Terahertz Characterization of DNA: Enabling a Novel Approach

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under contract W911NF-10-2-0074

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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.

**Subject Terms**
terahertz spectroscopy, DNA analysis, surface plasmon, DNA resonant peak, overlapping resonance
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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, \(\lambda\), of the propagating surface plasmon wave,

\[
\lambda = \frac{L}{\sqrt{m^2 + n^2}} \sqrt{\varepsilon},
\]

where \(\varepsilon\) 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](image)

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>
<thead>
<tr>
<th>Sample no.</th>
<th>DNA quantity (mg)</th>
<th>Solvent volume</th>
<th>Mass concentration</th>
</tr>
</thead>
</table>
| 1          | 0.23              | 1.0-mL TRIS buffer | 0.230  
| 2          | 0.23              | 0.33-mL TRIS buffer | 0.696  
| 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 spectroscopy. 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.

![Mesh vs DNA](image)

**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 \( \mu \)m.

\[
L = \frac{\lambda \sqrt{m^2 + n^2}}{\sqrt{\varepsilon}} = \frac{(\frac{v}{f})\sqrt{0^2 + 1^2}}{\sqrt{1}} = \frac{299792458 \frac{m}{s}}{0.58 \times 10^{12} \text{Hz}} = 5.16 \times 10^{-4} \text{ m} = 516 \mu \text{m}. \tag{2}
\]

Using \( L \) in the second-order peak calculation,

\[
\lambda = \frac{L \sqrt{\varepsilon}}{\sqrt{m^2 + n^2}} = \frac{516 \mu \text{m}}{\sqrt{1^2 + 1^2}} = 364 \times 10^{-4} \text{ m} \times \frac{1}{299792458 \frac{m}{s}} = 0.823 \text{ THz}. \tag{3}
\]

This approximation is close to the 500-\( \mu \)m pitch of the material’s circular holes, even though the approximation assumed square holes. The 516-\( \mu \)m 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](image-url)
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. The entire structure can be oriented parallel or perpendicular to the electromagnetic field of a terahertz system. 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.
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