DETECTION OF DNA-GOLD NANOPARTICLE HYBRIDS ON PATTERNED SURFACES

A. M. Weld[1], H. B. Yin[2], T. Melvin[1, 2]
[1]Optoelectronics Research Centre, University of Southampton, Highfield, Southampton, S017 1BJ,UK
[2]School of Electronics and Computer Science, University of Southampton, Highfield, Southampton, S017 1BJ,UK
amw@orc.soton.ac.uk

An optical diffraction based sensor has been developed for the detection of DNA-gold nanoparticle hybrids. Silicon substrates were patterned with oligonucleotide sequences in the form of a one-dimensional diffraction grating. Complementary sequences are detected by hybridisation with an oligonucleotide functionalised gold nanoparticle label. Detection of the gold nanoparticles is achieved by examining the diffraction efficiency of the diffraction gratings formed by laser illumination.

I. INTRODUCTION

A wide range of microarrays have been created for the analysis of DNA sequences. Commercially available systems have been developed containing arrays of up to 10000 different DNA sequences [1]. Many of these systems use fluorescence detection. Known DNA oligonucleotides are immobilised on a surface and act as probes for gene sequences. Samples are fluorescently labelled and the hybrids are located and quantified from the fluorescent images.

Much effort has been made to develop non-fluorescent methods such as forming DNA-nanoparticle aggregates or holography. Mirkin and co-workers have developed a colourimetric DNA detection assay [2]. DNA labelled gold nanoparticles act as probes and hybridise with complementary linker strands forming aggregates. This is observable by a change in colour of the nanoparticle-DNA solution from red to blue as aggregation occurs and particles become closer together, red-shifting the plasmon absorption band.

Interference based detection of oligonucleotides has been developed by Jenison *et al* [3]. Capture oligonucleotides were attached to optically coated silicon, which appears gold in white light. After successful hybridisation with DNA target sequences labelled with biotin, a biotin antibody was added causing precipitation of a thin film. The colour of the reflected light was dependant upon the film thickness – as a result of destructive interference. A diffraction based approach will offer potential for creating a highly sensitive DNA biosensor.

II. DIFFRACTION-BASED DNA SENSOR

A diffraction-based sensor for detecting DNA oligonucleotide sequences has been developed. A silicon wafer is patterned with oligonucleotide capture sequences in a diffraction grating pattern with a period of 20.0 microns. Target sequences are attached to gold nanoparticle probes. Successful hybridisation is detected by illuminating the sample with a laser and measuring the diffraction pattern produced.

III. METHODS

A. Micropatterning of Silicon with oligonucleotides

For the development of this sensor device, silicon wafers have been patterned with diffraction gratings featuring a 20.0 micron period (shown in figure 1). Methods for preparing DNA patterns on silicon substrates have previously been reported [4, 5].

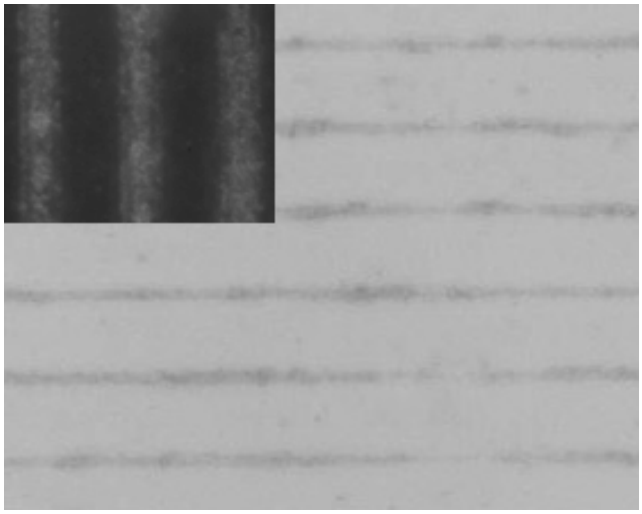

Figure 1) 20.0 micron period DNA grating on silicon

B. Gold Nanoparticle Hybridisation

Gold nanoparticles were functionalised with DNA oligonucleotide sequences. For the purpose of establishing the methodologies for optical detection of the nanoparticle labels, the sequences used were complementary to the oligonucleotides immobilised on the silicon wafer. The gold nanoparticles were hybridised directly to the DNA grating on the silicon wafer. The gold nanoparticles were placed on to the DNA grating in a hybridisation solution under a small cover-slip and incubated at room temperature in a humid chamber for 3 hours.

C. Diffraction Analysis

Fabricated DNA gratings on silicon were optically characterised by illumination with 1.0mW HeNe lasers with wavelengths of 632.8nm and 543.5nm. The incident laser beam was p-polarised (electric field vector parallel to the plane of incidence) and aligned at the Brewster angle for the

buffer environment of the DNA grating to minimise reflection from the surface. The diffraction order intensities were measured by scanning through the angular range of the first six diffraction orders with a power meter at an observation distance of 20.0cm. The experimental layout is shown in figure 2). A CCD camera was used to capture images of the diffraction pattern.

