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Abstract

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Identification of biological microparticles using ultrafast depletion spectroscopy

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1. Introduction

Rapid detection and identification of pathogenic aerosols from potential bioterrorism release and epidemic spreads are urgent safety issues. In order to efficiently protect populations, bioaerosol detection devices have to be very fast (typically minutes) and very selective to discriminate pathogenic from non-pathogenic particles and minimize false alarm rates. This very difficult task initiated recent major research and development efforts.

Biochemical identification procedures such as Polymerase Chain Reaction (PCR), antibiotic resistance determination, or matrices of biochemical micro-sensors are selective, but slow (at least some hours). On the other hand, optical techniques provide information in “real time” but until now lack in specificity. In particular, several optical systems, based on fluorescence and/or elastic scattering have been developed to distinguish bio- from non-bioaerosols. The most advanced experiments address individual aerosol particles, within which fluorescence is spectrally analyzed. The major drawback of these instruments is, however, frequent false alarms triggered by other organic aerosols, such as diesel particles or cigarette smoke. Fig. 1 shows, as an example, the similitude in the fluorescence spectra of diesel fuel, soot particles, the amino acid tryptophan (Trp) and Bacillus subtilis, which is a biosimulant for Bacillus anthracis. As shown in the figure, the major contribution in the UV-Vis fluorescence (around 340 nm) in biological

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Particles is due to the amino acid tryptophan. The longer wavelength tail of the fluorescence is attributed to the emission of nicotinamide adenine dinucleotide (NADH; around 450 nm) and flavins (riboflavin, flavin mononucleotide FMN and flavin adenine dinucleotide FAD around 560 nm). Due to the interference with Polycyclic Aromatic Hydrocarbons (PAHs) containing soot and diesel, the identification of bioaerosols in a background of traffic related particles (typical of urban conditions) is therefore extremely difficult. It is even more elusive to expect discrimination of different types of bacteria. An interesting approach was recently reported, which combines optical and biochemical analyses. A fluorescence/scattering device was used to sort "on-line" bioaerosols from other particles, which could then be subsequently chemically analyzed in situ.

Optical techniques are also attractive as they can provide information remotely. The Lidar (Light detection and ranging) technique allows for mapping aerosols in 3D over several kilometres, similar to an optical Radar. Lidars are able to detect the release and spread of potentially harmful plumes (such as pathogen releases from terrorists or legionella from cooling towers) at large distance and thus allow taking measures in time for protecting populations or identifying sources. So far, Lidar detection of bioaerosols has been demonstrated either using elastic scattering or UV-LIF. However, the distinction between bio- and non-bioaerosols was either impossible (elastic scattering only) or unsatisfactory for LIF-Lidars (interference with pollens and organic particles like traffic-related soot or PAHs).

To overcome these difficulties, there is an interest in exciting the fluorescence with ultrashort laser pulses in order to access specific molecular dynamical features. Recent experiments using coherent control and multiphoton ultrafast spectroscopy have shown the ability to discriminate between molecular species that have similar one-photon absorption and emission spectra. Two-Photon Excited Fluorescence (2PEF) and pulse shaping techniques should allow for selective enhancement of the fluorescence of one molecule versus another that has similar spectra. Optimal Dynamic Discrimination (ODD) of similar molecular agent provides the basis for generating optimal signals for detection.

In this paper, we demonstrate that femtosecond pump–probe spectroscopy allows for distinguishing biological microparticles from PAH-containing ones. More precisely, we could distinguish amino acids (Trp) and flavins (riboflavin RbF, FMN and FAD) from PAHs (naphthalene) and diesel fuel in the liquid phase using a “Pump–Pump Depletion” (PPD) technique. We also applied the technique to live bacteria.
and individual bioaerosol particles, for which PPD showed even higher selectivity than for molecules in the liquid phase.

