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Analyte-responsive holograms for (bio)chemical analysis

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Abstract

Analyte-responsive holograms comprise a holographic grating embedded in a smart hydrogel film. The grating acts as a reporter that enables analyte induced changes in the thickness of the associated polymer film to be accurately determined. Interaction of these materials with a specific analyte or stimuli leads to a change in the colour, image or brightness of the hologram and these changes can be visualised directly or quantified using a simple colour reader. Analyte-responsive holograms are inexpensive, robust and have proven suitable for detection of a wide range of clinically and industrially important analytes.

1. Introduction

The perceived utility of biosensors—analytical devices based on molecules that recognize specific biological substances—within the consumer, healthcare, biotechnology and biomedical industries is well established. However, commercial realization of these devices has been slow as early biosensor concepts were expensive, not sufficiently durable and unsuited to large-scale manufacture. These issues are now being addressed through the development of novel biorecognition systems coupled with transducer technologies amenable to mass-production techniques developed for the microelectronics, printing and photography industries. One such approach relies on the concept of using a simple reflection hologram as the interactive element in a truly mass-producible (bio)chemical sensor.

2. The holographic transduction principle

Holograms are of interest in a wide variety of fields such as artistic displays, optical security devices and holographic optical elements. Here we report on a remarkably simple, yet generic, approach for fabricating (bio)chemical sensors based on reflection holograms embedded within

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smart hydrogel films. Smart hydrogels consist of hydrophilic polymer networks which contain appropriate receptor groups and have the ability to undergo large reversible changes in solvent-swollen volume in response to specific stimuli including pH [1], ions [2, 3] temperature [4, 5], electric fields [6], solvents [7] and a number of chemical [8, 9] and biological analytes [10]. By coupling this swelling event with a suitable transduction system, a sensor can be constructed with the hydrogel acting as the analyte-responsive element.

Holographic gratings which are capable of exhibiting large effects as a result of volume changes need to be of the so-called 'Denisyuk' type [11]. Conventionally, Denisyuk holograms comprise a silver halide photographic emulsion coated onto suitable glass or plastic substrates, and are fabricated by passing a single, collimated laser beam through the film backed by a mirror [12]. Laser light reflected by the mirror interferes with the incident beam to establish a standing wave interference pattern within the thickness of the photosensitive polymer film. This interference pattern is recorded in the form of a latent image, which, after conventional development and fixing steps, manifests itself as a three-dimensional pattern comprising ultrafine grains of metallic silver, approximately 20 nm in diameter, distributed throughout the thickness of the polymer film. The silver grains lie in parallel planes, known as fringes, and are separated by a distance of approximately half the wavelength of the laser light used in their construction.

The interference planes are parallel to the substrate surface, much like the pages of a book, and together act as a Bragg grating. Under white light illumination the interference fringes reflect a specific narrow band of wavelengths, which recreates a monochromatic image of the original mirror used during hologram recording. Constructive interference between partial reflections from each fringe plane gives a characteristic spectral peak with a wavelength approximately governed by Bragg's law and determined by the spacing of the holographic fringes:

$$m\lambda = 2nd\sin\theta$$

where m is the diffraction order, λ is the wavelength of light *in vacuo*, n is the average refractive index of the system, d is the spacing of the diffracting plane and θ is the glancing angle between the direction of propagation of the incident light and the diffracting planes. Any physical, chemical or biological mechanism that changes the spacing of the fringes (d) or the average refractive index (n) will generate observable changes in the wavelength (colour) of the reflection hologram. For example, if a holographic grating is immersed in an aqueous sample, absorption of water by the hydrogel layer causes the grating to swell perpendicular to the plane of the substrate layer [13]. This swelling increases the holographic fringe separation and consequently red-shifts the diffraction wavelength with the embedded holographic grating acting as a sensitive reporter of film thickness, which can be easily quantified using a spectrometer and a white light source. Other types of hologram, for example, transmission or embossed holograms, do not behave like this as they possess an interference fringe structure which is perpendicular to the plane of the substrate surface and this makes them insensitive to changes in the thickness of the supporting polymer film [12]. The interference fringe structure of the sensor holograms are not permanently altered during such tests since the holograms can be swelled/de-swelled many times and always return to the same peak shape and reflectivity. Further work has shown that by incorporating holograms into smart hydrogel films that contain appropriate receptor systems it is possible to tune the reflection hologram to respond optically to the presence of specific target analytes such as specific ions or glucose.

