Submillimeter wave spectroscopy of biological macromolecules

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N5-Applications of THz Radiation
American Physical Society Meeting 2005, Los Angeles

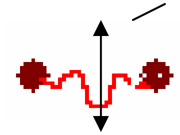
Absorption and Scattering Spectroscopy

Scattering and absorption spectroscopies utilize the **interaction** of an applied **electromagnetic field** with the phonon **(vibration) field** of the material to provide **useful structural information .**

Absorption

$h\nu_{light}$ $\mathbf{I_1}$

$\mathbf{h\nu_{vibr}}$



$h\nu_{light}$ $\mathbf{I_2}$

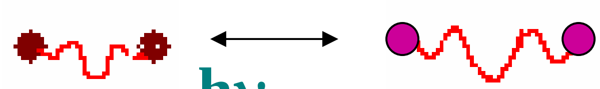
$h\nu_{vibr.} = h\nu_{light}$

Raman scattering

$h\nu_1$

Ground state

Exited state



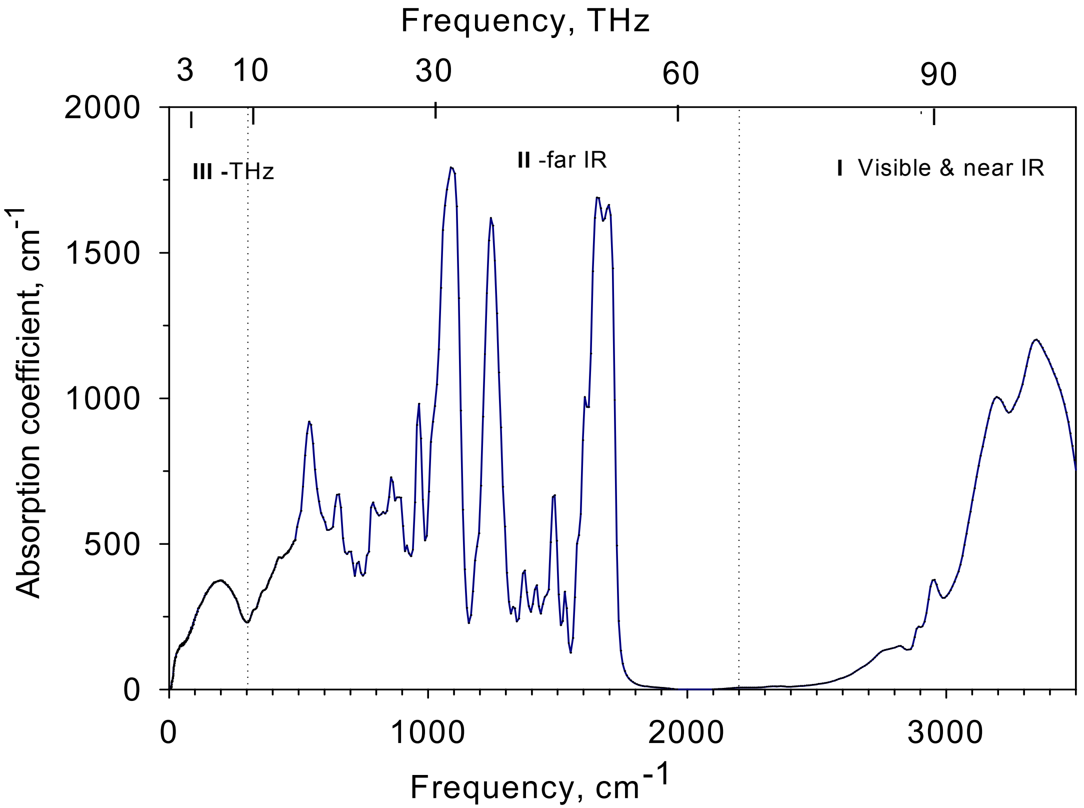
$\mathbf{h\nu_{vibr.}}$

$h\nu_2$

$h\nu_{vibr.} = h\nu_1 - h\nu_2$

$h\nu_1, h\nu_2 >> h\nu_{vibr.}$

DNA Absorption Spectrum

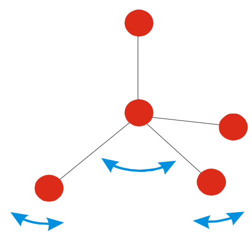
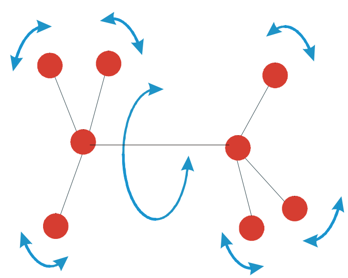


Absorption spectrum of DNA *(our results).*

- **Regions I & II** (Studied well by IR & Raman)- resonances due to short-range, **high energy interactions**
- **in THz Region III** – **<u>more species specific</u> spectral features of bio molecules are found**

FTIR spectroscopy produces high quality spectra **in the region II,** and can **separate overlapping subcomponents** in the spectra

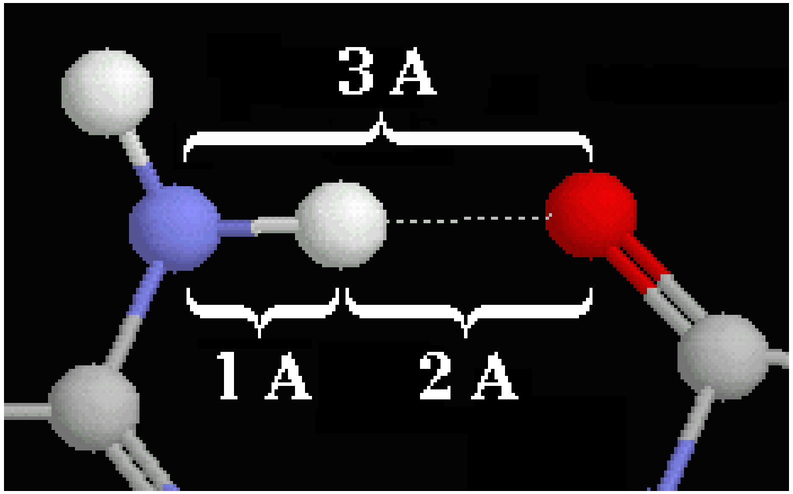
INTERNAL MOLECULAR VIBRATIONS

Vibrations of		**Frequencies**
Bonds		>21 THz (700 cm^{-1})
Bond angles		6-27 THz (200-900 cm^{-1})
Torsion angles		< 9 THz **(<300 cm^{-1})**

In the region III (0.1-10 THz **or 2 - 300 cm^{-1})**, **absorption spectra** reflect **low-frequency molecular internal motions or vibrations** involving **the weakest hydrogen bonds** and/**or non-bonded interactions** between different functional groups within molecules or even between molecules.

