Portable Fluorescence Detection System with Rayleigh Waves Removing Nonspecifically Bound Proteins

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***Abstract*—Immunofluorescence is one of the primary techniques for microbiological assays. Although it has existed for decades, its usages in medical and biological practices have limitations that need to be tackled with, especially the need of increasing its portability and specificity, and lowering its detection limit. A portable optical fluorescence system was designed to be combined with a direct digital synthesizer for surface acoustic wave generation. Rayleigh wave streaming generated on a piezoelectric device is able to prevent nonspecific binding on the biological interface. The miniaturized prototype, integrating acoustic wave removal of interfering proteins combined with optical detection, was developed to realize immune-detection on a portable platform.**

Keywords—Immunofluorescence; surface acoustic wave; Rayleigh wave; point of care; antibody-antigen; nonspecifically bound proteins

I. INTRODUCTION

Immunofluorescence (IF) assays are widely used because of their better capability to spatially visualize proteins and quantify them. This can be applied to quantify specific antigen/antibody interactions using fluorophores [1]. The significance of a portable device for rapid IF testing is valuable for time sensitive biotests, such as cancer biomarker tests [2]. However, the high false positive rate from background noise caused by the nonspecific adsorption of proteins is a problem [3, 4]. Therefore, techniques to prevent nonspecifically bound (NSB) proteins interfering with the target fluorescence signal are needed to be implemented in portable sensors. Compared to traditional techniques, such as self-assembled monolayers (SAMs) [5], blocking layers [6], and zwitterionic polymers [7], surface acoustic wave streaming enables less sample processing and easier surface modifications.

Our previous studies have characterized surface acoustic wave (SAW) [8] streaming induced removal of NSB of proteins in immunoassays [9]. We have utilized Rayleigh waves [10] generated by interdigital transducers (IDTs) using the inverse piezoelectric effect that propagate on the surface of a piezoelectric substrate to remove NSB proteins from sensor surfaces. As the specific binding force is much stronger than the nonspecific absorption force, the acoustic streaming is able to keep the antigen-antibody links intact by proper configuring of the streaming force [11].

Current measurement methods for the immunofluorescence system require large-scale and expensive equipment. We developed a mini-sized optical fluorescence system which integrates with a portable platform for Rayleigh waves power generation. The portable platform, based on a direct digital synthesizer (DDS) [12], is investigated for the acoustic wave driving of streaming phenomenon. It integrates signal synthesis and gain control in a small, portable electronic system. The system performance in sensing and NSB removal is evaluated, and shown to have potential for point-of-care realization of rapid and specific immunodetection.

II. EXPERIMENTAL

A. Reagents and Apparutus

The materials and reagent utilized in this experiments and their sources are: goat IgG, bull serum albumin (BSA), (3-aminopropyl) - triethoxysilane (APTES) were purchased from Sigma-Aldrich. Rabbit anti-goat IgG and mouse anti-rabbit IgG-CFL 488 were purchased from Santa Cruz Biotechnology. Reagent grade deionized (DI) water with 18.2 MΩ-cm resistivity was produced in the laboratory using a Millipore system.

The instrument utilized in the experiments were a network analyzer (Agilent 8753ES); and a Nikon FN1 fluorescence microscope.

B. Rayleigh wave devices

The Rayleigh wave devices were fabricated on the 4-inch 500 μm thickness 128° YX $LiNbO_3$ double-side polished wafers, which provided a transparent substrate. The IDT electrodes are placed on the left and right side of the device. Each IDT electrodes consist of 60 finger pair electrodes (width of 10 μm), that is a wavelength of 40 μm. The IDT is of 200 μm thickness, which is deposited via aluminum E-beam evaporation. The patterned wafers were diced into 20 mm×20 mm individual chips.

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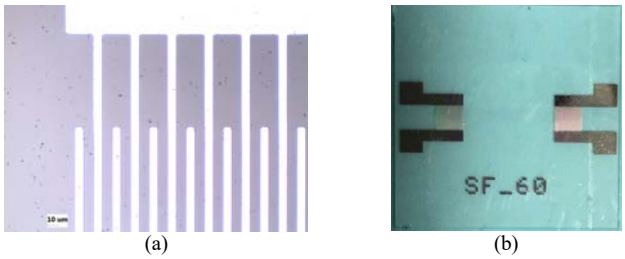


(a) (b)

Fig. 1. (a) Image of IDT pairs under microscope; (b) image of Rayleigh wave device.

C. Sandwich immunoassay

The fabricated Rayleigh wave device were modified with biomolecules as follows for the sandwich immunoassay: After solvent rinsing and drying with N_2 gas, the chips were soaked in 10 mM APTES in pure ethyl alcohol solution to silanize the surface. After 1-hour of soaking, the chips were washed with pure ethyl alcohol and dried with N_2 gas.

