



ARL-CR-0788 • Nov 2015



Terahertz Characterization of DNA: Enabling a Novel Approach

prepared by Sarah Stranieri

*University of Illinois at Urbana-Champaign
Champaign, IL*

Molleshree Karna

*Columbia University
New York, NY*

Daniel Shreiber

*Oak Ridge Institute for Science and Education
Belcamp, MD*

under contract W911NF-10-2-0074

Approved for public release; distribution is unlimited.

NOTICES

Disclaimers

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

Citation of manufacturer's or trade names does not constitute an official endorsement or approval of the use thereof.

Destroy this report when it is no longer needed. Do not return it to the originator.



Terahertz Characterization of DNA: Enabling a Novel Approach

prepared by Sarah Stranieri

*University of Illinois at Urbana-Champaign
Champaign, IL*

Molleshree Karna

*Columbia University
New York, NY*

Daniel Shreiber

*Oak Ridge Institute for Science and Education
Belcamp, MD*

under contract W911NF-10-2-0074

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE (DD-MM-YYYY) November 2015		2. REPORT TYPE Contractor Report		3. DATES COVERED (From - To) June–August 2015	
4. TITLE AND SUBTITLE Terahertz Characterization of DNA: Enabling a Novel Approach				5a. CONTRACT NUMBER W911NF-10-2-0074	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Sarah Stranieri, Molleshree Karna, and Daniel Shreiber				5d. PROJECT NUMBER R.0014684.1.82	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Illinois at Urbana-Champaign, Champaign, IL Columbia University, New York, NY Oak Ridge Institute for Science and Education, Belcamp, MD				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) US Army Research Laboratory ATTN: RDRL-WMM-E Aberdeen Proving Ground, MD 21005				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S) ARL-CR-0788	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The terahertz spectrum of radiation has been determined to be a promising candidate for the characterization of biological molecules, such as DNA. Several alternative methods, including fluorescent chromophore labeling and techniques that use terahertz radiation, have been proposed and are currently in use. Though established, they have disadvantages, such as alteration to the nucleic acid sequence, requirement of a thick DNA testing layer, and conductor structure complexity. This project enables a novel method to identify DNA in a more reliable and less procedurally complicated manner. The method involves the use of terahertz surface plasmon generated on the surface of a gold-coated stainless steel perforated foil. This approach is less expensive and requires smaller quantities of genetic testing material. Such advantages are due to overlapping resonance when the plasmon frequency generated by a foil coincides with that of the biological material. The interference of the impinging terahertz wave and surface plasmon produces spectral graphs, which can be analyzed to identify and characterize a DNA sample. This work sets the foundations of a systematic approach to successfully make the plasmon-based terahertz approach a promising candidate. The location and orientation of the samples were kept constant to obtain conclusive and comparative results. This work offers clear evidence that the DNA molecules under investigation interact with the terahertz wave.					
15. SUBJECT TERMS terahertz spectroscopy, DNA analysis, surface plasmon, DNA resonant peak, overlapping resonance					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 20	19a. NAME OF RESPONSIBLE PERSON Daniel Shreiber
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code) 410-306-4928

Contents

List of Figures	iv
List of Tables	iv
Acknowledgments	v
1. Introduction and Background	1
2. Experiment	2
3. Results	5
4. Discussion	8
5. Conclusions and Future Research	9
6. References	10
Distribution List	11

List of Figures

Fig. 1	Gold-sputtered metal mesh	2
Fig. 2	TERA K15 terahertz spectrometer. One lens in front of each antenna was used for focused beam testing. The iris was placed equidistant from the antenna.	3
Fig. 3	Focused beam setup (top view).....	3
Fig. 4	Rapid scan after aligning the antenna and lenses in free space	4
Fig. 5	Rapid scan with sample placed in the Iris. The signal height is significantly lower.	4
Fig. 6	Bare gold mesh compared to mesh coated in sample 2. A distinct DNA resonance is present at 1.4 THz.	5
Fig. 7	Bare gold mesh compared to mesh coated in sample 3. The effect of TRIS resonance is present at 1.5 THz.....	6
Fig. 8	Samples 2 and 3 on gold mesh. DNA has a distinct resonance effect from the TRIS buffer alone.....	6
Fig. 9	Interface of DNA layer and plain mesh in the corner of sample 2	7
Fig. 10	Side profile of sample 2 from a laser microscope. The DNA layer was determined to be about 27 μm thick.	8

List of Tables

Table.	DNA sample properties	2
--------	-----------------------------	---

Acknowledgments

The lead author of this report, Sarah Stranieri, would like to thank the US Army Research Laboratory and the College Qualified Leaders Program for allowing her to conduct research at the Rodman Materials Research Center throughout the summer of 2015. She would like to specifically thank Dr Mathew Ivill for providing texts on surface plasmon polaritons.

