A flash-lamp based device for fluorescence detection and identification of individual pollen grains

Denis Kiselev, Luigi Bonacina, and Jean-Pierre Wolf

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A flash-lamp based device for fluorescence detection and identification of individual pollen grains

Denis Kiselev,a) Luigi Bonacina, and Jean-Pierre Wolf
GAP-Biophotonics, Université de Genève, 22 chemin de Pinchat, 1211 Genève 4, Switzerland

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We present a novel optical aerosol particle detector based on Xe flash lamp excitation and spectrally resolved fluorescence acquisition. We demonstrate its performances on three natural pollens acquiring in real-time scattering intensity at two wavelengths, sub-microsecond time-resolved scattering traces of the particles’ passage in the focus, and UV-excited fluorescence spectra. We show that the device gives access to a rather specific detection of the bioaerosol particles. © 2013 American Institute of Physics. [http://dx.doi.org/10.1063/1.4793792]

I. INTRODUCTION

Aerosol influence on air quality and climate is subject of an increasingly large debate.1,2 From the public health standpoint, there is an urgent need for cost-effective real-time devices capable of detecting and recognizing aerosols in indoor and outdoor public areas. We present here a new transportable optical aerosol detector ready for in-field measurements. The device is able to simultaneously detect scattering and fluorescence from individual aerosol particles. The information collected can be used to rapidly classify airborne particles by their size and by their fluorescence spectrum. Moreover, we show that surface morphological information can be prospectively extracted.

The basic scheme we adopted for this development was originally conceived by Chang and co-workers from Yale University in collaboration with the U.S. Army Research Labs.3–5 According to this design (Fig. 1), aerosols are sucked with ambient air and led into a special nozzle generating a thin laminar flow. Two continuous wavelength (CW) lasers are focused and crossed at the nozzle outlet. The presence of an aerosol in the flow is recognized by the concomitant scattering of both CW lasers, which in turn generates a trigger signal for the UV source, a frequency-quadrupled Nd:YAG laser in the case of Chang’s apparatus. Most biological aerosols, like pollens and bacteria, and some non-biological organic aerosols, like diesel fuel particles, display characteristic fluorescence in the visible spectral region upon absorption of UV light. A real-time treatment of these spectra can provide a useful first level alarm for air monitoring applications. This laser-based approach has already proven successful,6–13 and a recent upgraded version based on dual wavelength excitation has demonstrated even higher discrimination power.14,15 The major disadvantage is related to the price of the laser source (about 1000 USD/μJ of UV) which can prevent the widespread use of devices based on this scheme. A possible cost-effective alternative is represented by flash lamps (about 100 USD/μJ). This approach has been followed by Kaye and co-workers from the University of Hertfordshire.16–18 They have realized an aerosol detector (now commercial) implementing one (respectively, two) Xenon flash lamps for fluorescence excitation. Contrary to Chang’s approach, fluorescence detection is not spectrally resolved, but instead performed by one (two) single detector(s) measuring the overall intensity of comparatively broad spectral bands.

Our solution combines the advantages of both methods, combining spectrally resolved detection with flash lamp excitation. One additional feature we implemented is the acquisition of time resolved scattering traces to get supplementary information for aerosol discrimination. We present the design and operation of the device in details, and discuss the results obtained with three pollens (Mulberry, Ragweed and Pecan) used here as reference bioaerosols.19