3. Sensor hologram manufacture

The desire to record reflection holograms within non-conventional materials led to the development of a new system to transform pre-formed polymer films into holographic recording

materials [14]. The holograms are fabricated by coating a thin layer (∼10 μm thick) of unsensitized polymer film on a silanized glass slide and then immersing the slide sequentially in solutions of a silver salt and a bromide salt containing a photosensitizing dye, 1,1′-diethyl-2,2′-cyanine iodide. In this way, ultra-fine grains of photosensitive silver bromide (<20 nm in diameter) are precipitated within the matrix of the film thus transforming it into a holographic recording material. It is worth noting that the finished holograms consist only of metallic silver (Ag^0) suspended in the polymer film and are 'absorption' rather than 'phase' gratings. Consequently, they rely on diffractive reflection from the silver grains and not on the difference in refractive index between two transparent phases.

The holograms are typically recorded in these films with a single, 10 ns, pulse from a frequency doubled Nd:YAG laser (532 nm). The films are positioned over a front surface mirror prior to exposure with a spacer positioned at one end to hold the film at an angle of approximately 4° in relation to its surface [15]. Crucially, this small displacement from the horizontal position prevents the finished hologram from diffracting the incident light at the same angle as specularly reflected light. After a conventional photographic development step, illumination of the grating under white light recreates the monochromatic image of the plane mirror used in its construction with the constructive interference at each fringe plane, resulting in a characteristic spectral peak with a wavelength governed by the Bragg equation. Reflection holograms created using this technique were found to have similar brightness under the same exposure and processing conditions to those made from commercially available holographic recording materials [14].

The significance of this technique for the field of holography is considerable as the diffusion method circumvents the traditional laborious, emulsion-forming, methods that are currently used to construct holographic recording materials [16]. Crucially, for the construction of sensor holograms, the diffusion method makes it possible to record silver halide volume holograms in a wide range of previously inaccessible natural and synthetic smart polymer films, even those that are somewhat hydrophobic [7] or would otherwise encourage very rapid grain growth [17]. Generally speaking, the finished holograms are robust and relatively unresponsive to changes in the physical environment. For example, the holograms are not light sensitive since the grating consists only of Ag^0 metal, and with a suitable choice of polymeric matrix do not change colour with temperature. Nevertheless, it is advisable to thermostat the temperature of the medium in which the analysis is performed to ±1 °C. The simplicity and rapidity of this technique, combined with the sensing capability of smart hydrogels, offers the possibility of constructing inexpensive optical sensors for a range of putative analytes.

4. Analyte-responsive holograms

Solvent-responsive holographic sensors

Given their swelling behaviour in water, holographic gratings have been proposed for the direct measurement of water activity in liquids [13]. Immersion of gelatine holograms in 'wet' hydrophobic liquids results in swelling due to the absorption of water. The resultant red-shift in diffraction wavelength is proportional to the water activity (a_w) of the liquid. Because holographic sensors generate a visually perceptible colour change across the visible spectrum, they offer the possibility of use in a semi-quantitative format without the need for additional instrumentation. These indicators suggest that these holographic water sensors may find widespread application as power-free immersible water activity sensors in the petrochemical, food, textile, electronics and pharmaceutical industries.

