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SCIENCE & TECHNOLOGY RESEARCH INSTITUTE
FOR DEFENCE

APPLICATION OF OPTICAL TECHNIQUES TO DETECT CHEMICAL AND BIOLOGICAL AGENTS

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ABSTRACT

Chemical or biological contamination, due to natural or man-made disasters, represents a severe concern for safety and security of people, and of the environment. Chemical agents (CAs) and biological agents (BAs) are commonly used for a number of civilian and military applications, and can be deployed as a weapon with terroristic purposes. Therefore, it is necessary to develop specific systems aimed at preventing or reducing the consequences of the spread of these agents. To this end, the authors have developed optical systems to detect (LIDAR) and identify (DIAL) CAs, and to detect BAs (fluorescence technique). These systems and technologies will be presented in this work together with the analysis and discussion of the results obtained.

Keywords: *Light detection and ranging (LIDAR); differential absorption of light (DIAL); fluorescence; chemical agents (CAs); biological agents (BAs).*

1. INTRODUCTION

Nowadays the perception of risks has completely changed as compared to that in the past. The geographical boundaries are thin, so too the geo-political ones. The social boundaries have totally disappeared leaving space to a world with different roles as compared to the past. Globalization and the simplicity to access information on the one hand has improved technologies and quality of life, but on the other hand, has created more opportunities to create terror. The cases of the dispersion of toxic gases in the past and in recent history (Okumura *et al.*, 1998; Singer *et al.*, 2005; Varney *et al.*, 2006), the DAESH progression in the Arabic countries (Hansen-Lewis & Shapiro, 2015; Fromson & Simon, 2015; Akbarzadeh, 2015) connected to the actual risk of toxin dispersion in water, Ebola diffusion through aerial transportation (Cenciarelli *et al.*, 2014, 2015a,b), and radioactive dispersion due to failures in fission or fusion devices (Gallo *et al.*, 2012; Cacciotti *et al.*, 2014; Malizia *et al.*, 2014; Koninhs *et al.*, 2015; Malins *et al.*, 2016) are all examples of how the new merge between the well-known risks together with the new “dangerous ideas” have exponentially increased the uncertainties of human health all around the world.

The University of Rome Tor Vergata, in collaboration with the Faculty of Engineering and Faculty of Medicine and Surgery, is facing these new problems with innovative working

approaches devoted to the use the expertise developed in classic scientific disciplines in an unconventional way. Currently several technologies have been investigated in order to develop systems based on optical sensing of chemical agents (CAs) and biological agents (BAs). BA and CA contamination are important not only for security reasons but also for safety issues. As a matter of fact, chemical contamination in actuality is a real menace, with the DAESH scaring the world by threatening the contamination of water. Biological epidemic spreads are relatively easy thanks to the worldwide aerial connection. The risks of chemical or biological contaminations can also be found in hospitals or in private factories and it is necessary to guarantee, in all these areas, a continuum monitoring to reduce the probabilities and the magnitude of those events.

The market is full of products that are able to detect and identify the CAs for both chemical warfare agents (CWA) and toxic industrial components or toxic industrial materials (TIC/TIM), and to identify BAs (such as biological warfare agents or similar), but these technologies work only in short range (chemical) or in the laboratory, after automatic or manual sample collection (biological). The current commercialized tools are only able to detect the putative variation of the environmental biological or chemical background in a defined confined area, providing an on/off response type. In this work, the authors go over simple detection through optical techniques, demonstrating how they are working to have a quasi-real time detection and/or identification of BAs and CAs that is fundamental during operation in a contaminated scenario, such as one characterized by an accidents, or terroristic or war events.

2 MATERIALS AND METHODS

This section is devoted to describing the experimental set-up (together with the methods) developed and used by the authors to detect (LIDAR) and identify (DIAL) CAs, and to detect BAs (fluorescence technique).

2.1 Laser-Diode based Remote LIDAR

The measurements described in the paper were performed with the LIDAR system developed by the Quantum Electronic Plasma Physics (QEP) research group at the University of Rome Tor Vergata. Furthermore, a ground-based mini LIDAR (Figure 1) station has been built and continuously upgraded at the CRATI s.c.r.l. of the University of Calabria. The measurements have been performed with a commercial Nd:Yag (neodymium-doped yttrium aluminium garnet; Nd:Y₃Al₅O₁₂) laser operating in so-called Q-switching mode. Q-switching, sometimes known as giant pulse formation or Q-spoiling, is a technique by which a laser can be made to produce a pulsed output beam. This technique allows for the production of light pulses with extremely high peak power, much higher than would be produced by the same laser if it were operating in a continuous wave (constant output) mode. A wavelength of 1,064 nm was used during the experimental campaign. This choice satisfies the need for developing a compact system that is robust enough to guarantee continuous operations. This technology has also become relatively standard and thus, it can be procured at reasonable costs. The receiver system is composed of an avalanche photodiode and a telescope, based on the Newtonian configuration. The entire apparatus is installed on a mobile system and thus, constitutes a fully mobile station, which can be used for in field campaigns in any location. The experimental campaign was conducted at Crotona, in the Calabria region in the south of Italy (Bellecci *et al.*, 2009, 2010; Gaudio *et al.*, 2013a, 2014, Gelfusa *et al.*, 2015, Parracino *et al.*, 2016).

