CN violet -band emission as a time-resolved optical probe of transient temperature, induced by laser ablation of type I collagen from bovine Achilles tendon

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ABSTRACT

The $B\ ^2\Sigma^+ \rightarrow X\ ^2\Sigma^+$ violet band emission spectrum of CN following laser ablation of type I collagen was exploited for the estimation of the transient temperature of the plasma plume and consequently of the sample surface considering thermodynamic equilibrium. The temperature dependence upon experimental parameters, such as laser intensity and wavelength as well as delay of gated detection was obtained. The temperature was found to increase with increasing laser intensity and decrease with increasing gated time delay and laser wavelength.

Keywords: Collagen, laser ablation, plasma emission, vibrational temperature

1. INTRODUCTION

The CN violet system ($B\ ^2\Sigma^+ \rightarrow X\ ^2\Sigma^+$) has been thoroughly studied by various techniques of molecular spectroscopy due to its importance as a free radical occurring in many emission sources even in the presence of traces of the elements involved. CN molecules were found to exist in the sun[1], stellar atmospheres[2], comets[3], dark and diffuse interstellar clouds[4,5].

In the recent years there has been a rapidly growing interest in the study of the composition and evolution of Laser Induced Plasma (LIP) from graphite in the effort towards deposition of diamond-like carbon thin films[6-9]. In this context species existing in the formed plasma, originating from recombination, such as C_2 and CN and their emission bands have served as potential plasma diagnostics. These excited diatomic species have shown to be formed by a secondary photolysis of the initial products[6]. Several attempts have also been made to investigate the spatial and temporal variations of the plasma based on the optical emission of C_2 Swan bands and the CN violet ($B\ ^2\Sigma^+ \rightarrow X\ ^2\Sigma^+$) and red ($A\ ^2\Pi \rightarrow X\ ^2\Sigma^+$) bands as observed and analyzed in different regions of the plasma plume[10-12].

Collagen is the most widespread structural protein amongst higher vertebrates and in particular collagen type I accounts for the 80% of the content of the dermis. It is thus the main means of structural support and during laser surgical ablation collagen is locally evaporated to achieve a lancet-like incision in the area of interest. The transient temperature achieved at the ablation spot is of utmost importance, as from this the equilibrium temperature can be derived depending on the temporal characteristics and the wavelength of the ablating laser irradiation.

In the current work, collagen type I from Achilles bovine tendon has been used as a tissue model for the study of CN violet bands optical emission during the ablation by nanosecond pulsed laser radiation. The vibrational temperature of the CN radical has been extracted for three laser wavelengths, namely 355, 532 and 1064nm for varying laser pulse energies and observation delay times. The results discussed in detail in the text are compatible with Local Thermodynamic Equilibrium (LTE) from a hot source (plasma) with a consistent temporal cooling pattern for varying

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laser parameters. The wavelength dependence, for various times of observation and various laser intensities, indicates higher vibrational temperatures with decreasing laser wavelength.

2. EXPERIMENTAL PROCEDURE

The ablation target was type I collagen from bovine Achilles tendon (Fluka 27662). A standard KBr pelletser (C30 Research and Industrial Instruments Company, UK) was used to exert 10-ton pressure on 100mg of collagen fibres, thus forming a uniform disc of 1cm diameter with thickness of about 0.5 mm. This target was mounted on a X-Y translation stage (NEAT) with computer-driven stepper motors. The computer program was programmed to execute a meander-like movement as a combination of 7 Π-like patterns. After each such meander sequence the stepper motor returned to its origin to execute the routine again. The purpose of this target movement was to avoid non-uniform pitting of the target surface.

