF), which represents a very rough proxy of the shape of the particle. Then the particle flies through the first xenon flash-tube, which is fired 10 μs after passing through the red, triggering laser (Figure 1.2). This flash-tube is optically filtered to a narrow band centered on 280 nm, which is tuned to excite the specific target biological molecule tryptophan, among other possibilities. Then the second xenon flash tube emits at 370 nm. Thus, simultaneous electrical pulses are recorded by the FL1 and FL2 detectors, proportional to the magnitude of fluorescence in the 310-400nm and 420-650nm bands, respectively at the 280 nm excitation. The FL3 detector channel detects 420-650 at 370 nm excitation. The FL1 detector channel is expected to show fluorescence from the biofluorophore tryptophan and the FL3 detector channel is expected to show fluorescence from the biofluorophore NADH. These are two fluorophores that are ubiquitous in biological cells and plant tissues; however, one should note many other biomolecules can also contribute fluorescence in these bandwidths. Because many of the fluorophores can be excited in several wavelengths, for example, some of the NAD+ can be excited at 280 nm and emits the similar wavelength as NADH at 420-650nm. The maximum flash rate of the Xenon lamps is approximately 125 Hz so not all particles passing through the cavity will be irradiated when particle concentrations are high. The instrument records both the number of particles that pass through the laser beam and the number of particles for which the lamps flash. Data presented here have been corrected to account for particles missed by the flash lamps. If a particle is missed the fluorescent detection, it will
be count as “missed particles” and their scattered size information can still be counted, but not the fluorescent information.

The WIBS returns size data in micrometers for particles in the size range of 0.5 to 15 µm and the fluorescence signal strength. Following Gabey et al. a baseline fluorescence signal value was determined for each channel due to low-level excitation of the optical components in the WIBS. This baseline value was determined by measuring fluorescent emissions following excitation of the cavity in the absence of particles. Baseline intensity in each channel is assumed to be normally distributed. To identify the baseline excitation threshold an equation was established:

$$E_{\text{Threshold}} (i) = \bar{E}_i + 3\sigma_i \quad (i = \text{fluorescence channels})$$  \hspace{1cm} \text{Eq 1}$$

$E_i$ bar is the modal baseline value for channel $i$, and $\sigma$ is the standard deviation of the signal counts at each fluorescing value. Each particle was grouped into a common fluorescence excitation and emission profile. Criteria for selecting the excitation and
emission profile involved analysis of the distribution of particles in each fluorescent channel. The letter A, B, and C was used to represent the fluorescent emission in channel Fl1, Fl2, Fl3, respectively. For example, if particles only emits fluorescent signal in Fl1 channel, it will be categorized in group “A”. Since the WIBS instrument provides the 3-fluorescent emission data for every single particle, use the combination of the fluorescent channel emission may potentially be a way to characterize the aerosols. A combined category “AB” means the particle emits the fluorescent signal in both Fl1 and Fl2 channel, but not has emission at Fl3 channel. Category “ABC” simply mean the particle have fluorescent emission in all 3 channels. In total there are 7 categories to characterize the fluorescent emission. Note there is an “any” category to represent the particles that emits fluorescence in any channel, which is the sum of all categorized fluorescent channels. The logical laws used to separate the criteria of common fluorescence excitation and emission profile are shown in Figure 1.3.
1.5.3 UV-APS

The Ultraviolet Aerodynamic Particle Sizer (UV-APS) spectrometer was the first commercially available instruments for real-time detection of biological aerosol particles using fluorescence. The original design and detailed description of the UVAPS can be found in the paper of Hairston et al. It continuously monitors aerodynamic size, scattered light intensity, and fluorescence of viable airborne microorganism’s particles.

The particle counting system of the UVAPS uses a double crest optical system. This means that the detection area consists of two broadly focused laser beams, which is collected by an elliptical mirror and sensed by a solid-state photodetector. This laser system provides both aerodynamic diameter and scattered light measurements for particles from 0.5 to 15 µm. A PMT is used to detect the fluorescence emissions.
Fluorescence is produced by exciting particles with a pulsed ultraviolet laser beam at an excitation wavelength of 355 nm and detected at emission region between 420 and 575 nm. Under this condition, many biological fluorophors emit a fluorescence signal, including NAD(P)H (excitation peak at 340 nm and emission peak at 470 nm) and flavins (excitation peak at 385 nm and emission peak at 525 nm), which are commonly found in living microorganisms. Fluorescence spectra are not typically selective enough by itself for identification of specific airborne microorganisms.

1.6 Aim of thesis

On planet earth approximately 70% area is ocean, and the concentration if IN in the marine environment plays an important role for climate and precipitation. However, few studies have been performed in a marine environment because sampling is relatively difficult. In 2013, Burrows et al. used a satellite observational data of chlorophyll, which as proxy of bioaerosols (Figure 1.4). From the satellite imaging the chlorophyll is obviously concentrated in coastal region than continental and marine region. The circles (bottom right of figure) show IN concentration measurements. These data predict that in Australia Ocean region the IN concentration is related with the chlorophyll. Thus, a major objective of the study discussed here is to link bioaerosols and IN in a Canadian coastal region. Fluorescent particles detected with the WIBS can be regard as a proxy of PBAPs, which will be compared with ambient IN. A 3-week field measurement on the west coast of Canada was performed in order to measure the local
PBAPs and IN data. After data analysis, we concluded that the fluorescent biological particle concentration correlated well with ice nuclei concentration.

Figure 1.4 Geographic distribution of chlorophyll and ship measured IN. Figure created by Susannah Burrows, used with permission from the author.

