DOI: http://dx.doi.org/10.18103/imr.v0i4.90

Label-free detection of single nucleotide polymorphism and DNA hybridization by terahertz spectrometry

Anis Rahman[1,*], Gulshan Ara[1], Aunik K Rahman[1], and Bruce Stanley[2]

[1]Applied Research & Photonics, 470 Friendship Road, Ste. 10, Harrisburg, PA 17111

[2]Hershey College of Medicine, 500 University Avenue, Hershey, PA 17033
*Corresponding author email: a.rahman@arphotonics.net

Abstract

Time-domain terahertz (THz) spectrometry has been used to analyze a 21-mer nucleotide polymorphism (SNP) sequence in a single and double stranded DNA in a label-free manner. THz temporal signal (or interferogram) converted to frequency domain constitutes a signature of a given molecular "event" (e.g., a vibrational or a conformational state). The temporal signal provides a means of probing a molecular event in an appropriate time window. This is a unique ability of this technique because different molecular events exhibit different time response based on their physical and chemical nature. Conformational difference of a given molecule results in different signature with an appropriate time response that can be accurately probed by a terahertz temporal signal. In this work using a single stranded and double stranded 21-mer oligonucleotides in pico-molar concentration with or without specific SNP we demonstrated that terahertz spectroscopic technique produces distinctly different spectral signature in each case. For each species, peaks were distinctly different allowing a meaningful comparison. Additionally, temporal transmission spectra of the DNA specimens were collected at normal temperature and atmosphere allowing easy handling of the samples. The results clearly demonstrate the ability of the spectrometer to detect a minute amount of biomolecules in a label-free fashion. Based on the results, it is assumed that the spectral signature can be used to identify SNPs as a diagnostic tool for certain disease states, for designing personalized medicine and also can be useful in plant genetics. This capability can be used as a diagnostic tool, as well as for studying molecular reactions such as mutation.

Keywords: Terahertz Spectrometry, Single Nucleotide Polymorphism, Label-free Detection, DNA Hybridization State, Fourier Analysis, Single- and Double-stranded DNA.

1. Introduction

Single nucleotide polymorphisms (SNPs) are DNA sequence variations that occur when a single nucleotide (i.e., A, T, C, or G) in the genome sequence is altered. SNPs play important role in human genetics and plant genetics. SNPs make up about 90% of all human genetic variations; therefore, SNP detection and genotyping assays are of fundamental importance in the identification of numerous genetic and hereditary diseases and also for the development of personalized medicine. SNPs are also the most abundant genetic marker in modern plant genetic analysis, such as diversity analysis, genetic trait mapping, association studies, and marker assisted selection.

The need for quick, reliable and affordable means for detecting SNPs from clinical samples and plant samples is crucial and could become a routine test for personalized medicine and also for improvement of various aspects of plant crops. Because a significant portion of the costs of existing (clinical) methods are incurred in the reagents, a label-free detection of SNPs via spectral analysis have a great potential for providing an alternative route for facilitating numerous SNP based quick diagnosis and enhancement.

1.1. SNPs as diagnostic marker

An example of a specific SNP linked to a disease state is the FCGR3A SNP (559T→G), which has been reported to play important role in the development of autoimmune disease, particularly as a diagnostic marker for development of Rheumatoid arthritis [1]. In addition, phosphatase non-receptor 22 (PTPN22) C1858T polymorphism allele confers susceptibility to various autoimmune diseases, e.g. Rheumatoid arthritis, Type I diabetes, Systemic lupus erythematosus (SLE or Lupus) and other autoimmune diseases and can be used as a diagnostic marker for autoimmune diseases [2]. Other well known examples are the A → T (Glu → Val) point mutation in human β-globin gene leading to sickle cell anemia [3], tau SNPs [17 (FTDP-17)] for development of Parkinson's disease [4, 5] and other SNPs relating to diabetes [6], cardiac disease [7, 8], etc.

1.2. *SNPs for the development of personalized medicine*

Various novel personalized treatments of cancer with monoclonal antibody (mAb) do not show expected results in patients having certain genotypes (SNPs) of FCGR3A [9–12]. Presence of a SNP in natural killer

(NK) cell receptor FCGR3A (4985 G→T) has been shown to be predictive of binding affinity of NK cells to mAb, hence predictive of efficacy of the mAb drug Rituximab to kill cancer cells [9–12]. These polymorphisms might also prove important to the efficacy of other IgG1 monoclonal antibody drugs currently in use and those in clinical development. Hence, detection of SNPs in cancer patients will help determine which subgroup of patients will be eligible to receive the mAb drugs.

