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RESEARCH PAPER

Development of Light Waveguide Resonance Light-Scattering Scanner for Microarray Detection

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Abstract: An optical waveguide resonance light-scattering scanner (RLS Scanner) was developed for microarray detection. The major components of the device included optical system, waveguide excitation system, photovoltaic conversion system, mechanical drive system and PC software. The minimum scanning resolution of the RLS scanner was 5 μm, and the maximum scanning range was 100 mm. The signal-to-noise ratio (SNR) of RLS was improved by the waveguide excitation mode. This device is capable of using a white light source as excitation source, thereby providing significant cost-savings. Several kinds of microarrays including carbohydrate microarray, DNA microarray and peptide microarray with gold nanoparticle (GNP) probes were used to demonstrate the detection performance of the device. Detection limits of 1 ng mL^{-1} and 100 pM in solution were obtained for concanavalin A (Con A) and DNA respectively. For the peptide on microarray spots, a detection limit of 100 fg was reported. The RLS scanner showed at least one to two orders of magnitude more sensitive than that of commercial scanner. Furthermore, taking advantage of the RLS technique, the device was characterized by simple construction and low cost.

Key Words: Microarray; Waveguide; Resonance light scattering; Gold nanoparticles

1 Introduction

Microarrays have emerged as one of the most powerful techniques for rapid, sensitive and high-throughput detection of biological targets including glycans, DNAs, proteins and cells[1–8]. In the microarray-based assays, the probe molecules are immobilized on the surface of the microarray substrates (e.g. glass microscope slide gold coated film, and polycarbonate membrane) by physical or chemical methods. After incubated with the test samples, target biomolecules are captured by the immobilized probe molecules through various affinity interactions, such as nucleic acid hybridization and specific antigen-antibody binding. Finally, the detection of biorecognition events is achieved by labeling specific signaling molecules. Currently, fluorescent dyes are standard labels of microarray techniques, which have several potential drawbacks including poor photoinstability of the dyes employed, relatively strong background noise and requirement of complex and expensive instruments[9–14]. Therefore, there is high desire to develop low cost non-fluorescence-based microarray detection system with high sensitivity.

Recently, it has been recognized that inorganic nanoparticles may be exploited in biological detection assays as an alternative to the use of fluorescent dyes[6–18]. In particular, the detection of resonance light scattering (RLS) by metal particles (e.g. gold nanoparticles (GNPs)) represents a great step forward, towards higher sensitivity, with the eventual goal of detecting single biomolecular binding events on microarrays[15–18]. The surface excitation is the most commonly used excitation mode for generating RLS of

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nanoparticles. However, the background noise is increased by increasing the excitation light intensity in the surface excitation mode. Therefore, effectively reduce background noise and maximize excitation light scattering become the key issues on the development of RLS-based microarray detection devices. Optical/light waveguide is a typically dielectric structure waveguide. The structure guides optical waves by total internal reflection because the dielectric material with high permittivity is surrounded by a material with lower permittivity in the dielectric structure. In this work, an optical waveguide RLS scanner was developed for microarray detection. In the device, glass microscope slide (i.e., microarray substrate) was used as optical waveguide medium, and GNPs were employed as detecting probes for sensing analytes. Compare to commercial scanner with surface excitation mode, the as-fabricated RLS scanner with optical waveguide excitation mode can significantly reduce the background noise caused by exciting light, and increase the sensitivity of assay.

2 Experimental

2.1 Materials and reagents

4-aminophenyl *α*-*D*-mannopyranoside (Man-*α*), biotin-Con A, sliver enhancer were purchased from Sigma-Aldrich Co. (USA). Avidin as fluorescein conjugate was purchased from Invitrogen (USA). Synthetic oligonucleotides (5'-TAA CAA TAA TCC-NH_2-3', 5'-GGA TTA TTG TTA AATATT GAT AAG GAT-3', 5'-HO$(CH_2)_6$-S-S-$(CH_2)_6$-T_{10} ATCCTT ATC AAT ATT-3') were purchased from Sangon Ltd. (Shanghai, China). Peptide CALNNGK(biotin)G was purchased from ChinaPeptides Ltd. (Shanghai, China). Aldehyde 3-D glass slides and polytetrafluoroethylene (PTFE) masker were purchased from CapitalBio Ltd. (Beijing, China). Motorized translation stage (NRT100-M) and CCD (DCC1240C, one megapixel) were purchased from Thorlabs Inc. (USA). Other chemicals were of analytical grade and used as received. Milli-Q water (18.2 MΩ cm) was used in all experiments.

