Low cost flatbed scanner label-free biosensor

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ABSTRACT

In this paper, we demonstrate utilization of a commercial flatbed document scanner as a label-free biosensor for high-throughput imaging of DNA and protein microarrays. We implemented an interferometric sensing technique through use of a silicon/oxide layered substrate, and easy to implement hardware modifications such as re-aligning moving parts and inserting a custom made sample plate. With a cost as low as 100USD, powered by a USB cable, and scan speed of 30 seconds for a 4mm x 4 mm area with ~10µm lateral resolution, the presented system offers a super low cost, easy to use alternative to commercially available label-free systems.

Keywords: Optical biosensor, label-free, DNA / protein microarray, flatbed scanner

1. INTRODUCTION

In low-resource setting and point of care applications, low-cost, portable, easy-to use and robust biosensors are highly demanded. In order to fulfill this need, a popular approach has been the application of novel biosensing ideas through utilization of powerful hardware of commercially available devices. Examples of this approach include several mobile phone based platforms introduced in the last decade, which benefit from advancements in sensor technologies and increased computational capacity of these devices[1,2]. On the other hand, flatbed scanners which are designed specifically for scanning documents or photographs have been successfully translated to low-resource clinical settings[3] for applications such as detection of chemical compounds using colorimetric arrays[4], quantification of virus plaque by scanning 96-well plates[5], and detection of proteins[6] and DNA arrays[7] with the help of metal nanoparticle labeling. In addition, they have also been adapted for fluorescence microscopy[8] and digital holographic microscopy[9] applications.

In recent years, several biosensors that rely on label-free detection have been introduced, which are suitable for low-resource settings as they reduce sample preparation and washing steps required by their label-based counterparts[10,11]. One such biosensor, called Interferometric Reflectance Imaging Sensor (IRIS), monitors biomolecule accumulation on a layered substrate by detecting changes in the interferometric reflectance in a high-throughput manner. The technique has been used for monitoring DNA hybridization and antibody-antigen binding[12,13]. IRIS also provides an elegant solution for quality control and calibration in fluorescence-based microarray detection through quantification of immobilized probe density of microarrays[14].

In this work, we mechanically modified a commercial flatbed scanner (Canon LIDE 210) with the aim of applying the detection principles of IRIS to create a label-free biosensor for low-resource settings. We have used the commercial scanner software for capturing images, and MATLAB for data processing. With this system, we have analyzed DNA spots in microarray format, and benchmarked the results with a standard IRIS system. Our modified scanner costs as low

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as 100USD, and has the ability to scan a 4mmx4mm microarray surface in 30 seconds with ~10µm lateral resolution, thus offering a low-cost, easy-to-use alternative to complex and lab-bench suitable label-free systems.

2. METHODS

2.1 Interferometric Imaging

Our detection method is based on the Interferometric Reflectance Imaging Sensor (IRIS)[13,15,16]. Shortly, in this method, a layered substrate creates a wavelength dependent reflectance curve due to reflections from layer interfaces (Si/SiO_2). Any biomass accumulation on chip introduces additional optical path length, hence creates a shift in this reflectance curve as shown in Figure 1a. The thickness of the biofilm can be quantified by sampling this reflectance curve at specific wavelengths and solving well-known Fresnel reflection formulas for layered substrates. In order to reduce the computation time required for the fitting algorithms, in this work, we have used a lookup table (LUT) approach[17]. In this approach, the reflectivity of a SiO_2 film is measured and by applying non-linear fitting, a reflection curve is determined. Using these fitting parameters, a LUT is populated by calculating predicted reflectivites corresponding to different thicknesses for a single wavelength. The generated LUT can be applied to any subsequent measurement to determine the optical thickness.

