

Optical characteristics of biological molecules in the Terahertz gap

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ABSTRACT

Terahertz Spectroscopy has been recently introduced as a promising technique for the collection of signature data in transmission spectra of biological materials including warfare agent simulants. To characterize material rather than sample, it is always desirable to obtain the material's optical properties as functions of frequency. In this work, we present results from parallel measurements of reflection and transmission spectra of biological molecules to enable detailed and direct calculation of refractive index and absorption coefficient spectra in the terahertz gap. DNA samples from herring and salmon as well as samples of Ovalbumin and Bacillus Subtillus spores have been characterized. The technique for simulation is described. Reflection spectra reveal resonance features similar to those demonstrated earlier for transmission, thereby affirming molecular vibrational modes in biological materials. The dispersion of refractive index and absorption coefficient is demonstrated within the Terahertz gap of 10 cm^{-1} to 25 cm^{-1} .

Keywords: Terahertz, spectroscopy, transmission, reflection, refractive index, absorption coefficient, dispersion, bio-materials

I. INTRODUCTION

Terahertz (THz) spectroscopy has been recently introduced as a promising technique for collection of signature data in transmission spectra of biological materials including warfare agent simulants^{1,2}. Although there are new THz techniques becoming available, including advent of THz time domain spectroscopy (TDS), a basic result obtained for DNA and other biological materials from pulsed TDS is increase of broadband absorption with frequency³. Fourier Transform IR (FTIR) spectroscopy is still one of the most fruitful techniques that provides information on resonant features in transmission spectra at this lowest frequency end of the very far IR called the THz-gap (frequencies between 0.1 and 3 THz). These features originate from interaction of THz radiation with vibrational modes of biological macromolecules.

The absolute level of transmission as well as the form of spectrum depends strongly on many details of sample preparation technique including amount of material, solvent, sample thickness and others. As shown in Figure 1, results of transmission measurements over broad range in frequency are very sensitive to the thickness of the film, d , due to multiple reflection at the film boundaries. For the determination of characteristic material properties versus those specific to contain biological samples, it is always desirable to obtain the material's optical characteristics in terms of the complex dielectric constant $\varepsilon(\omega)$, or its real and imaginary components (refractive index and absorption coefficient), as a function of frequency ω . This information can be very useful for many practical applications of THz spectroscopy, in particular for remote sensing of warfare and environmental agents. There is however very scarce information available on these optical characteristics of biological materials in the THz gap (see, for example the topical reviews^{4,5}), and in many cases absorption is presented only in the arbitrary units^{3,6-9}.

We have earlier reported¹⁰ sub-millimeter wave absorption spectra of DNA and several artificial RNA polymers in the spectral range between 10 cm^{-1} and 25 cm^{-1} . Measurements of optical characteristics in this spectral range where

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absorption is relatively weak requires samples with thickness in the range 1-200 μm , and the multiple reflection occurs at sample boundaries causing the interference pattern in both measured characteristics, transmission (T) and reflection (R). Each of these characteristics depends on absorption coefficient and refractive index that are varying with frequencies. In our work¹¹ the interference spectroscopy technique (IST) was used to extract the absorption coefficient spectra from transmission measured on films, after appropriately accounting for multiple reflection on the sample boundaries. However, although the procedure for accurate analysis requires the knowledge of exact refractive index spectra, in prior work its values were only roughly estimated from the position of transmission extrema on the wavelength scale for relatively thick film samples, with thickness $>100 \mu\text{m}$.

To address the issue, we present in this work results from parallel measurements of reflection and transmission spectra of biological molecules, for more detailed and accurate calculation of refractive index and absorption coefficient in the terahertz gap. We report the first results utilizing experimental reflection as a parameter for comparison in the IST model. Reflection spectra demonstrate resonance features that are similar to those of transmission. The samples chosen for study fall under the categories of DNA, proteins and spores. DNA samples from herring (H) and salmon (S), ovalbumin protein (Ov), and Bacillus Subtillus (BG) spores have been characterized. The technique for simulation is described. The dispersion of refractive index and absorption coefficient is derived. One particular issue of interest demonstrated in this work is the difference in optical characteristics of single and double stranded DNA materials, which could serve for monitoring DNA denaturation process. We have studied biological materials in both solid and aqueous form, the latter to present the potential of this technique for real-time in-situ analysis of real-life biological phenomena in liquid state.

