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CONTROLLED UNWINDING OF THE DOUBLE HELIX(U) ARIZONA
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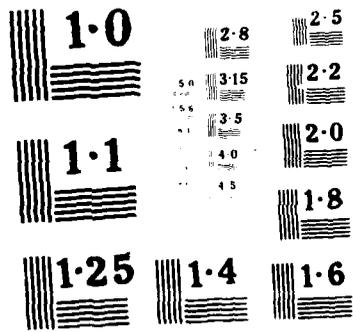
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Abstract.

We are assembling experiments₁ designed to pump vibrational modes of DNA in the 80 cm⁻¹ frequency region. Modes of this frequency stretch the bridging hydrogen bonds. We will pump these modes both with Stimulated Raman Scattering (SRS) and directly with radiation from a free electron laser (FEL). We will monitor ultraviolet absorption, as the DNA is pumped, in order to see if unwinding occurs.

Initial Raman studies indicate that interhelical contacts may influence the 'melting' modes of DNA.

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Controlled Unwinding of the Double Helix.

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Abstract.

We are assembling experiments designed to pump vibrational modes of DNA in the 80 cm^{-1} frequency region. Modes of this frequency stretch the bridging hydrogen bonds. We will pump these modes both with Stimulated Raman Scattering (SRS) and directly with radiation from a free electron laser (FEL). We will monitor ultraviolet absorption, as the DNA is pumped, in order to see if unwinding occurs.

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1. Introduction.

This annual report covers the first year of a study of the non-equilibrium vibrations of DNA in the $10\text{-}100\text{ cm}^{-1}$ region. It overlaps the last year of a study of the vibrational modes of DNA at gigahertz frequencies. That work has been described in a terminal report¹ which will be circulated with this report. Our study of the lower frequency motion outlined mechanisms of DNA-microwave interactions¹. However, in that report, we also pointed out that excitation at GHz. frequencies is unlikely to affect interactions of the DNA. This is because the microwave absorption cross section and phonon lifetimes are such as to result in just one non-equilibrium quantum of vibrational energy being pumped in typical experiments. At gigahertz frequencies, thermal energy will populate several thousand quanta.

At tens of wavenumbers the situation may be very different. The thermal population is just a few quanta. If even the smallest increase in the population of the modes that stretch the bridging hydrogen bonds can be achieved, calculations predict that the double helical structure may be destabilized². We do not know absorption cross sections or phonon lifetimes in this frequency region - it is one of the objects of this project to determine these parameters - however initial measurements of the far IR absorption, the Raman cross section and Raman spectra offer some hope that a significant non-equilibrium population can be pumped. This possibility is quite intriguing - not only because it may offer some insight into the mechanism of enzymes that unwind the

double helix, but also because calculations indicate that different phonon frequencies are associated with, for example, major and minor groove melting near a defect³, so that the direction of unwinding might be controlled by the frequency at which the double helix is pumped.

2. Background.

Prohofsky's calculations⁴ show that almost all the modes which contribute significantly to stretching the bridging hydrogen bonds are clustered around the 80-90 cm^{-1} region, exactly where a quite pronounced band is seen in the Raman spectrum⁵. This band disappears as the DNA is melted, so was indeed assigned to stretching modes of the bridging hydrogen bonds⁵. Very recent far IR studies show a pronounced infrared absorption band at about 85 cm^{-1} in poly (dA). poly (dT) films⁶. Since the band assigned to hydrogen bond breathing modes appears to be both IR and Raman active, we proposed two methods of pumping 85 cm^{-1} phonons. In the first, the Raman activity of the modes is exploited in an experiment in which two coherent dye laser beams are incident on the sample simultaneously, one at a frequency ω_1 , the other at a frequency ω_2 such that

$$\Omega = \omega_1 - \omega_2$$

where Ω is the frequency of the mode being pumped. The presence of a non-equilibrium population of coherent phonons is probed by Raman scattering from a third beam (phase matched to the stimulated phonons) in a technique known as Coherent Anti-Stokes Raman Scattering (CARS). By using picosecond laser pulses, and probing the CARS signal at various delay times after pumping the non-equilibrium phonons, the lifetime (both coherence and population) may be measured.

In the second approach, we will exploit the infrared activity of the molecule by pumping directly in the far IR. A tuneable source of significant power has just become available with the operation of the FEL at the University of California, Santa Barbara, and we are collaborating with the center for free electron laser studies in setting up this second experiment.

