“Smart” Holograms – A Novel Diagnostics Platform.

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ABSTRACT

“Smart” holograms are novel sensor systems which utilise diffraction as the transduction method for the detection of physical stimuli or (bio)chemical analytes. Interaction of these sensors with a specific analyte or stimulus changes the colour, image or brightness of the hologram and these changes can be visualised directly by the user or quantified using a simple colour reader. Sensor holograms are inexpensive, robust, label-free, format flexible systems which can be engineered to be sensitive to a wide range of analytes.

Keywords: Hologram, Sensor, Diffraction, Glucose

1 INTRODUCTION

Sensor holograms utilise the principles of volume holography with a Denisyuk holographic grating recorded within a “smart” polymer system. Unlike conventional holographic recording media, the “smart” polymers are rationally designed synthetic polymers [1] with receptors that allow them to interact with a highly specific stimulus or analyte. Interaction of the analyte with the hologram causes a change in the swelling state or cross-linking density of the polymer, which in turn results in a change in the recorded hologram. A range of sensors have been designed using these principles including systems for detecting pH [2], ionic strength [3], sodium ions, potassium ions [4], calcium ions [5], alcohol [6], water [7], glucose [8-10], a variety of enzymes [11, 12], bacterial cells [13] and physical stimuli.

2 SMART HOLOGRAM MANUFACTURE

Smart Holograms are constructed by coating a thin layer (~10 μm thick) of un-sensitised polymer film on a glass or plastic substrate and then immersing the film sequentially in solutions of silver salts and bromide salts containing a photosensitising dye [14]. This process precipitates ultra-fine grains of photosensitive silver bromide (< 20 nm in diameter) within the matrix of the film thus transforming it into a holographic recording material. Typically, to construct a Holographic Sensor, a hologram of an off-axis plane mirror is recorded in these films with a single, 10 ns, pulse from a frequency doubled Nd:YAG laser (532 nm) [2]. 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 approximately governed by Bragg’s Law:

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

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 incident light propagation direction and the diffracting planes. Any physical or (bio)chemical mechanism that changes 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 water, the film swells perpendicular to the plane of the substrate layer. This swelling increases the holographic fringe separation and consequently red-shifts the diffraction wavelength which produces a visually perceptible response which can be quantified using a spectrometer or a dual layer photodiode coupled with a simple white light source.

3 SMART HOLOGRAPHIC SENSORS

By incorporating holograms into smart polymer films that contain appropriate receptor systems, it is possible to produce holograms which optically respond to the presence of specific target analytes such as specific ions or glucose [12].

Highly sensitive water sensors can be fabricated which respond rapidly to small amounts of water in solvents [7] or even to moisture saturated air (Figure 1). By tailoring the hydrophobicity of the holographic film, it is possible to tailor the response of the sensors to solvents other than water [6] as well. A series of holograms were constructed in a range of different synthetic polymeric materials and tested for their sensitivity to ethanol. Holographic sensors prepared from crosslinked poly(hydroxyethyl methacrylate) 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% (volume) of their stated value [6]. Since these Smart Holograms generate a perceptible colour change across the visible spectrum, they offer the possibility for use as power-free, visual alcohol sensors.

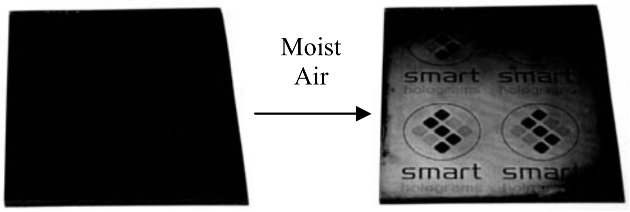

Figure 1. Appearance of a holographic image (right) on exposing a sensor hologram to moisture saturated air.

Crown ethers are well known to form strong complexes with metal ions in solution, and by incorporating these into appropriate holograms, sensors for K^+ and Na^+ have been developed [4]. Holograms constructed with 18-crown-6 were shown to respond linearly over the concentration range relevant to the physiological measurement of K^+. Additionally, they were shown to be virtually unaffected by normal physiological variations in background sodium ion levels, suggesting their potential for use as blood potassium sensors. Further work has shown that a methacrylated analogue of iminodiacetic acid (IDA) can be successfully incorporated into a polyHEMA hologram to confer sensitivity to the presence of Ca^{2+}, Mg^{2+}, Co^{2+} and Zn^{2+} ions [5]. Furthermore, sensor holograms for monitoring ionic strength can be fabricated from charged sulphonate and quaternary ammonium monomers, incorporated into a holographic matrix [3]. As the salt concentration of the bathing medium increases, the reversible electrostatic bonds between positively and negatively charged groups are broken, causing the hologram to swell.