II. EXPERIMENTAL PROCEDURE AND SAMPLE PREPARATION

For spectral studies, a commercial Bruker Fourier Transform Spectrometer (IFS-66) system utilizing a mercury-lamp source and a liquid Helium cooled Si-bolometer operating at 1.7 $^{\circ}\text{K}$ were used. The spectrometer was equipped with attachment for reflection measurements. The samples were measured in the same position several times to check reproducibility. The resolution used was 0.2 cm^{-1} and the spectral range of most interest 10 cm^{-1} to 25 cm^{-1} .

DNA sodium salts from herring and salmon testes and Ovalbumin from chicken egg (Ov) were purchased from Sigma Chemicals Co. Powder spore material, Bacillus subtilus (BG) var. Niger, was received from Dr. A. Samuels (U.S. Army SCBRD, Edgewood, MD). Film samples were prepared from water gel using the technique described earlier¹². Lyophilized DNA material was resuspended in deionized water at pH 7 with the water to dry material content ratio in the gel from 2:1 to 50:1. Double stranded DNA was transformed into a single stranded structure by increasing solution pH up to 13 ± 0.2 ¹³ after adding NaOH. Polycarbonate membranes of thickness 9-10 μm and around 98 % transmission (see Figure 2) were used as supporting substrates for air-dried film samples. Thickness of film varied from 1 to 250 μm . Samples of at least 0.5" diameter were fabricated.

To prepare samples in liquid phase, the gel-like solution was placed between two PE films of 5-10 μm thick (Saran wrap film, S.C. Johnson Co.) and sealed. The thickness of the sample was controlled by Teflon or stainless steel washers with thickness in the range 10-100 μm . A linear low-density polyethylene film (PE) is a highly transparent material with a very low reflection in the spectral range of interest and the transmission for support films was close to 100 %¹⁴.

DNA, as other typical of rod-like or semi-rigid polymer, spontaneously forms ordered liquid crystalline phases in aqueous solution above some critical concentration (exceeding 100 mg/ ml^{-1}) to minimize the polymer-excluded volume¹⁵. In drying process, DNA solution undergoes a series of transitions through multiple liquid crystalline phases and film samples are characterized by their microscopic textures with periodic variations in refractive index and fringe patterns observed in polarizing microscope. Gel-samples can be mechanically aligned to provide preferable orientation of long molecule axes in one direction. However, the film texture depends on the concentration of molecules in solution and on drying conditions as well¹⁰. All prepared samples were partially oriented. Measurements were performed with electric field of radiation perpendicular to the direction of orientation.

Samples used in this study are listed in the Table 1.

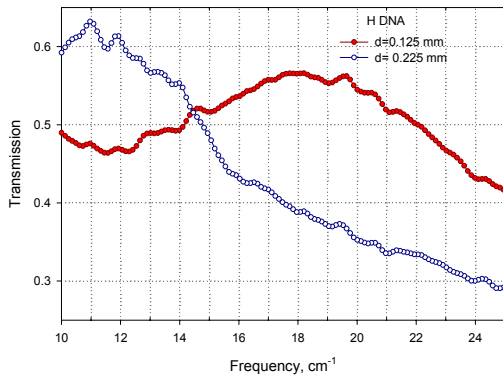


Figure 1: Interference pattern in transmission spectra for two samples of herring DNA with different thickness

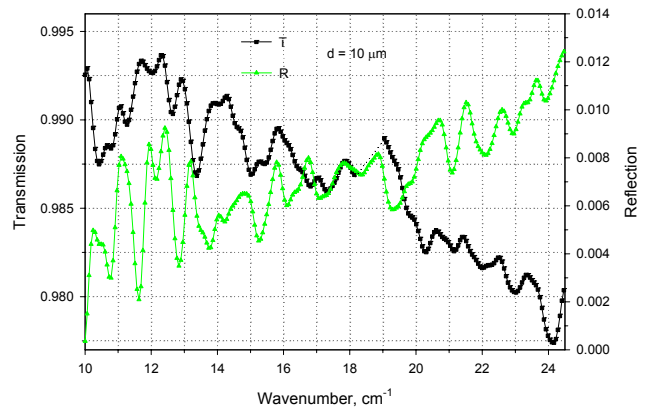


Figure 2: Transmission (T) and Reflection (R) spectra of polycarbonate (PC) membrane with thickness 10 μm

