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Nondestructive Label-Free Mapping of DNA Bioassay Using a Near-Field Scanning Microwave Microscope

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Abstract. Near-field scanning microwave microscope (NSMM) is used to readout and visualize homemade 10mer oligonucleotides microarrays of DNA. NSMM instrumentation does not require labeling of target sequences with fluorophores or other tagging groups. Sensitive examination of DNA coverage and high resolution mapping of DNA spots in the microarray were observed by measuring the change of microwave reflection coefficient (S_{11}) at about 4 GHz operating frequency. The linear dynamic range for the 10mer nucleotide is over at least 3 orders of magnitude from 0.001pM to 1pM with the detection limit estimated to be approximately 1fM, i.e. 2×10^{10} stands/m^2.

Keywords: microwaves, DNA microarray, hybridization

The DNA microarray format is a new integrated tool that allows an analysis of RNA and DNA homology for thousands of genes at the same time with highly effective data processing. Modern assays for DNA diagnostics have evolved into high-throughput microarray formats that typically rely on fluorescent labeling of the analyte [1-3]. Label-free diagnostics remain attractive, however, as they simplify sample preparation, decrease assay costs, and eliminate potential artifacts from label instability or perturbation of assay thermodynamics [4-7]. Ideally, identification of DNA spots in a bioassay would be sensitive yet not rely heavily on instrumentation and chemical reagents [8]. Recently, there have been a number of investigations using nanoparticle labels to detect DNA [9-11]. These detection methodologies strongly depend on the availability of a mechanism that transduces and amplifies specific DNA binding events to detectable signals. For example, DNA strands with a complementary sequence to that of target DNA can be chemically linked to gold nanoparticles and then the optical, electric, magnetic or other properties are used to detect the DNA utilizing oligonucleotide-functionalized Au nanoparticles [12-14].

The design, imaging performance and applications of a near-field scanning microwave microscope (NSMM) for noninvasive characterization of electrical properties of conducting and dielectric materials have been previously described [15-18]. The changes in intrinsic impedance and material characteristics (electrical conductivity, dielectric permittivity, magnetic permeability, volumetric and thin film properties, etc.) of various materials were investigated by NSMM by measuring the microwave reflection coefficient S_{11}. For example, the reflection coefficient depends on the complex dielectric permittivity profile ε, across the surface being probed. Difference in ε for

DNA and substrate, is reflected in shifts in resonance frequency f_r and reflection coefficient amplitude S_{11}, and can be mathematically modeled by transmission line theory within a material perturbation approach, assuming near-field dipole-dipole interactions [19-21]. Physically, the dielectric permittivity of a DNA-modified film surface is expected to depend on length of the strands, on arrangement of the molecules (e.g. single- vs. double-stranded, ordered vs. disordered) and presence of other species on the surface including, most prominently, physisorbed water [22]. To detect the DNA spots in microarray bioassay we imaged the sample surface by measuring the microwave reflection coefficient S_{11} at the resonance frequency of $f_r = 3.98$ GHz.

We demonstrate NSMM is able to measure the surface coverage analyte species at sensitivities comparable to conventional fluorescence bioassay devices. The NSMM approach does not require functionalization of DNA strands with flourophores, redox couples, nanoparticles or other labels. The technique provides a straightforward approach to multiplexed detection of DNA sequences with high sensitivity and selectivity.

A series of homemade DNA arrays with variable coverage was prepared for characterizing the detection response as a function of DNA coverage. 10mer oligonucleotides (5' HS-$(CH_2)_6$- CAA TAC GCA 3) and their unmodified complementary sequences were purchased from MWG Biotech and were purified with the company's HPSF process. The coverage of strands was controlled by varying the concentration of attaching oligonucleotide. 10mer oligonucleotides were spotted at a concentrations 0.1-6 μM in SSC1M buffer on an Affymetrix GMS 417 spotter, with spotted droplets allowed to dry completely (~2 min), followed immediately by a DI water wash and drying of the slide.