The main aims of the thesis were:

- To understand the Waveband Integrated Bioaerosol Sensor (WIBS-4) in a laboratory.
- To determine the ambient fluorescent particle concentrations in a rural, coastal environment as a proxy for marine aerosol by using WIBS-4.
- To analysis the data by resolving time, size, and fluorescence.
- To compare, for the first time, the size resolved and fluorescent resolved ambient fluorescent particles and IN, and find how they effect each other.
Chapter 2 Ucluelet Field Measurement

2.2 Experiment Details

2.2.1 Site position and local information

Site measurements were performed at Amphitrite Point (48.92° N, 125.54° W) on the west coast of Vancouver Island in British Columbia, Canada. This site is located approximately 2.2 km south of the town of Ucluelet (population of 1627 in 2011; Statistics Canada, 2012). The largest nearby population centers are Nanaimo, which is 120 km to the east, and Vancouver, which is 180 km to the east. Local vegetation includes western hemlock, western red cedar, and Douglas-fir, shrub including lavandula and euphorbia, and large amount of Fern. This region has a typical marine climate with mild summer and winter, and relatively high levels of humidity. Figure 2.1 is a satellite picture of the measurement site.
The aerosol instrumentation was located in a mobile laboratory (Fig 2.2). Aerosols were sampled through louvered total suspended particulate inlets (BGI Inc., Waltham, MA, USA) or louvered PM10 inlets (Thermos Scientific, Waltham, MA, USA) from the top of the laboratory. The laboratory was approximately 20 m above mean sea level and 100 m from the high tide line of the Pacific Ocean. A row of trees and shrubs approximately 2 m to 10 m in height stands between the laboratories and the rocky shoreline.
2.2.2 Fluorescent particle measurements

The total aerosol flow for WIBS4 was 2.3 L•min⁻¹. To generate a confined particle flow beam through the detection section, WIBS4 uses a split aerosol inlet system to redirect a part of main flow through a HEPA filter and then returned to sheath the remaining sample flow in the inlet. In this way a confined sample flow of 0.63 L•min⁻¹ is generated by the inlet. Note that because the WIBS4A instrument used was the first unit sold by DMT, it has a unique flow rate. Other commercially available WIBS4 have the same total inlet flow, but 0.23 L•min⁻¹ for sample flow.
The WIBS4 inlet was placed on the roof of the remote laboratory, approximately 5 m above the ground, 20 m above sea level. A total suspended particles (TSP) inlet was used to sample ambient aerosol. The TSP inlet is an omnidirectional inlet, which is widely used for general particulate pollution monitoring. In this study, a low-volume TSP inlet was used with a sample flow rate of 16.67 L min\(^{-1}\). With this sample flow rate the TSP inlet is specified to collect particles with a wide spectrum of sizes up to around 30–40 μm. The 13 mm inner diameter stainless steel sampling tube downstream from the TSP inlet penetrates the roof of the laboratory where it is fitted to a 40 mm inner diameter flow tube, which is operated at flow rate of 16.67 L min\(^{-1}\). At the far end of this tube another 13 mm inner diameter sampling tube penetrates the wider flow tube by about 40 cm. The final connection between WIBS4 and the TSP inlet system was made using an electrically conductive silicon rubber tube (13 mm inner diameter; Simolex Rubber Inc.). The overall length of the sampling line is about 3 m. Note that the sampling system is generally vertical, i.e. there are minimal horizontal sampling sections, which avoids sedimentation losses.

In this field measurement, the WIBS runs continuously to monitor the ambient aerosols. There’s a 48-hour measurement gap in between August 17 and August 19 because of the sea water atomization experiment. The other instrument, UV-APS was supposed to do the parallel measurement with the WIBS, however, it had a system malfunction during the campaign and its data were not usable. The WIBS trigger threshold setup was set to Th1=8, Th2=10. Forced trigger measurements for background calibration was performed 1-2 times per day, 5-10 minutes each time. The timing of the
forced trigger experiment was purposely distributed into dawn, morning, noon, afternoon, evening, and late night to acquire the average fluorescent background.

2.2.3 Fluorescent Particle Data Analysis

The instrument is controlled via a laptop connected over a USB 2.0 port. Manufacturer’s software is used to store the measured single particle data in comma separated value (CSV) files. The parameters that are recorded for each single particle are; particle arrival time, the forward and side scattering data, the power of the xenon lamps, the fluorescence intensities for the three different channels, the time of flight (TOF) values, the particle optical size in micrometers, the asymmetry factor values and the missed particle counts. Also, particles that have fluorescence intensity higher than the threshold in any channel (one or multiple) are considered to be fluorescent aerosols. Therefore the software records almost all parameters defined by the manufacturer for each particle. As discussed in Section 1.4, in the presence of too many particles (upper limit ≈ 4×10^4) or when a particle is detected while the UV lamps are being recharged, no fluorescence data can be provided for these particles. Such particles are marked as “missed particle” and will be taken into account when calculating total aerosol number concentration.

WASP Analysis

A WIBS data analysis program (WASP) based on Igor Pro 6 (Wavemetrics Inc, Portland, Oregon) program is used to process the single particle data and to characterize
their biological properties. The program was written by Niall Robinson at the University of Manchester and applied for WIBS-3 data analysis in previously studies\textsuperscript{67}. The instrument generates large amount of data very fast since it measures single particles. The data sets were segmented into 1-15 minutes time intervals and analyzed. According to Eq. (1), the threshold intensity can be automatically calculated and setup by loading all the forced trigger files, or they can be manually setup by user. All single particles having fluorescence signal above this threshold value and not saturating any fluorescence channel are accepted as fluorescent biological aerosol particles. The missed particle count is also estimated by the WASP.

WASP reads CSV files and saves each in different data folders which is a very useful feature of Igor Pro. Figure 2.4 shows the main panel of the WASP, the forced trigger and initial data loading can be finished in this panel. Each data folder is subsequently worked on individually and the results are presented in different forms such as time series of fluorescent biological aerosols or integrated number size distribution for any type of particle according to particle selection criteria. For each data file (consists of a maximum of $3 \times 10^4$ particle events), if available, background fluorescence intensity in each channel (F1, F2 and F3) is read, the mean ($E$-bar) and the standard deviation ($\sigma$) of fluorescence intensity are calculated. Background threshold is calculated from the background forced trigger fluorescence data according to Eq. (1), and any particle for which the measured fluorescence is greater than the background threshold is accepted as a fluorescent biological particle. If data do not contain any background information, the latest background threshold value is used. In Figure 2.5, the average fluorescent emission
and fluorescent threshold is automatically counted by the WASP, the circle dot represent
the averaged fluorescent baseline, and the cross mark is the fluorescent threshold for each
detection channel. During the data loading, particle which has fluorescence emission
lower than the corresponding threshold will not be count as fluorescent.

