

# Reflective Diffraction Gratings from Hydrogels as Biochemical Sensors

Chun-Li Chang, Zhenwen Ding, Venkata N. L. R. Patchigolla, Babak Ziaie, and Cagri A. Savran

**Abstract**—We report reflective diffraction gratings made from smart hydrogels for ultrasensitive biochemical detection. As an example for a stimuli-responsive hydrogel, we chose a pH-sensitive hydrogel to construct diffraction gratings that swell/shrink reversibly due to changes in pH. Interferometric analysis of the grating enabled detection of the hydrogel's motions with nanoscale precision and resulted in a resolution of  $6 \times 10^{-4}$  pH units. The developed system is remarkably simple both to fabricate and operate, and yet extremely sensitive. Moreover, the concept of the reflective hydrogel grating is generic and can be applied detection of a wide range of other stimuli.

**Index Terms**—Biochemical sensors, diffraction, gratings, smart hydrogels.

## I. INTRODUCTION

**H**YDROGELS that respond to stimuli, also known as “smart” hydrogels, have been investigated in recent years [1, 2]. Smart hydrogels are polymers that alter their properties in response to changes (stimuli) in the surrounding liquid such as concentration of the content molecules, temperature and pH [1-9]. Reversible volume change is one of the most common responses of the smart hydrogels to various stimuli. For example an antigen-responsive hydrogel's volume is reported to change based on the antigen concentration in the surrounding solution [7]. A glucose-sensitive hydrogel swells in the presence of glucose and its swelling ratio is dependent on the glucose concentration [8]. Similarly, an anionic pH-sensitive hydrogel is known to swell when the pH value of the aqueous environment increases and shrink if the pH decreases [9]. By combining the hydrogel's reversible swelling characteristics with modern detection technologies, it is possible to develop

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ultrasensitive sensors for various applications. A number of sensors have been developed by incorporating hydrogels into structures. Some examples are cantilever-based pH sensors, microgravimetric sensors, hydrogel microarray sensors and CO<sub>2</sub> gas sensors [9-14]. Very recently, researchers have also patterned smart hydrogels in the form of diffraction gratings for biosensing applications [15-18]. For example, Ye *et al.* have developed a transmission-based diffraction sensor, by patterning smart hydrogels in the form of diffraction gratings. In this method, various stimuli cause changes in shape and the refractive index of the hydrogel which modulate the light transmitted through the transparent grating [16-18].

In this paper we demonstrate a reflective diffraction grating fabricated directly from a biochemically responsive hydrogel. A laser beam illuminates and reflects from the grating surface without interacting with the grating material itself. Hence, the detection mechanism relies on detecting the phase of the grating which is induced only by grating height changes (not by the optical properties of the hydrogel itself.) The diffraction-based sensor presented here takes advantage of both the hydrogel's reversible swelling characteristic and the ability of reflective diffractometry to detect extremely small, nanoscale motions. Although our scheme is generic and can readily incorporate other hydrogels responsive to other stimuli, here we focus on a basic example; pH detection; to demonstrate the concept of robust yet extremely sensitive biochemical detection with hydrogel-based reflective diffraction gratings.

pH measurement is common in many laboratories that perform chemical/biological research because it is often critical to precisely control the reagent's pH at an optimal value in a chemical reaction [19]. Also, some biological molecules such as enzymes involved in pH-changing reactions can be detected by directly monitoring pH [20, 21]. pH shifts usually result from releasing of hydrogen (H<sup>+</sup>) or hydroxide (OH<sup>-</sup>) ions as by-products of chemical reactions. Accordingly, when H<sup>+</sup>/OH<sup>-</sup> ions are released in small amounts, the resulting pH shift is also expected to be small. Hence, a device that allows sensitive detection of pH shifts is of great significance. This need led researchers to develop various novel and sophisticated pH detectors with outstanding sensitivities between  $5 \times 10^{-4}$  and  $10^{-2}$  pH units range, such as light-addressable potentiometric sensors, scanning probe potentiometers, and ratiometric pH-responsive nanoensembles integrated on carbon nanotubes [22-24].