II. DESIGN CONCEPT

The basic operation concept of the device is reported in Fig. 1(b). To generate single aerosol particles, a few milligrams of dry pollens deposed on the bottom of a small reservoir equipped with a clean air 1 l/min inlet I1 are set in suspension by a magnetic stirrer. The rotation speed in combination with the length of outlet tube, allows selecting the cut-off size of the particles to be investigated. The suspended aerosols are then directed into the main inlet of a specially developed sheath nozzle. The latter features a 1 l/min outer inlet, I2. The two air flows are combined together at the nozzle outlet, to form a laminar flow of particles. Two laser diodes Y1 (Laser Components, model FP-78/20AAF-SD5) and Y2 (Laser Components, model FP-66/20AAF-SD5), centered at λY1 = 780 nm and λY2 = 655 nm respectively, are crossed and focused onto the aerosol flow at 1.5 mm from the nozzle outlet. The presence of an aerosol in the chamber is determined from the coincidence of the scatter signals detected by two photomultiplier tubes (PMTs) D1, D2 (Hamamatsu Photonics, model H6780-20) equipped with tailored band-pass interference filters (λD1 = 780 nm CWL, 10 nm FWHM, and λD2 = 658 nm CWL, 10 nm FWHM, Edmund Optics, models NT65-663 and NT65-656). The PMT signals are processed by the scatter analysis system, SSA. After detecting a coincidence among D1 and D2, the SSA triggers the Xenon flash lamp XFL (Perkin Elmer, model FX-4401) which generates a 3–4 μs light pulse. The rather broad Xenon lamp

References:

1) Electronic mail: Denis.Kiselev@unige.ch.
spectrum, is spectrally filtered through the filter set $F_2$ and focused on the particle.

A reflective objective $L_1$ (Newport Corporation, model 50105-02) collects the fluorescence emitted by the particle upon UV excitation and sends it through the filter set $F_1$ onto the diffraction grating $G_1$ ($d_{G_1} = 1200$ lines/mm). The diffracted spectrum is then imaged by a bi-convex lens $L_2$ (focal length 75 mm) onto the 32-anode PMT array (Hamamatsu Photonics, model H7260-03) for detection (resolution ≃8 nm) and the signal fed to the spectral acquisition system SAS.

A. Nozzle

The design of the focusing nozzle for aerosol injection was based on existing drawings already published by the U.S. Army Research Labs. Originally, it was supposed to be fabricated by electro-erosion, requiring a very specialized manufacturing process. By introducing some minor geometry modifications in order to simplify the fabrication, we could realize it “in house” by standard manufacturing tools. The nozzle is made of polished brass with a nickel surface coating to decrease its flow resistance. As reported in Fig. 2, it consists of four assembled pieces; two forming the external block for auxiliary flow, two used for the internal block for the main (aerosol) flow.

To ensure that the modified nozzle maintains the properties of the original design, in terms of creation of a thin laminar flow, we performed a complete numerical simulation with the CFD software (Comsol Multiphysics) in turbulent flow model. The resulting absolute velocity field obtained for typical working conditions ($I_1 = 1 \text{l/min}, I_2 = 1 \text{l/min}$) is shown in Fig. 2(a). Based on these simulations, particle tracing was performed using the Richardson-Kahn force model and it was calculated that $1–50 \mu m$ particles form a thin flow of around 300 $\mu m$ FWHM in the first 3 mm from the nozzle outlet. This simulation was confirmed by experimental measurements of the spot size (Fig. 2(b)) obtained by processing the images of water vapor droplets with size distribution $1–100 \mu m$ focused by the nozzle.

B. Detection geometry

The system (Figs. 3(a) and 3(b)) uses classical approach with two CW laser beams crossed onto the particle flow. Each beam is spatially shaped with a set of two lenses: spherical one, $L_s$ (BK7, $F = 75 \text{mm}$) and a cylindrical one, $L_c$ (BK7, $F = -150 \text{mm}$). An additional circular iris $I_r$ is placed right after the cylindrical lens to reduce beam size. Figures 3(c) and 3(d) report the beam shapes of 655 nm and 780 nm, respectively, without cylindrical lens at the working distance, where the particle flow passes. Figures 3(e) and 3(f) present the same lasers with additional cylindrical lens $L_c$. 
The shapes of laser beams obtained with this geometry allow a very precise (±25 μm) overlap in the vertical direction, collinear with the particle flow. At the same time, having large spots in the horizontal plane reduces particle losses due to flow divergence.

Scattering detection is based on two photomultipliers arranged as shown in Figure 4. Both detectors are placed in the same plane that corresponding CW laser beam and tilted in the vertical direction by 19°. This angle was chosen to keep detection axis as close as possible to the beam axis in order to obtain a higher forward scattering signal. A further reducing of this angle was not possible due to the parasitic scattering from input optical windows, Wi, and other mechanical constraints. The solid angle of view for each photomultiplier was kept minimal for the same reasons. No collimated optics was employed to reduce the influence of the uncertainty of particle position on the amplitude of scattering signal.

