



Autofluorescence of atmospheric bioaerosols – fluorescent biomolecules and potential interferences

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Abstract. Primary biological aerosol particles (PBAP) are an important subset of air particulate matter with a substantial contribution to the organic aerosol fraction and potentially strong effects on public health and climate. Recent progress has been made in PBAP quantification by utilizing real-time bioaerosol detectors based on the principle that specific organic molecules of biological origin such as proteins, coenzymes, cell wall compounds and pigments exhibit intrinsic fluorescence. The properties of many fluorophores have been well documented, but it is unclear which are most relevant for detection of atmospheric PBAP. The present study provides a systematic synthesis of literature data on potentially relevant biological fluorophores. We analyze and discuss their relative importance for the detection of fluorescent biological aerosol particles (FBAP) by online instrumentation for atmospheric measurements such as the ultraviolet aerodynamic particle sizer (UV-APS) or the wide issue bioaerosol sensor (WIBS).

In addition, we provide new laboratory measurement data for selected compounds using bench-top fluorescence spectroscopy. Relevant biological materials were chosen for comparison with existing literature data and to fill in gaps of understanding. The excitation-emission matrices (EEM) exhibit pronounced peaks at excitation wavelengths of ~280 nm and ~360 nm, confirming the suitability of light sources used for online detection of FBAP. They also show, however, that valuable information is missed by instruments that do not record full emission spectra at multiple wavelengths of excitation, and co-occurrence of multiple fluorophores within a detected sample will likely confound detailed molecular analysis. Selected non-biological

materials were also analyzed to assess their possible influence on FBAP detection and generally exhibit only low levels of background-corrected fluorescent emission. This study strengthens the hypothesis that ambient supermicron particle fluorescence in wavelength ranges used for most FBAP instruments is likely to be dominated by biological material and that such instrumentation is able to discriminate between FBAP and non-biological material in many situations. More detailed follow-up studies on single particle fluorescence are still required to reduce these uncertainties further, however.

1 Introduction

1.1 Primary biological aerosol particles

Primary biological aerosol particles (PBAP), also referred to as bioaerosols, are a diverse collection of small pieces of material emitted directly from the biosphere into the atmosphere (Després et al., 2012). They are globally ubiquitous, in some cases can dominate suspended particle concentrations, and comprise a diverse selection of particle types, including: whole organisms (e.g. bacteria, algae), reproductive entities (e.g. pollen, spores from fungi, bacteria, ferns, mosses), biopolymers (e.g. DNA, chitin, cellulose and other polysaccharides), plant debris, insect parts, and decaying biomass (e.g. Gregory, 1973; Madelin, 1994; Cox and Wathes, 1995; Andreae and Crutzen, 1997; Jones and Harrison, 2004; Jaenicke, 2005; Pöschl, 2005; Elbert et al., 2007; Jaenicke et al., 2007; Bauer et al., 2008; Pöschl et al., 2010; Després et al., 2012). PBAP can span several orders

of magnitude in particle diameter, from a few nanometers (e.g. viruses) to hundreds of micrometers (e.g. plant debris), making analysis challenging. A number of studies have indicated that PBAP may comprise large fractions of ambient particulate matter (PM) in many environments. Microscopic investigations have shown that PBAP can account for up to ~30 % of fine (<1 μm) and up to ~70 % of coarse (>1 μm) PM in rural and rain forest air, and estimates of PBAP emissions range from ~60 Tg a^{-1} of fine PM to ~1000 Tg a^{-1} in all size ranges (Penner, 1994; Jaenicke, 2005; Elbert et al., 2007). Fungal spores in particular account for a large proportion of PBAP with typical mass concentrations of ~1 $\mu\text{g m}^{-3}$ in continental boundary layer air and estimated global emissions in the order of ~50 Tg a^{-1} (Elbert et al., 2007).

The aerial dispersal and transport of biological aerosols, particularly reproductive material such as spores and pollen, is an important pathway for the spread of organisms within and across ecosystems (Gregory, 1973; Elbert et al., 2007). Bioaerosols may act as giant cloud condensation nuclei (CCN) and ice nuclei (IN) (e.g. Dingle, 1966; Schnell and Vali, 1972; Lohmann and Feichter, 2005; Sun and Ariya, 2006; Möhler et al., 2007; Christner et al., 2008), thus influencing global climate by affecting cloud formation and precipitation. They also play a crucial role in public health by spreading disease to humans (e.g. tuberculosis, influenza), animals (e.g. anthrax, brucellosis) and crops (e.g. rust, smut) and by causing allergies (e.g. Lacey and Dutkiewicz, 1994; Linskens and Cresti, 2000; Brown and Hovmoller, 2002; Douwes et al., 2003; Franze et al., 2005). As a result, quantification of local PBAP concentrations and identification of individual species or classes is essential for diverse scientific fields such as climatology, atmospheric science, human health, crop science and agriculture, biogeography, and others. Within the last two decades, especially, the detection of bioaerosols used as biological warfare agents (BWA) has also become important, both on the battlefield and with respect to domestic terrorism (e.g. Hill et al., 1995; Pinnick et al., 1995; Primmerman, 2000; Ho, 2002; Lim et al., 2005; Manninen et al., 2008).

Existing methods for PBAP measurement have historically been off-line techniques which suffer from poor time resolution (hours to days), requiring costly and time consuming sample analysis (Griffiths and Decosemo, 1994). As a result, much work has been done to report qualitative and semi-quantitative observations of certain ambient bioaerosol classes, but systematic efforts to quantify PBAP as a whole have been limited (e.g. Lighthart and Shaffer, 1995; Matthias-Maser et al., 2000). Counting methods based on cultivation of viable airborne organisms cause certain methodologically difficulties that make comparison with other techniques difficult. For example, the “great plate count anomaly” describes the well known problem that only a small fraction (typically <10 %, often <1 %) of airborne microorganisms form colonies on a typical culture media (Amann et al., 1995; Heidelberg et al., 1997; Chi

and Li, 2007), thus leading to a significant underestimation of the actual viable airborne bioaerosol concentration. The vast remaining number of airborne microorganisms can be described as viable but non-culturable (VBNC), indicating very low metabolic activity or resting dormant state (Roszak and Colwell, 1987). Even non-viable PBAP can be important for atmospheric, biological, and human health concerns, however.

Cultivation methods only attempt to count full, viable (living) organisms, while other physical or chemical techniques are able to selectively analyze non-viable cells or fragments of biological material. In recent years virtually every available chemical, biological, and physical technique of analysis has been applied to the measurement of ambient PBAP, and a full review of these attempts is well beyond the scope of this manuscript (e.g. Spurny, 1994; Ho, 2002; Georgakopoulos et al., 2009; Després et al., 2012; Xu et al., 2011). Mass spectrometry (MS), in its various forms, has been successful at detecting PBAP with high time resolution and detailed chemical identification of analyzed particles (e.g. Parker et al., 2000; Fergenson et al., 2004; van Wuijkhuijse et al., 2005). Among modern microbiological methods, polymerase chain reaction (PCR) and quantitative PCR (qPCR) are promising tools for identification and quantification of cells from a specific organism via identification of sampled DNA, although the techniques are not capable of providing an estimate of the total PBAP concentration (e.g. Després et al., 2007; Bowers et al., 2009; Fröhlich-Nowoisky et al., 2009; Bowers et al., 2011). Epifluorescence microscopy, particularly in combination with the use of fluorescent dyes, has been a frequently utilized technique for PBAP quantification and refers to the microscopic imaging of the distribution of fluorescent compounds in cells or tissue (e.g. Hobbie et al., 1977; Kepner and Pratt, 1994). By staining specific cell constituents (e.g. DNA, proteins, cell walls) PBAP can be analyzed regardless of the metabolic state of the organism, thus overcoming the problem of poor culturability. The commercial availability of a wide selection of fluorescent dyes allows a flexible and selective quantification of many classes of microorganisms (McFeters et al., 1995; Li et al., 2004). The method suffers from the need for time-consuming and, therefore, costly sample analysis, however. Instruments able to discriminate biological content in real-time based on the emission of laser-induced fluorescence (LIF) have recently become commercially available and are among the most promising techniques for ambient atmospheric PBAP analysis (e.g. Pinnick et al., 1995; Hairston et al., 1997; Kaye et al., 2000). While these do not offer the molecular specificity or detailed imaging capabilities of microscopy, many instruments are able to provide an estimate of PBAP properties in real-time, with high time and size resolution (e.g. Huffman et al., 2010).

1.2 Physical principles of fluorescence

Spectroscopy refers to a wide class of analytical techniques where an analyte is selectively interrogated through interaction with a region of the electromagnetic radiation, and has been widely applied to virtually every scientific field, including the measurement of biological aerosols. For the purpose of clarifying arguments later in the paper we present a brief overview of key spectroscopic principles here. Analyte molecules exposed to incident radiation can either absorb or scatter incoming photons. If photons are absorbed or inelastically scattered, energy is imparted to a molecule that can be dissipated either radiatively, non-radiatively, or through a combination of both (e.g. fluorescence, phosphorescence). Molecular fluorescence can be seen mechanistically as an inelastic light scattering process (i.e. energy is imparted to the molecule) with three distinct electronic steps: (I) Photon absorption causes an electron to transition from the lowest vibrational state of the electronic ground state of the molecule to a higher vibrational state of an excited electronic state. (II) This is followed by dissipation of energy through non-radiative decay (heat) and transition of the electron to the lowest vibrational level of the excited electronic state. (III) The electron can then return to the electronic ground state by radiative emission (fluorescence) or through a variety of non-radiative processes (quenching), depending largely on the local molecular environment. Many different quenching mechanisms are possible, such as collision quenching (i.e. by oxygen or water) and concentration quenching (i.e. self-quenching and inner-filtering effects) (Sinski and Exner, 2007). Phosphorescence is mechanistically similar to fluorescence, except that a spin change occurs due to an intersystem conversion in the excited electronic state. This leads to the practical distinction that the timescale of radiative emission for fluorescence (ns) is up to several orders of magnitude shorter than for phosphorescence (μs -ms) (RichardsKortum and SevickMuraca, 1996).

Both excitation (λ_{ex}) and emission wavelengths (λ_{em}) are characteristic for a given fluorophore, and the most likely electron transition pathways for that molecule are determined by the overlap integral of the corresponding vibrational wave functions. Usually spontaneous fluorescent emission is specific for a certain fluorophore and its molecular environment and occurs at relatively narrow, defined λ_{em} . Molecular excitation, however, can occur over a comparatively wide spectral range due to several accessible electron transition pathways, but is still an important piece of information for fluorophore assignment. The spectral difference between λ_{ex} and λ_{em} is referred to as the Stokes shift ($\Delta\lambda_{\text{Stokes}}$). An important feature of fluorescence is the independence of λ_{em} with respect to λ_{ex} which means that the qualitative appearance of the emission peak is similar for different excitation wavelengths. This is due to the fact that all non-radiative decay processes end in the lowest vibrational level of the first excited electronic state from where fluorescent

relaxation occurs. For further details see Lakowicz (1999), Kunnil (2005) and Ramanujam (2006).

The fluorescence properties of a molecule can be characterized by parameters of steady state fluorescence spectroscopy, such as λ_{ex} and λ_{em} or the quantum yield Φ (fluorescent emission events per photons absorbed). Time resolved measurements also analyze the lifetime τ of the excited state (average time the electron spends in the excited electronic state until it returns to the ground state). Large polymers, heterocyclic aromatic compounds, or molecules with conjugated double bonds are among classes of molecules known to be especially efficient at emitting fluorescence, particularly when nitrogenous substitutions are present. The incident light in the ultraviolet (UV) to visible range predominantly promotes electronic $\pi - \pi^*$ transitions. Accordingly the size of the conjugated π -bond system and the identity of adjacent electron donating or withdrawing groups determine the spectral properties of the fluorescence process.

Fluorescence spectroscopy and imaging have become tools of enormous analytical importance, especially within biological research. The techniques can be divided into the general classes of extrinsic and intrinsic fluorescence. Extrinsic fluorescence refers to the addition of exogenous fluorophores to a set of analyte molecules in order to highlight biological components not easily distinguishable otherwise. This is achieved by insertion of a synthetic fluorescent probe into an organism or material in order to perform selective labeling of a certain target. As a result of its wide use, a large variety of fluorescent probes have become commercially available for selective labeling of macromolecules, cell parts or tissue and for subsequent analysis by various imaging techniques. For example fluorescent dyes such as 4',6-diamidino-2-phenylindole (DAPI) are useful tools to visualize DNA and RNA. Fluorescence in-situ hybridization (FISH) allows the labeling of complete DNA sequences in order to selectively detect and quantify specific organisms. In molecular biology the gene of the green fluorescent protein (GFP) has been introduced in many organisms where GFP is expressed for selective in vivo labeling of certain cell parts. These methods are powerful, in part, because they enable the combination of highly selective labeling of a target molecule with tools for quick, quantitative and non-invasive detection of fluorescence signals within tissue and biological material. Intrinsic fluorescence, or autofluorescence, in contrast, is caused by fluorescent molecules (fluorophores) which exist as a natural component of the analyte material. A large number of biological molecules and compounds are known to exhibit fluorescence light emission. Certain amino acids and coenzymes are among the most commonly analyzed biofluorophores, but numerous other metabolites and biogenic molecules emit fluorescence. Biological samples most often contain a multi-component mixture of biofluorophores, and, accordingly, the overall fluorescent emission often comprises a superposition of individual signals.

Intrinsic fluorescence has been extensively utilized in various fields such as tissue diagnostic (e.g. Koenig and Schneck-enburger, 1994; Andersson Engels et al., 1997), bioprocess monitoring (e.g. Marose et al., 1998; Pons et al., 2004), plant physiology (e.g. Roshchina, 2003, 2004), and environmental studies (e.g. Hudson et al., 2007; Wedborg et al., 2007).

1.3 History of online detection of fluorescent aerosols

While the use of fluorescence techniques to image biological specimens under a microscope is well established, the application of fluorescence to biological aerosol measurement has received significant attention only within the last decades and increasingly so recently. In particular, much effort has been placed on the use of laser/light-induced fluorescence (LIF) for online particle detection, because it allows the non-invasive, in-situ detection of particles with high temporal resolution. Most of the progress in this field has been a result of scientific communities focused on the detection of biological warfare agents (BWA) (e.g. Hill et al., 1995; Cheng et al., 1999; Seaver et al., 1999; Kaye et al., 2000; Ho, 2002). BWA sensors are designed to detect suspicious agents reliably, even in low concentrations, with a low false-positive rate and with short response. Especially challenging is the rapid and selective recognition of specific organisms on top of a diverse and variable ambient aerosol background. Naturally occurring PBAP such as pollen and fungal spores as well as certain non-biogenic aerosols (e.g. soot, industrial plumes) could, in some cases, mimic the BWA signal and trigger a false-alarm. Reliable distinction of analyzed particles is not possible by single-wavelength LIF, and so many instrument developers have added the ability to monitor additional optical properties of the sampled particles, such as: LIF at a second wavelength (dual-wavelength LIF), optical size, shape factor or light absorption.

Most BWA agents are bacteria and bacterial spores (*Bacillus anthracis*, *Yersinia pestis*), viruses (ebola virus, Marburg virus) and toxins (ricin, botulinum toxin) (Hawley and Eitzen, 2001; Kamboj et al., 2006). It has been shown that amino acids (i.e. tryptophan) and some coenzymes (i.e. NAD(P)H, flavins, vitamin B₆ and B₉ compounds) dominate the fluorescence properties of these agents, particularly bacteria (Dalterio et al., 1987; Kocpzyński et al., 2005; Johansson and Liden, 2006; Włodarski et al., 2006), thus simplifying the list of molecules desired for online detection. Viable bacteria usually exhibit a coenzyme signal due to increased metabolic activity and an amino acid signal due to proteins and peptides. In contrast, viruses and peptide toxins exclusively show a protein signal. Accordingly, BWA sensors are individually optimized to match the spectral properties of these agents.