## II. DETECTION STRATEGY

The pH-hydrogel diffraction grating presented here is a simple yet sensitive system that allows detection of nanometer

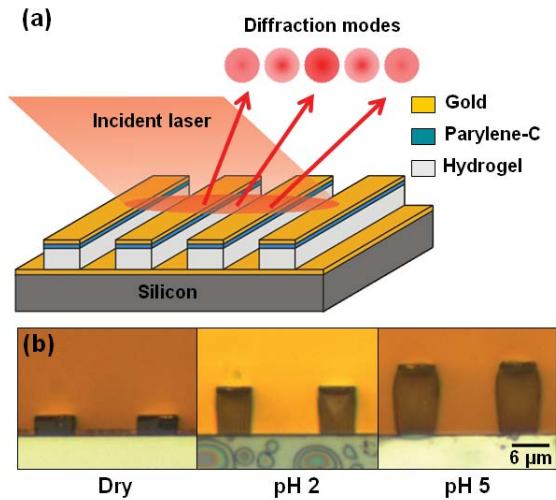


Fig. 1. (a) Schematic of the hydrogel diffraction grating. (b) Cross-sectional optical micrographs of hydrogel gratings in dry condition and in PBS buffer with pH 2 and 5.

level changes in grating heights caused by pH-induced hydrogel swelling/shrinking. Fig. 1(a) shows a schematic of the pH sensor and the detection strategy. The illumination of the grating with a coherent light source, i.e. a laser beam, produces diffraction modes whose intensities reveal the motion of the grating height due to hydrogel's swelling/shrinking. When compared with many other optical devices, the planar structure of the grating device relaxes the alignment requirements making it simple to operate. With this device, detection of small range pH variations (0.01) in liquid is demonstrated with a resolution of  $6 \times 10^{-4}$  pH units. The concept of the hydrogel grating is generic and can be applied to detection of other stimuli such as molecules of biochemical significance like glucose or antigens using hydrogels responsive to such stimuli.

Fig. 1 shows alternating hydrogel stripes defined on a gold-coated silicon chip by means of standard lithography. The top of the hydrogel stripes were also coated with a thin gold film to increase the reflectivity. The sidewalls of the hydrogel were open to the surrounding environment to enhance exposure to target analytes or ions. This fabrication method constrains the top and bottom planes of the hydrogel from stretching horizontally such that the main change in the grating due to pH shifts is in its height. Fig. 1(b) shows the optical micrographs of a dry hydrogel grating and those exposed to pH 2 and pH 5 phosphate buffered saline (PBS) solutions. The grating height increases dramatically from pH 2 to pH 5 while the width only increases slightly. Upon shining a laser beam on the grating surface, the reflected beams from the gold-coated hydrogel stripes and the silicon substrate interfere in a phase-sensitive manner that depends on the height of the grating, and produce a series of bright diffraction modes. The grating height can be determined by measuring the intensity of the diffraction modes with a photodiode. The operation theory of the diffraction measurement has been described in detail in a previous study [25]. The intensity of the first order diffraction mode ( $I_1$ ) can be represented by (1) [26]

$$I_1 \propto \sin^2 \left( 2\pi \frac{n\delta}{\lambda} \right) \quad (1)$$

where  $n$  represents the refractive index of the medium ( $\sim 1.33$  for PBS solution),  $\delta$  the height of the grating, and  $\lambda$  the nominal wavelength of the laser beam (632.8 nm). Equation (1) suggests that the intensity of the first diffraction mode is a periodic function of the grating height  $\delta$ . Moreover, the grating height shift that depends on the pH change in the solution, can be calibrated from the photodiode output with the knowledge of  $n$  and  $\lambda$ .

