Remote sensing for the detection of bio- and non-bioaerosols for defence applications

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The present study describes a novel approach to distinguish between bio- and fluorescing non-bioparticles from a stand-off distance of 5 m using laser-induced fluorescence technique. The variations of peak fluorescence intensities of bio- and non-bioaerosols with time were observed experimentally. Substantial decay of fluorescence peak intensities with time was observed in case of bioparticles, while it was negligible in case of non-bioparticles. This difference in decay of fluorescence peaks with time can form the basis for making a distinction between bio- and fluorescing non-bioparticles from stand-off distance. Further, this approach can be converted to a handy product for defence and security applications.

Keywords: Bio and non-bio agents, laser induced fluorescence, laser spectroscopy, UV radiation effect.

LASER-induced fluorescence (LIF) is fast emerging as a tool for stand-off detection of biological agents[1–3]. Stand-off detection of bioagents is gaining importance due to its capability of early warning of a possible biological warfare attack. Several researchers have shown the potential of LIF technique in detecting biomolecules from stand-off distances[4–6]. In an attempt to classify bioaerosols from stand-off distance, Hausmann *et al.*[7] used the LIF technique with two wavelengths (280 and 355 nm) from stand-off distance between 20 and 135 m. In a recent study, detection of bioaerosols from 1.0 km has been demonstrated by Kumar *et al.*[8] using 266 nm LIF with detection limit of 10^6 particles per litre during daytime. One of the main advantages of using the LIF-based detection technique is that it is much faster than traditional techniques that involve days of culture of bioagents. In early experiments, the differentiation between bioparticles and non-bioparticles was done by exploiting the notion that only the biological particles can be induced to fluoresce. In those experiments both scattered and fluorescence lights were used for detection and discrimination. One such system was developed by TSI Inc.[9]. However, now it is a well known fact that fluorescence can be induced in some non-bioparticles as well. Therefore, it is difficult to discriminate between bio- and fluorescing non-bio particles. The discrimination between biological and chemical clouds is done by depolarization measurements using a Glan–Thomson polarizer. The depolarization ratio of nearly 1 indicates the spherical shape of the particles present in the cloud, which in turn implies the presence of chemical species in the cloud. The deviation of depolarization ratio from 1 indicates the presence of biological particles in the cloud due to their non-spherical elliptical shapes[10,11].

In the present work, we have adopted a novel approach and used only LIF to discriminate between bio- and fluorescing non-bioparticles. We have studied the variation of peak intensities in the fluorescence spectra with time. In the study, we have selected four biomolecules and four chemicals which give fluorescence when excited with 266 nm wavelength. We found that the peak fluorescence intensity decays with time initially and attains an almost steady state after some time. In the case of chemicals, the decay in peak intensity is negligible in comparison to that in biomolecules.

Figure 1 shows the schematic of the experimental set-up. The fourth harmonic of Nd : YAG laser (model Brilliant B, make – Quantel) having pulse energy of 5 mJ was used as the excitation source. The wavelength, pulse duration and beam diameter at the exit were 266 nm, 6 ns and 10 mm respectively. The beam divergence of the laser was 0.6 milliradians. The pulse repetition frequency of the laser was 10 Hz. A CCD spectrometer (model QE65000, make – Ocean Optics, USA) having spectral range 200–1100 nm was used as the detector. This was coupled to a silver-coated collecting concave mirror (diameter 20 cm and focal length 45 cm) through a compatible optical fibre (diameter 600 μm). A 266 nm edge filter (optical density at 266 nm >6, average transmission the range 280–600 nm >90%) was mounted in front of the

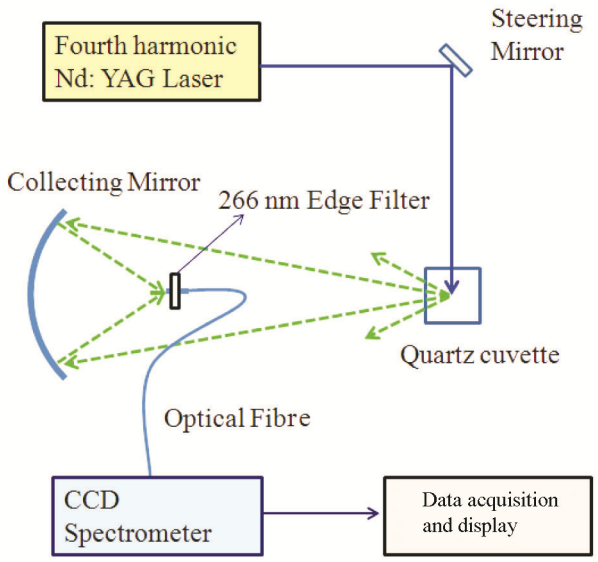

Figure 1. Schematic of the experimental set-up for the detection of bio and non-bio aerosols using UV laser-induced fluorescence (LIF).