![Figure 2.4 WASP control panel](image)

According to the forced trigger fluorescence and calculated threshold of the field
measurement, it shows clearly that the channel F2 and channel F3 have the greatest
variability, whereas the channel-F1 was rather stable. After determining the threshold for
all three channels, the WASP can load the CSV files and process the data.
As discussed in Section 2.3, the data analysis functions provided by the WASP were still very general. Separating fluorescence intensity by 3 channels was not specific enough to characterize biological aerosols. To better characterize ambient fluorescent aerosols, combined fluorescent emission of multiple channels are also considered. Most of the biological aerosols emitted fluorescence in one of the three channels, whereas numbers of particle emits fluorescence in two or three channels. For example, some biological aerosols have fluorescence emission in Channel Fl1 and Fl2 channel, but not in Fl3 channel. Therefore, this characteristic can be considered as a factor to classify the fluorescent particles. And the particle fluorescent emission was characterized into 7 categories. This 7-category analysis provides greater ability to characterize the fluorescent aerosols.

Category analysis

At present there is no existing data analysis program that can achieve this category separation automatically, so this analysis was performed by manual separation using Microsoft Excel. The WASP panel was used to load the data, in order to combine
numbers from individual WIBS data CSV files into one time series. This was then directly copied into a Microsoft Excel file. Fluorescent thresholds were calculated from the forced trigger file (see Section 1.4). To separate fluorescent categories the “advanced filter” function was used. In order to extract the AB category data, the filter function was used to select the particles have Fl1 and Fl2 that above their threshold, and Fl3 below the threshold. It takes time to use this method, because most of the commands and calculations need to be done manually, however, it is a reliable method and each of the steps is straightforward. In addition there are no software issues that need to be addressed for this method.

Figure 2.6 Worksheet for separate category AB

Figure 2.6 shows an example how to setup the filter function. The blue highlighted area is the raw fluorescence data for a single particle; they will be filtered by the criteria, which is the dashed line box. Note that the criteria for each fluorescent channel are not in the same row, which means the filtered data should match “greater
than threshold 1, greater than threshold 2, less than threshold 3” these 3 conditions at the same time.

2.2.4 Ice nuclei particle measurements

The micro-orifice uniform deposit impactor-droplet freezing technique (MOUDI-DFT) is an ice nuclei concentration measurement technique developed by the group of Allan Bertram at the University of British Columbia. The IN measurements was performed by Ryan Mason and Meng Si from the UBC.

Over the past few years, techniques for measuring the ice nuclei particles concentrations have changed rapidly. Many techniques aim to measure the IN concentration in real-time. These techniques typically provide the number concentration of IN smaller than a cut-off size that are active at a certain temperature. However, characterization of IN as a function of size could is also important, especially to provide improved inputs for modeling the transport of IN within the atmospheric system. Additionally, size-resolved measurements may be useful to determine if some current techniques for measuring the total concentration of IN miss some fraction of ice nuclei concentration. For example, instruments based on the continuous flow diffusion chamber (CFDC) design of Rogers et al.\textsuperscript{38} limit the size of particles to ≤ 2.4µm in most cases, whereas many classes of potential IN are much larger in size. Some traditional size resolving techniques to measuring the concentration of IN involve either particle size-selection by inertial separation\textsuperscript{67} or by filtration\textsuperscript{67} and followed by freezing
measurements. These freezing techniques usually have a temperature limitation of −25°C or higher, and the performance of size resolution was also limited by filters.

![Image of laboratory setup](image)

Figure 2.7 The laboratory setup of droplet freezing technique apparatus (Univ. BC). Photo courtesy of J. A. Huffman

To accurately measure the ice nuclei particles with better size resolution, Huffman et al.\(^7\) recently introduced the micro-orifice uniform deposit impactor-droplet freezing technique (MOUDI-DFT) for measuring the concentration of IN as a function of size\(^67\). The MOUDI is a cascade impactor for high accuracy aerosol sampling to collect size-fractionated particulate samples for chemical analysis. It contains a sample inlet to limit the size of the input particles. For this field measurement, MOUDI with six collection stages spanning a size range of 0.3-10.0 µm, and an outlet filter to collect remaining particles was used. There is a nozzle plate on each stage to direct the sample and an impaction receiver upon which substrates are located for collecting particles. Hydrophobic 50 mm glass cover slips were used as the collection substrates. Particles collected by the MOUDI were analyzed for their ability to act as ice nuclei in freezing droplet state. The droplet freezing technique uses a flow cell with temperature and
humidity control to convert the collected aerosols to freezing droplets which can then be analyzed by a microscope Charge-coupled device (CCD) camera. The structure of the cell and the flow path design are illustrated in Fig 2.7.

The sampling time was separated into day/night cycle and each exampling period takes 8-10 hours. This setup is optimized to collect enough aerosols and characterize them by diurnal activity. The details of sampling period for each IN experiment can be found in Appendix B.

2.2.5 Atomization measurement: Sampling Sea Microlayer Water

One important hypothesis of this field measurement is to find the relationship between seawater and marine aerosols. Various evidence shows that the biological content of aerosols in marine environment was from the ocean microlayer, brought into the atmosphere by oceanic activities. The microlayer samples were collected on continuous 3 days, at 20 different positions around the measurement site. To atomize the sample seawater, a 6-jet collision nebulizer with approximately 7 L/min inlet pump flow rate was used to generate the aerosols. Then the airflow was dried in the diffusion drying tube connected to an open end multi-branch path to deliver into different instrument for parallel measurement. If the hypothesis was correct, some common factors (size, fluorescent emission) should be found in both ambient aerosols and atomized seawater. However, by checking our result, we found that the background noise cannot be efficiently reduced by the diffusion drying tube and these signals totally block the signal of biological aerosols for both WIBS and MOUDI. One reason is the silica gel drying
agent was partially crashed during transmission and they generate a lot of particles to cause the noise. Hence we were unable to characterize the biological aerosols and active ice nuclei particles from the microlayer seawater.
Chapter 3 Data analysis and discussion