Using the LIDAR system, it is possible to detect anomalies in terms of variation of the normal backscattered LIDAR signal sent into the atmosphere through the application of Equation 1, which is the LIDAR equation, and Equation 2, which is the Klett inversion (Parracino *et al.*, 2016).

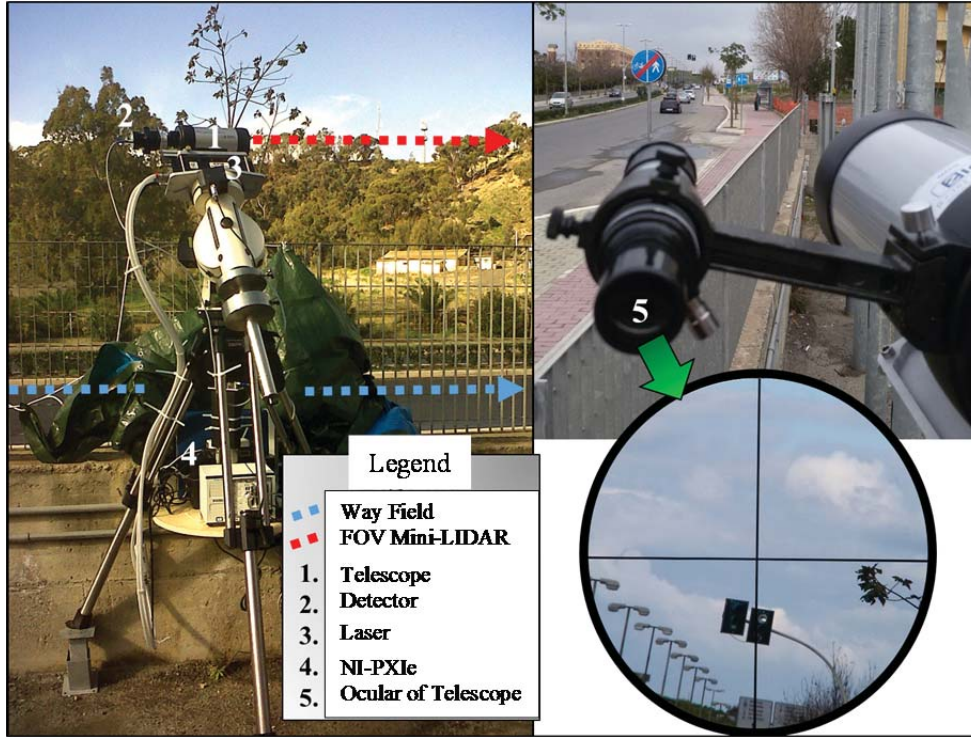


Figure 1: The deployed field LIDAR system.

$$P_r(\lambda, R) = E_1 \frac{c A_r}{2 R^2} \beta(\lambda, R) C(\lambda, R) \exp\left(-2 \int_0^R K(\lambda, R') dR'\right) \quad (1)$$

The LIDAR equation is the way to relate the light power backscattered by the atmospheric target with the signal collected by the LIDAR receiver. In the equation, $P_r(\lambda, R)$ is the backscattered power received from the distance R , at the specific laser operative wavelength λ (because the equation describes the elastic scattering); E_1 is the laser pulse energy, c is the speed of light; $A_r \cdot R^{-2}$ is the acceptance solid angle of the receiver optics with collecting area A_r ; and $C(\lambda, R)$ is a function determined by the geometric considerations of the receiver optics, the transmitter (set at 100%) and receiver efficiencies, and the overlap of the emitted laser beam with the field of view of the receiver. The last two terms are known as the optical parameters and are closely linked to the aerosols load. The backscattering coefficient $\beta(\lambda, R)$ is a measure of the scattering in the backward direction (i.e., towards the incident direction, at a scattering angle of 180°) for the light encountering the atmospheric aerosol particles and molecules. The extinction coefficient $K(\lambda, R)$ is a measure of attenuation of the light passing through the atmosphere due to the scattering and absorption by aerosol particles and molecules. Since it is reasonable to assume that molecular backscattering is negligible in the infrared region, and that particulate backscattering dominates in this region, the LIDAR signal gives particle backscatter information directly if laser is operating in this region. By assuming a relationship between relative variation of backscattering coefficient and particulate mass or number density, the LIDAR signal can be used to monitor and track atmospheric particles (Parracino *et al.*, 2016).