INTENTIONALLY LEFT BLANK.

1. Introduction and Background

Proper and detailed identification of short DNA oligonucleotides has been a long-sought goal of molecular biologists and medical researchers. These nucleic acid fragments are used to locate and analyze identical base pair sequences in full strands. Compilation of their nucleic acid sequences within a genetic library would pave the way for improved forensic analysis, genetic testing, and DNA production processes.¹ Other medical applications include earlier disease detection to initiate gene therapy, as well as a thorough analysis of metabolic behaviors dictated by mRNA.²

Within the submillimeter-wave frequency range of 0.01–10 THz, DNA molecules exhibit several types of internal vibrations. The nature of these movements dictates biological processes, such as transcription and replication of genetic material.³ Some occur between base pairs because of their weak hydrogen bonding interactions; others stretch, twist, and bend the entire double helix structure.³ These features, when measured via terahertz technology, reveal specific features of a DNA code.⁴ This terahertz spectral range has proved difficult to use with organic material. The high absorption of water in aqueous solution masks the weaker magnitude of absorption in biological materials.

An emitted terahertz electromagnetic wave released from a semiconductor antenna interacts with the array of metal holes along the metal mesh located on the path of the emitted terahertz wave. This interference generates surface plasmons—electron density waves that propagate laterally across the metal surface. The interference of this surface plasmon with the impinging terahertz wave is registered as a plasmon peak on a spectrum. The surface plasmon always propagates across the interface between the metal mesh and the dielectric (which is the DNA sample in our case). Its properties are a function of dielectric properties of both the metal and the dielectric. The electrical properties of the dielectric can be studied if those of the metal are known. This project seeks to detect the interaction of DNA with surface plasmons.

The Drude model approximation calculates the expected wavelength, λ , of the propagating surface plasmon wave,

$$\lambda = \frac{L}{\sqrt{m^2+n^2}} \sqrt{\varepsilon}, \quad (1)$$

where ε represents the dielectric constant of the DNA material, and m and n are the quantum orders of the propagating wave. Our DNA samples' resonant frequencies were obtained on a metal mesh with a pitch (L) of 500 μm .

2. Experiment

Two 16 cm² stainless steel metal meshes, with a pitch of 500 μm and a hole diameter of 200 μm, were sputtered with a 452-nm gold layer. The base pressure of 2 mTorr was achieved in the sputtering system. Upon reaching this minimum pressure, the chamber was filled with argon gas, and 3 min of presputtering was performed to clean the target. The gold thin film deposition time was 4 min and 43 s at a rate of 84.8 nm/min. A profilometer confirmed the 425-nm thickness of this gold layer at multiple surface spots, showing the uniformity of the deposition. The resulting perforated foil is depicted in Fig. 1.

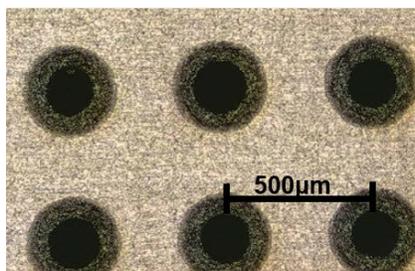


Fig. 1 Gold-sputtered metal mesh

After a day of air drying, the 4- × 4-cm squares were cut down to 2 × 2 cm. Solution containing 200 μL of DNA was spin coated onto the meshes at 203 rpm and dried at this rotational speed for 10 min. The DNA oligomer sequence used was 5'-CATTAACGAGTTACTCAATGAGT5CTTTCTG-3'. The following table details the solution parameters and concentrations of DNA in each sample that contains a tris(hydroxymethyl) (TRIS) buffer solution.

