

Automated pollen monitoring system using laser optics for observing seasonal changes in the concentration of total airborne pollen

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Abstract The development of a simple and automatic pollen measurement methodology is required to manage allergic problems caused by airborne pollen. We developed a device and algorithm to automatically monitor airborne pollen by using basic laser optics technology. The device measures the sideward and forward scattering intensities of laser light from each particle. Because this device provides detailed temporal variation in the pollen concentration, the dispersal dynamics of airborne pollen can be effectively analyzed. We compared the pollen counts obtained with the automated method and standard Hirst-type method. Scatter-plot graphs were constructed of the forward and sideward scattering intensities of the observed particles. An extract window methodology was used to estimate the

concentrations of the major allergenic pollens. The extract window parameters were obtained for major types of allergenic pollen. The results suggest the possibility of developing a device for monitoring several types of airborne pollen simultaneously. We determined the standard extract window to be used for estimating the concentration of all types of airborne pollen together. A field experiment was performed to evaluate the automated monitoring system with the standard extract window. The estimated temporal variation pattern of the total airborne pollen concentration agreed well with the observed temporal variation pattern for the whole pollen season. The pollen monitor was able to estimate the overall features of seasonal changes in the total airborne pollen concentration.

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1 Introduction

The number of pollinosis cases has increased rapidly worldwide in recent years, and the disease is becoming a significant social problem. In Japan, considerable aerobiological research related to cedar pollinosis has been conducted to clarify the spatial and temporal variations of airborne pollen concentrations (e.g.,

Kawashima and Takahashi 1995, 1999). Remedies do exist to ameliorate the allergy, such as treatment with antiallergic drugs; however, the most important measure is limiting exposure to the allergens. Therefore, an automatic real-time pollen measurement method is needed to promptly provide comprehensive pollen-related information and offer boundary conditions for forecasts.

Existing methods require significant amounts of labor and time for identifying and counting the subject pollen using a microscope. Despite the wide use of common standards, counting errors can occur depending on the skill level of the personnel (Galan et al. 2014). With respect to techniques for measuring airborne pollen, research has been conducted on providing prompt pollen reports. Such research has been based on automation and laborsaving techniques, such as developing new types of pollen collectors and investigating methods for automatic pollen monitoring.

The investigations on automated methodology for measuring airborne pollen had been launched to develop image recognition technologies based on microscopic image of sampled pollen (e.g., Bennett 1990; Bechar et al. 1997; Aronne et al. 2001). Progress in this field has been made in recent decades. Chen et al. (2006) focused on an automatic pollen categorization device that uses image processing of pollen grains for automated pollen identification. They considered the pollens of orchard grass (*Dactylis glomerata*), birch (*Betula*), and mugwort (*Artemisia vulgaris*) for computerized identification. The identification process consisted of five stages: image preprocessing, automatic pollen sorting, feature extraction, classification, and validation. A sample of 254 pollen grains with a mixture of three types of pollen was tested, and 97.2% of the pollen grains was correctly identified. Ranzato et al. (2007) presented a simple general-purpose system to identify the biological particles in microscopic images and demonstrated the possibility of categorizing airborne pollen into several groups by using image analysis. Gottardini et al. (2007) developed a method of identifying different varieties of pollen by using information obtained from infrared spectroscopy. Landsmeer et al. (2009) presented a method of detecting pollen in multifocal optical microscopy images of air samples collected by a Hirst-type pollen sampler. Wagner and Macher (2012) considered the automated identification and counting of fungal and bacterial spores by

employing an analysis of microscopic images and the automated detection of peaks in aerosol size distributions. In addition to pollen identification methods using microscopic image analysis, several other novel ideas have also been examined (Hinz et al. 1999; Takahashi et al. 2008; Longhi et al. 2009; Stanley et al. 2011; Rittenour et al. 2012; Healy et al. 2012; O'Connor et al. 2013, 2014; Marcos et al. 2015; Oteros et al. 2015).

In another attempt at automated measurement of pollen (Harder 1990; Young and Stanton 1990), a particle counter was used. However, because this approach is simply the application of a particle counter, only the particles within a given size range were counted. It was therefore difficult to distinguish pollen grains from dust particles of a similar size. Advances in laser optics in the last decade have enabled the application of this technology in various fields. We (Kawashima et al. 2007) developed a device and algorithm for monitoring airborne pollen automatically by using laser optics. Mitsumoto et al. (2010) attempted to construct a new real-time pollen variety counter that uses the autofluorescence of pollen to rapidly categorize and count different pollen varieties. Measurements with this collector showed a strong correlation with a Durham-type collector, but its collection efficiency was higher. Crouzy et al. (2016) examined the applicability of an air flow cytometer, the Plair PA-300 (Kiselev et al. 2011, 2013), to identifying pollen types in air. They showed that the device can be used to discriminate between different pollen taxa.

