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6. AUTHOR(S)
Yongle Pan, Stephen Holler, Richard K. Chang
Steven C. Hill, Ronald G. Pinnick, Stanley Niles,
Jerold Bottiger

7. PERFORMING ORGANIZATION NAMES(S) AND ADDRESS(ES)
Richard K. Chang
Dept. of Applied Physics
Yale University
P.O. Box 208284
New Haven, CT 06520

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13. ABSTRACT *(Maximum 200 words)*

Reproducible fluorescence spectra of individual 2- to 5-μm-diameter biological aerosol particles excited with a single shot from a Q-switched laser (266 or 351 nm) have been obtained with highly improved signal-to-noise ratios. Critical to the advance are crossed diode-laser trigger beams, which precisely define the sample volume, and a reflecting objective, which minimizes chromatic aberration and has a large N.A. for collecting fluorescence. Several allergens (red oak, meadow oat pollen, paper mulberry pollen, and puffball spores) have different fluorescence spectra. *Bacillus subtilis* fluorescence spectrum deteriorates at high 266-nm incident intensity. Dry riboflavin particles illuminated with a 351-nm light exhibit a new 420-nm fluorescence peak that grows nonlinearly with laser pulse energy.

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Single-shot fluorescence spectra of individual micrometer-sized bioaerosols illuminated by a 351- or a 266-nm ultraviolet laser

Yong-le Pan, Stephen Holler, and Richard K. Chang

Department of Applied Physics and Center for Laser Diagnostics, Yale University, New Haven, Connecticut 06520

Steven C. Hill, Ronald G. Pinnick, and Stanley Niles

U.S. Army Research Laboratory, Adelphi, Maryland 20783-1197

Jerold R. Bottiger

U.S. Army Edgewood Research, Development, and Engineering Center, Aberdeen Proving Ground, Maryland 21010

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Reproducible fluorescence spectra of individual 2- to 5-μm-diameter biological aerosol particles excited with a single shot from a Q-switched laser (266 or 351 nm) have been obtained with highly improved signal-to-noise ratios. Critical to the advance are crossed diode-laser trigger beams, which precisely define the sample volume, and a reflecting objective, which minimizes chromatic aberration and has a large N.A. for collecting fluorescence. Several allergens (red oak, meadow oat pollen, paper mulberry pollen, and puffball spores) have different fluorescence spectra. *Bacillus subtilis* fluorescence spectrum deteriorates at high 266-nm incident intensity. Dry riboflavin particles illuminated with a 351-nm light exhibit a new 420-nm fluorescence peak that grows nonlinearly with laser pulse energy. © 1999 Optical Society of America

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Laser-induced fluorescence in one or two emission bands has been shown to be useful for distinguishing, in real time, between biological and nonbiological aerosols.[1–3] Fluorescence spectra of aerosols[4,5] may be useful for additional discrimination among the many possible types of aerosol, particularly bioaerosols. Instruments that can measure good-quality, single-particle spectra of flowing aerosols are necessary for determining how well fluorescence spectra can be used to characterize biological aerosols.[6]

Previously measured single-particle, single-shot fluorescence spectra (266-nm excitation) of agglomerates of biological cells had spectral reproducibility problems and poor signal-to-noise (S/N) ratios. Accumulation of fluorescence spectra from ~100 particles was necessary for useful comparisons among particle types.[4,5] In previous research a UV laser and an intensified-CCD (ICCD) detector were both triggered when a particle traversed a single cw beam that was focused slightly upstream from the sample volume. The problem of too few fluorescence photons could not be solved simply by increasing the laser intensity, because higher intensities caused nonlinearities (resulting from, e.g., fluorescence saturation and laser-induced plasma formation).