The experimental set-up is shown in Fig. 1. The ablating radiation was provided by a Quantel Mo. YG581 nanosecond, Q-switched laser at 10Hz repetition rate. The laser was operated in its fundamental wavelength 1064 nm as well as in the second and third harmonics at 532 and 355 nm respectively. The laser beam was focused by a focal length FL=15 cm lens on the target surface, which was placed about 1.5 cm before the focal point of the lens. In this manner the circular beam spot on the sample had a diameter of 1.4 mm. The pulse energies used were 35 and 55 mJ at 1064 nm, 20, 35 and 55 mJ at 532nm and 20, 35 mJ at 355 nm. The optical plasma emission was collected by a 0.6 mm core quartz fibre (CVI) placed at a distance of ca 5 mm and at 45° to the normal of the ablated surface that coincided with the plasma propagation axis. In the present work, ablation of collagen took place under atmospheric conditions in contrast to the majority of the referred studies on graphite samples that were performed in vacuum. This was done to simulate the conditions during laser ablative surgery. The implication of this is that the plasma in our case was confined to a smaller area. Given the geometry of the fibre as described above, the latter was able to collect the overall optical signal of the plasma plume. The collected signal was directed and focused with use of a quartz lens into the 250 μm entrance slit of a HRP Jobin-Yvon monochromator. A 20 nm spectral window around the central wavelength (fixed throughout the experiments to 392.5nm) of the monochromator was detected by a Mo. 1420 vidicon detector of an EG&G Mo. 1460 optical multi-channel analyser (OMA) III. The detector was gated by a EG&G Mo. 1211 high voltage pulse generator. The gate delay with respect to the laser pulse and gate width, were displayed on a 2467 Tektronix oscilloscope. A fast photodiode recorded 10% of the laser signal and its output was fed into the second channel of the oscilloscope. Throughout the experiments delay times of 1, 2, 3 and 4 μs with respect to the laser pulse were selected while the gate width was kept fixed at 1μs.

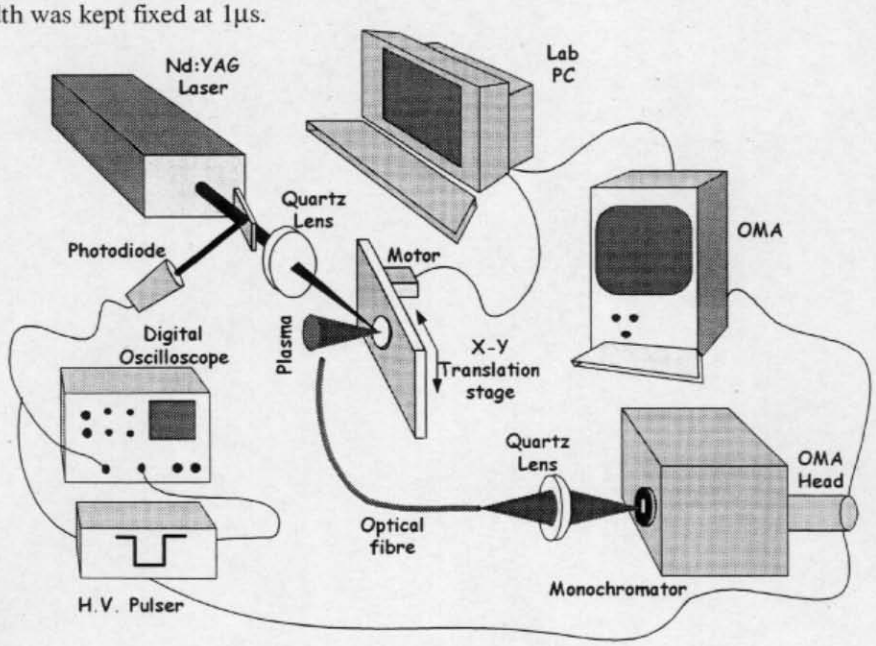

Fig. 1: Experimental set-up.

The OMA was operated in the accumulative mode: initially 150 scans were recorded with the laser beam blocked and the accumulated signal was stored in memory and served as background. Then another 150 scans were registered with the beam block removed and the background was subtracted from this signal pixel by pixel to yield the spectra for further processing. The spectra obtained in this manner were stored in a PC to which the OMA was interfaced.