In our first study (Kawashima et al. 2007), a high correlation was found between the automated method and traditional standard method. This device is used in the cedar pollen information network “Hanakosan,” which was established and managed by the Ministry of the Environment, Japan. Although this was a preliminary result for a limited period and few types of airborne pollen, this method became the basis for our research. Our final objective is to develop a generally applicable algorithm and device for monitoring and discriminating types of airborne pollen grains automatically. Our current objective is to establish a rapid measurement technique for airborne pollen that is easy to maintain. For an actual monitoring system, easy maintenance and low-cost devices are required to establish a network with a relevant number of observation points.

In this paper, we discuss the appropriate parameters for the detection algorithm of a laser pollen monitor to fit several types of allergenic airborne pollens. The detection parameters for monitoring airborne pollen concentration were applied to different sites separate from the location where the parameters were determined. The effectiveness of the automatic pollen monitoring system in observing the seasonal changes in the total airborne pollen concentration was demonstrated.

2 Materials and methods

2.1 Experimental sites and pollen measurements

Two series of field experiments were performed in the central region of Western Europe. One experiment was conducted to obtain basic data for improving the algorithm and parameters of the automatic pollen monitoring system, and the other was to test the actual performance of the improved pollen monitor. This paper discusses the daily airborne pollen concentration with a focus on considering the changes in the airborne pollen concentrations over a long period during pollen season.

The first experiment was conducted in 2010 at the Réseau National de Surveillance Aérobiologique (RNSA) near Lyon, France. The altitude of the town is approximately 480 m a.s.l., and it is located in a gentle hilly district in the western part of the city. A Hirst-type pollen sampler (Lanzoni s.r.l.) and our automatic monitoring device for airborne pollen were set up adjacent to each other in the garden of the RNSA institute near the hilltop area. Figure 1 shows a general diagram of the airborne pollen measurement system at RNSA. In this experiment, we used the original data acquisition type of pollen monitor. Meteorological data were measured at the same time in the region. The Hirst sampler used a 7-day sampling head. We obtained the pollen concentration data in pollen grains per cubic meter. We summarized the observed data as the daily average concentration. Several pollen taxa and groups of taxa were selected as subjects for the research based on their level of allergenicity and airborne quantity. The appropriate period for the pollen monitor estimation was determined for each type (specie, genus, or family) by comparing the temporal variations in the airborne

pollen concentrations obtained by the two methods (i.e., the Hirst sampler and pollen monitor).

The second experiment was conducted from 2013 to 2015 at Station Aérologique de Payerne, which is the MétéoSuisse center for meteorological measurement in Switzerland. The altitude of the site is approximately 490 m a.s.l. A Hirst-type pollen sampler (Burkard Manufacturing Co., Ltd.) and our automatic monitoring device for airborne pollen were set up adjacent to each other on the roof of the 2-storey building. The arrangement of the measurement system in Payerne was the same as that shown in Fig. 1. In this experiment, we used an actual operational type of pollen monitor. The meteorological data were measured at the same time onsite, and the data processing procedure for the Hirst sampler was the same as that in the RNSA experiment.

2.2 Outline of the automatic pollen monitor

The pollen monitors used in our experiments are special versions customized for the experiments based on the commercial version of the automatic pollen monitor (KH-3000-01, Yamatronics, Japan). The intake air is directed to the dust-filtering device set in the first part of the airflow system of the pollen monitor (Kawashima et al. 2007). Because the high-density particles in the air drop quickly, they can be removed from the airflow. The air containing the remaining particles, mostly small particles and pollen grains, is introduced into the optical system. The scattered light induced by each particle passing in the beam of a semiconductor laser (780 nm, 3 mW) is detected. The forward- and sideward-scattered pulses are measured and the peak intensities of them are sent to a personal computer. Automatic power control system for laser removes the effects of temperature on measurement. The output of the optical system is calibrated by using Japanese cedar (*Cryptomeria japonica*) pollen provided by the Japan Forest Tree Breeding Association.

The intensity of the light scattered by the particles is affected by the size, shape, color, surface roughness, and other characters related to the particles. The size of the pollen grains is the most important character for their identification. The difference between the sideward and forward intensities of the scattered light is mainly related to the particle shape and surface roughness (Xu 2000; Mishchenko et al. 2000). The