5415R centrifuge (Eppendorf Co., Germany), ChipMate centrifuge (Tomy Co., Janpan), SmartArrayer 136 system (CapitalBio Ltd., Beijing, China) and Colorimetric scanner (TeleChem International Inc., USA) were used in the experiments.

2.2 System frame

The major components of RLS scanner included optical system, waveguide excitation system, photoelectric conversion system, mechanical system, electric driver system and PC software. The schematic representation of waveguide excitation system was shown in Fig.1. XD-300 Xenon lamp (35 W, 400 nm to 1100 nm wavelength range and 6000 K color temperature), as the excitation light source, was purchased from Yanan special lighting factory (Nanjing, China). An optical fiber was used to connect the Xenon lamp and the optical shaping prisms. The use of fiber transmission effectively minimized the heating effect of Xenon lamp on the optical system. The optical shaping prism group produced a homogeneous and collimated laser line focused onto the edge of the waveguide medium (i.e., microarray substrate). As shown in Fig.1, after launched the light into the waveguide by coupling the evanescent field of the propagating modes, chromophores (GNPs) on the microarray spots was excited, which resulted in RLS. CCD was used as photovoltaic conversion part. The resolution of CCD was 1.95 μm since minimum scan field was 2 mm × 2 mm. Motorized translation stage was used as mechanical motion unit. Electric rails of motorized translation stage were controlled by PC software to achieve positioning and scanning functions. The minimum resolution of stage was 2 μm and maximum distance of movement was 100 mm. Therefore, the system minimum resolution could be down to 5 μm (CCD resolution × stage resolution). Home-written PC software was used to achieve

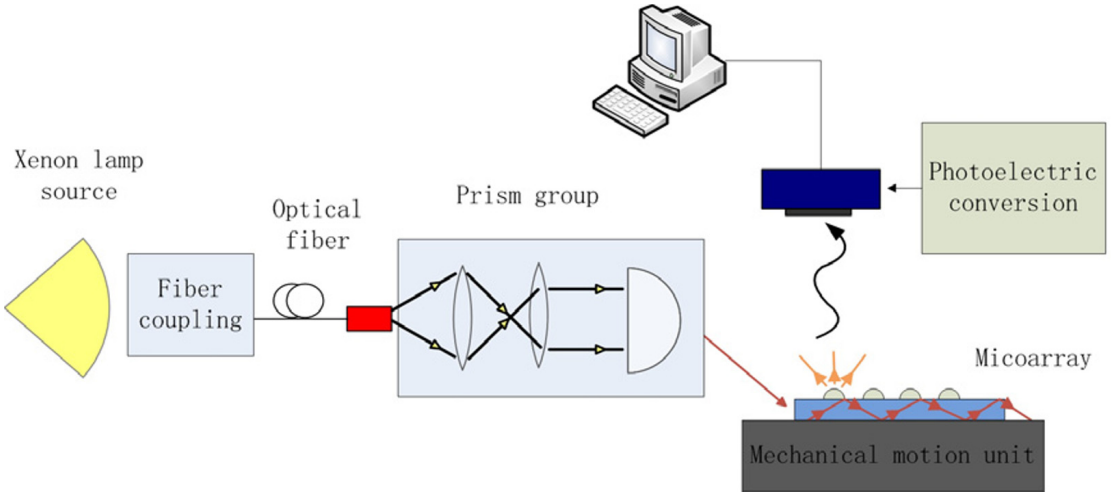

Fig.1 Schematic representation of waveguide excitation system

stage control, CCD data collection and data analysis and storage. Under the optical waveguide excitation mode, only RLS of GNPs were collected by CCD device because the exciting light indirectly into the view field of CCD. Therefore, the background noise could be decreased, resulting in greatly improvement of signal-to-noise ratio (SNR) and detection sensitivity. In additional, taking advantage of the RLS technique, the method is capable of using a broad spectrum white light source as the excitation light source, thereby providing significant cost-savings.