3. THEORETICAL BACKGROUND

At moderate resolution electronic transitions of diatomic molecules exhibit band structures that are attributed to numerous vibrational levels. Molecules which are in a certain vibrational level, v', of their excited electronic state, can decay to various vibrational levels of the lower electronic state, v'', each of these transitions rendering an experimentally measured intensity $I_{v'v''}$. When the transition probabilities are known, the intensity ratio of two vibronic transitions can yield the vibrational temperature, assuming a Boltzmann distribution as follows:

$$\frac{I_{v'v''}}{I_{w'w''}} = \frac{A_{v'v''}}{A_{w'w''}} \cdot \exp\left(-\frac{G(v') - G(w')}{k_B \cdot T_{vib}}\right) \Leftrightarrow T_{vib} = \frac{G(w') - G(v')}{k_B \cdot \ln \frac{I_{v'v''} \cdot A_{w'w''}}{I_{w'w''} \cdot A_{v'v''}}} \quad (1)$$

In the above equation $G(v')$ and $G(w')$ are the term values of the upper vibrational levels v' and w' respectively, calculated from ref. 13, k_B is the Boltzmann constant and $A_{v'v''}$ and $A_{w'w''}$ are the Einstein spontaneous emission coefficients. T_{vib} is the vibrational temperature of the diatomic molecule. In principle, in this equation, the integrated intensity over all the rotational substructure of the band should be used. However since in our experiments the rotational structure was not resolved, the band peak intensity was used instead. A correlation factor, or ratio of the integrated band intensity over the band peak intensity, $F_{corr}(v'v'')$, is defined in ref. 14. There it is also shown that for a band system for which the wavelength does not spread too far and for large instrumental broadening – as in our case – this correlation factor can be treated as a constant. Furthermore, any error deriving from this approximation should be minimised, since it would appear in the form of the ratio $F_{corr}(v'v'')/F_{corr}(w'w'')$, which is very close to unity.

4. RESULTS AND DISCUSSION.

A characteristic spectrum of the CN violet ($B\,^2\Sigma^+ \rightarrow X\,^2\Sigma^+$) as registered according to the previously described experimental set-up for 55 mJ laser pulse energy at 1064 nm appears in Fig. 2.

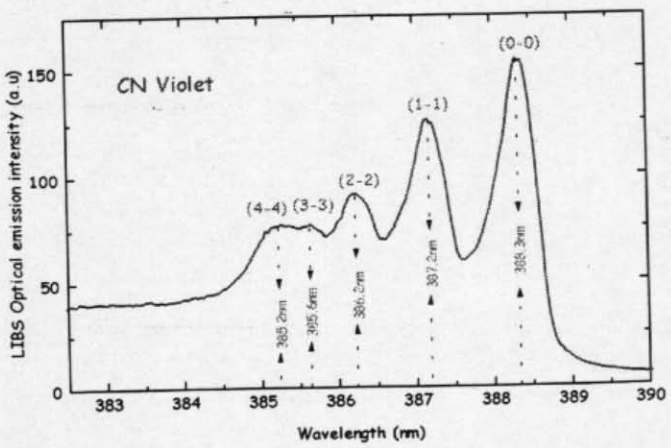

Fig. 2.: A characteristic CN violet band spectrum, as registered at 55mJ/pulse, 1064 nm laser irradiation.

The largest peak at 388.3 nm corresponds to the 0→0 vibronic transition of CN, the peak centered at 387.2nm corresponds to the 1→1 vibrational transition and the peaks at 386.2 and 385.6nm correspond to the 2→2 and 3→3 transitions respectively. From the intensity ratios of these peaks and their spontaneous emission Einstein coefficients as taken from ref. 15, one can derive the excited state vibrational temperature assuming Boltzmann statistics according to eq. (1). The vibronic temperatures were extracted from the intensity ratios I_{00}/I_{11}, I_{00}/I_{22} and I_{00}/I_{33}. The error was calculated as the standard deviation of the mean value obtained by the above three intensity ratios. This was found comparable to the value that would be derived by taking 10% error in the emission intensities and 5% uncertainty in the Einstein coefficients as given in ref 15. The calculated vibronic temperatures for the various experimental parameters selected, are listed in Table 1 below.

