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FUNDING NUMBERS W911NF0510033. REPORT NUMBER 47213ELCDP

There are two different parts in this project.

**Part I.** Our project "New Concepts for Detection Biological Targets: Terahertz Signature Data Base Generation" is in direct response to the BAA W911NF-04-R-0006 topic area II.B.5.a, CB simulants and correlation of physical and chemical properties to those of CB agents. This two year project was completed on January 31, 2007.

The most detailed report on this part I can be found in two Conference Proceedings and in the Interim Progress Report, Aug 30, 2007:

- T. Globus, A. Bykhovski, B. Gelmont, R. M. Weikle, J. O. Jensen, W. R. Loerop, "Enhanced Spectroscopy Signatures of Biological Molecules and Organisms in the Low THz Range". The 2007 Scientific Conference on Chemical & Biological Defense Research, Timonium, Maryland, 13-15 November 2007.
- T. Globus, A. Bykhovski, B. Gelmont, J.O.Jensen, "Terahertz Spectroscopic Signature of Biological Species", 7<sup>th</sup> Joint Conference on Standoff Detection for Chemical and Biological Defense, Oct. 23-27, Williamsburg, VA (2006).
- 3. Interim Progress Report Proposal Proposal Number: 47213ELCDP Agreement/Contract Number: W911NF0510033, Report Date: Aug 30, 2007.

#### Statement of the problem studied in Part I

Objective: To establish a realistic and credible foundation for the future detection technology of biological warfare agents in the form of aerosol and solutions. This new technology is based on combined experimental and modeling research on spectroscopic signature of bio- materials in the terahertz (THz) range.

The focus of this project was:

1) to construct a reliable experimental database for THz signature of bio-simulants;

2) to develop computational models for THz signature simulation of cellular component molecules (DNA, RNA, Proteins);

3) to develop component-based models for bio-simulants and agents;

4) to simulate THz vibrational spectra of bacterias, spores and their structural components;

5) to verify predicted spectral characteristics of bio-simulants by comparison calculated and measured spectra and to improve models;

6) to construct a predicted database for THz signature of bio-warfare agents.

#### Summary of the most important results of the Part I

During the two year period we developed a sensitive experimental technique for THz characterization of biological objects, conducted experimental data base collection for bio simulants, basically in the form of solution, and we developed a first round of computer simulation models for

THz signatures of simulant cells, spores, their constituents, and also signatures of bioagent –anthrax. We compared modeling results with experimental data for simulants and found significant correlations. However, we realize that the second round of modeling and experimental database generation is required to improve models for even more reliable prediction of bio-agent signatures.

For each particular bio-organism the set of absorption resonance frequencies has been determined in the spectral range of interest (10-25 cm<sup>-1</sup>) with the goal to obtain the reliable database that does not change depending on experimental conditions, such as a method of sample preparation (including aerosol, liquid suspension, or powder in inert matrix), sample thickness, concentration of biomaterial in matrix, substrate material. The complete set of these resonance features can be used for detection and identification of specific biological objects. The experimental database of simulants signatures (*Bacillus subtilis, Escherichia coli*) at THz was constructed in this project for developing the next generation sensing systems required to effectively counter potential biological attacks on the homeland (Reports #13&14 for March and Aril 2006 and publications [T. Globus, A. Bykhovski, B. Gelmont, J.O.Jensen, "Terahertz Spectroscopic Signature of Biological Species", 7<sup>th</sup> Joint Conference on Standoff Detection for Chemical and Biological Defense, Oct. 23-27, Williamsburg, VA (Nov. 2006); T.Globus, T. Khromova, D. Woolard and A. Samuels, "THz Resonance Spectra of Bacillus Subtilis Cells and Spores in PE Pellets and Dilute Water Solutions," SPIE Proc. V.6212-21, Defense and Security Symposium (DSS06); Conference: Terahertz for Military and Security Applications IV, Orlando, Florida, Apr.17-21, 2006.]).

