

Optical detection of nanoparticle-enhanced human papillomavirus genotyping microarrays

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Abstract: In this study, we propose a new detection method of nanoparticle-enhanced human papillomavirus genotyping microarrays using a DVD optical pick-up with a photodiode. The HPV genotyping DNA chip was labeled using Au/Ag core-shell nanoparticles, prepared on a treatment glass substrate. Then, the bio information of the HPV genotyping target DNA was detected by measuring the difference of the optical signals between the DNA spots and the background parts for cervical cancer diagnosis. Moreover the approximate linear relationship between the concentration of the HPV genotyping target DNA and the optical signal depending on the density of Au/Ag core-shell nanoparticles was obtained by performing a spot finding algorithm. It is shown that the nanoparticle-labeled HPV genotyping target DNA can be measured and quantified by collecting the low-cost photodiode signal on the treatment glass chip, replacing high-cost fluorescence microarray scanners using a photomultiplier tube.

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1. Introduction

Over the past decade, many advanced techniques have been developed to detect the bio information of various DNA, RNA and Protein chips. Typically biochips are prepared on a glass plate after they are labeled by fluorescence materials such as Cy3 or Cy5. Biochip microarrays have become extremely valuable tools in gene discovery, disease diagnosis, and drug discovery. After labeling, biochips are scanned using instruments specifically designed to detect fluorescent signals [1,2]. There are two major technologies for fluorescence detection. One uses laser for excitation and photodetectors for detection, and the other uses a CCD or CMOS array for image collection [3–5].

Conventional biochip detectors scan the fluorescent signals of spots with the different concentrations of dye, and then they analyze the fluorescence intensity to diagnose a disease. However this fluorescence-based detection technique suffers from weak emission signal, photochemical instability and environment-dependent quantum yield. Moreover the fluorescence-based biochip will not be able to keep a long time at room temperature due to degradation and instability [6]. Photodetectors for optical detection fundamentally operate on the transition of an electron from a lower energy state to a higher energy state as a result of the absorption of a photon. Most of commercial fluorescence scanners use a photomultiplier tube (PMT) for detection because it is the most sensitive photodetector in the visible light range [7]. However, a PMT is not the best choice for fluorescence detection because of its high operating voltages, fragility, size, and cost. An alternative approach employing wide field imaging such as CCD detectors has high quantum efficiency across the visible and near infrared and the ability to use broadband light sources.

In order to overcome the shortcomings of the fluorescence-based detection technique, many researcher adopt nanoparticles to label biomolecules instead of fluorescence dyes [6–9]. The use of nanoparticles as labels in biomolecule detection in place of conventional molecular fluorophores has led to improvements in sensitivity, selectivity, and multiplexing capacity. For example, it is reported that DNA microarray detection with Ag-deposited Au labels using a typical fluorescent scanner has achieved a sensitivity that is 100 times greater than that of conventional fluorescence-based detection [6]. However, in nanoparticle-based optical detection, the nanoparticle layers and depositions lead to a complicated process for manufacturing the nanoparticle-based microarrays. Therefore, further simplification is needed to take these nanoparticle-based technologies from the laboratory to point of care [7]. Recently, the authors have developed a fluorescent detection scanner for DNA microarrays using a commercial DVD optical pick-up with low cost and small size [10,11]. However, even though the DVD pick-up module includes a photodiode, the fluorescence scanner uses a PMT rather than the built-in photodiode in order to amplify weak fluorescent signals. The optical pick-up module is also used to detect fluorescent profiles in microfluidic channels [12].

Human papillomavirus (HPV) is classified into categories of low-risk types responsible for the most common sexually-transmitted viral infections and high-risk types which are crucial etiological factors for cervical cancer development [13]. It is extremely important to detect and genotype HPVs at an early stage of the infection so as to direct clinical treatment and reduce the incidence of HPV-related carcinomas, especially cervical cancer [14]. The traditional method for HPV detection, such as morphological and immunological methods, showed low specificity and sensitivity. Currently, the most frequently used methods for HPV detection are molecular biological methods, including nucleic acid hybridization-based and polymerase chain reaction (PCR)-based methods. Recently, several studies have been aimed at evaluating the usefulness of microarray technology for the simultaneous detection and typing of HPV in routine clinical specimens. A user-developed HPV DNA microarray for high-risk HPV genotyping was evaluated by using a panel of malignant and nonmalignant

cervical smears. This approach provides the potential to improve the clinical management of patients with cervical cytological abnormalities [15,16]. Several HPV DNA chips have been approved as a diagnostic reagent for use in Korea by the Korean Food and Drug Administration (KFDA). However, the high-cost biochip scanners prevent a wide spread in the DNA-chip market. Therefore, the low-cost optical detection technology becomes one of the essential factors to expand the DNA-chip market.