Decades of research by military agencies interested in detecting BWA have recently led to the development and commercialization of several instruments now available for civilian use and that have begun to contribute to atmospheric

measurement and research. The ultraviolet aerodynamic particle sizer (UV-APS), for example, provides aerodynamic particle sizing and fluorescence information via single-wavelength LIF by a pulsed Nd:YAG laser ($\lambda_{\text{ex}} = 355 \text{ nm}$; $\lambda_{\text{em}} = 420\text{--}575 \text{ nm}$) (Hairston et al., 1997; Brosseau et al., 2000) and is sold by TSI, Inc. (Shoreview, MN, USA). The University of Hertfordshire wide issue bioaerosol sensor (WIBS) optically provides particle sizing, an estimate of particle sphericity, and fluorescence information based on dual-wavelength LIF by a Xe-flashlamp ($\lambda_{\text{ex},1} = 280 \text{ nm}$, $\lambda_{\text{ex},2} = 370 \text{ nm}$; $\lambda_{\text{em}} = 310\text{--}400$ and $400\text{--}600 \text{ nm}$) (Kaye et al., 2000; Stanley et al., 2011). The Bristol Industrial and Research Associates Limited (BIRAL) aerosol fluorescence sensor (AFS) provides particle size and sphericity based on light scattering and fluorescence information after excitation from a UV flash lamp ($\lambda_{\text{ex}} = 280 \text{ nm}$; $\lambda_{\text{em}} = 330\text{--}650$ and $420\text{--}600 \text{ nm}$). Both, UV-APS and WIBS have been utilized for ambient measurements in urban European (Huffman et al., 2010; Gabey, 2011) and in pristine rainforest (Gabey et al., 2010; Pöschl et al., 2010; Huffman et al., 2011) environments. These instruments have provided the first continuous and quantitative measurements of fluorescent biological aerosol particles (FBAP) with both high temporal and size resolution, thus providing a lower limit of PBAP concentrations (Huffman et al., 2010). Similar future experiments will help provide crucial input estimates of PBAP flux for global transport models (e.g. Burrows et al., 2009; Heald and Spracklen, 2009; Hoose et al., 2010).

1.4 Fluorescence spectral maps and online FBAP detectors

Wavelength-dependent fluorescence emission spectra, recorded as a function of excitation wavelength, can be plotted as a three-dimensional landscape and thus referred to as an excitation-emission matrix (EEM). Such plots, accordingly, relay a broad collection of information about the steady-state autofluorescence properties of a compound and can be regarded as a unique, sample-specific fingerprint. Similar spectral summaries have been applied as valuable tools in various scientific fields, such as environmental (Kunnil, 2005; Hudson et al., 2007; Wedborg et al., 2007) and medical science (DaCosta et al., 2003; Chang et al., 2005), because they allow a quick, quantitative and non-invasive in-situ characterization with high sensitivity (detection limit: $10^{-8}\text{--}10^{-9} \text{ M}$) (Miao et al., 2003). EEMs may also help facilitate the qualitative assignment of spectral modes to known fluorophores, thus providing insights into the molecular origin of fluorescence. This kind of analysis in the laboratory, in complement to ambient PBAP detection, is particularly necessary for the identification of fluorophores responsible for fluorescence within atmospheric biological particles, and consequently may also allow the estimation of the fluorophore selectivity of a given real-time instrument.

Light scattering effects can complicate peak assignment of fluorescence spectra and, in particular, quantification of fluorescence. Three types of light scattering are typically important for laboratory fluorescence measurements. Rayleigh scattering can originate from both sample and solvent (i.e. water) molecules, which are smaller than the incident wavelength, and Tyndall scattering is a strongly wavelength-dependent reflection process from particulate matter in a suspension (Zepp et al., 2004). Both are types of elastic scattering, meaning that the incident wavelength is identical to the emitted or reflected wavelength ($\lambda_{em} = \lambda_{ex}$). Inelastic Raman scattering from solvent molecules (i.e. water O-H stretching mode) is a much weaker process and appears at slightly longer wavelengths than the elastic scattering signal in solution-phase samples (Ewald et al., 1983; Zepp et al., 2004; Bhartia et al., 2008). If present, each scattering effect can appear as peak in a given emission spectrum and can be further distorted by non-idealities in the spectrometer wavelength dispersion or filtering. For example, elastic scattering of 350 nm incident light will exhibit a sharp peak at the same wavelength in the emission spectrum, referred to as a first order effect ($\lambda_{em} = \lambda_{ex}$), but diffraction gratings can also introduce peaks at emission wavelengths of diffraction orders. Thus second order diffraction would allow the same 350 nm incident photons to appear at 700 nm ($\lambda_{em} = 2\lambda_{ex}$) in the emission spectra. Higher order (e.g. 2nd, 3rd) diffraction, as well as 0-th order diffraction are also possible, but less intense. Imperfect wavelength filtering also allows photons with shorter or longer wavelength than desired to pass through the instrument optics and this is referred to as a form of “light leakage”. This phenomenon broadens the incoming wavelength peak and is noticeable by size of the tail of elastic scattering signal. For samples in solution the emission spectrum of pure solvent can be subtracted to reduce the influence of these interferences. No similar subtraction is possible for suspended or solid-phase samples, however, and scattering effects can, therefore, introduce artificial features to spectra.

Because fluorescence is an inelastic light scattering process, emitted radiation is by definition red-shifted with respect to the incident radiation ($\lambda_{em} > \lambda_{ex}$). The Stokes shift is, therefore, also by definition positive. Typical $\Delta\lambda_{Stokes}$ values of naturally occurring fluorophores are usually approximately 10–200 nm, which means that fluorescence signals usually appear between the 1st and 2nd order diffraction peaks of elastically scattered light (Lakowicz, 1999). As an example, contour plots of three frequently investigated biological fluorophores (tryptophan, NADPH and riboflavin) are shown in Fig. 1. Fluorescence modes usually occur as broad and relatively featureless peaks. As discussed in Sect. 1.2, electron transition can occur to different vibrational energy levels. The resulting vibrational fine structure can be seen for samples in the gas phase where fewer molecular collisions occur, but are usually not resolvable for condensed phase samples. Instead, the appearance of multiple distinct excitation modes (i.e. for riboflavin) is due to different electron

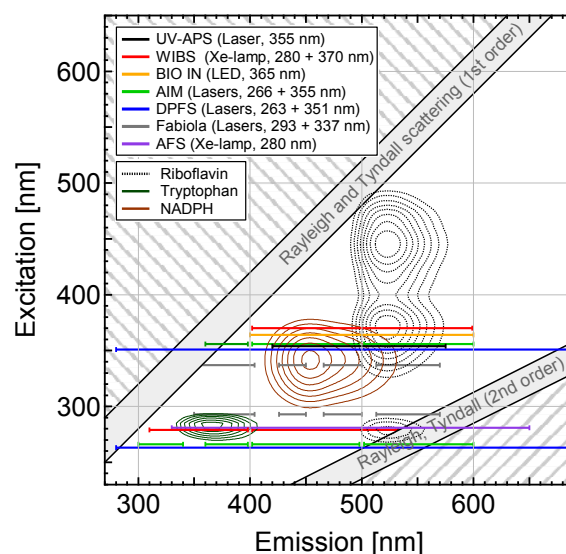


Fig. 1. Conceptual EEM displaying: (a) fluorescence data area (white), areas strongly influenced by Rayleigh and Tyndall light scattering (grey diagonal bars), areas without meaningful data (grey stripes); (b) contour lines for the fluorophores tryptophan, NADPH and riboflavin; (c) operational range of selected bioaerosol detectors represented by horizontal colored lines. Length of individual lines indicates measured emission band for a certain excitation wavelength shown as sharp line for purpose of clarity. Single-wavelength detectors are represented by one line, dual-wavelength detectors by two lines. Instruments shown with spectral specification: UV-APS (laser, $\lambda_{ex} = 355$ nm, $\lambda_{em} = 420$ – 575 nm) (Hairston et al., 1997); WIBS (Xe-lamps, $\lambda_{ex,1} = 280$, $\lambda_{em,280} = 310$ – 400 , 400 – 600 ; $\lambda_{ex,2} = 370$, $\lambda_{em,370} = 400$ – 600) (Foot et al., 2008; Gabey, 2011); DPFS (lasers, $\lambda_{ex,1} = 263$, $\lambda_{em,263} = 280$ – 700 ; $\lambda_{ex,2} = 351$, $\lambda_{em,351} = 280$ – 700) (Pan et al., 2011); AIM (lasers, $\lambda_{ex,1} = 266$, $\lambda_{em,266} = 300$ – 340 , 360 – 400 , 400 – 500 , 500 – 600 ; $\lambda_{ex,2} = 355$, $\lambda_{em,355} = 360$ – 400 , 400 – 500 , 500 – 600) (Sivaprakasam et al., 2004, 2009); Fabiola (lasers, $\lambda_{ex,1} = 293$, $\lambda_{em,293} = 350$ – 404 , 426 – 450 , 466 – 500 , 513 – 570 ; $\lambda_{ex,2} = 337$, $\lambda_{em,337} = 350$ – 404 , 426 – 450 , 466 – 500 , 513 – 570) (Feugnet et al., 2008); SPFA (laser, $\lambda_{ex} = 266$, $\lambda_{em,266} = 300$ – 400 , 400 – 600) (Snyder et al., 2004); BIO IN ($\lambda_{ex} = 365$, $\lambda_{em,365} = 400$ – 600) (Bundke et al., 2010); AFS ($\lambda_{ex} = 280$, $\lambda_{em,280} = 330$ – 650 , 420 – 650).

transition pathways (Lakowicz, 1999). Figure 1 also conceptually shows how EEMs can be utilized in this context. According to Thygesen et al. (2004) three different areas can be distinguished in an EEM: (I) the fluorescence data area (white), (II) areas dominated by strong light scattering interferences (gray diagonal bars), and (III) an area that lacks physical meaning because $\lambda_{em} < \lambda_{ex}$ (gray stripes in upper left corner). Additionally, we have removed data from the lower right corner of each EEM for data shown here. In this region spurious peaks may appear which are caused by second order diffraction of the fluorescent light at the emission grating. As mentioned, the Raman signal of solvents can also be observed in certain situations and would appear in Fig. 1

as a weak diagonal signal whose slope is 0.75 times that of the 1st order elastic scattering line. Raman scattering was not directly observed in any measurements reported here, however, and will not be discussed further.

Figure 1 also summarizes the spectral ranges over which several current bioaerosol instruments detect FBAP. Though somewhat independent in terms of instrument design, only two relatively narrow ranges of excitation (~ 280 nm and ~ 360 nm) have been utilized for most FBAP detectors. Excitation at ~ 280 nm has typically been chosen to highlight the presence of tryptophan as a marker for proteins, and has therefore been used to estimate the total biological fraction in environmental samples. Excitation at ~ 360 nm has typically been chosen to highlight NAD(P)H as a measure for the viable biological fraction, because they are directly linked to the cellular energy metabolism. These assumptions could potentially be problematically simplistic, as will be discussed further. Several dual-wavelength biodetectors have been designed to provide fluorescence from multiple wavelengths of excitation in an attempt to quantify both the whole FBAP fraction and an estimate of its viable subset (i.e. Kaye et al., 2005; Pan et al., 2010).

Selectivity, or the ability to separate fluorophore types within analyzed particles, is a key issue in the design of bioaerosol detectors. A reliable estimate of PBAP concentration requires clear distinction between biological and non-biological particles. It has been suggested that FBAP can be seen as a lower limit estimate for PBAP, because not all biofluorophores will fluoresce at a given wavelength, leading to an underestimation of PBAP concentration (Huffman et al., 2010). However, the exact relationship of FBAP and PBAP is complicated and still unknown. In addition, the ability to discriminate between viable and non-viable organisms based on LIF of key metabolic coenzymes (e.g. NADH) has been a key assumption in the development of many FBAP detectors. This separation and distinction has been successfully shown under certain defined conditions (e.g. in bioreactors and for aerosolized laboratory bacteria) but is highly uncertain under complex atmospheric conditions (Marose et al., 1998; Agranovski et al., 2003; Johansson and Liden, 2006; Li et al., 2011). Based on the amount of biological fluorophores that also fluoresce at wavelengths typically used to determine cell viability (see Table 1 and following discussion) it is almost certain that the signal at ~ 360 nm, typically assumed to indicate the presence of NAD(P)H, reflects more than just viable FBAP. In all cases, however, detailed EEM plots can be a helpful tool to visualize the combination of fluorophores emission signals and the instruments operational range.

1.5 Motivation of fluorophore literature synthesis

As discussed, much work has been performed by the BWA community and more recently by researchers interested in ambient PBAP analysis to utilize autofluorescence for the

search of bioaerosols in the atmosphere, taking advantage of the fact that biological material contains characteristic intrinsic fluorophores. Both biological and non-biological material can exhibit intrinsic fluorescence, however, and so the challenge of discriminating between the two requires a firm knowledge of the corresponding fluorophores. Amino acids with aromatic residues (tryptophan, tyrosine, phenylalanine) and several coenzymes (e.g. NAD(P)H, flavins, and vitamin B₉ compounds) have been generally used by these communities as the most important fluorophores in biological cells and particles (i.e. Davitt et al., 2005; Kaye et al., 2005; Wlodarski et al., 2006; Pan et al., 2010). Whereas amino acids occur in all organisms as building blocks of proteins, coenzymes such as NADH and flavins have only been shown to occur in actively metabolizing organisms and therefore may be specific markers for living cells. Living bacteria actively metabolize and are an important subset of ambient PBAP, but other essential constituents, such as fungal and bacterial spores as well as pollen, do not exhibit measureable metabolic activity (Setlow and Setlow, 1977). As such, they may not contain significant concentrations of NADH, though this is not well agreed upon within the scientific community and we are not aware that the concentration of NADH has even been measured with individual fungal spores or pollen grains (Sivaprakasam et al., 2004; Hill et al., 2009).

This paper investigates the identity and properties of biogenic fluorophores that are most likely responsible for autofluorescence within FBAP. Understanding of the molecular origin of bioaerosol autofluorescence is critical to the development of and measurements with real-time FBAP instrumentation. Moreover a detailed understanding of commonly occurring fluorophores will aid the estimation of the selectivity that can be achieved by FBAP measurement. Some initial studies provide fluorescence spectroscopic data for different classes of PBAP, pure fluorophores and interferences (Campbell et al., 2005; Kopczynski et al., 2005; Wlodarski et al., 2006; Jeys et al., 2007; Hill et al., 2009; O'Connor et al., 2011). In this study we provide a synthesis of literature data as well as original laboratory measurements, designed to give a broad overview of intrinsic fluorophores most likely to be important within ambient aerosols. While subsets of information regarding intrinsic fluorescence of classes of biological molecules have been previously reviewed elsewhere (see Sec. 2), each from a certain perspective, the current synthesis comprehensively summarizes material relevant to atmospheric aerosol research for the first time. Fluorophores are characterized by their steady state fluorescence properties and discussed in relation to a collection of current fluorescent bioaerosol detectors. Moreover, we estimate the relative importance of these fluorophores to atmospheric measurement, using fluorescence characteristics and atmospheric abundance as primary metrics. We also provide fluorescent EEM maps measured with a bench-top spectrofluorometer both for a variety of bulk solid-, suspended-, and solution-phase biological fluorophores and for selected non-biological

compounds which could be considered as important interfering species for FBAP detection and quantification in certain ambient situations. This combination of information will be crucial in the future investigation of the fluorescent properties of standard bioaerosol particles in the laboratory under varied environmental conditions.

2 Literature synthesis

Many fields of science have utilized intrinsic fluorescence for study of biological molecules. The following section serves as an overview of existing knowledge about biological fluorophores that have potential atmospheric relevance. Several existing reviews have collected similar information from the perspective of disparate scientific fields such as biology (Billinton and Knight, 2001; Roshchina, 2003, 2005), medical science (Koenig and Schneckenburger, 1994; Richards-Kortum and Sevick-Muraca, 1996; Ramanujam, 2000; Da-Costa et al., 2003) and defense research (Kopczynski et al., 2005; Wlodarski et al., 2006; Pan et al., 2010). Most of these overviews, however, are shorter and less exhaustive. For this reason we provide in this section a synthesis of literature values and knowledge that represent an extensive collection of fluorophores specifically relevant to atmospheric PBAP detection. Table 1 gives a summary of key parameters for each compound and a short characterization of the major fluorophore groups is given in the corresponding text section. Information about λ_{ex} and λ_{em} are based on data from different studies which are merged here into representative spectral ranges. Depending on data availability and measurement conditions, this can be seen as a general, but not exhaustive, characterization of the fluorescence properties of key fluorophores and classes. Moreover, as a first approach for general orientation the relevance of individual fluorophores for FBAP detection is estimated based on its fluorescence properties (category 1) and atmospheric abundance (category 2). Fluorophores were ranked as highly relevant if they show both intense fluorescence emission and are considered by the authors to be very abundant in PBAP. Fluorophores were ranked with medium relevance if one of the two categories was rated high and the other was considered weak/low. Low relevance means molecules are weakly fluorescent and show rare occurrence. While the terms listed here are uncertain and potentially contentious, we feel this language provides a qualitative means for discussion.