2. Experimental

PPD uses a sequence of two femtosecond pulses (one UV-Vis and the other at 810 nm), separated by a variable time delay $\Delta t$. The fluorescence is recorded as a function of $\Delta t$, which reflects the molecular dynamics within the intermediate state (see below). The experiments (Fig. 2) use a Kerr lens mode-locked Ti : sapphire oscillator and a chirped pulse amplifier that delivers 120 fs pulses at 810 nm. The output is frequency doubled and tripled in two consecutive $\beta$-Barium Borate (BBO) crystals so that the pulses at 270 nm for Trp, naphthalene and diesel (405 nm for Rbf, FMN and FAD) and at 810 nm are synchronously emitted. After splitting both pulses with a dichroic mirror, the second (near infrared) pump pulse is delayed using a stepper motorized delay line, with a resolution of 16 fs. Both beams are then recombined, carefully spatially superimposed and focused onto a 1 mm length quartz flow cell, leading to intensities up to $I_{270} = 4 \times 10^7$ W cm$^{-2}$ ($I_{405} = 9 \times 10^8$ W cm$^{-2}$) and $I_{810} = 2 \times 10^{11}$ W cm$^{-2}$. The fluorescence is dispersed by a Jobin Yvon Y10 spectrometer (2 nm resolution) or filtered with BandPass (BP) (10 nm) filters centred at 340 nm close to the maximum intensity of Trp and PAH fluorescence bands (or at 520 nm for flavins) and detected by PhotoMultiplier Tubes (PMT). Trp or flavins are solvated in water, whereas naphthalene is dissolved in cyclohexane.

The concentrations used are typically 0.2 g L$^{-1}$ for Trp, 0.3 g L$^{-1}$ for naphthalene, 0.1 g L$^{-1}$ for Rbf and from 0.1 to 1 g L$^{-1}$ for FMN and FAD. We checked that our results do not depend on the different solution concentrations. In the further experiments, solutions of bacteria (Escherichia coli, Bacillus subtilis, Enterococcus fæcalis) have been prepared by dissolving lyophilized cells and spores in water (Strain W ATCC 9637 for Escherichia coli, ATCC 6633 for Bacillus subtilis) or in a nutrient buffered (Symbioflor for Enterococcus fæcalis) solution at concentrations of $10^7$ to $10^9$ bacteria cm$^{-3}$. Diesel fuel has also been used to simulate the complex mixture of PAHs contained by organic particles emitted by diesel engines. A circulator ensures that solutions in the excitation volume are renewed at each laser pulse.

For the experiments performed in air, an aerosol generator based on a piezo-electric-driven nozzle is used. This allows producing the particles “on demand” and therefore to synchronize the generator with the incoming laser pulses, so that each droplet is irradiated by a single laser pulse pair (pump–probe type of experiment). The size of the droplets can be varied from 20 to 60 μm by tuning the appropriate voltage pulse on the piezoelectric element. The aerosol stream speed is about 1 m s$^{-1}$. The particle diameter was stable over hours within a few percent (measured by

**Fig. 2** Experimental set-up of the PPD experiments. (a) Optical layout used for the experiments in liquids. For the experiments in air, bioaerosols are generated with a droplet generator (b), which replaces the flow cell in (a). The droplet generator is synchronized with the femtosecond pulse pair.
forward Mie scattering with a Helium–Neon laser). The laser is slightly focused with a 1 m focal length lens, so that the beam waist remains 5–10 times larger than the droplet diameter. A (synchronized) pulsed LED and a CCD camera permanently monitor the droplet position and the laser waist.

3. Pump–probe spectroscopy to identify bacteria and biomolecules in liquids

As mentioned above, a major drawback inherent in LIF instruments is the lack of selectivity because UV-Vis fluorescence is incapable of discriminating different molecules with similar absorption and fluorescence signatures. While mineral and carbon black particles do not fluoresce significantly, aromatics and polycyclic aromatic hydrocarbons from organic particles and diesel soot strongly interfere with biological fluorophors such as amino acids. The similarity between the spectral signatures of PAH and biological molecules under UV-Vis excitation lies in the fact that similar π-electrons from carbonic rings are involved. Therefore, PAHs (such as naphtalene) exhibit absorption and emission bands similar to those of amino acids like Tyrosine or Trp. Some shifts are present because of differences in specific bonds and the number of aromatic rings, but the broad, featureless nature of the bands renders them almost indistinguishable (Fig. 1).