Table 1. The list of samples

Sample number	Material	Substrate, μm	Sample Thickness	Comments
ds H 712	Herring DNA	PC 1.0, 10 μm	16.3 μm	airdried, ds
ds S 1142	Salmon DNA	PC 0.2, 9 μm	24.3 μm	airdried, ds
ss S 1136	Salmon DNA	PC 0.2, 9 μm	5.8 μm	airdried, ss
ds S 1143	Salmon DNA	PE, 9 μm	8.4 μm	liquid, ds
ss S 1139	Salmon DNA	PE, 9 μm	5.7 μm	liquid, ss
Ov 948	Ovalbumin	PC 1.0, 10 μm	250 μm	airdried
BG1 milld#13	Bacillus Subtillus	PC 0.4, 9 μm	10.8 μm	airdried
BG2 milld#13	Bacillus Subtillus	PC 0.4, 9 μm	8.2 μm	airdried
BG1 DPG	Bacillus Subtillus	PTFE, 1 μm	16 μm	airdried
BG2 DPG	Bacillus Subtillus	PTFE, 1 μm	16.4 μm	airdried
BG 1048	Bacillus Subtillus	PE, 9 μm	65 μm	cells, liquid

III. THEORETICAL ANALYSIS

The Interference Spectroscopy Technique (IST) for the accurate determination of absorption coefficient within thin film materials has been implemented for the biological samples measured. This allows for exact extraction of absorption coefficient from transmission data as it goes through the rigors of modeling by accounting for the multiple reflection behavior. First the refractive index of the sample is estimated for approximate fit of experimental and theoretical reflection data. Then exact values of absorption coefficient are obtained through the second stage of modeling which gives the actual values of refractive index that would be appropriate for the two independently measured transmission and reflection data. The effect of substrates can also be taken into account.

As it is demonstrated in Figure 2 for a polycarbonate (PC) membrane substrate, the transmission of the substrates used in our study is generally close to unity throughout the entire spectrum and the reflection is close to 0. This is resulted from very low absorption coefficient of the substrate material and a very small thickness in comparison with wavelength of radiation. Hence with a good accuracy, the substrate can be neglected for modeling of the air/ material/ substrate system, meaning the absorption coefficient of the substrate is negligible (close to 0) and the refractive index is approximately equal to 1. The skewness around 18.6 cm^{-1} is a false feature due to absorption of water vapor in air.

As discussed in previous work¹¹, transmission, T_{13} , of an air/ film/ substrate system in the case of normal angle of incidence, without taking into account substrate multiple reflections is:

$$T_{13} = \xi T_{12} T_{23} \exp(-\alpha_2 d_2), \quad (1)$$

$$R_{13} = \xi (R_{12} - 2 \exp(-\alpha_2 d_2) \cos(2\delta_2) (R_{12} R_{23})^{1/2} + R_{23} \exp(-2\alpha_2 d_2))$$

$$\text{where } \xi = (1 - 2 \exp(-\alpha_2 d_2) \cos(2\delta_2) (R_{12} R_{23})^{1/2} + R_{12} R_{23} \exp(-2\alpha_2 d_2))^{-1}. \quad (2)$$

Index 2 refers to a film and index 3 to a substrate,

$\delta_2 = 2\pi n_2 d_2 / \lambda$ is the phase thickness of material at wavelength λ ; α_2 and n_2 are the absorption coefficient and refractive index of the material. Coefficients of transmission and reflection $T_{i, i+1}$ and $R_{i, i+1}$ at the medium i / medium $(i+1)$ interface (squares of the corresponding Fresnel transmission and reflection coefficients) are given by

$$T_{i, i+1} = 4(n_{i+1}/n_i)(n_i^2 + k_i^2) / ((n_i + n_{i+1})^2 + (k_i + k_{i+1})^2), \quad (3)$$

$$R_{i, i+1} = ((n_i - n_{i+1})^2 + (k_i - k_{i+1})^2) / ((n_i + n_{i+1})^2 + (k_i + k_{i+1})^2), \quad (4)$$

where n_i are refractive indices and $k_i = \lambda \alpha_i / 4\pi$ corresponding extinction coefficients. All coefficients are real and these expressions are valid for a relatively weak absorption coefficient of the material, i.e. $n_2^2 > k_2^2$.