2.1 Group I – amino acids

Amino acids are the basic structural constituent of proteins and peptides in all organisms. Three amino acids with aromatic side chains – tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe) – are known to UV or blue visible fluorescent emission when excited in the UV range of the

electromagnetic spectrum. All three are relatively rare in proteins (Trp: 1.1 % of total amino acid groups, average; Tyr: 3.5 %; Phe: 3.5 %)¹, though still structurally important and of sufficient concentration to cause the omnipresent intrinsic fluorescence of proteins (Klapper, 1977; Creed, 1984a,b). However, it is known that tryptophan is the dominant fluorophore in proteins (causing approx. 90 % of the signal), due to a relatively high quantum yield ($\Phi = 0.13\text{--}0.20$, depending on microenvironment) and efficient (non-radiative) resonance energy transfer (RET) from phenylalanine and tyrosine to tryptophan (energy transfer quenching of Phe and Tyr fluorescence). Tyrosine is the major source of fluorescence for proteins and peptides without tryptophan, whereas phenylalanine is of only minor importance in all cases due to its low quantum yield ($\Phi = \sim 0.02$) and efficient RET. Consequently, in most cases emission of fluorescence from proteins is dominated by tryptophan, though specific properties are also highly sensitive to the protein's molecular environment (i.e. conformational transitions, substrate binding, denaturation which leads to variations in emission maximum and quantum yield between proteins) (Dalterio et al., 1987; Permyakov, 1993; Lakowicz, 1999; Ladokhin, 2000).

2.2 Group II – coenzymes and vitamins

This group summarizes fluorophores that can be assigned to coenzymes (free or bound to an enzyme) and vitamins. They frequently exhibit fluorescence due to heterocyclic aromatic rings within their molecular structures. The major classes of fluorescent coenzymes are the pyridines, pteridines, pyridoxines and structurally related flavins, among which NAD(P)H and flavins are most frequently studied (Dalterio et al., 1987; Lakowicz, 1999; Wlodarski et al., 2006).

Two pyridine nucleotides, nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH), are well known fluorescent coenzymes which are often utilized as intrinsic fluorescent probes in various scientific fields (Bigio and Mourant, 1997; Huang et al., 2002). While similar in structure, the two coenzymes appear in different biochemical mechanisms. NADH is the universal coenzymatic redox carrier in the energy metabolism of cells, whereas NADPH is a ubiquitous reductant in biosynthesis processes (Klingenberg and Bucher, 1960; Michal, 1999; Voet and Voet, 2004). In contrast to the oxidized form NAD(P)⁺, only the reduced form NAD(P)H is fluorescent and can be utilized for an optical assessment of cell metabolic status (Eng et al., 1989; Li et al., 1991; Farabegoli et al., 2003; Wos and Pollard, 2006). Excitation occurs either at ~ 340 nm (direct excitation of the coenzyme) or at ~ 295 nm (light absorption by protein and RET to bound NAD(P)H) (Li and Lin, 1996). NADH and NADPH both show an emission signal at ~ 460 nm and thus cannot be spectrally separated. For this reason the two molecules are often represented

¹Based on sequences from 207 non-related proteins.

Table 1. Summary of atmospherically relevant biological fluorophores. Within the table the molecules are organized in six subgroups: (I) amino acids, (II) coenzymes and vitamins, (III) structural biopolymers and cell wall compounds, (IV) pigments, (V) secondary metabolites and (VI) others. Every fluorophore or family of fluorophores, respectively, is characterized by means of its natural occurrence (organism and metabolic function), its fluorescence properties (especially λ_{ex} and λ_{em} and quantum yield Φ if available) and an estimation of its importance (high, medium or low) to ambient FBAP detection. The selection is focused on the main constituents of PBAP (in particular, microorganisms and debris from larger organisms). References are listed below table.

Fluorophore	Organism	Cell part/ Metabolic function	λ_{ex} [nm]	λ_{em} [nm]	Remarks	Role in FBAP detection (<i>Estimated relevance</i>)
<i>I. Amino acids</i>						
Tryptophan (Trp)	All	Proteins	280–295 ^{2–6} 286 ¹	340–353 ^{2–6} 363 ¹	Amino acid (AA) with highest fluorescence in native proteins – responsible for ~90 % of signal; $\Phi = 0.13–0.2$; Efficient RET from Tyr and Phe to Trp ^{2–4,7}	Most important AA for FBAP detection; used as indicator for total biomass; protein fluorescence resembles Trp signal; main target for many FBAP detectors (<i>High</i>)
Tyrosine (Tyr)	All	Proteins	275 ^{2–6} 280 ¹	300–304 ^{2–6} 307 ¹	Signal in native proteins often quenched (RET: Tyr to Trp); responsible for ~10 % of proteins fluorescence; $\Phi = 0.1–0.14$ ^{2–4,7}	Responsible for protein fluorescence when no Trp present; in presence of Trp, signal usually not detectable (<i>Medium</i>)
Phenylalanine (Phe)	All	Proteins	260 ^{2–6} 270 ¹	280–282 ^{2–6} 296 ¹	Signal in native proteins often quenched (RET: Phe to Trp); Weakly fluorescent; $\Phi = 0.02–0.04$ ^{2–4,7}	Negligible for proteins overall fluorescence (<i>Low</i>)
<i>II. Cofactors, coenzymes, vitamins</i>						
Pyridine nucleotides: NADH and NADPH (Nicotinamide adenine dinucleotide (phosphate))	All	NADH: Ubiquitous coenzymatic redox carrier NADPH: Reductant in biosynthesis	290–295, 340–366 ^{2–5,8,9} 341 ¹	440–470 ^{2–5,8,9} 454 ¹	Only reduced form NAD(P)H fluorescent oxidized form NAD(P) ⁺ non-fluorescent, higher (fourfold) fluorescence intensity when bound to protein; spectral parameters sensitive to local environment ^{2,10,11}	Major contribution to fluorescence of metabolizing cells; used as indicator for viability main target for many FBAP detectors (<i>High</i>)
Flavins: Riboflavin, Flavin mononucleotide (FMN), Flavin adenine dinucleotide (FAD)	All	Ubiquitous coenzymatic redox carrier (in most organisms) and photoreceptor (in plants, fungi)	380, 450–488 ^{4,5,8,12,13} 280, 373, 445 ¹	520–560 ^{4,5,8,12,13} 523 ¹	Only oxidized form fluorescent; $\Phi = \sim 0.3$; Riboflavin and FMN show strong fluorescence, whereas FAD and flavoproteins (flavin non-covalently bound to protein) exhibit weak signal ^{2,4,10,14}	Indicator for cell metabolism; supposed to be a major source of cellular autofluorescence; target for many FBAP detectors (<i>High</i>)
Vitamin B ₆ compounds: Pyridoxine, Pyridoxamine, Pyridoxal, 4-Pyridoxic acid, Pyridoxal-5'- phosphate	All	Key coenzymes in amino acid, protein, carbohydrate, lipid and nucleic acid metabolism	315–345 ^{5,8,15,16} 280, 320–360 ¹	350–425 ^{5,8,15,16} 391–394 ¹	Fluorescence very sensitive to molecular environment and conditions (i.e. pH, ionization state, solvent polarity) ^{10,15,17}	Very abundant; strongly fluorescent; used as indicator for metabolic state in combination with other fluorophores (<i>High</i>)

Table I. Continued.

Fluorophore	Organism	Cell part/ Metabolic function	λ_{ex} [nm]	λ_{em} [nm]	Remarks	Role in FBAP detection (<i>Estimated relevance</i>)
Pteridine compounds: Folic acid (vitamin B ₉), Pterin, Bioplerin, Neopterin, Lumazine	e.g. microorganisms, plants, archaea, mammals	Various metabolic functions (i.e. oxygenation reactions, cellular electron transport, growth factor)	331–361 ^{4,18} 335–355 ¹	373–458 ^{4,18} 446–454 ¹	Significant contribution to autofluorescence of various organisms; properties highly influenced by conditions ^{4,10,12,19,20}	Ubiquitous coenzymes; highly fluorescent; used as specific marker fluorophores; considered as particularly important for microorganisms (<i>High</i>)
Vitamin A (retinol)	e.g. plants, mammals	Many, i.e. gene transcription, photodetection	327–340 ^{5,8}	490–510 ^{5,8,21}	Belongs to carotenoid compounds	Rarely mentioned in context of autofluorescence (<i>Low</i>)
Vitamin C (ascorbic acid)	e.g. plants, algae, fungi, mammals	Many, i.e. involved in various biological redox reactions	350 ⁸	430 ⁸	Non-aromatic compound showing weak fluorescence	Rarely mentioned in context of autofluorescence; weakly fluorescent (<i>Low</i>)
Vitamin D compounds: i.e. sterols: calciferol, ergosterol	e.g. plants, fungi, mammals	Many, i.e. involved in Ca ²⁺ and phosphate metabolism; cell wall components	390 ^{5,8}	470–480 ^{5,8}	Many sterol compounds known to be fluorescent; important cell wall fluorophores ^{22,23}	Ergosterol used as tracer for fungal spores; considered to be relative weakly fluorescent (<i>Low</i>)
Vitamin K and quinones: i.e. phyloquinone, menaquinone, anthraquinone	e.g. plants, bacteria, mammals	Many, i.e. involved in photosynthetic processes	335 ⁵	480 ⁵	n/a	Rarely mentioned in context of autofluorescence (<i>Low</i>)
<i>III. Structural biopolymers and cell wall compounds</i>						
Cellulose	Plants, algae, bacteria, oomycetes	Structural component of primary cell wall	250–350 ^{24–26} ~250, ~340 ¹	350–500 ^{24–26} 410–460 ¹	Nature of molecular fluorophores in cellulose not unequivocally identified; main contributor to cell wall fluorescence; weakly fluorescent compared to other fluorophores (i.e. secondary metabolites) ^{24–27}	Very abundant biopolymer (also in atmosphere); fluorescent emission over wide wavelength range; cell wall fluorescence common feature of FBAP (<i>Medium</i>)
Chitin	Fungi, algae, arthropods, crustaceans, mollusks, insects	Principal cell wall compound	335 ^{28–30} 254, 373 ¹	413 ^{28–30} 452–458 ¹	Very abundant; i.e. occurrence in many fungi; brightly fluorescent ^{28–30}	Referred to as main fluorophore in fungal spore cell walls (<i>High</i>)
Lignin	Plants, algae, (fungi)	Cell wall component; chief component of woody tissue for mechanical support and protection	240–320 ^{8,25,31} 282, 331–345 ¹	360, 470–510 ^{8,25,31} 419–458 ¹	Polyphenolic macromolecule based on coniferyl, coumaryl, and sinapyl alcohols; λ_{ex} and λ_{em} dependent on organism and microenvironment of fluorophores; potential fluorophores: ferulic acid, phenylcoumarones, cis-stilbene, quinines ^{32–34}	Fluorescent emission over broad spectral range; highly abundant in nature; occurrence in plant debris (<i>Medium</i>)

Table 1. Continued.

Fluorophore	Organism	Cell part/ Metabolic function	λ_{exc} [nm]	λ_{em} [nm]	Remarks	Role in FBAP detection (<i>Estimated relevance</i>)
Sporopollenin	Pollen, fungal spores, microspores	Cell wall constituent in exine	300–550 ^{35–37}	400–650 ^{35–37}	Complex biopolymer containing mixture of fluorescent compounds (i.e. phenolics, carotenoids, azulene) ^{35–38}	Origin of intense fluorescence from pollen (<i>High</i>)
Collagen and elastin	Mammals	Fibrous proteins; collagen: major extracellular matrix component; elastin: major component of elastic fibers	290, 325 ^{3,5}	340, 400 ^{3,5}	Cross links are origin of fluorescence (i) collagen: hydroxylylsyl pyridoline and lysyl pyridinoline; (ii) for elastin: tricarboxylic triimino pyridinium ⁵	No occurrence in microorganisms; low abundance in FBAP (<i>Low</i>)
Suberin and cutin	Plants	Suberin: cell wall constituents of underground organs; Cutin: waxy polymer coating outer cell walls	n/a	n/a	Three-dimensional hydrophobic network based on functionalized fatty acids and phenolic compounds; related to lignin ³²	Rarely mentioned in context of autofluorescence (<i>Low</i>)
Phospholipid	All	Major component of cell membranes	436 ⁵	540, 560 ⁵	Cell wall component in all cells	Rarely mentioned in context of autofluorescence (<i>Low</i>)
<i>IV. Pigments</i>						
Lipofuscin and ceroid (age-related pigments)	i.e. plants (pollen), bacteria, algae, fungi, mammals	Heterogeneous granules in residual body-type lysosomes in cytosol	260–280- 340–490 ^{39,40}	430–670 ^{39,40}	Strongly cross-linked lipid-protein pigmented substance; generated due to age, oxidative stress, antioxidant deficiency, UV-induced lipidic peroxidation ^{41–43}	Ubiquitous in biology; lipofus- cinogenesis induced by ozone or UV-light; known as fluorophore in pollen (<i>Medium</i>)
Advanced glycation end-products (AGEs) (age-related pigments)	i.e. mammals	Pigments caused by aging and oxidative stress	320–370 ^{8,41}	385–450 ^{8,41}	Products of non-enzymatic glycosylation (Maillard reaction); family of nitrogenous, cyclic and conjugated compounds ^{8,41}	(<i>Low-medium</i>)
Melanin	Mammals, fungi, bacteria	Accumulated in cell wall; protective response against adverse environmental conditions	469–471 ⁴⁴	543–548 ⁴⁴	Polymerized phenolic and/or indolic compounds; when non-oxidized: non-fluorescent, when oxidized (i.e. by ozone): shows bright fluorescence ^{44,45}	Ubiquitous in biology; in some cells highly concentrated; relatively low fluorescence intensity (<i>Low</i>)
Chlorophyll and pheopigments	Plants, cyanobacteria, purple and green bacteria, algae, cryptomonads	Photopigment for microbial and plant photosynthesis	390–470 ^{46–48}	630–730 ^{46–48}	Chlorophylls strongly fluorescent; pheopigments = chlorophyll degradation products; fluorescence of pheopigments weaker than of parent chlorophyll ^{7,32,46}	Very abundant in various organisms; (<i>Medium</i>)

Table 1. Continued.

Fluorophore	Organism	Cell part/ Metabolic function	λ_{ex} [nm]	λ_{em} [nm]	Remarks	Role in FBAP detection (<i>Estimated relevance</i>)
Phycobiliproteins: i.e. phycoerythrin, phycocyanin, red allophycocyanin	Plants, cyano- bacteria (= blue- green algae), red algae, cryptomonads	Photopigment in light-harvesting antenna complex for microbial and plant photosynthesis	480–650 ⁴⁹	580–660 ⁴⁹	High molar absorption coefficient ($\sim 10^6 \text{ M}^{-1} \text{ cm}^{-1}$); high quantum yield (up to 0.98); broad excitation range ⁴⁹	Highly fluorescent; very abundant in algae and cyanobacteria – sometimes highly enriched (up to 40% of proteins in cells) (<i>Medium</i>)
Flavonoids: i.e. anthocyanins, flavons, flavonols, isoflavones	Plants	Defense, attraction of pollinators, UV-shielding	365 ^{36,50}	440–610 ^{36,50}	Major class of colored pigments; oligophenolic compounds; spectral properties (coloration) span over wide range depending on functionalization; wide occurrence in plants ^{32,51}	Flavonol accumulated in pollen (2–4% of dry weight); known to be major fluorescent component in pollen (<i>High</i>)
Carotenoids: i.e. carotenes, xanthophylls, retinonoids	Plants, algae, bacteria, fungi	Accessory pigment in photosynthesis; UV-protection	400–500 ^{36,50}	520–560 ^{36,50}	Major compound family of colored pigments; (tetra)terpenoid compounds ^{32,51}	Widely distributed in plants; enriched in outer pollen wall (<i>High</i>)
<i>V. Secondary metabolites</i>						
Alkaloids: i.e. amino acid based, purine and polyketide alkaloids	e.g. plants, fungi, bacteria, insects, animals	Many, i.e. defense agents against predators and pathogens	360–380 ^{36,37,52}	410–600 ^{36,37,52}	Heterocyclic nitrogenous compounds; structurally highly diverse compound class; λ_{em} spans broad spectral range ^{32,36,52}	Occurrence in many (micro)- organisms; strongly autofluorescent (<i>High–medium</i>)
Phenolics: Simple compounds (i.e. coumarins), oligophenolics (i.e. flavonoids), polyphenolics (i.e. tannins)	e.g. plants, fungi, bacteria,	Many, i.e. defense agents, pollinator attractors, UV- shielding; constituent of supporting tissue	300–380 ^{36,52}	400–500 ^{36,52}	Chemically highly heterogeneous group; fluorescence mostly in blue range, although variety of structures ^{32,36,52}	Wide occurrence particularly in plants; many compounds fluoresce – some with high intensity; crucial for cell wall fluorescence; (<i>High–medium</i>)
Terpenoids: i.e. monoterpenes (e.g. menthol), sesquiterpenes (e.g. azulenes), diterpenes	e.g. plants, fungi	Many, i.e. pigments, growth factors, messengers, toxins	250–395 ³⁶	400–725 ^{36,37}	Fluorescent over wide spectral range depending on structure ^{32,36,52}	Very abundant; some compounds known to be highly fluorescent (<i>Medium</i>)
Other secondary metabolites: i.e. mycotoxins, siderophores, cytokinines, Mycosporins/ Mycosporine-like amino acids (MAAs)	Plants, bacteria, fungi	Various metabolic functions	n/a	n/a	Many heterocyclic aromatic compounds, hence potentially highly fluorescent; very broad range of reported fluorescence properties ⁵²	High diversity of compounds; individual compounds can be highly fluorescent and relevant for FBAP detection (<i>Medium</i>)

Table 1. Continued.