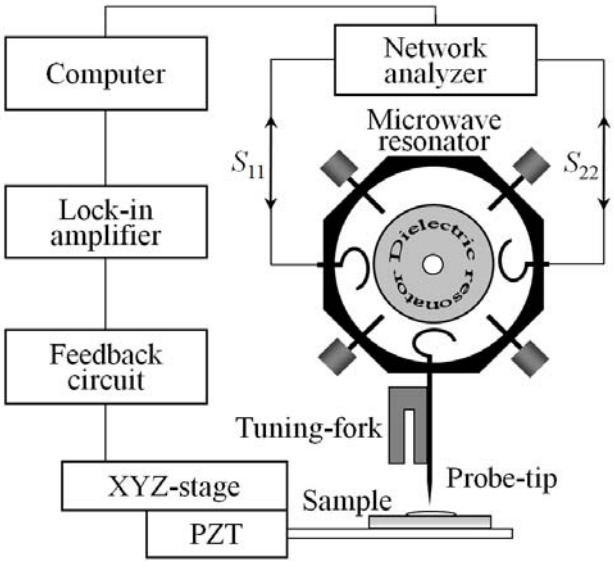

Fig. 1. The schematic of NSMM experimental setup.

The detailed description of the basic operation of a NFMM is presented in Ref. [19]. The schematic of the NSMM is shown in Fig. 1. Microwaves are generated by a source and input into the three-port cavity with the dielectric resonator inside. A tungsten tip is attached to the resonator

and extends outside the cavity. The other end of the tip is in close proximity to the sample surface (about of 10 nm). The cavity and resonator store electromagnetic energy with the resonant frequency depending strongly on material properties. The resonant frequency is sensitive to the near-field interaction of the tip and the sample. As the interaction changes (due to changes of material characteristics of the sample or external conditions), the coupled dipoles generate a large change in the electromagnetic energy in the resonator. In particular, dielectric permittivity changes in the sample will cause changes of both the central resonance frequency f_r and the quality factor Q of the resonator. By measuring the resonance frequency shift $\Delta f/f_r$ and the reflection coefficient change ΔS_{11} of the microwaves input into the cavity, the determination of the dielectric permittivity of the sample is possible. In order to optimize measurement sensitivity, both the quality of the resonator and the sensitivity of the probe must be maximized and matched, as a result, contrast of a NSMM image mainly depends on the interaction between the probe tip and the sample [20]. To extract quantitative information, it is essential to precisely control the probe tip-sample separation due to its strong influence on S_{11}.

The resonance frequency (f_r) and reflection coefficient (S_{11}) changes are related to the stored electric and magnetic energies in the original and perturbed cavity, so that the shift in resonant frequency can be related to the changes in stored energy of the perturbed cavity. In addition, the magnitude of the reflection coefficient S_{11} depends on the sample surface impedance. Minimization of the reflection coefficient without sample is the analogue of subtracting the background level in the measurement. A change in probe-sample interactions, due to the variation of material characteristic of the sample, perturbs the resonance condition and changes S_{11}. Thus, the shift of resonance frequency and the change in reflection coefficient correlate with the effective intrinsic impedance of the material. An expression for how the reflectivity S_{11} depends on sample impedance can be derived by using standard transmission line theory [21]

$$S_{11} = 20\log\left|\frac{Z_{in} - Z_0}{Z_{in} + Z_0}\right|. \qquad (1)$$

Here Z_0 is the effective impedance of the probe tip and Z_{in} is the complex intrinsic impedance of the DNA/substrate system. The complex input impedance of the sample then can be estimated as

$$Z_{in} = j\frac{Z_a k_a t_g}{1 - k_a^2 t_g t_{DNA} \varepsilon_{DNA}}, \qquad (3)$$

where Z_a and k_a is the characteristic impedance and wave number of the free-space, t_g is the thickness of glass slide and ε_{DNA} is the relative dielectric permittivity of DNA. Thus, the microwave reflection coefficient, S_{11} is affected by the relative dielectric permittivity of DNA.

