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**TITLE: BIOMEDICAL APPLICATIONS ON LASER TECHNOLOGY AT
LOS ALAMOS**

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Biomedical applications of laser technology at Los Alamos

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ABSTRACT

Los Alamos has a long history of research in laser technology, and over the past few years we have begun to build a program directing some of that technology to a variety of applications in biology and medicine. As examples, two of these activities will be described in this manuscript: 1) the use of a laser interferometer to measure the microwave absorption spectrum of DNA, and 2) spectral measurements of the secondary light produced during laser corneal ablation.

1. MEASUREMENTS OF THE LOW-FREQUENCY ABSORPTION SPECTRUM OF DNA

In a Physical Review Letter published in 1984¹, and in following work², Edwards, Davis, Saffer, and Swicord (EDSS) reported the observation of narrowband features in the 1-to-10 GHz microwave absorption spectrum of plasmid DNA in aqueous solution, leading the authors to infer the existence of vibrational modes of DNA with astonishingly longer relaxation times (~300 ps) than would otherwise be expected for a large molecule in intimate contact with a room temperature solvent. Theoretical workers have since suggested, on the one hand, that a nonlinear wave equation with stable soliton-like solutions might describe the acoustic excitations of the hydrogen-bonded DNA chain³, or on the other hand, that elastic coupling rather than the expected viscous coupling of the molecule to the surrounding solvent leads to an enhancement of the lifetimes of the molecule's conventional linear normal modes⁴.

The experimental technique employed by EDSS (a semi-automated network analyzer) is not inherently well-suited for measurements in samples of large dielectric constant and requires extreme care to avoid instrumental artifacts. Additional experimental work carefully performed by two independent groups using essentially similar instrumentation, however, failed to reproduce the EDSS observations.^{5,6}

Despite these negative results, the far-reaching implications for both physics and biology that the EDSS observations may hold have motivated us to conduct our own search for resonant dielectric absorption in dilute solutions of supercoiled circular DNA (pUC8c.2). In order to offer as definitive an experiment as possible, however, we have chosen to use an entirely different spectroscopic technique, and have developed our own microwave spectrometer based on laser interferometry. This new technique is especially suited to measurements of liquid samples with large dielectric constants. In the experiments of EDSS and others, purely dielectrometric measurements of the real and imaginary parts of the dielectric function are used to indirectly determine the absorption coefficient of the sample. This approach requires great attention to the details of the sample geometry and data reduction in order to avoid systematic artifacts. The spectrometer we have constructed uses a laser interferometer as a photothermal detector to *directly* measure the Beer's Law decay, along an open parallel-conductor transmission line, of the microwave power deposited in an absorbing sample. The accuracy of our system, which is described in detail in Ref. [7], is easily verified by comparison with well-established literature values for the dielectric constant of pure water. To press our

investigations further, we have not only taken data in a frequency range that overlaps the range where absorption features were observed by EDSS, but have also investigated the previously unexplored (by any authors) 10-20 GHz range, where EDSS expected the observation of additional harmonics of lower-frequency modes.