Drug metabolism is a complex biochemical process that consists of many different parts and reactions in the human organism. Some drugs are excreted unchanged in urine and feces without passing any metabolic treatment in the liver, but most of the drugs have a multi-step metabolism, which is mainly associated with Cytochrome P450 (CYP450). While a large percentage of patients respond in the intended medically beneficial way to a given drug; some smaller percentage might either have no response or have an adverse, life threatening response. For instance, warfarin is the most commonly prescribed anticoagulant for the treatment and prevention of arterial and venous thromboembolism. The most biologically active form of warfarin is metabolized by CYP2C9, one of the major cytochrome P450 drug-metabolizing enzymes. Warfarin has a narrow therapeutic index and improper dosing can result in bleeding events that may be severe or fatal. Polymorphisms in VKORC1 (c.-1639G→A) and CYP2C9 (alleles *2 and *3, also known as c.430C→T and c.1075A→C respectively) partly determines warfarin sensitivity accounting for 35–50% of the variability in warfarin dose requirement [13, 14]. Tardive dyskinesia (TD) is a common and potentially irreversible side effect associated with long-term treatment with typical antipsychotics. Approximately, 80% or more of patients with schizophrenia are smokers. Smoking is a potent inducer of the CYP1A2 enzyme, and is known to cause a significant decrease in plasma concentrations of some antipsychotics. Therefore, person-to-person differences in the extent of CYP1A2 induction by smoking may contribute to risk for the development of TD. Genetic polymorphism in the CYP1A2 gene (C→A) [15] may raise genetic risk factor for the development of TD in patients with schizophrenia, hence, implicating diagnostic application of SNP detection in schizophrenia patients.

By studying the polymorphism of drug metabolizing enzymes like CYP P450, it becomes possible to choose the most appropriate medicine and decide on the most appropriate amount of dosage for better results and less side effects. It may also lead to development of derivatives that differs little due to polymorphisms. Therefore, detection of known SNPs is an important task for personalized medicine.

1.3. SNPs in plant genetics

In the case of plant genetics SNPs hold tremendous promise. Potential applications include marker-assisted trait screening, marker-assisted plant breeding, accelerated marker-assisted backcrossing, quantitative trait loci (QTL) mapping and genetic fingerprinting [16, 17].

Tomato SNP marker consortium is one of a number of initiatives that DNA Landmarks is spearheading in its drive to deliver improved marker services to the entire agricultural value chain. SNPs also have a major impact on how the organism develops and responds to the environment.

Sugarcane has the most complex genome of any crop plant. It is now possible to identify candidate gene sequences that may underpin important traits in sugarcane and to characterize single nucleotide polymorphisms (SNPs) in these genes. Combinations of SNPs in a gene sequence act as signatures for individual gene haplotypes that may be considered as allele equivalents in the sugarcane genome [18]. Application of SNP markers for genetic diversity analysis in Brassica species is also being worked out [19].

1.4.How it is done now, what are the problems

Current methods for SNP detection are labor intensive and expensive, requiring PCR amplification, DNA denaturation, and fluorescent labeling. For example, Ambry Genetics uses

Sanger's method, Asuragen uses Taqman and Array processing. All of these methods need DNA amplification, denaturation and fluorescent labeling of the nucleotides. DNA sequences are identified by hybridization of unknown target DNA molecules to known single-stranded oligonucleotide probe molecules [20]. The dominant approach to detect hybridization is based on fluorescent labeling of the target denatured DNA. Although fluorescent labeling has given rise to extremely efficient diagnostic systems, especially since the emergence of gene chips [21,22], a strong interest exists in developing alternative label-free detection schemes. Labeling not only constitutes an additional

costly and time-consuming preparatory step, it can introduce modifications in DNA strand conformation that lower the accuracy of gene detection [23]. Additionally, label degradation, labeling yield fluctuations, fluorescence efficiency site dependence and fluorophore quenching inhibit the quantifiability of genetic diagnostics [24, 25].

2. Terahertz spectrometry

In contrast, none of the sample preparations steps associated with the above mentioned methods is needed in terahertz spectroscopic determination of SNP using TeraSpectra, thus aiding a quick and cost-effective route.

Sub-millimeter wave range of DNA absorption spectra (~2–1000 $^{-1}$) corresponding to ~ 0.2 THz –30 THz is fairly rich with spectral features which can give information regarding three dimensional structure and flexibility of DNA double helix and inter-backbone excitations of DNA . Theoretical calculations predict the presence of a number of resonances in the terahertz frequency range associated with inter-backbone excitations of DNA molecules [26–28]. Hence, a unique potential exists for the label-free detection of the DNA binding state and single nucleotide polymorphisms (SNPs) via THz spectroscopy [29–31]. Terahertz measurements are conducted in time domain where the temporal signal (or an interferogram) is acquired in the range of a few tens of femto-seconds to a few pico-seconds. The Fourier transform of this time-domain pulse constitutes the frequency domain spectrum. An optical delay is used to generate time delay on sub-pico second scale while the sample response is detected with a technique known as electro-optic sampling. Therefore, an important advantage of this technique is that it offers a useful means of probing a molecular "event" (e.g., a vibrational state or a conformational state, etc.) in an appropriate time window. This is an important ability because probing of different molecular event requires appropriate time window, based on the physical and chemical nature of each. For example, a molecular vibration occurs over a longer time scale compared to a bond vibration or torsion. Similarly, compositional or conformational difference of a given molecule results in different signature with appropriate time response that can be accurately probed by a THz temporal signal. The frequency domain signature of a given event is unique and represents a means of identifying the event, thus forms the basis of detection of molecular characteristics such

as polymorphism, chirality or other structural variations of DNA.

