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## Short-time dynamic patterns of bioaerosol generation and displacement in an indoor environment

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**Abstract** Short-time dynamics and distribution of airborne biological and total particles were assessed in a large university hallway by particle counting using laser particle counters and impaction air samplers. Particle numbers of four different size ranges were determined every 2 minutes over several hours. Bioaerosols (culturable bacteria and fungi determined as colony-forming units) were directly collected every 5 minutes on Petri dishes containing the corresponding growth medium. Results clearly show distinct short-time dynamics of particulate aerosols, both of biological and non-biological origin. These reproducible periodic patterns are closely related to periods when lectures are held in lecture rooms and the intermissions in between where students are present in the hallway. Peaks of airborne culturable bacteria were observed with a periodicity of 1 hour. Bioaerosol concentrations follow synchronously the variation in total number of particles. These highly reproducible temporal dynamics have to be considered when monitoring indoor environments with respect to air quality.

keywords: particulate aerosols, bioaerosols, aeromicrobiology, monitoring, particle counting, impaction sampling

## 1 Introduction

Airborne particulate matter is ubiquitous in the atmosphere and is present in outdoor as well as in indoor environments (Colbeck 1995). Due to their lightness in weight, airborne particles are readily transported, transferred, and displaced from an environmental compartment to another. Therefore, temporal and spatial variations with time occur easily within an environment (Fierer 2008). In many cases, depending on the location and the prevailing environmental conditions, regular patterns (daily, weekly, monthly, seasonally) can be observed (e.g. Johansson et al. 2007). However, regarding indoor locations, temporal and spatial fluctuations of airborne particle concentrations might occur even on very small time scales (within minutes) depending on the ventilation regime of the rooms as well as on occupant-related activities such as e.g. movement of people, smoking, cooking, or cleaning (Abt et al. 2000; Luoma 2001; Morawska et al. 2003;). However, reports rarely include information on temporal fluctuations or periodic patterns in time scales of <1 hour.

Bioaerosols are part of airborne particles found in the atmosphere. They are defined as aerosols (solid or liquid particles in a gas) of biological origin (Heikkinen et al. 2005). Bioaerosols occur naturally in outdoor and indoor environments and include viruses, viable organisms such as bacteria and fungi as well as products of organisms such as bacterial or fungal spores, plant parts or pollen (Colbeck 1995). It has been estimated that at certain locations and times of the year particles of biological origin can contribute with up to 50% to the total airborne particle numbers (Jaenicke 2005). Regarding indoor environments, airborne microorganisms might pose an environmental hazard when present in high concentrations resulting in health problems (Stetzenbach et al. 2004). This might mainly be the case in industrial or agricultural environments (Brandl et al. 2005; Dutkiewicz et al. 2000, Zollinger et al. 2006; Zormann 2008).

The indoor atmosphere is a very dynamic system where particles of biological and non-biological origin are distributed and displaced. As has been stated a long time ago (Tyndall 1876), that “myriads of germs are floating in our atmosphere”. Regarding indoor environments, it was already observed at this time that bioaerosols are heterogeneously distributed in the air and occur in “bacterial clouds” (Tyndall 1876). Microbial concentrations may fluctuate in time periods of minutes by several orders of magnitude (Brandl et al. 2005; Lighthart 1995). Indoor bioaerosol concentrations are related to climatic factors (e.g. outdoor temperature, humidity), room-related parameters (e.g. ventilation), and occupancy parameters (e.g. activities of persons present) (Bartlett et al. 2004). These parameters can be used to model and predict indoor bioaerosol concentrations (Green et al. 2003). In analogy to other ecosystems such as soil or water, only a small percentage (approx. 0.1 to 10%) of microorganisms present in the air can be grown in culture (Lighthart 1997).

The principal objective of this work was the investigation of temporal particle dynamics of aerosols of both biological and non-biological origin in an indoor location within a time scale of minutes. The focus was mainly on the determination of repetitive regularly occurring generation and distribution patterns of bioaerosols. In particular, the experimental goals were (i) to determine quantitatively the total number of airborne particles; (ii) to analyze the dynamics of the total particle number and composition of bioaerosols, and (iii) to study the correlation between the number of particles and human activities in an indoor environment.