In this paper, we propose an optical detector of nanoparticle-based microarrays using a photodiode (PD) sensor rather than a PMT with high cost and large size. In order to verify the feasibility the photodiode based detector, we use Au/Ag core-shell nanoparticles to label human papillomavirus genotyping DNA. Then the bio information of the DNA molecules is quantified by comparing the photodiode signals measured at DNA spots and background regions on the HPV microarray.

2. System design

2.1. Design of optical detection system

Figure 1(a) illustrates shows a schematic diagram of a DVD pick-up based detection system. The DVD optical pick-up is the commercial product (HPD-65) manufactured by Hitachi Electronics, as shown in Fig. 1(b). The optical detection system consists of a red laser diode, two photodiodes, a voice coil motor (VCM) and several optical parts including a collimating lens, a polarizing beam splitter and a dichroic filter. The numerical aperture of the focusing objective lens is 0.64 with a focal distance of 3.22 mm. The DVD optical pick-up has two laser diodes, one for reading compact discs (CD) and the other for reading denser DVD's. The DVD laser diode generates red light with the wavelength of 650 nm.

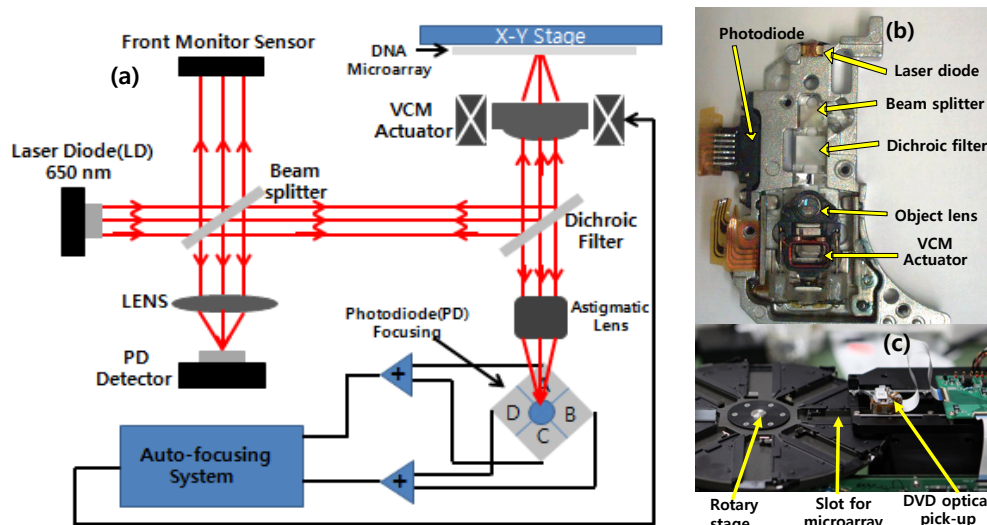


Fig. 1. (a) A schematic diagram of the DVD optical pick-up with photodiode sensor; (b) DVD pick-up used in experiments and its major components; (c) the experimental setup of photodiode based detection system

There are two different optical paths with two PDs in the diagram. One is for auto-focusing by a four-quadrant PD and the other is for optical detection by an additional PD. Small part of the laser beam passing through the beam splitter is used to regulate the laser power by a front monitor sensor. Most of the laser beam is transmitted through the beam splitter, then reflected by the dichroic filter and focused onto the surface of the DNA microarray chip. A part of reflected beam from the chip surface passes through the dichroic filter and it is finally focused onto the four-quadrant PD. The focus error signal is calculated by the distribution of the beam spot across the four divided regions of the PD. The focus error

signal is used to drive the VCM actuator in such a way that the objective lens is shifted to make light beam focused to the chip surface. The other part of laser beam reflected from the surface of the DNA chip is reflected from the surface of the dichroic filter. Then it is focused onto the additional PD sensor by an objective lens after the laser beam is reflected by the beam splitter. The analogue signal measured by the PD sensor is converted into the digital signal by an analogue digital converter. Finally, the digital signal is transmitted to a personal computer for analyzing the bio information of the DNA chip.

Figure 1(c) shows a photo of the experimental setup for the photodiode based microarray detection system. The rotary stage consists of a disk platform with slots loading DNA chips and a disk rotation motor. A maximum of eight slide glasses can be loaded on the disk platform, simultaneously. The rotation speed of the rotary stage is controlled by the disk rotation motor from 1 rpm to 600 rpm. A linear motor is used to move the optical pick-up detection module in the horizontal plane. The pick-up module moves along the radial direction of the rotary stage with the resolution on 2 ~10 μm as the stage rotates.