From Equation 1, and using the Klett inversion method (Klett, 1981), it is possible to extract both the extinction and backscattering coefficients. In this paper, the attention is focused on the particle backscattering coefficient, the primary atmospheric parameter that determines the strength of the LIDAR signal. The far boundary solution is reported into the following equation:

$$\beta(R, \lambda) = \frac{e^{[\mu(R, \lambda) - \mu_m(\lambda)]}}{\frac{1}{\beta_m(\lambda)} + \frac{2}{F_{\pi}(R, \lambda)} \int_R^{R_m} e^{[\mu(R', \lambda) - \mu_m(\lambda)]} dR'} \quad (2)$$

Development of a Laser-based LIDAR DIAL

The EP research group developed two dial carbon dioxide (CO₂) based laser systems. The first one is mounted on a truck. Today, it is used in experimental campaigns all around Italy for pollutants identification in the atmosphere (Gaudio *et al.*, 2011, 2013b; Gelfusa *et al.*, 2014a, 2014b). After that, a mini-CO₂ system (Figure 2) was developed to obtain remote, stand-off identification of CAs, TIMs and TICs. Both the systems work with the DIAL techniques that provide the capability to remotely measure the concentration and spatial distribution of compounds in the atmosphere. The ability to scan the optical measurement beam throughout the atmosphere enables pollutant concentrations to be mapped and emission fluxes to be determined.

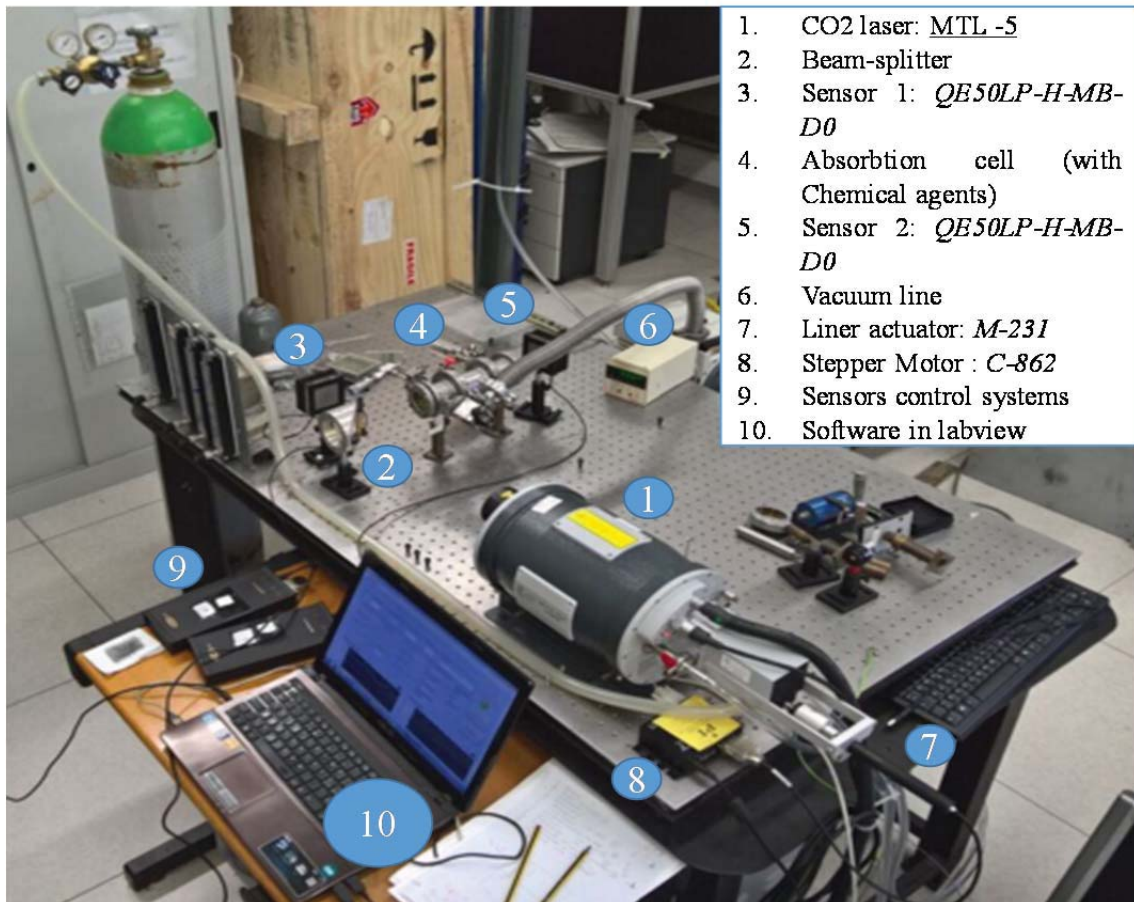


Figure 2: The developed mini-DIAL system.