## 2 Materials and Methods

### 2.1 Sampling location

Three independent experiments were carried out in an indoor environment (university hallway) at three different dates (April 19, 2005; October 27, 2005; April 20, 2006) to verify the reproducibility and generalization of the experimental observations (Tab. 1). As comparison and reference measurement, air was sampled on October 28, 2005 between 09:30 and 16:00 h in an outdoor location (roof of the building).

**Table 1** Summary of measurements of airborne particles and bioaerosols in an indoor environment (university hallway)

	Date		
	April 2005	October 2005	April, 2006
Measuring time	09:54 to 11:32	09:30 to 16:08	10:21 to 16:29
Overall time period (h)	1.6	6.6	6.1
Total particles (# of samples)	50	200	181
Sampling interval (min)	2	2	2
Culturable bacteria (# of samples collected by impaction)	10	16	39
Sampling interval (min)	10	10	5
Number of replicates	3	1	1
Culturable fungi (# of samples collected by impaction)	10	16	19
Sampling interval (min)	10	10	10
Number of replicates	3	1	1
Average temperature (°C)	24.1 ± 0.2	23.8 ± 0.8	25.3 ± 0.7
Average relative humidity (%)	33.9 ± 0.7	42.2 ± 1.5	29.7 ± 0.7

Air samplers (particle counters, impaction samplers) were placed in the center of the university hallway (dimensions 55 x 35 x 12 m; volume approx. 23100 m<sup>3</sup>; granite flooring, concrete ceiling, unfurnished) on tripods (1.2 m in height) in a close distance of approx. 2 m to ensure correlation of total particles numbers and culturable microorganisms. Doors from a lecture room (volume approx. 5800 m<sup>3</sup>, wood paneling (walls, ceiling), vinyl carpeting, no windows, 450 seats) lead to the hallway. Around 120 students were attending classes during the measuring period. The distance between the samplers and students (walking away from the samplers) was approx. 10 m.

### 2.2 Particle counting

Three “MetOne 227B” laser particle counters (SKAN AG, Allschwil, Switzerland) were used simultaneously to determine particle numbers. In total, particles of four different size ranges (0.3 to 0.5 µm, 0.5 to 1 µm, 1 to 5 µm, and >5 µm) were measured. Particles with aerodynamic sizes ranging from 0.3 to 0.5 µm were determined as triplicates to assess experimental standard errors. Generally, variations between the three instruments were within 5 to 8%. One of the particle counters was equipped with a temperature and humidity sensor. Samples were taken during the whole monitoring period for 21 seconds (corresponding to 1 liter of air) in intervals of 2 min resulting in 30 samples per hour (Table 1). Two particle size ranges can be simultaneously recorded by one particle counter. Single readings are stored in the internal memory which can be downloaded to a computer and subsequently be analyzed using the software Particle Vision PortAll 1.2.

### 2.3 Impaction sampling

“MAS-100 eco” (MBV, Littau, Switzerland) impaction samplers were used for the collection of bioaerosols. Routinely, 100 liters of air were collected in time intervals of 5 to 10 minutes

(Table 1). At certain occasions, up to three samplers were simultaneously operated. Standard 90 mm Petri dishes containing different selective standard growth media were used with the impaction sampler (Zollinger et al. 2006). Nutrient agar, NA, was used to determine the total number of culturable bacteria. NA medium consisted of meat extract (1 g/l), yeast extract (2 g/l), peptone (5 g/l) sodium chloride (5 g/l), and agar (15 g/l). The pH was adjusted to 7.4. After sterilization at 121°C for 20 min, cycloheximide (50 mg/l) was added through sterile filtration to prevent fungal growth. For the determination of fungi (molds and yeasts) malt extract agar, MEA, (malt extract, 20 g/l; yeast extract, 4 g/l; agar, 20 g/l) was used (Zollinger et al. 2006). pH was adjusted to 5.6 - 5.8 with HCl. After air sampling, NA plates were incubated at 30°C for 3 days, MEA plates at 27°C for 4 days. Colony forming units (cfu) were counted after visual inspection. Numbers were converted by the “positive hole correction” method and extrapolated to the effective counts (Feller 1950).

## **2.4 Particle residence times**

Displacement rates and residence times of airborne particles were modeled according to a first-order decay process (Cox 1995):  $N_t = N_0 e^{-kt}$ , where  $N_0$  represents the number of particles at time  $t = 0$ ;  $N_t$  represent the number of particles at time  $t$ ; and  $k$  represent the first-order decay constant ( $h^{-1}$ ). A linear regression plot of the natural logarithms of the particle concentration as a function of the time results in a straight line of negative slope ( $-k$ ). Residence times are calculated as  $k^{-1}$  (h).

