

Sub-terahertz Vibrational Spectroscopy with High Resolution for Biological Molecules and Cells Identification

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It's hard to accept, that with cellular, Wi-Fi, and Bluetooth connections so ubiquitous, parts of the electromagnetic spectrum remain available in our need to fill our lives with constant connection technology. Unused and, for the most part, ignored—electromagnetic radiation in the terahertz frequency range, however, are an untapped resource. This part of the spectrum, lying between microwaves (<100 gigahertz) and far IR radiation (>10 terahertz), span the cusp between electronics and photonics, and have a reputation for being difficult to use. One intriguing quality of terahertz radiation is its ability to excite low-frequency molecular vibrations between groups of atoms in biomolecules. Because the vibrational absorption frequencies are dependent on the molecular environment, each biomolecule produces its own vibrational signature, providing the potential to use terahertz excitation to identify unknown molecules [1].

For many years, theoretical studies predicted the existence of multiple resonances in absorption spectra of biological molecules in the THz range. THz radiation is absorbed by these molecules exciting low-frequency internal molecular resonance vibrations. These vibrations involve atoms within molecules connected by the weakest bonds: hydrogen bonds, van der Waals and/or other non-bonded interactions [2,3]. Intramolecular hydrogen bonds, like -C....H-N-, -C....H-O-, -N-H....O=C- determine the structure, function and kinetics of biopolymers. Although hydrogen bonds have only ~5% of the strength of covalent bonds, by working together, multiple hydrogen bonds stabilize the secondary structure conformation of polypeptides, and hold the two strands of the DNA double helix together. Experimental absorption spectra of bio-molecules and species in the sub-THz/THz region reveal these low frequency molecular motions. As hydrogen bonds determine the three-dimensional molecular structure, vibrational resonance frequencies are sensitive to conformational changes in molecules and to environmental effects that influence structure. It can be expected that each biomolecule possesses unique resonant absorption characteristics, a THz fingerprint, which could be utilized for identifying and characterizing objects based on THz spectroscopic measurements [4].

Vibrational resonance spectroscopy in the THz range is thus an emerging technique for bio-sensing, for examining bio-molecular structure and dynamics, and to characterize biological materials based on specific resonance features in absorption spectra of molecules or entire bacterial cells/spores. Several techniques, such as X-ray crystallography, electron crystallography, and NMR, are currently used for studying high resolution structure of biological molecules. Although these techniques can provide atomic resolution, there are many difficulties in realization including requirements of special sample preparation. Lower resolution optical techniques, like IR or THz Spectroscopy, can still provide valuable, global structural information, in particular by monitoring structural changes in response to a physiological stimulus [5].

Recently, it became clear that THz radiation can be extremely important for the life sciences related research because of the unique capability of these low energy electromagnetic waves to interact with

vibrations of atoms within biological molecules to produce specific molecular fingerprints [6,7]. THz spectroscopy is the only technique capable of directly detecting the weakest hydrogen bonds and other non-bonded interactions within biopolymers. Due to this unique capability of THz radiation to probe biomolecules for weak interactions, THz vibrational spectroscopy addresses very different properties than spectral measurements in the IR, Visible, or UV regions, and potentially with much higher specificity.

Traditionally, identification of pathogens from samples relied on the collection and isolation of species using culture methods. The identification process then utilized microscopic examination to determine phenotypic characteristics of bacterial colonies. Molecular biology and biochemical methods were then used to assist in final identification of microbial species. Cultivation process alone might take longer than one week. There is, therefore, an increasing need for alternative methods that allow for fast and reliable identification of microorganisms. Accurate identification of infectious agents (such as pathogenic bacteria) is critical for effective diagnosis and treatment of bacterial diseases. In addition, rapid monitoring and detection of pathogens in food products are also important for protecting human health.

Terahertz (THz) vibrational spectroscopy represents a relatively new experimental method, potentially more effective than standard methods, especially when the quantity of sample is limited. The emerging instruments for highly resolved THz vibrational spectroscopy provide an optical, label and reagent free technique, which can be utilized for examination, detection, and identification of bacterial cells to the level of strains.

According to theoretical predictions resonant frequencies of intramolecular motions, occurring below 300 cm^{-1} , are strongly dependent on the three-dimensional molecular structure, established by intramolecular hydrogen bonds and non-bonded interactions between functional groups [3]. As such, vibrational modes excitation energies are sensitive to conformational changes within molecules and to the environment. Absorption spectra in the THz region reveal the low frequency molecular motions of biomolecules and species. Each bio molecule possesses unique resonant absorption spectral features, a THz fingerprint. Thus, the THz vibrational spectroscopy technique for objects identification is based on the specificity of spectroscopic

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signatures at characteristic frequencies. The capability of recently emerged THz spectroscopy to detect directly low-frequency vibrations of the weakest atomic interactions is unique, providing different information than what is obtained from visible or IR spectroscopic characterization. Big advantages of THz spectroscopy are that it is an optical method, requires no labels or reagents, requires little sample preparation, and is non-destructive for living species.

