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Automatic detection of airborne pollen: an overview

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Abstract Pollen monitoring has traditionally been carried out using manual methods first developed in the early 1950s. Although this technique has been recently standardised, it suffers from several drawbacks, notably data usually only being available with a delay of 3–9 days and usually delivered at a daily resolution. Several automatic instruments have come on to the market over the past few years, with more new devices also under development. This paper provides a comprehensive overview of all available and developing automatic instruments, how they measure, how they identify airborne pollen, what impacts measurement quality, as well as what potential there is for further advancement in the field of bioaerosol monitoring.

Keywords Automatic monitor · Pollen · Fungal spores · Methods

1 Introduction

Automatic pollen sampling holds the promise of techniques that are easier to standardise, can identify

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targets in real- or near-real-time, and that provide information considerably faster to users. It also significantly reduces the labour-intensive identification that current manual methods require (Crouzy et al., 2016; Sauliene et al., 2019; Oteros et al., 2020; Sauvageat et al., 2020). Real- and near-real-time information about current pollen and fungal spore concentrations is useful to a wide range of stakeholders. Although largely overlapping, the requirements of end-users for medical purposes, climate change analysis, crop forecasting, pest disease control in agronomy, or forecast modelling are not necessarily identical (Tummon et al., 2021). As an automatic pollen and fungal spore monitoring network is established across Europe, the techniques and standards applied should serve the diverse needs of all stakeholders, whichever domain they focus on. Indeed, the development of the majority of automatic monitoring devices has to date been driven by user needs, for example, to identify human pathogens in real time (Douwes et al., 2003).

This paper aims to describe the range of techniques used to monitor airborne pollen grains and fungal spores automatically, the real-time instruments currently available on the market or in development, how they can be used to obtain concentrations, as well as what taxa they should measure and what affects instrument accuracy. We respond to the

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main questions that may emerge when establishing an automatic monitoring network and present a set of requirements that measurement systems should ideally comply with. The information provided here is based on published research, experience from early adopters, and feedback from instrument manufacturers. For simplicity, the focus of this text is on pollen monitoring, however, the same concepts also apply to the detection of fungal spores and other large biological aerosols. It is also important to note that no single method can reasonably be expected to be able to identify all bioaerosol present in the atmosphere since they cover a huge size range from viruses in droplets (micrometres in size) through to pieces of plant debris (millimetres in size). Furthermore, the term real time is used to describe all cases where measurement systems can provide data within a matter of a few hours from the time the observation was taken. This thus also includes near real-time provision of data and only the term real time is used from here on.

Other papers in this special issue describe in more detail the reasoning and aims of the EUMETNET AutoPollen Programme, standards that are under development for automatic monitoring, site and network requirements, as well as the impact that the provision of real-time information will have across various domains. While Huffman et al. (2020) provide a relatively extensive review of bioaerosol monitors, here we provide a much more focused discussion of those instruments that can, or have the potential, to identify airborne pollen. We provide a comprehensive overview of how each instrument functions, how they identify pollen, what impacts measurement quality, and what taxa, from an end-user perspective, should be monitored across Europe.

2 What taxa should be sampled?

Currently, trained analysts using the standard manual method (EN16868: 2019) for monitoring pollen can identify a larger range of taxa than available automatic systems. This is mainly because these systems are still relatively new and their respective software have not yet been extended to include identification of all pollen or other possible particle types.

When designing a strategy to develop an automatic pollen monitoring network, one thus has to accept, at least until the ongoing developments are implemented, a certain sacrifice when introducing automation. This is, however, partly offset by the advantages of having high temporal resolution data available in real time and with well-characterised quality. This section describes the needs of various end-user groups in terms of information about different pollen and fungal spore taxa, thus providing a basis upon which priorities can be set for choosing a suitable system as well as to steer further development of automatic instruments (Table 1).

It is important to note that one single instrument may not need to be able to accurately determine all taxa on the list; precise counting and identification of a limited list of taxa may be of more value than a long list with high classification errors.

2.1 Medical needs-respiratory diseases

Pollen allergies affect between 15 and 40% of the European population, with pollen taxa of the Poaceae, Betulaceae, Oleaceae, Asteraceae and Cupressaceae families counting among the most frequent causes of these allergies (D'Amato et al., 1998; Heinzerling et al., 2009, Zuberbier et al., 2017). However, the main allergenic pollen taxa can vary from one biogeographical region to another (Asam et al., 2015; Smith et al., 2014), thus a list of the most important taxa for the medical field will differ to a certain extent from one area to the other. Other aspects, such as the pattern of sensitization, also need to be considered since this varies widely regionally (Jäger, 2011). For example, in North America, oak pollen is considered a primary allergen (Bernstein et al, 2021) but in Central and Northern Europe, among trees, birch pollen elicits the most allergic sensitizations. However, because of the high IgE cross-reactivity between the major birch PR10 allergens and those of other Fagales (Asam et al., 2015; Ipsen & Hansen, 1991), it is necessary to also monitor oak and beech pollen since they can extend the seasonal symptoms of those allergic to birch (Pablos et al., 2016; Biedermann et al., 2019). In the Mediterranean region, sensitivity to olive pollen may favour development of sensitivity to other species from the Oleaceae family present in urban spaces, for example ash or privet, as a consequence of the priming effect (Vara et al., 2016), prolonging allergies during winter and into spring. Furthermore, people from the Mediterranean region who are sensitized to olive pollen risk developing allergic

Table 1 Inventory of taxa scoring according to purpose and importance in Europe based on a survey of European pollen networks and on Asam et al., 2015, EAACI Global atlas of allergy 2014, ANSES, 2014, D'Amato et al., 2007

	MED	CLI	FOR	AGR	
Major taxa for which identification is important					
Pollen	Amaranthaceae *	x	x		x
	Asteraceae: *Artemisia*	x	x		
	Asteraceae: *Ambrosia*	x	x		x
	Betulaceae: *Alnus, Betula, Carpinus, Corylus, Ostrya*	x	x	x	
	Cupressaceae *	x	x	x	
	Fagaceae: *Fagus, Quercus*	x	x	x	
	Platanaceae: *Platanus*	x	x	x	
	Poaceae *	x	x		x
	Oleaceae: *Olea europaea*	x	x		x
	Oleaceae: *Fraxinus excelsior*	x	x	x	
	Pinaceae: *Abies, Picea, Pinus, Larix, Cedrus*		x	x	
	Sapindaceae: *Acer*		x	x	
	Taxaceae: *Taxus*		x		
	Urticaceae: *Parietaria, Urtica* *	x	x		
	Vitaceae: *Vitis vinifera*				x
Spores	*Alternaria*	*x*	*x*		*x*
	Aspergillus	*x*			*x*
	Botrytis				*x*
	Cladosporium	*x*	*x*		*x*
	Fusarium				*x*
	Oidium			*x*	*x*
	Penicillium	*x*			*x*
	Uncinula			*x*	*x*
Secondary or regional important taxa (non exhaustive list)					
Pollen	Arecaceae		x	x	
	Casuarinaceae: *Casuarina*	x	x	x	
	Fagaceae: *Castanea*	x	x	x	
	Moraceae: *Morus*	x	x		
	Plantaginaceae*: Plantago*	x	x		
	Poaceae: Secale cereale	x			x
	Polygonaceae: *Rumex*	x	x		x
	Oleaceae: *Fraxinus ornus,* various Oleaceae taxa	x	x	x	
	Salicaeae: *Populus, Salix*	x	x	x	
	Urticaceae: *Urtica*		x		
	Ulmaceae: *Ulmus*	x	x	x	
Spores	*Didymella*	*x*			*x*
	Epicoccum	*x*			*x*
	Ganoderma	*x*		*x*	
	Stemphylium	*x*			*x*