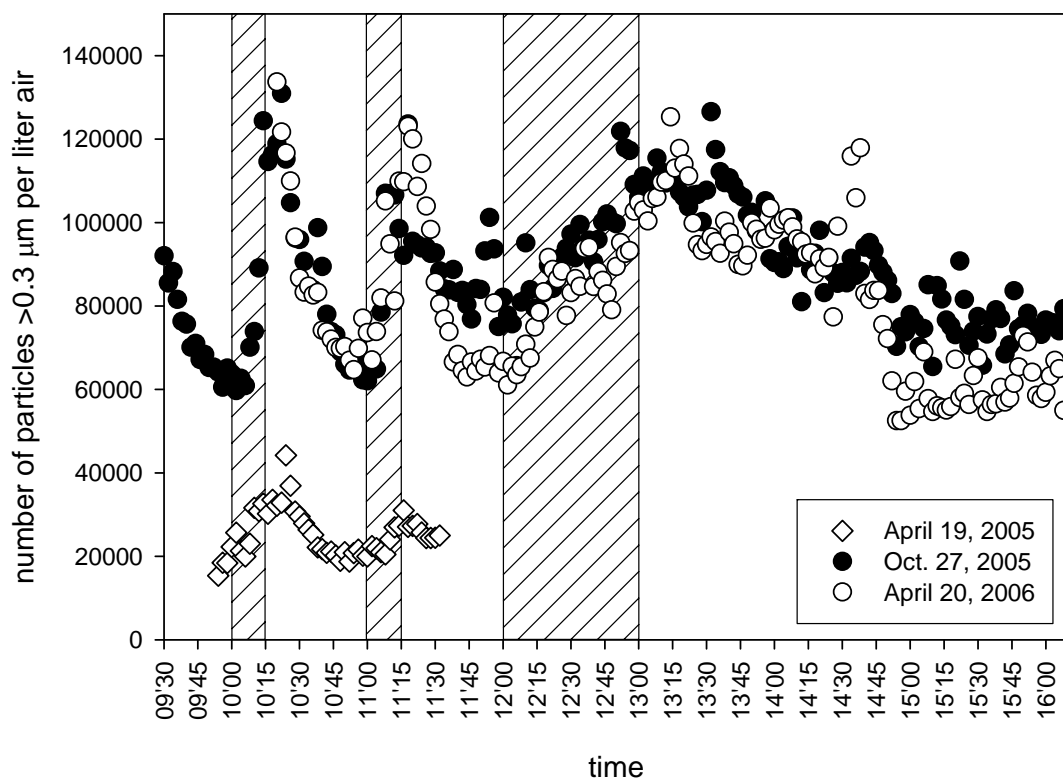
## **2.5 Air velocity**

Air velocity was measured with a Testo 400 ambient air velocity probe (Testo AG, Lenzkirch, Germany).

## **3 Results and Discussion**

Concentrations of airborne biological and total particles  $>0.3 \mu m$  showed distinct patterns closely related to anthropogenic activities, i.e. opening of doors and presence of students in the university hallway (Fig. 1). Lessons started always 15 min past the hour and lasted 45 minutes. After the end of the lectures doors opened and students entered the hallway. Fifteen minutes later doors are closed and lessons continue. A longer intermission (lunch break) was from 12:00 to 13:00 where students were present in the hallway. There were no lectures in the afternoon. In general, during breaks between lessons particle concentrations in the hallway increased within minutes by a factor of 2, whereas during lessons particle concentrations was reduced to background levels. Even in cases where total particle load (particles  $>0.3 \mu m$ ) was much smaller, temporal pattern was identical (Fig. 1).