The resonant frequencies of such motions – **phonon modes**- are strongly **dependent on molecular structure**

WEAK HYDROGEN BONDS



Weakest hydrogen bonds,
shown by dots:

-C-H ······O-
-C-H ······N-
-C ······ H-N-
-C ······ H-O-
-N-H······O=C-

- **weak** and have only ~ 5% of the strength of covalent bonds

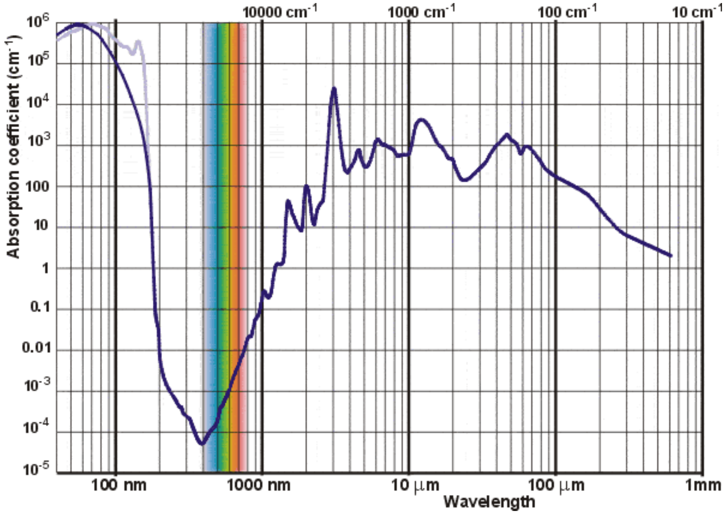
- **multiple** hydrogen bonds **stabilize the structure** of bio-polymers

- **hold** the **two strands** of the DNA double helix **together**, or hold polypeptides together in **different secondary structure conformations**.

Why THz?

- **THz spectroscopy reveals structural information quite different from all other methods since it can directly detect weakest hydrogen bonds and non-bonded interactions within biopolymers.**

Liquid water absorption



- **Less water absorption (at list 2 orders) compare to IR and far-IR. Less overlap** with water or other analytes **absorption bands. Liquid samples can be characterized.**
- **Absorption bands are more narrow in the THz range than in the IR and overlapping of neighboring bands is less.**

- **The availability of multiple resonances for the sensitive measurement of bio-molecule structure.**
- **Spectra are more species specific.**

"THE WORLD OF THE DEAD OR OF FUTURE PUNISHMENT"

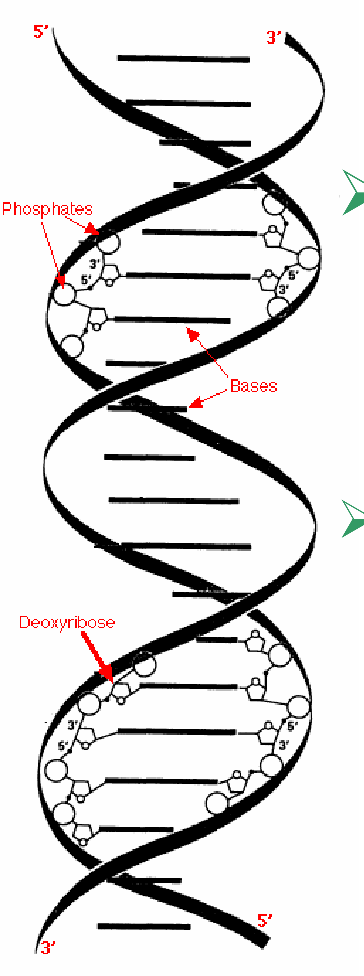
M .N. Afsar &K.J.Button, "Infrared and Millimeter Waves," V.12, Acad. Press, 1984

"Terahertz gap"

The spectral range between the upper end of the microwave and the lower end of the extreme far IR

- Low energy of sources.
- Low absorption of biological material requires samples with large area and thickness which is difficult to make because samples are too fragile.
- Poor reproducibility of experimental results due to multiple reflection in measurement systems, responsible for artificial features; difficulties in sample preparation, instability of material.
- The absence of good commercially available laboratory instruments
- Potentially promising laboratory techniques as time resolved spectroscopy and photomixing technology are only recently emerged

MOTIVATION and GOALS



- There is a general **need for faster and less expensive techniques** that can provide useful **structural information on bio-materials.**

- Our goal is **to demonstrate** that **THz spectroscopy** can be a **fruitful technique** even with all mentioned difficulties and by using **commercially available instruments**.

Questions to answer:

- Is there something in the very far IR spectra? (initial prediction of vibrational modes in polymer DNA in the 1-100 cm-1 frequency range [E.W.Prohofsky, K.C. Lu, L.L.Van Zandt and B. F. Putnam, Phys Lett., 70 a, 492 1979; K.V. Devi Prasad and E.W.Prohofsky, Biopolymers, 23,1795, 1984].

- What are the reasons why researchers for 20 years failed to achieve reproducible results? Experimental results are not reproducible and are contradictive. It was not clear what to expect. Can we improve the results?

- Can we use the observed features for DNA characterization, identification and discrimination between species?

- The key to answer all these questions: we need to know of what we are looking for.

THEORETICAL PREDICTION OF THz ABSORPTION SPECTRA

Maria Bykhovskaia, B. Gelmont

IR active modes are calculated directly from the base pair sequence and topology of a molecule.

Initial approximation was generated and optimized by the **program packages JUMNA and LIGAND** (group of Prof. Lavery, Inst.Biologie Phys.Chim.Paris).

Energy minimum → Normal modes → Oscillator strengths → Spectra

QUESTIONS:

- What do we expect to find in the submillimeter wave range?
- What is the predictive power of the method?
- How sensitive are far IR absorption spectra to DNA structure?

Normal mode analysis is applicable to molecules with less than 30 base-pairs

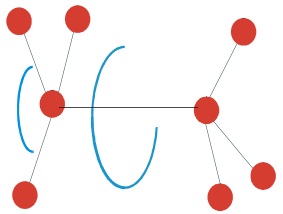
ENERGY MINIMIZATION AND NORMAL MODE ANALYSIS
(in internal coordinates of a molecule)

Maria Bykhovskaia, B. Gelmont

Molecular potential energy approximated as a function of dynamic variables(q): torsion and bond angles.

Conformational energy

including long distance interactions :



$$E_{total} = E_{Van\ der\ Waals} + E_{Electrostatic} + E_{HBonds} + E_{Torsion} + E_{Bond\ angles}$$

Van der Waals and electrostatic interactions; the energy of hydrogen bonds deformations; torsion rotation potentials; stretching deformations of bond angles and of bond length

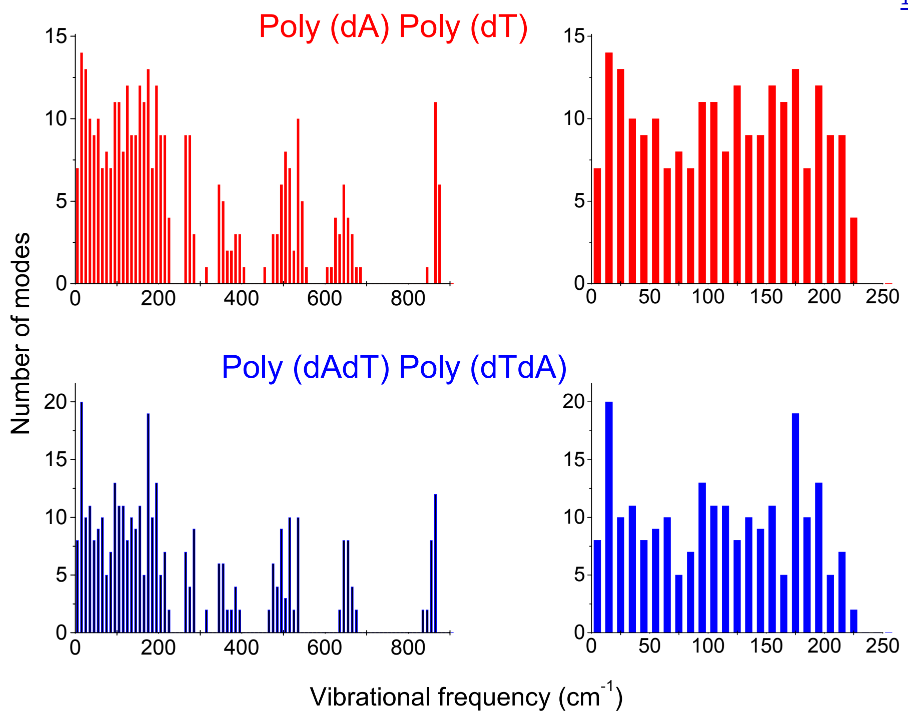
Two **B-helical conformation DNA** fragments **(TA)12** with different base pair sequences:

AAAAAAAAAAAA   ATATATATATAT
TTTTTTTTTTTT   TATATATATATA

and the **A-helix of double stranded RNA Poly[C]·Poly[G]**

LOW FREQUENCY NORMAL MODES

Maria Bykhovskaia, B. Gelmont

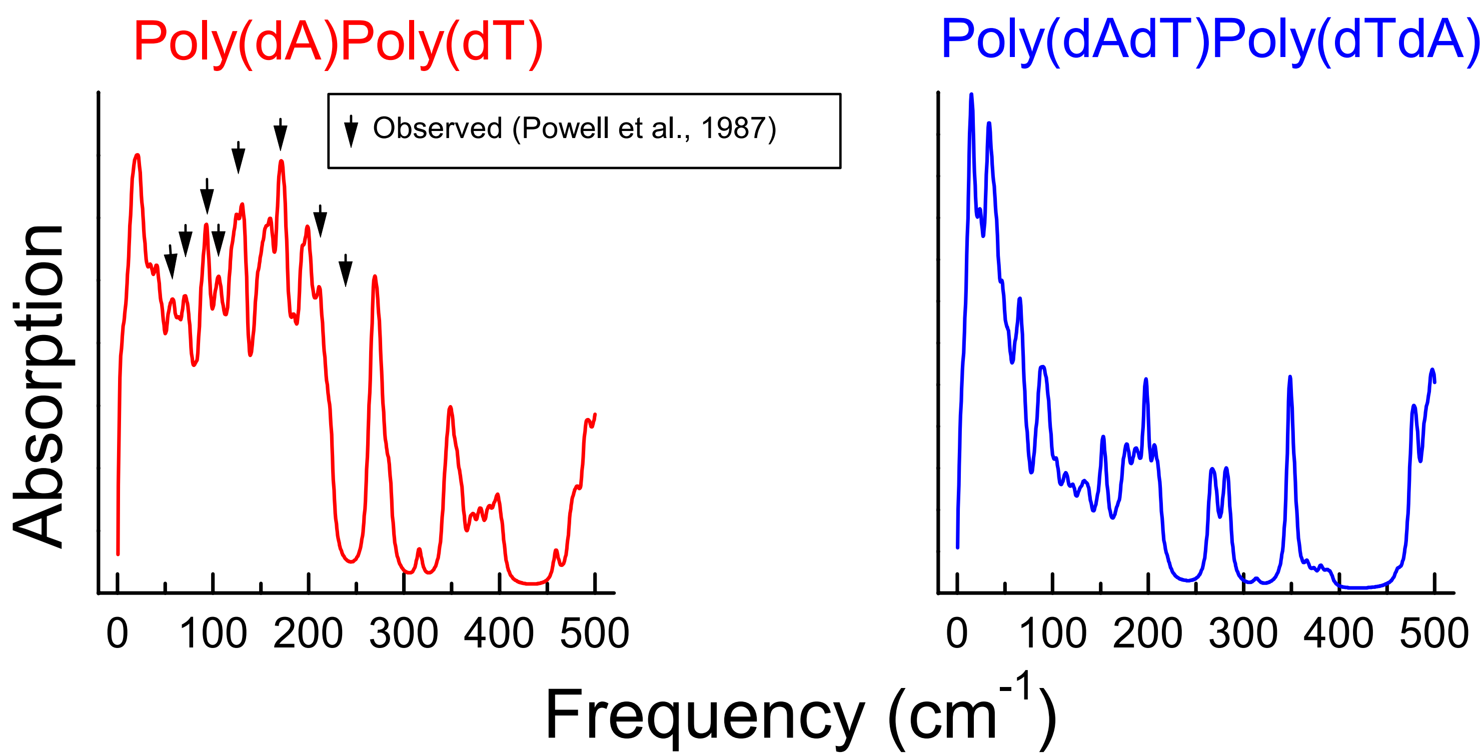


360 normal modes were found for each sequence with the density higher than 1 mode per cm^{-1}. There is almost no overlap of weak bond modes with vibrations of covalent bonds which have frequencies above 750 cm^{-1}.

Absorption Spectra vs. Base Pair Sequence

$\alpha(\omega) \sim \gamma\omega^2 \sum (p^k)^2 / ((\omega_k^2 - \omega^2)^2 + \gamma^2 \omega^2)$, the oscillator decay γ_k=2 cm^{-1} ,

the dipole moment $\mathbf{p} = \sum_i e_i \mathbf{a}_i / \sqrt{m_i}$,

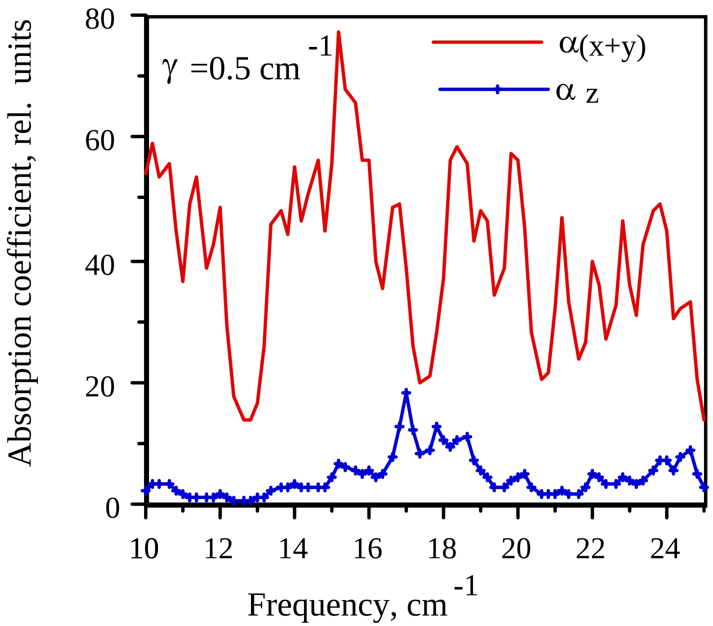


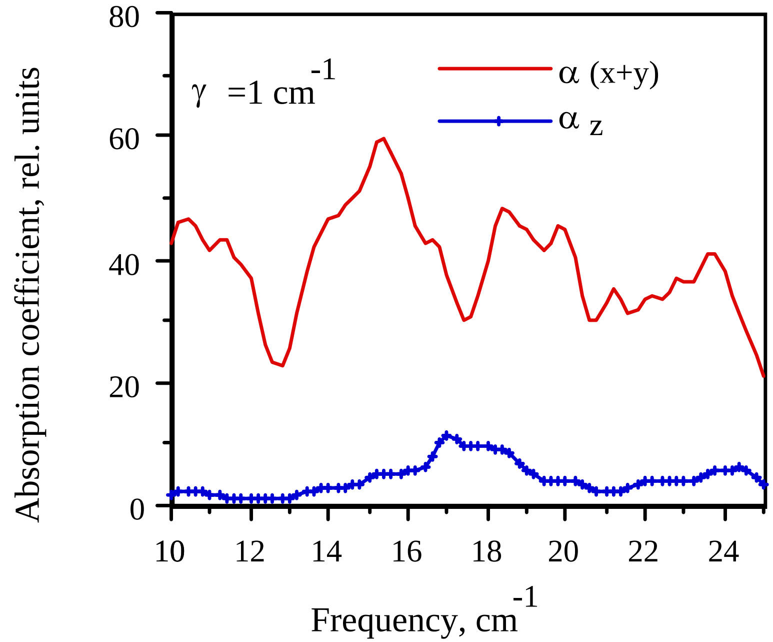
The **spectrum of optical activities** is very **sensitive to the DNA base pair sequence**