(* indicates that it is very difficult to distinguish the pollen of different genera or species by morphology, the respective contribution of different genera or species to allergy is therefore difficult to assess) (MED: medical; C/F climatology and/or forestry; AGR: agriculture). For comparison at the international level, it is important to measure across Europe (at least) a common list of aeroallergens

symptoms when exposed to ash (*Fraxinus excelsior L.)* pollen in countries throughout the European temperate zone where ash is widespread (Beck et al., 2016; Gassner et al., 2019; Mas et al., 2014; Schmid-Grendelmeier et al., 1994), or vice versa.

Pollen is not the only biological material important for health issues. Fungi represent a kingdom with a high diversity of taxa adapted for living in all environments, outdoor and indoor, with an important presence in home and workplace environments. The spores of *Alternaria, Cladosporium, Aspergillus, Penicillium, Leptosphaeria, Epicoccum, Stemphylium,* and *Didymella* are considered to be the most important allergenic taxa contributing to respiratory disease, particularly asthma and seasonal coughs (Denning et al., 2006; Larenas-Linnemann et al., 2016; Rick et al., 2016) as well as polysensitization (Gabriel et al., 2016). These genera are widespread across Europe, albeit at lower concentrations in the driest environments, such as Spain (Elvira-Rendueles et al., 2013) or Greece (Pyrri & Kapsanaki-Gotsi, 2015), whereas other spore types are found in high concentrations in the more humid parts of north-west Europe, particularly the UK (Sadyś et al., 2016; Skjoth et al., 2016). *Aspergillus fumigatus*, particularly associated with composting, is responsible for triggering bronchopulmonary aspergillosis in immune-compromised individuals (Denning et al., 2006). The most prevalent airborne spore types tend to be those that grow on cereal or brassica crops (Jedryczka et al., 2016), particularly *Alternaria* spp (Apangu et al., 2020). The prevalence of sensitization to fungi varies according to taxon and region considered (Barnes, 2019; Rodinkova et al., 2020) and is, in general, underestimated (Lehrer et al., 1994; Crameri et al., 2014). While many automatic monitors have not yet developed classification software to identify fungal spores, only a limited number of manual monitoring sites report spore concentrations, and usually only a very short list of taxa when they do so.

2.2 Ecology and environmental protection

Pollen networks need to be able not only to detect pollen from plants present in the area of interest, but also aeroallergens from long-range transport and new pollen types in an area, produced by shifting ranges, neophytes, or invasive species, all of which may only be present in small quantities. For example, a very early alder season was detected and attributed to newly planted Spaeth Alder (*Alnus x spaethii*; Gehrig et al., 2014; Polling et al., 2022), which flowers much earlier than native alder species, and further plantation of this species could be prevented. Another example is the spread and increase of ragweed (*Ambrosia artemisiifolia*; Taramarcaz et al., 2005; Buters et al., 2015), which was detected at an early stage in the mid-1990s in Switzerland, thus allowing effective control actions, an effort continued until now and effectively allowing the almost complete reduction of ragweed populations in the country. In the dynamics of an invasion, control measures are more effective and costs significantly reduced if action is taken at an early stage of the invasion. It is therefore important that automatic instruments can easily be trained to identify non-native or invasive taxa to better understand the dynamics of these species and survey their spread, to provide an opportunity to improve environmental protection efforts and avoid the development of new sensitizations in an area. This can be achieved by community efforts to produce training datasets for a range of taxa from different areas that can be used to further develop particle classification software. While monitoring for such purposes are certainly important, given the slower timescales of ecological processes it is not as imperative that it is done in real time. Nevertheless, automatic monitoring provides the possibility to monitor in remote locations and potentially to have much denser measurement networks, which are particularly of interest for micro-ecology.

2.3 Climate change, phenology, agriculture, and sylviculture

Pollen observations analysed together with meteorological data can be used to quantify changes in the timing and intensity of the pollen season resulting from climate change, which are expected to adversely affect public health and the quality of life of allergy sufferers (Anderegg et al., 2021; Höflich et al., 2016; Smith et al., 2014; Ziello et al., 2012; Ziska et al., 2019). These studies require the identification of pollen taxa that are relatively abundant in the air as well as carefully selected criteria for the pollen season definition (Gehrig & Clot, 2021; Glick et al., 2021).

Pollen data are also used in forestry research (Kasprzyk et al., 2014) or for studying changes in masting behaviour (Ascoli et al., 2017; Nussbaumer

et al., 2020; Ranta et al., 2005), particularly for species such as birch (*Betula* spp.), spruce (*Picea* spp.), pine (*Pinus* spp.), olive (*Olea europaea*), beech (*Fagus* spp.), and oak (*Quercus* spp). Climate change, in particular more extreme weather events and the consequently increased damage caused by changing species distributions, is affecting the composition of forests on a global scale. Informed decision-making based on such data is beneficial for improved forestry management despite the slow growth and long lifetime of trees, which results in significant inertia in their response to climate changes (Jones et al., 2009). Real-time observations allow the possibility to detect, track, and map fungal spores and thus to predict areas that may be at risk or that require treatment.

In addition, pollen observations are also used for crop forecasts. The relationship between the amount of airborne pollen and final yield is especially useful in regions characterized by climate uncertainties that magnify seasonal yield fluctuations (Cunha et al., 2016; Galán et al., 2008; Garcia-Mozo et al., 2015; Oteros et al., 2014). This represents an important competitive advantage by enabling the assessment of crop yield size at the flowering stage and therefore also crop management and transformation operations, commercial strategies, and policies regarding prices, stocks, or economic aid.

Also related to agriculture, the monitoring of specific phytopathogenic fungal spores is important in the context of crop protection since this makes it possible to more accurately forecast plant disease incidence, severity, and geographical distribution (Martinez-Bracero et al., 2019; Oliveira et al., 2009; Rodriguez-Rajo et al., 2010). The main potential infection risk periods can be determined as a function of airborne phytopathogenic spore concentrations and in turn more targeted fungicidal treatments can be applied, thus significantly reducing production costs and environmental impacts (Dedeurwaerder et al., 2011; Isard and Chamecki, 2016).