One can consider a single molecule of DNA attached to a surface. To make this more precise, one can appeal to the model of dielectric properties of DNA as proposed by Oosawa [23]. In Oosawa's model, a single DNA chain is treated as a sphere consisting of interpenetrating positive and negative charges, with overall charge neutrality. By considering fluctuations of the dipole moment in equilibrium, it is easy to calculate that the relative shift in the dielectric permittivity,

$(\varepsilon - \varepsilon_0)/\varepsilon_0 \sim \nu n$ where ν is the volume of a single DNA molecule and n is the coverage (concentration) of molecules. However, this spherical geometry is not very realistic. A more realistic model, also proposed by Oosawa, consists of a positively charged ellipsoid with an interpenetrating ellipsoid of negative charge. This model, arguably still not realize, might serve as a crude approximation for short DNA strands.

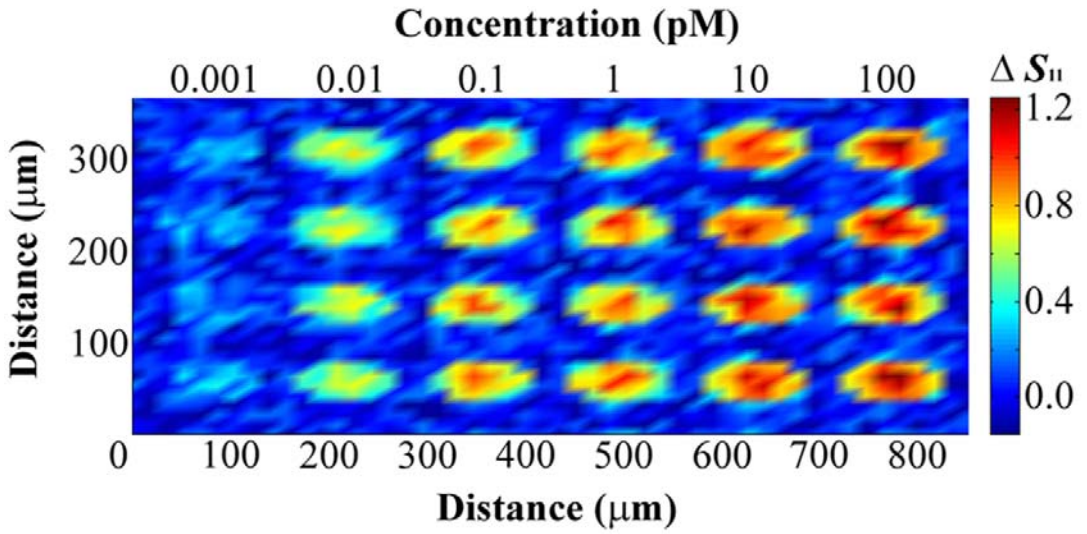

Fig. 2. The 350 μm × 850 μm NSMM image for 10mer oligonucleotide long sequence DNA in the concentration range of 0.001-100pM.

To illustrate application of NSMM to mismatch discrimination, capture oligonucleotides, consisting of a 10mer oligonucleotide long sequence derived from the anthrax lethal factor concatenated with a spacer, were immobilized by UV-crosslinking to aminosilanized slides. Figure 2 shows S_{11} traces from different coverage DNA surface ("capture" surface). From the data, using aminosilanized glass as reference, the reflection coefficient change for a spot with the lower coverage specimen was 0.47 dB compared to 1.33 dB for the highest coverage "capture" spot (2.85 times larger). This ratio can be compared to the value expected within the above discussed rudimentary theoretical model if all capture molecules were to bind target strands. The agreement is believed to be somewhat coincidental since not all probes are expected to be capable of binding target strands with different coverage. Moreover, target molecules with variable concentration can adsorb through sequence-nonspecific physical interactions, in this instance increasing the measured change. Such various possible contributions to S_{11}, representing important, but difficult-to-track aspects of surface modification with DNA molecules, merit a more detailed study separate from the current focus on the NSMM technique.

