

Self-Powered Forward Error-Correcting Biosensor Based on Integration of Paper-Based Microfluidics and Self-Assembled Quick Response Codes

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Abstract—This paper extends our previous work on silver-enhancement based self-assembling structures for designing reliable, self-powered biosensors with forward error correcting (FEC) capability. At the core of the proposed approach is the integration of paper-based microfluidics with quick response (QR) codes that can be optically scanned using a smart-phone. The scanned information is first decoded to obtain the location of a web-server which further processes the self-assembled QR image to determine the concentration of target analytes. The integration substrate for the proposed FEC biosensor is polyethylene and the patterning of the QR code on the substrate has been achieved using a combination of low-cost ink-jet printing and a regular ballpoint dispensing pen. A paper-based microfluidics channel has been integrated underneath the substrate for acquiring, mixing and flowing the sample to areas on the substrate where different parts of the code can self-assemble in presence of immobilized gold nanorods. In this paper we demonstrate the proof-of-concept detection using prototypes of QR encoded FEC biosensors.

Index Terms—Flexible electronics, forward error correcting biosensor, ink-jet printing, mobile health, paper-based microfluidics, self-assembly, self-powered sensing, silver enhancement, wireless biosensor.

I. INTRODUCTION

THE last decade has seen proliferation of smart-phones into the consumer market and across different parts of the world [1]. Not only do these mobile devices support multi-modal communications (voice and data), they can also be used for biosensing and measurement. For instance, by using the images acquired from the phone's integrated camera and by applying different image processing algorithms, the smart-phone has been used as a portable microscope [2], [3], as a heart-rate monitor [4], [5] and as an environmental sensor [6], [7]. Another optical sensing application of a smart-phone, which is relevant

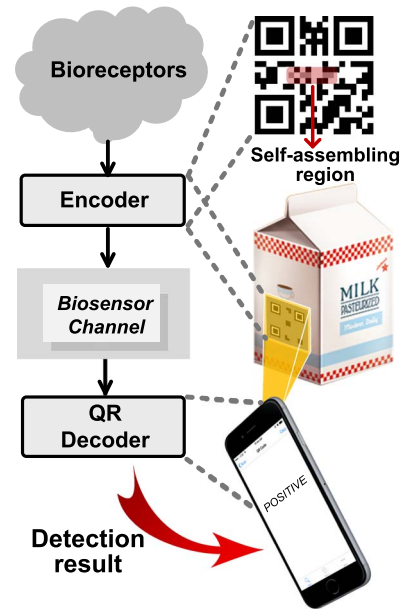


Fig. 1. Framework of a forward error-correcting biosensor specific to the proposed self-assembled QR code. A smart-phone is used for scanning and decoding the code and determining the presence or absence of target contaminants.

to this paper, is for scanning quick-response (QR) codes as shown in Fig. 1. A QR code could encode the location of a web-server from which the product specific information (e.g., active ingredients, side-effects and consumer ratings) could be retrieved. In the area of biosensing, QR codes have been used for static encoding of product information. In [8] Kubota used a QR code to describe the specifications of an electrochemical paper-based p-nitrophenol biosensor. Similarly, in [9] a QR code was used to encode platform and patient information on a lateral flow immunochromatographic sensor for detecting human immunodeficiency virus (HIV) and for measuring prostate-specific antigen (PSA). In [10] Zharnikov reported a method to pattern a 29×29 QR code using nanoparticles that were transferred using an ultra-thin hydrogel template.

However, if the information encoded by a QR code could also convey information about the concentration levels of target analytes in a product, then the smart-phone could be used to determine the freshness of a perishable product like milk (as shown in Fig. 1) before purchase. This ability would be useful for preventing food-borne disease outbreaks and product

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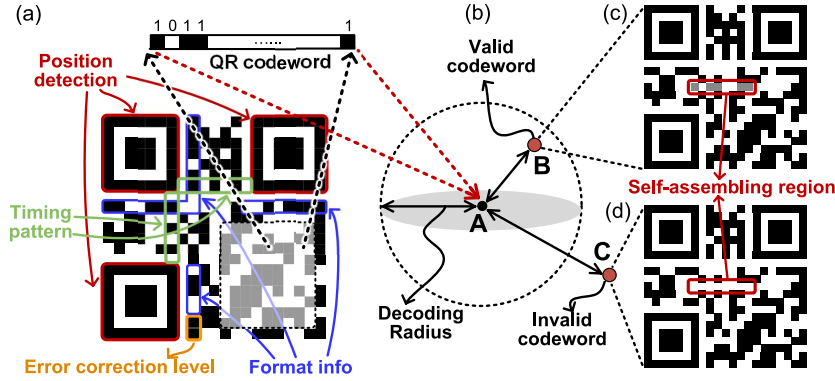