3 How can automatic pollen and fungal spore data be used?

The high temporal resolution of automatic pollen and fungal spore measurements provides the possibility for a quantum leap in terms of the research that is possible. Domains as diverse as epidemiology, atmospheric physics, and agronomy, amongst many others, would significantly benefit from such information (Tummon et al., 2021). The door is also open to a wide range of other fields where the application of these data has not yet been explored. Importantly, not only the measurements are themselves relevant, but as for forecasts, the uncertainty associated with each observation point is also important and should be systematically communicated with the data. Likewise, multifactorial information is important, i.e. the provision of meteorological or air quality observations from the same or closely situated sites.

3.1 New aerobiological knowledge

High temporal resolution observations provide the possibility to better understand the sub-daily -or even sub-hourly-variability of pollen and fungal spore concentrations, such as how they depend on meteorological factors (e.g. Chappuis et al., 2019) or how their emissions vary over the course of the day. This is intimately related to the establishment of biological functions for each taxon, which are crucial notably for forecast models.

In terms of health research, such data would be useful to study how the behaviour, symptoms, and overall health of allergic individuals vary in time compared to atmospheric pollen or fungal spore concentrations. Furthermore, such information would serve to better understand the relationship between symptoms and sensitization rates, with regard to exposure levels and duration, in the short and long term, as well as the complex biological mechanisms behind allergies. Other research questions related to new therapies as well as assessing the rate of medication consumption and self-management in response to the provision of real-time information and forecasts would also benefit from such measurements (e.g. when it would be best to aerate your home or good to start/stop taking medication. These studies would be very useful to establish better public health practices, to communicate more accurate risk levels, and to make recommendations to the general public about how to further reduce exposure thus reducing allergic symptoms.

The availability of high temporal resolution observations will also allow significant progress to be made in several other fields, including atmospheric sciences, ecosystem research, understanding the role

of pollen and fungal spore particles in the hydrological cycle as well as studies of biogeography and biodiversity (Burkart et al., 2021; Fröhlich-Nowoisky, et al., 2016). Pollen and fungal spores are present all over the globe and, as any atmospheric aerosol, they scatter and absorb radiation entering the Earth-atmosphere system. For example, in the city of Barcelona, in strong pollen events, pollen can represent up to 30% of the total optical depth (Sicard et al., 2016). Even though it is challenging for present remote sensing technology to identify individual pollen taxa, high temporal resolution surface observations are the only way to link surface and atmospheric column observations. High temporal resolution observations would thus significantly help advance understanding of various atmospheric processes in the vertical, which in turn would also help to further improve forecast models.

3.2 Pollen and fungal spore forecasts

Pollen and fungal spore forecasts can be based on either statistical and/or numerical models that require observations as a starting point (e.g. Grinn-Gofrón et al., 2019). Current forecast models use either climatological values or phenological observations, with some models being merged with pollen data which may be several days old. However, the availability of real-time data means that these measurements can be integrated into forecast models, as is routinely done with meteorological observations and weather forecasts (Sofiev, 2019; Sofiev et al., 2017). This is expected to significantly improve the quality of the forecasts since they will be based on actual conditions, in contrast to current predictions (many mobile applications are not even based on data and are consequently inaccurate). Real-time integration of pollen observations is also expected to allow for more accurate simulations of punctual events such as thunderstorm asthma epidemics, which have been shown to cause high numbers of emergency department visits and even fatalities (Thien et al., 2018).

4 What automatic technology is available?

A broad spectrum of measurement techniques has been applied to monitor pollen in real time, several of which have only very recently been commercialised or are still in the development phase. In addition, a number of instruments also have the potential to identify fungal spores, although classification algorithms still need to be developed to do so. A complementary and more general review of the full range of measurement techniques used to monitor bioaerosol can be found in Huffman et al. (2019).

It is important to note that all the methods currently in use do not directly produce pollen concentration values. Data analysis is required to interpret the measurements, whether it be digital images or electrical signals from various types of sensors. The specificities of data analysis are elaborated on in Sect. 8.

4.1 Digital microscopy

Digital microscopes differ from traditional optical microscopes only in the sense that a digital camera is used to obtain images of the surface upon which particles have impacted. Usually, images at various focal depths are taken and stacked to provide a single image with extended depth of field to improve particle identification. The images are analysed by a computer that isolates particles of interest using image recognition methods. Airborne concentrations are estimated by using the number of particles per deposition surface area and the air volume sampled. The sampling rate thus depends on the air volume and the proportion of the surface area analysed, as well as the impaction efficiency. This technique is used in the BAA500, Pollensense, ACPD, and Aerotrap (see instrument descriptions in Sect. 5).

4.2 Elastic light scattering

Elastic scattering occurs when light hits a particle and is reflected off the particle without changing wavelength; this occurs when the state of the scattering particle remains unchanged. The angle at which the light is scattered gives an indication about the size and surface of the particle itself and this feature is used to determine particle size and to some extent shape in most particle counters. This technique is used in the Rapid-E, Poleno, WIBS, and KH-3000.

4.3 Light-induced fluorescence

Certain molecules, called fluorophores, fluoresce when excited by light at a specific wavelength. This is

typically caused by the conjugated aromatic systems which they contain (e.g. tryptophan, riboflavin) and which can indicate that particles come from a biological source. This property is taken advantage of in light-induced spectroscopy where a light source, most frequently a monochromatic laser, is used to induce fluorescence in individual particles that pass through the measurement device. The wavelengths of the light sources are typically chosen to correspond to the excitation bandwidths of one or several fluorophores and the resultant fluorescence is measured across relevant emission bandwidths. What complexifies this technique is that many fluorophores may be present in biological particles at the same time or in variable amounts over a season, and thus emission signals contain information about all the molecules that are made to fluoresce. Furthermore, certain non-biological particles that contain polycyclic aromatic hydrocarbons, such as particles from incomplete combustion or microplastics, can also fluoresce, further complexifying the differentiation between particle types. In large part, this technique has been used simply to differentiate between biological and non-biological particles, although more recent instruments have been used to characterise various types of biological particles (e.g. Perring, 2016). These newer techniques often rely on multiple excitation wavelengths which can help to improve discrimination, although the characterisation of different pollen taxa using only this technique has so far not been possible using these methods. This technique is used in the Rapid-E, Poleno, and WIBS.

4.4 Holography

Holograms are recordings of a light field as an interference pattern rather than a lens-formed image. Typically, they are produced with a laser beam that illuminates an object, for example, a pollen grain, as well as the reference. The reference beam and the light scattered from the object together form an interference pattern which is recorded, in the case of digital holography, by a sensor array. The resultant interferograms can then be used to reconstruct a 3D image of the original object but also can provide additional information, for example particle size, potentially useful for discrimination between particles of interest. This technique is used in the Poleno.