2.2 Scanner Modifications

Document scanners can be classified into two groups depending on their sensor technology, namely reduction type linear sensors (or CCD based) and Contact Image Sensors (CIS). In CCD based scanners, the sample is illuminated with white light, and through demagnification optics, the image of the sample is projected onto the CCD sensor composed of red, green and blue elements. On the other hand, in CIS based scanners, the sample is sequentially illuminated with red, green and blue LEDs, and scattered light from the document is imaged to a monochromatic sensor with unit magnification by a self-focusing Gradient Index (GRIN) lens array. Although CCD based scanners have superior performance in terms of depth of field, which makes them the preferred method for document scanning, they are more expensive, have more complex optics, and consume more power. In this work, a CIS scanner (Canon LIDE 210, ~90 USD) has been chosen due to its similarities to the IRIS system.

The scanning head consists of LEDs, a waveguide, a GRIN lens array, and a line detector. During scanning, this head moves across the document and takes the image of the document line by line. A glass panel is placed above the head and pushes it down from its two ends to keep the scanner head stable during the scan. The thickness of the glass panel is optimized to keep the focal plane on the top surface where the document is placed. In contrast to scattering-based document scanning, our detection relies on reflection from Si/SiO_2 layered sensor chips, and thus the optical alignment of the system needs to be modified accordingly. To capture the reflected light from the sensor surface, placing the chip at an angle is not the ideal solution as the focal plane is at the top surface. Instead, we re-aligned the head inside the scanner by inserting a 3D printed wedge inside the moving platform. In order to prevent reflections from the glass surface, we replaced it with a custom sample plate made of aluminum fabricated with a CNC machine, which has holes at different heights for placing the sample. To form contact between the scanning head and the sample plate, two 3D-printed sample plate holders are placed at both ends of the scanning head as shown in Figure 1 c and d. The height of the sample plate and the holders are optimized to obtain the best focus.

The images are recorded with the scanner's commercial software, which allows capturing 48-bit color images, with a 4800 dpi resolution.

2.3 Sample Preparation

Si/SiO_2 substrates are purchased from Silicon Valley Microelectronics (Santa Clara, CA) and cut into 10mm x 10mm. Chips are first sonicated with acetone, then rinsed with methanol and DI water, and finally dried under nitrogen gas. Chip surfaces are coated with copoly(DMA-NAS-MAPS) as explained elsewhere[18], and stored in the dessicator until DNA spotting. 40–mer aminated DNA samples are purchased from Integrated DNA technologies and spotted using a Scienion S3 Flexarrayer (Berlin, Germany). Single-strand specific sequences of DNA are spotted with seven different concentrations from 0 (negative control) to 50 µM, spiked into non-specific DNA solution to keep the spotting concentration for all spots the same. Microarrays are kept in a 65% humid environment overnight. After this immobilization process, microarrays are washed 3 times in 2xSSC for 3 min, once in 0.2x SSC for 3 min, and once in 0.1x SSC for 10 sec. The chips are incubated with target oligonucleotides at 1 µM concentration in PBS at room temperature for 1 hour. Following hybridization, the surfaces are washed by applying the same washing protocol.

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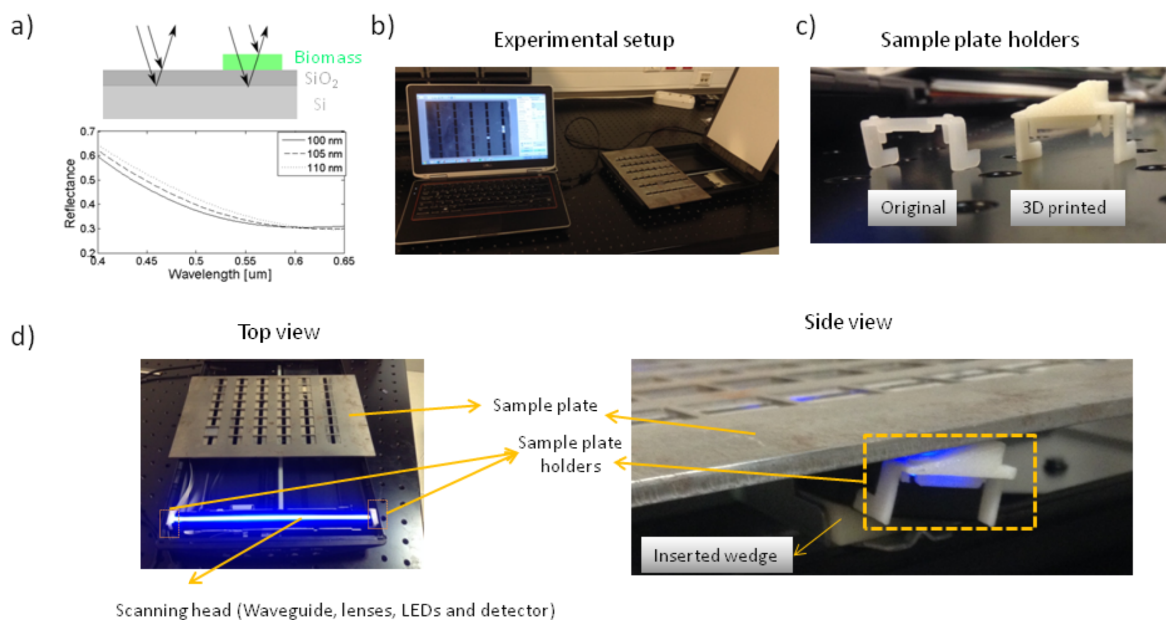