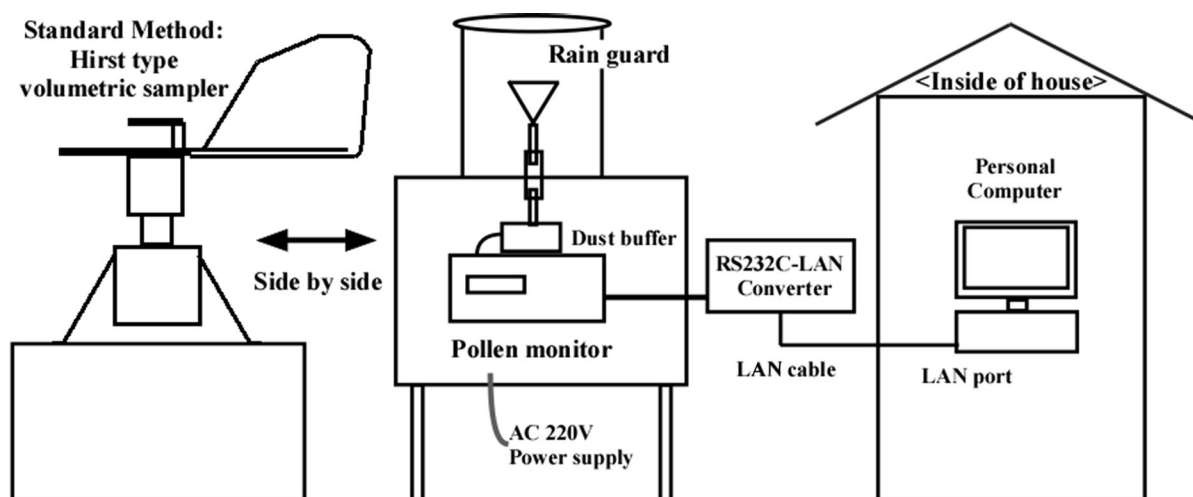


Fig. 1 Overall diagram of the airborne pollen measurement system in the RNA. The Hirst-type pollen sampler and automatic pollen monitor were set up adjacent to each other

intensities of the scattering light induced by a specific type of pollen can be observed although with statistical variability, even for a single type of pollen. There are two main reasons for the variability. First, the variation may be caused by individual differences in the pollen sample. Second, the variation may be caused by the measurement hardware. The measurement algorithm for the laser pollen monitor should consider the presence of variability in the intensity of the observed scattering data.

We used two types of automatic pollen monitor: the original data acquisition type used in the RNA experiment and the actual operational type used in *MeteoSwiss* experiment. The optical systems of the two types are the same with only the contents of the output data being different. The original data acquisition type outputs the raw data of scattering light intensities. The actual operational type outputs the number of particles that are obtained from the extract window algorithm. Both monitor types used in this study have the same optical systems which was improved from the one introduced in the prototype monitor (Kawashima et al. 2007).

2.3 Extract window and collection factor

The expected ranges for the two directions of scattering were used to define a rectangular area inside the scatter chart of sideward scattering intensity versus forward scattering intensity. Points in the scatter chart were extracted from the window of this rectangular area. We

call this rectangular area the “extract window” and this scatter chart the “scattering light diagram” (Fig. 2a). We assumed that there is an appropriate window for each type of subject pollen or each pollen group, and the subject pollen is counted within this window. The location and size of the extract window were determined by trial and error; we compared the monitored pollen counts with the observed concentrations from the Hirst sampler. The Pearson product-moment correlation coefficient between the daily values of the two methods was used as an index of appropriateness to optimize the extract window for each type of subject pollen or pollen group. The parameters for the extract window were determined to maximize the correlation coefficient.

The collection rate of the monitor was considered simultaneously with the process of searching for the parameters. We calculated the ratio of the sum of daily pollen concentrations observed by the Hirst sampler to the sum of daily pollen counts obtained from the pollen monitor. This ratio was obtained after the totalizing of each data. We call this ratio the “collection factor.” Extract window and collection factor were calculated using the data of the monitor estimation period determined for each type of subject pollen or pollen group.

To simplify the explanation, we consider only one direction of scattering light here. The histogram for the distribution of the scattered light intensity caused by one type of pollen is schematically shown in Fig. 2b. If the histogram pattern is specific, then, depending on the type of pollen, the ratio between the total count of the entire range and the count for a fixed extract window