Table. DNA sample properties

Sample no.	DNA quantity (mg)	Solvent volume	Mass concentration
1	0.23	1.0-mL TRIS buffer	0.230 $\frac{g}{L}$
2	0.23	0.33-mL TRIS buffer	0.696 $\frac{g}{L}$
3	0.00	0.33-mL TRIS buffer	...

A Menlo Systems TERA K15 terahertz time domain spectrometer (Figs. 2–5) was used to test each sample in transmission mode. A laser, with a wavelength of 1,560 nm and 120-fs pulse duration, was connected to 2 antennas via fiber optic cables. Each mesh was secured to an iris in between the 2 antennas. The DNA-coated side of each sample faced the same direction. Nitrogen gas was pumped into the testing chamber for 2 h to purge the water vapor. All samples were tested with a 3-mm-diameter focused beam. Plasmon peaks were detected and analyzed with respect to DNA resonance behavior.



Fig. 2 TERA K15 terahertz spectrometer. One lens in front of each antenna was used for focused beam testing. The iris was placed equidistant from the antenna.

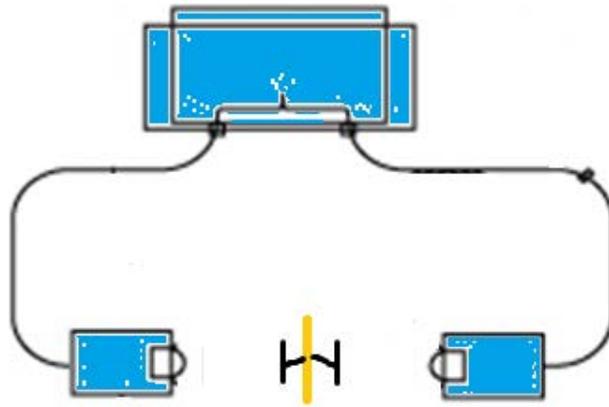


Fig. 3 Focused beam setup (top view)