A double stranded 12 base pair RNA homopolymer fragment

Poly[C]-Poly[G] (Maria Bykhovskaia, B. Gelmont)

Absorption spectra for two values of **oscillator decay** γ =0.5 cm $^{-1}$ and γ =1 cm $^{-1}$,



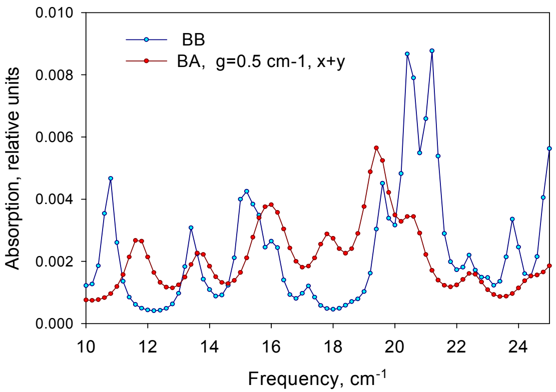


for electric field **E** perpendicular to the long axes of a molecule (α_{x+y}) and parallel to the long axes (α_z).

For this fragment, the maximum absorption corresponds to E perpendicular to the long molecular axis z.

Short artificial DNA (Maria Bykhovskaia, B. Gelmont)

Dry In gel High salt



Optical characteristics depend on conformation

B-form: 5'-d(CCGGCGCCGG)-3‘, **10 base pairs per turn,** right-handed, has major and minor grooves.
A-form: 5'-d(CCCGGCCGGG)-3‘is adopted by dehydrated DNA; **it has 11 base pairs per turn,** and the base pairs are tilted with respect to the helix axis. A-form is sensitive to humidity and can be changed to B-form.
Z-form: 5'-d(GCGCGCGCGC)-3‘- is a **left handed DNA** helix in a zig zag pattern **with12 base pairs per turn.** It adopted in solution at **high salt concentration** and when reduce salt content it can be changed from left-handed to right-handed.
It has no documented biological relevance. DNA exerts a regulatory activity when in Z conformation.

The two DNA strands are held together by base pairing (hydrogen bonding) between complementary bases. Cytosine (C) is always hydrogen bonded to guanine (G).

Experimental set up

- Bruker IFS-66 spectrometer (Hg- lamp source, He cooled Si-bolometer @ 1.7 º K). Vacuum systems are not shown.

- Attachment for reflection measurements.
- Resolution 0.2 cm^{-1}.
- Range of interest throughout 10 cm^{-1} – 25 cm^{-1}.

Martin-Pupplett Polarizing Spectrometer

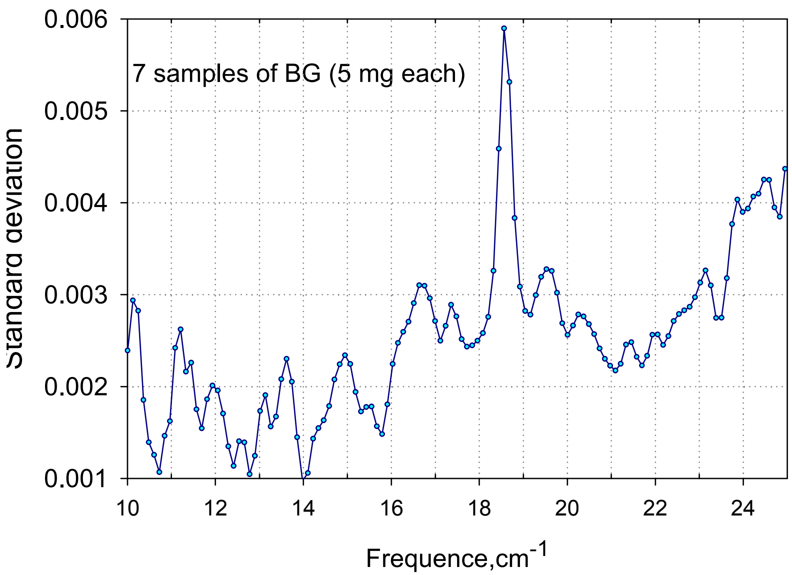
THz Fourier-Transform (FT) spectroscopy

What is important?

Good Instrument performance:

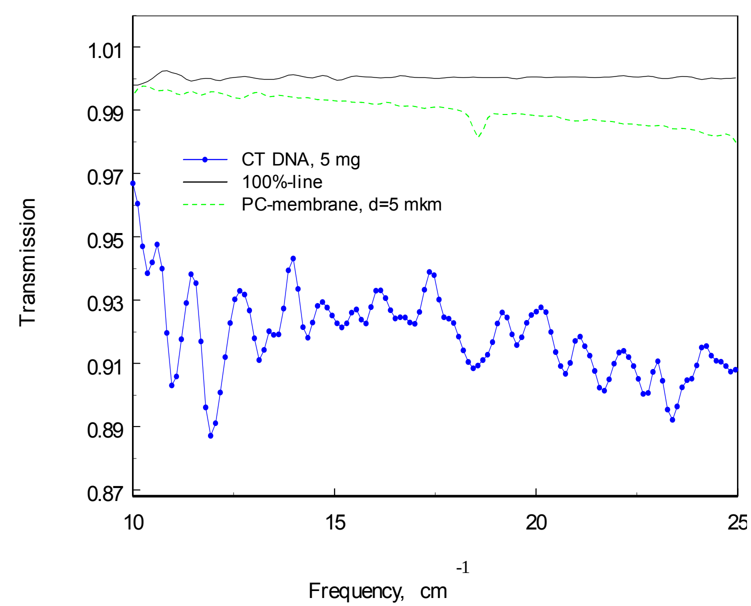
- **Spectral resolution at least 0.25 cm^{-1} to measure features with 0.5 cm^{-1} band width**
- **High sensitivity (signal to noise) and reproducibility to provide standard deviation better than 0.3% to measure small signals**

