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'Label-free' DNA analysis using a-Si:H UV sensors

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Abstract

In this work we present the first results on a 'label-free' DNA analysis system based on hydrogenated amorphous silicon (a-Si:H) thin film sensors. The detection mechanism is based on absorbance measurements performed on DNA molecules in a buffer solution. The experiments made with single-stranded 30-mer DNA at different concentrations showed an excellent linearity over three orders of magnitude. The extrapolated minimum quantity (expressed as concentration - optical path product) detectable by our set-up was 2 nmol×cm, limited by short term variations of the UV source intensity. Furthermore, relying on the hypo-chromic effect it is possible to distinguish between single- and double-stranded DNA chains. This was observed performing DNA hybridization experiment on a functionalized glass substrate.

Keywords: DNA chip, amorphous silicon, UV sensor, label-free

1 Introduction

Microarray technology, or DNA chip [1], has recently emerged as a powerful tool for genetic research. Most of the available DNA chips are based on the detection of fluorescence [2], determined by fluorochrome attached to the target DNA molecule. However, this 'labeled' technique shows some important limits due to the complicate sample preparation as well as to possible influence on the bio-molecular interaction. In alternative, 'label-free' techniques have been proposed, based on the measurement of the changes of the mass [3] or of the electrical charge [4] associated with the hybridization process. In this paper, we demonstrate a 'label-free' detection of DNA hybridization based on the variation of the absorption peak in the deep ultra-violet (UV) range (hypo-chromic effect). In particular a 30% increase of UV absorption at 260 nm is expected when strand separation occurs. The novelty of our system lies in the use of a hydrogenated amorphous silicon (a-Si:H) UV sensor to detect the DNA absorbance [5].

2 Experimental details

2.1 Device structure and operation

The device proposed for the DNA analysis is a a-Si:H n-i-p stacked structure grown on a glass substrate covered with Cr/Al/Cr metal layer (see figure 1) deposited by a Plasma Enhanced Chemical Vapor Deposition (PECVD) system. The details of the deposition parameters are reported in Table I. The top contact is a Al/Cr metal grid, whose spacing was optimized for charge collection according to the conductivity of the underlying p-layer. The impinging light is incident from the top-side, on the p-type region which acts as the device active layer: from here, the photo-generated electrons diffuse to the p-i interface where are swept toward the n-region by the electric field existing in the intrinsic region and collected at the contact.

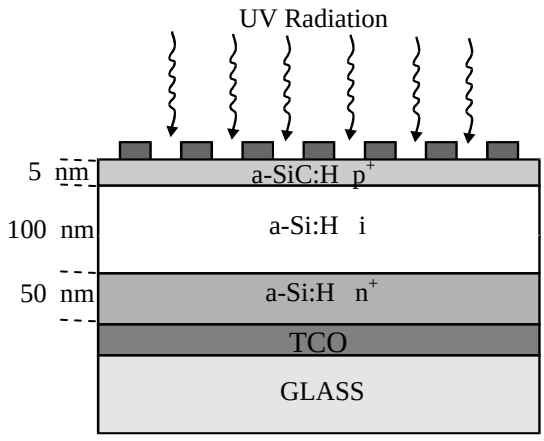

Figure 1. Structure of the device.

Table 1: Table I. Deposition parameters used in the PECVD process of the amorphous silicon p-i-n structure. The gas flows are: SiH_4 pure silane, PH_3 silane diluted (5%), B_2H_6 helium diluted (5%); P_D is the process pressure; P_{RF} is the power density of the plasma discharge; T_D is the substrate temperature; t_D is the deposition time. Thickness of the layers is calculated by the grow rates of the single film deposition on glass substrates with the same deposition parameters.

Layer	SiH_4 (sccm)	CH_4 (sccm)	PH_3 (sccm)	B_2H_6 (sccm)	P_D (Torr)	P_{RF} (mW/cm^2)	T_D (°C)	t_D (sec)	Thickness (Å)
p+	40	60	--	4	0.3	25	200	35	50
i	40	--	--	--	0.68	25	200	600	1000
n^+	40	--	10	--	0.3	25	230	300	500

According to the requirements of the present application, we optimized the structure of the amorphous silicon carbide (a-SiC:H) UV sensor presented in [5]. In fact, in DNA hybridization, low intensity levels are required for making inexpensive commercial products and/or compact analysis systems. These specs can be met by reducing the dark current of the device compared to the previous device. To this aim, we used hydrogenated amorphous silicon for the intrinsic layer instead of amorphous silicon-carbide. Indeed, the lower defect density of the pure amorphous silicon reduces the contribution to the dark current due to the thermal generation [6]. In particular, the minimum of the dark current has been achieved with a thickness of the intrinsic a-Si:H layer of 100 nm. Thinner intrinsic layers lead to an increase of the dark current due to tunnelling phenomena while thicker layers increase the thermal generation in the n-i-p structure. The lower energy gap of a-Si:H with respect to a-SiC:H, however, leads to enhanced response in the visible range. Therefore, the low dark current spec is fulfilled at the expenses of a reduced spectral selectivity. This, however, does not represent a limit for the proposed application, because the operation is based on the variation of absorption of a monochromatic light in the UV spectral range.