Investigations by Raman, Fourier-transform and time-domain THz techniques on hybridized DNA molecules have been performed in the past [32–34]; however, few experiments have addressed binding state specific analysis. It has been pointed out that the dependence of the binding state of DNA to its dielectric properties at THz frequencies can be used as a potential method for label-free gene detection [33, 34]. The idea has been experimentally verified by ARP (see [29] and the preliminary studies report section).

Previously we have reported that THz spectral pattern of a 25-mer oligonucleotide (ssDNA) is distinctly different from the spectra of hybridized DNA (dsDNA) [29]. Using THz spectroscopy, three different peaks were identified for both ssDNA and dsDNA of known sequence but the peaks were significantly shifted compared to each other thus allowing a meaningful comparison. We have also shown that THz spectra of dsDNA or ssDNA with native nucleotides can be distinguished from those containing SNPs [31].

An attractive advantage of this method is the ease of the procedure. Commercial sources such as Ambry Genetics and Affymetrix offer services for detection of SNPs from clinical samples. These techniques use DNA denaturation, amplification and fluorescence labeling. Ambry

Genetics uses Sanger's method, which is also referred to as dideoxy sequencing or chain termination. Asuragen uses Taqman and Affymetrix uses array method. For THz spectral analysis using TeraSpectra, there is no need for carrying out DNA denaturation, no PCR, no fluorescent labeling. It requires smaller quantity samples (femto-mol); sample preparation and spectral acquisition is quick (seconds/sample). Hence overall cost for SNP analysis is minimized.

3. Experimental

Here we describe a terahertz spectrometer (TeraSpectra, Applied Research & Photonics, Harrisburg, PA 17111) and its use for detection of SNPs. ARP's TeraSpectra uses a high power CW terahertz source based on electro-optic dendrimer [35]. TeraSpectra implements the time-domain spectrometry (or spectroscopy) (THz-TDS) via electro-optic route where the spectrometer is built around the above mentioned DFG source and detection is done via standard electro-optic sampling method. Unlike the pulsed systems

where a femto-second pulsed laser is the key component, ARP uses a CW source. A probing beam is used to interrogate the terahertz beam that carries the sample information due to terahertz interaction with the specimen. Consequently, a sharp interferogram is generated in the time-domain due to the interference of the scanning beam with the probing beam, whose intensity distribution is captured by the detection system. This is different than the so called difference frequency mixing where one pump laser is kept fixed while another pump laser is temperature tuned to generate the difference frequency. ARP's implementation allows a much better control of the spectrometer system with very high sensitivity (~ femto-molar) and a wider terahertz range (up to ~30 THz).

3.1.Absorption by individual nucleotides within the THz range

The nucleotides (A, T, G & C) were synthesized at the Molecular Synthesis Facility of the Penn State College of Medicine. Solutions of each nucleotide at a concentration of 2 mM in Tris-EDTA were used. Absorbance by the nucleotides within the entire THz range (0.1–20 THz) is shown in Fig. 1. This was estimated as absorbance = transmitted power of blank glass slide – transmitted power of the same slide with nucleotides on it. As depicted in Fig. 1, absorbance follows the order $A_A > A_G > A_C > A_T$.

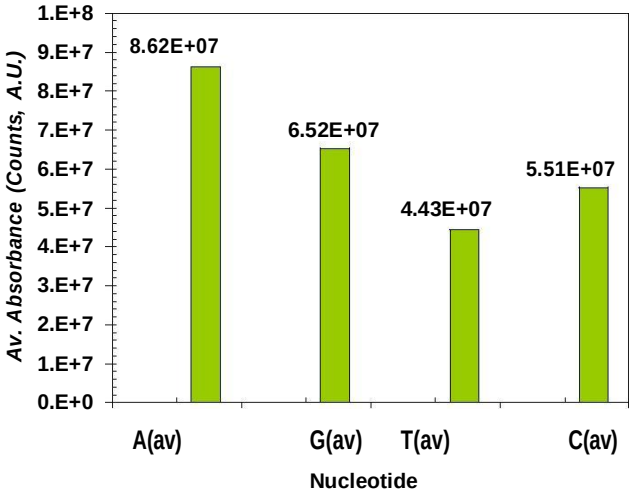

Fig. 1. Average absorbance by A, G, T, C were carried out three different times between 0.1–20 THz range and the mean absorbance is plotted. Average absorbance follows the order $A_A > A_G > Ac > A_T$.