In parallel with the experimental work, the computational modeling technique was developed and the component based models were created to simulate the THz spectra of entire bacterial cells or spores, their genetic and other components. All available information on the structure of cell components, such as DNA sequencing for genetic materials of organisms, X-ray data for atomic structures of membranes, specific proteins, RNAs, lipids, and pdb files from the protein databank and other sources with initial three-dimensional structures were used for simulations. In the computational procedures, the initial structures were optimized using available program packages for energy minimization and molecular dynamics (Amber). The realistic statistical approach was developed for modeling THz signatures of chromosome DNA for Bacillus subtilis, Escherichia coli, and Bacillus anthracis with actual base pair distributions and base pair ratios (C/G/A/T) [A. Bykhovski, X. Li, T. Globus, T. Khromova, B. Gelmont, D. Woolard, A. C. Samuels, and J. O. Jensen, "THz absorption signature detection of genetic material of E. coli and B. subtilis", SPIE Proceed. V 5995, pp.59950N-1:10, 2005, Optics East, Boston, Oct. 2005]. THz absorption spectra for *Bacillus subtilis*, *Escherichia coli*, and *Bacillus* anthracis and their components were simulated [A. Bykhovski, T. Globus, T. Khromova, B. Gelmont, D. Woolard, "Resonant Terahertz Spectroscopy of Bacterial Thioredoxin in Water: Simulation And Experiment", ISSSR 2006-67; Int. J. High Speed Electr. Systems; A. Bykhovski, T. Globus, T. Khromova, B. Gelmont, and D. Woolard. An Analysis of the THz Frequency Signatures in the Cellular Components of Biological Agents, Int. J. High Speed Electr. Systems (2006); A. Bykhovski, T. Globus, T. Khromova, B. Gelmont, D. Woolard, and M. Bykhovskaia, "An Analysis of the THz Frequency Signatures in the Cellular Components of Biological Agents", SPIE Defense and Security Symposium 2006, V 6212-18, p. 132-141. Orlando, Florida, April 2006].

Two most **important results** obtained in this research are:

### 1. The THz signatures unique for each bio-agent.

# 2. The genetic components (DNA and RNA) contribute significantly into the signature of the entire cell.

Comparison with experimental spectra was used to optimize models and to adjust simulation parameters. This process of adjustment requires significant time and efforts and has not yet been completed. The second round of optimization is required for reliable prediction of THz signature for *Bacillus anthracis* and other bio agents.

## Part II. Rapid, Reagent-less Detection and Discrimination of Biological Warfare (BW) Agents using Multi-Photon, Multi-Wavelength Processes within Bio-Molecular Architectures. Task: Molecular-Level Platforms for Spectral Characterization.

This work is the part of a broad research on bio-molecular-based device concepts for enhanced sensing Terahertz (THz) frequency bio-signatures. The overall Project Goal is discovering new effects and demonstrating reproducible results in the Terahertz active and passive spectral characterization of biological molecular structures with well defined composition and spatial orientation.

**Statement of the problem studied in the Part II.** Three major objectives of this research are: 1. Developing optical setup and collecting bio-signature information in the THz range from multiple states of biological molecules resulting from both electronic and geometrical transformations of biomolecules under the influence of photo-excitation in the visible or UV radiation.

2. To demonstrate THz detection of conformational changes in a sensor like structures with retinol, retinoic acid or stilbene.

3. To improve collection capabilities of THz signatures with increased levels of sensitivity and repeatability by utilizing well aligned bio-molecular arrays of natural and artificially designed structural elements, and to test and to verify THz-Optical properties associated with coupling of bio-molecules to other organic and inorganic systems.

**Impact of the Research:** The impact of this research is that it will derive THz spectral bio-signatures for organic molecules that are amenable to photo-induced metastable-state conformations, define THz properties associated with both electronic and geometrical transformations of bio-molecules under the influence of coupling to other organic and inorganic systems and will establish an initial scientific foundation and design blueprint for an enhanced THz bio-signature sensing capability.

**Merit of the Research:** The information collected in this project will be used for future development of THz photonic devices utilizing biological molecular electronic elements.

Detailed reports of Results from this Project have been presented at two US ARMY Meetings and in the Interim Progress Reports, Aug 2007 and 2008:

1. T. Globus, B. Gelmont, "Molecular-level platforms for THz spectral characterization", US ARMY Meeting, Duck Key, Florida, Dec. 2007.