Fig. 2. Principle of the proposed QR code self-assembly and decoding process. (a) Different parts of a QR code. (b) Decoding procedure where any assembled QR is successfully decoded if the code-word lies within the decoding radius. Examples of a QR code that can be (c) successfully decoded and (d) cannot be decoded.

recalls like the 2015 *Listeria* outbreak due to contaminated ice-cream [11]. The QR code also provides a platform to implement forward error-correcting (FEC) biosensors [12] which combines the process of biosensing with FEC to achieve higher reliability and throughput in analyte detection. In an FEC framework, shown in Fig. 1, the biosensor is visualized as a communication channel where the binding event between an analyte with its biological receptor (e.g., antibody or aptamer) is considered to be noisy and the source of error [13]. A channel encoder, comprising of different spatial and logical bioreceptor patterns [13], is used to mitigate the effect of channel noise and in the process enhance the channel capacity. As an example, in [14] we reported a low-density parity check type encoder that was constructed using antibody based logic gates. For this work, we propose to use the QR code as a bio-encoder and the smart-phone as a decoder, as shown in Fig. 2. The encoder is constructed by overlaying an unassembled QR code over a paper-based microfluidics assay which is used to sample the analyte and direct it into the regions where portions of the QR code could self-assemble. In this work self-assembly has been achieved using a process of silver-enhancement which is triggered only when target analytes are present in the sample. The self-assembly results in a change in the optical absorption in the specific areas of the QR code. This paper builds upon our previous work in the area of silver-enhancement [15]–[20] where we have demonstrated self-assembly of radio-frequency (RF) antenna structures and for implementing conductance based biosensors. In these previous studies, silver-enhancement procedure was used to amplify and measure the concentration of gold-nanoparticles which was then shown to be equivalent to measuring the concentration levels of the target analytes. In this paper we show that the QR decoding process can also measure the concentration levels of immobilized gold-nanoparticles with the assumption (based on our prior work) that this ability can be easily translated to measuring the concentration levels of target analytes.

This paper is organized as follows: Section II briefly introduces the basics of a QR code and self-assembly based on silver-enhancement and its integration with paper-based microfluidics devices. In Section III, we describe the materials and methods used for preparing the experimental setup. Section IV

presents measurements obtained using the proposed biosensor and Section V concludes the paper with discussions about future work in this area.

II. PRINCIPLE OF OPERATION

A. Quick Response (QR) Code

QR code is a two-dimensional barcode which was developed to enhance the data encoding capacity per unit area compared to a traditional barcode [21]. Since its inception for use in tracking automobile parts, QR codes have now been widely adopted in commercial tracking, entertainment, ticketing and labeling applications. While several references can be found in literature describing the theory and decoding of QR codes [22]–[25], for the sake of completeness we briefly describe the structure of the code without too much emphasis on different variants and versions of the code. Typically, four kinds of data, namely numeric, alphanumeric, byte/binary, and kanji, can be encoded and stored in the QR code. As an example Fig. 2(a) shows a version 1 (21×21 pixels) QR code encoding the word “POSITIVE.” The reader can readily verify this by using the QR decoder app on their smart-phone. The position detection pattern [highlighted by a red square in Fig. 2(a)] located at the three of the four corners are used for alignment, such that a QR code scanner can normalize and calibrate for any image scaling or misalignment. Format information [shown in Fig. 2(a)] contains the error correction rate and mask pattern of a QR code. This information is read first to determine the “strength of error-correction” before the code is decoded. The timing pattern helps the decoder to detect the position of each cell in the QR code. Data encoding and decoding use a Reed-Solomon error correction algorithm that can correct for corruption in parts of the scanned QR code. Like any FEC code, a QR code can embody different strengths of error correction (Low-7%, Medium-15%, Quartile-25% and High-30%) by trading-off data storage capacity. For example, in Fig. 2(a) the area marked in yellow encodes the error correction level “L,” which implies that the code can be reliably decoded even if approximately 7% of the data is corrupted or damaged. We will exploit this error-correcting capability in the proposed

FEC biosensor where parts of the QR code-word will self-assemble and become decodable based on the presence of target analytes in the sample.