3.1 General Analysis and discussion

Sampling was initiated August 6, 2013 and terminated August 28, 2013. The WIBS was operated almost continuously for 3 weeks. From August 18 to August 20 there was a 48 hour pause because of the atomization experiment.
Figure 3.1 Time series plots for particle concentration: (A: All particle; B: any fluorescent particle; C: Fl1 fluorescent particle; D: Fl2 fluorescent particle; E: Fl3 fluorescent particle; F: non-fluorescent particle)
Figure 3.1 is the time series plots for six different particle categories. The y axis is the particle concentration and the x axis is the measurement date. From these plots it is clear that the number concentration change along with time of fluorescent particle is quite different from the total particle. Concentration of total particle is approximately 1000 liter\(^{-1}\) average in 21-day measurement, and the fluorescent particle is approximately 80 liter\(^{-1}\) concentration. Hummel et al. use fungal spore simulate the bioaerosol and the concentration study applied was 26 liter\(^{-1}\), as the average level of central Europe\(^{68}\). Therefore, bioaerosol in this coastal region seems richer than other continental regions. There is also expected interference for the fluorescent measurement. Although one reason that to select this measurement site is because this rural region has relatively few human activities and pollution sources. However, the transportation activity, such as ships, cars, helicopters is still a potential source to generate soot, and mineral dust may be transported from farther away.

There are several high peaks that can be observed between 8/9 and 8/16 and the intensity of these peaks is similar. The concentration of total particles and fluorescent aerosols shows a similar trend between 8/19 and 8/22; from the plot there are 3 major peaks at this time. The change in fluorescent aerosols shows regular up and down wave after 8/24, although in this period the average particle concentration for fluorescent aerosols is higher than the beginning period, we don’t see any significant change for fluorescent aerosols. For total particles, there are two peaks in the last couple days: A major peak on 8/26 night to 8/27 morning and a lower peak on 8/27 noon. It is notable
that the higher peak drops very fast on 8/27 morning, and the fluorescent aerosols have a same significant drop at the same time.

The concentration trend for each fluorescent channel is very similar to that of the aerosols in the “any fluorescence” category. We have to look for some other data analysis method to relate the trend of biological to IN activity (See category analysis in section 3.2). The designation non-fluorescent particle is the difference between total and fluorescent particles. In this dataset particles exhibiting any fluorescence represent approximately 2.5% of the total aerosol concentration, so the trend of the non-fluorescent aerosol is similar to that of the total aerosol concentration. By analyzing the time series plot, we can see some a regular pattern of peaks and valleys. Figure 3.2 and 3.3 shows an image plot of total particle and biological particles highlighting the obvious diurnal trends over the course of the campaign.

![Figure 3.2 Time series image plot for total particle size and concentration](image1)

Figure 3.2 Time series image plot for total particle size and concentration

![Figure 3.3 Time series image plot for any fluorescent particle size and concentration](image2)

Figure 3.3 Time series image plot for any fluorescent particle size and concentration. Particle concentration shown as z-axis color scale. The measurement gap between 8/16
and 8/19 was caused by the instrument being taken offline for laboratory experiment; other small breaks were for forced trigger tests.

The particle size distribution is different for all particles and for fluorescent aerosols. The peak in the total aerosol concentration is typically at diameter < 1 µm, whereas the fluorescent aerosol peaks typically in the size range of 2.0-6.0 µm with occasional peaks in the < 1 µm size. The concentration modes of the total particle are relatively evenly distributed in time series, this regular pattern of dips/peaks present a diurnal effect of the particles.

Figure 3.4 Time series diurnal plot for total particle concentration (19 days average). Cross mark shows mean value, horizontal line shows median value. Top and bottom of box shows 75th and 25th percentile values, respectively and vertical lines show 10th and 90th percentile values.

Figure 3.5 Time series diurnal plot for any fluorescent particle concentration (19 days average)
The fluorescent particles show a more obvious diurnal trend than the total particles, though the concentration of the fluorescent particles is much lower than the total. One reason for the diurnal pattern is the regular influence of biological patterns influenced by local environment conditions. Figure 3.4 and 3.5 shows the averaged diurnal activity for total and fluorescent particles. From Figure 3.6, one can see that the fluorescent aerosols show a 19 days average diurnal pattern. The concentration reaches a peak in the early morning and the lowest concentration in the late afternoon. Similar diurnal trend have been observed previously and attributed to a mix of regular biological activity and atmospheric mixing and dilution due to varying boundary layer height\textsuperscript{7,10,23}. To investigate factors influencing the diurnal aerosol pattern, we compared the diurnal aerosol trend with that of air temperature, relative humidity (RH), and wind direction. The diurnally averaged air temperature exhibits a minimum of 12.9°C at 4:00 local time (LT) and a diurnal maximum of 14.1°C at 17:00 on 14.1°C. The average RH was approximately 60% for most of the day (2:00 pm) and increased regularly to approximately 90% at night (2:00 am). However, the wind direction did not show an obvious diurnal effect. The total particle concentration shows an opposite trend to that of...
the fluorescent aerosol. It reaches a peak in the afternoon, with the lowest concentration in the morning. This is likely because the main components of total particles are non-biologicals like black carbon, soot, or other secondary organic aerosols (SOA) caused by human activity. SOA peaks in the afternoon as a function of photochemical activity.