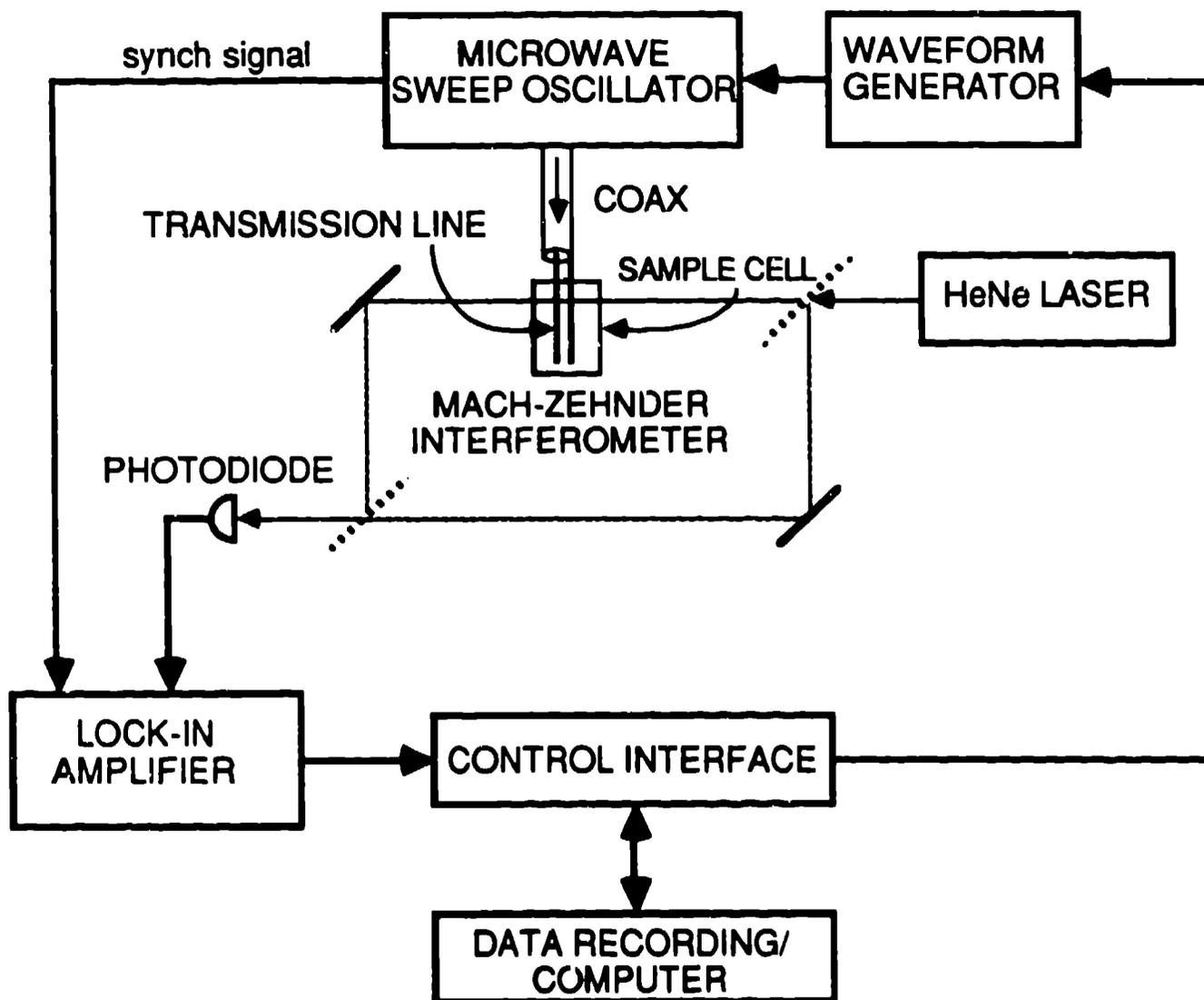


Figure 1. Schematic diagram of the laser-interferometer microwave spectrometer.

Our laser interferometer system is illustrated in Fig. 1. This system is an example of a "photothermal detector" capable of zero-background detection of weak absorbers, or conversely of weakly-illuminated strong absorbers (our case: less than 100 mW of peak microwave power reaches the sample, and the net temperature increase of the sample is less than 0.1 K). This spectroscopic technique stands in contrast to the conventional time-domain reflectometer and network analyzer techniques - not zero-background methods - that require the measurement of small changes in large signals. Another advantage of the spectrometer is that it can easily measure absorption coefficients in media with large ϵ' and ϵ'' (such as water) which generally presents difficulties for the traditional methods. It should also be noted that since a transmission line is used, the spectrometer is intrinsically capable of broadband measurements.

For these measurements the same plasmid DNA was used as in Refs. [1,2,5,6] and was produced in precisely the same manner. Plasmid pUC8c2 DNA (5480 bp), which is essentially a dimer of pUC8 (2740 bp), was prepared from a culture of host *E. coli*.