This FEC process is illustrated in Fig. 2 where a valid QR image (image that can be successfully decoded) is visualized as a vector (A) in a high-dimensional space [illustrated in three dimensions in Fig. 2(b)]. The decoding radius in Fig. 2(b) quantifies the error-correcting capability of the QR code. Any vector that differs from the valid QR code (A) by a certain degree (for instance Hamming distance or Euclidean distance) but lies within the hyper-sphere can be successfully decoded and any vector that lies outside cannot be decoded. This is illustrated in Fig. 2 which shows two examples of assembled QR codes (B and C) that differ from each other regions that is highlighted. The QR code B lies within the decoding radius and can therefore be successfully decoded whereas the code-word C lies outside the radius and hence cannot be decoded. In the proposed FEC biosensor, the sensing process (or the process of growing or assembling the QR code) will follow a trajectory starting from an unassembled QR codeword like C to an assembled codeword like B, if and only if target analytes are present. Also illustrated in Fig. 2 is that the decoding distance between the assembled QR codeword and the perfect QR codeword can be used to determine the concentration of the target analyte.

B. Self-Assembly and Analyte Detection Based on Silver-Enhancement

Silver-enhancement based analyte detection relies on the formation of a sandwich structure comprising of primary probes (e.g., antibodies), target and gold nanorod (AuNR) conjugated secondary probe, as illustrated in Fig. 3(a). In our previous work [19], [20] we have described the mechanism of the sandwich formation for different substrates and for detecting different target analytes, which we briefly summarize in this section. The AuNR conjugated secondary probes first hybridize with their target analytes which then bind with the primary probes that are immobilized on specific locations on a substrate (e.g., silicon or nitrocellulose paper). Any unbound secondary probes are washed away leaving the sandwich structure (labeled by AuNR) intact. Because the dimensions of AuNRs are in the scale of nanometers, they are not large enough to be optically visible or electrically detectable. Therefore, in literature a silver-enhancement procedure is used to stain the gold labels (nanoparticles or nanorods) and measure its concentration [26], [27].

When a silver enhancement solution (comprising of silver ions (Ag^+) and hydroquinone, a photographic developing solution) is applied, silver ions start reducing into metallic silver on the surface of the gold. This process is completely self-powered by the chemical activation energy and does not require any external biasing. Also, during this process, gold serves as a catalyst and facilitate further reduction of silver-ions. As time progresses, more silver ions are reduced and a chain of AuNRs cored silver micro-structures assemble, as shown in Fig. 3(c). This results in an equivalent increase in conductance [as shown in Fig. 3(e) and (f)] which can be measured to determine the concentration of the captured targets. In [15] and [16], we have

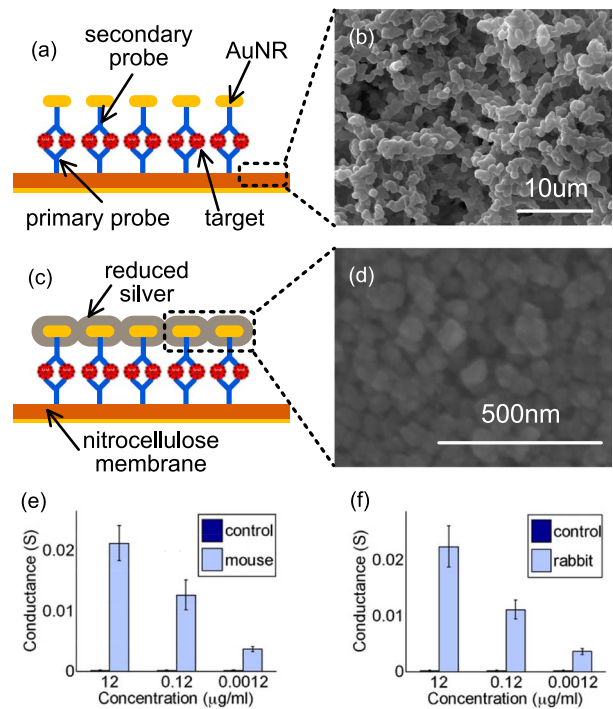


Fig. 3. Analyte detection using silver-enhancement. (a) Sandwich structure labeled using AuNR and before the process of silver-enhancement. (b) SEM image of nitrocellulose membrane surface. (c) After the silver-enhancement process, the silver ions reduce on the AuNR. (d) SEM image showing silver ions were reduced on the surface of AuNRs and grow the particle in size with silver-enhancement. (e) and (f) Summarize previous experimental results showing that the growth of silver-particles leads to a change in conductance which is proportional to the concentration level of mouse and rabbit IgG [19].

used the silver-enhancement principle to grow radio-frequency antennas in different antenna patterns. In this work we use the silver-enhancement principle to grow parts of a QR code that can be optically scanned. When sufficient silver enhancement solution is present, the increase of the particle size is monotonic [20] with respect to the time, which reduces the intensity of the scattered light. Thus, the regions where the silver-enhancement successfully occurs become darker. Note that besides silver enhancement, gold solution can also be reduced and deposited on the gold nanorod surface, and works in a similar way as the process of silver-enhancement. However, the time-scale for gold-enhancement is much longer than silver-enhancement, the relative change in intensity is much lower and the cost of using gold as an enhancer is much higher than silver.