The 200 µg/ml goat IgGs were assembled on the chip as capture antibodies and were incubated for 2 hours at room temperature. Then 1 % BSA in PBS solution was added for blocking the unbound sites on the substrate. Rabbit anti-goat IgG was detected as target and followed by the 4 µg/ml mouse anti-rabbit IgG-CFL 488 as the detection antibody was applied for fluorescence tests. The IgG conjugate Cruz Fluor™ 488 has an excitation peak of 491 nm, and an emission peak of 520 nm. The chips were rinsed with PBS solution and dried with N_2 gas between each step.

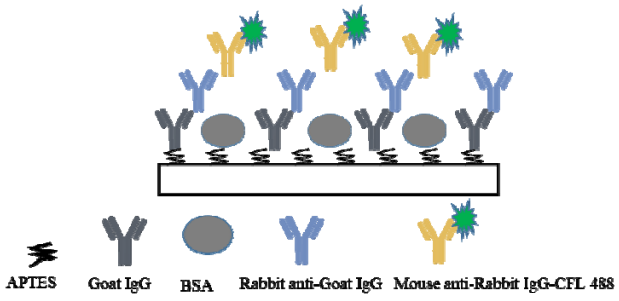
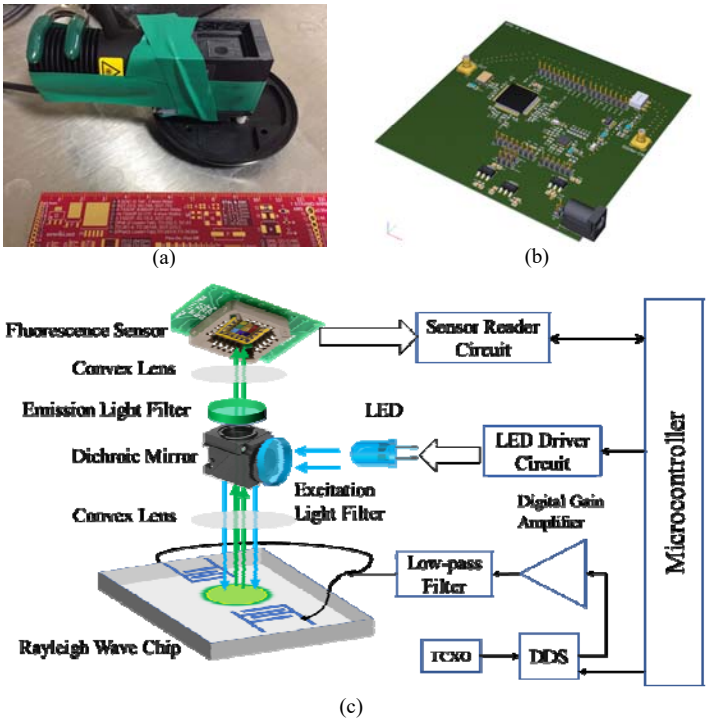


Fig. 2. Schematic diagram of chip modification for fluorescence immunoassays.

D. Optical system and Rayleigh wave driving system

The portable system consists of two main sections, the optical fluorescence detection system and the Rayleigh wave device driving circuit. In the fluorescence optical detection component, the light of 490 nm wavelength from LED source passes through the optical system. The specially designed filter cube allows the emission signal to be detected by the fluorescence sensor, which has a peak around 520 nm. The SAW driving component has a radio frequency (RF) signal generator using the direct digital synthesizer technique. After passing through a buffer circuit and digital gain control RF amplifier, the RF signal, with a working frequency of approximately 100 MHz, is sent to the Rayleigh wave chip to remove the NSB proteins. The intensity of the signal is converted to digital data, which are processed by the microcontroller. The sensor system hardware connects to a PC via USB interface. The application software includes a graphical user interface that is based on a simple design to allow for easy operation.



(a) (b) (c)

Fig. 3. (a) Photo of optical fluorescence detection system; (b) image of Rayleigh wave device driving circuit; (c) schematic diagram of portable fluorescence biodetecion system.

III. Results and Discusstion

A. Comparision of fluroscence microscope and portable opticle system

The results of fluorescence intensity from the prototype and fluorescence microscope were compared to verify the prototype's reliability. Fluorescence comes from the pre-assembled glass slides with sandwich immunoassay structure on them. The target rabbit anti-goat IgG is at concentration of 100 µg, 1 µg and 10 ng respectively. All chips were measured under Nikon FN1 fluorescence microscope. The expose time was 10s with the light dose at 100% and gain of 1X. The fluorescence images were collected and the green channel values of the RGB system (255,255,255) were calculated. Fig. 4 shows that the results from the designed prototype agree very well with those from the microscope.