Figure 1. Flatbed scanner label-free biosensor (a) IRIS detection principle: absorbed biomass on a layered substrate introduces an optical path length difference, and thus a quantifiable shift in reflectance curve. (b) USB powered modified scanner setup. (c) Glass panel holders are replaced with 3D printed holders to tilt the scanner head (d) Top view of the modified scanner when sample plate is not on scanning head and side view showing the inserted 3D wedge.

3. RESULTS AND DISCUSSION

The DNA chip is scanned with the modified flatbed scanner before and after hybridization. In order to remove the fringes visible in the raw images (Fig 2a), a clean chip is also scanned as a reference image (Fig 2b) and the original image is divided to this reference image to get a scaled image free of fringes (Fig 2c). To reduce noise, the chip is scanned 10 times and averaged (Fig 2d). In Figure 2, the white dots in the images are etched Si alignment marks. After frame averaging, all pixels are assigned to a thickness value and visualized as grayscale images. Figure 2e shows the thickness profile on a line cut. The height of each spot is then calculated by subtracting an average thickness of the neighboring oxide region from the average thickness of the spot's central area.

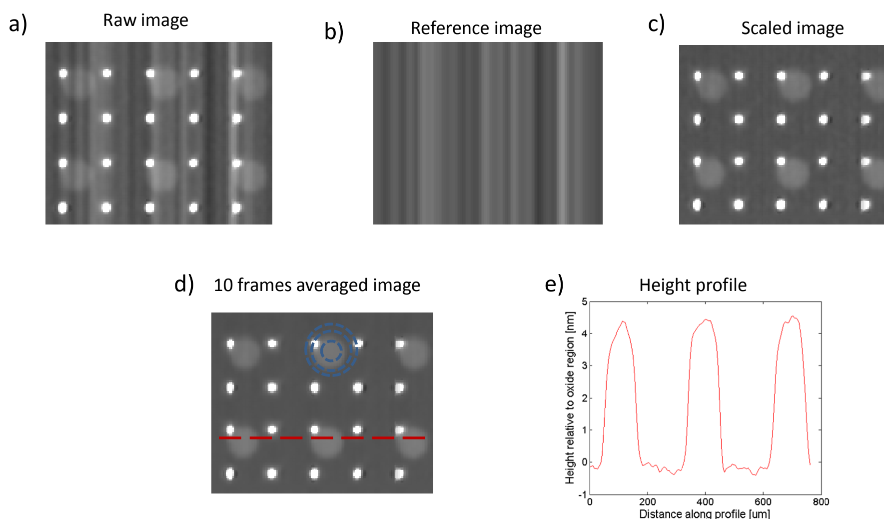

Figure 2: Data acquisition with the scanner. (a) Raw image (b) Reference image obtained by scanning a clean chip (c) Scaled image free of scanning artifacts (d) Frame averaged final image. (e) Height profile of the line-cut.