5 Real-time instruments

This section provides a brief overview of the instruments currently commercially available or under development that are capable of measuring pollen grains and/or fungal spores in real-time or near-real-time. The measurement principles and technical specifications from each device are based on published research or information provided by the instrument manufacturers. Further details about several of these systems as well as other bioaerosol monitors can be found in Huffman et al. (2019) and Santl-Temkiv et al. (2019).

5.1 Commercially available instruments

5.1.1 Helmut Hund BAA500

The first automatic pollen monitor to be made commercially available was the Helmut-Hund BAA500 (Oteros et al., 2015). This device is based on digital microscopy and essentially automates the manual process by impacting aerosols onto sampling slides (covered with gelatine) from an air flow. The system ensures that the sample does not contain particles smaller than 10 µm and, thus, the amount of non-pollen particles is reduced. The samples are then heated so that particles are embedded and re-hydrate in the gelatine substrate. After a sampling period specified by the end-user (typically three hours), the slide is moved underneath an automated light microscope. This microscope scans a portion of the slide area (usually 25%, but which is decreased when particle concentrations are high) and collects an image stack across a number of focal positions. The image stack is then collapsed to a final image with an extended depth of field so that it contains all sharp object details. An identification algorithm is then applied to first isolate and then classify pollen particles while the next sample is being taken. A magazine of sampling slides ensures autonomous operation over a time interval determined by the daily number of samples taken (usually 3 h, which means data are provided up to 6 h after the measurement has been made). With eight samples per day, the magazine needs to be replaced after 3 weeks of operation. By upgrading the BAA500 with a so-called magazine extension, the operation time of one magazine can be extended to 12 weeks. Collected samples are stored in an archive

magazine which are available together with the digital images for later quality control measures. Unknown pollen taxa can be labelled manually and added to the database for software training. The first version of this instrument was tested in 2006 after which it was further developed before being implemented as part of the ePIN (Electronic Pollen Information Network) automatic pollen monitoring network in Bavaria as of 2019 (Oteros et al., 2020). As such, it is one of the first instruments to be used operationally over an entire network and not just for research purposes. Helmut Hund GmbH has announced the launch of a smaller version of this instrument, the BAA500e, although it is not yet commercially available.

5.1.2 Plair Rapid-E

The Plair Rapid-E is a bioaerosol monitor that uses fluorescence laser spectroscopy to record a number of signals from each detected particle. A 400 nm laser is used to produce a scattering signal which is measured across 24 detectors at various angles from + 45° to − 45°. A second 337 nm laser is then used to excite fluorescent particles, the signals from which are detected across 32 channels from 350-800 nm and with eight sequential 0.5-microsecond acquisitions to estimate fluorescence lifetime (Kiselev et al., 2011, 2013). An initial version of the instrument, the PA-300 was able to provide real-time total and grass pollen concentrations (Crouzy et al., 2016) and the newer Rapid-E has been shown capable of distinguishing between a range of different pollen taxa (Sauliene et al., 2019; Tesendic et al., 2020; Daunys et al., 2021). The device is in use across a small operational monitoring network between Croatia and Serbia (Tesendic et al., 2020) but challenges remain in applying the same identification algorithm to different instruments (Sauliene et al., 2019). Plair has also recently launched an updated version of this instrument called the Rapid-E + .

5.1.3 PollenSense APS (Automated Pollen Sensor)

Also designed to mimic the manual process, the PollenSense Automated Pollen Sensor (APS) was first commercialised in 2018. The instrument uses a pump to draw air through an inlet located at the bottom of the instrument. Particles deposit onto a sticky tape which then passes below a high-resolution camera with a microscope integrated. The tape is moved below the camera every 7–10 min, depending on the density of particle deposition. This tape needs to be replaced every 2–3 months. A ring of light-emitting diodes (LEDs) are used to illuminate the sample from several angles and multiple images are taken. This provides more information to the identification algorithm, which is based on machine learning techniques, to characterise the particles present. Airborne pollen concentrations are available from half an hour to several hours after the sample is taken. A number of the APS devices have been installed across the USA, Europe, the Middle East, South America and Australia. No peer-reviewed publications are available at this time.

5.1.4 Swisens Poleno

The Swisens Poleno is an air flow cytometer that uses digital holography to identify particles. Two holographic images at 90° to one another are taken once a trigger laser has detected a particle in the airflow. Thereafter the fluorescence spectrum and lifetime are measured at three excitation wavelengths, namely 280, 365, and 405 nm (Sauvageat et al., 2020). Finally, a polarisation measurement is obtained before the particle exits the device. So far, just the holographic images have been used to identify up to ten different pollen taxa (Sauvageat et al., 2020). Three different versions of the Poleno currently exist on the market, the Jupiter which includes all the above aspects, the Neptune which contains only the holographic imager, but can be upgraded to Jupiter, and the Mars which is a miniaturised version of the Neptune. The Swisens Poleno is currently being installed across the Swiss national automatic pollen monitoring network, with the entire network to be operational by the pollen season of 2023.

6 Research/prototype instruments

6.1 Sextant Technologies Aerotrap

The Sextant Technologies Aerotrap operates similarly to the PollenSense APS. An airflow is established using a pump that runs at 10 L/min and aerosols present in the air stick to an adhesive tape. The pump runs for 10 min after which a camera equipped with a

microscopic lens takes pictures of the collected aerosols. The pictures are sent by FTP to a server where they are analysed by a neural network classification algorithm.

6.2 University of Graz ACPD (Affordable Continuous aerosol Particle Detector)

The ACPD is a research instrument designed and developed at the University of Graz, Austria (Nam et al., 2020). It consists of two sub-systems, an omnidirectional 6-input cyclone particle trap with a concentrator that deposits particles on a rotating glass disc covered with glycerine as well as a digital-transmitted light microscope with a layer-wise focus that takes images of the sample at various focal depths. The rotating glass plate is cleaned and re-covered with glycerine once the sample has been analysed. The device functions with air flow rates up to 150 L/min and evaluates over 90% of captured pollen grains with an average measurement delay of 1 h (maximum 2 h). The prototype collects raw time-stamped microscopic images with 5–60 layers per sample, depending on the number of particles per sample. The images are transmitted to a server where the identification algorithm is run. The glycerine supply needs to be replenished once per season.