	Vibrational Temperature of CN (°K)						
Del ay	**355nm**		**532nm**			**1064nm**	
	20mJ/pulse	*35mJ/pulse*	*20mJ/pulse*	*35mJ/pulse*	*55mJ/pulse*	*35mJ/pulse*	*55mJ/pulse*
1μs	27600±3000	38400±6950	29000±1750	32400±2050	-	25700±2500	38200±2900
2μs	18500±2100	25000±2650	19900±1800	21100±800	32400±6700	17200±1000	24700±1600
3μs	17200±1950	22000±2600	17000±2000	18100±1050	25900±2200	13900±1600	21300±1000
4μs	14500±2687	17700±2000	14000±1100	14900±1200	21200±1900	12700±1000	19400±1000

Table 1: Calculated vibrational temperatures of CN, for various experimental parameters.

In Fig. 3 the plot of the dependence of the calculated vibronic temperatures upon the laser pulse energies is presented for sample ablation by 532 nm laser radiation. The temperature values in this graph are normalised to the higher value, always corresponding to the pulse energy of 55 mJ and the data sets for different gating delays, namely 2, 3 and 4 μs are denoted by different symbols. The increase of the vibrational temperature of the CN molecules with laser irradiance shows that comparatively larger numbers of molecules are excited into higher vibrational levels with increasing laser irradiance.

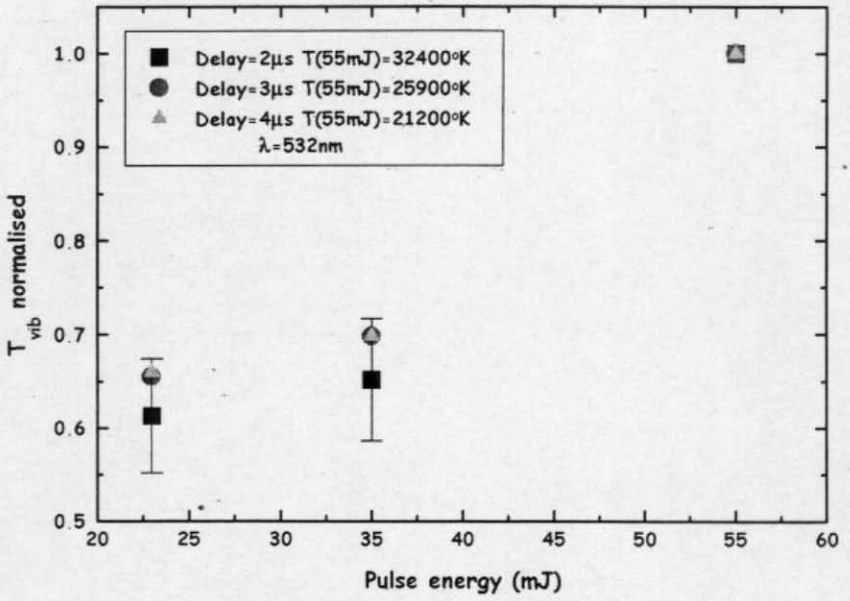

Fig. 3: Vibrational temperature dependence upon laser (λ=532 nm) pulse energy. The temperature values are normalised to the corresponding value for E=55 mJ/pulse.

