



Label-free real-time detection of biotinylated bovine serum albumin using a low-cost optical cavity-based biosensor

DONGGEE RHO AND SEUNGHYUN KIM*

Baylor University, One Bear Place #97356, Waco, TX 76798, USA

*Seunghyun_Kim@baylor.edu

Abstract: We have developed a low-cost optical cavity-based biosensor with a differential detection method for point-of-care medical diagnostics. To experimentally demonstrate its label-free real-time biosensing capability, we performed the detection of biotinylated bovine serum albumin (BSA). Streptavidin is introduced into the optical cavity structure and immobilized on 3-aminopropyltriethoxysilane (APTES) coated surface. After rinsing out unbound streptavidin with DI water, biotinylated BSA without any labeling is introduced. A CMOS camera captures the transmitted light of two different wavelengths passing through the optical cavity sensing area in real-time. Then, the differential values are calculated to enhance the responsivity. We successfully demonstrated the label-free real-time detection of biotinylated BSA, and the measurement results matched well with the simulation results. The limit of detection of the optical cavity-based biosensor for the biotinylated BSA detection with the sensing area of $180\ \mu\text{m} \times 180\ \mu\text{m}$ is estimated to be 2.82 pM, which could be reduced further for a smaller sensing area with the tradeoff of a longer sensing time.

© 2018 Optical Society of America under the terms of the [OSA Open Access Publishing Agreement](#)

OCIS codes: (140.3948) Microcavity devices; (280.1415) Biological sensing and sensors.

References and links

1. N. Howlader, A. Noone, M. Krapcho, D. Miller, K. Bishop, C. Kosary, M. Yu, J. Ruhl, Z. Tatalovich, A. Mariotto, D. Lewis, H. Chen, E. Feuer, and K. Cronin, "2017. SEER Cancer Statistics Review, 1975-2014," Natl. Cancer Institute: Bethesda, MD https://seer.cancer.gov/csr/1975_2014/
2. American Cancer Society, "Cancer Facts & Figures 2017," (American Cancer Society, 2017) <https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2017.html>
3. A. Thiha and F. Ibrahim, "A colorimetric enzyme-linked immunosorbent assay (ELISA) detection platform for a point-of-care dengue detection system on a lab-on-compact-disc," *Sensors (Basel)* **15**(5), 11431–11441 (2015).
4. S. D. Gan and K. R. Patel, "Enzyme immunoassay and enzyme-linked immunosorbent assay," *J. Invest. Dermatol.* **133**(9), e12 (2013).
5. P. K. Drain, E. P. Hyle, F. Noubary, K. A. Freedberg, D. Wilson, W. R. Bishai, W. Rodriguez, and I. V. Bassett, "Diagnostic point-of-care tests in resource-limited settings," *Lancet Infect. Dis.* **14**(3), 239–249 (2014).
6. F. J. Gruhl, B. E. Rapp, and K. Länge, "Biosensors for diagnostic applications," *Adv. Biochem. Eng. Biotechnol.* **133**, 115–148 (2013).
7. S. A. Soper, K. Brown, A. Ellington, B. Frazier, G. Garcia-Manero, V. Gau, S. I. Gutman, D. F. Hayes, B. Korte, J. L. Landers, D. Larson, F. Ligler, A. Majumdar, M. Mascini, D. Nolte, Z. Rosenzweig, J. Wang, and D. Wilson, "Point-of-care biosensor systems for cancer diagnostics/prognostics," *Biosens. Bioelectron.* **21**(10), 1932–1942 (2006).
8. S. Choi, M. Goryll, L. Y. M. Sin, P. K. Wong, and J. Chae, "Microfluidic-based biosensors toward point-of-care detection of nucleic acids and proteins," *Microfluid. Nanofluidics* **10**(2), 231–247 (2011).
9. R. W. Peeling, K. K. Holmes, D. Mabey, and A. Ronald, "Rapid tests for sexually transmitted infections (STIs): the way forward," *Sex. Transm. Infect.* **82**(Suppl 5), v1–v6 (2006).
10. D. Rho, S. Cho, C. Joy, and S. Kim, "Demonstration of an optical cavity sensor with a differential detection method by refractive index measurements," in *Proceedings of Wireless and Microwave Circuits and Systems* (Texas Symposium, 2015), pp. 1–4.
11. S. Cho, J. H. Brake, C. Joy, and S. Kim, "Refractive index measurement using an optical cavity based biosensor with a differential detection," *Proc. SPIE* **9332**, 93320Z (2015).
12. P. Cowles, C. Joy, A. Bujana, D. Rho, and S. Kim, "Preliminary measurement results of biotinylated BSA detection of a low cost optical cavity based biosensor using differential detection," *Proc. SPIE* **9725**, 972509 (2016).