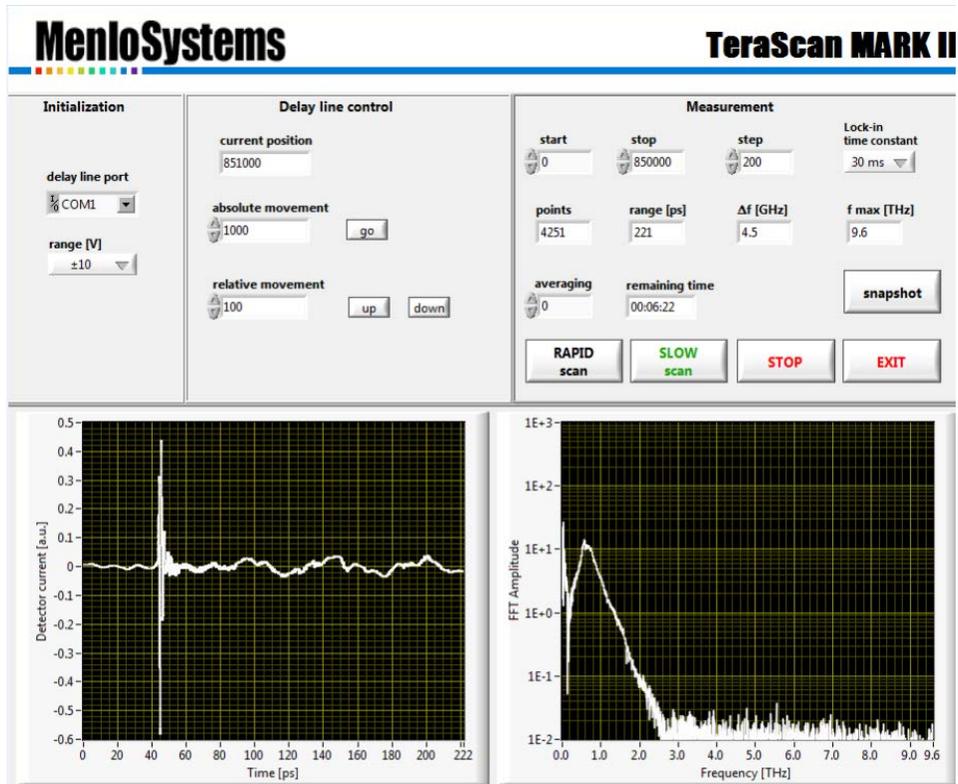


Fig. 4 Rapid scan after aligning the antenna and lenses in free space

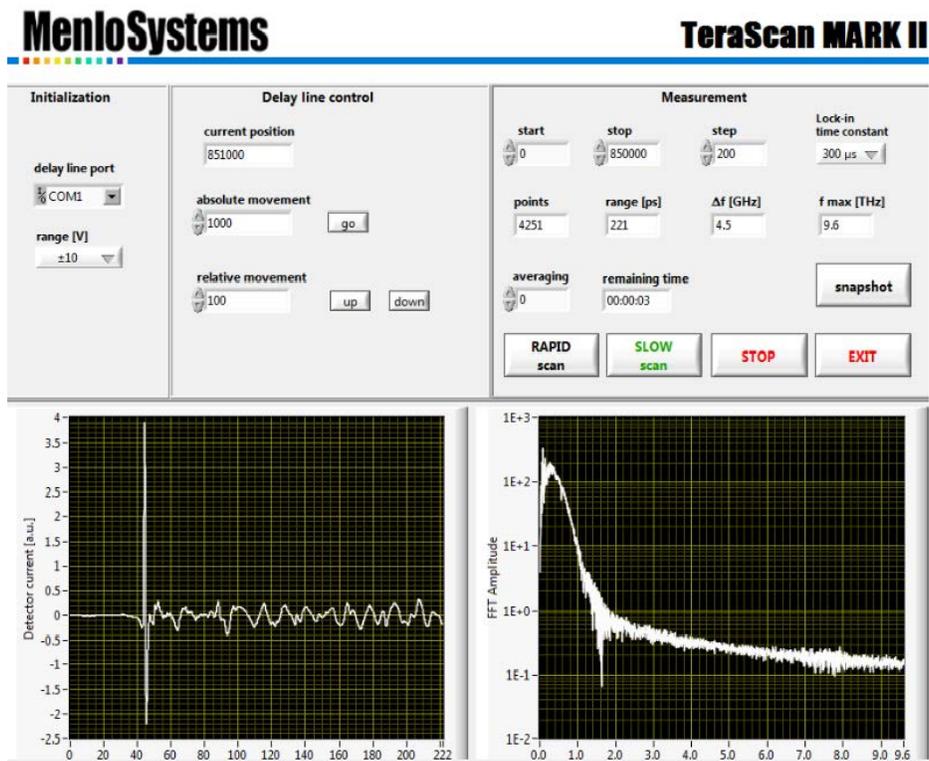


Fig. 5 Rapid scan with sample placed in the Iris. The signal height is significantly lower.

3. Results

Samples 2 and 3 were tested under focused beam in terahertz time domain spectroscope. The focused beam plots are shown in Figs. 6–8. A plain gold mesh without buffers or coatings was compared to each sample in the first 2 graphs. In Fig. 8, the samples were directly compared. All plots were normalized by division by free space.