The ability of THz spectroscopy to detect low frequency vibrations provides information quite different from other spectroscopic characterization methods. This uniqueness potentially opens applications for THz vibrational spectroscopy in many areas such as:

- Monitoring of biological processes in real time
- Portable instrumentation for biomaterial structure testing
- Evaluation of conformation change in biomedicine and pharmaceutical processes
- Monitoring drug-bacteria cell wall interactions in drug development
- Rapid testing of tissue and bio-cells for disease diagnosis, a promising approach toward discriminating between different tumor phenotypes
- Detection and identification of harmful biological species
- Water quality monitoring

Progress in THz spectroscopy and imaging techniques has however been impeded by a number of key issues that include low spatial resolution due to the relatively long wavelengths (~1 mm) associated with THz energy. Besides, to provide the needed information the spectral resolution of spectroscopic instruments has to be less than the spectral width of features to be measured. Although multiple resonance absorption lines in sub-THz region have been reported in measurements with appropriate spectral resolution, for example [8-13], successful application of THz spectroscopy for DNA, RNA and protein characterization requires deep understanding of relaxation processes of atomic dynamics (displacements) within a macromolecule. The dissipation time is one of the fundamental problems related to THz vibrational modes in biological molecules. There is, however, a large uncertainty regarding the widths of individual spectral lines. Both, the width and the intensity of resonance features observed in sub-THz spectra are sensitive to relaxation processes of atomic displacements within a macromolecule, but the mechanisms determining relaxation (dissipation) times in dynamics processes are not completely understood. It is clear, however, that the decay (or relaxation) time, τ , dictates the spectral width and intensity of vibrational modes, and thus the required spectral resolution, which eventually limits the discriminative capability of sub-THz spectroscopy.

Individual spectral line widths and the intensity of resonance features in sub-THz spectroscopy are sensitive to relaxation processes of atomic movements within a macromolecule. Thus, the knowledge of relaxation times of atomic oscillations will be critical for successful application of THz spectroscopy to characterization of hydrogen bonds [14].

The predicted range for molecular dynamics relaxation times without actual bio-molecular conformational change varies from approximately 1.5 ps to 1 ns in different studies [15,16]. Corresponding values for the dissipation factor, γ and the spectral line width, W , which are reciprocal to τ are between 6 cm^{-1} and 0.01 cm^{-1} . Values of W above 1 cm^{-1} would result in structure less sub-THz spectra, since resolution

of vibrational resonances would not be possible when there is a high density of low intensity vibrational modes [10].

The contribution from water into THz spectra of biological polymers is important. Solid thin film samples have at least some amount of water. In the case of biomolecules in aqueous solutions this contribution is crucial. However, the properties of hydrogen bonds within biological molecules are different from bulk water.

In [14], statistical analysis of molecular dynamic (MD) simulations of the *E. coli* protein thioredoxin (2TRX) was used to study relaxation vibrational dynamics of two intra-molecular H-bonds, O/H-N and O/H-C, within the structure. Two different complimentary techniques were employed in that study, one focused on analyzing the statistical distribution of relaxation time and dissipation factor values relevant to low frequency oscillations, while the second analyzed the autocorrelation function of low frequency quasi-periodic movements. The atoms were found to be involved in a number of collective oscillations characterized by varied relaxation time scales ranging from 2-3 ps to greater than 150 ps. Although short time oscillations have higher statistical probability, their contribution to absorption spectra is negligible because of low dipole moment change during vibration [17]. The existence of long-lasting dynamic processes is confirmed by the relaxation dynamics of side chains in thioredoxin, which were observed by time resolved fluorescence experiments [18]. These long lasting relaxation processes provide high intensity absorption peaks and open the possibility to directly observe and study the vibrational modes of hydrogen bonds in sub-THz absorption spectra of bio- molecules if spectra can be measured with the necessary spectral resolution.

Multiple vibrational resonance features do exist in absorption (or transmission) spectra of biological materials in the terahertz (THz) frequency range, 0.1-10 THz, including at the lowest frequency end of this band, 0.1 THz. THz spectroscopy opens new opportunities for detailed studies of the form and function of biological molecules as well as biomolecular interactions. It is fast, less expensive techniques that can provide useful genetically relevant information on biomaterials using relatively small database.

Bacteria and spores are very complex biological objects and one of the scientific goals of THz spectroscopy is to find out from what molecular components the THz signature of spores, bacteria, viruses and biological cells come from. Contributions from separate molecular components should provide insights into the features of the whole bacterial cell spectra. Genetic material, proteins, bacterial cell wall, cell membrane, proteinaceous coat might all contribute into the bacterial cell/spore THz signature. In particular, the genetic material of bacterial cells and spores might be essential for their THz signature. The understanding of contributions from major structural components into the signature of the whole object enables us to develop the modeling approach and to predict the spectral signature of a target.

Experimental results from measurements with high spectral resolution (1 GHz or 0.03 cm^{-1}) produce spectra with very intense and narrow spectral features from biological molecules and bacteria having peak widths between 0.05 cm^{-1} and 0.2 cm^{-1} [19-22]. These features were not evident in results from earlier measurements having a resolution of 0.25 cm^{-1} .