The experimental set-up is composed of a mini MTL-5 CO₂ TEA laser (1) (a manual tuned laser that uses CO₂ using transverse excited atmospheric pressure) that emits laser beams at different wavelengths obtained by a linear actuator (2) controlled by a stepper motor (8) on a beam-splitter (2) that divides the beam into two beams, one directed to energy meter 1 (3) and one that passes through an absorption cell (4) that contains the CA under investigation. This second beam is partially absorbed by the CA inside (4) and then goes to energy meter 2 (5). Both the sensors are connected to an analog to digital converter (ADC) board (9) to acquire the measurements. The absorption cell is connected to a vacuum line (6) to get different pressure conditions.

3.1 Fluorescence Apparatus

The EP research group, in collaboration with the Department of Industrial Engineering, is working on an experimental set-up (Figure 3) based on fluorescence techniques to detect BAs (Carestia *et al.*, 2014a, 2014b, 2015a, 2015b). Steady-state photoluminescence measurements on liquid samples in an optical cuvette were performed in a standard laboratory set-up with a photomultiplier optical detector located at the output port of a 25-cm monochromator. The excitation light beam was provided by the monochromatized output of a Hg(Xe) 200-W discharge lamp (that is a Mercury Xenon lamp). All measurements were performed at temperature of 20 ± 2°C. The two wavelengths selected for the excitation of the samples were 355 and 266 nm, referring to the third and fourth harmonics of a Nd:YAG laser source respectively. The experimental set-up is shown in Figure 3. All the components of the experimental apparatus are reported in Table 1. A schematic view of the experimental apparatus is shown in Figure 4.

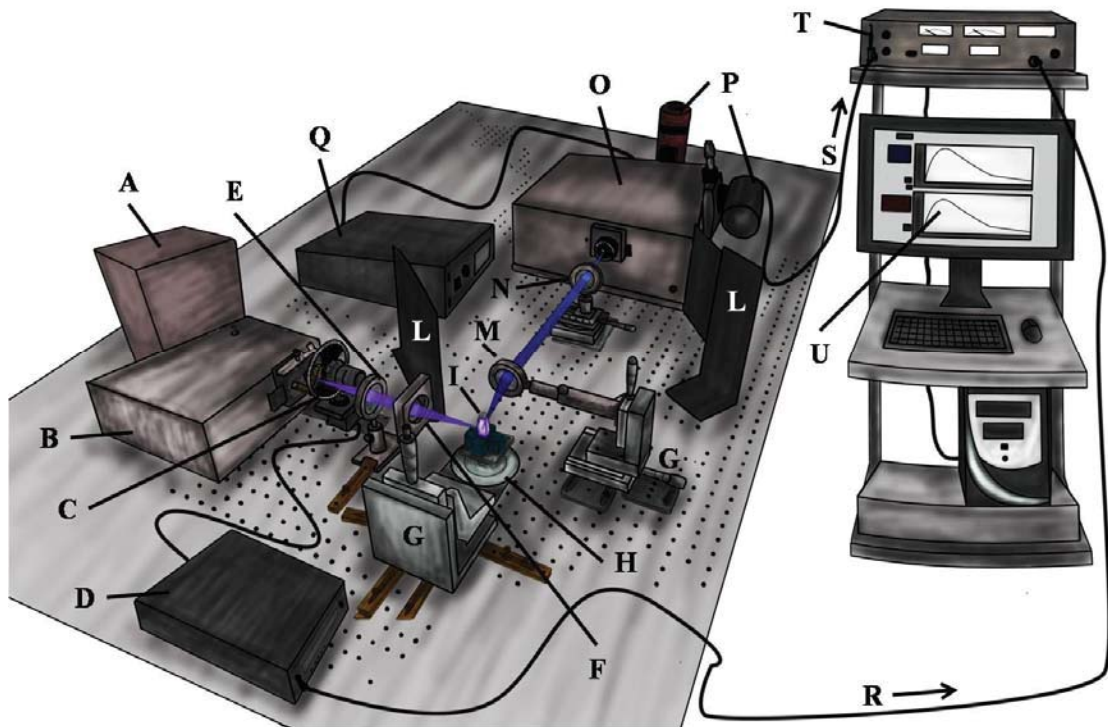
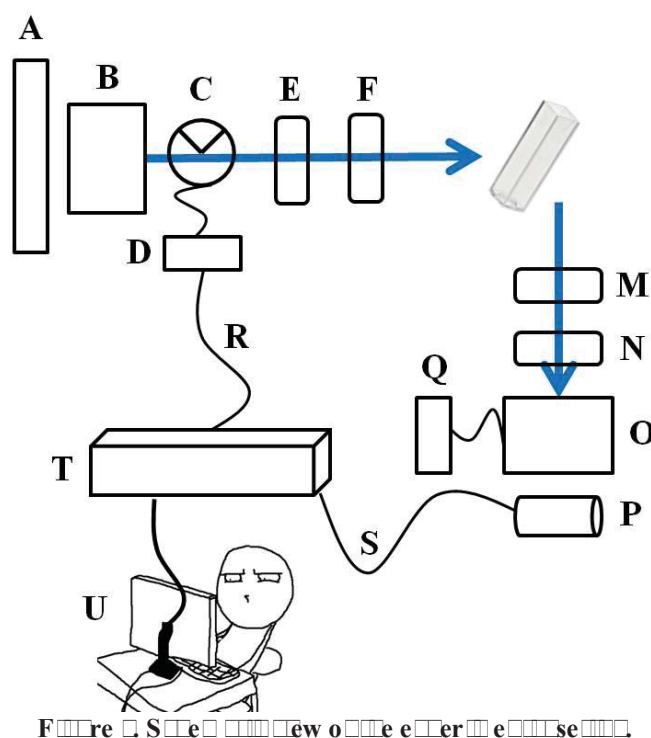