 T. Globus, B. Gelmont, A. Bykhovski, "Molecular-Level Platforms for THz Spectral Characterization", Presentation at US ARMY Meeting "Advanced Architecture", January 2007, Edgewood.Results.
 Interim Progress Report Proposal Proposal Number: 47213ELCDP. Agreement/Contract Number: W911NF0510033, Report Date: Aug 30, 2007.

4. Interim Progress Report Proposal Proposal Number: 47213ELCDP. Agreement/Contract Number: W911NF0510033, Report Date: Aug 2008

**Summary of the most important results of the Part II.** (All Figures for Part II, see Appendix) **Task 1.** A typical Fourier Transform Infrared system (IFS-66v) was modified to include a simple off axis excitation source inside of the systems sample chamber. This modification made it possible to optically illuminate a sample inside the FTIR systems sample chamber.

Two conformations of the retinal molecule have been studied in order to characterize the molecule's THz transmission spectra in both the ground and metastable states. (See T.J. Wright, Y. Luo, T. Globus, B. Gelmont, T. Khromova, N. Swami, and A. Isin, "THz Characterization of All-trans and 9-cis Retinal, Experiment and Modeling" Sensors-02087-2000; T. Globus, B. Gelmont, "Molecular-level platforms for thz spectral characterization", US ARMY Meeting, Duck Key, Florida, Dec. 2007).

We demonstrated the existence of lowest frequency vibrational modes within retinal molecules in the sub-THz (See Fig. 1). The experimental results are close to the modeling predictions. When subjected to an adequate external excitation the retinal molecule can experience a change in conformation and associated THz transmission spectra. Conclusion: It is possible to detect conformation change in retinal molecules using THz spectroscopy.

**Task 2**. The Spectroscopic system prototype developed by the Virginia Diodes has been tested and evaluated continuous-wave (CW) spectrometer for direct transmission measurements as a function of frequency at 390-465 GHz. The system is compact, lightweight, and fully computer controlled (Fig.2). A user friendly labview program with many advanced features allows the user to maximize accuracy and sweep time with a few simple settings.

## Conclusions for a Task 2.

This is potentially a very promising instrument. We believe that the performance can be significantly improved relatively easy in several aspects: a. The ripples probably can be removed with additional apperture.

b. The collection time can be significantly reduced by eliminating background measurements for each run with the sample. This will also create much more favorable conditions for the sample measurements, especially liquid samples, eliminating shacking of bio-sample.

c. Since there is no need to move the sample out of the beam at each point, the standard holder used in most spectrometers (including Bruker) can be easily incorporated in this spectrometer. This make it possible to measure biological and other samples (including samples in solutions) using well fixed and reproduciblesample position, which is the first requirement for any spectrometer.

d. Even with the ripple effect, the instrument still can be used for characterization providing good information (Fig. 3.).

Recommendations: Modify a sample holder. Modify software. Extend the spectral range.

**Task 3.** To improve collection capabilities of THz signatures with increased levels of sensitivity and repeatability by utilizing well aligned bio-molecular arrays of natural and artificially designed structural elements, and to test and to verify THz-properties associated with coupling of bio-molecules to other organic and inorganic systems (in several different projects).

a) Self-organized technique for bio sample preparation has been developed to improve collection capabilities of THz signatures with increased levels of sensitivity and repeatability by using complexes of bio- molecules and species with water or other solvents (See Figures 4,5).

b) New spectroscopic and imaging technique to enlarge THz signatures are under development. Thetechnique is based on electromagnetic field enhancement at the edges of a periodic structure.

c) In collaboration with the teams from several Universities and companies, THz tests and characterization of bio-materials, structural elements of bio-molecular electronics, of geometrically ordered DNA-based sequences were conducted:

• Terahertz characterization of DNA self-assembled mono-layers from Prof. M. Stroscio's Lab (Nano Engineering Research Group, College of Engineering, University of Illinois at Chicago) – see figures 6,7 in Appendix.

• Terahertz characterization of Plasmids from Prof. Michael Norton's Lab. (The Department of Chemistry, Marshall University, V. Virginia)-Results are presented at the ISSSR 2008 Meeting (to be published).