In the first stage of modeling, the experimental transmission data and the corresponding wave numbers are chosen as input and the refractive index pattern guessed for approximate fit of experimental and calculated reflection. The known transmission is also solved for absorption coefficient, which again is approximate. Furthermore, the product of refractive index and sample material thickness, nd , can be obtained from transmission extrema position, λ_{extr} , and is defined by the equation

$$nd = m \lambda_{\text{extr}} / 4, \quad (5)$$

where m is an integer starting from the zero frequency side and corresponding to the order of extremum, including maxima and minima. Equation (5) can be applied to guess refractive index of relatively thick samples for which the interference pattern can be observed in the spectral range of measurements. This pattern is usually better seen in reflection spectra as the fringes are more suppressed in transmission in the region of higher material absorption. Figure 3 demonstrates T and R spectra taken from Ovalbumin sample with thickness $250 \mu\text{m}$. Although absorption in such a thick sample is high, traces of the interference pattern are observed in reflection at the level of $R \sim 1\%-2\%$.

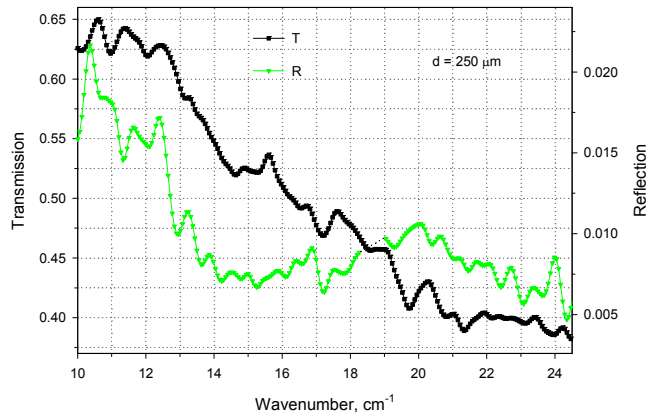


Figure 3: Experimental Transmission (T) and Reflection (R) spectra of Ovalbumin (Ov948) with thickness $250 \mu\text{m}$.

The second stage involves transmission data, wave numbers, guessed refractive index of sample and experimental reflection data as inputs. The exact value of refractive index is found by calculating corresponding reflection for a step-by-step change in index until calculated reflection equals the experimental data.

III. EXPERIMENTAL AND MODELING RESULTS

The spectral measurements yielded well-defined structures for vibrational modes in reflection data, with phase-correlation with transmission spectra as shown in Figures 4 and 5. In all experimental spectra we have to ignore features around 18.6 cm^{-1} since this spectral sub-range is disturbed by absorption of water vapor in air. It occurs since conditions for recording background and sample spectra can be slightly different, resulting in artificial structure between $18\text{-}19\text{ cm}^{-1}$. Wherever the artificial peaks are very sharp, we have used dotted lines to join the spectra in the $18\text{-}19\text{ cm}^{-1}$ regions. In many cases the reflection spectra yielded more clearly distinguishable mode features when compared to transmission spectra of the corresponding sample.

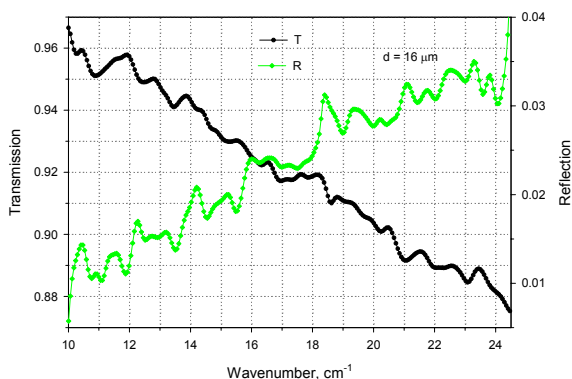
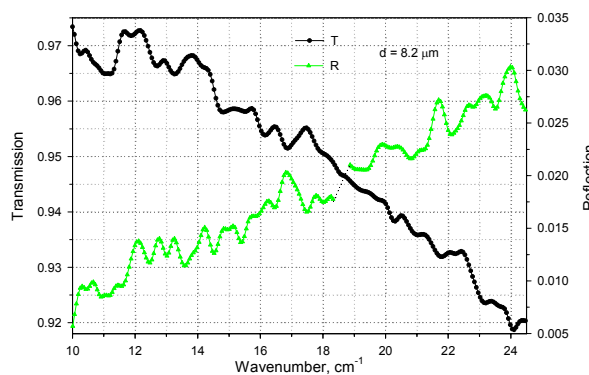