Sensitivity   Resolution



7 different samples

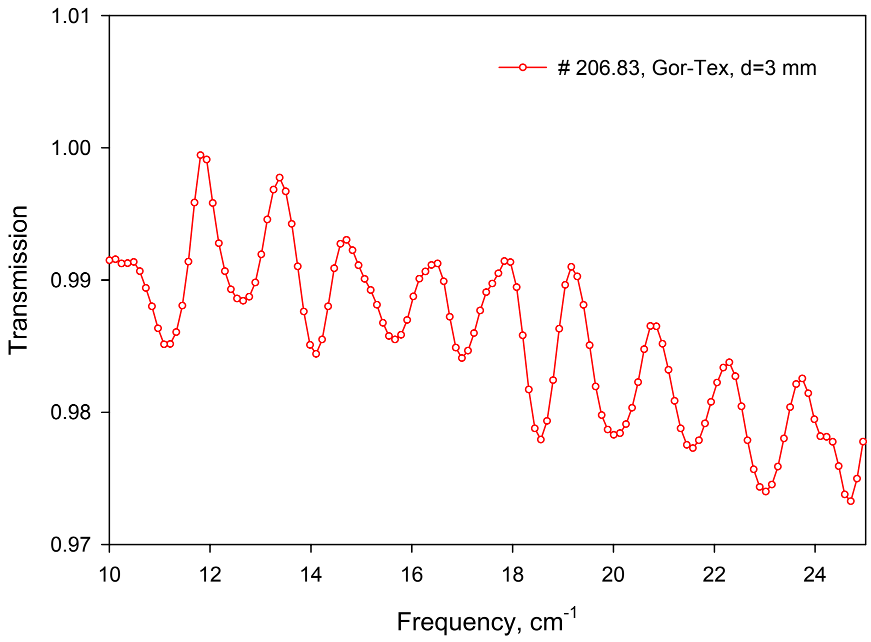
- **High transparency of substrates**



Challenging Problem

Serious problem at THz **- all kinds of multiple reflections** or **standing waves** in most of measurement systems because of large wavelengths of radiation.

These effects cause **artificial false resonances**.



Check for false resonances using thick plates from material with low absorption is important. Ideally spectrum is close to cos form.

Material for study

- Herring, salmon and calf thymus **DNA** sodium salts with 6 % Na content, from Sigma Chemical Co.

- Artificial **short-chained oligonucleotides of known base-pair sequences** from Sigma Chemical Co:

  • ***Single stranded RNA*** *- potassium salts with the different nucleotide composition:* ***poly (G), poly (C), poly (A), poly (U),***

  (Guanine (G), Cytosine (C), Adenine (A), Uracyl (U)).

  • ***Double helical RNA*** *- sodium salts:* ***Poly [C] * Poly [G]*** *and* ***Poly [A] * Poly [U].***

  • ***DNA, as 5'-d(CpCpGpGpCpGpCpCpGpGp)-3' and others with different sequencing, in A, B and Z conformation.***

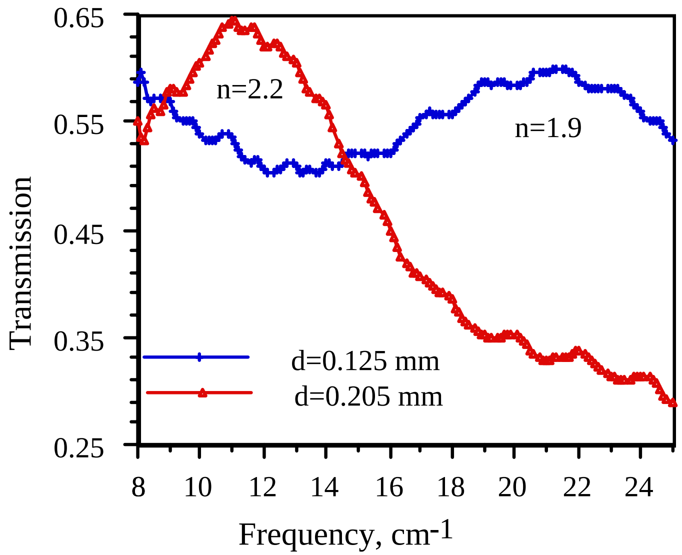
- **Spores, plasmids, proteins, cells**
- **Bio-materials in solutions**

Sample preparation

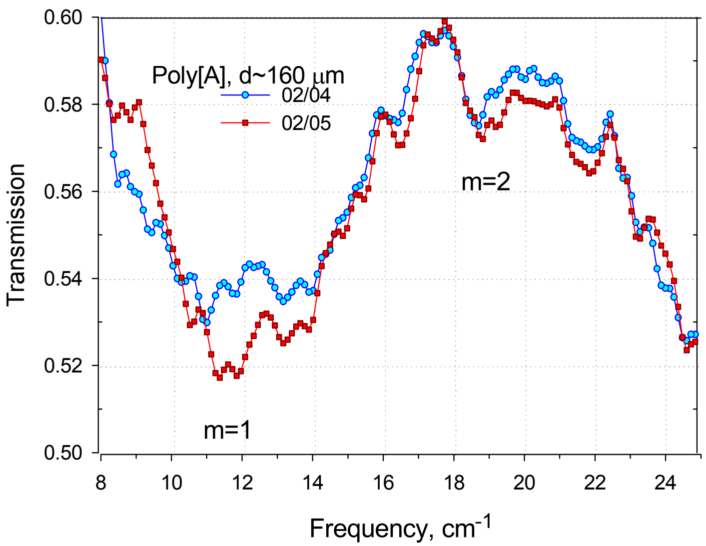
Simple techniques has been developed to fabricate large area, thin, stable samples

- Free-standing films and films on substrates are prepared from the water gel. Film thickness 2 µm - 250 µm.
- The ratio of water to dry material in the gel from 5:1 to 30:1.
- Thin polycarbonate membranes, polyethylene or Teflon films with ~98% transmission are used as supporting substrates in some cases.
- To receive good resolution, samples of at least 1/2" diameter are fabricated.
- Samples are aligned to receive preferable orientation of long molecule axes in one direction. Good alignment enhances the sensitivity.

Reproducibility vs. orientation and interference effects



Same orientation



Resonance features are resolved on the envelope of the wide **interference pattern.**

$$nd = \frac{m \quad \lambda extr}{4}$$

λextr - the wavelength of transmission extrema

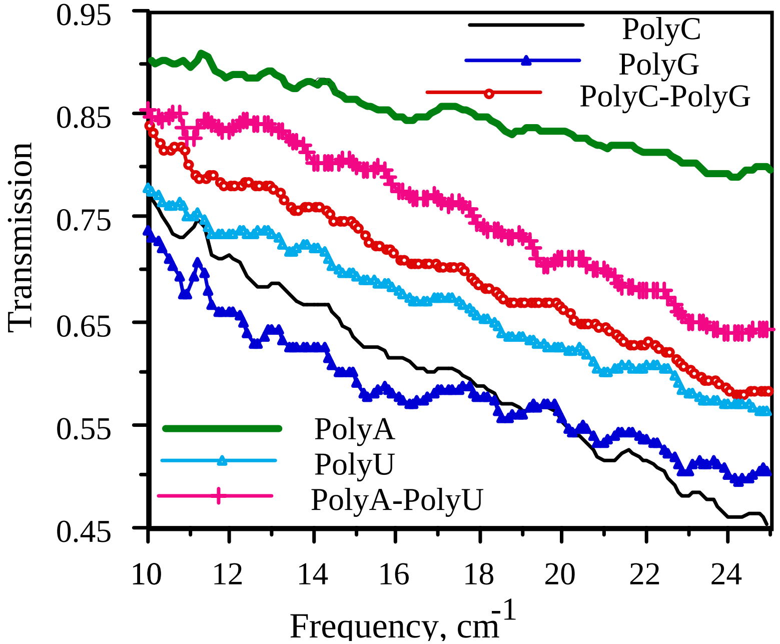
d - the film thickness

m - the order of extremum.

n- **refractive index** ~ 1.7 - 2.3

These kind of interference features were initially considered as resonance modes in bio materials.

Absorption coefficient



The interference pattern is not obvious in transmission of thin films (thickness between 15 and 70 µm).

Absorption coefficient spectra are derived by interference spectroscopy technique (IST) for proper modeling of the multiple reflection behavior.