We report a method for measuring significantly improved, reproducible, single-shot UV excited fluorescence spectra of individual biological particle agglomerates (as small as 2 μm in diameter). This advance enables us to discriminate the differences in fluorescence spectra from various types of each individual particle. We illustrate the improvement by showing single-shot 266-nm UV-laser-excited fluorescence spectra from nearly monodispersed (all ~5 μm in diameter) allergens and agglomerates of *Bacillus subtilis*. We show how the fluorescence spectra of *B. subtilis* deteriorate at high energies (incident 266-nm laser) and demonstrate in riboflavin aerosols a new peak at 420 nm that increases nonlinearly with illumination energy at 351 nm.

Figure 1 illustrates the experimental setup, which has two crucial improvements: First, two nearly orthogonal, different-wavelength diode-laser beams (diode lasers 1 and 2 emitting at 635 and 670 nm, respectively) are used to define an ≈15-μm-diameter focal volume at the focal plane of the reflecting objective that is used as a collection lens. As an aerosol particle flows through the focal volume of the

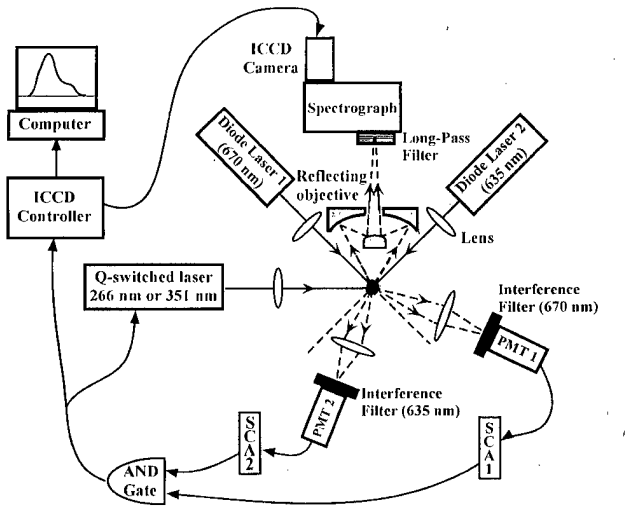

Fig. 1. Experimental setup used for detecting single-shot laser-induced fluorescence spectra from individual micrometer-sized aerosol particles. There are two critical improvements: two cw diode laser beams (at 635 and 670 nm), which define the sample volume, and a high-N.A. reflecting objective, which avoids chromatic aberration.

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crossed beams, the near-forward elastic scattering is measured with photomultipliers PMT 1 and PMT 2 (with narrow-band interference filters at 635 and 670 nm, respectively). The PMT signals are fed into two single-channel analyzers (SCA1 and SCA2) set to operate as discriminators in a window mode. The PMT pulses must fall between the preset upper and lower voltage levels before the SCA provides an output pulse. The two SCA outputs are fed into a logic AND gate, which produces an output pulse only when the outputs of the SCA's overlap for at least 3 ns. The AND gate output then sends a trigger pulse to the controller of the ICCD detector and the Q-switched laser [266-nm, fourth harmonic of a Nd:YAG laser, 30- or 70-ns pulse duration (Spectra-Physics) or 351-nm, third harmonic of a Nd:YLF laser, 120-ns pulse duration (Quantronix)]. The Q-switched laser fires within ~3 μs, during which time the particle travels (~10 m/s) less than 40 μm. This displacement is compensated for by a small vertical displacement of the focal volume of the two diode-laser beams from the UV laser beam, which is also aligned to focus at the focal point of the reflecting objective.

The second improvement is the use of a reflecting Schwarzchild objective (Ealing) with large N.A. (=0.5) that collects and focuses the fluorescence onto the spectrograph slit (1-mm; Acton SP-150 with a 300-groove/mm grating blazed at 500 nm; N.A., 0.125), without chromatic aberration. Because the depth of field of the reflecting objective is small (~1 μm), any substantial displacement of the particle outside this focal volume will deform the magnified image of the particle at the spectrograph entrance slit and cause an offset angle of the wave entering the spectrograph, resulting in a wavelength shift. The need for a precisely defined sample volume is met by the use of the two crossed trigger beams. A long-pass filter is placed in front of the spectrograph to block the elastic-scattered radiation, 266 or 351 nm, and to pass the fluorescence.