3.2 Fluorescent particle link to ice nuclei

Measurement of ice nuclei number for each MOUDI sample was provided by Ryan Mason at the University of British Columbia. To investigate the possible relationship between the fluorescent aerosol and IN we correlated the average fluorescent aerosol concentration for the period associated with each sample. We did this for each of the categories of aerosol fluorescence (Section 2.2.3) to achieve a slope and R2 value for linear fits between the variables. R^2 is a statistical value, defined as from 0 to 1, that evaluates how strongly one variable is correlated with a second variable. A value of 0 indicates no correlation and a value of 1 indicates a perfect. According to Dancey and Reidy’s (2007) classification, correlation values from 0.7 to 0.9 are considered strong, 0.4 to 0.6 moderate, and 0.1 to 0.3 are considered weak. The values associated with strong and weak correlations, however, are also a function of the type of data being correlated.
Figure 3.7 Linear fitting curve for 0.5-18µm averaged IN particles vs fluorescent particle (x error: uncertainty of IN concentration. Y error: standard deviation)

Figure 3.8 Linear fitting curve for 0.5-18µm IN particles vs non-fluorescent particle with error bars

Figure 3.7 and 3.8 show the results of this analysis. WIBS fluorescent particle data were time-averaged to match the MOUDI sampling period for each experiment. Thus, each data points represents the 8-10 hours sampling time for both IN and fluorescent particles. The correlation between IN number and fluorescent aerosol shows a relatively good linear relationship ($R^2=0.69$), whereas the linear coefficient for IN versus non-fluorescent particle is much lower at 0.152. The slope of the graph in Figure 3.5 is 0.02, which are 4 times that of the graph in Figure 3.6. This suggests that not only is the relationship between IN and fluorescent aerosol stronger than with the non-fluorescent
aerosol, but that the fluorescent particles that can serve as IN do so with much higher efficiency.

The next step was to look at the slope and $R^2$ value for fluorescent particle within different size ranges. Comparing the relationships at the different sizes (see Table C in Appendix C), one can see the linear relationship becomes weaker as particle size increases. Correlations with other MOUDI stages and as a function of different fluorescence categories are shown in tables in Appendix C.

Fluorescent category-resolved correlations with IN number from MOUDI stage 3 (0.56-1.0µm) are in Figure 3.9. The y-axis keeps shows the same number concentration in each plot, while the x-axis changes the fluorescent particle category depicted.
Figure 3.9 Categorized and size-resolved fluorescent particle vs size-resolved IN for MOUDI stage 3 (0.56-1.0µm) particles with error bars

Stage 3 collected the smallest particles (0.56-1.0µm), which correlated best with the fluorescent aerosol concentration ($R^2$ at 0.15-0.59). The best linear correlation for IN is with ANY fluorescent with $R^2=0.594$, whereas the AC category showed the lowest $R^2=0.169$. Category-resolved data show a higher $R^2$ value in smaller sizes bins of 0.5-1.8
µm. Except for any fluorescent category, category B ($R^2=0.513$) usually have the best linear dependence. Categories B and BC ($R^2=0.403$) have greater number concentration than others; this may be a reason for the good correlation. Category A, AB, ABC shows similar particle concentration, but the correlation of AB ($R^2=0.154$) is not as good as A ($R^2=0.300$) and ABC ($R^2=0.430$). The category AC shows the lowest number concentration and a low $R^2$ value in common. By comparing the correlations between fluorescent particle and IN, one can conclude the concentration of IN is significantly related to the activity of fluorescent particles. It should be noted the category AC has the best the slope value. Other than this, category ABC is exemplary because of the relative high $R^2$ and slope.

Figure 3.10 shows bar plots of IN and categorized fluorescent particles. Each plot corresponds to one ambient sampling period. These plots are useful for studying the distribution of fluorescent particles based on category characterizing. For example, it is notable that for 0.5-1.0 µm particles, category B and BC occupy the majority population in general. The occupation of B and BC becomes less with larger size. From 2.0-4.0 µm range, category AB and ABC are the largest population, whereas BC is significantly reduced at this size range.
Figure 3.10 Bar-plot for category and size resolved fluorescence along with ice nuclei concentration for sampling period. The x axis is the size distribution and y axis is the number concentration. For each size bin, 7 fluorescent categories are shown in the stacked bar plot, and each category has its own color code. The IN concentration is a rhombus mark. (A: Day 5 daytime; B: Day 10 daytime; C: Day 10 nighttime; D: Day 18 nighttime)

Figure 3.10 A-B are day time measurements. One can see the category B and BC is the major fluorescent category. Figure 3.10 C-D are night time measurements, the category distribution is different than daytime’s. Category AB has considerably higher population for the night. Note these four bar plots are randomly selected, so it merely provide a general idea about the fluorescent category distribution. Some individual experiment period may have discrepancy.
Chapter 4 Conclusion

As a part of the NETCARE project, we participated in a field campaign near the town of Ucluelet on the rural west coast of Vancouver Island in British Columbia, Canada in August 2013 to measure the number concentration and size-resolved properties of fluorescent particle. A WIBS-4 instrument was employed to characterize particles in real time. We worked with the Bertram group from the Chemistry Department at the University of British Columbia who collected particles via cascade impaction and analyzed their size- and temperature-resolved ice-forming properties. The study was designed to measure the bioaerosol and IN in the coastal region, and find the corresponding relationship between these factors. We investigated the total fluorescent particles and IN particles, as well as size resolved particles. In addition, by applying the different combination of 3 fluorescent channel data, a 7-category resolved fluorescent particle data was used to better characterize PBAPs and their possible link to and role as IN.

By general observation of the results, it can be seen that the number concentration of total particle and fluorescent particle is very different. For the 21-day average, total particle concentration is about 1000 liter\(^{-1}\), whereas fluorescent particle is approximately 80 liter\(^{-1}\) (8% of total). One can also see that fluorescent particle concentration reaches
their peak concentration at early morning and a minimum concentration in the afternoon. This trend is similar with other studies which suggested that the diurnal release patterns of certain fungal spore aerosols may partially explain this observation. The concentration trend of total particle is almost opposite to that of the fluorescent aerosols, with concentration lowest in the early morning and highest in the afternoon.