### III. EXPERIMENTAL PREPARATION

#### A. Fabrication of Hydrogel Diffraction Gratings

To fabricate the hydrogel gratings, a titanium/gold (Ti/Au, 20nm/80nm) film was first evaporated onto the surface of a silicon substrate. A layer of 50nm silicon dioxide ( $\text{SiO}_2$ ) was then deposited by plasma enhanced chemical vapor deposition (PECVD) and was subsequently silanized to create a chemical bond with the hydrogel with improved adhesion. To deposit a hydrogel layer on the  $\text{SiO}_2$  surface, two solutions (A and B) were prepared and mixed in a volume ratio of 5.9:1 to form a pregel solution: solution A was composed of 334.5 mg acrylamide (AAm, Sigma-Aldrich), 100.8  $\mu\text{L}$  methacrylic acid (Sigma-Aldrich, distilled to get rid of inhibitor after received), 16.25 mg  $N, N'$ -methylenebisacrylamide (Bis, Polysciences Inc.), 100  $\mu\text{L}$   $N, N, N', N'$ -tetramethylethylenediamine (TEMED, Sigma-Aldrich) and 1.2 mL deionized (DI) water while solution B consisted of 80 mg/mL ammonium persulfate (APS, Polysciences Inc.) in DI water. The pregel solution was then introduced to the silanized  $\text{SiO}_2$  surface and pressed to form a flat and uniform hydrogel film, following by dehydrating in acetone. To pattern a reflective hydrogel grating structure with a metallic top surface, we first deposited a 0.5  $\mu\text{m}$  thick parylene-C layer on the dried hydrogel film ( $\sim 2 \mu\text{m}$ ) to prevent soaking of the hydrogel and hence to avoid swelling during the subsequent wet processes. A metal layer of 20nm/80nm Ti/Au was then evaporated on top of the parylene layer. Photoresist AZ1518 (MicroChemical GmbH) was spin-coated onto the above structure following by a soft-bake at 95  $^\circ\text{C}$  for 1 minute. Subsequently, the sample was exposed and developed using AZ351:DI water = 1:4 by volume. Ti/Au layer on top of the parylene/hydrogel was then selectively etched by a gold etchant (Gold Etchant TFA, Transene Company, Inc., 28  $\text{\AA}/\text{sec}$ ) and 10% HF (for Ti etching). The patterned upper Au/Ti layer served as a mask and the chip was subjected to a reactive ion etch (RIE) in oxygen plasma ( $\text{O}_2$  flow rate: 50 sccm; Chamber pressure: 100mTorr; Power: 100W; Plasma Technology RIE) to remove the photoresist and pattern the parylene/hydrogel layer. Finally, the remaining photoresist (if any) was removed in acetone. Fig. 2 shows the fabricated devices imaged by optical microscope (from top view) and scanning electronic microscope (SEM, cross-section), respectively.

#### B. Buffers With Different pH Values

To observe the height changes of the hydrogel grating, PBS solutions with various pH values between pH 2 and pH 12

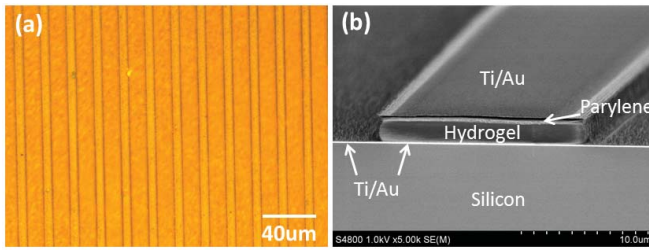


Fig. 2. (a) Optical and (b) SEM micrographs of the gold-coated hydrogel gratings from top view and cross-section, respectively. Narrower patterns in (a) are the hydrogel stripes ( $6 \mu\text{m}$ ).

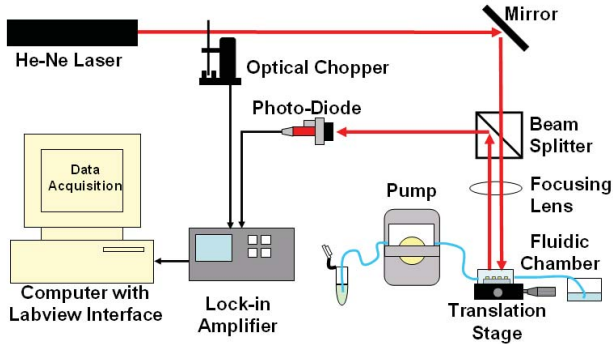


Fig. 3. Schematic of the experimental setup.

were prepared by adding HCl or NaOH to 1X PBS (pH 7.4) purchased from Invitrogen. The final pH values were measured using a commercially available pH meter (accumet AB 15, Fisher Scientific).