Figure 4: (a) Transmission and Reflection spectra of air-dried *Bacillus Subtillus* sample (BG1DPG) on PTFE substrate



(b) Transmission and Reflection spectra of air-dried *Bacillus Subtillus* sample (BG2mild#13) on PC substrate

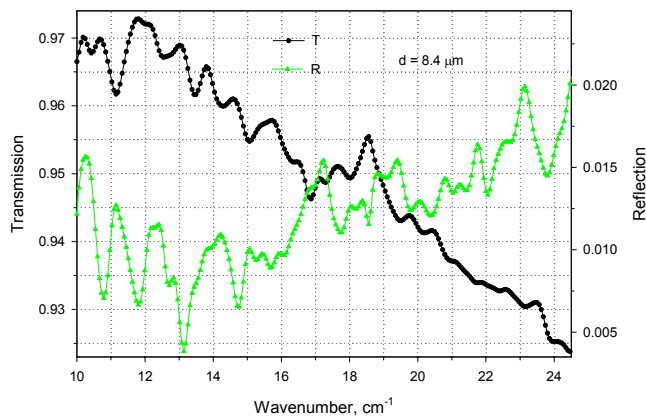


Figure 5: Transmission and Reflection spectra of liquid double stranded salmon DNA (dsS1143)

Comparisons of reflection spectra of two samples of the same material yielded similar structures (Figure 6a.), implying vibrational modes can serve as a unique fingerprint of the material. Note that BG spores in this experiment

come from different sources¹⁶. Different materials yielded different structures in reflection spectra as expected (Figure 6b). The absolute value of reflection is also different.

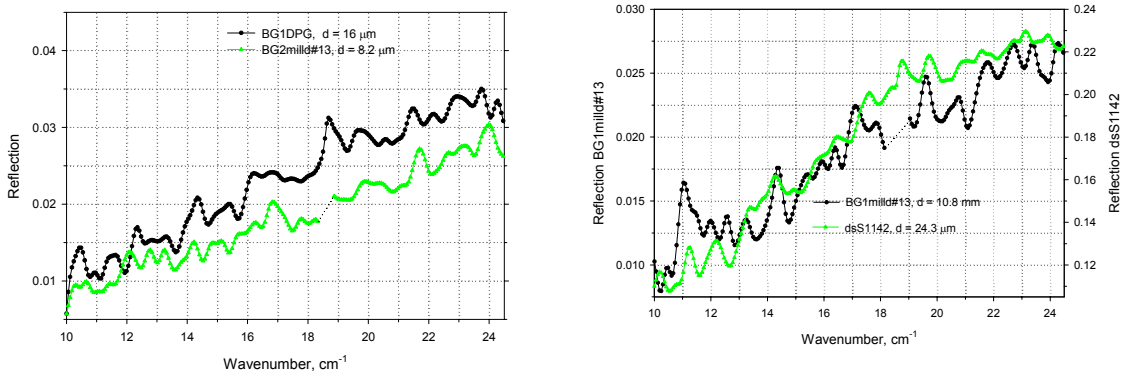


Figure 6: (a) Reflection spectra of two samples of *Bacillus Subtillus* from two different sources. (b) Reflection spectra of *Bacillus Subtillus* (BG1mild#13) and double stranded salmon DNA (dsS1142).

The IST technique of modeling was applied to samples of Herring DNA (air-dried), double (ds-) and single-stranded (ss-) Salmon DNA (air-dried and liquid), Ovalbumin (air-dried) and *Bacillus Subtillus* (BG) spores (air-dried and liquid) and yielded changes in refractive index with frequency and sharp changes in absorption coefficient around phonon resonances for transmission and reflection data collected in independent experiments. A 10% uncertainty in thickness measurements would result in a 4 % uncertainty in refractive index and ~10% uncertainty in absorption coefficient. Results on optical characteristics are discussed below.