C. Flash lamp spectral filtering

To obtain an acceptable signal-to-noise ratio, it was imperative to add both excitation end detection filtering ($F_1\lambda_{i}$ and $F_2\lambda_{j}$, respectively). The latter is a composite filter designed for high efficient transmission of the excitation band 250–290 nm and blockage of the spectral portion of the flash lamp leaking into the fluorescence detection band (300–600 nm). The filter set $F_2$ consists of six stages: $F_{2.1}$, $F_{2.2}$, and $F_{2.4}$ are

266 nm Nd:YAG laser mirrors (CVI Melles Griot, model Y4-1025-45-UNP); $F_{2.3}$ is a hard coated high-pass filter with cut-off wavelength $\lambda_{F_{2.3}} = 300$ nm (Semrock Inc., model FF01-300/SP-25); $F_{2.5}$ is a bandpass filter ($\lambda_{F_{2.5}} = 254$ nm CWL, 40 nm FWHM, Edmund Optics, model NT67-809); $F_{2.6}$ is a dichroic mirror (reflectance band 255–295 nm, transmission band 310–600 nm, Semrock Inc., model FF310-Di01-25×36).

The spectral intensity of the Xe lamp after filtering is presented in Figure 5. Starting with 0.5 J spectrally integrated over the whole emission range (180–1000 nm), we could typically obtain 30 μA output at the focal point of L1. Figure 5(c) shows the radiant sensitivity of the 32-anode PMT convoluted with the detection filter set ($F_2$) (lower plot).

D. Electronics

Detection electronics consists of two main blocks: the first for processing light scattering signals, the second one dedicated to spectral acquisition. Figures 6(a) and 6(c) show the implementation of the former unit for signals $P_I$ coming from the 655 nm and 780 nm PMTs. The analogue front-end is made of transimpedance amplifiers $TI$, which convert current signal to voltages (gain $-10^5$ V/A, bandwidth 3 MHz). These voltages are buffered and amplified in $B1$ (gain +20 dB, bandwidth 10 MHz), and digitized by $AC$ (16-bit resolution, 2.5 Msps). In parallel, $B2$ (gain +20 dB, bandwidth 10 MHz) drives input of voltage comparator $CO$ (rise
time 3.5 ns) with voltage reference VR. Field programmable gate arrays L1, L2 control corresponding VR, adjust gains of scattering detection PMTs via digital to analogue converters DC (12-bit resolution, 10 kbps) and communicate via the parallel bus BS.

The spectral acquisition unit has an analogue front-end composed of 32 integrating amplifiers IN (integration time $T_{int} = 10–1000 \mu s$), buffered by B3 (gain −6 dB, bandwidth 100 kHz) and digitized by 32 precision Delta-Sigma converters DS (24-bit resolution, 144 kbps). The output data are read and processed by L2, which is also used to synchronize the spectral detection on scattering events by sending the trigger signal DS and to ensure communication with host computer via USB UI or Ethernet EI interfaces.

E. External housing

Figure 7(a) shows the assembly of the nozzle alignment system in the measurement chamber, described more in details in Figure 7(b). Finally, a picture of the actual aerosol detector is presented in Figure 7(c). All components (nozzle, measurement chamber, detectors, control and acquisition electronics, air pump, particle filter) are housed in a rugged aluminium box (40 cm × 30 cm × 25 cm).

III. PERFORMANCE TESTS

The detector was tested on three well known natural pollens: Mulberry (Morus), Ragweed (Ambrosia), and Pecan Carya Pecan. The samples were purchased in dry powder form (Bonapol A.S.). For each detected aerosol particle, the system captures and digitizes two scattering traces composed of 64 data points over 25 μs, and a 32 points of fluorescence spectrum ranging from 295 nm to 570 nm.

An example of real-time measurement on Mulberry pollen is shown in Fig. 8. The first ten blank events serve as a reference for the background noise level, especially important for the fluorescence channel (panel b). We limited the data analysis to events for which all detection channels are unsaturated. A few exemplary saturated scattering traces not retained for the data treatment are visible in panel c.