By compare with total fluorescent particles and IN, relatively good linear correlation was observed, with correlation coefficient \( R^2 \) of 0.6. This result suggests that for the measurement period, the fluorescent particle are strongly related to the IN, and further that biological aerosol may be acting as a good source of IN in this region. In addition, the fluorescent particle data was size resolved to match the MOUDI data. The result shows the smaller fluorescent particles versus IN to have greater \( R^2 \) values than larger ones. However, it should be noted that the counting efficiently of the WIBS is reduced at the size range between 0.5-0.8 µm and so comparisons are relatively more uncertain. By comparing the fluorescent categories in same size range, it consequently shows that any fluorescent category has the highest \( R^2 \) value. Fluorescent categories which contain B type emission (e.g. BC, ABC) usually have the higher \( R^2 \) value than those ones do not have a B type emission, especially for category BC. Category AC has the lowest \( R^2 \) value and number concentration in common. Such a finding points towards the fluorescent particles, as a proxy of bioaerosols, have been seen to be efficient IN source. And there is possibility that the certain fluorescent particle could be potentially strong related to IN. The other purpose of the field measurement was to determine whether marine aerosol can serve as an efficient source of IN. Although the importance
of PBAPs has been recognized, the sources of biological aerosols can sometimes be difficult to identify using current instrumentation. This is because the source of the bioaerosols is broad and hard to identify. The current understanding of biological aerosols has been influenced by culture- and DNA-dependent, but the study of physical and chemical properties is developing rapidly. Even so, a lack of comparison between biological aerosols, IN, and CCN remains. So there is a current limitation of understanding the source, transformation, transmission, dissemination and climate effect of PBAPs.

In further study, the following problems are still need to be explained.

1. PBAP influence on the atmospheric quality and global climate change. As a unique class of the organic aerosols, there is a potential impact for biological aerosols on physical and chemical processes and global climate change.

2. Sources, sinks and transformation of PBAP. Different sources of biological aerosols make them have a diverse composition. They can be transmitted in long-distance and have some conversions occurs during the transmission.

3. An index that evaluates the effect of bioaerosol on air quality. At present there is not a relevant provision of bioaerosol exposure levels. For a more comprehensive, systematic evaluation of air quality, protection of human health effects, the study of biological aerosols on air quality and to develop appropriate evaluation is necessary.

4. Relationship with humans and plant epidemic diseases. PBAP diffusion in the atmosphere can cause the spread of acute and chronic diseases in humans as well as
animal and plant diseases. For example, case of the Severe Acute Respiratory Syndrome (SARS) in 2003 and Middle East Respiratory syndrome coronavirus (MERS) in 2015. There is a controversy about whether the virus can be transmitted as droplet nuclei or in an aerosol forma because of the lack of relevant research on the relationship between biological aerosols and epidemiology.

5. Spatial and temporal distribution. Several researchers have shown different types of PBAP have different concentrations and temporal distribution patterns in the atmosphere, but it’s still lack the global distribution information.

6. Impact of human activities on the formation and evolution of PBAP. With the developing usage of microbial technologies like bio-pesticides and microbial treatment of waste water, the biological aerosols caused by human activities is increasing fast in numbers and composition. How are these aerosols linked to ice nuclei and climate change?
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below a tropical forest canopy using a dual channel fluorescence spectrometer. *Atmospheric Chemistry and Physics* 2010, 10 (10), 4453-4466.


Appendix A List of frequently used acronyms

A  Corresponding particles fluoresce in channel F1 for category analysis
B  Corresponding Particles fluoresce in channel F2 for category analysis
C  Corresponding Particles fluoresce in channel F3 for category analysis
AB Particles fluoresce both in F1 and F2 at the same time
AC Particles fluoresce both in F1 and F3 at the same time
BC Particles fluoresce both in F2 and F3 at the same time
ABC Particles fluoresce both in all three channels at the same time
Fl1 Particles in WIBS fluorescent channel 1
Fl2 Particles in WIBS fluorescent channel 2
Fl3 Particles in WIBS fluorescent channel 3
IN Ice Nuclei
PBAP Primary biological aerosol particle
PSL Polystyrene Latex
UVAPS Ultraviolet Aerodynamic Particle Sizer
WIBS Wideband integrated bioaerosol sensor
WASP WIBS data analysis program
## Appendix B MOUDI sampling time for Ucluelet Field Study

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Appendix C Linear $R^2$ values for size resolved IN and fluorescent particles

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Appendix D Logical criteria for selecting fluorescent category

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Appendix E Lab Instrument Study: Ultraviolet Aerodynamic Particle Sizer

Early in my masters work I performed experiments using a different set of instruments in the laboratory. These experiments are briefly described here.

E.1 Introduction

Ultraviolet Aerodynamic Particle Sizer (UV-APS) is an advanced aerosol detecting device based on fluorescence spectroscopy principle\textsuperscript{66}. The system can separate air aerodynamic diameter between 0.5 and 15 μm microorganisms and ordinary dust particles and detect their concentration and size distribution in less than a minute. The UV laser system was designed to detect single particle fluorescence emission based after being trigger by particles above a certain aerodynamic diameters\textsuperscript{66}. As one of the earliest commercially available online aerosol instrument, the UV-APS was developed to characterize PBAPs\textsuperscript{71}. Among many early deployments, Huffman et al. used the UVAPS to detect the biologicals aerosols in Mainz, Germany in 2010 and the results showed the size distribution of bioaerosols have a clear diurnal cycle\textsuperscript{25}. The strongest fluorescent aerosol signal is at early morning before sunrise, particle size around dominant peak is 3–4 μm, which could be attributed to fungal spores or agglomerated bacterial cells.
The fluorescent sensitivity can be changed by the UV laser power settings and the PMT voltage gain settings. The UVAPS operates with an external computer with controlling software that offers instrument control and limited data analysis capabilities. In atomization experiment for particle characterization or instrument calibration, the physical geometry of the input path is another important factor that influences the detection performance. For example, the detection of particle concentration of vertical input path is greatly higher than the horizontal input path. The principle of atomization delivery path design is to make it smooth and fluent. The air flow rate should not encounter any bumping or resistance.

E.2 Experiment Setup

For atomization experiments, the aerosols were generated by a 6-jet Collison nebulizer (BGI, Inc.). It uses an oil-free air pump to generate 6 L/min air flow. Aerosolized particles were passed through a water trap and diffusion dryer in order to remove moisture, after which they were delivered into the instrument via electrostatic-free tubing.