By varying the hydrophobicity of the holographic film it is possible to tailor the response of the sensors to different solvents. A series of sensor holograms have been constructed in a

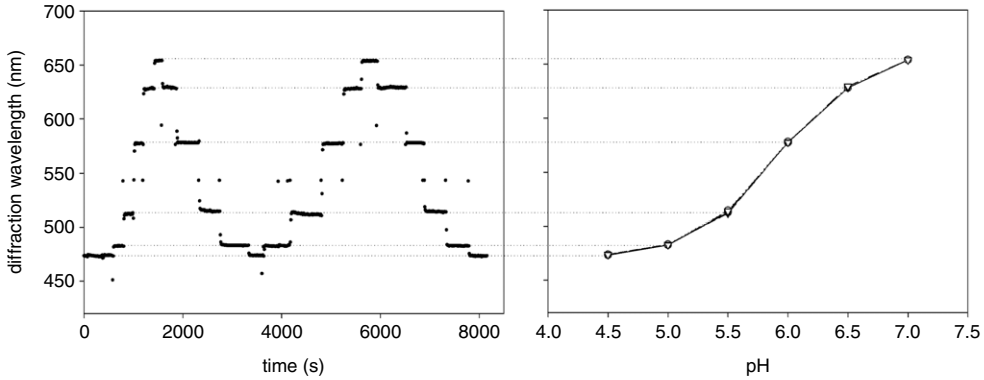

Figure 1. The effect of two cycles of step changes in pH (0.5 unit) within the range 4.5–7 on the replay wavelength response of a polyHEMA hologram containing 4 mol% methacrylic acid in 200 mM buffer at 30 °C.

range of different synthetic polymeric materials and tested for their sensitivity to ethanol in order to select one with the appropriate characteristics for use as an alcohol sensor [7]. Sensor holograms prepared in crosslinked poly(hydroxyethyl methacrylate) (polyHEMA) displayed a linear red-shift in diffraction wavelength across a wide range of alcohol concentrations. This sensor hologram was used to measure the alcohol content of a range of commercial alcoholic beverages such as wines and beers to within ±0.3% (vol) of their stated value. Crucially, as the reflected signal was monitored through the rear of the device that was not in contact with the test solution, the optical signal from the sensor hologram was unaffected by the highly coloured and/or turbid alcohol samples.

Ion-sensitive holographic sensors

The determination of pH and many ionic species is critical within the biomedical, environmental, food, beverage, security, agricultural and biotechnology industries. In the case of pH-sensitive sensor holograms the Bragg gratings are recorded within hydrogels containing acidic or basic monomers within the polymeric backbone [15]. Ionization of the pendant functional groups causes the grating to swell as a result of electrostatic and osmotic forces that draw in or expel counterions and water into or out of the gel phase. This increases the fringe separation and causes longer wavelengths to be selected for reflection from the holographic mirror, and hence the diffraction wavelength of the sensor hologram is dependent on the pH of the bathing medium (figure 1). To date, a series of pH sensor holograms have been constructed in two different hydrogel systems, polyHEMA and poly(acrylamide). Co-polymerization with monomers bearing ionizable groups and suitable crosslinkers has been shown to confer a characteristic pH sensitivity dependent on the individual functional monomer incorporated. Reversible and visually perceptible colour changes occur either side of an apparent dissociation constant (pK_a) as a function of pH and are governed by the nature of the ionizable monomer incorporated into the holographic matrix. Through selection of acidic or basic monomers with appropriate pK_a values, it is possible to tune the response of the resultant sensor hologram to the pH range of interest for a particular application.

The rational modification of polyHEMA with crown ethers was investigated to develop sensor holograms for K^+ and Na^+ [18]. Crown ethers are well known to form strong complexes with metal ions in solution [19]. Methacrylated crown ethers were incorporated into the polyHEMA film during photopolymerization and holograms containing either 12-crown-4, 15 crown-5 or 18-crown-6 were shown to respond to alkali and alkaline earth ions

with varying magnitudes and specificity. Holograms constructed with 18-crown-6 were shown to respond linearly over the concentration range relevant to the physiological measurement of K^+; furthermore, they were shown to be virtually unaffected by normal physiological variations in background sodium ion levels, highlighting their potential for use as blood potassium sensors [18]. Further work has investigated the use of holograms for the detection of divalent metal ions by incorporating chelating monomers into the holographic matrix. A methacrylated analogue of iminodiacetic acid (IDA) was successfully incorporated into a polyHEMA hologram to confer sensitivity to the presence of Ca^{2+}, Mg^{2+}, Co^{2+} and Zn^{2+} ions [20].