Figure 2 shows the absorption spectrum, from 5 to 20 GHz, of the buffered solution of DNA, plotted in terms of the percentage change in absorption, $\Delta\alpha/\alpha$, due to the addition of DNA to the buffer solution. [$\Delta\alpha = \alpha(\text{buffer} + \text{DNA}) - \alpha(\text{buffer})$, and in the denominator $\alpha = \alpha(\text{buffer})$]. In these measurements the concentration of DNA was 0.7 mg/ml, the same concentration as that used in Refs. [1,2]. It is clear, at this concentration, that there is no significant difference in excess of the 1-2% statistical fluctuations. The original data of EDSS is represented by the dashed line.

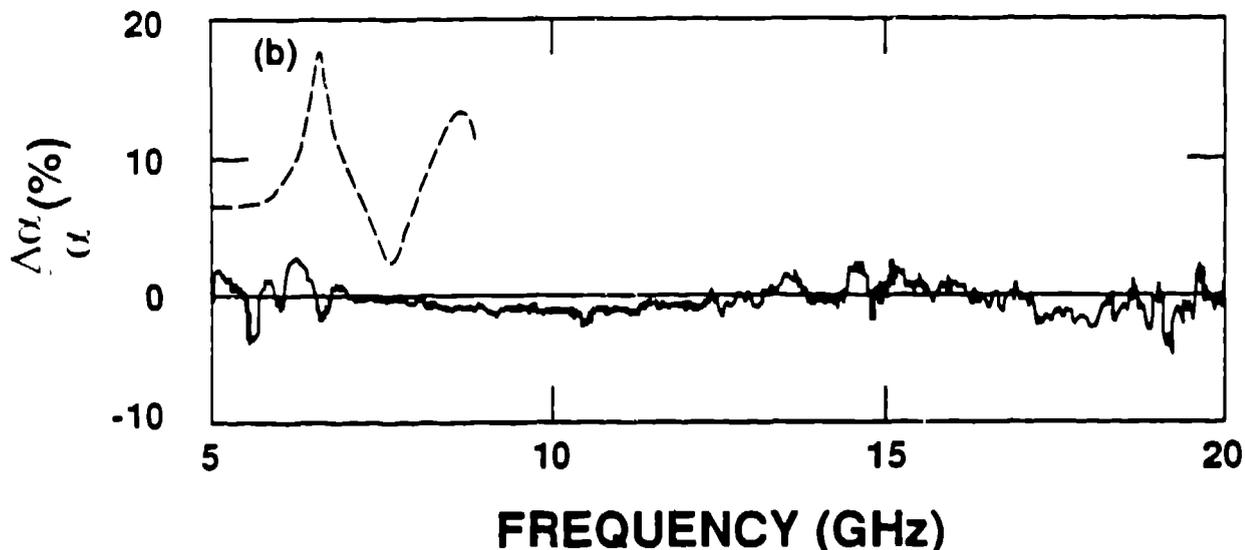


Figure 2. The absolute absorption spectrum of DNA. The dashed line represents the EDSS data for comparison

However, one interesting consequence of these studies has been the realization that while low-level electromagnetic radiation may not significantly change the bulk temperature of a solution of biological molecules, it does not mean that any physiological effects must be the consequence of "non-thermal" reactions. Given the large differences in salt concentration and/or pH that can exist between the intra-cellular and extra-cellular regions, or between different domains within the cell, it is quite reasonable to expect that there may be significant differences in the absorption coefficients and hence large temporary differences in temperature, even though that is not evidenced by measurement of the bulk temperature of the bath. Such local differences in temperature certainly can have physiological/biological consequences.

2. MEASUREMENTS OF THE SECONDARY LIGHT DURING ArF LASER CORNEAL ABLATION

Photochemical ablation of the cornea with 193-nm ArF excimer photons is being used successfully in human clinical trials to change the shape of the cornea for the correction of vision defects. This

fluorescence, etc.) that accompany tissue ablation. In this investigation we have measured the spectrum and energy density of the secondary emission to determine if this light is a threat to the safety of the corneal resculpting procedure.