### C. Experimental Setup

The experimental setup is demonstrated in Fig. 3. A pH-sensitive hydrogel grating was placed in a micro-fluidic chamber mounted on a translation stage. By using a peristaltic pump (Ismatec ISM596B), PBS solutions with various pH values were pumped into the fluidic chamber through an “open” loop: The inlet of the chamber was connected to the PBS source through the pump while the outlet drained into a waste container. The flow rate was kept constant ( $500 \mu\text{L}/\text{min}$ ) during the pH measurement. A Helium-Neon (He-Ne) laser beam (Newport R-30991,  $632.8\text{nm}$ ,  $5\text{mW}$ ) was focused onto the sample surface in the chamber by a convex lens (focal length  $60 \text{mm}$ ) to a beam diameter of approximately  $120 \mu\text{m}$ . The reflected laser beam was guided onto a silicon photodiode ( $12\text{V}$  reverse-bias, Thorlabs DET110) by a beam splitter (Thorlabs BS016). The photodiode was connected to a  $10 \text{K}\Omega$  resistor to convert the output current into voltage and was used in conjunction with an adjustable aperture (Thorlabs SM1D12) and a band-pass filter (Thorlabs FL632.8-10) for measuring the intensity of the first diffraction mode. An optical chopper (Thorlabs MC1000A), connected to a lock-in amplifier (Stanford Research SR830), was placed at the output of the laser source and was driven at  $100 \text{Hz}$  in order to reduce low-frequency noise. The signal measured by the

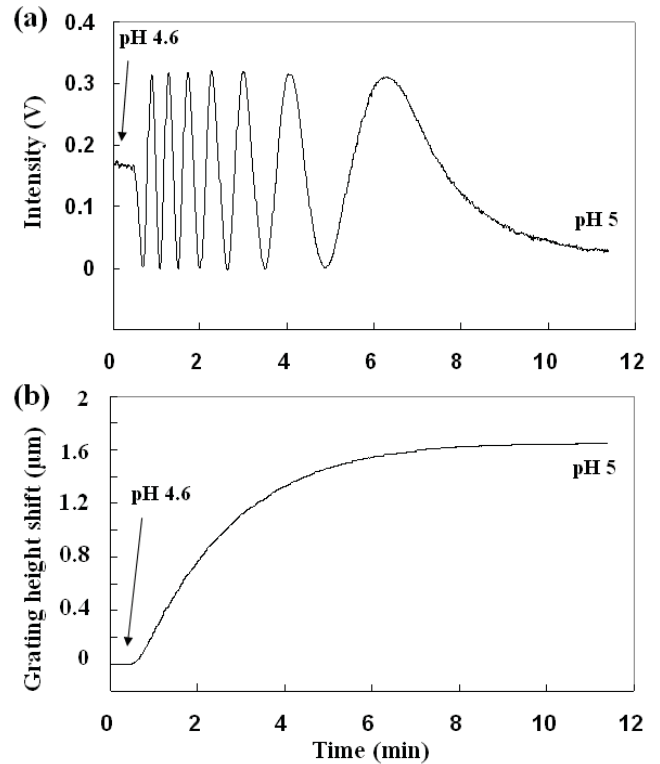


Fig. 4. Large pH change measurement (a) variation of the first diffraction mode and (b) corresponding grating height shift when pH changes from 4.6–5.

photodiode was collected by the lock-in amplifier and recorded by a computer with a National Instruments LabView interface.