Material texture

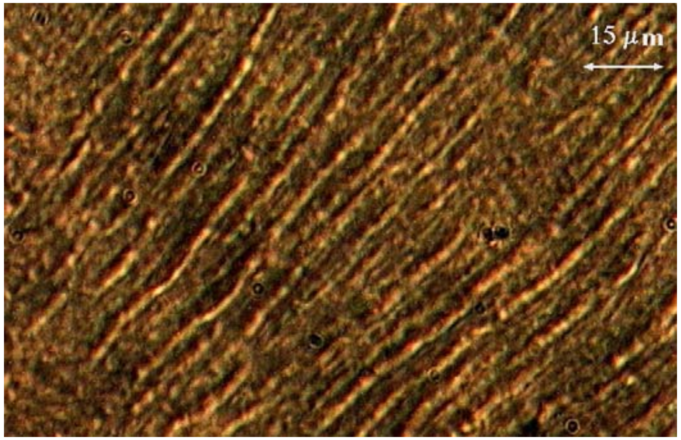


Image of the Salmon DNA sample in polarizing microscope (free standing).

Film **thickness** about **10 μm**.

Gel concentration 1:10.

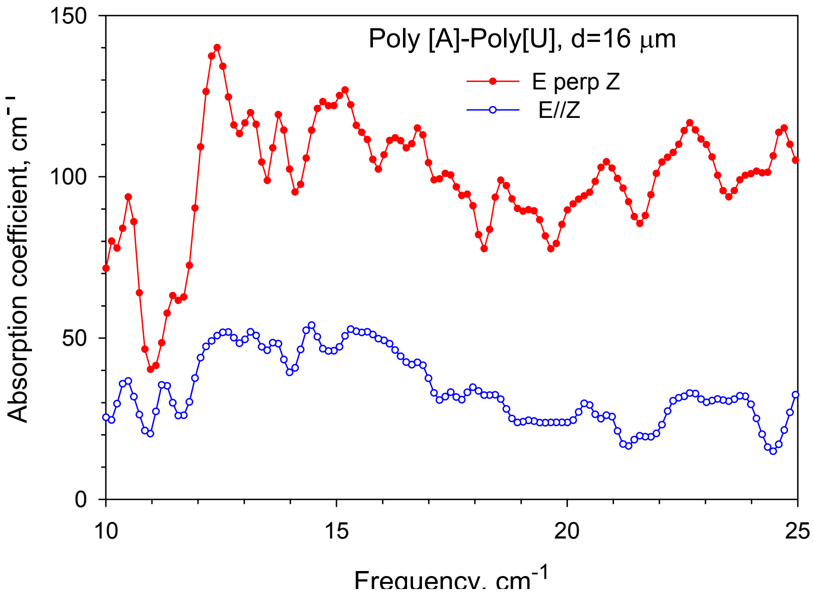
DNA, as a **rod-like polymer**, **spontaneously** forms **ordered liquid crystalline phases** in aqueous solution with the **long molecular axis preferentially aligned in one direction.**

In drying process, DNA solution undergoes a series of transitions and **film samples are characterized by their microscopic textures** with periodic variations in refractive index and **fringe patterns observed in polarizing microscope.**

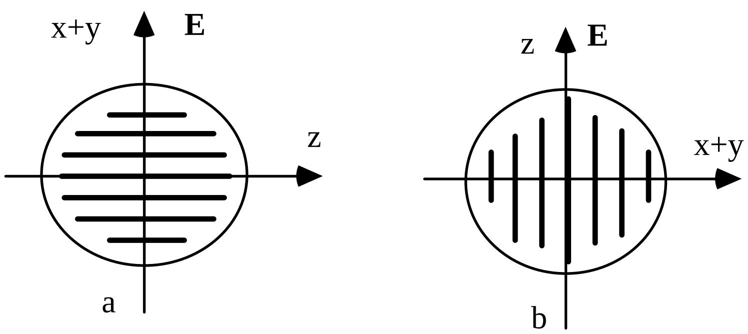
The film **texture** depends on the **concentration of molecules in solution and on drying conditions.**

Change with sample rotation.

- Documented **strong anisotropy of optical characteristics** of biological molecules at THz.



Absorption coefficient at two orientations



Sample position with electric field (a) perpendicular and (b) parallel to the long-axis of the molecule z.

For Poly[A]-Poly[U] fragments, absorption is higher and resonance structure is much more pronounced with electric field of radiation E perpendicular to the long axes of molecules Z.

Experiment and Modeling

Many of the initial successful measurements of the THz absorption properties of biological materials have been performed at the University of Virginia.

Evidence of multiple **resonances** in THz transmission **spectra** with a high degree **confidence in recognition of bio- molecules** has been demonstrated.

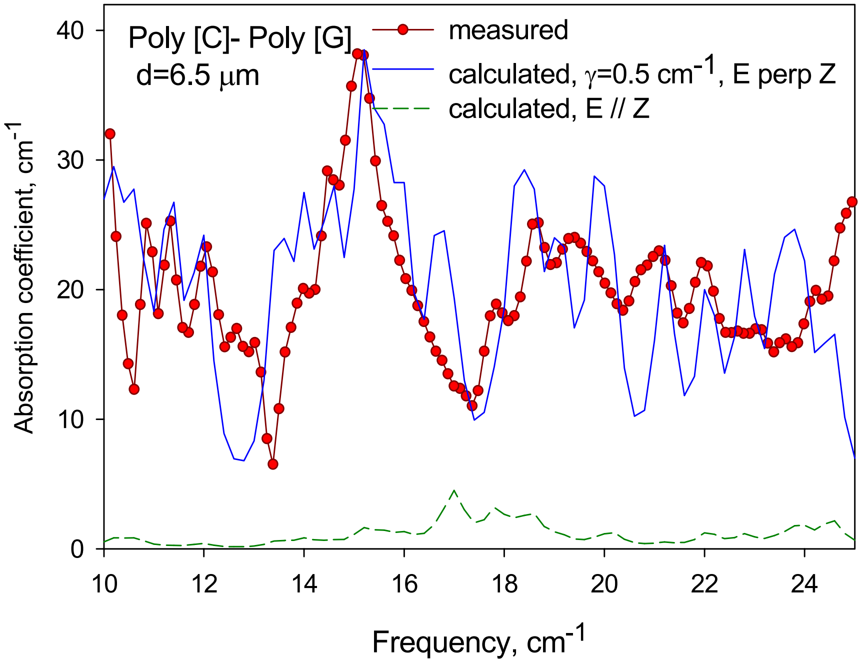
Direct comparison of experimental spectrum (red) **with theoretical prediction** (blue) **for a short chain DNA fragment with known structure.**

Reasonably **Good correlation validates both, experimental and theoretical results**.