![FIG. 6. (a) and (c) Scatter analysis system for 655 nm and 780 nm PMTs; (b) Spectrum acquisition system. Blocks: TI – transimpedance amplifier; B1, B2, B3 – buffers, AC – analogue to digital converter; L1, L2 – programmable logic; VR – voltage reference; CO – comparator; DC – digital to analogue converter; IN – 32-channel integrator scheme; DS – 32-channel analogue to digital conversion scheme. Analogue signals: PI – from scatter PMTs; AO – amplified output of scatter signal; SI – from 32-anode PMT; PP – gain regulation of scatter PMTs. Digital signals: DT – trigger from CO; BS – communication bus; UI – USB interface to host; EI – Ethernet interface to host.]

Table I reports the event statistics of the measurements. Valid spectra were selected by searching local maxima in a given spectral range. This range was defined from measurements with high signal levels and applied to the rest of the dataset.

<table>
<thead>
<tr>
<th>Pollen</th>
<th>Mulberry</th>
<th>Ragweed</th>
<th>Pecan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain size (μm)</td>
<td>12–13</td>
<td>18–20</td>
<td>44–48</td>
</tr>
<tr>
<td>Total number of events</td>
<td>383</td>
<td>216</td>
<td>71</td>
</tr>
<tr>
<td>Number of retained events</td>
<td>237</td>
<td>294</td>
<td>30</td>
</tr>
</tbody>
</table>

![FIG. 7. CAD drawing of hardware components of detector: (a) nozzle block and chamber assembly; (b) measurement chamber. (c) Photograph of actual aerosol detector (left) and its power supply (right).]
polyethylene microspheres UVPMSBG-1.025 (Cospheric Inc.) and Ragweed pollen, one can observe that, for the latter, the signal tail at long time-delays present sharp spikes while the microspheres yield a very smooth curve. A similar behavior, although less pronounced, was observed with the other two pollen species. Such time-resolved features can be explained by a combination of diffraction and refraction effects induced by irregular sample surface and shape. The two-photon fluorescence microscopy image in the inset\textsuperscript{22} indicates that this pollen species presents indeed a very sharp-edged surface. Up to now this discrimination approach has not been exploited by optical aerosol detectors, mainly because of the limited bandwidth of the scattering detection channels, and large angle scattering collection.

We also tried to evaluate the time of flight through the crossed laser beams. Based on the flow simulations presented before, the particle velocity was expected to be approximately 1 m/s. Taking into account the experimental beam profiles of CW lasers (Fig. 3), the expected time of flight should be at least a few dozens of microseconds. However, we measured a much shorter time indicating the presence of addition effects to be investigated. Moreover, if the particle velocity is nearly constant during the passage through the laser beams, there is no reason to obtain any asymmetry in the mean time-resolved scattering trace. In opposite to our expectations, we observed on all traces a short rising edge (1–2 μs) and longer falling edge (1–2 μs), e.g., Figure 10.

To understand these features, we simplified the problem and investigated the geometrical light refraction by a spherical water particle in transit through the laser beam. This approach can be justified by the fact that the typical particle size in our experiments is at least 15λ. More elaborated calculations based on Mie scattering theory can be found in literature.\textsuperscript{23} The simulation of double refraction angle (air-particle and particle-air interfaces) given in Figure 11(a) shows that the output angle changes nonlinearly as function of transit time. It also makes evident that refracted light is collected within the detector’s solid angle during a very short period of a few microseconds. At the same time, the static simulation Mie scattering of a plane wave (Fig. 11(b)) give a very narrow angular distribution that cannot contribute significantly to the detected signal time shape. As a result, the time modulated refraction can be seen as the main contribution to the observed scattering traces (Fig. 11(c)) and can explain both the short (few microseconds) time of flight and the asymmetry. It also fits well to the observed time-resolved traces reported in Figure 11(d).