## IV. RESULTS AND DISCUSSION

### A. Large pH Measurements

We first tested the hydrogel grating’s response to a relatively large pH change. We equilibrated the hydrogel in a pH 4.6 PBS solution, introduced a pH 5 solution into the fluidic chamber and monitored the first diffraction mode intensity ( $I_1$ ) in real-time. Fig. 4(a) shows the plot of the diffraction intensity versus time when pH was increased from 4.6 to 5. The ordinate’s minimum in fig. 4(a) was arbitrarily set to zero for the ease of grating height change calibration. The intensity, starting at a value of  $\sim 0.16 \text{V}$ , oscillates between a minimum ( $0 \text{V}$ ) and a maximum ( $\sim 0.32 \text{V}$ ) and slowly reaches equilibrium at  $0.03 \text{V}$  as the period of oscillations increases. This suggests that the height of the hydrogel grating increases upon introduction of pH 5 PBS which results in  $I_1$  to change as predicted by Equation (1). The corresponding grating height shift was calibrated according to Equation (1) and plotted in fig. 4(b) which indicates an increase of  $1.65 \mu\text{m}$  in height. This yields a sensitivity of over  $4000 \text{nm}/\text{pH}$  between pH 4.6 and pH 5.

To characterize the hydrogel swelling behavior over a wide pH range, we increased the pH value gradually from pH 2 to pH 9 and measured  $I_1$  in real-time. The dry hydrogel grating in the fluidic chamber was first rehydrated by exposing it to pH 2 which resulted in its height to increase from  $3 \mu\text{m}$  to approximately  $7 \mu\text{m}$  (Fig. 1(b)). Subsequently, pH was

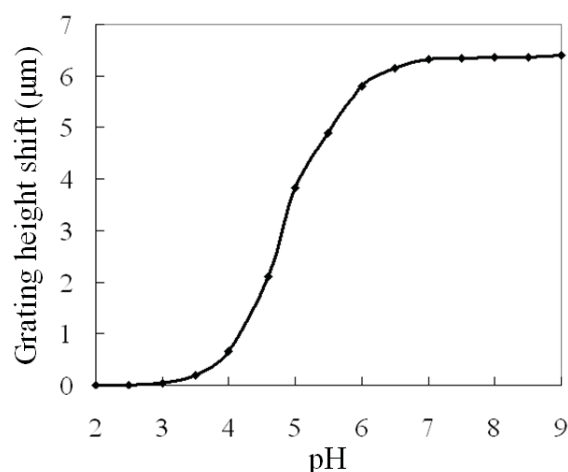


Fig. 5. Dependence of the hydrogel grating height shift (calibrated from the diffraction intensity) on pH.

increased in steps of  $\sim 0.5$  only when equilibrium had been achieved. The dependence of the grating height shift on pH between pH 2 and pH 9 was calibrated in the same way and plotted in Fig. 5. This plot suggests that the hydrogel grating is more sensitive between pH 4 and pH 6 and less sensitive in other regions, which agrees with the behavior of a similar pH-sensitive hydrogel reported in a previous study [9].

### B. Effect of pH on Refractive Index

In order to investigate the effect of possible refractive index ( $n$ ) changes in the PBS solution (induced by pH changes) on the grating response, a control experiment was conducted. A gold-coated non-responsive SU-8 grating with a similar spatial period to the hydrogel grating was fabricated with a grating height of  $9\ \mu\text{m}$  (optical micrograph shown in the inset of Fig. 6). We repeated the diffraction measurement using the SU-8 grating while PBS solutions with various pH values between pH 2 and pH 12 were introduced. The resulting diffraction intensity did not change significantly (as shown in Fig. 6), indicating that the effect of changes in refractive index on the same measurement system was negligible when pH changed from pH 2 to pH 12. These measurements also confirmed that changes in diffraction intensities were mainly due to variation in grating height.