13. D. Rho and S. Kim, "Large dynamic range optical cavity based sensor using a low cost three-laser system," in *2017 39th Annual International Conference of the IEEE Engineering in Medicine and Biology Society* (2017), pp. 1393–1396.
14. D. Rho and S. Kim, "Low-cost optical cavity based sensor with a large dynamic range," *Opt. Express* **25**(10), 11244–11253 (2017).
15. F. Liu, J. Z. Zhang, and Y. Mei, "The origin of the cooperativity in the streptavidin-biotin system: A computational investigation through molecular dynamics simulations," *Sci. Rep.* **6**(1), 27190 (2016).
16. B. M. Lowe, K. Sun, I. Zeimpekis, C. K. Skylaris, and N. G. Green, "Field-effect sensors - from pH sensing to biosensing: sensitivity enhancement using streptavidin-biotin as a model system," *Analyst (Lond.)* **142**(22), 4173–4200 (2017).
17. D. A. Bergstein, Resonant cavity enhanced multi-analyte sensing (Doctoral dissertation, Boston University) (2007).
18. M. Olivo and U. S. Dinis, *Frontiers in Biophotonics for Translational Medicine*, (Springer, 2016).
19. S. Satyanarayana, R. N. Karnik, and A. Majumdar, "Stamp-and-stick room-temperature bonding technique for microdevices," *J. Microelectromech. Syst.* **14**(2), 392–399 (2005).
20. J. Kim, E. C. Jensen, M. Megens, B. Boser, and R. A. Mathies, "Integrated microfluidic bioprocessor for solid phase capture immunoassays," *Lab Chip* **11**(18), 3106–3112 (2011).
21. X. Fan, *Advanced photonic structures for biological and chemical detection*, (New York: Springer, 2009).
22. C. M. Cheng, A. W. Martinez, J. Gong, C. R. Mace, S. T. Phillips, E. Carrilho, K. A. Mirica, and G. M. Whitesides, "Paper-based ELISA," *Angew. Chem. Int. Ed. Engl.* **49**(28), 4771–4774 (2010).
23. I. M. White and X. Fan, "On the performance quantification of resonant refractive index sensors," *Opt. Express* **16**(2), 1020–1028 (2008).

1. Introduction

For almost all cancers, 5-year survival rates drastically decrease as the cancers are diagnosed at advanced stages [1, 2]. The early detection of cancers is the most effective way to improve the survival rates by enabling patients to receive treatment early. However, for example, over 50% of lung cancer patients are diagnosed after the cancer has spread to distant parts of the body and their survival rates are less than 5% [2]. The current gold standard of diagnostic methods based on biomarker detection is enzyme-linked immunosorbent assay (ELISA), which is well-established technology with high sensitivity and selectivity. However, this technology has some limitations to be used for the early detection of diseases [3, 4]. A point-of-care (POC) biosensor has emerged as an alternative diagnostic method designed to be used near the patient to monitor and diagnose the patient's health [5–8] and has received much attention as an enabling tool for the early detection of diseases. The World Health Organization (WHO) outlined criteria for a POC device, known as ASSURED (affordable, sensitive, specific, user friendly, rapid and robust, equipment-free, and deliverable to end-users) [9].