2.2. HPV genotyping microarray

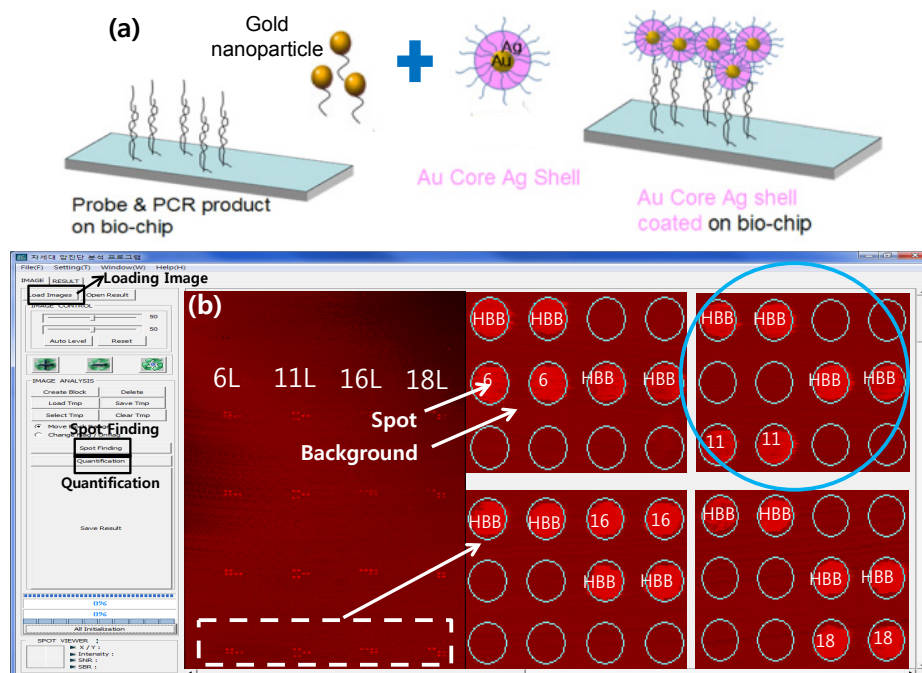


Fig. 2. (a) A process diagram of the HPV genotyping microarray preparation; (b) the analysis software to load the scanned image and calculate the signal to background ratio of the HPV genotyping DNA with the low risk 6, 11, 16 and 18 types.

The HPV genotyping DNA chip, labeled by Au/Ag core-shell nanoparticles for cervical cancer diagnosis, was prepared by the following process [17]. First, the HPV genotyping immobilized probe DNA was spotted on a treatment glass chip by using the pin array method. Then, the HPV genotyping target DNA for the low risk 6, 11, 16 and 18 types was labeled by gold nanoparticles with a diameter of 13 nm and the concentration of the target DNA was changed from 0.05 pmol/ μl to 0.5 pmol/ μl . In order to improve the sensitivity of optical detection, the gold nanoparticles, which label the HPV genotyping target DNA, were enhanced to 15 nm in diameter by conjugating with silver. Finally, the target DNA was hybridized with the HPV genotyping immobilized probe DNA in one well on the treatment glass chip, as shown in Fig. 2(a). The bio information of the HPV genotyping microarray can be quantified through collecting the optical signals with the wavelength of 650 nm which is

reflected from the chip surface. The density of the labeled nanoparticles is proportional to the concentration of the target DNA. Therefore the photodiode signal depending on the labeled nanoparticles increases with the concentration of the target DNA.

3. Experiments and results

In order to analyze the optical signal measured by the PD based detection system, we define the signal and background ratio (SBR) as the ratio of the mean value of digital signal for spot to the mean value of digital signal for background:

$$\text{SBR} = R_s / R_b, \quad (1)$$

where R_s is the mean value of digital signal of the spot, and R_b is the mean value of digital signal of the background. In order to calculate the SBR and analyze the optical signal depending on the HPV genotyping target DNA, we have developed the customized analysis software as shown in Fig. 2(b). The prepared HPV genotyping DNA chip was scanned by the proposed detection system. Then the scanned image was automatically in a personal computer connected to the scanner. After loading the scanned image into the analysis software, we execute the spot-finding algorithm, which calculates the signal intensity of the scanned image, determines the spot and background regions, measures the SBR and classifies the types of the HPV genotyping target DNA. The SBR is calculated by quantifying the signal intensities of the spot and background regions in Fig. 3(a). We execute the quantification menu of the software to generate the diagnostic results of the HPV genotyping target DNA in Fig. 3(b).