Within experimental errors, the data from the three different delay times seem to be consistent, following the same pattern and the temperature value corresponding to 35 mJ pulse energy seems to be 65-70% that corresponding to 55mJ pulse energy. Accordingly the temperature value corresponding to the 20mJ pulse energy seems to be 61-65% the value of that at 55mJ/pulse. It is evident from the figure that there is a knee in the corespondent curve after the value of 35 mJ/pulse and this nonlinear effect is consistent at various delay times. This effect indicates the onset of threshold non-linear phenomena such as self-focusing. The self-focusing of laser beams propagating in plasma occurs when the Debye length λ_D, which is the characteristic screening length of the plasma, is less than the laser diameter. More specifically when:

$$D_L > \lambda_D \Leftrightarrow D_L > \left[\frac{k_B \cdot T \cdot \varepsilon_0}{N \cdot e^2}\right]^{1/2} \quad (2)$$

In the above equation, k_B is the Boltzmann constant, T_e is the average electronic plasma temperature, N_e is the equillibrium concentration, ε_0 is the permeativity, e is the electron charge and D_L is the laser beam waist. Such self-focusing in the plasma increases the effective laser power density leading to enhanced optical emission.

The appearrance of the CN vibrational bands the in plasma plume is due to the recombination of ionic, molecular and atomic carbon with nitrogen molecules from the surrounding atmosphere. There have been numerous studies of the dependence of the formation of the CN radical upon the N_2 content of the ambient atmosphere[6,10,12] and in particular ref. [10] claims that the CN is formed by gas surface reactions between the dissociated nitrogen and particles ejected from the target. These CN complex radicals are born with a population distribution in high vibrational levels that corresponds to the ambient temperature of the surrounding plasma (assuming local thermodynamic equilibrium). This is supported by the almost identical dependence of the CN vibrational temperature upon the delay time of observation. Figure 4 denotes this dependence for all the wavelengths used in the present study, each wavelength data set appearing with a different symbol. In this graph the temperature values are normalised to the maximum value at the delay of 1μs and the temperature values were in all cases calculated for the pulse energy of 35 mJ. A similar result was obtained however for pulse energy values of 20 and 55 mJ as can be deduced from the data of table 1.

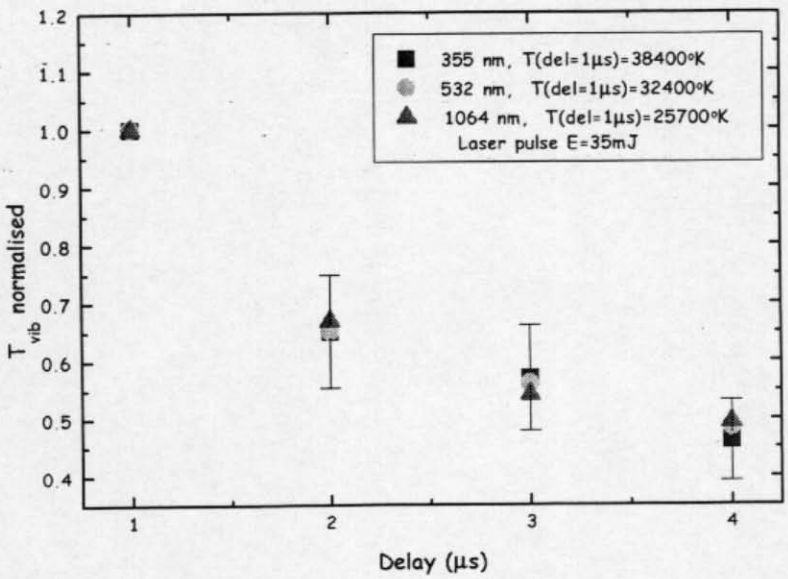

Fig. 4: Dependence of vibrational temperature upon gating delay. The values are normalized with respect to the value corresponding to the delay time of 1 μs.

The decay pattern appearing in Fig. 4 is consistent with energy (heat) dissipation to the surrounding atmospheric environment from a hot source in thermal equilibrium. The normalised values for the delay of 2μs seem to be 65-66% of the corresponding values at 1μs delay. The values of temperature at the delays of 3 and 4 μs were found to be 54-57% and 46-49% of the values at 1μs delay respectively. The consistence of this dissipation rate for various experimental parameters supports the existence of Local Thermodynamic Equilibrium (LTE): the CN vibrational temperatures calculated reflect the plasma temperature within each observation window.