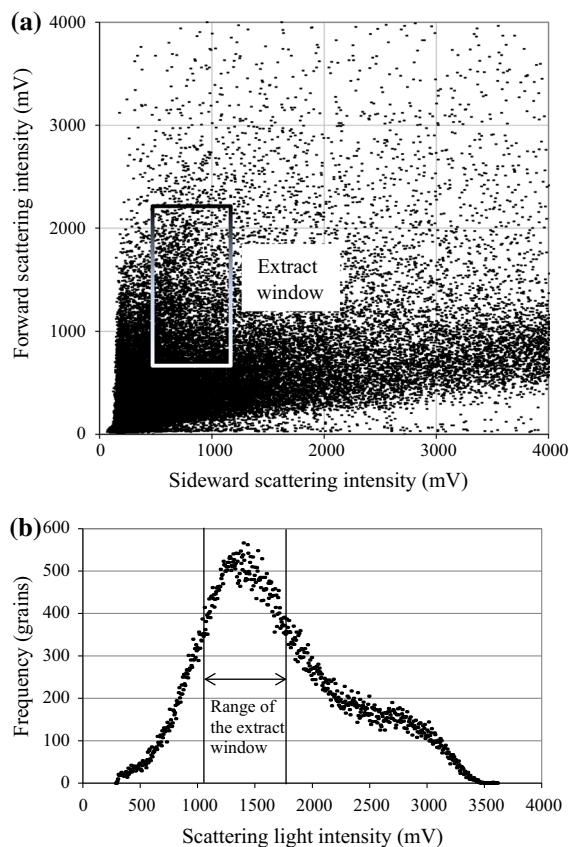


Fig. 2 **a** Example of an extract window located in a scatter chart between the sideward and forward scattering intensities; **b** example chart for the histogram distribution of the scattered light intensity caused by Japanese cedar pollen

would be constant. The collection factor includes this ratio in the histogram as well as the difference in collection efficiency between the two methods. The collection factor was used to convert the pollen monitor count to a concentration with the unit of the standard method: grains per cubic meter. In this study, we selected the conversion method which is recognized rationally as collection efficiency, and constructed the estimation method by using a collection factor.

3 Results

3.1 Features of temporal variation in the airborne pollen concentration at RNSA

We performed a basic experiment at RNSA to clarify the appropriate locations and ranges of the extract

windows for several pollen taxa and groups of taxa. The observation was conducted from March 22 to September 26, 2010. The pollen of Cupressaceae was observed from the end of March to the beginning of April. *Fraxinus*, *Quercus*, and *Betula* were dispersed from the end of March to the middle of May. These three types of highly allergenic pollen were dispersed together with peak concentrations in the following descending order: *Quercus*, *Fraxinus*, and *Betula*. The second highest total pollen concentration throughout the pollen season was *Poaceae*, which was observed from the beginning of May to the middle of July. The Urticaceae family typically dispersed pollen from the end of May to the late stages of the pollen season. *Ambrosia* pollen with high allergenicity was dispersed temporarily. The major types of pollen in the experimental region and their allergenicity levels are as follows: Cupressaceae, level 5 (max); *Poaceae*, level 5 (max); *Betula*, level 5 (max); *Ambrosia*, level 5 (max); *Fraxinus*, level 4; *Quercus*, level 4; Urticaceae, level 3; *Populus*, level 2; *Castanea*, level 2; *Pinus*, level 0. Allergenicity levels are based on the criterion suggested by RNSA.

3.2 Extract window and correlation between the two methods

Pollens with high allergenicity dispersed for a period of approximately 4 months from the end of March to the middle of July. The characteristics and advantages of the pollen monitor during the main pollen dispersal season were examined by considering the temporal variations in the airborne pollen concentrations of the major allergen pollens. Table 1 lists the obtained parameters for the major types of allergenic pollen.

3.2.1 Major types of allergenic pollen

The Cupressaceae pollen appeared mostly during the early stage of the pollen season. The extract window for the Cupressaceae pollen was located on the left-hand side of the scattering light diagram. For the major dispersal period of Cupressaceae, the correlation coefficient between the daily airborne pollen concentration from the Hirst sampler and the daily pollen count extracted by the monitor was high at 0.68. Figure 3a shows the temporal variations in the airborne pollen concentrations from the two observational methods for the Cupressaceae pollen. The

Table 1 Extract windows and collection factors for the major types of allergenic pollen

Subject pollen	Range of extract window		Collection factor	Period for monitor estimate	Correlation coefficient
	Sideward (mV)	Forward (mV)			
Cupressaceae	250–500	400–750	8.1	3/22–4/9	0.68
Fraxinus	570–950	270–480	6.6	3/26–4/17	0.73
Betula	1000–1500	900–1300	19.5	4/5–4/25	0.84
Quercus	500–760	500–850	21.5	4/21–5/13	0.68
Poaceae	670–900	870–2200	20.4	5/1–7/12	0.74
Spring major 3	500–780	300–850	11.4	3/26–5/14	0.68
Total pollen	300–900	300–2000	5.8	3/26–7/12	0.52

The plant species, range parameters for the extract window, collection factor, period of monitor estimation, and correlation coefficient between the observed pollen concentration from the Hirst sampler and estimated pollen concentration from the pollen monitor are listed