To understand how the silver-enhancement can be combined with a QR code, consider the modified QR code shown in Fig. 2(c) which is the same QR code as the one shown in Fig. 2(a). The only difference is that five black squares have been reduced in intensity to gray. This simulates the condition when different concentrations of analytes lead to different degrees of staining. The code in Fig. 2(c) can still be decoded (in this case with WeChat App on iPhone 6) and the reader can verify this using his/her smart-phone. However, when these five black modules/squares are completely removed from the code, as shown in Fig. 2(d), the code can not be decoded by a smart-phone because the code-word lies outside the decoding radius, as shown in Fig. 2(b).

C. Integration With Paper-Based Microfluidics

Paper-based microfluidics is used for sampling and directing the flow of analytes to the regions of the QR code that can self-assemble. Most of the paper-based microfluidics devices are fabricated by patterning hydrophilic channels and hydrophobic barriers on cellulose-based paper membranes, such as cellulose chromatography papers [28] and cellulose filter paper [29]. In this paper we have used nitrocellulose membranes (manufactured by Millipore) as our microfluidics substrate due to its high bioreceptor (e.g., antibodies) binding capacity and consistent pore size [30]. This is important because the length of the microfluidics channels is determined by the degree of penetration of the liquid through the porous nitrocellulose membrane, which is approximately modeled by the Washburn's equation [29] as

$$L = \sqrt{\frac{\gamma R \cos \theta}{2\eta} t} \quad (1)$$

where L is the liquid penetration distance in the paper, R is the average radius of pore size, γ is the surface tension of the liquid, η is the liquid viscosity and t is the time of the penetration. Both the parameters L and t can be used to optimize the size of the microfluidic channels as described in [17], [31]. When the silver enhancement solution is applied to the application pad, it diffuses through the nitrocellulose channel to the adsorption pad and in the process it interacts with the gold nanorods immobilized in the target area. For the proposed QR code, target specific primary probes could be immobilized in the specific regions of the code, for instance the highlighted region shown in Fig. 2(d). Similar to the operation principle of the lateral-flow immunoassay [32], target specific analyte will first conjugate with the AuNR labeled secondary probe to form a partial sandwich structure. When the partial sandwich conjugate diffuses to the regions where the primary probes are immobilized, a complete sandwich structure will be formed. Thus, the state of the QR code after the formation of the sandwich structure will be similar to that shown in Fig. 2(d) because the AuNR cannot be detected by the QR code reader/scanner. After silver-enhancement based staining the color of the target region will change and depending on the concentration of the AuNR could make the QR code decodable [as shown in Fig. 2(c)].

Since the focus of this paper is the integration of QR code with silver-enhancement based self-assembly, we will skip the process of forming the sandwich structure as shown in Fig. 3 and directly pattern gold nanorods at different concentration levels. If we can detect and measure different concentration levels of AuNR, we should be able to extend the approach towards detecting different concentration levels of target analyte based on our previously reported approaches [15]–[17].

III. MATERIALS AND METHODS

A. Materials and Apparatus

Gold chloride (HAuCl_4), Hexadecyltrimethylammonium bromide (CTAB), sodium borohydride (NaBH_4), silver nitrate

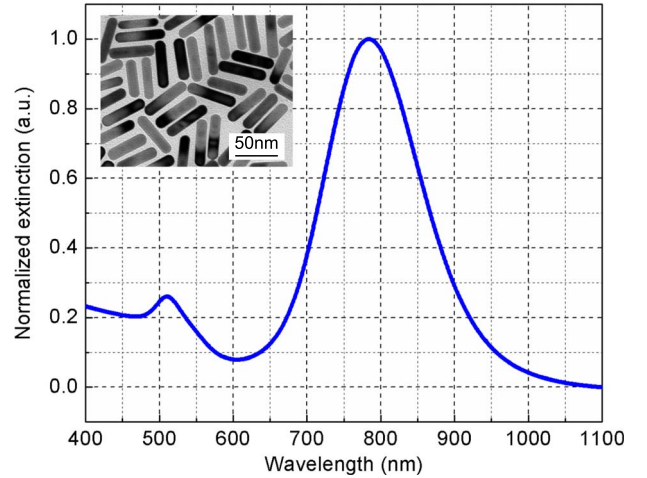


Fig. 4. Optical extinction spectrum of AuNR verifying the dimensions of the nanorods (inset: TEM image of AuNR).