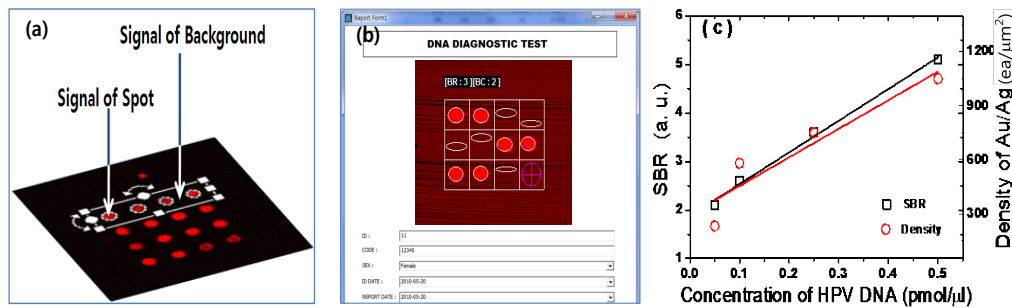


Fig. 3. (a) Optical detection of the spot and background of the DNA chip to calculate the SBR; (b) the diagnostic result of the DNA chip of low risk 11 type for the case of concentration of the target DNA of 0.1 pmol/μl; (c) the relationship between the concentration of the HPV genotyping target DNA and the SBR, and the density of Au/Ag core-shell nanoparticles.

Here we simply select the low risk 11 type to appraise the detector system. The feasibility of the detector system is demonstrated by measuring the hybridized information of the HPV genotyping microarray for the low risk 11 type. In each DNA microarray, the region for the target DNA of 11 type consists of six spots which are marked with a blue circle in Fig. 2(b). Four spots are for the housekeeping beta-globin (HBB) DNA and two spots are for the target DNA of the low risk 11 type. The size of each spot is 200 μm. All the samples of the HPV genotyping microarrays with different concentrations of the target DNA of 0.05, 0.1, 0.25 and 0.5 pmol/μl are scanned by the photodiode based detection system. We execute the analysis software for each scanned image to calculate the SBR and the diagnostic result as shown in Fig. 3(b). The relationship between the concentration of the target DNA and the SBR are plotted with square markers in Fig. 3(c). The curve-fitting graph shows a good linear relationship between the concentration of the target DNA and the SBR.

However, the photodiode-measured optical signal is actually laser beam reflected from the nanoparticle-enhanced spot of the target DNA of the low risk 11. Since the HPV genotyping target DNA is labeled by Au/Ag core-shell nanoparticles, it is necessary to investigate the relationship between the concentration of the target DNA and the density of the labeled nanoparticles. The density of Au/Ag core-shell nanoparticles was measured by a scanning

electron microscope (SEM). The SEM images for different concentrations of the target DNA (0.05, 0.1, 0.25 and 0.5 pmol/ μ l) are shown in Fig. 4. From each SEM image, the number of the labeled nanoparticles was counted. The density of the labeled nanoparticles on the glass substrate were increased from 230 ea/ μ m² to 1050 ea/ μ m² as the concentration of the target DNA of the low risk 11 type was changed from 0.05 pmol/ μ l to 0.5 pmol/ μ l. It is considered that the target DNA labeled nanoparticles was well hybridized with the HPV genotyping immobilized probe DNA on the glass substrate. The relationship between the concentration of the target DNA and the density of the labeled nanoparticles is plotted in Fig. 3(c), together with the SBR. It is shown that the concentration of the target DNA is in good agreement with the density of the labeled nanoparticles.

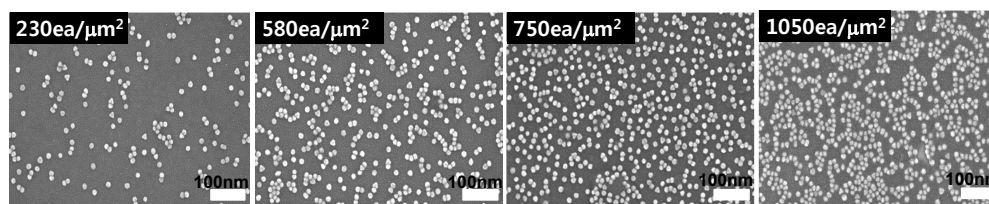


Fig. 4. SEM images of the Au/Ag core-shell nanoparticles for the HPV genotyping microarray with different concentrations of the target DNA (0.05, 0.1, 0.25 and 0.5 pmol/ μ l)

4. Conclusion

In this study, we propose a photodiode-based detection method of nanoparticle-enhanced human papillomavirus genotyping microarrays for cervical cancer diagnosis. In order to overcome the signal degradation and instability of the fluorescence based detection techniques, we adopt the Au/Ag core-shell nanoparticles to label the HPV genotyping target DNA molecules. The nanoparticle-enhanced HPV microarrays with different concentrations of the target DNA are analyzed by measuring the optical signal reflected from the spot with the DNA information and the background part. Experimental results show that the concentration of the target DNA has linear relationships with both the signal-to-background ratio and the density of Au/Ag core-shell nanoparticles. The nanoparticle-labeled HPV genotyping target DNA can be simply quantified by collecting the low-cost photodiode signal reflected from the chip surface, replacing high-cost fluorescence microarray scanners using a photomultiplier tube.

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