In order to discriminate these fluorescing molecules, we applied a novel femto-second PPD concept (Fig. 3). It is based on the time-resolved observation of the competition between Excited State Absorption (ESA) into higher lying excited states and fluorescence into the ground state. This approach makes use of two physical processes beyond that available in the usual linear fluorescence spectroscopy: (1) the dynamics in the intermediate pumped state ($S_1$) and (2) the coupling efficiency to higher lying excited states ($S_n$).

As sketched in Fig. 3, a first femtosecond pump pulse (at 270 nm for Trp and PAHs, 405 nm for flavins), resonant with the first absorption band of the fluorophores, coherently excites them from the ground state $S_0$ to a set of vibronic levels $S_1\{\nu\}$. The vibronic excitation relaxes by internal energy redistribution to lower $\{\nu\}$ modes. Fluorescence relaxation to the ground state occurs within a lifetime of several nanoseconds. Meanwhile, a second 810 nm femtosecond “repump” pulse is used to transfer part of the $S_1\{\nu\}$ population to higher lying electronic states $S_n$. The depletion of the $S_1$ population under investigation depends on both the molecular

![Fig. 3](image-url) Absorption spectra of Tryptophan (a) and Riboflavin (b). (c) PPD scheme in Trp, flavins and polycyclic aromatics. The pump pulse brings the molecules in their first excited state $S_1$. The $S_1$ population (and therefore the fluorescence) is depleted by the second pump pulse. Reprinted from ref. 32 © 2006, with permission from Elsevier.
dynamics in this intermediate state and the transition probability to $S_n$. The relaxation from the intermediate excited state may be associated with different processes, including charge transfer, conformational relaxation\textsuperscript{26,27} and intersystem crossing with repulsive $\pi\sigma^*$ states.\textsuperscript{28,29} $S_n$ states are both autoionizing\textsuperscript{29} and relaxing radiationlessly\textsuperscript{30} into $S_0$. By varying the temporal delay $\Delta t$ between the UV-Vis and the IR pulses, the dynamics of the internal energy redistribution within the intermediate excited potential hypersurface $S_1$ is explored. The $S_1$ population and the fluorescence signal is therefore depleted as a function of $\Delta t$. As different species have distinct $S_1$ hypersurfaces, discriminating signals can be obtained.

Fig. 4(a) shows the PPD dynamics of $S_1$ in Trp as compared to diesel fuel and naphtalene in cyclohexane, one of the most abundant fluorescing PAHs in diesel. While depletion reaches as much as 50% in Trp, diesel fuel and naphtalene appear almost unaffected (within a few percent), at least on these timescales.\textsuperscript{31} The depletion factor $\delta$ is defined as $\delta = (P_{\text{undepleted}} - P_{\text{depleted}})/P_{\text{undepleted}}$ (where $P$ is the fluorescence power). This remarkable difference allows for efficient discrimination between Trp and organic species, although they exhibit very similar linear excitation/fluorescence spectra (Fig. 1).

Two reasons might be invoked to understand this difference: (1) the intermediate state dynamics are predominantly influenced by the NH- and CO-groups of the amino acid backbone and (2) the ionization efficiency is lower for the PAHs. Further electronic structure calculations are required to better understand the process, especially on the higher lying $S_n$ potential surfaces.
Fluorescence depletion has also been obtained for RbF, FMN, and FAD (Fig. 4b). However, the depletion in this case is only about 15% (with a maximum intensity of $5 \times 10^{11}$ W cm$^{-2}$ at 810 nm). We then repeated the experiment but exciting the flavins at 270 nm, as for Trp. Flavins indeed absorb in both spectral regions (Fig. 3). The second excitation step by the 810 nm pulse then brings the molecules in the excited states around 200 nm. The fluorescence depletion observed with the 270 nm excitation reached 35%. This is an indication that the branching ratio to autoionization in the 270 nm band is much lower than around 200 nm. To get the depletion while exciting at 405 nm, two photons at 810 nm probably have to be used. This is confirmed by intensity dependencies, which show a quadratic dependence in $I_{810}$ in this latter case. A model based on rate equations was recently developed in order to quantitatively explain the PPD behaviour in Trp and RbF.  