As part of a broad study to survey and parse the fundamental spectroscopic features of double stranded DNA in the THz domain, we have performed a comparative study of a 2900 base pairs basic sequence, 3400 base pair basic sequence with inserts and 1000, 500 base pairs segment of the basic sequence over the range 10 to 25cm-1. Samples were generated by amplifying regions of the Lambda Phase DNA

sequence or Firefly Luciferase Gene using the Polymerase Chain Reaction (PCR). This mechanism of sample preparation addresses several potential problems associated with obtaining artifact free THz spectroscopy of biomolecules. The PCR process can rapidly yield 2 mg of sample with a defined sequence at relatively high concentrations on the order of 1 mg/ml. It is important to use a sample preparation method which provides the significant quantities of the uniform samples required for multiple duplicate experiments. An additional benefit of using an in-vitro method of DNA sample preparation is that only one enzyme (polymerase) is involved in the process, significantly limiting the number of potential macromolecular impurities in the sample. In contrast, biologically derived materials can contain many biomolecular impurities, potentially making spectra less reproducible and therefore more difficult to interpret.

Rich spectra have been obtained from each type of sample at varying concentrations (Figures 8-10). Major features appear to be relatively unaffected by concentration, over the range of concentrations studied.

Conclusion: The sensitivity of THz vibrational spectroscopy is enough to see both the effects of filtering and of insertions in plasmid DNA. We can conclude that we can detect contaminations and modifications of DNA in plasmids using THz spectroscopy.

• THz characterization of Plasmids from Prof. N. Stewart Lab (The Department of Plant Science and Landscape Systems, University of Tennessee) –See the Progress Report2007 and the US ARMY Meeting Presentation, Duck Key, Florida, Dec. 2007.THz vibrational spectroscopy can be used to detect and discriminate between different types of plasmids. This capability of THz fingerprinting could be important for particular applications since some plasmidsare able to pass easily from one cell to another and are capable of integrating into the host genome. Plasmids are widely used in genetic modification experiments to clone genes from other organisms and make large quantities of their DNA. Plasmids plays an important role in gene transfer and in infection. It typically carries one or several genes encoding proteins resistant to an antimicrobial agents (antibiotics). A number of artificially constructed plasmids are used as cloning vectors.

• THz characterization of nanofluidic cells from Dr. E. Mendosa (Rebondo Optics). (See the US ARMY Meeting Presentation, Duck Key, Florida, Dec. 2007).

We have measured THz spectra of E.coli cells in nanofluidic cells fabricated by the U. New Mexico (Dr. Steve Brueck) and Redondo Optics, Inc. (Dr.Edmund Mendoza). In Figure 12. rather good results reproducibility is demonstrated, although the signature is very weak (Results of averaging over 30 spectra for each device measured with the Teraview Spectrometer at room temperature and filtered at analysis). Tests of nanofluidic cells reveals serious problems. First, the etalon effect from the cell without and with bio-material show strong fringes due to thick silicon substrate. In most cases it is impossible to fill cell reproducibly with pure water and with solutions. As a result, the etalon effect is not compensated when spectra recalculated relatively to background, and in most cases this effect masks THz signature of biological material. Second, surface hydrofobic effects prevent appropriate filling of channels with solution of bio-material (and even with pure water). These results are used to optimize nanofluidic cell construction.

Recommendations: The thinner the substrate, and the smaller the refractive index, n, of substrate material - the better. Although reflection will be the same for thinner substrate, the fringes pattern will be stretched on the frequency scale, and it will less interfere with the signature pattern. Smaller n will reduce the reflection. Enlarge the channel cross section. Reduce hydrofobic effects inside the fluidic channels.

#### **Appendix (Figures)**

Attachment to the Final Report Febr 1, 2005 to Jul 31, 2008 New Concepts for Detection Biological Targets: Terahertz Signature Data Base Generation Tatiana Globus, Alexei Bykhovski, Boris Gelmont UVA

FUNDING NUMBERS W911NF0510033

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During the two years (02/2005 - 01/2007) we developed a sensitive experimental technique for THz characterization of biological objects, conducted experimental data base collection for bio simulants, basically in the form of solution. In parallel with the experimental work, the computational technique was developed , and we developed a first round of computer simulation models for THz signatures of simulant cells, spores, their constituents, and also signatures of bioagent –anthrax. We compared modeling results with experimental data for simulants and found significant correlations. Two most important results obtained in this research are: 1) the THz signatures are unique for each bio-organism, and 2) the genetic components (DNA and RNA) contribute significantly into the signature of the entire bio-organism. However, we realize that the second round of modeling and experimental database generation is required to improve models for even more reliable prediction of bio-agent signatures. See also the previous Interim Progress Report Aug 30, 2007, and monthly reports P-47213-EL-CDP.