An optical cavity-based biosensor using a differential detection method has been proposed as a POC biosensor, meeting most of the ASSURED criteria [10–14]. The optical cavity structure is created by a small gap between two partially reflective surfaces in parallel. When light propagates through the optical cavity, the light bounces back and forth at the two surfaces, creating a large number of transmitted waves. The multiple transmitted waves constructively or destructively interfere with each other and produce a resonance response. As a sample fluid is introduced into the optical cavity which also functions as a microfluidic channel, the target biomarkers in the sample fluid are immobilized on the receptor molecules. A very thin layer with a higher refractive index than the sample fluid is created, which results in a shift in the resonance response. The proposed system measures the changes in intensities of low-cost laser diodes using a CMOS camera instead of measuring a shift of resonance peak, which would require an expensive spectrometer and/or tunable laser. In addition to lowering cost, intensity-based measurement has other advantages in terms of being a POC biosensor such as label-free detection, multiplexability, small sample volume, and simple fabrication process. To achieve high sensitivity, the differential detection method is employed to enhance the responsivity compared to that of individual wavelength. Refractive index measurements were conducted as proof of concept tests using standard refractive index fluids from 1.3 to 1.395 to detect the change of bulk refractive index inside the optical cavity. The

measurement results were compared with the simulation results, and they matched very well [14].

We employed the biotin-streptavidin binding system to validate the label-free biosensing capability of the optical cavity-based biosensor. The biotin-streptavidin conjugate is one of the most commonly used protein-ligand interactions to assess the biosensing capability of devices because of its specific and strong affinity [15, 16]. In this paper, we demonstrated the detection of biotinylated BSA in real-time by the optical cavity-based biosensor. The optical cavity design with simulation results, fabrication process of the optical cavity, surface immobilization process, and measurement results are discussed in detail.

2. Simulation results

A schematic diagram of the proposed optical cavity-based biosensor is shown in Fig. 1(a). Two wavelengths ($\lambda_1 = 780$ nm, $\lambda_2 = 850$ nm) of low-cost laser diodes are collimated, combined with a beam splitter, and propagate through the optical cavity. Their intensities are then measured by a low-cost CMOS camera. Figure 1(b) shows the cross-sectional view of the optical cavity structure including a spin-on-glass (SOG) layer on top of top and bottom silver layers. SOG layers are used to immobilize streptavidin inside the optical cavity and protect the silver layers from being damaged by the sample/rinse fluid. For designing the optical cavity structure using FIMMWAVE/FIMMPROP (Photon Design), we employed the fixed index model [17]. The fixed index model uses a refractive index value for the sensing layer and its thickness corresponds to the number of the biomolecules on the sensing area. The initial sensing layer thickness is 0 (no biomolecule is bound) and it changes up to the monolayer thickness of the biomolecules (the sensing area is fully occupied by the biomolecules). We used 1.45 for simulations as the refractive index of the sensing layer [18].

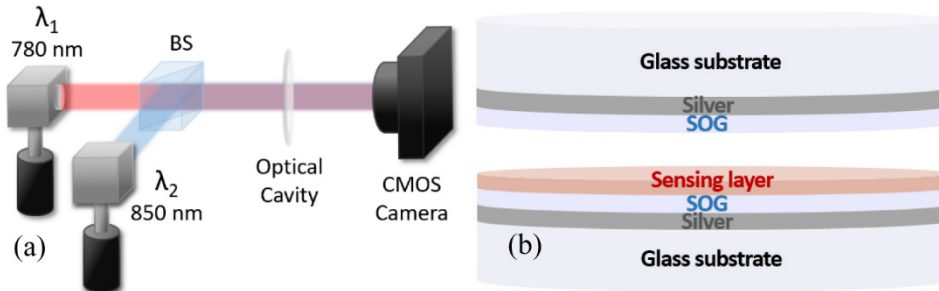


Fig. 1. (a) Schematic diagram of the proposed biosensor [14]. (b) The optical cavity structure.