In order to more closely approach the application of detecting and discriminating bioagents from organic particles, we applied PPD spectroscopy to live bacteria ($\lambda_1 = 270$ nm and $\lambda_2 = 810$ nm), such as *Escherichia coli*, *Enterococcus* and *Bacillus subtilis*. Artefacts due to preparation methods have been discarded by using a variety of samples, i.e. lyophilized cells and spores, suspended either in pure or in biologically-buffered water (i.e. typically $10^7$–$10^9$ bacteria cm$^{-3}$). The bacteria-containing solutions replaced the Trp- or flavin-containing solutions of the formerly described experiment. The observed pump-probe depletion results are remarkably robust (Fig. 5), with similar depletion values for all the considered bacteria (results for *Enterococcus*, not shown in the figure, are identical), although the Trp microenvironment within the bacteria proteins is very different from water.

On the other hand, the very similar depletion behaviour for all bacteria and Trp also shows the limitations of PPD spectroscopy in the present configuration. Biomolecules can be distinguished from other aromatics but PPD is unable to discriminate two different bacteria in solution. We are currently exploring new experimental ODD approaches to reach this goal (see Section 5).

### 4. Multi-Photon Excited Fluorescence (MPEF) and PPD in aerosol microparticles

Femtosecond lasers provide high intensity pulses with low energy, which allows inducing non-linear processes in particles without deformation due to electrostrictive and thermal expansion effects.

The most prominent feature of non-linear processes in aerosol particles is strong localization of the emitting molecules within the particle, and subsequent backward enhancement of the emitted light. 33,34 This unexpected behavior is extremely
attractive for remote detection schemes, such as Lidar applications. Localization is achieved by the non-linear processes themselves, which typically involve the $n$th power of the internal intensity $I^n(r)$ ($r$ for position inside the particle). The backward enhancement can be qualitatively understood by the reciprocity (or “time reversal”) principle: re-emission from regions with high $I^n(r)$ tends to return toward the illuminating source by essentially retracing the direction of the incident beam that gave rise to the focal points. This backward enhancement has been observed for both spherical and non spherical\textsuperscript{35} microparticles.

More precisely, we investigated, both theoretically and experimentally, incoherent multiphoton processes involving $n = 1$ to 5 photons.\textsuperscript{34} For $n = 1, 2, 3$ (at 800 nm incident wavelength), MPEF occurs in bioaerosols because of the absorptions of amino acids (tryptophan, tyrosin), NADH, and flavins. The strongly anisotropic MPEF emission was demonstrated on individual water micro-droplets containing tryptophan, riboflavin, or other synthetic fluorophors in ethanol.\textsuperscript{33–35} The experiment was performed with the aerosol source described in Section 2. MPEF angular distribution for the one- (400 nm), two- (800 nm) and three-photon (1, 2 $\mu$m) excitation show that the fluorescence emission is maximum in the direction towards the exciting source. The directionality of the emission is dependent on the increase of $n$, because the excitation process involves the $n$th power of the intensity $I^n(r)$. The ratio $R_f = P(180^\circ)/P(90^\circ)$ increases from 1.8 to 9 when $n$ changes from 1 to 3 ($P$ is the emitted light power). For 3PEF, fluorescence from aerosol microparticles is therefore mainly emitted backwards, which is ideal for Lidar experiments.