Figure 3: Experimental set-up for fluorescence measurements.

Figure 1: Components of a fluorescence spectrometer.

A	Hg(Xe) 200 nm discharge lamp	M	Collecting collimating lens
B	Excitation monochromator	N	Collecting focusing lens
C	Excitation modulator (chopper)	O	Emission monochromator
D	Chopper power supply	P	Photomultiplier
E	Excitation collimating lens	Q	Emission monochromator power supply
F	Excitation focusing lens	R	Modulation reference
G	Positioning	S	Signal
H	Sample holder	T	Lock in amplifier
I	Cuvette	U	Software analysis bench
L	Light shield		



The light emitted by the lamp (A) is focused on the input slit of an excitation monochromator (B), which selects the required wavelength. The monochromatic beam passes through an amplitude modulator (C) and through two lenses, collimating (E) and focusing (F); then it strikes the sample (I). The wavelength intensity distribution of the emitted light (emission spectrum) that passes through two lenses, collimating (M) and focusing (N), is analyzed by the emission monochromator (O). The light intensity is transduced to an electric signal by the photomultiplier (P). The emission monochromator is also necessary to avoid exciting radiation from reaching the detector. This can happen despite the emitted radiation being measured perpendicular to the exciting radiation, due to the scattering phenomena, in particular due to the Rayleigh and Tyndall scattering. The light scattering, as a result of the interaction of the light with the matter, can be defined as elastic (Rayleigh or Tyndall scattering) or inelastic (Raman scattering). In the first case, the scattered light has the same frequency of the incident beam, while in the case of inelastic scattering, the frequencies are different and are present in the sample spectrum. The Rayleigh scattering occurs when the radiation passes through a

transparent medium that illuminates particles with a diameter much lower (less than $1 \mu\text{m}$) than its wavelength. The scattered light has the same wavelength of the incident light and an intensity proportional to the fourth power of the frequency of the incident light. Tyndall scattering occurs in the presence of colloidal systems in which the particles have a diameter comparable with the wavelength of visible light. The intensity of the scattered light is proportional to the second power of the frequency of the incident radiation. Raman scattering, which is solvent-dependent, occurs when only a very small fraction of the light that is incident on a transparent medium is diffused. (Carestia *et al.*, 2015b)

2 EXPERIMENTS AND RESULTS

This section is devoted to describe the experimental results obtained with the experimental set-ups previously discussed.

2.1 LIDAR

The variations in atmosphere detected with the COmpact LIDAR system (COLI) can be resolved both in time (Figure 5) or space (Figure 6), and visualized through backscattering coefficient maps that essentially give the exact time and position of the detected anomaly. The maximum range reached by the system is 1.5 km, which is quite common. However for reasons of safety and due to the constraints of the experimental field investigated area, the far range boundary point of LIDAR system was reduced. For these reasons, the two backscattering maps reported in the following figures have been cut from 0 to 500-600m. In spite of these limitations, this improves the accuracy of experimental results, increasing the signal to noise ratio (SNR) level.