B. Fluorescence detection

Figure 12 reports the average fluorescence spectra of the pollens under investigation. The peak wavelength varies

![FIG. 8. Real-time detection of Mulberry pollens: (a) scattered light from 655 nm CW laser; (b) fluorescence spectrum; (c) scattered light from 780 nm CW laser.](image1)

![FIG. 9. Mean values (at least 30 events) of scattering maxima for 658 nm (green) and 780 nm (red) lasers. Error bars reports interval with 50% of events within it.](image2)

![FIG. 10. Normalized time-resolved scattering traces for (a) 25 μm microspheres and (b) Ragweed pollen.](image3)
among samples, which—in principle—ensures the possibility to discriminate among them based on spectrally resolved detection. However, the spectral shift measured between Pecan and Ragweed (6–7 nm) is not statistically significant since it is inferior than the spectral resolution allowed by the apparatus. On the other hand, Mulberry yields a fluorescence maximum 40–45 nm away from that of the other two samples. Thus, a simple comparison between fluorescence spectra allows its reliable discrimination. Interestingly, the Mulberry pollen, although it has also the smallest size among the tested species, shows the highest fluorescence intensity. This peculiarity can be used as an additional element of discrimination. On the other hand, the measurements points to the need of further statistical analysis to distinguish between Pecan and Ragweed pollen grains.

C. Principal component analysis

Principal component analysis (PCA) is a powerful mathematical tool generally employed to reduce the number of degrees of freedom in a dataset. The method treats each initial degree of freedom (in the case of our experiment the intensities measured by each pixel of the 32-channel PMT) as an independent variable and defines a rectangular matrix containing these variables as columns and their different values as rows. Each dataset is normalized, so any proportional change from one dataset to another is disregarded. The input matrix is then used to calculate cross-correlation coefficients among all its elements resulting in a cross-correlation (or covariance) matrix. This matrix projects the whole dataset into a new space formed by its eigenvectors (also called principal components). The eigenvalues corresponding to these vectors reflect the relative importance of each dimension on the data covariance.

Figure 13 represents raw data projections on the first principal component of fluorescence data and scattering trace maxima. An independent variable was included into PCA: the ratio between the scattering intensity maxima and the total

![Graphs and diagrams illustrating the refraction angle simulation and time-resolved scattering traces.](image-url)

FIG. 11. Refraction angle simulation of a particle passage through a laser beam (particle diameter 10 μm, beam diameter 10 μm, particle velocity 1 m/s): (a) double refraction angle as function of time, (b) Mie scattering profile, (c) particle passage through the beam. (d) Measured mean time-resolved scattering traces at 780 nm.

![Averaged fluorescence spectra for different pollens: Mulberry, Ragweed, Pecan.](image-url)

FIG. 12. Averaged fluorescence spectra (at least 30 events) for different pollens: (a) Mulberry; (b) Ragweed; (c) Pecan. Dashed lines show 96% confidence interval (±2σ).
photons in the fluorescence spectra for all three samples are well separated. Only a few events overlap the distributions from other samples.

Based on this statistical analysis and supposing that the distributions along both axis are Gaussian, one can calculate the respective probabilities of detection for each pollen sample. This estimation allows predicting the pollen species corresponding to any detected particle with a known probability. The contour plot in Figure 13 indicates the regions corresponding to 66%, 95%, and 99% of probability that a detected event lies within a given ensemble. If a particle is detected outside the three distributions, it will not belong to any of them with >99% probability.

IV. CONCLUSIONS AND OUTLOOK

We have presented a novel aerosol particle detector based on Xe flash lamp excitation and spectrally resolved fluorescence acquisition. We tested its performances on three natural pollens acquiring in real-time scattering intensity at two wavelengths, time-resolved scattering traces of the particles’ passage in the focus, and UV-excited fluorescence spectra. We have demonstrated that the collection of this information gives access to a rather specific detection of the bioaerosol particle. Moreover, we have shown that time-resolved scattering traces yield information about the surface morphology of particles, enabling an additional degree of discrimination not employed so far. Combining these results with PCA statistical analysis we could associate a probability to the attribution of each event to a known pollen species, determining that regions associated to 66% probability are not overlapped, so that relatively high accuracy can be achieved. The exposed method demonstrated that a device based on a cost-effective and reliable excitation source can be competitive with UV pulsed laser based apparatus.

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