# Integrated THz technology for label-free genetic diagnostics

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(Received 24 May 2001; accepted for publication 30 October 2001)

We report on a promising approach for the label-free analysis of DNA molecules using direct probing of the binding state of DNA with electromagnetic waves at THz frequencies. Passive THz resonator devices based on planar waveguides are used as sample carriers and transducers for THz transmission analysis. In comparison to a formerly used free-space detection scheme, this method provides a drastically enhanced sensitivity enabling analysis down to femtomol levels. We examine the potential of our approach on biologically relevant DNA samples and demonstrate the detection of single base mutations on DNA molecules. © *2002 American Institute of Physics.*
[DOI: 10.1063/1.1428619]

Genetic diagnostics has seen a dramatic capability increase with the development of DNA biosensors, gene chips, and lab-on-a-chip DNA analyzers.[1] Most approaches for identifying polynucleotide sequences are based on detecting the binding (''*hybridization*'') of unknown ''*target*'' DNA molecules to single-stranded oligo- or polynucleotide ''*probe*'' DNA molecules having a known base sequence, as hybridization occurs preferentially between molecules with complementary base sequences. Currently, hybridization detection is largely based on fluorescent labeling and detection of the target DNA molecules. Although fluorescent labeling has given rise to extremely efficient diagnostic systems, like gene chips,[2] alternative label-free detection schemes appear mandatory: Labeling not only constitutes an additional preparatory step, but can modify the DNA strand conformation lowering the precision of gene detection.[3] Additionally, fluorophore degradation, labeling yield fluctuations and fluorescence efficiency site dependence deteriorate the quantificability of fluorescence based diagnostics.[4,5] Theoretical calculations predict a multitude of intrinsic resonances in the THz frequency range associated with interbackbone excitations of DNA molecules,[6,7] providing THz-probing technologies with a unique potential for the label-free detection of the DNA binding state. Although investigations by Raman,[8] Fourier-transform,[9] or time-domain[10] THz techniques on hybridized DNA molecules have been performed in the past, only very few experiments have addressed its binding state, e.g., by evaluating temperature dependencies.[11] Recently, the interrelation of the binding state of DNA and its complex refractive index at THz frequencies was demonstrated, opening a way for label-free gene detection:[12] With a free-space detection scheme a broadband (0.4–2.2 THz) higher real and imaginary refractive index for samples with hybridized (double-stranded) DNA molecules was observed in comparison to denatured (single-stranded) DNA molecules. Despite of its proof-of-principle relevance, this free-space approach has the major drawback of requiring large amounts of DNA for yielding reliable results, which hinders its application for large throughput genetic analysis. Here, we present an integrated planar THz probing system using femtosecond laser based techniques that drastically enhances the analytic sensitivity and reduces the DNA material requirements, which consequently may open the path for the widespread diagnostic application of THz sensing by enabling the further development of integrated THz gene chips.

The integrated planar THz probing approach is based on the generation and detection of the involved THz signals directly on the chip where the DNA sample is placed. This integration allows one to enhance the analytic sensitivity by orders of magnitude by increasing the interaction length of THz signal and DNA sample using thin-film microstrip (TFMS) lines with an embedded resonator to guide the THz signal in plane along the very thin DNA samples. Figure 1(a) depicts a top view of a complete test structure consisting of a photoconductive switch to generate broadband THz signals, TFMS lines to guide the signals and a band-pass filter acting as the THz resonator which senses the DNA molecules. The photoconductive switch is based on low-temperature grown GaAs that has been van der Waals bonded to the dielectric layer below. The TFMS lines illustrated in Fig. 1(b) have been carefully designed in terms of geometry and material choice in order to support broadband single mode propaga-