We employed the differential detection method for which differential values (η) are calculated with an equation shown below, which is simply the difference of the intensity rate changes of two wavelengths.

$$\eta = \frac{I_1 - I_{1o}}{I_{1o}} - \frac{I_2 - I_{2o}}{I_{2o}} \quad (1)$$

I_1 and I_2 are the optical intensities at 780 nm and 850 nm wavelengths, respectively, and I_{1o} is the initial value of I_1 while I_{2o} is the initial value of I_2 . The optical cavity structure is designed specifically for two wavelengths' intensities to change in opposite directions as the sensing layer thickness increases. Therefore, the differential detection method enhances the responsivity (i.e., the change due to the increase of the sensing layer thickness). In addition to the enhanced responsivity, differential values always start at zero regardless of the intensities of two laser diodes by equalizing intensity levels of two wavelengths, which helps to obtain consistent results between tests.

The optical cavity structure is optimized to have the most changes in intensities of two wavelengths in opposite directions for the sensing layer thickness change from 0 nm to 20 nm. The final optimized design has a cavity width of 2.5 μm , a silver thickness of 14 nm, and a SOG thickness of 100 nm. The simulation results of the optimized optical cavity structure are shown in Fig. 2. As the sensing layer thickness increases, the efficiency of 780 nm increases linearly from 0.1613 to 0.1809 ($\Delta I_1 = 0.0195$), and the efficiency of 850 nm decreases linearly from 0.1859 to 0.1616 ($\Delta I_2 = 0.0243$). For the same range, the calculated differential value changes from 0 to 0.252, which is more than ten times greater than the changes in both wavelengths.

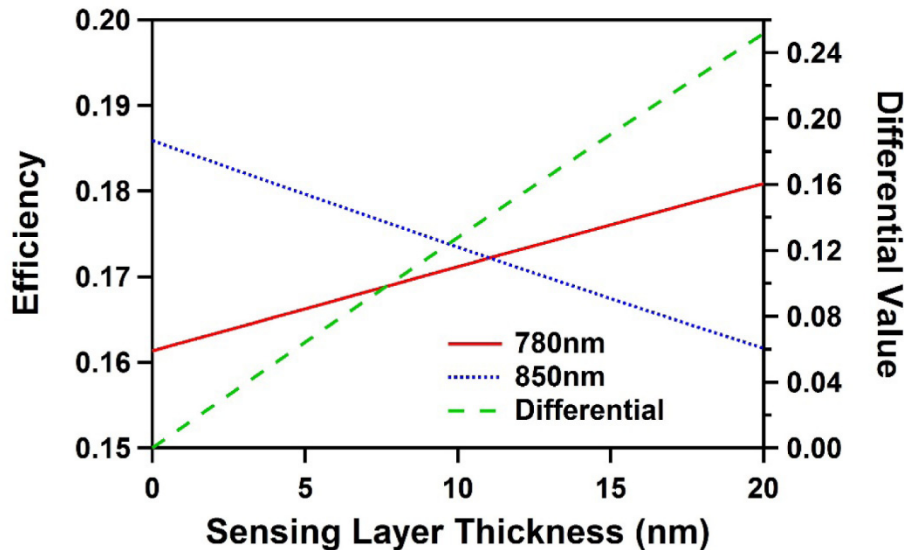


Fig. 2. Efficiencies of 780 nm (red) and 850 nm (blue) and differential value (green) vs. the sensing layer thickness.

3. Fabrication and experimental processes

The fabrication process of the optical cavity-based biosensor structure is shown in Fig. 3. A 3-inch glass substrate was drilled using a 1 mm drill bit to make a channel inlet and an outlet. Thin silver layers were deposited on both the drilled glass substrate and one plain glass substrate. SOG was spin-coated on the silver layer of both substrates and cured on a hot plate. On top of the SOG layer of the plain glass substrate, an SU8 layer was patterned using a photolithography process to define the microfluidic channel and the gap between SOG layers. We then used an UV curable epoxy (Norland Products) to bond the drilled and plain glass substrates. The UV epoxy was spin-coated on a glass substrate. The SU8 pattern was stamped on the UV epoxy layer, and then aligned and brought into contact with the drilled glass substrate followed by UV exposure [19].