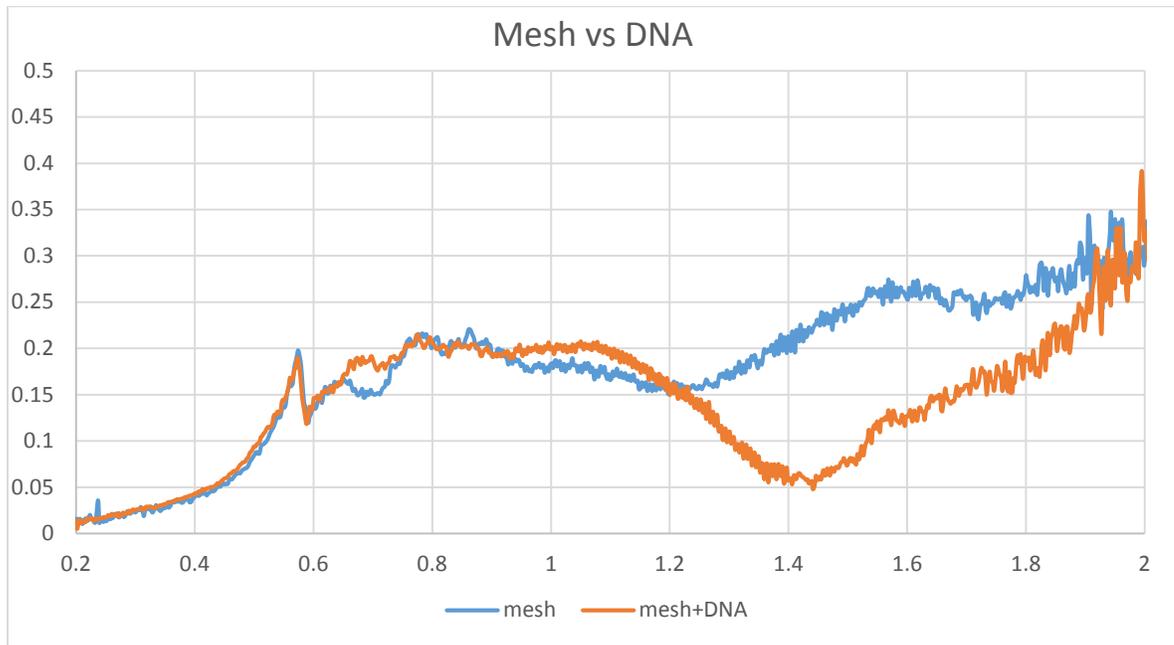


Fig. 6 Bare gold mesh compared to mesh coated in sample 2. A distinct DNA resonance is present at 1.4 THz.

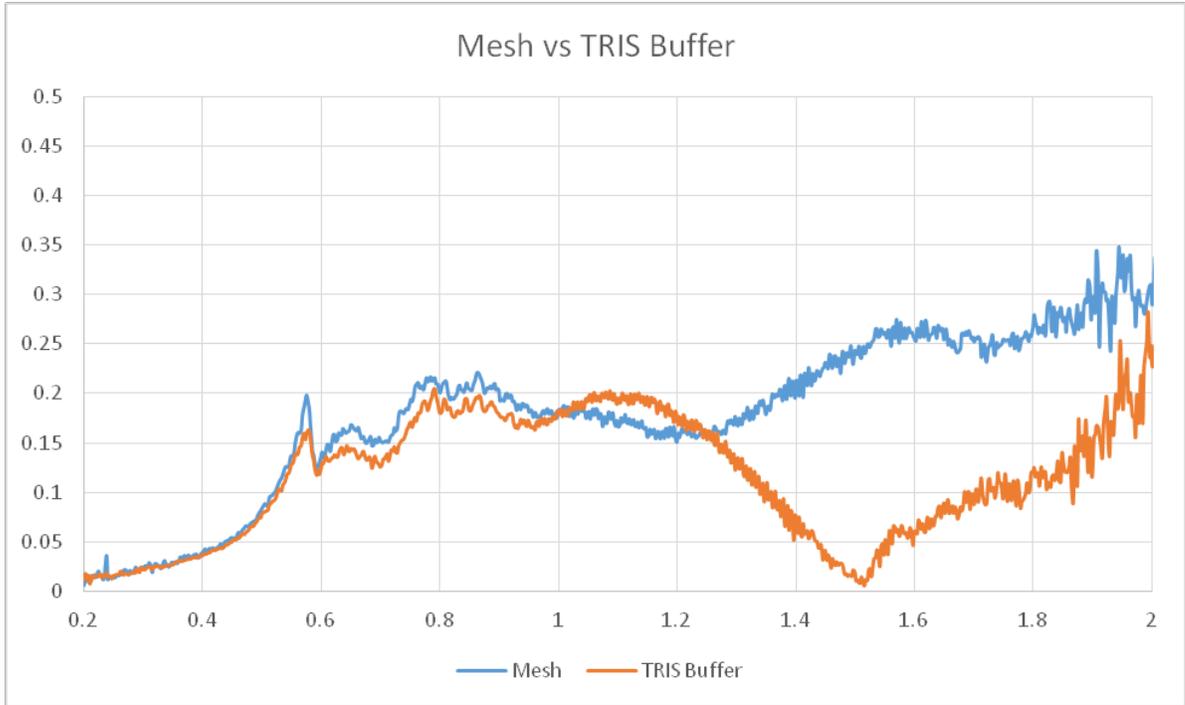


Fig. 7 Bare gold mesh compared to mesh coated in sample 3. The effect of TRIS resonance is present at 1.5 THz.