After hybridisation of the DNA gratings with gold nanoparticles, the diffraction orders were measured again. The diffraction data was analysed to examine the change in light distribution resulting from successful hybridisation of gold nanoparticles.

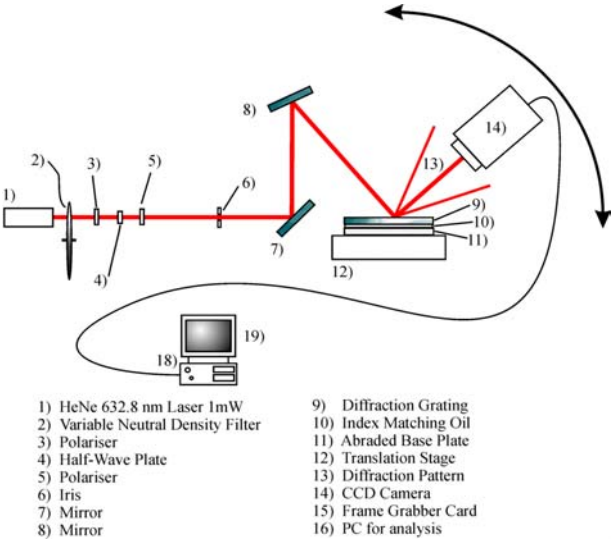

Figure 2) Experimental layout for diffraction analysis of DNA gratings

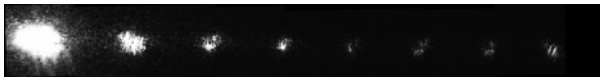

Figure 3) Diffraction pattern captured by scanning a CCD camera in an arc for a 20.0 micron DNA grating on silicon with hybridised 50nm gold nanoparticles. On the left is the m=0 diffraction order, with the diffraction orders m=1-7 to the right

IV. RESULTS

The DNA patterned lines made up a diffraction grating of DNA on the silicon wafer with a period of 20.0 microns. The difference in reflectivity between the DNA covered regions and bare silicon yielded a weak diffraction pattern when monitored using the analysis method described above. Upon hybridisation with gold nanoparticles of 1.4nm, 10nm and 50nm diameters, the diffraction efficiency ratio of the first order to the zeroth reflection (I_1/I_0) was observed to increase from 0.5% to 3.6, 6.4% and 9.5% respectively (see figure 4). The intensity of the diffraction orders was higher when gold nanoparticles hybridised to the grating, enabling detection of the DNA sequences. A typical diffraction pattern image is shown in figure 3).

10nm gold nanoparticles were found to give the highest intensity diffraction due to their larger size than the 1.4nm particles. 50nm particles did not hybridise very well due to their weight.

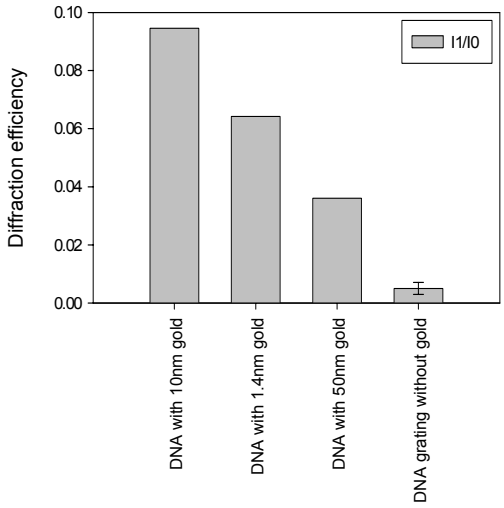

Figure 4) Diffraction efficiencies for DNA gratings with different size hybridised gold nanoparticles

V. CONCLUSIONS

DNA diffraction gratings have been fabricated on silicon wafers. Successful hybridisation of complementary DNA sequences labelled with gold nanoparticles was detected by increased diffraction efficiencies of the DNA gratings. This work will be extended to give quantification of the number of particles hybridised to the grating related to the intensities of the diffraction orders. The diffraction sensor will be developed further to enable the simultaneous analysis of a number of DNA sequences from genomic samples of specific relevance to hereditary disease.

REFERENCES

[1] Mike May, *Genotyping Scales Up and Packs in the Data*, Genomics & Proteomics, Vol. 3, No. 9, 2003, p46-48

[2] J. J. Storhoff, C. A. Mirkin, *Programmed Materials Synthesis with DNA,*Chem. Rev., 1999, Vol. 99, p1849-1862

[3] Robert Jenison, Shao Yang, Ayla Haeberli, Barry Polski, *Interference-based detection of nucleic acid targets on optically coated silicon,* Nature Biotechnology, Vol 19, 2001, p62-65

[4] H. B. Yin, T. Brown, R. Greef, J. S. Wilkinson and T. Melvin, *Chemical modification and micropatterning of Si(1 0 0) with oligonucleotides*, Microelectronic Engineering, Vol. 73-74, 2004, p830-836

[5] H. B. Yin, T. Brown, R. Greef, S. Mailis, R. W. Eason, J. S. Wilkinson, T. Melvin, *Photo-patterning of DNA oligonucleotides on silicon surfaces with micron-scale dimensions*, SPIE, Vol 5461, 2004