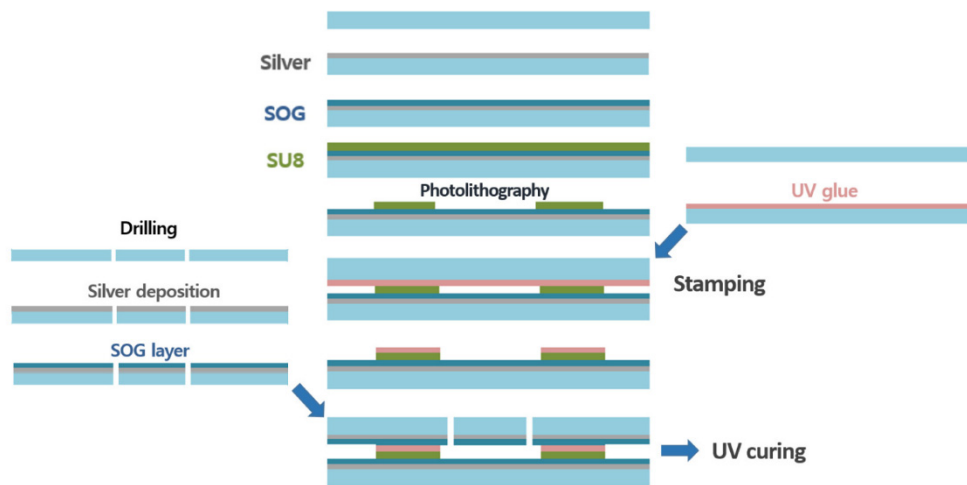


Fig. 3. Fabrication process of the optical cavity-based biosensor structure.

Before the UV bonding, the SOG surface of the glass substrate with the SU8 pattern was treated with oxygen plasma to increase the number of hydroxyl groups. Then, 5% of 3-aminopropyltriethoxysilane (APTES) was applied to the center area of the channel (i.e., sensing area) and incubated for 30 minutes to form APTES layer on the SOG surface. The unbound APTES molecules were then rinsed out with DI water. After the bonding, 2 μ l of streptavidin is introduced into the channel and incubated for an hour to allow bonding to the APTES via electrostatic interactions [20]. After the incubation, unbound streptavidin was rinsed out by flowing DI water with a vacuum pump. Finally, 2 μ l of biotinylated BSA with a concentration of 3 μ M was introduced and incubated for 50 minutes while we were performing the real-time measurements. Streptavidin and biotinylated BSA were diluted in DI water, and Tween-20 with a concentration of 0.2% was added to all fluids to facilitate fluid flows. Each functionalization step was experimentally confirmed by using fluorescent streptavidin and biotin with a fluorescent microscope beforehand.

4. Test setup

Figure 4 shows the test setup on an optical table including two laser diodes with collimators at the wavelength of 780 nm and 850 nm, a 50:50 beam splitter, a mirror, a fabricated optical cavity sample placed in a 3-D printed sample holder, a 3-D printed beam blocker operated by a servo motor, and a CMOS camera. The beam blocker rotates to alternately block the light from one laser diode at a time at one-second intervals. This allows us to monitor both intensities with a CMOS camera and calculate differential values in real-time. Total cost of the test setup with off-the-shelf products is ~\$1,000 (excluding optical mounts and posts). The fabrication process is very simple and does not require expensive equipment or materials as described in Fig. 3. Therefore, the proposed optical cavity-based biosensor is a low-cost system.

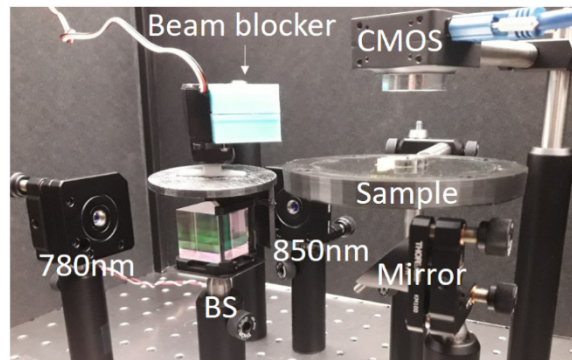


Fig. 4. Test setup for biosensing with the optical cavity-based biosensor.