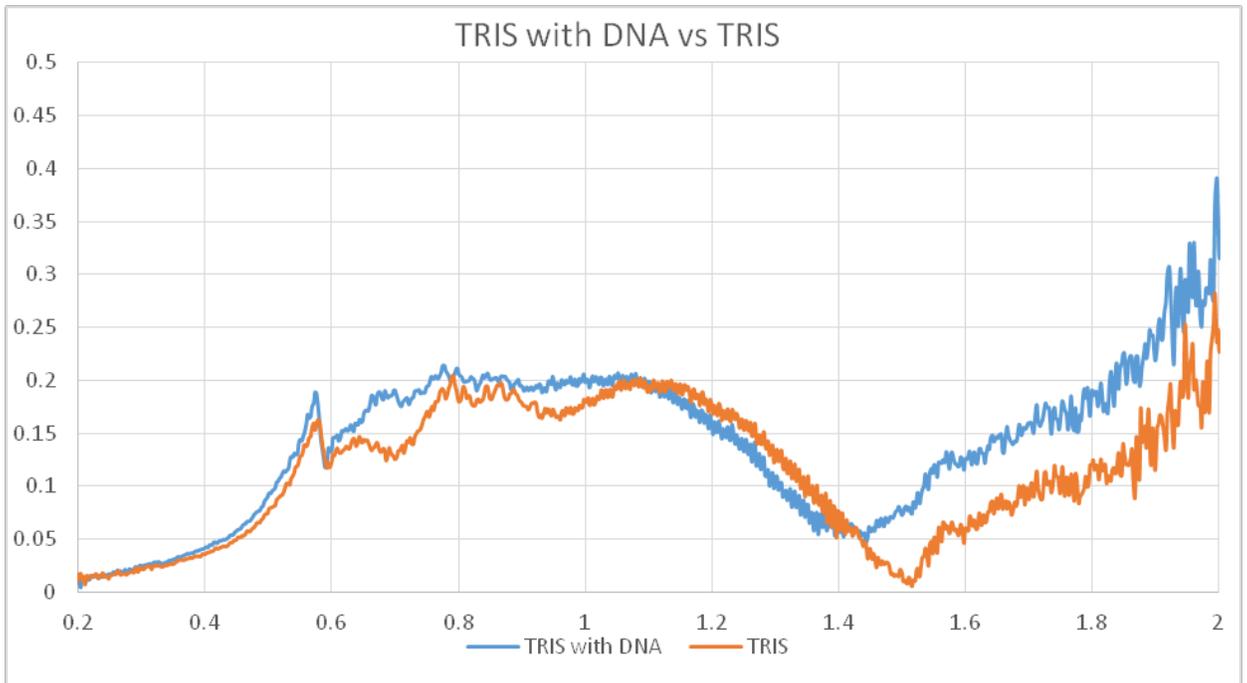


Fig. 8 Samples 2 and 3 on gold mesh. DNA has a distinct resonance effect from the TRIS buffer alone.

The Drude model approximation (Eq. 1) was used to first correlate with the pitch of the metal mesh. Using our first peak value of 0.58 THz, present on all 3 graphs, we calculated L to be 516 μm .

$$L = \frac{\lambda\sqrt{m^2+n^2}}{\sqrt{\epsilon}} = \frac{\left(\frac{v}{f}\right)\sqrt{0^2+1^2}}{\sqrt{1}} = \frac{299792458 \frac{\text{m}}{\text{s}}}{0.58*10^{12}\text{Hz}} = 5.16 \times 10^{-4} \text{ m} = 516 \mu\text{m} . \quad (2)$$

Using L in the second-order peak calculation,

$$\lambda = \frac{L\sqrt{\epsilon}}{\sqrt{m^2+n^2}} = \frac{516 \mu\text{m}}{\sqrt{1^2+1^2}} = 364 \times 10^{-4} \text{ m} \times \frac{1}{299792458 \frac{\text{m}}{\text{s}}} = 0.823 \text{ THz} . \quad (3)$$

This approximation is close to the 500- μm pitch of the material's circular holes, even though the approximation assumed square holes. The 516- μm value was used in subsequent calculations.