Periodic fluctuations of airborne particles is related to the ventilation regime of the building: Outdoor air is actively collected and enters the ventilation system on the roof of the building. Incoming air is filtered and distributed into the lecture room resulting in a slight overpressure when doors are closed. When the doors open, air is



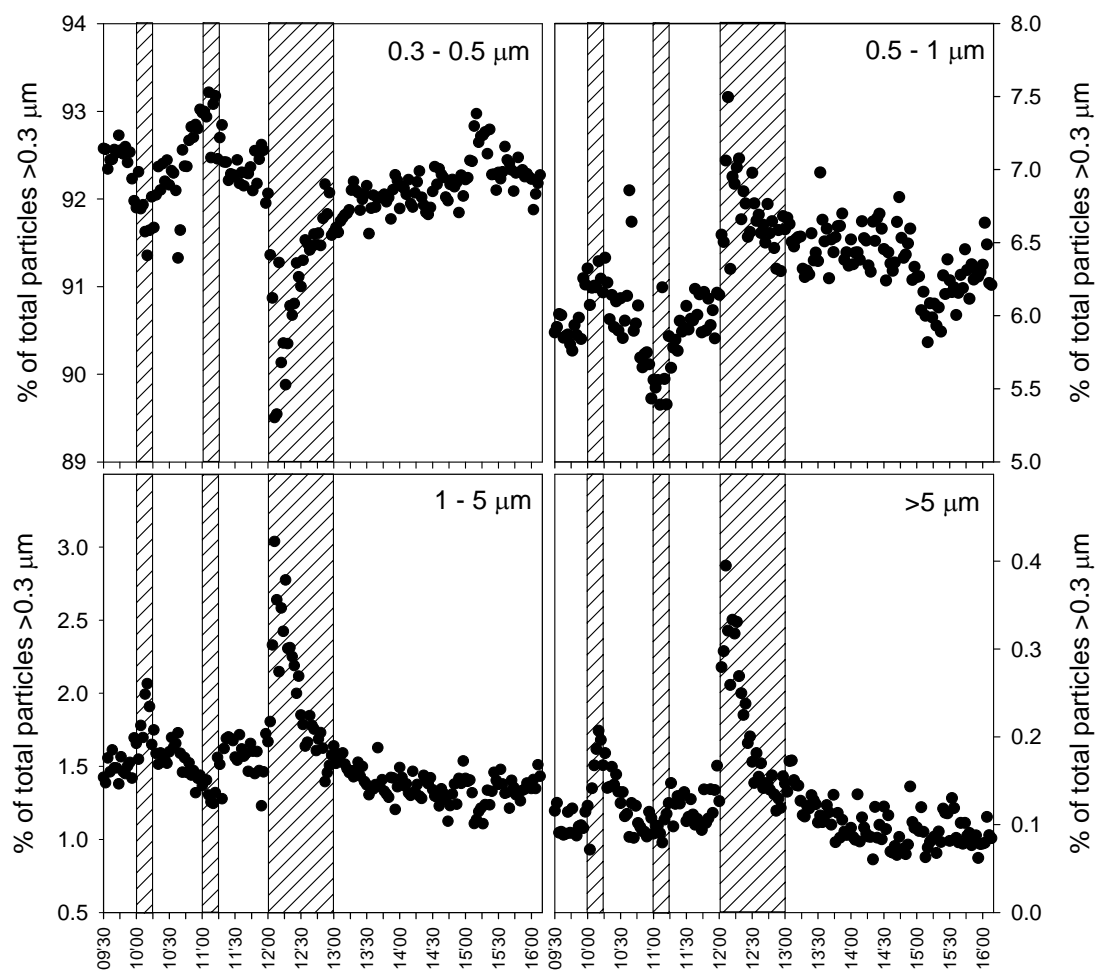
**Fig. 1** Time course of total airborne particles  $>0.3 \mu\text{m}$  in an indoor location (university hallway) between 09:00 and 16:00 h on three independent days. Shaded areas denote times of intermissions between lessons where lecture room doors are opened and students enter the hallway. Points represent mean values of triplicate measurements. Errors are  $<8\%$ .

transferred ( $0.35 \text{ m/s}$ ) from the lecture room into the hallway resulting in increased levels of airborne particles. These particles were rapidly displaced either by passive settling or due to air currents in the indoor environment as soon as intermissions between lesson are terminated and class is resumed. Residence times of particles are mainly between 0.9 and 2.5 hours (Table 2). At times with reduced anthropogenic activities (no afternoon lessons), particle residence time is approximately 6.5 hours.