3.2. THz spectral analysis of FCGR3A oligonucleotides with native oligo-nucleotide sequence or SNP

We examined whether any difference in spectra of the oligonucleotides with or without SNP FCGR3A SNP (559T → G) can be detected within the THz frequency domain. Two 21-mer oligonucleotides 'A' and 'B' of FCGR3A gene with and without SNP (559T → G) were synthesized using POLYGEN synthesizer as shown below:

A) 559T: Forward GGG GGC TTT TAC AGC GGC TCC

B) 559G: Forward GGG GGC TTG TAC AGC GGC TCC

The samples (~2.72 pMol) were applied onto ~1 cm spots on microscopic slides and THz time domain signals were collected by an automated setup. Fig. 2 exhibits temporal spectra of the oligonucleotides A & B. Fig. 3 shows the normalized absorbance spectra that were obtained by fast Fourier analysis of the temporal time domain spectra shown in Fig. 2. It is notable that due to a SNP (559T → G) both temporal spectra and frequency spectra show distinctly different characteristics. When thymine (T) was replaced by guanine (G), absorbance within the terahertz region was increased substantially both in temporal spectra (Fig. 2) and Fourier transformed spectra (Fig. 3). This observation was in agreement with the fact that absorbance by G within the THz range 0.1–20 was higher compared to that by T as shown in Fig. 1.

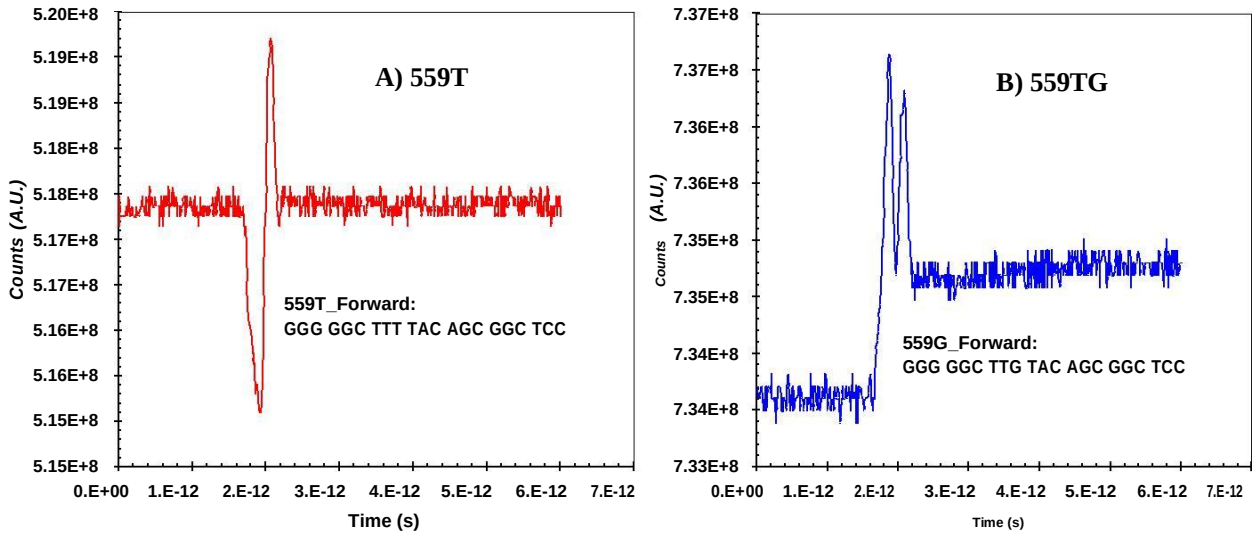

Fig. 2. Temporal pulses of 21-mer oligonucleotides of single strand FCGR3A with SNP (B) or without SNP (A) obtained by subtracting the blank substrate pulse from the respective samples. Temporal signal of sample B showed higher counts compared to that by native oligonucleotide A.

3.3. Distinct spectra of various ss DNA and hybridized ds DNA with or without SNPs

Previously we have reported that THz spectral patterns of a 25-mer oligonucleotide (ssDNA) are distinctly different from the spectra of hybridized DNA (dsDNA) (29). Using THz spectroscopy three different peaks were identified for both ssDNA and dsDNA of known sequence and the peaks were significantly shifted compared to each other thus allowing an easy comparison. We have also shown that FCGR3A oligonucleotides with or without SNP show distinctly different THz spectra (31). Here we show that THz spectra of dsDNA with native nucleotides can be distinguished from those containing SNPs. In that case, patients DNA samples can be characterized without any denaturation process hence cutting down on reagent, time and cost. We have synthesized complementary oligonucleotides C and D as C: 559T_Reverse: GGA GCC GCT GTA AAA GCC CCC or 559T_Reverse: D: GGA GCC GCT GTA CAA GCC CCC and examined the spectral patterns of ssDNA and that of the hybridized dsDNA.