DNA samples

The frequency variation of refractive index (n) and absorption coefficient (α) of air-dried Herring DNA (H712) sample of thickness 16.3 μm is shown in figure 7.

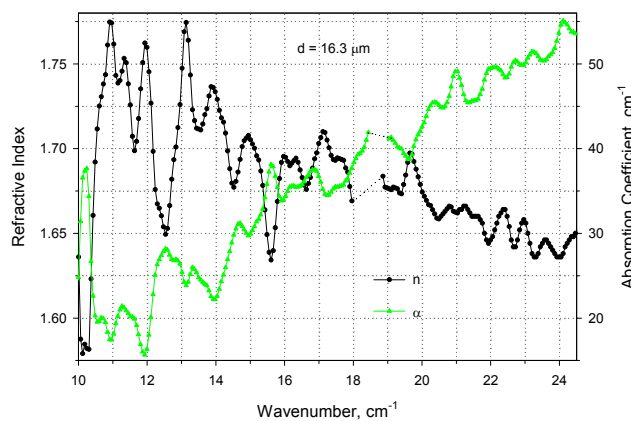


Figure 7: Dispersion of Refractive Index and Absorption Coefficient of Herring DNA (H712)

Absorption is higher at higher frequencies. Although peaks in absorption spectrum and in reflection are shifted in frequency, there is a definite correlation between transmission and reflection resonance features. Near peaks of absorption, minima of refractive index are observed. This can be expected since the optical constants are related to one another by Kramer-Kronig relations.

Figure 8 demonstrates comparison of THz characteristics of salmon DNA films in native (ds-) and denatured (ss-) forms. The absorption coefficient of ss-DNA is ~20% higher than that of ds-DNA. Additional sharp peaks at 11.2 cm⁻¹, 13.4 cm⁻¹ and 14.8 cm⁻¹ are observed for ss-DNA even though there is general similarity in the pattern for two conformations. This probably follows from the difference in three dimensional structure and flexibility of molecule in native and denatured conditions. We can expect that one strand of DNA is more flexible than the double stranded helix.

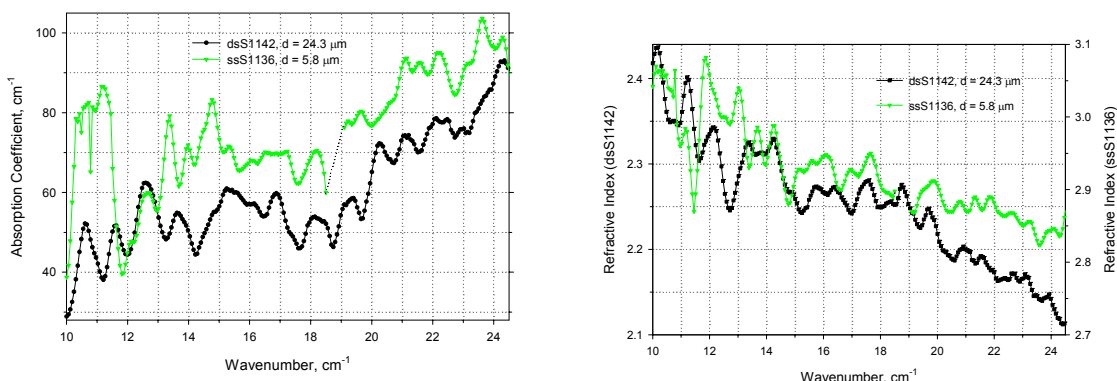


Figure 8: (a) Dispersion of a for air-dried ds- and ss- DNA (b) Dispersion of n for air-dried ds- and ss-DNA

Consequently, salmon DNA samples yielded higher refractive index for ss-DNA compared to the ds-conformation as indicated in Figure 8b. This is different from the result obtained by Brucherseifer et al⁹.

Liquid samples have higher absorption in comparison with airdried samples due to the effect of water (Figure 9a). Liquid ss-salmon DNA has ~33% higher absorption coefficient when compared to ds-salmon DNA. Consistent with airdried samples, the refractive index of liquid ss-salmon DNA was higher than that of the double stranded counterpart as seen in Figure 9b. The refractive index is much steeper for liquid samples. The absorption coefficient for ssS1139 does not have any sharp peaks distinct from dsS1143 like the case of air-dried samples. This result is however not 100% conclusive since optical data also depends on orientation and optical constants of DNA are anisotropic due to π -electrons in the bases¹⁷.