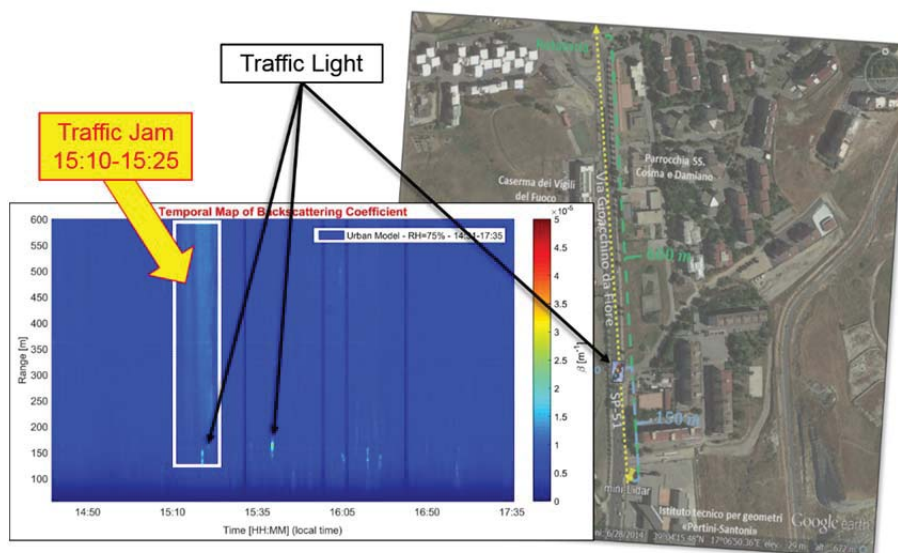


Figure 5: The COLI system resolution.