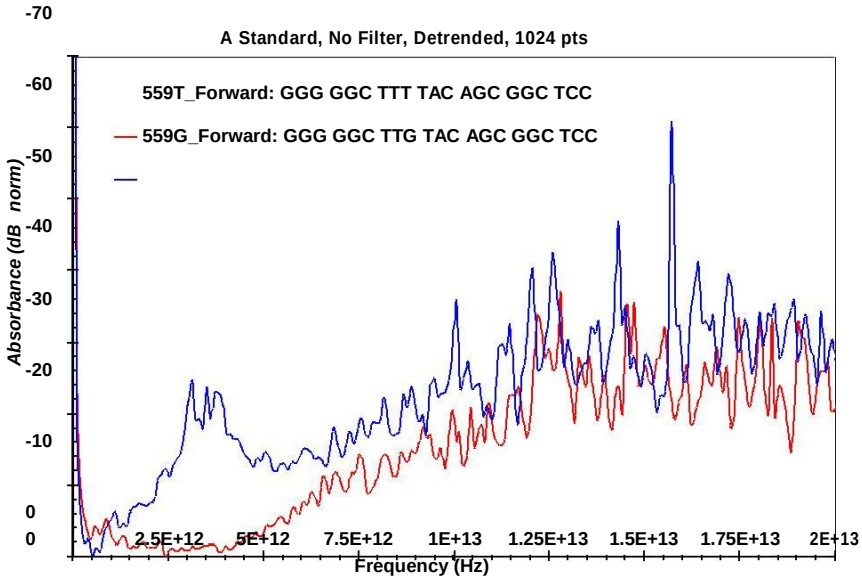

Fig. 3. Absorbance spectra were obtained by Fourier transform of temporal pulse of single strand oligonucleotides. Compared to sample A (red), sample B (blue) with SNP (T → G) showed substantially higher absorbance within 2–6 THz frequency.

Hybridization of complementary ssDNA probes was accomplished by heating solutions of 1:1 mixtures of the two oligos at 90°C, then slowly cooling to room temperature. Aqueous solution of these samples (~2 pmol) was applied onto microscope slides to make 1 cm spots. Temporal signals were acquired in sub-pico to pico-second range and converted to frequency domain via Fourier transform.

Spectral patterns of ssDNA (A, B, C, D) and double stranded DNA (A+C, B+C) are shown in figures 6 and 7, respectively. Figure 6 shows the spectral analysis in THz frequency domain of the following ss oligonucleotides:

A) 559T_Forward: GGG GGC TT**T** TAC AGC GGC TCC
B) 559G_Forward: GGG GGC TT**G** TAC AGC GGC TCC
C) 559T_Reverse: GGA GCC GCT GTA AAA GCC CCC
D) 559T_Reverse: GGA GCC GCT GTA CAA GCC CCC

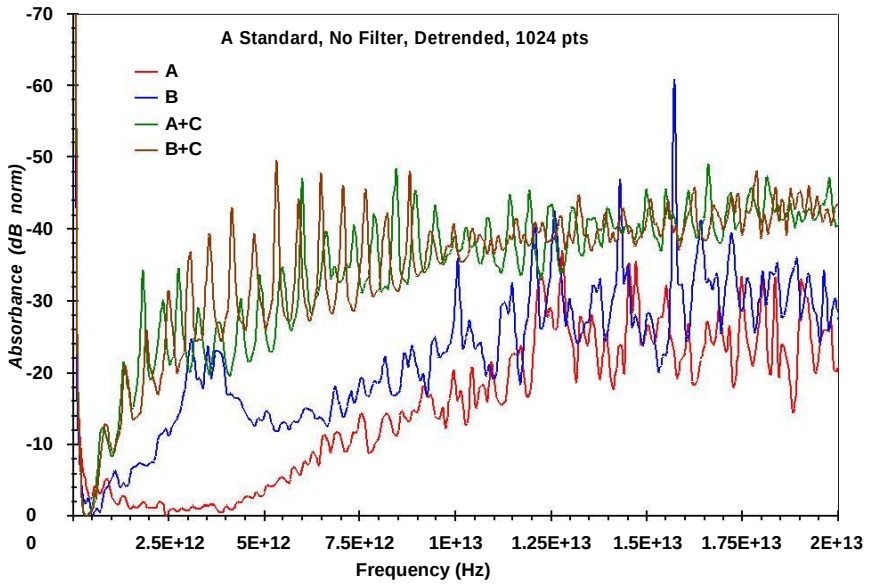

Fig. 4. Spectral patterns of oligonucleotide C and D are compared with those of A and B within the terahertz frequency range 1–15. Compared to those of A and B, absorbance by the oligonucleotide C and D were substantially higher over the entire range.

Absorbance by ssDNA 'C' and 'D' were significantly higher over the entire THz range compared to those by ssDNA 'A' and 'B'. Part of the reasons may be due to the fact that an overall absorbance by the nucleotide A is substantially higher within the THz range (0.1-20) compared to those by the nucleotides G, C, T (Fig. 1) and the ssDNA 'C' and 'D' contain more adenine (5 or 4) compared to by ssDNA 'A' and 'B' (2). Spectra of hybridized dsDNA with or without SNP (T → G) are also distinctly different showing (Fig. 7) higher absorption peaks by hybridized B+C compared to that by A+C as would be expected from the spectra of single stranded oligonicleotides A, B and C (Fig. 6). It is important to note that in a double stranded DNA, complementary base pairs G and C are bound by three hydrogen bonds and the base pairs A and T are bound by two hydrogen bonds and double stranded DNA with more G/C bonds become more tightly bound compared to A/T bonds. Hence due to a SNP (T → G) DNA acquires one more G-C pair and becomes more stable and resonates at different THz frequency.

