Remote time-resolved filament-induced breakdown spectroscopy of biological materials

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We report, for what we believe to be the first time, on the feasibility of remote time-resolved filament-induced breakdown spectroscopy (FIBS) of biological materials. The fluorescence from egg white and yeast powder, induced by femtosecond laser pulse filamentation in air, was detected in the backward direction with targets located 3.5 m away from the detection system. The remarkably distinct spectra of egg white and yeast allow us to propose that this technique, time-resolved FIBS, could be potentially useful for remote detection and identification of harmful biological agents. © 2006 Optical Society of America

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Remote detection and identification of biological agents is a subject of intense interest for early warning of biological threats to public and defense security and also for some civilian applications, such as online hygienic controls in food-processing industries. Recently, nanosecond laser-induced breakdown spectroscopy (LIBS) has been suggested to be a potential technique for identifying biological agents.1,2 It is based on the emission spectroscopy of material ablated into a small plasma by a tightly focused laser beam, generally a Q-switched Nd:YAG laser. However, a key aspect of remote LIBS design is to ensure that sufficiently high laser intensities are achieved at a remote position to induce a plasma breakdown on the sample. Clearly, because of diffraction, the proportionality of the focal diameter to the distance leads to difficulty in delivering high laser intensities over long distances, which limits the operational range of conventional LIBS.

Recently, remote filament-induced breakdown spectroscopy (R-FIBS), a special configuration of remote LIBS, has been developed to overcome the diffraction limit for remote elemental analysis of metallic samples.3,4 The FIBS scheme is based on the filamentation phenomenon induced by nonlinear propagation of femtosecond laser pulses in air (see, e.g., Ref. 5 and the references therein). Filaments appear as a result of a dynamic balance between Kerr self-focusing and defocusing of plasma produced by multiphoton or tunnel ionization of the air molecules. The equilibrium limits the laser intensity inside the filament core to about $5 \times 10^{13} \text{W/cm}^2$ (intensity clamping). Since it has been reported that filamentation could occur at a distance as far as a few kilometers in the atmosphere,6 the technique of R-FIBS would be superior to traditional long-pulse LIBS in terms of the ability to produce high laser intensity at a remote distance.3

However, remote femtosecond two-photon-excited fluorescence experiments on biological targets such as riboflavin gave rise to only broadband spectra,7 very similar to UV-laser-excited fluorescence of biomolecules.8 Such broadband fluorescence represents a weakness insofar as efficient and effective identification of biomolecules is concerned. In this work, using FIBS, we discovered that sharp distinctive fluorescence lines from biotargets could be revealed when a time-resolved technique is used to avoid the strong scattering of the pump and the white-light laser.5 The results allow us to propose that the characteristic time-resolved fluorescence spectra could be used as a fingerprint for remote identification of potentially harmful biological material. Extrapolation of the detection limit shows that this technique could be extended up to the kilometer range.

The pulses emitted from a Ti:sapphire femtosecond laser system9 (10 Hz, 45 fs) was focused in air by a fused-silica lens, L1 (f=3 m, thickness 6.3 mm; Fig. 1). The pulse spectrum was centered at 807 nm with a 23 nm bandwidth (FWHM). The laser beam radius was $a = 2.7 \text{mm}$ (1/e level of intensity). A dielectric mirror (M1, diameter 76.2 mm), with high reflectivity at around 800 nm and high transmission for UV light, was used to reflect the beam at a right angle. The distance between L1 and M1 was about 10 cm.

The filament started about 2 m away from dielectric mirror M1 and had a length of about 0.9 m when the input energy per pulse was about 7 mJ. The sample container was a cylinder with a diameter of 2 cm and a length of 20 cm. The front end was located 2.2 m away from mirror M1; i.e., the filament started about 0.2 m from the front end of the container. Yeast and egg white in the form of powders were investigated. In order to prevent any destruction of sample structures because of the sensitivity of the biological material to thermal shock induced by laser irradiation, and to allow noninvasive measurements, a motor was used to rotate the sample container with a slow speed (about 1 turn/min). The surface of the sample powder

![Fig. 1. Experimental setup used for remote time-resolved FIBS measurements.](https://example.com/fig1.png)
The powder on the surface self-mixed continuously as the motor turned, so that each laser shot hit a new sample. Although the site where the laser hit was not exactly the same from shot to shot, the intensity of the filament zone interacting with the target surface was roughly constant because of intensity clamping.5

The fluorescence signal was observed in the backward direction; it was collected and focused, with a fused-silica lens, L2 (\(f=10\) cm, diameter 50.8 mm), onto the entrance slit of an imaging spectrometer (Acton Research Corporation, SpectraPro-500i). The distance between collection lens L2 and mirror M1 was about 1.3 m. The total detection distance of 3.5 m is limited by the size and other constraints of our laboratory. The spectral resolution of the spectrometer was about 0.4 nm when a grating of 1200 grooves/mm (blazed wavelength at 500 nm) with 100 \(\mu\)m entrance slit width was used. The dispersed fluorescence was detected by using a gated intensified charge-coupled device (ICCD, Princeton Instruments Pi-Max 512), which can permit time-resolved measurement with nanosecond precision and thus prevent the acquisition of foreground white light due to self-phase modulation, self-steepening,5 and continuum emission associated with the recombination of free electrons and ions.