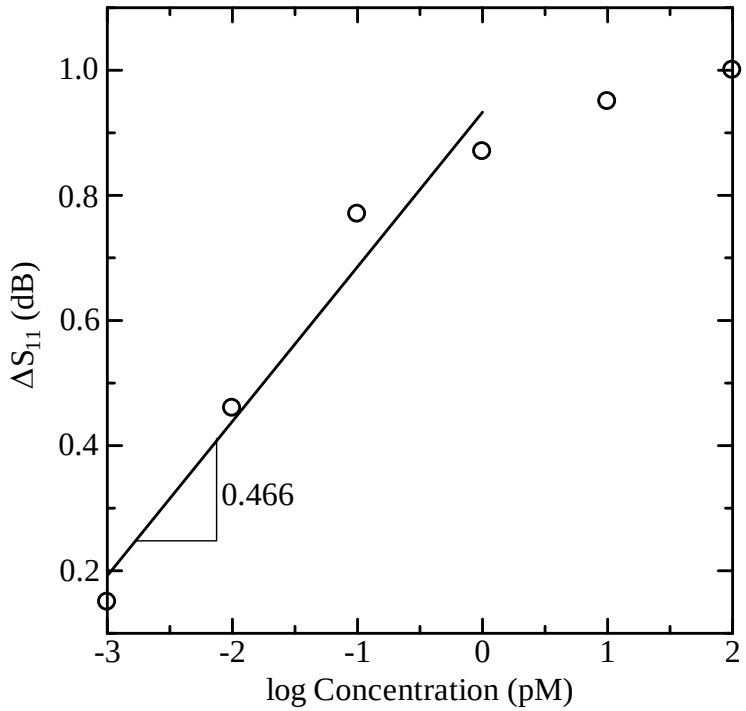

Fig. 3. The microwave reflection coefficient change, ΔS_{11} as a function of the target concentration of the 10mer oligonucleotide at the resonant frequency.

Assuming that full change corresponds to a difference of ~1.2 dB (between glass substrate and the highest coverage DNA), the NSMM method should be readily capable of detecting target coverage down to several percentage of the probe coverage. Furthermore, this estimate implies that the NSMM techniques can access decades of dynamic range as shown in Fig. 3. The microwave reflection coefficient change, ΔS_{11} is shown as a function of the target concentration in the 10mer oligonucleotide at the resonant frequency $f_r = 3.98$ GHz. The line is a linear fit through the first concentrations (0.001pM to 1pM). Note that the correlation coefficient was 0.466 for lower coverage (dynamic range) vs. 0.001 for higher coverage (1pM to 100pM). The smallest detectable change in coverage is 2×10^{10} strands/m^2 for 10mer nucleotides. This compares favorably with fluorescence-based scanners widely used in microarray applications. For example, an optimized home-built system was reported capable of detecting coverage down to 1×10^{13} fluorophores/m^2, or about 0.003pM in a 150 μm diameter microarray spot [24]. The above estimates show that even when multiple fluorophores are used per strand, label-free NSMM detection can approach if not exceed the sensitivity of conventional fluorescence diagnostics.

In summary, the main advantage of NSMM over other label-free detection methods is outstanding sensitivity and high spatial resolution (potentially less than 50 nm). The biophysical properties difference in the genomic DNA spots in the microarray chip leads to changes in the microwave reflection coefficient, which are measured by the NSMM. These changes are caused by bio-induced modification (hybridization) of the dielectric constant profile of the DNA strands. NSMM instrumentation does not require labeling of target sequences with fluorophores or other tagging groups. We presented the 3D NSMM image scanned over the patterned DNAs in

microarray format. These results clearly show the sensitivity and usefulness of that microwave microscope for those type biological investigations.

Acknowledgments

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