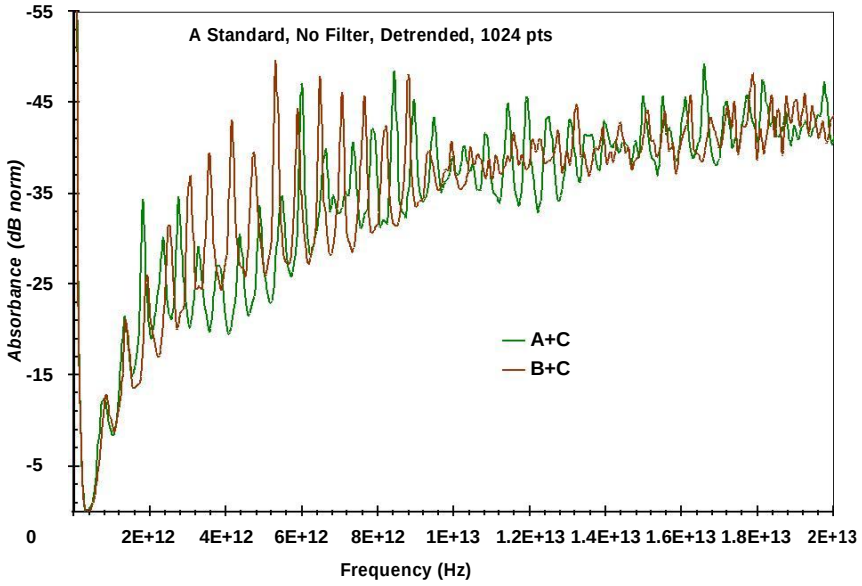

Fig. 5. Absorbance spectra of the dsDNA with samples A+C (green) and B+C (red). Higher absorbance peaks are observed with sample B+C compared to hat of A+C. These peaks are likely arising from unique vibrational and/or conformational states of the present oligos and the spectrum forms a unique signature of this particular sequence.

4. Conclusions

TeraSpectra offers a spectroscopic means for label free detection of SNPs. Detection and genotyping of SNPs are of fundamental importance in the identification of numerous genetic and hereditary diseases and also for the development of personalized medicine. SNPs are also the most abundant genetic marker in modern plant genetic analysis. Hence, there is a tremendous need for quick and affordable means for routine detection of SNPs. Existing molecular methods for detection of SNPs are very labor intensive and expensive requiring DNA denaturation, PCR and fluorescence labeling. TeraSpectra is a turn key spectrometer developed by Applied Research & Photonics spanning the frequency range 0.1–20 terahertz within which a multitude of DNA resonances can occur. The method does not require DNA denaturation, PCR or fluorescence labeling and spectral fingerprints of DNA with SNPs can be achieved within minutes. With the development of an automated 96-well high-throughput spectral scanning of DNA samples followed by data analysis, TeraSpectra constitutes a very efficient, quick and less expensive (compared to molecular methods) way of SNP detection. In addition, lesser sample processing requires lesser reagents implying lesser problem with disposal of biohazard materials.

5. References

1. Y.H. Lee, J. D. Ji and G. G. Song. Associations between *FCGR3A* Polymorphisms and Susceptibility to Rheumatoid Arthritis: A Metaanalysis J. Rheumatology, 35: 2129-35, 2008

2. Y. H. Lee , Y. H. Rho , S. J. Choi , J. D. Ji , G. G. Song , S. K. Nath and J. B. Harley. The PTPN22 C1858T functional polymorphism and autoimmune diseases—a meta-analysis' Rheumatology Advance Access published online on November 29, 2006

3. Neil Hanchard, Abier Elzein, Clare Trafford, Kirk Rockett, Margaret Pinder, Muminatou Jallow, Rosalind Harding, Dominic Kwiatkowski, and Colin McKenzie. Classical sickle beta-globin haplotypes exhibit a high degree of long-range haplotype similarity in African and Afro-Caribbean populations. BMC Genet, 8: 52, 2007.