From the width of the vibrational modes:

oscillator decay $\gamma = 0.5\ \text{cm}^{-1}$

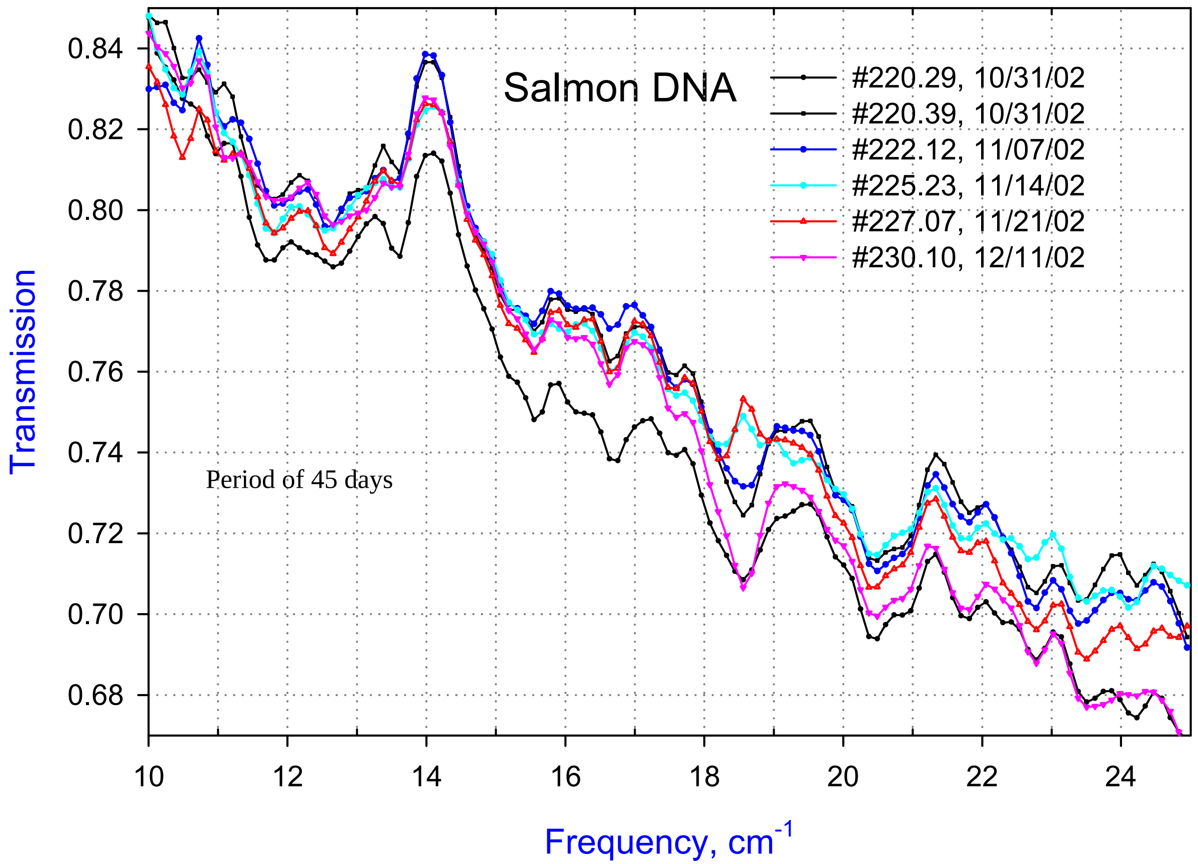
relaxation time $\tau = 7\ 10^{-11}$ **s**



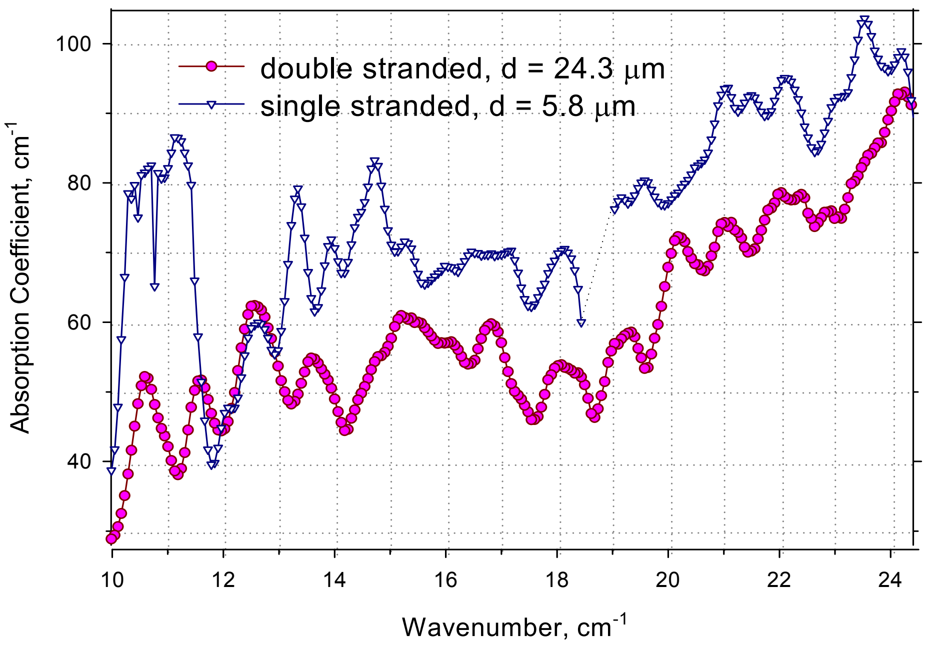
T.Globus, UVA

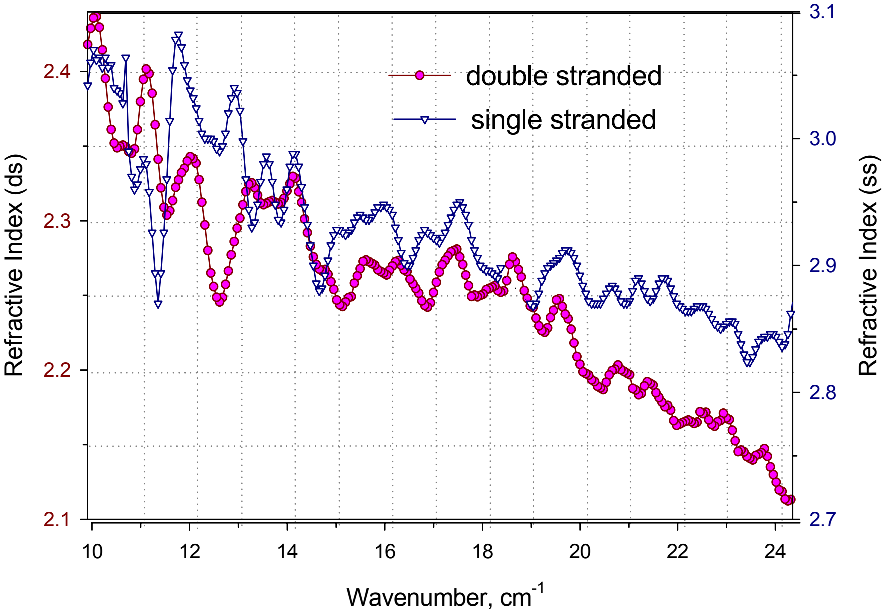
Long term reproducibility

- Improved technique for solid film sample preparation:
  - good alignment
  - reduced amount of material from 15-20 mg to 1- 3 mg for one sample
  - reproducibility better than 0.5%



Single- and double stranded Salmon-DNA



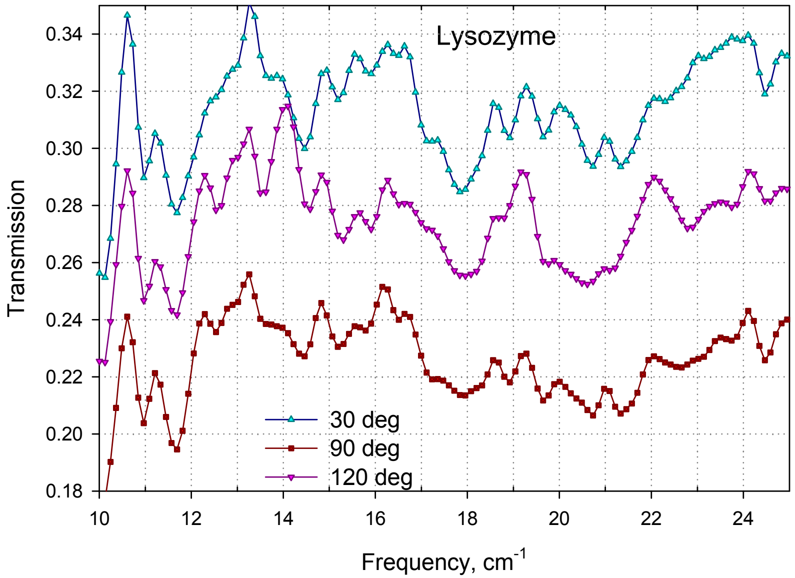


- Absorption for ss-DNA ~ 20 % higher than for ds-DNA.
- Additional peaks in α at 11.2 cm^{-1},13.4 cm^{-1} and 14.8 cm^{-1} for ss-DNA.
- Higher n for ss-DNA.