3. Progress in the stimulated Raman scattering experiments.

In collaboration with my colleague, K.T. Tsen, we have set up a dual pump beam system for CARS. Two dye lasers are used to give us complete control of the pumping frequency. We propose to sweep through the region of interest, looking for anomalies in the CARS signal as we pass through 80 cm^{-1} . Normally the CARS signal increases as the cube of the beam power. However, destabilization of the structure should produce a marked deviation from this behavior.

The low frequency of the phonons we are pumping has required a rather novel beam geometry for us to achieve the required phase matching. A schematic layout of our 'counter propagating beams' geometry is shown in Figure 1.

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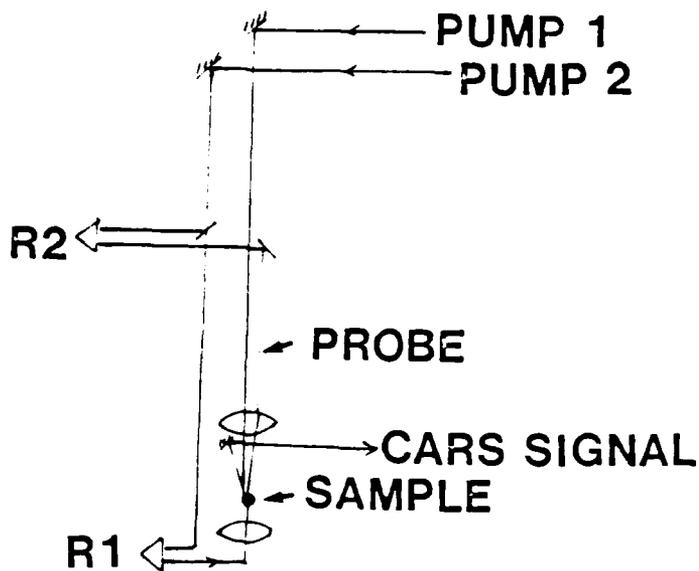


Figure 1. Geometry of the proposed CARS experiment. The retroreflector, R1, introduces a variable delay to allow the two pump beams to be made coincident in time. The second reflector (R2) delays the probe. In this geometry, the angle between the pump beams and the 85 cm^{-1} CARS signal is about 6° .

This geometry requires a large Raman cross section for a significant CARS signal (the interaction volume is small), and the ' 85 cm^{-1} ' band does indeed appear to have an anomalously large Raman cross section. We have been studying the normal thermal Raman scattering in this region in order to estimate cross sections, before embarking on the non-equilibrium studies. With a mixture of alarm and interest, we have found some rather unexpected behavior:

- The Raman signal does not fall linearly with dilution (though it does disappear at small enough concentrations!)
- The shape of the Raman band appears to change in going from a concentrated gel to a solution. In solution, the ' 85 cm^{-1} ' band appears to peak at about 65 cm^{-1} .
- The band is much more pronounced and somewhat sharper in solution.

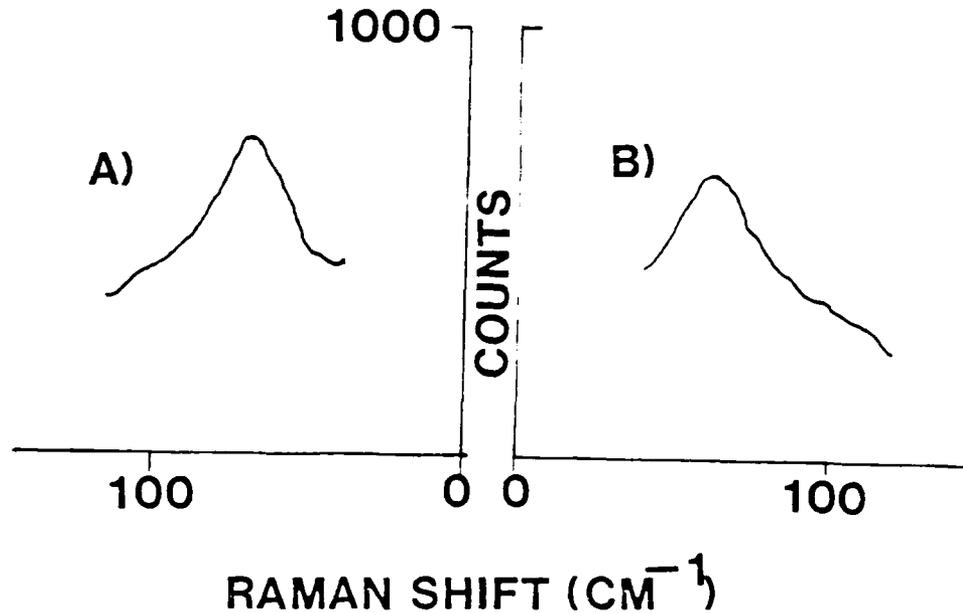


Figure 2. Low frequency Raman spectra (a) antistokes and (b) stokes for a 1 mg/ml solution of CT DNA ($A_{260}/A_{280} > 1.8$). The peak shift is about 66 cm^{-1} . Note that these are raw spectra (no background subtraction).