6.3 Other bioaerosol monitors with potential for pollen identification

6.4 Droplet Measurement Technologies WIBS (Wideband Integrated Bioaerosol Spectrometer)

The Wideband Integrated Bioaerosol Spectrometer (WIBS) was initially developed in the early 2000s and then later commercialised by Droplet Measurement Technologies. Several versions have been produced, revisions 3, 4, and the WIBS-5/Neo. The instrument uses both a 635 nm laser to produce an elastic scattering signal as well as two Xenon flash lamps at 280 and 370 nm which excite fluorophores. Two wideband channels, from 310-400 nm and 420-650 nm, are used to provide three fluorescent signals in addition to the elastic scattering which is used to determine particle size and asymmetry. The device has nearly exclusively been used for research purposes, with initial focus just on distinguishing bioaerosol from other aerosols (Foot et al., 2008; Kaye et al., 2005) but more recently a typing scheme has been developed to separate bioaerosol into seven different fluorescence categories. (Perring., 2016; Savage et al., 2017). The WIBS has also more recently been used in comparison with Hirst measurements for fungal spores at a number of rural and green waste facilities (O'Connor et al., 2015; Sodeau and O'Connor, 2016; Fenney et al., 2018; Fernández-Rodríguez et al., 2018).

6.4.1 Flir IBAC-2

The Instantaneous Bioaerosol Analysis and Collection 2 (IBAC 2), produced commercially by the American company FLIR, is a fully automated outdoor bioaerosol monitor that was developed to identify airborne biothreat agents in four classes: spores, bacteria, viruses, and toxins. A 405 nm continuous diode-laser is used to induce fluorescence to distinguish between biological and other particles. However, it should be noted that particles are not broken into these classes via the real-time sampling component of the instrument. The data are simply classified as fluorescent or non-fluorescent with an additional size division being applied thereafter. The device can detect particles in the size range of 0.7–10 µm (separated into two size regimes of 0.7–1.5 and 1.5–10 µm). The system allows for sampling of very high concentrations, with sampling rates of up to 500,000 particles/litre possible. The IBAC-2 also incorporates a secondary impaction sampler which can be set to run continuously or to initiate sampling once a threshold (of potentially biological particles) has been achieved. This secondary sampler physically collects particles onto a dry (polyester felt filters) or wet (buffer rinse fluid in premeasured vials) medium. Primary customers of the first IBAC have been described as homeland security and defence customers (DeFreez, 2009; Santarpia, 2013; Jonsson & Kullander, 2014). Thus, most work has focused on bioaerosol far smaller in size than the majority of pollen species. Given the size cut-offs of the sampling heads used in the IBAC-2, little to no pollen are expected to be sampled by the instrument, however, fragments of pollen or smaller fungal spores can likely be analysed.

6.4.2 *Yamatronics KH3000*

The first automatic monitor used to detect pollen operationally is the Yamatronics KH-3000. This instrument has been used since 2002 as the measurement device of the Japanese "Hanakosan" network. The target pollen taxa are somewhat particular in the country with the main allergenic taxa (Japanese cedar (*Cryptomeria japonica*) and cypress (Cupressaceae spp.)) being present essentially in spring and being relatively large and round so easily distinguishable from other airborne particles present. The device draws air in at a rate of 4.1 L/min and all airborne particles are targeted with a 780 nm laser which produces forward- and side-scattering signals (Kawashima et al., 2007). These are then used to estimate particle size and shape, from which it is assumed that all large round particles are pollen. Attempts to identify individual pollen taxa have met with mixed results (Kawashima et al., 2017).

7 What affects the accuracy of measurements?

The accuracy of instruments used to measure atmospheric properties, whether they be physical or chemical, is generally assured through systematic calibrations against a reference. Here, we discuss a range of topics related to the question of instrument accuracy and provide some recommendations on what parameters should be considered to fully address this issue for automatic pollen monitors.

To evaluate the performance of automatic instruments, one first has to be acquainted with the uncertainties of the manual technique currently used as a standard across much of the world.

7.1 Manual techniques

When it first came into use in the 1950s, the Hirst trap was a considerable leap forward in terms of observational capacity since prior to its development only passive samplers with neither flow control nor any time resolution were available (Hirst, 1952). Since then, the vast majority of aerobiological studies have used volumetric traps based on the Hirst design. It should be noted, however, that despite their widespread use, research since then has shown that there are a number of drawbacks of the Hirst sampler, including:

- Air flow: depending on the method used to adjust the air flow, a bias and/or error of approximately 30% is common. This error can be circumvented by using a resistance-free flowmeter to adjust the Hirst pump whenever this is required (Oteros et al., 2016).
- The distance between the inlet and the deposition surface is critical for an impactor; however, no procedure nor recommendation to periodically control this distance has been published.
- Tsunami-effect: during the preparation of a sample for the microscope the pollen can shift position or be eliminated entirely (Galán et al., 2014).
- Sensitivity of the adhesives to the ambient temperature—pollen stick differently depending on the temperature. Furthermore, different glues have different adhesive properties (Maya-Manzano et al., 2016; Rojo et al., 2019).
- The relative uncertainty decreases with the sampled area of the slide. An error of more than 30% is reported (Adamov et al., 2021; Smith et al., 2018), mainly when the sampled area is below the minimum recommendation of 10% (Comtois et al., 1999; Galán et al., 2014).
- During very high pollen peaks there may be several layers of pollen grains, some of which may not get to touch the adhesive surface and therefore get lost.
- Human error: it is generally accepted that the human error associated with the identification and counting of pollen grains is around 30% (Smith et al., 2018).
- Sampling efficiency varies with wind speed and physical properties of the particle, in particular size (e.g. Frenz, 2000; West & Kimber, 2015), because the approach is not isokinetic.

Parallel observations using Hirst-type instruments were made to estimate the integrated measurement uncertainty stemming from several of the above-mentioned error components. This yielded a general measurement error of between 76 and 98% for daily average values (VDI:45252, 2018).

There is currently no "gold standard" against which new, automatic instruments can be thoroughly tested and it is, therefore, important that the

above-mentioned errors are either corrected for or, at the very least, taken into account when any comparisons are made. This is particularly the case for sub-daily timescales.

7.2 Automatic measurements

By nature, automatic instruments have no human error associated with the measurement itself and often have higher sampling rates than the manual method (see Table 2). Nevertheless, there are a number of aspects that can potentially affect measurement accuracy. These include:

- Higher sampling rates mean a large number of particles to discriminate and this can lead to possible saturation of detectors or imaging processors. This can be circumvented by identifying only a fraction of the particles and then calculating real concentrations by applying a multiplication factor based on this fractional value; doing so means assuming a certain homogeneity in the sample.
- Human errors can affect the installation of the instrument or the development and deployment of identification software. These can be minimised by following manufacturer's instructions closely and by applying the standard methods being developed through the AutoPollen Programme (see companion paper in this special issue).
- Particle deposition in the instrument and later release of these particles (the so called "memory effect"), which could result in correct identification but at the wrong time of the day or season. This can be reduced through instrument design and manufacturing processes, and regular maintenance/cleaning.
- Certain instruments using laser technology are sensitive to environmental parameters such as temperature, although this is largely controlled through heating/cooling systems that adapt temperature in the instrument housing.
- Bias and random error may occur as for the manual method, however, the higher sampling rates result in better statistics and lower error rates.
- Aggregation, i.e. when two or more pollen/fungal spores stick together or when they are attached to other particles such as dust, which may make the automatic identification difficult.