2.3 Software design

Home-written PC software was used to operate the as-fabricated device. The features of software included CCD image acquisition, CCD exposure and gain control, multi-axis electric stage control, data extraction, region of interest (ROI) array data extraction, data analysis and data storage. The image acquisition area was responsible for previewing microarray information and positioning specific area for high precision scanning. Scanner control area was used to definite CCD position and image acquisition. The camera control region was used to set the exposure time and gain compensation of the camera. The guide control area provided real-time control of the rail. In addition of automatic scanning function, the electric rail could also be moved according to the instructions of the users.

2.4 Microarray fabrication and detection

The microarrays were manufactured by the standard procedure according to the previously reported methods[4–6]. The distance between two spots in microarray was 300 μm. The slides were detected by both commercial Colorimetric scanner (TeleChem International Inc., USA) and home-made RLS scanner, respectively. The surface excitation mode was employed for generating RLS of nanoparticles by the Colorimetric scanner. The background originating from the slide was recorded and subtracted from each image prior to evaluation. The mean value and standard deviation of the signal were determined for the 6 spot replicates per sample, respectively. The detection limit was determined to be the concentration where signal/standard deviation = 3 was reached.

3 Results and discussion

3.1 Carbohydrate microarrays detection

10 mM monosaccharide (4-aminophenyl *α-D*-mannopyranoside (Man-*α*)) was spotted and immobilized on the glass microscope slides to detect biotinylated Con A by our previously reported method[5]. 13 nm avidin-stabilized GNPs were used to label this recognition event by biotin-avidin reaction. The microarray was read by RLS scanner and Colorimetric scanner, respectively. As shown in Fig.2, the assay performance (signal intensity and background noise) of RLS scanner was better than that of Colorimetric scanner. The detection limit for Con A of the RLS scanner (1 ng mL^{-1}) was two orders of magnitude more sensitive than that of Colorimetric scanner (100 ng mL^{-1}).

3.2 DNA microarrays detection

DNA Probe (5'-TAA CAA TAA TCC-NH_2-3', 5 μM) was immobilized on the glass microscope slide and hybridized with target DNA (5'-GGA TTA TTG TTA AATATT GAT AAG GAT-3') by our previously reported method[4]. Thiolated DNA (5'-$HO(CH_2)_6$-S-S-$(CH_2)_6$-T_{10} ATCCTT ATC AAT ATT-3') conjugated 13 nm GNPs (DNA@GNPs) were used to label the hybridization events. Subsequently, a silver enhancement step was applied to the microarray for signal amplification[4]. Finally, the microarray was read by RLS scanner and Colorimetric scanner, respectively. As shown in Fig.3, RLS scanner showed relatively high signal intensity and low background. The detection limit for target DNA of the RLS scanner (100 pM) was one order of magnitude more sensitive than that of Colorimetric scanner (1 nM).

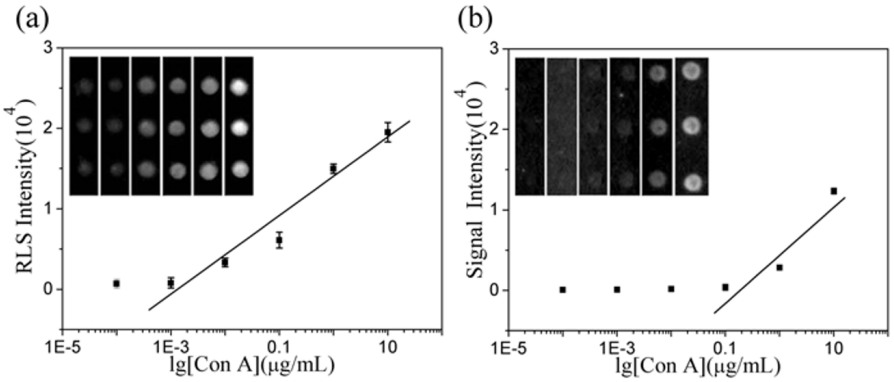

Fig.2 Logarithmic plot of (a) RLS scanner and (b) colorimetric scanner signal intensity as a function of the concentration of Con A in the probe solution. The insets are corresponding images of microarrays. The concentration of Man-α in the spotting solution was 10 mM