THz spectra are sensitive to conformation change that can be used for monitoring folding-unfolding of DNA

THz spectra of Biopolymers in water (gel)

Importance: **all living matter is in a liquid form**

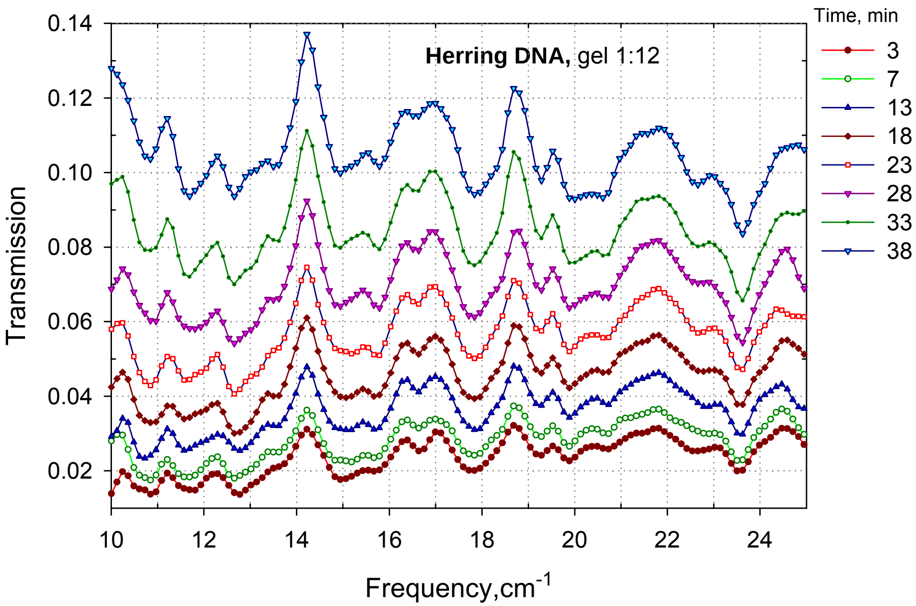


Disturbance at 18.6 cm^{-1} is due to absorption of water vapor in air.

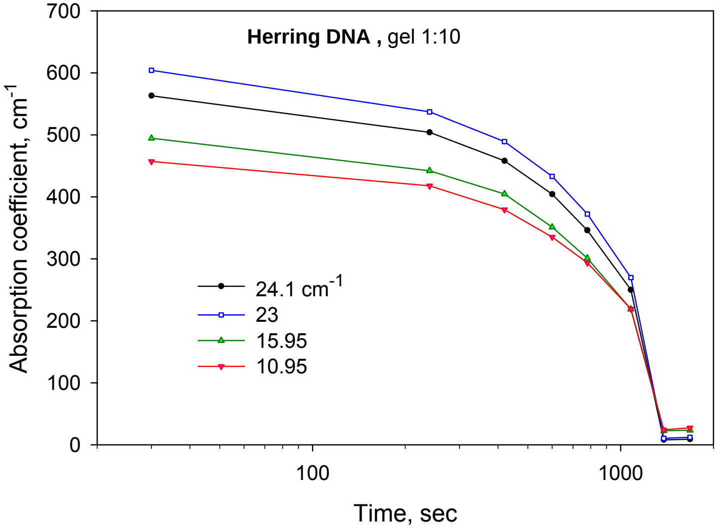
- Biological materials in **an aqueous form (or gel)** can be characterized as well as in solids.
- **Signature is strong.** Relative **change in the peak** up to 10-30%.
- **Spectra are not disturbed** by water absorption **at THz (except at 18.6 cm^{-1})**.
- **High sensitivity of spectra to orientation**.

- Possible applications: **structural characterization of proteins and DNA at THz; monitoring biological processes.**

Structural phase transition



Changing transmission spectrum of liquid herring DNA sample with time after defrosting.



Several vibrational modes

❖Temperature of **structural transitions** is close to RT and **is sensitive** to environmental conditions, including **humidity.**

❖Very **high reproducibility of resonant features** up to transmission level 90 %.

Sensitivity limit in aqueous form as well as the possibility to measure **polarization effects require clarification.**

Possible applications of THz spectroscopy

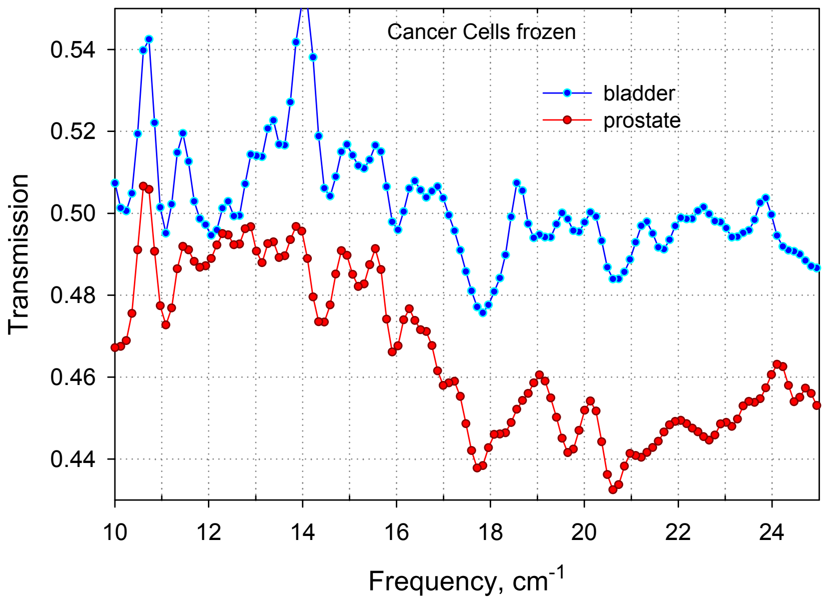
- Wide-range of biomedical applications based on close relationship between structure and spectra, including **monitoring changes in molecular conformation in real time**
  - real-time analysis of protein binding for transport
  - protein binding with antibodies,
  - specificity interactions between proteins and nucleic acids
  - binding stability studies of drug-protein and vitamin-protein systems
  - disease diagnostic

- Systems for bio-detection and identification

- Remote sensing of biochemical agents

Bio-Medical Application: Disease diagnostic

Cancer cells suspended in buffer solution (Phosphate-Buffered Saline) with the ratio of dry material to liquid ~ 1:10 were measured



Spectra of prostate and bladder cancer cells

THz spectroscopy appears to be **a promising approach** toward **discriminating between different tumor phenotypes.**

Can we use tissue for cancer characterization?

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