Typically, automatic measurements are evaluated using manual observations from Hirst-type traps. While this is currently the only way to validate real-time instruments, at least in terms of the pollen identification, it is important to keep in mind the large uncertainty associated with the manual observations (Adamov et al., 2021). Further methods will need to be developed to assess the precision and accuracy of identification algorithms since this is a source of error unique to automatic measurements. To date, metrics such as confusion matrices have been used (e.g. Crouzy et al., 2016; Sauliene et al., 2019), however, these can only be used to a certain extent to assess image-based instruments under real environmental conditions (Damialis et al., 2021; Oteros et al., 2020). Comparing automatic pollen monitors against a well-calibrated gold standard or assessing reproducibility by running several automatic pollen monitors in parallel (of the same type and of different types) could provide part of the missing information. It is essential that standardised metrics or methods are developed to ensure results are comparable across studies, instruments, and networks.

Overall, however, it is expected that properly calibrated automatic instruments, suffer from considerably less error than manual techniques, can provide more standardised measurements, and errors can be better quantified. Nevertheless, given their novelty, few studies yet exist exploring their accuracy or detection limits (Chappuis et al., 2019; Oteros et al., 2020; Lieberherr et al., 2021).

8 How can one ensure measurements are reliable and comparable?

Given the range of techniques used to detect pollen grains and fungal spores in real time, it is important not only that instruments of the same type but also that different methods provide comparable results. A companion paper in this special issue outlines a number of guidelines and methods that can be used to standardise instruments. This includes issues such as ensuring the right metadata are measured and reported, how to validate instruments in a traceable way, as well as physical constraints for instruments, amongst several others. Developers and manufacturers need to consider and evaluate these features when designing automatic instruments, while the

Table 2 Main technical characteristics of all commercially available and research automatic monitors, as well as for the manual Hirst-type method (for comparison). Information has been provided by instrument manufacturers

Instrument												
	Hirst-type (manual system, for comparison)	BAA500	Rapid-E	APS	Poleno-Mars	Poleno-Neptune	Poleno-Jupiter	AeroTrap	ACPD	WIBS	IBAC-2	KH3000
Manufacturer	Burkard Scientific (UK) / Burkard manufacturing (UK) / Lanzoni (IT) / Cavazza Anna (IT)	Helmut Hund GmbH (DE)	Plair SA (CH)	PollenSense (USA)	Swisens AG (CH)	Swisens AG (CH)	Swisens AG (CH)	Sextant Technologies (NZ)	University of Graz (AT)	Droplet Measurement Technologies (USA)	Flir (USA)	Yamatronics (JP)
Commercially Available	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes
Detection Technique	Impaction + Microscopy	Impaction + Digital Microscopy	Elastic scattering + Light-induced Fluorescence	Impaction + Digital Microscopy	Elastic scattering + Holography	Elastic scattering + Holography	Elastic scattering + Holography + Light-induced Fluorescence + Polarization	Impaction + Microscopy	Impaction + Digital Microscopy	Elastic scattering + Light-induced Fluorescence	Light-induced Fluorescence + Elastic scattering	Elastic scattering
Measured Parameters	Adhesive tape with collected particles	Microscopic image stacks and particle texture parameters	Particle Size, Scattering in 32 channels, Integrated fluorescence in 16 channels, Fluorescence lifetime	Particle Size, Microscopic Images	Particle Size, 2 holographic images, morphological characteristics	Particle Size, 2 holographic images, morphological characteristics	Particle Size, Morphological characteristics, 2 holographic images, Integrated fluorescence in 5 channels, Fluorescence lifetime, Polarization	Unknown	Microscopic videos	Particle Size, Auto-fluorescence, Peak fluorescence in 3 channels + Asphericity	Particle Size, Auto-fluorescence	Forward + Sideward scattering
Size without housing (LxBxH)	60×60x120cm	40×50x60cm	40×34x73cm	Not sold without housing	Not sold without housing	30×30x50cm	30×30x50cm	60×50x25cm	40×30x50cm	45×36x24cm	Not sold without housing	32×23x14cm
Size with housing (LxBxH)	No housing required	90×70x180cm	Not sold with housing	40×40x20cm	60×47x125cm	3×73x150cm	3×73x150cm	Unknown	No housing required	Not sold with housing	100×75x75cm	Not sold with housing
Weight without housing	15 kg	40 kg	30 kg + housing	Not sold without housing	Not sold without housing	26 kg	26 kg	Unknown	15 kg	13 kg	Not sold without housing	5 kg
Weight with housing	No housing required	260-290 kg	Not sold with housing	20 kg	34 kg	134 kg	134 kg	6 kg	No housing required	Not sold with housing	30 kg	Not sold with housing

Table 2 (continued)

Instrument												
	Hirst-type (manual system, for comparison)	BAA500	Rapid-E	APS	Poleno-Mars	Poleno-Neptune	Poleno-Jupiter	AeroTrap	ACPD	WIBS	IBAC-2	KH3000
Sampling Volume	10L/min	50-100L/min (non-continuous sampling; every 1 of 6–10 min, can be selected)	2.8L/min (20L/min with concentrator)	14L/min	40L/min (with concentrator)	40L/min (with concentrator)	40L/min (with concentrator)	10L/min	150L/min	0.3L/min (sample flow)	3.8 L/min (sample flow) and 100L/min (secondary impactor)	4.1L/min
Sampling Rate	10% of slide (CEN recommendation)	10–100% of slide, up to 6000 pollen grains/slide	Up to 1600 particles/litre	5% of deposition band	Up to 1′000 particles/m3	Up to 1′000 particles/m3	Up to 1′000 particles/m3	? % of band	50% of the band	Up to 1′300′000 particles /m3	Up to 500′000 particles/L	Up to 100′000 particles/min
Effective sampled volume	1 L/min	50-100L/min (non-continuous)	2.8 or 20 L/min * efficiency	0.7L/min	40 L/min * efficiency	40 L/min * efficiency	40 L/min * efficiency	Unknown	75 L/min	0.3 L/min * efficiency	3.8 L/min * efficiency	4.1 L/min
Standard Temporal Resolution	24 h	3 h (standard), 1-12 h (possible)	1 h	1 h	1 h	1 h	1 h	1 h	1 h	User selectable	1 h	1 h
Particle Size Range	> 5 µm	> 10 µm	~ 1–100 µm	> 3 µm	~ 1–300 µm	~ 1–300 µm	~ 1–300 µm	Unknown	> 5 µm	~ 0.5–30 µm	0.7–10 µm (split in 2 size regimes: (0.7–1.5 and 1.5–10 µm, no individual particle size)	> 10 µm
Particle discrimination capacity	Pollen, fungal spores	Pollen, fungal spores, other aerosol > 10 microns	Pollen	Pollen,fungal spores, microplastics, pet dander	Pollen, fungal spores	Pollen, fungal spores, other aerosol > 5 microns	Pollen, fungal spores, other aerosol > 5 microns	Pollen	Pollen	Fluorescent particles, 7 Types of fluorescence	(off-line analysis can add to discrimination)	Total pollen
Current # pollen taxa identified (claimed)	In Europe, lists of operationally counted taxa range from ~ 10 to 100	~ 40	~ 15	35	~ 10	~ 10	~ 10	7	~ 15	0	0	0

Table 2 (continued)

Instrument												
	Hirst-type (manual system, for comparison)	BAA500	Rapid-E	APS	Poleno-Mars	Poleno-Neptune	Poleno-Jupiter	AeroTrap	ACPD	WIBS	IBAC-2	KH3000
Potential to Identify & Count Particles other than Pollen & Fungal Spores	No	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No
Consumables required	Yes	Yes	No	Yes	No	No	No	Yes	Yes	No	(Yes, for analysis of secondary sampler)	No

community as a whole needs to develop methods and protocols for standards.