Fluorophore	Organism	Cell part/ Metabolic function	λ_{ex} [nm]	λ_{em} [nm]	Remarks	Role in FBAP detection (<i>Estimated relevance</i>)
<i>VI. Others</i>						
Dipicolinic acid (DPA) and salts CaDPA, NaDPA	Unique constituents of bacterial endospores	Accumulated in spore core; supposed role in dormancy, heat- and UV- resistance	278, 300–360 ^{53,54}	400–440 ^{53,54}	DPA = 2,6-pyridine-dicarboxylic acid (C ₇ H ₅ NO ₄); 5–17 % of dry weight; highly fluorescent after UV- exposure and/or when dry ^{53,54}	Highly fluorescent; high concentration in spores as important bacterial propagation vector in atmosphere (<i>Medium</i>)
DNA/RNA	All	Storage for genetic information	260 ^{52,55}	334 ^{52,55}	Quantum yield 100 times lower than for Trp ^{52,56–58}	Ubiquitous in organisms but weak fluorescence emission (<i>Low</i>)

¹ This report; ² Laskowicz (1999); ³ Richards Kortum and Sevcik Muraca (1996); ⁴ Kopeczynski et al. (2005); ⁵ Ramanujam (2000); ⁶ Teale and Weber (1957); ⁷ Voet and Voet (2004); ⁸ Billinton and Knight (2001); ⁹ Avi-Dor et al. (1962); ¹⁰ Michal (1999); ¹¹ Li and Lin (1996); ¹² Wlodarski et al. (2006); ¹³ Benson et al. (1979); ¹⁴ Albani et al. (1999); ¹⁵ Bridges et al. (1966); ¹⁶ Bueno and Encinas (2003); ¹⁷ Li et al. (1991); ¹⁸ Dalerio et al. (1987); ¹⁹ Tyagi and Benzkofer (2010); ²⁰ Rembold and Gyure (1972); ²¹ Zipfel et al. (2003); ²² McIntosh et al. (2008); ²³ Rosenheim (1927); ²⁴ Olmstead and Gray (1993); ²⁵ Olmstead and Gray (1997); ²⁶ Castellan et al. (2007); ²⁷ Jabajhare et al. (1984); ²⁸ Bonifantefasolo et al. (1990); ²⁹ Vriehelling et al. (1999); ³⁰ Dreyer et al. (2006); ³¹ He et al. (1999); ³² Taitz and Zeiger (2010); ³³ Donaldson et al. (1999); ³⁴ Albinsson et al. (1999); ³⁵ Roshchina et al. (1997); ³⁶ Roshchina (2003); ³⁷ Roshchina et al. (2004); ³⁸ Brooks and Shaw (1978); ³⁹ Eldred et al. (1982); ⁴⁰ Andersson et al. (1998); ⁴¹ Yin (1996); ⁴² Tsuchida et al. (1987); ⁴³ Roshchina and Karnaukhov (1999); ⁴⁴ Kayatz et al. (2001); ⁴⁵ Gomez and Nosanechuk (2003); ⁴⁶ French et al. (1956); ⁴⁷ Wekschmeyer (1994); ⁴⁸ Moberg et al. (2001); ⁴⁹ Glazer (1994); ⁵⁰ Roshchina and Mel'nikova (2001); ⁵¹ Wiernann and Vieh (1983); ⁵² Roshchina (2008); ⁵³ Alimova et al. (2003); ⁵⁴ Sarasamanarajah et al. (2005); ⁵⁵ Leblanc and Dufour (2002); ⁵⁶ Pan et al. (2009); ⁵⁷ Ammor et al. (2004); ⁵⁸ Ammor (2007)

by a short-hand NAD(P)H form, referring to both molecules at once. It has been shown that the fluorescence contribution of NADH dominates that of NADPH, due to differences in cellular concentration and fluorescence characteristics. Furthermore the NADH signal is highly influenced by the metabolic status whereas the weaker NADPH signal is rather constant (Vishwasrao et al., 2005). The excited electronic state is quenched by two mechanisms: collisional deactivation and stacking of the fluorescent nicotinamide ring to the adenine moiety. The extent of quenching is decreased when NAD(P)H is bound to a protein, thus explaining the fourfold increase in fluorescence intensity as compared to free NAD(P)H in solution (Li and Lin, 1996). Because of its links to active cell metabolism, NAD(P)H has often been used in the search for viable airborne microorganisms (i.e. Hairston et al., 1997; Bundke et al., 2010). Cell death is accompanied by NADH and NADPH depletion within the temporal range of hours (2–10 h) depending on various intracellular processes and extracellular conditions (Petit et al., 2001; Liang et al., 2007).

Riboflavin (vitamin B₂) is the precursor for both cofactors flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are prosthetic groups of flavoenzymes (Benson et al., 1979). Flavins are ubiquitous redox carriers in the energy metabolism of all organisms and act also as photoreceptors in plants and fungi (Galland and Senger, 1988; Billinton and Knight, 2001). In contrast to NAD(P)H, only the oxidized form of flavins is fluorescent and shows light emission at ~525 nm when excited at ~450 nm, although excitation has also been observed at ~280 nm and ~380 nm (Hill et al., 2009). It is known that riboflavin and FMN show intense fluorescence, whereas FAD and especially flavoproteins exhibit only a weak fluorescence signal (Albani et al., 1999). Flavins are also connected to the metabolic status of cells, but at different rates than other coenzymes. Accordingly it has been suggested that the ratio of fluorescence emission of NAD(P)H to flavins can be used as indicator for the cellular metabolic rate (Chance et al., 1979; Siano and Mutharasan, 1989; Heikal, 2010).

Vitamin B₆ acts as precursor for the coenzyme pyridoxine and related compounds (i.e. pyridoxal 5'-phosphate, pyridoxamine 5'-phosphate). Pyridoxine and related coenzymes are required by many enzymes that are involved in amino acid, protein, carbohydrate, lipid and nucleic acid metabolism in all organisms (Martell, 1989; Voet and Voet, 2004). Pyridoxine compounds comprise a substituted pyridine ring as structural fluorophore and its spectral characteristics are highly sensitive to its molecular environment (i.e. pH, ionization state, solvent polarity). Accordingly fluorescence occurs in a relatively broad spectral ranges depending on the conditions ($\lambda_{\text{ex}} = 315\text{--}345\text{ nm}$, $\lambda_{\text{em}} = 350\text{--}425\text{ nm}$) (Bridges et al., 1966; Bueno and Encinas, 2003). Pyridoxine compounds are key metabolites and along with NAD(P)H and flavins have been commonly utilized as indicators for metabolic activity, often in combination with other intrinsic

fluorophores (i.e. Trp, NAD(P)H) (Siano and Mutharasan, 1989; Li et al., 1991; Heikal, 2010).

Pteridine compounds are ubiquitous within most organisms and as a group are comprised by: folic acid (vitamin B₉), pterins, and lumazines, among others. They are involved in various biochemical processes, such as oxygenase reactions, single carbon transfer reactions and cellular electron transport. The bicyclic ring system is structurally related to purine and exhibits strong fluorescent emission (Rembold and Gyure, 1972). Pteridines have been considered as responsible for significant autofluorescence within bacterial cells (Dalterio et al., 1987; Kopczynski et al., 2005; Wlodarski et al., 2006), fungi (Hohl et al., 1992; Galland and Tolle, 2003) and other microorganisms (Galland et al., 1990; Uchiyama et al., 1997). Pteridines emit fluorescence peaking at ~450 nm when excited at ~350 nm, though this is partially dependent on environmental conditions such as pH and ionization state (Dalterio et al., 1987; Tyagi and Penzkofer, 2010).

Other vitamins, such as vitamin A, C, D and K, are sometimes mentioned in the context of cellular fluorescence (Billinton and Knight, 2001; Ramanujam, 2006). However, most of these are considered to comprise only minor contributions, due to low concentration, weak fluorescent emission, or both. In particular ergosterol (sterol compound, precursor for vitamin D) can be found in the cells wall of fungi and yeasts and has often been used as a tracer for fungal spores (Lau et al., 2006; Burshtein et al., 2011). Ergosterol and other sterols are known to fluoresce, though usually weakly, and this has been utilized in some cases for their detection (e.g. This work, Fig. 2l; Rosenheim, 1927; McIntosh et al., 2008).

2.3 Group III – structural biopolymers and cell wall compounds

In addition to fluorophores which actively participates in cellular metabolism, various structural compounds also emit fluorescent radiation. The biopolymers cellulose, chitin, lignin and sporopollenin are structural compounds crucial to many microorganisms and are abundant within the atmosphere (Kunit and Puxbaum, 1996; Winiwarer et al., 2009). The protein polymers elastin and collagen also exhibit fluorescent emission, however, they are regarded as of minor importance due to their absence in microorganisms.

Cellulose (β (1,4)-linked homopolymer of D-glucose) is one of the most abundant organic compounds in nature and, in particular, is an essential constituent in the cell wall of various organisms (such as bacteria, fungi, algae, plants) (Cannon and Anderson, 1991; Niklas, 2004; Voet and Voet, 2004). Cellulosic materials exhibit fluorescence over a broad spectral range with an emission maximum at ~420 nm and efficient excitation in the UV range (250–350 nm) (Olmstead and Gray, 1993, 1997; Castellan et al., 2007). The spectral properties of cellulose from different sources are well

characterized, however the molecular origin of the fluorescence is still debated (Olmstead and Gray, 1993). The fluorescence of cellulose is known to be highly increased by phenolic residues (e.g. ferulic acid) (Roshchina, 2008).

Chitin (β (1,4)-linked homopolymer of N-acetylglucosamine) is a highly abundant cell wall compounds which occurs in all fungi, various microorganisms and insects (Lenardon et al., 2010). It shows maximum fluorescent emission ~ 410 nm and an excitation maximum ~ 335 nm (Jabajihare et al., 1984). Several studies refer to chitin as a major fluorophore in fungal cell walls (Bonfantefasolo et al., 1990; Vierheilig et al., 1999; Dreyer et al., 2006).

Lignin is the chief compound in woody tissue and the most abundant polymer in nature, along with cellulose. It is a polyphenolic, branched, and disordered macromolecule derived by polymerization of monolignols in the cell wall of plants and algae (Donaldson, 2001; Boerjan et al., 2003). Lignin exhibits intense fluorescent emission of blue and green light when excited by radiation in the UV or blue spectral range, respectively (Harris and Hartley, 1976; He et al., 1999; Donaldson, 2001). It is assumed that light is absorbed by a number of chromophores (light-absorbing molecules) followed by intramolecular energy transfer to certain emission sinks (i.e. phenylcoumarone, stilbene) (Olmstead and Gray, 1997; Albinsson et al., 1999). In general, the fluorescence properties of these structural compounds are rather undefined and distributed over a broad spectral range, depending on organism and conditions (Billinton and Knight, 2001).

Sporopollenin is a rigid and complex biopolymer comprising a main constituent of the outer cell wall (exine) of pollen (Brooks and Shaw, 1978). Its intense fluorescent emission over a broad spectral range is caused by a mixture of different fluorophores, such as phenolic compounds ($\lambda_{em} = \sim 460$ nm), carotenoids ($\lambda_{em} = \sim 530$ nm), azulene ($\lambda_{em} = \sim 450$ nm), anthocyanin ($\lambda_{em} = \sim 460$ and 620 nm) and other compounds (Roshchina et al., 1997, 2003, 2004). However, the exact chemical composition of sporopollenin, including its fluorescence properties, is not well known.

The structural proteins collagen and elastin are important fluorophores within mammalian tissue which are frequently used for fluorescence-based tissue investigation. Both show similar fluorescent properties, respectively: $\lambda_{ex} = \sim 325$ nm and $\lambda_{em} = \sim 400$ nm (Billinton and Knight, 2001; Ramanujam, 2006). Due to their exclusive occurrence in mammalian tissues, however, these molecules are not expected to bare much significance on atmospheric FBAP.

2.4 Group IV – pigments

Two compound classes are summarized as fluorescent pigments in the following section: age-related pigments (i.e. lipofuscin, ceroid and melanin) and pigments that are involved in photoreceptor, photosynthesis and light

harvesting processes (i.e. (bacterio)chlorophylls, phycobiliproteins, flavonoids and carotenoids).

The following intracellular and extracellular compounds are usually summarized as age related pigments: lipofuscin, ceroid, advanced glycation end-products (AGEs) and age pigment-like fluorophores (APFs). The formation of these yellow-brown pigments is based on oxidation and peroxidation of lipids, proteins, nucleic acids, carbohydrates and other biomolecules, followed by cross-linking of the secondary oxidation products (particularly unsaturated carbonyls) with amino compounds (i.e. primary amines, amino acids). The generated fluorophores are mainly nitrogenous conjugated and cyclic compounds and polymers (Tsuchida et al., 1987; Yin, 1996). Fluorescent emission has been observed in a broad spectral range, when excited by UV or blue light. Usually fluorescent emission of cellular age related pigments is located between 450 and 640 nm, depending on the identity of the fluorophores, cellular environment and concentration (Eldred et al., 1982; Andersson et al., 1998).

Melanins are dark-colored hydrophobic pigments of high molecular weight which are assumed to consist of polymerized phenolic and indolic compounds. They are ubiquitous in nature and occur in various organisms, particularly fungi and bacteria have been found to show a high melanin content which is associated with microbial pathogenesis (Gomez and Nosanchuk, 2003; Nosanchuk and Casadevall, 2003). Compared to the other age-related pigments, melanin is regarded to show only a relatively weak fluorescence, but it has been shown that oxidation significantly increases fluorescence with a emission maximum at ~ 540 nm when excited at ~ 470 nm (Kayatz et al., 2001).

Fluorescent light emission from tetrapyrrolic compounds (i.e. protoporphyrin IX, chlorophylls, phycobilins) is well known and utilized in various applications (i.e. Lichtenhaler et al., 1986). Photosynthetic chlorophyll pigments such as chlorophyll-*a*, *b*, *c* and *d* in plants, many algae and cyanobacteria show fluorescent emission ~ 650 nm. Pheopigments are chlorophyll degradation products obtained by acidification which show fluorescent emission (630–730 nm) with weaker intensity than the parent chlorophylls (French et al., 1956; Welschmeyer, 1994; Moberg et al., 2001). Another important class of fluorescent tetrapyrrolic pigments are phycobiliproteins and bilins as their prosthetic groups. They are known to show intense fluorescent emission of red light (580–660 nm) with high quantum yields (up to 0.98) (Glazer, 1994).