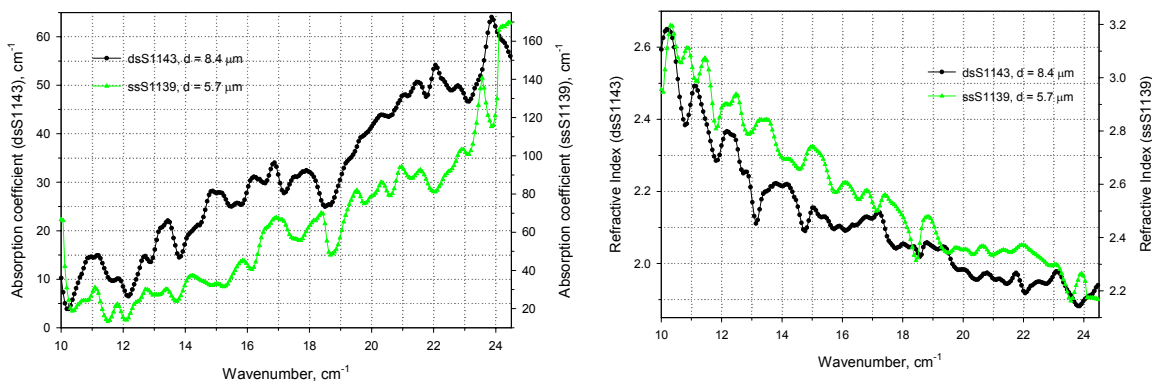


Figure 9: (a) Dispersion of a for liquid ds- and ss- DNA (b): Dispersion of n for liquid ds- and ss-DNA

Protein sample

As discussed previously, the experimental reflection of Ovalbumin shows the presence of fringing due to the large thickness (250 μm) and the first stage of modeling necessitates the use of equation (5) as a template for

approximately estimating refractive index (n). The product nd is a key factor in determining the positions of extrema. Reducing nd shifts the pattern to the direction of higher wave number. The integer m is equal to 3 in this case.

The results of modeling for optical characteristics of Ovalbumin are shown in Figure 10. We have met with difficulties when trying to extract optical characteristics from measurement results of this sample since we were unable to fit accurately two sets of experimental data (R and T) in the range of 14.3 cm^{-1} to 15.8 cm^{-1} . We have to note that measurements of this sample have been repeated independently several times, and the results were well reproducible. The measured reflection of Ovalbumin sample is very low, only 1-2%, and calculated refractive index is also very low. In the region around 15 cm^{-1} to 16 cm^{-1} abnormal behavior in refractive index spectrum is observed which probably could be explained by the phenomenon of anomalous dispersion based on the classical assumption of the solid as an assembly of oscillators that are set to forced vibration by the radiation¹⁸. Around the vicinity of the oscillation frequency of the electrons, refractive index falls from a maximum and with increased frequency is at some point less than 1 where the real part of the permittivity is also less than unity. Equations of IST (3, 4) that we have used for modeling are not valid in this region in the case of $n < 1$. Any reasonable corrections due to possible error in the sample thickness did not change the anomalous behavior in n .

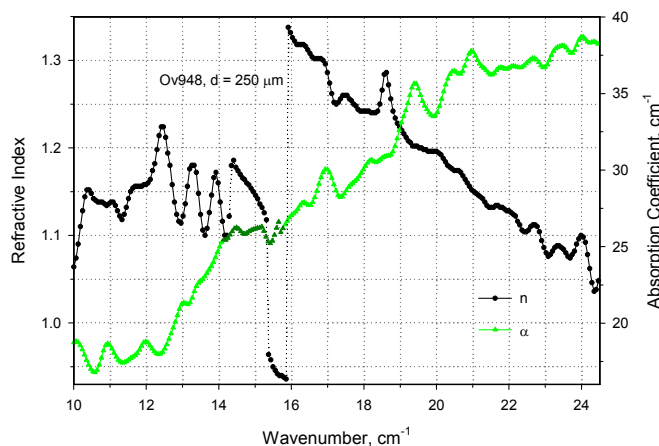


Figure 10: Absorption coefficient and refractive index spectra of Ovalbumin (Ov948)

Spore samples

Two air-dried samples of Bacillus Subtillus spores from different sources yielded close variations in absorption coefficient and refractive index as shown in Figure 11 regardless of the substrate used. BG2mild#13 has polycarbonate membrane as substrate while BG1DPG is held between Teflon films. The peaks in refractive index at 12.8 cm^{-1} and 16.8 cm^{-1} are sharper for BG2mild#13.