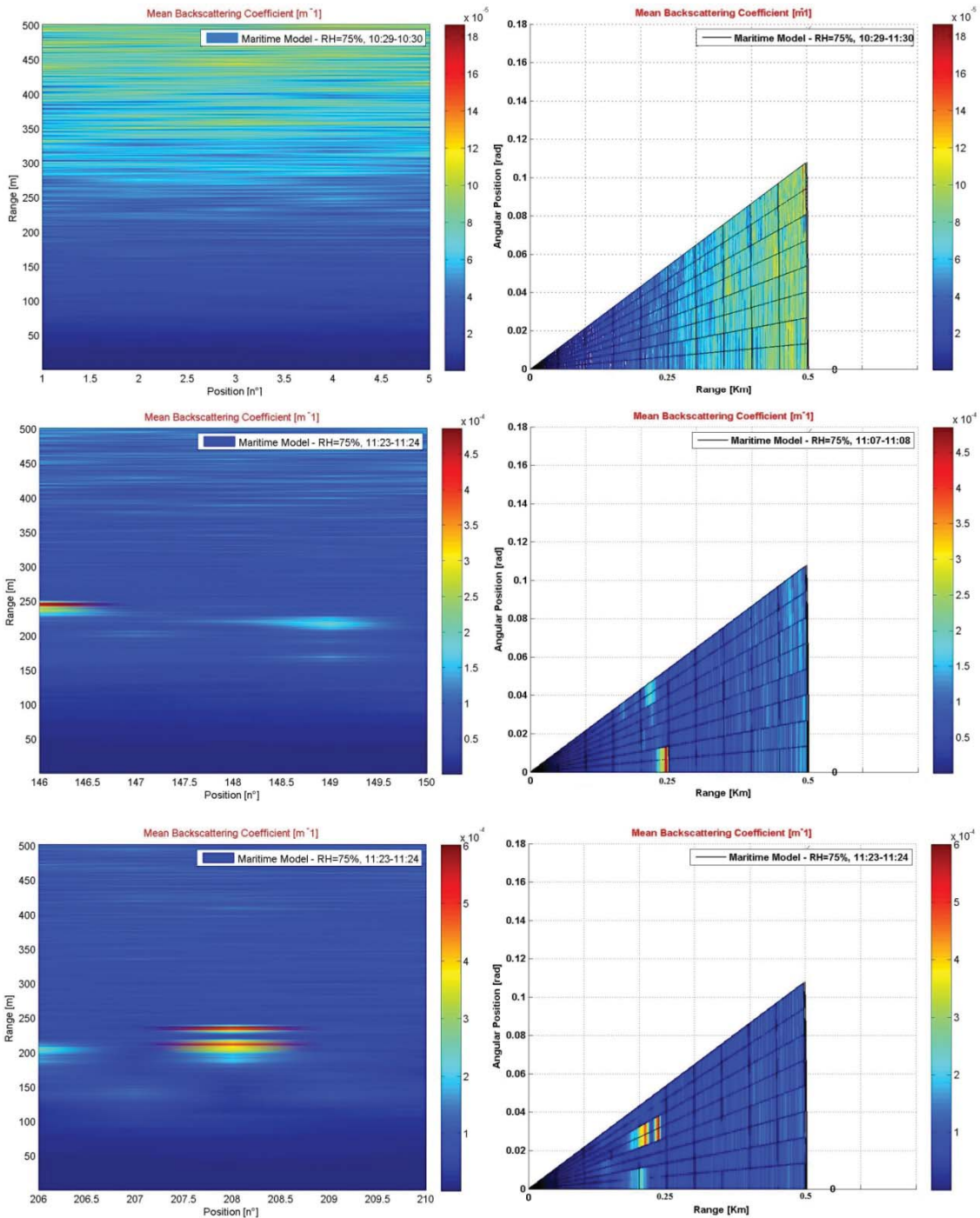


Figure 3: Backscattered coefficients resolved in space

3.2 DIAL

The CO₂ DIAL systems are used to obtain the absorption spectra, which are the characteristic of each CA, in order to obtain a standoff medium range (0-1 km) identification that can help the decision makers to understand the typology of contamination present in an open or closed environment. An experimental campaign, dedicated to test the DIAL system, was conducted in order to demonstrate the capability of this system to identify CAs, in order to use it for C₆A, TIC and TIM dispersion problems. In this experimental campaign, CEP used mainly TIC, some of them are official recognized as C₆A simulants. The capability to identify chemicals with the

optical system developed is demonstrated in this section. The experiments were performed not only to demonstrate the capability to identify chemicals through the optical apparatus developed but also to create a database of the absorption spectra of the chemicals to guarantee use on the field with a fast comparison between the spectra acquired and the one on the database.

The toxic component of a chemical weapon is called its CA. Based on their mode of action (i.e. the route of penetration and their effect on the human body), chemical agents are commonly divided into several categories (OPC, 201):

- Blister agents
- Blood agents
- Nerve agents
- Riot control agents
- Potential CA Agents
- Mustard agents
- Psychotomimetic Agents
- Toxins

All the experimental measures were conducted in two different conditions:

- Internal cell pressure of 50 mbar (high vacuum): This is a condition with a quasi-unique presence of analyte molecules.
- Internal cell pressure of 1,000 mbar (atmospheric pressure): in this condition, the air has little amount (in percentage) of analyte molecules (JRAMC, 2002).

The composition of air is variable in general depending on the considered quantity of analyte. For a fixed quantity, the ratio between the amount of nitrogen and the amount of oxygen contained in the air remains almost constant thanks to the balance between the consumption and continuous intake of these elements associated with the oxygen cycle and the cycle of nitrogen. Instead, the water vapor and carbon dioxide concentrations are variable. For this reason, they often indicate the properties of the air without water vapor (which is called "dry air", otherwise it is called "moist air"). Dry air at the ground level is composed of approximately 80% nitrogen (N₂), 21% oxygen (O₂), 0.96% argon (Ar) and 0.04% carbon dioxide (CO₂), plus other components in minor amounts, including solid particles in suspension, which constitute the so-called "atmospheric dust". Moist air can contain up to 1% of the volume composed by the water vapor; the percentage of water vapor in the air corresponds to the relative humidity of air and temperature. The maximum value of water vapor that the air can hold at equilibrium corresponds to the saturation conditions; above this maximum value, the water vapor tends to condense spontaneously. This maximum value is related to the temperature changing from values close to 0°C (to a temperature equal to -40°C), about 0.5% (0°C), up to 5% (around 40°C) (JRAMC, 2002).

It was experimentally verified that the variations of the absorption spectra between cell under vacuum and at atmospheric pressure cell (both with analyte) are not relevant. The differences between spectra in dry and moist air conditions are also negligible. In addition, the temperature variations also do not give considerable variation to the measuring spectra if we are in the temperature variation range present in nature.

The absorption spectra are all prepared with pure substance in the cell (black graph in Figure 1) and with the combination of substance+air (red graph in Figure 1) using all the wavelengths available with MTL-5. Figure 1 shows the absorption spectra of acetone that has no specific absorption values with the exception of short wavelengths, in particular 9,225 nm both for vacuum and air conditions and a peak at 9,425 nm just in vacuum conditions, which is probably an error during the acquisition.