Carotenoids and flavonoids comprise the most important colored pigments in plants (Mantoura and Llewellyn, 1983). Flavonoids are polyphenolic and carotenoids are terpene compounds. Because of their pronounced fluorescence characteristics and importance in FBAP detection, they are each listed here as independent fluorophore classes. Both pigments are known to be enriched in certain plant parts, particularly in pollen ($\mu\text{g kg}^{-1}$), constituting a main origin of

pollen autofluorescence (Roshchina and Karnaukhov, 1999, 2003; Taiz and Zeiger, 2010).

2.5 Group V – secondary metabolites

In addition to the given metabolites and intermediates (i.e. amino acids, sugars, nucleotides) in the primary metabolism (growth and development) of organisms, myriads of highly structurally diverse organic compounds, called secondary metabolites, are known, but which have no direct role in basic anabolic or catabolic processes. They are distinctive products with relatively narrow distribution among small groups of species and are active players in highly specialized metabolic processes of survival and competition: i.e. mycotoxins in fungi, terpenoids as attractants for pollinators, or metal-chelating siderophores in bacteria (Vining, 1990; Theis and Lerda, 2003; Keller et al., 2005; Taiz and Zeiger, 2010). Secondary metabolites usually comprise complex chemical structures, often with conjugated heterocycles containing oxygen atoms (O) or nitrogen atoms (N), or both. Many tend to be autofluorescent and are known to show a significantly higher intensity compared to structural fluorophores in cell walls and primary metabolites (Roshchina, 2003, 2008). In the following paragraph main classes of secondary metabolites and their autofluorescence properties (if known) are discussed.

Alkaloids are one of the most diverse groups of secondary metabolites and occur in plants, fungi, bacteria, insects and other organisms, though every species shows a specific genetically determined alkaloid pattern. Most of these compounds serve as defense agents against predators and pathogens. Alkaloids are defined as cyclic compounds containing N in low oxidation states and can be subdivided based on their molecular origin into four groups: alkaloids based on amino acids, purine alkaloids, aminated terpenes and polyketide alkaloids (Roberts, 1998). The high structural diversity is reflected into highly diverse spectral properties. According to Roshchina (2003, 2005) a wide spectral range (410–600 nm) of fluorescent emission is covered by plant alkaloids. For UV excitation (360–380 nm) emission in the shorter wavelength range (410–520 nm) has been found for structurally smaller alkaloids (e.g. atropine) whereas larger compounds (e.g. sanguinarine) exhibit further red-shifted emission (585–600 nm).

Phenolic compounds comprise a large variety of chemically heterogeneous substances including simple molecules (e.g. benzoic acid derivatives, coumarins), oligophenolics (e.g. flavonoids) and complex polyphenolics (e.g. lignin, tannins). The unifying chemical feature is a multiple hydroxyl functionalization of conjugated and non-conjugated aromatic structures. They serve as UV shielding, defense against pathogens and mechanically supportive tissue. Many phenolics are well known fluorophores which usually emit blue fluorescence light (400–500 nm) when excited in the UV range (Roshchina, 1997).

Terpenes (or terpenoids) comprise a diverse class of lipophilic secondary metabolites synthesized by plants. Structurally based on isoprene (C_5) units, this group includes monoterpenes (C_{10} compounds), sesquiterpenes (C_{15}), diterpenes (C_{20}) and other even larger compounds. They play important roles in various metabolic processes for examples as accessory pigments in photosynthesis (carotenoids, C_{20}), deterrent toxins (pyrethroids, C_{10}) or insect repellents (mono- and sesquiterpenes) (Theis and Lerda, 2003; Taiz and Zeiger, 2010). For several monoterpenes Roshchina (2003) reports fluorescent emission in the blue range of the electromagnetic spectrum (405–430 nm) when excited with UV light (310–380 nm). For larger terpenes fluorescent emission occurs red-shifted, depending on the size of the conjugated π -bond system.

In addition to the previously mentioned large classes of secondary metabolites several other families of compounds such as mycotoxins, siderophores, quinones and mycosporin-like amino acids (MAAs) are known to autofluoresce, although details are in most cases not yet understood. In certain cases these compounds can be relevant for the autofluorescence of an organism even in low concentrations. In general secondary metabolites can be considered as a highly structurally diverse class of compounds comprising autofluorescent emission over a wide spectral range. Depending on their occurrence and concentration in certain species they can act as important or even dominant fluorophores.

2.6 Group VI – other fluorophores

Fluorophores that do not fit in the previous groups are summarized here. The most important compounds here are DNA and RNA as well as dipicolinic acid (DPA). The genetic information of all organisms is stored in the sequence of purin and pyrimidine bases in deoxyribonucleic acid (DNA). In the expression of genes and in the biosynthesis of peptides and proteins ribonucleic acid (RNA) is responsible for the information transfer from the genome to the proteome. The nucleic acids DNA and RNA are known to be fluorescent due to their constitutive nitrogenous and heterocyclic bases adenine, cytosine, guanine and thymine. Different studies report about DNA and RNA autofluorescence in microorganisms. When excited at 260 nm DNA fluorescence can be observed at 330–335 nm, although the signal is merged with the protein emission peak (e.g. Leblanc and Dufour, 2002; Roshchina, 2008). Biological aromatic molecules tend to be fluorescent due to energetically accessible $\pi - \pi^*$ transitions, however, some heterocyclic aromatic compounds (i.e. the bases of DNA and RNA) are known to be very weakly fluorescent (Pan et al., 2009). The quantum yield of DNA/RNA is 100-times lower than tryptophan (Leblanc and Dufour, 2002; Estes et al., 2003; Ammor et al., 2004, 2007), however, and thus the overall relevance of DNA autofluorescence is considered to be negligible.

Dipicolinic acid (DPA or 2,6-pyridine-dicarboxylic acid) and its salts are synthesized during bacterial spore formation and enriched in the spore core (>10% of dry weight), where these compounds are assumed to be involved in affecting spore dormancy, heat resistance, and UV radiation protection (Paidhungat et al., 2000; Slieman and Nicholson, 2001). Medium fluorescence is observed for pure DPA in the dry state which is increased by UV-light exposure. The emission occurs around 440 nm, with an excitation maximum at 360 nm. The calcium salt CaDPA is also known to be fluorescent with peak emission at approximately 410 nm, when excited between 280 and 340 nm (Alimova et al., 2003; Sarasanandarajah et al., 2005). Since DPA occurs exclusively in bacterial endospores, it has been used as a spectral signature for spore detection (Bronk et al., 2000).

2.7 Possible interfering, non-biological compounds

Fluorescence is a widespread phenomenon common to a wide variety of chemical compounds and materials. As such it is not unique for biological materials, and aerosol of both anthropogenic and natural origin may also show emission of fluorescent radiation. This must be considered when performing fluorescence measurements on atmospheric samples, because certain non-biological compounds have the potential to cause positive fluorescence measurement artifacts and an overestimation of actual FBAP concentrations. We discuss here a selection of possible interferences in this context: (I) humic-like substances (HULIS), (II) secondary organic aerosols (SOA), (III) minerals/mineral dust, (IV) polycyclic aromatic hydrocarbons (PAH) and (V) soot. The following section briefly characterizes the fluorescence properties of each class, but is not represented in Table 1.

HULIS is a complex mixture of poorly defined heterogeneous compounds formed by biological material broken down through natural decay mechanisms and after subsequent atmospheric oxidation (Andreae and Gelencser, 2006; Graber and Rudich, 2006). It is terrestrially ubiquitous, but also constitutes an important subset of the organic aerosol fraction. While not chemically identical, humic and fulvic acids are generally considered to be appropriate HULIS surrogates. Various studies have investigated the fluorescence properties of humic and fulvic acids by means of EEMs. In particular the soluble fraction, referred to as dissolved organic matter (DOM), has been investigated in natural waters from rivers and oceans (Coble, 1996; Hudson et al., 2007) as well as rain and fog droplets (Duarte et al., 2004; Kieber et al., 2006; Birdwell and Valsaraj, 2010). Because humic-like material extracted from complex natural matter is not chemically pure it contains a mixture of many fluorophores. It has been shown that EEMs of DOM show two types of fluorescence modes: for humic-like and protein-like fluorescence, respectively. Humic-like fluorescence has been observed in three characteristic spectral positions ($\lambda_{\text{ex}}/\lambda_{\text{em}}$) at: (i) 237–260/380–460

(humic-like, UV excitation), (ii) 300–370/400–500 (humic-like, visible excitation), (iii) 312/380–420 (marine humic-like). Protein- or amino acid-like fluorescence, however, occurs at 275/310 (tyrosine-like) and at 275/340 (tryptophan-like) (Coble, 1996; Hudson et al., 2007; Muller et al., 2008). Occurrence, spectral properties, and relative intensity of these five modes are highly dependent on molecular identity and chemical properties of the sample, however, and vary from sample to sample. It is reported that the fluorescence spectra, particularly EEMs, of humic and fulvic acids provide chemical and structural information such as conformation and functional groups (Chen et al., 2003), molecular weight (Stewart and Wetzel, 1980), aromatic character (Miano et al., 1988) and oxidation state (Klapper et al., 2002). HULIS isolated from aerosols or cloud water tends to show farther blue-shifted fluorescence when compared to terrestrial samples and thus is more similar to marine humic-like material (e.g. Krivacsy et al., 2001; Duarte et al., 2004). This may indicate aerosol HULIS contains a lower molecular weight and a smaller content of aromatic and conjugated π -bond structures compared with terrestrial HULIS (Graber and Rudich, 2006).

Secondary organic aerosol (SOA) is also an important constituent of atmospheric particulate matter, dominating sub-micron aerosol in many environments and also capable of condensing as a liquid layer onto larger particles (Pöschl, 2005; Jimenez et al., 2009; Pöschl et al., 2010). SOA forms as a result of the atmospheric oxidation of ubiquitous volatile organic compounds (VOCs) generating myriads of differently functionalized organic molecules. Bones et al. (2010) investigated the autofluorescence properties of lab-generated limonene and γ -terpinene SOA which exhibits strongly increased fluorescent emission when aged in the presence of ammonium compounds or amino acids. They found four major fluorescence modes (280/420, 300/360, 340/440, 425/490 nm, respectively) similar to the autofluorescence characteristics of HULIS. In particular this study indicates that nitrogen-containing SOA could be an important source of autofluorescence within certain SOA. In a related study Chang et al. (2010) found fluorescent emission within 325–425 nm when excited at 280 nm for photooxidation products of various phenolic compounds.

Mineral dust is also a major constituent of the global atmospheric aerosol, occurring in both fine and coarse mode and dominating total particle mass concentrations at locations worldwide (Gillette and Walker, 1977; Andreae and Rosenfeld, 2008; Monks et al., 2009). The photoluminescence of minerals is a well known phenomenon, and this allows the analysis of the presence and identity of so-called luminescence centers within given minerals (i.e. transition metal cations, rare-earth cations, biological impurities, trapped electron-hole pairs). A variety of different naturally occurring minerals (e.g. silicate, carbonate, phosphate and sulfate minerals) have been found to show clearly resolved, narrow emission modes at \sim 500 and \sim 700 nm when excited at

different wavelengths, for example 266 or 355 nm, in which particularly transition metal cations such as Mn^{2+} , Eu^{2+} and/or Ti^{3+} originate these characteristic emission bands (Bozlee et al., 2005). The lifetime of these processes have been found to span a wide range from nanoseconds (10^{-9} s) to milliseconds (10^{-3} s), indicating the occurrences of both fluorescence and phosphorescence processes (Reisfeld et al., 1996; Gaft et al., 2001; Bozlee et al., 2005). The luminescence processes in minerals are not yet well understood due to complex atomic interactions within the crystal structures. However, because many isolated minerals have been shown to be capable of fluorescent emission they may contribute to fluorescence of certain types of dust within atmospheric aerosols and, therefore, should be considered as possibly introducing interference for FBAP detection in some situations. In many cases the timescale of emission (i.e. for phosphorescence) may be longer than detectable by online instrumentation, however, and so would not contribute significantly to detected FBAP numbers in these cases (e.g. Reisfeld et al., 1996).

PAHs are omnipresent in the environment and are known to have severe impacts on human health (e.g. Dix and Marrett, 1983; Nielsen et al., 1984). In the atmosphere they can occur primarily in the gas-phase as products of incomplete combustion (highly volatile 2-ring compounds, i.e. naphthalene), absorbed on organic aerosol particles such as soot, (low-volatile 5-ring compounds, i.e. benzo[*a*]pyrene) or as semi-volatile species present in both gas and particle phases (3- and 4-ring compounds, i.e. phenanthrene) (e.g. Finlayson-Pitts and Pitts, 2000; Slowik et al., 2007). LIF and a number of other methods have been utilized to identify and quantify PAHs in atmospheric aerosols (e.g. Niessner and Krupp, 1991; Panne et al., 2000). Pure PAHs are known to be highly fluorescent (quantum yields up to 1.0) over a wide spectral range (300–460/310–540). In the natural environment they usually occur as mixture of different compounds and produce complex EEMs (Kumke et al., 1995). PAHs are also known to be particularly enriched on the surface of soot particles from biomass burning and fuel combustion. When excited by strong laser light the mixture of PAHs in combustion aerosols can be analyzed and quantified (Aizawa and Kosaka, 2008, 2010). However, it has also been observed that fluorescent emission by PAH coatings on organic aerosols is weak due to efficient quenching by the bulk material (Lewitzka and Niessner, 1995; Panne et al., 2000).

3 Materials and methods

3.1 Chemicals and materials

Samples of humic acid (2S101H) and fulvic acid (1S101F) were purchased from International Humic Substance Standards (IHSS; St. Paul, MN, USA). Arizona test dust

(ISO 12103-1) was obtained from Powder Technology Inc (Burnsville, MN, USA). Kaolin was purchased from Sigma Aldrich (K7375). Ash was obtained from wood burning (spruce, specially designed wood burning facility). Diesel soot (SRM 2975) was purchased from National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA). Biomass burning soot was obtained by burning a sample of spruce and directly sampling the black smoke on a clean glass cover slide held at approximately 10 cm away from the flame in direct flow of the smoke. *Saccharomyces cerevisiae* was purchased as baker's yeast from a local supermarket and used immediately (within one day). Solvents were obtained from Merck (Darmstadt, Germany). All other chemicals were purchased from Sigma Aldrich. All purchased chemicals were used as delivered.

3.2 Fluorescence spectroscopy

All fluorescence spectra were recorded on a LS 45 Luminescence Spectrometer (Perkin Elmer, Inc.; Waltham, MA, USA). The instrument is equipped with a pulsed xenon lamp, two Monk-Gillieson-type grating monochromators², and a gated photomultiplier tube (PMT) detector. Wavelength-dependent variation in light source intensity (*I*) was corrected by an internal reference light path with dedicated PMT. Measurements were initiated after a minimum of approximately 30 min of instrument warm-up time to ensure lamp stability. The instrument was operated using FL WinLab software (Perkin Elmer, Inc.) and its performance was controlled (every ~10th spectrum analyzed) by means of a software validation utilizing Raman and Rayleigh signal positions as well as the signal-to-noise ratio. All spectra were recorded at a PMT voltage of 650 V, a scan speed of 600 nm min⁻¹, and using excitation and emission slit widths of 10 nm at room temperature. EEMs were recorded for excitation wavelengths from 230–650 nm (5 nm increments) and emission wavelengths from 270–700 nm (0.5 nm increments). To avoid detector saturation for highly fluorescent samples a blocking filter (Perkin Elmer, Inc.) was utilized to reduce the emission intensity. This filter is a metal plate with holes bored through to allow 1 % of light to be transmitted, but without introducing wavelength-dependent effects. Spectral analysis was performed using Igor Pro (Wavemetrics, Inc.; Portland, OR, USA).

Samples were investigated in the solid (powder), solution, or suspension states. Solvated and suspended samples were placed in a 10 × 10 × 40 mm UV quartz cuvette (Hellma Analytics, Müllheim, Germany) and measured immediately (within 15 min). The concentration of fluorophore within solution or suspension was deliberately low in order to avoid inner filtering and concentration-related quenching effects. For highly dilute samples the concentration is linearly correlated with fluorescence intensity according to the

²e.g. <http://gratings.newport.com/information/handbook/chapter6.as>

Beer-Lambert law. Suspensions of insoluble materials were prepared by adding fluorophore analyte ($0.2\text{--}0.4\text{ mg ml}^{-1}$) to phosphate-buffered saline (PBS) at pH 7.2. PBS was prepared from ultrapure water (MilliQ, $18.2\text{ M}\Omega$) and purified by sterile filtration before use. A magnetic stirrer was utilized to stir the sample in the cuvette during the measurement to avoid sedimentation. Solutions of most soluble fluorophores were prepared in PBS, however, pyrene, naphthalene, phenanthrene and chlorophyll-*b* were solvated in *n*-hexane due to their hydrophobicity. The concentration of analyte used was $3\text{ }\mu\text{mol l}^{-1}$, but was increased by a factor of 10 or 100 for weakly fluorescent samples until fluorescent emission was detectable. Background correction of solvated or suspended samples was performed by taking the difference of fluorescence spectra of solvent with and without sample.