4. Eden R. Martin, William K. Scott, Martha A. Nance, Ray L. Watts, Jean P. Hubble, William C. Koller, Kelly Lyons, Rajesh Pahwa, Matthew B. Stern, Amy Colcher, Bradley C. Hiner, Joseph Jankovic, William G. Ondo, Fred H. Allen, Christopher G. Goetz, Gary W. Small, Donna Masterman, Frank Mastaglia, Nigel G. Laing, Jeffrey M. Stajich, Rbert C. Ribble, Michael W. Booze, Allison Rogala, Michael A. Hauser, Fengyu Zhang, Rachel A. Gibson, Lefkos T. Middleton, Allen D. Roses, Jonathan L. Haines, Burton L. Scott, Margaret

   A. Pericak-Vance, Jeffery M. Vance. Association of Single-Nucleotide Polymorphisms of the Tau Gene With Late-Onset Parkinson Disease.. J*AMA*, 286:2245-2250 (2001)

5. Yi-Ju Li, Sofia A. Oliveira , Puting Xu,

Eden R. Marti, Judith E. Stenger, Clemens R. Scherzer, Michael A. Hauser, William K. Scott, Gary W. Small, Martha A. Nance, Ray L. Watts, Jean P. Hubble, William C. Koller, Rajesh Pahwa, Mathew B. Stern, Bradley C. Hiner, Joseph Jankovic, Christopher G. Goetz, Frank Mastaglia, Lefkos T. Middleton, Allen

D. Roses, Ann M. Saunders, Donald E. Schmechel, Steven R. Gullans, Jonathan L. Haines , John R. Gilbert , Jeffery M. Vance and Margaret A. Pericak-Vance. Glutathione S-transferase omega-1 modifies age-at-onset of Alzheimer disease and Parkinson disease.. Human Molecular Genetics, 2003, Vol. 12**,** 3259-3267, 2003

6. Henning Gohlke' Uta Ferrari, Kerstin Koczwara , Ezio Bonifacio , Thomas Illig, Anette-G. Ziegler. SLC30A8 (ZnT8) Polymorphism is Associated with Young Age at Type 1 Diabetes Onset. Rev. Diabet. Stud, 2008, 5(1):25-27

7. Scot J. Matkovich, Derek J. Van Booven, Anna Hindes, Min Young Kang, Todd E. Druley, Francesco L.M. Vallania, Robi D. Mitra, Muredach P. Reilly, Thomas P. Cappola and Gerald W. Dorn, II. Cardiac signaling genes exhibit unexpected sequence diversity in sporadic cardiomyopathy, revealing *HSPB7* polymorphisms associated with disease. J Clin. Invest. 2010;120(1):280–289.

8. Kristin J. Meyers; Thomas H. Mosley; Ervin Fox; Eric Boerwinkle; Donna K. Arnett; Richard B. Devereux; Sharon L.R. Kardia Genetic Variations Associated With Echocardiographic Left Ventricular Traits in Hypertensive Blacks. Hypertension. 2007; 49:992-999

9. Evdoxia Hatjiharissi, Lian Xu, Daniel Ditzel Santos, Zachary R. Hunter, Bryan T. Ciccarelli,

Sigitas Verselis, Michael Modica, Yang Cao, Robert J. Mannin, Xavier Leleu, Elizabeth A. Dimmock, Alexandros Kortsaris, Constantine Mitsiades[2], , Kenneth C. Anderson, Edward A.

Fox, and Steven P. Treon ; Increased natural killer cell expression of CD16, augmented binding and ADCC activity to rituximab among individuals expressing the Fc γRIIIa-158 V/V and V/F polymorphism. Prepublished online as Blood First Edition Paper, May 2, 2007

10. Cartron G, Dacheux L, Salles G, et al. Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor Fcgamma RIIIA gene. Blood 2002; 99:754–758

11. Weng WK and Levy R. Two immunoglobulin G Fc receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. J Clin Oncol 2003; 21:3940–3947

12. Treon SP, Hansen M, Branagan AR, et al. Polymorphisms in Fc γRIIIA (CD16) receptor expression are associated with clinical responses to rituximab in Waldenstrom's macroglobulinemia. J. Clin. Oncol. 2005; 23:474–481

13. Wadelius M et al. Blood. 2008 Jun 23. Epub ahead of print. DOI 10.1182/blood-2008-04-149070.

14. Gage BF, Lesko LJ. Pharmacogenetics of warfarin: Regulatory, scientific and clinical issues. J Thromb Thrombolysis. 2008: 25:45-51

15. V S Basile , V Özdemir , M Masellis , M L Walker , H Y Meltzer , J A Lieberman , S G Potkin , G Alva , W Kalow , F M Macciardi and J L

Kennedy A functional polymorphism of the cytochrome P450 1A2 *(CYP1A2)* gene: association with tardive dyskinesia in schizophrenia, , Molecular Psychiatry (2000) 5, 410–417.