At higher intensities, significant ionization occurs in water itself, involving $n = 5$ photons. The growth of the plasma is also a non-linear function of $I^n(r)$. We showed that both localization and backward enhancement strongly increases with the order $n$ of the multiphoton process, exceeding $R_f = P(180^\circ)/P(90^\circ) = 35$ for $n = 5$.\textsuperscript{36} Notice that the light emitted by the plasma has the potential of providing information about the aerosol composition, as recently demonstrated in LIBS (Laser Induced Breakdown Spectroscopy) experiments on bacteria.\textsuperscript{37,38} This unique backward emission behavior allowed us to demonstrate the first MPEF Lidar detection of biological aerosols\textsuperscript{39,40} using the “Teramobile” system. The Teramobile (www.teramobile.org) is the first femtosecond-terawatt laser-based Lidar, and was developed by a French–German consortium, formed by the Universities of Iena, Berlin, Lyon, and the Ecole Polytechnique (Palaiseau).

The bioaerosol particles, consisting of 1 $\mu$m-size water droplets containing 0.03 g l$^{-1}$ of Riboflavin were generated at a distance of 50 m from the Teramobile system. Riboflavin was excited by two photons at 800 nm and emitted a broad fluorescence around 540 nm. The broad fluorescence signature was clearly observed from the particle cloud (typically $10^4$ p cm$^{-2}$), with a range resolution of a few meters.

Primarily, MPEF-Lidar is advantageous as compared to linear LIF-Lidar for the following reasons: (1) MPEF is enhanced in the backward direction and (2) the transmission of the atmosphere is much higher for longer wavelengths. For example, if we consider the detection of Trp with 3-PEF, the transmission of the atmosphere is typically 0.6 km$^{-1}$ at 270 nm, whereas it is $3 \times 10^{-3}$ km$^{-1}$ at 810 nm (for a clear atmosphere, depending on the background ozone concentration). This compensates the lower 3-PEF cross-section compared to the 1-PEF cross-section at distances larger than a couple of kilometres. The most attractive feature of MPEF is, however, the possibility of using pump-probe techniques, as described in Section 3, in order to discriminate bioaerosols from background interferents, such as traffic-related soot or PAHs.

In order to get closer to the real application, we then performed ultrafast PPD spectroscopy in bioaerosols and, in particular, water microdroplets that contain Trp and/or FMN (Fig. 2). The droplet radius was about 25 $\mu$m, which is larger than the
size of single bacteria (1 μm) or even bacteria clusters (typically 10 μm), but still constitute an acceptable model.

The laser intensities at 810 nm and 270 nm for Trp (405 nm for FMN) that excited the microparticles were similar to the intensities used for the experiments in liquids.

The most impressive result of these experiments is the very high PPD efficiency as compared to depletion ratios in liquids. The depletion factor \( \delta \) reaches 80% for Trp droplets and 60% for FMN droplets (to be compared to 50% and 15% in liquids, respectively). Some tentative explanations could be invoked for this unexpectedly high efficiency, but the definitive reason is not clear yet: (1) The spatial overlap between pump and probe pulses might be enhanced by the shape of the droplet; (2) the spherical shape induces hot spots inside the droplet where intensities are up to 100 times higher than the incident one, but the total hot spot volume is rather small; (3) there might be some surface effects (orientation of molecules on the surface) that would enhance the two photon absorption.

Notice that these time-resolved pump–probe experiments could also potentially be used up to the size of the microparticle. Time-resolved two-photon absorption (and fluorescence) has indeed been used in droplets to observe the trajectories of the light bullets within the particle. Each time the femtosecond pulses crossed within the droplet, the fluorescence signal was enhanced. The time delay between two fluorescence maxima corresponds to the roundtrip time \( t_R = 2a m c \) and thus to the radius of the droplet \( a \) (\( m \) is the refraction index = 1.33). In our case, as we measure fluorescence depletion, the second pulse (at 810 nm) could lead to further depletion after a roundtrip. Unfortunately, this was not observed in our experiments (Fig. 6, with Fig. 6  PPD spectroscopy in bioaerosols: (a) Trp-containing microdroplets, (b) FMN-containing microdroplets. Depletion is as much as 80% for Trp and 60% for FMN. The peak in (b) shows the cross-correlation of both laser pulses, which indicates the time resolution of the experiment.