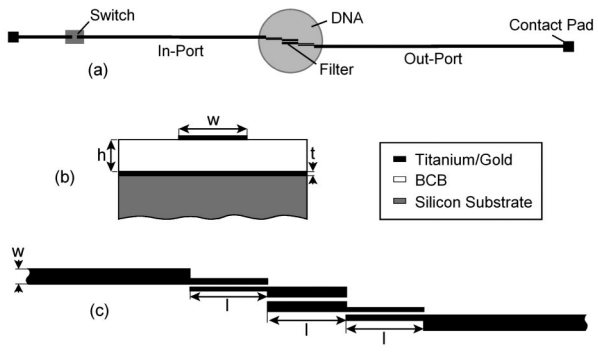


FIG. 1. Top view (not to scale) of the applied THz device structure (a) cross section of the TFMS line using gold for the signal lines and benzocyclobutene (BCB) as a low-$k$ dielectric material (b), and enlarged top view of the band-pass filter (c), $w=16\ \mu$m and $l=85\ \mu$m.

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tion with low attenuation and dispersion in the intended frequency range of 20–2000 GHz.[13] Using an embedded band-pass filter as a resonant structure has a further advantage in order to increase the probing sensitivity: sensing the resonance shift of a high-$Q$ resonator loaded with a dielectric material is a far more sensitive approach for characterizing dielectric properties than broadband probing methods.[14] Until now, however, the application of resonant probing has been limited to the lower frequencies that standard all-electronic equipment provide. Using optoelectronic techniques, we extend the application area of this method to the THz frequency range. In our case, as sketched in Fig. 1(c), a band-pass filter is used which consists of three parallel-coupled microstrip line resonators, where the basic length $l$ is a quarter wavelength at the first pass-band center frequency $f_c$ (for design details, see e.g., Ref. 15). The uncoated filter is designed to have a band-pass center frequency of 610 GHz by using commercial high-frequency simulation software. For the analysis, the resonator is coated with DNA samples, by pipetting the DNA material from an aqueous solution onto the device. After the water evaporation, a thin DNA film is formed above the resonator.

The experiments are conducted as follows: A sub-ps electrical pulse train is excited by a solid-state laser pumped 780-nm Ti:Sapphire laser with a repetition rate of 78 MHz at the biased photoconductive switch. The electric signal propagates along the TFMS lines and through the DNA-loaded resonator. Time-delayed pulses of a probe laser beam detect the electric-field transients on the TFMS at a distance of 2 mm before and behind the resonator with a freely positionable $LiTaO_3$ electrooptic probe tip.[16] This large distance is used to allow the temporal separation of incoming and reflected signals. The measured data is converted to the frequency domain by fast Fourier transformation to derive the frequency dependent transmission parameters ($S_{21}$).

To evaluate the potential of our method on biologically relevant samples, we conducted the following two sets of experiments. In a first set of experiments, the filters are coated with denatured or hybridized DNA solutions at a concentration of 5 $\mu$g/$\mu$l in bidestilled $H_2O$. As samples, we use a DNA molecule (vector pcDNA3, Invitrogen, Carlsbad, CA) with a length of 5400 b (nucleotide bases). One part of this solution is denatured by rising the temperature to 95 °C for 5 min and immediately quenched on ice. Renaturation is known to be small, due to the large DNA molecule size. 0.5 $\mu$l of denatured or hybridized pcDNA3 solution, respectively, is pipetted on two identical THz resonators fabricated at a distance of 1 mm on the same wafer. Thin films of DNA with a diameter of approx. 0.5 mm and a thickness of 40 nm and 80 nm are obtained for hybridized and denatured DNA, respectively, after water evaporation. A bulge with a height of ~5 $\mu$m is formed at the edge of each DNA film. Repeated experiments on devices prior to and after DNA pipetting are made. The magnitude of the measured transmission parameters ($S_{21}$) of a filter with and without DNA film are compared in Fig. 2. The measured and simulated data of the unloaded device (without DNA) are in good agreement. Measurements at several DNA loaded resonator pairs are taken at 40% room humidity at a constant time interval after sample pipetting, since the refractive index of DNA is known to depend on humidity.[10] Repeated analyses demonstrate a good reproducibility. We would like to emphasize, that the depicted resonances represent the inherent resonance of the transducer (i.e., the band-pass filter) that is detuned in proportion to the refractive index of the overlaid DNA samples—they are not a direct visualization of a DNA molecule resonance. Figure 2 demonstrates that hybridized DNA clearly generates a stronger shift from the original center frequency than denatured DNA. The observed frequency shifts amount to 124 GHz (20%) and 55 GHz (9%) for the hybridized and the denatured material, respectively. Also a lower quality factor $Q$ and a stronger damping of the hybridized sample is observed. The significant resonance frequency shift upon the degree of hybridization illustrates the capability of THz probing as a label-free detection scheme.