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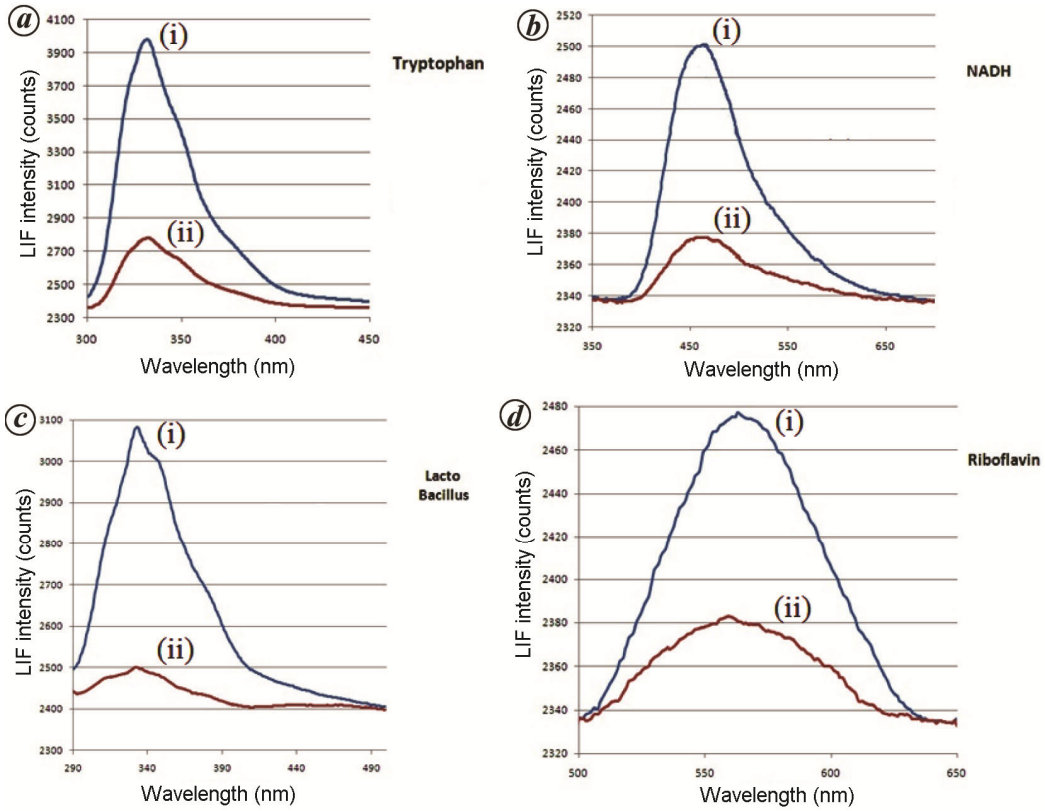

Figure 2. Spectra of biomolecules: (***a***) tryptophan, (***b***) NADH, (***c***) lacto bacillus and (***d***) riboflavin recorded from a distance of 5 m. Curves (i) and (ii) correspond to the spectra with exposure time of 5 sec and 300 sec respectively.

Table 1. Intensity of fluorescence peaks of biomolecules and percentage change in intensity of fluorescence peaks with exposure time

Biomolecule	Peak position (nm)	Intensity of fluorescence peaks for exposure time of 5 sec, I_0 (counts)	Intensity of fluorescence peaks for exposure time of 300 sec, I_T (counts)	% Change in peak intensity $\{(I_0 - I_T)/I_0\} \times 100$
Tryptophan	~332	3979.78	2780.78	30.13
NADH	~463	2500.89	2377.56	04.93
Bacillus lactose	~333	3079.11	2499.22	18.83
Riboflavin	~563	2477	2383	03.79

optical fibre tip to avoid any possible entry of 266 nm light in the detector. The receiving fibre tip (coupled to the detector) and sample cuvette were kept 5 m apart. The samples in the quartz cuvette were in the form of bioaerosols. Liquid sample was converted in aerosol form using the aerosol generator. In the experiments, the axes of laser beam and collecting mirror were kept in a transverse orientation. The fluorescence spectra were recorded and analysed using Spectra Suite software provided with the CCD spectrometer.