Vibrational resonance spectroscopy in the Terahertz (THz) frequency range (0.1-3 THz or $3\text{-}100 \text{ cm}^{-1}$) is a newly emerging technique for fingerprinting biological molecules and other materials. Multiple resonances-vibrational modes are available for sensitive characterization of bio-molecule structure, and spectra are more species

specific. THz spectra from biological molecules and organisms are specific to the molecular sequence and the three-dimensional structure, thus well-resolved spectra can be used for fast characterization and fingerprinting. Overlapping of neighboring absorption bands is less. Frequencies below 1 THz are especially attractive for practical applications because there is little absorption of this radiation from water or from water vapor absorption (sensors thus do not require evacuation or purging with dry nitrogen).

THz resonance spectroscopy was impeded in the past by the absence of THz sources of radiation, detectors and spectroscopic systems that effectively couple radiation and biological material samples, resulting in issues with sensitivity. In the last 10 years, the situation was changing rapidly due to new sources and detectors becoming available [23]. However, most of experimental data were obtained at frequencies above 1 THz where the power of sources is growing fast with frequency. Unfortunately, the strongest THz signature features for large macromolecules and organisms occur in the sub-THz region. Instrumentation that works in this frequency region is still limited, thus not a lot of research has been performed in this area [24].

Until recently, a Bruker Fourier transform (FT) transmission spectroscopy instrument (IFS66v) provided the most detailed sub-THz vibrational spectral signatures for biological molecules [5,10,25,26], but had only a moderate spectral resolution of 0.25 cm^{-1} . However, the low THz power provided by traditional sources, like a mercury lamp, required a detector cooled with liquid helium for reliable characterization. The 12 mm optical aperture of this instrument dictated a large area sample with mg quantities of material. Measurements in air were possible since there are almost no absorption lines from water vapors or oxygen in the $10\text{-}25\text{ cm}^{-1}$ spectral range except water vapor absorption band around 18.6 cm^{-1} . However, a spectral resolution of 0.25 cm^{-1} does not provide the required specificity of spectral features to discriminate between individual molecules or bacterial strains.

A number of instruments that are based on time domain or photomixing technologies have recently been introduced for producing and measuring THz radiation, however most of them do not have the sensitivity, or spectral and spatial resolution required for biological molecules and cells characterization [4,5].

A new, frequency domain, near field spectroscopic instrument with imaging capability combined with a microchip for sample material that permits characterization of traces amounts of biological materials at room temperature has been recently introduced [20]. This novel continuous wave, frequency domain instrument relies on a very strong local enhancement of the electromagnetic field, which allows increased coupling of the THz radiation with the sample biomaterials.

Over the last 3 years, this new, sub-THz spectroscopic instrument was used to measure transmission/absorption spectra from biomolecules and cells demonstrating very intense and narrow spectral features with widths between 0.05 cm^{-1} and 0.15 cm^{-1} [20,27-29] that reflect low frequency molecular motions. It was found that experimental spectra from biomolecules correlate reasonably well with computational predictions, that the spectra from different cells are sufficiently different, and that cells can be identified based on their spectral signature. Computational modeling results confirmed that the observed spectroscopic features from cells can be ascribed to fundamental physical interaction mechanisms between THz radiation and biological macro-molecules inside the cell [26-28]. In particular, the results show that spectroscopic signatures of microorganisms originate from combinations of modes or group of low frequency vibrational

modes at close frequencies (vibrational bands) within the molecular components inside the cells [26]. These types of spectral features were not observed in results obtained previously with a resolution of 0.25 cm^{-1} . The existence of multiple intense and specific resonance features provide the conditions required for reliable discrimination using sub-THz frequency domain spectroscopy, discrimination even to the level of strains of the same bacteria, which was not possible before.

Very recently the new THz spectroscopic technique and system having high spectral and spatial resolution [20] was demonstrated in application for potential quantification of molecules such as micro-RNAs in cancer cells and body liquids with further potential development leading to clinical analysis of cancer [29]. Combined experimental and computational approaches were used to investigate the ability of sub-THz spectroscopy to identify and quantify biological molecules. The initial study using this technique demonstrated spectroscopic signatures from ovarian cancer cells by interrogation of resonance features caused by atomic vibrations within biological molecules, which are absent in normal cells. Ovarian cancer is used for this first demonstration; however the method is very general and can be as well applied to other cancer types.

The application of sub-THz absorption/transmission technology for early cancer diagnostic and prognosis should be possible and simpler than current methods. Since THz radiation detects signatures from components inside the cancer cells, the complicated procedures of extracting biomarkers from the cells would not be necessary and could be eliminated. This spectroscopic method provides the opportunity to look for specific spectral signatures from molecules within each cell, to differentiate between cell types, or to look at influences of environmental factors on cellular function, representing new analytical research capabilities not provided by current instrumentation. The new technology can also significantly assist qPCR methods by providing quick preliminary data and making DNA and RNA analysis a more effective and targeted procedure.

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