Vibratess LLC for Vabio Application

Vibratess, LLC (Vibrational Terahertz Spectroscopic Sensors) was formed in 2007 as a spin-off from the University of Virginia to develop and commercialize low terahertz (THz) resonance spectroscopy for biological materials detection and characterization. Vibratess is a woman-owned small business that has received continuous funding from Small Business Innovation Research Grants and Contracts for 9 years.

Highly resolved sub-Terahertz (THz) spectroscopy is a novel analytical method being developed for molecular and cellular analysis of samples in a broad spectrum of life science related applications. THz waves are important for life science because of the unique capability of this radiation to interact with low energy vibrations of weak, so called hydrogen bonds between atoms within biological molecules. Although hydrogen bonds are very weak (~ 20 times) compared to covalent bonds, these weak bonds in biomolecules and cells do essential work. Hydrogen bonds determine the structure and properties of most biopolymers, including the structure of double stranded DNA and secondary structures of proteins, and therefore are vital for cellular processes and bio-molecule interactions relating to DNA, enzymes, proteins, ribosomes, and their functions. Absorption of radiation happens when a photon's energy coincides with the energy of the internal molecular vibrations. Because the resonant frequencies of these vibrations are sensitive to a molecule's 3D structure, the radiation interacts with each type of biomolecules differently, producing specific molecular absorption spectral fingerprints that can be used for identification and characterization. As sub-THz radiation propagates through biological cell, molecular components, like proteins and genetic material, all contribute to a detailed absorption spectral signature allowing cellular identification. Because THz spectroscopy is the only technique that can directly probe and detect the weakest hydrogen bonds and other weak non-bonded interactions within biopolymers, it can be used to address properties that spectroscopy using IR, Visible, or UV radiation cannot.

The recent introduction of new sources and detectors operating in the sub-THz frequency range has allowed Vibratess to design and build spectroscopic instruments for multiple applications of vibrational resonance spectroscopy for fingerprinting of bio-molecules and entire biological cells. Using Funding from DoD Contracts and self-research, Vibratess has developed and built 3 generations of automated sub-THz spectrometer prototypes ready to use for characterization of ultra-small amounts of sample materials in solutions (Fig. 1). The high sensitivity of these instruments and spatial resolution ~ 10-20 μm became possible via a novel approach to the fundamental problem of improving THz radiation coupling to biomolecules based on local electro-magnetic field enhancement through the use of metal array sample holders [2, 3].

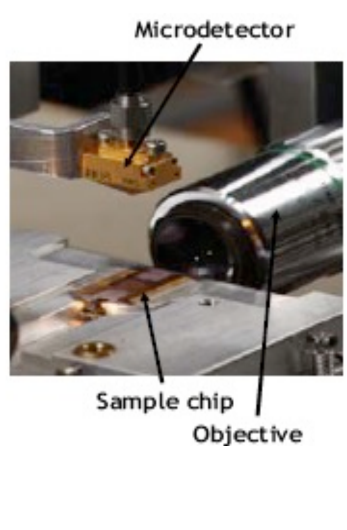

Fig.1. Sample chip in Vibratess' Spectrometer, Vibr-2, for general laboratory applications. [1]

The objective is used for visualization of a disposable chip device with a periodic structure for sample material along with the microdetector in a Vibratess' housing. Detector is in the upper position for loading sample using micropipette or micro-syringe.

A sample table is movable in 2 directions (0.13 μm step) and the detector movable in the Z direction.

The latest version of Vibratess' spectrometer, Vibr 4, is a rather small, fast (one scan 2-5 min), highly sensitive, simple and easy to use instrument for one touch operation between 315 and 490 GHz with a spectral resolution better than 1 GHz. All of our instruments have been intensively used for more than 6 years and tested for sensitivity, accuracy and reproducible results. The measurements procedures have been optimized, the required optimal amount of sample material and concentration in liquids have been found; software for using the instruments has been developed.

Problem that we are solving.