By incorporating acidic or basic groups into the holographic films it is possible to construct holograms which respond to pH [2]. Ionisation of acidic or basic groups causes the grating to swell as a result of electrostatic and osmotic forces that draw counterions and water into the interior of the hologram. This increases the fringe separation and hence, the diffraction wavelength of the sensor hologram is dependent on the pH of the bathing medium. Reversible and visually perceptible colour changes occur either side of an apparent dissociation constant (pK_a) as a function of pH and by selecting acidic or basic monomers with appropriate pK_a values, it is possible to tune the response of the hologram to the pH range of interest for a particular application. For example, by coupling an enzyme with a suitable smart hologram, it is possible to monitor the enzyme's reaction by the resulting pH change [15]. The concept of these enzyme-linked sensors was demonstrated for the clinically and industrially relevant metabolites urea and penicillin with the pH changes resulting from the enzymatic reactions being sensitively measured to determine the original levels of urea and penicillin [15]. This approach represents a generic system for producing a host of inexpensive/disposable sensors for a wide range of biochemical analytes. Another approach for monitoring enzyme reactions involves monitoring the degradation of a smart hologram by specific enzymes. For example, a red-shift in diffraction wavelength occurs when an enzyme cleaves bonds in the holographic film thus causing it to swell. Abnormal levels of trypsin or amylase within the human body are indicative of disease and sensor holograms have been successfully fabricated to detect both of these analytes at diagnostically relevant concentrations [11].

There are over 150 million diabetics worldwide and this number is expected to grow very rapidly over the next 20 years [16]. There is therefore a growing demand for simple, robust continuous measuring systems for blood glucose.

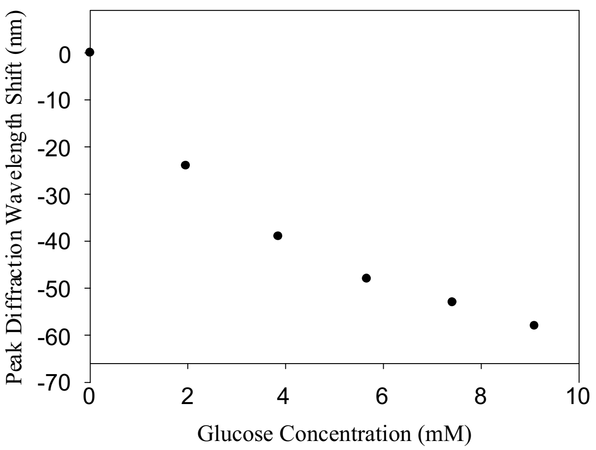

Figure 2. Response of an acrylamide co-polymer containing 20 mol% 3-acrylamidophenylboronic acid and 20 mol% *N*-[3-(dimethylamino)propyl]acrylamide to glucose in phosphate buffered saline at pH 7.4, 30º C.

Holographic glucose sensors are being developed in a variety of formats to be used in a similar fashion to commercial blood stick monitoring and as continuous monitors of blood or interstitial fluid glucose levels. Smart Holograms that respond to glucose levels have been constructed from holograms containing phenylboronic acid derivatives [8-10, 13, 17]. The ability of boronic acids to bind glucose has long been known [18, 19]. Acrylamide-based films 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 [8, 10, 17]. These holographic sensors display a monotonic 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). The reaction of glucose with boronic acids is unusual since the covalent bond formed between the two molecules is reversible [9]. When glucose is removed from the bathing medium, the hologram returns to its original diffraction wavelength value indicating that these sensors are suitable for continuous, real-time sensing of dynamic changes in glucose concentration. Initial *in vitro* testing in human plasma samples has shown that these sensors can function within such media. Glucose sensitive holograms have also been incorporated into contact lenses [20, 21]. A successful initial trial on a human subject [21] has shown that these sensors can be used as a non-invasive monitor for tear glucose which can then be related to the blood glucose concentration [22].

A rapid detection system for various pathogens is being developed [12]. This can be used within clinical, home, industrial or military fields and allows for rapid detection of pathogens. The system utilises a disposable microfluidics chip alongside an electronic reader system (Figure 3). The sample is placed within the chip and is automatically processed. Growth of live pathogens is then registered by a change in the embedded holographic sensor which detects changes in the metabolite or enzyme content of the growth media within the chip. Much of the work to date has been focused on spore detection systems. Calcium ion sensor holograms have been utlized to monitor the germination of *Bacillus megaterium* spores [5]. The organism is a model for *Bacillus anthracis*, and current work is focused on further characterising this approach and assessing its potential for deployment as a rapid anthrax detection system.

Figure 3. Prototype electronic reader and disposable chip with incorporated sensor hologram.

4 SUMMARY

Smart Holograms represent a novel and generic sensing technology platform for a wide variety of diagnostics applications. The sensor hologram interacts with the target analyte causing a change in its swelling state and a concurrent change in the optical properties of the holographic image. The change in the image can be used to quantify the target analyte. The simplicity and inexpensiveness of this approach suggests that these Smart Holograms may be highly suitable for the construction of (bio)chemical sensors that can be easily deployed at the point-of-need.

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