Under the focused beam, the TRIS buffer exhibits resonant behavior at 1.5 THz, whereas the resonance frequency is closer to 1.4 THz for the sample that contains DNA.

The presence of DNA on the spin-coated samples was confirmed using a laser microscope image, magnified by a $5\times$ lens (Figs. 9 and 10).

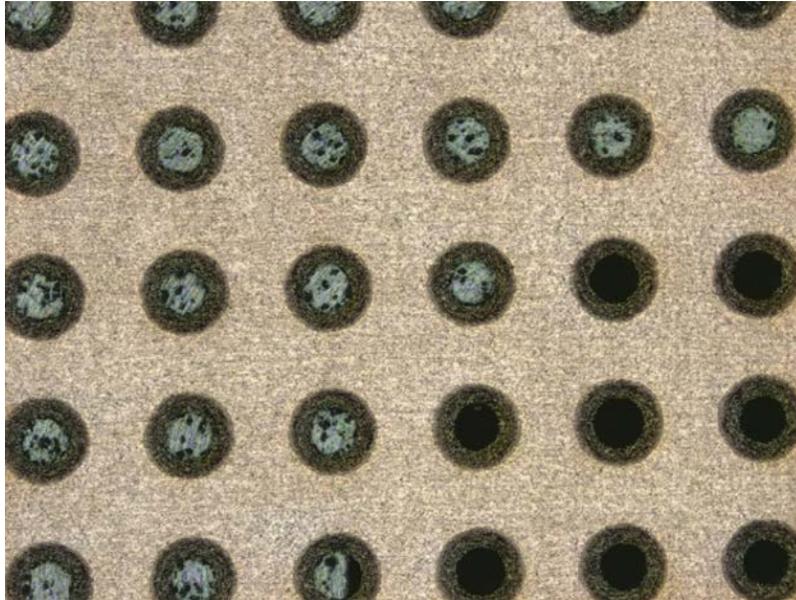


Fig. 9 Interface of DNA layer and plain mesh in the corner of sample 2

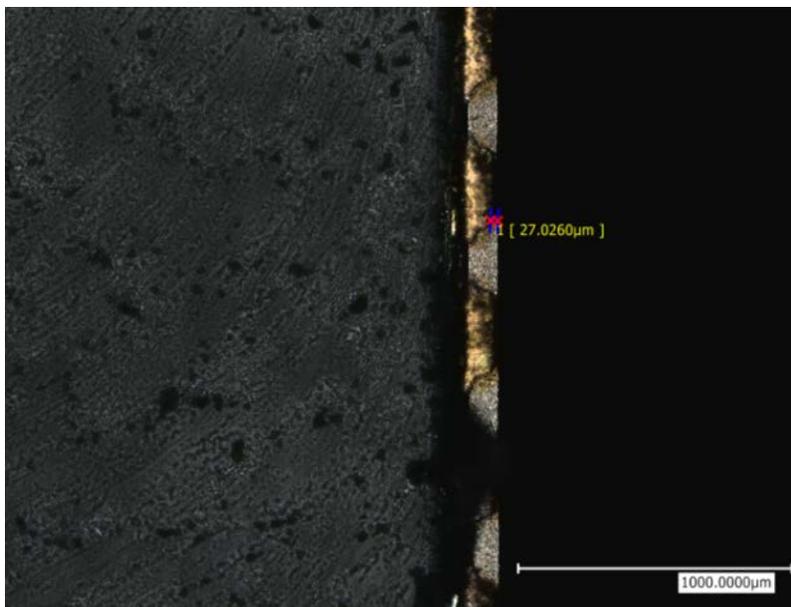


Fig. 10 Side profile of sample 2 from a laser microscope. The DNA layer was determined to be about 27 μm thick.

4. Discussion

Surface plasmons were successfully generated on both the DNA-coated and plain metal mesh samples, indicated by the spectral peaks. A nonlinear interaction, generated by application of a focused terahertz beam, to a sample that contains both DNA strands and TRIS, caused the resonance peak to shift from 1.5 to 1.4 THz. This result indicates that the low terahertz spectrum is a good candidate for DNA testing in this experimental configuration. The result can be significantly enhanced if the idea of overlapping resonances is applied to investigate the DNA samples where the plasmon frequency of the terahertz surface plasmon is matched with the resonant frequency of the DNA sample. This was not the case in the current configuration, where the first-order surface plasmon peak has been demonstrated at 0.58 THz.