Particulate aerosol composition was closely correlated to anthropogenic activities. During times of anthropogenic activities (opened doors, students present in the hallway) relative amount of particles (in % of total particles  $>0.3 \mu\text{m}$ ) in the size range 1 to  $5 \mu\text{m}$  and  $>5 \mu\text{m}$  increased maximally by factors of approximately 2 and 4, respectively (Fig. 2). The relative concentration of very small particles (size range 0.3 to  $0.5 \mu\text{m}$ ) concurrently decreased. Variations in the composition (in percent contribution of different size ranges) were already observed at other occasions where temporal fluctuations of indoor airborne particle concentrations have been triggered by anthropogenic activities such as unpacking of mail or unloading of agricultural products in industrial environments as well as smoking, cooking, and cleaning in residential locations (Brandl et al. 2006; Branis et al. 2005; Morawska et al. 2003; Stetzenbach et al. 2004). Diurnal variations have been observed; these were correlated to human activities (Morawska et al. 2003; Abt et al. 2000).

Bioaerosol concentrations are closely related to temporal variations of total particles and followed these patterns synchronously (Fig. 3). With a periodicity of 60 minutes a peak of airborne culturable bacteria was observed. During intermissions between lesson concentration of airborne bacteria were increasing, whereas during lessons (with doors closed), bacterial numbers reached minimal values in the hallway. Maximally  $1200 \text{ cfu/m}^3$  were detected;

minimal values were around 300 cfu/m<sup>3</sup>. Temporal patterns of airborne fungi are less pronounced (Fig. 3). Generally, fungal cfu are smaller compared to bacterial numbers. In outdoor environments fungal spores occur mainly in summer and fall (Anonymous 1993). Since the outdoor air is the main source of fungi in the indoor air, this might also be reflected in airborne fungal concentrations determined in October 2005.



**Fig. 2** Composition of airborne particles in four size ranges (0.3 to 0.5 µm, 0.5 to 1 µm, 1 to 5 µm, >5 µm) in percent of total number of airborne particles >0.3 µm. Sampling date was October 27, 2005 (see Table 1).

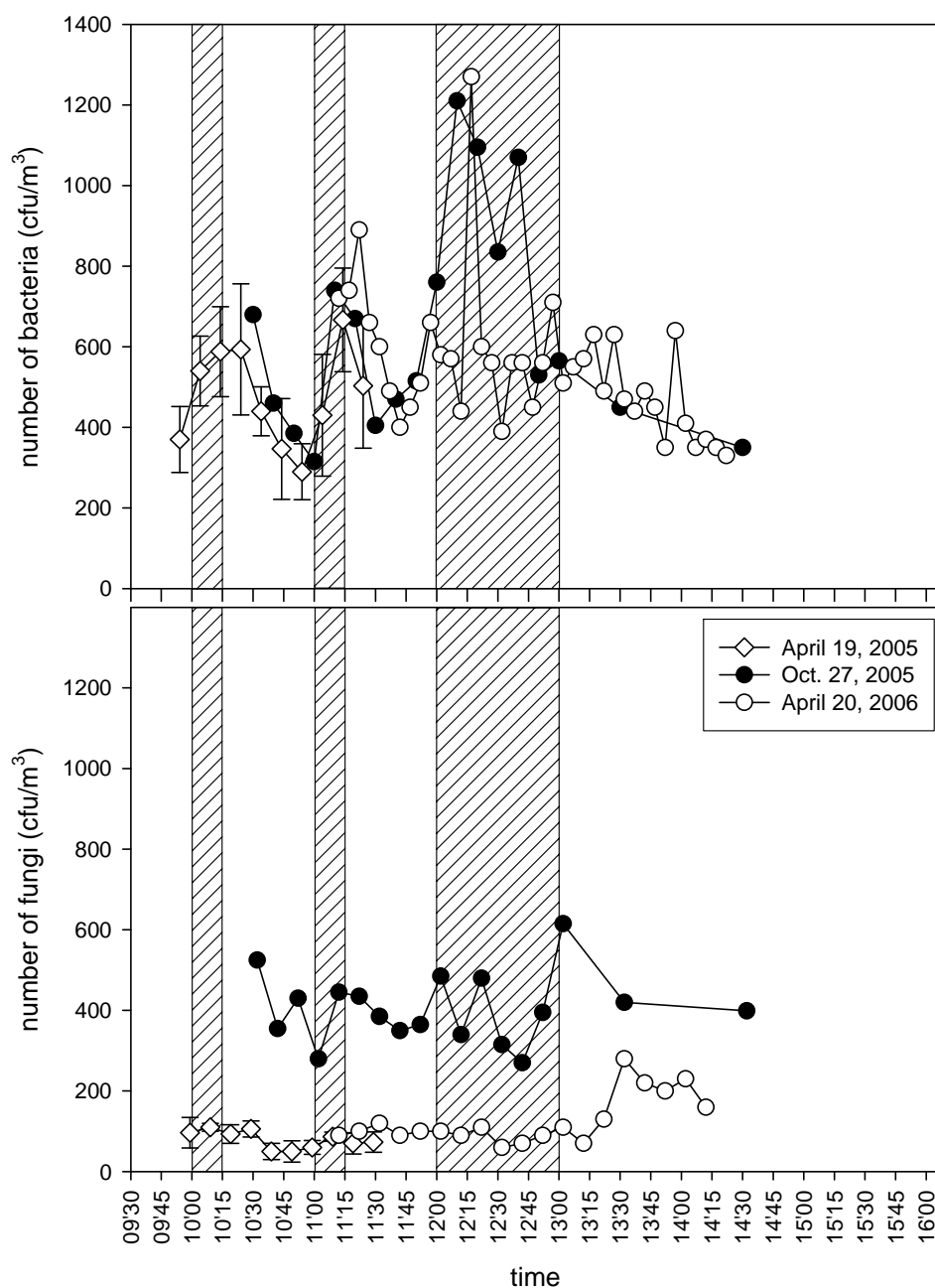
**Table 2.** Displacement rates (h<sup>-1</sup>) and residence times (h) of particles >0.3 µm in an indoor environment (university hallway) at different times during the day when lecture room doors are closed during the lessons. na: not applicable, because no samples were taken