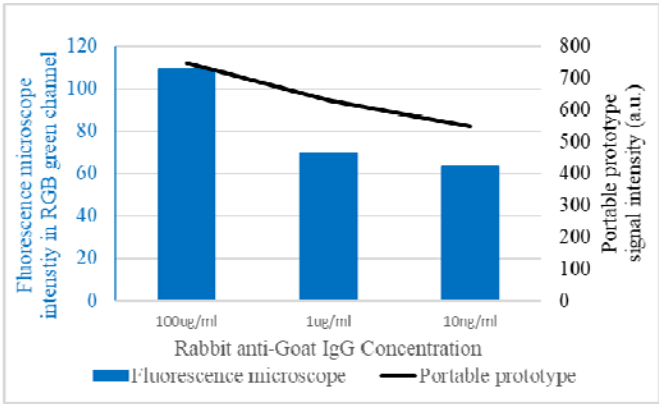


Fig. 4. Comparision of fluroscence microscope and portable system .

B. *Acoustic wave streaming removal of NSB proteins*

The electrical properties of the Rayleigh wave device were measured using a network analyzer. The S_{21} parameter of the device is as shown in Fig. 5. The center frequency of the device is approximately 99.3 MHz.

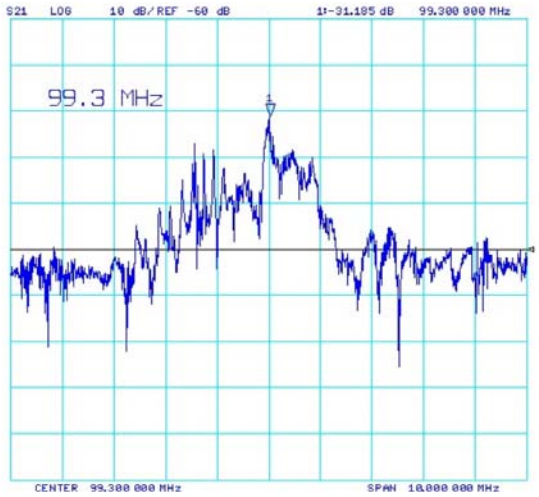


Fig. 5. S_{21} parameter of Rayleigh wave device

The effect of Rayleigh wave removal of NSB antibodies was tested as follows: the 200 µg/ml goat IgGs were assembled on the chip as capture antibodies, and were incubated for 2 hours at room temperature. Then 1 % BSA in PBS solution was added for blocking the unbound sites on the substrate. 4 µg/ml mouse anti-rabbit IgG-CFL 488 as the detection antibody was applied for fluorescence tests. As the mouse anti-rabbit IgG would not specifically bind with goat IgG, the remaining fluorescent antibodies are almost owing to nonspecific adsorption. PBS solution was used to rinse the nonspecific adsorbed carefully, and the intensity signal was measured using the portable prototype. Then, the output of the signal generator was set to 20 dbm and 25 dbm, respectively to load on the Rayleigh wave device's double side IDTs. After rinsed again with PBS solution, the intensity results were measured by the prototype. The fluorescence intensities are listed in Table I. The background intensity, which is the intensity reading from the sample when no fluorescence labelled antibody was assembled, is about 124 a.u. After the acoustic wave streaming removal, the intensity value was reduced from 260 a.u. to 164 a.u., which indicates that the prototype has excellent NSB removal capability.

TABLE I. RESULTS OF NSB ANTIBODIES REMOVAL

	Before removal	***Power: 20 dbm***	***Power: 25 dbm***	***Background***
Intensity (a.u.)	260	184	164	124

C. *Acoustic wave Streamig effect on sandwich immunoassay*

In the sandwich immunoassay, the goat IgGs/rabbit anti-goat antibody, and rabbit antibodies/mouse anti-rabbit antibodies could bind to each other specifically. However, there are still a certain amount of non-specifically adsorbed antibodies remaining on the biological interface, which present weak linking forces. These excess fluorescent antibodies remaining on the sensor surface may cause indicated value to be higher than the actual concentration. The Rayleigh wave streaming in the sandwich immunoassay could effectively solve this issue. The effect of the Rayleigh wave streaming on the specific antigen-antibody binding is demonstrated in Table II. The target antibody – rabbit anti-goat IgG of various concentrations were detected on the Rayleigh wave chip. After the acoustic wave streaming loading on the chips, the average intensity of the fluorescent was decreased, which indicates that the adsorbed weak binding antibodies were removed.

TABLE II. RESULTS OF REMOVAL EFFECT ON SANDWICH IMMUNOASSAY

Intensity (a.u)	**Concentration**			
	1ng/ml	***10ng/ml***	***10µg/ml***	***100µg/ml***
Before removal	325	363	368	535
After removal	303	313	350	434

I. CONCLUSION

A portable optical fluorescence system was constructed and evaluated, and it demonstrates comprehensive and competitive performance compared to a fluorescence microscope. The miniaturized Rayleigh wave driving circuit can provide effective removal of nonspecific binding proteins on the sensing chips. Such a small-size, light-weight, low-cost, low-power integrated system has great prospect to become a cutting edge detection instrument for immunofluorescence assays. Furthermore, this system has great potential for point-of-care testing, with the indicated next step to develop a biosensor for point-of-care operation directly from body fluids such as blood and urine.

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