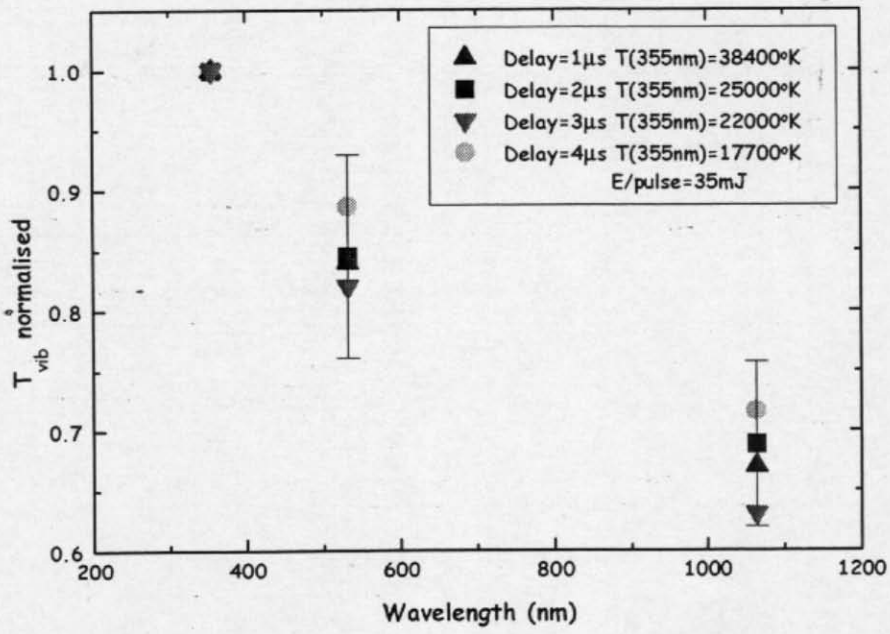

Fig. 5: Dependence of vibrational temperature upon laser wavelength. The temperature values are normalized with respect to the ones for λ=355 nm.

Another striking result of the present study was the vibronic temperature dependence upon the laser wavelength for fixed laser intensity. In Fig. 5 this dependence is shown for the pulse energy of 35 mJ/pulse. In this graph, different gate delay values appear as different symbols and the temperatures are each time normalised to their highest value always measured at the 355 nm wavelength.

This dependence of temperature upon the ablation wavelength at a fixed laser fluence indicates that the temperature rises with decreasing laser wavelength and the normalised temperature values corresponding to 532 and 1064nm laser wavelengths were found to be 88-84% and 71-67% of the values at 355 nm wavelength for various parameters. This result was reproducible and consistent for all delay times of observation and indicates a thermal ablation mechanism, supported by Burns *et al*[16] and the arguments therein. In this context the higher the photon energy, the higher the vibrational level in which the CN radicals are formed, resulting of course in higher plasma vibrational temperature.

The present study offers a new diagnostic tool to laser ablative surgery with respect to the transient temperature induced to the ablated tissue. It has been shown here, that LTE conditions are valid since for different gate delays we observed a hot source thermal dissipation pattern unchanged for varying parameters such as laser intensity and wavelength. From this fact and the fact that thermal equilibrium exists between the plasma and the sample, since they are in direct contact, the transient temperature could potentially yield the average temperature induced to the ablated sample. Further experimental work is required to promote this novel diagnostic method that seems to have the advantage of utilizing the ablating laser beam itself to probe the transient character of the average electronic temperature induced.

One additional outcome of the current work considered to be important towards the clarification of the mechanisms involved in laser ablation, is that UV photons seem to induce bigger photothermal effects in the ablation plasma than visible or infrared photons. This does not rule out the possibility of photochemical interaction in the UV region as well.

However it corroborates the thermal interaction as a common mechanism in all optical spectral regions during ablation, with the more energetic photons raising the plasma temperature to higher values.

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