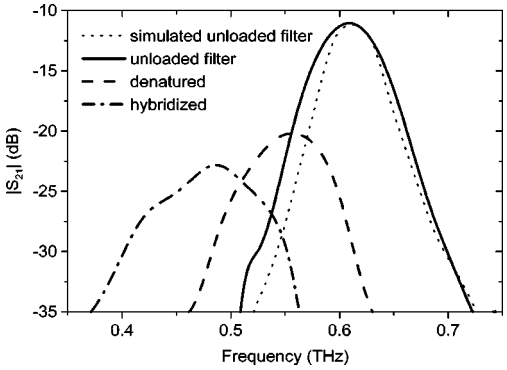


FIG. 2. Magnitude of the transmission parameter $S_{21}$ of the band-pass filters loaded with denatured or hybridized DNA films on top (5.4 kb vector pcDNA3). In comparison, the measured and simulated reference data of the unloaded filter.

In a second set of experiments, we evaluate the potential of THz probing to detect even single base mutations. We analyzed a gene (termed ''HFE'') that is frequently mutated in patients with hereditary hemochromatosis—one of the most common genetic diseases. For the analysis, we use non-mutated (*wt*: ''wild-type'') HFE DNA molecules with a length of 164 b that cover the region where this single-base mutation occurs. We prepare three different DNA samples with a concentration of 0,52 $\mu$g/$\mu$l in bidistilled $H_2O$ by mixing solutions of the double-stranded *wt* HFE gene fragment with equimolar solutions of three different single-stranded DNA molecules (oligos) with a length of 15 b. In the first sample, the *wt* HFE gene is mixed with an oligo having a base sequence that exactly complements the center region of *wt* HFE gene (''*oligo wt*''), in the second sample, it is mixed with a similar oligo having the single-base mutation responsible for hemochromatosis (''*oligo mut*'') and in the third sample with an oligo having a completely different base sequence (''*oligo control*'') but with matching size and average base composition. All these mixtures are denatured and subsequently cooled down to room temperature to permit rehybridization before they are deposited on the THz resonators. The experimentally measured transmission parameters of all three samples are compared in Fig. 3. The lowest shift of $\Delta f = 37$ GHz (6%) from the original resonance frequency $f_{c0}$ is observed for the exact complementary sample, while a significant higher shift of $\Delta f = 62$ GHz (10%) is introduced by the sample containing the single-base mutation. The larg-

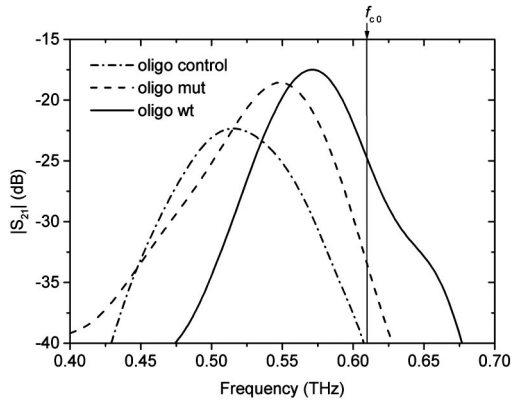


FIG. 3. Magnitude of the transmission parameter $S_{21}$ of the band-pass filters loaded with three different DNA samples. All samples are composed of the same double-stranded HFE DNA molecules with a length of 164 b but different 15 b single-strands as probes.

est shift $\Delta f = 93\,\mathrm{GHz}$ (15%) and significant frequency broadening arises from the control sample.