2.2 Measurement set-up

In figure 2 we report the set-up used in our experiment.

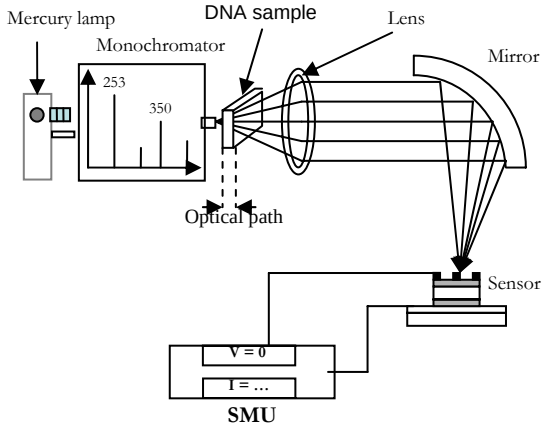

Figure 2. Experimental measurement set-up.

The light, coming from a mercury lamp, is filtered by a Jobin-Yvon SPEX-H10 monochromator, passes through a quartz lens and then is focused on the sensor by means a mirror. The DNA sample is placed just in front at the monochromator exit in order to avoid defocusing of the UV radiation, thus ensuring that all the light impinges on the sample. Sensor current has been measured by a Keithley Source Unit Measure 236.

3 Results

In figure 3 the current-voltage characteristics in dark and under monochromatic radiation at 253.4 nm of a 2×2 mm^2 device are shown. The metal grid of the device had a pitch of 200 µm and the width of the fingers was 50 µm. The incident power was 0.35 µW.

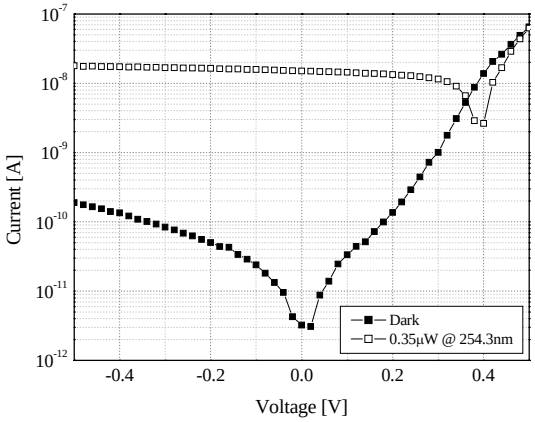

Figure 3. Current-voltage characteristics for the presented device in dark condition and under monochromatic light.

From the analysis of the curve, we calculated that the sensor responsivity is around 45mA/W and observe that is depending on the applied voltage. In particular, the efficiency collection increase with the reverse applied voltage indicates an increase of the electric field in the structure.

In order to evaluate the performances of the a-Si:H UV sensor for DNA analysis, different concentrations of 30-mer single-stranded oligonucleotide chain were placed in the cuvette and the sensor current was measured with DNA (I_{DNA}), without DNA (I_{BUFFER}) and without cuvette (I_{LAMP}). This last measure was

used to compensate for long term variability of the intensity of the mercury lamp. The DNA absorbance defined as:

$$DNA\ Assorbance = -\log \frac{I_{DNA}}{I_{BUFFER}} \qquad (1)$$

is reported in Figure 4 versus the concentration-optical path product. The data points reported in a double logarithmic suggest a good linearity for the investigated range.

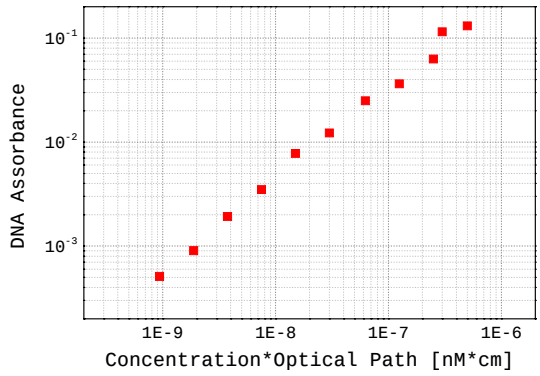

Figure 4. DNA assorbance as function of DNA concentration-optical path product.