5. Measurement results

The fabricated optical cavity sample is aligned for both collimated beams of two wavelengths to pass through the center area of the microfluidic channel. The area of a 50×50 pixel array from the CMOS images near the center of the microfluidic channel was selected as a sensing area, and further processing was performed only in that area. Because the size of a pixel of CMOS camera is $3.6 \mu\text{m} \times 3.6 \mu\text{m}$, the total sensing area is $180 \mu\text{m} \times 180 \mu\text{m}$. Figure 5 shows the average intensity values of 780 nm and 850 nm in the sensing area with the calculated differential values during the biotinylated BSA incubation time. There was not much change in the first 10 minutes. However, both 780 nm and 850 nm show changes after 10 minutes where we believe the binding of biotinylated BSA on streptavidin started. As expected in the simulation results shown in Fig. 2, the intensity of 780 nm is increased while the intensity of 850 nm is decreased. The total change of differential value over 50 minutes incubation time is 0.179. Compared with the simulation results, the differential value of 0.179 corresponds to the sensing layer thickness of 14.1 nm. Considering the estimated size of a BSA molecule is $4 \text{ nm} \times 4 \text{ nm} \times 14 \text{ nm}$ [21], the measurement results of biotinylated BSA sensing match very well with the simulation results.

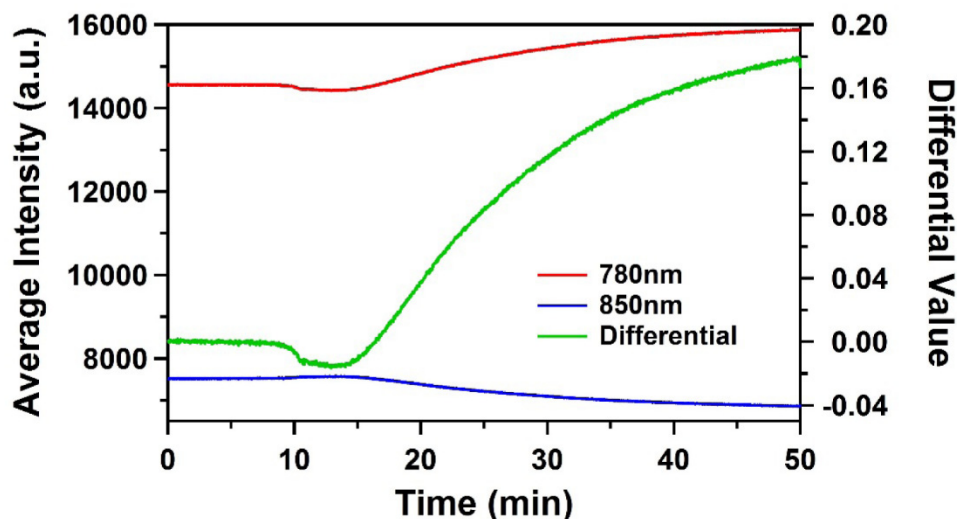


Fig. 5. Measured average intensities for 780 nm (red) and 850 nm (blue) and corresponding differential values (green).

Based on the measurement results, we attempted to estimate the limit of detection (LOD). Even though we used a high concentration of biotinylated BSA ($3\text{ }\mu\text{M}$) for this experiment, we only need enough molecules to fill the sensing area to get the exactly same results we got. Since the sensing area is $180\text{ }\mu\text{m} \times 180\text{ }\mu\text{m}$, the required number of biotinylated BSA molecules to fill the sensing area is then 2.025×10^9 , assuming $4\text{ nm} \times 4\text{ nm}$ side of the biotinylated BSA molecules are densely packed and create a monolayer on the surface. This corresponds to 3.36 femtomole based on the Avogadro's number, 6.022×10^{23} . By using the volume of the sample fluid containing biotinylated BSA used ($2\text{ }\mu\text{l}$), the minimum concentration to fill the sensing area assuming all biotinylated BSA reach to the sensing area is 1.68 nM . The minimum detectable change is usually considered as three times standard deviation of the blank measurements [22, 23]. Based on the measurement results shown in Fig. 5, the calculated standard deviation for the first 5 minutes is 1×10^{-4} and the corresponding minimum detectable change is 3×10^{-4} . Therefore, based on the differential value change of 0.179 , the minimum concentration of 1.68 nM , and the minimum detectable change of 3×10^{-4} , the estimated LOD is 2.82 pM . In summary, if we use $2\text{ }\mu\text{l}$ of sample fluid with 1.68 nM concentration of biotinylated BSA, we will be able to see the differential value change of 0.179 as long as we incubate long enough for all biotinylated BSA molecules reach to the sensing area. However, even a concentration as small as 2.82 pM will produce a detectable change with the optical cavity-based biosensor for the $180\text{ }\mu\text{m} \times 180\text{ }\mu\text{m}$ sensing area, assuming all biotinylated BSA reaches to the sensing area. This LOD can go down further with a smaller sensing area, even in the fM range, with the tradeoff of a longer incubation (i.e., sensing) time.