The experiments were performed with eight samples (four of biomolecules and four of non-biomecules). In case of biomolecules, tryptophan, bacillus lactose, riboflavin and NADH (reduced form of nicotamide adenine dinucleotide) were selected. These molecules are responsible for fluorescence in bioparticles[12,13]. From non-biomolecules, ethanol, acetic acid, acetone, and dimethylenediphosphatase (DMMP) were selected as they exhibit fluorescence when excited using appropriate light.

Fluorescence spectra of all the molecules were recorded using the experimental set-up. Figure 2 shows the recorded spectra of all biomolecules. Table 1 provides the characteristic fluorescence peaks of these biomolecules. Before recording the spectra, the laser was allowed to stabilize for half an hour. The first set of recordings was performed with 5 sec exposure time. Averaging of 10 pulses was set for recording. The second set of recordings was done after 5 min of first exposure. While analysing the spectra, it was observed that in the case of biomolecules, the fluorescence intensities decay fast initially and attain saturation after around 5 min (Figure 2). Table 1

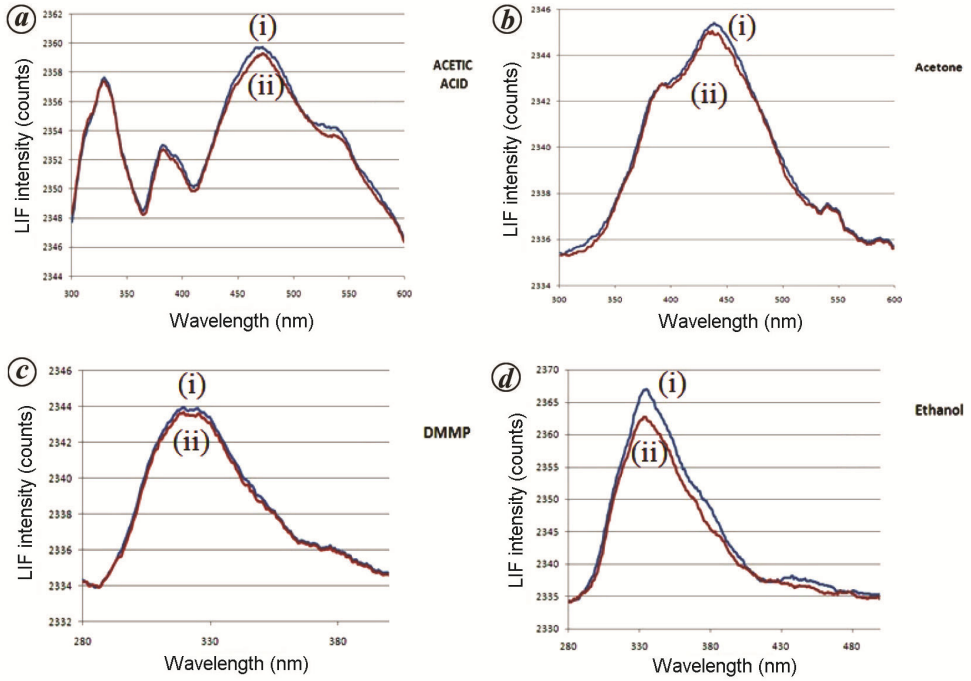

Figure 3. Spectra of chemicals: (***a***) acetic acid, (***b***) acetone, (***c***) DMMP and (***d***) ethanol recorded from a distance of 5 m. Curves (i) and (ii) correspond to spectra with exposure time of 5 sec and 300 sec respectively.

Table 2. Intensity of fluorescence peaks of non-biomolecules and percentage change in intensity of fluorescence peaks with exposure time

Non-biomolecule	Peak position (nm)	Intensity of fluorescence peaks for exposure time of 5 sec, I_0 (counts)	Intensity of fluorescence peaks for exposure time of 300 sec, I_T (counts)	% Change in peak intensity $\{(I_0 - I_T)/I_0\} \times 100$
Ethanol	335	2366.93	2362.6	0.1829
Acetic acid	330	2357.57	2357.37	0.0085
	380	2352.97	2352.68	0.0123
	470	2359.66	2359.28	0.0161
	540			
Acetone	392	2342.78	2342.69	0.0038
	440	2345.37	2344.89	0.0205
	540	2337.62	2337.59	0.0013
DMMP	320	2343.96	2343.66	0.0128

also gives the percentage change in the fluorescence peaks of biomolecules during the two exposures.