Figure E.1 Collision Nebulizer (left) and Silica-filled Sorbent Drying Tube (right)
A different atomization apparatus was also tested in the lab. The aerosols were generated by an electronic spray aerosol nozzle, which applies a high frequency vibration onto droplets to break it into aerosols. It uses a syringe pump with 2.0 L/min liquid flow rate to apply droplets. Aerosolized particles were dried by an electronic heat drying tube, and then delivered into the UVAPS.

![Figure E.2 Syringe Pump (left), Wide Spray Atomizer Nozzle (middle), and Electricity Drying Tube (right)](image)

E.2.1 Aerosols

E.2.1.1 Polystyrene Latex (PSL) Particles

The aerosols were generated using monodispersed Polystyrene Latex (PSL) particles with diameters of 0.53 µm, 2.01µm, 5.94 µm, 9.98 µm and Green Fluorescent Microspheres with a diameter of 0.50 µm. All particles have a density of 1.05 g/cm³ that corresponded to aerodynamic equivalent diameters of 0.60 µm, 2.1 µm, 6.2 µm and 10.5 µm, and 0.53 µm, respectively. Aliquots of stock suspensions were diluted with sterile distilled deionized water (SDW) to provide suspensions with the concentrations in order of $10^7$ particles/ mL.
E.2.1.2 Pollen aerosols

It has been generally believed that due to its relatively large size, pollen cannot reach the periphery of the lung, and thereby induce an asthmatic attack. Mechanisms have recently been discovered which explain how allergens housed in large pollen grains can trigger asthma. Study has been established that the pollen, produced during flowering in rye-grass, mostly remains on the open anthers in the absence of wind or other physical disturbances. If wetted, rye-grass pollen can rupture within minutes. Fragmented cytoplasm is emitted through the pore region of the pollen grain. Drying winds release this cytoplasmic debris directly from the flowers as a respirable allergen-loaded aerosol. The implications of this work are that pollen allergens can be contained in respirable aerosols after moist weather, and these aerosols might deposit into the lower airways where they would be a potent asthma trigger. Pollen grains rupture and the formation of an aerosol of allergen-laden debris of respirable size (less than 2.5 microns in diameter) is common to all the highly allergenic plants so far examined. In this experiment we put the pollen samples into the water to make them ruptured (water-culture)

Pollen test aerosols were generated using two types of microorganisms: Paper Mulberry (~11μm) and Brassica Napus (~23μm). These microorganisms are commonly found in the ambient air environment and were selected to represent pollens that are robust or sensitive to environmental stresses.
E.3 Results and Discussion

PSL with 0.60 µm, 2.1 µm, 6.2 µm and 10.5 µm diameters were tested under the same collision nebulizer setup. The size calibration curve is shown below:

![Calibration Curve of UV-APS](image)

Figure E.3 UVAPS size calibration curve

As was discussed earlier, the sensitivity can be adjusted by setting two parameters: the gain of the fluorescence sensing PMT and the UV laser pulse energy. In accordance with the instrument instruction manual, when the fluorescence sensitivity is set high, the UVAPS internal fluorescence baseline will result in measured fluorescence even from particles that do not contain fluorophores. Thus, the instrument fluorescent baseline should be occasionally checked using non-fluorescent test particles.

The three-dimensional spectra of the aerosols are presented in Fig 1. The particle aerodynamic diameters are presented in 52 size channels, with the respective concentrations recorded in number of particles per cubic centimeter. The fluorescence intensity is presented at 64 channels representing gradually increasing fluorescence intensity of the particles. Data on non-fluorescing particles are presented in the first channel and the data on fluorescing particles in channels from 2 to 64. Total particle
concentration, therefore, corresponds to the sum of particle concentrations from channel 1 to 64 and should be equal to the total particle concentration obtained in the APS mode (spectra are not shown). Nevertheless, the APS data were always higher than the corresponding data recorded in the UVAPS mode.

Figure E.4 0.5 μm PSL at svp-429 (left) and svp-550 (right)

Figure E.5 Paper Mulberry at svp-429 (top) and svp-550 (bottom)
For comparative tests, the pollen aerosols were simultaneously sampled with the UVAPS (for monitoring both total and fluorescent particles) and the collision nebulizer. Paper Mulberry (~11μm) and Brassica Napus (~23μm) pollen with fresh and 3 day water-cultured atomization sample are tested. Vacuum filtration was also tried to remove the concentrated super small particles (less than 1 μm) in order to reduce background noise. Though the UVAPS measures pollen particles rather than individual microorganisms and water-culture is known to break particles and, thus, measure ruptured pollens, it was appropriate to compare their data since the majority of the airborne pollen aerosols generated by Collision nebulizer were single microorganisms, which was evident from the size distribution of the aerosols.

Brassica Napus

Figure E.6 Water and Fresh Filtrate of Brassica Napus
Brassica Napus has better ruptured grade than Paper Mulberry. And this shows better ruptured solutions have more obvious fluorescence intensity. Particles have about 2 microns size shows larger concentration in unfiltered samples than filtered samples. And particle around 2 microns also show increase the concentration in three days.
The disrupted pollen solution has greater fluorescence intensity. Most particles around 0.9 microns are caused by something outside of pollens that can be washed away by filtration. The filtered pollen shows a little bit cleaner graph with less small particle noise. By just look at the UVAPS results, Paper Mulberry doesn’t have a good disruption after 3 days in water. And there is not a significant fluorescence emission difference between fresh and disrupted pollen. To compare with water, filtrate has a large numbers of particles around 2 microns. But there’s nothing showed under the microscope.
Pollens have different rupture properties and there’s soluble substance outside of pollens. Most of the soluble substance and ruptured substance concentrate at 0.7-2.0 μm. UV-APS is not an ideal instrument to quantitative analysis and characterization ruptured pollens. In contrast, the PSL particles started to produce fluorescence at the settings close to the instrument default conditions (PMT gain at 500 V and UV laser pulse energy at 50 %). The PSL particle size was found to have no effect on the instrument settings.
Appendix F Cluster Analysis: Method, Results, and Discussion

A new method to characterize the biological aerosols is called cluster analysis. This method is to separate large amounts of data into several clusters based on the five different measurements that WIBS provides of each particle, which is used in subsequent analyses herein: optical size, asymmetry factor, and three fluorescence measurements. The detailed introduction of the cluster analysis for WIBS can be found in the study of N. H. Robinson et al.\textsuperscript{74}

The operation of the cluster analysis is performed by the WASP. The initial loading procedure is the same as regular analysis. After loading the forced trigger and measurement data, WASP can do a single particle distribution (SPD) calculation. This procedure acquires every single particle’s information so that the software can do the subsequent analyses. There are two obvious issues for this step: 1) the calculation capacity of the Cluster analysis is 10,000 single particles. So for Ucluelet data, I have to only load 0.04% of SPD data for cluster analysis. 2) Calculating SPD for Ucluelet data takes at least 8 hours.