Fraxinus pollen appeared after the Cupressaceae pollen. This pollen dispersed together with the *Betula* pollen during April and together with the *Quercus* pollen from the end of April to the beginning of May. The extract window was located at the lower left of the scattering light diagram. The correlation coefficient related to the major dispersal period was high at 0.73. Figure 3b shows the temporal variations in the airborne pollen concentrations from the two observational methods for the *Fraxinus* pollen. The *Betula* pollen, which is one of the most problematic in many countries, dispersed mainly during April. The extract window appeared at the lower left near the center of the scattering light diagram. The correlation coefficient related to the major dispersal period was very high at 0.84. Figure 3c shows the temporal variations in the airborne pollen concentrations from the two observational methods for the *Betula* pollen. The *Quercus* pollen appeared just after the peak period of the *Betula* pollen and had relatively high allergenicity during the spring. The extract window appeared with a wider range at the lower left of the scattering light diagram. The correlation coefficient related to the major dispersal period was high at 0.68. Figure 3d shows the temporal variations in the airborne pollen concentrations from the two observational methods for the *Quercus* pollen. Poaceae is a representative pollen with a high level of allergenicity and disperses during the summer (from May to July). Although many other types of pollen dispersed at the same time, the amount of airborne Poaceae pollen was dominant. The dispersal of Urticaceae pollen started in the latter half

of the Poaceae dispersal period. An extract window with a wider range appeared on the left-hand side of the scattering light diagram. The correlation coefficient related to the dispersal period was high at 0.74. Figure 3e shows the temporal variations in the airborne pollen concentrations from the two observational methods for Poaceae pollen.

3.2.2 Three main types of pollen and total airborne pollen

We examined the extract window for a combination of subject pollens. In spring (from April to May), several types of pollen with strong allergenicity were dispersed together. It was possible for the pollen monitor to identify and distinguish these types of pollen to some degree by changing the position and size of the extract window. However, it is convenient to estimate these pollen types together with a fixed extract window. Consequently, the total concentration of the three types of representative pollen (*Fraxinus*, *Betula*, and *Quercus*) should be monitored together as a practical method of utilizing the pollen monitor. The extract window for the sum of these three types of pollen was located at the lower left of the scattering light diagram. The correlation coefficient related to the dispersal period was high at 0.68. Figure 4a shows temporal variations in the airborne pollen concentrations from the two observational methods for the three major types of pollen during spring.

In actual pollen monitoring, it is convenient to use the monitor with a single fixed extract window