In general, few studies have focused on testing and extending methods that are used as standard for air quality networks (e.g. Lieberherr et al., 2021; Sauvageat et al., 2020). Present techniques use polymer microspheres with certified parameters, e.g. diameter, which are tested under known concentrations through reference calibrations. These are, however, presently limited in terms of the particle size that can be tested (maximum 10 µm in diameter) and the types of particles tested. Currently it is not possible to aerosolise pollen or fungal spores at known concentrations (Lieberherr et al., 2021; Sauvageat et al., 2020), but work is ongoing with the metrology community to establish standard methods to do so both in the laboratory and in the field.

A further aspect to consider is device comparability across entire monitoring networks. Standardisation methods based, for example, on similar procedures as used in meteorology, are currently being developed for automatic pollen and fungal spore monitoring networks. This starts with standardised laboratory calibrations that need to be performed at the end of the production process. Travelling standards should then be used at regular intervals to ensure comparability of results obtained with different instruments. Thorough laboratory calibrations could then be performed to sort out the issues identified using the travelling standards. This will help reduce error across and between networks; a significant advantage of automatic techniques and one that serves many end-user groups.

9 Obtaining pollen/spore concentrations from raw measurements

No matter the technique applied to obtain measurements, automatic monitors require the application of various data analysis methods to identify specific groups or types of particles (such as pollen or fungal spores) and then obtain their airborne concentrations. At present, most instruments have not been validated in all biogeographical regions. The identification algorithms that they use thus need to be tested in each new environment into which an instrument is placed since there is large variability of biological particles and possible confounding aerosol from one region to another.

9.1 Differentiation of bioaerosols from other aerosols

To differentiate between particles of biological origin and other aerosols, analysis usually focuses on the size, shape, or fluorescence of particles. These techniques vary in complexity depending on how each one functions. Instruments such as the Yamatronics KH-3000 use a relatively simple filtering window on the scattering signal collected from each particle, thus removing particles of unwanted size (see Kawashima et al., 2007 for further details). A slightly more complex typing scheme is applied to data from the Droplet Measurement Technologies WIBS device, where in addition to size analysis from laser scattering data, additional fluorescence characteristics can be used to identify particles of biological origin (Perring, 2016; Duflot et al., 2019). Instruments based on digital microscopy or holography usually segment the images obtained to separate pollen or fungal spore particles from other particles (Oteros et al., 2020; Sauvageat et al., 2020). Yet other technologies use a combination of laser spectroscopic and fluorescence signals in combination to identify particles. It is only in recent years, with advances in robotics, computer vision, and data science that it has become possible to operationalise these processes (Sevillano et al., 2020).

9.2 Pollen classification in commercially available systems

The majority of image-based systems developed for airborne monitoring combine three tasks to identify particles: segmentation, feature extraction, and classification (Holt & Bennett, 2014; Marcos et al., 2015). Segmentation is the process of partitioning a digital image into several smaller parts with the aim of simplifying an image into something that is easier to analyse. Feature extraction consists of defining a set of quantitative measurements derived from the images or signals used for classification (Marcos et al., 2015; Sevillano et al., 2020). These features can be divided into discriminant morphological and geometric descriptors such as symmetry, area, perimeter, particle sphericity, mean distance to the centroid, or discriminant texture-based descriptors such as grey-scale co-occurrence matrices or entropy features (e.g. Arias

et al., 2017; Kubik-Komar et al., 2018; Manikis et al., 2019); the number of extracted features varies considerably from one device to another. Thereafter, statistical, machine learning, or deep learning classification algorithms are applied to automatically assign the isolated target particle to a taxonomic group (family, genera, or species). The most frequently used algorithms include linear discriminant analysis (LDA), k-nearest neighbour (KNN), support-vector machines (SVM), multilayer perceptron neural networks (MLP-NN), recurrent neural networks (RNN), and most recently convolutional neural networks (CNN) (e.g. Arias et al., 2017; Manikis et al., 2019; Mokin et al., 2017; Sevillano & Aznarte, 2018).

This section covers the instruments for which scientific studies have been published. To date no information is available for the other devices presented in Table 2.

9.2.1 Hund-Wetzlar BAA500

The Hund-Wetzlar BAA500 uses traditional image recognition techniques to identify pollen particles. The instrument can currently classify over 30 pollen taxa, however, this depends on the number of taxa for which the identification software has been trained. Of 15 pollen types with sufficient numbers to evaluate performance, the overall classification accuracy was 91% and comparison against daily manual observations over an entire pollen season showed strong positive correlations of >0.84 for 12 of these taxa (Oteros et al., 2020). For some important allergenic pollen taxa, the system needs improvement to reduce the number of false-negative detections (e.g. about 20% of *Betula* pollen were misclassified as unknown), however, overall accuracy is relatively good (Oteros et al., 2020). The results from a study at a nearby location have shown similar results with the application of machine learning techniques, achieving an overall classification rate (recall) of $>95\%$ for the nine most abundant taxa (Schaefer et al., 2021).

9.2.2 Plair Rapid-E

Although the Plair Rapid-E does not record images, the analysis of its signals (i.e. laser-induced scattering, UV-induced fluorescence lifetime, and spectra) also rely on processing with machine learning techniques (Crouzy et al., 2016; Sauliene et al. 2019; Tesendic et al., 2020). When performing side-by-side comparisons with the standard manual method (EN16868: 2019), the Rapid-E compared well for the main pollen season, showing a positive correlation (>0.7) for 11 out of 24 pollen taxa tested over a 7-month sampling period from February to September (Tesendic et al., 2020). However, it should be noted that for some pollen taxa the results can be notably better under different conditions, e.g. location, season, device, ANN architecture, classes to be identified (Sauliene et al. 2019; Chappuis et al., 2019 ; Daunys et al., 2021).