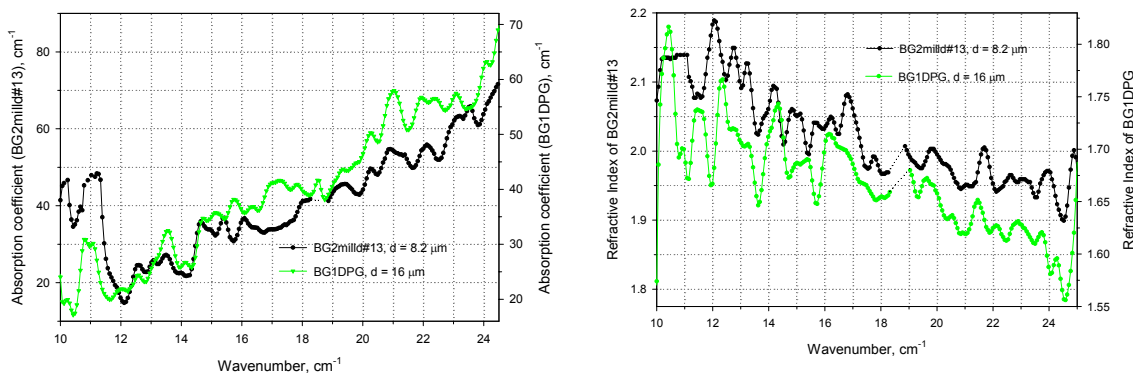


Figure 11: (a) Dispersion of α of air-dried Bacillus Subtillus (BG) samples from different sources

(b) Dispersion of n of air-dried BG samples

As liquid DNA, liquid BG samples have higher absorption compared to solid films due to water absorption as shown in Figure 12. There are sharp changes in α and n beginning 11.2 cm^{-1} . As before, refractive index has minima near peaks of absorption. There are some similarities in resonance features of n for liquid and solid samples.

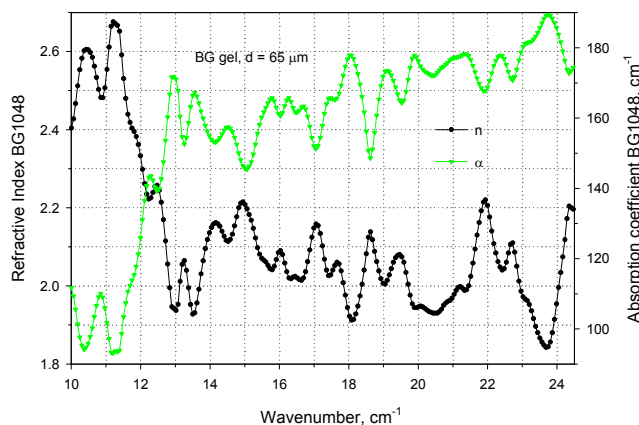


Figure 12: Optical constants (n and α) for liquid BG sample (BG1048)

IV. CONCLUSIONS

This paper reported first results of optical constant calculations of several biological samples of DNA, protein and spores using interference spectroscopy technique to fit independently measured experimental reflection and transmission data in the THz gap. The constants were similar for materials of the same kind regardless of thickness and substrate used. The important result of this study is that reflection has the capability to provide spectral features of biological materials similar to those revealed earlier in transmission that are resonance modes due to interaction of THz radiation with intra-molecular vibrational motions. In many cases reflection has even more pronounced features as compared to transmission, with both of them being correlated. This fact can be used to enlarge the sensitivity for remote bio-detection. The optical calculations based on this data yielded higher refractive index and absorption coefficient for single stranded Salmon DNA than for the double stranded counterpart with several different vibrational modes. This uniqueness has a potential application in monitoring denaturation process. Gel samples have higher absorption in comparison with solid films. Additional work is needed to confirm the results on anomalous dispersion of optical constants in protein sample.

ACKNOWLEDGMENTS

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