### C. Small pH Measurements

To demonstrate the ability of the sensor to measure small pH variations, PBS solutions were prepared with pH values that differ by 0.01 pH units between pH 4.6 and 4.7. It was challenging to prepare PBS solutions with such small pH differences by using the commercially available pH-meter due to its detection limit. Hence the pH values were approximated mathematically: PBS solutions with 0.01 pH difference were prepared by mixing pH 4.6 and pH 4.7 PBS solutions (by using an Accumet AB 15 pH meter) with a volume ratio of from 9:1, 8:2, ..., to 1:9, respectively. The resulting theoretical pH values are around 4.609, 4.618, 4.628, 4.637, 4.647, 4.657,

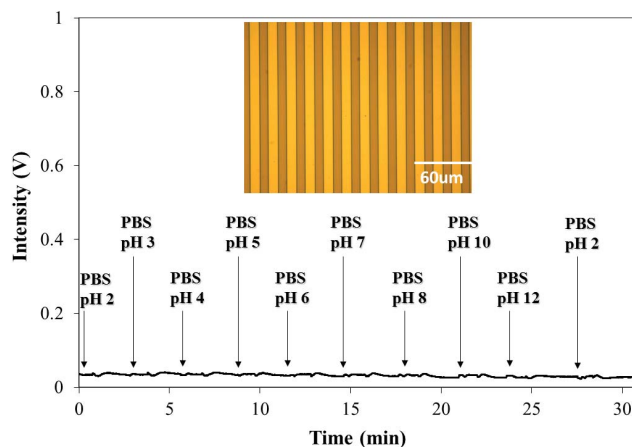


Fig. 6. Diffraction intensity measured using a gold-coated SU-8 grating (with a fixed height of  $9\ \mu\text{m}$ ), while the pH changed between 2 and 12. Arrows indicate the injection of each pH solution. Inset: optical micrograph of the gold-coated SU-8 grating from the top.

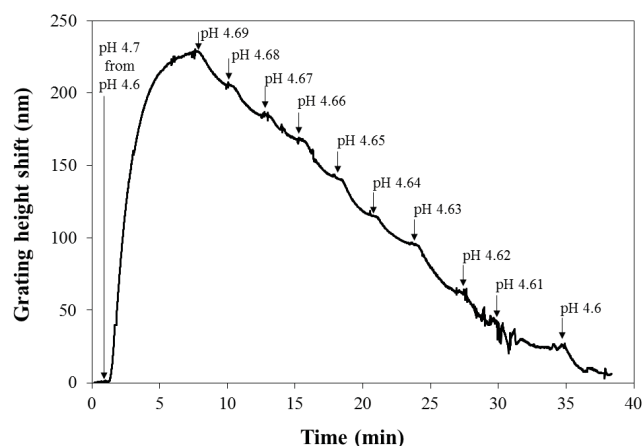


Fig. 7. Hydrogel grating height shift upon changes in pH from 4.7 to 4.6 in increments of 0.01. Arrows indicate the injection of pH solutions.

4.668, 4.678, and 4.689, which are reasonable approximations of 4.61, 4.62, ..., 4.69. The hydrogel grating was first immersed in a pH 4.6 PBS solution for rehydration and then pH 4.7 PBS was introduced into the chamber. Afterwards the pH value was decreased from 4.7 to 4.6 in increments of 0.01. The diffraction intensity was monitored in real-time and the pH value was changed to the next level when the intensity response had stabilized.

The corresponding grating height shift was again calibrated according to (1) and plotted against time in Fig. 7. When pH first increased from 4.6 to 4.7 (between 2nd and 8th minutes), the grating height increased by 228 nm. When pH gradually descended from 4.7 to 4.6 with 0.01 intervals, the grating height shrank accordingly and by similar intervals. The overall height decrease was 224 nm which resulted in an average decrease of 22.4 nm with a standard deviation of 4.06 nm for every incremental pH change. There is a 4-nm-difference in the grating height at pH 4.6 before and after the experiment. This could be attributed to trace amounts of residual ions remaining in the hydrogel or solutions in the