	09:15 to 10:00		10:15 to 11:00		11:15 to 12:00		13:00 to 16:00	
	rate	residence	rate	residence	rate	residence	rate	residence
date	(h <sup>-1</sup> )	time (h)	(h <sup>-1</sup> )	time (h)	(h <sup>-1</sup> )	time (h)	(h <sup>-1</sup> )	time (h)
April 19, 2005	na	na	-0.904	1.11	-0.758	1.32	na	na
October 27, 2005	-0.661	1.51	-0.923	1.08	-0.388	2.58	-0.154	6.49
April 20, 2006	na	na	-1.092	0.92	-1.097	0.91	-0.150	6.67

No measures have been taken to identify bacteria or fungi on the genus or species level. It was our intention to monitor and compare counts of both total particles and particles

of bacterial or fungal origin in relation to a periodic pattern of anthropogenic activities in an indoor environment. Detailed investigation of the organismic composition of the bioaerosols collected is in the focus of further work.

Neither temporal fluctuations of indoor airborne particles nor variations in bioaerosol concentrations are reflected by outdoor conditions. Outdoor concentration of particles  $>0.3\mu\text{m}$  remained more or less constant over the experimental period of 6.5 hours with a slight linear decrease of approximately 13 %. In contrast to indoor locations, regular periodic variations were not observed. No periodic fluctuations were observed as well for both outdoor bacterial and fungal aerosol concentrations, which were in the range of  $321 \pm 103$  and  $418 \pm 137$  cfu/m<sup>3</sup> (n = 13), respectively.



**Fig. 3.** Time course of bioaerosols (culturable bacteria, culturable fungi) in an indoor location (university hallway) between 09:40 and 14:45 h on three independent days. Shaded areas denote times of intermissions between lessons where doors of lecture rooms are opened and students enter the hallway. Error bars represent standard deviations of triplicate measurements. Single measurements were performed where no error bars are shown.



## 4 Conclusions

Results clearly show the occurrence of distinct and reproducible short-time dynamics (in a time scale of minutes) of total particles and bioaerosols related to periods of anthropogenic activities (presence/absence of people) in the hallway i.e. when lectures are held in lecture rooms and the intermissions in between. As soon as lectures are terminated students enter the hallway resulting in distinct changes of particle distribution patterns. A periodic generation and displacement during the course of a day can be observed. Bioaerosol concentrations follow synchronously the variation in total number of particles  $>0.3\ \mu\text{m}$ . In our case, the temporal patterns were highly reproducible. In general, when monitoring air quality of indoor environments regarding the occurrence of both biological and total particles, these short-time temporal dynamics have to be considered.

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