Although with the present set-up it was not possible to further dilute the DNA solution, an estimate of the minimum detectable DNA quantity can be made by extrapolating the measured data toward lower concentrations and by determining the intercept with the noise current (or twice its value to ensure a minimum signal to noise ratio of 6dB). For our set-up the noise current was actually dominated by short term variations of the UV light intensity intrinsic to the radiation source and therefore the actual limit set by the shot noise of the sensor photocurrent (~60×10 ^{15}A) and the input equivalent noise of the Keithley 236 SMU (~10×10 ^{15}A) could not be reached. The measured standard deviation of the sensor current was 10 pA, which sets the absorbance limit to about 6×10 $^{-4}$, corresponding to a 2nM concentration for short oligonucleotides and an optical path of 1cm. This result is very encouraging, because, even though the experimental set-up is not optimized, our detector shows better resolution capabilities compared to most crystalline silicon photodiodes used in standard spectrophotometers [7] . Furthermore, by using differential measurement techniques (e.g. using two sensors, one without DNA as reference and the other with the DNA solution) it should be possible to decrease this limit of at least one order of magnitude.

In order to detect the DNA hybridization, however, it is necessary to measure small variations of the UV absorption: typically the absorbance of the double strand molecules is 30% lower than the single strand ones. In this case, the noise limit of our system has to be compared with the photo-current variations associated with the hybridization process.

The minimum detectable absorbance variation is calculated as a function of the absorbance by assuming a constant intensity P_0 incident on a DNA sample of absorbance A_0. The photo-current induced in the sensor with responsivity (Resp) is then:

$$I_1 = Resp \cdot P_0 \cdot 10^{-A_0} \qquad (2)$$

A variation of absorbance can be detected if the corresponding variation of the photo-current is 2 times the noise current (6dB SNR). The minimum detectable absorbance variation is thus given by:

$$\Delta A_{\min}(A_0) = -\log\left(\frac{I_1 - 2 \cdot I_{noise}}{I_1}\right) = -\log\left(1 - \frac{2 \cdot I_{noise}}{Resp \cdot P_0 \cdot 10^{-A_0}}\right) \qquad (3)$$

In general I_{noise} is a function of A_0 as well, because of the shot noise of the photo-current but in our case, from what said above, it is a constant equal to about 10 pA. The value of ΔA_{min} derived from equation (1), using the experimental value of I_{noise}, Resp and P_0, has to be compared with the 30% absorbance variation expexted from the hybridization process. We found that, in our experiment, the minimum DNA absorbance needed to detect the hybridization process is somewhat less than 10^{-3} which corresponds to about 3nM concentration of 30-mer oligonucleotides in a 1 cm pathlength cuvette.

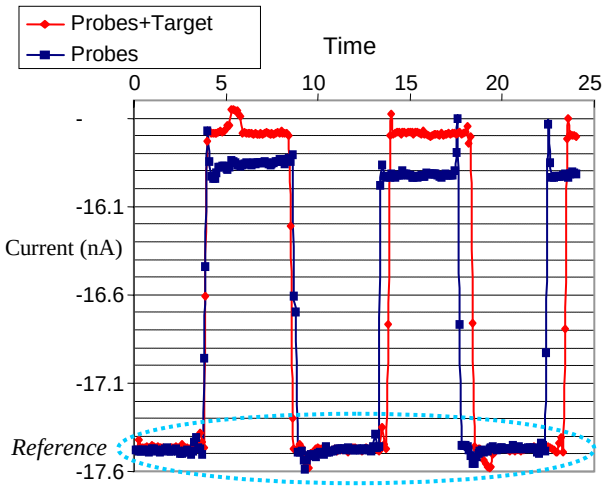

Figure 5. Sensor photocurrent as a function of time for single strand and hybridized DNA. Single-strand oligonucleotides has been functionalized on a glass substrate.

Finally, we tested the sensor ability to distinguish between single- and double-stranded molecules. In

particular, a quartz substrate has been funcionalized with a 25-mer single-stranded oligonucleotides used as probes. Half substrate has then been exposed to linearized and denaturated pBR 322 4162-mer single-stranded oligonucleotides used as target in order to achieve hybridization. Results are summarized in figure 5, where sensor photocurrent is reported as a function of time for hybridized (diamonds) and not-hybridized (squares) probes. The reference current represents the sensor response to the direct radiation (i.e. not passing through any DNA sample). This normalization is useful to reduce the effect of lamp variability.

It is evident a different DNA absorption in the two cases, indicating that our sensor is able to distinguish between single- and double-stranded molecules functionalized as single layer on a surface. A quantitative analysis of this last experiment will be presented at the conference.

4 Conclusions

In conclusion, we have presented a 'label-free' DNA analysis system based on a-Si:H UV sensor showing good performances in terms of both signal linearity and detection limit. The UV sensor, whose structure has been optimized for this application, allows to detect very low absorbances of DNA molecules (down to 6×10^{-4}, which corresponds to a 2 nM concentration) in a quartz cuvette. Furthermore, the sensor is able to distinguish between single- and double-stranded molecules functionalized as single layer on a surface. This was demonstrated by performing DNA hybridization experiments on a quartz substrate.

5 References

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