Part II. "Rapid, Reagent-less Detection and Discrimination of Biological Warfare (BW) Agents using Multi-Photon, Multi-Wavelength Processes within Bio-Molecular Architectures. Task: Molecular-Level Platforms for Spectral Characterization". Starting from Aug 2006 Amendments No P00002- P00004. (See also Report Duck Key, Florida, Dec. 2007).

Detection of conformational changes in a sensor-like structures with retinal has been demonstrated. Collection capabilities of THz signatures has been improved with increased levels of sensitivity and repeatability. Spectroscopic system prototype developed by the Virginia Diodes Inc has been evaluated.

In this research the following tasks have been performed.

The optical setup was developed for collecting bio-signature information in the THz range from multiple states of biological molecules resulting from both electronic and geometrical transformations of bio-molecules under the influence of photo-excitation in the visible or UV radiation.

The existence of lowest frequency vibrational modes within retinal molecules in the sub-THz was demonstrated with the experimental results that are close to the modeling predictions. The possibility of using THz spectroscopy to detect conformation change in retinal molecules under the external excitation with UV light was demonstrated.

Spectroscopic system prototype developed by the Virginia Diodes Inc has been evaluated.

Self-organized technique for bio sample preparation using complexes of bio- molecules and species with water or other solvents has been developed to improve spectroscopic data collection capabilities.

In collaboration with the teams from several Universities and companies, THz tests and

characterization of bio-materials, structural elements of bio-molecular electronics, of geometrically ordered DNA-based sequences, and of THz sensing systems were conducted.

## Figures (Part II)



Characterization of THz spectra of biological molecules in the ground and excited states

Fig. 1. Two modes were detected in all trance retinal (ATR) samples: at frequencies ~ 14 cm  $^{-1}$  and 12.2 cm $^{-1}$  although modeling predicted only one mode at 14 cm  $^{-1}$ . The material is actually a mixture of several metastable conformations.

Evaluating the Spectroscopic system prototype developed by the Virginia Diodes



Fig. 2. Spectroscopic system prototype developed by the Virginia Diodes

Salmon DNA in water, drying



Fig.3. Two Salmon DNA samples measured using different spectrometers. Samples are different but prepared using the same technique. Vibrational modes in biological materials detected by Bruker can be detected using VDI spectrometer as well.

Improve collection capabilities. Self-organized technique for bio sample preparation: complexes of bio- molecules and species with water or other solvents.



Fig.4. Transmission spectra of 3 different samples (S DNA) in the form of gel (50 mg/ml): reproducible shape line and resonance frequencies and strong features



Fig. 5. Discriminate easily the difference between double and single stranded DNA molecules. Useful for monitoring bioprocesses THz tests and characterization of bio-materials, structural elements of bio-molecular electronics, of geometrically ordered DNA-based sequences were conducted:



Fig. 6. DNA self-assembled monolayers (SAM) samples grown on poly methyl methacrylate substrate (PMMA): 5'- Amino AC TCG AGT ACA GCG ACC CAA CAT GAG AGA AC-'3.



Fig. 7. Reproducibility of spectral features in transmission spectra of DNA self-assembled monolayers (SAM) samples grown on poly methyl methacrylate substrate (PMMA) in the laboratory of Professor M. Stroscio. Results were presented at the 2007 NanoDDS Meeting.

Fig. 8. 3 samples of pGEM, 500 bp, were measured. The spectrum from one sample compared with the averaged result over all 3 samples. A very good reproducibility of spectral features is demonstrated.



Fig.9 .Samples with 2 different concentrations were prepared from the 1000 bp material (0.4 and 2.9 mg/ml). Figure 9 compares the results for two concentrations and demonstrates a good reproducibility of results for two concentrations.

Fig. 10 compares spectra from materials 500 bp and 1000 bp. In the low frequency portion (10-20 cm<sup>-1</sup>), spectra are very similar. However, there is significant difference around 21-23 cm<sup>-1</sup>.