16. Dubcovsky, J. 2004. Marker assisted selection in public breeding programs: The wheat experience. Crop Sci. 44:1895–1898

17. Dekkers, J.C.M., and F. Hospital. 2002. The use of molecular genetics in the improvement of agricultural populations. Nat. Rev. Genet. 3:22–32

18. John Manners, Lynne McIntyre, Rosanne Casu, Giovanni Cordeiro, Mark Jackson, Karen Aitken, Phillip Jackson, Graham Bonnett, Slade Lee and Robert Henry. Can genomics revolutionise genetics and breeding in sugarcane? Genet. Mol. Biol. vol.32 no.3 São Paulo 2009 Epub June 19, 2009

19. Feng Li, Hiroyasu Kitashiba, Kiyofumi Inaba † and Takeshi Nishio* A *Brassica rapa* Linkage Map of EST-based SNP Markers for Identification of Candidate Genes Controlling Flowering Time and Leaf Morphological Traits**.** DNA Research 2009 16(6):311-323

20. Drmanac, R., Drmanac, S., Strezoka, Z., Paunesku, T., Labat, I., Zeremski, M., Snoddy, J., Funkhouser, W., K., Koop, B., Hood, L., & Crkvenjakov, R. DNA-sequence determination by hybridization – a strategy for efficient large scale sequencing. Science 260:1649-1653, 1993.

21. Guo, Z., Guilfoyle, R. A., Thiel, A., J., Wang, R. F. & Smith, L. M., Direct fluorescence analysis of genetic polymorphisms by hybridization with oligonucleotide arrays on glass supports. Nucl. Acids Res. 22: 5456-5465, 1994.

22. Chee, M., Yang, R., Hubbel, E., Berno, A., Huang, X. C., Stern, D., Winkler, J., Lockhart, D. J., Morris, M. S., & Fodor, S., A., Accessing genetic information with high density DNA arrays. Science 274: 610-614, 1996.

23. Ozaki, H. & Mclaughlin, L.W., The estimation of distances between specific backbonelabeled sites in DNA using fluorescence resonance energy transfer. Nucl. Acids Res. 20: 5205-5214, 1992

24. Zhu, Z. & Waggoner, A., S., Molecular mechanism controlling the incorporation of fluorescent nucleotides into DNA and PCR. Cytometry 28: 206-211, 1997.

25. Larramendy, M. L., El-Rifai, W. & Knuutila, S., Comparison of fluorescein sothiocyanateand Texas red-conjugated nucleotides for direct labeling in comparative genomic hybridization. Cytometry 31: 174-179, 1998.

26. VanZandt, L. L. & Saxena, V. K., Millimeter-microwave spectrum of DNA – six predictions for spectroscopy. Phys. Rev. A39: 2672-2674, 1989.

27. Zhuang, W., Feng, Y., & Prohofsky, E. W., Self-consistant calculation of localized DNA vibrational properties at a double-helix-single-strand junction with anharmonic potential. Phys. Rev. 1990: A41, 7033.

28. Bolivar, P. H., Nagel, M., Richter, F., Brucherseifer, M., Kurz, H., Bosserhoff, A., and Buttner, R., Label-free THz sensing of genetic sequences: towards 'THz biochips, Phil. Trans.. R. Soc. Lond.. A (2004) 262, 323-335.

29. Anis Rahman, Bruce Stanley, Aunik K. Rahman, "Ultrasensitive label-free detection and quantitation of DNA hybridization via terahertz spectrometry,"Paper Number: 7568-8, SPIE conference on Imaging,

Manipulation, and Analysis of Biomolecules Cells, and Tissues VIII, January 23-28 2010, SPIE Photonics, San Francisco, CA

30. Markelz, A. G., Roitberg, A., & Heilweil, E. J., Pulsed terahertz spectroscopy of DNA, bovine serum albumin and collagen between 0.1 and 2.0 THz. Chem. Phys. Lett. 320: 42-48, 2000.

31. Terahertz Spectral Analysis of FCGR3A Genotypes. Gulshan Ara, Aunik K. Rahman, Bruce A. Stanley, Anis Rahman, ACS , 41st Mid-Atlantic Regional Meeting. April 10-13, 2010

32. Urbe, H. & Tominaga, Y., Low frequency Raman spectra of DNA. Biopolymers 21: 2477-2481, 1982.

33. Woolard, D., Koscica, T., Rhodes, D. L., Cui, H. L., Pastore, R. A., Jensen, J. O., Jensen, J. L., Loerop, W. R., Jacobsen, R. H., Mittleman, D., & Nuss, M. C., Millimeter wave induced modes in DNA as a possible alternative test to animal tests to probe for carcinogenic mutations. J. Appl. Toxicol. 17: 243, 1997.

34. Brucherseifer, M., Nagel, M., Bolivar, P. H., Kurz, H., Bosserhoff, A. and Buttner, R., Label-free probing of the binding state of DNA by time-domain terahertz sensing. Appl. Phys. Lett. 77: 4049-4051, 2000.

35. (a) Rahman, A. "Stimulated emission of terahertz radiation from electro-optic dendrimer" SPIE Proceedings Vol. 7601 Terahertz Technology and Applications III, Laurence P. Sadwick and Creidhe M. M. O'Sullivan Editors, vol. **7601**: 76010C-1 – 76010C-5, (2010).

(b) Rahman, A., Rahman, A. K., & Tomalia, D. A., "Dendrimer Dipole Excitation: A New Mechanism for Terahertz Generation," J. Biosens. Bioelectron. 2016, 7:1. DOI: 10.4172/2155-6210.1000196.