To produce nearly monodispersed dry particles, we used a laboratory-built ink-jet aerosol generator.[7] The particles to be converted to an aerosol in this generator are first suspended in water. The ink jet generates ~50-μm-diameter droplets of the suspension and sprays them into the filtered carrier gas (~5 mL/s), which passes through a drying chamber. The dried particles are carried through a tapered exit nozzle (1-mm diameter) and then through the focal region of all the laser beams as well as the collection optics (~2 mm below the nozzle tip). The volume of the dried particle is proportional to the initial sample concentration in the water suspension. Particle size distributions were determined with a TSI Aerodynamic particle sizer. In some cases scanning-electron micrographs were also made of filter collections.

Allergens were converted to aerosols (aerodynamic diameter, ~5 μm) by the ink-jet aerosol generator. The observed single-particle single-shot fluorescence spectra (Fig. 2; all spectra are not corrected for the instrument spectral sensitivity) of different allergens have different line shapes. The 635- and 670-nm peaks are caused by elastic scattering from the two diode lasers. The extent to which these differences can be used to distinguish and identify the allergens needs further investigation.

The uniformity of the fluorescence signal from ~5-μm particles of dried *B. subtilis* (vegetative cells, Sigma) with consecutive single shots of the 266-nm laser triggered by crossed beams is revealed in Fig. 3(a). The broad fluorescence peak (at 350 nm) comes mainly from tryptophan, and the tail from 400 to 500 nm is attributed to fluorescence from residues of the nutrient growth material but may have contributions from reduced nicotinamide compounds. The sharp 532-nm peaks are from leakage of the 532-nm beam that generates the 266-nm beam. The 590-nm peak may be the laser-induced Na plasma, which appeared only at high laser intensity. Similar

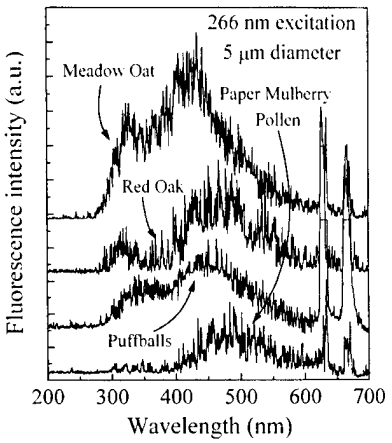

Fig. 2. Single-shot fluorescence spectra of four allergens, excited by a UV laser.

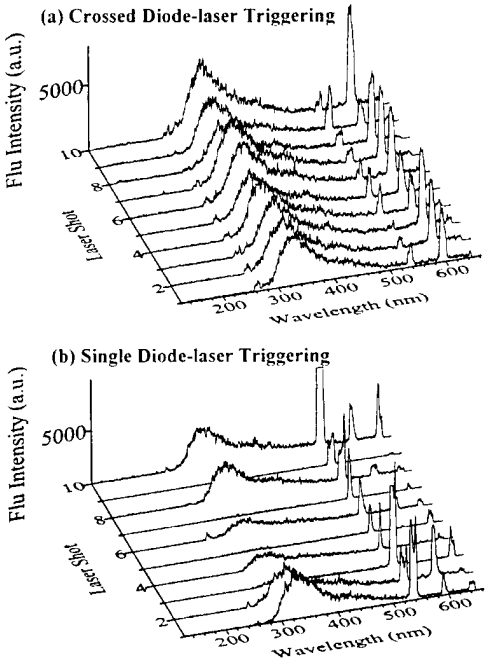

Fig. 3. Fluorescence spectra of ten consecutive single shots of a 266-nm laser beam on dried vegetative *B. subtilis* aggregates (all ≈5 μm in diameter).