9.2.3 PollenSense APS

Image analysis is also used by the PollenSense APS device (https://pollensense.com), with the instrument making use of a machine learning algorithm to identify different pollen taxa. Up to now, results with this instrument have only been published as conference abstracts: in a side-by-side comparison with manual rotorod devices, the APS showed positive correlations (>0.7) for *Ambrosia* pollen over its main season when performance for its identification was tested for daily averages (Dalan et al., 2020). The results for total pollen, however, showed limited matches, with peak periods being longer compared to the manual Hirst and rotorod observations for daily averages over a period of 3 months (Lucas & Bunderson, 2019).

9.2.4 Swisens Poleno

Depending on the version, the Swisens Poleno records a different combination of holographic images, UV-induced fluorescence lifetime and spectra, as well as time-resolved optical polarization that can be utilized separately or combined for particle identification. Recent results using only the holographic images and a deep learning algorithm showed that the identification of eight chosen pollen taxa was very good, with six of the eight exceeding 90% accuracy (Sauvageat et al., 2020). A comparison with manual observations (EN16868: 2019) is ongoing.

9.2.5 Droplet Measurement Technologies WIBS

A typing scheme can be applied to data from the Droplet Measurement Technologies WIBS device to differentiate between biological particles (Perring,

2016). Pollen is discriminated from other bioaerosols using fluorescence intensity thresholds in each of the four channels and by selecting particles only larger than 15 µm in size (O'Connor et al., 2014). In comparison with daily standard manual measurements, a strong positive correlation (> 0.9) was observed for total pollen over test periods of 8–12 days.

9.2.6 Yamatronics KH-3000

The Yamatronics KH-3000 is designed to quantify particles in a predefined size range (Kawashima et al., 2007) which makes it of limited use for the classification of different pollen types where size overlaps notably between taxa. In an experimental setup, adjustments of the ranges for the rectangular area inside the scatter chart of sideward vs. forward scattering intensity indicated a possibility to discriminate six pollen types (Kawashima et al., 2017), however, comparison with the standard method (EN16868: 2019) showed a positive correlation (> 0.7) for just three out of these six taxa when daily averages over the season (3–9 weeks) of each pollen was considered. Performance for total pollen was somewhat better for total pollen, with correlations for daily averages ranging from 0.78 to 0.87 for three separate years with monitoring periods of 2–5 months (Kawashima et al., 2017).

9.3 What influences the performance of classification models?

All of the above-mentioned methods require sets of training data which are produced either by manually labelling a sufficient number of images sampled during operational measurements, or by using only pollen of a particular taxon which has been collected from the source of interest and fed into the instrument using the best-adapted technique to obtain data from a sufficient number of particles. The quality (purity) of the sample to feed into the device is of importance, as are the environmental conditions (temperature, humidity, etc.) that can affect particle properties such as shape and size. Little work has been done on how atmospheric conditions, such as humidity levels, may alter the shape and size of pollen grains in flight. Pollen are fully hydrated in the anthers, but can dehydrate within minutes once airborne and exposed to the ambient atmosphere, thus changing their shape (collapsing) and later potentially rehydrating (Katifori et al., 2010). It is, however, completely unknown to what extent and how this varies across different pollen taxa. This is particularly important for instruments based on flow cytometry that measure particles in an airflow without affecting them (i.e. they do not land on a substrate and thus do not re-hydrate). Similarly, the fluorescence signal may change as pollen grains age (Könemann et al., 2019), however, it is unknown how long this takes. Systematic studies are needed to better ensure the quality of identification.

Pollen identification algorithms also need to be developed to take several other aspects into account. For example, individual particles are analysed one at a time. In regions with periods of very high particle concentrations, whether it is pollen or not, (e.g. in polluted cities or areas affected by desert dust), this issue is of particular importance. Aggregation of particles can also affect the shape and size of what is detected, which can, in turn, affect instruments using both image recognition and measuring optical particle properties. Similarly, ambient debris adhering to the surface of pollen may affect their identification by changing their morphological structure. It is currently not known how pollution affects the optical properties when other small aerosols attach to pollen grains; this could impact all systems using optical properties as part of the detection and identification. The influence of environmental conditions, other particles present in the atmosphere, as well as the aggregation of pollen particles are all aspects that need to be considered when developing instruments and the detection algorithms that they use.

It is also important to note that the performance of classification usually decreases with the number of classes that are to be identified. This needs to be kept in mind when evaluating the performance of different algorithms or different instruments. To make a fair comparison, at least the same number of taxa should be considered, ideally even exactly the same taxa. Again, guidelines for how to do this more consistently are provided in the companion paper on standards in this special issue.

10 What can we conclude?

New technologies and methods have allowed the development of a range of different instruments that

can monitor bioaerosol and, in certain cases, specific pollen and fungal spore taxa automatically. The number of such instruments only continues to increase, so working together with instrument developers and manufacturers will help grow expertise across the community and ensure more standardised results, whether it be related to data formats, algorithm development or instrument validation. Likewise, it is equally important to work with end-users who require the information such instruments and networks provide, to ensure that products and services are developed to better meet their needs.

Compared to the current manual standard in use across most pollen and fungal spore monitoring networks, automatic instruments offer a number of advantages. These include the provision of data in real time at considerably higher temporal resolutions (up to hourly), as well as requiring considerably less manual intervention (e.g. less frequent replacement of consumables or maintenance). Nevertheless, such technologies are new and not yet capable of identifying as large a range of pollen or fungal spore taxa as manual methods. In the long term, automatic instruments should be capable of identifying a wide range of taxa at concentrations that meet the needs of all end-user groups. This includes for numerical forecast models, as well as for medical, climate change, forestry, and agricultural applications. Measurement techniques and identification algorithms will need to be improved to enlarge the number of taxa that can be discriminated, in particular, this includes fungal spores. Furthermore, standards need to be developed regarding the data and metadata provided, in addition to how particle identification algorithms are developed and validated. This will enable traceable data quality across the entire measurement chain. Changes to instrumentation as well as identification algorithms will improve device performance over time, but care needs to be taken to ensure homogenous datasets can be produced. This will require detailed protocols to be kept, analysis software is versioned, and that all data are stored in case reanalyses need to be carried out at a later date. At the community level, new expertise covering topics such as software development, machine learning techniques, and dealing with big data is required. In addition, guidelines need to be developed for routine instrument and algorithm testing, as well as those related to assessing data quality. All these tasks are being carried out by a number of groups, most of them through or as part of the EUMETNET AutoPollen Programme.

While no automatic instrument can yet identify all the pollen and fungal spore types required by end users (see Table 1) and no standardised method to calibrate automatic instruments has been established, there is continual development in the field and these monitors have already proven their worth–both in terms of the higher temporal resolution information they can provide and their ability to do so in real time. Indeed, a number of sites or networks across Europe already have enough confidence in these technologies to publish their data in real-time online. As the technology further develops and matures, automatic instruments can be expected to provide more and better quality data which can be used across a growing number of domains.

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