(AgNO_3), ascorbic acid and silver enhancement kit were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nitrocellulose (NC) membranes with flow-time (measure of flow rate) of 135 sec/4 cm was purchased from Millipore (Billerica, MA, USA). Deionized (DI) water used in the experiment was obtained through Millipore water purification systems (Billerica, MA, USA). An EPSON stylus C88+ ink-jet printer was used to print the QR code. The printing substrate, the empty cartridge and aqueous inkjet vehicle were purchased from Novacentrix (Austin, TX, USA). Paper mate profile retractable ballpoint pens were bought from Amazon.com, Inc. All the chemicals were used as received without further purification. The experiments were carried out in a certified Biological Safety Level II laboratory.

B. Synthesis of Gold Nanorods

Gold nanorods were synthesized by using seed-mediated method [33], [34]. Seed solution was synthesized by adding 0.6 ml of an ice-cold NaBH_4 (10 mM) solution into 10 ml of HAuCl_4 (0.25 mM) and CTAB (0.1 M) solution under vigorous stirring at room temperature. The color of the seed solution changed from yellow to brown. Growth solution was prepared by mixing 5 ml HAuCl_4 (10 mM), 95 ml CTAB (0.1 M), 1 ml AgNO_3 (10 mM) and 0.55 ml ascorbic acid (0.1 M), consecutively. The solution was homogenized by gentle shaking. To the resulting colorless solution, 0.12 ml of freshly prepared seed solution was added and kept undisturbed in the dark for 14 h. Prior to use, the AuNR solution was centrifuged twice at 8000 rpm for 10 min to remove excess CTAB and re-dispersed in nanopure water. UV-Vis extinction spectra were measured using Shimadzu UV-1800 UV-Vis spectrophotometer and shown in Fig. 4. The extinction spectrum of AuNR exhibits two characteristic bands at 510 nm and 784 nm each corresponding to the transverse and longitudinal plasmon resonances. These represent unique signatures for a nanorod with a specific length and diameter. The length and diameter of AuNR were respectively measured to be 52.6 ± 4.4 nm and 13.1 ± 0.8 nm using Transmission electron microscopy (TEM) (inset



Fig. 5. Experiment showing different stages of QR self-assembly. (a) An example of QR code printed on a white paper (information encoded in this QR code: Adaptive Integrated Microsystems Laboratory <http://aimlab.seas.wustl.edu/>). (b) The same QR code printed on a plastic substrate using AuNR ink. (c) The plastic substrate after 20 min of silver-enhancement. (d) Information in the QR code decoded with a smart-phone app.

in Fig. 4). TEM image was collected using a JEOL JEM-2100F field emission microscopy.

C. Ink-Jet Printed QR Code and Silver-Enhancement

We verified the process of encoding and decoding QR codes self-assembled using the silver-enhancement process in the following experiment. QR code in Fig. 5(a) was ink-jet printed on the mesoporous polyethylene substrate using AuNR solution as ink and was developed with silver enhancement solution, as shown in Fig. 5. Before loading the cartridge filled with the AuNR solution to print the QR code, the printer head was purged by printing the MIS purge document using aqueous inkjet vehicle. Around 10–20 pages were printed by selecting “Heavyweight Matte paper” and “Best Photo” in the printer preferences until pigment disappeared. Then the cartridge filled with AuNR solution was loaded. Another 5 pages (or more) of the MIS purge document were printed on Heavyweight Matte paper and Best Photo using AuNR ink until the ink appeared consistent. Then the mesoporous substrate was loaded and the QR code was printed. After the QR code printed on the substrate dries up, the code is not visible as shown in Fig. 5(b). This is because the size of the AuNR is in nanometer. The mesoporous substrate was then treated with silver enhancement solution (composing of the initiator and enhancer with volume ratio of 1 : 1). After a 20 min development process, more and more silver ions get reduced on the surface of AuNR. The size of the particle gets increased from nanometer scale to micrometer scale. The area on the substrate where is originally printed with AuNR can be visually seen in black, as shown in

Fig. 5(c). Then the substrate was rinsed with DI water gently to remove the remaining silver enhancement solution and stop the process of silver enhancement. The developed code can then be decoded with QR code scanner, as illustrated in Fig. 5. It should be noted in Fig. 5(c) that the area where no AuNR is printed gets darker after silver enhancement compared to that in Fig. 5(b). It is because the AuNR solution printed on the mesoporous is not firmly captured by the substrate. Once the substrate is treated with silver enhancement solution for development, some of those AuNRs are washed away from their original spots and are captured again in other spots by the substrate. These AuNRs also participate in the silver enhancement process since they are also exposed to the solution, which turns the substrate darker. Fortunately, due to the error-correcting capability (15% of codewords can be restored in this case), the QR code can still be correctly decoded.