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For benchmarking, the same chip is also imaged with the IRIS system. Figure 3 shows microarray images captured with both the scanner (a) and IRIS (b). For each spot, the corresponding height is calculated. Figure 3c shows a comparison of the calculated thicknesses. The errorbars correspond to the standard deviation of the thickness in replicated spots. Although at lower concentrations the deviation in scanner data is higher, the errorbars are comparable as the spot height increases.

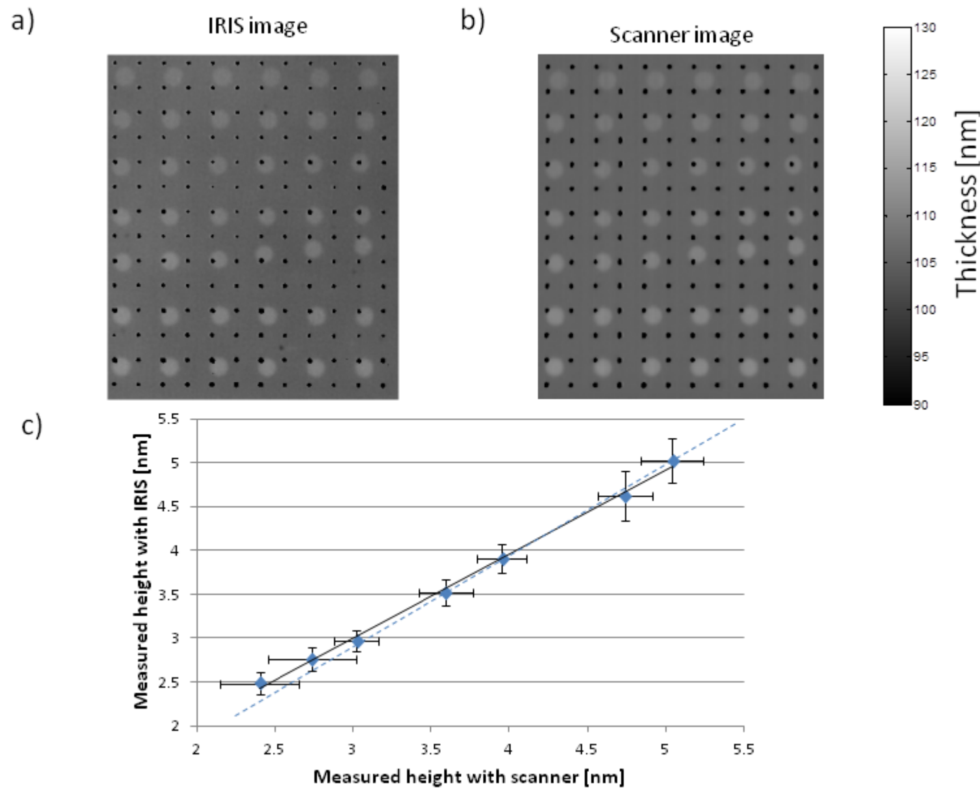

Figure 3: (a)DNA microarray image captured with IRIS. (b) Image of same chip obtained with the modified scanner (c) Comparison of calculated height with the two methods. Blue dashed line represents equivalence (y=x), and black line is linear fitted curve y=0.96x+0.12, R^2=0.9974. Height is calculated by subtracting average thickness of neighboring oxide region from the average thickness of spot's central region.

4. SUMMARY

In this work, we demonstrated the implementation of a label-free interferometric sensor (IRIS) by modifying a commercial flatbed scanner. With this USB powered instrument, we have shown detection of DNA hybridization in a microarray format with comparable performance to an IRIS system. Our instrument costs as low as 100 USD, and is capable of scanning a 4mm x 4mm area (scalable to 19cm x 28cm), in 30 seconds with ~10µm lateral resolution. We expect the developed system will find interest for label-free molecular diagnostics applications in low-resource settings and as a low-cost lab-bench quality control device for fluorescence microarray users.

5. ACKNOWLEDGEMENTS

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