Figure 2 shows a filament-induced spectrum of yeast in a semilogarithmic representation with a gate width of 500 ns and a delay time of \(t=-1\) ns. Note that the laser pulse arrives on the target at \(t=0\). The data were averaged over 200 shots. It can be seen that the nitrogen fluorescence induced inside the filament10 and the white light have masked the fluorescence signals from the sample. The band around 540 nm results from the grating’s second-order diffraction of the third harmonic of the pump laser.11 It makes the sensing of the biomolecules via the backscattered fluorescence difficult, although the spectral lines of atomic sodium12 (Na I, 588.995 and 589.592 nm) and calcium (Ca I, 422.673 nm) can still be observed. However, clean UV–visible fluorescence spectra of yeast were obtained when we switched to time-resolved measurements (Fig. 3). These two spectra were acquired with a fixed gate width of 500 ns and varying delay times of \(t=10\) and \(t=40\) ns for Figs. 3(a) and 3(b), respectively. The spectral lines and bands are assigned to atomic sodium and atomic and ionic calcium (Ca II, 393.366 and 396.847 nm) and molecular cyan radical (CN, 375–390 nm).13 Taking into account the fact that the spectra in Fig. 3 are plotted on a linear scale, the signal-to-background ratio has been improved by many orders of magnitude. In addition, a very broad low-intensity spectral band can be clearly observed in Fig. 3(a), which might possibly come from the continuum emission of the plasma. The spectral lines and bands in Fig. 3 have also been observed in the spectra of classical LIBS by using a nanosecond-laser system.1 Figure 4 presents the time-resolved fluorescence spectra of egg white under the same experimental conditions, except for the delay times of the ICCD, which are \(t=10\) and 320 ns for Figs. 4(a) and 4(b), respectively. To our knowledge, there are no classical LIBS spectra of egg white available in the literature. The spectral lines and bands in Fig. 4 are assigned to atomic sodium, molecular cyan radical, phosphino radical (PH2, 433–439 nm) and amino radical14 (NH2, 538–565 nm and 605–640 nm). A very broad low-intensity spectral band was also observed for this sample, which is, however, different from that of Fig. 3(a), since it appears in both parts of Fig. 4 even after a much longer delay time. The long decay time of this spectral band suggests that it might result from a specific large molecule. Note that the cyan radical, one of the basic components of amino acids, protein, and enzymes, can be seen in cases of both yeast and egg white and that the phosphino and amino radicals can only be observed for egg white. Although there is much interest in studying the mechanisms of dissociation of bio-

![Figure 2](image-url)  
*Fig. 2. FIBS spectrum obtained for yeast with a delay of \(t=-1\) ns with respect to the laser pulse on the target (\(t=0\)). Laser pulse energy, 7 mJ; ICCD gate width, 500 ns.*

![Figure 3](image-url)  
*Fig. 3. Time-resolved FIBS spectra obtained for yeast with delays of (a) \(t=10\) ns and (b) \(t=40\) ns. Note the different intensity scales used for each spectrum. Inset, time-resolved FIBS spectra obtained for yeast at a distance of 50 m outside the laboratory with a delay of \(t=350\) ns.*

![Figure 4](image-url)  
*Fig. 4. Time-resolved FIBS spectra obtained for egg white with delays of (a) \(t=10\) ns and (b) \(t=320\) ns.*
logical molecules interacting with ultraintense femtosecond pulses, that is beyond the scope of the present letter.

From Figs. 3 and 4, we can see that the spectra of yeast and egg white are remarkably different, which implies that these spectra can be used to identify different biological species. Because of intensity clamping, the single-filament-induced FIBS signal is expected to be independent of the propagation distance of the laser pulse before the filament hits the sample. This allows us to estimate an ultimate detection distance based on the data obtained in our experiment. We assume that the collection and detection system could be improved, e.g., by using a larger telescope or by replacing the ICCD with a photomultiplier tube, with an signal enhancement by a factor of 100. Moreover, if multifilamentation occurs, the signal could be enhanced by at least a factor of 10, which is a reasonable assumption, since more than 30 filaments have been observed across the beam profile when a practical terawatt system was used. Thus, according to the LIDAR equation $I \propto 1/R^2$ (where $I$ is the signal intensity and $R$ is the distance between the sample and the detector$^{15}$) and the parameters obtained in Fig. 4(b) ($I=14,450$ for the NH$_2$ band integrating between 538 and 565 nm at $R=3.5$ m), the detection limit of $3\sigma$ will be reached at about 1.6 km; here $\sigma$ is the standard deviation of the background noise level. Note that the above extrapolation is based on optimistic conditions. In practical applications, there are certain questions we have to consider. For instance, if the sample is much more diluted in a real application, it will lead to the reduction of the signal. Therefore, the enhancement of the detection sensitivity will become a technical challenge in the future. However, our experimental results demonstrate the feasibility of using FIBS for bioagent material remote detection for the first time to our knowledge. In addition, the same fluorescence spectrum of yeast as shown in Fig. 3 has been obtained successfully in our experiment when the sample is illuminated by filaments at a distance 50 m away in a corridor outside the laboratory. The result is shown as the inset of Fig. 3. This experiment was performed with a laser energy of 100 mJ and a negatively chirped pulse duration of 10 ps. The time delay is $t=350$ ns, and the gate width is 500 ns. The diameter of the laser beam is 2.5 cm (FWHM). This further demonstrates the feasibility of FIBS for remote sensing of biological agents.

In summary, FIBS is shown to be a potentially good technique to remotely detect and identify biological species, when combined with time-resolved measurements. With reasonable laser energies, it is possible to extend the detection limit up to the kilometer range. In view of the complexity of biological matters and of fragmentary knowledge of FIBS spectra, it is clear that much additional effort is needed for practical applications of this technique in remote analysis of potentially hazardous biological agents.

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