This low-cost optical cavity-based biosensor not only has label-free real-time detection capability with high sensitivity, but also multiplexability. Figure 6 show differential values of a 50×50 pixel array at $t = 0, 17, 30$, and 50 minutes. These images show the biotinylated BSA binding does not happen uniformly across the sensing area over time, and 50 minutes incubation time may not be sufficient to cover the entire sensing area. In addition to that useful information, these images show we can extract information in a local pixel area as small as one single pixel. Therefore, these images clearly prove that, by properly immobilizing various biomarker-specific receptors on different locations within the sensing area, we can detect the multiple target biomarkers in the sample fluid simultaneously.

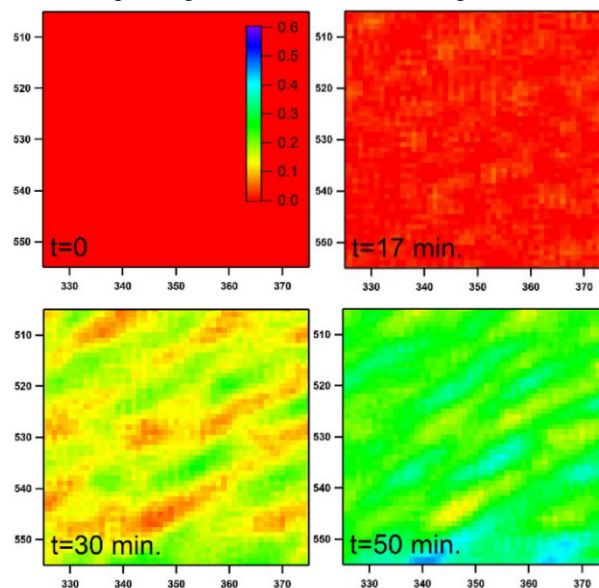


Fig. 6. The intensity changes in the sensing area for the differential value at 0, 17, 30, and 50 minutes.

6. Conclusion

We successfully demonstrated the biosensing capability of the low-cost optical cavity-based biosensor with a differential detection method using biotinylated BSA. The optimized optical cavity structure has a cavity width of 2.5 μm , a silver thickness of 14 nm, and a SOG thickness of 100 nm. As the sensing layer thickness increases inside the optical cavity, the intensities of 780 nm and 850 nm are linearly increased and decreased, respectively. The corresponding differential value change shows ten times greater responsivity than that of an individual wavelength. Biotinylated BSA with a concentration of 3 μM was introduced and immobilized on the streptavidin-coated surface. The real-time detection results match very well with the simulation results. The total change of 0.179 in the differential value corresponds to a sensing layer thickness of 14.1 nm which is reasonable considering the dimension of BSA. Finally, we estimated the LOD of the optical cavity-based biosensor to be 2.82 pM, which could be reduced further into fM range for a smaller sensing area with the tradeoff of a longer sensing time. We also discussed the multiplexability of the optical cavity-based biosensor.

Funding

National Science Foundation (NSF) (CBET-1706472)

Acknowledgment

Publication was made possible, in part, by support from the Open Access Fund sponsored by the Baylor University Libraries.