D. QR Code Biosensor Prototype Fabrication

Fig. 6 illustrates the fabrication procedure for the QR code FEC biosensor. QR code shown in Fig. 2(c) was first ink-jet printed on both the mesoporous polyethylene substrate and the plain printing paper [this will be used after step shown in Fig. 6] with EPSON C88+ printer using normal black ink as shown in Fig. 6(a). The five gray squares can be distinguished from other black squares. Disposable Miltex Scalpel #11 blade is used to cut through the plastic substrate along the edges of those five gray modules to remove them from the substrate to form three open windows on the substrate as shown in Fig. 6(b). Nitrocellulose (NC) membrane with desired shape is attached on the back side of the substrate using transparent tape with nitrocellulose side facing the open windows created in the last step [Fig. 6(c)]. Then the single biosensor piece is aligned to the alignment marks on the letter sized printing paper which is printed in the first step and is fixed using transparent tape. For better alignment we used a transparent PET instead of an opaque substrate for printing and fabrication. When the QR code is printed on the transparent substrate, the biosensor can be aligned to the QR code which is printed on a plain printing paper. We can then adjust the position of biosensor by matching the alignment pattern of the QR code on the PET substrate to the alignment pattern of the QR code on the white paper. The black ink cartridge is replaced with the one filled with aqueous inkjet vehicle. The printer was first initialized (to ensure uniform printing of AuNR patterns) using the procedure described in the previous section. The printing paper (with biosensor attached) is loaded in the printer, and the yellow bar area [shown in Fig. 6(d)] which covers the three open windows is printed with AuNR solution. The ink-jet printed AuNRs are captured by the porous nitrocellulose surface. The AuNR printing is a self-alignment process, which only allows the AuNR solution printed in the area facing to the open windows of the substrate to be captured by the nitrocellulose. All the rest ink-jet printed AuNR solution is printed on the surface of the plastic substrate, which improves the yield because normally the commercial printer (e.g., EPSON C88+ we used in our experiment) is not able to provide a very precise alignment for

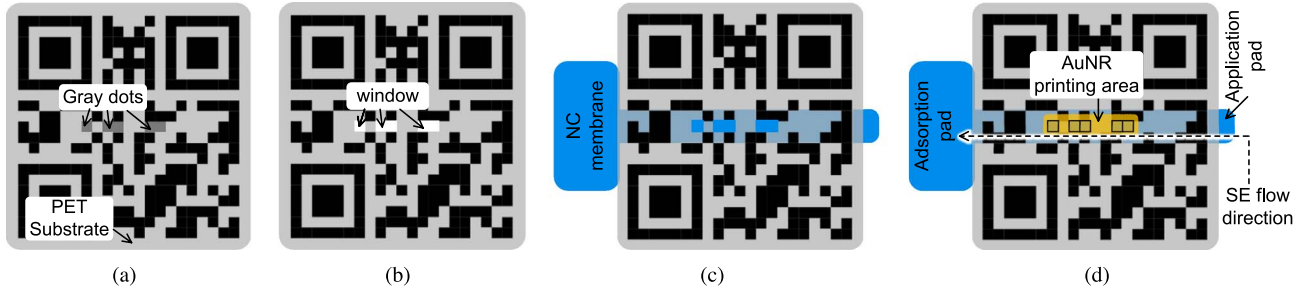


Fig. 6. Illustration of QR code FEC biosensor fabrication procedure. (a) QR code printed on the mesoporous substrate using normal ink. (b) The plastic substrate in the gray colored square dots area removed. (c) NC membrane is attached on the back side of the QR code. (d) AuNR ink is printed on the yellow area.