We exposed enucleated human eyes to ArF laser beams of uniform energy density in the 25-1000 mJ/cm² range, which is from below the ablation threshold to well above operational resculpting values. Spectra were collected with both fiber and imaging systems, dispersed by a 1/3-m spectrometer, and detected and recorded by an intensified diode array. The spectra were corrected for the wavelength responses of the complete detection systems.

The time behavior of the secondary emission was obtained by replacing the spectrometer with a photodiode detector. Adding band-pass filters before the photodiode gave the time response in various spectral bands, which proved to be of interest. The energy density of the secondary light on the cornea was derived from the photodiode signal amplitudes by comparison to the signals generated by measured energy densities of 308-nm (XeCl laser) light. This known beam was scattered from a ground quartz plate in the plane of the cornea. The 308-nm laser line was conveniently close to the peak of the secondary light spectrum.

While the emission appeared faintly sodium-orange to the observer, the spectrum was actually like that of Figure 3, with a very strong ultraviolet peak tailing into the visible. In the corrected spectrum of the figure, the sodium lines are barely discernible. The peak wavelength moved around slightly with the age (time since enucleation) of the eye, but was always close to 310 nm.

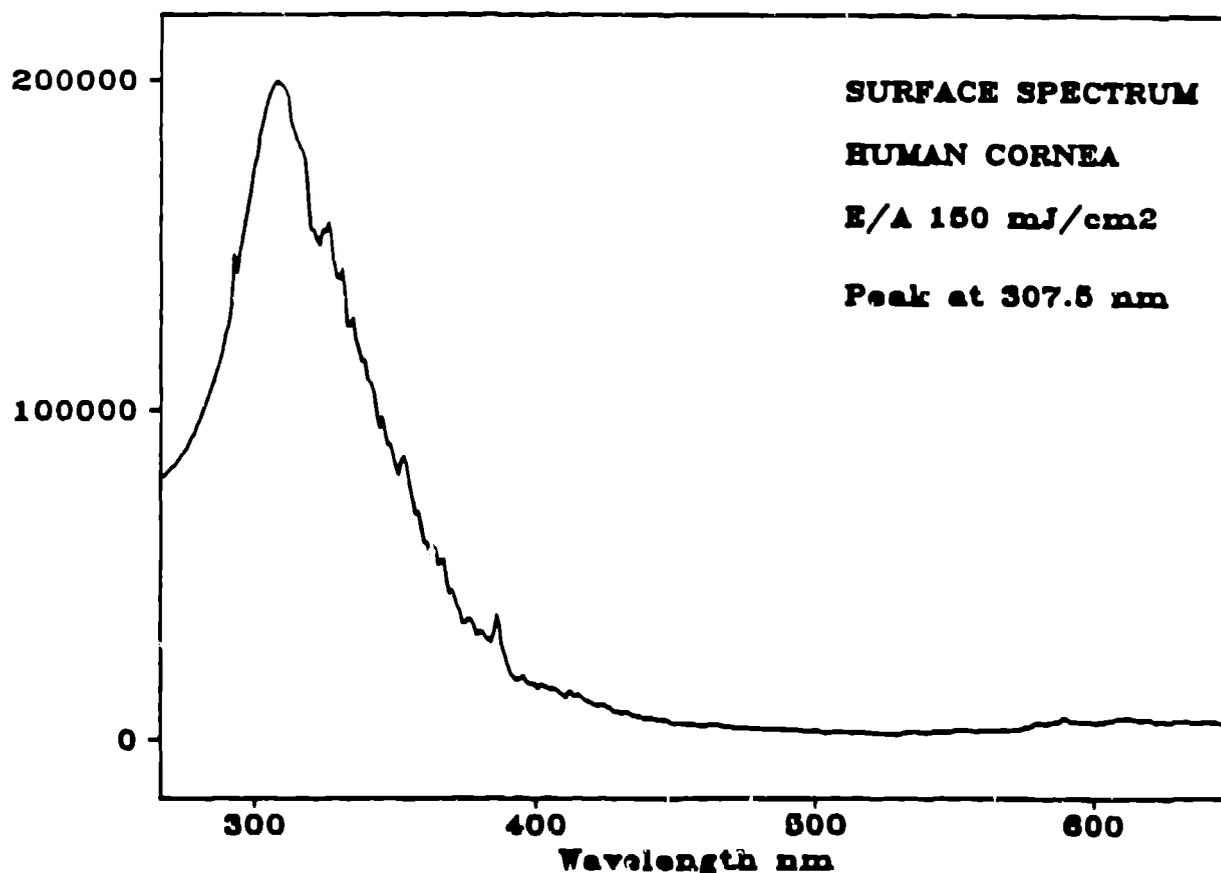


Fig. 3 Normalized spectrum of the secondary light during ArF laser ablation of human corneal tissue. The short-wavelength limit of the data was due to detector limitations.

There was also an eye age factor observed in the time behavior of the secondary light. The older eyes had a strong correlation between ablative energy densities and a sharp 2-3 ns leading edge spike at a wavelength shorter than 320 nm. In fresher eyes (1-2 days) this correlation between ablation and uv spike was less clear. The integrated energy density of the secondary emission on the surface of the cornea also varied with age, from about $10 \mu\text{J}/\text{cm}^2$ in older eyes to a maximum of $50 \mu\text{J}/\text{cm}^2$ in the fresh eyes.