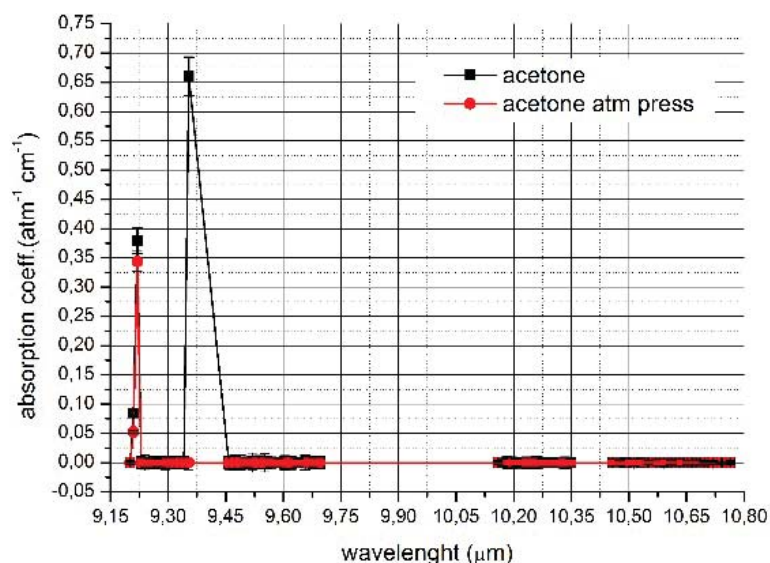


Figure 8: Acetone absorption coefficient

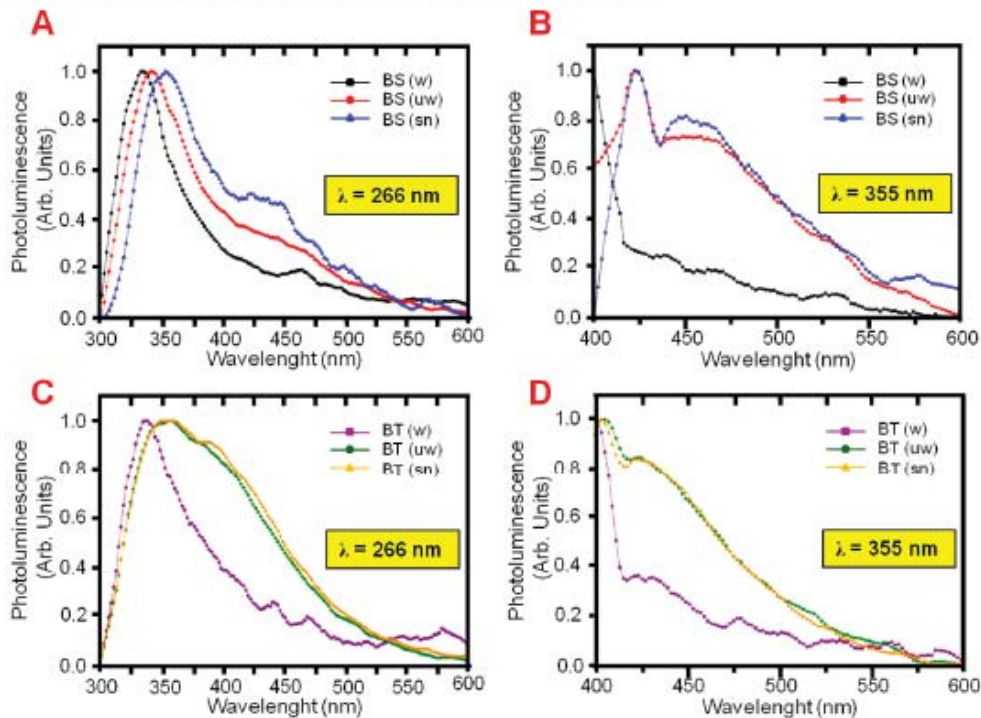
2.2 Fluorescence Measurements of BAs

The capability to obtain fluorescence spectra (Figure 8) was investigated by the authors using bacillus spores samples (washed, unwashed and supernatant). These results clearly show the potentiality of this technique merged with statistical methods, such as the universal multi-event Locator (UMEL), a statistical tool based on support vector regression, to improve the ability to discriminate different BAs emission spectra, to achieve early detection of BAs. It can be used to detect the contamination of surfaces, in liquid samples or in the atmosphere. The experimental and data analysis methods have to be improved in order to reduce the false positives, increase the field of view (FOV) of the system, and to speed up and automate the detection process.

3 CONCLUSION AND FUTURE DEVELOPMENTS

The authors in the last fifteen years have demonstrated that laser-based optical techniques can enable continuous and quasi real-time monitoring of CAs (CA, TIC, TIM) in the air at medium-long distances. In particular, the capability of the LIDAR systems to detect CAs for the early warning phase and of the DIAL systems to identify at short-medium range certain CAs for the decision making phase has been demonstrated. Using the same approach (optical techniques for standoff detection) but changing the method (fluorescence), the authors assessed its potential for BA detection. The challenges for the future are to optimize the systems by reducing the dimensions and cost, while at the same time improving the performances in terms of reduction of false alarms, increase of robustness and acquisition speed, and increase reliability and determinism.

NORMALIZED AT THEIR PEAK VALUES



F *re* *Nor* *ed* *or* *s* *e* *r*: *Bacillus subtilis* **A** *B* *Bacillus thuringiensis* **C** **D**

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