Sensor holograms for monitoring ionic strength have been fabricated from charged sulphonate and quaternary ammonium monomers, incorporated into the holographic matrix. As the salt concentration of the bathing medium is increased, the reversible electrostatic bonds that exist between positively and negatively charged groups are broken and hence the hologram swells as the effective crosslinking density is decreased. Unlike sensors that rely on monomers of a single polarity, these sensors could quantify ionic strength independent of the identity of the ionic species present in the test solution. This system was successfully used to quantify the ionic strength of milk solutions which contain a complex mixture of ions and biological components [21].

Sensor holograms for enzyme and enzymatic reactions

Holographic Bragg gratings can also be exploited as quantitative sensing elements for measuring enzyme activity [17, 22–24] or substrate concentration [25]. For example, a red-shift in diffraction wavelength occurs when an enzyme cleaves bonds in the holographic matrix. The holographic layer swells as the effective crosslinking density of the polymeric network is decreased by the action of the enzyme. This approach was initially demonstrated with a gelatine-based Bragg reflector which was used to quantify the protease trypsin which cleaves peptide bonds within the gelatine film [22, 23]. A quantitative optical response of the holographic element constructed in gelatine and designed to replay with a narrow range of peak wavelengths (665–696 nm) was shown for a range of trypsin concentrations down to 25 nM with a response time of 20 min. Later work involved constructing poly(vinyl alcohol) (PVA) holograms which were modified with the trypsin substrate, poly(L-lysine). The incorporation of poly(L-lysine) rendered the otherwise unresponsive hologram sensitive to the protease trypsin, whilst remaining unresponsive to other proteases such as chymotrypsin [24]. A similar approach has been used to quantify α-amylase activity, but this time using a starch-based Bragg grating [17]. The enzyme α-amylase degrades the starch-based matrix resulting in a decrease in both diffraction efficiency and wavelength. Abnormal levels of trypsin or α-amylase activity within the human body are indicative of pancreatic disorder and this behaviour demonstrates the potential of the sensor technology to detect trypsin or amylase in pancreatic or salivary extracts.

An alternative approach to monitoring enzymatic reactions relies on the sensor hologram detecting the products of that reaction. The concept of these enzyme-linked holographic sensors was demonstrated for the clinically and industrially relevant metabolites urea and penicillin [25]. The action of the enzymes urease and penicillinase on their respective substrates is known to cause acidification or alkalinzation of a test solution. Each enzyme was immobilized to an appropriate holographic pH sensor and the pH changes resulting from the enzymatic reactions were sensitively measured and correlated with the original levels of urea and penicillin. These particular devices are representative of a generic system whereby enzymes can be integrated with holograms to generate a series of inexpensive/disposable sensors for a wide range of biochemical analytes.

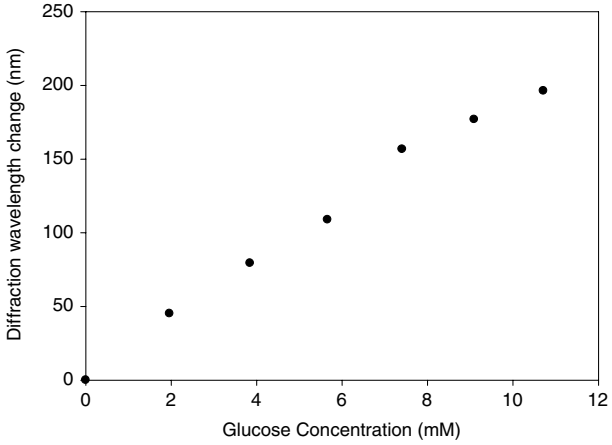

Figure 2. The change in diffraction wavelength of a 20 mol% 3-APB hologram immersed in pH 7.4 phosphate buffered saline (I = 150 mM NaCl) solutions of varying glucose concentration at 30 °C.