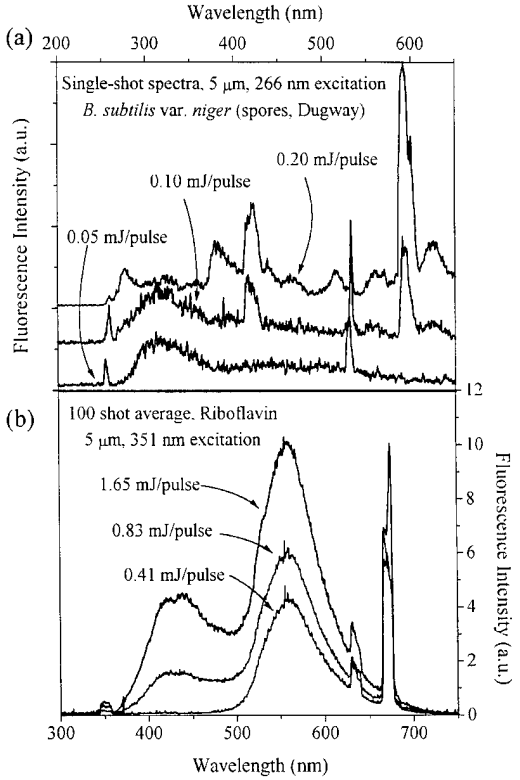

Fig. 4. (a) Fluorescence spectrum of *B. subtilis* var. niger spore aggregate irradiated by a single-shot-266-nm laser with three pulse energies. (b) Fluorescence spectra of dried riboflavin particles irradiated at three different pulse energies of a 351-nm laser. The 560-nm peak increased linearly with input-pulse energy, whereas the 420-nm peak increased nonlinearly with input-pulse energy.

single-shot-excited fluorescence spectra from individual flowing dried particles (as small as 2 μm in diameter) of vegetative *B. subtilis* with good S/N ratios have also been observed.

Figure 3(b) shows that the shot-to-shot intensity and spectral fluctuations increase dramatically when only one diode laser is used for triggering. The fluorescence signal is decreased or is even zero for those particles that cross one diode-laser beam and are outside the focal volume of the reflecting objective but still in the UV beam or are outside the UV beam altogether.

Effects of increasing the UV intensity on fluorescence from biologicals (saturation effects and irreproducibility) were mentioned previously.[8,9] Figure 4(a) shows the spectral change of *B. subtilis* var. niger spore aggregate (5-μm diameter, $\sim 10^2$ spores; Dugway Proving Grounds) illuminated with a 266-nm laser (70 ns). When the laser pulse energy (focused to $\sim$40 μm) is increased from 0.05 to 0.1 mJ, additional lines appear in the emission spectra at 385, 425, and 590 nm. At 0.2 mJ the main peak at 300 nm decreases, and these additional sharp peaks become more intense. Although the mechanism for spectral change may be laser-induced plasma, the present observations imply that increasing the laser intensity does not improve the S/N ratio but degrades the quality of the fluorescence spectra.

A new peak near 420 nm appears with 351-nm illumination of dried riboflavin aerosols ($\sim$5-μm diameter) as the pulse energy increases from 0.41 to 1.65 mJ [Fig. 4(b)]. The fluorescence intensity of the main 560-nm peak increases with input-laser pulse energy, but the peak at 420 nm, which apparently is absent at low pulse energies, increases nonlinearly with at higher pulse energies. The origin of this new band needs further investigation.

In summary, reproducible single-shot fluorescence spectra from individual dry 2–5-μm-diameter particles of bacteria cells, spores, and pollen allergens have been detected by use of a low-energy pulse (less than 8 J/cm^2) from a UV laser. The key improvements that permit spectra with good S/N ratios are the implementation of the cross-beam triggering scheme and a high-N.A. reflecting objective to collect fluorescence. The fluorescence spectra of *B. subtilis* aggregates are found to deteriorate at high 266-nm laser energy. For dry riboflavin particles a new $\sim$420-nm peak grew nonlinearly with the 351-nm input pulse energy.

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