Powdered materials were analyzed with a Front Surface Accessory for the spectrometer (Perkin Elmer, Inc.). With this technique several milligrams of powder were placed onto the black surface holder and placed as a vertical plane into the spectrometer such that the illumination angle shining horizontally onto the sample surface shows maximum fluorescence intensity. The illumination spot size on the samples was $\sim 60\text{ mm}^2$. For weakly fluorescent powders, which exhibited no clear emission peaks, fluorescence properties were qualitatively dominated by light leakage (e.g. NaCl, Fig. S6 in the Supplement) and appear as steep-walled valleys of emission between the 1st and 2nd order elastic scattering lines. The magnitude of “emission” on the left and right of the 1st order scattering peak are similar, however, confirming that most of the signal in the fluorescence region is spurious. The magnitude of this effect correlated with the color of the sample powder, with white samples exhibiting a higher background than colored samples. This suggests that light absorption within the samples reduces internal reflection and mitigates the background effect. We, therefore, assume that all radiation arriving to the detector at wavelengths shorter than the incident wavelength is due either to stray background light, or light leakage past the monochromators. EEMs of dry powder samples were normalized by the intensity of light leakage in order to provide a comparable scale of fluorescence intensity across all solid samples. Often fluorescence spectra of this type are normalized to the height of the 1st order scattering peak, but this was not possible here, because the PMT was saturated at this wavelength for some samples. For each EEM, a constant normalization factor (NF) was determined by taking the mean of the measured light leakage intensity values along a line 40 nm above the center of the excitation line (as shown in Fig. S1 in the Supplement) and dividing the entire matrix by this NF. Doing so preserves all qualitative features within measured EEMs, but normalizes the absolute intensity of fluorescence for comparison. Each 1-D spectrum shown with single excitation wavelength (Fig. 6) was extracted from the normalized EEMs.

As a result of differing normalization procedure quantitative comparison of emitted fluorescence intensity between

wet and dry samples is not possible by this technique, while qualitative features are consistent and comparable. Further details concerning the normalization procedure can be found in the supplement (Fig. S1 in the Supplement) along with examples of non-normalized spectra (Figs. S4 and S5 in the Supplement).

3.3 Fluorescence microscopy

The fluorescence microscopy images were taken on a BZ-9000 Fluorescence Microscope (Keyence, Inc., Osaka, Japan). The instrument was equipped with a super-high-compression mercury lamp (120 W) and a 2/3-inch, 1.5 mega pixel monochrome CCD. The following fluorescence filters were used to take images in different spectral ranges: OP-66834 DAPI-BP ($\lambda_{\text{ex}} = 360/20\text{ nm}$, $\lambda_{\text{Dichroic}} = 400\text{ nm}$, $\lambda_{\text{Absorp}} = 460/25$), OP-66836 GFP-BP ($\lambda_{\text{ex}} = 470/20\text{ nm}$, $\lambda_{\text{Dichroic}} = 495\text{ nm}$, $\lambda_{\text{Absorp}} = 535/25$), OP-66838 TexasRed ($\lambda_{\text{ex}} = 560/20\text{ nm}$, $\lambda_{\text{Dichroic}} = 595\text{ nm}$, $\lambda_{\text{Absorp}} = 630/30$). Filter specifications are represented as wavelength and peak width (λ/FWHM).

3.4 Ambient sampling

Ambient samples of airborne particulate matter were taken using a single stage impactor (PIXE International Corp. Tallahassee, FL, USA; nozzle diameter 0.65 mm, distance between nozzle and impaction plate 3 mm, flow rate 1 l min^{-1} , cut-point $\sim 0.6\text{ }\mu\text{m}$) (Russell et al., 2002). Samples were taken on the roof of the Max Planck Institute for Chemistry building on the university campus in Mainz, Germany. Sampling was performed on a clean round glass cover slide (diameter 15 mm, thickness 0.13–0.15 mm) and immediately used for microscopy investigation.

4 Results and discussion

4.1 EEMs of pure biological standards

Following on the summary presented in Table 1 we applied fluorescence spectroscopy to a selection of pure biological fluorophores, particularly those molecules that are considered to be most relevant within ambient PBAPs. Figure 2 shows EEMs for a selection of biological fluorophores, and spectra for further compounds can be found in Fig. S2 in the Supplement. The selection covers protein autofluorescence by means of all three fluorescent amino acids and two common proteins: bovine serum albumin (BSA) and ovalbumin (OVA). Coenzymes constitute a diverse class of highly fluorescent compounds and are represented by several examples (i.e. pyridoxine, NADPH, folic acid and riboflavin). The major biopolymers are represented by cellulose and chitin as well as ferulic acid as a proxy for lignin. As an example for vitamins the sterol compound ergosterol

is shown. Chlorophyll-*b* is an example of the group of porphyrin fluorophores.

It is well known that the fluorescence properties of a fluorophore (i.e. spectral parameters and intensity) are highly dependent on its molecular environment, particularly: pH, fluorophore concentration and interaction with other compounds. All measurements in the present study were, therefore, performed under consistent, defined conditions. Pure compounds were utilized for solid state investigations. For samples in solution or suspension, defined pH and fluorophore concentrations were chosen to ensure comparability of the spectral parameters (see Sect. 3.2 for more details). The molecular environment within biological samples, such as cells and tissue, is much more dynamic and complex than in a defined test environment. As a result the position and intensity of fluorescence modes shown may differ from in situ measurements within actual biological samples. Bearing this complexity in mind, however, we suggest that the presented results can be seen as a general characterization and overview, providing a collection of fluorescence landmarks for different compounds relevant to atmospheric PBAP.

Under atmospheric conditions aerosols can contain wide ranges of moisture content, from extremely dry particles to nuclei suspended in cloud water droplets. Hydration state has also been found to significantly influence the fluorescence properties of a fluorophore (Lakowicz, 1999; Pöhlker et al., 2012). Accordingly, spectra for most materials in this study were each recorded in solid, solution, and suspension phases, respectively. All EEMs of solvated or suspended samples show clearly defined fluorescence modes with only weak interference by light scattering effects. In EEMs of solid state compounds fluorescence modes, by contrast, are usually less clearly resolved and exhibit stronger light scattering, broader fluorescence modes, and an elevated fluorescent background. This background effect is more pronounced in white samples with very weak fluorescence modes, and we propose that the light detected as fluorescence is simply a function of light leakage (i.e. the tails of the 1st and 2nd order elastic scattering peaks cause a significant enhancement in signal in the absence of true molecular fluorescence). For this reason, the absolute intensity of all powdered samples have been normalized, as discussed in Sect. 3.2. Moreover a shift in location of emission peaks from solid- and solution-phase samples (e.g. cellulose in Figs. 2m and S2k in the Supplement, riboflavin in Figs. 2f and S2f in the Supplement) was also observed.

Molecular fluorescence generally provides less characteristic spectra than other methods of spectroscopy, making detailed chemical analysis impossible in most cases. Mixing of emission peaks from different fluorophores has been particularly well described for bacteria (Marose et al., 1998). Different compounds also exhibit large variations in the intensity of fluorescent emission. Compounds such as pyridoxine and neopterin emit intensely even in low concentrations, for example, whereas fluorescent emission of

phenylalanine and ferulic acid exhibited weak emission in all cases. The intensity of fluorophores frequently discussed within the bioaerosol detection community, such as tryptophan, NADPH and riboflavin, can be characterized among the compounds shown as having a medium level of fluorescent emission. However, fluorescence intensity is a complex function of various parameters such as concentration, extinction coefficient at λ_{ex} and quantum yield at λ_{em} as well as influences by the molecular environment. Accordingly, only semi-quantitative comparison of intensity levels is possible based on the presented results.

4.2 Spectral mapping of biological fluorophores

As a way of summarizing the properties of key biofluorophores, Fig. 3 highlights fluorescent emission peaks (and corresponding mode widths), but ignores differences in intensity among the compounds. Most compounds chosen exhibit peaks at relatively short (<500 nm) wavelengths of excitation and emission and appear in the lower left of Fig. 3 (i.e. $\lambda_{\text{ex}} = 250\text{--}400$ nm, $\lambda_{\text{em}} = 300\text{--}500$ nm). Riboflavin and chlorophyll-*b*, however, uniquely exhibit significantly larger $\Delta\lambda_{\text{Stokes}}$ (i.e. the right side of the plot). Furthermore, it is obvious that the modes of many fluorophores cluster in two “emission hotspots”. Hotspot I ($\lambda_{\text{ex}} = \sim 280$ nm; $\lambda_{\text{em}} = 300\text{--}530$ nm) is dominated by amino acids and proteins, biopolymers such as cellulose and chitin, and several coenzymes (i.e. pyridoxamine, riboflavin). Hotspot II ($\lambda_{\text{ex}} = 330\text{--}360$ nm; $\lambda_{\text{em}} = 390\text{--}460$ nm) shows emission from the majority of coenzymes (i.e. NADPH, pyridoxine, folic acid), the proxies for lignin (ferulic acid, phenylcoumarin) and DPA. Note that experimental data for DPA are not available through the present study, but were added to Fig. 3 from Sarasanandarajah et al. (2005) because of its importance in bacterial spores. Both emission hotspots directly correspond with the operational range of most bioaerosol detectors, as shown in Fig. 1. Excitation at ~ 280 and ~ 360 nm are particularly important wavelengths to excite many atmospherically important biofluorophores. Beyond these established excitation bands, however, the development of relatively cheap diodes lasers for consumer electronic technology (e.g. Blu-Ray DiscTM, $\lambda_{\text{ex}} = 405$ nm) within the last several years has opened up new possibilities for the design of FBAP detection instrumentation. Extensive laboratory studies have not yet been performed to validate the use of these new sources for FBAP detection and will be necessary before interpretation of complex aerosol samples will be meaningful. Figure 3 shows that the 405 nm excitation band is located in a spectral region where only relatively few biofluorophores (e.g. riboflavin, chlorophyll-*b* and chitin) are efficiently excited (see also Fig. S6 in the Supplement). Riboflavin, however, is ubiquitous within PBAP and the relative selectivity of $\lambda_{\text{ex}} = 405$ nm may eventually prove particularly successful at PBAP detection.

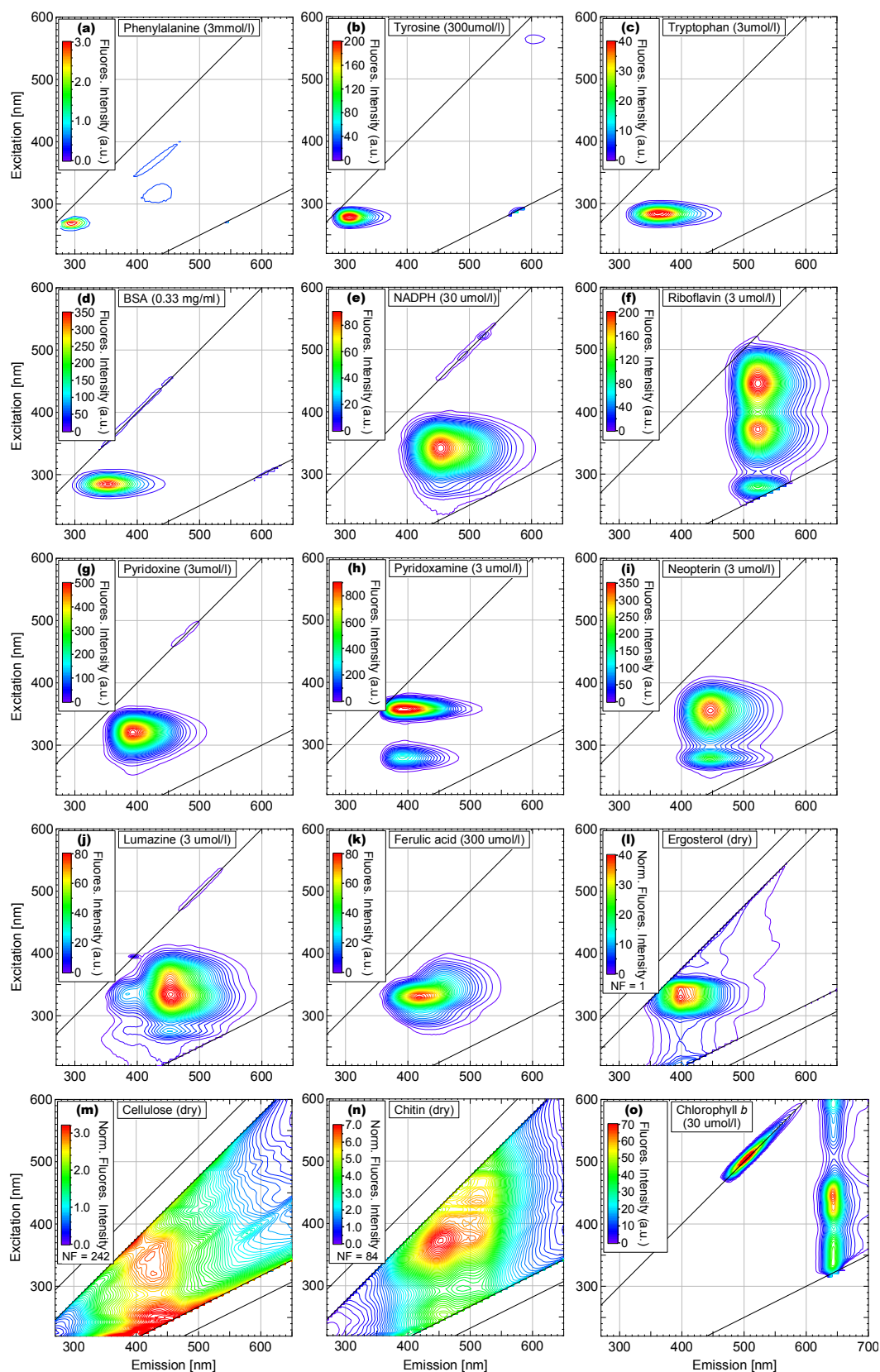


Fig. 2. EEM contour profiles for selected pure biological fluorophores in solid or solvated state. Intensity color scale has been adjusted to intensity of individual components. All EEMs are normalized as discussed in text (Sect. 3.2). Normalization factor (NF) is reported for each solid-state sample. Lower NF indicates higher fluorescence intensity. Note change in x-axis scale for chlorophyll-*b*.

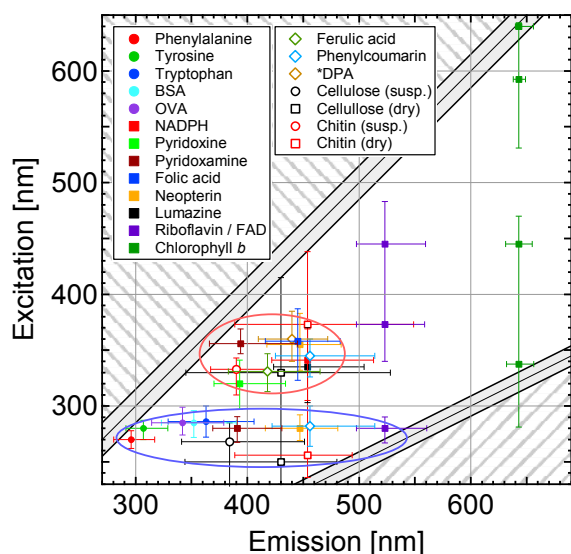


Fig. 3. Overview EEM mapping the spectral position of biological fluorophores. Protein fluorophores are represented by solid circular markers, coenzyme fluorophores by solid square markers and structural fluorophores by open markers. Full width half max (FWHM) of emission peaks is represented by horizontal and vertical bars. Differences in relative fluorescence intensity are neglected. For multimodal fluorophores all modes are shown. Data refer to measurements of solved fluorophores in PBS. For cellulose and chitin results of solid state and suspension state spectra are shown. * Data for DPA are taken from Sarasanandarajah et al. (2005).