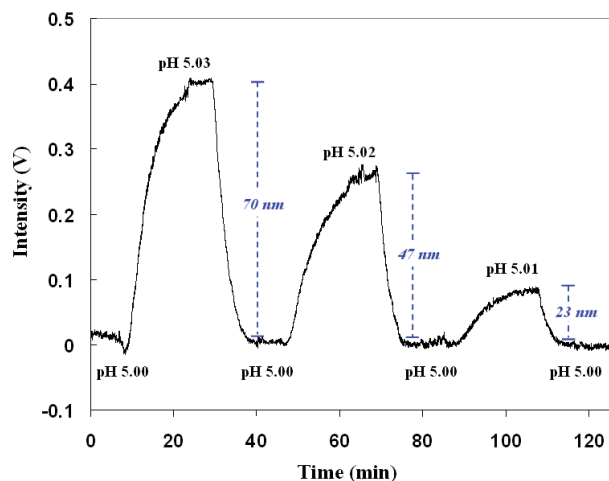


Fig. 8. Reversible variation in diffraction intensity due to small pH changes.

fluidic chamber after intended pH changes. The fluctuation between 29th and 32nd minutes resulted from bubbles in the chamber that occurred during the measurement.

To demonstrate that the hydrogel gratings can be used to measure small pH variations reversibly, measurements were performed with longer incubation times to ensure equilibrium. PBS solutions with pH 5.03, pH 5.02 and pH 5.01 were again prepared by mixing PBS with pH 5.0 and pH 5.1 with a volume ratio of 7:3, 8:2 and 9:1, respectively. The hydrogel grating was first immersed in pH 5 PBS solution for rehydration. Afterwards PBS solutions with pH 5.03, pH 5.02 and pH 5.01 were introduced by alternating with pH 5 between each step. The pH was changed to the next level only after the response had remained stable for at least 10 minutes.

The resulting diffraction intensity was plotted against time in Fig. 8. Again, the ordinate of the plot was arbitrarily set to zero and the plot was intentionally inverted to represent swelling with increasing pH. The diffraction intensity increased by 0.40, 0.26 and 0.08 V when pH changed by 0.03, 0.02 and 0.01, respectively. The corresponding grating height shifts are calibrated to be 70, 47 and 23 nm, which are in good agreement with those in Fig. 7. We attribute the small dip that occurred around the 8th minute and the nonzero slope of the curve before that to the possibility that pH 5.03 PBS was introduced too early, before the equilibrium was achieved during hydrogel rehydration. This did not affect the measurement since equilibrium was achieved for every pH measurement thereafter. The root mean square (rms) noise of this system is calculated to be around 5 mV over a 10 minute-period, which yields a signal-to-noise ratio of 16 at pH 5 for the 0.01 pH variation measurement. Hence the pH resolution of this diffraction sensor is about  $6 \times 10^{-4}$  pH units around pH 5. We would like to point out that this is a conservative estimate due to the diffractometer being at a relatively insensitive point (close to the bottom of the oscillating response in fig 4(a)) at pH = 5. For example, we expect the sensitivity to be even better around pH 4.6, where the diffractometer is more sensitive to motions. Alternatively, the diffractometer can be biased by means other

than changing the pH such as changing the illumination wavelength or controlling the effective grating height before the experiments. We also believe that the diffusion limited nature of the system's response time can be improved by reducing the width of the hydrogel stripes.

## V. CONCLUSION

We have demonstrated a smart hydrogel-based reflective diffraction grating as an ultrasensitive biochemical sensor. The sensor is easy to operate because of its relatively relaxed tolerance to alignment errors (since a number of stripes are illuminated in a grating) and its significantly more sturdy nature e.g. in comparison with many other micro-machined sensors with fragile components. During a measurement, the incident laser beam does not pass through the hydrogel grating but reflects back from its gold-coated top surface. Since the phase of the reflected laser only depends on changes in grating height and not in other optical changes in the hydrogel itself, calibration process to reveal a height change is reliable and straightforward. Despite its relatively simple nature, the sensor possesses remarkable sensitivity mainly due to the ability of reflective diffractometry to detect extremely small motions. Detection of small pH shifts (0.01 pH) was presented with a resolution of  $6 \times 10^{-4}$  pH units. Since the gratings presented here can be fabricated by replacing the pH hydrogel with other smart hydrogels responsive to various stimuli, we envision this concept to be applicable to a wide variety of target entities including glucose and antigens.

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