The strong ultraviolet peak, which lies just where the cornea begins to transmit the ultraviolet, would appear to be most threatening to the lens. Two factors combine to make it the cornea that is at risk. The first is geometry; the secondary emission is basically isotropic, so the energy density that reaches the lens is greatly reduced from the value on the cornea. The second factor is that the corneal damage threshold at these wavelengths⁸ is lower than that for the lens (based on data for rabbits). To take an extreme example, if the procedure took 1000 shots the total radiant exposure would be $0.05 \text{ J}/\text{cm}^2$, which is comparable to the measured damage threshold for the human cornea at 310 nm. A more typical 100-shot procedure would then have considerable safety margin, about a factor of 10 for the cornea and more than 100 for the lens (without considering the geometrical factors that reduce the radiant exposure). Note in Reference [8] that the damage threshold is an exponential function of the wavelength, being a factor of 10 higher at 320 than at 310 nm.

These preliminary data do not challenge the safety of the current procedures, but do suggest that this matter is worthy of further investigation. We suggest that an authoritative answer can only be obtained from measurements taken during a clinical procedure to a living eye.

REFERENCES

1. G.S. Edwards, C.C. Davis, J.D. Saffer, and M.L. Swicord, "Resonant microwave-absorption of selected DNA molecules", *Phys. Rev. Lett.* **53**, 1284-1287(1984).
2. G.S. Edwards, C.C. Davis, J.D. Saffer, and M.L. Swicord. Microwave-field-driven acoustic modes in DNA. *Biophys. J.* **47**,799-807(1985).
3. A.C. Scott, "Anharmonic analysis of resonant microwave absorption in DNA", *Physica Scripta* **32**, pp. 617-623 (1985).
4. L.L. Van Zandt, "Resonant microwave absorption by dissolved DNA", *Phys. Rev. Lett.* **57** (16), pp. 2085-2087 (1986).
5. C. Gabriel, E. H. Grant, R. Tata, P. R. Brown, B. Gestblom and E. Noreland, "Microwave absorption in aqueous solutions of DNA", *Nature* **328**, 145(1987).
6. K. R. Foster, B. R. Epstein and M. A. Gealt, "Resonances in the dielectric absorption of DNA?", *Biophys. Jour.* **52**, 421(1987).
7. Pritish Mukherjee, Timothy R. Gosnell, and Irving J. Bigio, "Broadband microwave absorption spectrometer for liquid media", *Rev. Sci. Instrum.* **59** (12), pp. 2577-2582 (1988).
8. D.G. Pitts, A. Cullen, P. Hacker, and W.H. Parr; "Ocular Ultraviolet Effects from 295 nm to 400 nm in the Rabbit Eye", DHEW (NIOSH) Publication #77-175, October, 1977.