Vibratess Terahertz resonance spectroscopy technology and spectroscopic sensors are a fast, optical, label-free and reagent-free, highly innovative approach to improve molecular and cellular characterization analysis for life science applications, including but not limited to early detection, diagnosis, prognosis and therapeutic treatment of cancers and other diseases that are currently difficult to detect. The experimental measurements are performed in conjunction with molecular dynamic (MD) simulations to predict, understand, and analyze the molecular absorption signatures produced by radiation in the sub-THz frequency range. Our group has developed computational modeling techniques to predict and study THz spectroscopic signatures of large DNAs, RNAs, proteins and other molecular components of cells using energy minimization, normal mode analysis and Molecular dynamics (MD) approaches [4-7].

Figures 2 & 3 give examples that compare measured and simulated signatures. This comparison provides both confirmation of experimental results, and ways to predict spectra that can be used to analyze for the presence of specific compounds in samples.

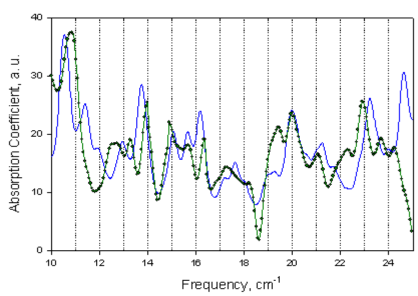

Fig. 2. Absorption of tyrosine transfer RNA, MD simulation (blue) and experiment (green) measured with a Bruker Fourier Transform Spectrometer and a liquid helium cooled detector, spectral resolution ~ 0.5 cm $^{-1}$ [1].

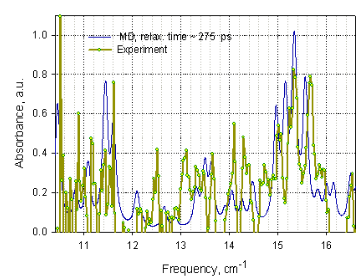

Fig. 3. Absorbance spectra of protein thioredoxin from *E. coli*, MD simulation and experiment. Vibr-2, room temperature, spectral resolution ~0.1 cm $^{-1}$ [4].

The observed resonances in absorption spectra of biological molecules due to hydrogen bonds vibrations have been confirmed by computational modeling, which has demonstrated the existence of long-lasting relaxation processes within molecules, that result in detectable absorption peaks [8]. Combining simulations with experimental techniques improves analysis and understanding of measured spectra and even provides predictive capabilities from modeling (see also our published book chapters [9, 10]).

Current Marketable Products:

-Automated, simple optical spectroscopic instrument prototypes (Systems) for fast operation at room temperature with high sensitivity, high spectral resolution (selectivity), spatial resolution below diffraction limit (~150 μm).

-Customized THz Biosensor Prototypes for Specific Life Science Related Applications.

-User friendly Operational Software for Spectroscopy Systems and Biosensors.

-Customized THz Sample chips for Dry and Nano-Fluidic Samples

-Sub-THz radiation microdetectors in a Customized Waveguide Housing

Marketable Services:

• THz Spectroscopic characterization of biological and organic materials: macromolecules, cells and microorganisms.
• Development of Experimental databases for THz spectroscopic signatures from certain biological molecules and objects.
• Computational modeling databases for prediction and analysis of spectroscopic signatures from bio-materials.
• Consulting services on potential applications of this new technology.

Biomedical Applications: microRNAs and Cancer

The sub-THz resonance spectroscopy technology and innovative spectroscopic systems Vibratess introduced have recently being applied in Oncology where cancer is studied at the molecular level.

Advances in sequencing technologies have led to an increased focus on blood-derived nucleic acid-based approaches for biomarker discovery. If appropriate biomarkers are available, human serum and other body fluids could be measured in clinical diagnosis of cancer. Multiple evidence described in medical publications suggest that microRNAs are involved in cancer and other diseases development. MicroRNAs in serum are sufficiently stable to serve as clinical biomarkers, and serum microRNA profiles reflect physiological conditions. ***Quantification,*** however, remains a problem with extracting and analyzing circulating microRNA using traditional approach. These are multi-step, time consuming procedures yielding terabytes of data requiring bio-informatics analysis capabilities. On the contrary, THz absorption spectra can give rich visual information (frequencies and intensities of multiple absorption resonances) about molecules circulating in blood or other bodily liquids.

In our work we introduced and validated sub-THz resonance spectroscopy combined with MD computation as promising technology for optical analysis and potential quantification of micro-RNA molecular biomarkers for ovarian cancer (OC) and normal control (NC) samples (see examples in Figures 4 and 5). This allowed us to identify micro-RNA as the molecular components that mostly contribute to the experimental signature observed for ovarian cancer cells [11-14].