Quite unexpectedly, we can easily see the band at concentrations of 1 mg/ml. This is a part in a thousand concentration, and suggests an anomalous Raman cross section. Other measurements seem to indicate molecular weight effects. Most strangely, we see the band in H-V polarization, but not in H-H. This (for a solution) is a violation of the so called Placzek invariance. We have no explanation for this. The features certainly do not appear to be artifacts.

A typical run with a 1 mg/ml sample of calf thymus DNA is shown in Figure 2. We went to some lengths to ensure no protein contamination of the sample. These bands are not seen in the buffer. It will be important to understand the solid state effects that cause these changes between solutions and gels, because calculations based on the measured Raman cross section (approximately $2 \cdot 10^{-30} \text{ cm}^2/\text{sr}$) indicate that we will have to use fairly concentrated samples to achieve significant pumping.

4. Progress in the free electron laser experiments.

The FEL will be used to deliver a pulse of a few μs duration every 3 seconds. Over the range of interest ($10\text{-}120 \text{ cm}^{-1}$) the

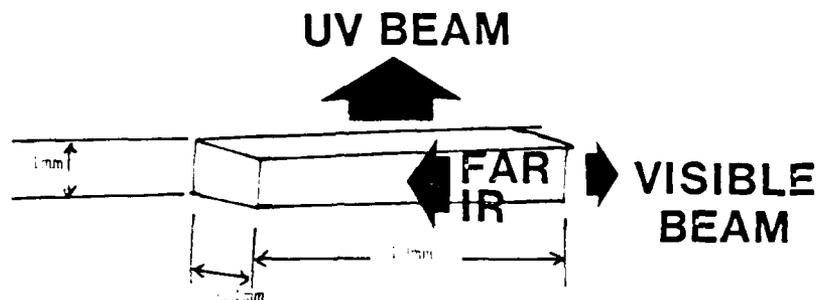


Figure 3. Schematic layout of the FEL absorption cell showing the path of the far IR pump beam (1), the UV probe of melting (2) and the visible probe of temperature (3).

absorption length in water varies from $\sim 1\text{mm}$ to $120\mu\text{m}$. Thus the sample cell has to be designed in such a way as to allow probing of a thin layer of solution near the irradiated surface. Furthermore, at full laser power output, it may be possible to heat the sample by as much as 40° , enough to cause thermal melting. For this reason, a probe of temperature is needed. The apparatus we are constructing consists of the following parts:

- A far IR focussing system and cell (flushed with He gas to minimize absorption and give thermal contact to the cell face). The IR is incident as a line image about 1mm high and 1cm long on the quartz cell window.

- A UV probe of melting. The hyperchromism will be measured at 260nm in the usual manner. The only unusual aspect is the beam optics - a cylindrical lens is used to focus a line image of the UV beam within $100\mu\text{m}$ of the cell window (and traveling perpendicular to the IR beam). Since only about 1mm thickness of sample will be traversed in this geometry (with an area $100\mu\text{m}$ by 1cm), concentrations as high as 1mg/ml can be used. Our measurements show that we can very easily detect as little as 0.1% melting (i.e. one base-pair in a thousand open). The UV signal will be recorded on a digital oscilloscope as the far IR pulse arrives, and subsequently transferred to a small computer.

- A visible probe of temperature in the sample volume. A visible beam will be focussed into a narrow pencil (approximately 1 cm long, about $60\mu\text{m}$ at the waist, and $100\mu\text{m}$ at each end) and passed through the sample as one arm of a Mach-Zender interferometer. Temperature changes will be measured by counting fringe shifts, for the refractive index of water changes by more than a part in 10^4 per $^\circ\text{K}$. The sample path is much more than 10^4 wavelengths, so small fractions of a degree of heating will be detected. The output of the interferometer will go via a fringe counting system into another channel of the digital oscilloscope to be recorded for later transfer to the computer.

We illustrate this confusing array of optics with the very schematic Figure 3 which shows the path of the various probe beams through the cell. This apparatus is under construction at present with a target date of October for the first trials on the FEL.

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