Accordingly, a variety of fluorophores within atmospheric particles are potential targets for these instruments. It is clear that PBAPs comprise potentially many different fluorescent constituents at the same time (see Table 1). For example, bacteria have been shown to contain a mixture of fluorescent coenzymes such as pyridine, flavin, pyridoxine and pteridine compounds (Dalterio et al., 1987; Wlodarski et al., 2006). It can thus be assumed that ambient FBAP detection based on autofluorescence usually detects a mixed signal from potentially many different fluorophores. In contrast to technical applications where autofluorescence of NADH is directly used to monitor the metabolic status of yeast or bacteria in bioreactors (Marose et al., 1998; Johansson and Liden, 2006), the high diversity of organisms, debris and other compounds in the atmosphere makes a selective assignment of the measured fluorescence to a certain fluorophores difficult. This suggestion is not new, however, and several groups have investigated the use of varied post-detection techniques (e.g. principle component analysis) to elucidate further information from ambient PBAP (e.g. Pinnick et al., 2004; Pan et al., 2010, 2011). The only coenzyme which is largely free from interference by other biofluorophore is riboflavin and related flavoproteins (Koenig and Schneckenburger, 1994).

Figure 3 also shows that the fluorescence signal of the proteins sampled (BSA, OVA) is very similar to the tryptophan

signal. Because the fluorescent emission of tyrosine and phenylalanine have a smaller $\Delta\lambda_{\text{Stokes}}$ than tryptophan this may suggest that these amino acids do not contribute significantly to the overall fluorescence signal in native proteins where they occur (BSA: 30 Phe residues, 21 Tyr, 3 Trp; Hirayama et al., 1990; OVA: 15 Phe, 10 Tyr, 3 Trp; Nisbet et al., 1981). In addition, it can be seen that the modes of BSA and OVA are slightly red-shifted with respect to tryptophan, which is due to the specific influence of the microenvironment of the folded protein (Lakowicz, 1999). The position of each individual fluorescence mode in Fig. 3 is determined by the structural nature of the corresponding fluorophore, such as the extent of π -bond conjugation or influence of electron donating or withdrawing groups. Aizawa et al. (2008) provides an overview EEM map showing the spectral position of PAHs depends on the size of their π -bond system. Figure 3 displays a similar map for biofluorophores presented here. Sampled compounds that contain small monocyclic fluorophore groups such as phenyl (phenylalanine), phenol (tyrosine) and indol (tryptophan) are excited by radiation in the UV and blue visible range ($\lambda_{\text{ex}} = 260\text{--}290$ nm; $\lambda_{\text{em}} = 290\text{--}370$ nm). Compounds with larger fluorophores such as pyridine (i.e. pyridoxine and dipicolinic acid), nicotinamide (NAD(P)H) and pteridine (i.e. folic acid, lumazine) are spectrally more red-shifted ($\lambda_{\text{ex}} = 280\text{--}370$ nm; $\lambda_{\text{em}} = 390\text{--}470$ nm). Compounds containing large aromatic structures such as flavins and porphyrins (chlorophyll-*b*) are even further red-shifted ($\lambda_{\text{ex}} = 280\text{--}640$ nm; $\lambda_{\text{em}} = 510\text{--}660$ nm). In addition, increasing the size of the π -bond system opens more potential excitation pathways for electron transition, thus extending the fluorescence band over a much broader range of excitation wavelengths (i.e. for riboflavin and chlorophyll-*b*).

In addition to the investigation of fluorescence properties of pure fluorophores, the analysis of biological standard compounds (i.e. bacteria and pollen) promises to help to explain the selectivity of online FBAP detection. A full investigation of microorganism fluorescence is beyond the scope of this text, but *Saccharomyces cerevisiae* (baker's yeast) is shown in Fig. 4 as an example of an actively metabolizing organism and surrogate for atmospheric PBAP. The fluorescence properties of this organism are well described, due to its various industrial applications. According to Marose et al. (1998) tryptophan, NAD(P)H, pyridoxine, pyridoxal 5'-phosphate and flavins are the dominating fluorophores in *S. cerevisiae*. The ratio of their relative emission intensities has been used to monitor the metabolic status of the cells and to estimate the overall biomass. The EEM in Fig. 4 shows a fluorescence pattern corresponding with several of the previously mentioned fluorophores. A strong peak at 285/350 nm ($\lambda_{\text{ex}}/\lambda_{\text{em}}$) is likely caused by proteins, particularly tryptophan residues, whereas the signal at 230/350 nm appears to be also protein fluorescence due to deep UV excitation (Bhartia et al., 2008). An emission signal occurs at 350/450 nm that is most probably NAD(P)H, although pteridin coenzymes may also

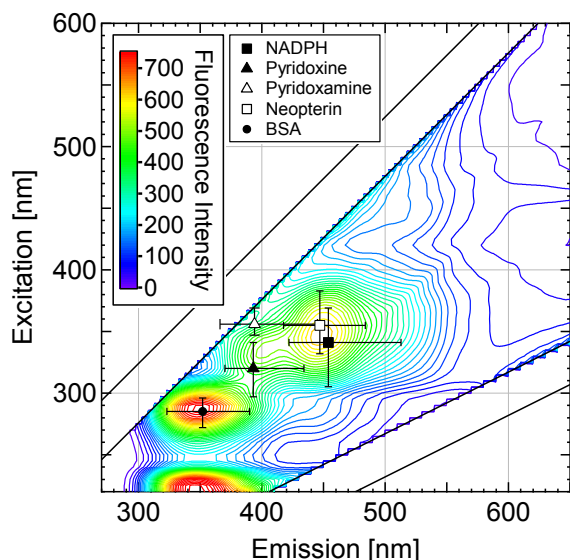


Fig. 4. EEM contour profile for *Saccharomyces cerevisiae* (baker's yeast). Spectral positions of selected pure fluorophores are shown for comparison. BSA is chosen as a general proxy for protein.

contribute to this signal (Michal, 1999; Kopczynski et al., 2005). A shoulder at 340/410 nm is likely caused by pyridoxine coenzymes. However, no flavin signal was observed. For purpose of illustration the spectral positions of the corresponding fluorophores (based on the measurements in Fig. 2) are projected into the EEM. In further studies EEMs will be used to characterize the fluorescence properties of various bioaerosol standard particles.

4.3 EEMs of potential interferents

In order to estimate the relative interfering potential of the compound types discussed in Sect. 2.7 fluorescence spectra of HULIS (humic and fulvic acid), PAHs (pyrene, naphthalene, phenanthrene, fluoranthene), mineral dust (kaolin and Arizona test dust) and soot (biomass burning and diesel soot) were investigated. Figure 5 shows EEMs of selected compounds, with additional samples shown in Fig. S3 in the Supplement, and can be grouped into two qualitatively distinct categories: (I) EEMs with clearly resolved fluorescence modes (e.g. all solutions and suspensions, dry PAHs) and (II) EEMs without clear modes, but with significant light emission over a wide spectral “plateau” (all dry samples with the exception of PAHs).

EEMs of HULIS surrogates which show defined fluorescence modes are consistent with results from DOM analysis. Humic and fulvic acids in solution exhibit broad modes (390/490, 450/520), but with relatively low intensity and resemble fluorescence behavior of DOM (e.g. Chen et al., 2003; Wedborg et al., 2007). In contrast, PAH compounds show strongly fluorescent emission, the peak location of which corresponds to the size of the π -bond

system. In general, PAH samples in the solid state can be excited efficiently over a wide wavelength range (230–390 nm), whereas samples in solution show relatively narrow, defined excitations.

In contrast to compounds that show well defined modes of fluorescent emission, EEMs of dry powders such as mineral dust (kaolin and Arizona test dust) and HULIS (humic and fulvic acid) exhibit broad, featureless emission over an extended spectral area (230–500/350–600) with steep increase near the 1st and 2nd order scattering signals. We observed that the relative intensity level of this plateau correlates with the visible color of the samples. For example, the broad emission intensity of kaolin (white, Fig. S5h in the Supplement) is higher than that of fulvic acid (yellow, Fig. S5b in the Supplement), which is higher still than that of humic acid (brown, Fig. S5a in the Supplement). We further tested additional white powder compounds (i.e. NaCl and glass microspheres) not expected to show any classical fluorescent emission in this range. These also exhibit EEMs that are qualitatively almost identical with the other dry powder samples, with intensity levels similar to that of the white kaolin powder. This suggests that the featureless emission observed from these powder samples is spurious and does not originate as molecular fluorescence. We thus suggest that the “plateau” effect is a result of light leakage due to imperfect monochromators within the spectrofluorometer and that light arriving at the detector at wavelengths in between the dominant scattering lines largely represents the transmission tails of the elastic scattering signals. The presence of this elevated fluorescence background is minimal for compounds that exhibit defined fluorescence modes, is magnified in powdered samples with high surface area for reflection, and is highest in samples whose color reduces light absorption. In order to qualitatively eliminate these effects from EEMs of dry materials the spectra have been normalized as described in Sect. 3.2. Normalized spectra are shown in Figs. 5 and S3 in the Supplement, while a selection of raw EEMs can be found in Figs. S4 and S5 in the Supplement. According to De Souza Sierra et al. (1994) it is critical to correct fluorescence spectra of solid particulate matter for stray light transmitted by the excitation monochromator and scattered by the sample to ensure comparability. These normalized spectra offer the ability to semi-quantitatively compare fluorescent intensities from different materials. While quantitative comparisons within the list of dry materials or within the list of wet materials (solution, suspension) is possible, differences in normalization procedure between the two is quantitatively challenging by this technique.

4.4 Comparison of autofluorescence from biofluorophores and interfering species

Figure 6 shows a summary of selected biological and non-biological compounds, excited at one of two wavelengths: either 280 nm (Fig. 6a and c) or 355 nm (Fig. 6b and d). These

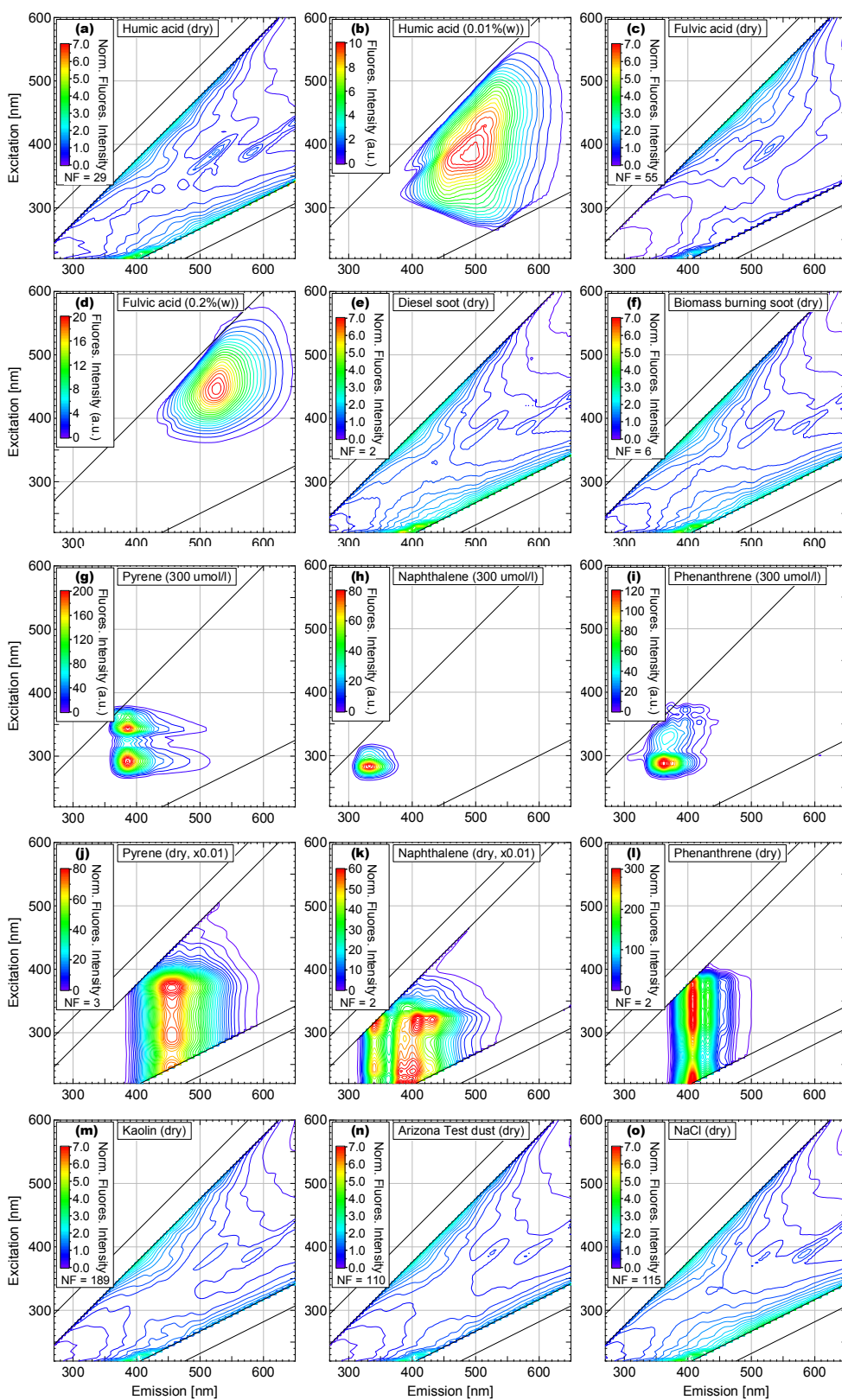


Fig. 5. Normalized EEM contour profiles for selected interferences in solid state and/or solution. Intensity color scale has been adjusted to intensity of individual components. All EEMs are normalized as discussed in text (Sect. 3.2). Normalization factor (NF) is reported for each solid-state sample. Lower NF indicates higher fluorescence intensity.

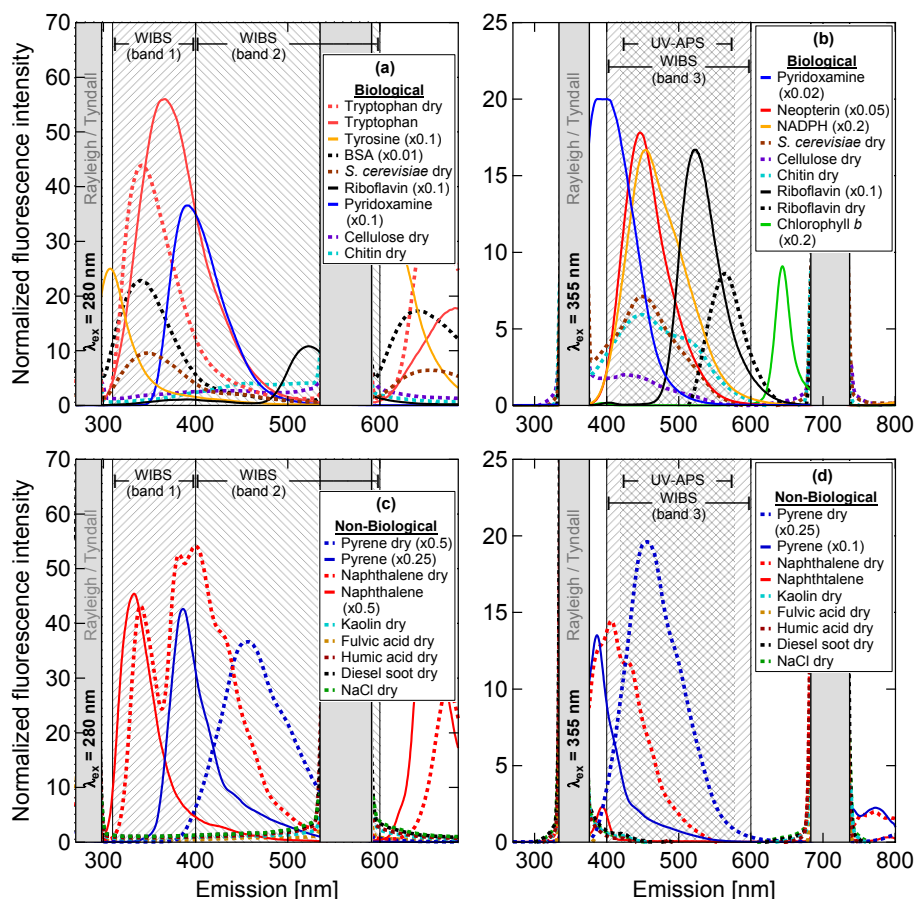


Fig. 6. Normalized fluorescence emission spectra of biofluorophores and potential interferences for selected excitation wavelengths λ_{ex} : (a) emission spectra of biological fluorophores at $\lambda_{\text{ex}} = 280$ nm; (b) emission spectra of biological fluorophores at $\lambda_{\text{ex}} = 355$ nm; (c) emission spectra of potential interferences at $\lambda_{\text{ex}} = 280$ nm; (d) emission spectra of potential interferences at $\lambda_{\text{ex}} = 355$ nm. Dashed lines indicate samples in dry state, solid lines indicate samples in solution. Spectra were scaled in some cases to fit on same y-axis. For example, pyridoxamine in (a) peaks at ~ 350 a.u., and not ~ 35 a.u. as shown.

wavelengths of excitation were chosen to highlight observed fluorescent emission within the two primary bands of fluorophores discussed in Sects. 4.1–4.2 and because they represent or approximate the excitation sources of two commercially available FBAP-detection instruments (UV-APS and WIBS). Detected emission ranges for both instruments are shown by shaded regions on the plot, although it is important to note that the second WIBS excitation source produces radiation at 370 nm and not at 355 nm (corresponding to UV-APS) as shown in Fig. 6b and d. Colored traces show individual fluorophores selected to represent key molecules for FBAP detection from Table 1. This set of summary plots enables a semi-quantitative comparison of fluorophore importance, in particular regarding the influence of potential interferences. Fluorescence properties of certain biofluorophores have been investigated in various studies, however, much less information is available regarding the intercomparison between very different compound types (in particular biofluorophores and non-biological fluorophores). Figure 6 provides a simple approach to fill this gap.