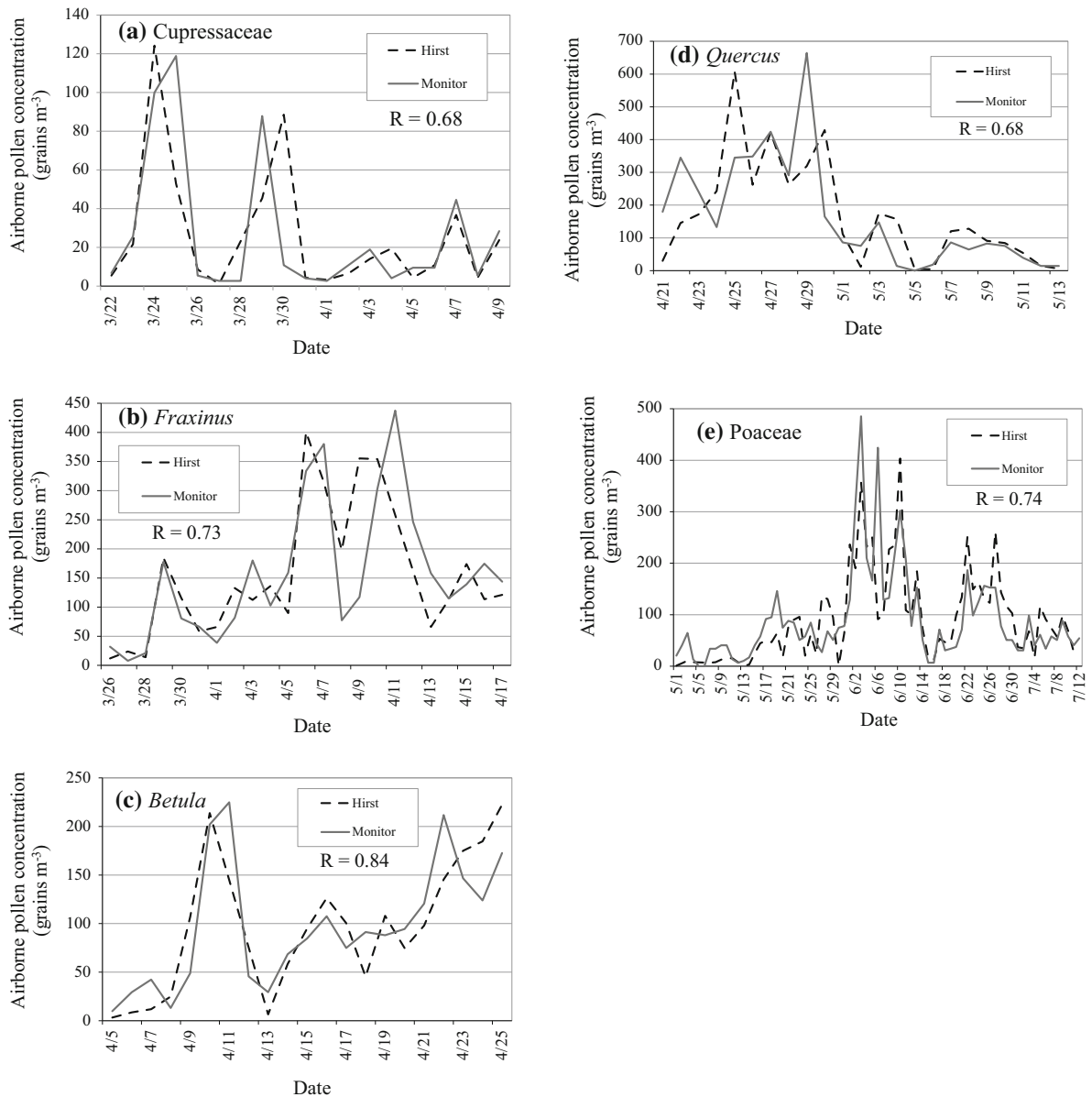


Fig. 3 Temporal changes in the airborne pollen concentrations according to the two observational methods at the RNSA in 2010: **a** Cupressaceae, **b** *Fraxinus*, **c** *Betula*, **d** *Quercus*, and **e** Poaceae

throughout the main pollen season. We searched for an appropriate extract window for the total airborne pollen concentration. The extract window was located at the lower left of the scattering light diagram. The correlation coefficient related to the dispersal period was moderately positive at 0.52. Figure 4b shows the temporal variations in the airborne pollen concentrations from the two observational methods for the total airborne pollen.

3.3 Performance of real-time pollen monitoring

A validation experiment was conducted by using a pollen monitor with a fixed extract window to estimate the actual applicability of the pollen monitor to obtaining the real-time concentration of total airborne pollen at Payerne about 200 km far from Lyon. Based on the results at RNSA, the range of the extract window for total pollen was fixed at 300–900 mV for

sideward scattering and 300–2000 mV for forward scattering. We call this extract window the “standard extract window.”

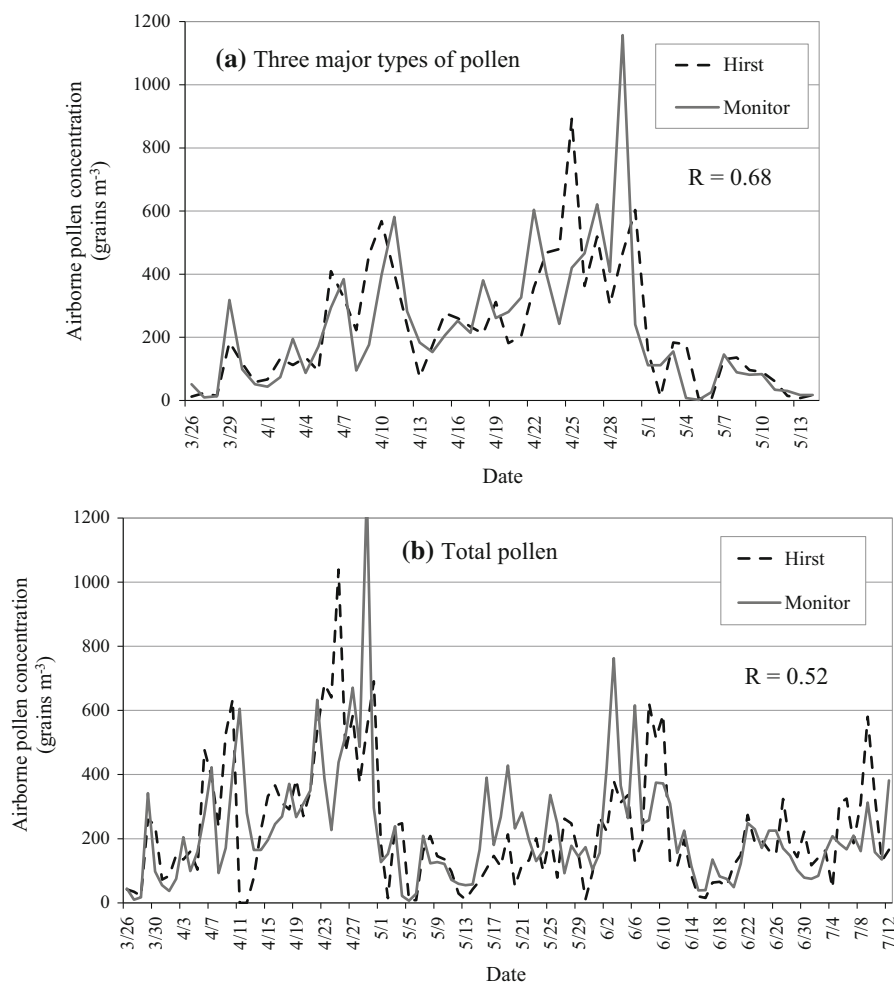
In 2013, an observation was performed from May 25 to July 25. Figure 5a shows the temporal variations in the daily airborne pollen concentrations from the two observational methods for total airborne pollen. The correlation coefficient between the Hirst data and monitor data was very high at 0.87. The collection factor in this case was 4.5. The dashed line labeled as “Monitor” shows the monitor data estimated by the collection factor for this year. The dotted line labeled as “Monitor unite” shows the monitor data estimated with the collection factor of 4.2, which is the mean collection factor for the 3-year period of 2013–2015. The collection factor in 2013 was close to the mean collection factor for the 3 years. A large peak was