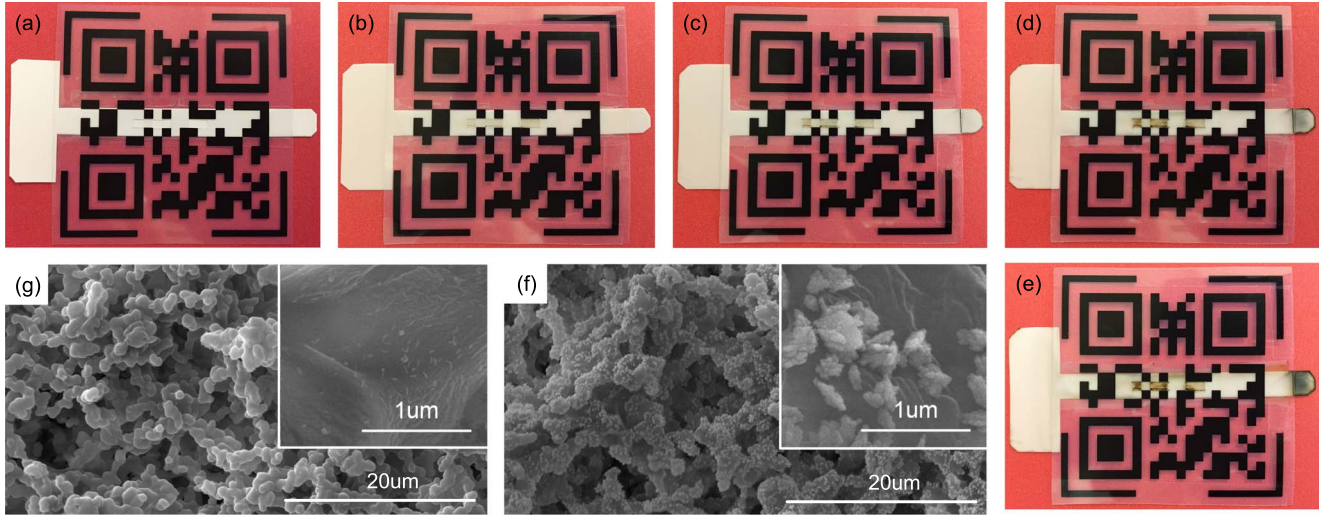


Fig. 7. (a) AuNR solution printed on the nitrocellulose membrane where faces to the open windows on the substrate before silver enhancement solution applied. (b)–(e) Show the color changes of the NC membrane with silver enhancement for 5 min, 10 min, 20 min, and 25 min, respectively, (f) AuNR on NC membrane after silver enhancement, (g) AuNR captured on the NC membrane before silver enhancement.

multiple loadings of a single sheet of paper. Silver enhancement solution is then applied in the “Application pad” of the NC membrane shown in Fig. 6(d) and the solution will flow all the way down to the “Adsorption pad” due to the capillary force of the membrane. During this process, the AuNR is exposed to the silver enhancement solution. Silver ions start to get reduced into metallic silver on the surface of the AuNR, which will result in a visible color changing from white to brown or even black. Note that the Adsorption pad is designed to have a sufficiently large surface area so that it can continuously absorb silver enhancement solution diffusing from the application pad and to facilitate continuous evaporation of the reagent to self-power the diffusion process.

IV. MEASURED RESULTS

The fabricated biosensor prototype is shown in Fig. 7(a)–(e). AuNR is ink-jet printed on the NC membrane surface where faces to the open windows on the plastic substrate created by disposable scalpel. As it can be seen in Fig. 7(a), the printed AuNR is not visible and hence the QR code is not decodable. Fig. 7(b)–(e) shows a set of photos illustrating the process

of silver enhancement as the QR code assembles. The color change can be visually observed even in just 5 min after silver enhancement. In this set of photos the color turns into brown (even black) as time progresses. The word “POSITIVE” can be correctly decoded after 20 minutes’ silver enhancement [Fig. 7(d)], which the reader can verify by using the QR code scanner on their smart-phones. The process of silver-enhancement has been verified through an SEM analysis of the enhancement region as shown in Fig. 7(g) and (f), where the scans were obtained after 30 minutes of enhancement time. The SEM images were obtained using a FEI Nova 2300 Field Emission SEM at an accelerating voltage of 5 kV. The porous surface texture feature of the nitrocellulose membrane provides strong AuNR capturing capability as shown in the inset in Fig. 7(g).

A. Quantitative Measurement Using QR Codes

In the next set of experiments we verified if the procedure of QR decoding could be used to infer the concentration level of target analytes. Since in our previous papers we have already demonstrated that measuring the concentration of gold

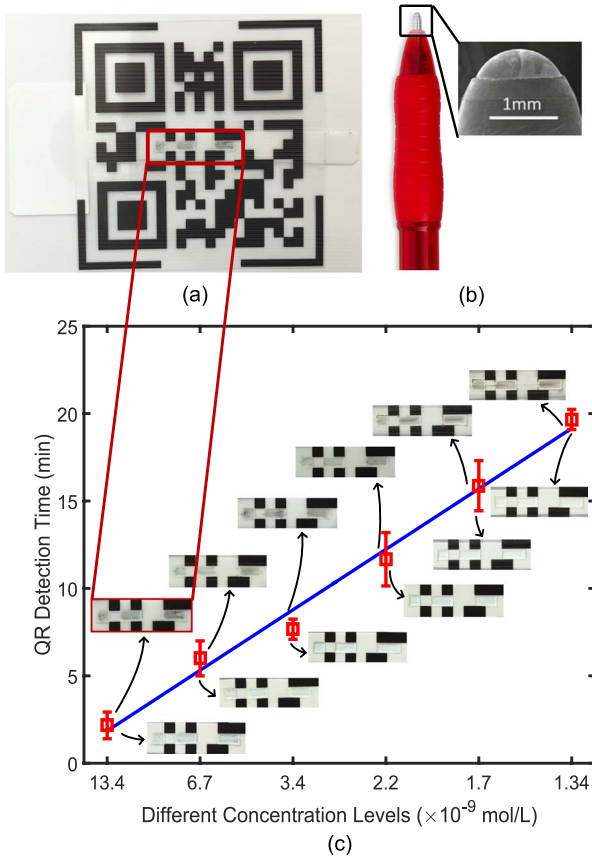


Fig. 8. Experiments to measure different AuNR concentration levels using the example (a) assembled QR code; where the AuNR ink is dispensed using a (b) retractable ballpoint pen with an SEM image of the tip. (c) Time measured to assemble the QR code using silver enhancement for different concentration levels of AuNR solution (Inset shows the sensing region before and after QR segments assembled).