After loading SPD data, it needs to be checked that the total particle number is less than 10,000 by clicking the “view input stats”. Use “do cluster analysis” function to
start calculations of all possible combination to make clusters. This calculating process takes about 15 hours for analyzing the Ucluelet data, the progress can be found at left bottom corner. However, it’s inconvenient that the WASP does not provide a warning message after done of the calculation.

After finishing cluster analysis, click “Calc stats” for plotting the graph with number of clusters, their corresponding root mean square and $R^2$ value. Note this function lets the user select how many clusters to separate the data into. Commonly 6-20 cluster solutions were used in this thesis. However, there are not a suggested number of clusters. A user can separate as much as needed.

![Figure F.1 A 20-cluster solution stats plot](image)

This figure shows a 20 cluster solution for Ucluelet data. According to Robinson, it is to select the concomitant drop in $R^2$ and N, and the rise in root mean square (RMS); it shows that a 15 cluster solution is suggested. “View solution” is a function to let the user quickly check the particle number concentration for each cluster. And “assign remaining” is the last step; this procedure lets the user plot the selected cluster back to the time series plot. Note this procedure will take another 10+ hours to process.
To show the result, I loaded all 15 clusters. The graph below shows the time series plot of total, fl, and cluster 1 particle.

![Time series plot for total, fluorescent particle and cluster 1](image)

**Figure F.2 Time series plot for total, fluorescent particle and cluster 1**

It is very obvious that the cluster 1 mostly follows the trend of total particles. So cluster 1 is not an effective separation. Note that however many cluster solutions the user selects; the cluster 1 is always similar with total particles. This is either because of some mathematical mistake or program error.

![Time series plot for cluster 1, 2, and 3](image)

**Figure F.3 Time series plot for cluster 1, 2, and 3**

The figure above is cluster 1, cluster 2, cluster 3 time series plot. One can see the number concentration drops fast with different clusters. The time series plot for each
fluorescent channels can be found in chapter 3. The next plots show the cluster 2 and cluster 3 with fluorescent channels respectively.

![Figure F.4 Time series plot for Fl1, Fl2, Fl3 channel and cluster 2](image)

![Figure F.5 Time series plot for Fl1, Fl2, Fl3 channel and cluster 3](image)

The average number concentration of cluster 2 is significantly higher than any fluorescent channels, whereas the number concentration of cluster 3 is lower than the fl3, which is the lowest channel with number concentrations. This tells me the cluster 2 is still a separation from the total particles. And after cluster 3 it starts to separate from the fluorescent particles.
Cluster 3, 6, and 9 have relatively higher number concentration than other clusters (expect cluster 1 and 2). Cluster 3 was selected as a standard to compare with other clusters.

Figure F.7 shows cluster 3 with cluster 4, 5, 7, 8. Looks like these four clusters are in the same number scale. But their activities are different to each other.
Figure F.8 Time series plot for cluster 3, 10, 11, and 12

Figure F.9 Time series plot for cluster 3, 13, 14, and 15

Cluster 10-15. Cluster analysis is stopped at the time series analysis. Because: 1) The mechanism for separating clusters is not clear, not sure how well each cluster can present the real bioaerosol activity. 2) Uncertainty, one can see from cluster 3-15 each cluster has its unique activity trend, but I don’t know what causes the difference. Also, the use of different cluster number solutions gives completely different results. 3) Time consuming. To complete the whole cluster analysis method usually takes 18+ hours. Here are the questions that I encountered during the cluster analysis:

1. The 10,000 limitation is a very critical shortage. As I mentioned in the operation part, only 0.04% of particle information can be load for cluster analysis. How can
we tell this 0.04% amount of particles present can present enough information of the area? Additionally, if there were longer duration measurements, or aerosol-rich environment measurements, does cluster analysis provide the information precisely?

2. The mechanism of the cluster is still not clear, or at least cannot be found during the WASP cluster analysis. For example, if I chose an 8-cluster solution and generate 8 category of biological aerosol, how do I identify the central information of each category? If all 8 clusters were separated by size, do each cluster’s particles shares similar diameter? Or are the clusters using different information for grouping, like one cluster shares the fluorescent information and another cluster shares similar AF?

3. Time consuming. Load measurement data, load SPD data, and calculate the possible cluster solution, each of these procedures takes 6-8 hours for Ucluelet data analysis.

4. Theory. The unique technique for online aerosol measurement is to gain the information of the single particles. However, the cluster analysis method is still performing the average analysis, which is same as the traditional offline method. Is this correct?

Cluster analysis may be a good potential analysis method for separating WIBS data. It provides some interesting idea for better characterizing the fluorescence data based on the optical size, asymmetry factor, and three fluorescence emissions. However, the current state of the cluster analysis on WIBS is not very successful. The main issue is
that too little particle data can be loaded, and it’s not sure this amount of particles contains enough information to can precisely present the bioaerosol activity. In addition the analysis procedure is very time consuming. I would suggest adding a message function for the WASP, that use some sound and pop-up window to remind the user analysis is done. And at this point, the data analysis method is not suitable for long-time, massive data analysis. Information provided by cluster analysis is a sort of the averaged particle data. But compared with the traditional agar collection, and offline average analysis, its data pool is too small. Therefore, the current study of using cluster analysis for WIBS data is still early. It needs to be improved on both theoretical and software-developing sides.