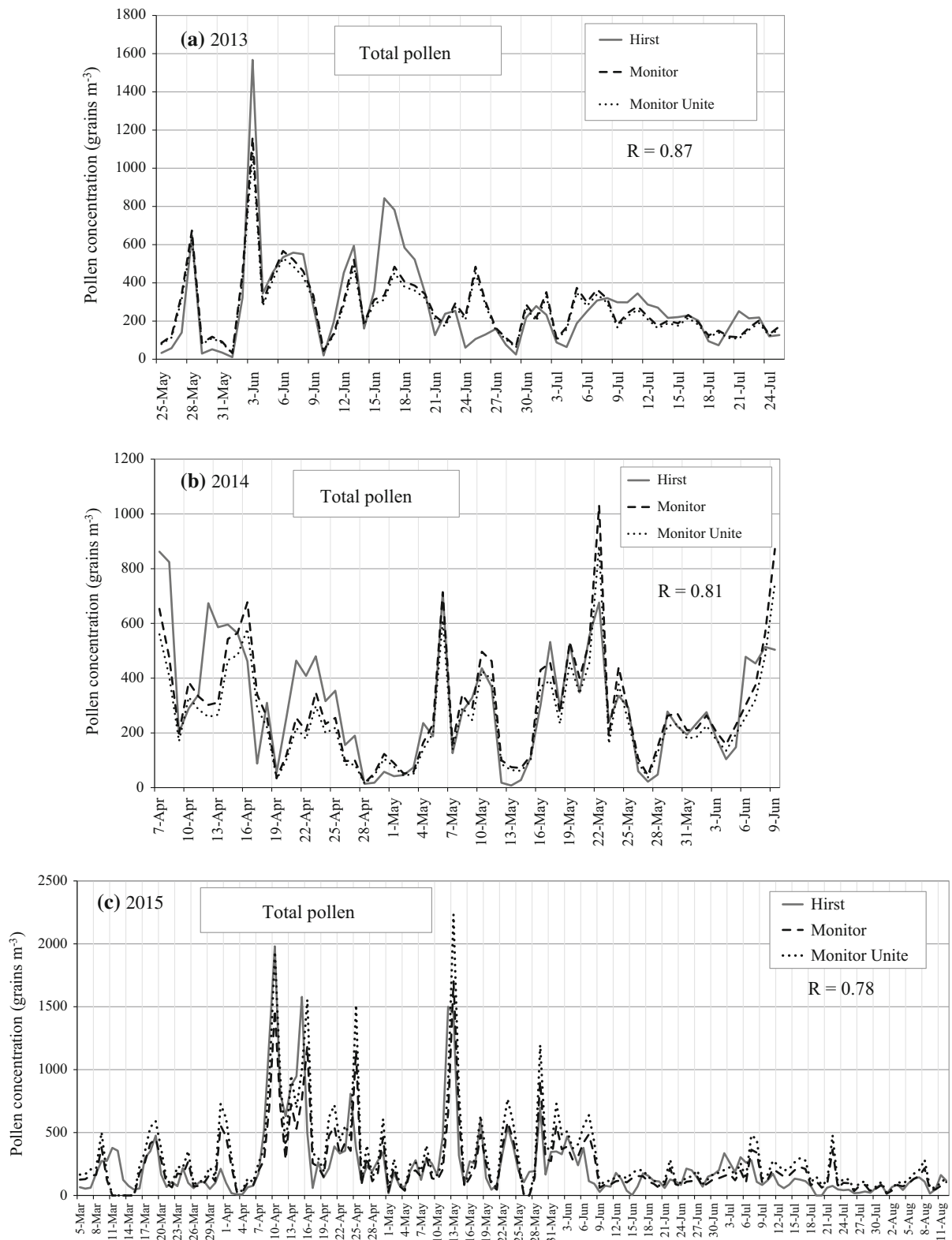
Fig. 5 Temporal changes in the total airborne pollen concentrations according to the two observational methods at Station Aérologique de Payerne. “Hirst” is the concentration obtained by the Hirst sampler, “Monitor” is the concentration estimated from the monitor using the collection factor for each year, and “Monitor Unite” is the concentration estimated from the monitor using the mean collection factor for the 3 years: **a** 2013, **b** 2014, and **c** 2015

observed at the beginning of June, and the pollen monitor detected this peak well. The pollen monitor estimated increases and decreases in the daily pollen concentration, as well as the gradual decreasing trend after the large peak.