We have also tested saliva, as an easily collected proximal biofluid containing numerous microRNAs that presents an additional attractive noninvasive diagnostic tool in detecting diseases, determining prognosis, and effects of therapy [12]. Experiments have also demonstrated the difference between ovarian and breast cancer signatures.

This approach, which is very general, can be used for molecular diagnostic of other difficult to detect cancers and other diseases [12, 15].

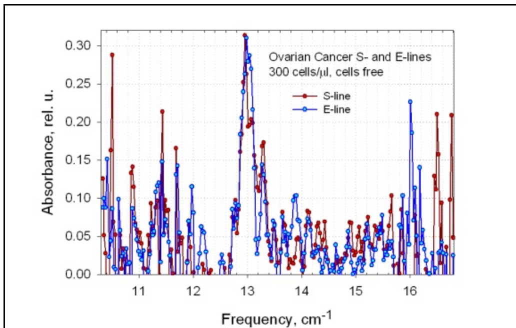

Fig. 4. Comparison of absorbance spectra from two ovarian cancer lines, SKOV3 (ATCC® HTB-77™) and ES-2 [11].

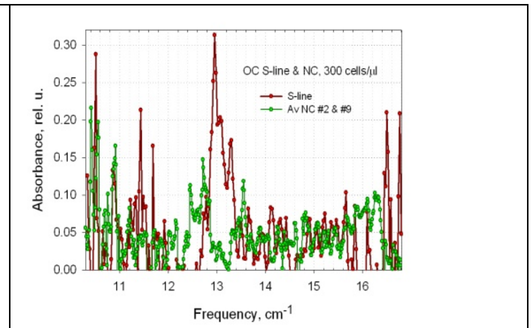

Fig. 5. Comparison of absorbance from cancer S-line (brown) and normal control (green) samples [11].

microRNAs and Neuroscience

As confirmed by molecular dynamic simulations of spectra from these molecules, we demonstrated that microRNAs are the molecules present in body liquids, which we are mostly detecting. These molecules dominate since they are rather small and easily penetrate from inside cells into blood and exhibit strong absorption bands in this range. These molecules are very stable but deregulated in the bodies of patients, with changes occurring in miRNA profiles in response to changes in the physiological conditions in the body.

The review of recent medical publications reveals increasing evidence of the involvement of miRNAs with pathogenesis in many neurodegenerative disorders including Parkinson's disease, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis. All of these diseases are characterized by the progressive loss of neuronal function and structure, which eventually leads to neuronal death in the nervous system [16]. Many studies have shown a significant association between blood brain barrier (BBB) dysfunction and neurological diseases. However the molecular mechanisms of BBB dysfunction that is essential for therapeutic treatments is not yet understood.

Even a fast review of resent publications on neuronal MicroRNAs indicate the importance of molecules from this class, specifically microRNA327 in Brain Tumors, 11 microRNAs implicated in Neuronal Fate of Neural Stem cells, microRNA137 and microRNA181C identified as important in Alzheimer, and microRNA137 involved in Schizophrenia [17]. The authors of a recent review paper call it "Decoding the ubiquitous role of microRNAs in neurogenesis".

In more recent overview of microRNAs as Biomarkers of Amyotrophic lateral sclerosis [18] the authors emphasize that "**while the properties of miRNA as biomarkers of ALS ... are ideal...**" there are challenges that could be related to the methodology and difficulty in quantifying the concentration when using miRNA RT-qPCR kits due to the minimal and varied amount of RNA in biofluids, among other reasons. In addition the presence of one of the most commonly used biomarkers in ALS, the neurofilament proteins that has been found in serum, can be detected using Vibratess spectrometer if needed.

The introduction and use of a simple and fast approach for visualization and quantification of microRNAs that transfer genetic information from RNAs to proteins and modulate both neuronal and immune processes, promises to significantly facilitate and accelerate understanding by scientists at the molecular level toward neurodegenerative prognosis and therapeutic treatment selection.

In conclusion: We are engineers and we built the tools. We are interested in collaboration with neuroscientists who can and wish to use these tools to make the discoveries in their research more straightforward. The latest version of our instrument is very easy to use. In addition we can provide initial database - THz signatures of specific microRNAs important for you.

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