Glucose-sensitive holographic sensors

Diabetes represents one of the largest health concerns of the 21st century [26]. Sensor holograms that detect glucose have been fabricated from hydrogel films containing chemical ligands based on phenylboronic acid derivatives. The ability of boronic acids to bind glucose has long been known, although this normally occurs only at alkaline pH [27, 28]. More recently, new boronic acid derivatives that bind glucose at physiological pH values have been developed and these have been utilized in the fabrication of sensor holograms. Acrylamide-based hydrogels containing the monomer 3-acrylamidophenylboronic acid (3-APB) have been fabricated and the chemical composition of the films optimized for glucose detection using embedded reflection holograms [29, 30].

The sensor holograms display a monotonic red-shift in diffraction wavelength as a function of glucose concentration across the normal glucose concentration range (2–10 mM) at physiological pH and ionic strength values (figure 2). Glucose diffuses into the hologram from the bulk medium and binds to the pendant boronic acid groups. This effectively decreases the pK_a of the boronic acid–glucose complex by stabilizing the charged tetrahedral phenylboronate anion, and the presence of these charged groups within the polymer generates a Donnan potential resulting in an osmotic pressure that causes the hologram to imbibe more water thereby red-shifting the diffraction wavelength. The reaction of glucose with boronic acids is unusual since the covalent bond formed between the two molecules is reversible [31] and when the glucose is removed from the bathing medium, the hologram contracts and returns to its original diffraction wavelength. These observations suggest that the sensor is suitable for continuous real-time sensing of dynamic changes in glucose concentration. Current work in glucose detection is focused on assessing the performance of these sensor holograms for the detection of glucose in complex biological media and their incorporation into a suitable sensor format for continuous glucose monitoring in humans.

Sensor holograms for monitoring bacterial fermentations

There is much interest in the use of small-scale bioprocesses ($\sim\mu$l volumes) for fast, reliable and inexpensive high-throughput bioprocessing to enable early process development studies

and the screening of drug candidates in drug discovery and development. However, in small volumes and in a parallel format it is difficult to use standard industrial sensors because of their dimensions. Due to the ease of miniaturizing the sensor holograms and the fact that they can be sterilized *in situ* by autoclaving, they have been successfully utilized to monitor the metabolic products of small-scale (<1 ml) microbial fermentations. For example, sensor holograms have been used to monitor the homolactic fermentation of milk by *Lactobacillus casei* thorough the change in pH generated during the fermentation process [15]. In other work, the depletion of glucose during the growth of *Bacillus subtilis* has been monitored holographically using glucose sensor holograms [30]. In this case, these sensor holograms offer the considerable advantage that they do not consume glucose, unlike conventional enzyme-based systems, and thereby do not reduce substrate levels in small-volume bio-reactors which can lead to alterations in the metabolism of the living cells being monitored. Calcium ion sensor holograms have been applied to monitor the germination of *Bacillus megaterium* spores by measuring the efflux of calcium into the medium in real time [20]. The organism is a model for *Bacillus anthracis*, and current work is focused on further characterizing this approach and assessing its potential for deployment as a rapid anthrax detection system.

5. Summary

The combination of responsive-polymeric matrices, which incorporate selective recognition elements, with holographic gratings provides an inexpensive and generic sensor technology. The gratings act as an optically interrogatable reporter that enables analyte-induced changes in the thickness of the supporting polymer layer to be accurately determined. Recent advances in hologram fabrication techniques have enabled the use of a wide range of natural, synthetic or rationally designed films that would not ordinarily be suitable for use as holographic recording materials. The resultant sensors have proven suitable for use in opaque and very turbid samples and are very stable with respect to calibration, as, unlike optical sensing methodologies involving fluorescent dyes, the gratings do not suffer problems associated with photobleaching. Furthermore, the sensors display comparable selectivities and sensitivities to sensors currently in use which utilize similar recognition phenomena. These characteristics suggest that these sensor holograms may be highly suitable for the construction of simple, inexpensive and robust (bio)chemical sensors that can be readily deployed in the field for use at the point of need.

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