In 2014, an observation was performed from April 7 to June 9. Figure 5b shows the temporal variations in the daily airborne pollen concentrations from the two observational methods for the total airborne pollen.

Fig. 4 Temporal changes in the airborne pollen concentrations according to the two observational methods at the RNSA in 2010: **a** three major types of pollen in spring and **b** total pollen





The correlation coefficient between the Hirst data and monitor data was very high of 0.81. The collection factor in this case was 4.9. The collection factor in 2014 was larger than the mean collection factor for the 3 years.

In 2015, an observation was performed from March 5 to August 12. Long-period verification could be performed. Figure 5c shows the temporal variations in the daily airborne pollen concentrations from the two observational methods for the total airborne pollen. The correlation coefficient between the Hirst data and monitor data was very high at 0.78. The collection factor in this case was 3.2. The collection factor in 2015 was smaller than the mean collection factor for the 3 years. As indicated by this figure, the estimated temporal variation pattern of the total airborne pollen concentration agreed well with the observed temporal variation pattern for almost the entire pollen season. Although the agreement between the estimated and observed values was not good over several days, the pollen monitor was able to estimate the overall features of the seasonal changes in the total airborne pollen concentration.

4 Discussion

The principle of our pollen monitor is not based on detecting all of the grains of the subject pollen. Instead, we estimate the airborne concentration of the subject pollen based on a dataset observed during a time interval of 1 day or 1 h. The dataset consists of a group of observed data, such as a cluster, mainly of the subject pollen. Variability is indicated by the intensity of the scattering light for each sample of pollen, even when the type of pollen is the same species. The frequency distribution for the same type of pollen shows the approximate shape of a second-order normal distribution. In the actual data, the distribution of points is a complex overlapped result of a number of individual clusters. Each individual cluster is thought to correspond with a specific type of pollen or specific airborne material. To observe a specific type of pollen as the subject of scattering light diagram, we need to clarify the area in which the cluster density of the subject pollen is high and the cluster density of the other types of particles is low. We set the extract window in such an area and count the particles observed inside the extract window. The

probability of the subject pollen appearing in the extract window among the pollen clusters is an important factor. A similar interpretation is possible when the total airborne pollen is the subject. Although the cluster for a single type of pollen shows an occurrence frequency corresponding to a normal distribution, the cluster for the total pollen can become a complex distribution of occurrence frequencies with multiple peaks. However, the measurement methodology of using the extract window for a single type of pollen can be applied to measuring the total airborne pollen.

The primary merit of our automated pollen monitoring system is easy maintenance and lower cost of the system compared with recent microscopic methods (Marcos et al. 2015; Oteros et al. 2015) and the recent laser-induced fluorescence method (Crouzy et al. 2016). Comparing the effectiveness of these methods for real-time usage is difficult because the experimental conditions and hypotheses were different in each study. However, we could perform verification experiments for 3 years of actual operation. The verification was made in different place from the place where we decided the parameters of the device. The results might be evidence of the effectiveness of our automated pollen monitoring system using laser optics. The collection factor changed slightly in the 3 years during the experiments. Although the mean collection factor of the 3 years may be applicable for actual operation, studies to theoretically clarify the change in the collection factor are needed in order to improve the accuracy for practical usage of pollen monitor. After the pollen season, the device should be cleaned and calibrated. The accuracy of the pollen monitor will degrade without maintenance procedure.

In the future, we are planning to develop a practical methodology for distinguishing between two or more types of pollen in real-time. The measurement accuracy needs to be improved for the monitor to be applied throughout the pollen season. This is mainly related to technological improvements for minimizing the statistical variability in the intensities of the scattering light. If the range areas of the plots for each type of pollen in the scattering light diagram are smaller, setting the extract window for the subject pollen can be determined with less mixing of non-subject pollens. Moreover, performing simultaneous measurements of several subject pollens will be easier.

5 Conclusions

The aim of our study was to develop a device and algorithm to automatically monitor airborne pollen by using basic laser optics technology. The sideward and forward scattering intensities of laser light from each particle were measured. We compared the pollen counts obtained by the automated method with that obtained by the standard Hirst-type method. An extract window methodology was used to estimate the concentrations of the major allergenic pollens. The extract window parameters were obtained for major types of allergenic pollen. The results suggest the possibility of developing a device for monitoring several types of airborne pollen simultaneously. We could determine the standard extract window to be used for estimating the concentration of all types of airborne pollen together throughout the pollen season in the experiment at RNSA in 2010. Field experiments were performed for 3 years from 2013 in a location 200 km away from RNSA to verify the effectiveness of the automated monitoring system with the standard extract window. The laser optical methodology was demonstrated to be applicable to estimating the overall features of seasonal changes in the total airborne pollen concentration.

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