In the raw (non-normalized) spectra (Figs. S4, 5 in the Supplement) the “plateau-like” emission of materials such as fulvic acid, kaolin, NaCl, and cellulose can be easily seen, while the normalized spectra of such materials (Fig. 6) show only weak fluorescence. The normalized spectra of NaCl can be used as a surrogate post-normalization background, because it is not expected to fluoresce at wavelengths used here and because it exhibits the most fluorescence intensity of the “non-fluorescent” powders. As such, the emission spectrum for NaCl retains a measureable background when excited at 280 nm, increasing from a minimum $I \sim 1.0$ at $\lambda_{\text{em},350}$ to ~ 2.0 at $\lambda_{\text{em},550}$, while it exhibits a steep valley profile when excited by 355 nm radiation, but with $I < 0.5$ for all emission between 400 and 650 nm. Small peaks in emission spectra of weakly fluorescing compounds can consistently be seen at ~ 340 , 422, 459, and 485 nm for $\lambda_{\text{ex},280}$ and at 420 and 485 nm for $\lambda_{\text{ex},355}$. The most prominent of these appear as subtle diagonal lines between the 1st and 2nd order elastic scattering lines on EEMs of weakly fluorescing dry powders (e.g. Figs. 2m and 5a,c,m–o). These

do not appear for fluorescent compounds, however, and are assumed to be a product of weakly interfering elastic scattering processes. In contrast to weakly fluorescing molecules, emission by biofluorophores and PAH compounds show clearly resolved peaks before and after normalization, and we roughly categorize these to be fluorescent in all cases. In general it can be stated that position and linewidth of fluorescence emission peaks in EEMs are independent of λ_{ex} due to radiative electron relaxation from the lowest vibrational level of the first excited electronic state (Sect. 1.2). Thus, fluorescence signals are indicated by vertical lines or individual peaks in EEMs as presented here. Further, the only parameter that varies along the excitation axis (vertical) is the fluorescence intensity, which does so as a function of the absorption spectrum of the fluorophore. In contrast, signals of other light scattering effects (i.e. Rayleigh, Tyndall and Raman) are a function of λ_{ex} and accordingly can be seen as diagonal lines within the EEMs (Coble et al., 1990).

Figure 6a and b highlight the fact that a wide variety of molecules associated with biological organisms fluoresce. Moreover the compounds shown in Fig. 6, which were chosen to represent larger groups of molecules from Table 1, underline the previously mentioned suggestion that a large variety of fluorophores contribute to the overall fluorescence signal detected by online FBAP instrumentation. Among the nine biomolecules chosen, fluorescence is generally high. The only exceptions to this are cellulose and chitin. Cellulose shows essentially no fluorescence (similar emission profile of NaCl) at $\lambda_{\text{ex},280}$, but weak fluorescence at $\lambda_{\text{ex},355}$ ($I \sim 1$ at $\lambda_{\text{em},426}$). Chitin shows only marginally increased fluorescence for $\lambda_{\text{ex},280}$ compared to cellulose, but significant emission for $\lambda_{\text{ex},355}$ ($I \sim 5.9$ at $\lambda_{\text{em},426}$ and ~ 4.2 at $\lambda_{\text{em},515}$). All other samples of biological fluorophores and material chosen exhibit relatively high intensity peaks in each emission spectrum shown in Fig. 6. Amino acid peaks, especially tryptophan, showed fluorescent emission among the most intense of compounds sampled. Samples of isolated protein and of the *S. cerevisiae* each showed similar peak placement with tryptophan indicating that tryptophan is the major and crucial fluorophore in proteins.

In contrast, most of the seven non-biological samples shown in Fig. 6c and d show very weak fluorescence. Pyrene and naphthalene, shown here as examples of PAH fluorescence, are the only exceptions to this trend and show sharp peaks in the range of 333–456 nm in the emission spectra for both excitation wavelengths, where I : 54–172 for $\lambda_{\text{ex},280}$ and 2–140 for $\lambda_{\text{ex},355}$. Strong fluorescent emission of pyrene is observed for $\lambda_{\text{ex},280}$ and $\lambda_{\text{ex},355}$ whereas naphthalene shows only fluorescence when excited at 280 nm. Moreover, large differences in λ_{em} can be observed between solid state and solution samples, for example pyrene in *n*-hexane exhibits $\lambda_{\text{em},280} = 387$ nm, while in the solid state exhibits $\lambda_{\text{em},280} = 457$ nm. As mentioned, NaCl is expected to exhibit no emission of classically-termed fluorescence, but four of the other compounds chosen show fluorescent emission

levels at or below the level of NaCl for each excitation wavelength chosen in Fig. 6. This indicates that, while mineral dust has been shown to fluoresce in some circumstances, kaolin fluorescence is insignificantly weak compared with the intensity of the biofluorophores measured here. Fulvic and humic acids analyzed in this study also exhibited very weak fluorescence. This could indicate that HULIS may not cause significant interference within FBAP measurements because of a comparatively low level of fluorescent intensity compared to biological material. However, these effects are complicated and contain a high degree of uncertainty (see Sect. 2.7). In particular, fluorescence from single particles may exhibit different trends from that of the bulk. While pure PAH compounds were observed to fluoresce intensely, diesel and biomass burning soot that likely contain PAH molecules both show emission levels below the surrogate NaCl background. While still a preliminary determination, the spectra of these soot samples corroborate suggestions by Lewitzka and Niessner (1995) and Panne et al. (2000) that soot exhibits only weak fluorescence due to inner quenching effects.

For excitation at 355 nm fluorophores such as NAD(P)H, pyridoxamine, neopterin, riboflavin, chitin and cellulose likely contribute to wavelength-integrated fluorescence detection by UV-APS ($\lambda_{\text{em}} = 420\text{--}575$ nm) and WIBS ($\lambda_{\text{em}} = 400\text{--}600$ nm) instruments, but this depends largely on exact instrumental optics and operational emission thresholds. Moreover a large number of other molecules (i.e. secondary metabolites) emit in the same spectral region, as discussed in Sect. 2. Accordingly we suggest that within this spectral range an integrated fluorescence signal provides no clear selectivity for certain coenzymes (e.g. NAD(P)H) as indicators for viability, and users of such instrumentation should avoid the assumption that these molecules dominate the fluorescence signal in all cases. Neither UV-APS nor WIBS instrument is likely to detect chlorophyll, because of its large $\Delta\lambda_{\text{Stokes}}$. The WIBS channels measuring emission after $\lambda_{\text{ex},280}$ may be more sensitive to influence from PAHs when the molecular environment in the sampled atmospheric particle approximates that of the solution-phase PAHs presented here. However, for excitation at 280 nm, the region exemplified by the first WIBS emission band ($\lambda_{\text{em}} = 310\text{--}400$ nm) suggests that fluorophores such as proteins and particularly tryptophan-containing proteins will dominate the emission signal in most cases, with some coenzymes (i.e. pyridoxamine, riboflavin) also important. The region exemplified by the second WIBS emission band ($\lambda_{\text{em}} = 400\text{--}600$ nm) at $\lambda_{\text{ex},280}$ will also likely detect protein and coenzyme fluorescence, however, to a lower extent.

The emission spectrum observed for *S. cerevisiae* suggests by extrapolation that viable PBAP can, not surprisingly, be expected to exhibit clear signals in both FBAP instruments. Given the fluorescence spectra shown, however, the WIBS may be more likely to see higher intensity for this organism in the first of two channels at $\lambda_{\text{ex},280}$.

5 Conclusions and outlook

In this study a systematic literature survey of key biological fluorophores which are relevant for atmospheric science is presented. The individual compounds are organized into major fluorophore classes and characterized by their biological role and abundance as well as by certain fluorescence properties. As a first approach the relative atmospheric importance of individual fluorophores or classes of fluorophores have been estimated. In addition, emission spectra have been measured as a function of excitation wavelength for various representative compounds. An overview of individual fluorophores shows that unique autofluorescence properties can indeed be useful for discrimination of biological material by online FBAP methods. Moreover key biological fluorophores can be collected into two relatively narrow excitation bands (~ 280 and ~ 360 nm). Each of these bands has been utilized by designers of FBAP instruments for bioaerosol detection.

Amino acids and proteins are among the most important fluorophores at $\lambda_{\text{ex},280}$, while some coenzymes (e.g. pyridoxamine, riboflavin) are also potentially important. Coenzymes such as NAD(P)H, pyridoxine compounds and pteridines are among the most highly fluorescent compounds at $\lambda_{\text{ex},355}$, but structural biopolymers (e.g. chitin, lignin) are also important. The complexity of overlapping spectra for fluorophores expected to be present in many classes of PBAP highlights the difficulty in making molecular determination of fluorescence origin from spectrally integrated signals detected in broad emission bands. Care should be taken, therefore, not to assume molecular identity from signals from instruments such as the UV-APS and WIBS. Further, the ability to determine cell viability for an unknown biological particle arriving at a detector may be difficult by single wavelength fluorescence alone, as has been suggested in depth previously (e.g. Pinnick et al., 2004). More work investigating standard particles in the laboratory are needed before uncertainties in these suggestions can be reduced, however.

A collection of atmospherically relevant non-biological compounds have also been analyzed and presented here. These data suggest that some non-biological materials could potentially contribute to fluorescence at wavelengths used by FBAP detectors (e.g. PAHs and SOA), whereas the majority of compounds suggested to cause interference (e.g. HULIS, mineral dust, soot) are less likely to contribute signal due to extremely weak intensity. SOA were not investigated here, because experimental procedures for producing oxidized aerosol in environmental chambers are beyond the scope of this text. Lab-generated SOA have been shown to fluoresce under certain conditions (Bones et al., 2010), though this has not been documented systematically within scientific literature. While SOA can dominate submicron particle concentrations, its contribution to supermicron particle mass is probably small. As such, its influence on the fluorescence signal of supermicron FBAP is likely also very

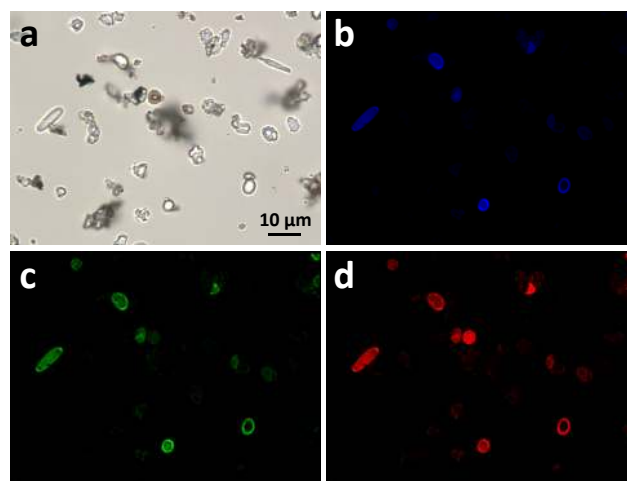


Fig. 7. Microscopy pictures of ambient aerosol sample. Illumination source: (a) brightfield, (b) $\lambda_{\text{ex}} = 340\text{--}380$ nm, $\lambda_{\text{em}} = 435\text{--}485$, (c) $\lambda_{\text{ex}} = 465\text{--}495$ nm, $\lambda_{\text{em}} = 510\text{--}560$, (d) $\lambda_{\text{ex}} = 540\text{--}580$ nm, $\lambda_{\text{em}} = 600\text{--}660$.

small, although this should be investigated further. PAHs, which often are intensely fluorescent, are largely present in atmospheric aerosols on the surface of soot particles, but the complex chemical environments of soot have been shown to largely quench fluorescence. As a result of this and the fact that soot also often peaks at submicron sizes PAHs are considered to contribute only minimally to FBAP number, though they could cause potential interference in some cases. Despite uncertainties based on this bottom-up approach of addressing fluorescent properties of selected compounds the results of this laboratory study suggest that fluorescent properties of ambient FBAP are likely to be dominated by biological material in most cases. However, the authors are aware of the fact that the results presented in this study can only serve as a first general approach to aspects of selectivity in FBAP detection. In particular, further investigation must address differences between bulk and single particle fluorescence (Hill et al., 2009), the characteristic fluorescence fingerprints of authentic bioparticles and the impact of different atmospheric conditions and aging.

As a proof of concept study we collected ambient aerosol in Mainz, Germany and other locations for investigation of autofluorescence via fluorescence microscopy. Initial observations show that particles that fluoresce most strongly also exhibit characteristic biological morphologies, based on visual evidence presented here (Fig. 7). These ambient studies need to be furthered in much greater detail, but again provide a similar conclusion as given by the laboratory fluorescence study presented here that ambient FBAP appear to be comprised at least heavily of particles from biological origin, consistent with previous observations reported by Pöschl et al. (2010) and Huffman et al. (2012). Further work is necessary and forthcoming in order to fill in important gaps in

understanding. For example, the fluorescence properties of standard microorganism types have been investigated in the laboratory under varying environmental conditions and will be presented in a follow-up study.

List of frequently used acronyms.

Acronym	Description
AA	amino acid
AFS	aerosol fluorescence sensor
AGE	advanced glycation end-product
APF	age pigment-like fluorophore
BP	band-pass
BSA	bovine serum albumin
BWA	biological warfare agent
CCN	cloud condensation nuclei
DAPI	4',6-diamidino-2-phenylindole
DNA	deoxyribonucleic acid
DOM	dissolved organic matter
DPA/CaDPA	dipicolinic acid/calcium dipicolinic acid
EEM	excitation-emission matrix
FAD	flavin adenine dinucleotide
FBAP	fluorescent biological aerosol particles
FISH	fluorescence in-situ hybridization
FMN	flavin mononucleotide
FWHM	full width half max
GFP	green fluorescent protein
HULIS	humic-like substances
IN	ice nuclei
LIF	light/laser-induced fluorescence
MAA	mycosporin-like amino acid
MS	mass spectrometry
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NAD(P)H	NADH and NADPH
Nd:YAG	neodymium-doped yttrium aluminium garnet
NF	normalization factor
OVA	ovalbumin
PAH	polycyclic aromatic hydrocarbons
PBAP	primary biological aerosol particles
PBS	phosphate-buffered saline
PCR/qPCR	polymerase chain reaction/quantitative polymerase chain reaction
Phe	phenylalanine
PM	particulate matter
PMT	photomultiplier tube
RET	resonance energy transfer
RNA	ribonucleic acid
SOA	secondary organic aerosols
Trp	tryptophan
Tyr	tyrosine

UV	ultraviolet
UV-APS	ultraviolet aerodynamic particle sizer
VBNC	viable but non-culturable
VOC	volatile organic compound
WIBS	wide issue bioaerosol sensor

Supplementary material related to this article is available online at:
<http://www.atmos-meas-tech.net/5/37/2012/amt-5-37-2012-supplement.pdf>.

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