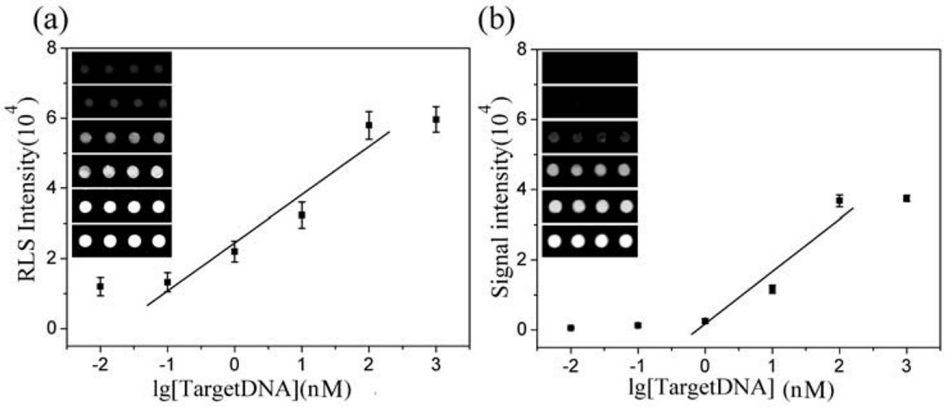

Fig.3 Logarithmic plot of RLS scanner (a) and colorimetric scanner signal intensity as a function of the concentration of target DNA oligomers in the probe solution (b). The insets are corresponding images of microarrays. The concentration of the probe DNA oligomers in the spotting solution was 5 μM

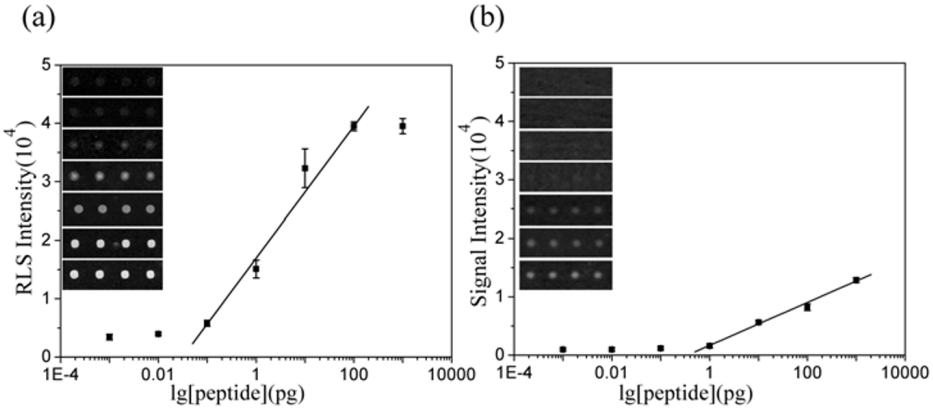

Fig.4 Logarithmic plot of RLS scanner (a) and colorimetric scanner signal intensity as a function of the concentration of peptide on the microarray spots (b). The insets are corresponding images of microarrays

3.3 Peptide microarrays detection

Biotinylated peptides (CALNNGK(biotin)G) with various concentrations were spotted and immobilized on the glass microscope slides by our previously reported method[6]. 13 nm avidin-stabilized GNPs were used to label the biotinylated peptides by biotin-avidin reaction. Then, a silver enhancement step was applied to the microarray for signal amplification[6]. Finally, the microarray was read by RLS scanner and Colorimetric scanner, respectively. As shown in Fig.4, the detection limit for the immobilized peptides of the RLS scanner (100 fg) was one order of magnitude more sensitive than that of Colorimetric scanner (1 pg).

4 Conclusions

An optical waveguide RLS scanner has been fabricated for microarray detection. Carbohydrate microarray, DNA microarray and peptide microarray are chosen here to demonstrate the detection performance of the RLS scanner. In comparison with commercial scanner with surface excitation mode, the home-made RLS scanner has better detection performance (e.g., high signal intensity and low background noise) and reasonably lower detection limits for Con A and DNA in solutions and immobilized peptide on microarray spots. In additional, in comparison with conventional confocal fluorescent laser scanner, the RLS has several advantages including simple instrumentation structure and low production cost because the RLS scanner using a broad spectrum white light as exciting light source. Therefore, the device has great promising applications in microarray-based assays.

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