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Because of the limited signal-to-noise ratio (the intensity of the pulse after 1 roundtrip is reduced by at least one order of magnitude, see ref. 42 and 43).

We finally applied this technique to 20 μm water droplets containing typically 100 live bacteria (Escherichia coli). As shown in Fig. 7, the depletion factor δ is again greatly enhanced as compared to bacteria in bulk water: 60% depletion in the microdroplet and 20% in solution (λ₁ = 270 nm, λ₂ = 810 nm).

This experiment is also interesting for field applications, as bacteria and viruses are efficiently transmitted by droplets of saliva (coughing, breathing, speaking, etc.).

We propose to use the unique discrimination capability of PPD as a basis for a novel selective bioaerosol detection technique that avoids interference from background (traffic-related) organic particles in air. For instance, let us consider a mixture of N_B bacteria and N_D diesel particles. The excitation shall consist of a PPD sequence (270 nm and 810 nm pulses). The fluorescence power P emitted by the mixture shall be measured as the second laser (at 810 nm) is alternately switched on and off (denoted as P_on and P_off). Without the second laser pulse, the fluorescence cross-sections are σ_D(I₈₁₀ = 0) = σ_D and σ_B(I₈₁₀ = 0) = σ_B, while with the probe laser the cross-sections will be reduced by a factor R_B (=0.2) and R_D (=0.98) for the biological and the diesel particles, respectively. This differential procedure allows for determining the concentration of the two types of particles:

\[ N_B = \frac{R_D P_{\text{off}} - P_{\text{on}}}{I_{270} \sigma_B (R_D - R_B)} \quad \text{and} \quad N_D = \frac{R_B P_{\text{off}} - P_{\text{on}}}{I_{270} \sigma_D (R_B - R_D)} \]

The method’s performance strongly depends on the difference R_B − R_D between the two species to be discriminated, which is large in our case. Notice that in order to precisely quantify the concentration of the diesel particles, quantitative knowledge of the cross-sections is required, which might be difficult to determine because of the variety of possible organic particles.

The PPD-technique will be especially attractive for active remote sensing techniques such as Lidar, where the lack of discrimination between bioaerosols and transportation related organics is currently most acute. The implementation of the PPD-technique in the Teramobile system is therefore presently under consideration.

5. Towards a coherent identification of bacteria in air

PPD spectroscopy is an attractive technique for discriminating bioaerosols from other organics. However, it is unable to discriminate one type of biological aerosol from another (Fig. 5). A possible reason is that the averaged dynamics in the excited states of the bio-fluorophors (embedded in complex proteins) are quite similar in all.

![Fig. 7 PPD spectroscopy in 20 μm droplets containing about 100 E. coli bacteria.](image-url)
living organisms. A natural approach is therefore to extend the PPD technique to coherent control, where the amplitude and phase of every spectral component of the exciting pulses are shaped in order to best-fit the potential hypersurfaces. The method is then extremely sensitive to the details of the potential hypersurfaces, which provides unprecedented selectivity. For this, a large number of parameters (corresponding to the amplitude and phase of each spectral component within the exciting laser pulse(s)) has to be controlled. This “pulse shaping” technique is usually performed by introducing a liquid crystal array in the Fourier plane between two gratings (4f arrangement). In 1992, the concept of “optimal control” was introduced, in which a feedback loop optimizes the laser pulse characteristics to reach most efficiently the desired target. Excellent results have been obtained using coherent control schemes in atomic and molecular systems (mostly in the gas phase). 46

A major technical limitation in quantum control experiments is the relatively narrow spectral region available (often around 800 nm) and the limited available bandwidth. This is a major drawback for large molecules exhibiting broad and featureless absorption bands. We therefore intend to develop a spectrally-broad laser source with a full control of the laser parameters. This will allow for the first time to coherently populate or depopulate a broad energy domain of the electronically excited intermediate state. This is the basis of the “Optical Dynamic Discrimination” (ODD) technique. The physical processes that we intend to use to get the selectivity are extensions of the PPD-technique, namely shaped PPD, intrapulse pump–dump with detection of the fluorescence and stimulated emission, and CARS (Coherent Antistokes Raman Scattering).