In order to understand the experimental observations, one should be aware, that the characteristic parameters of the sensing THz resonator like center frequency ($f_c$), quality factor ($Q$) and loss depend sensitively on the relative permittivity and the absorption of the attached dielectric medium. Generally, an increase of the effective permittivity ($\varepsilon_{r\,\mathrm{eff}}$) of a microstrip line will lead to a decrease of the supported phase velocity. As a consequence the resonance frequency ($f_c$) of the filter decreases in the same way. In our experiments $\varepsilon_{r\,\mathrm{eff}}$ of the resonator is increased by the attached DNA samples. Of course, the shift of $f_c$ to lower frequencies is stronger for a material with a higher refractive index, which means that the resonator is detuned in proportion to the hybridization degree of the DNA molecules. The data presented here are in good agreement with the free-space measurements where the hybridized samples exhibited an increased refractive index and higher absorption in comparison to the denatured samples.[12] By denaturing a DNA solution, the viscosity of this solution increases. Thus, the thickness of the denatured DNA films was on average twice as high as the hybridized DNA films. We can exclude, however, that we only probe thickness variations, because the thinner hybridized films always resulted in a higher frequency shift. This variation implies that the sensitivity of THz hybridization probing will be even larger than currently observed in the case of identical sample thickness. As our DNA spots exceed the THz resonator size by far, more DNA material is applied than necessary. The resonance frequency shift, however, stems from the extremely small DNA volume defined by the resonator size ($265\times 50\,\mu\mathrm{m}^2$) and the DNA film thickness. It corresponds to 1.1 femtomol of probed DNA molecules in the first experiment. If adequate pipetting and immobilization techniques are introduced, this femtomolar sensitivity makes this integrated THz technique comparably sensitive to standard commercial fluorescence-based systems.[17] In comparison to free-space THz sensing,[12] we have reduced the amount of probed DNA molecules by a factor of $>10^3$.

The presented detection of a single-base defect in the second experiment takes advantage of the specificity of DNA to hybridize. It is well known that shorter DNA segments hybridize faster than larger segments.[18] As a consequence, rehybridization of the longer HFE fragments is effectively blocked by the faster affiliating short wild-type *oligo* single-strands matching exactly a complementary part on one of the HFE fragments. The mutated oligo single-strand, however, does affiliate worse as it does not have an exact complementary part on the HFE gene fragment and therefore a stronger rehybridization of the HFE fragments takes place. The control strand is entirely incompatible with the DNA sample. Therefore, rehybridization of the HFE fragment occurs unhindered. The *wt* sample (*oligo wt*) contains thus the lowest, the mutated (*oligo mut*) an intermediate and the control sample (*oligo control*) the highest amount of rehybridized DNA base pairs, which lead to correspondingly increasing resonance frequency shifts. The distinct frequency shifts are reproduced very well in repeated experiments and confirm conclusively the capability of THz methods not only to identify, but, to quantify the hybridization degree of polynucleotides. Due to the lower concentration of this samples, however, the signal response is slightly reduced in comparison to the previous data of Fig. 2.

In summary, our analysis demonstrates that integrated THz technologies can efficiently probe the hybridization of nucleic acids. A detection sensitivity reaching femtomol levels and the capability for single-base mutation detection are demonstrated. One of the inherent advantages of the applied planar guided-wave approach is its scalability, leaving plenty of room for developing high-throughput genetic diagnostic systems. By integrating two-dimensional arrays of THz transducers, a large number of genes could simultaneously be analyzed. Further refinement of the technique, e.g., by improving the rudimentary pipetting and immobilizing probe molecules is, however, required before this measurement principle can be transferred to large scale gene analysis.

The authors gratefully acknowledge useful discussions with A. Stahl, H. P. M. Pellemans, O. Voigt, and C. Mittermaier for continuous encouragement, and financial support by the DFG and by the *Ministerium für Schule Wissenschaft und Forschung des Landes Nordrhein-Westfalen*.

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