The proposed approach uses a set of standard sample preparation techniques, such as gold thin film sputtering and a standard spin coating technique for DNA deposition. The sputtering and spin-coating process could be scaled up to larger batches to test for consistency among identically prepared samples.

In this proof-of-principle experiment, only the resonant frequency of a specific DNA sample in the terahertz spectrum was identified. By tuning the surface plasmon frequency of the sample by adjusting the specific pitch between the holes, we could achieve greater details and more specific terahertz readings to identify molecular movements and nucleic acid sequences. Comparison of DNA samples

with minute differences in base pairs or molecular motions with this surface plasmon technique could identify resonant frequencies of specific DNA features. A surface plasmon library of genetic spectral data could be compiled to study oligomers in greater detail.

5. Conclusions and Future Research

Spin coating of DNA and gold sputtering is a successful preparation method to fabricate DNA samples for terahertz investigation. The proposed terahertz technique is a promising method to identify DNA molecules due to prevalent DNA resonant behavior in these frequencies. This work lays the foundations to a relatively inexpensive and precise technique to classify nucleic acid.

The next objective is to prepare samples embedded with resonant gold nanoparticles in order to investigate the resonant behavior changes in the DNA sample as a function of the gold nanoparticles' shape or plasmonic activity.

The random orientation of DNA with spin coating does not allow for specific alignment of the sample with the polarized electromagnetic field produced by the terahertz spectrometer. Using nanopores, carbon nanotubes, and other electrically conducting porous structures, DNA strands can be guided through these holes with an electric field.⁵ The entire structure can be oriented parallel or perpendicular to the electromagnetic field of a terahertz system.⁶ Aligning the structures parallel or perpendicular to the terahertz field polarization will significantly enhance the response of the DNA structure under investigation and analysis capabilities of the proposed setup.

6. References

1. Brucherseifer M, Nagel M, Haring Bolivar P. 2000. Label-free probing of the binding state of DNA by time-domain terahertz sensing. *Applied Physics Letters*. 2000;77:4049 [accessed 2015 Oct 30].
<http://scitation.aip.org/content/aip/journal/apl/77/24/10.1063/1.1332415>
2. Chernev AL, Bagraev NT, Klyachkin LE, Emelyanov AK, Dubina MV. DNA detection by THz pumping. *Semiconductors*. 2015;49(7):977–948 [accessed 2015 Oct 30].
<http://arxiv.org/abs/1407.6520>
3. Globus T, Khromova T, Woolard D. Terahertz Fourier transform characterization of biological materials in solid and liquid phases. *Proc. SPIE 5268, Chemical and Biological Standoff Detection*; 2004 Feb 27; p. 10. doi:10.1117/12.519172; <http://dx.doi.org/10.1117/12.519172>
4. Woodlard et al. Submillimeter-wave phonon modes in DNA macromolecules. *Physical Review E*. 2002;65:051903 [accessed 2015 Oct 30].
<http://journals.aps.org/pre/pdf/10.1103/PhysRevE.65.051903>
5. Muthukumar M, Plesa C, Dekker C. Single-molecule sensing with nanopores. *Physics Today*. 2015;68(8):40 [accessed 2015 Aug 06].
<http://dx.doi.org/10.1063/PT.3.2881>
6. Globus TR, Woolard DL, Khromova T. THz-spectroscopy of biological molecules. *Journal of Biological Physics*. 2003;29(2–3):89–100 [accessed 2015 Oct 30].
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3456403/pdf/10867_2004_Article_5121570.pdf

1 DEFENSE TECHNICAL
(PDF) INFORMATION CTR
DTIC OCA

2 DIRECTOR
(PDF) US ARMY RESEARCH LAB
RDRL CIO LL
IMAL HRA MAIL & RECORDS
MGMT

1 GOVT PRINTG OFC
(PDF) A MALHOTRA

1 UNIV OF ILLINOIS
(PDF) S STRANIERI

1 DIR USARL
(PDF) RDRL WMM E
D SHREIBER

INTENTIONALLY LEFT BLANK.