In order to adapt the pulses to the very broad absorption bands of the biological fluorophors, and to the related short coherence time, spectral broadening of the laser pulses by filamentation in a gas cell may be used. This technique was recently successfully applied to generate pulses at 800 nm as short as 5 fs, by recompressing the broadened pulse with chirped mirrors. Filaments arise in the non-linear propagation of ultrashort, high-power laser pulses in transparent media. They result from a dynamic balance between Kerr-self-focusing and defocusing by a self-induced plasma. These spatio-temporal solitonic structures are capable of generating an extraordinary broad supercontinuum by Self-Phase Modulation (SPM) and Four Wave Mixing (FWM), spanning from the UV to the IR. First broadening experiments at 400 nm using filamentation have been conducted in order to obtain a broadband laser source for flavins (and NADH) excitation. Filamentation was produced in an Ar gas-filled cell (7 bars) of 100 cm length. While the incoming laser bandwidth was only 3 nm (0.5 to 1 mJ, 150 fs), it reaches 15 to 20 nm (FWHM) after filamentation, as shown in Fig. 8.

![Fig. 8](image_url)
In a later phase, the same approach will be applied in the UV, in order to coherently excite the amino acids, such as Trp.

At present, pulse shaping techniques are mainly based on Liquid Crystal Display (LCD) arrays and acousto-optic modulators, but their transmission is rather limited in the UV-Vis domain. We therefore intend to adopt as an active element a novel and versatile generation of Micro Electro Mechanical Systems (MEMS) represented by an array of movable micro mirrors. Recently, this technology has been applied for phase-only shaping of femtosecond pulses at 400 nm.\textsuperscript{49} Within this approach, shaping of the UV-Vis pulses is carried out in the experimental arrangement shown in Fig. 9, which acts as a 4-\textit{f} zero dispersion compressor, with the mirror array placed in the Fourier plane. The pulses are first dispersed by a reflection grating; successively, a cylindrical lens focuses the spectrum on the mirror array. The phase modulation is accomplished by varying the overall optical path length of the different components, acting on the pistons of the mirror elements. Note that the unique spectral range of the apparatus is represented by the maximum travel of the mirror pistons, which limits the maximum phase shift at a given wavelength $\Delta \Phi(\lambda)$.

The new device that we are currently designing should allow both phase and amplitude shaping of pulses in a wide spectral region, ranging from 250 to 900 nm. An additional degree of freedom will be added to the mirrors, in that the intensity attenuation will be performed by tilting the mirrors in the vertical plane.

These developments should allow evaluating the ultimate capabilities of ODD for discriminating bioaerosols from other organics, bacteria from pollen, and bacteria among each other.

**Conclusion**

Femtosecond spectroscopy opens new ways for the optical detection and identification of microorganisms in water and in air. Its unique capability of distinguishing molecules that exhibit almost identical absorption and fluorescence signatures is a key feature for identifying bacteria in a background of urban aerosols. The technique can also be applied for the remote detection of microorganisms in air, if a non-linear Lidar-based configuration is used, as for the Teramobile system. A more difficult task will be the distinction of one bacteria species from another, and in particular the identification of pathogen from non-pathogen bioaerosols. A possible way to reach this difficult goal might be coherent control and ODD. Significant technical improvements are currently developed, which should provide a definitive answer on the potential of coherent excitation schemes. Another option could be

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{setup.png}
\caption{Set-up for the shaping of broadband fs pulses.}
\end{figure}
provided by LIBS, as the wealth of emission lines under femtosecond excitation might allow the targeting of some biological process that is characteristic from one type of bacteria.

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