Figure 3 shows the recorded spectra of chemicals, while Table 2 gives the corresponding characteristic fluorescence peaks and percentage change.

As earlier, the spectra were recorded after exposing the samples for 5 sec and then after 5 min of exposure. In the case of non-biomolecules, the decays in the fluorescence peaks were not significant (almost negligible, as visible in Figure 3) in comparison to those for biomolecules.

The reason for the decay in fluorescence peak in biomolecules is attributed to the following: (i) covalent modification and (ii) singlet–triplet transition. Due to continuous incidence of photons on the samples, the chemical bonds between the molecules get damaged leading to irreversible covalent bond modifications, and hence the fluorescence peak decreases in case of biomolecules[14]. Secondly, the transition from an excited singlet state to an excited triplet state occurs as a consequence of irreversible covalent-bond modification. The excited triplet state is long-lived compared to the singlet state, and the same process is repeated thousand or million times, which is more dominant in case of biomolecules, and hence the decay in biomolecules is more compared to non-biomolecules[15].

Thus, by just looking at the decay of fluorescence peaks in the spectra with time, one can distinguish between bio- and non-biomolecules. If the decay is significantly large, the molecule under examination can be classified as a biomolecule, while if the decay is insignificant, then the fluorescing molecules are non-biomolecules. In Figure 4, the temporal LIF signal of non-bioaerosols

(ethanol) is shown. The initial (recorded after 5 sec exposure time) and final (recorded after 5 min exposure time) LIF signal intensities are almost similar, but in the case of biomolecules, for which the temporal fluorescence peaks are shown in Figure 5, the initial and final LIF signal intensities differ significantly.

The variation of peak fluorescence intensities with exposure time have been studied for bio- and non-biomolecules from stand-off distance using LIF. The percentage decays in the intensities of fluorescence peaks ($\{[I_0 - I_T]/I_0\} \times 100$), after fixed exposure time, were evaluated for both the bio- and non-biomolecules. It was observed that the values of ΔI for biomolecules were very large in comparison to those for non-biomolecules. The large value of ΔI ($= I_0 - I_T$) can be utilized as a marker of biomolecules, which can form the basis for differentiation between bio- and fluorescing non-biomolecules from stand-off distances. This is a novel approach based on radiative relaxation using the LIF technique for discrimination between bio- and fluorescing non-biomolecules. In future, this technique will be used to develop a system to discriminate between of bio- and fluorescing non-biomolecules from stand-off distances for defence and security applications. The methodology has demonstrated detection and discrimination between bio- and non-bioaerosols. With detection limit up to 10^3 particle per litre (ppl).

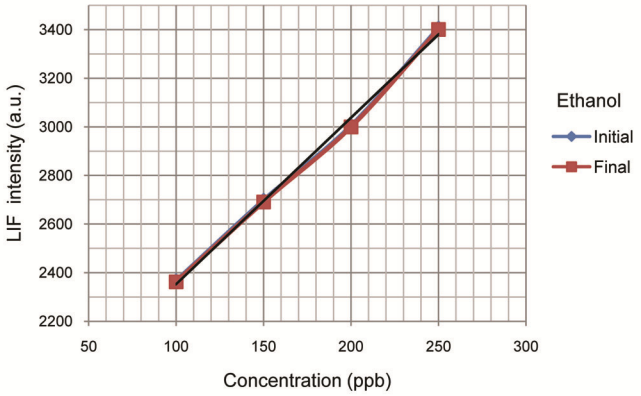

Figure 4. Time-dependent LIF signal of non-bioaersols.

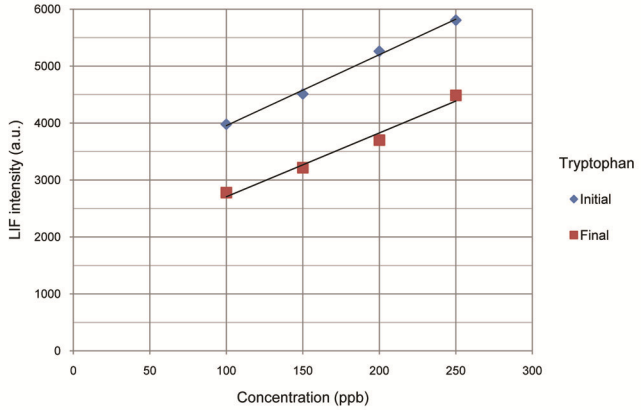

Figure 5. Time-dependent LIF signal of bioaerosols.

Disclosure: The authors declare no conflict of interest.

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