nanoparticle labels to the concentration of target analytes, in this experiment we only measure the concentration levels of immobilized AuNR. We found that direct dispensing of AuNR ink in the enhancement regions is not consistent for different concentration levels. The printer was unable to dispense equal amount of ink for different printing cycles. The primary reasons could be: 1) unequal distances between the tip of the printer head and the surface of the PET substrate and the nitrocellulose membrane creates different printing areas; and 2) the physical properties of the AuNR (e.g., viscosity, surface tension) is not optimized for the hybrid substrate. Due these limitations, we resorted to calligraph the AuNR ink on the QR assembly regions using a regular ballpoint pen [35], [36]. Fig. 8(b) shows the retractable ballpoint pen (from PaperMate) that was used and the inset shows the SEM graph of the pen's dispensing tip. The original ink in the pen's refill container was pushed out using nitrogen gun after the tip and the refill are separated. Then the ballpoint pen refill and the tip are cleaned with ethanol by sonication for 2 days in a centrifuge tube. The ethanol was replaced every 3 hours. Different dilution levels (1:2 to 1:10) of AuNR solution were prepared and the AuNR concentration level was confirmed using ultra-violet (UV) measurement [37], [38] according to

$$A = \epsilon bC \quad (2)$$

where A is absorption, ϵ is extinction coefficient, b is the light path in the UV-cuvette, and C is molar concentration of nanoparticle solution. The concentration of AuNRs was determined from the optical extinction of colloidal suspension of AuNR. Extinction coefficient ϵ of AuNR is about $3 \times 10^9 \text{ M}^{-1} \text{ cm}^{-1}$. The extinction of $20\times$ diluted AuNR solution in our study is 2.0, light path is 1 cm, so the molar concentration of AuNR solution ($20\times$ diluted) is $6.7 \times 10^{-10} \text{ M}$. The diluted AuNR solution was injected into the cleaned ballpoint pen refill and the pen was then used for writing and dispensing the AuNR ink in the QR assembly regions.

The time to successfully decode the QR code after the silver-enhancement solution was applied was measured for different dilution levels. Each measurement was repeated three times and the relative error-bars are also shown in Fig. 8(c). As it can be seen from Fig. 8(c), the QR code detection time, or assembly time increases monotonically with respect to the AuNR dilution levels, thus demonstrating the use of QR decoding for measuring concentration levels of target analytes.

V. CONCLUSION

In this article we demonstrated the integration of paper-based microfluidics with silver-enhancement based self-assembly for designing a QR encoded FEC biosensor. The silver enhancement process self-assembles segments of a QR code that can be scanned and decoded using a standard smart-phone. Similar to our previously reported approach for self-assembling RF antennas, this method can also be viewed as a process that transitions from a high-entropy state (random codeword) to a low-entropy state (valid QR codeword) which is opposite to the effect due to environmental artifacts. Thus, we believe that similar to the spirit of FEC biosensing principle the proposed method should yield lower false-positives and higher reliability. Based on the QR based decoding, there are two use cases for the proposed biosensor in real-world applications. In the first use case, a consumer triggers the sampling of the analyte using a capillary channel (a dipstick configuration) and then waits for the result of silver-enhancement. Our measurement results show that the detection time will provide an estimate of the concentration of the target analyte which then could be used as a contamination flag. The second use case, which is more challenging is that the embedded biosensor continuously samples the analyte and updates the QR code. In this case the key challenges lie in the packaging of the biosensor and accurately controlling the flow of different reagents so that its operation matches the shelf-life of the product. Another challenge is to reproduce the quality of the biosensor and reduce its production cost when fabricated in large volumes. To address this issue the research focus will be to investigate inexpensive approaches for developing silver-enhancement reagents and to investigate reliable dispensing of the AuNR ink and other reagents using the inkjet printing technique. Future work will also focus on enhancing the shelf-life of the biosensing platform and on optimizing the amount of reagent used for analysis. Also, future work will focus on leveraging cloud-based computation and data-fusion capabilities to improve the reliability of detection and to provide updated feedback to the end user.

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